2012

THE POTENTIAL OF INDUSTRIAL WASTE AND AGRICULTURAL FEEDSTOCK TOWARDS SUSTAINABLE BIOFUELS PRODUCTION: TECHNO-ECONOMIC AND ENVIRONMENTAL IMPACT PERSPECTIVES

Felix K. Adom
Michigan Technological University

THE POTENTIAL OF INDUSTRIAL WASTE AND AGRICULTURAL FEEDSTOCK TOWARDS SUSTAINABLE BIOFUELS PRODUCTION: TECHNO-ECONOMIC AND ENVIRONMENTAL IMPACT PERSPECTIVES

By

Felix K. Adom

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Chemical Engineering

MICHIGAN TECHNOLOGICAL UNIVERSITY
2012

This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Chemical Engineering

Department of Chemical Engineering

Dissertation Advisor:	*Robbins Professor. David R Shonnard*
Committee Member:	*Professor. Susan T Bagley*
Committee Member:	*Dr. Tony N Rogers*
Committee Member:	*Dr. Wenzhen Li*
Department Chair:	*Professor Komar Kawatra.*

Table of contents

List of figures

List of tables

Preface

This dissertation titled "The potential of industrial waste and agricultural feedstock towards sustainable biofuels production: Techno-economic and environmental impact perspectives," centers on the efficient utilization of biomass feedstock for the production of value added bioproducts and carbon footprint analysis. This PhD research work in its entirety comprises of three components; characterization studies, hydrolysis experiments and sustainability analysis.

All laboratory experiments and computer simulation works were implemented in consultation with my PhD advisor Professor David Shonnard and periodic interaction with other PhD committee members. With the help of Jiqing Fan (PhD candidate), Jamie Davis (undergraduate researcher) and Paul Dunn (undergraduate researcher), various analytical experiments were conducted to understand the chemical and structural components of defatted corn syrup from a dry corn mill facility for the characterization studies.

The hydrolysis experiments required the development of an optimized hydrolysis pathway to produce fermentable sugars and amino acid platform using defatted corn syrup as a feedstock. After experimental design in consultation with my advisor, Jiqing Fan, Jamie Davis and Amanda Taylor (undergraduate researcher) assisted in conducting various experiments as well as analyzing data for the sugar platform optimization. For the amino acid platform optimization using the syrup, after experimental design in consultation with my advisor, Paul Dunn and Stefan Ruccins supported in implementing various experiments as well as analyzing data.

The sustainability analysis component is comprised of three subcomponents namely techno-economic analysis (TEA) and life cycle assessment (LCA), carbon footprint of dairy feeds for milk production in the U.S., and carbon footprint of a dairy feed from a dairy mill in Michigan, U.S. The TEA and LCA models were constructed in close

collaboration with Dr. Tony Rogers (PhD committee member). Both the research on carbon footprint analysis of dairy feed and a feed mill were a collaboration study with University of Arkansas. Ashley Maes and Charles Workman (undergraduate researchers) lent support to collected data as well as construct the carbon footprint models in excel spreadsheet. Collaborators from University of Arkansas (Greg Thoma and Zara Clayton-Niederman) helped analyze some other dairy feeds (forage crops) and provided timely feedback.

Finally, chapters 2, 3, 4, and 5 are planned manuscripts for future submission. Chapter 6 and 7 have already been published in International Journal and Life Cycle Assessment (Springer) and Internation Dairy Journal respectively (Elsevier). With the kind permission of both Springer (see Figure D-1) and Elsevier (see Figure E-1), this work has been reproduced for use in this dissertation.

Acknowledgements

This project reflects the work of a great many people and has been completed successfully through the individual contribution and moral support of individuals and some corporate sponsors. The author is indebted to his primary advisor, Dr. David Shonnard who supervised this Ph.D. research, provided insights, academic and technical support in this study. Sincere gratitude is due to members of the supervisory committee: Dr. Susan Bagley, Dr. Tony Rogers and Dr. Wenzhen Li without whose support this study would have been defective. The author would also like to gratefully acknowledge the funding sources that made my Ph.D. work possible. The Dairy Management Incorporated and the Michigan Energy Development Center funded me. I am appreciative of my fellow graduate students particularly Michael Brodeur-Campbell, Jiqing Fan, Jordan Klinger and Jifei Liu for their support. The author wishes to express appreciation to his family: my parents Samuel and Elizabeth Adom, brother Augustus Adom, sister Patricia Adom, fiancée Carolyn Adama and friends for their support during this period of study. I would also like to express my sincere gratitude to Jerry Anane Frimpong and Nicholas Kyei-Baffour for their fatherly support and advice. Their unwavering love, encouragement and belief in me have brought me this far. Finally, my biggest gratitude goes to Lord God Almighty for giving me the strength and intelligence to complete my PhD study successfully. May His name be forever extolled.

List of abbreviations

AAA - Amino acid analysis
ABE - Acetone Butanol Ethanol
Ar/Mn - Arabinose/mannose
ASL - Acid soluble lignin
AIL - Acid insoluble lignin
Ala - Alanine
Arg - Arginine
Asn - Asparagine
Asp - Aspartic acid
BSA - Bovine Serum Albumin
CF - Correction factor (Anhydrous)
Cys - Cysteine
DAH - Dilute acid hydrolysate
DOE - Department of Energy
DAP - Dilute acid pretreatment
DCS - Defatted corn syrup
DDGS - Dried distillers grains with solubles
DM - Dry matter
DWG - Distillers wet grains
EH - Enzymatic hydrolysis
EAA - Essential amino acids
FMOC - 9-Fluorenylmethyl chloroformate
Gln - Glutamine
Glu - Glutamic acid
Gly - Glycine
GHG - Greenhouse gas
GWP – Global warming potential
His - Histidine
HPLC - High-performance liquid chromatography
HMF - Hydroxymethylfurfural
Ile - Isoleucine
LAP - Laboratory analytical procedures
Leu - Leucine
LPS – Low pressure steam
Lys - Lysine
Met - Methionine
M - Million
NEAA - Non-essential amino acids
NREL - National Renewable Energy Lab
NSC - Non-starch carbohydrates
OPA - O-phthalaldehyde
Otd - Ornithine
PES - Potato extracted starch
Phe - Phenylanlanine
Pro - Proline
SD - Sustainable development
Ser - Serine
SRS - Sugar recovery standards
%Starch - Percentage of starch
%R starch - Starch recovery standards
T - Thousand
TBC - Target bio-based chemical
Thr - Threonine
TMS: Total monomer sugars
Try - Tryptophan
TS - Thin stillage
Tyr - Tyrosine
Val - Valine
VOC – Volatile organic compounds
X - Yield

Abstract

This Ph.D. research is comprised of three major components; (i) Characterization study to analyze the composition of defatted corn syrup (DCS) from a dry corn mill facility (ii) Hydrolysis experiments to optimize the production of fermentable sugars and amino acid platform using DCS and (iii) Sustainability analyses. Analyses of DCS included total solids, ash content, total protein, amino acids, inorganic elements, starch, total carbohydrates, lignin, organic acids, glycerol, and presence of functional groups. Total solids content was 37.4% (± 0.4%) by weight, and the mass balance closure was 101%. Total carbohydrates [27% (± 5%) wt.] comprised of starch (5.6%), soluble monomer carbohydrates (12%) and non-starch carbohydrates (10%). Hemicellulose components (structural and non-structural) were; xylan (6%), xylose (1%), mannan (1%), mannose (0.4%), arabinan (1%), arabinose (0.4%), galatactan (3%) and galactose (0.4%). Based on the measured physical and chemical components, bio-chemical conversion route and subsequent fermentation to value added products was identified as promising. DCS has potential to serve as an important fermentation feedstock for bio-based chemicals production.

In the sugar hydrolysis experiments, reaction parameters such as acid concentration and retention time were analyzed to determine the optimal conditions to maximize monomer sugar yields while keeping the inhibitors at minimum. Total fermentable sugars produced can reach approximately 86% of theoretical yield when subjected to dilute acid pretreatment (DAP). DAP followed by subsequent enzymatic hydrolysis was most effective for 0 wt% acid hydrolysate samples and least efficient towards 1 and 2 wt% acid hydrolysate samples. The best hydrolysis scheme DCS from an industry's point of view is standalone 60 minutes dilute acid hydrolysis at 2 wt% acid concentration.

The combined effect of hydrolysis reaction time, temperature and ratio of enzyme to substrate ratio to develop hydrolysis process that optimizes the production of amino

acids in DCS were studied. Four key hydrolysis pathways were investigated for the production of amino acids using DCS. The first hydrolysis pathway is the amino acid analysis using DAP. The second pathway is DAP of DCS followed by protein hydrolysis using proteases [Trypsin, Pronase E (*Streptomyces griseus*) and Protex 6L]. The third hydrolysis pathway investigated a standalone experiment using proteases (Trypsin, Pronase E, Protex 6L, and Alcalase) on the DCS without any pretreatment. The final pathway investigated the use of Accellerase 1500® and Protex 6L to simultaneously produce fermentable sugars and amino acids over a 24 hour hydrolysis reaction time.

The 3 key objectives of the techno-economic analysis component of this PhD research included; (i) Development of a process design for the production of both the sugar and amino acid platforms with DAP using DCS (ii) A preliminary cost analysis to estimate the initial capital cost and operating cost of this facility (iii) A greenhouse gas analysis to understand the environmental impact of this facility. Using Aspen Plus®, a conceptual process design has been constructed. Finally, both Aspen Plus Economic Analyzer® and Simapro® sofware were employed to conduct the cost analysis as well as the carbon footprint emissions of this process facility respectively.

Another section of my PhD research work focused on the life cycle assessment (LCA) of commonly used dairy feeds in the U.S. Greenhouse gas (GHG) emissions analysis was conducted for cultivation, harvesting, and production of common dairy feeds used for the production of dairy milk in the U.S. The goal was to determine the carbon footprint [grams CO_2 equivalents (gCO_2e)/kg of dry feed] in the U.S. on a regional basis, identify key inputs, and make recommendations for emissions reduction. The final section of my Ph.D. research work was an LCA of a single dairy feed mill located in Michigan, USA. The primary goal was to conduct a preliminary assessment of dairy feed mill operations and ultimately determine the GHG emissions for 1 kilogram of milled dairy feed.

Chapter 1
1 Introduction

1.1 Motivation

Perhaps the most widely used definition of sustainable development (SD) is the Brundtland Commission's version, which states that "ability to make development sustainable-to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs (Bruntland 1987)." In addition, SD was defined as "development without growth-that is, qualitative improvement in the ability to satisfy (needs and desires) without quantitative increase in throughput beyond environmental carrying capacity (Daly and Farley 2010). Carrying capacity is the population of humans that can be sustained by a given ecosystem at a given level of consumption, with a given technology." Generally, SD is viewed as some combination of the "triple bottom line" of economic development, social development, and environmental / resource sustainability (Solomon 2010).

Driven mostly by population and gross domestic product (GDP), the annual energy consumption in the U.S. has increased steadily by more than 200% since 1950 (Krupnick et al. 2010). Atmospheric concentrations of CO_2 have increased from pre-industrial levels of about 280 parts per million (ppm) to their current levels of about 395 ppm and this increase over pre-industrial levels is mainly due to anthropogenic emissions (Pachauri 2007). Key global sustainability challenges facing the Earth's population in the 21st century are related to the nearly total complete reliance on fossil fuel for energy consumption, energy's environmental consequences, and finally the impact of the rapid development of the four major developing continents: Africa, Asia, Latin America and other small island developing states.

Transition to bio-based raw materials as opposed to fossil resources has long been touted as the key to addressing some of these challenges (Mowrey and Spain 1999; Simmons et al. 2008; Hallac et al. 2009; Solomon 2010). The primary drivers for the

use of biomass as a renewable feedstock includes, but is not limited to, decreasing reliance on fossil fuels (energy security), and as a means of addressing concerns over the contribution of fossil-fuel consumption by the transport sector to global warming (McKendry 2002). In the USDA-DOE billion ton update report (United States. Dept. of Energy 2011), it was established that the U.S. has enough biomass to sustainably displace about 1/3 of its petroleum demand. The development of environmentally benign technologies to tap biomass resources as well as policies to promote the use of renewable energy should be complemented with the development of science-based sustainability metrics and indicators to measure progress.

The Climate Change Technology Program (CCTP) established administratively in 2002 with authorization by the Energy Policy Act in 2005 (Congress 2005), has the mandate of accelerating the development and deployment of technologies that can reduce, avoid, or capture and store greenhouse gases (GHG). Four key goals of technology strategy were identified as important; ***end-use efficiency and infrastructure***, ***energy supply***, ***carbon capture and sequestration*** and ***non-CO_2 GHG's abatement technologies***. End-use efficiency and infrastructure emphasized on four major sectors; transportation, buildings, industry, and the electric grid. Improved vehicle efficiency, electric-fuel engine hybrids ("hybrid-electric" vehicles and "plug-in hybrids"), and clean diesel engines are a few examples under transportation. CCTP also emphasized on two key areas for industry. Firstly, technologies should be developed to improve efficiency of process heating and enhanced industrial plant design. These technologies should have the capability of reducing waste and material use intensity through material and waste energy recycling processes. Secondly, process technologies should increase the use of industrial by-products and waste materials as a potential energy sources and raw materials. Doing this will create an industry that can self-generate clean energy, making it more sustainable and less dependent on other sources of energy.

CCTP has also identified energy supply as a potential for large-scale GHG mitigation. It emphasizing four major sectors namely; **i.** emission reduction from energy supply, **ii.** fossil-based fuels and power, **iii.** hydrogen, renewable energy & fuels, and **iv.** nuclear fission. Integrated gasification combined system and oxy-fuel combustion, hydrogen production from natural gas and biomass, low-speed wind turbines, biochemical reactors for conversion of sugar to ethanol, the bio-refinery concept and gasification or pyrolysis to produce bio-fuels are some proposed sustainable energy technologies.

Carbon Sequestration focuses on carbon capture, geologic storage and terrestrial sequestration. Amine scrubbing, CO_2 injection with oil or methane recovery and cropland, forestland management with advanced information technologies are examples of some of the technologies that are currently available for deployment. However, there are still some economic, environmental and political challenges. Other non-CO_2 GHG such as methane, nitrous oxide (N_2O), and the halocarbons (e.g. HFCs, CFCs and HCFCs typically contained in coolants) contributes to warming the atmosphere. Some technologies proposed by CCTP include; aerobic and anaerobic bioreactor treatment, advance agricultural sensors, nitrogen transformation inhibitors, controlled release fertilizers, and N_2O abatement technologies for nitric acid production.

In line with the CCTP strategic goals, there is an urgent need to develop technologies capable of reducing waste and material use intensity through material recycling processes. Human beings generate tons of wastes daily, and there is also the need to increase usage of industrial by-products. Another underlying factor for SD is the establishment of scientific based sustainability metrics and indicators as a means of tracking progress in developing sustainable products / processes for various industries. In addition to the internationally established methods for measuring sustainability impacts (ISO 2006a; 2006b; Sinden et al. 2008), other researchers (Allen and Shonnard 2001; Reinhard et al. 2011; Hennecke et al. 2012) have to a great extent reported on this in the literature.

1.2 PhD research objectives

The primary direction of this Ph.D. research was defined by three major components. The first component of the research is the characterization study to analyze the composition of an industrial process residue [defatted corn ethanol mill syrup (DCS)] to evaluate its suitability for conversion to biofuels and bio-products. A second research component is hydrolysis experiments which were focused on developing processing conditions and techniques to optimize the release of fermentable intermediate products (sugars & amino acids). These intermediates may serve as a platform for the production of higher value products. The third component of this Ph.D. research program includes sustainability analyses, and has three further subcategories. The aim of subcategory "a" was to develop a conceptual process design for the production of two intermediate products; fermentable sugars and amino acids using DCS as the feedstock to investigate how the interplay between the economic and environmental impacts will influence commercial scale up in the future. The aim of subcomponent "b" was to understand the environmental impact of commonly used dairy feeds such as grains, forage crops and other co-products like soybean meal and distiller's grain cultivated and harvested across the U.S. Finally, the aim of subcomponent "c" was to analyze the carbon footprint (GHG emissions) of producing dairy feed from a feed mill in the U.S. and including transport to local dairy markets.

Specific objectives of this Ph.D. project are highlighted below;

1. A compositional analysis of DCS to investigate the following; total solids, ash content, protein and amino acids, inorganic elements, starch, structural and soluble carbohydrates, lignin, organic acids, glycerol, and functional groups.
2. Optimization of the release of fermentable sugars from DCS via dilute acid pretreatment (DAP) and enzymatic hydrolysis (EH) by varying taking into account the following process variables: reaction time, temperature and acid catalyst concentrations was also investigated.

3. Optimization of the release of fermentable amino acids from DCS using DAP and proteases for protein hydrolysis (PH) taking into account the following process variables: temperature, reaction time, and enzyme/substrate ratio was also investigated.
4. Application of process simulation software (Aspen plus ®) and environmental life cycle assessment software (Simapro ®) to model the optimized hydrolysis pathway and to investigate the initial capital cost and associated environmental impacts.
5. Determination of the carbon footprint (GHG emissions) from the cultivation and harvesting of U.S. dairy feeds on a basis of 1 kg of feed harvested or produced in units of grams CO_2 equivalents (gCO_2e) / kg of dry feed.
6. The final task was to develop Life Cycle Assessment (LCA) methodology applicable to the animal feed mill industry to accommodate a large number of inputs and activities associated with dairy mill operations and to help understand its environmental impacts [Greenhouse gas (GHG) emissions only].

1.3 Dissertation outline

This dissertation comprise of eight chapters. The first chapter introduces the "triple bottom line" concept of sustainability and further identifies various technologies for addressing global sustainability challenges. This section further identifies what industry needs to do (a key motivation for this Ph.D. project) in order to address sustainability issues, and it emphasizes on the need to use internationally established metrics and indicators as a means of tracking progress in developing sustainable products / processes. Chapters 2-4 present the methods, results, and analyses for DCS compositional analyses, hydrolysis optimization of carbohydrates to produce sugars, and hydrolysis optimization of protein to produce amino acids. Chapter 5 reports on results from the process simulation and LCA analyses to produce sugar and amino acids as intermediate products. Chapters 6 and 7 report the LCA analyses for the various dairy feed crops as well as the mill impact analyses focusing on GHG emissions. Finally, in chapter 8, a summary of all findings from the Ph.D. research are reported, conclusions are drawn and potential future research projects have been

recommended. Some repetition may be observed given that each major chapter has been prepared as a "stand-alone" article for publication in peer reviewed journals.

1.4 References

Allen DT, Shonnard DR, Green engineering: environmentally conscious design of chemical processes. Prentice Hall: **2001**.

Bruntland G, Our common future: the world commission on environment and development. Oxford: Oxford University Press: **1987**; Vol. 1.

Congress U. 2005. Energy Policy Act of 2005. Public Law 109:58.

Daly HE, Farley J, Ecological economics: principles and applications. Island Pr: **2010**.

Hallac BB, Sannigrahi P, Pu Y, Ray M, Murphy RJ, Ragauskas AJ. 2009. Biomass characterization of Buddleja davidii: a potential feedstock for biofuel production. Journal of agricultural and food chemistry 57(4):1275-1281.

Hennecke AM, Faist M, Reinhardt J, Junquera V, Neeft J, Fehrenbach H. 2012. Biofuel greenhouse gas calculations under the European Renewable Energy Directive–A comparison of the BioGrace tool vs. the tool of the Roundtable on Sustainable Biofuels. Applied Energy.

ISO E. 2006a. 14040: 2006. Environmental management–Life cycle assessment–Principles and framework.

ISO E. 2006b. 14044: 2006. Environmental Management–Life Cycle Assessment–Requirements and Guidelines.

Krupnick AJ, Parry IWH, Walls M, Knowles T, Hayes K. 2010. Toward a new national energy policy: assessing the options. Washington, DC: Resources for the Future.

McKendry P. 2002. Energy production from biomass (part 1): overview of biomass. Bioresource technology 83:37-46.

Mowrey A, Spain J. 1999. Results of a nationwide survey to determine feedstuffs fed to lactating dairy cows. Journal of dairy science 82(2):445-451.

Pachauri RK. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Change 446(November).

Reinhard J, Emmenegger MF, Widok AH, Wohlgemuth V. A LCA Tool for the Assesment of Biofuels Sustainability. 2011.

Simmons BA, Loque D, Blanch HW. 2008. Next-generation biomass feedstocks for biofuel production. Genome Biol 9(12):242.

Sinden G, Skea S, Jefferiss P, Clift R, Jackson T, Ilot T. 2008. PAS 2050: 2008, Specification for the Assessment of the Life Cycle Greenhouse Gas Emissions of Goods and Services.

Solomon BD. 2010. Biofuels and sustainability. annals of the New York Academy of Sciences 1185(1):119-134.

United States. Dept. of Energy. 2011. US Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Perlack, R.D., Stokes, B.J. (Leads). Oak Ridge National Laboratory.

Chapter 2

2 Compositional Analysis of Defatted Syrup from a Corn Ethanol Dry Mill as a Feedstock for Bio-Based Products[1]

2.1 Introduction

Depletion of non-renewable fossil fuels and increasing greenhouse gas (GHG) emissions continue to raise economic and environmental concerns. As a result, research on bio-based fuels and chemicals has gained worldwide momentum. Lignocellulosic biomass and processing residues are two types of feedstocks which could be used to produce bio-based fuels and chemicals, while not competing with the production of food.

The USDA-DOE billion ton update report (United States. Dept. of Energy 2011) identified forest and agricultural resources as major potential sources of biomass with the potential of sustainably displacing about 1/3 of U.S. petroleum demand. The potential of feedstock such as switch grass, willow and hybrid poplar have been extensively studied (Tharakan et al. 2003; Sannigrahi et al. 2010). The investigation of process residues such as municipal solid waste, sewage sludge, defatted corn ethanol dry mill syrup (DCS), dried distillers grains with solubles (DDGS), and food processing wastes from dairy and sugar industry as potential feedstocks for bio-based products has received less attention.

Biomass characterization is an important first step in evaluating the feasibility of biomass as a potential feedstock for conversion to biofuels and bio-based products. Apart from informing the choice of conversion platform such as thermochemical, chemical and bio-chemical, it is vital for many other reasons (McKendry 2002). For

[1] *This chapter will be submitted to the Journal of Agricultural and Food Chemistry.* Citation: Adom, F., Fan, J., Davis, J., Dunn, P., Shonnard, D. (2012). Compositional Analysis of Defatted Syrup from a Corn Ethanol Dry Mill as a Feedstock for Bio-Based Products. Journal of Agricultural and Food Chemistry.

example, quantification of cellulose, hemicellulose and lignin is crucial as it affects the overall economics of biorefining, especially for wet biomass conversion processes. Inorganic elements (macro & micronutrients) analysis provide useful information on nutrients depletion of soil (Sannigrahi et al. 2010) while lignin can be used as process heat energy (Xu et al. 2006).

Figure 2-1 summarizes the basic steps for the dry-grind corn mill process, and more details are reported in another study (Rausch and Belyea 2006b). Thin stillage (TS) which is the parent stream of syrup [referred to as DCS in this article (Figure 2-1)] is the feedstock in this study. DCS stream results from the dewatering of TS through the multiple effect evaporators. DCS is golden brown in color with a slightly fermented aroma, and it is viscous compared to water. Due to the high fiber, carbohydrates and protein content it is usually added to DDGS for drying and use as a feed additive (Rausch and Belyea 2006b).

Literature review on prior work done on DCS identified a number of studies to be relevant (Wilkie et al. 2000; Rausch and Belyea 2005; Belyea et al. 2006; Morey et al. 2006; Rausch and Belyea 2006a; Kim et al. 2008; Kim et al. 2010; Reaney et al. 2011). One study (Belyea et al. 2006) focused on characterizing the elemental concentrations of primary process streams from dry-grind ethanol plants with focus on tolerable levels of these elements as a source of animal feed. In another study, the authors (Morey et al. 2006) investigated the fuel and emission characteristics of co-products such as distillers wet grains (DWG), condensed distillers solubles (referred to as “syrup” or “DCS” in this study), DDGS, and corn stover. Technical evaluation of stillage treatment and by-product recovery in the ethanol industry focusing on the viability of anaerobic digestion for stillage treatment was another relevant study identified (Wilkie et al. 2000). However, no single study was identified in the literature on DCS focusing on detailed characterization and evaluation of its potential towards production of bio-based products & biofuel. This study fills this gap by contributing to the knowledge of

the potential utilization of DCS as a renewable feedstock. Three key objectives were identified in this study;

- Conduct an expansive composition analysis on DCS (i.e. total solids, ash content, protein, amino acids, inorganic elements, starch, structural and soluble carbohydrates, lignin, organic acids, glycerol, and functional groups)
- Recommend the most suitable conversion technology, i.e., thermochemical, chemical and bio-chemical for DCS
- Conduct a high level analysis of potential market for which DCS can serve as a feedstock for the production of biofuels and bio-based products

This study evaluates whether the components of DCS can serve as an important fermentation media for bio-based chemicals, pharmaceuticals, food, beverages and many other products.

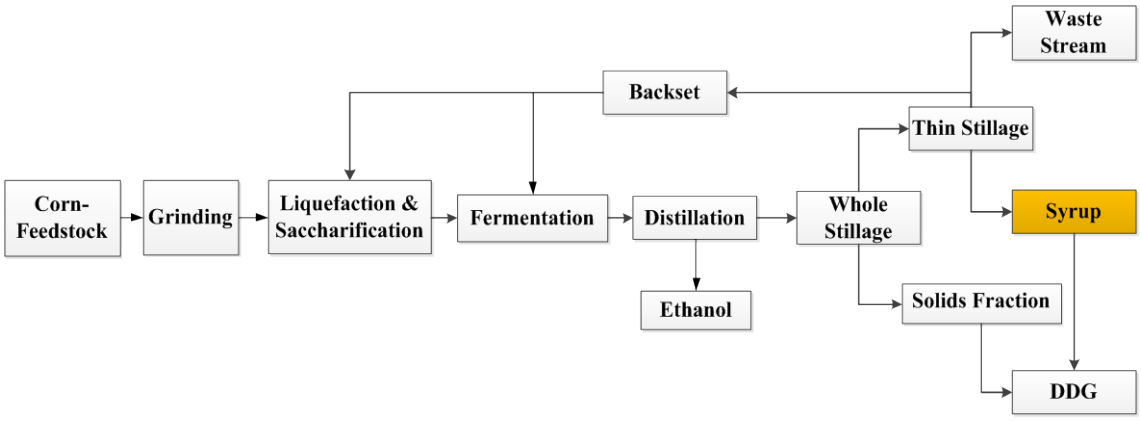


Figure 2-1 Schematic diagram of the dry-grind corn mill facility
(Adapted from Reaney et al, 2011)

2.2 Materials and methods

Six different samples in a 500 ml centrifuge flasks labeled “A” through to “F” were received from a dry-grind corn ethanol milling facility and stored in a refrigerator at 5^{o}C prior to any analysis.

2.2.1 Total solids analysis

The total solid percentage in DCS was estimated by drying the sample in a convection-drying oven at 105°C following an NREL protocol (Sluiter et al. 2008a). Remaining solid residues were sealed in Ziploc bags and stored in a desiccator for ash content analysis. DCS samples "A" through to "F" were all analyzed. All experiments were conducted in duplicate and equation (1) below was used for the analysis.

$$\textit{\% Total Solids} = \frac{[\textit{Weight}_{(\text{dry pan + dry DCS})}] - [\textit{Weight}_{(\text{dry pan})}]}{\textit{Weight of DCS}} \quad (1)$$

2.2.2 Ash content analysis

The NREL protocol for ash analysis (Sluiter et al. 2008b) was used to estimate the total ash content of syrup using a Thermolyene 2000 muffle furnace (Thermo Scientific, West Palm Beach, FL). The percentage composition of ash was estimated by conducting duplicate trials at 575^{o}C. All samples labeled "A" through to "F" were analyzed. The quantity of ash in syrup was analyzed using equation (2):

$$\textit{\% Ash} = \frac{\textit{Weight}_{(\text{ash})}}{\textit{Weight}_{(\text{DCS sample})}} \times 100 \quad (2)$$

2.2.3 Inorganic element profile

1g of oven dried DCS ground to powder was digested in 10ml of 1% HNO_3 (v / v) solution (Zarcinas et al. 1987). The solution was heated to 90^{o}C for 45 minutes and subsequently increased to 140°C with occasional swirling until approximately 1ml of the solution was remaining. After cooling, 20mL of 1N nitric acid was added; the solution was further diluted with deionized water (~30-60x. dilution) for analysis using the Inductively Coupled Plasma Spectrometry (Perkin Elmer Optima 7000DV ICP-OES, Waltham, MA). DCS samples "D" and "E" were analyzed for the following elements; Ca, Fe, Mg, Na, K, P, Al, Cu, Zn, Mn & S. All experiments were conducted in duplicate.

2.2.4 Protein content analysis

Bradford reagent (St. Louis, MI) was used for this analysis. A detailed experimental procedure is reported in the technical bulletin (Sigma-Aldrich 2011). Using Bovine Serum Albumin (BSA) as reference protein, standards were prepared by serially diluting 100 mg / ml BSA stock solution: 0 (blank solution), 0.10, 0.25, and 0.8 mg / ml of BSA with deionized water. To 100 µl of DCS solution (5x diluted), 3ml of Bradford reagent was added in a 16 X 100 mm test tube, vortexed and allowed to settle between 10-30 minutes at room temperature. Absorbance of standards and syrup solutions were measured at 595nm using a Milton Roy, Spectronic 21D spectrophotometer (Champaign, IL).

2.2.5 Amino acid analysis of syrup

Amino acid analysis (AAA) technique by Agilent Technologies (Henderson et al. 2000) was used to analyze DCS. Briefly, 0.5ml of DCS was transferred into 1.5ml centrifuge vial using a micropipette and diluted three fold with distilled water. Ensuring uniform solution mixture by shaking with the hand, the vials were then subsequently centrifuged using VWR, Galaxy 16 Microcentrifuge (Batavia, IL) at 10,000 RPM for 25 minutes. A 0.22-µm membrane was used to filter the supernatant into high-performance liquid chromatography (HPLC) vials. Samples were analyzed using an HPLC (Agilent 1200 series) equipped with Zorbax Eclipse column (4.6×150×5µm) at an operating temperature of 40^{o}C.

2.2.6 Total carbohydrate analysis

The total carbohydrate analysis (not including lignin) of DCS was comprised of three major components namely; ***(i) starch assay*** *and* ***(ii) soluble carbohydrate analysis (iii) non-starch carbohydrate analysis.*** Starch assay focused on glucose sugars generated from the starch hydrolysis enzyme taking into account the initial glucose present. Soluble carbohydrate analysis analyzed for water-soluble C_5 and C_6 sugars (non-structural bound) in DCS. Non-starch carbohydrate analysis considered polymeric carbohydrates such as cellulose and hemicelluloses and any other oligomers in the DCS.

2.2.6.1 Starch assay

Detailed experimental method for the starch assay adopted for DCS is reported in an NREL report (Sluiter and Sluiter 2005). Briefly, 0.1g of oven dried DCS was hydrolyzed using α-amylase (Sigma-Aldrich, St. Louis, MI, USA) and amyloglucosidase (Sigma-Aldrich, St. Louis, MI, USA). Hydrolysate was centrifuged, filtered (0.22 μm) and analyzed for glucose using an Aminex HPX-87P column (Bio-Rad Life Sciences, Hercules, CA) in the HPLC. A starch recovery standard was run under the same conditions simultaneously to account for unhydrolyzed starch using pure potato extracted starch (St. Louis, MI, USA). The equations (3) & (4) were used to estimate the starch recovery standards (%R_{starch}) and the percentage of starch (%Starch) in DCS respectively.

$$\%\,R_{(Starch)} = \frac{Conc._{(glucose,\,PES)} \times Volume_{(PES)}}{Weight_{(PES)}} \times 100 \quad (3)$$

$$\%\,Starch = \frac{[Conc.\ (glucose,\ DCS) \times Volume\ (DCS)] - [Mass\ of\ free\ glucose\ (oven\ dried,\ DCS)]}{\%R\ (Starch) \times 1.11 \times Weight\ (oven\ dried,\ DCS)} \times 100 \quad (4)$$

where **%R $_{(starch)}$**: Starch recovery standard, **Conc $_{(glucose,\ PES)}$**: Concentration of glucose measured from the potato extracted starch (PES) hydrolysate, **Conc $_{(glucose,\ DCS)}$:** Concentration of glucose measured from DCS hydrolysate, **Volume $_{(PES)}$:** Volume of glucose solution for PES hydrolyzate, **Volume $_{(DCS)}$:** Volume of glucose solution for DCS hydrolyzate, **Weight $_{(PES)}$:** Weight of PES measured & **Weight $_{(oven\ dried,\ DCS)}$:** Weight of oven dried DCS measured. "1.11" represents the glucose to starch oligomer correction factor. The mass of free glucose in the oven dry sample before application of α-amylase and amyloglucosidase was measured using soluble carbohydrate analysis methods (in next section), total solid content analysis of DCS previously discussed, and syrup density of approximately 1000 mg / ml of syrup.

2.2.6.2 Soluble carbohydrate analysis

The concentrations of soluble carbohydrates (cellobiose, xylose, glucose, galactose, mannose, and arabinose) and fermentation inhibitors [furfural and hydroxymethylfurfural (HMF)] in DCS were determined by HPLC, (Agilent 1200, Santa Clara, CA), using Aminex HPX-87P column (Bio-Rad Life Sciences, Hercules,

CA). Both the refractive index detector (RID) and diode array detector (DAD) were used. A 10x dilution of DCS was prepared using distilled water and mixed then filtered (0.22-μm membrane) into HPLC vials. Standards for both sugars and inhibitors were analyzed to generate four-point calibration curves. Duplicate samples were analyzed.

2.2.6.3 Non-starch carbohydrate analyses

A detailed experimental procedure for this analysis is reported in another report by NREL (Sluiter et al. 2008c). This analysis was conducted by measuring the total structural carbohydrate sugars (Sluiter et al. 2008c) and then subtracting from this the starch carbohydrate and soluble monomer sugars. Briefly, oven-dried DCS were taken through a two-step pretreatment procedure using H_2SO_4. To 0.3g of oven dried DCS, 3ml of 72%wt H_2SO_4 was added and incubated in a water bath (30^oC) for 60 minutes for the first stage pretreatment step. Hydrolysate was subsequently brought to 4%wt H_2SO_4 acid using distilled water and autoclaved at 121^oC for 60 minutes. For sugar recovery standards (SRS), monomer sugars of known concentration were run through the second step of the two-step procedure to account for sugar degradation and percent sugar recovered (% $R_{(sugar)}$) using HPLC and equations (5) & (6).

$$\%R\ (sugar) = \frac{Concentration\ of\ sugar\ in\ SRS\ measured\ by\ HPLC\ (after\ pretreatment)}{Concentration\ of\ sugar\ in\ SRS\ measured\ by\ HPLC\ (before\ pretreatment)} \times 100 \quad (5)$$

$$\%\ Total\ Structural\ Carbohydrate = \frac{Conc_{(DCS)} \times CF \times Volume_{(DCS)}}{\%R\ sugar \times Weight_{(oven\ dried,\ DCS)}} \times 100 \quad (6)$$

In the equation (6), **Conc $_{(DCS)}$** is the sugar concentration measured from DCS hydrolysate following two-step pretreatment while **Volume $_{(DCS)}$** is the volume of DCS hydrolysate. Finally, anhydrous correction factor **(CF)**, which is the molecular mass ratio of the polymeric sugars to their monomeric units, was applied in the equation above; 0.9 was assigned for glucose and galactose (C_6-sugars) while 0.88 was used for xylose and arabinose (C_5-sugars).

2.2.7 Lignin analyses

The method for acid soluble lignin (ASL) and acid insoluble lignin (AIL) analysis by NREL (Sluiter et al. 2008c) was adopted for this study. Similar to the total carbohydrate analysis previously described, the oven-dried DCS biomass was run through a two-step pretreatment stage. The hydrolysate was separated by filtration using a membrane filter (VWR, polycarbonate membrane filter, 25mm dia., 0.2 µm pore size) into two fractions: a liquid fraction and an insoluble fraction. The liquid fraction containing the soluble lignin was analyzed using a UV–Visible spectrophotometer (Genensys™ 10, Thermo Electron Corp., West Palm Beach, FL) at a wavelength of 240nm. AIL concentrations were corrected for protein by subtracting protein concentrations estimated under protein content analysis of DCS. The insoluble fraction was ashed at 575^{o}C until constant weight and the final weight of residues was measured. Both ASL and AIL were estimated using equations (7) & (8) below:

$$\%\ AIL = \frac{Weight_{(residue)} - Weight_{(ash)} - Weight_{(protein)}}{Weight_{(sample,\ DCS)}} \times 100 \quad (7)$$

$$\%\ ASL = \frac{Absorbance_{(240nm)} \times Volume_{(filtrate)} \times Dilution}{Absorptivity \times Weight_{(sample,\ DCS)} \times Pathlength} \times 100 \quad (6)$$

Absorptivity was 55 L / g / cm

2.2.8 Glycerol analysis

DCS samples were diluted five-fold using distilled water. The diluted samples were filtered into HPLC vials (0.22 µm membrane) and analyzed using HPLC with an Aminex HPX-87P column and a refractive index detector. Calibration standards were run with known concentrations of glycerol (Macron Fine Chemicals ^{TM}., Batavia, IL). Duplicate samples were analyzed.

2.2.9 Total organic acid analysis

Samples of DCS (2ml) were transferred into a 10ml vial. Distilled water (2ml) was added to dilute samples by two-fold. The syrup solution was vortexed to ensure uniform mixture. A 0.22 µm membrane was used to filter the solution into an HPLC vial for organic acid analysis in the HPLC. The Rezex ROA-organic H+ (8%) column

(Phenomenex., Torrance, CA) was used for this analysis. The mobile phase was 0.005N H_2SO_4 with a flow rate of 0.6 ml / min and an operating temperature of 80^oC. Both standards and diluted syrup were analyzed using the RI detector. The following standards were analyzed on the column: oxalic acid, citric acid, succinic acid, acetic acid and lactic acid. Assuming that acetic acid in the sample was from acetate, 0.983 conversion factor of acetic acid to acetate(Sluiter et al. 2008c) was used to estimate the acetate content of DCS.

2.2.10 Functional group analysis using FTIR-ATR

A Fourier Transform Infrared Attenuated Total Reflectance (FTIR ATR-PerkinElmer., Waltham, MA) spectrophotometer equipped with a clean diamond ATR crystal was used to investigate the functional group components of the syrup. Oven dried DCS (at 105^oC) was ground into fine powder using Norpro 696 round porcelain mortar and pestle, 1/4 Cup. Using a detection resolution of 4cm^{-1} and 32 scans per sample, oven dried DCS were analyzed for their spectra. Duplicate samples each of "A", "B" & "C" was analyzed for their functional groups. Using Speckwin32 software, (Menges 2011) observed spectra for all samples analyzed was averaged and used to represent DCS.

2.3 Results and discussion

Apart from amino acid analysis where samples received in the year 2010 and 2011 were averaged to represent DCS, all other reported results were for samples received in 2011. The following results will be accompanied by short discussions of potential conversion processing challenges and other issues.

2.3.1 Total solids and ash content

Total solids concentration was consistent in all samples ranging between 37-38% wt., on average DCS was estimated to contain 37.4% (±0.4%) wt. of total solids [i.e. 63% (±0.4%) wt. of moisture content]. Ash percentage composition in DCS on a dry solid basis ranged from 11-12% wt. For both analyses the average of samples (See Figure 2-2) "A" through to "F" was used to represent DCS. In a separate studies, the authors reported 60-70% of moisture and approximately 30-40% wt. of total solids (Morey et

al. 2006; Kent Rausch and Belyea 2006a) and 15% wt. of ash in DCS (Morey et al. 2006) on dry solid basis.

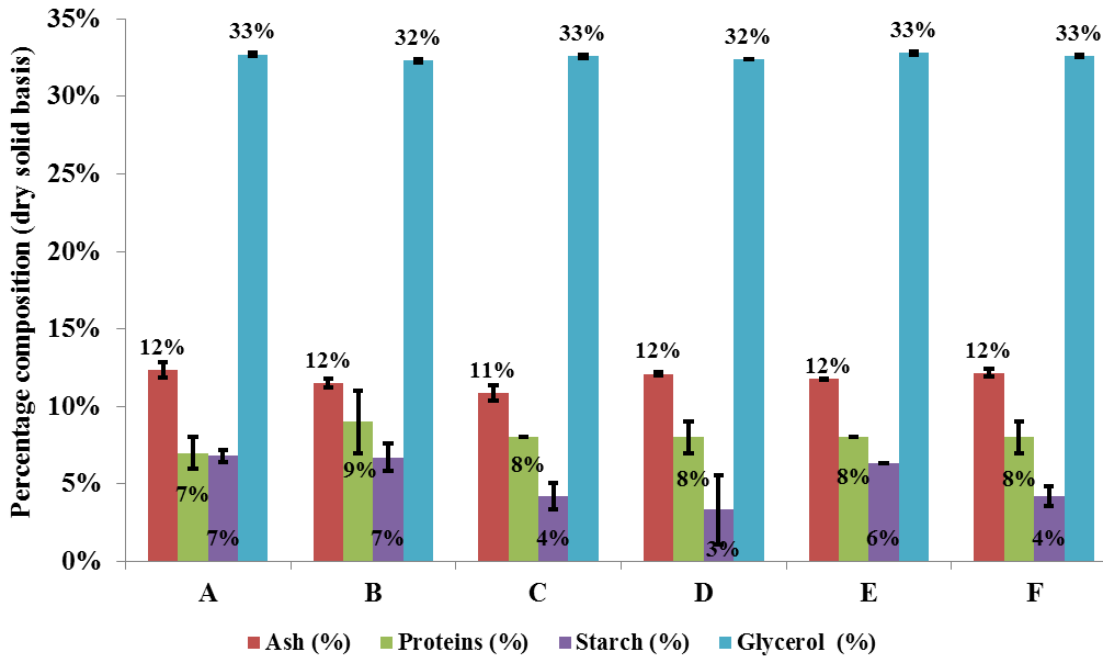


Figure 2-2 Percentage composition on a dry syrup solids basis of total ash, protein, starch and glycerol for samples "A" through to "F" (Duplicates reported as mean standard deviation)

Thermochemical (pyrolysis or gasification) conversion requires low moisture content feedstock (typically <50%) while bio-convention technology can utilize higher moisture content feedstock (McKendry 2002) making the latter more suitable for DCS. Dilute acid and enzymatic hydrolysis followed by fermentation, to produce biofuels, bio-chemicals, or other bio-products may be more suitable. Another possible implication during biochemical conversion processes such as acid pretreatment is higher consumption of acid due to the alkaline nature of ash. Finally, high ash content will likely influence the overall cost of handling and processing solid residues from non-biodegradable carbon in DCS in the downstream processing and should be considered during the biorefinery concept stage.

2.3.2 Inorganic element profile

Table 2-1 summarizes the elemental composition of DCS for duplicate samples. Final concentrations accounted for any dilutions made prior to analysis on the ICP, and variability between samples "E" and "F" was insignificant. From Table I, S, K and P are the dominant elements in DCS, the authors (Rausch and Belyea 2006a) in their study reported Na, K, and P as dominant in their analysis of syrup.

Table 2-1 Summary of inorganic element profile. Percent is based on syrup solids content (Duplicates reported as mean standard deviation)

Elemental	Average of Sample (mg / ml)	Composition in syrup(%)
Ca	0.016 (±0.0004)	0.03%
Fe	0.003 (±0.0001)	0.01%
Mg	0.267(±0.002)	0.56%
Na	0.114 (±0.0039)	0.24%
K	0.884 (±0.0015)	1.86%
P	0.642 (±0.0093)	1.35%
Al	0.002 (±0.0001)	0.003%
Cu	0.0001 (±0.00001)	0.0002%
Zn	0.003 (±0.0001)	0.01%
Mn	0.001 (±0.0000)	0.002%
S	0.955 (±0.0199)	2.01%
Total	2.889 (±0.0246)	6.07%

The reactive nature of alkali metals with silica in biomass results in the formation of "slag" during thermal conversion processes, which blocks airways in furnace and boiler plants (McKendry 2002). This may be an issue during processing of high-throughput DCS via thermal conversion. Finally, large scale processing needs to consider emission

control device such as scrubbers since ***S*** (see Table 2-1) has the potential to produce harmful emissions such as SOx.

2.3.3 Protein content analysis

Protein concentrations in DCS ranged from 5-7 mg / ml representing 7-9% wt. of syrup on a dry basis (see Figure 2-2). Duplicate samples were analyzed for sample "A" through to "F" and averaged. Average protein concentration was 6.06 (±0.85) mg / ml of proteins representing 8% (±0.6%) wt. of DCS on a dry basis. In a separate study, the authors (Rausch and Belyea 2006a) reported relatively higher protein concentration (29.8 g / 100 g on DM basis) in the syrup stream, while crude protein content of DDGS and wet distiller's grain (see Figure 2-1, solid fraction) were reported to be 30.1 (± 1.4 %) and 33.1 (± 3.2 %) (Kim et al. 2010). The higher protein concentration in DDGS and wet distillers' grains as oppose to DCS is expected. After centrifugation of the whole stillage (see Figure 2-1), the solid fraction (containing most of the proteins) goes into making the DDGS and wet distiller's grain while the supernatant goes into making the TS (parent stream of syrup).

Few studies on integrated biorefinery scenarios have considered the technical feasibility, cost and environmental impact of protein recovery (Dale et al. 2009; Laser et al. 2009) using biomass feedstock. DCS is yet to be subjected to such analysis, and any attempt to extract protein from DCS makes the use of the thermochemical technologies unsuitable.

2.3.4 Glycerol analysis

Figure 2-2 summarizes the glycerol concentrations of sample "A" through to "F". By averaging all glycerol results, it was estimated that DCS contained approximately 24.4 mg / ml (±0.25) of glycerol, representing 33% (± 0.2%) wt. in DCS on a dry solids basis. Glycerol percentage compositions were significant and consistent in all samples analyzed as displayed in Figure 2-2.

Glycerol production has increased significantly from 113 million kg of glycerol in the U.S. from biodiesel in the year 2006 to 272 million kg currently (Johnson and Taconi 2007). A glycerol glut in the market has stimulated research into its potential use as a feedstock for the production of value-added products. The production of co-products such as 1,3-propanediol, acetic acid, butanol, acetone, etc through anaerobic fermentation of glycerol by clostridia have been reported (Johnson and Taconi 2007). Also, the production of succinic acid, a value-added chemical (Werpy et al. 2004), using glycerol as a feedstock has been successfully demonstrated (Vlysidis et al. 2011). This is another potential use of the glycerol component in DCS to improve processing plant profitability. Future conversion route for DCS should explore the optimization of the sugar platform via acid hydrolysis and enzymatic saccharification to serve as a fermentation media for the bio-based platform chemicals.

2.3.5 Total carbohydrate content analysis of DCS

2.3.5.1 Starch assay result

Figure 2-2 exhibits the starch content of DCS on a dry solids basis for duplicate samples of "A" through to "F". The starch content of DCS dry solids ranged from 2-8% wt., and by averaging the results obtained from samples "A" through to "F", it was estimated that DCS contained 5.6% (± 2%) wt. of starch.

2.3.5.2 Soluble monomer carbohydrate analysis results

Duplicate samples of vials "A" and "E" were analyzed and their results were averaged to represent DCS. Glucose monomer concentration was highest in DCS being 36.9 mg / ml (± 1.95) followed by cellobiose at 23.7 mg / ml (± 1.95). Relatively smaller concentrations of xylose (3.55 ± 0.17), galactose (1.40 ± 0.09), arabinose / mannose (2.76 ± 0.14) mg / ml were detected. Fermentation inhibitors in DCS were measured to be 0.27 (± 0.02) and 0.26 (± 0.01) mg / ml of furfural and HMF, respectively.

2.3.5.3 Non-starch carbohydrates (NSC) results

Duplicate samples of "A", "B" and "D" were analyzed, and their results were averaged to represent DCS. NSC components comprised of the following; cellulose, and

structurally bound hemicellulose components (xylan, galactan, arabinan, & mannan) after accounting for the starch and water-soluble carbohydrate components. Cellulose was a small fraction of DCS, with the highest estimated value being approximately 1% wt. (±0.01%) on a dry solid basis. Overall hemicellulose components were approximately 9% wt., specifically; xylan 5% wt. (±1%), galactan, 2% wt. (±0.6%), arabinan 0.65 wt. (±0.3%) & mannan, 1 wt. (±0.5%).

Table 2-2 compares the total structural carbohydrate components results of DCS from our study to TS. In summary, the total carbohydrates (starch + soluble monomer carbohydrates + NSC) content of DCS averaged 27% (±5%) wt. Results are compared to another study (Kim et al. 2008) in Table 2-2, and apart from galactan and mannan for which the authors did not detect any, the results are comparable.

Table 2-2 Summary of total carbohydrate content of DCS and thin stillage. Percent is based on syrup solids content (ND: No data)

Components	**Syrup (This study)- Percentage composition**	**Thin Stillage - Percentage composition** (Kim et al. 2008)
Glucan (soluble glucose+starch+cellulose)	16% (15-16%)	16%
Xylan & Xylose	6% (4-6%)	5%
Arabinan & Arabinose	1% (0.1-1%)	1%
Galactan & Galactose	3% (0-3%)	ND
Manann & Mannose	1% (0-1%)	ND

2.3.6 Acid soluble and acid insoluble lignin analysis

Figure 2-3 summarizes results obtained from lignin analysis of DCS. AIL ranged from 6-9 % wt. on a dry solids basis for DCS while ASL varied from 1-3% wt. Averaging all samples analyzed, it was estimated that DCS contained 8% (± 2%) wt. and 2% (± 1%) wt. of AIL and ASL, respectively.

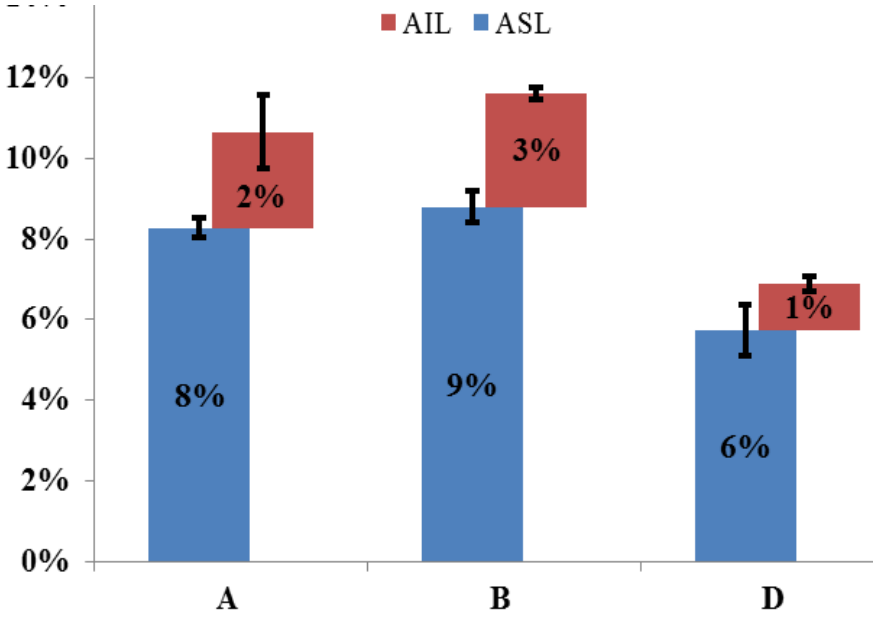


Figure 2-3 . Results for acid soluble & acid insoluble lignin of syrup on a dry solids basis

As previously stated, lignin can further be incinerated for use as process heat (Xu et al. 2006) and should be considered in this regard for future biorefinery scale-up operations.

2.3.7 Amino acid analysis

A summary of the amino acid profile of DCS is displayed in Figure 2-4. Total amino acid concentrations were measured to be 3.51 (±0.24) and 3.38 (±0.35) mg / ml for DCS analyzed in the year 2011 and 2010 respectively. The amino acid profile comprised of the following primary amino acids: aspartic acid, glutamic acid, asparagine, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine and lysine. No secondary amino acids were detected. Averaging all the samples (2010 & 2011) analyzed, it was estimated that the free amino acids in DCS were approximately 3.45% (±0.3 %) wt. on a dry basis.

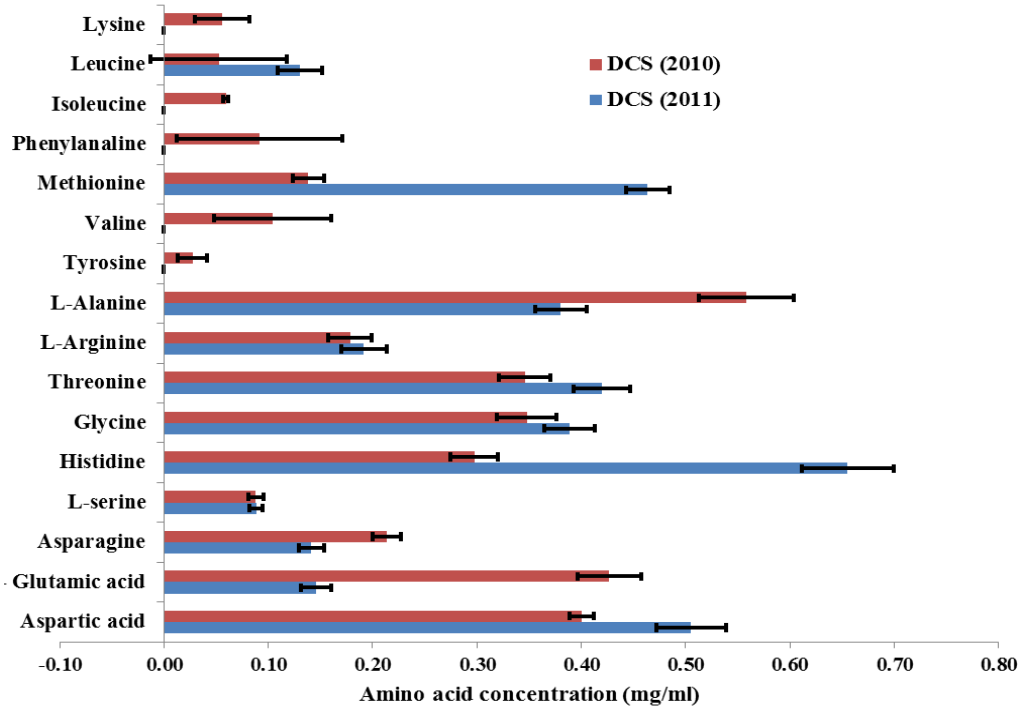


Figure 2-4 Free amino acid content of syrup on a dry solids basis

The total amino acids of TS on a dry solids basis were reported to be 1.1% (Kim et al. 2008). We expected the amino acid profile for TS to be comparable to DCS since it is the parent stream. Table 2-3 compares the amino acid profile for DCS analyzed in this study to TS reported in another study (Kim et al. 2008).

Table 2-3 Comparison of amino acid profile for syrup and thin stillage (TS), [EAA-Essential Amino Acids & NEAA-Non Essential Amino Acids]. Numbers are percent of dry solids. Source of TS data. (Refer to list of abbreviations for others)

EAA	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Thr**	**Try**	**Val**	**Pro**	**Ser**	**Tyr**
Syrup	0.14	0.01	0.03	0.01	0.09	0.01	0.1	0.0	0.02	0.0	0.03	0.004
TS	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.0
NEAA	**Ala**	**Arg**	**Asn**	**Asp**	**Cys**	**Glu**	**Gln**	**Gly**	**Otd**			
Syrup	0.14	0.05	0.05	0.13	0.0	0.08	0.0	0.11	0.0			
TS	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0			

In both samples, tryptophan was not identified, while histidine, methionine, tyrosine, and asparagine were identified in DCS, but these were missing in TS. A possible explanation could be that these amino acid residues detected in the DCS were below the detection limit in the TS given the extremely high moisture content of 92.3% (Kim et al. 2008). The presence of proteins in DCS presents an opportunity to produce more amino acids through hydrolysis reactions. Future research should explore the potential of amino acid production by hydrolysis of DCS.

2.3.8 FTIR-ATR analysis

Figures 2-5 shows the spectra obtained from the FTIR ATR spectrophotometer of oven-dried DCS. Spectra for all samples were averaged using Speckwin32 software, (Menges 2011) and the blue colored spectra represents DCS. About 12 major peaks were identified labeled "A" through to "L". Table 2-4 presents the various peaks identified and relates them to the expected functional groups as identified in the literature.

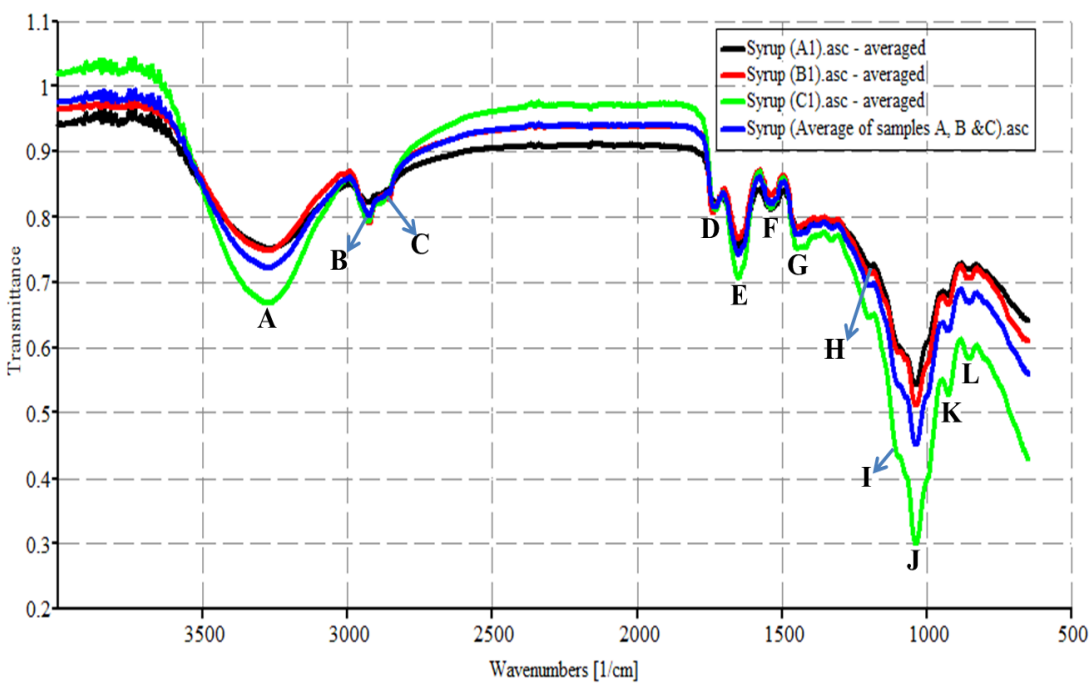


Figure 2-5 FTIR spectra of oven-dried syrup for samples A, B & C

Table 2-4 Results for functional group analysis of oven dried syrup

FTIR-ATR analysis (DCS)			**Findings from Literature Review**	
Peak	**λ (cm^{-1})**	**Transmittance**	**Reported Range from Literature**	**Assignment**
A	3271-3625	0.7228-0.9535	3200-3600	**O-H** (in H-bonded ROH and ArOH)(Meislich 1999)
B	2927	0.8026	2927	**C-H** stretching (indicates rupture of methyl/methylene)(Theerarattananoon et al. 2010)
C	2857	0.836	2500-3000	**O-H** in COOH (Meislich 1999)
E	1736	0.8148	1740	**C=O** Acetyl group (Mascarenhas et al. 2000)
			1738	**C=O** ester; strong carbonyl groups in branched hemicellulose (Pandey 1999)
F	1653	0.7432	1653 and 1549	Protein strong band of amide I and amide II, respectively (Meislich 1999)
G	1540	0.8196	1650-1440	**C=C** vibrations due to the presence of benzene ring (Meislich 1999)
H	1447	0.7734	1453-1456	Syringyl absorption of hardwoods (**C-H** methyl vibrations and methylene deformation) (Corredor et al. 2008)
I	1328	0.7832	1315-1317	**C-O** vibration of syringyl ring of lignin (Corredor et al. 2008)
J	1099	0.7036	1098-1109	C-O vibration of crystalline cellulose; glucose ring stretch from cellulose(Corredor et al. 2008)
K	1039	0.4521	1050, 1030	Cellulose **C-OH** (Mascarenhas et al. 2000)
			1060 and 1035	**C-O** vibrations of cellulose(Corredor et al. 2008)
N	927	0.6252	1106, 1045, 994, 926, 852	Major glycerol absorption peaks(Petibois et al. 2002)
L	855	0.6697	915, 840	α-D Glucose (Mascarenhas et al. 2000) &(Tul'chinsky et al. 1976)

Generally, FTIR as a semi-quantitative tool was useful in confirming most of the chemical components, previously identified using other methods, based on functional group absorbance. For example, peak "F" indicated the presence of proteins strong band of amide I and amide II. Functional group analysis results presented in Figure 2-5 & Table 2-4 strongly confirms the presence of chemical components measured using other analytical wet chemistry techniques in this study. FTIR is also useful to follow changes in functional groups in solid samples as a result of conversion reactions, though we deemed this beyond the scope of this characterization study.

2.3.9 Mass balance closure of DCS

The overall mass closure, which totaled 101%, was calculated by summing the results reported in this section for components analyzed on a dry solid basis. This included the following; ash (12%), protein (8%), amino acids (3%), glycerol (33%), lignin (ASL & AIL-10%), oxalic acid (1%), succinic acid (1%), lactic acid (4%) acetate (1%) and total carbohydrates (28%). Figure 2-6 summarizes these results showing the various components.

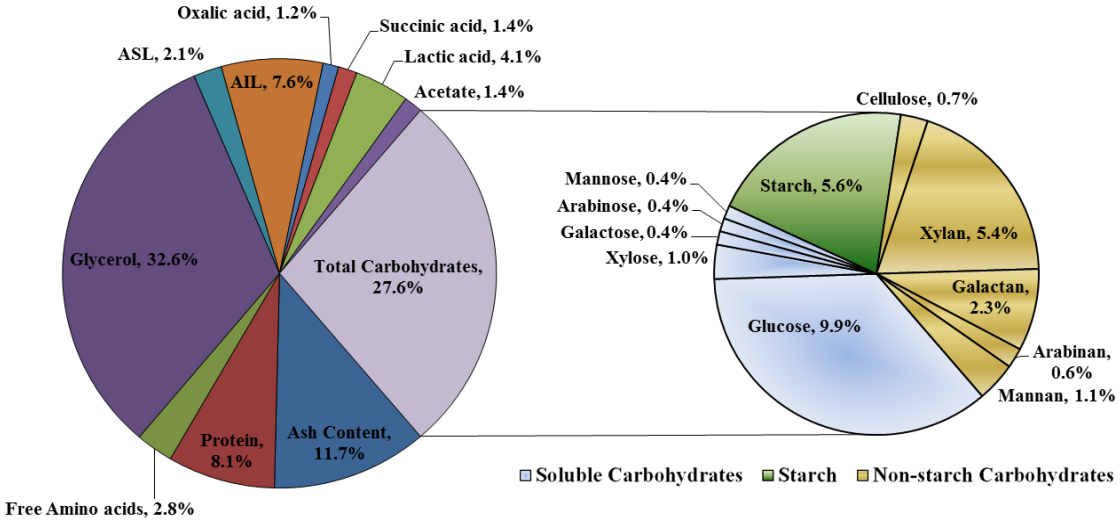


Figure 2-6 Summary of the compositional analysis result for oven-dried syrup

Process conditions such as elevated temperature and the presence of acids are capable of rendering hemicellulose and cellulose soluble (Harmsen et al. 2010). Acid pretreatment should be investigated as a potential conversion route for producing the

sugar platform using DCS as the feedstock. Apart from the fact that a significant amount of soluble sugars of DCS is in solution (~40wt percent of the total carbohydrates), dilute acid pretreatment may be advantageous given the prevalence of starch as compared to cellulose. In addition to acid hydrolysis, future work could also investigate milder process conditions through the use of cellulases and starch hydrolyzing enzymes. Ultimately, the cost and quantity of available feedstock (DCS), usable fermentable sugars, concentration of fermentation inhibitors and conversion yields will influence any intended use towards bio-based specialty chemical. The next section elaborates more on the potential of DCS as a feedstock for some bio-based chemicals.

2.4 Potential yields from biorefining using syrup as a feedstock

In this section, and using the characterization results from this study, we estimate the potential quantity of target chemical products that can be produced using DCS as a feedstock. Production of DCS averaged 59 million kg per month (~708 million kg per year) in the U.S. (O'Brien 2010). A summary of our analysis is displayed in Table 2-5. Apart from ethanol, which was estimated using the theoretical yield calculator (DOE),(U.S. 2006) all other target bio-based chemicals (TBC) yields using fermentable carbohydrates were estimated using equation (9), where ***X*** represents yield of TBC on carbohydrate:

$$\text{TBC (kg)} = 708 \times 10^{6} \frac{\text{kg syrup}}{\text{Year}} \times \frac{37.4}{100} \frac{\text{kg syrup DM}}{\text{kg syrup}} \times \frac{27}{100} \frac{\text{kg carbohydrate}}{\text{kg syrup DM}} \times \boldsymbol{X} \quad (9)$$

In the case of glycerol as a potential feedstock, the necessary adjustment was made by applying the ratio of 33/100 in the place of 27/100 in equation (9). The key highlight from this analysis is that DCS has a potential to meet current U.S. demand for succinic acid, and future research should investigate the feasibility of utilizing both fermentable sugars as well as glycerol for the production of succinic acid. ***Escherichia coli*** and ***Actinobacillus succinogenes*** strains have been successfully used for succinic acid production using glucose and glycerol as feedstock (Lennartsson 2005; Vlysidis et al. 2011). It was also interesting to note that even without any form of hydrolysis and based only on the concentration in DCS, histidine could be recovered (potential of

370,000 kg) and could meet global demand of 360,000 kg (Ikeda 2003). From our analysis, DCS seem less promising to displace significant transportation fuels through production of ethanol and ABE (Acetone-Butanol-Ethanol). We recommend future research to investigate the feasibility of using DCS in a sugar platform approach as a feedstock for bio-based chemicals production.

Table 2-5 Potential yields of bio-based chemicals using DCS as a feedstock (M: Million & T: Thousand)

TBC	**DCS component**	**Current demand**	**Potential with utilization of DCS**	**Yield (X)**
Succinic acid	Fermentable carbohydrates	20-30 **M** kg (Cukalovic and Stevens 2008)	51 **M** kg	0.71 (Lennartsson 2005)
Ethanol	Fermentable carbohydrates	14-**billion** gal (RFA 2005)	51 **T** m^3 (13**M** gal)	172.83[a] & 176.86[b] (U.S. 2006)
Acetone Butanol Ethanol **(ABE)**	Fermentable carbohydrates	25 **M** gal (butanol)(Cascone 2008) &(Pfromm et al. 2010)	9 **T** m^3 (2.3**M** gal)	0.31 (Qureshi 2010)
			17 **T** m^3 (4.5**M** gal)	
			3 **T** m^3 (0.8**M** gal)	
Succinic acid	Glycerol	20-30 **M** kg (Cukalovic and Stevens 2008)	110 **M** kg	1.23 (Vlysidis et al. 2011)
Threonine	Amino acid	3.6 **M** kg (Ikeda 2003)	.30 **M** kg	1.0
Tyrosine	Amino acid	110 **T** kg (Ikeda 2003)	10 **T** kg	
Histidine	Amino acid	360 **T** kg (Ikeda 2003)	370 **T** kg	
Protein	Protein	5 trillion kg (Dale et al. 2009)	21 **M** kg	

[a] 172. 83 gallons per dry ton of C_6 sugar (7.21 x 10^{-4} m^3 of ethanol / kg C_6 sugar)

[b] 176. 86 gallons per dry ton of C_5 sugar (7.38 x 10^{-4} m^3 of ethanol / kg C_5 sugar)

Ultimately, detailed economic analyses considering feedstock cost, plant capacity, technology maturity, etc. will be required to analyze the profitability of using DCS as a bio-based feedstock. Furthermore, there are many other processing challenges to be addressed such as; toxicity / inhibitory levels of hydrolysate components that influence fermentation yields, product separation and recovery costs, scale-up, and system integration issues.

2.5 Conclusions

DCS is a co-product of the dry-grind corn ethanol process and no previous studies have investigated the potential utilization as a renewable feedstock for bio-based chemicals and products. In this study, DCS was analyzed for its physical and chemical characteristics. With total solids of 37.4% wt., a mass balance closure on all components of DCS was 101%. Total carbohydrates (28% of dry wt.) comprised of starch components (6%), soluble carbohydrates (12%) & non-starch carbohydrates (10%). Structural and non-structural bound hemicellulose components included; xylan (6%), mannan (1%), arabinan (1%) and galatactan (3%). The ash content comprised of 12% wt. DM basis while protein, glycerol and amino acids were 8% wt., 33%, and 3% wt. on DM basis, respectively. Syrup has good potential as a renewable feedstock for bio-chemicals production through either fermentation or separation of various compounds directly from the syrup.

2.6 References

Belyea RL, Clevenger TE, Singh V, Tumbleson M, Rausch KD. 2006. Element concentrations of dry-grind corn-processing streams. Applied Biochemistry and Biotechnology 134:113-128.

Cascone R. 2008. Biobutanol: a replacement for bioethanol? In: Chemical Engineering Progress Special Edition Biofuels 104:S4–S9.

Corredor D, Sun X, Salazar J, Hohn K, Wang D. 2008. Enzymatic hydrolysis of soybean hulls using dilute acid and modified steam-explosion pretreatments. Journal of Biobased Materials and Bioenergy 2:43-50.

Cukalovic A, Stevens CV. 2008. Feasibility of production methods for succinic acid derivatives: a marriage of renewable resources and chemical technology. Biofuels, Bioproducts and Biorefining 2:505-529.

Dale BE, Allen MS, Laser M, Lynd LR. 2009. Protein feeds coproduction in biomass conversion to fuels and chemicals. Biofuels, Bioproducts and Biorefining 3:219-230.

Harmsen P, Huijgen W, López LMB, Bakker R. 2010. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Wageningen UR, Food & Biobased Research. No. 1184.

Henderson J, Ricker RD, Bidlingmeyer BA, Woodward C. 2000. Rapid, accurate, sensitive, and reproducible HPLC analysis of amino acids. Application bulletin 5980.

Ikeda M. 2003. Amino acid production processes. Microbial production of l-amino acids 79:1-35.

Johnson DT, Taconi KA. 2007. The glycerin glut: Options for the value-added conversion of crude glycerol resulting from biodiesel production. Environmental Progress 26:338-348.

Kim Y, Mosier NS, Hendrickson R, Ezeji T, Blaschek H, Dien B, Cotta M, Dale B, Ladisch MR. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresource technology 99:5165-5176.

Kim Y, Hendrickson R, Mosier NS, Ladisch MR, Bals B, Balan V, Dale BE, Dien BS, Cotta MA. 2010. Effect of compositional variability of distillers' grains on cellulosic ethanol production. Bioresource technology 101:5385-5393.

Laser M, Jin H, Jayawardhana K, Dale BE, Lynd LR. 2009. Projected mature technology scenarios for conversion of cellulosic biomass to ethanol with coproduction thermochemical fuels, power, and/or animal feed protein. Biofuels, Bioproducts and Biorefining 3:231-246.

Lennartsson A. 2005. Production of Succinic Acid by E. coli from Mixtures of Glucose and Fructose. Thesis work 2005. Lulea University of Technology, Div. Biochemical and Chemical Process Engineering.

Mascarenhas M, Dighton J, Arbuckle GA. 2000. Characterization of plant carbohydrates and changes in leaf carbohydrate chemistry due to chemical and enzymatic degradation measured by microscopic ATR FT-IR spectroscopy. Applied spectroscopy 54:681-686.

McKendry P. 2002. Energy production from biomass (part 1): overview of biomass. Bioresource technology 83:37-46.

Meislich H, Nechamkin. H., Sharefkin. J., , Schaum's Outline of Theory and Problems of Organic Chemistry (Schaum's Outline Series). Second ed.; McGraw-Hill: New York, **1999**.

Menges F. 2011. Spekwin32- optical spectroscopy software. version 1.71.6, 201x ed.

Morey RV, Hatfield DL, Sears R, Tiffany D. 2006. Characterization of feed streams and emissions from biomass gasification/combustion at fuel ethanol plants. ASABE Meeting Paper(064180).

O'Brien D. 2010. Updated trends in U.S wet and dry corn milling production. Kansas State University.

Pandey K. 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. Journal of applied polymer science 71:1969-1975.

Petibois C, Cazorla G, Déléris G. 2002. Triglycerides and glycerol concentration determinations using plasma FT-IR spectra. Applied spectroscopy 56:10-16.

Pfromm PH, Amanor-Boadu V, Nelson R, Vadlani P, Madl R. 2010. Bio-butanol vs. bio-ethanol: A technical and economic assessment for corn and switchgrass fermented by yeast or Clostridium acetobutylicum. Biomass and Bioenergy 34:515-524.

Qureshi N. 2010. Agricultural residues and energy crops as potentially economical and novel substrates for microbial production of butanol (a biofuel).

Rausch K, Belyea R. 2006a. The future of coproducts from corn processing. Applied Biochemistry and Biotechnology 128:47-86.

Rausch KD, Belyea RL. 2005. Coproducts from bioprocessing of corn. ASAE Annual International Meeting(057041).

Rausch KD, Belyea RL. 2006b. The future of coproducts from corn processing. Applied Biochemistry and Biotechnology 128:47-86.

Reaney MJ, Jia Y, Shen J, Ratanaparlyanuch K. 2011. Recovery of multiple compounds and recyclable water from thin stillage. EP Patent 2,297,291.

RFA. 2005. Homegrown for the homeland: Ethanol industry outlook 2005.

Sannigrahi P, Ragauskas AJ, Tuskan GA. 2010. Poplar as a feedstock for biofuels: A review of compositional characteristics. Biofuels, Bioproducts and Biorefining 4:209-226.

Sigma-Aldrich. 2011. Bradford Reagent Technical Bulletin. No. RC,JDS,MAM 06/11-1.

Sluiter A, Sluiter J. 2005. Determination of Starch in Solid Biomass Samples by HPLC. Laboratory Analytical Procedure. NREL/TP-510-42624. National Renewable Energy Laboratory, Golden, Colorado.

Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Wolfe J. 2008a. Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory Analytical Procedures, National Renewable Research Laboratory, Technical Report TP-510-42621, Golden, Co.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 2008b. Determination of ash in biomass. Laboratory Analytical Procedures, National Renewable Research Laboratory, Technical Report TP-510-42622, Golden, Co.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. 2008c. Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure NREL/TP-510-426i8. National Renewable Energy Laboratory, Golden, Colorado.

Tharakan P, Volk T, Abrahamson L, White E. 2003. Energy feedstock characteristics of willow and hybrid poplar clones at harvest age. Biomass and Bioenergy 25:571-580.

Theerarattananoon K, Wu X, Staggenborg S, Propheter R, Madl R, Wang D. 2010. Evaluation and characterization of sorghum biomass as feedstock for sugar production. Transactions of the ASABE 53:509–525.

Tul'chinsky VM, Zurabyan SE, Asankozhoev KA, Kogan GA, Khorlin AY. 1976. Study of the infrared spectra of oligosaccharides in the region 1,000-40 cm-1. Carbohydrate research 51(1):1-8.

U.S. DoEEERE. 2006. (Biomass Program).Theoretical Ethanol Yield Calculator. Theoretical Ethanol Yield Calculator. .

United States. Dept. of Energy. 2011. US Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Perlack, R.D., Stokes, B.J. (Leads). Oak Ridge National Laboratory.

Vlysidis A, Binns M, Webb C, Theodoropoulos C. 2011. Glycerol utilisation for the production of chemicals: Conversion to succinic acid, a combined experimental and computational study. Biochemical Engineering Journal 58-59:1-11.

Werpy T, Petersen G, Aden A, Bozell J, Holladay J, White J, Manheim A, Eliot D, Lasure L, Jones S. 2004. Top Value Added Chemicals From Biomass. Volume 1-Results of Screening for Potential Candidates From Sugars and Synthesis Gas. DTIC Document.

Wilkie AC, Riedesel KJ, Owens JM. 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy 19:63-102.

Xu F, Sun J, Liu C, Sun R. 2006. Comparative study of alkali-and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse. Carbohydrate research 341:253-261.

Zarcinas B, Cartwright B, Spouncer L. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Communications in Soil Science & Plant Analysis 18(1):131-146.

Chapter 3

3 Optimization of the Dilute Acid and Enzymatic Pretreatment of Defatted Syrup from a Corn Ethanol Dry Mill[2]

3.1 Introduction

Lignocellulosic biomass refers to plants and plant derived-organic material that contain cellulose, hemicellulose, and lignin as major components (de Wild et al. 2011). Considered the most abundant biopolymer on Earth, lignocellulosic biomass constitutes 50% of the world's biomass with an annual production of 10-50 billion tonnes (Claassen et al. 1999). Cellulose and hemicellulose are both potential sources of fermentable sugars. Unlike hemicellulose, which can be hydrolyzed under mild acid or alkaline conditions, cellulose is more resistant (Harmsen et al. 2010) and requires specialized enzymes or very high acid concentrations to de-polymerize to yield glucose.

Pretreatment involves the conversion of lignocellulosic biomass from its native form, in which it is recalcitrant to cellulase enzyme systems, into a form for which cellulose hydrolysis is much more effective (Zheng et al. 2009). The primary goals of pretreatment are (Brodeur et al. 2011) ; (i) production of highly digestible solids that enhances glucose yields during enzyme hydrolysis, (ii) avoiding the degradation of sugars (mainly pentoses) including those derived from hemicellulose, (iii) minimizing the formation of inhibitors of subsequent fermentation steps, (iv) recovery of lignin for conversion into bioenergy or valuable co-products, and (v) to be cost effective by operating in reactors of moderate size and by minimizing heat and power requirements.

[2] *This chapter will be submitted to Biomass and Bioenergy for publication.* Citation: Adom, F., Fan, J., Davis, J., Taylor, A., Shonnard, D. (2012). Optimization of the Dilute Acid and Enzymatic Pretreatment of Defatted Syrup from a Corn Ethanol Dry Mill. Biomass and Bioenergy.

A generalized classification of biomass pretreatment methods include; physical, physicochemical, chemical, and biological. Physical pretreatment involves the breakdown of biomass size and crystallinity through milling or grinding to enhance subsequent hydrolysis by improving mass transfer characteristics from reduction in particle size. Energy requirement for this pretreatment method is high and the overall process is expensive (Sun and Cheng 2002). Physicochemical pretreatment includes a majority of pretreatment technologies including: steam pretreatment, liquid hot water pretreatment, wet oxidation pretreatment, ammonia fiber / freeze explosion, ammonia recycle percolation, aqueous ammonia pretreatment and Organosolv pretreatment (Agbor et al. 2011). Generally, this pretreatment method utilizes conditions and compounds capable of affecting the physical and chemical properties of biomass.

Chemical pretreatment involves the use of chemicals through the initiation of chemical reactions to disrupt biomass structure (Harmsen et al. 2010). Acids, alkali, organic solvents, and ionic liquids have been reported to have significant effect on native lignocellulosic materials (Agbor et al. 2011). Both weak and strong acids have been reported for the pretreatment procedure. Two categories of weak acid hydrolysis (Harmsen et al. 2010) are; (i) high temperature and continuous flow for low-solids loading ($T>160°C$, 5-10 wt% substrate concentration) and (ii) Low temperature and batch process for high-solids loading ($T\leq160°C$, 10-40 wt%. substrate concentration). The method (i) is more suitable for low lignin containing biomass. The use of strong acid is a less desirable approach given the comparatively higher corrosive nature and the need to recycle acids in order to lower cost (Harmsen et al. 2010; Agbor et al. 2011).

Pretreatment processes are capital intensive and are estimated to be about 20% of the total cost of the biorefinery (Kootstra et al. 2009). The primary economic drivers of pretreatment costs are; yield of both five and six carbon sugars, solids concentration, enzyme loading and hemicelullase activity (Eggeman and Elander 2005). Careful optimization of lab scale pretreatment process taking into account various processing

variables is essential to making its integration into the biorefinery concept economical. Biological pretreatment uses microorganisms (mainly fungi) to degrade lignin, hemicellulose and polyphenols but leave the cellulose intact (Sun and Cheng 2002; Agbor et al. 2011). White and soft-rot fungi and brown-rot fungi uses have been reported (Lee 1997; Sun and Cheng 2002). Slow rate of biological pretreatment, the requirement of careful growth conditions and the large amount of space for biological pretreatment have made this approach unattractive from an industrial perspective (Agbor et al. 2011).

In Chapter 2 was presented a detailed characterization study on defatted corn syrup (DCS) with an overall mass balance closure of about 101 wt%. It was recommended to further study hydrolysis of DCS via acid pretreatment and cellulase application. It was further estimated that approximately 27 wt% on dry solid basis of the syrup is attributable to carbohydrates comprising of the following; (i) soluble carbohydrates (ii) starch and (iii) non-starch carbohydrates (cellulose, xylan, galactan, mannan & arabinan). The primary goal of this chapter's research is to optimize the production of sugars using DCS via dilute sulfuric acid hydrolysis and enzymatic saccharification using cellulase enzymes. The sugar platform can then serve as a source of feedstock for the production of higher value bio-based chemical products such as succinic acids and polymer products (Werpy et al. 2004).

3.2 Materials and methods

3.2.1 Hydrolysis scheme and experimental matrix

The hydrolysis experiments involved dilute sulfuric acid (H_2SO_4) pretreatment of the DCS followed by enzymatic hydrolysis. The experimental matrix was comprised of six experiment sets with different reaction times. Table 3-1 summarizes the set of experiments conducted. For example, in experimental set 1, DCS was pretreated with varying dilute acid concentrations (0, 1, & 2 wt%) for 1 minute (in an autoclave) at 121^{o}C with subsequent enzymatic hydrolysis for the following reaction times; 0, 24, 48, and 72 hours at 50^{o}C (in an incubator). The reaction time (0) refers to sampling of

the hydrolysate right after adding the cellulase enzymes. The solid loading of biomass was kept at 10 wt% for all pretreatment experiments conducted while the enzymatic hydrolysis experiments were conducted at approximately 2% solids.

Table 3-1 Experimental matrix for dilute acid hydrolysis (10% solids) and enzymatic saccharification (~2% solids) of DCS

Experiment Set	**Dilute Acid Pretreatment (Minutes, Temperature)**	**Acid Concentrations**	**Enzymatic Hydrolysis**
1	1min, 121^{o}C	0, 1, & 2wt. %	0hr, 24hrs, 48hrs, 72hrs, 50^{o}C
2	30min, 121^{o}C		
3	45min, 121^{o}C		
4	60min, 121^{o}C		
5	75min, 121^{o}C		
6	90min, 121^{o}C		

3.2.2 Materials

The feedstock (DCS) used in this study was received from a dry-grind corn mill ethanol facility in a 500ml centrifuge flask. DCS was stored in a refrigerator at 5^{o}C prior to any analysis. Reagent carbohydrates: D(+)Glucose; D(+)Xylose; D(+)Arabinose; D(+)Cellobiose; D(+)Galactose); and the fermentation inhibitors hydroxymethylfurfural (HMF) and furfural were purchased from Sigma Chemical Company (St. Louis, MO). Sulfuric acid (96 wt%) and NaOH pellets were purchased from Fisher Scientific (Pittsburgh, PA). The cellulase enzyme formulation used for this study was Accellerase1500© from Genencor®. Other materials used in enzymatic hydrolysis include sodium citrate buffer for pH stabilization, tetracycline and cycloheximide, which were all purchased from Sigma Chemical Company.

3.2.3 Equipment

An autoclave (New Burnswick Scientific AC-48) was used for the pretreatment experiment for controlling the temperature and time of reaction. A 14ml Ace® glass reactor (Ace Glass Inc., 8648-124) equipped with a PTFE seal was used as the reaction

vessel. Enzymatic saccharification reactions were conducted in an incubator (Lab-Line Orbit Environ Shaker) at 50°C. A combination of litmus paper and electronic pH meter (Accumet® pH Electrode) were used for monitoring pH of solutions. Finally, High Perfomance Liquid Chromatography-HPLC (Agilent 1200 series) was used for sugar detection. The sugar concentrations were measured by the use of a refractive-index detector and furfural and HMF were measured with a diode-array detector, combined with suitable calibration of the detectors using known standards.

3.2.4 Characterization of soluble sugars and inhibitors

Using HPLC, the concentrations of soluble carbohydrates (cellobiose, xylose, glucose, galactose, mannose, arabinose) and fermentation inhibitors (furfural and HMF) were quantified prior to hydrolysis. To 1g of syrup, distilled water (4g) was added to prepare a 5x dilution. To ensure a uniform mixture, the solution was swirled using a votex mixer. Using a membrane filter purchased from VWR (Polycarbonate membrane filter, 25mm dia., 0.2 μm pore size), the solution was filtered into clear a HPLC vial for sugar analysis in the HPLC using an Aminex HPX-87P column (Bio-Rad). Filtered distilled water was used as the mobile phase at a flow rate of 0.6 ml $minute^{-1}$ and the column temperature was set to 80^{o}C. Duplicate samples were analyzed.

3.2.5 Dilute acid pretreatment (DAP) procedure (First pretreatment stage)

The dilute acid hydrolysis experiments were conducted according to the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al. 2008) with slight modification (Figure A-1). 10g of the DCS sample was first added in each 100ml beaker, and then each syrup sample was diluted with 10 wt% H_2SO_4 and distilled H_2O to designated acid concentration of 0, 1 and 2 wt% and to a total solid level of 10 wt%. The diluted syrup solutions were transferred into the Ace® glass reactors for autoclaving. The autoclave time (temperature at 121°C) was varied from 1 to 90 minutes to evaluate the impact of different pretreatment times on sugar recovery performance. These reaction times do not include the periods of normal autoclave heat up and cool down (to 80°C when contents were removed). After the DAP, 5 ml of well-mixed dilute acid hydrolysate (DAH) was transferred from each Ace® bottle for

subsequent enzymatic hydrolysis and approximately 2.5ml of the remaining hydrolysate was subjected to another acid hydrolysis for the oligomer analysis.

3.2.6 Oligomer analysis (Second pretreatment stage)

The oligomer analysis procedure was adopted from the NREL laboratory analytical procedures (LAP) protocol (Sluiter et al. 2008). Approximately 2.5 ml of the remaining hydrolysate was filtered through 0.2 μm membrane into 1.5 ml micro centrifuge vial (VWR) to collect 1 ml DAH filtrate for the oligomer analysis. The acid concentration of filtrate in centrifuge vial was then brought to 4 wt%. by adding different required volumes of 96 wt% H_2SO_4 followed by autoclaving for another 60 minutes at 121^oC to hydrolyze oligomer components to monomer sugars. 1ml of the retrieved hydrolysate after autoclaving were neutralized to 5-8 pH range using 10N NaOH, and filtered using 0.2 μm membrane into HPLC vials for sugar analysis. Duplicate samples were analyzed. A "sugar recovery" standard of known sugar concentration was subjected to the same autoclave procedures as above and a sugar recovery factor was applied as per the NREL LAP (Sluiter et al. 2008) to calculate the oligomeric sugar concentrations in the samples.

3.2.7 Enzymatic hydrolysis (EH)

Five ml of the well-mixed DAH sample (after first pretreatment stage) was transferred into a 50 ml Erlenmeyer flask, and then the hydrolyzate was adjusted to pH 5 using 10N NaOH. This was followed by the addition of the following: 1.5 ml of 1M sodium citrate buffer to stabilize the pH, 120 μl tetracycline (10 mg ml^{-1} in 70 vol% ethanol) and 90 μl cycloheximide (10 mg ml^{-1} in H_2O) to all flasks as antimicrobial agents. The flasks were covered with parafilm and thin aluminum foil and allowed to equilibrate in an incubator for one hour at 50^oC. After one hour, dosages of the enzymes (75 μl Accellerase®1500 and 15 μl of Accellerase® XY) and pre-warmed (50°C) distilled water were added into the flask until the total volume was 30 ml. These enzyme dosages were in accordance with the recommended optimum dosage levels by Genencor® (0.25 ml per gram of biomass). The pH meter was used to ensure a pH range of 4.5-5 prior to the addition of enzymes. After 0, 24, 48 and 72 hours, duplicate

samples were drawn using pipette for HPLC analysis. The approach is similar to the NREL LAP protocol (Selig et al. 2008).

3.3 Results

All reported final concentrations have been "back calculated" to the syrup by accounting for all dilution factors (e.g., acids, bases, distilled H_2O, and antibiotics). Concentrations for enzyme blank for all experiments using Accellerase® 1500 and XY were also accounted.

3.3.1 Results for soluble carbohydrates and inhibitors in DCS

Soluble carbohydrate analysis results (prior to any hydrolysis) obtained for DCS are as follows: cellobiose [15.8 (±1.7) mg ml^{-1}], glucose [11.4 (±0.1) mg ml^{-1}], xylose [1.7 (±0.02) mg ml^{-1}], galactose [1.0 (±0.03) mg ml^{-1}], arabinose [2.0 (±0.1) mg ml^{-1}], and mannose [2 (±0.6) mg ml^{-1}]. In addition, furfural and HMF were measured to be 0.22 (±0.02) and 0.04 (±0.01) mg ml^{-1}, respectively. Total monomer sugars (TMS), which is the sum of glucose, xylose, galactose, arabinose, and mannose concentration, was approximately 18 mg ml^{-1}. DCS sample used for this hydrolysis experiment contained 28% dry solids and 12 wt% ash in dry solids (Figure A-2).

3.3.2 Results for experiment set 1

3.3.2.1 Dilute acid pretreatment & oligomer analysis - 1 minute

Figure 3-1 shows the monomer sugar concentrations after 1 minute DAP (first stage pretreatment) and oligomer analysis (second stage pretreatment) for DCS solutions of 0, 1, and 2 wt% acid concentrations. The deep blue, red, and green represents the first pretreatment stage for 0, 1 and 2 wt% acid concentrated hydrolysate, respectively, while the lighter shades represent the net monomer sugar increase after oligomer analysis. While the duration of time at target temperature of 121°C is only 1 minute, the time spent heating up from room temperature to 121°C (about 1 hour) and cooling down to 80°C prior to opening up the autoclave (about 0.5 hour) must be kept in mind. This will affect the overall hydrolysis reaction time and subsequently the total monomer sugars and inhibitors generated over time.

Cellobiose concentration increased because of the breakdown of more polymers into oligomers, due to increasing acid intensity. The relatively short hydrolysis reaction time (1 minute) was not enough to further degrade the cellobiose into its glucose monomer units as was observed in other experiment sets. Glucose concentration peaked at around 23 mg ml^{-1} with the 2 wt% acid concentrated DCS after the first pretreatment stage. Overall, the TMS concentration was 13.4, 16.3 and 36 mg ml^{-1} for 0, 1 and 2 wt% acid sample, respectively, after the first pretreatment stage (1 minute). A two-fold (2x) increase in TMS was observed when comparing 2 wt% acid sample (36 mg ml^{-1}) with the initially present soluble carbohydrates (see section 3.3.1).

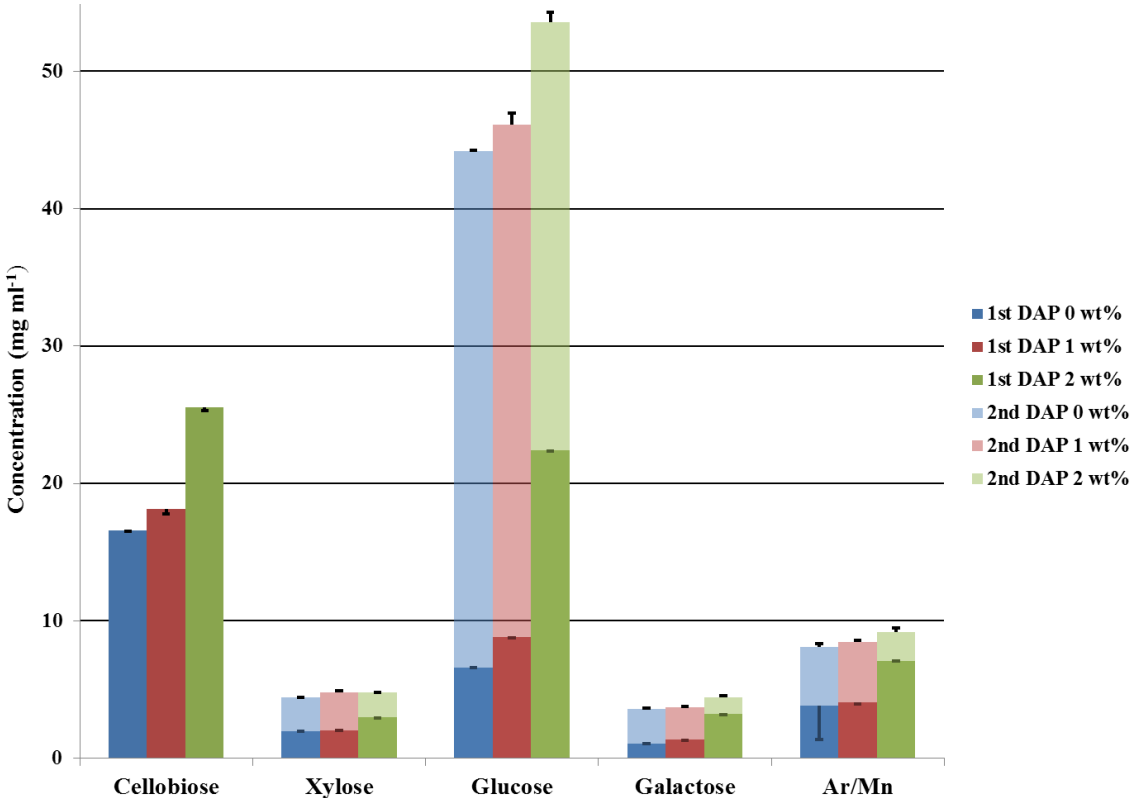


Figure 3-1 Average carbohydrates (cellobiose, xylose, glucose,galactose, mannose & mannose) sugar concentration trend after 1 minute DAP (first & second pretreatment stage)

Figure 3-1 shows the net increase in monomer sugars after oligomer analysis for a previously pretreated DCS at 1 minute. The net increase in monomer sugar

concentrations (after the "sugar recovery" factor) after oligomer analysis (see Figure 3-1) was comparatively higher for both 0 and 1 wt% acid concentrated samples. On average, an approximate net increase of 37 mg ml^{-1} of glucose were measured for both 0 and 1 wt% acid concentration with only about 31 mg ml^{-1} detected for the 2 wt% acid concentrated DCS. Another observation (Figure 3-1) is that the monomer sugar (xylose, galactose, arabinose and mannose) concentrations for 0 & 1 wt% acid-concencetrated hydrolysate doubled after oligomer analysis. TMS (first stage dilute acid hydrolysis + oligomer analysis) were estimated to be 60, 63 and 70 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated samples respectively (Figure A-3). Glucose accounted for 71-78% of the TMS (first stage dilute acid hydrolysis + oligomer analysis) indicating its dominance over other monomer sugars.

3.3.2.2 Enzymatic hydrolysis - 1 minute DAH

In Table 3-2, results for monomer sugars, cellobiose, furfural and HMF measured for DCS (0, 1 and 2 wt% acid concentrations) pretreated for 1 minute with subsequent EH at different incubation times are displayed. The general trend is an increase in TMS and decrease in cellobiose concentration over increasing reaction time. The t=0 hr TMS results are slightly higher than those shown in Figure 3-1 presumable due to the effect of adding enzymes. Glucose concentrations increased (~2x) with a corresponding decrease in cellobiose within the first 24 hour incubation period, an indication that most of the EH occurs within this reaction time. On average, about 17.5 mg ml^{-1} increase in glucose concentrations was observed within the first 24 hours in all cases. Higher acid concentrations resulted in comparatively higher TMS concentrations over increasing reaction time, indicating the effectiveness of the acid catalyst.

Additionally, higher acid concentrations resulted in relatively higher concentrations of inhibitors (Figure A-4). Inhibitors concentrations after DAP (first stage pretreatment), specifically for the 2 wt% acid concentrated DCS sample were high, 0.62 and 0.22 mg ml^{-1} for furfural and HMF respectively. These unusually high concentrations detected may be artifacts of the HPLC analysis for this one experiment set. During EH,

concentrations of HMF remain fairly constant or decreases slightly over time as a result of volatilization from the reaction flask.

Table 3-2 Average (and Standard Deviations) TMS, furfural and HMF concentrations (mg ml^{-1}) for Enzymatic Hydrolysis on 1 minute DAH Samples

Enzymatic Hydrolysate sample	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid- (1 min, DAH)	0	12.4 (±5.7)	2.0 (±1.4)	16.4 (±5.75)	0.9 (±0.61)	1.6 (±1.0)	0.1 (±0.0)	0.1 (±0.01)	19.4 (±10.4)
	24	5.4 (±0.2)	3.5 (±0.4)	38.2 (±1.08)	2.0 (±0.22)	2.4 (±0.42)	0.2 (±0.01)	0.03 (±0.0)	46.1 (±2.0)
	48	3.0 (±0.1)	3.2 (±0.1)	40.0(±1.02)	1.8 (±0.05)	5.6 (±0.18)	0.2 (±0.01)	0.03 (±0.0)	50.7 (±1.3)
	72	3.2 (±0.4)	3.3 (±0.0)	41.2 (±0.43)	1.5 (±0.07)	5.2 (±0.21)	0.2 (±0.01)	0.03 (±0.0)	51.2 (±0.15)
1% wt. Acid- (1 min, DAH)	0	20.2 (±0.3)	2.8 (±0.02)	23.1 (±0.73)	1.3 (±0.02)	3.6 (±0.02)	0.21 (±0.0)	0.21 (±0.0)	30.8 (±0.75)
	24	6.0 (±0.6)	4.1 (±0.1)	39.3 (±0.01)	2.2 (±0.09)	6.4 (±0.67)	0.2 (±0.0)	0.03 (±0.0)	52.0 (±0.86)
	48	4.9 (±0.19)	4.1 (±0.2)	41.3 (±1.10)	2.1 (±0.08)	6.8 (±0.11)	0.2 (±0.0)	0.03 (±0.0)	54.3 (±1.49)
	72	3.8 (±0.04)	4.3 (±0.1)	43.1 (±0.49)	1.9 (±0.03)	6.5 (±0.01)	0.2 (±0.01)	0.03 (±0.0)	55.8 (±0.58)
2% wt. Acid- (1 min, DAH)	0	11.4 (±1.2)	3.7 (±0.0)	26.7 (±0.11)	2.3 (±0.06)	3.8 (±0.09)	0.2 (±0.01)	0.2 (±0.01)	36.6 (±0.25)
	24	9.7 (±0.3)	4.2 (±0.1)	40.6 (±0.15)	2.7 (±0.02)	6.3 (±0.02)	0.2 (±0.01)	0.04 (±0.0)	53.9 (±0.24)
	48	5.0 (±0.3)	4.4 (±0.1)	43.0 (±0.57)	2.8 (±0.05)	6.3 (±0.15)	0.2 (±0.0)	0.04 (±0.0)	56.5 (±0.83)
	72	6.4 (±0.3)	4.6 (±0.0)	43.8 (±0.52)	2.6 (±0.01)	6.0 (±0.07)	0.2 (±0.01)	0.04 (±0.0)	57.0 (±0.58)

3.3.3 Results for experiment set 2

3.3.3.1 Dilute acid pretreatment & oligomer analysis - 30 minutes

Figure 3-2 displays the results of monomer sugar concentrations after 30 minutes of DAP (first & second stage pretreatment) for 0, 1 and 2 wt% acid concentrated samples. Worth noting is the cellobiose trend, which peaks around 26 mg ml^{-1} at 1 wt% acid concentration followed by degradation drop to 11 mg ml^{-1} at 2% acid. The extended reaction time (30 minutes) and the 1 wt % acid concentrated DCS are effective in hydrolyzing more oligomers into cellobiose and subsequent degradation into glucose especially for the 2 wt% hydrolysate. For all sugars, there is less oligomer remaining in solution for the 30 min. reaction period for all acid levels than for the 1 min. results shown in Figure 3-1.

When comparing only glucose concentrations for the first stage pretreatment for 1 and 30 minutes, 7, 9 and 23 mg ml^{-1} were measured for 0, 1 and 2 wt% acid concentration for 1 minute, respectively (see Figure 3-1). For 30 minutes, we measured 7, 18 and 41 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated sample (see Figure 3-2) respectively. The doubling of the glucose concentration indicates the effectiveness of increasing acid

catalyst concentration with increasing reaction time. Reaction temperature (121°C) coupled with increasing reaction time (30 minutes) was not effective in degrading the carbohydrate component into monomer sugars for the 0% acid concentrated sample. Finally, TMS were estimated to be 12.4, 27.1 and 52.7 mg ml^{-1} respectively for the 0, 1 and 2 wt% acid DCS (first stage pretreatment, 30 minutes).

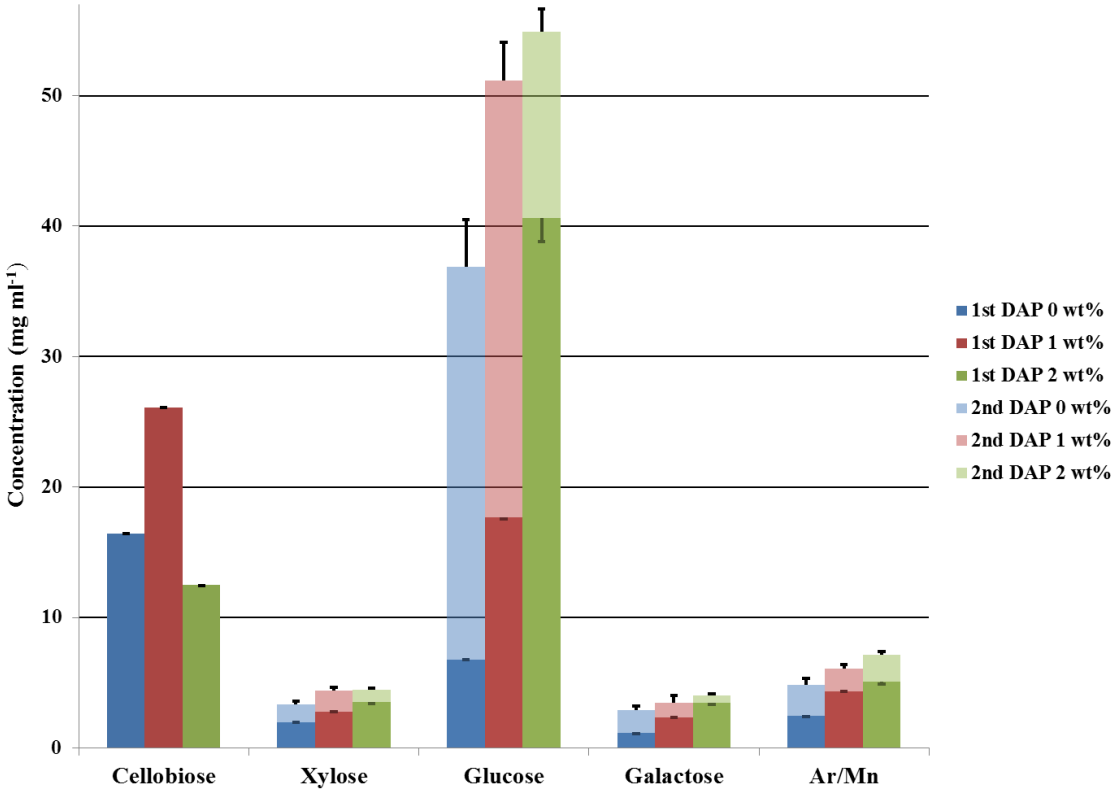


Figure 3-2 Average carbohydrates (cellobiose, xylose, glucose, galactose, mannose & mannose) sugar concentration trend after 30 minutes DAP (first & second pretreatment stage)

The light color shades in Figure 3-2, indicate the net increase in monomer sugars during oligomer analysis (30 minutes DAP) at different acid concentrations. Net glucose concentrations of approximately 30 and 33 mg ml^{-1} were estimated from the oligomer analysis for 0 and 1 wt% samples respectively. Using glucose concentration from the first pretreatment as the basis (see Figure 3-2) indicates an increase by a factor of about 4 and 2 for 0 and 1 wt%. acid concentrated DCS respectively when glucose results from oligomer analysis are considered. The 2 wt% acid hydolyzed sample

yielded only 14 mg ml^{-1} of net glucose after oligomer analysis, as this may indicate that the first hydrolysis step was effective in hydrolyzing most of the glucan component of DCS. TMS (first stage hydrolysis + oligomer analysis) was estimated to be 48, 65 and 69 mg ml^{-1} for 0, 1 and 2 wt% acid DCS, respectively (see Figure A-5).

3.3.3.2 Enzymatic hydrolysis - 30 minutes DAH

Table 3-3 summarizes the results of EH for 30 minutes pretreated DCS. TMS increased with acid concentration over increasing reaction time though this trend was not significant for the 2 wt% acid concentrated DCS. These observations also indicate that the 2 wt% acid concentration was effective in the first stage pretreatment. HMF and furfural concentrations for 1 and 2 wt% acid concentrated samples for the first stage acid pretreatment [(DAP) - see Figure A-6] seems to be unusually low compared to the 0 hour incubation period. However, concentrations decreased overtime due to volatilization and remain relatively constant. The highest conentrations were detected at the 0 hour incubation time for 2 wt% acid concentrated DCS at, 0.2 and 1.2 mg ml^{-1} of HMF and furfural respectively.

Table 3-3 Average TMS, furfural and HMF concentrations (mg ml^{-1}) for EH on 30 minutes DAH

Enzymatic Hydrolysate sample	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid- (1 min, DAH)	0	12.4 (±5.7)	2.0 (±1.4)	16.4 (±5.75)	0.9 (±0.61)	1.6 (±1.0)	0.1 (±0.0)	0.1 (±0.01)	19.4 (±10.4)
	24	5.4 (±0.2)	3.5 (±0.4)	38.2 (±1.08)	2.0 (±0.22)	2.4 (±0.42)	0.2 (±0.01)	0.03 (±0.0)	46.1 (±2.0)
	48	3.0 (±0.1)	3.2 (±0.1)	40.0(±1.02)	1.8 (±0.05)	5.6 (±0.18)	0.2 (±0.01)	0.03 (±0.0)	50.7 (±1.3)
	72	3.2 (±0.4)	3.3 (±0.0)	41.2 (±0.43)	1.5 (±0.07)	5.2 (±0.21)	0.2 (±0.01)	0.03 (±0.0)	51.2 (±0.15)
1% wt. Acid- (1 min, DAH)	0	20.2 (±0.3)	2.8 (±0.02)	23.1 (±0.73)	1.3 (±0.02)	3.6 (±0.02)	0.21 (±0.0)	0.21 (±0.0)	30.8 (±0.75)
	24	6.0 (±0.6)	4.1 (±0.1)	39.3 (±0.01)	2.2 (±0.09)	6.4 (±0.67)	0.2 (±0.0)	0.03 (±0.0)	52.0 (±0.86)
	48	4.9 (±0.19)	4.1 (±0.2)	41.3 (±1.10)	2.1 (±0.08)	6.8 (±0.11)	0.2 (±0.0)	0.03 (±0.0)	54.3 (±1.49)
	72	3.8 (±0.04)	4.3 (±0.1)	43.1 (±0.49)	1.9 (±0.03)	6.5 (±0.01)	0.2 (±0.01)	0.03 (±0.0)	55.8 (±0.58)
2% wt. Acid- (1 min, DAH)	0	11.4 (±1.2)	3.7 (±0.0)	26.7 (±0.11)	2.3 (±0.06)	3.8 (±0.09)	0.2 (±0.01)	0.2 (±0.01)	36.6 (±0.25)
	24	9.7 (±0.3)	4.2 (±0.1)	40.6 (±0.15)	2.7 (±0.02)	6.3 (±0.02)	0.2 (±0.01)	0.04 (±0.0)	53.9 (±0.24)
	48	5.0 (±0.3)	4.4 (±0.1)	43.0 (±0.57)	2.8 (±0.05)	6.3 (±0.15)	0.2 (±0.0)	0.04 (±0.0)	56.5 (±0.83)
	72	6.4 (±0.3)	4.6 (±0.0)	43.8 (±0.52)	2.6 (±0.01)	6.0 (±0.07)	0.2 (±0.01)	0.04 (±0.0)	57.0 (±0.58)

3.3.4 Results for experiment set 3

3.3.4.1 Dilute acid pretreatment & oligomer analysis - 45 minutes

Result of the DAP (first & second stage pretreatment) for 45 minutes is displayed in Figure 3-3. In a similar trend as before, cellobiose increased to about 28 mg ml^{-1} for 1 wt% acid concentrated DCS and decreased to about 11 mg ml^{-1} (about 17 mg ml^{-1} degraded) in the 2 wt% samples. Glucose concentrations after the first pretreatment stage were measured as 6, 18 and 52 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated sample (see Figure 3-3). Comparing with glucose concentrations after first pretreatment stage for 30 minutes (7, 18 and 41 mg ml^{-1} for 0, 1 and 2 wt% acid) in section 3.3.3.1 indicates that approximately 11 mg ml^{-1} of additional glucose was generated for 2 wt% acid sample. In the case of 0 & 1 wt% acid samples there was no significant increase. All other monomer sugars (xylose, mannose, arabinnose and galactose) follow the stepwise increase in concentration with increasing acid concentration as observed for other experiment sets.

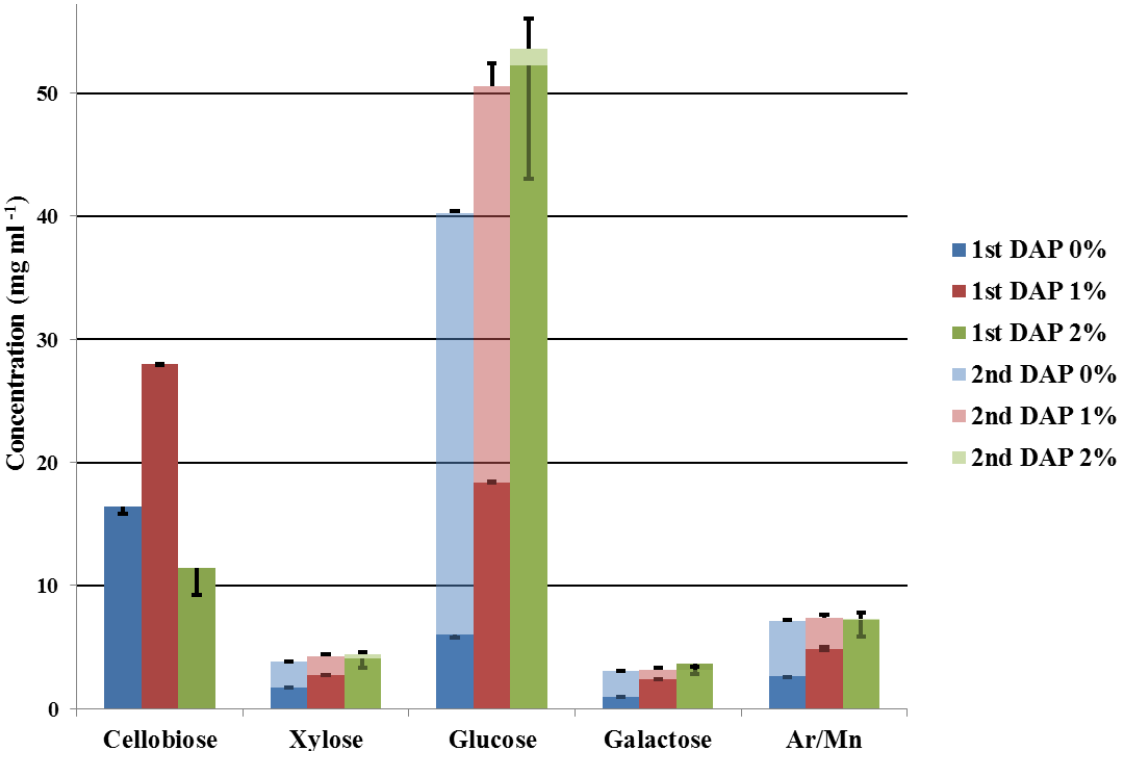


Figure 3-3 Average carbohydrates (cellobiose, xylose, glucose,galactose, mannose & mannose) sugar concentration trend after 45 minutes DAP (first & second pretreatment stage)

Finally, TMS were measured to be 11, 28 and 67 mg ml-1 respectively for the 0, 1 and 2 wt% acid DCS (first stage pretreatment, 45 minutes). Even though the TMS concentration in the 2 wt% acid concentrated DCS was the highest observed so far for all first stage acid pretreatment, there was a high level of uncertainty (67 ± 12 mg ml-1).

Figure 3-3 also shows oligomer analysis of 0, 1 and 2 wt% acid concentrated DCS initially acid pretreated for 45 minutes. The net increase of glucose concentrations measured after oligomer analysis were 34, 32 and 1.43 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated DCS. We observed a decreasing trend in the net increase of glucose concentration with increasing initial pretreatment time after oligomer analysis (2 wt% acid concentrated DCS): 31 mg ml^{-1} (see Figure 3-1, for 1 minute), 14 mg ml^{-1} (see Figure 3-2, for 30 minute) and 1.43 mg ml^{-1} (see Figure 3-3, for 45 minutes). We also observed degradation of galactose sugars (< 1 mg ml^{-1}) for 2 wt% acid concentrated sample (see Figure 3-3). Net increases in TMS were significant for both 0 and 1 wt% acid hydrolysates. Unlike 1 and 30 minutes oligomer analysis (see Figures 3-1 & 3-2) where we observed 35 and 17 mg ml^{-1} (net glucose concentration) for 2 wt% acid concentrated DCS, a rather low yield (1.3 mg ml^{-1}) for 45 minutes oligomer analysis (see Figure 3-3) was observed in addition to galactose (< 1 mg ml^{-1}) degradation. The TMS for both the first stage acid pretreatment and oligomer analysis were estimated to be 54.2, 65.4 and 68.5 mg ml^{-1} (Figure A-7).

3.3.4.2 Enzymatic hydrolysis - 45 minutes DAH

Table 3-4 shows the general trend of increasing TMS and a corresponding decrease in cellobiose concentrations over time for 45 minutes DAH. Glucose concentrations were the most dominant for all hydrolysate analyzed (see Table 3-4). Arabinose/manose (Ar/Mn), HMF and furfural remain approximately constant over time. From Table 3-4, it is indicative that higher acid concentration results in increase in TMS. HMF and Furfural for EH averaged 0.13 (± 0.05) and 0.2 (± 0.04) mg ml^{-1} respectively (see Figure A-8).

Table 3-4 Average TMS, furfural and HMF concentrations (mg ml^{-1}) for EH on 45 minutes DAH

Enzymatic Hydrolysate sample	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid- (30 min, DAH)	0	6.0 (±0.0)	1.9 (±0.07)	25.9 (±1.17)	2.1 (±0.09)	1.1 (±1.63)	0.1 (±0.01)	0.5 (±0.05)	31.0 (±2.9)
	24	5.6 (±0.15)	3.6 (±0.01)	39.1 (±0.5)	1.9 (±0.11)	3.2 (±0.10)	0.2 (±0.003)	0.04 (±0.0)	47.8 (±0.7)
	48	3.4 (±0.03)	3.6 (±0.14)	42.2 (±0.49)	1.9 (±0.08)	5.9 (±0.27)	0.2 (±0.01)	0.04 (±0.001)	53.7 (±1.0)
	72	3.5 (±0.12)	3.7 (±0.06)	42.7 (±0.31)	1.7 (±0.04)	5.3 (±0.50)	0.2 (±0.02)	0.04 (±0.0)	53.4 (±0.84)
1% wt. Acid- (30 min, DAH)	0	5.6 (±0.0)	2.6 (±0.08)	30.3 (±0.48)	3.5 (±0.06)	6.0 (±3.08)	0.2 (±0.01)	1.1 (±0.03)	42.5 (±2.6)
	24	7.0 (±0.24)	5.2 (±0.12)	42.5 (±0.80)	3.3 (±0.01)	5.2 (±0.0)	0.3 (±0.002)	0.05 (±0.002)	56.2 (±0.9)
	48	3.3 (±1.15)	3.3 (±1.84)	35.8 (±14.5)	2.4 (±0.9)	5.7 (±1.66)	0.2 (±0.08)	0.04 (±0.02)	47.2 (±18.9)
	72	4.2 (±0.1)	4.8 (±0.12)	47.2 (±0.97)	2.8 (±0.07)	6.7 (±0.52)	0.3 (±0.01)	0.05 (±0.001)	61.5 (±1.7)
2% wt. Acid- (30 min, DAH)	0	5.9 (±0.13)	2.7 (±0.0)	42.3 (±0.31)	4.5 (±0.03)	10.6 (±0.47)	0.2 (±0.002)	1.8 (±0.04)	60.1 (±0.8)
	24	4.1 (±0.17)	5.3 (±0.02)	49.4 (±1.52)	5.0 (±0.01)	7.0 (±0.08)	0.4 (±0.02)	0.09 (±0.002)	66.7 (±1.6)
	48	6.0 (±0.27)	4.3 (±0.11)	48.8 (±1.06)	4.5 (±0.06)	7.1 (±0.11)	0.4 (±0.01)	0.09 (±0.001)	64.7 (±1.3)
	72	3.8 (±0.12)	4.3 (±0.17)	50.2 (±2.0)	4.0 (±0.11)	6.7 (±0.25)	0.3 (±0.01)	0.09 (±0.003)	65.3 (±2.5)

3.3.5 Results for experiment set 4

3.3.5.1 Dilute acid pretreatment & oligomer analysis – 60 minutes

Results of first & second stage DAP for 60 minutes are displayed in Figure 3-4. As previously observed, cellobiose increased from 18 mg ml^{-1} for 0 wt% acid concentrated sample peaking at 27 mg ml^{-1} (1 wt% acid) followed by subsequent degradation to 10 mg ml^{-1} for 2 wt% acid concentrated sample. (see Figure 3-4).

The glucose concentration after first pretreatment stage was measured to be 6.8, 22 and 49 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated sample (see Figure 3-4). Comparing with glucose concentration for the first pretreatment after 45 minutes (6, 18 and 52 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated sample, see section 3.3.4.1) indicates a slight improvement for 1 wt% acid concentrated sample after 60 minutes DAP. Finally, TMS were estimated to be 13.6, 36 and 66 mg ml^{-1} respectively for the 0, 1 and 2 wt% acid DCS (first stage pretreatment, 60 minutes). Even though TMS of 2 wt% acid concentrated DCS for 45 minutes (see Figure 3-3) was 67 (±12) mg ml^{-1} as opposed to 66 (±2) mg ml^{-1} for the 60 minutes (see Figure 3-4), the high level of uncertainty for the former makes the 60 minute result a more confident choice.

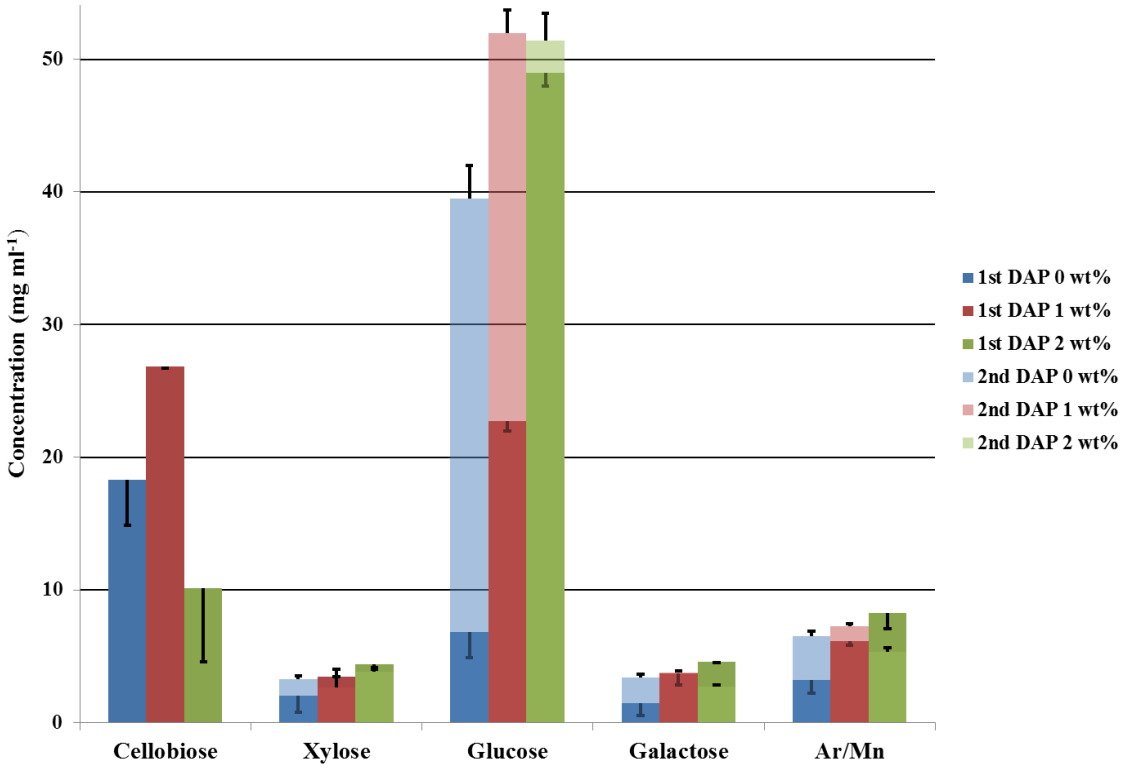


Figure 3-4 Average carbohydrates (cellobiose, xylose, glucose,galactose, mannose & mannose) sugar concentration trend after 60 minutes DAP (first & second pretreatment stage)

The net increase in monomer sugars due to 60 minutes oligomer analysis is also displayed in Figure 3-4. The increase in glucose concentrations measured after oligomer analysis were 32, 29 and 2.4 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated DCS as shown in Figure 3-4 indicating the effectiveness of the first pretreatment stage in the case of 2 wt% acid sample. Apart from glucose, all other monomer sugars (xylose, galactose, arabinose and mannose) degraded in the 2 wt% acid hydrolysate (concentration became lower). Glucose once again showed significant net increase especially for 0 & 1 wt% acid concentrated DCS, an indication that the first hydrolysis stage was not effective in hydrolyzing the glucan component in DCS. Finally, TMS for both the first stage acid pretreatment and oligomer analysis combined was estimated to be 52.7, 65.6 and 65.7 mg ml^{-1} (see Figure A-9).

3.3.5.2 Enzymatic hydrolysis - 60 minutes DAH

Table 3-5 shows the TMS, furfural and HMF concentrations after varying times of EH (60 minutes first stage DAH). A trend of increasing xylose, glucose and galactose production over time was observed. Glucose is the dominant sugar, and it showed the largest increase in the first 24 hours of EH (0 wt% acid concentrated DCS). Arabinose/manose, HMF and furfural remained approximately constant over time, and cellobiose showed a decreasing trend over time with none detected in some cases. Results for inhibitors (HMF and Furfural) concentrations generated for the 60 minutes hydrolysis scheme fluctuate with no clear pattern, they were all below 0.3 mg ml^{-1} (see Figure A-10).

Table 3-5 Average TMS, furfural and HMF concentrations (mg ml^{-1}) for EH on 60 minutes DAH

Components	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid- (60 min, DAH)	0	19.0 (±1.9)	2.0 (±0.9)	17.3 (±0.9)	0.6 (±0.6)	2.3 (±0.5)	0.1 (±0.0)	0.1 (±0.0)	22.2 (±2.9)
	24	9.1 (±0.0)	2.2 (±0.0)	32.3 (±0.3)	0.5 (±0.0)	2.2 (±0.0)	0.1 (±0.0)	0.1 (±0.0)	37.3 (±0.2)
	48	3.1 (±0.1)	3.2 (±0.0)	40.8 (±0.2)	0.9 (±0.4)	2.8 (±0.8)	0.1 (±0.0)	0.2 (±0.0)	47.8 (±1.4)
	72	(±0.0)	3.7 (±1.2)	44.8 (±0.2)	2.6 (±2.8)	2.9 (±0.7)	0.1 (±0.0)	0.1 (±0.0)	53.9 (±4.9)
1% wt. Acid- (60 min, DAH)	0	9.7(±0.2)	3.4 (±0.3)	29.6(±0.8)	2.2 (±0.1)	4.2 (±0.2)	0.1 (±0.0)	0.2 (±0.0)	39.4 (±1.3)
	24	9.4 (±0.0)	2.8 (±0.3)	40.7 (±1.1)	1.2 (±0.5)	3.3 (±0.4)	0.1 (±0.0)	0.2 (±0.0)	48.0 (±0.2)
	48	2.9 (±0.9)	3.4 (±0.4)	48.9 (±7.0)	1.7 (±0.7)	4.0 (±0.9)	0.2 (±0.1)	0.2 (±0.0)	58.0 (±9.1)
	72	(±0.0)	4.3 (±0.1)	47.4 (±0.9)	3.0 (±0.0)	4.6 (±0.2)	0.1 (±0.0)	0.2 (±0.0)	59.2 (±0.8)
2% wt. Acid- (60 min, DAH)	0	6.7 (±0.3)	3.9 (±0.2)	48.7 (±0.7)	2.8 (±0.1)	5.2 (±0.1)	0.2 (±0.0)	0.2 (±0.0)	60.7 (±1.0)
	24	(±0.0)	3.8 (±0.0)	51.9 (±0.3)	3.0 (±0.0)	5.6 (±0.1)	0.2 (±0.0)	0.2 (±0.0)	64.3 (±0.5)
	48	(±0.0)	4.1 (±0.3)	49.6 (±4.9)	2.9 (±0.4)	5.4 (±0.8)	0.2 (±0.1)	0.2 (±0.0)	62.0 (±5.8)
	72	(±0.0)	3.3 (±0.0)	53.3 (±0.0)	3.1 (±0.1)	5.2 (±0.1)	0.2 (±0.0)	0.2 (±0.0)	64.9 (±0.2)

3.3.6 Results for experiment set 5

3.3.6.1 Dilute acid pretreatment - 75 minutes

Sugar concentrations measured after 75 minutes of dilute acid hydrolysis (first & second stage pretreatment) are shown in Figure 3-5. Cellobiose increased from 15 mg ml^{-1} (0 wt% acid concentrated sample) peaking at 27 mg ml^{-1} (1 wt% acid concentrated sample). It further degraded due to increased acid catalyst concentration and reaction time to 6 mg ml^{-1} (2 wt% acid) as shown in Figure 3-5.

Glucose concentration after the first acid pretreatment stage was 6, 23 and 51 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated sample (see Figure 3-5) for 75 minutes. There was no significant increase in monomer sugars especially (glucose) when compared to the previous hydrolysis stage at 60 minutes (see section 3.3.5.1). TMS were estimated to be 11.5, 34.0 and 65.2 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated DCS respectively, slightly lower compared to 60 minutes first stage pretreatment (see Figure 3-4). After 75 minutes, little monomer sugars were generated for the second stage acid pretreatment.

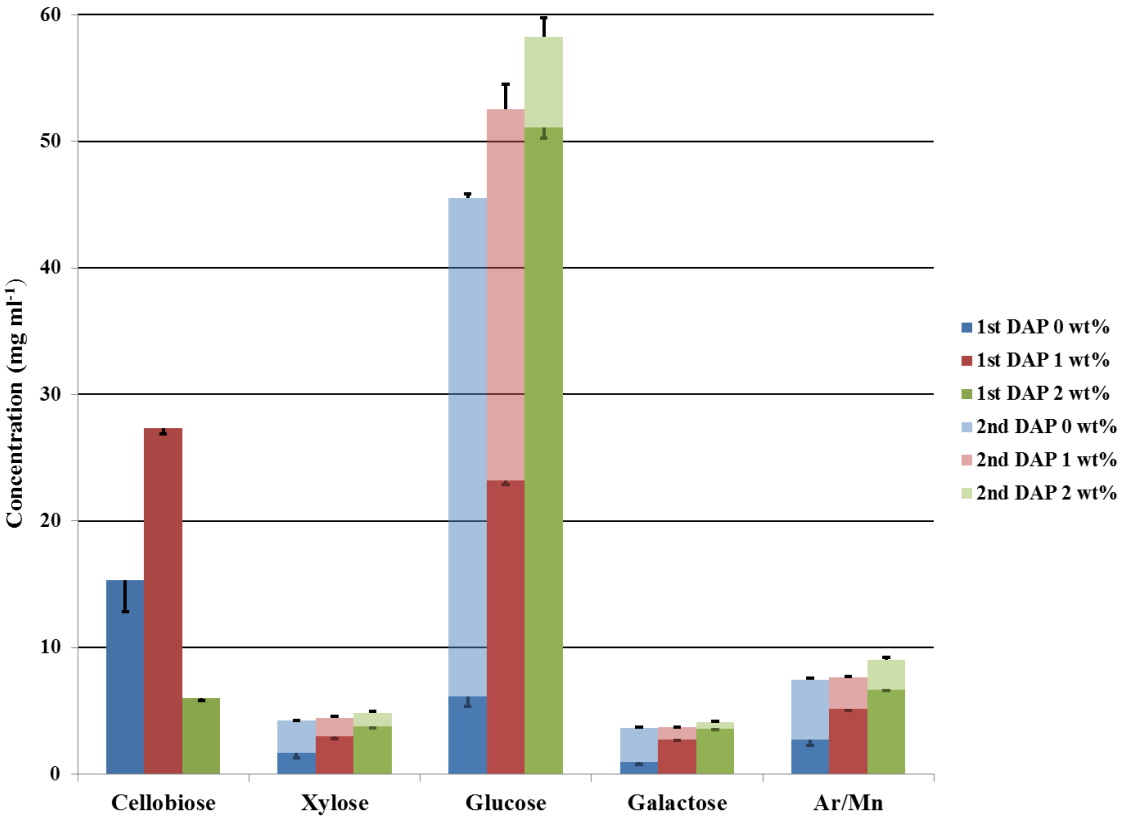


Figure 3-5 Average carbohydrates (cellobiose, xylose, glucose, galactose, mannose & arabinose) sugar concentration trend after 75 minutes DAP (first & second pretreatment stage)

The net increase in monomer sugars due to oligomer analysis of hydolysate previously subjected to 75 minutes DAP (first stage) is also shown in Figure 3-5. Interestingly,

there was no degradation of monomer sugars for 75 minutes oligomer analysis, as observed for the 60 minutes (section 3.3.5.1). The TMS for both hydrolysis (first stage pretreatment + oligomer analysis) is displayed in Figure A-11, 60.8, 68.3 and 74.8 mg ml^{-1} of TMS were measured for 0, 1 and 2 wt% acid DCS respectively.

3.3.6.2 Enzymatic hydrolysis - 75 minutes DAH

Table 3-6 shows the results for monomer sugar and inhibitors concentrations for samples treated for 75 minutes with 0, 1 and 2 wt% DAP (first stage) respectively followed by varying times of EH. The general trend, which is similar to previous experiments, shows a sharp increase in glucose concentration within the first 24 hour incubation period, especially for 0 wt% acid DCS, with a simultaneous decline in cellobiose. When comparing the three acid treatment intensities, the results indicated that nearly all concentrations are higher at higher acid concentrations. Slightly higher concentrations of inhibitors were observed for 75 minutes DAH (see Figure A-12) compared to 60 minutes (see Figure A-10). After the first stage DAP, inhibitor concentration for 2 wt% acid content remains constant over time after a slight increase during the first 24 hour incubation period.

Table 3-6 Average TMS, furfural and HMF concentrations (mg ml^{-1}) for EH on 75 minutes DAH

Components	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid- (75 min, DAH)	0	18.4 (±0.8)	2.0 (±0.0)	20.5 (±0.3)	0.8 (±0.0)	2.9 (±0.0)	0.1 (±0.0)	0.1 (±0.0)	26.2 (±0.3)
	24	5.6 (±0.1)	2.6 (±0.0)	37.7 (±0.0)	0.9 (±0.0)	2.8 (±0.0)	0.2 (±0.0)	0.2 (±0.0)	43.9 (±0.1)
	48	3.4 (±0.1)	3.0 (±0.1)	40.8 (±0.1)	1.0 (±0.0)	2.9 (±0.0)	0.1 (±0.0)	0.2 (±0.0)	47.8 (±0.0)
	72	2.6 (±0.2)	3.2 (±0.0)	40.7 (±0.3)	1.0 (±0.0)	2.7 (±0.0)	0.1 (±0.0)	0.2 (±0.0)	47.6 (±0.4)
1% wt. Acid- (75 min, DAH)	0	12.0 (±0.2)	3.0 (±0.1)	29.2 (±0.6)	2.3 (±0.0)	4.4 (±0.1)	0.2 (±0.0)	0.3 (±0.0)	38.9 (±0.8)
	24	5.7 (±0.0)	3.6 (±0.1)	43.6 (±0.5)	2.3 (±0.0)	4.4 (±0.1)	0.2 (±0.0)	0.3 (±0.0)	54.0 (±0.6)
	48	3.9 (±0.1)	4.0 (±0.1)	46.3 (±0.9)	2.5 (±0.1)	4.4 (±0.1)	0.1 (±0.0)	0.3 (±0.0)	57.2 (±1.1)
	72	3.2 (±0.0)	4.1 (±0.1)	45.9 (±0.9)	2.5 (±0.0)	4.3 (±0.1)	0.1 (±0.0)	0.3 (±0.0)	56.8 (±1.0)
2% wt. Acid- (75 min, DAH)	0	4.3 (±0.1)	3.3 (±0.0)	49.8 (±0.4)	2.9 (±0.1)	5.4 (±0.0)	0.3 (±0.0)	0.3 (±0.0)	61.3 (±0.3)
	24	1.4 (±0.1)	3.5 (±0.1)	53.3 (±0.6)	2.9 (±0.1)	5.4 (±0.1)	0.3 (±0.0)	0.3 (±0.0)	65.1 (±0.9)
	48	1.3 (±0.0)	3.7 (±0.0)	53.6 (±0.1)	3.0 (±0.1)	5.5 (±0.1)	0.2 (±0.0)	0.3 (±0.0)	65.7 (±0.3)
	72	1.3 (±0.0)	3.6 (±0.0)	52.8 (±0.1)	3.0 (±0.0)	5.3 (±0.1)	0.2 (±0.0)	0.3 (±0.0)	64.7 (±0.0)

3.3.7 Results for experiment set 7

3.3.7.1 Dilute acid pretreatment - 90 minutes

Figure 3-6 summarizes both the first & second stage acid pretreatment results of DCS for 90 minutes. There is a general increase in monomer sugar concentrations with increasing acid concentration. As previously observed, while the 1 wt% acid content tends to breakdown more polymers into oligomers, the 2 wt% is effective in further hydrolyzing cellobiose into glucose units hence a corresponding decrease in concentration of cellobiose (see Figure 3-6).

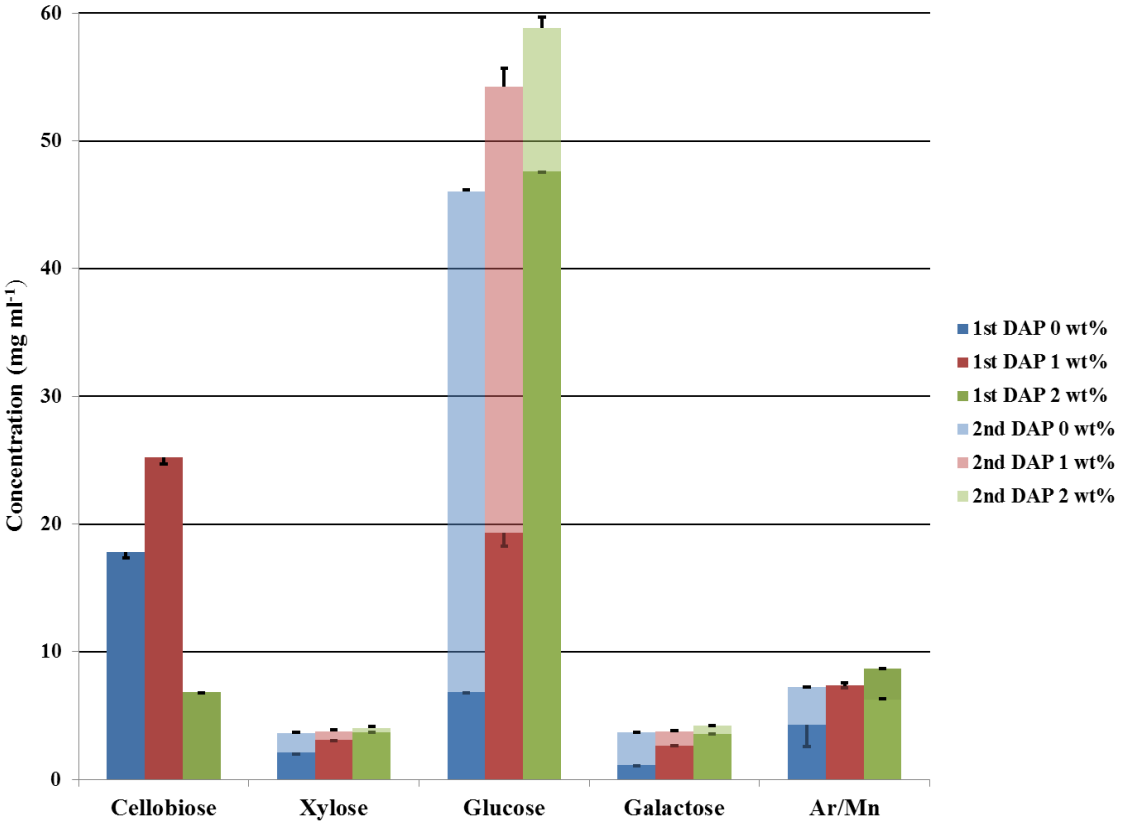


Figure 3-6 Average carbohydrates (cellobiose, xylose, glucose,galactose, mannose & mannose) sugar concentration trend after 90 minutes DAP (first & second pretreatment stage)

Glucose concentrations after the first pretreatment stage (90 minutes) were 7, 19 and 48 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated DCS respectively. Comparing these results to glucose concentrations after first stage pretreatment for 60 and 75 minutes (see Figures 3-4 & 3-5) indicates no increase for 0 and 2 wt% acid concentrated

samples but further decline in the 1 wt% acid concentrated sample. TMS after first stage pretreatment was 14.3, 32.4 and 63.6 mg ml^{-1} for 0, 1 and 2 wt% acid concentrated DCS respectively with glucose being the highest as observed in all experiments for the first stage acid pretreatment (see Figure 3-6).

In the oligomer analysis results for 90 minutes DAP (see Figure 3-6), more monomer sugars were detected in the 0 and 1 wt% DCS generating a total of 46 and 37 mg ml^{-1} (net increase) of TMS respectively. Glucose concentrations increased and peaked at approximately 39 mg ml^{-1} for 0 wt% acid concentrated DCS. The TMS for the two pretreatment stages (first acid hydrolysis + oligomer analysis) were estimated to be 60.5, 69.0 and 74.3 mg ml^{-1} (Figure A-13).

3.7.2 Enzymatic hydrolysis - 90 minutes DAH

Table 3-7 summarizes the sugar, furfural and HMF concentrations for samples pretreated for 90 minutes with 0, 1 and 2 wt% wt. acid and subsequent EH at varying reaction times. A similar trend of increasing xylose, glucose and galactose over time was observed.

Table 3-7 Average TMS, furfural and HMF concentrations (mg ml^{-1}) for EH on 90 minutes DAH

Components	Time (hours)	Cellobiose	Xylose	Glucose	Galactose	Ar/Mn	HMF	Furfural	TMS
0% wt. Acid, 90 min DAH	**0**	18.1 (±0.2)	2.2 (±0.5)	11.1 (±0.1)	1.1 (±0.3)	8.7 (±4.1)	0.1 (±0.0)	0.1 (±0.0)	23.1 (±3.4)
	24	4.9 (±0.3)	2.9 (±0.6)	39.1 (±1.0)	1.6 (±0.4)	3.0 (±0.1)	0.1 (±0.0)	0.1 (±0.1)	46.8 (±2.1)
	48	2.8 (±0.0)	2.9 (±0.0)	41.2 (±0.5)	1.6 (±0.0)	3.2 (±0.0)	0.08 (±0.0)	0.1 (±0.0)	48.8 (±0.5)
	72	3.1 (±0.1)	3.8 (±0.1)	42.3 (±0.1)	1.3 (±0.1)	3.3 (±0.2)	0.08 (±0.0)	0.1 (±0.0)	50.7 (±0.3)
1% wt. Acid, 90 min DAH	**0**	25.5 (±0.9)	2.7 (±0.2)	23.9 (±0.2)	2.2 (±0.0)	9.3 (±0.2)	0.1 (±0.0)	0.2 (±0.1)	35.4 (±0.0)
	24	4.4 (±0.3)	3.3 (±0.1)	44.8 (±1.1)	2.9 (±0.3)	4.3 (±0.3)	0.2 (±0.0)	0.3 (±0.0)	55.4 (±1.3)
	48	6.6 (±0.3)	6.4 (±0.9)	48.5 (±1.9)	4.2 (±0.8)	5.9 (±0.5)	0.2 (±0.1)	0.2 (±0.1)	65.1 (±4.1)
	72	(±0.0)	4.5 (±0.8)	51.3 (±4.6)	3.1 (±0.2)	5.4 (±0.2)	0.2 (±0.1)	0.3 (±0.0)	64.4 (±3.9)
2% wt. Acid, 90 min DAH	**0**	6.6 (±0.5)	3.3 (±0.1)	46.6 (±0.5)	3.6 (±0.2)	10.9 (±0.2)	0.2 (±0.0)	0.3 (±0.0)	61.2 (±0.0)
	24	1.1 (±0.0)	3.2 (±0.0)	53.4 (±0.1)	3.3 (±0.0)	5.3 (±0.0)	0.4 (±0.0)	0.4 (±0.0)	65.2 (±0.1)
	48	4.3 (±0.5)	4.5 (±0.2)	53.1 (±0.4)	3.7 (±1.0)	5.8 (±0.6)	0.1 (±0.0)	0.4 (±0.0)	67.2 (±2.2)
	72	(±0.0)	4.1 (±0.2)	48.7 (±4.7)	2.7 (±0.5)	4.9 (±0.8)	0.2 (±0.1)	0.2 (±0.0)	60.5 (±5.8)

Glucose is the dominant sugar once again, and it shows the largest increase within the first 24 hours of enzymatic hydrolysis, with little increase after 24 hours. Inhibitors

generated for the 90 minutes hydrolysis scheme are shown in Figure A-14. Comparatively, slightly higher concentrations were measured with 0.37 (HMF) and 0.40 mg ml^{-1} (furfural) estimated as the highest for 2 wt% acid concentrated DCS for the first 24 hour incubation period. HMF and furfural concentrations decreased over time after the 72 hour incubation period due to volatilization.

3.4 Discussion

The average total carbohydrate (starch, soluble sugars & cellulose) content of DCS is 27 wt% on a dry solid basis. The maximum theoretical yield assuming total hydrolysis (i.e., 100% solubilization of carbohydrate component in DCS into monomer sugars) was estimated to be 76 mg ml^{-1} (see Appendix A for detailed calculations).

First stage acid pretreatment: Figure A-16 summarizes the effect of residence time on the TMS yields (first stage acid pretreatment) at various acid concentrations (0, 1 & 2 wt%). The effect of 0 wt% acid on the yield of TMS overtime generally was very low, the highest yield observed was 14 mg ml^{-1} for both 60 and 90 minutes, representing 18% (=14/76) of the theoretical TMS yield. Clearly, the application of temperature alone over increasing reaction time was not effective in degrading the polymeric component of the carbohydrates in DCS into monomer sugars. Apart from 1 minute DAP (first stage pretreatment), TMS yield increased two fold (2x.) with the application of 1 wt% acid concentration, peaking at 60 minutes with a reported yield of 36 mg ml^{-1} (Figure A-16). This represents only 47% of the theoretically available carbohydrates, a clear indication of the effectiveness of the presence of acid catalyst as compared to the 0 wt% acid concentrated DCS. The TMS yield continues to increase with the application of 2 wt% acid. Estimated yields at 2 wt% acid concentration for 45 and 60 minutes were 67 (87%) and 66 (86%) mg ml^{-1} respectively, in parenthesis are the theoretical yields. However, the high level of uncertainty associated with the glucose peak at 45 minutes (Figure 3-3) makes 60 minutes a better option for reaction time. The high acid concentration requirement for increase in TMS is due to the high ash content (alkaline in nature) which continues to neutralize the effect of the acid catalyst. The signature for 1 and 2 wt% acid concentrated hydrolysate was very similar

(Figure A-16), there was an increase in concentrations of TMS overtime, peaking around 45 & 60 minutes and consequently experiencing a decline in TMS due to the degradation of monomer sugars into inhibitory products over extended reaction time. The key highlight from the first stage pretreatment is that, 2 wt% acid concentrated DCS was the most effective in hydrolyzing the carbohydrates into monomer sugars, especially for 45 and 60 minutes. This is most likely because the bulk of the carbohydrate components comprise of starch, soluble monomer sugars and non-starch components (xylan, mannan, galactan, mannan, & cellulose)(Adom et al. 2012). Apart from cellulose, all other components are easily susceptible to degradation in the presence of acid catalyst at elevated temperatures.

Oligomer analysis (second stage acid pretreatment): The application of the second stage acid pretreatment (oligomer analysis) in principle should be capable of hydrolyzing all the carbohydrates into monomer sugars. This however can increase the concentration of inhibitors due to the increase in acid concentration and consequently affect TMS yield. From an industrial perspective, it is not attractive because the increase in acid requirement adversely affects pretreatment reactors and subsequently results in increase cost of maintaining reactors. The key highlights of the oligomer hydrolysis is that, 2 wt% acid concentrated samples pretreated initially at 75 and 90 minutes [see Figures A-11 and A-13] followed by oligomer analysis yielded 75 (97%) and 74 (96%) mg ml^{-1} of TMS. This represents near full recovery of the TMS, there was however, a comparatively high concentrations of inhibitors observed most likely due to the increased acid concentration and extended reaction time. Second stage pretreatment was most effective for 0 wt% acid concentrated in terms of TMS yield (during DAP) but less effective for 1 and 2 wt% acid concentrated DCS (especially for 2 wt%).

Enyzmatic hydrolysis: A summary of the enzymatic hydrolysis results for the different acid concentrations versus time are displayed in Figures A-17, A-18 & A-19. In almost all cases, increase in monomer sugars occurs simultaneously with decreasing cellobiose concentrations, a clear indication of the effectiveness of the cellulase

enzymes. It was also observed that enzymes were predominantly active within the first 24 hour incubation period resulting in drastic increase in concentrations of monomer sugars especially for glucose. Another observation was that, while cellulase enzymes were most efficient in hydrolyzing 0 wt% acid pretreated DCS, since the first stage acid pretreatment were comparatively efficient for the 1 and 2% acid pretreated DCS samples. We also observed from the various experimental results that extended reaction times and reaction temperature were not effective for 0 wt% hydrolysate for the first stage acid pretreatment. TMS increased by a factor of four in all cases for 0 wt% acid concentrated DCS subjected to EH (see Tables A-1 & Figure A-20) after 72 hours of incubation time. Enzymes were less efficient in hydrolyzing both 1 and 2 wt% acid concentrated samples (see Tables A-2 and A-3; Figures A-21 and A-22) probably because a significant portion of the carbohydrate component has been hydrolysed after the first stage pretreatment. TMS increased by a factor of 2 and 1 after 72 hours EH for 1 and 2 wt% acid pretreated samples respectively, confirming the hypothesis that increasing acid concentration (especially 2%) was efficient in hydrolyzing the carbohydrate component.

Inhibitors: The concentrations of HMF and furfural generated over time during the first stage acid pretreatment are displayed (Figure A-23). Furfural shows unusually high concentrations for 2% wt. acid at 1 minute, 0.6 mg ml^{-1} was the highest concentration observed. The next highest level of furfural was at 1 wt% acid concentration and 30 minutes, estimated at 0.38 mg ml^{-1}. Also, the highest observed HMF concentration was 0.25 mg ml^{-1} at 45 minutes. We compared these estimated inhibitors generated after the first pretreatment stage (Figure A-23) to various acceptable level of inhibitor concentrations for a strain of *E.coli* anf three different types of yeast (Tables A-4). It can be inferred that levels of inhibitors generated in these experiments are below inhibitory levels likely to affect the efficiency of fermentation with *E.coli* into biobased chemicals.

3.5 Conclusions

In our choice of optimal condition, our goal was to identify the condition that maximizes yield of total monomer sugars within the shortest possible time as well as producing low concentrations of inhibitors. Avoidance of the application of enzyme will be ideal if at all possible given the significant portion of the costs associated with bio-based chemical production. From our analysis, we observed that contribution of cellulase enzymes to the TMS yield was not so significant. With high level of certainty, we determined that the first stage acid pretreatment for 60 minutes at 2 wt% acid was efficient in producing approximately 86% of the theoretically available carbohydrates with acceptable low inhibitory level.

3.6 References

Adom F, Fan J, Davis J, Dunn P, Shonnard D. 2012. Compositional Analysis of Defatted Syrup from a Corn Ethanol Dry Mill as a Feedstock for Bio-Based Products. Journal of agricultural and food chemistry (in revisions).

Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. 2011. Biomass pretreatment: fundamentals toward application. Biotechnology advances 29(6):675-685.

Brodeur G, Yau E, Badal K, Collier J, Ramachandran K, Ramakrishnan S. 2011. Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. Enzyme research 2011.

Claassen P, Van Lier J, Lopez Contreras A, Van Niel E, Sijtsma L, Stams A, De Vries S, Weusthuis R. 1999. Utilisation of biomass for the supply of energy carriers. Applied Microbiology and Biotechnology 52(6):741-755.

de Wild P, Reith H, Heeres E. 2011. Biomass pyrolysis for chemicals. Biofuels 2(2):185-208.

Eggeman T, Elander RT. 2005. Process and economic analysis of pretreatment technologies. Bioresource technology 96(18):2019-2025.

Harmsen P, Huijgen W, López LMB, Bakker R. 2010. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Wageningen UR, Food & Biobased Research. No. 1184.

Kootstra AMJ, Beeftink HH, Scott EL, Sanders JPM. 2009. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochemical Engineering Journal 46(2):126-131.

Lee J. 1997. Biological conversion of lignocellulosic biomass to ethanol. Journal of biotechnology 56(1):1-24.

Selig M, Weiss N, Ji Y. 2008. Enzymatic saccharification of lignocellulosic biomass. Golden, CO: National Renewable Energy Laboratory; 8 p. Report No.: NREL/TP-510-42629.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Golden, CO: National Renewable Energy Laboratory;14 p. Report No.: NREL/TP-510-42623.

Sun Y, Cheng J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource technology 83(1):1-11.

Werpy T, Petersen G, Aden A, Bozell J, Holladay J, White J, Manheim A, Eliot D, Lasure L, Jones S. 2004. Top Value Added Chemicals From Biomass. Volume 1-Results of Screening for Potential Candidates From Sugars and Synthesis Gas. DTIC Document.

Zheng Y, Pan Z, Zhang R. 2009. Overview of biomass pretreatment for cellulosic ethanol production. International Journal of Agricultural and Biological Engineering 2(3):51-68.

Chapter 4

4 Optimization of the Protein Hydrolysis Scheme of Defatted Syrup from a Corn Ethanol Dry Mill Facility[3]

4.1 Introduction

As the building blocks of life, amino acids have long played an important role in human, animal nutrition and health maintenance (Leuchtenberger et al. 2005; Bercovicil and Fuller 2008). Amino acids have applications such as animal feed additives (lysine, methionine, threonine and tryptophan), flavor enhancers (monosodium glutamate, serine, aspartic acid) and as specialty nutrients in the medical field. Contributing the largest share by weight (56%) of the total amino acids sold globally, animal feed additives were estimated at approximately $4.5 billion in terms of the market volume in 2004 (Leuchtenberger et al. 2005). Protein hydrolysis into constituent amino acids has applications in areas such as biochemistry, food science, microbiology, clinical studies, food industries, and diagnostic studies. Amino acid analysis (AAA) involves breaking down of protein to free the peptides/amino acids followed by quantitative measurements using chromatographic instruments. Here, a summary of literature review findings in relations to protein hydrolysis are discussed with emphasis on the following; i) types of protein hydrolysis (ii) enzymatic hydrolysis and the different types of proteases and (iii) separation, detection techniques and quantification of amino acids.

Protein hydrolysis can be classified into two major groups as chemical and enzymatic (Fountoulakis and Lahm 1998). Each hydrolysis route has been widely reported in the literature (Ozols 1990; I. Davidson 1997; Irvine 1997; Fountoulakis and Lahm 1998; Smith 2003) highlighting advantages and disadvantages. Factors such as *temperature*,

[3] *This chapter will be submitted to Biofuels, Bioproducts and Biorefining for publication.* Citation: Adom, F., Dunn, P., Ruccins, S., Shonnard, D. (2012). Optimization of the Protein Hydrolysis Scheme of Defatted Syrup from a Corn Ethanol Dry Mill Facility. Biofuels, Bioproducts and Biorefining.

reaction time, *hydrolysis agent and additives* affect the completeness of protein hydrolysis reactions (Fountoulakis and Lahm 1998). Due to its convenience, acid hydrolysis is the most commonly used method and two types have been reported; liquid- and gas-phase mode (Fountoulakis and Lahm 1998). In the liquid-phase mode, samples are dried to prevent the dilution of acid by water prior hydrolysis and subsequently hydrolyzed in a tube at 110^{o}C for 24 hours (Fountoulakis and Lahm 1998). Vapor-phase mode hydrolysis involves the use of acid vapor to hydrolyze the dried samples in hydrolysis solution under an inert atmosphere; it is suitable when only smaller quantities of sample are available. Vapor phase hydrolysis is also conducted around 110^{o}C for a 24 hour period with the advantage of reducing contamination of sample due to the use of acid reagent (Fountoulakis and Lahm 1998).

Typical acid hydrolysis reagents reported in the literature included HCl, (Badadani et al. 2007) methanesulfonic acid, (Malmer and Schroeder 1990) toluenesulfonic acid, HCl-propionic acid, and mercaptoethanesulfonic acid (Fountoulakis and Lahm 1998). One advantage of methanesulfonic acid is that it allows for the determination of tryptophan and methionine sulfoxide, which are usually destroyed during the conventional hydrolysis with HCl (Fountoulakis and Lahm 1998). Liquid-phase mode hydrolysis has some setbacks however, because asparagine and glutamine are completely hydrolyzed to aspartic and glutamic acids but tryptophan and cysteine are destroyed. Vapor-phase mode hydrolysis though relatively fast, presents the danger of exploding of vials due to high-pressure requirement (Ian Davidson and O'Connor 2008).

Alkaline hydrolysis requires the use of a basic medium for the hydrolysis to proceed. The stable nature of tryptophan in a basic medium makes this approach suitable for its determination (Fountoulakis and Lahm 1998). This type of hydrolysis specifically uses aqueous solutions of NaOH or KOH to degrade proteins into peptides and amino acids. The use of heat at elevated temperatures of approximately 150^{o}C accelerates the hydrolytic process (Thacker 2004). Apart from tryptophan determination, alkaline

hydrolysis has been used to determine phosphoamino acids (e.g., phosphohistidine), sulfated tyrosine and also for the release of phosphate from phosphor-serinyl and threonyl residues (Fountoulakis and Lahm 1998). There are also reported drawbacks associated with the use of alkaline hydrolysis approach such as the complete destruction of some amino acids, e.g. arginine, asparagine, glutamine, and serine. Additionally, other amino acids become racemized (Thacker 2004).

A number of studies (Pickering and Newton 1990; Fountoulakis and Lahm 1998; Weiss et al. 1998) report on the use of microwave radiation-induced hydrolysis. This method of hydrolysis is usually conducted in a specially designed pressurized apparatus with the transfer of energy microwave radiation. This form of hydrolysis can be conducted in either the liquid- or the gas-phase with hydrolysis reagent such as HCl and methanesulfonic acid (Fountoulakis and Lahm 1998). Complete hydrolysis can be attained from 30-45 minutes depending on the mode of hydrolysis being used. While the process is rapid, conditions required for hydrolysis are extreme increasing the dangers of exploding vials (Davidson and O'Connor 2008).

Enzymatic hydrolysis requires the use of proteases to catalyze the amide or peptide bond during hydrolysis of protein or peptide substrates. One major advantage of enzymatic hydrolysis is that it allows for the quantification of asparagine and glutamine (Fountoulakis and Lahm 1998). There are many types of proteases available for conducting enzymatic hydrolysis. We reviewed the literature and identified studies that reported on various proteases using different substrates under different hydrolysis conditions. This included the following; Alcalase, Pepsin, Trypsin, Protamax, Papain and Favourzyme (Mota et al. 2004; Claver and Zhou 2005). Like other hydrolysis methods, the proteolytic activity is affected by factors such as temperature, pH range and the enzyme dosages. It was clear from our review the superiority of Alcalase enzyme in hydrolyzing a wide range of protein residues resulting in completion of total protein degradation. For this study on DCS, the two major methods of hydrolysis were applied (acid and enzymatic hydrolysis).

Protein hydrolysis is subsequently followed by separation, detection and quantification of amino acids. Different separation techniques exist; however pre-column derivatization has gained prominence over the post –column derivatization because of the ability to use a broader range of reagents (Sigma-Aldrich). An example is the pre-column o-phthaladehyde (OPA) followed by reverse-phase HPLC separation with fluorometric detection or diode array. Pre-column (dimethylamino) azobenzenesulfonyl chloride (DABS-CL) followed by reversed-phase HPLC separation with visible light detection is another example. Other available techniques include pre-column 9-fluorenylmethylchloroformate (FMO-CI), precolumn phenylisothiocyanate (PITC) and post-column ninhydrin detection (Fürst et al. 1990).

Defatted corn syrup (DCS) from a dry mill facility has been characterized in this dissertation to identify both the chemical and physical components in a previous chapter and study (Adom et al. 2012a). The fermentable carbohydrate component has also been optimize to release fermentable sugars through dilute acid pretreatment and enzymatic saccharification (Adom et al. 2012b). This study aims to study the combined effect of hydrolysis reaction time, temperature, and ratio of enzyme to substrate ratio to develop hydrolysis process that optimizes the amount of usable amino acids available in DCS.

4.2 Materials and methods

4.2.1 Protein hydrolysis scheme and experimental matrix

Protein content and free amino acids in DCS prior to hydrolysis have been reported in a previous chapter (2) and study (Adom et al. 2012a). Free amino acids characterization was subsequently followed by the development of hydrolysis pathways to investigate the release of amino acids. Figure 4-1 summarizes hydrolysis pathways. Pathway 1 (DAP-Dilute Acid Pretreatment) investigated the amount of amino acids recovered from protein at the previously determined optimum condition reported for dilute acid and enzymatic saccharification of DCS (Adom et al. 2012a). In pathway 2, we investigated DAP followed by subsequent protein hydrolysis using 3 different types of

proteases namely; Pronase E (Sigma-Aldrich), Protex 6L (Genencor®), and Trypsin (Sigma-Aldrich®). Pathway 3 investigated a separate standalone experiment on both the recovery of monomeric fermentable sugars and amino acids on the hydrolysis of biomass in DCS without any prior pretreatment. Alcalase (Calbiochem), Pronase E, Protex 6L, and Trypsin were used to investigate the recovery of amino acids. In the case of FS, the enzymes used included Accellerase 1500®, Accellerase XY® (Genencor,USA), α-amylase (Sigma, St. Louis, MI, USA) and amyloglucosidase (Sigma, St. Louis, MI, USA). This experiment was necessary because the results obtained served as a basis for designing the experiments for hydrolysis pathway 4. Finally, in hydrolysis pathway 4, simultaneous hydrolysis using both cellulases (Accellerase 1500 and XY) and a protease (Protex 6L) was investigated to quantify the release of both FS and amino acids in the same hydrolysis solution. The subsequent sections explain into more details the experiment matrixes and methods used in this study.

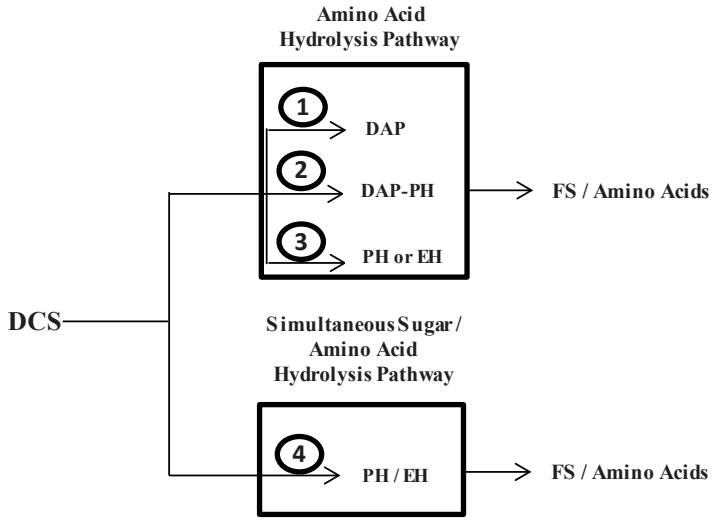


Figure 4-1 Hydrolysis pathways for the release of fermentable sugars and amino acids (DAP: dilute acid pretreatment, EH: enzymatic hydrolysis of cellulose, PH: Protein hydrolysis, FS: Fermentable Sugars)

4.2.2 Amino acid analysis of crude DCS

Characterization of free amino acids and proteins in DCS prior to protein hydrolysis was conducted previously on the crude DCS and results have been reported (Adom et

al. 2012a). Briefly, DCS samples were diluted (3x dilution), centrifuged and filtered (VWR, polycarbonate membrane filter, 25mm dia., 0.2 μm pore size) into separate High Pressure Liquid Chromatography (HPLC) sample vials. Samples were analyzed in the HPLC according to the amino acid analysis procedures outlined section 2.7. Protein content analyses were conducted using the Bradford assay (Sigma-Aldrich 2011).

4.2.3 Hydrolysis pathway 1: Experiment description of amino acid hydrolysis using DAP

Two hydrolysate solutions namely "A" and "B" were prepared. Briefly, a 10g sample of DCS was diluted with distilled water (20 ml) and sulfuric acid (6.9 ml) to bring the acid concentration to 2wt% and total solid loading in solution to 10wt% [see Figure B-1, (Adom et al. 2012b)]. The unpretreated defatted corn syrup (UPDCS) was analyzed for amino acids prior to autoclaving using the HPLC by transferring 1ml into 1.5ml centrifuge vials. The pH of UPDCS was then adjusted with 10N NaOH into the range of 6-8 (i.e. the suitable pH range for separation of amino acids on the Zorbax Eclipse Column) and centrifuged for 25 minutes at 10,000 RPM. Using a 0.2 μm membrane filters, the supernatant was filtered into HPLC vials and analyzed in the HPLC for amino acids. Another 2wt% acid concentrated DCS solutions for "A" and "B" was prepared and transferred into the glass reactors according to the conditions described above (see Figure B-1). After the DAP for 1 hour, the pretreated defatted corn syrup (PDCS) was adjusted to a pH range of 6-8 and treated in the same manner as UPDCS prior to analysis in the HPLC. Duplicate samples of hydrolyzates "A" and "B" were all analyzed for amino acid concentrations, which were back calculated to account for all dilution factors (e.g. acids, bases, and distilled H_2O).

4.2.4 Hydrolysis pathway 2: Experiment description of DAP followed by protein hydrolysis using proteases

The experiment matrix for hydrolysis pathway 2 comprised of four experiment sets (see Table 4-1) with different reaction times. A number of factors such as extreme heat, pH and the presence of heavy metals could result in denaturation of protein. DCS as a

co-product of the dry corn mill facility undergoes a lot of processing which are likely to denature the proteins in DCS. Three proteases, Pronase E, Protex 6L and Trypsin, were chosen because of their ability to hydrolyze both native and denatured proteins (Haurowitz et al. 1945; Genencor 2011). Phosphate buffer was chosen because it had the required buffering range (pH 5.8-8) needed for the optimal performance of the proteases. 5ml of well-mixed PDCS (see Figure B-1) were measured into 8 different labeled 50 ml Erlenmeyer flasks and diluted with 23.2ml of distilled water. The solution was neutralized by adding 10N NaOH until pH 6 or 7. This was followed by the addition of 1.5 ml of 0.2M phosphate buffer to stabilize the pH. The flasks were covered with parafilm and aluminum foil and allowed to equilibrate in an incubator for one hour at 34^{o}C. The control solutions were prepared in the same manner without the addition of any proteases (see Figure B-2).

Table 4-1 Experiment matrix for protein hydrolysis scheme: pathway 2

Experiment Sets	**Hydrolysis Solution**	**pH Investigated**	**Enzymatic Hydrolysis Reaction times (Temperature)**
1	Control	6, 7	1min, 2hrs, 3hrs,5hrs, 34^{o}C
2	Pronase	6, 7	1min, 2hrs, 3hrs,5hrs, 34^{o}C
3	Trypsin	6, 7	1min, 2hrs, 3hrs,5hrs, 34^{o}C
4	Protex 6L	6, 7	1min, 2hrs, 3hrs,5hrs, 34^{o}C

After one hour, dosages of the enzymes (~300µl) were added into the flask to make 1% [(V/V)-enzyme added] of the total hydrolysis solution. The pH meter was used to ensure the solution had a pH 6 or 7 before putting them back in the incubator for protein hydrolysis. After 1 min, 2 hours and 5 hours, samples were drawn (duplicates) using micropipette into labeled 1.5ml centrifuge vials. This was followed by enzyme inactivation by heating the centrifuge vials containing sampled hydrolyzates in a water bath at 90^{o}C for 15 minutes. The vials were then centrifuged for 25 minutes at 10,000 RPM and filtered with a 0.2µm filter membranes into HPLC vials for amino acid analysis.

4.2.5 Hydrolysis pathway 3: Experiment description of amino acid hydrolysis on unpretreated DCS using proteases, cellulases, α-amylase and amyloglucosidase

The experimental matrix for hydrolysis pathway 3 comprised of seven experiment sets. Process variables investigated included: pH, enzyme dosages, temperature, and reaction time. Table 4-2 below summarizes the experimental matrix used in these sets of experiments.

Table 4-2 Experiment matrix for protein hydrolysis scheme of unpretreated DCS (10% wt. DCS): Pathway 3. H = High (1.5 v/w), M = Medium (1.0 v/w), L = Low (0.5 v/w) (Volume of enzyme solution / weight of protein hydrolysate solution)

Experiment Sets	Enzyme	pH	Buffer	Enzyme dosage	Hydrolysis Temperature	Reaction times
1	Alcalase	7, 8 & 9	Tris	H, M & L	45 & 55^{o}C	1hr, 2hrs, 3hrs,5hrs, 24hrs & 48hrs
2	Pronase E	6 & 7	Phosphate	H	34^{o}C	
3	Trypsin	6 & 7	Phosphate	H	34^{o}C	
4	Protex 6L	6 & 7	Phosphate	H	34^{o}C	
5	Accellerase 1500 & XY	6	Phosphate	H	40^{o}C	
6	Amylase	6	Phosphate	H	40^{o}C	
7	Amylase & AMG	6	Phosphate	H	40^{o}C	

In experiment sets 1 through to 4, proteases (Alcalase, Pronase E, Protex 6L and Trypsin) were solely used to investigate amino acid production on the sample (on an as- received basis) without any form of pretreatment. In experiment sets 5 through to 7, cellulases, α-amylase and AMG were used to investigate sugar recoveries over time. These sets of experiments as previously stated were necessary in designing experiments in hydrolysis pathway 4.

Experiment set 1: At the sample preparation and conditioning stage, 10g of the DCS was measured using an electronic weighing balance and transferred into a 50 ml

beaker. 18ml of distilled water were added to the DCS. In this experiment, amino acid hydrolysis was investigated at pHs 7, 8, and 9 (see Table 4-2) because Alcalase proteolytic activity increases and remains fairly stable within this pH range. After the addition of distilled water, 10N NaOH was added to the DCS solution using a micropipette to adjust the solution to the required pH. 1.5ml of the 0.1M Tris buffer were then added to stabilize the pH and the flask was covered with parafilm and aluminum foil and allowed to equilibrate in an incubator for one hour at 45^{o}C or 55^{o}C.

The choice of enzyme loading was investigated at 3 different loading levels as shown in Table 4-2. They are high, medium and low enzyme loading. The high enzyme loading ratio of 1.5% v/w (volume of enzyme solution / weight of protein hydrolysate solution), which was approximately 428 µl alcalse enzyme solution. The medium and low were 1 and 0.5 % v/w respectively representing approximately 284 and 141 µl alcalse enzyme solution. Hydrolysates were transferred at various reaction times into centrifuge vials for AAA in the HPLC. After AAA, sampled hydrolyzates were swirled in a water bath at 90^{o}C for 15 minutes to inactivate enzyme activity. After the enzyme inactivation stage, the hydrolysates were centrifuged for 25 minutes at 10,000 RPM followed by the filtration of supernatant into a labeled HPLC vial using 0.2µm membrane filter for AAA at room temperature.

Experiment sets 2, 3 & 4: In experiment sets 2, 3 and 4, three proteases were used; Pronase E, Protex 6L and Trypsin. Additionally, phosphate buffer was chosen because it had a buffering range of 5.8-8, which was ideal for the pH of this experiment. Similar to experiment set 1, 10g of DCS was measured into a 50ml beaker and 18ml of distilled water was then added and followed with pH neutralization to 6 and 7 with 10N NaOH. 1.5ml of 0.2M phosphate buffer was added to stabilize pH and the hydrolysis solution was allowed to equilibrate at 34^{o}C for 1 hour prior to the addition of proteases. Dosages of the enzymes (~300µl) were added into the flask to make 1% (v/v) of the total solution. The same procedure for enzyme inactivation, centrifugation and

filtration processes previously described above was applied to hydrolysates prior to AAA in the HPLC.

Experiment sets 5, 6 & 7: 10g of DCS was measured into a 50ml Erlenmeyer flask and neutralized by adding approximately 380 µl of 10N NaOH to adjust pH to 6. This was followed by the addition of 1 ml of 0.2M phosphate buffer at pH 6 to stabilize the pH of the hydrolysis solution. 370 µl tetracycline (10 mg / ml in 70% EtOH) and 280 µl cycloheximide (10 mg / ml in H_2O) were added to all hydrolysis flasks. The flasks were covered with parafilm and aluminum foil and allowed to equilibrate in an incubator for one hour at 34^{o}C.

After one hour, dosages of the enzymes and pre-warmed (34°C) distilled water were added into the flasks until the total volume was 37 ml. Enzyme dosages were added as follows; 925 µl and 46 µl of accellerase 1500 and XY based on the recommended dosages by Genecor® in experiment set 5 (see Table 4-2). In experimental set 6, 137 µl of amylase was added, while 137 µl each of amylase and AMG was added in experiment set 7. The pH meter was used to ensure the solution had a pH ~6 before putting them back in the incubator for enzymatic hydrolysis. After 1 min, 24, 48 and 72 hours, samples were drawn using micropipette for HPLC analysis.

4.2.6 Hydrolysis pathway 4: Experiment description of amino acid hydrolysis using protease & cellulases (Simultaneous Hydrolysis)

A combination of Accellerase 1500, XY and Protex 6L were used to investigate the simultaneous production of sugar and amino acids at 34^{o}C for 24 hours. Protex 6L was chosen because compared to other proteases used in this study, it had a very wide range of temperature activity from 25-70^{o}C (Genencor 2011). Two hydrolysis solutions were prepared, with both having highest enzyme dosage of cellulases, i.e., Accellerase 1500 and XY, however the loading of Protex was varied from 1% and 2% (v/v) enzyme dosage of the total solution.

To 10 g of DCS measured into a 50 ml Erlenmeyer flask, approximately 380 µl of 10N NaOH was used to adjust pH to 6. This was followed by the addition of the following:

1 ml 0.2M phosphate buffer at pH 6, 370 µl tetracycline (10 mg/ml in 70% EtOH) and 280 µl cycloheximide (10 mg/ml in H_2O). After covering the hydrolysis flask with parafilm and aluminum foil, the flask was allowed to equilibrate for one hour at 34^oC before enzyme loading. After one hour, dosages of the enzymes and pre-warmed (34°C) distilled water were added into the flask until the total volume was 38 ml (10% w/w DCS in solution). Enzyme dosages were added as follows; 925 µl and 46 µl of Accellerase 1500 and XY. For 1% (v/v) and 2% (v/v) Protex solution 380 µl and 760 µl Protex 6L enzyme solution were added in addition to the cellulases (Accellerase 1500 and XY) respectively. The pH meter was used to ensure the hydrolysis solutions had a pH ~6 before putting them back in the incubator for protein hydrolysis to proceed. After 1 min, 6, 12 and 24 hours, samples were drawn using micropipette for HPLC analysis prior to enzyme inactivation at 90^oC as previously described. After analysis with the Aminex HPX-87P column (Bio-Rad Life Sciences, Hercules, CA) for monomer sugars concentrations, the HPLC column was replaced with a Zorbax Eclipse column, 4.6×150×5µm for AAA and the hydrolysates were rerun.

4.2.7 HPLC analysis of amino acids

AAA protocol by Agilent Technology was adopted for this study (Henderson et al. 2000). This is an analytical technique with automated derivatization using o-phthalaldehyde (OPA) for primary amino acids and 9-fluorenylmethyl chloroformate (FMOC) for secondary amino acids. Two mobile phases were used in the gradient elution, mobile phase "A" comprised of 40mM Na_2PHO_4 adjusted to pH of 7.8 while mobile phase "B" was a mixture of acetonitrile, methanol and water (45:45:10, v/v/v). Prepared amino acids standards were separated on the Zorbax Eclipse column (4.6×150×5µm) at temperature of 40^oC for calibration purposes. All other analytes were analyzed in a similar manner.

4.3 Results and discussion

In addition to "back calculating" all final concentrations by accounting for all dilution factors (e.g. acids, bases, distilled H_2O, antibiotics, and enzymes), concentrations for

enzyme blank for all experiments using Alcalase, Pronase E, Protex 6L and Trypsin were also accounted (see Table B-1 through to B-8).

4.3.1 Results for protein content and free amino acid analysis of crude DCS

Protein concentrations in DCS ranged from 5-7 mg/ml representing 7-9 wt% of syrup on a dry basis. The average protein concentration was 6.06 (±0.85) mg/ml of proteins representing 8 wt% (± 0.6%) of DCS on a dry basis (Adom et al. 2012a). Amino acid profile of DCS is displayed in Figure 4-2 and was measured to be 3.51 (±0.24) and 3.38 (±0.35) mg / ml for DCS samples obtained at different times from the corporate sponsor and analyzed in the year 2011 and 2010 respectively. It was estimated that the free amino acids in DCS were approximately 3.45% (±0.3 %) wt. on a dry basis by averaging all samples (2010 & 2011), (Adom et al. 2012a).

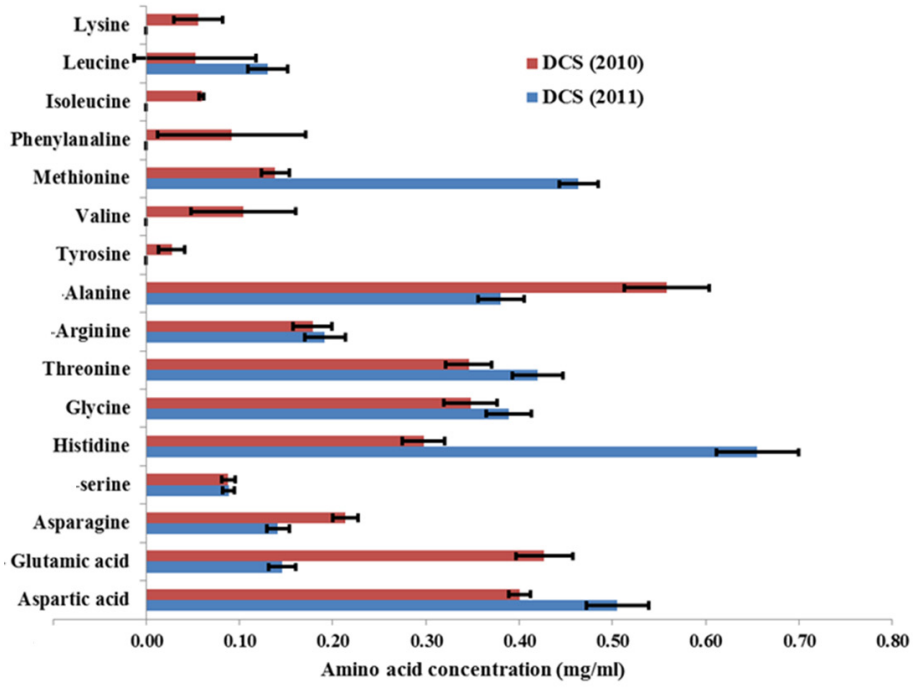


Figure 4-2 Average amino acid concentrations for DCS received in year 2010 and 2011 [Standard deviation of duplicate samples (Adom et al. 2012a)]

Taking into account the free amino acids (~3.4 mg / ml) and the available proteins (~7-9 mg/ml), a total of about ~10-12 mg/ml was estimated to be the maximum theoretical

yield assuming 100% conversion of protein to total amino acids in DCS. Apart from some differences between samples analyzed in 2011 & 2012, there were also some differences between samples analyzed within the same year considering the error bars (see Figure 4-2). For example while tyrosine was present in 2010, it was not in the 2011 sample. The reasoning behind this observation was not clear; however, we think the cultivation practices of corn in addition to various processing techniques prior getting DCS could influence the amino acid profile.

4.3.2 Results for hydrolysis pathway 1: Amino acid analysis using DAP

Table 4-3 summarizes the total amino acid yields using DAP (2% acid, 121°C, 60 min) for hydrolysis pathway 1. Yields generally averaged around 8.2 (± 0.4) mg / ml corresponding to 82-68% [=8.2/ (10 or 12)] of the theoretically available amino acids. Figure 4-3 displays the amino acid profile results for UPDCS (before DAP) and PDCS (after DAP). As previously described in section 4.2.3, two hydrolyzates "A" and "B" were prepared for pretreatment in the autoclave and each hydrolyzate was sampled in duplicates before and after the DAP autoclaving process for AAA. Clearly, the addition of H_2SO_4 2 wt% even before pretreating at 121^{o}C for 60 minutes in the autoclave liberates approximately 2 mg / ml of additional amino acids. This was estimated by subtracting the concentration of free amino acid in the crude sample (see Figure 4-2) from amino acid concentration of hydrolyzates A and B before autoclaving. Specifically, total amino acid concentration for UPDCS was estimated to be 5.8 and 5.3 mg/ml for samples A and B respectively (see Figure 4-3).

Table 4-3 Summary of total amino acid recovery using hydrolysis pathway 1 (DAP)

Samples	**Amino Acid Concentration (mg /ml)**
H_1 A	7.8
H_2 A (duplicate)	8.5
H_1 B	8.2
H_2 B (duplicate)	7.8

After dilute acid pretreatment in the autoclave, total amino acid concentrations increased to approximately 7.8–8.5 mg/ml (see Figure 4-3). Specifically, aspartic acid

and glutamic acid concentrations in UPDCS averaged around 0.6 and 0.4 mg / ml respectively. Both aspartic acid and glutamic acid increased to approximately 2 (~3x) and 1 (~2x) mg / ml in all hyrolyzate samples. Taking into account the margin of error, serine remained stable without any major degradation for both hydrolyzates.

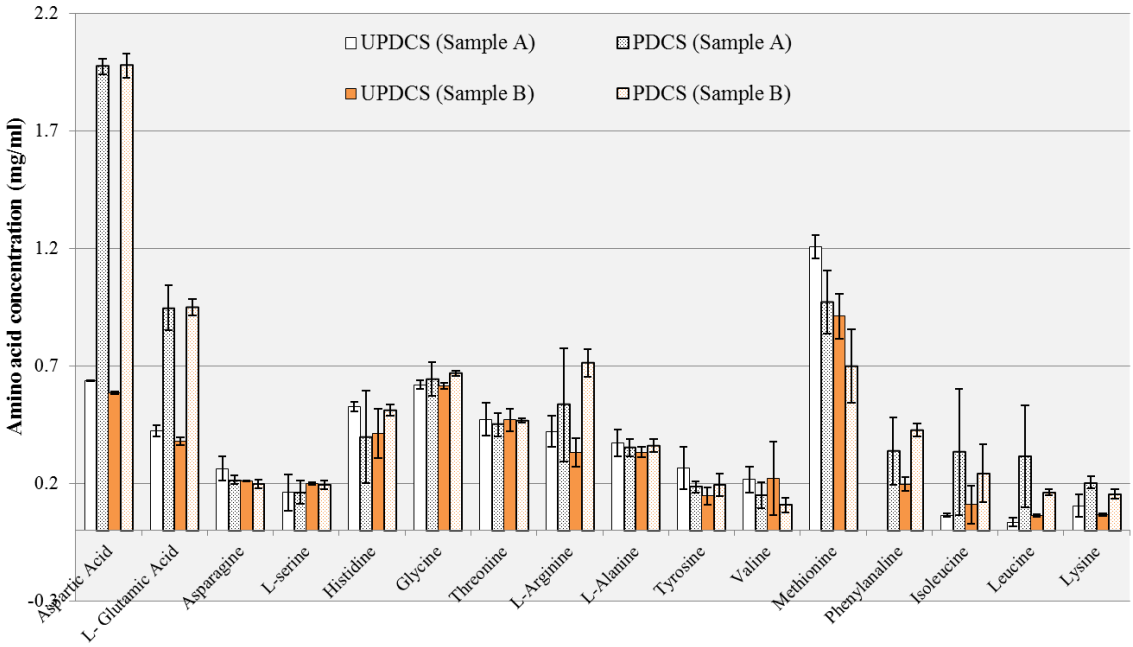


Figure 4-3 Amino acid analysis of hydrolyzate "A & B" before (UPDCS) and after (PDCS) dilute acid pretreatment. (Standard deviation of duplicate samples)

4.3.3 Results for hydrolysis pathway 2: DAP followed by protein hydrolysis using proteases

Table 4-4 summarizes the average results from replicates and including standard deviations obtained for amino acid hydrolysis of DCS using pathway 2. Total amino acid concentration of the control remained fairly constant and with some degradation observed after 5-hour hydrolysis for both solutions (pH 7 and 6). For both control pH 7 & 6, about 25% of aspartic acid was degraded after the 5 hour period of hydrolysis, this was not observed in any of the protease inoculated solutions. Other amino acids like glutamic acid, serine, glycine, threonine, arginine and isoleucine remained fairly constant with little or no degradation over the hydrolysis time for both control solutions pH 6 and 7 (see Figure B-3 and B-4). Another interesting observation was the total

degradation of asparagine and lysine after the 5 hours hydrolysis period for both solutions. Apart from aspartic acid, degradation of alanine, tyrosine and phenylalanine were relatively small ranging from 2-5% (recovery for 1 minute used as basis for comparison).

Table 4-4 Summary of results for hydrolysis pathway 2: amino acid analysis of dilute acid pretreated syrup followed by protease hydrolysis

Enzyme & pH	**Reaction times & total amino acid concentrations (mg / ml)**		
	1 min	**2 hrs**	**5 hrs**
Control-pH 7	7.5 (± 0.14)	7.6 (± 0.23)	6.5(± 0.25)
Control- pH 6	7.5 (± 0.29)	8.4 (± 0.53)	6.5 (± 0.11)
Pronase E-pH 7	8.0 (± 0.20)	9.1(± 0.03)	9.5 (± 0.50)
Pronase E-pH 6	10.0 (± 0.10)	9.0 (± 1.22)	10.7 (± 0.07)
Protex 6L -pH 7	11.2 (± 1.21)	11.1 (± 0.32)	9.6 (± 0.31)
Protex 6L-pH 6	10.4 (± 0.29)	9.3 (± 0.89)	13.5 (± 0.28)
Trypsin pH 7	8.6 (± 0.14)	8.0 (± 0.33)	9.5 (± 0.13)
Trypsin pH 6	7.9 (± 0.07)	8.0 (± 0.08)	9.4 (± 0.29)

Pronase and Protex results are generally high, however all these reported results accounts for any concentration of any free amino acids in the enzyme solutions. Additionally, there were cases where we observed more that 100% amino acid production after hydrolysis. We think this is probably due to water of hydration for amino acids.

Hydrolysis with Pronase E at pH 7 and 6 yielded 9.5 (95-79%) and 10.7 (100-89%) mg / ml of total amino acids respectively (see Table 4-4). For Pronase E. hydrolysis solution conditioned at pH 7, the following amino acid concentrations increased over time: histidine, glycine, arginine, valine, methionine, phenylalanine and isoleucine (see Figure B-5). The highest increase was isoleucine and leucine which increased about a factor of 8 and 2 respectively. All the other amino acids increased by factor of 1. Surprisingly, some degradation was observed for aspartic acid, glutamic acid, asparagine, serine, alanine and tyrosine. Aspartic acid was the least degraded (~1%

loss) with total degradation of tyrosine after the 5 hour hydrolysis period. In a previous study (Fountoulakis and Lahm 1998), the authors reported complete hydrolysis of asparagine and glutamine to aspartic acid and glutamic acid via conventional acidic hydrolysis of a protein substrate. They also reported partial degradation of tyrosine, serine and threonine. It is therefore most likely that these observed degradations are as a result of the initial acid pretreatment step. A similar trend was observed for hydrolysis solution conditioned at pH 6 with some degradation of valine and methionine (see Figure B-6).

Results for individual amino acid recovery over time using Protex 6L are presented in Figure B-7 and B-8. Aspartic acid, histidine, arginine, and leucine all increased in concentration over time depending on the pH of the enzyme solution. The highest increase observed was histidine which increased by a factor of 4 for hydrolysis solution conditioned at a pH of 7 and about a factor of 10 for hydrolysis solution of pH 6 after the 5 hour reaction time. Asparagine increased by a factor of 3 in hydrolysis solution of pH 6 after 5 hours but the concentration remained fairly constant in hydrolysis solution of pH 7 taking into account the magnitude of error bars. Some amino acids also degraded over time; these included glycine, threonine, tyrosine, valine, methionine and phenylalanine. Degradation ranged from 33-77% loss in amino acid concentration depending on the pH of the solution. Final reported yields after 5 hours were 9.62 (100-96%) and 13.51 (112%) for pH 7 and 6 respectively (see Table 4-4).

Figure B-9 and B-10 summarizes amino acid trends over time due to hydrolysis by trypsin. For both pH 7 & 6 hydrolysis solutions, amino acid concentrations of aspartic acid, glutamic acid, arginine, tyrosine, valine, methionine, isoleucine and leucine increased. The highest gain was observed for arginine and threonine that increased by a factor of 2 and 3 respectively for hydrolysis solution conditioned at pH 7 after 5 hours. Leucine also increased by a factor of 5 for hydrolysis solution at pH 6 after 5 hours of hydrolysis. Alanine, phenylalanine and lysine all exhibited some loss with total loss of

lysine in hydrolysis solution at pH 7. Final reported yields after 5 hours were 9.5 (95-79%) and 9.4 (94-78%) for pH 7 and 6 respectively (see Table 4-4).

4.3.4 Results for hydrolysis pathway 3: Hydrolysis of unpretreated DCS using proteases, cellulases, α-Amylase and AMG

This sections reports on amino acid recovery for hydrolysis pathway 3 over time for experiment sets 1 through to 4 (see section 4.2.5) where proteases (Alcalase, Pronase E, Protex 6L and Trypsin) were solely used on DCS without any form of pretreatment. Results for experimental sets 5 through to 7 (see section 4.2.5) where cellulases, α-amylase and AMG were applied to investigate sugar, recoveries over time are also reported.

4.3.4.1 Results for hydrolysis pathway 3: Alcalase at 45^{o}C and 55^{o}C

Table 4-5 summarizes the results obtained from hydrolysis of DCS with Alcalase enzyme. The effect of enzyme loading and temperature on early amino acid production (1 min) shows higher concentrations with higher loading and temperature. The effect of time on amino acid production shows increased amino acid concentrations with increasing time. The effect of pH on either initial production or ultimate increase in amino acid concentration exhibits no clear trend.

Surprisingly, the highest amino acid concentrations at 48 hr are from hydrolysis reactions at 45°C. Production of amino acids from the protein fraction of DCS ranges between 60-100% depending on reaction conditions, and therefore Alcalase® hydrolysis appears to be an effective means for production of amino acids from unpretreated DCS.

Alcalase hydrolysis at pH 7 (55^{o}C) yielded 9.62, 9.87, and 8.92 mg / ml of total amino acids after 48 hours for high, medium and low enzyme loadings, respectively. These results correspond to a theoretical amino acid yields of 96-80%, 98-82% and 89-74% for high, medium and low enzyme loading, respectively and were generally high comparatively.

Table 4-5 Summary of results for hydrolysis pathway 3: Amino acid analysis of DCS using Alcalase without DAP at temperatures of 45 & 55^{o}C.

Enzyme, pH, & T	Reaction times & amino acid concentrations (mg / ml)					
	1 hr	2 hrs	3 hrs	5 hrs	24 hrs	48 hrs
H-pH 7-55^{o}C	7.28	8.18	8.83	9.64	8.47	9.62
M-pH 7-55^{o}C	7.89	8.03	7.88	7.73	7.75	9.87
L-pH 7-55^{o}C	6.34	6.35	6.48	6.61	7.82	8.92
H-pH 8-55^{o}C	6.68	6.96	6.35	9.07	9.50	9.62
M-pH 8-55^{o}C	6.68	6.37	8.99	8.39	9.44	8.60
L-pH 8-55^{o}C	6.17	6.33	6.70	7.35	7.97	7.22
H-pH 9-55^{o}C	7.31	7.66	8.17	8.03	8.73	9.21
M-pH 9-55^{o}C	6.11	7.32	8.32	8.42	9.60	11.03
L-pH 9-55^{o}C	5.92	8.63	7.80	8.31	7.82	10.14
H-pH 7-45^{o}C	5.84	6.42	6.59	6.51	8.81	10.20
M-pH 7-45^{o}C	4.97	5.49	5.80	6.32	8.45	11.73
L-pH 7-45^{o}C	4.69	4.90	4.90	5.39	9.44	10.67
H-pH 8-45^{o}C	5.89	7.38	7.20	7.45	9.28	8.13
M-pH 8-45^{o}C	5.33	6.23	6.31	5.59	9.32	9.01
L-pH 8-45^{o}C	5.13	5.49	5.42	6.22	7.97	7.22
H-pH 9-45^{o}C	5.33	5.72	7.36	7.79	8.69	10.67
M-pH9-45^{o}C	5.13	5.24	5.93	7.56	8.40	11.60
L-pH 9-45^{o}C	4.05	5.43	5.30	5.16	7.74	9.39

4.3.4.2 Results for hydrolysis pathway 3: Pronase, Protex and Trypsin at 34^{o}C

Results for a standalone experiments using proteases (Trypsin, Pronase E, and Protex 6L) on the DCS without any pretreatment at 34°C are reported in this section. The process variables investigated included the following; pH (6 & 7), temperature (34^{o}C) and hydrolysis reaction time up to 48 hours [(1 minute, 2, 5, 24 and 48 hours), see section 4.2.5]. Table 4-6 summarizes the hydrolysis results obtained from these experiments.

Total amino acid concentrations for the controls at pH 6 and 7 appear to remain fairly constant, with some fluctuations that are bounded by the standard deviation error bounds, over the 48 hour hydrolysis reaction time, indicating that little to no hydrolysis occurred in the absence of enzymes. However, when comparing amino acid concentrations (Control) reported in Table 4-6 with that of the crude sample on an as received basis (~3.5 mg / ml-see section 4.3.1) is indicative that the neutralization step

of the hydrolysis solution liberates some amino acids. Concentrations reported in Table 4-6 (Control pH 7) are approximately 43-71% higher than total amino acid concentrations of crude DCS.

Table 4-6 Summary of results for hydrolysis pathway 3: Amino acid analysis of syrup without DAP using Pronase, Protex and Trypsin at low temperature (34^{o}C). Control contains no enzymes. Standard deviation of duplicate samples in parenthesis

Enzyme & pH	Reaction Times & Amino Acid Concentrations (mg / ml)				
	1 minute	2 hrs	5 hrs	24 hrs	48 hrs
Control-pH 7	5.28 (0.07)	5.88 (0.93)	4.92 (0.81)	4.50 (0.11)	4.72 (0.10)
Control-pH 6	6.82 (0.49)	5.88 (1.01)	4.92 (0.17)	4.25 (0.28)	6.88 (0.10)
Pronase-pH 7	6.14 (0.99)	7.03 (1.03)	6.73 (1.39)	5.39 (0.73)	6.60 (1.25)
Pronase-pH 6	7.54 (0.12)	4.59 (0.02)	6.54 (0.91)	6.72 (0.66)	5.50 (0.08)
Protex-pH 7	5.13 (0.01)	5.19 (0.01)	6.63 (0.33)	8.82 (0.19)	12.47 (0.07)
Protex-pH 6	5.78 (0.35)	6.55 (0.41)	5.51 (0.13)	6.12 (0.32)	9.91 (0.26)
Trypsin pH 7	4.14 (0.11)	3.98 (0.04)	4.51 (0.21)	4.92 (0.14)	6.19 (0.14)
Trypsin pH 6	4.12 (1.32)	4.08 (1.03)	4.73 (1.39)	5.64 (0.73)	6.09 (0.66)

Yields reported for hydrolysis solutions at pH 7 and 6 using Pronase E. were generally low (see Table 4-6). Total amino acid concentrations for solution pH 7 peaked at 2 hours yielding approximately 7 mg/ml representing approximately 70-58% of theoretically available amino acids. Total amino acid concentration of hydrolysis solution degraded slightly over time giving a final yield of 6.60 mg/ml after 48 hours. Similarly, for hydrolysis solution of pH 6, total amino acid concentration at 1 minute was quantified to be approximately 7.5 mg/ml. This concentration reduced over time to a total of 5.5 (55-46%) mg/ml after 48 hours.

Hydrolysis solution of pH 7 using Protex 6L showed a gradual rise in total amino acid concentration over time peaking after 48 hours with total amino acid yields of 12.5 mg/ml (see Table 4-6). This represents more than 100% yield in the theoretically available amino acids. Similarly, for hydrolysis solution of pH 6 using Protex 6L, total amino acid recovery over time was a steady rise after 5 hours as can be seen in Table 4-6. This concentration peaked after 48 hours yielding approximately 10 mg / ml of total

amino acid concentrations representing about 100-83% of the theoretically available amino acids in DCS.

Comparatively, the reported yields for the use of Trypsin were low (see Table 4-6). For both hydrolysis solutions of pH 6 and 7, recovery increased steadily over time peaking after 48 hours. Total yields were quantified to be 6.20 and 6.10 mg/ml of total amino acids for solutions of pH 7 and 6 respectively. The use of Trypsin yielded approximately 51-62% of the theoretically available amino acids and was comparatively less effective. The key highlight from this study was the efficiency of Protex 6L (pH 7) in bringing into completeness the protein hydrolysis reaction ultimately achieving 100% of the theoretically available amino acids.

4.3.4.3 Results for hydrolysis pathway 3: Sugar analysis using Accellerase 1500 and XY, α-Amylase and a combination of α-Amylase and AMG (40^{o}C)

Figure 4-4 shows the general trend of total monomer sugar production over 72 hours for hydrolysis at pH 6. Characterization of the total monomer sugars for unpretreated DCS ranged from 21-23 mg/ml. Control and α-amylase hydrolysis solutions behaved very similar, with total monomer sugar concentrations ranging between 41-43 mg/ml over the 72 hour period of hydrolysis (see Figure 4-4). These results are an indication that Amylase by itself is not effective in hydrolyzing the starch and other carbohydrate components of DCS.

Hydrolysis reaction containing a combination of Accellerase 1500/XY exhibited increased total monomer sugar concentrations and peaked at 24 hours with 64.50 mg/ml. Thereafter to 48 hours, concentration started to decrease rapidly after 48 hours. The total monomer sugar concentration for enzyme solution containing a combination of α-amylase / AMG increased slowly but peaked at 72 hours yielding a total of 63.05 mg / ml. In addition to α-amylase, AMG was required to complete the hydrolysis by further converting maltodextrins into monomer sugars. Finally, concentrations of HMF for all solutions ranged from 0.03-0.30 mg/ml while that of furfural ranged from 0.17-0.32 mg/ml (see Figure B-9).

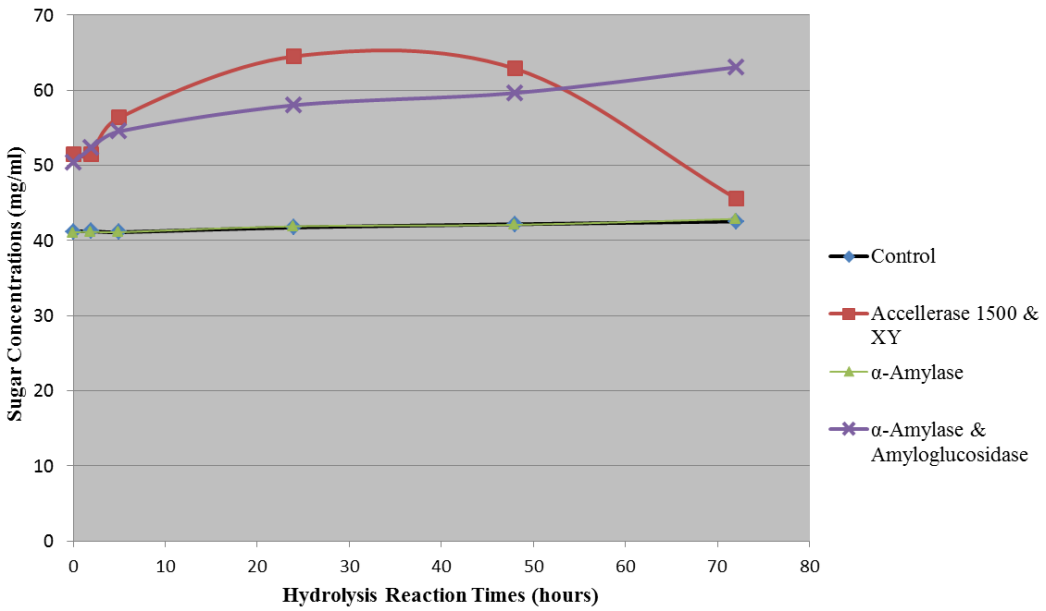


Figure 4-4 Total monomer sugar recovery using a combination of Accellerase 1500 / XY, α-amylase / AMG and α-amylase alone

4.3.5 Results for hydrolysis pathway 4: Simultaneous hydrolysis of unpretreated DCS using Protease (Protex 6L) & Cellulases (Accellerase 1500 and XY)

Figure 4-5 shows the results obtained from the simultaneous hydrolysis of unpretreated DCS using Protex 6L, Accellerase 1500 and XY. Total monomer sugar concentrations for the control solution remained stable over the 24 hour hydrolysis period. Total sugar yields of hydrolysis solution containing 1% Protex increased steadily over time peaking after the 24 hour period at 56 mg/ml. A similar trend was observed for hydrolysis solution containing 2% of Protex. These quantified total monomer sugars presented in Figure 4-5 were relatively small compared to the standalone hydrolysis of unpretreated DCS solution using just Accellerase 1500 and XY (see Figure 4-4) where total monomer sugars peaked at 24 hours yielding 63 mg/ml.

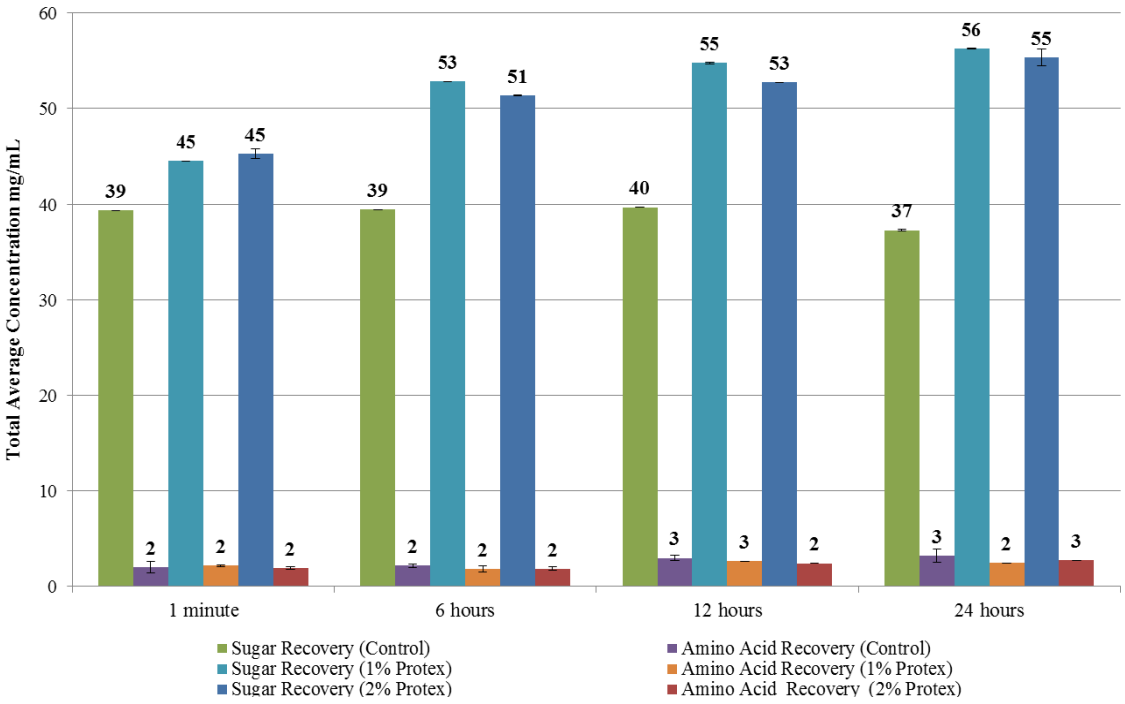


Figure 4-5 Total monomer sugar and amino acid recovery using a combination of Protex 6L and Accellerase 1500 / XY for simultaneous hydrolysis

No increase in total amino acid concentrations was observed for both 1 and 2% Protex enzymatic hydrolysis solutions. Control solution compares closely to the amino acid analysis of DCS on as received basis (see Section 4.3.1). The use of protease and cellulases together seem to have a significant effect the proteolytic activity of Protex 6L and hence was not effective in the protein hydrolysis. Total amino acid concentration for 1 and 2% (v/v) loaded enzymatic hydrolysis solutions ranged between 2-3 mg/ml representing only 18-27% of the theoretically available amino acids in DCS biomass.

4.4 Conclusions & recommendations

The goal of this research was to study the combined effect of hydrolysis reaction time, temperature, and ratio of enzyme to substrate ratio to develop hydrolysis process that optimizes the amount of usable amino acids available in DCS. Hydrolysis pathway 1, which is DAP alone at "optimum carbohydrate hydrolysis conditions (60 min, 2% acid)" yielded 68-82 % of the theoretically available amino acids. Hydrolysis pathway

2, which is DAP of syrup followed by subsequent protease hydrolysis was also investigated using Trypsin, Pronase E (streptomyces griseus) and Protex 6L. Overall, reported yields ranged from 100-78% of the theoretically available amino acids (pH 6 & 7). For this pathway, Pronase E at pH 7 resulted in the highest yield of 10.7 mg/ml (100-89%) of total amino acids. Hydrolysis pathway 3 which was a standalone experiment using proteases Trypsin, Pronase E (Streptomyces griseus) and Protex 6L on the unpretreated DCS reported yields ranging from 46-100% of the theoretically available amino acids. Protex at pH 7 yielded a total amino acid concentrations of 12.5 mg/ml (100% yield) which was the highest for pathway 3. Pathway 4 (simultaneous hydrolysis with cellulase and protex) generally reported the lowest yields for both amino acids and total monomer sugars. Total amino acid concentration for 1 and 2% (v/v) loaded enzymatic hydrolysis solutions ranged between 2-3 mg/ml representing only 18-27% of the theoretically available amino acids in DCS biomass. Apart from hydrolysis pathway 4, varying hydrolysis reaction times, investigated temperature and various enzyme loadings resulted in nearly quantitative recovery of amino acids from the protein contained in DCS. Since different alternate pathways could result in quantitative recovery of amino acids, a techno-economic analysis taking into account these routes will be important to help understand the economic impacts of these hydrolysis routes. This research topic is covered in Chapter 5.

4.5 References

Adom F, Fan J, Davis J, Dunn P, Shonnard D. 2012a. Compositional Analysis of Defatted Syrup from a Corn Ethanol Dry Mill as a Feedstock for Bio-Based Products. Journal of agricultural and food chemistry (in revisions).

Adom F, Fan J, Davis J, Taylor A, Shonnard D. 2012b. Optimization of the Dilute Acid and Enzymatic Pretreatment of Defatted Syrup from a Corn Ethanol Dry Mill. . Biomass and Bioenergy in revisions.

Badadani M, Babu SVS, Shetty KT. 2007. Dinitrophenyl derivatization of imino acids, spectral characteristics and HPLC analysis: application in urinary peptide-derived hydroxyproline and proline assay. Annals of clinical biochemistry 44(2):164-172.

Bercovicil D, Fuller MF. 2008. 6 Industrial amino acids in nonruminant animal nutrition. Biotechnology in Animal Feeds and Animal Feeding:93.

Claver IP, Zhou H. 2005. Enzymatic hydrolysis of defatted wheat germ by proteases and the effect on the functional properties of resulting protein hydrolysates. Journal of food biochemistry 29(1):13-26.

Davidson I. 1997. Hydrolysis of samples for amino acid analysis. Methods in molecular biology (Clifton, NJ) 64:119.

Davidson I, O'Connor P, Amino Acid Analysis Molecular Biomethods Handbook. Walker JM, Rapley R, Eds. Humana Press: **2008**; pp 793-808.

Fountoulakis M, Lahm HW. 1998. Hydrolysis and amino acid composition analysis of proteins. Journal of Chromatography A 826(2):109-134.

Fürst P, Pollack L, Graser T, Godel H, Stehle P. 1990. Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. Journal of Chromatography A 499:557-569.

Genencor. 2011. Protex 6L Bacterial Alkaline Protease. REV0301 1654 PRO44AX 02:2.

Haurowitz F, Tunca M, Schwerin P, Göksu V. 1945. The action of trypsin on native and denatured proteins. Journal of Biological Chemistry 157(2):621-625.

Henderson J, Ricker RD, Bidlingmeyer BA, Woodward C. 2000. Rapid, accurate, sensitive, and reproducible HPLC analysis of amino acids. Application bulletin 5980.

Irvine GB. 1997. Amino acid analysis. Precolumn derivatization methods. Methods in molecular biology (Clifton, NJ) 64:131.

Leuchtenberger W, Huthmacher K, Drauz K. 2005. Biotechnological production of amino acids and derivatives: current status and prospects. Applied Microbiology and Biotechnology 69(1):1-8.

Malmer MF, Schroeder LA. 1990. Amino acid anslysis by high-performance liquid chromatography with methanesulfonic acid hydrolysis and 9-fluorenylmethylchloroformate derivatization. Journal of Chromatography A 514:227-239.

Mota M, Ferreira I, Oliveira M, Rocha C, Teixeira J, Torres D, Gonçalves M. 2004. Enzymatic hydrolysis of whey protein concentrates: Peptide HPLC profiles. Journal of liquid chromatography & related technologies 27(16):2625-2639.

Ozols J. 1990. [44] Amino acid analysis. Methods in enzymology 182:587-601.

Pickering MV, Newton P. 1990. Amino acid hydrolysis: old problems, new solutions. LC-GC 8(10).

Peptide Analysis [Internet]. [updated cited.

Sigma-Aldrich. 2011. Bradford Reagent Technical Bulletin. No. RC, JDS,MAM 06/11-1.

Smith BJ, Protein sequencing protocols. Springer: **2003**; Vol. 211.

Thacker H. 2004. Alkaline hydrolysis. Carcass disposal: A comprehensive review. National Agricultural Biosecurity Center, Kansas State University.

Weiss M, Manneberg M, Juranville JF, Lahm HW, Fountoulakis M. 1998. Effect of the hydrolysis method on the determination of the amino acid composition of proteins. Journal of Chromatography A 795(2):263-275.

Chapter 5

5 Modeling of Dilute Acid Pretreatment Process using Defatted Corn Syrup as Feedstock: Techno-economic Analysis and Life Cycle Assessment[4]

5.1 Introduction

Defatted corn syrup (DCS) from a dry corn mill facility is a processed residue from the dry corn mill facility. Production of DCS averaged 59 million kg per month (~708 million kg per year) in the U.S. (O'Brien 2010) and is expected to increase given the continuous expansion of the dry-grind mill facility across the U.S. Rich in carbohydrates and amino acids, DCS has potential as a feedstock for bio-products. Currently, it is dried and added to distillers dried grains with solubles (DDGS) as a feed additive. DCS as a complex process residue consisting of various soluble and non-soluble carbohydrate polymers making it ideal feedstock for bioproducts via the bio-chemical conversion route and subsequent fermentation to value-added products (Adom et al. 2012b). Separate studies (Adom et al. 2012a; Adom et al. 2012c) investigating the combined effect of hydrolysis reaction time, temperature, and ratio of enzyme to substrate ratio to develop hydrolysis process that optimizes the amount of usable fermentable sugars and amino acids using DCS have been conducted. This developed platform can serve as a building block for high value chemicals such as lactic acid, glycerol, and amino acids (lysine, aspartic acid, etc.).

The aim of this work was to investigate the economic feasibility of an industrial process of the sugar and amino acid platform from DCS via dilute acid pretreatment. This constructed model will serve as a platform for the investigation of specific

[4] *This chapter will be submitted to Industrial & Engineering Chemistry Research for publication.* Citation: Adom, F., Rogers, T., Shonnard, D. (2012). Modeling of dilute acid pretreatment process using defatted corn syrup as feedstock: Techno-economic analysis and life cycle assessment. Industrial & Engineering Chemistry Research

bioproduct using the sugar and amino acid platform while considering additional downstream processes in future research analyses. The process was simulated using Aspen Plus ® (Aspen Technologies, Cambridge, MA, USA) based on experimental data in the lab. Originally developed for the Department of Energy (DOE) by the Massachusetts Institute of Technology in 1987, Aspen Plus is capable of solving steady state material and energy balances, calculating phase equilibria, and estimating physical properties for thousands of chemical compounds as well as capital costs of equipment (Jayawardhana and Walsum 2004). Process simulation procedures primarily involve; defining chemical components, selecting the thermodynamic model, choosing proper operating conditions (flow rate, temperature, pressure, etc.) (Fasahati and Liu 2012). Physical properties selection is important, and the successful implementation of a process design starts with selecting the appropriate physical property method (Carlson 1996). For example, GRAYSON is recommended for hydrogen components and Peng Robingson is useful for gas processing coupled with binary parameters (Peris Serrano 2012). The Non-random, Two Liquids (NRTL) property method is capable of estimating the vapor-liquid phase equilibria by using the binary interaction coefficients for chemical components and has been mostly adopted for biomass pretreatment processes (Aden and Foust 2009; Humbird and Aden 2009; Kazi et al. 2010). Specific study objectives for this chapter are enumerated below:

- Development of a process design for the production of the both the sugar and amino acid platform via dilute acid pretreatment
- A preliminary cost analysis to estimate the initial capital cost and operating cost of this facility using Aspen Plus Economic Analyzer
- A greenhouse gas analysis to understand the environmental impact of this facility

5.2 Methods

5.2.1 Process description

A plant with a total capacity of processing 27,329 kg/hr (wet basis) [~10,240 kg/hr (dry basis)] of DCS is simulated. The Non-random, Two Liquids (NRTL) property method

was selected for this process design. Assuming a plant uptime of 80% (7000 hours per year), the cost component of the analysis is simulated using Aspen Process Economic Analyzer®.

5.2.2 Feed composition of DCS

DCS broadly comprise of: (i) Fermentable carbohydrates & Lignin (35 wt%) (ii) Protein & Amino acids (10.9 wt%) (iii) Organic acids, glycerol & Ash (52 wt%). Table 5-1 summarizes the DCS composition and their corresponding flow rates on an hourly basis used in this process design.

Table 5-1 Summary of the DCS composition and their corresponding flow rates on an hourly basis used. (FAA: Fermentable amino acids, FS: Fermentable sugars, SA: Succinic acid, AIL Acid insoluble lignin, and ASL: Acid soluble lignin)

FAA	Flow rate (kg/hr)	% Dry basis	FS and Lignin	Flow rate (kg/hr)	% Dry basis	Others	Flow rate (kg/hr)	% Dry basis
Aspartic acid	37	0.004	Cellulose	71	0.007	Ash	1,185	0.117
Glutamic acid	23	0.002	Xylan	547	0.054	Glycerol	3,302	0.326
Asparagine	14	0.001	Galactan	233	0.023	Oxalic acid	122	0.012
Serine	7	0.001	Arabinan	61	0.006	SA	142	0.014
Histidine	39	0.004	Mannan	111	0.011	Lactic acid	415	0.041
Glycine	30	0.003	Glucose	1003	0.099	Acetate	142	0.014
Threonine	31	0.003	Xylose	101	0.01			
Arginine	15	0.002	Galactose	41	0.004			
Alanine	38	0.004	Arabinose	41	0.004			
Tyrosine	1	0.0001	Mannose	41	0.004			
Valine	4	0.0004	Starch	567	0.056			
Methionine	24	0.002	ASL	213	0.021			
Phenylalanine	4	0.0004	AIL	770	0.076			
Iso-leucine	2	0.0002						
Leucine	7	0.001						
Lysine	2	0.0002						
Protein	821	0.081						
Total	1,101	0.109		3,799	0.375		5,308	0.524

5.2.3 Process description

Figure 5-1 shows a process flow diagram for the pretreatment design process. The pretreatment section comprise of 3 key subsections namely (i) ***DCS viscosity reduction and preheating section*** (ii) ***Dilute acid pretreatment and flash cooling section*** and (iii) ***Neutralization and unreacted residues separation section***. For approximately 27,329 kg/hr (wet basis), 74,661 kg/hr of H_2O and 209 kg/hr of 98wt% H_2SO_4 is required to bring the solution to 10wt% solid loadings and 2wt% acid concentration.

DCS viscosity reduction and preheating section: Pretreatment proceeds (see Figure 5-1) by mixing (MIXER-1) DCS with a water stream (24,887 kg/hr) to reduce viscosity of DCS for subsequent transfer of stream (S-1) to the pretreatment reactor (RSTOIC-1) via a centrifugal pump. Both streams (water & DCS) have pressure and temperature of 1 atm and 25^{o}C. Positive displacement and special effect types such as venture eductors are used but by far the most common type of slurry pump is the centrifugal pump (Warman International 1994). Assuming 85% pump efficiency and power requirement of 3728 Watts or 5 horsepower (Lardy G 2004) for a centrifugal slurry pump (PUMP-1), Aspen Plus® was run to estimate parameters such as net positive suction height, brake power, and the pressure of outlet of stream (S-2). A mixing valve (VALVE-1) to regulate the amount of dilute DCS stream (S-3) going into the preheater (PREHEAT1) operating at an adiabatic flash with no pressure drop was used. DCS solution (S-3) is preheated to 60^{o}C with an incoming low-pressure steam (STEAM-1) at 2.96 atm and 134^{o}C (Towler and Sinnott 2012). Using the design specification capability in Aspen Plus®, STEAM-1 was varied between 1000-3000 using the following design specification expressions; Spec (S1), Target (60) and Tolerance (0.001). Aspen Plus® estimated 2,574 kg/hr of low-pressure steam (LPS) required to reach the 60^{o}C target temperature in the preheater (PREHEAT1), this was used in this model. Preheated stream (S-4) is subsequently pumped to the dilute acid pretreatment and flash cooling section.

Dilute acid pretreatment and flash cooling section: The preheated stream (S-4) with biomass solid loadings of approximately 20 wt% is pumped (PUMP-2) to the pretreatment reactor (RSTOIC-1) prior to mixing with another LPS (STEAM-2) and 98wt% H_2SO_4. Given the pretreatment reactor conditions (see Table 5-2) in the RSTOIC-1, it was estimated that approximately 47,558 kg/hr of LP steam (STEAM-2) is required to bring total solid loadings in stream (S-7) to 10 wt%. RSTOIC-1 is used because of the unavailability of kinetic data for the pretreatment reactions.

Table 5-2 Pretreatment Reactor Conditions (RSTOIC-1) (Adom et al. 2012c)

Processing Variables	**Conditions**
Sulfuric acid loading	2 wt%
Residence Time	60 minutes
Temperature	121^{o}C
Total solids loading	10wt%

However, experimentally validated conversion yields measured and reported in chapters 3 and 4 of this dissertation were used (see Table 5-3). DCS optimization focusing on protein degradation into amino acids reported 82-68% (Average = 75%) of theoretical amino acids liberated at the pretreatment reactor conditions (see Table 5-4). A protein model ($CH_{1.99}O_{0.61}N_{0.32}S_{0.01}$) in addition to a protein degradation hydrolysis reaction model was developed based on the reported amino acid yields at the pretreatment condition using a mass balance approach (see Table C-1 and C-2). This was necessary to model protein degradation at the pretreatment conditions reported in Table 5-2. Aspen Plus® model was initially run by inputting the reactions (see Table 5-3) in RSTOIC-1 assuming a heat duty (0 kWhr) and pressure (2.96 atm). This initial run estimated the RSTOIC-1 reactor conditions to be approximately 135^{o}C. Design specification was therefore necessary to achieve the specified reactor conditions of 121^{o}C.

Design specification analysis was implemented in Aspen Plus® by estimating the required pressure in RSTOIC-1 to achieve the target temperature (121^{o}C). The following design specification expressions; Spec (S2), Target (121) and Tolerance (0.001) were used to estimate the required pressure in RSTOIC-1 to achieve the target temperature. Manipulated variable (pressure in RSTOIC-1) specified in Aspen Plus® ranged from 1 to 5 atm. Aspen Plus® calculated the required pressure (1.95 atm) to reach the 121^{o}C reaction conditions, and this was used in our process design. The final unit operation in this section is the application of a flash tank (FLASH-1) to flash cool (110^{o}C) the slurry (S-8) to volatilize some inhibitors like HMF, furfural and acetic acid into volatile organic compounds (VOC's). The carbohydrate and amino acid rich stream (S-10) is sent to the neutralization and unreacted residues separation section for further detoxification.

Table 5-3 Pretreatment Hydrolysis Reactions (Refer to list of abbreviations for the meaning of 3-letter amino acid)

Reaction	Reactant	Conversion
H_2O + Xylan (Cisolid) --> Xylose	Xylan	90%
H_2O + Galactan (Cisolid) --> Galactose	Galactan	85.0%
Xylan (Cisolid) --> Furfural + 2 H_2O	Xylan	0.05%
Mannan (Cisolid) --> HMF + 2 H_2O	Mannan	15.7%
Arabinan (Cisolid) --> 2 H_2O + Furfural	Arabinan	0.6%
Starch (Cisolid) + H_2O --> 2 Glucose	Starch	100%
Acetate (Cisolid) --> Acetic acid	Acetate	100%
Lignin (Cisolid) --> Soluble lignin	Lignin	50%
H_2O + Mannan (Cisolid) --> Mannose	Mannan	60%
H_2O + Arabinan (Cisolid) --> Arabinose	Arabinan	49.5%
Galactan (Cisolid) --> HMF + 2 H_2O	Galactan	1%
Cellulose (Cisolid) + H_2O --> Glucose	Cellulose	100%
PROTEIN(Cisolid) + .09138 H_2O --> .24 ASP + .12 GLU + .03 ASN + .02 SER + .06 HIS + .08 GLY + .06 THR + .06 ARG + .04 ALA + .02 TYR + .02 VAL + .1 MET + .05 PHE + .04 ILE + .03 LEU + .02 LYS	Protein	75%

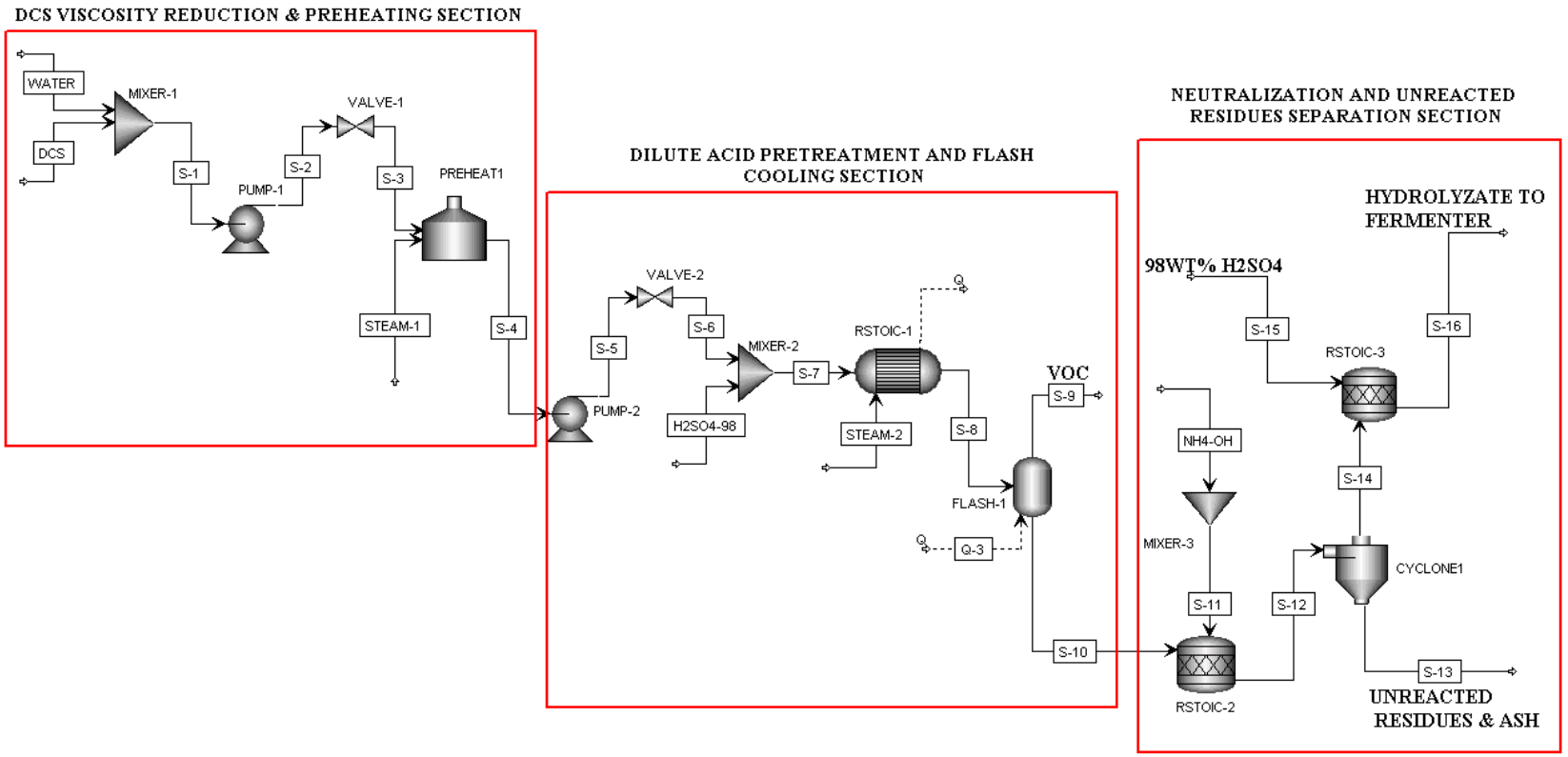


Figure 5-1 ASPEN-Plus process flow diagram of dilute acid pretreatment process as analyzed in this study. The entire pretreatment section comprise of (i) DCS viscosity reduction and preheating section, (ii) Dilute acid pretreatment and flash cooling section and (iii) Neutralization and unreacted residues separation section

Neutralization and unreacted residues separation section: Though more expensive than lime, reduction in both sugar loss and overall capital cost make the use of ammonia a more attractive neutralizer (Jennings and Schell 2011). Ammonia loading of 4.8 g/l of hydrolysate (Humbird and Aden 2009) was assumed for the neutralization of acetic acid and H_2SO_4 into ammonium acetate ($C_2H_3O_2NH_4$) and ammonium sulfate [$(NH_4)_2SO_4$] respectively. The neutralized slurry (S-12) is run through a cylone (CYCLONE1) to separate the unreacted residues (S-13) from the liquid hydrolyzate (S-12). Stream (S-14) which is the hydrolyzate containing excess NH_3 is further neutralized with 98wt% H_2SO_4 to subsequently precipitate more $(NH_4)_2SO_4$ prior to fermentation. Design specification analysis was used to estimate the amount of 98wt% H_2SO_4 (5580.134 kg/hr) required to have less than 0.1 kg/hr of NH_3 in the STREAM-16 (hydrolyzate to fermenter). Operation conditions of the unit operations for the entire pretreatment section are summarized in Table 5-4.

Table 5-4 Parameters used for modeling the process flow diagram

Unit Operation	Modeling Parameters used
Mixers (Mixer-1 & 2)	Valid phases specified; Liquid & Vapor phase. No outlet pressure was specified allowing mixer to use minimum pressure from the inlet streams to determine outlet stream conditions.
Pumps (Pump-1 & 2)	Efficiency: 85% Power required : 3.7 kWh
Valves (Valves 1 & 2)	Adiabatic flash with zero pressure drop used to estimate outlet stream temperature and phase conditions
Reactor (RSTOIC 1, 2 & 3)	RSTOIC 1: Pressure 1.95 atm, Heat duty: 0 kW RSTOIC 2 & 3: Pressure 1.37 atm, Heat duty: 0 kW
Flash tank (Flash-1)	Temperature: 110^{o}C, modeled as adiabatic flash
Cyclone (CYCLONE1)	MIXED (Split fraction : 1) CISOLID (Split fraction: 0.01)

5.3 Process simulation and economic calculations

Aspen Plus was used to conduct rigorous material and energy balance calculations by choosing equipment models, specifying key input flow rates and allowing the software to determine resultant stream composition and energy flow. Standard Aspen Plus in-house databank lacks the physical properties of typical lignocellulosic biomass components. Thermodynamic database for components such as cellulose, lignin, xylose, etc were obtained from an NREL technical report (Wooley and Putsche 1996).

Using Aspen Plus Economic Analyzer® (APEA) version 7.3, the capital cost of equipment such as pumps, reactors, vessels, cyclones, mixers and valves were estimated. The cost basis for this version of APEA is first quarter of 2010. APEA has the capability of analyzing the cost of other auxiliary equipment such as piping, electrical equipment, instrumentation, etc., all these cost components were included in the final cost analysis. Results from the Aspen Plus® simulation file for the mass and energy balances were used by APEA for sizing calculations and subsequent mapping of equipment. The stoichiometric reactors in this process were all considered agitated tanks with enclosed jackets.

For this cost analysis, startup and construction period of 2 years, in addition to a plant life of 15 years, was assumed. Annual maintenance and insurance expenditures were assumed to be 2% and 1% of FCI respectively. Assuming that 10 persons operate the plant, we further assumed default labor wage in Aspen Plus Economic Analyzer ($20/operator and $35/supervisor) for this analysis. Since plant, location has not been determined; this figure has been reported to be enough to cover labor expenses in Europe and USA (Sassner et al, 2008; Lohrasbi et al, 2010). 30% taxation rate has been reported to be reasonable for most places and hence assumed for this model (Lohrasbi et al, 2010). Finally, a straight-line depreciation method and 5% salvage value of the initial fixed capital cost was assumed for this process economic evaluation. Table 5-5 summarizes the additional cost elements used in the evaluation of this processing facility.

Table 5-5 Cost of raw materials and utilities used in process simulation

Inputs	Price	Unit (Source)
Raw material (DCS)	0.01	$/kg (Agri-Energy 2012)
Sulfuric acid	0.17	$/kg (Lohrasbi et al. 2010)
Ammonia	0.33	$/kg (Wingren et al. 2003)
Steam	0.0013	$/lb (Jayawardhana and Walsum 2004)
Electricity	0.05	$/kWhr (Lohrasbi et al. 2010)
Maintenance	2	% of fixed capital
Insurance	1	% of fixed capital

5.4 Greenhouse gas analysis of the dilute acid pretreatment processing facility

A GHG analysis was conducted on this processing facility to estimate the overall greenhouse gas emissions on an annual basis. The environmental impact (GHG) was analyzed by identifying the input requirements for this process facility. A key assumption is that DCS has no environmental burden and is treated as "waste" given the fact that it is a low value product in the market. Other key inputs included in this analysis are; 98 wt% H_2SO_4 (5789 kg/hr), NH_4OH (6.830 kg/hr), electricity (7.46 kWh), steam (50,132 kg/hr) and water (24,882 kg/hr). Using the global warming potential method (CO_2=1, CH_4=25, N_2O=298) in SimaPro®, the emission factors of the corresponding inputs were identified and applied on these inputs. Specifically, the following emission factors from SimaPro® were used for this analysis: sulfuric acid, liquid, at plant/RER S (0.123), Ammonia, steam reforming, liquid, at plant/RER S (1.91), Water, completely softened, at plant/RER S ($2.43x10^{-5}$) and Steam, for chemical processes, at plant/RER S (0.234) all on a basis of $kgCO_2$ equivalent (e) / kg of input. Emision factor for Electricity, U.S. national grid (0.823 $kgCO_2e$/ kWh) from Adom et al. (2012d) was used.

5.5 Results and discussion

5.5.1 Mass and energy balances for process simulation in Aspen Plus®

Table 5-6 summarizes the composition of streams for hydrolyzate to fermenter (S-16) and unreacted residues stream (S-13) for this simulated processing facility. Generally, the stream (S-16) is rich in both carbohydrates and amino acids. Glucose production is approximately 1,697 kg/hr while glycerol is 3,338 kg/hr. This stream (S-16) subsequently goes into the fermentation section for the fermentation process to produce high value products. The two major energy intensive unit operations were the preheater (PREHEAT-1) and pretreatment reactor (RSTOIC-1). Overall steam demand amounted to 50,100 kg/hr (351,000,000 kg/yr).

Table 5-6 Composition of streams for hydrolyzate to fermenter (S-16) and unreacted residues (S-13) [GLU-ACID: Glutamic Acid and ASP-ACID: Aspartic Acid]

Components	**Mass Flow kg/hr**		**Components**	**Mass Flow kg/hr**	
	S-13	**S-16**		**S-13**	**S-16**
H_2O	-	48,326	XYLAN	52	-
GLUCOSE	-	1,697	ARABINAN	30	-
GALACTOS	-	263	LIGNIN	385	-
MANNOSE	-	116	ACETATE	-	-
XYLOSE	-	668	ASH	1,186	-
ARABINOS	-	76	LYSINE	-	13
LIGNIN-SOLUBLE	-	604	LEUCINE	-	22
HMF	-	42	I-LEUCIN	-	21
FURFURAL	-	0	PHENYLAL	-	24
ACETIC ACID	-	-	METHIONI	-	87
LACTIC ACID	-	417	VALINE	-	13
XYLITOL	-	-	TYROSINE	-	10
GLYCEROL	-	3,338	ALANINE	-	63
SUCCINIC ACID	-	143	ARGININE	-	3
OXALIC ACID	-	123	THREONIN	-	54
NH_3	-	-	GLYCINE	-	96
H_2SO_4	-	-	HISTIDIN	-	86
NH_4SO_4	278	-	SERINE	-	22
NH_4ACETATE	112	-	ASPARAGINE	-	36
CELLULOSE	-	-	GLU-ACID	-	100
GALACTAN	33	-	ASP-ACID	-	211
MANNAN	27	-	PROTEIN	205	-
Total Flow kg/hr	**596**	**55,077**		**2,322**	**861**

5.5.2 Results for economic analysis

Table 5-7 summarizes the total cost components of the facility under investigation. From this analysis, raw materials and plant operational cost were the most significant. The most expensive streams were identified to be (see Figure 5-1); STEAM-2 (1,048 $/hr), S-15 (98wt% H_2SO_4-967 $/hr), DCS (274 $/hr) and NH_4OH stream (67 $/hr). Future analysis should investigate the potential of regulating the ammonia content in the hydrolysis stream to serve as a source of nitrogen during fermentation to value added products.

In another study (Jayawardhana and Walsum 2004), the authors reported the capital cost of a facility using H_2SO_4 as the main catalyst to be $5,847,005. In our study, operating cost is particularly high because of the high requirement of steam, sulfuric acid and ammonia for neutralization, however because of the minimization of sugar loss this is expected to improve overall process economics depending on target bio-product (e.g. succinic acid, lysine, and aspartic acid).

Table 5-7 Summary of results from economic analysis from Aspen Plus® (United States Dollars: USD)

Name	Summary
Total Capital Cost [USD]	4,700,000
Total Operating Cost [USD/Year]	22,100,000
Total Raw Materials Cost [USD/Year]	19,300,000
Total Utilities Cost [USD/Year]	70,200

Finally, Figure 5-2 summarizes the direct cost for all the unit operations assembled for the process facility. The direct cost comprises of equipment cost and auxilliary equipment as well as the building requirements associated with process and installation (Wingren et al. 2003). The three stoichiometric reactors were the largest cost component contributing approximately 62% towards total direct cost for all unit

operations combined. The next major contributors were the cyclone (CYCLONE1), flash vessel (FLASH-1), and preheater (PREHEAT1) contributing approximately 13%, 11% and 9% towards the direct cost respectively. For detailed cost analysis for each unit operation, see Figures C-1 through to C-7.

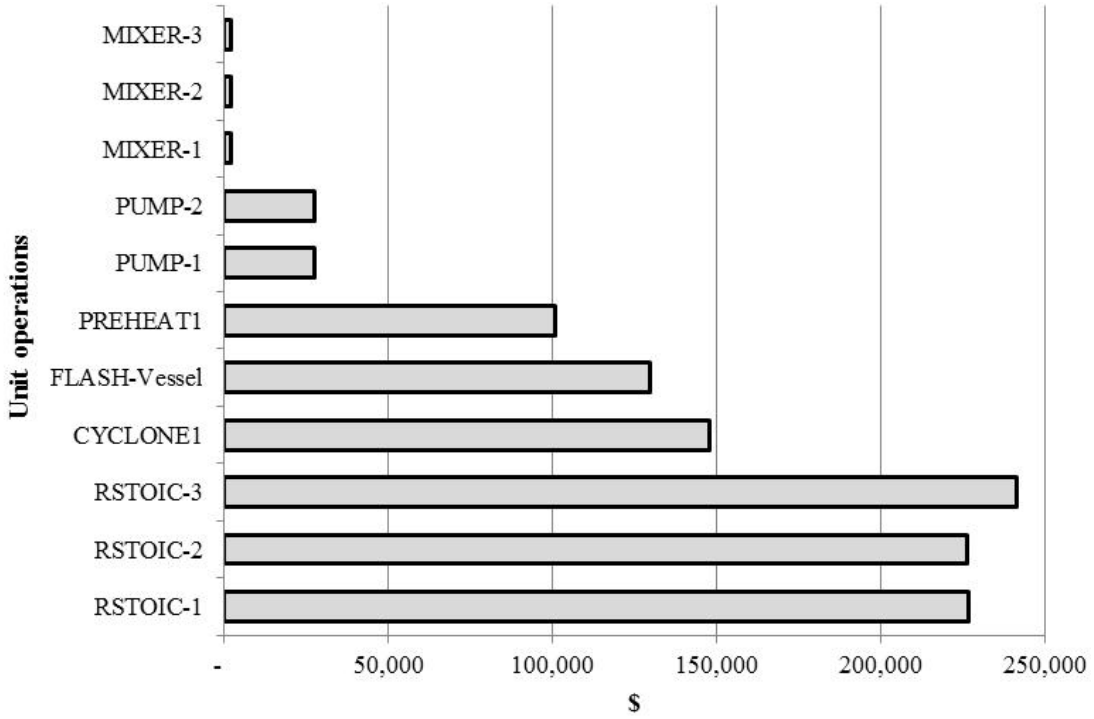


Figure 5-2 Summary of cost components for all equipment used in this facility

Results from the mass balance of this constructed process facility indicate that 2,844 kg fermentable carbohydrates/hr (see Figure 5-6) could be potentially available for fermentation. Fermentable carbohydrate here refers to all the monomer sugars (glucose, xylose, galactose, arabinose and mannose). Assuming the reported yield of 0.71 for succinic acid (Lennartsson. 2005) it was calculated that approximately, 2004 kg succinic acid per hour could be theoretically produced after fermentation. Taking into account total plant uptime, capital and operataion cost, about 14,000,000 kg of succinic acid could be produced at a cost of 0.5 $/kg succinic acid.

Wholesale price of succinic acid varies significantly ranging from 2-25 $/kg (Alibaba, 2012) depending on grade and intended use. Additional detailed economic analysis such as fermentation, product recovery and purification is required to fully understand the overall cost per production of succinic acid using DCS. However, the high level estimated value of 0.5 $/kg succinic acid is promising in terms of making a biorefinery using DCS as a feedstock potentially profitable and attractive.

5.5.3 GHG results

Table 5-8 summarizes the GHG impact of this processing facility. Overall, this facility will emit approximately 114,000,000 kgCO_2e/yr (114,000 MT CO_2e/yr). The 2 key drivers were identified to be steam and ammonia contributing 72 and 24% towards GHG emission respectively. All other inputs contribution with the exception of ammonia and steam to GHG were approximately 4%.

Table 5-8 Summary of GHG results from carbon footprint analysis

Input data	**KgCO_2e/yr**
H_2SO_4	4,980,000
NH_4OH (NH_3 gas)	27,200,000
NH_4OH (Water)	815
Electricity	43,000
Steam	82,100,000
Water	4,000
Total	114,000,000

5.6 Conclusions & recommendations

The key objectives of this PhD research work are (i) Development of a process design for the production of the both the sugar and amino acid platform via dilute acid pretreatment (ii) A preliminary cost analysis to estimate the initial capital cost and operating cost of this facility using Aspen Plus Economic Analyzer® and (iii) A greenhouse gas analysis to understand the environmental impact of this facility. A conceptual process design has been constructed to produce the carbohydrate and amino acid rich stream. The initial capital cost was estimated to be $4,700,000 with

substantial operational ($22,100,000) and raw material cost ($19,300,000) on an annual basis. This is mainly attributable to the high steam and 98wt% H_2SO_4 requirement. Finally, GHG emissions from this facility were estimated to be 114,000,000 $kgCO_2e/yr$ (114,000 MT CO_2e/yr) with steam and ammonia contributing 72 and 24% while all other inputs contributed 4% or less.

5.7 References

Alibaba, 2012.Available at: http://www.alibaba.com/showroom/succinic-acid.html

Aden A, Foust T. 2009. Technoeconomic analysis of the dilute sulfuric acid and enzymatic hydrolysis process for the conversion of corn stover to ethanol. Cellulose 16(4):535-545.

Adom F, Dunn P, Ruccins S, Shonnard D. 2012a. Optimization of the Protein Hydrolysis Scheme of Defatted Syrup from a Corn Ethanol Dry Mill Facility. Biofuels, Bioproducts and Biorefining (in revisions).

Adom F, Fan J, Davis J, Dunn P, Shonnard D. 2012b. Compositional Analysis of Defatted Syrup from a Corn Ethanol Dry Mill as a Feedstock for Bio-Based Products. Journal of agricultural and food chemistry (in revisions).

Adom F, Fan J, Davis J, Taylor A, Shonnard D. 2012c. Optimization of the Dilute Acid and Enzymatic Pretreatment of Defatted Syrup from a Corn Ethanol Dry Mill. . Biomass and Bioenergy in revisions.

Adom F, Maes A, Workman C, Clayton-Nierderman Z, Thoma G, Shonnard D. 2012d. Regional carbon footprint analysis of dairy feeds for milk production in the USA. The International Journal of Life Cycle Assessment 17(5):520-534.

Agri-Energy. 2012. [updated cited. Available from: http://www.commonwealthagrienergy.com/pricing.

Carlson EC. 1996. Don't gamble with physical properties for simulations. Chemical Engineering Progress 92(10):35-46.

Fasahati P, Liu JJ. 2012. Process simulation of bioethanol production from brown algae. Cellulose 6:6.

Humbird D, Aden A, Biochemical production of ethanol from corn stover: 2008 state of technology model. National Renewable Energy Laboratory: 2009.

Jayawardhana K, Peter Van Walsum G. 2004. Modeling of carbonic acid pretreatment process using ASPEN-plus Ⓡ. Applied Biochemistry and Biotechnology 115(1):1087-1102.

Jennings EW, Schell DJ. 2011. Conditioning of dilute-acid pretreated corn stover hydrolysate liquors by treatment with lime or ammonium hydroxide to improve conversion of sugars to ethanol. Bioresource technology 102(2):1240-1245.

Kazi FK, Fortman JA, Anex RP, Hsu DD, Aden A, Dutta A, Kothandaraman G. 2010. Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. Fuel 89:S20-S28.

Lardy G. 2004. Handling Liquid Feed Commodities. NDSU Extension Service. NDSU Department of Animal and Range Sciences.

Lennartsson A. 2005. Production of Succinic Acid by E. coli from Mixtures of Glucose and Fructose. Thesis work 2005. Lulea University of Technology, Div. Biochemical and Chemical Process Engineering.

Lohrasbi M, Pourbafrani M, Niklasson C, Taherzadeh MJ. 2010. Process design and economic analysis of a citrus waste biorefinery with biofuels and limonene as products. Bioresource technology 101(19):7382-7388.

O'Brien D. 2010. Updated trends in U.S wet and dry corn milling production. Kansas State University.

Peris Serrano R. 2012. Biogas Process Simulation using Aspen Plus [Masters]. University of Southern Denmark.

Sassner P, Galbe M, Zacchi G. 2008. Techno-economic evaluation of bioethanol production from three different lignocellulosic materials. Biomass and Bioenergy 32(5):422-430.

Towler G, Sinnott RK, Chemical engineering design: principles, practice and economics of plant and process design. Butterworth-Heinemann: 2012.

Warman International I. Warman Slurry Pumping Handbook [Internet]. 1994.

Wingren A, Galbe M, Zacchi G. 2008. Techno-Economic Evaluation of Producing Ethanol from Softwood: Comparison of SSF and SHF and Identification of Bottlenecks. Biotechnology progress 19(4):1109-1117.

Wooley RJ, Putsche V, Development of an ASPEN PLUS physical property database for biofuels components. National Renewable Energy Laboratory Golden, CO: 1996.

Chapter 6

6 Regional Carbon Footprint Analysis of Dairy Feeds for Milk Production in the United States[5]

6.1 Introduction

The issue of environmental sustainability has become a prominent factor in decision-making for industries in addressing environmental challenges, such as global climate change. The United States (U.S.) dairy industry inaugurated a study to analyze greenhouse gas (GHG) emissions from milk production in the U.S. The U.S. dairy milk supply chain can be divided into the following major stages: (a) feed production, (b) milk production, (c) milk delivery to processor, (d) processing, (e) packaging, (f) distribution, (g) retail activities, (h) milk consumption, and (i) disposal. In a comprehensive report, within which this article is a part, each stage was analyzed independently and combined to provide the carbon footprint for the dairy supply chain (Thoma et al. 2012). This article here focuses on the production of dairy feed in the U.S. using sources of data at the level of individual states and then aggregates that information into five dairy regions.

While there have been a number of life cycle assessment (LCA) studies on crops in Europe, there have been relatively few in the U.S. The Ecoinvent™ (PRé Consultants 2006) database contains many food and forage crop inventory profiles, but these are from European data sources. Hayashi et al. (2006) reviewed the progress of LCA studies in Europe for areas like renewable energy, animal production, and horticulture. In the U.S., there were several LCA studies conducted on single crops such as

[5] *This chapter has been published as an article in International Journal of Life Cycle Assessment.* Figure D-1 shows copyright clearance allowing for use in dissertation. *Citation*: Adom, F., Maes, A., Workman, C., Clayton-Nierderman, Z., Thoma, G., & Shonnard, D. (2012). Regional carbon footprint analysis of dairy feeds for milk production in the USA. The International Journal of Life Cycle Assessment, 17(5), 520-534. doi: 10.1007/s11367-012-0386-y

switchgrass, soybeans, and corn associated with bioenergy product analyses, including studies by Kim et al. (2009 a, b), Spatari et al. (2005) Landis et al. (2007), Shapouri et al. (2003), Sheehan et al.(1998), Pradhan et al. (2009) and Rotz et al. (2010). A review of this literature indicated that no previous LCAs considered a large number of crops and dairy feeds, and therefore, our study fills an important gap in the U.S. with respect to updated analyses for agricultural crops and other dairy feeds.

6.2 Life cycle assessment methodology

6.2.1 Dairy feeds, goal, and scope

In this study, ISO protocols were followed and all GHG emissions were expressed as equivalent emissions of carbon dioxide (CO_2e.). Commonly used feeds for U.S. dairy production were identified based on a recent literature source (Mowrey and Spain 1999) and information obtained from a nationwide dairy producer survey regarding the composition of dairy feeds (and other related topics) [(Thoma et al. 2012)—see Tables D-1 through to Table D-10 and Figures D-2 through to D-5)]. Over 5,000 surveys were sent to dairy farmers through their Co-ops from January to May 2009 and a second mailing was conducted in June 2009. Of those surveyed, 531 responded. The main relevancy of this survey to this carbon footprint study was the identification of commonly used dairy feeds in the U.S. Responses from the dairy farmer survey and the collection of other crop data were organized on the basis of five regions as shown in Figure 6-1. The definition of dairy milk production regions was done through consultation with dairy experts (Thoma et al. 2012). The basis for selection of these regions was a combination of production practices and climatic conditions. There are over 130 distinct dairy feedstuffs included in the results of that survey.

Goal*:* The main goal of this study was to determine the carbon footprint from the cultivation and harvesting of U.S. dairy feeds on a basis of 1 kg of feed harvested or produced in units of grams CO_2 equivalents (gCO_2e) / kg of dry feed. An additional goal was to identify dairy feed inputs with the highest environmental impact to serve as a source of information for improvement in production and as a benchmark against which progress can be measured in the dairy industry.

Scope: The scope was a cradle-to-farm gate analysis. In this article, we report on grain, forage crops, and other co-products e.g., dried distillers grains with soluble (DDGS) and soybean meal for which inventory data were available from U.S. government and university extension sources. In this study, we did not consider all of the 130 or so dairy feeds identified in the survey by Thoma et al. (2012). Table 6-1 shows the three major categories of dairy feeds considered in this study, including grain crops, forage crops, and co-products. This study includes application of inorganic fertilizers, effects of crop residues, manure application, crop protection chemicals, and energy inputs required for cultivation and harvesting. According to the study by Landis et al. (2007), seed production comprised less than 1% of GHG emissions for corn and soybean.

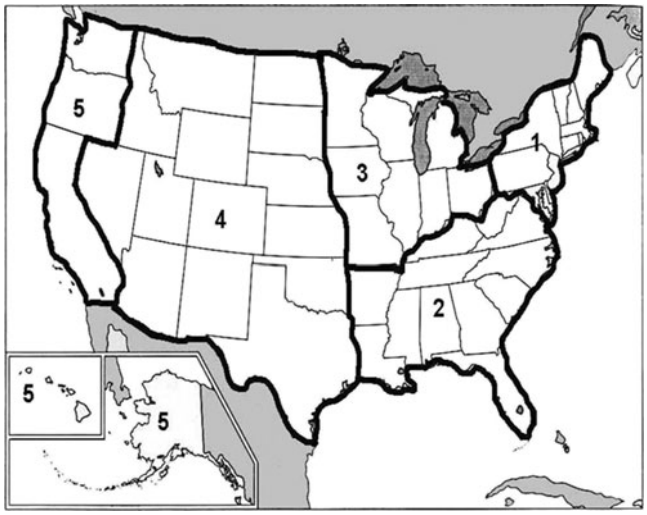


Figure 6-1 Dairy production regions used for this study

This result was generalized for all dairy feeds analyzed in this study by assuming all associated inputs for seed production were below cutoff criteria, and hence were excluded. Also, the scope of this carbon footprint analysis does not include incidental effects such as emissions from employee travel to or from the farm. Infrastructure elements, such as construction of buildings and farm equipment, were also excluded.

6.2.2 Functional unit

The functional unit for this carbon footprint study was 1 kg of dairy feed (grains, forage crops, and other co-products) harvested or processed on dry matter basis.

Table 6-1 Dairy feeds analyzed in this study. Shown in parenthesis are the percentage moisture content for all feed analyzed in this study (NDSU 2011)

Grain Crops	**Forage Crops**	**Co-products**
Oats (14%) Soybean (13%) Corn silage (65%) Corn grain (15.5%) Winter wheat (13.5%)	Alfalfa hay (16%) Alfalfa silage (16%) Forage mix (16%) Grain mix (15%) Grass hay (16%) Grass pasture (16%) Grass silage (16%)	DDGS, dry mill (10%) DDGS, wet mill (60%) Soybean meal (11%)

6.2.3 Geographical boundaries

The geographical context of this carbon footprint study is the U.S. for dairy feeds grown and produced in the U.S.

6.2.4 Allocation procedure

Most dairy feeds produced no co-products, but for certain feeds, it was not possible to avoid allocation. For those feeds, allocations based on market value were used, as shown in Table 6-2. Section 6.3.1 explains the basis for allocation of nitrogen (N) inputs to corn and corn silage. Sections 6.3.1.1 and 6.3.1.2 explain in more detail the economic allocation to soybean oil and meal as well as wet and dried distillers grains with solubles. Five-year average commodity cost data from Illinois were used for economic allocation of soybean oil and meal, which was assumed to be representative of the national commodity market (USDA-IL 2010). Also, mass allocation based on a 5-year average yield provided by the National Oilseeds Processing Association was used for testing scenario cases, while economic allocation was adopted as the base case. Economic and mass allocation values for dried distillers grains with solubles from the thesis by Kodera (2007) were used in this study.

6.2.5 Inputs versus inventory data and possible limitations

Inputs such as fertilizer and fuel used for each crop production system were obtained from U.S. government sources and the U.S. literature. Inventory data underlying those inputs are largely from the Ecoinvent™ database (PRé Consultants 2006), which mostly represents European production. This presents a possible limitation to this study. However, European inventory data, while not geographically relevant, are technologically relevant for the inputs used in this U.S. study because both U.S. and European production uses modern technology. In addition, inventory data for many study inputs are simply not available yet based on U.S. production.

Table 6-2 Summary of allocation ratios and types used in this study

Co-product	Economic allocation	Mass allocation
Soybean oil: Meal: Hulls	**56.7:41.2:2.1**	19.4:74:6.6 [d]
DDGS dry: Ethanol	**30:70**	52:48
DDGS wet: Ethanol	**24:76**	51:49
Dairy Feed: Corn		**Causal Allocation**
Corn: Corn Silage [e]		
Region 1		59:41
Region 2		91:9
Region 3		96:4
Region 4		95:5
Region 5		No data
		Causal relationship based on crop nitrogen requirements

[d] (CGB 2010)

[e] The large differences between regions are primarily determined by the relative production of each crop. More silage is grown in region 1 compared to corn grain than the other regions, and therefore, the allocation of shared inputs is not nearly equal

6.3 Life cycle inventory analysis

A life cycle diagram describing the key inputs for each crop production system is shown in Figure 6-2. The major inputs included: inorganic and organic fertilizer application on the farm, agrochemicals used to control pests, and farm energy use. Lime application on the farm was considered for some of the crops where data were

available as well as effects of crop residues on direct and indirect nitrous oxide (N_2O) emissions. Energy use included gasoline, diesel, liquefied petroleum gas (LPG), natural gas, and electricity. GHG emissions for this analysis included: carbon dioxide (CO_2), methane (CH_4), N_2O, solvents, and refrigerants. Solvents and refrigerants were not directly included as system inputs, rather these were incorporated by the use of Ecoinvent™ ecoprofiles (PRé Consultants 2006) for the various crop inputs. N_2O emissions from nitrogen fertilizer application for the degradation of crop residues and manure application were accounted for using guidelines (IPCC 2006) for national GHG inventories (tier 1).

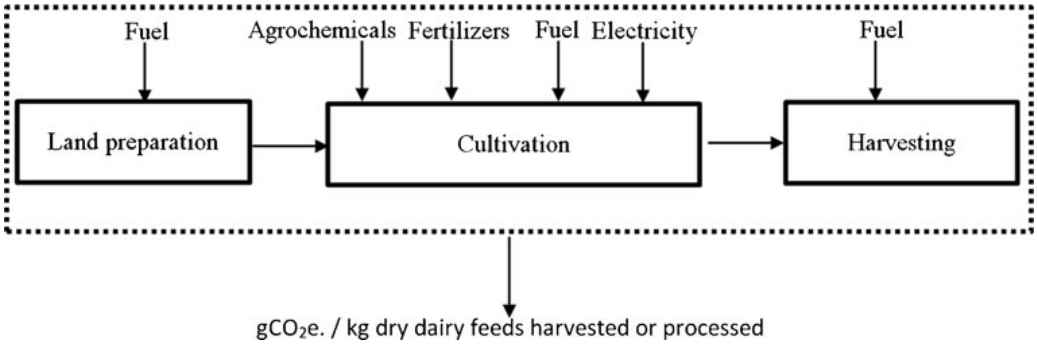


Figure 6-2 Life cycle diagram for the cultivation and harvesting of dairy feed crops. Dotted lines represent the system boundary considered in this carbon footprint analysis

6.3.1 Production inputs and inventory for grains: corn, oats, soybeans, and winter wheat

Every year, the U.S. Department of Agriculture (USDA) National Agricultural Statistical Service (NASS) conducts hundreds of farm surveys on cropping practice, chemical use, farm costs, and income. It is usually structured in a three-phase annual survey with specific goals. Phase I screens various farms for commodities and for potential inclusion in phases II and III, and this is done usually on a state-by-state basis. Phase II collects data on chemicals, fertilizer, and pesticides and has only one collection mode—personal interviews via-face-to-face contact. Phase III focuses on detailed economic information about the agricultural operation and the operator's household. Response rate from farmers has been highest for phase II with an average

response rate of 80% from 2002 to 2006 and an average sample size of 5,465 (National Research Council 2008).

Table 6-3 summarizes the major crop databases and sources of the dairy grain crops. The USDA NASS databases were the primary source of crop production data for this study. Specific data obtained on a state-by-state basis included area harvested, yield, and total production. Average values for the harvested acres, yield, and production over the 5-year production period (2004–2008) were calculated and used. Annual crop production data for soybean, oats, wheat, corn grain, and silage for the 5-year period were obtained from the crop production summary reports from (USDA NASS 2007a; USDA NASS 2009). Tables D-11 through to D-17 shows the computational spreadsheets of the major crops discussed here (USDA NASS 2006).

Mac Donald et al. (2009) established that about 5% of U.S. cropland receives animal manure, with corn land receiving over half of this applied manure. The percentage of planted acres receiving manure (manure share) was highest for corn and oats, being 11.6% and 9%, respectively. For all other grain crops, this area percentage for manure was approximately 1% or less. Therefore, we assume that only corn and oats receive manure as a fertilizer supplement. Dairy production regions needing supplementation with manure were identified by estimating the growth nitrogen requirements to meet crop production yields and comparing these with reported inorganic nitrogen inputs from the USDA NASS databases. The following sections explain in more details how the manure inputs were determined.

Corn: Combined corn and silage input data for fertilizer and chemical application rates were obtained for states in regions 1, 2, 3, and 4, but no data were reported for region 5. USDA NASS database reported separate productivity data for corn grain and silage. Agrochemical chemical input data such as inorganic fertilizers and herbicides were reported for combined corn and silage land area. Productivity data indicated that region 5 contributed less than 1% toward the total corn production in the U.S. The

authors (Mac Donald et al. 2009) in their report on manure usage for fertilizer estimated that 408 million kg of manure nitrogen was applied to corn grain and silage in the U.S. in 2007. The USDA NASS data for nitrogen application rates do not include manure contributions. In addition, the reported inorganic N application rates do not meet known crop requirements of approximately 0.54 kg N/bushel (bu) for corn grain and 5.19 kg N/mt for silage as defined by numerous crop production budgets. The amount of manure N required to reach the crop requirement was determined on a state-by-state basis using this equation: **manure N = corn N growth requirement − synthetic N fertilizer application − residual N following rotation with soybeans**. Using crop budgets for a corn–soybean rotation, it was estimated that approximately 23 kg N/ac was supplied in soybean residue (MSU 2010). The organic N from manure was applied in a manner to force the total N per crop to match the growth requirements mentioned above. Using a causal allocation based on the crop nitrogen requirements for both grain and silage, other crop inputs were allocated. Table 6-2 shows the allocation ratios used in this model for the various dairy production regions. Using this method, the total manure nitrogen applied to corn was approximately matched to the reported annual application rate of 408 million kg within a 4% margin. Specific inputs (e.g., lime) for the various crops are further explained in subsequent sections.

Soybeans In the case of soybeans, the USDA NASS (2009a, 2007 a, b) had data such as quantity of inorganic fertilizer used, area harvested, crop productivity, chemical use, and other information for states in regions 2, 3, and 4. Soybean energy inputs and lime application rate data were obtained from another study Sheehan et al. (1998) and Pradhan et al. (2009), respectively. Inorganic nitrogen input data from USDA NASS (2007b) were included, while manure inputs were not because Mac Donald et al. (2009) reported a manure share of approximately 1% of acres for soybean. Section 6.3.1.1 provides the sources of inventory data for the soybean meal–oil. The average of the carbon footprint in regions 2, 3, and 4 was used to represent regions 1 and 5 for which there were no data available.

Table 6-3 Crop databases and data sources for dairy grains

Summary of Crop inventory and data source	
Soybean & Soybean meal Area harvested /production data Fertilizer and agrochemical inputs Lime input	**Data Sources:** USDA NASS (2009a, 2007a) USDA NASS (2007b) Pradhan et al. (2009)
Oats Area harvested /production data Fertilizer and agrochemical inputs Lime input Energy inputs	 USDA NASS (2009a, 2007a) USDA NASS (2006) Pradhan et al. (2009) Dartt and Schwab (2001)
Wheat Area harvested /production data Fertilizer and agrochemical inputs Energy inputs	 USDA NASS (2009a, 2007a) USDA NASS (2007b) Piringer and Steinberg (2006)
Corn Area harvested /production data Fertilizer and agrochemical inputs Energy inputs	 USDA NASS (2009a, 2007a) USDA NASS (2006) Shapouri et al. (2002)
DDGS (dried distillers grains with solubles)	Hill et al. (2006) Wang (2001) Kodera (2007) Kim and Dale (2002)

Oats The primary source of data for fertilizer and chemical inputs for oats was from USDA NASS (2006). However, no input data (e.g., inorganic fertilizer and crop protection chemical) were reported for the states in region 2, and this is due to its relatively low oats productivity (5% of oats production). Due to the unavailability of input data for lime application for oat-producing states, the national average lime application rate for soybeans was assumed for the oats analysis (extension documents validated this estimate). Based on the N requirement recommendation of 0.5 kg N/ bu and 40 kg N/ac (Beegle 1997), NASS reported inorganic N input data for dairy production regions 1 and 3 were low, requiring supplementation with manure. The reported inorganic N input data for regions 4 and 5 were sufficient to meet N requirement of oats. An estimated 20 kg N/ac of additional N from manure meets the reported yields, and this was applied to regions 1 and 3 on a state-by-state basis. This

method does not take into account any nitrogen credit from prior rotation unlike in the case of manure GHG impact estimation for corn. Section 6.3.5 provides the details on energy inputs. Finally, due to lack of data for region 2, the inventory for this region was estimated by averaging regions 1, 3, 4, and 5.

Winter wheat This study focused on winter wheat because it accounts for 70% to 80% of the total wheat produced in the U.S. (USDA NASS 2009b) as compared to other types like durum and spring wheat. Productivity data were obtained from USDA NASS (2009a, 2007a); however, no data were available for the energy inputs on a state-to-state basis. Energy estimates for the production of wheat in the U.S. on a per hectare basis was obtained from Piringer and Steinberg (2006) for the wheat analysis. Manure impact was not considered for wheat primarily because it has less than 1% of acres applied with manure Mac Donald et al. (2009).

For all crops, input data for fuel and electricity consumption on the farm for crop production were obtained from the technical literature, state agricultural extension services, the U.S. Department of Energy, the USDA, and other academic institutions (see Table 6-3). There are three regional interconnection grids in the U.S., namely, Eastern Interconnection, Western Interconnection, and the Electric Reliability Council of Texas Interconnection. GHG emission factors (in gCO_2e/kWh) were constructed using Ecoinvent™ unit processes (PRé Consultants 2006) based on regional fuel mixes. Additionally, pre-combustion emissions and the transmission and distribution losses were included in the emission factor using regional interconnection grid data reported by (Deru and Torcellini 2007). Section 6.3.5 of this article explains in more detail the assumptions and data sources for the specific crops for which energy input data were not available.

6.3.1.1 Soybean meal–oil–hull allocation

In the soybean meal analysis, additional processes were considered including transporting soybean to the crusher and crushing to recover oil and meal. The impact of transporting soybean to the crusher was estimated as well as the impact of crushing

with the use of data obtained from a separate study Sheehan et al. (1998), Pradhan et al. (2009) and Pollak (2010). The crushing and extraction energy required were updated based on a more recent study Pradhan et al. (2009). Allocation to meal and oil were based on economic value of the co-products from price data averaged over 2004–2008. The primary data source for prices was from Illinois, but is expected to be representative of the national commodity markets during the time period (USDA-IL 2010). Soybean meal allocation factors are shown in Table 6-2.

6.3.1.2 Dried distillers grains with solubles

Articles from the technical literature representing work done by LCA experts with corn ethanol and DDGS were used in this analysis. A thesis by Kodera (2007) performed a review of the effects of allocation method on LCA impacts of corn ethanol production by the dry milling process, for example, mass, energy, and value allocation as well as system expansion. Based on the allocation factor summary in this thesis and another study Kim and Dale (2002), an allocation of the GHG burdens for corn ethanol production was made to DDGS in our model. As shown in Table 6-4, allocation factors varied widely and this resulted in some uncertainty for DDGS carbon footprint analysis. The DDGS GHG emissions values in this table were obtained using the allocation factors shown combined with GHG emissions for corn ethanol from three studies (Wang 2001; Shapouri et al. 2003; Hill et al. 2006) and DDGS production data from Hill et al. (2006). Detailed analysis of wet mill and dry mill DDGS can be found in Tables D-16 and D-17.

6.3.2 Production inputs and inventory for forage crops: alfalfa, alfalfa silage, grass hay, grass pasture, and grass silage

To estimate the inventory for cattle forage production, crop production budgets produced by state agriculture extension specialists were collected and used as the primary source of input data. These budgets estimated the inputs needed to produce alfalfa, grass hay, silage, and pasture. These are not actual production records, but estimates prepared by agricultural extension agents with detailed knowledge of agronomic conditions in specific states. For this analysis, inventory data on fuel,

electricity, fertilizers, soil amendments (N, P, K, sulfur, boron, and lime), and crop protection chemicals were used. When only purchase price for inputs was given, price was converted to quantity using information from budgets published on the same year that provided both price and quantity for the inputs in question. Pesticide application rates varied widely, depending on the type of pesticide. For budgets where only estimated pesticide purchase price was provided, available cost data were used to convert to quantities (Schnitkey 2004). Mac Donald et al. (2009) reported that 6.9% (manure share) of hay and pasture land received manure as fertilizer. Because the budgets used to create the unit processes for these forage feeds report recommended total organic and inorganic nitrogen application rates together, it was assumed that 6.9% of the fertilizer applied was in the form of manure. In several cases, budgets provided total quantity of fertilizer, but did not specify the percentage breakdown for each. In this case, a ratio of 20:40:40 NPK for alfalfa was chosen, as it is a nitrogen fixer. For grass, we used 50:25:25.

Some budgets included custom costs for contracted services such as tilling, planting, or harvesting rather than providing explicit input estimates for each of these processes. Using figures from MSU Extension (MSU 2010) that showed custom costs per acre and fuel cost per acre for different practices, it was found that 16% of custom costs for tillage went to fuel, 12% to planting, 18% to fertilizers, and 18% of harvesting costs went to fuel. Over a 5-year period, a typical field is tilled and planted once, fertilized five times and harvested twice per year (10×); thus each practice was weighted by these estimated rates, giving tillage and planting a value of 1, fertilizing a value of 5, and harvesting a value of 10. As a result, a weighted average of 18% of custom costs was attributed to the consumption of diesel fuel.

There is a large difference in diesel use for hay, silage, and pasture. Most states provided budgets for hay, but fewer for pasture or silage. Using those few states that provided diesel use data for both (primarily regions 2 and 3), the average difference in diesel used to harvest hay or silage per short ton of crop was calculated. We assumed

the dry matter yield was equivalent for pasture, hay, or silage. The only difference was harvesting and hauling. After finding the mean diesel use for hay for each region, we added ~1 gal per dry short ton of crop if harvested as silage and subtracted ~3 gal if kept as pasture.

Table 6-4 Allocation factors and GHG intensity of DDGS (See Tables D-16 and D-17)

	Energy	Mass	Economic	System Expansion	References
Allocation Factor to Ethanol	0.57	0.48	0.70	0.80	Kim and Dale (2002); Kodera (2007)
Allocation Factor to DDGS	0.43	0.52	0.30	0.20	
DDGS GHG Emissions [kg CO_2e / kg DDGS (dry)]	1.60	2.30	0.91	0.53	
Corn Ethanol (kg CO_2 eq / MJ ethanol)					Hill et al. (2006)
					Wang (2001)
					Shapouri et al. (2003)

6.3.3 Direct/indirect N_2O emissions

The (IPCC 2006) tier 1 method was used to calculate direct and indirect N_2O emissions from managed soils for inputs such as synthetic and manure N fertilizer, N in crop residues (above and below ground residues) as well as CO_2 released by lime and urea-containing fertilizer. Direct N_2O release was estimated as 1% of N applied to soil released as N in N_2O. For indirect N_2O emissions, two major pathways were included. The first is the volatilization of N as NH_3 and oxides of N at a rate of 10% of applied N, and redeposition of these gases on water bodies where N_2O–N is emitted at a rate of 1% of the redeposited N. Leaching and runoff is the second pathway with a default leaching factor of 30% of applied N and an emission factor for N_2O–N of 0.75% of leached N. When urea ($CO(NH_2)_2$) is applied, it can be converted to ions like ammonium (NH_4^+) and bicarbonate (HCO_3^-) in the presence of urease enzymes and release CO_2. GHG emission from lime application is dealt with in Section 6.3.6. In this study, dinitrogen monoxide (N_2O) emissions for manure application is a

combination of direct and indirect mechanisms as discussed above (see Tables D-18, D-19 and D-20) including emissions from manure management systems (MMS).

The USDA NASS database does provide N fertilizer input data for crops (see Table 6-3); however, this database does not indicate the type of nitrogen fertilizer applied to crops. The production of different nitrogen fertilizers results in very different quantities of GHG emissions from their production. Therefore, an average US nitrogen fertilizer production profile was created for this study. Data on fertilizer consumption in the U.S. from the period of 2004–2007 was obtained and used to create the synthetic N ecoprofile for this analysis (see Tables D-21 and D-22). One of the N fertilizers, nitrogen solutions, was comprised of urea (35%), ammonium nitrate (40%), and water (25%) (Dyno Nobel Inc ; Vitosh 1996).

For phosphorus fertilizer, a similar approach as for N fertilizer was taken by basing the mixture of phosphate fertilizers in proportion to their U.S. production (USDA ERS 2009) as reported in Table D-23. Potassium and sulfur fertilizers as well as lime were treated similarly.

6.3.4 Crop protection chemicals

Insecticides, herbicides, and fungicides applied on the farm were considered in our analysis. In cases where the ecoprofile of a pesticide was not found in the Ecoinvent™ database in SimaPro 7.1© (PRé Consultants 2006), the chemical class was used. For instance, tebupirimphos which was not directly listed in the Ecoinvent™ database belongs to the organophosphorous class of compounds (PAN Pesticides Database 2009) and this was the ecoprofile used in our model. Rate of crop protection chemical application for soybean and winter wheat were all obtained from USDA NASS (2007b) while that of corn and oats were obtained from USDA NASS (2006). Forage crop protection data were obtained from state extension budgets as mentioned earlier.

6.3.5 On-farm energy

This analysis accounted for the following energy inputs on the farm: electricity, gasoline, diesel, LPG, and natural gas. Due to the lack of energy input information in the USDA NASS database, other sources were used to fill in the required data for the crop analysis. Energy input data for forage energy were from state extension documents as mentioned previously. Soybean energy input data were obtained from Sheehan et al.(1998) and represented 14 soybean-producing states, which together accounted for about 86% of the soybean produced in the U.S. Additionally, energy input data for corn producing states were obtained from Shapouri et al.(2003) and represented about 80% of corn produced in the U.S. In the case of oats, data for diesel use were obtained from Dartt and Schwab (2001). Due to lack of data on gasoline consumption for oats cultivation and harvesting, it was assumed that gasoline consumption was equal to one third of diesel consumption, based on diesel and gasoline inputs for other field crops, for example corn and soybeans. To fill data gaps, LPG and electricity inputs for corn and soybean were then averaged on a regional basis and used as an estimate for oats. Energy estimates for production of wheat in the USA on a per hectare basis was obtained from another study Piringer and Steinberg (2006).

6.3.6 Lime application

Lime application rates for soybean were obtained from Pradhan et al. (2009. In the case of oats, the national average of lime application rate for soybeans was assumed, which in our study (358 lb lime/acre) falls within the recommended range from two budgets that were obtained from KSU (2003) and Crozier et al. (2004). Lime application data for corn grain and silage were estimated using a crop production budget (MSU 2010). While data on lime application rate were not available for wheat production, it appeared that lime was seldom used. For example, only 9% of wheat land area has ever been treated with lime based on a 1997 survey by USDA (Heimlich 2003). According to a U.S. Geological Survey (USGS 2007) approximately 10.8 billion and 32 million kg of limestone and quicklime were applied in the U.S. agricultural sector, respectively. As a result, every kilogram of an average U.S. lime

comprises 0.997 kg $CaCO_3$ and 0.003 kg of CaO. Final GHG intensity of lime accounts for both the production and its application on the field. Due to the on-farm application of calcium carbonate to acidic soils, CO_2 is released, which was accounted for in this study using the emission factor from the IPCC (2006) (see Section 6.3.8 for emission factor).

6.3.7 Crop residue effects on direct/indirect N_2O emissions

In this study, the 2006 IPCC guidelines for national GHG inventories (tier 1) was used to account for the N_2O emissions from the degradation of crop residues above and below ground. The average regional yields for various dairy feeds were converted on a dry weight basis to obtain a kilogram dry crop per harvested area. In addition, other parameters like the N content and weight of dry matter residue above and below ground allowed for the final estimation of kilogram N above and below ground of crop residue per kilogram of crop harvested. Tables D-24 through to D-28 shows the detailed analysis of N_2O emissions of crop residues.

6.3.8 Emission factors for fertilizer, crop protection chemicals, and energy input

The emission factors are shown in Table 6-5 for the production and use of various fertilizers, lime, and energy inputs. Emission factors for pesticides are listed in the Tables D-29 through to D-32.

Table 6-5 Emission Factors for Farm Input: Fertilizer, agro-chemical and energy

Farm input	Emission Factors			Sources
Fertilizer	N	3.871	kg CO_2 eq/kg N in U.S mix N fertilizer due to manufacturing of N fertilizer	USDA ERS[f]
		0.633	kg CO_2 eq/kg N in U.S urea in U.S mix of N fertilizer due to field emissions CO_2	IPCC (2006)
		6.205	kg CO_2 eq/kg N in U.S mix of N fertilizer due to direct and indirect N_2O field emissions	EcoInvent database (SimaPro)
	P	3.028	kg CO_2 eq/kg P in U.S mix P fertilizer due to manufacturing of P fertilizer (applied as P)	USDA ERS EcoInvent database (SimaPro)

		K	0.573 kg CO_2 eq/kg K in U.S mix K fertilizer due to manufacturing of K fertilizer	USDA ERS EcoInvent database (SimaPro)
		S	3.855 kg CO_2 eq/kg S in fertilizer	EcoInvent database (SimaPro)
Agro-chemicals		Lime	0.0158 kg CO_2 eq/kg lime due to manufacturing 0.4400 kg CO_2/kg $CaCO_3$ due to application on farm	USGS (2007)[g]
Energy Input	**Fuel**	Gasoline	10.96 kg CO_2 eq./gallon	Deru & Torcellini (2007) SEIT (2006)
		Diesel	11.89 kg CO_2 eq./gallon	
		LPG[h]	7.66 kg CO_2 eq./gallon	
		NG[i]	7.72 kg CO_2 eq./ CCF	
	Electricity	**U.S Region**	**kg CO_2 eq./kWh**	**Sources**
		U.S Avg[j]	0.823	Deru & Torcellini (2007)
		Eastern	0.867	
		Western	0.653	
		ERCOT	0.928	

[f]Source:http://www.ers.usda.gov/Data/fertilizeruse/,[g]http://minerals.usgs.gov/minerals/pubs/commodity/stone_crushed/myb1-2007-stonc.xls & http://minerals.usgs.gov/minerals/pubs/commodity/lime/myb1-2007-lime.xls, [h]LPG: Liquefied Petroleum Gas, ERCOT: Electric Reliability Council of Texas,[i]NG: Natural Gas, [j] Avg: Average

6.3.9 Data quality

The pedigree matrix derived from Frischknecht et al. (2007) was used to assess the quality of data, primarily fertilizer and other N_2O emissions, crop protection chemicals, and energy inputs. Six characteristics of data quality were included: *reliability*, *completeness*, *temporal correlation*, *geographic correlation*, *further technological correlation*, and *sample size*. This was done by assigning a set of scores from 1 to 5 after a careful analysis of each data source (see Tables D-33 through to D-44). Using some basic uncertainty (U_7) factors provided in Table 7.2 of Frischknecht et al. (2007) and assessing the data sources according to the six characteristics mentioned above, the square of geometric standard deviation (SD_{g95}) was calculated using the equation below (SD_{g95}): For calculating SD_{g95}

$$SD_{g95} = \sigma_g^2 = \exp^{\sqrt{[\ln(U_1)]^2+[\ln(U_2)]^2+[\ln(U_3)]^2+[\ln(U_4)]^2+[\ln(U_5)]^2+[\ln(U_6)]^2+[\ln(U_7)]^2}}$$

where U_1 = uncertainty factor of reliability, U_2 = uncertainty factor of completeness, U_3 = uncertainty factor of temporal correlation, U_4 = uncertainty factor of geographic correlation, U_5 = uncertainty factor of other technological correlation, U_6 = uncertainty factor of sample size, and U_7 = basic uncertainty factor.

By assuming a log-normal distribution of uncertainty, the estimated SD_{g95} was used to calculate an upper and a lower bound of the 95th percentile confidence interval for the various dairy feeds on a national basis (Table 6-6). The geometric mean (in micrograms) was used to estimate the lower and upper bound (gCO_2e/kg feed) using equations below (Frischknecht et al. 2007). Equations below were used for calculating the lower and upper bound values of carbon footprint.

$$Upperbound = \mu_g \times \sigma^2_g \qquad Lowerbound = \frac{\mu_g}{\sigma^2_g}$$

6.4 Life cycle greenhouse gas impact assessment and interpretation of results

6.4.1 General assumptions for life cycle impact analysis

In estimating the carbon footprint, the GHG emissions were converted to CO_2 equivalents using global warming potentials (GWP) in the ‘‘IPCC 2006 100a’’ method in SimaPro 7.1© (PRé Consultants 2006); GWP is 1 for CO_2, 298 for N_2O, and 25 for CH_4 (Forster et al. 2007). The effects of other greenhouse gases emitted in minor amounts such as refrigerants, halons, and certain chlorinated solvents were also accounted for.

6.4.2 Regional greenhouse gas emissions of dairy feeds

Table 6-6 summarizes the regional GHG emissions of dairy feeds on a per dry kilogram basis. Careful examination of the table reveals that there is significant variability among the regions for several feeds. Nearly all of the highest values are associated with region 2, and this appears to be driven primarily by greater nitrogen

and lime inputs. The exception is the production of oats in region 5, which is nearly double the lowest value. This is as a result of much higher application rates for N reported in California; approximately three times the rates applied in other areas. This is partially offset by larger yields; however, the yield is only 1.5 to 1.7 times that of other regions. Grass has a higher carbon footprint than other forage crops and nearly as high as corn grain. Regional results for each feed analyzed were combined to estimate the national carbon footprint (see Table 6-6). Overall, processed co-products like wet mill and dry mill DDGS and soybean meal show higher GHG emissions.

Results in Table 6-6 can be compared to recent literature values, though some of these studies occurred in different geographic contexts. Landis et al. (2007) modeled the agro-system material flows for U.S. corn and soybean by employing the greenhouse gases, regulated emissions, and energy use in transportation (GREET) model. The following results were obtained by Landis et al. (2007): 310–680 gCO_2e/kg of dry corn and 120–290 gCO_2e/kg of dry soybean. The carbon footprint results for corn and soybean at the farm stage from GREET (2010) were 290 and 200 gCO_2e/kg of dry crop, respectively. Two separate studies by Kim and Dale (2009a—40 counties in the U.S.) and Kim et al. (2009b—eight counties in the U.S.) estimated 360 ± 100 and 540 ± 290 gCO_2e/kg of dry corn grain, respectively, for U.S. corn-producing counties. In our study, the national carbon footprint of corn grain was estimated to be 390 gCO_2e/ kg of dry corn grain, with upper and lower bounds of 270 and 560 gCO_2e/kg of dry corn grain. Additionally, a value of 300 gCO_2e was estimated for 1 kg dry corn at field using the United States Life Cycle Inventory database in SimaPro (PRé Consultants 2006). The GHG emissions of 1 kg corn silage at the farm gate for the Swiss production processes using Ecoinvent Database was 190 gCO_2e/kg of dry corn silage, a value close to corn silage for our study in Table 6-6. A value of 620 gCO_2e/kg dry soybean was obtained from the Denmark LCA food database in SimaPro (Denmark LCA Food 2011 and PRé Consultants 2009). Dalgaard et al. (2008), using the EDIP 97 database (a Danish LCA methodology) in SimaPro

(PRé Consultants 2006), analyzed the GWP of 1 kg (dry) of soybean meal to be 721 gCO_2e while Pelletier (2008) in the study of the environmental performance in the U.S. broiler poultry sector estimated 297 gCO_2e.

Table 6-6 Cradle to farm gate carbon footprint results of commonly used feeds by region and on national basis (g CO_2 e. / kg dry feed).

	Production Region							
	1	2	3	4	5	Lower bound	Central bound (Geometric mean)[k]	Higher bound
Alfalfa hay	190	270	140	140	150	140	170	210
Alfalfa silage	200	280	150	150	160	150	180	220
Corn grain	360	440	370	440	**400**	270	390	560
Corn silage	160	260	190	220	**210**	140	200	290
DDGS, dry mill	910	910	910	910	910	590	910	1400
DDGS, wet mill	670	670	670	670	670	430	670	1400
Forage mix	160	260	140	140	150	130	160	200
Grain mix	530	590	520	570	550	450	550	670
Grass hay	300	470	280	270	330	260	320	390
Grass pasture	240	410	250	220	280	130	270	560
Grass silage	310	480	290	280	340	270	330	410
Oats	800	**800**	580	1000	1140	580	850	1240
Soybean	**410**	520	330	390	**410**	270	390	580
Soybean meal	460	540	400	430	450	420	460	490
Winter wheat	380	400	510	500	390	300	430	600

For crops with data presented in bold, no data for production was available; the average of results from other regions was adopted. [k] The geometric mean represents the US national greenhouse gas profiles for the various dairy feed with their respective ranges (lower/upper bound) estimated using the square of geometric standard deviation.

Finally, another European study by Van der Werf et al. (2005) estimated the GHG emissions for the production of 1 kg of wheat and barley to be 375 and 400 gCO_2e/kg of dry crop, respectively, while the Denmark LCA food database (PRé Consultants 2006) estimates 710 and 570 gCO_2e for 1 kg of dry wheat and oats, respectively. Taking into account the differences in modeling tools, study scope, and geographical context for the different studies, results from the literature are generally comparable to those obtained in this study. The following sections will display the results in more

detail with regard to the relative importance of specific crop life cycle stages and inputs.

6.4.2.1 Soybean

Soybean showed a lower carbon footprint than some crops due to lower inorganic nitrogen fertilizer application, and this was largely due to the fact that it is a nitrogen-fixing crop. However, significant contributors to the various regional results are: lime application, gasoline, diesel, and N_2O emissions from soybean residues, as shown in Figure 6-3. Together, they contributed about 70–86% of the overall GHG emissions in each productive dairy region. Interesting was the relative impact of lime input on the overall regional footprints. Lime input data for regions 2 and 3 for the soybean-producing states were relatively comprehensive (60% and 100% of states reporting, respectively). For region 4, data for lime application were available for just two states out of the six soybean-producing states. Another probable reason could have been the acidic nature of soils in regions 2 and 3 requiring more lime to increase soil pH for plant growth.

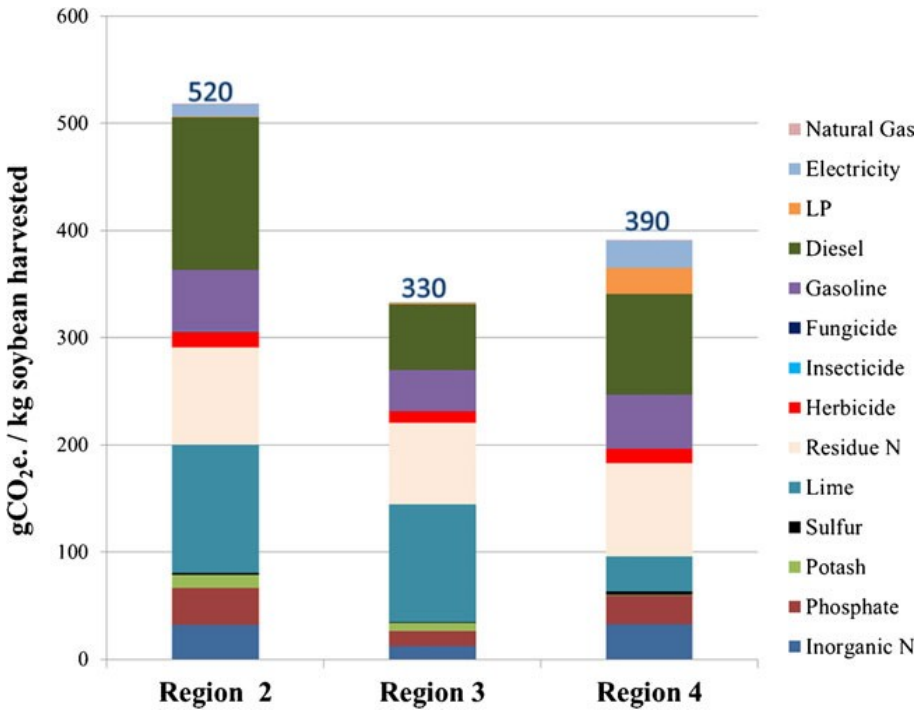


Figure 6-3 Carbon footprint profile of soybeans harvested in the U.S.

Emissions of N_2O from crop residues were large compared to N_2O released from the application of N fertilizers for soybeans, a distinctly different feature compared to other crops. Approximately 65% of GHG emissions from N fertilizers were due to field application, with about 35% from manufacture, as also seen from the data in Table 6-5. Although it was not exactly clear why the states in the midwest (region 3) used relatively lower amounts of diesel, one possible reason was the effect of the Midwest Clean Initiative Diesel (EPA, 2011) which encourages operational changes, technological improvements, and use of cleaner fuels for powering equipment. Finally, using the pedigree matrix, the standard deviation with 95% confidence interval for inorganic fertilizer, crop protection chemicals, and energy inputs was estimated to be 1.51, 1.21, and 1.57, respectively (see Table D-43).

6.4.2.2 Oats

The major contributors to the oats carbon footprint in the U.S. (Figure 6-4) were identified to be inorganic nitrogen and phosphate fertilizers, manure, lime application, diesel, and the impact of N_2O emissions from oat residues, which together makes up approximately 72–92% of the overall footprint in each region.

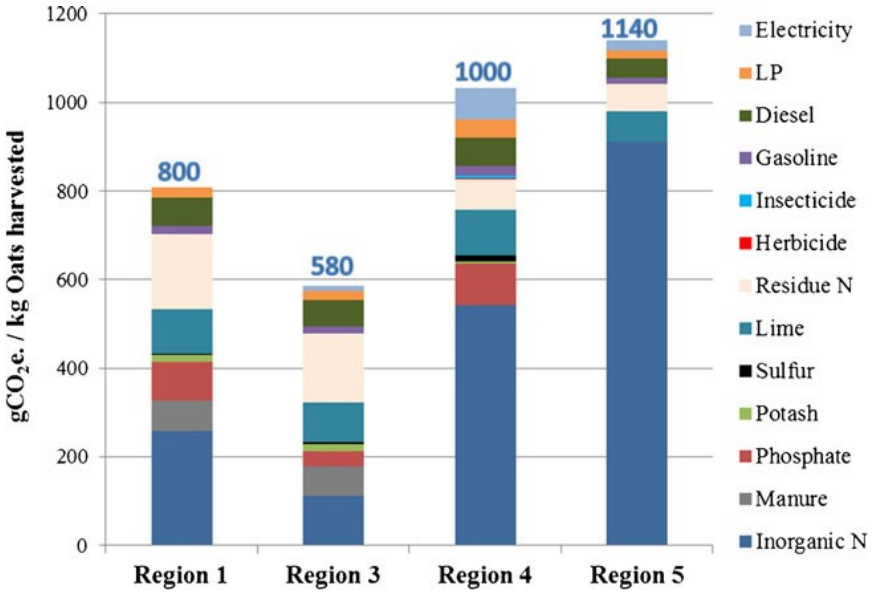


Figure 6-4 Carbon footprint profile of Oats harvested in the U.S

The regional variation in carbon footprint was due to the impact of fertilizer application rate. For example, dairy region 5 shows an unusually high carbon footprint of 1,100 gCO_2e/kg of oats harvested, due to high fertilizer N application. Furthermore, results from California in region 5 may not be representative of the other states in this region. About 65% of inorganic N fertilizer GHG emissions was from field application and 35% was due to manufacture. The impact of crop residues remains fairly constant across the various regions for oats, contributing about 9% on national average towards the carbon footprints reported. However, the use of manure to supplement inorganic fertilizers in regions 1 and 3 contributed 21% and 26%, respectively, towards the regional footprints. Finally, in the case of oats, the standard deviation with 95% confidence for inorganic fertilizer, chemical protection, and energy inputs was estimated to be 1.51, 1.24, and 1.36, respectively (see Table D-43).

6.4.2.3 Corn grain and silage

Inorganic fertilizers, manure, phosphates, lime, diesel as well as the impacts of grain drying and N_2O emissions due to residues contributed approximately 80–90% towards the regional carbon footprint of corn grain (see Figure 6-5).

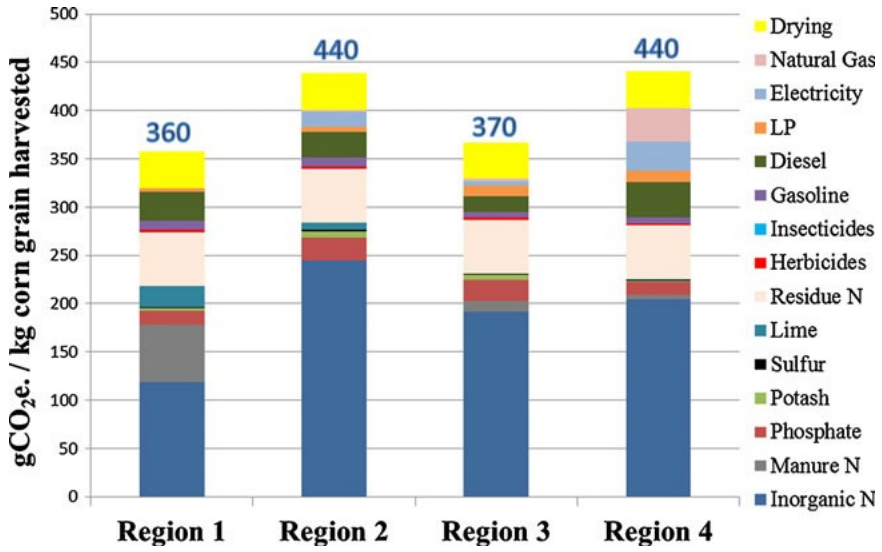


Figure 6-5 Carbon footprint profile of Corn grain harvested in the U.S

In the corn silage analysis in Figure 6-6, inorganic fertilizers, manure, phosphates,

lime, diesel as well as the impacts of drying and N_2O emissions due to residues contributed about 73–90% towards the corn silage footprint for each dairy region. The contribution of the MMS to the GHG emissions for both crops was small (always <2%). Generally, the GHG emissions for corn grain with respect to the various dairy regions were about two times greater than for the corn silage. The comparatively larger emissions for corn grain compared to silage were mainly due to the allocation method applied from Section 7.3.1, under "Corn". Figure 6-5 shows high contributions of inorganic fertilizer from region 2, as this is the reason why additional manure was not added to supplement plant growth in this region. Interestingly, Figure 6-6 shows a relatively high contribution for the use of natural gas for region 4 and this was primarily due to extremely high level of energy requirements from corn farms in Texas. In the final analysis, the standard deviation with 95% confidence for fertilizer, chemical protection, and energy inputs was estimated to be 1.51, 1.21, and 1.26, respectively, (see Table D-43) using the pedigree matrix.

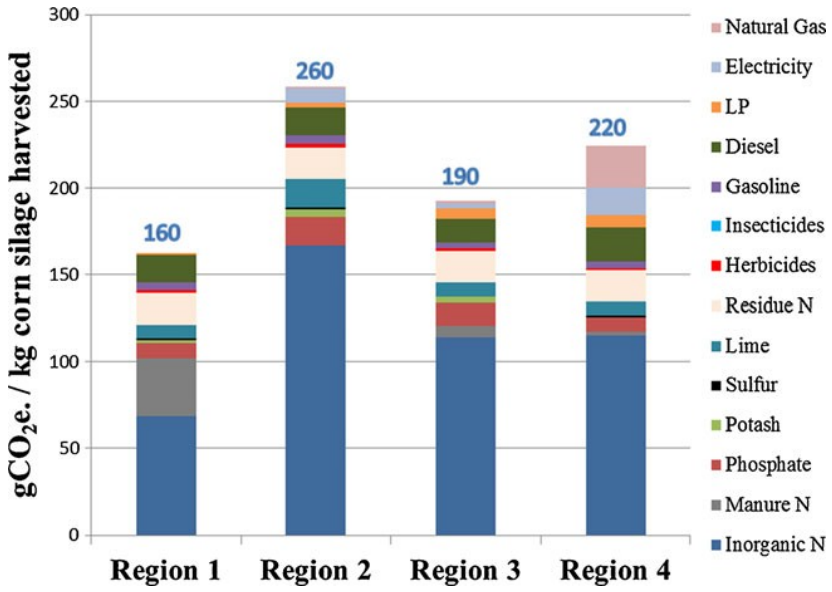


Figure 6-6 Carbon footprint profile of Corn silage harvested in the U.S.

6.4.2.4 Winter wheat

Regions 3 and 4 showed the highest carbon footprint (Figure 6-7), largely due to the high rate of application of inorganic nitrogen fertilizers by farmers. Inorganic nitrogen and phosphate fertilizers, diesel, and the impact of N_2O releases contributed 93–95% of the overall GHG emissions in each dairy region. As in other crops, about 65% of inorganic N fertilizer GHG emissions was from field application and 35% was due to fertilizer manufacture.

On the whole, the carbon footprints for all dairy feed crops analyzed in this study were within the range 160–1140 gCO_2e/kg of dry feed. Various contributions of different farm inputs varied on a regional basis and this was mainly due to the different fertilizer, liming, and energy requirements depending on location, soil properties, and climate.

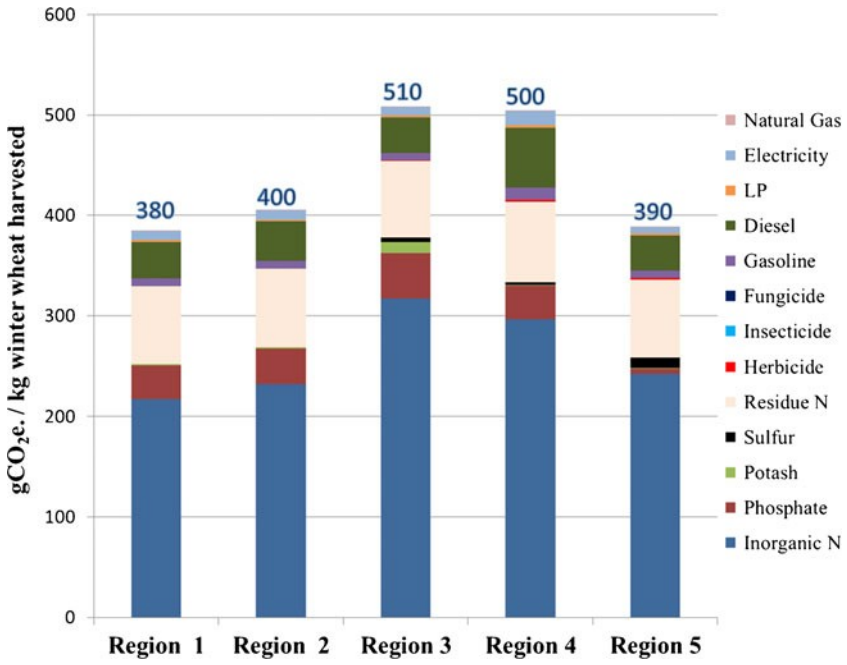


Figure 6-7 Carbon footprint profile of winter wheat harvested in the U.S

6.4.2.5 Forage crops: alfalfa hay, alfalfa silage, grass hay, grass pasture, and grass silage

The major contributors towards the regional footprints for both alfalfa hay and silage were identified to be due to crop residue, phosphate, lime, diesel, and electricity. In all regions, these factors contributed between 80% and 90% toward the overall regional footprint. However, impacts due to the application of potash, boron, crop protection chemicals, and use of gasoline were minimal ranging between 4% and 14% toward the carbon footprint for both alfalfa hay and silage. Contributions to carbon (GHG) footprint due to the application of inorganic fertilizer for both alfalfa hay and silage was less than 10% in all dairy production regions for which input data were available, and this low result was not surprising given that alfalfa is a nitrogen-fixing crop.

Grass showed a higher carbon footprint than other forage crops and nearly as high as the corn grain. Grass typically requires less maintenance and inputs, but produces lower yields than many other crops. In addition, there is much higher variability and uncertainty in actual yield than for other commodity crops. Region 2, which has the highest carbon footprint for grass and hay production, also had higher fuel, lime, and nitrogen use based on the available budget information. In all the different types of grass analyzed, inorganic fertilizers were the major contributors ranging from 34% to as high as 90% toward the footprint in the case of grass pasture. Lime contributions were significant for regions 1, 2, and 3, ranging between 13% and 19% for all grasses analyzed, but under 10% for regions 4 and 5. This reflects the acidic nature of soil in regions 1 to 3.

Finally, the standard deviation with 95% confidence for all inputs of alfalfa and grass were both estimated to be 1.22. Emission ranges varied significantly on a regional basis. The ranges reported in gCO_2e/kg dry forage feed were as follows: 140–270 (alfalfa hay), 150–280 (alfalfa silage), 270–470 (grass hay), 220–410 (grass pasture) and 280–410 (grass silage). The GHG emissions of 1 kg grass hay and silage at the farm gate for the Swiss production processes using Ecoinvent™ database (PRé Consultants 2009) were analyzed to be 180 and 220 gCO_2e/kg of dry feed,

respectively, and somewhat lower than our results.

6.5 Conclusions and recommendations

In this carbon footprint study, the main goal was to estimate the GHG emissions from the cultivation and harvesting of dairy feeds on a basis of one dry kilogram of dairy feed harvested or produced (gCO_2e/kg of dry dairy feed). Table 6-6 shows the cradle-to-farm gate carbon footprint results obtained for all dairy feeds analyzed in this study. There were large differences in GHG emissions among the different dairy crops, with corn silage showing the lowest, while oats and DDGS displayed the highest. This variability was largely driven by fertilizer and energy utilization intensity as shown in Figures: 6-3, 6-4, 6-5, 6-6, and 6-7. There was some variability in carbon footprint for any crop from region to region, driven by regional differences in energy and lime use, but this variability was smaller than inter-crop variability.

The highest contributor to carbon footprint was the on-farm application of inorganic N fertilizer except for the leguminous feeds, whereas the fertilizer input categories P, K, and S accounted for relatively small impacts for all crops. About 65% of inorganic N fertilizer GHG emissions was due to N_2O release upon application, whereas 35% was from fertilizer manufacture. N_2O emission contribution from crop residues was also significant for most crops. With N fertilizer input being the largest contributor to GHG emissions, much effort should be targeted toward lowering emissions associated with their production and use on the farm. Additionally, the efficient transfer of knowledge to farmers with regards to fertilizer best management practices might help reduce emissions on the farm. The use of crop protection chemicals was not so significant however, and energy use impacts varied widely from region to region, likely due to differences in climate, energy conservation programs, and need for crop drying. Finally, on the energy front, there is the need to promote the use of safe and cleaner forms of energy to help reduce climate active GHG emissions associated with the energy input needed by farmers.

This study highlights key crop inputs that are the drivers for emissions of greenhouse gases from the cradle-to-gate cultivation and harvesting for US dairy grain and forage crops. These crop results are equally applicable for uses other than dairy products; for example food production in general and bioenergy. Hopefully, these results will be useful for reducing GHG emissions by guiding efforts to modifying agricultural practices with respect to fertilizer application, use of manure, and energy consumption.

6.6 References

Beegle D (1997) Estimating manure application rates. Agronomy fact sheet 55. Available at: http://juniataccd.org/programs/NMP/agronomy55.pdf

CGB (2010) CGG Enterprise Inc. http://www.cgb.com/browse.asp?page0339. Accessed 23 Jan 2010

Crozier CR, Osmond DS, Dunphy EJ, Fisher L, Heiniger RW, Johnson SE, Jordan DL, Weisz R, Hardy DH (2004) Lime and fertilizer suggestions—field, pasture, and hay crops. In 2012 North Carolina agricultural chemicals manual. The College of Agric. And Life Sci, North Carolina State Univ, Raleigh, p 40 Available: http://ipm.ncsu.edu/agchem/4-toc.pdf. Accessed 1 Dec 2010

Dalgaard R, Schmidt J, Halberg N, Christensen P, Thrane M, Pengue W (2008) LCA of soybean meal. Int J Life Cycle Assess 13:240–254

Dartt A, Schwab G (2001) Crops and livestock budgets estimates for Michigan. Agricultural economics report 609. Available at: http://www.aec.msu.edu/aecreports/budgets01.htm

Denmark LCA Food Database (2011). Available at http://www.lcafood.dk/products/crops/cashcrops.htm. Accessed 16 Jan 2011

Deru M, Torcellini P (2007) Source energy and emission factors for energy use in building, technical report NREL/TP-550-38617. Available at: http://www.nrel.gov/docs/fy07osti/38617.pdf

Dyno Nobel Inc, MSDS (2010) Material safety data sheet, Dyno Nobel Inc. MSDS # 1138. Available at: http://www.irmteam.com/Product_Sheets/MSDS-dynonobel-UAN32.pdf

EPA (2011) Environmental Protect Agency. USA http://www.epa.gov/midwestcleandiesel/. Accessed 23 Feb 2011

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW,Haywood J, Lean J, Lowe DC, Myhre G, Nganga J, Prinn R, Prinn G, Raga G, Schulz M, Van Dorland R (2007) Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin Min D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge. Available at: http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf

Frischknecht R, Jungbluth N, Althaus HJ, Doka G, Heck T, Hellweg S, Hischier R, Nemecek T, Rebitzer G, Spielmann M, Wernet G (2007) Overview and methodology. Ecoinvent report no 1. Swiss Centre for Life Cycle Inventories, Dübendorf

GREET (2010) The greenhouse gases, regulated emissions, and energy use in transportation model. Argonne National Laboratory, U.S. Department of Energy. Available at: http://greet.es.anl.gov/main.Version 1.8d. Accessed 10 Jan 2011

Hayashi K, Gaillard G, Nemecek T (2006) Life cycle assessment of agricultural production systems: current issues and future perspectives. In: Hu SH, Bejosano-Gloria C (eds) Good agricultural practice (GAP) in Asia and Oceania. Food and Fertilizer Technology Center, Taipei, pp 98–110

Heimlich R (2003) Agricultural resources and environmental indicators.Agriculture handbook no. AH722. Washington, DC: USDA Economic Research Service. Available at:

http://www.ers.usda.gov/publications/arei/ah722/arei4_4/AREI4_4nutrientmgt.pdf

Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006) Environmental, economic, energetic costs and benefits of biodiesel and ethanol biofuels. Proc Natl Acad Sci 103:1206–11210

IPCC (2006) Guidelines for national greenhouse gas inventories, prepared by the National Greenhouse Gas Inventories Programme. In: Eggleston H, Buendia L, Miwa K, Ngara Tand Tanabe K (eds). Available at: http://www.ipcc-nggip.iges.or.jp/public/2006gl/index. htm

Kim S, Dale B (2002) Allocation procedure in ethanol production system from corn grain: I. System expansion. Int J Life Cycle Assess 7:237–243

Kim S, Dale B (2009) Regional variations in greenhouse gas emissions of biobased products in the United States—corn-based ethanol and soybean oil. Int J Life Cycle Assess 14:540–546

Kim S, Dale B, Jenkins R (2009) Life cycle assessment of corn grain and corn stover in the United States. Int J Life Cycle Assess 14:160–174

Kodera K (2007) Analysis of allocation methods of bioethanol LCA, M.Sc. Thesis, CML, Leiden University, Netherlands, pp 23

KSU (2003) http://www.ksre.ksu.edu/library/crpsl2/mf2586.pdf. Accessed 1 Jan 2010

Landis E, Miller A, Theis L (2007) Life cycle of the corn−soybean agroecosystem for biobased production. Environ Sci Technol 41:1457–1464

MacDonald J, Ribaudo M, Livingston M, Beckman J, HuangW(2009) Manure use for fertilizer and for energy: report to congress. Administrative publication number: 037. Available at: http://www.ers.usda.gov/publications/ap/ap037/

Mowrey A, Spain N (1999) Results of a nationwide survey to determine feedstuffs fed to lactating dairy cows. J Dairy Sci 82:445–451

MSU (2010) Michigan State University Extension. https://www.msu.edu/user/steind/. Accessed 1 Jan 2010

National Research Council (2008) Understanding American agriculture: challenges for the agricultural resource management survey. Panel to review USDAs

agricultural resource management survey. Committee on national statistics, division of behavioral and social sciences and education. The National Academies Press, Washington, DC

NDSU (North Dakota State University, USA) (1992) Fertilizing corn grain, popcorn, silage corn, and sweet corn. Available at: http://www.ag.ndsu.edu/pubs/plantsci/soilfert/sf722w.htm. Accessed 23Feb 2011

NDSU (North Dakota State University, USA) (2011). Equivalent weights of grains and oilseeds. Available at: http://www.ag.ndsu.edu/pubs/ageng/machine/ae945w.htm. Accessed 23 Jan 2011

PAN Pesticides Database (2009) Available at: http://www.pesticideinfo.org/Search_Chemicals.jsp. Accessed 1 Jan 2009

Pelletier N (2008) Environmental performance in the US broiler poultry sector: life cycle energy use and greenhouse gas, ozone depleting, acidifying and eutrophying emissions. Agric Syst 98:67–73

Piringer G, Steinberg LJ (2006) Reevaluation of energy use in wheat production in the United States. J Ind Ecol 10:149–167

Pollak J (2010) Data update of soybean and soy feedstocks production and life cycle assessment of soy-based products and petroleum based alternatives, peer reviewed final report. Omni Tech International and Four Elements Consulting. Available at: http://www.biodiesel.org/resources/reportsdatabase/reports/gen/20100201_GEN-422.pdf

Pradhan A, Shrestha S, Mc Aloon A, Yee W, Haas M, Duffield A,Shapouri H (2009) Energy life cycle assessment of soybean biodiesel.U.S. Department of Agriculture, Office of Energy Policy and New Uses. Agricultural economic report no. 845. 10/2002

PRé Consultants (2009) SimaPro 7.1 LCA software. http://www.pre.nl/. Accessed 1 Jan 2009

Rotz CA, Montes F, Chianese DS (2010) The carbon footprint of dairy production systems through partial life cycle assessment. J Dairy Sci 93:1266–1282

Schnitkey G (2004) Estimated cost of crop production in Illinois. Farm business management handbook. Available at: www.farmdoc.illinois.edu/manage/enterprise_cost/2004_crop_budgets.pdf

Shapouri H, Duffield JA, Wang M (2002) The energy balance of corn ethanol: an update: U.S. Department of Agriculture, Office of Energy Policy and New Uses. Agricultural economic report no.813. 7/2002. Available at: http://www.transportation.anl.gov/pdfs/AF/265.pdf

Shapouri H, Duffield JA, Wang M (2003) The energy balance of corn ethanol revisited. U.S. Department of Agriculture, Office of Energy Policy and New Uses. Agricultural economic report number 813

Sheehan J, Camobreco V, Duffield JA, Graboski M, Shapouri H (1998) Life-cycle inventory of biodiesel and petroleum diesel for use in an urban bus. A joint study sponsored by U.S. Department of Agriculture and U.S. Department of Energy. NREL/SR-580-24089 Golden, CO: National Renewable Energy Laboratory. U.S. Department of Energy, 5/1998

Simplified Emission Inventory Tools - SEIT (2006). SEIT calculation workbook; v1.0. US Energy Information Administration, Washington, July 2006

Spatari S, Zhang Y, Maclean HL (2005) Life cycle assessment of switch and corn stover derived ethanol-fueled automobiles. Environ Sci Technol 39:9750–9758

Thoma G, Popp J, Shonnard D, Nutter D, Ulrich R, Matlock M, Kim D, Neiderman Z, East C, Adom F, Kemper N, (2012) Greenhouse gas emissions from production and consumption in the United States: A cradle-to-grave life cycle assessment circa. International Dairy Journal (2012), http://dx.doi.org/10.1016/j.idairyj.2012.08.013

USDA NASS (2006) U.S. Department of Agriculture, National Agricultural Statistics Service. Agricultural chemical usage-field crops summary (2005). Available at: http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC//2000s/2006/AgriChemUsFC-05-17-2006.pdf

USDA NASS (2007a) U.S. Department of Agriculture, National Agricultural Statistics Service. Crop production 2006 summary. U.S. Department of Agriculture, 11 Jan 2008. Available at: http://usda.mannlib.cornell.edu/usda/nass/CropProdSu//2000s/2007/CropProdSu-01-12-2007.pdf

USDA NASS (2007b) U.S. Department of Agriculture, National Agricultural Statistics Service. Agricultural chemical usage-field crops summary, 2006. Available at: http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC//2000s/2007/AgriChemUsFC-05-16-2007_revision.pdf

USDA ERS (Economic Research Service) (2009) U.S. fertilizer use and price. Available at: www.ers.usda.gov/Data/FertilizerUse. Accessed 1 Jan 2009

USDA NASS (U.S. Department of Agriculture, National Agricultural Statistics Service) (2009a) Crop production 2008 summary. U.S. Department of Agriculture, 11 January 2008. http://usda.mannlib.cornell.edu/usda/nass/CropProdSu//2000s/2009/CropProdSu-01-12-2009.pdf

USDA NASS (2009b) U.S. Wheat classes. Available at: http://www.ers.usda.gov/briefing/wheat/background.htm. Accessed 14 Apr 2009

USDA-IL (2010) Illinois Department of Agriculture. Available at: http://www.agr.state.il.us/marketing/index.html. Accessed 23 Feb 2011

USGS (2007) United States Geological Survey 2007 available at: http://minerals.usgs.gov/minerals/pubs/commodity/stone_crushed/myb1-2007-stonc.xls http://minerals.usgs.gov/minerals/pubs/commodity/lime/myb1-2007-lime.xls

Van der Werf H, Petit J, Sanders J (2005) The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. Agric Syst 83:153–177

Vitosh ML (1996) N-P-K fertilizers, Michigan State University Extension Bulletin. Available at: http://fieldcrop.msu.edu/sites/fieldcrop/files/E0896.pdf

Wang M (2001) Development and use of GREET 1.6 fuel-cycle model for transportation fuels and vehicle technologies. Tech. Rep. ANL/ESD/TM-163, Argonne National Laboratory, Argonne, IL, 2001. Available at: www.transportation.anl.gov/pdfs/TA/153.pdf

Chapter 7

7 Carbon Footprint Analysis of Dairy Feed from a Mill in Michigan, U.S.[6]

7.1 Introduction

Key business decisions should take into account environmentally-benign processes and products as a means of addressing environmental issues. It was on this premise that the U.S. dairy industry embarked on a project to study the GHG emissions from the production of milk in the U.S. dairy industry. Findings from this dairy study were presented in a report by Thoma et al. (2010). Subsequently, Thoma et al. (2012) reported nine major stages comprising the U.S. dairy industry as follows: i. feed production stage (cultivation of grain and forage crops and other mill feed ingredients plus mill operations and all transportation steps), ii. milk production, iii. delivery to processor; iv. processing, v. packaging, vi. distribution, vii. retail, viii. consumption and ix. disposal. Analyzing each stage separately and then combining all stages provided the carbon footprint of the U.S. dairy milk supply chain. The analysis reported here however required a carbon footprint study of a U.S. dairy feed mill as part of "i. feed production stage" listed above. Additionally, a detailed literature review by the authors revealed that no previous studies were found with regard to carbon footprint analysis of any animal feed mills in the U.S. Shaw et al. (1998)., investigating the development of emission factors for unloading grain and loading feed at mills for cattle feed yards. A recent global dairy sector GHG emissions life cycle assessment [LCA] (Gerber et al. 2010) compared impacts of fat- and protein-corrected milk production and processing for different countries and agricultural cultivation settings, but did not include an analysis of dairy feed mills. Therefore, our

[6] *This chapter has been published as an article in International Dairy Journal.* Figure E-1 shows copyright clearance from Elsevier. *Citation*: Adom, F., Workman, C., Thoma, G., Shonnard, D., Carbon Footprint Analysis of Dairy Feed from a Mill in Michigan, U.S., International Dairy Journal (2012), doi: 10.1016/j.idairyj.2012.09.008.

study makes a contribution in understanding the GHG emissions of dairy feed mills and identifies major mill inputs contribution to the carbon footprint.

The American Feed Industry Association [AFIA], which represents the U.S. animal feed industry, is a trade association which estimates that approximately 3,000 feed mills exist in the U.S. and these mills produced between 107, 000 to 112,000 million kg of animal feed over the last ten years (Balal et al. 2008). The feed mill sector is a very important part of the agricultural industry for the U.S. from an economic perspective because the sector directly employs about 110,000 individuals and contributes approximately $35 billion from feed sales towards the U.S. economy annually (International Feed Federation Industry, 2009). The mandatory reporting of GHG emissions proposed by the U.S. Environmental Protection Agency (USEPA) requires industrial facilities emitting more than 25 million kg of CO_2 equivalents each year to report to the USEPA. This study calculates the magnitude of GHG emissions expected from a dairy feed mill, whose facilities have yet to be subject to such analysis in the U.S. Specific study goals are:

- Develop an LCA methodology applicable to the animal feed mill industry to accommodate a large number of inputs and activities associated with dairy mill operations, and
- Gain an understanding of the relative importance of milled dairy feed inputs and activities on the GHG emissions of the outputs of the mill (which are themselves inputs to dairy milk production) through the application of these developed methodologies.

7.2 Materials and methods

7.2.1 Goal and scope definition

This is an analysis of a single dairy feed mill including transport of milled dairy feed to various dairy farms in Michigan. The scope of this carbon footprint analysis did not include biogenic carbon removals and emissions, emissions from employee travel to

or from the mill, the impacts of manufacturing the mill itself, and other passenger vehicles used on the milling premises.

- **Goal.** Estimation of GHGs emitted from feed mill operations on the basis of one kilogram of dairy feed output from the mill (kg CO_2-eq kg^{-1} of milled dairy feed), including delivery to local dairy farms.
- **Scope.** The scope specifically included GHG emissions only (see Figure 7-1). The study authors acknowledge that different formulations for dairy feed are possible depending on animal age and other factors. Indeed, the mill under study produces custom formulation of dairy feeds for specific customers. However, this analysis was meant to determine the impacts of producing dairy feed averaged over a typical year, by extrapolating the data provided over an annual cycle.

7.2.2 Audience

This study was a subsystem of a larger study undertaken for the U.S. dairy industry sector, yet the results are relevant to animal feed mill industry sector, the general public and federal government agencies responsible for the regulation of emissions from industrial operations.

7.2.3 Functional unit

The functional unit was 1 kg of milled dairy feed at its exit moisture content (an average feed formulation for dairy animal nutrition at this mill).

7.2.4 System boundaries

System boundaries included production and transport of feed inputs (grain crops, processed feed components, nutrients and other additives, and energy use) to the mill, for milling of the feed ingredients, to the delivery of milled feed to dairy farms. Figure 7-1 shows a schematic diagram (red line indicates the system boundaries) for the stages considered in this analysis. The green ellipses represent the various inputs at each stage while the red rounded squares represent corresponding emissions.

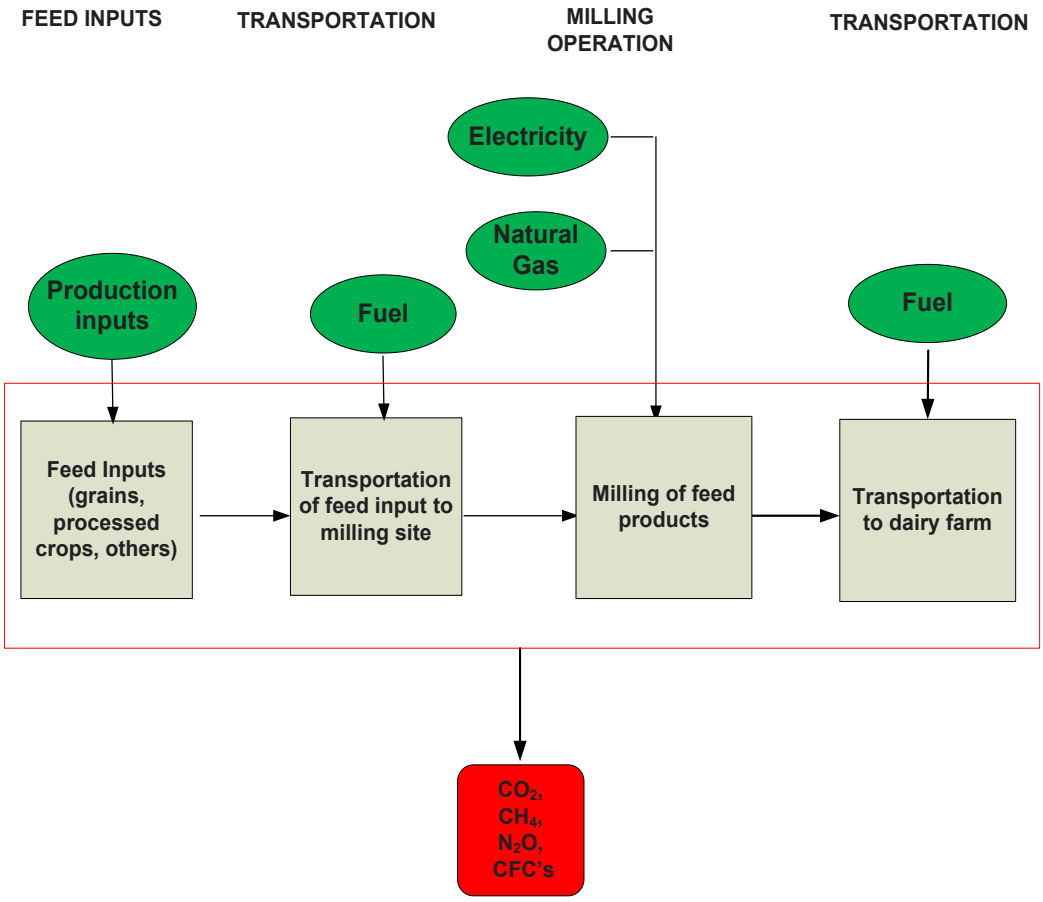


Figure 7-1 Schematic diagram of various stages for dairy feed mill carbon footprint analysis

To the extent possible, ecoinvent™ unit processes (PRé Consultants., 2009) have been used. The ecoinvent^{TM} data are mostly based on European conditions, whereas the geographic context of our study was the U.S. This situation introduced a geographic-relevance conflict; however, technology relevance is still strong because both E.U. and U.S. manufacturers use modern production technology. For major crop and agricultural by-product inputs to this study, we have developed inventories based on our own research using U.S. data sources. There were many inputs for which unit processes were modeled using Open input-output (IO) data (Sustainability Consortium, 2011) and also some data were obtained from peer reviewed journal articles. Differences in system boundaries, particularly between input-output and process-based models will result in inconsistent system boundaries. This is because

Open IO models in essence have no specific boundary cut-off criteria. However, in this study, a relatively small fraction of the mass of feed inputs to the mill has been modeled with the IO approach. The specific items for which IO data have been used are restricted to nutritional supplements for feed ingredients in category 3. Section 7.2.9.1 provides more details on the different categories of feed ingredients.

7.2.5 Geographical boundaries

This mill, located in the lower peninsula of Michigan, is the geographical context for this carbon footprint study. It is a modern milling site with the bulk of its milled animal feed being dairy feed. Results from this mill carbon footprint analysis may not be representative of other dairy feed mills in the U.S. However, in an attempt to model mills from other locations in the U.S., sensitivity analyses in section 7-4 of this article model GHG emissions of milled dairy feeds with a predominance of dry distillers grains and solubles (DDGS), soybean meal, and oats, respectively in separate scenarios.

7.2.6 Allocation procedures

The ISO guidelines were followed for co-product allocation in this carbon footprint study. Specifically, ISO standards 14040:14044 (ISO, 2006 a, b) and Sinden et al, (2008) recommend the avoidance of allocation by using system expansion. However, system expansion was not possible in our study given that LCA results are not currently available to credit the non-dairy feed products from this mill. Apart from this, it has been stated in section 7.2.2 that this study was a subsystem of a larger study (Thoma et al., 2012). In the overall study, economic and mass allocations were used, and hence to be consistent we used both of these allocation approaches. An economic allocation factor of 0.90 was used for milled dairy feed based on consultation with the mill manager who indicated that 90% of total mill revenue generated was attributable to the sale of dairy feed output. A mass allocation factor of 0.88 was used based on the fact that 88% of the mill outputs were dairy feed while the remaining outputs were non-dairy feed products.

7.2.7 Collection of input data

Data collection efforts have been a combination of a survey instrument developed for the mill manager, internet searches (e.g., ISI, Google scholar, ProQuest, etc), peer-reviewed journal articles, a mill site visit, and direct communication with the feed mill manager. Inputs such as types of feed, mass of each feed ingredient, transportation distances, as well as unit and total cost of feed ingredients were all obtained from the purchase history documents of the milling facility, provided by the mill manager. The next sections show how input data were collected and organized as well as some sensitivity analyses considered in this study.

7.2.8 Developing a data collection spreadsheet (Survey)

The life cycle inventory stage of this project required gathering input and output data for the milling operation. A survey instrument was created and used to collect data from the mill facility (see Appendix E). This survey instrument can broadly be categorized into three major sections. Questions in Table E-1 sought information on the various types of fuel used in the milling operations, types of feed produced aside from dairy feed, and the annual energy consumption for the milling processes. The main objective in Table E-2 of the survey instrument was to determine the kind and amount of feed that go into producing starter, lactating and dry feed for dairy cattle. In the transportation section, Table E-3, questions specifically targeted the transportation of feed inputs to the milling site, including modes of transportation, the kind of road vehicles used, and distances covered in transporting feed ingredients to the milling site. The data obtained was collected between March 1 and June 30, 2009. The feed mill manager confirmed that this dataset was representative of annual production.

7.2.9 Organization of input data for carbon footprint analysis

As identified in Figure 7-1, input data from this mill facility were organized for this carbon footprint analysis into feed ingredients, transport of feed ingredients to milling site, mill electricity and natural gas use, and milled product transportation.

7.2.9.1 Categories of feed ingredients and sources of inventory data

The feed ingredients were organized into three categories based on i. specific functions, ii. source of emission factors, and iii. environmental impact modeling approach. The total 4 month input of feed ingredients to the mill was approximately 9,683,000 kg, and this was increased to an annual input (three-fold increase) in consultation with the feed mill manager. The mill manager confirmed that inputs equal to mill feed outputs. The first category of mill inputs was the majority of feed ingredients on a mass-input basis (Category 1). Inventory data for these ingredients were obtained primarily from unit processes in the ecoinvent™ database and also from the study by Adom et al., (2012). This first feed category was comprised mainly of soybean co-products, DDGS, and other high-mass inputs. Table 7-1 shows the individual feed components, their overall percentage contributions towards the feed mill inputs, and organizes these components into major feed types for which inventory data were available. Reported feed types in both tables 7-1 & 7-2 were obtained from the purchase history document obtained from the feed mill manager. The percentage composition of the individual components making up the total 4 month input were estimated by dividing their individual masses (kg) of feed types by the total (9,683,000 kg)**.** For this particular feed mill, soybean meal-type feed alone accounted for approximately 59% of the mill inputs while DDGS contributed close to 17%. Category 1 of the feed ingredients contributed about 84% of the mill's total feed input by mass.

Miller, Ramsey, & Madsen (1988) and Siciliano-Jones, Socha, Tomlinson, & DeFrain (2008) established that trace minerals such as Zn, Mn, Cu, and Co plays a very important role in overall health of dairy animals. For example, these trace minerals help in protein synthesis, vitamin metabolism, formation of connective tissue, and immune function in animals.

Table 7-1 Major feed inputs on a 4-month basis: soybean, dried distiller grain and other co-products (Category 1)

FEED TYPE	FEED INPUTS (T = TRUCK, R = RAIL)	UNITS PURCHASED (1000 kg)	PERCENTAGE
COTTONSEED	Fuzzy Cottonseed (T)	124	1.28%
DRIED DISTILLER GRAIN (DDG)	Corn Gluten Feed Bulk (T)	578	5.97%
	Distillers Bulk (T)	843	8.70%
	Corn Gluten Direct (T)	127	1.31%
	Direct Distillers (T)	89	0.91%
SOY MEAL	Canola Meal (T)	304	3.14%
	Heifer Concentrate 35% (T)	6	0.07%
	Heifers Edge Direct (T)	27	0.28%
	Soybean Meal 48% Direct (T)	83	0.86%
	Chief Beef Finisher 36 (T)	25	0.26%
	Dairy Beef Finisher (T)	3	0.03%
	Bran Meal 50# (T)	0.05	0.0005%
	Bulk 48% Soy 50# (T)	1,915	19.78%
	Heifers Edge Bulk (T)	46	0.48%
	Soy Chlor 16 50# (T)	11	0.11%
	Soy Plus Bulk 50# (R)	3,271	33.78%
	Vita Soy Bulk (T)	6	0.06%
SUGAR	Dairy Sugar 38(T)	53	0.54%
	Dairy Sugar 38(T)	8	0.08%
SOY HULLS	Direct Soy Hulls (T)	22	0.23%
	Direct Soy Plus (T)	21	0.22%
	Soy Hulls Bulk (T)	189	1.95%
ANIMAL MEAL	Blood Meal 50# (T)	0.005	0.0005%
	Fish Meal 50# (T)	4	0.04%
	Pork and Bone Meal Bulk (T)	108	1.12%
FAT	A/V Blend Fat Bulk (T)	94	0.97%
	Choice White Grease Bulk (T)	79	0.82%
	Energy Booster 100 50# Bag (T)	30	0.31%
	Megalac 50# (T)	2	0.02%
MOLASSES	Dry Molasses 50# (T)	7	0.07%
	Liquid Molasses-Bulk (T)	29	0.30%
	Molasses Tub-16% (T)	1	0.01%
	Molasses Tub-25% (T)	1	0.01%
	Direct Molasses (T)	5	0.06%
OATS	Rolled Oats 50# (T)	2	0.02%
UREA	Feed Urea Bag 50# (T)	41	0.43%
WHEY	Dried Whey 50# (T)	2	0.02%
		8,158	84%

The second category of feed ingredients (see Table 7-2, Category 2) was comprised of mineral ingredients and other feed components, contributing approximately 12% by mass to the feed mill inputs. These are highly-processed feed ingredients. For

example, dairy base mix (Hubbard Feeds, 2007) provides calcium, phosphorous, magnesium, and other trace minerals.

Table 7-2 Feed inputs on a 4-month basis: minerals and others (Category 2)

FEED TYPE	FEED INPUTS (T = TRUCK, R = RAIL)	UNITS PURCHASED (1000 kg)	PERCENTAGE
GYPSUM	Cal Sulfate Bag 50# (T)	27	0.276%
LIME	Hydrated Lime 50# BAG (T)	2	0.019%
LIMESTONE	Cal Carb Bulk (T)	281	2.903%
	Cal Carb 50# (T)	13	0.131%
	Dical Bag 50# (T)	1	0.009%
MAGNESIUM OXIDE (MgO)	Mag Oxide Bag 50# (T)	19	0.197%
MAGNESIUM SULFATE ($MgSO_4$)	Mag Sulfate 50# Bag (T)	4	0.044%
OTHER TRACE MINERALS	24-12 Mineral 50# (T)	2	0.023%
	Copper Sulfate –Fine 50# (T)	2	0.019%
	Copper Sulfate –Cryb 50# (T)	0.3	0.004%
	Dairy Base Mix Bulk (T)	85	0.879%
	DCAD Plus-Potasm Carb 50# (T)	10	0.103%
	Dical/Monocal Bulk (T)	49	0.505%
	Iodine 50 50# (T)	0.05	0.001%
	Manganese Sulfate 50# (T)	2	0.019%
	Propnos Mineral W/Altosiu (T)	0.005	0.002%
	Minerals Mixture	38	0.392%
SALT (NaCl)	Mixing Salt Bag (T)	14	0.149%
	Tm Blocks W/Sel (T)	4	0.041%
	Tm Salt Bag (T)	10	0.103%
	Mixing Salt Bulk (T)	136	1.405%
	White Salt Blocks (T)	2	0.026%
	White Salt 50# (T)	2	0.023%
	TM Blocks (T)	7	0.072%
SODA POWDER	Bicarb Bulk (R)	445	4.596%
	Bicarb-Bag (T)	9	0.090%
		1,165	12%

The largest input to Category 2 ingredients was soda powder, contributing approximately 5% towards total feed mass. In addition to serving as a source of sodium, soda powder also offers buffering qualities that help stabilize rumen pH by reducing acid conditions. Finally, feed input labeled mineral mixture contributed less than 0.5% towards the feed milling input by weight even though it was comprised of

41 different ingredients (Table E-4). These ingredients contain varying concentrations of trace minerals such as selenium, copper, zinc, among others, which were grouped and referred to as minerals mixture. Inventory data for Category 2 dairy feed inputs were obtained from ecoinvent™.

The third category for the feed mill inputs (Category 3) was comprised of 66 different components with much smaller amounts on a weight basis (see Table E-5). This category mainly included highly-processed ingredients like vitamins and amino acids such as lysine 98.5%, methionine, aureomycin 50, among others. This category however contributed approximately 4% towards the mill inputs by mass. Inventory data for Category 3 dairy feed inputs were obtained from the Open IO database because the ecoprofiles for them were not available in ecoinvent™ or any other literature sources.

Open IO is a comprehensive analytical database developed and created by staff of the Applied Sustainability Center at the Walton College of Business, University of Arkansas for the Sustainability Consortium (2011). In analyzing feed inputs in Category 3, the economic sector most closely related to these mill input ingredients was identified as "other food manufacturing" (sector-311119) and was used to complete the inventory. This sector ecoprofile was imported into SimaPro and modified to remove the contribution of Category 1 and 2 inputs, and the outputs re-normalized so that the relative contribution of all other sectors would be proportionally increased.

7.2.9.2 Onsite energy

For the energy analysis in this study, two major inputs were identified using data obtained from the mill operation survey: electricity and natural gas. The total electricity used (kWh) for three electricity meters was obtained for an eleven month period (see Table 7-3). Electricity consumption averaged over the eleven month period was used as an estimate for the twelfth month to obtain the total annual electricity used.

Table 7-3 Summary of electricity inventory data for milling site from 2008-2009

Electricity (11 month.)	
Meter #	kWh
10988145	21,940
7838695	42,514
83157581	38,270

In the case of natural gas, annual average for natural gas used at the site for 2007 and 2008 were used in the calculations. Data for natural gas inputs are presented in Table 7-4.

Table 7-4 Summary of natural gas inventory data for milling site from 2008-2009 Note: The ecoinvent profile used for natural gas is: Heat, natural gas, at boiler modulating <100kW/RER S. The emission factor for electricity assuming Michigan grid was modified according to the study by Deru & Torcellini, 2007

Natural Gas (1 year)	
Year	Cubic meter (m^3)
2008	125,826
2009	180,401
Total (Average)	153,115

7.2.9.3 Transportation

The goal for the transportation analysis was to model the GHG emissions of transportation of feed ingredients to the mill site as well as the milled products to the various local dairy farms. For this section of the analysis, the site manager provided the required data inputs for assessing both steps. Appendix E shows transportation data of all the feed ingredients input to the mill facility. These data included the miles traveled, amount transported, and transportation mode. Tables E-6, E-7 and E-8 show the transportation inputs in terms of miles travelled for feed ingredients in categories 1, 2 and 3, respectively. Using this information, ecoinvent™ ecoprofiles most closely

matching transport mode were used. A 16,257-32,514 kg European road transport ecoprofile and a U.S. freight train ecoprofile were selected from the ecoinvent™ database. The freight train emission factor used was 3.8 x 10^{-5} kg CO_2-eq (kg km)$^{-1}$, and multiplying this by the corresponding payload–distance (kg km) values for each ingredient, the total GHG emissions for each ingredient transported were estimated. Using a similar approach for a 16,257-32,514 kg capacity road transport, with emission factor of 1.7 x 10^{-4} kg CO_2-eq (kg km)$^{-1}$, the GHG results were estimated for road transport of feed ingredients.

Inputs for the transportation of milled dairy feed products using the mill fleet of trucks to local dairy farms were provided by the mill manager in terms of the diesel use. These transport inputs are summarized in Table 7-5. Data covered the period January 2007 to August 2009; however, the average amount of diesel used for transportation in 2007 and 2008 was used in this analysis due to the incomplete data reported in 2009. Using diesel density of 840 kg m^{-3} and heating value of 42.8 MJ kg^{-1} of diesel (Edwards et al, 2006), the total mass (kg) as well as the total amount of energy (MJ) were estimated. Inventories of GHG emissions for production and combustion of diesel were obtained using the ecoinvent™ profile "diesel, burned in diesel-electric generating set/GLO S" (90 gCO_2 MJ^{-1}), which closely approximates diesel emissions from use in trucks.

Table 7-5 Summary fuel usage input data (average for 2007 and 2008) for road transport of milled feed product from mill to Michigan dairy farm

Transportation Fuel Usage Input From Dairy Feed Mill to Dairy Farms			
Date	Diesel (m^3)	Total Mass (kg)	Total Amount of Energy (Mega Joule- MJ)
1/1/2009-8/31/2009	58.94	49,512	2,119,123
1/1/2008-12/31/2008	145.11	121,903	5,217,443
1/1/2007-12/31/2007	135.28	113,648	4,864,142
Average (2007-2008)	140.19	117,776	5,040,792

7.3 Life cycle impact assessment

The IPCC GWP 100a method in SimaPro 7.3 was used to convert GHG inventory data into equivalent emissions of CO_2. This method uses global warming potentials [GWPs] of 1 for CO_2, 25 for CH_4, and 298 for N_2O. In addition to these three greenhouse gases, the analysis included emissions of refrigerants and of other chemicals with high GWPs that were included in the inventory data from ecoinvent™ and the open IO model.

7.3.1 Emission factors for GHG analysis

Table 7-6 summarizes the GHG emission factors used in this mill analysis. The majority of GHG emission factors for inputs to the feed mill were obtained using ecoprofiles™ in the ecoinvent database or were generated from original crop inputs from another study (Adom et al., 2012). In the case of sugar and animal meal, emission factors for these inputs were obtained from LCA Food Database (Nielsen, Weidema, Dalgaard & Halberg., 2003). Also, the emission factor for "other trace minerals" was a unit process comprising of all the commonly used minerals in feed input category 2. Emission factors used for electricity and natural gas from the ecoinvent™ database were 0.82 kg CO_2-eq kWh^{-1} assuming a Michigan grid mix and 0.075 kg CO_2-eq MJ^{-1} of natural gas.

Table 7-6 Emission factors and mill greenhouse gas analysis
(MA: Mass allocation and EA: Economic Allocation)

	Emission Factors ($kgCO_2eq$ / kg feed input)		
Category 1	MA	EA	Source
Cottonseed	1.27	0.39	PRé Consultants (2009)- Ecoinvent database (Cotton seed, at regional storehouse/US U)
DDGS (Dry mill)	2.30	0.91	Adom et al., (2012)
DDGS (Wet mill)	2.21	0.67	Adom et al., (2012)
Soy meal	0.54	0.41	Adom et al., (2012)
Sugar (Cotton seed, at regional storehouse/US U UA Dairy)	0.51	0.51	Nielsen, Weidema , Dalgaard & Halberg (2003)

Soy hulls	0.50	0.41	Thoma et al, (2010)
Animal meal	0.07	0.07	Nielsen, Weidema , Dalgaard & Halberg (2003)
Fat (Tallow, at plant/CH U)	0.66	0.66	PRé Consultants (2009)-Ecoinvent database
Molasses	0.11	0.11	PRé Consultants (2009)-Ecoinvent database
Oats	0.58	0.58	Adom et al., (2012)
Urea (Urea, as N, at regional storehouse/RER U)	3.30	3.30	PRé Consultants (2009)-Ecoinvent database
Category 2	MA	EA	Source
Gypsum (Gypsum, mineral, at mine/CH U)	0.002	0.002	PRé Consultants (2009)-EcoInvent database
Lime (Lime, hydrated, loose, at plant/CH U)	0.75	0.75	
Limestone (Limestone, milled, loose, at plant/CH U)	0.013	0.013	
Magnesium Oxide (Magnesium oxide, at plant/RER U)	1.05	1.05	
Magnesium sulfate (Magnesium sulphate, at plant/RER U)	0.30	0.30	
Other Trace Minerals (Minerals mixture, at factory/US U)	1.59	1.59	
Sodium Chloride (Sodium chloride, powder, at plant/RER U)	0.18	0.18	
Soda powder (Soda, powder, at plant/RER U)	0.44	0.44	
Category 3	MA	EA	Source
Supplements	1.07	1.07	Open IO database

7.4 Sensitivity analyses

Sensitivity analyses were performed to compare three major scenarios to the base case study (the MI mill inputs). In the base case, soybean meal dominated the ingredients on a mass-input basis by contributing 59% (wt.), while DDGS from dry corn mill facility contributed 17% (wt.). In Scenario 1, we investigated the feed mill's GHG impacts when using DDGS from a wet corn mill facility as oppose to a dry mill, without changing the mass input contributions of any other feed inputs. Scenarios 2 and 3 investigated the impact of input grain crop type by modifying the major crop inputs. To investigate a DDGS dominant case, DDGS from a dry corn mill and soybean meal were assumed to contribute 59% and 17%, respectively to the total feed input in scenario 2 (the inverse of the MI mill). In scenario 3, oats was assumed to contribute 42% and DDGS (from dry mill facility) and soybean meal were assumed to

each contribute 17% to the total feed input on a mass-input basis. These scenarios reflect the geographical preferences for the feed inputs. For example, DDGS is likely to be dominant over soybean and soybean meal in regions with high production of DDGS such as Iowa (scenario 2). Scenario 3 is more relevant for regions where oats is more prevalent in the local grain-crop supply, such as North and South Dakota. In section 7-6 of this manuscript, results obtained from the various scenarios investigated are presented

7.5 LCA results and discussion of base case

7.5.1 GHG impact of a dairy feed mill in Michigan, U.S.

In Figure 7-2, the GHG footprint contributions of various inputs and activities for the base case study are presented. The pie charts compare the effect of allocation choice on the resultant carbon footprint for the mill output. For both mass and economic allocation [Figure 7-2(A) and 7-2(B)], the majority of the GHG footprint of the dairy feed mill products were due to the input crops and other major ingredients to the mill (Category 1 inputs contributed approximately 84% of mill inputs by mass). Depending on allocation used, 73 to 82% of the total feed mill's GHG footprint was attributable to feed inputs in category 1. Category 1 impact was lower (73%) in the feed mill's GHG footprint when economic allocation was used. This was because the emission factors (Table 7-6) for co-products such as cottonseed, DDGS and soybean meal on economic allocation basis were smaller given the lower value of these co-products in the market compared to those estimated using a mass allocation.

The next largest category for GHG emissions were mineral ingredients (Category 2) which contributed approximately 6 to 9% to total mill carbon footprint (and 12% of total feed mass). The next largest category for GHG emissions were supplements (Category 3), which contributed 4 to 7% of the carbon footprint depending on allocation method (approximately 4% of total feed mass). Category 3 feed input GHG impact was estimated using Open IO data, and thus has a different system boundary than other inputs, as discussed in section 2.1.3.

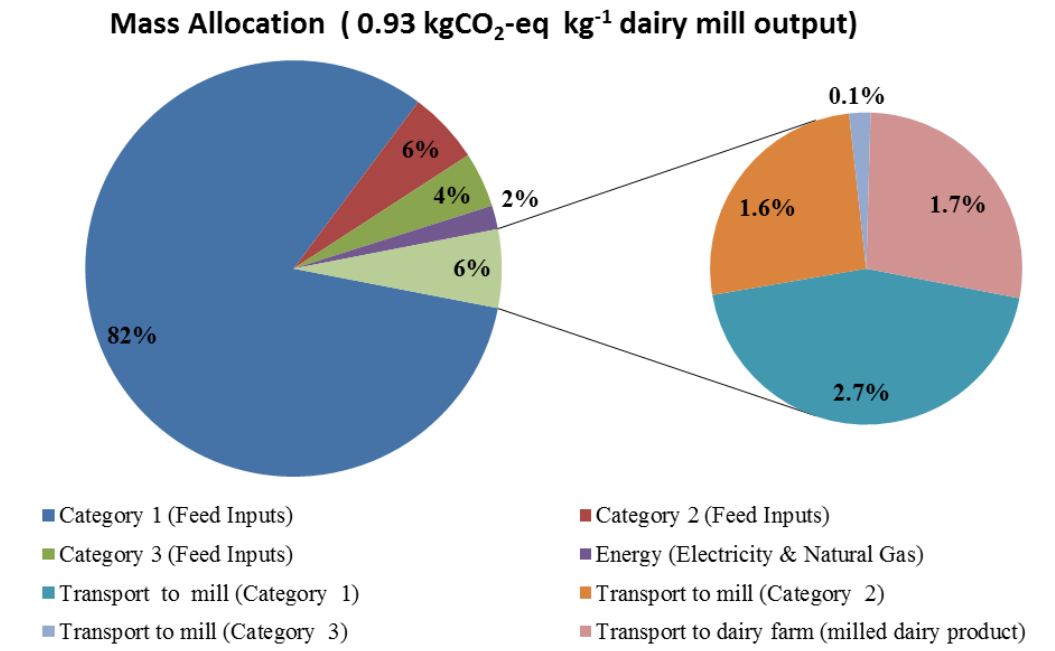


Figure 7-2 Relative contribution to GHG emissions of milled dairy feed (Base case analysis). Panel A

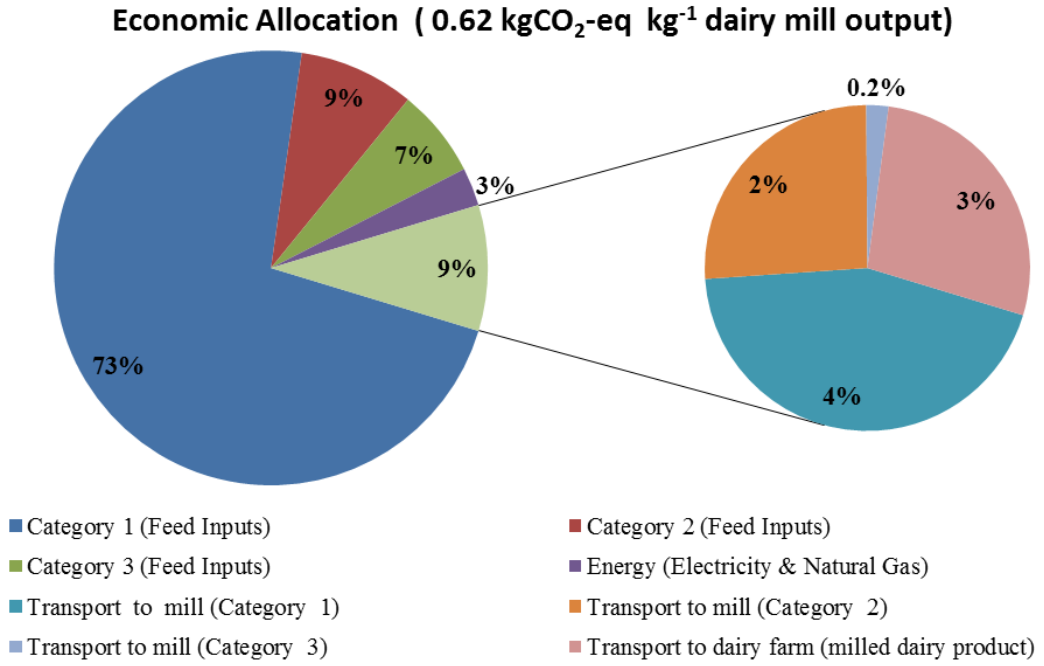


Figure 7-2 Relative contribution to GHG emissions of milled dairy feed (Base case analysis). Panel B

Nonetheless, this larger GHG intensity (per unit mass of Category 3 input) was expected given that many of these inputs (e.g., amino acids) were subjected to much more processing compared to the major crop inputs (e.g., oats, soybean meal, DDGS).

An analysis of all unit processes contributing to the feed mill showed that the economic IO data represents about 4% of the total mill carbon footprint, and thus system boundary inconsistencies do not have substantial influence on the final GHG results. On-site energy consumption at the mill contributed only about 2 to 3% (see Figure 7-2) to the total GHG emissions depending on allocation, and natural gas for crop drying accounted for 80% of this energy impact. All transportation, both raw material delivery and distribution of the feed to local MI dairy farms, contributed approximately 6 to 9% of the footprint depending on allocation method, as shown in Figure 7-2. Section 7.5.2 provides details of the transportation impacts.

7.5.2 Discussion of base case LCA results for annual emissions

Category 1 feed inputs contributed approximately 19 and 11 million kg CO_2-eq $year^{-1}$ for mass and economic allocation, respectively. This was due to high mass input rate and differences in emission factors based on economic and mass allocation as previously explained in section 7.3.1. Category 2 inputs contributed 1.3 and 1.4 million kg CO_2-eq year-1 for both allocation methods considered. Category 3 input contributions were approximately one million kg CO_2-eq yr^{-1} for both allocation methods considered. In the final analysis, the total GHG emission of all feed inputs of this milling site was estimated to be approximately 22 and 14 million kg CO_2-eq $year^{-1}$ for mass and economic allocation, respectively.

A total of approximately 1.4 and 1.5 million kg CO_2-eq $year^{-1}$ for mass and economic allocations, respectively, was the estimated GHG emissions due to fuel inputs associated with transportation. This accounted for GHG burdens due to transport of all feed ingredients to the milling site as well as the transportation of the processed dairy feed to various dairy farms. Figure 7-2 provides more details on the transportation

impact. GHG burdens due to the transportation of feed ingredients to the milling site were about three times more than the impact due to the transport of milled dairy output to the various dairy farms. Transportation impact of feed ingredients (all feed categories) was estimated to be about one million kg CO_2-eq $year^{-1}$ whereas transportation to various dairy farms was estimated to be 400,000 kg CO_2-eq $year^{-1}$. The reason for this difference is that this milling site serves mainly the local market and is located at a distance close to customers whereas purchased mill inputs are transported much further.

Annual GHG emissions as a result of onsite energy use at this mill facility were approximately 450,000 kg CO_2-eq $year^{-1}$ (economic allocation). Natural gas was the largest contributor, accounting for 80% of this annual total, with electricity consumption accounting for the remaining 20%. Natural gas is used in drying corn grain, which arrives at the milling site with relatively high moisture content that is typical of a northern U.S. mill location. Mills in southern locations of the US generally receive corn that is of lower moisture content and hence tend to use much less energy in drying (based on communication with a mill manager).

Cradle-to-dairy farm GHG annual emissions were approximately 16 and 24 million kg CO_2-eq yr^{-1} for the milled dairy feed product system including all inputs and transport activities using economic and mass allocations, respectively. When restricting the mill inputs to those directly consumed in mill operations, such as electricity, natural gas, and diesel fuel for transport of feed to dairy farms, annual milled dairy feed-related GHG emissions were much lower (860,000 kg CO_2-eq yr^{-1} using economic allocation). Total annual emissions from the MI feed mill, including dairy and non-dairy products are 860,000 / 0.90 = 950,000 kg CO_2-eq yr^{-1}, where 0.90 is the economic allocation factor for this mill.

7.6 Discussion of results from sensitivity analyses

As described in section 7-4, sensitivity analyses were conducted to investigate three major scenarios for comparison with the base case GHG analysis. Figure 7-3 summarizes the GHG results estimated for all the scenarios considered. Figures E-2 through E-4 present GHG profile pie charts of the scenario results based on mass and economic allocations. In scenario 1, use of DDGS from a wet mill facility reduces the overall footprint of this mill by just 2 to 6% depending on allocation method (see Figure E-2). This is because the differences in emission factor values for DDGS from a wet mill relative to those from a dry mill were minor, especially for mass allocation (see Table 7-6).

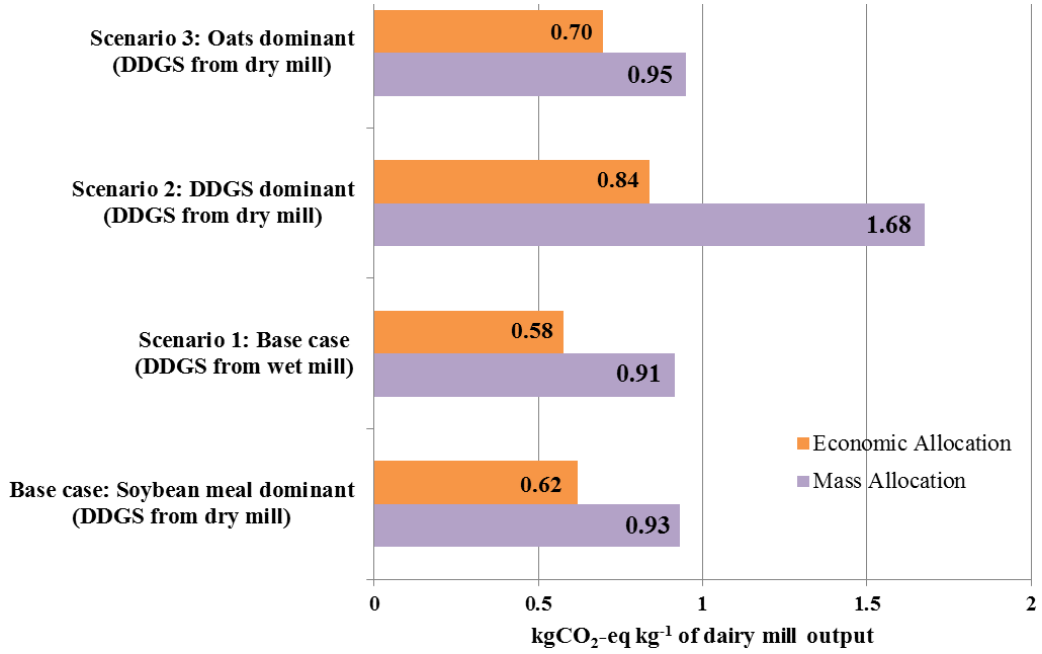


Figure 7-3 Sensitivity analysis of feed inputs to dairy feed mill greenhouse gas profile

Scenario 2, in which DDGS (from the dry mill facility) was considered to be dominant, resulted in a substantial increase in the mill GHG emission (1.70 $kgCO_2$-eq. kg^{-1} dairy mill output based on mass allocation) which was about two times that of the base case using a mass allocation (see Figure E-3). The feed mill GHG burdens increased by approximately 35%, from 0.62 to 0.84 kg CO_2-eq kg^{-1} dairy mill output, based on

economic allocation. This was due to the relatively high emission factors for DDGS as opposed to soybean meal (See Table 7-6).

In scenario 3 (oats dominant), the GHG profiles for this mill were calculated to be 0.69 and 0.95 kg CO_2-eq. kg^{-1} dairy mill output for both economic and mass allocation, respectively (see Figure E-4). This resulted in a small increase relative to the base case of between 2 and 11% in the feed mill's GHG profile, depending on allocation method. This was not surprising given that the emission factor for oats reported in Table 6 is comparable to the base case in which soybean meal is the dominant feed ingredient.

These scenario analyses demonstrate that geographic differences in dairy feed mill GHG impacts can be substantial, especially for mill locations that predominantly process GHG-intense ingredients such as DDGS.

7.7 Conclusions & recommendations

The goals of this carbon footprint study were to i. develop an LCA methodology applicable to the animal feed mill industry to accommodate a large number of inputs and activities associated with dairy mill operations, and ii. gain an understanding of the relative importance of milled dairy feed inputs and activities on the GHG emissions of the outputs of the mill (which are themselves inputs to dairy milk production) through the application of these developed methodologies. Our methods were able to accommodate a very large number of system inputs using a variety of inventory data sources, including existing databases, new LCA results for U.S. crops and agricultural co-products, and industry sector IO data on highly processed ingredients for which no ecoprofiles currently exist.

GHG emission values of 0.62 and 0.93 kg CO_2-eq kg^{-1} milled dairy feed were calculated based on economic and mass allocations, respectively. Overall, the highest contributors to the mill feed carbon footprint were agricultural co-product feed inputs (e.g., DDGS, soybean meal), contributing between 88 to 92% of the carbon footprint

depending on the allocation method (see Figure 7-2). Mill energy use and transportation of mill inputs and of mill products together contributed 8 to 12%. In the final analysis, this mill facility emits approximately 16 to 24 million kg CO_2-eq $year^{-1}$ (depending on allocation method) assuming the study system boundary of cradle-to-dairy farm gate. Annual GHG emissions directly attributable to dairy and non-dairy feed mill activities, including on-site electricity use, process heat demands, and road transport of mill feed to local farms, totals 950,000 kg CO_2-eq yr^{-1}. It is very clear from scenarios 2 and 3 that the type of feed crop greatly affects the feed mill GHG emissions. Crop inputs are likely to vary from U.S. region depending on local supply of feed crops.

This study is of a single dairy feed mill, and therefore further study is required to investigate location-specific differences in dairy feed mill inputs and resulting effects these differences have on GHG emissions for the mill feed products. Mill site energy consumption and transportation fuel emissions are under the control of mill operators. Suggested measures to reduce dairy feed mill GHG emissions will center on the use of cleaner sources of electricity and low carbon fuels, such as biodiesel and renewable hydrocarbon diesel from biomass. It is also recommended that further studies be conducted to increase the mill sample size and to include several facilities from southern U.S. locations. Finally, given the large number of ingredients to the mill, we also recommend further studies of other highly processed supplements to help improve the accuracy of estimating the GHG burdens of milled dairy feeds.

7.8 References

F. Adom, A. Maes, C. Workman, Z. Clayton-Nierderman, G. Thoma, D. Shonnard. Regional carbon footprint analysis of dairy feeds for milk production in the USA. The International Journal of Life Cycle Assessment, 17 (5) (2012), pp. 520–534 http://dx.doi.org/10.1007/s11367-012-0386-y

Batal, A., Dale, N., Waller, J., Pettigrew, J. E., Hollis, G. R., Baker Hagler Jr., W. M. (2008). Feedstuffs 2009: Reference issue & buyers guide. Minnetonka, MN,

USA: Sarah Muirhead. URL http://fdsmagissues.feedstuffs.com/fds/Reference_issue_2010/Reference_issue_2009/Section1_2008.pdf

British Standards Institute (BSI). (2008). Specification for the assessment of the life cycle greenhouse gas emissions of goods and services (PAS 2050: 2008). BSI.

Deru, M. P., & Torcellini, P. A. (2007). Source energy and emission factors for energy use in buildings (NREL/TP-550-38617). Golden, CO, USA: National Renewable Energy Laboratory.

Edwards, R., Larive, J. F., Mahieu, V. & Rouveirolles, P. (2006). Well-to-wheels analysis of future automotive fuels and powertrains in the European context: Well-to-wheels report Version 2b [online report]. URL http://ies.jrc.ec.europa.eu.

Gerber, P., Vellinga, T., Opio, C., Henderson, B., & Steinfeld, H. (2010). Greenhouse gas emissions from the dairy sector, a life cycle assessment. Rome: FAO Food and Agricultural Organisation of the United Nations Animal Production and Health Division.

Google Scholar. (2009). Google Scholar [Online search engine]. URL http://scholar.google.com/

Hubbard Feeds. (2007). Hubbards Feeds [WWW page]. URL http://www.hubbardfeeds.com/Default.aspx

International Feed Industry Federation, 2009

International Feed Industry Federation. (2009). International Feed Industry Federation Annual Report 2009. Brussels, Belgium: International Feed Industry Federation.

ISI. (2009). Web of Knowledge [online search engine]. URL http://apps.webofknowledge.com

ISO. (2006a). 14040: 2006 Environmental management–life cycle assessment–principles and framework. Geneva, Switzerland: ISO.

ISO. (2006b). 14044: 2006 Environmental management–life cycle assessment–requirements and guidelines. Geneva, Switzerland: ISO.

Miller, J.K., Ramsey, N., & Madsen, F.C. (1988). The trace elements. In D.C. Church (Ed.), The ruminant animal-digestive physiology and nutrition (pp. 342–401). Englewood Cliffs, NJ, USA: Prentice-Hall.

Nielsen, P.H., Nielsen, N. A., Weidema, B.P., Dalgaard, R., & Halberg, N. (2003). LCA food database [Online database]. URL www.lcafood.dk

PRé Consultants. (2009). SimaPro 7.1 LCA software [Computer software]. URL http://www.pre.nl/

ProQuest. (2009). ProQuest [online search engine] URL http://www.proquest.com/en-US/

B. Shaw, P. Buharivala, C. Parnell Jr., M. Demny Emission factors for grain receiving and feed loading operations at feed millsTransactions of the ASAE, 41 (3) (1998), pp. 757–765

J. Siciliano-Jones, M. Socha, D. Tomlinson, J. DeFrain Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle Journal of Dairy Science, 91 (5) (2008), pp. 1985–1995

The Sustainability Consortium. (2011). Open-IO Version Beta 1.5 [Online database]. URL http://www.sustainabilityconsortium.org/open-io/

Thoma, G., Popp, J., Nutter, D., Shonnard, D., Ulrich, R., Matlock, M., Adom, F. (2010). Regional analysis of greenhouse gas emissions from milk production practices in the United States. Rosemont, IL, USA: Innovation Centre for US Dairy.http://www.usdairy.com/Public%20Communication%20Tools/USDairy_Sustainability_Report_12-2010%20(4).pdf

Thoma, G., Popp, J., Nutter, D., Shonnard, D., Ulrich, R., Matlock, M., Adom, F. (2012). Greenhouse gas emissions from milk production and consumption in the U.S.: A cradle to grave life cycle assessment. International Dairy Journal (2012).

Chapter 8

8 Summary, Conclusions, and Recommendations for Future Work

8.1 Summary and conclusions

Compositional analysis of defatted syrup from a corn ethanol dry mill as a feedstock for bio-based products: DCS which is a co-product of the dry-grind corn ethanol process was analyzed for its physical and chemical characteristics. With total solids of 37.4% wt., a mass balance closure on all components of DCS was 101%. Total carbohydrates (28% of dry wt.) comprised of starch components (6%), soluble carbohydrates (12%) & non-starch carbohydrates (10%). Structural and non-structural bound hemicellulose components included; xylan (6%), mannan (1%), arabinan (1%) and galatactan (3%). The ash content comprised of 12% wt. DM basis while protein, glycerol and amino acids were 8% wt., 33%, and 3% wt. on DM basis, respectively. Syrup has good potential as a renewable feedstock for bio-chemicals production through either fermentation or separation of various compounds directly from the syrup.

Optimization of the dilute acid and enzymatic pretreatment of defatted syrup from a corn ethanol dry mill: The sugar platform optimization using DCS investigated the use of different acid concentrations (0, 1 & 2%) and subsequent enzymatic hydrolysis over a range of hydrolysis reaction time. Dilute acid pretreatment and enzymatic hydrolysis were conducted at 121^{o}C and 50^{o}C respectively. In our choice of optimal condition, our goal was to identify the condition that maximizes yield of total monomer sugars within the shortest possible time as well as producing low concentrations of inhibitors. Avoidance of the application of enzyme will be ideal if at all possible given the significant portion of the costs associated with bio-based chemical production. From our analysis, we observed that contribution of cellulase enzymes to the TMS yield was not so significant. With high level of certainty, we determined that the first stage acid pretreatment for 60 minutes at 2% acid was efficient

in producing approximately 86% of the theoretically available carbohydrates with acceptable low inhibitory level.

Optimization of the protein hydrolysis scheme of defatted syrup from a corn ethanol dry mill facility: The protein optimization experiments of my Ph.D. research investigated the combined effect of hydrolysis reaction time, temperature, and ratio of enzyme to substrate ratio to develop hydrolysis process that optimizes the amount of usable amino acids available in DCS. Apart from hydrolysis pathway 4, experimental results show nearly quantitative recovery amino acids from the protein contained in DCS. Hydrolysis pathway 1, which is DAP alone at "optimum carbohydrate hydrolysis conditions (60 min, 2% acid)" yielded 82-68% of the theoretically available amino acids. Hydrolysis pathway 2, which is DAP of syrup followed by subsequent protease hydrolysis was also investigated using Trypsin, Pronase E (*streptomyces griseus*) and Protex 6L. Overall, reported yields ranged from 100-78% of the theoretically available amino acids (pH 6 & 7). For this pathway, Pronase E at pH 7 resulted in the highest yield of 10.7 mg/ml (100-89%) of total amino acids. Hydrolysis pathway 3 which was a standalone experiment using proteases Trypsin, Pronase E (*streptomyces griseus*) and Protex 6L on the unpretreated DCS reported yields ranging from 100-46% of the theoretically available amino acids. Protex at pH 7 yielded a total amino acid concentrations of 12.5 mg/ml (100% yield) which was the highest for pathway 3. Pathway 4 (simultaneous hydrolysis with cellulase and protex) generally reported the lowest yields for both amino acids and total monomer sugars. Total amino acid concentration for 1 and 2% (v/v) loaded enzymatic hydrolysis solutions ranged between 2-3 mg/ml representing only 18-27% of the theoretically available amino acids in DCS biomass.

Modeling of dilute acid pretreatment process using defatted corn syrup as feedstock: Techno-economic analysis & life cycle assessment: A preliminary cost analysis to estimate the initial capital cost and operating cost of this facility using Aspen Plus Economic Analyzer® and (iii) A greenhouse gas analysis to understand the

environmental impact of this facility. A conceptual process design has been constructed to produce the carbohydrate and amino acid rich stream. The initial capital cost was estimated to be $4,682,000 with substantial operational ($22,100,000) and raw material cost ($19,300,000) on an annual basis. This is mainly attributable to the high steam and 98wt sulfuric acid requirement. Finally, GHG emissions from this facility was estimated to be 114,000,000 kgCO_2e/yr (114,000 MT CO_2e/yr) with steam and ammonia contributing 72 and 24% while all other inputs contributed 4% or less.

Regional carbon footprint analysis of dairy feeds for milk production in the United States: The next objective of my Ph.D. research work is the LCA of dairy feeds in the U.S. The main goal was to estimate the GHG emissions from the cultivation and harvesting of dairy feeds on a basis of one dry kilogram of dairy feed harvested or produced (gCO_2e/kg of dry dairy feed). There were large differences in GHG emissions among the different dairy crops, with corn silage showing the lowest, while oats and DDGS displayed the highest. This variability was largely driven by fertilizer and energy utilization intensity. There was also some variability in carbon footprint for any crop from region to region, driven by regional differences in energy and lime use, but this variability was smaller than inter-crop variability.

The highest contributor to carbon footprint was the on-farm application of inorganic N fertilizer except for the leguminous feeds, whereas the fertilizer input categories P, K, and S accounted for relatively small impacts for all crops. About 65% of inorganic N fertilizer GHG emissions was due to N_2O release upon application, whereas 35% was from fertilizer manufacture. N_2O emission contribution from crop residues was also significant for most crops. With N fertilizer input being the largest contributor to GHG emissions, much effort should be targeted toward lowering emissions associated with their production and use on the farm. Additionally, the efficient transfer of knowledge to farmers with regards to fertilizer best management practices might help reduce emissions on the farm. The use of crop protection chemicals was not so significant

however, and energy use impacts varied widely from region to region, likely due to differences in climate, energy conservation programs, and need for crop drying.

This study highlights key crop inputs that are the drivers for emissions of greenhouse gases from the cradle-to-gate cultivation and harvesting for US dairy grain and forage crops. These crop results are equally applicable for uses other than dairy products; for example food production in general and bioenergy.

Carbon footprint analysis of dairy feed from a mill in Michigan, U.S: The final objective of my Ph.D. research work was GHG analysis of a dairy feed mill. The goals of this carbon footprint study were to i. develop an LCA methodology applicable to the animal feed mill industry to accommodate a large number of inputs and activities associated with dairy mill operations, and ii. gain an understanding of the relative importance of milled dairy feed inputs and activities on the GHG emissions of the outputs of the mill (which are themselves inputs to dairy milk production) through the application of these developed methodologies. Our methods were able to accommodate a very large number of system inputs using a variety of inventory data sources, including existing databases, new LCA results for U.S. crops and agricultural co-products, and industry sector IO data on highly processed ingredients for which no ecoprofiles currently exist.

GHG emission values of 0.62 and 0.93 kg CO_2-eq kg^{-1} milled dairy feed were calculated based on economic and mass allocations, respectively. Overall, the highest contributors to the mill feed carbon footprint were agricultural co-product feed inputs (e.g., DDGS, soybean meal), contributing between 88 to 92% of the carbon footprint depending on the allocation method. Mill energy use and transportation of mill inputs and of mill products together contributed 8 to 12%. In the final analysis, this mill facility emits approximately 16 to 24 million kg CO_2-eq $year^{-1}$ (depending on allocation method) assuming the study system boundary of cradle-to-dairy farm gate. Annual GHG emissions directly attributable to dairy and non-dairy feed mill activities,

including on-site electricity use, process heat demands, and road transport of mill feed to local farms, totals 950,000 kg CO_2-eq yr^{-1}. It is clear from the scenarios (2 and 3) investigated that the type of feed crop greatly affects the feed mill GHG emissions. Crop inputs are likely to vary from U.S. region depending on local supply of feed crops.

This study is of a single dairy feed mill, and therefore further study is required to investigate location-specific differences in dairy feed mill inputs and resulting effects these differences have on GHG emissions for the mill feed products. Mill site energy consumption and transportation fuel emissions are under the control of mill operators. Suggested measures to reduce dairy feed mill GHG emissions will center on the use of cleaner sources of electricity and low carbon fuels, such as biodiesel and renewable hydrocarbon diesel from biomass.

8.2 Recommendations for future work

All the optimization experiments (Chapters 3 & 4) focused on producing sugar and amino acid platform for subsequent production of value added products via fermentation. The glycerol glut on the marketed has stimulated research into using glycerol as a feedstock for bio-products. Future research work should investigate the potential of DCS in this regard given the significant amount of glycerol component. Specifically, the potential of using both the fermentable carbohydrates and glycerol component to simultaneous produce succinic acid is important and needs further investigation. Theoretically, they both can meet global demand for succinic acid (Chapter 2). Additionally, since different alternate pathways could result in quantitative recovery of amino acids, a techno-economic analysis taking into account these routes will be important to help understand the economic impacts of these hydrolysis routes.

The techno-economic analysis focused on just a previous determined optimum carbohydrate hydrolysis conditions (60 min, 2% acid). Future research work should investigate other hydrolysis pathways like dilute acid pretreatment followed by protein hydrolysis or just the standalone scenario where only proteases were recovered.

Current process design should be improved upon by investigating the effect of heat integration using heat exchangers heated hydrolysate streams from pretreatment reactor to preheat incoming DCS streams to the facility. This may help reduce cost of utilities. Finally, there is the need to investigate the potential combusting the unreacted residues as a source of heat and power generation to this facility.

The LCA on dairy feeds is a comprehensive GHG analysis of commonly used dairy feeds in the U.S. On the energy front, there is the need to promote the use of safe and cleaner forms of energy to help reduce climate active GHG emissions associated with the energy input needed by farmers. Also, there is the need to investigate other environmental impacts besides carbon footprint. For example, future studies should investigate impacts such as eutrophication, land use intensity, water use impact among others. Hopefully, results from the dairy feed LCA will be useful for reducing GHG emissions by guiding efforts to modifying agricultural practices with respect to fertilizer application, use of manure, and energy consumption.

For the mill GHG analysis, it is also recommended that further studies be conducted to increase the mill sample size and to include several facilities from southern U.S. locations. Finally, given the large number of ingredients to the mill, we also recommend further studies of other highly processed supplements to help improve the accuracy of estimating the GHG burdens of milled dairy feeds.

Appendix

Appendix A: Supplementary information for sugar platform optimization experiments

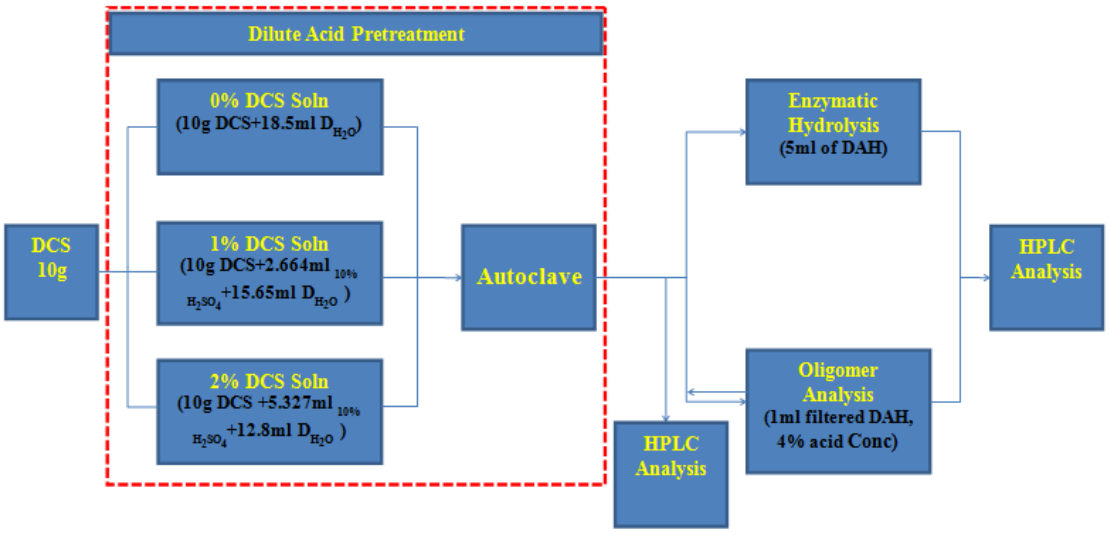


Figure A-1 Flow diagram of dilute acid hydrolysis and enzymatic saccharification of DCS

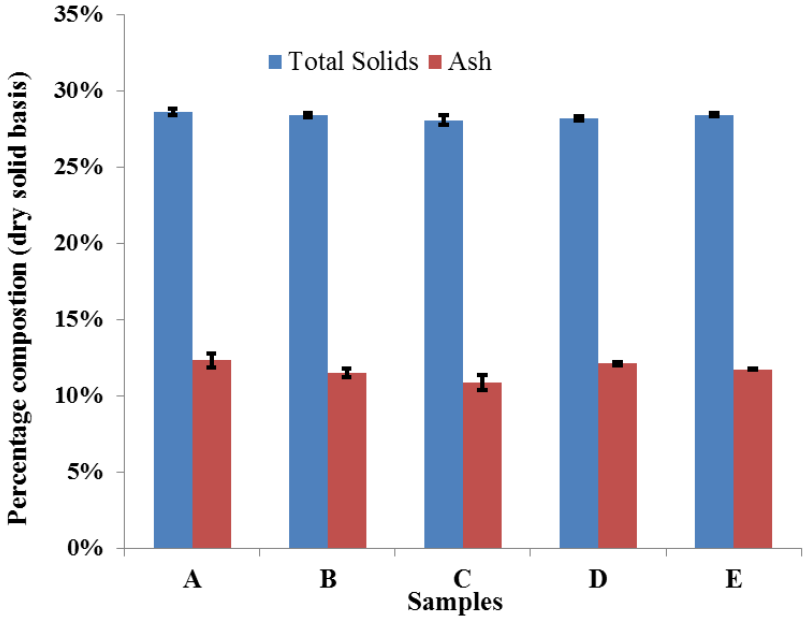


Figure A-2 Total Solids and Ash Content for DCS used for hydrolysis

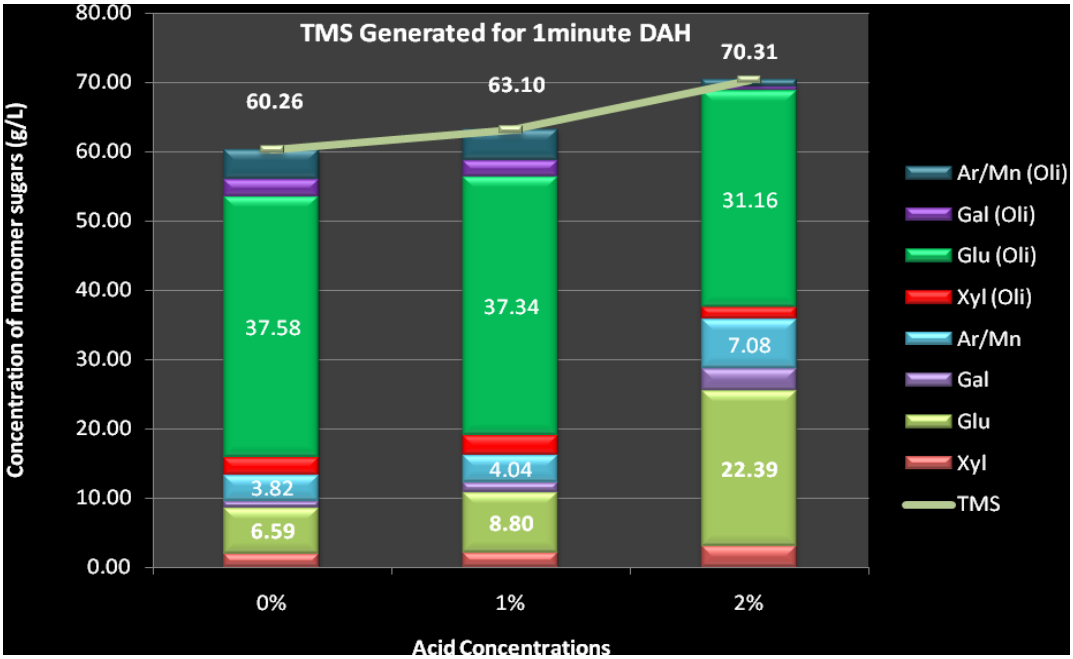


Figure A-3 TMS for 1-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

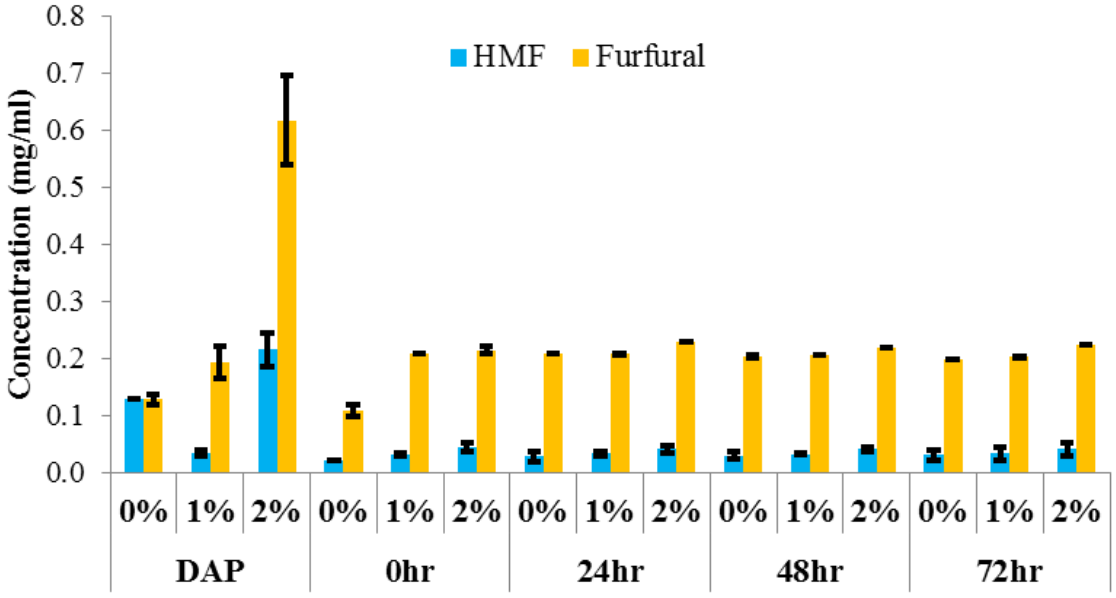


Figure A-4 Concentrations of inhibitors generated for 1 minute hydrolysis scheme

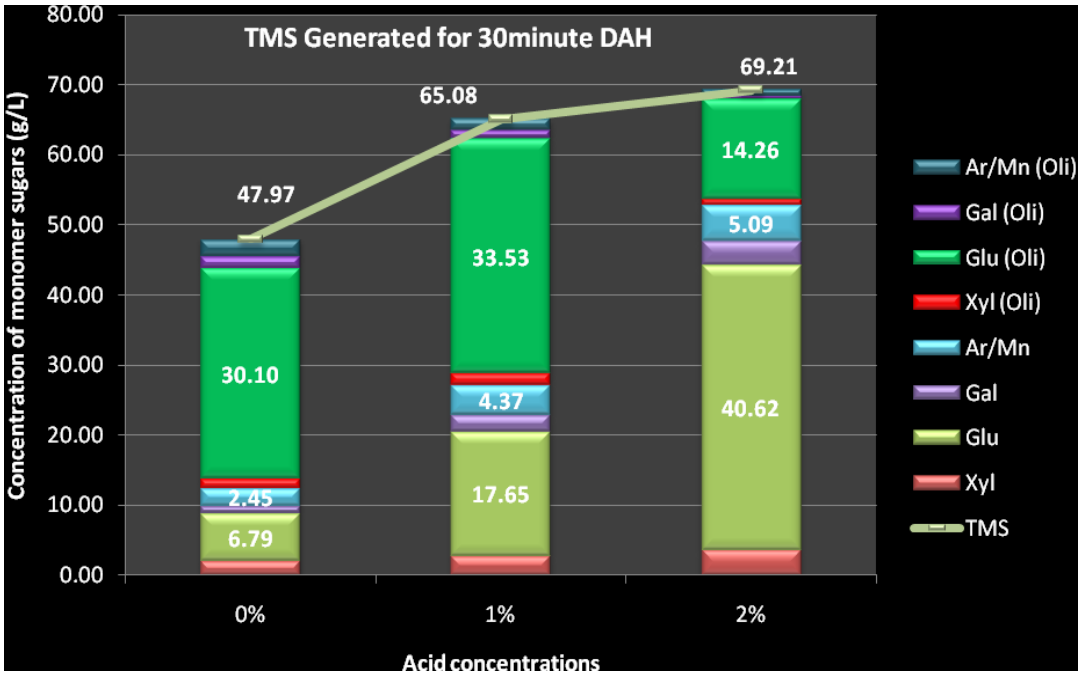


Figure A-5 TMS for 30-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

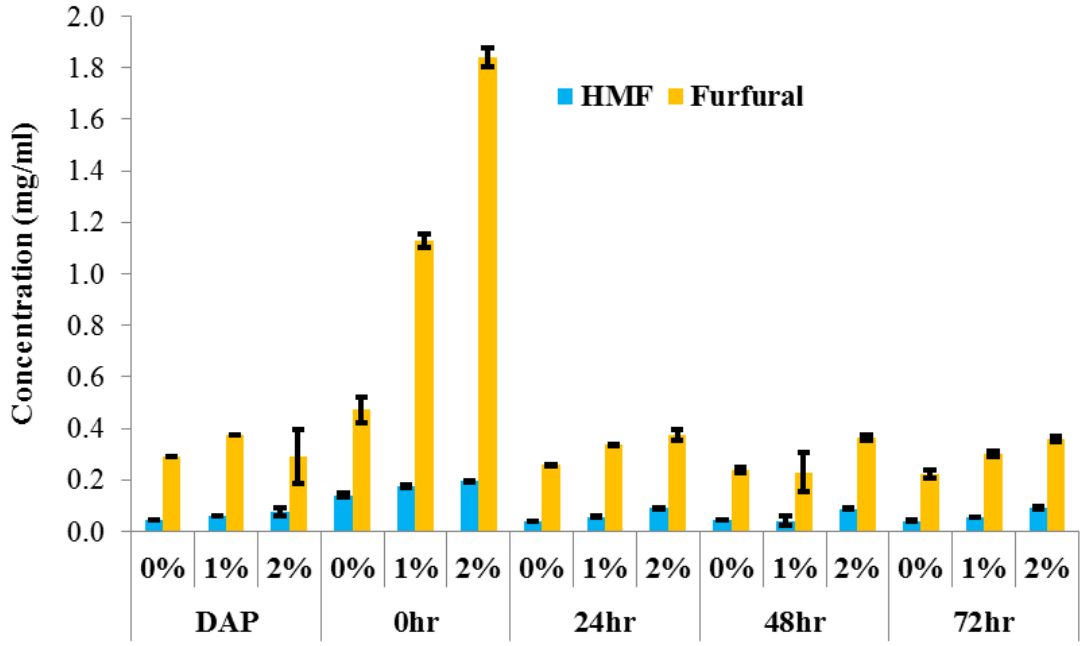


Figure A-6 Concentrations of inhibitors generated for 30 minutes hydrolysis scheme

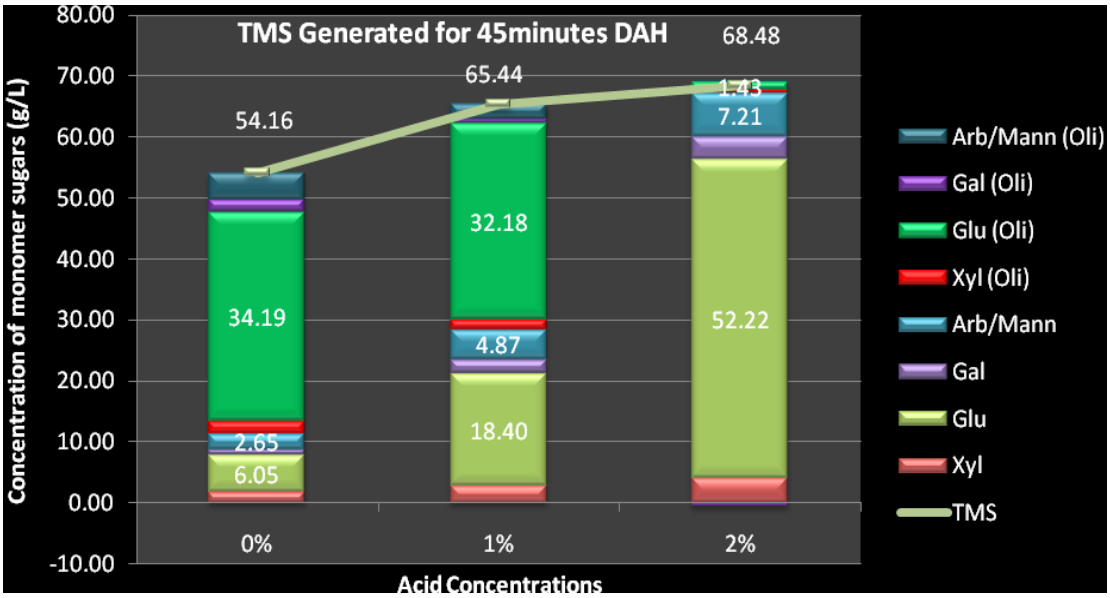


Figure A-7 TMS for 45-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

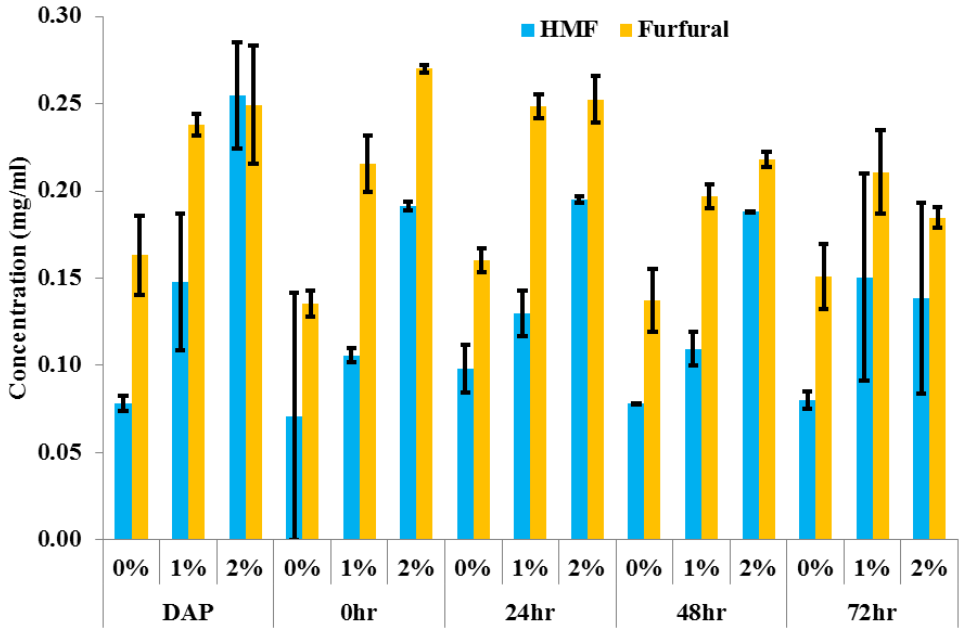


Figure A-8 Concentrations of inhibitors generated for 45 minutes hydrolysis scheme

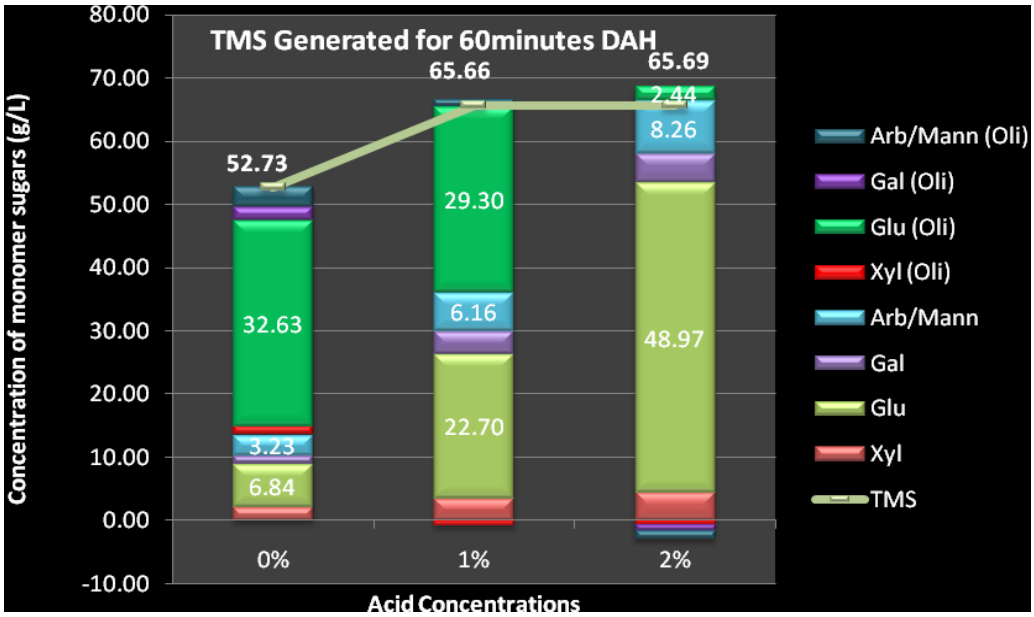


Figure A-9 TMS for 60-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

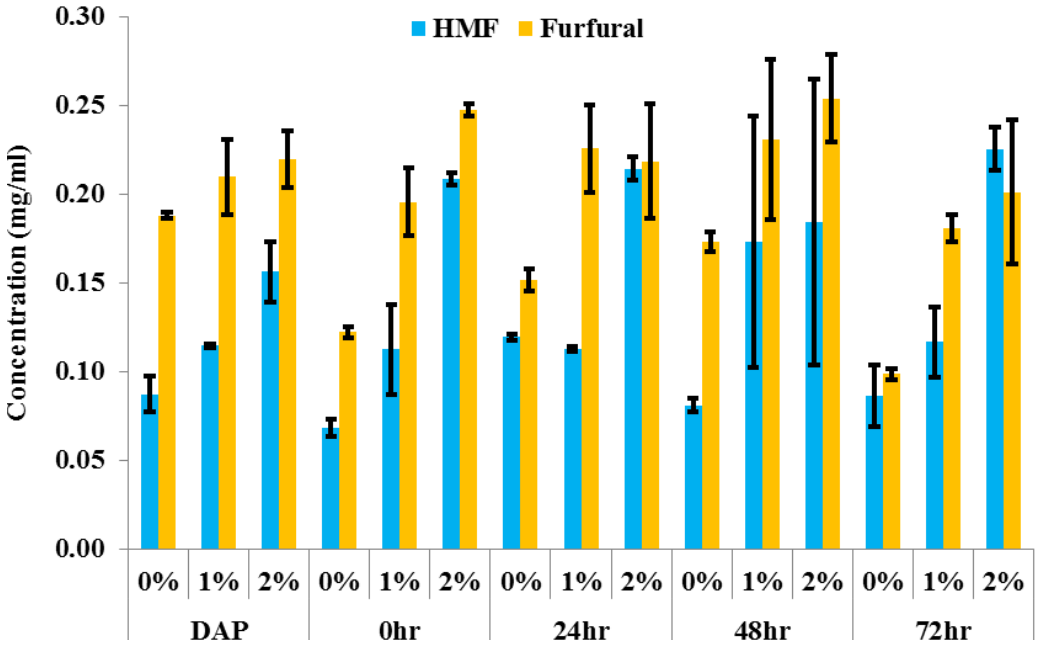


Figure A-10 Concentrations of inhibitors generated for 60 minutes hydrolysis scheme

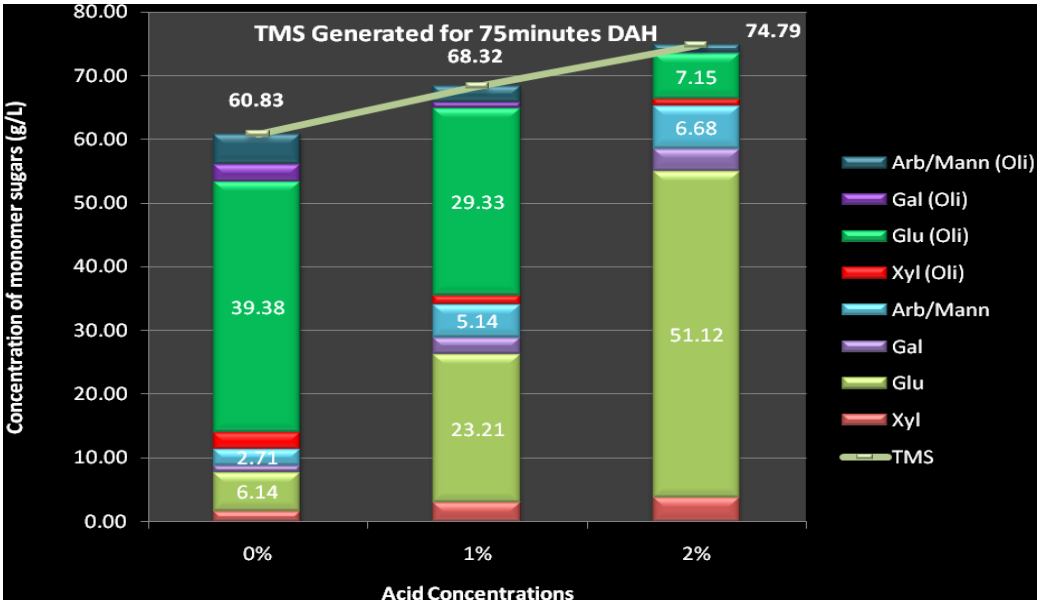


Figure A-11 TMS for 75-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

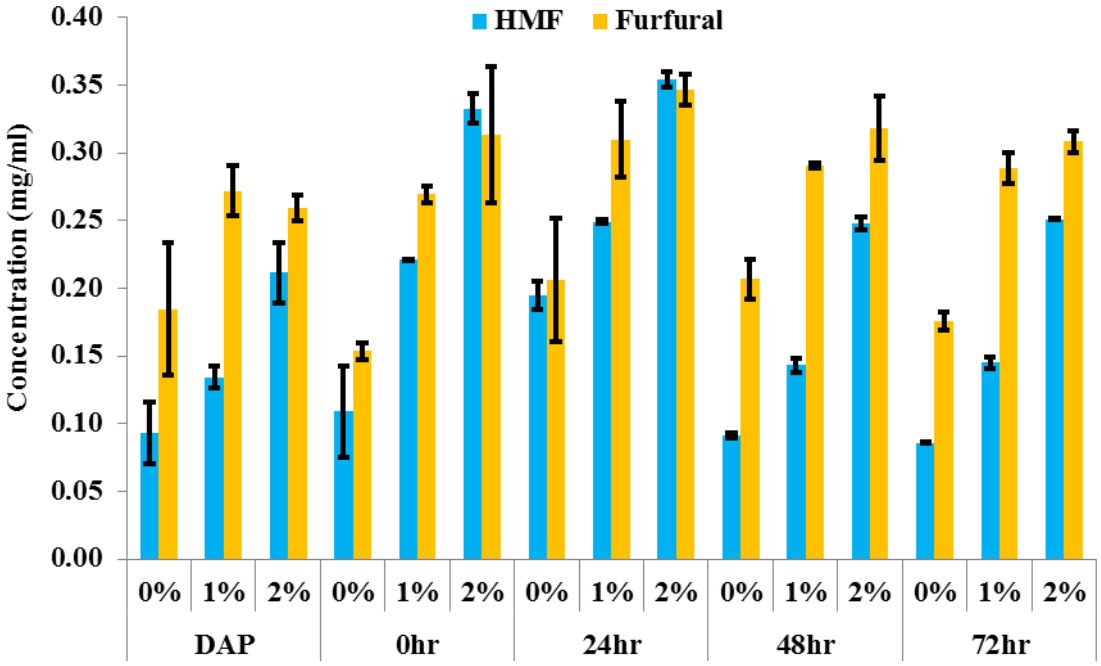


Figure A-12 Concentrations of inhibitors generated for 75-minute hydrolysis scheme

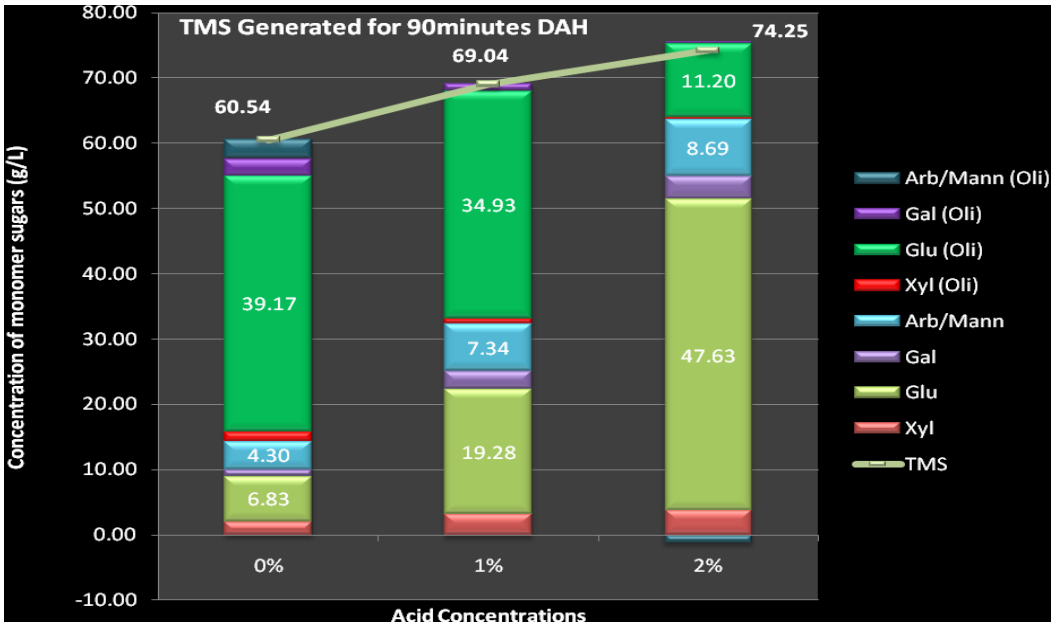


Figure A-13 TMS for 90-minute hydrolysis (first stage dilute acid hydrolysis +oligomer analysis)

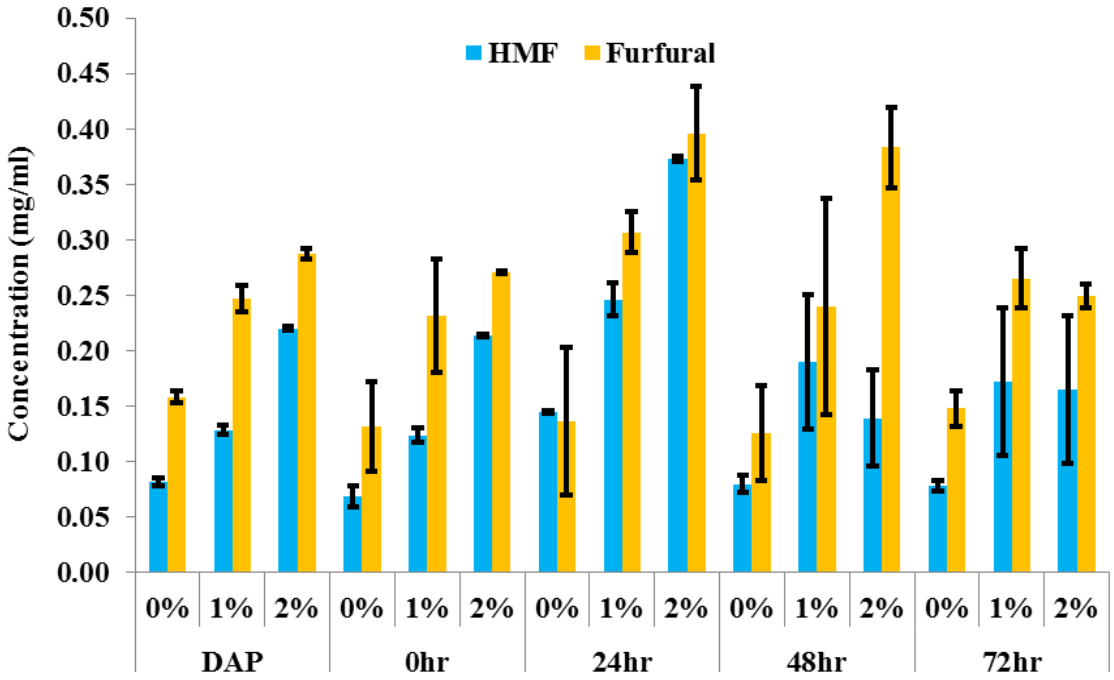


Figure A-14 Concentrations of inhibitors generated for 90 minute hydrolysis scheme

In the another study (Adom et al. 2012), the authors estimated the average total carbohydrates to be be [27% (± 5%) wt.]. Total carbohydrates comprised of the following; starch, soluble sugars (glucose, xylose, galactose, mannose, & arabinose) and cellulose. Using the total carbohydrates, we estimated the maximum theoretical TMS (**TMS $_{max}$**) that can be obtained from this hydrolysis as follows;

Weight of syrup used in hydrolysis = 10 g, Total solids in DCS (Appendix A. Figure A-15) = 28% wt.

Total carbohydrates = 27% wt. (of total solids), Syrup density = 1000 mg/ml

$$\mathbf{TMS\ (max)} = \frac{10g\ DCS \times 28\%\ (total\ solids) \times 27\%\ (carbohydrates) \times 1000\ mg/ml}{10\ \ ml\ DCS}$$

$$= 76\ mg/ml$$

The maximum TMS expected (assuming all carbohydrates was hydrolyzed to monomer sugars) was estimated to be 76 mg/ml.

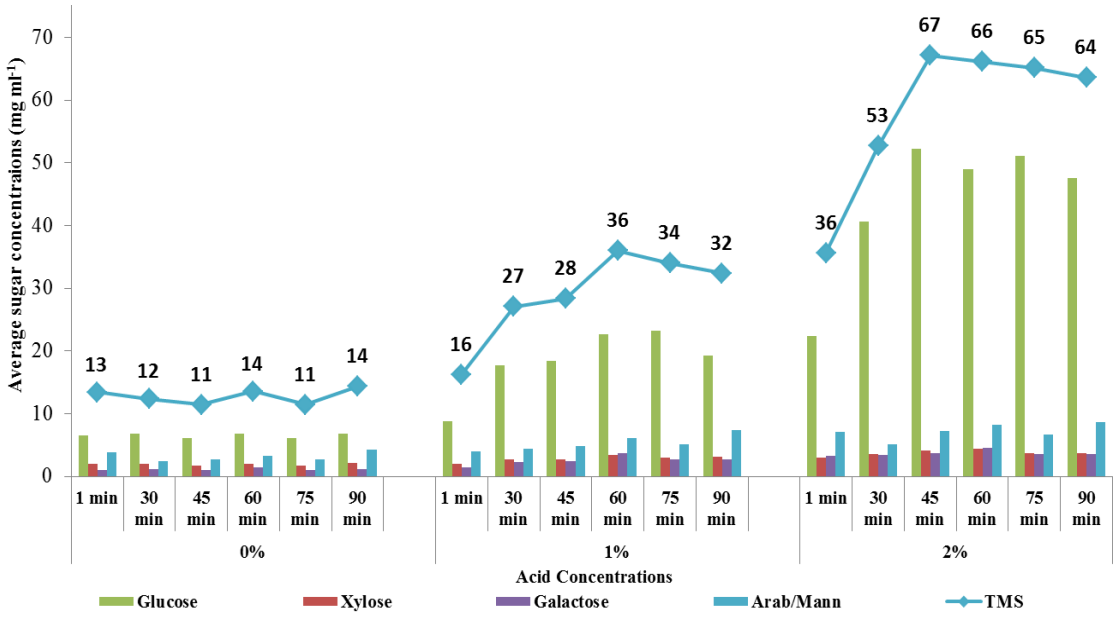


Figure A-16 Effect of time and 0, 1 & 2 wt% acid concentration on the yield of total monomer sugars (first stage acid pretreatment)

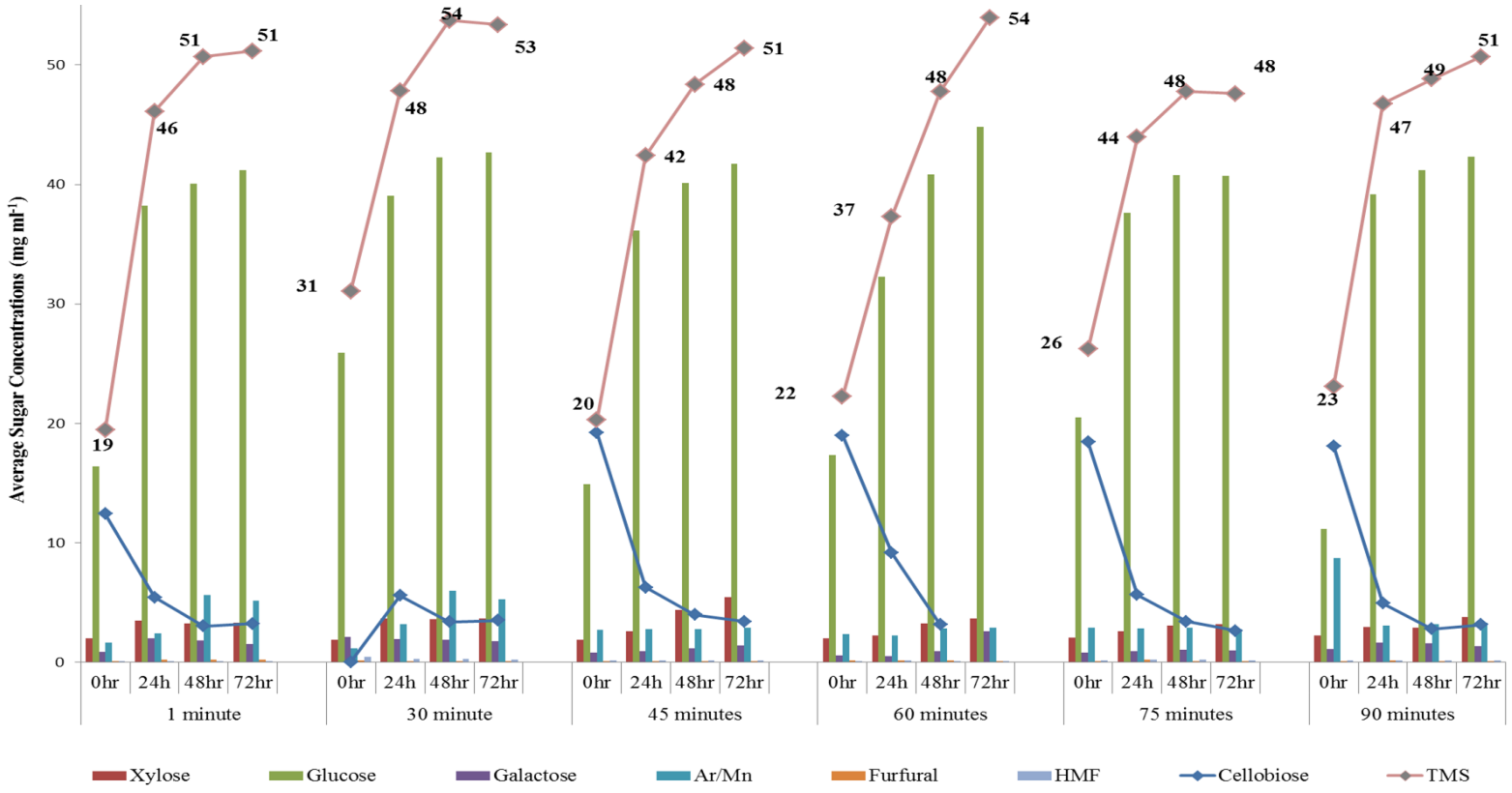


Figure A-17 Effect of 0 wt% acid concentration and time on the yield of monomer sugars with enzymes (Ar/Mn = arabinose)

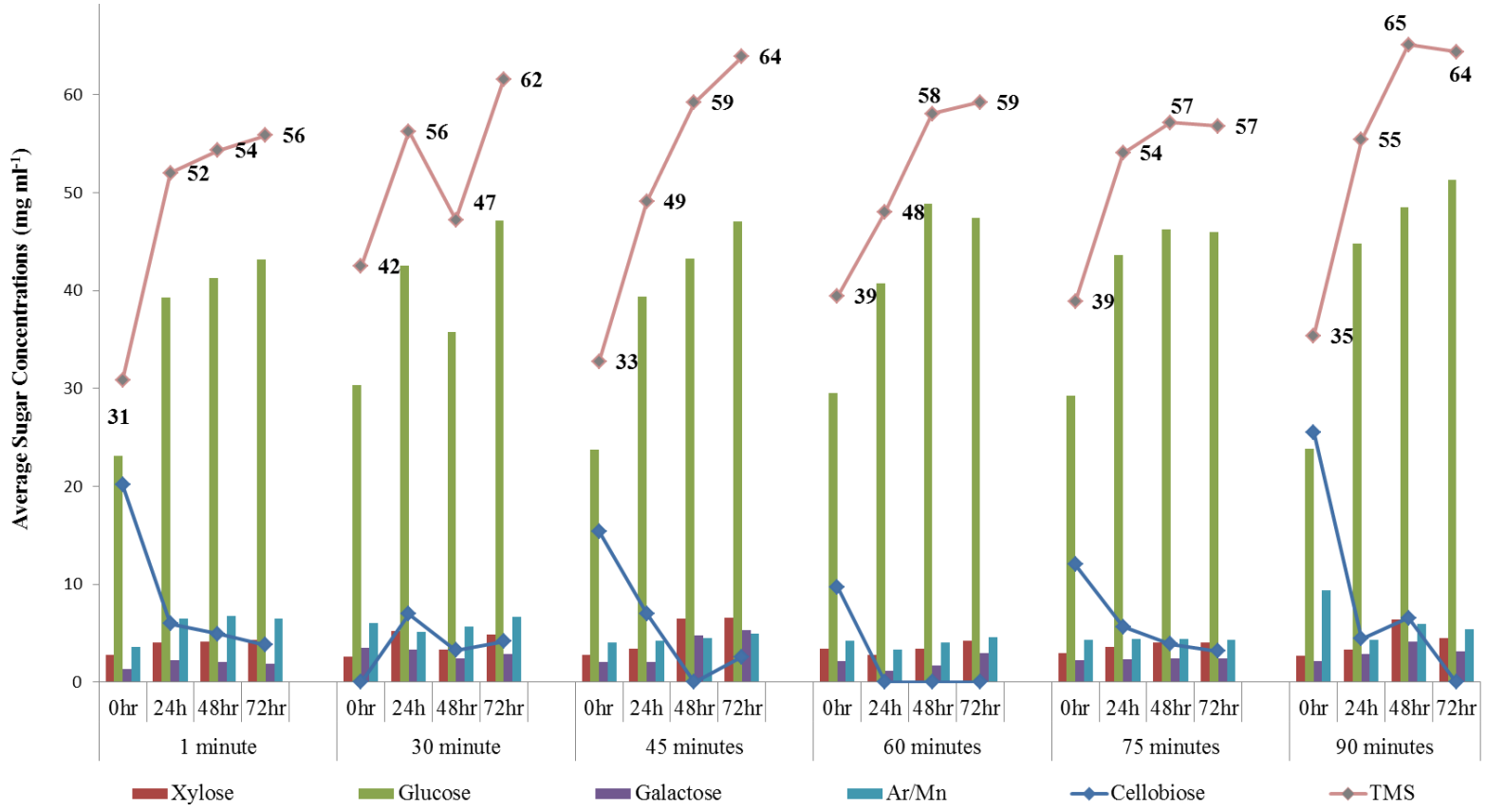


Figure A-18 Effect of 1 wt% acid concentration and time on the yield of monomer sugars with enzymes (Ar/Mn = arabinose)

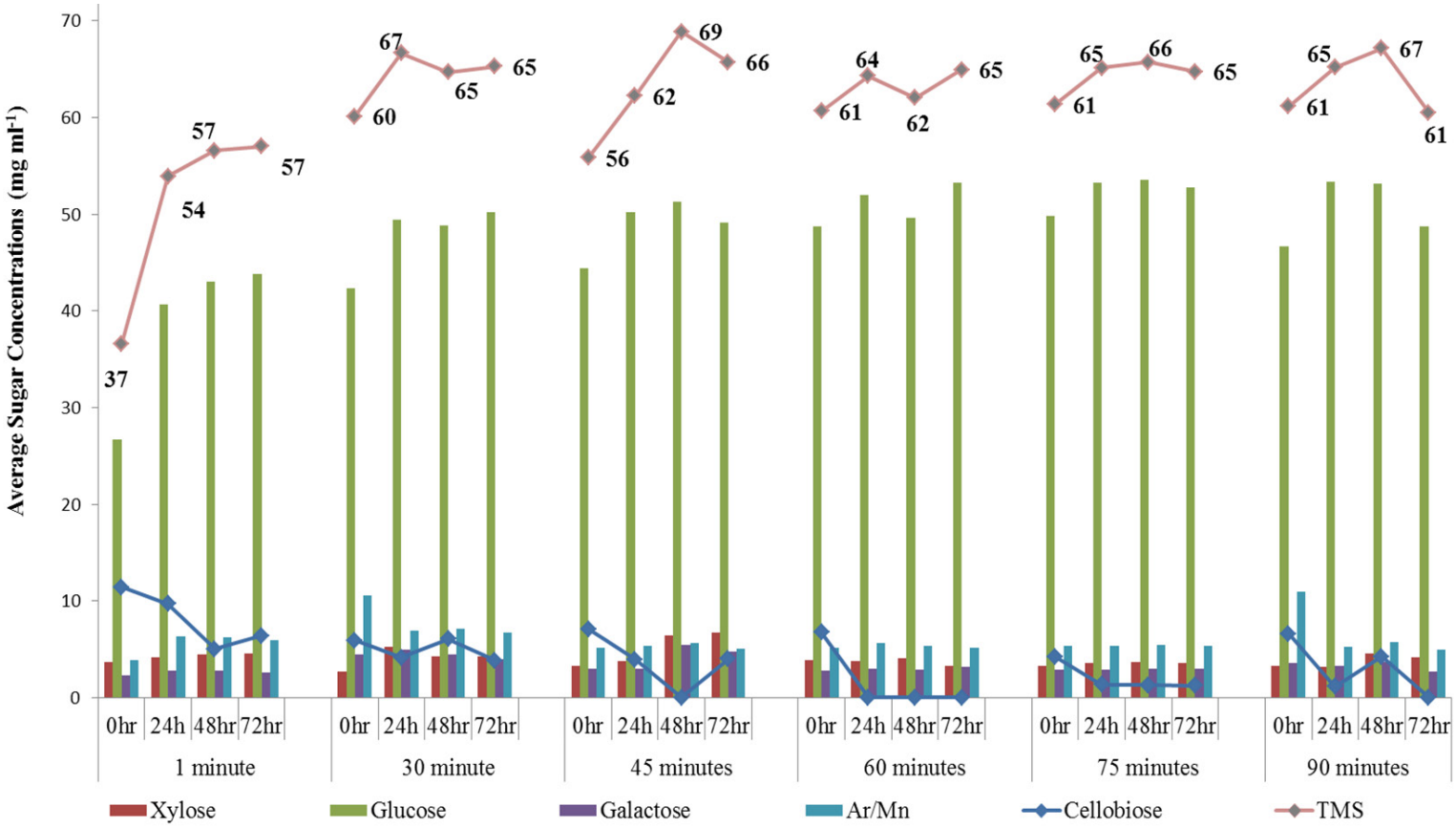


Figure A-19 Effect of 2 wt% acid concentration and time on the yield of monomer sugars with enzymes (Ar/Mn = arabinose)

Table A-1 Comparison of DAP (first stage) with 72 hours EH for 0 wt% acid concentration [Min: Minute(s)]

0 wt% (Total Sugars in mg ml^{-1})						
Incubation time	1 min	30 min	45 min	60 min	75 min	90 min
0hr	19.44	31.05	20.31	26.21	26.21	23.10
24h	46.12	47.80	42.39	43.94	43.94	46.78
48hr	50.69	53.73	48.39	47.77	47.77	48.83
72hr EH	51.17	55.00	51.40	54.00	47.59	50.71
TMS due to DAP (0 wt%)-First stage hydrolysis	13.40	12.36	11.44	13.55	11.46	14.32
Factor of increase after 72hr EH	3.8	4.4	4.5	4.0	4.2	3.5

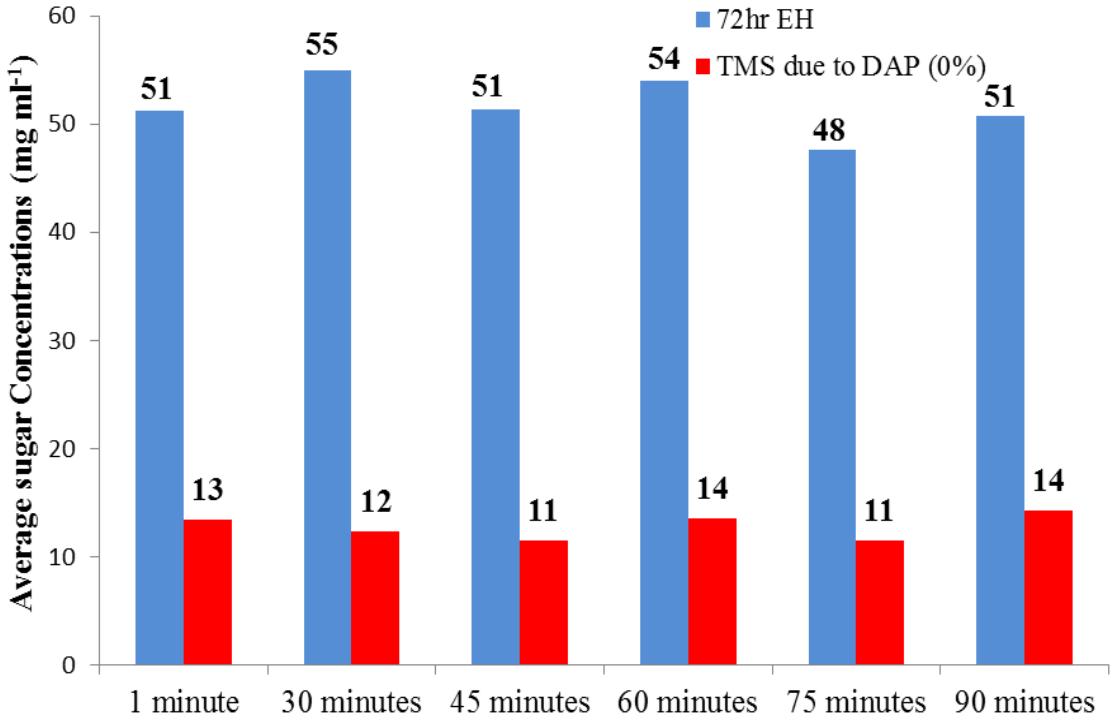


Figure A-20 Comparison of DAP (first stage) with 72 hours EH for 0 wt% acid concentration DCS

Table A-2 Comparison of DAP (first stage) with 72 hours EH for 1 wt% acid concentration [Min: Minute(s)]

1 wt% (Total Sugars in mg ml^{-1})						
Incubation time	1 min	30 min	45 min	60 min	75 min	90 min
0hr	30.85	42.48	32.71	39.41	38.87	35.40
24h	52.03	56.26	49.05	48.05	54.04	55.38
48hr	54.30	47.20	59.20	58.06	57.18	65.10
72hr	55.85	61.53	63.91	59.25	56.82	64.39
TMS due to DAP (1 wt%)	16.26	27.11	28.40	36.03	34.03	32.39
Factor of increase after 72hr EH	3.4	2.3	2.3	1.6	1.7	2.0

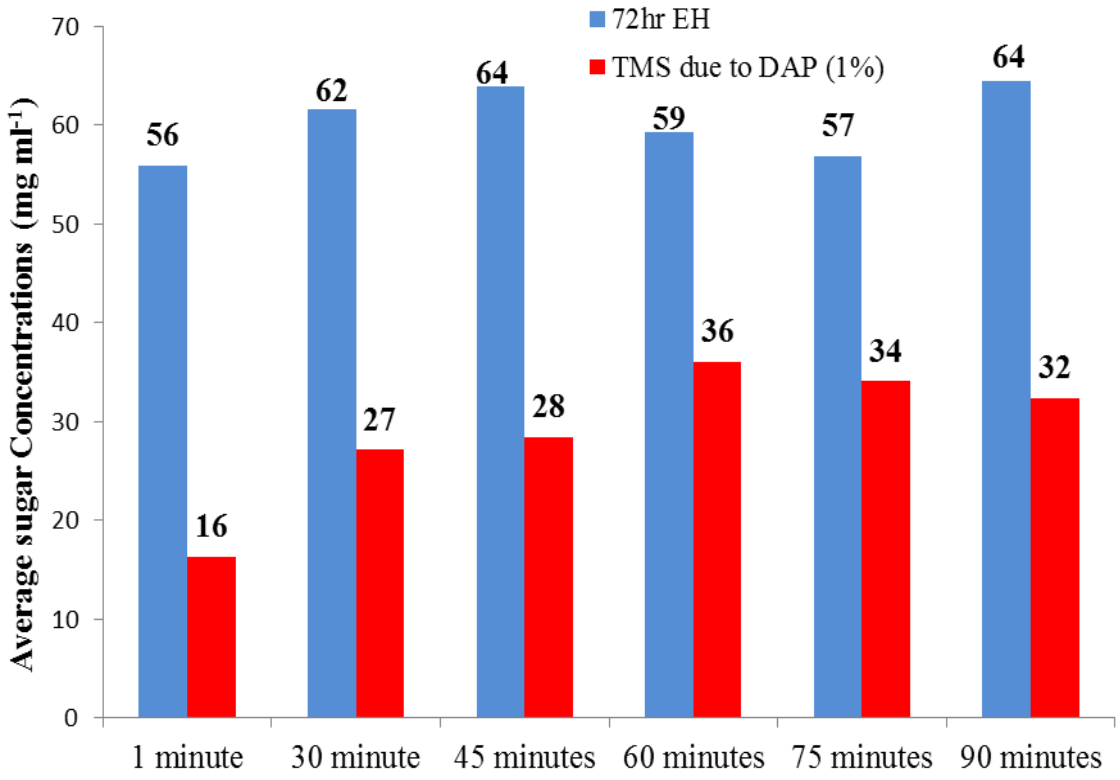


Figure A-21 Comparison of DAP (first stage) with 72 hours EH for 1 wt% acid concentration DCS

Table A-3 Comparison of DAP (first stage) with 72 hours EH for 2 wt% acid concentration [Min: Minute(s)]

2 wt% (Total Sugars in mg ml^{-1})						
Incubation time	1 min	30 min	45 min	60 min	75 min	90 min
0hr	36.60	60.13	55.82	60.67	61.35	61.17
24h	53.95	66.67	62.23	64.32	65.13	65.18
48hr	56.56	64.67	68.89	62.02	65.73	67.18
72hr	57.03	65.31	65.74	64.95	64.74	60.53
TMS due to DAP (2 wt%)	35.65	52.70	67.19	66.20	65.15	63.61
Factor of increase after 72hr EH	1.6	1.2	1.2	1.0	1.0	0.9

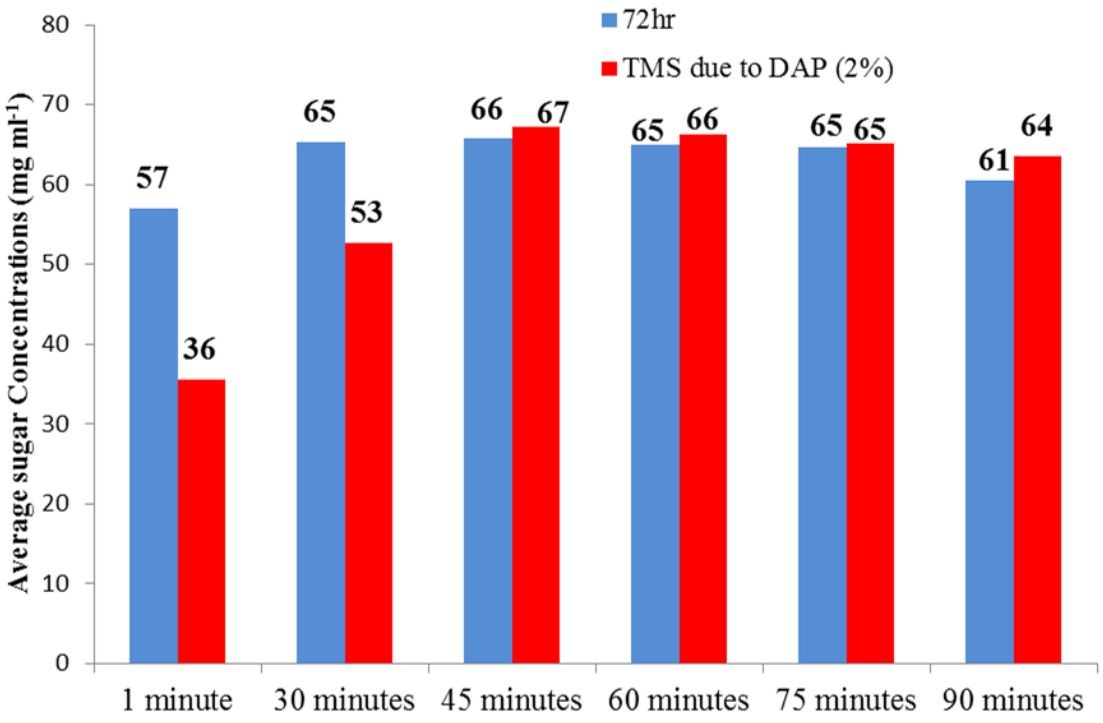


Figure A-22 Comparison of DAP (first stage) with 72 hours EH for 2 wt% acid concentration DCS

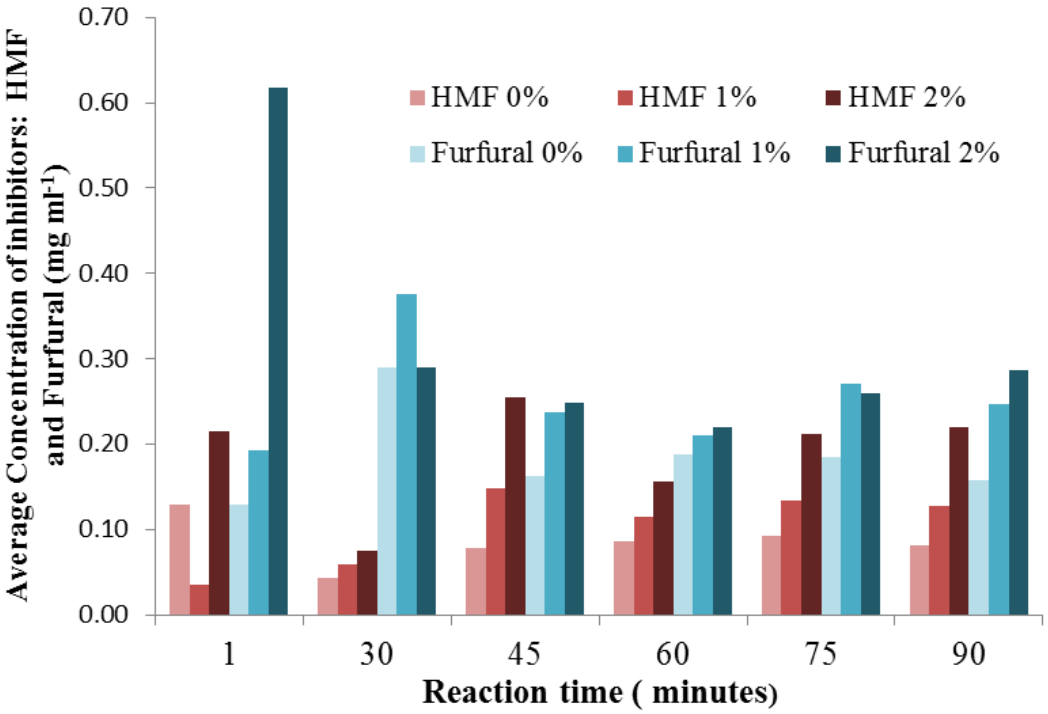


Figure A-23 The concentrations of HMF and Furfural generated overtime

Table A-4 Inhibitory concentrations of furfural and HMF for three types of yeast and *E. coli* KO11

Organism	**Furfural (mg ml^{-1})**	**5-HMF (mg ml $^{-1}$)**
Pichia stipitis	2.0-2.5 (Delgenes et al. 1996)	5.0 (Delgenes et al. 1996)
Kluveromyces marxianus	2.0-2.5 (Oliva et al. 2003)	4.0- 4.2 (Oliva et al. 2003)
Pachysolen tannophilus	0.35-0.7 (Almeida et al. 2009)	n/a
***Escherichia coli* KO11**	3.5 (Zaldivar et al. 1999)	4.0 (Zaldivar et al. 1999)

Almeida JRM, Bertilsson M, Gorwa-Grauslund MF, Gorsich S, Lidén G. 2009. Metabolic effects of furaldehydes and impacts on biotechnological processes. Applied Microbiology and Biotechnology 82(4):625-638.

Delgenes J, Moletta R, Navarro J. 1996. Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by Saccharomyces cerevisiae, Zymomonas mobilis, Pichia stipitis, and Candida shehatae. Enzyme and microbial technology 19(3):220-225.

Oliva JM, Saez F, Ballesteros I, Gonzalez A, Negro MJ, Manzanares P, Ballesteros M. 2003. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast Kluyveromyces marxianus. Applied Biochemistry and Biotechnology 105(1):14.153-1

Zaldivar J, Martinez A, Ingram LO. 1999. Effect of selected aldehydes on the growth and fermentation of ethanologenic Escherichia coli. Biotechnology and bioengineering 65(1):24-33.

Appendix B: Supplementary information for protein platform optimization experiments

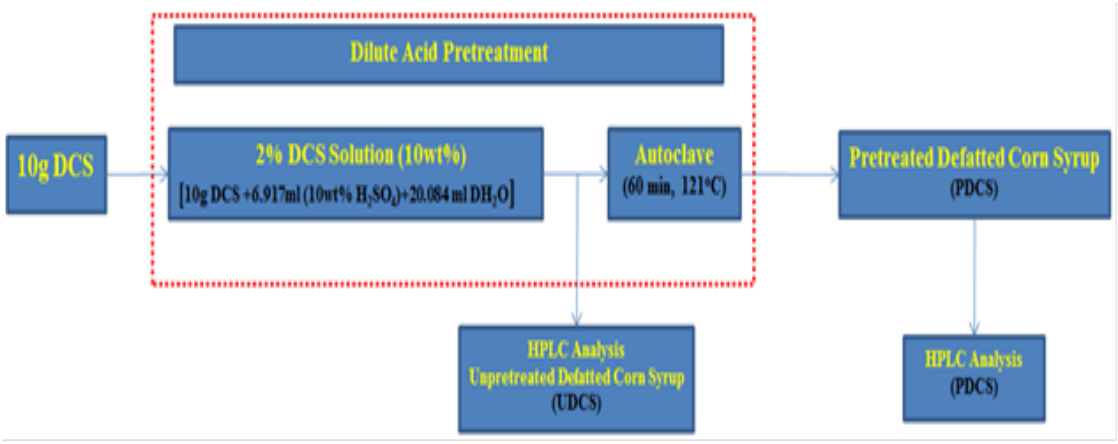


Figure B-1 Flow diagram of dilute acid hydrolysis at optimum conditions

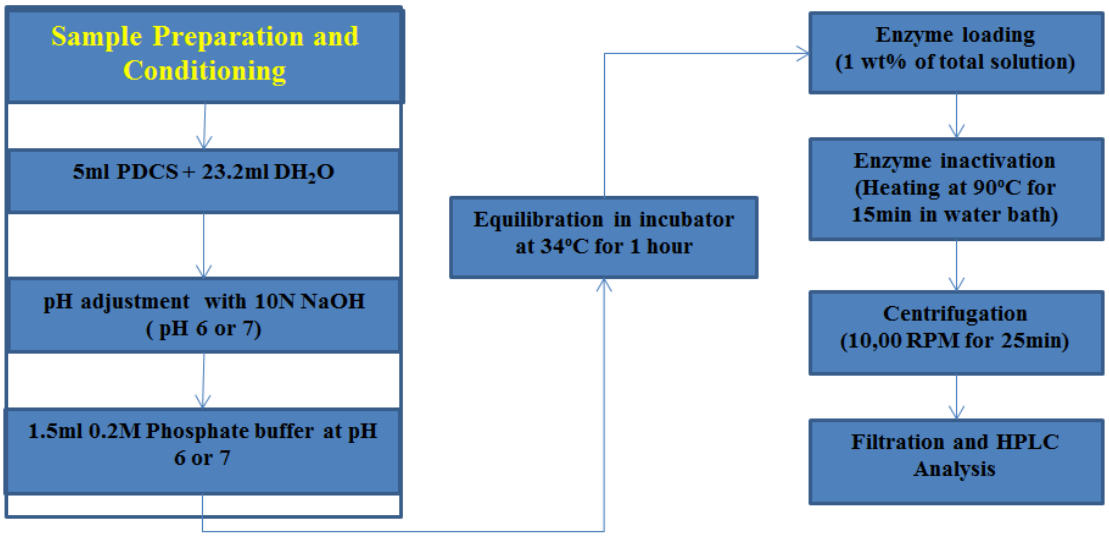


Figure B-2 Flow diagram for dilute acid pretreatment followed by protein hydrolysis using proteases (Hydrolysis Pathway 2)

Table B-1 Amino acid concentrations of enzyme blanks (Pathway 2)

Pronase E, Protex 6L and Trypsin (34^{o}C)			
Hydrolysis pathway 2-pH 7		**Hydrolysis pathway 2-pH 6**	
Amino acids	**Amino acid concentrations**	**Amino acids**	**Amino acid concentrations**
Aspartic Acid L- Glutamic Acid Asparagine L-serine Histidine Glycine Threonine L-Arginine L-Alanine Tyrosine Valine Methionine Phenylanaline Isoleucine Leucine Lysine	**None detected**	Aspartic Acid L- Glutamic Acid Asparagine L-serine Histidine Glycine Threonine L-Arginine L-Alanine Tyrosine Valine Methionine Phenylanaline Isoleucine Leucine Lysine	**None detected**

NB: Enzyme blank for all hydrolysis solutions comprised of all reagents (e.g. distilled water, base, tetracycline, etc) except for the protease or cellulase enzyme.

Table B-2 Amino acid concentrations of enzyme blanks (Pathway 3) experimental set 1

Enzyme Blank (Alcalase ®)		
Blank	Retention Time	Peak Area
pH 7,8,9 H	Serine	202.50
pH 7,8,9 M	Serine	198.60
pH 7,8,9 L	Serine	195.23

Table B-3 Amino acid concentrations of enzyme blanks (Pathway 3) experimental sets 2, 3 & 4

Enzyme Blank for Hydrolysis Pathway 3			
Pronase E, Protex 6L and Trypsin			
Hydrolysis Pathway 3-pH 7 (34^{o}C)		**Hydrolysis Pathway 2-pH 6 (34^{o}C)**	
Amino Acid Concentration	**Concentration**	**Amino Acid Concentration**	**Concentration**
Aspartic Acid L- Glutamic Acid Asparagine L-serine Histidine Glycine Threonine L-Arginine L-Alanine Tyrosine Valine Methionine Phenylanaline Isoleucine Leucine Lysine	None Detected	Aspartic Acid L- Glutamic Acid Asparagine L-serine Histidine Glycine Threonine L-Arginine L-Alanine Tyrosine Valine Methionine Phenylanaline Isoleucine Leucine Lysine	None Detected

Table B-4 Amino acid concentrations of enzyme blanks (Pathway 3) experimental sets 5, 6 & 7 (ND: None detected)

Sugars/HMF/Furfural	Accellerase 1500 : Peak area (nRIU*S)	Amylase Peak area (nRIU*S)	Amylase & AMG Peak area (nRIU*S)
Cellobiose	ND	ND	ND
Glucose	25546.8		5.68E+04
Xylose			
Galactose			
Arab	ND		ND
Mann			
HMF			
Furfural			

Table B-5 Amino acid concentrations of enzyme blanks (Pathway 4): 1% v/v Protex 6L

Enzyme Blank for Hydrolysis Pathway 4		
1% v/v Protex 6L		
Hydrolysis Pathway 2-pH 6 (40^{o}C)		
# Peaks	**Name of AA**	**mg/ml**
1	Aspartic Acid	0.04
2	L- Glutamic Acid	0.05
3	Asparagine	0.02
4	L-serine	0.01
5	Histidine	0.00
6	Glycine	0.00
7	Threonine	0.00
8	L-Arginine	0.02
9	L-Alanine	0.04
10	Tyrosine	0.01
11	Valine	0.00
12	Methionine	0.00
13	Phenylanaline	0.08
14	Isoleucine	0.00
15	Leucine	0.06
16	Lysine	0.00

Table B-6 Amino acid concentrations of enzyme blanks (Pathway 4): 2% v/v Protex 6L

Enzyme Blank for Hydrolysis Pathway 4		
2% v/v Protex 6L		
Hydrolysis Pathway 2-pH 6 (40^{o}C)		
# Peaks	**Amino Acid Component**	**(mg/ml)**
1	Aspartic Acid	0.03
2	L-Glutamic Acid	0.05
3	Asparagine	0.02
4	L-serine	0.02
5	Histidine	0.00
6	Glycine	0.01
7	Threonine	0.01
8	L-Arginine	0.03
9	L-Alanine	0.05
10	Tyrosine	0.02
11	Valine	0.00
12	Methionine	0.00
13	Phenylanaline	0.00
14	Isoleucine	0.02
15	Leucine	0.09
16	Lysine	0.00

Table B-7 Sugar concentrations of enzyme blanks (Pathway 4): 1% v/v Protex 6L

Enzyme Blank for Hydrolysis Pathway 4		
1% v/v Protex 6L		
Hydrolysis Pathway 2-pH 6 (40^{o}C)		
Sugars/HMF/Furfural	Peak area (nRIU*S)	**mg/ml**
Cellobiose	ND	0
Glucose	24851.1	0.635
Xylose		0
Galactose		0
Arab		0
Mann		0
HMF		0
Furfural	ND	0
Total Sugar = Glucose + Xylose + Galactose + Arabinose + Mannose =		0.635

Table B-8 Sugar concentrations of enzyme blanks (Pathway 4): 2% v/v Protex 6L

Enzyme Blank for Hydrolysis Pathway 4		
2% v/v Protex 6L		
Hydrolysis Pathway 2-pH 6 (40^{o}C)		
Sugars/HMF/Furfural	Peak area (nRIU*S)	**Dilution Factor (DF=3.8)-mg/ml**
Cellobiose	ND	0
Glucose	23424.4	0.598
Xylose		0
Galactose		0
Arab		0
Mann		0
HMF		0
Furfural	ND	0
Total Sugar = Glucose + Xylose + Galactose + Arabinose + Mannose =		0.598

Table B-9 HMF and Furfural concentrations for hydrolysis pathway 3

Hydrolysis solution	**Inhibitors**	**1 minute**		**2 hours**		**5 hours**	
		Conc (mg/ml)	**Std Dev**	**Conc (mg/ml)**	**Std Dev**	**Conc (mg/ml)**	**Std Dev**
Control	HMF	0.26	0.0143	0.25	0.00001	0.25	0.00211
	Furfural	0.25	0.0019	0.28	0.0007	0.27	0.0008
Accellerase	HMF	0.25	0.0040	0.26	0.0001	0.26	0.0004
	Furfural	0.24	0.0012	0.27	0.0002	0.27	0.0003
Amylase	HMF	0.26	0.0046	0.27	0.0007	0.26	0.0097
	Furfural	0.28	0.0004	0.28	0.0012	0.28	0.0041
Amylase and AMG	HMF	0.30	0.000	0.30	0.0035	0.28	0.0333
	Furfural	0.32	0.002	0.32	0.0019	0.31	0.0104
Hydrolysis solution	**Inhibitors**	**24 hours**		**48 hours**		**72 hours**	
		Conc (mg/ml)	**Std Dev**	**Conc (mg/ml)**	**Std Dev**	**Conc (mg/ml)**	**Std Dev**
Control	HMF	0.20	0.0147	0.01	0.0172	0.18	0.1213
	Furfural	0.26	0.0063	0.17	0.0089	0.25	0.0391
Accellerase	HMF	0.25	0.0593	0.24	0.0000	0.25	0.0000
	Furfural	0.26	0.0185	0.26	0.0023	0.23	0.0405
Amylase	HMF	0.47	0.0808	0.47	0.0130	0.47	0.0130
	Furfural	0.32	0.0215	0.18	0.0060	0.17	0.0019
Amylase and AMG	HMF	0.24	0.099	0.10	0.018	0.03	0.000
	Furfural	0.31	0.054	0.22	0.007	0.20	0.007

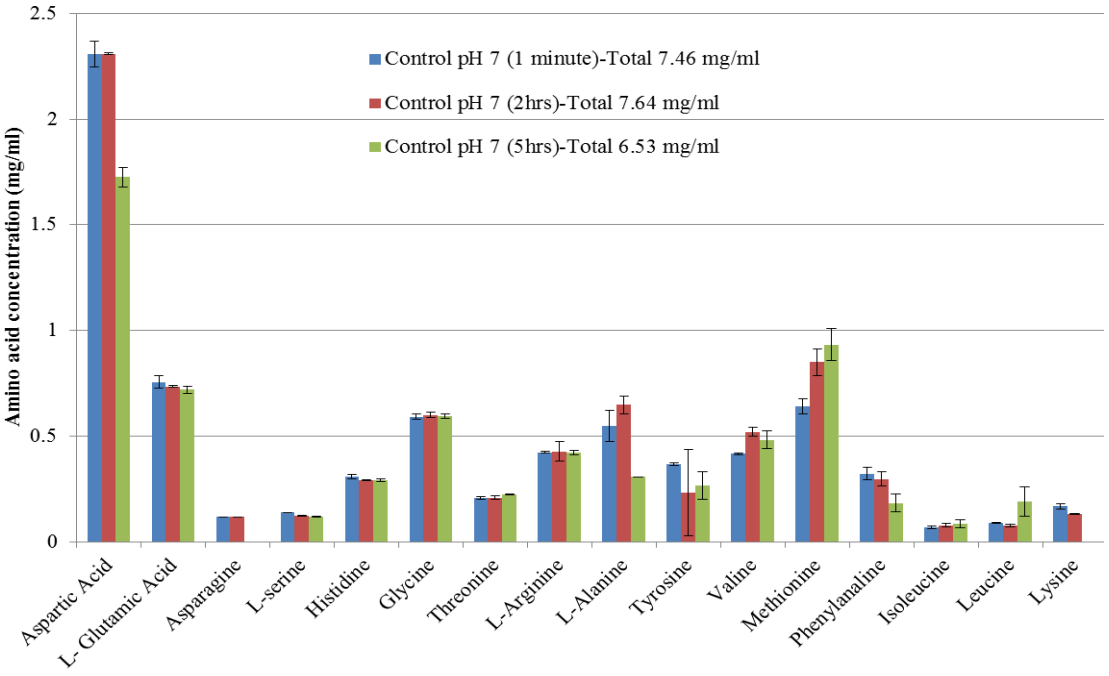


Figure B-3 Results for Hydrolysis Pathway 2: DAP followed by protein hydrolysis using proteases

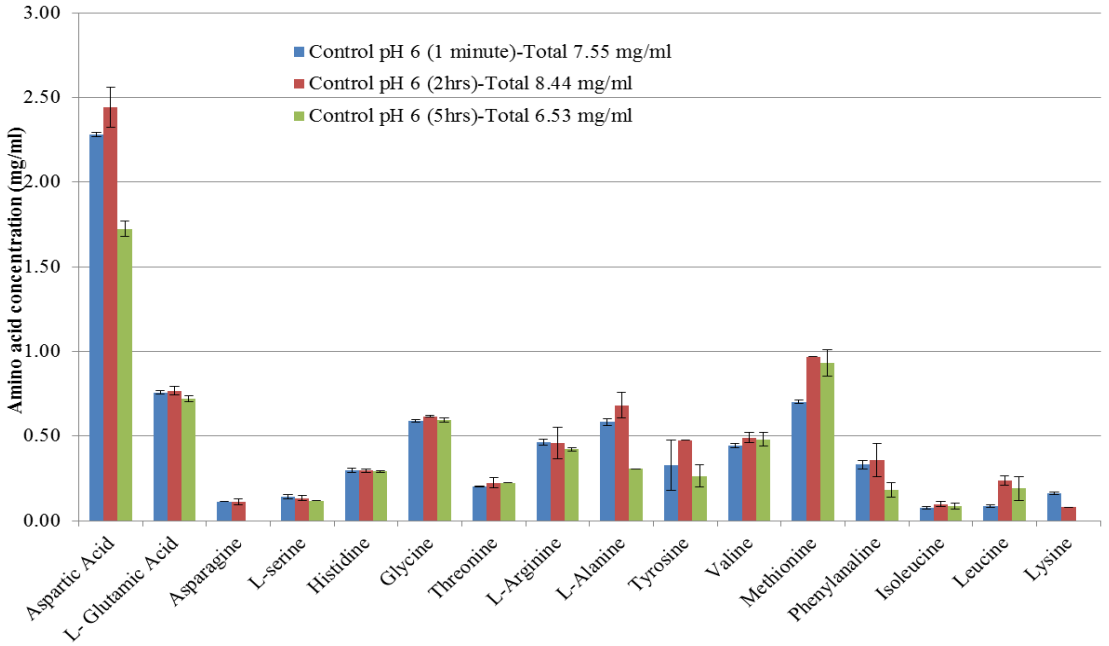


Figure B-4 Amino acid hydrolysis trends of individual amino acids for control pH 6

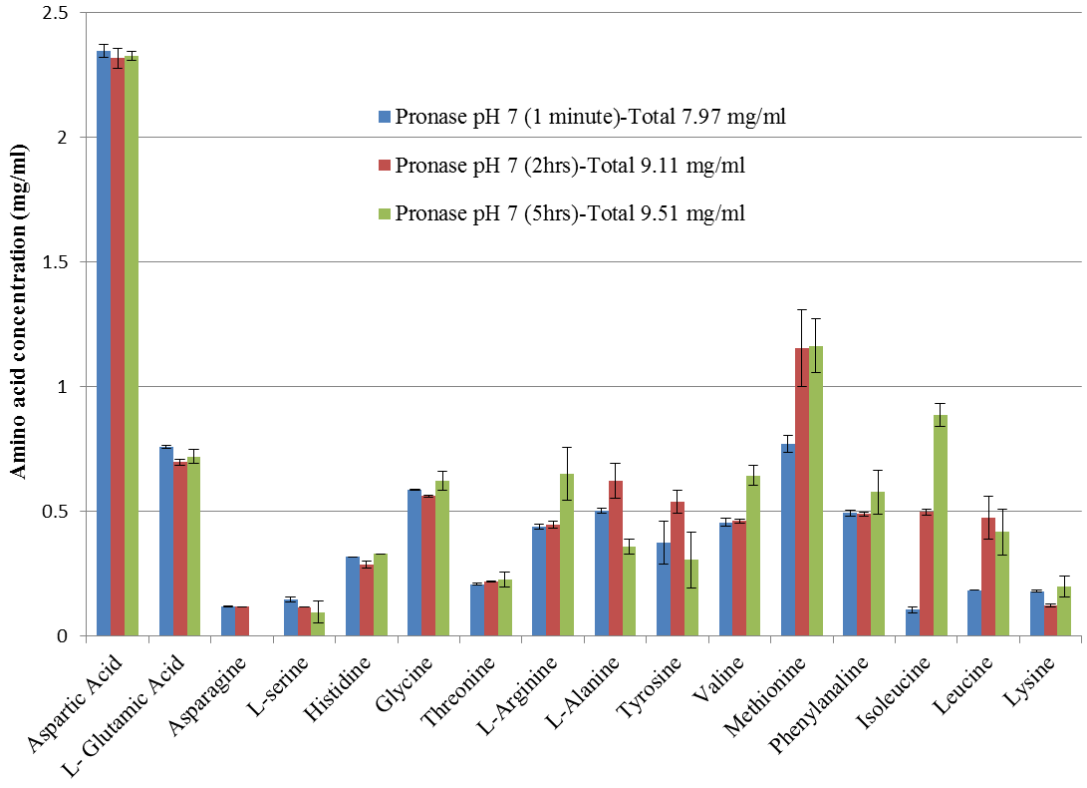


Figure B-5 Amino acid hydrolysis trends of individual amino acids for Pronase pH 7

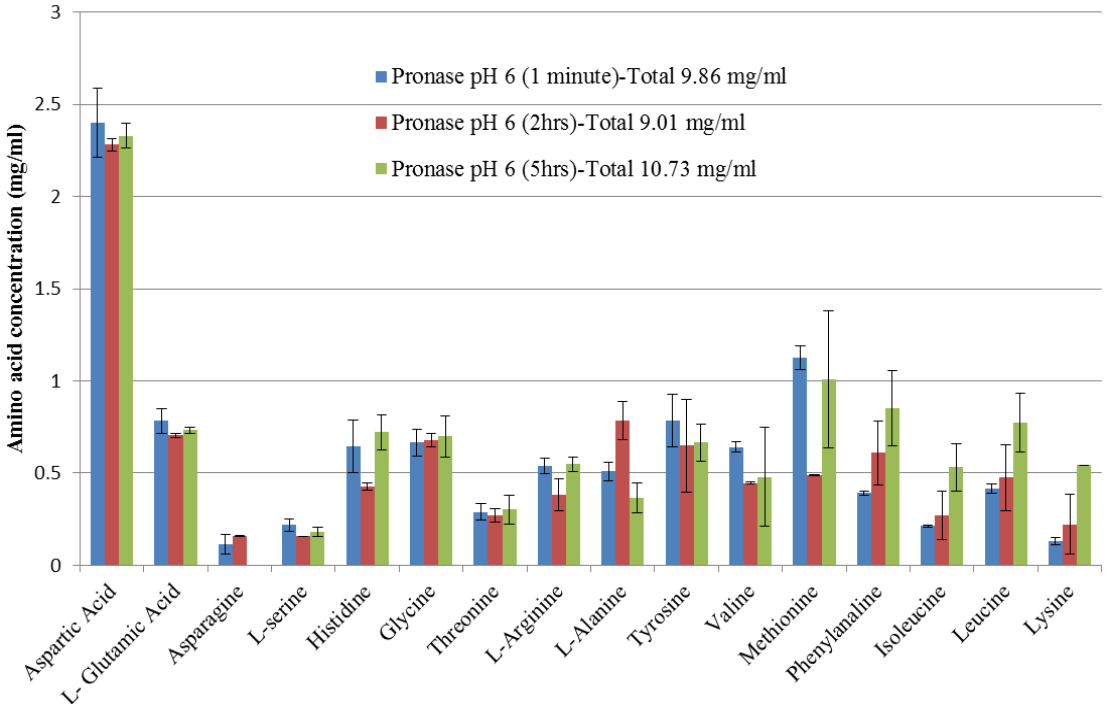


Figure B-6 Amino acid hydrolysis trends of individual amino acids for Pronase pH 6

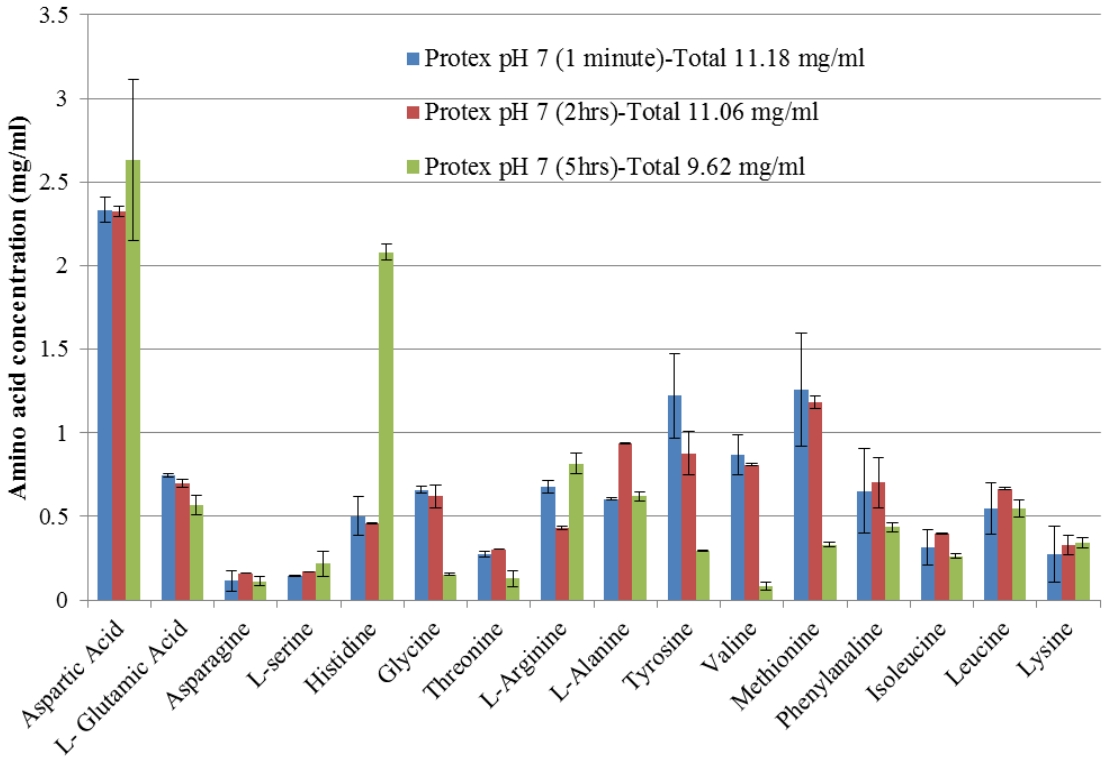


Figure B-7 Amino acid hydrolysis trends of individual amino acids for Protex pH 7

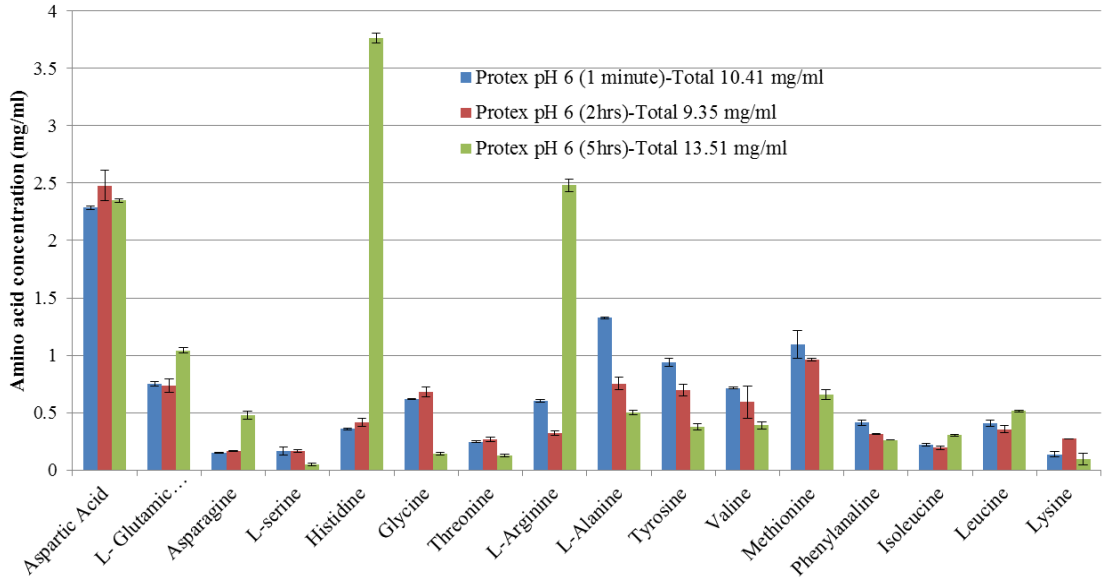


Figure B-8 Amino acid hydrolysis trends of individual amino acids for Protex pH 6

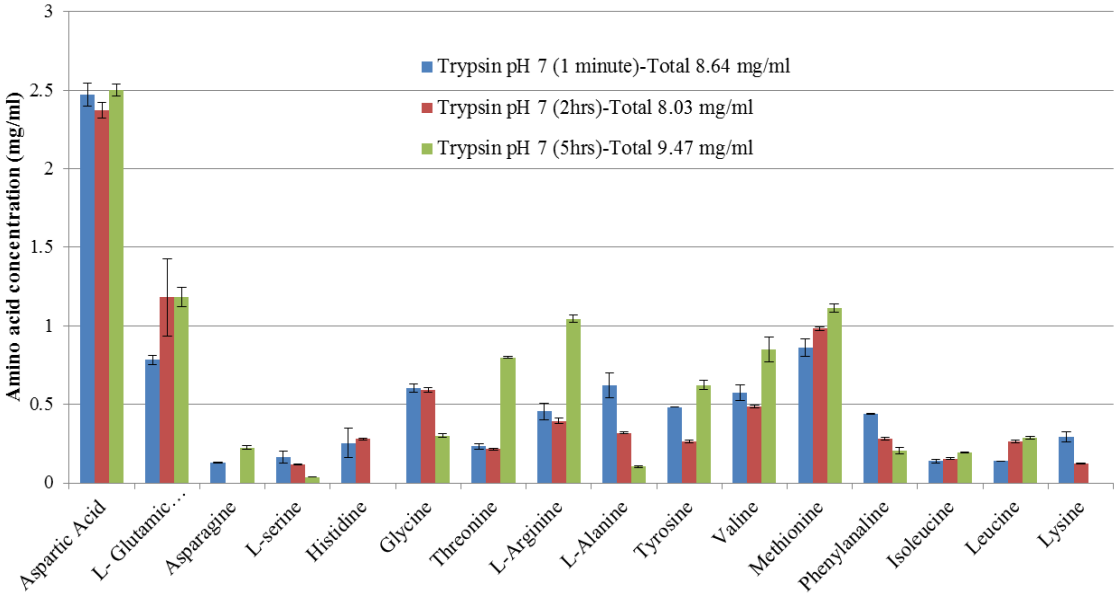


Figure B-9 Amino acid hydrolysis trends of individual amino acids for Trypsin pH 7

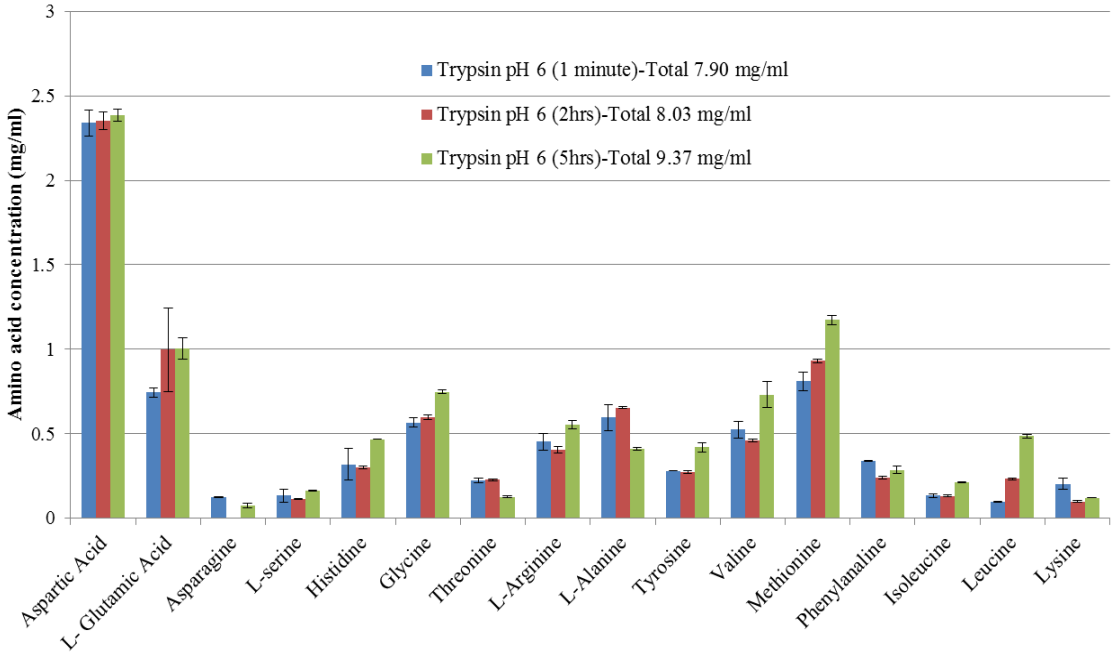


Figure B-10 Amino acid hydrolysis trends of individual amino acids for Trypsin pH 6

Appendix C: Supplementary information for modeling of dilute acid pretreatment process using defatted corn syrup as feedstock.

Table C-1: Reactant components and chemical formular for protein model

Reactants				
#Mole	Component	Formular	MW	(#Mole* MW) (amount in solution, g)
1	Protein	**$CH_{1.99}O_{0.61}N_{0.32}S_{0.01}$**	28.55	28.55
0.09	H_2O	**H_2O**	18	1.6
0	Acid catalyst	**H_2SO_4**	0	0
	Total			**30.19**

Table C-2 Product components and chemical formular for protein model

Products				
#Mole	Component	Formular	MW	(#Mole* MW) (amount in solution, g)
0.24	**Aspartic acid**	**$CH_{1.75}ON_{0.25}$**	33.25	7.98
0.12	**Glutamic acid**	**$CH_{1.8}O_{0.8}N_{0.2}$**	29.4	3.528
0.03	**Asparagine**	**$CH_2O_{0.75}N_{0.5}$**	33.0	0.99
0.02	**Serine**	**$CH_{2.33}ON_{0.33}$**	34.95	0.699
0.06	**Histidine**	**$CH_2O_{0.75}N_{0.7}$**	35.8	2.148
0.08	**Glycine**	**$CH_{2.5}ON_{0.5}$**	37.5	3
0.06	**Threonine**	**$CH_{2.25}O_{0.75}N_{0.75}$**	36.75	2.205
0.06	**Arginine**	**$CH_{2.33}O_{0.67}N_{0.33}$**	29.67	1.7802
0.04	**Alanine**	**$CH_{2.33}O_{0.67}N_{0.33}$**	29.67	1.1868
0.02	**Tyrosine**	**$CH_{1.22}O_{0.33}N_{0.11}$**	20.04	0.4008
0.02	**Valine**	**$CH_{2.2}O_{0.4}N_{0.2}$**	23.42	0.4684
0.1	**Methionine**	**$CH_{1.22}O_{0.4}N_{0.2}S_{0.2}$**	28.82	2.882
0.05	**Phenylalanine**	**$CH_{1.22}O_{0.22}N_{0.11}$**	18.28	0.914
0.04	**Isoleucine**	**$CH_{2.17}O_{0.33}N_{0.17}$**	21.83	0.8732
0.03	**Leucine**	**$CH_{2.17}O_{0.33}N_{0.17}$**	21.83	0.6549
0.02	**Lysine**	**$CH_{2.33}O_{0.33}N_{0.33}$**	24.23	0.4846
	Total			**30.19**

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'CYCLONE1'.					
CT - 1	BATCH AUTO	CYCLONE1	Material	A285C	91700
		CODE OF ACCOUNT: 193	Centrifuge diameter	609.60 MM	
		TAG NO.: CYCLONE1	Centrifuge capacity	0.0566 M3	
			Driver power	15.00 KW	
			Total weight	3400 KG	

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING:	91700.	1.0000	750.	0.0082	27	0.008
PIPING	9039.	0.0986	17943.	0.1957	597	1.985
CIVIL	728.	0.0079	1207.	0.0132	52	1.657
STRUCTURAL STEEL	0.	0.0000	0.	0.0000	0	0.000
INSTRUMENTATION	13841.	0.1509	5591.	0.0610	189	0.404
ELECTRICAL	837.	0.0091	1199.	0.0131	41	1.433
INSULATION	0.	0.0000	0.	0.0000	0	0.000
PAINT	1317.	0.0144	3295.	0.0359	153	2.503
SUBTOTAL	117461.	1.2809	29984.	0.3270	1059	0.255

TOTAL MATERIAL AND MANPOWER COST =USD 147400. INST'L COST/PE RATIO = 1.607

Figure C-1 Detailed cost analysis of unit operation: Cyclone

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'FLASH-1'.					
VT - 2	CYLINDER	FLASH-1-flash vessel	Shell material	A 516	26100
		CODE OF ACCOUNT: 113	Liquid volume	15.64 M3	
		TAG NO.: FLASH-1-flas	Vessel diameter	2.286 M	
			Vessel tangent to tangent height	3.810 M	
			Design temperature	151.06 DEG C	
			Design gauge pressure	243.67 KPAG	
			Application	CONT	
			Base material thickness	8.000 MM	
			Total weight	2900 KG	

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING:	26100.	1.0000	785.	0.0301	25	0.030
PIPING	24873.	0.9530	12896.	0.4941	430	0.518
CIVIL	3283.	0.1258	3783.	0.1449	162	1.152
STRUCTURAL STEEL	8314.	0.3185	1563.	0.0599	57	0.188
INSTRUMENTATION	24020.	0.9203	4837.	0.1853	160	0.201
ELECTRICAL	835.	0.0320	559.	0.0214	20	0.669
INSULATION	7864.	0.3013	6491.	0.2487	295	0.825
PAINT	701.	0.0269	1662.	0.0637	76	2.371
SUBTOTAL	95990.	3.6778	32577.	1.2482	1225	0.339

TOTAL MATERIAL AND MANPOWER COST =USD 128600. INST'L COST/PE RATIO = 4.927

Figure C-2 Detailed cost analysis of unit operation: Flash tank

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'PUMP-1'.					
CP - 7 ANSI	PUMP-1	Casing material	CS		7600
	CODE OF ACCOUNT: 161	Liquid flow rate	14.37	L/S	
	TAG NO.: PUMP-1	Fluid head	22.30	M	
		Design temperature	125.00	DEG C	
		Speed	1800.00	RPM	
		Driver power	5.500	KW	
		Fluid viscosity	0.912	MPA-S	
		Design gauge pressure	243.67	KPAG	
		Driver type	MOTOR		
		Seal type	SNGL		
		Total weight	340	KG	

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING	7600.	1.0000	859.	0.1131	27	0.113
PIPING	7432.	0.9779	4131.	0.5436	138	0.556
CIVIL	276.	0.0363	820.	0.1079	35	2.968
STRUCTURAL STEEL	0.	0.0000	0.	0.0000	0	0.000
INSTRUMENTATION	284.	0.0373	86.	0.0113	3	0.303
ELECTRICAL	830.	0.1092	1196.	0.1574	41	1.442
INSULATION	1843.	0.2425	1668.	0.2194	76	0.905
PAINT	155.	0.0205	417.	0.0549	19	2.686
SUBTOTAL	18420.	2.4236	9178.	1.2076	339	0.498

TOTAL MATERIAL AND MANPOWER COST =USD 27600. INST'L COST/PE RATIO = 3.632

Figure C-3 Detailed cost analysis of unit operation: Pump-1

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'PUMP-2'.					
CP - 8 ANSI	PUMP-2	Casing material	CS		7700
	CODE OF ACCOUNT: 161	Liquid flow rate	15.70	L/S	
	TAG NO.: PUMP-2	Fluid head	21.26	M	
		Design temperature	125.00	DEG C	
		Speed	1800.00	RPM	
		Driver power	5.500	KW	
		Fluid viscosity	0.467	MPA-S	
		Design gauge pressure	590.83	KPAG	
		Driver type	MOTOR		
		Seal type	SNGL		
		Total weight	340	KG	

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING	7700.	1.0000	859.	0.1116	27	0.112
PIPING	7432.	0.9652	4131.	0.5365	138	0.556
CIVIL	276.	0.0359	820.	0.1065	35	2.968
STRUCTURAL STEEL	0.	0.0000	0.	0.0000	0	0.000
INSTRUMENTATION	284.	0.0368	86.	0.0112	3	0.303
ELECTRICAL	830.	0.1077	1196.	0.1554	41	1.442
INSULATION	1843.	0.2393	1668.	0.2166	76	0.905
PAINT	155.	0.0202	417.	0.0542	19	2.686
SUBTOTAL	18520.	2.4051	9178.	1.1919	339	0.496

TOTAL MATERIAL AND MANPOWER COST =USD 27700. INST'L COST/PE RATIO = 3.597

Figure C-4 Detailed cost analysis of unit operation: Pump-2

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'RSTOIC-1'.					
AT - 9 REACTOR	RSTOIC-1	Shell material	A285C		118200
	CODE OF ACCOUNT: 132	Liquid volume	7.881 M3		
	TAG NO.: RSTOIC-1	Vessel diameter	1.372 M		
		Vessel tangent to tangent height	5.334 M		
		Agitator power	30.00 KW		
		Impeller speed	575.00 RPM		
		Design temperature	164.00 DEG C		
		Design gauge pressure	243.67 KPAG		
		Application	CONT		
		Base material thickness	20.00 MM		
		Fluid depth	5.029 M		
		Jacket type	FULL		
		Jacket design gauge pressure	620.00 KPAG		
		Total weight	8500 KG		

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING:	118200.	1.0000	891.	0.0075	28	0.008
PIPING	15392.	0.1302	16529.	0.1398	553	1.074
CIVIL	2317.	0.0196	2648.	0.0224	113	1.143
STRUCTURAL STEEL	6615.	0.0560	1265.	0.0107	46	0.191
INSTRUMENTATION	33289.	0.2816	11146.	0.0943	371	0.335
ELECTRICAL	2267.	0.0192	2095.	0.0177	72	0.924
INSULATION	7904.	0.0669	7430.	0.0629	338	0.940
PAINT	630.	0.0053	1604.	0.0136	74	2.546
SUBTOTAL	186614.	1.5788	43609.	0.3689	1595	0.234

TOTAL MATERIAL AND MANPOWER COST =USD 230200. INST'L COST/PE RATIO = 1.948

Figure C-5 Detailed cost analysis of unit operation: RSTOIC-1

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA		PURCHASED EQUIPMENT COST USD
Equipment mapped from 'RSTOIC-2'.					
AT - 10 REACTOR	RSTOIC-2	Shell material	A285C		118200
	CODE OF ACCOUNT: 132	Liquid volume	7.881 M3		
	TAG NO.: RSTOIC-2	Vessel diameter	1.372 M		
		Vessel tangent to tangent height	5.334 M		
		Agitator power	30.00 KW		
		Impeller speed	575.00 RPM		
		Design temperature	140.00 DEG C		
		Design gauge pressure	980.57 KPAG		
		Application	CONT		
		Base material thickness	20.00 MM		
		Fluid depth	5.029 M		
		Jacket type	FULL		
		Jacket design gauge pressure	620.00 KPAG		
		Total weight	8500 KG		

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING:	118200.	1.0000	891.	0.0075	28	0.008
PIPING	15392.	0.1302	16529.	0.1398	553	1.074
CIVIL	2317.	0.0196	2648.	0.0224	113	1.143
STRUCTURAL STEEL	6615.	0.0560	1265.	0.0107	46	0.191
INSTRUMENTATION	33289.	0.2816	11146.	0.0943	371	0.335
ELECTRICAL	2267.	0.0192	2095.	0.0177	72	0.924
INSULATION	6819.	0.0577	6393.	0.0541	291	0.937
PAINT	630.	0.0053	1604.	0.0136	74	2.546
SUBTOTAL	185530.	1.5696	42571.	0.3602	1548	0.229

TOTAL MATERIAL AND MANPOWER COST =USD 228100. INST'L COST/PE RATIO = 1.930

Figure C-6 Detailed cost analysis of unit operation: RSTOIC-2

ORIGIN	ITEM TYPE	ITEM DESCRIPTION	DESIGN DATA			PURCHASED EQUIPMENT COST USD
Equipment mapped from 'RSTOIC-3'.						
AT - 11 REACTOR	RSTOIC-3		Shell material	A285C		130000
	CODE OF ACCOUNT: 132		Liquid volume	8.107	M3	
	TAG NO.: RSTOIC-3		Vessel diameter	1.372	M	
			Vessel tangent to tangent height	5.486	M	
			Agitator power	37.50	KW	
			Impeller speed	583.00	RPM	
			Design temperature	224.59	DEG C	
			Design gauge pressure	243.67	KPAG	
			Application	CONT		
			Base material thickness	20.00	MM	
			Fluid depth	5.182	M	
			Jacket type	FULL		
			Jacket design gauge pressure	620.00	KPAG	
			Total weight	8900	KG	

ITEM	MATERIAL USD	MATERIAL FRACTION OF PE	MANPOWER USD	MANPOWER FRACTION OF PE	MANHOURS	L/M RATIO USD/USD
EQUIPMENT&SETTING:	130000.	1.0000	949.	0.0073	30	0.007
PIPING	15456.	0.1189	16626.	0.1279	556	1.076
CIVIL	2317.	0.0178	2648.	0.0204	113	1.143
STRUCTURAL STEEL	6651.	0.0512	1273.	0.0098	46	0.191
INSTRUMENTATION	33289.	0.2561	11146.	0.0857	371	0.335
ELECTRICAL	2268.	0.0174	2097.	0.0161	73	0.925
INSULATION	8607.	0.0662	7724.	0.0594	351	0.897
PAINT	633.	0.0049	1610.	0.0124	74	2.544
SUBTOTAL	199220.	1.5325	44074.	0.3390	1614	0.221

TOTAL MATERIAL AND MANPOWER COST =USD 243300. INST'L COST/PE RATIO = 1.872

Figure C-7 Detailed cost analysis of unit operation: RSTOIC-3

Appendix D: Supplementary information for regional carbon footprint analysis of dairy feeds for milk production in the USA

This is a License Agreement between Felix Adom ("You") and Springer ("Springer") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Springer, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3054331358205
License date	Dec 22, 2012
Licensed content publisher	Springer
Licensed content publication	The International Journal of Life Cycle Assessment
Licensed content title	Regional carbon footprint analysis of dairy feeds for milk production in the USA
Licensed content author	Felix Adom
Licensed content date	Jan 1, 2012
Volume number	17
Issue number	5
Type of Use	Thesis/Dissertation
Portion	Full text
Number of copies	1
Author of this Springer article	Yes and you are a contributor of the new work
Order reference number	None
Title of your thesis / dissertation	THE POTENTIAL OF INDUSTRIAL WASTE AND AGRICULTURAL FEEDSTOCK TOWARDS SUSTAINABLE BIOFUELS PRODUCTION: TECHNO-ECONOMIC AND ENVIRONMENTAL IMPACT PERSPECTIVES
Expected completion date	Dec 2012
Estimated size(pages)	318
Total	**0.00 USD**

Figure D-1: Copyright clearance from Springer

Figure D-1 above applies to chapter 6 including all supplementary materials in Appendix-D

Table D-1 Region 1 Grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating	Dry
alfalfa hay	0.26	1.46	1.12	2.08	1.87	2.12	1.74
alfalfa silage	0.95	5.28	7.93	3.36	7.39	6.86	4.48
canola meal	0.00	0.00	0.15	0.14	0.97	0.96	0.00
corn	0.08	0.45	0.09	1.56	5.37	5.86	0.29
corn silage	0.76	4.26	7.47	10.78	16.03	17.04	12.27
corn, hm	0.01	0.08	0.08	0.05	1.24	1.13	0.14
ddg, dry	0.03	0.15	0.54	0.57	1.14	1.14	0.44
grain mix	0.15	0.84	0.08	0.49	3.89	3.90	0.85
grass hay	0.06	0.31	0.89	1.57	0.09	0.09	1.80
grass silage	0.01	0.03	0.34	1.86	0.22	0.39	0.67
oat silage	0.00	0.01	0.46	0.00			0.00
protein mix	0.03	0.19	0.09	0.48	3.09	2.81	0.18
soybean meal	0.03	0.19	0.08	0.62	1.31	1.48	0.75
supplement	0.08	0.42	0.45	1.24	1.94	2.38	1.04
wheat silage	0.15	0.81	0.72			0.40	
wheat straw			0.12	2.01	0.11	0.17	2.36

Table D-2 Region 1 Non-grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating	Dry
alfalfa hay	1.15	0.48	0.72	0.96	3.42	3.15	0.09
alfalfa silage		0.54	0.24	0.65	0.45	0.37	0.10
corn	2.36	1.69	0.46	0.74	2.71	3.39	0.30
corn silage	0.96	1.32	1.00	2.66	1.83	2.50	0.24
corn, hm	0.05		0.11	0.07	1.96	1.97	
grain mix	0.33	0.31	0.18	0.58	2.75	2.76	0.06
grass hay	0.61		0.06	0.73			
pasture	20.02	8.74	17.28	17.72	25.18	26.09	1.57
pmr	0.15			1.20	1.14	1.89	
soy hulls	0.07	0.02	0.01	0.25	1.14	0.61	0.00
soybean meal	0.20	0.77	0.16	0.65	0.89	1.07	0.14
supplement	1.12	0.56	0.31	0.63	2.05	2.32	0.10
wheat midds		0.05	0.09		1.14	0.61	0.01

Table D-3 Region 2 Grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	0.23	1.19	4.44		1.53	1.07
alfalfa silage			0.70	0.31	2.70	2.48
bermudagrass hay				1.74		
citrus pulp				0.30	0.19	1.83
corn	0.04	0.20	3.26	0.41	2.81	1.64
corn silage	0.63	3.26	2.12	3.99	9.02	7.09
corn, hm	0.03	0.14	0.71	0.21	1.77	1.75
corn, hominy	0.07	0.36		0.56	0.40	2.54
cottonseed				0.16	0.61	1.31
cottonseed hulls			1.91	0.24	0.61	1.17
ddg, dry	0.65	3.36	0.15	0.52	3.48	3.13
grain mix					1.09	1.10
grass hay	0.38	1.97	1.39	12.90	0.56	0.57
grass silage	0.41	2.15			1.55	0.52
protein mix	0.00	0.02	0.03	0.69	0.43	0.48
rye haylage						1.82
sorghum silage			2.24			
soy hulls	0.08	0.39	0.11	3.31	0.78	0.80
soybean meal	0.05	0.25	2.09	0.62	1.73	2.36
supplement	0.05	0.24	0.32	1.13	1.16	1.20
wheat straw					0.95	0.41

Table D-4 Region 2 Non-grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
citrus pulp	1.98	1.32	1.56	2.77	4.86	4.46
corn	0.72	0.56	2.05	1.26	2.40	2.95
corn, hominy		0.59			1.32	1.74
cottonseed					2.80	2.37
cottonseed hulls	0.57	0.38		0.79	1.21	1.21
ddg, dry	1.11	1.04	0.48	1.10	1.50	2.45
grain mix		0.08			0.94	0.73
grass hay	1.30	1.83	5.72	0.91	0.88	0.62
pasture	15.35	6.01	7.73	17.46	10.45	12.16
soy hulls	0.39	0.63	0.06	0.27	2.04	1.44
soybean meal	1.46	0.83	1.35	1.85	2.48	2.44
supplement	0.59	0.27	0.54	0.69	0.50	0.71

Table D-5 Region 3 Grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	0.37	2.19	1.61	0.71	1.56	1.45
alfalfa silage	0.67	3.89	4.40	1.45	8.54	9.35
corn	0.12	0.72	0.04	0.81	6.89	6.64
corn gluten feed	0.05	0.28	0.62	0.97	2.04	2.21
corn silage	0.76	4.44	7.70	10.26	15.31	16.26
corn, hm	0.05	0.29	0.05	0.43	2.57	3.31
ddg, dry	0.06	0.36	0.46	0.91	1.66	1.24
grain mix	0.09	0.53	0.18	0.27	1.02	0.95
grass hay	0.21	1.20	0.98	1.42	0.11	0.22
oat silage	0.01	0.08	0.46			
protein mix	0.03	0.15	0.75	1.25	3.22	3.21
soybean meal	0.04	0.25	0.71	1.29	1.70	1.83
soybean, roasted	0.00	0.01	0.00	0.02	0.92	1.00
supplement	0.08	0.45	0.74	1.57	2.24	2.48
wheat straw	0.06	0.34	3.12	5.18	0.69	0.71

Table D-6 Region 3 Non-grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	0.83	0.07	0.07	0.40	0.55	0.62
corn	1.02	0.43	0.39	0.34	3.94	3.52
corn gluten feed	3.22		0.05		1.31	1.43
corn silage			1.06	0.26	0.62	0.36
corn, hm		0.72	0.67		4.84	5.74
cottonseed					1.89	2.03
ddg, dry				4.32	3.61	3.28
grain mix	1.07	0.73	0.62	0.60	1.47	1.77
pasture	21.01	12.82	18.54	18.65	26.99	28.41
protein mix		0.12	0.11	0.03	0.99	1.44
soybean meal		0.12	0.02	1.11	0.91	0.80
supplement	0.19	0.16	0.28	0.84	1.34	1.46

Table D-7 Region 4 Grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	0.81	5.51	5.74	6.88	10.29	10.11
alfalfa silage	0.26	1.75	3.53	1.08	6.24	5.91
barley				1.01	1.83	2.57
bermudagrass hay	0.01	0.06	0.26	0.27		
canola meal	0.02	0.14	0.13	0.49	0.84	1.17
corn	0.06	0.41	0.14	2.37	5.15	5.62
corn gluten feed	0.14	0.95	0.67	0.29	0.77	0.75
corn silage	0.24	1.65	1.46	7.21	9.70	10.13
corn steep liquor	0.08	0.55	0.66			
corn, hm	0.01	0.06	0.01	0.28	2.86	2.73
cotton gin trash	0.13	0.86	1.62			0.13
cottonseed			0.03	0.64	2.49	2.65
ddg, dry	0.13	0.90	0.96	0.42	2.57	2.58
grain mix	0.00	0.01	0.01	0.19	0.72	1.07
grass hay	0.12	0.83	1.03	2.14	0.02	0.10
molasses	0.01	0.04	0.13	0.16	1.00	0.85
oat hay			0.75	0.19		
oat silage	0.06	0.39	2.35	0.01		
oat straw	0.07	0.46				
protein mix	0.00	0.00	0.34	0.58	1.44	1.22
ryegrass silage	0.05	0.32	0.30		0.42	0.41
sorghum silage	0.23	1.58	1.82	0.77	0.76	0.61
soybean meal	0.00	0.01	0.01	0.37	0.82	0.51
sudangrass hay	0.01	0.05	0.13	0.10		
supplement	0.04	0.26	0.30	1.48	1.31	2.28
wheat hay			0.33	0.23	0.18	0.12
wheat straw	0.14	0.92	0.33	0.90	0.15	0.08

Table D-8 Region 4 Non-grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	1.73	1.37	0.50		3.66	2.07
alfalfa silage					4.58	2.56
barley		6.27	6.53			
corn	0.82	0.14	0.27	4.02	8.43	13.50
corn gluten feed		0.56	0.24			
corn silage	1.15	0.92	0.28		4.42	2.47
cotton gin trash		1.16	0.93			
ddg, dry	0.68	0.04	0.24	0.01	2.35	1.55
pasture	26.28	6.95	12.69	21.98	15.57	23.05
protein mix	0.03	0.01		0.04	1.62	2.14
sorghum grain	0.14	0.01	0.24		4.87	
soybean meal	0.14	0.01	0.01	0.15	0.72	2.14
soybean, extruded	0.12				2.44	1.36
supplement	0.32	0.28	0.41	1.87	0.91	0.76
wheat hay			0.73			

Table D-9 Region 5 Grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
alfalfa hay	0.50	2.60	3.52	4.85	7.68	7.74
alfalfa silage	0.01	0.03	0.82	0.07	0.88	0.87
almond hulls	0.11	0.60	1.45	0.39	2.44	2.87
barley					0.54	0.66
canola meal	0.09	0.47	0.61	0.54	2.64	2.26
citrus pulp	0.07	0.36	0.34		0.46	0.49
corn	0.21	1.09	0.11	2.53	5.00	5.91
corn dust						
corn gluten feed			0.27	0.54	1.27	1.35
corn screenings					0.81	0.12
corn silage	0.35	1.83	1.91	8.58	8.71	8.98
corn stover	0.01	0.04	0.45			
corn, hominy					0.55	0.44
cottonseed				0.20	0.92	1.00
ddg, dry	0.13	0.71	0.44	0.84	3.59	3.19
grain mix	0.07	0.34	0.07	0.47	1.12	1.55
grape pomace	0.01	0.05	0.82			
grass hay			0.32	0.04	0.18	0.06
oat hay	0.13	0.70	1.22	1.51	0.35	0.33
oat silage	0.23	1.20	2.17	0.21		0.24
pea silage						
soybean meal	0.02	0.10	0.03	0.20	0.37	0.64
sugar	0.02	0.13	0.26		0.09	0.16
supplement	0.11	0.56	0.33	2.63	1.32	2.59
wheat hay				0.45	0.03	
wheat midds	0.06	0.32			0.16	0.14
wheat mill run				0.33	0.92	0.66
wheat silage	0.28	1.46	2.37	0.43	0.48	0.53
wheat straw	0.19	1.00	1.83	0.25	0.21	0.17
whey			0.01		0.80	0.85

Table D-10 Region 5 Non-grazing season average ration (All values reported as pounds of dry matter intake per day)

Feed	Calves	Open Heifers	Bred Heifers	Springers	First Calf Heifers	Lactating
grain mix				1.20	2.90	6.53
pasture	5.12	4.60	10.34	5.87	20.50	19.00
pmr	22.39	9.00	9.00	17.98	18.13	18.25

Section D: Animal Feedstuffs and Grazing Practices

D1. Please print (or ask your nutritionist to provide) and attach all of your fed rations for each of the following animal classes during both grazing and non-grazing seasons. Please write the animal class name (see list below) and "non-grazing season" or "grazing season" on each attached ration. If you cannot provide details of your ration, please attach feed composition information (feed tags) and label with each animal class/season.

Please list the average number of head in each animal class, corresponding to the rations (or feed compositions). Note: The numbers may be the same across non-grazing and grazing seasons.

Animal Class	Non-Grazing Season	Grazing Season
Newborn Calves	head	head
Open Heifers	head	head
Bred Heifers	head	head
Springers and Close-ups	head	head
First-Calf Heifers	head	head
Mature Cows	head	head
Dry Cows	head	head

D2. Do the rations you printed for question D1 above include the pounds per day fed to each class of animal on either an as-fed or dry-matter basis (DMI)?

☐ yes (skip to question D4) ☐ no (continue with question D3)

D3. For each ration, indicate how many pounds were fed daily to each class of animal. Do not include forage consumed while grazing, which is covered in another question. Indicate below whether you are reporting rations on an as-fed basis or a dry-matter basis. In the table on the following page, if you have more than one ration to report per animal class, use a separate line in each box for each ration, and be sure to name the ration. If you need more space, attach a separate sheet or use the space at the end of section D.

As-Fed vs. Dry-Matter Rations: Rations contain water, whether they are concentrate feedstuffs or forages. As-fed means the feed or forage as it is fed to animals, including the moisture content. Dry-matter intake (DMI) is the feed on a moisture-free basis. Use whichever format is easiest, but indicate which you are using:

☐ as-fed basis ☐ dry-matter (DMI) basis

Figure D-2 Dairy producer life cycle assessment survey and How-To guide (a)

Animal Class	Non-Grazing Season Rations (lbs per day per animal class)	Grazing Season Ration (lbs per day per animal class)
Newborn Calves (0-3 months) Please describe milk and milk replacer feeding regimes (amounts given and units)		
Newborn Calves (0-3 months) Feed rations (not milk or milk replacer)	(lbs/day)	(lbs/day)
Open Heifers	(lbs/day)	(lbs/day)
Bred Heifers	(lbs/day)	(lbs/day)
Springers and Close-ups	(lbs/day)	(lbs/day)
First-Calf Heifers	(lbs/day)	(lbs/day)
Mature Cows	(lbs/day)	(lbs/day)
Dry Cows	(lbs/day)	(lbs/day)

D4. In 2008 did you feed any agricultural byproducts (cottonseed, citrus pulp, DDGs, etc.) that are not included in the rations you described/attached in questions D1 through D3?

☐ yes (continue with question D5)

☐ no (skip to question D6)

D5. If you also fed agricultural by-products (cottonseed, citrus pulp, DDGs, etc.) and they were NOT included in your fed rations described above, please describe the average by-product feeding regime for each class of animal here. Please list each average by-product ration for each class of animal (in pounds per day) throughout the year. Attach additional paper (or use the space below question D8), if necessary.

Are you reporting pounds per day fed on an as-fed basis or dry-matter basis?

☐ as-fed basis

☐ dry-matter (DMI) basis

Figure D-3 Dairy producer life cycle assessment survey and How-To guide (b)

Animal Class	By-Product	Non- Grazing Season (lbs per day per animal class)	Grazing Season (lbs per day per animal class)
Newborn Calves	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
Open Heifers	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
Bred Heifers	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
Springers and Close-ups	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
First-Calf Heifers	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
Mature Cows	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)
Dry Cows	_____	_____(lbs / day)	_____(lbs / day)
	_____	_____(lbs / day)	_____(lbs / day)

D6. In 2008, how many acres were dedicated primarily to grazing?

_____ acres

If the answer to this question is zero (0), please move to question D8.

Figure D-4 Dairy producer life cycle assessment survey and How-To guide (c)

D7. Please indicate with an X the months in 2008 during which your cattle were receiving the majority of their forage intake from pasture. For example, if your cattle are kept on pasture all year, only check months during which your cattle are feeding on forage actually growing on the pasture. If you feed baled hay November through February, but no forage is being eaten off the pasture, do not check those months.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Open Heifers												
Bred Heifers												
Springers and Close-ups												
First-Calf Heifers												
Mature Cows												
Dry Cows												

D8. Please list any additional details regarding your feeding regimen that could help us understand the attached feed information and/or how you fed your cattle in 2008.

Figure D-5 Dairy producer life cycle assessment survey and How-To guide (d)

Table D-11 Soybean (GHG profile lb CO_2 eq per lb dry soybean)

Region 1-Inventory Data For Soybean										
Dairy Production States	**Productivity Data**			**Soil Amendment Chemical Input Data**						**GHG Profile**
	Area Harvested (1000 acres)	**Yield (Bushels / acre)**	**Production (1000 lbs)**	**Nitrogen-N (Million lbs)**	**Nitrogen in Residue / mass soybean**	**Phosphorous-P (Million lbs)**	**Potash-K (Million lbs)**	**Sulfur-S (Million lbs)**	**Lime (1000 lbs)**	**lb CO_2 eq./lb Soybean**
Maine										0.410
New Hampshire										
Vermont										
Massachusetts										
Rhode Island										
Connecticut										
New York	197	42	420,250							
Pennsylvania	426	42	886,000							
New Jersey	90	33	149,740							
Delaware	183	31	282,930							
Maryland	461	34	783,500							
Total	**1,357**	**37**	**2,522,420**							
lb Input/lb Soybeans										
lb CO_2 eq./lb Soybean										
Contribution to regional GHG profile										

Region 1-Inventory Data For Soybean (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gal./acre)**	**Diesel (Gal./acre)**	**LP (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
Maine								
New Hampshire								
Vermont								
Massachusetts								
Rhode Island								
Connecticut								
New York								
Pennsylvania								
New Jersey								
Delaware								
Maryland								
Total								
lb Input/lb Soybeans								
lb CO_2 eq./lb Soybean								
Contribution to regional GHG profile								

Region 2-Inventory Data For Soybean										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass soybean	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (1000 lbs)	lb CO_2 eq./lb Soybean
Virginia	524	32	837,700	3.90		7	15	0	403,323	
West Virginia	17	39	32,950							
Kentucky	1,278	39	2,479,700	14.60		35	45		1,106,237	
Tennessee	1,176	34	2,030,800	12.00		28	64	1	974,081	
North Carolina	1,474	30	2,194,100	11.00		26	51		962,375	
South Carolina	462	25	593,300							
Georgia	257	28	374,200							
Florida	14	31	23,590							
Alabama	204	29	305,700							
Mississippi	1,656	36	2,991,550	1		9	26		198,720	
Arkansas	3,058	36	5,573,200	0.90		60	94		164,215	**0.520**
Louisiana	846	36	1,481,200	0.40		5	9		59,812	
Total	**10,966**	**34**	**18,917,990**	**44**		**171**	**304**	**1**	**3,868,762**	
lb Input/lb Soybeans				**0.0025**	**0.0146**	**0.0097**	**0.0173**	**0.0005**	**0.21996**	
lb CO_2 eq./lb Soybean				**0.0267**	**0.0905**	**0.0294**	**0.0099**	**0.0019**	**0.10025**	
Contribution to regional GHG profile				**6%**	**20%**	**7%**	**2%**	**0.4%**	**22%**	

Region 2-Inventory Data For Soybean (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Virginia	842	4						
West Virginia	1,385	9						
Kentucky	1,978	1	40					
Tennessee	1,866	8	43					
North Carolina	1,968	30	12					
South Carolina				2.55	8.54	0.01	0.08	
Georgia				3.4	6.84	0.11	21.83	
Florida								
Alabama				4.27	6.75	0.002		
Mississippi	3,770	65	30	2.52	7.44	0.24	4.3	0.02
Arkansas	4,317	96	26	4.18	9.19	0.07	6.81	0.01
Louisiana	1,664	499	66					
Total	**16,405**	**703**	**217**					
lb Input or gallon or kWh /lb Soybeans	**0.00093**	**0.00004**	**0.00001**	**0.00199**	**0.00456**	**0.00005**	**0.00485**	**0.00001**
lb CO_2 eq./lb Soybean	**0.01261**	**0.00031**	**0.00004**	**0.04808**	**0.11949**	**0.00086**	**0.00928**	**0.000002**
Contribution to regional GHG profile	**3%**	**0.07%**	**0.01%**	**10.70%**	**26.60%**	**0.19%**	**2.06%**	**0.0003%**

Region 3-Inventory Data For Soybean										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass soybean	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (1000 lbs)	lb CO_2 eq./ lb Soybean
Minnesota	6,868	41	13,890,750	15.3		53.2	57.4	0	1,248,602	
Wisconsin	1,548	40	3,066,950	7.4		18	74.2	2.3	587,156	
Michigan	1,928	40	3,829,350	5.9		19.5	96.7	0.2	623,322	
Ohio	4,448	44	9,870,400	11.9		40.5	171.4	1.7	1,755,181	
Indiana	5,360	48	12,965,900	15.2		54.6	177.4		3,584,768	
Illinois	9,356	47	22,047,050	18.1		96	290.2		5,566,820	
Missouri	4,936	39	9,653,150	10.9		45.7	76.2		4,040,116	
Iowa	9,710	50	24,259,800	10.8		64.4	172.6	0.9	2,780,944	
Total	**44,154**	**45**	**99,583,350**	**95.5**		**391.9**	**1116.1**	**5.1**	**20,186,910**	**0.330**
lb Input/lb Soybeans				**9.59E-04**	**1.23E-02**	**3.94E-03**	**1.12E-02**	**9.29E-05**	**0.2027**	
lb CO_2 eq./lb Soybean				**1.03E-02**	**7.63E-02**	**1.19E-02**	**6.42E-03**	**3.58E-04**	**0.0924**	
Contribution to regional GHG profile				**3.52%**	**26.15%**	**4.08%**	**2.20%**	**0.12%**	**31.66%**	

Region 3-Inventory Data For Soybean (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Minnesota	9715	896		3.03	5.68		0.21	
Wisconsin	2058	13						
Michigan	2390							
Ohio	6871	23	19	3.6	4.41	0.03	0.82	
Indiana	8,910		44	2.95	4.49	0.01	0.51	
Illinois	13,794	141	12	2.72	4.1	0.35		
Missouri	6577	28	70	2.97	3.84	0.41		
Iowa	13946	127		2.77	4.35			
Total	**64,261**	**1,228**	**145**					
lb Input or gallon or kWh /lb Soybeans	**6.45E-04**	**1.48E-05**	**2.66E-06**	**1.33E-03**	**1.98E-03**	**7.06E-05**	**1.13E-04**	
lb CO_2 eq./lb Soybean	**8.74E-03**	**1.17E-04**	**7.93E-06**	**0.032**	**0.052**	**1.19E-03**	**2.17E-04**	
Contribution to regional GHG profile	**3.0%**	**0.04%**	**0.003%**	**10.99%**	**17.76%**	**0.41%**	**0.07%**	

Region 4-Inventory Data For Soybean										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass soybean	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (1000 lbs)	lb CO_2 eq./lb Soybean
Texas	190	29	267,100							
Oklahoma	270	25	339,650							
Nebraska	4,626	49	11,266,700	20.2		70.4	15.8	8	573,161	
Kansas	2,900	36	5,215,000	10.5		32	8.8		425,430	
North Dakota	3,432	31	5,203,900	22.6		58.3	1.9			
South Dakota	3,824	36	6,798,500	19.7		49.4	8.6			
Montana										
Idaho										
Colorado										
Wyoming										
Utah										0.390
New Mexico										
Arizona										
Nevada										
Total	**15,242**	**38**	**29,090,850**	**73**		**210**	**35**	**8**	**998,591**	
lb Input/lb Soybeans				**2.5628E-03**	**1.4011E-02**	**7.38E-03**	**1.23E-03**	**7.10E-04**	**6.06E-02**	
lb CO_2 eq./lb Soybean				**2.74E-02**	**8.69E-02**	**2.23E-02**	**7.06E-04**	**2.74E-03**	**2.76E-02**	
Contribution to regional GHG profile				**8.03%**	**25.43%**	**6.53%**	**0.21%**	**0.80%**	**8.08%**	

Region 4-Inventory Data For Soybean (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Texas								
Oklahoma								
Nebraska	7,837	129		4.09	9.63	4.96	50.44	1.15
Kansas	4,386	7		3.95	3.98		1.98	0.28
North Dakota	4,982	480						
South Dakota	5,620	111		2.76	4.85	0.12	18.93	
Montana								
Idaho								
Colorado								
Wyoming								
Utah								
New Mexico								
Arizona								
Nevada								
Total	**22,825**	**727**						
lb Input or gallon or kWh /lb Soybeans	**8.01E-04**	**2.55E-05**		**1.76E-03**	**3.00E-03**	**1.24E-03**	**1.16E-02**	**3.49E-04**
lb CO_2 eq./lb Soybean	**1.09E-02**	**2.01E-04**		**0.042**	**0.079**	**2.09E-02**	**2.10E-02**	**5.94E-05**
Contribution to regional GHG profile	**3.17%**	**0.06%**		**12.41%**	**23.00%**	**6.12%**	**6.15%**	**0.02%**

Region 5-Inventory Data For Soybean										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass soybean	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (1000 lbs)	lb CO_2 eq./lb Soybean
California										
Oregon										
Washington										
Alaska										
Hawaii										0.410
Total										
lb Input/lb Soybeans										
lb CO_2 eq./lb Soybean										
Contribution to regional GHG profile										

Region 5-Inventory Data For Soybean (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gal./acre)**	**Diesel (Gal./acre)**	**LP (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
California								
Oregon								
Washington								
Alaska								
Hawaii								
Total								
lb Input/lb Soybeans								
lb CO_2 eq./lb Soybean								
Contribution to regional GHG profile								

Table D-12 Corn grain (GHG profile lb CO_2 eq per lb dry corn grain)

Region 1-Inventory Data For Corn grain											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/acre)	lb CO_2 eq./lb Corn grain
Maine											0.360
New Hampshire											
Vermont											
Massachusetts											
Rhode Island											
Connecticut											
New York	526	129.4	3836224	62.2	82		33.2	34.9		500	
Pennsylvania	952	128.2	6832000	108.4	65		40.7	37.4	3	500	
New Jersey	70.8	126.8	502521.6								
Delaware	161	132.8	1186505.6								
Maryland	423	130.4	3077088								
Total	**2133**	**129**	**15,434,339**	**171**	**147**		**74**	**72**	**3**	**1000**	
lb Input/lb Corn grain				**0.0094**	**0.0081**	**0.00759**	**0.0041**	**0.0040**	**0.0002**	**0.041**	
lb CO_2 eq./lb Corn grain				**0.1005**	**0.0500**	**0.04709**	**0.0123**	**0.0023**	**0.0008**	**0.018**	
Contribution to regional GHG profile				**33.29%**	**16.57%**	**15.59%**	**4.08%**	**0.75%**	**0.26%**	**6.1%**	

Region 1-Inventory Data For Corn grain (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**		**Energy Input Data**					
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Diesel (Gal./acre)**	**Gasoline (Gal./acre)**	**LPG (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**	**Storage moisture content per SimaPro model**
Maine								
New Hampshire								
Vermont								
Massachusetts								
Rhode Island								
Connecticut								
New York	2,325		8.2	3.1	0.4			
Pennsylvania	3,346	154	6.5	1.6	1.5			
New Jersey								
Delaware								
Maryland								
Total	**5,671**	**154**						
lb Input or gallon /lb Corn grain	**0.000312**	**0.000008**	**0.000986**	**0.000296**	**0.000154**			
lb CO_2 eq./lb Corn grain	**0.0024**	**0.0001**	**0.0259**	**0.0072**	**0.0026**			**0.03234**
Contribution to regional GHG profile	**0.78%**	**0.04%**	**8.56%**	**2.37%**	**0.86%**	**0.00%**	**0.00%**	**10.71%**

Region 2-Inventory Data For Corn Grain											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/acre)	lb CO_2 eq./lb Corn Grain
Virginia	362	116	41,526								0.440
West Virginia	27	123	3,270								
Kentucky	804	109	86,800								
Tennessee	1,164	139	160,944								
North Carolina	299	128	38,364	90.5			25.5	53.1	1.1	1000	
South Carolina	626	124	76,806	210.5			75.5	86.9		1000	
Georgia	311	98	30,165	38.7			16.1	24.5	2.5	1000	
Florida	32	92	2,959								
Alabama	215	99	21,189								
Mississippi	548	133	75,071								
Arkansas	347	148	53,094								
Louisiana	454	144	66,652								
Total	**5,189**	**127**	**656,840**	**340**			**117**	**165**	**3.6**	**3000**	
lb Input/lb Corn grain				**0.0193**		**0.0076**	**6.6E-03**	**9.3E-03**	**4.5E-04**	**0.0213**	
lb CO_2 eq./lb Corn grain				**0.0584**		**0.0471**	**0.0201**	**0.0053**	**0.0017**	**0.0097**	
Contribution to regional GHG profile				**55.1%**		**12.6%**	**5.4%**	**1.4%**	**0.5%**	**2.6%**	

Region 2-Inventory Data For Corn Grain (cont.)								
Dairy Production States	Crop Protection Chemical Input Data		Energy Input Data					
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Diesel (Gal./acre)	Gasoline (Gal./acre)	LPG (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)	Storage moisture content per SimaPro model
Virginia								
West Virginia								
Kentucky								
Tennessee								
North Carolina	1,669	130	6.2	2.3	2.2			
South Carolina	3,187	26	3.6	2	2.5	58.7	67.5	
Georgia	495	25	14.8	2.5	0.5	29.6		
Florida								
Alabama								
Mississippi								
Arkansas								
Louisiana								
Total	**5,351**	**181**						
lb Input or gallon or kWh/lb Corn grain	**3.04E-04**	**1.03E-05**	**8.44E-04**	**3.06E-04**	**3.00E-04**	**6.90E-03**	**8.68E-03**	
lb CO_2 eq./lb Corn grain	**0.0023**	**0.0001**	**0.022**	**0.007**	**0.005**	**0.013**	**0.001**	**0.032**
Contribution to regional GHG profile	**0.6%**	**0.04%**	**5.9%**	**2.0%**	**1.4%**	**3.5%**	**0.4%**	**8.6%**

Region 3-Inventory Data For Corn Grain											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/ acre)	lb CO_2 eq./lb Corn Grain
Minnesota		161	64,317,120	954	128		378.1	400.3	8.2	500	
Wisconsin	2,892	140	22,630,272	381	102		118.8	191.7	9.1	500	
Michigan	2,074	137	15,858,864	278	1		89.6	148.4	3.7	500	
Ohio	3,210	149	26,762,064	552			224.9	264.5	3.2	500	
Indiana	5,702	158	50,532,832	869			420.2	648.2	8.1	500	
Illinois	11,930	168	112,315,280	1,728	88		780.4	1160.5	14.9	500	
Missouri	2,880	139	22,383,984	490	1		149.5	180.1	10	500	
Iowa	12,790	172	123,454,240	1,653	386		579	762.3	4.5	500	**0.370**
Total	**48,638**	**161**	**438,254,656**	**6,905**	**386**		**2740.5**	**3756**	**61.7**		
lb Input/lb Corn grain				**0.0151**	**0.0015**	**0.0076**	**0.0060**	**0.0082**	**0.0001**	**0.0013**	
lb CO_2 eq./lb Corn grain				**0.1621**	**0.0096**	**0.04709**	**0.01819**	**0.00471**	**0.00052**	**0.00061**	
Contribution to regional GHG profile				**52%**	**3.09%**	**15.16%**	**5.86%**	**1.52%**	**0.17%**	**0.20%**	

Region 3-Inventory Data For Corn Grain (cont.)								
Dairy Production States	Crop Protection Chemical Input Data		Energy Input Data					
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Diesel (Gal./acre)	Gasoline (Gal./acre)	LPG (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)	Storage moisture content per SimaPro model
Minnesota	10,361	214	5.4	1.7	8.5	26.8	45.8	
Wisconsin	6,369	134	7.4	1.4	1.9	6.6	124	
Michigan	5,145	153	7.2	2.5	3.6	25.5	223.1	
Ohio	9,322	215	4.3	1.6	5.6	10	164	
Indiana	14,136	722	4.6	2.1	3.2	28.3	144.2	
Illinois	30,967	1,426	3.7	1.5	2.8	9.6	76.9	
Missouri	7,707	41	5	2.5	3.6	25.5	223.1	
Iowa	24,726	187	4.6	1.2	7.2	16.8		
Total	**108,733**	**3,092**						
lb Input or gallon or kWh/lb Corn grain	**2.4E-04**	**6.8E-06**	**5.30E-04**	**1.80E-04**	**5.61E-04**	**1.96E-03**	**1.29E-02**	
lb CO_2 eq./lb Corn grain	**0.00180**	**0.00010**	**0.01389**	**0.00435**	**0.00947**	**0.00375**	**2.19E-03**	**3.23E-02**
Contribution to regional GHG profile	**0.58%**	**0.03%**	**4.47%**	**1.40%**	**3.05%**	**1.21%**	**0.71%**	**10.41%**

Region 4-Inventory Data For Corn Grain											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/ acre)	lb CO_2 eq./lb Corn Grain
Texas	1,796	129	13,050,016	282.0	28		73.9	10.6	6.9	500.0	
Nebraska	8,340	159	74,299,120	1162.5	38		237.3	38.8	35	500.0	
Kansas	3,328	134	25,054,512	482.1			112.7	34.9	5.3	500.0	
South Dakota	4,020	119	27,168,064	477.7			154.2	41.9	5.5	500.0	
Colorado	998	143	7,969,024	126.2	19		24.4	4.2	3.3	500.0	
North Dakota	1,680	117	11,074,000	169.3	0.4		58.8	13.3	0.9	500.0	
Montana	25	143	194,533								
Idaho	77	170	733,040								
Oklahoma	252	126	1,767,360								
Wyoming	51	133	379,938								
Utah	17	156	150,035								0.440
New Mexico	53	180	537,712								
Arizona	21	186	210,056								
Nevada											
Total	**20,658**	**140**	**162,587,410**	**2,826**	**104**		**686**	**148**	**60**		
lb Input/lb Corn grain				**0.01617**	**0.00051**	**0.00754**	**0.00396**	**8.61E-04**	**3.41E-04**	**2.40E-03**	
lb CO_2 eq./lb Corn grain				**0.1732**	**0.0032**	**0.0468**	**1.20E-02**	**4.93E-04**	**1.31E-03**	**1.10E-03**	
Contribution to regional GHG profile				**46.42%**	**0.85%**	**12.54%**	**3.22%**	**0.13%**	**0.35%**	**0.29%**	

Region 4-Inventory Data For Corn Grain (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**		**Energy Input Data**					
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Diesel (Gal./acre)**	**Gasoline (Gal./acre)**	**LPG (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**	**Storage moisture content per SimaPro model**
Texas	3,344	236	8.9	1.1	1.5	49.4	6236.8	
Nebraska	18,416	456	12.4	2.1	4.1	152.5	964	
Kansas	1,094	89	9.2	1.3	13.1	16.9	1531.7	
South Dakota	6,036	239	4.4	1.5	0.5	27.4	7	
Colorado	1,494	252	9	1.6	3.8	390.3		
North Dakota	6,036		4.5	2.1	7.1			
Montana								
Idaho								
Oklahoma								
Wyoming								
Utah								
New Mexico								
Arizona								
Nevada								
Total	**37,914**	**1,763**						
lb Input or gallon or kWh/lb Corn grain	**2.18E-04**	**7.62E-06**	**1.16E-03**	**2.21E-04**	**6.21E-04**	**0.013**	**0.175**	
lb CO_2 eq./lb Corn grain	**1.65E-03**	**1.08E-04**	**0.0305**	**0.0053**	**0.0105**	**0.0247**	**0.0298**	**0.0323**
Contribution to regional GHG profile	**0.44%**	**0.03%**	**8.19%**	**1.43%**	**2.81%**	**6.63%**	**7.99%**	**8.67%**

Region 5-Inventory Data For Corn Grain											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/ acre)	lb CO_2 eq./lb Corn Grain
California											
Oregon											
Washington											
Alaska											
Hawaii											
Total											
lb Input/lb Corn grain											**0.400**
lb CO_2 eq./lb Corn grain											
Contribution to regional GHG profile											

Region 5-Inventory Data For Corn Grain (cont.)								
Dairy Production States	Crop Protection Chemical Input Data		Energy Input Data					
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Diesel (Gal./acre)	Gasoline (Gal./acre)	LPG (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)	Storage moisture content per SimaPro model
California								
Oregon								
Washington								
Alaska								
Hawaii								
Total								
lb Input or gallon or kWh/lb Corn grain								
lb CO_2 eq./lb Corn grain								
Contribution to regional GHG profile								

Table D-13 Corn Silage (Yield: wet short tons/ acre & GHG profile Ib CO2 eq per lb dry corn silage)

Region 1-Inventory Data For Corn Silage											
Dairy Production States	**Productivity Data**			**Soil Amendment Chemical Input Data**							**GHG Profile**
	Area Harvested (1000 acres)	**Yield (tons / acre)**	**Production (1000 lbs)**	**Nitrogen-N (Million lbs)**	**Nitrogen From manure (Million lbs)**	**Nitrogen in Residue / mass corn grain**	**Phosphorous-P (Million lbs)**	**Potash-K (Million lbs)**	**Sulfur-S (Million lbs)**	**Lime (lbs/ acre)**	**lb CO_2 eq./lb Corn silage**
Maine	25	18	896,000								**0.160**
New Hampshire	14	20	560,400								
Vermont	87	18	3,176,000								
Massachusetts	16	20	647,200								
Rhode Island	2	20	80,800								
Connecticut	25	20	1,007,600								
New York	480	18	17,038,000	62.2	82		33.2	34.9		500	
Pennsylvania	408	18	14,520,000	108.4	65		40.7	37.4	3	500	
New Jersey	13	17	448,800								
Delaware	6	16	202,000								
Maryland	61	13	1,567,200								
Total	**1137**	**17.67**	**40,144,000**	**171**	**147**		**74**	**72**	**3**		
lb Input/lb Corn silage				**0.0022**	**0.0019**	**0.001027**	**0.000967**	**0.00094**	**0.000110**	**0.0058**	
lb CO_2 eq./lb Corn silage				**0.0239**	**0.0119**	**0.0064**	**0.0029**	**0.0005**	**0.0004**	**0.0026**	
Contribution to regional GHG profile				**42.06%**	**20.93%**	**11.21%**	**5.15%**	**0.95%**	**0.75%**	**4.66%**	

Region 1-Inventory Data For Corn Silage(cont.)							
Dairy Production States	**Crop Protection Chemical Input Data**		**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Diesel (Gal./acre)**	**Gasoline (Gal./acre)**	**LPG (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
Maine							
New Hampshire							
Vermont							
Massachusetts							
Rhode Island							
Connecticut							
New York	2,325		8.2	3.1	0.4		
Pennsylvania	3,346	154	6.5	1.6	1.5		
New Jersey							
Delaware							
Maryland							
Total	**5,671**	**154**					
lb Input or gallon/lb Corn silage	**0.000074**	**0.000002**	**0.000208**	**0.000068**	**0.000025**		
lb CO_2 eq./lb Corn silage	**0.00056**	**0.00003**	**0.0055**	**0.0016**	**0.0004**		
Contribution to regional GHG profile	**0.99%**	**0.05%**	**9.61%**	**2.88%**	**0.76%**	**0.00%**	**0.00%**

Region 2-Inventory Data For Corn Silage											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (tons / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs /acre)	lb CO_2 eq./lb Corn silage
Virginia	129	17	4,368,000								
West Virginia	17.6	16	564,800								
Kentucky	56	16	1,794,000								
Tennessee	75	16	2,388,400								
North Carolina	41	18	1,438,000	90.5			25.5	53.1	1.1	1000	
South Carolina	52.4	16	1,670,800	210.5			75.5	86.9		1000	
Georgia	15.6	14	400,800	38.7			16.1	24.5	2.5	1000	
Florida	29.6	18	1,051,600								
Alabama	11	13	288,000								
Mississippi	13	14	360,000								**0.260**
Arkansas	4.4	14	123,600								
Louisiana	5	15	152,000								
Total	**449.6**	**16.26**	**14,600,000**	**340**			**117**	**165**	**3.6**	**3000**	
lb Input/lb Corn silage				**0.0055**		**0.0010**	**0.0019**	**0.0026**	**0.0001**	**0.0028**	
lb CO_2 eq./lb Corn silage				**0.0584**		**0.006**	**0.00569**	**0.0015**	**0.0005**	**0.001**	
Contribution to regional GHG profile				**68.0 %**		**7.4%**	**6.6%**	**1.8%**	**0.6%**	**1.5%**	

Region 2-Inventory Data For Corn Silage(cont.)							
Dairy Production States	**Crop Protection Chemical Input Data**		**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Diesel (Gal./acre)**	**Gasoline (Gal./acre)**	**LPG (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
Virginia							
West Virginia							
Kentucky							
Tennessee							
North Carolina	1,669	130	6.2	2.3	2.2		
South Carolina	3,187	26	3.6	2	2.5	58.7	67.5
Georgia	495	25	14.8	2.5	0.5	29.6	
Florida							
Alabama							
Mississippi							
Arkansas							
Louisiana							
Total	**5,351**	**181**					
lb Input or gallon or kWh/lb Corn silage	**8.59E-05**	**2.90E-06**	**2.2E-04**	**6.8E-05**	**5.9E-05**	**1.46E-03**	**2.11E-03**
lb CO_2 eq./lb Corn silage	**0.000648**	**0.000041**	**0.0057**	**0.0016**	**0.0010**	**0.0028**	**0.0004**
Contribution to regional GHG profile	**0.8%**	**0.0%**	**6.6%**	**1.9%**	**1.2%**	**3.3%**	**0.4%**

Region 3-Inventory Data For Corn Silage											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (tons / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/acre)	lb CO_2 eq./lb Corn silage
Minnesota	410	15	12,510,000	954	128		378.1	400.3	8.2	500	
Wisconsin	856	16	27,841,200	381	102		118.8	191.7	9.1	500	
Michigan	254	17	8,397,200	278	1		89.6	148.4	3.7	500	
Ohio	164	17	5,576,000	552			224.9	264.5	3.2	500	
Indiana	112	20	4,482,000	869			420.2	648.2	8.1	500	
Illinois	106	18	3,726,000	1,728	88		780.4	1160.5	14.9	500	
Missouri	68	14	1,874,000	490	1		149.5	180.1	10	500	
Iowa	226	19	8,714,000	1,653	386		579	762.3	4.5	500	0.190
Total	**2196**	**16.66**	**73,120,400**	**6,905**	**386**		**2740.5**	**3756**	**61.7**		
lb Input/lb Corn silage				**0.0037**	**0.0004**	**0.0010**	**0.0015**	**0.0020**	**3.33E-05**	**0.0006**	
lb CO_2 eq./lb Corn silage				**0.03994**	**0.0024**	**0.00637**	**0.00448**	**0.002**	**0.00013**	**0.0003**	
Contribution to regional GHG profile				**61.54%**	**3.64%**	**9.82%**	**6.91%**	**1.79%**	**0.20%**	**0.42%**	

Region 3-Inventory Data For Corn Silage(cont.)							
Dairy Production States	Crop Protection Chemical Input Data		Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Diesel (Gal./acre)	Gasoline (Gal./acre)	LPG (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Minnesota	10,361	214	5.4	1.7	8.5	26.8	45.8
Wisconsin	6,369	134	7.4	1.4	1.9	6.6	124
Michigan	5,145	153	7.2	2.5	3.6	25.5	223.1
Ohio	9,322	215	4.3	1.6	5.6	10	164
Indiana	14,136	722	4.6	2.1	3.2	28.3	144.2
Illinois	30,967	1,426	3.7	1.5	2.8	9.6	76.9
Missouri	7,707	41	5	2.5	3.6	25.5	223.1
Iowa	24,726	187	4.6	1.2	7.2	16.8	
Total	**108,733**	**3,092**					
lb Input or gallon or kWh/lb Corn silage	**5.87E-05**	**1.67E-06**	**1.82E-04**	**4.95E-05**	**1.29E-04**	**4.70E-04**	**3.84E-03**
lb CO_2 eq./lb Corn silage	**0.00044**	**0.00002**	**0.00478**	**0.00120**	**0.00218**	**0.00090**	**6.54E-04**
Contribution to regional GHG profile	**0.68%**	**0.04%**	**7.37%**	**1.84%**	**3.36%**	**1.39%**	**1.01%**

Region 4-Inventory Data For Corn Silage											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (tons / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/acre)	lb CO_2 eq./lb Corn Silage
Texas	146	20	5,904,000	282.0	28		73.9	10.6	6.9	500.0	
Nebraska	208	16	6,570,000	1162.5	38		237.3	38.8	35	500.0	
Kansas	190	16	5,728,000	482.1			112.7	34.9	5.3	500.0	
South Dakota	484	10	9,148,000	477.7			154.2	41.9	5.5	500.0	
Colorado	108	22	4,762,000	126.2	19		24.4	4.2	3.3	500.0	
North Dakota	201	9	3,687,600	169.3	0.4		58.8	13.3	0.9	500.0	
Montana	45	22	2,034,400								
Idaho	189	27	10,182,000								
Oklahoma	30	18	1,092,400								
Wyoming	33	22	1,431,600								
Utah	45	22	1,980,000								0.220
New Mexico	79	25	3,936,400								
Arizona	30	13	853,600								
Nevada	5	24	223,200								
Total	**1794**	**16**	**57,533,200**	**2,826**	**104**		**686**	**148**	**60**		
lb Input/lb Corn silage				**0.00377**	**0.00012**	**0.00103**	**0.00092**	**2.01E-04**	**7.95E-05**	**6.96E-04**	
lb CO_2 eq./lb Corn silage				**0.0404**	**0.0007**	**0.0064**	**0.0028**	**1.15E-04**	**3.06E-04**	**3.17E-04**	
Contribution to regional GHG profile				**52.98 %**	**0.97%**	**8.36%**	**3.67%**	**0.15%**	**0.40%**	**0.42%**	

Region 4-Inventory Data For Corn Silage (cont.)							
Dairy Production States	Crop Protection Chemical Input Data		Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Diesel (Gal./acre)	Gasoline (Gal./acre)	LPG (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Texas	3,344	236	8.9	1.1	1.5	49.4	6236.8
Nebraska	18,416	456	12.4	2.1	4.1	152.5	964
Kansas	1,094	89	9.2	1.3	13.1	16.9	1531.7
South Dakota	6,036	239	4.4	1.5	0.5	27.4	7
Colorado	1,494	252	9	1.6	3.8	390.3	
North Dakota	6,036		4.5	2.1	7.1		
Montana							
Idaho							
Oklahoma							
Wyoming							
Utah							
New Mexico							
Arizona							
Nevada							
Total	**37,914**	**1,763**					
lb Input or gallon or kWh/lb Corn silage	**5.09E-05**	**1.78E-06**	**2.60E-04**	**5.85E-05**	**1.52E-04**	**2.93E-03**	**4.93E-02**
lb CO_2 eq./lb Corn silage	**3.84E-04**	**2.51E-05**	**6.82E-03**	**1.41E-03**	**2.57E-03**	**5.57E-03**	**8.39E-03**
Contribution to regional GHG profile	**0.50%**	**0.03%**	**8.95%**	**1.86%**	**3.38%**	**7.31%**	**11.01%**

Region 5-Inventory Data For Corn Silage											
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data							GHG Profile
	Area Harvested (1000 acres)	Yield (tons / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen From manure (Million lbs)	Nitrogen in Residue / mass corn grain	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (lbs/acre)	lb CO_2 eq./lb Corn Silage
California											
Oregon											
Washington											
Alaska											
Hawaii											
Total											
lb Input/lb Corn silage											**0.210**
lb CO_2 eq./lb Corn silage											
Contribution to regional GHG profile											

Region 5-Inventory Data For Corn Silage (cont.)							
Dairy Production States	**Crop Protection Chemical Input Data**		**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Diesel (Gal./acre)**	**Gasoline (Gal./acre)**	**LPG (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
California							
Oregon							
Washington							
Alaska							
Hawaii							
Total							
lb Input or gallon or kWh/lb Corn silage							
lb CO_2 eq./lb Corn silage							
Contribution to regional GHG profile							

Table D-14 Oats (GHG profile: lb CO_2 eq per lb dry oats)

Region 1-Inventory Data For Oats										
Dairy Production States	**Productivity Data**			**Soil Amendment Chemical Input Data**						**GHG Profile**
	Area Harvested (1000 acres)	**Yield (Bushels / acre)**	**Production (1000 lbs)**	**Nitrogen-N (Million lbs)**	**Nitrogen in Residue / mass Oats**	**Phosphorous-P (Million lbs)**	**Potash-K (Million lbs)**	**Sulfur-S (Million lbs)**	**Lime (Million lbs)**	**lb CO_2 eq./lb Oats**
Maine										
New Hampshire										
Vermont										
Massachusetts										
Rhode Island										
Connecticut										
New York	63	63	127,757	1.9	2.8	2.7	2.8		22.6256	
Pennsylvania	98	58	180,864	4.5	4.4	4.9	5.1	0.1	35.084	
New Jersey										
Delaware										**0.800**
Maryland										
Total				**6.4**	**7.254**	**7.6**	**7.9**	**0.1**	**57.7096**	
lb Input/lb Oats				**0.0207**	**0.0318**	**0.0246**	**0.0256**	**0.00055**	**0.18699193**	
lb CO_2 eq./lb Oat				**0.2221**	**0.1971**	**0.0746**	**0.0147**	**0.0021**	**0.0852**	
Contribution to regional GHG profile				**32.24%**	**28.61%**	**10.83%**	**2.13%**	**0.31%**	**12.37%**	

Region 1-Inventory Data For Oats (cont.)							
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**			
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gallons)**	**Diesel (Gallons)**	**LPG (Gallons)**	**Electricity (kWh)**
Maine				39,200	117,600	69,219	
New Hampshire							
Vermont							
Massachusetts							
Rhode Island							
Connecticut							
New York	23			84,267	252,800	148,798	2373.60
Pennsylvania	46			130,667	392,000	230,731	3680.58
New Jersey							
Delaware					0		
Maryland					0		
Total	**69**	**0**		**254,133**	**762,400**	**448,749**	**6,054**
lb Input/lb Oats	**0.00022358**	**0**					
lb CO_2 eq./lb Oat	**0.0015**	**0**		**0.0165**	**0.0548**	**0.0203**	**3.75E-05**
Contribution to regional GHG profile	**0.21%**	**0%**		**2.39%**	**7.95%**	**2.95%**	**0.01%**

Region 2-Inventory Data For Oats										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass Oat	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (Million lbs)	lb CO_2 eq./lb Oat
Virginia	58		7,456							
West Virginia										
Kentucky										
Tennessee										
North Carolina	69		53,402							
South Carolina	54		31,898							
Georgia	58		47,648							
Florida										
Alabama	51		25,139							
Mississippi										
Arkansas	0		0							**0.800**
Louisiana										
Total	**59**		**165,542**							
lb Input/lb Oats				-		-	-	-		
lb CO_2 eq./lb Oat										
Contribution to regional GHG profile										

Region 2-Inventory Data For Oats (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gallons)**	**Diesel (Gallons)**	**LPG (Gallons)**	**Electricity (kWh)**	**Natural Gas (C.F)**
Virginia								
West Virginia					16,000			
Kentucky								
Tennessee								
North Carolina					95,200			
South Carolina					72,800			
Georgia					104,000			
Florida								
Alabama					60,800			
Mississippi								
Arkansas								
Louisiana								
Total	**0**	**0**			**348,800**			
lb Input/lb Oats	-	-						
lb CO_2 eq./lb Oat								
Contribution to regional GHG profile								

Region 3-Inventory Data For Oats										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass Oat	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (Million lbs)	lb CO_2 eq./lb Oat
Minnesota	190	63	383,424	4.2	8.6	2.4	5.9	0.2	68.02	
Wisconsin	201	64	412,160	2.1	9.0	3.9	15.1	0.4	71.958	
Michigan	64	63	128,416	2.6	2.9	2.8	3.4		22.912	
Ohio	53	66	111,840							
Indiana	10	70	22,016							
Illinois	34	72	78,579	0.4	1.5	0.4	1.7		12.1004	
Missouri	15	57	28,800							
Iowa	103	73	242,861	1.8	4.7	2.5	6.9		37.0172	**0.580**
Total	**670**	**66**	**1,408,096**	**11.1**		**12**	**33**	**0.6**	**212**	
lb Input/lb Oats				**0.0089**	**0.0321**	**0.0096**	**0.0265**	**0.00075**	**0.170**	
lb CO_2 eq./lb Oat				**0.0954**	**0.1991**	**0.0292**	**0.0152**	**0.0029**	**0.078**	
Contribution to regional GHG profile				**18.61%**	**38.83%**	**5.69%**	**2.96%**	**0.57%**	**15.13 %**	

Region 3-Inventory Data For Oats (cont.)							
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data			
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gallons)	Diesel (Gallons)	LPG (Gallons)	Electricity (kWh)
Minnesota				253,333	760,000	451,250	1,819,329
Wisconsin	25			268,000	804,000	477,375	1,924,659
Michigan	26			85,333	256,000	152,000	612,827
Ohio				70,667	212,000	125,875	507,497
Indiana				12,800	38,400	22,800	91,924
Illinois	1			45,067	135,200	80,275	323,649
Missouri				20,000	60,000	35,625	143,631
Iowa	2			137,867	413,600	245,575	990,098
Total	**54**	**0**	**0**	**893,067**	**2,679,200**	**1,590,775**	**6,413,614**
lb Input/lb Oats	**6.26E-05**	**-**		**6.34E-04**	**1.90E-03**	**1.13E-03**	**4.55E-03**
lb CO_2 eq./lb Oat	**0.0004**	**0.0000**	**0.0000**	**0.0153**	**0.0499**	**0.0191**	**0.009**
Contribution to regional GHG profile	**0.08%**	**0.00%**	**0.00%**	**2.99%**	**9.72%**	**3.72%**	**1.70%**

Region 4-Inventory Data For Oats										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass Oat	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (Million lbs)	lb CO_2 eq./lb Oat
Texas	114	42	152,512	45.4		12.7	4.9	1.7	40.8	
Oklahoma	12	36	13,248							
Nebraska	45	63	92,096	4.5		1.3	0.1	0.0	16.1	
Kansas	36	49	56,448	4.4		1.4	0.8		12.9	
North Dakota	194	55	352,832	15.8		5.7	0.7	0.1	69.5	
South Dakota	139	71	322,842	11.8		5.6	1.7		49.8	
Montana	33	52	55,290	2.0		1.0	0.4	0.1	11.7	
Idaho	20	68	43,264	1.6		1.4	0.1	0.2	7.2	
Colorado	12	65	25,376							
Wyoming	12	51	19,552							
Utah	6	77	14,682							**1.00**
New Mexico										
Arizona										
Nevada										
Total	**623**	**57**	**1,148,141**	**40.1**		**29.1**	**8.7**	**2.1**	**207.9**	
lb Input/lb Oats				**0.0435**	**0.011**	**0.027**	**0.008**	**0.003**	**0.225**	
lb CO_2 eq./lb Oat				**0.4654**	**0.0692**	**0.082**	**0.0046**	**0.012**	**0.102**	
Contribution to regional GHG profile				**51.79%**	**7.70%**	**9.12%**	**0.52%**	**1.29%**	**9.91%**	

Region 4-Inventory Data For Oats (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gallons)	Diesel (Gallons)	LP (Gallons)	Electricity (kWh)	Natural Gas (C.F/acre)
Texas	80	35		152,000	456,000	430,730	8,611,750	
Oklahoma				15,467	46,400	43,829	876,283	
Nebraska	4			60,000	180,000	170,025	3,399,375	
Kansas	13			48,000	144,000	136,020	2,719,500	
North Dakota	167			258,667	776,000	732,997	14,655,083	
South Dakota	52			185,333	556,000	525,188	10,500,292	
Montana	18			43,733	131,200	123,929	2,477,767	
Idaho	17			26,667	80,000	75,567	1,510,833	
Colorado				16,533	49,600	46,851	936,717	
Wyoming				15,733	47,200	44,584	891,392	
Utah				8,000	24,000	22,670	453,250	
New Mexico								
Arizona								
Nevada								
Total	**351.0**	**35.0**		**830,133**	**2,490,400**	**2,352,390**	**47,032,242**	
lb Input/lb Oats	**0.0003264**	**0.0002295**						
lb CO_2 eq./lb Oat	**0.0021270**	**0.0045990**		**0.0174732**	**0.0566437**	**0.0346060**	**0.0622566**	
Contribution to regional GHG profile	**0.24%**	**0.51%**		**1.94%**	**6.30%**	**3.85%**	**6.93%**	

Region 5-Inventory Data For Oats										
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data						GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen in Residue / mass Oat	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	Lime (Million lbs)	lb CO_2 eq./lb Oat
California	22	85	60,160	4.4					7.9	
Oregon	19	90	54,067							
Washington	7	75	17,523							
Alaska										
Hawaii										
Total	**48**	**85**	**131,750**	**4.4**		**0**	**0**	**0**	**7.9**	
lb Input/lb Oats				**0.07314**	**0.01**				**0.15**	**1.14**
lb CO_2 eq./lb Oat				**0.7832**	**0.061**	**0.000**	**0.00**	**0.000**	**0.069**	
Contribution to regional GHG profile				**79.21%**	**6.21%**	**0.00%**	**0.00%**	**0.00%**	**6.09%**	

Region 5-Inventory Data For Oats (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gallons)**	**Diesel (Gallons)**	**LP (Gallons)**	**Electricity (kWh)**	**Natural Gas (C.F)**
California	59			29,333	88,000	51,797	826,252	
Oregon				25,067	75,200	44,263	706,070	
Washington				9,867	29,600	17,423	277,921	
Alaska				-	-	-	-	
Hawaii					-			
Total	**59**	**0**		**64,267**	**192,800**	**113,482**	**1,810,242**	
lb Input/lb Oats	**0.001**	**0.00**						
lb CO_2 eq./lb Oat	**0.006**	**0.00**		**0.0118**	**0.0384**	**0.0145**	**0.0198**	
Contribution to regional GHG profile	**0.00%**	**0.00%**		**1.19%**	**3.88%**	**1.47%**	**2.00%**	

Table D-15 Winter wheat (GHG profile: lb CO_2 eq per lb dry wheat)

Region 1-Inventory Data For Winter Wheat									
Dairy Production States	**Productivity Data**			**Soil Amendment Chemical Input Data**					**GHG Profile**
	Area Harvested (1000 acres)	**Yield (Bushels / acre)**	**Production (1000 lbs)**	**Nitrogen -N (Million lbs)**	**Nitrogen Residues**	**Phosphate (Million lbs)**	**Potash (Million lbs)**	**Sulfur (Million lbs)**	**lb CO_2 eq./lb Wheat**
Maine									**0.380**
New Hampshire									
Vermont									
Massachusetts									
Rhode Island									
Connecticut									
New York	99	57	340992	6.3616					
Pennsylvania	154	57	529500	9.856					
New Jersey	26	54	85296	1.664					
Delaware	55	68	229608	3.5456					
Maryland									
Total	**335**	**59**	**1,185,396**	**21**		**12**	**2**		
lb Input/lb Wheat				**1.81E-02**	**1.2E-02**	**9.89E-03**	**1.98E-03**		
lb CO_2 eq./lb Wheat									
Contribution to regional GHG profile				**42**	**17**	**6**	**0.25%**		

Region 1- Inventory Data For Winter Wheat (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gal./acre)**	**Diesel (Gal./acre)**	**LP (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
Maine								
New Hampshire								
Vermont								
Massachusetts								
Rhode Island								
Connecticut								
New York				1.01	4.41	0.3	15.01	0.10
Pennsylvania				1.01	4.41	0.3	15.01	0.10
New Jersey				1.01	4.41	0.3	15.01	0.10
Delaware				1.01	4.41	0.3	15.01	0.10
Maryland								
Total								
lb Input or gallon or kWh /lb Wheat				**2.84E-04**	**1.24E-03**	**8.46E-05**	**4.24E-03**	**2.83E-05**
lb CO_2 eq./lb Wheat				**6.86E-03**	**3.26E-02**	**1.43E-03**	**8.11E-03**	**4.81E-06**
Contribution to regional GHG profile				**1%**	**7%**	**0.31%**	**2%**	**0.001%**

Region 2- Inventory Data For Winter Wheat									
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data					GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen Residues	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	lb CO_2 eq./lb Wheat
Virginia	196	64	762240	12.544		6.86	1.372		
West Virginia	6	58	20976	0.384		0.21	0.042		
Kentucky	342	62	1299600	21.888		11.97	2.394		
Tennessee	280	55	932400	17.92		9.8	1.96		
North Carolina	507	53	1629300	32.448		17.745	3.549		
South Carolina	162	46	453240	10.3424		5.656	1.1312		
Georgia	216	48	639720	13.824		7.56	1.512		
Florida	12	48	36060	0.768		0.42	0.084		**0.400**
Alabama	85	54	301584	5.4528		2.982	0.5964		
Mississippi	218	56	759144	13.9264		7.616	1.5232		
Arkansas	553	53	1732140	35.392		19.355	3.871		
Louisiana	195	52	629280	12.48		6.825	1.365		
Total	**2771**	**55**	**9195684**	**177**		**97**	**19**		
lb Input/lb Wheat				**1.93E-02**	**1.24E-02**	**1.05E-02**	**2.11E-03**		
lb CO_2 eq./lb Wheat				**0.207**	**0.077**	**0.032**	**0.001**		
Contribution to regional GHG profile				**45%**	**17%**	**7%**	**0.3%**		

Region 2- Inventory Data For Winter Wheat (cont.)								
Dairy Production States	**Crop Protection Chemical Input Data**			**Energy Input Data**				
	Herbicides (1000 lbs)	**Insecticides (1000 lbs)**	**Fungicides (1000 lbs)**	**Gasoline (Gal./acre)**	**Diesel (Gal./acre)**	**LP (Gal./acre)**	**Electricity (kWh/acre)**	**Natural Gas (C.F/acre)**
Virginia				1.01	4.41	0.3	15.01	0.10
West Virginia				1.01	4.41	0.3	15.01	0.10
Kentucky				1.01	4.41	0.3	15.01	0.10
Tennessee				1.01	4.41	0.3	15.01	0.10
North Carolina				1.01	4.41	0.3	15.01	0.10
South Carolina				1.01	4.41	0.3	15.01	0.10
Georgia				1.01	4.41	0.3	15.01	0.10
Florida				1.01	4.41	0.3	15.01	0.10
Alabama				1.01	4.41	0.3	15.01	0.10
Mississippi				1.01	4.41	0.3	15.01	0.10
Arkansas				1.01	4.41	0.3	15.01	0.10
Louisiana				1.01	4.41	0.3	15.01	0.10
Total								
lb Input or gallon or kWh/lb Wheat				**3.03E-04**	**1.33E-03**	**9.02E-05**	**4.52E-03**	**3.01E-05**
lb CO_2 eq./lb Wheat				**7.32E-03**	**3.48E-02**	**1.52E-03**	**8.65E-03**	**5.13E-06**
Contribution to regional GHG profile				**2.0%**	**9.4%**	**0.4%**	**2.3%**	**0.001%**

Region 3- Inventory Data For Winter Wheat									
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data					GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen Residues	Phosphorous -P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	lb CO_2 eq./lb Wheat
Minnesota	43	47	128040						0.510
Wisconsin	247	66	971820						
Michigan	624	67	2529480	57.6		22.2	33.9	3	
Ohio	900	66	3576480	86.2		53	57.5	7.2	
Indiana	434	66	1714320						
Illinois	890	61	3278640	82.1		49.8	68.4	0.5	
Missouri	884	50	2642160	90.7		35.5	44.8	1.8	
Iowa	24	53	75384						
Total	**4046**	**61**	**14916324**	**317**		**161**	**205**	**13**	
lb Input/lb Wheat				**2.63E-02**	**1.24E-02**	**1.33E-02**	**1.70E-02**	**1.04E-03**	
lb CO_2 eq./lb Wheat				**0.282**	**0.077**	**4.04E-02**	**9.74E-03**	**4.01E-03**	
Contribution to regional GHG profile				**61%**	**17%**	**9%**	**2%**	**1%**	

Region 3- Inventory Data For Winter Wheat (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Minnesota								
Wisconsin								
Michigan	148		17	1.01	4.41	0.30	15.01	0.10
Ohio	93			1.01	4.41	0.30	15.01	0.10
Indiana								
Illinois	62		7	1.01	4.41	0.30	15.01	0.10
Missouri	49	12	10	1.01	4.41	0.30	15.01	0.10
Iowa								
Total	**352**	**12**	**34**					
lb Input or gallon or kWh/lb Wheat	**2.93E-05**	**4.54E-06**	**4.02E-06**	**2.76E-04**	**1.21E-03**	**8.21E-05**	**4.12E-03**	**2.74E-05**
lb CO_2 eq./lb Wheat	**3.10E-04**	**2.30E-07**	**2.11E-05**	**6.66E-03**	**3.17E-02**	**1.39E-03**	**7.87E-03**	**4.67E-06**
Contribution to regional GHG profile	**0.067%**	**0.00005%**	**0.005%**	**1.4%**	**7%**	**0.3%**	**1.7%**	**0.001%**

Region 4- Inventory Data For Winter Wheat									
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data					GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen Residues	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	lb CO_2 eq./lb Wheat
Texas	3000	27	7833600	152.1		47.3	20.8	5.3	
Oklahoma	4020	31	7663200	283.4		130.9	9.8		
Nebraska	1748	40	4183800	73.3		34	1.4	1.9	
Kansas	8920	36	19506000	493		197.5	29	5.3	
North Dakota	337	43	879480						
South Dakota	1552	45	4298880	78.7		28.1	4.7	1.1	
Montana	2052	41	5057880	96.8		46.2	9.9	2	
Idaho	730	81	3551160	80.9		13.7	2.2	9.6	
Colorado	2010	28	3447000	36.8		13.5		0.7	
Wyoming	135	27	220920						
Utah	125	44	327600						**0.500**
New Mexico	226	30	407520						
Arizona	5	89	26400						
Nevada	8	93	44172						
Total	**24868**	**36**	**54447612**	**1295**		**511**	**78**	**26**	
lb Input/lb Wheat				**2.46E-02**	**1.29E-02**	**9.73E-03**	**1.58E-03**	**5.77E-04**	
lb CO_2 eq./lb Wheat				**0.264**	**0.080**	**2.95E-02**	**9.08E-04**	**2.22E-03**	
Contribution to regional GHG profile				**58%**	**17%**	**6%**	**0.2%**	**0.49%**	

Region 4- Inventory Data For Winter Wheat (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
Texas	1299	92		1.01	4.41	.30	15.01	.10
Oklahoma	495	138		1.01	4.41	.30	15.01	.10
Nebraska	399		8	1.01	4.41	.30	15.01	.10
Kansas	2600			1.01	4.41	.30	15.01	.10
North Dakota				1.01	4.41	.30	15.01	.10
South Dakota	749		27	1.01	4.41	.30	15.01	.10
Montana	2315			1.01	4.41	.30	15.01	.10
Idaho	349		3	1.01	4.41	.30	15.01	.10
Colorado	1018			1.01	4.41	.30	15.01	.10
Wyoming								
Utah								
New Mexico								
Arizona								
Nevada								
Total	**9224**	**230**	**38**					
lb Input or gallon or kWh/lb Wheat	**1.76-04**	**1.84-05**	**3.16-06**	**4.60E-04**	**2.01E-03**	**1.37E-04**	**6.87E-03**	**4.58E-05**
lb CO_2 eq./lb Wheat	**1.86-03**	**9.31-07**	**1.66-05**	**1.11E-02**	**5.28E-02**	**2.31E-03**	**1.25E-02**	**7.79E-06**
Contribution to regional GHG profile	**0.41%**	**0.0002%**	**0.004%**	**2%**	**12%**	**1%**	**3%**	**0.002%**

Region 5- Inventory Data For Winter Wheat									
Dairy Production States	Productivity Data			Soil Amendment Chemical Input Data					GHG Profile
	Area Harvested (1000 acres)	Yield (Bushels / acre)	Production (1000 lbs)	Nitrogen-N (Million lbs)	Nitrogen Residues	Phosphorous-P (Million lbs)	Potash-K (Million lbs)	Sulfur-S (Million lbs)	lb CO_2 eq./lb Wheat
California	307	76	1425180						
Oregon	757	57	2603520	46.2		2.8	1.4	4.9	
Washington	1752	63	6671400	140.8		12	3.5	18	
Alaska									
Hawaii									
Total	**2816**	**63**	**10700100**	**187**		**15**	**5**	**23**	**0.390**
lb Input or gallon or kWh/lb Wheat				**2.02E-02**	**1.23E-02**	**1.60E-03**	**5.28E-04**	**2.47E-03**	
lb CO_2 eq./lb Wheat				**0.216**	**0.077**	**4.83E-03**	**3.03E-04**	**9.52E-03**	
Contribution to regional GHG profile				**61%**	**22%**	**1%**	**0.1%**	**3%**	

Region 5-Inventory Data For Winter Wheat (cont.)								
Dairy Production States	Crop Protection Chemical Input Data			Energy Input Data				
	Herbicides (1000 lbs)	Insecticides (1000 lbs)	Fungicides (1000 lbs)	Gasoline (Gal./acre)	Diesel (Gal./acre)	LP (Gal./acre)	Electricity (kWh/acre)	Natural Gas (C.F/acre)
California								
Oregon	366		3	1.01	4.41	0.30	15.01	0.10
Washington	1077		5	1.01	4.41	0.30	15.01	0.10
Alaska								
Hawaii								
Total	**1443**		**8**					
lb Input or gallon or kWh/lb Wheat	**1.56E-04**		**8.63E-07**	**2.72E-04**	**1.19E-03**	**8.10E-05**	**4.06E-03**	**2.71E-05**
lb CO_2 eq./lb Wheat	**1.65E-03**		**4.53E-06**	**6.57E-03**	**3.12E-02**	**1.37E-03**	**5.85E-03**	**4.61E-06**
Contribution to regional GHG profile	**0.5%**	**0%**	**0.001%**	**2%**	**9%**	**0.4%**	**2%**	**0.001%**

Table D-16 Wet mill / Dry mill dried distillers grains with solubles (DDGS)

	Energy	Mass	Value	System Expansion	References
Allocation to Corn Ethanol (Wet mill)	0.61	0.49	0.76	0.8	Kim. S., Dale. B. E., (2002)
Allocation to Corn Ethanol (Dry mill)	0.57	0.48	0.7	0.8	Kodera, K. (2007)
Allocation to DDGS (Wet mill)	0.39	0.51	0.24	0.2	Estimated from corn and ethanol allocation
Allocation to DDGS (Dry mill)	0.43	0.52	0.3	0.2	
Corn Ethanol	0.0849 kg CO_2 eq / MJ EtOH				Hill, Tillman et al. PNAS 2006
	0.07 kg CO_2 eq / MJ EtOH				Wang , M. GREET 1.6 report, 2001
	0.062 kg CO_2 eq / MJ EtOH				Shapouri et al. 2003
0.914 kg dry DDGS / kg corn ethanol					Hill, Tillman et al. PNAS 2006 Table 9
26.8 MJ / kg ethanol					CONCAWE WTT Appendix 1 pg 11.

Table D-17 GHG emissions on basis of kgCO_2e/MJ Ethanol

	GHG Emissions kg CO_2eq/MJ Ethanol			
	Energy	Mass	Value	System
DDGS (WET MILL)	0.054	0.088	0.027	0.021
	0.045	0.073	0.022	0.018
	0.040	0.065	0.020	0.016
DDGS (DRY MILL)	0.064	0.092	0.036	0.021
	0.053	0.076	0.030	0.018
	0.047	0.067	0.027	0.016
Wet Mill, DDGS GHG Emissions			2.2065	kg CO_2 eq / kg dry DDGS -Mass
			1.3554	kg CO_2 eq / kg dry DDGS -Energy
			0.6695	kg CO_2 eq / kg dry DDGS -Value
			0.5300	kg CO_2 eq / kg dry DDGS -System
Dry Mill, DDGS GHG Emissions			2.2966	kg CO_2 eq / kg dry DDGS -Mass
			1.5993	kg CO_2 eq / kg dry DDGS -Energy
			0.9086	kg CO_2 eq / kg dry DDGS -Value
			0.5300	kg CO_2 eq / kg dry DDGS -System

Table D-18 Estimation of N emissions for corn from manure management system (MMS) using IPCC Tier I model: Region 1

			Number of animals	Default N excretion rate	Typical animal mass for livestock category	Annual N excretion per head of species/livestock category3	Fraction of total annual nitrogen excretion managed in MMS for each species/ livestock category	Total nitrogen excretion for the MMS 4	Emission factor for direct N_2ON emissions from MMS	Annual direct N_2O emissions from Manure Management	**Total kg N_2O per yr for Region 1**
MMS		Species	Heads	[kg N (1000 kg animal)-1 day-1]	(kg)	(kg N /animal/ year)	(-)	(kg N per yr)	[kg N_2O-N/ (kg N in MMS)]	kg N2O per yr	
Region 1	NY	Dairy Cows	566,277	0.44	604	970.024	0.263	144,466,464.09	0.005	703,758	**1,382,988**
		Beef Cattle	8,338	0.31	389	440.1535	0.184	675,276.74	0.02	3,820	
		Swine	24,337	0.24	196	171.696	0.042	175,500.11	0.005	179	
		Poultry	5,171,500	1.1	0.9	3.6135	0.015	280,308.23	0.005	110	
		Other Cattle	256,748	0.31	389	440.1535	0.184	20,793,534.85	0.02	13,070	
	PA	Dairy Cows	499,295	0.44	604	970.024	0.263	127,378,304.10	0.005	620,514	
		Beef Cattle	58,357	0.31	389	440.1535	0.184	4,726,198.56	0.02	26,737	
		Swine	377,386	0.24	196	171.696	0.042	2,721,416.63	0.005	2,780	
		Poultry	56,919,750	1.1	0.9	3.6135	0.015	3,085,192.75	0.005	1,212	

Table D-19 Estimation of N emissions for corn from MMS using IPCC Tier I model: Region 3

MMS		Species	Heads	[kg N (1000 kg animal) -1 day-1]	(kg)	(kg N /animal/ year)	(-)	(kg N per yr)	[kg N_2O-N/ (kg N in MMS)]	kg N2O per yr	Total kg N_2O per yr for Region 3
Region 3	MN	Dairy Cows	488,493	0.44	604	970.024	0.263	124,622,593	0.005	607,090	4,455,402
		Beef Cattle	190,965	0.31	389	440.1535	0.184	15,465,905	0.02	87,493	
		Swine	2,167,437	0.24	196	171.696	0.042	15,629,888	0.005	15,965	
		Poultry	95,382,250	1.1	0.9	3.6135	0.015	5,169,956.41	0.005	2,031	
		Other Cattle	418,556	0.31	389	440.1535	0.184	33,898,132	0.02	21,307	
	WI	Dairy Cows	1,207,453	0.44	604	970.024	0.263	308,040,910	0.005	1,500,599	
		Beef Cattle	76,039	0.31	389	440.1535	0.184	6,158,276	0.02	34,838	
		Swine	243,913	0.24	196	171.696	0.042	1,758,909	0.005	1,797	
		Poultry	17,646,250	1.1	0.9	3.6135	0.015	956,471	0.005	376	
		Other	601,035	0.31	389	440.1535	0.184	48,676,759	0.02	30,597	
	MI	Dairy Cows	270,175	0.44	604	970.024	0.263	68,925,983	0.005	335,768	
		Beef Cattle	72,828	0.31	389	440.1535	0.184	5,898,192	0.02	33,367	
		Swine	377,786	0.24	196	171.696	0.042	2,724,304	0.005	2,783	
		Poultry	16,531,000	1.1	0.9	3.6135	0.015	896,021	0.005	352	
		Other	162,819	0.31	389	440.1535	0.184	13,186,421	0.02	8,289	

	OH	Dairy Cows	499,295	0.44	604	970.024	0.263	127,378,304	0.005	620,514	
		Beef Cattle	58,357	0.31	389	440.1535	0.184	4,726,198.56	0.02	26,737	
		Swine	377,386	0.24	196	171.696	0.042	2,721,416.63	0.005	2,780	
		Poultry	56,919,750	1.1	0.9	3.6135	0.015	3,085,192.75	0.005	1,212	
		Other	212,306	0.31	389	440.1535	0.184	17,194,257.74	0.02	10,808	
	IN	Dairy Cows	74,812	0.44	604	970.024	0.263	19,085,705.41	0.005	92,975	
		Beef Cattle	56,053	0.31	389	440.1535	0.184	4,539,606.50	0.02	25,681	
		Swine	1,436,671	0.24	196	171.696	0.042	10,360,167.38	0.005	10,582	
		Poultry	47,717,750	1.1	0.9	3.6135	0.015	2,586,421.34	0.005	1,016	
		Other	89,444	0.31	389	440.1535	0.184	7,243,949.75	0.02	4,553	
	IL	Dairy Cows	102,111	0.44	604	970.024	0.263	26,050,212.35	0.005	126,902	
		Beef Cattle	144,581	0.31	389	440.1535	0.184	11,709,320.81	0.02	66,241	
		Swine	1,698,950	0.24	196	171.696	0.042	12,251,525.27	0.005	12,514	
		Poultry	9,254,750	1.1	0.9	3.6135	0.015	501,630.59	0.005	197	
		Other	143,810	0.31	389	440.1535	0.184	11,646,895.88	0.02	7,321	
	MO	Dairy Cows	109,680	0.44	604	970.024	0.263	27,981,116.28	0.005	136,308	
		Beef Cattle	66,583	0.31	389	440.1535	0.184	5,392,427.29	0.02	30,506	
		Swine	1,224,646	0.24	196	171.696	0.042	8,831,208.08	0.005	9,020	
		Poultry	64,484,7	1.1	0.9	3.6135	0.015	3,495,234.66	0.005	1,373	

			50								
		Other	502,776	0.31	389	440.1535	0.184	40,718,950.22	0.02	25,595	
	IO	Dairy Cows	190,726	0.44	604	970.024	0.263	48,657,344	0.005	237,031	
		Beef Cattle	583,963	0.31	389	440.1535	0.184	47,294,115	0.02	267,550	
		Swine	4,912,752	0.24	196	171.696	0.042	35,426,994	0.005	36,186	
		Poultry	37,957,000	1.1	0.9	3.6135	0.015	2,057,364	0.005	808	
		Other	360,281	0.31	389	440.1535	0.184	29,178,537	0.02	18,341	

Table D-20 Estimation of N emissions for corn from MMS using IPCC Tier I model: Region 3

MMS		Species/ Livestock Category	Heads	[kg N (1000 kg animal)-1 day-1]	(kg)	(kg N /animal/ year)	(-)	(kg N per yr)	[kg N_2O-N/ (kg N in MMS)]	kg N2O per yr	Total kg N_2O per yr for Region 4
Region 4	TX	Dairy Cows	279,197	0.44	604	970.024	0.263	71,227,663.04	0.005	346,980	3,838,343
		Beef Cattle	2,416,683	0.31	389	440.1535	0.184	195,722,878.46	0.02	1,107,232	
		Swine	198,677	0.24	196	171.696	0.042	1,432,708.61	0.005	1,463	
		Poultry	80,790,750	1.1	0.9	3.6135	0.015	4,379,060.63	0.005	1,720	
		Other Cattle	753,441	0.31	389	440.1535	0.184	61,019,859.50	0.02	38,355	
	NE	Dairy Cows	53,580	0.44	604	970.024	0.263	13,669,055.46	0.005	66,588	
		Beef Cattle	1,937,040	0.31	389	440.1535	0.184	156,877,477.88	0.02	887,478	
		Swine	1,222,030	0.24	196	171.696	0.042	8,812,339.17	0.005	9,001	
		Poultry	16,404,250	1.1	0.9	3.6135	0.015	889,151.36	0.005	349	
		Other	514,090	0.31	389	440.1535	0.184	41,635,267.41	0.02	26,171	
	KS	Dairy Cows	68,694	0.44	604	970.024	0.263	17,525,010.96	0.005	85,372	
		Beef Cattle	2,101,666	0.31	389	440.1535	0.184	170,210,269.59	0.02	962,904	
		Swine	534,029	0.24	196	171.696	0.042	3,851,009.68	0.005	3,934	

		Poultry	5,031,000	1.1	0.9	3.6135	0.015	272,692.78	0.005	107
		Other	272,055	0.31	389	440.1535	0.184	22,033,225.14	0.02	13,849
	SD	Dairy Cows	75,946	0.44	604	970.024	0.263	19,375,114.45	0.005	94,384
		Beef Cattle	207,355	0.31	389	440.1535	0.184	16,793,284.94	0.02	95,002
		Swine	473,068	0.24	196	171.696	0.042	3,411,402.59	0.005	3,485
		Poultry	8,786,250	1.1	0.9	3.6135	0.015	476,236.72	0.005	187
		Other	475,814	0.31	389	440.1535	0.184	38,535,341.14	0.02	24,222
	CO	Dairy Cows	221	0.44	604	970.024	0.263	56,447.04	0.005	275
		Beef Cattle	644	0.31	389	440.1535	0.184	52,164.53	0.02	295
		Swine	676	0.24	196	171.696	0.042	4,871.26	0.005	5
		Poultry	24,000	1.1	0.9	3.6135	0.015	1,300.86	0.005	1
		Other	16,783	0.31	389	440.1535	0.184	1,359,203.83	0.02	854
	ND	Dairy Cows	42,447	0.44	604	970.024	0.263	10,828,957.81	0.005	52,752
		Beef Cattle	30,982	0.31	389	440.1535	0.184	2,509,159.96	0.02	14,195
		Swine	57,976	0.24	196	171.696	0.042	418,081.13	0.005	427
		Poultry	4,339,000	1.1	0.9	3.6135	0.015	235,184.65	0.005	92
		Other	12,991	0.31	389	440.1535	0.184	1,052,083.45	0.02	661

Table D-21 U.S. annual consumption of selected nitrogen materials from 2004-2007 (short tons N fertilizer)

	2004	2005	2006	2007
Anhydrous Ammonia	4,068,586	3,857,891	3,821,691	4,249,988
Aqua Ammonia	521,181	420,879	397,647	373,817
Ammonium Nitrate	1,527,964	1,420,653	963,710	1,056,148
Ammonium Sulfate	1,229,569	1,181,609	1,218,964	1,382,310
Nitrogen solutions	11,195,765	10,499,854	10,104,319	11,970,556
Sodium Nitrate	16,798	21,353	17,219	13,041
Urea	5,644,619	5,211,665	5,369,913	5,722,579
Other	2,752,062	2,629,043	2,839,576	2,491,535

Table D-22 Fertilizer Mixtures used in this study (N fertilizer). Note that the values do not add to 1.0 because ammonia is on a total compound weight basis while all others are on weight of N only basis

	US Nitrogen fertilizer Mix	kg N in N fertilizer / kg N in national mix of N	EcoInvent Unit Process
Nitrogen Fertilizers	Ammonia Anhydrous	0.308	Ammonia, liquid, at region storehouse/RER U
	Ammonia Aqua	0.008	Ammonia, liquid, at regional storehouse/RER U
	Ammonium nitrate	0.038	Ammonium nitrate, as N, at regional storehouse/RER
	Ammonium sulfate	0.025	Ammonium sulfate, as N, at regional
	Nitrogen Solution	0.166 0.132	Urea, as N, at regional storehouse/RER U Ammonium nitrate, as N, at regional storehouse/RER
	Sodium Nitrate	0.0003	Potassium nitrate, as N, at regional storehouse/RER
	Urea	0.237	Urea, as N, at regional storehouse/RER U
	Other	0.086	Liquid ammonia, ammonium nitrate and urea
Source: www.ers.usda.gov/Data/FertilizerUse/			

Table D-23 Fertilizer mixtures used in this study (P.K.S fertilizer)

	Amount (kg)	EcoInvent Unit Process
Phosphorous (P)	0.0104 0.2767 0.3741 0.3178 0.0210	Single superphosphate, as P_2O_5 /RER U Ammonium nitrate phosphate, as P_2O_5 /RER U Diammonium phosphate, as P_2O_5 /RER U
Potassium (K)	0.90 0.05 0.05	Potassium chloride, as K_2O /RER U Potassium hydroxide /RER U
Sulfur (S)	1	Potassium sulfate, as K_2O /RER U Sulfur is applied as K_2SO_4. (32 g S applied per 110g K_2O, equivalent to 174 g K_2SO_4.)

NB: **Potassium (K)** - Consumption data of potash in the US comprise of potassium chloride and other single nutrients. Personal communication with USDA indicates that single nutrients are made up of lime-potash mixture and manure salts. A 50: 50 composition was assumed between KOH and lime for this analysis

Table D-24 Estimation of nitrogen emission from major crop residues using IPPC Tier I model soybean (SB)

IPCC Tier 1 Model Crop Residue Estimation For Soybean

	Nitrogen Emissions for SB Residue		
	Region 2	**Region 3**	**Region 4**
Bushel of SB/acre	29	45	32
kg dm SB / ha	1791	2757	1964
Mg dm SB / ha	1.791	2.757	1.964
Mg dm SB residue / ha	3.015	3.914	3.177
Mg dm residue / Mg dm harvested SB	1.684	1.420	1.617
kg N content / kg SB dm residue above ground	0.008	0.008	0.008
kg N content / kg SB dm residue below ground	0.008	0.008	0.008
kg below ground dm SB residue / above ground dm SB residue	0.190	0.190	0.190
kg N in above and below ground dm SB residue / kg dm SB harvested	0.016	0.014	0.015
g N/bushel	436	368	419
kg N in above and below ground dm SB residue / kg SB harvested	**0.015**	**0.012**	**0.014**

Conversion factors

	slope	intercept	ac/ha	lb/bu	lb/kg	kg dm/kg SB harvested
Soybean	0.93	1.35	2.47	60	2.205	0.91

Table D-25 Estimation of nitrogen emission from major crop residues using IPPC Tier

IPCC Tier 1 Model Crop Residue Estimation For Corn Grain						
		Nitrogen Emissions for Corn Grain Residues				
		Region 1	**Region 2**	**Region 3**	**Region 4**	**Region 5**
Bushel corn grain/ac		129	126	161	140	189
kg dm corn grain / ha		7115	6967	8882	7755	10427
Mg dm corn grain/ ha		7.115	6.967	8.882	7.755	10.427
Mg dm corn grain residue / ha		8.636	8.474	10.562	9.333	12.245
Mg dm residue / Mg dm harvested corn grain		1.214	1.216	1.189	1.203	1.174
kg N content / kg corn grain dm residue above ground		0.006	0.006	0.006	0.006	0.006
kg N content / kg corn grain dm residue below ground		0.009	0.009	0.009	0.009	0.009
kg below ground dm corn grain residue / above ground dm corn grain residue		0.220	0.220	0.220	0.220	0.220
kg N in above and below ground dm corn grain residue / kg dm corn grain harvested		0.010	0.010	0.009	0.010	0.009
g N/bu		246	247	241	244	238
kg N in above and below ground dm corn grain residue / kg corn grain harvested		**0.009**	**0.009**	**0.008**	**0.008**	**0.008**
Conversion factors						
Corn grain	Slope	Intercept	Ac/Ha	lb/Bu	lb/kg	kg dm/kg Corn Grain Harvested
	1.09	0.88	2.47	56	2.205	0.88

I model corn grain

Table D-26 Estimation of nitrogen emission from major crop residues using IPPC Tier I model corn silage

IPCC Tier 1 Model Crop Residue Estimation For Corn Silage						
		Nitrogen Emissions for Corn silage Residues				
		Region 1	**Region 2**	**Region 3**	**Region 4**	**Region 5**
Ton corn silage/ac		18	16	17	17	
kg dm corn silage / ha		13879	12703	13017	13095	
Mg dm corn silage / ha		13.879	12.703	13.017	13.095	
Mg dm corn silage residue / ha		4.164	3.811	3.905	3.928	
Mg dm residue / Mg dm harvested corn silage		0.300	0.300	0.300	0.300	
kg N content / kg corn grain dm residue above ground		0.006	0.006	0.006	0.006	
kg N content / kg corn silage dm residue below ground		0.007	0.007	0.007	0.007	
kg below ground dm corn grain residue / above ground dm corn silage residue		0.54	0.54	0.54	0.54	
kg N in above and below ground dm corn grain residue / kg dm corn silage harvested		0.003	0.003	0.003	0.003	
kg N in above and below ground dm corn grain residue / kg corn silage harvested		**0.00103**	**0.0010**	**0.0010**	**0.0010**	
Conversion factors						
Corn silage	Slope	Intercept	Ac/Ha		lb/kg	kg dm/kg Corn Silage Harvested
	0.3	0	2.47		2.205	0.88

Table D-27 Estimation of nitrogen emission from major crop residues using IPPC Tier I model oats

IPCC Tier 1 Model Crop Residue Estimation						
		Region 1	**Region 2**	**Region 3**	**Region 4**	**Region 5**
Bushel oats/ac		60	59	66	58	85
kg dm oats / ha		1909	1893	2097	1846	2726
Mg dm oats/ ha		1.909	1.893	2.097	1.846	2.726
Mg dm oats residue / ha		2.627	2.612	2.798	2.570	3.371
Mg dm residue / Mg dm harvested oats		1.376	1.380	1.334	1.392	1.236
kg N content / kg oats dm residue above ground		0.007	0.007	0.007	0.007	0.007
kg N content / kg oats dm residue below ground		0.008	0.008	0.008	0.008	0.008
kg below ground dm oats residue / above ground dm oats residue		0.25	0.25	0.25	0.25	0.25
kg N in above and below ground dm oats residue / kg dm oats harvested		0.0123	0.012422	0.01201	0.01253	0.011128
g N/bu		179.76	180.28	174.30	181.84	161.50
kg N in above and below ground dm oats residue / kg oats harvested		**0.01102**	**0.01106**	**0.01069**	**0.0111**	**0.00990**
	Conversion factors					
	Slope	Intercept	Ac/Ha	lb/Bu	lb/kg	kg dm/kg Oats Harvested
Oats	0.91	0.89	2.47	32	2.205	0.89

Table D-28 Estimation of nitrogen emission from major crop residues using IPPC Tier I model winter wheat

IPCC Tier 1 Model Crop Residue Estimation						
		Nitrogen Emissions for winter wheat Residue				
		Region 1	**Region 2**	**Region 3**	**Region 4**	**Region 5**
Bushel winter/ac		59.01	55.30	60.78	36.44	61.61
kg dm winter wheat / ha		3530	3308	3636	2180	3685
Mg dm winter wheat / ha		3.530	3.308	3.636	2.180	3.685
Mg dm winter wheat residue / ha		6.083	5.726	6.253	3.909	6.334
Mg dm residue / Mg dm harvested winter wheat		1.723	1.731	1.720	1.794	1.719
kg N content / kg winter wheat dm residue above ground		0.006	0.006	0.006	0.006	0.006
kg N content / kg winter wheat dm residue below ground		0.009	0.009	0.009	0.009	0.009
kg below ground dm winter wheat residue / above ground dm winter wheat residue		0.23	0.23	0.23	0.23	0.23
kg N in above and below ground dm winter wheat residue / kg dm winter wheat harvested		0.0139	0.0140	0.0139	0.0145	0.0139
g N/bu		378.43	380.10	377.70	393.84	377.38
kg N in above and below ground dm winter wheat residue / kg winter wheat harvested		**0.0124**	**0.0124**	**0.0124**	**0.0129**	**0.0123**
	Conversion factors					
	slope	intercept	ac/ha	lb/bu	lb/kg	kg dm/kg winter wheat harvested
Winter Wheat	1.61	0.4	2.47	60	2.205	0.89

Table D-29 GHG Emission Factors of Pesticides for some major crops (Soybean)

GHG Emission Factors for Soybean	
Pesticides	**kg or lb CO_2 eq./kg or lb pesticide**
Herbicide	
Glyphosate, at regional storehouse/CH U	13.84
2,4-D, at regional storehouse/CH U	2.99
Total (Weighted)	**13.54**
Insecticides	
Organophosphorus-compounds, at regional storehouse/CH U (**Chlorpyrifos**)	6.69
Pyretroid-compounds, at regional storehouse/CH U (**Esfenvalerate**)	20.04
Pyretroid-compounds, at regional storehouse/CH U (**Lambda-Cyhalothrin**)	20.04
Total (Weighted)	**7.89**
Fungicide	
Nitrile-compounds, at regional storehouse/CH U (**Azoxystrobin**)	4.52
Nitro-compounds, at regional storehouse/CH U (**Pyraclostrobin**)	2.26
Total (Weighted)	**2.98**

Table D-30 GHG Emission Factors of Pesticides for some major crops (corn grain/silage)

GHG Emission Factors for Corn grain/silage	
Pesticides	**kg or lb CO_2 eq./kg or lb pesticide**
Herbicide	
Glyphosate, at regional storehouse/CH U	13.84
Atrazine, at regional storehouse/CH U	4.88
S-Metolachlor, at regional storehouse/CH U	7.72
Acetochlor, at regional storehouse/CH U	7.72
Total (Weighted)	**7.55**
Insecticides	
Organophosphorus-compounds, at regional storehouse/CH U (**Tebupirimphos**)	6.69
Pyretroid-compounds, at regional storehouse/CH U (**Tefluthrin**)	20.04
Pyretroid-compounds, at regional storehouse/CH U (**Cyfluthrin**)	20.04
Total (Weighted)	**14.15**

Table D-31 GHG Emission Factors of Pesticides for some major crops (oats)

GHG Emission Factors for Oats	
Agrochemical	**kg or lb CO2 eq./kg or lb pesticide**
Herbicide	
Glyphosate, at regional storehouse/CH U	13.84
2,4-D, at regional storehouse/CH U **(2,4-D,2-EHE)**	2.99
2,4-D, at regional storehouse/CH U **(2,4-D,dimeth salt)**	2.99
Total (Weighted)	**6.52**
Insecticides	
Pyretroid-compounds, at regional storehouse/CH U (**Lambda-Cyhalothrin**)	20.04
Total(Weighted)	**20.04**

Table D-32 GHG Emission Factors of Pesticides for some major crops (winter wheat)

GHG Emission Factors for winter wheat	
Pesticides	**kg or lb CO2 eq./kg or lb pesticide**
Herbicide	
Glyphosate, at regional storehouse/CH U	13.84
2,4-D, at regional storehouse/CH U **(2,4-D,2-EHE)**	2.99
2,4-D, at regional storehouse/CH U **(2,4-D,dimeth salt)**	
[sulfonyl]urea-compounds, at regional storehouse/RER U **(Metsulfuronmethyl)**	9.23
Total (Weighted)	**10.594**
Insecticides	
Organophosphorus-compounds, at regional storehouse/RER U **(Chlorpyrifos)**	6.69
Total	**0.0506**
Fungicide	
Nitro-compounds, at regional storehouse/RER U **(Pyraclostrobin)**	**2.26**
cyclic N-compounds, at regional storehouse/RER U **(Propiconazole)**	**4.53**
acetamide-anillide-compounds, at regional storehouse /RER U **(Azoxystrobin)**	**7.44**
Total (Weighted)	**5.25**

Table D-33 Pedigree matrix for Soybean

Data Quality Indicator (DQI) For Inorganic Fertilizer inputs		
Indicator	**Score**	**Explanation**
Reliability	2	The data source is official from the USDA which was obtained by NASS through the annual Agricultural Resource Management Survey (ARMS)
Completeness	2	Out of 31U.S states producing Soybean, 19 of them had their N, P, K, S input reported representing over 50% for year period considered. This is adequate to cover fluctuations due to different farm practices.
Temporal Correlation	1	Data covers year of study (2006). Original goal was to obtain chemical/fertilizer input for the most recent year (2009), but the most recent obtained was for 2006.
Geographical correlation	1	Data was obtained from soybean producing farms in the United States.(Our interest here is soybean producing farms in the United States where this LCA was conducted)
Further Technological Correlation	1	The data represents the estimated sum of N, P, K, S used by some soybean producing states. NPKS being used in 2006 is basically the same being used with regards to our reference year.
Sample size	1	This survey was conducted for farms in 31 states that produces soybean.
DQI for Crop Protection Chemical Inputs		
Reliability	2	The data source is official from the USDA which was obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews with farmers.
Completeness	2	Once again, out of the 31 potentially soybean producing states, data on about 20 was reported representing over 50%.
Temporal Correlation	1	Data covers year of study (2006). Original goal was to obtain chemical/fertilizer input for the most recent year (2009), but the most recent obtained was for 2006.
Geographical correlation	1	Data was obtained from soybean producing farms in the United States.
Further Technological Correlation	1	The same pesticides are still on the market with no significant changes..
Sample size	2	Sample size greater than ten
DQI for Energy inputs		
Reliability	2	Data obtained from a report for DOE USA prepared by Sheehan and his group. The input was originally obtained from Farm Costs and Returns Survey (FCRS), FCRS was the main precursor to ARMS and was conducted annually from 1985-1995
Completeness	3	14(<<50%) Soybean producing states was considered in the FCRS survey. This represents only some sites which are relevant for our consideration
Temporal Correlation	5	Data from FCRS was for the year 1990, hence 19 years less than the reference year (2009).

Geographical correlation	1	Data was obtained from 14 different soybean producing states in the United States.
Further Technological Correlation	3	Though data is coming from same farms, due to technological advancement over the years some farming practices might have changed.
Sample size	2	Sample size greater than ten

Table D-34 Pedigree matrix for Corn grain/silage

DQI For Inorganic Fertilizer inputs		
Indicator	**Score**	**Explanation**
Reliability	2	The data source is official from the USDA obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews with farmers.
Completeness	3	Out of 41 states producing corn, 19 of them had their N, P, K, S input reported representing less than 50% for the 2005 year period. This is fairly adequate to cover fluctuations due to different farm practices.
Temporal Correlation	2	Data covers year of study (2005). Original goal was to obtain chemical/fertilizer input for the most recent year (2009). We are within 6 years of difference to our reference year.
Geographical correlation	3	Data was obtained from corn producing farms in the United States. However, some of the GHG emission factors were obtained from a similar area (Europe) using values from Simapro.
Further Technological Correlation	1	The data represents the estimated sum of N, P, K, S used by several corn producing states. Life cycle of N, P, K, S in 2005 is relatively identical to today's processes.
Sample size	1	3,300 reports were summarized accounting for 93% of the total US acreage.
DQI for Crop Protection Chemical Inputs		
Reliability	2	The data source is official from the USDA obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews.
Completeness	3	Out of 41 states producing corn, 19 of them had their pesticide inputs reported representing less than 50% for the 2005 year period. This is fairly adequate to cover fluctuations due to different farm practices.
Temporal Correlation	2	Data covers year of study (2005). Original goal was to obtain chemical/fertilizer input for the most recent year (2009). We are within 6 years of difference to our reference year.
Geographical correlation	3	Data was obtained from corn producing farms in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	1	The same pesticides are still on the market. Production processes and materials for these pesticides are nearly identical.
Sample size		3,300 reports were summarized accounting for 93% of the total US acreage.
DQI for Energy inputs		

Reliability	2	Data was obtained from Shapouri et al 2001. This paper is based on straightforward methodology and highly regarded quality data from the 2001 Agricultural Resource Management Survey (ARMS), Economic Research Service, ERS/USDA, 2001 Agricultural Chemical Usage, and 2001 Crop Production, National Agricultural Statistics Service, NASS/USDA, and the 2001 survey of ethanol plants.
Completeness	3	Data is representative of nine major corn producing states. However, this is less than 50% of the total market considered for dairy cattle feed.
Temporal Correlation	3	Data was assembled from 2001 ARMS. Therefore, we are within 10 years of difference to our reference year.
Geographical correlation	3	Data is from a smaller area than the total countrywide area under study.
Further Technologica l Correlation	3	Although data is coming from farms with similar farming practices, technological advancement over the years may account for some change in these practices. This model does not account for differences such as no-till, conventional till, or other tillage practices.
Sample size	3	The figures are significantly aggregated although a portion of the sample size used in these calculations stems from 2,989 reports in a 2001 NASS Agricultural Chemical Usage survey accounting for 93% of the total US acreage.

Table D-35 Pedigree matrix for Oats

DQI For Inorganic Fertilizer inputs		
Indicator	**Score**	**Explanation**
Reliability	2	The data source is official from the USDA obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews.
Completeness	3	Out of 31 states producing oats, 15 of them had their NPKS input reported representing less than 50% for the 2005 year period. This is fairly adequate to cover fluctuations due to different farm practices.
Temporal Correlation	2	Data covers year of study (2005). Original goal was to obtain chemical/fertilizer input for the most recent year (2009). We are within 6 years of difference to our reference year.
Geographical correlation	3	Data was obtained from oat producing farms in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	1	The data represents the estimated sum of NPKS used by some oat producing states. Life cycle of NPKS in 2005 is relatively identical to today's processes.
Sample size	1	1,592 reports were summarized.
DQI for Crop Protection Chemical Inputs		
Reliability	2	The data source is official from the USDA obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews.

Completeness	4	Out of 31 states producing oats, 1 of them had their pesticide inputs reported representing less than 50% for the 2005 year period. This is fairly adequate to cover fluctuations due to different farm practices.
Temporal Correlation	2	Data covers year of study (2005). Original goal was to obtain chemical/fertilizer input for the most recent year (2009). We are within 6 years of difference to our reference year.
Geographical correlation	3	Data was obtained from oat producing farms in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	1	The same pesticides are still on the market. Production processes and materials for these pesticides are nearly identical.
Sample size	1	1,592 reports were summarized.
DQI for Energy inputs		
Reliability	3	Data was obtained from an extension program at Michigan State University. Fuel information was obtained from Michigan State University, was based on a regional study for selected farms in the state of Michigan with assistance from county and regional Extension staff for specialized crops.
Completeness	4	Data is representative of only the state of Michigan. This is one site relevant for the market considered.
Temporal Correlation	3	Data was assembled in Winter/Spring 2000-2001 and represent an estimate of 2000-2001 conditions. We are within 10 years of difference to our reference year.
Geographical correlation	3	Data is from a smaller area than the area under study.
Further Technological Correlation	3	Although data is coming from farms with similar farming practices, technological advancement over the years may account for some change in these practices.
Sample size	5	Unknown sample size.

Table D-36 Pedigree matrix for Winter wheat

DQI For Inorganic Fertilizer inputs		
Indicator	**Score**	**Explanation**
Reliability	2	The data source is official from the USDA obtained by NASS through the annual Agricultural Resource Management Survey (ARMS) and Conservation Effects Assessment Project (CEAP).
Completeness	2	Data reported for all the wheat accounted well over 50% percent of the total of the relevant sites. This is fairly representative.
Temporal Correlation	1	Data covers year of study (2006). Original goal was to obtain chemical/fertilizer input for the most recent year (2009), but the most recent obtained was for 2006.
Geographical correlation	1	Data was obtained from wheat producing farms in the United States.
Further Technological Correlation	1	The data represents the estimated sum of NPKS used by some wheat producing states. NPKS being used in 2006 is basically the same being used with regards to our reference year.
Sample size	1	This survey was conducted for farms in over 20 states that produces wheat
DQI for Crop Protection Chemical Inputs		
Reliability	2	The data source is official from the USDA obtained by NASS through the annual ARMS and Conservation Effects Assessment Project (CEAP). Data for pesticides usage was obtained from personal interviews.
Completeness	2	Once again, data for all the wheat producing states, accounted for over 50% of the relevant sites.
Temporal Correlation	1	Data covers year of study (2006). Original goal was to obtain chemical/fertilizer input for the most recent year (2009), but the most recent obtained was for 2006.
Geographical correlation	1	Data was obtained from wheat producing farms in the United States where we are conducting our LCA study
Further Technological Correlation	1	The same pesticides are still on the market and technology used in for their production has not seen any changes.
Sample size	1	Sample size greater than twenty
DQI for Energy inputs		
Reliability	2	Data obtained from a paper by researchers Piringer. G., and Steinberg. L. J., (2006).The energy and fuel input were originally obtained from a USDA-ERS 2003.
Completeness	3	This paper cited the work of Briggle, in Briggle's system for his energy analysis 8 wheat producing states were considered.
Temporal Correlation	2	With reference to the original energy data source from USDA-ERS 2003, this was about 6 years less than our reference year.
Geographical correlation	2	Data was obtained from wheat producing farms in the United States where we are conducting our LCA study
Further Technological Correlation	3	Though data is coming from same farms, due to technological advancement over the years some farming practices might have changed. This paper cited work done as far back as 1980.
Sample size	3	Sample size greater than ten.

Table D-37 Pedigree matrix for DDG

DQI For Inorganic Fertilizer inputs		
Indicator	**Score**	**Explanation**
Reliability	3	The inventory data in the references for DDGS GHG emissions are a combination of measurements and estimates based on best engineering judgment.
Completeness	3	The references are unclear on this topic, so we assume a middle value for completeness indicator.
Temporal Correlation	3	Data covers inputs from near the year 2000 for one reference and up to 2006 for another, so we choose a middle value.
Geographical correlation	2	The inventory data in the references was national in geographic extent, and therefore do not represent the regional differences that are being sought in this dairy study.
Further Technological Correlation	1	Inventory data from references are from processes that produce ethanol from corn and DDGS, and therefore the correlation is good.
Sample size	3	The references are unclear on this topic, so we assume a middle value for sample size indicator.

Table D-38 Pedigree matrix for Alfalfa Hay and Silage

DQI For Alfalfa Hay & Silage inputs and yield		
Indicator	**Score**	**Explanation**
Reliability	4	The data source is based upon production budgets from state extension specialists. These budgets are based upon best or expected practices, and do not represent actual data or average data for a county, state or region
Completeness	3	This data represents 15 states, and although in some cases multiple budgets per state
Temporal Correlation	1	Data covers primarily the year of study, but nearly all are within 3 years of study
Geographical correlation	3	Data was obtained from Alfalfa producing states in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	4	The data represents the estimated sum of inputs from alfalfa producing farms. In some cases input quantities are based upon conversions from prices, and hence highly dependent upon fluctuating price levels.
Sample size	2	There are 39 production budgets. While they do not represent actual data, they are expected to represent approximate average production methods

Table D-39 Pedigree matrix for Grass Hay and Silage

DQI For Grass Hay & Silage inputs and yield		
Indicator	**Score**	**Explanation**
Reliability	4	The data source is based upon production budgets from state extension specialists. These budgets are based upon best or expected practices, and do not represent actual data or average data for a county, state or region
Completeness	3	This data represents 17 states, and although in some cases multiple budgets per state
Temporal Correlation	1	Data covers primarily the year of study, but nearly all are within 3 years of study
Geographical correlation	3	Data was obtained from grass producing states in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	4	The data represents the estimated sum of inputs from grass producing farms. In some cases input quantities are based upon conversions from prices, and hence highly dependent upon fluctuating price levels.
Sample size	2	There are 44 production budgets. While they do not represent actual data, they are expected to represent approximate average production methods

Table D-40 Pedigree matrix for Grass Pasture

DQI For Grass Pasture inputs and yield		
Indicator	**Score**	**Explanation**
Reliability	**4**	The data source is based upon production budgets from state extension specialists. These budgets are based upon best or expected practices, and do not represent actual data or average data for a county, state or region
Completeness	**3**	This data represents 17 states, and although in some cases multiple budgets per state
Temporal Correlation	**1**	Data covers primarily the year of study, but nearly all are within 3 years of study
Geographical correlation	**3**	Data was obtained from grass producing states in the United States. However, the GHG data comes from a similar area (Europe) using values from Simapro. Furthermore only the states with reported inputs are averaged or summed.
Further Technological Correlation	**5**	The data represents the estimated sum of inputs from grass hay producing farms, but does not represent grass pasture
Sample size	**2**	There are 44 production budgets. While they do not represent actual data, they are expected to represent approximate average production methods

Table D-41 Pedigree matrix for Soybean Meal

DQI For Soybean Meal inputs and yield		
DQI For DDGS Results	**Score**	**Explanation**
Reliability	2	The inventory data in the references for SBM/O GHG emissions are a combination of conversations with industry representatives and modeled data.
Completeness	2	14(>>50%) Soybean producing states was considered in the model by Sheehan et al 1998.
Temporal Correlation	2	The crushing and extraction energy required were updated based on a more recent study by Pradhan et al., (2009)
Geographical correlation	2	The inventory data in the references was national in geographic extent, and therefore do not represent the regional differences that are being sought in this dairy study.
Further Technological Correlation	1	Inventory data from references are from processes that produce soybean meal and oil from soybean, and therefore the correlation is good.
Sample size	2	The sample size represents the major soybean producing states in the US

Table D-42 Geometric standard deviation estimation (SBM: Soybean meal)

Estimation of the square of geometric standard deviation (SDg_{95})				
	Inputs	**Data Quality Index**	**Uncertainty factors (U_n)**	**$SD_{g\,95}$**
Corn	Inorganic Fertilizer	(2, 3, 2, 3, 1, 1)	(1.05, 1.05, 1.03, 1.02, 1.0, 1.0, 1.50)	1.51
	Crop Protection Chemical	(2, 3, 2, 3, 1, 1)	(1.05, 1.05, 1.03, 1.02, 1.0, 1.0, 1.2)	1.22
	Energy	(2, 3, 3, 3, 3, 3)	(1.05, 1.05, 1.10, 1.02, 1.20, 1.05, 1.05)	1.26
Soybean	Inorganic Fertilizer	(2, 2, 1, 1, 1, 1)	(1.05, 1.02, 1.0, 1.0, 1.0, 1.0, 1.5)	1.51
	Crop Protection Chemical	(2, 2, 1, 1, 1, 2)	(1.05, 1.02, 1.0, 1.0, 1.0, 1.02, 1.2)	1.21
	Energy	(2, 3, 5, 1, 3, 2)	(1.05, 1.02, 1.0, 1.0, 1.0, 1.02, 1.05)	1.57
SBM	For all input data	(2, 2, 2, 2, 1, 2)	(1.05, 1.02, 1.03, 1.01, 1, 1.02, 1.05)	1.08
Oats	Inorganic Fertilizer	(2, 3, 2, 3, 1, 1)	(1.05, 1.05, 1.03, 1.02, 1.0, 1.0, 1.50)	1.51
	Crop Protection Chemical	(2, 4, 2, 3, 1, 1)	(1.05, 1.10, 1.03, 1.02, 1.0, 1.0, 1.2)	1.24
	Energy	(3, 4, 3, 3, 3, 5)	(1.10, 1.10, 1.10, 1.02, 1.20, 1.20, 1.05)	1.36
Wheat	Inorganic Fertilizer	(2, 2, 1, 1, 1, 1)	(1.05, 1.02, 1.0, 1.0, 1.0, 1.0, 1.5)	1.51
	Crop Protection Chemical	(2, 2, 1, 1, 1, 2)	(1.05, 1.02, 1.0, 1.0, 1.0, 1.02, 1.2)	1.21
	Energy	(2, 3, 2, 2, 3, 3)	(1.05, 1.05, 1.03, 1.01, 1.20, 1.05, 1.05)	1.23
DDGS	For all input data	(3, 3, 3, 2, 1, 3)	(1.1, 1.05, 1.1, 1.01, 1.0, 1.02, 1.5)	1.54
Alfalfa	For all input data	(4, 3,1, 3, 1, 3)	(1.2, 1.05, 1.0, 1.02, 1.0, 1.05, 1.05)	1.22
Grass Hay	For all input data	(4, 3,1, 3, 1, 2)	(1.2, 1.05, 1.0, 1.02, 1.0, 1.02, 1.05)	1.22
Grass Pasture	For all input data	(4, 3,1, 3, 5, 3)	(1.2, 1.05, 1.0, 1.02, 1.0, 1.05, 1.05)	2.06

Table D-43 Estimation of upper/lower bound values of grain crops

		Geometric mean equivalent emission ($kgCO_2eq$ /kg feed)	Inorganic fertilizers	Crop protection chemicals	Energy sources
Soybean	Square of Geometric Standard Deviation GSD^2 (σg^2)		1.510	1.210	1.570
	minValue (2.5%) equivalent emission=$\mu g/\sigma g^2$	0.237	0.088	0.063	0.086
	Geometric mean (μg) equivalent emission	0.344	0.133	0.076	0.135
	maxValue equivalent emission (97.5%)=$\mu g*\sigma g^2$	0.505	0.200	0.092	0.212
Oats	Square of Geometric Standard Deviation GSD^2 (σg^2)		1.510	1.240	1.360
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.507	0.366	0.063	0.077
	Geometric mean (μg)	0.736	0.553	0.078	0.105
	maxValue (97.5%)=$\mu g*\sigma g^2$	1.075	0.834	0.097	0.143
Corn grain	Square of Geometric Standard Deviation GSD^2 (σg^2)		1.510	1.220	1.260
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.231	0.160	0.005	0.066
	Geometric mean (μg)	0.331	0.242	0.006	0.084
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.478	0.365	0.007	0.105
Corn silage	Square of Geometric Standard Deviation GSD^2 (σg^2)		1.510	1.220	1.260
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.049	0.036	0.003	0.010
	Geometric mean (μg)	0.071	0.055	0.004	0.012
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.103	0.083	0.005	0.015
Winter wheat	Square of Geometric Standard Deviation GSD^2 (σg^2)		1.510	1.210	1.230
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.270	0.225	0.001	0.043
	Geometric mean (μg)	0.395	0.340	0.001	0.053
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.581	0.514	0.001	0.066
Wet,DDG	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.540			
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.174			
	Geometric mean (μg)	0.268			
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.412			
Dry,DDG	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.540			
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.531			

	Geometric mean (μg)	0.818
	maxValue (97.5%)=μg*σg^2	1.259
Soybean meal	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.080
	minValue (2.5%)'=μg/σg^2	0.376
	Geometric mean (μg)	0.406
	maxValue (97.5%)=μg*σg^2	0.438

Table D-44 Estimation of upper/lower bound values of forage

		Geometric mean equivalent emission ($kgCO_2eq/kg$ feed)
Alfalfa hay	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.224
	minValue (2.5%) equivalent emission=$\mu g/\sigma g^2$	0.120
	Geometric mean (μg) equivalent emission	0.147
	maxValue equivalent emission (97.5%)=$\mu g*\sigma g^2$	0.180
Alfalfa silage	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.224
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.127
	Geometric mean (μg)	0.156
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.191
Forage mix	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.224
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.112
	Geometric mean (μg)	0.137
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.167
Grain mix	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.224
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.365
	Geometric mean (μg)	0.446
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.546
Grass hay	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.218
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.223
	Geometric mean (μg)	0.272
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.331
Grass pasture	Square of Geometric Standard Deviation GSD^2 (σg^2)	2.058
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.110
	Geometric mean (μg)	0.226
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.466
Grass silage	Square of Geometric Standard Deviation GSD^2 (σg^2)	1.218
	minValue (2.5%)'=$\mu g/\sigma g^2$	0.230
	Geometric mean (μg)	0.280
	maxValue (97.5%)=$\mu g*\sigma g^2$	0.341

Appendix E: Supplementary information for carbon footprint analysis of dairy feed from a mill in Michigan, USA

	Preprint version (with a few exceptions- see below *)	Accepted Author Manuscript	Published Journal Articles
Use for classroom teaching by author or author's institution and presentation at a meeting or conference and distributing copies to attendees	Yes	Yes	Yes
Use for internal training by author's company	Yes	Yes	Yes
Distribution to colleagues for their research use	Yes	Yes	Yes
Use in a subsequent compilation of the author's works	Yes	Yes	Yes
Inclusion in a thesis or dissertation	Yes	Yes	Yes
Reuse of portions or extracts from the article in other works	Yes	Yes with full acknowledgement of final article	Yes with full acknowledgement of final article
Preparation of derivative works (other than for commercial purposes)	Yes	Yes with full acknowledgement of final article	Yes with full acknowledgement of final article
Preprint servers	Yes	Yes with the specific written permission of Elsevier	No
Voluntary posting on open web sites operated by author or author's institution for scholarly purposes	Yes (author may later add an appropriate bibliographic citation, indicating subsequent publication by Elsevier and journal title)	Yes, with appropriate bibliographic citation and a link to the article once published	Only with the specific written permission of Elsevier
Mandated deposit or deposit in or posting to subject-oriented or centralized repositories	Yes under specific agreement between Elsevier and the repository	Yes under specific agreement between Elsevier and the repository**	Yes under specific agreement between Elsevier and the repository
Use or posting for commercial gain or to substitute for services provided directly by journal	Only with the specific written permission of Elsevier	Only with the specific written permission of Elsevier	Only with the specific written permission of Elsevier

Figure E-1 Copyright clearance for Elsevier (http://www.elsevier.com/authors/author-rights-and-responsibilities)

Figure E-1 above applies to chapter 7 including all supplementary materials in Appendix-E

Table E-1 Survey questions for milling operations

Sustainable Technologies Laboratory

Sustainable Futures Institute
Michigan Technological University

MichiganTech

SURVEY FOR THE ANALYSIS OF FEEDMILL GREENHOUSE GAS EMISSIONS

Company Name:	
Date:	
Address:	
Name of Contact Person:	
Phone:	
Email:	
Year of data:	

Contact:
David R. Shonnard, Ph.D.
Robbins Professor
Department of Chemical Engineering
Sustainable Futures Institute
Michigan Technological University
Houghton, MI 49931USA
Ph. 906-487-3468
Email: drshonna@mtu.edu

Total Annual Mill Energy Input (1 year of mill operation)	
Annual Fuel Input for Feed Mill	**Quantity**
Diesel (gallons/yr)	
Gasoline (gallons/yr)	
Kerosene (gallons/yr)	
Liquified Petroleum Gas (lb or volume/yr)	
Natural Gas/ Propane (scf or m3 or BTU / yr)	
Fuel oil (gallons / yr)	
Coal (lb / yr)	
Electricity (kWh / yr)	

Total Annual Animal Feed Production (1 year production)	
Type of Feed	**Short Tons/year**
Dairy feed; all types Other animal feed; all types	

Dairy Feed Production (1 year production)	
Type of Feed	**Short Tons/year**
Starter period	
Starter Feed birth *Open Heifer Feed* *Bred Heifer Feed*	
Lactation period	
High Ration *Low Ration*	
Dry period	

Routine Lubrication and Maintenance of Feed Mill	
Frequently used Lubricant/ Fluid	**Amount (Gallons/year)**
Grease Others (please list)	

Table E-2 Survey Questions for feed ingredients inputs

Feed Type		
Starter/Lactating/Dry Feed		

Starter/Lactating/ Dry Feed-Based on 1 short ton production		
Kind of ingredients used in feed	**Amount (lb/ kg / Ton)**	**% composition**
Alfalfa Product		
Alfalfa meal, dehydrated, 13%		
Alfalfa meal, dehydrated, 17%		
Alfalfa meal, suncured, 13%		
Others (please list)		
Animal Products		
Blood meal		
Blood flour		
Meat meal		
Others (please list)		
Barley Products		
Rolled barley		
Barley		
Others (please list)		
Brewers Products		
Brewers dried grains		
Malt sprouts		
Others (please list)		
Citrus Products		
Dried citrus pulp		
Others (please list)		
Corn Products		
Corn, whole shelled		
Corn meal		
Corn bran		
Others (please list)		

Table E-3 Survey questions for feed transportation inputs

Sustainable Technologies Laboratory

Sustainable Futures Institute
Michigan Technological University

MichiganTech

Crop Input To The Mill

Dairy Feed Crop	Mode of Transport										
		Vehicle	DC 1	% Used		% Used	DC 1		Vehicle	DC 1	% Used
	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		Other		%							
	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		Other		%							
	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		Other		%							

Mill Product (Output)

Feed	Mode of Transport										
		Vehicle	DC 1	% Used		% Used	DC 1		Vehicle	DC 1	% Used
Starter, Early	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							
Starter, Open Heifer	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							
Starter, Bred Heifer	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							
Lactation, High Ration	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							
Lactation, Low Ration	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							
Dry Feed	Road	Large Semi		%	Rail	%		Water	Ship		%
		Small Semi		%							
		Flatbed		%					Barge		%
		delivery Van		%							

[1] **Please enter the following information for the Distance Code:**

DC=Distance Code

A	0-50 miles
B	50-100 miles
C	100-150 miles
D	150-200 miles
E	Specify number of miles if greater than 200 miles

Table E-4 Components of Minerals Mixture (Category 2)

Minerals Mixture		
DESCRIPTION (T = TRUCK, R = RAIL)	**Kg**	**PERCENTAGE**
SAFEGUARD 1000 10# (T)	20	0.0002%
IVOMEC 20# (T)	50	0.0006%
MAXI CARE 25# (T)	110	0.0012%
COBAN 90G 50# (T)	20	0.0002%
SAFE-GUARD 5% 25# (T)	60	0.0006%
RABON BLOCK 33.3# (T)	420	0.0044%
SUPER MICRO (T)	110	0.0012%
AVAILA-ZM 55.115# (T)	120	0.0013%
OYSTER SHELLS 50# (T)	910	0.0094%
CHOLINE CHLORIDE55.115# (T)	520	0.0054%
TYLAN 10G 50# (T)	110	0.0012%
ALTOSID TUB 225# (T)	410	0.0042%
PURELY NAT 9T PMX 50# (T)	230	0.0023%
BEL 90 50# (T)	1,810	0.0187%
MGA 200 50# BAG (T)	20	0.0002%
PEAK PLUS 37 50# (T)	910	0.0094%
GRIT - MEDIUM 50# (T)	2,720	0.0281%
HY-D 55.115 #BAG (T)	120	0.0013%
SAFE-GUARD PIG WORMER 50# (T)	50	0.0005%
BMD 60 -50# BAG (T)	110	0.0012%
ACID-I-FRESH RUM 50# (T)	910	0.0094%
COW'S MATCH JERSEY 50# (T)	450	0.0047%
BOVATEC 91 50# (T)	70	0.0007%
SEL 270 50# (T)	3,630	0.0375%
MAXI CARE 50# (T)	680	0.0070%
PURINA SUPP 2 20-05 50# (T)	1,810	0.0187%
ECOCARE PAK 50# BAG (T)	910	0.0094%
COBAN 90 (T)	160	0.0016%
DRY COW MICRO PAK 50#	340	0.0035%
LDH FORTIFIER 50# (T)	270	0.0028%
NATURA PORK SOW96 48# (T)	2,090	0.0216%
REASHURE CHOLINE 25# (T)	910	0.0094%
AVATEC (T)	250	0.0026%
EN140P 50# BAG (T)	450	0.0047%
SWINE MICRO 4 50# (T)	910	0.0094%
CROP N RICH 1000 (T)	230	0.0023%
S-700 CRUMBS 50# (T)	3,630	0.0375%
SELENO SOURCE 2000 50# (T)	910	0.0094%
AVAILA-4 55# (T)	2,000	0.0206%
MEPRON 85 55# BAG (T)	1,000	0.0103%
DAIRY FORTA PLUS -50# (T)	7,260	0.0750%
TOTAL	37,700	0.39%

Table E-5 Feed Inputs; 4-Month Purchase History (Category 3)

SUPPLEMENTS :SECTOR 311119 (ANIMAL FOOD MANUFACTURING					
DESCRIPTION (T = TRUCK, R = RAIL)	kg	PERCENTAGE	DESCRIPTION (T = TRUCK, R = RAIL)	kg	PERCENTAGE
19% PIG 50# (T)	910	0.01%	HEIFER MIN R1600 50# (T)	11,750	0.12%
AKEY GOLD MILK REPLACER 50# (T)	910	0.01%	HIGH LYSINE BYPASS BULK (T)	21,450	0.22%
ALFALFA CUBES 50# (T)	140	0.00%	LACT-GOAT MIX (T)	270	0.00%
AMPLI-CALF 22% 50# (T)	1,360	0.01%	LAYER PMX S 50# (T)	340	0.00%
ASP-250 50# (T)	140	0.00%	L-LYSINE 98.5%-55.115# BAG (T)	7,000	0.07%
AUREOMYCIN 50 50#	910	0.01%	METHIONINE DL 55# (T)	2,990	0.03%
AURO 50 FULL BAG (T)	910	0.01%	MGA 2000 50# BAG (T)	70	0.00%
AURO CRUMBS 10G 50# (T)	1,130	0.01%	OMNIGEN AF 50# (T)	9,070	0.09%
AURO CRUMBS 10GR 50# (T)	910	0.01%	PHYTASE 1200 50# (T)	880	0.01%
BEEF MICRO PREMIX (T)	520	0.01%	PIG 2000 50# (T)	90	0.00%
BIOTIN 640 50# BAG (T)	2,720	0.03%	PRESTART PIG 550 50# (T)	230	0.00%
BLOOD MEAL 50# (T)	50	0.00%	PURINA RACE READY 50# (T)	1,130	0.01%
BOAR PLUS 25# (T)	450	0.00%	RU-MAX 40# (T)	470	0.00%
BUNKLIFE 50 # (T)	1,130	0.01%	RUMENSIN 80-50# (T)	910	0.01%
CALF 16 PELLET B60 (T)	102,670	1.06%	SUNGLO FINAL CONTROL 35# (T)	50	0.00%
CALF PRIMER DIRECT(T)	38,070	0.39%	SUNGLO FULL TANK (T)	180	0.00%
CALF"O"LA PELLET 16%(T)	21,830	0.23%	SUNGLO SUMO (T)	30	0.00%
CROP N RICH G (T)	450	0.00%	THIAMINE10GM 50# (T)	20	0.00%
D/B FINISHER 35/15 50# (T)	8,190	0.08%	THREONINE 55# (T)	270	0.00%
DEER PELLET 20%(T)	2,670	0.03%	TMR 30 (T)	22,780	0.24%
DRIED WHEY 50# (T)	1,814	0.02%	TURKEY PMX W/PHITASE 50# (T)	1,840	0.02%

DRY COW AKEY 50 # (T)	1,790	0.02%	TURKEY STARTER BREEDER 50# (T)	910	0.01%
ENERGY BOOSTER 100 50# BAG (T)	29,940	0.31%	ULTRA MET 50# (T)	910	0.01%
EXT NUGGET CONC BULK (T)	22,600	0.23%	VIT A-30 50# (T)	450	0.00%
FISH MEAL 50# (T)	3,630	0.04%	VIT ADE 50# (T)	5,420	0.06%
FREMONT GF PREMIX (T)	910	0.01%	VIT E - 125000-50# (T)	1,810	0.02%
GLDN CALF CH(T)	16,270	0.17%	VITA PLUS MILK 50# (T)	1,470	0.02%
GRO BOOSTER 45# (T)	180	0.00%	VITAMIN D3 4MIL IU/ 50# (T)	450	0.00%
GRO FIN 46-37 46# (T)	1,670	0.02%	VITAMIN E 20000 50# (T)	230	0.00%
GRO N GLOW CALF(T)	20,050	0.21%	VITA-SOY BULK (T)	5,730	0.06%
HAY PRO 2 DRUM 50 GA (T)	3,020	0.03%	WHITE MILLET 50# (T)	50	0.00%
HAY PRO 2 TOTE 200 GA (T)	3,020	0.03%	XPC YEAST 50# (T)	8,160	0.08%
HEIFER CONCENTRATE 35% 50# (T)	1,810	0.02%	YEAST, XP 50# BAG (T)	1,810	0.02%
HEIFER CONCENTRATE 35%(T)	6,400	0.07%	TOTAL	408,420	4%

Table E-6 Transportation Inputs for Category 1 Feed Ingredients

TRANSPORTATION INPUT DATA TO MILLING SITE (CATEGORY 1) [Emission Factors from SimaPro: 0.0385x10^{-3} kg CO_2 eq kgkm^{-1} EI US trains & 0.168x10^{-3} kg CO_2 eq tkm^{-1} EUR 16,257-32,514 kg lorry]						
CATEGORY 1 FEED INPUT DESCRIPTION (T = TRUCK, R = RAIL)	**UNITS PURCHASED (kg)**	**MILEAGE (MILES)**	**kg**	**kgkm^{-1} (x10^3)**	**TOTAL kgCO_2eq EMITTED**	**kg CO_2eq / kg DAIRY FEED OUTPUT**
FUZZY COTTONSEED(T)	124,000	100	124,284	20,022	3,363.71	3.95E-04
CORN GLUTEN FEED BULK (T)	578,000	100	577,877	93,019	15,627.23	1.83E-03
DISTILLERS BULK (T)	843,000	200	842,775	271,225	45,565.78	5.35E-03
CORN GLUTEN DIRECT(T)	127,000	100	127,006	20,469	3,438.76	4.04E-04
DIRECT DISTILLERS(T)	89,000	100	88,904	14,257	2,395.11	2.81E-04
CANOLA MEAL(T)	304,000	200	303,907	97,959	16,457.07	1.93E-03
HEIFER CONCENTRATE 35%(T)	6,000	300	6,350	3,092	519.49	6.10E-05
HEIFERS EDGE DIRECT(T)	27,000	100	27,216	4,413	741.34	8.70E-05
SOYBEAN MEAL 48% DIRECT(T)	83,000	50	82,554	6,672	1,120.91	1.32E-04
CHIEF BEEF FINISHER 36 (T)	25,000	30	25,401	1,216	204.34	2.40E-05
DAIRY BEEF FINISHER(T)	3,000	100	2,722	438	73.52	8.63E-06
BRAN MEAL 50 #(T)	500	100	907	73	12.26	1.44E-06
BULK 48% SOY 50#(T)	1,915,000	50	1,915,067	154,134	25,894.58	3.04E-03
HEIFERS EDGE BULK (T)	46,000	100	46,266	7,436	1,249.25	1.47E-04
SOY CHLOR 16 50#(T)	11,000	300	10,886	5,256	882.99	1.04E-04
SOYPLUS-BULK 50#(R)	3,271,000	500	3,271,308	2,632,250	101,341.62	1.19E-02
VITA-SOY BULK(T)	6,000	300	5,443	2,768	465.04	5.46E-05
DAIRY SUGAR 38(T)	53,000	100	52,617	8,485	1,425.54	1.67E-04

DAIRY SUGAR 35(T)	8,000	100	8,165	1,320	221.73	2.60E-05
DIRECT SOYHULLS(T)	22,000	100	21,772	3,568	599.45	7.03E-05
DIRECT SOYPLUS(T)	21,000	100	21,772	3,434	576.96	6.77E-05
SOY HULLS BULK (T)	189,000	100	188,694	30,340	5,097.07	5.98E-04
ALFALFA CUBES 50# (T)	100	100	136	22	3.68	4.32E-07
BLOOD MEAL 50# (T)	0	300	45	22	3.68	4.32E-07
FISH MEAL 50# (T)	4,000	300	3,629	1,752	294.33	3.45E-05
PORK & BONE MEAL - BULK (T)	108,000	100	107,955	17,415	2,925.64	3.43E-04
A/V BLEND FAT BULK (T)	94,000	100	93,440	15,100	2,536.88	2.98E-04
CHOICE WHITE GREASE BULK (T)	79,000	100	78,925	12,760	2,143.71	2.52E-04
ENERGY BOOSTER 100 50# BAG (T)	30,000	300	29,937	14,454	2,428.23	2.85E-04
MEGALAC 50# (T)	2,000	300	1,814	876	147.17	1.73E-05
DRY MOLASSES 50# (T)	7,000	100	7,257	1,168	196.22	2.30E-05
LIQUID MOLASSES-BULK (T)	29,000	100	29,030	4,687	787.33	9.24E-05
MOLASSES TUB-16% (T)	1,000	100	907	117	19.62	2.30E-06
MOLASSES TUB-25% (T)	1,000	100	907	117	19.62	2.30E-06
DIRECT MOLASSES(T)	5,000	100	5,443	881	148.02	1.74E-05
ROLLED OATS 50# (T)	2,000	100	1,814	281	47.22	5.54E-06
FEED UREA BAG 50# (T)	41,000	300	41,730	19,929	3,348.01	3.93E-04
DRIED WHEY 50# (T)	2,000	300	1,814	876	147.17	1.73E-05
Total (4 months)-Unallocated					242,470.28	2.85E-02

Table E-7 Transportation Inputs for Category 2 Feed Ingredients

	TRANPORTATION INPUT DATA TO MILLING SITE (CATEGORY 2) [Emission Factors from SimaPro: 0.0385x10^{-3} kg CO_2 eq kgkm^{-1} EI US trains & 0.168x10^{-3} kg CO_2 eq kgkm^{-1} EUR 16,257-32,514 kg lorry]						
	CATEGORY 2 FEED INPUT DESCRIPTION (T = TRUCK, R = RAIL)	**UNITS PURCHASED (kg)**	**MILEAGE (MILES)**	**kg**	**kgkm^{-1}(x10^3)**	**TOTAL (kg CO_2eq.EMITTED)**	**kg CO_2eq / kg DAIRY FEED OUTPUT**
gypsum	CAL SULFATE -BAG 50# (T)	26,708	300	26,308	12,894	2,166.27	2.54E-04
lime	HYDRATED LIME 50# BAG (T)	1,810	100	1,814	292	49.06	5.76E-06
limestone	CAL CARB BULK (T)	281,109	300	281,227	135,720	22,801.04	2.68E-03
limestone	CAL-CARB-50# (T)	12,700	100	12,701	2,044	343.39	4.03E-05
limestone	DICAL BAG 50# (T)	910	100	907	146	24.53	2.88E-06
MgO	MAG OXIDE BAG 50# (T)	19,051	150	19,051	4,599	772.62	9.07E-05
$MGSO_4$	MAG. SULFATE 50# BAG (T)	4,218	150	4,536	1,018	171.08	2.01E-05
minerals	24-12 MINERAL 50# (T)	2,250	100	1,814	361	60.71	7.12E-06
minerals	COPPER SULFATE - FINE 50# (T)	1,814	100	1,814	292	49.06	5.76E-06
minerals	COPPER SULFATE-CRYB 50# (T)	340	100	0	55	9.20	1.08E-06
minerals	DAIRY BASE MIX BULK (T)	85,162	300	85,275	41,116	6,907.57	8.11E-04
minerals	DCAD PLUS-POTASM CARB 50# (T)	9,979	400	9,979	6,424	1,079.21	1.27E-04
minerals	DICAL/MONOCAL BULK (T)	48,852	300	48,988	23,586	3,962.42	4.65E-04
minerals	IODINE 50 50# (T)	70	300	0	33	5.52	6.48E-07

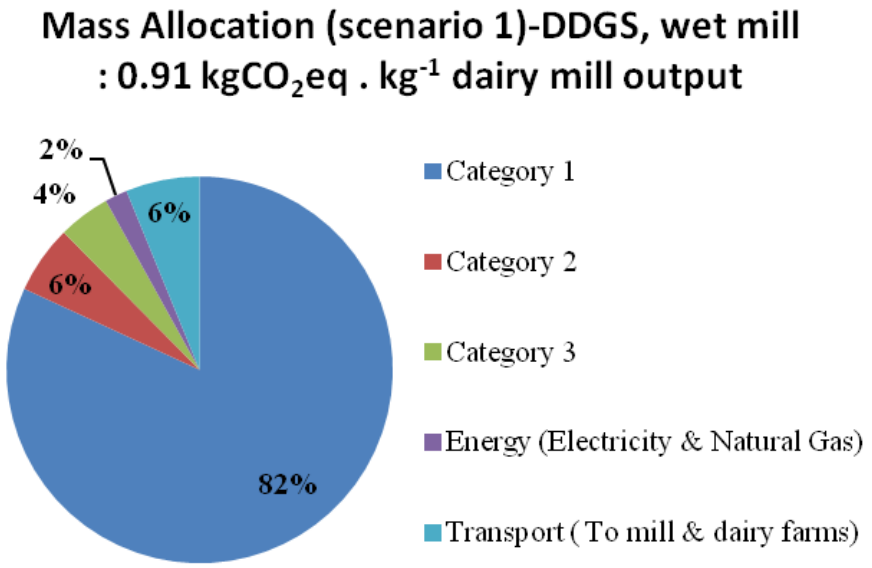
minerals	MANGANESE SULFATE (T)	1,814	150	1,814	438	73.58	8.64E-06
minerals	PROPNOS MINERAL W/ALTOSIU (T)	230	200	0	73	12.26	1.44E-06
NaCl	MIXING SALT BAG (T)	14,450	50	14,515	1,163	195.30	2.29E-05
NaCl	TM BLOCKS W/SEL (T)	3,990	50	3,629	321	53.96	6.33E-06
NaCl	TM SALT BAG 50# (T)	10,000	50	9,979	805	135.21	1.59E-05
NaCl	MIXING SALT BULK (T)	136,029	50	136,078	10,946	1,838.90	2.16E-04
NaCl	WHITE SALT BLOCKS (T)	2,495	50	2,722	201	33.73	3.96E-06
NaCl	WHITE SALT-50# (T)	2,223	50	1,814	179	30.05	3.53E-06
NaCl	TM BLOCKS (T)	6,990	50	7,257	562	94.43	1.11E-05
	Mineral Mixture	38,180	9000	38,102	553,066	92,915.13	1.09E-02
soda powder	BICARB BULK (R)	445,047	300	445,428	214,870	8,272.49	9.71E-04
soda powder	BI-CARB-BAG (T)	8,730	100	9,072	1,405	236.08	2.77E-05
Total (4 months)						142,292.78	1.67E-02

Table E-8 Transportation Inputs for Category 3 Feed Ingredients

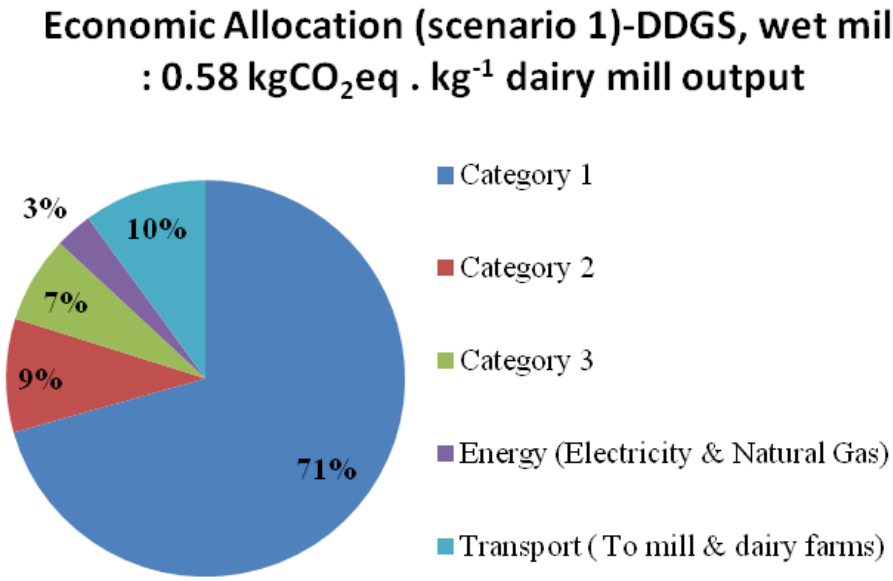
CATEGORY 3 FEED INPUT DESCRIPTION (T = TRUCK, R = RAIL)	UNITS PURCHASED (kg)	MILEAGE (MILES)	kg	$kgkm^{-1}$ ($x10^3$)	TOTAL (kg CO_2eq. EMITTED)	kg CO_2eq / kg DAIRY FEED OUTPUT
19% PIG 50# (T)	910	200	907	292	49	5.76E-06
AKEY GOLD MILK REPLACER 50# (T)	910	300	907	438	74	8.64E-06
AMPLI-CALF 22% 50# (T)	1,360	100	1,361	219	37	4.32E-06
ASP-250 50# (T)	140	300	136	66	11	1.30E-06
AUREOMYCIN 50 50#	910	200	907	292	49	5.76E-06
AURO 50 FULL BAG (T)	910	300	907	438	74	8.64E-06
AURO CRUMBS 10G 50# (T)	1,130	100	1,134	182	31	3.60E-06
AURO CRUMBS 10GR 50# (T)	910	100	907	146	25	2.88E-06
BEEF MICRO PREMIX (T)	520	300	522	252	42	4.97E-06
BIOTIN 640 50# BAG (T)	2,722	100	2,722	438	74	8.64E-06
BOAR PLUS 25# (T)	450	300	454	219	37	4.32E-06
BUNKLIFE 50 # (T)	1,130	300	1,134	547	92	1.08E-05
CALF 16 PELLET B60 (T)	103,000	100	102,673	16,524	2776	3.26E-04
CALF PRIMER DIRECT(T)	38,000	100	38,075	6,128	1029	1.21E-04
CALF"O"LA PELLET 16%(T)	22,000	30	21,827	1,054	177	2.08E-05
CROP N RICH G (T)	454	300	454	219	37	4.32E-06
D/B FINISHER 35/15 50# (T)	8,190	100	8,187	1,318	221	2.60E-05
DEER PELLET 20%(T)	3,000	30	2,667	129	22	2.54E-06
DRY COW AKEY 50 # (T)	1,790	300	1,792	865	145	1.71E-05
EXT NUGGET CONC BULK (T)	22,600	100	22,603	3,638	611	7.17E-05
FREMONT GF PREMIX (T)	910	100	907	146	25	2.88E-06

GLDN CALF CH(T)	16,000	100	16,273	2,619	440	5.16E-05
GRO BOOSTER 45# (T)	180	300	184	89	15	1.75E-06
GRO FIN 46-37 46# (T)	1,670	300	1,669	806	135	1.59E-05
GRO N GLOW CALF(T)	20,000	30	20,047	968	163	1.91E-05
HAY PRO 2 DRUM 50 GA (T)	3,020	100	3,019	486	82	9.58E-06
HAY PRO 2 TOTE 200 GA (T)	3,020	100	3,019	486	82	9.58E-06
HEIFER CONCENTRATE 35% 50# (T)	1,810	30	1,814	88	15	1.73E-06
HEIFER MIN R1600 50# (T)	11,750	100	11,748	1,891	318	3.73E-05
HIGH LYSINE BYPASS BULK (T)	21,446	100	21,446	3,451	580	6.80E-05
LACT-GOAT MIX (T)	270	200	272	88	15	1.73E-06
LAYER PMX S 50# (T)	340	300	340	164	28	3.24E-06
L-LYSINE 98.5%-55.115# BAG (T)	7,000	300	7,000	3,380	568	6.66E-05
METHIONINE DL 55# (T)	2,994	300	2,994	1,445	243	2.85E-05
MGA 2000 50# BAG (T)	70	300	68	33	6	6.48E-07
OMNIGEN AF 50# (T)	9,072	300	9,072	4,380	736	8.64E-05
PHYTASE 1200 50# (T)	885	300	885	427	72	8.42E-06
PIG 2000 50# (T)	90	300	91	44	7	8.64E-07
PRESTART PIG 550 50# (T)	230	300	227	109	18	2.16E-06
PURINA RACE READY 50# (T)	1,130	100	1,134	182	31	3.60E-06
RUMENSIN 80-50# (T)	907	300	907	438	74	8.64E-06
SUNGLO FINAL CONTROL 35# (T)	50	300	48	23	4	4.53E-07
SUNGLO FULL TANK (T)	180	200	181	58	10	1.15E-06
SUNGLO SUMO (T)	30	300	34	16	3	3.24E-07
THIAMINE10GM 50# (T)	23	30	23	1	0	2.16E-08
THREONINE 55# (T)	272	200	272	88	15	1.73E-06

TMR 30 (T)	23,000	100	22,784	3,667	616	7.23E-05
TURKEY PMX W/PHTTASE 50# (T)	1,837	300	1,837	887	149	1.75E-05
TURKEY STARTER BREEDER 50# (T)	907	300	907	438	74	8.64E-06
ULTRA MET 50# (T)	907	300	907	438	74	8.64E-06
VIT A-30 50# (T)	454	300	454	219	37	4.32E-06
VIT ADE 50# (T)	5,420	300	5,420	2,617	440	5.16E-05
VIT E - 125000-50# (T)	1,814	300	1,814	876	147	1.73E-05
VITA PLUS MILK 50# (T)	1,470	300	1,474	712	120	1.40E-05
VITAMIN D3 4MIL IU/ 50# (T)	454	300	454	219	37	4.32E-06
VITAMIN E 20000 50# (T)	227	300	227	109	18	2.16E-06
WHITE MILLET 50# (T)	50	100	45	7	1	1.44E-07
XPC YEAST 50# (T)	8,165	400	8,165	5,256	883	1.04E-04
YEAST, XP 50# BAG (T)	1,814	400	1,814	1,168	196	2.30E-05
Total (4 months)					12,083	1.42E-03

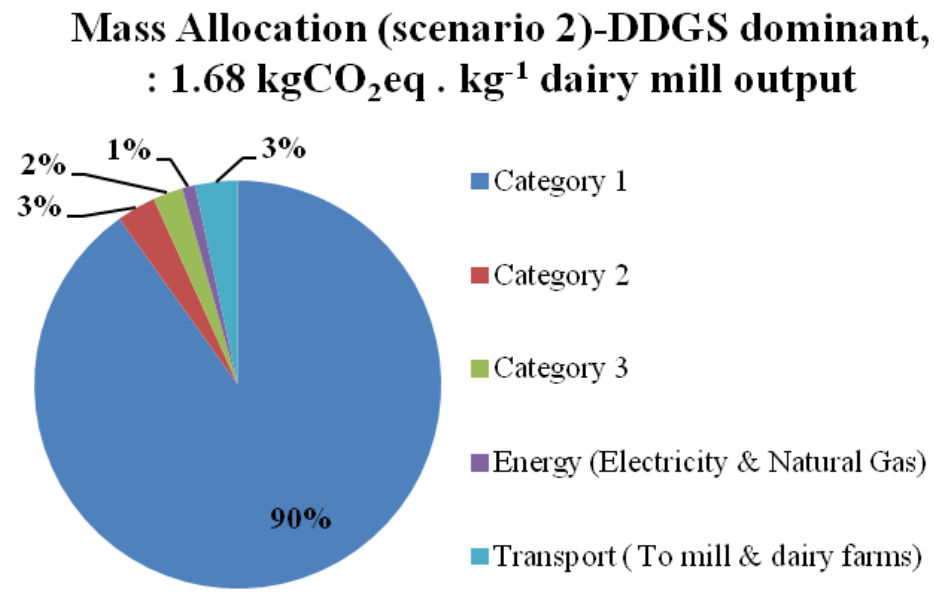


Panel A

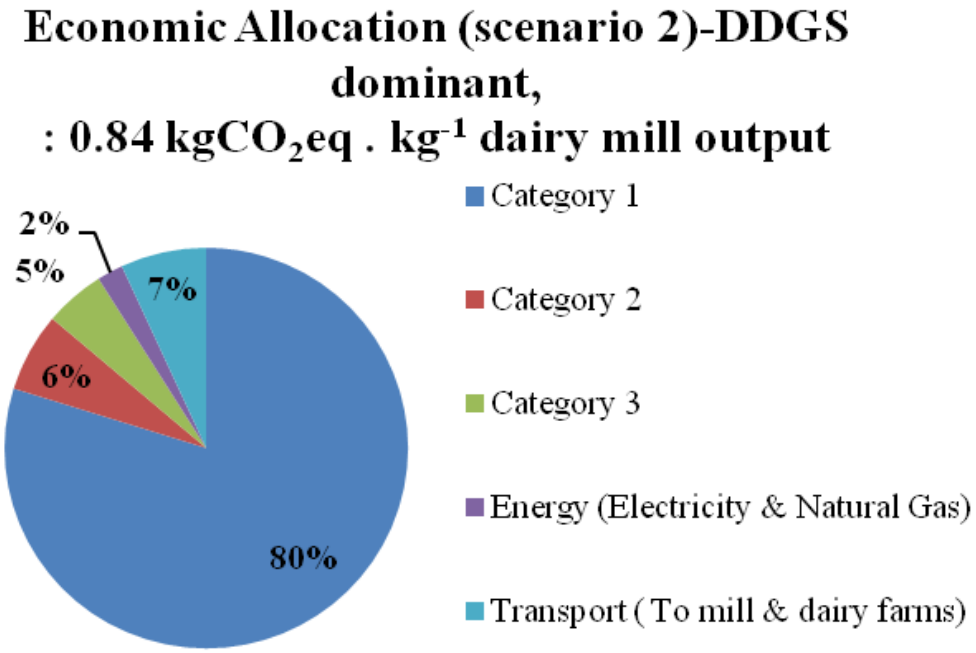


Panel B

Figure E-2 Relative Contribution to GWP of Feed Mill Dairy Feed for Scenario 1

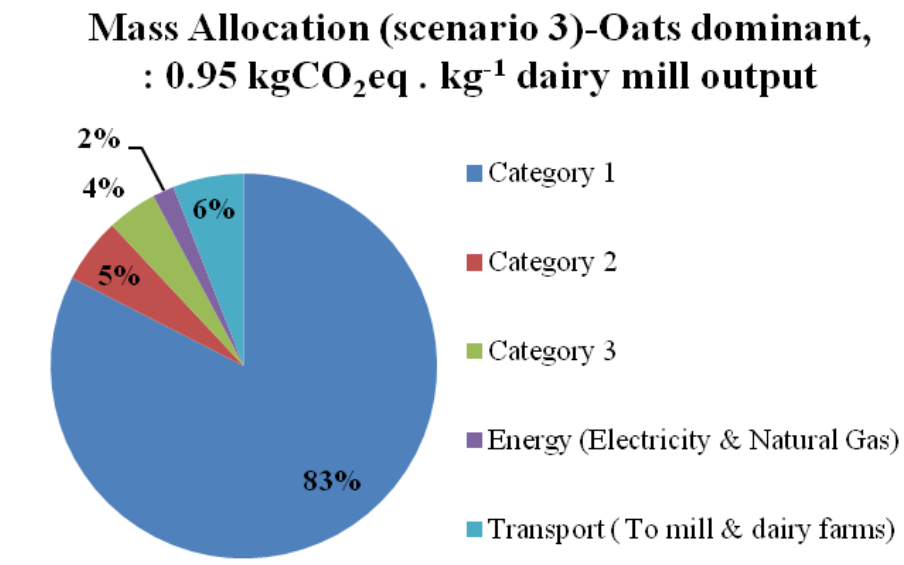


Panel A

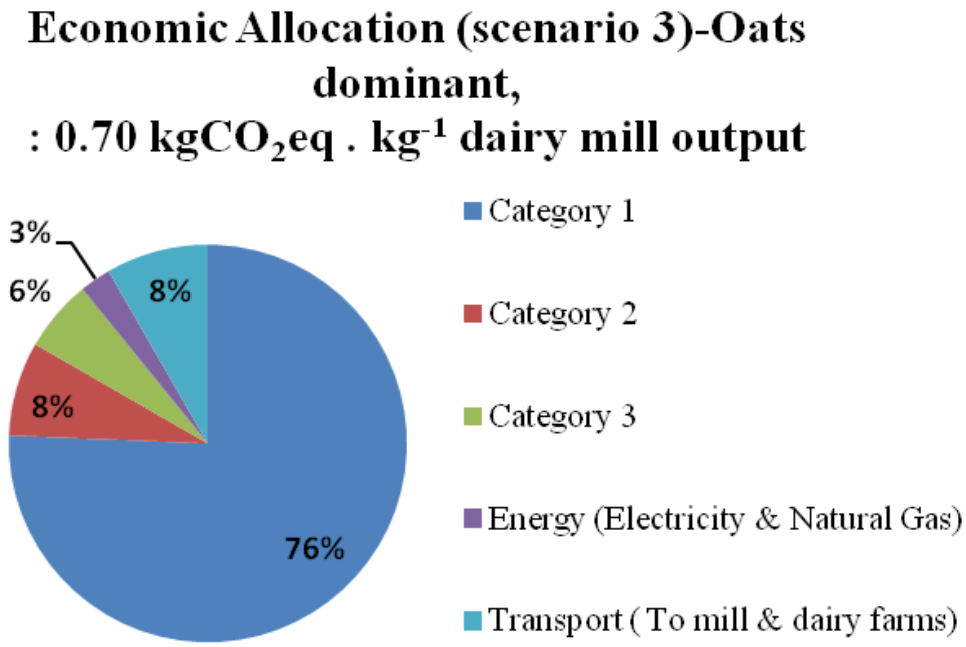


Panel B

Figure E-3 Relative Contribution to GWP of Feed Mill Dairy Feed for Scenario 2



Panel A



Panel B

Figure E- 4 Relative Contribution to GWP of Feed Mill Dairy Feed for Scenario 3