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BIOMASS PRODUCTION OF *Saccharomyces cerevisiae* (BAKER'S YEAST) USING THE CACTUS CLADODES EXTRACT AS A CULTURE MEDIUM

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains neither materials previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institutes, except where due acknowledgment has been made in the text.

Majed Shaban Hammad

May - 2008

IN MEMORY OF

MY FATHER

(1933-1997)

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Abstract

This study aims to evaluate *Cactus opuntia* cladodes (*COC*) extract as an alternative medium for producing *Saccharomyces cerevisiae (*baker's yeast) (*BY*), and to optimize the environmental conditions of its growth (pH, temperature). The study design was a comparative study. *COC* were obtained from the boarders of a farm in Northern governate at the age of 1-2 years. The crude *COC* extract was diluted to 50% before using. The preactivated *BY* sample used also diluted to 10^{-7}. Experiment was carried out on both surface and submerged *COC* extract cultures. The control media were both potatoes dextrose agar and broth (PDA, PDB). SPSS system was used to analyze the obtained data. The results showed that the *BY* can grow and proliferate in *COC* extract (50% dilution) where the average specific growth rate (μ) was 0.21 $h.^{-1}$ The optimum pH for yeast growth was about (4.0) and the optimum temperature for good specific growth rate was 30 ^{0}C. The average specific growth rate of BY in PDB was 0.25 h^{-1} and the optimum pH and temperature were 4.0 and 30 ^{0}C, respectively. It was found also that 29.0 g/L of yeast (dry weight) was produced in *COC* extract, while 37.0 g/ L was produced in PDB after 72 hours growth. It was found also that the total yeast dry weight that produced in *COC* extract was about 41.3 g/L, while an equal quantity of PDB produced about 54.1 g/L. The study found also that 1000g fresh weight of COC can produce about 62.0 g of BY. It can be concluded that *COC* extract could be a good medium for producing BY under restricted economical conditions.

Key words: Biomass production, *Cactus opuntia* cladodes, Potato dextrose broth *Saccharomyces cerevisiae*, Specific growth rate.

إنتاج خميرة الخبز باستخدام عصير سيقان التين الشوكي كوسط للنمو.

الخلاصة

تهدف هذه الدراسة إلى تقييم استخدام عصير سيقان التين الشوكي كوسط بديل لانتاج خميرة الخبز و ضبط الظروف المحيطة بنموها خصوصا ما يتعلق بحموضة الوسط ودرجة حرارته .

إن أكثر الأوساط شيوعا لانتاج خميرة الخبز في العالم هو المولاس المحضر من قصب السكر والبنجر، لانه متوفر بكثرة ورخيص الثمن، ويحتوي على جميع المكونات اللازمة لنمو الخميرة. إن السكريات المناسبة لنمو الخميرة في قطاع غزة محدودة جداً و غالية الثمن وتستورد من خارج البلاد، في المقابل فالتين الشوكي متوفر بكثرة في القطاع وعصيره غني بالسكريات القابلة للتخمير مثل الجالاكتوز، الارابينوز والراميnوز التي تستخدمها الخميرة للنمو والتكاثر. أحضرت عينة الدراسة من التين الشوكي من حدود مزرعة في شمال قطاع غزة، وكان عمرها يتراوح بين 1-2 سنة، وقد تم تخفيف عصيرها إلى النصف قبل استخدامه في تنمية الخميرة، وكذلك الخميرة نفسها المنشطة لمدة 48 ساعة قد خففت بمعامل تخفيف إلى 10^{-7}. لقد تم تنمية الخميرة على الوسطين الصلب والسائل من عصير التين الشوكي واستخدم نشا البطاطس السائل والصلب أيضاً كوسط ضابط لنمو الخميرة .

أظهرت هذه الدراسة أنه يمكن تربية وتنمية خميرة الخبز على عصير التين الشوكي المخفف 50 % بمعدل نمو خاص μ = 0.21 / ساعة ، وأن أفضل وسط حمضي لنموها هو (pH=4.0) وتحت درجة حرارة 30م^0، في حين أن تنمية الخميرة على محلول نشا البطاطس كان بمعدل نمو خاص 0.25 / ساعة وفي وسط درجة حامضيته pH=4.0 ودرجة حرارة 30م^0. ووجدت الدراسة أيضاً أن لتر واحد من عصير التين الشوكي المخفف 50 % يستطيع إنتاج حوالي 29.0 جرام من الخميرة (وزن جاف)، أما نفس الكمية من محلول نشا البطاطس فقد أنتجت حوالي 37.0 جرام (وزن جاف) تحت نفس الظروف بعد 72ساعة من النمو ، وقد بلغ أكبر معدل لإنتاج هذه الخميرة في لتر واحد من عصير التين الشوكي حوالي 41.3 جرام (وزن جاف) في زمن الزراعة الكلي ، أما نفس الكمية من محلول نشا البطاطس فقد أنتجت حوالي 54.1 جرام (وزن جاف) .وأظهرت الدراسة كذلك أنه يمكن إنتاج 62.0 جرام من الخميرة لكل كيلو جرام واحد من سيقان التين الشوكي الطازجة .

استنتجت الدراسة انه يمكن اعتبار عصير سيقان التين الشوكي وسط جيد لإنتاج خميرة الخبز تحت الظروف الاقتصادية الصعبة.

الكلمات المفتاحية : إنتاج الكتلة الحيوية ، سكارومايسيز سيريفيسيا، سيقان التين الشوكي، محلول نشا البطاطس، معدل النمو الخاص.

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LIST OF SYMBOLS AND ABBREVIATIONS

COC**:** *Cactus opuntia cladodes*

PDA**:** Potato Dextrose Agar

PDB**:** Potatoes Dextrose Broth

O.D**:** Optical Density

D.W**:** Dry Weight

BY**:** Baker's Yeast

μ**:** Specific Growth Rate

IUG: Islamic University of Gaza

TMTC: Too Much To Count

v.n : Vernacular

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CHAPTER 1

INTRODUCTION

1.1 Overview

The best known of all yeasts is *Saccharomyces cerevisiae*, its natural habitat is the surface of fruit, but it has been used by man for thousands of years to produce alcoholic beverages and breads [1].

Yeast reproduces vegetatively by budding, a process during which new buds grows from the side of the existing cell wall. These buds eventually breaks away from the mother cell to form a separate daughter cells. Each yeast cell, on average, undergoes this budding process 12 to 15 times before it is no longer capable of reproducing. During commercial production, yeast is grown under carefully controlled conditions on a sugar containing media typically composed of beet and cane molasses. Under ideal optimum growth conditions, a yeast cell reproduces every two to three hours [2].

S. cerevisiae is able to grow rapidly in rich media under anaerobic conditions. However, since the energy yield from fermentation is low, about 98% of the glucose present is metabolized to provide energy and 2% is incorporated into cell materials. Cell yield per gram of sugar metabolized is hence low. A high level of glucose in the cell will suppress aerobic metabolism even if oxygen is present, a phenomenon known as catabolite repression or the Crabtree effect. Hence on a rich medium with abundant sugar, fermentation of glucose to ethanol and carbon dioxide and water occurs even in aerobic conditions. However, when the glucose supply is exhausted, ethanol is converted to pyruvate. Some of the pyruvate is then metabolized to carbon dioxide and water via the tricarboxylic acid cycle to yield energy. In addition some of it is converted back to the sugars needed for wall synthesis by gluconeogenesis. Since aerobic metabolism yield much more energy than fermentation, approximately 10% instead of 2% of the glucose supplied is converted to cell material. This

increased cell yield under aerobic conditions per gram of sugar supplied is known as the Pasteur effect [1].

Glucose utilization in laboratory lays essentially on the fact that it has a general repressive and inhibitory effect on the assimilation of other sugars and some other compounds utilized by yeasts as carbon sources. The ability to use many different compounds as carbon and energy sources allows yeasts to colonize and proliferate in the most diverse niches from water, flowers and food to humans [3].

Opuntia genus is widely known for its mucilage production. Mucilage, a complex carbohydrate with a great capacity to absorb water, should be considered a potential source of industrial hydrocolloid. Mucilage contains varying proportions of arabinose, galactose, rhamnose, and xylose, as well as galacturonic acid. The mucilage content found in the *Cactus opuntia* cladodes is influenced not only by the management of the crop but also depends on the temperature, irrigation and the rain [4]. Ruiz-Feria, et al, reported that, the chemical composition of cactus was, 5.7% dry matter, 9.3 % crude protein, 10.5 % crude fiber, 5.9% ether extract, 24.1% ash, 50.2% nitrogen-free-extract, 32.8% neutral detergent fiber, and 19.5% acid detergent fiber [5].

Many and various compounds can be used as a source of carbon and energy by yeasts, glucose representing the easiest and the most widely used source of carbon followed closely by fructose, mannose and then galactose. Yeast requirements for minerals are similar to that of other cells with a supply of potassium, magnesium and several trace elements being necessary for growth. K^{+} and Mg^{+2} are regarded as bulk or macro elements, which are required in millimolar concentrations to establish the main metallic cationic environment in the yeast cell. Concerning potassium, the most prevalent cation in the yeast cytoplasm, where yeasts have an absolute growth requirement for this mineral which is essential as a cofactor for a wide variety of enzymes involved in oxidative phosphorylation, protein biosynthesis and carbohydrate metabolism. Yeasts vary widely in their essential elements. Yeast essential elements

include: vitamins (as coenzymes), purines and pyrimidines, nucleosides, amino acids, fatty acids, sterols and other miscellaneous compounds. When a yeast species is said to have essential element this indicates that it cannot synthesize that particular element, resulting in the impairment of growth and key metabolic processes until its addition to the culture medium be performed [6].

1.2 Significance of the study

Although *Cactus opuntia* spp is very available in large quantities in the Gaza strip and its extract is rich in fermentable sugars that the yeast can use to grow. To date no scientific studies have been carried out using this local raw material for growing the yeast in a great economical reward for the local community**.** However**,** a preliminary study using cactus extract was carried out previously in the biology department of IUG and it was found that cactus extract was promising to be a good medium for production of baker's yeast (unpublished data).

1.3 Main objective of the study:

To evaluate *Cactus opuntia cladodes* extract for biomass production of baker's yeast .

1.4 Specific objectives of the study

The specific objectives of the present study were :

1- To determine the specific growth rate of an industrial BY grown on cactus extract cultures aerobically.

2- To optimize the environmental conditions of the BY growth on COC extract

3- To determine the BY mass produced per ml of liquid culture of COC extract.

4- To determine the BY mass produced per kg of fresh COC weight.

CHAPTER 2

LITERATURE REVIEW

2.1. Yeast

2.1.1 Yeast growth and nutrition

Yeasts are a growth form of eukaryotic microorganisms classified in the kingdom Fungi, with approximately 1,500 species described [7]. Most reproduce a sexually by budding, although a few do by binary fission. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or true hyphae as seen in most molds [6]. Yeast size can vary greatly depending on the species, typically measuring 3–4 µm in diameter, although some yeasts can reach over 40 µm [8].

Yeasts are chemoorganotrophs as they use organic compounds as a source of energy and do not require light to grow. The main source of carbon is obtained by hexose sugars such as glucose, or disaccharides such as sucrose and maltose. Some species can metabolize pentose sugars such as fructose, alcohols, and organic acids. Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic but also have aerobic methods of energy production (facultative anaerobes). Unlike bacteria, there are no known yeast species that grow only anaerobically (obligate anaerobes). Also, because they are adapted to them, yeasts grow best in a neutral pH environment. Yeasts are ubiquitous in the environment, but are most frequently isolated from sugar-rich samples. Some good examples include fruits and berries (such as grapes, apples or peaches), and exudates from plants (such as plant saps or cacti). Some yeasts are also found in association with soil and insects [9].

Yeast are generally grown in the laboratory on solid growth media or liquid broths. Common media used for the cultivation of yeasts include; potato

dextrose agar (PDA) or potato dextrose broth (PDB), Wallerstien Laboratories Nutrient agar (WLN), Yeast Peptone Dextrose agar (YPD), and Yeast Mould agar or broth (YM) [10].

Most yeasts form small circular colonies when grown on agar media. Growth will at first be exponential until a mass of cells is produced. At this stage only the cells at the edge of the mass will have access to nutrients so the marginal growth zone will result, and exponential growth in this zone will lead to a linear increase in radius with time which will be slowed or even halted by nutrients depletion or by the accumulation of toxic metabolites such as ethanol [11].

2.1.2 Yeast reproduction

Through yeast reproduction, a small bud, or daughter cell, is formed on the parent cell. The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell. The bud continues to grow until it separates from the parent cell, forming a new cell. The bud can develop on different parts of the parent cell depending on the genus of the yeast [12].

The yeast species *Saccharomyces cerevisiae* has been used in baking and fermenting alcoholic beverages for thousands of years. It is also extremely important as a model organism in modern cell biology research, and is the most thoroughly researched eukaryotic microorganism. Researchers can use it to gather information into the biology of the eukaryotic cell and ultimately human biology [13].

When grown in liquid media wild-type *S. cerevisiae* grows well at 30°C with good aeration and with glucose as a carbon source. When using culture tubes, vortex the contents briefly after inoculation to disperse the cells. Erlenmeyer flasks work well for growing larger liquid cultures, and baffled-bottom flasks to increase aeration are especially good. It is important that all glassware be detergent-free. When grown on solid media, yeast cells can be streaked or spread on plates. When a dilute suspension of wild-type haploid yeast cells is spread over the surface of a YPD plate and incubated at 30 ^{0}C, single colonies

may be seen after 24 hr but require 48 hr before they can be picked or replica plated [14].

The density of cells in a culture can be determined spectrophotometrically by measuring its optical density(OD) at 600nm. For reliable measurements, cultures should be diluted such that the OD_{600} is <1. In this range, each 0.1 OD_{600} unit corresponds to ~3 × 10^6 cells/ml. Thus, an OD_{600} of 1 is equal to ~3 × 10^7 cells/ml. It is advisable to calibrate the spectrophotometer by graphing the OD_{600} as a function of the cell density that has been determined by some other means, such as direct counting in a hemacytometer chamber or tittering for viable colonies [14]. Active dry yeast consists of pure, dried yeast cells with viability counts of 15 billion live yeast cells or colony forming units (cfu) per gram [15].

Yeast cells often encounter a mixture of different carbohydrates in industrial processes. However, glucose and sucrose are always consumed first. The presence of these sugars causes repression of gluconeogenesis, the glyoxylate cycle, respiration and the uptake of less-preferred carbohydrates. Glucose and sucrose also trigger unexpected, hormone-like effects, including the activation of cellular growth, the mobilization of storage compounds and the diminution of cellular stress resistance [16].

When microorganisms are grown in a homogeneous batch culture, for example in liquid media in Erlenmeyer flasks on a rotary shaker, three phases of growth can be distinguished – the lag, exponential and stationary phases. The exponential phase is the period during which a constant specific growth rate (μ) is maintained and biomass increase is exponential. The specific growth rate is maximal ($\mu = \mu_{max}$) when all the nutrients required for growth are in excess and no inhibitor of growth is present. If any nutrient is present in sub optimal amounts then the value of μ relative to μ_{max} is determined by the affinity of the organism for the limiting nutrient and by the concentration of the nutrient. The specific growth rate (μ) when plotted against nutrient concentration(s) yields a rectangular hyperbola, just as does the rate of an enzyme reaction when plotted

against substrate concentration. The affinity of the organism for a nutrient is expressed as the saturation constant K_s, the nutrient constant at which μ is half μ_{max}. The K_s for glucose in *Saccharomyces cerevisiae* is 25 mg l^{-1} (140 μ M) and the maximal specific growth rate on glucose is 0.47 h^{-1} while the doubling time is 88 minute. The growth rate equation used to calculate the specific growth rate is; $\mu = 2.3 (\log 10\ x - \log 10\ x_0) / \Delta t$, where x and x_0 are, biomass or O.D and Δt is the difference of time. Doubling time is obtained from the relationship $t_d = 0.69/\mu_{max}$ [11].

Hoek, Van Dijken, and Pronk reported that, baker's yeast was cultured on a medium of glucose, salts, sodium citrate, yeast extract, vitamin and mineral solutions, in a 50 L bottom-driven fermentor interfaced with a single board computer. A fed-batch mode was employed, and fermentation was effected at 30 ^oC and pH 4.8-4.9. The system included a 30 L substrate tank, an oxygen cylinder and an air compressor. A mixture of pure oxygen and air was supplied to the fermentation. Results obtained under conditions of dissolved oxygen, CO_2 and feed rate control were presented. The cell concentration increased from 5 to 118 g/L and cell productivity was 7.6 g/L.hr. Ethanol concentration was kept at low levels by feed rate control. Results showed that the production of baker's yeast could be effectively performed using microcomputer-controlled fermentors [17].

A recent study investigated the effect of the specific growth rate on the physiology and fermentative capacity of an industrial *Saccharomyces cerevisiae* strain in aerobic, glucose-limited chemostat cultures. At specific growth rates (dilution rates, D) below 0.28 h^{-1}, glucose metabolism was fully respiratory. Above this dilution rate, respirofermentative metabolism set in, with ethanol production rates of up to 14 mmol of ethanol. g of $biomass^{-1}$. h^{-1} at D = 0.40 h^{-1}. A substantial fermentative capacity (assayed offline as ethanol production rate under anaerobic conditions) was found in cultures in which no ethanol was detectable (D < 0.28 h^{-1}). This fermentative capacity increased with increasing dilution rates, from 10.0 mmol of ethanol/ h. g of dry yeast biomass at D = 0.025 h^{-1} to 20.5 mmol of ethanol/ h. g of dry yeast biomass at D = 0.28 h^{-1}. At even

higher dilution rates, the fermentative capacity showed only a small further increase, up to 22.0 mmol of ethanol/ h. g of dry yeast biomass at D = 0.40 h^{-1}. The activities of all glycolytic enzymes, pyruvate decarboxylase, and alcohol dehydrogenase were determined in cell extracts. Only the in vitro activities of pyruvate decarboxylase and phosphofructokinase showed a clear positive correlation with fermentative capacity. These enzymes are interesting targets for over expression in attempts to improve the fermentative capacity of aerobic cultures grown at low specific growth rates [18].

Optimization of biomass productivity requires that the specific growth rate and biomass yield in the fed-batch process be as high as possible. In the early stage of the process, the maximum feasible growth rate is dictated by the threshold specific growth rate at which respirofermentative metabolism sets in. In later stages, the specific growth rate is decreased to avoid problems with the limited oxygen transfer and/or cooling capacity of industrial bioreactors [19].

2.1.3 Substrates required by yeast

Yeast which is to be used for baking purposes is produced on a commercial scale by a multi-stable operation. This multi-stage operation includes, generally, a preparation of the basic ingredients needed by the yeast for growth. The basic ingredients may include sterilized cane sugar and/or molasses, corn steep liquor and an acid or alkali to adjust the pH of the mixture to the range of 4.0 to 5.0. The mixture is diluted with water, fortified by the addition of inorganic nitrogen and phosphorus-containing compounds and, when necessary, the pH of the mixture is again adjusted to a range of 4.0 to 5.0 [20].

A new control strategy was experimentally assessed on a laboratory scale by means of a two-stage fermentation for yeast production. The two-stage fermentation includes a batch culture to grow inoculum and a subsequent fed-batch culture. The strategy was based on controlling biomass concentration throughout the two-stage fermentation by manipulating the glucose feed rate. An L/A algorithm continuously tuned by ethanol concentration and respiratory quotient was used with constant external settings. The L/A controller performed

satisfactorily throughout the two-stage fermentation and was able to move smoothly from one phase of operation to another. An average specific growth rate of 0.25 h^{-1} and a cellular yield of 0.5 $g \cdot g^{-1}$ were achieved during the fed-batch phase while over 40 $g \cdot l^{-1}$ biomass was obtained [21].

BY is normally produced from molasses, grains or potatoes. Feed yeast usually utilizes brewer's or distiller's stillage. These raw materials are not sufficiently rich in assimilable nitrogenous and phosphorus compounds and, usually the addition of inorganic ammonium compounds and phosphoric acid is necessary.

Molasses is the final effluent obtained in the preparation of sugar by repeated crystallization; it is the residual syrup from which no crystalline sucrose can be obtained by simple means. The yield of molasses is approximately 3.0 percent per tone of cane but it is influenced by a number of factors and may vary within a wide range (2.2 to 3.7 percent). The specific gravity varies between 1.39 and 1.49, with 1.43 as indicative average. The composition of molasses and other resources used by yeast to grow and proliferate in comparable with COC extract are found in table 2.1.

Table 2.1 : The chemical composition of different carbon resources used to produce BY comparable to COC extract [22-26].

The content	Molasses [22]	Maize[23, 25]	Potatoes[24]	COC extract[26]
Moisture%	24	12.2	18	84
Total carbohydrates%	47	75.9	23	7.0
Crude protein%	4.5	5.8	2.0	8.7
Total fat%	0.0	11.0	0.1	0.02
Crude fibers%	0.0	0.8	1.0	2.9
Ash%	8.4	1.2	1.3	1.2
Calcium,mg/100gm	50.0	4.8	6.5	1.1
Phosphorus,mg/100gm	50	300	90	60
Copper, mg/100gm	2.4	1.3	1.7	0.9
Iron, mg/100gm	18.3	4.8	10.8	1.3

About 4 kg of molasses would be required to produce 1 kg of active dry BY (92 percent dry matter). Yeast is used in bread production at about 1 percent by weight of flour. On a dry matter basis, it contains about 44 percent protein. About 4 kg of molasses would also be required to produce 1 kg of feed yeast (92 percent dry matter) which generally contains about 50 percent of crude protein. In both processes adequate and fine aeration is important and some 15 m^5 per kg of dry yeast are usually required [27].

The principal raw materials used in producing baker's yeast are the pure yeast culture and molasses. The yeast strain used in producing compressed yeast is *Saccharomyces cerevisiae*. Cane and beet molasses are used as the principal carbon source to promote yeast growth. Molasses contains 45 to 55 percent fermentable sugars by weight in the forms of sucrose, glucose, and fructose. This sugar by-product is the least expensive source of sugar known. Other sources, such as corn grits, raisins, or sugar-containing wastes of the confectionary industry are also effective, but for various reasons (mostly economic), these alternatives were found to be unsuitable as carbon and energy substrates for baker's yeast production [28].

Other required raw materials are a variety of essential nutrients and vitamins. Mineral requirements include nitrogen, potassium, phosphate, magnesium, and calcium. Nitrogen is normally supplied through the addition of ammonium salts, aqueous ammonia, or anhydrous ammonia to the feed stock. The molasses normally provides sufficient quantities of potassium and calcium. Phosphates and magnesium are added in the form of phosphoric acid or phosphate salts and magnesium salts. Iron, zinc, copper, manganese, and molybdenum are also required in trace amounts. Several vitamins are required for yeast growth (biotin, inositol, pantothenic acid, and thiamine). Yeast will not grow in the absence of biotin. Thiamine is not required for yeast growth but is normally added to the feed stock because it is a potent stimulant for fermenting doughs. Both cane and beet molasses usually provide enough inositol and pantothenic acid for yeast growth. However, if beet molasses, which is deficient in biotin, is

used, biotin must be added or a mixture of cane and beet molasses is required [28].

Yeast fermentation vessels are operated under aerobic conditions (free oxygen present, or excess air) because under anaerobic conditions (limited or no oxygen available) the fermentable sugars are consumed in the formation of ethanol and carbon dioxide, which results in low yeast yields. Yeast yields under anaerobic conditions are often less than 10 percent-by weight of fermentable sugars, whereas yeast yields of up to 50 percent-by-weight of fermentable sugars are obtained under aerobic conditions [28].

2.1.4 Yeast enzymes

Enzymes in yeast consist of invertase, maltase, zymase and protease. There are others, but they are not important in bread production. The protease enzyme in yeast is only active if the cell wall of the yeast is damaged in some way. That is the only time it can penetrate the cell wall of the yeast. There are always a few damaged yeast cells especially in dry yeast where some cells are damaged during the drying process, or in yeast that has gotten a little old. If the protease enzyme does penetrate the cell wall of the yeast, it will weaken the gluten. Invertase enzyme is an intercellular enzyme. Sucrose, (cane or beet sugar) when dissolved enter the cell wall and are changed to dextrose and fructose, which are later changed by the enzyme zymase. Zymase is the enzyme which changes the simple sugars, dextrose and fructose into carbon dioxide gas and alcohol, and several esters which result in producing the unique aroma flavor and taste of breads [29].

2.2 Cactus

2.2.1 Cactus cladodes extracts

Prickly pear cactus (*Opuntia spp*.) is a fast growing xerophytic plant well adapted to arid conditions. The genus appears to have its center of genetic

diversity in Mexico where it is widely used as fodder, forage, fruit and green vegetables [30].

Prickly pear cactus is native to New World, where it grows well in dry regions, such as the Southwestern United States and Northern Mexico [31].(see the photograph in Figure 2.1.

Figure 2.1: A photograph for *prickly peer cactus* at the borders of a farm western jabalia camp - Gaza strip.

Nowadays, *Opuntia* plants are grown in more than 30 countries on about 1,00000 ha. Among others Mexico, the Mediterranean (Egypt, Italy, Greece, Spain, Turkey), California, South America (Argentina, Brazil, Chile, Columbia, Peru), the Middle East (Historical Palestine, Jordan), North Africa (Algeria, Marocco, Tunisia), South Africa, and India [32].

For cladodes, a mean hectare yield of 30–80 ton can be achieved annually [17]. Mexico is the only country planting cladodes for commercial use on 10,000 ha with a total production of 600000 tons per year [33].

The cladode mucilage was also proposed as a protecting agent against corrosion [15]. In the countries of origin, the cladodes are added to building material to improve stability and compressibility [20]. Further uses such as clarification of waste water with cactus hydrocolloids are compiled elsewhere [4].

Opuntia ficus indica Mill (*Cactaceae*) is cultivated in Chile for fruit production. In some countries the young stems (*nopalitos*) it is also used for human consumption [34].

The cactus stems are composed of a white medullar parenchyma (core tissue) and the chlorophyll containing photosynthetically active parenchyma (cortex tissue). The latter is covered with spines (modified leaves) and multicellular hairs or trichomes, both forming the so-called areole, which is characteristic of members from the *Cactaceae* family. The subfamily *Opuntioideae* is further characterized by having short, sharp, barbed, deciduous spines, called glochids. The areoles are also the origin of the flowers, i.e., short shoots with specialized leaves [35].

The *cactus Opuntia* (genus *Opuntia*, subfamily *Opuntioideae*, family *Cactaceae*) is a xerophyte producing about 200–300 species and is mainly growing in arid (less than 250 mm annual precipitation) and semi-arid (250–450 mm annual precipitation) zones [36].

Due to their remarkable genetic variability, *Opuntia* plants show a high ecological adaptivity and can therefore be encountered in places of virtually all climatic conditions; North, Central and South America, the Mediterranean, North, Central and South Africa, the Middle East, Australia and also in India [37].

The genus *Opuntia* (v.n “nopal“ Cactaceae) is characterized by the production of a hydrocolloid commonly known as mucilage, which forms molecular networks that are able to retain large amounts of water [38].

The raw plant contains abundant mucilage, which is a complex carbohydrate that may delay absorption of glucose. The cactus also contains fiber, which is known to delay glucose absorption [39].

The plant is rich in energy and water and poor in protein and has been used in livestock feeding since the nineteenth century [40].

On the basis of wet weight, a master degree study was done in Gaza strip to evaluate the COC as a partial feed for growing rabbits found that the chemical composition of COC in percents was, Moisture 84.0, Crude protein 8.7, Crude fibers 2.9, Fat contents 0.02, Carbohydrates 7.0, Nitrogen free extract 10.9, Ash 1.2, Calcium (mg/ kg) 11.0, Iron (mg/ kg) 1.3 [26]. Table 2.2 shows the chemical composition of COC extract in Gaza strip comparable with other places .

Table 2.2: Shows the percent chemical composition of COC extract in Gaza strip (study sample) comparable with other studies on basis of wet weight.

Item	Study sample [26]	Other studies [41]
Moisture %	84.0	85-92
Total carbohydrates %	7.0	4-6
Crude protein %	8.7	1.0
Total fat %	0.02	0.2
Crude fibers %	2.9	1.0
Ash %	1.2	1.6
Calcium,mg/kg	11.0	7.7
Iron, mg/kg	1.3	1.6

2.2.2 Mucilages

Mucilages are complex polymeric substances of carbohydrate nature, with a highly branched structure. Mucilages contains varying proportions of L-arabinose, D-galactose, L-rhamnose and D-xylose, as well as galacturonic acid in different proportions. The mucilage structure is proposed as two distinctive water-soluble fractions. One is a pectin with gelling properties with Ca^{2+}, and the other is a mucilage without gelling properties [42].

Within pads tissues, in the chlorenchyma and parenchyma, there are mucilaginous cells that store mucilage; this material is commonly known as ‘‘nopal dribble’’ [43].

Tissue studies on *Cactus cladodes* have demonstrated that the mucilage is present only in the Golgi Apparatus, and the mucilage synthesis takes place probably in it and in the vesicles are derived from it [44].

It is reported that in *Opuntia ficus indica,* the water-soluble polysaccharide fraction with thickening properties represents less than 10% of the water-soluble material [45].

The chemical composition of *O. ficus indica* mucilage from cladodes (pads), commonly named nopal, has been the subject of various studies:

The dried mucilage had in average 5.6% moisture; 7.3% protein; 37.3% ash; 1.14% nitrogen; 9.86% calcium and 1.55% of potassium. The color analysis showed a high light value and the chromatic co-ordinates were in the yellow-greenish spectrum [46].

On the other hand, it is reported that the extract from *O. ficus indica* was a mixture of a neutral glucan, glycoproteins and an acidic polysaccharide composed of L-arabinose, D-galactose, L-rhamnnose, D-xylose and D-galacturonic acid [47].

The main sugar component of mucilage is arabinose followed by xylose. The percentage wt of these sugar components are: Arabinose 44.04, Galactose 20.43, Xylose 22.13, Rhamnose 7.02, Galacturonic acid 6.38**.** From elution profiles, a 2.3 x10^4 molecular weight for the mucilage was determined [48].

On a fresh weight basis, these values translate into 3–7 g carbohydrates, 1–2 g minerals, 0.5–1 g proteins, 0.2 g lipids, and 1 g fibrous substances per 100 g plant material [49].

Younger cladodes show higher carbohydrate, protein, and water contents. Interestingly, fertilization low in nitrogen lead to an increase in the crude protein content, while for use as feed for lactating cows, nitrogen doses of 224 kg/ha were recommended. Phosphate supplementation of 112 kg/ha improved the low phosphate content of the cladodes [36]. The water content of 88–95% makes cladodes a low-calorie food with 27 kcal/100 g [37].

Potassium is the main mineral amounting to about 60% of the total ash content (166 mg/100 g fresh weight), followed by calcium (93 mg/100 g fresh weight), sodium (2 mg/100 g fresh weight), and iron (1.6 mg/100 g fresh weight) while magnesium was not detected [35]. In more recent studies, the mineral composition was reported to be 50, 18–57, and 11–17 mg/100 g dry weight for potassium, calcium, and magnesium, respectively, followed by manganese (62–103 µg/g), iron (59–66 µg/g), zinc (22–27 µg/g), and copper (8–9 µg/g) on a dry weight basis [50]. The free sugar content of cactus cladodes extract was reported to reach 0.32 g/100 g fresh weight [35].

Teles, et al, reported that, the reducing sugar fraction of *Cactus cladodes* extract was to be 0.64–0.88 g/100 g dry weight increasing with development but also varying with species [50].

The crude protein content was reported to reach 11 g/100 g on a fresh or 0.5 g/100 g on a dry weight basis, respectively [51].

Total vitamin C (ascorbic and dehydroascorbic acid) in 100 g fresh weight amounted up to 22 mg, beta-carotene to 11.3–53.5 µg, thiamine to 0.14 mg, riboflavin to 0.6 mg, and niacine to 0.46 mg, respectively [49].

Majdoub et al, investigated the polysaccharide fraction of the chlorophyll-containing stem parenchyma and also of the peeled cladode. Based on an identical yield of 85 mg/100g fresh weight, the chlorenchyma was composed of 53.7% rhamnose, and 46.3% galacturonic acid. At pH 6.3, the polysaccharide fraction of the chlorenchyma exhibited a methoxylation degree of 88% and a molecular weight of $3.53X10^5$ g/mol while the core tissue afforded 8.8% and $6.12X10^6$ g/mol, respectively [52].

Corrales-Garcia, et al, found that the average pH of the young cladodes is 4.08 and for old cladodes 3.83, while the Ca and P contents of the young and old cladodes were 7.72 mg/100gm, 0 .60 mg/100gm and 6.56 mg/100gm, 0 .5 mg/100gm, respectively [53].

According to a recent study on crassulacean acid metabolism (CAM) active cladodes, the acid content was not only dependent on the time of harvest during the day but also on the respective *Opuntia spp*. variant (0.28– 0.76%), and the post harvest conditions applied [53].

In addition, cool storage maintained the acidity or even amplified it. The sugar content and pH were decreased while protein increased [36].

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Chemicals and Instruments

See Annex B.5.

3.2 Methods

3.2.1 Study design

The present study was a comparative study between the growth of BY on *COC* extract and PDB as culture medium.

3.2.2 Study hypothesis

There is no difference between the growth promoting ability of COC extract and PDA for *S.cerevisiae*.

3.2.3 Setting of the study

The experiments were carried out at the faculty of science laboratories in the Islamic university- Gaza.

3.2.4 Duration of the study

The study started at July, 2007 and ended at April, 2008.

3.2.5 Pre- activation of BY

To activate the yeast, an autoclaved PDB medium (100 ml) at a pH (3.5) was prepared then inoculated by a small amount of the dry yeast (0.1 gm). The container used was sealed by a piece of cotton and left in a shaker incubator (120 r.p.m) for 48 hours at 30 ^{0}C [54].

3.2.6 Dilution of BY sample

One ml of the pre-activated BY was taken into a small test tube and completed to 10 ml by sterile normal saline and vortexed well. Serial dilution of 10^{-1} to 10^{-8} was performed [55].

3.2.7 Preparation of the *COC* extract

The harvested *COC* were picked off from spines and glochids and washed well by tap water, then peeled by a sharp knife. The peeled cladodes were cut off into small cubes then blended. The resulted extract was filtrated by a piece of clothes then centrifuged by Sorvall centrifuge at 5000 r.p.m for 10 min at 30 ^{0}C. The clear extract is then transferred to a clean flask, and sealed by a Para-film then left at home refrigerator (4 ^{0}C) till used [46].

3.2.8 Preparation of PDA dishes

Potato dextrose agar (PDA) medium was prepared by dissolving 39 g of PDA powder in distilled water to a final volume of one litter, then autoclaved at 121^{0}C, 15 PSI, for 15 minutes. Any ingredients added to the medium were carried out under normal sterile conditions after autoclaving. The media pH of 3.5 was adjusted using tartaric acid, and then 20-25 ml aliquots of the medium were poured into a set of Petri dishes, and left to cool, then stored in the home refrigerator at 4^{0}C till used. This medium was used for surface cultures as a control medium [modified from ref.56].

3.2.9 Preparation of *COC* extract dishes

The *COC* were homogenized using normal home blender and filtrated using a piece of cheese cloth. The filtrate of *COC* extract was transferred to a clean flask, diluted to the needed percentage using distilled water, and a suitable amount of agar was added (15 g/l). A pellet of NaOH was added to the extract while heated to prevent destruction of the agar. After autoclaving for the proper time, the pH was adjusted to the value 3.5 using 1M HCL and 1M NaOH ,then it was poured into dishes. Addition of peptone to the medium was done before

autoclaving. The dishes were left to cool then stored in the home refrigerator at 4^0C till used [modified from ref. 56].

3.2.10 Preparation of PDB

Potato dextrose broth media were prepared by dissolving the PDB (27g) media in distilled water to a final volume of one litter, then autoclaved at 121^0C, 15 PSI, for 15 minuets. Any ingredients additions to the PDB medium was carried out after autoclaving. The pH of the medium was adjusted using tartaric acid to 3.5, and left to cool then stored in the home refrigerator at 4^0C till used. This medium was used for liquid cultures as a control medium, for yeast activation and for yeast growth calibration [modified from ref. 56].

3.2.11 Preparation of COC extract broth.

The same steps carried out in item (3.2.9.) were repeated here but without adding agar.

3.2.12 Cultivation on solid media

Inoculation of PDA, *COC* extract and *COC* extract plus peptone dishes that have six different concentrations of peptone (1 up to 6 gm/100 ml) with diluted activated BY was carried out in triplicates. Inoculation of all kinds of dishes was done by 0.1 ml of the diluted BY samples. Different dilutions of the activated yeast were as follows: 10^{-1},10^{-2},10^{-3},...,10^{-7}. All the dishes were incubated at 30 ^{0}C for two days.

3.2.13 Cultivation in broth media

COC extract that prepared previously in item (3.2.7) was transferred to a clean flasks, some of them were inculcated with different concentrations of peptone. All flasks were inoculated with 3% by volume of preactivated BY (dilution 10^{-7}). The control medium (PDA) also was inoculated with the same activated and diluted BY quantity (3% of the total medium volume). The inoculated flasks were plugged with a piece of cotton and left in a shaker incubator (120 r.p.m) at 30 ^{0}C for one week.

3.3 Experimentations

3.3.1 The surface cultures.

3.3.1.1 Determination of BY ability to grow on *COC* extract.

The prepared Petri dishes of PDA and pure *COC* extract with agar were inoculated with 0.1ml of diluted yeast samples of the dilutions 10^{-1} up to 10^{-8}. The pH of both media was 5.0. The PDA dishes were used as control. The experiment was done in triplicates, and the dishes were left in the incubator for 48 hours at 30 ^{0}C. The yeast colonies were counted visually.

3.3.1.2 Growth of BY on PDA and COC extract with Peptone.

The prepared Petri dishes of pure *COC* extract with agar and pure *COC* extract with agar plus peptone concentrations of 1g up to 6g/100 ml were inoculated with 0.1 ml of diluted BY samples of the dilutions 10^{-1} to 10^{-7}. The pH of media was 5.0. PDA Petri dishes were used as control. The experiment was done in triplicate, and the dishes were left in the incubator for 48 hours at 30 ^{0}C. The BY colonies were also counted visually.

3.3.1.3. Optimizing the pH of the yeast growth medium

Several concentrations of the *COC* extract dishes have been prepared (100%, 75%, 50%, 25%,10%) in triplicates with different pHs (3.5, 4.0, 4.5, 5.0, 5.5) and inoculated with 0.1 ml of pre-activated yeast of the dilution 10^{-7}. The dishes were incubated at 30 ^{0}C for 48 hours. The produced colonies were also counted visually.

3.3.2. The submerged cultures

3.3.2.1.Growth curve of BY on PDB.

One tenth (0.1) gm of dry yeast was added to 100 ml of autoclaved PDB flask (500 ml) with pH 4.0. The flask was plugged with a piece of cotton and incubated in a shaker incubator with a shaking rate of 120 r.p.m at 30 ^{0}C. A sample of 1 ml was taken at variant times in four days and the optical density

(O.D) at 660 nm was measured by a spectrophotometer. The experiment was done in duplicate.

3.3.2.2 Calibration curve of BY

(1.0 $x10^{-4}$ up to 2.0 x 10^{-3} gm) weights of dry BY was dissolved in a suitable normal a saline solution quantity and measured at 660 nm at room temperature. The optical densities were obtained in duplicates and graphed by Microsoft Excel to find the calibration line for yeast concentration [50].

3.3.2.3 Growth of BY in PDB and in COC extract with peptone

An enough volume of COC extract at pH 5.0 was poured into 7 flasks, (100 ml for each flask). Each flask was 500 ml in volume. The flasks were autoclaved for 15 min at 121 ^{0}C and 15 PSI. The first six flasks were inoculated with different concentrations of peptone (1g/100ml up to 6g/100ml), the seventh one contained pure COC extract. An eighth flask contained PDB as control. Three percents (3% v/v) of the preactivated yeast at the dilution 10^{-7} (3.0ml) was added to every flask. The flasks were incubated in a shaker incubator (120 r.p.m) for a week at 30 ^{0}C. One ml from each flask was taken every 24 hours, and the O.D at 660 nm was measured. The experiment was done in triplicates.

3.3.2.4. Growth curve of BY on *COC* extract

COC extract (125 ml) with the concentration of 50% was poured into each of 9 flasks (500 ml in volume). The flasks were autoclaved for 15 min at 121 ^{0}C and 15 PSI. Every three flasks were setted up at the same pH where the pHs were (4.0, 4.5, 5.0) using 1M HCL and 1 M NaOH. Three percents (3% v/v) of preactivated yeast at the dilution of 10^{-7} was added to every flask. The flasks were incubated in a shaker incubator for a week at 30 ^{0}C. One ml from each flask was taken every 24 hours and the O.D at 660 nm was determined. PDB flasks of the same pHs as the cactus ones were used as control. The experiment was done in triplicate.

3.3.2.5. Growth curve of BY on COC extract at pH 4.0

The previous method mentioned in item (3.3.2.4**)** was repeated just only with a pH of 4.0. The experiment was done in duplicate.

3.3.2.6. Optimization of temperature for BY growth

One tenth (0.1) gm of dry yeast was cultivated on 100 ml of PDB and *COC* extract (50% conc.) at pH 4.0, and at different temperatures range of (25 ^{0}C up to 40 ^{0}C) with an increment of 5 ^{0}C for each trial. One ml of the medium was taken every one hour, diluted to 25% by adding it to a triplicate quantity of the same medium then the O.D was measured.

3.4 Data Analysis

Data obtained were analyzed using SPSS system (version 11) and microsoft excel. Student t-test and ANOVA test were carried out at a significance level of 5%, for two and more than two numerical means, respectively. Any difference between variables was considered statistically significant if P- value is less than 0.05.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Growth of BY on the surface cultures.

4.1.1. Determination of BY ability to grow on *COC* extract

This first experiment was carried out in order to investigate the ability of BY to grow in COC extract in a comparable manner with PDA as a control medium. Table 4.1 shows that BY can grow in COC extract as good as on PDA. Number of BY colonies that were grown on both media at the same conditions (T=30 0C, pH 5) were too much to count (TMTC) up to dilution factor of 10-6. There was no growth of BY at 10-8 dilution. The best dilution that gave good and measurable results was 10-7 where the size of the colonies was small and countable. Thus, the effect of adding nitrogen to the medium should also be studied .

Table 4.1: BY colonies count on COC vs. PDA. PDA was a control medium.

Media	Dilution of BY	BY colonies No.			BY average colonies No.(SD)
		1	**2**	**3**	
PDA	10^{-1}	TMTC	TMTC	TMTC	TMTC
	10^{-2}	TMTC	TMTC	TMTC	TMTC
	10^{-3}	TMTC	TMTC	TMTC	TMTC
	10^{-4}	TMTC	TMTC	TMTC	TMTC
	10^{-5}	TMTC	TMTC	TMTC	TMTC
	10^{-6}	TMTC	TMTC	TMTC	TMTC
	10^{-7}	55	48	51	51(3.0)*
	10^{-8}	< 25	< 25	< 25	< 25
COC Extract + Agar	10^{-1}	TMTC	TMTC	TMTC	TMTC
	10^{-2}	TMTC	TMTC	TMTC	TMTC
	10^{-3}	TMTC	TMTC	TMTC	TMTC
	10^{-4}	TMTC	TMTC	TMTC	TMTC
	10^{-5}	TMTC	TMTC	TMTC	TMTC
	10^{-6}	TMTC	TMTC	TMTC	TMTC
	10^{-7}	45	40	38	41(3.0)*
	10^{-8}	< 25	< 25	< 25	< 25

***** Means of BY colonies on PDA and COC extract differ significantly ($p<0.05$).

4.1.2. Growth of BY on PDA and *COC* extract with peptone.

In order to study the effect of adding peptone as a source of nitrogen onto the COC extract, the BY was cultivated on several COC media with different concentrations of peptone and the PDA medium was also used as a control (table 4.2). It was shown that, adding peptone to the BY growth medium have no statistical significance at the first six dilutions of BY(10-1 to 10-6) on all media, where the colonies number where uncountable (Annex A.1–A.6). Moreover, the size of the colonies on pure COC extract was medium and slightly large size was obtained on COC extract plus peptone. The BY growth at all media was not noticeable at the dilution of 10-8. At the BY dilution of 10-7 there was no statistical significance differences between addition of peptone up to 4g/100ml on COC extract with pure COC extract alone (Table 4.2). Moreover, BY growth on PDA was significantly better than the growth of BY on pure COC extract and COC extract plus peptone (Figure 4.1). The infectivity of peptone addition to the medium on BY growth might indicate that the a available quantity of nitrogenous compounds in COC extract was enough for BY to grow. In contrast, table 4.2 also shows that more addition of peptone (5g/100ml and 6 g/100ml) has inhibitory effect on the BY growth. This inhibitory effect was increased as peptone increases.

Table 4.2: BY growth colonies on COC extract plus peptone vs. COC extract. PDA as control medium (BY dilution was 10^{-7})

Media	BY dilution	Peptone conc. g/100 ml	BY colonies No.			Average BY colonies No.* (SD)
			1	2	3	
PDA	10^{-7}	0	55	48	51	51 (3)[a]
Pure *COC extract*	10^{-7}	0	45	40	38	41 (3)[b]
COC extract + Peptone	10^{-7}	1	42	38	35	38 (3)[b]
		2	40	36	36	37 (2)[b]
		3	44	39	37	40 (3)[b]
		4	39	35	36	37 (2)[b]
		5	25	27	23	25 (2)[c]
		6	19	16	17	17 (1)[d]

*Means with different superscripts in the final column differ significantly($p<0.05$)

a **b** **c**

Figure 4.1: (a) BY colonies on COC extract plus peptone, **(b)** on COC extract, **(c)** on PDA as control.

4.1.3. Optimizing the pH of BY growth medium.

Optimizing pH of the BY growth was carried out on Petri dishes using of COC extract with different dilutions (10% up to 100%) and with different pHs (3.5, 4.0, 4.5, 5.0, 5.5) at 30 0C. Table 4.3 shows the best pH and COC extract dilution that the BY needs to grow and reproduce. By counting BY colonies and observing their size then through statistical measurements between BY growth on different pHs at the same COC extract concentration, it was found that the best pH was about 4.0 and the best COC extract concentration was 75% of the raw material (figure 4.2). However, these results meant that, there was no need to set up the COC extract pH values because its normal pH is already slightly acidic (4.1) upon its harvesting in the morning (6:00 AM). Moreover, the slight difference between the number of BY colonies upon 75% and 50% COC extract concentrations might indicate that it was possible to use 50% COC extract concentration from economical point of view. The growth of BY on 100% and 50% COC concentration was very identical. Thus, the viscosity of 100% crude COC extract might limits the BY growth. Table 4.3 shows also that there was on growth of BY colonies on COC extract at pH 5.5 and most COC concentrations which may indicate that the accumulation of the two factors on the BY growth have inhibitory effect, specially at the COC concentration of less than 50% where the carbon source goes to negligible amount .

Table 4.3: Effect of pH values on BY growth at different COC extract dilutions**.

COC extract conc.		pH of the *COC* Medium					F- value	p- value
		3.5	4	4.5	5	5.5		
100%	Mean of colonies(SD)	31(2)^{c}	44(2)^{a}	35(1)^{b}	20(2)^{d}	8(2)^{e}	116.38	0.00
75%		35(1)^{c}	50(2)^{a}	38(1)^{b}	9(1)^{d}	0	472.82	0.00
50%		15(1)^{c}	45(2)^{a}	31(2)^{b}	3(1)^{d}	0	302.67	0.00
25%		10(1)^{b}	20(1)^{a}	10(1)^{b}	3(1)^{c}	0	74.75	0.00
10%		10(1)^{a}	7(1)^{b}	9(2)^{a}	0	0	35.55	0.00

Means with different superscripts in the same raw differ significantly ($p<0.05$)
** The values given were an average of triplicate samples.

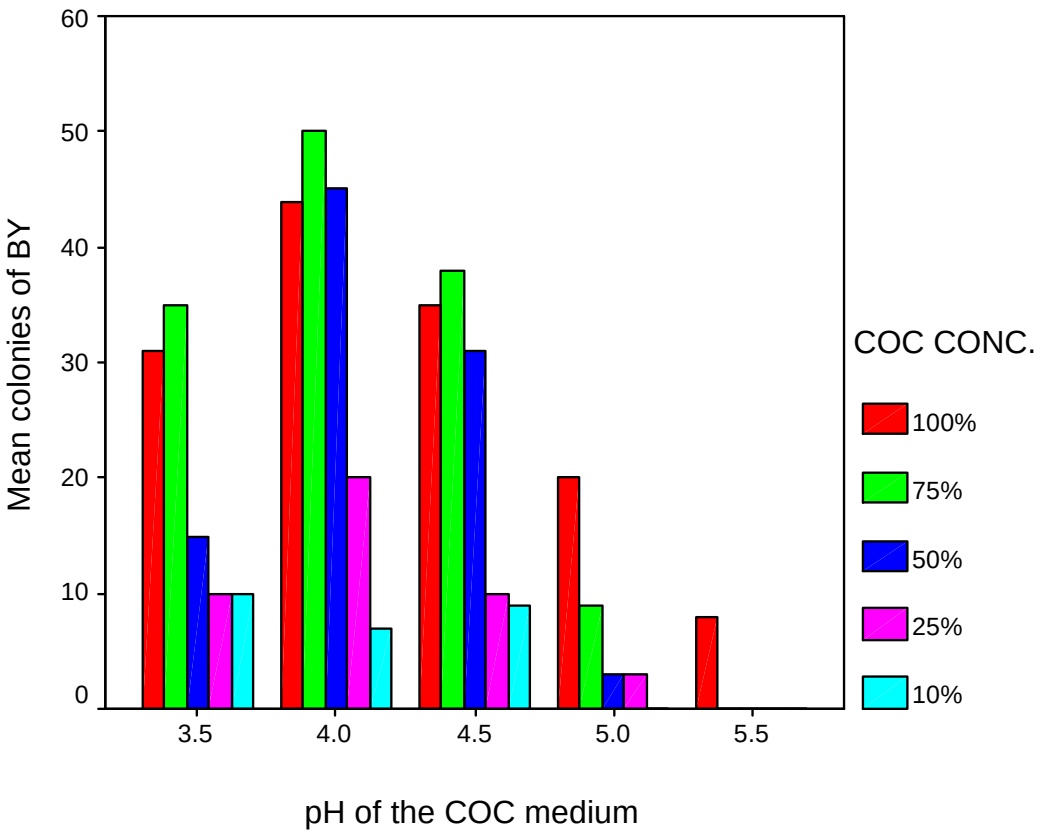


Figure 4.2: Multiple bar chart showing effect of pH on the growth of BY at different COC extract concentrations .

4.2 Growth of BY in submerged cultures.

4.2.1 Growth curve of BY in PDB

Growth curve of BY was performed in a 500 ml flask, where 0.1 gm of dry BY was added to a 100 ml sterile PDB with a pH of (4.0). The experiment was carried out in duplicates and the flasks were incubated in a shaker incubator (120 r.p.m) at 30 0C. A sample of 1 ml was taken at different times from each flask and measured by spectrophotometer at 660 nm and the data were recorded (Annex B.1, table 1). Figure (4.3) shows all growth phases of BY on the PDB. It was observed that the lag, log and stationary phases were completed through the first three days and the death phase began in the fourth day as an indication for the exhaustion of the medium substrate. The specific growth rate (μ) and the doubling time (td = ln2/ μmax) of BY in PDB was 0.25 h-1 and 83 minutes, respectively, while they were 0.24 h-1 and 88 minutes in glucose as reported in the literature [29]. These slight differences in the specific growth rate and then the doubling time may be was due to difference in carbohydrates contents between both media. The maximum O.D obtained was 22.91 which was equivalent to 54.1 g/L (D.W) of BY can be produced.

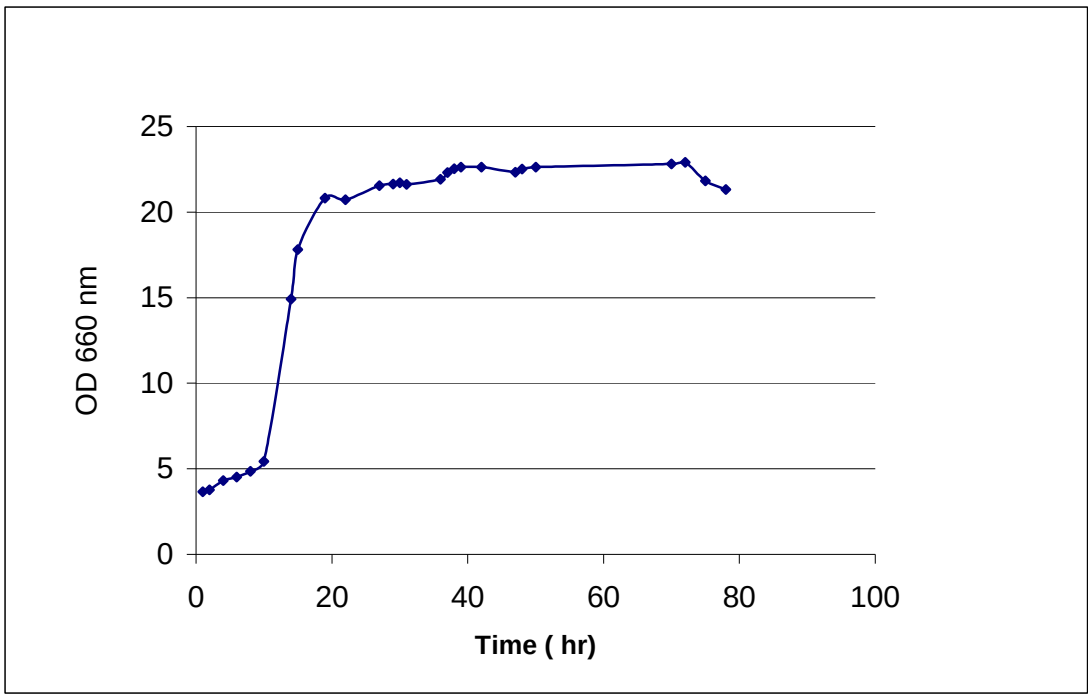
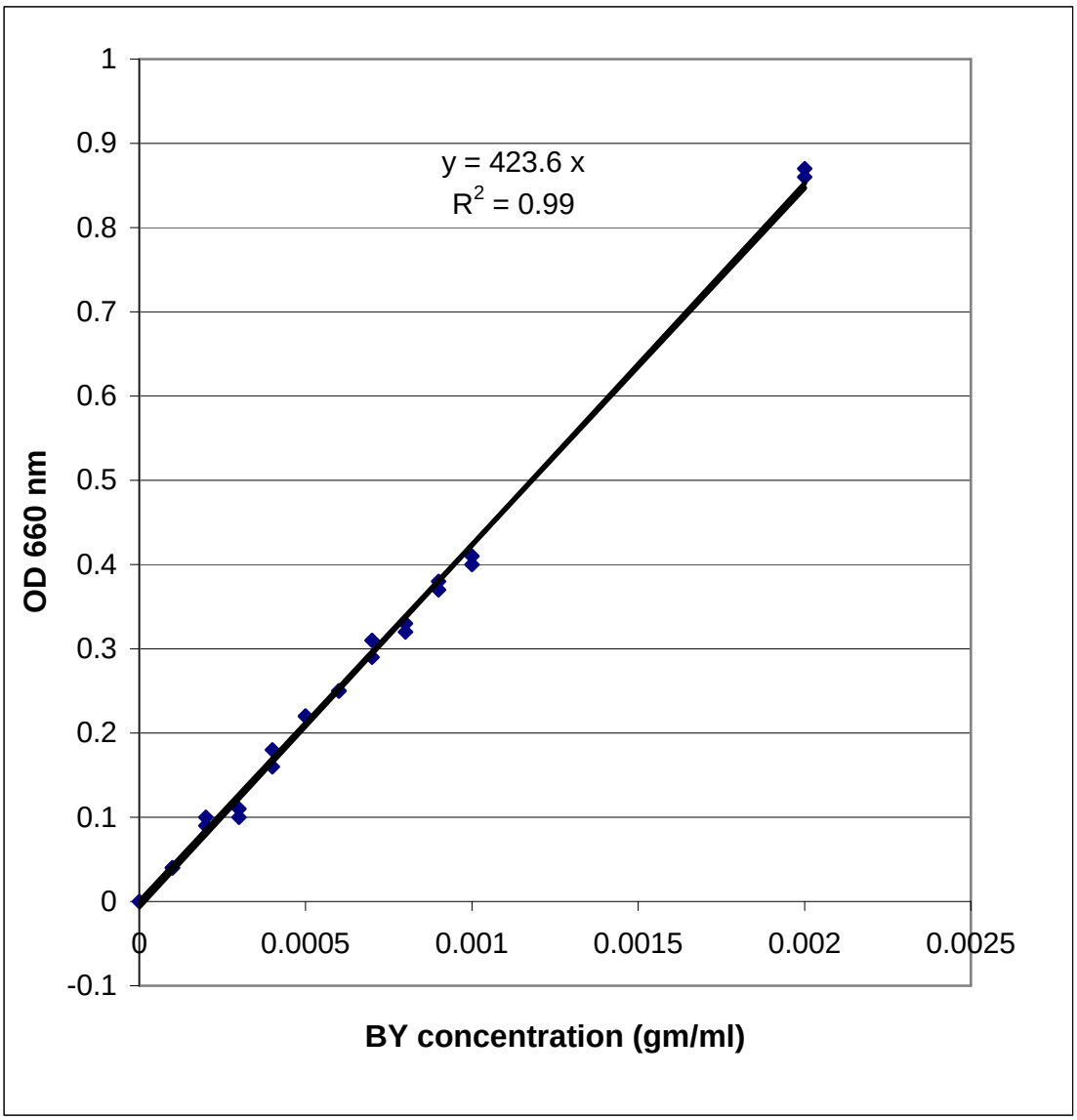


Figure 4.3: Growth curve of BY in PDB.

4.2.2 Calibration curve of BY dry weight

(Annex B.2, Table 1) shows the data of BY growth in PDB, (measured in duplicates). Figure 4.4 shows the resultant calibration curve of BY growth at 660 nm and room temperature. According to the figure, the extension coefficient $Є_{660}$ nm of BY was 423.6 $gm^{-1}cm^{-1}ml$ and each O.D unit corresponded to about 2.2 $x10^{-3}$ g BY dry weight or about 3 x 10^7 cells/ ml if we considered that each one gram of BY counts about 15 billion live cells. These results were very consistent with those results reported by Treco and winston, 1992 (14) and stone,1998 (15).



F igure 4.4: Calibration curve of BY cells at 660nm and room temperature.(in g/ml dry weight).

4.2.3 Growth of BY in COC extract with different peptone concentrations

In order to study the effect of adding peptone as a source of nitrogen onto the COC extract, the BY was cultivated in several COC media with different concentrations of peptone (0g/100ml up to 6g/100dl) and the PDB medium was used as a control (table 4.4). At the BY dilution of 10-7 there was no statistical significant differences between addition of peptone up to 4g/100ml in COC extract when compared with pure COC extract alone (Table 4.4). Moreover BY growth in PDB was significantly better than the growth of BY in pure COC extract and COC extract plus peptone. In contrast, table 4.5 shows that the addition of more peptone (5g/100ml and 6g/100ml) has inhibitory effect on the BY growth if compared with the less concentrations of peptone ($p>0.05$). It is noticeable that the specific growth rate (μ) of BY in COC extract increased slightly at (1g/100ml) peptone concentration comparably with pure COC extract but decreased as the peptone concentration increased (table 4.5, figure 4.5). These results indicates that, COC extract proteins might not be enough for BY growth and proliferation so, adding some nitrogen ingredients to the COC extract medium may improve the original COC extract for BY growth continuity.

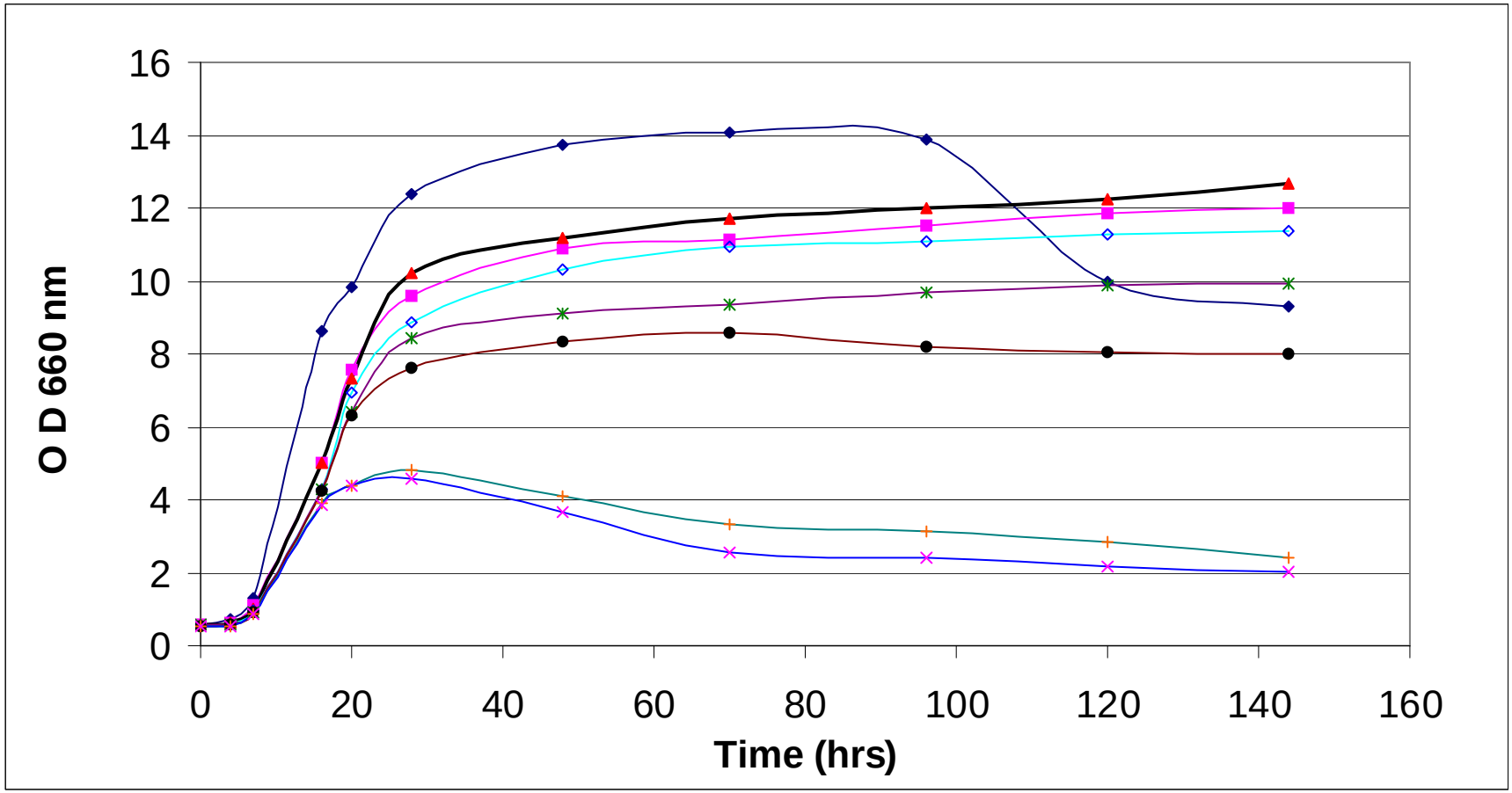


Figure 4.5: Growth of BY in COC extract .With peptone conc. 0g/100ml(■), 1g/100ml(▲), 2g/100ml(◊), 3g/100ml(ж), 4g/100ml (●), 5g/100ml (+), 6g/100ml (×), PDB the control(♦).

Table 4.4: BY growth In COC extract at different peptone concentrations. PDB as control medium (BY dilution was 10^{-7})

Time (hrs)	Media Average O.D* at 660 nm(SD)							
	PDB	COC extract	COC+pep. 1g/100ml	COC+pep. 2g/100ml	COC+pep. 3g/100ml	COC+pep. 4g/100ml	COC+pep. 5g/100ml	COC+pep. 6g/100ml
0	0.57(0.01)	0.56(0.01)	0.56(0.01)	0.54(0.03)	0.56(0.02)	0.55(0.02)	0.54(0.01)	0.52(0.01)
4	0.72(0.02)	0.64(0.01)	0.64(0.01)	0.59(0.01)	0.58(0.01)	0.57(0.01)	0.55(0.01)	0.54(0.01)
7	1.30(0.02)	1.10(0.02)	1.01(0.02)	0.97(0.02)	0.93(0.01)	0.93(0.02)	0.89(002)	0.88(0.01)
16	8.62(0.01)	5.01(0.01)	5.02(0.01)	4.30(0.01	4.29(0.02)	4.23(0.03)	3.91(0.02)	3.85(0.01)
20	9.85(0.06)	7.55(0.02)	7.33(0.03)	6.94(0.07)	6.41(0.05)	6.33(0.03)	4.44(0.02)	4.42(0.03)
28	12.40(0.04)	9.62(0.02)	10.23(0.01)	8.85(0.04)	8.45(0.04)	7.63(0.04)	4.84(0.03)	4.64(0.02)
48	13.73(0.03)	10.90(0.05)	11.21(0.07)	10.31(0.09)	9.09(0.03)	8.34(0.02)	4.12(0.03)	3.65(0.08)
70	14.08(0.03)	11.15(0.06)	11.72(0.03)	10.92(0.09)	9.36(0.04)	8.62(003)	3.34(0.09)	2.57(0.06)
96	13.86(0.09)	11.54(0.02)	12.01(0.04)	11.12(002)	9.70(0.04)	8.21(0.04)	3.15(0.04)	2.41(0.03)
120	9.96(0.02)	11.87(0.07)	12.24(0.03)	11.26(0.05)	9.88(0.04)	8.03(0.03)	2.84(0.02)	2.16(0.02)
144	9.32(0.04)	11.98(0.03)	12.68(0.03)	11.37(0.01)	9.93(0.03)	7.98(0.02)	2.41(0.02)	2.02(0.02)

*The values given were an average of triplicate samples.

Table 4.5: Specific growth rate of BY in COC extract at different peptone conc.(PDB as control).

	(μ)*(SD) h^{-1}						
	COC extract + Peptone						
PDB	Pure COC	1g/100ml	2g/100ml	3g/100ml	4g/100ml	5g/100ml	6g/100ml
0.210 (0.016)	0.168^{b} (0.012)	0.178^{a} (0.022)	0.165^{c} (0.016)	0.169^{b} (0.016)	0.168^{b} (0.024)	0.164^{c} (0.016)	0.164^{c} (0.016)

*The values given were an average of triplicate samples

* Means with different superscripts differ significantly

4.2.4 Growth curve of BY in COC extract medium .

A sample of 1 ml was taken every 24 hours from each of the flasks that mentioned in the section (3.3.2.4), measured by spectrophotometer at 660 nm and data were recorded in table 4.5. The D.W of each O.D value was calculated from the extension coefficient Є of the previous calibration curve (Figure 4.4). The best medium's pH for BY to grow was 4.0 in both COC extract and PDB (Figures 4.5 and 4.6). It can be noticed in both figures that BY production decreased at the beginning of the fifth day of cultivation which means that, yeast cells began to die because the medium ingredients decreased as a result of cell competition or because wastes accumulation in the medium. In addition it was noticed that 29.0 mg/ml of BY was produced in COC extract after 72 hours of cultivation at pH 4.0 compared with 37.0 mg/ml produced in PDB at the same conditions. However, the best specific growth rate (μ) of BY in COC extract medium was 0.21 h^{-1} and 0.18 h^{-1} at the pHs of 4.0 and 4.5, respectively (Table 4.6). Table 4.5 also shows that (μ) of BY at different pH values in PDB medium was not significantly more than in COC extract specially at the pH values of 4.0 ($p>0.05$). The reason for this finding is that, the COC extract medium has BY fermentable sugar ingredients that might be considered as equal productive as to glucose in the PDB medium. From another view, (μ) value of BY in PDB was significantly more than (μ) value in the COC extract at the pHs 4.5 and 5.0 ($p<0.05$). These results and the previous ones on the surface culture (Item 4.1.3) showed that the growth of BY on PDB and the COC extract was identical at optimum pH 4.0.

Table 4.6: Growth of BY in *COC* extract and PDB.

Time (hr)	pH	Average $O.D_{660nm}$ on COC (SD)	D.W of BY on COC gm/ml	Average $O.D_{660nm}$ on PDB (SD)	D.W of BY on PDB gm/ml	Student t - value	P-value
0	4.0	0.57(0.07)	0.001	0.59(0.01)	0.001	0.29	0.79
	4.5	0.58(0.01)	0.001	0.59(0.00)	0.001	3.15	0.03
	5.0	0.56(0.01)	0.001	0.57(0.02)	0.001	0.80	0.47
8	4.0	0.98(0.03)	0.002	1.02(0.01)	0.002	1.68	0.17
	4.5	1.09(0.08)	0.003	1.15(0.04)	0.003	0.97	0.39
	5.0	1.08(0.03)	0.003	1.19(0.08)	0.003	1.79	0.15
18	4.0	8.15(0.08)	0.019	11.95(0.08)	0.028	46.59	0.00
	4.5	6.63(0.16)	0.016	11.54(0.11)	0.027	34.84	0.00
	5.0	5.45(0.18)	0.013	9.73(0.16)	0.023	25.06	0.00
24	4.0	9.59(0.196)	0.023	12.22(0.17)	0.029	14.26	0.00
	4.5	9.18(0.11)	0.022	11.90(0.16)	0.028	19.75	0.00
	5.0	8.83(0.12)	0.021	12.28(0.11)	0.029	31.15	0.00
48	4.0	9.98(0.07)	0.024	15.35(0.16)	0.036	43.18	0.00
	4.5	10.75(0.16)	0.025	15.35(0.11)	0.036	33.23	0.00
	5.0	10.36(0.07)	0.024	14.97(0.08)	0.035	64.14	0.00
72	4.0	12.11(0.09)	0.029	15.74(0.13)	0.037	31.88	0.00
	4.5	11.51(0.07)	0.027	15.35(0.11)	0.036	43.02	0.00
	5.0	11.13(0.06)	0.026	14.96(0.09)	0.035	51.01	0.00
96	4.0	12.05(0.11)	0.028	15.50(0.14)	0.037	27.19	0.00
	4.5	11.13(0.07)	0.026	14.97(0.09)	0.035	53.46	0.00
	5.0	10.36(0.09)	0.024	14.58(0.18)	0.034	29.72	0.00
120	4.0	11.51(0.16)	0.027	13.43(0.09)	0.032	15.10	0.00
	4.5	10.75(0.16)	0.025	12.28(0.15)	0.032	9.85	0.00
	5.0	8.83(0.07)	0.021	10.36(0.10)	0.029	17.51	0.00
144	4.0	9.98(0.02)	0.024	12.67(0.08)	0.030	45.69	0.00
	4.5	9.60(0.16)	0.023	11.51(0.17)	0.027	11.55	0.00
	5.0	8.06(0.02)	0.019	9.60(0.14)	0.023	15.17	0.00

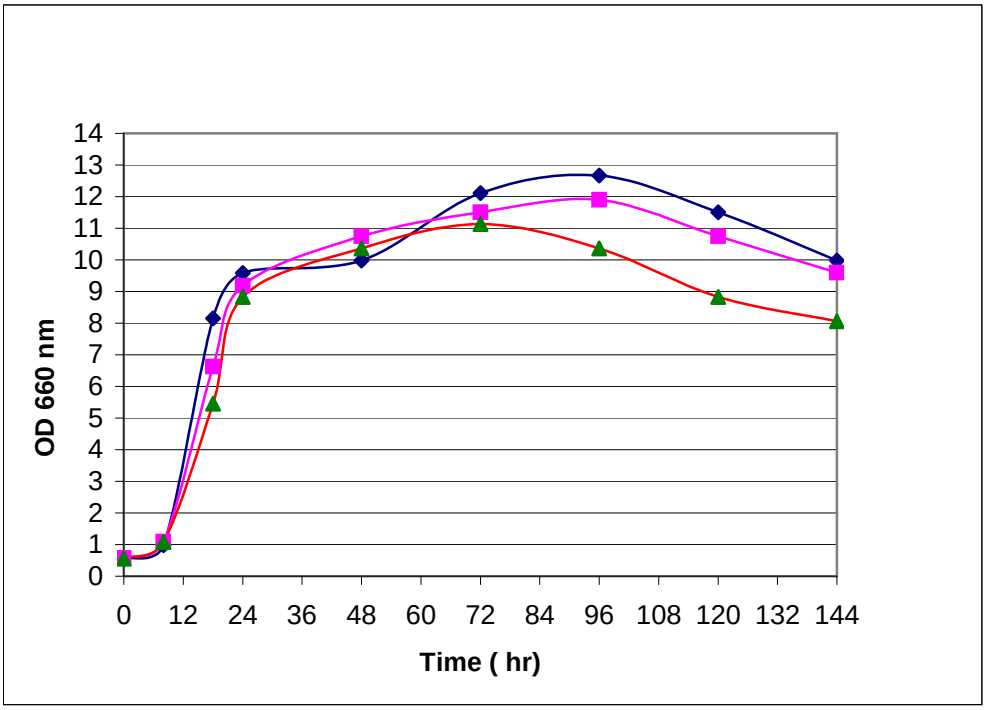


Figure 4.6 : Growth of BY in *COC* extract .At pH 4.0(♦),pH 4.5(■), pH 5.0(▲).(in D.W)

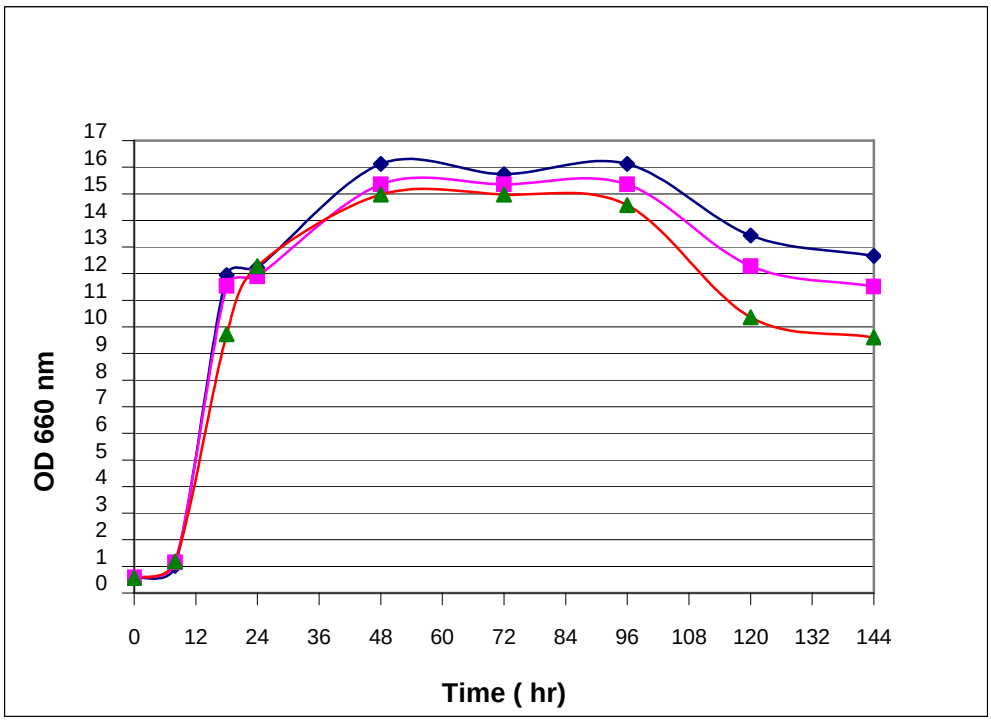


Figure 4.7: Growth of BY in PDB. At pH 4.0(♦),pH 4.5(■), pH 5.0(▲) (in D.W)

Table 4.7: Specific growth rate of BY in COC extract at different pH values (PDB as control).

pH	μ On COC extract (SD) h^{-1}	μ On PDB (SD) h^{-1}	Student t-test	P- value
4.0	0. 21 (0.01)	0.25 (0.02)	2.62	0.06
4.5	0. 18 (0.01)	0.23 (0.01)	4.33	0.01
5.0	0. 16 (0.02)	0.21 (0.01)	3.27	0.03

4.2.5 Overall growth curve of BY on the *COC* extract at pH 4.0

The experiment was repeated in COC extract at pH 4.0 and 30 0C. The amount of BY inoculants was 3% of the COC extract volume used. The starting dilution was 10-7. A sample of 1 ml was also taken every 60 min from each of the flasks that mentioned in section (3.3.2.4), measured by spectrophotometer at 660 nm and data were given in Annex B.3 table 1. Figure 4.7 shows that the growth curve of BY has more than one log phase because there was more than one carbon source that BY can use to grow and proliferate (diauxic growth behavior). The largest optical density measured was 17.48 at the beginning of the seventh day of cultivation where 41.3 g/L of BY was produced in COC extract diluted up to 50%. The O.D was decreased to 13.2 in the last 3 hours of the BY growth, the second stationary phase was not noticed. It seems that the amount of the last fermented carbon source was very small in such away that, the second stationary phase was not started. In addition, the specific growth rate of BY in the second exponential phase was so small. According to figure 4.7, μ1 and μ2 of BY in COC extract were 0.21 h-1 and 0.01 h-1, respectively. Hence the doubling times of BY were 1.64 and 34.7 hours, respectively. The over all growth rate curve of BY showed that there were two log phases and one clear stationary phase. The second phase was not completed and followed with fast death of the BY. It was found also that each 1000g fresh weight of COC produced about 62.0 g (DW) of BY, where as each 1000g fresh weight COC produced 750 ml of concentrated extract.

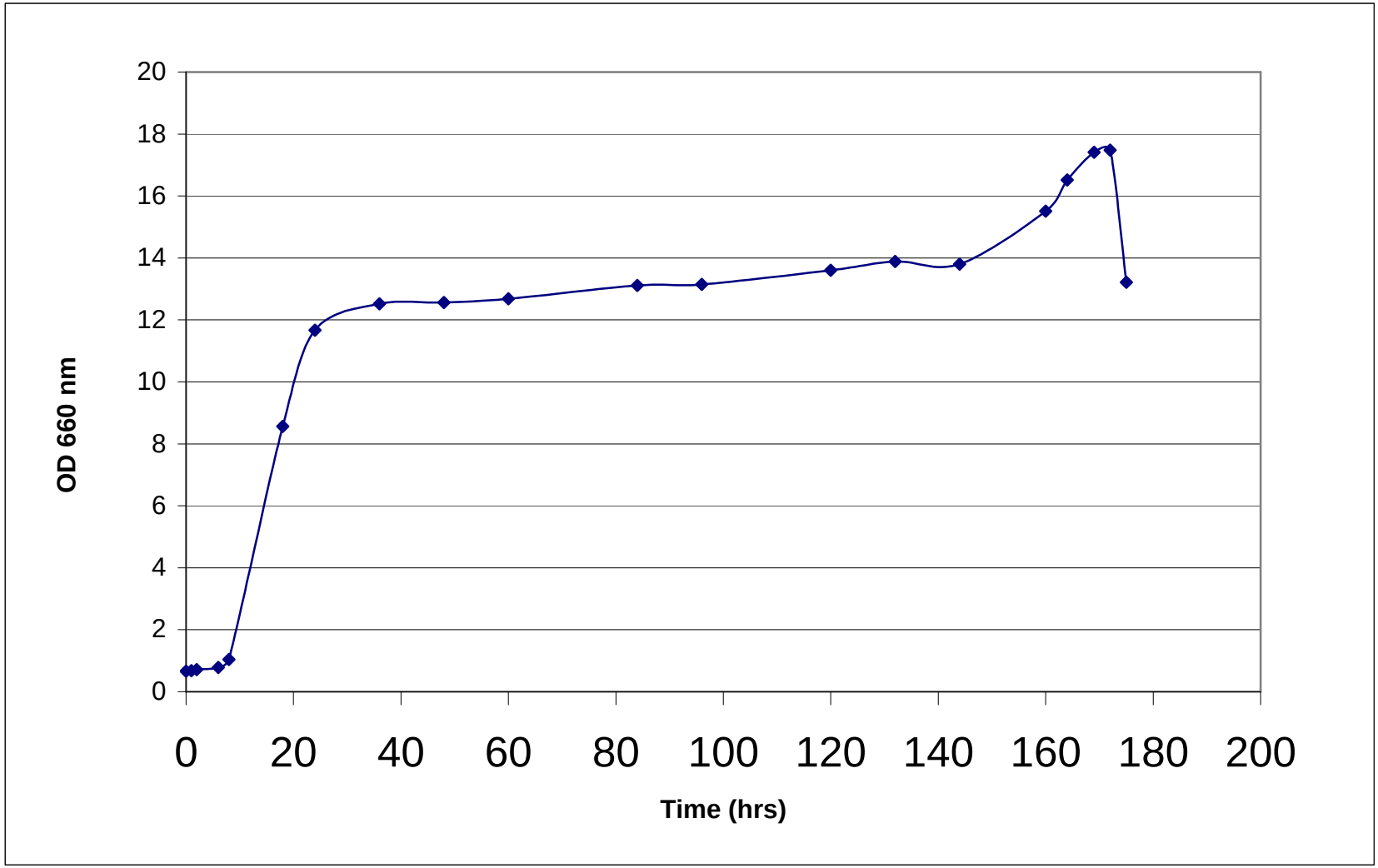


Figure 4.8 : Growth curve of BY on *COC* extract at 30 ^{0}C and pH 4.0 .

4.2.6 Optimizing the temperature of the BY growth medium

A sample of 1 ml was also taken every 60 min from each of the flasks as mentioned in the item (3.3.2.6**)** and measured by spectrophotometer at 660 nm, where the medium's temperature varied from 25 ^{0}C up to 40 ^{0}C (Annex B.4, tables 1- 4). Annex B.4, table 3 shows the data at 30 ^{0}C. Figure 4.8 shows the growth curve of BY at 30 ^{0}C using PDB as a control. Figure 4.9 also shows the growth curves of BY in *COC* extract medium and PDB as control at different temperatures 25 ^{0}C, 35 ^{0}C ,40 ^{0}C. It was shown that the optimum temperature for BY growth in both *COC* extract and PDB media was at 30 ^{0}C. At this temperature value the growth of BY in COC extract continued for about 158 hours before exhaustion of the medium. From one side, the longer time that the BY growth took place in COC extract compared with the BY growth in PDB might indicate that the first stationary phase interfered with the second lag phase, from the other side, there might be a production of some substances that the BY can use in the fermentation process. From a third side, there might be a certain difficulty for BY enzymes to deal with the second carbon source easily. Table 4.7 shows the specific growth rate of BY in both COC extract and PDB at the mentioned temperatures. It was noticed that the effect of high temperature equals the effect of low temperature on the BY growth, where both effects disturb the enzyme function inside the BY cells leading to the decrease in growth rate.(See Figure 4.9) .

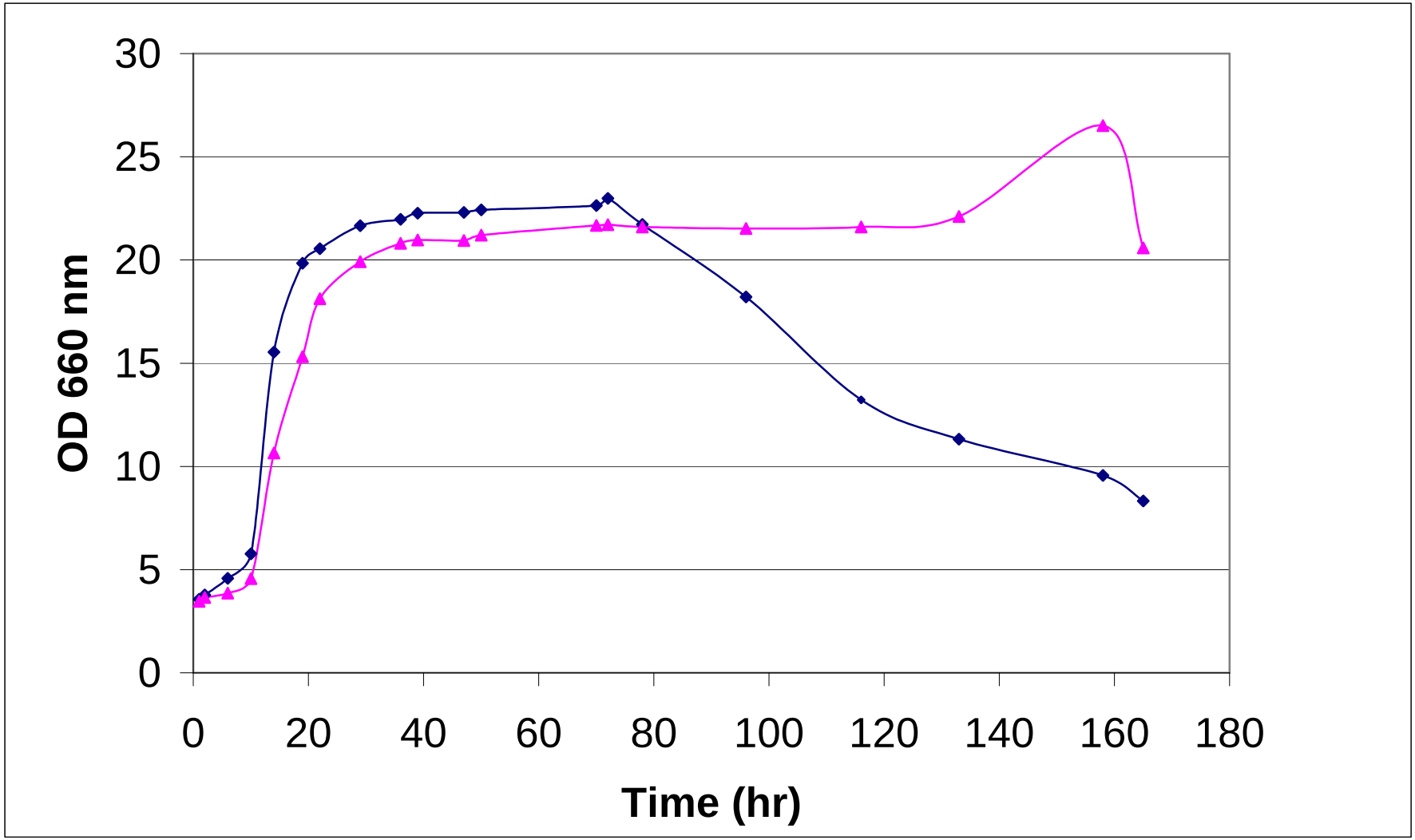
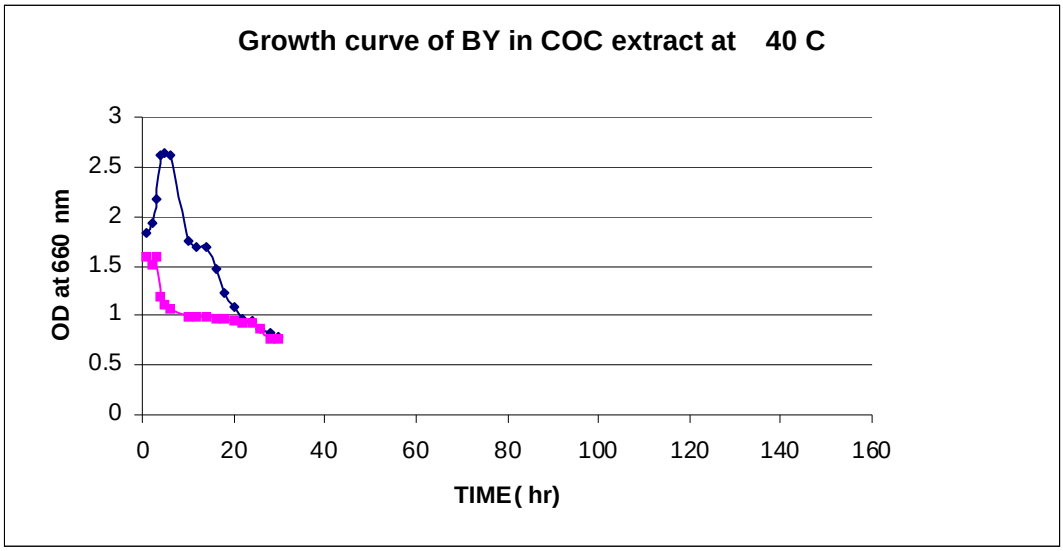
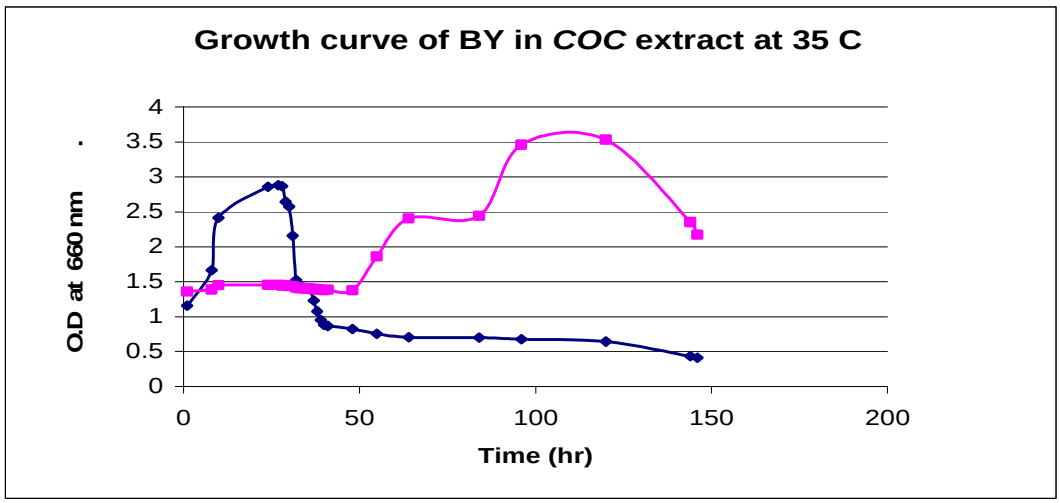


Figure 4.9: BY growth at temperatures (30 C^0). in PDB(control) (♦) and in *COC* extract (▲).





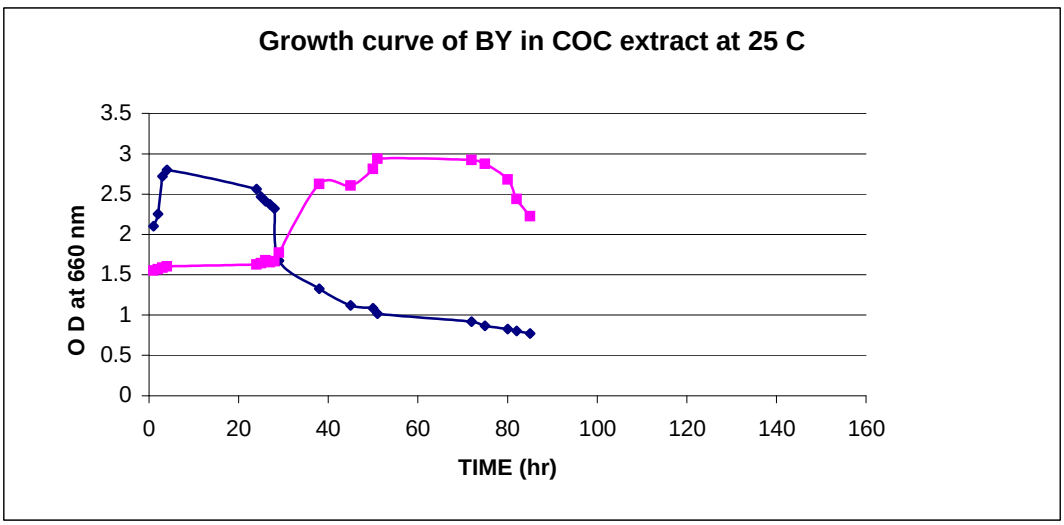


Figure 4.10: BY growth at (400C,350C,250C). in PDB (control) (♦), and in COC extract (■).

From the Figures 4.8 and 4.9 above it was found that the specific growth rates of BY in COC extract and in PDB as control at the different temperatures (25 ^{0}C up to 40 ^{0}C) as follows in Table 4.6 .

Table 4.8: Specific growth rate (μ) of BY vs temperature on COC extract, PDB is control.

Temp. ^{0}C	μ of BY on COC extract (h^{-1})	μ of BY on PDB (h^{-1})	Student t-test	P - value
25	0.04 (0.05)	0.19 (0.01)	3.13	0.04
30	0.21 (0.01)	0.25 (0.02)	3.91	0.02
35	0.04 (0.01)	0.19 (0.01)	3.68	0.02
40	0.05 (0.01)	0.18 (0.01)	23.31	0.00

CHAPTER 5

CONCLUSIONS and RECOMMENDATIONS

5.1 Conclusions

Saccharomyces cerevisiae (the BY) is currently fed an expensive PDB medium in vitro and grown commercially on sugar molasses which are not available in the Gaza strip. Therefore, the available low cost *COC* extract was tested to replace these media. The BY was grown well on *COC* extract as a complete medium, the control BY was grown on PDB, and the conclusions of the study were as the following :

1-Through following up the growth curve of BY on *COC* extract it was found that, BY showed diauxic growth behavior where it can use more than one carbon source to gain energy and to perform its growth and proliferation.

2-The optimum pH and temperature values for good growth of BY on *COC* extract medium were found to be about 4.0 and 30 ^{0}C, respectively.

3- The specific growth rate of BY on *COC* extract was found to be 0.21 h^{-1} at pH 4.0 and 30 ^{0}C. Whereas on PDB it was 0.25 h^{-1} at the same conditions with no significant differences.

4-The resultant growth of BY on the *COC* extract, the availability and the low cost of the COC gave an impression that the *COC* extract can be used to grow BY in a good economical manner.

5- The overall growth period of BY on COC extract was longer than that of PDB which indicate that, the longer fermentation time may lead to better results in By growth in COC extract.

6- It was possible to produce protein rich BY biomass from low protein COC extract.

7- One kg of fresh COC produced about 62.0 g of BY (D.W) at pH 4.0 and 30 ^{0}C.

5.2 Recommendations

After carrying out these preliminary experiments on using COC extract medium as an alternative medium for BY growth, further studies have been recommended which are:

1- Studying BY growth on COC extract in presence of natural rich protein additives.

2- Studying the ability of preparing powdered media using dried COC cladodes as a cheap alternative to PDA or other high expensive media of the BY.

3- Large scale studies might be carried out in the future on COC extract to produce BY in a commercial scale.

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APPENDICES

Annex A: Growth of BY on surface culture.

Table 1 : Growth of the BY on COC extract plus peptone vs PDA was a control.(yeast dilution 10^{-1}).

Media	BY dilution	Peptone conc. .(per 100ml)	Colonies No.			Average colonies No.
			1	2	3	
PDA	10^{-1}	*******	TMTC	TMTC	TMTC	TMTC
Cactus Extract	10^{-1}	*******	TMTC	TMTC	TMTC	TMTC
Cactus Extract + Peptone	10^{-1}	1g	TMTC	TMTC	TMTC	TMTC
		2g	TMTC	TMTC	TMTC	TMTC
		3g	TMTC	TMTC	TMTC	TMTC
		4g	TMTC	TMTC	TMTC	TMTC
		5g	TMTC	TMTC	TMTC	TMTC
		6g	TMTC	TMTC	TMTC	TMTC

Table 2 : Growth of the BY on *COC* plus peptone vs PDA . PDA was a control.(yeast dilution 10^{-2}).

Media	BY dilution	Peptone conc. .(per 100ml)	Colonies No.			Average colonies No.
			1	2	3	
PDA	10^{-2}	**********	TMTC	TMTC	TMTC	TMTC
COC Extract	10^{-2}	**********	TMTC	TMTC	TMTC	TMTC
COC Extract + Peptone	10^{-2}	1g	TMTC	TMTC	TMTC	TMTC
		2g	TMTC	TMTC	TMTC	TMTC
		3g	TMTC	TMTC	TMTC	TMTC
		4g	TMTC	TMTC	TMTC	TMTC
		5g	TMTC	TMTC	TMTC	TMTC
		6g	TMTC	TMTC	TMTC	TMTC

Table 3 : Growth of the BY on *COC* plus peptone vs PDA . PDA was a control.(yeast dilution 10^{-3}).

Media	BY dilution	Peptone conc. (per 100ml)	Colonies No.			Average colonies No.(SD)
			1	2	3	
PDA	10^{-3}	*********	TMTC	TMTC	TMTC	TMTC
COC Extract	10^{-3}	*********	TMTC	TMTC	TMTC	TMTC
COC Extract + Peptone	10^{-3}	1g	TMTC	TMTC	TMTC	TMTC
		2g	TMTC	TMTC	TMTC	TMTC
		3g	TMTC	TMTC	TMTC	TMTC
		4g	90	92	95	92 (2)
		5g	TMTC	TMTC	97	TMTC
		6g	84	75	52	70 (13)

Table 4 : Growth of the BY on *COC* plus peptone vs PDA . PDA was a control.(yeast dilution 10^{-4}).

Media	BY dilution	Peptone conc. (per 100ml)	Colonies No.			Average colonies No.
			1	2	3	
PDA	10^{-4}	*********	TMTC	TMTC	TMTC	TMTC
COC Extract	10^{-4}	*********	TMTC	TMTC	TMTC	TMTC
COC Extract + Peptone	10^{-4}	1g	TMTC	TMTC	TMTC	TMTC
		2g	TMTC	TMTC	TMTC	TMTC
		3g	TMTC	TMTC	TMTC	TMTC
		4g	TMTC	TMTC	TMTC	TMTC
		5g	TMTC	TMTC	TMTC	TMTC
		6g	TMTC	TMTC	TMTC	TMTC

Table 5 : Growth of the BY on *COC* plus peptone vs PDA .PDA is a control. (yeast dilution 10^{-5}).

Media	BY dilution	Peptone conc. (per 100ml)	Colonies No.			Average colonies No.(SD)
			1	2	3	
PDA	10^{-5}	**********	TMTC	TMTC	TMTC	TMTC
COC Extract	10^{-5}	**********	153	141	162	152 (8)
COC Extract + Peptone	10^{-5}	1g	TMTC	TMTC	TMTC	TMTC
		2g	TMTC	TMTC	TMTC	TMTC
		3g	TMTC	TMTC	TMTC	TMTC
		4g	TMTC	TMTC	TMTC	TMTC
		5g	TMTC	TMTC	TMTC	TMTC
		6g	TMTC	TMTC	TMTC	TMTC

Table 6: Growth of the BY on *COC* plus peptone vs PDA .PDA is a control.(yeast dilution 10^{-6}).

media	BY dilution	Peptone conc. (per 100ml)	Colonies No.			Average colonies No.(SD)*
			1	2	3	
PDA	10^{-6}	********	TMTC	TMTC	TMTC	TMTC
COC Extract	10^{-6}	********	106	136	106	116 ^{a}(14)
COC Extract + Peptone	10^{-6}	1g	107	96	96	100 ^{c}(5)
		2g	97	111	89	99 ^{c}(9)
		3g	114	103	97	105 ^{b}(7)
		4g	69	71	72	71 ^{d}(1)
		5g	65	70	72	69 ^{d}(3)
		6g	56	54	77	62 ^{e}(1)

*Mean with difference superscripts in the final column differ significantly $p<0.05$)

Annex B.1: Standard growth curve of BY in PDB.

Table 1: Growth of BY in PDB.

Time (hr)	O.D (660 nm)	Time (hr)	O.D (660 nm)
1	3.66	31	21.62
2	3.78	36	21.92
4	4.32	37	22.31
6	4.52	38	22.54
8	4.86	39	22.62
10	5.43	42	22.63
14	14.91	47	22.33
15	17.81	48	22.51
19	20.82	50	22.62
22	20.72	70	22.82
27	21.55	72	22.91
29	21.63	75	21.82
30	21.72	78	21.32

Annex B.2: Growth of BY in broth culture.

Table 1: Calibration curve data of BY dry weight

Cell Conc. (gm/ ml)	O.D 660 nm
0.00	0.00
0.0001	0.04
0.0001	0.04
0.0002	0.10
0.0002	0.09
0.0003	0.10
0.0003	0.11
0.0004	0.16
0.0004	0.18
0.0005	0.22
0.0005	0.22
0.0006	0.25
0.0006	0.25
0.0007	0.29
0.0007	0.31
0.0008	0.33
0.0008	0.32
0.0009	0.37
0.0009	0.38
0.0010	0.40
0.0010	0.41
0.0020	0.86
0.0020	0.87

Annex B.3 : Growth curve of BY in COC extract .

Table 1: Growth of BY in *COC* extract *, at pH 4.0, T=30 ^{0}C.

Time (hr)	Average O.D at (660 nm)*
0	0.66
1	0.68
2	0.71
6	0.78
8	1.04
18	8.56
24	11.67
36	12.52
48	12.56
60	12.68
84	13.11
96	13.15
120	13.60
132	13.89
144	13.80
160	15.51
164	16.52
169	17.41
172	17.48
175	13.22

*The values given were an average of triplicate samples.

Annex B.4 : Optimizing the temperature of BY growth media.

Table 1: The O.D of BY in COC extract and PDB at 40 ^{0}C .

Time (hr)	Average O.D of BY	
	in PDB at 660nm	in COC extract at 660 nm
1	1.83	1.59
2	1.95	1.52
3	2.18	1.60
4	2.61	1.18
5	2.63	1.10
6	2.61	1.08
10	1.75	0.10
12	1.68	0.99
14	1.70	0.98
16	1.47	0.97
18	1.23	0.97
20	1.08	0.94
22	0.98	0.93
24	0.95	0.92
26	0.86	0.86
28	0.82	0.77
30	0.79	0.76

Table 2: The O.D of BY in COC extract and PDB at 35 ^{0}C .

Time (hr)	Average O.D of BY	
	in PDB at 660nm	in COC extract at 660 nm
1	1.16	1.36
8	1.66	1.39
10	2.41	1.45
24	2.86	1.45
27	2.88	1.46
28	2.87	1.44
29	2.65	1.45
30	2.58	1.44
31	2.16	1.44
32	1.52	1.41
33	1.44	1.41
34	1.42	1.41
35	1.41	1.40
36	1.39	1.40
37	1.23	1.39
38	1.08	1.40
39	0.95	1.39
40	0.89	1.38
41	0.86	1.39
48	0.83	1.38
55	0.76	1.86
64	0.70	2.41
84	0.70	2.44
96	0.68	3.46
120	0.64	3.53
144	0.44	2.35
146	0.41	2.17

Table 3: The O.D of BY In COC extract and PDB at 30 ^{0} C.

Time (hr)	Average OD of BY	
	in PDB at 660nm	in COC extract at 660 nm
1	3.56	3.47
2	3.77	3.65
6	4.58	3.86
10	5.76	4.56
14	15.54	10.65
19	19.84	15.31
22	20.55	18.12
29	21.66	19.92
36	21.97	20.81
39	22.27	20.97
47	22.31	20.95
50	22.43	21.21
70	22.64	21.68
72	22.98	21.71
78	21.72	21.60
96	18.21	21.52
116	13.23	21.60
133	11.32	22.11
158	9.56	26.51
165	8.32	20.58

Table 4: The O.D of BY in COC extract and PDB at 25 ^{0} C.

Time (hr)	Average O.D of BY	
	in PDB at 660nm	in COC extract at 660 nm
1	2.10	1.55
2	2.25	1.57
3	2.72	1.59
4	2.80	1.60
24	2.56	1.63
25	2.47	1.64
26	2.41	1.68
27	2.37	1.65
28	2.32	1.67
29	1.67	1.77
38	1.33	2.63
45	1.12	2.61
50	1.08	2.81
51	1.01	2.94
72	0.92	2.93
75	0.87	2.88
80	0.83	2.68
82	0.80	2.44
85	0.77	2.23

Annex B.5 : Materials and Instruments

1. Chemicals:

The compounds sodium chloride, sodium hydroxide were purchased from Frutarom Co.; Haifa, tartaric acid, hydrochloric acid were purchased from Ghadot Co. ; "Israel."

The media potato dextrose agar, broth and peptone were purchased from Himedia; India.

The bacteriological agar type (E) was from Biokar Diagnostics Co. ; France.

For the calibration of pH meter, the buffers of 4.0 and 7.0 were obtained from Ventura; Spain.

Petri dishes used for surface cultures are from Miniplast EIN – Shemer;" Israel".

The sample of *COC* used was at the age of 1-2 years and harvested at 6:00 AM from a farm borders at west Jabalia Refugee Camp.

The BY Spp. (*Saccharomyces cerevisiae)* strain was from the local market which produced in Maya Co.; Turkey.

2. Instruments

Instrumentation and devices used in this study were: Blender, pH meter, Spectrophotometer, Sorvall centrifuge, Eppendrof table top centrifuge Stirrer, Vortex, Digital electric balance, Benson burner, Test tubes, Eppendrof tubes, Micropipettes, Autoclave, Shaker incubator, Spreader, Droppers and they were all from Islamic university of Gaza laboratories.