SIMULTANEOUS DEGRADATION OF TOXIC AND VOLATILE SUBSTRATES BY TWO PHASE PARTITIONING BIOREACTOR SYSTEMS: PERFORMANCE CHARACTERIZATION AND RATIONAL POLYMER SELECTION

by

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Abstract

The degradation of toxic and volatile contaminants in aqueous streams is considered a challenge using conventional bioremediation strategies. At moderate concentrations, toxic contaminants induce microbial inhibition, which results in an overall decrease of reaction rates. On the other hand, volatile compounds are often stripped out of solution into the atmosphere during aeration in conventional wastewater treatments, and are not treated.

The addition of a second non-aqueous phase with affinities for the contaminants can reduce aqueous concentrations to sub-inhibitory levels and also decrease contaminant volatilization, while still allowing controlled release of contaminants back to the microbial population; such systems have been denoted as Two Phase Partitioning Bioreactor (TPPB). The current work examined and compared the performance of solid-liquid TPPB to a liquid-liquid TPPB and a single phase system. The systems were compared in the simultaneous degradation of phenol and butyl acetate, two substrates known for their relatively high levels of toxicity and volatility, respectively.

The solid-liquid TPPB, using 2 polymers selected heuristically, showed an improvement of 40 and 54 % in phenol degradation rates compared to the single phase and the liquid-liquid systems. Additionally, the solid-liquid system presented a 55 and 11 % enhancement in the amount of butyl acetate degraded. At higher initial substrate concentration the solid-liquid TPPB showed an improvement in the phenol degradation rate and the amount of butyl acetate degraded of 44 and 94 % respectively, compared to the single phase system.

In order to rationalize polymer screening for solid-liquid TPPBs, selection criteria based on first principles were developed, and were based on consideration of polymer accessibility and polymer-solute thermodynamic affinity. Polymer accessibility was evaluated by considering glass transition temperature (T_g) and degree of crystallinity, while polymer-solute thermodynamic affinity was assessed using three different methods, Hildebrand solubility parameters, Hansen

Solubility Parameters (HSP) and activity coefficients at infinite dilution. It was found that the HSP method gave the best trends and its predictions had better agreement with the experimental results. Consequent biodegradation experiments with a single, rationally selected polymer, and a mixture of waste polymers, demonstrated the superior performance of rational selected polymers.

Co-Authorship

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Lists of Major abbreviations

4CP	4-chlorophenol
4NP	4-nitrophenol
DO	Dissolved Oxygen
EVA	Poly(ethylene-co-vinyl acetate)
EVOH	Poly(ethylene-co-vinyl alcohol)
GC	Gas Chromatography
HDPE	High Density Polyethylene
HPLC	High-performance liquid chromatography
HSP	Hansen Solubility Parameter
LLDPE	Linear Low Density Polyethylene
Log P	Octanol-water Partitioning Coefficient
MEK	Methyl ethyl ketone
MTBX	Mixture of MEK, toluene, butyl acetate and xylene
NAP	Non-Aqueous Phase
NBR	Nitrile Butadiene Rubber
NRTL	Non-Random Two Liquid
OD	Optical density
PAH	Poly Aromatic Hydrocarbons
PC	Partitioning Coefficient
Ro	Interaction radius
SBR	Styrene-Butadiene Rubber
SO	Silicone Oil
T_g	Glass Transition Temperature
TPPB	Two Phase Partitioning Bioreactor
UNIFAC	UNIQUAC Functional-group Activity Coefficients
UNIQUAC	Universal Quasichemical
VOC	Volatile Organic Compounds

Chapter 1

Introduction

1.1 Background

Due to its inherent nature, biological treatment is considered a “greener” alternative in comparison with other remediation platforms, and its main mineralization products of CO_2 and water, can be easily re-accommodated in the environment with minimal harmful effects. Bioremediation is also a cost-effective strategy, as it involves relatively low capital out-lay and most of its processes can take place at ambient conditions of pressure and temperature. Finally, bioremediation allows the complete destruction and not just relocation of the contaminants, as is the case for landfilling and other strategies [1].

Despite all of its benefits one of the main limitations of bioremediation technologies is the microbial inhibition that can arise at sufficiently high concentrations of toxic substrates. Microbial inhibition is detrimental for reaction rates and can even result in the complete termination of microbial activity. As a consequence the search for viable technologies to overcome substrate inhibition has become critical for bioprocess design [2].

The effective removal of volatile organic compounds has also become a challenge for conventional bioremediation strategies especially in aerated activated sludge systems, as most volatile compounds are stripped out the liquid phase into the atmosphere before being consumed by the microorganism; such a situation cannot be described as being real remediation but just a mere transfer of the contaminants [3, 4].

Two Phase Partitioning Bioreactors (TPPBs) have become an attractive technology that has proven to be an effective means to overcome substrate inhibition and volatilization [2, 5].

TPPBs are self-regulating processes in which a hydrophobic Non-Aqueous Phase (NAP) absorbs and progressively rereleases the substrate to a cell containing aqueous phase. This process is primarily governed by the NAP-solute relative thermodynamic affinity and the cell metabolic demand.

Originally, organic solvents were used as the sequestering phase in TPPBs, although more recently they have been replaced by solid polymers. The practical and performance advantages of polymers over organic solvents have been already pointed out in the literature [6], however, despite all their benefits, polymers possess complex structures and, in contrast to immiscible organic liquids their thermodynamic properties are somewhat difficult to predict [7-9].

As a consequence, one of the current challenges facing solid-liquid TPPBs related research is the development of criteria for effective polymer selection. In one recent report polymer absorption was characterized in terms of polymer accessibility, which was correlated to the physical properties of the polymers, and the solute-polymer thermodynamic interaction. Nevertheless, these criteria were developed primarily in a study of extremely hydrophobic polyaromatic hydrocarbons [9]. Thus, it is not yet clear if such criteria can be universally applied to the selection of polymers for a broader range of target solutes.

The current study examined the biodegradation performance and polymer selection of a TPPB system containing two substantially different substrates: phenol, a highly inhibitory compound, and butyl acetate, a volatile ester. It was demonstrated that solid-liquid TPPB systems can effectively be used to overcome substrate volatility and inhibition and that a first principle's approach to polymer selection can be extended to systems with clearly distinct properties.

1.2 Objectives

The first part of this project aimed to demonstrate the superior performance of solid-liquid TPPB with respect to single phase and liquid-liquid TPPB systems in the degradation of mixtures of butyl acetate and phenol. The comparisons were done in terms of the amount of butyl acetate degraded and volatilized and the phenol degradation rates.

The second part of this study aimed to develop a polymer selection criteria based on first principles' for the butyl acetate-phenol system. In order to accomplish this, three different thermodynamic methods were compared: Hildebrand solubility parameter, Hansen solubility parameters and activity coefficients at infinite dilution. The effectiveness of each method was assessed based on its ability to make relative predictions of the polymer-solute uptake capacity for each substrate. The most effective method was then used to identify promising TPPB polymers among commercial and waste materials.

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Chapter 2

Literature review

2.1 Two Phase Partitioning Bioreactors (TPPBs)

TPPBs are characterized by a cell-containing aqueous phase and the addition of an immiscible non-aqueous phase (NAP) that absorbs and subsequently re-releases the substrate to the aqueous phase depending on the cells' biological demand. As soon as the non-aqueous phase is added to the bio-treatment system the target molecule partitions between the two phases until equilibrium conditions are met. Substrate consumption by the cells disrupts this equilibrium resulting in the transfer of substrate from the organic to the aqueous phase. As a result, a continuous controlled substrate supply based on the cells' metabolic requirements is achieved [1]. To date TPPB applications can be generally divided into two main groups; liquid-liquid TPPBs and solid-liquid TPPBs; their main difference relies on the nature of the organic phase used.

2.1.1 Liquid-Liquid TPPBs

These represent the first approach developed for partitioning bioreactors. In these types of reactors an immiscible organic solvent is used as the NAP for the controlled extraction/ release of the target molecule [2].

Solvent selection is carried out by considering a variety of different aspects. The solvent must be biocompatible; biocompatibility is assessed by comparing the microorganisms' critical octanol-water partition coefficient (Log P) with the solvent Log P. A solvent will be biocompatible if its corresponding Log P is larger than the microorganisms' critical value [3]. Effective solvents should also not be bio-available, which means that they should not be consumed by the biocatalyst. Finally the solvent must have high affinity for the target molecule in

order to ensure partitioning. Regarding this last aspect the “Extractant Screening Program” developed at Queen’s University was a UNIFAC based software program that predicted analyte partitioning coefficients, providing a good insight into the system behavior at equilibrium conditions [4].

Liquid-liquid TPPBs have shown considerably advantages compared to single phase reactors for a wide variety of applications [2], nevertheless, it is difficult to meet all of the criteria described above for all cases especially when working with mixed populations of microorganisms. Silicone oil has proven to be one of the most versatile solvents due to its non-bioavailability that enables the use of a wide variety of different microbial strains, although its potential advantages come with a series of drawbacks related to its practical handling, such as high viscosity, foaming, emulsion formation, internal adhesion, high cost, among others [2]. In addition silicon oil is a relatively hydrophobic solvent and possesses negligible affinity for many molecules of interest [5].

2.1.2 Solid-Liquid TPPBs

Solid- liquid TPPBs are characterized by the use of polymers as the sequestering phase. Polymers are, in most cases, biocompatible, non-volatile and easy to separate from the bulk solution. They are also non-biodegradable and considerably cheaper compared to organic solvents [6, 7]. Critically polymers can accommodate a wider range of different functionalities broadening the spectrum of possible applications. Finally they can also be shaped and easily reused. These advantages make polymer based systems a superior configuration.

Polymer based systems are especially useful in biodegradation applications because they allow the use of microbial consortia which enables the degradation of multiple substrates and enhances degradation rates [8]. Despite their benefits, polymers possess smaller surface areas

available for transfer compared to organic solvents simply because polymer beads are usually larger than the solvent droplets that are formed with liquid-liquid TPPBs [9]. Additionally, due to their solid nature, solute diffusion is slower than in liquid based systems. As a consequence polymer absorption requires longer equilibration times in contrast to organic liquids where mass transfer is virtually instantaneous [5].

Rational selection of polymers is challenging because solute-polymer interactions are complex and difficult to predict. Physical properties of polymers must also be considered because they have an impact on the target molecules' diffusion rates. Some criteria for polymer screening have already been developed and applied in previous TPPB applications. Nevertheless, such criteria have been rather empirical and qualitative and may not be successfully applied to more challenging solid-liquid TPPB applications. One of the main objectives of the present work is to develop a more systematic and first principles' approach to polymer selection.

2.2 Early polymer selection for solid-liquid TPPBs

Initial polymer screening was carried out considering potential functional group interactions and some characteristic parameters such as the octanol-water partition coefficient (Log P) and polymer hardness.

2.2.1 Functional group interaction

Functional groups were thought to have a direct impact on material properties and behavior, and it was suggested that certain moieties could enhance polymer-solute interactions resulting in higher uptakes. A relevant example was presented by Prpich *et al*, [1] who suggested that the presence of ester groups in the Hytrel polymer family allowed hydrogen bonding interactions with phenol hydroxyl group, thus enhancing the uptake capacities compared to other

polymer families. Further explanation on the phenol absorption mechanism is given in section 2.6.2.

There is also a general consensus within the literature that polarity plays a crucial role in the absorption mechanism [1, 10-12]. Polar molecules will have stronger interactions with relatively polar polymers, while non-polar molecules will be better absorbed by non-polar polymers. This is in agreement with the fundamental chemical principle of "like seeks like".

Consideration of polymer-molecule interactions in TPPB applications has led to the objective of trying to identify the key mechanism(s) responsible for sorption. This knowledge can then be extended to the area of polymer formulation, as it has been suggested that specific functional groups could be grafted onto polymers in order to achieve the desired interactions for given TPPB applications [10].

2.2.2 Octanol-water coefficient (Log P) and hydrophobicity

The log P is a dimensionless quantity defined as the logarithm of the concentration ratio of a given compound in equal volumes of octanol and water when both phases have reached equilibrium, as shown in Equation 2-1. Thus the Log P can be seen as a measure of the hydrophobicity of a compound

$$LogP_{oct/wat} = Log\left(\frac{[solute]_{octanol}}{[solute]_{water}}\right) \quad 2\text{-}1$$

In the context of solid-liquid TPPBs the octanol-water partition coefficient has been used to explain the affinity of molecules for polymers [8, 12]. Frequently, molecules with high Log P are expected to partition better into polymers.

The Log P until now has been the only quantitative measure utilized to describe the polymer-molecule affinity. Unfortunately, it has been suggested recently that the Log P cannot

be used as a characteristic parameter when the target molecule does not interact with the auxiliary phase in the same way as it does with octanol [13].

2.2.3 Polymer hardness

Polymer hardness has been correlated with the uptake capacity [10]. Gao and Daugulis, studied the uptake of cis-1,3-indandiol, iso-butanol, succinic acid, 3-hydroxy-butyrolactone and 2-phenylethanol for different polymer families and grades. It was found that softer polymer grades had greater uptake capacity than harder grades of the same polymer family. At the same time, the polymer hardness was believed to increase with the crystalline domain proportion within the polymer composition.

The degree of hardness showed negligible effect on the uptake for polymer families that presented supposedly poor affinity to a given target solute, suggesting that no absorption could be possible without a proper group functionality match between polymer and solute. The chemical affinity in this case was assessed by doing a qualitative analysis over the polymers and solutes species and their possible interactions [10]. Polymer hardness was vaguely related in this work to crystallinity, although in-depth analysis on the effect of crystallinity over the uptake was not carried out.

2.3 Rational approach to polymer selection

The rationale described above for polymer selection was applied to some biotransformation and bio-treatment applications [9, 14, 15]. Nevertheless, as mentioned before, it falls short when it comes to more challenging applications such as simultaneous removal and delivery of species or degradation of multiple substrates. Thus, a more systematic and theoretically based methodology was necessary to match a suitable polymer for these rather

complex applications. Recent approaches for polymer selection are described in the following sections.

2.3.1 Degree of Crystallinity

The influence of the crystal domain on polymer absorption has been briefly discussed in early polymer selection studies [1, 10]. Nevertheless, the first rigorous study on the effect of polymer crystallinity in solid-liquid TPPBs was recently performed by Parent *et al*, [13]. Part of this work studied the absorption of different polyaromatic hydrocarbons (PAH) into low density polyethylene (HDPE), 63 % crystallinity, and linear low density polyethylene (LLDPE), 39 % crystallinity. It was found that the polymer/water partitioning coefficients (PC) for all the PAHs absorbed into LLDPE were almost double compared to HDPE. Considering that highly regular crystalline domains are impenetrable even by small molecules, it was understandable that the PC decreased with the percentage of crystalline domain. Thus, it was inferred that only the amorphous part of the polymer took part in absorption. Promising polymers for TPPBs applications must therefore possess only enough crystalline phase in order to provide the required mechanical properties for handling and separations.

It is important to note that the glass transition temperature values for the LLDPE and HDPE used in this study were considerably lower than the operation temperature ($T_g \approx -100$ °C), so contributions to the crystalline domain coming from the amorphous phase were negligible.

2.3.2 Glass transition temperature

The glass transition temperature (T_g) describes the temperature at which a polymer transforms from a rubbery to a glassy brittle state. Promising polymers for TPPB applications must possess T_g values lower than temperature required for its application. Under these conditions their amorphous phase possesses the long-range segmental motion required to allow

relatively fast permeation. In contrast, polymers working above their T_g do not possess enough thermal energy to ensure significant chain mobility [13]. This last aspect has a significant impact on the permeabilities which decrease several orders of magnitude.

Polymer T_g can be also be affected by the water content. Parent *et al* [13], noticed a change in T_g for poly(ethylene-co-vinyl alcohol) (EVOH) samples that were stored at ambient humidity conditions. Moreover, they could not detect any appreciable T_g for samples previously immersed in water for several days. The T_g broadening was attributed to a water plasticization effect. Water can plasticize glassy polymers increasing their chain mobility translating into higher permeability and lower T_g. These two effects combined suggest that the plasticization effect could be desirable for TPPBs purposes, although no formal investigation has been carried out on this topic yet.

2.3.3 Hildebrand solubility parameter and polymer solution theory

Parent *et al* [13], characterized polymer-solute interaction in terms of their Hildebrand solubility parameter distance. Hildebrand *et al* [16], developed the first approach to solute-polymer mutual solubility. The Hildebrand solubility parameter was defined as the energy of vaporization (E_v) divided by the molar volume of the compound (V), as presented in Equation 2-2 [16].

$$\delta = \sqrt{\frac{E_v}{V}} \qquad 2\text{-}2$$

This value also represents the cohesion energy density of a compound. The solubility parameters were extended to polymer solutions through the Flory-Huggins theory. The corresponding free energy expression for this approach is shown in Equation 2-3 [17]

$$\frac{\Delta G_m}{RT} = n_1 \cdot \ln(\Phi_1) + n_2 . \ln(\Phi_2) + \chi \cdot \Phi_1 . \Phi_2 (n_1 + m \cdot n_2 .) \quad 2\text{-}3$$

where ΔG_m is the polymer-solvent free energy of mixing, R is the Universal constant of gases, T is the system temperature, n_1 and n_2 refer to the number of solvent and polymer moles respectively, χ is the Flory interaction parameter, Φ_1 and Φ_2 correspond to the volume fractions of solvent and polymer respectively defined as shown in Equation 2-4 [17]

$$\Phi_1 = \frac{n_1}{n_1 + m \cdot n_2}, \quad \Phi_2 = \frac{m \cdot n_2}{n_1 + m \cdot n_2} \quad 2\text{-}4$$

where m is the ratio of molar volumes of polymer.

Polymer-solvent mixing will be spontaneous if the free energy of mixing is negative. The first two terms in Equation 2-3 correspond to the combinatorial contribution, which is directly related to the entropy of mixing. Since the mixing process tends to favor an increase in entropy these two terms are always negative. The last term in Equation 2-3 corresponds to the residual contribution and accounts for the energetic interactions of the molecules. This last term must be small (if positive) or negative for the mixing process to be spontaneous.

The parameter χ correspond to a free energy parameter and therefore it also comprises two types of contributions as shown in Equation 2-5

$$\chi = \chi_s + \frac{V_1(\delta_1 - \delta_2)^2}{RT} \quad 2\text{-}5$$

where χ_s corresponds to the entropic contribution, V_1 is the solvent molar volume, δ_1 and δ_2 correspond to the solubility parameters of solute and polymer respectively.

Blank and Prausnitz [17] found that values between 0.3 and 0.4 for χ_s provide good agreement with the experimental data for interactions between polar and non-polar molecules and as a result χ_s is frequently set to 0.35. The second term in Equation 2-5 corresponds to the

residual contribution and its magnitude is highly governed by the relative difference between the solubility parameters.

From the set of equations presented above it is possible to note that small differences in solubility parameters minimize the Gibbs energy of mixing, translating into higher polymer-solute thermodynamic affinity.

In the context of TPPBs solubility parameter matching could be used as a new ground-breaking tool to match polymers to a specific applications. As seen in Equation 2-2, the concept of solubility parameters is based on the energy of vaporization which is specific to the chemical structure of the compound. In this sense the solubility parameter can be used to quantify the thermodynamic affinity between solvent and polymer, as opposed to the early polymer selection in which polymer-solute affinity was assessed by a merely qualitative analysis of the possible favorable interactions between polymer and solute moieties.

2.3.4 Hansen solubility parameter (HSP)

Hansen solubility parameters (HSP) have demonstrated to be a useful tool in industry especially for solvent selection for coating-related applications of polymers. HSPs have also been used to correlate the diffusion of molecules into polymers, as pure solvents with HSP values close to the HSP of a given polymer are expected to swell or dissolve it and in low concentrations the diffusion process should be favorable [18].

Unlike the Hildebrand approach, the HSPs accounts for three different forms of interactions; dispersion forces, dipole- dipole interactions and molecular hydrogen bonding; accordingly the overall solubility parameter can be broken down into these three different contributions, as shown in Equation 2-6 [18].

$$\delta = \sqrt{\delta_D^2 + \delta_P^2 + \delta_H^2} \qquad 2\text{-}6$$

where δ_D represents the dispersion solubility parameter, δ_P is the polar solubility parameter and δ_H represents the hydrogen bonding solubility parameter.

Consequently, the solubility parameters can be represented by a three dimensional space where the axis represent the magnitude of each of the mentioned contributions. This representation is known as Hansen space and is particularly useful when considering solvent-polymer interactions. Figure 2-1 depicts a typical plot in the Hansen space [18].

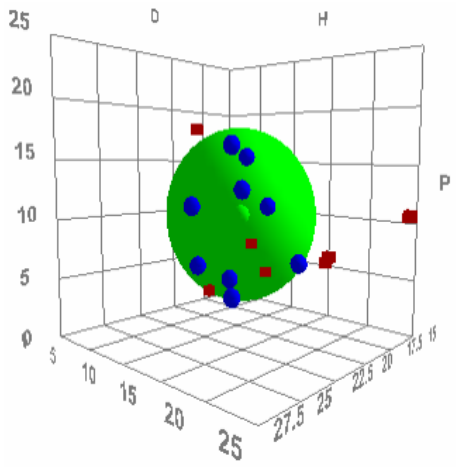


Figure 2-1: **Polymer-solvent interaction in the Hansen space [18]**

When polymers are characterized using the Hansen approach [18], an interaction radius (R_o) is obtained along with the solubility parameters; this fact makes 3-D representations very convenient as shown in Figure 2-1.

Ideally all the solvents located within the sphere will interact to a greater or lesser extent with the polymer, whereas no interaction is expected for those solvents located outside the sphere. Hence the interaction radius can be seen as the "degree of interaction forgiveness" for a polymer.

In the HSP context the parameter "distance" between two different materials has been defined by Hansen and Skaarup as [18]

$$(Ra)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \quad 2\text{-}7$$

where the indexes 1 and 2 correspond to different materials.

When studying polymer-solvent systems the Ra value obtained through Equation 2-7 can be divided by the corresponding polymer Ro in order to know if the solvent lies within the polymer sphere. This parameter is knows as RED number and it is defined as shown in Equation 2-8.

$$RED = \frac{Ra}{Ro} \quad 2\text{-}8$$

RED numbers smaller than one indicate good affinity, while RED equal to one represents a boundary condition corresponding to those solvents located at the edge of the sphere, and RED numbers greater than one indicate poor affinity between the polymer-solvent pair.

Contrary to some coating applications that aim to increase the chemical resistance of the materials, for solid-liquid TPPBs fairly good interaction between solvent and polymer is desired in order to ensure proximity and then diffusion of the species through the polymer. Thus polymers should be screened by considering RED numbers less than one.

2.3.4.1 Solvent characterization

Most of the relevant organic solvents have been characterized and their respective HSP can be found in the literature [18]. Some of the correlations employed to obtain the Hansen values are shown below.

2.3.4.1.1 Dispersion parameter

The dispersion solubility parameter is calculated using a group-contribution method where the cohesion energy of all the contributing functionalities is first calculated and then a

weight average based on the number of significant atoms is taken. For a good simplified calculation, Hansen recommends the use of Barton inspired plots; such plots allow the calculation of the cohesive energy of simple molecules just by knowing the reduced temperature and the molar volume. An example of such plots is shown in Figure 2-2.

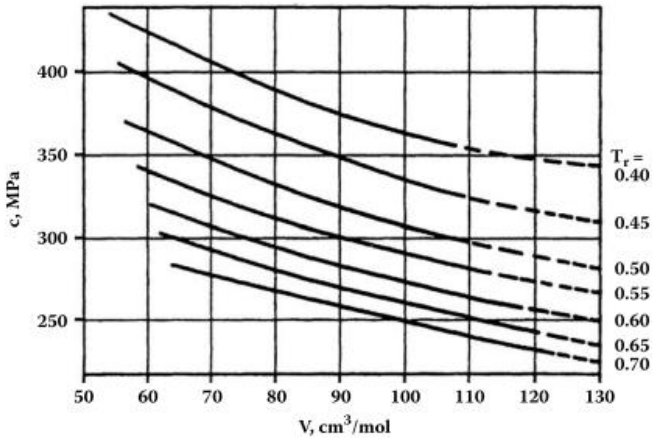


Figure 2-2: **Cohesive energy of cyclo-alkanes as a function of the molar volume and the reduce temperature [18].**

Plots of these types for calculating the contribution of aliphatic and aromatics compounds are also given in [18]. A correlation for the calculation of the critical temperature is also given for cases in which the latter is not known.

Polar parameter

For the polar contribution calculation of a solvent Hansen recommends the use of Equation 2-9

$$\delta_p = \frac{37.4}{V^{1/2}} \cdot DM \qquad 2\text{-}9$$

where DM correspond to the dipole moment of the molecule and V the molar volume. An extensive list of dipolar moments values can be found in [19]. In situations in which the dipolar

moment cannot be found Hansen recommends the use of the Beerbower group contribution method [18].

2.3.4.1.2 Hydrogen bonding parameter

The hydrogen bonding contribution can be found by subtracting the polar and the dispersion energies of vaporization from the total energy of vaporization. Nevertheless, Panayiotou and coworkers have now developed studies based on statistical thermodynamics that allow the calculation of the hydrogen bonding solubility parameter individually [18].

An equation of state approach that can be used to calculate all the aforementioned contributions has been also given by Hansen. This equation was first developed to calculate the hydrogen bonding contribution and was later modified to also fit the other contributions. According to Hansen the approach allows calculation of the solubility parameters for a wide range of temperatures and pressures with relative good accuracy [18].

2.3.4.2 Polymer characterization

In contrast to organic solvents, polymers HSPs listings are not as complete, and this is due to their wide diversity of compositions and properties. Usually polymers must be characterized in each case, although, polymer characterization can be performed without knowing the exact structure of the polymer. This last aspect offers a great improvement over the former approach to polymer selection, especially in cases where the exact polymer structure is unknown due to proprietary reasons.

Group contribution methods have been proposed to carry out polymer characterization [20], although their accuracy is still uncertain. The most reliable method is to evaluate their relative solubility or degree of uptake in a series of well-defined solvents [18]. The degree of swelling as defined by Hansen ranges from 0 to 6 where 0 corresponds to “poor” interaction and

6 to a “good” interaction. These numbers can be then use as entry data in computer software to obtain the corresponding polymer solubility parameter. The computer program, Hansen solubility parameter in Practice (HSPiP) developed by Hansen himself and coworkers, becomes especially useful for this purpose, however similar programs have been developed by other authors as well [21].

The definition of “poor” and “good” interactions is arbitrary and may vary depending on the application; Hansen himself, for instance, uses a very strict criterion where a good interaction is limited to those solvents that dissolve or visibly swell a given polymer. Such a criterion is appropriate for coating and paint applications to which the HSPs were originally intended. However, less strict criteria could be also applied in order to suit a particular application. In the case of solid-liquid TPPBs a high degree of swelling and polymer solvation within the bio-treatment system is actually not desirable because it hinders polymer separation and reuse. Furthermore given the nature of biological systems, the reactions always take place in aqueous solutions possessing rather low organic solvent concentrations, e.g 1-3000 mg/L making solvation highly unlikely. Thus, such strict criteria might not be necessary for these applications. On the contrary, a relative high uptake of the target molecule without considerable polymer modifications could be considered as a “good” polymer-target molecule interaction. It is important to note that HSPs obtained this way correspond to the polymer amorphous phase which, as mentioned previously, is the most likely to participate in the absorption mechanism. Despite its practicality and simplicity, the HSP approach might fall short in TPPB applications because it considers just solute-polymer interaction rather than the actual three components system, water-solute-polymer characteristic of the solid-liquid TPPBs.

2.3.5 Activity coefficients

Activity coefficient estimations can be employed to predict the actual substrate partition coefficient in a system, providing direct insight into polymer effectiveness to absorb a given solute. The main drawback of this approach is the complexity involved in the calculations. Moreover due to the complex structure of the polymers the accuracy of the predictions might also be dubious.

2.3.5.1 Chemical potential

Formally, the chemical potential is defined as the change of energy produced when a certain amount of mass is introduced or extracted from a system at a constant entropy volume, charge etc. The concept is depicted by Equation 2-10 [22, 23].

$$\left(\frac{\partial E}{\partial N}\right)_{S,V,Q\ldots} \qquad 2\text{-}10$$

where E corresponds to the system energy and N to the amount of mass.

In a practical sense the chemical potential is known as the quantity that determines the tendency of diffusion and the rate of change in a reaction. The following discussion will be presented in terms of the diffusion phenomenon, which is more relevant for the case of solid-liquid TPPBs.

The chemical potential is the driving force for mass transport between two interacting phases. Similar to the heat and temperature flow, the rate of transport will increase with the difference of chemical potential between phases, and it will occur spontaneously from high to low chemical potential until both quantities are equal; this last state corresponding to equilibrium. [23].

For relatively low concentration solutions the chemical potential in a given phase "I" can be defined as shown in Equation 2-11

$$\mu^{I}_{solute} = \mu^{\circ I} + RT \cdot \ln(a^{I}_{solute}) \quad 2\text{-}11$$

where $\mu^{\circ I}$ is the chemical potential at the standard state for the corresponding phase *I*, R is the universal constant of gases, T is the system temperature and a^{I} is the activity of the compound in the corresponding phase *I*. The activity can be expressed as a function of the activity coefficients as shown in Equation 12-12

$$a^{I}_{solute} = x^{I}_{solute} \cdot \gamma^{I}_{solute} \quad 2\text{-}12$$

where x^{I}_{solute} is the solute mole fraction in phase *I* and γ^{I}_{solute} is the activity coefficient in the corresponding phase.

As has already been explained, solid-liquid TPPBs are comprised of an aqueous phase in which all the biological activity takes place, and a polymer phase that serves as a reservoir for the controlled released of the substrate (solute). In order to ensure equilibrium, the chemical potential of the substrate must be equal in both the polymer and the aqueous phases.

$$\mu^{poly}_{subs} = \mu^{aq}_{subs} \quad 2\text{-}13$$

It is important to note that thermal and mechanical equilibrium between phases, ($T_{poly} = T_{aq,}\, P_{poly} = P_{aq,}$) are also required in order to achieve global equilibrium. In the case of TPPBs, this condition is easily met due to the nature of biological processes which mostly occur at room conditions.

Finally Equation 2-14 is obtained by combining equations 2-11, 2-12 and 2-13 and making some simplifications

$$\frac{x_{subs}^{poly}}{x_{subs}^{aq}} = \frac{\gamma_{subs}^{aq}}{\gamma_{subs}^{poly}} = PC \qquad 2\text{-}14$$

where x_{solute}^{poly} and x_{solute}^{aq} are the solute mole fraction in the polymer and in the aqueous phase respectively , γ_{solute}^{aq} and γ_{solute}^{poly} are the solute activity coefficients in their corresponding phases.

As shown above, predicting the partition coefficient for a given solute becomes a problem of determining its activity coefficient in each of the equilibrating phases. To date, there have been several different thermodynamics models available to perform such calculations, and some of the most popular are mentioned in the following sections. A similar derivation for the partitioning coefficient based on the fugacity coefficients can be found in [24].

2.3.5.2 Activity coefficient at infinite dilution

As the mole fraction of a solute in solution approaches zero, its activity coefficient in solution approaches a fixed value known as the activity coefficient at infinite dilution [25], as seen in Equation 2-15

$$\gamma_{i\,x\to 0} = \lim_{x\to 0}(\gamma_i) \qquad 2\text{-}15$$

The activity coefficient at infinite dilution provides meaningful insights about the vapor-liquid equilibrium at low concentration regions. It is particularly useful for the design of processes that involve very dilute solutions, such as aroma recovery from food streams or azeotropic distillations [25, 26]. They are also used to developed thermodynamic models and for the evaluations of interaction parameters in vapor-liquid equilibria predicting methods such as UNIFAC and NRTL [27].

In the current work the activity coefficients at infinite dilution can be considered due to the relatively low molar fractions of solute present in both the aqueous and in the polymer phase

(<0.01). These values are used for predicting the solutes partitioning coefficient in polymer-water systems.

2.3.5.3 Polymer solution thermodynamics

There are mainly two types of models for predicting polymer properties in solution, lattice models and van der Waals Models, and hybrid models with mixed characteristics can also be found. Regardless, of the type of model, the molecules' behavior is usually described by two different contributions, the combinatorial and residual terms as shown in Equation 2-16.

$$\ln(a) = \ln(a^C) + \ln(a^R) \qquad 2\text{-}16$$

where a is the activity of the material in solution, is the activity's combinatorial contribution and $\ln(a)^R$ is the activity's residual contribution.

The combinatorial contribution accounts for the shape and size of the molecules and it is dominant at high temperature where the kinetic energy of the molecules is large compared to the interaction between them [24]. In lattice models the combinatorial contribution is calculated from the statistical possible number of arrangements within the lattice; on the other hand for van der Waals models the combinatorial contribution is associated with the molecules free volume term, which is calculated by subtracting the molecules volume from the total volume of the system.

The second contribution is commonly referred to as the enthalpic contribution. This contribution accounts for the molecular interactions which at the same time are responsible for the heat of mixing [24].

Some of the most elaborate models also possess local contribution corrections for the combinatorial terms which account for the non-random distribution that result from the molecular interactions.

2.3.5.4 Flory-Huggins Model

The Flory-Huggins Model has been the cornerstone of the polymer solution theory. It corresponds to a lattice model that accounts for different arrangements and sizes for the polymer and solvent molecules. Initially the activity of a given solvent in a polymer solution was given by

$$\ln(a_1^C) = \ln(1 - \Phi_2) + \left(1 - \frac{1}{m}\right)\Phi_2 \qquad 2\text{-}17$$

where a_1^C corresponded to combinatorial contribution of the solvent activity in solution, Φ_2 and m correspond to the polymers volume fraction and the ratio of molar volumes as described previously in Equation 2-3.

In 1942 Flory noted that this term was inadequate to fully describe the thermodynamic behavior of molecules in polymer solutions; consequently a residual term was added to account for the interactions between the lattice sites.

$$\ln(a_1^R) = \chi \cdot {\Phi_2}^2 \qquad 2\text{-}18$$

where a_1^R is the residual contribution for the solvent activity in solution, χ and Φ_2 correspond to the Flory interaction parameter and the polymer volume fraction as mentioned previously.

The relationship between the solubility parameter and the interaction parameter χ was already explained in Section 2.4.3. Although, one of the deficiencies of this model is that the Hildebrand-Scot solubility parameter used accounts only for the interaction arising from dispersive forces in the definition of the cohesive energy density [24]. This last factor translates into poor results for predictions performed on solutions containing polar and hydrogen bonding compounds. An expression for the Flory interaction parameter (χ) as a function of Hansen Solubility Parameters has been proposed in order to correct for this deficiency [28].

$$\chi = \alpha \frac{v_1}{RT}\left(\left(\delta_{1,d} - \delta_{2,d}\right)^2 + 0.25\left(\delta_{1,p} - \delta_{2,p}\right)^2 + 0.25\left(\delta_{1,hb} - \delta_{2,hb}\right)^2\right) \qquad 2\text{-}19$$

where v_1 is the molar volume of the material 1, R is the universal constant of gases, T correspond to the system's temperature and α is an empirical constant that ranges from 0 to 1.

Hansen showed that a α value of 1 works particularly well for systems where dispersion forces dominate, nevertheless Lindving *et al* [28], demonstrated that the optimal value for α it is not necessarily 1, and that it varies depending on the nature of the system studied.

Several equations of states such as Sanchez-Lacombe, Panayiotou-Vera, Kumar and other important models like UNIQUAC and UNIFAC have been developed based on the lattice model introduced by Flory-Huggins, corroborating its important contribution to the theory of polymer solutions.

2.3.5.5 UNIFAC free volumes models (UNIFAC-FV)

Oishi and Prauztnitz noted that the combinatorial contribution of the UNIFAC models did not explicitly account for the differences in free volume between solvents because in ordinary liquid mixtures distant from critical conditions the components are approximately equally expanded. Nevertheless, in polymer-solute systems since the polymer molecules are more tightly packed than the solvent molecules their difference in free volumes are greater and cannot be neglected [29].

Thus, the addition of a free volume contribution was proposed that could be significant for polymer-solvents systems as seen in Equation 2-20.

$$\ln(a_1) = \ln(a_1)^C + \ln(a_1)^R + \ln(a_1)^{FV} \qquad 2\text{-}20$$

This new free volume term was based on a simplified version of the Flory equation of state. Recently it was noted that the free volume contribution proposed by Oishi and Prautnitz [29] always resulted in a positive correction due to the nature of its empirical parameters which were regressed from data where solvents always possessed greater free volumes than the

polymers. Nevertheless, a negative correction is necessary when the solvents' free volume percentage is smaller than the polymers', being the most critical example polymer-aqueous systems. Kannan *et al*, developed a new free volume contribution term directly derived from the generalized van der Waals partition function. Such term was capable of given the appropriate corrections for such systems [30].

Despite the fact that the solubility parameters and the activity coefficients have been presented as two different approaches, they are both part of the same thermodynamic theory for polymer solutions. As mentioned before, similar solubility parameters are but one relevant condition to ensure affinity. Researchers have used this approach to obtain fairly good approximations without performing complex thermodynamic calculations, which in some cases are not even necessary. Nevertheless, as shown previously the solubility parameters are part of the residual contribution that accounts only for the solute-polymer energetic interactions. Thus, when using the solubility parameters approach other relevant contributions and considerations such as the entropic, free volume, are neglected. For some industrial applications that required detailed calculations, these simplifications are not valid and a complete thermodynamic treatment must be carried out [18].

The main concern about using any solubility parameter approach in TPPBs applications is the presence of water for all applications. As mentioned above the solubility parameter simplification accounts only for solute-polymer interactions and does not consider the respective water interactions. Given the complexity of TPPB systems and the diverse types of possible interactions that water may possess, the solubility parameter "shortcut" could be not enough to make useful predictions.

Furthermore, solubility parameter analysis over water-solute or water-polymer interactions cannot be assessed due to the fact that the solubility parameters for water are not well defined and usually different calculation criteria results in different values [18].

A more rigorous approach might be necessary to effectively select polymers for TPPB applications. As shown in Equation 2-14, PC predictions can be obtained by estimating the respective solute activity coefficient for each phase. This approach requires a more thorough interaction analysis over the complete three component system: water-solute-polymer characteristic of TPPBs.

In the present work, Hildebrand solubility parameter, HSP and activity coefficient methods are compared on their effectiveness to select polymers for specific TPPB applications. As it will be shown in Chapter 4, the methods will be applied to two distinct TPPB substrates, butyl acetate and phenol, in a wide variety of polymer systems. The predictions obtained are contrasted with the experimental PCs.

2.4 Butyl acetate degradation

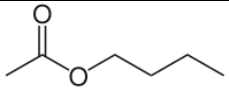
Butyl acetate biodegradation is rarely found in the literature, perhaps due to its relatively low toxicity compare to others contaminants such phenol, xylenes etc, although it is one of the solvents frequently found among industrial waste for the coating industry. Some of its properties are listed in Table 2-1.

One of the few works carried out in this topic was done by Pauss *et al*, in 1999, [31]; in that study a 10 g/L xylene (70%) - butyl acetate (30%) mixture was degraded within 96 hours resulting in an overall degradation rate of 63 mg L^{-1} h^{-1}. The microorganisms used in this work were obtained by means of selective enrichment, with the effective microorganisms being

selected after performing ten subcultures [31]. The degradation was performed in a liquid-liquid TPPB with silicone oil as the sequestering phase.

Some of the observations made by Pauss are relevant for the present discussion given the similarity of the system studied. In the first place, butyl acetate degradation occurred more readily than did xylene. The acetate was completely degraded after 24 hours. On the other hand, butyl acetate degradation was accompanied by a dramatic pH drop, from 7 to 3, that seemed to be detrimental for the growth and therefore the further degradation of the aromatic compounds, pH control was then applied at a value of 6. The results showed an improvement both in rates and cell growth [31].

Table 2-1: Butyl acetate properties

Property	Butyl Acetate
Structure	
Formula	$C_6H_{12}O_2$
CAS number	123-86-4
Molecular weight (g/mol)	116.16
Density (kg/m^3)	880
Octanol-Water Partition Coefficient (Log P)	1.54
Water solubility at 20 ◦C (g/l)	10
LD50 oral, for rats (mg/kg)	10,768
Henry's Constant (H_{cc}, C_{gas}/C_{aq})	$1.14 \cdot 10^{-2}$

Individual degradation for each substrate was also carried out. Xylene degradation occurred with a medium color change which at some point turned yellow. According to Pauss this color has been attributed in previous studies to the formations of semi-aldehydes within the xylene metabolic pathways [31].

Few other studies on butyl acetate degradation have been performed. Kurmar *et al*, [32] isolated the most actives microbes from samples of a biotrickling filter that operated to degrade a mixture of methyl-ethyl ketone (MEK), toluene, butyl acetate and o-Xylene (MTBX). Seven different colonies were obtained. One of them, identified as *Shewanella putrefaciens*, showed the highest degradation capacity for the mixture. Optimal operation ranges of temperature and pH were determined at 25-35 °C and 6-8 respectively. Out of these ranges the degradation performance of the *S. putrefaciens* was severally affected.

Studies of the degradation for each individual compound were also carried out in this work. The results showed that butyl acetate and MEK were degraded more readily than xylene and toluene; 500 mg/L of butyl acetate could be completely degraded in less than 70 hours whereas toluene took more than 100 hours, and in the case of o-xylene just 70 % removal could be achieved. The authors suggested that this was due to the fact that straight carbon chains were easier to degrade than substituted aromatic hydrocarbons [32].

Another important reference regarding butyl acetate degradation corresponds to the work done by Vandenberg in 1988, as a part of the "Bacterial method and composition for degrading hydrocarbons". However this set of procedures are patents of Microlife Techniques and are not readily available.

2.5 Biodegradation of volatile species

Bioremediation of volatile organics, such as butyl acetate, require constant monitoring in order to account for the losses due to volatility [33]. Bioremediation occurs when the major part of the pollutant is removed due to microbial activity and not from stripping.

The treatment of materials by stripping is usually characterized by further treatment of the venting gases [34]. Nonetheless, stripping may also occur spontaneously during aerated

biological treatment process such as activated sludge [35]. Further remediation of the air stripped in these cases is often impractical due to the large dimensions and the nature of these facilities which are usually open to the environment [35], therefore, volatile losses must be avoided or minimized in order to ensure proper treatment of the material and not transfer of the pollutants into the atmosphere.

As discussed previously one of the main features of solid-liquid TPPBs is the decrease in the target molecule apparent concentrations in the aqueous phase. Such decrease translates into benefits regarding the microbial inhibition but may also prove to be advantageous when trying to minimize volatility losses.

For dilute solutions the equilibrium between the amount of solute present in the liquid phase and its corresponding partial vapor pressure is described by the Henry's law as shown below.

$$\mathrm{p} = k_H \cdot C \qquad 2\text{-}21$$

where p is the partial pressure of the component in the gas phase, k_H is the corresponding Henry's constant and C corresponds to the liquid concentration of solute.

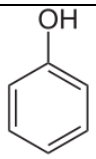
As seen in Equation 2-21, the apparent decrease in concentration in the liquid phase characteristic of the TPPBs translates into lower partial pressures which at the same time results in a reduction in the volatility losses by stripping.

2.6 Phenol degradation

In contrast to butyl acetate, phenol degradation has been extensively studied in a wide variety of systems. Phenol, which is relatively water soluble, is commonly found in sources such as rivers and lakes located near industrial factories or petroleum processing plants [8]. This compound has been categorized as being highly toxic by the Agency of Toxic Substances and

Disease Registry. It possesses a rather low LD_{50} value of 317 mg/kg (for rats) and is able to kill humans in consumptions ranging from 1 to 32 g [8]. Due to these reasons, there is an obvious concern and interest in the bioremediation of phenol contaminated soils and water sources. Phenol properties are listed in Table 2-2.

Table 2-2: Phenol properties table.

Property	Phenol
Structure	
Formula	C_6H_6O
CAS number	108-95-2
Molecular weight (g/mol)	94.11
Density (kg/m^3)	1070
Octanol-Water Partition Coefficient (Log P)	1.46
Water solubility at 20 ◦C (g/l)	83
LD50 oral, for rats (mg/kg)	317
Henry's Constant (H_{cc}, C_{gas}/C_{aq})	$3.11 \cdot 10^{-5}$

2.6.1 Phenol degradation by solid-liquid TPPB

Phenol and substituted phenols have been by far the most common substrates utilized in this research area. In fact, the first report of a solid-liquid TPPB system was on phenol degradation in 2003 by Amsdem *et al*, [14]. In this work a phenol solution of 2000 mg/L was reduce in concentration to sub-inhibitory levels (750 mg/L) using a poly (ethylene-co-vinyl acetate) (EVA). Phenol was completely degraded after 63 h. During the exponential growth phase the medium took on a characteristic yellow color, and it was suggested that this was due to formation of 2-hydroxy muconic semi-aldehide which is known to be a by-product of phenol degradation by the meta-cleavage pathway. It is probable that the intermediate mentioned above in [31] is of a similar nature.

After this work, it became clear that polymers could be successfully used as sequestering phases in order to regulate the concentration of highly inhibitory compounds. To date many other studies with more complex features such, a tailored mixtures of polymers, culture consortia, simultaneous degradation of different substrates, simultaneous substrate-release and product in situ removal (PISR), among others, have been envisioned [9, 36].

2.6.2 Absorption mechanism

As mentioned before, one of the most appealing features of the solid-liquid TPPBs is their potential to use either tailored polymers or mixture of polymers to regulate the species concentration in the aqueous phase. A good understanding of the compound-polymer interaction mechanism is then necessary in order to identify the relevant functional groups that participate in absorption.

Prpich *et al*, have suggested that phenol absorption into the polymer matrix occurs through the formation of hydrogen bonds between the phenol hydroxyl group and electro-donor moieties present in the polymer [37]. This notion is supported by past investigations done on the extraction of aromatics and organic compound by polyurethane foams [38], [39]. L. Shack *et al*, studied and compared extractions with several organic compounds containing OH groups using polyether and polyester foams. The partitioning coefficients for the solutes studied were in most cases considerably greater for polyether foams than for polyesters foams. This was attributed to the higher tendency of polyethers to form hydrogen bonds due to their greater protonation constant. Exceptions to this general tendency were found with compounds that had electro donor moieties in ortho positions relative to the hydroxyl group e.g. o-nitrophenol, o-methoxyphenol. It was suggested that intramolecular hydrogen bonding prevented hydrogen bond formation with the polyurethane foams translating into lower partitioning [40].

Anjaneyulu *et* al [39], studied the absorption of aromatic compounds with increasing numbers of hydroxyl groups into polyurethane foams. It was found that the phenol extractions decreased with the number of hydroxyl groups. It was suggested that greater number of hydroxyl groups per molecule would block more potential active sites for hydrogen bonding within the foam, thus decreasing the total amount of molecules extracted. This last supports the hydrogen bonding mechanism for the extraction of phenolic species into polymers.

Prpich *et al*, also pointed out that the degree of hydrophobicity may play a role in absorption [41]. This is in agreement with the discussion presented in Section 1.2.2 regarding the Log P parameter. Anjaneyulu *et al*, presented data comparing the absorption of phenol and different isomers of dimethyl phenol. The dimethyl phenols isomers (log P $\approx$ 2.7) showed absorption values 50 % greater than phenol's (log P $\approx$ 1.77), supporting the idea that the degree of hydrophobicity has an impact over the polymer-solute affinity.

Identification of a dominant absorption mechanism has a clear impact in the polymer screening process.

2.7 Microbial Consortia

For biosynthesis applications, pure cultures are usually required in order to maximize production rates, but for degradation purposes consortia of mixed populations have proven to be more effective [8], especially when the substrate to be degraded comprises a mixture of different types of compounds. This fact represents a challenge for liquid-liquid TPPB applications due to the existence of very few solvents that would not be preferentially degraded by any of the microorganisms present within a consortium. The use of polymers in TPPBs has enabled the utilization of microbial consortia in biodegradation processes.

It is believed that the wider enzymatic complex possessed by the consortia relative to pure cultures, has a synergistic effect that allows the depletion of toxic inhibitory intermediates produced. Furthermore, one of the practical advantages of microbial consortia is that they do not require rigorous aseptic techniques, such as autoclaving e.g., because any microorganism that could potentially enter into the culture could either enhance the reaction performance or in the worst case just perish.

Comparisons between pure culture and microbial consortia performance on phenol degradation can be found in the scientific literature [42]. Ambujom *et al*, studied the degradation performance of a large bacterial consortium that comprised ten members and compared it with the individual performance of each and different combinations of isolates. The best performance was found when all of the ten isolates were present, being able to degrade 500 mg/L of phenol in a 24 h period, on the other hand the single best isolate was able to degrade only 350 mg/L under the same conditions. The experiments were carried out in 500 ml Erlenmeyer flask filled with 100 ml of medium. It is interesting to note that Ambujom et al, identified two non-phenol degrading bacteria within the consortium. Even though these bacteria were not able to degrade phenol they could stand relative high concentrations (700 mg/L); furthermore they seemed to have a synergic role within the consortium [42].

Consortia and pure cultures have also been compared within the TPPB context. Prpich *et al*, [8] used an enriched consortium to degrade 2000 mg/L in a solid-liquid TPPB using EVA polymer as the sequestering phase. The conditions used in this work were chosen to be similar to the ones used in [14], where a single organism, *Pseudomonas putida* ATCC 11172, was employed. A decreased lag time of 10 hours, a higher specific rate of phenol degradation of 0.71 g phenol g^{-1} cell.h and a higher cell yield was achieved compared to [14].

Prpich *et al*, also noticed that slight growth takes place after phenol is fully depleted suggesting the further degradation of intermediates; this is in accordance with the synergic nature of the consortia [8].

2.8 Degradation of multiple substrates

One of the first concerns when degrading multiple substrates is whether the partitioning coefficient of the individual species will be different in the bulk solutions. Few reports have addressed this issue. Prpich *et al*, showed that phenol partitioning coefficient does not vary considerably when present in a mixture with other aromatics compounds [41], and such a result is relevant because it can support the idea that the polymer uptake mechanism occurs through absorption rather than adsorption. Nevertheless, a similar discussion for the other species present in the mixture studied was not shown in this report [41].

Another interesting phenomenon seen when degrading mixtures is co-metabolism. Co-metabolism occurs in simultaneous degradation when one of the species cannot be degraded in the absence of a primary substrate, which is preferentially degraded by the microorganism. Prpich *et al*, found that cometabolism took place when degrading a phenolic mixture of phenol, o-cresol and 4-chlorophenol (4CP). 4CP, the non-growth substrate, could be degraded only by the consortium used in the presence of phenol [41].

Another common phenomenon is competitive inhibition in which moities degraded by the same enzyme "compete" for the active sites of the latter. In such cases the secondary substrate is degraded after the preferential substrate is almost completely depleted, which results in a characteristic sequential degradation. Vandermeer *et al*, observed this phenomenon when degrading a mixture of low and high molecular weight poly-aromatics [43]. In this study, the degradation of the low molecular weight aromatics naphthalene and phenanthrene, occurred

readily during the first 15 hours; once these compounds were completely depleted the degradation of pyrene started. When the concentration of the latter had considerably decreased benzo[a]pyrene was finally be degraded [43].

It is worth noting that co-metabolism and competitive inhibition can be related effects and be present simultaneously during the degradations as appeared in [41].

2.9 Solid-liquid TPPBs vs other systems

It is already well established that TPPBs perform better than their corresponding single phase counterparts, and there are many examples in the literature in which either degradation or bioproduction rates have been enhanced by the addition of a secondary phase for solute partitioning. Nevertheless, when it comes to deciding what type of TPPB platform (liquid-liquid or solid-liquid), works best, the answer is usually not as straight forward. As mentioned before in Section 1.1.2 solid-liquid configurations provide a series of practical advantages that make them appealing, although the lower absorption/desorption rates compared to liquid-liquid systems, could be important. In fact there are some authors who are skeptical about whether the concentration profiles obtained for these reactors are actually representative of the intrinsic biodegradation reaction kinetics [44, 45]. It has also been noted that liquid-liquid systems can be especially advantageous for applications that require large absorption capacities [8]. Under this situation it is fair to say that for all new application, both types of TPPB configurations should be tried and compared.

In the specific case of phenolic compounds degradation, polymer systems have proven to be superior to other biotreatment strategies. Tomei *et al*, modeled the 4-nitrophenol (4NP) biodegradation kinetics for both cases liquid-liquid and solid-liquid TPPBs, assuming substrate inhibited kinetics [45]. The maximum removal rates estimated in each case were 0.05 mg 4NP

mg VSS^{-1} h^{-1} for liquid-liquid and 0.09 mg 4NP mg VSS^{-1} h^{-1} for solid-liquid, demonstrating the superior performance of the solid-liquid configuration [45].

One of the reasons solid-liquid TPPBs seem to outperform liquid-liquid applications in this case is that it is hard to find adequate organic solvents with good affinity to rather polar compounds such as phenol. Polymers on the other hand possess a larger and more complex structure that can easily accommodate a wider variety of functional groups. This allows polymers to interact with target molecules through many different mechanism (polar interaction, hydrogen bonding etc), increasing their affinity.

An alternative idea to overcome the limitations of organic solvents has been to use ionic liquids (ILs) as auxiliary phases [46]. These solvents comprise salts that are liquid at room temperature; they are non-flammable, possess no measurable vapor pressure and are metal and halogen free. All these features make them a more environmentally friendly options compared to organic solvents. In addition, their ionic nature provides them with a better affinity towards polar molecules.

Despite their potential advantages, ILs tend to be non-biocompatible thus biocompatibility test are required to select the appropriate candidate for a given strain [46]. In addition ILs are considerable more expensive compared to organic solvents especially with respect to polymers. As an example 50 g of trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl) amide, (an ionic liquid successfully used in phenol degradation), can be purchased for $400, (Sigma Aldrich Catalog) whereas rubber tires polymers are free as waste material.

2.10 Tailored polymer mixtures for TPPBs

To date there are just two studies in which tailored mixtures of polymers have been employed. Hernandez *et al*, studied the biodegradation of a volatile organic compounds (VOC) mixture that comprised species with different hydrophobicities. The compounds within the mixture were MEK, (low hydrophobicity), toluene (moderate hydrophobicity), hexane (high hydrophobicity) [36].

Polymers used in this study were selected by considering their corresponding two hours partitioning coefficient, which was thought to be a more realistic estimation for the VOC transfer into solid polymers compared to the equilibrium partitioning coefficient. The polymer selected this way were Hytrel 8206, Recycled Rubber Tires (RRT), Engage 8842 and Engage 8100. The corresponding partitioning coefficients reported in this work are shown in Table 2-3

Table 2-3: Partitioning coefficient for relevant polymers presented in [36]**.**

Polymer	**P.C Hexane**	**P.C Toluene**	**P.C MEK**
Hytrel 8206	99 ± 14	51 ± 3	0.67 ± 0.058
Engage 8100	3885 ± 999	100 ± 3	0.088 ± 0.059
Engage 8842	1442 ± 571	150 ± 8	0.65 ± 0.30
Recycled Rubber tire	3284 ± 559	151 ± 13	0.26 ± 0.19

Two tailored polymer mixtures were assessed for the capacity to improve the rate of degradation compared to the single phase reactor. Mixture A contained 50 % Hytrel 8206, (with good affinity for MEK), and 50 % RRT, (that showed relatively good affinity for both hexane and toluene). Mixture B comprised 33% Hytrel 8206, 33 % Engage 8842 (with affinity for toluene) , 33% Engage 8100 (with affinity for hexane).

Polymer toxicity towards the microbial community was also assessed. It was found that none of the polymers was toxic to the microbial consortium.

The results obtained indicated that the use of the polymer mixtures did not enhance the reactor steady state performance, although the removal efficiency during transient loads was slightly improved with the addition of the corresponding polymer mixture.

Polymer mixtures have been also tried for biosynthesis applications [9]. Morrish *et al,* used a polymer mixture to improve the reactor's performance and operability in the bio-production of carvone from trans-carveol by *Rhodococcus erythropolis*. Initial polymer screening was carried out by considering the equilibrium partitioning coefficient; polymers selected were those with relative high affinity for carvone and trans-carveol. Promising polymers were autoclaved at the same conditions used for the reactor in order to assess their thermal-stability.

The polymers selected were Hytrel 8206, which presented a good partitioning coefficient for carveol isomers and styrene/butadiene rubber (SBR) which showed a good affinity for carvone. The partitioning coefficients obtained in each case are presented in Table 2-4.

Table 2-4: Relevant partitioning coefficients presented in [9]**.**

Polymer	**PC for carvone**	**PC for trans-carveol**
Hytrel 8206	49	36
Styrene-butadiene rubber	118	5

Three different reactor configurations were tried. In the first experiment SBR was used as the only NAP for the in situ removal of carvone. In the second experiment, a mixture of 50% Hytrel and 50% SBR was placed directly in the reaction bulk with the intention to achieve simultaneous removal of cis-carveol isomer and carvone. The polymer mixture did not show a

considerable improvement on the volumetric productivity compared to the 100% SBR system, moreover the total amount of carveol that could be added before the reaction stopped was 40% less. In the third configuration, Hytrel 8206 was packed in an external column and SBR was directly added to the reaction bulk. This last configuration did not show an improvement in the volumetric productivity but increased the amount of carveol that could be added by 40% allowing for a longer reaction time.

The feature of polymer mixtures has not been yet fully exploited for solid-liquid TPPBs probably due to the lack of effective selection rationale. The aim of this work is to broaden the scope of this application by developing a deeper understanding of polymer-solute interactions that govern the absorption phenomena and to apply this knowledge for effective polymer selection for TPPBs.

2.11 References

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Chapter 3

Simultaneous Biodegradation of Volatile and Toxic Contaminant Mixture by Solid-Liquid Two-Phase Partitioning Bioreactors

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3.1 Preface

As noted in Chapter 2 microbial inhibition, and stripping of volatile compounds, are two common problems encountered in the bio-treatment of contaminated wastewaters. Potentially, both problems can be addressed through the addition of a hydrophobic auxiliary phase that can absorb the substrate, thus lowering its initial concentration, and subsequently re-release it based on the cell metabolic demand. Such systems have been described as Two Phase Partitioning Bioreactors (TPPBs). Particularly, solid-liquid TPPBs configurations have proven to be superior mostly due to the great versatility of polymers. Nevertheless polymer based systems have yet to be tested in the degradation of mixtures of clearly distinct substrates.

The current chapter quantitatively compared the performance of solid-liquid TPPBs to common (single phase and liquid-liquid TPPB) bioremediation strategies in the simultaneous degradation of phenol and butyl acetate. An analysis of each degradation profile was carried out, as well as a performance comparison based on the amount of butyl acetate degraded and volatilized, as well as the phenol degradation rates.

3.2 Abstract

The current chapter studied and compared the performance of a solid-liquid TPPB, a liquid-liquid TPPB and a single phase reactor for the simultaneous degradation of butyl acetate (the volatile component) and phenol (the toxic component) at different initial concentration levels. The auxiliary phase used in the solid-liquid TPPB was a 1:1 polymer mixture of styrene-butadiene rubber and Hytrel® 8206, with high affinities for butyl acetate and phenol, respectively. The liquid-liquid TPPB employed silicone oil which has fixed physical properties, and had no capacity to absorb the toxic contaminant (phenol). Butyl acetate degradation was enhanced in both TPPBs relative to the single phase showing an improvement of around 50% in the amount of

butyl acetate degraded although, the solid-liquid TPPB also showed a substantial increase in phenol degradation rate of 53% and 40 % relative to the silicone oil and the single phase systems respectively. The findings of the study demonstrated the superiority of polymer- based systems in the degradation of multiple substrates with different properties.

3.3 Introduction

Phenols are common water pollutants found in the effluents of oil refineries, and the plastics and steel industries. They are also found in suburban and agricultural runoff as they are frequently used as pesticides [1]. Phenol concentrations in natural contaminated sources can range from 20-2000 mg/L, far exceeding the tolerance limit in surface water of 0.5 mg/l [2]. Accordingly, phenol has been designated by the US EPA as a priority pollutant [3].

Bioremediation has become an effective strategy for the treatment of phenol contaminated sources, although one of the main limitations of biotreatment processes is the microbial inhibition that can arise even at moderate concentrations of inhibitory substrates such as phenol. In order to overcome this, challenge traditional biological treatments have often relied on prior chemical treatment or dilution [4].

Other very different types of organic contaminants, volatile organic compounds (VOC), also present a challenge to conventional biotreatment strategies. In some cases the disappearance of target VOCs is often wrongfully attributed to biodegradation when in reality it may be caused by abiotic stripping. In the case of wastewater treatment, air stripping usually takes place in aerated biological treatment processes, such as activated sludge aeration basins [5]. Unfortunately, the transfer of pollutants to the atmosphere is unacceptable despite their apparent "treatment" because even small airborne concentrations of some VOCs have been related to environmental deterioration and public health hazards [6].

Two Phase Partitioning Bioreactors (TPPBs) are an efficient and practical means of biologically treating high concentrations of toxic pollutants while providing sub-inhibitory substrate delivery to microbial populations [4]. TPPBs have also been used to minimize volatility losses during the treatment of soils contaminated with xenobiotics compound [7]. TPPBs consist

of a cell containing aqueous phase and an immiscible sequestering phase, which can preferentially absorb inhibitory target molecules, and then progressively release them based on the microbial metabolic demand. This dynamic process of absorption and release is governed by thermodynamic equilibrium and the relative affinity between the solutes and the auxiliary phase [8]. TPPBs have been operated as liquid-liquid and solid-liquid configurations, mainly differentiated by the nature of the auxiliary phase; the first type utilizes immiscible organic solvents while the second makes use of amorphous polymers. Solid-liquid TPPBs systems have repeatedly proven to outperform their liquid-liquid counterparts due to the versatility and large variety of existing polymers [9-11]. In contrast to organic solvents whose properties are fixed, polymers possess a wide variety of functional groups and can often be formed into chemical mixtures, as co-polymers, or as mixtures of individual polymers [11-13]. These features translate into a greater number of possible interactions with many different solutes, thus broadening the scope of possible applications [14].

TPPBs have been used to treat phenol-containing streams and also streams containing mixtures of substituted phenolics [15]; however, these target contaminants possess very similar chemical structures as well as closely-related physical/chemical and toxicological characteristics. In actual wastewaters, it can be expected that the contaminant mixtures would vary widely in these properties, thus posing challenges in designing single, effective treatment systems. The main objective of the present work was to assess and compare the performance of solid-liquid TPPBs to single phase and organic-aqueous platforms in the treatment of aqueous mixtures of phenol and butyl acetate, two contaminants of widely different toxicity and volatility. As will be explained later, these substrates also pose two different problems during the bio-treatment:

microbial inhibition at moderate phenol concentrations and volatility losses of butyl acetate. It will be shown how these two relevant issues can be addressed by the use of solid-liquid TPPBs.

3.4 Materials and methods

3.4.1 Chemicals and polymers

All medium components were purchased from Fisher Scientific (Guelph, Canada). Silicone oil (poly(dimethylsiloxane)) with a viscosity of 5cSt and density of 0.98 g/ml, phenol (99%) and butyl acetate (>99%) were obtained from Sigma-Aldrich. Hytrel® 8206 and 3548 were supplied by DuPont (Kingston, Ontario, Canada), the various grades of Nylon were supplied by DuPont (Kingston, Ontario, Canada), Pebax® 1657 was obtained from Arkema (Burlington, Ontario, Canada), and the remaining polymers were purchased from Scientific Polymer (Ontario, New York, U.S.A). Important butyl acetate and phenol properties were already presented in Tables 2-1 and 2-2, polymer properties are shown in Table 3-1.

3.4.2 Selective enrichment

A microbial consortium to degrade phenol and butyl acetate was obtained via selective enrichment. Initial seeds included contaminated soil from tar sands deposits in Alberta, Canada and a microbial consortium previously used for phenol degradation [16]. The medium consisted of 2 g/l $(NH_4)_2SO_4$, 0.75 g/l $MgSO_4 \cdot 7H_2O$, 2 g/l K_2HPO_4, 2g/l KH_2PO_4, 0.1 g/l yeast extract, 1ml/l trace element solution, prepared as referred in [17], in tap water. Three grams of soil and 1 ml of microbial consortium were placed in a shake flask containing 50 ml of medium with concentrations of 400 mg/l, increasing to 700 mg/l, of phenol or butyl acetate. Aliquots of 5ml of the microbial population obtained were transferred to subsequent flasks containing fresh medium and different substrates concentrations. This last process was repeated for approximately one

month until the consortium proved to consistently degrade both substrates. Growth in serum bottles was undertaken towards the end of the enrichment to ensure that the disappearance of butyl acetate was due to microbial activity and not abiotic losses (Appendix C).

Table 3-1: **Polymer basic properties and partitioning coefficients**

Commercial name	Chemical name	Glass transition temperature (T_g, ◦C)	Density (g/ml)	Butyl acetate PCs ($g/kg_{poly}/g/kg_{aq}$)	Phenol PCs ($g/kg_{poly}/g/kg_{aq}$)
Hytrel 8206	Polyether-ester copolymer	-59	1.17	9.2 ± 0.4	37.40 ± 0.6
Pebax 1657	Polyether-amide copolymer	-40	1.14	3.8 ± 1.8	13.0 ± 0.4
Poly(ethylene succinate)		-1	1.08	1.1 ± 0.7	2.9 ± 0.4
Poly(ethylene adipate)		0	1.18	7.3 ± 0.4	10.9 ± 0.3
Polycaprolactone		-60	1.15	8.2 ± 1.0	9.8 ± 0.1
Poly(1,4-butylene adipate)		-68	1.02	8.1 ± 0.3	12.0 ± 0.40
Poly(ethylene alcohol)		72	1.2	0.0 ± 0.1	0.3 ± 0.1
Nylon-6	Polyamide	47	1.13	0.2 ± 0.2	2.2 ± 0.11
Nylon-12	Polyamide	41	1.02	0.7 ± 0.4	1.7 ± 0.3
Styrene-butadiene rubber		- 60	0.91	34.0 ± 1.7	0.8 ± 0.3
Silicone oil	Poly(dimethyl siloxane)	NA	0.98	27[a]	0[a]

[a] Experimental values obtained from [18]

3.4.3 Partitioning coefficients

All partitioning coefficient (PC) experiments were performed in quadruplicate with four different polymer masses which usually ranged from 1 to 4 g. The desired polymer mass was placed in 20 ml scintillation vials, following the addition of 18 ml of a solution containing known concentrations of both butyl acetate and phenol. The vials were sealed and shaken at 30ºC and 180 rpm overnight to ensure equilibrium. Final aqueous concentrations were measured for both

analytes and the PC values were obtained through mass balances. For the sake of consistency all PC experiments were performed using ultra-pure water using a Mili-Q plus system.

3.4.4 Abiotic volatility tests

Three liters of tap water were added to a 5-L New Brunswick Scientific BioFlo II bioreactor along with phenol or butyl acetate, with aeration and agitation at 1 l/min and 400 rpm respectively. Samples were periodically taken and analyzed. Volatility tests in single phase was performed for both phenol and butyl acetate. Butyl acetate volatility was additionally tested in a system containing 250 g of butadiene-styrene copolymer.

3.4.5 Toxicity tests

An initial seed was grown for 24 hours in a shake flask containing 50 ml of medium with 500 mg/l phenol and butyl acetate. Subsequently, 5 ml were transferred to six other flasks containing 70 ml of media with different initial concentrations of phenol or butyl acetate. Substrate concentrations and cell growth were monitored to evaluate the impact of the initial substrate concentration on biodegradation activity.

3.4.6 Biodegradation tests

Inoculum was prepared in 50 ml of medium and 500 mg/l phenol and butyl acetate in 125 ml flasks. After incubating at 30 ºC and 180 rpm for 24 hours, 30 ml of the resulting of broth were transferred and equally divided into 6 flasks containing 50 ml of fresh medium and 500mg/l of phenol and butyl acetate. The flasks were incubated for an additional 24 hours, and used as bioreactor inocula.

Biodegradations tests were undertaken in sequencing batch mode. For the initial cycle, 2.67 liters of fresh medium were placed in a sterile 5-l New Brunswick Scientic BioFlo III with

500 mg/l of phenol and butyl acetate, followed by inoculation. The pH was controlled at 6.9 using a KOH solution of 3M , the temperature was maintained at 30 ºC. The dissolved Oxygen (DO) calibration was carried out prior to the addition of microorganisms or secondary phases for the sake of consistency. For the purpose of the calibration the agitation and aeration were set to 500 rpm and 4 l/min respectively, and the DO reading at these conditions was arbitrarily set to 100%. The DO profile was qualitative registered throughout the whole reaction as a percentage of the initial (maximum) calibration point. Agitation and aeration during the reaction were maintained at 400 rpm and 1 l/min unless the dissolved oxygen (DO) percentage decreased to a value less than 40 %, in which case both were increased to 500 rpm and 4 l/min.

A typical operation cycle lasted 22 hours divided as follows: fill phase 1 minute, reaction phase approximately 10 hours, idle phase 12 hours, decant phase 5 minutes. Prior the start of a new cycle, broth was pumped from the reactor quickly in order to avoid biomass settling, leaving 600 ml of broth, equivalent to an exchange factor of 80% v/v. The reactor was subsequently filled with 2400 ml of fresh medium with the various phenol and butyl acetate concentrations. The reaction proceeded with the inoculum obtained from the previous cycle.

For TPPB operation, the sequestering phases were added immediately after removing the broth from the reactor, prior to the addition of fresh medium. In each case, 150 g of the selected auxiliary phase was added (silicone oil or polymer). For the polymer experiment, a 1:1 w/w mixture of styrene-butadiene rubber and Hytrel® 8206 was utilized.

3.4.7 Analytical procedures

Reactor samples were centrifuged for 5 min at 16000 G and 5ºC to avoid volatilization losses. Samples were filtered through 2ml vials using teflon syringe filters. In the case of silicone

oil containing samples, silicone oil supernatant was removed by aspiration to avoid damage to chromatography equipment.

Phenol and butyl acetate concentrations were measured using a Varian Pro Star HPLC with UV/VIS detection, with dual wavelength scans performed to measure the concentration of both analytes simultaneously (butyl acetate: 190 nm, phenol: 260 nm). A mixture of 1:1 v/v water/acetonitrile was used as the mobile phase at 1ml/min and a total running time of 7 min.

Butyl acetate headspace concentration was measured using a Varian CP 3800 Gas Chromatograph (GC) equipped with a 30 m WCOT fused silica coated capillarity column (Model CP 8771), and FID detector. The carrier gas was helium at 1.5 ml/min. The method was: injector and detector temperatures at 250ºC, initial oven temperature at 30ºC and a temperature program of hold for 2 min followed by a ramp of 30 ºC/min until reaching the final temperature of 200ºC, a split ratio of 10 was used.100µl samples were taken from the reactor headspace using a Hamilton gas-tight syringe and manually injected. Occasionally gas phase butyl acetate concentrations were determined from aqueous phase concentrations, using Henry's law constant, and this method was validated in separate experiments (Appendix B).

Cell concentration was determined by optical density using a UV/VIS spectrophotometer at 600 nm, and converted to dry cell weight concentrations by means of a calibration curve.

3.5 Results and discussion

3.5.1 Polymer Selection

Polymer selection was based on the PCs for both substrates, which are shown in Table 3-1 and are a measure of the polymer absorption capacity for a given solute. Prospective polymers must possess moderate PC values to ensure effective performance in TPPBs; that is, polymers with low PCs do not provide enough uptake to significantly decrease the substrate aqueous

concentration, while too high PCs may not re-release sufficient substrate to respond to the microbial demand [15]. PCs in the range of 20 to 50 have proven to perform well in solid-liquid TPPB applications [3, 11, 19]. Table 3-1 shows that the only two polymers meeting this criterion were Hytrel® 8206 with a phenol PC of 37.4, and styrene-butadiene copolymer with a butyl acetate PC of 34. These polymers possess low T_g values and a relative large portion of soft active segment available for substrate absorption, 95 % in the case of styrene-butadiene rubber and more than 50 % for Hytrel® 8206, properties that favor higher substrate uptake capacities [20].

A polymer mixture was therefore used in the solid-liquid TPPBs in order to target both substrates simultaneously. Interestingly, the same polymer mixture has been used previously to improve the performance in the bioproduction of important aromas [11] showing the versatility and adaptability of polymer based systems, particularly as mixtures. Silicone oil (SO) is overwhelmingly the most common organic solvent used in liquid-liquid TPPBs [21]. Previous work has shown that SO provides good affinity for butyl acetate with a PC of 27, nevertheless its phenol partitioning was shown to be literally negligible [18]. Clearly, silicone oil, unlike polymers selected from the many tens of thousands available, will be limited to a small number of TPPB applications. A liquid-liquid TPPB experiment was also carried out using silicone oil as the auxiliary phase in order to compare its performance with its solid-liquid counterpart.

3.5.2 Substrates volatility

A previous study attempted to overcome volatility problems by performing biodegradation experiments in leak-free sealed systems [22]. This approach, however, has little resemblance to real industrial conditions given that most wastewater treatments processes take place in large aeration tanks completely open to the environment and are subject to high levels of aeration and mixing [5]. In the current work, experiments were performed to assess the volatility

of each of the substrates via abiotic losses. Figure 3-1 shows the time course for butyl acetate and phenol, and in all cases the decrease in the aqueous concentration was attributed to volatility losses and/or polymer absorption.

As seen in Figure 3-1 phenol showed virtually no losses due to volatility even at the aggressive conditions of aeration and agitation employed. In contrast, the butyl acetate concentration decreased rapidly in both the single phase and the polymer systems. The experiment with polymers showed a more pronounced decrease at the beginning of the test compared to the single phase system, due to the combined action of polymer absorption and volatilization. Butyl acetate volatility losses were reduced by 30% with the use of polymers. Based on thermodynamic principles, a decrease in the aqueous concentration, in this case due to polymer sorption, will translate into a corresponding reduction in the amount of substrate lost to volatilization [23].

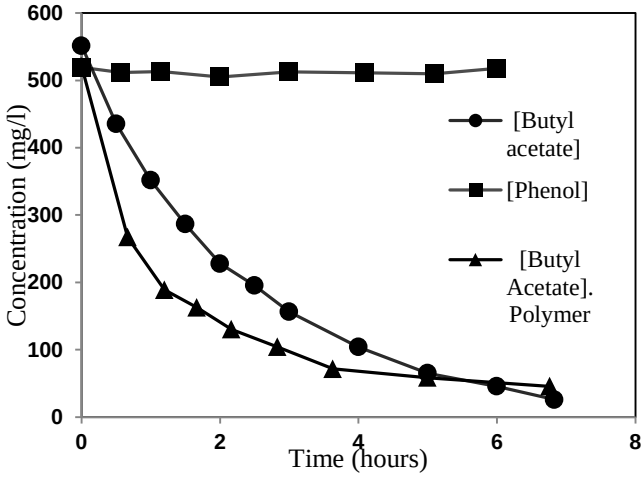

Figure 3-1: Time course of phenol and butyl acetate in different volatility experiments.

3.5.3 Substrate toxicity

Previous research has shown that inhibition thresholds are highly variable, and dependent on the biomass used (e.g. pure strains or consortia) and on the type of contact (free cells, immobilized cells, biofilms, etc.) [21, 24]. A toxicity test was therefore performed to determine the effect of the initial substrate concentrations on the growth and activity of the microbial consortium employed here. The toxicity tests were done independently for each substrate. Figure 3-2a shows the butyl acetate and cell concentrations at three different initial substrate levels. Butyl acetate disappearance occurred readily, and such disappearance is likely due to simultaneous degradation and volatilization, as noted previously.

At best, inhibition caused by butyl acetate was observed only at the highest initial concentration studied, 2420 mg/l; even at this initial concentration cell growth was reduced only slightly compared to the other two lower concentration cases, suggesting minimal inhibition by butyl acetate. To support this observation initial specific rates for the first 5.5 hours of reaction were calculated at the initial concentration of 800,1640 and 2430 mg/l and were estimated to be 940, 1500, 1440 mg butyl acetate/mg cell.h, respectively. Cell growth at the lowest initial butyl acetate concentration continued even after the complete disappearance of the acetate, presumably due to the further degradation of intermediates and metabolites.

Phenol disappearance was significantly slower, as seen in the concentration profiles in Figure 3-2b, and was attributed entirely to biodegradation. Cell inhibition was clearer in this case, being present even at moderate concentrations, as seen by the longer degradation times. Initial specific rates for phenol at the initial concentrations of 500, 700, 950 mg/l were estimated as 67.3, 54.7 and 35.0 mg phenol/mg cell.h, respectively.

In contrast to butyl acetate, phenol initial specific rates presented a monotonic decrease with increasing initial substrate concentrations. Inhibition effects are especially noticeable at 950 mg/l at which concentration the initial specific rate is about half that for 500 mg/l.

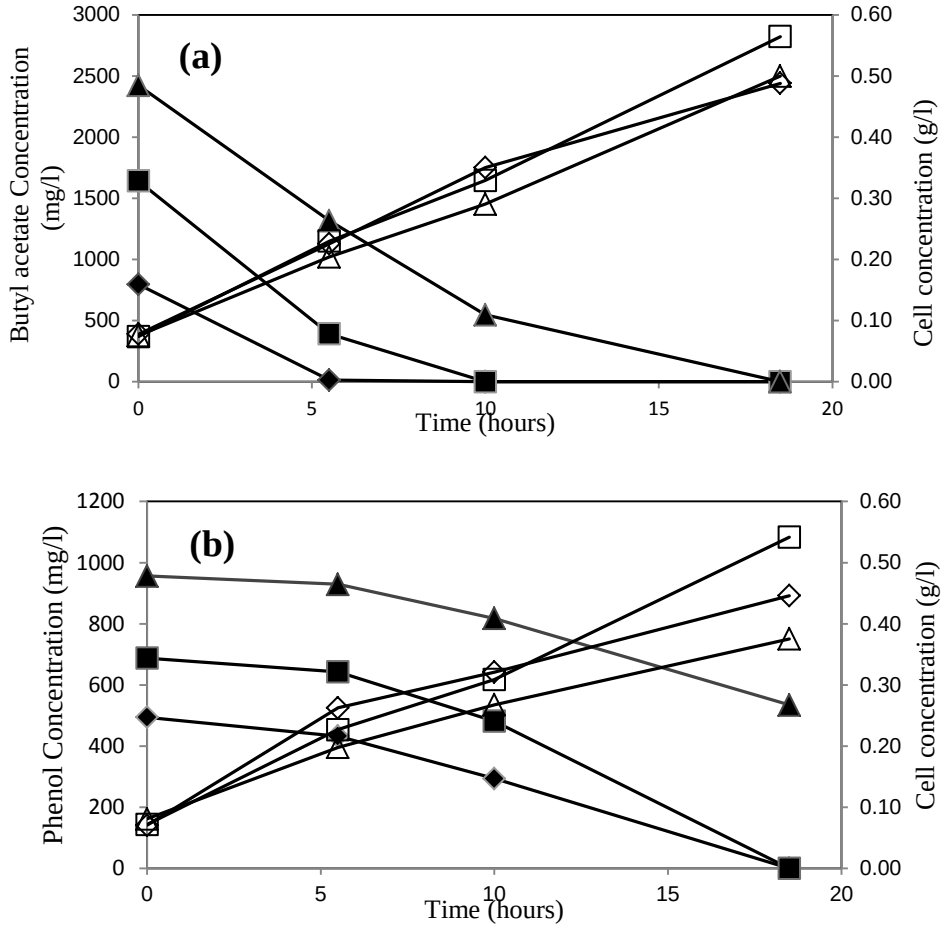

Figure 3-2: **(a) Butyl acetate time course (solid symbols) and cell concentration profile (open symbols) ▲-initial concentration 2420 mg/l. ■- initial concentration 1640 mg/l. ♦- initial concentration 800 mg/l. (b) Phenol time course (solid symbols) cell concentration profile (open symbols) ▲-initial concentration 960 mg/l. ■- initial concentration 690 mg/l. ♦- initial concentration 500 mg/l.**

These results reflect the nature of the 2 substrates selected for this work, butyl acetate and phenol, whose principal properties (in terms of this study) were high volatility and minimal toxicity for butyl acetate, and low volatility and high toxicity for phenol.

3.5.4 Single phase degradation

Sequencing Batch Reactor (SBR) mode was used in this work, as it has been shown to be effective in treating phenolic contaminants [25]. To demonstrate the reproducibility of SBR operation, single phase degradation was repeated for four consecutive cycles with an initial concentration of 500 mg/l of both substrates. Cycle 1 was not considered to be characteristic of subsequent cycles because the inoculum conditions were different. Figure 3-3 shows the time course, cell concentration profile and Dissolved Oxygen (DO) trace of a typical single phase cycle.

As seen previously butyl acetate disappearance occurs readily at the beginning of the reaction due to cell consumption and volatility; the gradual decrease in the DO profile and the increase in biomass at the beginning of the reaction are evidence of butyl acetate degradation. In contrast, phenol degradation is slower and begins near the end of butyl acetate disappearance. This type of sequential degradation has been observed previously in systems containing multiple substrates such as acetates and phenolic compounds [26, 27], and strongly suggests the presence of diauxic growth.

The DO profile shows a steep increase near the last hour of reaction which coincides with the depletion of phenol. It is important to remark that the wide band observed for the DO profile is presumably due to noise in the data acquisition system. Single phase characterization is presented in Table 3-2. The percentages of butyl acetate degraded and volatilized presented in Table 3-2 do not add up to 100% because the complete mass balance also considered losses of

butyl acetate due to sampling, and such losses were not ascribed to either degradation or volatilization in Table 3-2. The total reaction time coincided with complete phenol degradation.

Single phase experiments were also conducted at 1000 mg/l of butyl acetate and phenol in order to test the system's limitations, and the performance is also presented in Figure 3-3. The trends are consistent with the experimental results found at 500 mg/l, and are characterized by an immediate decrease in butyl acetate concentration followed by phenol degradation only after the complete depletion of butyl acetate. Sampling was stopped after 11.5 hours, and the phenol profile was therefore not entirely determined. Nevertheless, it is clear from the data obtained at 500 mg/l, that the DO trace is a reliable representation of the microbial activity for the single phase system. The DO trace at 1000 mg/l showed a steep increase in the DO around the twentieth hour of reaction characteristic of the end of microbial activity and the depletion of the substrate. Figure 3-3a and b show the strong inhibitory effect of phenol at higher concentrations. At 1000 mg/l phenol starts being consumed only after the eighth hour of reaction and, additionally, cell growth is clearly hampered compared to the lower concentration experiment.

Table 3-2: Single phase reactor performance parameters

Performance comparison	Cycle 2	Cycle 3	Cycle 4	Average values
Total reaction time (h)	10.3	9.0	10.5	9.9 ± 0.7
Butyl acetate rate of disappearance (mg/l.h)	101.6	106.8	106.8	105.1 ± 2.5
Phenol rate of degradation (mg/l.h)	47.8	56.3	48.2	50.8 ± 3.9
Total mass of butyl acetate volatilized (mg)	787.1	841.2	817.6	815.3 ± 22.2
Total mass of butyl acetate volatilized (%)	52.5	56.1	54.5	54.4 ± 1.5
Total mass of butyl acetate degraded (mg)	673.3	616.8	642.2	644.1 ± 23.1
Total mass of butyl acetate degraded (%)	44.9	41.1	42.8	42.9 ± 1.5

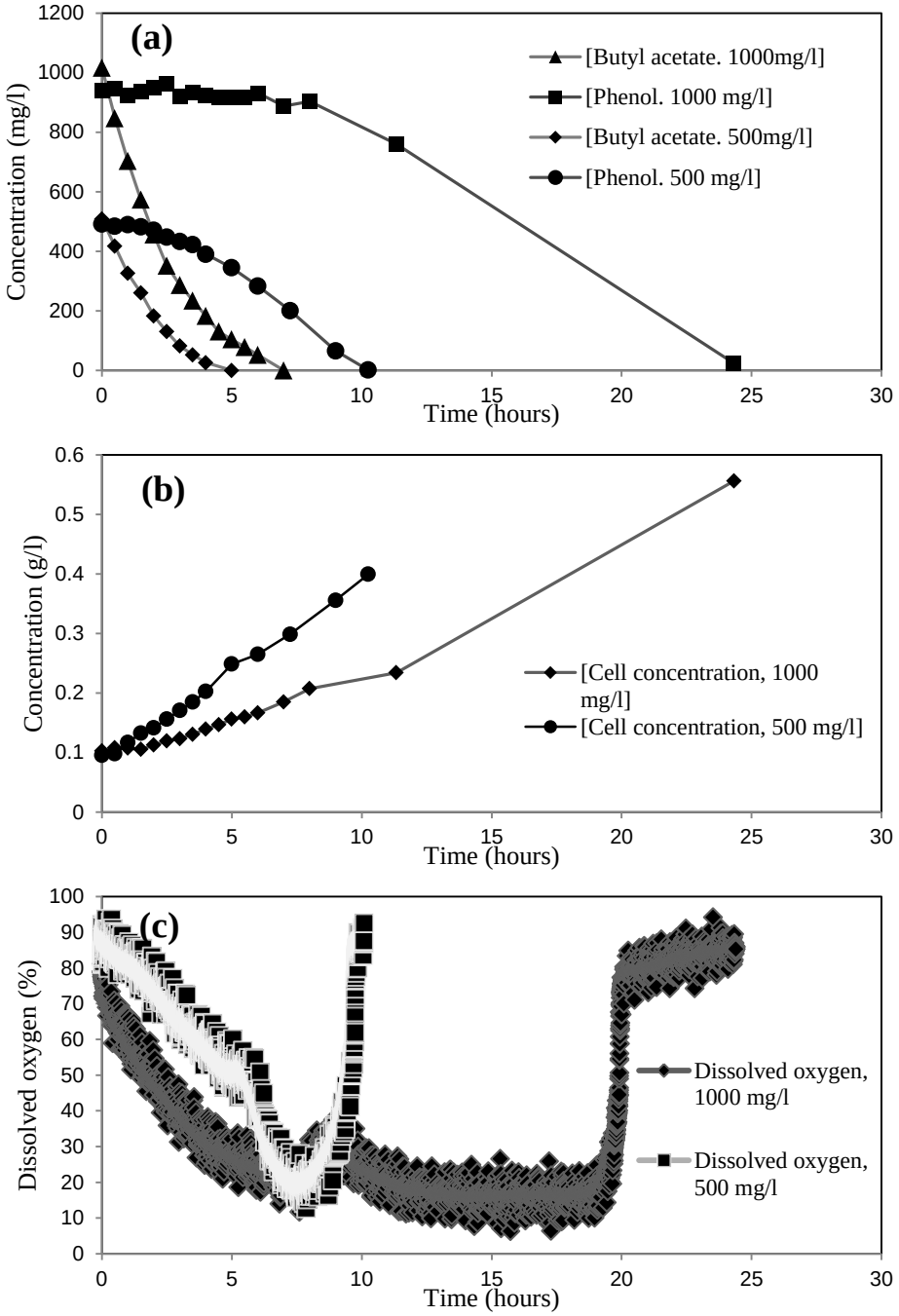

Figure 3-3: **Single phase biodegradation at 500 and 1000 mg/l. (a) Time course (b) Cell concentration profile (c) DO trace**

3.5.5 Two phase degradation: Liquid-Liquid

Silicone oil was chosen as the auxiliary phase due to its overwhelming popularity in liquid-liquid TPPB applications. This organic solvent is non-toxic and non-bioavailable for a wide variety of organism, thus making it suitable for many applications including those using microbial consortia. Nevertheless, in contrast to polymers, SO properties are fixed and its thermodynamic affinity to many target molecules is exceedingly low, in addition to possessing a number of practical problems related to its handling [28]. Lastly, SO is considerably more expensive than polymers with prices of up to 386 $/l, (Sigma-Aldrich catalog price), compared to about $ 6/kg for polymers [21]. Some authors have claimed to have found lower cost suppliers of SO [29] but until biodegradation experiments are published showing equivalent performance using such low-cost materials, the relative price differences remain as stated above.

Figure 3-4 shows the biodegradation using SO. Butyl acetate concentration decreased immediately at the beginning of the reaction from 500 to 208 mg/l due to its immediate absorption into the SO. This is in agreement with the observations in [18] where it was also shown that the partitioning of butyl acetate into silicone oil occurs instantaneously. A PC of 27.9 was calculated assuming an initial concentration of 500 mg/l based on the amount of butyl acetate added and the reactor volume. On the other hand initial phenol concentrations remained near the original value of 500 mg/l given the poor affinity of SO for this substrate, PC $\approx$ 0.

The butyl acetate time course had some differences compared to the single phase experiment, showing a straight line trend, with complete degradation being delayed by one hour compared to the single phase system. These changes in the concentration profile have been ascribed in previous studies to the added system complexity arising from the absorption-desorption phenomenon of the substrate into the auxiliary phase [15]. The concentration profile of

phenol remained almost unchanged compared to the single phase case, and this was expected given the negligible affinity between phenol and SO. The cell concentration profile could not be successfully tracked, as the presence of the SO promoted emulsion formation which strongly affected the optical density measurements.

The DO profile remained almost constant during the entire reaction, contrary to the single phase experiment. This changed profile can be attributed to an enhanced oxygen transfer capacity provided by the addition of SO. Previous research on the transfer of hydrophobic substrates in TPPBs systems, has shown that the addition of a non-aqueous phase enhances the gas/water transport vector by means of reducing the surface tension and hence air droplets coalescence. The latter allows a greater gas/water interfacial area that translates into an enhanced oxygen transfer capacity. Bubble size distribution and overall gas holdup data presented in some of these studies seem to support this argument, although the interaction between the auxiliary hydrophobic phase and air and the exact transfer mechanism are not yet fully understood [30-32].

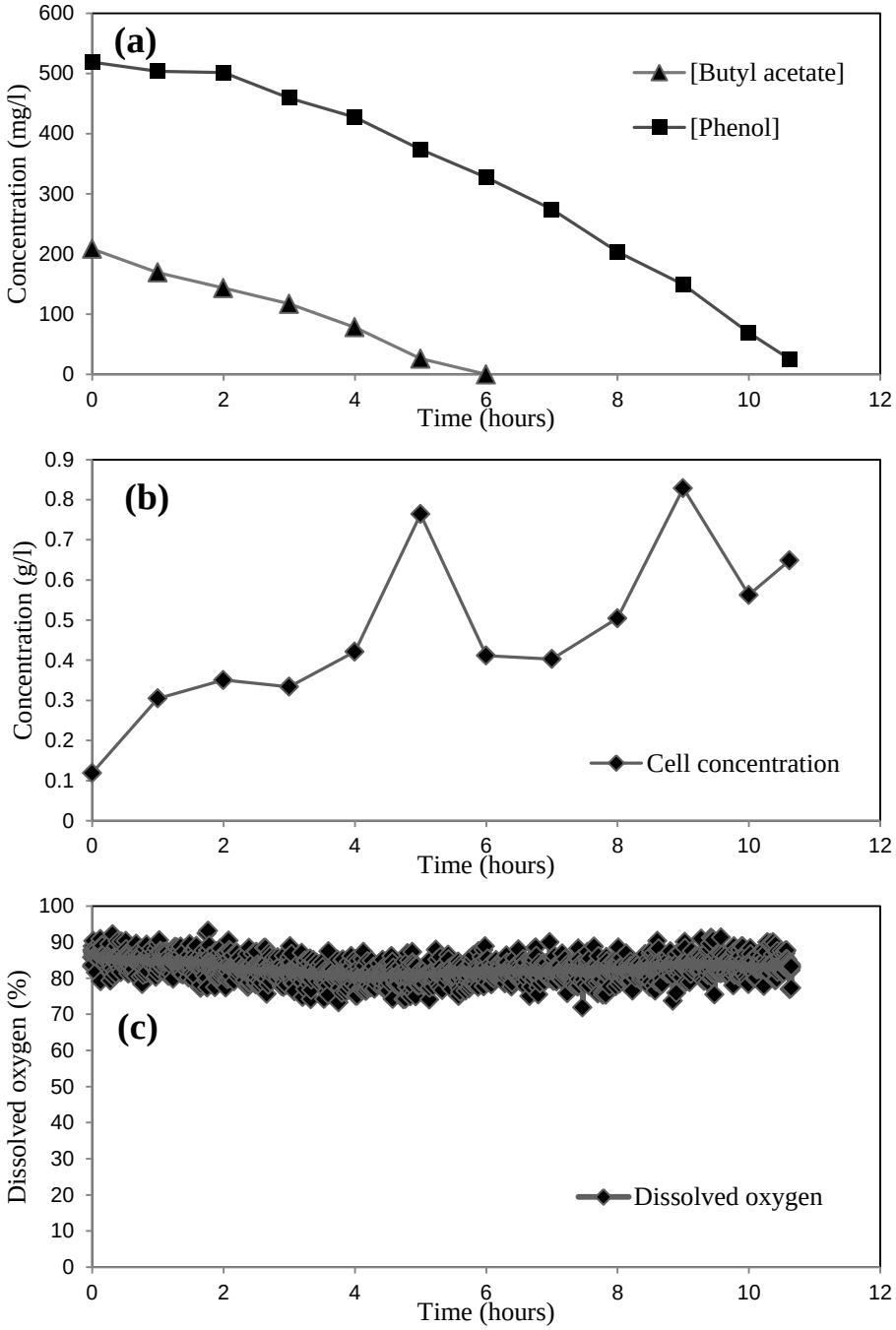

Figure 3-4: **Liquid-liquid TPPB using silicone oil as the sequestering phase. (a) Time course. (b) Cell concentration profile (c) DO trace**

3.5.6 Two phase degradation: Solid-liquid TPPB

Figure 3-5a and b show the time course and the cell concentration profiles for the degradation of phenol and butyl acetate at 500 and 1000 mg/l. In the experiment carried out at initial concentrations of 500 mg/l, the butyl acetate concentration decreased at the beginning of the reaction due to the combined effect of three phenomena: polymer absorption, volatilization and degradation. In contrast to the SO experiment, the absorption of butyl acetate into the polymers did not occur instantaneously, as the concentration decreased sharply during the first 30 minutes and then adopted a decreasing linear trend similar to the SO experiment. The phenol concentration showed a gradual decrease at the beginning of the reaction attributed to its absorption into the Hytrel® 8206 beads. After the complete disappearance of the acetate at 4.5 hours the phenol concentration began to decrease more sharply until complete depletion at 7 h. The total reaction time was considerably less compared to the previous systems confirming that the partial phenol absorption greatly enhanced the microbial activity through reduction in inhibition. The cell concentration profile again suggested the presence of diauxic growth.

In the experiment carried out at initial concentrations of 1000 mg/l the butyl acetate concentration decreased until its complete depletion at 4.5 hours, while the phenol time course showed a gradual decrease at the beginning due to polymer absorption and a sharper decrease at 6.5 hours until its complete depletion at around 15 hours.

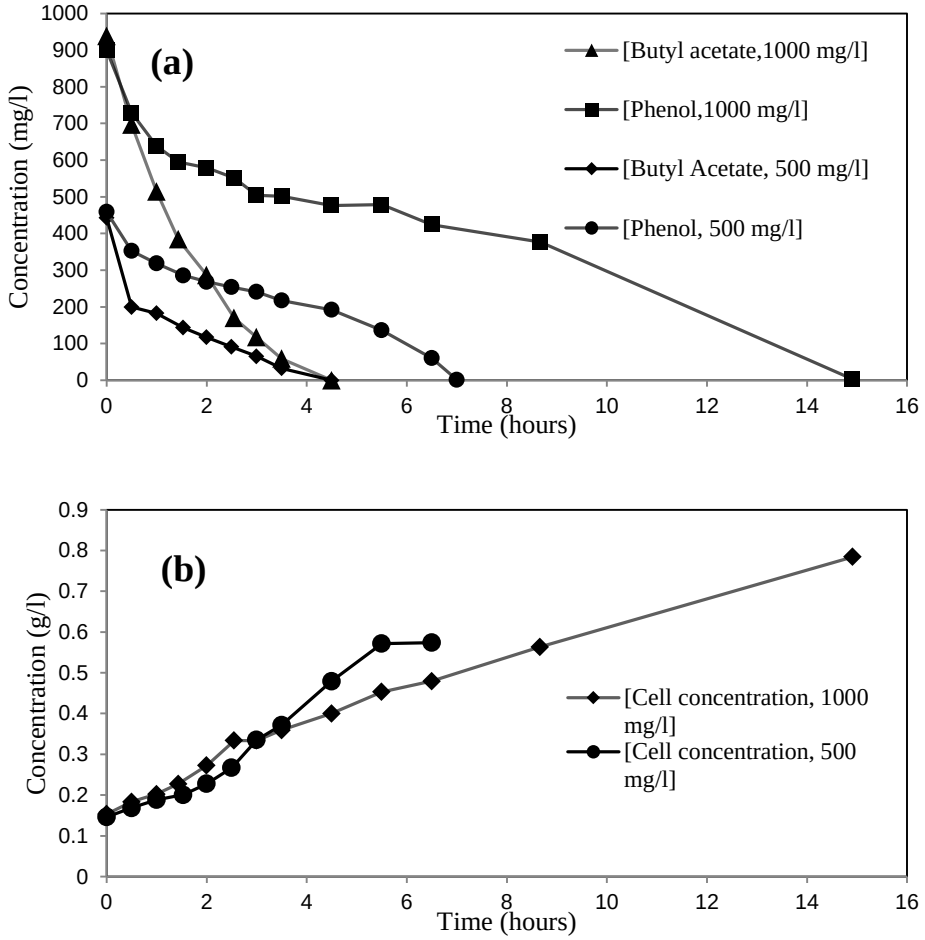

Figure 3-5: Solid-liquid TPPB using a polymer mixture of 50:50 SBR and Hytrel® 8206 at 500 and 1000 mg/l. (a) Constituents time course (b) Cell concentration profile.

3.5.7 Performance comparison

The biodegradation experiments were characterized in terms of total reaction time, the degradation rates for each substrate and the amount of butyl acetate degraded and volatilized and are summarized in Table 3-3. The improvement percentages relative to the single phase system were calculated by subtracting the corresponding polymer performance and the single phase metrics. The absolute value of the subtraction was then divided by the corresponding single phase

performance metric and multiplied by 100. Similar calculations were performed to estimate the improvements relative to the SO system.

The SO system provided a considerable improvement in the amount of butyl acetate degraded relative to the single phase system, due to the reduction in volatility losses. Nevertheless, as expected, there were no significant difference in the total reaction time and in the phenol degradation rate, due to the negligible affinity of SO for phenol.

The polymer system showed faster degradation compared to the other systems, and the reaction time was reduced by 30 and 35 % relative to the single phase and the SO systems, respectively. In terms of butyl acetate degraded and volatilized, no significant enhancements were obtained compared to SO, although great improvement was seen relative to the single phase system with an increase of more than 50 % in the amount of butyl acetate degraded and a decrease of around 40 % in the total amount of butyl acetate volatilized. Butyl acetate rates of disappearance varied, and the highest rate of disappearance was obtained for the polymer system while the lowest was seen in the SO system. An increase in the rate of butyl acetate disappearance is not necessarily associated with an enhancement given the complexity of the system where degradation and volatility occur concurrently.

The relatively high rate of disappearance observed for the single phase system can be mostly attributed to volatilization. For the two phase systems the absorption phenomenon plays an important role in the retention of the butyl acetate within the system, allowing for additional biodegradation. In the case of the SO system the apparently slow butyl acetate disappearance is related to the relatively high affinity between it and the solvent; this causes a slower re-release and ultimately delays the degradation [15]. In the polymer system, the apparent higher butyl acetate disappearance is presumably associated with an enhanced microbial activity as a

consequence of the lower phenol aqueous concentrations. This is reflected in an increase of the amount of butyl acetate degraded and also in the higher values of cell concentration. By the time butyl acetate is completely depleted in the single phase system, around 5 hours, the cell concentration is 0.25 g/l, whereas for the polymer system the cell concentration at butyl acetate depletion time, 4.5 hours, is 0.37 g/l. As discussed earlier phenol disappearance unlike butyl acetate, can be mostly attributed to degradation. The polymer system showed a remarkable improvement with respect to the phenol degradation rate with a 40.7 and 53.4 % improvement relative to the single phase and the SO systems, respectively.

Table 3-3: Performance comparison for all cases studied

Performance comparison at 500 mg/l					
Performance parameter	Single phase	Silicone Oil	Polymer mixture	Polymer Improvement relative to single phase (%)	Polymer Improvement relative to SO (%)
Total reaction time (h)	9.9	10.9	7.0	29.3	35.8
Butyl acetate rate of disappearance (mg/l.h)	105.1	83.3	125.0	----------	----------
Phenol rate of degradation (mg/l.h)	50.8	46.4	71.4	40.6	53.9
Total mass of butyl acetate volatilized (mg)	815.3	576.5	476.4	41.6	17.4
Total mass of butyl acetate degraded (mg)	644.1	901.3	997.8	54.9	10.7
Performance comparison at 1000 mg/l					
Total reaction time (h)	19.8	------	14.9	24.7	----------
Butyl acetate rate of disappearance (mg/l.h)	145.1	------	222.2	----------	----------
Phenol rate of degradation (mg/l.h)	46.3	------	66.8	44.2	----------
Total mass of butyl acetate volatilized (mg)	2014.0	------	1214.6	39.7	----------
Total mass of butyl acetate degraded (mg)	886.8	------	1722.1	94.2	----------

For the degradation carried out at 1000 mg/l improvements of over 24 % were obtained for all the performance metrics studied. The highest enhancement was seen for the amount of butyl acetate degraded which was increased by almost 100% relative to single phase.

3.6 Conclusion

The solid-liquid TPPB platform was successfully used to overcome two clearly distinct limitations commonly found in bioremediation applications: stripping losses and microbial inhibition. A polymer mixture of styrene-butadiene rubber and Hytrel® 8206 was chosen for the TPPB experiments due to their high absorption capacity for butyl acetate and phenol, respectively. The degradation patterns for butyl acetate and phenol presented strong evidence of diauxic growth and in the case of the single phase experiment the dissolved oxygen trace provided good insights into the microbial activity variations over the course of the biodegradation. For the liquid-liquid TPPB, SO was found to enhance oxygen transfer, presenting almost no noticeable changes in the DO trace during the whole reaction.

Performance comparisons at two different initial concentrations 500 mg/l and 1000 mg/l were undertaken. At 500 mg/l both TPPB versions clearly outperformed the single phase reactor by decreasing the amount of butyl acetate volatized. The solid-liquid TPPB also outperformed its SO counterpart; in this case the relative improvement in volatility was not as marked but the phenol degradation rate was substantially increased as SO has zero affinity for phenol. Experiments carried out at 1000 mg/l showed a clear superiority of the solid-liquid TPPB relative to the single phase reactor especially in the amount of butyl acetate degraded which was improved by almost 100 %.

In the current work, polymer selection was carried out based solely on the absorption capacities of the polymer represented by their corresponding PC values. A more systematic

protocol founded on fundamental thermodynamic principles is currently being developed with the aim of optimizing the selection of effective polymers for a variety of applications.

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Chapter 4

A comparison of three first principles' methods for predicting solute-polymer affinity, and the simultaneous biodegradation of phenol and butyl acetate in a two-phase partitioning bioreactor

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With minor editorial changes to fulfill formatting requirements, this chapter was summited to the *Journal of Chemical Technology and Biotechnology* and is currently under peer review.

4.1 Preface

The work presented in Chapter 3 demonstrated the practical and performance superiority of solid-liquid TPPB systems compared to other bioremediation strategies. Nevertheless, the polymer selection carried out in Chapter 3 was done solely based on the partitioning coefficients for butyl acetate and phenol. In order to enhance the polymers selection efficiency and effectiveness a more systematic approach is required. A polymer selection guide for TPPB applications had already been suggested [1], although it is still unclear if such guide could be extended to systems with widely different target molecules.

The current chapter examined different first principles' approaches to polymer selection. Three different methods to evaluate polymer-solute thermodynamic affinity were compared based on prediction of partitioning coefficients and experimental data. Ultimately, the work aimed to develop a more universal polymer selection methodology to be effectively employed in future TPPB applications.

4.2 Abstract

The present study examined three thermodynamic methods for predicting the polymer-solute thermodynamic affinity of butyl acetate and phenol containing systems. The methods investigated were Hildebrand solubility parameters, Hansen solubility parameters and activity coefficients at infinite dilution. The Hansen solubility parameter method presented the best trends and its relative predictions had better agreement with the experimental results compared to the other methods. An effective polymer Pebax® 2533 was selected due its low degree of chrystallinity and glass transition temperature and its increased affinity to the target solutes. Biodegradation experiments carried out with the selected polymer at 1000 mg/l presented a 40 %

improvement in the phenol degradation rate with respect to the previous polymer mixture used, additionally it presented a 100 % improvement compared to the single phase system in all the performance metrics. The Hansen solubility parameter method was then used for the selection of effective TPPB polymers among waste materials resulting in the selection of a polymer mixture of rubber tires and acrylonitrile. Biodegradation experiments performed with this polymer mixture showed a comparable performance to the Pebax® 2533 system demonstrating that inexpensive waste materials can be effectively employed in TPPB applications. The findings in this work can be extended to other systems due to its fundamental approach.

4.3 Introduction

Two Phase Partitioning Bioreactors (TPPBs) utilize a non-aqueous phase that acts as a reservoir for high concentrations of inhibitory substrates. This sequestering phase reduces the aqueous substrate concentration to sub-cytotoxic levels, and progressively re-releases it to the cell-containing aqueous phase in order to respond to the microbial metabolic demand. This dynamic process is regulated by thermodynamic equilibrium (the relative affinity of the substrate between the aqueous and sequestering phases) and cell metabolism [2].

Although immiscible organic solvents were originally used as the partitioning phase in TPPBs it has been shown that polymer-based systems can match and even outperform their liquid-liquid counterparts in many different types of applications[3-5]. Polymers are in virtually all cases biocompatible, non-bioavailable and easy to handle; critically, polymers are also much less expensive (circa $5-6/kg) than non-aqueous phase liquids [6]. Perhaps most importantly, polymers possess a large variety of different chemical structures that can be combined into chemical mixtures as blends of individual monomers or as co-polymers, allowing a wider range of solute affinities [7]. Nevertheless, despite their versatility, polymers' complex structure makes solute-polymer interactions difficult to predict, and as a consequence rational selection of polymers for specific TPPBs applications is challenging, certainly more so than predicting solvent-solute interactions, which have been well-described by classical thermodynamic methods [8].

In Chapter 3 it was showed that it is possible to find 2 polymers to target 2 distinct substrates, phenol and butyl acetate, whose chemical and physical properties differ considerably [9]. Phenol is a relatively polar and toxic aromatic compound possessing moderate aqueous solubility, low volatility, and high capacity for hydrogen bonding; butyl acetate is a considerably

more hydrophobic and less toxic linear molecule, with lower aqueous solubility and high volatility. The selected polymers were identified by trial-and-error screening of a number of polymers available in our group but, although TPPB tests were highly effective, such random polymer testing does not provide a basis for polymer selection for other TPPB applications.

In an attempt to generate a more rational approach for polymer identification, properties such as the polymer hardness, solute octanol-water partitioning coefficient (log P) and polymer crystallinity, have previously been considered to try to explain polymer-solute absorption [10], although it has been shown that such parameters are inadequate when attempting to describe or predict all possible polymer-solute interactions [1]. Recently, a first principles' approach for polymer selection for TPPBs was proposed [1] in which polymer absorption of highly hydrophobic solutes (polyaromatic hydrocarbons) was initially described in terms of polymer accessibility, which was correlated with the degree of crystallinity and the glass transition temperature of the polymer (T_g). Additionally, specific polymer-solute thermodynamic affinity was characterized using the overall Hildebrand solubility parameter method. Hildebrand solubility parameters (SPs) were originally intended for non-polar non-electrolyte systems [11] and are commonly used to describe polymer-solute binary interactions in several industrial applications (e.g. protective coatings) due to their widespread availability and simplicity [12]. However, solid-liquid TPPBs are complex three components systems, (polymer-solute-water), which can provide a wide range of possible interactions. Moreover, the Hildebrand solubility parameter approach can account only for the polymer-solute interactions, completely neglecting the presences of water and its corresponding effects.

The current work has provided an extension of the above polymer selection criterion: three different methods used to characterize polymer-solute interactions were compared,

Hildebrand SPs, Hansen Solubility Parameters (HSPs) and activity coefficient predictions. Such methods, to best of our knowledge, are the only thermodynamic approaches available to describe polymer-solute interactions [1, 13]. The methods were tested against two TPPB substrates, phenol and butyl acetate whose chemical and physical properties, as noted, differ considerably [9] thereby providing a good test of the three predictive methods. The methods were used to identify a single polymer with affinity for both target molecules that could operate satisfactorily in a TPPB, and also to identify waste polymers for the same purpose. TPPB biodegradation experiments were then undertaken to demonstrate the subsequent performance of the identified polymers.

4.4 Materials and methods

4.4.1 Chemicals and polymers

All medium components and powder free nitrile rubber examination gloves were purchased from Fisher Scientific (Guelph, Canada). High density polyethylene, phenol (99%) and butyl acetate (>99%) were obtained from Sigma-Aldrich. The various grades of Pebax® were purchased from Arkema (Burlington, Ontario, Canada), Hytrel® 3548 was supplied by DuPont (Kingston, Ontario, Canada), polyisoprene and nitrile butadiene rubber (21 % acrylonitrile content) were purchased from Scientific Polymer (Ontario, New York, U.S.A), neoprene and nitrile rubber tubing were obtained from Rubber Sheet Roll Inc. (Shippensburg, Pennsylvania, U.S), nitrile rubber O-rings and polypropylene tubing were purchased from Cole-Parmer (Montreal, Quebec, Canada), and nitrile O-rings were purchased from Levac Supplied Limited (Kingston, Ontario, Canada). Used tire rubber was obtained from Recovery Technologies Canada Inc. (Cambridge Ontario), in the form of rubber “crumble”. Crumble is obtained by a cryogenic process in which tires are cooled to a temperature lower than –80 °C. Below this glass transition

temperature rubber becomes brittle, and size reduction can be accomplished by crushing and grinding, after which steel and fibre separation is easily accomplished. The crumble is claimed to have an unaltered chemical composition relative to the original tires, and is currently used for rubber modified asphalt, moulded rubber products and sports surfaces [14].

Table 4-1: Relevant properties for commercial polymers

Polymer name	Structure arrangement	Fraction of active component (%)	(T_g, ◦C)
Hytrel® 8206	Block copolymer	~ 50[a]	-59
Hytrel® 3548	Block copolymer	~ 50[b]	-45
Pebax® 2533	Block copolymer	80	-65
Pebax® 1074	Block copolymer	55	-40
High density polyethylene (HDPE)	Homopolymer	≈ 25[c]	≈ -124[d]
Styrene-butadiene rubber (5 % Styrene, SBR)	Random copolymer	100	≈ -76[d]
Trans-Polyisoprene (P.I)	Homopolymer	≈ 67[e]	-68
Acrylonitrile/butadiene rubber (21 % ACN, NBR)	Random copolymer	100	≈ -60[d]

[a] Obtained from [7]
[b] Assuming similar soft segment composition as 8206
[c] Obtained from values presented in [15]
[d] Obtained from values presented in [16]
[e] Obtained from values presented in [17]

4.4.2 Partitioning coefficients measurements

The Partitioning Coefficients (PC) measurements were performed as described in Chapter 3. PCs were calculated in quadruple varying the amount of polymer mass used in each case. Aqueous concentrations were determined in each case and the PCs were obtained through mass balances.

4.4.3 Solid-liquid biodegradation test

TPPB biodegradation tests were undertaken in a sequencing batch mode as previously described in Chapter 3. Operation using a single polymer or waste polymer mixtures was carried out through consecutive cycles varying the initial substrate concentration for each cycle. At the

end of each cycle 80% of the reaction broth was pumped out and replaced with fresh medium and substrate to start a new cycle. Each cycle proceeded with the inoculum obtained from the previous cycle except for the initial cycle whose inoculum was prepared separately in shake flasks.

In the first biodegradation test 150 g of Pebax® 2533 were added, corresponding to a polymer fraction of 5% w/w. For the second biodegradation test a polymer mixture of 75 g of rubber tire and 250 g of nitrile rubber was employed, corresponding to a total polymer fraction of 10.8 % w/w.

4.4.4 Analytical procedures

Phenol, butyl acetate and cell concentrations were determined as described previously describe in Chapter 3. Phenol and butyl acetate aqueous concentrations were determined through HPLC and cell concentration was obtained through optical density measurements.

4.5 Results and discussion

4.5.1 Polymer Selection

In early research involving solid-liquid TPPBs polymer absorption capacity was explained in somewhat qualitative terms based on general information available such as polymer hardness, and the possible interactions that may be occurring between the polymer and target molecule [7, 10], such as polarity, hydrogen bonding potential, among others. Recently a more rational approach has been proposed using simple first principles' considerations to better understand the structural and chemical interactions that govern polymer absorption in the context of TPPBs. As a result, polymer absorption has been characterized by means of two main factors: polymer accessibility and thermodynamic affinity [1].

Polymer accessibility is determined primarily by the degree of crystallinity and the glass transition temperature of the polymer. In the case of block copolymers and partially crystalline homopolymers, high degrees of crystallinity generally translate into poor uptake capacities simply because the crystalline domain is unable to participate in absorption due to its greater rigidity and resistance to solute penetration. In addition, if the amorphous segment of these polymers possesses glass transition temperatures (T_g) greater than the operating temperatures, the polymer will lack significant chain and segmental mobility to allow the diffusion of even small molecules at such operating conditions. Thus, effective block copolymers and homopolymers must possess just enough crystalline domains to provide acceptable mechanical properties (i.e. they make the polymer solid), and a larger amorphous phase capable of participating in the absorption process. Additionally, the amorphous phase must have a considerable lower T_g than the operation temperature to ensure enough segmental and chain motion [1].

In contrast to block copolymers, random copolymers possess a non-regular distribution of the monomers along their structure preventing the formation of clearly defined soft and hard segments [18]. Owning to this structural irregularity, crystallization is more difficult in random copolymers and in addition they tend to present intermediate properties to the corresponding homopolymers. [19].

The polymers used in this work were initially selected using the accessibility criteria described above. As seen in Table 4-1, all of the polymers selected, except for HDPE, possessed relatively small amounts of a hard crystalline phase. Polymers shown in Table 4-1 also had T_g values considerably lower than the operating temperature of the experiments (30◦C). In the case of styrene butadiene rubber and nitrile butadiene rubber which are random copolymers, it is suspected that these polymers do not possess multiple structural domains as opposed to the other

commercial block copolymers studied, and for the purpose of this work they are considered completely amorphous with structural and thermodynamic contributions arising from all of their constituents [19].

The accessibility criteria are an initial guideline to predict whether a given polymer has the structural characteristics to provide general solute absorption, nevertheless such criteria say little about the specific polymer solute interactions that confer solute-polymer affinity. In order to effectively select polymers for a given TPPB application the thermodynamic affinity between candidate polymers and target molecules must be assessed. Polymer solute interactions can be characterized by using the three thermodynamic tools available: Hildebrand SPs, Hansen Solubility Parameters (HSPs), and activity coefficient methods [12, 20, 21]. In the current work the performance of each method was assessed using the normalized PCs for butyl acetate and phenol presented in Table 4-2. The normalized PCs were obtained by dividing the experimentally-determined PCs by their corresponding amorphous polymer fraction; thus the normalized PCs account only for the mass of polymer which was actively participating in the absorption process, that is, which has an affinity for the solute.

Table 4-2: Partitioning coefficients for different polymers and materials

Material	Butyl acetate PC		Phenol PC	
	Experimental	Normalized	Experimental	Normalized
Hytrel® 8206[f]	9.2 ± 0.4	17.9 ± 0.7	37.4 ± 0.6	73.3 ± 1.1
Hytrel® 3548	22.9 ± 0.7	41.6 ± 1.3	49.8 ± 0.9	90.5 ± 1.7
Pebax® 2533	32.7 ± 3.6	40.9 ± 4.5	50.0 ± 0.6	62.5 ± 0.8
Pebax®1074	7.7 ± 1.4	13.9 ± 2.6	36.8 ± 0.9	66.8 ± 1.7
High density polyethylene	2.1 ± 0.03	8.4 ± 0.1	0 ± 0.07	0 ± 0.3
Styrene-butadiene rubber[f]	34.0 ± 1.8	34.0 ± 1.8	0.8 ± 0.3	0.8 ± 0.3
Trans-Polyisoprene	7.8 ± 0.3	11.6 ± 0.4	0.1 ± 0.06	0.2 ± 0.1
Acrylonitrile/butadiene rubber	34.8 ± 0.6	34.8 ± 0.6	6.0 ± 0.5	6.0 ± 0.5
Rubber tire	18.0 ± 1.5	----------	1.2 ± 0.1	----------
Nitrile rubber glove	26.6 ± 2.9	----------	15.0 ± 1.0	----------
Nitrile rubber tubing	20.2 ± 0.8	----------	5.1 ± 0.2	----------
Nitrile rubber O-ring (Levac Supplies Inc)	23.0 ±1.8	----------	7.3 ± 0.8	----------
Nitrile rubber O-ring (Cole-Parmer)	11.7 ± 1.7	----------	4.4 ± 0.9	----------
Poly propylene tubing	18.5 ± 1	----------	0.8 ± 0.05	----------

[f] Data from [9]

4.5.1.1 Hildebrand solubility parameter

The first approach using solubility parameters was developed by Hildebrand and Scot [11], and they defined solubility parameters (SPs) as shown in Equation 4-1

$$\delta = \sqrt{\frac{\Delta H_v}{V}} \qquad 4\text{-}1$$

where ΔH_v is the heat of vaporization and V corresponds to the molar volume of the given molecule.

The SPs represent a measure of the cohesive energy density and provide a quantitative estimate of the potential interaction between materials [12]; according to traditional polymer solution theory smaller differences in SPs minimize the activity of a solute in a given polymer.

This ultimately translates into greater uptake capacities and therefore the smaller the differences in SPs between polymer-solute pairs the higher the expected partitioning coefficient [1].

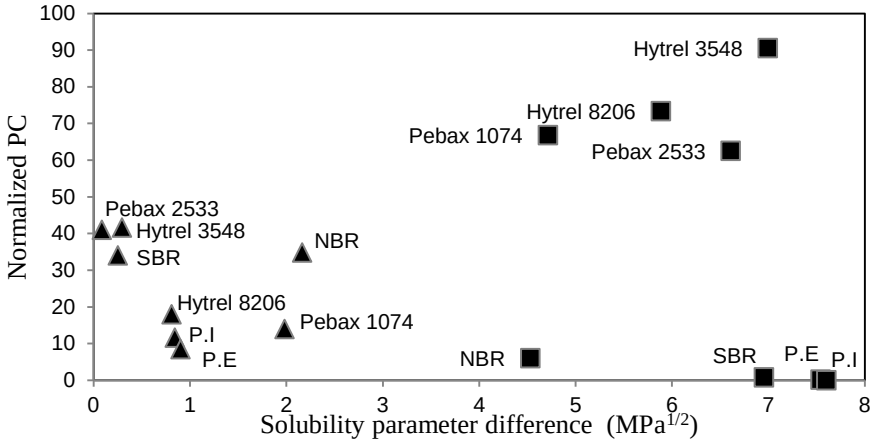

Figure 4-1: Hildebrand solubility parameter method assessment based on normalized partitioning coefficient and solubility parameter difference. ▲- Butyl acetate, ■-Phenol.

Figure 4-1 shows the normalized PCs as a function of the differences in the corresponding Hildebrand SPs between polymers and solutes. Hildebrand solubility parameters for the polymers amorphous phase were obtained from [22]. Ideally smaller solubility parameters differences should translate into higher partitioning coefficients, which would be seen as a plot with a curve sloping upward to the left. This trend is observable for butyl acetate, however the trend is less clear for phenol as the data for phenol are considerably more scattered. In general, the solubility parameter difference between the polymers tested and phenol was greater than with butyl acetate. As will be described later, this may be due to the higher polarity and hydrogen bonding character of phenol, which is not captured by the "lumped" single solubility parameter as defined by Hildebrand and which therefore cannot be used to explain this observation given that Hildebrand SPs make no distinction between different types of interactions.

4.5.1.2 Hansen Solubility Parameter (HSP)

Hansen proposed an extension of the Hildebrand solubility parameters that could better capture the nature of the polar and hydrogen bonding interactions between materials [12]. In the HSP approach the solubility parameter is divided into three main contributions: atomic dispersion forces (δ_D), molecular dipole interactions (δ_P) and molecular hydrogen bonding (δ_H). Each contribution corresponds to a coordinate in the three dimensional "Hansen Space". The total solubility parameter for a substance can be calculated as the Euclidean norm of the vector arising from these three contributions, as shown in Equation 4-2.

$$\delta_{tot} = \sqrt{\delta_D{}^2 + \delta_P{}^2 + \delta_H{}^2} \qquad 4\text{-}2$$

The relative HSP "distance" between two different materials has been defined by Hansen and Skaarup [12] as

$$(Ra)^2 = 4 \cdot (\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \qquad 4\text{-}3$$

Similar to the Hildebrand approach smaller HSP distance (Ra) will translate into higher miscibility between materials and, again, a plot of PC vs Ra would result in a curve sloping upward to the left. HSPs for the polymer amorphous part were obtained from the HSPiP software v3.1. (Charles M. Hansen (http://www.hansen-solubility.com)).

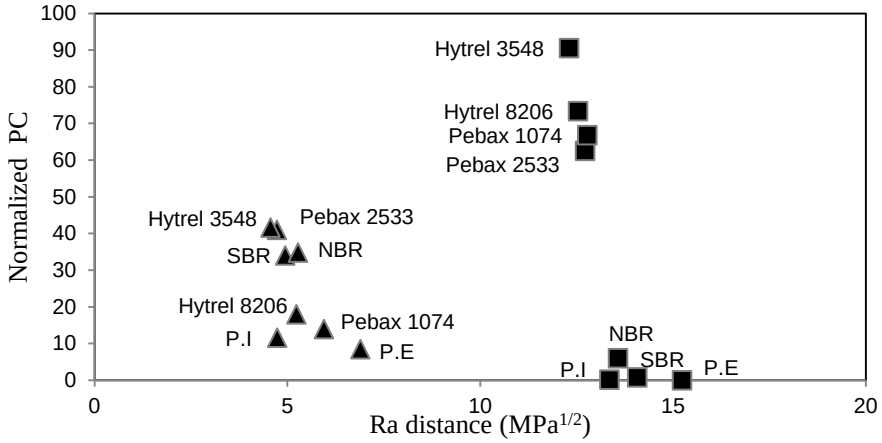

Figure 4-2: Hansen solubility parameter method assessment based on normalized partitioning coefficients and Ra values. ▲- Butyl acetate, ■-Phenol.

Figure 4-2 shows the relationship between the normalized partitioning coefficients and the HSP distance between the polymers and both solutes. In this case, both solutes followed the expected trend showing higher PCs at smaller Ra distances. The only clear outliner was seen for polyisoprene for which the HSP method predicts a higher relative affinity compared to the other polymers; nonetheless significant improvement was found in the data for phenol compared to the Hildebrand method. This may be due to the fact that the HSP concept provides an explanation for the greater differences in solubility parameters observed between phenol and the studied polymers. As seen in Table 4-3, the hydroxyl group in phenol greatly enhances its polar and hydrogen bonding character, thus increasing the total HSP values. These solubility parameter values are considerably greater than those of most polymers which usually possess long non-polar carbon chains. Polymer thermodynamic affinity to high SP solutes, such as phenol, could be potentially enhanced by the addition of polar or hydrogen bonding groups into the polymer structure, nevertheless the introduction of these groups reduces chain mobility and causes an increase in T_g which can be detrimental for polymer accessibility, as mentioned previously [1].

Table 4-3: Hansen Solubility Parameters for relevant compounds and materials

Compound/Polymer	Hansen solubility parameter ($MPa^{1/2}$)				Ra distances ($MPa^{1/2}$)	
	Dispersion	Polar	Hydrogen bonding	Overall	To butyl acetate	To phenol
Butyl acetate	15.8	3.7	6.3	17.4	----------	----------
Phenol	18.5	5.9	14.9	24.5	----------	----------
Styrene-butadiene	17.1	3.4	1.6	17.5	5.4	13.8
Nitrile rubber	16.7	5.8	1.8	17.8	5.3	13.6
Polypropylene	16.7	2.9	0.6	17.0	6.0	15.0

4.5.1.3 Infinite dilution activity coefficient predictions

Activity coefficients can account for the non-idealities of a chemical substance in a mixture, and can be defined as shown in Equation 4-4

$$\gamma_i \equiv \frac{a_i}{x_i} \tag{4-4}$$

where γ_i is the activity coefficient of the substance i, a_i corresponds to the activity of the substance i in the mixture and x_i is the molar fraction of the compound in the mixture.

[23]. As the molar fraction of the compound approaches zero the activity coefficient reaches a defined fixed value that can be expressed by the following limit equation [24],

$$\gamma_{i\,x\to 0} = \lim_{x\to 0}(\gamma_i) \tag{4-5}$$

For solid-liquid TPPBs systems if infinite dilutions conditions are met, the corresponding PC can be estimated as the ratio of the independently determined infinite dilution activity coefficients, as shown in Equation 4-6

$$PC = \frac{\gamma^{aq}_{solute,x\to 0}}{\gamma^{polymer}_{solute,x\to 0}} = \frac{\Omega^{aq}_{solute,x\to 0}}{\Omega^{polymer}_{solute,x\to 0}} \tag{4-6}$$

where $\gamma^{aq}_{solute,x\to 0}$ is the molar based infinite dilution activity coefficient of the target solute in the aqueous phase, $\gamma^{polymer}_{solute,x\to 0}$ corresponds to the molar based infinite dilution activity coefficient of the target solute in the polymer phase. The Ω coefficients are the equivalent mass basis activity coefficients. For the system studied infinite dilution conditions were assumed for the aqueous phase given that the maximum molar fraction of solute during the PC measurements was approximately 4 x 10^{-4}. Infinite dilution coefficients for butyl acetate and phenol in water were obtained from [25]. For the polymer phase, infinite dilutions coefficients were estimated using a UNIFAC-vdW-FV model developed in [26], which is a modified version of the original Oishi-Prausnitz model that allows predictions for polymer-aqueous systems. It was found that the activity coefficient for both phenol and butyl acetate in all polymers systems were constant (data not shown) throughout the range of concentration studied, suggesting that they also corresponded to infinite dilution activity coefficients [24].

Figure 4-3 compares the UNIFAC predictions to the normalized PCs for butyl acetate and phenol, and in this case a perfect correlation would be a 45° straight line with the same axis values. The butyl acetate results show a coarse trend of this nature, although most of the predicted PCs are overestimated. On the other hand, phenol predictions are more scattered and PC estimates are greatly underestimated. At best, the predictions obtained with the UNIFAC methods seem to be useful only for performing a relative assessment of the effectiveness of the polymers due the great difference between the experimentally and the predicted PCs.

The solubility parameter methods, however, presented clear trends for the normalized PCs as a function of their corresponding SP differences/distance. In particular, the HSP method showed only slight scattering and gave a more direct insight into the relationship between the

molecules' structures and the occurring interactions in the system, proving to be superior to its Hildebrand counterpart. The activity coefficient at infinite dilution method showed the correct trends although the predictions obtained were considerably more scattered compared to the SP methods. Moreover the PCs predicted differed greatly from the normalized values making this method useful only for relative predictions, and the results may not justify the higher level of rigor employed in this case.

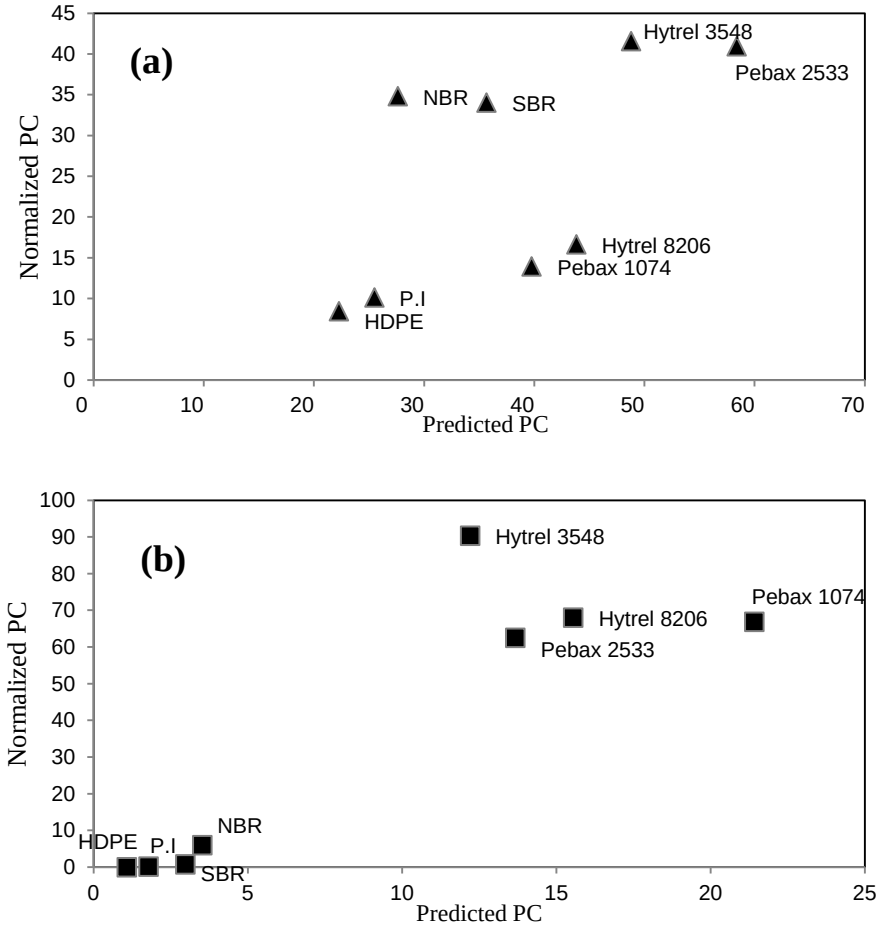

Figure 4-3: UNIFAC method assessment based on normalized partitioning coefficients and predicted partitioning coefficients. a) Butyl acetate, b) Phenol

For the purposes of polymer selection, butadiene copolymers and Pebax® 2533 showed the highest experimental PCs for butyl acetate while all the polyether based copolymers showed relatively large PCs for phenol. Importantly, these polymers also had the smallest Ra in the HSP method, as seen in Figure 4-2. Pebax® 2533 was therefore identified as the most suitable single polymer for both solutes studied due to the predicted high thermodynamic affinity (particularly seen in the HSP approach) and its high amorphous fraction, 80%, and low T_g. In our earlier work a mixture of Hytrel® 8206 and SBR were used for the simultaneous degradation of phenol and butyl acetate in a TPPB platform [9]. The selection of the single polymer Pebax® 2533 with increased uptake capacities (Table 4-2) through the HSP approach represents a considerable practical improvement in that only a single target polymer needed to be used. To test the performance of Pebax® 2533, corresponding TPPB biodegradation experiments were carried out.

4.5.2 Solid-liquid TPPB using Pebax® 2355

Figure 4-4 shows the time course for the simultaneous biodegradation of phenol and butyl acetate using Pebax® 2355 at two different initial concentrations of 500 and 1000 mg/l.
The key performance metrics of the degradation of these substantially different substrates were related to the higher toxicity of phenol (and therefore the resulting phenol degradation rate), and the higher volatility of butyl acetate (and therefore the proportion of butyl acetate degraded vs volatilized). In the 500 mg/l experiment the concentrations of both substrates decreased at the beginning of the reaction and, as has been demonstrated previously, such a decrease corresponds to polymer absorption in the case of phenol and a combined effect of volatilization, polymer absorption and degradation in the case of butyl acetate [9]. As a consequence the butyl acetate concentration profile always remained lower than that of phenol. The phenol concentration

profile showed a sharp decrease after 3.5 hours suggesting an increase in the phenol degradation rate. Both substrates were completely degraded after 6.7 hours of reaction.

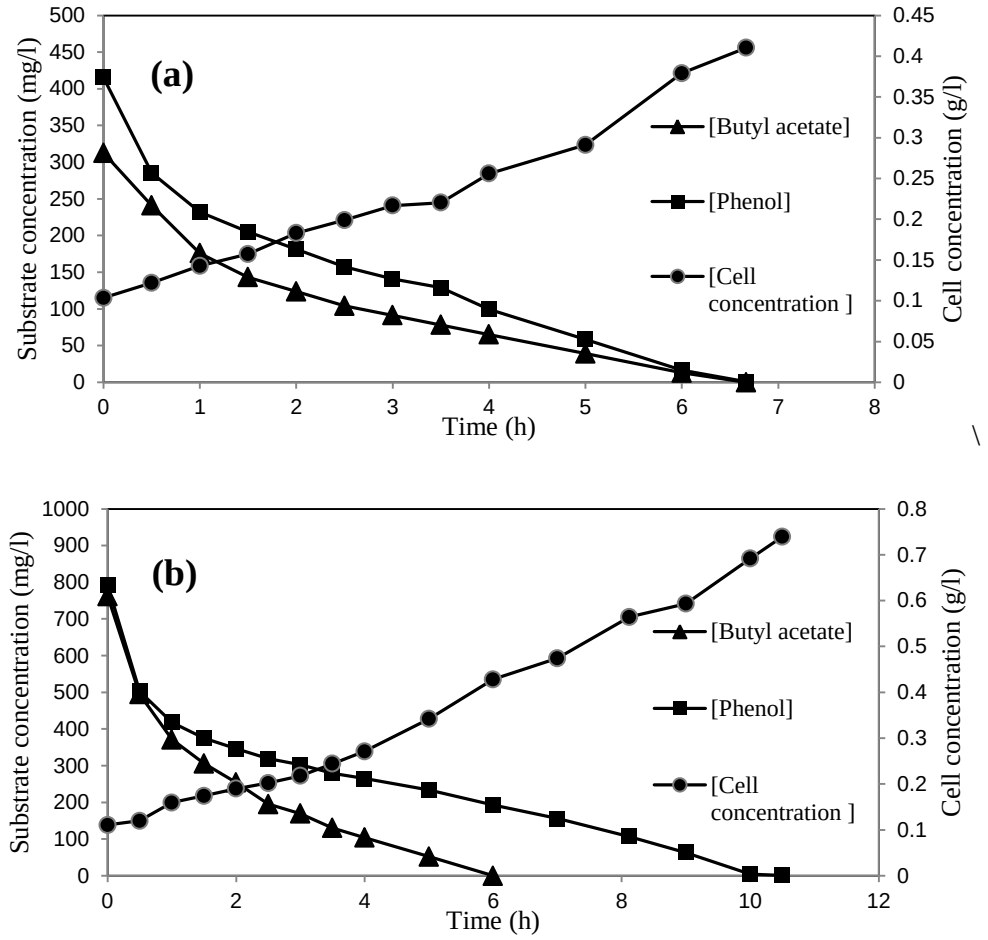

Figure 4-4: Compounds time course and cell concentration profile for the solid-liquid TPPB using Pebax® 2355. a) Initial substrate concentration 500 mg/l, b) Initial substrate concentration 1000 mg/l.

The 1000 mg/l experiment also presented a sharp initial decrease in concentration with butyl acetate disappearing more quickly with complete removal from the reactor after 6 hours. The rate of phenol degradation increased considerably after butyl acetate's depletion until its

complete degradation at 10 hours. This trend is characteristic of a diauxic type of degradation as has been suggested before for this system [9].

4.5.3 Polymer selection for waste materials

To further test the capabilities of the HSP method, it was used to predict the relative effectiveness of rubber tire and common laboratory waste polymers to absorb phenol and butyl acetate, and therefore to potentially act as the sequestering phase in a TPPB. The predictions were carried out considering the following model waste polymers: SBR (tires), NBR (nitrile rubber gloves, tubing and O-rings) and polypropylene (tubing), which are commonly found in many industrial and research applications. Their PC values and their corresponding Ra values to phenol and butyl acetate are presented in Tables 4-2 and 4-3, respectively.

According to Table 4-3 nitrile rubber based materials would have the highest butyl acetate and phenol uptake capacities followed by SBR based materials. Lastly polypropylene materials were expected to have the smallest uptake capacities for both substrates. In terms of measure PC values, the NBR materials followed the expected trend having the highest affinity for both components, nevertheless polypropylene tubing showed a slightly higher PC for butyl acetate than did the rubber tires contrary to what was expected. It is important to note that the exact composition of the materials tested is not provided due to proprietary reasons, and therefore some of the PCs determined experimentally may be influenced by additive that are not considered in the predictions.

4.5.4 Solid-liquid TPPB using a polymer mixture

TPPB experiments were conducted using a polymer mixture of rubber tire and NBR. The use of scrap tires in a TPPB system is appealing due to their significant global accumulation [14],

and scrap tires and its main constituent (styrene-butadiene rubber) have already been successfully employed in solid-liquid TPPBs applications [9, 14]. Nevertheless, scrap tires have a negligible uptake affinity for phenol, thus NBR containing material had to be added to the mixture in order to ensure sub-inhibitory aqueous phenol concentrations. Pure NBR rubber was chosen as representative of these waste polymers in order that results not be skewed by a particular supplier's formulation.

Figure 4-5 shows the phenol, butyl acetate and cell concentration time course at different initial substrate concentrations using a polymer mixture of rubber tire and NBR. A higher polymer mass fraction was employed this time to compensate for the lower phenol uptake capacity of nitrile rubber compared to other commercial polymers such as Pebax® 2533 and Hytrel® 8206. Figure 4-5a presents the biodegradation carried out at an initial concentration of 500 mg/l for both substrates, and the concentration profile shows an initial decrease for both substrates due to polymer absorption, although the extent and rate of phenol sorption was not as pronounced as it was for the Pebax® single polymer system. Butyl acetate disappearance occurred at four hours and it was followed by a sharp decrease in the phenol concentration.

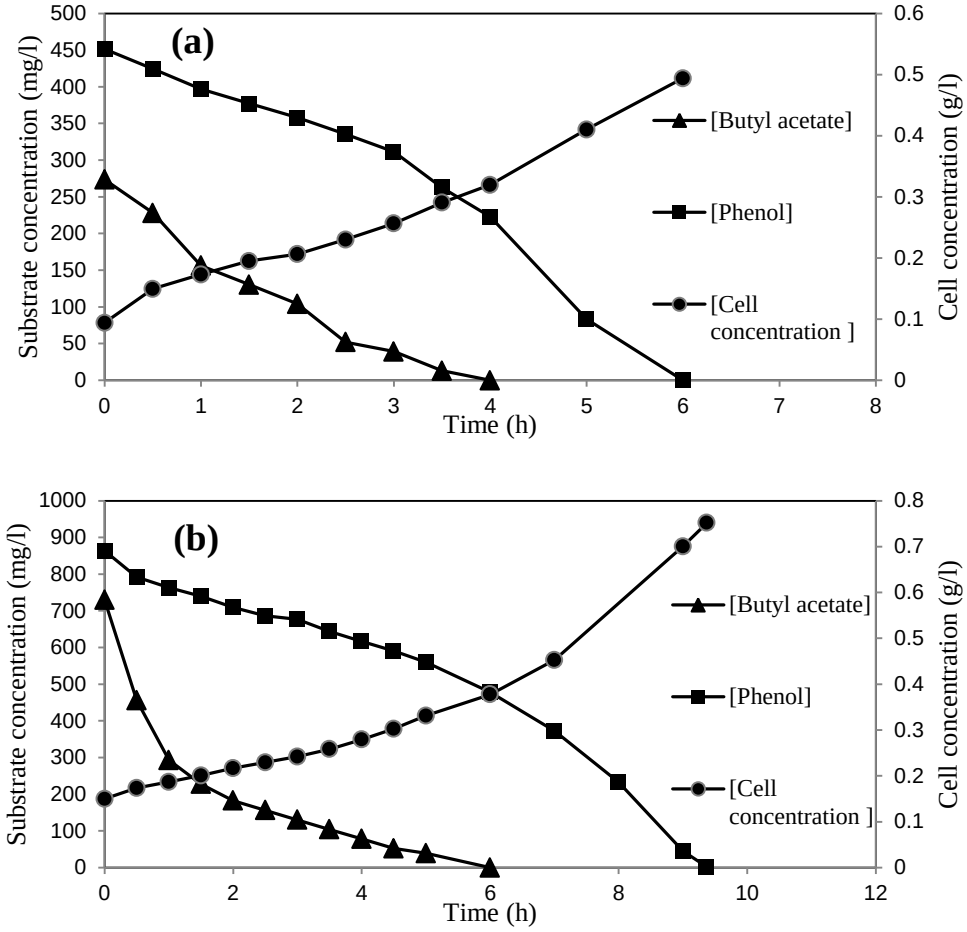

Figure 4-5: Compounds time course and cell concentration profile for the solid-liquid TPPB using a polymer mixture of NBR and tire rubber. a) Initial substrate concentration 500 mg/l, b) Initial substrate concentration 1000 mg/l.

The degradation carried out 1000 mg/l presented in Figure 4-5b showed a similar profile, with less extensive and less rapid phenol sorption, but with very rapid and complete butyl acetate disappearance. The relevant performance parameters for the removal of the two distinct substrates by the waste polymer mixture are shown in Table 4-4, and are comparable to the Pebax® single polymer system. These are encouraging results and demonstrate the capacity of waste polymers to also perform effectively in TPPBs, although at higher polymer fractions. It is important to remark

that the percentages of butyl acetate degraded and volatilized shown in Table 4-4 do not add up to 100% because the complete mass balanced considered losses due to sampling that were not ascribed either to volatility of degradation.

Table 4-4: Performance parameters for solid-liquid TPPBs with Pebax® 2533, a mixture of rubber tire and NBR, a mixture of Hytrel® 8206 and SBR and a single phase system at different initial substrate concentrations

Performance for Pebax® 2533		
Performance parameter	**500 mg/l**	**1000 mg/l**
Total reaction time (h)	6.7	10.5
Phenol rate of degradation (mg/l.h)	75.0	95.2
Butyl acetate volatilized (%)	39.5	38.6
Butyl acetate degraded (%)	58.6	59.5
Performance for polymer mixture of rubber tire and NBR		
Total reaction time (h)	6.0	9.4
Phenol rate of degradation (mg/l.h)	83.3	106.7
Butyl acetate volatilized (%)	25.9	30.4
Butyl acetate degraded (%)	72.8	68.0
Performance for polymer mixture of Hytrel® 8206 and SBR[f]		
Total reaction time (h)	7.0	14.9
Phenol rate of degradation (mg/l.h)	71.4	66.8
Butyl acetate volatilized (%)	31.7	40.5
Butyl acetate degraded (%)	66.5	57.4
Performance for a single phase reactor[f]		
Total reaction time (h)	10.0	19.8
Phenol rate of degradation (mg/l.h)	50.7	46.4
Butyl acetate volatilized (%)	54.3	67.1
Butyl acetate degraded (%)	43.0	30.0

[f]Data from [9]

4.6 Conclusion

Polymer selection criteria based on first principles' thermodynamic methods were tested against measured partition coefficient values to determine which method(s) could effectively identify polymers for the simultaneous sequestration and degradation of phenol and butyl acetate in solid-liquid TPPBs. The criteria were based on polymer accessibility (polymers' T_g and crystalline fractions) and the thermodynamic affinity between the polymers and the target molecule, as predicted by Hildebrand SPs and Hansen HSP, and activity coefficient estimates. HSP presented the best trends and offered some important insights into the resulting interactions between the polymers and the target molecules. The previously reported Hildebrand SP method showed greater scatter of the data and gave little insight about the possible occurring interactions present in the system, perhaps due to the fact that it does not capture individual molecular interactions in the manner of the HSP method. Nevertheless, if applied to hydrophobic non-hydrogen-bonding solutes, it may still be a valuable tool for rational polymer selection [1]. Finally the activity coefficient method provided the anticipated trend, although the predicted PC values did not match actual measured PC values well, and perhaps does not justify the use of this more complex method. It may, however, prove to be the best choice for systems in which truly ternary phase behavior is anticipated, such as in the case of polymers that have some water-absorbing capacity or systems with extremely hydrophilic solutes, and for which binary interaction predictions (Hildebrand SPs and HSP) may be inadequate.

Pebax® 2355 was identified as the most suitable polymer due to its high PCs for both butyl acetate and phenol. This result was consistent with the high amorphous fraction and the results obtained with the HSP method. Biodegradation TPPB experiments performed with Pebax® 2533 showed improved performance compared with a previous report which used two target

polymers for the two solutes, and suggests that the Hansen HSP method may effectively select polymers for other applications.

The HSP method was applied to different waste polymers commonly found in commercial and laboratory environments. The polymer mixture of rubber tire and NBR selected proved to be very effective in consequent biodegradation experiments at higher polymer fractions, approaching the performance found with Pebax® 2355.

We continue to evaluate these three thermodynamic methods for predicting effective polymers over a range of solutes/polymers, with the goal of arriving at an overarching guide for other researchers who may wish to rationally select polymers for their TPPB applications.

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Chapter 5

Conclusions and Recommendations for Future Work

5.1 Conclusion

Domestic and industrial wastewater consists of mixtures of contaminants possessing various properties including high volatility and microbial toxicity. Although Two Phase Partitioning Bioreactors (TPPB) has been used to treat a variety of solutes, they have not included mixtures possessing such diverse properties. The present work studied the simultaneous biodegradation of butyl acetate and phenol, two substrates known for their high volatility and cytotoxicity respectively.

A preliminary set of experiments was conducted in order to compare the performance of solid-liquid TPPBs with other existing platform: a liquid-liquid TPPB and a single phase control system. For the solid-liquid system a mixture of Hytrel® 8206 and styrene-butadiene rubber was chosen among randomly selected polymers, and silicone oil was employed in the liquid-liquid system due to its almost universal use in liquids-liquid TPPB applications. The solid-liquid TPPB system showed considerable enhancements compared to the single phase systems in both the amount of butyl acetate degraded and in the phenol degradation rates at all initial concentration levels studied. The silicone oil system showed some enhancements in the amount of butyl acetate degraded and volatized relative to the single phase system, but it did not present any considerable improvement in the phenol degradation rates due to its negligible uptake capacity for that compound. In addition, silicone oil required considerable more handling, and formed emulsion within the reaction broth impeding accurate cell density measurements. These

results demonstrated the superiority of solid-liquid TPPBs for the degradation of toxic and volatile compounds mixtures in solution from a performance and practical standpoint of view.

In order to more rationally select polymers, first principles' polymer selection criteria were developed. This resulted in a methodology that contained two main guidelines: polymer accessibility and polymer-solute thermodynamic affinity. Polymer accessibility was correlated to the polymer's glass transition temperature and degree of crystallinity and evaluated whether a polymer could effectively participate in absorption. The polymer-solute thermodynamic affinity was used to carry out relative predictions on the polymers' uptake capacity for a given molecule. The thermodynamic affinity was evaluated using three distinct methods: Hildebrand Solubility Parameters, Hansen Solubility Parameters (HSP) and infinite dilution activity coefficients.

It was found that the HSP method gave the best trends and had better agreement with the experimental results. A single candidate polymer, Pebax® 2533, was identified using the newly developed criteria. Biodegradation experiments carried out demonstrated the superiority of the selected polymer compared to the randomly selected polymer mixture previously employed.

The HSP method was subsequently applied to identify effective TPPB polymers among waste materials resulting in the selection of a polymer mixture of rubber tire and acrylonitrile-butadiene. The polymer mixture showed comparable results to Pebax® 2533 demonstrating the ability of waste materials to effectively perform in TPPB systems.

5.2 Recommendation and future work.

The present work examined the degradation of substrates in concentrations ranging from 500 mg/l to 1000 mg/l, nevertheless the dissolved oxygen data and the relatively high uptake capacity of the polymers studied suggest that the system would be able to effectively degrade higher concentrations of substrate.

Additional mixtures of contaminants with higher number of constituents could be explored in order to better recreate industrial-like conditions. These contaminants must also possess critical differences in their level of toxicity, volatility, hydrophobicity and water solubility in order to make a complete assessment on the process capacity to treat contaminants with various properties.

Different methods of operation could also be studied; especially relevant is the continuous-flow stirred tank reactor which is commonly used in real industrial type of applications [1], and the handling of the polymers under such continuous flow operation would also need to be addressed.

Regarding polymer selection, it remains a challenge especially for applications that contemplate the removal of extremely hydrophilic molecules (e.g ethanol, butanol, succinic acid, tri(2-chloroethyl)phosphate etc.) from the reaction broth. In this case, the use of polymer selection criteria becomes crucial to facilitate the initial polymer screening given that most polymers possess negligible uptake capacities for these molecules [2-4].

The HSP approach proved to be effective for the study case presented in this project, although as explained in previous chapters, polymer-solute solubility parameter matching considers only the possible occurring interactions between the polymer-solute pair, completely neglecting the corresponding water interactions. Given the strong affinity between hydrophilic molecules and water, such interactions become more relevant. Thus, it is not really clear whether the HSP approach is suitable for this type of application or if a more rigorous approach is needed. Future research should then corroborate that the existing criteria can effectively be applied to systems containing highly hydrophilic compounds.

Another important aspect is polymer water uptake and its consequent plasticization. Some polymers with hydrophilic character are able to uptake considerable amount of water and become plasticized during the process [5]. Polymer water plasticization might be desirable because it produces depression of the polymers' T_g [5], nevertheless it may also change the polymers' properties and consequently their corresponding interaction with the target solutes. Polymer plasticization and its effects over the polymers absorption capacity in the context of TPPBs systems has yet to be studied.

Finally the effect of polymer molecular weight (M_w) on the uptake capacity must also be considered in future work. Different M_w may translate into structural differences such as free volume changes that can also affect the general capacity of the polymer to participate in absorption.

5.3 References

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[5] J. Scott Parent, M. Capela, J.T. Dafoe, A.J. Daugulis, A first principles approach to identifying polymers for use in two-phase partitioning bioreactors, J. Chem. Technol. Biotechnol. 87 (2012) 1059-1065.

Appendix A

Calibrations Curves

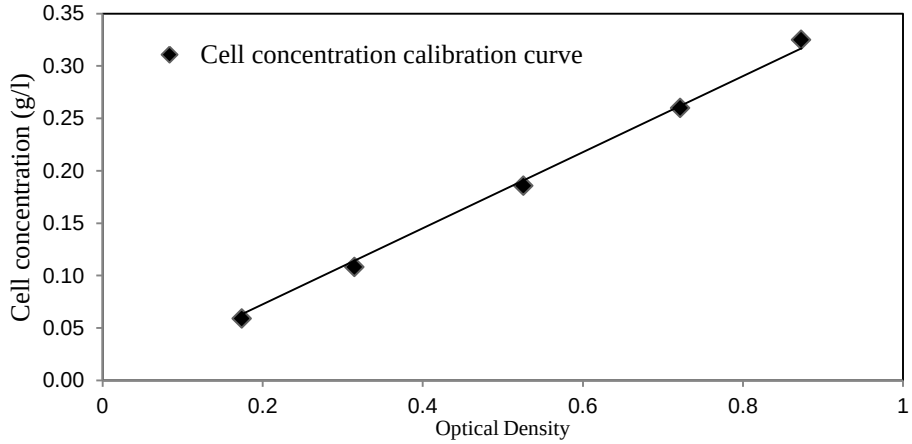

Figure A-1: Cell concentration calibration curve

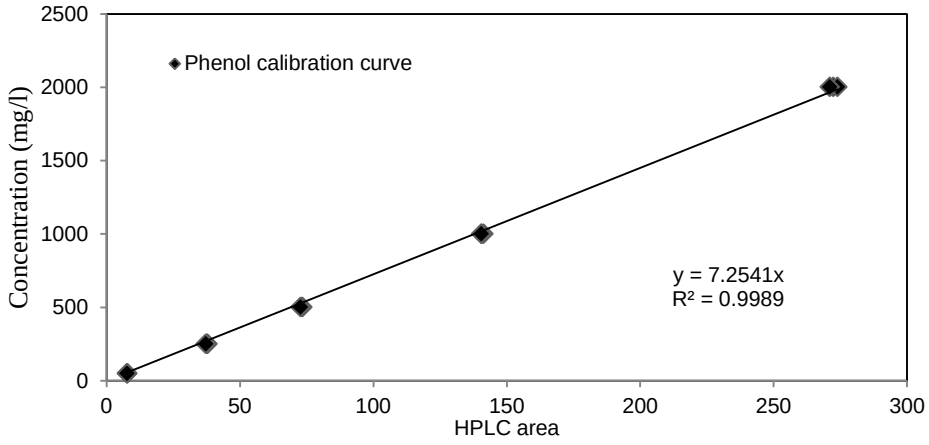

Figure A-2: Phenol calibration curve

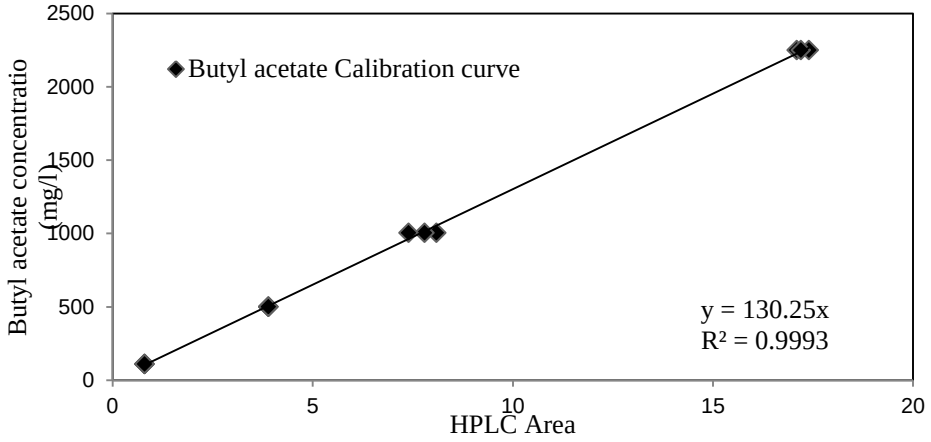

Figure A-3: Butyl acetate calibration curve

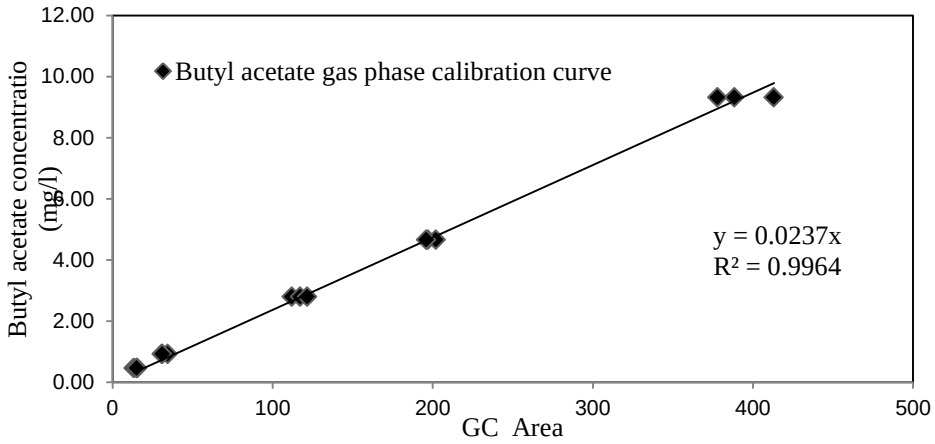

Figure A-4: Butyl acetate head space calibration

Appendix B

Comparison between Henry's Law estimates and GC measurements

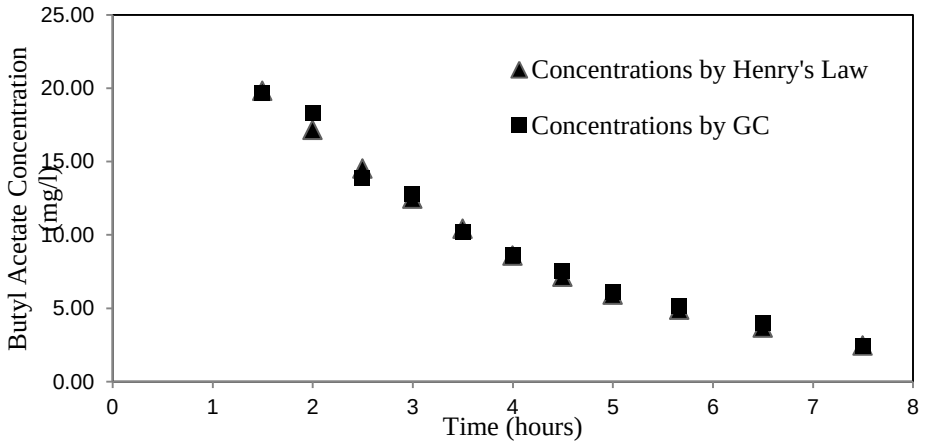

Figure B-1: Butyl acetate head space concentration estimated through Henry's Law and GC during a bench-scale stripping experiment.

Appendix C

Butyl acetate degradation in sealed serum bottles

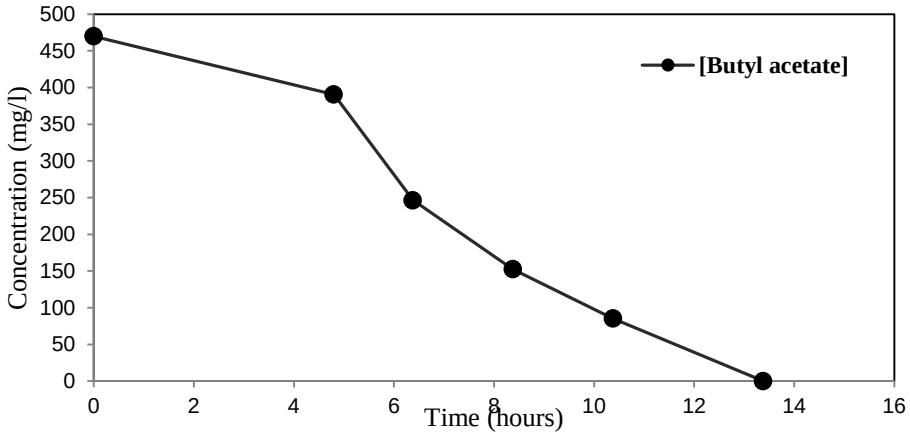

Figure C-1: Butyl acetate time course in degradation carried out in sealed serum bottles.