Review Article

Solid-liquid biphasic distributive bio systems; a novel method for removal of contaminants

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ABSTRACT

Solid-liquid biphasic biosystems contains two phases; aqueous phase containing the microorganisms and polymeric solid phase. Non aqueous phase has various applications including faster removal of hydrophobic contaminants and release them to the aqueous environment for better decomposition by microorganisms, controlling the concentration of toxic and inhibitor substrates and isolation of the limiting reagents of the reaction from the environment. The major advantages of polymer use are nontoxicity for microorganisms, improbability to be used as a carbon source and very low price compared to the organic liquid phase. Using cheap, non-volatile, inflammable and easily felxible polymers as the second phase leads to design a biological process with high efficiency and low cost which is called the green process without the solvent.

In thisreview, the mechanism of action of this type of biphasic systems and the methods to choose the solid phase was introduced followed by the various applications of these systems.

Keywords: Solid-liquid biphasic biosystems, polymeric solid phase, hydrophobic contaminant, inhibitor substrate

INTRODUCTION

The concept of biphasic systems was used in extractive fermentation, where a non-miscible solvent is circulated from a tube in order to remove the inhibiting reagents from the aqueous phase. Suitable solution for extractive fermentation process should have specific features including bioadaptibility, nondegradability by the microorganisms, non-toxic nature, high distribution coefficient for target molecules, low solubility in water and high selectivity. The advantages of using extractive fermentation process compared to the common continuous fermentation process could be high productivity without limiting reagents, reduction of water consumption to use compact food and reduction of isolation cost. The concept of extractive fermentation was expanded to biphasic distributive bioreactors where the insoluble solvent was used as controlling matter of the limiting substrate. In these bioreactors, the limiting substrate is distributed selectively between insoluble organic phase and aqueous phase containing the microorganism. Selection of organic solvent, the substrate is distributed in the aqueous phase that will not limit the microorganism. This is the major advantages of these type of systems when materials are toxic in high concentrations. Moreover, biphasic systems are automatically adjusted in response to metabolic activities of the cell until a microbial degradation occurs by a concentration gradient [1]. In brief, biphasic systems and their advantages and disadvantages can be categorized as Table 1.

Theoretical definitions, basics and principals

In this part, the common terms in biphasic systems including aqueous phase, non-aqueous phase, solid-liquid phase and dissociation coefficient are defined.

Aqueous phase

A phase which usually consists of water molecules and the microorganisms for survival.

Non-aqueous phase

In the system, solid phase is a polymer which the substrate or product is trapped in the network depending on the type of the process. But in general, it could be the organic solvent.

Biphasic solid-liquid biosystem

Biphasic system which the second phase is the polymer.

Dissociation coefficient

Dissociation coefficient or distribution coefficient is the ratio of the concentration of distributive matter between two phases. In biphasic systems it is the ratio of concentration of the desired substance in polymeric solid phase to the concentration of the same substance in aqueous phase and is shown by Ks/w. Distribution coefficient of various common polymers for biphenyl molecule is shown in Table 2.

It should be noticed that one of the main effective parameters in choosing the solid phase is dissociation coefficient and the affinity of target cell to different partitions should be studied before testing.

Table. 1. Some of the common biphasic systems

Biphasic systems	Advantages	Disadvantages
Biphasic aqueous-organic systems	Clarity of physical parameters, suitable bed for industrial processes	Lack of bioadaptablity, inflammability, on-volatility and toxicity of organic solvent
Aqueous biphasic system	High bioadaptibility, environment friendly	High cost of the solvent, high viscosity in high concentrations of the solvent
Reverse micelle	High contact area, low price of the solvent	Analysis and control of the problem of the process, weak bioadaptibility
Ionic liquid at room temperature	Substrate/product, green solvent for lack of volatility	Shortage of basic physical-chemical parameters

Table. 2. Distribution coefficient of common various polymers in biphenyl removal

Polymer	Kraton	Nucrel	Nylon	Desmopan	Elvax	Hytrel
Distribution coefficient	7072	2049	184	10987	4781	3234

Distributive biphasic solid-liquid biosystem

The ability of polymers to extract the selective constituents from aqueous solutions has been studied for decades. Using polymer as attractive and repulsive substance for target cell provides the possibility to overcome the limits in biphasic systems. The polymers can attract materials with low molecular weight is not deniable. This is the basis for many drug delivery systems using polymers. Polymers are cheap and can be turned into different shapes, sizes and non-destructive. As the design is in such way that resists against disintegration, therefore in contrast to biphasic liquid systems there is no concern for losing polymer in these systems. This feature causes ability for the system to use mixed microorganisms which is essential for BTX (bezene, toluene, xylene) operation [3]. Organic molecules which are in aqueous solutions such as aromatic compounds can significantly be absorbed by specific polymers and easily be separated. Recently, it has been clarified that organic solvent phase can be replaced by polymeric particles which absorb and release volatile contaminating compounds by the same method. These systems are known as distributive solid-liquid biphasic systems. Microorganisms cannot be used solid polymers as substrate. Therefore, polymers can be used with microbialuse. There is no possibility for absorption by reactor equipments such as washers, tubes and lids [4]. Moreover, there is a wide variety of polymers with different basic groups. This polymer to be chosen logically based on the molecules to be destructed. Using polymers as the second phase in TPPBs (two phase partitioning bioreactor) compared to organic liquid solvents is less dangerous for environment. As polymers can be made of recycled polymeric products, they are considered less toxic. Studies were conducted before 2010, SL-TPPB (solid liquid two phase partitioning bioreactor) were limited to toluene destruction which it has been proven that polymers can load toluene vastly and destruct in high concentrations [5].

Many liquid and solid phases have been used TPPB which silicon and Hytrel oil (a polar polymeric elastomer) are the most common non aqueous phases that have been studied. It is necessary to mention that the performance of TPPB made of a non-aqueous phase for the treatment of a complex mixture of contaminants depends on the similarity of the properties of each contaminants. If the properties of the contaminants including polarity or being in consistent with Henry's Law are significantly different, more than one organic phase is required for optimized microbial degradation process [6,7].

Mechanism of biphasic bioreactors

The basis for isolation of substances is the variety of the solubility of substances in phases. When two phases are in contact with each other's, the desired substance is moved into liquid phase from the solid phase based on the equilibrium between two phases and then the suitable reaction iscarried out. Therefore the concentration of the substance reduces in aqueous phase and mass transfer occurs from the solid phase to liquid phase to compensate this shortage. This action continues until the substances were used in both phases.

Estimated flow rates for gas substrate (organic hydrophobic volatile compounds and oxygen) in the common system (without the second phase) and biphasic system are shown in Fig 1A and 1B, respectively.

Substrate flow rate is shown as the product of mass transfer coefficient and gradient concentration between contact surface and substrate concentration in a specific volume. In the common system microbial activity (and the removal of volatile organic contaminant) is limited by the low speed of substrate transfer to aqueous phase. In biphasic system (the concentration of substrate is maintained in very low amounts for microbial consumption and high tendency of the substrate to the solid phase which causes an increase in transferring the substrate to aqueous phase. Some microorganism cantransfer to solid phase surface and directly absorb the contamination without transferring the contamination to the aqueous phase

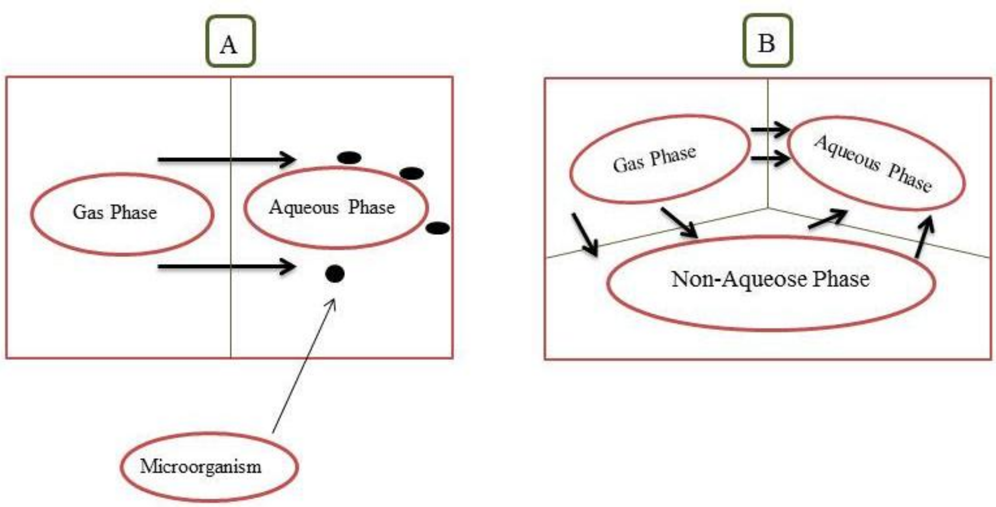

Fig. 1. Difference of mass transfer in biphasic and monophasic systems [8].

Depending on the solubility of the substrate and product in phases, there is a possibility of different mass transfers between two phases. For example, the product can stay in aqueous phase or transfer to solid phase again and separate from the phase containing the substrate by this transfer. If the substrate and the product are in two separate phases, primary purification has also been done.

Stages of choosing polymeric solid phase

Polymeric phase is available in various shapes and sizes but are usually used in cylindrical or spherical shapes with a diameter of 2-5 mm. Polymers have various properties which are resistance to microbial degradation and non-toxicity for microorganisms. Specie of microorganisms produces bio surfactants which are degrading hydrophobic compounds [9]. The production of this substances in liquid-liquid biphasic systems leads to the production of emulsion state and causes many problems during the process. While in solid phase systems such phenomenon does not occur which provides the possibility to use a wide variety of microorganisms and specifically a mixture of some types of microorganisms which are considered very important factors in removal of a mixture of contaminants including polycyclic aromatic hydrocarbons or polychlorinated biphenyl. Biphasic systems with organic liquid phase with a mixture of microorganisms can be chosen a non-toxic solvent for all the microorganisms used in the process. Therefore, the liquid organic phase in liquid-liquid biphasic systems is limited to some cases of silicon oil and branched alkenes [10,11].

Distribution of target cell between water and solid phase

The accuracy of choosing the suitable polymer as the carrier phase can be determined by distribution coefficient of target molecule between water and polymer. In all studies, if the goal is to transfer the hydrophobic or toxic substrate, target molecule has to be solved in a suitable solvent and then added to a system containing polymer and water. The amount of the target molecule which has entered the solid phase is measured by spectrophotometer.

Loading the carrier phase with target molecule

In this step, target molecule is added to a plate with solvent and solid polymer and the measurements and calculations for determining the amount of target molecule in solid carrier phase are carried out after a specific time.

Transferring target molecule from polymeric solid phase to the aqueous environment containing microorganism

This stage occurs to transfer of toxic hydrophobic substrate to the aqueous environment. The goal of this step, to find a polymer which can transfer all the loaded target molecules in its polymeric matrix into the liquid phase.

Various applications of liquid-solid biphasic systems

Gas treatment

The process of removal of volatile organic compounds (VOC) encounters the limitation of mass transfer. In the case which the contaminant is insoluble and toxic, the capacity of removal will decrease. The available low concentration gradient of transferring very hydrophobic species such as hexane or alpha-pinen significantly limits the transfer of contaminant to aqueous phase containing microorganism and its availability. For example, as for low affinity of hexane to water (Kg/w=71) the maximum driving force for mass transfer of 3 g/m^3 hexane to aqueous environment is 0.04 g/m^3. In return, maximum concentration gradient for acetone (hydrophilic contaminant with Kg/w=0.001) can reach up to 70000. On the other hand, long duration of contact of microorganism with aromatic compounds such as toluene and benzene can reduce microbial activity. According to the report of Monoz et al., in a continuous reactor for removal of toluene by pseudomonas, cells reduced from 100% to 51% in the first 4 days which leads to decrease in removal capacity from 134 g/m^3h to 10 g/m^3h [12].

Biphasic systems have the ability to overcome these limitations by high affinity of second phase to organic volatile contaminants. Recent studies have proven that using desmopan polymer as the second phase in gas/aqueous system increases the surface of gas mass transfer up to 3 fold. On the other hand, low concentration of contaminant has beneficial effects on cellular activity of microorganism because of the presence of polymeric phase. Another advantage of biphasic system in gas treatment is the ability of this system to create a buffer state when the intensity of contaminant changes. Therefore, polymeric phase reduces the contaminant concentration in high loading intensities [11].

It should be mentioned that in solid-liquid biphasic system, VOC treatment is carried out in higher loading intensities compared to common processes.

Sewage treatment

Biological methods do not have good performance in the presence of toxic substrates such as chlorinated phenyls or mono-aromatic hydrocarbons (benzene, toluene, xylene or BTX) in high concentrations. There are performance limitations in the presence of polycyclic aromatic hydrocarbons (anthracene, phenanthrene, naphtalene, benzopyrene), as these compounds are significantly toxic and therefore their solubility in water is somewhat acceptable. High toxicity of phenolic and polycyclic compounds for microorganism can be associated with the ability of this substance. In addition to membrane immersion, other effects such as inactivation and change in protein gradient can be observed. Also, when the contaminants are in high concentrations, a large amount of toxic intermediate compounds (even more toxic than the primary molecule) aggregate in culture media and decreases microbial activity which leads to decrease in degradation [11].

Biphasic systems are introduced as an effective method for microbial treatment of toxic aromatic contaminants. This technology is based on low concentration of contaminant in aqueous environment for high distribution of contaminant in solid phase and also controlling the substrate in aqueous phase. Therefore, transferring the trapped contaminant to solid phase depends on distribution coefficient of aqueous/solid phase and also the microbial consumption of the contaminant. Therefore, microbial degradation occurs in a state which the aqueous concentration of toxic substrate in biphasic system is lower than the inhibition state [14]. Similar to gas treatment by biphasic system, a sudden change in the concentration of contaminant is controlled and neutralized by solid phase which is considered a performance advantage of these systems [15].

The presence of solid phase in biological degradation process significantly increases oxygen transfer to sewage treatment process. The process of changing organic contaminants such as aromatic hydrocarbons requires a lot of oxygen. 260% increasing in oxygen transfer in the presence of second organic phase compared to monophasic state has been reported in references [16].

Different non- aqueous phases have been tested for degradation of toxic substrate which silicon oil and Hytrel polymer have been mostly used. Transferring toxic substance from polymer to aqueous state depends on two factors: The condition of mixing and contact surface of polymer/water.

Subsequently, contact surface depends on the amount of the solid phase [11].

The available intensity of degradation in biphasic systems is clearly highcompared to common systems in the same conditions.

Soil treatment

Contamination of soil to hydrocarbons is drop of oil and its derivatives in the transferring process. Among the existing methods, in order to reduce soil contamination, biological treatment has low cost and the capability to reuse the soil. With the current advantages, there are still serious limitations. The first limitation is absorption and distribution of the contaminants in soil matrix for its hydrophobic nature. This limitation causes inaccessibility of contaminant to the microorganism and therefore its death. The second limitation for the microorganism is the toxicity of soil contaminants (for example BTXs, PAHs, polycarbonates and biphenyls) especially in high concentrations of the contaminant. Another limitation is the steam evaporation during drilling soil which can be caused genetic mutations and cancer [17]. The requirements to overcome the mentioned limitations lead to studies over biphasic devices. In these system the polymer can extract the contaminant distributed inside the soil and also can prevent the evaporation of dangerous contaminants and death of microorganisms. Using biphasic systems generally consist of two steps:

As it has been shown in Fig. 2, at first the soil is extracted from soil because of its high affinity to the polymer (extraction step) and in the next step, biological degradation occurs under controlled conditions (degradation step).

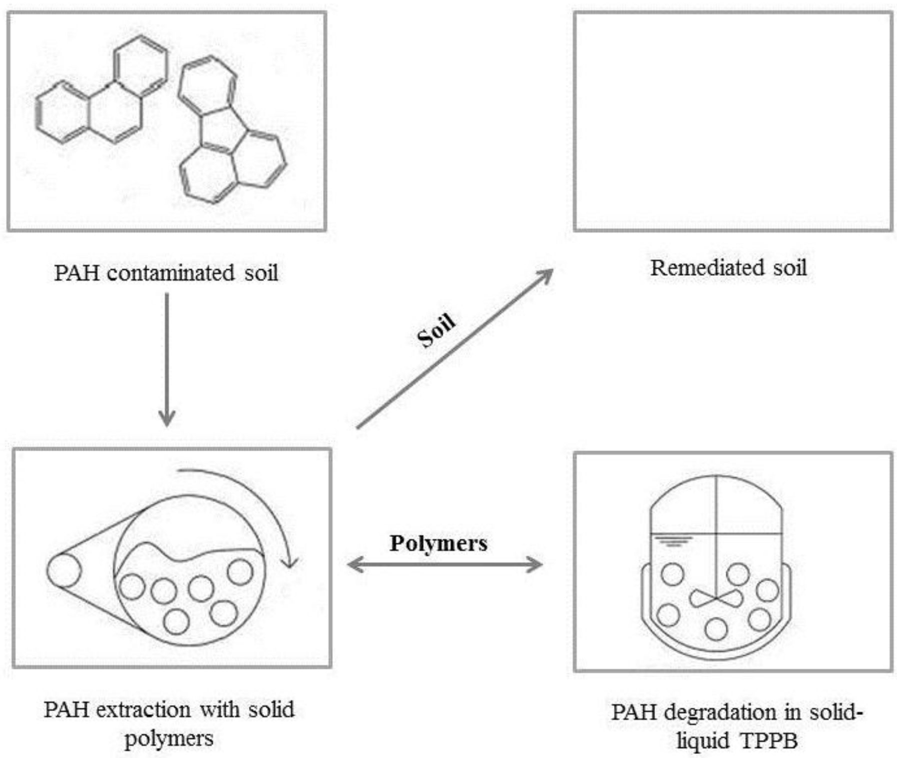

Fig. 2. The process of removal of contaminated soil with PAH, treatment process includes PAH recycling from the soil followed by its removal from biphasic system [20].

In soil treatment, degradation and removal depends on choosing the solid polymeric phase in which the target contaminant has a high affinity compared to the soil matrix so that a more efficient extraction can be observed [18]. Contaminant extraction from soil and its transfer into the solid phase can be increased significantly using carriers such as ethanol, isopropyl alcohol, hexane and octane. Desmopan (polyurethane) and Hytrel (polyester) are considered as common polymers which are used in extraction of soil contaminant. In the case of Hytrel, extraction yield of 96% has been obtained for phenol in the absence of solvent. In some cases, using solvent, a low efficiency has been obtained which emphasizes on the importance of choosing a suitable solid phase [19].

The intensity of microbial degradation of the contaminant in biphasic system is significantly higher than the common biphasic bioreactor. For example the intensity of the removal of phenanthrene in biphasic systems with desmopan polymer is about 1.7 mg/L.h which is 12 fold higher than the highest degradation intensity in common biphasic bioreactors [20]. Using surfactants in biphasic bioreactors increases microbial degradation up to 0.45 mg/L.h but is still 4 fold less than the accessible amount in solid-liquid biphasic system. With the mentioned advantages using solid-liquid biphasic systems in soil treatment, there is a lack of study in choosing the suitable solid phase. Choosing the suitable solid phase for soil treatment is commonly based on the high affinity of solid phase to the target contaminant regardless of the interaction between solid phase, soil matrix and the contaminant [21]. Researchers have found that not only the extraction of contaminant depends on its affinity to solid phase, but also depends on soil properties. Therefore, more studies are required considering the use of solid-liquid biphasic system in soil treatment [11].

The process of fermentation and production

Biological processes have two major disadvantages compared to chemical processes: low productivity and low concentration of final product.

The reasons for these problems can be related to the toxicity of the product or the substrate. Biphasic systems are introduced as a possible solution for the transfer of substrate and isolation of the product *In situ*. One type of biphasic systems is the solid-liquid biphasic systems which are used solid polymer properties such as non-toxicity, inaccessibility for the microorganism and the possibility for recycling. At first, polymer was used as the second phase for the isolation of product i*n situ* for the production of 3-methylcathechol from toluene which the polymer had overcome the toxicity of the final product and the concentration of the product and then productivity had been increased [22].

For example, in a research, changing carveol to carvone was studied. In this case both the substrate and final product had limiting properties for the microorganism and these were toxic. Using one type of polymer (copolymer of styrene/butadiene) with another polymer (Hytrel

8206) in an external column through which the culture media returns from it. Results have been shown in Table 3 in summary [23].

Table. 3. A summary of bioreactor performance for carvone production

System /variable	Input carveol (ml)	Time(h)	Volumetric production (mg/L.h)
Monophasic	5	15.25	31
Liquid-liquid biphasic	13	28.75	29
Type 1: solid-liquid biphasic	25	34.25	102
Type 2: solid-liquid biphasic	15	24	106
Type 3: solid-liquid biphasic	35	48.75	99

It should be mentioned that the microorganism used in this test, was *R.eurythropolis* and the organic phase for liquid-liquid state was silicon oil.

CONCLUSION

After proving the probability of replacement of organic phase with solid polymer, many studies were conducted using this system in biosynthesis and biological treatments. The major advantage of using this polymer is nontoxicity for the microorganism, not be used as carbon source and very low price compared to organic liquid phase. For example, Hytrel polymer costs less than 5 pounds for a kilogram. Using cheap, non-volatile, inflammable polymers with easy plasticity as the second phase leads to design a biological process with high efficiency and low costs which is called the green process without solvent. Substances such as toluene, phenol, BTX, 4-nitrophenol, hexan and payene have been degraded by biphasic systems with the polymer. Solid-liquid system has been shown a good performance. Liquid biphasic systems are generally limited to using silicon or HMN (hexa methyl disilazane), especially when we are facing a mixture of microorganisms for removal.

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