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Development of a Supported Emulsion Liquid Membrane System for Propionic Acid Separation in a Microgravity Environment

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Abstract Perstractive fermentation is a good way to increase the productivity of bioreactors. Using *Propionibacteria* as the model system, the feasibility of using supported emulsion liquid membrane (SELM) for perstractive fermentation is assessed in this study. Five industrial solvents were considered as the solvent for preparing the SELM. The more polar a solvent is, the higher the partition coefficient. However, toxicity of a solvent also increases with its polarity. CO-1055 (industrial decanol/octanol blend) has the highest partition coefficient toward propionic acid among the solvents that has no molecular toxicity toward *Propionibacteria*. A preliminary extraction study was conducted using tetradecane as solvent in a hydrophobic hollow fiber contactor. The result confirmed that SELM eliminates the equilibrium limitation of conventional liquid-liquid extraction, and allows the use of a non-toxic solvent with low partition coefficient.

Keywords: microgravity, supported emulsion liquid membrane, hollow fiber contactor, extraction, toxicity

INTRODUCTION

Bioreactors can be used aboard spacecraft to overcome gravity-induced limitations in cell growth. Such conditions are particularly useful for the formation of three-dimensional tissue. Currently, these space bioreactors are not integrated into an overall bioprocess scheme which allows water reuse and byproduct removal. High ground-to-orbit launch costs as well as the utility costs of refrigeration for storage makes the media requirement quite substantial in space experiments. It is clear that purification and reuse of complex media are needed for long duration cell culture in outer space. On earth, media is typically discarded after a single use, which usually leads to expensive wastewater treatment afterwards; therefore, it is also beneficial to recycle media on earth as well. In addition, if the unconverted substrate is recycled rather than discarded, the recycling of growth media should maximize the conversion of nutrient substrate.

A key step in media recycle is the removal of inhibitory compounds from the fermentation broth, which can be accomplished via extraction. When extraction is conducted online during the fermentation, the process is referred to as extractive fermentation. In extractive fermentation, the media is brought to contact with an organic solvent. The inhibitory compound partitions into the solvent and the rejuvenated media is returned to the bioreactor. The effectiveness of extractive fermentation has been demonstrated for various fermentations including ethanol, acetic acid, and propionic acid [1-4].

The main design consideration for extractive fermentation is the selection of solvent. An ideal solvent for extractive fermentation must have a favorable partition coefficient for the desired compounds, a low solubility in water, and a minimal toxic effect on the microorganism. Unfortunately, these conditions often compete with each other and a compromise must be made. Organic solvents are often toxic to cells and prevent growth or cause cell death. The organic solvent can rupture the cell membrane, causing massive leakage of the intercellular constituents [5]. The contact of the cell with a bulk organic phase can cause mechanical damage to the cell due to the relatively high surface tension of the solvent-water interface [6]. Toxicity caused by direct contact between the microorganism and the solvent is termed "phase" toxicity while the toxicity caused by trace amounts of organic compound dissolved in water is commonly referred to as "molecular" toxicity [7]. Phase toxicity may be avoided by using a barrier between the cells and the solvent such as a membrane contactor. Molecular toxicity is, in general, a greater concern. Unfortunately, solvents with favorable partition property often possess high molecular toxicity [8]. If a solvent of low partition coefficient is used, the extraction will soon reach equilibrium and stop removing the inhibitory compound from the media.

Supported emulsion liquid membrane (SELM) extraction is an ideal way to carry out extractive fermentation because it alleviates the constraints in solvent selection. SELM extraction has been successfully used in

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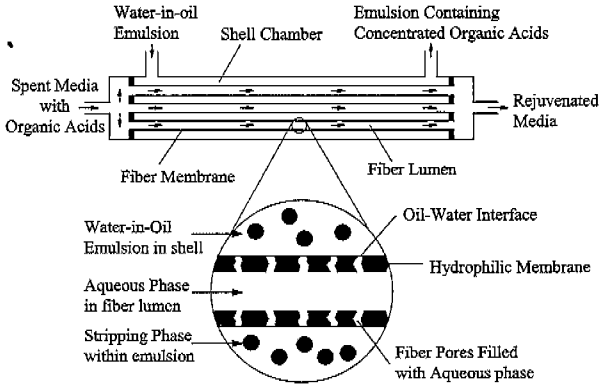

Fig. 1. Propionic acid extraction in a hydrophilic HFC. Spent media with organic acid passes through the lumen side of the membrane while ELM passes through the shell side of the HFC. Because the fiber material is hydrophilic, the aqueous feed phase will penetrate the membrane pores. By applying a higher pressure in the ELM side, a stable interface can be maintain between the feed phase and ELM while providing a large contacting area.

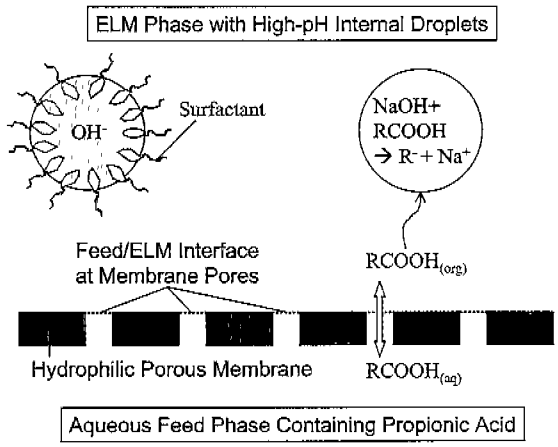

Fig. 2. Propionic acid extraction by SELM. Propionic acid molecule in the feed phase first partition into the organic solvent at the membrane pores. The molecule then diffuses through the liquid membrane and reaches the internal droplet of the ELM. Propionic acid is stripped into the droplet phase because the internal phase is highly basic.

the extraction of heavy metals and organics [9-13]. The SELM extraction employs a polymer membrane contactor (*e.g.* flat sheet or hollow fiber unit) to facilitate the transfer of the solute from the feed stream to an emulsion liquid membrane (ELM) phase (see Fig. 1 and Fig. 2). The ELM phase contains numerous microscopic aqueous droplets stabilized by surfactant in a continuous organic phase [14]. In SELM extraction, the solute first partition into the organic solvent at the membrane surface then diffuses through the liquid membrane. Once the solute reaches the surface of an internal droplet, it is immediately stripped into the internal phase by an appropriate chemical reaction. In the case of propionic acid, a basic internal phase such a sodium hydroxide solution may be used as the internal phase reagent. Because of the presence of the internal phase and the stripping reaction, the concentration of the solute in the organic phase does not achieve equilibrium levels. Since the equilibrium limitation of extraction is no longer a concern for SELM systems, a solvent of low molecular toxicity (yet low partition coefficient) may be used.

An additional benefit of SELM extraction in the microgravity environment is that the usage of surfactant may be eliminated. Typically, surfactant is required to stabilize the internal aqueous phase in the organic solvent in ELM due the density difference between the two phases. However, use of surfactant leads to problems such as swelling and makes the product recovery more difficult [12]. In microgravity, the internal droplets can be formed by simple mechanical agitation; density difference will not result in phase separation and a large contacting area will be available to the stripping reaction without the use of surfactant.

The goal of this study is to establish the feasibility of using SELM technique to extract propionic acid from the fermentation media. Five organic solvents are tested for toxicity. Preliminary propionic acid extraction experiments using plain solvent and SELM are conducted and the efficacy is evaluated.

MATERIALS AND METHODS

Microbial System and Media

Three *Propionibacteria* strains (P15, P20, P127) were obtained from Iowa State University through the courtesy of Professor B. Glatz. These three strains are known to produce the growth inhibitory compound propionic acid during their growth. Stock cultures are maintained at -80°C in sodium lactate broth with 50% glycerol. The broth (1 liter) is made by dissolving 10 mL sodium lactate 60% syrup, 10 g yeast extract and 10 g trypticase soy broth in deionized water. Working cultures are maintained at 4°C on sodium lactate agar plates [4]. In batch fermentation, cells from a working culture are transferred to sodium lactate broth in a sponge-capped flask and incubated at 32°C in an anaerobic incubator.

Organic Chemicals

CO-898 (octyl alcohol), CO-1055 (decyl/octyl alcohol) and CO-1270 (fatty alcohol having 68-74% C12 alcohol, 24-30% C14 alcohol) were generously provided by Procter & Gamble Chemicals (New Milford, CT, USA). Mineral oil and propionic acid (99%), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 10 N sodium hydroxide solution, used as the stripping phase, was purchased from Fisher Scientific, Fair Lawn, NJ, USA. Solvent *n*-tetradecane (technical grade) was

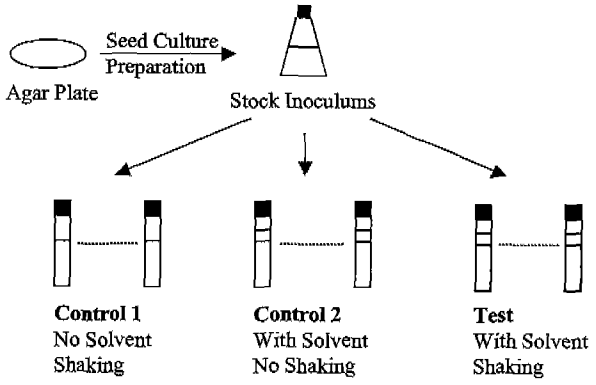

Fig. 3. Toxicity test for organic solvents. Various solvents are brought into contact with the culture in culture tubes. Without shaking, the cell is less likely to have direct contact with the solvent and any toxicity is mostly attributed to molecular toxicity. When the culture tube is shaken, both molecular toxicity and phase toxicity become factors. Cell viability is assessed by mesuring the optical density of the culture over time.

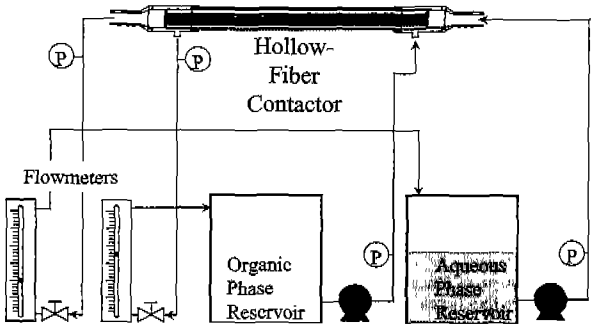

Fig. 4. A typical configuration for HFC extraction. In this case, both phases are delivered by a pump in total-recycle mode. The organic/ELM phase flows through the tube side while aqueous phase flows through the shell side of the HFC. The flow rate is controlled by the speed of the peristaltic pumps while pressure is controlled by the openness of the needle valves downstream of the HFC.

purchased from Zeeland Chemicals, Inc. (Zeeland, MI, USA). Paranox 106, which is the surfactant to stabilize the emulsion, was provided by Exxon Chemical Company (Houston, TX, USA).

Solvent Toxicity

Mineral oil, fatty alcohols and their combinations have proven successful in related work [5] and have been studied as part of this research. First, the seed culture was prepared by inoculating an isolated colony from a working culture plate into a 250 mL flask, which contains 200 mL sodium lactate broth, and incubating it at 32°C for approximately 60 h (until obvious turbidity is observed). Then a 1.0% (v/v) inoculum of this broth was added to 800 mL fresh medium, which was distributed to 50 mL sterilized culture tubes afterwards. Each tube contains 30 mL medium broth and all the tubes are divided into three groups (Fig. 3). One of these groups serves as control 1, it has no solvent and is shaken at 250 rpm. The other two groups have solvent; one of them is shaken at 250 rpm (test group) while the other is incubated without shaking (control 2). Every 12 h, one tube from each group was withdrawn and its optical density measured at 660 nm.

The comparison between control 1 and test groups gives the effect of solvent and the comparison between control 2 and the test groups gives the effect of shaking when solvent is present. If there is visually obvious difference in the optical densities between the two comparisons, then it can be concluded that the effect is strong; otherwise, the effect is negligible.

Concentration Analysis

Concentration of the propionic acid in the aqueous solution was analyzed by high-performance liquid chromatography (Aminex® HPX-87H HPLC organic acid analysis column 300 × 7.8 mm, Bio-Rad). The method used in this part of research is a modification of that in the literature [15]. The HPLC system was operated at 65°C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 mL/min. Peaks were detected with a UV detector at 210 nm. The propionic acid concentration versus peak area was calibrated by standard solutions and the retention time for the propionic acid is found to be 20.3 ± 0.1 min.

Determination of Partition Coefficients

A 20 mL aqueous sample containing a known concentration of propionic acid (either measured by HPLC, or prepared as a standard) is mixed with 20 mL organic solvent, and shaken over 8 h to allow full extraction. Then, 10 mL after-extraction solvent is back extracted by 10 mL 1 M NaOH solution for at least 8 h. The propionic acid concentration in the sample after extraction with solvent and in the NaOH after back extraction is analyzed by the HPLC method. Since the NaOH solution after back extraction is too caustic to inject directly onto the HPLC column, 0.1 mL 98% H_2SO_4 is used to neutralize 5 mL NaOH before HPLC analysis.

Simple Extraction and SELM Extraction

The experimental set up is that illustrated in Fig. 4. In a simple extraction experiment, 500 mL tetradecane was circulated through the tube side of the hydrophobic module, while 2,500 mL 5% propionic acid passed through the shell side. Both sides were run in the total recycle mode. 1 mL aqueous sample was collected every few hours from the shell side, and the change in propionic acid concentration was monitored by HPLC analysis. In SELM separation, 500 mL Tetradecane was mixed

Table 1. Specifications of Enka LM-2P06 hollow-fiber contactor

Material	Length	Shell ID	Fiber Number	Pore Size	Fiber OD	Fiber ID
Polypropylene	24 cm	1.5 cm	85	0.2 μm	0.1 cm	0.06 cm

Table 2. The toxicity study on organic solvents and their mixtures (Test, Control 2 vs. Control 1)

	Shaking (Test)			No Shaking (Control 2)		
	P15	P20	P127	P15	P20	P127
CO-898 (C-8 alcohol)	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
CO-1055 (C-10/11 alcohol)	Toxic	Toxic	Toxic	Nontoxic	Nontoxic	Nontoxic
CO-1270 (C-12/14 alcohol)	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic
Mineral oil	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic
CO-1270/Mineral oil (3/2, v/v)	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic

with 214 mL 1 N NaOH (Tetradecane/NaOH, v/v=7:3) and 5% (w%) paranox 106 was added to stabilize the resulting emulsion. The emulsion was then pumped through the tube side of the hydrophobic module in total recycle mode, while 2,500 mL 5% propionic acid ran through the shell side in total recycle mode. The HFC used is Enka LM-2P06 (Microdyn Technology, Raleigh, NC, USA). The specifications of the HFC are listed in Table 1.

RESULTS AND DISCUSSION

Toxicity Study

CO-1270, mineral oil, CO-1270/mineral oil (3/2, v/v) mixture, CO-898, and CO-1055 were evaluated for toxicity by the method described above. Fig. 5 shows the growth curves from the toxicity studies of two representative solvents. Plot (a) is the study of mineral oil. Since mineral oil is insoluble in water, molecular toxicity is not expected. The "No Shake" curve, which is intended to measure molecular toxicity, does show a lower cell density than the control experiment. Because shaking facilitates the delivery of nutrient to cells, the lower cell density observed here is more likely due to nutrient deficiency than solvent toxicity. Phase toxicity of mineral oil is insignificant as well, as reflected by the "Shake" curve. Plot (b) shows the effect of CO-898 as a solvent. It is obvious the solvent has detrimental effect on cell viability. The molecular toxicity alone has completely prohibited cell growth so phase toxicity is inconsequential. The results of the toxicity studies are summarized in Table 2. It can be seen that CO-1055 is very toxic to the microbial system under shaking conditions while it is tolerable under non-shaking conditions. CO-1055 is a typical example of solvent with low molecular toxicity but high phase toxicity; shaking increases the direct contact of the solvent with the cells, hence phase toxicity increases. Another possible explanation is that shaking also increases the amount of solvent dissolved in the media, which causes higher molecular toxicity. However, given the length of the study (60 h), dissolution of solvent should have reached equilibrium early in the experiment and should not play a role here.

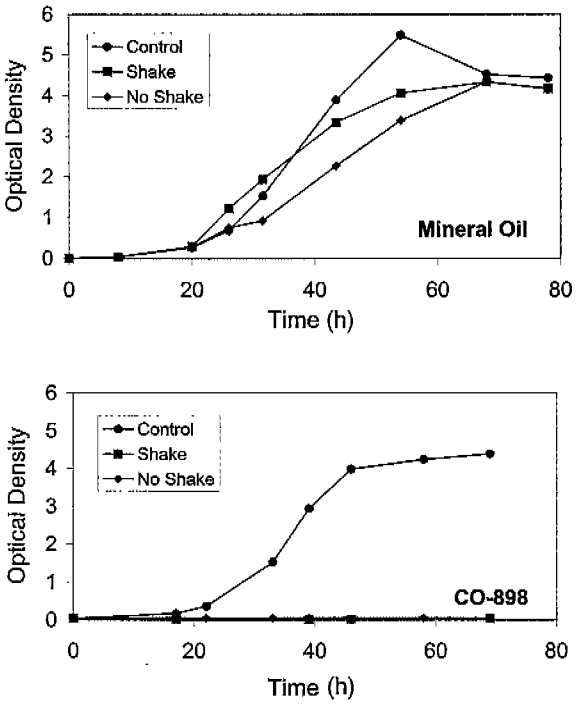

Fig. 5. Growth curves from toxicity studies. Toxicity of two representative solvents is shown: (a) mineral oil, which does not show significant impact on cell viability; (b) CO-898, which is highly toxic.

The results in Table 2 are consistent with that reported in the literature, *i.e.* less polar solvents are less toxic [5]. Laane *et al.* has established a correlation between solvent toxicity and the log *P* value of the solvent [8]. Log *P* is the logarithm of the partition coefficient of a given compound in the standard octanol-water two-phase system (*P*=[Solvent in Octanol]/

Table 3. Partition coefficients for some organic solvents

Solvent	K_p
Mineral oil	0.0174
CO-1270/Mineral oil (1/1, v/v)	0.596
CO-1270/Mineral oil (3/2, v/v)	0.779
CO-1055	1.590

Where $K_p = [RCOOH]_{org}/[RCOOH]_{aq}$.
The unit of concentration is g/mL.

[Solvent in Water]). It quantitatively reflects the polarity of the solvent – the higher the log *P* value, the less polar the solvent. Increased toxicity is associated with increased polarity of the organic solvent, and it is reported that the onset for toxicity is in the log *P* range of 4-6; solvents with log *P* values less than 4 are most likely to be toxic to microorganisms, while solvents with log *P* values greater than 6 will have minimal toxic effect on cells. For those solvents whose log *P* values are between 4 and 6, the toxicity depends on the microorganism used [16,17]. CO-898, whose main component is octanol, has a log *P* of 2.86 and is toxic to the microorganism [5]. As the length of the carbon chain increases, polarity of fatty alcohol decreases. Dodecanol, which is the main ingredient of CO-1270, is reported to have a log *P* of 7.69 [5]. The currnet experiments confirm that CO-1270 does not display molecular toxicity toward the *Propionibacteria* cells. Yabannavar *et al.* showed a nontoxic solvent can be made by mixing a toxic solvent (log $P < 4$) and a nontoxic solvent (log $P > 6$) together [18]. However, the partition coefficient is likely to decrease with such practice.

Partition Coefficient Measurement

An important advantage of the SELM technique is that it allows the utilization of low toxicity solvents, which, as mentioned before, may have low partition coefficients for organic acids. The binding capability of the internal aqueous stripping phase with the organic acids moves the overall partitioning in a favorable direction, and therefore makes the need for a high partition coefficient less important.

In Table 2, the measured partition coefficients of several organic solvents are listed. The partition coefficient is defined as the ratio of propionic acid concentration in the organic phase to that in the aqueous at equilibrium. As expected, solvents that have stronger polarity also have higher partition coefficient. Mineral oil, which is a non-polar solvent, has a partition coefficient of only 0.0174. CO-1270 did not show any toxicity in this study. However, due to its high freezing point, using pure CO-1270 as the solvent for extraction is impractical. Thus, CO-1270 was diluted in mineral oil, which does not have toxicity either, for this part of the study. As the concentration of CO-1270 increases, polarity of the solvent increases and the partition coefficient also increases. CO-1055 has the same –OH functional group as CO-1270 does but has a shorter carbon chain. As a result, CO-1055 has the strongest polarity and the highest partition coefficient of 1.590 among the solvents tested. Although CO-1055 shows phase toxicity toward the cells, molecular toxicity is the main concern in membrane-based extraction because solvent will not contact the cells directly.

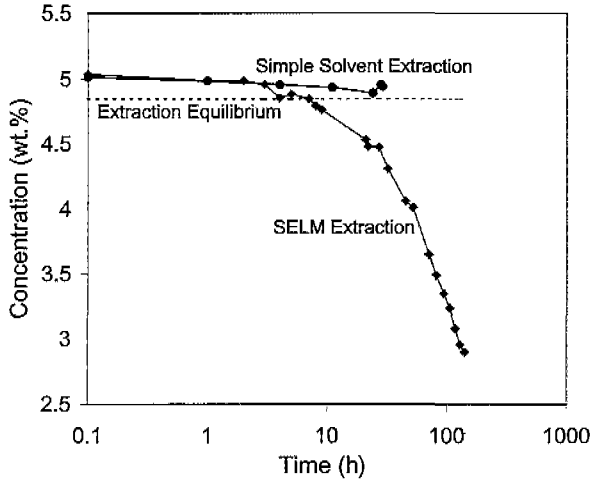

Fig. 6. Comparison between simple and SELM extraction. Simple extraction is limited by the equilibrium partitioning.. With SELM extraction, the concentration level in the aqueous phase is reduced to values much lower than the partition equilibrium because of the presence of the stripping phase (NaOH).

Simple and SELM Extraction

Propionic acid extraction using plain solvent and SELM were conducted and compared. It should be noted that in this preliminary experiment, the findings in the toxicity study and partition coefficient study were not incorporated. Tetradecane, which has a similar polarity as mineral oil, is used as the solvent. The purpose is to demonstrate the potential of the SELM technique in extractive fermentation. Toxicity and partition coefficient information will be taken into account in future extraction experiments.

Fig. 6 compares the extraction of propionic acid from an aqueous feed phase (initial concentration of 5 wt.%) with plain tetradecane and SELM. In both cases, the same amount of tetradecane (500 mL) was used. The volume of the aqueous feed phase is 2,500 mL. The equilibrium concentration of propionic acid in the aqueous phase was determined to be 4.85 wt. % under these conditions, as represented by the dash line in the figure. As expected, simple solvent extraction cannot decrease the feed concentration beyond the equilibrium concentration as indicated by cicular data points. SELM extraction, on the other hand, can reduce the feed concentration to far below the equilibrium line even though tetradecane has a relatively small partition coef-

ficient of 0.13. The stripping phase in SELM removes propionic acid from the organic phase and provides it with a very high extracting capacity. This observation confirms that a solvent of high partition coefficient is not necessary with SELM extraction and less toxic solvent may be used without hampering the extraction capability.

It is very important to note that the SELM extraction was terminated prematurely even though the rate of extraction was steady. The overall slow extraction kinetics was due to the fact that membrane material has not been optimized in this preliminary experiment. As a result, the membrane resistant is high and it took about 140 hours to reduce the propionic concentration from 5 wt. % to 2.9 wt. %.

Resistance of mass transfer in membrane devices may be broken down into three terms: resistance in the aqueous phase, resistance in the membrane, and resistance in the organic phase [9]. When partition coefficient is greater than 1.0, a hydrophobic membrane is preferred, when it is less than 1.0, a hydrophilic membrane should be used to minimize the resistance of mass transfer. The overall slow extraction kinetics observed in the current study is a result of using a hydrophobic membrane on a system with small partition coefficient.

We expect the efficiency of the SELM extraction to increase dramatically once the membrane material and solvent are optimized for propionic acid extraction. In the current study, the propionic acid concentration was reduced to half of its initial concentration in 173 h while it took *Propionibacteria* about 40 h to reach growth inhibition due to propionic acid accumulation. If a continuous, perstractive fermentation is desired, the current SELM system does not provide enough capacity for the online extraction of propionic acid. Because membrane resistance is the main hurdle in the current study, increasing partition coefficient of solvent alone should greatly improve the extraction kinetics. For example, if CO-1055 (K_p = 1.59) was used instead of tetradecane (K_p = 0.13), the extraction kinetics will improve by more than an order of magnitude. The SELM system will have more than enough capacity for online extraction. Increasing the membrane area and changing to appropriate material (hydrophobic or hydrophilic depending on solvent) will also improve the overall extraction kinetics.

CONCLUSION

Bioreactors have been widely used aboard spacecraft to overcome gravity-induced limitations in cell growth. However, these space bioreactors are not currently integrated into an overall bioprocess scheme which allows water reuse and byproduct removal. The goal of this research is to develop a SELM system to remove low molecular weight organic acids, which usually inhibit cell growth, from fermentation media to facilitate water reuse.

We have screened several solvents for SELM extraction by assessing their toxicity and partition coefficient. Mineral oil, the least polar solvent studied, has no toxicity to *Propionibacteria* but has a partition coefficient of only 0.0174 toward propionic acid. Other solvents studied are fatty alcohols or mixtures of fatty alcohol and mineral oil. As the length of carbon chain in fatty alcohol decreases, polarity, toxicity and partition coefficient increases. Octanol is highly toxic to the microorganisms; decanol has phase toxicity but no molecular toxicity, while dodecanol and tetradecanol are not toxic. These results are consistent with the literature, *i.e.* partition coefficient and toxicity increase with solvent polarity. Because the cells do not directly contact the solvent in SELM extraction, molecular toxicity is the main concern. Thus, decanol is the most suitable solvent for SELM extraction of propionic acid.

In a preliminary study, tetradecane was utilized as the solvent for simple extraction and SELM extraction in a hydrophobic HFC. The results indicate that SELM is capable of extracting propionic acid to levels much lower than the partition equilibrium concentration. However, the data also suggest that proper membrane selection is crucial in maximizing the mass transfer rate As the partition coefficient of tetradecane is less than 1 for propionic acid, a hydrophilic membrane should yield faster extraction since the mass transfer resistance within the membrane is minimized. Future studies will incorporate the results from toxicity and partition coefficient studies presented here. The potential and feasibility of using SELM for perstractive fermentation is clearly demonstrated. Once the solvent and membrane material are optimized, SELM extraction is expected to be a highly efficient means to separate inhibitory compounds during fermentation.

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