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Ionic liquid-based \textit{in situ} product removal (ISPR) design exemplified for an ABE fermentation

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Abstract

Selecting an appropriate separation technique is essential for the application of \textit{in situ} product removal (ISPR) technology in biological processes. In this work, a three-stage systematic design method is proposed as a guide to integrate ionic liquid (IL)-based separation techniques into ISPR. This design method combines the selection of a suitable ISPR processing scheme, the optimal design of an IL-based liquid-liquid extraction (LLE) system followed by process simulation and evaluation. As a proof of concept, results for a conventional acetone-butanol-ethanol (ABE) fermentation are presented (40,000 tone/year butanol production). In this application, ILs tetradecyl(trihexyl)phosphonium tetracyanoborate ([TDPh][TCB]) and tetraoctylammonium 2-methyl-1-naphthoate ([TOA][MNaph]) are identified as the optimal solvents from computer-aided IL design (CAILD) method and reported experimental data, respectively. The dynamic simulation results for the fermentation process show that, the productivity of IL-based \textit{in situ} (fed-batch) process and \textit{in situ} (batch) process is around 2.7 and 1.8-fold that of base case. Additionally, the IL-based \textit{in situ} (fed-batch) process and \textit{in situ} (batch) process also have significant energy savings (79.6% and 77.6%) when compared to the base case.

Keywords: ISPR, ionic liquid, ABE fermentation, CAILD, process design

1. Introduction

Many bioprocesses involve the use of living microorganisms to produce chemical and biochemical products, and in the last decade in particular they have received considerable attention since the conditions for biosynthesis are usually mild. More importantly, they also provide the possibility of using sustainable feedstocks. However, there are also some limitations, and these are mostly related to product inhibition, giving a low productivity and highly diluted product stream leading to difficult downstream separation. Here, improved reaction conditions as well as rapid inhibitory product removal from the fermentation broth may bring the possibility to overcome these constraints. Indeed, in the past few decades, \textit{in-situ} product removal (ISPR) technology has been introduced as one of the potential tools for mitigating the problem of product inhibition. The applications of ISPR have been widely studied in whole-cell biotechnology. In general, different ISPR-integrated fermentation schemes can be applied, dependent on the location (internal or external) of the product removal (direct or indirect). Among all the ISPR processing schemes, the internal product removal has lower equipment costs due to both reaction and product removal taking place in the same vessel. While external product removal allows easier process control and reduces the contact time between biocatalyst and organic solvent. In addition, direct product removal does not need a physical barrier such as membrane, while indirect product removal reduces solvent problems such as biocompatibility and toxicity. Further details on different ISPR schemes using IL solvents are provided in Section 2.1.

Currently, ISPR technologies are being used in bioprocesses for the production of biofuels, antibiotics, drugs and food additives. In ISPR, both product capacity and product removal rate are critical since they...
both significantly affect the process operating and capital costs. Therefore, the selection of an appropriate separation method is of great importance for application of ISPR technology.\textsuperscript{1} To date, various separation methods (e.g. membrane-based,\textsuperscript{6,7} crystallization-based,\textsuperscript{8} chromatographic\textsuperscript{9}) have been studied and applied dependent upon the nature of the product to be removed. Organic solvents such as heptane and toluene are typically used for the recovery of small molecules because of their immiscibility with aqueous media.\textsuperscript{10} However, most of the conventional organic solvents are volatile and toxic. Unlike most commonly used organic solvents, ionic liquids (ILs) have a low-volatility and are non-flammable, providing alternatives for the chemical, biochemical and other industries.\textsuperscript{11,12} Until now, ILs have been widely studied as solvents for gas separations\textsuperscript{13,14} (e.g. CO\textsubscript{2} capture,\textsuperscript{15} shale gas purification\textsuperscript{16}) and energy intensive liquid-liquid separation.\textsuperscript{17-19} Additionally, applications of ILs in biotechnology have also been in focus over the past few decades.\textsuperscript{20,21} For example, they have been used as extractants for the recovery of various small molecules (e.g. biofuels,\textsuperscript{22-24} alkaloids,\textsuperscript{25,26} amino acids,\textsuperscript{27-30} phenolic compounds,\textsuperscript{31} isoprene\textsuperscript{32}) produced by fermentation or by biosynthetic routes. More applications of IL-based techniques for the extraction and separation of bioactive compounds have also been summarized in a critical review from Ventura and co-workers (2017).\textsuperscript{33} Recently, poly(ionic liquids) (PILs) have also attracted interest in biotechnology due to their unique properties.\textsuperscript{34} For example, polymerization of cytotoxic ILs can successfully yield biocompatible absorbents with excellent absorptive performance for the recovery of biobutanol.\textsuperscript{35} In this work, PILs are not considered due to the lack of suitable property models to describe systems containing these polyelectrolytes.

Although ILs are usually labeled as "green" solvents, their biological effects and toxicity towards microorganisms has been reported.\textsuperscript{36} Nonetheless, the IL physicochemical properties can be tailored by tuning their chemical structure,\textsuperscript{20} which makes it possible to design low/non-toxic and biocompatible ILs at the molecular level. However, for the same reason, it is very challenging to find suitable ILs with the desired properties from the numerous potential candidates. Currently, the selection of ILs for downstream separation processes is mainly based on a trial-and-error approach, which is time consuming and expensive. Furthermore, most of the previous studies on bioproduct recovery using IL-based liquid-liquid extractions (LLE) have focused on the downstream separation process only. It is therefore a three-stage systematic design method for such purposes that is presented in this work. The method combines the selection of suitable ISPR processing schemes, identification of the optimal design of IL-based LLE systems followed by process simulation and evaluation.

Generally, bioprocess unit operations vary significantly from one product to another. The proposed design method in this work focuses on small-molecule fermentation processes. Most of the fermentation processes are carried out in dilute aqueous conditions and this often leads to a water-rich feed to the downstream process. Therefore, hydrophobic water-immiscible ILs are initially preferred considering their applications may allow the combination of extraction, purification, and concentration in a single step. On the other hand, the use of water-miscible ILs is presented via IL-based aqueous biphasic systems (ABS) by adding a salting-out agent to create a second liquid phase.\textsuperscript{37} It is already well accepted that the toxicity of ILs mainly depends on their hydrophobicity, and therefore hydrophilic water-miscible ILs generally exhibit low toxicities.\textsuperscript{38} Owing to these advantages, IL-based ABS have recently have been used for the recovery and purification of different bioproducts.\textsuperscript{38-41} Furthermore for design, besides experimental studies, it is also important to have a good thermodynamic model to describe and predict liquid-liquid equilibria. In this work, a proposed design method is demonstrated through its application on a conventional acetone-butanol-ethanol (ABE) fermentation, since the IL-ABE systems has been widely
studied experimentally\textsuperscript{23,24,42} and the thermodynamic model (UNIFAC-IL) has also been extended to these systems.\textsuperscript{43}

2. Design Methodology

The IL-based ISPR design method includes three design stages, as shown in Figure 1. First, a suitable ISPR processing scheme is selected according to the properties of the studied small-molecule fermentation process. Afterwards, suitable IL candidates with desired properties are identified by experimental/model-based solvent screening methods, and then thermodynamic calculations of the studied LLE systems are performed. This design stage is the most challenging and important part of the proposed method. Finally, the IL-based ISPR process is simulated and further evaluated by performing energy and/or economic assessments.

Figure 1: Design methodology of IL-based \textit{in-situ} product removal for small molecule fermentation.

2.1. ISPR Selection

\textbf{Step 1. ISPR Scheme Selection.} Similar to conventional organic solvents, ILs can also be classified as water-miscible (where IL-based ABS can be formed), and water-immiscible. Using either water-miscible or water-immiscible ILs as solvents, different forms of ISPR-integrated fermentation schemes dependent on the location (internal or external) of the product removal (direct or indirect) can be considered (see Figure 2). Scheme-a represents internal product removal with direct IL contact, where both reaction and product removal are completed in the same vessel and therefore the number of processing units is reduced. Scheme-c represents external product removal with direct IL contact, where contact time between the solvent and the cells is decreased as the product removal takes place in the external separation unit. On the other hand, Scheme-b represents internal product removal with indirect IL contact, where cells are isolated from the solvent by a physical barrier such as a membrane.\textsuperscript{44} Finally, scheme-d represents external product removal with indirect IL contact, where the cells are separated from the product before feeding to the downstream separation unit. In all these cases, two-liquid phase systems containing ILs will improve the reaction yield/productivity as well as the product concentration fed to the downstream processing unit. Generally, ABS that mainly composed of water are more attractive for bioreaction systems because of their biocompatibility as media for cells, and have therefore been widely studied for the purification and recovery of bioproducts.\textsuperscript{33,37}

Figure 2. Conceptual design of IL-based \textit{in-situ} product removal for small-molecule fermentation.

When water-immiscible ILs are used as solvents in the ISPR design, the concentrated product stream fed to the downstream separation unit(s) generally contains no water. In this case, it is followed by both product(s) recovery and IL regeneration through evaporation in a flash unit. On the other hand, in the ISPR schemes using water-miscible ILs as solvents, where the product is concentrated in an IL-rich aqueous phase, the water should be removed from the concentrated product stream followed by product recovery. For water removal, reverse osmosis (RO) using a partially permeable membrane to remove ions, molecules and larger particles can be considered as a dehydration unit. In RO, an applied pressure is used to overcome osmotic pressure which is a colligative property driven by chemical potential differences of the solvent, and a thermodynamic parameter. RO can remove many types of dissolved and suspended chemical species from water. For example, RO is extensively used in both industrial processes and the production of potable water. The result is that the solute is retained on the pressurized side of the membrane and the solvent is allowed to pass to the other side. To be “selective”, this membrane should not allow large molecules or ions through the pores (holes) but allow water to pass freely.

For both internal and external ISPR schemes with direct solvent contact, the cells are directly in contact with the IL (see Figure 2a and c). Conversely, in both internal and external ISPR schemes with indirect
solvent contact, the cells are prevented from contact with the IL (see Figure 2b and d). For this reason, the selection of IL for a two liquid-phase system with direct contact is more difficult compared to those with indirect contact schemes. Also, it must not be toxic and deactivate the cells. Additionally, in the internal configurations (Figure 2a and b), both reaction and product removal are carried out in the same processing unit which can lower the investment cost, while in the external configurations, the contact time between the solvent and the cells is reduced by introducing a separate loop containing an external unit.\textsuperscript{1,44} A suitable ISPR scheme can be selected according to the properties of the studied small-molecule fermentation process.

2.2. IL-based LLE systems design

**Step 2. Criteria and Design Targets of ILs.** Considering the numerous applicable ILs to industrial processes, it is necessary to narrow down the list of potential ILs by evaluating their suitability in a given application. Therefore, reasonable criteria for IL selection need to be considered at the design stage of IL-based LLE systems. As with organic solvents, the selected ILs should fulfill the requirements of environmental sustainability, health and safety concerns. Although ILs typically present high thermal and chemical stability, systematic understanding of their toxicity and biodegradability is still limited; thus, a better structure-based knowledge of these properties is critical. The toxicity of ILs is an important property that directly relates to health and safety concerns. For the purpose of advanced design of ‘green’ ILs, numerous studies regarding the toxicity of IL groups have been performed. As reported, the antimicrobial properties of ILs (defined as minimal inhibitory and bactericidal concentrations against microbial strains) relevant for human health is observed to increase with the length of the side alkoxy chain (from 2 to 12 carbon atoms), and the anion’s effect on the toxicity was observed secondary to the cation’s effect.\textsuperscript{45-47} Together with these observations, non-aromatic head groups were generally found less toxic than their corresponding aromatic analogues.\textsuperscript{48} For the major properties associated with the evaluation of environmental impact, biodegradability of ILs has also been studied and discussed recently.\textsuperscript{49-53} It is observed that the presence of certain functional groups (e.g. amides, esters, hydroxyls) in the side chain of some cations such as imidazolium,\textsuperscript{54} and pyridinium\textsuperscript{55,56} can significantly enhance the biodegradability of their corresponding ILs. Meanwhile, some studies show that ILs having alkyl chains containing four carbon atoms are poorly biodegradable while those which are cholinium (cation)-based ILs are observed to be readily biodegradable. Nonetheless, the knowledge of mechanism and biodegradation pathways remains limited. Therefore, further experimental studies are necessary for the specific system under investigation.

When choosing an IL as the solvent for bioproduct recovery, it should be easy to separate from the dilute aqueous phase, as well as remove the desired products from the IL rich phase. Separation performance is generally described by solvent properties at infinite dilution such as selectivity ($S$), infinite dilution distribution coefficient ($D$), solvent loss ($SL$) and solvent power ($SP$), defined variously as:

$$S = \frac{\gamma_{H,O,IL}}{\gamma_{i,IL}}$$

$1$
\[ D = \frac{\gamma^\infty_{i,aq} M_{w, H_2O}}{\gamma^\infty_{i,IL} M_{w, IL}} \]  
\[ SL = \frac{1}{\gamma^\infty_{i,IL,aq}} \]  
\[ SP = \frac{1}{\gamma^\infty_{i,IL}} \]

Where \( \gamma^\infty_{i,aq} \) and \( \gamma^\infty_{i,IL,aq} \) are the infinite dilution activity coefficient of solute \( i \) and solvent (IL) in aqueous raffinate phase, while \( \gamma^\infty_{i,IL} \) and \( \gamma^\infty_{i,IL,w} \) are the infinite dilution activity coefficient of solute \( i \) and water in IL-rich extract phase, respectively. In Eq. 2, \( M_{w, H_2O} \) and \( M_{w, IL} \) denote the molecular weights of water and IL, respectively.

For a given IL-water-solute system, the distribution coefficient of solute \( i \) \( (D_i) \) and water \( (D_{H_2O}) \) are expressed by their equilibrium weight fraction in IL-rich extract phase \( (x^i_{IL}, x^{H_2O}_{IL}) \) and aqueous raffinate phase \( (x^i_{aq}, x^{H_2O}_{aq}) \), respectively. The selectivity \( (S_i) \) is given as the ratio of the distribution coefficient \( D_i \) and \( D_{H_2O} \).

\[ D_i = \frac{x^i_{IL}}{x^i_{aq}} \]  
\[ D_{H_2O} = \frac{x^{H_2O}_{IL}}{x^{H_2O}_{aq}} \]  
\[ S_i = \frac{D_i}{D_{H_2O}} \]

Distribution coefficient is a measure of the difference in solubility of the solute (desired product) in two split phases at equilibrium and the product is usually supposed to be concentrated in the water-immiscible phase. Selectivity is the ratio of the infinite dilution activity coefficients of solute \( i \) and water in the hydrophobic solvent phase. Most commonly, the selectivity must exceed unity for a possible separation. Solvent loss is the amount of solvent in aqueous raffinate phase. It is desired for this amount to be almost zero, in order for less solvent to be used. Likewise, solute loss is the amount of solute \( i \) that remains dissolved in the aqueous raffinate phase. In order to obtain a successful extraction, the value of this parameter should be as small as possible. For water-miscible ILs, the IL-based ABS is formed by adding a salting-out agent to form the second phase. When using water-immiscible ILs, two phases already exist before the addition of any salt, and one of the phases is far from being aqueous-rich due to the low solubility of these ILs in water. In addition to the properties associated to biocompatibility and separation performance, properties such as viscosity and melting point also needed to be taken into account considering their significant impact on industrial operation.

**Step 3. IL Design/Selection.** As a successful IL-based LLE system largely depends upon the selection of suitable ILs, this is an important step. Here both conventional solvent screening methods (i.e. experimental data-based methods) and model-based design methods (i.e. computer-aided ionic liquid design (CAILD) methods) are considered. With sufficient experimental data for a certain case under study, the conventional method is more attractive since it can provide more reliable results. In CAILD, ILs...
containing desirable properties (e.g. high selectivity, high distribution coefficient) can be tailored via tuning their chemical structures. In this method, IL functional groups (i.e. cations, anions and substituents) are systematically combined and suitable ILs with desired thermodynamic, physical and/or chemical properties can be generated according to a particular set of structural and property constraints. Compared to conventional selection methods that are usually cost intensive and time consuming, CAILD is more systematic and effective.

When using CAILD to search high performance ILs, reasonable constraints and the design objective should be set for its corresponding optimization problem. In all possible cases of IL-based ISPR, the solvent loss should be as low as possible. Thus, the selectivity of ILs is employed as a design target while other thermodynamic associated properties such as distribution coefficient, solvent power and solvent loss are used as design constraints. Meanwhile, the constraints for chemical structures and physical properties are also included in the considerations for structural feasibility, complexity, and their operability in industry.

Step 4. Thermodynamic Calculation of LLE. For the application of IL-based LLE approaches, it is important to have a good thermodynamic model to predict and describe LLE conditions for design. To date, several models have been used such as COSMO-RS, COSMO-SAC, PC-SAFT, UNIQUAC, NRTL, and UNIFAC. Usually, to obtain global and reliable parameters for thermodynamic models, phase equilibrium data is sufficient for this purpose.

It has been shown that, in most cases, that the UNIFAC-IL model can provide reliable thermodynamic predictions and also has good group extendibility. The activity coefficient calculation in UNIFAC is based on the functional group information of involved components and therefore proper decomposition of the IL molecule is needed for use of this thermodynamic method. Among commonly used decomposition approaches, the method in which IL is decomposed separately into cation, anion and substituents is preferred because of its improved design space and flexibility. It should be noted that the salting-out agent should also be included in the thermodynamic calculation when using IL-based ABS as an extraction system.

2.3. ISPR Evaluation

Step 5. Process Simulation and optimization. An optimal IL for a specific design should be identified based on the best process performance. However, it is unrealistic to simulate the process considering all possible ILs as solvents and therefore only optimal ILs designed/selected in the Step 2 should be simulated. In this design stage, both dynamic-state simulation of fermenter and steady-state simulation of downstream separation processes are performed. Meanwhile, in order to obtain optimal processing configurations, process optimization is also included.

In the steady-state simulation, no variations of temperature, pressure, composition and reaction rate with respect to time are considered. On the other hand, the dynamic-state that describes the reaction behavior is simulated with ISPR, where the model equation is generally derived from the differential mass balance since the composition and reaction rate vary with respect to time in the reactor. To date, the simulation of processes involving ILs is still a challenging task since ILs are still not included in the component database of common process simulators (e.g. Aspen Plus, PRO/II) and the required information of IL-containing systems for calculating their thermodynamic behavior is also limited. Nonetheless, works regarding process simulation of IL-containing systems have been reported recently. Among these works, Aspen Plus
is the most widely used simulation tool, where ILs are introduced as pseudo-components by specifying their critical and/or physical properties. These properties can be calculated by group contribution (GC)-based methods from previously published data.77-80

**Step 6. Performance Evaluation.** Finally, based on the reaction and separation simulation results from Step 6, the process performance of the ISPR schemes can be evaluated with the applicable energy and economic models. In the ISPR schemes with IL-based ABS, the product is concentrated in the IL-rich aqueous phase and therefore water should be removed from the concentrated product stream prior to product recovery. For the water removal process, reverse osmosis (RO) that uses a partially permeable membrane to remove ions and molecules from the water can be considered as dehydration unit. Therefore, the cost model of the membrane unit may also be considered in some cases.

3. **Applied IL-based ISPR in ABE fermentation**

Butanol has been identified as an important biofuel since it offers several advantages (e.g. high energy content, lower hygroscopic nature and volatility) over ethanol and other fermentation derived fuels.81 Butanol can be produced from renewable resources via the ABE fermentation route to create biobutanol or from fossil resources to create petro-butanol. Many new technologies and methods have been applied recently to further develop the biobutanol route. These include strain engineering to increase butanol production (e.g. as done by Green Biologics) and using genetically-modified yeast to produce iso-butanol from glucose (e.g. as done by Gevo).82 Although significant advances in biobutanol production by ABE fermentation have been achieved recently, the market penetration of bio-butanol is still hindered due to its high production costs compared to those for petro-butanol. The inhibitory effect associated with the ABE fermentation process mainly comes from the butanol toxicity to the culture. For example, only 22 g/L of total fermentation products with a butanol concentration below 13 g/L are typically obtained during a batch process,84 which results in a high process cost due to large volumes and high energy demand in the downstream product recovery. For this reason, separation methods that enable effective and rapid removal of toxic components (in particular butanol in this case) from the fermentation broth are essential to improve the economic competitiveness of bio-butanol production via the ABE fermentation.

Many separation techniques including distillation, absorption, gas stripping, solvent extraction and pervaporation have been used for the product recovery from ABE fermentations.85,86 Besides these, enhanced or hybrid process designs allowing energy efficient operations have also been proposed.87-91 Meanwhile, in-situ recovery of butanol from broth during ABE fermentations have also been reported,92-100 demonstrating the reduction of inhibition and improvement of butanol productivity. Table 1 summarizes the recent experimental reports regarding the application of in-situ product recovery strategies with different separation methods in ABE fermentations. Results show that in general in-situ product removal processes have higher productivity and yield compared to the batch process. Nevertheless, problems of in-situ product removal strategies using conventional separation methods also exist. For example, when combining gas stripping with in-situ product removal, the energy demand of the whole process will increase due to the duty required at the condenser for product recovery from the gas stream.97 As mentioned previously, ISPR with IL-based LLE has the potential to improve the productivity, as well as lower the energy consumption in downstream product removal processes. In this work, we are trying to use this strategy for the butanol production via ABE fermentation, and the proposed three-stage systematic design method is applied. In this work, the conventional ABE fermentation that makes n-butanol by *Clostridium acetobutylicum* is used considering the ISPR technology has been widely studied on this process, thereby making comparison easier, while evaluating the performance of the proposed IL-based ISPR design method. It is to be noted that this design method can also be used in other small-molecule fermentation processes including iso-butanol production from glucose using genetically modified yeast.

Table 1. Experimental work of using In-situ product removal strategies using different separation methods in ABE fermentation.
3.1. ISPR scheme selection

As stated in section 2.2, different schemes of IL-based ISPR can be generated for using water-immiscible ILs. Extractive fermentation with in-situ product removal may not be suitable for large-scale production for various reasons, such as poor mass transfer into the solvent, cell damage by solvent (interface toxicity), loss of cells at the interface and difficult process control. In contrast, these shortcomings can be avoided by using an external product removal scheme. Therefore, product removal in an external extraction column with a recycle of water back to the fermenter has been the preferred scheme for large-scale production of bio-butanol.\textsuperscript{107,108}

Figure 3. IL-based ISPR scheme for bio-butanol production from ABE fermentation.

Figure 3 shows the proposed IL-based ISPR scheme for the production of bio-butanol from conventional ABE fermentation. The fermentation broth is withdrawn from the fermenter and fed to the extractive column (E-C1) at a time when the fermentation products start to inhibit the microorganism, and then the water is recycled to the fermenter. It should be noted that a membrane unit (M-U1) is required between the fermenter and extractive column in order to retain the microorganisms and/or some intermediates inside the fermenter. After leaving the extractive column, the product rich phase is sent to a flash unit (F-U1), where the most of fermentation products together with the extracted water are evaporated and then separated from the IL solvent, which is recycled back to the extractive column after being cooled to the fermentation temperature. Afterwards, light products acetone and ethanol are separated from water and butanol in a distillation column (D-C1). Next, the butanol-water mixture, which can form a heterogeneous azeotrope, is further separated using a two-column distillation system with a decanter (D-U1). In this system, almost pure water is obtained from the bottom of the distillation column (D-C1), which is then cooled to the fermentation temperature before being recycled to the fermenter. Meanwhile, a high purity butanol product can be obtained from the bottom of the second distillation column (D-C2). A solvent make-up stream is also considered for the IL losses occurring in the whole process. Together with process optimization, heat integration that allow for energy savings are also considered in this process. The detailed information of these designs is given in section 3.3.

3.2. Screening IL for LLE systems design

Solvent plays an important role as removal and concentrating agent for biomolecules from dilute aqueous solutions. In order to obtain an effective recovery of butanol from the dilute broth, a good solvent with a high affinity for butanol combined with low water co-extraction is highly desired. Several organic solvents with high selectivity and/or high capacity for butanol-water system have been studied as extractive solvents in ABE fermentation processes,\textsuperscript{42,88,100,109,110} and oleyl alcohol (OA) is the most widely reported due to its non-toxicity and low volatility. Recently, some water-immiscible ILs with high distribution coefficients and selectivity for this binary mixture were also reported.\textsuperscript{111} These solvents are listed in Table S3 (Supporting Information), together with their experimental distribution coefficient and selectivity in the butanol-water system. Among them, tetraoctylammonium 2-methyl-1-naphthoate ([TOA] [MNaph]) has the highest selectivity (21) and D_{butanol} (274) for butanol-water system, which are much higher than that of OA (3.42 and 194, respectively). Figure 4 gives the structure of [TOA] [MNaph] and Figure 5 presents the UNIFAC-IL calculated liquid-liquid equilibria of the water-butanol-[TOA] [MNaph] ternary system.
Besides the IL selection from experimental data involving distribution and selectivity, CAILD is also employed to search suitable ILs for the butanol recovery in ABE fermentation process. In this work, CAILD is formulated as a mixed-integer non-linear programming (MINLP) problem, where the maximization of selectivity is set as the objective function and multiple (design) constraints are imposed on the IL structure, thermodynamic properties (i.e. distribution, solvent power and solvent loss) and physical properties (i.e. melting point, viscosity). In this case, an optimal IL, tetradecyl(trihexyl)phosphonium tetracyanoborate ([TDPh][TCB]), is identified by solving the formulated CAILD-based MINLP problem in the modelling system GAMS by using a deterministic global optimization solver, LINDOGLOBAL. The detailed information of this CAILD-based MINLP problem is provided in Table S3 (Supporting Information).

The structure of [TDPh][TCB] is given in Figure 6 and the UNIFAC-IL calculated liquid-liquid equilibria of [TDPh][TCB]-water-butanol ternary system is presented in Figure 7. In this work, the used UNIFAC-IL model was extended from 39,358 experimental infinite dilution activity coefficient data and has been verified by large experimental liquid–liquid and vapor–liquid equilibria data. The mean absolute percentage error (MAPE) of the predictions for IL-alcohol-water systems (893 data points) is about 5.7%, which allows reliable thermodynamic calculations for these ternary systems. Remarkably, [TDPh][TCB] was also proposed by Domańska and Królikowski for possible use in separation of butanol from aqueous phase based on their experimental work, which largely supports the reliability of our proposed CAILD method.

3.3. Process simulation and performance evaluation

In this work, water-immiscible ILs, [TOA][MNaph] and [TDPh][TCB], are finally selected as the extractive solvents for the studied ABE fermentation process. In order to evaluate the proposed IL-based ISPR scheme for ABE fermentation, process simulations including dynamic simulation of the fermentation process and steady-state simulation of the downstream separation process are performed and illustrated. In this work, a kinetic model developed by Mulchandani and Volesky (see Appendix A) is employed to simulate the fermenter, where the accumulation of butanol (B) and butyric acid (BA) accounts for the process inhibition, as shown in Eqs. 8 and 9.

\[
f(I) = \exp(-0.01BBA) \quad BBA \leq 8.0 \text{ g/L} \quad 8
\]

\[
f(I) = -0.153BBA + 2.16 \quad 8.0 \leq BBA \leq 13.9 \text{ g/L} \quad 9
\]

\[
BBA = C_B + C_{BA} \quad 10
\]
where concentration of butanol ($C_B$) and butyric acid ($C_{BA}$) as well as other involved components (i.e. substrate, microorganism, acetic acid, acetone, ethanol and water) can be calculated from the reactor model, as expressed in Eq.11.

$$\frac{dC_i}{dt} = r_i - \vartheta(C_i + C_{i,IN})$$  \hspace{1cm} 11

Here, $r_i$ is the rate of reaction and $\vartheta$ is the dilution factor that corresponds to the ratio between the volumetric flowrate withdrawn from the reactor and the reactor volume. $C_i$ and $C_{i,IN}$ denote the concentration of the component $i$ in the recycle stream and inside the fermenter, respectively. Clearly, the reactor model of a typical batch process can be expressed by Eq.11 with no product removal ($\vartheta = 0$), while Eq.11 with no substrate make-up stream ($C_{i,IN} = 0$) represents another special reactor model of the fermentation process. Here, we compare the dynamic behavior of three different processing schemes, i.e., batch process (base case), in situ (batch) process and in situ (fed-batch) process. The ordinary differential equation (ODE) system from Eq.11 is solved in MATLAB by using ODE45 algorithm. The dynamic simulation results of all three processing schemes are given in Figures 8, 9 and 10, respectively.

Figure 8. Component concentration tracking of batch process.

Figure 9. Component concentration tracking of in situ (batch) process.

Figure 10. Component concentration tracking of in situ (fed-batch) process.

As shown in Figure 8, the growth of cells is significantly inhibited after 13 h due to the accumulation of butanol and butyric acid, resulting in a reduction in the rate of sugar consumption. After 50 h, the fermentation is almost stopped as the concentration of ABE reaches 38.5 g/L with a corresponding butanol concentration of 20.4 g/L. In in situ (batch) process, the ABE products are removed (after 12 h) through filtration from the fermenter, thereby reducing the inhibition of the fermentation process. The rate of product removal can be controlled by changing the flowrate of the recycle and filtered streams. In order to obtain a stable butanol concentration in the reactor during the whole fermentation process, a dilution ratio of 0.1 h$^{-1}$ is used. Unlike the situation of batch case, sugar can be fully converted to the product in this scheme. Moreover, less than 23 h is needed to achieve a 100% sugar conversion (see Figure 9), which means that more than 54% of the fermentation time can be saved, compared to the batch fermentation process. Therefore, based on the same fermenter volume, the butanol productivity of in situ (batch) process is around 1.8-fold that of batch case.

For both batch and in situ (batch) cases, the fermentation process will end after a time either due to the toxic effect of products or the full consumption of the substrate. In order to achieve a stable butanol production in the fermenter, a processing scheme of ISPR with a substrate make-up stream, i.e. in situ (fed-batch) process, can be used. In this scheme, the ABE products are removed through the filtrate stream after 12 h, while a substrate make-up stream is added to the recycled water before it is sent to the fermenter.
By varying the dilution ratio, it was found that the butanol concentration is kept around 10 g/L with a dilution ratio of 0.11 h⁻¹, which allows a stable butanol production between 12 and 100 h (see Figure 10) and the butanol productivity of this scheme is around 2.7-fold that of the normal batch scheme. The broth composition of both in situ (batch) and in situ (fed-batch) processes is given in Table 2. It is to be noted that we treat the filtrate fermentation broth and fermentation broth remained in the fermenter as a whole in in situ (batch) process, while the average composition of the filtrate stream between 12 and 100 h is applied in in situ (fed-batch) process.

Table 2. Broth composition for downstream separation.

In this work, the downstream separation process including product recovery and purification is simulated in Aspen Plus (V8.6), where the IL solvent is defined as a pseudo-component by specifying its properties (e.g. molecular weight, heat capacity, critical properties) and model parameters of thermodynamic method (UNIFAC). The missing thermodynamic parameters of [TOA][MNaph]-acetone are obtained by regressing the infinite dilution activity coefficient data of acetone in [TOA][MNaph]. These activity coefficient data are generated from the COSMO-RS model. As reported 43, the MAPE of COSMO-RS for the IL-ketone systems is about 30%. Although such prediction performance is rather low, the concentration of acetone has a relatively small inhibition impact on the studied ABE fermentation process. For this reason, using the thermodynamic parameters calculated from COSMO-RS for the [TOA][MNaph]-acetone system is acceptable. All parameters used in the process simulation are provided in Table S6 (Supporting Information).

As shown in Figure 3, E-C1 is modelled by the Extract column block, while F-U1 and D-U1 are modelled by Flash and Decanter separator block, respectively. Meanwhile, all distillation columns (i.e. D-C1, D-C2, and D-C3) are modelled by the RadFrac block. By comparing Figure 5 and Figure 7, we know that [TOA][MNaph] has a higher butanol capacity than [TDPh][TCB]. For this reason, only processes using [TOA][MNaph] are further simulated and demonstrated for the studied ABE downstream separation. The optimized simulation results to recover 5t/h butanol (99.8% mass purity) from the fermentation broth of both in situ processing schemes are given in Table 3. Meanwhile, a heat exchanger network (HEN) design is also included for this downstream separation process, as shown in Figure 11.

Table 3. Optimized simulation results of the ABE downstream separation for in situ processing schemes.

As expected, the IL-based in situ (fed-batch) process has better energy (11.25 MJ/kg butanol) and solvent performance (124 t/h) than that of in situ (batch) process (12.38 MJ/kg butanol, 155t/h). For in situ (batch) process, we can obtain a stable downstream operation by processing the filtrate fermentation broth and fermentation broth remained in the fermenter as a whole in downstream separation, although a buffer tank is needed between E-C1 and F-U1. However, process control of downstream operation is needed in in situ (fed-batch) process for achieving a stable operation. Both IL-based in situ (fed-batch) process and in situ (batch) process have better energy performance than their counterparts using 2-butyl-1-octanol or vegetable oil as solvent, as shown in Table 4. In addition, the IL-based in situ (fed-batch) process and in situ (batch) process have significant energy savings (79.6% and 77.6%) when compared to the base case (55.2 MJ/kg butanol).100 As reported,114 butanol has a high energy density of 36.05 MJ/kg, the energy demand of butanol recovery in either in situ (batch) process or in situ (fed-batch) process is very attractive. Furthermore, the in-situ product removal from the fermenter allows to reduce the fermentation time or lengthen the process operation and thereby increasing the formation rate of butanol. As previously stated, the productivity of IL-based in situ (fed-batch) process and in situ (batch) process is around 2.7 and 1.8-fold that of conventional batch process.
When solvents are used in the fermentation process, their biocompatibility becomes a very important property that needs to be considered. Unfortunately, no studies on biocompatibility of [TOA][MNaph] and [TDPh][TCB] have been reported so far. However, an external-indirect processing scheme is used in this case and therefore IL doesn’t appear in the fermenter, which means their cytotoxic impacts on the fermentation process are very limited. Nonetheless, these impacts need to be taken into account when direct processing schemes are applied. Moreover, their environmental properties such as biodegradability and ecotoxicity should also be considered before taking them to the industry. Although, currently these properties are not included due to the lack of suitable models to describe them, they can easily be integrated into this design method once the property models are available. To date, high viscosity and high cost of ILs are two major impediments to their utilization. In this work, selected ILs are highly viscous as most ILs and this should be considered in the equipment design. On the other hand, the current cost of IL is suggested as 30 $/kg by BASF from IL bulk production,\textsuperscript{115} which is higher than many organic solvents. However, the cost of ILs are decreasing with the development of the cost-effective synthetic methods and the application of inexpensive raw materials.\textsuperscript{116,117} As reported, large scale production of triethylammonium hydrogen sulfate ([HNEt\textsubscript{3}][HSO\textsubscript{4}]) will be as low as 1.24 $/kg,\textsuperscript{118} which shows that ILs have potential to compete with conventional organic chemicals in terms of solvent cost.

4. Conclusions

This work presents a three-stage systematic design method that combines IL-based LLE systems design and ISPR schemes generation for small-molecule fermentation. In this method, selection criteria of ILs (e.g. selectivity, distribution coefficient), IL solvent screening methods (i.e. experimental data-based method, CAILD), process design, optimization and evaluation are all discussed and investigated. The conceptual design of IL-based ISPR that is able to reduce the inhibition on cells and increase the volumetric productivity is exemplified for butanol production from ABE fermentation. In this case, ILs [TDPh][TCB] and [TOA][MNaph] are identified as the optimal solvents for the recovery of biobutanol. The dynamic simulation results of the fermentation process show that, compared with the batch process, both IL-based in situ (fed-batch) process and in situ (batch) process can significantly improve the process productivity, which offers great advantage for the fermentation process, especially for the production the high-value bioproducts. In addition, the IL-based in situ (fed-batch) process and in situ (batch) process, respectively, have 79.6% and 77.6% savings in energy when compared to the base process.

Although experimental verification of these IL schemes is required for their further industrial application, the results of the studied ABE fermentation process demonstrate that IL-based ISPR is able to afford higher productivity and energy performance of bioprocesses when compared to batch operations. In this work, the design method is only tested by a conventional ABE fermentation process, but it can potentially be a guide for the other small molecule fermentation processes. Nevertheless, some issues need to be addressed for its widespread use in bioprocesses. First, the current experimental database is still limited to a few well-known ILs and small molecules. Therefore, more experimental work covering new IL-bioprocess systems is necessary. Secondly, the separation method of IL-based ABS has many advantages, but its optimal design is very difficult due to limited thermodynamic models. For this reason, modelling studies
of IL-based ABS are highly desired. In addition, although the major properties associated to the evaluation of environmental impact and biodegradability of ILs have been recently studied, the knowledge of their modes of toxicity, and biodegradation pathways is still limited. Therefore, further experimental investigation and theoretical studies are essential and should be the focus of attention in the coming years. Finally, in terms of the ISPR technology itself, the robustness of the design to industrial conditions is critical. Controlling the conditions is more difficult for an ISPR process than for a non-integrated process and thereby efforts for improving the robustness of the design are highly desirable.

Acknowledgments

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Notation: List of Abbreviations

ABE acetone-butanol-ethanol
ABS aqueous biphasic systems
BA butyric acid
\([\text{HMIM}][\text{Tf}_2\text{N}]\) 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
CaLB Candida antarctica lipase B
CAILD computer-aided ionic liquid design
COSMO-RS conductor-like screening model - real solvents
COSMO-SAC conductor-like screening model - segment activity coefficient
Dnb di-n-butylphthalate
GC group contribution
HEN heat exchangers network
MAPE mean absolute percentage error
ILs ionic liquids
ISPR in situ product removal
LLE liquid-liquid extraction
MINLP mixed integer nonlinear programming
\([\text{N}_{1,8,8,8}][\text{Oct}]\) 1-methyltriocylammonium octanoate
NRTL non-random two liquid
OA oleyl alcohol
PC-SAFT perturbed chain statistical associating fluid theory
RO reverse osmosis
TP tricresyl phosphate
\([\text{TDA}][\text{Mchb}]\) tetrakis(decyl)-ammonium 1-methyl-1-cyclohexanoate
\([\text{TDPb}][\text{TCB}]\) tetradecyl(trihexyl)phosphonium tetracyanoborate
\([\text{TDPb}][\text{phos}]\) tetradecyl(trihexyl)phosphonium bis-2,4,4-trimethylpentyl-phosphinate
\([\text{TOA}][\text{MNaph}]\) tetraoctylammonium 2-methyl-1-naphthoate
UNIFAC universal quasichemical functional-group activity coefficients
UNIQUAC universal quasichecmical

References


(94) Haigh, K. F.; Petersen, A. M.; Gottumukkala, L.; Mandegari, M.; Naleli, K.; Görgens, J. F.: Simulation and comparison of processes for biobutanol production from
lignocellulose via ABE fermentation. *Biofuels, Bioproducts and Biorefining* 2018, 12, 1023-1036.


1.1. ISPR Selection

Figure 1: Design methodology of IL-based in-situ product removal for small molecule fermentation.

<table>
<thead>
<tr>
<th>Internal configurations</th>
<th>External configurations</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td>c)</td>
</tr>
<tr>
<td>IL with direct IL contact</td>
<td>IL + P + (H₂O)</td>
</tr>
<tr>
<td>Feed</td>
<td>B/C + (H₂O)</td>
</tr>
<tr>
<td>IL</td>
<td>Feed</td>
</tr>
<tr>
<td>H₂O + P + US + B/C</td>
<td>IL + P + (H₂O)</td>
</tr>
<tr>
<td></td>
<td>d)</td>
</tr>
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</table>
Figure 2. Conceptual design of IL-based in-situ product removal for small-molecule fermentation.

Table 1. Experimental work of using In-situ product removal strategies using different separation methods in ABE fermentation.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Substrate</th>
<th>Productivity increase*</th>
<th>Yield increase*</th>
<th>Separation method</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>C-B BA101</td>
<td>Glucose</td>
<td>229%</td>
<td>5%</td>
<td>Gas stripping</td>
<td>101</td>
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<tr>
<td>C-A JB200</td>
<td>Glucose</td>
<td>33%</td>
<td>25%</td>
<td>Two-stage gas stripping</td>
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<td>C-A ATCC 824</td>
<td>Glucose</td>
<td>28%</td>
<td>10%</td>
<td>Extraction</td>
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<td>C-A ATCC 824</td>
<td>Glucose</td>
<td>56%</td>
<td>8%</td>
<td>Extraction-gas stripping</td>
<td>103</td>
</tr>
<tr>
<td>C-B CC101</td>
<td>Glucose</td>
<td>32%</td>
<td>15%</td>
<td>Adsorption</td>
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</tr>
<tr>
<td>C-A B3</td>
<td>Glucose</td>
<td>40%</td>
<td>15%</td>
<td>Permeating-heating-gas stripping</td>
<td>104</td>
</tr>
</tbody>
</table>
C-A ATCC 55025  Glucose  15%  3%  Pervaporation  105
C-A ATCC824  Glucose/xlycose  126%  67%  Pervaporation  106

C-B: *Clostridium beijerinckii*, C-A: *Clostridium acetobutylicum*, †: compared with a batch process.

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**Figure 3.** IL-based ISPR scheme for bio-butanol production from ABE fermentation.

**Figure 4.** Structure of tetraoctylammonium 2-methyl-1-naphthoate ([TOA] [MNaph]).

**Figure 5.** UNIFAC-IL calculated liquid-liquid equilibria of water-butanol-[TOA] [MNaph] (mass fraction).
Figure 6. Structure of trihexyltetradecylphosphonium tetracyanoborate ([TDPh][TCB]).

Figure 7. UNIFAC-IL calculated liquid-liquid equilibria of water-butanol-[TDPh][TCB] (mass fraction).
Figure 8. Component concentration tracking of batch process.

Figure 9. Component concentration tracking of \textit{in situ} (batch) process.
Figure 10. Component concentration tracking of *in situ* (fed-batch) process.

Table 2. Broth composition for downstream separation.

<table>
<thead>
<tr>
<th>Component</th>
<th>In situ (batch) Mass fraction</th>
<th>In situ (fed-batch) Mass fraction</th>
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<tbody>
<tr>
<td>Butanol</td>
<td>8.10E-03</td>
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<tr>
<td>Acetone</td>
<td>5.19E-03</td>
<td>8.00E-03</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5.61E-04</td>
<td>4.41E-04</td>
</tr>
<tr>
<td>Water</td>
<td>0.9862</td>
<td>0.9813</td>
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Table 3. Optimized simulation results of the ABE downstream separation for *in situ* processing schemes.

<table>
<thead>
<tr>
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<th>In situ (batch)</th>
<th>In situ (fed-batch)</th>
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<tr>
<td></td>
<td>Pressure (bar)</td>
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<tr>
<td></td>
<td>Number of stages</td>
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<td>36</td>
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<td>F-U1</td>
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<td></td>
<td>Temperature (°C)</td>
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<td>135</td>
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<tr>
<td></td>
<td>Energy input (MW)</td>
<td>5.98</td>
<td>5.09</td>
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<tr>
<td>D-C1</td>
<td>Pressure (bar)</td>
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<td>Number of stages</td>
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<td>56</td>
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<td>Feed location</td>
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<td>1</td>
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<td></td>
<td>D-C3</td>
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<tr>
<td>Reboiler duty (MW)</td>
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<td>Number of stages</td>
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<td>Feed location</td>
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<td>1</td>
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<td>Reboiler duty (MW)</td>
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<td>Energy demand (MW)</td>
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<td>Energy demand (MW)</td>
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<td>-0.10</td>
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<td>Energy demand (MW)</td>
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<td>-1.87</td>
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</tr>
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<tbody>
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<td>Energy demand (MW)</td>
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<td>-1.13</td>
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<th></th>
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<tbody>
<tr>
<td>Energy demand (MW)</td>
<td>-1.16</td>
<td>-1.04</td>
</tr>
</tbody>
</table>

| Total energy demand (MW) | 17.20 | 15.63 |
| Energy demand (MJ/kg of butanol produced) | 12.38 | 11.25 |

<table>
<thead>
<tr>
<th>Solvent</th>
<th>in situ (batch)</th>
<th>in situ (fed-batch)</th>
<th>Ref.</th>
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<tr>
<td>IL</td>
<td>12.38</td>
<td>11.25</td>
<td>This work</td>
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<tr>
<td>2-butyl-1-octanol</td>
<td>26.80</td>
<td>16.20</td>
<td>100</td>
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<tr>
<td>Vegetable oil</td>
<td>36.90</td>
<td>16.00</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 11. Heat exchangers network (HEN) design for the ABE downstream separation process.

Table 4. Energy performance of the *in situ* (fed-batch) or (batch) process with different solvents.