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REVIEW

In situ product removal in fermentation systems: improved process performance and rational extractant selection

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Abstract The separation of inhibitory compounds as they are produced in biotransformation and fermentation systems is termed in situ product removal (ISPR). This review examines recent ISPR strategies employing several classes of extractants including liquids, solids, gases, and combined extraction systems. Improvement through the simple application of an auxiliary phase are tabulated and summarized to indicate the breadth of recent ISPR activities. Studies within the past 5 years that have highlighted and have discussed "second phase" properties, and that have an effect on fermentation performance, are particular focus of this review. ISPR, as a demonstrably effective processing strategy, continues to be widely adopted as more applications are explored; however, focus on the properties of extractants and their rational selection based on first principle considerations will likely be key to successfully applying ISPR to more challenging target molecules.

Keywords Absorption · Adsorption · Biocatalysis · Extractive fermentation · Ionic liquids · Reactive extraction · Product removal (in situ)

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Introduction

The incorporation of an extractant phase for in situ product removal (ISPR) of inhibitory fermentation and biotransformation molecules is a powerful tool to alleviate the effect of high aqueous-phase concentrations of target molecules, improving bioreactor productivity. Such a processing configuration has been termed a "two-phase partitioning bioreactor" (TPPB) reflecting the presence of distinct aqueous and sequestering phases, as well as the fact that the target molecule will differentially partition between these two phases. In addition to reducing toxic product concentrations, ISPR can be applied to shift unfavourable reaction equilibria, reduce the number of downstream processing steps, favour the accumulation of an intermediate in multistep reaction systems, and prevent product losses due to degradation or volatility.

The affinity of a target molecule for the extractant phase is measured by the distribution (partition) coefficient at equilibrium, while selectivity is measured by the distribution coefficient towards the target molecule relative to another species, which can be another fermentation product molecule, or water itself. An auxiliary phase possessing a high distribution coefficient is desirable to minimize the amount of sequestering phase required, which has economic consequences throughout the design, extraction, recovery, and recycling aspects of a bioprocess. Selectivity towards the target molecule relative to

water is also important for concentrating the target molecule in the extractant phase. The extractant phase may be an immiscible liquid, a solid or a gas; consideration of target molecule properties, in conjunction with those of the extractant phase and the particular biocatalytic system, largely determine the applicability of a particular extraction strategy. Nevertheless, several different types of extractant have been utilized for a few common target molecules, demonstrating the flexible applicability of ISPR as a generic processing strategy.

Scope

Although the operational configuration of the sequestering phase in contact with the aqueous fermentation medium can vary, the direct removal of target molecules from the fermentor during operation, termed ISPR, is the focus of this review. To illustrate the breadth of recent work, reports over approximately the last 5 years of process improvements with the addition of an extractant phase, and reports which emphasize biological aspects of a biphasic transformation are tabulated in Table 1. Several authors have additionally provided insights into the characteristics and sorption mechanisms of the extractant phases, and have identified promising strategies for targeted extractant selection. These reports are of particular interest and form the basis of this review.

Studies investigating the pre-treatment of streams prior to fermentation have also been considered here, because these operations have been shown to have a direct effect on the subsequent fermentation and require similar considerations to true ISPR processes. Conversely, downstream processing operations which do not require any consideration of the bioreactor itself are not included. Protein, peptide, and antibiotic products are not included in this review due to their unique requirements for stability; such molecules are almost exclusively extracted by ISPR using aqueous two-phase systems (ATPS). Finally, TPPB systems in which the sequestering phase is used to *deliver* inhibitory substrates, rather than *remove* fermentation products are, although very similar in operation, also not considered here, and have recently been reviewed (Muñoz et al. 2012).

Liquid–liquid systems

Liquid extractants are typically water-immiscible organic solvents, immiscible liquid polymers, or liquid polymer-based aqueous two-phase systems (ATPS); however, ionic liquids (ILs) have also recently gained attention as potential extractants, and have been used as both immiscible solvents and as ATPS components in TPPBs. Although Table 1 provides a complete list of ISPR/TPPB systems utilizing immiscible organic solvents, we report below on recent examples using liquid extraction where novel features of the extractants are identified and discussed, and which have a bearing on fermentation performance.

Water-immiscible solvents have been conventionally used to extract hydrophobic fermentation products. The extractant's log $K_{o/w}$ value (octanol–water partition coefficient) must be sufficiently high to maintain a biphasic system with water; additionally, a solvent's log $K_{o/w}$ value is also a measure of biocompatibility, as above an organism-specific critical log $K_{o/w}$ value, all solvents are considered to be biocompatible (Garikipati et al. 2009). A third requirement mainly specific to liquid organic solvents is non-bioavailability, which is important for extractant stability and biocatalyst performance, ensuring that the intended carbon source, rather than the solvent, is exclusively metabolized. Generally, an organism's critical log $K_{o/w}$, as well as a solvent's bioavailability, must be experimentally determined for each specific system.

Because ISPR solvent extractants are generally limited to hydrophobic liquids, their extraction potential is related to the target molecule's hydrophobicity and chemical affinity towards the extractant, generally described by the principle of "like dissolves like". The hydrophobic, liquid polymer, silicone oil (polydimethylsiloxane) has enjoyed widespread use for its non-volatility, non-bioavailability, and high hydrophobicity providing good biocompatibility, which has led to its adoption as a default hydrophobic liquid extractant in TPPBs. However, it shares many of the same drawbacks as organic solvents including high cost, and emulsion formation which hampers reuse. Critically, its affinity is strictly towards hydrophobic target molecules. To our knowledge, there are no reports providing insight into the properties of silicone oil for use as an extractant, likely because it is

Table 1 Other applications of ISPR

Target molecule	Extractant class	Remarks	Reference
p-Hydroxystyrene	Organic solvent	Solvent selected from thirteen screened for product affinity and biocompatibility based on enzyme activity	Jung et al. (2013)
p-Hydroxystyrene		1-Decanol selected without rationale given. Solvent presence negatively affected product yield on substrate attributed to toxicity, yet productivity improved via extraction	Verhoef et al. (2009)
Taxa-4,11-diene and 6-deoxyerythronolide B		*N*-dodecane selected based on a previous report as an extractant for a similar (hydrophobic) product, without further rationale. Success attributed to the hydrophobic nature of these biotransformation products	Boghigian et al. (2011)
(R)-epichlorohydrin		Cyclohexane selected based on screening ten solvents for product yield and enantiomeric excess. Attributed success to solvent hydrophobicity and toxicity to solvent hydrophilicity	Jin et al. (2013)
2,3-Butanediol		Eleven solvents screened hierarchically for biocompatibility, bioavailability, and product capacity. Oleyl alcohol chosen based on miscibility	Pahlavanzadeh et al. (2009)
2,3-Butanediol		Eleven solvents screened based on high log P (biocompatibility), high boiling point, high product partition coefficient, low water solubility, bioavailability, and product capacity. Oleyl alcohol chosen based on miscibility	Anvari and Khayati (2009)
Styrene oxide	Organic solvent blend	Solvent blend of bis(2-ethylhexyl)phthalate containing 1 % (v/v octane) and 8 % (v/v) styrene (substrate for delivery) employed without providing rationale	Julsing et al. (2012)
Methane	Liquid polymer solvent (silicone oil)	Silicone oil used as hydrophobic phase for methane delivery from air stream without rationale provided, but speculated to improve substrate availability by reducing Henry's constant	Zúñiga et al. (2011)
Lactic acid	Natural adsorbent (activated carbon)	Activated carbon selected because of low cost, high surface area, and product affinity at low pH, prompting pH-uncontrolled mode of operation	Gao et al. (2011)
2-Phenylethanol	Adsorbent resin	Non-polar macroporous resin selected from eight tested based on productivity enhancement; attributed to hydrophobicity and surface area	Mei et al. (2009)
2-Phenylethanol		Non-polar macroporous resin selected from eight tested (rationale not shown) including ion-exchange, polar, and non-polar macroporous	Wang et al. (2011)
2-Phenylethanol		Non-polar macroporous resin selected from eight tested based on product capacity and selectivity with respect to substrate	Hua et al. (2010)
2-Phenylethanol		Resin selection criteria unclear. Semi-continuous operation by alternating adsorption/elution with two resin columns improved volumetric productivity	Wang et al. (2011)
2-Phenylethanol		Non-polar macroporous resin selected from seven tested based on high adsorption ratio, good biocompatibility, and quick product recovery. No data shown	Rong et al. (2011)
Poly-L-lysine		One resin chosen from four tested based on adsorption capacity; no rationale provided	Liu et al. (2011)
Propionic acid and vitamin B12		Resin selected from nine tested based on adsorption capacity. Ranking of resin capacities in model solution differed from that in fermentation broth, affecting selection	Wang et al. (2012)
(S)-1-phenyl-1,2-ethanediol		Resin selected from six tested based on rate of adsorption/desorption and capacity. Compromise in affinity required because substrate affinity was too high to permit biotransformation	Hu et al. (2010)

Table 1 continued

Target molecule	Extractant class	Remarks	Reference
Epichlorohydrin		Resin selected from seven tested based on adsorption capacity and product selectivity relative to substrate; no rationale provided	Zou et al. (2013)
Butanol		Adsorbent resin selected from five tested, including adsorbents and absorbents, based on capacity. Solid polymer extractants outperform organic solvents	Choi and Yeom (2011)
Linear and branched 2C–5C alcohols	Adsorbents and absorbent	Compared solute extraction efficacy of five adsorbent resins and one absorbent polymer. Solute hydrophobicity improved extraction via hydrophobic interactions, allowing prediction of extraction extent based on solutes' relative hydrophobicity. Did not differentiate adsorption/absorption mechanisms	Nielsen et al. (2010)
Propionic acid	Anion-exchange resin	Anion-exchange resin chosen, no selection criteria stated. Compared different feeding strategies and modes of contact with ion-exchange resin. External fluidized bed found to provide greatest ISPR improvement due to resin regeneration step	Wang et al. (2012)
Succinic acid		Macroporous polystyrene anion-exchange resin used in two parallel columns for expanded bed adsorption and elution in fed-batch operation. No resin selection rationale provided	Li et al. (2011a)
Phenylacetylcarbinol	Absorbent polymer	Absorbent polymer chosen for product partitioning was saturated with substrate and used for substrate delivery and simultaneous product removal	Khan and Daugulis (2010)
2-Pheylethanol		Absorbent polymer chosen based on product partitioning was used for selective product removal; hydrophilic substrate was not absorbed	Gao and Daugulis (2009)
1,2-Indandiol		Absorbent polymer chosen based on product and by-product partitioning was loaded with substrate via solvent partitioning and used for simultaneous substrate delivery and product/by-product removal	Dafoe and Daugulis (2011)
Benzaldehyde		Absorbent polymer selected based on product selectivity via maximizing the product/substrate partition coefficient ratio	Jain et al. (2010)
Hexanoic acid	Reactive extractant	Trioctylamine 10 % (v/v) in oleyl alcohol was selected based on previous reports of its use extracting organic acids. Toxic effects on biocatalyst noted	Jeon et al. (2013)
Ethylene glycol and glycolic acid	Combined membrane/anion-exchange resin	Hollow-fiber membrane used to protect cells from non-biocompatible anion-exchange resin. Resin selected from three tested based on product capacity	Wei et al. (2009)

a single, defined material with the only possible variation being viscosity, which is a function of molecular weight. In light of this, we believe that new silicone oil ISPR applications are unlikely to emerge beyond their facile use for hydrophobic target molecules.

Organic solvents

In cases involving system-specific constraints, extractant phase selection requires considerations beyond the basic aspects of biocompatibility, non-bioavailability and target molecule affinity. Recent experimental studies discussed below have identified additional desirable/necessary extractant properties, which introduce considerations of ecological impacts arising from solvent selection, mass transfer rate, or impose restrictions on extractant properties to enable its incorporation into a final product. Additionally, advances in the understanding of extractant-target molecule interactions have enabled more effective rational extractant selection strategies using simulations requiring no experimental data, described below.

Organic solvents as extractant phases have been compared on the basis of ecological impacts, in addition to economic considerations, in order to minimize the environmental footprint of the

biocatalytic epoxidation of styrene. Renewably-sourced ethyl oleate, a component of biodiesel, had an ecological cost which was 9 % lower than the conventional solvent, bis(2-ethylhexyl)phthalate, derived from petroleum. This was determined by considering comprehensive environmental criteria of the two solvents including their production, handling risks, and environmental fates (Kuhn et al. 2012). Comparison of solvents on an ecological basis is an important consideration to ensure process sustainability, and this should be considered in parallel with economic and performance aspects, especially with the use of solvents which cannot be recycled indefinitely.

Garikipati et al. (2009) found that in extractant selection, the high viscosity of dioctyl phthalate imposed a mass transfer limitation during mixing thereby rationalizing the choice of lauryl acetate as a lower-viscosity solvent despite it having a slightly lower affinity towards the product, 1-naphthol. In this case, the volumetric productivity was improved by increasing the mass transfer rate, rather than the extent, of extraction. An ideal extractant would provide both benefits simultaneously, however a priori predictions of such diverse properties are not currently available.

Solvent extraction ISPR has been effective in reducing end-product toxicity in the fermentative production of transportation biofuels and several groups have recognized the potential to use the extractant itself as a component of the fuel mixtures. The examples below show that hydrophobic products provide flexibility in selecting a liquid extractant which may satisfy additional criteria beyond the basic requirements of biocompatibility, non-bioavailability and affinity, sometimes at the expense of compromised performance. The inclusion of an auxiliary solvent phase which itself is a useful product may reduce or remove the requirement for downstream processing or product recovery, as demonstrated by the extraction of acetone, butanol, and ethanol from fermentation broth using biodiesel, resulting in improved diesel fuel properties (Li et al. 2010). Similarly, the use of farnesane as an extractant for microbially-produced limonene resulted in moderate alleviation of monoterpene toxicity relative to other solvents. In another study, farnesane was used to extract limonene, producing a solvent blend that had properties similar to jet fuel and could potentially be used without further processing (Brennan et al. 2012). Other researchers added an enzyme for the esterification of butanol (the inhibitory fermentation product) to butyl butyrate, a more hydrophobic species, and improved the partition coefficient in the auxiliary phase nearly 1000-fold. Its extensive extraction by hexadecane, a model diesel fuel compound, maintained a favorable equilibrium position towards esterification at fermentation pH while improving fuel quality (van den Berg et al. 2013). That is, in this approach, the improvement in product extraction concurrently increased its contribution to improved fuel properties of the blended auxiliary phase. The above solvent extraction approaches share the similar objective of providing process simplification by incorporating the extracted product within the auxiliary phase as a solvent blend having desirable fuel properties, reducing the number of subsequent downstream separation steps.

Hydrophilic products require more careful consideration of extractant interactions to facilitate adequate extraction because favourable interactions with the extractant are required in order to overcome their affinity for water, and a high distribution coefficient is desirable to reduce process expense by decreasing the amount of extractant required in a TPPB configuration. In an attempt to improve extractant selection strategies for ethanol, a water-miscible product which is difficult to extract, Keasler et al. (2013) examined specific interactions between extractants and the solute using molecular simulation techniques. The extraction of ethanol from water by several similar 10-carbon alcohols was predicted using computationally-intensive Monte Carlo simulations, and a trade-off was found between extractant capacity and selectivity relative to water. The ability of primary alcohols to form large hydrogen-bonded ethanol clusters also promoted the co-extraction of water, whereas branched alcohols had lower ethanol capacity and also had the highest ethanol selectivity relative to water (Keasler et al. 2013). Insights into the mechanistic differences between similar extractants improve our understanding of such interactions, and these findings may be applicable to new extractant materials. Detailed investigations of extractant-target molecule interactions such as this are necessary to understand subtle extractant behaviours and may be useful in fine-tuning processes for minor improvements in capacity/selectivity, but ultimately, primary

selection criteria such as biocompatibility and non-bioavailability must remain at the forefront.

The above reports are advances in the application of liquid solvent extractants to ISPR that go beyond merely describing a new system, and offer novel insights. Other recent demonstrations of improvements using liquid solvent extractants, compiled in Table 1, show the breadth of applications, and a recent patent application filed on the subject suggests that ISPR liquid solvent extraction may be approaching commercialization (Grady et al. 2010). While the use of organic solvent extractants in ISPR situations is now fairly mature, a focus on identifying and characterizing relevant solvent properties, from first principles, will likely be a source of inspiration for advances in future systems.

Ionic liquids

A more recent class of materials with potential for use as liquid extractants is ILs. ILs are salts that are liquid at room temperature and are comprised of a pair of counter-ions, opening a range of possible compositions and properties, making them potentially attractive as extractants. There is considerable flexibility in "constructing" the anion/cation pairs comprising ILs, providing the range of properties seen within the IL family; however, one of the more representative features of ILs is their very low volatility. Additionally, many ILs are toxic to biocatalysts or perform poorly relative to organic solvents with respect to target molecule affinity and selectivity and, perhaps most importantly, ILs are generally very expensive, an order or more higher in price than organic solvents. The lack of straightforward IL applications in Table 1 indicates that their use for ISPR requires more rigorous considerations than alternative ISPR extractants. Nevertheless, the reports reviewed below demonstrate recent advances in the use of ILs and, in some cases, suggest that ILs could surpass conventional solvents in extraction performance by providing a greater opportunity for fine-tuning the structure–property relationships towards the goals of biocompatibility, affinity, and selectivity.

In order to improve on the state of IL extractants for butanol recovery via ISPR, Garcia-Chavez et al. (2013) reviewed previous investigations and found that anion carboxylate functionality improved the distribution coefficient of butanol, while its hydrogen-bonding ability played a significant role in water uptake, hampering selectivity. Using this information, the authors designed a new IL containing a carboxylate functionality with two aromatic rings in the anion and long alkyl chains in the cation to improve its hydrophobicity. The new task-specific IL (TSIL) outperformed the distribution coefficient of the benchmark solvent, oleyl alcohol, by a factor of six with a 30 % improvement in selectivity. The entire process, including recovery and re-use, was simulated using various extractants, and for the new IL, a solvent: feed ratio of 0.071 performed equivalently to a ratio of 0.456 for oleyl alcohol in terms of butanol recovery. This represented a significant economic improvement in the energy required to heat the solvent for recovery and re-use. The decrease in amounts of solvent being used is motivation to seek specialized materials but their additional cost must be justified, and this report concedes that biocompatibility is an additional, important factor in their application which has not yet been investigated.

The application of ILs to fermentation systems certainly requires considering biocompatibility, and the lack of tabulated IL properties (e.g. log $K_{o/w}$) makes standardized a priori prediction, as can be done for organic solvents, currently impossible. In the fungal hydroxylation of epoxyprogesterone, Mao et al. (2012) screened seven ILs, only one of which demonstrated nearly-full biocompatibility, yet all ILs had some deleterious effect on cell growth. The inhibitory effect was attributed to the anion, while improvements in distribution coefficient were attributed to the cation structure, gained by increasing alkyl chain length and increasing the surfactant nature of the cation. The effect of ILs on substrate conversion mirrored their toxicity profiles and only the biocompatible IL improved productivity relative to the control. The distribution coefficients of the substrate and product were both exceptionally high, an expected result based on their hydrophobicity, such that the primary effect of ILs on the biotransformation was IL toxicity. The authors suggest that a lower phase ratio could reduce harmful interfacial contact of the biocatalyst with the IL phase, and may be a strategy to optimize extraction and biocompatibility (Mao et al. 2012). Despite their toxicity, the high distribution coefficients of the ILs offered some protection from substrate inhibition in

every case; however biocompatibility should be an absolute requirement of extractant phases.

ILs are valued for their solvation power and non-volatility, which could substantially decrease energy costs of ethanol distillation if ILs were to be used as ISPR components in ethanol production. Neves et al. (2011) undertook a detailed study of seven phosphonium-based ILs in ternary phase systems with ethanol and water, creating phase diagrams that were extremely well-predicted by the NRTL model. The COSMO-RS model was less accurate in predicting exact phase compositions but was able to rank the ILs correctly without requiring experimental data, and was also used to estimate the performance of two additional ILs for which data are not available. These authors also considered the cost and biocompatibility of the chosen ILs which compare favorably to imidazolium-based ILs, but note that by using tabulated EC_{50} values as an indication of biocompatibility, interactions with cell membranes are not represented and biocompatibility with whole cells would require additional experimentation. This report (Neves et al. 2011) underscores the comprehensive nature of extractant selection requirements, where multiple, unrelated parameters must be considered simultaneously, and the properties of IL extractants, at this stage of our understanding, may prevent characterization to a similar degree as organic extractants.

The relatively high cost of ILs may be acceptable if their performance enhancements surpass the cost-effectiveness of alternative extractants, particularly if the value of the recovered product is high. However, the relative scarcity of literature discussing the application of ILs in ISPR suggests that the understanding of IL biocompatibility is heuristic at best, and progress must be made in that regard, as well as in reducing IL cost, to exploit their attractive performance as immiscible solvents. Overall, our general understanding of IL properties and their interactions with cells is somewhat limited, and an important future area of research, focusing on defining extractant properties, will be in addressing these aspects.

Aqueous two-phase systems

Aqueous two-phase systems (ATPS) are a subset of TPPBs comprised of water-soluble polymers and/or salts in aqueous solutions at concentrations at which two primarily aqueous phases can form. ATPS have traditionally been favored for the recovery of proteins and antibiotics, whose labile structures can be preserved in such an aqueous environment; however, examples of the recovery of small molecules are more rare, and recent examples are described below. The reports reviewed below were selected as they demonstrate ATPS applications which provide insight into the effects of selected ATPS components (i.e. the composition of the two aqueous phases) and operating parameters on biocompatibility, product partitioning, and selectivity.

The recovery of extracellular cyanobacterial products, beta-carotene and lutein, was investigated as single-step ISPR using an ATPS rather than a downstream extraction process as had been previously proposed. The ATPS composition consisting of polyethylene glycol (PEG)/potassium salts was found to prevent cyanobacterial growth, while a PEG/dextran system allowed growth only at lower dextran molecular weights, attributed to the potential occlusion of aqueous nutrients in a dextran phase with a very high excluded volume at high molecular weight. This effect, arising solely from the molecular weight of an ATPS component, suggests that determining biocompatibility in these systems may be less systematic and more complicated than for solvent-based TPPBs because of the complex interactions with water occurring in both phases, which may cause more direct interactions with the biocatalyst, such that water activity may be an additional criterion in determining ATPS biocompatibility. Biomass partitioning is an important factor in ATPS design, and can provide preliminary separation of the product from the biocatalyst as a subsequent downstream operation. In this particular case, the biomass partitioned to the phase interface, but its presence in the PEG phase required centrifugation prior to product recovery with hexane (Chavez-Santoscoy et al. 2010). While this study extends the applicability of ATPS to valuable small molecule products, it is questionable whether an ATPS system would be advantageous, given the complications of system tuning for biomass viability, product partitioning, and the requirement for ultimate solvent extraction, while the hydrophobic product would likely partition effectively into a biocompatible organic solvent.

ISPR using surfactant micelles is another ATPS configuration, termed a cloud-point system for its

phase separation, which is induced at certain operating conditions (typically at an elevated temperature) rather than being inherently biphasic as with a TPPB. Five amphiphilic copolymers of polyethylene oxide (PEO) and polypropylene oxide (PPO) in varying proportions were investigated as non-ionic surfactants for the extractive fermentation and subsequent cloud-point extraction of butanol from model fermentation broth. The butanol capacity was very high for the hydrophobic surfactants, although a water-insoluble surfactant was unable to capture any of the butanol. Of the five surfactants studied, only the more hydrophobic ones proved to be biocompatible, attributed to the hydrophile-lipophile balance, with the more hydrophilic surfactants exhibiting toxicity. The combined effects of surfactant biocompatibility and butanol capacity resulted in only one surfactant improving the butanol titer. Micelle formation was ruled out, indicating that butanol was associating with free, dissolved surfactant molecules and this was sufficient to reduce its toxicity. Butanol was recovered by cloud-point separation of the surfactant at 70 °C, demonstrating a butanol partition coefficient of 3.5, followed by butanol evaporation at elevated temperature (Dhamole et al. 2012). This partition coefficient value is not particularly high relative to other reports of butanol ISPR (Barton and Daugulis 1992; Oudshoorn et al. 2009). While the application of a carefully-selected surfactant reduced butanol toxicity, It is conceivable that similar improvements (and similar complications) would arise from butanol ISPR using a biocompatible solvent having similar affinity, but would offer a simpler operation as an inherently biphasic system.

Two inhibitory molecules sufficiently different in polarity that they would not be effectively extracted together using a hydrophobic liquid prompted the investigation of cloud-point extraction of L-phenylacetylcarbinol produced from benzaldehyde, an inhibitory substrate, using a hydrophilic non-ionic surfactant. This required downstream solvent extraction with an equal volume of butyl acetate, followed by water extraction to separate the product from the surfactant, resulting in high recovery of components and alleviation of substrate/product inhibition (Wang et al. 2010). This configuration was efficient in terms of productivity, yet the requirement for subsequent extraction steps using large amounts of solvent represents an unattractive processing step and expense. Ideally, an extractant could be recovered directly, requiring only a single recovery step prior to re-use, potentially avoiding the direct contact of toxic solvent with the fermentation medium.

For the recovery of intracellular pigments from the fungus, *Monascus*, the use of an organic solvent (vegetable oil), an IL and several surfactants were each compared for their extraction performance in a combined perstraction/cloud-point extraction approach (Hu et al. 2012). In this system, the extractant intentionally permeabilized the cell membrane to promote the secretion of intracellular pigments, while micelles formed in the aqueous phase partitioned and concentrated the products separate from the aqueous phase. The use of a surfactant was preferable to immiscible organic solvents, which must compromise extraction efficiency with biocompatibility, while amphiphilic surfactants could fulfill both roles. One surfactant was found to outperform the others in improving productivity by alleviating intracellular inhibition, while the IL failed to promote any pigment secretion. This result was presumed to be due to the ILs potential toxicity of the IL, as biocompatibility was evaluated solely based on production. Concerns about widespread toxicity of ILs discussed above are obvious here in comparison to other ISPR materials. Additionally, the production profile was skewed, attributed to the relative polarity of the various pigments affecting their extent of extraction and hence level of inhibition. At high surfactant concentrations, the growth morphology became filamentous and productivity dropped, suggesting a possible biocompatibility problem (Hu et al. 2012). This system showed that intracellular products may require additional extractant features, such as a surfactant nature, in order to facilitate secretion and subsequent extraction. This aspect must be considered simultaneously with the conventional requirements of biocompatibility and product affinity; furthermore, such features may be mutually exclusive, narrowing the array of candidate materials.

Aqueous two-phase systems have been applied to the extraction of small molecules where conventional liquid solvents may not satisfy both biocompatibility and product extraction requirements; however, in some cases the motivation to adopt ATPS rather than alternative extraction schemes is not obvious, as simpler operation may be achieved using conventional solvents which are biocompatible. Phase separation at elevated temperature, typically requiring centrifugation of biomass prior to product recovery, is energy-

intensive and imposes logistical constraints on process continuity. The relatively complicated nature of selecting the phase-forming components for this extraction approach to meet both general (target molecule affinity, biocompatibility) and system-specific requirements means that such systems may be more suited to the high-value products which are typically extracted using ATPS, rather than commodity products. This is likely why we have been unable to provide any ATPS reports in Table 1; similar to ILs, their complexity requires considerable characterization which generally makes ATPS less accessible as a simple ISPR strategy.

Solid–liquid systems

Solid extractants (particularly as polymers) provide more options for selecting ISPR materials because their complex chemistry and related structure–property relationships enable wider variation in thermodynamic properties, which have an effect on target molecule affinity (e.g. homopolymer/co-polymer composition, degree of crystallinity, glass transition temperature, etc.) than liquid extractants while generally also being biocompatible, as well as being non-bioavailable. As solids, they are mechanically stable and do not dissolve in the fermentation medium, making them largely inert with respect to cellular toxicity, and immune to losses in TPPB configurations associated with water-solubility. The nature of solid extractants differs depending on systems' requirements for cost (more on cost later) and performance, ranging from natural materials such as zeolites and activated charcoal to synthetic macroporous or gel-type resins, and, recently, soft amorphous polymers.

Porous resins are typically hard, glassy solids in order to maintain their porous configuration and surface morphology. Resins may be functionalized with surface chemistry to impart properties such as polarity, and acid/base functionality may be incorporated on the surface of porous materials, or within the bulk in the case of gel-type ion exchange resins.

Adsorbents

Adsorbent resins have become a popular choice of ISPR extractant, typically selected by screening many commercial materials using trial-and-error methods, and subsequently comparing product capacity and selectivity, or similar parameters, in order to select an extractant. That is, there does not appear to have been any attempt to this point: to predict which types of resin adsorbents would be effective from first principles' considerations; rather, observations tend to be made in hindsight. Nevertheless, their relative simplicity of implementation has led to adsorbent resins representing the majority of recent ISPR studies, shown in Table 1. Several studies of particular interest are discussed below for their initial insight into extractant selection strategies, which may be useful in guiding selection for future investigations. These reports consider additional process parameters, such as tiers of selectivity for multiple target molecules, sequential ion-exchange resins in series, product/substrate selectivity, or characteristics of the resins which introduce an effect on system operation and performance.

Several aspects of adsorbents, beyond their distinct mechanisms of sorption, distinguish them from absorbents. The performance of adsorbents in fermentation medium is generally lower than in model solutions due to the presence of other solutes which compete for the finite number of surface sorption sites (Mirata et al. 2009; Ranjan et al. 2009). Furthermore, the presence of abundant surface area on macroporous resins provides access for biofilm formation and fouling by cells (Mirata et al. 2009; Wang et al. 2012; Wei et al. 2009). The difference in surface morphology between a macroporous adsorbent resin and an absorbent polymer is shown in Fig. 1.

Neutral adsorbents, such as zeolites, activated charcoal and non-functionalized resins, interact with target molecules through hydrophobic interactions, where affinity and selectivity arise from the solutes' relative affinity between the aqueous and adsorbent phases. Beyond relative hydrophobicity, other adsorbent characteristics can be used to provide selectivity. As an example, in a recent study three hydrophobic zeolites, having different pore sizes selected for the removal of three inhibitory hydrolysate components by size exclusion, enabled the selective, individual removal of three valuable co-products (hydroxymethylfurfural, furfural, and vanillin) which would inhibit ethanol fermentation, while preserving fermentable sugars (Ranjan et al. 2009). Such selectivity among several small molecules, based on resin pore size

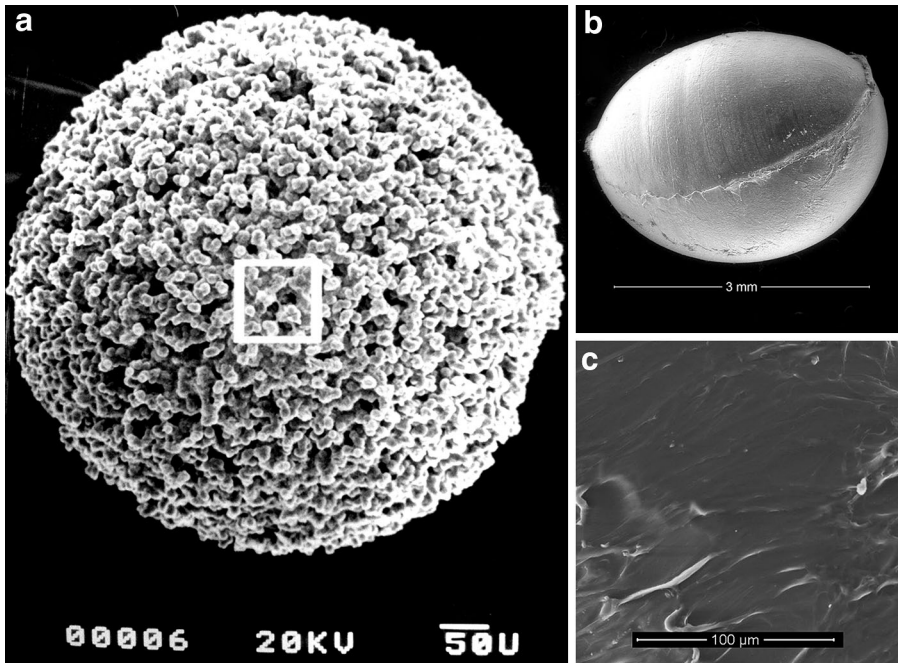

Fig. 1 Scanning electron micrographs of **a** a macroporous adsorbent resin bead (Rohm & Haas Amberlite IRA938) (50 µm scale indicated) (Krug and Daugulis 1983). **b** An absorbent polymer bead (Pebax 2533) (3 mm scale indicated). **c** The smooth, monolithic surface of Pebax 2533 (100 µm scale indicated)

being matched to molecular dimensions, is a promising tool for recovery of fermentation products as well.

The extractive fermentation of organic acids is gaining attention to improve process economics for commodity production, yet carboxylic acids typically carry a negative charge at fermentation pH due to their low pK_a values, and will not engage in hydrophobic interactions for product recovery using conventional hydrophobic resins. The extraction of acids in their ionic form may be accomplished using ion-exchange resins which engage in ionic bonding, but must be pre-equilibrated prior to and regenerated after use with salt solutions which pose a problem for waste generation.

The production of perillic acid by limonene oxidation is a suitable system for ion-exchange ISPR, where the carbon source, glycerol, was found to not adsorb, in contrast with alternative, ionic carbon sources, which would be expected to have similar affinity towards the resin as the product itself and could introduce a nutrient limitation. Furthermore, from seven anion-exchange resins which were screened, only two did not affect the pH of the medium appreciably, while product affinity was the final resin selection criterion. However, the hydrophobic substrate, limonene, was adsorbed significantly by all resins, signifying a loss of yield on substrate, and necessitating a fed-batch feeding strategy using a limonene-saturated air stream and limonene-saturated resin (Mirata et al. 2009). In this case, the extractant was secondary to the selection of carbon source in terms of process viability, yet the substrate, which cannot be similarly substituted, imposed a limitation on yield by its extensive retention.

Facing a similar challenge, an ISPR strategy designed to overcome inhibition from (*R*)-(−)-mandelic acid sought a resin which had high product selectivity relative to the substrate, (*R*,*S*)-mandelonitrile, to ensure the substrate was supplied without limitation. The authors determined that strong base functionality provided the best affinity towards the acid product, while gel-type architecture provided greater selectivity than porous architectures (Xue et al. 2010). These insights, considering properties of polymer architecture that relate to affinity, may help improve adsorbent affinity and selectivity in future studies.

The use of adsorbent and ion-exchange resins for product recovery is effective for hydrophobic compounds, and target molecules which carry a charge at fermentation pH, and these systems are well-

represented in Table 1, indicating their straightforward implementation. However, recovery of strongly-adsorbed molecules often requires heating or concentrated salt solutions to regenerate the extractant, which introduce significant energy and waste stream demands. The literature indicates that trial-and-error is still widely employed in selection to characterize adsorbent properties, and a compromise among several selection criteria will usually be necessary. As noted, few attempts have been made to approach the rational selection of ISPR resins via first principles considerations, which will hamper progress in expanding their application.

Absorbents

A distinct class of materials, soft (having a low glass transition temperature), amorphous (lacking significant crystallinity) polymers, has been used as an alternative to the above-mentioned adsorbents, primarily in the authors' group, as "solid solvents" because they operate by passive solution and diffusion of the target molecule within the polymer structure itself, a distinct mechanism from surface adsorption or strong ionic interactions seen with conventional resins. An important aspect of absorptive ISPR is the rate of target molecule extraction relative to the rate of biocatalytic production, which is governed by the diffusivity of the target molecule within the polymer, and should be sufficient to keep pace with the biotransformation rate. If mass transfer rate was limiting, the diffusive path length could be reduced by decreasing the size of absorbent polymer particles, thereby improving the overall mass transfer rate (Fam and Daugulis 2012). This kinetic aspect of absorbent ISPR has received less attention than polymer-target molecule affinity at equilibrium, possibly because it has only rarely been identified as a problem (Rehmann and Daugulis 2007). [This aspect is a focus of ongoing work within the authors' group.]

In contrast to surface-area-based adsorbents, the performance of absorbents in fermentation medium is generally higher than in model solutions due to the presence of additional solutes in the fermentation medium, thereby acting to increase the target molecule's activity in the aqueous phase, resulting in a higher proportion in the extractant at equilibrium, similar to what is seen with liquid extractants (Dafoe and Daugulis 2013b). The effect of competition for site occupation, as seen with adsorbents, does not occur because the absorption mechanism permits internal permeation and swelling of the polymer, giving a solute capacity which can vary depending on the solute's compatibility with the polymer (Parent et al. 2012). This results in typically linear isotherms across the concentration ranges of interest. Since the entire amount of amorphous polymer is used to sorb target molecules, it is the mass of the polymer, rather than the surface area available, that determines overall extent of uptake with these materials. This fundamental difference in uptake mechanism was confirmed by changing the specific surface area of fixed masses of absorbent polymer beads, and demonstrating that the absorption capacity remained unchanged, meaning that these materials' performance is mass, not surface area, dependent (Craig and Daugulis 2012). The lack of macroporous architecture in these materials also means that a relatively small surface area is exposed to the fermentation culture, such that cell attachment has not been observed, in contrast to many reports of resins and solvents becoming fouled by entrapped cells, preventing their re-use.

Although many earlier successful examples of soft-polymer ISPR systems have now been published (some recent examples are provided in Table 1), the selection of such polymers has historically been via trial-and-error, substantially as has been the case for hard resin testing and selection. Recently, however, the selection of soft polymers for absorptive extraction has been examined from a first-principles' perspective, where the polymers were treated as solvents in judging the relative affinity towards target molecules through the use of solubility parameters, a useful tool which moves polymer selection from a heuristic approach towards rational selection (Parent et al. 2012).

Absorbent polymers have been utilized for selective product removal and also for simultaneous substrate delivery and product removal. Recent examples demonstrating their utility are given in Table 1. Absorbed molecules are easily recovered by contacting the polymers with a solvent which typically has a much higher affinity for the target molecule than water, requiring a relatively small volume for complete recovery and regeneration of the polymer. Alternatively, volatile products could be recovered

by heating the polymer, avoiding the use of solvents altogether and providing significant energy savings.

Absorbent polymers exhibit similar overall trends to other extractants with respect to hydrophilic target molecules partitioning modestly, because affinity for water decreases the extent of extraction. However, the availability of hydrophilic polymers can somewhat address this shortfall, while solvent extractants are necessarily strictly hydrophobic. Block copolymers containing hydrophilic chain segments may be stabilized against dissolving in water by incorporating hard, glassy or crystalline segments within the polymer chain. In this way, the biocompatibility requirement of the relatively water-soluble polymer extractant is circumvented because it remains in a separate, solid, phase. This feature of absorbent copolymers is currently the focus of more in-depth investigation in the authors' group as a potentially useful tool for target molecule selectivity.

Such hydrophilic block copolymers exhibit greater affinity towards relatively hydrophilic target molecules which would be impossible to extract using hydrophobic solvents, as was demonstrated for the simultaneous removal of a relatively polar pharmaceutical intermediate, 1,2-indandiol, and its toxic by-product, 1-indenol, both of which a hydrophobic liquid solvent (silicone oil) was unable to extract (Dafoe and Daugulis 2011). Additionally, less-polar target molecules had a higher affinity towards the hydrophobic polymers, while the more polar product had preferential affinity towards a hydrophilic polymer relative to hydrophobic ones, resulting in different biocatalytic production profiles as a result of preferential removal by each polymer (Dafoe and Daugulis 2013b). The extent of water uptake by hydrophilic polymers must be carefully considered from a selectivity standpoint, to avoid the co-extraction of other water-soluble medium components (Dafoe and Daugulis 2013a). Also, when comparing target molecule affinity, polymers with different water uptake levels must be analyzed on an equal mass basis, and should be in equilibrium with water prior to measuring target molecule partitioning to avoid simply transferring aqueous volume and skewing partition coefficients.

Being commercially-produced materials on a commodity scale, these absorbent polymers cost much less than either specialized resins, ILs, or biocompatible solvents (Quijano et al. 2010), typically in the range of $5–7 per kg. Despite their ease of use and good performance, polymer selection remains in a less-developed state than selection schemes for solvents due to the complex structure–property relationships with polymers. The advantages of soft polymers relative to other extractants has motivated a more thorough understanding of the interactions between the polymer and the target molecule, as well as other fermentation components such as water, to enable more effective polymer selection, and such thermodynamic approaches are currently underway (Poleo and Daugulis 2013). The number of reports demonstrating the straightforward application of soft absorptive polymer ISPR to diverse biocatalytic systems, shown in Table 1, indicates that this strategy is both effective and simple to implement.

Other systems

Reactive extraction

In order to overcome the often modest distribution coefficients which impede the effective extraction of relatively hydrophilic products, the inclusion of a reactive extractant, a species with functionality complementary to a target molecule's reactive group, typically as a component in an immiscible organic phase solution, can improve uptake by stoichiometrically binding the product in the extractant phase, enhancing its solubility and providing a high driving force for removal. The reactive mechanism ensures that only compounds with complementary functionality will interact, providing a basis for selectivity among target molecules with different functionalities. However, the covalently-bound complex may subsequently require increased energy and material inputs for ultimate product recovery. The following reports demonstrate recent advances in reactive extraction, which include imparting reactive functionality to a solid-phase support, using a blended extraction mechanism for a wider target molecule profile, or using commercial materials to guide the synthesis of effective reactive extractant solvents. The system-specific considerations required in conceiving a reactive extraction scheme has limited the breadth of straightforward adoption; only a single application appears in Table 1.

A reversible binding process was sought for the removal and recovery of 3-hydroxypropionaldehyde

(3-HPA), a toxic product which is non-volatile, hydrophilic, and heat-sensitive, and for which conventional extractants fail or perform poorly. By binding reactive sulfite ligands to a chloride-functionalized ion-exchange resin, a 3-HPA-sulfite adduct could be formed on the resin surface. The presence of the modified resin had a negative effect on cell growth and viability and up to 35 mol% of bound 3-HPA leaked out of the resin as the adduct. Such cytotoxicity has often been reported for the use of reactive extractants as ISPR materials. Furthermore, the adduct was eluted rather than the free product and required several complex steps for product recovery. This represented a significant additional processing expense (Ruetti et al. 2011). The modification of resins for reactive, reversible product binding is an example of designing complementary functionality towards target molecule into the extractant, however the viability suffers from adduct leakage from the reactive ligand.

For the detoxification of biomass hydrolysate intended for the production of ethanol, reactive extraction with trioctylamine (TOA) was examined for the removal of five inhibitors (formic acid, acetic acid, levulinic acid, 5-hydroxymethylfurfural, and tetrahydrofuran) from a model solution using solvents containing TOA (Jeong et al. 2013). *n*-Octanol was the most appropriate diluent compared to alkanes of various chain lengths, as it was speculated that solvent polarity owing to its hydroxyl functionality would promote solubility of the polar amine complexes relative to the non-polar alkanes. For each target molecule, the extent of extraction depended on its relative affinity towards the aqueous phase, with only 2–4 % of formic acid removed. The extraction of acids was found to occur via reactive extraction, while extraction of the small neutral species was found to occur via non-reactive solvent extraction in the organic phase, which was improved by the addition of 10 % kerosene as a non-polar diluent. Despite being a pretreatment step rather than a classical example of ISPR, this strategy provides insight for ISPR applications which use reactive extraction for multiple target molecules, showing that a compromise in extraction performance across all targets must be reached by adjusting extractant composition.

As TOA has long been the benchmark extractant for carboxylic acids, there may be potential to improve on its binding capacity using rational means. A recent study examined several commercial functionalized silica compounds in order to design a strategy to synthesize novel liquid extractants for lactic acid (Leeman et al. 2013). Based on results of carboxylic acid affinity for the functionalized silicas, an extractant synthesized with dimethylaminopyridine functionality for extraction and tridecyl alkyl chains for hydrophobicity was found to significantly exceed the capacity of TOA at all temperatures and lactic acid concentrations; however, being a solid, its maximum loading in the diluent 1-octanol was only 20 %. Two other extractant candidates were found to increase the pH and form emulsions in water upon complexation with lactic acid, suggesting that these complexes were soluble in the aqueous phase and were leaching adsorbed lactic acid. Although only one candidate met the process requirements and had less than optimal capacity due to its solid state, the use of readily-available commercial materials to guide synthesis of extractant candidates is a rapid and cost-effective approach to extractant design and synthesis, and proved to be an effective strategy in outperforming the current benchmark extractant, TOA.

Reactive extraction demonstrates its greatest potential in the removal of reactive, hydrophilic molecules which are difficult to extract using conventional means; however, reactive extractants are often non-biocompatible and additional steps must be taken for their implementation without harming the biocatalyst. The additional affinity towards the extractant makes the product equally more difficult to recover, which should also be considered in evaluating process viability.

Combined systems

The establishment of several distinct ISPR categories described above has led to recent examples combining different approaches in order to exploit the advantages, or to reduce the drawbacks, of individual methods. Consequently, these combined systems have increased complexity and, due to their additional, system-specific considerations, reports of straightforward applications do not appear Table 1. These strategies strive to improve on limitations inherent in established processes, such as mass-transfer rate, or to separate the potentially adverse interactions between extractant and cells in the fermentation medium. In all cases, continuous extraction is preserved through the

use of membranes in conjunction with conventional solvent extraction, or by using alternative, biologically inert modes of extraction such as gas stripping or electrodeionization.

To circumvent solvent toxicity, the use of hollow-fiber membranes, to physically separate the aqueous and extractant phases, reduced the requirement for strict biocompatibility thereby allowing cytotoxic pentane to be used to aid in the recovery of 1-phenylethanol. This configuration required a compromise between extraction efficiency and biocompatibility. Less hydrophobic solvents which were non-biocompatible, but which possessed higher solute affinity, were prone to leak through the membrane into the aqueous phase, while more hydrophobic, biocompatible solvents with higher affinity could dissolve the membrane itself (Mihal et al. 2012). As noted, the chosen solvent (pentane) was the compromise solvent balancing solute affinity with membrane compatibility. The addition of a membrane enabled the use of a non-biocompatible solvent but still introduced additional factors (i.e. membrane compatibility and leaching) which required specific considerations during process design. Nevertheless, this configuration performed well over multiple extractant re-use cycles, and was well-predicted by a mathematical model for future applications.

Using a porous poly(tetrafluoroethylene) (PTFE) membrane to separate the extractant from the fermentation medium enabled the use of 1-dodecanol, a solvent which was non-biocompatible towards the organism, *Clostridium saccharoperbutylacetonicum*, for the extraction of butanol (Tanaka et al. 2012). Dodecanol out-performed the benchmark biocompatible solvent, oleyl alcohol, in this configuration due to its higher partition coefficient. Furthermore, the porous PTFE membrane enabled 20-fold greater butanol productivity per specific membrane area than a previous report using a monolithic silicone membrane, indicating that the membrane surface area could be significantly reduced with this configuration. The remarkable improvement in specific productivity resulting from simply replacing the membrane material indicates that there is potential for subsequent improvements using a rational material selection approach, however the different mechanisms of membrane permeation, either sorption and diffusion or conduction through pores, means that a comprehensive selection strategy may not be available as with soft polymers, which all operate via absorption.

The use of a silicone membrane contacting the fermentation medium with a vacuum on one side was used to remove, and subsequently condense, volatile products from the acetone/butanol/ethanol fermentation (Li et al. 2011b). The mechanism of membrane permeation followed the sorption and diffusion mechanism, enabling characterization with a mathematical model. Butanol mass-transfer coefficients across the membrane decreased upon increasing the complexity pf the solution due to reduced solute activity, while fouling was apparently not a significant problem. This pervaporation configuration enabled higher and more rapid butanol production with continuous extraction, without requiring solvent recycling; however, the amount of co-extracted water greatly exceeded the amount of butanol, producing a dilute extract. This demonstrates that the introduction of vacuum in combination with the membrane imposes an additional degree of complexity, and membrane material selection efforts under this constraint may be necessary to maximize product selectivity.

The use of a liquid membrane consisting of TOA dissolved in dichloromethane at varying concentrations, held in a U-shaped cell separating the feed aqueous phase from a solution of NaOH for back-extraction, was investigated to improve the separation of succinic acid from model fermentation medium which additionally contained acetic and formic acids (Galaction et al. 2013). [Please refer to this article for further information on the apparatus employed.] The acid co-products were extracted sequentially into TOA based on their relative acidity, and TOA concentrations, beyond those which could stoichiometrically bind the smaller acids, were required for succinic acid extraction. In addition to suffering from diffusion limitation through the liquid membrane, reactive extraction combined with membrane permeation also introduces the kinetic limitation of complex formation and subsequent re-extraction into the extractant phase. The results of this study suggest that combining reactive extraction with membrane permeation may be favorable to selectively remove other acid by-products, leaving succinic acid, with the most favorable selectivity in this regard occurring with the highest pH gradient between the feed and extractant phases and at a relatively low TOA concentration in

dichloromethane (Galaction et al. 2013). A high extraction capacity was provided by the back-extraction step in this configuration; however a final separation step for recovery of succinic acid, the desired product, would be required.

The accumulation of butanol has been addressed with intermittent gas stripping in order to design an economical process with few steps (Xue et al. 2012). Relatively high aqueous butanol titers above 8 g l^{-1} were tolerated by an immobilized, solvent-tolerant *Clostridium* strain in repeated batch cultures, which increased the driving force for mass transfer to the stripping gas and greatly improved butanol recovery. Collection and condensation of the gas stream yielded a 15 % (w/v) butanol aqueous solution which spontaneously phase-separated into an organic phase containing >60 % (w/v) butanol, 4 % (w/v) acetone, 1 % (w/v) ethanol, with the remainder presumably being water. A very effective first extraction step. The presence of free cells adversely affected the stripping efficiency, such that the immobilization technique may likely be necessary for both continuous cultivation and product recovery. This study has shown that improvements to biocatalyst solvent tolerance open new options for ISPR which can take advantage of more intensive production, but also impose new considerations for process configuration.

In a continuous adsorption/desorption process, a membrane bioreactor with ion-selective membranes was packed with a mixture of acid and base ion-exchange resins, and a 15 V DC field was applied across the fermentation medium. This configuration continually removed adsorbed lactate from the resin which accumulated in a membrane-separated phase through electrodeionization (Boontawan et al. 2011). Cell deactivation by lactate exposure was reduced fivefold, and fed-batch operation was maintained for 10 days. The coupling of resin adsorption and electrodeionization permitted the use of a relatively small amount of resin which could be chosen mainly based on its selectivity, as capacity was not a limiting factor in this configuration.

A similar electroextractive configuration was used for the removal of inhibitory organic acids in the production of H_2 by separating the cell-containing vessel from an electrolyte solution using a combination of ion-selective membranes. Electrokinetic removal of organic acids successfully regulated culture pH and alleviated inhibition, while H_2 was measured in the off-gas. The culture continued for 25 days until ethanol accumulation was presumed to become inhibitory as it was not removed by electrodeionization (Redwood et al. 2012). The above examples of electrodeionization demonstrate efficient removal of charged species, at the expense of system complexity and accumulation of neutral inhibitors, which may require additional, combined extraction approaches. Nevertheless, the use of a mild electric current for the removal of ions through selectively-permeable membranes is a non-invasive, continuous strategy which could be applied to many fermentation systems.

Careful consideration of a particular system's requirements is necessary when combining different ISPR strategies in order to achieve improvements, and these configurations are not "one-size-fits-all" solutions due to their complexity; therefore there are no examples of combined ISPR systems contained in Table 1. In the above examples, combined approaches were conceived in order to counteract a drawback arising from a property of a particular system (e.g. biocompatibility problems); however, additional complexity and drawbacks are inevitably introduced. The degree of complexity permissible depends on the value of the product, and development of combined ISPR processes may be worthwhile in cases where biocatalysis is an attractive route for production of a valuable product and conventional ISPR strategies are ineffective.

Conclusions and future directions

The shape of recent literature discussing ISPR indicates that the field is expanding in both breadth, through an increase in the number of applications, and depth, through advances in technical know-how and constantly improving benchmarks. Emphasis on improving extractant capacity and selectivity will intensify processes without requiring dramatic alterations to equipment or biocatalysts. Nevertheless, expected improvements to engineered biocatalysts in the areas of enzyme selectivity and tolerance to inhibitory molecules will also benefit from better extractant performance in more intense processes. While a highly-selective extractant with a low capacity would suit a continuous process, many bioprocesses

operate in batch mode for logistical reasons, and a high capacity enables simpler batch operation with less frequent turnaround.

Cost is an important factor in extractant selection, especially for high-volume, low-cost products. The use of exotic, task-specific materials or complex process configurations must improve performance sufficiently to justify the inevitable increase in cost. Consideration of all relevant extractant properties in order to arrive at a rational choice for a particular system remains a challenge; one must consider the basic requirements (affinity, biocompatibility, and non-bioavailability) simultaneously with system-specific ones (e.g. operability concerns or the ultimate fate of the product) and system complexity introduces additional considerations. Understanding the interactions which govern target molecule-extractant affinity is of utmost importance for any class of extractant to gain widespread adoption, and recent strides have been made in this regard in the case of absorbent polymers; although promising, ILs currently appear to lack systematic approaches. With the above aspects considered, it is our opinion that soft, absorbent polymers are an attractive yet under-recognized class of ISPR materials having advantages over all alternatives in the areas discussed: biocompatibility, non-bioavailability, phase-stability, cost, simplicity of implementation, and affinity towards a wide range of target molecules. We further believe that these are the most promising materials for many future ISPR applications.

References

Anvari M, Khayati G (2009) In situ recovery of 2, 3-butanediol from fermentation by liquid–liquid extraction. J Ind Microbiol Biotechnol 36:313–317

Barton WE, Daugulis AJ (1992) Evaluation of solvents for extractive butanol fermentation with *Clostridium acetobutylicum* and the use of poly (propylene glycol) 1200. Appl Microbiol Biotechnol 36:632–639

Boghigian BA, Myint M, Wu J, Pfeifer BA (2011) Simultaneous production and partitioning of heterologous polyketide and isoprenoid natural products in an *Escherichia coli* two-phase bioprocess. J Ind Microbiol Biotechnol 38: 1809–1820

Boontawan P, Kanchanathawee S, Boontawan A (2011) Extractive fermentation of l-(+)-lactic acid by *Pediococcus pentosaceus* using electrodeionization (EDI) technique. Biochem Eng J 54:192–199

Brennan TC, Turner CD, Krömer JO, Nielsen LK (2012) Alleviating monoterpene toxicity using a two-phase extractive fermentation for the bioproduction of jet fuel mixtures in *Saccharomyces cerevisiae*. Biotechnol Bioeng 109: 2513–2522

Chavez-Santoscoy A, Benavides J, Vermaas W, Rito-Palomares M (2010) Application of aqueous two-phase systems for the potential extractive fermentation of cyanobacterial products. Chem Eng Technol 33:177–182

Choi JM, Yeom SH (2011) Simulation study of biobutanol production in a polymer-loaded two-phase partitioning bioreactor (PL-TPPB): model development. Korean J Chem Eng 28:2170–2177

Craig T, Daugulis AJ (2012) Polymer characterization and optimization of conditions for the enhanced bioproduction of benzaldehyde by *Pichia pastoris* in a two-; phase partitioning bioreactor. Biotechnol Bioeng 110:1098–1105

Dafoe JTS, Daugulis AJ (2011) Bioproduction of cis-(1S, 2R)-indandiol, a chiral pharmaceutical intermediate, using a solid–liquid two-phase partitioning bioreactor for enhanced removal of inhibitors. J Chem Technol Biotechnol 86:1379–1385

Dafoe JT, Daugulis AJ (2013a) Production of 4-valerolactone by an equilibrium-limited transformation in a partitioning bioreactor: impact of absorptive polymer properties. Bioprocess Biosyst Eng. doi:10.1007/s00449-013-1020-5

Dafoe JT, Daugulis AJ (2013b) Manipulating the composition of absorbent polymers affects product and by-product concentration profiles in the biphasic biotransformation of indene to *cis*-1, 2-indandiol. Biochem Eng J 77:7–14

Dhamole PB, Wang Z, Liu Y, Wang B, Feng H (2012) Extractive fermentation with non-ionic surfactants to enhance butanol production. Biomass Bioenergy 40: 112–119

Fam H, Daugulis AJ (2012) Substrate mass transport in two-phase partitioning bioreactors employing liquid and solid non-aqueous phases. Bioprocess Biosyst Eng 35:1367–1374

Galaction A, Poştaru M, Caşcaval D, Kloetzer L (2013) Selective separation of carboxylic acids obtained by succinic acid fermentation using facilitated pertraction. Solvent Extr Ion Exch 31:171–183

Gao F, Daugulis AJ (2009) Bioproduction of the aroma compound 2-phenylethanol in a solid–liquid two-phase partitioning bioreactor system by *Kluyveromyces marxianus*. Biotechnol Bioeng 104:332–339

Gao MT, Shimamura T, Ishida N, Takahashi H (2011) pH-uncontrolled lactic acid fermentation with activated carbon as an adsorbent. Enzyme Microb Technol 48:526–530

Garcia-Chavez LY, Garsia CM, Schuur B, de Haan AB (2013) Bio-butanol recovery using non-fluorinated task specific ionic liquids (TSILs). Ind Eng Chem Res 52:4902–4910

Garikipati SJ, McIver AM, Peeples TL (2009) Whole-cell biocatalysis for 1-naphthol production in liquid–liquid biphasic systems. Appl Environ Microbiol 75:6545–6552

Grady MC, Jahic M, Patnaik R (2010) Method for producing butanol using two-phase extractive fermentation. Patent application US 20100221802 A1

Hu Q, Xu Y, Nie Y (2010) Enhancement of *Candida parapsilosis* catalyzing deracemization of (R, S)-1-phenyl-1, 2-ethanediol to its (S)-enantiomer by a highly productive

"two-in-one" resin-based in situ product removal strategy. Bioresour Technol 101:8461–8463

Hu Z, Zhang X, Wu Z, Qi H, Wang Z (2012) Perstraction of intracellular pigments by submerged cultivation of Monascus in nonionic surfactant micelle aqueous solution. Appl Microbiol Biotechnol 94:81–89

Hua D, Lin S, Li Y, Chen H, Zhang Z, Du Y, Zhang X, Xu P (2010) Enhanced 2-phenylethanol production from L-phenylalanine via in situ product adsorption. Biocatal Biotransfor 28:259–266

Jain AN, Khan TR, Daugulis AJ (2010) Bioproduction of benzaldehyde in a solid–liquid two-phase partitioning bioreactor using *Pichia pastoris*. Biotechnol Lett 32:1649–1654

Jeon BS, Moon C, Kim B, Kim H, Um Y, Sang B (2013) In situ extractive fermentation for the production of hexanoic acid from galactitol by *Clostridium* sp. BS-1. Enzyme Microb Technol 53:143–151

Jeong G, Kim S, Park D (2013) Detoxification of hydrolysate by reactive-extraction for generating biofuels. Biotechnol Bioprocess Eng 18:88–93

Jin H, Liu Z, Hu Z, Zheng Y (2013) Production of (R)-epichlorohydrin from 1, 3-dichloro-2-propanol by two-step biocatalysis using haloalcohol dehalogenase and epoxide hydrolase in two-phase system. Biochem Eng J 74:1–7

Julsing MK, Kuhn D, Schmid A, Bühler B (2012) Resting cells of recombinant *E. coli* show high epoxidation yields on energy source and high sensitivity to product inhibition. Biotechnol Bioeng 109:1109–1119

Jung D, Choi W, Choi K, Jung E, Yun H, Kazlauskas RJ, Kim B (2013) Bioconversion of *p*-coumaric acid to *p*-hydroxystyrene using phenolic acid decarboxylase from *B. amyloliquefaciens* in biphasic reaction system. Appl Microbiol Biotechnol 97:1501–1511

Keasler SJ, Lewin JL, Schultz NE, Ross RB, Gryska NM, Nakamura M, Siepmann JI (2013) Molecular insights for the optimization of solvent-based selective extraction of ethanol from fermentation broths. AIChE J 59:3065–3070

Khan TR, Daugulis AJ (2010) Application of solid–liquid TPPBs to the production of L-phenylacetylcarbinol from benzaldehyde using *Candida utilis*. Biotechnol Bioeng 107:633–641

Krug TA, Daugulis AJ (1983) Ethanol production using *Zymomonas mobilis* immobilized on an ion exchange resin. Biotechnol Lett 5:159–164

Kuhn D, Julsing MK, Heinzle E, Bühler B (2012) Systematic optimization of a biocatalytic two-liquid phase oxyfunctionalization process guided by ecological and economic assessment. Green Chem 14:645–653

Leeman M, Vossebeld F, Visser TJ, Schuur B, de Haan AB (2013) Novel extractants for the recovery of fermentation derived lactic acid. Sep Purif Technol 111:82–89

Li Q, Cai H, Hao B, Zhang C, Yu Z, Zhou S, Chenjuan L (2010) Enhancing clostridial acetone–butanol–ethanol (ABE) production and improving fuel properties of ABE-enriched biodiesel by extractive fermentation with biodiesel. Appl Biochem Biotechnol 162:2381–2386

Li Q, Wang D, Hu G, Xing J, Su Z (2011a) Integrated bioprocess for high-efficiency production of succinic acid in an expanded-bed adsorption system. Biochem Eng J 56: 150–157

Li SY, Srivastava R, Parnas RS (2011b) Study of in situ 1-butanol pervaporation from A–B–E fermentation using a PDMS composite membrane: validity of solution-diffusion model for pervaporative A–B–E fermentation. Biotechnol Prog 27:111–120

Liu S, Wu Q, Zhang J, Mo S (2011) Production of epsilon-poly-L-lysine by *Streptomyces* sp. using resin-based, in situ product removal. Biotechnol Lett 33:1581–1585

Mao S, Hua B, Wang N, HU X, Ge Z, Li Y, Liu S, Lu F (2012) 11α hydroxylation of 16α, 17-epoxypro-gesterone in biphasic ionic liquid/water system by *Aspergillus ochraceus*. J Chem Technol Biotechnol 88:287–292

Mei J, Min H, Lü Z (2009) Enhanced biotransformation of L-phenylalanine to 2-phenylethanol using an in situ product adsorption technique. Proc Biochem 44:886–890

Mihal M, Markoš J, Annus J, Štefuca V (2012) Intensification of 1-phenylethanol production by periodical membrane extraction of the product from fermentation broth. J Chem Technol Biot 87:1017–1026

Mirata MA, Heerd D, Schrader J (2009) Integrated bioprocess for the oxidation of limonene to perillic acid with *Pseudomonas putida* DSM 12264. Proc Biochem 44:764–771

Muñoz R, Daugulis AJ, Hernández M, Quijano G (2012) Recent advances in two-phase partitioning bioreactors for the treatment of volatile organic compounds. Biotechnol Adv 30:1707–1720

Neves CMSS, Granjo JFO, Freire MG, Robertson A, Oliveira NMC, Coutinho JAP (2011) Separation of ethanol–water mixtures by liquid–liquid extraction using phosphonium-based ionic liquids. Green Chem 13:1517–1526

Nielsen DR, Amarasiriwardena GS, Prather KLJ (2010) Predicting the adsorption of second-generation biofuels by polymeric resins with applications for in situ product recovery (ISPR). Bioresour Technol 101:2762–2769

Oudshoorn A, van der Wielen LAM, Straathof AJJ (2009) Assessment of options for selective 1-butanol recovery from aqueous solution. Ind Eng Chem Res 48:7325–7336

Pahlavanzadeh H, Khayati G, Vasheghani-Farahani E, Ghaemi N (2009) Extractive capacity of oleyl alcohol on 2, 3-butanediol production in fermentation process with use of *Klebsiella pneumoniae* PTCC 1290. Iran J Chem Chem Eng -Int Engl Ed 28:103–109

Parent JS, Capela M, Dafoe JT, Daugulis AJ (2012) A first principles approach to identifying polymers for use in two-phase partitioning bioreactors. J Chem Technol Biotechnol 87:1059–1065

Poleo EE, Daugulis AJ (2013) A comparison of three first principles methods for predicting solute–polymer affinity, and the simultaneous biodegradation of phenol and butyl acetate in a two-phase partitioning bioreactor. J Chem Technol Biotechnol. doi:10.1002/jctb.4116

Quijano G, Hernandez M, Villaverde S, Thalasso F, Muñoz R (2010) A step-forward in the characterization and potential applications of solid and liquid oxygen transfer vectors. Appl Microbiol Biotechnol 85:543–551

Ranjan R, Thust S, Gounaris CE, Woo M, Floudas CA, Keitz MV, Valentas KJ, Wei J, Tsapatsis M (2009) Adsorption of fermentation inhibitors from lignocellulosic biomass hydrolyzates for improved ethanol yield and value-added product recovery. Microporous Mesoporous Mater 122: 143–148

Redwood MD, Orozco RL, Majewski AJ, Macaskie LE (2012) Electro-extractive fermentation for efficient biohydrogen production. Bioresour Technol 107:166–174

Rehmann L, Daugulis AJ (2007) Biodegradation of biphenyl in a solid-liquid two-phase partitioning bioreactor. Biochem Eng J 36:195–201

Rong S, Ding B, Zhang X, Zheng X, Wang Y (2011) Enhanced biotransformation of 2-phenylethanol with ethanol oxidation in a solid–liquid two-phase system by active dry yeast. Curr Microbiol 63:503–509

Ruetti DP, Lacroix C, Jeremic T, Mathis M, Die A, Vollenweider S (2011) Development of a reversible binding process for in situ removal of 3-hydroxypropionaldehyde during biotechnological conversion of glycerol. Biochem Eng J 55:176–184

Tanaka S, Tashiro Y, Kobayashi G, Ikegami T, Negishi H, Sakaki K (2012) Membrane-assisted extractive butanol fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 with 1-dodecanol as the extractant. Bioresour Technol 116:448–452

van den Berg C, Heeres AS, van der Wielen LA, Straathof AJ (2013) Simultaneous clostridial fermentation, lipase-catalyzed esterification, and ester extraction to enrich diesel with butyl butyrate. Biotechnol Bioeng 110:137–142

Verhoef S, Wierckx N, Westerhof R, De Winde JH, Ruijssenaars HJ (2009) Bioproduction of p-hydroxystyrene from glucose by the solvent-tolerant bacterium *Pseudomonas putida* S12 in a two-phase water-decanol fermentation. Appl Environ Microbiol 75:931–936

Wang Z, Liang R, Xu J, Liu Y, Qi H (2010) A closed concept of extractive whole cell Microbial transformation of benzaldehyde into L-phenylacetylcarbinol by *Saccharomyces cerevisiae* in novel polyethylene-glycol-induced cloud-point system. Appl Biochem Biotechnol 160:1865–1877

Wang H, Dong Q, Guan A, Meng C, Shi X, Guo Y (2011a) Synergistic inhibition effect of 2-phenylethanol and ethanol on bioproduction of natural 2-phenylethanol by *Saccharomyces cerevisiae* and process enhancement. J Biosci Bioeng 112:26–31

Wang H, Dong Q, Meng C, Guo Y (2011b) A continuous and adsorptive bioprocess for efficient production of the natural aroma chemical 2-phenylethanol with yeast. Enzyme Microb Technol 48:404–407

Wang P, Wang Y, Su Z (2012a) Microbial production of propionic acid with *Propionibacterium freudenreichii* using an anion exchanger-based in situ product recovery (ISPR) process with direct and indirect contact of cells. Appl Biochem Biotechnol 166:1–13

Wang P, Wang Y, Liu Y, Shi H, Su Z (2012b) Novel in situ product removal technique for simultaneous production of propionic acid and vitamin B12 by expanded bed adsorption bioreactor. Bioresour Technol 104:652–659

Wei G, Yang X, Zhou W, Lin J, Wei D (2009) Adsorptive bioconversion of ethylene glycol to glycolic acid by *Gluconobacter oxydans* DSM 2003. Biochem Eng J 47: 127–131

Xue Y, Liu Z, Xu M, Wang Y, Zheng Y, Shen Y (2010) Enhanced biotransformation of (R, S)-mandelonitrile to (R)-(−)-mandelic acid with in situ production removal by addition of resin. Biochem Eng J 53:143–149

Xue C, Zhao J, Lu C, Yang S, Bai F, Tang I (2012) High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol Bioeng 109:2746–2756

Zou S, Du E, Hu Z, Zheng Y (2013) Enhanced biotransformation of 1, 3-dichloro-2-propanol to epichlorohydrin via resin-based in situ product removal process. Biotechnol Lett 35:937–942

Zúñiga C, Morales M, Le Borgne S, Revah S (2011) Production of poly-β-hydroxybutyrate (PHB) by *Methylobacterium organophilum* isolated from a methanotrophic consortium in a two-phase partition bioreactor. J Hazard Mater 190:876–882