

Direct Capture of Products from Biotransformations

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Forward

Vision2020 is an industrial-lead collaborative process to accelerate innovation and technology development for the chemical industry by leveraging financial and technical resources to produce a successful, sustainable, internationally competitive chemical industry and satisfy the expectations of shareholders, employees, communities and government. Their goal is to establish R&D collaborations in pre-competitive areas that will result in widespread implementation of continuous improvement and/or breakthrough technologies to help chemical companies enhance their competitiveness and meet societal needs by 2020.

Based on input from senior managers of major chemical companies, Vision2020 is pursuing advanced separations as one of its top priority technology development areas. A Vision2020 collaborative industrial team, presently consisting of Air Products, Cargill, Dow, Dow Corning, DuPont, Eastman Chemicals, ExxonMobil, FairField Resources, GlaxoSmithKline, Practical Sustainability, Praxair, and Tate & Lyle, has been established to develop novel separations technologies to improve energy efficiency, economics, the environment, and sustainability. The team has undertaken a bioseparations initiative with emphasis on direct capture of products and water removal from fermentation broths. In support of this effort, the DOE OIT Chemicals Industry of the Future has funded Argonne National Laboratory, with assistance from Oak Ridge National Laboratory, to perform a state of the industry assessment of direct capture of products from biotransformations. This document summarizes the results of this study.

1 Executive Summary – The Promise of Direct Capture of Products from Biotransformations

This report summarizes the results of a technology assessment for direct capture of products from biotransformations. It focuses on less mature technologies where the bank of knowledge is less mature including separations based on volatility, electrical, or specific interactions. Membrane technology offers some of the most promising breakthroughs if selectivity could be enhanced without reducing flux. Much of the improvements in separations technologies will develop from new materials or new modifications of generic materials including resins, membranes, and solvents. Removal of impurities such as cell debris, proteins, sugars, and salts improves all subsequent separations technologies and should be considered in all processes. Particularly important areas that warrant R&D in biocatalysis are: 1) working with minimal fermentation media to reduce impurities, and 2) enhancing microorganism tolerance to products, acids, and salts to increase titer. Integrating biocatalysis with separations offers the potential for greatly

simplifying separations. Two issues appearing repeatedly are pH control of fermentations and continuous removal of products. There is already a large knowledge base covering separations technologies (and materials) for biotransformations. This knowledge base could be better applied if there was an increased R&D focus on the screening, modeling, and databasing requirements in processes and materials. The ultimate key to improved separations is to optimize biocatalysis and separations in an integrated process; simultaneously enhancing both selectivity and flux while reducing impurities and fouling.

2 Introduction

In the 21st century, a sustainable U.S. chemical industry faces increasing challenges balancing public demand for superior energy and environmental performance with the market demand for superior financial performance (Technology Vision 2020, 1996). In addition, our own domestic security requirements challenge us to reduce our dependence on foreign, fossil fuel-based feedstocks. Chemicals produced from biobased feedstocks or by bioprocesses provide an opportunity to address these challenges and to capture a significant portion of the U.S chemical industry market by the year 2020 (Technology Vision 2020, 1996; The Technology Roadmap, 1999; New Biocatalysts; 1999). Vision 2020 has produced a series of roadmaps to identify and address the critical barriers to the chemical industry in general and to the emerging “biobased” chemical industry in specific (Technology Vision 2020, 1996; The Technology Roadmap, 1999; New Biocatalysts; 1999; Vision 2020, 2000). The “*vision*” of these roadmaps is to achieve a tenfold increase in biobased product use (25% market penetration) by 2020, and a 50% market penetration by 2050.

Frequently the cost of product separations drives the economics in the biobased chemical industry. Therefore in order for biobased products to compete with fossil fuel-based feedstocks and bioprocessing to compete with chemical catalysis, breakthroughs in separation technologies are essential. This report summarizes the results of a technical assessment of the state of the art of bioseparations and identifies R&D which could overcome critical barriers in the biobased chemical industry. A summary of emerging technologies based on the separations approach is appended. Also appended is a summary of stretch goals that separations R&D should endeavor to achieve. In preparing this report, the authorssupplemented their own expertise with literature reviews and input from experts in the field.

Several technical areas considered ripe for discovery R&D were evaluated, and the most promising were coalesced into the following approaches.

- **Removal of impurities** – For example removal of proteins, sugars, and salts from organic acids can prevent fouling of membrane-based and ion exchange separations.
- **New Materials** – Resins, membranes, and solvents that provide higher selectivity, specificity, or flux with increased stability and robustness provide some of the most promising avenues to enhance separations.
- **Modification of existing generic materials** – Frequently separations are too specific to warrant development of new materials. Modification of existing generic materials to meet performance requirements could be more effective than designing new materials.
- **New approaches to screening** – The body of knowledge about separations technologies is extensive, but frequently not directly relevant to the target or process of interest. Computational modeling and database comparisons to existing materials and processes are required to extrapolate data and supplement screening for desired performance.
- **Advancements in biocatalysis and fermentation** – The revolution in genomics, proteomics, and bioinformatics enables new approaches to biocatalysis and fermentation. Enzymes and microorganisms can be engineered to increase product yield and feedstock conversion efficiency, as well as increase robustness under more demanding environments or higher products concentrations.

Three product targets that represent important process streams with unique separations technology requirements were evaluated in this study. These targets were considered to represent a significant breadth of the separations requirements while not involving proprietary industrial projects or information.

- **Organic acids** (and esters) – major metabolic products and platforms for other products
- **ABE** – acetone-butanol-ethanol as a significant example of solvent systems
- **Biobased oils** (biodiesel and biolubricants) – important potential targets as a representative of fat-soluble products

Technologies that could enhance separation of these three product streams are identified and opportunities for breakthrough R&D in direct capture of products from biotransformations are highlighted. Other biobased targets including proteins and enzymes, while significant separations targets, were not considered germane to the more commodity-based focus of this report.

3 Description of Model Systems

Organic Acids

Organic acid production via fermentation is a well-explored technology. A problem with this technology has been pH control, the neutralization required for the microorganisms result in the salt not the desired acid product, along with the cost of the caustics. For example, research into acetic acid production from synthesis gas with the cell *C. ljungdahlii* (Phillips et al., 1994) has indicated a total product yield in the 1% range. If base (e.g., NaOH) is added to neutralize this acid, this leaves a two-fold problem. First, in many bioprocessing systems, product inhibition limits conversion. Second, even in the absence of product inhibition, the desired acid product is now a salt that requires additional processing.

Acetone-Butanol-Ethanol (ABE) System

The traditional ABE system uses *Clostridium acetobutylicum* for the conversion of corn mash and wood hydrolyzates to ABE (Marlatt and Datta, 1986). A typical reaction yields a product with about 1.5% butanol, 0.5% acetone, and 0.2% ethanol. Other products include dilute amounts of organic acids (acetic, butyric, etc.) and gases (CO₂ and H₂). This reaction halts at the low yields because the butanol poisons the enzymatic conversion at this concentration.

Biobased oils (Biodiesel/Biolubricants)

Biodiesel production is an example of a potential large volume application of capture of biobased oils. In biodiesel technology, biobased feedstocks (e.g., rapeseed, soybean oil) are converted into alkyl esters (and glycerols) for use in diesel engines (Knothe et al., 1997). The advantage of these fuels is they can perform with existing engine configurations, have little to no SO₂ formation, and provide a renewable energy resource replacement for fossil fuels. Currently, the process is based on a base-catalyzed transesterification in the presence of methanol. Enzymatic transformations have also been explored. The reaction yields a self-induced two-phase formation of an ester phase (biodiesel) and an aqueous phase (with the glycerols and catalyst). The ester phase must be, neutralized, desalted and dried, and the aqueous phase must undergo neutralization and treatment to recover and/or destroy the glycerols.

4 Removal of Impurities

In biological fermentation broths there are desired products as well as a large variety of impurities. In this section, we focus more on residual impurities from media and fermentation than co-products (such as ethanol with butanol). The residual impurities include sugars, salts, solids, and proteins from the starting media as well as lysed microbes, denatured enzymes, lipids or other components from a biological conversion. As we use more complex mixed feedstocks (i.e., biomass), these impurities may increase; such as when a mixed feedstock is used over a pure feedstock (e.g., xylose and glucose over just glucose in lactic acid production). These impurities will impact efficient separations. Proteins and solids cause

membrane and even resin fouling; salts compete for binding sites in ion exchange; lipids and other surfactants can form stable rag layers in liquid/liquid extraction. The impurities in commodity fermentation broths are much less than the desired product but can still be in the g/L concentration range. This challenge links with the use of cleaner more defined media under biocatalysis below.

The problems due to these impurities usually surface late in development as initial tests and literature data are typically reported on pristine samples. These impurities can also be carried through the separation process scheme and may impact the usability of the final product. The key R&D question: can these impurities be removed early in the separation scheme to prevent later problems? This is already part of some separation trains. Most begin with centrifugation to remove cells (perhaps for recycling) and residual solids. A challenge with bacteria over yeast is the two to tenfold greater centrifugal separation difficulty due to their smaller size. Microfiltration can remove higher molecular weight proteins and some lipids along with cells. Nanofiltration performs better at protein removal but at a cost. Flootation, flocculation, or coagulation has been borrowed from the waste treatment area for some specific applications. Any of the other separation methods discussed could be used here if selective for the impurity and not for the product.

Organic acids

Here impurities can have additional impact on the product. Very high purity is needed for polymer applications (e.g., polylactide properties are very dependent on purity including chiral form). Residual sulfate may poison subsequent catalysts; anion exchange polishing may be effective for this cleanup. The presence of ammonia may allow other reactions to occur (e.g., pyrrolidinones from succinate, Kanetaka, 1975). Crystallization is effective at removing proteins, sugars, and even other organic acids; however it is neither rapid nor energy efficient. Salts are a problem for organic acids: they compete for sorbent binding sites, they buffer the solution making neutralization or acidification more difficult and costly, and they compete for electrons in membrane electrodialysis.

With biological approaches to organic acid production, other impurities include other acids that are lesser coproducts. These impurities include acetic acid in the case of succinic acid production and succinic acid in the case of lactic acid production. This, of course, is when whole cells are used for production (with enzyme production there is typically a very high selectivity). Crystallization offers high selectivity (e.g., up to 99% in a single stage) but with loss, handling and material costs. Other processes offer some selectivity to the desired acid but generally the relative specificity between organic acids is within an order of magnitude (e.g., for sorbents or extractants) (Atkinson, 1991).

ABE

The ABE fermentation broths will share the problems of protein and sugars as discussed for organic acids. If distillation is used, these compounds will degrade and decompose into the bottoms – adding a manageable fouling problem in the distillation. This is a complex mixture of primary products (acetone, butanol, ethanol as well as acetic and butyric acids). If membranes are used for protein removal, the stability against solvents must be tested. Loss of solvent product is likely in these clean-up separations. In addition, the solvents will likely interfere with any water removal methods.

Biobased oils (biodiesel)

The feedstock triglycerides are a mixture dependent on the source. If waste oils (grease, etc.) are used some amount of polishing may be required to remove free fatty acids and salts as well as solid matter. The fatty acid salt may resist caustic esterification with methanol and end up in the aqueous phase, unreacted. These may lead to “soap” formation, which again can interfere with phase separation, neutralization, and polishing of the phases.

5 New Highly Selective Materials

The development of new materials could make a significant, step change impact on several technologies. These are pervaporation for the ABE system and possibly the biodiesels, electrodialysis (ED) and electrodeionization (EDI) for the separation of organic acids, extraction for ABE and biodiesels, and adsorption, which may work well with all three model systems. Further, size based membrane separation technologies could have a big impact if molecular weight cut-offs are tightened. We will discuss all of these technologies with respect to the model systems below.

Volatility-Based Separations

Volatility-based separations (e.g., distillation, evaporation, etc.) use differences in vapor pressure of components to drive the separations. For instance, Mathys et al. (1998) reported a process for separation of 1-octanol by extraction and subsequent distillation. Although distillation and evaporation are extremely important in de-watering, significant research is unlikely to make a large impact on direct product capture because these technologies are very mature and there exist few examples of their value for direct capture (Lye and Woodley, 1999). However, incremental changes are possible in the areas of entrainers and packing design with possibly bigger impacts in the areas of electrodistillation and reactive distillation.

Classical research on the ABE process has revealed butanol recovery by staged distillation to yield high butanol recovery at reasonable, but not significantly lower costs (Marlatt and Datta, 1986) with new butanol-resistant cells lines leading to even lower costs (Qureshi and Blaschek, 2001). However, pervaporation, a membrane-based process based on a vapor pressure driving force and a membrane selectivity driving force, may lead to great technical and economical benefits. For instance, Qureshi and Blaschek (1999) studied the separation of butanol from an ABE process. In a combined reactor fermentation broth, they found a silicone pervaporation membrane had a permeate concentration range of 26 – 95 g/L with fluxes $<215 \text{ g/m}^2$ butanol. Although this is a promising result, there is still a need for distillation of the permeate stream. Other research has also been reported for ethanol recovery by pervaporation (O'Brien et al., 2000).

Several key parameters must be examined before the full potential of pervaporation for butanol recovery in an ABE process can be realized. First, most pervaporation membranes are designed to be either highly water selective, or highly selective towards extremely hydrophobic (e. g., MTBE, TCE, etc.) organic compounds. The selectivities, in water permeation from ethanol, can be as high as 1000 with polyvinyl alcohol, with performance based on hydrogen bonding interactions. However, in the ABE process and perhaps the biodiesel technology, it is desirable to have permeation of an alcohol. Yet, ethanol, propanol, and butanol lack a long enough alkyl chain to take advantage of hydrophobic interactions. Thus, design of new membrane materials with high ethanol-butanol/water (>100) selectivity may enable either direct capture of butanol from the mixture or separation of all the organics from the water and organic acids. For a reasonable system to be applied, butanol fluxes of $>0.5 \text{ kg/m}^2$ are necessary. Either way, if done in direct product capture mode, the enzyme/cell-based reaction could continue for a much longer period of time, drastically reducing cost.

The major advantages of pervaporation in this process are the possibilities for direct product capture, where the membrane operating conditions are similar to the fermentation conditions. The membranes are also modular in design making scale-up straightforward. However, the disadvantages include relatively poor membrane selectivity for alcohols, low membrane flux, and high degrees of membrane fouling by whole cell systems and enzymes.

Steric-Based Separations

Separations based only on steric factors, size or molecular weight include most forms of filtration, including basic membrane and some chromatography (i.e., gel permeation). They typically function by a differential movement or exclusion from pores of specified size. The challenge here is to increase flux without limiting high selectivity. The single pass separation factor for closely sized molecules is small. Steric-based separations are frequently enhanced by the addition of other effects (charge or specific

affinity interactions) as discussed below. Membrane materials with extremely high selectivities (e.g., 99% selectivity of 110 MW vs. 100 MW) at reasonable fluxes do not currently exist but their development could make a major impact in direct product capture. Some of the most promising materials for this development are zeolites and ceramics since they are more rigid than traditional polymeric membranes.

Electrical-Based Separations

Electrical-based separations use the charge and size of an ion to separate species. Three technologies that hold promise to direct product capture are electrodialysis (ED), electrodeionization (EDI), and electrodispersion. There have been several examples in literature of researchers attempting to combine electrodialytic processes for product separation of organic acids. These include the production of gluconic acid via whole cell production (Ferraz et al., 2001), the production of lactic acid (Bailly et al., 2001), and a combined processing application for L-leucine production (Weuster-Botz, 1996). As a more detailed example, Lee et al. (1998) used a two stage ED process for lactic acid recovery. The two-stage process used a conventional ED and a water splitting ED. Using this two stage process, they were able to obtain concentrations of lactic acid in the feed of >100 g/L while reducing the concentration in the permeate to <1 g/L. The experiments allowed for an overall current efficiency of 81 – 84% for feed lactate concentration of 100 – 200 g/L with a specific energy consumption of 0.54 – 0.71 (kWh/kg lactic acid). Other examples include using EDI technology for the separation of a charged organic acid (e.g., acetic acid) through a cation exchange resin, which increased the conductivity 40 fold (Narebska et al., 1998). In all cases, the current efficiency of the process is extremely important.

There are several approaches to designing new ED and EDI systems. First, most of the materials are designed for transporting small ions (e.g., Na^+ and Cl^-) and thus have very low transport numbers with organic acids. Thus, new materials that demonstrate selectivity for the desired product could enhance the specific separation and increase the overall conductivity. Second, the processes could be redesigned (including resins for EDI), to perform on more dilute organic acids and salts. This may enhance acid and salt yield as well as enable continuous product removal avoiding product inhibition (which will be discussed later). It should be noted that the prohibitive factor in electrodialysis separations is often the capital costs and energy costs. Although capital costs may remain high, energy costs could be reduced significantly with the design of better performing materials.

Extraction-Based Separations

Liquid-liquid extraction is a fairly mature technology that uses a second liquid phase to remove one component from another. Several examples of this exist in literature including the extraction of 1-octanol (Mathys et al., 1998), ABE (Wayman and Parekh, 1987), etc. Very often this process requires additional separation, but it still has been proposed for the large-scale production of succinic acid (Zeikus et al., 1999). A variation on an extraction process is extraction using a membrane contactor (Lee et al., 2001). These membrane contactors provide surface area where the two immiscible phases can exchange the product. In this technique, there is not as much organic adsorption in the liquid phase but the overall extraction can be lower. Supercritical solvent extraction offers the added advantage that after the extraction is completed, the pressure drop changes in solvation behavior avoiding the need for an additional separation step (Condoret et al., 1997).

With all of these extraction procedures, several important factors must be considered in the design: 1) whether the extractant is entrained in the feed stream, 2) what is the efficiency of the extraction, 3) what is the selectivity of the extraction, 4) what is the biotoxicity of the solvent and, 5) does the system form rag layers? Since liquid-liquid extraction processes are well documented, these considerations can be readily determined. Far less is known about membrane contactors and supercritical extractions. For membrane contactors, there is a need for further studies into membrane materials for the contactors and refinement of those materials for efficient separations. A membrane contactor, for instance, could extract the glycerols from the water stream, leaving the KOH in water, which may be concentrated and recycled.

Adsorption/Ion-Exchange

Most ion exchange systems are comprised of columns containing resin beads that separate the product based on affinity. Charged organics, such as acids, provide important separation challenges because the reaction is not only limited by product inhibition, but also by reactor acidification by the product. Roddick and Britz (1997) used ion exchange for product separation of hexanoic acid produced in whole cell configuration by *Megasphaera elsdenii*. In a case going from no pH control to pH control with ion exchange they increased product concentration from 2 – 3 g/L to 11 g/L. Modeling of these processes requires knowledge of equilibrium and kinetic isotherms as well as the dependence on processing conditions (Sosa et al., 2000). A bead format has been used for most adsorbents, but they also have been used in a gel format, which limits non-specific adsorption with proper choice of the gel material (Nigam et al., 1990).

Ion-exchange/adsorption research also may be particularly interesting in the ABE and biodiesel processes. If an adsorbent could be designed to concentrate butanol selectively, and be regenerated easily, or if an adsorbent could remove glycerols from the water stream in biodiesels, for instance. One advantage is that adsorption and ion exchange are economical for dilute separations. Disadvantages include necessary regeneration (pressure, temperature, solvent stripping), the relative size of the column (footprint of the systems are large), and non-specific binding of proteins (fouling). Electrosorption, where the sorbent only binds the solute under an electric field may have promise for easier regeneration (Stuart et al., 1991; Horanyi, 1995). Therefore new materials are needed to improve selectivity, increase loading, decrease fouling, and allow the processes to be redesigned for easier regeneration.

6 Modification of Generic Materials

As well as the design of new materials, the modification of generic materials also holds promise in direct product capture. Although these materials may not have the same selectivities and fluxes, that could be balanced by inexpensive, easy to integrate modifications that can be introduced quickly into existing industrial technology. These materials often take advantage of the same selective groups as new materials so the design could advance simultaneously and could leverage expertise developed with new materials.

The most promising strategies are: 1) attachment of chelating groups onto ion-exchange resins for better adsorption and EDI applications, 2) use of traditional organic solvents for liquid-liquid extraction spiked with highly-selective extracting agents, and 3) modification of the surfaces of pervaporation and ED membranes to allow for a higher concentration of the transporting component on the surface.

7 Improved Approaches to Screening

With the design of new materials, processes, and technologies, as well as the modification of existing ones, modeling and databasing of this information becomes critical. Methods for screening the performance of new or modified materials without requiring full in-process testing would greatly enhance the identification of the most promising candidates. A high-quality screening process enables the prediction of a wide-class of compounds separation from single compound research.

There are several examples of improved screening processes that already exist in literature. For instance, using fluorescence for multiple array sensing, used for detecting the selectivity of membrane materials. Research in this field has incorporated a platform utilizing fiber optics for combining extremely high densities of sensor material in a small area (Michael et al., 1998). Other work in this area includes the use of a 64-point fiber optic fluorescence array for the measurement of Rhodamine-6G down to 40 ppb (Perkins and Jones, 1989). Another way of screening materials is by studying the thermodynamics of interactions between extractant groups and compounds. Because they are simpler in chemical make-up, more work has been done with metal ions, (e.g. Jensen and Nash, 2001). The integration of screening

procedures with computer modeling and databasing could open up this field for the design of extractants for the ABE and biodiesel processes.

The rapidly expanding genomics and proteomics databases enable use of bioinformatic tools to search for enzymes and entire metabolic pathways with novel or enhanced activity. These screens can be used to identify novel organisms, metabolic pathways, or specific enzymes that should be isolated and investigated. The biological targets could be used in natural form or engineered to enhance separations.

Methods to extrapolate from existing data and materials to new processes would also be helpful. For example, new models would be needed to use data on specific sorbents for lactic acid and to predict which are the best candidates to test for acetic or succinic acids.

8 Biocatalytic approaches

One way to deal with separation challenges is to minimize and avoid them. All biocatalytic approaches will have powerful but indirect effects on separations. Most directly, we can change the catalyst to decrease the separations need - such as chiral reactions (to avoid chiral separations), use defined media (to reduce problems with impurities), or use high product tolerant microbe. However, the biocatalyst should function within a closed loop - e.g., biotoxicity of agents in the separation recycle loop. In addition, novel biocatalysis can bring new separations challenges - like the recycle of required cofactors, or the providing of trace metals. Biocatalyst improvement to increase product titer is an obvious part of any process improvement strategy; substantial improvements in product titer are frequently achievable with effort. This may be accomplished via genetic engineering, culture optimization, or even process configuration (e.g., immobilized cells are nongrowing and thus can tolerate higher product levels).

Several of the bioconversions are limited by conditions such as inhibitory product concentrations despite the dilute aqueous streams. Several proposed processes seek to alleviate these types of limitations by combining separations and conversion. Almost every method of separation has been tried for integrated reaction and separations. Possibilities for *in situ* product removal or simultaneous fermentation and separation (SFS) include vacuum distillation, pervaporation and the use of hollow-fiber reactors, solid adsorbents and an immiscible extractive solvent (Schurgerl, 2000; Lipenski et al., 1999). Generally SFS has been shown in principle to allow higher conversions, higher rates and sometimes higher yields when the inhibitory product is removed from the ongoing bioconversion. In concept, proposal for complete water recycle are related to this where regeneration and accumulation of trace toxic compounds is important.

The modification of the bioconversion to allow use of minimal fermentation media (e.g., cleaner or more defined media) is critical for improved separations for the reasons discussed previously regarding removal of impurities. Minimal media usually increases the cost as specific vitamins, amino acids and trace metals are substituted for LSW (Light steep water) or yeast extract. This has impacts in the pharmaceutical industry with the replacement of serum in tissue culture. Approaches to media design have been investigated by trial and error testing for critical components and traditional microbiology and adaptation. Metabolic engineering approaches have great general promise for improving media requirements. For example, as we can make amino acid deficient mutants, can we modify a microbe to become a complete autotroph?

Enzymes have been used on many processes but have not been able to replace fermentation on large scale. By working directly with enzymes rather than fermentations, removal of impurities and coproducts can largely be avoided. Critical areas to address include: enzyme stability and robustness in harsh environments, immobilization of enzymes to facilitate product separation, the use of multistep pathways, cofactor requirements, and enzyme kinetics and specificity. The use of bioinformatics tools and genetic engineering offer many opportunities to improve enzyme properties (Powell et al., 2001; Jaswal et al., 2002)

Organic acids

- a. When a bioreactor operates without pH control, the pH drops rapidly and conversion is halted. Further, if base is used for pH control, feedback inhibition often exists and the separation often becomes more difficult. Particular, as the organic acid is usually the desired form – not the salt. Microorganisms with improved pH tolerance need to be developed. Contrary to this approach is the fact that the protonated acid form is generally an order of magnitude more inhibitory than the unprotonated salt. Product concentrations of 50 to >100 g/L have been achieved for lactic, succinic, and acetic acid by process and culture optimization.
- b. Simultaneous fermentation and separation – Thus, a bioreactor combined with the separation of the organic acid makes good sense. For this bioreactor to be effective, it needs to remove most of the acid from solution, controlling pH, at very low concentrations (for instance, <1 mg/L organic acid drops the pH from 7 to <6). This pH control must occur in the presence of sugars and products in the enzyme production case and sugars, products, and cell media in the case of whole cell production. This has been shown for lactic acid with solid sorbents (Kaufman et al. 1994). The simplest configuration uses a sidestream loop to pass through a separation; however, direct addition and removal of sorbent has been demonstrated using immobilized cells. Membrane electrodialysis is possible for a sidestream but would be extremely difficult in situ. Many of the liquid extractants (i.e., quaternary amines) suggested for organic acids are biotoxic. Vacuum distillation or pervaporation are conceivable but require handling of acid vapors. SFS's additional advantage of pH control for organic acids must occur in the presence of sugars, products, and cell media in the case of whole cell production. Coadsorption of the substrate can be another concern in the selection of materials. Ideally the product will be directly captured from a dilute stream avoiding product inhibition or reactor acidification.
- c. Use of minimal media – This depends on the fermentation, but *Lactobacillus* are well known for requiring a very rich media to achieve high yield (up to 1 g lactic/g glucose) (Atkinson, 1991). It is not clear what novel methods might decrease this requirement. Since some cells (see Lactic acid production) require high amounts of media, this makes the separation very difficult. Thus, approaches that combine production with cells requiring limited media, makes the separation possible and narrows the gap to continuous fermentation.

ABE

Butanol is the primary product of the fermentation of sugars by various bacteria, in particular *Clostridium acetobutylicum*. This is a complex fermentation, with, first, an acidogenic phase producing butyric and acetic acids and, then, a solventogenic phase producing butanol, acetone, and ethanol. Both the products and the lowered pH can be inhibitory to the continued fermentation. Typically, this has limited final butanol concentrations to a maximum of 15 g/L in batch culture, with generally much lower yields (Awang, 1988). Other than the butanol, acetone, and ethanol, the main coproducts are organic acids. The production is a complex interaction of product concentrations, carbon and redox balances, substrate levels, pH, and culture state. If biocatalytic systems are designed having higher butanol tolerance, the removal of organic acids may be very important to pH control of the system and further conversion. A combination of biocatalytic and separation improvements may make the process economically feasible again. (Nimcevic, 2000; Gappes, 2000, Qureshi and Blaschek, 2001)

- a. New cell and enzyme design – Since the butanol is the main product and the main inhibitor at relatively low concentrations (<20 g/L), research into cell strains and enzyme strains with more butanol resistance would be one of the main objectives. Blaschek has shown some improvements to *Clostridia acetobutylicum* in this area (Qureshi and Blaschek, 1999). Other desired biocatalyst features would be to control of the ABE ratio, decrease in organic acid production, control of spore formation and culture degeneration.

- b. Simultaneous fermentation and separation – The removal of the inhibitory product from the ongoing fermentation has been suggested by many researchers as a method to alleviate the product inhibition and improve the process. Several reviews of extractive butanol fermentation exist (Durre, 1998; Park 1992; Groot, 1990). The key advantages suggested for extractive bioconversion are higher feed concentrations leading to less process waste and reduced product recovery costs compared to distillation. Removal of the solvent does allow the conversion to proceed further; there is also some evidence of a shift in the yield and product ratios to more solvents. Possibilities for in situ product removal include pervaporation and the use of hollow-fiber reactors, solid adsorbents, and an immiscible extractive solvent. The use of sidestream contactors and cell recycle are also possible. Key issues are the extractant toxicity and capacity as well as the actual contacting scheme devised and its operability. Many solvents have been tested for the acetone-butanol fermentation. Oleyl alcohol has been commonly used based on its low toxicity, reasonable distribution coefficient, and selectivity for butanol (Park, 1992; Davison, 1993).

If the product solvents are not removed, the co-product organic acids may be removed, if the cells/enzymes were more resistant to butanol. Processes for performing this separation have already been described.

Most of these extraction-based technologies have shown improvements in productivity, due to the advantages of continuous operation. Several liquid extraction reports have shown improvements in yield as well. Perstraction is seen to have major operational difficulties and will not be considered. Adsorption also has difficulties in situ, and the current adsorbents are poor for neutral solvents. Pervaporation and extractive fermentation are seen as the most promising for further research. If the product solvents are not removed, the co-product organic acids may be removed, if the cells/enzymes were more resistant to butanol. Processes for performing this separation have already been described.

- c. Minimal media – Since the product here is particularly dilute, a cell line that operates in limited media may be very important to make the separation steps viable. Also, the media choice could be part of the design consideration based on which separation technique is targeted.

Biobased oils (biodiesel)

Biocatalysis – A goal may be to replace the caustic transesterification process with a biocatalytic method in the aqueous or organic phase. Esterifications are carried out by lipases, enzymes that can be used in free solution and may seek out the aqueous/lipid interface (Sonntag, 1998). Enzymatic hydrolysis of the triglycerides is also possible using aqueous organic systems. The advantages of enzymatic conversion are lower energy and capital costs and decreased caustic costs and the need to neutralize the two product streams. The difficulties for esterification include the cost of the enzyme; and the need for minimum water content, pH control, and high conversions from an equilibrium reaction. The enzymes may also be destabilized at oil-water interfaces (Sang and Rhee, 1993; Han and Rhee, 1986). Decrease of enzyme costs may require the separation and reuse of the enzymes; these enzymes have also been employed in immobilized forms. Lipases have also been used and/or modified to catalyze reactions directly in an organic phase (Khalaf et al., 1996).

Liquid extraction may be possible for integrated reaction and separation; however removal of the extractant from the methyl-fatty acid will likely be difficult. This may be better for other lipid/water systems, such as production of single fatty acids. Membrane system might allow caustic recycle. The use of minimal media is not appropriate to this system.

9 Conclusions

Direct capture of products from biotransformations is a technology area where R&D investment and collaboration could provide significant energy, environmental, security, and economic benefit to the U.S. biobased chemical industry. The existing and potential products are varied and include targets that are

produced from fossil fuel feedstocks and/or by chemical catalysis means. Separations are usually the economic driver for implementation of biotransformations for large-volume or commodity products. This report outlined several technologies that, with targeted R&D investment and collaborations, could reduce the costs of “*bioseparations*”. Reducing the separations costs will expand the market penetration of biobased products. The authors considered the following areas as most suitable for pre-competitive R&D: 1) removal of impurities, 2) the design of new materials, 3) the modification of generic materials, 4) enhanced screening, modeling, and databasing of materials and processes, and 5) improvements to biocatalysis. The key to improving separations is to simultaneously enhance both selectivity and flux while reducing impurities and preventing fouling.

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Appendix 1 – Summary of Separations Technologies – New and emerging technologies

Topic-Technology	Advantages	Challenges	Relevance to Direct Capture or Dewatering
Volatility Based Separations	Distillation and evaporation are well-developed technologies. Further, mechanical recompression can be economically attractive.	Thermodynamic limits, components may degrade with heat. With pervaporation, membrane fouling is a concern.	Distillation and evaporation are used for mainly dewatering. Pervaporation is more useful for product capture.
Solubility Based Separations	Well-developed, low capital requirements, already commercial in some cases (e.g., citric acid). Often highly selective.	Very difficult for extreme dilute separations, organic phase contaminates water phase.	Has some potential applications to direct capture and dewatering.
Steric Based Separations	Modular design of modules in the case of membranes, low temperature, relatively high throughput rates.	Membranes often vary significantly in pore sizes and thus extremely tight steric selectivity difficult. Fouling a concern.	Reverse osmosis is most relevant to dewatering, and nanofiltration and ultrafiltration may be relevant to direct product capture.
Electrically Based Separations	Electrodialysis: Very good separation of acids from unconverted sugars. Can produce significant product concentrations (30-35%). Electrodialysis good if high surface area needed for liquids.	Does not discriminate among charged species. Fouling and degrading lowers flux. Membranes don't like poly-cations. Electrically assisted sorption less studied.	Can produce significant product concentrations, so may be relevant to both dewatering and direct product capture.
Adsorption Based Separations	Based on chemical properties, many different types of ion-exchange resins and adsorbents already exist.	Performance prediction more empirical than other separation technologies. Regeneration difficult. Challenge to concentrate product not increase dilution.	Quite relevant to direct product capture and dewatering, if the component of interest can be regenerated at high concentrations.
Biocatalytic Approaches	Prevents some difficult downstream process steps, may make other separations possible (e.g., if cells can handle lower pH).	Each cell-line must be designed separately since all behave very differently.	May make direct product capture and dewatering easier.
Integrated Fermentation/ Separation	Improves rates, yields, may provide pH control.	Complexity, biotoxicity, fouling, incomplete separations.	Direct capture only.

Appendix 2 - Long-term Conceptual (Stretch) Goals

- A perfect simultaneous fermentation/separation process with for highly selective, high capacity separation of only the desired product (e.g., acid, oil, protein). Speculation included volatile cations, temperature swing systems for rapid regeneration/recycle of the “extractant”, simplified pH control.
- A method to fractionate narrow MWt ranges (e.g., to <10% for small molecules & 10-100 Da for large molecules range) of closely related organics, proteins, or lipids of similar functionality from aqueous or organic streams; this also should provide 99% selective fractionation with 20% reduction in energy from today’s status.
- Membrane transport of dilute components (e.g., <10 ppb of butanol by pervaporation) at similar flux and selectivities compared to concentrated streams.
- Membrane flux increase of >500% at current selectivity and fouling characteristics
- Sorbents with extremely high affinity (ppb) with comparable usable capacity (>0.1 g/g) and with easy regeneration (e.g. via a small temperature, pressure, or electric field shift).
- A method to remove proteins completely from fermentation broth w/o removal of other products (solvents, acid, oil) to prevent downstream interferences. Incomplete removal is accomplished by agglomeration, membranes, etc.
- The ability to modify a robust low cost mass separation media (membrane/sorbent/extractant) to be highly selective/specific at low ppm concentrations for a variety of low MW organics (in presence or absence of cells). This would require:
 - Tools for rational design of separation interactions
 - New binding sites using skills from polymer design or proteomics
 - Model effect of specific functional groups added to surfaces
 - Control of water hydration layers to enhance separation effects
- Eliminate whole cell fermentation by use of an effective “artificial cell” or nanocomposites using “in vitro” multistep enzyme or biomimetic pathways immobilized on robust supports
- Microorganisms designed to augment separation via active transport, excretion of product, or product formation as purified inclusion bodies that can be removed without cell lysis.

Appendix 3. Participants in Vision2020 Advanced Separations Team and Chemicals Plus Project

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