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Downstream of the bioreactor: Advancements in recovering fuels and commodity chemicals

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Downstream processing aims at recovering the target product at the required specifications from the bioreactor effluent. Research and development in this field relies on experimental and mathematical tools at the levels of chemical components, unit operations and processes. Recently, advances have been made in addressing the broth mixture complexity early on, in incorporating high-throughput experimentation for data generation and mechanistic understanding of the separation processes, in improving the materials and scalability of specific unit operations, as well as establishing the potential of process integration concepts. Further developments are expected considering the variety of (non-sugar) feedstocks currently under research, the need to transition to renewable energy sources, and the opportunities for improved scale-up through initiatives as Big Data and digital manufacturing.

Introduction

Biotechnology offers options to produce a wide variety of desired organic chemicals with high selectivity from a wide variety of feedstocks [1]. The focus of biotechnology research is on development of the microbial or enzymatic conversion, but this conversion typically leads to rather dilute and impure product streams. Downstream processing (DSP) should recover the product from such streams. The importance of DSP is clear from its 15-90% contribution to the overall production costs in commercialized biotech processes [2]. For fuels and commodity chemicals produced using biotechnology, this contribution is 15-50%. These are simpler compounds than specialty chemicals and biopharmaceuticals, and are produced at higher concentrations. Energy costs can easily dominate DSP costs [3,4].

Aims of DSP

Downstream processing aims to recover the target product at required specifications from the bioreactor effluent. This should be accomplished at minimum costs and environmental burden per amount of recovered product. Such minimization should be performed for the overall process; not for the DSP or any of its steps in isolation. Therefore, DSP research should provide feedback to the development of fermentation or enzymatic conversion, and to operations that are further upstream. Upon early identification of impurities that will lead to high DSP costs, the upstream processes may be adapted to decrease the level of such impurities, such that overall production costs and environmental burden can be minimized. Hence, DSP development relies on experimental and theoretical methods at component, unit operation and process levels (see Figure 1).

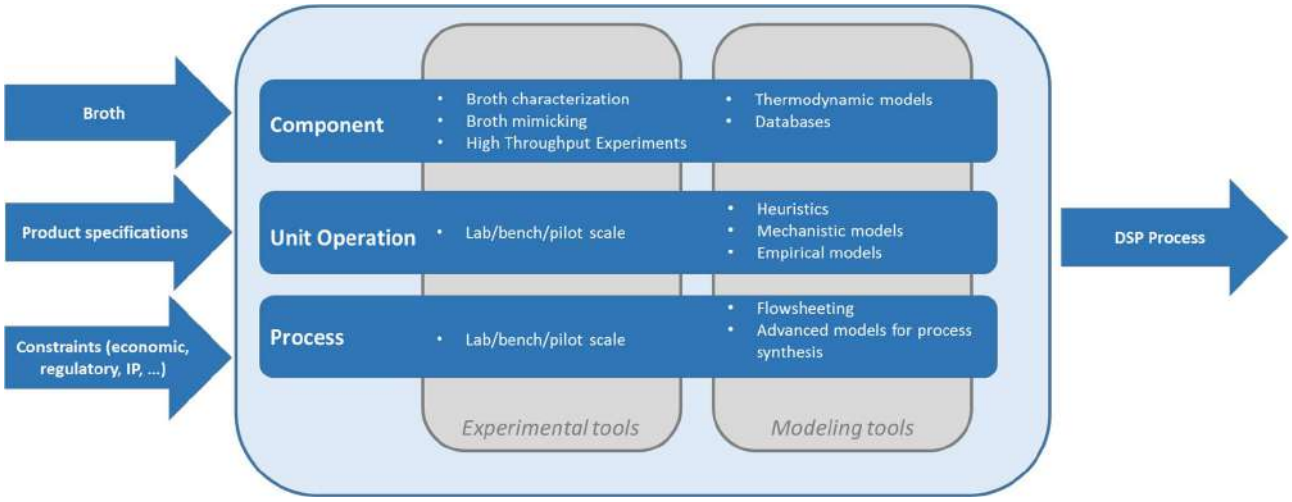

Figure 1. Elements in DSP synthesis and development.

Scope of this review

This review covers important developments from the past few years for DSP of fuels and commodity chemicals produced in (enzymatic or microbial) bioreactors. For such compounds, including food/feed ingredients, the industrial DSP status can be seen in Table 1. Remarkably, liquid/liquid extraction does not show up in this Table, despite ample associated academic research.

Table 1. DSP of biotechnology products with a production capacity exceeding ~20000 t/a.

Product	F/E [a]	Main DSP operations in typical processes [b]	Ref.
Ethanol	F	Centrifugation – Distillation – Water adsorption	[3]
1-Butanol	F	Distillations	[5]
Isobutanol	F	In-situ vacuum evaporation – L/L splitting – Distillation	[c]
1,3-Propanediol	F	MF – UF – IX – Water evaporation – Distillation	[d]
1,4-Butanediol	F	MF – NF – UF – IX – Water evaporation - Distillation	[6]
Lactic acid	F	Filtration – Acidification - $CaSO_4$ removal – Water evaporation – Distillation	[7]
Succinic acid	F	All commercial processes differ significantly	[7]
Itaconic acid	F	Filtration – C-treatment - Water evaporation – Crystallization	[7]
Gluconic acid [e]	F	Filtration – C-treatment – Water evaporation – IX	[7]
Citric acid	F	Filtration – Precipitation as Ca salt – Acidification - Crystallization	[7]
Lysine	F	Filtration - Water evaporation – Spray drying	[8]
Glutamate	F	Centrifugation or UF – Water evaporation - Crystallization	[8]

Polyhydroxyalkanoate	F	Cell disruption – S/L extraction – Precipitation - Drying	[9]
Xanthan	F	Precipitation – Dewatering - Drying - Milling	[10]
Acrylamide [e]	E	Filtration – C-treatment	[11]
Glucose [e]	E	IX – C-treatment - Water evaporation	[f]
Fructose [e]	E	IX – C-treatment - Water evaporation - Adsorption	[f]
Galacto-oligosaccharides [e]	E	C-treatment – Water evaporation	[12]

a) Abbreviations: F, fermentative production; E, enzymatic production.
b) Abbreviations: C, activated carbon; IX, ion-exchange; L/L splitting, liquid/liquid splitiing; MF, microfiltration; NF, nanofiltration; S/L extraction, solid/liquid extraction; UF, ultrafiltration
c) *www.chemicalprocessing.com/articles/2016/bio-based-isobutanol-beckons/*
d) *https://ec.europa.eu/jrc/sites/jrcsh/files/BISO-EnvSust-Bioproducts-13PDO_140930.pdf*
e) Aqueous solution, for example 50%, as desired in most applications.
f) https://www.vogelbusch-biocommodities.com/technology/starch-sugar-process-plants/

DSP development

Mimicking the broth composition

DSP development for bio-based fuels and commodity chemicals is intrinsically challenging because of the complexity of the mixtures, which include a broad range of impurities, equally broad in their physicochemical properties. These impurities originate from nonselective fermentation or bioconversion, from the feedstock, or from upstream addition (e.g. titrants, antifoams). Academic and industrial research approach this challenge in different ways, varying in the complexity of the mixtures used for experimentation and whether the studies are supported by mathematical models or not. In general, model mixtures or broth "mimics" are used in academic research since this approach allows developing mechanistic understanding of the effect of impurities, with the additional advantages of enhanced reproducibility, material availability and speed. Such studies, however, are not often validated on (high performance) fermentation broth. Conclusions drawn with fermentations only, on the other hand, are not easily reproduced by others, nor easily translated to other fermentation systems. Such studies are useful as proof of principle or bespoke manufacturing solutions, but do not add to our general and extensible understanding of impact of impurities on DSP. Recent examples include the evaluation of alternative DSP routes for succinic and propionic acid recovery from fermentations on lignocellulosic feedstock [13], and the recovery of volatile fatty acids (VFA) from fermented waste water by adsorption [14]. We know no studies in which DSP development for bio-based fuels and commodity chemicals was done using mathematical modelling only, and in which fermentation broth subsequently behaved exactly as predicted.

In systematic studies on removal of impurities, a pre-selection between impurities is often based on experience with the specific product, and on the type of compounds that can become problematic for the unit operation in question. For example, studies on ethanol pervaporation have focused on the impact of fermentation by-products such as glycerol, carboxylic acids and diols, lignocellulosic feedstock components such as furfural, or expected medium components such as salts. Sugars and salts have been found to affect the vapor pressure of water and/or ethanol, while the effect of glycerol, diols and carboxylic acids is largely membrane dependent due to the interactions of the impurity with the membrane material (e.g. by sorption or by altering the membrane hydrophobicity) [15]. In a similar fashion, Raganati et al. [16] evaluated the effect of several impurities on the adsorption of butanol, where glucose and carboxylic acids were found to compete for adsorption sites. Such effect is likely resin dependent, and single component isotherms were not appropriate to predict multicomponent ones. Both studies were performed with model mixtures; Raganati et al. [16] validated their findings on actual fermentation broth as well. In other cases no specific impurities, but rather the effect of the total impurity matrix is studied. Comparing succinic acid crystallization from

fermentation broth and a model mixture showed that prior nanofiltration was effective in rejecting compounds (e.g. other carboxylic acids, colloids, and proteins) that were otherwise detrimental [17].

Separating aqueous and organic liquids, for example in bioproduct extraction, or when the bioproduct is a nonpolar liquid for diesel and jet fuel replacement [18], can be affected by known medium components [19],[20]; advances have been made in demonstrating the role of the microorganism [21,22] and antifoams in stabilizing the emulsions that are usually formed in such systems (Ref: Cuellar, M.C., Steinbusch, K. 2017. Integration and scale-up of multiphase fermentations. Symposium on Biotechnology for Fuels and Chemicals). Understanding this is relevant, considering that emulsion stability changes during fermentation [23], and hence, recovery performance becomes dependent on harvest and holding times.

High-throughput experimentation

In the last years, high-throughput experimentation (HTE) and high-throughput process development (HTPD) have been broadly adopted by both academic and industrial research, albeit mostly for biopharmaceutical applications. The main reasons for this lie in the inherently limited amount of material in early stages of process development, the need for fast process development for registration purposes and IP protection, and by regulatory initiatives such as Quality by Design (QbD). Independent of the type of product, however, the value of HTE is the generation of (large) data sets in a short time and with limited amount of material for: a) characterization of physical-chemical and thermodynamic properties from the target compound and/or the impurity matrix (see previous section); b) screening of a broad range of operating conditions and/or auxiliary materials for a specific recovery step; and c) overall accelerated process development. Considering that fermentation development for bio-based fuels and chemicals is also shifting to smaller scales (< 1 L), it is to be expected that DSP development in this field can benefit from HTE. For HTE to be possible, miniaturization is required and has been achieved so far by the following approaches:

- Robotic liquid handlers. Broadly used for equilibrium experiments, hydrolysis, flocculation and stability studies, and steadily being adopted by industrial R&D [24].
- Microfluidics. Examples include L/L extraction and subsequent phase separation [25], studying the effect of impurities in biphasic systems [19], and crystallization studies (nucleation and crystal growth, solubility and metastability zone determination, polymorph screening) [26].
- Ultra Scale Down approaches. Typically at the mL scale, these have been used for centrifugation, flocculation and filtration studies [27], also for processes with microorganisms relevant for industrial biotechnology [28].

Novel and improved separation methods

Developments at the unit operation level are being geared towards improving material selectivity without compromising processing rates, and on improving hardware scalability. Some examples from the last years include:

- Improvements in synthetic membranes are leading to a wider applicability of membrane separations. Nowadays, for dewatering of bioethanol, pervaporation can compete with azeotropic distillation [29], while the increased availability of high-quality ion exchange membranes enables the recovery of carboxylic acids by bipolar membrane electrodialysis [30]. It has been noticed that pores in biological membranes such as ion channels and aquaporins have the potential to combine high selectivity with high permeability at low energy requirements [31], which has not been demonstrated yet in synthetic membranes for industrial applications.
- Commercial recovery of intracellular compounds, such as polyhydroxyalkanoates and some lipid biodiesel precursors, requires efficient cell disruption methods at low costs on equipment and energy, as well as elimination of the use of chemicals that may lead to waste, safety and product quality concerns. In the field of food processing, ultrasonic technology is progressing, with commercial devices being up to 16 kW and 1 m^3 [32]. Lipid recovery for biodiesel production requires larger scales, such as achievable for high-pressure homogenization and subcritical water hydrolysis [33].

- Expanded bed adsorption (EBA) has traditionally been explored for recovering proteins from fermentation broth, to bypass centrifugation or filtration of cells. Now this unit operation is being further developed and scaled-up, and gets in scope already for lower-value products. Upon integration of EBA with simulated moving bed chromatography (SMB), ≥92% pure γ-aminobutyric acid was recovered from unclarified fermentation broth in one step [34].
- Dividing-wall columns, industrially applied since 1985 for the separation of three or more components in a single distillation [35], are now entering the field of biofuels. To overcome the high downstream processing costs for butanol recovery from acetone-butanol-ethanol (ABE) fermentation, butanol extraction followed by divided-wall distillation was proposed [36], resulting in reductions in the total annualized cost of up to 22%; a heat pump (vapor recompression)-assisted azeotropic dividing-wall column lead to energy requirement reduction by 58% when compared to conventional distillation [37].

Furthermore, the integration of product recovery during the bioconversion or fermentation, referred to as in-situ product recovery (ISPR), remains an active field of research [38]. In the production of biodiesel, the use of centrifugal contactors and membrane reactors allow for enhanced mass transfer and improved phase separation, while reactive distillation and reactive absorption have been shown to lead to enhanced conversion rates, lower energy requirements and a reduced footprint [39]. In the case of fermentation processes, integrated stripping has been demonstrated for 2-butanol and ABE, and solvent extraction has been applied for diesel and jetfuel replacements such as sesquiterpenes and alkanes [40]. For excreted soluble products, a simple mathematical model weighs the benefit of longer production per fermentation versus the drawback of product recovery at lower titer [40]. Such analysis can be used for a priori deciding on the usefulness of ISPR as a process configuration alternative.

Regarding equipment sizing, systematic research on optimization of dimensions of recovery equipment has been traditionally limited by the commercially available sizes. In-house construction of test equipment with varying dimensions was usually costly and slow. However, using 3D printing, the dimensions of mini-hydrocyclones were optimized for the recovery of yeast from aqueous suspension [41]. Similarly, hollow fiber membrane printing has been used [42], for example.

Downstream process design

Selection of the best types and sequence of unit operations, and selection of their operational settings, are major challenges in the recovery of any biobased product, considering the large number of components and the nonideal thermodynamics of the liquid mixtures that typically need to be separated. Trends include incorporating more mechanistic/molecular detail in the mathematical models used for process simulation. For example, to optimize distillation processes downstream in the biorefining area, algorithms have been developed to deal with the topology of complex process superstructures involving rigorous thermodynamic models and 230 decision variables [43]. For mixtures such as obtained from in-situ pervaporation during ABE fermentation, others (e.g. [44]) rather use insight in the separation problem to choose a separation sequence. Shortcut methods, however, are useful to decide if pursuing a separation is worthwhile. Lange [45] has proposed an easy method to estimate distillation costs from the boiling points and mass fractions of the volatile components in a mixture. The heat transfer duty can be estimated from these data, and hence energy costs. A correlation between energy duty and capital investment was obtained from petrochemical data. Subsequently was calculated, for instance, that the product concentration needs to exceed 3 mass% for lactic acid to keep recovery cost below 0.10 $/kg product at 200 kt/a capacity. Shortcut models have also been published for selecting microorganism-liquid separation method [46] and for selecting extraction solvent [47]. A shortcut model has even been formulated to synthesize separation networks for recovery of any type of liquid or solid chemical produced by fermentation [48]. This is no simple model though, considering the superstructures and hundreds to thousands variables.

Merchan et al. [49] reviewed the state of the art in process systems engineering, which encompasses model-based process simulation, optimization and control. Process design and subsequent plant design involve several modelling steps that, for propagation and iteration at various levels, require many types of

bidirectional information exchange. There is a need to integrate the various steps within one unifying computational framework. That might involve millions of variables and constraints. Computational power is not seen as key bottleneck; rather the fact that many different types of underlying problems need to be solved, for which highly specialized software exist already, each with specific advantages to their particular goal. Often, these applications are not compatible, and may use proprietary databases, such as for thermodynamic properties. Therefore, the CAPE-OPEN industry standard has been set for interoperability between process simulation software. This standard is well known in process engineering [50] but has not yet received particular interest in the field of bioprocess engineering. Model management and understanding the underlying computational problems should receive proper attention at an early stage of bioprocess development, though.

Future trends

Upcoming separation challenges

Lignocellulosic feedstocks have a large impact on fermentation, which has been widely studied. Much fewer studies have been devoted to the impact of lignocellulosic fermentation on DSP [13,15]. Lignocellulosic impurities are hard to remove from nonvolatile fermentation products. Another upcoming complex mixture stems from anaerobic mixed culture fermentation of waste streams to short-chain carboxylate salts ("VFA") [14,51]. Then, low product concentrations and neutralization requirements complicate recovery of pure carboxylic acids or their derivatives. Microbial electrosynthesis [52] can lead to similar product mixtures, but use a cleaner feedstock (CO_2). Other gas fermentations use syngas [53], biogas, or electrolytic H_2, for example, and may require DSP of off-gas, in addition to feed gas cleaning. When products are low molecular and not excreted, but polymeric or intracellular, each new product type may pose a large new DSP challenge.

Electrification

Using renewable rather than fossil energy sources may become a focal point of industrial biotechnology [1]. Such a trend may favor (renewable) electricity-driven separations (see Table 2). Thus, in-situ vacuum stripping of 1-butanol [54], becomes more favorable as compared to conventional distillation, and especially for charged products, electro-membrane processes will receive much more attention in the future [55]. Electric power might also be used to achieve the high DSP temperatures currently achieved by fossil-fuel derived steam.

Table 2. Main energy source of important unit operations.

Key function	Unit operation	Energy utility
Solid-liquid separation	Centrifugation	Electricity
Solid-liquid separation	Filtration / microfiltration	Electricity
Primary recovery	Evaporation (+ condensation)	Steam
Primary recovery	L/L Extraction (+ solvent recovery)	Steam
Primary recovery	Reverse osmosis/ nanofiltration	Electricity
Primary recovery	Pervaporation + vapor compression	Electricity
Primary recovery	Vacuum stripping (+ vapor condensation)	Electricity
Primary recovery	Electrodialysis	Electricity
Primary recovery / purification	Adsorption / chromatography	Depends on desorption and regeneration methods
Purification	Distillation	Steam[a]
Purification	Cooling crystallization	Steam + electricity
Purification	Antisolvent crystallization + solvent recovery	Steam
Formulation	Drying	Steam

[a] Considerably less steam when using (electricity-driven) mechanical vapor recompression

Big data and digital manufacturing

Artificial intelligence (AI) and internet of things (IoT) are entering the process industry. In bioprocess industry, however, the examples are still rather scarce, and seem to be driven by the biopharmaceuticals sector given the well-established use of Process Analytical Technologies (PAT) at commercial scale. Nevertheless, the bioprocess industry and DSP development in particular will benefit from the following advances:

- Digital twins combines advanced (first principles and multiscale) models for specific unit operations and equipment, and data from laboratory, pilot or production scale. Typical applications include new process development and scale-up, and existing plant optimization. This field benefits from collaborations between providers of advanced modelling software and providers of plant automation and control systems (e.g. www.pseenterprise.com and www.siemens.com, respectively).
- Industrial advanced analytics uses (historic) plant and process performance data with advanced tools for data analysis, to improve operations performance and enhance scale-down and scale-up methodologies. Additionally, companies aim at using client plant data to tailor product development (e.g. Novozymes website; URL: https://www.novozymes.com/en/news/news-archive/2016/10/unlocking-big-data-for-ethanol-producers). However, often the amount and quality of DSP manufacturing data in the production processes for biofuels and biobased chemicals is not recognized as "Big Data", limiting the opportunities for process improvement by data mining.

Conclusions

Developments and opportunities in downstream processing in biotechnology do not so much originate from new separation principles, but rather from better hardware (membranes, 3D printed devices) as well as from better software (incorporating better understanding and better mathematical modelling). Many new separation problems arise due to changes in the feedstock landscape. Key challenges are to define a separation problem in molecular terms (composition and properties of *all* components and their mixture) early on, and to solve the huge combinatorial problem of finding the best types and sequence of unit operations for the required separation.

Conflict of interest statement

The authors have no conflict of interest.

Annotations

- Park, et al. [31]
  A good starting point for understanding the state-of-the-art of membrane separations and the opportunities for further developments. Effectively illustrates the impact of membrane properties on key performance indicators such as permeability and selectivity.
- Górak and Stankiewicz [38]
  Comprehensive compilation of process integration and process intensification works on bioprocessing, covering several application fields and both experimental and modelling studies.
- Lange [45]
  A shortcut method that allows to quickly get a rough estimate of distillation costs.
- Merchan, et al. [49]
  Provides insight in software tools used at various levels of process development – and what is needed to integrate these tools.

- Handojo, et al. [55]
  Overview of options to use electricity-driven membrane permeation for carboxylic acid recovery – also useful for other charged products.

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