**Improving n-butanol production in batch and semi-continuous processes
through integrated product recovery**

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Abstract

Although it represents a promising biofuel, n-butanol production by conventional batch fermentation is limited as a result of its cytotoxic effects. To address this limitation and facilitate semi-continuous fermentation, *in situ* n-butanol removal has proven to be an effective approach. Exploiting the phenomena of solvent extraction, adsorption, or vaporization, numerous integrated bioprocess configurations have been developed to facilitate selective n-butanol recovery. The objective of this review is to provide a broad overview of different technology options and process configurations to this end, highlighting notable achievements and recent developments. In each case, relevant design considerations critical for improving key production metrics will be discussed, with particular emphasis given to studies that, as a result of relieved product toxicity, have successfully demonstrated further enhanced n-butanol production through semi-continuous operation.

Keywords: n-butanol; *in situ* product recovery; adsorption; extraction; gas stripping; pervaporation; continuous bioprocessing.

Introduction

Produced by microbial biocatalysts from biomass-derived feedstocks, biofuels represent a promising solution to the growing global need for sustainable liquid transportation fuels [1-3]. In light of inherent shortcomings associated with ethanol, however, which include its high water solubility and low energy density (attributes that collectively diminish its compatibility with conventional engines and fuel distribution infrastructure) [1], interest continues to shift to the alternative fermentative production of higher (i.e., >2-carbon) alcohol biofuels [2, 3], including n-butanol [4-6]. With more similar physical and thermodynamic properties, n-butanol represents a potential ‘drop in’ compatible gasoline replacement [7]. Naturally synthesized by many *Clostridium* sp. [8], n-butanol is produced as the major end-product along with acetone and ethanol as part of the so-called ABE fermentation. Efficient and commonly employed n-butanol production strains include, for example, the type strain *C. acetobutylicum* ATCC 824 and the hyper-producer *C. beijerinckii* [9]. Notably, the novel and focal biosynthesis of n-butanol has also recently been engineered in a number of other heterologous microbes [10-12].

Despite its promising attributes, however, several inherent limitations surround the n-butanol fermentation process, challenging its overall commercial prospects. Chief among these is product toxicity, and the resultant feedback inhibition it causes against producing microbes at relative low aqueous n-butanol concentrations [8, 13, 14]. As with other alcohols and solvents, microbial activity is reduced as a result of n-butanol partitioning into the cytoplasmic membrane [15, 16]. As the membrane structure becomes disrupted [13], increased membrane fluidity [14, 17] and diminished function of several essential cellular functions results [13]. Consequently, maximal n-butanol titers of only 13 g/L are typical for wild-type *C. acetobutylicum* [8], whereas strains engineered or selected for enhanced solvent tolerance fair only slightly better (e.g.,

maximal titers reaching no more than 20-24 g/L have been reported [18-21]). At this output, large-scale bioreactors and high turnover rates are required to produce n-butanol in quantities sufficient for biofuel applications. Accordingly, there remains keen interest in the development of effective, process-level solutions for addressing n-butanol toxicity and promoting high rates of volumetric productivity.

As with many other toxic bioproducts, *in situ* product recovery has been demonstrated as an effective strategy for improving n-butanol production by circumventing its inhibitory effects [22-25]. Utilizing an integrated bioprocess to synchronously incorporate fermentation and separation operations, n-butanol can be selectively removed from the fermentation medium as it is produced. With typical yield coefficients of up to about 0.3 g-butanol/g-glucose [26-29], in traditional batch cultures the n-butanol toxicity threshold is readily reached when *Clostridium* sp. are provided with as little as 60 g/L glucose, with excess amounts typically going unused. Alternatively, by incorporating a second, product recovery phase to concentrate and sequester n-butanol away from susceptible cells, greater net substrate utilization is then possible as is n-butanol accumulation to above would be inhibitory levels. However, as high sugar concentrations can themselves be inhibitory (e.g., >100 g/L glucose [30], >250 g/L lactose [31]), this is typically best accomplished through fed-batch operation. This semi-continuous approach to n-butanol production provides further practical benefits, including minimal wastewater generation, decreased frequency of culture turnover, and reduced between-run downtime for cleaning and sterilization. Time saved as a result of the latter features is often translated into rates of volumetric productivity, surpassing the 0.2-0.6 g/L-h typically achieved under conventional batch conditions [8].

The selective separation of n-butanol from aqueous solutions can be achieved by exploiting of one of several unique physicochemical attributes. Effective strategies for *in situ* n-butanol recovery, however, must also satisfy several important prerequisites:

1. Cost: minimal energy and/or material costs to maintain favorable energetic and economic yields.
2. Biocompatibility: microbial activity and viability should not be adversely impacted by the conditions or materials required for the separation.
3. High affinity: effective n-butanol separation should be possible under dilute concentrations.
4. Regeneration: full regeneration possible with minimal energy input, enabling both facile release of recovered n-butanol and reuse of materials.
5. Robustness: separation materials and design should be resistant against non-ideal interactions with medium components and/or cells, as these can result in performance losses due to inhibition, (bio)fouling, concentration polarization, and degradation.
6. Modularity: important for improving implementation in retrofit applications and enabling different modes of operation.

Despite these stringent design considerations, numerous effective integrated bioprocesses have been developed to date and applied to enhance n-butanol production through its *in situ* recovery.

The objective of this review is to provide a broad overview of different technologies, recent applications, and novel developments to this end (note: for earlier, complementary reviews see also the works of Schugerl *et al.* [23], Ramaswamy *et al.* [32], and Ezeji *et al.* [33]). In each case, as appropriate, specific emphasis is also given to relevant design considerations. Meanwhile, in light of known challenges related to the development of continuous n-butanol fermentations [34], of particular interest will be to highlight studies which, as a result of this

approach, have led to the successful development of semi-continuous bioprocesses with further enhanced n-butanol production metrics (e.g., titer, yield, and/or rate of productivity). While it is of course appreciated that n-butanol is synthesized alongside both acetone and ethanol (minor yet important co-products) to maintain tractability and focus, descriptions and discussions provided here will be confined to the production of n-butanol and, in some cases, total solvents. As perhaps the most promising strategies, specific focus will be given to processes employing solvent extraction, perstraction, adsorption, gas stripping, vacuum stripping and pervaporation. To facilitate the discussion, the structure of this review has been organized on the basis of the different separation media and equilibrium driving forces used to affect n-butanol separation in each case, namely solvent-based, adsorbent-based, and vapor-based processes. As a point of reference, the major advantages and disadvantages of the various technology options to be discussed are compared in Table 1.

Solvent-based Processes

Among the earliest integrated bioprocesses developed for *in situ* n-butanol recovery are those that exploit solubility differences to extract n-butanol from its aqueous environment and concentrate it within an immiscible solvent phase [35]. Accordingly, such processes must be coupled with a second, downstream separation process (typically distillation) to ultimately recover and purify n-butanol, regenerating the solvent in the process [36]. As a relatively mature technology, several useful algorithms have been developed to aid in designing LLE-based processes [35]. A key design variable is the solvent partitioning coefficient (K), defined as:

$$K = \frac{c_S}{c_A} \quad (1)$$

Where C_S and C_A are the equilibrated n-butanol concentrations in the solvent and aqueous phases, respectively. In general, large K -values are desired to minimize material requirements, with several prior screening [26, 37] and modeling [38-40] studies having identified effective solvent candidates for n-butanol extraction. For example, a classic screening study by Barton and Daugulis found that, among 63 screened solvents, 28 were found to be biocompatible, with 14 of those having $K > 2$ [26]. Commonly employed solvents include oleyl alcohol [41, 42] and 1-dodecanol [43], whose K -values have been reported as 3.56 and 5.14, respectively.

Direct Solvent Addition

In the simplest configuration, solvents are added directly to the bioreactor (Figure 1A). To provide sufficient capacity for n-butanol accumulation, solvent-aqueous phase ratios of around 0.4 and up to 2 (by vol.) have commonly been employed [44, 45]. In an early study by Roffler *et al.*, for example, following an initial screening, oleyl alcohol was selected for n-butanol extraction from batch cultures, resulting in 25% greater glucose conversion and a 60% increase in volumetric n-butanol productivity (from 0.58 to 0.72 g/L-h) [46]. Ishizaki *et al.* later compared the utility of oleyl alcohol against that of methylated crude palm oil ($K = 0.9$) as solvents for n-butanol recovery from batch cultures of *C. saccharoperbutylacetonicum* N1-4. Even despite its lower K -value, net n-butanol and total solvent production in cultures with methylated crude palm oil were found to be increased by 32% (15.9 to 20.9 g/L) and 41% (21.2 to 29.8 g/L), respectively [47]. A similar result was also obtained by Li *et al.*, who explored the potential of biodiesel (a mixture of oleic, palmitic, stearic, linoleic, linolenic, arachidic and myristic methyl esters) as the solvent phase [48].

Meanwhile, building upon the successful performance of oleyl alcohol in batch cultures, Roffler *et al.* later explored its effect on n-butanol production under fed-batch conditions. Further improvements in volumetric n-butanol productivity were demonstrated, in this case reaching up to 1.5 g/L-h (a 208% increase), due largely to the afforded ability to convert twice as much glucose substrate [45]. Fed-batch operation was also explored by Baron and Daugulis, in this case, however, using poly(propylene glycol) 1200 as the solvent phase. Adding a concentrated glucose solution to the culture at an average frequency of every 13.5 h, it was demonstrated that the duration of solvent production phase could be increased from 70 to 202 h, during which time net glucose consumption and total solvent production both increased by ~360% (53.8 to 194.7 g/L and 15.9 to 58.6 g/L, respectively).

Re-circulation through an External Solvent Extractor

Despite its straightforward implementation (i.e., requiring no additional equipment), direct solvent addition presents several practical challenges, particularly with respect to process scale-up. Achieving rapid mass transfer, for instance, requires large interfacial areas and thus high energy inputs for agitating the biphasic mixture [49]. Alternatively, improved separation conditions, as well as enhanced n-butanol production, have been realized with designs that integrate an external solvent extractor. In this case, the cell containing media is continuously re-circulated between the bioreactor and external extractor. Roffler *et al.*, for example, also developed a looping process for n-butanol recovery in an external solvent extractor by employing a Karr reciprocating plate extraction column (Figure 1B) containing oleyl alcohol [49]. Although the per pass extraction efficiency of n-butanol in the column was low (only 15-

40%), continuous re-circulation of the culture through the column maintained aqueous n-butanol concentrations below 8 g/L throughout 55 h of fed-batch fermentation. In that time, a total of 214 g/L glucose was consumed with 40 g/L n-butanol produced (67 g/L total solvents) at an overall productivity of 1.0 g/L-h – a 70% increase over traditional batch cultures.

Membrane-Assisted Extractive Fermentation (Perstraction)

In addition to the risk of productivity losses due to solvent-induced toxicity [50], solvent entrainment due to mutual solubility and stable emulsion formation commonly leads to costly material losses [50, 51]. These shortcomings have been addressed through the development of membrane perstraction, wherein an n-butanol-permeable membrane barrier is additionally incorporated to provide physical separation between the culture and solvent phases [51-53]. Moreover, by shielding cells from the solvent, better performing and/or cheaper solvents can be employed, independent of their potential biocompatibility. In contrast to direct addition, this approach further promotes facile solvent recovery and replacement with minimal disruption of the culture. However, as the membrane introduces an additional mass transfer resistance, potentially slowing rates of n-butanol uptake, the selection of effective membrane materials and dimensions (e.g., thickness, area) are important design considerations. In most case, membranes composed of polydimethylsiloxane (PDMS; i.e., silicone) have performed well in perstraction designs due to their high n-butanol diffusivity. Tanaka *et al.*, meanwhile, also demonstrated enhanced n-butanol production metrics in a perstraction design using a polytetrafluoroethylene (PTFE) membrane [43].

In general, membrane perstraction can be performed according to one of two prototypical configurations, differing with respect to the circulated phase: culture vs. solvent (Figures 1C and 1D, respectively). An early study by Groot *et al.* provided a comparison of both designs, using ethylene glycol or isopropyl myristate as solvents in an external reservoir [52]. In the case of solvent circulation (Figure 1D), however, it was found that the high solvent permeability through the silicone tubing membrane lead to its significant accumulation in the fermentation broth. Reaching levels of up to 80 g/L in the case of ethylene glycol, this represents not only a significant material loss, but also a point of concern should more toxic solvents instead be employed. In this case, this limitation was addressed by modifying the process to instead circulate the culture through the external solvent reservoir (Figure 1C). As a result, n-butanol accumulation in the isopropyl myristate phase was found to top 20 g/L after 160 h of operation, with a total of 100 g/L glucose consumed in the process.

Incorporating the lessons learned from Groot *et al.*, subsequent studies have predominantly been directed towards the development of perstraction processes employing culture circulation. Also using silicone tubing as a membrane and oleyl alcohol as solvent, a perstraction process was developed by Qureshi *et al.* wherein the entire contents of the bioreactor were circulated through the extractor an average of every ~3.3 min [31]. Meanwhile, to maintain a high extraction driving force, solvent removal and replacement (i.e., with fresh solvent) was performed whenever the extracted n-butanol concentration exceeded 6.65 g/L. By producing and removing n-butanol from the bioreactor in this semi-continuous manner, the n-butanol content in the bioreactor was maintained below 3 g/L for the duration of the 391 h fermentation, during which time total solvent production reached 136.58 g, including 66.4 g n-butanol. Jeon *et al.* demonstrated similarly impressive results under fed-batch conditions using

oleyl alcohol and a membrane composed of silicone tubing [53]. In this case, a total of 147.7 g/L n-butanol was produced from 601 g/L glucose over course of 209 h of continuous operation [53].

Adsorbent-based Processes

As an alternative to the use of solvents, increasing focus continues to be directed towards the development of novel process configurations and materials for *in situ* n-butanol recovery via adsorption [6, 54-56]. Adsorbents are generally more biocompatible than solvents (promoting maximal biocatalyst activity), as well as fully immiscible and unsusceptible to emulsification – features that facilitate their separation from cultures and improve prospects for repeated regeneration and reuse [57]. As with solvents, additional downstream processing is required to ultimately recover the adsorbed n-butanol product, as has been achieved by thermal treatment [58], steam stripping [59], gas stripping [60], vacuum application [61], or solvent elution [58]. Combined thermal treatment (heating to 100°C) and vacuum application, for example, proved an effective strategy for recovering up to 85% of adsorbed n-butanol from Dowex® Optipore L-493, a polymer resin adsorbent, as a condensable vapor [30]. Through the same process, the adsorbent was also thereby regenerated for reuse without loss of separation performance. Several prior studies have demonstrated that successive reapplication of regenerated adsorbents is possible with little to no loss in separation performance [62, 63]. Adsorption-based processes have been suggested to be among the most energy efficient for n-butanol recovery, with estimates of at least a 10% decrease in required energy relative to other approaches (specifically, conventional distillation, extraction/perstraction, gas stripping, pervaporation) [55, 59].

Typically selected via *ad hoc* materials screening, numerous classes and types of adsorbents have been characterized and employed for n-butanol separations [51], in forms ranging from powders to pellets [64]. Particularly promising n-butanol adsorbents include silicalite and other inorganic zeolites [65-70], activated [62, 67, 71] and mesoporous [72] carbons, as well as polymer resins [30, 62, 63, 73]. Key attributes of an effective adsorbent include a high capacity and affinity for n-butanol, along with biocompatibility, as well as chemical, thermal and mechanical robustness. Adsorption capacity and affinity are determined via the equilibrium isotherm, typically represented by either the Langmuir or Freundlich isotherm models (given by Equations 2 and 3, respectively).

$$q = \frac{q_{max}k_l C_A}{1+k_l C_A} \quad (2)$$

$$q = k_f C_A^{1/n} \quad (3)$$

Where q is equilibrium capacity, q_{max} is maximum equilibrium capacity, k_l and k_f are Langmuir and Freundlich constants, respectively, n is the Freundlich exponent, and C_A is defined as above. In most cases, adsorbent behavior has been characterized using model solutions (e.g., n-butanol in water). It should be noted, however, that adsorption capacity is often decreased in fermentation broths as a result of competitive inhibition by other adsorbed species (e.g., broth components or co-products), with n-butanol capacity reductions of up to 29% having been reported [64]. Meanwhile, whereas whole cell biofouling of the adsorbent surface is a common concern in such applications, several recent studies have found such occurrences to be minimal with little to no impact on either separation performance or n-butanol production [61, 62].

The separation performance of several previously investigated n-butanol adsorbents is compared in Figure 2. As a result of van der Waals interactions [59, 74, 75], adsorbents with

hydrophobic surface chemistries display some of the highest capacities and affinities [30, 65-67, 73]. Effective polymer adsorbents are those that include more hydrophobic monomer substituents (e.g., long n-alkyl acrylates and aromatic groups [76]), whereas zeolite hydrophobicity has been improved by increasing the Si/Al ratio [64, 65]. Separation performance is also strongly influenced by adsorbent structure; high specific surface areas offer more adsorption sites and thus high adsorption capacity [30, 66, 73, 77, 78] while regular and interconnected pores facilitate intraparticle transport to promote enhanced uptake rates [72, 79].

Direct Adsorbent Addition

As with solvents, the simplest adsorbent-based designs also involve their direct addition to cultures (Figure 3A). An early study by Yang *et al.* explored the use of a poly(vinyl pyridine) ion exchange resin (Reillex 425) to adsorb n-butanol in batch cultures, demonstrating a 56% increase in glucose consumption and 54% increase in total solvent production (reaching up to 29.8 g/L). Volumetric productivity was also improved to 0.92 g/L-h, a 130% increase over conventional batch culture. However, the low specific surface area of the selected adsorbent (only ~90 m²/g) resulted in only a modest n-butanol loading capacity (~52 mg/g), thus necessitating the use of a high adsorbent phase ratio (300 g-adsorbent/L-culture) to achieve the requisite total level of product removal. While adsorbent phase ratios of 50 to 400 g/L are common in such designs [59, 63], this design parameter should be minimized to reduce material costs. Moreover, owing to their low specificity (which can deplete the media of essential nutrients), excessive adsorbent addition has in some cases also led to reduced growth and productivity [30, 63]. A lower adsorbent phase ratios is possible when using a higher capacity

adsorbent. As seen in Figure 2, among the top performing adsorbents identified to date are those notably capable of adsorbing n-butanol at up to ~300 mg/g (i.e., ~30% of their dry weight) by the time the aqueous toxicity threshold is reached (i.e., 13 g/L). This includes, for example, both Dowex® Optipore L-493 and SD-2 (macroporous, poly(styrene-*co*-DVB)-derived resins with specific surface areas of 1100 and 800 m²/g, respectively) [30]. Based on their high adsorption affinities and capacities, said resins were identified as the most promising n-butanol adsorbents amongst a pool of 17 candidates with different polymer chemistries and functionalities. Ultimately, the in a batch culture containing just 50 g/L Dowex® Optipore SD-2, net n-butanol production reached 22.2 g/L, nearly doubling the titers achieved in conventional batch culture [30].

Fixed Bed Adsorption

In contrast to their direct addition, adsorbents may also be housed in external adsorption beds through which the culture broth is re-circulated (continuously or intermittently; Figure 3B). With increased accessibility external to the fermenter, adsorbent removal and replacement is facilitated with reduced risk for compromising the integrity of the culture. Early adoptions of this approach focused on the application of adsorbents in fixed beds (i.e., packed columns); an approach that affords efficient n-butanol recovery. Abdehagh *et al.*, for example, showed that packed column containing activated carbon F-400 could be used to recover up to 80% of n-butanol from a model ABE mixture [80]. The approach has also been integrated with cultures. For example, using a poly(vinyl pyridine) resin (Reillex 425) as the adsorbent, Yang and Tsao developed a fixed bed process to enhance n-butanol production by *C. acetobutylicum* [81]. The

contents of the 0.7 L bioreactor were continuously circulated through a 0.3 L cylindrical column packed with 423 g resin. However, due to the presence of the cells (which can become entrained in and block flow through the bed), the process further incorporated microfiltration to remove and recycle cells prior to entering the adsorbent bed [82]. Operating in a fed-batch manner for ~240 h, n-butanol and total solvent production reached 38.7 and 47.2 g/L, with total solvent productivity reaching 1.69 g/L-h. Increased modularity also enables facile integration of adsorbent beds with different bioreactor configurations, including those poorly compatible with direct adsorbent addition (e.g., non-planktonic cultures). For example, Liu *et al.* recently coupled a fixed bed adsorption column containing macroporous KA-I resin with a *C. acetobutylicum* biofilm reactor [83]. With the culture immobilized, upstream microfiltration was no longer required. Through repeated batch culture, n-butanol production ultimately reached 92.6 g/L at a productivity of 1.51 g/L-h, representing 4- and 3-fold improvements over traditional batch fermentation using planktonic culture, respectively.

Expanded Bed Adsorption

In contrast to fixed bed operation, alternative operation of the adsorption column in an expanded bed mode provides sufficient void space between adsorbent particles to accommodate direct cell passage (Figure 3C). Fluidization is achieved in this case by circulating the culture through the bed in an up-flow manner; however, it should be noted that maintaining a stable bed and avoiding inversion imposes restrictions on the circulation rate of the media through the adsorption column. While this approach eliminates the requirement for upstream microfiltration, reduced separation efficiency is experienced relative to packed bed operation [84]. Initially developed and applied to improve the fermentative production of both succinic [85] and

propionic acid [86] (using ion exchange resins as adsorbents in both cases), the authors recently applied expanded bed adsorption to improve n-butanol production by *C. acetobutylicum* ATCC 824 [61]. In this case, the expanded bed consisted of 0.17 L glass column containing 75 g of Dowex® Optipore L-493 (thereby leaving ~55% of the column volume unoccupied to accommodate for bed expansion). The contents of the 1 L culture were continuously recirculated between the bioreactor and adsorption bed at a rate of about 100 mL/min, expanding the bed height by ~50%. Fed-batch operation with this design ultimately enabled volumetric n-butanol and total solvents production to be increased to 27.1 and 40.7 g/L, respectively, each ~2.2-fold improvements over batch culture [61]. The modular nature of the adsorbent bed design was further exploited in said study during the regeneration cycle, wherein direct vacuum application to the liquid-drained column (i.e., without adsorbent transfer) was used to recover 81% of the produced n-butanol [61]. By facilitating the cycle of adsorbent recovery, regeneration, and reuse, such increasingly modular designs will remain crucial to the continued optimization of semi-continuous n-butanol production. Meanwhile, through further process evolution, continuous n-butanol production will also likely be realized through the future incorporation of simulated moving bed (SMB) operation (Figure 3D) [87]. A staple of the modern biopharmaceutical industry, to facilitate the continuous production and recovery of proteins, SMB is an unexplored yet particularly promising technology for enhancing n-butanol production.

Vapor-based Processes

Solvent- and adsorbent-based processes expose cultures to foreign chemicals/materials, a practice that, in spite of careful material selection, presents increased risk of phase induced

toxicity while also introducing opportunities for biofouling. Vapor-based processes circumvent these challenges by exploiting relative volatility for *in situ* n-butanol removal by vaporization, with condensation subsequently used to trap the evolved vapors. Operating under dilute conditions, Henry's Law describes n-butanol's vapor-liquid equilibrium behavior (Equation 4), with differences from this equilibrium condition providing the driving force for separation.

$$p = HC_A \quad (4)$$

Where p is the partial pressure of n-butanol in the vapor phase, H is the Henry's Law coefficient, and C_A is defined as above. In general, however, vapor-based processes for n-butanol recovery are commonly challenged by its relatively low vapor pressure relative to that of water (e.g., 0.0109 vs. 0.0312 atm at 25°C). In vapor-based processes, the ability of n-butanol to be selectively removed over water is determined by the separation factor:

$$\alpha_{12} = \frac{C_1^v/C_2^v}{C_1^l/C_2^l} \quad (5)$$

Where C_1 and C_2 are concentrations of vaporized species (i.e., n-butanol and water, respectively) and superscripts v and l indicate the vapor and liquid phases, respectively.

Gas Stripping

Requiring only a carrier gas to be sparged through the fermentation media (Figure 4A); gas stripping is an easily implemented and effective *in situ* recovery technique for n-butanol [18, 59, 88]. For example, with stripping rates on the order of 4-4.5 g/L-h having been reported [20, 88] – nearly an order of magnitude greater than typical production rates – aqueous accumulation

of n-butanol to inhibitory levels is readily precluded. To maintain anaerobic conditions, however, an inert carrier gas (typically a mixture of H₂ and CO₂) must be utilized [89, 90], the generation of which increases capital and operating costs. Pre-humidification of the carrier gas, meanwhile, is also typically performed to reduce culture volume losses [90].

Using an engineered strain of *C. acetobutylicum* (developed to produce isopropanol in lieu of acetone), Lee *et al.* recently demonstrated the utility of gas stripping for promoting solvent production under fed-batch conditions [91]. With 132.9 g/L glucose consumed in 45 h, 35.6 g/L total solvents were produced, a ~75% increase relative to batch mode operation [91]. In earlier works, Ezeji *et al.* likewise employed continuous gas stripping under fed-batch conditions, in this case to improve the ABE fermentation [89]. Although productivity was continuously maintained for an initial period of over 201 h, accumulating 152 g/L n-butanol and 233 g/L total solvent from 500 g/L glucose, production was ultimately and unduly halted as a result of gradually declining culture conditions. Though uncharacterized, it was postulated that said limitation perhaps resulted from either the depletion of an essential micronutrient or the accumulation of non-viable cells, acids, and/or other byproducts in the aqueous phase.

As most separation strategies are selective towards only the target product, such productivity-limiting factors are a common challenge faced by *in situ* product recovery designs, particularly in long-term applications (e.g., fed-batch). In many cases, however, this problem has been effectively addressed by performing periodic exchanges of the culture medium, thereby replenishing nutrients while diluting byproducts. In a follow-up study, for example, Ezeji *et al.* successfully demonstrated that by adopting a medium exchange protocol, their semi-continuous gas stripping process could be operated under fed-batch conditions for an uninterrupted period of up to 504 h [90]. Specifically, at an average dilution rate of 0.03 h⁻¹, a portion of the bioreactor

contents were continuously exchanged for a glucose-rich feed solution (containing 250-500 g/L glucose). By this approach, 461 g/L total solvents were produced from 1125 g/L of glucose, representing a 3-fold further improvement relative to their initial gas stripping/fed-batch design and a 25-fold increase over traditional batch culture [90].

The ease of implementation of gas stripping makes it an amenable approach for integration with a variety of bioreactor configurations. In a recent study by Chen *et al.*, for example, its utility was explored in fed-batch cultures of immobilized *C. acetobutylicum* [88]. Following 175 h of continuous operation, 290 g/L glucose was consumed resulting in the production of 66 g/L n-butanol and 106 g/L total solvents. The approach is equally effective for use with cultures grown on complex, biomass-derived substrates. Lu *et al.*, for example, demonstrated a 56% improvements in n-butanol production from wood pulping hydrolysate as a result of integrated gas stripping [92]. Xue *et al.*, also studied the use of gas stripping in fed-batch cultures of immobilized *C. acetobutylicum* [20]. In this case, however, gas stripping was applied intermittently, and only when aqueous n-butanol concentrations exceeded >8 g/L. This approach not only maximized achievable separation factors, but also reduced energy input and operating costs. Ultimately, 113 g/L n-butanol (nearly a 6-fold increase) along with 172 g/L total solvents were produced from 475 g/L glucose over the course of 326 h of continuous operation. To further enhance their design by reducing energy requirements associated with n-butanol purification, Xue *et al.* later investigated two-stage gas stripping protocol [25, 93]. While the first stage served to remove n-butanol from the fibrous bed bioreactor, the second served to then recover n-butanol at further increased purity from the initial condensate. In the final design, n-butanol selectivity of up to 33.2 was achieved, rendering a recovered product phases containing 515.3 g/L n-butanol and 671.1 g/L total solvents [25].

410

411 *Vacuum Stripping*

412 In contrast to evolving n-butanol vapors from the culture via its displacement into a
413 sparged gas, vacuum stripping renders a similar outcome by reducing pressure above the
414 bioreactor headspace (Figure 4B). As it is not limited by poor gas solubility at physiological
415 temperatures [88], vacuum stripping has been reported to support n-butanol removal rates as
416 much as 10-fold faster than by gas stripping [94]. However, whereas a study by Mariano *et al.*
417 showed that vacuum stripping can be used to increase n-butanol and total solvent production by
418 as much as ~31% under batch conditions, to date, vacuum stripping has seen limited application
419 with respect to semi-continuous operation [94].

420

421 *Pervaporation*

422 Despite their proven ability to enable enhanced n-butanol production, the fundamental
423 weakness of both of gas and vacuum stripping ultimately lies in their lack of selectivity. In
424 vapor-based processes, low separation factors are undesirable as significant co-removal of water
425 vapor results in a hydrous solvent product in need of additional downstream purification [20]. In
426 practice, separation factors for n-butanol are inversely proportional to its concentration in the
427 media [90], with typical values for gas stripping having been reported to range between 4 to 12
428 [90] and up to 20 for vacuum stripping [94]. One effective approach for improving the
429 separation factor in vapor-based process involves the use of a membrane barrier to reject evolved
430 water vapor. In pervaporation, for example, an n-butanol permselective membrane is contacted
431 directly with the fermentation broth while a sweep gas or vacuum conditions (more common due

to improved mass transfer rates [95]) are applied on the permeate side to provide the separation driving force. Following permeation into the membrane, n-butanol diffuses through it, becoming vaporized as it reaches the permeate side (Figure 4C) [95]. The overall process requires minimal energy input, prompting the U.S. Department of Energy to label pervaporation as among the most efficient methods recovering bio-alcohols [96].

Separation factor and flux are important measures of membrane performance and as such serve as key design parameters for the overall process [51]. Whereas separation factor remains as defined in Equation 5, flux, is determined as:

$$J = \frac{m_p}{A_m \Delta t} \quad (6)$$

Where m_p is mass of the permeate, A_m is membrane area, and t is time. Higher fluxes support efficient pervaporation designs by minimizing the total membrane surface area necessary to achieve rapid n-butanol recovery. It should be appreciated, however, that, as with most membrane separations, an inverse relationship exists between pervaporation flux and separation factor [97].

Hydrophobic, non-porous polymer membranes have been the most intently studied for n-butanol pervaporation, the most common being those composed of PDMS [98]. Although high fluxes have been reported using PDMS membranes (ranging between 10-400 g/m²-h [99]), achievable separation factors typically remain <20 [99]. Both using PDMS disk membranes, for example, Groot *et al.* reported an n-butanol separation factor of ~11 and an average flux of 10.4 g/m²-h using a PDMS disk membrane [100], while Van Hecke *et al.* reported values of ~14.7 and ~367 g/m²-h, respectively [99]. It has been suggested, however, that to offset the increased capital and operating cost requirements associated with pervaporation and achieve economically

viable n-butanol recovery, a minimum separation factor of at least 30 should be achieved [51]. Thus, although it remains a commonly employed material in research studies, commercial prospects of PDMS membranes remain limited [51]. Accordingly, there remains keen interest in the development of novel pervaporation membranes with improved separation performance, including those composed of poly(tetrafluoroethylene) (PTFE), poly(ether-block-amide) (PEBA) [101], and other related tuned block copolymers [18, 101-103]. Meanwhile, more recent studies have also explored the potential of various nanoporous inorganic (e.g., zeolites [104]) and ‘composite’ membranes (e.g., PDMS coated silicalite [102] and PEBA mixed with carbon nanotubes [103]), materials with improved prospects for enhanced and tunable separation performance [97, 105, 106]. Key performance metrics of select polymer, inorganic, and composite membranes are compared in Table 2. It should be noted, however, that in addition to differences in polymer chemistry [97], performance also varies due to membrane thickness [101], feed temperature [101], level of vacuum (or sweep gas flow rate) [95], and other process/environmental conditions.

A recent study by Yen *et al.* provided a direct comparison of the relative performance of two different polymer membranes, namely PDMS and PEBA, and their resultant impact on n-butanol separation and production [101]. PEBA membranes ultimately supported the greatest separation factor and flux in model solutions (17.4 and 9.98 g/m²-h vs. 14 and 3.91 g/m²-h, respectively) and, when applied to fed-batch fermentation, enabled n-butanol and total solvent production at up to 16.6 and 27.3 g/L, respectively (from a total of 130 g/L glucose) [101]. Further improvements to PEBA membrane performance were made by the same authors in a subsequent study, notably through the incorporation of 5%wt. multi-walled carbon nanotubes (CNT) [103]. Using this composite membrane, flux was improved to 16.1 g/m²-h and the

separation factor to 19.4, along with a 20% further increase in volumetric productivity. The efficacy of composite membranes for n-butanol pervaporation was similarly explored by Li *et al.*, in this case using silicalite-1 filled PDMS on a poly(acrylonitrile) support [106]. Using model solutions, separation factors of 30-33 were demonstrated, along with fluxes ranging from 40 to 173 g/m²-h. When applied to fed-batch fermentation, after 288 h of continuous operation a biphasic solution was ultimately recovered in the condensed permeate. Owing to the high separation factor (>30), the n-butanol content in the condensate exceeded its solubility in water, leading an organic-rich phase with 446 g/L n-butanol along with an aqueous phase containing 89.6 g/L n-butanol [106]. Spontaneous phase separation by the condensate is advantageous as it reduces energy requirements associated with further downstream purification.

One challenge commonly faced in pervaporation designs is diminished separation performance due to membrane fouling. Surface fouling can occur in the presence of other medium components, biomolecules, and/or whole cells. For example, using a tubular silicalite-1 membrane coated with silicone rubber, Ikegami *et al.* compared relative n-butanol separation performance between both binary solutions and cell-free fermentation broths [102]. Separation factor averaged 294 and 413 between model solutions and broths, whereas average flux was maintained at 35 and 22.3 g/m²-h, respectively [102]. Though detrimental to performance, fouling effects can be addressed through additional process improvements. For example, Qureshi *et al.* used ultrafiltration to eliminate potential foulants from the fermentation broth prior to contacting it with a silicalite-PDMS composite membrane [97]. As a result, separation performance was sustained throughout the course of a 36 day fed-batch fermentation, enabling n-butanol separation factor and flux to be maintained at average values of 203 and 89 g/m²-h,

respectively. By this approach, a total of 445 g/L glucose was consumed to yield 105 g/L n-butanol and 155 g/L total solvents [97].

Future Trends and Directions

Although *in situ* n-butanol removal has proven to be an effective approach for circumventing toxicity and facilitating semi-continuous fermentation, the field remains ripe for further innovation. Future advancements in the field will be facilitated by the development of novel materials and/or process configurations, the likes of which will continue to address some of the key inherent shortcomings identified here within. With respect to novel separation materials, the objective will be to develop and/or identify those with enhanced separation performance, including in terms selectivity, recovery rate, fouling resistance, and robustness, while also simultaneously reducing material costs and energy requirements. Such efforts are already underway, and leading to promising new candidates. For example, as a departure from the traditional focus on organic solvents in solvent-based designs, non-ionic and liquid polymer surfactants have recently emerged as effective alternatives [107]. Ionic liquids, meanwhile, offer a wide range of physical and separation properties along with an unprecedented degree of tunability [50, 108]. Furthermore, with nearly negligible vapor pressure, volatile losses of these solvents during fermentation are virtually eliminated while also streamlining downstream processing [36]. Tunability is a similarly attractive feature with respect to hybrid or composite membrane materials. Combining the processability of polymer membranes with the selectivity of inorganic membranes, such materials are expected to draw increasing interest for n-butanol pervaporation. Increased development and application of engineered adsorbent materials is

likewise expected, in particular with respect to the development of increasingly hydrophobic materials with defined pore structures (e.g., mesoporous carbons).

Meanwhile, the development of increasingly modular process will continue to provide several important benefits, including: *i*) improved control over the separation conditions (maximizing the return on material costs), *ii*) protection of the cells from the separation media (and vice versa), *iii*) ease of semi-continuous operation with minimal process disruption for phase separation/regeneration, and *iv*) improved prospects for process integration, especially in retrofit applications. (e.g., packed or expanded bed adsorption vs. direct addition). This trend can already be seen in the each of solvent-, adsorbent-, and vapor-based processes, and is likewise expected to extend into the future.

Conclusions

By circumventing the toxic effects associated with the in culture accumulation of n-butanol, *in situ* product recovery has emerged as an effective strategy for enhancing its key production metrics. Whereas multiple effective process strategies have been developed for this purpose, it should be appreciated that each approach carries its own unique pros and cons. Future advancements in terms of both materials and process development will ultimately enable *in situ* product recovery to evolve from its current laboratory bound incarnations into a viable strategy for enhancing commercial-scale n-butanol production.

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821 **List of Tables**

822 **Table 1.** Summarizing the major advantages and disadvantages of the different separation
823 technologies discussed in this review.

Approach	Configuration	Advantages	Disadvantages
solvent-based	Direct addition	Easily implemented, maximal phase contact	High material costs, limited phase recovery, solvent toxicity, energy input required for phase dispersion
	External extractor	Improved control over separation conditions, increased modularity	Increased capital and energy costs, high material costs, limited phase recovery, solvent toxicity
	Perstraction	Reduced solvent toxicity, greater solvent options (cheaper and/or more effective), improved phase recovery	Solvent diffusion into media, mass transfer restriction (additional diffusion resistance, reduced surface area)
adsorbent-based	Direct addition	Easily implemented, maximal phase contact, high biocompatibility, low energy requirements	High material costs, high adsorbent load required, difficult phase recovery
	Packed bed	Modular design (amenable to retrofit applications), efficient separation conditions, high biocompatibility, low energy requirements	High operating pressure, pre-filtration required for cell removal
	Expanded bed	Modular design (amenable to retrofit applications), low operating pressure, no pre-filtration required, high biocompatibility, low energy requirements	Flow rate restrictions, larger column dimensions required
vapor-based	Gas stripping	Easily implemented amenable to retrofit applications), maximal biocompatibility, low capital and operating costs (no special equipment and few moving parts)	Significant co-removal of water, poor separation factor, must generate anaerobic carrier gas
	Vacuum stripping	Easily implemented amenable to retrofit applications), maximal biocompatibility	Significant co-removal of water, poor separation factor, increased capital and utility costs
	Pervaporation	Modular design (amenable to retrofit applications), increased separation factor, high biocompatibility, low energy requirements	Membrane fouling results in performance loss, higher capital cost

Table 2. Comparing the *n*-butanol separation performance of different pervaporation membranes and under different test conditions. ‘Binary’ refers to a binary mixture of *n*-butanol in water; ‘ABE’ refers to a model solution of acetone, *n*-butanol, and ethanol in water; ‘Broth’ refers to a *Clostridium* sp. fermentation broth, typically cell-free and applied as a downstream separation.

Membrane	Separation factor	Flux (g/m ² -h)	Feed type	Reference
Polymer Membranes				
PDMS tubing	45-64	6.2-14.5	Binary	[109]
PDMS	15-37	70-1000	Binary	[95]
PTFE	2.7-4.8	35-2100	Binary	[95]
PDMS	14	3.91	Binary	[101]
PEBA	17.4	9.98	Binary	[101]
Polypropylene	6.3	1400-1600	Fermentation broth	[95]
Composite Membranes				
CNT filled PEBA	19.4	16.1	Binary	[103]
CNT filled PDMS	32.9	244.3	Binary	[110]
5% ZSM-5-PEBA	~21.8-33.3	190-719.3	Binary	[111]
Silicalite filled PDMS	30-33	40-173	Binary	[106]
Silicalite filled PDMS	10-180	12.5-240	ABE	[97]
PDMS-PVDF composite	35.2	769.6	ABE	[112]
PDMS-PVDF composite	14.4–21.2	93.3–108.8	Fermentation broth	[112]
Liquid Membranes				
Polymer supported ionic liquid	11	560	Binary	[113]
Inorganic Membranes				
Phenyltrimethylsiloxane (PhTMS)/PDMS Coated Alumina	~10-30	200-900	Binary	[114]
Methylated Silica	~0.0001	~10-200	Binary	[115]

Figure Captions

Figure 1. A comparison of different solvent-based design configurations used for *in situ* *n*-butanol recovery, including: A) direct solvent addition, B) use of an external extraction unit, C) perstraction with culture circulation, and D) perstraction with solvent circulation. Legend: 1. bioreactor, 2. solvent, 3. pump, 4. extraction unit (reciprocating column shown), 5. tubular membrane.

Figure 2. Comparing equilibrium isotherms for various *n*-butanol adsorbents. Polymer resins: Dowex® Optipore L-493 (open diamonds), Dowex® Optipore SD-2 (open circles), Diaion HP-20 (open upright triangles), poly(4-ethylstyrene-*co*-divinylbenzene) (open squares), Dowex® M43 (open inverted triangles), all Nielsen and Prather (2009); Amberlite XAD-4 (solid circles), [62, 63]; Amberlite XAD-16 (solid squares), Ennis et al. (1987); Amberlite XAD-8 (solid upright triangles), [62]; Amberlite XAD-2 (solid diamonds), [62]; Silica aerogels: TLD302-330 ('+'), Wiehn *et al.* (2013); Zeolites: silicalite-1 (solid squares), Milestone and Bibby (1981); Activated carbon: AC F-400 (open left triangles), AC F-600 (solid stars), both Abdehagh *et al.* (2013). Mesoporous carbon: FDU-16-800 ('X'), CS-81-800 (open stars), both Levario *et al.* (2012). Trend lines, shown for reference and indicated by dotted lines, represent the best fit Freundlich isotherm models for each of (in decreasing order): Dowex® Optipore L-493, Dowex® Optipore SD-2, Diaion HP-20, Dowex® M43, and Amberlite IRA-900. Solid line indicates $y = x$.

854

855 **Figure 3.** A comparison of different adsorbent-based design configurations used for *in situ* n-
856 butanol recovery, including: A) Direct adsorbent addition, B) packed bed adsorption, C)
857 expanded bed adsorption, and D) simulated moving bed operation. Legend: 1. bioreactor, 2.
858 adsorbent, 3. microfiltration unit, 4. regeneration media, 5. n-butanol-rich recovery stream.

859

860 **Figure 4.** A comparison of different vapor-based design configurations used for *in situ* n-butanol
861 recovery, including: A) gas stripping, B) vacuum stripping, and C) pervaporation. Legend: 1.
862 bioreactor, 2. vapor condensation trap, 3. sparged carrier gas, 4. vacuum pump.

863

864

fig 1

fig 2

fig 3

fig 4