Minireview: High-Productivity and Low-Cost Biobutanol Production by Integrated Process Development

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Abstract—**Butanol is an important chemical in industry and a potential substitute for gasoline as liquid fuel. Over the past decades, numerous attempts have been made to produce biobutanol effectively. This article reviews the progress in high-productivity and low-cost butanol production process development. We first describe the solventogenic Clostridia strains and the construction of high butanol producing strains engineered using metabolic engineering and random mutation techniques. To reduce biobutanol production cost, the hydrolysate of low-value lignocelluloses has been applied to replace the traditional food-based substrates. The advances in fermentation technologies, such as cell immobilization, cell recycle, fed-batch and continuous fermentations, for high butanol titer, yield and productivity are then discussed. Various recovery techniques developed to remove butanol in situ, improve butanol production and reduce the energy requirement in product separation are then reviewed. Finally we discuss how to efficiently produce biobutanol from lignoceluloase by integrating fermentation with online butanol recovery.**

Keywords-biobutanol; feedstock; fermentation; recovery

I. INTRODUCTION

Butanol is an important raw material in the production of lacquer and latex surface coating, a widely used solvent in the manufacturing of hormones and vitamins, and a superior liquid biofuel with great potential to directly replace gasoline due to its excellent fuel properties. Butanol was predominately produced from Acetone-Butanol-Ethanol (ABE) fermentation and used as a solvent between 1920 and 1980, but the biological production gradually slowed down because of the rise of cheaper petrochemical synthesis of butanol from crude oil and the high cost of fermentation raw materials [1,2,3,4]. However, the renewed interests and trends towards using green energy have been increasing due to the high price of crude oil, the environmental concern about using fossil fuel, and the growing demand for non-fossil based bioproducts. Compared to ethanol and methanol, butanol is a better fuel candidate due to the higher energy content and lower volatility. Importantly, butanol can directly replace gasoline or work as a fuel additive in the current internal combustion engine without any modification.

Because of all the great features of biobutanol as a potential next generation liquid biofuel and raw material in biotechnology, the revisit on ABE fermentation over the past few decades has made significant advances and breakthroughs in the bioproduction of butanol. Novel strain construction and high-efficient process development can significantly improve butanol titer and productivity, while the application of low-cost biomass as substrate and the development of energy-efficient butanol recovery techniques can greatly reduce the cost of butanol production. Developing a high butanol producing strain is the first key step to achieve high biobutanol production. Metabolic engineering has been used to reprogram the metabolic pathways in microorganism using genetic engineering tools [5,6,7,8], and synthetic biology is another efficient cell engineering strategy which introduces a series of heterologous enzymes or metabolic pathway to produce biochemical or biofuel [9,10,11]. With engineered strain, the further fermentation process development can achieve high-productivity, high-quality, robust and scalable butanol production. Various powerful process development strategies have been applied in butanol and other biochemical production, including bioreactor parameters optimization via precise bioreactor

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controllers, dynamically monitoring metabolic parameters with in situ sensors, and rational process development based on the knowledge of systems biology [12,13,14,15].

The application of low-value biomass as substrate is an efficient strategy to reduce the cost of butanol production [16,17]. Lignocellulosic biomass represents the most abundant and sustainable carbon source on earth. Intensive research has been conducted to understand and improve the efficiency of lignocellulose hydrolysis to release fermentable sugars [18,19]. Moreover, the butanol separation from ABE fermentation broth is usually an energy-intensive and costly process. Therefore, the biggest challenge of reducing biobutanol production cost is how to effectively recover butanol. To address this issue, various butanol separation technologies, such as adsorption, liquid-liquid extraction, pervaporation and gas stripping, have been developed and integrated with ABE fermentation.

In this article, we review the advanced technologies to develop a high-productivity and low-cost butanol production process, including strain construction, low-cost feedstock application, fed-batch and continuous fermentation development, product recovery development, and integrated biobutanol production development.

II. SOLVENTOGENIC STRAIN AND NEW STRAIN CONSTRUCTION

A. Solventogenic Clostridial Strains

The solvents, such as butanol, acetone, ethanol and isopropanol, can be naturally produced by genus Clostridia bacteria [4,20,21], which are rod-shaped, spore-forming, gram-positive and obligate anaerobe. The solvent fermentation processes have been developed using *C. acetobutylicum* ATCC 824, *C. carboxidivorans* P7, *C. saccharoperbutylacetonicum* N1-4, *C. saccharobutylicum* NCP 262, *C. butylicum* NRRL B592, *C. beijerinckii* NCIMB 8052, *C. aurantibutyricum* ATCC 17777, and *C. pasteurianum* ATCC 6013 [22,23,24,25,26,27,28,29]. Most of these species produce butanol as the main solvent product, although some species also produce significant amounts of 1,3-propanediol and isopropanol at pHs of 5.5-6.5 and temperatures of 35-37^{o}C. ABE fermentation using Clostridia is biphasic process, in which acids and energy are first produced in the acidogenic phase, and solvents are generated from acids in the following solventogenic phase [4,20,30]. A detailed metabolic pathway in *C. acetobutylicum* ATCC 824 with genes and enzymes for reactions during acidogenesis and solventogenesis is shown in Fig. 1. Acids (e.g. acetate and butyrate) and carbon dioxide are produced as the main products at low pH during acidogenic phase. The produced acids are converted to solvents (acetone, butanol and ethanol) after solventogenic phase is triggered by a series of gene regulations [21,31].

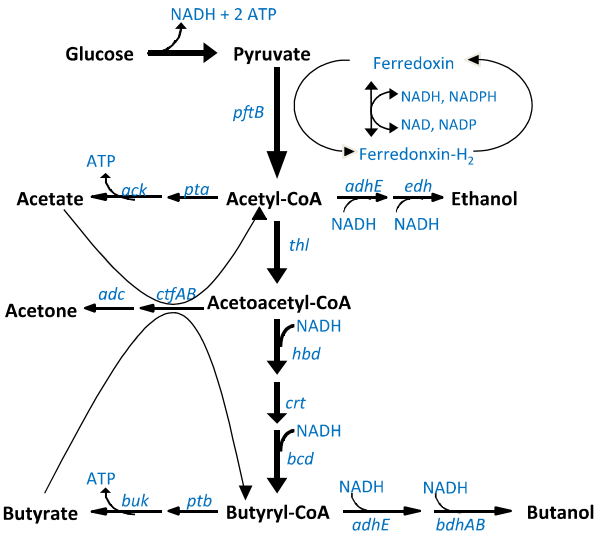

Figure 1. Metabolic pathway of *Clostridium acetobutylicum* ATCC 824 [21]. *pftB*: pyruvate-ferredxin oxidoreductase; *ack*: acetate kinase; *pta*: phosphotrans acetylase; *adhE*: aldehyde dehydrogenase; *edh*: ethanol dehydrogenase; *thl*: thiolase; *adc*: acetoacetate decarboxylase; *ctfAB*: CoA-transferase; *hbd*: 3-hydroxybutyryl-CoA dehydrogenase; *crt*: crotonase; *bcd*: butyryl-CoA dehydrogenase; *buk*: butyrate kinase; *ptb*: phosphotrans butyrylase; *bdh*: butanol dehydrogenase.

B. Strain Modification by Metabolic Engineering

As a cytotoxin, butanol can alter cell membrane structure, compromise membrane fluidity, and affect many membrane-bound transport activities [4,30], resulting in severe inhibition to fermentation [32]. Various cell engineering strategies, such as mutation, genetic engineering and metabolic engineering, have been developed to enhance the microbial butanol tolerance and thereby improve butanol production. The mutant SA-1 of *C. acetobutylicum* ATCC 824 and mutant BA101 of *C. beijerinckii* NCIMB 8052 are two representative mutants

that have been successfully developed using chemical mutagens [33,34,35]. SA-1 has been obtained through a serial culture transfer into medium containing increased amounts of butanol (mutagen), whereas BA101 has been developed using N-methyl-N9-nitro-N-nitrosoguanidine (mutagen) along with selective enrichment on glucose analog 2-deoxyglucose. The tolerance of butanol by *C. beijerinckii* BA101 mutant and *C. acetobutylicum* SA-1 mutant were significantly improved as compared to the parental strains [33,34].

Metabolic engineering is a powerful approach to generate novel strains with the advances in gene knockout, overexpression and synthetic biology. The sporulation transcription factor (*Spo0A*) has been identified as a positive regulator to enhance solvent production. Harris et al. reported that the inactivation of *Spo0A* resulted in 1.0 g/L butanol, whereas the overexpression of *Spo0A* led to a 10.2 g/L butanol in *C. acetobutylicum* ATCC 824 [36]. The overexpression of the solvent up-regulating genes in *C. acetobutylicum* ATCC 824, such as CoA-transferases (*ctfA* and *ctfB*), acetoacetate decarboxylase (*adc*) or aldehyde/alcohol dehydrogenase (*aad/adhE1*), has generated high butanol titer of 13.2 g/L. The production of byproducts (e.g., acetone, acetate and butyrate) has been decreased by overexpressing aldehyde/alcohol dehydrogenase (*aad*) and thiolase (*thl*) coupled with down-regulation of CoA transferase with antisense RNA in *C. acetobutylicum*. More recently, a metabolically engineered mutant of *C. tyrobutyricum* ATCC 25755 overexpressing aldehyde/alcohol dehydrogenase (*adhE2*) gene has produced >10 g/L of butanol from glucose and 16 g/L of butanol from manitol [37]. The knockout of multiple genes *via* homogenous integration, including butyrate kinase (*buk*), phosphotrans acetyrylase (*pta*), *adhE* or transcriptional activator protein (*solR*), has been applied to improve butanol production by down-regulating the metabolic pathways that generate byproducts [38]. The ratio of butanol to acetone (i.e. selectivity of butanol) has been enhanced by knocking out the *adc* gene and altering electron flow with the addition of methyl viologen [39]. However, the low efficiency of homogenous integration and the instability of mutation have hindered the application of gene knockout. The recently developed ClosTron, a Group II intron directed mutagenesis system, can address these issues in gene knockout. The ClosTron plasmid contains a mini-intron derivative and element facilitating plasmid conjugal transfer [40]. In addition to traditional gene knockout and overexpression, synthetic biology has been applied to create new strains by heterogeneously expressing n-butanol-producing pathway from *C. acetobutylicum* into *E. coli, P. putida* and *B. subtilis* [10,11,41,42]. The n-butanol titer of 14 g/L was produced from glucose by the recombinant *E. coli* with a ~80 % theoretical yield [43,44], but these mutants still suffer from low butanol tolerance and productivity.

III. SUBSTRATE AND FEEDSTOCK IN BUTANOL FERMENTATION

The cost of fermentation substrate is one of the important factors impacting the economic production of butanol. Traditionally, corn starch, molasses and glucose were the major substrates utilized in commercial ABE fermentation [1,20,45,46]. The concerns with sustainability and cost-effectiveness, research motives have been driven in the direction to search for low-value substrates for butanol production.

Clostridia can utilize a variety of carbohydrates as fermentation substrates, including glucose, xylose, arabinose, fructose, mannose, sucrose, lactose, cellobiose, starch, glycerol and dextrin, but not trehalose, rhamnose and melibiose [1,2,4,46]. This feature makes it feasible to produce butanol by Clostridia from low-cost substrates, for example, lignocellulosic biomass feedstocks. Lignocellulose is the largest reservoir of solar energy stored in the form of carbon source on earth, representing a large group of feedstocks suitable for many bioconversion processes. The composition and current use of some common lignocellulosic feedstocks is summarized in Table I. Lignocellulose includes a variety of agro-industrial residues (e.g., corn fiber and corn stover), energy crops (e.g. switchgrass), forestry products (e.g. wood chips), and municipal solid wastes [47,48,49,50]. Lignocellulose mainly consists of cellulose (35-50%), hemicellulose (25-35%) and lignin (10-25%), a small amount of protein, ash and some extractives [48,51]. Most of these feedstocks are currently used as animal feed, soil conditioner and burnt as fuel.

The cellulose and hemicellulose present in the lignocellulosic feedstocks can't be directly consumed by solventogenic Clostridia because the enzymes breaking down the biomass are not available in most high butanol producing Clostridia. To utilize lignocellulosic biomass in fermentation process, all the sugars stored in the form of hemicellulose and cellulose need be released using an efficient pretreatment and hydrolysis approach. Due to the lignin protection and crystalline cellulose microfibrils, lignocellulosic materials are usually resistant to enzymatic hydrolysis, so additional pre-treatment becomes critical [47,51,52]. To extract fermentable sugars from lignocellulosic biomass, the most commonly used pretreatment method is dilute acid. Enzymatic hydrolysis can maximize the sugar yield in the hydrolysate for the subsequent fermentation step. Various hydrolysis studies have been developed to convert lignocellulose to value-added products, such as enzymes, biofuels and chemicals [53,54,55,56,57]. Several lignocellulosic materials, such as corn fiber, wheat straw and switchgrass, have been successfully applied in ABE fermentation, which produced high levels of butanol at concentration of 19-26 g/L [24,58,59].

It has been estimated that the net energy generated from corn-to-butanol is 6.53 MJ/L, which could be significantly improved to 15.90 MJ/L if lignocellulosic biomass is used [15]. Therefore, it is of great interest to study the biobutanol production using domestically produced lignocellulosic feedstocks as potential substrates. Assuming that the butanol yield of 0.42 g/g is produced from ABE fermentation and the current crop harvest

yield is achieved, it is expected that 8.27 billion gallons of biobutanol can be annually produced from the bioconversion of renewable and sustainable lignocellulosic biomass, such as corn stover and switchgrass. This biobutanol production capability can replace the current gasoline production of 7.55 billion gallons per year [15].

TABLE I. COMPOSITIONS OF DIFFERENT LIGNOCELLULOSIC BIOMASS AND THEIR CURRENT USE

	Composition (%, dry basis)				Current use	Ref.
	Cellulose	**Hemicellulose**	**Lignin**	**Starch**		
Corn fiber	15	23- 64	8	12-32	Animal feed, burnt as fuel, compost, soil conditioner	[2,47,48,49,50,59,60,61,62,63]
Corn cob	45	35	15	---		
Corn stover	38- 40	25- 28	7- 21	---		
Rice straw	28- 36	23- 28	12- 14	---		
Wheat straw	35- 40	20- 30	17- 19	---		
Fresh bagasse	33.4	30	18.0	---	Burnt as fuel, landfill	
Sugarcane bagasse	40- 50	24- 25	25	---		
Grass	25- 40	25- 50	10- 30	---	Burnt	
Hardwood stems	40-55	24-40	18-25	---	Soil conditioner, burnt	
Softwood stems	45-50	25-35	25-35	---		
Newspapers	40- 55	25- 40	18-30	---	Partially recycled	
Waste papers from chemical pulps	60-70	10-20	5-10	---		

IV. FERMENTATION PROCESS DEVELOPMENT

Conventional ABE fermentation had been operated with free cells in the batch mode, but free-cell batch fermentation suffers from low cell density and low reactor productivity due to end product toxicity [30,45,46,64]. As a result, the butanol yield in traditional ABE fermentation is low, typically around 0.20-0.25 g/g, and cell density is about 3-4 g/L. Due to the low cell density and severe product inhibition, the reactor productivity is usually around 0.25-0.4 g/L·h and up to 0.5-0.6 g/L·h. Therefore, advanced fermentation processes have been developed to increase butanol production and cell growth.

A. Immobilized-Cell Fermentation

In immobilized-cell fermentation, microorganism cells are fixed on a support material through adsorption or entrapment. Cell immobilization by adsorption allows cell renewal, which can maintain a highly viable cell density in the bioreactor. Multiple materials such as sponge, brick, fibrous matrix, cotton towel, and corn stalk have been applied as potential support materials for cell immobilization. Among all the materials, cotton towel is the most commonly available and inexpensive material. Yang has designed a novel spiral wound cell immobilization system (i.e. fibrous-bed bioreactor, FBB) that contains stainless steel mash and cotton towel to offer large contact surface area and good mass transfer [65]. The spaces between fibrous matrices in FBB provide large void volume to allow the fermentation gases and particles to easily pass through the bioreactor, which can avoid pressure build-up and reduce the clogging problems. In FBB, constant cell renewal is achieved by reversible adsorption that can maintain high viable cell density. Enhanced reactor productivity and final product concentration have been reported in several processes employing this cell immobilization system [66,67]. Moreover, the increased productivity in immobilized-cell fermentation offers harsh environment for cell mutation and evolution over an extended period of time [67,68].

To further improve cell retaining efficiency, cell recycle technology using membrane has been integrated with cell immobilization. This integrated technology can recycle cells and prevent cell loss happened in the immobilized-cell bioreactors. The increased cell density per reactor volume can significantly improve bioreactor productivity and eliminate reactor downtime. For example, reactor productivity as high as 12.43 g/L·h and average productivity of 4.0-6.0 g/L·h have been achieved using brick as support material in the integrated immobilization-recycle system. Lu et al. has developed a novel fed-batch FBB fermentation using cotton towel coupled with butanol stripping and cell recycle, which has produced 108.5 g/L of biobutanol from ABE fermentation [69]. The highest butanol yield of 0.44 g/g was obtained in FBB fermentation [70].

B. Fed-Batch, Continuous and Simultaneous Saccharification Fermentations

Fed-batch fermentation is a popular fermentation technology by adding highly concentrated substrates into bioreactor at intervals to maintain a desirable substrate concentration to avoid substrate inhibition [1,30]. Fed-batch fermentation usually starts with a certain level of substrate and a small volume of highly concentrated substrate is fed into bioreactor to replace the consumed substrate. Fed-batch technology can significantly improve the volumetric productivity and thereby reduce the reactor volume, lowering the capital cost and thus improving the process efficiency [4,21,45,60]. However, the accumulation of end product can inhibit cell growth and further production improvement. This issue can be addressed by the incorporation of online product recovery. It has been reported that 500 g/L glucose was utilized in fed-batch fermentation coupled with gas stripping for product recovery, resulting in ABE titer of 232.8 g/L and productivity of 1.16 g/L·h [1,71].

Another effective butanol production process is continuous fermentation. Fresh medium is continuously fed into reactor at the same rate of product stream flowing out the reactor, keeping a constant volume in the reactor [1]. Due to the dilution by fresh medium, end product inhibition is prevented, and dead cells and toxic metabolites are removed from continuous fermentation, leading to a longer fermentation life. Continuous fermentation can be operated with free cells, or operated with cell immobilization or cell recycle in order to achieve higher cell density. Continuous ABE fermentation can achieve high solvent productivity, but at the expense of lower product concentration due to dilution. A productivity of 12.4 g/L·h has been reported in a continuous immobilized-cell fermentation, with a maximum ABE concentration of 8.8 g/L [72]. In addition, continuous butanol fermentation can eliminate the downtime of bioproduction operation and simplify the downstream process, which can lower the process cost and increase biobutanol production efficiency [73].

Simultaneous saccharification and fermentation (SSF) has been recently proposed as another feasible technology for ABE fermentation [46,74]. The traditional separate hydrolysis and fermentation (SHF) process has been employed to produce butanol using lignocellulosic biomass as substrate. In SHF, the hydrolysis process and fermentation process can be operated under the optimal conditions for both enzymatic hydrolysis (e.g. pH 5.0 and Temp 50^{o}C) and butanol production (e.g. Temp 30-37^{o}C) [75]. However, the end products of the hydrolysis (i.e. sugars) inhibit the hydrolysis enzyme activity and lower its efficiency. SSF can solve this problem by integrating the enzyme hydrolysis of lignocellulose and butanol production in the same bioreactor at a compromised condition, i.e. pH 5.0 and 37^{o}C. Specifically, enzyme inhibition by sugars is relieved due to the simultaneous utilization of the released sugars by the microorganism. SSF is commonly used in ethanol fermentation from lignocellulosic biomass, which can reduce process energy requirement and improve enzyme efficiency and ethanol production [75]. With wheat straw as substrate, 13.12 g/L of ABE was produced from SHF by *C. beijerinckii* P260, whereas similar ABE titer of 11.93 g/L was obtained from SSF [46]. This study shows that SSF is capable to achieve high ABE fermentation from lignocellulosic biomass.

V. PRODUCT RECOVERY AND SEPARATION

Butanol recovery is the most energy-intensive and expensive operation in the whole biobutanol production because of the relatively low butanol concentration in fermentation broth. Developing an energy-efficient butanol recovery and separation strategy is the key to low-cost butanol production development. Over the years, some integrated techniques have been developed to directly recover solvents from fermentation broth, including gas stripping, pervaporation, liquid-liquid extraction, and adsorption [1,45,60]. A comparison of energy input for ABE separation using these recovery technologies have been reported by Qureshi et al., and adsorption was projected to be the most energy-efficient, followed by liquid-liquid extraction, pervaporation, and gas stripping [76]. The schematic designs of these four butanol recovery processes are summarized in Fig. 2. The detailed technologies of these recovery methods are discussed below.

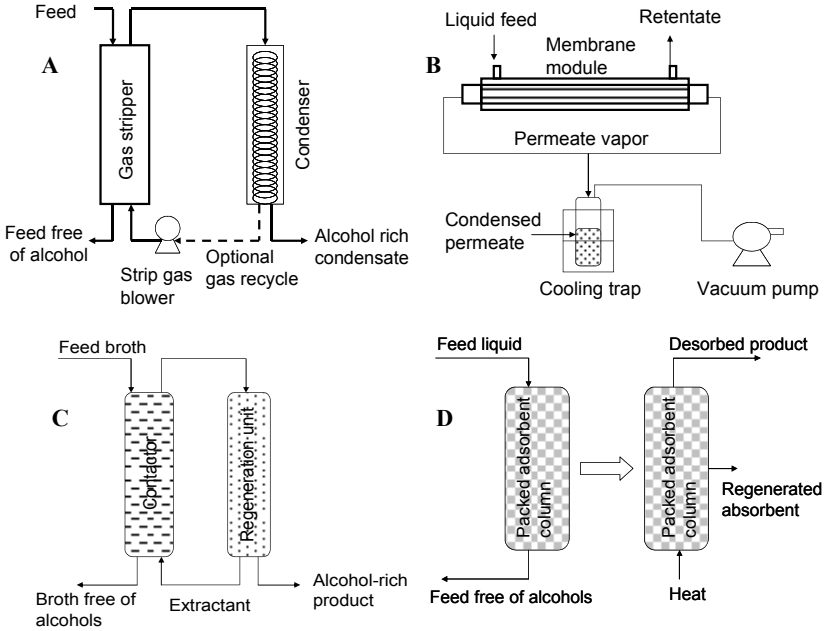

Figure 2. Schematic diagram of biobutanol recovery processes: (A) gas stripping [1], (B) pervaporation [80], (C) liquid-liquid extraction [61], and (D) adsorption [77].

A. Gas Stripping

Fig. 2A depicts the schematic diagram of a typical gas stripping process. Gas stripping is an easy-to-operate technique to recover butanol from fermentation broth, which can be either integrated with fermentation in the bioreactor or performed in an individual stripping column. Nitrogen, hydrogen and carbon dioxide are used as stripping gases and also maintain the anaerobic condition [1]. In the integrated fermentation-butanol recovery scenario, the volatile butanol and other solvents in fermentor broth are captured by the introduced stripping gas, which is subsequently passed through a condenser to condense and enrich butanol in the condensate stream. In the scenario of using a separate gas stripper, the butanol feed stream (i.e. fermentation broth) enters into the stripper where the butanol is captured by stripping gas, and the fermentation broth is recycled and feed back to the fermentor. The stripping gas flow can be operated either in single-pass mode where the used gas is released into open air, or in recycle mode where the stripping gas is recycled back to bioreactor post condense and assist further butanol capture. As a product recovery technology, gas stripping offers many advantages when it is integrated with fermentation, such as feasibility to recycle fermentation gases as stripping gas and ability to operate under fermentation temperature [77]. The gas stripping only removes solvents, so it is highly selective towards butanol over acetone. The presence of cells in fermentation broth decreases butanol removal efficiency [78], but the integrated gas stripping neither affects cell growth nor causes the loss of any nutrients from the fermentor [79].

B. Pervaporation

As the schematic diagram in Fig. 2B shown, pervaporation is a membrane-based separation technique, where liquid feed containing volatile species flows on one side of the membrane while the other side of the membrane is under vacuum. Components in the liquid feed that have similar properties to the selective membrane material can diffuse through the membrane and enrich in the permeate side through cold trap. When the selected components diffuse to and enrich in the permeate side, their concentration is reduced in the liquid feed, so the retentate leaving the module has low concentration of the selected components [80,81]. The concentration gradient between feed and permeate is the driving force for a component to transport across the membrane, and the transport flux is inversely proportional to the overall resistance and proportional to the concentration gradient [82]. In the case of butanol separation from water by pervaporation, a hydrophobic membrane is used to get butanol-rich condensate.

Currently, the poly(dimethyl siloxane) membrane (i.e. PDMS or silicone rubber membrane) is the benchmark of hydrophobic membrane commonly used in alcohol/water separation by pervaporation [77,80]. PDMS membrane enables a separation factor of 4.4-10.8 for ethanol/water system, and 40-60 for butanol/water separation [80]. The Poly [1-(trimethylsilyl)-1-propyne] (i.e. PTMSP) is another excellent membrane providing high alcohol/water separation factor as high as 70 [83]. The disadvantage of PTMSP is that its flux and selectivity gradually decrease over pervaporation time [84,85]. Inorganic zeolite materials (e.g. silicalite and Ge-ZSM-5) have also been used as hydrophobic membranes in pervaporation process [86] to fabricate a mixed matrix PDMS membrane, which resulted in a high butanol/water separation factor of up to 209 in ABE fermentation [35,87,88,89]. In addition, liquid membrane (e.g. oleyl alcohol) has been evaluated in pervaporation process and achieved a high butanol/water separation factor of 180, but the liquid leaks into the fermentation broth over time and the liquid membrane has to be regenerated [81,90]. Pervaporation is an emerging membrane-based product recovery technology that could efficiently recover alcohol from the aqueous fermentation broth.

C. Liquid-Liquid Extraction

Liquid-liquid extraction is an alternative butanol separation technique (Fig. 2C) that could be integrated with fermentation. The extractant liquid contacts with fermentation broth via mixing or membrane and directly extracts butanol due to the solubility difference of butanol between extractant and fermentation broth [1,77]. Enriched with alcohols, the extractant is regenerated by recovering alcohols using distillation, vacuum evaporation, and pervaporation. As Madox and Van discussed before [64,77], a solvent can work as a suitable extractant to recover butanol via liquid-liquid extraction when it meets the following requirements: 1) high selectivity of alcohol to water (separation factor), 2) high distribution coefficient to reduce the volume of extractant, 3) immiscible, non-emulsifying, clear phase separation from aqueous solution, 4) non-toxic to microorganisms, non-reactive with fermentation components, and non-flammable to ensure operation safety, and 5) inexpensive to use and easily available. The most commonly studied traditional extractants are usually long-chain alcohols, alkanes, esters, fatty acids and oils, for example, poly(propylene glycol) (PPG) 1200, PPG 2000, oleyl alcohol, isophytol, eutanol G and triethyl citrate. High butanol productivity (19.3-63.0 g/L) has been achieved using oleyl alcohol in extractive fed-batch ABE fermentation. In addition, some novel materials such as ionic liquid (IL) or biodiesel have also been evaluated as potential extractant for butanol recovery via liquid-liquid extraction because of their thermally and chemically stable properties [91,92].

D. Adsorption

Adsorption is another efficient separation process for butanol recovery (Fig. 2D). In adsorption process, butanol is adsorbed by the packed adsorbent materials in column and then desorbed to obtain a concentrated butanol solution during the regeneration cycle [77]. The high separation factor and distribution coefficient are two key parameters to select proper adsorbent materials. The most commonly used adsorbent materials for alcohol

recovery have been evaluated, such as hydrophobic zeolites, especially silicalite-1 [76,93]. Oudshoorn et al. have reported that the zeolite with the lowest SiO_2/Al_2O_3 ratio has the highest capacity for butanol adsorption (i.e. high distribution coefficient) [93]. Silicalite shows the most appealing adsorbent performance, e.g. concentrating butanol to 810 g/L from a 5 g/L dilute feed solution with a complete butanol recovery [76]. The major concern for for applying adsorption technology to recover butanol is the adsorbent fouling by cells and adsorption of nutrients and acid products, which could be addressed by the membrane-assisted cell recycle system [77,94,95].

VI. INTEGRATED BUTANOL FERMENTATION AND ONLINE PRODUCT RECOVERY

The advances in metabolic engineering or synthetic biology have generated various new microorganism strains that have the capability to produce high-level biobutanol. The fermentation process development has significantly improved butanol yield and productivity. The development of biomass hydrolysis technology enables the low cost of butanol fermentation. However, the toxicity of butanol (i.e. end product inhibition) inhibits cell growth and thereby results in low concentration, low yield and low productivity of butanol fermentation. In addition, the post-fermentation butanol recovery is an energy consuming process. All these challenges have hampered the economic butanol fermentation. Therefore, the development of an efficient fermentation process is desired, which can be achieved by integrating online butanol recovery with ABE fermentation using low-cost lignocellulosic feedstocks as substrate. The new advances in butanol recovery techniques such as liquid-liquid extraction, pervaporation and gas stripping have been integrated with butanol fermentation in an effort to develop a commercial biobutanol production process [78,80,90]. These integrated fermentation processes significantly improved sugar consumption efficiency and butanol concentration and productivity from the feedstocks such as whey permeate, wheat straw, corn fiber, cassava bagasse, wood chips, etc.

As summarized in Table II, high ABE production by various Clostridia strains has been obtained from the integrated fermentation process, with titer of 10-380 g/L, yield of 0.26-0.44 g/g and productivity of 0.14-2.3 g/L·h. Some integrated ABE production processes even exhibit the potential to commercialize in biofuel industry. For example, Yang lab has generated a high butanol producing mutant, *C. acetobutylicum* JB 200, which consumes ~80 g/L glucose to produce up to 22 g/L butanol in batch fermentation without online butanol recovery [96]. This super butanol-producing mutant strain utilizes 600 g/L glucose and produces a total of 172.1 g/L ABE with yield of 0.36 g/g and productivity of 0.53 g/L·h from fed-batch fermentation coupled with intermittent gas stripping. Over 8-fold increase in solvent production has been achieved in the integrated process comparing to batch operation. In addition, the bioreactor volume and water usage was significantly reduced in their process, consuming only 3.6 L of water per kg of ABE produced without any water recycle. Another successful case study performed by Ezeji et al. has showed that an integrated continuous fermentation with gas stripping utilizes 1163 g/L glucose and produced a total of 460.0 g/L ABE [73]. Qureshi and Blaschek have reported a total ABE production of 165 g/L from whey permeate in a pervaporation-integrated fed-batch process [99]. In addition, Yang lab has developed a two-stage gas stripping process, where the butanol saturated aqueous phase (butanol concentration of 101 g/L) from the first stage was further enriched by the second stage gas stripping, resulting in a highly concentrated stream containing ~420 g/L butanol. This process would reduce the energy requirement in the following distillation process to less than 5 MJ/kg of butanol [100]. All these studies illustrate that the integrated ABE fermentation with simultaneous product recovery is a promising butanol production strategy to achieve high titer and productivity. The butanol inhibition issue can also be addressed by timely removing butanol from fermentation broth so that the butanol in fermentor never reaches the inhibitory level. The enriched solvent in the recovered stream can also significantly cut down the purification and separation cost.

TABLE II. INTEGRATED ABE FERMENTATION PROCESSES

Integrated Production	Substrate	Strain	Ferment. Mode	Titer (g/L)	Yield (g/g)	Produc. (g/L· h)	Ref.
ABE Ferment./ Gas stripping	Whey permeate	*C. acetobutylicum* P262	Batch	70.0	0.35	0.32	[97]
		C. acetobutylicum P262	Continuous	69.1	0.38	0.26	[98]
	Wheat straw	*C. beijerinckii* P260	Batch	47.6	0.37	0.36	[59]
	Wood pulp	*C. beijerinckii* CC 101	Batch	17.7	0.44	0.25	[70]
	Cassava bagasse	*C. acetobutylicum* JB 200	Fed-batch	108.5	0.32	0.41	[69]
	Glucose	*C. acetobutylicum* JB 200	Fed-batch	172.1	0.36	0.53	[96]
ABE Ferment./ Pervaporation	Whey permeate	*C. acetobutylicum* P262	Continuous	42.0	0.34	0.14	[98]
	Glucose	*C. beijerinckii* BA101	Fed-batch	165.0	0.43	0.98	[99]
		C. acetobutylicum ATCC 824	Fed-batch	155.0	0.35	0.18	[35]
ABE Ferment./ Liquid-liquid extraction	Whey permeate	*C. acetobutylicum* P262	Continuous	23.8	0.35	0.14	[98]
	Glucose	*C. acetobutylicum* ATCC 824	Fed-batch	50.5–96.5	0.33–0.36	1.4–2.3	[101]
ABE Ferment./ Adsorption	Whey permeate	*C. acetobutylicum*	Fed-batch	59.8	0.32	1.33	[95]
			Repeated fed-batch	387.3	0.32	1.69	

VII. SUMMARY AND PERSPECTIVE

The development of high-productivity and low-cost butanol production process can be achieved via strain construction, feedstock application, fed-batch and continuous fermentation development, product recovery development and integrated biobutanol production development. Among these advanced technologies, the in-situ recovery of butanol is crucial for improving butanol production performance because simultaneous butanol recovery can relieve the product inhibition and lead to an effective carbon metabolism. In addition, it allows the usage of a concentrated feed and extends the fermentation period. Moreover, online butanol recovery also simplifies the downstream separation process, which lowers the energy consumption and brings down the whole process cost. Taken together all the effective achievements in biobutanol production can benefit the commercialization of biobutanol production, which can replace the petroleum-based butanol production.

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