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Recent advances on biobutanol production

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Abstract

Recent studies have shown that butanol is a potential gasoline replacement that can also be blended in significant quantities with conventional diesel fuel. However, biotechnological production of butanol has some challenges such as low butanol titer, high cost feedstocks and product inhibition. The present work reviewed the technical and economic feasibility of the main technologies available to produce biobutanol. The latest studies integrating continuous fermentation processes with efficient product recovery and the use of mathematical models as tools for process scale-up, optimization and control are presented.

Keywords: Biobutanol, Engineered microorganism, Separation, Fermentation

Introduction

During the last decade the interest in the production of chemicals and fuels from renewable resources has increased. Reasons for this trend include growing concerns about global warming and climatic change, volatility of oil supply, increasing price of crude oil and legislation restricting the use of nonrenewable energy sources. Furthermore, the generation of biofuels may improve the local employment opportunities and contribute to the reduction of CO_2 emissions [1-3]. Among the alternative fuels, biobutanol has shown promise as its properties are similar to gasoline [4] and, in comparison with ethanol, it has a longer carbon chain length as well as higher volatility, polarity, combustion value, octane rating [5] and is less corrosive [6]. It can also be a substitute for gasoline without alteration in current vehicle or engine technologies [7]. In addition, it has less ignition problems since the heat of vaporization of butanol is less than half of that of ethanol, hence an engine running on butanol should be easier to start in cold weather than the one running on ethanol or methanol [8].

Commercial butanol fermentation processes have been developed by some companies [2]. There is an expectation that the number of companies devoted to biobutanol production will increase worldwide as well as the development of new technologies to increase the yield [9]. A difficulty in butanol fermentation is the inhibition caused by the product as butanol concentrations around 20 g/L inhibit microbial growth [5]. In addition, the clostridium species are strictly anaerobes [10] and the anaerobic conditions need to be established before the beginning of the fermentation and the reactor must be remain closed during the process [11].

The cost of the plant for butanol production depends on the price of the feedstock and is extremely sensitive to any price fluctuation [12]. Thereby, the commodity price is still very dependent of feedstock price and an expensive raw material generates an expensive product. Agricultural residues and wastes are demonstrated to be cheaper than other sources [13]. However, the hydrolysis of these materials can generate fermentation inhibitors, which is another problem to be solved [14].

Another important point in butanol production is the separation techniques and their application, mainly for in situ continuous recovery [15]. Distillation is the unit operation widely used in separation of aqueous solution from butanol fermentation. However, the problem in this process is the formation of an azeotrope that increases the energy cost [16]. Alternative methods are reported with the objective to promote a cheaper and efficient separation. More recently, mathematical models have been developed to design the process as well as to simulate its behavior on an industrial scale without the need to carry out experiments to optimize the operational conditions of the reactor [17-19].

Although there are excellent reviews available in the literature concerning butanol production [12,20-29], the present work is focused on the presentation of the technical and economic feasibility of the main technologies

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that have been developed to produce biobutanol, complementing the existing literature about the topic. For this purpose, the latest studies reporting the microorganism used in butyric fermentation, as well as the integration of continuous fermentation processes with efficient product recovery and the use of mathematical models as tools for process, optimization and control are reviewed.

Microorganism

According to Liu et al. [30], the most common microbial strains employed for butanol fermentation are the mesophiles *Clostridium acetobutylicum* and *Clostridium beijerinckii,* where *Clostridium acetobutylicum* is the most reported in acetone–butanol–ethanol (ABE) fermentation, which are the major products obtained in the process [7]. Furthermore, *Clostridium acetobutylicum* was the first bacterium used for ABE fermentation [2]. However, other *clostridium* sp. have also been reported, for example; *C. pasteurianum* [31], *C. sporogenes* [32], *C. saccharoperbutylacetonicum* [33] and *C. saccharobutylicum* [26]. The main strains used for biobutanol production are reported in Table 1.

ABE fermentation is one of the oldest known industrial fermentations with a history of more than 100 years [4]. However, this fermentation is not widely used as butanol is highly toxic to microorganisms and, for this reason, less than 13 g/L of butanol are produced during batch fermentation. In general, fermentation using Clostridia sp. results in the ABE production of around 15–25 g/L with a yield of 0.25–0.4 g ABE/g sugar [2]. Substrate inhibition has not been the major concern in ABE fermentation when glucose is used as a carbon source [53]. However, Chen et al. [54] reported inhibition of butanol production at high substrate concentrations. Ezeji et al. [55] also reported that *Clostridium* sp. showed a catabolic inhibition for sugar concentration higher than 162 g/L.

The pH of the fermentation broth, initially at 6.8–7.0, drops to 4.5–5.0 during the acidogenic phase. This phase is associated with the fast growth of cells and the secretion of the carboxylic acids, acetate, and butyrate [4]. According to Napoli et al. [34], the pH varies between 4.0 - 5.0 for butanol production. Li et al. [53] verified that pH 4.3, maintained constant during fermentation, was optimal for butanol production using *C. acetobutylicum*. Similar results were also reported previously by Bahl et al. [56]. On the other hand, Qureshi [14] reported that the pH is self controlled at approximately 5.2 ± 0.2 during the solventogenic stage of *C. beijerinckii*. These different ranges of pH are due to different clostridium species used in the process.

The metabolism of Clostridia strains has two distinct phases, acidogenesis and solventogenesis. The acidogenesis is characterized by substrate conversion into acids (acetic and butyric acids) and exponential cell growth with ATP formation. This is a fundamental step of fermentation, without which the number of viable cell would be greatly reduced, making the normal solvents production difficult. The solventogenesis phase is characterized by conversion of substrate and acids into solvents (ABE) [23,34]. Solventogenic clostridia can utilize a wide range of carbon sources, such as starch, sucrose, glucose, fructose, galactose, cellobiose, xylose, arabinose, glycerol, and syngas as fermentation substrates for the ABE production.

According to Jang et al. [57], it is very important to have a better understanding of the genes that are the basis of microbial metabolism for the production of butanol, since it will be possible to obtain modified strains able to improve biomass conversion [4], increase oxygen tolerance, increase the cell density, prolong cell viability, direct the utilization of cellulosics and provide high solvent tolerance and high butanol selectivity [58]. Genetic modification of *Clostridium* is widely used, by inserting some heterogenetic genes or over expressing or knocking out/down some relative endogenous genes, to improve butanol production. Some researchers are working with genetic tools which are being used to manipulate their metabolism by introducing the genes that are responsible for butanol production from *Clostridium acetobutylicum* into *E. coli* and yeast (commonly *Saccharomyces cerevisiae*) [24]. This manipulation can enable the microbial strain to increase the production of butanol in the medium, without inhibition by products. The details of genetic engineering for butanol production can be observed in these papers e.g. [59,60].

According to Lütke-Eversloh and Bahl [61], the modifications in the strains of the genus *Clostridium* can be achieved in the following ways: disruption of the pathway that synthesizes the unwanted products or changing the pathway for formation of acetate and butyrate (acidogenic stage). The disruption of the pathway for acetone production increased the butanol production from 71% to 80% [62]. Conversely Isar and Rangaswamy [50] reported an increase in the tolerance of solvents from 18 g/L to 25 g/L using *Clostridium beijerinckii* adapted to solvent. It indicated that the strain had adapted to butanol and become solvent tolerant in the absence of any mutation.

In the study of Abd-Alla and El-Enany [41], the authors described an alternative method to maintain the anaerobic medium during the clostridial fermentation. The culture of *Bacillus subtilis* DSM 4451 was used to maintain strict anaerobic conditions for *C. acetobutylicum* ATCC 824. Thus, fermentation does not need N_2 flushing to remove the oxygen so the costs decreased. The highest butanol production obtained was 21.7 g/L (this is similar to that reported by clostridium production) just using the consortium of microorganism to maintain

Table 1 Microorganism, substrate, yield/production and main aspects in the butanol production reported

Microorganism	Substrate	Yield/Production	Technology	Reference
C. acetobutylicum (immobilized)	Cheese whey (lactose)	Yield: 15% to 0.54 h^{-1} of dilution and 28% to 0.97 h^{-1} of dilution	Reactor (PBR) with immobilized clostridium	[34]
C. beijeirinckii ATCC 55025	Hydrolysate of wheat bran	Yield: 32%/Production: 8.8 $g.l^{-1}$ of biobutanol	Acid hydrolysis	[30]
C. beijerinckii	Cassava flour	Production: 23.98 $g.l^{-1}$ of butanol	Enzymatic treatment with yield of 9.12% to Reducing sugar	[35]
C. beijerinckii P260	Wheat straw	Yield: 42%	Acid pretreatment and enzymatic hydrolysis	[14]
	Barley straw	Yield: 43%/Production: 26,64 $g.l^{-1}$ of total solvents	Dilute sulfuric acid hydrolysis/overliming	[36]
	Corn stover	Yield: 43%/Production: 18.04 $g.l^{-1}$ of total solvents	Acid and enzymatic steps of hydrolysis/overliming	[37]
	Switchgrass	Yield: 37%/Production: 8.91 $g.l^{-1}$ of total solvents		
	Glucose	Production: 17.54 $g.l^{-1}$ of butanol	Intermittent vacuum application	[10]
C. saccharobutylicum DSM 13864	Sago starch	Yield: 29%	Free microorganism fermentation	[26]
C. acetobutylicum	Cassava bagasse	Yield: 32%/Production: 76.4 $g.l^{-1}$ of butanol	Hydrolyze by enzymes fibrous bed bioreactor/ Gas stripping	[38]
	Palm empty fruit bunches	Production: 1.262 $g.l^{-1}$ of butanol	Acid pretreatment/ enzymatic hydrolysis	[39]
C. beijerinckii BA101	Liquefied corn starch	Butanol production: 81, 3 $g.l^{-1}$ (with gas stripping)/18.6 $g.l^{-1}$ (without gas stripping)	Bath reactor/gas stripping/ enzymatic hydrolysis	[40]
C. acetobutylicum ATCC 824 and Bacillus subtilis DSM 4451	Spoilage date palm fruits	Yield: 42%/production: 21.56 $g.l^{-1}$ of Solvents	Bacterial consortium (anaerobic conditions)	[41]
C. beijerinckii NCIMB 8052	Tropical maize stalk juice	Production: 0.27 g-butanol/g-sugar	Optimization of pH, agitation, sugar concentration	[42]
C. acetobutylicum ATCC824	Sugar maple Hemicellulosic material	Production: 7 $g.l^{-1}$ of butanol	Alkali pretreatment/acid hydrolysis/overliming	[43]
C. saccharoperbutylacetonicum N1-4	Rice bran	Yield: 57% to sugar generated.	Acid hydrolysis	[44]
	De-oiled rice bran	Yield: 44% to sugar generated.	Acid pretreatment/ enzymatic Hydrolysis	
C. acetobutylicum XY16	Glucose	Production: 20.3 $g.l^{-1}$ of butanol	pH steps in the fermentation	[45]
C. sporogenes BE01	rice straw	Production of 3.49 g/L and 5.32 g/L of butanol and total solvents respectively	Acid pretreatment/enzymatic Hydrolysis/Overliming	[32]
C. saccharoperbutylacetonicum N1-4	rice straw	Maximum butanol production of 6.6 g/L and butanol yield 0.2 g/g of total sugar.	Absence of pretreatment/ enzymatic hydrolysis/ Non-sterile conditions	[33]
C. pasteurianum	Glycerol	Maximum butanol production of 8.8 g/L and butanol yield 0.35 g/g of glycerol at initial substrate concentration of 25 g/L.	Immobilized cells/Bath fermentation	[31]
C. acetobutylicum NCIM 2337	Rice straw	Butanol production of 13.5 g/L and butanol yield 0.34 g/g of total sugar generated.	Acid treatment with shear stress	[46]
C. acetobutylicum MTCC 481	Rice straw	Butanol production of 1.72 g/L.	Steam explosion	[47]
		Butanol production of 1.6 g/L	Acid treatment	
		Butanol production of 2.1 g/L	Acid pre-treatment/ enzymatic hydrolysis	
C beijerinckii NCIMB 8052	Corncob	Butanol production of 8.2 g/L	Alkali pre-treatment/ enzymatic hydrolysis/ overliming	[48]

Table 1 Microorganism, substrate, yield/production and main aspects in the butanol production reported *(Continued)*

C. acetobutylicum JB200	Glucose	Yield: 21%/Production: 172 $g.l^{-1}$ of solvents	Gas stripping	[49]
C. beijerinckii ATCC 10132	Glucose	Production: 20 $g.l^{-1}$ of butanol	Bath reactor	[50]
C. acetobutylicum CICC 8008	Corn straw	Production: 6.20 $g.l^{-1}$ of butanol	Enzymatic hydrolysis/ bath reactor	[51]
C. acetobutylicum P262	Whey permeate medium	Yield: 44%/Production: 98.97 $g.l^{-1}$ of solvents	Perstraction/bath reactor	[52]

the anaerobic medium. The main objective of using the microorganism in the butanol fermentation is to attempt to create an engineered microbe that can overcome the limitations of *Clostridium*. The increase of solvent tolerance, butanol titer, and tolerance towards traces of oxygen are the most desirable characteristics in the engineered microorganism for it to be viable for industrial process application.

Substrates

The prices of substrates for biobutanol production influence the economic competition with the petrochemical industry [34]. The cost of feedstock represents over 70% of the total production costs of biobutanol [63]. At the beginning of butanol fermentation, substrates based on sugars and starch were used, but these are expensive and the process becomes unfeasible. One of the strategies to decrease the production cost is to use cheap and renewable feedstocks, such as lignocellulosic materials (e.g. agricultural waste, paper waste, wood chips), which are abundant. The production of alcohol using lignocelluloses follows an integrated process involving basically three steps: pre-treatment, hydrolysis and fermentation [7]. The main substrates used for biobutanol production are reported in Table 1.

The molasses are used for biobutanol production. However, these kinds of substrate are more expensive than agricultural residues. On the other hand, molasses can be used directly in the fermentation. Thus, it is not possible to assert that the cellulosic residues will be cheaper than molasses. Van der Merwe et al. [64] reported analyses of the energy efficiency and economics of biobutanol production using sugarcane molasses. Another important point to be analyzed related to the choice of substrate and its availability throughout the year. The major sources of this kind of raw material are agricultural residues and wastes, such as rice straw, wheat straw, wood (hardwood), byproducts left over from the corn milling process (corn fiber), annual and perennial crops, waste paper [14] and sweet sorghum [65]. These raw materials consist of three types of polymers: cellulose, hemicellulose, and lignin. Cellulose has strong physical-chemical interaction with hemicelluloses and lignin. Cellulose, a linear glucose polymer (that is broken in the hydrolysis), is a highly ordered polymer formed of glucose representing about 50% of the wood mass. Hemicellulose is a short, highly branched heteropolymer formed mainly of xylose, plus glucose, mannose, galactose and arabinose and sometimes uronic acids. Lignin consists of phenylpropanoid units derived from the corresponding p-hydroxycinnapyl alcohols. Lignin is hydrophobic and highly resistant to chemical and biological degradation [66]. *Clostridium beijerinckiii* is being explored as a promising strain to produce biobutanol from cellulosic materials [26].

The problems in the use of cellulosic or lignocellulosic materials for butanol production are the processes for production of these hydrolysates, resulting in the generation of chemical byproducts that inhibit cell growth and fermentation. Such inhibitors include salts, furfural, hydroxymethyl furfural, acetic, ferulic, glucuronic, r-coumaric acids, and phenolic compounds. Lignocellulosic materials are difficult to hydrolyse biologically [39]. Furthermore, the hydrolytic process can generate significant amounts of waste and hence increase the cost of the butanol produced [36]. Moreover, with the fermentation of any of these substrates (mainly cellulosic and starchy after a hydrolysis treatment) there is the need for nutritional supplementation. Lee et al. [67] reported the use of KH_2PO_4, K_2HPO_4, ammonium acetate, para-aminobenzoic acid, thiamin, biotin, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $FeSO_4 \cdot 7H_2O$, NaCl, and yeast extract as supplements for biobutanol production.

The pretreatment for starchy and cellulosic materials is a limiting step and needs to be optimized for a satisfactory production of butanol. Liu et al. [30] pretreated wheat bran using sulfuric acid at high temperature followed by neutralization with Ca $(OH)_2$ for biobutanol production by *C. beijerinckii 55025*. This procedure increased the cost of the butanol produced, but this cost can be considerably decreased if a large amount of a cheap source of raw material is used. Lépiz-Aguilar [35] used HCl 1 M combined with high temperature for 2 h or enzymatic hydrolysis (using α-amylase and β-glucoamylase) to hydrolyze the cassava flour. The best results in terms of butanol production were 23.98 and 13.78 $g.L^{-1}$ using enzymatic and acid hydrolysis, respectively. Qureshi et al. [7] studied the pretreatment of wheat straw with a mix of enzymes (cellulose, β-glucosidase and xylanase) at pH 5.0, 45°C for 72 h and 80 rpm,

obtaining a butanol production of 12.0 g/L. Alternative technologies to hydrolyze the raw material such as microwave-assisted pre-treatment processes, steam explosion, ozonolysis, oxidative delignification, pulsed-electric-field pretreatment were also reported [68-70].

Qureshi et al. [36] believed that barley straw can be used for butanol production. However, there is the presence of inhibitors in this substrate and hence pretreatment (with lime called overliming) is necessary for an effective fermentation. After the pretreatment, the production of butanol was higher than when using glucose as substrate. Similarly, Qureshi et al. [37] evaluated corn stover and switchgrass hydrolysates as substrates for butanol production. The production of butanol using corn stover hydrolysates was similar to that presented in previous work using barley [36].

Al-Shorgani et al. [44] reported the formation of inhibitors during the acid pretreatment of cellulosic raw material (rice bran and de-oiled rice bran). Similar to other studies, the authors used overliming treatment and extraction of inhibitors with a nonionic polymeric adsorbent resin. These procedures improved butanol production and yield. Qureshi et al. [71] concluded that the formation of fermentation inhibitors after hydrolysis of cellulosic raw material is substrate and pretreatment dependent. Thus, it is necessary for a specific study to be carried out for each substrate and treatment.

Lin et al. [51] reported the use of corn straw as a raw material for butanol production after hydrolysis using alkali pre-treatement. The sugar concentration obtained was around 44 g/L and this represents approximately 400 grams of sugar per kg of corn straw, producing 6.54 g/L (65 g/kg of corn straw) of butanol in the fermentation. Using another residue from corn production (corncob), Zhang et al. [48] reported production of 16 g/L of solvents using the enzymatic hydrolyzed corncob pretreated and detoxified with Ca $(OH)_2$. Several authors have stated that biobutanol production will only be feasible industrially if a low cost substrate can be employed. However, it is important to consider the total cost involved in the substrate utilization. In these scenarios, the tendency is for the diversification of substrates and the use of regional crops (molasses, starch or cellulosic one) for butanol production.

Bioreactors for biobutanol production

According to Kumar and Gayen [26], the operation of bioreactors for biobutanol production can be accomplished in batch, fed-batch, and continuous modes. Continuous processes offer various advantages such as reduction in sterilization and inoculation time, high productivity, and reduction in butanol inhibition, but this reactor presents high product recovery costs due to low concentration of biofuel [26]. Fed-batch fermentation is started with a low substrate concentration. When the fermentation culture consumes the substrate, more substrate is added to maintain the fermentation process while not exceeding the detrimental substrate level [53]. The usage of a continuous packed bed reactor (PBR) is reported as an alternative for fermentation using immobilized microorganism [34]. Lu et al. [38] used a fibrous bed bioreactor (FBB). This reactor is interesting because the microorganism is immobilized in the bed enabling the process to recover products in situ without the loss of cells. However, the reactor most reported is the batch one (as can be verified in table 1). This preference can be explained because it is easy to handle, maintain the anaerobic medium, control the temperature and pH, and take samples. Furthermore, this reactor has less difficulties when coupling to a separation unit.

Mariano et al. [15] reported the use of batch-bioreactor containing 7 litres of medium. The anaerobic medium was maintained by oxygen free nitrogen. Parekh et al. [72] reported the use of a pilot-scale of 200 litres using corn steep liquor as the raw material, obtaining 17.8 g/L of butanol. The same size of bioreactor was used by Lee et al. [59]. These bioreactors are larger than the others reported, thus, these studies are very important to predict the behavior of clostridial fermentation after the scale up.

Separation

The main separation process used for purification of biobutanol from the fermentation broth is the distillation. However, the butanol-water system at 101.3 kPa has an azeotrope at 55.5 wt% butanol. The greatest difficulty in this process is the low solubility of butanol in water (maximum of 7.7 wt%). As the azeotrope occurs above this solubility limit, two liquid phases are formed at the azeotrope. The upper phase contains 79.9 wt% butanol whereas the lower phase contains 7.7 wt% butanol [16], which boils at a lower temperature [10]. The recovery of low concentration butanol by traditional distillation is energy intensive and thus, economically unfeasible [73].

Mariano et al. [74], Secuianu et al. [75] and Ezeji [3] reported some of the most commonly used techniques for continuous recovery of butanol from the fermentation broth, namely adsorption, gas stripping, ionic liquids, liquid-liquid extraction, pervaporation, aqueous two-phase separation, supercritical extraction, and flash fermentation. Adsorption should allow separation of butanol from the bulk aqueous fermentation broth. Hydrophobic adsorbents potentially show high selectivity for butanol over water [5]. In adsorption, alcohol is preferentially transferred from the feed liquid to a solid adsorbent material [16]. Dhamole et al. [76] used a non-ionic surfactant to decrease butanol toxicity and its separation from the non-ionic surfactant micelle aqueous solution by cloud point extraction. Thus, the fermentation is not

inhibited and butanol concentration is increased in the micelles.

Ezeji [3] used gas stripping for *in situ* separation because it is a simple technique that is free of emulsion formation and it does not require a membrane or expensive chemicals. The production of ABE using gas stripping in the fermentation of 500 g/L of sugar using the strain *C. beijerinckii* BA101 was 232.8 g/L compared to a control batch reactor, where 17.6 g/L ABE was produced. Moreover, gas stripping was more selective in removing butanol than acetone and ethanol [38]. Gas stripping is more efficient when butanol concentration in the fermentation broth is higher than 8 g/L [49]. According to Ezeji et al. [40], the production of ABE increased from 18.6 g/L to 81.3 g/L, whereas sugar consumption increased about 487% (compared with the control of the same substrate without gas stripping) using gas stripping in the fermentation of liquefied corn starch with *C. beijerinckii* BA101.

Pervaporation technique using membranes with high product selectivity is one of the most promising alternatives to conventional distillation. Without heating energy, the pervaporation process enables the efficient separation and concentration of the product in a single step, and maintains the productivity of the microorganism as a result of preventing product inhibition [77]. Yen et al. [78] tested a membrane of poly (ether-block-amide) and 5% and 10% (w/v) of carbon nanotubes for pervaporation. The productivity and yield increased about 20% in comparison with the pervaporation using a poly (ether-block-amide) membrane.

In the perstractive separation, the fermentation broth and the extractant are separated by a membrane. The membrane contactor provides a surface area where the two immiscible phases can exchange butanol, thus, the toxicity of solvent for the cells does not occur [79]. According to Qureshi and Maddox [52], in the control experiment 28.6 g/L lactose was used while in the fermentation perstraction experiment 227 g/L lactose was utilized in ABE fermentation.

The removal of butanol or ABE from the fermentation broth by liquid–liquid extraction is considered an important technique. Usually, a water-insoluble organic extractant is mixed with the fermentation broth. The main problem concerned with the use of this technique is related to the toxicity of the solvent to the cells [79]. The major limitation is that the extractant with high partition coefficient often leads to microbial toxicity because of direct contact between the fermentation broth and the extractant [80].

In the membrane-assisted extractive fermentation, two phases of extractant and fermentation broth are separated by a porous membrane. The membrane can be either hydrophilic or hydrophobic and the interface is immobilized by the impregnation of its pores with one of the two phases depending on the membrane affinity. Thus, the microbial toxicity of the extractant can be reduced. Tanaka et al. [80] using this approach reported an increase in the glucose consumption from 66% to 100% due to the absence of inhibition for butanol in the medium.

Mariano et al. [15] reported the use of a cyclic vacuum applied in a bioreactor during fermentation. Using a vacuum is a good method to remove butanol from the fermentation medium, resulting in decreased product inhibition. Furthermore, in this study the continuous and intermittent vacuum has been tested. The use of intermittent vacuum showed a reduction of 39% in the energy expenditure without product inhibition because of low butanol concentration. Moreover, this process results in pre-concentration of the aqueous solution of butanol, which decreases the energy expenditure in the purification of the butanol. The same authors reported the use of a flash fermentation for *in situ* butanol recovery. Flash fermentation is a good way to decrease the butanol concentration in the fermentation broth. In this technology, a partial separation of the solvents and water occurs in the flash tank separator, where the liquid fraction returns to the fermentor and the vapor fraction (after condensation) plus the purge and permeate streams will compose the final stream that is sent to distillation. Thus, butanol concentration is always less than the critical concentration (for inhibition by product) [17]. Furthermore, they developed a mathematical model to predict the behavior of the process. This consisted of a batch fermentation reactor, and a vacuum flash vessel (with a filter to remove any solids before it gets in the flash vessel). The schematic design of the process is showed in the Figure 1. For the development of the model the differential equations for the batch reactor assume constant volume and factor in the removal of butanol during the process. The objective of this work was to demonstrate that the use of flash fermentation was able to decrease product inhibition [17]. The Figure 1 demonstrates the cyclic process that can be generated using this technology. The volatile compounds are removed in the flash tank and the liquid fraction returns to the fermentation. The feed is used to control the sugar concentration in the reactor, the purge is used to control the level and allow renewal of cell mass with removal of old cells and the filter is used to prevent solids entering the flash tank.

Mariano et al. [81] proposed a mathematical model for a continuous flash fermentation and used this model to optimize the process using response surface techniques. In other work, Mariano et al. [82] used the same model proposed by Mariano et al. [81], but the process was optimized using the method of particle swarm optimization to obtain the best operating conditions for butanol

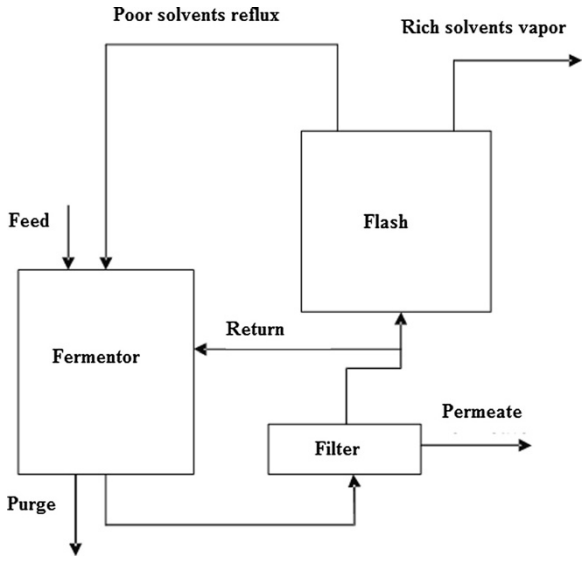

Figure 1 Schematic design of flash fermentation process.

production. The same authors proposed the utilization of a servo control in flash fermentation [18]. This work was carried out because in previous studies it has been proved that this process can be used to improve butyric fermentation. So it becomes necessary to control the removal of butanol, since natural oscillations can occur during the dynamic process. The mathematical modeling is similar to the one used in [17] with some minor changes in the differential equations due to the alteration of the reaction volume. The objective of the control was to keep sugar and butanol concentrations constant in the fermentor. The controller was shown to efficiently regulate the operating conditions. Thus, the use of a controller in flash fermentation is able to enlarge the process, and suit this to an industrial application.

Similarly, Liu et al. [19] propose a mathematical model to simulate the process consisting of a fermentor, gas striping, and a purification process for the condensate from gas striping. The objective was to simulate a process to produce 150.000 tons of butanol per year with purity of 99 wt%, and to evaluate the energy demand of all parts of this process. The authors concluded that ABE fermentation has lesser liquid fuel production (energy basis) using corn as a substrate than the ethanol production process. However, this scenario could change very quickly with the development of the process and genetic engineering.

Clearly, the use of *in situ* separation techniques in butanol production is promising for industrial applications by decreasing the product inhibition problems of the fermentation process. Furthermore, it can be considered a pre-separation process and decrease the quantity of butanol to be purified. The use of mathematical models to simulate the behavior of a fermentation process linked to any of these separation processes is important to predict behavior and production costs. With this it is possible to represent experimentally the best theoretical conditions and predict the adjustment necessary to scale up the process to industrial applications.

Concluding remarks

In this review, biobutanol production was discussed in terms of microorganisms, substrates, types of bioreactors used in the process, separation techniques with special attention to *in situ* separation leading to decrease butanol inhibition and, the development of mathematical models to represent the *in situ* separation techniques. The engineering of microorganisms for butanol production has been reported over recent years. These changes in the bacterium decrease acetone production as well as increasing the resistance of microorganisms to high concentrations of butanol with consequent improvement in the use of sugar as a substrate for biobutanol production. Concerning substrates, there is a trend to use lignocellulosic materials, but the inhibitors generated during hydrolysis imposed difficulties for the industrial usage of this raw material for biobutanol production. The *in situ* separations associated with low energy expenditure during the removal of biobutanol are the technologies that will predominate in the future.

Competing interests

The authors declared that they have no competing interests.

Authors' contribution

All authors participated in the compilation of data, discussion, data interpretation and manuscript drafting. All authors read and approved the final manuscript.

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References

1. Sorensen B: **A sustainable energy future: construction of demand and renewable energy supply scenarios.** *Int J Energy Res* 2008, **32:**436–470.
2. Cheng CL, Che PY, Chen BY, Lee WJ, Chien LJ, Chang JS: **High yield bio-butanol production by solvent-producing bacterial microflora.** *Bioresour Technol* 2012, **113:**58–64.
3. Ezeji TC, Qureshi N, Blaschek HP: **Microbial production of a biofuel (acetone–butanol–ethanol) in a continuous bioreactor: impact of bleed and simultaneous product removal.** *Bioprocess Biosyst Eng* 2012, doi:10.1007/s00449-012-0766-5.
4. Ranjan A, Moholkar VS: **Biobutanol: science, engineering, and economics.** *Int J Energ Res* 2012, **36:**277–323.
5. Knoshaug EP, Zhang M: **Butanol tolerance in a selection of microorganisms.** *Appl Biochem Biotechnol* 2009, **153:**13–20.
6. Oudshoorn A, van der Wielen LAM, Straathof AJJ: **Adsorption equilibria of bio-based butanol solutions using zeolite.** *Biochem Eng J* 2009, **48:**99–103.
7. Cheng CL, Che PY, Chen BY, Lee WJ, Lin CY, Chang JS: **Biobutanol production from agricultural waste by an acclimated mixed bacterial microflora.** *Applied Energy* 2012, doi:10.1016/j.apenergy.2012.05.042.

8. Sullivan L, Cates MS, Bennett GN: **Structural correlations of activity of Clostridium acetobutylicum ATCC 824 butyrate kinase isozymes.** *Enzyme Microb Technol* 2010, **46:**118–124.
9. Pfromm PH, Amanor-Boadu V, Nelson R, Vadlani P, Madl R: **Bio-butanol vs. bio-ethanol: a technical and economic assessment for corn and switchgrass fermented by yeast or Clostridium acetobutylicum.** *Biomass Bioenerg* 2010, **34:**515–524.
10. Mariano AP, Qureshi N, Maciel Filho R, Ezeji TC: **Bioproduction of butanol in bioreactors: new insights from simultaneous in situ butanol recovery to eliminate product toxicity.** *Biotechnol Bioeng* 2011, **108:**1757–1765.
11. Shinto H, Tashiro Y, Yamashita M, Kobayashi G, Sekiguchi T, Hanai T, Kuriya Y, Okamoto M, Sonomoto K: **Kinetic modeling and sensitivity analysis of acetone–butanol–ethanol production.** *J Biotechnol* 2007, **131:**45–56.
12. Green EM: **Fermentative production of butanol- the industrial perspective.** *Curr Opin Biotechnol* 2011, **22:**337–343.
13. Taylor G: **Biofuels and the biorefinery concept.** *Energy Policy* 2008, **36:**4406–4409.
14. Qureshi N, Saha BC, Cotta MA: **Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*.** *Bioprocess Biosyst Eng* 2007, **30:**419–427.
15. Mariano AP, Maciel Filho R, Ezeji TC: **Energy requirements during butanol production and in situ recovery by cyclic vacuum.** *Renew Energy* 2012, **47:**183–187.
16. Vane LM: **Separation technologies for the recovery and dehydration of alcohols from fermentation broths.** *Biofuels Bioprod Bior* 2008, **2:**553–588.
17. Mariano AP, Angelis DF, Maugeri F, Atala DIP, Maciel MRW, Maciel Filho R: **An alternative process for butanol production: continuous flash fermentation.** *Chem Prod Process Mode* 2008, **3:**34.
18. Mariano AP, Costa CBB, Maciel MRW, Maugeri Filho F, Atala DIP, Angelis DF, Maciel FR: **Dynamics and control strategies for a butanol fermentation process.** *Appl Biochem Biotechnol* 2010, **160:**2424–2448.
19. Liu J, Wu M, Wang M: **Simulation of the process for producing butanol from corn fermentation.** *Ind Eng Chem Res* 2009, **48:**5551–5557.
20. Jurgens G, Survase S, Berezina O, Sklavounos E, Linnekoski J, Kurkijärvi A, Väkeva M, Heiningen AV, Granström T: **Butanol production from lignocellulosics.** *Biotechnol Lett* 2012, **34:**1415–1434.
21. Qureshi N, Ezeji TC: **Butanol, 'a superior biofuel' production from agricultural residues (renewable biomass): recent progress in technology.** *Biofuels Bioprod Bior* 2008, **2:**319–330.
22. Qureshi N: **Agricultural residues and energy crops as potentially economical and novel substrates for microbial production of butanol (a biofuel.** *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2010, **5:**59.
23. Jang YS, Lee J, Malaviya A, Seung DY, Cho JH, Lee SY: **Butanol production from renewable biomass: rediscovery of metabolic pathways and metabolic engineering.** *Biotechnol J* 2012, **7:**186–198.
24. Huang H, Liu H, Gan YR: **Genetic modification of critical enzymes and involved genes in butanol biosynthesis from biomass.** *Biotechnol Adv* 2010, **28:**651–657.
25. Gheshlaghi R, Scharer JM, Moo-Young M, Chou CP: **Metabolic pathways of clostridia for producing butanol.** *Biotechnol Adv* 2009, **27:**764–781.
26. Kumar M, Gayen K: **Developments in biobutanol production: New insights.** *Appl Energ* 2011, **88:**1999–2012.
27. Dürre P: **Fermentative production of butanol- the academic perspective.** *Curr Opin Biotechnol* 2011, **22:**331–336.
28. Jin C, Yao M, Liu H, Lee CF, Ji J: **Progress in the production and application of n-butanol as a biofuel.** *Renew Sustain Energy Rev* 2011, **15:**4080–4106.
29. Moholkar VS, Ranjan A, Mayank R: **Economics of biobutanol: a review.** *Res J Pharm Biol Chem Sci* 2012, **3:**901–913.
30. Liu Z, Ying Y, Li F, Ma C, Xu P: **Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran.** *J Ind Microbiol Biotechnol* 2010, **37:**495–501.
31. Khanna S, Goyal A, Moholkar VS: **Production of n -butanol from biodiesel derived crude glycerol using *Clostridium pasteurianum* immobilized on Amberlite.** *Fuel* 2013, **112:**557–561.
32. Gottumukkala LD, Parameswaran B, Valappil SK, Mathiyazhakan K: **Biobutanol production from rice straw by a non acetone producing Clostridium sporogenes BE01.** *Bioresour Technol* 2013, **145:**182–187.
33. Chen WH, Chen YC, Lin JG: **Evaluation of biobutanol production from non-pretreated rice straw hydrolysate under non-sterile environmental conditions.** *Bioresour Technol* 2013, **135:**262–268.
34. Napoli F, Olivieri G, Russo ME, Marzocchella A, Salatino P: **Butanol production by *Clostridium acetobutylicum* in a continuous packed bed reactor.** *J Ind Microbiol Biotechnol* 2010, **37:**603–608.
35. Lépiz-Aguilar L, Rodríguez-Rodríguez CE, Arias ML, Lutz G, Ulate W: **Butanol production by *Clostridium beijerinckii* BA101 using cassava flour as fermentation substrate: enzymatic versus chemical pretreatments.** *World J Microbiol Biotechnol* 2011, **27:**1933–1939.
36. Qureshi N, Saha BC, Dien B, Hector RE, Cotta MA: **Production of butanol (a biofuel) from agricultural residues: Part I – Use of barley straw hydrolysate.** *Biomass Bioenerg* 2010, **34:**559–565.
37. Qureshi N, Saha BC, Hector RE, Dien B, Hughes S, Liu S, Iten L, Bowman MJ, Sarath G, Cotta MA: **Production of butanol (a biofuel) from agricultural residues: Part II – Use of corn stover and switchgrass hydrolysates.** *Biomass Bioenerg* 2010, **34:**566–571.
38. Lu C, Zhao J, Yang ST, Wei D: **Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping.** *Bioresour Technol* 2012, **104:**380–387.
39. Noomtim P, Cheirsilp B: **Production of Butanol from Palm Empty Fruit Bunches Hydrolyzate by *Clostridium acetobutylicum*.** *Energy Procedia* 2011, **9:**140–146.
40. Ezeji TC, Qureshi N, Blaschek HP: **Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping.** *J Ind Microbiol Biotechnol* 2007, **34:**771–777.
41. Abd-Alla MH, El-Enany AWE: **Production of acetone-butanol-ethanol from spoilage date palm (Phoenix dactyliferaL.) fruits by mixed culture of *Clostridium acetobutylicum* and *Bacillus subtilis*.** *Biomass Bioenerg* 2012, **42:**172–178.
42. Wang Y, Blaschek HP: **Optimization of butanol production from tropical maize stalk juice by fermentation with *Clostridium beijerinckii* NCIMB 8052.** *Bioresour Technol* 2011, **102:**9985–9990.
43. Sun Z, Liu S: **Production of n-butanol from concentrated sugar maple hemicellulosic hydrolysate by *Clostridia acetobutylicum* ATCC824.** *Biomass Bioenerg* 2012, **39:**39–47.
44. Al-Shorgani NKN, Kalil MS, Yusoff WMW: **Biobutanol production from rice bran and de-oiled rice bran by *Clostridium saccharoperbutylacetonicum* N1-4.** *Bioprocess Biosyst Eng* 2012, **35:**817–826.
45. Guo T, Sun B, Jiang M, Wu H, Du T, Tang Y, Wei P, Ouyang P: **Enhancement of butanol production and reducing power using a two-stage controlled-pH strategy in batch culture of *Clostridium acetobutylicum* XY16.** *World J Microbiol Biotechnol* 2012, **28:**2551–2558.
46. Ranjan A, Khanna S, Moholkar VS: **Feasibility of rice straw as alternate substrate for biobutanol production.** *Appl Energ* 2013, **103:**32–38.
47. Ranjan A, Moholkar VS: **Comparative study of various pretreatment techniques for rice straw saccharification for the production of alcoholic biofuels.** *Fuel* 2011, **112:**567–571.
48. Zhang WL, Liu ZY, Liu Z, Li FL: **Butanol production from corncob residue using Clostridium beijerinckii NCIMB 8052.** *Lett Appl Microbiol* 2012, **55:**240–246.
49. Xue C, Zhao J, Lu C, Yang ST, Bai F, Tang IC: **High-titern-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping.** *Biotechnol Bioeng* 2012, **109:**2746–2756.
50. Isar J, Rangaswamy V: **Improved n-butanol production by solvent tolerant *Clostridium beijerinckii*.** *Biomass Bioenerg* 2012, **37:**9–15.
51. Lin Y, Wang J, Wang X, Sun XH: **Optimization of butanol production from corn straw hydrolysate by *Clostridium acetobutylicum* using response surface method.** *Chin Sci Bull* 2011, **56:**1422–1428.
52. Qureshi N, Maddox IS: **Reduction in Butanol Inhibition by Perstraction: Utilization of Concentrated Lactose/Whey Permeate by *Clostridium acetobutylicum* to Enhance Butanol Fermentation Economics.** *Food Bioprod Process* 2005, **83:**43–52.
53. Li SY, Srivastava R, Suib SL, Li Y, Parnas RS: **Performance of batch, fed-batch, and continuous A–B–E fermentation with pH-control.** *Bioresour Technol* 2011, **102:**4241–4250.
54. Chen Y, Zhou T, Liu D, Li A, Xu S, Liu Q, Li B, Ying H: **Production of Butanol from Glucose and Xylose with Immobilized Cells of Clostridium acetobutylicum.** *Biotechnol Bioprocess Eng* 2013, **18:**234–241.

55. Ezeji TC, Qureshi N, Blaschek HP: **Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping.** *Appl Microbiol Biotechnol* 2004, **63:**653–658.
56. Bahl H, Andersch W, Braun K, Gottschalk G: **Effect of pH and Butyrate Concentration on the Production of Acetone and Butanol by Clostridium acetobutylicum Grown in Continuous Culture.** *Eur J Appl Microbiol Biotechnol* 1982, **14:**17–20.
57. Jang YS, Park JM, Choi S, Choi YJ, Seung DY, Cho JH, Lee SY: **Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches.** *Biotechnol Adv* 2012, **30:**989–1000.
58. García V, Päkkilä J, Ojamo H, Muurinen E, Keiski RL: **Challenges in biobutanol production: How to improve the efficiency?** *Renew Sustain Energy Rev* 2011, **15:**964–980.
59. Lee J, Jang YS, Choi SJ, Im JA, Song H, Cho JH, Seung DY, Papoutsakis T, Bennett GN, Lee SY: **Metabolic Engineering of Clostridium acetobutylicumATCC 824 for Isopropanol-Butanol-Ethanol Fermentation.** *Appl Environ Microbiol* 2012, **78:**1416–1423.
60. Collas F, Kuit W, Clément B, Marchal R, López-Contreras AM, Monot F: **Simultaneous production of isopropanol, butanol, ethanol and 2,3-butanediol by Clostridium acetobutylicumATCC 824 engineered strains.** *AMB Express* 2012, **2:**45.
61. Lütke-Eversloh T, Bahl H: **Metabolic engineering of *Clostridium acetobutylicum*: recent advances to improve butanol production.** *Curr Opin Biotechnol* 2011, **22:**634–647.
62. Jiang Y, Xu C, Dong F, Yang Y, Jiang W, Yang S: **Disruption of the acetoacetate decarboxylase gene in solvent-producing *Clostridium acetobutylicum* increases the butanol ratio.** *Metab Eng* 2009, **11:**284–291.
63. Gu Y, Jiang Y, Wu H, Liu X, Li Z, Li J, Xiao H, Shen Z, Dong H, Yang Y, Li Y, Jiang W, Yang S: **Economical challenges to microbial producers of butanol: feedstock, butanol ratio and titer.** *Biotechnol J* 2011, **6:**1348–1357.
64. Van der Merwe AB, Cheng H, Görgens JF, Knoetze JH: **Comparison of energy efficiency and economics of process designs for biobutanol production from sugarcane molasses.** *Fuel* 2013, **105:**451–458.
65. Whitfield MB, Chinn MS, Veal MW: **Processing of materials derived from sweet sorghum for biobased products.** *In Crops Prod* 2012, **37:**362–375.
66. Adsul MG, Singhvi MS, Gaikaiwari SA, Gokhale DV: **Development of biocatalysts for production of commodity chemicals from lignocellulosic biomass.** *Bioresour Technol* 2011, **102:**4304–4312.
67. Lee J, Seo E, Kweon DH, Park K, Jin YS: **Fermentation of rice bran and defatted rice bran for butanol production using *Clostridium beijerinckii* NCIMB 8052.** *J Microbiol Biotechnol* 2009, **19:**482–490.
68. Kumar P, Barrett DM, Delwiche MJ, Stroeve P: **Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production.** *Ind Eng Chem Res* 2009, **48:**3713–3729.
69. Ooi BG, Rambo AL, Hurtado MA: **Overcoming the Recalcitrance for the Conversion of Kenaf Pulp to Glucose via Microwave-Assisted Pre-Treatment Processes.** *Int J Mol Sci* 2011, **12:**1451–1463.
70. Thirmal C, Dahman Y: **Comparisons of existing pretreatment, saccharification, and fermentation processes for butanol production from agricultural residues.** *Can J Chem Eng* 2012, **90:**745–761.
71. Qureshi N, Saha BC, Hector RE, Cotta MA: **Removal of fermentation inhibitors from alkaline peroxide pretreated and enzymatically hydrolyzed wheat straw: production of butanol from hydrolysate using *Clostridium beijerinckiiin* batch reactors.** *Biomass Bioenerg* 2008, **32:**1353–1358.
72. Parekh M, Formanek J, Blaschek HP: **Pilot-scale production of butanol by Clostridium beijerinckii BA101 using a low-cost fermentation medium based on corn steep water.** *Appl Microbiol Biotechnol* 1999, **51:**152–157.
73. Zheng YN, Li LZ, Xian M, Ma YJ, Yang JM, Xu X, He DZ: **Problems with the microbial production of butanol.** *J Ind Microbiol Biotechnol* 2009, **36:**1127–1138.
74. Mariano AP, Costa CBB, Angelis DF, Maugeri Filho F, Atala DIP, Maciel MRW, Maciel FR: **Dynamics of a continuous flash fermentation for butanol production.** *Chem Eng Trans* 2010, **20:**285–290.
75. Secuianu C, Feroiu V, Geana D: **High-Pressure Vapor -Liquid Equilibria in the System Carbon Dioxide + 1-Butanol at Temperatures from (293.15 to 324.15) K.** *J Chem Eng Data* 2004, **49:**1635–1638.
76. Dhamole PB, Wang Z, Liu Y, Wang B, Feng H: **Extractive fermentation with non-ionic surfactants to enhance butanol production.** *Biomass Bioenerg* 2012, **40:**112–119.
77. Nakayama S, Morita T, Negishi H, Ikegami T, Sakaki K, Kitamoto D: **Candida krusei produces ethanol without production of succinic acid; a potential advantage for ethanol recovery by pervaporation membrane separation.** *FEMS Yeast Res* 2008, **8:**706–714.
78. Yen HW, Chen ZH, Yang IK: **Use of the composite membrane of poly (ether-block-amide) and carbon nanotubes (CNTs) in a pervaporation system incorporated with fermentation for butanol production by *Clostridium acetobutylicum*.** *Bioresour Technol* 2012, **109:**105–109.
79. Ezeji TC, Qureshi N, Blaschek HP: **Bioproduction of butanol from biomass: from genes to bioreactors.** *Curr Opin Biotechnol* 2007, **18:**220–227.
80. Tanaka S, Tashiro Y, Kobayashi G, Ikegami T, Negishi H, Sakaki K: **Membrane-assisted extractive butanol fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 with 1-dodecanol as the extractant.** *Bioresour Technol* 2012, **116:**448–452.
81. Mariano AP, Costa CBB, Angelis DF, Maugeri Filho F, Atala DIP, Maciel MRW, Maciel FR: **Optimisation of a continuous flash fermentation for butanol production using the response surface methodology.** *Chem Eng Res Des* 2009, **88:**562–571.
82. Mariano AP, Costa CBB, Angelis DF, Maugeri Filho F, Atala DIP, Maciel MRW, Maciel FR: **Optimisation of a fermentation process for butanol production by particle swarm optimisation (PSO).** *J Chem Technol Biotechnol* 2010, **85:**934–949.

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