

REVIEW

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# Recent advances on biobutanol production

Luiz J Visioli, Heveline Enzweiler, Raquel C Kuhn, Marcio Schwaab and Marcio A Mazutti\*

## 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<sub>2</sub> 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

\* Correspondence: mazutti@ufsm.br  
Department of Chemical Engineering, Federal University of Santa Maria,  
97105-900 Santa Maria, Brazil

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  $5.2 \pm 0.2$  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 cellulose and provide high solvent tolerance and high butanol selectivity [58]. Genetic modification of *Clostridium* is widely used, by inserting some heterogenous 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<sub>2</sub> 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
<i>C. acetobutylicum</i> (immobilized)	Cheese whey (lactose)	Yield: 15% to 0.54 h <sup>-1</sup> of dilution and 28% to 0.97 h <sup>-1</sup> of dilution	Reactor (PBR) with immobilized clostridium	[34]
<i>C. beijerinckii</i> ATCC 55025	Hydrolysate of wheat bran	Yield: 32%/Production: 8.8 g.l <sup>-1</sup> of biobutanol	Acid hydrolysis	[30]
<i>C. beijerinckii</i>	Cassava flour	Production: 23.98 g.l <sup>-1</sup> of butanol	Enzymatic treatment with yield of 9.12% to Reducing sugar	[35]
<i>C. beijerinckii</i> P260	Wheat straw	Yield: 42%	Acid pretreatment and enzymatic hydrolysis	[14]
	Barley straw	Yield: 43%/Production: 26,64 g.l <sup>-1</sup> of total solvents	Dilute sulfuric acid hydrolysis/overliming	[36]
	Corn stover	Yield: 43%/Production: 18.04 g.l <sup>-1</sup> of total solvents	Acid and enzymatic steps of hydrolysis/overliming	[37]
	Switchgrass	Yield: 37%/Production: 8.91 g.l <sup>-1</sup> of total solvents		
	Glucose	Production: 17.54 g.l <sup>-1</sup> of butanol	Intermittent vacuum application	[10]
<i>C. saccharobutylicum</i> DSM 13864	Sago starch	Yield: 29%	Free microorganism fermentation	[26]
<i>C. acetobutylicum</i>	Cassava bagasse	Yield: 32%/Production: 76.4 g.l <sup>-1</sup> of butanol	Hydrolyze by enzymes fibrous bed bioreactor/ Gas stripping	[38]
	Palm empty fruit bunches	Production: 1.262 g.l <sup>-1</sup> of butanol	Acid pretreatment/ enzymatic hydrolysis	[39]
<i>C. beijerinckii</i> BA101	Liquefied corn starch	Butanol production: 81, 3 g.l <sup>-1</sup> (with gas stripping)/18.6 g.l <sup>-1</sup> (without gas stripping)	Bath reactor/gas stripping/ enzymatic hydrolysis	[40]
<i>C. acetobutylicum</i> ATCC 824 and <i>Bacillus subtilis</i> DSM 4451	Spoilage date palm fruits	Yield: 42%/production: 21.56 g.l <sup>-1</sup> of Solvents	Bacterial consortium (anaerobic conditions)	[41]
<i>C. beijerinckii</i> NCIMB 8052	Tropical maize stalk juice	Production: 0.27 g-butanol/g-sugar	Optimization of pH, agitation, sugar concentration	[42]
<i>C. acetobutylicum</i> ATCC824	Sugar maple Hemicellulosic material	Production: 7 g.l <sup>-1</sup> of butanol	Alkali pretreatment/acid hydrolysis/overliming	[43]
<i>C. saccharoperbutylacetonicum</i> 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	
<i>C. acetobutylicum</i> XY16	Glucose	Production: 20.3 g.l <sup>-1</sup> of butanol	pH steps in the fermentation	[45]
<i>C. sporogenes</i> 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]
<i>C. saccharoperbutylacetonicum</i> 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]
<i>C. pasteurianum</i>	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]
<i>C. acetobutylicum</i> 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]
<i>C. acetobutylicum</i> MTCC 481	Rice straw	Butanol production of 1.72 g/L. Butanol production of 1.6 g/L. Butanol production of 2.1 g/L.	Steam explosion Acid treatment Acid pre-treatment/ enzymatic hydrolysis	[47]
<i>C. beijerinckii</i> NCIMB 8052	Corn cob	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)**

<i>C. acetobutylicum</i> JB200	Glucose	Yield: 21%/Production: 172 g.L <sup>-1</sup> of solvents	Gas stripping	[49]
<i>C. beijerinckii</i> ATCC 10132	Glucose	Production: 20 g.L <sup>-1</sup> of butanol	Bath reactor	[50]
<i>C. acetobutylicum</i> CICC 8008	Corn straw	Production: 6.20 g.L <sup>-1</sup> of butanol	Enzymatic hydrolysis/ bath reactor	[51]
<i>C. acetobutylicum</i> P262	Whey permeate medium	Yield: 44%/Production: 98.97 g.L <sup>-1</sup> 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 beijerinckii* 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<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, ammonium acetate, para-aminobenzoic acid, thiamin, biotin, MgSO<sub>4</sub>·7H<sub>2</sub>O, MnSO<sub>4</sub>·H<sub>2</sub>O, FeSO<sub>4</sub>·7H<sub>2</sub>O, 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)<sub>2</sub> 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-glucosylase) to hydrolyze the cassava flour. The best results in terms of butanol production were 23.98 and 13.78 g.L<sup>-1</sup> 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-treatment. 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 (corn cob), Zhang et al. [48] reported production of 16 g/L of solvents using the enzymatic hydrolyzed corn cob pretreated and detoxified with  $\text{Ca}(\text{OH})_2$ . 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.

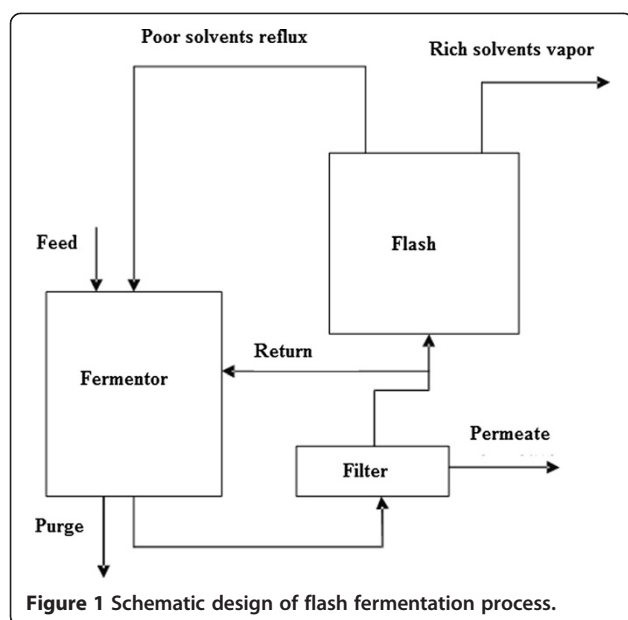
The removal of butanol or ABE from the fermentation broth by liquid–liquid extraction is considered an important technique. Usually, a water-insoluble organic extractant is mixed with the fermentation broth. The main problem concerned with the use of this technique is related to the toxicity of the solvent 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



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 stripping, and a purification process for the condensate from gas stripping. 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.

## Acknowledgements

The authors thank CAPES for the scholarships and SCIT-RS for the financial support of this work.

Received: 18 November 2013 Accepted: 11 June 2014

Published: 19 June 2014

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doi:10.1186/2043-7129-2-15

**Cite this article as:** Visioli et al.: Recent advances on biobutanol production. *Sustainable Chemical Processes* 2014 **2**:15.

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