Bioresource Technology 123 (2012) 653–663

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

Butanol production from renewable biomass by clostridia

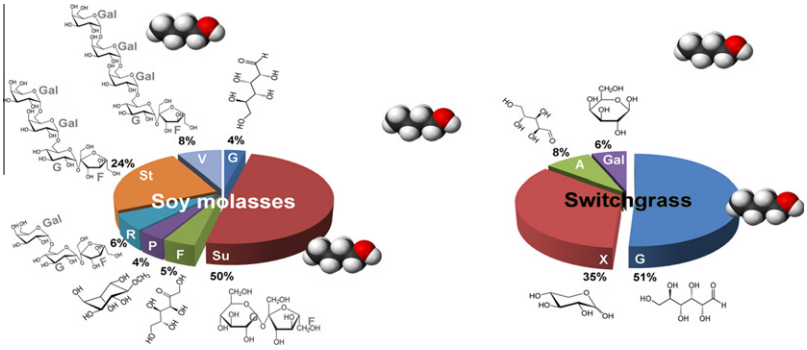
Yu-Sin Jang [a,1], Alok Malaviya [a,1], Changhee Cho [a], Joungmin Lee [a], Sang Yup Lee [a,b,*]

[a] Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering (BK21 Program), BioProcess Engineering Research Center, Center for Systems and Synthetic Biotechnology, Institute for the BioCentury, KAIST, Republic of Korea
[b] Department of Bio and Brain Engineering and Bioinformatics Research Center, KAIST, Republic of Korea

HIGHLIGHTS

- Butanol is an important industrial chemical as well as an advanced biofuel.
- We review the use of various carbon sources on fermentation performance.
- We review advanced fermentation and recovery processes for butanol production.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 May 2012
Received in revised form 25 July 2012
Accepted 26 July 2012
Available online 7 August 2012

Keywords:
Biomass
Butanol
Clostridium
Fermentation
Recovery

ABSTRACT

Global energy crisis and limited supply of petroleum fuels have rekindled the worldwide focus towards development of a sustainable technology for alternative fuel production. Utilization of abundant renewable biomass offers an excellent opportunity for the development of an economical biofuel production process at a scale sufficiently large to have an impact on sustainability and security objectives. Additionally, several environmental benefits have also been linked with the utilization of renewable biomass. Butanol is considered to be superior to ethanol due to its higher energy content and less hygroscopy. This has led to an increased research interest in butanol production from renewable biomass in recent years. In this paper, we review the various aspects of utilizing renewable biomass for clostridial butanol production. Focus is given on various alternative substrates that have been used for butanol production and on fermentation strategies recently reported to improve butanol production.

1. Introduction

Butanol is an important industrial chemical and is also considered as a superior liquid fuel with a potential to replace gasoline (Dürre, 2007; Lee et al., 2008; Papoutsakis, 2008). Research effort towards the efficient production of butanol from sustainable and renewable carbon sources has steadily been progressing. Based on advances in biotechnology and process engineering, new fermentation processes are being developed for converting the abundantly available biomass to butanol (Dürre, 2007; Lee et al., 2008; Papoutsakis, 2008). Butanol has traditionally been produced by anaerobic fermentation of sugar substrates using various species of solventogenic clostridia (Jones and Woods, 1986). Except for the solventogenic clostridia, it is believed that no genus among the bacteria, archea and eucarya is efficient enough to naturally produce butanol (Qureshi et al., 2008).

Treatment of biomass for the extraction of fermentable sugars results in a mixture of sugar substrates, mainly pentoses, hexoses, and disaccharide. *Clostridium* sp. are known to have the capability of utilizing simple and complex sugars, including both pentose and

* Corresponding author at: Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305–701, Republic of Korea. Fax: +82 42 350 3910.
E-mail address: leesy@kaist.ac.kr (S.Y. Lee).
[1] These authors contributed equally to this work.

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.07.104

hexose, as well as CO_2, H_2 and CO (Jang et al., 2012a; Kopke et al., 2010; Munasinghe and Khanal, 2010; Tracy et al., 2012). Hence, application of *Clostridium* sp. for butanol production using extracted sugars obtained from treatment of cellulosic biomass has been suggested to the best strategy to allow economical production of butanol (Jones and Woods, 1986; Lee et al., 2008; Qureshi et al., 2008; Zverlov et al., 2006). However, in addition to utilization of inexpensive and renewable substrates, there are some other process related issues which need to be considered for sustainable and economical production of butanol.

The performance of butanol fermentation process using clostridial sp. is severely limited by various factors, such as high cost of fermentation substrate, substrate inhibition, low butanol concentration due to low solvent tolerance, sluggish growth and low cell density achievable during solventogenic fermentation. These limitations result in low butanol productivity with low yield due to the production of additional by-products, high downstream processing cost for butanol recovery due to the formation of mixed solvents and low butanol concentration. Researchers have attempted to solve these problems thorough various studies including microbial strain development for improved butanol titer and tolerance (Green, 2011; Jang et al., 2012a; Jiang et al., 2009; Lee et al., 2009b), development of efficient *in situ* product recovery technologies to overcome the butanol toxicity to fermenting microorganism (Ezeji et al., 2003; Li et al., 2011; Lu et al., 2012; Mariano et al., 2011; Nielsen and Prather, 2009; Roffler et al., 1988; Xue et al., 2012), and the application of various fermentation strategies to increase the cell density (Tashiro et al., 2005) as well as butanol titer, yield and productivity (Ezeji et al., 2003).

In particular, strain improvement by metabolic engineering will have a high impact on the overall performance of bio-based butanol production. However, this review will not cover metabolic engineering aspects as there have recently been excellent papers published on the features of clostridial pathway and alternative pathways for butanol production (Jang et al., 2012a; Jones and Woods, 1986; Lutke-Eversloh and Bahl, 2011; Tracy et al., 2012; Yu et al., 2011), and on metabolic engineering of butanol producers (Jang et al., 2012b; Kim et al., 2008; Krivoruchko et al., 2011; Tan and Liao, 2012; Wahrheit et al., 2011). Readers are encouraged to refer to the above papers.

Once the production strain is selected, application of low-cost plant materials for bio-based production of butanol along with the process optimization becomes important to achieve better economics of butanol production. In this paper, we review on the recent advances in bio-based butanol production with focuses given on the process technologies used for the conversion of renewable biomass into butanol.

2. Renewable fermentation substrates for butanol production

2.1. Sucrose and starch

Butanol has traditionally been produced by ABE fermentation using carbon substrates obtained from corn, sugar beets and sugar cane, potatoes, tapioca and millet (Jones and Woods, 1986; Qureshi et al., 2008). As *Clostridium* sp. possess strong amylase activities, they can effectively utilize starchy substrates without the need for hydrolysis. Molasses, whey permeate, cassava and Jerusalem artichokes have also been used as fermentation substrates for butanol production. In South Africa, cane molasses was successfully used for butanol production at industrial scale until 1980s (Jones and Woods, 1986). Soy molasses has also been evaluated for butanol production using *C. beijerinckii* BA101, and about 8 g/l butanol with a yield of 0.1 g/g substrate and a volumetric productivity of 0.08 g/l/h could be produced using 80.0 g/l spray dried soy molasses (Qureshi et al., 2001). As a part of French research program on production of fuel extenders from biomass, application of Jeruselum artichokes as a substrate has been evaluated with *C. acetobutylicum* isolated from Jeruselum artichokes tubers. Under optimized condition, 14.8 g/l of butanol with a yield of 0.23 g/g total sugar could be produced in 33 h from inulinase treated Jerusalem artichoke hydrolysate without any additional nutritional supplement (Marchal et al., 1985). This process was scaled up to pilot scale (Marchal et al., 1985).

Starch based packing peanuts has also been used for butanol production by ABE fermentation using *C. beijerinckii* BA101. Total solvent including butanol of 18.9 g/l was produced from 80.0 g/l packing peanuts in 110 h (Ezeji et al., 2003). Madihah et al. (2001) reported the application of gelatinized sago starch as fermentation substrate for butanol production by *C. acetobutylicum*. They found that fermentation of this starch resulted in 16.0 g/l butanol production with a yield of 0.24 g/g glucose and a volumetric productivity of 0.21 g/l/h) as compared to those obtained with potato and tapioca starch, and the performance of which was equivalent to that obtained with corn starch (Madihah et al., 2001). Similarly, liquefied corn starch is another commercially available traditional substrate that has been used for butanol production by *C. beijerinckii* BA101 at concentrations comparable to those obtained with glucose (Ezeji et al., 2007).

Recently, cassava starch and chips have been used as fermentation substrates for butanol fermentation (Thang et al., 2010). In one such study, *C. saccharoperbutylacetonicum* N1–4 possessing inherently hyper amylolytic activity was used for butanol fermentation directly from starch material without additional hydrolysis step. Batch fermentations of cassava starch and cassava chip hydrolysate resulted in 16.4–16.9 g/l butanol with a yield of 0.26–0.35 g/g substrate and a volumetric productivity of 0.35–0.46 g/l/h (Thang et al., 2010). Although sucrose and starch are good substrates for butanol fermentation, increasing demand for these substrates for biofuel production has increased the prices of these feedstocks as well. Importantly, these crops potentially compete with food supply causing food versus fuel debate (Fig. 1). In this sense, lignocellulosic biomass described next is certainly the substrate of choice to be employed.

2.2. Lignocellulosic biomass

The possible answer for obtaining enough fermentable carbon substrates without getting into the competition with food supplies resides in the efficient utilization of plentiful lignocellulosic biomass available on the earth. Cellulose, hemicellulose and pectin are the most abundant polysaccharides (Fig. 1), which form the major fraction of agricultural, industrial, forest, and wood waste residues (Buschke et al., 2011). These polysaccharides are attracting much attention as cheap and readily available substrates that do not compete with food supply (Fig. 1). Biofuels obtained from lignocellulosic biomass has the additional advantage of lowering greenhouse gas emission as compared to those biofuels produced from corn (Cai et al., 2012). However, although abundant, it is rather difficult to obtain fermentable sugars from these lignocellulosic materials (Blanch et al., 2011). It requires physical, chemical, or biological pretreatment or their combinational treatments, before being utilized as fermentation substrates. Additionally, these treatments result in a hydrolysate that contains a mixture of different kinds of sugars as well as associated compounds. However, it is noteworthy that the solventogenic clostridia can utilize a wide range of carbohydrates, in particular both pentose and hexose (Tracy et al., 2012). Thus, considerable research effort has been made towards utilization of lignocellulosic hydrolysate for butanol fermentation (Al-Shorgani et al., 2012; Ezeji and Blaschek, 2008; Ezeji et al., 2007; Lu et al., 2012; Qureshi et al., 2008; Qureshi et al., 2007; Qureshi et al., 2010a; Qureshi et al., 2010b).

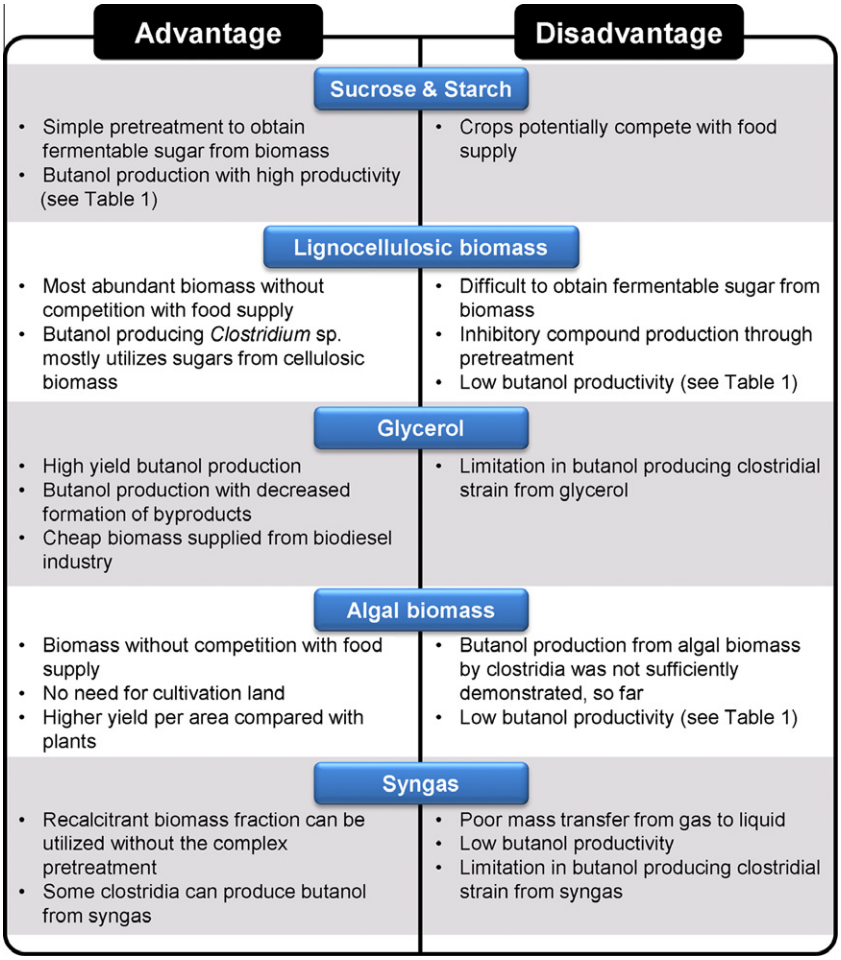


Fig. 1. Significance of renewable biomass for butanol production.

2.2.1. Agricultural residues

Among the various lignocellulosic substrates used for butanol fermentation, agricultural residues seem to be most interesting (Lopez-Contreras et al., 2000; Qureshi et al., 2008; Qureshi et al., 2007; Qureshi et al., 2010a; Qureshi et al., 2010b). In one such study, saccharides obtained from domestic organic waste (DOW) were utilized as substrate for butanol production by *C. acetobutylicum* ATCC 824 (Lopez-Contreras et al., 2000). The fresh and dried domestic wastes were treated by extrusion and the total sugar content in the polymeric fraction varied from 27.7% to 39.3% (w/w), in which glucose represented 18.4% and 25.1% of the materials for fresh and dried DOW respectively (Lopez-Contreras et al., 2000). Without any further nutrient supply, the *C. acetobutylicum* cells were grown on a suspension of 10% (w/v) extruded fresh DOW, which allowed production of 3 g/l butanol with a volumetric productivity of 0.03 g/l/h from 100 g DOW (Lopez-Contreras et al., 2000). The butanol titer and productivity was improved to 4.2 g/l and 0.09 g/l/h, when the DOW hydrolysed by combination of commercial cellulases and β-glucosidases was used (Lopez-Contreras et al., 2000). In another study, wheat straw hydrolysate has been used as a fermentation substrate for butanol production by *C. beijerinckii* P260 (Qureshi et al., 2007). Experiment was started with 60.2 g/l total sugar obtained by hydrolysis of 86.0 g/l wheat straw, which at the end resulted in the production of 12.0 g/l butanol with a productivity and yield of 0.29 g/l/h and 0.20 g/g total sugar (Qureshi et al., 2007). The obtained results were superior to the corresponding control experiments carried out with glucose (Qureshi et al., 2007). The hydrolysates of lignocellulosic biomass obtained from dried distiller's grains and soluble (DDGS) (Ezeji and Blaschek, 2008), rice bran (Lee et al., 2009a), corn fiber (Qureshi et al., 2008), and cassava bagasse (Lu et al., 2012) have also been evaluated as fermentation substrates for butanol production, which are summarized in Table 1. Similarly, barley straw hydrolysates (Qureshi et al., 2010a), corn stover hydrolysates (Qureshi et al., 2010b) and switchgrass hydrolysates (Qureshi et al., 2010b) have also been successfully used for butanol fermentation by *C. beijerinckii* P260 (Table 1). Similarly, wheat bran, a by-product of wheat milling industry, has been used as a fermentation substrate for butanol production using *C. beijerinckii* ATCC 55025 (Liu et al., 2010). Using the wheat bran hydrolysate containing 53.1 g/l total reducing sugar, including 21.3 g/l glucose, 17.4 g/l xylose and 10.6 g/l arabinose, 8.8 g/l of butanol could be produced in 72 h (Liu et al., 2010). Recently, rice bran and de-oiled rice bran have been used as the substrates for butanol production using *C. saccharoperbutylacetonicum* N1–4, which resulted in the production of 7.7 g/l butanol with a yield of 0.27 g/g total sugar and a volumetric productivity of 0.06 g/l/h (Al-Shorgani et al., 2012). The results obtained in the above studies suggest that lignocellulosic biomass can be efficiently utilized as a fermentation substrate for butanol production.

2.2.2. Effect of inhibitory compounds of lignocellulosic hydrolysates on butanol production

In the use of lignocellulosic biomass, the inhibitory compounds present in the lignocellulosic hydrolysate are major problems affecting fermentation performance (Ezeji et al., 2007). During

Table 1
Production of butanol from fermentation of varied carbon sources.

Carbon source	Microorganism	Strain	Fermentation time (h)	Substrates or consumed sugar	BuOH or (ABE) (g/L)	BuOH yield (g/g)		Productivity (g/l/h)	Reference
						on total sugar	on substrate		
Sugar and starch	*C. beijerinckii*	BA101	96	80 g/l spray dried soy molasses (34.7 g/l sugar)	8 (10.7)	0.23	0.10	0.08	(Qureshi et al., 2001)
	C. acetobutylicum	IFP904	33	65 g/l total sugar from inulinase treated Jerusalem artichoke hydrolysate	14.8 (22.1)	0.23	–	0.45	(Marchal et al., 1985)
	C. beijerinckii	BA101	110	80 g/l packing peanuts	–[a] (18.9)	–	–	–	(Ezeji et al., 2003)
	C. acetobutylicum	P262	75	60 g/l sago starch	16.0 (18.0)	0.24	–	0.21	(Madihah et al., 2001)
	C. beijerinckii	BA101	120	Liquefied cornstarch, LCS (44.9 g/l glucose)	13.4 (18.4)	0.30	–	0.11	(Ezeji et al., 2007)
	C. beijerinckii	BA101	78	Saccharified liquefied cornstarch, SLCS (45.7 g/l glucose)	13.4 (18.2)	0.29	–	0.17	(Ezeji et al., 2007)
	C. saccharoperbutylacetonicum	N1–4	36	62.6 g/l cssava chip hydrolysate	16.4 (23.1)	0.25	0.26	0.46	(Thang et al., 2010)
	C. saccharoperbutylacetonicum	N1–4	48	48.5 g/l cassava starch	16.9 (21.0)	0.33	0.35	0.35	(Thang et al., 2010)
Lignocellulosic biomass	*C. acetobutylicum*	ATCC 824	120	10% domestic organic waste (DOW)	3.0 (3.3)	–	–	0.03	(Lopez-Contreras et al., 2000)
	C. acetobutylicum	ATCC 824	48	10% DOW hydrolysate	4.2 (6.1)	–	–	0.09	(Lopez-Contreras et al., 2000)
	C. beijerinckii	P260	42	86 g/l wheat straw hydrolysate (60.2 g/l total sugar)	12.0 (25.0)	0.20	0.14	0.29	(Qureshi et al., 2007)
	C. saccharobutyli-cum	262	120	Dilute acid pretreated dried distiller's grains and soluble (DDGS)	7.3 (12.1)	0.21	–	0.06	(Ezeji and Blaschek, 2008)
	C. butylicum	592	72	Liquid hot water pretreated DDGS (48.8 g/l sugar)	7.0 (12.9)	0.17	–	0.10	(Ezeji and Blaschek, 2008)
	C. butylicum	592	72	AFEX pretreated DDGS (41.4 g/l sugar)	7.0 (11.6)	0.19	–	0.10	(Ezeji and Blaschek, 2008)
	C. beijerinckii	ATCC 55025	72	Wheat bran hydrolysate containing 53.1 g/l total sugar	8.8 (–)	0.17	-	0.12	(Liu et al., 2010)
	C. saccharoperbutylacetonicum	N1–4	128	Rice bran	7.7(–)	0.27	–	0.06	(Al-Shorgani et al., 2012)
	C. beijerinckii	BA101	88	Dilute sulfuric acid treated corn fiber (23.6 g/l sugar)	6.4 (9.3)	0.27	–	0.07	(Qureshi et al., 2008)
	C. acetobutylicum	JB200	40	cassava bagasse (44.8 g/l glucose)	9.71 (15.4)	0.22	–	0.24	(Lu et al., 2012)
	C. beijerinckii	P260	68	Lime treated barley straw (63.4 g/l sugar)	18.0 (26.6)	0.29	–	0.26	(Qureshi et al., 2010a)
	C. beijerinckii	P260	96	Corn stover hydrolysate (37.3 g/l sugar)	10.4 (16.0)	0.28	–	0.11	(Qureshi et al., 2010b)
	C. beijerinckii	P260	85	Lime treated switchgrass hydrolysates	14.5 (26.3)	–	–	0.17	(Qureshi et al., 2010b)
Glycerol	*C. pasteurianum*	–	–	60 g/l glycerol	17.0 (–)	0.20	0.28	-	(Biebl, 2001)
	C. acetobutylicum	ATCC 4259	20	13 g/l glycerol and 30 g/l glucose mixture; continuous culture	8.6 (9.2)	0.20	–	0.42	(Andrade and Vasconcelos, 2003)
	C. pasteurianum	ATCC 6013	240	21.5 g/l glycerol	7.8 (18.3)[b]	0.36	–	0.03	(Taconi et al., 2009)
	C. pasteurianum	DSM 525	41	24 g/l glycerol	6.2–7.2 (–)	~0.27	–	~0.16	(Ahn et al., 2011)
	C. pasteurianum	MBEL_GLY2		82.0 g/l glycerol	17.8(27.0)[b]	0.30	–	0.43	(Malaviya et al., 2012)
Algal biomass	*C. pasteurianum*	–	–	Mixture of algal biomass with 4% glycerol	– (14–16.0)	–	–	-	(Jones and Woods, 1986)
	C. saccharoperbutylacetonicum	N1–4	96	10% acid/base pretreated algae	2.3 (2.8)	0.20	–	0.02	(Klasson et al., 1991)
Syngas	*C. carboxidivorans*	$P7^T$	96	Carbon monoxide	0.002 (0.009)	–	–	-	(Vega et al., 1989)

[a] Not determined.
[b] Total solvents including butanol, ethanol, and 1,3-PDO.

the pretreatment of lignocellulosic materials, a number of associated compounds such as salts, furfural, hydroxymethylfurfural, acetic, ferulic, glucuronic, *p*-coumaric acids and phenolic compounds are also generated (Ezeji et al., 2007). Some of these compounds are known to be inhibitory to butanol fermentation (Fig. 1). Although generation of these inhibitors is substrate and pretreatment specific, methodologies for removal of these inhibitors need to be developed for the efficient utilization of lignocellulosic hydrolysates. In one such attempt, Ezeji et al. (2007) evaluated the impact of degradation products from agricultural biomass on the growth and butanol production by *C. beijerinckii* BA101. It was found that *C. beijerinckii* BA101 can utilize the individual sugars present in lignocellulosic hydrolysates such as cellobiose, glucose, mannose, arabinose and xylose (Ezeji et al., 2007). The *p*-coumaric acid and ferulic acids were found to be inhibitory at the concentration of as low as 0.3 g/l while furfural and hydroxymethyl furfural showed stimulatory effect on the growth of microorganism and butanol fermentation (Ezeji and Blaschek, 2008; Ezeji et al., 2007). Qureshi et al. (2008) have also reported that removal of fermentation inhibitors from alkaline peroxide pretreated and enzymatically hydrolyzed wheat straw significantly improved the butanol production by *C. beijerinckii* P260. Similarly, Cho et al. (2009) have investigated the effects of model phenolic compounds in lignocellulosic hydrolysates (*p*-coumaric acid, ferulic acid, 4-hydroxybenzoic acid, vanillic acid, syringaldehyde, and vanillin) on butanol production by *C. beijerinckii* NCIM 8052. The phenolic compounds tested at the concentration of 1 g/l were found to inhibit cell growth by 64–75% while production of butanol was completely inhibited. However, removal of phenolic compounds by peroxidase treatment resulted in improved cell growth and butanol production to the levels achieved with the control (Cho et al., 2009). Similarly, treatment of barley straw hydrolysate with lime has been reported to improve butanol production from about 4.5 g/l with a yield of 0.21 g/g total sugar and a productivity of 0.06 g/l/h (with untreated hydrolysate) to 18.0 g/l, 0.29 g/g and 0.26 g/l/h (Qureshi et al., 2010a). Similar results were obtained when corn stover and switch grass hydrolysates were used as fermentation substrate for butanol production (Qureshi et al., 2010b) (Table 1). These results suggest that detoxification of lignocellulosic hydrolysates is indeed required in order to maximize the butanol production from these substrates.

Currently the immediate factor impeding the utilization of cellulosic biomass as fermentation substrate for biofuel production on large scale is the high cost of pretreatment processing, rather than the cost or availability of feedstock. Thus, development of cost effective technologies to obtain fermentable sugar from lignocellulosic biomass is urgently needed to have a cost advantage over easily hydrolyzable raw materials such as corn. This can be achieved by several strategies such as development of improved cellulose hydrolyzing organisms as well as enzymes, development of improved biomass pretreatment process, development of metabolically engineered biomass that can be more easily hydrolyzed with less inhibitory compounds, and development of metabolically engineered microorganisms that are tolerant to the inhibitory compounds present in lignocellulosic hydrolysates.

2.2.3. Consolidated bioprocessing of cellulosic biomass

An alternative approach to produce butanol is the consolidated bioprocessing (CBP) of cellulosic biomass, which offers the advantage of cost effectiveness and higher efficiency with respect to processes involving dedicated cellulase production, biomass hydrolysis and finally butanol production (Lynd et al., 2005). In this respect, it is noteworthy that butanol producing *C. acetobutylicum* possesses a large extracellular enzyme complex called the cellulosome. As detailed in a recent review (Jang et al., 2012a), there has been effort to develop functional cellulosome for the efficient cellulose metabolism in *C. acetobutylicum*. Recently, Bokinsky et al. (2011) has demonstrated the coordinated engineering of all the genes involved in biomass degradation and biofuel production in a single organism, *Escherichia coli*. Initially, the cellulolytic and hemicellulolytic strains of *E. coli* were developed, which was followed by further engineering of these strains with three biofuel synthetic pathways. They demonstrated the fuel substitutes and precursors suitable for gasoline (butanol), diesel and jet fuel could be produced directly from the ionic liquid treated switchgrass without externally supplied hydrolase enzymes (Bokinsky et al., 2011). This demonstration can be considered as a major advance towards realization of the idea of biofuel production by CBP of lignocellulosic biomass. It has also been reported that during 1980s, a full scale continuous ABE fermentation plant was operated at industrial scale by exclusively using the lignocellulosic hydrolysate obtained from agricultural wastes (Zverlov et al., 2006). Thus, it is expected that butanol fermentation process will again be developed in an industrial scale using lignocellulosic biomass as a fermentation substrate in the near future.

2.3. Glycerol as an alternative substrate

Glycerol is an important renewable carbon source and has widely been used as a fermentation substrate for the production of industrially important products such as 1,3-propanediol, dihydroxyacetone, succinic acid, propionic acid, citric acid, ethanol, polyhydroxyalkanoates, and biosurfactants (da Silva et al., 2009). Also, there are a few reports on utilization of glycerol as fermentation substrate for butanol production (Biebl, 2001; Dabrock et al., 1992; Taconi et al., 2009). When used as a fermentation substrate, the highly reduced nature of glycerol provides it additional advantages over traditionally used C6 and C5 sugar. For example, conversion of glycerol into the glycolytic intermediates phosphoenolpyruvate (PEP) or pyruvate generates twice the amount of reducing equivalents compared with that generated from glucose or xylose (Yazdani and Gonzalez, 2007).

Clostridium pasteurianum has been reported as the only native producer of butanol by glycerol fermentation (Fig. 1). A maximum of 17.0 g/l butanol production has been reported by glycerol fermentation using *C. pasteurianum* (Biebl, 2001). This corresponds to an average butanol yield of 0.20 g/g. Several byproducts including 1,3-PDO, ethanol, acetate, butyrate and lactate were also detected in the fermentation broth under various fermentation conditions. A similar product tolerance and a better product yield were claimed by glycerol fermentation as compared to conventional starch or molasses based butanol fermentation. When this study was done, the higher glycerol price was suggested as a major disadvantage for the development of glycerol based butanol production process (Biebl, 2001). This scenario has been changed recently as glycerol is produced as a major co-product of biodiesel industry. The rapidly expanding market for biodiesel is dramatically altering the cost and availability of glycerol (Taconi et al., 2009); abundant availability of glycerol has resulted in significant decrease in the market price of glycerol. Once considered as an important byproduct of biodiesel industry, the crude glycerol generated during biodiesel production has now become a financial benefit for the biodiesel companies (Fig. 1). Hence, the need for advanced development and commercialization of innovative waste conversion technologies is more demanded than ever.

It has been reported that the feedstock cost for sugar based fermentation process is usually 60–80% of the total production cost and represents one of the most important factors affecting the cost of butanol production as well (Taconi et al., 2009). Similarly, Yazdani and Gonzalez (2007) have reported the reduction in feedstock as well as operational cost by using glycerol as compared to dry mill corn for ethanol production; the cost for ethanol production

can be reduced from US $1.05 per gallon to US $0.66 per gallon by replacing dry mill corn with glycerol. Literature reports suggest that if the butanol fermentation process can be integrated with a biodiesel production facility, there would essentially be no cost for the feedstock. This can result in significant decrease in the production cost for butanol as well as improvement in the economy of associated biodiesel production facility (Taconi et al., 2009).

During the continuous fermentation of *C. acetobutylicum* for 70 days, Andrade and Vasconcelos (2003) found that a mixture of glycerol and glucose can be used as a good substrate for butanol production. It was observed that low grade glycerol (65% w/v purity) can be efficiently used to replace the commercial grade glycerol (87% w/v purity). A maximum butanol yield of 0.34 (mol/mol) and butanol productivity of 0.42 g/l/h was obtained during this study (Andrade and Vasconcelos, 2003). Similarly, Taconi et al. (2009) has reported the utilization of glycerol obtained from biodiesel industry for butanol production using *C. pasteurianum*. During this study, they could achieve a maximum butanol titer of 7.8 g/l with a yield of 0.36 g/g. However, the productivity of this process was rather low at 0.03 g/l/h. Utilization of glycerol present in thin stillage obtained from ethanol industry has also been reported for butanol production using *C. pasteurianum* DSM 525; it was possible to produce a maximum of 6.2–7.2 g/l butanol (Ahn et al., 2011). Recently, Malaviya et al. (2012) reported the development of an efficient process for butanol production with significantly reduced byproduct formation using glycerol as a fermentation substrate. Under the optimized condition, the *C. pasteurianum* mutant strain MBEL_GLY2 was able to produce a maximum of 17.8 g/l butanol with the yield and productivity of 0.30 g/g and 0.43 g/l/h, respectively. The obtained results are comparable to those reported using glucose as a carbon source. Thus, it can be concluded that glycerol can efficiently be used as a fermentation substrate for butanol production.

3. Algae derived butanol production

3.1. Algae as an alternative substrate

Biofuel production using terrestrial biomass will only be beneficial if the biomass can be produced in a sustainable way without affecting environment and biodiversity; if not, the food *vs*. fuel debate surges again. Fuels based on terrestrial plants are controversial because they require cultivation area that could otherwise be used for growing food crops intended to supply food, feed and fiber to an expanding world population. A possible solution for this is the utilization of abundantly available algal biomass for sustainable biofuel production. Some of the advantages of using algal biomass over terrestrial biomass include no need for cultivation land, no requirement of fresh water due to their growth ability in sea water and wastewater, higher growth rate and higher yield per area compared with the terrestrial plants (Fig. 1). Polysaccharides in the cell wall account for a large proportion of the carbon contained in algae. Several algal species can produce more carbohydrates, which can be used as good fermentation substrate for butanol production. The suitability of the halophilic microalgae *Dunaliella* as a fermentation substrate has already been demonstrated; approximately 14.0–16.0 g/l mixed solvent including butanol could be produced by *C. pasteurianum* when a mixture of this algal biomass with 40 g/l glycerol was used as a fermentation substrate (Jones and Woods, 1986). Recently, pretreated algal biomasses such as, *Nannochloropsis* sp., *Arthrospira platensis,* and wastewater algae have been tested as substrates for butanol production by clostridia (Efremenko et al., 2012; Ellis et al., 2012). In one such study using 10% acid/base pretreated algae, batch fermentation of *C. saccharoperbutylacetonicum* N1–4 showed 2.3 g/l butanol production with a yield of 0.20 g/g total sugar and a volumetric productivity of 0.02 g/l/h (Ellis et al., 2012). The utilization of algal biomass for butanol production is getting more attractive. Thus, research effort needs to be exerted to use abundantly available algal biomass for the production of butanol.

3.2. Algal host for butanol production

Recently, the '*Photonol*' concept has been proposed for direct conversion of solar energy into the reducing power of fermentation end products such as biofuels by photosynthetic algae. According to this concept, a metabolic network is constructed by merging the phototrophic and fermentative metabolisms by using the tools and methods of synthetic biology. As a result, H_2O, CO_2 and solar energy are used as input for the production of fermentation products as output. This is referred as 'photofermentation', which will make the biofuel production economically attractive when the photosynthetic efficiency is further improved (Angermayr et al., 2009; Hellingwerf and Teixeira de Mattos, 2009). This strategy was employed for ethanol production by expressing two genes encoding pyruvate decarboxylase and alcohol dehydrogenase II in cyanobacteria, *Synechocystis* sp. PCC 6803 (Dexter and Fu, 2009). The Photonol concept is not limited to ethanol and other products including butanol can theoretically be produced by this technology (Angermayr et al., 2009). In another study, *Synechococcus elongates* PCC7942A has been metabolically engineered to directly capture CO_2 to produce isobutanol (Atsumi et al., 2009). The results were in agreement with those described in Photonol concept. Thus, it will be possible to develop a butanol producing photoautotrophic cyanobacterial strain by introducing the butanol producing pathway in a similar way. Again, it is expected that such butanol producing photoautotroph will revolutionize the entire biofuel sector when the photosynthetic efficiency can be further improved.

4. Advances in fermentation process development for improved butanol production

In order to establish a highly productive and cost competitive butanol fermentation system, attempts have been made to develop new fermentation strategies using inexpensive carbon substrates. Efforts to reduce the cost of fermentation substrates by utilization of alternative substrates have been reviewed in the previous section. In the present section, some of the innovative strategies recently developed to overcome the problems in butanol production by ABE fermentation are described (Table 2). Combination of these strategies can be applied for the development of a highly efficient butanol production process.

4.1. Advances in batch and fed-batch fermentation strategies

Batch fermentation offers simple operation and reduced risk of contamination (Lee et al., 2008). Much research effort has been exerted to improve the batch performance of butanol fermentation by applying various fermentation strategies. Simultaneous hydrolysis of biomass, such as wheat straw and cassava, to sugars and their conversion to butanol by ABE fermentation reported as an attractive option for the replacement of purified glucose (Marchal et al., 1984; Tran et al., 2010). Simultaneous hydrolysis and fermentation of wheat straw resulted in the butanol productivity of 0.27 g/l/h, which was similar to that (0.30 g/l/h) obtained with the control system using glucose as a fermentation substrate (Marchal et al., 1984). In another study, improvement in butanol production from starch has been reported by the co-culture of amylase producing *Bacillus subtilis* WD 161 strain with *Clostridium butylicum* TISTR 1032 (Tran et al., 2010). The microbial co-culture resulted in 10-fold increase in amylase activity while the butanol

Table 2
Recent fermentation and recovery process development for improved butanol production (since 2009).

Fermentation and recovery process	Microorganism	Strain	Strategy	Performance	Reference
Batch	*C. butylicum*	TISTR 1032	Co-culture of amylase producing *Bacillus subtilis* WD 161 with solventogenic clostridia	10-fold increase in amylase activity; butanol production from starch was improved by 5.4–6.5-fold	(Tran et al., 2010)
Fed-batch	*C. saccharoperbutylacetonicum*	N14	pH stat continuous lactic acid and glucose feeding	Production of 15.5 g/l butanol with 1.76 g/l/h productivity	(Oshiro et al., 2010)
Continuous	*C. pasteurianum*	MBEL_GLY2	High cell recycling using glycerol	710 h fermentation without strain degeneration, 7.8 g/l/h of butanol productivity	(Malaviya et al., 2012)
In situ product recovery	*C. acetobutylicum*	ATCC 824	*In situ* recovery of butanol by adsorption on polymeric resins	By application of 0.05 kg/L DowexOptipore SD-2 resin, 22.2 g/l butanol was obtained	(Nielsen and Prather, 2009)
	C. acetobutylicum	ATCC 824	Pervaporation processby application of a new polydimethylsiloxane (PDMS)/dual support compositemembrane	Max. volumetric glucose consumption rate increased 3.15 from 2.64 g/L/h (no pervaporation condition); average volumetric glucose consumption rate increased up to 2.11 from 1.62 g/L/h	(Li et al., 2011)
	C. acetobutylicum	JB200	Fibrous bed bioreactor with gas stripping	76.4 g/l butanol with a yield of 0.23 g/g substrate and a volumetric productivity of 0.29 g/l/h	(Lu et al., 2012)
	C. acetobutylicum	JB200	Gas stripping applied in fed-batch fermentation	Production of 113.3 g/L butanol from 474.9 g/L glucose during 326 h	(Xue et al., 2012)
	C. acetobutylicum	PJC4BK (pIPA3-Cm2)	Gas stripping applied in fed-batch fermentation	Production of 26 g/L butanol (35.6 g/L of IBE) during 45 h	(Lee et al., 2012a)
	C. beijerinckii	P260	Vacuum process (continuous, 4-h, 6-h, 8-h interval vacuum) with 7-L fermentation volume	76.4 g butanol production with productivity of 0.23 g/l/h (continuous); 85.1 g butanol production with productivity of 0.27 g/l/h (4-h interval); 103.0 g butanol production with productivity of 0.23 g/l/h (6-h interval); 84.6 g butanol production with productivity of 0.19 g/l/h (8-h interval)	(Mariano et al., 2011)

production was improved by 5.4 and 6.5 times as ABE of those obtained by using soluble and cassava starch, respectively (Tran et al., 2010). Additionally, it was also found that during this co-culture mediated butanol fermentation process, neither the addition of expensive reducing agent nor the flushing of nitrogen gas was required to ensure the anaerobic condition (Tran et al., 2010). Such new fermentation strategies can add significant value to the economy of anaerobic butanol fermentation process utilizing renewable biomass.

Fed-batch operation is an industrially preferred fermentation strategy as it allows higher concentration and productivity of the desired product. Several innovative fed-batch fermentation strategies have been developed for butanol production. In order to overcome the substrate and product inhibition during butanol fermentation, Ezeji et al. (2004) had applied an integrated fed-batch fermentation-gas stripping product recovery system with concentrated substrate feeding. The fed-batch fermentation was operated for 201 h and a total of 500 g glucose was utilized to produce 151.1 g butanol with the yield and productivity of 0.30 g/g and 0.75 g/l/h, respectively (Ezeji et al., 2004). Similarly, the fermentation strategy was used for the production of butanol from liquefied corn starch (Ezeji et al., 2007). Tashiro et al. (2004) has reported a pH-stat fed-batch strategy for butanol production using *C. saccharoperbutylacetonicum* N1–4. A mixture of glucose and butyric acid was used as a feed to maintain a constant pH and butyric acid concentration in the fermentation broth. Finally, a maximum of 16.0 g/l butanol production could be achieved at the butyrate to glucose ratio of 1.4 (Tashiro et al., 2004). Similarly, Oshiro et al. (2010) reported an efficient conversion of lactic acid to butanol by *C. saccharoperbutylacetonicum* N1–4 using the pH-stat continuous lactic acid and glucose feeding method; 15.5 g/l butanol with 1.76 g/l/h productivity could be achieved (Oshiro et al., 2010).

4.2. Continuous fermentation at high cell density

Continuous fermentation is an attractive fermentation strategy as it reduces the preparation time lag phase as compared to typical batch and fed-batch processes, and often results in the improved productivity (Lee et al., 2008). However, traditional continuous butanol fermentation system has an inherent limitation of cell wash-out, which seriously affects the productivity of the process. The problem of cell wash-out at higher dilution rate can be addressed by high cell density cultivation. High cell density continuous fermentation is particularly well suited when the microorganism grows slowly or is strongly affected by product inhibition. Owing to the good potential for operation at a higher dilution rate as well as reduced product toxicity, high cell density continuous fermentation by either cell immobilization or cell recycling offers higher productivities than batch, fed batch and free cell continuous fermentations. Due to the prevention of cell wash-out during high cell density continuous fermentation, the required fermentor volume to achieve the same productivity can be reduced. Three different reactor systems have been reported for the high cell density continuous butanol fermentation: biofilm-based bioreactors (Qureshi et al., 2005), membrane cell recycling bioreactors (Tashiro et al., 2004), and packed-bed bioreactors (Lienhardt et al., 2002).

Biofilm formation is a natural process where microbial cells attach to the support (adsorbent) or aggregates without the use of chemical and form thick layer of cells. It has been reported that such biofilm-based reactors exhibit higher productivities than those obtained by other fermentation strategies (Qureshi et al., 2005). Various types of biofilm reactor systems such as vertical packed bed reactor, horizontal packed bed reactor, plug flow reactor, compartmentalized reactor, double series reactor and fluidized bed reactors have been evaluated for butanol production. Among the various types of reactors examined, the highest butanol productivity was obtained with a continuous plug flow biofilm reactor, inoculated with *C. beijerinckii* BA101 cells (Lienhardt et al., 2002). To achieve high cell density by the formation of biofilm, *C. beijerinckii* BA101 cells were immobilized on clay bricks by adsorption. After the optimization of fermentation conditions with pervaporation, a maximum solvents productivity of 16.2 g/l/h could be achieved (Lienhardt et al., 2002).

Another important strategy used for achieving high cell density is the membrane cell recycling bioreactors (MCRB), where a hollow fiber ultramembrane filter is used to separate and recycle the cells into the bioreactor. Such reactors have the advantages of broth homogeneity and complete cell recycling as compared to the biofilm reactors containing immobilized cells. Thus, MCRB can significantly improve the volumetric productivity of a fermentation process. The high efficiency of MCRB for butanol production has been demonstrated by Tashiro et al. (2005). During continuous fermentation with total cell recycling of *C. saccharoperbutylacetonicum* N1–4, cell concentration of as high as 100 g/l could be achieved (Tashiro et al., 2005). Using this system, a maximum solvents (including butanol) productivity of 11.0 g/l/h at a dilution rate of 0.85 h^{-1} was obtained. Through the examination of the effects of cell bleeding along with cell recycling on fermentation performance, the optimal operation condition was found; the fermentation could be run for more than 200 h without strain degeneration and the overall volumetric productivity of 7.6 g/l/h could be achieved (Tashiro et al., 2005). Recently, Malaviya et al. (2012) have also reported the high cell density continuous butanol production using glycerol as a fermentation substrate. They were able to successfully operate this fermentation for 710 h without strain degeneration. The maximum volumetric productivity of butanol was found to be 7.8 g/l/h (Malaviya et al., 2012). The results of the above studies suggest that high cell density continuous fermentation has an excellent potential for improved butanol production for viable economic reasons.

4.3. Fermentation coupled with in situ product recovery

Butanol toxicity is one of the most critical problems of ABE fermentation. This limits the concentration of carbon substrate that can be used during fermentation and eventually results in low butanol concentration and productivity (Lee et al., 2008). In order to overcome the problem of butanol toxicity, *in situ* product recovery (ISPR) techniques have been developed with an aim to improve the process performance by reducing the product toxicity. As reviewed before (Lee et al., 2008), several ISPR techniques including adsorption, liquid–liquid extraction, perstraction (a combined membrane permeation and extraction by contacting the fermentation broth with an extracting solvent), reverse osmosis, pervaporation, and gas stripping have been evaluated for their efficiency and performance with respect to butanol production and recovery during ABE fermentation.

4.3.1. Pervaporation

Toxicity of extractant butanol towards microbial cells, loss of extractant during extraction, formation of emulsion and the accumulation microbial cells at the extractant and fermentation broth interphase limits the application of liquid–liquid extraction process. Recently, pervaporation based *in situ* butanol recovery during ABE fermentation has been reported (Li et al., 2011), in which a new polydimethylsiloxane (PDMS) dual support composite membrane was used. The performance of butanol fermentation using the integrated pervaporation fermentation process showed higher biomass concentration and glucose consumption rates than the control system (Li et al., 2011). The overall mass transfer coefficient during the separation of butanol from fermentation culture broth of *C. acetobutylicum* ATCC 824 was found to be 1.08 mm/h (Li et al., 2011). Irrespective of the impressive results obtained by membrane-based processes such as perstraction, reverse osmosis and pervaporation, membrane fouling and clogging problems are still common.

4.3.2. In situ product recovery by adsorption

Hence, ISPR by adsorption and gas stripping (see next section) can be considered as alternatives. Recently, Nielsen and Prather (2009) have reported the *in situ* recovery of butanol by adsorption on polymeric resins. Several resins were evaluated for their capabilities for butanol recovery during ABE fermentation by *C. acetobutylicum* ATCC 824. By the application of 0.05 kg/L Dowex Optipore SD-2 resin, it was possible to produce 22.2 g/l butanol, which is well above the inhibitory butanol concentration for *C. acetobutylicum* ATCC 824 (Nielsen and Prather, 2009). Butanol was recovered by mild thermal treatment and the resin was regenerated without loss of butanol affinity or structural integrity (Nielsen and Prather, 2009). It is expected that implementation of resin based *in situ* recovery process can result in dramatic increase in productivity and lowered operation costs associated with product purification (Nielsen and Prather, 2009).

4.3.3. Gas stripping

Irrespective of the initial encouraging results obtained by resin based ISPR process for butanol recovery, literature reports suggest gas stripping as one of the best techniques for butanol recovery (Ezeji et al., 2004). The advantages of gas stripping over other ISPR techniques used for butanol recovery include no harmful effect on fermentation, no requirement for membrane and expensive and harmful extractants, and no loss of nutrients and intermediates during the stripping. During the gas stripping, carrier gas such as H_2, CO_2 or N_2 is passed through a sparger resulting in the formation of gas bubbles in the bioreactor. Vaporized butanol through gas sparging is passed through condenser. While passing through a condenser, butanol becomes condensed and the carrier gas is recycled back to the bioreactor to recover more butanol (Ezeji et al., 2003; Ezeji et al., 2005; Lee et al., 2012a; Lee et al., 2008). Using this technique, the butanol productivity and yield could be improved by 200% and 118% as ABE, respectively, compared with the control batch fermentation (Ezeji et al., 2003). Similarly, the performance of butanol fermentation using liquefied corn starch was compared in batch fermentation carried out with and without *in situ* product recovery by gas stripping (Ezeji et al., 2007). When butanol was recovered by gas stripping during the fed-batch fermentation on saccharified liquefied corn starch, 56 g/l butanol was produced (Ezeji et al., 2007). Recently, concentrated cassava bagasse hydrolysate has been used for butanol production by *C. acetobutylicum* JB200 with gas stripping. The fed-batch fermentation with ISPR resulted in 76.4 g/l of butanol with a yield of 0.23 g/g substrate and a volumetric productivity of 0.29 g/l/h (Lu et al., 2012), while in the glucose fermentation 113.2 g/l of butanol produced from 474.9 g/l of glucose during 326 h (Xue et al., 2012).

A metabolically engineered strain has been successfully applied to this system to produce isopropanol-butanol-ethanol (IBE) (Lee et al., 2012a). The *sadh* gene of *C. beijerinckii* NRRL B-593 and the synthetic acetone operon were introduced into the *buk*-mutant, PJC4BK strain (Harris et al., 2000) to construct an IBE producer (Lee et al., 2012a). The resulting PJC4BK (pIPA3-Cm2) strain produced about 26 g/L of butanol (35.6 g/L of IBE) during the fed-batch fermentation with gas stripping in 45 h (Lee et al., 2012a).

4.3.4. Vacuum-based in situ product recovery

A vacuum-based ISPR process has also been reported for butanol recovery during ABE fermentation using *C. beijerinckii* P260 (Mariano et al., 2011). Application of vacuum-based integrated ISPR system resulted in complete substrate utilization, higher productivity, and improved cell growth in addition to the recovery of a concentrated butanol stream (Mariano et al., 2011). Comparison of the above results suggest that gas stripping might be, at least for now, the most economical and effective technology for *in situ* butanol recovery. It is evident that fermentation processes with inte-

grated ISPR techniques will be the choice of operation for fermentative butanol production.

4.4. Syngas and bioreactors suitable for gaseous substrates

Some clostridia can produce butanol from syngas, a mixture of carbon monoxide, carbon dioxide, and hydrogen (Jang et al., 2012a; Kopke et al., 2010; Munasinghe and Khanal, 2010; Tracy et al., 2012). Currently, syngas is produced by catalytic reforming of natural gas. Syngas production from biomass will also become an important technology in the future, because the recalcitrant biomass fraction such as lignin can be utilized without the complex pretreatment process. This is one of the merits of syngas fermentation for butanol production. Thus, bioreactors suitable for fermentation of gaseous substrates are briefly discussed in this section. Syngas fermentation for butanol production by clostridia is limited due to the poor mass transfer from gas to liquid. In one such study, *Clostridium carboxidivorans* P7 produced only 2.2 mg/l butanol in 4 days (Bruant et al., 2010). To enhance the production of butanol from syngas, the gas-phase substrates should be efficiently transferred and diffused through the culture medium to the cells. Bioreactors suitable for fermentation of gaseous substrates should maximize mass transfer from syngas to clostridial cells through liquid medium. Such suitable reactors well known for syngas fermentation are the stirred-tank reactor (Klasson et al., 1991), packed-bubble column (Shah et al., 1982), and hollow-fiber membrane reactor (Lee et al., 2012b). Continuous stirred-tank reactor has continuous gas flow into a liquid phase reactor, in which the agitation rate should be relatively high in order to promote transfer of the gas-phase substrate to the clostridia cells through the liquid medium. Packed-bubble columns provide high interfacial area and high mass transfer coefficients, although a large liquid retention time is needed. Recently, reactor equipped with a hollow fiber membrane diffuser which is a potential alternative to enhance the mass transfer into liquid media from gaseous substrate, has been adopted in syngas fermentation (Lee et al., 2012b). It is expected that development of reactors for butanol fermentation from syngas will be further addressed for industrial applications.

5. Future perspective on butanol production

Butanol has emerged as a 'superior biofuel' compared with other contemporary biofuels such as ethanol and biodiesel. In order to become cost competitive and used as a biofuel, its overall production cost needs to be reduced. Reduction in feedstock cost by utilization of renewable lignocellulosic biomass offers the best possible solution to ensure the sustainable production of low cost butanol. Utilization of lignocellosics is more applicable to butanol production using *Clostridium* sp. considering the broad substrate range and capability to utilize C6 as well as C5 sugars. This will improve the butanol productivity per unit lignocellosic biomass utilized and hence improve the economy of process. However, before switching to industrial scale butanol production using lignocellulosic biomass, further improvement to efficiently extract fermentable sugars from lignocellulosic biomass needs to be established. Additionally, integration of butanol production process with biodiesel production has been suggested to utilize the primary byproduct of biodiesel industry, crude glycerol, as fermentation substrate. This has been suggested to bring the economy for industrial production of both biodiesel as well as butanol. Alternatively, utilization of sugars from algal biomass can reduce the feedstock cost for butanol production. It is suggested that utmost care should be taken towards choice of strain as it determines the fermentation performance with a particular substrate. The chosen strain also influences the method of butanol recovery as well as the methods used for pre-treatment and hydrolysis of biomass. The selected strain can further be improved by the application of systems metabolic engineering that integrated synthetic biology and systems biology with metabolic engineering. Application of advanced bioprocess engineering techniques can further improve the process performance during process development for butanol production using renewable biomass. Development of a process for photoautotrophic production of butanol is an alternative concept that, if successfully developed, can ensure the sustainable production of this superior biofuel. Considering the worldwide energy demand, potential of butanol to act as fossil fuel substitute and worldwide efforts on various aspects of butanol production from various renewable biomasses, it is envisaged that sustainable butanol production on industrial scale can be realized in the near future. Summarizing, cost effective preparation of fermentable sugars from the least expensive and most abundant biomass, increased butanol titer, productivity and yield, increased tolerance to butanol, and development of cost effective purification method will be continuously pursued for the development of overall cost effective butanol process.

6. Conclusions

Butanol can be used as a fuel as well as an important industrial chemical. In order to make butanol production economically viable, it is important to use inexpensive non-food carbon substrates obtained from renewable biomass and develop efficient fermentation and downstream processing strategies. Through the optimization of the upstream (strain development by metabolic engineering and use of inexpensive carbon substrate), midstream (innovative fermentation strategies), and downstream (*in situ* recovery and other cost effective recovery) processes in an integrated manner, it is expected that a sustainable and cost effective process for butanol production will be realized in the near future.

Acknowledgements

This work was supported by the Technology Development Program to Solve Climate Changes (systems metabolic engineering for biorefineries) from the Ministry of Education, Science and Technology (MEST) through the National Research Foundation of Korea (NRF-2012-C1AAA001-2012M1A2A2026556) and by the Advanced Biomass R&D Center of Korea (2011-0028386) through the Global Frontier Research Program of the MEST. Further support by BioFuelChem and EEWS program of KAIST are appreciated.

References

Ahn, J.H., Sang, B.I., Um, Y., 2011. Butanol production from thin stillage using Clostridium pasteurianum. Bioresour. Technol. 102, 4934–4937.

Al-Shorgani, N.K., Kalil, M.S., Yusoff, W.M., 2012. Biobutanol production from rice bran and de-oiled rice bran by Clostridium saccharoperbutylacetonicum N1–4. Bioprocess. Biosyst. Eng. doi. http://dx.doi.org/10.1007/s00449-011-0664-2.

Andrade, J.C., Vasconcelos, I., 2003. Continuous cultures of Clostridium acetobutylicum: culture stability and low-grade glycerol utilisation. Biotechnol. Lett. 25, 121–125.

Angermayr, S.A., Hellingwerf, K.J., Lindblad, P., de Mattos, M.J., 2009. Energy biotechnology with cyanobacteria. Curr. Opin. Biotechnol. 20, 257–263.

Atsumi, S., Higashide, W., Liao, J.C., 2009. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat. Biotechnol. 27, 1177–1180.

Biebl, H., 2001. Fermentation of glycerol by Clostridium pasteurianum: batch and continuous culture studies. J. Ind. Microbiol. Biotechnol. 27, 18–26.

Blanch, H.W., Simmons, B.A., Klein-Marcuschamer, D., 2011. Biomass deconstruction to sugars. Biotechnol. J. 6, 1086–1102.

Bokinsky, G., Peralta-Yahya, P.P., George, A., Holmes, B.M., Steen, E.J., Dietrich, J., Soon Lee, T., Tullman-Ercek, D., Voigt, C.A., Simmons, B.A., Keasling, J.D., 2011. Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered Escherichia coli. Proc. Natl. Acad. Sci. USA 108, 19949–19954.

Bruant, G., Levesque, M.J., Peter, C., Guiot, S.R., Masson, L., 2010. Genomic analysis of carbon monoxide utilization and butanol production by Clostridium carboxidivorans strain P7. PloS one 5, e13033.

Buschke, N., Schroder, H., Wittmann, C., 2011. Metabolic engineering of Corynebacterium glutamicum for production of 1,5-diaminopentane from hemicellulose. Biotechnol. J. 6, 306–317.
Cai, Z., Zhang, B., Li, Y., 2012. Engineering Saccharomyces cerevisiae for efficient anaerobic xylose fermentation: reflections and perspectives. Biotechnol. J. 7, 34–46.
Cho, D.H., Lee, Y.J., Um, Y., Sang, B.I., Kim, Y.H., 2009. Detoxification of model phenolic compounds in lignocellulosic hydrolysates with peroxidase for butanol production from Clostridium beijerinckii. Appl. Microbiol. Biotechnol. 83, 1035–1043.
Dürre, P., 2007. Biobutanol: an attractive biofuel. Biotechnol. J. 2, 1525–1534.
da Silva, G.P., Mack, M., Contiero, J., 2009. Glycerol: a promising and abundant carbon source for industrial microbiology. Biotechnol. Adv. 27, 30–39.
Dabrock, B., Bahl, H., Gottschalk, G., 1992. Parameters affecting solvent production by Clostridium pasteurianum. Appl. Environ. Microbiol. 58, 1233–1239.
Dexter, J., Fu, P., 2009. Metabolic engineering of cyanobacteria for ethanol production. Energy Environ. Sci. 2, 857–864.
Efremenko, E.N., Nikolskaya, A.B., Lyagin, I.V., Senko, O.V., Makhlis, T.A., Stepanov, N.A., Maslova, O.V., Mamedova, F., Varfolomeev, S.D., 2012. Production of biofuels from pretreated microalgae biomass by anaerobic fermentation with immobilized Clostridium acetobutylicum cells. Bioresour. Technol. 114, 342–348.
Ellis, J.T., Hengge, N.N., Sims, R.C., Miller, C.D., 2012. Acetone, butanol, and ethanol production from wastewater algae. Bioresour. Technol. 111, 491–495.
Ezeji, T., Blaschek, H.P., 2008. Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia. Bioresource technology 99, 5232–5242.
Ezeji, T.C., Groberg, M., Qureshi, N., Blaschek, H.P., 2003. Continuous production of butanol from starch-based packing peanuts. Appl. Biochem. Biotechnol. 105–108, 375–382.
Ezeji, T.C., Karcher, P.M., Qureshi, N., Blaschek, H.P., 2005. Improving performance of a gas stripping-based recovery system to remove butanol from Clostridium beijerinckii fermentation. Bioprocess. Biosyst. Eng. 27, 207–214.
Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2004. 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. 63, 653–658.
Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2007. 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. 34, 771–777.
Green, E.M., 2011. Fermentative production of butanol–the industrial perspective. Curr. Opin. Biotechnol. 22, 337–343.
Harris, L.M., Desai, R.P., Welker, N.E., Papoutsakis, E.T., 2000. Characterization of recombinant strains of the Clostridium acetobutylicum butyrate kinase inactivation mutant: need for new phenomenological models for solventogenesis and butanol inhibition? Biotechnol. Bioeng. 67, 1–11.
Hellingwerf, K.J., Teixeira de Mattos, M.J., 2009. Alternative routes to biofuels: light-driven biofuel formation from CO2 and water based on the 'photanol' approach. J. Biotechnol. 142, 87–90.
Jang, Y.S., Lee, J., Malaviya, A., Seung, D.Y., Cho, J.H., Lee, S.Y., 2012a. Butanol production from renewable biomass: Rediscovery of metabolic pathways and metabolic engineering. Biotechnol. J. 7, 186–198.
Jang, Y.S., Park, J.M., Choi, S., Choi, Y.J., Seung, D.Y., Cho, J.H., Lee, S.Y., 2012b. Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. Biotechnol. Adv. 30, 989–1000.
Jiang, Y., Xu, C., Dong, F., Yang, Y., Jiang, W., Yang, S., 2009. Disruption of the acetoacetate decarboxylase gene in solvent-producing Clostridium acetobutylicum increases the butanol ratio. Metab. Eng. 11, 284–291.
Jones, D.T., Woods, D.R., 1986. Acetone-butanol fermentation revisited. Microbiol. Rev. 50, 484–524.
Kim, T.Y., Sohn, S.B., Kim, H.U., Lee, S.Y., 2008. Strategies for systems-level metabolic engineering. Biotechnol. J. 3, 612–623.
Klasson, K.T., Ackerson, M.D., Clausen, E.C., Gaddy, J.L., 1991. Bioreactors for synthesis gas fermentations. Resour. Conserv. Recy. 5, 145–165.
Kopke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., Ehrenreich, A., Liebl, W., Gottschalk, G., Durre, P., 2010. Clostridium ljungdahlii represents a microbial production platform based on syngas. Proc. Natl. Acad. Sci. USA 107, 13087–13092.
Krivoruchko, A., Siewers, V., Nielsen, J., 2011. Opportunities for yeast metabolic engineering: Lessons from synthetic biology. Biotechnol. J. 6, 262–276.
Lee, J., Jang, Y.S., Choi, S.J., Im, J.A., Song, H., Cho, J.H., Seung do, Y., Papoutsakis, E.T., Bennett, G.N., Lee, S.Y., 2012a. Metabolic engineering of Clostridium acetobutylicum ATCC 824 for isopropanol-butanol-ethanol fermentation. Appl. Environ. Microbiol. 78, 1416–1423.
Lee, J., Seo, E., Kweon, D.H., Park, K., Jin, Y.S., 2009a. Fermentation of rice bran and defatted rice bran for butanol 5 production using Clostridium beijerinckii NCIMB 8052. J. Microbiol. Biotechnol. 19, 482–490.
Lee, J.Y., Jang, Y.S., Lee, J., Papoutsakis, E.T., Lee, S.Y., 2009b. Metabolic engineering of Clostridium acetobutylicum M5 for highly selective butanol production. Biotechnol. J. 4, 1432–1440.
Lee, P.-H., Ni, S.-Q., Chang, S.-Y., Sung, S., Kim, S.-H., 2012b. Enhancement of carbon monoxide mass transfer using an innovative external hollow fiber membrane (HFM) diffuser for syngas fermentation: Experimental studies and model development. Chem. Eng. J. 184, 268–277.
Lee, S.Y., Park, J.H., Jang, S.H., Nielsen, L.K., Kim, J., Jung, K.S., 2008. Fermentative butanol production by clostridia. Biotechnol. Bioeng. 101, 209–228.
Li, S.Y., Srivastava, R., Parnas, R.S., 2011. Study of in situ 1-butanol pervaporation from A-B-E fermentation using a PDMS composite membrane: validity of solution-diffusion model for pervaporative A-B-E fermentation. Biotechnol. Prog. 27, 111–120.
Lienhardt, J., Schripsema, J., Qureshi, N., Blaschek, H.P., 2002. Butanol production by Clostridium beijerinckii BA101 in an immobilized cell biofilm reactor: increase in sugar utilization. Appl. Biochem. Biotechnol. 98–100, 591–598.
Liu, Z., Ying, Y., Li, F., Ma, C., Xu, P., 2010. Butanol production by Clostridium beijerinckii ATCC 55025 from wheat bran. J. Ind. Microbiol. Biotechnol. 37, 495–501.
Lopez-Contreras, A.M., Claassen, P.A., Mooibroek, H., De Vos, W.M., 2000. Utilisation of saccharides in extruded domestic organic waste by Clostridium acetobutylicum ATCC 824 for production of acetone, butanol and ethanol. Appl. Microbiol. Biotechnol. 54, 162–167.
Lu, C., Zhao, J., Yang, S.T., Wei, D., 2012. Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresour. Technol. 104, 380–387.
Lutke-Eversloh, T., Bahl, H., 2011. Metabolic engineering of Clostridium acetobutylicum: recent advances to improve butanol production. Curr. Opin. Biotechnol. 22, 634–647.
Lynd, L.R., van Zyl, W.H., McBride, J.E., Laser, M., 2005. Consolidated bioprocessing of cellulosic biomass: an update. Curr. Opin. Biotechnol. 16, 577–583.
Madihah, M.S., Ariff, A.B., Sahaid, K.M., Suraini, A.A., Karim, M.I.A., 2001. Direct fermentation of gelatinized sago starch to acetone–butanol–ethanol by Clostridium acetobutylicum. World J. Microbiol. Biotechnol. 17, 567–576.
Malaviya, A., Jang, Y.S., Lee, S.Y., 2012. Continuous butanol production with reduced byproducts formation from glycerol by a hyper producing mutant of Clostridium pasteurianum. Appl. Microbiol. Biotechnol. 93, 1485–1494.
Marchal, R., Blanchet, D., Vandecasteele, J.P., 1985. Industrial optimization of acetone-butanol fermentation: a study of the utilization of Jerusalem artichokes. Appl. Microbiol. Biotechnol. 23, 92–98.
Marchal, R., Rebeller, M., Vandecasteele, J.P., 1984. Direct bioconversion of alkali-pretreated straw using simultanesous enzymatic hydrolysis and acetone-butanol fermentation. Biotechnol. Lett. 6, 523–528.
Mariano, A.P., Qureshi, N., Filho, R.M., Ezeji, T.C., 2011. Bioproduction of butanol in bioreactors: new insights from simultaneous in situ butanol recovery to eliminate product toxicity. Biotechnol. Bioeng. 108, 1757–1765.
Munasinghe, P.C., Khanal, S.K., 2010. Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. Bioresour. Technol. 101, 5013–5022.
Nielsen, D.R., Prather, K.J., 2009. In situ product recovery of n-butanol using polymeric resins. Biotechnol. Bioeng. 102, 811–821.
Oshiro, M., Hanada, K., Tashiro, Y., Sonomoto, K., 2010. Efficient conversion of lactic acid to butanol with pH-stat continuous lactic acid and glucose feeding method by Clostridium saccharoperbutylacetonicum. Appl. Microbiol. Biotechnol. 87, 1177–1185.
Papoutsakis, E.T., 2008. Engineering solventogenic clostridia. Curr. Opin. Biotechnol. 19, 420–429.
Qureshi, N., Annous, B.A., Ezeji, T.C., Karcher, P., Maddox, I.S., 2005. Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microb. Cell Fact. 4, 24.
Qureshi, N., Ezeji, T.C., Ebener, J., Dien, B.S., Cotta, M.A., Blaschek, H.P., 2008. Butanol production by Clostridium beijerinckii. Part I: use of acid and enzyme hydrolyzed corn fiber. Bioresour. Technol. 99, 5915–5922.
Qureshi, N., Lolas, A., Blaschek, H.P., 2001. Soy molasses as fermentation substrate for production of butanol using Clostridium beijerinckii BA101. J. Ind. Microbiol. Biotechnol. 26, 290–295.
Qureshi, N., Saha, B.C., Cotta, M.A., 2007. Butanol production from wheat straw hydrolysate using Clostridium beijerinckii. Bioprocess. Biosyst. Eng. 30, 419–427.
Qureshi, N., Saha, B.C., Dien, B., Hector, R.E., Cotta, M.A., 2010a. Production of butanol (a biofuel) from agricultural residues: Part I - Use of barley straw hydrolysate. Biomass Bioenerg. 34, 559–565.
Qureshi, N., Saha, B.C., Hector, R.E., Dien, B., Hughes, S., Liu, S., Iten, L., Bowman, M.J., Sarath, G., Cotta, M.A., 2010b. Production of butanol (a biofuel) from agricultural residues: Part II - Use of corn stover and switchgrass hydrolysates. Biomass Bioenerg. 34, 566–571.
Roffler, S.R., Blanch, H.W., Wilke, C.R., 1988. In situ extractive fermentation of acetone and butanol. Biotechnol. Bioeng. 31, 135–143.
Shah, Y.T., Kelkar, B.G., Godbole, S.P., Deckwer, W.-D., 1982. Design parameters estimations for bubble column reactors. AIChE J. 28, 353–379.
Taconi, K.A., Venkataramanan, K.P., Johnson, D.T., 2009. Growth and solvent production by Clostridium pasteurianum ATCC® 6013™ utilizing biodiesel-derived crude glycerol as the sole carbon source. Environ. Prog. Sust. Energy 28, 100–110.
Tan, Y., Liao, J.C., 2012. Metabolic ensemble modeling for strain engineers. Biotechnol. J. 7, 343–353.
Tashiro, Y., Takeda, K., Kobayashi, G., Sonomoto, K., 2005. High production of acetone-butanol-ethanol with high cell density culture by cell-recycling and bleeding. J. Biotechnol. 120, 197–206.
Tashiro, Y., Takeda, K., Kobayashi, G., Sonomoto, K., Ishizaki, A., Yoshino, S., 2004. High butanol production by Clostridium saccharoperbutylacetonicum N1–4 in fed-batch culture with pH-Stat continuous butyric acid and glucose feeding method. J. Biosci. Bioeng. 98, 263–268.

Thang, V.H., Kanda, K., Kobayashi, G., 2010. Production of acetone-butanol-ethanol (ABE) in direct fermentation of cassava by Clostridium saccharoperbutylacetonicum N1–4. Appl. Biochem. Biotechnol. 161, 157–170.

Tracy, B.P., Jones, S.W., Fast, A.G., Indurthi, D.C., Papoutsakis, E.T., 2012. Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications. Curr. Opin. Biotechnol. 23, 364–381.

Tran, H.T.M., Cheirsilp, B., Hodgson, B., Umsakul, K., 2010. Potential use of Bacillus subtilis in a co-culture with Clostridium butylicum for acetone-butanol-ethanol production from cassava starch. Biochem. Eng. J. 48, 260–267.

Vega, J.L., Antorrena, G.M., Clausen, E.C., Gaddy, J.L., 1989. Study of gaseous substrate fermentations: carbon monoxide conversion to acetate. 2. Continuous culture. Biotechnol. Bioeng. 34, 785–793.

Wahrheit, J., Nicolae, A., Heinzle, E., 2011. Eukaryotic metabolism: measuring compartment fluxes. Biotechnol. J. 6, 1071–1085.

Xue, C., Zhao, J., Lu, C., Yang, S.T., Bai, F., Tang, I.C., 2012. High-titer n-butanol production by Clostridium acetobutylicum JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol. Bioeng.. http://dx.doi.org/10.1002/bit.24563.

Yazdani, S.S., Gonzalez, R., 2007. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18, 213–219.

Yu, M., Zhang, Y., Tang, I.C., Yang, S.T., 2011. Metabolic engineering of Clostridium tyrobutyricum for n-butanol production. Metab. Eng. 13, 373–382.

Zverlov, V.V., Berezina, O., Velikodvorskaya, G.A., Schwarz, W.H., 2006. Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. Appl. Microbiol. Biotechnol. 71, 587–597.