Preamble

This chapter presents a critical review of various aspects of biobutanol production starting from the selection of a suitable feedstock to the separation of the end-product that is butanol. Development taking place at every step of the butanol production i.e. feedstock selection, pretreatment/hydrolysis to get large amount of sugar content, fermentation of sugar to butanol and its recovery from fermentation broth are discussed along with the possible techniques for further improvement in the product yield. Various fermentation and downstream modeling aspects with mass balance calculations are also included for data validation and feasibility of large scale butanol production.

2.1 Introduction

The biomass based fuels are receiving increasing attention because their economically viable and environmentally safe nature. Among various biofuels (biobutanol, bioethanol, biodiesel, etc.), biobutanol is being considered as a suitable and sustainable fuel capable of replacing petroleum derived gasoline (Dutta et al. 2014a). Currently butanol, which is being used as solvent, is produced using propylene through the Oxo process. This involves hydrogenation in addition to hydroformylation whereas gasoline is derived through simple fractionation of crude petroleum (Ndaba et al. 2015). Hence replacement of gasoline by petro-derived butanol is certainly not a viable alternative, whereas butanol production through fermentation is a benign process and is also potentially an economical process. Lignocellulosic biomass is the commonly used raw-material at industrial scale for biobutanol production through ABE fermentation. Now the focus is shifting to a new route "Green Technology Route" i.e. utilization of algal biomass as raw-material for biobutanol production as it does not suffer from the disadvantages of lignocellulosic biomass (Efremenko et al. 2012). Further, applications of different nanocatalysts to overcome the existing challenges in the biobutanol field are also attracting the interest of researchers. Though algal biomass seems a potential viable feedstock, still a lot more research and developments is required to optimize the process parameters, select a suitable downstream processing strategy and scale-up the process.

2.2 ABE Production through Fermentation

2.2.1 Feedstocks

Available reported literature on butanol production through different feedstocks is summarized in Table 2.1. This table lists the optimum operating conditions and the challenges associated with feedstocks and process. Cheese whey, a dairy industry waste, has already been proved as an excellent feedstock over other lactose substrates for fermentative production of butanol (Becerra et al. 2015; Foda et al. 2010). The requirement of an edible biomass is the greatest roadblock for the popularization of the first generation biobutanol technology.

Recent reports reveal that due to large availability of lignocellulosic materials the sharp increase in biobutanol production has been achieved in countries having large cultivated area (Kumar et al. 2012). The non-food feedstock (agricultural wastes, wood cheaps, grains residues, etc) reduce the dependency on food materials and even minimize the cost of production (Swana et al. 2011). Utilization of the lignocellulosic biomass even at industrial scale seems economically viable for ABE production through fermentation (Kumar et al. 2012). Different agricultural residues such as rice, wheat and barley straw are easily available and are a good source of sugars that can be utilized for such a purpose. Gottumukkala et al. (2013) used 4%(w/w) H_2SO_4 pretreated rice straw and obtained biobutanol using *Clostridium sporogenes* BE01, a non-acetone producing bacteria. They reported that large concentration of inhibitors (formic acid and furfurals) is released due to the pre-processing of rice straw and obtained only 3.43 g/L of butanol yield while 5.52 g/L butanol was achieved from the detoxified (Amberlite resins) hydrolysate. Presence of the

large lignin content and thereby production of various inhibitors during pretreatment adversely affects the fermentation process (Lynd 1996). Qureshi et al. (2007) have reported a better butanol production from wheat straw without performing any detoxification process. Nearly 25.0 g/L of ABE (12.0 g/L of butanol) production was obtained from 1%(v/v) H_2SO_4 pretreated wheat straw (60.2 g/L sugars from 86.0 g/L of wheat straw) while increased solvent concentration (47.6 g/L of ABE) was obtained when the media was supplemented with 60.0 g/L glucose with continuous product recovery through gas stripping. The cellulosic and hemicellulosic contents of different lignocellulosic biomass vary largely. The activity of any pretreatment agent for releasing sugars depends upon the crystallinity of the structure and this could be the possible reason of the lower release of sugars from rice straw compared to wheat straw resulting in lower ABE production. The major disadvantages associated with lignocellulosic materials are geographical and seasonal variations, requirement of large arable land and water supply and their higher lignin content (Sun and Cheng 2002).

The problems associated with first and second generation feedstock have shifted the focus of researchers towards the evaluation of the third generation feedstock i.e. algal biomass which is available in large quantity throughout the world without the use of any arable land. Algae are the potential source of green renewable energy as they can assimilate CO_2 and remove inorganic nutrients from the effluent containing large concentrations of nitrogen and phosphorus (Oswald 2003). Algal biomass harvested from the effluent can also be used for the production of various liquid and gaseous biofuels (Ellis et al. 2012; Ullah et al. 2015). Microalgal carbohydrates are easily accessible and convertible to alcohols than macroalgae

(presence of alginate is the major hurdle) hence selection of a suitable algal strain with large carbohydrate content is an important step due to the variation in carbohydrate composition and other metabolites (Rangel-Yagui et al. 2004). Though some studies have been performed on the growth optimization of various cyanobacterial and green algal strains for increased carbohydrate content (Depraetere et al. 2015; Ho et al. 2012; Sun et al. 2014), however, no information is available on the utilization of such biomass for butanol fermentation and search for suitable algal species is still on for commercial production.

Wang et al. (2016) tested microalgae *Chlorella vulgaris* JSC-6 for efficient butanol production and achieved 13.1 g/L of butanol concentration using biomass pretreated with 1% NaOH and hydrolysed with 3% H_2SO_4. Nearly 97.5% glucose consumption proved the efficiency of the fermentation without any detoxification process. Various workers have reported the efficient utilization of lipid extracted algal residue for butanol fermentation that has proved the viability of the process for producing different biofuels (Cheng et al. 2015). Gao et al. (2016) used lipid extracted microalgal biomass of *Chlorella vulgaris* UTEX 2714 and reported the highest butanol concentration of 8.05 g/L with acid hydrolysates of hexane extracted microalgae. Comparative study of the fermentation process by using hydrolysate of ionic liquid extracted algae and hexane extracted algae has been performed and it has been concluded that detoxification is required with hexane extracted algae to support butanol production. Table 2.2 lists various cyanobacterial biomasses and the type of biofuels produced from them. Sufficient published information is also available on the production of other biofuels and value-added products from cyanobacteria while scanty information is available on the biobutanol production.

Table 2.1: Butanol production from various feedstocks

Feedstock	Microorganisms	Pretreatment & Hydrolysis	ABE Production (g/L)/time (h)	Remarks	References
Restaurant food waste	*C. beijerinckii* P260	Blending, autoclaving at 121°C for 15 mins	18.9/41	Maximum sugar utilization, integrated vaccum stripping system to avoid butanol toxicity to cells	Huang et al. (2015)
Corn steep liquor	*C. beijerinckii* BA101	1M HCl	81.3/120	Enhanced production due to the use of saccharified liquor and integrated fermentation-recovery technique	Ezeji et al. (2007b)
Palm kernel cake	*C. saccharoperbutylacetonicum* N1-4	Enzymatic hydrolysis (mannanase)	3.27 butanol/126	-	Shukor et al. (2016)
Sweet sorghum	*C. acetobutylicum*	Multi-stage hot water treatment (75°C, 7 stages)	18	Multi-stage hot water treatment for tannins removal, no need to add supplements for fermentation	Mirfakhar et al. (2017)
Pineapple peel	*C. acetobutylicum* B 527	Thermal & acidic (120°C & 1.3% (v/v) H_2SO_4)	5.23	Inhibitors (HMF, furfural, phenolics) removal using activated carbon, 95-97% phenolics reduction	Khedkar et al. (2017)
Distiller's dried grains	*C. beijerinckii* BA101	1% H_2SO_4 2.5% NaOH & enzymatic	5.46/96	Use of electrolyzed water reduces the concentration of toxins	Wang et al. (2013)

Wheat straw	*C. beijerinckii* P260	1% H_2SO_4 & enzymatic	21.42/72	Comparative analysis of different processes proves the efficiency of simultaneous saccharification and fermentation over others	Qureshi et al. (2008d)
	*C. beijerincki*i P260	Alkaline peroxide (1.2 M NaOH & 30% H_2O_2)	22.17/72	Inhibitors removal by electrodialysis technique using sodium sulfate as electrolyte and NaCl to trap ions	Qureshi et al. (2008c)
Corn fiber	*C. beijerinckii* BA101	0.5% H_2SO_4 & enzymatic	9.3/72	Lower yield due to the presence of inhibitors	Qureshi et al. (2008a)
Degermed corn	*C. beijerinckii* BA101	1 M HCl & enzymatic	14.28/110	Better yield from saccharified corn with long-term sustainability of bacteria in continuous mode	Ezeji et al. (2007a)
Barley straw	*C. beijerinckii* P260	1% H_2SO_4 & overliming & enzymatic	26.64/68	Use of $Ca(OH)_2$ followed by 1 g/L Na_2SO_3 to reduce toxicity	Qureshi et al. (2010a)
Corn stover	*C. beijerinckii* P260	1% H_2SO_4 & enzymatic	26.27	Large ABE production due to the overliming of hydrolysate to reduce effect of inhibitors on fermentation	Qureshi et al. (2010b)
	C. acetobutylicum CICC 8008	NaOH & enzymatic	6.2/70	Lower yield due to the presence of inhibitors	YouSheng et al. (2011)
Switchgrass	*C. acetobutylicum* ATCC 824	Hydrothermolysis followed by enzymatic	17/-	Efficient inhibitors (HMF, furfural, coumaric acid, syringic acid, vanillic acid, vanillin and cinnamaldehyde) removal using activated carbon	Liu et al. (2015a)
Wheat bran	*C. beijerinckii* ATCC 55025	0.75% H_2SO_4	11.8/72	Better feedstock due to the lower impact of inhibitors on fermentation process and microorganisms	Liu et al. (2010)

Rice straw	*C. acetobutylicum* NCIM 2337	1% H_2SO_4	13.5 butanol/288	Combined (physical & chemical) pretreatment results higher glucose release	Ranjan et al. (2013a)
	C. saccharo-perbutylacetonicum N1-4	-	7.9 butanol/240	High butanol production under non-sterile condition with high initial cell loading	Chen et al. (2013b)
	C. acetobutylicum NRRL B-591	Organosolv (75% v/v ethanol & 1% w/w H_2SO_4) & enzymatic	10.5/72	Organosolv pretreatment reduces the use of detoxification and improves the production efficiency	Amiri et al. (2014)
Sugarcane bagasse	*C. acetobutylicum* GX01	1% NaOH (60°C, 3days) & enzymatic	21.11/60	Nearly complete hydrolysis of pretreated biomass using extracted enzymes from *Thermoascus aurantiacus*,	Pang et al. (2016)
	C. acetobutylicum XY16	Microwave-alkali pretreatment (400 W & 1% NaOH)	14.26/60	Large fermentable sugar release with the gamma-valerolactone (GVL) assisted hydrolysis	Kong et al. (2016)
	C. acetobutylicum CH02	1% H_2SO_4 (140°C, 1 h) & oxidate ammonolysis & enzymatic	12.12/120	-	Li et al. (2017)
Sweet sorghum bagasse	*C. acetobutylicum* ABE 1201	0.2% w/v CH_3COOH	20.9/72	94.5% of toxin (furfural) reduction by pervaporation and 87.5% phenolic compounds degradation by laccase detoxification	Cai et al. (2013)
Bamboo	*C. beijerinckii* ATCC 55025-E604	Simultaneous pretreatment & saccharification (laccase & cellulase)	6.45 butanol/73	Lower production due to the absence of initial pre-processing of biomass	Kumar et al. (2017)

Willow biomass	*C. beijerinckii* NCIMB 8052	72% H_2SO_4	Stem – 4.5 Bark – 4.3/96	Hindrance in phase conversion from acidogenic to solventogenic	Han et al. (2013)
Arthrospira platensis	*C. acetobutylicum*	Thermal & 0.1mM H_2SO_4	0.43 butanol/96	Lower butanol yield due to the lower soluble carbohydrate	Efremenko et al. (2012)
Ulva lactuca	*C. acetobutylicum* *C. beijerinckii* *C. saccharoperbutylicum* ATCC 27021	6% NaOH & 7.5% H_2SO_4 & enzymatic 6% NaOH & 7.5% H_2SO_4 & enzymatic 1% H_2SO_4 (125°C, 30 min)	0.8/140 4.5/140 4 g/L butanol	Inability to consume total released sugar (glucose & rhamnose) Comparatively large production due to rhamnose consumption More suitable for field experiments due to higher air tolerant capacity	Vander Wal et al. (2013) Vander Wal et al. (2013) Potts et al. (2012)
Wastewater algae	*saccharoperbutylacetonicum* ATCC 27021	Autoclave (121°C, 1 h)	0.13 g butanol/g biomass	Lower yield due to the presence of more non-fermentable sugars	Jernigan et al. (2013)
Laminaria digitata	*C. beijerinckii* DSM-6422	Enzymatic hydrolysis (10% v/w, 24 h)	8.13/100	Efficiently utilized lactic acid and alginate from biomass hydrolysate	Hou et al. (2017)

HMF: 5-hydroxymethyl-furfural

Table 2.2: Cyanobacteria used for various biofuels production

Cyanobacterial Biomass	Biofuel	Titer	Reference
Synechocytis sp. PCC 6803	Acetone	36 mg/L	Zhou et al. (2012)
Synechocystis sp. PCC 6803	Ethanol	5.5 g/L	Gao et al. (2012)
Synechococcus elongatus PCC 7942	Ethanol	182 mg/L	Lan et al. (2013)
Synechococcus elongatus PCC 7942	Isobutanol	450 mg/L	Atsumi et al. (2009)
Synechococcus elongatus PCC 7942	n-butanol	14.5 mg/L	Lan and Liao (2011)
Synechococcus elongatus PCC 7942	n-butanol	30 mg/L	Lan and Liao (2012)
Synechococcus elongatus PCC 7942	n-butanol	317 mg/L	Lan et al. 2013

2.2.2 Pre-processing of feedstocks

Pretreatment of the raw-biomass is one of the most important upstream operations which significantly improves the fermentation efficiency through improved release of sugars (Durre 2007). Different pretreatment techniques such as physical, chemical and physico-chemical have been used by different workers. The selection of these techniques heavily depends on the characteristic and composition of feedstock. The easy accessibility and larger amount of sugar makes the first generation feedstock the most attractive raw-material. This is the main reason of nearly no requirement of complex pretreatment techniques for these biomass (Mohanram et al. 2013). Only basic pretreatment processes such as dilute acid treatment, heat sterilization and tyndallization etc, have been utilized for these feedstocks for improved sugar release into fermentation broth (Li et al. 2013). Raganati et al. (2013) used cheese whey in a biofilm packed bed reactor for butanol production by using the bacteria *Clostridium acetobutylicum* DSM 792. Different pretreatment techniques (heat sterilization, wet tyndallization, dry tyndallization, and deproteinization) have been used for cheese whey, among those deproteinization has resulted in maximum butanol concentration of 8.9 g/L.

Heat sterilization and tyndallization are good pretreatment techniques for food crops but with cheese whey these led to clot formation causing lower butanol yield (Raganati et al. 2013).

The complexity associated with the second generation feedstock sometimes imposes limits on its utilization as it contains different complex molecules such as cellulose, hemicellulose, lignin etc, hence a comprehensive pretreatment is required to alter these complex structures into accessible sugar (Moxley et al. 2008). The pretreatment helps in the removal of lignin, reduction of cellulose/hemicelluloses, improves the porosity of the feedstock and facilitates the formation of sugar, minimization of carbohydrate losses and the elimination of byproducts formation inhibitory to fermentation and thus makes the process more cost effective (Sun and Cheng 2002). On the other hand relatively simple nature of third generation feedstock and amenability to simple pretreatment processes make it viable contender for biobutanol production.

2.2.2.1 Physical pretreatment

Physical pretreatment techniques are almost universally employed for every feedstock prior to subjecting them to subsequent treatment strategies such as chemical and biological/enzymatic (Table 2.3). This strategy increases the surface area of the raw-material by reducing their particle sizes and results in the separation of important botanical parts of the feedstock into different fractions which can then be used as feedstock for relevant applications (Barakat et al. 2015). Physical treatment is a dry process which eliminates water usage and hence effluent discharge facilitating sustainability. Among the physical

techniques listed in Table 2.3, extrusion technique seems to be the most effective due to its large cellulose and lignin alteration that increases the efficiency of other treatment (chemical/biological) vis-à-vis butanol production. The important factors on which applicability of physical pretreatment depends are, characteristics of biomass, energy demand, moisture content, particle size and degree of modification required in the tissues (Barakat et al. 2015). The greatest disadvantage of physical pretreatment processes is their high specific energy requirement.

2.2.2.2 Chemical pretreatment

Various chemical pretreatment agents such as ozone, acid, alkali, peroxides and organosolvs have been used for the pretreatment of raw-materials as summarized in Table 2.4. This technique alters the structure of raw-materials and large lignin removal improves the enzymatic hydrolysis of the materials (Mood et al. 2013). Use of combined pretreatment technique is a better approach to alter the complex structure and improve the effectiveness of further processes.

2.2.2.2.1 Acid pretreatment

Acidic pretreatment is highly efficient as it reduces hemicelluloses to xylose. Dilute acids (H_2SO_4, HCl, etc.) can be used as concentrated acids are highly toxic, corrosive and harmful (Mosier et al. 2005). Acid pretreatment can be carried out either at high temperature with low solids loading (5-10%) or at lower temperature with high solids loading (10-40%) for increasing the cellulose hydrolysis significantly (Esteghlalian et al. 1997). Up to 83% sugar yield has been achieved with 1% H_2SO_4 treatment at 160-180°C within 1-5 min followed by

enzymatic (β-galactosidase) hydrolysis confirming the higher rate of reaction and increased sugar yield at higher temperature (Hsu et al. 2010). Kudahettige-Nilsson et al. (2015) examined the suitability of the xylose recovered from Kraft black liquor for ABE fermentation and found the efficient utilization of xylose (95%) for the production of total 9.4 g/L of ABE that confirmed the efficiency of acid hydrolysis of both cellulose and hemicellulose. In the case of algal biomass dilute acid pretreatment is sufficient to destruct the complex polymeric structure due to the negligible lignin content. Native Clostridial *sp*. can tolerate about 1 g/L of furfural and 2 g/L of 5-hydroxymethyl furfural. The algal hydrolysate contains very small quantity of these toxins so detoxification is not required with this biomass (Wang et al. 2015). Castro et al. (2015) optimized the sugar release from wastewater algae using H_2SO_4 (0-1.5 M) treatment. Nearly 166.1 g of sugar per kg of dry algae was obtained by treating the biomass with 1 M H_2SO_4 at 80-90°C for 120 min, subsequent fermentation of released sugar resulted in 3.74 g/L of butanol production.

2.2.2.2.2 Alkali pretreatment

Alkali pretreatment of raw-materials facilitates delignification and solubilization of hemicelluloses in large quantities (Zhao et al. 2008). Only drawback is the longer time of treatment for the release of sufficient quantity of sugar to be used for fermentation (Kumar et al. 2009; Zhu and Pan 2010). Dilute acid pretreatment is a successful strategy but only with the low lignin containing raw-material such as hardwood and algal biomass while alkali pretreatment is more suitable for softwood that yields comparatively higher cellulose and hemicelluloses for enzymatic hydrolysis (Zhu and Pan 2010).

Table 2.3: Physical pretreatment of biomass

Technique	Solubilization	Crystallinity	Lignin structure	Toxin release	Others	References
Comminution (wet & dry milling)	-	Gets reduced	-	Nil	Increases surface area, Particle size: 0.003-30 mm	Hendriks and Zeeman (2009)
Extrusion	-	Increased cellulose digestibility	Large alteration	Low (furfural/HMF)	Pressure: 0.45-3.5 MPa; Temperature: 40-150°C; Time: 4-12 min	Zheng and Rehmann (2014)
Microwave irradiation	Less solubilization of hemicellulose	Gets reduced	-	Low (furfural/HMF)	Temperature: 160-250°C; Time: few minutes to hours	Li et al. (2016)
Pyrolysis	-	Gets reduced	Lower impact	Low	Temperature: 500°C High heat transfer	Das and Sarmah (2015)
γ-irradiation	Less solubilization of hemicellulose	Gets reduced	Lower impact	Low	Costly process and industrially nonfeasible	Kumar et al. (2009), Liu et al. (2015b)

Table 2.4: Chemical pretreatment of biomass

Technique	Solubilization	Crystallinity	Lignin structure	Toxin release	Others	References
Acidic	High	Increased cellulose digestibility	Moderate alteration	High	Neutralization of filtrate to avoid corrosion	Carvalheiro et al. (2008)
Alkali	High	-	Large alteration	High	Costly and additional neutralization of the filtrate	Zhao et al. (2008), Hendriks and Zeeman (2009)
Peroxides	Moderate	-	Large alteration	-	-	Sun and Cheng (2002)
Ozonolysis	Moderate	-	Moderate alteration	Nil	Industrially unfeasible due to large ozone requirement	Kumar et al. (2009),
Organosolvs	-	-	Efficient destruction of lignin-carbohydrate matrix	High	Inhibitory effect on enzymatic hydrolysis, costly	Chen et al. (2015)

Alkali pretreatment of raw-material involves the saponification of intermolecular bonds and increases the porosity of material by disrupting the complex polymeric structure (Zheng et al. 2014). Comparative evaluation of different alkaline agents such as NaOH, KOH, aqueous ammonia and sodium carbonate has been investigated for various feedstocks such as rice straw, eucalyptus residue, pinus and barley straw, etc for accessing the efficacy of the treating agents towards the digestibility of the material for sugar release (Park and Kim 2012). Higher enzymatic digestibility (95.0%) has been reported for barley straw soaked in 15% aqueous ammonia. However, NaOH was found to be more efficient alkali for increasing the internal surface area of the cellulose, rupturing the lignin and reducing the degree of polymerization and crystallinity of the biomass structures. Gao et al. (2014) used 1% (w/v) NaOH for the pretreatment of switchgrass and phragmites biomass. Lignin content in switchgrass was reported as 24.49 and in phragmites as 28.83 g/100g of raw biomass. The reducing sugar release of 365 and 385 g/kg of raw biomass from switchgrass and phragmites, respectively, were reported that proved the effectiveness of the pretreatment conditions.

2.2.2.3 Other pretreatment techniques

Various other pretreatment strategies such as physico-chemical and organosolv have also been employed prior to ABE fermentation. The steam explosion is the most viable physico-chemical treatment that involves two basic steps i.e. auto-hydrolysis and de-pressurization. Auto-hydrolysis involves the formation of acetic acid at high temperature and de-pressurization ruptures the bonds present in the complex structure (Liu et al. 2013). One drawback with this process is the release of large amount of inhibitory compounds due to

incomplete break down of lignin-carbohydrate matrix (Sun and Cheng 2002). Wang and Chen (2011) used steam-exploded corn stover (SECS) for the analysis of effect of inhibitory compounds and the soluble lignin content was found to be inhibitory above the concentration of 1.77 g/L. Activated charcoal was used to adsorb the soluble lignin and alkaline peroxide treatment was adopted to reduce the inhibitors formation during the hydrolysis. Use of acids in steam explosion decreases the formation of inhibitors significantly by improving the hydrolysis efficiency of raw-material (Kumar et al. 2009). Other physico-chemical pretreatment techniques (liquid hot water, ammonia fiber explosion, supercritical CO_2 explosion, wet air oxidation) are less commonly used due to their limitations of lignin and hemicelluloses solubilization (Sun and Cheng 2002).

Organosolv process involves an organic or aqueous organic phase with an inorganic catalyst (HCl, H_2SO_4, NaOH) and helps in delignification. Ethanol is commonly used for organolv pretreatment due to its lower toxicity, higher boiling point and low combustion potential. Amiri et al. (2014) prepared hydrolysate from rice straw by using 75% (v/v) aqueous ethanol with 1% (w/w) H_2SO_4 as catalyst at 150°C for 1 h and obtained 31 g/L of sugar release after enzymatic hydrolysis and 10.5 g/L of ABE production. Salapa et al. (2017) performed the comparative evaluation of different solvents (ethanol, methanol, butanol, acetone, diethylene glycol) along with 23 $mol \cdot m^{-3}$ H_2SO_4 as catalyst for the treatment of wheat straw. Similarly ethanol pretreated biomass resulted in maximum cellulose conversion (89%) at 180°C within 40 min and nearly 60% of lignin removal. Tang et al. (2017) also used ethanol (60% v/v) with NaOH (4% w/w) as catalyst for cornstalks. Higher lignin removal (more than 80%) and lower hemicelluloses degradation were achieved at 110°C while enzymatic

hydrolysis resulted in 85% of cellulose and 82% of hemicelluloses conversion to fermentable sugars and subsequent 12.8 g/L of total ABE production. Effectiveness of the further processes is increased significantly after the organosolv pretreatment. Still it is economically unattractive due to the high cost of the solvent and need for an elaborate detoxification process (Chen et al. 2015).

2.2.2.4 Enzymatic hydrolysis

It is important to have an enzymatic hydrolysis step after the pretreatment of lignocellulosic biomass while it is optional for algal biomass for completing the pre-processing step. Enzymatic hydrolysis is highly efficient due to the availability of component specific enzymes for the breakdown of large complex structure into its monomers. According to the available literature utility cost of enzyme is much lower compared to other treatment options and is also free of the corrosion problem (Duff and Murray 1996). Enzymatic hydrolysis results into increase in sugar release by several folds and up to 8 fold increase in ABE production has been reported with the use of cellulase enzyme, signifying its utility and economics (Ponthein and Cheirsilp 2011). It is the intrinsic properties of fungi to release some specific enzymes such as cellulases, β-glucosidases, hemicellulases, etc and the exploitation of this capability of microorganisms could be an excellent approach to reduce the cost of enzymatic treatment for butanol production (Saritha et al. 2012). Higher FPase (0.25 FPU/mL), CMCase (0.18 IU/mL), xylanase (5.8 IU/mL) and β-glucosidase activities were found in *Trichoderma atroviride* fungal strain isolated from switchgrass bales that enhanced the saccharification efficiency for butanol production (Jain et al. 2014). Various factors such as biomass particle size and loading, cellulose crystallinity and degree of

polymerization, lignin percentage and its distribution, reaction heterogeneity, enzyme binding onto the surface and thermal inactivation of enzymes, etc, affect the hydrolysis efficiency (Esteghlalian et al. 2000). Yoshida et al. (2008) reported a large monosaccharide yield (90% cellulose and nearly 100% hemicellulose hydrolysis from delignified biomass) with decrease in biomass crystallinity of *Miscanthus sinensis*. A linear relationaship between biomass loading and enzymatic hydrolysis was observed by Cara et al. (2007) for olive tree biomass and by Ramachandriya et al. (2013) for Eastern redcedar biomass. Hydrolysis of pretreated olive tree residue was done with commercially available cellulase and β-glucosidase by changing the solids loading in the range of 2–30% (w/v). Large glucose release was found with 30% solid loading of hot water pretreated and delignified biomass (73.0 g/L) while only 61.0 g/L was found with steam-exploded and delignified biomass at 20% solids loading within 72 h. Delignification increases the susceptible sites for enzymes on polysaccharide structure resulting in better sugar release. Incubation temperature is another important factor that affects the enzyme activity and stability. Bravo et al. (2000) studied the effect of temperature (40-70°C) on cellobiose hydrolysis and found enzyme deactivation above 60°C.

Solid State Fermentation (SSF) is an efficient technique accepted industrially for the production of various enzymes (Sajith et al. 2016). Robledo et al. (2016) reported the production of extracellular thermostable xylanase by SSF using isolated thermophilic (>55°C) fungal strains of *Aspergillus* and *Rhizomucor* and corn cob as the support substrate material. *Rhizomucor pusillus* SOC-4A strain produced thermostable xylanase possessed better enzymatic activity (nearly 824 U/g) at 70°C and thermally stable up to 75°C. Mahajan

et al. (2016) efficiently extracted lignocellulolytic enzymes (glycosyl hydrolases, polysaccharide lyases, carbohydrate esterases and cellobiose dehydrogenase) from thermophilic fungus *Malbranchea cinnamomea* using sorghum straw as C-source. Nearly 5.7 fold increased saccharification efficiency was reported in the presence of Mn^{2+} on the alkali treated carrot grass due to the presence of metal dependent enzymes.

Enzyme activity and stability are of major concern during the processing of pretreated biomass, and application of nanoparticles for such purpose has aroused much interest (Dutta et al. 2014b). Different metal nanoparticles have been reported to function as a support material for enzyme immobilization that also increases the enzymatic activity and stability (Srivastava et al. 2016). Srivastava et al. (2014) used $NiCo_2O_4$ nanoparticles for cellulase enzyme production from *Aspergillus fumigatus* NS and obtained improved thermal stability of enzyme under the studied conditions. Nearly 40% increased filter paper activity was observed with addition of 1 mM $NiCo_2O_4$ nanoparticles in the media. Other enzymatic activities such as endoglucanase, β-glucosidase and xylanase were also affected by 49, 53 and 19.8%, respectively. Thermal stability of the produced enzyme increased up to 7 h at 80°C (with nanoparticles) while the control sample was stable only for 4 h at the same temperature. These results confirm the usefulness of nanoparticles for enhanced bioconversion processes as well as improved enzymatic activity and stability. Reusability of the enzymes due to the immobilization over the nanoparticles surface proves the cost-effectiveness of the process as well.

2.2.3 Microorganism and metabolic pathway

An obligate anaerobe, gram positive and spore forming bacteria *C. acetobutylicum* commonly known as Weizmann's organism is the earliest utilized microbe at industrial scale for acetone and butanol fermentation from sugar and starchy grains (Garcia et al. 2011). Various carbohydrates like glucose, fructose, mannose, sucrose, lactose, starch and dextrins are completely consumed while galactose, xylose, arabinose, raffinose and mannitol are partially fermented by Clostridial bacteria, whereas this strain cannot ferment carbohydrates like trehalose, rhamnose, melibiose and glycerol (Kumar and Gayen 2011). The most widely used and efficient butanol producing Clostridial strains are *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum*. These natural strains cannot tolerate butanol concentrations beyond 13-20 g/L (varies from species to species) (Garcia et al. 2011).

Fermentative butanol production completely depends on the metabolic activity of microorganisms and it is better to understand the metabolic mechanism to enhance the solvent productivity. Native clostridium strains (*C. acetobutylicum* and *C. beijerinckii*) follow a similar solvent production pathway (Figure 2.1) that can be broadly categorized into two phases the acid producing phase and the solvent producing phase (Kumar and Gayen 2011). Exponential bacterial growth takes place during the acidogenesis phase along with the formation of various intermediary acids (mainly acetic and butyric), leading to decrease in media pH to ~4.5 (Gheshlaghi et al. 2009). Prime precursor of this sugar conversion pathway is acetyl-CoA for the synthesis of acetone, butanol, ethanol, acetate and butyrate under anaerobic condition. Synthesis of acetyl-CoA and butyryl-CoA is controlled

by various enzymes viz., thiolase, dehydrogenase and crotonase that further direct the synthesis of acetate and butyrate, respectively under the control of different enzymes i.e. transferase and kinase (Jiang et al. 2009). Shift in metabolic activity from acidogenic to solventogenic occurs to compensate for the lower pH condition. Slow microorganism growth was found during solventogenesis while exponential increase in ABE concentration and slight increase in pH has been reported due to the consumption of acetate and butyrate (Kumar and Gayen 2011). However, metabolic activity of ABE fermentation and duration of phases differ from species to species.

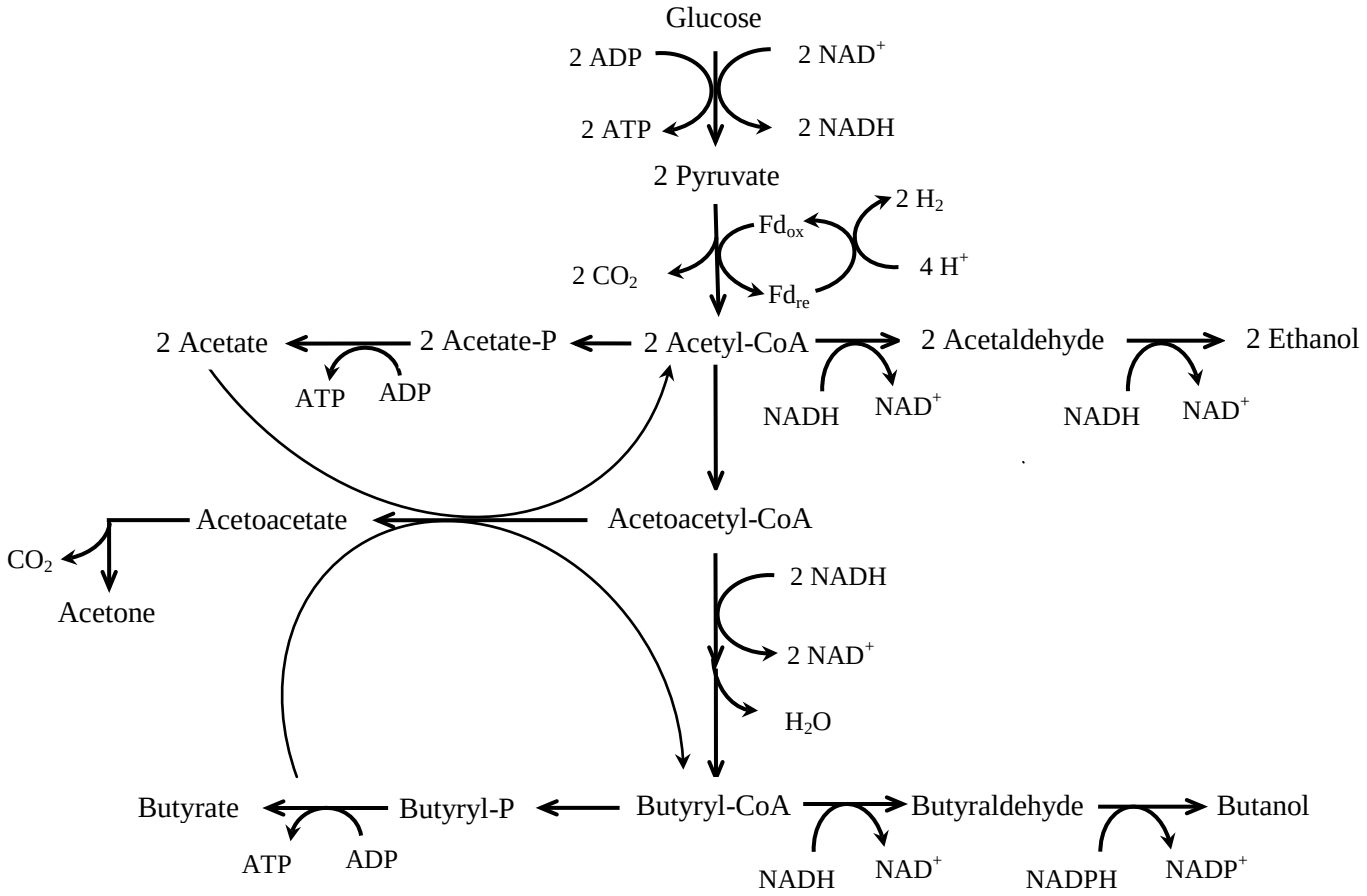


Figure 2.1: General metabolic pathway for ABE fermentation

2.2.3.1 Butanol toxicity and yield

The biggest roadblock of butanol fermentation by native strains is the product inhibition at higher butanol concentration, inability of the microorganisms for industrial level production and acid accumulation during fermentation (Garcia et al. 2011). Repeated sub-culturing of Clostridia *sp.* reduces its butanol forming ability due to the hydrophobicity of butanol that increases the cell membrane fluidity and changes the trans-membrane pH gradient, affecting intracellular ATP level and glucose uptake capability, etc (Huffer et al. 2011). Clostridia *sp.* can tolerate lower butanol concentrations (2%, v/v) and nearly 20–30% increase in the membrane fluidity was found in *C. acetobutylicum* strain by exposing the cells to 1% butanol (Knoshaug and Zhang 2009; Liu and Qureshi 2009). To evaluate the effect of butanol over these processes and cell growth, mixed cultures of Clostridial *sp.* (*C. saccharoperbutylacetonicum, C. butylicum, C. acetobutylicum*) were grown in an optimal growth media and dose response analysis was performed (Chen et al. 2012). The mixed culture was found tolerant upto 1.6% butanol level. This suggests that butanol production at industrial scale by using natural strain is not a viable option.

In order to improve the performance of microorganisms for the production of biobutanol attempts have also been made to use metabolic engineering tools. Various butanol tolerant Clostridium strains have been developed by random and targeted mutagenesis of butanol producing natural strains and non-butanol producing microorganisms to improve productivity that can be attractive industrially (Connor et al. 2010; Cooksley et al. 2012). Development of engineered acid tolerant *C. acetobutylicum* ATCC 824 strain was a great success in the field of metabolic engineering for ABE fermentation (Borden et al. 2010).

2.2.4 ABE Fermentation

2.2.4.1 Effect of different operating parameters

ABE Fermentation is one of the oldest recognized industrial fermentations and has its economic importance. However, the performance of the fermentation depends on a number of operating parameters such as agitation, media pH, incubation time, temperature and toxicity effect, etc. Agitation plays an important role in maintaining the homogeneity of nutrients and microbes in the fermentation broth. Higher agitation speed improves broth homogeneity, reduces temperature gradient, and favors butanol production. But very high agitation creates adverse impact due to cell damage hence an optimum speed for better production is a must (Kumar and Gayen 2011). Fermentation media pH is another factor that influences the productivity to a great extent and its effect has been investigated over a wide range and maximum production has been reported at pH 4.5-5.5 (Zhu and Yang 2004). Biebl (1999) confirmed the effect of pH on the production and obtained the maximum production (9.0 g/L) at pH 4.5. Availability of nitrogen to the growing bacterial culture is less at lower pH, controlling the solvent production through solventogenesis process (Li et al. 2011). All the reported results have shown that lower pH favors the butanol production but impact and yield both get affected with varying microbial culture. It also prevents acid-crash due to excess acid formation during acidogenic phase (Maddox et al. 2000).

Another important parameter affecting production is the incubation time that divides the whole fermentation pathway into two phases (acidogenic and solventogenic). Acidogenesis starts some time after inoculation and extends for a longer period for the production of acetic and butyric acids (nearly 30 h) and then the process enter into the solventogenic phase that

extends for 90 h (Kumar et al. 2013). These two phases depend on the initial availability of glucose at a particular temperature and media pH. Incubation temperature mainly affects the membrane fluidity of the native micro-organisms, while butanol tolerant strains can remain unaffected even in the presence of large butanol titer and varying temperature. Baer et al. (1987) subjected both, butanol-tolerant strain SA-2 and native *C. acetobutylicum* ATCC 824 to different butanol concentrations (0, 1, 1.5%, v/v) and temperatures (22, 37, 42°C), and found that the mutant strain remained unaffected while increased membrane fluidity was observed in the native strain with resultant increase in butanol concentration. The lower temperature (22 & 37°C) increased the saturated to unsaturated fatty acid ratio for both the strains, while inhibitory effect was observed at higher temperature (42°C). It means incubation temperature largely affects the microorganism's membrane in developing a stable membrane mechanism against butanol concentration.

2.2.4.2 Fermentation modes

The batch, fed-batch and continuous modes of fermentations have been evaluated extensively by various workers. Their basic features and merits are compared in Figure 2.2. Though continuous fermentation is having a number of advantages over batch and fed-batch such as reduction in sterilization, butanol inhibition and re-inoculation of micro-organisms, still batch mode has attracted the attention of researchers due to high yield (Dolejs et al. 2014; Li et al. 2011). Jiang et al. (2014) used repeated batch mode with *C. acetobutylicum* JB200, a butanol tolerant species having great potential for use at large scale. Immobilized cells were used in a fibrous-bed bioreactor for 16 consecutive batches for 800 h and nearly 16-20 g/L butanol production was achieved. Production of biobutanol from different algal

biomass has been analyzed by various workers in batch mode (Castro et al. 2015; Cheng et al. 2015; Efremenko et al. 2012). Ellis et al. (2012) reported the maximum ABE production (9.74 g/L) from wastewater algae after enzymatic hydrolysis with cellulase and xylanase enzymes while only 0.73 g/L of ABE was obtained from untreated algal biomass. Other modes of fermentation i.e. fed-batch and continuous, have also been used for efficient and economic production of butanol (Dolejs et al. 2014; Ni et al. 2012). In the case of substrate inhibition or catabolite repression fed-batch mode of fermentation is considered as the most suitable option, however, it is the least preferable option due to large solvent accumulation so it is advisable to operate fed-batch mode integrated with the end product separation process (Song et al. 2010). Qureshi and Blaschek (2001b) used fed-batch fermentation of ABE integrated with the pervaporation technique for product recovery using silicalite-silicone composite membrane. Large solvent recovery (154.97 g/L ABE in 870 h) proved the efficiency of integrated fed-batch system over batch fermentation.

For both batch and fed-batch processes, there are certain limitations such as need for reactor sterilization after every cycle, re-inoculation and solvent inhibition etc (Kumar and Gayen 2011). To overcome the limitations of batch/fed-batch, continuous mode of fermentation was adopted due to the possibility of use of free cells, immobilized cells and recycles of cell mass (Bankar et al. 2012; Liew et al. 2006; Survase et al. 2012). Zheng et al. (2013) reported high butanol productivity (3.32 g/L/h) from xylose fermentation with continuous cell recycling (17.4 g/L) at a dilution rate of 0.78 h^{-1}. To improve the productivity Tashiro et al. (2005) used cell recycling along with cell bleeding due to the large cell growth (>100 g/L) in continuous system and obtained maximum productivity of 7.55 g/L/h. Table 2.5 compares

different fermentation processes in terms of their total solvent yield and productivity. It can be concluded that large scale continuous ABE fermentation is a viable approach but at laboratory scale batch mode is preferable due to almost similar solvent productivity and less chances of contamination.

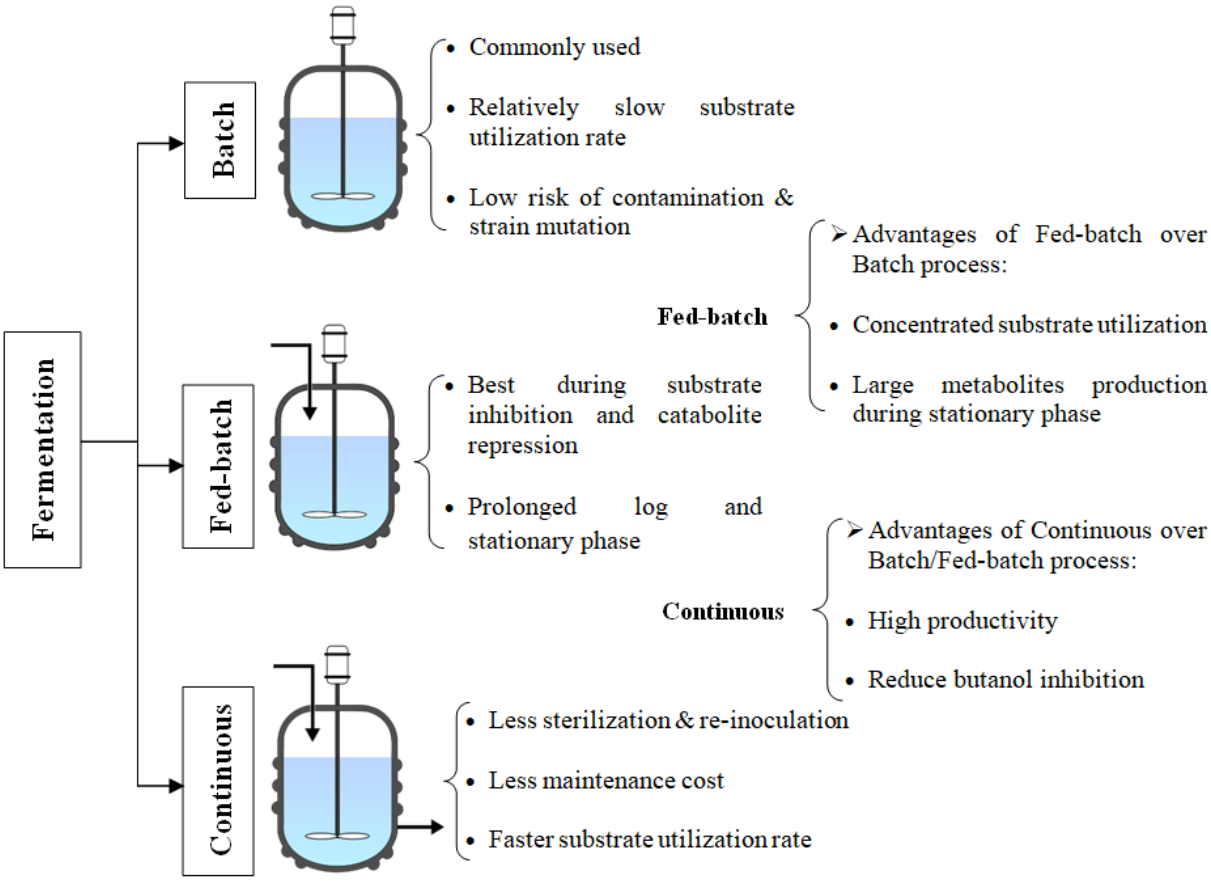


Figure 2.2: Various modes of ABE fermentation

Table 2.5: Comparison of different fermentation modes for butanol production

Fermentation mode	Feedstock	Total Solvent yield (g/g)/productivity (g/L/h)	Butanol or (ABE) (g/L)	References
Batch fermentation	Wheat straw	0.41/0.31	(21.42)	Qureshi et al. (2008d)
	Switchgrass	0.37/0.09	(14.61)	Qureshi et al. (2010b)
	Barley straw & gelatinized grain slurry	0.29	7.8 (13.5)	Yang et al. (2015)
	Microalgae biodiesel residue	0.13 (butanol)	3.86 (-)	Cheng et al. (2015)
Fed-batch fermentation	Glucose	0.24/1.91	9.12 (14.53)	Dolejs et al. (2014)
	Wheat straw	-/0.36	(16.59)	Qureshi et al. (2008b)
	Cassava bagasse	0.32(ABE)/0.32 (butanol)	76.4(108.5) (integrated gas-stripping)	Lu et al. (2012)
Free cell continuous fermentation	Degermed corn	-/0.3	(14.28)	Ezeji et al. (2007a)
	Sago starch	0.29/0.85	(9.1)	Liew et al. (2006)
Immobilized cell continuous fermentation	Glucose	0.35/2.5	16.9 (25.32)	Bankar et al. (2012)
	Glucose	0.4/13.66	(14.32)	Survase et al. (2012)
	Corn	0.42/4.6	12.5	Huang et al. (2004)
Continuous fermentation with cell recycling	Xylose	-/3.32 (butanol)	4.26	Zheng et al. (2013)

2.3 Downstream operation

The separation and purification steps are most critical aspects of any production process. Various downstream techniques such as adsorption, gas-stripping, liquid-liquid extraction, perstraction and pervaporation, currently being evaluated are listed in Table 2.6. A viable separation technique should have high solvent selectivity, efficient removal rate and cost effectiveness (Abdehagh et al. 2014; Garcia et al. 2011). Recovery of butanol from the fermented media is a challenge because of its lower concentration and higher boiling point than water. It's separation from the low concentration broth requires highly sophisticated recovery techniques that involve high energy input to operate the process efficiently (Qureshi and Blaschek 2001b). This step needs more attention of researchers from various branches of engineering and science to improve upon the butanol productivity and purity at industrial scale. Integration of butanol recovery with fermentation step might lead to a cost effective approach as it lowers down the toxic effect of butanol resulting into enhanced solvent productivity (Zheng et al. 2009). Merits and demerits of various downstream techniques and comparison between them on the basis of energy requirement for butanol recovery are listed in Table 2.6.

2.3.1 Liquid-liquid extraction

Separation of end-product through liquid-liquid extraction is an effective approach to achieve maximum recovery from fermented broth. Major advantage of the process is the high selectivity of the used solvent towards the desired product and possibility to integrate the separation with the fermentation process inside the fermenter itself (Ha et al. 2010). Use of highly selective immiscible solvent as extractant increases the recovery of desired product

Table 2.6: Comparison of different downstream operations for butanol recovery

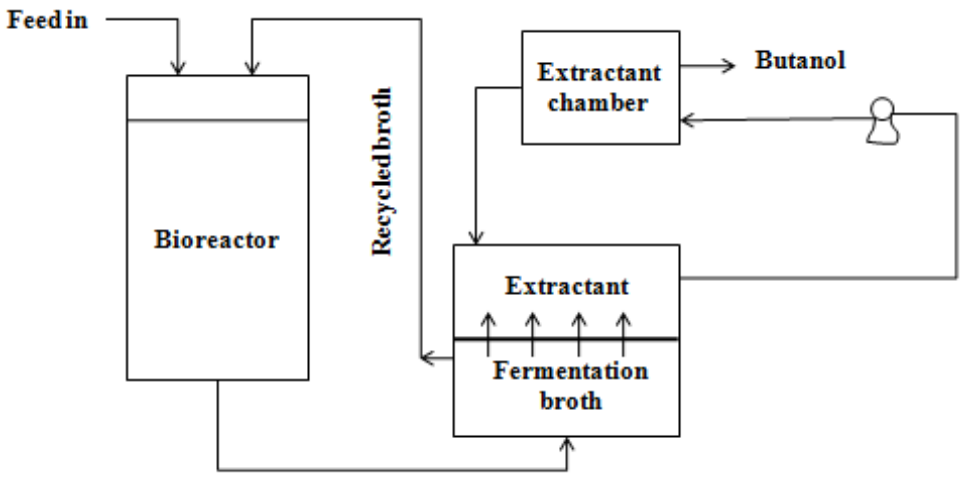
Recovery technique	Merits	Demerits	Energy requirement (kcal/kg butanol)	Butanol or (ABE) (g/L) with online recovery	References
Adsorption	Less energy requirement, reuse of the adsorbents	Not feasible at industrial scale	1948	54.6	Xue et al. (2016)
				(59.8)	Qureshi et al. (2005)
				58.3	Liu et al. (2014)
Gas-stripping	Easy operation, prevents fouling, better butanol productivity	Lower selectivity, high operation cost	5220	(81.3)	Ezeji et al. (2007b)
				444.8	Rochon et al. (2017)
				(232.8)	Ezeji et al. (2004)
Liquid-liquid extraction	Flexible with properties of solvent, facilitate stage-wise phase contact	Extractant toxicity to microorganisms, emulsion formation, loss of extractant	1840	16.9	Bankar et al. (2012)
				13.58	Bankar et al. (2013)
Perstraction	-	Membrane fouling, costly	1840	(136.58)	Qureshi and Maddox (2005)
Pervaporation	Efficient in butanol recovery	Membrane fouling and lower durability, low fluxes, membrane swelling, costly	3295	(142)	Wu et al. (2015)
				451.98	Cai et al. (2017)

and avoids removal of any undesired components (Ezeji et al. 2007b). Qureshi and Maddox (1995) performed continuous production of ABE in packed-bed and fluidized bed reactors and adapted an integrated liquid-liquid extractive recovery technique using three extractants (oleyl alcohol, benzyl benzoate and dibutyl phthalate). Among these oleyl alcohol was found to be the most efficient extractant to recover butanol. Kurkijarvi et al. (2014) reported increased rate of extraction by adapting the dual extraction method. In the first extraction step non-biocompatible solvent (octanol, nonanol, decanol, undecanol, isodecanol) with high distribution coefficient for butanol was used and in the second step a non-toxic extractant (mesitylene) was used to remove traces of ABE and non-biocompatible solvent from the broth. Combination of decanol and mesitylene has been found to be an excellent extractant. Various ionic liquids (melting point<100°C) have also been utilized as an alternative for liquid-liquid extraction (Cull et al. 2000). Ionic liquids are organic salts composed of organic cations and organic/inorganic anions (Simoni et al. 2010). These solvents are also known as “designer solvents” and possess some unique properties such as negligible vapor pressure and nonflammable nature and are stable over a wide range of temperature (-70–400°C) (Fredlake et al. 2004; Ha et al. 2010). Combination of ionic liquids (1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([bmim][Tf2N]) and 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([hmim][Tf2N])) has been used for butanol-water separation (Davis and Morton 2008). High selectivity of solvents for butanol was found at lower butanol concentrations in the aqueous phase. In another experiment different imidazolium based ionic liquids were used for butanol recovery and a maximum recovery of 74% could be achieved by 1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide from binary solution with maximum butanol distribution

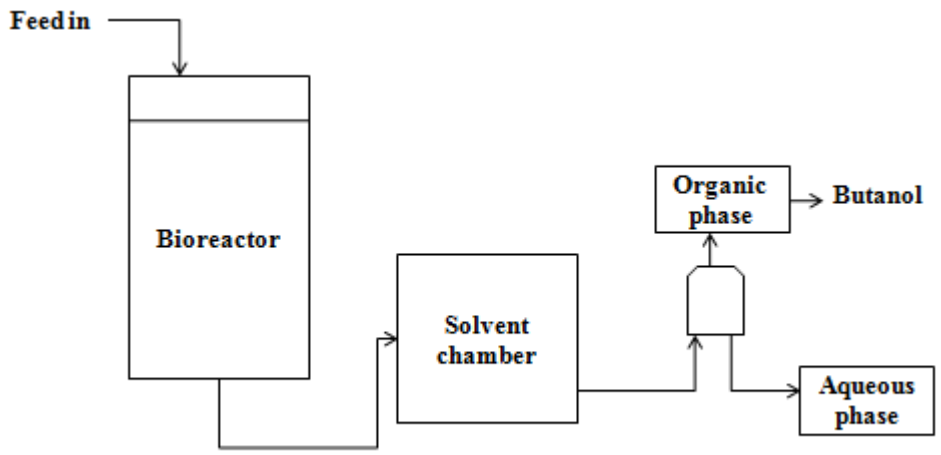
coefficient of 1.939 and selectivity of 132 at 323.15 K (Ha et al. 2010). Apart from the various advantages of using ionic liquid for recovery processes there are certain disadvantages associated with it. Toxicity, corrosive nature of certain ionic liquids and their high preparation cost make them unsuitable for industrial scale recovery therefore, organic solvents are still preferred for butanol recovery. Apart from the demerits of liquid-liquid extraction and perstraction, these two techniques require minimum energy for butanol separation, schematic diagram of these two techniques is shown in Figures 2.3(a) and (b) (Morone and Pandey 2014).

2.3.2 Perstraction/Membrane-assisted solvent extraction

Perstraction technique was adopted to overcome the problems of liquid-liquid extraction requiring direct contact of extractant with the fermented broth, toxicity of solvent to microorganisms and emulsion formation during the extraction (Abdehagh et al. 2014).



(a)



(b)

Figure 2.3: Techniques for butanol recovery **(a)** Liquid-liquid extraction **(b)** Perstraction

It involves the selective permeation through an appropriate membrane and then extraction of the desired product using a suitable solvent. Butanol specific extractants used in perstraction process are polypropylene glycol, oleyl alcohol, 1-octanol, 1-dodecanol, 2-ethyl-1-hexanol and tributyrin, etc (Abdehagh et al. 2014). Economics of the process depends on the selection of specific membrane for the butanol separation and extractants with high diffusion coefficient (Xue et al. 2014). Several researchers have explored this area to evaluate the process efficiency (Grobben et al. 1993; Groot et al. 1990; Qureshi et al. 2005). Tanaka et al. (2012) used the membrane-assisted butanol recovery from the batch fermentation system using polytetrafluoroethylene membrane and 1-dodecanol as the extractant. Combination of membrane and extractant increased the total butanol production from 16 to 20.1 g/L and glucose consumption from 59.4 to 86.0 g/L. This result proved the efficiency of perstraction over liquid-liquid extraction by using a toxic extractant efficiently for butanol recovery with improved productivity (from 0.817 to 0.979 g/L/h). Similarly Qureshi et al. (2005) used

whey permeate supplemented with lactose for ABE fermentation in a batch reactor integrated with perstraction. Oleyl alcohol was used as the extractant and maximum concentration of ABE in the extractant was found as 9.75 g/L. It was concluded that the ratio of acids to solvents was lower in the coupled system than the control batch process (possibly due to the conversion of acids to solvents). In spite of these improvements certain limitations (membrane clogging and fouling, lower solvent flux) are still associated with this process that makes it industrially nonviable (Qureshi and Maddox 2005, Xue et al. 2014).

2.3.3 Other butanol recovery techniques

Several other butanol recovery techniques such as adsorption, gas stripping and pervaporation have also been evaluated by various researchers and are listed in Table 2.6. An energy intensive adsorption technique has gained much attention. Various adsorbents such as silicalite, resins, bone charcoal, activated charcoal, polyvinylpyridine and bonopore have been tested for their suitability for butanol recovery. The maximum adsorption capacity was exhibited by the activated carbon (252 mg/g) followed by bone charcoal (206 mg/g) and the lowest (97 mg/g) for silicalite, however, silicalite offers the advantage of complete desorption (Qureshi et al. 2005). The major disadvantage of this technique is the unavailability of quality adsorbents with good desorption capacity.

Gas-stripping is another separation technique that uses various gases viz., oxygen free nitrogen gas, hydrogen and carbon dioxide for recovery and recycling back for the next cycle (Abdehagh et al. 2014). Its efficiency depends on gas flow rate, media composition and foam formation due to the large gaseous flow (Ezeji et al. 2005). Use of high superficial

velocity of gas permits reduction in liquid side mass transfer coefficient hence higher recovery (Liao et al. 2014). For increasing the productivity and selectivity for butanol researchers are now adapting the two-stage gas-stripping or two-stage fermentation integrated with gas-stripping technique. Though the process is capable of increasing the recovery of butanol by several folds but requires very high energy input (Ezeji et al. 2004).

Pervaporation is widely used for separating desired products from fermentation broth using a selective membrane and exploiting the difference in the partial vapor pressure of the mixture components (Abdehagh et al. 2014). Fermentation coupled with pervaporation is the best way to reduce the butanol toxicity in the broth in conjunction with ultrafiltration to sustain the microorganisms in the fermenter (Li et al. 2014a). Thus pervaporation has found favor with researchers as it possess a high separation factor and permeate flux making it suitable at industrial level while less viable for lab scale production due to the large energy requirement and cost involvement.

2.4 Mathematical models for ABE fermentation process

Several research groups have attempted to develop mathematical models for ABE fermentation. These models can be broadly grouped as kinetic, physiological, and extractive-fermentative models. Ranjan and Moholkar (2012) and Mayank et al. (2013) in their comprehensive review on biobutanol have given a detailed account of various mathematical models. Here only a brief account of various models is presented.

Papoutsakis (1984) developed a comprehensive model based general equation for ABE fermentation by butyric acid bacteria using stoichiometric balance for carbon, hydrogen, oxygen, and nitrogen involving 12 reactions of EMP pathway and 16 variables. He used elemental composition of organic substrate, microbial biomass, and extracellular products. The values for various elements in the empirical formula of biomass were obtained from its elemental analysis. He also incorporated the concept of the degree of reductance of various compounds defined as the number of equivalents of electrons per carbon atom of the substrate, biomass and extracellular products and a representative composition of biomass. He further assumed that the weight fraction of carbon in the biomass and the degree of reductance of biomass are nearly constant and glucose, pyruvate, and acetyl-CoA were assumed to have concentrations > 0. Chauvatcharin et al. (1998) applied this model for analyzing microbial metabolism of AB fermentation to obtain different physiological states of fermentation. Papoutsakis and Meyer (1985a, 1985b) extended the model to propionic acid and butanediol and mixed acid fermentation. Singularity due to interacting pathways for some products is the limitation of this model and makes calculation of *in vivo* fluxes difficult. In order to overcome this they grouped together acetone pathway by replacing *in vivo* fluxes with net production rate of acetone, acetate and butyrate. This however, resulted in the loss of information pertaining to physiologically important *in vivo* fluxes. Efforts have been made to overcome this limitation by measuring one of the *in vivo* fluxes and introducing optimality principle.

Yerushalmi et al. (1986) and Votruba et al. (1986) developed a process kinetic model based on the AB fermentation dynamics and a physiological state model that considered major

process parameters and the extent of their quantitative influence on the control of biosynthesis. The physiological model took into account the mass transfer phenomena across the cell membrane. The cell membrane permeability and number of active sugar transport sites were considered as the major parameters affecting the biosynthesis. The batch fermentation was limited by glucose and unaffected by nitrogen source. The physiological model accounted for both cellular and extracellular culture conditions as well as transport parameters for solvent at cellular level. The model comprised 8 ordinary differential equations involving 8 variables. The rate of butyric acid synthesis, conversion of butyric acid to butanol, diffusion of butyric acid out of the cell and transport of acid due to electrical potential gradient and change in acid concentration with changing volume of biomass during growth were considered in the mass balance. Monod model was used to express butyric acid to butanol conversion. Votruba et al. (1986) used parametric sensitivity analysis approach and showed that kinetics of butanol and butyric acid formation and biomass growth depend on culture performance and butanol production. Mulchandani and Volesky (1986) also developed a model for AB fermentation in a cell retention reactor. The governing equations are similar to those used for the process kinetics model. The inhibition caused by butyric acid/butanol was accounted for an inhibition function depending on butanol and butyric acid concentrations. It was concluded that the steady state could not be attained in the reactor for glucose concentration > 52 g/L.

Desai et al. (1999) developed a new model considering the fact that the rate of uptake of acetic and butyric acids is catalyzed by the same enzyme and obtained a relation between *in vivo* uptakes through the butyrate-acetone pathway to that of acetate-acetone. Since the

concentrations of acetate and butyrate are functions of their respective rates of formation, the model presented a nonlinear constraint. Under such a situation, Papoutsakis (1984) stoichiometric approach was used to determine *in vivo* metabolic fluxes for describing the metabolism of ABE producing clostridia. The advantages of this model are- i) it is possible to resolve the singularity in the stoichiometric model using a physiologically based nonlinear constraint, ii) it permits incorporation of nonlinear equations in the stoichiometric models, and iii) a single metabolic network describes the metabolism of a range of substrate mixtures without a priori determination of respective fluxes.

Shi et al. (1990) developed a general frame work for extractive fermentation applicable for batch, sequential batch and repeated fed-batch modes. The model used governing differential equations for biomass concentration, substrate utilization, and concentration of various products. A product of inhibition coefficient (depending on the production rate of an inhibitory product and the inhibition constant for the product) and the average growth rate obtained from Monod kinetics was taken as the growth rate. Honda et al. (1987) extended this model to repeated batch and repeated fed-batch fermentations. Shi et al. (1990) used this model to evaluate the performance of AB fermentation with the addition of oleyl alcohol as extractant for butanol and benzyl benzoate as that for acetone. Yang et al. (1994) developed a model for cell growth under synergistic inhibition of multiple products/byproducts using a Monod type relation under product inhibition conditions. They obtained a relation for the ratio of the specific growth rates under uninhibited and inhibited situations using experimental data. The model considered the inhibition caused by acetone, butanol, ethanol, acetate, and butyrate. It was observed that the presence of inhibitors like acetate and butyrate

augmented the inhibition of butanol. On the other hand acetone and ethanol neither caused much inhibition nor interacted with other products. The pH affected the extent of inhibition through the ionization of species and also the cell membrane and other physiological functions. In view of these observations pH was considered as an independent parameter in the model.

A model for fed-batch butanol fermentation with simultaneous pervaporation was developed by Park and Geng (1996). The pervaporation module was mounted inside the fermenter system. They used Monod kinetics with inhibition. The results of simulation indicated that glucose concentration decreased slowly in the lag phase regime of growth. With the onset of solventogenesis, it decreased rapidly until the cell growth was inhibited at higher butanol concentration in the broth. In presence of pervaporation module the glucose consumption rate increased instantaneously with the onset of solventogenesis. A saw-tooth type behavior was observed with varying membrane thickness. The glucose consumption rate increased with decreasing membrane thickness.

2.5 Economics of butanol production

The feedstock, production process and separation techniques control the process economics. The global demand for butanol increased at the rate of 2.7% per annum during 2005-13 and the current demand is more than 1.2 billion gallons (DUBLIN, March 10th, 2017, PRNewswire). The global butanol market is projected to grow more rapidly during 2014-2019 driven primarily by the Asia-Pacific region countries valued at $3.0 billion in 2013 and is expected to attain $4.3 billion by 2018. Presently China is expanding the

butanol market and probable demand is projected as 1.64 million tonnes by 2021 (MicroMarket Monitor, July 2nd, 2014, PRWEB). The cost of biobutanol is higher ($1.87/kg n-butanol) than that from the petrochemical route ($1.52/kg n-butanol) (Jiang et al. 2015). Production cost for butanol has been estimated as $4.41 and $2.71/gal for corn and soy molasses, respectively (Dong et al. 2014). Genetically modified microorganisms and utilization of algal feedstocks could allow further price reduction. DuPont and Bio Architecture Lab, have invested around $8.8 million on R&D activities for commercial butanol production from seaweed biomass.

2.6 Challenges in biobutanol production

Though the commercial scale biobutanol production from lignocellulosic biomass is growing rapidly, however, industries are still facing a number of challenges that need to be overcome for economical production of biobutanol. The lignocellulosic biomass, a renewable and sustainable energy source possess drawbacks such as need for large area for cultivation and release of toxins, thus the focus has shifted to the third generation algal biomass. Out of several biobutanol production processes using various algal biomasses as feedstock, the cyanobacteria-based biobutanol production is still at the developing stage. Despite of all its advantages there are several bottlenecks that limit the use of cyanobacterial biomass at commercial scale, in particular, unavailability of suitable cyanobacterial species with large carbohydrate content and lower biomass yield leading to lower butanol yield. Though continuous improvement has been achieved both in upstream and downstream processes at low volume, improved R&D and scale-up strategies are still required to make cyanobacterial biomass an attractive feedstock for biobutanol production. Application of

different nanomaterials for processing of biomass has been explored and shown immense prospects. Still research is needed to explore their uses during preprocessing of cyanobacterial biomass and fermentation to enhance the sugar as well as butanol yield.

2.7 Specific objective of present work

On the basis of the drawbacks associated with the cyanobacterial biomass and research gaps that exist in the field the objectives of the present research work have been finalized.

- Growth optimization of cyanobacterial strains (*L. limnetica* and *O. obscura*) and their carbohydrate content enhancement
- Optimization of pretreatment conditions using Response Surface Methodology (RSM)
- Production of enzyme from cyanobacterial biomass in shake flasks and comparative analysis with commercial enzymes
- Optimization of process parameters for biobutanol production using glucose as carbon source through shake flask experiments
- Production of biobutanol using cyanobacterial hydrolysate as carbon source at optimized conditions
- Study of biobutanol production in a Continuous stirred tank reactor (CSTR) at optimized conditions with glucose as well as cyanobacterial hydrolysate
- Mass balance for biobutanol production
- Estimation of Mercier's kinetic model parameters
- Recovery of biobutanol from fermentation broth