INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–1259/2014/16–5–1019–1024
http://www.fspublishers.org

*Review Article*

# A Review on Bio-butyric Acid Production and its Optimization

**Ajay Kumar Jha[1,2], Jianzheng Li[1*], Yixing Yuan[1], Nawraj Baral[1] and Binling Ai[1]**
[1]*State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, P. R. China*
[2]*Kathmandu Engineering College, Tribhuvan University, Kathmandu, Nepal*
*For correspondence: ljz6677@163.com

## Abstract

Butyric acid is treated as one of the renewable green fuels of tomorrow due to its high energy content, and it can reduce health and environmental issues including emission of greenhouse gases, global warming and climate change. The production of bio-butyric acid by microbial fermentation is not cost-effective and economically competitive due to its production at a relatively low concentration, yield, and rate. In order to enhance the economics of the fermentation method, the butyrate production should increase using economical substrate, favorable pretreatment techniques, multicultural stains and fermentation conditions. In order for further investigation and improvement in butyric acid production, batch, repeated batch, fed batch and continuous type butyric acid fermentation of biomass have been discussed in this review. 

**Keywords:** Butyric acid; *Clostridium;* Fermentation; Renewable biomass

## Introduction

Energy has been regarded as one of the main elements of human life, social civilization and techno-socio-economic progress. However, the advances in energy technologies have brought various revolutions throughout the world. Present global energy supply-consumption chain is obviously unsustainable from the technical, environmental, economic and social points of view due to limited availability of fossil fuels and inevitably be depleted (Barnes and Floor, 1996). The emissions of greenhouse gases and their consequences including global warming, acid rain, climate change and other environmental issues force us to think about alternative fuels, although conventional fossil fuels is the world's vital energy resource (Jha and Jha, 2010). The application and development of bio-fuels can reduce the consumption of fossil fuels and alleviate energy crisis to some extent. The recent advances in the fields of biotechnology and microbial fermentation technologies have resulted in a renewed attention in bio-butyric acid production from low cost renewable biomass (Zhang *et al*., 2009; Dwidar *et al.*, 2012).

A four-carbon short chain ($CH_3CH_2CH_2COOH$) bio-butyric acid and its derivatives have numerous potential applications in chemical, textile, plastic, food, beverage, dairy and pharmaceutical industries (Zigová and Šturdík, 2000). They are extensively used as solvent, diluents, drugs, plasticizer, perfumes, fiber, additive and raw materials (Zhang *et al*., 2009). Bio-butyric acid is regarded as a prospective chemical building-block to make chemicals. It is also regarded as a promising specialty chemical as it can be converted to bio-butanol. But its commercial production is dominated by chemical synthesis. However industrial production of butyric acid mainly depends upon crude oil due to comparatively lower production cost and large scale supply, consumers prefer butyric acid of natural origin, especially for foodstuff additives or pharmaceutical products (Zigová and Šturdík, 2000). With decreasing availability of crude oil, growing demand for natural products and rising concerns over environment, microbial fermentation technology for butyric acid production from renewable biomass has been paying attention of many researchers because bio-butyric acid could be one of the most promising sustainable bio-fuels to meet the desires of green energy supply for replacing fossil fuels. In addition, presence of profuse lignocellulose biomass as low-value agricultural commodities or obligation of apt disposal of bio-wastes to pass up pollution tribulations have been creating favorable business climate for butyric acid fermentation.

Although butyric acid is a significant bio-fuel to develop sustainable green society, its production through fermentation of biomass has been regarded as very complex and hard to control. The microbial butyric acid fermentation is affected with several process dilemmas including self-inhibitory effect of end products, increasing inhibition due to pretreatment, slow rate of strain development (Zigova´ and Šturdík, 2000). The production strains for butyric acid fermentation also produce other types of acids, which are very difficult to separate. Higher substrate cost, degeneration of the butyric acid-producing strains, limited productivity, lower concentration, lower yield and higher products recovery cost are main factors, which limit the fermentation routes as well. Limited

**To cite this paper**: Jha, A.K., J. Li, Y. Yuan, N. Baral and B. Ai, 2014. A review on bio-butyric acid production and its optimization. *Int. J. Agric. Biol.,* 16: 1019–1024

studies on production of bio-butyric acid by anaerobic fermentation have been reported in association to bio-refinery perspectives including selection and growth of strains, and physiological examination for greater yield, productivity and selectivity. A higher demand in contrast to lower production through the microbial fermentation routs gives rise to the necessity of addressing and solving the problems related to the butyric acid fermentation process. In order to improve butyric acid fermentation and reduce overall production cost, extensive efforts are needed for strains improvement, metabolic pathways, bioreactor design, feed stocks, effect of inhibitors, fermentation process control parameters and optimization techniques.

## Microbial Strains

Appropriate microorganisms selection is the base of a successful fermentation process. Numerous bacterial strains, which are suitable to produce bio-butyric acid, are mainly isolated from waste water, excess sludge, soil, contaminated dairy and food products, meats, and animal digestive systems. Altogether, more than ten butyrate-producing bacterial strains, belonged to the genera *Clostridium*, *Butyrvibrio*, *Butyribacterium*, *Eubacterium*, *Fusobacterium Megasphera* and *Sarcina*, are reported (Zigová and Šturdík, 2000). They are Gram positive, chemoorganotrophic, strictly anaerobic, and spore-forming bacteria. The strains of genera *Clostridium* have been extensively used and studied microorganisms due to their high productivities and relatively higher stability, followed by *Butyrivibrio* and *Butyribacterium*. Among them, *C. butyricum*, *C. beijerinckii*, *C. acetobutylicum*, *C. tyobutyricum*, *C. populeti* and *C. thermobutyricum* are superior strains. Favorable culture temperature ranges from 30-37°C for *C. butyricum*, *C. populeti* and *C. tyrobutyricum*, while 55°C is considered as optimal culture temperature for *C. thermobutyrium.* Although an extensive range of carbon source is able to be utilized by clostridia, glucose is common substrate for bio-butyrate production. *Clostridium* bacteria can utilize different types of sugars including hexoses, several pentoses, oligo- and polysaccharides for bio-butyric acid production while *C. butyricum* is able to utilize glycerol, pentose, hexose, molasses, lignocellulose, cheese-whey permeat, and potato starch as carbon sources. However, *C. tyrobutyricum* can only make use of glucose, xylose, and fructose (Matijasic *et al*., 2007). Baroi *et al.* (2013) presented *C. tyrobutyricum* has the ability to transfer both pentose and hexose sugars but the xylose uptaking speed is lower than that of glucose. *C. thermobutyricum*, which is isolated from horse dung, can mainly use monomeric sugars (glucose, fructose, maltose, xylose, and ribose, but not arabinose, galactose, and mannose), dimeric (cellobiose), oligomeric and polymeric sugars.

## Metabolic Pathway and Inhibition in Bo-butyric Acid Fermentation

Extensive studies have illustrated metabolic pathways and regulations for fermentation of glucose to produce bio-butyric acid using clostridia (Zhang *et al*., 2009; Zigová and Šturdík, 2000; Ramey and Yang, 2004). Glucose fermentation by *C. butyricum* (Eq. 1) and *C. tyrobutyricum* (Eq. 2) follows the stoichiometric equations below (Zhang *et al.*, 2009):

$$\text{Glucose} \rightarrow 0.8\ \text{Butyrate} + 0.4\ \text{Acetate} + 2.4\ H_2 + 2\ CO_2 \quad \text{(Eq. 1)}$$

$$\text{Glucose} \rightarrow 0.85\ \text{Butyrate} + 0.1\ \text{Acetate} + 0.2\ \text{Lactate} + 1.9\ H_2 + 1.8\ CO_2 \quad \text{(Eq. 2)}$$

The metabolic route of glucose fermentation is presented in Fig. 1. Butyric acid is produced from glucose during acetogenesis stage whereas in solventogenesis phase, butyrate is converted into butanol (Ramey and Yang, 2004). High ATP concentration and minimal NADH:NAD ratio should be maintained in order to prevent solventogenesis (Zigová and Šturdík, 2000). Glucose is metabolized to pyruvate by means of Embden–Meyerhof–Parnas and generates ATP and NADH. Afterwards, acetyl-CoA, acetoacetyl-CoA and butyryl-CoA are formed from pyruvate as key intermediates in the main branch (Jones and Woods, 1986). Butyric acid can be produced consequently in case of presence of high levels of enzymes that are concerned with the pathway of butyryl-CoA to butyrate. During the conversion of acetyl-CoA into butyryl-CoA, thiolase, crotonase, 3-hydroxybutyryl-CoA dehydrogenase and butyryl-CoA dehydrogenase are played vital roles as key enzymes. The bio-butyrate-producing clostridia not only produce bio-butyric acid but also several possible by-products, including acetate, $H_2$, $CO_2$, lactate and other products. The conversion of acetyl-CoA is firstly occurred into acetyl phosphate, which is converted into acetate. Similarly, butyryl-CoA is firstly converted into butyryl phosphate and butyrate is produced from butyryl phosphate. The acetyl-CoA is catalyzed by phosphotransacetylase (PTA) whereas phosphotransbutyrylase (PTB) catlalyzes butyryl-CoA. Correspondingly, acetate kinase (AK) and butyrate kinase (BK) catalyze acetyl-phosphates and butyryl- phosphates for the production of acetate and butyrate, respectively.

In fact, the metabolic pathway of a microorganism during anaerobic fermentation is affected by several factors. In case of bio-butyrate-producing clostridia, mainly glucose concentration, pH, hydrogen partial pressure, acetate, and butyrate are able to influence the growth rate, final products concentration and distribution of the products (Kong *et al*., 2006; Jo *et al*., 2008; Rodriguez *et al*., 2006). It is necessity to build up an appropriate and healthy process parameters that yields low amount of acetic acid (greater selectivity), has a superior yield and a greater productivity of bio-butyric acid from lignocellulosic renewable biomass.

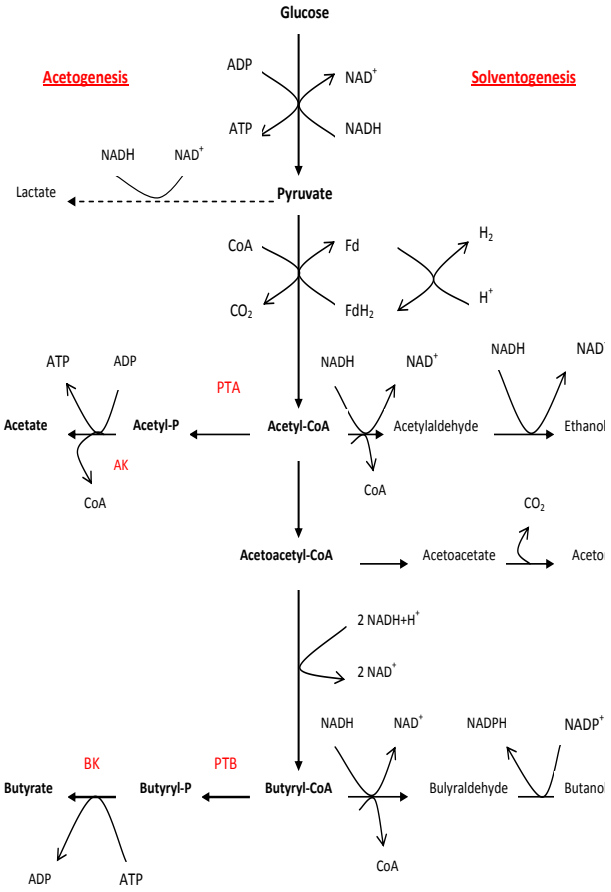


**Fig. 1:** Pathways for bio-butyric acid fermentation (Ramey and Yang, 2004; Jones and Woods, 1986).
(AK: acetate kinase, BK: butyrate kinase, PTA: phosphotransacetylase, PTB: phosphotransbutyrylase)

Surplus carbon supplies often have an effect on osmotic dehydration of microorganisms in an anaerobic fermentation process. A momentous rise in the ratio of butyrate to acetate is found in bio-butyric acid fermentation process with limited amount of glucose and *C. butyricum* as working microorganism (Saint-Amans and Soucaille, 1995). It is noteworthy that various pH values can influence not only the sharing of produced organic acids but also cell membrane transfer behavior, and cell lysis (Zigová and Šturdík, 2000). Relatively higher pH (e.g. >6.0) is useful for cell development and biosynthesis of butyric acid, especially in the case of *C. butyricum* (He *et al*., 2005). Furthermore, media pH also has an effect on the particular growth rate, bio-butyric acid production rate and less sugars utilization rate. For *C. tyrobutyricum*, various pHs are able to change the distribution of the metabolic flux. At pH 6.3, the highest bio-butyrate production is observed, compared to that at pH 6.0 and 6.7 (Zhu and Yang, 2003; Jo *et al*., 2008). Bio-butyrate production was lower at lower pH, with acetate and lactate as the main acid products at pH 5.0. The metabolic shift from butyrate formation at pH 6.3 to lactate and acetate formation at pH 5.0 is associated with decreased activities of PTB and independent lactate dehydrogenase (iLDH), and increased activities of PTA and LDH (Zhu and Yang, 2003). In butyrate-producing strains, PTA, AK, PTB, BK, iLDH, and LDH are the main enzymes relevant to acetate, butyrate, and lactate production. Their products distribution is affected by media pH significantly. AK and BK in the direction of acyl-phosphate formation were not significantly affected by the pH between 5.0 and 7.0. However, in the acyl-phosphate-forming direction, the activity of PTA increased while PTB decreased with increasing the pH (Zhu and Yang, 2003). It has also been reported that under a low partial pressure of $H_2$, the ratio of acetate to butyrate increased with a decrease in hydrogen partial pressure, accompanied by an increase of ATP yield during bio-butyric acid production fermentation by *C. butyricum* (van Andel *et al*., 1985). The production of bio-butanol from butyric acid is also affected by inadequate pH. Soni and Jain (1997) studied the consequence of pH on bio-butyrate uptake as a result of the transformed strain of *clostridium acetobutylicum* and found that lower pH (<4.6) unfavorably exaggerated overall metabolic activity. Minimum pH 5.2 was needed for uptake of bio-butyrate at a concentration of 4 g/L. They also observed that a straight connection among minimum pH prerequisite, butyrate concentration and allied anaerobic fermentation temperature. The end product inhibition is a challenge for the researchers, working to enhance product concentration. Undissociated bio-butyric acid gets ahead of through the bacterial membrane and detaches within the cell. It impinges on the transmembrane pH gradient and declines the sum of existing energy for biomass growth (Zigová and Šturdík, 2000). One of the approaches to solve this problem is to develop a combination of butyric acid tolerated strains while other technique may be online separation or *in situ* product removal, especially extraction and pertraction.

The concomitant production of acetic acid as by-product with butyrate production creates problems for the recovery of bio-butyric acid in downstream processing (Zhang *et al*., 2009). For example, in the immobilized cell anaerobic fermentation process, a greater quantity of 0.27 mol/mol of acetate was formed along with 0.95 mol/mol of butyrate from glucose (Ramey and Yang, 2004). It is obvious that reducing biomass formation and acetate production increases butyrate yield significantly. Ramey and Yang (2004) reported that complete elimination of acetate formation could increase butyric acid yield more than 1 mol/mol for glucose and 0.83 mol/mol for xylose as the substrate for immobilized cell fermentation. Knocking out the acetate-producing pathway can increase the butyrate production.

## Optimization of Butyric Acid Bio-production

The conventional bio-butyric acid production technique is not yet cost-effective and economically competitive

**Table 1:** Some examples of butyrate-production by fermentation

| Feedstocks | Pretreatment | Culture design | Strain | Cul. temp. (°C) | pH | Butyrate Conc. (g/L) | Reference |
|---|---|---|---|---|---|---|---|
| Cane molasses | Sulfuric acid | Fed batch/Immobilized fibrous bed bioreactor | *C. tyrobutyricum* | 37 | 6.0 | 55.2 | Jiang *et al*., 2009 |
| Cheese whey | - | Batch | *C. beijerinckii* | 37 | 5.5 | >12 | Alam *et al*., 1988 |
| Corn stalk | 5-8 mm size, 1% (v/v) Hydrochloric acid | Immobilized continuous reactor | *C. thermobutyricum* | 55 | 6.0 | 15.82 | Li *et al*., 2011 |
| Jerusalem artichoke | 0.01M Sulfuric acid | Fed batch/Immobilized fibrous-bed bioreactor | *C. tyrobutyricum* | 37 | 6.0 | 60.4 | Huang *et al*., 2011 |
| Sugarcane bagasse hydrolysate | 0.1–0.5 M HCl and enzymatic hydrolysis with cellulases | Fed batch culture/Immobilized fibrous bed bioreactor | C. tyrobutyricum | 37/ 200 rpm | 6.0 | 20.9 | Wei *et al*., 2013 |
| Glucose from wheat straw | Pretreated and hydrolyzed | Batch | C. tyrobutyricum | | 6.0- 7.0 | 71.6 | Baroi *et al.*, 2013 |
| Xylose from wheat straw | pretreated and hydrolyzed | Batch | C. tyrobutyricum | | 6.0- 7.0 | 55.4 | Baroi *et al*., 2013 |

due to lower concentration, lower productivity and lower yield of the bio-butyrate. The production of byproducts such as acetic acid, propionic acid and ethanol causes further reduction in butyric acid concentration and increases the costs for product recovery and purification. It means the complicated and expensive isolation process also limits its commercialization. In order to increase the economics of butyrate production, various optimizing techniques may be useful.

## Feed stocks

The cost of feed stocks is regarded as a key issue for economical production of bio-butyric acid by microbial fermentation process. Considering economics of the bio-butyric acid fermentation, it can be pointed out that agricultural products would not be feasible substrates due to high cost and direct uses for human beings and other animals. In such situations, agricultural residues and other industrial wastes are the favorable substrates based on its composition, availability, cost, good water retention capacity and ease of pretreatment. Lignocellulosic materials such as maize straw, rice straw, barley, wheat straw, molasses and dairy wastes have potential to serve as low cost renewable raw materials for bio-butyric acid fermentation, mainly in agriculture based countries. They are readily available and inexpensive renewable biomass. In fact, they consist of cellulose, hemi-cellulose, lignin and smaller quantities of pectin, protein, extractives and ash. Li *et al*. (2011) observed that corn stalk has a great potential to immobilize *C. thermobutyricum* for bio-butyric acid fermentation. Similarly, Jiang *et al*. (2009) and Vandák *et al*. (1995) have used cane molasses for bio-butyric acid fermentation using *C. butyricum* and *C. tyrobutyricum* respectively. Baroi *et al.* (2013) reported the main contents in the sugars yielded from wheat straw haves glucose (71.6 g/L) and xylose (55.4 g/L) respectively.

Hemicelluloses, the second most abundant available polysaccharides in nature, represent around 20-35% of lignocellulosic biomass (Ezeji *et al*., 2007). Enough fermentable carbon substrates can be obtained from lignocellulosic biomass while the cellulosic and hemicellulosic hydrolysates and starch can be used to produce butyric acid production. However, these lignocellulosic residues are abundant available in the developing countries, they are being used inefficiently and consequently cause considerable environmental evils. The beauty of cellulosic materials is that significant amounts of different types of sugar can be obtained through hydrolysis process, which are useful for bio-production of butyric acid and bio-butanol and consequently minimize waste generation (Li *et al*., 2011).

## Pretreatment

The degradation of lignocelluloses is the major obstacle for utilizing renewal and cost-effective biomass including agricultural residues to produce bio-butyric acid. Pretreatment methods are considered as one of the approach to solve this problem. The treatment of molasses by sulfuric acid increased butyric acid concentration by 32.6%, yield by 31.8% and sugar utilization 12.3%, compared to untreated molasses (Jiang *et al*., 2009). In contrast, high costs of hydrolyzing cellulose into simple monomeric sugars and formation of inhibition products during the hydrolysis are the major limitation for pretreatment techniques. The effect of different pretreatment methods including acid, base, thermal, thermal-acid and thermal-alkali with various pretreatment time, temperature and concentration for higher glucose production and low quantity of inhibition products, and consequently higher butyrate production will be focus for the future researches. In addition, physical pretreatment such as appropriate size for substrate, and biological treatment are very significant for higher butyric acid production as well.

## Multicultural Strains

Inability of most of the single butyrate-producing strains to grow and degrade substrate at different fermentation

conditions is one of the major barriers for butyrate fermentation. The identification of a combination of multicultured microbes and their metabolic pathways will accelerate fermentation process to produce higher rate of butyric acid because (i) different conditions and process parameters might be favorable for different microbes; (ii) the inhibition problems due to hydrolysis and fermentation products will be decreased by having better tolerance to butyric acid inhibition for the microbes. The depth insight for finding combinations of microbial strains will be major focus for future researches.

## Reactor Design and its Operation

Batch, repeated batch, fed batch, continuous and cell recycle anaerobic fermentation processes are widely utilized for the researches on butyric acid bio-production (Table 1). It was observed that greater butyrate concentrations could be achieved in batch culture while higher productivity might be obtained in continuous cultures (Michel-Savin *et al*., 1990). Repeated batch culture could eliminate the lag phase by providing adaptation period required for cells to survive in a changed environment. Adaptation of bacterial cultures and shift in metabolic pathways in fed batch fermentation could increase further butyrate concentration and consequently butyrate/acetate ratio. The productivity can be enhanced using cell recycling process or cell immobilization. Jiang *et al*. (2009) studied performance of a fibrous bed bioreactor (FBB) with immobilized *C. tyrobutyricum* under batch, repeated batch and fed batch fermentation systems in order to optimize bio-butyric acid production from cane molasses. The fed-batch fermentation produced 61.9% higher butyrate concentration and reduced 50.9% acetic acid but yield and productivity were decreased by 16.4 and 46.9%, compared to the batch fermentation process. Similarly, Mitchell *et al*. (2009) found that the continuous culture system with immobilized cells has improved butyric acid productivity and consequently reduced product separation cost. The fibrous bed bioreactors due to their regeneratives and higher mass transfer capabilities can maintain the productivity over a longer period. Huang *et al*. (2002) reported that cells immobilized FBB with packed fibrous matrix is successful in producing most of the commercially used organic acids.

## Fermentation Conditions

The identification of relatively optimum process parameters will solve the problems related to efficient butyrate production to some extent. It is believed that thermophilic microorganisms can produce higher fermentation products due to higher rate of hydrolysis, improved mass transfer and reduced susceptibility to contamination. The pH value and acetic acid concentration have also significant effects on the total organic acids and butyric acid production, productivity, and yield. As overall metabolic activity of *Clostridium bacterium* is decreased at low pH, it is considered that a pH range from 4.5 to 7.0 is favorable for butyric acid fermentation (Zigová *et al*., 1999). According to Jiang *et al*. (2009), the optimum pH for butyric acid production and its microorganism growth is 6.0. They further explained that lowering pH to 5.0 brings a change in metabolic shift for acetate production from butyric acid production route and consequently acetate becomes the major product. Alam *et al*. (1988) noted that a constant pH 5.5 provides highest level of butyric acid from cheese whey using *C. beijerinckii* at 37°C in batch culture. Similarly, Li *et al*. (2011) observed highest butyric acid yield from corn stalk at pH 6.0 while pH 7.0 was favorable for maximal butyrate/acetate ratio in a continuous type immobilized cell reactor using *C. thermobutyricum* at 55°C.

## Separation of Butyrate

Difficulty in separating butyric acid from anaerobic fermentation broth is considered as one of the major bottlenecks for butyrate production. Solvent extraction and distillation processes are extensively applied to separate butyric acid from other byproducts, mainly acetic acid. The main drawbacks of these processes are solvent toxicity in extraction and huge energy consumption in distillation, which prohibit their uses in the separation of bulk chemicals from bio-production. Wu *et al*. (2010) reported "salting out" separation method based on an aqueous two-phase system with inorganic salts such as calcium chloride for effective separation of bio-butyric acid from anaerobic fermentation broth and consequently increase butyrate/acetate ratio.

## Conclusion

It is possible to significantly obtain butyric acid from renewable biomass by anaerobic fermentation using different strains of the genera *Clostridium*, *Butyrivibrio*, *Butyribacterium*, *Sarcina* and others but low productivity, rate and concentration are questions for its economical production. Isolation of butyrate tolerate strains, cost effective lignocellulosic materials, optimum fermentation condition, low cost culture media materials, and economical hydrolysis are the key factors, which could reduce challenges of butyric acid bio-production and accelerate its research and commercial production.

## Acknowledgements

The authors gratefully acknowledge the National Natural Science Foundation of China (Grant No. 51178136) and the State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (Grant No. HCK201206) for valuable financial support.

## References

Alam, S., D. Stevens and R. Bajpai, 1988. Production of butyric acid by batch fermentation of cheese whey with *Clostridium beijerinckii*. *J. Indust. Microbiol.*, 2: 359–364

Barnes, D.F. and W.M. Floor, 1996. A challenge for economic development. *Ann. Rev. Ener. Environ.*, 21: 497–530

Baroi, N.B., P. Westermann and H.N. Gavala, 2013. Butyric acid fermentation from pretreated and hydrolyzed wheat straw by *C. tyrobutyricum. In: Industrial biotechnology-meeting the challenges*. International Conference, September 12–13, Lund, Sweden

Dwidar, M., J.Y. Park, J.R. Mitchell and B. Sang, 2012. The future of butyric acid in industry. *Sci. World J.*, 2012: 471417

Ezeji, T., N. Qureshi and H.P. Blaschek, 2007. Butanol production from agricultural residues: Impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. *Biotechnol. Bioeng.*, 97: 1460–1469

He, G.Q., Q. Kong, Q.H. Chen and H. Ruan, 2005. Batch and fed–batch production of butyric acid by *Clostridium butyricum* ZJUCB. *J. Zhejiang Univ. Sci.*, 6B: 1076–1080

Huang, J., J. Cai, J. Wang, X. Zhu, L. Huang, S.T. Yang and Z. Xu, 2011. Efficient production of butyric acid from Jerusalem artichoke by immobilized *Clostridium tyrobutyricum* in a fibrous–bed bioreactor. *Bioresour. Technol.*, 102: 3923–3926

Huang, Y.L., Z. Wu, L. Zhang, C. Ming, S.T. Yang, 2002. Production of carboxylic acids from hydrolyzed corn meal by immobilized cell fermentation in a fibrous-bed bioreactor. *Bioresour. Technol.*, 82: 51–59

Jha, A.K. and S. Jha, 2010. Potential biogas resources in Nepal. *Nepal Eng. Assoc. Tech. J.*, 28: 16–22

Jiang, L., J. Wang, S. Liang, X. Wang, P. Cen and Z. Xu, 2009. Butyric acid fermentation in a fibrous bed bioreactor with immobilized *Clostridium tyrobutyricum* from cane molasses. *Bioresour. Technol.*, 100: 3403–3409

Jo, J.H., D.S. Lee and J.M. Park, 2008. The effects of pH on carbon material and energy balances in hydrogen–producing *Clostridium tyrobutyricum* JM1. *Bioresour. Technol.*, 99: 8485–8491

Kong, Q., G.Q. He, F. Chen and H. Ruan, 2006. Studies on a kinetic model for butyric acid bioproduction by *Clostridium butyricum. Lett. Appl. Microbiol.*, 43: 71–77

Li, W., H.J. Han and C.H. Zhang, 2011. Continuous butyric acid production by corn stalk immobilized *Clostridium thermobutyricum* cells. *Afr. J. Microbiol. Res.*, 5: 661–666.

Matijasic, B.B., M.K. Rajsp, B. Perko and I. Rogelj, 2007. Inhibition of *Clostridium tyrobutyricum* in cheese by Lactobacillus gasseri. *Int. Dairy J.*, 17: 157–166

Michel-Savin, D., R. Marchal and J.P. Vandecasteele, 1990. Butyrate production in continuous culture of *Clostridium tyrobutyricum*: Effect of end–product inhibition. *Appl. Microbiol. Biotechnol.*, 33: 127–131

Mitchell, R.J., J.S. Kim, B.S. Jeon and B. I. Sang, 2009. Continuous hydrogen and butyric acid fermentation by immobilized *Clostridium tyrobutyricum* ATCC 25755: Effects of the glucose concentration and hydraulic retention time. *Bioresour. Technol*., 100: 5352–5355

Ramey, D. and S. Yang, 2004. *Production of butyric acid and butanol from biomass*. Final Report, U.S. Department of Energy, Morgantown, West Virginia, USA

Rodriguez, J., R. Kleerebezem, J.M. Lema and M.C.M. van Loosdrecht, 2006. Modeling product formation in anaerobic mixed culture fermentations. *Biotechnol. Bioeng.*, 93: 592–606

Saint-Amans, S. and P. Soucaille, 1995. Carbon and electron flow in *Clostridium butyricum* grown in chemostat culture on glucoseglycerol mixtures. *Biotechnol. Lett.*, 17: 211–216

Soni, B.K. and M.K. Jain, 1997. Influence of pH on butyrate uptake and solvent fermentation by a mutant strain of *Clostridium acetobutylicum. Bioprocess Eng.*, 17: 329–334

van Andel, J., G. Zoutberg, P. Crabbendam and A. Breure, 1985. Glucose fermentation by *Clostridium butyricum* grown under a self generated gas atmosphere in chemostat culture. *Appl. Microbiol. Biotechnol.*, 23: 21–26

Vandák, D., M. Telgarský and E. Šturdík, 1995. Influence of growth factor supplements on butyric acid production from sucrose by *Clostridium butyricum*. *Folia Microbiol.*, 40: 669–672

Wei, D., X. Liu and S. Yang, 2013. Butyric acid production from sugarcane bagasse hydrolysate by *Clostridium tyrobutyricum* immobilized in a fibrous–bed bioreactor. *Bioresour. Technol.*, 129: 553–560

Wu, D., H. Chen, L. Jiang, J. Cai, Z. Xu and P. Cen, 2010. Efficient separation of butyric acid by an aqueous two–phase system with calcium chloride. *Chin. J. Chem. Eng.*, 18: 533–537

Zhang, C., H. Yang, F. Yang and Y. Ma, 2009. Current progress on butyric acid production by fermentation. *Curr. Microbiol.*, 59: 656–663

Zhu, Y. and S.T. Yang, 2003. Adaptation of *Clostridium tyrobutyricum* for enhanced tolerance to butyric acid in a fibrous–bed bioreactor. *Biotechnol. Prog.*, 19: 365–372

Zigová, J. and E. Šturdík, 2000. Advances in biotechnological production of butyric acid. *J. Ind. Microbiol. Biotechnol.*, 24: 153–160

Zigová, J., E. Sturdik, D. Vandak and S. Schlosser, 1999. Butyric acid production by *Clostridium butyricum* with integrated extraction and pertraction. *Process Biochem.*, 34: 835–843

Zones, D.T. and D.R. Woods, 1986. Acetone–butanol fermentation revisited. *Microbiol. Rev.*, 50: 484–524

**(Received 25 October 2013; Accepted 24 February 2014)**