

Original Research Article

doi: <http://dx.doi.org/10.20546/ijcmas.2016.502.070>

Studies on Biobutanol Production and Comparison of its Downstream Processes

Bhatnagar Tripti^{1*}, Aggarwal Meetu² and Jaiswal Alok¹

¹Codon Biotech Pvt. Ltd, Noida, Uttar Pradesh, India

²Noida International University, Noida, Uttar Pradesh, India

*Corresponding author

ABSTRACT

Keywords

Biobutanol,
Lignocellulosic
wastes,
Fermentation,
Distillation,
Pervaporation.

Article Info

Accepted:

29 January 2016

Available Online:

10, February 2016

The depletion of natural resources, petroleum products and rising prices of raw materials tend to look for fuels from renewable energy sources and biofuels. Biobutanol is a very attractive energy source because it is non-hygroscopic, does not cause corrosion and has a higher calorific value. The basic problem of wider use of biobutanol depends on its production with sufficient efficiency and this in turn is limited by separation of butanol from fermentation broth. The present research work is concerned with the production of Butanol and synthesis of such a process that results in highest purification fold and also minimizes the cost of the downstream processing. Thus, the process involves 1) production of Biobutanol from Lignocellulosic wastes by *Clostridium acetobutylicum* 2) study of different separation processes to separate butanol from fermentation broth and identify a economically viable process.

Introduction

Biobutanol has properties similar to gasoline which makes it the most attractive alternative fuel. When compared with ethanol, it has a longer carbon chain length, a higher volatility, polarity, combustion value, octane rating and is less corrosive. (Honig et al, 2014). These properties make butanol a better a substitute for gasoline without any modification in vehicle or engine technologies. Furthermore, it has less ignition complications because the heat of vaporization of butanol is less than half of that of ethanol, thus an engine functioning

on butanol is easier to start in cold weather than the one working on ethanol or methanol. Two main obstacles in butanol fermentation and production are the expenditure on the substrate or raw material and the inhibition caused by the product as butanol concentrations around 20 g/L inhibit microbial growth. (Claassen et al, 2000). The clostridium species are strictly anaerobes and for maintaining the anaerobic conditions the reactor must remain closed during the process.

To make the raw material cheaper and cost effective different Lignocellulosic materials have been utilized for Butanol production. But with these substrate new problems arise like: production of fermentation inhibitors on hydrolysis of these materials and problems with the separation techniques and their application. Distillation is the unit operation extensively used in separation of aqueous solution from butanol fermentation but the complication in this process is the formation of an azeotrope that increases the energy cost. (Visioli et al., 2014).

This paper deals with production of butanol using cheap and economical lignocelluloses wastes which are thrown out of every house. After production different purification or butanol separation methods were tried in order to map out a cost effective process flow. Process synthesis is one of the most, if not the most, effective means of reducing chemical production cost (Qureshi et al, 2008). Butanol, ethanol, and acetone are major chemicals consumed in bulk in a variety of processes, e.g., as fuels, fuel additives, solvents, or precursors for synthesizing various other organic compounds.

Production of butanol, ethanol, and acetone by fermenting different starch containing sources have shown that grains, rice husk and other cellulosic material are good raw material for Butanol production by *Clostridium acetobutylicum*. The conversion of grains has been significantly enhanced through genetic engineering of enzymes (Oudshoorn et al, 2009). Nevertheless, the attainable conversion at present remains extremely low, thereby rendering the products exception-ally dilute in the aqueous effluent streams. The downstream processing to separate and purify the products is very expensive. Thus, reducing the downstream processing cost will lead to reduction of the total production

cost. In this study different separation methods have been tested to analyse the percentage purification.

Materials and Methods

Clostridium acetobutylicum NCIM 2878 was obtained from NCIM, Pune. Fresh inoculum suspension was prepared by maintaining *C.acetobutylicum* in reinforced Cooked Meat Media. Anaerobic handling and incubation of the culture were performed in anaerobic chamber with nitrogen gas. The culture was incubated at 37°C for 48 hours until active growth was observed.

Medium was prepared as described by Xue et al (2012) but with various modifications. Lignocellulosic wastes (mostly house hold and kitchen green waste) 20 – 30 g/L, yeast extract 1g/L, KH₂PO₄ 0.5g/L, K₂HPO₄ 0.5g/L, Ammonium acetate 2.2g/L, MgSO₄.7H₂O 0.2g/L, MnSO₄.H₂O 0.01g/L, FeSO₄.7H₂O 0.01g/L, NaCl 0.01 g/L. In this experiment soyabean powder and animal wastes was used as a substitute for yeast which is very expensive. The medium was autoclaved at 121°C at 15 psi for 20 minutes.

The inoculum size of 10% was used to inoculate the fermentation medium. Shake flask and 3 litre fermenter were used for butanol production. Anerobic condition was maintained by providing culture media with the supply of oxygen free nitrogen gas. Fermentation was carried out for 4-5 days. Butanol in the fermentation broth was analyzed by GC.

Separation Methods

In the present study different methods of purification were used to analyse or select a purification method which would decrease the cost of the whole process. There is a

systematic removal of this butanol from the fermentation broth. The traditional method of product recovery is distillation.

Distillation

Fermentation broth was taken in the distillation unit and heated at 100°C and the residue was collected. Butanol was then estimated from gas chromatography. Butanol and water forms a azeotropic mixture. As butanol has a higher boiling point than water, therefore, this process consumes much energy, and therefore it increases the cost of the whole process, especially at low concentration of butanol in the broth. Distillation is a high energy utilizing process, so more energy loss occurs in this method. Distillation method leads low productivity and high costs for purification of butanol.

Adsorption

Adsorption using membranes was used for butanol purification from fermentation media. Membranes of Silicates and active charcoal were used for adsorption because it is removal of organic impurities which can affect color, taste, and odor. It is used for removal of butanol from fermentation broth in laboratory scale because the separation process is not suitable on an industrial or semi-technical scale.

Filtration

Two filtration steps were used for filtration. First, microfiltration was used to remove the cells from the reactor broth and second ultra filtration membranes were used to filter out proteins with a molecular weight of 5,000 Daltons and higher. The feed pressure of ultra filtration membrane was 60 psi.

Gas stripping

Gas stripping technique was used for

separation of butanol from fermentation broth. Gas stripping is an easy to operate technique for butanol recovery from fermentation broth. The fermentation broth is first filtered or centrifuged to remove solid material. Then the feed is injected at the bottom of the stripping jar and the hot gases are introduced so as to help in separation of acetone, ethanol and butanol at different levels. This technique utilizes the differences of volatilities among compounds. The volatile compounds can be obtained by lowering the pressure, heat, and use of inert gas. This technique is only used for lab scale.

Pervaporation

Pervaporation method was used for separation of butanol from fermentation media. In this method, different membranes were used to act as a selective barrier between the two phases: the liquid phase feed and the vapor phase permeate. The membranes used were: Zeolite membranes, PDMS (Polydimethylsiloxane) with or without Ceramic membrane and PTFE were used for molecular separations of Butanol from acetone and ethanol which are co-produced in the ABE process. The membrane was placed in contact with the fermentation broth and the volatile liquids or solvents diffuse through the membrane as a vapor which are recovered by condensation. A vacuum was applied to the side of permeate. The permeated vapors were condensed on low pressure side. The membrane acted as a selective barrier between the two phases. When vacuum is applied the separation occurs at different rates, if different parameters were changed. Initially different temperatures were tried for checking the butanol separation flux. Then, the feed flow and feed concentration was also varied to obtain higher flux of separation. Here, glucose was used as feed.

Analytical Analysis

Butanol in the fermentation broth was estimated by gas chromatography method. A computer related Nuchrome series gas chromatograph equipped with flame ionization detector (FID) was employed for the separation and quantification of ethanol. A stainless steel column (5m × 2mm) was fitted into the instrument to provide on column injection. The column packing was PorapakQ. The detector and injector temperature was maintained at 200°C. The gas chromatograph was connected to an integrator and computer system to determine area of ethanol and internal standard peak. For analysis of butanol the following program has been standardized. Butanol was determined in gm/l.

Results and Discussion

Butanol production

In the present study *C.acetobutylicum* NCIM 2878 was used for butanol production. The strain was maintained cooked meat media. *C.acetobutylicum* was successfully culture in the cooked meat media and active growth was observed after incubation of 48 hrs at 37°C. Batch fermentation was used for producing butanol using *C.acetobutylicum*. Fermentation was carried out for 4-5 days. The highest production was observed in the broth where orange peel was used as carbon source i.e 3.06gm/L whereas Baggassae produces 2.61gm/L of butanol. As seen in the Table:1 and Figure:1 the butanol production was less when carbon source was used as compared to some of the cellulosic wastes like orange peel and pulp.

But when Soyabean and animal wastes were used as nitrogen source (Table:2 and Figure:2) it was seen that the production increased to 7.6 gm/L - 8.0 gm/ L which is a sufficiently high production levels when

lignocellulosic wastes were used. Thus, productions studies led to identification of cost effective and economical fermentation media and raw material. The productivity of the whole process was a little less (0.24 g/l/h) as the highest amount of butanol is produced on the 4th day i.e actually after 65 hours.

This study thus shows that the media raw materials like the carbon source and nitrogen sources can be changed into very cost effective constituents thus reducing the cost of the whole fermentation process.

Effective Separation of Butanol from Fermentation Broth

Recovery or separation process for the biobutanol produced was done first by distillation. Before distillation, the production of butanol was 2.4 gm/l when Glucose was used as carbon source. The fermentation broth was centrifuged to remove the cell debris and proteins. Fermentation broth was then taken in the distillation unit and after distillation, purified butanol was obtained as residue was 92 % pure and recovery was very less. When 100 ml was used as starting material only 9.6 ml pure butanol was left.. It is showing in fig no 1 and tables no 1.

The traditional method of product recovery is distillation. Distillation is a process which requires high input cost of energy, as the boiling point of water is lower than the maximum concentration of butanol and butanol present in the fermentation broth is 3% by weight. This leads to low productivity and high costs of separation and purification of butanol.

Next, filtration of fermentation media was done to remove solid material. The two filters were used for filtration. First, microfiltration was used to remove the cells

from the reactor broth and second ultra filtration membranes were used to filter out proteins. *C. acetobutylicum* cells and proteins were removed from fermentation media by micro filter and ultrafilter. Once the solid materials were removed, separation of Butanol from the fermentation broth was tried out by adsorption process. Silicalite, a form of silica with a zeolite-like structure and hydrophobic properties was used for adsorption to selectively adsorb small organic molecule like like butanol etc from dilute aqueous solutions. Active charcoal was also used for adsorption. This step removes the colour in the broth.

Gas stripping was done for butanol recovery under various operating condition. The gases were utilized from fermentation media. The butanol was recovered from fermentation broth by gas stripping. Being a volatile compound separation was good and 30% recovery was obtained.

Pervaporation was used for separation of butanol from broth. Zeolite membranes, PDMS (Polydimethylsiloxane) with or without Ceramic membrane and PTFE were used for molecular separations of Butanol from acetone and ethanol which are co-produced in the ABE process. The membrane is placed in contact with the fermentation broth and the volatile liquids or solvents diffuse through the membrane as a

vapor which is recovered by condensation. Recovery was 60 % of the total production and the Butanol flux through the membrane was 43 - 700 gm⁻² h⁻¹ depending on different parameters. The flux changed as and when the temperature, the fermentation feed input rate and feed concentration is varied. Three aspects were studied for optimization of pervaporation. Firstly, Effect of temperature was observed by changing the temperature during the filtration process.

- a) Effect of Operating Temperature: During pervaporation when temperature of the unit was increased it was found that PTFE gave the best results. As the temperature increased the amount of purified permeate travelling across the membrane increased. Thus the separation was best at 60⁰C. (Table :4)
- b) Effect of Feed Concentration: The same kind of results was obtained when the feed concentration was increases from 1ml/min to 3.5ml/min. The highest separation was done when PTFE membrane was used.(Table :5)
- c) Effect of Feed flow rate: Another parameter taken to analyze the capacity of PTFE membrane was feed flow. This means that the separation of butanol from Broth depends upon the flow of the feed injected in the port. (Table :6)

Table.1 Production of Butanol when Different Lignocellulosic Waste were used as Carbon Source

S.No.	Broth containing different Lignocellulosic waste	Biobutanol production in gm/l			
		1 st Day	2 nd Day	3 rd Day	4 th Day
1	Glucose	0.33	0.41	1.3	2.4
2	Baggassae	0.4	0.88	1.45	2.61
3	Orange peel and pulp	0.2	0.75	2.43	3.01
4	Vegetable waste	-	0.36	0.81	0.97
5	Weeds /grasses	-	0.5	0.72	1.01

Table.2 Production of Butanol when Soyabean Powder and Animal Waste were used as Nitrogen Source

S. No.	Broth containing different Lignocellulosic waste	Biobutanol production in gm/l			
		1 st Day	2 nd Day	3 rd Day	4 th Day
1	Glucose (20 %) + Soyabean pd.	0.43	0.52	2.3	8.4
	Glucose (20 %) + animal waste	0.35	0.75	2.6	7.0
2	Bagassae + Soyabean pd.	0.45	0.68	2.45	7.6
	Bagassae + animal waste	0.36	0.77	2.05	7.4
3	Orange peel and pulp + Soyabean pd.	0.2	0.75	2.76	8.01
	Orange peel and pulp + animal waste	0.33	0.67	2.58	7.45
4	Vegetable waste + Soyabean pd.	0.23	0.56	0.79	2.47
	Vegetable waste + animal waste	0.12	0.63	0.71	1.96
5	Weeds /grasses + Soyabean pd.	-	0.32	0.49	1.25
	Weeds /grasses + animal waste	-	0.41	0.49	1.06

Table.3 Butanol Production Before and After Distillation

Sample	Butanol
Before distillation	2.4 gm/l
After distillation	9.6 gm/l

Table.4 Effect of Operating Temperature on Total Flux of Butanol During Pervaporation in Gm-2h-

Temperature	PDMS	ZEOLITE	PDMS+CERAMIC	PTFE
30°C	50	43	43	235
35°C	53	50	50	305
40°C	60	57	57	342
45°C	66	65	65	423
50°C	72	68	68	490
55°C	78	74	74	570
60°C	84	80	80	680

Table.5 Effect of Feed Concentration on Total Flux in gm-2h⁻¹ during Pervap

Feed conc. gm/L	PDMS	ZEOLITE	PDMS+CERAMIC	PTFE
1	50	41	150	300
1.5	75	45	187	478
2	89	49	238	508
2.5	97	66	342	585
3	120	84	402	652
3.5	142	107	539	704

Table.6 Effect of Feed Flow on Total Flux in gm-2h⁻¹

FEED FLOW	PDMS	ZEOLITE	PDMS+CERAMIC	PTFE
30	50	62	142	192
40	63	76	202	212
50	78	82	294	294
60	90	94	340	348
70	97	101	398	402
80	115	122	410	439
90	136	134	490	520
100	160	155	554	580

Figure.1 Bar Diagram Showing Comparative Butanol Production when Different Carbon Source were Used

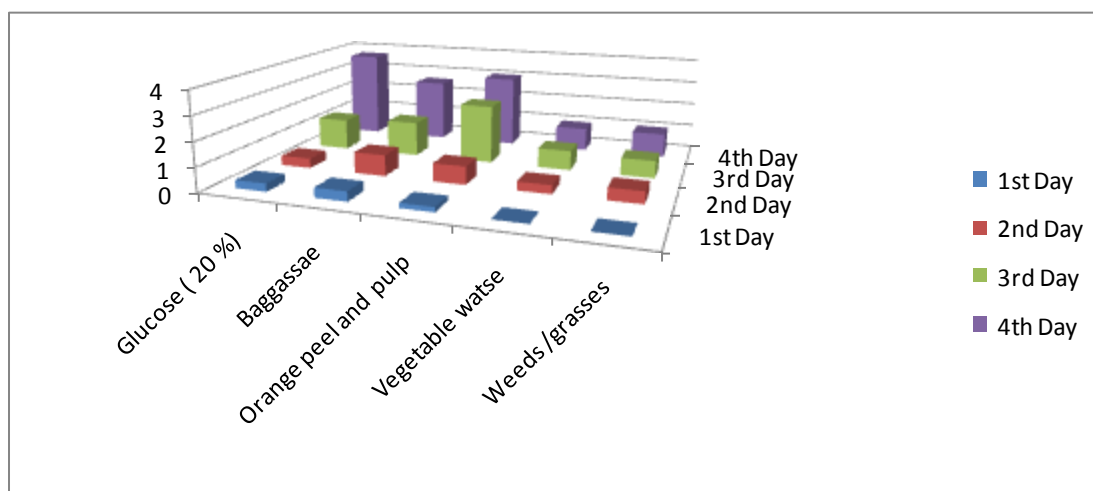


Figure.2 Bar Diagram Showing Comparative Butanol Production when Different Nitrogen Sources were Used

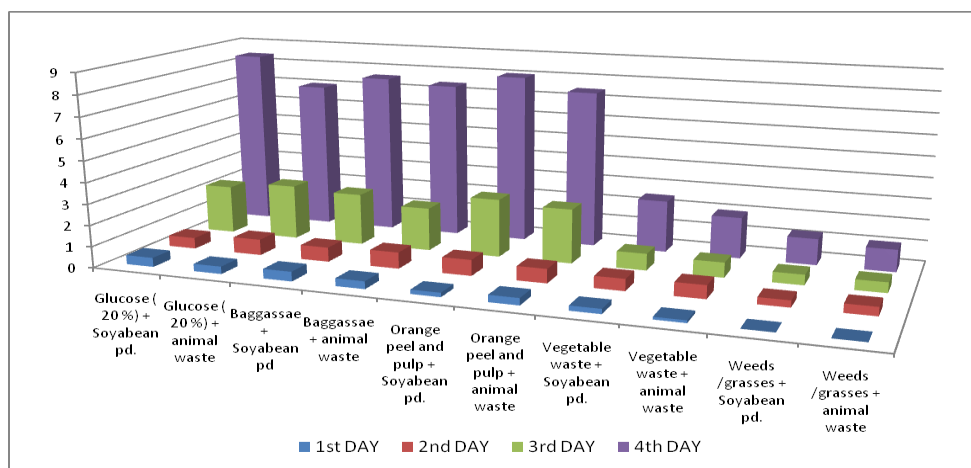


Figure.3 Effect of Operating Temperature during Pervaporation on Total Flux in gm-2h⁻¹

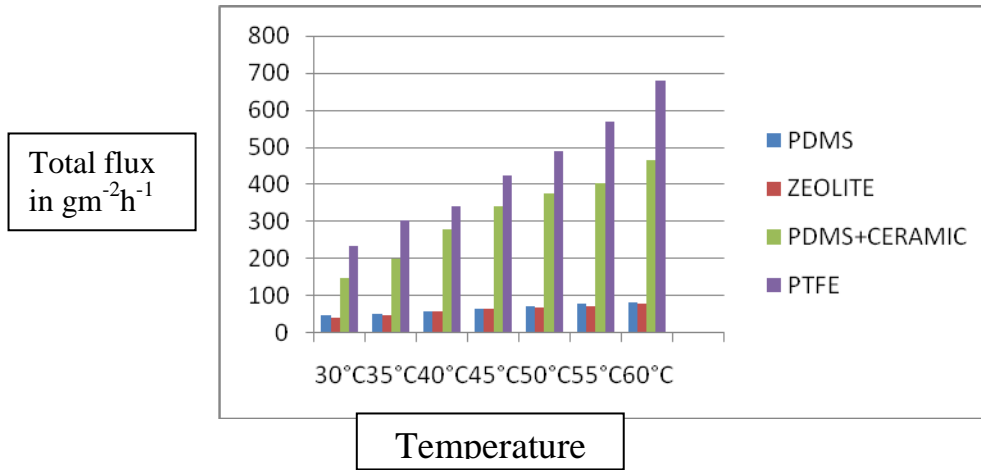


Fig.4 Effect of Feed Concentration on Total Flux in gm-2h⁻¹ during Pervaporation

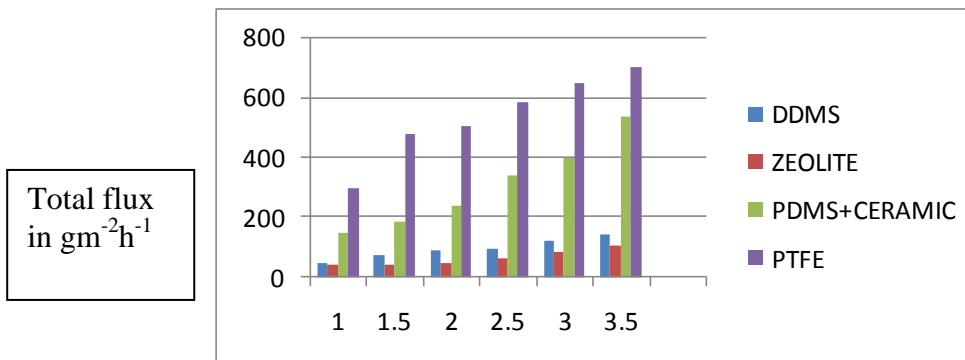
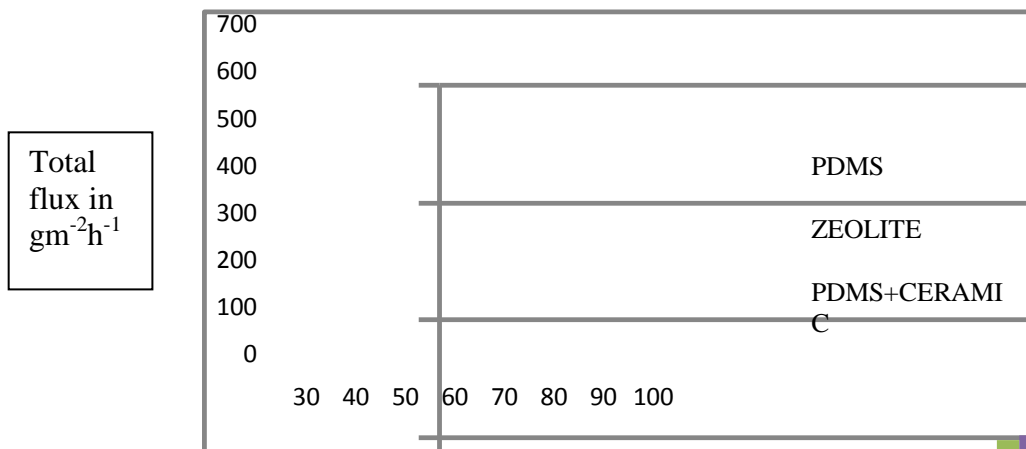


Fig.5 Effect of Feed Flow on Total Flux in Gm-2h⁻¹ during Pervaporation



Butanol produced was 2.4 gm/L when glucose was the main carbon source, while it increased to 3.04gm/L when orange peel and

pulp were used as carbon source. This production was then further enhanced when orange pulp and peel were used along with

soyabean powder as nitrogen source. The residual acid concentration at the end of fermentation was 1.26 g/L. *C. acetobutylicum* displayed similar pattern in all the fermentations.

The concentrations of both the ABE and the butanol measured at the end of the fermentation in soyabean supplemented media were higher than that measured during the fermentation in standard medium. The butanol produced was 2.4 gm/L, 3.8 gm/L, 7.6gm/L respectively for glucose, baggassae and soyabean + baggassae. Therefore, the beneficial effects of soyabean supplementation improve the economic advantage of the process.

Recovery of butanol through the process of pervaporation using PTFE membrane was 60 % of the total production and the Butanol flux through the membrane was 43 - 700 gm⁻² h⁻¹. The flux changed as and when the temperature, the fermentation feed input rate and feed concentration is varied.

Acknowledgement

All the research work was performed in the laboratories of Codon Biotech Pvt. Ltd., Noida, India.

References

Claassen, P. A., Budde, M. A., & Lopez-Contreras, A.M. (2000). Acetone, Butanol and Ethanol production from domestic organic waste by solventogenic clostridia. *Journal of Molecular Microbiology and Biotechnology*, 2(1), 39 – 44.

Demirbas, E., M. Kobya, and M. T. Sulak (2008) Adsorption kinetics of a basic dye from aqueous solutions onto apricot stone activated carbon.

Bioresource Technology. 99(13): 5368-5373.

Evasn P.J. and Wang H.Y.: (1988) *Enhancement of butanol fermentation by Clostridium acetobutylicum in presence of decanol-oleyl alcohol mixed extractions*. *Appl. Environ. Microbiol.*, 54, 1662-1667.

Honig, V., Kotek, M., & Marik, J. (2014). Use of Butanol as a fuel for internal combustion engines. *Agronomy Research*, 12(2), 333 – 340.

Lee Joung Min , Yu-Sin Jang , Sung Jun Choi , Jung AeIm, Hyohak Song , JungHee Cho , Do Young Seung , E. Terry Papoutsakis, George N. Bennett and Sang Yup Lee (2012) *Metabolic Engineering of Clostridium acetobutylicum ATCC 824 for Isopropanol-Butanol-Ethanol Fermentation*. *Appl. Environ. Microbiol.* vol. 78 no. 5 1416-1423.

Lee, S. Y., Park, J. H., Jang, S. H., Nielsen, L. K., Kim, J., & Jung, K. S. (2008). Fermentative butanol production by Clostridia. *Biotechnology and Bioengineering*, 101(2), 209 – 28.

Oudshoorn A., Van der Wielen L.A.M., Straathof A.J.J (2009) Assessment of options for selective 1-butanol recovery from aqueous solution. *Ind. Eng. Chem. Res.*, 2009, 48, 7325-7336.

Qureshi N., Saha B.C., Hector R.E., Hughes S.R. and Cotta M.A (2008) Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part I – Batch fermentation. *Biomass Bioener.*, 32, 168-175.

Roffler S.R., Blanch H.W. and Wilke C.R. (1988) *In situ extraction*

- fermentation of acetone and butanol*.
Biotechnol. Bioeng., 31, 135-143.
- Visioli, L. J., Enzweiler, H., Kuhn, R. C.,
Schwaab, M., & Mazutti, M. a.
(2014). Recent Advances on
Biobutanol production. Blucher
Chemical Engineering Proceedings,
p. 2670-2677.
- Xue Chuang, Jingbo Zhao, Congcong Lu,
Shang-Tian Yang, Fengwu Bai, I-
Ching Tang (2012), High-Titer n-
Butanol Production by *Clostridium*
acetobutylicum JB200 in Fed-Batch
Fermentation With Intermittent Gas
Stripping, Biotechnol. Bioeng.
2012;109: 2746–2756.

How to cite this article:

Bhatnagar Tripti, Aggarwal Meetu and Jaiswal Alok. 2016. Studies on Biobutanol Production and Comparison of its Downstream Processes. *Int.J.Curr.Microbiol.App.Sci.* 5(2): 627-636. doi: <http://dx.doi.org/10.20546/ijcmas.2016.502.070>