Optimization of Biobutanol production from different lignocellulosic wastes

Aggarwal Meetu
Noida International University,

Jaiswal Alok
Noida International University,

DR.Tripti Bhatnagar
Codon Biotech Pvt. Ltd., Noida

ABSTRACT

Butanol has been considered as a potential fuel or fuel additive and an alternate to Ethanol addition to fuel. The present study used ABE fermentation process to produce biobutanol. In this study, the sustainability of four different biobutanol feedstock (lignocellulosic) materials is assessed, and it was observed that the biobutanol production from lignocellulosic waste as almost same or higher than the production when only Glucose was used as carbon source. The production of butanol when only glucose was used as carbon - was 2.4. gm/l on 4th day where as butanol from baggassae was 2.61gm/l and from orange pulp and peel was 3.01gm/l.

Key words: Butanol, Biofuels, Lignocellulose wastes.

Introduction

Bioethanol and biodiesel are now the most used biofuels in the transportation sector, but even then new alternatives such as biobutanol are needed to fulfill the fuel demands. The other important aspect is that, it is important that biofuels are produced in as sustainable a way as possible. In particular, production based on non-food feedstocks such as lignocellulosic materials and wastes/by-products is considered and sustainability assessment is performed to evaluate different feedstocks. The hypothesis is that lignocellulosic and waste-based biobutanol can be a sustainable transportation fuel.

Butanol is an aliphatic saturated alcohol with the molecular formula C_4H_9OH. It is superior to ethanol with regard to having higher energy content, lower volatility, being less hydroscopic and less corrosive (Sang Yup Lee *et al*, 2009). Butanol is an important industrial solvent and advanced biofuel that can be

produced by biphasic fermentation by *Clostridium acetobutylicum* (Yu-Sin Jang *et al*, 2012),a process more commonly known as the ABE fermentation.

ABE fermentation process includes two phases. The first phase is known as the acidogenic phase, where the acid formation pathways are activated in which carbohydrate substrates particularly glucose, are fermented to organic acids. Acetate, butyrate, hydrogen and carbon dioxide are the major products of this phase. This acidogenic phase usually occurs during the growth phase of the Clostridium species. The second phase is the solventogenic phase in which acid reassimilation occurs (Emran I. Khamaiseh*et al*, 2014, Qureshi et al,2000; Leeet al ;2008; Napoli et al,2010) and final Butanol is produced.

The main perspective of this research is to study the effect of the type of lignocellulosic material being used for Butanol production.

2. Material and Method

Clostridium acetobutylicum NCIM 2878 was obtained from NCIM, Pune. Fresh inoculum suspension was prepared by maintaining *C.acetobutylicum* in reinforced Cooked Meat Media. Sodium thioglycolate was added to the autoclaved Cooked Meat Media for anaerobic condition. The experiments were performed in anaerobic chamber with nitrogen gas. The culture was incubated at 37°C for 48 hours until active growth was observed.

2.1 Media preparation for butanol production

Normally cell growth of *C. acetobutylicum*is is dependent on the presence of Mg, Fe and K in the medium (Frederic Monot *et* a*l*, 1982). P2 medium as described by Xue et al (2012) was prepared with certain modifications. It consisted of glucose (80 – 100g/L), yeast extract (1g/L), KH_2PO_4 (0.5g/L), K_2HPO_4 (0.5g/L), ammonium acetate (2.2g/L), vitamins (1mg/L- *para* amino benzoic acid, 1mg/L thiamin and 0.01 mg/L biotin) and mineral salts (0.2g/L $MgSO_4.7H_2O$, 0.01g/L $MnSO_4.H_2O$, 0.01g/L $FeSO_4.7H_2O$, 0.01 g/L NaCl) and autoclaved at 121^0c at 15 psi for 20 minutes. The above media was further modified using different lignocellulosic waste and Butanol production was estimated accordingly. Different medias were then used to optimize the production of Butanol.

The lignocellulosic wastes which were used were baggassae, Orange peel, vegetable waste, and agricultural waste like weeds and grasses. Shake flask method of fermentation was used for the production of butanol using *C.acetobutylicum* as the bacterial inoculum. For depolymerization and hydrolysis of the lignocellulosic waste, methods like acid hydrolysis, heat hydrolysis and enzymatic hydrolysis was used.

Pyrex screw capped bottle were filled with 100 ml each of fermentation media having different lignocellulosic material as carbon sources and inoculated with 10% of the *C. acetobutylicum*is culture. The bottles were kept in a desiccators and anaerobic condition was provided with the supply of oxygen free nitrogen gas into unit. Fermentation was carried and Butanol concentration was estimated with Gas chromatograph having a FID detector. A computer related Nuchrome series gas chromatograph equipped with flame ionization detector (FID) was employed for the separation and quantification of Butanol. Optical density was measured at 600 nm for cell growth.

3. Results and Discussion

In the present study the *C.acetobutylicum* NCIM 2878 was used for initial study of butanol production. The strain was maintained on cooked meat media. *C. acetobutylicum* was successfully culture in the cooked meat media and active growth was observed after incubation of 48 hrs at 37^0C. The growth curve of *C.acetobutylicum* showed that stationary phase after 3 days. When the curve was co - related to Butanol production it was seen that the maximum production of Butanol was also seen on the 4^{th} day after inoculation.

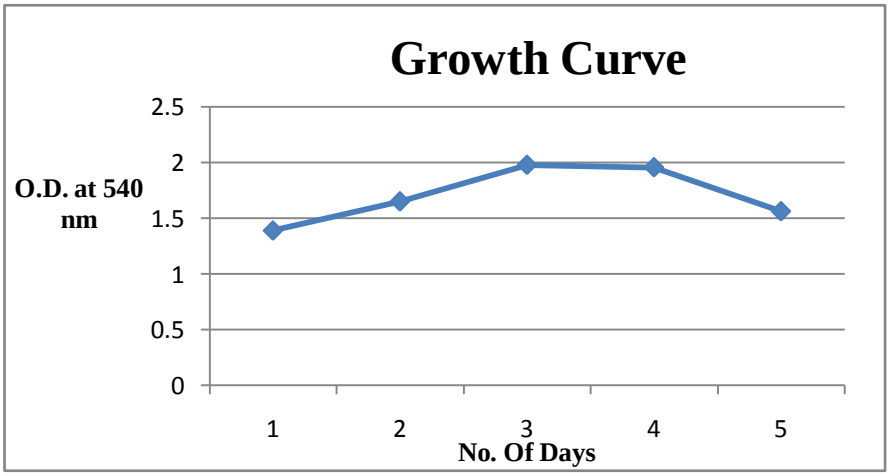

Fig no. 1 Growth curve of *C.acetobutylicum*

S.No.	Broth containing different Lignocellulosic waste	Biobutanol production in gm/l			
		1st Day	2nd Day	3rd Day	4th Day
1	Glucose (20 %)	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 watse	-	0.36	0.81	0.97
5	Weeds /grasses	-	0.5	0.72	1.01

Table no. 1 Butanol production on different days after inoculation

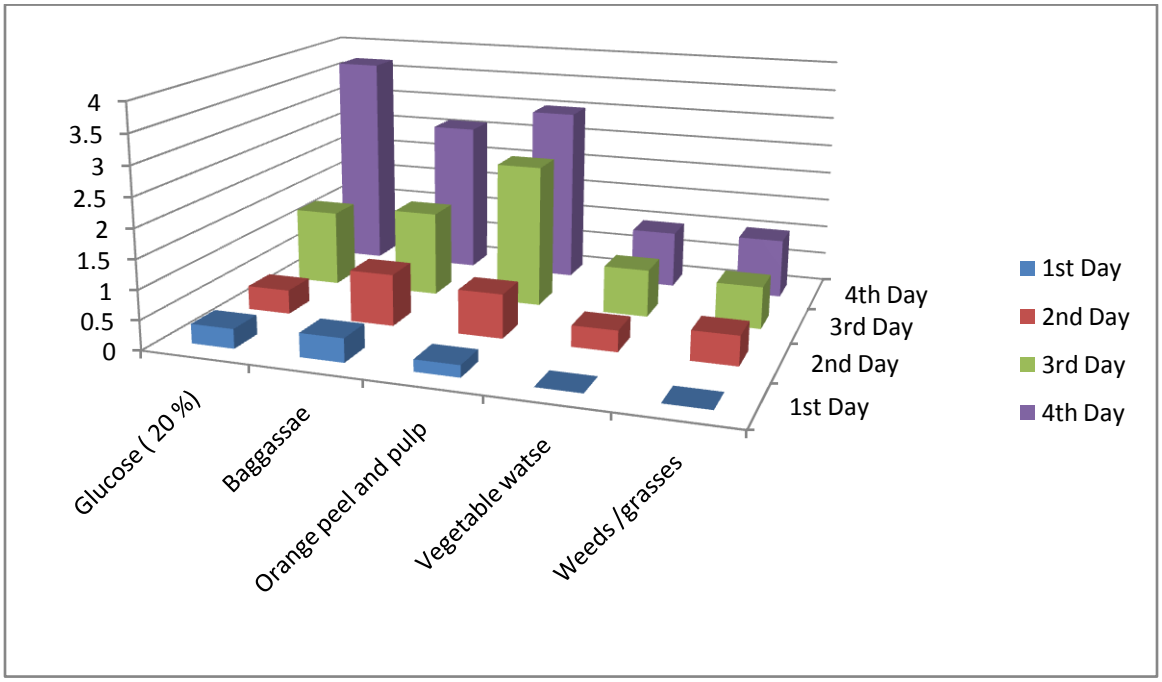

Fig no. 2 Bar diagram depicting the relative production quantities of butanol.

The production of butanol was estimated by gas chromatography. Butanol was found to be produced highest on the 4th day. When calculation were done utilizing the standard of butanol, there were a vast difference between the production of 1st, 2nd ,and 3rd day, as seen in fig (2). The production of butanol when only glucose was used as carbon - was 0.033 gm/l on 1st day, 0.041 gm/l on 2nd day, 1.3 gm/l 3rd day, and 2.4. gm/l on 4th day. This pattern of production is seen to be common for all types of fermentation media. Even though the concentration of butanol produced in different medias differ.

Butanol production occurs during stationary phase (solventogenesis) and is not considered to be associated with cell growth. In a different study, living C. acetobutylicum ATCC 824T immobilized to beechwood shavings exhibited the maximum ABE productivity of 1.19 g/L/h at a dilution rate of 0.374 h–1 using glucose as a substrate. Note that this value is much lower than those observed using high-density immobilized growing cells and cell recycling. A drastic decrease in ABE productivity over time was also observed, presumably due to the lack of enzyme regeneration under nitrogen-limited conditions. To maintain the activity of living cells, the growth medium is intermittently supplied cells during continuous butanol production [Reardon and Bailey , 2013]. By this method, the operational period could be prolonged to more than 30 days by intermittent dosing of nutrient medium for 15 min every 7 h. In another study, Batch, fed-batch, and continuous A-B-E fermentations were conducted and compared with pH controlled at 4.5, the optimal value for solvent production. It has been shown that the continuous mode was preferential in terms of butanol yield and productivity. The highest butanol yield and productivity found in the continuous fermentation at dilution rate of 0.1 h^{-}

Thus, the production from lignocellulosic waste seems to be a cost effective technique, to a certain extent, even though the yield of butanol is very less. Butanol production by ABE fermentation could not compete economically with petrochemical synthesis. In order to introduce an economically competitive biological process, there are major drawbacks in typical batch fermentation like I. High substrate costs when glucose and molasses was used. II. Low final butanol concentration. III. Low butanol productivity. IV. High cost of butanol recovery from low-concentration yields. Thus, if lignocellulosic wastes are used the cost of raw material decreases, studies are required for increasing the butanol production.

4. Conclusion

Over all study and result indicated that the Optimization of butanol production from fermentation media using *C. acetobutylicum* was done wherein initial production on the fourth day of fermentation was 2.4 gm/l to 3.01gm/l .

5. Acknowledgement:

The entire research work has been done in the laboratories of Codon Biotech Pvt. Ltd.

REFERENCE

1. Chuang Xue, Jingbo Zhao, Congcong Lu, Shang-Tian Yang, Fengwu Bai, 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.

2. Qureshi N., Schripsema J, Lienhardt J, Blaschek H P., (2000) Continuous solvent production by *Clostridium beijerinckii*BA101 immobilized by adsorption onto brick, World J MicrobBiot., 16, 337-382.

3. Napoli F, Olivieri G., Russo M.E., Marzocchella A., Salatino P., (2012), Optimization of solvent recovery in the production of butanol by fermentation. Environmental Eng and Management J, 11, 1499-1504.

4. Napoli F., Olivieri G., Russo M.E., Marzocchella A., Salatino P., (2010) Butanol production by *Clostridium acetobutylicum*in a continuous packed bed reactor. J Ind. Microbiol. Biotechnol., 37, 603–608.

5. Liu J., Fan L.T., (2004) downstream process synthesis for biochemical production of butanol, ethanol, and acetone from grains: generation of optimal and near-optimal flowsheets with conventional operating units, Biotech. Progress, 20, 518-1527

6. Lee S., Cho M., Park C., (2008) Continuous butanol production using suspended and immobilized *Clostridium beijerinckii*NCIMB 8052 with supplementary butyrate, Energy & Fuels, 22(13), 3459-3464.

7. Ezeji T.C., Qureschi N., Blaschek H.P., (2007) Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii*coupled with product recovery by gas stripping, J Ind. Microbiol. Biotechnol., 34, 771–777.

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

9. Jin Young Lee1, Yu-Sin Jang1, Joungmin Lee1, Eleftherios Terry Papoutsakis3and Sang Yup Lee (2009) Metabolic engineering of *Clostridium acetobutylicum* M5 for highly selective butanol productionArticle first published online: 14 OCT 2009DOI: 10.1002/biot.200900.

10. EZEJI THADDEUS C ET AL: "Butanol fermentation research: upstream and downstream manipulations." CHEMICAL RECORD (NEW YORK, N.Y.) 2004, vol. 4, no. 5, 2004, pages 305-314, XP002502399 ISSN: 1527-8999**.**

11. Emran I. Khamaiseh,[1] Aidil Abdul Hamid,[1] Peyman Abdeshahian,[2] Wan Mohtar Wan Yusoff,[1] and Mohd Sahaid Kalil (2014) Enhanced Butanol Production by Clostridium acetobutylicum NCIMB 13357 Grown on Date Fruit as Carbon Source in P2 MediumThe Scientific World Journal Volume 2014

12. Monot F, Martin JR, Petitdemange H, Gay R. (1982) Acetone and butanol production by Clostridium acetobutylicum in a synthetic medium. Appl Environ Microbiol. ;2:1318–1324

13. Journal of Sustainable Development of Energy, Water and Environment Systems

Year 2013 Volume 1, Issue 2, pp 58-77

14. Reardon KF, Bailey JE. 2013 Effects of pH and added metabolites on bioconversion by immobilized non-growing Clostridium acetobutylicum. Biotechnol. Bioeng. 1989;34:825-837.