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Direct fermentation of citrus fruit waste by *Clostridium acetobutylicum* for production of Butanol

Jaiswal Alok[1], Bhatnagar Tripti*[1], Aggarwal Meetu[2]

1 Codon Biotech Pvt. Ltd., Noida
2 Noida International University, G.Noida, Uttar Pradesh, India.

E-mail : tripti.codonbt@gmail.com Contact No. : +91-9844037008

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Abstract

Butanol has been considered as a potential fuel or fuel additive and an alternate to Ethanol addition to fuel. Single-culture Clostridial fermentation was conducted to obtain Butanol from Citrus fruit waste as a low cost feedstock. Different inoculum concentrations and different nitrogen source were utilized for the fermentation. The fermentation resulted in 2.4. gm/l of butanol when only glucose was used as carbon source while production of Butanol increased to 3.01gm/l when orange pulp and peel was utilized. To further increase the production, low cost raw material like soyabean powder and animal waste were also used which resulted in 8gm/L of Butanol production.

Key words : Butanol, Biofuels, Citrus, Fermentation, Clostridium .

INTRODUCTION

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.[1] Butanol is an important industrial solvent and advanced biofuel that can be produced by biphasic fermentation by *Clostridium acetobutylicum* [2], 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. The second phase is the solventogenic phase in which acid reassimilation occurs [3][4] and final Butanol is produced.

For high and continuous use of Biofuels it is necessary that biofuels are produced in a sustainable way. [5][6] 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 main perspective of this research is to analyze the effect of culture concentration, use of Citrus waste material and use of soyabean powder as low cost raw material for Butanol production.

MATERIALAND 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.

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.[7] P2 medium [8] was prepared with certain modifications. It consisted of glucose (20g/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^{0}c at 15 psi for 20 minutes. The above media was further modified using different Citrus fruit pulp and peel waste (20% to 50%) and Soyabean and animal waste (6%-10%) as low cost raw material instead of Glucose and Yeast extract.

Shake flask method of fermentation was used for the production of butanol using *C.acetobutylicum* as the bacterial inoculum. Pyrex screw capped bottle were filled with 100 ml each of fermentation media having citrus fruit waste as carbon sources and inoculated with 5%, 10%, 15%,20% 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 Nuchrome series gas chromatograph equipped with flame ionization detector (FID) was employed for quantification of Butanol.

RESULTS

In the present study *C.acetobutylicum* NCIM 2878 was used for initial study of butanol production. The strain was maintained on cooked meat media. *C. acetobutylicum* was successfully cultured in the cooked meat media and active growth was observed after incubation of 48 hrs at 37^{0}C. The growth curve of *C.acetobutylicum* showed that stationary phase after 3 days. When the curve was correlated to Butanol production it was seen that the maximum production of Butanol was also seen on the 4th /90-96 hours day after inoculation.

The production of butanol was estimated by gas chromatography. Butanol was found to be produced highest after 90 hours. When different concentrations of *C.acetobutylicum* were used, it was observed that 20% concentration gave the highest butanol production of 3.01gm/l with Citrus waste as carbon source and yeast as nitrogen source (Table: 1, Fig: 1).

Batch fermentation was used for producing butanol using *C.acetobutylicum*. Fermentation was carried out for 4-5 days.

Table 1 : Butanol production on different days after inoculation using different Culture concentrations

S. No.	Broth containing Citrus fruit waste + different culture concentration.	Biobutanol production in gm/l			
		1st Day	2nd Day	3rd Day	4th Day
1	5%	0.33	0.41	1	1.23
2	10%	0.4	0.88	1.26	1.94
3	15%	0.2	0.75	1.78	2.56
4	20%	0.9	1.21	2.33	3.01

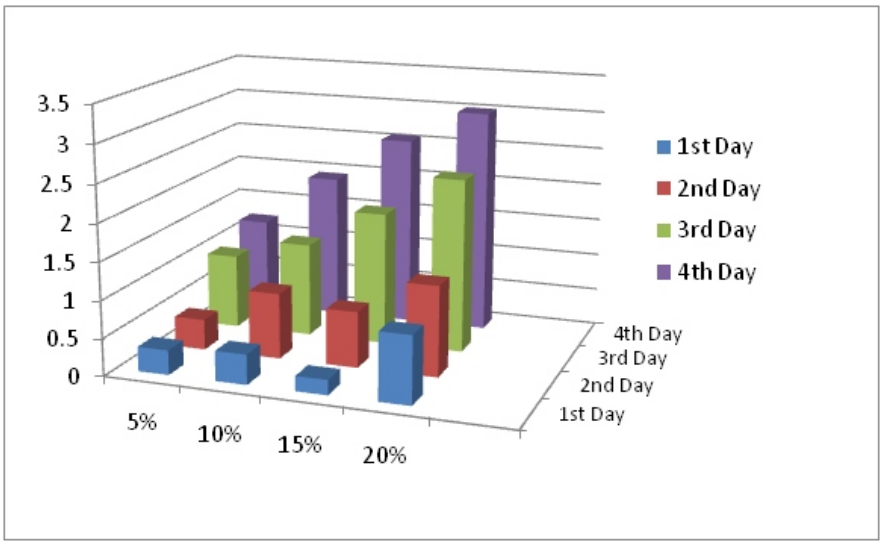

Figure 8 : Effect of Moisture content for Maximum Growth and Enzyme production

Table 2 : Production of Butanol when different lignocellulosic waste were used as carbon source.

S.No.	Broth containing different Lignocellulosic waste	Biobutanol production in gm/l			
		1st Day	2nd Day	3rd Day	4th Day
1	Glucose	0.33	0.41	1.3	2.4
2	Baggassae	0.4	0.88	1.45	2.61
3	Citrus Wastes	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

The production of butanol when only glucose was used as carbon source 2.4 gm/l on 4th day. This pattern of increase in production of Butanol from 1st to 4th day is seen to be common for all types of fermentation media. Even though the concentration of butanol produced in different Medias differ. (Table: 2, Fig: 2) In case of weeds/grasses the production starts on the 2nd day. The citrus waste shows the most amount of butanol production when the culture concentration was 20%.

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 (Table:2 and Figure:2) it was seen that the production increased to 7.6

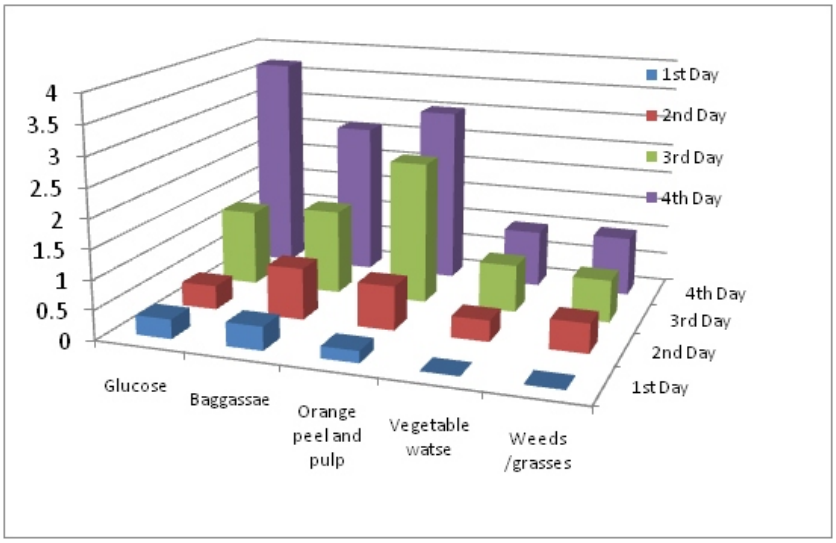

Figure 2 : Bar diagram showing comparative Butanol production when different carbon source were used

Table 3 : 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			
		1st Day	2nd Day	3rd Day	4th Day
1	Glucose + Soyabean pd.	0.43	0.52	2.3	8.4
	Glucose + animal waste	0.35	0.75	2.6	7.0
2	Baggassae + Soyabean pd.	0.45	0.68	2.45	7.6
	Baggassae + animal waste	0.36	0.77	2.05	7.4
3	Citrus peel and pulp + Soyabean pd.	0.2	0.75	2.76	8.01
	Citrus 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

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 90 hours.

DISCUSSION

The present 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.

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 h1 using glucose as a substrate. [9] 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. [10][11] 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.

Thus, the production of Butanol from lignocellulosic waste specially citrus wastes and using low cost nitrogen source seems to be a cost effective technique, even though the yield of butanol is very less. Butanol production by ABE fermentation at present does not compete economically with petrochemical synthesis.

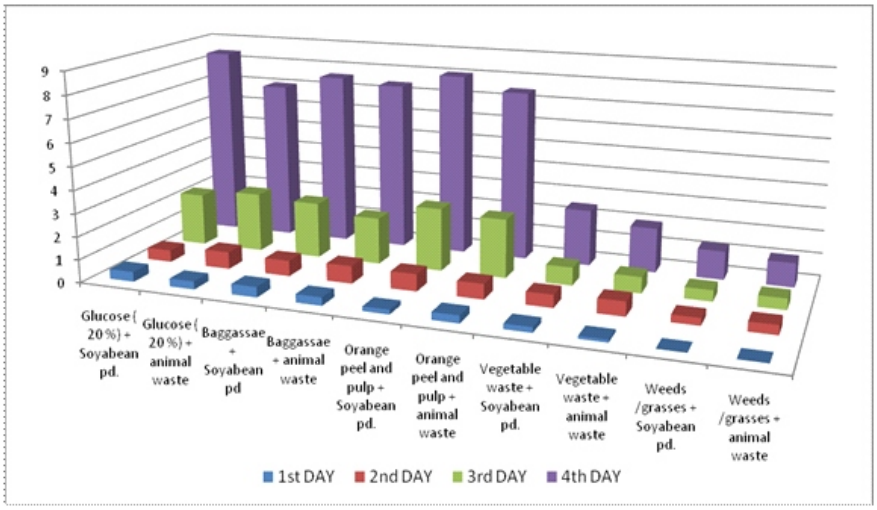

Figure 3 : Bar diagram showing comparative Butanol production when different nitrogen sources were used

Thus, to make butanol production economically competitive biological process, lignocellulosic wastes and cheaper nitrogen sources should be used. As the cost of raw material decreases, the whole process becomes economical even if the amount of Butanol produced is low.

CONCLUSION

Over all study and result indicated that the Citrus fruit waste can be a very cost effective and high producer of butanol when *C. acetobutylicum* was used in a concentration of 20% and soyabean powder was used as nitrogen source. The use of low cost nitrogen sources like soyabean powder resulted in increasing the Butanol production.

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