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Ethanol Production: By Using Yeast Immobilized Cells

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Abstract

Ethanol is a renewable fuel because it is produced from biomass. Ethanol also burns more clearly and completely than gasoline or diesel fuel and reduces greenhouse gases (GHG). It is one of the best tools to fight vehicular pollution and also reduces particulate emissions that pose a health hazard. Maximum ethanol 58.6 % yield were obtained from the fermentation medium with 1.3-1.7 mm diameter beads prepared from 3% (w/v) Na-alginate solution. At higher substrate concentration, substrate was recirculated through the fermentation vessel to increase the yield. The pH and temperature of 35-40 C was maintained to balance the process.

Keywords: Immobilized cells, Methanol, Yeast extract, sodium alginate beads.

Introduction

In more general way Ethyl alcohol (C2H5OH) or 'Ethanol' may be referred to other name e.g. the alcohol may be named to indicate the source of raw material from which it is manufactured or to indicate the general purpose for it is to be used. It is made by fermenting any biomass high in carbohydrate (starches, sugars, or cellulose) through a process similar to brewing beer.

Ethanol is a natural component of alcohol beverages and its use has been continued growth since the late 1970's, when it was used as a product extender due to Gasoline shortages caused by the OPEC Oil Embargoes. Ethanol is mostly used as a fuel additive to cut down a vehicle's carbon monoxide and others smog causing emissions.

With other fuel commodities such as coal and nuclear fuel alcohol (Methanol & Ethanol) will play a role of increasing and promising potential. Methanol and ethanol have almost all the advantage of liquid fuel. Flexible –fuel vehicle's which run on mixtures of gasoline and up to 85% ethanols are now available. Due to the dwindling of fossil fuel, microbial production of bio-fuel from organic by products has acquired significance in recent years.

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Immobilized cells exhibit many advantages over free cells such as relative ease of product separation, reuse of biocatalysts, high productivity and reduced susceptibility of cells to contamination (Goksungur and Guvenc, 1999). The simple and mild immobilization technique involves the drop wise addition of cells suspended in sodium-alginate onto a solution of calcium chloride when the cells immobilized in precipitated calcium-alginate gel in the form of beads (Rosevcar, 1984).

In the present study ethanol was produced using the fermentation medium. Yeast cells immobilized in Calcium-alginate and beads of 1.3-1.5mm diameter were used.

Materials and Methods

In order to produce the ethanol yeast cells were used which was immobilized in sodium alginate beads. Salt medium or fermentation medium was used for the growth of immobilized cells. The medium contained Glucose, (1.0gl-1), (NH4)2 SO4, (.20 gl-1), Na2HPO4, (0.66 gl-1), KH2PO4, (0.66 gl-1), Yeast extract (0.1 gl-1). pH of the medium was adjusted to 5.5 before sterilization. Fermentation medium (100ml) was inoculated with loop full of culture and incubated in shaker incubator at 45 C for 18-24 hours. After the growth removed the flask, centrifuged the medium at 10,000 rpm for 5 minutes. Discard the supernatant and washed the cells with sterile distill water.

Now immobilized the yeast cells for immobilization prepared the sodium alginate (3% w/v) by dissolving in distill water autoclaved it at 121 C at15 lbs for 15 minutes. 3 ml of cells were dissolved in alginate gel. Calcium chloride solution is prepared and cooled it in deep freeze. Filled up the syringe with alginate gel containing the cells and allowed it to drop in cool calcium chloride solution to form the beads. Protein content of bead was estimated with Folin-Lowry method at 750nm, as described by Lowry et al. (1951).

For the production of ethanol continuous fermentation process has been done, sterilized the jacketed column, openings and all the joints of the vessel. Packed the column with alginate beads to a bed height of 22 cm and a diameter of 2.5 cm. Allow to pass the fermentation medium from bottom to top of column with a peristaltic pump at a flow rate of 75 ml/hour. To collect the evaporated vapors a condenser was also attached to the top of the column.

The temperature was maintained at 45-50 C by the circulation of hot and cold water. The samples were collected at different time intervals, centrifuged at 2500 rpm; the supernatant obtained was used for the estimation of glucose, ethanol and carbon dioxide. Glucose was estimated by DNS method at 575 nm as described (Miller, 1959). Ethanol was estimated by colorimetric method at 584 nm. For the recovery of ethanol mixed the equal amount of fermentation broth with distill water and distillate it at 80 C and recovered the final product i.e. ethanol.

Results and Discussion

Effect Of pH

Ethanol concentration and theoretical yield as a function of intial pH. Maximum ethanol concentration of 58.6% and productivity of 12.3g/l were obtained at pH 3.5. At pH values of 4.5 and pH 4.0 concentration of 6.2% and ethanol productivity of 4.3g/l and at pH 4.0 16.8% and productivity of 6.2g/l were observed.

Effect of Bead Diameter

Beads of different diameter 1.3-1.7mm, 2.0-2.6mm and 2.8-3.0mm were prepared by using different syringes of different diameter. Beads of smaller diameter 1.3-1.7mm and 2.0-2.6mm were produced more ethanol. The highest ethanol production of 12.3g/l with yield of 58.6% was obtained from beads of 1.3-1.7mm diameter beads.

Colak and Hamamci (1991) stated that Ca- alginate gels were somewhat compressible and production of Co_2 during ethanol production caused the beads to be compressed and the result was a high pressure drops and phase separation together with a increase in ethanol productivity.

Recirculation Of Substrate

To increase the ethanol concentration during the process substrate was recirculated through the process. Substrate was recirculated through the column. For each substrate concentration ethanol, ethanol yield, carbon dioxide, glucose values were obtained after each circulation.

Effect On Carbon Dioxide

For every m^3 of ethanol formed about 760 kg of Carbon dioxide is liberated from the fermentation broth of the total 70-80% can be recovered and can be used in fire extinguisher, refrigerant and in chemical industry. During the process with increase in time and ethanol concentration the amount of Co_2 was also observed to be increased the maximum Co_2 produced is 10.4 gm at 75 hrs of fermentation process.

Table1: Estimation of Carbon dioxide.

S.No	Time (hrs)	Intial reading of $Ba(OH)_2$	Final reading of $Ba(OH)_2$	Carbon dioxide production (gm)
1.	10	67.6	26.0	3.2
2.	19	24.6	3.1	1.5
3.	58	67.6	21.8	3.6
4.	64	22.0	2.0	1.2
5.	75	67.6	21.2	3.6

Table 2. Estimation of Ethanol

S.No	Time	OD	EC (%V/V)	(g/L)

1.	0	-	-	-
2.	6	0.248	0.54	4.3
3.	10	0.359	0.79	6.2
4.	14	0.418	0.91	7.2
5.	19	0.500	1.09	8.6
6.	30	0.564	1.25	9.9
7.	34	0.568	1.35	10.7
8.	39	0.622	1.36	10.7
9.	54	0.586	1.275	10.1
10.	58	0.473	0.813	6.4
11.	64	0.492	1.075	8.5
12.	75	0.544	1.5625	12.3
13.	Final	0.409	1.175	9.3

Where, OD=optical density, EC= ethanol concentration

Table 3. Estimation of Glucose

S.No	Time	OD	GC	DF	G (g/l)	pH
1.	0	-	-	-	-	5.5
2.	6	0.751	0.28	240	67.5	4.0
3.	10	0.782	0.29	240	69.6	3.5
4.	14	0.825	0.30	240	72.0	3.5
5.	19	0.882	0.3225	240	77.4	3.5
6.	30	0.854	0.3725	240	75.0	3.5
7.	34	0.827	0.30	240	72.0	3.5
8.	39	0.889	0.3230	240	77.5	3.5
9.	54	0.855	0.3125	240	75.0	3.5
10.	58	0.744	0.275	300	83.25	3.5
11.	64	0.762	0.2375	300	85.5	3.5
12.	75	0.733	0.275	300	83.5	3.5
13	Final	0.781	0.29	300	87.0	3.5

Where OD= optical density, GC= glucose concentration, DF= dilution factor

Table 4. Glucose concentration, glucose consumed, Ethanol yield and Co_2 produced

Time(hrs)	Glucose conc.(g/l)		Glucose consumed (g/l)	Ethanol produced (g/l)	Yield(%)	CO_2 Produced (gm)	pH
	Inlet	Outlet					
0	106.5	-	-	-	-	-	5.5
6		67.5	39.0	4.3	6.2	-	4.5
10		69.6	36.9	6.2	16.8	3.5	4.0
14		72.0	34.5	7.2	20.9	-	3.5
19		77.4	29.1	8.6	29.6	5.2	3.5
30		75.0	31.5	9.9	31.4	-	3.5
34		72.0	34.5	10.7	31.0	-	3.5
		77.5	29.0	10.7	36.9	-	3.5

39							
54	103.5	75.0	28.5	10.1	35.4	-	3.5
58		83.25	20.2	6.4	31.7	9.0	3.5
64		85.5	18.0	8.5	47.2	-	3.5
75		82.5	21.0	12.3	58.6	10.4	3.5

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