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Countercurrent Extraction of 2,3-Butanediol

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***Abstract*—Biotechnological production of 2,3-butanediol (BD), a value added chemical from wastes and excessive biomass is a promising and attractive alternative for traditional chemical synthesis. A BD concentration of 0.61% has been achieved form 2.35% lactose utilized after 96 hrs. of incubation period using deproteinated whey as substrate. This corresponds to BD production of 0.259 g/g lactose utilized. Adding of 50 mM acetate increases BD production to 0.84% from 2.3% lactose utilized corresponding to a yield of 0.365g/g lactose utilized. At solvent to feed ratio of 5:1 an overall mass transfer coefficient of 0.1554 x10^{-3} sec^{-1} and extraction of 84.47 % was achieved with glucose fermentation broth. When concentrated DPW broth was used for continuous countercurrent extraction higher K_da of 0.239 x10^{-3} sec^{-1} and extraction of 94 % and equilibrium stages of 1.346 was achieved.**

***Index Terms*—2,3-Butanediol, butylene glycol, *K.oxytoca*, deproteinated whey, whey permeate, solvent extraction, countercurrent extraction.**

I. Introduction

Whey is generated at a rate of 9 kg for every 1kg cheese or 6 kg for every 1 kg of cottage cheese manufactured. One hundred kg of whey is equivalent to the sewage produced by 5 people [1] In many countries including India, most of the whey is discarded as waste creating severe environmental pollution problems due to its high chemical oxygen demand (COD) and biological oxygen demand(BOD) [2] This necessity has been heightened, in recent years, by the increased volume of whey being produced, the increased centralization of diary manufacturing at fewer processing sites and more stringent legislative requirements for effluent quality.

The most recent approach for whey utilization is ultra filtration to separate proteins from the permeate. The protein fraction is used as food but the permeate or deproteinated whey (DPW) still exerts a BOD greater than 30kg/m^3 and is normally disposed of without any treatment [3]. A number of options have been proposed to convert permeate to value added products and other more profitable alternatives, one of which is production of potentially value added chemical 2,3-butanediol having diverse applications in chemical industries. The main constraint in the commercialization of 2,3-butanediol production is its economic recovery from fermentation broth due to its high boiling point and great affinity with water. Also effective scale up of both difficulties encountered in the design of the separation or recovery system. Thus efficient large scale recovery processes with potential for continuous operation should be considered and developed. Liquid- liquid countercurrent extraction of 2,3-butanediol was undertaken in this study.

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II. Materials and Methods

a) All the solvents and salts used in the study is of analytical grade. *Klebsiella oxytoca NRRL-13-199* was obtained from the Biochemical Engineering Research Centre, IIT, New Delhi. The medium of *Klebsiella oxytoca* used in the experiment was described by Pirt and Callow and was called as PC medium [4]. The pH of the medium was adjusted to 6.5.

b) Whey was obtained from a local dairy. The pH of whey was adjusted to 7.0 by using 1N NaOH and they it was steamed for 30 minutes to precipitate protein. It was then cooled and kept at 4oC and filtered through ordinary filter paper. This filtrate called deproteinated whey (DPW), was further used for experimentation.

c) Reducing sugar was estimated by dinitrosalicylic acid method [5]. BD, acetoin, acetic acid and ethanol in fermentation broth were determined by Gas chromatography (Perkin Elmer Sigma 3B) using chromosorb 101 coated with 3% FFAP. Lactose was estimated by HPLC using waters Microbondapack carbohydrate analysis column [6].

III. Experimental Methods

a) Preparation of Inoculum for K.oxytoca: 100 ml solution of glucose or lactose(0.1%) were autoclaved at 0.7kg/cm2 pressure for 30 mins. Ammonium salt solution and nutrient medium which were previously sterilzed were added to the flask followed by inoculation form a 24 hrs. old slant culture of *K.oxytoca* and incubated on a shaker at 30oC for 24hrs. This culture was used as inoculum (1% v/v).

b) Production of BD: 100 ml portion of glucose solutions (3%) were autoclaved in 250 ml flask. PC minerals were added and then inoculated with *K.oxytoca*. When DPW was used for fermentation, PC minerals were not added. Autoclaved whey was directly inoculated with *K.oxytoca*. 1ml of 5M sodium acetate was added to get 50mM of acetate in fermentation broth. Agitation was provided throughout the experimentation. Samples were withdrawn after every 24hrs. incubation, centrifuged and analyzed for residual sugar, BD, acetoin, acetic acid and ethanol.

c) Counter current experimental set up: The essential parts of the equipment used for continuous countercurrent

extraction consisted of the extraction column, two feed reservoirs, and two peristaltic pumps. The column was made of 4.0 cm internal diameter and 47 cm long glass tube. The column had a packed length of 28.2 cm and two disengaging spaces at the top ad bottom where from the light and heavy phases respectively were withdrawn. The dispersed phase (3-methyl-1-butanol) was fed from a 3 litre flask to the column through a distributor at the bottom of the column. The distributor consists of a diffuser having pore size of 200 to 300 micron. The diffuser tube as connected to a silicon tubing which was attached to a peristaltic pump so that the flow of the dispersed phase could be regulated. Solvent left the column from the top through a silicon tube by gravity. A complete set up of the experiment is shown in Fig. 1.

The continuous phase (aqueous phase) was fed to the top of the packing through a tube with a nozzle of 1.5 mm internal diameter which was connected to a peristaltic pump though silicon tube to control the flow rate of the aqueous phase. The column was randomly packed with ceramic raschig rings having dimensions (Table1).

d) Extraction of 2,3-butanediol: Continuous countercurrent extraction CCE of 2,3-butanediol produced by fermentation was carried out to test the efficiency of method developed After 96 hrs of incubation fermentation broth was centrifuged to remove cells, then it was concentrated by simple distillation to 3% 2,3-butanediol. 10% sodium carbonate was added and the broth was saturated with 3-methyl-1-butanol. Similarly 3-methyl-1-butanol was saturated with water and than used as extracting solvent.

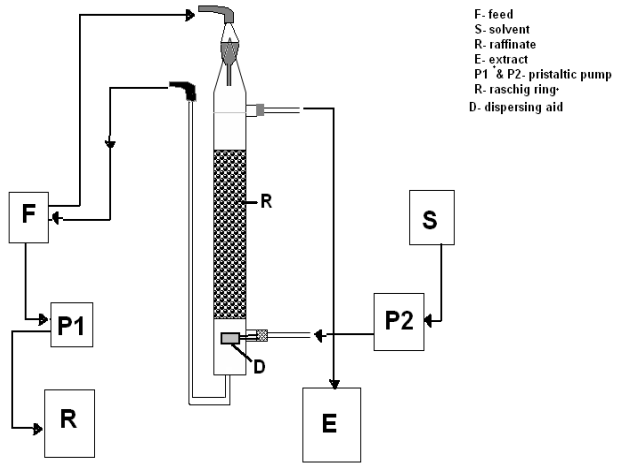


Fig. 1: Continuous Countercurrent Extration Column

TABLE: I DIMENSIONS OF RASCHIG RINGS

Outer Diameter	5.5mm
Internal Diameter	2.0mm
Height	4.5mm
No. the raschig rings used	1500

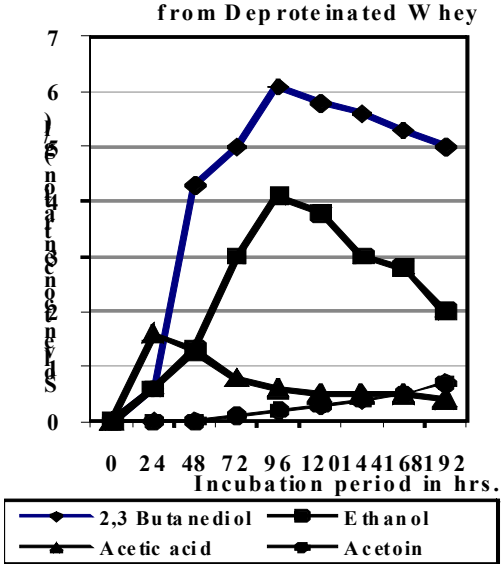
Solvent and fermentation broth were used as dispersed phase and continuous phase respectively. The effect of dispersed phase flow rate on mass transfer coefficient and percent extraction was studied.

IV. RESULT AND DISCUSSION

a) Production of BD: Fig 2 depicts production of BD from DPW. A BD concentration of 0.61% has been achieved form 2.35% lactose utilized after 96 hrs. of incubation period. This corresponds to BD production of 0.259 g/g lactose utilized. The results are found to be higher than reported by Speckman [7] who used *Bacillus polymyxa* for production from whey containing 4.9% lactose. The yield of BD achieved by these authors was 0.06 and 0.15 g/g lactose utilized after 72 and 168 hrs. respectively. Similarly a butanediol yield of 0.207 gg^{-1} was obtained by Kadathur B [8] using *Kelbsiella oxytoca* which is comparatively lower than the present study.

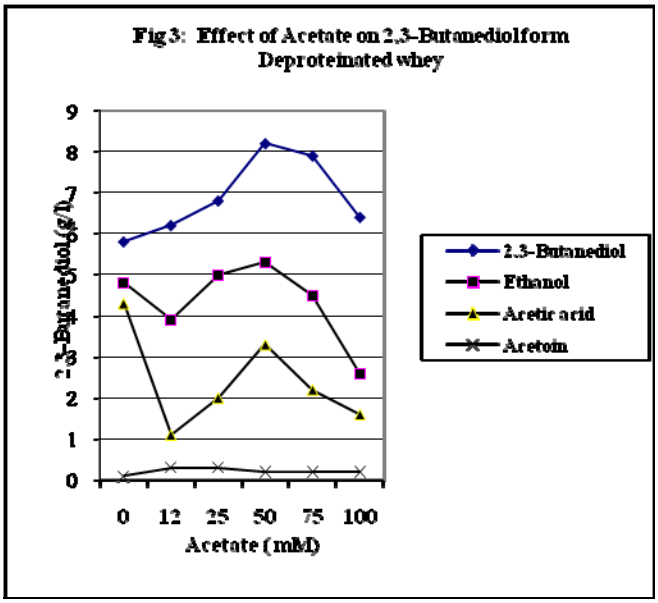
Whereas, Barrette et al [9] achieved 1.6% of BD from neutralized acid whey containing 5% lactose after 48 hrs. incubation period which is higher than the present study.

Fig 2: Production of 2,3-Butanediol from Deproteinated Whey



Here lactose concentration and the lower concentration of the minerals in the whey used in the present study might be one of the reason of lower product concentration.

Adding of 50 mM acetate increases BD production to 0.84% from 2.3% lactose utilized corresponding to a yield of 0.365g/g lactose utilized (Fig. 3).



Acetate induces three enzymes viz acetoacetate forming enzyme [10] acetolactate decarboxylase and dactyl relocates [11] involved in the conversion of pyruvate to BD, activates the acetoacetate forming enzyme and regulates the balance between acetoin and BD. Ethanol(0.43%), acetic acid (0.09%), and acetoin (0.02%) are also produced as byproducts during fermentation. Further it has been observed that *K.oxytoca* produces a higher concentration of BD from DPW as compared to PC medium with 3% lactose.

b) Continuous countercurrent extraction using fermentation broth: Various runs were carried out during continuous countercurrent extraction of 2,3-butanediol from fermentation broth and the results calculated were tabulated in Table 2.

The effect of dispersed phase flow rate on overall mass transfer coefficient as shown in Fig 4 and Fig 5 showed that increase in $K_d a$ with increase in dispersed phase flow rate. But overall mass transfer coefficient was found to be lower than that obtained with commercial BD solution. At solvent to feed ratio of 5:1 an overall mass transfer coefficient of 0.1554 $x10^{-3}$ sec^{-1} and percent extraction of 84.47 was achieved with glucose fermentation broth. Number of theoretical or equilibrium stages achieved during extraction was found to be 0.885. When concentrated DPW broth was used for continuous countercurrent extraction higher $K_d a$ of 0.239 $x10^{-3}$ sec^{-1} and percent extraction of 94 and equilibrium stages of 1.346 was achieved. This might be attributed to the fact that during concentration of whey broth the various minerals or salts present in DPW broth get concentrated, contributing to the salting out effect and resulting in high recovery. Othmer et.al[p12]have investigated extraction of BD using methyl viny carbinol acetate as solvent, from dilute fermentation broth after decolourization with lime and activated charcoal and concentration of broth to 20% BD, at solvent to feed ratio of 5:1, temperature 75°C and number of equilibrium units of 3 and 5. Recovery of BD was reported to be 84 % and 96 % with 3 and 5 equilibrium units respectively.

V. Conclusion

A BD concentration of 0.61% has been achieved form 2.35% lactose utilized after 96 hrs. of incubation period using DPW as substrate. This corresponds to BD production of 0.259 g/g lactose utilized. Adding of 50 mM acetate increases BD production to 0.84% from 2.3% lactose utilized corresponding to a yield of 0.365g/g lactose utilized.

At solvent to feed ratio of 5:1 an overall mass transfer coefficient of 0.1554 $x10^{-3}$ sec^{-1} and percent extraction of 84.47 was achieved with glucose fermentation broth. When concentrated DPW broth was used for continuous countercurrent extraction higher $K_d a$ of 0.239 $x10^{-3}$ sec^{-1} and percent extraction of 94 and equilibrium stages of 1.346 was achieved.

TABLE II Summary of Calculated Results for ContinuCountercurrent Extraction of BD in Packed Column from Fermentation Broth

No. of runs	Flow rates, cm/sec		Nav	Concentration, gmol/cm^3 $x10^{-4}$			Cd	Mass transfer coefficient
	$U_c x10^{-2}$	$U_d x10^{-2}$	gmol/sec $x10^{-7}$	C_{c1} water in	C_{c2} Water out	C_{d1} solvent out	Mole/ $cm^3 x10^{-4}$	$K_d a$ sec^{-1} $x10^{-3}$
1	0.531	0.557	4.37	4.37	2.26	2.11	3.89	0.110
2	0.531	1.062	12.85	4.37	1.80	1.28	3.84	0.010
3	0.531	1.593	15.12	4.37	0.97	1.13	3.01	0.145
4	0.531	2.190	14.51	4.37	0.87	0.85	2.98	0.141
5	0.531	2.655	14.77	4.37	0.68	0.74	2.75	0.155
6	0.531	3.186	14.53	4.37	0.63	0.74	2.72	0.155
7*	0.531	2.655	12.54	3.33	0.20	0.63	1.52	0.239

U_c – superficial velocity of continuous phase, cm/sec

U_d - superficial velocity of disperse phase, cm/sec
Nav - aerage rate of solute transfer, gmol/sec
C_{c1} , C_{c2} and C_{d1} solute concentration in continuous and disperse phase at 1 and 2 ends of column where solutions are concentrated and dilute resp.
Cd – solute concentration in disperse phase
$K_d a$- overall mass transfer coefficient, sec^{-1}

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