

## Liquid - Liquid Extraction of 2, 3-Butanediol from Fermentation Broth

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**ABSTRACT:** Recovery of metabolites from fermentation broth by solvent extraction can be used to optimize fermentation processes. End-product reutilization, low product concentration, large volumes of fermentation broth and the requirements for large bioreactors, in addition to the high cost largely contributed to the decline in fermentative 2, 3-butanediol production. Extraction can successfully be used for in-situ alcohol recovery in 2, 3-butanediol fermentations to increase the substrate conversion. In the present work organic extraction of 2, 3-butanediol produced by *Klebsiella pneumoniae* fermentation was studied to detect solvent effect on 2, 3-butanediol production and determination of efficient volume of solvent. The aim of this research was liquid-liquid extractive fermentation systems evaluation as an alternative to overcome the end product effect and to increase of 2, 3-butanediol production by *K. pneumoniae* because conventional fermentative production of 2, 3-butanediol by *K. pneumoniae* has the disadvantage of product reutilization by the organism. The highest 2, 3-butanediol production (23.01 g/L) achieved when 20% oleyl alcohol was used.

**KEY WORDS:** Extractive fermentation, 2,3-Butanediol, Oleyl alcohol, Liquid-liquid extraction.

### INTRODUCTION

With the extractive fermentation system, the product concentration in the extractant phase is higher as compared

to the fermentation broth and this helps to reduce down-stream separation costs. Extractive fermentation

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is advantageous, because the product recovery is not associated with a water flow through the bioreactor and solid-liquid separation of cells is not necessary if the organic solvent comes in direct contact with the culture broth [1].

Conventional fermentative production of 2,3-butanediol by *Klebsiella pneumoniae* has the disadvantage of product reutilization by the organism. In situ solvent extraction of 2, 3-butanediol during cultivation of *K. pneumoniae* appears to be a potentially viable alternative to overcome product reutilization. The in situ recovery of 2, 3-butanediol from fermentation broth has gained considerable attention in recent years [2-3]. It is considered that *in situ* recovery is one of the major developments that may attribute to the commercial revival of the 2, 3-butanediol fermentation. It will reduce the effect of product accumulation and will enable the conversion of a concentrated feed and lead to a high productivity, hence lowering the production costs. Another aim in research on in situ recovery is development a separation method which consumes less energy than the conventional distillation [4].

The purpose of this study was to survey the effects of oleyl alcohol on 2, 3-butanediol formation by *K. pneumoniae*.

## EXPERIMENTAL SECTION

### Organism and growth conditions

#### Microorganism

Bacterial strain used in this study was *K. pneumoniae* PTCC 1290, obtained from the Iranian Research Organization for Science and Technology (IROST). The strain was maintained on nutrient agar slants at 4 °C and sub-cultured monthly. The pre-culture medium was nutrient broth containing 2.0 g/L yeast extract, 5.0 g/L peptone, 5.0 g/L NaCl, and 1.0 g/L beef extract, sterilized at 121°C for 15 min. Cells in exponential growth were used as inoculums.

#### Fermentation

Submerged fermentation experiments were carried out in 1-L fermentor with shaking 180 rpm at 34°C. It was operated at working volume of 400 mL that included 10% inoculums. The extractant was then aseptically added to the surface of the broth after 10 h of cultivation. A control experiment was carried out without the addition of an extractant. Also we studied the effect of different solvent volume on product extraction.

All experiments were repeated at least three times in order to acquire high accuracy. This procedure gave consistent and reproducible results.

#### LLE measurements

The LLE measurements for the quaternary system water + 2, 3-butanediol + oleyl alcohol + ammonium sulfate were made at atmospheric pressure at 34°C. The equilibrium data were determined using an experimental apparatus of a 250 mL glass cell (as described in previous work) [5-6], where the temperature of the apparatus was controlled by a water jacket and maintained with an uncertainty of within  $\pm 0.1$  K. The prepared mixtures were then introduced into the extraction cell and stirred for 4 h. The mixtures were then left to settle for 8 h for phase separation. The samples of organic-rich phase were taken by syringe (1  $\mu$ L) from the upper layer and that of the water-rich phase from a sampling tap at the bottom of the cell.

#### Analytical methods

The composition of the sample was analyzed using a Konik Gas Chromatograph (GC) equipped with a Thermal Conductivity Detector (TCD) and C-R2AX Shimadzu Crop. integrator. A 2 mm (i.d.) Porapak QS packed column was used to separate the components. The TCD's response was linear and calibrated with 1-butanol as in internal standard.

All experiments were repeated at least three times in order to acquire high accuracy. This procedure gave consistent and reproducible results.

## RESULTS AND DISCUSSION

In extractive fermentation system product fermentation and extraction are synchronized. In the other word substrate and cells are isolated in aqueous phase and product is isolated in organic phase. So because of product extraction, its inhibition effect on bacterial growth is removed [1, 7-8].

Our previous works showed that oleyl alcohol appears to be the best suitable solvent for the in situ extraction of 2, 3-butanediol [9]. It has good relative solubility for 2, 3-butanediol, is biocompatible but not bioavailable, shows good phase stability (i.e., no emulsion-forming tendencies), and it has a log P value significantly above the critical log P value.

Table 1: Liquid-liquid equilibrium data of the system water + 2, 3-butanediol + oleyl alcohol + ammonium sulfate at 34°C.

Aqueous phase			Organic phase		
Water	2,3-butanediol	Oleyl alcohol	Water	2,3-butanediol	Oleyl alcohol
0.9672	0.0315	0.0005	0.0239	0.2175	0.7585
0.9394	0.0591	0.0007	0.0498	0.3229	0.6269
0.9183	0.0803	0.0007	0.1017	0.3898	0.5080
0.8992	0.0991	0.0010	0.1603	0.4314	0.4076
0.8783	0.1199	0.0011	0.2608	0.4387	0.3000
0.8431	0.1548	0.0014	0.3331	0.4347	0.2315

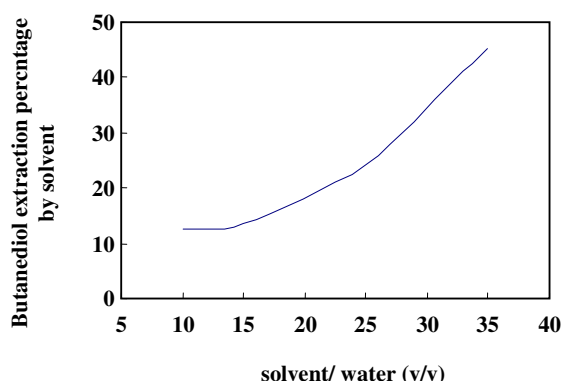


Fig. 1: Different consumed solvent volume for 2, 3-butanediol extraction based on model prediction.

Although previous works showed that, product accumulation after 25h of fermentation, but in 17.6 g/L it converted to acetoin and then acetoin was increased [10]. So 2, 3-butanediol production was restricted because of product reutilization. Acetoin is an intermediate metabolite immediately prior to the formation of 2,3-butanediol during fermentation. The metabolic conversion of acetoin to 2, 3-butanediol is reversible [11]. However, on longer incubation the level of butanediol subsequently declined. This appeared to be due to its reoxidation to form acetoin (or acetyl methyl carbonyl), which progressively increased during prolonged fermentation [2-3, 12].

Recovery of 2, 3-butanediol from fermentation broth by solvent extraction can resolve this problem.

In addition of solvent selection, determination of solvent volume is economically important. Equilibrium data can predict effective solvent volume for 2, 3-butanediol extraction (Table 1). In Table 1, the results

are given for the liquid-liquid equilibrium of the quaternary system. All mole fractions have been normalized to sum to 1. The correlation experimental equilibrium data was obtained on the basis of electrolyte UNIQUAC model at the previous works [5-6]. On the basis of this correlation, efficient solvent volume in order to removal of concentration inhibition effect is predictable (Fig. 1).

Our experiments showed that 1.76% accumulated concentration of product inhibited 2, 3-butanediol production but 23.4% 2, 3-butanediol extraction could be effective for removal of accumulation effect. Results obtained by model prediction also showed 24.6% of solvent volume was needed (Fig. 1).

Oleyl alcohol was added to fermentation broth as solvent in the basis of model prediction results in 15, 20 and 25% after 10h of fermentation. Results of solvent addition on extractive fermentation are shown in Table 2. The results showed that 2, 3-butanediol production and extraction percentage with 15% oleyl alcohol was 20.72 g/L and 18.9% respectively. Increasing of solvent to 20% resulted in increased 2, 3-butanediol production and extraction but higher solvent range only increased 2, 3-butanediol extraction not production. *Ghoandzadeh et al.* [13] also reported that with increasing of solvent to water ratio extraction percentage and 2, 3-butanediol distribution coefficient was directly increased.

With 2, 3-butanediol extraction from liquid phase to organic phase by oleyl alcohol (20%), product concentration was decreased in aqueous phase and reutilization of 2, 3-butanediol by microorganism was impossible. Our findings showed that there is no direct relationship between the increased solvent volume and 2, 3-butanediol production. So consumed solvent range is completely related to economically decisions and process costs.

**Table 2: Effect of solvent percentage on production 2, 3-butanediol and extraction percentage.**

Solvent percentage	2,3-butanediol production (g/L)	Extraction percentage
15	20.72±0.05	18.9
20	23.01±0.05	27.0
25	23.02±0.05	31.7

Our results also showed that 20% solvent also decreased 2, 3-butanediol concentration in culture medium and inhibited product accumulation in aqueous phase. This inhibited 2, 3-butanediol conversion to acetone because of different salts persistence in fermentation broth. Salts could cause organic phase enrichment and so product extraction increasing from aqueous phase in mixed aqueous-organic solvent medium.

Compared to regular batch fermentation in batch extractive fermentation using oleyl alcohol 2, 3-butanediol productivity and glucose consumption were increased 32 and 28%, respectively.

In general, it was concluded from the experiments that liquid-liquid extraction can successfully be used for in situ recovery, which is in agreement with literature [8, 13-16].

## CONCLUSIONS

Recovery of metabolites from fermentation broth by solvent extraction can be used to optimize fermentation processes. Extraction can successfully be used for in-situ alcohol recovery in 2,3-butanediol fermentations to increase the substrate conversion. Conventional fermentative production of 2,3-butanediol by *K. pneumonia* has the disadvantage of product reutilization by the organism. Extractive fermentation has been shown to solve this problem. In situ removal of end products from *K. pneumoniae* resulted in increased productivity.

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