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An efficient aqueous two phase systems using dual inorganic electrolytes to separate 1,3-propanediol from the fermented broth

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ABSTRACT

An aqueous two phase extraction using K_2CO_3:K_2HPO_4/Isoproponal was investigated for the recovery of 1,3-propanediol from the fermented broth. Initially, the concentration of K_2CO_3 on phase formation, the partition co-efficient and recovery of 1,3-PDO was evaluated with a optimum salt concentration of 60%. Later the partition co-efficient was improved using dual inorganic salts, K_2CO_3 and K_2HPO_4 with an optimum concentration of 45% and 15% respectively. Using Central Composite Design, pH and temperature on partition and recovery of 1,3-PDO was evaluated. With the optimized physical conditions and inorganic salts concentration, ATPS extraction was carried out in synthetic solution as well as fermented broth resulting in maximum 1,3-PDO partition co-efficient value of 42.46 and 56.93 and recovery yield of 97.69 and 98.27% respectively. A fair partition was observed with organic acids and 1,3-PDO, with removal of lactic acid and acetic acid up to 93.29 and 90.42% respectively.

1. Introduction

Polytrimethylene terephthalate (PTT) is a potential bio-based polymer with excellent physical and mechanical properties compared to commercially available polyethylene terephthalate (PET) and polybutylene terephthalate (PBT). This polymer has various applications in carpet making, textile industries, and polymer & coating materials. PTT is synthesized through polycondensation of monomeric 1,3-propanediol (1,3-PDO) and a dicarboxylic acid i.e., terephthalic acid. The characteristic of the polymer depends on the purity of the raw materials and the commercialization of the polymer depends on the availability of the raw materials (Bhatia and Kurian, 2008; Kaur et al., 2012).

Traditional chemical synthesis of 1,3-PDO from ethylene oxide (Shell Process), Acrolein (Degussa process) and catalytic hydrogenolysis of glycerol resulting in higher yields and productivity. But the processes have its major limitations of expensive catalysts, harsh reaction conditions like high temperatures and pressures (Liu et al., 2010a,b). Now people were much aware about the energy conservation and impact of these chemical pollutants derived from the mentioned processes. Hence there is requirement of efficient technologies which are environmentally friendly. Biological process for 1,3-propanediol production address the limitations of chemical synthesis like usage of toxic raw materials, expensive catalysts and unfavorable process conditions. From the literature, back in a decade August Freund first reported the production of 1,3-PDO by *Clostridium pasteurianum*. Till date several microorganisms of different genera like *Clostridium*, *Klebsiella*, *Citrobacter*, *Lactobacillus*, and *Shimwellia* were reported to produce 1,3-propanediol from glycerol (Vivek et al., 2017a). The development of sustainable and renewable energy sources for fuels led to the development of biodiesel industry, which reduced the price of glycerol. Almost 10% (w/w) of glycerol is formed with biodiesel synthesis as the main by-product. The market cost of crude glycerol was reported as $0.05 per pound in 2007, and the global biodiesel production increased by 17 percent since 2012, with this scenario, the cost of crude glycerol has been declined drastically. Whereas 1,3-PDO market is in an exponential phase with $310.5 MM in 2014 and estimated to be $621 MM by 2021.

One of the major limitations in the biological process for the production of 1,3-PDO is downstream processing. Low titers of 1,3-propanediol in the fermentation broth, high boiling point, and strong hydrophilic nature makes the process relatively difficult in separation and purification of 1,3-PDO from the fermented broth. The downstream processing of 1,3-PDO from the fermented broth involves following steps (i) removal of microbial cells, mostly by centrifugation, membrane filtration, or flocculation (ii) removal of impurities like proteins, nucleic acids, polysaccharides, salts, residual glycerol, by-products like lactic acid, acetic acid and separation of 1,3-PDO (iii) the final step is

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purification of 1,3-PDO by vacuum distillation or preparative chromatographic techniques (Anand et al., 2011). Several strategies evaluated for separation of 1,3-PDO from the fermented broth were liquid-liquid extraction (Malinowski, 1999), reactive extraction (Malinowski, 1999; Hao et al., 2005), ion exchange resins (Rukowicz et al., 2014), pervaporation (Kaur et al., 2015), ionic liquids (Lee et al., 2015; Müller & Górak, 2012), molecular distillation (Wang et al., 2013) and electro dialysis (Wu et al., 2011). Each of the strategy mentioned above has its pros and cons and none of the process was mentioned as the effective and efficient downstream process. Hence designing a novel process or performing improvements or combinations to the known conventional process in regard to increase the end product yield, purity with lower energy consumption would be desirable.

Since 1956 an aqueous two phase system (ATPS) was mentioned as the effective system for separation of biomolecules. In ATPS method, an inorganic electrolyte was used as salting out agent and an organic solvent as an extractant targeting the hydrophilic molecule in the aqueous solution (Li et al., 2013). The separation of 1,3-PDO was carried out in three different ATPS systems hydrophilic solvent/inorganic salt, hydrophobic solvent/inorganic salt or amphiphatic chemicals/inorganic salts based extraction (Li et al., 2015; Aydoğan et al., 2010). The salting out extraction leads to phase separation with the aid of organic solvents. Inorganic salts added to the mixed solution lead to decrease in the solubility of 1,3-propanediol in the aqueous solution and then the solute attains the solubility in the organic phase (Li et al., 2015). The hydration efficiency of organic solvents and cationic behavior or acidity of inorganic electrolytes greatly affects the formation of two phases in ATP system. The top phase of the ATPS extraction is rich in diols and bottom phase with sugars, salts, acids and residual glycerol. The process scheme representing the salting out extraction of 1,3-PDO is showed in Fig. 1. The ATPS extraction is more environmental friendly as no energy is required for heating the broth which is required in distillation, however to make the process more economical the inorganic salts can be precipitated by evaporating the water, that requires energy.

It is well known that usage of K_2CO_3 was efficient in enrichment of tertiary butanol aqueous solutions, similarly potassium salts used to attain liquid–liquid equilibrium. Hence two different salts K_2CO_3 and K_2HPO_4 were selected.

In the present study the broth obtained after the fermentation of *Lactobacillus brevis* N1E9.3.3 for 1,3-PDO was evaluated for downstream processing. The major end products in the broth were lactic acid and acetic acid in addition to 1,3-PDO. This work aimed at optimization of potassium carbonate concentration, evaluation of dual inorganic electrolyte combinations for salting out in regard to partition co-efficient (K) of 1,3-PDO and recovery percentage (Y). Every component of the process like effect of physical conditions were performed in order to promote the efficacy of ATPS system in separation and purification of 1,3-PDO.

2. Materials and methods

2.1. Chemicals and solvents

Crude glycerol (90%, Moisture 10%, oil 0.5%, ash 3% slightly yellow color with specific gravity 1.25) was purchased from Pasand Speciality Chemicals (Rajkot, India). Standards of 1,3-PDO was purchased from Sigma Chemical Co. (USA) and other chemicals used in this study were analytical grade and obtained from SRL chemicals (India).

2.2. Microorganism and fermentation conditions

Lactobacillus brevis N1E9.3.3 strain was isolated through onsite enrichment technique and evaluated as potent 1,3-PDO producer (Vivek et al., 2016). The production media for fermentation experiments was (grams/liter):22.9 g beef extract; 12.5 g yeast extract; 3 g peptone; 0.37 g $MgSO_4{\cdot}7H_2O$; 0.058 g $MnSO_4{\cdot}H_2O$; 6 mg Vitamin B_{12}; 1 g K_2HPO_4; 5 g sodium acetate; 3 g tri-sodium citrate; 80 g glycerol and 80 g glucose (Narisetty et al., 2017).

Pre-inoculum was prepared in sterile MRS (De Man, Rogosa and

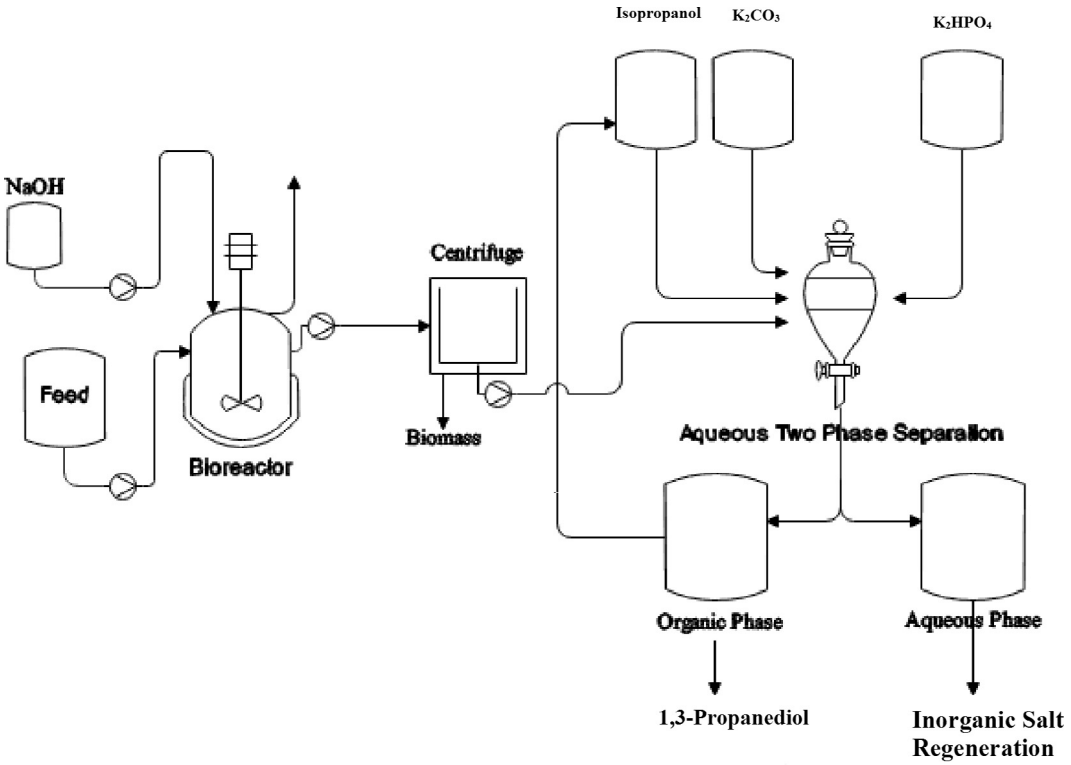

Fig. 1. Schematic representation of fermented broth obtained from the upstream process and aqueous two phase extraction of 1,3-propanediol using K_2CO_3 + K_2HPO_4/Isopropanol system.