Natural Resources, 2025, 16(5), 173-191
https://www.scirp.org/journal/nr
ISSN Online: 2158-7086
ISSN Print: 2158-706X

Waste Glycerol Valorization and Recovery of 1,3-Propanediol: Focus on Process Optimization

Pragya Gupta, Prakash C. Sahoo, Ravi Prakash Gupta, Manoj Kumar*

Industrial Biotechnology Department, Research and Development Centre, Indian Oil Corporation Limited, Faridabad, India
Email: *upretim@indianoil.in

How to cite this paper: Gupta, P., Sahoo, P.C., Gupta, R.P. and Kumar, M. (2025) Waste Glycerol Valorization and Recovery of 1,3-Propanediol: Focus on Process Optimization. *Natural Resources*, **16**, 173-191.
https://doi.org/10.4236/nr.2025.165009

Received: March 24, 2025
Accepted: May 17, 2025
Published: May 20, 2025

Abstract

This study investigates upscaling of bio-diesel industry crude glycerol to 1,3-propanediol at a 10 L scale, using *C. butyricum* L4. This strain showed higher potential for glycerol valorization having various toxins, affecting overall fermentation process. A COD mass balance of the 10 L fermentation with crude glycerol as a sole carbon source, showed a close balance of 90.02%, with 1,3-propanediol as the main metabolite (68.3% of total COD distribution). Three different aqueous two-phase extractions (SOE) were evaluated for 1,3-propanediol recovery. When $(NH_4)_2SO_4$ and ethanol were used, the best outcomes were achieved (87.6% recovery, 19.6% by-products removal). The purification process is multi-step involving biomass removal, activated charcoal treatment, vacuum distillation, and isocratic chromatography. Vacuum distillation aids crude broth concentration with simultaneous removal of low distillates. Through isocratic chromatography in the final purification stage, approximately 82.22% yield and a purity exceeding 97% for 1,3-propanediol were achieved. The objective of this study is to advance environmental sustainability within the production process.

Keywords

Recovery, 1,3-Propanediol, Fermentation, Downstream Processing, Crude Glycerol

1. Introduction

The current trajectory of the bioeconomy signals a noteworthy surge, actively contributing to sustainable development and poised to attain a valuation of USD 150 billion by 2025. One of the bio-produced green chemicals is 1,3-Propanediol

(PDO). It is a significant platform chemical that serves as a precursor for the synthesis of several industrially relevant chemicals like polytrimethylene terephthalate (PTT), other polyesters, polycarbonates, polyethers, polyurethanes and various cosmetic products etc. Polytrimethylene terephthalate (PTT), derived from 1,3-propanediol (1,3-PDO), is the most dominant product, representing approximately 72% of the total 1,3-PDO market. Incorporating 1,3-PDO into polyesters enhances properties such as thermal and hydrolytic stability, as well as flexibility [1]. The production of 1,3-PDO is linked to glycerol as every ten gallons of biodiesel produced generates a gallon of glycerol. Its demand extends to multiple fields due to virtue of its versatility and the annual price for 1,3-PDO is expected to increase by 14% through 2027 [2]. According to open sources, the bulk pricing for 1,3-PDO is between 3.8 - 4.2 $/kg at present.

Until recently, it has been manufactured by non-sustainable chemical route under various chemical and energy intensive processes involving use of valuable catalysts, high operating pressure and temperature, presence of toxic intermediates, with feedstock obtained from petrochemical industries and other environmental concerns [3]. To overcome these drawbacks, commercial route via microorganisms has been developed, using a recombinant *Escherichia coli* from glucose by CovationBio PDO (previously Dupont Tate & Lyle) and another via fermentative reforming of glycerol [4]. Microbial conversion of the waste glycerol derived from biodiesel industry to 1,3-propanediol is a unique opportunity to convert waste to value added product using various microbial strains like *Klebsiella pneumoniae, Citrobacter freundii, Clostridium pasteurianum, Clostridium butyricum, Clostridium beijerinckii, Rhodosporidium toruloides* and several others [5]-[7].

The downstreaming has been inundated with some difficulties namely—it's highly hydrophilic nature, high boiling point (214˚C at atmospheric pressure), difficult separation from low titer biological broths, high concentration of inorganic mineral salts, pigments, residual feedstocks, bio-macromolecules etc. In addition, the fermentative reforming of 1,3-PDO generates various organic acids along with it and their removal is one of the major steps of overall downstream processing. Accordingly, the costs of recovering can contribute 20% - 40% to the total production costs, thus the focus of this study is to improve recovery process that has application in different fermentation processes. The higher purity grade required at the end-use also accounts for significant cost and hence commercial viability. Various purification techniques have been evaluated in literature to refine biologically produced 1,3-PDO, with ion exchange, electrodialysis, and two-phase salting-out extraction being the most widely discussed. These methods effectively remove inorganic and organic impurities from the fermentation broth before the final distillation and separation of pure 1,3-PDO. But both ion exchange and electrodialysis application have been limited by the generation of huge volumes of wastewater. Dupont filed a patent that described the use of a strong acid cation exchange resin followed by a weak base anion exchange resin for the removal of inorganic salts (>98%) resulting in 1,3-PDO with 99.5% purity post-

distillation. However, a significant drawback of this method is the rapid saturation of the resins, necessitating large amounts of NaOH and HCl for regeneration [8]. Few of the other methodologies studied in combination or as stand-alone were either energy intensive, cost intensive, complicated, required regeneration of target compounds from its dioxolane derivatives; result in reaction with by-products present in real streams, used massive amounts of reagents, difficult to scale-up along with low yield or low purity due to lower selectivities like reactive extraction, column chromatography, Salting-out technology, zeolite membrane based, liquid-liquid extraction, phase separation using differences in miscibility [9]-[13]. The recovery and reuse inorganic salts/organic solvents are inevitable to make the salting-out process feasible for the commercial application. Another limitation to their reproducibility with real biological broths is that most investigations are conducted using model/simulated fermentation broths. This cannot predict how a developed purification process performs with complex real fermentation broth, which becomes a major bottleneck. Due to above technological challenges and economical constraints, large scale 1,3-propanediol purification limits the opportunity of utilizing wastes generated from biodiesel plants and producing valuable platform chemicals for supporting sustainable green economy [14].

Here, the previously isolated biocatalyst *Clostridium butyricum* L4 [15] was used to produce fermentation broths, under continuous anaerobic mode in a 10 L bioreactor with 7.5 L working volume. *Clostridium butyricum* L4 produced fermentation broth having 1,3-propanediol, which was used for development of a novel purification process for its recovery. In the developed process, 1,3-propanediol was achieved by four simple steps: biomass removal via centrifugation, colored/biomolecules (like proteins, nucleic acids, polysaccharides, salts etc) impurities removal using activated carbon, removal of low distil by-products via vacuum distillation, and fourth is extraction of 1,3-propanediol by chromatography with silica resin. The study successfully achieved separation of 1,3-propandiol from fermentation by-product produced by *Clostridium butyricum* L4 *i.e.*, acetic acid, biomass, colored impurities, and fermentation generation biotechnological biomolecules with higher recovery yields. The study aims to aid by approaching towards green future.

2. Material & Methods

2.1. Material

Glycerol, glucose, acetic acid, 1,3-Propanediol, Amberlite (cation exchange Na+ form), Amberlite (anion exchange Cl-form), DEAE cellulose (anion exchange), Silica gel (high-purity grade, pore size 60 Å, 70 - 230 mesh), bovine serum albumin (BSA) and Pt-Co/Hazen/APHA Colour Reference Standard were purchased from Sigma Chemicals (India).

2.2. Fermentation in 10 L Reactor

The broth was obtained from biodiesel derived crude glycerol fermentation with

C. butyricum L4 and characterized. *C. butyricum* is a well-known natural producer of 1,3-PDO from glycerol and was isolated in-house after rigorous experimentation. This strain likely exhibits higher yields of 1,3-PDO based on previous study [15] from residual waste glycerol obtained from biodiesel industries. Furthermore, it is capable of surviving in a wide range of conditions making industrial-scale fermentation easier. In the study, fermentation experiments were conducted in in-house 10 L reactor having 7.5 L working volume under strict anaerobic continuous stirred bioreactors at 30˚C, 75 rpm, pH = 7.0, having 72 hours of hydraulic retention time. The bioreactor was sparged with nitrogen gas (2 vvm) to achieve zero dissolved oxygen level and then inoculated (10% v/v). The medium was then supplemented with 6% (w/v) of glycerol. The reactor medium (per litre) consisted of: K_2HPO_4—3.4 g; KH_2PO_4—1.3 g; $(NH_4)_2SO_4$—15 g; $MgSO_4{\cdot}7H_2O$—0.2 g; $CaCl_2{\cdot}2H_2O$—0.02g; $FeSO_4{\cdot}7H_2O$—5 mg; yeast extract—2 g; L-cysteine—1 g; trace element solution SL7—1 ml (DSMZ). The in-house system has automatic pumping of 5 M NaOH for maintenance of medium pH at 7.0. The final fermentation broth (15 L) consisted of 1,3-propanediol (33.9 g/L), residual glycerol (0.45 g/L), acetic acid (6.5 g/L), butyric acid (1.39 g/L), proteins (3.1 g/L), inorganic salts, water, other unknown residual components, and biomass (4.1 g/L) in continuous mode. The volume of gas was calculated using water displacement, based on which 0.29 g/L of hydrogen was measured in the gaseous phase by mass balance equation [16] [17].

Mass Balance

The COD balance was carried out, where glycerol, 1,3-propanediol and other fermentative products were converted to the COD concentration (g-COD/L). For microbial biomass, $C_4H_7O_2N$ was assumed as an elemental composition. The COD distribution of each component was calculated by multiplying the respective COD concentration with 100 and then divided by the COD concentration of glycerol consumed [16]. Where, COD distribution for glycerol consumption was set to −100. The equation for COD balance was as:

$$\begin{aligned}\text{COD Balance}(\%) = {} & \text{COD distribution of substrate consumption} \\ & + \text{COD distribution of fermentative products and biomass}\end{aligned} \quad (1)$$

2.3. Recovery and Purification

2.3.1. Cell Free Broth and Decolorization

Biomass was removed using centrifugation (Thermoscientific Sorvall Lynx 4000) at 12,000 × g for 20 min. The obtained supernatant was transferred to another flask and stored at 4˚C for future use. Following which activated carbon was used for decolorization and biomolecules removal. Centrifugation is highly efficient for separating fine biomass particles quickly. Compared to microfiltration, which struggles with fine particles, and flocculation, which is slower and requires chemicals, centrifugation offers better versatility [18]. On the other hand, flotation and sedimentation are unsuitable options for biomass removal from fermentation broth. Despite its higher energy use, centrifugation excels in precision and speed.

It ensures high recovery rates without shear stress or frequent maintenance, making it ideal for large-scale 1,3-PDO production [19] [20]. The performance evaluation has been studied elsewhere in literature [18].

Activated carbon is the most effective method for protein and color removal due to its strong adsorption capacity, broad applicability, and minimal impact on broth composition. In contrast, precipitation requires extra filtration and possibly alters broth properties, while membrane filtration is quite effective, but is costly and very prone to clogging. Limitation with Ion exchange resins includes strict pH control and frequent regeneration, making them complex to use. In view of simplicity, and cost-effectiveness, activated carbon remains the preferred choice for industrial purification [21]. Thu, PVC based filtration column was fabricated. The length of the column is 50 cm with 5 cm internal diameter and 7 cm external diameter (refer e-supplementary data). The bottom of the column is kept closed but has a sieved plate. The liquid flows from top to bottom with help of peristaltic pump (**Figure 1**). Eight different columns were filled with different concentrations of activated carbons (2 to 30 g/L) and clarified broth was passed at 1ml/min through each one of them. Due to different concentrations of activated carbon the height of filtration bed (having activated carbon) differed. The obtained flow through were analyzed for color and protein loss.

2.3.2. Salting Out Extraction (SOE)

To remove by-products from the above broth salting-out extraction step was initiated in 50 mL graduated test tubes. For this 12 ml of cell free broth was mixed with 6 g of inorganic salt and vortex until the mixture was solubilized. Further to above add 6 ml of organic solvent, and again vortex until homogenized. This complete mixture was then held for 8 h at room temperature. Based on laboratory results obtained with simulated fermentation broth (data not shown), three different salting-out extraction (SOE) systems were evaluated for phase separation viz—1.) $(NH_4)_2SO_4$ and Ethanol; 2.) K_2CO_3 and Ethanol; 3.) K_2CO_3 and Isopropanol.

2.3.3. Phase Separation Using Ethyl Acetate

Another method used was phase separation using ethyl acetate as per Cho, Mi Hae *et al.* protocol [11]. Two hours after the occurrence of phase separation, both bottom and top phase were collected and analyzed.

2.3.4. Vacuum Distillation

Here, the cell free decolorized broth was concentrated five times in a rotating vacuum distillation unit (Hei-CHILL 600—Heidolph Instruments) at 70 ± 0.5˚C, 90 mbar vacuum. The distillate and retentate (condensed broth) were collected for analyses. The obtained retentate was incubated below 10˚C overnight for salt crystallization and later filtered using Whatman no., 1 filter paper. Vacuum distillation efficiently concentrates fermentation broth by lowering the boiling point. Also, it is energy-efficient, simultaneously preserving product integrity, and effectively removing excess water and volatile impurities. In contrast, filtration avoids

heat but faces clogging issues, while freeze drying slow and costly. Additionally, precipitation requires chemical additives, making vacuum distillation the best choice for balancing efficiency and product quality.

2.3.5. Chromatographic Purification

For purification purposes, column chromatography was employed, where the resin silica gel was used as stationary phase. A mixture of silica gel in chloroform and methanol (80:20) was made to pack a glass column. This was further eluted with sufficient amount of above. 50 mL of vacuum distilled retentate was loaded onto the column. Mobile phase was eluted at 10 ml/min and column fractions were collected after 10 min each for analyses. After the elution of 1,3-propanediol the column was re-equilibrated with hexane.

2.4. Analytical Methods

Quantitative analysis of glycerol, PDO production and other by-products was performed using high liquid performance chromatography (HPLC) technique as in previous works [15]. Samples were collected at regular time intervals and cell free supernatants were stored at 4˚C until use. Biomass was measured using optical density analyses at 600 nm (OD600) with spectramax i3x multi-mode microplate reader (Molecular Devices, LLC., USA). For estimation of protein concentrations bradford assay (based on protein-dye binding and shifting of absorbance from 465 to 595 nm) was used. Biogas composition was determined by gas chromatography (GC, Shimadzu 2014, Japan) equipped with thermal conductivity detector (TCD) as per protocol mentioned in literature [22]. ICP-MS (ELAN6100, Perkin–Elmer SCIEX, USA) was used for metal analysis of purified samples at after every step. Platinum-Cobalt Color, ASTM-D1209 is used for the visual measurement of the color of light colored fluids. The color generated by 1 mg of platinum cobalt in 1 L of water is called as one unit colour in platinum-cobalt scale. This visual color scale is based on color standards made from stock of chloroplatinate solutions (ranging from 0 for distilled water to 500 ppm) [23]. ASTM 1209 was used to measure decolorization of fermentation broth using activated carbon. All the experiments carried out in this study were performed in triplicates and the average values were presented ($p < 0.05$, one way student's t-test). The 1 H NMR and 13 C NMR spectra were confirmed with JEOL 400 spectrometer where D_2O was used as solvent (δ scale-for chemical shift values).

3. Results & Discussion

3.1. Metabolites Production and COD Balance

COD mass balances were conducted for a 10 L reactor operating in continuous mode (**Figure 1**). The initial glycerol concentration was maintained at 60 g/L. The COD mass balance was close, at 90.02%, with 1,3-PDO as the main metabolite, accounting for 68.3% of the total COD. This indicates that no major metabolic by-products were overlooked. Acetate (8.3% of total COD) and butyrate (3.0% of to-

tal COD) were the primary by-products, while H_2 represented approximately 2.7% of the total COD. The PDO yield obtained was 0.68 mol PDO/mol glycerol, corresponding to ~93% of the maximum theoretical yield, with a final concentration of 33.9 g/L. For comparison with theoretical yields for maximum 1,3-PDO production, equation (2), as previously described was used [24]:

$$68\ \text{Glycerol} + 3NH_3 \rightarrow 3C_4H_7O_2N + 15\ \text{Acetate} + 15CO_2 + 49\ \text{PDO} + 40H_2O \quad (2)$$

Thus, maximal theoretical 1,3-PDO yield (0.72 mol/mol) was obtained with only acetate as the by-product according to Equation (2).

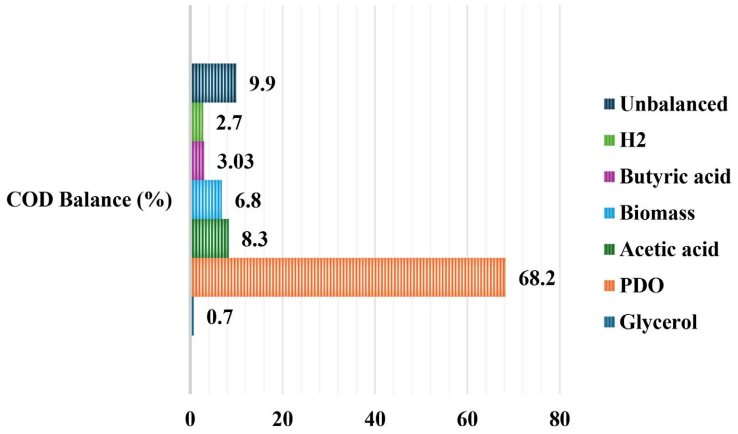

Figure 1. Metabolites distribution in Chemical oxygen demand (COD) equivalent.

3.2. Recovery and Purification of 1,3-Propanediol from Crude Fermentation Broth

The fermentation broth (FB) used in this study was produced in-house using a 10 L fermentor, yielding a total of approximately 15 L after the process. Downstream processing (DSP) and purification are critical aspects of any biologically produced chemical. Various economic indicators have been evaluated by Janković *et al.* (2024) through a case-by-case study on 1,3-PDO production from glucose and glycerol. The study applied the NREL method for cost calculation, which includes total capital costs (CAPEX), covering equipment purchase and installation, using a Marshall and Swift cost index. In another study, key sustainability metrics—such as energy intensity, water consumption, greenhouse gas emissions, wastewater generation, and pollutant and toxic emissions—were assessed to evaluate the environmental impact of the new recovery processes [25]. Another study provided a detailed analysis, for the first time, of the investments required for various glycerol valorization processes. Using the Guthrie, Taylor, and Aspen capital cost estimation methods, the study concluded that diols would be profitable if produced alongside glycerol carbonate [26]. There is a study which also detailed the procedures for calculating production costs and estimating profits from different processes. Their findings indicate that, with the technology available at the time and assuming a crude oil price of $25 per barrel, the production of 1,3-Propanediol (PDO) via white biotechnology was considered economically viable, given fer-

mentable sugar prices ranging from €70 to €400 per ton. The analysis suggests that within this price range, PDO production remained competitive with its petrochemical counterparts [27]. Additionally, a more recent 2023 study explores the integration of PDO production within existing sugar mills. This approach aims to diversify revenue streams and enhance the economic outlook of sugar mills, highlighting the ongoing interest and potential in bio-based PDO production [28]. The analysis evaluates three PDO production pathways: direct production from molasses (PDO-D), indirect production via glycerol (PDO-I), and a combined molasses-lignocellulosic approach (PDO-1G2G). PDO-D is the most cost-effective, with a production cost of $0.83/kg and a high Internal Rate of Return (IRR) of 75.4%. In contrast, PDO-I has a higher cost of $2.24/kg and a low IRR of 11.1%, making it less viable. PDO-1G2G balances cost and scalability, with a moderate cost of $1.02/kg and an IRR of 33.5%. These findings emphasize the importance of feedstock selection and process integration in optimizing bio-based PDO production. The study using SuperPro Designer®, confirms 1,3-PDO economic viability [29]. A 23,000 MT/year plant, selling PDO at $2.00/kg, could achieve a 6.9% gross margin, 13.3% ROI, and a 7.5-year payback period, with a $55 million investment and $43 million in annual costs (55% from raw materials). Assuring cost reductions could come from using lignocellulosic sugars or glycerol. The study highlights feedstock selection and process optimization as key to improving sustainability.

For technical processing, FB is first neutralized and subsequently acidified, producing gypsum, which has several associated drawbacks. In this study, the fermentation broth was centrifuged at 12,000 × g for 20 minutes (volatile solids = 0.48% ± 0.01% TS) to remove biomass and other insoluble impurities. The supernatant was then transferred to a PVC fabricated column and treated with activated carbon for decolorization and protein removal. Activated carbon is highly porous possessing high surface area capable of adsorption through non-covalent interactions thus, attracting impurities and other small molecules like protein, biomolecules to the surface of the charcoal [30]. This is further based on binding capacities of individual polymers and proteins influenced by differences in their size and effective charge.

The PVC based filtration column was used and is illustrated in E-supplementary data. Eight different columns were filled with different concentrations of activated carbons (2 to 30 g/L) and clarified broth was passed through each at a flow rate of 1 mL/min (**Figure 2**). However, as the concentration of activated carbon increased, a decrease in 1,3-propanediol recovery was observed [23]. The results illustrate maximum removal using 20 g/L of activated charcoal while minimizing 1,3-propanediol loss. The treated broth had a clear appearance, measuring 30 Pt-Co units. At this concentration, 91.7% of protein was removed, with a 12.9% loss of 1,3-propanediol. The color change from untreated (dark yellow) to treated broth (almost colorless) was evaluated using the Pt/Co scale and the Pt-Co/Hazen/APHA Color Reference Standard (Sigma). Other by-products were only partially removed on treating with activated charcoal (20 g/L) with reductions of

62.2% for glycerol, 21.8% for acetic acid and 47.4% for butyric acid. Similar results were illustrated elsewhere, where it was recommended that the optimal concentration of the activated charcoal treatment be between 20 - 30 g/L to limit 1,3-PDO loss [18].

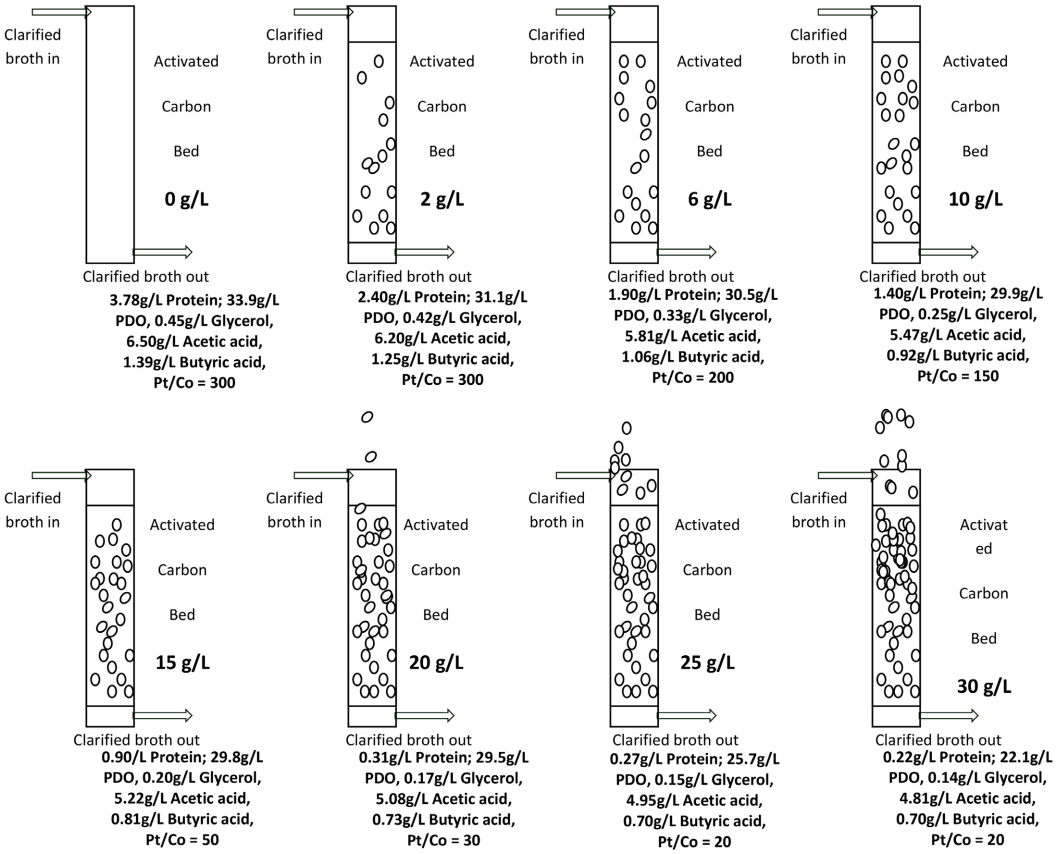

Figure 2. Effect of different concentration of activated carbon on 1,3-propanediol recovery and removal of unwanted impurities.

3.3. Outcomes of Salting-Out Extraction

Salting-out extraction (SOE) is a partition method to take out hydrophilic product from aqueous solution using organic solvent as the extractant and inorganic salt for salting it out. With hydrophillic solvent the SOE is aqueous two-phase extraction (ATPE) [30]. Herein, 1,3-PDO, acetic acid and butyric acid are in clarified broth and these are mainly distributed in the top (having organic solvent) and bottom phase (having salt and water). Volatile acids are partitioned to the top phase because of their higher affinity to organic solvents. Bottom phase is mostly enriched with glycerol and salts. The presence of salt type and solvent type affects the phases and thus, partition coefficient. Herein three different salt solvent systems have been used and the results are detailed in **Table 1**. With K_2CO_3 and Isopropanol maximum by-products were removed with minimum recovery yield (%). These organic solvent has hydration ability which differs due to its structures [31]. Wu and Wang (2012) achieved 72% 1,3-propanediol recovery using sodium

phosphate and pentanol in a single salt system. Interestingly, with two-salt system using sodium phosphate and sodium sulfate, recovery improved to 92.5% but volatile acids removal was not focused.

Table 1. The analyses of 1,3-propanediol and other unwanted impurities (acetic acid, butyric acid etc) after salting-out extraction (SOE).

S. No	Salting-out extraction (SOE)	1,3-propanediol-recovery (%)	Removal of acetic acid, butyric acid and glycerol from top phase (%)	Remarks
1	$(NH_4)_2SO_4$ and Ethanol	87.6%	19.6%	Minimum removal of by-product but maximum 1,3-propanediol recovery
2	K_2CO_3 and Ethanol	82.3%	8.1%	-
3	K_2CO_3 and Isopropanol	75.2%	79.2%	Maximum removal of by-product but minimum 1,3-propanediol recovery

In another study, two-step salting-out extraction method was investigated for separation of 1,3-propanediol from lactic acid in real fermentation broth of *Klebsiella pneumoniae* from biodiesel-derived crude glycerol. In the first step using isopropanol and K_2CO_3 (30% each) about 92.4% of 1,3-PDO was recovered. Subsequently, 28% ethanol was added to the salt phase to recover lactic acid (73.8%) [12].

In this study, using fermentation broth obtained from *Clostridium butyricum* L4, 75.2% 1,3-propanediol was recovered after separation of acetic acid with K_2CO_3 and Isopropanol SOE system. Literature suggests that 1,3-propanediol distribution in their common extraction solvents resulted in significant discrepancies between the predicted and experimental values making this technique unpredictable and not good enough for scaling up as simple and efficient extraction procedure [32]. Li *et al.* (2013) evaluated an aqueous two-phase system by ethanol/sodium carbonate system and recovered >95% 1,3-propanediol but the purity of target obtained was quite low [33]. Simple recovery of 1,3-propanediol by liquid–liquid extraction system with K_3PO_4/ethanol was successful using fermentation broth obtained from fed-batch of *Clostridium beijerinckii DSM* 791 *A*1 [34]. Effective recovery of 1,3-PDO from butyl acetate was achieved using two-step salting-out extraction, providing separation of 1,3-PDO, butyl acetate and acetic acid [10]. Furthermore, various investigations use screening software to evaluate purification of 1,3-propanediol with different extractants. The study indicated superior performance of fatty alcohols and aldehydes but, the experimental extraction varied from the simulated value, and the outcomes were again unsatisfactory. The strong polarity of 1,3-propanediol causes the extraction system not to act effectively and compromising its recovery from broth solution. Its poor distribution coefficients in both phases further decrease overall yields when using SOE. Studies have shown that predicted recovery efficiencies for 1,3-PDO can be overestimated

by 15% - 30% due to solvent polarity mismatches and phase distribution errors [35]. To summarize, discrepancies between predicted and actual extraction occur due to the complex composition of real fermentation broth, non-ideal phase behavior, and the strong polarity of 1,3-propanediol, which affects its partitioning. Predictive models also often assume ideal conditions and may not account for solvent-solute interactions, salt effects, or kinetic limitations. Variability in experimental conditions and raw materials further contributes to deviations in expected yields.

3.4. Phase Separation

Based on Cho, Mi Hae *et al*. protocol which was evaluated on model mixture in 2006 [10], the protocol used ethyl acetate for phase separation of 1,3-propanediol to remove glycerol and other by-products before chromatography from the real fermentation broth. The advantages reported were that the impurities overlap with target products in the feed during subsequent chromatographic separation (like glycerol). The results elucidated extraction of 1,3-propanediol (31.7 g/L), residual glycerol (0.40 g/L), acetic acid (5.1 g/L), butyric acid (1.0 g/L) in top layer. The experiments were repeated thrice to confirm the findings. Thus, the phase separation protocol for by-products removal didn't work with the fermentation broth produced from *Clostridium butyricum* L4 in present study. The possible explanation of which could be difference in salt concentration, alkalinity, pH of the broth from model mixture used in previous study. Another explanation may be higher 1,3-propanediol concentration as a prerequisite [10]. Saxena *et al*. (2009), reported similar evidence stating that the protocol using ethyl acetate have very low partition coefficient of the target product (<1.9); with requiring huge quantities of solvents [32].

3.5. Vacuum Distillation and Low Temperature Treatment

The above cell free decolorized broth was condensed as mentioned in materials and methods. Both the distillate and retentate were collected. Further, the retentate was incubated below 10˚C overnight for crystallizing the salts. The salts were removed using filtration with whatman's filter paper no. 1. The salts resulting in the broth tend to precipitate lead to undesired effects and conjugated by-products. The retentate was estimated with 146.5 g/L of 1,3-propanediol and 0.82 g/L of residual glycerol; while distillate was rich in lower distil products (24.8 g/L of acetic acid and 3.51 g/L butyric acid). This step helps remove lower chain by-products which can later be recovered from distillate. Thus, 1,3-Propanediol was easily extracted and concentrated without any significant loss (~0.67% only). In the study fractional distillation was not evaluated due to limitations. Otherwise, according to the Antoine equation, vacuum distillation at 25 mbar would save energy with the boiling points of 1,3-PDO and glycerol as 98˚C and 163˚C, respectively [36]. Moreover, before separation of glycerol and other impurities from 1,3-propanediol desalination and de-proteinization are required, else the soluble macromolecules salt out leading to low efficiency of evaporation/distillation [37].

3.6. Chromatography

Table 2. The average compositional analyses post each step of down streaming (with a tolerance of ± 0.5).

	~Volume (L)	1,3-propanediol (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Butyric acid (g/L)	Na+ (ppm)	K+ (ppm)	Mg+ (ppm)	Ca+ (ppm)
Fermentation broth	15	33.9	0.45	6.5	1.39	2364	1945	321	765
Fermentation broth after biomass removal	14.89	33.8	0.45	6.5	1.38	2119	1534	309	698
Decolorization	14.47	29.5	0.17	5.08	0.73	1472	1076	221	418
Concentrated vacuum distillation-retentate after crystallization (at low temperature)	2.88	146.5	0.82	0	0	415	102	64	56
Post Chromatography	1	418.91	0.03	0	0	203	43	18	19

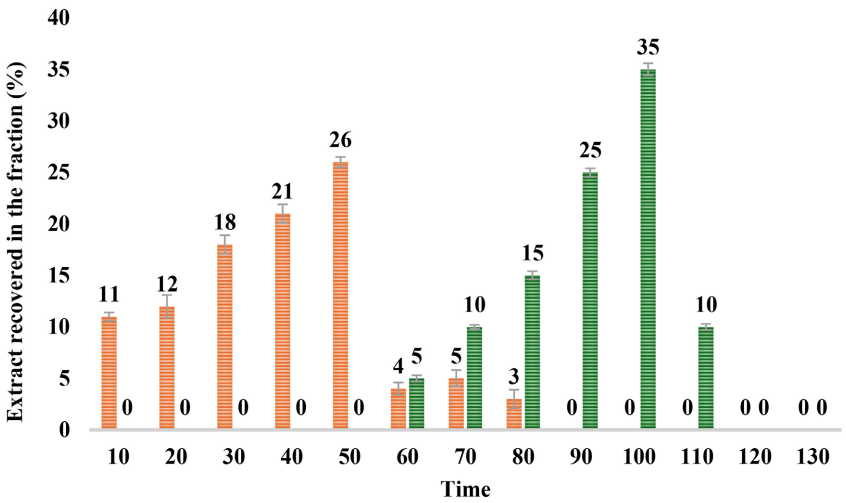

Figure 3. The elution profile in isocratic chromatography for 1,3-propanediol (product) and glycerol (substrate) using chloroform and methanol (80:20) as mobile phase.

Isocratic column chromatography-based purification was employed, where the resin silica gel was used as stationary phase. A mixture of silica gel in chloroform and methanol (80:20) was made to pack a glass column. These were selected on basis of laboratory based experiments with various resins. To maximize the 1,3-propanediol recovery with minimum glycerol overlap was the target of purification. The mobile phase was a mixture of polar and non-polar solvents. However, as the amount of glycerol left after fermentation was very less in comparison to other studies no overlapping was detected with chloroform and methanol (80:20) as mobile phase. Superior resolution was achieved in isocratic chromatography, without any significant overlapping between target and impurities. The adsorption depends on physical binding of target compound with silica gel, and this was

attributed to the presence of hydroxyl groups which enhances its interaction [10]. During first five fractions about 88% of 1,3-propanediol was recovered (**Figure 3**) with fractions between 60 to 80 minutes were contaminated with glycerol and after that were found rich in glycerol (even though the quantitative amount of glycerol was very low). The collected fractions were evaporated to remove the mobile phase. The left retentate was diluted in milli Q water and then prepared as other HPLC samples. The overall cumulative 1,3-propanediol extracted yield was 82.22%. In this case most of the loss (~12.9%) was the result of decolorization step. As it is necessary and inevitable step to remove colour and other biomolecules, more experimental work is required for further improvement. The separation of inorganic salts represents an exacting problem along with production of bio-based valuable substances, so the methods used for downstream processing play a very important role. **Table 2**, shows variation in concentration of ions and volumes at each step of purification. The product obtained after vacuum distillation and low temperature treatment had experienced reduction of about 88% in metal ion concentrations.

For ruling out other impurities NMR is usually performed. However, NMR spectra reveals that end fermentation broth has alcoholic impurities which seems to be around ~14% (mostly glycerol-spectra is not absolute) while, post chromatography product with purified 1,3-propanediol was almost pure as per the spectra.

Various downstreaming ways of PDO extraction have been discussed in literature [37]. Efforts were narrowed towards whole process evaluation involving instrument and cost calculations [38]. Most of purification strategies are trade-offs between purity, yield, scaling-up scale, cost and applications. Cost calculations most necessary step before a developed downstream process is scaled up to industry scale [8]. Previously, focus was on separation and extraction of 1,3-propanediol from fermentative broth obtained using pure glycerol as raw material. But as biodiesel derived crude glycerol is very readily available at much cheaper price it is the substrate of choice [15]. It contains impurities mainly aromatic in range and biological broth obtained from this crude glycerol is practical and realistic to be used for any purification-based study. *Clostridium butyricum D43* based crude glycerol fermentation to 1,3-propanediol was purified where, integrated flocculation, decolorization using activated carbon, cation exchange resin adsorption, and vacuum distillation was developed to obtain purity and yield of 99.2% and 80.8%, respectively. Molecular distillation or short path distillation equipment used for separation of 1,3-propanediol, which converts the low boiling compounds into vapor under vacuum and heating conditions. Where, two stages of a distillation process were used and the process was highly energy intensive in nature [39]. In similar study, glycerol fermentation by *C. freundii YRL 11* downstream processing of 1,3-PDO was developed where the overall 1,3-PDO yield was only 75.47% [9]. Kaeding et al. (2015) developed purification from fermentation broth obtained from *Clostridium pasteurianum K1* involving a two-step rectification for final purification. This was validated by a miniplant scale and Aspen plus simulation with the overall yield of only 57%. In addition, crude glycerol was desalinated using

electrodialysis before fermentation to minimize salt input, which is a very expensive process [38].

Desalination can be performed with electrodialysis or Amine-based extraction. However, it is limited by high cost of ion exchange membranes, high energy requirement to drive electricity, toxicity of the amine reagent etc [40]. recently, Zhang *et al.* (2021), developed a process for recovering 1,3-propanediol from biological broth of fed-batch fermentation of raw glycerol by *Clostridium pasteurianum* by vacuum distillation with continuous supplementation of glycerol as a supportive agent, so as to crystallize inorganic impurities without their precipitation and using alkaline hydrolysis to remove ester impurities, having yield of 76% with 99.63% purity. Hydrolysis involved stirring and heating the mixture at 60˚C for 1 hour. The biomass and proteins were removed by ultrafiltration. Overall the process is highly energy intensive and requires cost calculations for whole process evaluation. In another study, the biogenic produced 1, 3-PDO was obtained from pure glycerol fermentation by *Klebsiella pneumoniae DSMZ* 2026. The study used strong acidic ion exchange resin in H+ form and flocculation resulting in recovering biogenic 1,3-PDO [41]. Sui *et al.* (2022) developed a synergistic extraction process for recovering 1,3-propanediol from biological broths with multi-alcohol extractants [42].

Salting out extraction (SOE system) and phase separation were evaluated with same fermentation broth; however, both illustrated much lower yields and purity. With K_2CO_3 and isopropanol SOE system only 75.2% of target compound was recovered which was accompanied with removal of 79.2% acetic acid. When evaluated using $(NH_4)_2SO_4$ and ethanol about 87.6% of 1,3-propanediol was recovered but only 19.6% acetic acid was removed from broth. Additionally, to make the salting out process economical and more environment friendly recyclability of salts and solvents is very crucial. In contrast, using vacuum distillation 100% acetic acid was removed due to its' lower distillation point, with 98.3% of 1,3-propanediol (*i.e.*, 146.5 g/L) recovered in concentrated retentate. The ethyl acetate based phase separation illustrated nil removal of impurities from target compound in the present study.

Literature reports also use of other cationic/ acidic ion exchange resins like Amberlite IR-120H, 001 × 7 H-form, XAD-7 and XAD-16, H-ZSM-5 zeolite, polystyrene sulfonic acid type or divinylbenzene-based cation-exchange resins etc; and gradient chromatography for better elution and separation of impurities [8]-[10] [13] [41] [43] [44]. There are still research gaps related to ion exchange resins' adsorption behavior as it varies widely and is influenced by number of factors in fermentation broth like substrate used, salt medium, pH, temperature, by-products etc [45]. Few of these have limitation *i.e.*, the eluted product is diluted around 50 times with water, requiring high energy de-watering step along with expensive ion exchange resins and frequent regeneration requirement because of anionic and cationic moieties present in broth. Any industrial facility requiring ion exchange technology assesses not only the cost of ion exchange resins cost but the associated economic cost for maintaining and disposing them. It also vary depending on the manufacturer, type, quality, and resin size [46]. A comparison of

1,3-PDO recovery achieved through various available recovery techniques—Reactive solvent extraction, Ion-exchange chromatography, Esterification with lipids, Liquid-liquid extraction, and Aqueous Two-Phase System (ATPS)—was studied by Yeow *et al.* (2024). The results, compared across different studies, showed recovery rates of 87% for Reactive solvent extraction, 99% for Ion-exchange chromatography, 96% for Esterification with lipids, 90% for Liquid-liquid extraction, and 98% for ATPS. The present study utilizes a cheaper and easy to regenerate alternative *i.e.*, silica gel (having ability to bind over a wide pH range) resulting in cost effective polishing during the downstream processing [46]. Anand *et al.* (2011), gradient chromatography was used to overcome the issues associated with overlapping of glycerol and 1,3-propanediol elution resulting in overall recovery yield of 75.47% from fermentation broth having 98% of purity. Herein, cell free broth was achieved by centrifugation which was followed by decolorization and protein removal via activated charcoal treatment. The decolorized broth was vacuum distilled for removal of low boiling point impurities like acetic acid and simultaneous concentration via water removal. The last step of purification involved isocratic chromatography using silica gel and chloroform: methanol (90:20). The overall process was simple with recovery of about 82.22% 1,3-propanediol and >97% purity, further confirmed by NMR (1 H and 13 C).

The high-yield (82.22%) and high-purity (>97%) recovery of 1,3-propanediol (1,3-PDO) supports its use in bioplastics/PTT, cosmetics, and pharmaceuticals, enhancing large-scale bio-based PDO production. The optimized process can improve efficiency in fermentation-based industries and be adapted by others (**Figure 4**). High-purity 1,3-PDO has applications in sustainable polymers like polytrimethylene terephthalate (PTT) with applications in textiles, automotive parts, and biodegradable plastics. Additionally, its application in personal care products and pharmaceuticals can also be expanded due to its biocompatibility and low toxicity. Regulatory agencies may consider updating bio-chemical production guidelines to facilitate industrial adoption of this purification approach. The study aligns with circular economy principles and sustainability goals, supporting the market adoption of bio-based PDO.

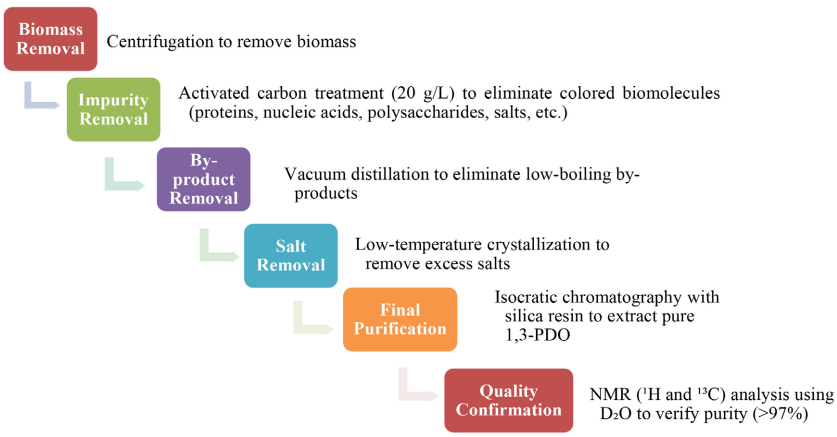

Figure 4. 1,3-propanediol recovery process flowchart.

4. Concluding Remarks

Purification of pure 1,3-propanediol from complex biological broth still stands as a major production issue in sustainable bio-refinery development. Herein, a novel purification process to recover and separate 1,3-PDO (high yield and purity were achieved) from biodiesel plant derived crude glycerol was elucidated. The derived crude glycerol was characterized with multiple impurities affecting the overall process and making purification complex. The stepwise final recovery illustrated herein involved-biomass removal with centrifugation, activated charcoal treatment (using 20 g/L) for decolorization, vacuum distillation for removal of low distil products succeeded by low temperature crystallization for excess salts removal. The process ended with isocratic chromatography resulting in overall recovery yield of 82.22% (with >97% purity). NMR (1 H and 13 C) further confirmed purity of targeted product in post chromatography liquid using D_2O as solvent. Thus, the study provides useful insights in down streaming process for developing greener, futuristic resolves.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Marr, A.C. (2024) 1,3-Propanediol, an Exemplary Bio-Renewable Organic Platform Chemical. *Advanced Synthesis & Catalysis*, **366**, 4835-4845. https://doi.org/10.1002/adsc.202401066

[2] Da Silva, A.B., de Menezes, C.A., Lovato, G., Rodrigues, J.A.D. and Silva, E.L. (2021) Enhanced Fermentative Production of 1,3 Propanediol by Employing Ethanol Industry Wastewater. *Bioresource Technology Reports*, **16**, Article 100865. https://doi.org/10.1016/j.biteb.2021.100865

[3] Veras, S.T.S., Rojas, P., Florencio, L., Kato, M.T. and Sanz, J.L. (2019) Production of 1,3-Propanediol from Pure and Crude Glycerol Using a UASB Reactor with Attached Biomass in Silicone Support. *Bioresource Technology*, **279**, 140-148. https://doi.org/10.1016/j.biortech.2019.01.125

[4] Janković, T., Straathof, A.J.J. and Kiss, A.A. (2024) Eco-Efficient Downstream Processing of 1,3-Propanediol Applicable to Various Fermentation Processes. *Process Biochemistry*, **143**, 210-224. https://doi.org/10.1016/j.procbio.2024.04.040

[5] Asopa, R.P., Ikram, M.M. and Saharan, V.K. (2022) Valorization of Glycerol into 1,3-Propanediol and Organic Acids Using Biocatalyst Saccharomyces Cerevisiae. *Bioresource Technology Reports*, **18**, Article 101084. https://doi.org/10.1016/j.biteb.2022.101084

[6] Alawi, A., Kiyoshi, K., Matsumoto, M., Yamaguchi, T., Narita, T., Morita, T., *et al.* (2022) Metabolic Engineering of a Newly Isolated *Citrobacter braakii* Strain to Produce 1,3-Propanediol from Glycerol. *Bioresource Technology Reports*, **20**, Article 101271. https://doi.org/10.1016/j.biteb.2022.101271

[7] Schoch, T., Baur, T., Kunz, J., Stöferle, S. and Dürre, P. (2023) Heterologous 1,3-Pro-

panediol Production Using Different Recombinant Clostridium Beijerinckii DSM 6423 Strains. *Microorganisms*, **11**, Article 784. https://doi.org/10.3390/microorganisms11030784

[8] Zhang, C., Sharma, S., Wang, W. and Zeng, A. (2021) A Novel Downstream Process for Highly Pure 1,3-Propanediol from an Efficient Fed-Batch Fermentation of Raw Glycerol by *clostridium Pasteurianum*. *Engineering in Life Sciences*, **21**, 351-363. https://doi.org/10.1002/elsc.202100012

[9] Anand, P., Saxena, R.K. and Marwah, R.G. (2011) A Novel Downstream Process for 1,3-Propanediol from Glycerol-Based Fermentation. *Applied Microbiology and Biotechnology*, **90**, 1267-1276. https://doi.org/10.1007/s00253-011-3161-2

[10] Cho, M., Joen, S.I., Pyo, S., Mun, S. and Kim, J. (2006) A Novel Separation and Purification Process for 1,3-Propanediol. *Process Biochemistry*, **41**, 739-744. https://doi.org/10.1016/j.procbio.2005.11.013

[11] Wang, X., Zhou, J., Sun, Y. and Xiu, Z. (2019) Bioconversion of Raw Glycerol from Waste Cooking-Oil-Based Biodiesel Production to 1,3-Propanediol and Lactate by a Microbial Consortium. *Frontiers in Bioengineering and Biotechnology*, **7**, Article 14. https://doi.org/10.3389/fbioe.2019.00014

[12] Song, Z., Sun, Y., Wei, B. and Xiu, Z. (2013) Two-Step Salting-Out Extraction of 1,3-Propanediol and Lactic Acid from the Fermentation Broth of *Klebsiella pneumoniae* on Biodiesel-Derived Crude Glycerol. *Engineering in Life Sciences*, **13**, 487-495. https://doi.org/10.1002/elsc.201200154

[13] Sun, Y., Shen, J., Yan, L., Zhou, J., Jiang, L., Chen, Y., *et al*. (2018) Advances in Bioconversion of Glycerol to 1,3-Propanediol: Prospects and Challenges. *Process Biochemistry*, **71**, 134-146. https://doi.org/10.1016/j.procbio.2018.05.009

[14] de Sousa e Silva, A., Sales Morais, N.W., Maciel Holanda Coelho, M., Lopes Pereira, E. and Bezerra dos Santos, A. (2020) Potentialities of Biotechnological Recovery of Methane, Hydrogen and Carboxylic Acids from Agro-Industrial Wastewaters. *Bioresource Technology Reports*, **10**, Article 100406. https://doi.org/10.1016/j.biteb.2020.100406

[15] Gupta, P., Kumar, M., Gupta, R.P., Puri, S.K. and Ramakumar, S.S.V. (2022) Fermentative Reforming of Crude Glycerol to 1,3-Propanediol Using *Clostridium butyricum* Strain L4. *Chemosphere*, **292**, Article 133426. https://doi.org/10.1016/j.chemosphere.2021.133426

[16] Sittijunda, S. and Reungsang, A. (2020) Valorization of Crude Glycerol into Hydrogen, 1,3-Propanediol, and Ethanol in an Up-Flow Anaerobic Sludge Blanket (UASB) Reactor under Thermophilic Conditions. *Renewable Energy*, **161**, 361-372. https://doi.org/10.1016/j.renene.2020.07.053

[17] Kongjan, P., Jariyaboon, R., Reungsang, A. and Sittijunda, S. (2021) Co-Fermentation of 1,3-Propanediol and 2,3-Butanediol from Crude Glycerol Derived from the Biodiesel Production Process by Newly Isolated Enterobacter Sp.: Optimization Factors Affecting. *Bioresource Technology Reports*, **13**, Article 100616. https://doi.org/10.1016/j.biteb.2020.100616

[18] Yeow, T.A., Said Ismail, A.A., Elly Agustin, Y., Ho, S.S., Yeap, S.K., Hui, Y.W., *et al*. (2024) Review on the Downstream Purification of the Biologically Produced 1,3-propanediol. *Applied Microbiology: Theory & Technology*, **5**, 72-92. https://doi.org/10.37256/amtt.5120244440

[19] Dobrovszky, K. and Ronkay, F. (2014) Alternative Polymer Separation Technology by Centrifugal Force in a Melted State. *Waste Management*, **34**, 2104-2112. https://doi.org/10.1016/j.wasman.2014.05.006

[20] Zhu, J., Wakisaka, M., Omura, T., Yang, Z., Yin, Y. and Fang, W. (2024) Advances in Industrial Harvesting Techniques for Edible Microalgae: Recent Insights into Sustainable, Efficient Methods and Future Directions. *Journal of Cleaner Production*, **436**, Article 140626. https://doi.org/10.1016/j.jclepro.2024.140626

[21] Ghaedi, M., Nasab, A.G., Khodadoust, S., Rajabi, M. and Azizian, S. (2014) Application of Activated Carbon as Adsorbents for Efficient Removal of Methylene Blue: Kinetics and Equilibrium Study. *Journal of Industrial and Engineering Chemistry*, **20**, 2317-2324. https://doi.org/10.1016/j.jiec.2013.10.007

[22] Gupta, P., Sreekrishnan, T.R. and Ahammad, S.Z. (2015) Acclimatization of Anaerobic Sludge to Treat Cr (VI) and 4-CP Present in Industrial Effluents and Their Effect on Microbial Communities. *Journal of Hazardous, Toxic, and Radioactive Waste*, **19**, Article 4015007. https://doi.org/10.1061/(asce)hz.2153-5515.0000279

[23] Drews, A. (2008) Standard Test Method for Color of Clear Liquids (Platinum-Cobalt Scale). In: Drews, A.W., Ed., *Manual on Hydrocarbon Analysis*, 6th Edition, ASTM International.

[24] Moscoviz, R., Trably, E. and Bernet, N. (2016) Consistent 1,3-Propanediol Production from Glycerol in Mixed Culture Fermentation over a Wide Range of pH. *Biotechnology for Biofuels*, **9**, Article No. 32. https://doi.org/10.1186/s13068-016-0447-8

[25] Sheldon, R.A. (2017) Metrics of Green Chemistry and Sustainability: Past, Present, and Future. *ACS Sustainable Chemistry & Engineering*, **6**, 32-48. https://doi.org/10.1021/acssuschemeng.7b03505

[26] D'Angelo, S.C., Dall'Ara, A., Mondelli, C., Pérez-Ramírez, J. and Papadokonstantakis, S. (2018) Techno-Economic Analysis of a Glycerol Biorefinery. *ACS Sustainable Chemistry & Engineering*, **6**, 16563-16572. https://doi.org/10.1021/acssuschemeng.8b03770

[27] Hermann, B.G. and Patel, M. (2007) Today's and Tomorrow's Bio-Based Bulk Chemicals from White Biotechnology: A Techno-Economic Analysis. *Applied Biochemistry and Biotechnology*, **136**, 361-388. https://doi.org/10.1007/s12010-007-9031-9

[28] van Heerden, C., Farzad, S. and Görgens, J.F. (2023) Techno-Economic and Environmental Assessments of 1,3-Propanediol and Xylooligosaccharide Production Annexed to a Typical Sugar Mill. *ACS Sustainable Chemistry & Engineering*, **11**, 16453-16468. https://doi.org/10.1021/acssuschemeng.3c03338

[29] Petrides, D. and Ferrerira, R. (2020) Production of 1,3-Propanediol (PDO) via Fermentation—Process Modeling and Techno-Economic Assessment (TEA) Using SuperPro Designer. Intelligen, Inc.

[30] Li, Z., Yan, L., Zhou, J., Wang, X., Sun, Y. and Xiu, Z. (2019) Two-Step Salting-Out Extraction of 1,3-Propanediol, Butyric Acid and Acetic Acid from Fermentation Broths. *Separation and Purification Technology*, **209**, 246-253. https://doi.org/10.1016/j.seppur.2018.07.021

[31] Wu, H.S. and Wang, Y.J. (2012) Salting-Out Effect on Recovery of 1,3-Propanediol from Fermentation Broth. *Industrial & Engineering Chemistry Research*, **51**, 10930-10935. https://doi.org/10.1021/ie300404t

[32] Saxena, R.K., Anand, P., Saran, S. and Isar, J. (2009) Microbial Production of 1,3-Propanediol: Recent Developments and Emerging Opportunities. *Biotechnology Advances*, **27**, 895-913. https://doi.org/10.1016/j.biotechadv.2009.07.003

[33] Li, Z., Sun, Y., Zheng, W., Teng, H. and Xiu, Z. (2013) A Novel and Environment-Friendly Bioprocess of 1,3-Propanediol Fermentation Integrated with Aqueous Two-Phase Extraction by Ethanol/Sodium Carbonate System. *Biochemical Engineering*

Journal, **80**, 68-75. https://doi.org/10.1016/j.bej.2013.09.014

[34] Wischral, D., Fu, H., Pellegrini Pessoa, F.L., Pereira, N. and Yang, S. (2018) Effective and Simple Recovery of 1,3-Propanediol from a Fermented Medium by Liquid-Liquid Extraction System with Ethanol and K 3 PO 4. *Chinese Journal of Chemical Engineering*, **26**, 104-108. https://doi.org/10.1016/j.cjche.2017.06.005

[35] Fu, C., Li, Z., Sun, Z. and Xie, S. (2020) A Review of Salting-Out Effect and Sugaring-Out Effect: Driving Forces for Novel Liquid-Liquid Extraction of Biofuels and Biochemicals. *Frontiers of Chemical Science and Engineering*, **15**, 854-871. https://doi.org/10.1007/s11705-020-1980-3

[36] Matugi, K., Chiavone-Filho, O., de Arruda Ribeiro, M.P., de Pelegrini Soares, R. and de Campos Giordano, R. (2018) Vapor-Liquid Equilibrium Calculation for Simulation of Bioethanol Concentration from Sugarcane. *Brazilian Journal of Chemical Engineering*, **35**, 341-352. https://doi.org/10.1590/0104-6632.20180352s20160278

[37] Xiu, Z. and Zeng, A. (2008) Present State and Perspective of Downstream Processing of Biologically Produced 1,3-Propanediol and 2,3-Butanediol. *Applied Microbiology and Biotechnology*, **78**, 917-926. https://doi.org/10.1007/s00253-008-1387-4

[38] Kaeding, T., DaLuz, J., Kube, J. and Zeng, A. (2014) Integrated Study of Fermentation and Downstream Processing in a Miniplant Significantly Improved the Microbial 1,3-Propanediol Production from Raw Glycerol. *Bioprocess and Biosystems Engineering*, **38**, 575-586. https://doi.org/10.1007/s00449-014-1297-z

[39] Wang, Z., Wu, Z. and Tan, T. (2013) Studies on Purification of 1,3-Propanediol by Molecular Distillation. *Biotechnology and Bioprocess Engineering*, **18**, 697-702. https://doi.org/10.1007/s12257-012-0804-9

[40] Gong, Y., Tang, Y., Wang, X., Yu, L. and Liu, D. (2004) The Possibility of the Desalination of Actual 1,3-Propanediol Fermentation Broth by Electrodialysis. *Desalination*, **161**, 169-178. https://doi.org/10.1016/s0011-9164(04)90052-5

[41] Mitrea, L., Leopold, L.F., Bouari, C. and Vodnar, D.C. (2020) Separation and Purification of Biogenic 1,3-Propanediol from Fermented Glycerol through Flocculation and Strong Acidic Ion-Exchange Resin. *Biomolecules*, **10**, Article 1601. https://doi.org/10.3390/biom10121601

[42] Sui, W., Sun, Y., Wang, X. and Xiu, Z. (2022) Synergistic Extraction of 1,3-Propanediol from Fermentation Broths Using Multialcohol Extractants. *ACS Sustainable Chemistry & Engineering*, **10**, 11891-11901. https://doi.org/10.1021/acssuschemeng.2c02853

[43] Patent Application Publication (2021) US 2021/0071208 A1.

[44] Vivek, N., Pandey, A. and Binod, P. (2018) An Efficient Aqueous Two Phase Systems Using Dual Inorganic Electrolytes to Separate 1,3-Propanediol from the Fermented Broth. *Bioresource Technology*, **254**, 239-246. https://doi.org/10.1016/j.biortech.2018.01.076

[45] Ahmad, A., Othman, I., Taher, H. and Banat, F. (2021) Lactic Acid Recovery from Date Pulp Waste Fermentation Broth by Ions Exchange Resins. *Environmental Technology & Innovation*, **22**, Article 101438. https://doi.org/10.1016/j.eti.2021.101438

[46] Wołowicz, A. and Wawrzkiewicz, M. (2021) Screening of Ion Exchange Resins for Hazardous Ni(II) Removal from Aqueous Solutions: Kinetic and Equilibrium Batch Adsorption Method. *Processes*, **9**, Article 285. https://doi.org/10.3390/pr9020285