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Review Article

Review on the Downstream Purification of the Biologically Produced 1,3-Propanediol

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Abstract: The blooming of biodiesel, oleochemical, and agricultural industries gives rise to the spiking availability of fermentable carbon sources for 1,3-propanediol (1,3-PDO) production from their by-products. Despite the availability of such sustainable feedstocks, the downstream process of bio-based 1,3-PDO which contributed to 50-70% of the total production cost remained a key bottleneck towards economic viability. Judging from the complex and aqueous content of the fermented broth, low volatility, and polar nature of 1,3-PDO, one-step refining is unachievable to obtain highly purified 1,3-PDO as required by the market. Herein, the purification of fermented 1,3-PDO involves multiple steps which include solids removal, flocculation, desalination, recovery, and final thermal separation step. This article gives an overview of the downstream processes with a main focus on dissecting resin adsorption and ion-exchange chromatography as the main polishing steps. Despite high efficiency, chromatography methods are impeded by their complicated procedures and the involvement of expensive resins, which are among the challenges to commercialization.

Keywords: 1,3-propanediol (1,3-PDO), ion-exchange chromatography, resin adsorption, cation resin, silica gel

1. Introduction

The global biodiesel production was estimated to be 48 billion liters in 2020 [1]. For every ton of biodiesel produced, 100 kg of crude glycerol is expected to be generated [2]. Furthermore, 110 kg of crude glycerol will also be generated from every ton of palm fatty acid produced through hydrolysis in the oleochemical industry [3]. Compounding these two streams and its derivation from other industries has driven the surplus of crude glycerol supply in the market. The price of crude glycerol has been reduced from USD 240 per ton in 2014, to USD 170 per ton in 2019 [4, 5]. The falling price of crude glycerol has attracted attention from researchers to valorize such carbon sources for

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the production of valuable chemicals. One of the major breakthroughs is to convert it into 1,3-propanediol (1,3-PDO) through microbial conversions such as by *Klebsiella pneumoniae*, *Clostridium pasteurianum*, *Citrobacter freundii*, *Escherichia coli* and *Clostridium butyricum*, which have achieved high yields [6-9].

Today, the biological production of 1,3-PDO is not a theoretical topic but has moved into the commercialization stage. Dupont, one of the largest global chemical producers has successfully manufactured fermentative 1,3-PDO in the plant based in Illinois, Chicago in the United States [10]. The fermentation process has saved Dupont up to 40% of the energy consumption due to milder operating conditions required for fermentation as compared to the conventional fossil fuel-based production route [11]. Besides that, the biological production route offers a more sustainable option for 1,3-PDO production as it does not involve hazardous catalysts which tend to be the root cause of industrial pollution [12, 13]. However, the downstream process for fermentative 1,3-PDO remains a bottleneck as it contributes to 50-70% of the overall production cost [14]. This is mainly due to the complexity of the bioreactor effluent and its low titer of 1,3-PDO. The fermented broth is highly aqueous and its composition varies with the microbial strains inoculated. Typically, water contributes to the major portion of the broth, followed by residual glycerol, organic acids, alcohols, macromolecules, and inorganic salts [15]. The aqueous nature of the broth and the existence of these impurities increase the difficulty for the purification of 1,3-PDO mainly due to the hydrophilic interaction between these compounds.

Additionally, the low volatility of 1,3-PDO (boiling point of 213 °C) would result in high energy consumption through thermal separation such as distillation [16-18]. The high energy requirement makes the one-step thermal separation of 1,3-PDO through thermal separation neither economically nor environmentally friendly [19]. Hence, several steps would need to be introduced before thermal separation to refine the composition of the broth. These steps are namely the solids removal, desalination, and the recovery step. Figure 1 illustrates the main causes for the difficulty faced in the purification of fermented 1,3-PDO and the steps required to achieve a purified product. Herein, debottlenecking the downstream process is essential to make fermentative 1,3-PDO production more economically viable.

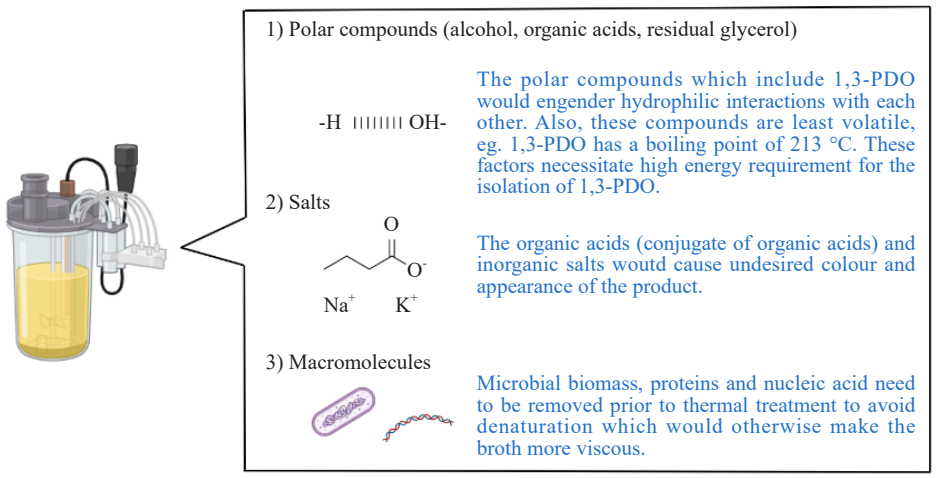

Figure 1. The overview of the fermented broth components and their associated issues impacting the downstream processes

The available technologies in each step (solids removal, flocculation, desalination, and recovery step) will be briefly introduced in the following context. The method of resin adsorption and ion-exchange chromatography applied in the refinery of fermentative 1,3-PDO will be thoroughly reviewed as the focus of this review article in terms of its technological feasibility, sustainability, and future outlook. Readers interested solely in the studies related to resin adsorption and ion-exchange chromatography are suggested to refer to section 3.3 onwards.

2. Pre-ion exchange treatment

2.1 *Solids removal*

Macromolecules including biomass and proteins are imperative to be removed from the broth prior to subsequent downstream processing. These macromolecules would otherwise be denatured due to high shear stress and temperature in the downstream process, thus contributing to a more viscous broth. Eventually, this would increase the difficulty of subsequent purification steps [15]. Centrifugation and filtration are the available methods for macromolecule removal from the broth. For instance, Anand and peers successfully removed 98.8% of the biomass from 1 L of the fermented broth through centrifugation rotated at 10,000 g for 20 minutes [20]. On the other hand, the study also examined the use of microfiltration for biomass removal and achieved a similar biomass removal rate of 98.7%.

Both methods suffered from their respective limitations. The centrifugation suffered from its low operating efficiency compared to filtration methods which allowed higher processing capacity. To filter 10 L of the broth, centrifugation would consume 3 hours which otherwise could be achieved by microfiltration within 30 minutes [20]. In addition to that, no studies thus far have shown the ability of centrifugation to remove protein whereas filtration methods have shown remarkable ability in protein removal (shown in Table 1). Due to this, centrifugation would need to be coupled with an additional flocculation step subsequently for protein removal. On the other hand, 1,3-PDO loss was the main issue facing the filtration method. When a membrane with a smaller molecular weight cut-off (MWCO) such as a hollow fiber polyacrylonitrile ultrafiltration membrane was applied, 25% of the 1,3-PDO was lost despite 99% of the biomass and protein being screened [16]. Furthermore, the filtration method generally requires higher energy input for pressure generation [21] and the membranes are prone to degradation due to the constant cleaning required [22].

Table 1. The performance of different macromolecules removal methods

Method	Biomass removal (%)	Protein removal (%)	1,3-PDO loss (%)	Reference
Centrifugation	98.8%	-	0%	[20]
Microfiltration	98.7%-100%	-	0%	[20]
Ultrafiltration (1 kDa) (with no preliminary filtration)	99%	99%	25%	[16]
Ultrafiltration (5 kDa)	100%	72%	0%	[23]
Ultrafiltration (6 kDa) (with no preliminary filtration)	99%	99%	0.8%	[16]
Nanofiltration	100%	90%	0%	[23]

2.2 *Flocculation step*

The residual protein and colour impurities upon centrifugation and filtration would need to be further removed by the flocculation step. Flocculation stands as one of the most popular methods for solution clarification due to the low material cost, energy input required, and its ability to be applied for high throughput operation. Several studies have demonstrated the effectiveness of applying activated carbon powder as the flocculating agent in treating fermented 1,3-PDO broth. For instance, Annand and the team attempted to add 5, 30, and 80 g/L of activated charcoal powder into the fermented broth and found that 25%, 96%, and 98% of the proteins were removed from the supernatant [20]. However, increasing 1,3-PDO loss was also observed as shown in Table 2.

Other researchers have enhanced the flocculation treatment by integrating chitosan as one of the flocculating agents. In a study, the pH value of the fermented broth was first adjusted to 5 with HCl followed by the addition of chitosan, calcinated kieselguhr, and 20 g/L activated charcoal powder into the fermented broth - the result showed 100% protein removal [24]. However, 7% of 1,3-PDO was lost. Similarly, another study that also applied 20 g/L of activated

charcoal powder together with chitosan reported 88.4% protein removal with only 1.09% of 1,3-PDO loss [25]. Notably, 98.8% of the residual biomass and 91.9% of the colour impurities were simultaneously removed, turning the originally yellowish broth transparent. The compiled information related to the charcoal concentration applied to protein removal has been summarised in Table 2. Based on the table, it was recommended that the optimal concentration of the activated charcoal would be between 20-30 g/L to avoid excessive loss of 1,3-PDO.

Table 2. The effect of the activated charcoal concentrations on both protein removal and 1,3-PDO loss

Activated charcoal concentration (g/L)	Protein removal (%)	1,3-PDO loss (%)	Reference
5	25.0	2.6	[20]
20	88.4	1.1-7.0	[24, 25]
30	96.0	15.2	[20]
80	98.0	59.1	[20]

2.3 *Desalination*

The salts resulting in the broth tend to precipitate and lead to the undesired colour and odor of the final product [26]. In this case, the term 'salts' refers to both inorganic and organic salts. Inorganic salts are often added during fermentation for pH level adjustment, such as potassium [26] and sodium salts [27], and thus they would need to be removed upon fermentation. On the other hand, organic salts are formed from the conjugate acids of fermented products such as organic acids. For instance, acetate and butyrate salts would be produced as the by-products of *C. butyricum* during the fermentation process [28]. Hence, the incorporation of desalination as the third step is vital to prevent these salts from precipitating.

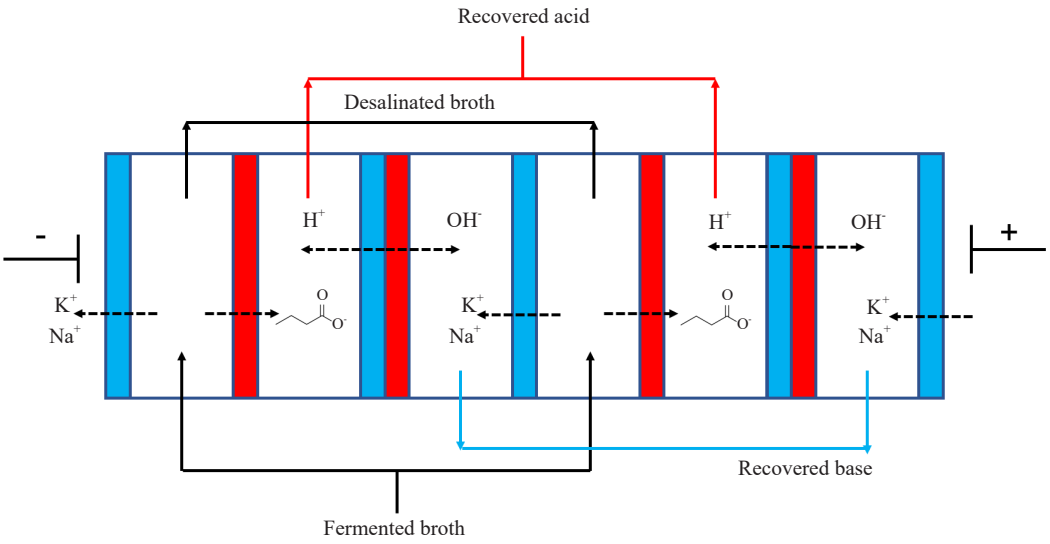

Figure 2. The conceptual working mechanism of broth desalination through electrodialysis. When electricity is driven across the membranes, the salts would be removed from the broth as the anion-exchange membrane (blue) and cation-exchange membrane (red) would allow the penetration of anions and cations, respectively. Acids and bases could be recovered at the designated compartments. The illustration is modified from [27]

Desalination could be achieved mainly through electrodialysis with the use of ion-exchange membranes as shown in Figure 2. Studies showed that applying an anion exchange membrane [26] and bipolar membrane [27] could help to reject 95% and 71.5% of the salts. However, similar to the filtration method, the use of a membrane would result in 1,3-PDO loss. In an electrodialysis process, 5.8% of the 1,3-PDO loss was experienced despite up to 90% of the organic acids being screened [29]. In addition to that, the high cost of ion exchange membranes and the high energy requirement to drive electricity are the main hurdles impeding the commercialization of this technique [30]. Due to this, other techniques are also being investigated to overcome the limitations of electrodialysis. Amine-based extraction could remove up to 98% of salts but the toxicity of the amine reagent remained the main concern facing the technique [11]. The more efficient ion-exchange chromatography technique with a Strong Acid Cation-Weak Base Anion (CACA) configuration could effectively remove salts and other impurities simultaneously [31]. This technique is thoroughly discussed in section 3.3.4.

3. Recovery step

Water, alcohols, and organic acids found in the fermented broth exhibit compatible chemical properties with 1,3-PDO. These compounds are hydrophilic and thus, they tend to form strong interacting bonds with 1,3-PDO which increases the difficulty of its isolation. Direct thermal separation of 1,3-PDO from these compounds would require a high amount of energy due to the existence of impurities and its low volatility. Therefore, a step that first concentrates 1,3-PDO by removing other hydrophilic compounds in the broth would be required to ease the final thermal separation step. Based on the literature survey, three main techniques are available i.e. selective solubilization, extraction, and resin adsorption and ion-exchange chromatography. The following part aims to introduce the limitations of the selective solubilization and extraction techniques to the readers before discussing the detailed studies of the ion-exchange chromatography method.

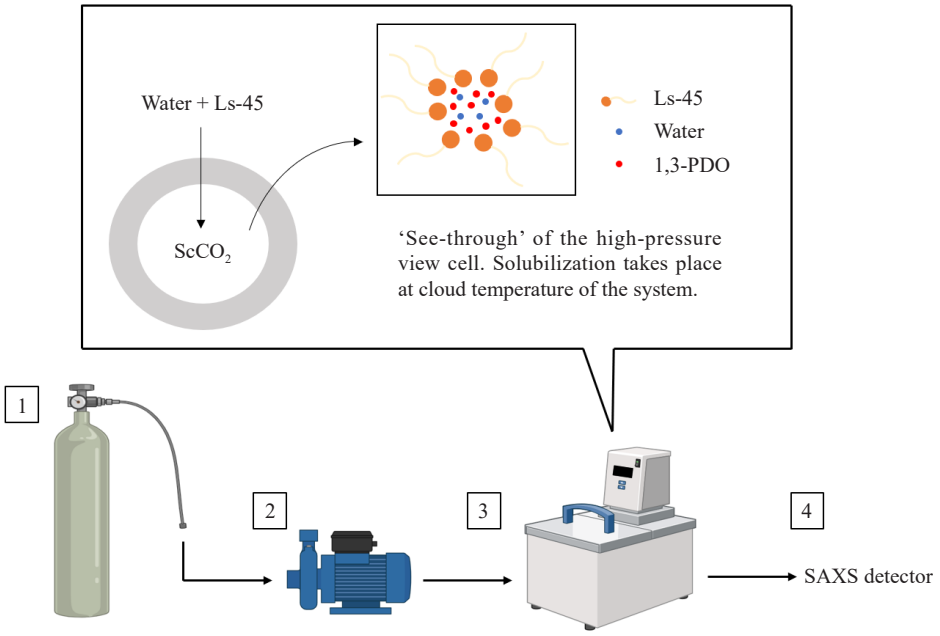

Figure 3. Simplified working procedures of $ScCO_2$ solubilization for 1,3-PDO, modified from [32, 33]. (1) CO_2 would first be transferred from the gas tank and pressurized in the series of cooling and high-pressure liquid pumps as in (2). (3) The solubilization would take place in a thermostat water bath. (4) The analyte would be analyzed with a SAXS detector

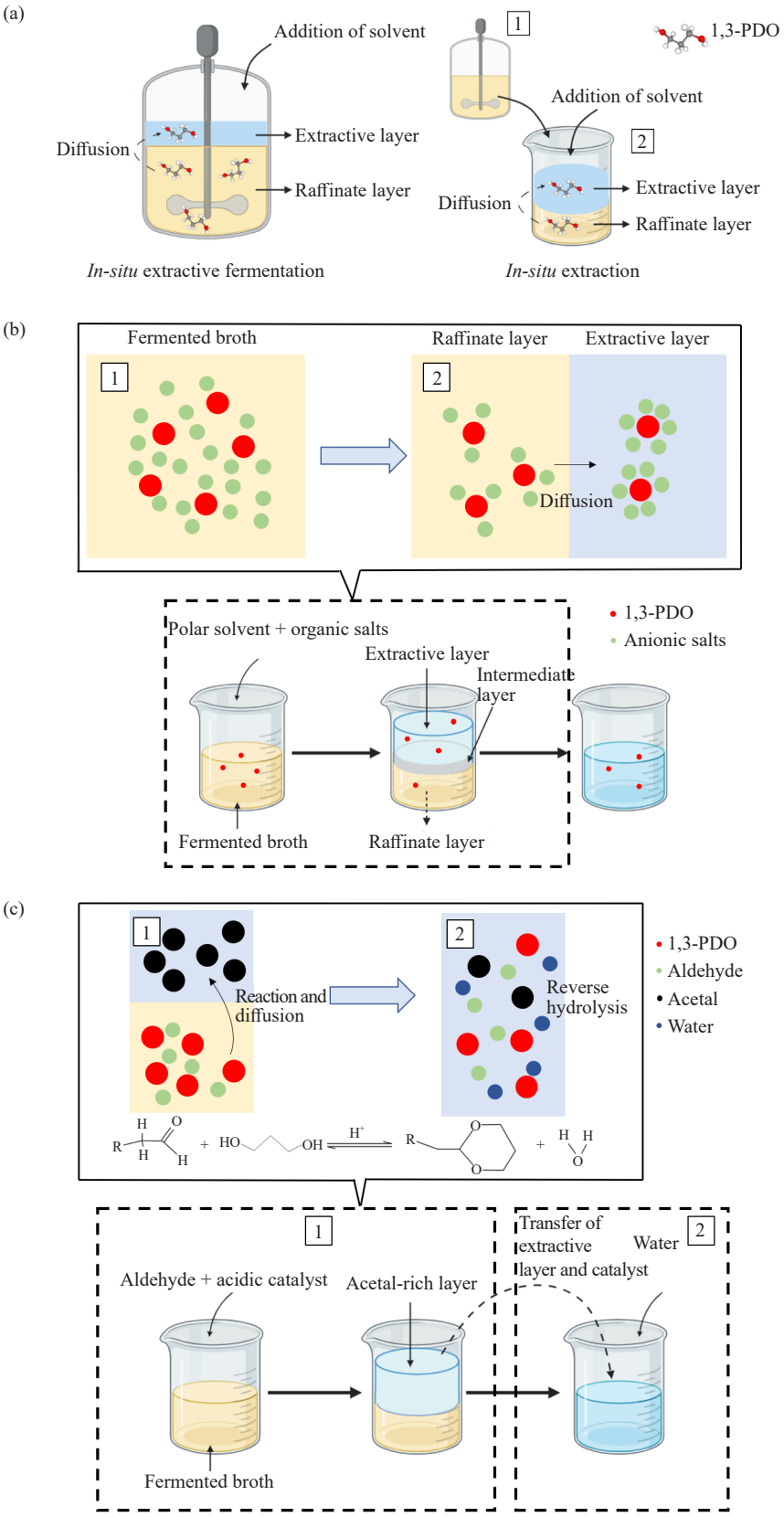

Figure 4. (a) The techniques available for liquid-liquid extraction. (b) (1) The salt agents increase the hydrophobicity of 1,3-PDO through entrapment and (2) the hydrophobic groups are salted out from the aqueous broth into the extractive layer. (c) (1) The acetals are formed upon reaction between 1,3-PDO and aldehyde and (2) acetals are hydrolyzed back to 1,3-PDO and aldehyde in the reverse reaction

3.1 *Selective solubilization technique*

Supercritical carbon dioxide ($scCO_2$) has received vast attention from researchers to replace organic solvents for the selective solubilization of hydrophilic compounds such as 1,3-PDO due to its moderate critical temperature and pressure, high abundance, low cost, toxicity, and flammability. A recent study showed that the headgroup of the surfactant, Ls-45 could emulsify 1,3-PDO forming a microemulsion system in the $scCO_2$ phase [32] as shown in Figure 3. Subsequently, the microemulsion system could be extracted and undergo distillation to obtain purified 1,3-PDO. However, the studies regarding the selective solubilization of 1,3-PDO in $scCO_2$ were limited, making this technique less promising as a recovery step for commercialization. In addition to that, the lack of dipole moment in CO_2 was believed to cause low selective solubilization performance due to poor interaction with 1,3-PDO as only weak van der Waal forces were exhibited [33].

3.2 *Extraction technique*

To our best knowledge, there are three available methods for the recovery of 1,3-PDO through extraction techniques, i.e.liquid-liquid extraction, aqueous two-phase extraction, and reactive solvent extraction. Each of these methods will be briefly elaborated to give an overview to the readers regarding their limitations in the following context.

3.2.1 *Liquid-liquid extraction*

Liquid-liquid extraction is one of the most economical and simplest methods for product recovery due to its high throughput and low energy consumption. Technically, liquid-liquid extraction of 1,3-PDO could be achieved through either *in-situ* or *ex-situ* methods as shown in Figure 4(a). The application of *in-situ* extraction is usually associated with problems such as the emulsion in broth which causes contamination risk, the formation of a precipitating layer, and undesired phase separation [34]. Therefore, it is desirable to carry out *ex-situ* extraction for 1,3-PDO to avoid these associated problems.

Applying a non-polar organic solvent could hardly extract 1,3-PDO. This is because polar 1,3-PDO does not have a high affinity towards the non-polar solvent and thus, could not achieve a high extraction rate. In a study where ethyl acetate (non-polar) was applied as the solvent, the distribution coefficient of 1,3-PDO was only 0.22 [35]. The distribution coefficient of 1,3-PDO could be increased to 0.31 by incorporating 10% ethanol (polar) as the co-solvent. In fact, the ionic liquid was also investigated by scientists in the hope of replacing organic solvents for 1,3-PDO extraction. One of the advantages of ionic liquid in the extraction of 1,3-PDO would be its higher design flexibility than the organic solvents. For instance, scientists could design ionic liquid with specified thermal stability which facilitates solvent recycling upon thermal separation. In a study that applied ionic liquid, $[P_{66614}]$ $[C_8CO_3]$ was found to extract 41% of 1,3-PDO with almost no loss of 1,3-PDO [36]. However, significant amounts of polar solutes (glycerol, acetic acid, lactic acid, and butyric acid) were also extracted causing unideal isolation of 1,3-PDO. However, the manufacture of ionic liquid is thus far not cost-effective - impeding their use for commercialization purposes. Therefore, instead of liquid-liquid extraction, several studies looked into the use of polar solvents for 1,3-PDO extraction.

3.2.2 *Aqueous two-phase extraction*

Aqueous two-phase extraction could be more efficient than liquid-liquid extraction for 1,3-PDO recovery due to the higher affinity of the polar solvent towards 1,3-PDO [37], shorter phase separation time required and resulting in less viscous broth upon extraction [38]. In addition to that, an intermediate layer would be formed as illustrated in Figure 4(b) which would help to extract the residual macromolecules and thus achieve better clarification of the broth [39-42]. Salts and sugars are often incorporated with polar solvents to destroy the interaction between water and 1,3-PDO to achieve better separation [43].

A study applied ethanol (polar) containing sodium carbonate successfully recovered 98% of the 1,3-PDO through *in-situ* extraction [19]. About 89% of the carbon dioxide produced during fermentation was absorbed by the salt-enriched (bottom) phase. In addition to that, 94% of the sodium carbonate was able to be recovered from the salt-enriched phase upon the extraction process. The ability in carbon dioxide mitigation and salt recovery made aqueous two-phase extraction a more sustainable recovery technique as compared to conventional liquid-liquid extraction.

Another study proved that methanol incorporated with phosphate salts was also able to recover 98% of 1,3-PDO with a distribution coefficient reaching 38.3 [44]. More than 90% of the macromolecules gathered at the intermediate layer and were removed subsequently.

Despite the high extraction efficiency of aqueous two-phase extraction in recovering low titer 1,3-PDO from the fermented broth, Li and team suggested that the involvement of salts in the extraction process would potentially denature the residual macromolecules and thus raise the viscosity of the broth [15]. Also, polar solvents such as ethanol and methanol may form hydrophilic bonds with polar solutes (water, alcohol, and organic acids) in the broth, resulting in higher energy requirements for their separation in the subsequent distillation. Therefore, another extraction method that converts 1,3-PDO into a non-polar intermediate followed by hydrolysis of the enrich phase without the use of polar solvent has been widely investigated. This technique is known as the reactive solvent extraction and it will be discussed in the following part.

3.2.3 *Reactive solvent extraction*

Reactive solvent extraction provides an alternative way of extraction for 1,3-PDO which aims to overcome the limitations of both liquid-liquid and aqueous two-phase extraction. The reactive solvent extraction is conducted in two steps in the presence of an acidic catalyst as shown in Figure 4(c), i.e. (1) the forward reaction which converts 1,3-PDO into intermediate acetal through the reaction with aldehyde. (2) The intermediate which is non-polar could be extracted together with the extract phase from the aqueous broth followed by reverse hydrolysis to regain 1,3-PDO.

Several studies have proven the success of reactive solvent extraction for 1,3-PDO. For instance, Boonoun and team applied acetaldehyde and ethylbenzene as the solvent at 35 °C successfully converted 92% of the 1,3-PDO into acetal with 83% of them being recovered in the extract phase [45]. Subsequently, the extract phase was hydrolyzed at 90 °C which converted more than 99% of the acetal back to 1,3-PDO in just 20 minutes. On the other hand, Hao and the team converted 1,3-PDO using butyraldehyde without solvent and recovered more than 99% of the converted acetal [46]. About 99% of the acetal was then converted back to 1,3-PDO after 8 hours of hydrolysis at 120 °C.

Reactive solvent extraction, while demonstrating commendable conversion and recovery rates of 1,3-PDO from fermentation, faces significant barriers to larger-scale implementation. The toxic nature of aldehydes and solvents such as toluene [47] and o-xylene [48] poses hazards to both organisms and the environment, necessitating specialized personnel for safe handling and increasing operational complexity. Apart from that, corrosion from acidic catalysts such as sulfuric acid [49] not only escalates equipment maintenance costs but also raises environmental concerns due to potential sulfate leaching [50]. These challenges, coupled with the need for stringent safety measures and compliance, underscore the economic and ecological limitations of the process.

3.3 *Resin adsorption and ion-exchange chromatography*

Resin adsorption and ion exchange chromatography techniques have emerged as highly promising methods for the separation of 1,3-PDO as opposed to other separation techniques. They have demonstrated exceptional product yield and purity, exceeding 98% [51, 52]. One of the significant advantages of resin adsorption processes is their low operating costs. These processes involve low energy consumption and utilize less toxic chemicals. Furthermore, with adequate maintenance, the resin beads can remain functional for many operating cycles before necessitating replacement. Resin adsorption and ion exchange chromatography mainly differ in the mechanism for solute separations. The former relies mainly on physical adsorption while the latter utilizes the difference in net charges exhibited at the surface of the resin beads for the separation of solutes (The detailed mechanism will be explained in the following context). The background of both techniques would first be introduced together with the respective literature reviews followed by critical discussions.

3.3.1 *1,3-PDO adsorption on silica gel resin*

Resin adsorption takes place within a column where it is packed with solid resin beads (stationary phase) having a specific functional group as shown in Figure 5. The broth containing the analyte, 1,3-PDO, would be loaded from the top of the column where the liquid would flow downwards along the gravity through the stationary phase. 1,3-PDO and

other compounds in the broth would be adsorbed onto the stationary phase. Upon adsorption, an eluent (mobile phase) would be loaded to displace the adsorbates out of the column. Since 1,3-PDO and other compounds exhibit different physiochemical properties, they would show different extents of affinity towards the stationary phase when they are being eluted [53]. Due to this, these compounds would be eluted at different rates thus, creating isolation of 1,3-PDO from other compounds.

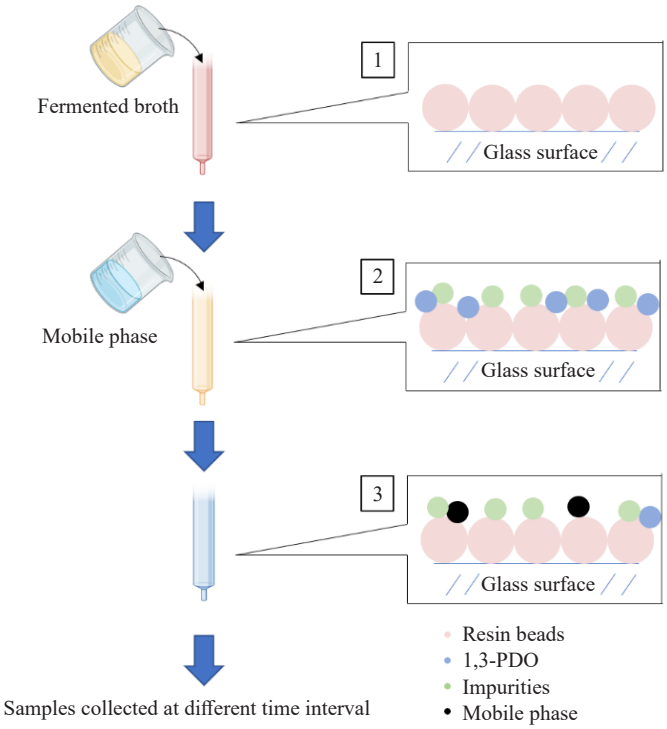

Figure 5. The working mechanisms of resin adsorption for 1,3-PDO purification. (1) The fermented broth is loaded into the column packed with resin beads. (2) The solutes in the fermented broth are adsorbed to the resin beads. (3) The mobile phase is applied to elute most of the impurities. Upon elution, the resin beads need to be washed to elute the adsorbed mobile phase molecules

In one of the studies conducted by Anand and team [20], where different resin types were investigated to separate 1,3-PDO from a complex fermentation broth containing 1,3-PDO, glycerol, ethanol, and organic acids. They found that DEAE cellulose resin was sensitive to pH changes, leading to a 48% decrease in adsorption performance. Other tested resins showed negligible adsorption performance. In contrast, silica gel resin demonstrated the best adsorption performance, yielding 89% 1,3-PDO recovery with 98% purity through gradient chromatography eluted with the mixture of chloroform and methanol. The choice of stationary phase for resin adsorption is limited, as silica gels seem to emerge as the only suitable candidate out of other types of resins. Silica gels outperformed the commercial ion-exchange resins such as DEAE cellulose resin, Clions, Dowex Monosphere resin, Amberlite XAD-7 resin [20] and ostadecylsilylated, C1-C18 (ODS) [51] in 1,3-PDO isolation. In addition to that, silica gel is more tolerant towards fluctuation in pH value as seen in its steady performance in solutions with a wide range of pH values. Silica gel was effective in isolating 1,3-PDO from 1,2-PDO, and possibly from other diols that are highly compatible. Moreover, silica gel is less expensive as compared to other commercial resins and it is highly reusable. About 70% recovery of 1,3-PDO was still able to achieve after 80 runs of chromatography on silica gel [51]. The high reusability of silica gel showed its feasibility of being upscaled to an industrial scale. Table 3 provides a summary of the relevant studies on the adsorption of 1,3-PDO using silica gel beads under various operating conditions.

Table 3. Performance and analysis parameters of silica gel resins for 1,3-PDO adsorption

Silica gel particle size (mesh)	Mobile phase	Feed loading (mL/min)	Eluent flow rate (mL/min)	Packing volume (mL)	Loading (mL)	Duration (min)	Temperature (K)	Yield (%)	Purity (%)	Reference
N.D.	Methanol/Chloroform 10:90	10	10	3,977.6	20	180	333	96	N.D.	[20]
					80			89	98	
N.D.	Ethyl acetate/Methanol 98:2 (during elution) 80:2 (during cleaning)	10	10	565	40	150	N.D.	98	82	[51]
635-403	Ethyl acetate/Methanol 98:2	N.D.	10	565	40	70	N.D.	96	94	[56]
						75		92	95	
						80		82	98	
						85		64	100	
					60	65	N.D.	81	91	
						70		78	94	
						75		66	98	
						80		42	100	
					20	60	N.D.	68	93	
						65		60	98	
						70		50	100	
	Methyl ethyl ketone/Methanol 98:2	N.D.	10	565	40	45	N.D.	79	94	
						50		76	95	
						55		48	96	
						60		26	98	
						65		13	100	
					60	45	N.D.	91	91	
						50		81	92	
						55		51	98	
						60		26	100	
					80	45	N.D.	80	91	
						50		78	92	
						55		50	98	
						60		29	100	

3.3.2 *1,3-PDO separation through ion-exchange chromatography*

On the other hand, ion-exchange chromatography relies on resin beads that are capable of exchanging ions with solution without undergoing physical change themselves. The resin beads used in ion exchange processes are typically spherical and have a diameter ranging from 0.5 to 1.0 mm. Ion-exchange resin beads are mainly made of crosslinked polymers and their properties are primarily distinguished by the functional groups. Figure 6 illustrates the contrast between a strongly acidic cation exchange resin and a strongly basic anion exchange resin in terms of the functional groups contained.

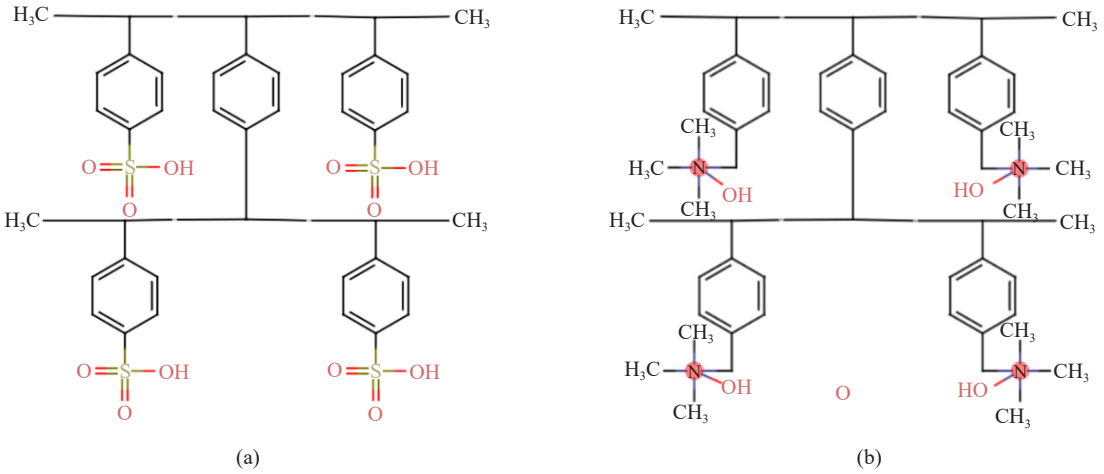

Figure 6. Structure of the ion-exchange resins. (a) Cationic exchange resin and (b) anionic exchange resin

The procedure for ion exchange chromatography is to some extent, similar to that of resin adsorption. The solutes would first be adsorbed through the exchange of ions. To recover the adsorbed solutes, an eluent is introduced to displace the adsorbates from the resin beads. The solutes would be eluted at different rates and thus could be separated. After elution, the surface of the resin beads would need to be recharged with ionic forms. This is mainly achieved by charging the reins with a solution containing insoluble acids and bases [54].

The summary of the studies conducted for 1,3-PDO separation using cationic ion-exchange resin beads attached with different ion forms and under various operating conditions has been tabulated in Table 4. One of the studies was performed by Mitrea and team where cationic Amberlite resin charged with H^+ ions within a glass column to capture 1,3-PDO was employed [24]. Upon eluting with ethanol solution as the mobile phase, 91% of 1,3-PDO was recovered. However, challenges arose where a significant amount of 1,3-PDO was released along organic acids, glycerol, and 2,3-butanediol (2,3-BDO) after loading 40% of the total eluent. Hence, this would suggest the need for another separation-purification procedure to further recover 1,3-PDO. In another research, Rukowicz and team harnessed cross-linked polystyrene sulfonate resins, each charged with various ions to separate 1,3-PDO from fermentation broth or a binary solution mixture containing glycerol and 2,3-BDO [55]. Their findings revealed that the choice of resin type played a pivotal role in achieving successful separation. When the Na^+, Ag^+, and Zn^{2+} resin forms were employed, they were incapable of achieving any separation of 1,3-PDO. The finding also showed that the H^+ resin form yielded the best result for separating 1,3-PDO from glycerol in the binary solution. However, the H^+ resin form was ineffective in separating 1,3-PDO from 2,3-BDO.

3.3.3 *Optimization of performance*

Several attempts have been made to identify the optimum conditions for the operation of the resin purification step. Those attempts are mainly focussed on finding the optimum resin particle sizes, ionic forms, flow rate, sample loading, resin stack height, resin particle size, temperature, suitable mobile phase, and the eluent flow rate.

Table 4. Performance and analysis parameters of cationic ion-exchange resins for 1,3-PDO separation

Stationary phase	Ionic form	Particle size (mesh)	Mobile phase	Feed flowrate (mL/min)	Eluent flowrate (mL/min)	Packing volume (mL)	Loading (mL)	Duration (min)	Temperature (K)	Recovery (%)	Purity (%)	Reference
Polystyrene sulfonate resin		45	Water	N.D.	2.6	100	10	N.D.	298	96.7	88.10	[52]
	Ca^{+}	30	75% ethanol	7.3	32.5	300	65.7	N.D.		99.5	89.4	
		60-70		N.D.	2.6	100	10	N.D.		98.4	92.4	
	Na^{+}	60-70		N.D.	2.6	100	10	9		95.7	87	
	Na^{+}	100-400		350	N.D.	10,500	1,050	N.D.	293	95	N.D.	[57]
	H^{+}	N.D.	Water	N.D.	7	212	10	30	296	91	N.D.	[24]
	H^{+}	N.D.		1	1		N.D.	N.D.	298	80.8	99.2	[25]
	Pb^{2+}	N.D.		N.D.	3	350	2.3	N.D.	338	84.5	N.D.	
	La^{3+}	N.D.		N.D.		350	2.3	N.D.	338	89.8	N.D.	[31]

3.3.3.1 *Influence of resin bead size on the performance of ion-exchange chromatography*

The resin bead size is one of the influencing factors for 1,3-PDO separation through adsorption. Figure 7 compares the performance affected by the resin bead sizes. For the selection of resin particle size, the use of a smaller particle size resin packing is recommended to achieve higher purity and recovery of 1,3-PDO. Rukowicz and Alejski examined the use of strongly acidic cationic polystyrene-divinylbenzene ion-exchange resin containing both Na^+ and H^+ forms with two ranges of mesh size, i.e. 200-335 and 100-200 mesh for 1,3-PDO isolation [57]. It was found that for H^+ form of the strongly acidic cation resin with particle sizes of 200-325 mesh and 100-200 mesh was able to separate 1,3-PDO from glycerol when eluting with water at 2 mL/min flow rate. However, a much better separation performance was achieved by the smaller resin particle size of 200-325 mesh. As for the Na^+ form of the strongly acidic cation resin, effective desalination of the fermentation broth (up to 90%) was achieved. Despite this, a poor separation of 1,3-PDO from glycerol was noticed with a recovery of only 55%. Moreover, Hilaly and Binder shed light on the critical role of resin particle size in the quest for purer and more efficient 1,3-PDO extraction [52]. The study revealed that employing a smaller particle size resin packing led to notable improvements in both the purity and recovery of 1,3-PDO from the product fraction. The use of 45 mesh resins in Ca^+ form showed lower recovery and purity of 1,3-PDO (96.7% and 88.1% respectively) as compared to 60-70 mesh resins (98.4% and 92.4%).

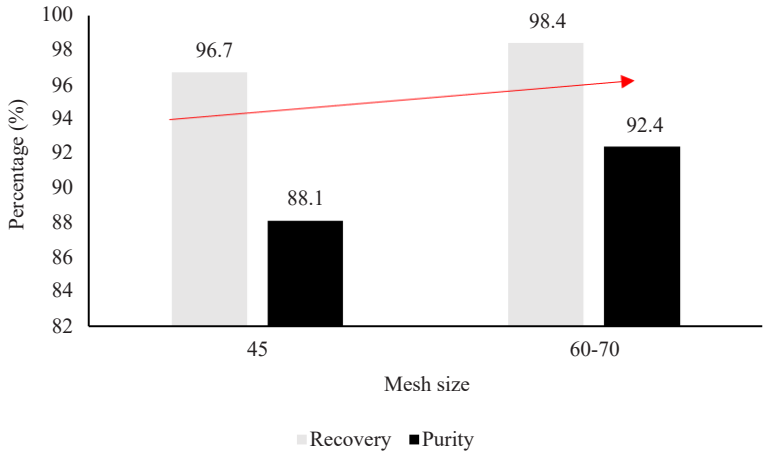

Figure 7. The effect of particle size of polystyrene-divinylbenzene ion-exchange resin on 1,3-PDO yield and purity

The significance of the resin bead sizes is also investigated through adsorption kinetic studies. For instance, a study hypothesized that the small bead size of resins not only provided a larger surface area for adsorption but also allowed more rid for 1,3-PDO to diffuse internally [55]. The freely diffuse 1,3-PDO would able to be adsorbed to the available sites thus, achieving resin saturation more quickly. To validate such a hypothesis, another study applied the Shell Progressive Approach (SPA) to investigate the rate-limiting step of 1,3-PDO adsorption [58]. The study found that the particle diffusion (internal diffusion) step was indeed the rate-limiting step as the experimental data fitted the limiting model better than other models (external diffusion and adsorption steps). Thus, smaller resin bead sizes would reduce the limiting effects for 1,3-PDO in internal diffusion and thus is preferable for higher adsorption.

3.3.3.2 *Influence of ionic forms on the performance of ion-exchange chromatography*

As illustrated in Figure 8, resins charged with Ca^{2+} forms exhibited the highest recovery for 1,3-PDO, followed by Na^+ and H^+ forms. Both the Pb^{2+} and La^{3+} forms showed appreciable recovery for 1,3-PDO but their abilities in isolating 1,3-PDO from other secondary metabolites such as glycerol and 1,3-BDO was rather poor, as indicated by the relatively low purity of the extracted 1,3-PDO which was 47% and 31.9% for the Pb^{2+} and La^{3+} forms, respectively as summarised in Table 4. The retention of 1,3-PDO is believed to be driven by affinity differences in the ionic forms. These variances

result from the various ion forms attached to the resin's functional group, particularly the SO_3^- group in the case of the strong cationic ion-exchange resins. It is worth noting that the various ions attached to the SO_3^- group have diverse impacts on the solute's elution profile. Dorfner determined that the order of affinity for certain common ions in the SO_3^- functional group could be understood as follows [59]:

$$Hg^{2+} < Li^{+} < H^{+} < Na^{+} < K^{+} = NH_4^{+} < Cd^{2+} < Cs^{+} < Ag^{+} < Mn^{2+} < Mg^{2+} < Zn^{2+} < Cu^{2+} < Ni^{2+}$$

$$< Co^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Al^{3+} < Fe^{3+}$$

From there, it is clear that the calcium ion has a stronger attraction or affinity towards the functional group when compared to the hydrogen or sodium ions. This heightened affinity can be attributed to the divalent nature and larger size of the Ca^{2+} ion in contrast to monovalent ions such as H^+ and Na^+. The increased size and higher charge of Ca^{2+} would lead to more potent electrostatic interactions with the negatively charged SO_3^- group on the resin. In essence, in the presence of hydrogen or sodium ions attached to the functional group, the components of the broth would take more time to appear in the eluting solvent. This delay might be due to the result of these broth components readily displacing the attached ion from the SO_3^- functional group of the resin. Subsequently, the components are strongly absorbed for an extended duration due to their heightened and robust electrostatic interactions with the functional group.

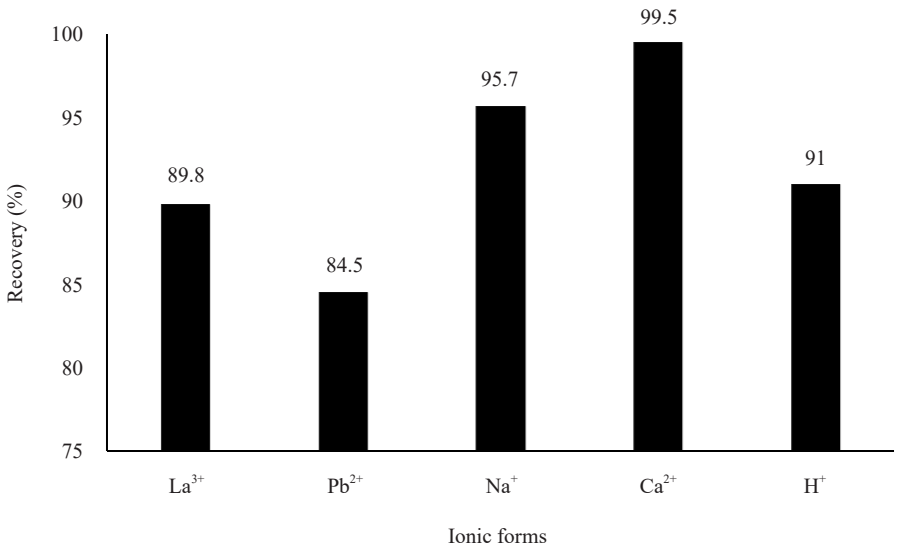

Figure 8. The effect of ionic forms charged to polystyrene-divinylbenzene ion-exchange resins on 1,3-PDO recovery

3.3.3.3 *Resin beads stack height in the column*

The impact of stack height on a strong cation exchange H^+ form resin was investigated in a study [25]. The resin was packed to the heights of 10 cm, 15 cm, and 30 cm. The findings revealed that increasing the stack height from 10 cm to 30 cm resulted in the delay of 1,3-PDO elution. The findings from Rukowicz and the team further supported this observation [55]. Their study demonstrated that increasing the packing height of various ion forms of a strong cation resin from 25 cm to 50 cm resulted in improved separation of mixtures. This suggested that a longer packing height may enhance the performance in achieving better separation outcomes.

The primary factor behind this observation was that as the stack height increased, longer period of contact between the resin beads and solutes was allowed, leading to improved adsorption. By adding more resin beads to the adsorption column (increasing stack height), the adsorption capacity for 1,3-PDO was enhanced consequently prolonging the time

it took to reach resin saturation [25]. On the contrary, shorter resin heights were found to have inadequate adsorption performance due to the reduced duration of contact between the resin and the solution. This limited contact time caused competing adsorption between 1,3-PDO and glycerol which shared highly compatible chemical properties, resulting in decreased adsorption efficiency.

3.3.3.4 *Broth loading flow rate through the column*

The effect of the flow rate of the broth sample through the silica gel resin beads was investigated by varying between 5, 10, and 15 mL/min at a fixed sample loading of 40 mL [20, 51]. The result showed that 1,3-PDO recovery was improved when the sample flow rate was adjusted from 5 to 10 mL/min. Oppositely, the 1,3-PDO recovery dropped when the flow rate was increased further to 15 mL/min. Figure 9 shows the effect of the flowrate on the 1,3-PDO recovery in both studies.

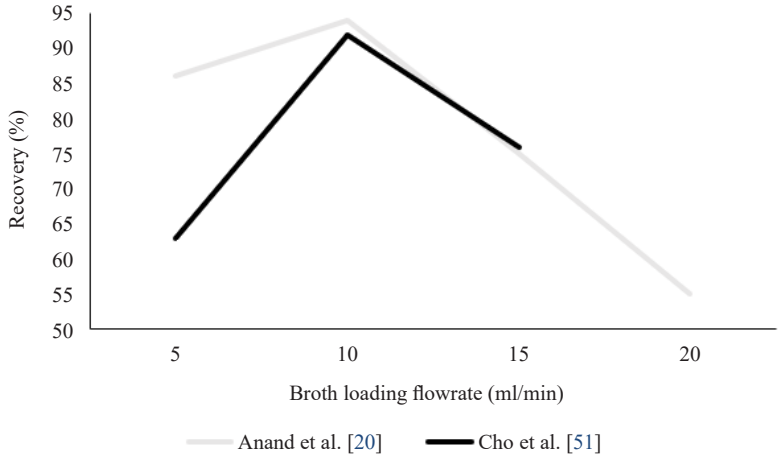

Figure 9. The effect of broth loading into the adsorption column on the recovery of 1,3-PDO

The explanation for this phenomenon lies in the impact of flow rate on the interaction time between 1,3-PDO and the resin. As the flow rate increases, the interaction time between the two decreases. Consequently, the saturated point of 1,3-PDO is reached more rapidly while the mass transfer zone expands [25]. This indicates a lower adsorption efficiency. However, it is important to note that a lower flow rate is not necessarily advantageous. It may lead to a delay in reaching the saturated point of 1,3-PDO and result in a lower adsorption efficiency which could be unfavorable for industrial-scale production. Therefore, finding the optimal flow rate is crucial to balance adsorption efficiency and production requirements which based on a literature survey, 10 mL/min was regarded as the optimum flow rate.

3.3.3.5 *Loading amount of broth*

Several studies showed that the loading amount of the broth sample could affect 1,3-PDO yield. To determine the optimal feed amount for the packed column, a series of experiments were conducted. The loading amount was varied at a fixed flow rate of 10 mL/min, using volumes of 20 mL, 40 mL, and 60 mL [57]. From the obtained results, it was found that the highest yield of 1,3-PDO, approximately 95%, was achieved when loading 40 mL of feed. This indicated that loading 40 mL of feed was the most effective in terms of maximizing the yield of 1,3-PDO during the purification process. In a similar vein, experiments with variations in loading amount at a fixed flow rate of 10 mL/min were conducted [20]. However, their study encompassed a wider range of volumes, specifically 20 mL, 40 mL, 60 mL, 80 mL, and 100 mL. The outcomes revealed that the highest yield of 1,3-PDO was attained when loading 20 mL of the sample (recovery of 96%). Notably, when the loading amount was increased to 80 mL, there was only a negligible loss of 1,3-propanediol observed (recovery of 89%). Taking into account the economic aspects, 80 mL was identified

as the optimal loading amount, as it provided a high yield while minimizing any significant loss. In a novel approach, Rukowicz and Alejski employed computer software to simulate the impact of loading amount on a 111 cm long column packed with sodium-form cation resin [57]. By fixing the flow rate at 2 h^{-1}, they investigated the loading amount of the sample as a percentage of the column volume required for optimal 1,3-PDO yield. Remarkably, the results demonstrated that a loading amount of 10% of the total column volume resulted in the highest yield of 1,3-PDO, achieving a yield of 100%. Notably, it can be observed that selecting an optimal loading amount of approximately 10% of the total bed volume yields favourable results.

3.3.3.6 *Mobile phase*

Several solvents have emerged as possible mobile phases for elution of the adsorption process. For instance, combined mobile phase (consisting of polar and non-polar solvents) such as methanol/ chloroform (10:90 v/v) [20], methanol/ethyl acetate (2:98 v/v) [51] and methanol/methyl ketone acetone (2:98 v/v) [56] recorded recovery and purity of 96% and 98%, 82% and 98%, 91% and 91% respectively - these performances were comparable with sole water with recovery and purity of 98.4% and 92.4%. However, the combined solvent is usually preferred over sole water as it is believed that having a combined solvent could enhance the ionic interaction between the compounds in the broth, mobile, and stationary phases. The non-polar component in the combined mobile phase could facilitate the elution of highly polar impurities such as glycerol, leaving only 1,3-PDO being adsorbed to the stationary phase and thus, achieving isolation. Apart from that, the sole water mobile phase would theoretically increase the total cost required for downstream processing as more energy would be needed to overcome the high miscibility between 1,3-propanediol and water [52].

3.3.3.7 *Mobile phase elution flowrate*

To date, the study conducted by Wang and the team stands as the only explicit investigation into the impact of the elution flow rate of the mobile phase on 1,3-PDO yield [25]. In this study, strong cation exchange H^+ form resins were eluted with 75% ethanol elution. The elution flow rate was varied from 1 to 2 mL/min to investigate its impact on 1,3-PDO and glycerol separation. The findings showed that increasing the flow rate led to a decrease in the elution peaks of both 1,3-PDO and glycerol. However, the elution of 1,3-PDO exhibited a more significant decrease compared to glycerol, indicating an improved separation of glycerol. Moreover, the mass ratio of 1,3-PDO to glycerol decreased from 14.6 to 9.88 when the flow rate increased from 1 mL/min to 2 mL/min. This suggested that an increased elution rate may be unfavourable for the effective separation of 1,3-PDO. Additionally, when the flow rate of the 75% ethanol was further decreased to 0.5 mL/min, better separation efficiency was observed as the overlapping degree of the 1,3-PDO and glycerol peaks after HPLC analysis reduced. However, the efficiency decreased as a significantly longer time was required for the separation process.

In conclusion, the study found that the separation of 1,3-PDO could be significantly improved by slowing down the elution rate and increasing the contact time between the eluent and 1,3-PDO in the resin. However, further decreasing the flow rate would otherwise enhance separation efficiency at the cost of reduced production efficiency [25]. In addition to the aforementioned study, another study put forward a suggestion regarding the total eluting amount of the eluent [24]. It was proposed that the total amount of eluent used for elution should be equivalent to the volume of the packed portion of the column. In other words, the eluent volume should match the volume of the resin-filled section of the column. This recommendation aims to ensure optimal elution efficiency and effective separation during the chromatography process.

3.3.4 *Industrial applications of ion-exchange resin for 1,3-PDO purification*

In fact, ion-exchange chromatography has already been patented by Dupont as part of the downstream process for 1,3-PDO purification [23]. According to the patent, the feed broth would first undergo a series of filtrations, to remove proteins and other large impurities. Then, the filtered broth would be passed into a repeated series of columns packed with strong cation resin (Dowex 88) and weak anion resin (Dowex 77). The cationic resin would drop the pH value of the broth. After passing through anionic resins, water would be formed due to neutralization between H^+ and OH^- ions from the resins. The water generated is capable of eliminating the salts from the broth and regenerating ions

for the resins. Then, the broth would be collected and evaporated to reduce the water content down to 20% which simultaneously raises the 1,3-PDO composition to 85% (dry weight basis). The broth would subsequently be carried over into a mixed bed column packed with both strong cation (Dowex 88) and anion (Dowex 22) resins. After that, the eluent broth would go through four distillation columns for fractionation. Eventually, with significant salt removal, 1,3-PDO was produced reportedly possessing a purity of over 99% with reduced colour and odour. This configuration of ion-exchange chromatography is named the Strong Acid Cation, Weak Base Anion, and Strong Acid Cation (CACA).

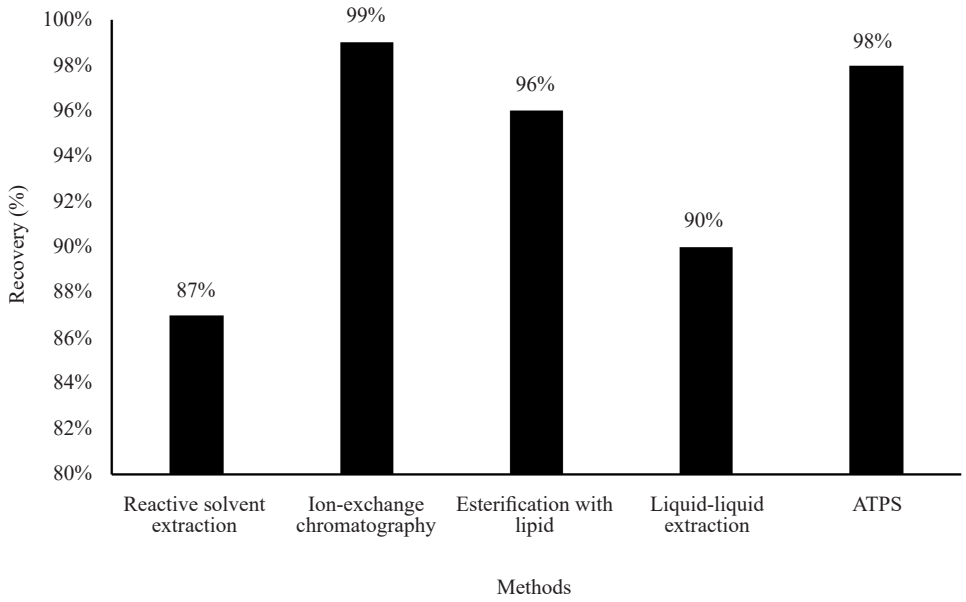

Figure 11. Comparison of 1,3-PDO recovery achieved by various available recovery techniques [20, 35, 44, 52, 60, 61]

4. Future outlook and conclusion

In a nutshell, resin adsorption and ion-exchange chromatography techniques are perceived as one of the most promising techniques in the recovery of 1,3-PDO as indicated by product recovery rate comparison among a few techniques as shown in Figure 11. Several advantages of resin adsorption and ion-exchange chromatography include its lower energy requirement and the use of less toxic reagents as compared to the extraction-based technique. This could help to gear towards the development of the greener downstream process. However, the technique itself faces scepticism in terms of its feasibility for large-scale developments. The expensive resins, long duration, and complicated procedures are the main hurdles that prevent the scaling up of the process. Therefore, intensive studies shall be conducted to overcome the aforementioned shortcomings. Several suggestions have been concluded upon compiling this review: 1) Cheaper synthetic or natural resins shall be discovered. This could potentially be achieved through studying the structures of the predominant resins such as the silica gels and commercial ion-exchange resins. 2) It is suggested to explore different resin compositions or modifications that could potentially enhance the separation efficiency and selectivity for 1,3-PDO. One promising avenue is the use of composite resins, which combine various functional groups or materials. For instance, a composite resin consisting of both cation and anion exchange functionalities may offer improved selectivity and separation efficiency for complex fermentation broth matrices. 3) Computer-Aided Design (CAD) could be utilized to discover the use of cheap material for the synthesis of resins. Through these, it is expected that with a better understanding of the adsorption process, a more efficient adsorption strategy could potentially be designed in the hope of reducing the overall duration and complexity of the process. 4) It is suggested to apply Nuclear Magnetic Resonance (NMR) spectroscopy (1 H and 13 C) to confirm the purity of 1,3-PDO eluted. This is as 1 H and 13 C NMR spectroscopy provides detailed information about the chemical structure, relative abundance of different nuclei,

and chemical shifts, thereby confirming the purity of 1,3-PDO. The absence of unexpected peaks and the agreement of peak ratios with expected values contribute to the confirmation of purity using NMR spectroscopy.

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Conflict of interest

The authors declared no conflict of interest regarding this publication.

Reference

[1] Armylisas AHN, Hoong SS, Tuan Ismail TNM. Characterization of crude glycerol and glycerol pitch from palm-based residual biomass. *Biomass Convers Biorefinery*. 2023. Available from: https://doi.org/10.1007/s13399-023-04003-4.

[2] Eleanor S, Abel R, Palm M, Board O, Loh SK, Palm M, et al. Bio-based 1,3-propanediol production from crude glycerol. *Palm Oil Engineering Bulletin*. 2018; 123.

[3] Tan HW, Abdul Aziz AR, Aroua MK. Glycerol production and its applications as a raw material: A review. *Renewable and Sustainable Energy Reviews.* 2013; 27: 118-127.

[4] Ciriminna R, Pina CD, Rossi M, Pagliaro M. Understanding the glycerol market. *European Journal of Lipid Science and Technology*. 2014; 116: 1432-1439.

[5] Buckner CA, Lafrenie RM, Dénommée JA, Caswell JM, Want DA, Gan GG, et al. Market prospecting and assessment of the economic potential of glycerol from biodiesel. *Advanced Biometric Technologies*. 2016; 11: 13. Available from: https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

[6] Deckwer WD. Microbial conversion of glycerol to 1,3-propanediol. *FEMS Microbiology Reviews*. 1995; 16: 143-149. Available from: https://doi.org/10.1016/0168-6445(94)00050-9.

[7] Papanikolaou S, Fakas S, Fick M, Chevalot I, Galiotou-Panayotou M, Komaitis M, et al. Biotechnological valorization of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil. *Biomass and Bioenergy*. 2008; 32: 60-71. Available from: https://doi.org/10.1016/j.biombioe.2007.06.007.

[8] Wang K, Hawley MC, DeAthos SJ. Conversion of glycerol to 1,3-propanediol via selective dehydroxylation. *Industrial & Engineering Chemistry Research*. 2003; 42: 2913-2923.

[9] Zeng AP, Biebl H. Bulk chemicals from biotechnology: the case of 1,3-propanediol production and the new trends. *Advances in Biochemical Engineering/Biotechnology*. 2002; 74: 239-259.

[10] Kanjilal B. *Fermentative Production of 1,3-Propanediol from Industrial Glycerol and Its Pervaporative Enrichment from Aqueous Mixtures*. 2015.

[11] Hong YK. Purification of 1,3-Propanediol for production of polytrimethylene terephthalate (PTT) from biomass. *Advanced Materials Research*. 2011; 320: 191-195.

[12] Da Silva GP, De Lima CJB, Contiero J. Production and productivity of 1,3-propanediol from glycerol by *Klebsiella pneumoniae* GLC29. *Catalysis Today*. 2015; 257: 259-266. Available from: http://dx.doi.org/10.1016/j.cattod.2014.05.016.

[13] Saxena RK, Anand P, Saran S, Isar J. Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. *Biotechnology Advances.* 2009; 27: 895-913. Available from: http://dx.doi.org/10.1016/j.biotechadv.2009.07.003.

[14] Xiu ZL, Zeng AP. Present state and perspective of downstream processing of biologically produced 1,3-propanediol and 2,3-butanediol. *Applied Microbiology and Biotechnology*. 2008; 78: 917-926.
[15] Li Z, Jiang B, Zhang D, Xiu Z. Aqueous two-phase extraction of 1,3-propanediol from glycerol-based fermentation broths. *Separation and Purification Technology*. 2009; 66: 472-478.
[16] Zhang C, Sharma S, Wang W, Zeng AP. 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*. 2021; 21: 351-563.
[17] Wu Y, Chen S, Zhu J, Chen K, Wu B, Ji L. The thermodynamics and separation process design for the ternary system 1,3-propanediol + 1,3-butanediol + 2,3-butanediol. *Separation Science and Technology*. 2020; 55: 61-67. Available from: https://doi.org/10.1080/01496395.2018.1562471.
[18] Wang Z, Wu Z, Tan T. Studies on purification of 1,3-propanediol by molecular distillation. *Biotechnology and Bioprocess Engineering*. 2013; 18: 697-702.
[19] Li ZG, Sun YQ, Zheng WL, Teng H, Xiu ZL. 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*. 2013; 80: 68-75. Available from: http://dx.doi.org/10.1016/j.bej.2013.09.014.
[20] Anand P, Saxena RK, Marwah RG. A novel downstream process for 1,3-propanediol from glycerol-based fermentation. *Applied Microbiology and Biotechnology*. 2011; 90: 1267-1276.
[21] Tomczak W, Gryta M. Comparison of polypropylene and ceramic microfiltration membranes applied for separation of 1,3-PD fermentation broths and saccharomyces cerevisiae yeast suspensions. *Membranes (Basel)*. 2021; 11(1): 1-26.
[22] Staszak K, Woźniak MJ, Karaś ZA, Staniewski J, Prochaska K. Application of nanofiltration in the process of the separation of model fermentation broths components. *Polish Journal of Chemical Technology*. 2013; 15: 1-4.
[23] Adkesson D, Alsop A, Ames T, Chu LA. *Process for the purification of 1,3-propanediol from a fermentation medium*. 2005. p. 10-12. Available from: https://patentimages.storage.googleapis.com/28/9f/b7/775e2c8af318b3/US20050104716A1.pdf [Accessed 17 October 2023].
[24] Mitrea L, Leopold LF, Bouari C, Vodnar DC. Separation and purification of biogenic 1,3-propanediol from fermented glycerol through flocculation and strong acidic ion-exchange resin. *Biomolecules*. 2020; 10: 1-10. Available from: https://doi.org/10.3390/biom10121601.
[25] Wang S, Qiu L, Dai H, Zeng X, Fang B. Highly pure 1,3-propanediol: Separation and purification from crude glycerol-based fermentation. *Engineering in Life Sciences*. 2015; 15: 788-796.
[26] Gong Y, Dai LM, Wang XL, Yu LX. Effects of transport properties of ion-exchange membranes on desalination of 1,3-propanediol fermentation broth by electrodialysis. *Desalination*. 2006; 191: 193-199. Available from: https://doi.org/10.1016/j.desal.2005.07.023.
[27] Wu RC, Xu YZ, Song YQ, Luo JA, Liu D. A novel strategy for salts recovery from 1,3-propanediol fermentation broth by bipolar membrane electrodialysis. *Separation and Purification Technology*. 2011; 83: 9-14. Available from: http://dx.doi.org/10.1016/j.seppur.2011.06.028.
[28] Biebl H. Glycerol fermentation of 1,3-propanediol by *Clostridium butyricum*. Measurement of product inhibition by use of a pH-auxostat. *Applied Microbiology and Biotechnology*. 1991; 35: 701-705.
[29] Gong Y, Tang Y, Wang XL, Yu LX, Liu DH. The possibility of the desalination of actual 1,3-propanediol fermentation broth by electrodialysis. *Desalination*. 2004; 161: 169-178.
[30] Kaeding T, DaLuz J, Kube J, Zeng AP. An integrated study of fermentation and downstream processing in a mini- plant significantly improved the microbial 1,3-propanediol production from raw glycerol. *Bioprocess and Biosystems Engineering*. 2015; 38: 575-586.
[31] Malinowski J, Broekhuis RR, Broekhuis RR, Tate PER. *Process for the purification of 1,3-propanediol from a fermentation medium*. 2002. Available from: https://patents.google.com/patent/US6428992B1/en [Accessed 30 September 2023].
[32] Yu W, Liu Y, Yin J. Separation of 1,3-propanediol using supercritical CO_2 microemulsion with non-ionic surfactant Ls-54: Experiment and simulation. *Chemical Engineering Journal*. 2015; 273: 197-204. Available from: http://dx.doi.org/10.1016/j.cej.2015.03.082.
[33] Yu W, Zhou D, Yin JZ, Gao JJ. Selective solubilization of 1,3-propanediol using a water-supercritical CO_2 microemulsion with Ls-45 as surfactant. *RSC Advances*. 2013; 3: 7585-7590.
[34] Kaur G, Srivastava AK, Chand S. Debottlenecking product inhibition in 1,3-propanediol fermentation by *In-Situ* Product Recovery. *Bioresource Technology*. 2015; 197: 451-457. Available from: http://dx.doi.org/10.1016/j.biortech.2015.08.101.

[35] Boonsongsawat T, Shotipruk A, Tantayakom V, Prasitchoke P, Chandavasu C, Boonnoun P, et al. Solvent extraction of biologically derived 1,3-propanediol with ethyl acetate and ethanol cosolvent. *Separation Science and Technology*. 2010; 45: 541-547.
[36] Liu XH, Rebroš M, Dolejš I, Marr AC. Designing ionic liquids for the extraction of alcohols from fermentation broth: phosphonium alkanesulfonates, solvents for diol extraction. *ACS Sustainable Chemistry & Engineering*. 2017; 5: 8260-8268.
[37] Dai JY, Sun YQ, Xiu ZL. Separation of bio-based chemicals from fermentation broths by salting-out extraction. *Engineering in Life Sciences*. 2014; 14: 108-117.
[38] Fu H, Sun Y, Xiu Z. Continuous countercurrent salting-out extraction of 1,3-propanediol from fermentation broth in a packed column. *Process Biochemistry*. 2013; 48: 1381-1386. Available from: http://dx.doi.org/10.1016/j.procbio.2013.06.017.
[39] Rukowicz B, Alejski K, Miesiąc I. Salting-out extraction of 1,3-propanediol from fermentation broth. *Polish Journal of Chemical Technology*. 2014; 16(2): 82-86.
[40] Wischral D, Fu H, Pellegrini Pessoa FL, Pereira N, Yang ST. Effective and simple recovery of 1,3-propanediol from a fermented medium by liquid-liquid extraction system with ethanol and K_3PO_4. *Chinese Journal of Chemical Engineering*. 2018; 26: 104-108. Available from: http://dx.doi.org/10.1016/j.cjche.2017.06.005.
[41] Li Z, Yan L, Zhou J, Wang X, Sun Y, Xiu ZL. Two-step salting-out extraction of 1,3-propanediol, butyric acid and acetic acid from fermentation broths. *Separation and Purification Technology*. 2019; 209: 246-253. Available from: https://doi.org/10.1016/j.seppur.2018.07.021.
[42] Vivek N, Pandey A, Binod P. An efficient aqueous two-phase systems using dual inorganic electrolytes to separate 1,3-propanediol from the fermented broth. *Bioresource Technology*. 2018; 254: 239-246. Available from: https://doi.org/10.1016/j.biortech.2018.01.076.
[43] Wu HS, Wang YJ. Salting-out effect on recovery of 1,3-propanediol from fermentation broth. *Industrial & Engineering Chemistry Research*. 2012; 51: 10930-10935.
[44] Li Z, Teng H, Xiu Z. Extraction of 1,3-propanediol from glycerol-based fermentation broths with methanol/phosphate aqueous two-phase system. *Process Biochemistry*. 2011; 46: 586-591. Available from: http://dx.doi.org/10.1016/j.procbio.2010.10.014.
[45] Boonoun P, Laosiripojana N, Muangnapoh C, Jongsomjit B, Panpranot J, Mekasuwandumrong O, et al. Application of sulfonated carbon-based catalyst for reactive extraction of 1,3-propanediol from model fermentation mixture. *Industrial & Engineering Chemistry Research*. 2010; 49: 12352-12357.
[46] Hao J, Xu F, Liu H, Liu D. Downstream processing of 1,3-propanediol fermentation broth. *Journal of Chemical Technology & Biotechnology*. 2006; 81: 102-108.
[47] Malinowski JJ, Daugulis AJ. The effective approach for recovery of methyl-substituted 1,3-dioxane from aqueous media. *Separation Science and Technology*. 2002; 37: 2659-2667.
[48] Fang YJ, Zhou P. Study on reactive extraction kinetics of 1,3-propanediol in dilute aqueous solutions. *Separation Science and Technology*. 2006; 41: 329-340.
[49] Matsumoto M, Nagai K, Kondo K. Reactive extraction of 1,3-propanediol with aldehydes in the presence of a hydrophobic acidic ionic liquid as a catalyst. *Solvent Extraction Research and Development Japan*. 2015; 22(2): 209-213.
[50] Wu M, Li CL, Zhang J, Miao CC, Zheng YP, Sun YM. ZrO_2-MoO_3 for the acetalization of 1,3-propanediol from dilute solutions. *Industrial & Engineering Chemistry Research*. 2012; 51: 6304-6309.
[51] Cho MH, Joen SI, Pyo SH, Mun S, Kim JH. A novel separation and purification process for 1,3-propanediol. *Process Biochemistry*. 2006; 41: 739-744.
[52] Hilaly A, Binder T. *Method of recovering 1,3-propanedol from fermentation broth*. 2002. Available from: https://patents.google.com/patent/US6479716B2/en [Accessed 17 October 2023].
[53] Acikara ÖB. Ion-exchange chromatography and its applications. *Column Chromatography*. 2013. Available from: http://dx.doi.org/10.5772/55744.
[54] Alexandratos SD. Ion-exchange resins: A retrospective from industrial and engineering chemistry research. *Industrial & Engineering Chemistry Research*. 2009; 48: 388-398.
[55] Rukowicz B, Miesic I, Alejski K. Separation of 1,3-propanediol from aqueous solutions by ion exchange chromatography. *Polish Journal of Chemical Technology*. 2014; 16: 82-86.
[56] Cho K, Kang S, Kim J, Examiner P, Price EO. *Method of isolating 1,3-propanediol or 1,3-propanediol and 1,2-propanediol from solution containing 1,3-propanediol, 1,2-propanediol, glycerol, and glucose*. 2009. Available from: https://patents.google.com/patent/US7488855B2/en [Accessed 17 October 2023].

[57] Rukowicz B, Alejski K. A biologically-derived 1,3-propanediol recovery from fermentation broth using preparative liquid chromatography. *Separation and Purification Technology*. 2018; 205: 196-202. Available from: https://doi.org/10.1016/j.seppur.2018.05.041.
[58] Wang S, Dai H, Yan Z, Zhu C, Huang L, Fang B. 1,3-Propanediol adsorption on a cation exchange resin: Adsorption isotherm, thermodynamics, and mechanistic studies. *Engineering in Life Sciences*. 2014; 14: 485-492.
[59] Dofner K. *Ion Exchangers*. Endeavor; 1991.
[60] Hao J, Liu H, Liu D. Novel route of reactive extraction to recover 1,3-propanediol from a dilute aqueous solution. *Industrial & Engineering Chemistry Research*. 2005; 44: 4380-4385.
[61] Cui C, Zhang Z, Chen B. Environmentally-friendly strategy for separation of 1,3-propanediol using biocatalytic conversion. *Bioresource Technology*. 2017; 245: 477-482. Available from: http://dx.doi.org/10.1016/j.biortech.2017.08.205.