

A review of salting-out effect and sugaring-out effect: driving forces for novel liquid-liquid extraction of biofuels and biochemicals

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Abstract Biofuels and bio-based chemicals are getting more and more attention because of their sustainable and renewable properties and wide industrial applications. However, the low concentrations of the targeted products in their fermentation broths, the complicated components of the broths and the high energy-intensive separation and purification process hinder the competitiveness of biofuels and biochemicals with the petro-based ones. Hence, the production and the separation of biofuels and bio-based chemicals in energy-saving, low-cost and greenness ways become hot topics nowadays. This review introduces the separation technologies (salting-out extraction, salting-out, sugaring-out extraction, and sugaring-out) that extract biobutanol, 1,3-propanediol, 2,3-butanediol, acetoin, organic acids and other bio-based chemicals from fermentation broths/aqueous solutions. Salting-out/sugaring-out extraction and salting-out/sugaring-out technologies display the high separating efficiency and the high targeted product yields. In addition, they are easy to operate and require low cost for separating products. Hence, they are the effective and potential technologies for separating targeted products in the wide industrial applications. The successful research into the salting-out/sugaring-out and salting-out/sugaring-out extraction not only affords biofuels and biochemical but also opens a door for the development of novel separation methods.

Keywords salting-out, sugaring-out, extraction, biofuels, biochemicals, application

1 Introduction

The overall evolution of nature is a process of increasing entropy that causes chaos. Contrarily, understanding and transforming nature is an entropy reduction process, which consumes energy and human labor with no essential difference. The traditional chemical engineering methods of separation and purification are often used to separate chemical products in chemical industries. The emerging technologies, such as distillation, absorption, evaporation and drying, require tremendous amount of energy [1]. Developing energy-saving technologies becomes essential in the chemical industry. An energy-efficient representative operating unit is extraction.

The traditional liquid-liquid extraction technologies have already been mature. Liquid-liquid extraction, also known as solvent extraction, is a mass transfer separation process that uses liquid extractants to deal with two or more than two components solution that are not miscible with extractants and then achieve component separation. Due to the advantages of operation with room temperature, energy saving, simple operation, and excluding solids and gases, liquid-liquid extraction is one of the most widely used unit operations and widely used in chemical and biological fields. However, it is hard to extract some polar targeted products, especially the products from fermentation broths. Othmer et al. [2] tried to extract 2,3-butanediol by using ethanol and esters as extractants in the early years, however it still needed to distill and concentrate the fermentation broth before this method could have a certain effect. Subsequently, Tsao et al. [3] used different solvents to purify 2,3-butanediol, and it was found that ether, ethyl acetate, *n*-butanol, dodecanol and oleyl alcohol had ordinary extraction effects. The recovery of 2,3-butanediol reached 75% by using ether as the extractant, and the recovery of ethanol and acetoin only reached 25% and

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65%, respectively. However, due to the shortcomings of large solvent consumption and high cost, this method is not suitable for large-scale production in industries. Malinowski et al. [4] used the extraction screening software ESP to evaluate the extraction of 1,3-propanediol with different extractants. The results showed that fatty alcohols and aldehydes were superior to other extractants in the extraction, but in the subsequent experiments, it was found that the extraction effect was quite different from the simulated value, and the results are not satisfactory. Because of the strong polarity of 1,3-propanediol and 2,3-butanediol, the traditional liquid-liquid extraction system cannot effectively extract them from their fermentation broths. The distribution coefficients of the products in both phases are poor, which makes the concentration of the products in the extractants much lower than that in fermentation broth, and the energy consumption for separation increased.

With the development of chemical industry, new extraction technologies have emerged, such as supercritical fluid extraction [5], aqueous two-phase extraction [6], ultrasound/microwave enhanced extraction [7], chelating extraction [8] and complex extractions [9]. These technologies are constantly developing in the era of biotechnology due to their unique advantages. With the development of gene engineering, protein engineering, cell culture engineering, metabolic engineering and other high and new biotechnology, varieties of new biochemical products are emerging. The low concentrated biological products have biological activities that cause their separating and environmental conditions become harsh [10–12]. The traditional liquid-liquid extraction in organic solvent can destroy the biological activity, so it cannot meet the separation requirements [13]. An emerging aqueous two-phase extraction separation technology can solve the problem [14]. The extraction agents for the traditional two-phase aqueous extraction method are water-soluble polymers, the substances belonging to surfactants, or either of them plus salt. When the concentrations of phase-forming materials are high, the system is easily divided into two phases. For example, the traditional aqueous two-phase extraction can be used to separate chloroplasts [15].

A lot of researches have been done on the extraction of organic compounds by traditional aqueous two-phase extraction [6]. The whole system of traditional two-phase aqueous extraction is featured with 70%–90% water in both phases. The extraction is conducted under the conditions of physiological environment of biological substances that will not cause the inactivation or denaturation of biological active substances. However, the traditional aqueous two-phase extraction is not suitable for recovering the targeted products and removing water due to the difficulty of separating the phase-forming materials.

For separating and recovering water-soluble organics, using the traditional organic solvents as extraction solvents

in liquid-liquid extraction technologies are not feasible [16]. For example, biobutanol is concomitant with ethanol, acetone and other polar organic compounds [17]. Organic compounds residue in the raffinate due to the compatibility between organic target products and water [18]. In the traditional aqueous two-phase systems, the water content in the extract phase is also relatively high, resulting in poor separation of the target products. Ghosh et al. reported that the distribution coefficient was only 1.15 in the extraction of 2,3-butanediol by an aqueous two-phase system composed of PEG6000/dextran 4000 [19]. The low distribution number indicates that the traditional aqueous two-phase system is not suitable for separating 2,3-butanediol from the fermentation broth.

Inspired by the salting-out effect in the traditional salt-induced aqueous two-phase systems, the amphipathic solvents, such as methanol, ethanol, acetonitrile, propanol, acetone, butanol, ethyl acetate, pentanol, etc. are used as the advanced phase-forming agent [20]. When a salt and an amphipathic solvent are mixed in solution, the solvent is affected by the changes of salt-induced surface properties, electric charge and various forces such as hydrophobic bonds, hydrogen bonds and ionic bonds [21,22]. As a result, a solvent-rich phase forms as the upper phase and a water + salt-rich phase forms as the bottom phase, generating a salting-out extraction (SOE) system. The SOE can be used to separate and recover bio-based chemicals from fermentation broths [20]. SOE is regarded as a new double-layer extraction technology. Based on the traditional aqueous two-phase system, the polymer is replaced by some common hydrophilic and hydrophobic organic solvents, which are applied for separating and purifying biological chemicals. The SOE reduces the amount of inorganic salts and organic solvents, reduces operating cost, and simplifies the separation steps.

The high concentrated products can be separated with the addition of salts in the fermentation broth/aqueous solution as well as forming the targeted product-rich new phase, namely the targeted products [23,24]. Salting-out can separate miscible organic compounds, such as 1,3-propanediol [25], 2,3-butanediol [26,27], or biobutanol [28] from their aqueous solutions. Additionally, salting-out does not require of adding extra organic solvents, so the cost of solvent is decreased. However, a little higher water content of the top phase must be taken into account for the subsequent purification of the target product, which means additional energy costs. Since there is a large amount of inorganic salt in the raffinate layer, the most economical and effective method is to reuse the salting-out agent [29].

High concentrated salt may deactivate the biological activities of products in SOE and salting-out [30,31]. The corrosions or fouling caused by proteins, remaining starches, remaining sugars and cells often occur in SOE and salting-out equipment. Thus a monomeric sugar or a disaccharide-based novel aqueous two-phase systems were also developed with the amphipathic solvents, which is

defined as sugaring-out extraction [32]. The difference of sugaring-out is the addition of a sugar rather than a salt. Similarly, sugaring-out refers to the separation of a miscible organic compound from fermentation broth/ aqueous solution and the formation of a two-phase system caused by the addition of a sugar. Sugars, the sugaring-out agents competed with the solute to attract water molecules so as to form a new phase for small organic molecules with lower polarity. The small molecules were predominant in the new phase, and the sugars mainly resided in the water phase. Therefore, the sugaring-out system renders the separation of small molecules achievable. The mildness and the fermentable sugar-enriched phase make sugaring-out stand out, because the sugaring-out extraction/sugaring-out can be coupled with fermentation [33,34]. The advantages of using sugars as the sugaring-out agents include its low corrosion to extraction equipment, mild extraction condition, and service as the substrate in fermentation.

The productions of bio-based C2–C6 platform chemicals have been attracted much attentions [35]. The platform chemicals are hydrophilic so that the recovery of these platform chemicals is the critical step in the fermentation industry. Due to the challenges associated with separating organics with large polarity in fermentation broth/ aqueous solution, the novel liquid-liquid extraction technologies are suitable for these systems and thus summarized in this review. We mainly introduced the theories of salting-out/ sugaring-out extraction and salting-out/sugaring-out, and their applications for the recovery of biobutanol and other biochemical such as 1,3-propanediol, 2,3-butanediol, acetoin, and organic acids, etc. The new separation technologies can be applied flexibly according to the characteristics of extraction system, so as to achieve the purpose of good separation, energy-saving effect and environmental protection.

2 Salting-out extraction

The polar micro-molecules, including butanol, pentanol and butanone can respectively formed two-phase system with water. Because butanol is partially soluble in water, the butanol aqueous solution forms two-phase systems. 2,3-butanediol can be extracted from fermentation broth through the two-phase system composed of butanol and

water [36]. However, the saturated solubility of butanol in water is relatively high. The polarity of 2,3-butanediol is greater than that of butanol which made 2,3-butanediol completely soluble in water. As a result, the butanol + water system showed poor extraction efficiency on the 2,3-butanediol separation. Therefore, the addition of dipotassium phosphate can enhance the repulsion effect on the micro-molecules in water and increase extraction efficiency [36]. Because some of organic extractants are slightly soluble or partially soluble in water, the process of adding a salting-out agent to enhance the extraction efficiency is called SOE. Salting out extraction is a new type of extraction technology. Due to the salting-out effect, many organic substances that are miscible with water, such as ethanol and methanol, can form an organic phase as long as the salt concentration reaches a certain value, thereby forming a salting-out system.

SOE system is used to recycle the extractives by adding extraction agents and salting-out agents. In reality, split-phase process of SOE is the process of contending the water molecules between organics and salting-out agents, mainly depending on the hydration of organics and salting-out agents. The effect of salting-out enhanced extraction efficiency resulted from the powerful capacity to preempt water molecules of salting-out agents. Generally, the split-phase ability of extraction agents with strong hydration is weak, but the split-phase ability of salting-out agents with strong hydration is strong. Four types of SOE systems based on the extractants are listed as the followings: 1) polymer-based; 2) hydrophilic solvent-based; 3) hydrophobic solvent-based; 4) ionic liquid-based SOE system. Table 1 lists some applications of different types of SOE systems.

As can be seen from Table 1, most of the SOE systems are formed due to the salting-out ability of the salting-out agents, which leads to good phase separation ability, thereby achieving the recovery of the target products. Since some target product concentration is too low, such as that of testosterone or phycocyanin, it is necessary to add an extractant to achieve a good phase separation. The mechanism of SOE is shown in Fig. 1.

2.1 SOE of biofuels

Compared with ethanol and methanol, *n*-butanol shows more advantages as a fuel, such as higher heating value,

Table 1 Some applications of SOE

Type	Extractant/salting-out agent	Target products	Ref.
1	Polyethylene glycol (4000)/potassium phosphate	Cphycocyanin	[37]
2	Ethanol/dipotassium hydrogen phosphate (K_2HPO_4)	Lactic acid	[38]
	Acetone/ K_2HPO_4	Acetoin	[39]
3	Pentanol/sodium phosphate	1,3-Propanediol	[40]
4	1-Butyl-3-methylimidazolium chloride/ K_2HPO_4	Testosterone and epitestosterone	[41]

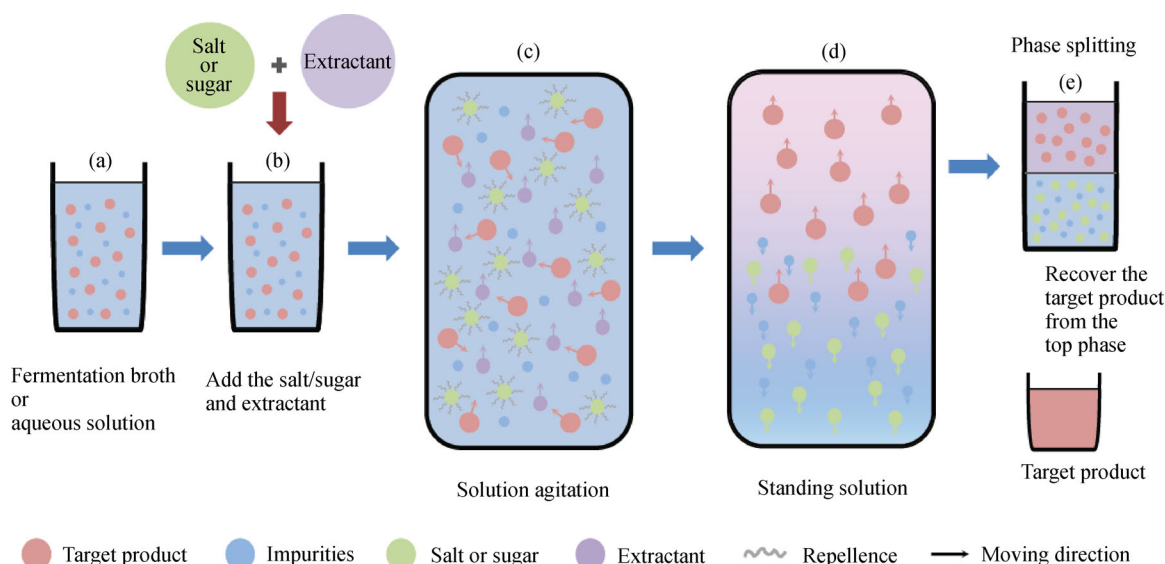


Fig. 1 Flow chart and mechanism of salting-out/sugaring-out extraction. (a) fermentation broth/aqueous solution containing target product(s) and impurities; (b) add salt(s)/sugar(s) and extractant(s) to fermentation broth/aqueous solution; (c) the attractive effect of extractant(s) impel the target product(s) to move toward extractant(s), meanwhile, the repulsive interaction between salt(s)/sugar(s) and target product(s) drives target product(s) to move away from fermentation broth/aqueous solution; (d) due to the changes in solubility of different solvents, the whole system will form two phase gradually; and the target product(s) will move to the upper phase (organic phase) with extractant(s) while the salt(s) and most of impurities will move to the bottom phase (aqueous phase); (e) phase splitting, target product(s) will be obtained by a series of separation operations from upper phase.

higher energy density, lower vapor pressure and better mixing ability with gasoline (mixing with gasoline in any ratio) [42]. The bottlenecks of biobutanol are mainly the low butanol titer in the biological butanol fermentation broth and the high separation cost. Therefore, the development of new separation techniques to reduce the cost of biobutanol production can increase the competitiveness of biobutanol. The SOE systems to recover biobutanol are summarized in Table 2. The recovery of ABE (acetone + butanol + ethanol) can be achieved by adding extraction agents and salting-out agents directly into ABE fermentation broth. Malinowski and Daugulis. [43] extracted the mixture of butanol, acetone, and ethanol solution by adding extraction agents, cyclopentanol, *n*-pentaldehyde, tertiary pentanol and ADOL85NF and they found that the distribution coefficients and selectivities of targeted products were significantly increased after the addition of CaCl_2 , KCl , NaCl , CH_3COOK and CH_3COONa in a broad range of concentrations. The presence of the salts reduces the solubility of the extractant in the aqueous phase, thereby helping to reduce the loss of solvent.

In order to recover butanol from simulated fermentation broth and unfiltered fermentation broth, Na_2CO_3 was added as a salting-out agent, and acetone or ethanol was added as an extractant. With 41.7% acetone and 25% Na_2CO_3 in the system, the distribution coefficient and recovery of butanol from simulated fermentation broth

were 55.70 and 98.38%, respectively; with 39.6% ethanol and 25% Na_2CO_3 in this system, the distribution coefficient and recovery of butanol were 35.06 and 97.87%, respectively. On the other hand, with 33% acetone and 30% Na_2CO_3 being added into the unfiltered fermentation broth system, the results showed that the distribution coefficient and recovery of butanol were 32.17 and 95.99%, respectively, while the removal ratios of proteins and cells were 87.13% and 99.92%, respectively [44].

Besides Na_2CO_3 , 50% K_2HPO_4 was also added to the simulated fermentation broth while 47.5% ethanol was added as an extractant to recover butanol [44]. The distribution coefficient and recovery of butanol were 25.03 and 96.84%, respectively. In addition, the system consisting of 40% acetone and 60% K_2HPO_4 was used to recover butanol from unfiltered fermentation broth, and the recovery of butanol was almost 100%, and the removal ratios of cells and proteins were 99.86% and 91.21%, respectively. According to the study of Sun et al. [45], the acetone/ K_2HPO_4 system can recover biobutanol from the ABE fermentation broth. The optimum conditions of this SOE system are 17.51% w/w acetone (extractant), 21.44% w/w K_2HPO_4 (salt), and ABE concentration of $17.3 \text{ g} \cdot \text{L}^{-1}$, and the partition coefficient, extraction yields, and concentration fold of butanol reached 63.13, 98.10% and 1.53, respectively. Additionally, the removal ratio of cells and proteins reached 99.12% and 95.71%, respectively.

Table 2 SOE of biobutanol from simulated fermentation broths or real fermentation broths

SOE systems	Type of fermentation broth	Partition coefficient	Recovery	Removal		Ref.
				Cells	Proteins	
Cyclopentanol, <i>n</i> -pentanaldehyde, tertiary pentanol, and ADOL85NF + CaCl ₂ , KCl, NaCl, CH ₃ COOK, CH ₃ COONa	Simulated ABE fermentation broth	–	–	–	–	[43]
41.7% Acetone + 25% Na ₂ CO ₃	Simulated ABE fermentation broth	55.71	98.38%	–	–	[44]
39.6% Ethanol + 25% Na ₂ CO ₃	Simulated ABE fermentation broth	35.06	97.87%	–	–	[44]
33% Acetone + 30% Na ₂ CO ₃	Unfiltered ABE fermentation broth	32.17	95.99%	99.92%	87.13%	[44]
47.5% Ethanol + 50% K ₂ HPO ₄	Simulated ABE fermentation broth	25.03	96.87%	–	–	[44]
40% Acetone + 60% K ₂ HPO ₄	Unfiltered ABE fermentation broth	–	~100%	99.86%	91.21%	[44]
17.51% Acetone + 21.44% K ₂ HPO ₄	ABE fermentation broth	63.13	98.10%	99.12%	95.71%	[45]
2-Ethyl-1-hexanol/cyclopentanol/ 2-methyl-2-butanol + K ₄ P ₂ O ₇	Simulated isobutanol fermentation broth	–	~100%	–	–	[47]
2-Ethyl-1-hexanol/cyclopentanol/ 2-methyl-2-butanol + K ₂ HPO ₄	Simulated isobutanol fermentation broth	–	~100%	–	–	[47]
2-Ethyl-1-hexanol/cyclopentanol/ 2-methyl-2-butanol + K ₃ PO ₄	Simulated isobutanol fermentation broth	–	~100%	–	–	[47]
2-Ethyl-1-hexanol/cyclopentanol/ 2-methyl-2-butanol + K ₂ CO ₃	Simulated isobutanol fermentation broth	–	~100%	–	–	[47]

The acetone/K₂HPO₄ system was demonstrated to be efficient and viable for recovering butanol from ABE fermentation broth.

Beside ABE, bio-based isobutanol has particularly received strong attention due to its attributes to potentially substitute gasoline or to serve as a gasoline supplement [46]. The SOE of isobutanol in the aqueous solutions was investigated by the combination of K₄P₂O₇, K₂HPO₄, K₃PO₄, K₂CO₃, K₂SO₄, KCl, Na₂CO₃, Na₂SO₄, NaCl and the organic solvents 2-ethyl-1-hexanol, cyclopentanol, 2-methyl-2-butanol [47]. The recovery of isobutanol depends on the salt concentration and types and organic content and types. Using K₄P₂O₇, K₂HPO₄, K₃PO₄, K₂CO₃ as the salting-out agents can achieve 100% recovery of isobutanol, and more than 91.62% removal of water for the isobutanol solution.

2.2 SOE of bio-based chemicals

Polymer-based SOE system is the traditional aqueous two-phase system and is mainly used for extracting biological products. Since the recoveries of hydrophilic chemicals by ATP system are quite low [48], the hydrophilic solvent-based SOE system was used and displayed a prominent advantage compared to polymer-based SOE system. The SOE systems for recovering bio-based chemicals are summarized in Table 3.

Because of the attractive chemical and physical properties [49], 1,3-propanediol is widely used as a chemical intermediate in organic synthesis, and is also an important monomer for the production of high performance polyester which has excellent tensile elastic recovery and dye ability

for the textile and fiber industries [50,51]. 1,3-Propanediol can be separated from fermentation broth through the SOE system composed of hydrophilic solvents and inorganic salts. Using pentanol as the extractant and 0.4 g·mL⁻¹ of sodium phosphate resulted in the highest distribution coefficient and recovery of 1,3-propanediol from fermentation broth produced using *Klebsiella pneumonia* up to 3.72 and 72%, respectively while using 0.4 g·mL⁻¹ of sodium sulfate gave out lower results (2.52% and 65.6%, respectively) [40]. The ethanol/ammonium sulfate (NH₄)₂SO₄ system was investigated to recover 1,3-propanediol, and the results revealed that under the condition using 46% (v/v) ethanol/(NH₄)₂SO₄ system, the highest partition coefficient and recovery of 1,3-propanediol were 4.77 and 93.7%, respectively [25]. In addition, the removal ratio of proteins and cells were 79.0% and 99.7%, respectively. The methanol/K₂HPO₄ system was also studied to separate the 1,3-propanediol from fermentation broth [29]. With the 35% (v/v) methanol and saturated concentration of K₂HPO₄ at a pH 10.7, the partition coefficient and recovery of 1,3-propanediol reached 38.3 and 98.1%, respectively. The recycling of K₂HPO₄ in the bottom phase reached 94.7% after adding 1.5 volume of methanol and the pH was adjusted to 4.5. Additionally, the removal ratios of proteins and cells were 92.4% and 99.85%, respectively. 90.30% of 1,3-propanediol (partition coefficient = 10.86) was recovered from the real fermentation broth via the SOE system composed of ethanol and 37.5% K₂HPO₄, and 94.4% of phosphate could be recycled after adding 0.2 volume of anhydrous ethanol into the bottom phase at pH 4.0 [52]. Moreover, in the extraction system composed of 34 wt-% K₃PO₄,

Table 3 SOE of bio-based chemicals

Target products	SOE systems	Partition coefficient	Recovery	Removal		Ref.
				Cells	Proteins	
1,3-Propanediol	46% Ethanol + (NH ₄) ₂ SO ₄ (saturated)	4.77	93.7%	99.7%	79.0%	[25]
	35% Methanol + K ₂ HPO ₄ (saturated)	38.3	98.1%	99.85%	92.4%	[29]
	Ethanol + 37.5% K ₂ HPO ₄	10.86	90.30%	—	—	[52]
	28% Ethanol + 34% K ₃ PO ₄	33	97%	—	—	[53]
	Pentanol + 0.4 g · L ⁻¹ Na ₃ PO ₄	3.72	72%	—	—	[40]
	Pentanol + 0.4 g · L ⁻¹ Na ₂ SO ₄	2.52	65.6%	—	—	[40]
	Ethanol + Na ₂ CO ₃	—	97.9%	99.1%	81.9%	[54]
	1) 30% Isopropanol + 30% K ₂ CO ₃ 2) 28% Ethanol	9.81	92.4%	100%	98.5%	[55]
2,3-Butanediol	24% Ethanol + 25% K ₂ HPO ₄	28.34	98.13%	99.63%	85.9%	[26]
	21% Ethanol + 17% K ₂ HPO ₄	13.4	99%	—	89%	[60]
	32% Ethanol + 16% (NH ₄) ₂ SO ₄	7.1	91.7	99.7%	91.2%	[72]
	2-sec-Butyl phenol + 10 wt-% K ₂ HPO ₄	3.04	—	—	—	[58]
	1-Butanol + 10 wt-% K ₂ HPO ₄	2.41	—	—	—	[58]
	1-Butanol + 10 wt-% K ₂ HPO ₄	2.26	—	—	—	[36]
	34% (w/w) 2-Propanol and 20% (w/w) (NH ₄) ₂ SO ₄	9.9	93.7%	99%	85%	[61]
	Tetrahydrofurn + K ₂ CO ₃	—	92.2%	—	—	[59]
Acetoin	Ethyl acetate + K ₂ HPO ₄ (v/v = 2:1)	5.47	91.3%	—	—	[64]
	100 mL Ethyl acetate + 20% ethanol + 50% K ₂ HPO ₄	18.5	95.3%	—	—	[64]
	40% 1-Propanol + K ₄ P ₂ O ₇ (saturated)	—	~100%	—	—	[65]
	6% EOAB + 38% K ₃ PO ₄	40.54	92.7%	—	—	[66]
Lactic acid	30.23% Ethanol + 18.40% K ₂ HPO ₄	2.26	87%	—	—	[38]
	30% Ethanol + 14% K ₂ HPO ₄	3.23	90.6%	100%	85.9%	[69]
	26% Methanol + 25% K ₂ HPO ₄	4.01	86%	100%	85.9%	[69]
	28% Ethanol + 30% K ₂ CO ₃	1.27	73.8%	100%	98.5%	[55]
Succinic acid	30% Acetone + 20% (NH ₄) ₂ SO ₄	8.64	90.05%	99.03%	90.82%	[70]
Butyric acid	26.7% Ethanol + 20% NaH ₂ PO ₄	106.2	99.5%	—	—	[71]

28 wt-% ethanol, and 38 wt-% fermentation broth that containing 23.0 g · L⁻¹ 1,3-propanediol, 97% of 1,3-propanediol can be recovered and the partition coefficient of 1,3-propanediol reached up to 33 [53]. Multi-stage counter-current SOE of 1,3-propanediol by ethanol/sodium carbonate system was also designed to recover 97.9% of 1,3-propanediol, and simultaneously remove 99.1% cells, 81.9% proteins, 75.5% organic acids and 78.7% water [54]. Two-step SOE was adopted for the sequential separation of 1,3-propanediol and lactic acid from the fermentation broth produced using *Klebsiella pneumonia* [55]. In the first stage, using 30% isopropanol and 30% K₂CO₃ resulted in the partition coefficient and recovery of 1,3-propanediol up to 9.81 and 92.4%.

2,3-Butanediol is used as liquid fuel and chemical raw materials in wide applications of fumigants, perfumes,

moistening, printing inks, plasticizers, and pharmaceutical carrier [56]. 2,3-Butanediol can be readily dehydrogenated into acetoin and diacetyl and can be dehydrated into methylethyl ketone [57]. The salting-out effects of various salts on the solvent extraction of 2,3-butanediol in simulated and real fermentation broth showed hydrophobic solvent types, salt types and salt concentrations determined the distribution and selectivity of 2,3-butanediol [58,59]. Jiang et al. [26] separated 2,3-butanediol from fermentation broth through SOE system. The system consists of ethanol and K₂HPO₄, and the results showed that the partition coefficient and recovery of 2,3-butanediol reached 28.34 and 98.13%, respectively. Simultaneously, the removal ratio of cells and proteins reached up to 99.63% and 85.9%, respectively [26]. Another study showed that 99% of 2,3-butanediol was obtained from the

viscous Jerusalem artichoke-based fermentation broth by using the system composed of 21% ethanol and 17% K_2HPO_4 (mass fraction), and the partition coefficient of 2,3-butanediol reached 13.4 [60]. In addition, 89% of soluble proteins and 98% of solid matters were removed from the broth. If the ethanol and K_2HPO_4 were replaced by isopropanol and $(NH_4)_2SO_4$, the highest partition coefficient and recovery of 2,3-butanediol reached 9.9 and 93.7%, respectively, and more than 99% of the cells and about 85% of the soluble proteins were removed [61]. Continuous countercurrent liquidliquid extraction of 2,3-butanediol from simulated and real fermentation broth in a bubble column using *n*-butanol as an extractant solvent and phosphate salts as the salting-out agents [36]. The results showed that the salting-out effect of K_2HPO_4 increased the 2,3-butanediol extraction efficiency by 81.8% for simulated solution and by 30% for fermentation broth at 2.5 wt-% 2,3-butanediol in feed.

Acetoin (3-hydroxy-2-butanone or acetyl methyl carbinol) is an important chemical found in fruit, cheese, wines, vinegar [62]. In addition, acetoin is also a precursor in the manufacture of acetyl butanediol, diacetyl, pyrazines and optically active α -hydroxyketone derivatives [63]. The 91.3% yield of acetoin (partition coefficient = 5.47) was obtained via the SOE system consisting of 100 mL ethyl acetate and 50 g K_2HPO_4 (50% (m/v) concentration) in 100 mL fermentation broth, whereas 95.3% of acetoin (partition coefficient = 18.5) was obtained via the system consisting of 100 mL ethyl acetate, 50 g K_2HPO_4 and 20% ethanol in 100 mL fermentation broth [64]. Most of the organic acids, residual glucose and coloring matters were removed from the upper phase. Another study showed that almost 100% of acetoin could be extracted by using the SOE system composed of 1-propanol, $K_4P_2O_7$ and water, with almost all of the salt being kept in the bottom phase and more than 99% of 1-propanol being recovered [65]. Moreover, ionic liquids-based SOE was investigated for the recovery of acetoin. 92.7% of acetoin was obtained from fermentation broth with the SOE system composed of 6% EOAB and 38% (w/w) K_3PO_4 , in which the partition coefficient of acetoin was 40.54 [66]. In addition, the AIR-IR spectra showed that the important role in the effective separation of acetoin from broth is the hydrogen bond between acetoin and O–H, N–H, and $-COO^-$ of hydroxylamine IL.

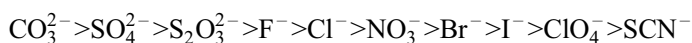
Organic acids, including lactic acid, succinic acid and butyric acid can be recovered via the SOE system. The organic acids have been used in food, polymer, pharmaceutical, biodegradable plastics industry, and other fields [67]. Most of these organic acids can be produced via fermentation and some of them can be found in low titer wastewater. However, hydrophilicity, pH-dependence and structural diversity create problems for recovering these organic acids [68]. Therefore, it is desired to seek an economical and effective method to recover organic acids.

With the optimal conditions of 30.23% (w/w) ethanol and 18.40% (w/w) K_2HPO_4 , the maximum partition coefficient and recovery of lactic acid from simulated fermentation broth were 2.26 and 87%, respectively [38]. Another study showed that the partition coefficient (3.23) and recovery (90.6%) of lactic acid were obtained with the optimal condition of 30% (w/w) ethanol and 14% (w/w) K_2HPO_4 , while the partition coefficient (4.01) and recovery (86%) of lactic acid were obtained with 26% (w/w) methanol and 25% (w/w) K_2HPO_4 [69]. 67.3% of glucose, 100% cells and 85.9% proteins were removed from the upper phase. In addition, under the optimum condition of 28% (w/w) ethanol and 30% (w/w) K_2CO_3 at pH 6.5, the partition coefficient and recovery of lactic acid reached up to 1.27 and 73.8%, respectively, and 100% of cells and 98.5% of proteins were removed [55]. Besides lactic acid, 90.05% of succinic acid was extracted via SOE and the highest partition coefficient reached up to 8.64 through the system of 30% (w/w) acetone and 20% (w/w) $(NH_4)_2SO_4$ at pH 3.0, and the removal ratio of cells, proteins, and glucose reached up to 99.03%, 90.82%, and 94.89%, respectively [70]. With the SOE system consisting of 20% monosodium phosphate (NaH_2PO_4) and 26.7% ethanol, the partition coefficient, extraction efficiency and total recovery yield of butyric acid were 106.7, 99% and 99.5%, respectively [71].

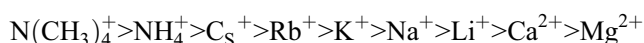
After the SOE, the salt dissolved in the aqueous phase can be recycled by adding an anti-solvent to precipitate out the salt. For example, the recovery of $(NH_4)_2SO_4$ in the aqueous phase reached 97.14% if two volumes of methanol was used as the anti-solvent [72].

3 Salting-out

Salting-out agents can be mixed with high concentrated targeted products, and it causes target products splitting from water albeit the absence of extraction agents [73]. Salting-out system is a process of recycling the extractives soluble in water by adding salting-out agents directly. According to the Hofmeister sequence [74], the salting-out effect of anions can be followed by:



Chloridion is the demarcation point of salting-in and salting-out effect. The anions on the left of Chloridion have strong hydration and salting-out ability, while the anions on the right have weak hydration and salting-out ability. The salting-out effect of cations on non-charged systems, such as protein skeletons, can be expressed as [75]:



The cations on the left showed the salting-out effect on proteins or biomolecules, while the cations on the right

showed the salting-in effect. Generally, the strong cationic hydration shows the salting-in effect, while the weak cationic hydration shows the salting-out effect. This trend is opposite to anions.

The phase splitting ability of salting-out agents depends on the types of solutes and the intrinsic properties of salting-out agents. Methanol, ethanol, propanol and acetone can form homogeneous system with water as they are soluble in water. Salting-out agents reduce the solubility of solute in aqueous solution, and then new organic phase is formed [76–78]. The applications of salting-out are partly listed in Table 4.

Table 4 shows NaCl facilitates the phase separation of organics in their aqueous solutions, such as acetone, acetonitrile, 1,4-dioxane, tetrahydrofuran, propanol and isopropanol. The purposes of split-phase can be divided into two types: 1) the water soluble organics were salted out to realize the application of SOE, for example, acetonitrile + H₂O + NaCl system showed superior extraction efficiency on *p*-nitroaromatic compounds/tetra-nitrotoluidine and nitrate esters than CH₂Cl₂ + H₂O + NaCl system [23]; 2) realizing the recovery and purification of water soluble organics, for example, 2,3-butanediol + H₂O + K₂CO₃ system is to achieve the recovery of 2,3-butanediol [27]. In this section, we will discuss the second type.

Salting-out avoids adding organic extraction agents, so it is easier to operate and achieve split-phase. The advantages of salting-out and SOE are easy magnification, time- and energy-efficient, and short duration of reaching phase equilibrium. However, recycling extraction agents and salting-out agents are still challenging. The mechanism of salting-out is shown in Fig. 2.

3.1 Salting-out of biofuels

The four main components, namely water and ABE in the biobutanol fermentation broth are miscible. The water + ethanol and water + 1-butanol azeotropes are formed during the distillation so that the energy consumption is

very high. Removing water through salting-out reduces the amount of azeotropes thus reduces energy consumption.

In order to obtain pure acetone or 1-butanol or ethanol from ABE fermentation broth, multi distillations are applied to separate most of the byproducts [84]. The concentrated ABE fermentation broth with 50–60 wt-% water will be obtained at the top of a prefractionator. Adding salting-out agents into the concentrated ABE fermentation broths can induce phase separation and recover ABE with only a small amount of water in the top phase [85]. The salting-out effects of various salts on biobutanol separation in the concentrated broth were studied in Qiu's group [86] and they found that the high energy consumption of biobutanol separation via industrial traditional distillation can be reduced remarkably through the removal of water by the salting-out effect. The results showed that NaAc, MgCl₂, Na₂CO₃ and other salts significantly changed the mutual solubility of *n*-butanol, acetone, and water system at room temperature. The mixed extractants composed of water and salt lead to increased distribution coefficients and selectivity coefficients of biobutanol which is beneficial for the separation and concentration of ABE. In the meanwhile, the effect of salting-out parameters on separation efficiency was studied, and the combination of salting-out and distillation showed that this hybrid method is more energy-saving than the traditional one [84]. The increasing salt concentration facilitated the separation of *n*-butanol to the organic phase [87]. NaNO₂ was found to have the best separation efficiency on *n*-butanol and increased *n*-butanol fraction from 20.8% to 65.24%. The purification sequence of various salts to *n*-butanol is followed by: NaNO₂ > NaAc > K₂CO₃ > NaNO₃ > NaCl > MgCl₂ > KCl > CaCl₂ > Na₂CO₃ > K₂C₂O₄. The purification sequence of acetone is followed by: K₂CO₃ > Na₂CO₃ > NaAc > NaCl > NaNO₂ > KCl > NaNO₃ > K₂C₂O₄ > CaCl₂ > MgCl₂. The purification sequence of various salts to ethanol is followed by: K₂CO₃ > Na₂CO₃ > NaNO₂ > NaCl > NaAc > CaCl₂ > NaNO₃ > KCl > K₂C₂O₄ > MgCl₂. The dehydration sequence of various salts is

Table 4 Some applications of salting-out

System	Target products	Ref.
Acetonitrile + H ₂ O + LiCl, + NaCl, + KCl	Acetonitrile Acetone	[79]
Acetone + H ₂ O + NaCl	2-Propanol	
2-Propanol + H ₂ O + NaCl		
1-Methyl-2-pyrrolidone + Na ₂ CO ₃ + H ₂ O	1-Methyl-2-pyrrolidone Acetonitrile	[80]
1-Methyl-2-pyrrolidone + K ₂ CO ₃ + H ₂ O		
Acetonitrile + Na ₂ CO ₃ + H ₂ O		
Acetonitrile + K ₂ CO ₃ + H ₂ O		
H ₂ O + ethanol + acetone + NaCl	Ethanol + acetone	[81]
2,3-Butanediol + H ₂ O + K ₂ CO ₃	2,3-Butanediol	[82]
NaCl + H ₂ O + acetone, acetonitrile, 1,4-dioxane, tetrahydrofuran, 1-propanol, or 2-propanol	Acetone, acetonitrile, 1,4-dioxane, tetrahydrofuran, 1-propanol, or 2-propanol	[83]

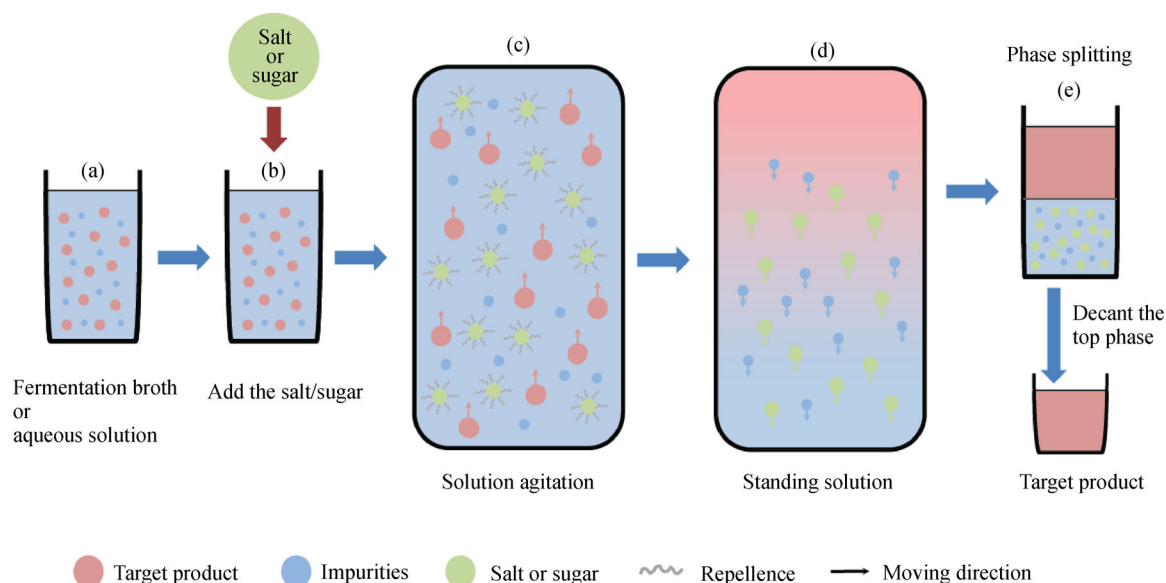


Fig. 2 Flow chart and mechanism of salting-out/sugaring-out. (a) fermentation broth/aqueous solution containing target product(s) and impurities; (b) add salt(s)/sugar(s) to fermentation broth/aqueous solution; (c) the repulsive interaction between salt(s)/sugar(s) and target product(s) drives target product(s) to move away from fermentation broth/aqueous solution; (d) due to the changes in solubility of different solvents, the whole system will form two phase gradually; and the target product(s) will move to the upper phase (organic phase) because of its(their) low density, while the salt(s) and most of impurities will move to the bottom phase (water phase); (e) phase splitting; target product(s) will be obtained by decanting the upper phase.

followed by: $\text{K}_2\text{CO}_3 > \text{NaAc} > \text{NaNO}_2 > \text{NaCl} > \text{Na}_2\text{CO}_3 > \text{NaNO}_3 > \text{KCl} > \text{CaCl}_2 > \text{MgCl}_2 > \text{K}_2\text{C}_2\text{O}_4$. However, the salts with low solubilities in water or the insufficient addition of the highly soluble salt resulted in the loss of ABE, the poor removal of water or the interfusion of salt in the organic phase [76]. The traditional production of biobutanol was improved by using potassium fluoride, but the new method saves 20% energy than the traditional one [88]. The separation of ABE system using potassium fluoride is technically flexible, but potassium fluoride is toxic and expensive [89].

Salting-out process for extracting 1-butanol from ABE fermentation broth is energy-efficient, low-cost and greenness. The intermolecular interactions of solvent, ion, and nonelectrolyte are the mechanisms of salting-out [90], and they are considered of causing hydration and hydrogen-bond breaking. The nonelectrolyte repulses solvent in addition to electrolyte (like salts) because the affinity of electrolyte is stronger than that of nonelectrolyte. This repelling movement can be explained of solvent molecules moving away from the nonelectrolyte ions to electrolyte ions due to the increase in hydration and the decrease in the solubility of nonelectrolyte. Therefore, salting-out attributes to the preferential movement of solvent molecules to electrolyte ions from their role as the solvent for ions of the nonelectrolyte [90]. In addition, salting-out is a process of losing entropies due to increasing temperature. The elevation of temperature and the enhancement of salt concentration are conducive to improve the salting-out

effect [84,91]. The highly soluble salts used for recovering biobutanol from fermentation broth are summarized in Table 5.

Twenty five compounds including neutral salts LiCl , CaCl_2 , KCl , MgCl_2 , NaCl , NaNO_3 , K_2SO_4 , acidic salts EDTA-2Na , AlCl_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl , NaH_2PO_4 , FeSO_4 , NaHSO_4 , $\text{Al}_2(\text{SO}_4)_3$, MnSO_4 , and basic salts $\text{K}_2\text{C}_2\text{O}_4$, NaAc , KAc , NaNO_2 , K_2CO_3 , Na_2CO_3 , Na_3PO_4 , HCOONa , Na_2SiO_3 have been investigated as possible salting-out agents to recover (10% acetone + 26% butanol + 4% ethanol) from a prefractionator [84]. Only K_2CO_3 was demonstrated to be the outstanding salting-out agent because of its inorganic property, its strongest salting-out effect and its high solubility in water. After the introduction of K_2CO_3 , the water content of the concentrated ABE was reduced from 60% to 4.91%, and the content of organic compounds in the aqueous phase reduced to 0%. Increasing the salt concentration and temperature speed up the equilibrium of phase separation and boost the salting-out effect of K_2CO_3 .

Inspired by the surprising salting-out performance of K_2CO_3 on ABE from the concentrated ABE fermentation solution, searching for the inorganic salts with strong salting-out effect and high solubility is critical for the further improvement in the ABE separation performance. K_2HPO_4 is another excellent salting-out agent for separating 1-butanol from the concentrated ABE fermentation broth [91]. K^+ and HPO_4^{2-} ionized from K_2HPO_4 are strong electrolytes. A “hydration shell” formed as water

Table 5 Salting-out of biofuel

Type of ABE fermentation broth	Type of salts	Water content of the organic phase	Recovery/%			Ref.
			Acetone	Butanol	Ethanol	
Concentrated ^{a)}	K ₂ CO ₃	6.83%	99	98.3	98	[84]
Concentrated ^{a)}	K ₂ HPO ₄	5.50%	~100	~100	~100	[91]
Concentrated ^{a)}	K ₃ PO ₄	7.11%	~100	~100	~100	[92]
Concentrated ^{a)}	K ₄ P ₂ O ₇	5.21%	~100	~100	~100	[93]
Dilute model solution ^{b)}	K ₂ CO ₃	5.59%	70.2	93.7	60.8	[94]
Dilute model solution ^{b)}	K ₂ HPO ₄	10.38%	96.1	100	85.6	[94]
Dilute model solution ^{b)}	K ₃ PO ₄	7.19%	89.4	100	72.2	[95]
Dilute ^{c)}	K ₃ PO ₄	7.0%	72.9	100	42.1	[95]
Dilute model solution ^{b)}	K ₄ P ₂ O ₇	7.76%	84.1	100	55.9	[96]

a) Concentrated ABE fermentation broth: ethanol, 4 wt-%; acetone, 26%; 1-butanol, 10%; others, water; b) model solution: ethanol, 0.18 wt-%; acetone, 0.54%; 1-butanol, 1.08%; others, water; c) real fermentation broth: the concentration of acetone, 1-butanol and ethanol were 0.39, 1.00 and 0.04 wt-%, respectively.

molecules surrounding K⁺ that has a high polarity index. Thus, K₂HPO₄ showed a prominent repellent effect, and 1-butanol showed salting-out effect [97]. According to the research [91], the residues of K₂HPO₄ in the organic phase were less than 200 mg·kg⁻¹ and gradually steady when the initial concentrations of K₂HPO₄ exceeded 400 g·kg⁻¹.

According to the recovery of ABE and the loss of organic after the salting-out process [91], the salting-out performance of K₂HPO₄ solution is superior to K₂CO₃

solution. Integrated salting-out-distillation process are recommended for the purification of ABE from dilute fermentation broth, as shown in Fig. 3 [84,98]. The dilute ABE fermentation broth was concentrated in a prefractionator. The concentrated ABE solution was salted out by K₂HPO₄ solutions at 80 °C. Then the ABE mixture was purified in the acetone column, butanol column and ethanol column respectively. The optimum value of the total energy requirements for the integrated process was

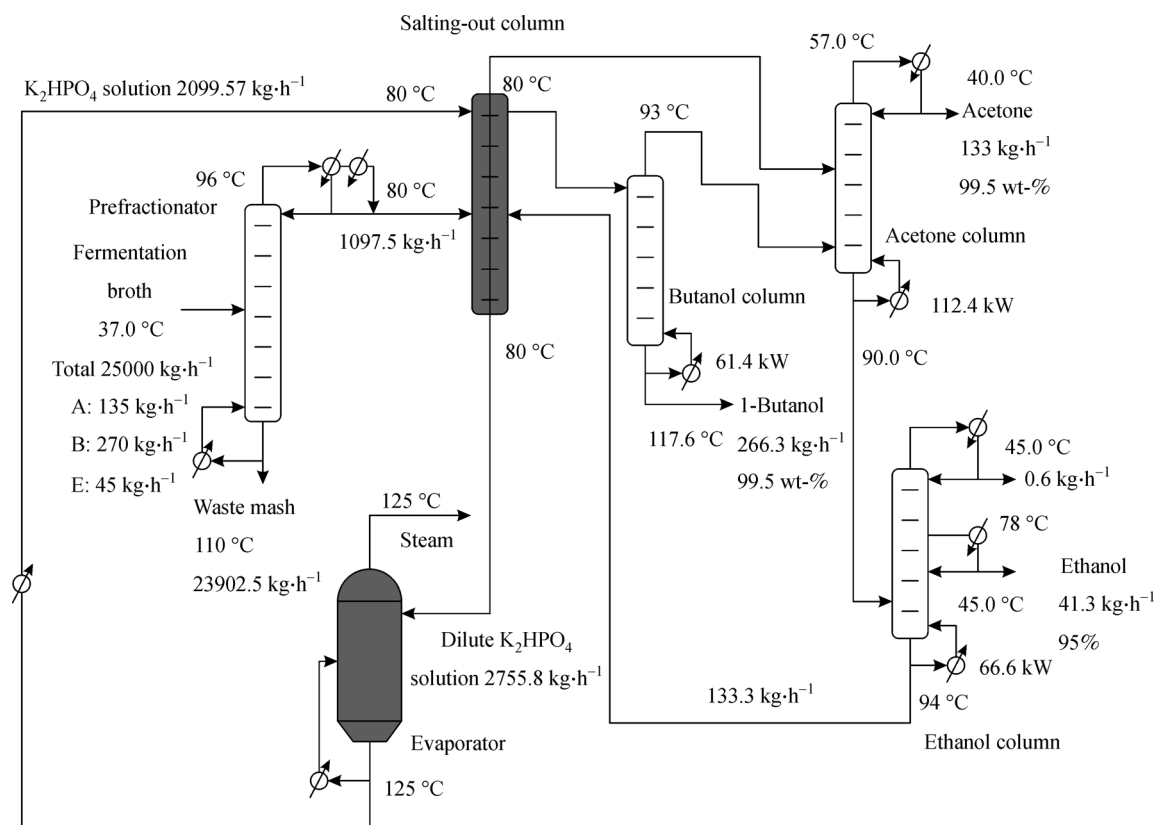


Fig. 3 Entire process of the salting-out and distillation method. Rate, composition, and temperature were based on scenario B at 80 °C. A: acetone; B: 1-butanol; E: ethanol. Reprinted with permission from Ref. [98]. Copyright 2015, John Wiley and Sons.

21.87 MJ·kg⁻¹ butanol, which turned out to be more energy-saving than the conventional one.

K₃PO₄ is another great salting-out agent for recovering 1-butanol from the concentrated ABE fermentation broth [92]. After the saturated condition of K₃PO₄ (510 g·kg⁻¹) at 298.15 K, the water content of the organic phase was reduced from 60.00% to 7.11%, and the mass fraction of 1-butanol increased from 26.00% to 60.37% in the organic phase and decreased from 26.00% to 0 in the aqueous phase. K₄P₂O₇ was also selected as a salting-out agent to extract 1-butanol from the concentrated ABE fermentation broth [93]. The residues of K₄P₂O₇ in the organic phase were less than 300 mg·kg⁻¹ when the initial concentration of K₄P₂O₇ exceed to 350 g·kg⁻¹. Under the saturated condition of K₄P₂O₇ at 298.15 K, the water content of the organic phase was reduced to 5.21%, and the mass fraction of 1-butanol in the aqueous phase was negligible, indicating that among all the investigated salts, K₄P₂O₇ showed the best salting-out performance on the ABE separation.

After the ABE fermentation broth is concentrated by the fractionator, the concentrated ABE fermentation broth is obtained at the top of this column whereas the energy consumption of this column accounts for about 1/3 of the energy consumption of the entire production workshop. Thus we developed the salting-out of ABE directly from dilute aqueous solution or fermentation broth [24,95,99]. The salting-out of simulated ABE fermentation broth and the real ABE fermentation broth only achieve 100% recovery of 1-butanol whereas the loss of ethanol and acetone was obvious. The salted-out ABE from the concentrated ABE fermentation broth can be treated as the extractants. By contrast, the amount of ABE from the dilute solution/fermentation is very small, indicating the

solvent extraction effect in this case is negligible. Thus the salting-out of concentrated solution additionally features the solvent extraction effect.

As the concentration of salt increases, the salting-out effect becomes stronger, suggesting that the mass fraction of water in the organic phase decreases. The water content in the organic phase is less than 10 wt-%, which means that most of the water in the dilute ABE fermentation broth is removed. No matter which salting-out agent is selected, *n*-butanol is preferentially recovered. The single component recovery rate is in the following sequence: recovery (butanol) > recovery (acetone) > recovery (ethanol). When the K₃PO₄ solution was used to recover the ABE real fermentation broth, more than 90 wt-% of ABE can be recovered, and more than 99.75% of water can be removed. The larger the total amount of ABE in the fermentation broth, the more helpful it is to improve the recovery of ABE, indicating improving the solvent titre is critical for the downstream process [90].

3.2 Salting-out of bio-based chemicals

Salting-out of bio-based chemicals is listed in Table 6. 1,3-propanediol and 2,3-butanediol can be also separated by salting-out in the absence of extractants. According to the study of Xie et al. [100], the salting-out effects of K₄P₂O₇, K₂CO₃, K₃PO₄ on the recovery of 1,3-propanediol were investigated. With increasing salt concentrations in the system or the initial 1,3-propanediol concentration in the aqueous solution at 298.15 K, the distribution coefficient and selectivity coefficient of 1,3-propanediol improved prominently, resulting in higher recovery of 1,3-propanediol. Moreover, among the tested salting-out agents, the salting-out effects of different salts on the 1,3-propanediol

Table 6 Salting-out of bio-based chemicals

Target products	Salts	Partition coefficient	Recovery/%	Water content of the organic phase/%	Ref.
1,3-Propanediol					
150 g·L ⁻¹	50% K ₂ CO ₃	22.6	—	20.5	[100]
150 g·L ⁻¹	50% K ₃ PO ₄	65.1	—	28.3	[100]
150 g·L ⁻¹	55% K ₄ P ₂ O ₇	300.9	95.7	17.3	[100]
2,3-Butanediol					
50 g·L ⁻¹	50% K ₂ HPO ₄	—	67.8	30.6	[59]
50 g·L ⁻¹	50% K ₃ PO ₄	—	54.5	18.2	[59]
50 g·L ⁻¹	50% K ₂ CO ₃	—	44.9	16.6	[59]
50 g·L ⁻¹	50% KF	—	36.0	23.8	[59]
100 g·L ⁻¹	50% K ₂ CO ₃	236.6	94.9	15.72	[27]
100 g·L ⁻¹	50% K ₃ PO ₄	723.6	97.5	22.5	[27]
100 g·L ⁻¹	50% K ₂ HPO ₄	277.6	93.7	31.1	[27]
100 g·L ⁻¹	50% K ₄ P ₂ O ₇	1791.1	99.0	19.0	[27]
100 g·L ⁻¹	50.79% K ₂ CO ₃	313	96.28	—	[101]
99.8 g·L ⁻¹ a)	67.9% K ₂ CO ₃	—	94.6	—	[101]

a) The real fermentation from saccharified starch contained 99.8 g·L⁻¹ 2,3-butanediol, 1.7 g·L⁻¹ acetoin and 4.3 g·L⁻¹ ethanol.

aqueous solutions were ordered in the sequence: $K_4P_2O_7 > K_3PO_4 > K_2CO_3$, which revealed that $K_4P_2O_7$ is ranked the highest salting-out effect followed by K_3PO_4 and K_2CO_3 [100].

When 100 g salt/100 g water was added to the $50 \text{ g} \cdot \text{L}^{-1}$ 2,3-butanediol aqueous solution, K_3PO_4 , K_2HPO_4 , K_2CO_3 and KF can cause the phase separation by the salting-out effect, while $K_3C_6H_5O_7$ cannot induce the phase separation due to its weak salting-out effect [59]. Using K_2HPO_4 as the salting-out agent recovered 67.8% of 2,3-butanediol from the dilute aqueous solution while reduce the water content of the organic phase remarkably. Furthermore, 2,3-butanediol can be recovered from aqueous solutions by some salting-out systems with different highly soluble salts, namely $K_4P_2O_7$, K_3PO_4 , K_2HPO_4 , K_2CO_3 [27]. The distribution coefficient and selectivity coefficient of 2,3-butanediol increased with increasing initial salt concentration and were affected significantly by the salt composition. The salting-out effects were followed the orders as $K_4P_2O_7 > K_3PO_4 > K_2CO_3 > K_2HPO_4$ under the same initial concentrations. Increasing the initial 2,3-butanediol concentration in the fermentation broth can improve the distribution and selectivity of 2,3-butanediol, resulting in higher recovery of 2,3-butanediol. Moreover, 100% of 2,3-butanediol from the aqueous solution was recovered and more than 97.5% of water was removed when the initial concentration of $K_4P_2O_7$ was higher than or equal to $550 \text{ g} \cdot \text{kg}^{-1}$, which could be concluded that $K_4P_2O_7$ was the most suitable salting-out agent for recovering 2,3-butanediol from aqueous solution [27]. Most of 2,3-butanediol from the aqueous solutions ($100 \text{ g} \cdot \text{L}^{-1}$) can be separated by salting out with K_2CO_3 , and the salting-out strategy can be also applied to bioprocess media with very good results. After precleaning the 2,3-butanediol fermentation broth from molasses or saccharified starch conversion process, it was possible to separate 94%–96% of the 2,3-butanediol using K_2CO_3 [101]. Without pretreatment, the concentration of the 2,3-butanediol in the top phase was 73.6 wt-% and much lower than the that separated from the simulated aqueous solution due to the presence of ethanol byproduct. Precleaning the medium and removing the ethanol by brief

boiling enable to separate 94 wt-% 2,3-butanediol with 53 wt-% K_2CO_3 and concentrate the 2,3-butanediol in the top phase up to 97 wt-%.

4 Sugaring-out extraction

Sugars as the sugaring-out agents competed with solute for attracting water molecules as forming a new phase for small organic molecules with lower polarity. The small molecules were predominant in the new phase, and the sugar mainly resided in the water phase. Therefore, the sugaring-out system renders separation of small molecules became achievable [32]. The advantages of using sugar as the sugaring-out agents include its low corrosion to extraction equipment, mild extraction condition, and service as the substrate in fermentation. The sugaring-out operates at mild condition and does not destroy the activity of biological macromolecules. Tasi et al. [102] extracted and detected the sulfonamides in honey by using sugaring-out technology. Dhamole et al. [103] found that over 60 wt-% acetonitrile had been removed by using sugaring-out, and that over 95 wt-% of water-soluble proteins (bovine serum albumin, trypsin and pepsin) had been recovered. Sugaring-out can also be used to separate Pt, Pd and Rh [104], however, the application of sugaring-out to biofuels or bio-based chemicals recovery remains to be studied.

As a novel separation method, sugaring-out extraction can also recover biomolecules [105], metal ions [104], drugs [102], and proteins [103], and the sugaring-out system includes but not limits to propylene glycol-sugar [106], ethanol-sugar [106], and acetonitrile-sugar [32]. In particular, it demonstrates high efficiency for recovering low hydrophilic chemicals, including syringic acid, ferulic acid, para-coumaric acid [32], and phenolic compounds [106]. The systems of sugaring-out extraction to recover bio-based chemicals are summarized in Table 7, and the mechanism of sugaring-out extraction is shown in Fig. 1.

Succinic acid is an important and valuable C4 building chemical from biomass [107], and it has wide applications

Table 7 Sugaring-out extraction of bio-based chemicals

Target products	Sugaring-out extraction systems	Type of fermentation broth	Partition coefficient	Recovery	References
Succinic acid	27% Glucose + 40% <i>t</i> -butanol	Synthetic fermentation broth	5.14	88.15%	[33]
	27% Glucose + 40% <i>t</i> -butanol + 9% $(NH_4)_2SO_4$	Synthetic fermentation broth	–	89.55%	[33]
	30% Glucose + 45% [Bmim]BF ₄	Simulated fermentation broth	2.53	75.96%	[110]
	30% Glucose + 45% [Bmim]BF ₄	Filtered fermentation broth	–	73.71%	[110]
	30% Glucose + 45% [Bmim]BF ₄	Unfiltered fermentation broth	–	72.53%	[110]
Lactic acid	12% Glucose + 40% isopropanol	Simulated fermentation broth	1.39	84.27%	[112]
2,3-butanediol	30% Glucose + 1.8% $(NH_4)_2HPO_4$ + 100% <i>t</i> -butanol	Filtered fermentation broth	–	75.6%	[113]
Acetoin	Glucose + ethyl acetate (v/v = 1:2)	Unfiltered fermentation broth	–	61.2%	[114]

in foods, biodegradable polymers, pharmaceuticals, fine chemicals [108]. In addition, succinic acid is produced from abundant biomass [109]. Sugaring-out extraction consisting of 27% (w/w) glucose and 40% (w/w) *t*-butanol was used to extract succinic acid [33]. Under the conditions: pH 3 and temperature 25 °C, the partition coefficient and extractability of succinic acid were 5.14 and 88.15%, respectively. 96.72% of *t*-butanol was distributed to the top phase while 94.69% of glucose into the bottom phase. In the presence of (NH₄)₂SO₄, the results of *t*-butanol-glucose system demonstrated that the extractability of succinic acid increased from 87.64% to 89.55% when the mass fraction of (NH₄)₂SO₄ was enhanced from 1% to 9% (w/w). The gradient crystallization results in 73% yield and 98% purity of succinic acid, respectively [33]. Another study reported that ionic liquids-base sugaring-out extraction is efficient to recover succinic acid from fermentation broth [110]. In the [Bmim]BF₄/glucose system consisting of 45% (w/w) [Bmim]BF₄ and 30% (w/w) glucose at pH 2.0, the partition coefficient and extractability of succinic acid from simulated fermentation broth were 2.53 and 75.96%, respectively. Moreover, the extractability of succinic acid from actual fermentation broth were 73.71% and 72.53% for filtered and unfiltered fermentation broth, respectively [110].

Lactic acid is widely used in the pharmaceutical and comestic industries. Lactic acid receives more attention in the manufacture of biodegradable poly-lactic acid [111]. In the study of Yan e al. [112], isopropanol/glucose system was chosen to recover lactic acid from fermentation broth. The optimum condition of this system was 12% (w/w) glucose and 40% (w/w) isopropanol, and under this condition, the partition coefficient and recovery of lactic acid were 1.39 and 84.27%, respectively. The bottom phase with rich glucose could be used to produce lactic acid after extracting isopropanol [112].

2,3-butanediol can be also recovered via the sugaring-out extraction system. The study of Dai et al. [113] showed that the sugaring-out extraction system of *t*-butanol/glucose/water was selected to recover 2,3-butanediol

from fermentation broth. The results revealed that 75.6% 2,3-butanediol was recovered from the fermentation broth (60.3 g·L⁻¹ 2,3-butanediol) which was mixed with 30% (w/v) glucose, 1.8% (w/v) (NH₄)₂HPO₄ and equal volume of *t*-butanol. Additionally, 78.0% soluble proteins and 86.8% lactic acid were removed from 2,3-butanediol [113].

Furthermore, Dai et al. [114] investigated the recovery of acetoin from fermentation broth via the system consisting of fermentation broth, glucose (100%, m/v) and two-fold volume of ethyl acetate. The results showed that 61.2% acetoin was distributed into top phase without coloring matters and organic acids while close to 100% glucose was in the bottom phase. Pure acetoin could be acquired by vacuum distillation of concentrated solution in the top phase [114].

5 Sugaring-out

As we known, compared to sugaring-out extraction, sugaring-out is a system without extractants and the sugar as a sugaring-out agent will be added in the fermentation broth/aqueous solutions directly to extract the target products, which eliminates the process of recycling extractants. Different sugars used to recover chemicals are summarized in Table 8, and the mechanism of sugaring-out is shown in Fig. 2.

The study of Xie et al. [34] investigated the sugaring-out effect of glucose and sucrose on the concentrated ABE system. The results demonstrated that the sugaring-out effect dependeds on the initial concentration of sugars. At the temperature 300.15 K, the water content in the top phase decreased from 60.00 to 14.31 and 16.72 wt-% by adding sucrose and glucose, respectively. In addition, the mass fraction of 1-butanol, ethanol, and acetone in the top phase was increased from 41.27 to 64.52 wt-%, 4.41 to 5.60 wt-% and 11.27 to 15.57 wt-% with increasing the initial sucrose concentration from 100 to 650 g·kg⁻¹ (saturated condition), respectively. The mass fraction of 1-butanol, ethanol and acetone in the top phase was

Table 8 Sugaring-out of fuels and chemicals

Target products	Type of sugar	Type of system	Initial concentration of sugars	Concentration of target products	References
Butanol	Sucrose	Concentrated ABE system	650 g·kg ⁻¹	64.52%	[34]
	Glucose	Concentrated ABE system	500 g·kg ⁻¹	62.62%	[34]
Ethanol	Sucrose	Concentrated ABE system	650 g·kg ⁻¹	5.60%	[34]
	Glucose	Concentrated ABE system	500 g·kg ⁻¹	5.61%	[34]
Acetone	Sucrose	Concentrated ABE system	650 g·kg ⁻¹	15.57%	[34]
	Glucose	Concentrated ABE system	500 g·kg ⁻¹	15.05%	[34]
Acetonitrile	Glucose	Acetonitrile aqueous solution	50 g·L ⁻¹	95.4%	[119]
	Xylose	Acetonitrile aqueous solution	50 g·L ⁻¹	90.9%	[119]
	Arabinose	Acetonitrile aqueous solution	40 g·L ⁻¹	94.9%	[119]
	Fructose	Acetonitrile aqueous solution	50 g·L ⁻¹	89.1%	[119]
	Sucrose	Acetonitrile aqueous solution	50 g·L ⁻¹	90.4%	[119]

increased from 44.68 to 62.62 wt-%, 4.46 to 5.61 wt-% and 11.71 to 15.05 wt-% with increasing the initial glucose concentration from 100 to 500 g·kg⁻¹ (saturated condition), respectively.

acetonitrile is used as a solvent for recovering high value products because of its physicochemical properties, and it is also used as a starting material for the syntheses of organic and inorganic chemicals [115] and wide applications in electrochemistry, liquid chromatography [116], and the increase of catalytic efficiency of enzymes [117]. In particular, process engineers and industrial chemists pay extra attention on extracting high purity acetonitrile from its aqueous solution. Dhamole et al. [118] showed that the concentration of acetonitrile in the top phase was increased as increasing the glucose concentration under constant temperature. The concentration of acetonitrile was decreased with increasing temperature at constant glucose concentration. The results of the tie-line length and its slope revealed that the best extraction conditions are 279 K and glucose concentration 180 g·L⁻¹ [118]. On the other hand, Wang et al. [119] investigated the separation of acetonitrile from its aqueous solution through the sugaring-out system. The initial concentrations of acetonitrile in the top phase were 87.6%, 74.5%, 91.1%, 81.4% and 81.7% by adding glucose (25 g·L⁻¹), xylose (15 g·L⁻¹), arabinose (25 g·L⁻¹), fructose (25 g·L⁻¹) and sucrose (30 g·L⁻¹), respectively. As increasing sugar concentration (50 g·L⁻¹), the concentrations of acetonitrile in the top phase was increased to 95.4% (glucose), 90.9% (xylose), 89.1% (fructose) and 90.4% (sucrose), respectively, whereas the concentration of acetonitrile decreased to 89.1% by adding arabinose [119].

6 Conclusions

Over the past decade, the development of biorefinery technology facilitates the production of a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat) from renewable biomass by microorganisms. However, the composition of fermentation broth is still complex. The procedures of traditional separation method include pretreatment, removal of residual sugar, bacteria, protein, and organic acid in the fermentation broth, concentration treatment, followed by repeated distillation, condensation and fractional equalization. They are complicating technology; require high energy consumption and cost; obtain low separation efficiency and product purity; and follow strict requirements on operating conditions and equipment. Salt/sugar induce novel liquid-liquid extraction system can be divided into salting-out/sugaring-out and salting-out/sugaring-out extraction according to the types of extractants.

This review introduces SOE, salting-out, sugaring-out extraction, and sugaring-out that extract biobutanol, 1,3-

propanediol, 2,3-butanediol, acetoin, organic acids and other bio-based chemicals from fermentation broths/ aqueous solutions. Salting-out/sugaring-out extraction and salting-out/sugaring-out technologies show the high separating efficiency and the high targeted product yields. In addition, they are easy to operate and require low cost for separating products. The salting-out agents in the lower phase can be recycled through simple evaporation or membrane filtration and the sugaring-out agents in the lower phase can be coupled with the fermentation process. These methods have good selectivities to the targeted products and reduces the energy consumption by 20%–70% compared to the conventional distillation method. Hence, they are the effective and potential technologies for separating targeted products in the wide industrial applications.

Due to the advantages of high efficiency and easy scale-up, salting-out/sugaring-out and salting-out/sugaring-out extraction are widely used in the separation of biofuels and bio-based chemicals. There novel liquid-liquid extraction systems integrate solid-liquid separation, partial impurity removal and target product concentration into one step. Thus these liquid-liquid extraction methods are a simple and effective technique for the separation of hydrophilic fermentation products, such as glycols and organic acids. However, the high cost caused by the use of large amounts of salts, solvents or polymers limits their industrial application. There are still some problems that need further study. Firstly, recycling of solvents and salts is one problems. Currently, the recovery of the solvents is not a problem, but the difference in the water content and different boiling points of the solvents in the upper phase cause the difference in energy consumption. The recovery and recycling of the salts need to be further explored to achieve a much lower energy consumption. For example, developing a facile membrane technology for the concentrated saline solution will help reduce the cost on salt recycling remarkably. Secondly, pigment is another problem. Fermentation broth usually contains a certain amount of pigment, and the color of some components of the feedstock such as molasses and other raw materials is darker. The salting-out/sugaring-out and salting-out/sugaring-out extraction systems currently studied are mostly hydrophilic solvent-based systems, and the pigments are almost evenly distributed in the upper and lower phases. The pigments gradually accumulate as the post-processing proceeds, which will increase the difficulty of decolorization. In addition, there is no corresponding industrial equipment currently for these newly developed technologies.

The successful research into the salting-out/sugaring-out and salting-out/sugaring-out extraction not only affords biofuels and biochemical but also opens a door for the development of novel separation methods. Understanding how to save energy using these novel methods is a classic challenge in industries. The integration of salting-out and

distillation shows an example for the energy-saving effect on the target products recovery from their fermentation broth. The salting-out/sugaring-out and salting-out/sugaring-out extraction were demonstrated to be promising separation technologies in the downstream separation process of bio-based chemicals from the perspective of operation and efficiency. More technical understanding and techno-economic analyses are needed for different fermentation broths, which will be crucial in evaluating which novel liquid-liquid extraction will ultimately be the most promising in separating biofuels and biochemicals.

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