Vol. 3, No. 3 Science and Engineering June, 2024
第 3 卷第 3 期 科学与工程 2024 年 6 月

Bio-based Isobutanol: An Emerging Attractive Biofuel

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Abstract: Increasingly serious energy and environmental problems have led to a growing interest in the study of biomass energy. Isobutanol (2-methyl-1-propanol) has become a new generation of biofuel with excellent characteristics such as high octane number, high energy density, low vapor pressure, low hygroscopicity, etc., and it is an ideal component for gasoline blending. However, this important compound is mainly produced by chemical synthesis, which not only consumes limited non-renewable human resources but also pollutes the environment. Compared with traditional chemical synthesis, microbial fermentation has the advantages of mild conditions, easy operation, few by-products, environmental protection, energy saving, and cost reduction, especially the easy availability of raw materials and the utilization of renewable resources indicating its promising development prospect. This review summarized the production of isobutanol by fermentation, especially the pioneer work on improving the isobutanol titer in the fermentation broth. For the bioisobutanol fermentation, another challenge is to recover this compound from the fermentation broth efficiently. The current methods using distillation have the disadvantage of being too energy-intensive, raising the cost of the fermentation method. Adsorption, gas stripping, membrane osmotic vaporization, membrane extraction, and liquid-liquid extraction, including salting-out, are new methods for the separation of bio-based isobutanol, which are very important for more efficient recovery of bio-isobutanol. The advantages and disadvantages of each separation technique were summarized. The large amount of water in the isobutanol fermentation broth results in high energy consumption for its separation, and also reduces the sugar load of the fermentation, and increases the amount of water used for fermentation. Further development of microorganisms that can increase solvent titer or methods for in-situ product removal should be developed to improve the market competitiveness of bioisobutanol..

Keywords: Bio-based Isobutanol; Biofuel; Fermentation; Separation; Salting-out

DOI: 10.57237/j.se.2024.03.002

1 Introduction

Biofuels have become a popular area of research, especially fuels obtained from liquid fermentation techniques. Currently, non-renewable energy sources, such as fossil energy, dominate the overall consumption of energy in the world. It has contributed to the high rate of modern economic development and provided an important basis for the production and development of human society [1]. The global population is expected to reach 8.92 billion by 2050, while world energy use will increase by 53% by 2035. Therefore there is an urgent need to develop new sources of energy to fill possible shortages and potentially replace our fast depleting oil reserves. Biomass energy as a renewable clean energy with its green and recyclable characteristics has entered our vision. Therefore, the vigorous development of bioenergy is of great significance to our energy security and is in line with the sustainable de-

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Received: 25 March 2024; Accepted: 17 May 2024; Published Online: 23 May 2024

velopment strategy [2].

The two most widely used biofuels as energy are ethanol and biodiesel, which are important renewable bioenergy sources [3, 4]. Bioethanol can be used as a biofuel or fuel additive and is currently booming industrially [5]. Bioethanol is mainly obtained from corn, molasses, and straw through fermentation degradation [6]. Biomass resources (e.g., crop residues, forestry wastes, and other lignocellulosic feedstocks) are abundant in China, and the production of bioethanol from these inexpensive waste resources not only overcomes the dependence of chemical ethanol on petroleum resources but also meets the demand for bioethanol for the future production of transportation fuels. Biodiesel, on the other hand, can be produced from vegetable oils or animal fats by pyrolysis, microemulsification, or transesterification, but there are problems such as expensive equipment and imperfect processes [7]. As an alternative to biofuels, isobutanol, with its longer carbon chain, has greater energy density and octane number, lower vapor pressure, and lower hygroscopicity than ethanol, which makes it superior to ethanol and biodiesel [8]. Isobutanol's 30% higher performance as a renewable energy source compared to ethanol and its higher fuel value-added make it a more promising alternative to other gasoline blending components [9]. Moreover, isobutanol can achieve higher blending ratios with gasoline, so isobutanol gasoline fuel may become one of the renewable and clean energy pathways to supply automotive energy [10].

2 Isobutanol Overview

2.1 Physicochemical Properties of Isobutanol

Isobutanol (2-methyl-1-propanol), its isomer is n-butanol. The boiling point is 108 ℃, the melting point is -108 ℃, relative density is 0.806 at 15 ℃. Vapor pressure is 10.43 mm Hg / 13.9 hPa at 25 ℃. Water solubility is 85.0 g/L [11, 12]. These properties reveal that isobutanol is lighter and soluble in water. It is a four-carbon achiral branched organic alcohol with molecular formula $C_4H_{10}O$, chemical structural formula $(CH_3)_2CHCH_2OH$, molecular weight 74.12, a colorless transparent liquid with a peculiar odor, which is miscible with many solvents, such as water, alcohols, and ethers.

2.2 Uses of Isobutanol

One of the major uses of isobutanol is the production of isobutyl acetate, which is mainly used in surface coatings and adhesives. Isobutanol has a wide range of downstream markets [8]. Isobutanol is an important raw material for the synthesis of plasticizers, antioxidants, artificial musks, essential oils of fruits, and pharmaceuticals, and is an important ingredient in the production of paints and varnishes, and as a surrogate for n-butanol. In addition, isobutanol is a highly potential new generation bioenergy [13]. In addition, isobutanol has the advantages of low vapor pressure, low corrosiveness, and easy pipeline transportation.

2.3 Synthesis Method of Isobutanol

2.3.1 Chemical Synthesis of Isobutanol

Chemical synthesis is currently the main production route for industrial production of isobutanol, which includes the carbonyl synthesis of isobutanol from propylene, the preparation of isobutanol from syngas [14], the production of isobutanol from isobutylene [15], the preparation of isobutanol from catalyzed low carbon alcohols [16], etc., of which the carbonylation of propylene is the main technology used in the industry at present. Industrially, isobutanol is produced by the carbonylation of propylene (organic/inorganic compounds doped with carbon monoxide). Propylene carbonylation can be carried out under high, medium, and low-pressure conditions. Currently, low-pressure synthesis catalyzed by rhodium catalysts has largely replaced high-pressure synthesis. However, the raw material for the chemical synthesis of propylene mainly comes from petrochemical raw materials, mainly olefins separated by distillation during the refining of petroleum in refineries. This not only consumes the limited non-renewable resources of human beings but also causes environmental pollution.

2.3.2 Biological Synthesis of Isobutanol

Biological isobutanol can be obtained by fermentation, and its substrates can be molasses, straw wood fiber, etc [17–19]. The production of isobutanol by fermentation can be achieved economically in two ways: (1) the development of highly tolerant or productive strains, where increasing the solvent tolerance of the strain facilitates the extraction and recovery of isobutanol, and (2) the use of energy-efficient process engineering techniques to isolate and purify isobutanol while removing toxic products. It is noteworthy that the first method for fermentation for isobutanol production was reported by Baez et al. in 2011 with great success. Many attempts have been made to improve the performance of strains for butanol production. The highest concentration of isobutanol fermentation broth that can be obtained so far reaches 22 g/L. However, the industrial fermentation-extraction of n-butanol is mature from a process engineering point of view, and we can use existing installations to improve them to produce isobutanol in an integrated and centralized manner with integrated fermentation-extraction, where the fermentation and the recovery of the product are carried out at the same time. Using such an integrated system (gas stripping and osmotic extraction), the concentration of isobutanol obtained is 51.9 g/L.

The traditional chemical synthesis method not only consumes petroleum and is highly polluting, compared with the microbial fermentation method, but the reaction conditions also are mild, the production process does not need to provide additional heat, environmental protection, and energy saving; and easy to operate, no need to add metal catalysts, which can overcome the dependence of the chemical method on petroleum resources. In addition, the use of engineered bacteria modification can make the reaction targeted, less by-products, and reduce costs. Its biggest advantage is the use of renewable resources, in line with the requirements of sustainable development, and raw materials from a wide range of easily accessible and inexpensive. In China, biomass resources such as lignocellulosic biomass can be used to ferment the production of isobutanol, so that it has the potential to become a petroleum substitute or as a supplement to gasoline, to achieve the purpose of turning waste into treasure. Lignocellulosic biomass, which may contain xylan, arabinoglycan, galactan, acetic acid, ferulic acid, and coumaric acid, is one of the most abundant renewable resources on the planet and has great potential as a fermentation substrate for the production of isobutanol, which has been receiving increasing attention recently for the production of biofuels. Because of its low energy requirement and high biomass yield. Sugar conversion in lignocellulosic biomass fermentation is preceded by pretreatment. Mosier et al. in 2005 pointed out that the goal of pretreatment is to disrupt the structure of the biomass and to unravel the lignocellulosic carbohydrate complex so that the corresponding enzymes can hydrolyze the carbohydrates converted to monosaccharides more rapidly to obtain greater yields. Economic analyses of current pretreatment methods indicate that the relatively high cost of biofuels from lignocellulosic biomass is primarily related to three factors: (1) their use of harsh pretreatment conditions (high temperatures, high pressures, use of acids or bases, long residence times, allowing for the formation of inhibitors, etc.); (2) the overuse of costly enzymes, which renders the recovery stage uneconomical; and (3) the recycling of the end product. Improved technology in any of these areas would help make the production of isobutanol from energy crops more economically viable. In addition, energy crops can be genetically modified to increase biomass production.

Given that microbial fermentation for isobutanol production has been developing at a rapid rate, the use of energy crops such as lignocellulosic biomass as a feedstock for the production of isobutanol is achievable. The production of isobutanol from low-cost lignocellulosic biomass, which does not compete with food crops, solves the problem of food security and reduces production costs. So low-cost fermentation substrates may become the key to economically efficient isobutanol fermentation production. To make microbial fermentation production of isobutanol a potential development trend, the use of non-food raw materials such as lignocellulose to produce bio-isobutanol should be vigorously developed. In addition, the production of isobutanol from fermentation broth has advantages over n-butanol: 1. The microorganisms producing isobutanol have a higher tolerance compared to n-butanol. 2. The boiling point of isobutanol is lower compared to n-butanol, 108 ℃ compared to 118 ℃, respectively, so it will be more economical in the recycling stage.

3 Progress in Bioisobutanol Research

3.1 Market Background and Industrialization Applications

At present, the foreign carbonylated production market of isobutanol is mature, of which the United States, Western Europe, Japan, and other countries are particularly obvious. in 2006, the total production capacity of foreign isobutanol was 473,000 t/a, of which Asia is the largest production region.

With the booming development of China's chemical industry, China's demand for isobutanol is getting higher and higher, and it has become the largest consumer of isobutanol. In recent years, many new isobutanol reactors have been built in China, which has reduced some of the domestic supply and demand conflicts. In 2006, the total production capacity of domestic isobutanol was 81,500 t/a, but the domestic production of isobutanol is still in a state of demand exceeding the supply, and China needs to import a larger amount of isobutanol every year. Since the production of domestic isobutanol mainly involves the carbonylation of propylene, with the continuous increase of oil prices and the unstable international oil situation, it is crucial to improve and increase the production of domestic isobutanol and to find a way to produce isobutanol by using renewable energy. Cheng Jia et al. predicted that the shortage of domestic isobutanol will further expand.

3.2 Progress in the Biosynthesis of Isobutanol

Bioethanol and n-butanol have been produced industrially by fermentation of Saccharomyces cerevisiae and acetone butanol carboxylic acid bacteria [20, 21], whereas so far scientists have not found the original strains that can directly synthesize isobutanol. The Ehrlich pathway was invented in 1904 when Ehrlich discovered that the addition of amino acids to a yeast fermenter could increase the yield of the corresponding alcohols. The pathway for the catabolism of amino acids to produce higher alcohols is commonly referred to as the Ehrlich pathway. The Ehrlich pathway is currently our primary method of biosynthesizing isobutanol through biosynthesis [22]. A more detailed study of the Ehrlich pathway was done by Neubauer and Fromherz in 1911, which concluded that the precursors in the metabolism of this amino acid are 2-keto acid analogs, which undergo catalytic decarboxylation to produce aldehydes and eventually alcohols.

According to the Ehrlich pathway, the 2-keto acid decarboxylase KDC (encoded by the kivd gene) and the dehydrogenase ADH (encoded by the adh2 gene) have a crucial role in the catalysis of 2-keto acids and the production of isobutanol in the production of higher alcohols. When the kivd gene from Lactobacillus and the adh2 gene from Saccharomyces cerevisiae were cloned and expressed in Escherichia coli, they were able to produce six-carbon long-chain alcohols including 1-propanol, 1-butanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol (Atsumi et al. 2008). 2008 Atsumi et al. found that this strategy utilized the ketoacid pathway, a highly active amino acid biosynthesis pathway present in the host microorganism, and that the host was able to reset the 2-ketoacid intermediate to synthesize higher alcohols. The metabolic pathway can vary in different hosts, with the main organisms being Saccharomyces cerevisiae and Escherichia coli.

3.2.1 The Pathway of Isobutanol Metabolism by SACCHAROMYCES Cerevisiae Yeast

Indeed, the catabolic amino acids of Saccharomyces cerevisiae have been extensively studied. The biological production of isobutanol was first reported by Dickinson et al. in 1998, who demonstrated that Saccharomyces cerevisiae was able to utilize C13-labeled valine as a substrate for the production of isobutanol. It has been hypothesized that there are four possible routes by which the product of valine amino transfer, α-ketoisovaleric acid, can be obtained as isobutanol, including (1) branched-chain α-ketoacid dehydrogenase catalyzes α-ketoisovaleric acid as a means of generating isobutyryl-coenzyme A, which is then reduced to isobutanol; (2) pyruvate decarboxylase (PDC) catalyzes the generation of isobutanol from α-ketoisovaleric acid; (3) α-ketoisovaleric acid reductase reduces α-ketoisovaleric acid to α hydroxyisovaleric acid; and (4) use of an enzyme encoded by YDL080c to produce isobutanol. Later experiments confirmed that α-ketoisovaleric acid is catalyzed by pyruvate decarboxylase PDC to isobutanol [23]. Scientists have since studied the metabolism of various amino acids by Saccharomyces cerevisiae, among which isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine are metabolized by Saccharomyces cerevisiae via the Ehrlich pathway to produce 2-methylbutanol (reactive pentanol), 3-methylbutanol (isoamyl alcohol), methylmercaptan, 2-phenylethanol, tryptophan, p-hydroxyphenylethanol and isobutanol, respectively. The Ehrlich pathway is prevalent in yeast fermentation production and is particularly active when yeast is grown in a medium where the carbon source is a single amino acid. In 2012, Lee et al. hypothesized that the inefficient production of isobutanol by Saccharomyces cerevisiae is due to a lack of KIV, the precursor 2-ketoisovaleric acid for the valine biosynthesis pathway, and subsequently they added exogenous KIV to the growth medium at 0.5 g/L. As expected, the growth medium was supplemented with exogenous KIV. expected, supplementation of KIV in the substrate increased isobutanol production, suggesting that the endogenous pathway of KIV production by Saccharomyces cerevisiae is the rate-limiting step in the isobutanol production pathway. Next, Chen et al. evaluated the effect of overexpression of genes ILV2, ILV3, ILV5, ILV6, and BAT2 in different combinations in host brewer's yeast with respect to isobutanol production. The recombinant strain

ILV235_XCY561 produced 0.97 mg isobutanol/ g glucose, which is six times higher than the control strain when the ILV2, ILV3, ILV5 overexpression strain (ILV235_XCY561) and the reference strain (CEN.PK113-5D) were cultured under anaerobic conditions. Thus, demonstrating the fact that overexpression of genes ILV2, ILV3, and ILV5 may increase the concentration of KIV, allowing an increase in the production of isobutanol.

3.2.2 Metabolism of Isobutanol by Escherichia Coli

Using E. coli as a host, Atsumi et al. in 2008 demonstrated that KIV is an important precursor for the biosynthesis of isobutanol. In addition, in order to obtain heterologous overexpression of genes for the production of highly efficient isobutanol-producing microorganisms, they removed six genes, adhE, ldhA, frd, fnr, pta, and pflB, whose deletion led to an increase in the amount of pyruvic acid, which favored the production of isobutanol. The yield of isobutanol at this time was 0.21 g isobutanol/g glucose. They then used the alsS gene from Bacillus subtilis and the ilvCD gene from E. coli for overexpression in E. coli and found that the strains could accumulate significant amounts of 2-ketoisovaleric acid, KIV, in fermentation broth. They introduced two heterologous enzymes into E. coli, 2-ketoisovaleric acid decarboxylase, kivD, from Lactobacillus, and ethanol dehydrogenase, adhA, from Saccharomyces cerevisiae, which were used to synthesize valine in E. coli. an intermediate metabolite of the E. coli valine synthesis pathway, 2-ketoisovaleric acid KIV node, shunts the carbon flux into the isobutanol synthesis pathway, realizing the biosynthesis of isobutanol in E. coli [21]. In addition, they increased the expression of 2-ketoisovaleric acid in the upstream pathway by applying the PLlacOI promoter to overexpress the ilvIHCD gene, enabling E. coli to produce fermented isobutanol at a yield of 22 g/L [24]. When kivd and adh2 were co-expressed in this recombinant strain, 22 g/L of isobutanol was produced after 112 hours of fermentation [16]. It is very important for improving the fermentation titers at shorter fermentation time [25].

4 Separation process for the Biological Isobutanol

Methods for the production of higher alcohols such as isobutanol using E. coli have been developed and have shown promising results. The isobutanol fermentation broth obtained using microbial fermentation without any treatment optimization has a very low concentration of isobutanol, generally below 22 g/L, and the main components are water, organic acids, and bacterial cells [24]. Firstly, the current industrial production of bioethanol, bio-n-butanol separation, and extraction process has been mature; secondly, the current research on the separation and extraction of bio-isobutanol is very little, but we summarize some novel methods for the extraction of bio-isobutanol. Adsorption [26–29], gas stripping [30], membrane osmotic vaporization [30–35], membrane extraction, and liquid-liquid extraction [36–39], including salting-out [40–47] for the separation of bio-based isobutanol were summarized in this review.

4.1 Gas Stripping

Here, Baez et al. [30] expanded the E. coli strain from shake flask production to produce isobutanol in a 1L bioreactor. Isobutanol was then extracted from the bioreactor in situ using vapor extraction and the results obtained were that the E. coli engineered strain (JCL260) produced more than 50 g/L of isobutanol in 72 hours. The ability to produce isobutanol using vapor extraction is approximately twice that of n-butanol and at a concentration 9% higher than that of n-butanol. However, the current work on this method is not yet complete, and the results they have produced are a simple strategy with no optimization studies.

4.2 Membrane-Based Separation

Omidali used hybrid hydrophobic/hydrophilic osmotic vaporization to separate and purify isobutanol from dilute solutions [48]. For this purpose, they first used a composite polydimethylsiloxane membrane to recover isobutanol by hydrophobic osmotic vaporization. Then the hydrophilic composite poly (vinyl alcohol) membrane was used to separate the water from the organic phase by osmotic vaporization. At the same time, they investigated the effect of feed flow rate on the performance of osmotic evaporation. They also applied a series of models to calculate the transport resistance through the composite membrane. It was observed that an increase in feed flux resulted in higher permeate flux and greater selectivity of the permeate component, while the flux of the impermeable component was almost constant. In addition, by increasing the feed flow rate, the resistance ratio from the liquid boundary layer to the film layer was reduced. Concentrations of up to 99% of isobutanol were obtained from a 2% aqueous solution of isobutanol by hybrid hydrophobic/hydrophilic osmotic vaporization. It can be seen that the membrane permeation vaporization method can separate the mixed aqueous solution efficiently and can selectively separate the products. However, the use of this method is limited by the high cost of membrane materials and the difficulty of cleaning and contamination.

A comprehensive comparison of these two separation methods, the vapor extraction method, which can make full use of the fermentation process of carbon dioxide, hydrogen, and other gases, and effectively remove the fermentation process on the fermentation of inhibition of the product, which is beneficial to the fermentation. However, the separation effect is not as good as that of membrane permeation vaporization. Membrane permeation vaporization allows butanol, acetone, and ethanol, which are below the boiling point of water, to pass through the membrane module under vacuum conditions and then be vaporized, making it easy to recover them and selectively separating the products by using the selectivity of the membrane. However, the current difficulty is that it is difficult to select inexpensive membrane materials that can be widely used in industry. Therefore, the separation and extraction process for the biological production of isobutanol needs to be further explored, which is directly related to whether the bioengineered production of isobutanol can be industrially produced.

4.3 Distillation

Distillation is a simple operation and industrialization is a widely used purification method. In biological fermentation purification, the United States and other developed countries have used distillation for bioethanol purification has done a lot of research work. 2004 July, the United States bioethanol production capacity of 3.3 billion gallons per year. They mainly use evaporative distillation to purify and recover ethanol fermentation broth. However, this method is energy intensive and consumes almost 18% to 22% of the combustion value of the ethanol produced. Although evaporative distillation is well established in industry, it is too energy-intensive to be economically viable. With the reaction, the tower kettle temperature, and salt concentration will be higher and higher, the bottom of the kettle is easy to coke, and the equipment has a certain impact. Therefore, from the direction of sustainable development, the evaporative distillation method can not meet the requirements of economic energy saving.

4.4 Adsorption

Because the evaporation method is too energy-consuming, the former proposed the use of resin adsorption to separate n-butanol. Adsorption refers to the selective adsorption of an adsorbent on a component of a liquid or gas, which plays the role of enrichment. Adsorption has become a well-established unit operation. Resin adsorption is easy to regenerate and has advantages such as high selectivity. At present, the use of resin for adsorption of bio-n-butanol has been realized in industrial production. The simulated fermentation broth in the experiment can accurately predict the actual fermentation. To ensure the practicality, scientists also conducted desorption experiments. The results showed that about 95% of the fermentation products could be recovered under the condition of 140 ℃ steam.

In summary, the key to resin adsorption is to find a suitable adsorbent for isobutanol with high selectivity and wear resistance. Large-scale application of this technology is difficult. Therefore, the separation of bioisobutanol by resin has some limitations.

4.5 Liquid-liquid Extraction

The liquid-liquid extraction method is also called the solvent extraction separation method, which is a process of separating isobutanol from the fermentation broth of isobutanol by mainly utilizing the difference in solubility or partition coefficient of isobutanol in the two phases that are not miscible (or slightly soluble) [31]. The essence of the extraction process is to convert isobutanol from hydrophilic to hydrophobic, so that it enters the organic phase, thus achieving the separation process. Extraction can be categorized into physical and chemical extraction.

Other important criteria are safety (flammability and toxicity), environmental risk factors (wastewater, solid waste, or solvent volatilization), and so on [29]. If the extractant used is in direct contact with the fermentation broth, it must not inhibit the fermentation of the strain and be non-toxic to the fermenting strain. Oleyl alcohol is considered to be the best solvent for the extraction of butanol due to its high affinity for butanol and its lack of toxicity to the cells over a short period of time [30]. However, high-purity oleyl alcohol is too expensive, making the cost of extraction to recover butanol less economically feasible.

4.6 Salting-out-based Extraction

In the process of extraction experiments carried out by previous generations, the extractant's role in the extraction of solutes is the first factor that people consider in the selection of the extractant. In the past, they concentrated on the study of the extraction process, and the role of the mechanism in the extraction process between the extractant and the solute. The main reason for the bad effect of extraction and separation of some systems is that the extractant can dissolve a higher concentration of solvent components, which reduces the selectivity of the extractant to the solute, thus affecting the separation effect of the system. Therefore, to make these systems get better separation, people must reduce the solubility of the solvent component of the extractant. However, people have not paid attention to the study of the process of extractant-solvent component interaction.

Among the polar extractants, water, which exists in large quantities in nature, has the advantages of being cheap, simple to obtain, easy to procure, non-toxic, harmless, stable, and polar, and is often prioritized as a polar extractant [44, 47, 49, 50]. However, due to the water extractant extraction process is often dependent on the competition for solute dissolution of the physical extraction, extraction efficiency is not high, in the actual production of the application is not large. If some suitable ionic groups can be added to the water to increase the chemical extraction of the solute extractant, it can strengthen the extraction effect of the extractant in general [40, 41, 51]. After research, it was found that the addition of one or more salts [52–59], dispersants, and complexing agents to the water composed of a composite extractant is an excellent composite extractant [58–60]. As this solvent extraction extractant is used by the composite salt, complexing agent, dispersant, and water, its extraction mechanism is not only the traditional solvent extraction of physical extraction but also contains the chemical extraction and the composite extractant to strengthen the extraction process. It mainly lies in the exclusion of the solvent, which will be used in the process of this composite extractant extraction, called the salting-out-based extraction process [61, 62].

The results of the salting-out experiments showed that $K_4P_2O_7$ had the best separation and dehydration effects [63]. When the initial molar salt concentration of $K_4P_2O_7$ reached 1.63 $mol \cdot kg^{-1}$, the recovery of isobutanol could reach 100% and the dehydration rate was up to 97.19%. Compared with the salt/solvent extraction method, when the isobutanol was completely recovered, the amount of salting-out agent required by the salt precipitation method was much higher than that of the salt/solvent extraction method with 1.01 $mol \cdot kg^{-1}$ $K_4P_2O_7$, and the higher salt content increased the cost and energy consumption of the subsequent salt recovery [64, 65].

The results of the salt/solvent extraction experiments showed that the recovery and dehydration of isobutanol increased significantly with the increase of the initial molar salt concentration. When the initial molar salt concentration of $K_4P_2O_7$ was equal to or higher than 1.01 $mol \cdot kg^{-1}$ and the mass ratio of isooctanol to dilute aqueous solution was 1:1, the recovery of isobutanol was able to reach 100%, and the dehydration rate was able to reach 91.62%, which was satisfactory. As the salt concentration continued to increase, the dehydration rate also increased significantly, and when the concentration of $K_4P_2O_7$ reached saturation, the dehydration rate could reach up to 97.19%, with an ideal dehydration effect. $K_4P_2O_7$ was demonstrated to be superior to other salts [66–70].

5 Conclusion and Outlook

This paper reviews the research progress of isobutanol production by fermentation. For the bioisobutanol fermentation, one of the challenges is the low titer of bioisobutanol. Another challenge is the recovery of this compound from the fermentation broth efficiently. The current methods using distillation have the disadvantage of being too energy-intensive, raising the cost of the bio-based method. Adsorption, gas stripping, membrane osmotic vaporization, membrane extraction separation, and liquid-liquid extraction, including salting-out, are new methods for the separation of bio-based isobutanol, which are very important for more efficient recovery of bio-isobutanol.

The large amount of water in the isobutanol fermentation broth results in high energy consumption for its separation, and also reduces the sugar load of the fermentation, and increases the amount of water used for fermentation. Further development of microorganisms that can increase solvent titer or methods for in-situ product removal should be developed to improve the market competitiveness of bioisobutanol..

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