Recent Advancements and Strategies of Improving CO₂ Utilization Efficiency in Bio-Succinic Acid Production

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Abstract: The production of bio-based succinic acid through microbial CO₂ fixation and conversion has gained significant attention as a promising approach to mitigate greenhouse gas emissions. However, the low CO₂ utilization efficiency limits the efficient biosynthesis of succinic acid. Therefore, it is crucial from environmental and economic perspectives to enhance the efficiency of CO₂ utilization in bio-succinic acid production. This review comprehensively covers the introduction of biosynthetic pathways for microbial CO₂ fixation and the conversion of CO₂ to succinic acid, as well as the challenges associated with CO₂ supply and utilization effectiveness. Moreover, strategies including genetic and metabolic engineering for CO₂ fixation, extracellular supply methods of CO₂ and some potential technical approaches for CO₂ capture (such as micro-nano bubbles, CO₂ adsorption material and biofilm) are summarized and presented.

Keywords: bio-succinic acid; microbial CO₂ fixation; CO₂ utilization efficiency; bioprocess optimization

1. Introduction

The large-scale emission of CO₂ is believed to be a major cause of global climate change and is taking a serious toll on human lives and livelihoods. The Chinese government has put forward the goal of “strive to peak CO₂ emissions by 2030 and strive to achieve carbon neutrality by 2060”. Recent technological advances in CO₂ capture, utilization, and storage are expected to significantly contribute to carbon removal [1]. In these methods, carbon utilization shows the greatest potential, by recycling captured CO₂ and harnessing it as a resource to produce fuels and chemicals. Third-generation biorefineries have been widely considered due to their mild conditions, good selectivity and environmental friendliness; they aim to utilize microbial cell factories to convert renewable energies and atmospheric CO₂ into fuels, chemicals and biodegradable plastic [2], thereby offsetting the cost of CO₂ capture and generating economic benefits [3]. At the same time, the transition from fossil fuels to biofuels and bio-based chemicals would greatly reduce carbon emissions [4–6].

Succinic acid (SA), a four-carbon dicarboxylic acid, is one of the most promising bio-based platform chemicals. It is used as a starting material for industrially important chemicals such as adipic acid, 1,4-butanediol, γ-butyrolactone and tetrahydrofuran and as feedstock for the production of biodegradable polybutylene succinate [7]. The production of SA by microbial fermentation has attracted widespread attention in recent years because of its advantages of utilizing renewable resources and fixing CO₂. In the reduction tricarboxylic acid pathway, the synthesis of 1 mol SA can theoretically fix 1 mol CO₂. Some studies have shown that the CO₂ fixation rate of the microbial anaerobic synthesis of SA was hundreds of times that of microalgae [8]. The strains used for anaerobic carbon sequestration synthesis of SA are usually divided into natural strains and metabolically engineered strains such as Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens, Basfia succiniciproducens, Escherichia coli and Corynebacterium glutamicum,
as shown in Table 1. As the main producing succinate strains, *E. coli*, *A. succinogenes* and *C. glutamicum* have been gaining more attention [9].

Table 1. Bio-based SA production using different bacterial species.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Fermentation type</th>
<th>Titer (g L(^{-1}))</th>
<th>Productivity (g L(^{-1}) h(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. succiniciproducens</em></td>
<td>Anaerobic, continuous culture</td>
<td>83</td>
<td>10.4</td>
<td>[10]</td>
</tr>
<tr>
<td><em>A. succinogenes</em> FZ53</td>
<td>Anaerobic batch</td>
<td>105.8</td>
<td>1.36</td>
<td>[11]</td>
</tr>
<tr>
<td><em>A. succinogenes</em> ATCC 55618</td>
<td>Anaerobic fed-batch</td>
<td>151.44</td>
<td>3.22</td>
<td>[12]</td>
</tr>
<tr>
<td><em>B. succiniciproducens</em> JF4016</td>
<td>Anaerobic batch</td>
<td>19</td>
<td>1.9</td>
<td>[14]</td>
</tr>
<tr>
<td><em>E. coli</em> JW1021</td>
<td>Dual-phase, fed-batch</td>
<td>114.0</td>
<td>3.25</td>
<td>[15]</td>
</tr>
<tr>
<td><em>E. coli</em> (Tang1527)</td>
<td>Dual-phase batch</td>
<td>89.4</td>
<td>1.24</td>
<td>[16]</td>
</tr>
<tr>
<td><em>C. glutamicum</em></td>
<td>Anaerobic batch</td>
<td>64.16</td>
<td>1.07</td>
<td>[17]</td>
</tr>
</tbody>
</table>

Molina Grima et al. [18] mentioned that the cost of compressed CO\(_2\) can be up to 41% of the total raw material costs in biomass production. Some studies have suggested that the flue gas and flaring gas generated from industrial exhaust gases, which typically contain 5–90% CO\(_2\), could be used as raw materials for microbial carbon sequestration. In general, these industrial exhaust gases are free, which would greatly reduce the cost of CO\(_2\) feedstock [19,20]. Wu et al. [21] used succinate-producing *E. coli* 261 to fix CO\(_2\) from ethylene oxide off-gas (80–85 vol% CO\(_2\)); an SA titer of 68.12 g L\(^{-1}\) with a CO\(_2\) fixation rate of 4.7 mmol L\(^{-1}\) h\(^{-1}\) was achieved. He et al. recycled the off-gas (59.58% CO\(_2\), 39.89% H\(_2\), 0.21% N\(_2\)) of acetone–butanol–ethanol (ABE) fermentation as a co-substrate for SA production with *E. coli* BSM209 [22]; the fermentation results showed that a maximum SA concentration of 65.7 g L\(^{-1}\) and a high yield of 0.86 g g\(^{-1}\) glucose were achieved; these results were similar to the fermentation performance of pure CO\(_2\) gas.

Therefore, the use of microbial anaerobic carbon sequestration to synthesize SA can not only reduce CO\(_2\) emissions in chemical synthesis but also achieve the high-value conversion of industrial waste gas CO\(_2\); this is a green and sustainable carbon emission reduction production method with important environmental protection and economic value. In this review, the CO\(_2\) fixation metabolic pathways of the microbial synthesis of SA and the bottlenecks in CO\(_2\) utilization in bio-SA production are introduced, the research progress for improving the CO\(_2\) utilization efficiency of SA production strains is expounded, and the related technologies for enhancing the extracellular CO\(_2\) supply level to achieve the efficient synthesis of SA are also analyzed and prospected. This paper provides some strategies and ideas to promote the research and application of microorganisms to produce chemicals with efficient fixations of CO\(_2\).

2. Microbial Fixation of CO\(_2\) to Synthesize SA

CO\(_2\) availability plays a crucial role in driving the metabolic pathway to produce succinate [23]. CO\(_2\) exists in the form of free CO\(_2\), HCO\(_3^-\) and CO\(_3^{2-}\) simultaneously in the aqueous phase. CO\(_2\) is a non-polar small molecule that enters the cell by free diffusion, while HCO\(_3^-\) and CO\(_3^{2-}\) are ionic compounds that have difficulty crossing cell membranes [24]. Carbonic anhydrases (CAs) are zinc metalloenzymes classified into five structurally different classes α, β, γ, δ and ζ on the basis of their protein sequence [25]. The α-CA derived from mammals and some bacteria is used as a human pathological and therapeutic target [26]. The uses of β-CA derived from plants, algae, bacteria and archaea include CO\(_2\) transport, CO\(_2\) fixation and the global carbon cycle [27]. The γ-CA in methanogenic archaea can also be used as a therapeutic target [28]. The δ-CA and ζ-CA in diatoms affect CO\(_2\) concentrations for photosynthesis [29]. The CAs in *E. coli* belong to β-CA, which catalyzes CO\(_2\) hydration and dehydration [30].

Seven natural CO\(_2\) fixation pathways in microorganisms have been identified, including the Calvin cycle [31], the 3-hydroxypropionate (3HP) cycle [32], the reductive tri-
carboxylic acid (r-TCA) cycle [33], the 3-hydroxypropionate-4-hydroxybutrate (3HP/4HB) cycle [34], the reductive acetyl-CoA (rAc-CoA) pathway [35], the dicarboxylate/4-hydroxybutyrate (DC/4HB) cycle [36] and the reductive glycine pathway [37]. Among these, the r-TCA cycle could be used to synthesize SA (Figure 1). CO2 entering the cell is reversibly hydrated into HCO3− by Cas [38], then HCO3− and phosphoenolpyruvate (PEP) are catalyzed by PEP carboxylase (PPC) or PEP carboxykinase (PCK) into the reductive branch of the TCA cycle and finally converted into succinate [39]. PPC is the key enzyme and rate-limiting enzyme for the biosynthesis of SA, which is affected and regulated by the concentration of CO2, and a higher concentration of CO2 can improve the activity of PPC [40]. The PPC activity of A. succiniciproducens ATCC 29305 at pH 6.2 was 35.6 times as much as that at pH 7.2 because of excess-CO2-HCO3− growth conditions [41]. PCK catalyzes the formation of oxaloacetate (OAA) plus ATP from PEP, ADP and CO2, which has a low affinity for bicarbonate, a relatively low catalytic velocity, and is activated only under conditions of gluconeogenesis [42–44]. In the glucose metabolism of other organisms, pyruvate carboxylase (PYC) is another enzyme to fix CO2 besides PPC and PCK. OAA is synthesized by the carboxylation of pyruvate with PYC after PEP is converted to pyruvate [45]. Mckinlay and Vieille reported that the presence of CO2 could suppress the decarboxylation of OAA and malate into pyruvate, resulting in higher net flux into the C4-pathway and thus augmenting the final yield of SA [46].

**Figure 1.** CO2 delivery into cells and bio-SA production pathways. Abbreviations: PEP, Phosphoenolpyruvate; PPC, Phosphoenolpyruvate carboxylase; PCK, Phosphoenolpyruvate carboxykinase; OAA, Oxaloacetate; CA, Carbonic anhydrase.

**3. CO2 Supply Bottleneck in the Process of Microbial Carbon Sequestration to Synthesize SA**

As the main carbon backbone source of microbial anaerobic carbon sequestration synthesis, the CO2 concentration level and supply capacity are limiting factors affecting the synthesis efficiency of SA. The dissolution of CO2 gas in the fermentation broth is affected by temperature, gas partial pressure and agitation. The solubility of gas decreases with increasing temperature. The solubility of CO2 in water at 20 °C is 32% higher than that at 30 °C because as the temperature rises, the movement rate of gas molecules accelerates and the gas is easy to overflow. The solubility of a slightly soluble gas in solution at a given temperature is directly proportional to the partial pressure of the gas according to Henry’s Law because the vapor–liquid equilibrium shifts to the liquid phase as the partial pressure increases [47]. Song et al. [48] reported that the dissolved CO2 concentrations in the medium were 5.83 mM and 17.3 mM when CO2 partial pressures were 25.32 kPa and 75.97 kPa, respectively; CO2 was dispersed into water via stirring and the solubility of CO2
increased with the increasing stirring speed. Xi et al. [49] showed that increasing the stirring speed could promote mass transfer between the CO\textsubscript{2} gas and fermentation liquid. When the stirring speed was increased to 200 r·min\textsuperscript{-1}, the CO\textsubscript{2} fixation rate and SA production rate became stable at 0.53 g·L\textsuperscript{-1}·h\textsuperscript{-1} and 1.41 g·L\textsuperscript{-1}·h\textsuperscript{-1}, respectively. Moreover, the presence of carbonate and bicarbonate salts in the medium also affects the dissolved CO\textsubscript{2} concentration. Zou et al. [40] reported that the maximum dissolved CO\textsubscript{2} concentration was 20.22 mM without the addition of MgCO\textsubscript{3}, and the maximum dissolved CO\textsubscript{2} concentration was 159.22 mM when gaseous CO\textsubscript{2} and MgCO\textsubscript{3} were supplied.

During the anaerobic fermentation process, a significant portion of input CO\textsubscript{2} gas remains undissolved and quickly overflows in the form of bubbles, resulting in the low efficiency of CO\textsubscript{2} gas utilization by strains [50]. It was reported that CO\textsubscript{2} gas was selected as the only CO\textsubscript{2} donor during SA production with \textit{A. succinogenes} N\textsubscript{113}, the CO\textsubscript{2} ventilated rate was controlled at 0.75 L·min\textsuperscript{-1} (or 0.25vvm), the agitation rate was 200 r·min\textsuperscript{-1}, and finally the CO\textsubscript{2} fixation rate could reach 0.6 g·L\textsuperscript{-1}·h\textsuperscript{-1}, but only 2.2% of the CO\textsubscript{2} gas was captured by microorganisms and converted into SA [51]. Moreover, Lee et al. [52] found that the sparging of CO\textsubscript{2} did not significantly improve the yield of SA when the fermentation was controlled at pH 6.2, because the solubility of CO\textsubscript{2} was up to three times lower than pH 6.5, indicating that the pH can affect the solubility of CO\textsubscript{2} in the fermentation broth and as a consequence affect the availability of CO\textsubscript{2} for microorganisms. SA-producing strains have optimal fermentation pH values. A high SA production of 116.2 g·L\textsuperscript{-1} was achieved by engineered \textit{E. coli} SD121 in the range of pH 6.4–pH 6.8 [53]. \textit{A. succinogenes} ATCC 55618 produced 146.0 g·L\textsuperscript{-1} at pH 6.8 [12], and 134.25 g·L\textsuperscript{-1} SA was synthesized by \textit{M. succiniciproducens} PALK at pH 6.5 [13]. Liu et al. [54] compared different pH control methods on SA production by \textit{A. succinogenes}; the results showed that cells grew well throughout the whole process of anaerobic fermentation, and the yield of SA reached 81.5% when MgCO\textsubscript{3} was used as a pH buffer, which was superior to NaOH. Alkaline carbonates supply CO\textsubscript{2} and regulate pH simultaneously, the HCO\textsubscript{3}\textsuperscript{−} and CO\textsubscript{2}\textsuperscript{−} dissolved from carbonates can exist in the liquid phase for a long time and they are directly utilized by SA-producing microorganisms (Figure 1).

Due to the extremely high rate of CO\textsubscript{2} spillage in water, various carbonates or bicarbonates such as MgCO\textsubscript{3}, NaHCO\textsubscript{3}, Na\textsubscript{2}CO\textsubscript{3} and CaCO\textsubscript{3} have been employed as indirect CO\textsubscript{2} donors, to make CO\textsubscript{2} directly available in the liquid phase [23]. Moreover, when only MgCO\textsubscript{3} was added (without CO\textsubscript{2}) and N\textsubscript{2} gas was used to establish anaerobic conditions, an SA titer of 22.6 ± 0.5 g·L\textsuperscript{-1} was obtained from an initial sugars concentration of 40 g·L\textsuperscript{-1} by \textit{A. succinogenes} [55]. Therefore, carbonates are commonly used in bio-refineries for the preparation of bio-SA [11]. However, large amounts of carbonates are employed as indirect CO\textsubscript{2} donors for the biosynthesis of SA; this would increase the material cost greatly compared with the free CO\textsubscript{2} exhausts from industry. Moreover, approximately equal amounts of inorganic acids would be consumed to re-acidify succinate into SA in the separation process. A large number of inorganic salts were even generated during acidification, which would not only increase the separation cost but also damage the environment [56,57]. Overall, the use of carbonates as CO\textsubscript{2} donors does not meet the original intention of microbial carbon fixation. Improving the supply and conversion efficiency of CO\textsubscript{2} are key problems in SA production with microbial CO\textsubscript{2} fixation.

4. Research Progress of Improving the Efficiency of Microbial CO\textsubscript{2} Fixation

4.1. Research Progress in Intracellular Regulation to Improve Carbon Sequestration Efficiency

In recent years, many studies have been conducted to improve CO\textsubscript{2} utilization via metabolic engineering. For example, phosphoribosyltransferase (NAPRTase) is a rate-limiting enzyme of the NAD(H) synthesis system, which can enhance the CO\textsubscript{2} fixation ability of PYC by increasing the NAD(H) pool size when NAPRTase is overexpressed. Engineered \textit{E. coli} BA207 (a pflB, ldhA and PPC deletion strain with co-expression of PYC and nicotinic acid NAPRTase) showed good CO\textsubscript{2} fixation and SA production abilities. The CO\textsubscript{2} fixation rate of 83.48 mg·L\textsuperscript{-1}·h\textsuperscript{-1} and SA productivity of 223.88 mg·L\textsuperscript{-1}·h\textsuperscript{-1} were
achieved under 0.10 MPa of CO₂ partial pressure [45]. PCK is a powerful CO₂-fixing enzyme and plays a vital role in directing more carbon flow towards SA-producing C₄ pathways. Kim, Laivenieks, Vieille and Zeikus [44] reported that overexpression of A. succinogenes PCK in mutant E. coli K-12 ppc:kan led to a 6.5-fold-increased SA production. Moreover, CA is the key enzyme that reversibly catalyzes the conversion of CO₂ to HCO₃⁻; hence, improving the properties of CA could also improve CO₂ capture capacity [58,59]. Plasmids carrying the CA gene from cyanobacterium Anabaena sp. 7120 were constructed and overexpressed in E. coli BL21, carrying pET-cyanoa; the final SA concentration was increased from 1.624 g/L to 3.486 g/L and the activity of PPC was also five-fold increased, which was derived from the availability of higher concentrations of HCO₃⁻ [60]. Furthermore, a recombinant E. coli strain (SGJS120) overexpressing a codon-optimized CA gene derived from Hahella chejuensis KCTC 2396 and a PEPC gene was constructed to hydrate gaseous CO₂ to HCO₃⁻ and enhance carbon flux via oxaloacetate synthesis using HCO₃⁻; the amount of succinate was 4.09-fold higher than the control strain. This result demonstrates succinate production derived from CO₂ gas directly without the addition of carbonate [61]. However, the contact of intracellular CA with the substrate is still restricted to a large extent by the cell membrane [62]. In summary, the development of genetically engineered strains is essential for improving the efficiency of microbial CO₂ fixation, but metabolic modification cannot change the extracellular CO₂ supply level.

4.2. Research Progress to Promote Extracellular CO₂ Supply

Although there have been many successful cases of improving carbon sequestration efficiency through metabolic modification, these modifications do not change the extremely low level of extracellular CO₂ distribution in broth. Therefore, the external supply of CO₂ is also an important factor affecting the CO₂ utilization efficiency. Increasing the CO₂ partial pressure is a straightforward method to increase the gas transfer rate and solubility [63]. The effects of the dissolved CO₂ levels on cell growth and SA production by M. succiniciproducens were studied in pH-controlled batch fermentation at 39 °C and 1 atm under various CO₂ partial pressures; the maximum specific growth rate obtained at a dissolved CO₂ concentration of 23.0 mM under 101.3 kPa was 1.12 h⁻¹, which was 1.43 times higher than that obtained at a dissolved CO₂ concentration of 8.74 mM under 37.98 kPa; the yield of SA also increased from 0.389 g g⁻¹ to 0.460 g g⁻¹ [48]. These results show that higher dissolved CO₂ concentrations in the medium have positive effects on cell growth and SA production. In addition, Amulya et al. [3] evaluated the impact of different CO₂ partial pressures on SA production in a high-pressure gas fermentation reactor; the SA-specific productivity and SA concentration were 0.46 g L⁻¹ h⁻¹ and 14 g L⁻¹ at 2 bar, respectively, 3.83 and 6.76 times higher than that obtained at 0.6 bar. It is worth noting that high pressures may have a significant influence on cellular and molecular systems, specifically in the protein structure, cell permeability, enzymatic activity, formation of metabolic end products, etc. [64]. As an example, Cao et al. [39] reported that further increasing the bioreactor pressure above 0.4 bar inhibited the biosynthesis of SA by A. succinogenes 130 Z. High pressure can promote CO₂ dissolution in water, but it increases the manufacturing costs of bioreactors and makes the control of fermentation more complex. Furthermore, excessively high pressures may have a negative effect on SA-producing strains.

Moreover, it could also promote the dissolution of CO₂ in water by changing the stirring method. A self-inducing agitator was used as a pump to draw gas above the liquid phase in a reactor; the induced gas could be distributed to the liquid to obtain perfect gas dispersion [65,66], and the gas was recycled inside the reactor so that it could extend the contact time of the microbe with the gas. Wu et al. [50] studied SA production and CO₂ fixation using a metabolically engineered E. coli NZN111 in a bioreactor equipped with a self-inducing agitator: the final SA yield was 1.33 mol mol⁻¹, which was similar to the process supplied with carbonates and CO₂ sparging (1.35 mol mol⁻¹); the overall SA production rate was 1.1 times higher than that when CO₂ was supplied by carbonate and sparging. However, some microorganisms cannot tolerate high shear stress, which
causes damage to cells and affects metabolite production. Cai et al. [67] reported that high shear stresses caused by mechanical force usually destroyed the normal metabolisms of Aspergillus glaucus HB1-19. Moreover, the economic issues of large amounts of energy consumption from the use of the agitator also need to be considered.

Cao et al. [39] developed an integrated fermentation and membrane separation process that effectively converted CO$_2$ into SA via A. succinogenes using NaOH as the neutralizer under mild pressure (0.4 bar) and a completely closed exhaust pipe with self-circulating CO$_2$ sparging at 0.1 vvm in the bioreactor. the self-circulation of CO$_2$ sparging redistributed the spilled gas into the medium via vacuum pumps and a gas sparger. The final SA concentration reached 37.8 ± 1.4 g·L$^{-1}$. However, membrane fouling is also a problem that cannot be ignored.

In summary, extending the residence time of CO$_2$ gas in water would decrease gas release and enhance the CO$_2$ supply in bio-SA production, which could improve CO$_2$ utilization efficiency.

5. Potential Strategies to Improve the Efficiency of Microbial CO$_2$ Fixation

5.1. Micro-Nano Bubbles

The key problem in improving the efficiency of microbial CO$_2$ fixation is to prolong the residence time of CO$_2$ gas in water. Micro-nano bubbles (MNBs) refer to bubbles with a diameter of less than 100 µm; they have the characteristics of slow rising speed, large specific surface area, very long time stability (Figure 2), negative surface charge and spontaneous radical generation capacity [68]. MNBs can be generated by physical approaches such as hydrodynamic cavitation or gas dispersion. Orifice and venturi hydrodynamic cavitation reactors have low operating and maintenance costs for MNBs generation, but they are easily blocked and eroded and have strong shearing forces. MNBs generated from microporous structures (e.g., membranes) are homogeneous and have high concentrations, which is more applicable to improving the gas supply of the bioreactor, although it will inevitably increase the manufacturing costs and energy consumption of the gas distributor [69].

![Figure 2. Main properties of MNBs.](image)

In wastewater treatment, MNBs were used to increase the solubility of oxygen in water and strengthen the mass-transfer efficiency of oxygen [70]. Typically, nano bubbles (NBs) can offer 2–30-fold higher gas solubility than gas sparging in the aqueous system [71]. When the dissolved-oxygen (DO) concentrations of oxygen gas NBs and air NBs exceeded
40 mg·L\(^{-1}\) and 10 mg·L\(^{-1}\), respectively, these DO levels could be maintained for one day [72]. NBs in water can persist for a longer duration with adequate dissolved gases, which could promote the better growth of microbes and their productivity [73]. Ye et al. [74] proposed to boost oxygen diffusion via MNBs; the oxygen transfer coefficient and rate of MNBs were 0.160 min\(^{-1}\) and 0.382 kg·m\(^{-3}\)·h\(^{-1}\) (about four times and five times greater than those of conventional aeration, respectively). For example, in the work of Guo et al., CO\(_2\)-MNBs had a promoting effect on the growth of Sinomicrobium oceani WH-15 at MNB concentrations of 5–10% in water, whereas the inhibitory effect appeared from 20% [75] because a high concentration of MNBs produced a large number of hydroxyl radicals and other reactive oxygen components, which was unfavorable to the microorganisms [76]. Additionally, CO\(_2\)-MNBs may also facilitate CO\(_2\) fixation by autotrophic microorganisms because more carbon sources are provided [73].

Haapala et al. observed that in papermaking-process waters, the presence of dry refined pine kraft pulp fibers (average fiber length: 1.52 mm) remarkably decreased the size of bubbles measured during a 30 s period after de-pressurizing the air-saturated suspension from 300 kPa to normal atmospheric pressure. Moreover, adhesion of the kraft wood fiber material to the air bubbles further decreased the bubble rise velocities. The bubble (size: 0.4 mm) rise rate in the model suspension with wood fibers was about 0.04 m·s\(^{-1}\), and the bubble rise rate in the suspension without wood fibers was about 0.55 m·s\(^{-1}\) [77].

5.2. CO\(_2\) Adsorption Material

In liquid fermentation systems, the adsorption of CO\(_2\) in water can reduce gas spillage. Adsorbents such as activated carbons, zeolites, nanotubes, metal–organic frameworks (MOFs) and carbon nanotubes are widely used for CO\(_2\) physical adsorption in gas purification. Among these adsorbents, zeolites and several MOFs, as hydrophilic adsorbents, offer high adsorption capacities and selective CO\(_2\) adsorption. Nevertheless, these adsorbents have low CO\(_2\) adsorption capacities when they are exposed to moisture [78]. For some types of MOFs, the sample with 4 wt% water adsorbs more CO\(_2\) than the dry sample, but less CO\(_2\) is adsorbed by the sample with 8 wt% water, indicating that a higher water content often has a negative impact on the CO\(_2\) adsorption [79]. Activated carbon is a hydrophobic adsorbent, Xu et al. [80] experimentally studied a simple one-bed, three-step vacuum swing adsorption (VSA) cyclic system with activated carbon for capturing CO\(_2\) from wet flue gas. The CO\(_2\) adsorption rate was 87.5%, which was close to the CO\(_2\) adsorption rate of 89.1% from dry gas. The results showed that the water resistance of activated carbon has a relatively minor impact on the process performance of CO\(_2\) capture.

The amine-functionalized porous adsorbent is one type of important material in CO\(_2\) capture due to its stability and efficiency. Organic amines adsorb a large amount of CO\(_2\) through chemical interactions between the functional groups and CO\(_2\) [81]. Qi et al. [82] reported that amine-functionalized mesoporous silica exhibited fast adsorption and ultrahigh CO\(_2\) adsorption capacities up to 7.9 mmol·g\(^{-1}\) under simulated flue gas conditions. Amines react rapidly with CO\(_2\) to form carbamate and bicarbonate structures under anhydrous and hydrous conditions (Figure 3) [83–85]. Fiber is easy to obtain, has high mechanical stability, a large surface area and is rich in hydroxyl content; it can be used for modification such as etherification, esterification, silane treatment and grafting copolymerization [86,87]. Materials containing hydroxyl can generate electrostatic interactions with CO\(_2\) to produce physical adsorption [88]. If the fiber is modified with amine groups, the physical adsorption and chemical reaction of CO\(_2\) will be enhanced. It has been reported that diethanolamine was loaded onto the cellulose aerogel by impregnation; the adsorption capacity of CO\(_2\) in the gaseous phase reached 1.99 mmol·g\(^{-1}\) under optimal conditions. However, the chemical reaction of amines and CO\(_2\) would form toxic carbamates in the absence of water, and the theoretical adsorption capacity of CO\(_2\) is only 0.5 mol·mol\(^{-1}\) of amine. However, in the presence of water, the adsorption capacity can break through this limit [89]. Meanwhile, amines can convert CO\(_2\) into HCO\(_3^-\) that is directly available in the liquid phase. Furthermore, the conversion of CO\(_2\) to HCO\(_3^-\) not only reduces the escape of gas from the aqueous
phase but also facilitates the mass transfer of CO\textsubscript{2} to cells. In contrast, CO\textsubscript{2} gas must be first dissolved in the fermentation medium to be utilized by microorganisms. Once CO\textsubscript{2} reacts with water to form HCO\textsubscript{3}\textsuperscript{−}, it will permeate through the cell membrane and take part in the internal metabolic pathways of the specific microorganisms [55]. In conclusion, the adsorption of CO\textsubscript{2} by amine-modified cellulose carriers in the aqueous phase could avoid the damage of carbamate to microorganisms and also provide bicarbonate for the synthesis of SA.

\[
\text{CO}_2 + 2\text{RNH}_2 \rightarrow \text{RNHCOO}^- + \text{RNH}_3^+ \\
\text{CO}_2 + 2\text{R}_1\text{R}_2\text{NH} \rightarrow \text{R}_1\text{R}_2\text{NCOO}^- + \text{R}_1\text{R}_2\text{NH}_2^+ \\
\text{CO}_2 + \text{RNH}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{RNH}_3^+ \\
\text{CO}_2 + \text{R}_1\text{R}_2\text{NH} + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{R}_1\text{R}_2\text{NH}_2^+ \\
\text{CO}_2 + \text{R}_1\text{R}_2\text{R}_3\text{N} + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{R}_1\text{R}_2\text{R}_3\text{N}^+
\]

Figure 3. Mechanism of reaction between organic amines and CO\textsubscript{2}.

5.3. Biofilm

When a large amount of CO\textsubscript{2} dissolves in water, the pH of the broth is reduced to an acidic level, and most of the anaerobic SA-production strains are particularly sensitive to the acidic environment. Biofilms are highly organized microbial community structures composed of exopolysaccharides (EPS), proteins, and eDNA produced by strains immobilized on the surface of a carrier [90,91]. Biofilm formation proceeds as a developmental process with distinct stages: “initial adhesion”, where microorganisms bind to carrier surfaces through cell-surface-associated adhesins; “early biofilm formation”, where they begin to divide and produce EPS (which enhances adhesion) and the forming matrix embeds the cells; “biofilm maturation”, where the three-dimensional development of the matrix provides a multifunctional and protective scaffold, and cells in an established biofilm are glued together by the EPS (which resists mechanical stresses and detachment of the community from the surface of the substrate); and finally “dispersal”, where some cells leave the biofilm to disperse into the bulk fluid [92]. Compared with the traditional fermentation by free cells, biofilm fermentation has the advantages of strong resistance to the harsh environment, high yield and continuous fermentation, and it is conducive to improving the fermentation performance [93]. Zhuang et al. [94] found that the bacteria converted carbon sources into organic acids in the first step of ABE fermentation, which reduced the pH of the fermentation broth and acted as a stress on the bacteria. However, the rate of butanol production and glucose consumption were less affected during immobilized fermentation than suspended fermentation, and cells in the biofilm were more capable of maintaining their morphology, indicating that the biofilm enhanced the tolerance levels of cells in acidic environments. Additionally, Wang et al. [95] showed that \textit{E. coli} O157:H7 (J29) was more resistant than \textit{E. coli} O157:H7 (CICC 21530) to lactic acid, which could be explained by the fact that J29 has a more complex micro-construct of biofilm when compared to CICC 21530.

EPS is secreted by cells to form a complex biofilm through interactions with carrier materials. In general, the biofilm carrier should meet the following requirements: a large surface area with multiple functional groups; physical, chemical and biological stability; good biocompatibility; and easy to obtain and operate [96]. Chen et al. [97] reported that \textit{A. succinogenes} CCTCC M2012036 was immobilized on positively charged polypropylene microfiber membranes, which could immobilize more cells through electrostatic interaction; the yield and productivity of SA achieved 0.82 g·g\textsuperscript{−1} and 1.04 g·L\textsuperscript{−1}·h\textsuperscript{−1} in this microfiber membrane bioreactor. Ding et al. [93] modified cotton fiber with succinic anhydride; the cotton fiber surface roughness increased, and the decrease in hydrophilic groups and negative charge on the surface enabled cotton-succinic anhydride to absorb more cells. It was reported that a packed-bed biofilm reactor filled with Tygon support was constructed...
for continuous SA fermentation by *A. succinogenes*. A visible biofilm layer formed on the carriers in 3 days. Finally, the reactor was successfully run for more than 5 months with a SA productivity of 35.0 g·L⁻¹·h⁻¹ and a glucose conversion of 88% [98].

On the other hand, the structural formation and development of biofilm might be benefitted by the MNBs. One study indicated that applying NB effectively provided extra oxygen for microbial aggregates and achieved a 10.58% improvement in total nitrogen removal; the structure of microbial aggregates was enhanced, where extracellular protein and polysaccharides respectively increased as much as 3.40 and 1.70 times in biofilm and activated sludge, accompanied by the development of activated sludge floc size and the thickness of the biofilms [99]. Zheng et al. [100] reported that gas bubbles could aggregate and adhere to the hydrophobic and rough surface; the bubble behaviors caused the biofilm to be porous (with a microporosity of 9.43–20.94%), which would facilitate the transport of gases and substances to the biofilm interior. Moreover, when the biofilm was covered with a large number of bubbles, the mass transfer distance between the cells and the bubbles was also reduced. Chen et al. [101] observed that floc sludge attached to the carrier gradually, and the microbes were not lost from the reactor when the MNBs entered the sponge interspace and interacted with the microbes of floc sludge; this indicates that MNBs promoted the conversion from suspended floc sludge to biofilm with the enhanced removal of chemical oxygen demand, NH₄⁺ -N and total nitrogen. However, some studies found that the intensive MNB blowing might damage biofilm formation on the membrane. The internal pressure of the bubble depended on the diameter of the bubble. A smaller diameter of bubble led to a higher internal pressure and subsequent bubble collapse, resulting in higher energy. The high energy generated allowed more detachment of the biofilm, and the pressure waves were distributed over the domain of the self-collapsing bubbles and dispelled the fixed biomass from the membrane surface [102]. Agarwal et al. [103] reported that air MBs generated pressure waves through shrinking and subsequent self-collapsing phenomena, which could remove nearly all extracellular polysaccharides and proteins from the nylon membrane surface after 1 h air microbubbling, indicating a complete disruption of the extracellular polymeric matrix of biofilms.

In conclusion, the biofilm could improve the tolerance of bacteria to acidic environments and facilitate the adsorption of bubbles. The modest CO₂ MNBs might cause the biofilm to be porous, which would facilitate mass transfer between the bubbles and the cells and enhance the utilization of CO₂. However, excessive supply of MNBs might cause damage to biofilms.

### 6. Conclusions

Due to the extremely low solubility of CO₂ in water and its short residence time in the fermentation broth, the actual utilization efficiency of CO₂ gas in the biosynthesis of SA is extremely low. Therefore, enhancing the rate of CO₂ conversion in the cell, improving the extracellular CO₂ supply efficiency and reducing the gas spillage are important for bio-SA production. Moreover, relevant studies have shown that MNBs and CO₂-adsorbent materials have the potential to reduce CO₂ spillage in the medium, and the biofilm is conducive to enhancing cell activity and resistance. At the same time, the adsorption of bubbles by the biofilm may be beneficial to the gas mass transfer on its surface.

In the future, the utilisation efficiency of CO₂ gas via SA-producing microorganisms needs to be improved in several ways, including intracellular and extracellular process. On the one hand, the expression of enzymes related to CO₂ transport and succinic acid synthesis needs to be enhanced by metabolic engineering, which would promote the transport and conversion of CO₂ gas into cells. On the other hand, an extracellular CO₂-controlled-release system could be constructed by combining MNBs and CO₂-adsorbent materials. At the same time, the biofilm is used to enhance cell activity; it can resist the acidic environment created by the high concentration of CO₂ and hydroxyl radicals produced by MNBs. In conclusion, enhancing the efficiency of CO₂ utilization through the
integration of multiple technologies will further promote the research and application of microbial carbon sequestration for chemical production.

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