Selective Conversion of *Scenedesmus* into Lactic Acid over Amine-Modified Sn-β

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**ABSTRACT:** Amine-modified Sn-β was synthesized to improve the yield of lactic acid produced from *Scenedesmus*. After studying the growth of *Scenedesmus*, we selected *Scenedesmus* with the highest sugar content of 46.7% after 8 days of culture as the reaction substrate. The results showed that the yield of lactic acid from *Scenedesmus* was greatly increased after being catalyzed by 3-aminopropyltrimethoxysilane (APTMS)-modified Sn-β. After the pretreatment of *Scenedesmus* in an ice bath ultrasound, under the optimal reaction conditions (190 °C and 5 h), the yield of lactic acid reached the highest (37%). The acid–base characterization results of the catalyst confirmed that there are both Lewis acidic sites and medium-strength basic sites in the catalyst. Both of these sites can promote the hydrolysis of *Scenedesmus*, while the Lewis acidic sites can promote the production of lactic acid and the basic sites can effectively inhibit the production of the byproduct 5-hydroxymethylfurfural (HMF). This study proved that this amination catalyst is a useful strategy to increase the yield of lactic acid.

1. INTRODUCTION

Biomass is considered as the most potent alternative source for disposable energy because of its wide distribution, low price, environmental friendliness, and sustainable use. At present, to cope with the increasingly serious energy crisis and environmental pollution, all countries are stepping up research on sustainable biomass resources and their conversion to high value-added chemicals.1–5

Among the chemicals obtained from the conversion of biomass resources, lactic acid is considered to be one of the most promising platform molecular compounds due to its wide application in fuels, materials, biology, medicine, etc.6,7

At present, the preparation methods of lactic acid mainly include three methods: microbial fermentation, chemical synthesis, and catalytic conversion. Industrially, lactic acid is mainly produced by lactic acid bacteria using rice, wheat, corn, and other cereals as substrates and fermented under anaerobic conditions. The process has a few byproducts, and the yield of lactic acid is high, but the growth conditions required for lactic acid bacteria culture are harsh and depend on nutrition such as carbohydrates, amino acids, nucleotides, and minerals.7 In the fermentation process, as the lactic acid continues to accumulate, to maintain the activity of the microorganism, it is necessary to add a base as a neutralizing agent to maintain the pH of the fermentation broth. At present, the most commonly used neutralizing agent is calcium hydroxide. The calcium lactate obtained by the process can obtain crude lactic acid after acidification of concentrated sulfuric acid, and the crude lactic acid is esterified into lactate and then purified and hydrolyzed to obtain high-purity lactic acid. Therefore, the fermentation process for the preparation of lactic acid and its subsequent separation process is very complicated and inefficient.7,9 The chemical synthesis method of lactic acid mainly includes a lactonitrile method, an acrylonitrile method, and a propionic acid method. These methods require the use of hazardous chemicals such as hydrocyanic acid in the implementation process, and the crude lactic acid esters obtained still require further rectification and hydrolysis to produce lactic acid and thus cannot be used as a large-scale industrial production method.7,10 Compared with biofermentation and chemical synthesis, biomass chemical catalysis has higher-scale production potential and higher production efficiency, and a wide variety of raw materials are available that can provide a more acceptable route for lactic acid preparation.

Lewis acid sites play a key role in isomerization, trans-aldol condensation, and 1,2-hydrogen transfer. Therefore, the development of Lewis acid catalysts is currently a hot spot in the production of lactic acid from biomass. Hammond et al.
proposed the solid-state ion-exchange (SSIE) method to synthesize Sn-β zeolites. The SSIE method can quickly and efficiently introduce Lewis acidic sites into the framework of dealuminated β zeolite through mechanical grinding and roasting processes.\(^1\)

Although Sn-β zeolite synthesized by the solid-state ion-exchange method has a strong Lewis acidic center, it still cannot obtain a high lactic acid yield in the catalytic conversion of biomass and is accompanied by the production of a large amount of the byproduct 5-hydroxymethylfurfural (HMF). Further organic or inorganic modification of Sn-β zeolites can improve their reaction performance in the conversion of biomass to lactic acid and increase the yield of target products. Surface amination refers to the use of silanizing reagents containing amine groups to interact with the silanol on the surface of the catalyst and the introduction of various organic amine groups on the surface of the catalyst through the formation of Si–O–Si bridges, thereby increasing the alkalinity of the catalyst,\(^12\) which in turn can inhibit side reactions during the catalytic conversion of biomass to lactic acid. Xu et al.\(^13\) reported in 2015 that ethylenediamine was used to modify sulfonic acid cation-exchange resin and catalyze the self-condensation reaction of cyclohexanone. The results of CHNS elemental analysis and Fourier transform infrared spectroscopy showed that the skeleton structure of the resin after modification did not change much. After the amino group was successfully immobilized on the resin, the alkalinity of the catalyst was enhanced, making the hydrogen resin a dual function of acid and alkali.

The catalytic preparation of lactic acid using biomass such as cellulose as a substrate is an important goal in the laboratory research stage. By improving the catalyst and catalytic system, it is expected to obtain a higher cellulose conversion rate and lactic acid yield. Catalytic conversion of actual agricultural waste biomass such as corn cobs and straws is the application stage of cellulose research. In this paper, we first researched the daily changes of intracellular substances commonly used in the chemical breaking method are hydrolysis of sugar-rich microalgae based on the La\(_2\)O\(_3\) catalyst. When Zan et al. used glucose as a model experiment, after 2 h of reaction at 483 K, the glucose conversion rate was 99%. We used aminated Sn-β zeolite as a catalyst and reacted at 463 K for 2 h, and the glucose conversion rate reached 100%.\(^18\) In 2018, Liu et al. established a system for preparing lactic acid based on the hydrolysis of sugar-rich microalgae based on the La\(_2\)O\(_3\) catalyst. The reaction temperature is 200 °C, the reaction time is 120 min, the initial pressure of helium is 4 MPa, and the amount of catalyst is 0.37 M; the yield of lactic acid in the preparation of liquid-phase products by hydrothermal catalysis of sugar-rich microalgae under the La\(_2\)O\(_3\) system reached the highest value of 29.80%.\(^19\) This reaction did not undergo any pretreatment of microalgae; although, it is very simple, the yield is not ideal.

Because most carbohydrate substances exist in microalgal cells and are surrounded by cell walls, it is necessary to pretreat Scenedesmus to break down the cell walls to make the carbohydrates in algae reach the catalyst before using Scenedesmus as the substrate for catalytic reaction to produce lactic acid.\(^20–25\) Common algae cell wall-breaking methods are physical wall-breaking method, chemical wall-breaking method, and biological wall-breaking method.\(^26–45\) The physical wall-breaking method refers to the removal of cell walls by mechanical force, including grinding method,\(^26\) ultrasonic method,\(^27\) swelling method,\(^28\) and freeze–thaw method.\(^29\) The chemical breaking method refers to the use of chemical substances to dissolve components such as proteins and carbohydrates in the cell wall, thereby achieving the purpose of releasing intracellular substances.\(^30–33\) The chemical substances commonly used in the chemical breaking method are sodium hydroxide, hydrochloric acid,\(^34–40\) and sulfuric acid.\(^41–45\) The biological cell wall-breaking method refers to the use of some biological enzymes to break the cell wall of algae cells.\(^46–48\) The commonly used biological enzymes are cellulase and pectinase.\(^49,50\) The biological methods are not considered here due to the high cost of enzymes.

In this paper, we first researched the daily changes of biomass and carbohydrate content of Scenedesmus under the conditions of the BG11 medium. Second, the effects of Scenedesmus pretreated by grinding, ultrasonication, and acid addition on the yield of lactic acid were discussed. Then, we compared the catalytic performance of β, deAl-β, Sn-β, deAl-β-xNH\(_2\)\(_x\)(V), and Sn-β-xNH\(_2\)\(_x\)(V). Finally, the effects of amination reagents, dosage, reaction time, and catalyst amount on the yield of lactic acids from Scenedesmus over amine-modified Sn-β catalyst were investigated. By solid-state ion-exchange (SSIE) method, Lewis acidic centers can be introduced into the structure of deAl-β zeolite to provide essential acidic sites for the conversion of biomass to lactic acid.

2. RESULTS AND DISCUSSION

2.1. Analysis of Acid–Base Properties. It can be seen from Figure S1 that the H-β zeolite did not collapse after being
dealuminated by concentrated nitric acid and high-temperature roasting and doped with tin, but the relative crystallinity has decreased, which is specifically manifested at \( 2\theta = 22.5-22.6^\circ \).

The characteristic diffraction peak intensity tends to decrease. Sn-\( \beta \)-\( \text{NH}_3 \) and Sn-\( \beta \)-\( \text{NH}_2 \) zeolites have similar X-ray diffraction (XRD) patterns to Sn-\( \beta \) zeolites, indicating that the grafting process of organosilane did not cause significant damage to the crystal structure of Sn-\( \beta \) zeolites. At the same time, weak SnO2 characteristic peak signals (2\( \theta \) = 26, 34, 51°) were found in the Sn-\( \beta \) and Sn-\( \beta \)-\( \text{NH}_3 \) zeolites, indicating that a small amount of SnO2 was formed on the catalyst surface during the solid-state ion-exchange process. This may be due to a large amount of tin acetate used in the grinding process.34

The pyridine adsorption infrared spectrum can characterize the type and strength of acidic sites in the catalyst.

Figure S2 shows a pyridine adsorption infrared spectrum of different \( \beta \) zeolites desorbed at 150 °C for 30 min under vacuum. It can be seen from the figure that the deAl-\( \beta \) zeolites obtained after concentrated nitric acid treatment did not show characteristic absorption peaks corresponding to the Brønsted acid sites (1544 and 1633 cm\(^{-1}\)) and the Lewis acid sites (1448 and 1610 cm\(^{-1}\)). The subsequent introduction of metal tin caused the appearance of the pyridine vibrational absorption peak corresponding to the strong Lewis acidic sites, which indicates that the Sn-\( \beta \) zeolite prepared by the SSIE method has a strong Lewis acidic site. With the connection of the organosilane and the silicon wall on the surface of the zeolite, the Lewis acidic sites in the Sn-\( \beta \)-\( \text{NH}_3 \) zeolite have decreased. The number of sites was 0.40 mmol/g. When the dosage of 3-aminopropyltrimethoxysilane (APTMS) was 200 \( \mu \)L, the number of Lewis acidic sites in Sn-\( \beta \)-\( \text{NH}_3 \) zeolite decreased to 0.20 mmol/g, which was caused by the basicity of the amino group. At the same time, because the average distance between large-volume organosilanes on the surface of zeolites is short, the introduction of aminopropylsilane also masked the tetraaluminum tin in the pores of the zeolite to a certain extent, limiting some of the Lewis acids. The function of the locus leads to the decrease of lactic acid yield. The number of masked acid sites increases with the increase in the amount of APTMS added; therefore, compared with Sn-\( \beta \)-\( \text{NH}_3 \), the number of Lewis acid sites in Sn-\( \beta \)-\( \text{NH}_2 \) zeolite is less.

Figure S3 shows the \( \text{CO}_2 \) desorption isotherms of different \( \beta \) zeolites. It can be seen from the figure that the thermal conductivity detector (TCD) signal of the Sn-\( \beta \) zeolite mainly appears at 400 °C after APTMS is functionalized, which agrees with the reports in the literature. A TCD signal between 300 and 600 °C indicates that this site corresponds to a medium-intensity alkaline site. \( \beta \)-type zeolites without surface amination have no obvious basic site signal. The above results further confirm that the grafting method has successfully introduced basic groups (\( -\text{NH}_2 \)) into zeolites.

Figure S6 shows the transmission electron microscopic image of the \( \beta \) zeolite. It can be seen that the pores of the zeolite have not collapsed and all have a clear crystal lattice. At the same time, highly dispersed small balls can be seen in Sn-\( \beta \) and Sn-\( \beta \)-\( \text{NH}_2 \), and may be SnO2.

Figure S7 shows the nitrogen adsorption/desorption curves of the catalyst. It can be seen from the figure that the \( \beta \) molecular sieve has two hysteresis loops at a relative pressures of \( P/P_0 < 0.01 \) and 0.6 < \( P/P_0 < 0.9 \), which belong to the typical I and IV isotherms, which are in line with the microporous molecular sieve. The characteristic of adsorption is the presence of capillary aggregation in the mesoporous pores. The most probable pore size of \( \beta \) molecular sieve in this article is about 3.9 nm (Table S3), which shows that the grafting of organic functional groups does not affect the pore structure of the Sn-\( \beta \) molecular sieve.

2.2. Diurnal Variation of Biological Indicators of \textit{Scenedesmus}. From the date of inoculation, the biomass and carbohydrate content of \textit{Scenedesmus} were measured at the same time every day to obtain the daily variation trend of carbohydrate content with the number of days of inoculation. Figure 1 shows the daily variation trend of biomass and carbohydrate content of \textit{Scenedesmus}. When just inoculated...
need to be broken. There are many ways in which microalgae break through the wall, including enzymatic hydrolysis, acid hydrolysis, and ultrasound. This chapter compares the effect of grinding, ultrasonic methods of physical wall breaking, and acid hydrolysis method of chemical wall breaking on the wall-breaking effect of *Scenedesmus*, and the optimal conditions of lactic acid yield are screened by the final lactic acid yield. The results are shown in Figure 2. In the blank test, when the catalyst was Sn-β-NH₂(50) zeolite, no lactic acid was produced. When the freeze-dried *Scenedesmus* powder was used as a substrate, the lactic acid yield was 7.8%. After grinding the grids for 30 min, a yield of 29% lactic acid was obtained. The lactic acid yield in this reaction system is 30%, which is similar to the lactic acid yield obtained by grinding and breaking the wall. Since re-hydrolysis of the byproduct HMF in the reaction system can also produce formic acid, it is hard to know the actual production and consumption of formic acid in the system. In the determination of *Scenedesmus* carbohydrate content, an ultrasonic cell crusher ice bath was used to break the wall to release the carbohydrate in *Scenedesmus* cells. In this section, the same method was used to carry out the chemical catalytic reaction under the reaction conditions of 190 °C and 5 h, and the yield of lactic acid was 37%. Therefore, the following is a catalytic reaction using an algae solution having a concentration of 30 mg/mL after being broken in an ice bath.

### 2.5. Reaction Pathways in the Conversion of *Scenedesmus* to Lactic Acid over Sn-β-NH₂ Catalyst

Since most of the reducing sugar in *Scenedesmus* is glucose, the possible reaction pathways are shown in Figure S4. It mainly includes two parts: the main reaction and the side reaction. The main reaction is the isomerization of glucose and fructose and the conversion of fructose to lactic acid under Lewis acidic conditions, and the side reaction is the conversion of glucose and fructose to HMF and the rehydration of HMF under acidic conditions.50,49 Glucose and other pyran-type sugars generate furan-type sugars through isomerization reaction; furan-type sugars undergo reverse aldol condensation reaction under the action of a catalyst to break the C=C bond to generate three carbon compounds such as 1,3-dihydroxyacetone and glyceraldehyde; the carbon compound finally generates lactic acid through hydrogen transfer and dehydration steps. The glucose isomerization reaction is a common step for the preparation of lactic acid, 5-hydroxymethylfurfural, and levulinic acid and other carbohydrate degradation products. It is generally believed that the isomerization reaction of glucose has gone through the steps of pyran ring → chain → furan ring. Similar to the route of preparing lactic acid, the isomerization reaction of glucose to fructose is catalyzed by Lewis acid or base, and the conversion of HMF is realized by the formation of carbonium ion intermediates.30

According to the above research results, acid-base bifunctionalization can be achieved using deAl-β through the immobilization of metal tin and the grafting of aminopropyl silane, providing the required Lewis acid sites for the main reaction of the process and inhibiting the basic groups that occur in side reactions.

### 2.6. Effect of Amine Reagent Types on the Catalytic Performance of Sn-β-xNH₂ Zeolite

Given the higher number of amino groups in the molecular structure formula, the more basic the amination reagent will be, in this section, based on the amination reagent APTMS selected in the previous two chapters. 3-(2-aminoethylamino)propyltriethoxysilane (AEPTMES) and diethylenetriamine (DETA) are selected according to the number of amino groups in the molecular structure formula to explore different alkaline strengths in the process of amination reagents and their modified additive quantity of *Scenedesmus* lactic acid production. The influence of the results is shown in Figure 3.

When APTMS was selected as the alkaline functionalizing agent, the lactic acid yield increased first and then decreased with the increase of APTMS dosage. When the APTMS dosage was 0 μL, that is, when the catalyst was an Sn-β zeolite, the lactic acid yield was 16%. When the APTMS dosage was increased to 50 μL, the lactic acid yield reached a maximum of 37%. Subsequently, the lactic acid yield began to decrease and the lactic acid yield decreased to 28% when the APTMS dosage was 120 μL. When AEPTMES was used as the modifying reagent, the tendency of lactic acid yield change was different from the trend of lactic acid yield obtained by using APTMS as an aminating agent. When the dosage of AEPTMES was increased to 30 μL, the lactic acid yield reached a maximum value under this condition, then decreased and stabilized, and at about 23%. The volume of the organosilane AEPTMES molecule is much larger than that of

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**Figure 2.** Comparison of different cell wall-breaking methods (reaction conditions: 300 mg of substrates, 10 mL of H₂O, 240 mg of Sn-β-NH₂(50) catalyst, 190 °C, 5 h) (M1: blank; M2: freeze-drying; M3: freeze-drying + grinding; M4: freeze-drying + 75 mg formic acid; M5: ice bath ultrasound).

**Figure 3.** Lactic acid yields obtained from *Scenedesmus* over Sn-β-xNH₂(50) catalysts (reaction conditions: 10 mL of *Scenedesmus* solution with a concentration of 30 mg/mL, 240 mg of Sn-β-xNH₂(50) catalyst, 190 °C, 5 h).
APTMS and it is easy to cross-link on the surface of the zeolite, which may cause large-scale masking of the Lewis acid sites inside the zeolite and hinders the conversion of *Scenedesmus* into the lactic acid reaction system, and part of the main reaction occurs. With DETA as the amination reagent, the lactic acid yield jumped at a DETA dosage of 10 μL, and the yield was similar to that obtained with 30 μL of AEPTMES as the amination reagent and then decreased to about 10%. The reason for this phenomenon is that DETA is strongly alkaline. Excessive dosage during surface amination modification may inhibit Lewis acid center in Sn-β zeolite and convert Sn-β-xNH₂ zeolite to act as a catalyst without obvious acidity and alkalinity. In summary, the selection of amination reagent in the process of surface amination modification is not as strong as possible; the stronger the alkalinity of the aminating agent, the smaller the amount of aminating reagent to be added to obtain the maximum lactic acid yield. However, when the maximum lactic acid yield obtained was compared, the yield of lactic acid obtained was the highest when APTMS was used as the amination reagent.

2.7. Effect of Amino-Modification on the Performance over Sn-β. Figure 4 compares the effects of several β zeolites on the yield of lactic acid and HMF produced by catalytically transformed *Scenedesmus*.

![Figure 4](image)

**Figure 4.** Lactic acid yields obtained from *Scenedesmus* over β-type catalysts (reaction conditions: 10 mL of 30 mg/mL *Scenedesmus* solution, 240 mg of catalyst, 190 °C, 5 h).

From the type and strength of the acidic sites in the infrared spectrum of pyridine adsorption, we can see that when deAl-β and deAl-β-NH₂(50) zeolites are used as catalysts, the yields of lactic acid and HMF are both low (<10%). This is due to the absence of Brønsted acidic sites and Lewis acidic sites in the catalyst. Commercial-grade β zeolites gave 10% lactic acid and 11% HMF yields at 190 °C and 5 h reaction conditions. The main reason for the increase in lactic acid yield is the increase of the Lewis acid site in the system, and the Brønsted acidic site contained in the β sieve promotes the breakage of the cell wall of *Scenedesmus*, which is in agreement with the results of Zan et al. However, similar to the above results, Brønsted acid also promotes the formation of the byproduct HMF in the reaction system in which the biomass is converted to lactic acid. Compared with the commercial-grade zeolite, the Sn-β zeolite obtained a 16% lactic acid yield due to the enhancement of Lewis acidity, and the HMF yield was also reduced to 9.8%. Under the same conditions, using aminated Sn-β zeolite, namely Sn-β-NH₂(50) zeolite, the lactic acid yield was as high as 37% and the HMF yield was greatly reduced to 1.3%. This is mainly due to the quantitative balance of the acid—basic sites in the catalyst so that it retains a part of the Lewis acidity and has an appropriate amount of basic sites, which inhibits the production of HMF. In an aqueous solution, HMF can continue to combine with water to produce levulinic acid and formic acid.

2.8. Optimization of Reaction Conditions. Reaction time is an indispensable factor in the chemical catalytic reaction system. Figure 5 shows the effect of reaction time on lactic acid yields obtained from *Scenedesmus* over the Sn-β-NH₂(50) catalyst.

![Figure 5](image)

**Figure 5.** Effect of reaction time on lactic acid yields obtained from *Scenedesmus* over the Sn-β-NH₂(50) catalyst (reaction conditions: 10 mL of 30 mg/mL *Scenedesmus* solution, 240 mg of catalyst, 190 °C).

As can be seen from Figure 5, as the reaction time is prolonged, the lactic acid yield also increases. When the reaction temperature was 5 h, the lactic acid yield reached a maximum of 37%. As the reaction temperature continues to increase, the lactic acid yield changes less. Therefore, for this reaction system, the optimum reaction time condition is 5 h.

It can be seen from Figure 6 that the dosage of the Sn-β-NH₂(50) zeolite catalyst has a great influence on the yield of lactic acid. When the catalyst dosage was less than 240 mg, the

![Figure 6](image)

**Figure 6.** Effect of catalyst dosage on lactic acid yields obtained from *Scenedesmus* over the Sn-β-NH₂(50) catalyst (reaction conditions: 10 mL of 30 mg/mL *Scenedesmus* solution, 190 °C, 5 h).
yield of catalyst to lactic acid increased from 5.6% of 120 mg to 37% of 240 mg, and lactic acid increased from 200 to 240 mg, with the greatest yield increase. When the dosage of the catalyst exceeds 240 mg, the lactic acid yield does not change significantly, and it is maintained at more than 35%. This indicates that in the reaction system in which the Sn-β-NH2/[50] zeolite catalyzes the production of lactic acid from Scenedesmus, the optimal dosage of the catalyst is 240 mg.

Compared with the same content of glucose as the reaction substrate, the lactic acid yield of glucose should be 23.5%, but the lactic acid yield of Scenedesmus can reach 37%, indicating that Sn-β zeolite has a good catalytic effect on Scenedesmus, which may be related to the cell structure of Scenedesmus. The pretreatment method of the ice bath ultrasound destroyed the cell wall so that the reducing sugar in the cell can be released at a concentration suitable for the reaction.

3. CONCLUSIONS

In this paper, the effect of surface amination of Sn-β zeolite on the performance of catalyzed production of lactic acid by Scenedesmus is discussed. The conclusions are as follows.

On the 6th day from the date of inoculation, the biomass reached a maximum of 3.2 mg/mL, and on the 8th day from the date of inoculation, the carbohydrate content reached a maximum of 46.7%. The ice bath ultrasound has the best effect on the breakage of Scenedesmus cells.

Under the optimal reaction conditions of 50 μL of APTMS, 240 mg of catalyst, the substrate Scenedesmus liquid concentration is 30 mg/mL, the reaction temperature is 190 °C, and the reaction time is 5 h of the optimum reaction conditions; using the one-step catalysis, a 37% lactic acid yield can be obtained, which is much higher than the 16% lactic acid yield obtained by Sn-β zeolite under the same conditions, while the yield of HMF is only 1.3%. The effects of amination modification on the production of lactic acid from Scenedesmus catalyzed by Sn-β zeolite were demonstrated.

4. EXPERIMENTAL SECTION

4.1. Materials. Scenedesmus was purchased from the freshwater algae species library of the Chinese Academy of Sciences.

Commercial β zeolite with a Si/Al ratio of 25 (Catalyst Plant of Nankai University) was used as the catalyst supporter. The reagents used in the experiments were tin(II) acetate (95%) (Alfa Aesar), 3-aminopropyltrimethoxysilane (APTMS) (98%) (Wako), 3-(2-aminoethylamino)propyltriethoxysilane (AEPTMES) (96%) (J&K), diethylenetriamine (99%) (Alfa Aesar), phosphoric acid (high-performance liquid chromatography, HPLC) (Sigma-Aldrich), phenol (AR, Aladdin), sulfuric acid (AR, SCRC), and hydrochloric acid (AR, SCRC).

4.2. Preparation of Scenedesmus. 4.2.1. Cultivation of Scenedesmus. The initial inoculation was carried out with 160 mL of algal seed solution into a 500 mL Erlenmeyer flask containing 160 mL of BG11 medium and cultured in a light incubator. The temperature and pH were maintained at 25 ± 0.2 °C and 7.0–8.0, respectively.

The culture process of the T8 double-layer single-sided photo-bioreactor was as follows: before the culture, the BG11 medium was sterilized in a high-temperature sterilization pot for 30 min, cooled to room temperature, and placed in an ultraclean aseptic table for 20 min. The operating tools were aseptically treated at the time of inoculation, and the procedures were carried out in an ultraclean aseptic bench.

The algae solution and BG11 medium were placed in a ratio of 1:5 into an 800 mL glass column with a working volume of 600 mL and then transferred to a T8 double-layer single-sided photo-bioreactor, which was passed with compressed air containing 3% CO2 (v/v) at a light intensity of 150 μmol/m²/s. The cells were harvested subsequently by centrifugation at 3000 rpm for 5 min after culturing for a certain number of days; the collected Scenedesmus cells were washed several times with deionized water until no white colonies attached to the upper layer were observed and freeze-dried in a vacuum freeze dryer. Freeze-dried cells were ground into a powder and stored at a temperature below 0 °C.

4.2.2. Determination of Biomass of Scenedesmus. The method for determining the biomass of Scenedesmus in this article is improved by the method of Yi et al.12

The biomass standard curve drawing process was as follows: 20 mL of algae solution cultured in the light incubator for 6 days was taken, centrifuged at 7500 rpm for 5 min, and the white fungus attached to the upper layer with deionized water was washed off. Then, the algae were washed several times with deionized water, dried in a vacuum freeze dryer, and weighed. The biomass concentration in the algae solution was calculated. A certain amount of the same batch of the bacterium was taken and diluted at a dilution factor of 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, and 0.01, and the OD625 value was measured at a wavelength of 625 nm with a spectrophotometer. A calculated standard curve was drawn for the biomass concentration.

The biomass determination of Scenedesmus was determined as follows: from the date of the inoculation of algae to the T8 double-sided single-sided photo-bioreactor, 1 mL of the algae solution to a volume of 10 mL was taken at the same time every day. After shaking, the OD625 value was determined and the marking line was recorded. It was multiplied by a dilution factor of 10 to obtain the biomass concentration in the algae solution.

4.2.3. Determination of the Carbohydrate Content of Scenedesmus. The method for determining the carbohydrate content of Scenedesmus in this article is improved by the method of Xiao et al.30

The glucose standard curve drawing process was as follows: first, a certain amount of glucose powder was dried in an oven at 80 °C to a constant weight. Two hundred milligrams of glucose powder was weighed and dissolved in a certain amount of distilled water to a volume of 1000 mL to obtain a 0.2 mg/mL glucose solution. The resulting 0.2 mg/mL glucose solution was diluted stepwise at a dilution factor of 0.8, 0.6, 0.4, 0.2, 0.1, and 0.05. Five grams of phenol dissolved in a certain amount of distilled water was weighed and diluted to 100 mL to obtain a 5% phenol solution. Two milliliters of 0.2 mg/mL glucose solution and 25% of its diluted solution was weighed, and then 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid were quickly added, shaken well, and the solution was placed in a water bath for 10 min at 80 °C. The OD490 value was measured at 490 nm after standing at room temperature for 20 min, and a standard curve was drawn.

The total carbohydrate concentration of Scenedesmus was determined as follows: from the date of the inoculation of algae to the T8 double-sided single-sided photo-bioreactor, 1 mL of the algae solution was taken at the same time every day to remove the supernatant, and then 10 mL of distilled water was
added and shaken well. The algae solution was placed in an ultrasonic cell pulverizer for 30 min in an ice bath with an ultrasonic gap of 3 s. The ultrasonicated algae solution was placed in a water bath at 80 °C for 2 h and then taken out and cooled to room temperature. Two milliliters of the cooled algae solution was taken, and then 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid were quickly added, shaken well, and the solution was placed in a water bath at 80 °C for 10 min. The OD\text{490} value was measured after standing at room temperature for 20 min in the dark. The OD\text{490} value was substituted into the standard glucose curve and multiplied by the dilution factor of 10 and then divided by 2 to obtain the total carbohydrate concentration in the algae solution. The percentage of the total carbohydrate concentration in the biomass concentration of the day was the carbohydrate content in the algae solution of the same day.

4.2.4. Determination of the Type and Content of Reducing Sugar of Scenedesmus. Lyophilized algae powder (0.4 g) was weighed in a round-bottom flask, and about 50 mL of 5% dilute sulfuric acid solution was added to the flask, and the flask was placed in a 100 °C oil bath and stirred for 4 h to fully hydrolyze the microalgae. After the reaction was over, the reactant was centrifuged and the supernatant was taken, the residue was washed with ultrapure water several times, and the cleaning solution was mixed with the supernatant. Then, HPLC was used to analyze the types and content of reducing sugars.

4.3. Catalyst Synthesis Procedure. Commercial-grade β(H-β) zeolites were placed in a three-neck round-bottom flask containing concentrated nitric acid (20 mL of concentrated nitric acid per 1 g of H-β). The round-bottom flask was placed in an oil bath, and the temperature was controlled. The mixture was dehydrated and deaerated at 80 °C for 20 h at a stirring rate of 200 rpm. The dealuminated solid—liquid mixture was centrifuged by a high-speed centrifuge (3000 rpm, 20 min), and then the centrifuged solid components were washed several times with deionized water until the pH of the eluent was neutral, and the washed solid was dried overnight at 150 °C to obtain the dealuminized β zeolite (referred to as deAl-β).

The Sn-β zeolite was prepared by a solid-state ion-exchange (SSIE) method. The specific steps were as follows: 0.2 g of tin acetate and 1.0 g of deAl-β were placed in an agate mortar and mixed for 30 min. It was calcined in a box-type electric resistance furnace (550 °C, 6 h) to obtain an Sn-β zeolite.

The amination-modified Sn-β zeolite (Sn-β-xNH\text{2(\text{v})}) was prepared as follows: Sn-β zeolite was pretreated at 120 °C for 2 h to remove impurities such as surface moisture. Sn-β zeolite (0.5 g) was dispersed in a 500 mL three-neck round-bottom flask containing 250 mL of absolute ethanol, and then a certain amount of aminating reagent was added. The mixture was condensed and refluxed at 80 °C for 6 h and cooled to room temperature. The mixture was centrifuged, and the white solid after centrifugation was washed with a large amount of absolute ethanol and dried at 80 °C overnight to obtain Sn-β-xNH\text{2(\text{v})}.

x represents the number of amino groups in the molecular formula of the amination reagent 3-aminopropyltrimethoxysilane (APTMS), \(3 \cdot (2\text{-aminoethylamine})\)-propytriethoxysilane (AEPTMES), and diethylenetriamine (DETA), where x is 1 (here omitted), 2, and 3, respectively, and V represents the amount of aminating reagent, in units of (μL), of three amination reagents.

4.4. Catalyst Characterizations. The catalytic performance of the catalyst is closely related to its physical and chemical properties. To investigate the effect of surface organic amination modification on the catalytic performance of Sn-β zeolites, a variety of characterization methods were used to characterize the β zeolite catalyst before and after amination modification. The amination reagent used in this paper is 3-aminopropyltrimethoxysilane (APTMS) because it has only one amino group.

Powder X-ray diffraction (XRD) is commonly used for the analysis of catalyst phase structure and crystallinity; the measurements were performed using a Bruker D8 ADVANCE X-ray powder diffractometer with Cu Ka radiation (λ = 1.54 Å) at a voltage of 40 kV and a current of 40 mA over a 2θ range of 10°–90° with a scan speed of 2°/min at room temperature.

Pyridine infrared spectroscopy (Py-IR) can be used for the acid analysis of catalysts. Pyridine infrared spectroscopy was measured by the frontier Fourier infrared spectrometer (Perkin Elmer, USA). The spectrometer has a recording range of 1400–1700 cm\(^{-1}\) and a resolution of 2 cm\(^{-1}\). Before the test, 10 mg of the catalyst was weighed and compressed under a pressure of 10 MPa, placed in an infrared sample cell, vacuum-prepared at 400 °C for 2 h, then cooled to room temperature and scanned to obtain a background map. Excess pyridine vapor was passed into the sample chamber to allow the sample to fully adsorb pyridine for 0.5 h, and then the temperature of the sample chamber was controlled at 150, 250, 350, and 450 °C and continued to stabilize for 1 h at each temperature. The infrared spectrum of the pyridine adsorption sample was recorded on the infrared spectrometer.

The specific surface area and pore size distribution of the catalyst were analyzed by nitrogen adsorption/desorption test, using the Brunauer–Emmett–Teller (BET) specific surface area measurement method. It was measured at −196 °C using an American Micromeritics ASAP 2460 instrument. Before nitrogen adsorption, the sample was vacuum-treated at 150 °C for 6 h. Through the BET test, the specific surface area is calculated according to the relative pressure P/P\(_0\) in the range of 0.05–0.25; the adsorption isotherm desorption branch is used, and the pore size distribution of the catalyst is calculated by the Barrett–Joyner–Halenda (BJH) method and analyzed by the t-plot micropore analysis to obtain its micropore volume.

Transmission electron microscopy (TEM) is used to observe the apparent morphology and structure of materials, and its observation accuracy can reach the nanometer level. In this study, the morphological structure of the catalyst was observed by a Tecnai G2F20S-TWIN transmission electron microscope (FEI Company, USA) under a 200 kV acceleration voltage. During the test, a small amount of sample was added to ethanol for ultrasonic treatment for 60 min and then the sample was dropped on the copper microgrid, and the test was performed after the ethanol was completely volatilized.

4.5. Catalytic Reactions and Product Analysis. Three hundred milligrams of Scenedesmus, 10 mL of distilled water, and a certain amount of Sn-β-xNH\text{2(\text{v})} catalyst were added to a 50 mL p-polyphenol (PPL) container. The container was placed in a stainless steel high-pressure reactor, sealed, and placed in an oven for high-temperature and high-pressure reactions. After the reaction, the reaction product was analyzed by high-performance liquid chromatography (HPLC). Each group of experiments was repeated three times in parallel, and
the data was processed and analyzed by Origin 8.5 software, which was expressed as the measured mean ± standard deviation.

The detection of organic acids (lactic acid, formic acid, acetic acid, and levulinic acid) and HMF in the product was carried out using a Gemini-NX 5 μm C18 column, where a 0.1% aqueous phosphoric acid solution with a flow rate of 1 mL/min was used as the mobile phase. All of the experiments were performed more than three times, and the quantitative data were the average values of the analytical results. The relative errors were less than 10% for all experiments.

The conversion of the substrate and the yield of the product were calculated using the following formula (calculated in terms of carbon moles)

\[
\text{carbohydrate conversion} \% = \left(1 - \frac{\text{the amount of carbohydrate in the remaining Scenedesmus}}{\text{the amount of carbohydrate in the Scenedesmus}}\right) \times 100
\]

product yield

\[
\% = \frac{\text{moles of carbon in the product}}{\text{moles of carbohydrate carbon in the Scenedesmus}} \times 100
\]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04561.

Powder XRD patterns of the samples; Py-IR spectra of the samples; CO2-temperature-programmed desorption (TPD) profiles of the samples; proposed reaction pathways in the conversion of glucose to lactic acid over Sn-β-NH2 zeolites; the yields of lactic acid converted by different substrates over the Sn-β-NH2[10] catalyst; TEM images of the samples; N2 sorption isotherms of the samples; scanning electron microscopy (SEM) images of the samples; reaction behaviors of 5-hydroxymethylfurfural under water (5 mL 5-hydroxymethylfurfural aqueous solution); physicochemical properties of the β-type zeolites; and particle size distribution table of the β-type zeolites (PDF)

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Notes

The authors declare no competing financial interest.

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