

Research and Mechanism of Two-step Preparation of Acetamide from Microalgae under Hydrothermal Conditions

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Abstract. A two-step synthesis of acetamide under hydrothermal condition from microalgae, is presented. results showed that the best yield of acetamide and selectivity of acetic acid were 9.5 % and 60.1 % at 320 °C for 8 min with a NH₃ supply of 30. Algae such as spirulina, cyanobacteria and autotrophic chlorella could also acquire acetamide, and lactic acid was found to be an important intermediate during the exploration of reaction pathways. These results demonstrated that it is possible to develop a process for conversion of microalgae biomass into acetamide.

1 Introduction

Diminishing fossil fuel reserves and ever-increasing CO₂ (carbon dioxide) concentration have prompted the exploration for an alternate renewable and sustainable energy source [1]. Meanwhile, the treatment of algae mud caused by algae bloom has been the focus of research in recent years [1]. As a result, microalgae are thought to be a candidate for future fuel. Microalgae is indeed a promising feedstock for advanced biofuels production owing to many compelling reasons [2], such as faster growth rate [3, 4], shorter multiplication cycle [5, 6], higher photosynthetic efficiency [3, 4] and maximum productivity [5, 6]. Microalgae can also be cultivated in saline and alkaline land or wastewater, and it is a good way to preserve conventional cropland and remove nitrogen, phosphorus, and heavy metals in wastewater [2]. Microalgae has the excellent ability to capture CO₂ for itself growth and finally can be converted into non-toxic and degradable biodiesel, which relieve environmental problems [7, 8].

There has been many researches committing to convert various biomass into biofuel and value-added chemicals [1, 8-10], and hydrothermal process is one of the most promising methods [11-14] because of its unique inherent properties when HTW (high-temperature water) behaves as reaction medium. HTW above the critical point undergoes drastic changes in its physical properties such as dielectric constant, density, ionic product, viscosity, and solubility [15]. Ionic product (K_w) of HTW, about 1000 times higher than that of ambient liquid water [16], and the low dielectric constant, are both favourable to promote reactions without catalysts and have a good selectivity on the products [12, 14]. HTW also has the good transport property to mix small organic compounds completely and break down hydrocarbons and

carbohydrates [17-19]. Most importantly, compared to other ways, the hydrothermal treatment of microalgae does not require dehydration, which could save a great deal of energy in the period of pretreatment [1, 19].

Acetic acid is one of main and stable intermediates during hydrothermal oxidation of organics [1], which contributes it to be the target product for organic waste utilization [20]. Acetamide is widely used in various industries and plays an important role in our daily life. For instance, acetamide is an excellent solute due to its higher dielectric constant, a promising PCMs (Phase-change-material) for its good stability during thermal cycles [21] and a wetting agent. At the same time, Acetamide pesticides are important compounds of selective herbicides, can effectively control phytopathogenic fungi (peronosporales) in potatoes, sugar beets, and other crops [22]. Besides, the traditional industrial method for acetamide is to make glacial acetic acid mixed with enough NH₃, and pure acetamide is received through a series of pyrolysis, dehydration, crystallization, and separation process.

The two-step production of acetamide from microalgae under hydrothermal conditions seems to be a good way to utilize waste and satisfy the requirement of low carbon. A series of experiments to study the effects of reaction temperature, NH₃ supply and reaction time on the acetamide production were performed, and possible reaction pathways were proposed.

2 Experimental

2.1 Materials

Hydrogen peroxide (GR, ≥30 %) used as H₂O₂ supply, ammonia (GR, 25-28 %) used as NH₃ apply, both convenient for handling, were supplied by Sinopharm

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Chemical Reagent Co., Ltd, China. The Spirulina, Cyanobacteria and Autotrophic chlorella were chosen to be the representative algae. The food grade Spirulina powder was obtained from Shandong BinzhouTianjian Biotechnology Co., Ltd., China. The powder was dried for 1 h at 105°C in a drying oven to eliminate error before each experiment; Cyanobacteria was the manufactured material of algae from Taihu Lake, which has been pretreated with a series of procedures such as cleaning, sedimentation, centrifugation, drying and trituration; Autotrophic chlorella was cultivated in SE medium. Table 1 lists the elemental composition of three kinds of microalgae analysed on a dry basis.

Table 1. Elemental analysis of representative algae.

Category	C (%)	N (%)	H (%)	O (%)	100% H ₂ O ₂ supply
Spirulina	46.84	8.37	6.96	37.83	0.71ml
Cyanobacteria	46.56	5.74	7.73	40	0.69ml
Autotrophic chlorella	36.18	7.33	5.36	48.14	0.50ml

2.2 Definition of terms

The definition of terms is shown as follows. Yield of acetic acid (mol%):

$$Y = \frac{\text{Moles of carbon in lactic acid produced}}{\text{Moles of carbon in loaded microalgae sample} \times 100\%} \quad (1)$$

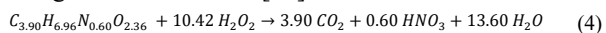
Yield of acetamide (mol%):

$$Y = \frac{\text{Moles of carbon in acetamide produced}}{\text{Moles of carbon in loaded microalgae sample} \times 100\%} \quad (2)$$

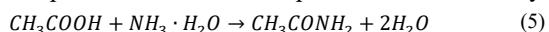
Selectivity of acetic acid (mol%):

$$Y = \frac{\text{Moles of acetamide produced in step 2}}{\text{Moles of lactic acid produced in step 1}} \times 100\% \quad (3)$$

100% H₂O₂ supply was defined as the volume of hydrogen peroxide (provide 30%wt H₂O₂) used to completely oxidize microalgae, where microalgae (approximated by C_{3.90}H_{6.96}N_{0.60}O_{2.36}) was converted to carbon dioxide, nitric acid and water, assuming 1 mol of H₂O₂ gives 1/2 mol of O₂[23].



Correspondingly, 100% NH₃ supply was defined as the volume of ammonia (provide 26%wt NH₃) used to convert acetic acid produced in the first step into acetamide totally.



2.3 Experimental procedure

The schematic drawing of the experimental set-up can be found elsewhere [1, 23-27]. 1) In a typical hydrothermal experiment, 0.067 g microalgae and 0.3-1.1 mL hydrogen peroxide were loaded into a batch-type reactor, which was made of stainless steel SUS316 tube (3/8-inch diameter, 1-mm wall thickness, 120-mm length) with two-end fittings, providing an inner volume of 5.7 mL. 2) The reactor was immersed into a salt bath preheated to a

desired temperature and kept shaking horizontally. 3) After the reaction, the reactor was placed into a cold-water bath to quench the reaction. 4) For the second-step reaction, the reactor was opened quickly and added into 0.3~0.98 mL ammonia immediately after cooling to room temperature and finally sealed again. 5) Repeat the above-mentioned procedures 2) and 3), samples of the liquid phase in the reactor were filtrated with a 0.45 μm filter and collected into the sample bottles for the subsequent detection. All the second-steps were directly conducted on optimal reaction conditions and yields of acetic acid respectively from representative algae based on previous research (Table 2). So, we took Spirulina powder as the key object and referred it as microalgae due to a higher yield of acetic acid among these representative algae. In all experiments, a loading of a mixture of deionized water, hydrogen peroxide and ammonia was usually set at 30 %, namely 1.7 mL. The reaction time was defined as the time that the reactor was kept in the salt bath, and the actual reaction time was shorter than the apparent reaction time.

Table 2. Optimal reaction conditions and yields of acetic acid of representative algae.

Category	Temperature (°C)	H ₂ O ₂ supply (%)	Time (S)	Yields (%)
Spirulina	300	100	80	14.9
Cyanobacteria	300	160	60	12.1
Autotrophic chlorella	320	160	100	12.0

3 Results and discussion

3.1 Preliminary experiments

To verify the feasibility of the study, the preliminary experiments were carried on at 80 s, 300 °C and 0.067 g (NH₄)₂CO₃ with first-step products of Spirulina. The pretreated samples were analysed by HPLC, and the results were shown as Fig.1. It was evident that acetamide was formed after the second-step reaction due to the peak.

Next, to explore whether there were other substances produced during the second-step reaction, we compared the products of the acetic acid sample and ammonia at 300 °C and 500% NH₃ supply for 1 minute and 2 minutes. In Fig.2, the total moles of acetic acid and acetamide were equal to the initial concentration of acetic acid sample (6800 mg/L, the probable amount of acetic acid generated in the first step) in control group 1 and 2. Results indicated when put NH₃ into acetic acid, we can stably produce acetamide as the only product. Therefore, acetamide could be regarded as the target product.

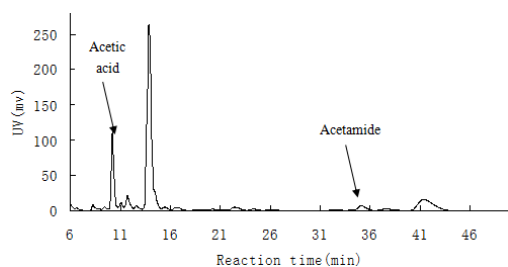


Fig. 1. HPLC of chlorella hydrothermal reaction products.

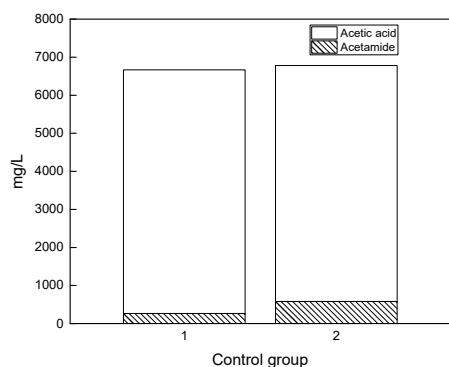


Fig. 2. Reactions of acetic acid sample and ammonia (UV detector Wavelength: 210nm, Column temperature: 80 °C, Mobile phase: 1mM HClO₄).

3.2 Influence of reaction temperature on acetamide yields

To examine the effects of reaction temperature on yields of acetamide from microalgae, a series of experiments were performed with a NH₃ supply of 20 and reaction time for 8 min in the second step. Fig.3 showed the effects of reaction temperature on yields of acetamide in the two-step process between 250-350 °C. The increase on temperature from 250 °C to 320 °C resulted in a monotonous increase on acetic acid selectivity from 21.1 % to 53.5 %, acetamide yields from 3.2 % to 8.1 % respectively, indicating that acetic acid produced in the first step failed to react completely with newly supplied NH₃. With temperature rising, yields of the acetamide boosted correspondingly. However, there was a slight negative effect on the production of acetamide when the temperature exceeded 320 °C. As the figure shown, the selectivity of acetic acid and yield of acetamide declined to 48.2 % and 7.3 % due to decomposition of production at this point. Therefore, it should be more suitable to take 320 °C as reaction temperature in the two-step process.

3.3 Influence of NH₃ supply on acetamide yields

Excess NH₃ was required because ammonia could volatilize easily. Subsequently, experiments on effects of NH₃ supply were conducted with 320 °C, 8 min and a NH₃ supply of 10~35 in the second step. The influence of NH₃ supply on yields of acetamide in the two-step process was shown in Fig.4. In the beginning, the selectivity of acetic acid and yield of acetamide was only 41.6 % and 6.3 % when the NH₃ supply was 10 folds. As NH₃ supply

increased, yields grew continuously and especially when the NH₃ supply was 30 folds, the selectivity and yield obtained 60.1 % and 9.1 % as a maximum value. However, more NH₃ supply did not result in an increase of the yields since then, indicating that reactions had reached a state of saturation. Furthermore, when NH₃ supply was 10 folds, there was still a smell of ammonia after reaction according to the experimental phenomenon, showing that NH₃ supply remained substantial. So, the recycling of NH₃ should be considered when it comes to application in industry.

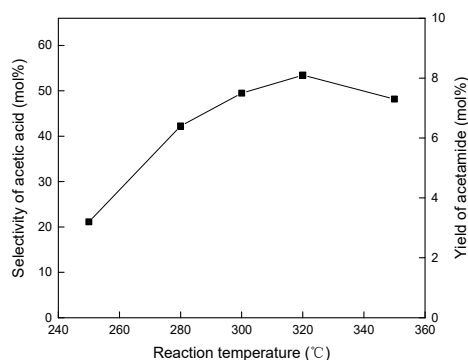


Fig.3. Effects of reaction temperature on selectivity and yield in the two-step process (step 1: 0.067 g microalgae; 100 % H₂O₂ supply; 300 °C; 80 s; step 2: 20 folds NH₃ supply; 8 min).

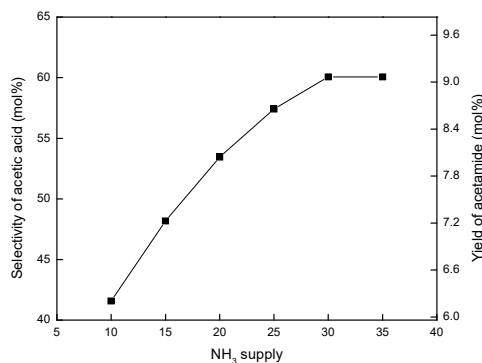


Fig.4. Effects of NH₃ supply on selectivity and yield in the two-step process (step 1: 0.067 g microalgae; 100 % H₂O₂ supply; 300 °C; 80 s; step 2: 320 °C; 8 min).

3.4 Effect of reaction time on acetamide yields

The effect of reaction time on yields of acetamide from microalgae was investigated under conditions of 20 folds NH₃ supply, 320 °C and 5-15 min. As illustrated in Fig.5, the yields of acetamide monotonously increased with the reaction time, and the selectivity and yield reached 60.1 % and 9.1 % after 8 min. However, yields decreased slightly when the reaction time was up to 10 min and then began smooth gradually. It could be seen from that, acetamide was relatively steady or the speed of decomposition was equivalent to that of composition at the temperature of 320 °C. In the same way, the selection of 8 min as reaction time should be more desirable on yields of acetamide in the two-step process.

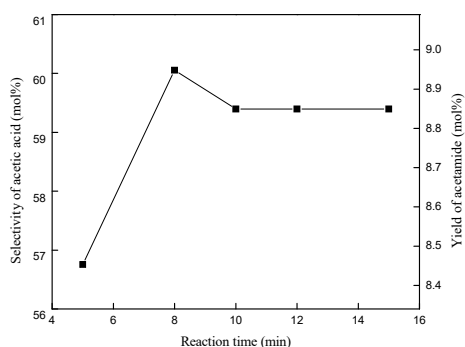


Fig.5. Effects of reaction time on selectivity and yield in the two-step process (step 1: 0.067 g microalgae; 100 % H₂O₂ supply; 300 °C; 80 s; step 2: 20 folds NH₃ supply; 320 °C).

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3.5 Possible pathway

The yield of acetic acid from cellulose was only 6.3% based on related literatures, while yields were 12-14.9 % from three kinds of representative algae in this research. Compared to composition analysis of cellulose, high protein content in microalgae may be the main reason. Besides, according to protein-derived amino acids could be converted into acetic acid under hydrothermal conditions, basic amino acids were regarded as the objects and were conducted the following research. A sequence of reaction conditions was discussed, and alanine and aspartic acid, the two common amino acids, were considered as the main targets due to the higher yields of acetic acid. The optimum conditions were presented in Table 3.

Table 3. Optimal reaction conditions and yields of alanine and aspartic acid.

Category	Temperature	H ₂ O ₂ supply	Time	Yields
alanine	300 °C	70 %	80S	36.9 %
aspartic acid	320 °C	160 %	80S	16.5 %

There was a big difference between the yields of alanine and aspartic acid, 36.9 % and 16.5 % respectively from Table 4, probably because three-carbon alanine could proceed a simpler oxidative decomposition than four-carbon aspartic acid. HPLC and GC-MS analyses were used to identify acetic acid, propionic acid, acetamide and formic acid as the major products, and they could be explained by the proposed reaction mechanism

in Fig.6 and Fig.7. In the first step, alanine undergoes a deamination reaction to lactic acid under hydrothermal conditions. And next, lactic acid will be oxidized to plenty of acetic acid, some propionic acid and trace formic acid in the presence of H₂O₂. Finally, some of acetic acid can be transformed to acetamide with NH₃, which stems from the deamination reaction of alanine in the first step. While aspartic acid will proceed a more complicate decomposition and produce a variety of organic acids, such as alanine, malic acid, and lactic acid. On one hand, aspartic acid could afford alanine via a decarboxylation process, and the subsequent pathway is identical to the Fig.6. On the other hand, aspartic acid can also be converted into malic acid through a deamination reaction under hydrothermal conditions. There exists the tautomerism between malic acid and oxaloacetic acid, and the latter can be decarboxylated into pyruvic acid, eventually into formic acid. Similarly, formic acid decomposed by malic acid can be converted into formamide with NH₃.

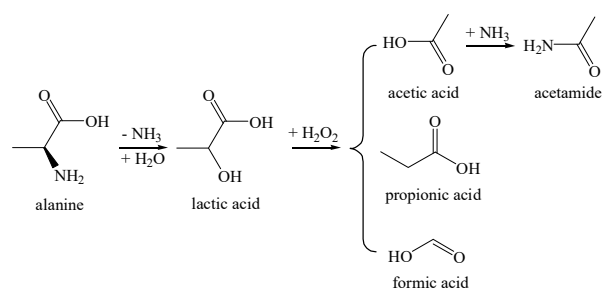


Fig.6. The possible pathway of alanine under hydrothermal conditions.

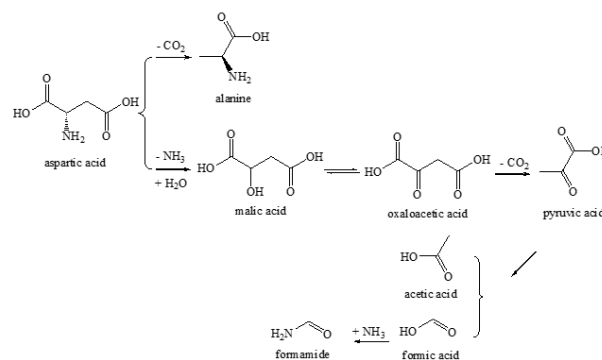


Fig.7. The possible pathway of aspartic acid under hydrothermal conditions.

So, the reason for lower yield in aspartic acid is that aspartic acid can undergo more reaction pathways and afford more products. More importantly, the pathways for aspartic are inclined to formic acid, because much formic acid was detected in the product of aspartic acid while no formic acid produced in alanine. During the production of acetic acid from microalgae, lactic acid is an important intermediate product and it can be converted into acetic acid under this hydrothermal condition. so how to improve the yield of lactic acid is crucial to the amount of acetic acid, and then acetamide.

4. Conclusion

In conclusion, a two-step production of acetamide from three kinds of representative algae under hydrothermal conditions was discussed. Results showed that the best yield of acetamide and selectivity of acetic acid were 9.5 % and 60.1 % at 320 °C for 8 min with a NH₃ supply of 30 from Spirulina, 8.0 % and 66.9 % at 320 °C for 8 min with a NH₃ supply of 40 from Cyanobacteria, 8.1 % and 67.5 % at 320 °C for 8 min with a NH₃ supply of 40 NH₃ supply from Autotrophic chlorella respectively. The results not only provide a green and sustainable process to produce acetamide from microalgae, but also facilitate the utilization of microalgae biomass. On the other hand, based on the reaction pathway of alanine and aspartic acid, lactic acid is an important intermediate during the two-step production of acetamide from microalgae under hydrothermal conditions. So, increasing the yield of lactic acid is the key to improve the amount of acetic acid and acetamide.

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