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 ℃ 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 (Kw) 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 haves 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
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% **H2O2** supply |
|----------------|-------|-------|-------|-------|----------------------|
| Spirulina      | 46.84 | 8.37  | 6.96  | 37.83 | 0.71 ml              |
| Cyanobacteria  | 46.56 | 5.74  | 7.73  | 40    | 0.69 ml              |
| Autotrophic chlorella | 36.18 | 7.33  | 5.36  | 48.14 | 0.50 ml              |

**2.2 Definition of terms**

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

\[
Y = \frac{\text{Moles of carbon in loaded microalgae sample}}{\text{Moles of carbon in acetic acid produced}} \times 100\% \tag{1}
\]

Yield of acetamide (mol%):

\[
Y = \frac{\text{Moles of carbon in acetamide produced}}{\text{Moles of carbon in loaded microalgae sample}} \times 100\% \tag{2}
\]

Selectivity of acetic acid (mol%):

\[
Y = \frac{\text{Moles of acetic acid produced in step 2}}{\text{Moles of acetic acid produced in step 1}} \times 100\% \tag{3}
\]

100% **H2O2** supply was defined as the volume of hydrogen peroxide (provide 30%wt H2O2) used to completely oxidize microalgae, where microalgae (approximated by C10.42H6.96N0.69O2.36) was converted to carbon dioxide, nitric acid and water, assuming 1 mol of H2O2 gives 1/2 mol of O2[23].

\[
C_{10.42}H_{6.96}N_{0.69}O_{2.36} + 10.42 H_2O_2 \rightarrow 3.90 CO_2 + 0.60 HNO_3 + 13.60 H_2O \tag{4}
\]

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.

\[
CH_3COOH + NH_3 \rightarrow CH_3CONH_2 + 2H_2O \tag{5}
\]

**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.
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 ℃, 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 declined to 48.2 % and 7.3 % due to decomposition of production at this point. Therefore, it should be more suitable to take 320 ℃ as reaction temperature in the two-step process.

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 ℃ 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 ℃. 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.
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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.

| Category  | Temperature | H2O2 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 exits the tautomerism between malic acid and oxaloacetic acid, and the latter can be decarboxylolated into pyruvic acid, eventually into formic acid. Similarly, formic acid decomposed by malic acid can be converted into formamide with NH3.

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 microalgal 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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