



Recovery of Organic and Amino Acids from Sludge and Fish Waste in Sub Critical Water Conditions

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Abstract

The possibility of organic and amino acid production from the treatment of sludge and fish waste using water at sub critical conditions was investigated. The results indicated that at sub-critical conditions, where the ion product of water went through a maximum, the formation of organic acids was favorable. The presence of oxidant favored formation of acetic and formic acid. Other organic acids of significant amount were propionic, succinic and lactic acids. Depending on the type of wastes, formation of other organic acids was also possible. Knowing the organic acids obtained by hydrolysis and oxidation in sub-critical water of various wastes are useful in designing of applicable waste treatment process, complete degradation of organic wastes into volatile carbon and water, and also on the viewpoint of resource recovery. The production of lactic acid was discussed as well. The results indicated that temperature of 573 K, with the absence of oxidant, yield of lactic acid from fish waste was higher than sewage sludge. The maximum yield of total amino acids (137 mg/g-dry fish) from waste fish entrails was obtained at subcritical condition ($T = 523$ K, $P = 4$ MPa) at reaction time of 60 min by using the batch reactor. The amino acids obtained in this study were mainly alanine and glycine.

Keywords: organic acids, amino acids, sub-critical water, hydrothermal, resources recovery

1. Introduction

Waste disposal is a serious problem in the world since the amount of waste increases every year. The presence of many industries that produce hazardous materials causes pollution and disturbs the ecosystem balance. Many processes have been done to treat the waste more efficiently without side effect. Technologies that would treat these wastes or recover some useful organic materials before disposal are necessary to prevent pollution. Thus, the recycling technology is needed to overcome these problems. The production of useful material from waste is becoming important recently in order to keep the environment clean and applicable recycling technologies offer better solution. Recovery or production of useful material may also compensate for the operating cost of treatment process.

Regarding treatment of organic wastes, recently, the use of sub-supercritical water treatment process (Hydrothermal treatment process) has attracted many researchers due to its potential as a solvent and as a catalyst for some organic reactions (Savage *et al.*, 1995; Goto *et al.*, 1998; Faisal *et al.*, 2005; Faisal *et al.*, 2007a;). Reactions in hydrothermal conditions have been gaining interests recently because of

the fascinating properties of water as a reaction media at elevated temperatures and pressures (Holgate *et al.*, 1995; Shanableh, 2000; Faisal *et al.*, 2007b; Faisal *et al.*, 2008; Faisal *et al.*, 2012). At room temperature and atmospheric pressure, water has a dielectric constant of 80 and ion product (K_w) of 10^{-14} . These values can be controlled by manipulating temperature and pressure, and could greatly affect the reactivity of various compounds in water. The dielectric constant expresses the affinity of water as a solvent to reaction substances especially for non-polar materials. In addition, ion product of water can also be adjusted to control the ability of hydrolysis. High ion product is good for hydrolysis. Under saturated vapor pressure, water has a maximum ion product at around 523 K.

The hydrothermal treatment process has been widely applied to various reactions such as reduction, pyrolytic, decomposition and dehydration (Goto *et al.*, 1997; Faisal *et al.*, 2008; Russell, *et al.*, 1998). Examples are oxidation of phenols, pyridine, and methanol; and hydrolysis of esters and thiodiglycol, among others. The technique was also applied to chemical recycling processes such as hydrolysis of polyethylene terephthalate (PET) into ethylene glycol and terephthalic acid. The most significant

applications are those in environmental processes such as decomposition of municipal sewage sludge and alcohol distillery wastewater (Goto *et al.*, 1998) and oxidation and decomposition of toxic organic compounds such as PCB and dioxins. However, there were only few studies reported on the application from the viewpoint of resources recovery. Shanableh (2000) studied the production of useful organic matter from sludge by applying super critical water oxidation. In the area of resource recovery from seafood processing wastes, a preliminary study on the application of sub-critical water to produce amino acids from waste by hydrolysis has been studied (Kang *et al.*, 2001; Yoshida *et al.*, 1999).

Among various compounds that can be obtained from the treatment of organic wastes, organic acids are of significant importance. In high temperature and pressure water oxidation of organic wastes, low molecular weight carboxylic acids are usually the intermediate products prior to complete degradation to volatile carbon and H₂O. Oxidation of these compounds is reported to be the rate-controlling step during oxidation of organic wastes in supercritical water (Krammer and Vogel, 2000; Luck, 1999; Gloyna and Li, 1993; Robert Shaw *et al.*, 1991). In the study of oxidation of organic compounds in supercritical water, oxidation of these low-molecular weight organic acids serves as model (Li *et al.*, 1999; Sasaki *et al.*, 1998). Thus, it is important to have basic knowledge on what and how much organic acids can be obtained from various wastes. This information can be useful in design of process for the treatment of organic wastes. Aside instead of knowing the reaction intermediates useful for process design, on the point of view of resource recovery, this would also give information on what compounds we could recover from the treatment process. One possible product of interest is lactic acid, a compound useful in various applications such as pharmaceutical, production of biodegradable plastics, etc.

This paper reports recovery of useful organic and amino acids from sludge and fish waste by using sub-supercritical water treatment process. The results are useful in design of applicable process for supercritical water oxidation of various organic wastes. The temperature and time dependence of organic acid production is investigated as well.

2. Methodology

In this work, sewage sludge and fish waste were chosen as tested materials. Sewage sludge was obtained from wastewater treatment plant of Toyohashi University of Technology. The samples were settled using gravitation system, then centrifuged for 10 minutes (High speed refrigerated centrifuge, RS 206, 7000 rpm) to separate the water from solid materials. The solid part was freeze-dried for about 8 hours using Freeze Drier (FDU-506). Fish meat was obtained from a seafood-processing factory. The samples were homogenized using a mixing cutter (Toshiba CGM-NI) for 10 min, and then stocked in a freezer at -21°C. Water used in this experiments was doubly distilled and pass through an ion exchange unit.

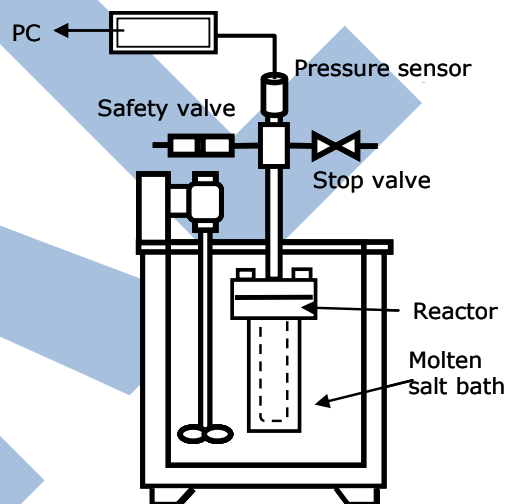


Figure 1. Schematic diagram of experimental apparatus.

The experimental apparatus (batch reactor, volume = 6 ml) constructed of stainless steel was used. The sample to water weight ratio was 1:50. After placing the sample and corresponding amount of water, the reactor was sealed and then the air inside was purge using argon gas. The reactor was immersed in a preheated molten salt bath containing of 45% of potassium nitrate and 55% of sodium nitrate. The reaction was carried out for 30 - 60 min at desired temperature conditions and pressure at the reaction tube was determined from steam tables (saturated vapor pressure). After a predetermine time, the reactor was remove from the bath and quenched in a 293 K water bath. Schematic diagram for the experimental apparatus is illustrated in Figure 1.

2.1. Analysis of Organic and Amino Acids

Organic acids and amino and contents of reaction products were determined using organic acid analyzer (LC-10A, Shimadzu Corp.) and amino acid analyzer (LC-10AD, Shimadzu Corp.), respectively. The organic acid analysis system consists of an ion exclusion column (Shim-Pack SCR-102H) and electroconductivity detector. The objects to be analyzed are aliphatic carboxylic acids, hydroxycarboxylic acids, ketocarboxylic acids, and other organic acids having dissociation constant (pKa) of 2 to 5 and carbon number of 5 or less.

On the other hand, the amino acid analyzer is a combination of an ion exclusion column (Shim-pack Amino-Na, Shimadzu Corp.) and post-column labeling methods with spectrofluorophotometer (RF-10A, Shimadzu Corp.). In sample preparations for amino acid analysis, filtration was done using ultrafiltration membrane (30,000 fractional molecular weight, Millipore Ultra Free C3) to keep good performance of the chromatographic system. The quantities of 17 amino acids – Aspartic acid (Asp), Threonine (Thr), Serine (Ser), Glutamine (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cystine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ileu), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His), Lysine (Lys), and Arginine (Arg), could be determined in each sampling run.

3. Results and Discussion

3.1. Organic Acids Production

This discussion is focused on the production of useful organic acids from sewage sludge and fish waste. The wastes were chosen since they contain relatively high percentage of protein that might have higher possibility for organic matter recovery. The properties of sewage sludge and waste fish entrails are shown in Table 1.

To verify the presence of proteins in these wastes, acid hydrolysis was performed and the results are shown in that the protein in fish waste (59 wt %) is much more than the amount in sewage sludge (16 wt %).

Figure 2 shows the HPLC chromatogram of organic acids product from fish waste at 573 K. Depend on operation conditions, other organic compound might possible exist. There are many parameters influencing the efficiency high temperature and high

pressure water treatment towards formation of organic acids, one of these parameters is temperature.

Table 1. Properties of sewage sludge and fish waste

Element	Sewage Sludge	Fish Waste
C	27.5%	49%
H	5.28%	6.38%
N	4.09%	11.32%
Others	63.13%	33.3%
Water-content	56%(After vacuum drying)	80%(without drying)

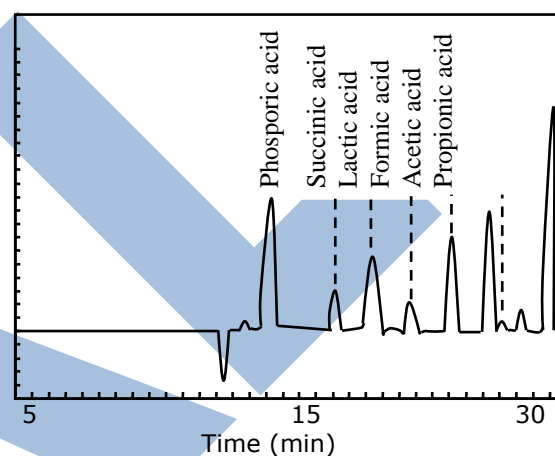


Figure 2. HPLC Chromatogram of product Containing organic acids from fish waste at 573 K and reaction time of 30 min.

Results show that the amount of organic acids increases with increasing temperature at subcritical condition and decreases as it approaches critical point of water (647 K, 22.1 MPa). At sub-critical condition, compared to supercritical condition, the ion product of water increase significantly. The ion product of water at temperature of 573 K (9 MPa) is about 10^{-11} moles²/L². Higher dielectric constant and the existence of highly polar water molecules at sub critical water promotes the formation of organic matters, at supercritical water conditions, the dielectric constant of water and the inorganic solubility begin to drop off markedly.

Figure 3 shows the production of organic acids from fish waste (a) and sewage sludge (b). As shown in the figure, both hydrolysis and oxidation favor formation of organic acids. Oxidation of fish waste and sewage sludge is favorable to formation of formic and acetic acid. The compounds are usually the major intermediate products prior to complete degradation to volatile carbon and

water. As shown in Figure 3, due to the stability of acetic acid, formation of acetic acid increases while formic acid decomposes more readily with increasing temperature. The production of OH radical from H_2O_2 might assist in the production of low molecular carboxylic acids. Among organic acids obtained, acetic acid is the major product. This is due to its relative stability or refractory behavior.

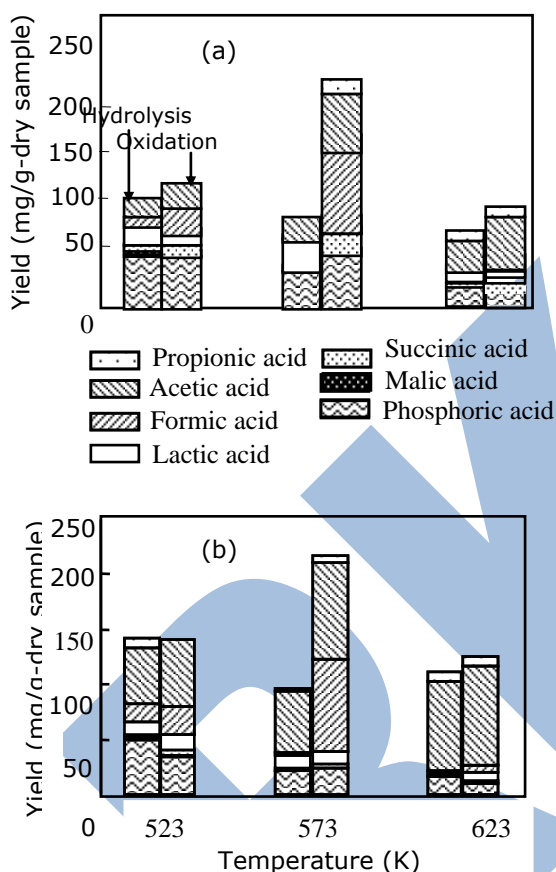


Figure 3. Temperature dependence of organic acid production by hydrolysis and oxidation of fish waste (a) and sewage sludge (b), ratio water to sample = 1:50, reaction time = 30 min.

In preliminary experiment, for reaction time of 60 minutes and sample to water weight ratio of 1:20, the optimum condition for production of lactic acid is 15.93 mg/g-dry fish at 573 K (9 MPa). In case of sludge, the same optimum condition was obtained at reaction time of 30 min and sample to water weight ratio of 1:80. At this condition, 18.12 mg of lactic acid/g-dry sludge was obtained. The production of lactic acid decreases as it approaches critical point. This decrease in lactic acid production is probably due to decomposition of lactic acid to other organic acids such as acetic acid, etc. and volatile compounds (CO_2 , etc.). Figure 3 also describes that the formation of lactic acid is

favoured in the absence of oxidant (H_2O_2). Recycling is not limited to lactic acid but also includes other organic products of thermal decompositions in high temperature and high pressure water reactions. On the other hand, this technique is also useful for supporting improvement biodegradability and enhancing biological phosphorus removal (Sanableh, 2000). Manipulating the temperature and pressure might increase the yield. As an example, (hydrolysis of fish waste, Figure 3 a), adjusting the ratio water to sample to 1:50, 573K, 9 MPa resulting on the increasing of lactic acid production at about 40%, or 27 mg/g-dry fish. Without the ignorance of cost factor, selecting an effective process and finding the optimum condition are very important to obtain a higher yield for the production of lactic acid. In order to generate of that, comparison of several processes is necessary.

So far the information for reaction pathways on decomposition of wastes into useful products such as lactic acid and its mechanism is not enough yet. Since organic wastes contain many chemical compound, we have to know which one of them play a major role as a reaction pathways and mechanism to produce lactic acid. Knowing reaction pathways and mechanism play a major role to develop and to support engineering design.

3.2. Amino Acid Production

In this study, we also investigate the possibility of amino acids obtained by hydrolysis of the waste at sub-supercritical conditions. As commonly known the amino acids have wide uses and applications in pharmaceuticals, food products, animal nutrition and cosmetic industries. As medicine, they are being used for the treatment of various diseases such as renal, gastrointestinal, endocrinal, dermal among others. In food industry, amino acids can be used as taste enhancers (e. g. sweeteners – glycine (Gly) and alanine (Ala), sourness – glutamine (Glu) and asparagine (Asp), bitterness – arginine (Arg)) and as animal feeds. If each amino acids could be separated individually, these could also be reagents for synthesis of new materials including electronic-related chemicals (e. g. for liquid crystals, exposure liquids for color copiers).

Figure 4 shows the effect of reaction temperature under sub- and supercritical conditions on the yield of amino acids from fish waste. All reactions were carried out at a

constant sample to water ratio of 1:20. At subcritical conditions, the reaction pressure corresponds to the saturated vapor pressure of water at each temperature (i. e. 2 MPa (at 473 K), 4 MPa (at 523 K) and 9 MPa (at 573 K)). At supercritical conditions, pressures of 45 and 35 MPa were set for temperatures of 653 and 723 K, respectively. The amount of amino acids at 298 K, obtained from the solution prepared by soaking sample in water for 30 min, corresponds to the free amino acids originally present in the sample. The temperature dependence of ion product of water (K_w) at each pressure (represented by open circles) is also shown in Figure 4.

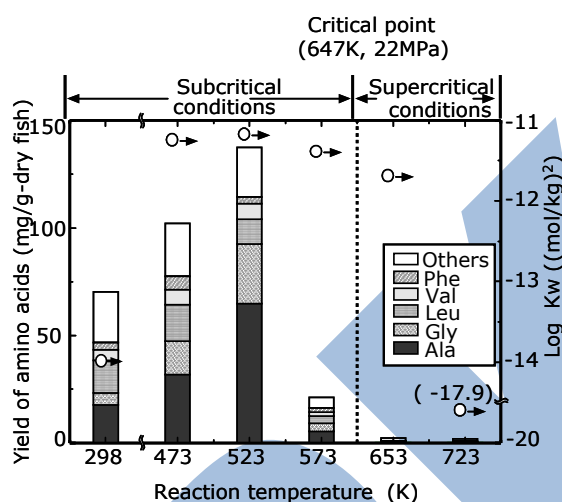


Figure 4. Effect of reaction temperature at suband supercritical conditions on the yield of amino acids in a batch reactor (reaction time = 60min).

As shown in Figure 4, among the amino acids, only alanine and glycine increased with increasing reaction time up to the temperature of 523 K. The highest yield of total amino acids can be obtained at $T = 523$ K ($P = 4$ MPa). This result agrees with that obtained from the preliminary experiment using a semi-batch reactor. This is near the optimum conditions ($T = 543$ K, 5.51 MPa) obtained by Yoshida *et al.* (1999) in the study of hydrolysis of fish meat. As stated in their work, the reason for high yield of amino acids by hydrolysis at this condition is the high ion product of water. The temperature that gives the maximum yield of amino acids agrees to that with the maximum K_w . At temperatures below 523 K and at high pressure and temperature ($T > 523$ K), the values of K_w are relatively low. This is expected to be the reason for the low yield of amino acids at these temperature ranges. At high temperature, the yield of amino acids is low because decomposition is favored over production of amino acids. At 573 K and reaction time of 60 min, almost all amino

acids are decomposed to organic acids and ammonia by decarboxylation and deamination, respectively.

Based on the results in Figure 4, the maximum yield of amino acids was obtained at $T = 523$ K (4 MPa). Under this condition, the effect of reaction time on the yield of amino acids was further studied. The reaction time was varied while maintaining the temperature of the salt bath at 523 K. As an intrinsic characteristic of a batch reactor, it took about 5 min for the temperature inside the reactor to reach the temperature of the salt bath (i. e. 523 K). During the first 5 min, at non-isothermal condition, the increase in the yield of amino acid is not significant. In this work, reaction time starts when the reactor is soaked into the salt bath.

At reaction time greater than 5 min, the yield starts to increase then reaches a peak at 60 min (137 mg/g-dry fish). At longer reaction time, the yield tends to decrease. This result implies that production of amino acids is favored over decomposition at reaction time less than 60 min.

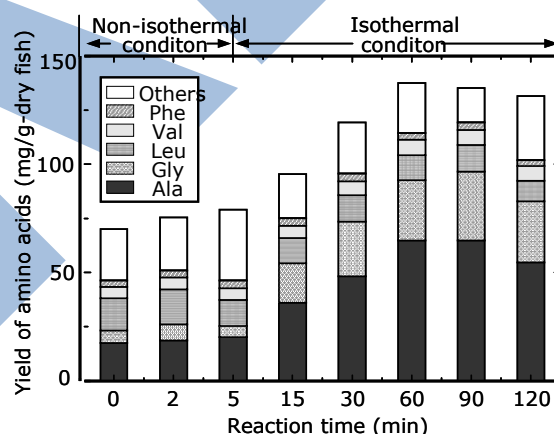


Figure 5. Effect of reaction time on yield of various amino acids. ($T = 523$ K, $P = 4$ MPa).

The specific amounts of various kinds of amino acids produced from the hydrolysis of proteins at different times are also shown in Figure 5. The main products are Alanine (Ala), Glycine (Gly), Leucine (Leu) and Valine (Val) with molecular weight in the range of 75.1 – 165.2. The yield is in the following order – Ala > Gly > Leu at 65, 28, and 12 mg/g-dry fish, respectively. It could be observed that only the amount of alanine changes significantly with increasing reaction time.

4. Conclusion

Production of useful organic matters from fish waste and sewage sludge has been

investigated by hydrolysis and oxidation in subcritical water. Depend on varying of operation conditions, various organic acids were found. The formation of lactic acid is favored in the absence of oxidant. At Subcritical conditions, where the ion product goes to maximum, the formation of useful organic matters is favorable. Recycling is not limited to lactic acid but also includes other organic products of thermal decompositions in high temperature and high pressure water reaction. The study on the effect of temperature showed that the maximum yield of total amino acids (137 mg/g-dry fish) from waste fish entrails was obtained at subcritical condition (T = 523 K, P= 4 MPa) at reaction time of 60 min by using the batch reactor. The amino acids obtained in this study were mainly alanine and glycine (65 and 28 mg/g-dry fish, respectively). Under supercritical conditions (e.g. T = 653 K, P = 45 MPa), the yield of amino acids decreases because of higher decomposition compare to production rate of amino acids at high temperatures and pressures.

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