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Ultrasonic Pretreatment for Thermophilic Aerobic Digestion in Industrial Waste Activated Sludge Treatment

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Abstract In order to enhance the degradation efficiency of waste activated sludge (WAS) by thermophilic aerobic digestion, an ultrasonic pretreatment was examined. It was observed that ultrasonic pretreatment increased the solubilization of organic matter in the WAS and that the solubilization ratio of the organics increased during the first 30 min but did not extensively increase thereafter. Therefore, a pretreatment time of 30 min was determined to be the economical pretreatment time from the experimental results. From the digestion experiments, which was conducted using the WAS collected from an oil refinery plant in Inchon, Korea, investigating the effects of an ultrasonic pretreatment on thermophilic aerobic digestion, it was confirmed that the proposed ultrasonic pretreatment was effective at enhancing the release of the cellular components in WAS and the degradation of released components in the thermophilic aerobic digestion.

Keywords: thermophilic aerobic digestion, waste activated sludge, ultrasonic pretreatment, optimization

INTRODUCTION

The activated sludge process is the most widely used biological treatment process for municipal and industrial wastewater. It allows the transformation of dissolved organic pollutants in the wastewater into biomass, or into carbon dioxide and water by microorganisms [1]. The major by-product of this process is the waste activated sludge (WAS), which mainly consists of microbial biomass. As a result of quantitative and qualitative expansion of the municipal and industrial wastewater treatment, WAS has also increased.

In order to minimize the quantities of WAS, anaerobic mesophilic digestion processes were used as conventional WAS biotreatment methods [2]. These processes, however, have some disadvantages, which include the incomplete inactivation of pathogenic organisms and a low WAS degradation rate [3]. Therefore, a thermophilic aerobic digestion (TAD) process has recently emerged as a possible candidate for the more effective sludge degradation and a method of reducing the number of pathogenic organisms [4]. Several researchers have studied the thermophilic aerobic digestion process using municipal WAS and found significant results with respect to total suspended solids and pathogen reduction [5,6]. In the thermophilic aerobic digestion process, the potentially pathogenic microorganisms are deactivated by temperature shock and autolytic mechanisms, but also by exo-enzymes, which are produced by the thermophilic bacteria [7,8]. The thermophilic aerobic digestion process, however, has not been successfully applied to the reduction of industrial WAS having high organic matters, with a study from our laboratory related with the thermophilic aerobic digestion of industrial WAS, collected from oil refinery plant, being the only one reported [9]. This study was focused on the selection of thermophilic bacteria for the effective treatment of industrial WAS. The degradation efficiency of industrial WAS is not as high as that of municipal waste activated sludge because of the relatively slow degradation rate and the high contents of recalcitrant organic compounds. During the digestion processes, it was thought that the hydrolysis of proteins and carbohydrates in particulate forms, which is the rate-limiting reaction, was not sufficiently achieved by the exo-enzymes produced by the thermophilic aerobic bacteria.

Some studies on sludge pretreatment effects on digestion achieved an enhanced hydrolysis rate [10,11]. These pretreatments led to the rupture of the cell wall and membranes of the bacteria in the WAS, resulting in the release of the cellular components. These organic substances, mainly proteins and carbohydrates, can be easily hydrolyzed to unit molecules by the extracellular enzymes excreted from thermophilic aerobic bacteria. However, those studies focused on applications with anaerobic mesophilic sewage sludge digestion, and pretreatment studies related with the thermophilic aerobic digestion of industrial WAS have not been reported. The order of pretreatment efficacy for the enhancement of anaerobic mesophilic digestion treatment performance was investigated by Wang *et al.* [10], and is as follows: ultrasonic lysis (20 W, 9 Hz, 30 min) > thermal

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pretreatment by autoclaving (120°C, 30 min) > thermal pretreatment with hot water (60°C, 30 min) > freezing (-10°C, 15 h). From their results, the ultrasonic treatment was selected as the best pretreatment method for anaerobic mesophilic digestion.

In this study, the possibility of using an ultrasonic pretreatment for an enhanced degradation performance within a thermophilic aerobic industrial WAS digestion was examined. The results were evaluated with regards to the effect of the ultrasonic pretreatment on the rupture and solubilization of WAS in terms of total suspended solids (TSS), dissolved organic carbon (DOC), extracellular protein, and extracellular carbohydrate concentrations. The dependency of WAS solubilization on the pretreatment time was also investigated to optimize the time of operation at a constant ultrasonic power. Finally, the degradation efficiency of the thermophilic aerobic digestion without an ultrasonic pretreatment was compared to that with a pretreatment. The effects of the ultrasonic pretreatment on the thermophilic digestion were verified from these comparison experiments.

MATERIALS AND METHODS

Ultrasonic Pretreatment Experiments

The industrial WAS used in this study was collected from a gravity thickener in an activated sludge process at an oil refinery plant in Inchon, Korea. The characteristics of the WAS were controlled as follows; TSS concentrations in the range of 11-15 g/L and DOC concentrations in the range of 130-200 mg/L.

A 50-mL sample of WAS was placed in a beaker (inner diameter 4.5 cm, occupied height 3.5 cm) and pretreated with an ultrasonicator (Kumsung Sonication Co., Korea, 150 W, 20 kHz). The contact surface area of the WAS was 65.4 cm^2. The temperature was maintained at 30-35°C during the pretreatment experiments using a cooling water. The ultrasonic pretreatment experiments were carried out using the same batch WAS for 10, 20, 30 or 40 min. Samples were analyzed immediately after collection, and then compared to the pretreatment efficiency. The results of the pretreatment experiments were obtained from an average of three experiments performed under identical conditions.

Thermophilic Aerobic Digestion Experiments

All digestion experiments were performed in a 3-L laboratory scale bioreactor (BIOFLO III, New Brunswick Scientific Co., NJ, USA) with a working volume of 2.5 L. The monitoring of temperature, pH, dissolved oxygen (DO) concentration, and impeller speed was fully auto-mated. A temperature of 60°C, a pH between 7.0 and 8.0, a dissolved oxygen concentration above 1.0 mg/L, and an impeller speed of 300 rpm were maintained throughout all experiments. To maintain aerobic conditions, the air, humidified by bubbling it through water, was supplied into the bioreactor through a silicone tube membrane with a flow rate of 1.0 vvm.

In the batch digestion experiments, the thermophilic bacteria, *Bacillus stearothermophilus* (ATCC 31197), which was used in our previous work [9], was collected from a flask culture during the late-exponential growth phase (15 h old). The bacteria were then concentrated by micro-filtration using a cellulose ester membrane filter (MFS membrane filter, pore size 0.2 μm, ADVANTEC Inc., Pleasanton, CA, USA) and then the concentrated thermophilic bacteria were inoculated into a bioreactor with 2.5 L of prepared WAS. The inoculum concentration in batch experiments was 4.0 g FCW (fresh cell weight)/2.5 L. The bioreactor was operated up to 100 h with either untreated or ultrasonic pretreated WAS, while 10 mL samples were collected every 3 h, using the sampling port, for TSS, DOC, TOC, protein, and carbohydrate concentration analyses. Evaporative loss was estimated by measuring the culture volumes, and sterilized distilled water was added daily to compensate for this loss and to assure a constant culture volume. The pretreated WAS was prepared by ultrasonic pretreatment with the optimized operation time.

Analytical Procedures

The total suspended solids concentration was determined using the following procedure and is expressed as g/L. Samples, 5 mL, were filtered through a pre-weighed glass microfiber filter (GF/C, Whatman Ltd., Maidstone, UK) and then the filter was dried at 80°C for 24 h. The dried filters were re-weighed and the difference was used to calculate the total suspended solids concentrations.

For analysis of the dissolved organic carbon (DOC) concentrations, 1-mL samples were centrifuged at 10,000 g and the supernatants were collected. Appropriate dilutions of the supernatants were made and the DOC concentrations were measured with a total organic carbon analyzer (TOC-5050A, Shimadzu Co., Tokyo, Japan). The total organic carbon concentrations were directly measured with a total organic carbon analyzer using the samples.

Samples were centrifuged at 10,000 g and the supernatants were collected to analyze the extracellular protein and carbohydrate concentrations. Extracellular protein concentrations were measured using the Bradford method with bovine serum albumin as a standard [12]. In order to measure the extracellular carbohydrate concentrations, a spectrophotometric method was used [13]. Measurements were performed at 489 nm with glucose as the standard. All protein and carbohydrate concentrations were calculated as the average of three analyses.

RESULTS AND DISCUSSION

Effects of Ultrasonic Pretreatment on WAS Degradation

The ultrasonic pretreatment experiments were carried out with various treatment times: 10, 20, 30, and 40 min. The TSS, DOC, extracellular protein, and carbohydrate concentrations were measured in the control and all pretreatment experiments, and are shown in Fig. 1(a) and (b). In Fig. 1(a), the TSS concentration remained fairly constant, but DOC concentration significantly increased according to the pretreatment time. Extracellular protein and carbohydrate concentrations also significantly increased, as shown in Fig. 1(b). From these results, it was verified that the bacterial cells, main components of WAS, were broken open by ultrasonic pretreatment and their intracellular components were released to the outside of the cells, primarily since the pressure waves generated by the ultrasonicator could break down flocs and individual bacterial cells.

The TSS concentration showed no change with any pretreatment time, but DOC concentration rapidly increased until a pretreatment time of 30 min, but showed a slight increase with pretreatments over 30 min, as shown in Fig. 1(a). Increase in the extracellular protein and carbohydrate concentration had a similar pattern to that of the DOC, but extracellular protein concentration showed fairly linearly increased with pretreatment time as shown in Fig. 1(b). From the above results, it was found that bacterial cell disruption and intracellular organic substance release were proportionally enhanced with the pretreatment time up to 30 min, and that there was no significant change in WAS solubilization with a treatment time of greater than 30 min. Therefore, 30 min was chosen as the economical ultrasonic pretreatment time.

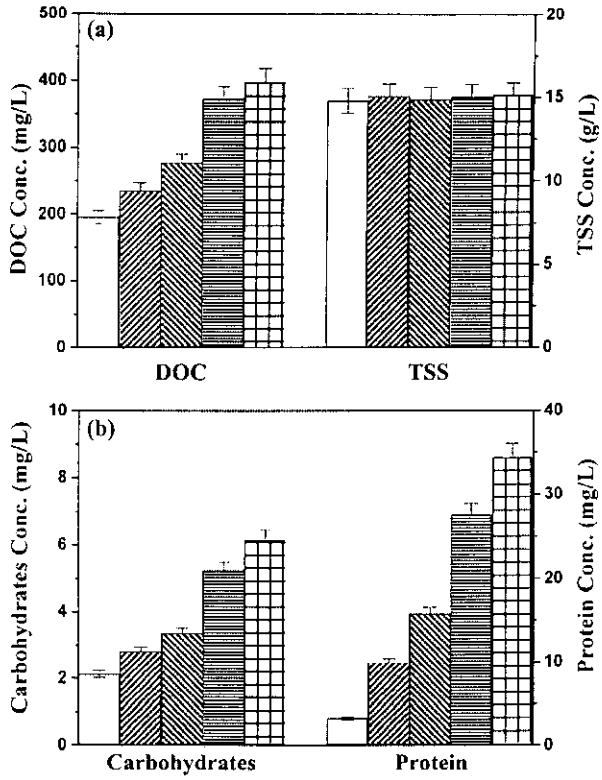

Fig. 1. The effect of ultrasonic pretreatment for various length of time; (a) changes in the TSS, and DOC concentration, (b) changes in the extracellular protein and carbohydrate concentrations (□: control, ▨: 10 min, ▧: 20 min, ▤: 30 min, ▦: 40 min).

Effect of Ultrasonic Pretreatment on Thermophilic Aerobic Digestion

Experiments on the digestion of WAS by a thermophilic aerobic process were carried out with the addition of *Bacillus stearothermophilus* (ATCC 31197). The performance of the experiments for industrial WAS degradation was estimated by changes in TSS, DOC, TOC, protein and carbohydrate concentrations. A control experiment, without ultrasonic pretreatment of the WAS, was performed to compare with the digestion experiments using the pretreated WAS. The time course behaviors of TSS, DOC, TOC, extracellular protein and carbohydrate concentrations are shown in Fig. 2 through Fig. 5, respectively.

As shown in Fig. 2, the TSS concentrations were reduced in both digestion experiments. In the control experiment (without ultrasonic pretreatment), the TSS concentrations continuously decreased until a time of 96 h. The decrease in the TSS could be explained by two mechanisms; one is the decomposition of the particulate solids by a thermal effect, and the other is due to cell lysis by the proteolytic enzymes secreted from thermophilic aerobic bacteria. In control experiment, it is thought that the initial rapid decrease in TSS concentrations is due to a thermal effect and the decrease seen during the rest of the experiment was induced by the extracellular enzymes having proteolytic activity. In contrast to control digestion experiment, the experiment using ultrasonic pretreated WAS showed a more rapid decrease in the TSS concentrations early on, about 30 h, and then a similar behavior to that of the control during the rest of experiment. This is probably due to the combined effects of thermophilic digestion and ultrasonic pretreatment. Consequently, the reduction in both of the thermophilic aerobic digestions, with and without ultrasonic pretreatment, showed a similar decomposition efficiency, 46.3% and 44.5% after 96 h, respectively.

The results of DOC reduction during the WAS digestion experiments are shown in Fig. 3. The accumulation of the solubilized product, which was caused by disruption of microorganisms, was confirmed by the rapid increase in DOC concentrations during the early stages of the experiments, and both the hydrolysis and the solubilization of the released compounds might result in the increase of DOC concentration [14]. However,

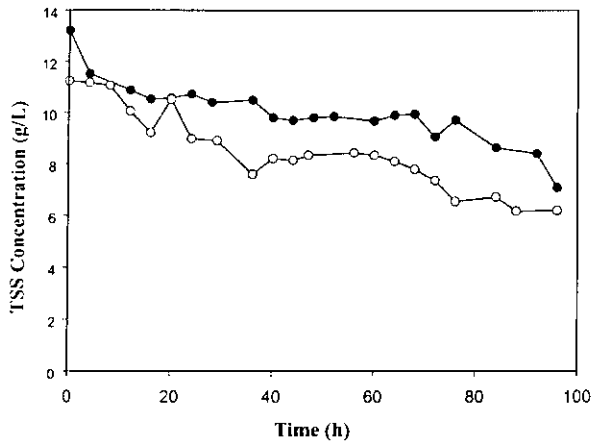

Fig. 2. Comparison of TSS concentrations over a period of 96 h during thermophilic aerobic digestion of WAS with or without pretreatment (o: control (without pretreatment), ●: with pretreatment).

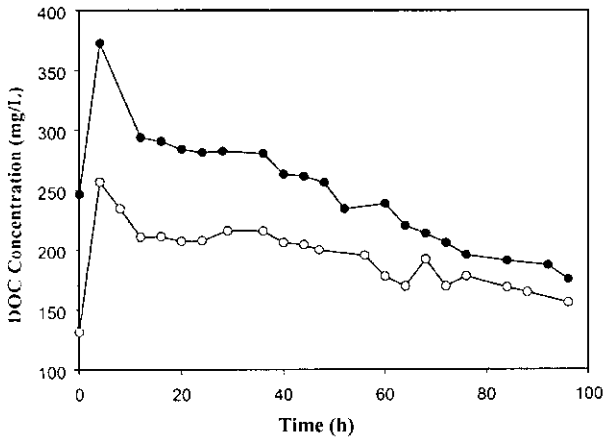

Fig. 3. Comparison of DOC concentrations over a period of 96 h during thermophilic aerobic digestion of WAS with or without pretreatment (o: control (without pretreatment), ●: with pretreatment).

rate at which the DOC concentration increased was significantly different from each other. In the case of the thermophilic aerobic digestion without pretreatment, DOC concentrations within 4 h increased by about 95% of the initial DOC concentration. But in the case of thermophilic aerobic digestion with ultrasonic pretreatment, initial DOC concentrations were similar to that of the DOC at 4 h in thermophilic aerobic digestion experiment without pretreatment, because of the solubilization of WAS by ultrasonic pretreatment. Then, the DOC concentration further increased by about 51%. Consequently, the highest DOC concentrations in the cases of thermophilic aerobic digestion with and without ultrasonic pretreatment were 195% and 283% of the unpretreated raw WAS, respectively. On the other hand, the final DOC concentrations in both digestion experiments did not show significant differences. Although the final DOC concentration was similar to

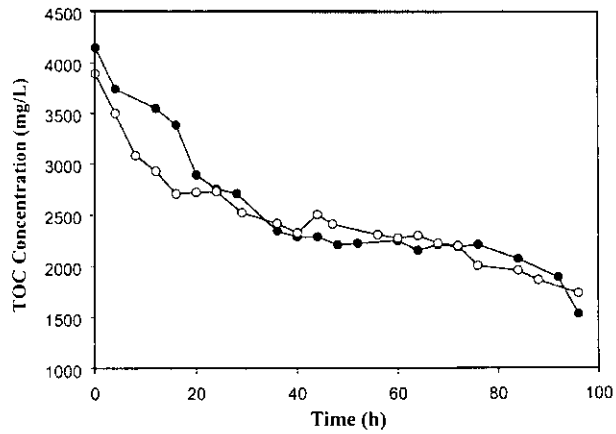

Fig. 4. Comparison of TOC concentrations over a period of 96 h during thermophilic aerobic digestion of WAS with or without pretreatment (o: control (without pretreatment), ●: with pretreatment).

each other, the digestion with pretreatment can be considered as the better process because the DOC degradation efficiency of the digestion with pretreatment, 53.6%, was much higher than that of the digestion without pretreatment, 39.5% (DOC degradation efficiency was calculated based on maximum DOC concentration). From the these results, it was verified that the solubilization of organic substances was induced by ultrasonic pretreatment and that an increased amount of DOC compounds could be successfully degraded by the thermophilic aerobic bacteria.

It was reported that about 70% of the WAS is composed of organic matter and this organic fraction can be stabilized by digestion [15]. In order to achieve TOC degradation, the solubilization of particulate organic carbon and the degradation of DOC must occur simultaneously. The time course behavior of TOC concentrations in both WAS digestion experiments is shown in Fig. 4. While the reduction of TOC was achieved to about 55.4% of the initial TOC concentration in the control digestion experiment without pretreatment, a slightly higher reduction of TOC, 63.3%, was observed in digestion experiments that had a pretreatment. This might due to the additional solubilization of particulate organic substances by the ultrasonic pretreatment.

The time course of extracellular protein and carbohydrate concentrations are shown in Fig. 5(a) and (b), respectively. The protein concentration rapidly increased during the first 16 h in both digestion experiments. The increased protein concentration was maintained until the end of the experiment using unpretreated WAS, but the increased protein concentrations in the pretreated WAS decreased until the time of 28 h and then was maintained nearly constant until the end of the experiment. Therefore, the increased amount of released protein, which was induced by ultrasonic pretreatment, was successfully removed by thermophilic aerobic digestion.

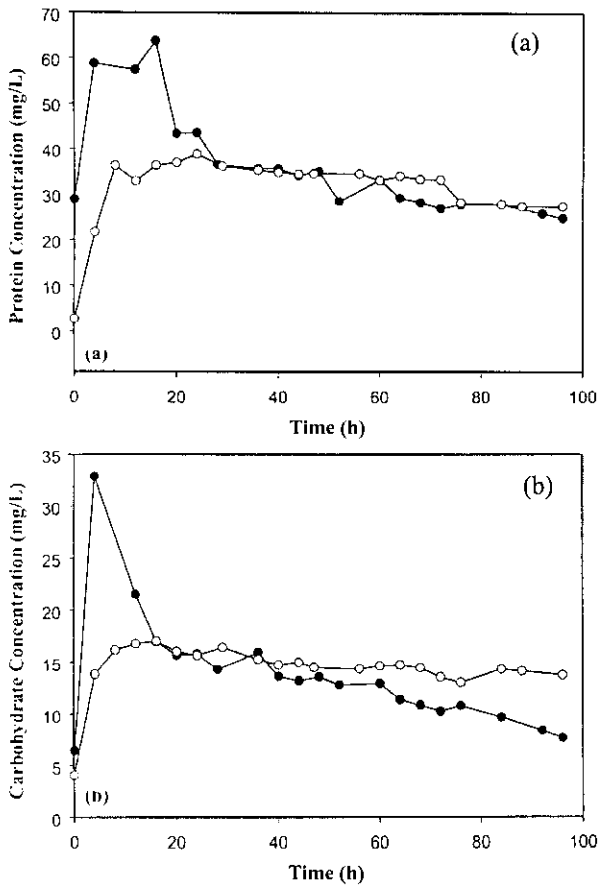

Fig. 5. Comparison of (a) extracellular protein concentrations and (b) extracellular carbohydrate concentrations over a period of 96 h during thermophilic aerobic digestion of WAS with or without pretreatment (o: control (without pretreatment), ●: with pretreatment).

The extracellular carbohydrate concentrations rapidly increased during the initial 4-8 h, as shown in Fig. 5(b). In the case of the digestion using ultrasonic pretreated WAS, the increased level of carbohydrates rapidly decreased until 20 h, and gradually decreased thereafter, while the increased carbohydrate concentration was not seen to decrease in the control experiment. It is regarded that the release and decomposition rates of the carbohydrates were equal after 20 h in the control experiment. From these results, ultrasonic pretreatment could enhance the release of carbohydrates, followed by the carbohydrate degradation by the thermophilic aerobic digestion.

For the overall comparison of the effect of an ultrasonic pretreatment, the reduction efficiencies of TSS, DOC, TOC, extracellular protein, and carbohydrate are shown in Table 1. From these results, it was verified that ultrasonic pretreatment with an optimal operation time could enhance the performance of thermophilic aerobic digestion. Our research will be extended to consider the enhancement of thermophilic aerobic WAS digestion by other pretreatment processes based on author's previous studies.

Table 1. The effects of ultrasonic pretreatment on the degradation performance of the thermophilic aerobic digestion

Degradation efficiency (%)	TSS[a]	DOC[b]	TOC[a]	Protein[b]	Carbohydrates[b]
Control	44.5	39.5	55.4	42.0	19.3
With pretreatment	46.3	53.1	63.3	61.1	76.6

[a] based on initial concentration, [b] based on maximum concentration

CONCLUSION

It was verified from experimental results that the solubilization efficiency depended on the ultrasonic pretreatment time. The solubilization of organic substances increased with the time of pretreatment up to 30 min, but there was no extensive increase over 30 min. Thus, a pretreatment time of 30 min was determined as the economical condition. From the experimental results investigating the effect of the ultrasonic pretreatment on thermophilic aerobic digestion, which was conducted using the WAS collected from an oil refinery plant in Inchon, Korea, it was observed that an ultrasonic pretreatment would enhance the release of the cellular components and the degradation of the released components in the thermophilic aerobic digestion.

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