J. Microbiol. Biotechnol. (2001), **11**(4), 570–576

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Application of a Thermophilic Aerobic Digestion Process to Industrial Waste Activated Sludge Treatment

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Received: December 27, 2000
Accepted: May 7, 2001

Abstract Thermophilic aerobic bacteria were applied in the degradation of industrial waste activated sludge (WAS) on a laboratory scale experiment. The performance of digestion was estimated by measuring the reduction of total suspended solids (TSS), dissolved organic carbon (DOC), and total organic carbon (TOC). Among three strains of *Bacillus stearothermophilus* and three strains of *Thermus* species, *B. stearothermophilus* ATCC 31197 showed the best overall efficiency level for the degradation of industrial WAS, which was collected from a wastewater treatment plant in an oil refinery factory. Industrial WAS could be successfully degraded in a batch digestion with ATCC 31197. The stability of the digestion process with ATCC 31197 was successfully verified by semi-continuous (fill-and-draw) digestion experiment. From the results of this study, it was shown that the thermophilic aerobic digestion process with ATCC 31197 could efficiently be applied to the degradation of industrial WAS.

Key words: Thermophilic aerobic digestion, waste activated sludge, *Bacillus stearothermophilus*, *Thermus* species

The activated sludge process has been the most widely used biological treatment process for municipal and industrial wastewaters. It allows the transformation of dissolved organic pollutants from wastewater into biomass, and the organic matters are finally converted into carbon dioxide and water by microorganisms [17]. The major by-product of this process is the waste activated sludge (WAS), which consists mainly of microbial biomass. As a result of quantitative and qualitative expansion of the municipal and industrial wastewater treatment, the production of the major by-product, WAS, has also been increased.

Laws governing wastewater treatment require both economical and environmentally safe disposal as a result of increase of this waste. Recently, USA, EC, and other countries have introduced stringent regulations for the disposal of WAS produced from wastewater treatment works [4, 7, 20]. From the viewpoint of resource recycling, the reuse of WAS to agricultural land was suggested as one of the most attractive methods for ultimate sludge disposal. However, the application to agricultural land has been restricted, because of the presence of potentially hazardous components in WAS treated by conventional mesophilic anaerobic digestion [10].

The thermophilic aerobic digestion (TAD) process has recently emerged as a possible candidate for assuring a more effective sludge disposal and satisfying the reuse regulations [9]. Several researchers have studied the thermophilic aerobic digestion process by using municipal WAS and found significant results with respect to total suspended solids and pathogen reductions [2, 15]. The potentially pathogenic microorganisms are deactivated by temperature shock, autolytic mechanism, and the activities of exo-enzymes, which are produced by thermophilic bacteria in the thermophilic aerobic digestion process [16]. These studies were focused on the reduction of potential pathogens for agricultural reuse of municipal WAS to land.

On the other hand, the thermophilic aerobic digestion process has not been successfully applied to the reduction of industrial WAS, and successful studies related to the thermophilic aerobic digestion of industrial WAS have not been reported. The degradation efficiency of industrial WAS is not satisfactory compared with that of municipal waste activated sludge, because of the relatively slow degradation rate and high contents of recalcitrant organic compounds. During the digestion process, it has been suggested that the hydrolysis of proteins and carbohydrates in particulate form, which is a rate-limiting reaction, is not sufficiently achieved by exo-enzymes produced by thermophilic aerobic bacteria. But *Bacillus* species, the thermophilic aerobic bacteria, are known to secrete extra-cellular proteolytic enzymes [12, 14] and thermo-stable α-

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amylases to hydrolyze carbohydrate compounds [5]. Also, Chen and Tayler [3], reported that two *Thermus* bacterial strains could degrade benzene, toluene, and xylene and *Thermus* species could secrete thermo-stable amylase [18, 21]. Therefore, some *Bacillus* and *Thermus* species can enhance the hydrolysis of proteins and carbohydrates in industrial WAS.

In order to apply thermophilic aerobic digestion to industrial WAS, the efficiency of biomass destruction in WAS and the degradation of recalcitrant organic matters in WAS must be enhanced. In this study, the thermophilic aerobic digestion of industrial WAS was performed with thermophilic aerobic bacteria to achieve these purposes. The six strains of thermophilic bacteria, three strains of *Bacillus* species and three strains of *Thermus* species, were used for performance enhancement of the thermophilic aerobic digestion process. In the series of experiments, the reduction of the total suspended solids and organic compounds were investigated to compare the digestion performance of each species, and eventually the most powerful bacterium was chosen. Also, a semi-continuous (fill-and-draw) process operation using the chosen bacterium was carried out with the intermittent feeding of raw WAS to investigate its feasibility of field application.

MATERIALS AND METHODS

Microorganisms and Culture Media

Six strains of thermophilic aerobic bacteria were used as the model strains to evaluate the performance of digestion. These were three strains of *Bacillus stearothermophilus* (ATCC 39536, 31197, and 31196), two strains of *Thermus* species (ATCC 27737 and 43814), and *Thermus aquaticus* (ATCC 25104). An appropriate nominal medium was used for the routine growth of each cell line. Cell growths of all *Bacillus* species were optimum at 55°C, but all the *Thermus* species were optimum at 70°C [1]. Therefore, *Bacillus* and *Thermus* species were cultivated in a shaking incubator (VS-8480SR, Vision Scientific Co., Seoul, Korea) with rotational speed of 220 rpm at 55°C and 70°C, respectively. All the cultures were transferred every 36 h.

The WAS used in this study was collected from the thickening tank of the wastewater treatment plant of Inchon Oil Refinery Co. in Korea. The total suspended solids (TSS), the dissolved organic carbon (DOC), and the total organic carbon (TOC) concentrations of industrial WAS used in the experiments were 17.57–31.07 g/l, 81.5–608.85 mg/l, and 5,925–9,697 mg/l, respectively.

Bioreactor and Operating Conditions

All digestion experiments were performed in a 3-l laboratory scale bioreactor (BIOFLO III, New Brunswick Scientific Co., NJ, U.S.A.) with a working volume of 2.5 l. Temperature, pH, dissolved oxygen (DO) concentration, and impeller speed were monitored. 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 the experiments. To maintain an aerobic condition, the air, humidified by passing through water, was supplied into the bioreactor through a silicone tube membrane with a flow rate of 1.0 vvm (gas vol/liquid vol min).

In the batch digestion experiments, the thermophilic bacteria were collected from a flask culture at the late-exponential growth phase, that was 15 h old. The collected bacteria, about 2.5 l, was concentrated by the micro-filtration process using a cellulose ester membrane filter (MFS membrane filter, pore size: 0.2 and 0.45 µm, ADVANTEC Inc, Pleasanton, CA, U.S.A.) and then the concentrated thermophilic bacteria were used to inoculate a bioreactor with prepared WAS of 2.5 l. The inoculum concentration in the batch experiments was 4.0 g FCW/2.5 l (FCW means fresh cell weight). To investigate complete digestion, a bioreactor was operated in a batch mode for up to 10 h. After inoculation, 10 ml of each sample were withdrawn every 3 h using a sampling port, for analysis.

Semi-continuous digestion was also carried out under the same initial conditions employed for batch digestion. However, one-third volume of treated waste activated sludge was removed from a bioreactor by using a sampling port and replaced with an equivalent volume of raw WAS every 48 h. Loss due to evaporation was estimated by measuring the culture volumes. Sterilized water was added daily to compensate for the evaporative loss and to assure the constant culture volume. A bioreactor was operated in a semi-continuous mode for up to 20 days, and samples of 10 ml were withdrawn every 6 h.

Analytical Methods

For a dry cell weight measurement, the suspended cell samples were centrifuged at 15,000 ×g for 15 min. The solids were washed twice with distilled water and resuspended in distilled water. The resuspended solutions were poured into tared aluminum dishes and dried at 80°C for 24 h. The aluminum dishes were re-weighed and dry cell weights were calculated as g/l.

The total suspended solids concentration was determined by following a procedure and expressed as g/l. Two-ml samples were filtered through a pre-weighed cellulose ester membrane (MFS membrane filter, 0.45 µm pore size, ADVANTEC Inc., Pleasanton, CA, U.S.A.) and then the membranes were dried at 80°C for 24 h. The membranes were re-weighed and total suspended solids concentrations were calculated.

For analysis of dissolved organic carbon (DOC) concentrations, 1 ml of samples were centrifuged at 15,000 ×g and the supernatant was removed for measurement. Appropriate dilutions of supernatants were made and then

Table 1. Characteristics of industrial waste activated sludge for batch digestion experiments with thermophilic bacteria.

Experiment	Control	ATCC 39536	ATCC 31197	ATCC 31196	ATCC 27737	ATCC 43814	ATCC 25104
TSS (g/l)	31.07	17.57	22.24	24.34	25.53	24.00	21.20
DOC (mg/l)	382.40	81.53	196.73	196.70	608.85	482.60	474.37
TOC (mg/l)	8659	5925	7813	8522	7792	7292	9697
PH	7.04	7.14	7.21	7.11	7.07	7.17	7.09

Data represent averages of three measurements.

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 by using the WAS samples.

Results and Discussion

Growth Behavior of Thermophilic Aerobic Bacteria

In order to characterize the growth behavior of the six strains of thermophilic aerobic bacteria, which were used as the inoculum to induce the thermophilic aerobic digestion, the routine culture of each cell was carried out in flasks, and the time course behavior is shown in Fig. 1. After inoculation, the lag phase of 3–6 h appeared as a result of the adaptation to environment change. After the lag phase, cells exponentially grew up to 12–15 h and the stationary phase then appeared. It was also observed that the growth rates of the *Bacillus* species were generally higher than those of the *Thermus* species. The maximum dry cell weights were found as 1.6, 2.5, 1.7, 1.1, 1.4, and 1.5 g/l in cultures of ATCC 39536, 31197, 31196, 25104, 27737, and 43814, respectively. From these results, the concentrated cell suspensions of 15-h old were inoculated to all the further digestion experiments.

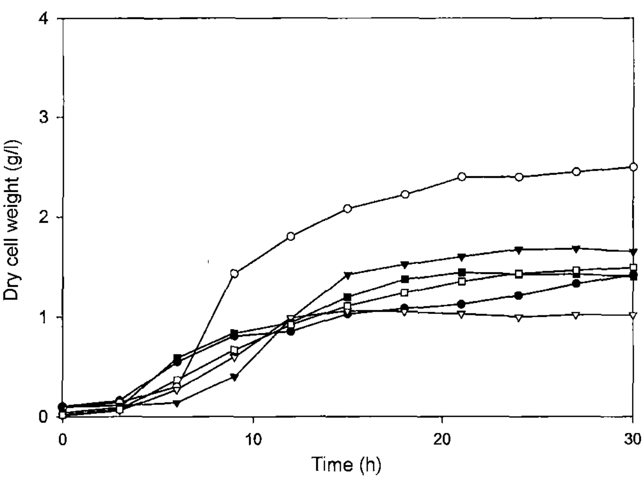

Fig. 1. Growth behavior of six strains of thermophilic bacteria. ●: ATCC39536; ○: ATCC31197; ▼: ATCC31196; ▽: ATCC 25104; ■: ATCC27737; □: ATCC43814.

Waste Activated Sludge (WAS) Characteristics

The waste activated sludge was periodically collected from a wastewater treatment plant in an oil refinery factory. The characteristics of WAS varied with the operating condition of wastewater treatment plant, which was dependent on the characteristics of the wastewater. The properties of WAS were averaged with the values of three measurements. The TSS, DOC, TOC, and pH of WAS are shown in Table 1.

Batch Bioreactor Operation for Waste Activated Sludge Digestion

The digestion of WAS by a thermophilic aerobic process was carried out in a batch mode with thermophilic aerobic bacteria. The performance of digestion for industrial WAS degradation was estimated by the reduction of TSS concentration, DOC concentration, and TOC concentration. The thermophilic aerobic digestion without thermophilic aerobic bacteria was compared with the experiments with thermophilic bacteria.

As shown in Fig. 2(a), TSS concentrations were reduced in all digestion experiments. In the control experiment (digestion without thermophilic bacteria), the TSS concentration was linearly decreased by 21.5% of the initial concentration in 40 h. It was most likely that the decrease of TSS was due to decomposition of the particulate solids by the thermal effect. The proteolytic activity of enzyme was not expected, since the activities of thermophilic aerobic bacteria which could secrete proteolytic enzymes was not expected in the WAS digestion experiment for DOC and TOC degradation. In contrast to the control experiment, WAS digestion with thermophilic bacteria showed a more rapid decrease of TSS in the initial 10 h of experiment and a linear decrease up to the end of the experiment. This might be caused by the combined effects of thermal decomposition and proteolytic activity of enzyme produced by thermophilic aerobic bacteria [11]. It was suggested that the initial rapid decrease of TSS was induced by the thermal effect and proteolytic activity, and the linear decrease of TSS was induced by extracellular proteolytic enzyme activity. Consequently, the TSS reduction in the cases of *Thermus aquaticus* ATCC 25104 and *Bacillus stearothermophilus* ATCC 31197 showed a better decomposition efficiency, which was 39% and 42%, respectively. The time course behavior of TSS reduction with ATCC 25104 and ATCC 31197 represented a different trend. In the case of ATCC

25104, the TSS reduction rate was markedly decreased with time, however, in the case of ATCC 31197, the TSS reduction rate was almost sustained up to the end of experiment. This might be due to the fact that cell activity of ATCC 31197 was sustained during the entire period of the experiment. This fact could also be supported by the highest DOC removal efficiency. In Fig. 2(a), the TSS data were normalized for convenience of comparison, based on the initial value, because of the different initial TSS concentration for each experiment.

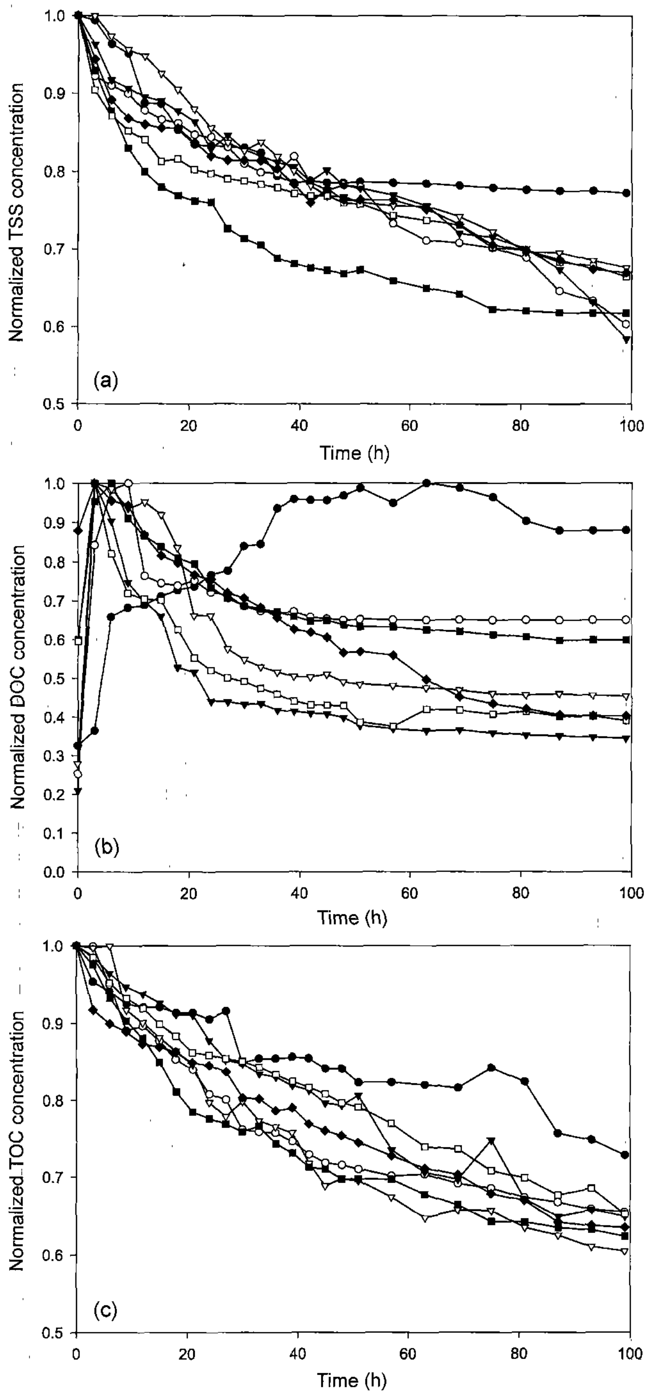

Fig. 2. Time course behavior of WAS degradation in a batch bioreactor.
(a) Total suspended solids; (b) dissolved organic carbon; (c) total organic carbon (●: Control; ○: ATCC39536; ▼: ATCC31197; ▽: ATCC31196; ■: ATCC 25104; □: ATCC27737; ◆: ATCC43814).

The results of DOC reduction in the batch WAS digestion experiments are shown in Fig. 2(b). The accumulation of the solubilized product, which resulted from destruction of microorganisms in WAS, was confirmed by the rapid increase of DOC concentration in the initial period of the experiments, as shown in Fig. 2(b). Both the hydrolysis and the solubilization of released compounds could result in the increase of DOC concentration [11]. But, the increasing ratios of DOC concentration to an initial value were markedly different with each other. This difference might be mainly caused by the difference in the amounts of the proteolytic extracellular enzymes produced by each thermophilic aerobic bacterium. For carrying out a convenient comparison of the degradation of DOC by thermophilic bacteria, DOC concentrations were normalized by the maximum value. In the experiment without the addition of thermophilic bacteria, the DOC concentration was not decreased in all periods of the experiment. The negligible reduction of DOC was observed only in the late period of the control experiment. However, the significant reduction of DOC was observed in experiments with thermophilic bacteria. It is considered that the dissolved organic carbon compounds produced by cell disruption could be degraded by the added thermophilic aerobic bacteria. The experiment with *Bacillus stearothermophilus* ATCC 31197 showed the best degradation efficiency with 66% of the maximum amounts of DOC, as shown in Fig. 2(b). The higher efficiency of DOC degradation could be explained by higher cell activity. Therefore, it is concluded that ATCC 31197 has the highest cell activity among the thermophilic bacteria used in these experiments.

The municipal WAS is composed of approximately 70% of organic matter and this organic fraction can be solubilized by digestion [13]. In the case of the industrial WAS, which was produced from an oil refinery plant, the total organic carbon fraction of WAS (5,925–9,697 mg/l) is larger than that of municipal WAS (about 3,500 mg/l) in a study conducted by a different researcher [11]. In order to achieve TOC degradation, solubilization of particulate organic carbon and degradation of DOC must occur simultaneously. In the digestion process, the first step of digestion is to disrupt large tissue or aggregates of microbial cell into small fragments. In the second step of digestion, the fragments need to be digested and to give rise to large molecular polymers or macromolecules. In the third step of digestion, these matters are solubilized and hydrolyzed into oligomers and monomers. Finally, these small dissolved organic compounds are consumed as carbon source by thermophilic aerobic bacteria. For this sludge digestion mechanism, the thermophilic aerobic bacteria must secrete extracellularly proteolytic and carbohydrate hydrolytic enzymes. It was reported that the thermophilic *Bacillus* species and *Thermus* species produce the heat stable extracellular protease [6, 13, 19] and the heat stable amylases to cleave polysaccharides or oligosaccharides [8]. The time course behavior of TOC concentrations in batch WAS digestion experiments is shown in Fig. 2(c). To make a convenient comparison of the TOC reduction of each digestion experiment, TOC concentrations were normalized by the initial TOC concentration. In the control experiment without thermophilic bacteria being added, the reduction of TOC was achieved by only 17% of the initial TOC concentration. However, significant reductions of TOC (about 35–40%) were observed in other experiments by adding thermophilic bacteria. These results verified that microorganisms used in the thermophilic aerobic digestion are capable of reducing TOC. It was also deduced that all microorganisms used in these experiments secreted extracellular lytic enzymes which were usefully applied to the thermophilic aerobic digestion processes. From the comparison of TOC reduction efficiency of each digestion experiment, *Bacillus stearothermophilus* ATCC 31196 showed the best degradation performance. ATCC 31197, which had the highest cell activity, did not show highest TOC reduction because higher cell activity was not directly related to the solubilization capacity of particulate organic substances.

For an overall comparison of the digestion performance of different thermophilic bacteria, the reduction efficiencies of TSS, DOC, and TOC for each experiment are shown in Table 2. As for TSS and DOC reduction, ATCC 31197 showed much higher removal efficiencies than others. The TOC degradation efficiency of ATCC 31197 was similar to those of others. Based on these results, *Bacillus stearothermophilus* ATCC 31197 was considered to be the best thermophilic bacteria for the thermophilic aerobic digestion of WAS, where the reductive ability of TSS, DOC, and TOC is concerned. Therefore, the continuous WAS digestion experiment was carried out by using ATCC 31197 as an inoculum.

Continuous Bioreactor Operation for Industrial Waste Activated Sludge Digestion

In the semi-continuous operation mode, the bioreactor was operated on a fill-and-draw cycle with a one-third volumetric fraction replacement. During 20 days of experiments, a cycle time of 48 h was employed. Through the semi-continuous laboratory scale experiments, the feasibility to field application was investigated. The performance of the semi-continuous digestion process was judged by the reduction efficiency of TSS concentration, DOC concentration, and TOC concentration. From the results of the batch digestion experiment, *Bacillus stearothermophilus* ATCC 31197 was used in the concentration of 4.0 g FCW/2.5-l as an inoculum for the semi-continuous digestion experiment.

The TSS concentration of industrial WAS was rapidly decreased up to about 80 hours and it was then maintained in spite of the intermittent feeding of raw industrial WAS, as shown in Fig. 3(a). At the time of each feeding, the rapid increase of TSS concentration was naturally observed, and the feeding points of raw industrial WAS are indicated by arrows in Fig. 3(a). The pseudo steady-state of TSS concentration was closely maintained after 80 h of experiment. The initial TSS concentration of raw industrial WAS was 14.8 g/l. At the pseudo steady-state condition, the TSS concentrations were maintained in the range of 7.0–8.0 g/l. Therefore, the average reduction efficiency of TSS was calculated to be approximately 49.3%.

The result derived from the DOC reduction in the semi-continuous industrial WAS digestion experiment is shown in Fig. 3(b). A rapid increase of DOC concentration in the initial experiment period naturally occurred, which was similar to the batch experiments. The DOC concentration was continuously decreased up to 200 h, and then maintained at pseudo steady-state value. The feeding points of raw industrial WAS are indicated with arrows in Fig. 3(b). In the semi-continuous digestion experiment, the initial DOC concentration was 900.6 mg/l and the maximum DOC concentration was 1,241 mg/l. At the pseudo steady-state condition, DOC concentrations were maintained at 250 mg/l, and the average reduction efficiency of DOC was calculated to be 72.2% and 79.9%, based on the initial DOC and maximum DOC concentrations, respectively.

The time course behavior of the TOC reduction in the semi-continuous digestion of industrial WAS is shown in Fig. 3(c). The reduction of TOC rapidly occurred up to approximately 80 h, and then the TOC concentration in the range of 3,500–4,000 mg/l was nearly constant. In this semi-continuous digestion experiment, the initial TOC concentration of raw industrial WAS was 7,598 mg/l, thus

Table 2. Overview of the degradation performance of batch digestion experiments.

Experiment	Control	ATCC 39536	ATCC 31197	ATCC 31196	ATCC 27737	ATCC 43814	ATCC 25104
TSS (%)[a]	22.7	39.7	41.6	32.4	33.6	33.0	38.3
DOC (%)[b]	21.8	35.0	65.5	54.6	61.0	59.7	40.1
TOC (%)[a]	27.2	34.6	35.1	39.6	34.8	36.5	37.7

[a]Based on initial concentration.

[b]Based on maximum concentration.

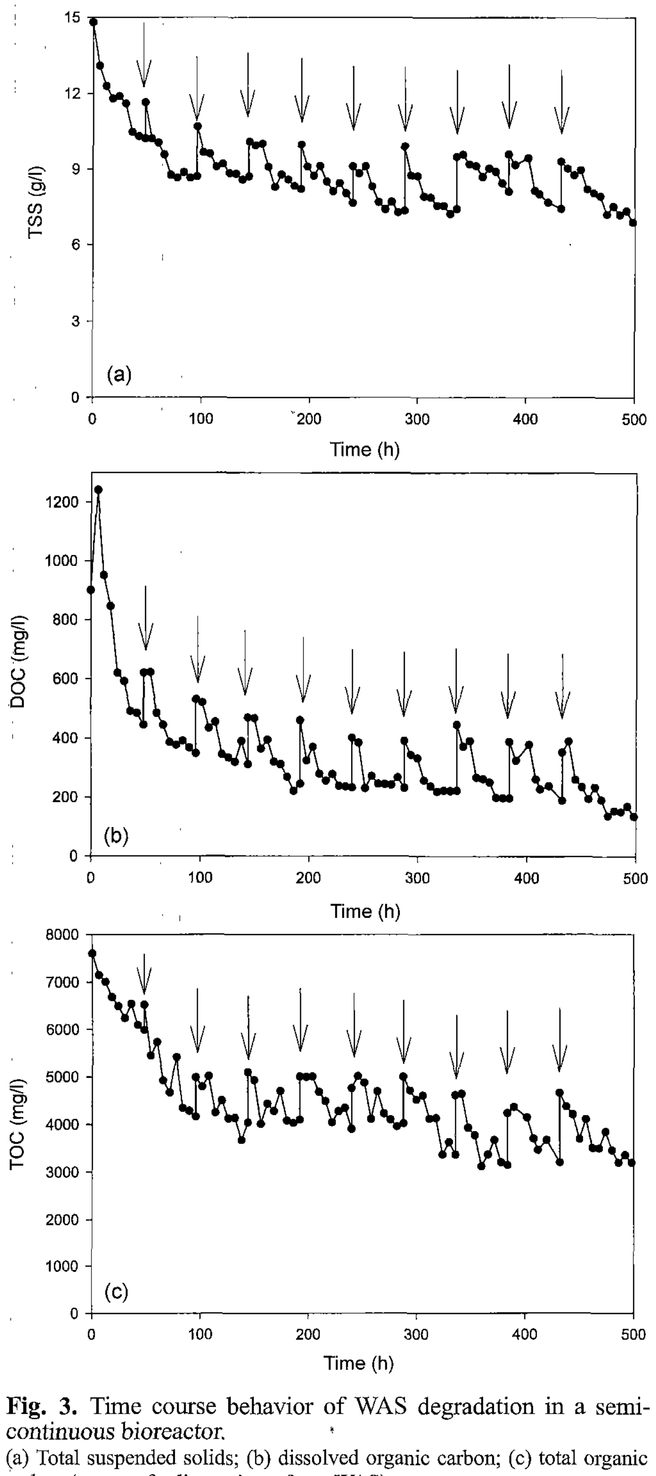

Fig. 3. Time course behavior of WAS degradation in a semi-continuous bioreactor.

(a) Total suspended solids; (b) dissolved organic carbon; (c) total organic carbon (arrows: feeding points of raw WAS).

the average reduction efficiency of TOC was calculated to be about 50.6%.

In the comparison of TSS, DOC, and TOC reduction efficiencies between the batch and semi-continuous experiments, the TSS reduction efficiency of the semi-continuous experiment (49.3%) was nearly identical to that of the batch experiment (42.0%). On the other hand, the DOC and TOC reduction efficiencies of the semi-continuous digestion were 79.9% and 50.6%, respectively, while they were slightly higher than those of the batch experiment, which were 66.0% and 35.0%, respectively. It is likely that *Bacillus stearothermophilus* ATCC 31197 microorganisms were adapted to the environments of WAS during the semi-continuous digestion experiment, and the utilization of organic carbon substrate was enhanced by adapted ATCC 31197 microorganisms.

CONCLUSIONS

The thermophilic aerobic digestion process was applied to treat WAS produced from a municipal wastewater treatment plant. Unfortunately, however, this process could not be successfully applied to the industrial WAS treatment, especially when WAS was produced from an oil refinery and petrochemical plant, because of the extremely slow degradation rate. For the enhancement of degradation efficiency of industrial WAS, several thermophilic bacteria (three strains each of *Bacillus* species and *Thermus* species) were used to investigate the performance of batch digestion experiments. Through batch digestion experiments, *Bacillus stearothermophilus* ATCC 31197 was chosen to be a proper microorganism for thermophilic aerobic digestion of industrial WAS. Also, the digestion of industrial WAS produced from an oil refinery plant was successfully achieved by the semi-continuous (fill-and draw) thermophilic aerobic digestion employing *B. stearothermophilus* ATCC 31197. In conclusion, the thermophilic aerobic digestion process with ATCC 31197 could successfully be applied to achieve the reduction of industrial WAS, especially the ones produced from oil refinery factory.

Acknowledgment

This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the Advanced Environmental Monitoring Research Center at Kwangju Institute of Science and Technalogy, and by the Sogang University Research Grants in 2000.

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