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Minimization of excess sludge and cryptic growth of microorganisms by alkaline treatment of activated sludge

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Abstract−Sludge solubilization was induced by the alkaline-thermal treatment to investigate the cryptic growth and reduction of a large amount of activated sludge produced from wastewater treatment. Activated sludge was divided into lysate, supernatant fraction and particulate fraction for a biodegradability test by cryptic growth. Sludge was reduced up to 78% at pH 13 and 44% at pH 10 using the single alkaline-thermal treatment. Also, it was found that alkaline-thermal treatment at pH 13 increased the quantity of intracellular components generated by cell lysis and promoted the power of significant cell destruction. The neutralization of pH after the solubilized activated sludge led to high biodegradability of organic carbon sources generated by cell lysis. This can be utilized in minimizing activated sludge.

Key words: Activated Sludge, Alkaline-thermal Treatment, Sludge Reduction, Cell Lysis, Cryptic Growth

INTRODUCTION

Activated sludge is produced during biological treatment of domestic and industrial wastewater. Biological treatment is based on the transformation of dissolved organic energy substrate from wastewater into CO_2, water and microbial biomass. Biomass includes a microbial population consisting of living cells, dead cells, and viable but non-culturable (VBNC) cells in an agglomerate of cells forming a floc or biofilm.

Reduction of excess sludge production has been successfully applied in practice by sludge lysis, cryptic growth and uptake of bacteria by predators in aerobic wastewater treatment [1-3]. Cells consisting of microbial biomass generally produce autochthonous substrates such as organic energy substrates through cell lysis as a result of cell death. This autochthonous substrate is reused in microbial metabolism. This process, known as "cryptic growth" [4,5], is remarkably stimulated in a wastewater treatment system. Therefore, overall biomass yield is reduced by such repetitive microbial metabolism. For the promotion of sludge lysis, some sludge breakage techniques have been developed by physical, chemical and physicochemical methods, such as alkaline-thermal, ozonation, and chlorination treatment [6,7,9].

Several studies have reported that reduction of sludge and cell lysis can be promoted by combination of chemical and thermal treatment [8,10]. Addition of NaOH combined with thermal treatment allowed reduction to about 37% of sludge as well as proving the most efficient method to result in cell lysis [8]. Another study attempted to investigate the reduction of sludge by thermo-chemical treatment at 90 °C for 3 hrs [11]. Most biological wastewater is sensitive to low and high pH, and high temperature. It is thus possible to achieve significant degradability of microbial cells and efficient sludge reduction.

This study proposes a process of recirculating the lysed sludge in the aeration tank, after inducing cell lysis by alkaline-thermal treatment prior to returning the excess sludge to the aeration tank. During the process, the biomass may be reduced, cryptic growth may increase and result in reduction of the effluent total organic carbon (TOC) by the cryptic growth process explained above. Thus, in the study, the objectives were to investigate the growth process of cells by cryptic growth and the reduction of overall biomass yield by the enhanced sludge solubilization using alkaline-thermal treatment. In addition, fluorescent dyes in terms of carboxyfluorescein diacetate (CFDA) and 4′,6-diamidino-2phenyl indole dihydrochloride (DAPI) were used to examine the process of the cryptic growth.

EXPERIMENTAL

1. Activated Sludge Samples

Activated sludge collected from an aeration tank at a domestic wastewater treatment plant (Gwangju, Korea) was harvested by centrifugation at 9,700 ×g for 5 min at room temperature to remove dissolved organic carbon in activated sludge and to adjust initial mixed liquor suspended solids (MLSS) concentration to about 7,000 mg l^{-1}. The precipitates were resuspended with deionized water.

2. Preparation of Cryptic Growth Medium

Chemical hydrolysis was carried out by placing 500 ml of activated sludge in a 500 ml medium to induce cell lysis and organic biomass breakage. 10 N NaOH was added to adjust pH to a value of 10 and 13, respectively. For the solubilization study, 0.12 g l^{-1} and 1.46 g l^{-1} concentrations of NaOH were used to reach pH values of 10 and 13, respectively. To promote the chemical degradation of certain compounds, the bottle was incubated at 60 °C for 12 hrs. The solution was neutralized to pH 7 with 0.1 N H_2SO_4. The sub-

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strate obtained by this process is defined as lysate. Fresh sludge samples were centrifuged to remove dissolved organic carbon sources and resuspended with deionized water. 10% of fresh sludge samples was used as a predator source to evaluate the reduction of the biomass yield and the cryptic growth.

3. Experimental Set-up

3-1. Single Alkaline-thermal Treatment

To investigate the effect of cryptic growth and the reduction of overall biomass by cell lysis, the biodegradability test (600 ml of sludge) was divided into three samples: supernatant fraction, particulate fraction, and lysate. The supernatant fraction was obtained from the supernatant by centrifugation at 1,750 ×g for 5 min at 4 ºC with 200 ml of lysate. The precipitates were resuspended in 200 ml of deionized water (particulate fraction). The batch experiments were carried out in 500 ml baffle flasks. The flasks were inoculated with 10% v/v of fresh sludge sample, and incubation period was conducted for 2 days at 28 ºC and 120 rpm under aerobic conditions.

3-2. Repeated Alkaline-thermal Treatment

To investigate the reduction of overall biomass by repeated solubilization, alkaline-thermal pretreated sludge samples were incubated in 2 L baffle flask with 10% v/v of fresh sludge sample for 6 days at 28 ºC at 120 rpm under aerobic conditions. The alkaline-thermal treatment experiment was carried out at intervals of two days.

4. Analytical Methods

MLSS was weighed after drying at 105 ºC for 2 hrs [12]. All chemical analyses were carried out with analytical grade. After NaOH treatment and H_2SO_4 neutralization, the remaining Na^+ and SO_4^{2-} produced Na_2SO_4. Na_2SO_4 was washed twice with deionized water. Before chemical analysis, samples were centrifuged for 5 min at 1,750 ×g. The supernatant was filtered through a mixed cellulose-ester membrane filter with a 0.45 μm pore size to measure the TOC. TOC concentration was measured by TOC analyzer (TOC-V_{CPH}; Shimadzu, Kyoto, Japan). The variations of sludge due to cell lysis were observed by optical microsope (BX60; Olympus Co. Ltd., Tokyo, Japan).

5. Bacterial Enumeration

The culturable cell count of aerobic heterotrophic bacteria in activated sludge was determined by R2A medium [13] containing (per a liter of deionized water) 0.5 g yeast extract, 0.5 g glucose, 0.5 g protease pepton No. 3, 0.5 g casamino acids, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4 and 0.05 g $MgSO_4 \cdot 7H_2O$ after homogenization treatment for 15 min at 15,000 rpm to break down the floc. Colonies were enumerated after 72 hrs of incubation at 28 ºC in triplicate. Viable cell counts were performed by CFDA, which shows green fluorescence when an esterified fluorogenic substrate enters a cell with an intact membrane and there is intracellular esterase activity [14]. The supernatant fraction, particulate fraction, and lysate samples were resuspended in phosphate buffer (pH 7.0) to a turbidity of 0.1 at 660 nm after homogenization. Cells in 1 ml of suspension from one of the microcosms were stained with CFDA in the presence of glutaraldehyde. The total cell number, including VBNC, culturable, and dead cells, was determined after

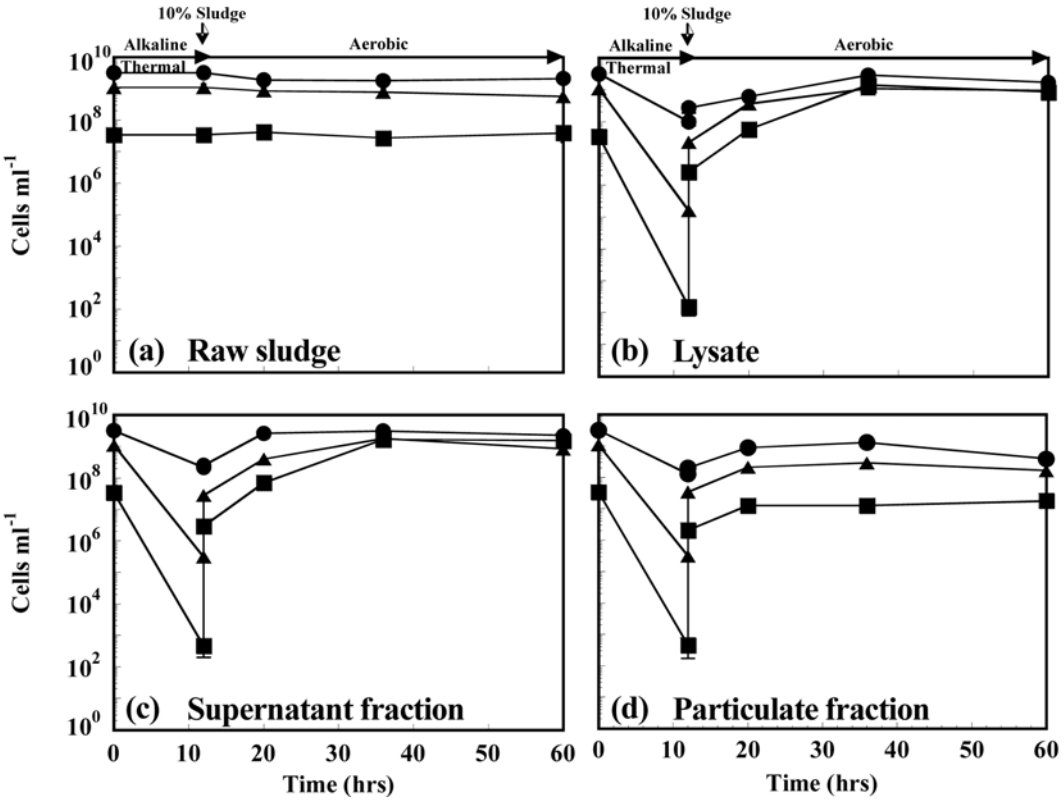

Fig. 1. Cryptic growth curve of 10% fresh sludge on a mixture of carbon sources derived from cell lysis after alkaline-thermal treatment at pH 13 (60 ºC). (a) Raw sludge (without alkaline-thermal treatment), (b) Lysate, (c) Supernatant fraction, and (d) Particulate fraction. ●, total cells counted by DAPI method; ▲, Viable cells counted by CFDA method; and ■, colony forming unit (CFU) on R2A plates.

staining with DAPI solution. CFDA/DAPI stained cells were appropriately diluted with phosphate buffer and then filtered through a black polycarbonate membrane filter with a 0.22 μm pore size. VBNC cell count was defined as a differential value of CFDA viable cell count and R2A medium.

RESULTS AND DISCUSSION

1. Microbial Growth

To investigate the availability of a growth substrate for microbial metabolism of the fresh sludge sample, sludge solubilized through cell lysis by alkaline-thermal treatment was divided into supernatant fraction, particulate fraction and lysate. The samples were monitored for a 60-hr period to compare the total cell number with the viable cell count. During the incubation, the data was based on a culturable cell count by R2A medium, a viable cell count using CFDA and a total cell count by DAPI. Also, the time profile of the cryptic growth under aerated conditions was conducted to evaluate the degree of cell growth on the basis of substrate produced by cell lysis as shown in Fig. 1.

In the case of culturable cells, the initial cell number of 3.4×10^7 cells ml^{-1} rapidly declined to 10^2 cells ml^{-1} after alkaline-thermal treatment and increased to about 10^6 cells ml^{-1} after addition of 10% fresh sludge sample. Following the incubation at 120 rpm for 36 hrs, the cell number of the lysate and the supernatant fraction significantly increased to about 10^9 cells ml^{-1}, whereas the cells of the particulate fraction showed only a slight variation to about 10^7 cells ml^{-1}.

The total cell of 3.2×10^9 cells ml^{-1} measured by DAPI analysis declined about 1log unit after alkaline-thermal treatment, to 3.0×10^8 in the case of lysate, to 2.1×10^8 in the case of the particulate fraction and to 1.5×10^8 in the case of the supernatant fraction, but gradually increased after fresh sludge sample was added.

Viable cells measured by the CFDA method changed in parallel with loss of culturability. In the case of lysate and supernatant fraction, the total, viable, and culturable cell number remained at the same level after incubation for 36 hrs. The previous study verified that *E. coli* cells existed in VBNC states in activated sludge [15]. As shown in Fig. 1, the initial 1.1×10^9 cells ml^{-1} by CFDA and 3.4×10^7 cells ml^{-1} by R2A medium of microorganisms were detected. The number of VBNC cell was estimated by the difference between the culturable cells (R2A medium) and viable cells (CFDA). A VBNC cell level of 1.5 log units was identified at 0 hrs. But after 36 hrs, no VBNC cells were detected. This result may be due to the growth of culturable cells and resuscitation of VBNC cells by the uptake of carbon sources generated by cell lysis. However, the VBNC cells in particulate fraction still exhibited a constant level of 1.5 log units. This result implies that the particulate fraction substrate does not participate in a significant part in cell growth.

As shown in Fig. 2, viable cells could not be observed by CFDA due to cell destruction through lysis after treatment. However, during aerobic incubation after addition of the fresh sample, an increase in the viable cell population which consisted of rod and cocci cells

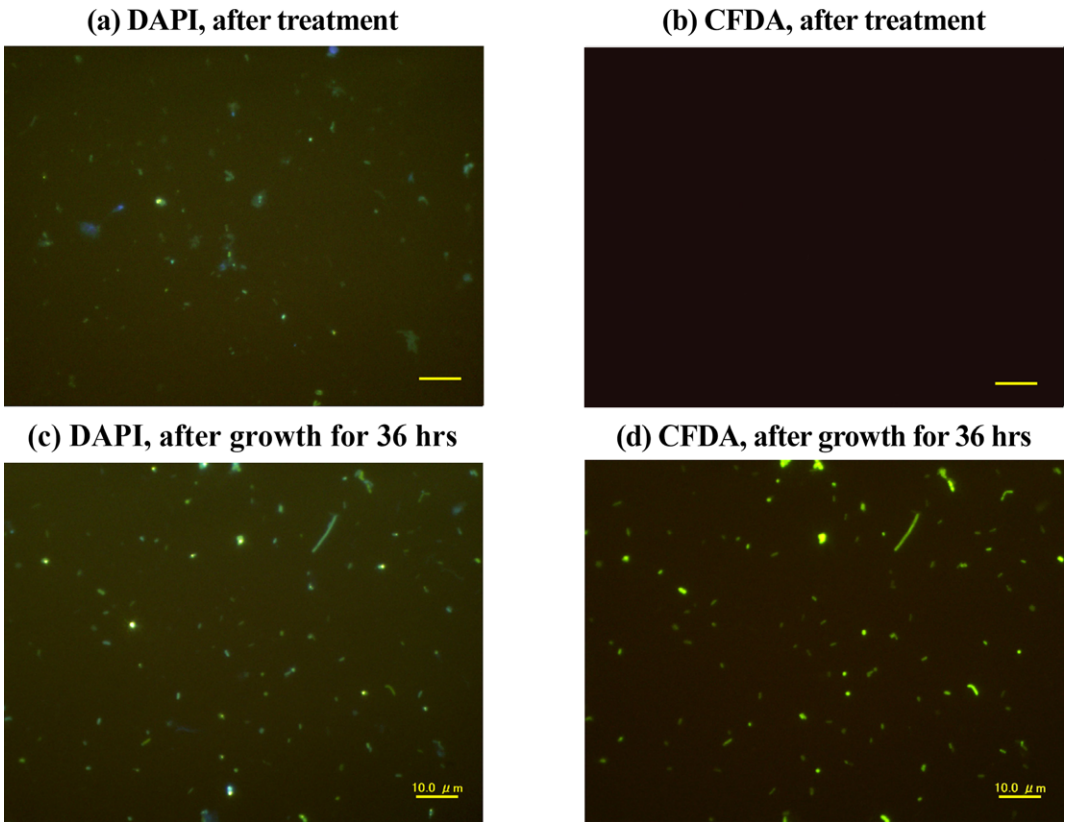

Fig. 2. Microscopy of excess sludge samples stained by 4′,6-diamidino-2phenyl indole dihydrochloride (DAPI) and 6-carboxyfluorescein diacetate (CFDA). (a), (b) Total and viable cells in absence of 10% fresh sludge sample after alkaline-thermal treatment at pH 13 (60 °C). (c), (d) Total and viable cells after 36 h incubation with 10% fresh sample. All bars indicate 10 μm.

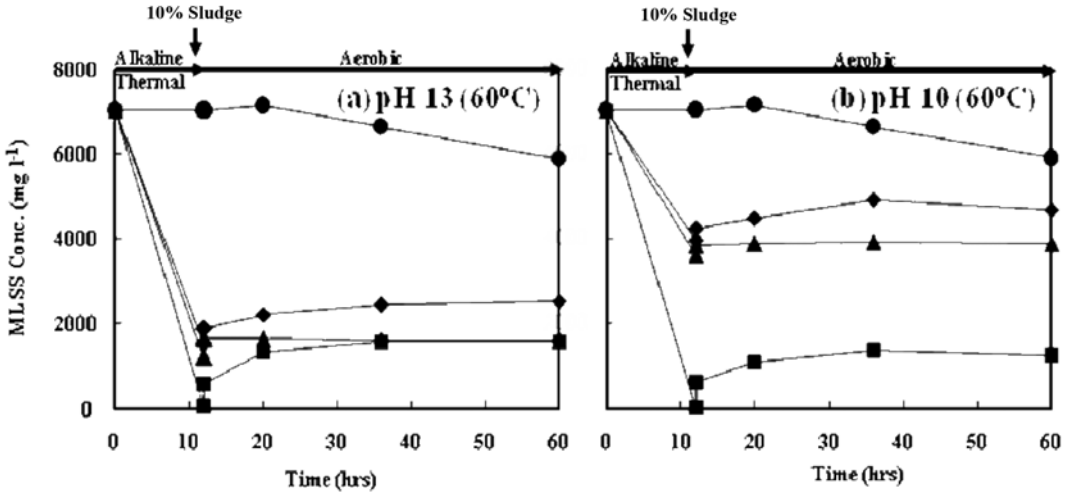

Fig. 3. Variation of MLSS due to cryptic growth after solubilization. (a) sludge solubilized at pH 13 (60 °C), (b) sludge solubilized at pH 10 (60 °C). ●, Raw sludge (Control); ◆, Lysate; ■, Supernatant fraction, and ▲, Particulate fraction.

was observed. The increase in viable cells was due to an increase in total cells (Fig. 2).

These phenomena are consistent with the cryptic growth described above. Therefore, these results imply that carbon compounds and organic matter released from the lysed cells can be utilized as a source of energy for microbial metabolism during the aerobic operations period.

2. Reduced Sludge Production Rates during Incubation Period

During the 60-hr operation period, the initial concentration of MLSS was always set at 7,000 mg l^{-1} and the MLSS reduction efficiency was monitored after alkaline-thermal treatment of activated sludge (Fig. 3). The MLSS of pH 13-lysate and pH 10-lysate was reduced to 78% and 44%, respectively. After alkaline-thermal treatment at 60 °C for 12 hrs, it increased after the addition of the fresh sample. Furthermore, the MLSS of particulate fraction exhibited the similar MLSS concentration of lysate after alkaline-thermal treatment, whereas the MLSS of supernatant fraction was almost non-existent (Fig. 3).

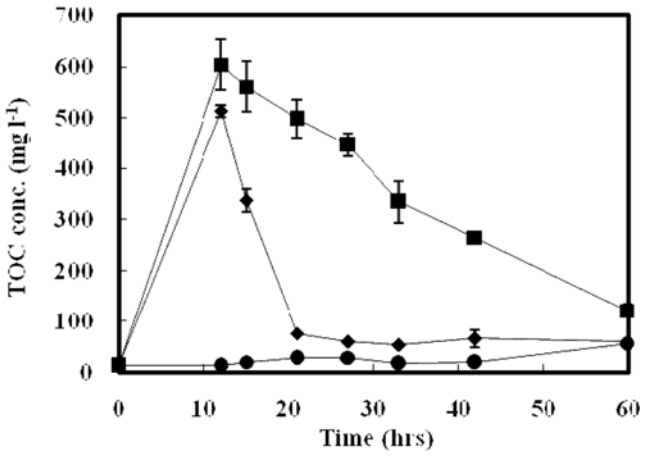

Fig. 4. Variation of TOC concentration under various conditions. ●, Raw sludge without alkaline-thermal treatment (Control); ■, sludge solubilized at pH 13 (60 °C); and ◆, sludge solubilized at pH 10 (60 °C).

The cryptic growth appears to be the most important factor during the condition of a biomass reduction. The initial TOC was measured to 10 ppm before alkaline-thermal treatment but increased to 603 ppm at pH 13-lysate and 513 ppm at pH 10-lysate after treatment. This indicates that alkaline-thermal treatment led to an increase in quantity of organic matter and TOC with a decline of the MLSS due to cell disruption. On the basis of TOC biodegradability, the cryptic growth was examined by using fresh sludge sample for 48 hrs. The results showed that TOC declined to a level of 60 ppm at pH 10-lysate and of 121 ppm at pH 13-lysate (Fig. 4). On the other hand, the biomass increased up to about 2,500 mg l^{-1} at pH 13-lysate, 4,700 mg l^{-1} at pH 10-lysate, 1,600 mg l^{-1} at pH 13-supernatant fraction, and 1,300 mg l^{-1} at pH 10-supernatant fraction. However, the MLSS of the particulate fraction was almost constant (Fig. 3). In addition, from the substrate test using solubilized pellets, neither great growth of cells resulting from cryptic growth nor any change in the MLSS concentration could be detected from particulate fraction. The particulate fraction was composed of colloidal fragments of cellular origin that arise from the lysis of culturable, VBNC and intact dead cells. The total MLSS concentration decreased due to solubilization. Furthermore, as shown in Fig. 1, the particulate fraction displayed a weak growth after the addition of the fresh sample. Therefore, the particulate fraction was not suitable for a nutrient for the cryptic growth of cells and recirculation. This led to 78% and 44% of sludge reduction at pH 13 (60 °C) and pH 10 (60 °C), respectively, after dehydration. 19% of inorganic matter which could not be completely eliminated through the cell destruction technique was present during the initial state of the raw sludge (data not shown).

As shown by the result in Fig. 3, organic matter produced by cell disruption can be reused in microbial metabolism as carbon sources are liberated as products. This resulted in the increase in biomass yield caused by cell growth and decrease of TOC. In the case of raw sludge, the MLSS was reduced to about 5,900 mg l^{-1} (16% reduction) with TOC increase by self digestion for 60 hrs (Fig. 3). As can be seen in the results, the longer aerobic treatment is maintained, the more effluent TOC increases with sludge reduction. How-

ever, the effluent TOC of alkaline-thermal treatment may be slightly lower than that of the conventional biological processes. Hence, a further study on cryptic growth and predation of bacteria is required for efficient sludge reduction.

To observe the degree of cell breakage by different lysing techniques, samples were examined by microscopy prior to fresh sludge addition. The biomass was observed to contain the flocculated formation with dense cell aggregate, protozoa and filamentous bacteria. It is well known that the presence of protozoa in aerobic wastewater system can be used as an indicator to maintain efficient effluent quality [16]. Accordingly, the raw sludge result in Fig. 3 implies that the MLSS reduction may be due to the effect of cryptic growth by microbial growth and predation of bacteria by higher bacteriovoric organisms.

In the case of pH 10 (60 ºC), the flocs were almost completely destroyed, but living bacteria, dead bacteria and higher organisms still remained, whereas the flocs and higher bacteriovoric organisms at pH 13 (60 ºC) were completely degraded (data not shown). As sludge solubilization generated cell lysis by the destruction of cell walls, the structure of the cell wall is crucial. Eukaryotic cells such as protozoa only possess cell membranes, so their destruction is relatively easy. On the other hand, the cell walls of prokaryotic cells are multi-structured with peptidoglycan layer so they have very high strength. The presence of eukaryotic cells yet to be destroyed with prokaryotic cells could be confirmed at pH 10 (60 ºC). On the other hand, at pH 13 (60 ºC), it was observed that most of the cells were destroyed. Thus, it can be concluded that alkaline-thermal treatment requires a high NaOH concentration such as pH 13 to induce cell lysis.

3. Minimization of Sludge Production by Repeated Alkaline-thermal Treatment

To minimize sludge reduction, a cell disruption technique using alkaline-thermal was effectuated at 60 ºC combined with pH 10 and pH 13 for 12 h at on interval of 48 h, and then operated at 28 ºC for 180 h under aerobic conditions with a fresh microbial community. The operative conditions of the three samples (pH 13-lysate, pH 10-lysate, and raw sludge) were all the same.

As shown in Fig. 5(a), after repeated alkaline-thermal treatment at pH 13 (60 ºC), and then initial treatment, a reduction rate of 78% from 7,000 mg l^{-1} to 1,527 mg l^{-1} was obtained, which increased to 2,342 mg l^{-1} through cryptic growth after aerobic incubation for 48 h. The second treatment yielded a reduction rate of 40% to 1,406 mg l^{-1}, which increased to 1,867 mg l^{-1} after 48 h. Finally, after the third treatment, the MLSS was reduced by 31% to 1,294 mg l^{-1} and increased to 1,728 mg l^{-1} by cryptic growth after 48 h. Also, after repeated alkaline-thermal treatment at pH 10 (60 ºC), the initial treatment achieved a reduction rate of 44% (from 7,000 mg l^{-1} to 3,955 mg l^{-1}) and increased to 4,685 mg l^{-1} through cryptic growth after 48 h of aerated incubation. The second treatment yielded a reduction rate of 30% to 3,288 mg l^{-1}, which increased to 3,685 mg l^{-1} after 48 h. Finally, after the third treatment, the MLSS was reduced by 25% to 2,788 mg l^{-1} and increased to 3,235 mg l^{-1} by cryptic growth after 48 h. Conclusively, after incubation for 180 h under aerated conditions, the total reduction rate of MLSS reached a level of 40% by self digestion in raw sludge, 54% from 7,000 mg l^{-1} to 3,235 mg l^{-1} in pH 10 (60 ºC), and 75% from 7,000 mg l^{-1} to 1,728 mg l^{-1} in pH 13 (60 ºC).

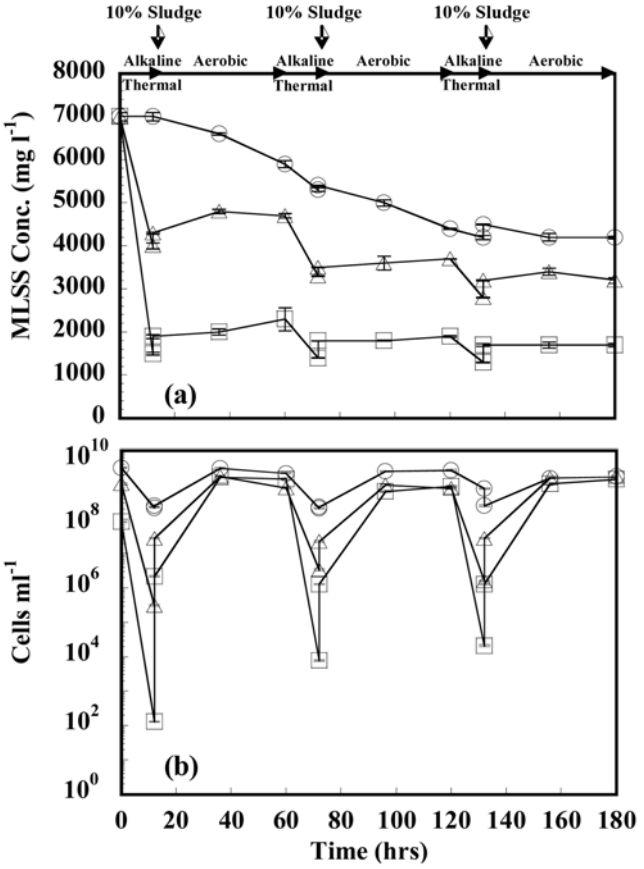

Fig. 5. The correlation between cell growth and MLSS concentration in repeated solubilization systems. (a) reduction of MLSS by cell lysis. Symbols are as follows: ○, Raw sludge (Control); △, pH 10-lysate; and □, pH 13-lysate, (b) cell growth curves due to cryptic growth: symbols are as follows: ○, total cells counted by DAPI method; △, Viable cells counted by CFDA method; and □, colony forming unit (CFU) on R2A plates.

The MLSS reduction rate of pH 10 (60 ºC) did not display a significant difference from raw sludge after 180 h, but there was a remarkable difference after the initial treatment at 60 ºC for 12 h. Therefore, although alkaline-thermal treatment has major problems including corrosion, odor and total running costs relative to aerobic incubation [17], it has merit in that it could dramatically sludge reduce through the cell destruction technique within a short period. Moreover, in the case of pH 13 (60 ºC), alkaline-thermal treatment was used three times, but the reduction rate was almost the same as that of a first alkaline-thermal treatment. Thus, repeated alkaline-thermal treatment should not yield substantial significance.

As shown in Fig. 5(b), the initial R2A medium in treated samples declined to 1.3×10^2 cells ml^{-1} in the first treated sample, 7.8×10^3 cells ml^{-1} in the second, and 2.1×10^4 cells ml^{-1} in the third. This result implies that the increase of culturable cells may be due to growth of thermophilic and resistant bacteria generated by repeated high alkaline-thermal treatment. Furthermore, the more alkaline-thermal treatment is practiced, the more the thermophilic and resistant bacteria gradually increase after treatment and thus trigger a gradual decrease in cell destruction by alkaline-thermal treatment.

CONCLUSIONS

Alkaline-thermal treatment with the activated sludge as a cell breakage technique was investigated in terms of cryptic growth and sludge reduction. Alkaline-thermal treatment at pH 13 is necessary to increase the quantity of intracellular components generated by cell lysis and to promote the power of significant cell destruction. The addition of acid after alkaline-thermal treatment increased assimilated sludge which could be utilized by microorganism. Biodegradability of organic matter produced by cell disruption showed that the culturable and VBNC cells increased by the uptake of carbon source generated by cell lysis (cryptic growth). This led to increase of the MLSS concentration and decrease of TOC. Repeated alkaline-thermal treatment in order to significantly decrease MLSS was used, but we confirm almost similar result of MLSS reduction after single alkaline-thermal treatment. Furthermore, culturable cells as assessed by R2A medium increased gradually due to growth of thermophilic and resistant bacteria generated by repeated high alkaline-thermal treatment. We expect that the amount of the generated sludge can be remarkably reduced, maintaining the wastewater treatment performance by applying the activated sludge hydrolysis process based on alkaline-thermal treatment, which is the core of this technology, to the biological wastewater treatment process.

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