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Microbial Evaluation for Biodegradability of Recalcitrant Organic in Textile Wastewater using an Immobilized-cell Activated Sludge Process

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Abstract

Textile wastewater is difficult to be treated because it contains recalcitrant matters. This study evaluated the performance of an immobilized-cell process using polyethylene glycol media and microbial properties of the immobilized-cells for biodegradation of recalcitrant organics. The immobilized-cell process could remove hardly-biodegradable soluble COD more than 50% at various Hydraulic Retention Times (HRTs) over 8~24 hours. Active microbial distribution was fluctuated at the start-up operation, but became stable at both lower and upper part of the reactor after 92 days of operation. Cell mass in the media at the bottom was higher than at the middle or top parts of the reactor. The microbial decay in the media was more dependent on oxygen than organics. *Stentrophomonas* sp. and *Pseudomonas putida*, known as aromatic and aliphatic compound degraders, were identified in the media, confirming spontaneous selection and growth of cells that could oxidize the hardly biodegradable contaminants in the textile wastewater.

Keywords: *activated sludge, immobilized-cell process, PEG media, recalcitrant organics, textile wastewater*

1. Introduction

Textile wastewaters contain pollutants which mainly are recalcitrant organics, color, toxicants, surfactants, chlorinated compounds and salts. The textile industry is looking for effective wastewater treatment technologies because government legislation regarding the disposal of textile wastewater is becoming more stringent in most developed countries. Despite the existence of a variety of physical-chemical treatment technologies, biological methods are generally economic and simple to apply for the removal of organics and color of textile wastewater (Banat *et al.*, 1996; André *et al*., 2007; Bahadir and Abdurrahman, 2008; Zhou *et al*., 2009; Katarzyna *et al.*, 2009).

Major aspects of the treatment of textile wastewater are the removal of colors and organic compounds. Many biological processes have been proposed as having potential application in textile wastewater treatment (Bustard *et al*., 1998). Among them, activated sludge process is the most commonly applications for textile wastewater treatment due to being efficient and cost effective (Iqbal *et al*., 2007; Yeh *et al*., 2012). In this process, the organic matter is oxidized by a suspended growth of different microbes in the presence of oxygen. However, the most important environmental problem of textile wastewater is related to recalcitrant matter such as Polyvinyl Alcohol (PVA), Ethylene Glycol (EG) and reactive dye, which are difficult to remove by conventional treatments based on aerobic biodegradation. In addition, the BOD_5 to COD ratio is around 0.25 in most cases that implies large amount of hardly biodegradable organic matter included in the textile wastewater (Adel *et al.*, 2004). Therefore, the conventional activated sludge systems have found difficulty to biodegrade complex structure of organic compounds (Vandevivere *et al*., 1998).

A review paper has reported that bacteria have the potential to degrade textile dyes aerobically (McMullan *et al*., 2001). Furthermore, aerobic biodegradation of organic dyestuff has been confirmed by a number of studies (Jian and Bishop, 1994; Wong and Yuen, 1996; Coughlin *et al*., 1997; Adedayo *et al.*, 2004). The biological process could grow recalcitrant organic degraders to remove specific contaminants from textile effluent, but the conventional activated process has difficulty retaining the slow-growing degraders at a sufficient level in the system.

Immobilized microbial cells could improve the efficacy of biodegradation and bioreactor operation compared to freely suspended cells. A higher cell loading, higher hydraulic loading, relatively reduced sludge generation, and easier control of the bioprocess could increase process stability and maintain the

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activities of slow-growing microbes (Pasukphun and Vinitnantharat, 2003; Chen *et al*., 2003; Galai *et al.*, 2010). Thus, the conventional activated sludge process can be improved for effective degradation of recalcitrant matter from textile industrial effluent using an immobilized-cell process.

An immobilized-cell process is effective for many applications in bioreactor systems. Even with the considerable research on immobilized-cells aimed to optimize microbial processes, there are still questions and mechanisms that need further elucidation (Martinez-Trujillo and Garcia-Rivero, 2012). It is important to understand the diffusion of substrates through the immobilized matrix, alterations in physiology or morphology of immobilized-cells, cell growth rates and the role of water activity. These areas have shown wide variations in results, and hence it is difficult to make generalizations, although a clear understanding of these areas is important for process operation. Furthermore, in terms of evaluation of microbial activity the immobilized-cell activated sludge process has not been specifically studied for real textile wastewater treatment. Development in this field may expand the environmental applications of immobilized-cells.

In the present study, activated sludge was immobilized into pelleted Polyethylene Glycol (PEG) media and used for textile wastewater treatment. The immobilized-cells were filled in a lab-scale aerobic column-type reactor to evaluate their performance and microbial characteristics. Furthermore, microbiological characteristics (oxygen and substrate utilization rate, cell distribution, cell growth and cell decay) of the immobilized-cells were studied to determine their effects on biodegradation ability.

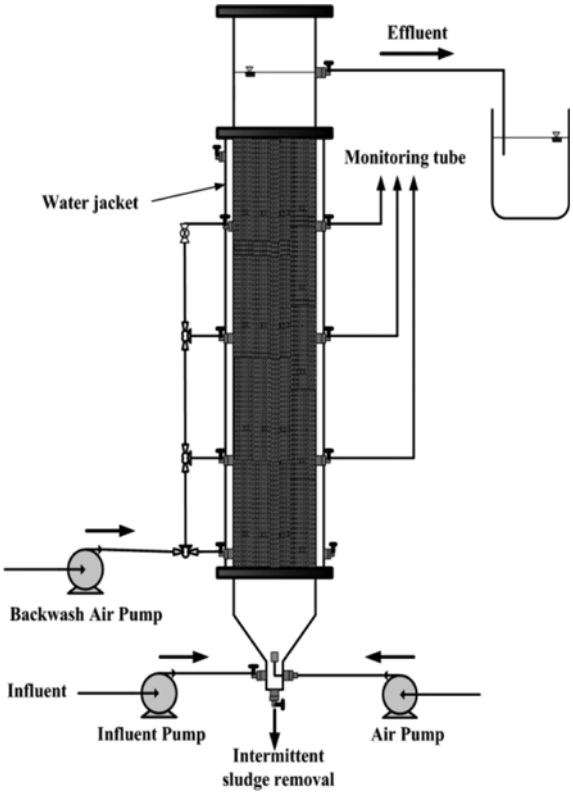

Fig. 1. Schematic Diagram and Photograph of the Aerobic Column Shape Reactor

2. Material and Methods

2.1 Wastewater for the Immobilized-cell Process

Wastewater from textile companies in an industrial complex, Korea has been treated by an activated sludge process in the treatment plant. Subsequently, the aerobically treated effluent was continuously fed through the immobilized-cell process in this study. Soluble Chemical Oxygen Demand (SCOD) is 334 mg L^{-1}, but only 21 mg L^{-1} of Soluble Biochemical Oxygen Demand (SBOD) is in the wastewater, which implying that most organic matter is deemed to be hardly biodegradable. pH, Temperature, TCOD, and SS are 7.5, 28ºC, 455 mg L^{-1}, and 85 mg L^{-1}, respectively.

2.2 Reactors

A lab-scale reactor was made of acrylic plastic with column shapes (Fig. 1). The column had the dimensions of D 7.5 cm × H 1,500 and the effective volume of each reactor was 5.5 L. The temperature was controlled at 30ºC. Immobilized-cells of 4 mm diameter × 4 mm height were charged with 75% of the reactor volume. Hydraulic retention time (HRT) was 24, 12, and 8 hr. Feed flow rate, COD volume loading, and process air flow rate were 1.01~4.32 $m^3/m^2/d$, 0.34~1.67 kg $SCOD/m^3/d$, and 0.13 air $m^3/m^2/min$, respectively. Media run off could be blocked by mesh filters that were set at the reactor's top and bottom. The reactor was operated by an up-flow system that injected wastewater and air from the bottom.

2.3 Immobilized-cell Media

Mixed microbes were used to treat textile wastewater. Spontaneous alternative species can arise in the media when using mixed microbes. For this reason, the activated sludge from the textile wastewater treatment plant was immobilized into the synthetic resin media at a microbial concentration of 0.5 w/v % (weight/volume percentage concentration).

Polyethylene Glycol (PEG) was chosen to prepare the synthetic resin media. PEG diacrylate prepolymer (molecular weight of 700) was used in a flask connected to a stirring motor and temperature sensor. The polymer synthesis of hydrophilic media was accomplished by radical polymerization. Potassium persulfate was interfused as an initiator. After that, additive (0.5 w/v %), promotor (1 w/v %) and AcOH were included and completely mixed with the activated sludge. The 4 mm PVC tube was then filled with the mixture for maturing in the 25ºC tube for 10 min. The completed media was cut into 5 mm sections. Both substrate and oxygen can be transferred to the inside of the media because microbes could be locked in the PEG media and formed about 0.3 μm fine pores.

2.4 Biomass Detection in the Media

The amount of biomass was estimated by determining protein

mass in the media. The standard curve between biomass and protein concentration was plotted according to the proposed method (Chen *et al.*, 2003). The biomass as Volatile Suspended Solids (VSS) was measured according to the standard method (APHA, 2005). The calibration curve of protein was created at 562 nm, and each protein in VSS was analyzed based on a Bicinchoninic Acid (BCA) protein assay (Smith *et al*., 1985).

To determine cell mass in the media, the media was cut into 30~40 pieces and collected in a test tube. After adding 2% Sodium Dodecyl Sulfate (SDS) solution, a 20 min sonication treatment was performed for protein extraction. Equipment for the experiment included Pre-Diluted Protein Assay Standards: a Bovine Serum Albumin (BSA) set, a BCA™ Protein Assay kit (PIERCE), a VCX 130 (Sonics & Materials) and a Synergy HT reader (Bio-Tek).

2.5 Test for Decay Rate of Immobilized-cells

The microbial decay rate of immobilized-cell process could be different along with the reactor height because both organics and oxygen are being supplied by up-flow. There are sufficient organics but little oxygen at the lower part of reactor. On the contrary, biodegradable organics were insufficient at the upper part of reactor but oxygen was abundant. Therefore, the decay rate in the media and the rate of COD production by cell lysis from the medias to the bulk phase in the reactor under the conditions of either substrates or oxygen deficient were evaluated to find out which condition is more important. Calculation for the decay rate followed the equation suggested by Leenen et al., 1997. Glucose (500 mg L^{-1} as COD) and dyeing material reactive red 2 (50 mg L^{-1}) were used as substrates for synthesis wastewater with the composition of nutrients (in mg L^{-1}, $NaHCO_3$ 4200, $NaNO_3$ 34.3, K_2HPO_4 112.5, $MgSO_4 \cdot 7H_2O$ 128, $MnSO_4 \cdot H_2O$ 40, $FeSO_4 \cdot 7H_2O$ 29.8, $CaCO_3$ 47.5).

2.6 Analysis

Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), color, Suspended Solids (SS) and Volatile Suspended Solids (VSS) were determined according to the standard method (APHA, 2005). The water sample was filtered by GF/C (Whatman, England) to determine soluble COD (SCOD). Recalcitrant organic concentration was determined by deducting soluble ultimate BOD from SCOD. The morphology of interior part of the media was examined by scanning electron microscopy (S-520 SEM, Hitachi, Japan, operated at 10 kV). Gram staining was carried out using the method proposed by Yazdankhah *et al.*, 2001. All the studied morphological characteristics were retrieved from the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/).

The proteins of sludge were extracted by cold aqueous extraction techniques after crushing the PEG media (Zhang *et al.*, 1998). A 20 mL sample of the biomass was centrifuged at 10000 rpm for 10 min and the supernatant was removed. The remaining particle was resuspended in an 8.5% NaCl solution containing 0.22% formaldehyde. After removing the residual solids by high-speed centrifugation for 30 min, the supernatant was used to determine the proteins using TKN analyzer (BUCHI Digestion Unit K-424).

A fluorescence microscope was used to investigate active microbe distribution in the media, which was prepared in a vial that contained a 0.02% Fluorescence Diacetate (FDA) solution of 2 mL for 70 min to diffuse FDA into the immobilized media. The center of the sample media was cut into 1-mm-thick disc shapes with a razor blade. Fluorescence was determined at 490 nm using a fluorescence microscope.

3. Results and Discussion

3.1 Organic Removal by an Immobilized-cell Process

The result of organic removal is shown in Fig. 2. The influent wastewater had extremely low biodegradability of 6% (SBOD/SCOD ratio) because most of the readily biodegradable organics had been removed by the previous activate sludge process. Accordingly, the organic matter in the wastewater could be counted as bio-recalcitrant. The average organic (SCOD) removals in HRT of 24 hr, 12 hr and 8 hr were 60.5, 53.3 and 51.5%, respectively. The recalcitrant organic removal was fluctuated due to changes of influent wastewater characteristics. All in all, the immobilized-cell process could remove hardly-biodegradable soluble COD more than 50% (recalcitrant organic removal efficiency) which failed to biodegrade in the conventional activated sludge process.

The classical activated sludge treatments failed to remove diverse recalcitrant organics from textile wastewater because aerobic treatment of dye wastes has proven ineffective in most cases (Zissi *et al*., 1997; Shaul *et al*., 1991; Pagga and Brown, 1986). On the contrary, the results of this study expressed significant organic removal from wastewater which contains high concentrated recalcitrant organics. This means microbial activity for recalcitrant organic degradation was improved in the media.

3.2 Cell distribution and Cell Mass in the Media

Active microbial distribution of the inside media was investigated

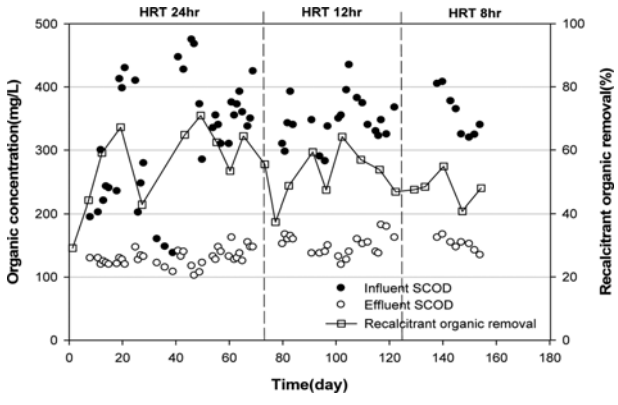

Fig. 2. Recalcitrant Organic Removal by Immobilized-cell Process

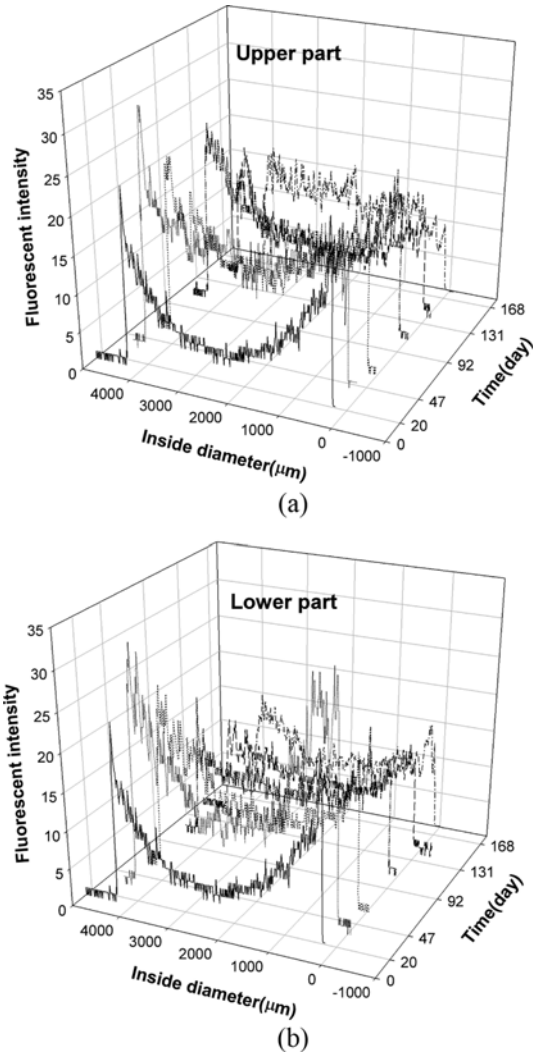

Fig. 3. Microbial Distribution of Immobilized-cells: (a) Upper Part, (b) Lower Part

using a fluorescence microscope. Fig. 3 shows line profiles for microbial distribution in the media at the upper part of the reactor and the lower part of reactor.

For the upper part of the media during the initial synthetic process, as compared to suspended-growth, a small quantity of oxygen was fed into the media by immobilizing the suspended-growth microbe before microbial death and media synthesis. As a result, the low initial intensity (0 day) was caused by microbial death in the center of the media. However, intensities within and external to the media increased after 20 days and maintained regular levels after a small decrease after 47 days. Even though activity and growth rate of microbes increased, the microbial distribution maintained regular levels because of spontaneous selection and cell-lysis. The initial intensity of the lower part was low for the same reason as the upper part. The intensity of the external media in the lower part of the reactor was very high after 20 days compared to that in the upper part because of difficulty of DO dispersion into the inner part of the media due to low DO concentration in the lower part despite of high concentration of the organics. The lower intensity of the center and external media was maintained compared to that of the upper part until 47 days. After that, the intensity was similar to that of the upper part, indicating that organic and oxygen dispersion were easily achieved in the media.

Figure 4 shows the SEM microphotographs of interior part of the media. The media provided pathways for oxygen and substrates between outside and inside, thus many aggregates of bacteria were founded in the interior of the media. The bacterial developed as attached growth to form biofilm that is propitious to biodegrade recalcitrant organics and prevent attack of toxicant. At day 92, the interior part was covered with filamentous, club and helical shaped bacterial which were suspected to be the heterotrophic organic oxidizer. In conclusion, the active microbial distribution was fluctuated at the start-up operation, but became stable at both lower and upper part of the reactor after 92 days of operation.

During media synthesis, the initial immobilized microbial concentration was 0.23 mg media^{-1}. For media collected from the bottom of the one-year operated reactor fed by textile wastewater with HRT of 8 hours, the average protein mass for initial time, bottom, middle and top of the reactor were 59.28,

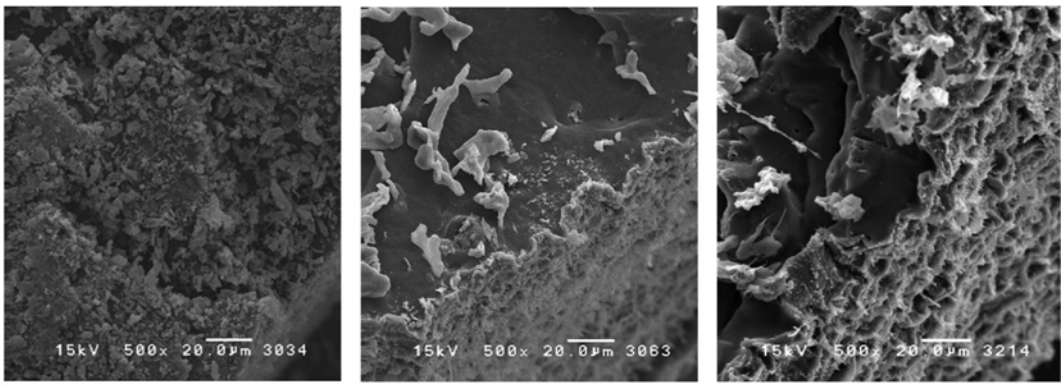

Fig. 4. SEM Microphotograph of Interior Part of Media: (a) 0 Day, (b) 47 Day, (c) 92 Day

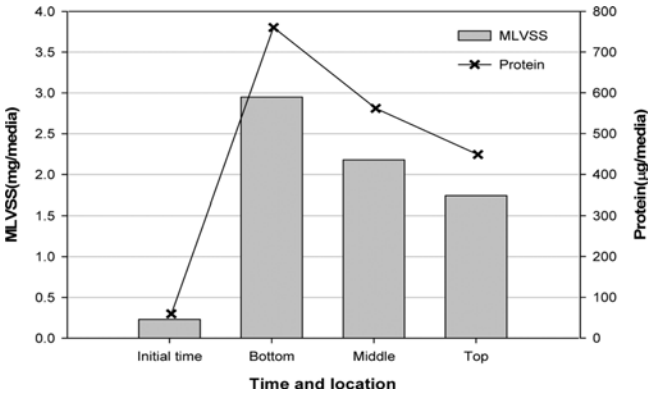

Fig. 5. MLVSS and Protein Concentrations in a Media for Initial Time (0 Day) and Different Locations at 380 Day

760.31, 561.85 and 448.45 μg media^{-1}, respectively, and the average microbial concentration was 2.95 mg media^{-1}. Assuming 2.95 mg media^{-1} was the maximum microbial concentration of capable immobilization collected from the most active point of substrate utilization, the microbial concentration increased 12.8-fold from 0.23 mg media^{-1} when the initial concentration was 4,600 mg L^{-1}.

According to the monitored results of the concentration distribution change at different locations in the reactor using textile wastewater, average microbial concentrations were 2.18 mg media^{-1} in the middle part and 1.74 mg media^{-1} at the top part, while the bottom part had the highest concentration at 2.99 mg media^{-1} (Fig. 5).

The microbial concentration decreased with reactor height. At the bottom, the inflow of biological treatment water contained residual organics, and the oxygen supply was continual because of the characteristics of up-flow operation. Hence, the inner microbial accumulation was higher than at the middle or top part of the reactor. Furthermore, reduction of recalcitrant materials could be performed by facultative as well as obligate anaerobes (Franciscon *et al*., 2009). A facultative anaerobe sustains activity by aerobic respiration if oxygen is present but is also capable of switching to fermentation. Therefore, the facultative anaerobes could be developed and contribute to organic removal under the conditions of the immobilized-cell process.

3.3 Identification of Selected Species

The microbes immobilized in the media were activated sludge from a wastewater treatment plant treating textile wastewater. The microbes from activated sludge reactor that used not to degrade recalcitrant materials were immobilized into the media and adapted by hardly-biodegradable textile wastewater. As a result, the spontaneous selection was induced in the media. Results of Gram staining of the immobilized media after 150 days of operation with HRT of 8 hours showed 10 species of Gram-negative bacteria and 4 species of Gram-positive bacteria. Morphologic features showed 2 species of cocci and 12 species of rod-shaped bacteria. By investigating microbe properties in the media using the National Center for Biotechnology Information (NCBI), it was determined that the isolation/identification microbes were species that degrade recalcitrant materials such as TCE, phenol and aliphatics (Table 1).

The *stenotrophomonas sp.* and the *Pseudomonas Putida* were dominant in the media. It is known that *stenotrophomonas sp.* degrades PAH, PVA and phenol and *Pseudomonas Putida* degrades nonylphenol. Therefore, the species that degrade recalcitrant materials grew in the media according to elapsed time.

3.4 Decay of Immobilized-cells

Immobilized microbes of the inner media grow and die with time. Dead microbes can be used as an energy source for other microbial growth. Organics by cell lysis can be transferred outside of the media. Investigation of microbial growth and decay in the media is important for determining the efficiency and stability of the media as well as identifying the operating factors of the process. In particular, in terms of the lower part of

Table 1. Microbe Isolation/identification Results in Media after 150 Days of Reactor Operation

Morphology	Strain	Microbe property (NCBI)
Gram staining -Gram(+): 4 species -Gram(–):10 species Morphologic feature -Cocci: 2 species -Rod: 12 species	1. *Comamonas aquatica*	Deep radioactive liquid waste repository
	2. *Stenotrophomonas sp.*	Polyphosphate kinase
	3. *Staphylococcus sp.*	A deep subsurface environment
	4. *Stenotrophomonas sp.*	Biodegraded Canadian oil reservoir
	5. *Brevundimonas diminuta*	Trichloroethylene degradation
	6. *Flavobacterium-like sp.*	Oxidation and reduction of arsenic in an unsaturated soil
	7. *Granulella daejeonensis*	Isolated from granule
	8. *Z.ramigera*	Quaternary ammonium alcohols
	9. *Rhodococcus sp.*	Methylotrophic bacteria
	10. *Flavobacterium*	Distribution of biosurfactant producing bacteria
	11. *Burkholderia sp.*	Diversity of Burkholderia cepacia complex
	12. *Pseudomonas putida*	Phenol degradation
	13. *Comamonas aquatica*	Deep radioactive liquid waste repository
	14. *Ochrobactrum sp.*	Isolates from soil samples and wheat roots

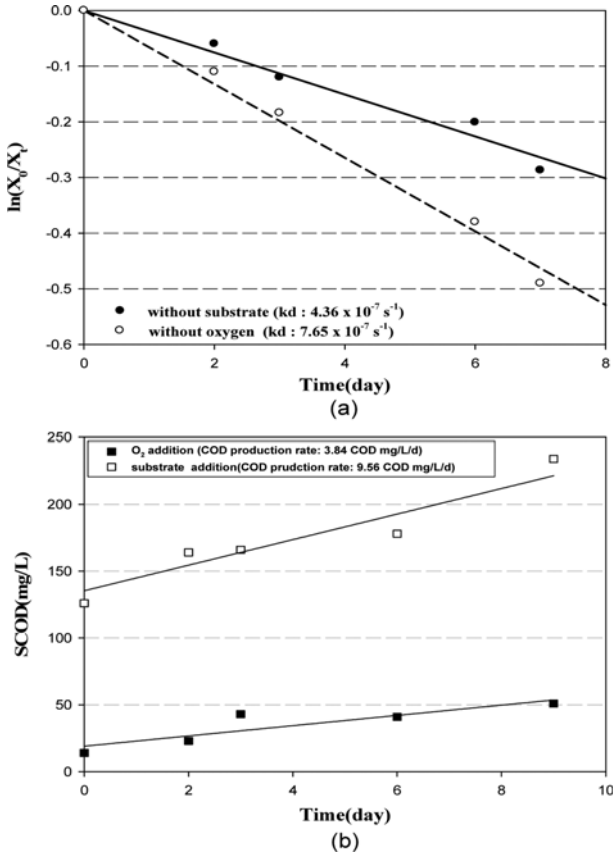

Fig. 6. Decay Rate of Microbes: (a) COD Variation of the Bulk Phase, (b) in the Absence of Either Oxygen or an Easily Biodegradable Substrate

reactor, oxygen dispersal into the media is very scarce because suspended-growth microbes may consume oxygen to remove influent organic matter. At higher locations in the reactor, degradable substrates can be insufficient in quantity because most of the organics are recalcitrant after degradable organics have been removed.

As shown in Fig. 6(a), the decay rate (kd) was determined by calculating $\ln(X_0/X_t)$ by time change. The decay rate (7.65×10^{-7} s^{-1}) without oxygen but using substrates (glucose) was higher than the decay rate without substrates but using oxygen (4.36×10^{-7} s^{-1}). Dyeing material hardly degraded at both test neither oxygen nor Glucose. Thus, the release of internal cellular material by microbial decay resulted in a COD increase (Fig. 6(b)). The COD increase rate of O_2 addition (9.56 mg L^{-1} d^{-1}) was 2.5 times higher than the COD increase rate of substrate addition (3.84 COD mg L^{-1} d^{-1}). The internal microbes might use dead cells as an energy source with oxygen.

The microbial growth was directly affected by oxygen transfer into the 4000 μm diameter of media. The microbial decay in the media was dependent on oxygen more than organics. The oxygen deficient might result in increase of COD concentration by accelerating release of biological substance from microbial decay. In addition, the released organics could be used as substrate again with the presence of oxygen.

4. Conclusions

The experimental results in this study apparently imply that the immobilized-cell process remove the hardly-biodegradable organics aerobically treated textile wastewater effluent efficiently at short retention time verified by the growth of the degraders in the media. Considering the shortcoming of the conventional activated sludge process that cannot remove dye compounds, the immobilized-cell process using activated sludge could be an appropriate alternative due to economic feasibility, effective degradation of recalcitrant organics and environmental-friendliness. However, further study might be required to improve removal at short retention times and to understand the more specific capability of microbial degradability in the media.

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