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BIOFILM GROWTHS WITH SUCROSE AS SUBSTRATE

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ABSTRACT: This study was conducted to: (1) Evaluate the effect of DO on cell yield in a fixed film reactor using 1,000 mg/L sucrose as a substrate; (2) evaluate the correlations of the biofilm thickness and density with DO and their resultant substrate stabilization rates; and (3) examine the response of biofilm communities as a result of DO and biofilm thickness changes. Data obtained from this study indicate that DO has only a minor effect on the cell yield. However, the thickness of aerobic biofilm is definitely related to DO, or thickness (mm) = (2.08 × DO)/(9.2 + DO). The biofilm density is also related to its thickness. At a DO of 5 mg/L or lower, the biofilm texture is firm and has a wet density of 27–48 mg/cm^3. At a higher DO (5–16 mg/L), the biofilm becomes porous and filled with air pockets, with its density being reduced to 25 mg/cm^3. The biological community in biofilm at a high DO environment (16 mg/L) is predominantly short rods grouped in a chain structure. At a low DO environment (0.5 mg/L), however, the prevalent forms are large rods, none of which are in chain grouping.

INTRODUCTION

Fixed film biological treatment processes have gained more attention recently due to their low energy consumption, ease of operation and low maintenance requirements. However, the dynamic nature of biofilms and their kinetic characteristics have not been fully understood. Designs of the fixed film treatment system are empirically oriented so far, which in many cases cannot achieve expected efficiencies.

Studies by Hoehn and Ray (3), Williamson and McCarty (16), Sanders (13), Kornegay and Andrews (7), Characklis (2), and other investigators (9,11) have described the concepts of fixed film development through physical, chemical, and biological aspects. Hoehn and Ray (3) indicated that the fixed film development was influenced by the molecular diffusion of both dissolved oxygen and substrate. Since the concentration of substrate is normally 50–100 times greater than that of dissolved oxygen in a typical fixed film treatment system, diffusion of oxygen was considered as the rate-limiting factor in many previous studies (1,9,11,15). Williamson & McCarty (16) also suggested that the oxygen to glucose ratio in a bulk solution must be at least equal to 1:9 to avoid any oxygen limitation in a fixed film system. Matson, et al. (10) found a similar ratio, or 0.125, for their suspended biofloc system. Generally, the maximum biofilm thickness under an atmospheric operating condition was found

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to vary from a few hundred microns to several millimeters (2,3,7,9, 13,15,16), depending on the substrate concentration and hydraulic shearing stress. However, for a thicker biofilm, an aerobic condition cannot be maintained throughout its depth under atmospheric environment. It was further observed by Huang and his co-workers (4,5) that aerobic biofilms were more effective in oxidizing organic matters and had a better settleability than the anaerobic counterpart.

In designing a fixed film treatment system, not only the rate of organic stabilization is of concern, but the cell yield is equally important. Unfortunately, most of the cell yield coefficients reported in the literature are for suspended growth systems. Little information is available for fixed-film biological reactors.

Objectives

The specific objectives of this study were to:

1. Evaluate the impact of dissolved oxygen on the cell yield in a fixed film biological reactor using sucrose as a substrate.
2. Correlate the substrate stabilization rate with biofilm thickness and DO concentration in the reactor.
3. Examine the response of biofilm communities as a result of DO changes. Sucrose was used because it is soluble and relatively easy to prepare a large volume of solution each day in the laboratory for the experimental need.

Experimental Materials

Annular Reactor and Experimental Setup.—The reactor used in this study was made of acrylic plastic. It consisted of a concentric outer cyl-

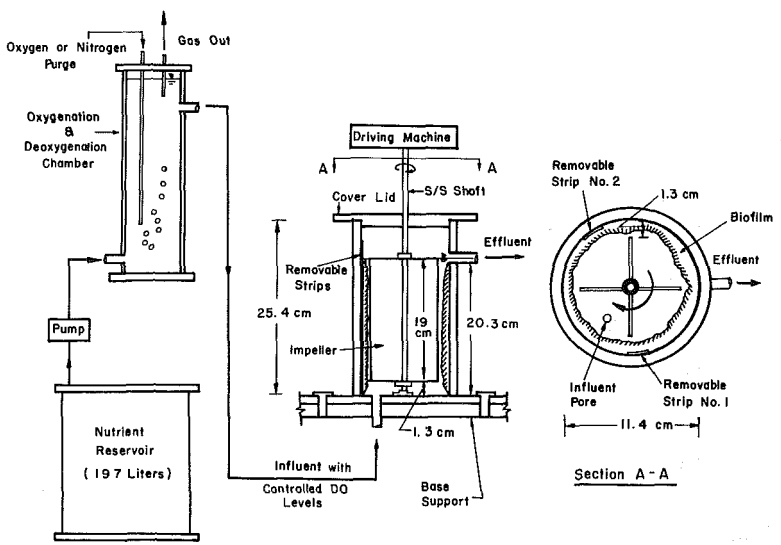

FIG. 1.—Schematic Diagram of Annular Reactor Used in Research Study

TABLE 1.—Physical Data and Operating Conditions of Annular Reactor

Category (1)	Parameter (2)	Value (3)
Physical data	Reactor volume	2,100 cm^3
	Wetted surface area	730 cm^2
	Reactor height	20.3 cm
	Inside diameter of reactor	11.4 cm
	Surface area of removable strip #1	30.5 cm^2
	Surface area of removable strip #2	26.4 cm^2
Operating conditions	Flow rate	140 ml/min
	Hydraulic retention	15 min
	pH	7.2
	Temperature	20° C
	Impeller peripheral velocity	18.3 cm/s

inder (11.4 cm in diam) and a rotating inner impeller (8.8 cm in diam), with a total height of 25.4 cm (Fig. 1). This type of annular reactor has the advantage of providing constant shearing throughout the stationary reactor surface. The reactor was designed for upflow operation. The effluent was withdrawn from an outlet located 20.3 cm above the base. Two removable thin plastic strips were inserted inside the reactor wall. These could be taken out from time to time for the measurement of biofilm thickness and density. The rotating impeller was controlled at a peripheral velocity of 18.3 cm/s (0.6 ft/sec), which was about two thirds of the upper limit normally used in the full-scale rotating biological contactor (RBC) operation.

During each testing, a 1,000 mg/L sucrose solution which was "charged" with a predetermined amount of dissolved oxygen was fed. The oxygenation was accomplished using either air or pure oxygen, while deoxygenation (if needed) was provided by purging N_2 gas through the substrate solution. The hydraulic detention time in the reactor was 15 min so that the factor of suspended growth could be neglected in the correlation of biofilm development and substrate reduction. From a preliminary study, this 15 min detention time was sufficient to achieve a significant degree of substrate stabilization by the biofilm system. Other

TABLE 2.—Characteristics of Feed Solution

Constituent (1)	Concentration (mg/L) (2)
$C_{12}H_{22}O_{11}$ (Sucrose)	1,000.0
Soluble Organic Carbon (SOC)	421.1
$(NH_4)_2SO_4$	192.0
K_2HPO_4	28.5
KH_2PO_4	13.2
$MgSO_4 \cdot H_2O$	100.0
$MnSO_4 \cdot H_2O$	10.0
$CaCl_2$	7.5
$FeCl_3 \cdot 6H_2O$	0.5
pH	adjusted to 7.2

pertinent physical data and operating conditions are listed in Table 1.

Substrate Solution.—The sucrose substrate solution was prepared daily using dechlorinated tap water. The exact composition of the substrate solution is listed in Table 2.

Experimental Methods

Initial Startup.—In order to startup the biofilm development, a mixture of sucrose solution and fresh primary effluent from a local sewage treatment plant was used daily on a fill-and-draw basis. Following several days of batch operation, a thin layer of biofilm became evident. Thereafter, straight sucrose solution was used on a continuous basis.

Experimental Procedures.—Four separate experimental runs, each with a predetermined DO inside the reactor, were conducted in this study. The first experimental run was conducted using a DO concentration of 5 mg/L. The second experimental run employed an oversaturated DO of 16 mg/L, while the third run had a DO of only 3 mg/L. The last experimental run had a DO of 0.5 mg/L. It was intentionally designed to have such an up-and-down variation of DO in the sequential experimental runs so that the corresponding changes of microbial communities could be assessed. In all of these tests, the reactor temperature was controlled at 20° C.

Experimental Parameters

Cell Yield.—The cell yield in this study was expressed as the amount of biomass produced per gram of SOC removed. Measurement of SOC was accomplished using a Beckman carbonaceous analyzer (Model IR 315). The suspended and attached biomass were determined by gravimetric method (14). For each measurement of attached biomass, a biofilm sample was removed from a 1.0 cm^2 area on the removable plastic strip and its wet weight as well as dry weight were determined. Also, prior to the removal of the biofilm, its thickness was measured using a micrometer (its procedure will be described in detail later). Thus, the biofilm density became calculable. Since the hydraulic detention time was only 15 min, the suspended biomass found inside the reactor was mainly due to the sloughing of the attached biomass. The combination of suspended and attached biomass was considered as the total biofilm growth in the reactor and this was used for the determination of the cell yield coefficient.

Biofilm Thickness.—The biofilm thickness was measured using a modified micrometer and a microscope. After each removable strip was taken out from the reactor, the excess water was allowed to drain for 5 min. Then the strip was mounted on the stage of a microscope (Fig. 2). The microscope was first focused at the biofilm surface. After the sharp tip of the micrometer just touched the biofilm surface, the reading on the micrometer was taken. Then the tip was forced through the biofilm to touch the plastic surface. At this time, a second reading was taken. The difference of these two readings gave the biofilm thickness. This measurement method was simple and reasonably accurate. Since the thickness of biofilm varied slightly from one place to another at a given

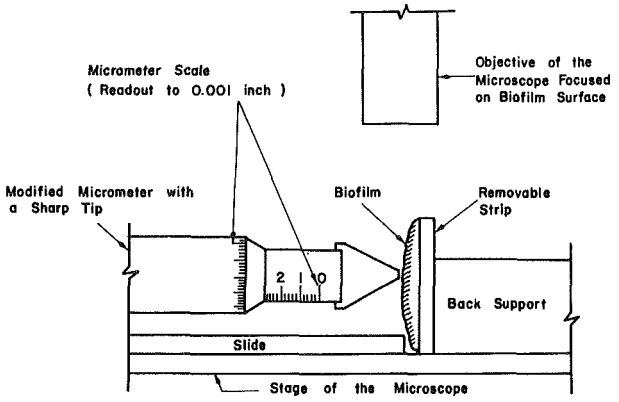

FIG. 2.—Biofilm Thickness Measurement by Modified Micrometer

time, normally about 20–25 thickness measurements were taken at random from each strip. The average value of these measurements was used in the data calculation.

Biofilm Wet Weight and Dry Density.—The total weight of the removable strip plus the attached biofilm was measured after it had been drained for 5 min. The difference between this value and the net weight of the plastic strip was taken as the wet weight of the biofilm, from which the wet density was calculated. To determine the dry density, the biomass was scraped from the strip and its dry weight was determined. The dry density was then determined by dividing the dry weight by the volume of the removed biofilm.

Reduction Rate of SOC by Biofilm.—To measure the SOC reduction rate by the attached biofilm, the reactor content was first emptied and then refilled with a fresh substrate solution. The reduction of SOC over a test period of 15 min under a batch condition was measured. In doing each test, the temperature was rigidly controlled at 20° C.

Results and Analysis

Total Cell Yield.—The impact of DO on the cell yield was evaluated over a DO range from 0.5–16 mg/L. There were good linear correlations between the biofilm growth and the SOC reduction in each testing DO condition, as shown in Fig. 3. The cell yield values were found to be 1.16, 1.36, 1.10, and 1.40 g of biomass per gram of SOC reduction when the testing DO were 0.5, 3, 5, and 16 mg/L, respectively. Although these data seem to suggest that the cell yield increases slightly with DO, the trend of such a correlation is not definite. Further work needs to be done in this area. It is interesting to compare these cell yield data with those reported by Ramanathan and Gaudy (12) for complete-mixed, suspended growth systems in the presence of different carbon sources. For a carbon source of sucrose, they reported an average cell yield of 0.53 g-biomass/g-COD, and the range was from 0.33–0.77 with a standard deviation of 0.14. These values are only slightly higher than the range

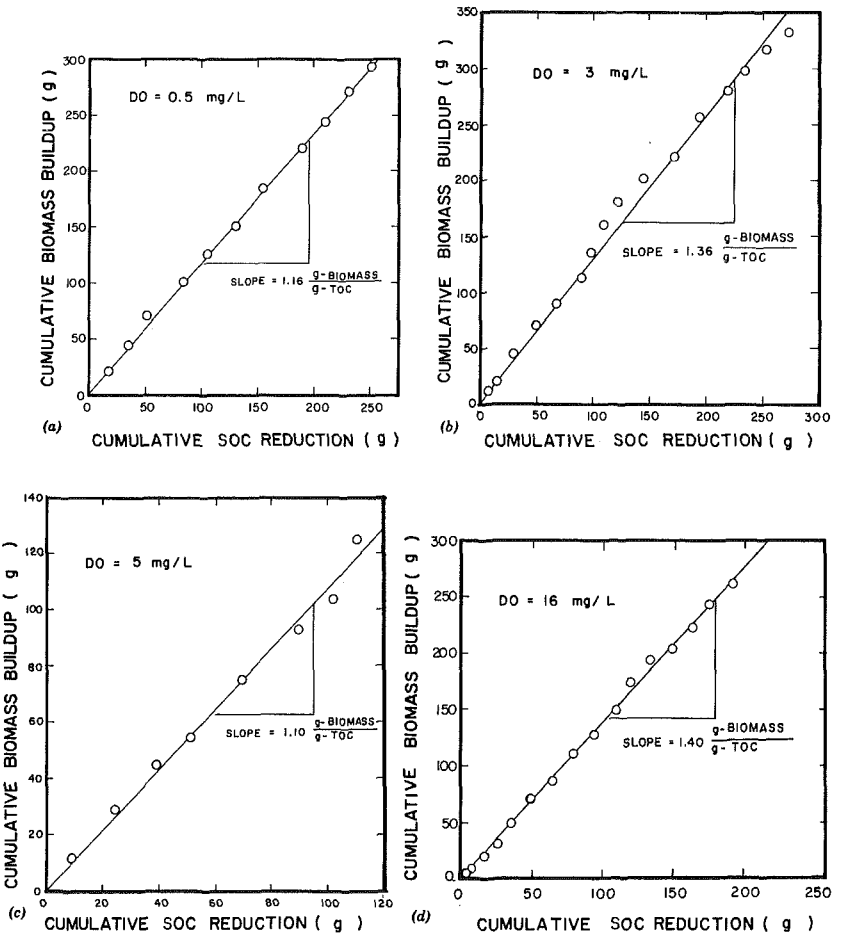

FIG. 3.—Linear Relationship between Biomass Growth and SOC Reduction at Different Testing DO Conditions

of 0.39–0.52 g-biomass/g-COD observed in this study.

Biofilm Thickness and Density.—The average thickness of aerobic biofilm was much dependent on DO, and their correlations were as follows: 100 μm at 0.5 ± 0.2 mg/L DO; 500 μm at 3 ± 0.5 mg/L DO; 700 μm at 5 ± 0.5 mg/L DO; and 1,350 μm at 16 ± 0.5 mg/L DO (Fig. 4). These correlations can be expressed as:

$$\text{Aerobic Biofilm Thickness (mm)} = \frac{(2.08 \times \text{DO})}{(9.2 + \text{DO})} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

It was further found that the wet density of the biofilm was also a function of DO (Fig. 5). The average densities were approximately 789, 660, 540, and 470 mg/cm^3, respectively, when the DO values were 0.5, 3, 5,

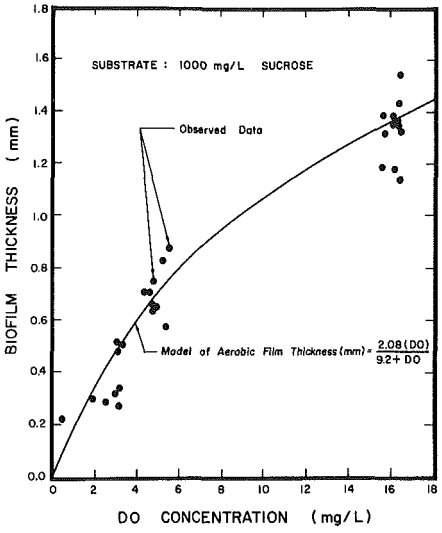

FIG. 4.—Correlations of Aerobic Biofilm Thickness with DO

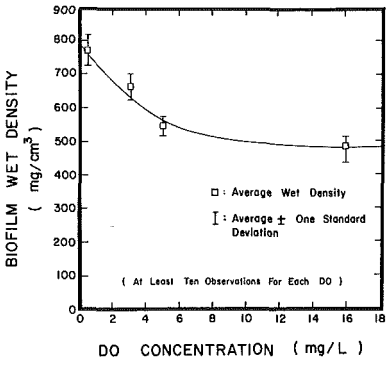

FIG. 5.—Effects of DO on Biofilm Wet Density

and 16 mg/L. Thus, the wet density of biofilm decreases with increasing DO.

During the study it was observed that whenever the test DO was higher than 5 mg/L, the biofilm texture was loose and porous. Air and water mixture were present inside the biofilm. This was largely responsible for the lower wet density at high DO. On the other hand, when the test DO was at a lower range, the biofilm appeared to be dense in structure without much porosity. Thus, the wet density became much higher at a lower DO condition.

The reported values of the biofilm dry density are quite varied among different investigators. Kornegay and Andrews (7) reported a dry density of approximately 90 mg/cm^3 when the film thickness was less than 300 μm. Tomlinson and Snaddon (15) found a somewhat lower value, about 50 mg/cm^3, for a film thickness of 100 to 1,100 μm. Hoehn and Ray (3) also found a dependence of the dry density on the film thickness. The maximum density was 110 mg/cm^3, which occurred at a film thickness of 200 μm, but a sharp decrease to approximately 20 mg/cm^3 was observed when the film thickness became either greater or smaller. In this study, the dry density was found to vary from a high of 48 mg/cm^3 at a DO of 0.5 mg/L, to a low of 25 mg/cm^3 when the DO was increased to 16 mg/L (Fig. 6). The corresponding biofilm thicknesses were 100 and 1,350 μm, respectively. It was further observed that the most significant reduction of biofilm density occurred when the DO was increased from 0.5 to 5 mg/L (Fig. 6). Such an observation of a greater volumetric density at a lower DO had also been reported by Jewell (6) from his studies of the aerobic fluidized bed process. The variations of the biofilm density and its structural matrix as a function of DO are prob-

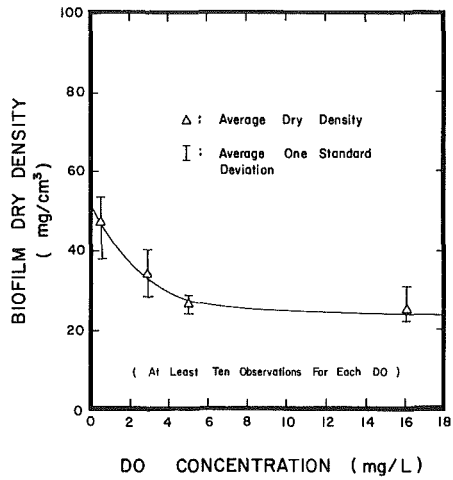

FIG. 6.—Effects of DO on Biofilm Dry Density

ably caused by the changes of microbial species. Throughout this study, microscopic examinations of the biofilm communities were conducted periodically. At the high DO environment (16 mg/L), the prevailing microorganisms were short rods, some of which were grouped together in a chain. On the other hand, when the DO was reduced to a low level (0.5 mg/L), the prevailing microorganisms were mainly large individual rods, none of which appeared to be in chain groupings. The difference in the microbial predominance and its associated metabolic patterns and products are probably responsible for the variations in biofilm density and structural porosity. Also noteworthy was the fact that the dry film densities obtained from this study and others (3,7,15) were considerably greater than the value normally found in suspended bioflocs, which had been reported (8) to have a maximum value of only 10 mg/cm^3.

Biofilm Thickness versus Substrate Stabilization.—It has been reported by Maier, et al. (9), Tomlinson and Snaddon (15), Kornegay and Andrews (7), Hoehn and Ray (3), and Characklis (2) that the rate of substrate stabilization increases with the biofilm thickness, but the maximum rate is reached by a film thickness of generally less than 200 μm (0.2 mm). Beyond that thickness, no significant improvement of the stabilization rate would take place. Attempts had also been made in this study to correlate the rate of substrate stabilization with the observed film thickness, and the data are shown in Fig. 7. Apparently, the SOC reduction rates were increased linearly with an increasing biofilm thickness, at least for a thickness up to 1,000 μm, in all test DO conditions. At a DO concentration of 16 mg/L, the substrate stabilization rate continued to increase even at a film thickness of 1,500 μm (or 1.5 mm). This thickness was almost seven times greater than the previously mentioned value (200 μm). This variation was mainly attributed to the high substrate (1,000 mg/L sucrose) and high DO concentrations used in this study, which allowed organic molecules and dissolved oxygen to pen-

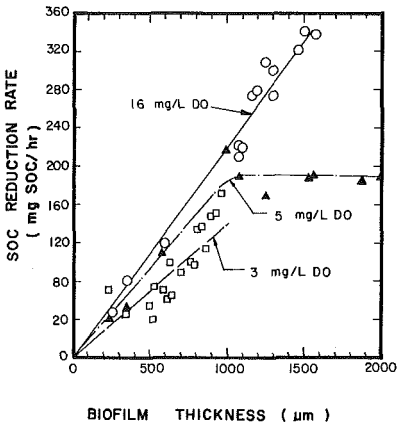

FIG. 7.—Correlations of SOC Reduction Rate with Biofilm Thickness

etrate deeper into the biofilm to support a thicker layer of active aerobic microorganisms. Another probable reason was the use of the sucrose substrate, which is readily oxidizable by microorganisms. If a complex substrate were used, which either contained larger organic molecules or had lower biodegradability than sucrose, the SOC stabilization rate might not be able to increase with the biofilm thickness to as high as 1,500 μm. Therefore, it is important to bear in mind that these SOC reduction rates were found for a sucrose substrate. It is reasonable to extend these data to other substrates of similar molecular size and biodegradability.

During the test, most of the biofilm inside the reactor appeared to be light brown in color as long as the film thickness was less than 250 μm. This light brown biofilm was considered aerobic. Beyond this thickness, some dark gray or black films started to appear, particularly when the test DO was below 3 mg/L. When the test DO was increased to 5 mg/L, the aerobic layer increased to 650 μm or greater. As the DO was further increased to 16 mg/L, the thickness of the aerobic film reached a level of 1,600 μm without showing any dark anaerobic spot. The corresponding SOC reduction rate at this thickness was 340 mg/h, which was almost two times the value of 190 mg/h observed for a DO of 5 mg/L. Thus, it becomes clear that a higher DO was able to maintain a thicker aerobic biofilm, which in turn achieved a higher rate of organic stabilization as long as the substrate concentration was not limiting.

Conclusions

In a fixed film system, accumulation of biomass on the support-media is a complicated matter, which depends on many factors such as oxygen availability, substrate nature and concentrations, hydraulic shearing and microbial species. The observations from this study using sucrose as a substrate have led to the following conclusions which will aid our understanding of some fundamental aspects of the biofilm system. Although these conclusions are derived from a specific substrate of su-

crose, the writer feels that they can be extended to other substrates so long as their molecular sizes and biodegradability are comparable to sucrose. They include:

1. The concentration of dissolved oxygen in a fixed-film reactor has only a minor effect on the cell yield coefficient. Although the observed data showed a slight increase of the cell yield with an increasing DO, the trend of such a correlation is not quite definite.
2. A higher DO will support a thicker layer of aerobic biofilm. The mathematical relationship can be expressed as in Eq. 1. From this model and for a 1,000 mg/L sucrose substrate, the thickness of aerobic layer which can be maintained by different DO levels are as follows: 500 μm for a DO of 3 mg/L, 730 μm for a DO of 5 mg/L, and 1,380 μm for a DO of 16 mg/L.
3. The biofilm density and microbial community are related to its thickness. A low DO of 5 mg/L or less tends to develop a thin biofilm with a firm texture and high density (about 540–780 mg/cm^3 for wet density and 27–48 mg/cm^3 for dry density). As the DO increases beyond this level, the biofilm becomes porous and filled with air and water pockets, thus its density is much reduced (about 480 mg/cm^3 for wet density and 25 mg/cm^3 for dry density in the DO range of 5–16 mg/L). The biological community at a high DO environment (16 mg/L) is predominantly short rods grouped in a chain structure. At a low DO environment (0.5 mg/L), however, the prevalent forms are large rods, none of which are in chain grouping.
4. At a sucrose concentration of 1,000 mg/L, the SOC reduction rate is related linearly with the biofilm thickness to a certain maximum limit, which depends on the DO concentration. At a biofilm thickness of 1,000 μm, the SOC reduction rates were found to be 130, 170, and 210 mg/h, respectively, at the DO concentrations of 3, 5, and 16 mg/L.

Acknowledgments

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