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ORIGINAL PAPER

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Adaptive response of microbial communities to soluble microbial products

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Abstract We carried out two experiments to study the influence of soluble microbial products (SMP) on biomass concentration [defined as mixed liquor suspended solids (MLSS)] and removal of soluble biological and chemical oxygen demands ($sBOD_5$ and sCOD): (1) SMP were allowed to accumulate, and (2) SMP content was artificially reduced by washing the biomass. The daily initial sCOD in both experiments was kept constant at 859 ± 6 mg/l for 16 days. In experiment 1, the highest sCOD removal (80%) occurred during the first day. Thereafter, it decreased successively to 40% [sludge retention time (SRT), 12 days], after which it increased steadily to 50 ± 4%. Variations in residual sCOD were accompanied by variations in $sBOD_5$, showing that the biodegradability of the accumulated SMP components was changing. MLSS fluctuated within the range $1{,}200 \pm 25$–$1{,}993 \pm 58$ mg/l. We attributed the irregular accumulation of the biomass to variations in the biodegradability of SMP components. The initial $sBOD_5$/MLSS ratio varied according to variations in initial $sBOD_5$ and MLSS, whereas the residual ratio was constant at 0.025 ± 0.008. This indicated a direct relationship between the concentrations of biomass and SMP produced. In experiment 2, MLSS increased from $1{,}200 \pm 25$ to a constant value ($2{,}810 \pm 16$ mg/l; SRT, 12 days). After this time, no decrease or increase in MLSS was observed. Correspondingly, sCOD and $sBOD_5$ removal increased from 80–97 to 84–99%. A stable microbial community that could consume organic matter efficiently was developed under these conditions.

Keywords Activated sludge · Biodegradation · Wastewater treatment · Microbial growth · Soluble microbial products

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Introduction

Biological wastewater treatment (e.g., the activated sludge process) is the most widely used process for treatment of domestic and industrial wastewater. Many factors affect the process and the quality of the treated wastewater [16]. The presence of soluble microbial products (SMP) is one of the important factors that affect the quality of the effluents from biological wastewater treatment systems. Because of their many characteristics, including flocculating and toxic properties [6, 8], SMP affect not only microbial activities, but also the performance of treatment processes.

Although many researchers have studied the characteristics of SMP and the kinetics of their production and biodegradation [3, 5, 17, 19], the exact behavior of SMP and their influence on microbial activities are not well understood and require further investigation. In their review paper, Laspidou and Rittmann [13] indicated that SMP are biodegradable. However, results presented by Schniener et al. [23] show that SMP are refractory to biodegradation. Moreover, Aquino and Stuckey [2] showed that SMP comprise a series of alkenes, alkanes and aromatic compounds, which are resistant to biodegradation. Recently, Shin and Kang [24] reported that accumulation of SMP in membrane bioreactors does not inhibit microbial activity. However, earlier studies by Huang et al. [11] show that accumulation of SMP is inhibitory towards the metabolic activity of activated sludge. Their results agree with those of Chudoba [6], who reported that refractory organic matter produced by activated sludge microorganisms negatively affects microbial activity and flocculation. Therefore, the literature shows conflicting evidence of the biodegradation of SMP and their effects on microbial activities. Depending on the system being studied, SMP can be either biodegradable or refractory to biodegradation, which leads to their accumulation in environmental surface waters [12].

The adaptive response of microorganisms to SMP in wastewater has not been reported. The literature does not report the behavior of the biomass and the subsequent activity of the microbial community under the influence of increases and decreases in SMP concentration. Therefore, the aim of the present study was to identify how factors such as substrate supply and accumulation of SMP influence microbial growth [i.e., changes in biomass concentration, defined as mixed liquor suspended solids (MLSS)] and removal of soluble chemical oxygen demand (sCOD) and soluble biological oxygen demand ($sBOD_5$). Two experiments were essentially performed. In the first experiment, SMP were allowed to accumulate, whereas washing the biomass artificially reduced SMP concentration in the second experiment.

Materials and methods

Source of microorganisms

Activated sludge used in the experiments was collected from the local municipal wastewater treatment plant (Gdansk, Poland). It was a mixture of activated sludge collected from all the biological reactors, which function according to the modified UCT process to enhance biological nutrient removal. A 1,000-ml sample of activated sludge was washed several times until the resulting supernatant was clear. Washing of activated sludge was necessary in order to reduce the amount of dissolved substances in the mixed liquor. The supernatant was discarded by decanting. Then, 100 ml washed sludge was mixed with synthetic wastewater in the bioreactor.

Bioreactor operation and microbial acclimation

Two approaches for studying how factors such as food supply and accumulation of SMP influence MLSS and the removal of sCOD and $sBOD_5$ included the following two independent experiments.

In experiment 1, aerobic biodegradation tests were conducted in three laboratory-scale batch bioreactors (2 l with working volume of 1 l). Initially, the bioreactors were fed with synthetic wastewater containing the following ingredients per liter (suppliers are shown in brackets): 150 mg tryptic soy broth, 50 mg soy peptone (Scharlau Chemie, Barcelona, Spain); 75 mg Tween 80, 50 mg starch, 10 mg sodium acetate, 30 mg urea (Sigma-Aldrich, Munich, Germany); 50 mg potassium soap, 7 mg $CaCl_2$, 7 mg $MgSO_4$ (POCH, Gliwice, Poland); and 225 mg refined rapeseed oil (Olvit, Gdansk, Poland). The ingredients were dissolved in distilled water by using a laboratory homogenizer. The ingredients were then mixed with 100 ml washed activated sludge. Table 1 shows the characteristics of the synthetic wastewater after mixing with washed activated sludge in the bioreactors.

The bioreactors were run as follows. Air was continuously supplied at constant rate (1,500 cm^3/min) by using an aquarium-type pump and diffuser. The airflow rate was uncontrolled with respect to oxygen consumption requirements. The air movement also helped in keeping the contents of the bioreactors well mixed. All the bioreactors were operated at ambient temperature (20 ± 1°C) at a sludge retention time (SRT) of 16 days. The bioreactors were fed daily with synthetic wastewater and distilled water to supplement the sCOD removed during each day and to keep the volume of the wastewater in the bioreactors constant at 1 l. Moreover, the bioreactors were covered with perforated aluminum foil to prevent evaporation of water. Addition of sCOD loadings was based on the results of preliminary experiments, which were aimed at determining the sCOD to be supplemented each day. Before withdrawing samples for analysis, the air supply was switched off and the bioreactors were thoroughly shaken by hand for a few seconds. Immediately, 50 ml mixed liquor was withdrawn by decanting for determination of MLSS in accordance with standard methods [1]. The contents of the bioreactors were then allowed to sediment before withdrawing 100 ml supernatant by careful decanting or by using a pipette. All three bioreactors were run simultaneously and the results presented are mean values.

In experiment 2, three laboratory-scale bioreactors were operated under the same conditions as described above. However, in experiment 2, after withdrawing samples for analysis, all the treated wastewater was discarded. Next, the biomass was washed with distilled water to remove the SMP and any residual initial substrates. We used laboratory separatory funnels to separate the washed biomass from the resulting wastes. Care was taken to minimize loss of biomass. The bioreactors were then fed with washed biomass and fresh synthetic wastewater.

Analytical methods

The supernatant was immediately centrifuged for 15 min at 2,332 *g* to remove suspended solids and microbial

Table 1 Characteristics of the synthetic wastewater after mixing with washed activated sludge. *COD* Chemical oxygen demand, *BOD* biological oxygen demand, *MLSS* mixed liquor suspended solids (= biomass concentration)

Parameters					
COD (mg/l)	BOD (mg/l)	MLSS (mg/l)	Total Kjeldahl N (mg/l)	PO_4^{2-} (mg/l)	pH
859 ± 6	515 ± 5	1,200 ± 25	38.7 ± 1.5	10.33 ± 1.5	7.06 ± 0.21

cells. Next, the sCOD and $sBOD_5$ of the supernatant were measured after filtration through 0.45 μm membrane filters. The values obtained represent the organic substrates or SMP. Many other researchers [4, 5, 11, 24] have also used this procedure. The initial and residual sCOD were determined using the colorimetric method in Hach COD vials and reactor. Noguera et al. [20] has also used this method for the analysis of SMP. We used the Hach spectrophotometer DR/2000. All samples were diluted with distilled water (1:5) before digestion in the Hach COD reactor. The following standard methods [1] were used to determine the remaining parameters: method 5210 B (5-day BOD test), BOD_5 (fresh activated sludge from the wastewater treatment plant was used as the seed source); and method 2540 D (drying at 105°C), biomass concentration (determined in terms of MLSS [16, 24]). Analytically pure reagents were used in all experiments. Dissolved oxygen concentration and pH were not controlled during the course of the experiments. All data were calculated using computer programs (Microsoft Excel 2000 and Microcal Origin 6; Microcal Software, Northhampton, Mass.).

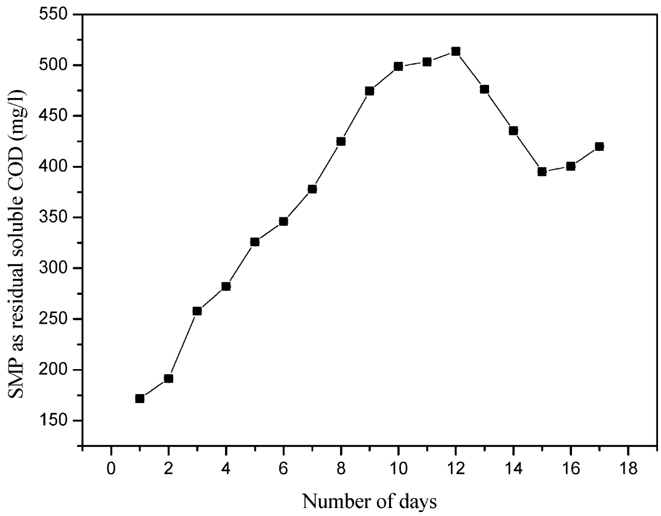

Fig. 1 Residual soluble chemical oxygen demand (sCOD) after 24 h of each day of experiment 1. The daily initial sCOD was 859 ± 6 mg/l. Error bars are not given for data points because the standard deviation was so small

Results and discussion

Experiment 1: initial sCOD loadings and variations in residual sCOD

In experiment 1, SMP were allowed to accumulate while the daily initial sCOD loadings were maintained at an approximately constant level (859 ± 6 mg/l sCOD). Figure 1 shows the variation in the residual sCOD values. Because the residual substrate cannot be measured (in terms of sCOD or $sBOD_5$), the SMP concentrations presented may also include any remaining initial substrates. These non-metabolized components of the feed may also have influenced our results. However, a study conducted by Daigger and Grady [7] shows that the residual organic materials from a biological treatment system are mostly of microbial origin—microbial activity and lysis. Using a fixed biofilm system, Namkung and Rittmann [17] also showed that residual soluble organics contain about 85% of microbial by-products. Therefore, in this study, we assumed that the residual sCOD and $sBOD_5$ represented the concentration of SMP. High sCOD removal (50–80%) occurred during the first 7 days. The highest removal (80%) occurred during the first day. Thereafter, the residual sCOD increased steadily until it reached its maximum level on day 12 (Fig. 1); the sCOD removal decreased to as low as 40%. After day 12, the sCOD removal increased steadily. The increase and decrease in residual sCOD indicate a variation in the accumulation of SMP.

Other researchers [14, 18, 21, 24] have also reported similar variations in accumulation of SMP. Parkin and McCarty [21] attributed the increase and decrease in SMP concentration to SRT and initial MLSS concentration. They found that there was an SRT at which the SMP concentration reached a minimum and beyond which it increased. Microbial decay was reported to be primarily the cause of the SMP production, which was linear to the initial MLSS concentration. Namkung and Rittmann [18] attributed changes in SMP concentration to changes in the concentration of the available influent substrates. Their study showed that the concentration of SMP increases linearly with the influent substrate concentration. On the other hand, at low substrate concentration there is relatively less accumulation of biomass. Hence, the amount of by-products formed either by microbial activity or lysis is reduced, thus causing an overall decrease in the SMP concentration. According to Lee et al. [14], however, longer SRT leads to higher biomass concentration, and the generated SMP are taken up more actively as substrates to maintain the larger biomass population; as a result the amount of SMP in the effluent decreases.

In this study, the decrease and increase in sCOD removal and the subsequent increase and decrease in residual sCOD were attributed to four possible causes. First, the increase in residual sCOD between days 1 and 12, as a result of decrease in sCOD removal, indicates that the residual sCOD was less biodegradable than the initial substrate. Second, the increase in sCOD removal (between days 12 and 14) indicated that some components of the feed were not metabolized and thus accumulated, and there was an increase in the number of microorganisms capable of consuming these components. Third, the increase in sCOD removal shows that there was an increase in the content of biodegradable SMP components. Fourth, the increase in sCOD removal indicated that there was an increase in the number of microorganisms capable of degrading the SMP. In fact, the latter three causes may all occur simultaneously.

Significance of variations in $sBOD_5$

Considering that characteristics of both the SMP and biomass change with SRT, we determined the corresponding $sBOD_5$ values in order to confirm the first three possible causes mentioned above. From day 1 to 12, residual sCOD increased (Fig. 1), whereas initial $sBOD_5$ decreased (Fig. 2). Therefore, since $sBOD_5$ represents the amount of degradable soluble organics, the decrease in initial $sBOD_5$ confirms that the residual soluble organic matter was less biodegradable. Moreover, comparison of Figs. 1 and 2 reveals that the increase in residual sCOD was predominantly due to accumulation of SMP rather than accumulation of non-metabolized initial substrates. This is so because any accumulation of biodegradable materials, e.g., initial substrates, should be accompanied by an increase in initial $sBOD_5$. However, this was not the case in this study (from day 1 to 12). This observation also justifies our assumption that residual sCOD and $sBOD_5$ were mainly due to the presence of SMP. Therefore, the possible growth of microorganisms on residual organic components of the feed was insignificant. After day 12, it should be noted that the observed decrease in residual $sBOD_5$ occurred for only 2 days, after which it increased (Fig. 3). This fluctuation in residual $sBOD_5$ at long SRT indicates the occurrence of changes in the composition of degradable components of the accumulated SMP. Furthermore, the initial $sBOD_5$ increased steadily during this period (Fig. 2), thus confirming the third cause, i.e., that the content of easily biodegradable compounds increased in the system.

Shin and Kang [24] have reported similar results. However, they indicated that at even longer SRT (day 79), the content of the accumulated less biodegradable high molecular weight compounds (> 10,000 Da) decreased from 73.1 to 58.2%, whereas

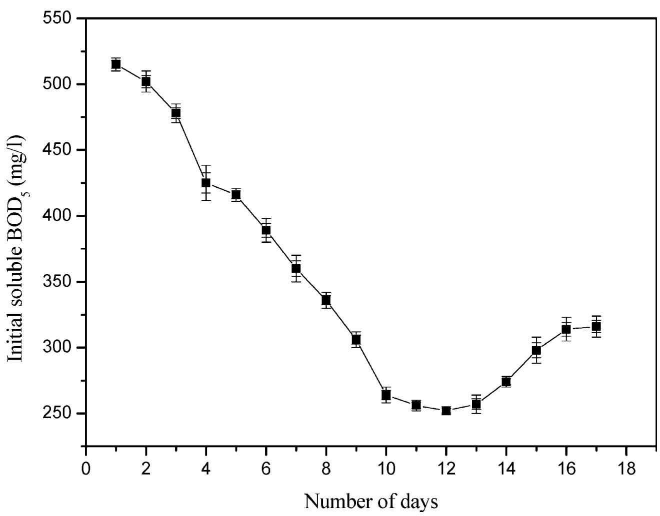

Fig. 2 Initial soluble biological oxygen demand ($sBOD_5$) loadings on each day of experiment 1. Error bars indicate three standard deviations of the data

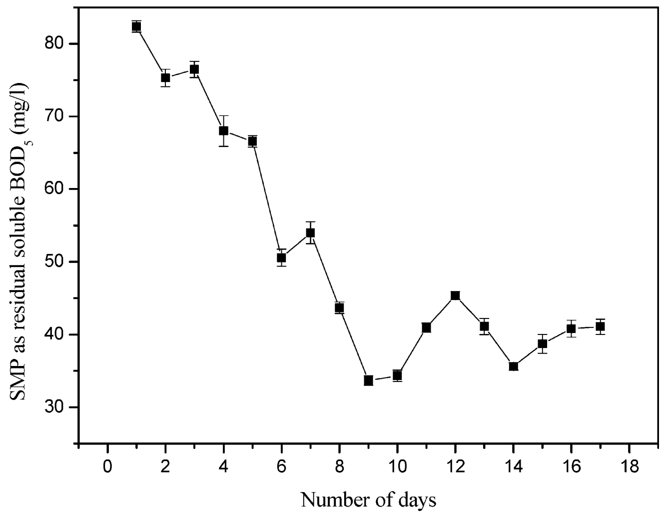

Fig. 3 Residual soluble BOD_5 after 24 h of each day of experiment 1. Error bars show three standard deviations of the data

that of the easily biodegradable small molecular weight compounds (< 1,000 Da) increased from 15 to 25%. Although the study of Shin and Kang [24] was conducted in membrane bioreactors at long SRT (up to 180 days), the results are comparable with those of this study, which was conducted in suspended growth bioreactors with an SRT of 16 days. Other researchers [11, 22] have similarly reported that SMP are degradable at long SRT. However, they indicated that, with extended SRT, accumulated SMP could not be fully decomposed in the reactors within the study period. In this study, when the residual sCOD was high (Fig. 1) (e.g., 500 mg/l), the corresponding low residual $sBOD_5$ (Fig. 3) (35 mg/l) did not imply that there was good removal of organic matter, but rather the presence of high levels of refractory residues of organic matter [10]. This change in the biodegradability of organic matter components was probably a result of changes in the characteristics of the biomass as discussed below.

Significance of variations in biomass concentration

Figure 4 shows the initial biomass concentration for each day. Considering that $sBOD_5$ represents the amount of organic matter that can be decomposed by microorganisms for growth, the reduction in initial $sBOD_5$ (Fig. 2) implied that organic substrates were the limiting factor. Under such conditions, the biomass concentration should reduce because of the decreased content of easily biodegradable organic substrates. Certainly, Fig. 4 shows that this was the case in this study. Interestingly, two distinctive regions were observed. Region 1 is a typical profile of microbial growth [16], which shows that after stationary phase the biomass enters endogenous phase and the biomass concentration reduces because of depleted substrates. Furthermore, it can be seen that the late stages of

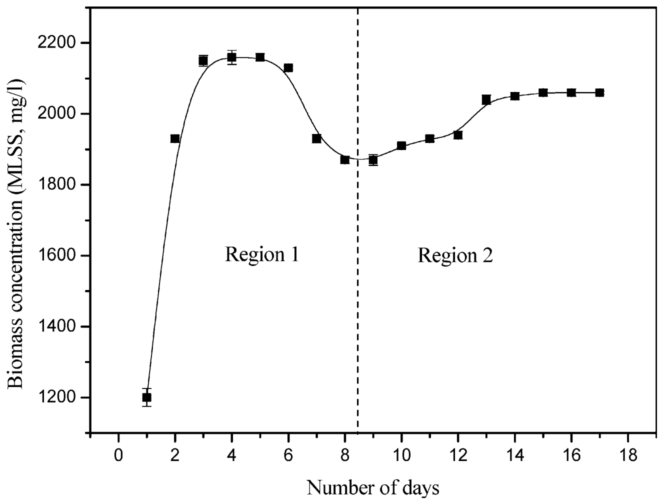

Fig. 4 Daily biomass concentration in bioreactors from which soluble microbial products (SMP) were not removed. Error bars are not shown for some data points because their standard deviations were so small

Region 1 correspond to a lag phase of microbial growth in Region 2, which shows that acclimation of microorganisms to SMP occurred. These data confirm the fourth cause mentioned above, i.e., that the increase in sCOD removal was also due to an increase in the population of microorganisms that could degrade SMP. Moreover, an increase in biomass concentration due to microbial consumption of insoluble BOD was also possible. Another possibility was an increase in biomass concentration due to growth of microbes on accumulated non-metabolized substrate components in the feed, i.e., possibility 2, which was apparently insignificant as described above.

Results reported by other researchers support these observations. Yoon et al. [28] showed that changes in substrate composition cause shifts in the microbial species structure and population (owing to competition among microorganisms), resulting in an increase or decrease in biomass concentration. In addition, Topalova et al. [25] reported that, at long SRT, the number of higher microorganisms (i.e., protozoa) increases. Similarly, Ekulund et al. [9] reported that populations of heterotrophic flagellates, amoebae and ciliates in ecosystems increase with increase in retention time. Many other results, including those of this study, have shown that the concentration of SMP increases with SRT. When all these results are considered together, they evidently show that higher microorganisms proliferate at high SMP concentration. Therefore, the shift towards a lower molecular weight distribution of SMP [24] might have been caused by the influence of higher microorganisms, which proliferate at high SMP concentration; mere acclimation of the initial microorganisms was not the cause, as the conclusions of other authors [14, 24] seem to suggest. In this respect, Fig. 4 suggests that the presence of SMP causes irregular accumulation of biomass due to possible changes in microbial species and population. This effect was more pronounced at high SMP concentration (over 50% of the initial substrate in terms of sCOD).

To examine the relationship between the concentrations of SMP and biomass, we determined the food-to-microorganisms (F/M) ratio in terms of $sBOD_5$/MLSS (data not shown). Variations in the daily initial F/M ratios were similar to the variations in the daily initial $sBOD_5$ values (Fig. 2). However, the corresponding ratios after 24 h were constant at 0.025 ± 0.008 although the daily residual $sBOD_5$ values changed significantly (Fig. 3). These data show that the daily residual $sBOD_5$ values were directly proportional to the daily biomass concentrations in the bioreactor. This is in agreement with data in the literature [17], which show that initial substrate concentration is proportional to effluent SMP concentration. Therefore, our results suggest that SMP play a significant role in influencing microbial activity and viability. However, detailed studies aimed at examining changes in the population and the function of microbial communities in relation to the accumulation of SMP are needed to confirm this conclusion.

Experiment 2: $sBOD_5$ and sCOD removal and biomass accumulation

To reduce the effect of SMP on the microbial community, we carried out experiments to reduce the content of these compounds in wastewater. The daily initial $sBOD_5$ and sCOD values were approximately constant ($sBOD_5$, 515 ± 5; sCOD, 859 ± 6 mg/l). Figure 5 shows $sBOD_5$ and sCOD removal from wastewater. The removal of $sBOD_5$ and sCOD increased with increase in SRT. This

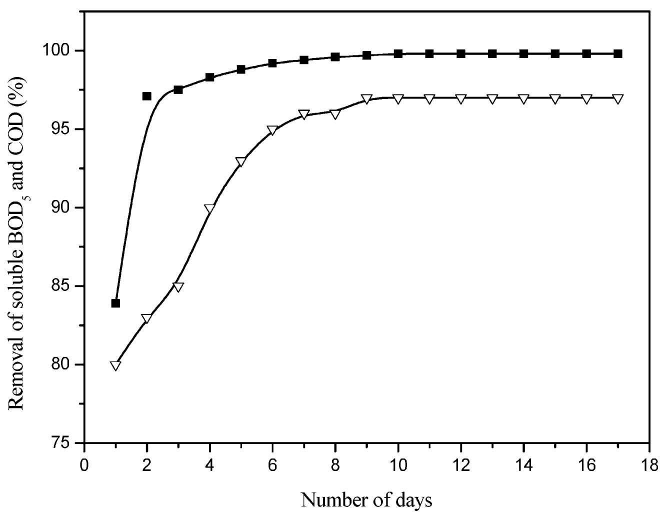

Fig. 5 $sBOD_5$ (■) and COD (▽) removal after 24 h of each day of experiment 2. The daily initial $sBOD_5$ and sCOD were 515 ± 5 and 859 ± 6 mg/l, respectively

shows that microbial communities developed in the bioreactors with reduced SMP concentration could sufficiently remove organic matter from wastewater. Consequently, the accumulation of the biomass (MLSS concentration) was steady under these conditions (Fig. 6). Unlike in the unwashed system described above, the low residual $sBOD_5$ and sCOD values also showed that production and release of SMP into the wastewater were minimal. At long SRT (after day 12), no increase or decrease in MLSS concentration was observed; it remained stable at slightly over 2,800 mg/l (Fig. 6).

A concept described in the literature can be used to explain this observation. According to the growth-death concept [26], net biomass production is limited by the available substrates (including secondary substrates from cell lysis) that allow a part of the biomass to grow, and the other part decays due to loss of cell viability. Thus, a steady state biomass concentration was maintained because of slow cell growth and death rates. Consequently, microbial growth and death were approximately zero.

Witzig et al. [27] have similarly reported that a steady state biomass concentration was reached in bioreactors with submerged membranes. Since membrane bioreactors are known to remove SMP from wastewater [15], we think that the membranes used by Witzig et al. [27] were effective in removing SMP. This is analogous to washing the biomass as in our experiments. Consequently, no irregularity in the accumulation of biomass was observed, unlike in our unwashed systems described above. Therefore, comparison of the results from the two systems in our study shows that the decrease in sCOD removal in biological treatment systems is not only a result of increase in SMP concentration, but also because of their influence on the microbial communities.

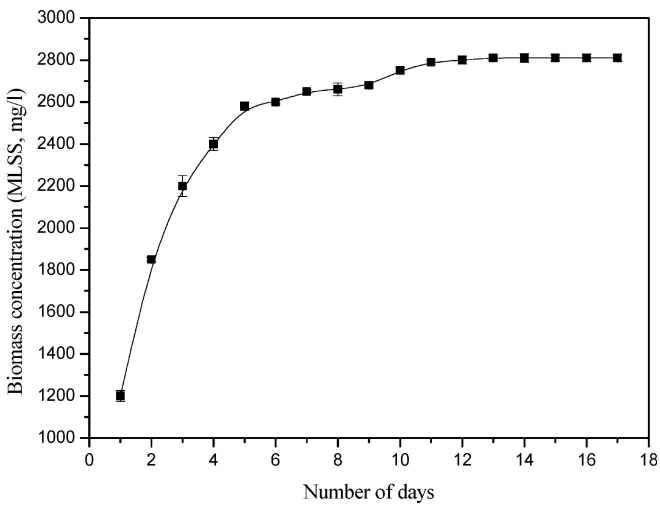

Fig. 6 Daily biomass concentration in the bioreactors in which the level of SMP was reduced. Error bars are not shown for some data points because their standard deviations are so small

Conclusions

In this study, the influence of organic substrates, including SMP, on microbial growth and removal of sCOD and $sBOD_5$ has been demonstrated. In summary, the conclusions of the study are as follows:

1. sCOD removal decreased with the increase in residual sCOD. This showed that the initial organic materials in the feed were more biodegradable than those in the treated wastewater. Changes in $sBOD_5$ values indicated a variation in the biodegradability of SMP components. Accumulation of SMP consequently caused irregular biomass accumulation.
2. Residual $sBOD_5$ was directly proportional to the daily biomass concentration. Thus, accumulation of SMP is linearly linked with microbial growth.
3. When the effect of SMP was reduced by washing the biomass, removal of $sBOD_5$ and sCOD increased and biomass concentration increased steadily to a constant value. Hence, a microbial community that could sufficiently consume organic matter was developed under these conditions.

These results suggest that adjustment of the accumulation of SMP could serve as an operational method for stabilizing not only the microbial community, but also the biodegradation process. The results also emphasize the need for concurrent determination of microbial population structure and by-products to understand the relative importance of SMP in relation to the microbial changes that occur during biodegradation processes.

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