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ORIGINAL PAPER

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Behavior of microbial communities developed in the presence/reduced level of soluble microbial products

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Abstract Soluble microbial products (SMP) are organics produced by microorganisms as they degrade substrates. The available literature does not reveal how SMP affect and regulate microbial activities. In this study, we monitored variations in pH, dissolved oxygen concentration, soluble biological and chemical oxygen demands ($sBOD_5$ and sCOD) as a measure of microbial activity in synthetic wastewater. Aerobic degradation tests were carried out under the following conditions: aeration, 1,500 cm^3 /min; initial $sBOD_5$, 515 ± 5 mg/l; initial sCOD, 859 ± 6 mg/l; initial biomass concentration (defined as mixed liquor suspended solids), $1{,}200 \pm 25$ mg/l; sludge retention time, 24 h; and temperature, 20 ± 1°C. The study involved non-acclimated biomass (R0 flora), biomass developed in the presence of SMP (R1 flora), and biomass developed in reduced level of SMP (R2 flora). We also determined which of these flora produced more refractory SMP. The results showed that R2 flora utilized the synthetic feed more quickly, and produced less refractory organic matter than R0 and R1 flora. The production of more refractory organics by R0 and R1 flora shows that not all the biomass was active. R1 flora degraded the substrates irregularly, suggesting that some microbes were dependent on the metabolic products of those that could utilize the feed components. These results show that production of SMP also depends on the prior substrates and on the ability of the flora to respond to changes in substrate composition.

Keywords Biodegradation · COD removal · Microbial activity · Wastewater treatment · Soluble microbial products

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Introduction

Soluble microbial products (SMP) are organic compounds produced by microorganisms as they degrade substrates. Evidence for this phenomenon has been proved experimentally since 1961 [8]. According to Namkung and Rittmann [14], SMP arise from two microbial processes: growth-associated and non-growth-associated. The resulting products are classified, respectively, as: (1) utilization-associated products (UAP), formed from substrate metabolism and microbial growth, and (2) biomass-associated products (BAP), derived from lysis and decay of microorganisms. SMP are reported to comprise a variety of organic compounds, such as humic acids, polysaccharides, proteins, nucleic acids, organic acids, antibiotics, steroids, exocellular enzymes, structural components of microbial cells, and metabolic products [5]. Additionally, Aquino and Stuckey [4] showed that SMP also contain a series of alkenes, alkanes and aromatic compounds.

Many researchers have studied the kinetics of production and biodegradation of SMP [5, 9, 14]. To what extent these additional organics introduced into the wastewater cycle play a part in biological processes is not yet fully understood. The question of whether SMP affect the microbial community has not been considered. The available literature sheds no light on how microbial activities are affected and regulated by these organic products. Therefore, we carried out experiments to compare the behavior of microbial communities developed in the presence and reduced level of SMP with fresh activated sludge. To measure microbial community activities, we monitored changes in pH, dissolved oxygen (DO) concentration, as well as soluble biological and chemical oxygen demands ($sBOD_5$ and sCOD). The originality of this procedure is that we compared the experimental data to determine which of the microbial communities produced more refractory SMP.

Materials and methods

Bioreactor operation and microbial acclimation

The activated sludge used was collected from the local municipal wastewater treatment plant (Gdansk, Poland). It was a mixture of activated sludge from all the biological reactors, which function according to the modified UCT process. A full description of this plant is given elsewhere [6]. The activated sludge microorganisms were acclimated to multiple substrates in synthetic wastewater. Characterization of the synthetic wastewater and a full experimental description of the adaptation of microbial communities are given elsewhere [7]. Two experiments were carried out. Briefly, in experiment 1, SMP were allowed to accumulate while the daily influent sCOD loadings were maintained constant at 859 ± 6 mg/l. As a result microbial communities in these bioreactors developed in the presence of SMP. To reduce the effect of SMP on microbial communities, SMP in the second experiment were removed by washing the biomass daily. The bioreactors were subsequently fed with fresh synthetic wastewater containing sCOD loadings of 859 ± 6 mg/l. Microbial communities in these bioreactors developed in the presence of reduced level of SMP. Three 2-l bioreactors (working volume 1 l) were used in each experiment. All the bioreactors were operated at a sludge retention time (SRT) of 16 days at ambient temperature (20 ± 1°C).

After the final day, treated wastewater was discarded from all the bioreactors after sedimentation. Next, the biomass and the bioreactors were washed with distilled water. Equal amounts [100 ml; mixed liquor suspended solids (MLSS), $1{,}200 \pm 25$ mg/l] of washed biomass were used in subsequent steps. The following bioreactors were set up in triplicate. R0 (washed non-acclimated biomass) contained fresh activated sludge collected from the local municipal treatment plant, R1 (washed acclimated biomass) contained microbial communities developed in the presence of SMP (described in experiment 1), and R2 (washed acclimated biomass) contained microbial communities developed in the presence of reduced level of SMP (described in experiment 2).

Experiments to monitor microbial activity during a 24-h period (with a break between the 11th and 17th hour) were carried out. Initial $sBOD_5$ and sCOD were 515 ± 5 and 859 ± 6 mg/l, respectively. The 24-h period was the actual time during which each bioreactor was continuously aerated (1,500 cm^3 /min). The aeration also helped to keep the contents of the bioreactors well mixed. All the bioreactors were operated at ambient temperature (20 ± 1°C) and DO concentration and pH were not controlled. Therefore, any changes in these variables were attributed to microbial community activities.

The biomass was not withdrawn from the bioreactors during the experiment. The total experimental time (including 15-min intervals for sedimentation and taking samples for sBOD and sCOD determination) was 28 h and 25 min.

Analytical methods

The pH values and DO concentration were measured hourly during aeration. pH was measured by inserting a microcomputer pH-meter into the bioreactors (CP-251, Elmetron, Zabrze Grzybowice, Poland). DO concentration was measured using an oxygen-sensing probe coupled to microprocessor DO-meter (Handy OxyGuard, OxyGuard International, Birkerod, Denmark). To withdraw samples for determination of $sBOD_5$ and sCOD, the air supply was switched off to allow sedimentation of the biomass. Next, 25 ml supernatant was withdrawn from each bioreactor using a pipette. Sedimentation and sampling were completed within 15 min. Samples were then centrifuged at 2,332 *g* for 15 min and filtered through a 0.45 μm membrane filter before storing them at 4°C in accordance with standard methods [3].

The initial and hourly residual sCOD were determined using the colorimetric method in Hach COD vials and reactor. We used the Hach spectrophotometer DR/2000. All samples were diluted with distilled water (1:5) before digestion in the Hach COD reactor. The initial and hourly $sBOD_5$ were determined according to standard methods [3]: method 5210 B (fresh activated sludge from the wastewater treatment plant was used as the seed source). Analytically pure reagents were used in all experiments.

Data analysis

The data from each triplicate experiment were calculated to obtain the arithmetic mean values and standard deviations using computer programs (Microsoft Excel 2000, Microsoft, Redmond, Wash.; Microcal Origin 6, Microcal Software, Northampton, Mass.). The arithmetic mean values ($n = 3$) were used for plotting the graphs.

Results and discussion

Variations in pH and DO concentration

Many methods have been developed for characterizing microbial growth and activities. These methods include standard plate counting, determination of enzyme activities, analysis of metabolic activity in situ by acridine orange staining, and measuring adenosine triphosphate concentration and thymidine incorporation. Molecular methods such as fluorescent in situ hybridization have also been used to analyze microbial growth rates and activities [1, 13]. These methods are expensive, labor intensive and time consuming. Therefore, the

methods that have been traditionally used to study the activity of activated sludge include measurements of pH, DO concentration, BOD and COD. In this study, we used these traditional, simpler methods to investigate microbial community activities.

Microbial growth and activity are a function of many physicochemical and biological factors. First, Menzl et al. [12] and Yoon et al. [19] showed that pH is a good indictor of microbial activities because variations in pH indicate biochemical activity. Second, Weddle and Jenkins [17], who studied variations in sludge metabolic activity and viable microorganism percentage as a function of growth rate, found that metabolic activity was directly proportional to the number of viable microorganisms. Third, Yoong et al. [20] showed that microbial growth and substrate removal are directly linked to the rate at which oxygen is consumed. Therefore, variations in DO concentration in aerobic systems link oxygen utilization with consumption of substrates by microbes. Combining these concepts, both pH and DO concentration can be taken as measures of microbial activity.

Figures 1 and 2 show variations in pH and DO concentration during the biodegradation of organic compounds in the synthetic wastewater. The magnitude of variations in pH was different in each bioreactor. We considered microbial consumption of organic matter (including hydrolysis and metabolism of the organic substrates) as the cause of the variations in pH. The following simplified equation describes the consumption of organic matter by microorganisms [10]:

$$n(CH_2O) + n(O_2) \rightarrow n(CO_2) + n(H_2O) \quad (1)$$

In addition to the effect of hydrolysis of organic matter, which results in the release of organic acids, the CO_2 produced reduces the pH in the bioreactor because of carbonic acid formed in accordance with following carbonate equilibria equations [2]:

$$CO_2 + H_2O \overset{K_1}{\leftrightarrow} H_2CO_3 \quad (2)$$

$$H_2CO_3 \overset{K_2}{\leftrightarrow} HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- \overset{K_3}{\leftrightarrow} CO_3^{2-} + H^+ \quad (4)$$

By combining Eqs. 2 and 3, the following relationship is obtained:

$$K_4 = \frac{[H^+][HCO_3^-]}{[CO_2]} \quad (5)$$

In this study, either effect might have caused a decrease in the concentration of CO_2 in the wastewater. First, aeration could cause the escape of CO_2 to air. Second, with a decrease in the content of biodegradable organic matter or reduced microbial action, the concentration of CO_2 could be correspondingly reduced, as Eq. 1 shows. Both effects would cause a decrease in the $[H^+][HCO_3^-]$ product by the same factor. Thus, the concentration of hydrogen ions is reduced. Therefore, when production of CO_2 is low due to a decrease in the content of biodegradable organic matter, or due to a decrease in the microbial substrate consumption rate, the pH increases correspondingly so that the quotient $([H^+][HCO_3^-]/[CO_2])$ is constant.

Comparing the pH values in all the bioreactors showed that hydrolysis in R0 was slower than that in either R1 or R2 (Fig. 1). Moreover, DO concentration at inoculation in R0 was the highest (Fig. 2), indicating a brief lag phase because non-acclimated activated sludge microorganisms were inoculated into a fresh medium [10]. The lowest DO concentration in all the bioreactors was at inoculation (Fig. 2), and DO levels

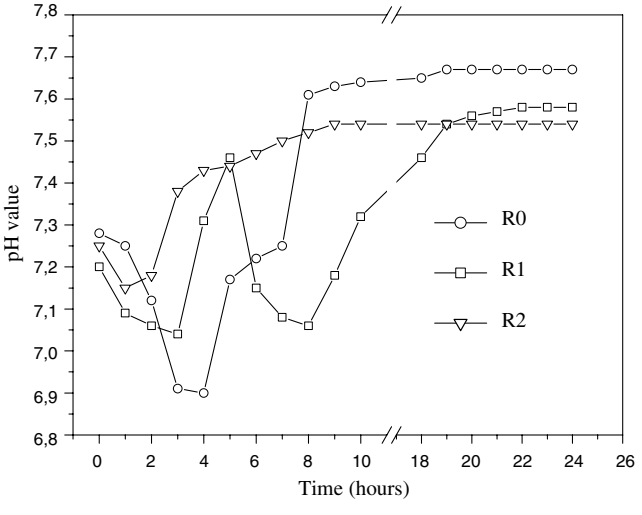

Fig. 1 Variations in pH values. *R0* Bioreactor with non-acclimated microbial communities, *R1* bioreactor with acclimated microbial communities, developed in the presence of soluble microbial products (SMP), *R2* bioreactor with acclimated microbial communities, developed in the presence of reduced level of SMP

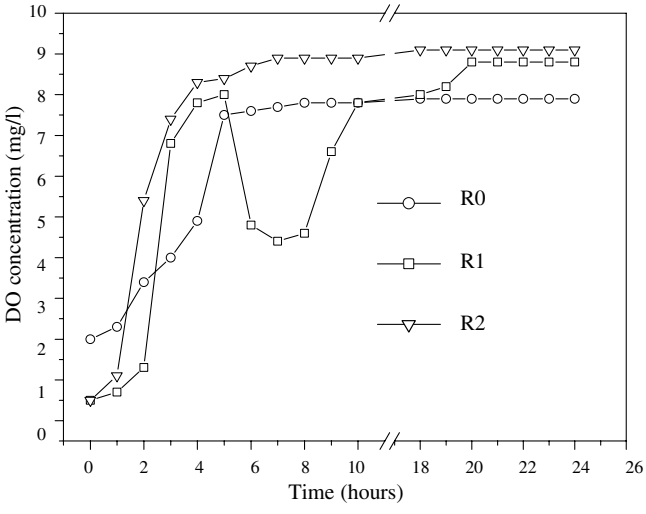

Fig. 2 Variations in dissolved oxygen (DO) concentration. *R0*, *R1* and *R2* as in Fig. 1

then increased with time. This showed that microbial activity was high during the first hours of the experiments when the concentration of organic substrates was high. The decrease in pH and the increase in DO concentration in bioreactors R1 and R2 occurred very quickly in the first 3 h (Figs. 1 and 2). This shows that hydrolysis and subsequent oxidation of organic substrates and production of CO_2 in R1 and R2 occurred immediately after inoculation. After 2 h, the pH in bioreactor R2 increased steadily and reached a constant value after 8 h, indicating that microbial uptake of organic substrates was predominant over hydrolysis. Because hydrolysis and uptake of organic substrates in R2 were quicker than in either R0 or R1, this also shows that the microbial community in R2 effected good removal of organic matter.

After 3 h, the pH in bioreactor R1 increased until 5 h. Considering Eqs. 1, 2, 3 and 4 given above, this shows that microbial uptake (oxidation) of organic substrates was predominant over hydrolysis during this period. Interestingly, pH and DO concentration decreased again between 5 and 8 h. In this case, the fluctuations in these variables were possibly due not only to microbial growth, but also to microbial death and lysis [16], which caused an extra organic loading, resulting in increased microbial activity.

Since air supply rates were the same, we assumed that the effect of aeration on stripping out CO_2 from the bioreactors was also the same. Therefore, the observed differences in the magnitude of variations in pH and DO concentration in the bioreactors indicated that the rates at which degradation of organic compounds occurred were different. Moreover, since initial substrates and their concentrations were the same in all the bioreactors, the differences in substrate consumption rates can be attributed to differences in microbial activities. The irregular variations in pH and DO concentration in R1 suggest that the biodegradability of organic matter released into the mixed liquor was also different from that in R0 and R2. To confirm this observation, we compared the residual sCOD and $sBOD_5$ values to determine which microbial communities produced more refractory SMP.

Variations in sCOD and $sBOD_5$

Figures 3 and 4 show the residual sCOD and $sBOD_5$ values. The initial sCOD and $sBOD_5$ values were as indicated at time 0. In bioreactor R1, more than 80% sCOD removal occurred between 3 and 10 h, and after 10–24 h. However, we also observed an increase in residual sCOD in R1 between 6 and 10 h (Fig. 3). We attributed the variations in organic substrate flux to microbial cell death and lysis [16]. The irregular variation in residual sCOD in R1 also shows that the biodegradability of residual organics and, possibly, the functional behavior of R1 flora were changing, as shown in Figs. 1 and 2.

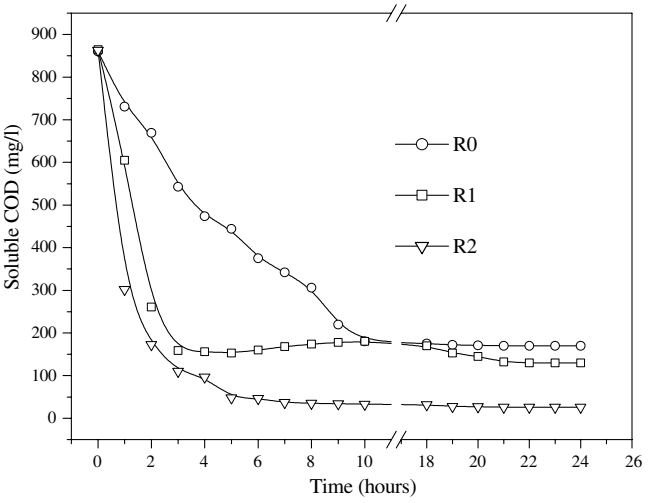

Fig. 3 Variations in soluble chemical oxygen demand (sCOD). *R0*, *R1* and *R2* as in Fig. 1

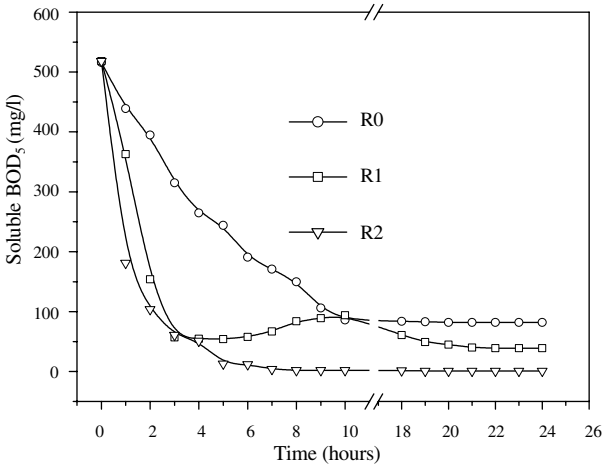

Fig. 4 Variations in soluble biological oxygen demand ($sBOD_5$). *R0*, *R1* and *R2* as in Fig. 1

Mamais et al. [11] reported that soluble COD of effluents from a biological wastewater treatment system filtered through a 0.45 μm membrane filter represents residual organic matter. Moreover, Wentzel et al. [18] reported that a decrease in oxygen utilization rate indicates that readily biodegradable COD is completely removed. Hence, the residual sCOD encountered when the oxygen utilization rate has decreased represents refractory organic matter. In the present study, decreased oxygen consumption rate (as shown by increase in DO concentration, Fig. 2) occurred when sCOD removal was 80% or more (Fig. 3). Consequently, we assumed that the residual sCOD and $sBOD_5$ encountered when there was 80% sCOD removal represented refractory SMP.

Determination of refractory SMP in the bioreactors

In order to determine which microbial community produced more refractory SMP, we compared the residual

sCOD values at times when 80% sCOD removal occurred in the bioreactors (R0, 18 h; R1, 8 h; and R2, 2 h). We also compared the corresponding residual $sBOD_5$ values. Although the residual sCOD in the bioreactors were approximately equal (about 174 mg/l), the corresponding $sBOD_5$ values were different from each other (Fig. 4). The $sBOD_5$ values were in the following increasing order: $R1 < R0 < R2$. Since $sBOD_5$ represents readily biodegradable components of the SMP, microorganisms in R0 and R1 produced more refractory organic materials than those in R2. This is so because the $sBOD_5$ value in R2 was the highest at the sCOD value similar to that in R0 and R1.

Furthermore, considering that R1 was characterized by irregular sCOD and $sBOD_5$ removal (Figs. 3, 4), we compared the after-18 h sCOD values in R0 and R1 (there was 80% sCOD removal in both bioreactors after 18 h). Although their sCOD values were similar, the $sBOD_5$ value in R1 was lower than that in R0, indicating that microorganisms in R1 produced more refractory organic compounds than those in R0.

In conclusion, these results have shown that the non-acclimated biomass in R0 was slow to degrade the synthetic feed. The biomass in R2, which contained a flora that had been developed in the presence of reduced level of SMP, utilized the substrates in the feed directly and hence did so more quickly and effectively than the R1 flora, which included some microbes that were dependent on the metabolic products of those that could utilize the feed components. Some of the R1 flora produced refractory organics, probably BAP, that are reported to be more difficult to degrade than UAP [15], thus explaining their accumulation in the medium.

The R2 flora produced less refractory organic matter. The lack of significant SMP accumulation in R2 means either that SMP production was minimal or nonexistent, or its subsequent biodegradation was very rapid. However, our results cannot differentiate between these three possibilities. This observation can be explained by using the model reported by Laspidou and Rittmann [9], which shows that when most of the biomass is active, microbial utilization of metabolites (UAP and BAP) does not result in the formation of new UAP and BAP. Hence, the R2 flora utilized the produced metabolites as substrates, resulting in less accumulation of SMP in the medium than that in R0 and R1. Since the initial biomass concentration was equal in all bioreactors, the results show that production of SMP depends not only on the biomass concentration but also on the prior substrates and on the ability of microorganisms to respond to changes in substrate composition. Further research is needed to identify how the presence of SMP influences the stability of microbial communities and their diversity.

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