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The influence of soluble microbial products on microbial community composition: hypothesis of microbial community succession

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Abstract

Soluble microbial products (SMP) are organic compounds produced by activated sludge microorganisms as they degrade substrates. They include by-products of microbial activity, death and lysis. The available literature does not reveal how SMP influence microbial community composition. In this regard, we microscopically studied changes in composition of microbial communities, especially protozoa and metazoa, under the influence of increased as well as reduced levels of SMP. The presence of SMP at high level significantly caused changes in microbial community composition. Microbial species shifted from attached ciliates (12–175 μm) to free-swimming and crawling ciliates (35–330 μm) and then invertebrates, which included rotifers (0.2–1 mm) and nematodes (1–50 mm). The shift of small-size microorganisms to large ones was observed as one of the most significant influences of SMP. Attached ciliates reappeared when we removed the SMP that had accumulated in the bioreactors – we have called this as the resurrection phenomenon of microorganisms. Such rapid changes in microbial community composition were not observed in the experiment with low concentration of SMP. Overall, the results suggest that accumulation of SMP is one of the intrinsic regulatory mechanisms that control viability and dormancy of microbial communities in activated sludge.

Key words: activated sludge, microbial community composition, protozoa, soluble microbial products, biological wastewater treatment

Introduction

Microorganisms used in the activated sludge process, which is commonly used for treatment of industrial and municipal wastewater, include bacteria, protozoa and metazoa (Amann *et al*., 1998; Amaral *et al.*, 2004). The interactive relationships among these microorganisms during the biodegradation of organic compounds are diverse and complex. It is well established that microbial communities in biological wastewater treatment systems undergo changes in structure, population as well as activity and viability (Kucnerowicz and Verstraete, 1983; Wagner *et al.*, 2002; Wanner, 1994). Many factors regulate microbial community composition. In activated sludge, it is believed that protozoan grazing on bacteria stimulates the activity of microbial communities. To explain this phenomenon, Ratsak *et al*. (1996) reviewed the hypotheses that give some insights: (1) protozoa excrete mineral nutrients, thus changing the C:N:P ratio, resulting in an increased rate of utilization of carbon sources; (2) protozoa excrete growth-stimulating compounds which enhance bacterial activity; and (3) protozoan grazing leads to the selection of species and release of nutrients contained in inactive biomass, and this enables the remaining microbial population to grow faster and maintain higher levels of activity resulting in increased use of carbon sources.

In biological wastewater treatment, another important factor that affects the quality of the effluents and the overall removal of organic matter is the presence of soluble microbial products (SMP), which are by-products of microbial activity, death and lysis. 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 as well as a series of alkenes, alkanes and aromatic compounds (Aquino and Stuckey, 2002; Barker and Stuckey, 1999). Although wastewater effluents contain SMP, little is known about these compounds playing a role in controlling microbial

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community compositions and the subsequent survival of microbial species.

Understanding factors that determine the stability of microbial community compositions and populations is crucial for consistent removal of pollutants from wastewater. Many mechanisms can theoretically cause changes in microbial community composition, making it difficult to identify a single explanation for the changes or maintenance of microbial community compositions and populations. Although many researchers have studied the characteristics of SMP and the kinetics of their production Laspidou and Rittmann, 2002; Namkung and Rittmann, 1996; Namour and Muller, 1998), there are no studies directly linking microbial community successions to accumulation of SMP. Since the concentration of SMP in activated sludge systems varies during various applications it is a worthwhile endeavor to study the influence of varying levels of SMP on the microbial community composition.

Recently, we have shown that SMP have a strong impact on microbial growth and utilization of the available substrates (Chipasa and Mędrzycka, 2004a; 2004b), suggesting that SMP play a significant role in influencing microbial activity. Considering these findings, we hypothesized that SMP are one of the major factors that cause changes in microbial community composition in activated sludge. In this study, the objective was to test this hypothesis. We therefore examined whether accumulation of SMP could cause changes in the microbial community composition in activated sludge. Experiments were set up to observe the occurrences of microorganisms developing in the presence of high and artificially reduced levels of SMP. Changes in the composition of the microbial community, mainly comprising protozoa and metazoa, were studied microscopically.

Experimental

Materials and Methods

Bioreactor operation and microbial community acclimation. Activated sludge used in the experiments was collected from the local municipal wastewater treatment plant (Gdańsk, 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 full description of this plant is given elsewhere (Chipasa, 2003). A 1000-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. The activated sludge microorganisms were acclimated to multiple substrates in synthetic wastewater. Three approaches for studying how factors such as food supply and accumulation of SMP influence mixed liquor suspended solids (MLSS) and the removal of soluble chemical and biological oxygen demands (sCOD and $sBOD_5$) included the following three 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, Gdańsk, 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 I 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 (1500 ml/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 ($20 \pm 1^{o}C$) 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. Feeding the bioreactors with fresh synthetic wastewater daily made it possible to maintain the bioreactors for 16 days without a breakdown of the ecosystem in bioreactors due to other conditions other than the accumulation of SMP. Since the bioreactors were fed with fresh synthetic wastewater daily, they can be described as fed-batch bioreactors. 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 (Amann *et al.*, 1998). 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 separating 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.

In experiment 3, after day 16, 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, 1200 ± 25 mg/l) of washed biomass were used in subsequent steps. The following bioreactors were set up: (1) (washed acclimated biomass) contained the microbial community developed in the presence of high level of SMP (described in experiment 1), and (2) (washed acclimated biomass) contained the microbial community developed in the presence of reduced level of SMP (described in experiment 2). Initial sBOD5 and sCOD were 515±5 and 859±6 mg/l, respectively. The purpose of washing the biomass used in experiment 3 was to especially monitor the behavior of the microbial community after removing the SMP that had accumulated during experiment 1.

Microbiological examinations. 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 high level of SMP. To reduce the effect of SMP on microbial communities, SMP in the second experiment were artificially 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. Monitoring of microbial community changes during the 16 days (experiments 1 and 2) and the 24-h period (experiment 3) was carried out as described below.

We initially examined the microbial community composition of the activated sludge (freshly collected from the local wastewater treatment plant) before the acclimation tests. All samples were collected in accordance with standard methods (APHA *et al*., 1996). Next, we carried out microbiological examinations to qualitatively determine the changes in compositions of microbial communities during their development in the presence of high and artificially reduced levels of SMP (experiments 1 and 2, respectively). Further, we examined changes in the microbial community composition, which had developed in the presence of high level of SMP, after washing the biomass (experiment 3). Samples were drawn from the well-mixed bioreactors using a pipette for microscopic examination using a phase contrast microscope equipped with a camera (Polish Optical Company, Poland). Microorganisms were observed at × 100 magnification. Small flagellates and amoeba were observed at × 400 magnification. Microorganisms were identified according to guide books (APHA *et al.*, 1996; Berk and Gunderson, 1993; Jenkins *et al*., 1993) and laboratory materials supplied by the authorities of the local municipal wastewater treatment plant.

It was possible to observe and identify most of the dominant species using this method. Other researchers have also studied the population of protozoa in activated sludge by microscopic methods (Amaral *et al.*, 2004). Microscopic observation of protozoa and metazoa is a common practice in wastewater treatment plants and provides key information on the overall performance of the treatment process and final effluent quality (Amaral *et al.*, 2004; Curds and Cockburn, 1970; Da Motta *et al*., 2001). The exact number of protozoa and metazoan species in activated sludge is large, varying and remains unknown. Based on literature reports (Amaral *et al*., 2004; Da Motta *et al*., 2001; Madoni, 1994) as well as on our own experience in handling activated sludge samples from wastewater treatment plants, protozoa are classified into three major groups (flagellates, sarcodines and ciliates) whereas metazoa are classified into two groups (rotifers and nematodes). Ciliates are the most abundant and they are further divided into three groups: attached ciliates (*i.e.*, sessile ciliates attached to the sludge flocs by stalk structures), free-swimming ciliates (*i.e.*, ciliates moving freely in the mixed liquor), and crawling ciliates (*i.e.*, ciliates moving on the surface of the sludge flocs). In this study, we grouped the microorganisms into three categories: attached ciliates, free-swimming and crawling ciliates, and invertebrates (including rotifers and nematodes). We recorded the occurrences of dominant individual microorganisms in these categories as common, frequent, occasional, and rare or not observed.

Results

The data to show the accumulation and reduction of SMP and how they influence microbial growth and activity (*i.e.*, changes in biomass concentration and removal of soluble COD and BOD) are reported in our recent publications (Chipasa and Mędrzycka,

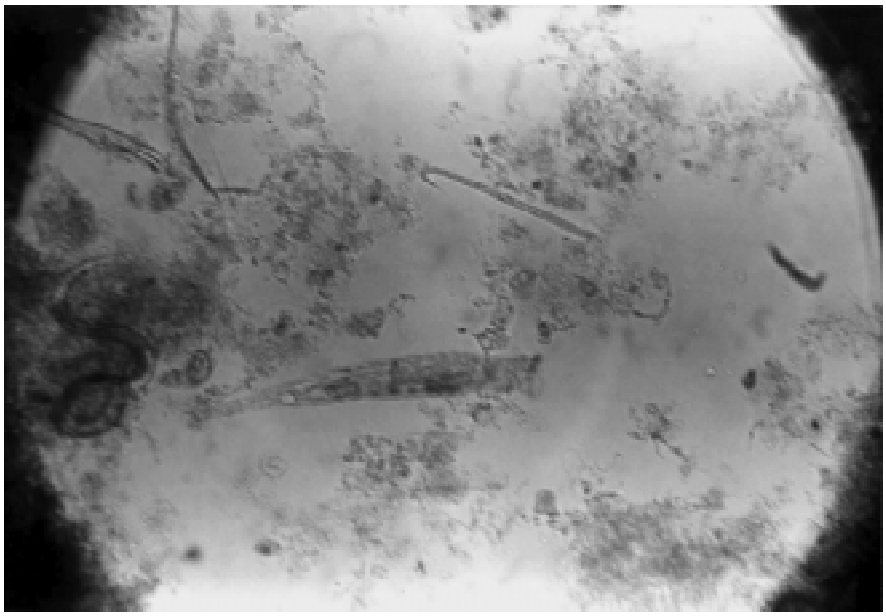

Fig. 1. Microphotograph showing the presence of higher microorganisms (rotifers: *Rotaria trisecata*, *Proales decipiens*; nematodes: *Plectus*, *Tripyla*). Ciliates and filamentous bacteria disappeared. Sample was taken from experiment 1 after day 7. Magnification ×100.

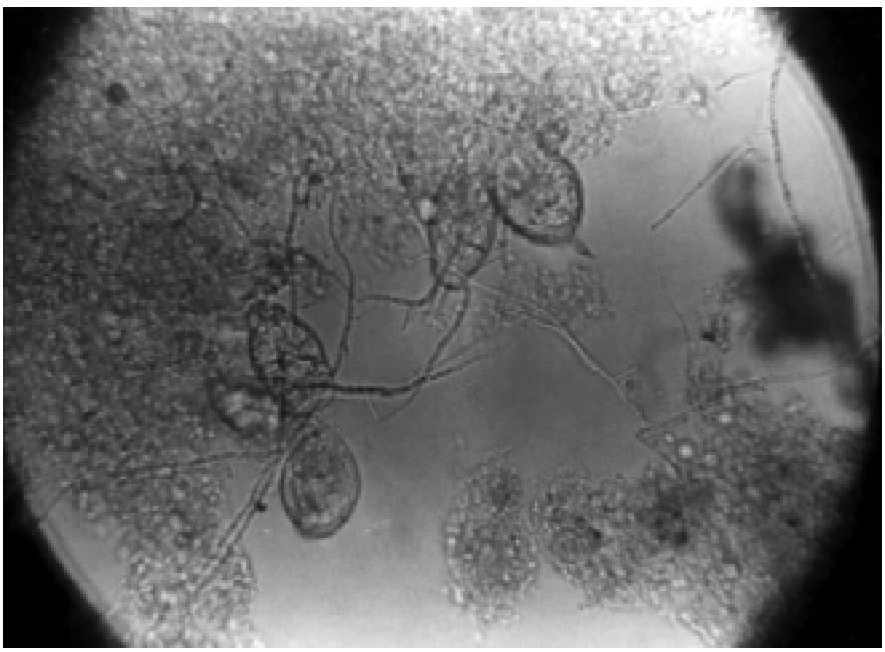

Fig. 2. Microphotograph showing the presence of ciliated protozoa, flagellates (seen as bright dots on the photo), and floc-forming bacteria. Sample was taken from experiment 2 after day 7. Magnification ×100.

2004a; 2004b). Tables II and III show occurrences of individual microorganisms in experiments 1 and 2, respectively. Day 0 shows the microbial community composition of the activated sludge collected from the wastewater treatment plant before the acclimation tests. We observed that, in experiment 1, where the SMP were allowed to accumulate, the microbial community composition changed rapidly with many microorganisms only appearing for a short period. All the attached ciliates (except *Vorticella*) and floc-forming bacteria disappeared by day 6. After day 6, invertebrates including rotifers, tardigrades, arachnoidea and nematodes decisively dominated the biomass (Table I and Fig. 1).

Table I
Characteristics of the synthetic wastewater after mixing with washed activated sludge.

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	1200±25	38.7±1.5	10.33±1.5	7,06±0.21

COD – Chemical oxygen demand; BOD – biological oxygen demand; MLSS – mixed liquor suspended solids (= biomass concentration)

Changes in the microbial community composition in experiment 2, where SMP were artificially removed, were slow. Small attached ciliates (*Tocophrya, Podophrya*, *Acineta*; 35–50 μm) disappeared by day 7, whereas most of the attached, free-swimming and

Table II
Occurrence of microorganisms developing in the presence of high level of soluble microbial products

Microorganisms		*Occurrence of microorganisms developed in the presence of high level of soluble microbial products																
		Number of days																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Attached ciliates	*Carchesium*	+	+	++	+	++	–	–	–	–	–	–	–	–	–	–	–	–
	Epistylis	++++	++++	++	+	++	–	–	–	–	–	–	–	–	–	–	–	–
	Opercularia	+	+	++	++	++	–	–	–	–	–	–	–	–	–	–	–	–
	Vorticella	+++	+++	++	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	Tocophrya	++	++	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Podophrya	++	++	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Acineta	++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Free-swimming and crawling ciliates	*Litonotus*	+++	+++	+++	++	+	++	+	+	+	+	+	–	–	–	–	–	–
	Aspidisca	++++	++++	++	+	+	++++	++	++	++	+	+	–	+	–	–	–	–
	Euplotes	++	++	+++	++++	++++	++++	++	++	++	+	+	+	+	+	+	–	–
	Chilodonella	++	++	++	+++	+++	+++	+	+	+	+	+	+	+	+	+	–	–
	Colpidium	+	+	+++	++	++	++	+	–	–	–	–	–	–	–	–	–	–
	Paramecium	++	++	+++	++++	++++	++++	++	++	++	++	++	+	+	+	+	–	–
	Amphileptus	+	+	+	++	+	+	–	–	–	–	–	–	–	–	–	–	–
	Trachelophyllum	+	+	+	++	+	+	–	–	–	–	–	–	–	–	–	–	–
	Prorodon teres	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Glaucoma	++	++	++	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	Tetrahymena	+	+	++	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Invertebrates	*Cephalodella*	+	+	++	++	+++	+++	+++	+++	++	+	+	+	+	+	–	–	–
	Monostyla	+	+	++	+++	++++	++++	++	+++	++	+	+	++	+	++	++	++	++
	Dicranophorus	++	++	++	++	++	++	+	+	–	–	–	–	–	–	–	–	–
	Habrotrocha	+	+	+	++	++	+++	+	++	+	–	–	–	–	–	–	–	–
	Lecane	+	+	+	+++	+++	+	+	++	+	++	++	++	++	+	+	++	++
	Philodina	–	–	–	–	–	–	–	++	++	++	++	++	+++	++	+++	++	+++
	Rotaria	++	++	–	–	–	–	–	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Tardigrades	–	–	–	–	–	–	–	++	+++	++	++	++	++	++	++	++	++
	Arachnoidea	–	–	–	–	–	–	–	++	+	+	+	+	+	+	+	+	+
	Nematodes	–	–	–	–	–	–	–	++	+++	+++	+++	+++	+++	+++	++++	+++	++++
Total number of species		24	24	22	19	19	14	12	15	14	13	13	11	12	11	10	7	7

* Occurrence: common (++++), frequent (+++), occasional (++), rare (+), not observed (–).

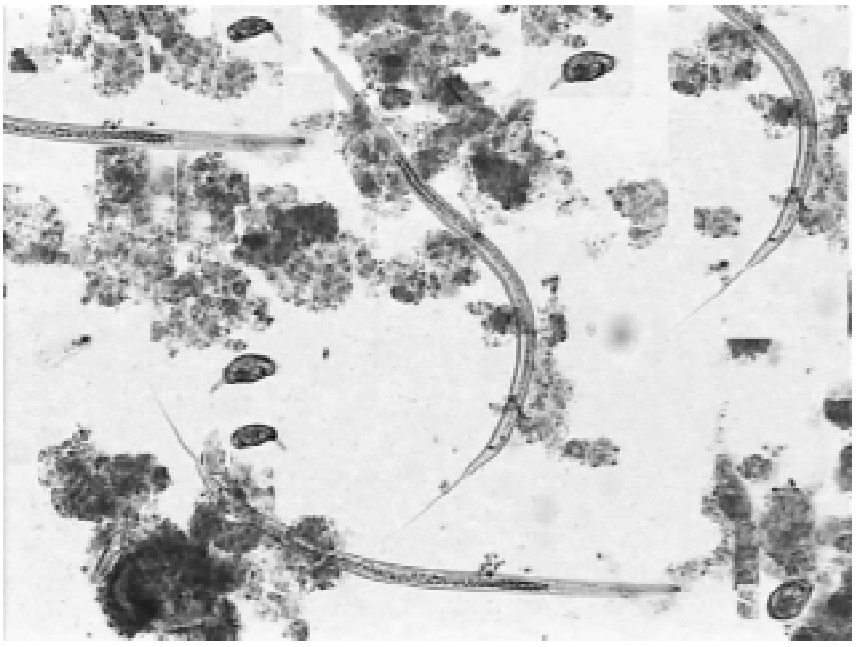

Fig. 3A. Microphotograph showing the presence of invertebrates (nematodes: *Plectus, nematode egg*; rotifer: *Cephalodella*). Sample was taken from experiment 1 before washing the biomass. Magnification ×100.

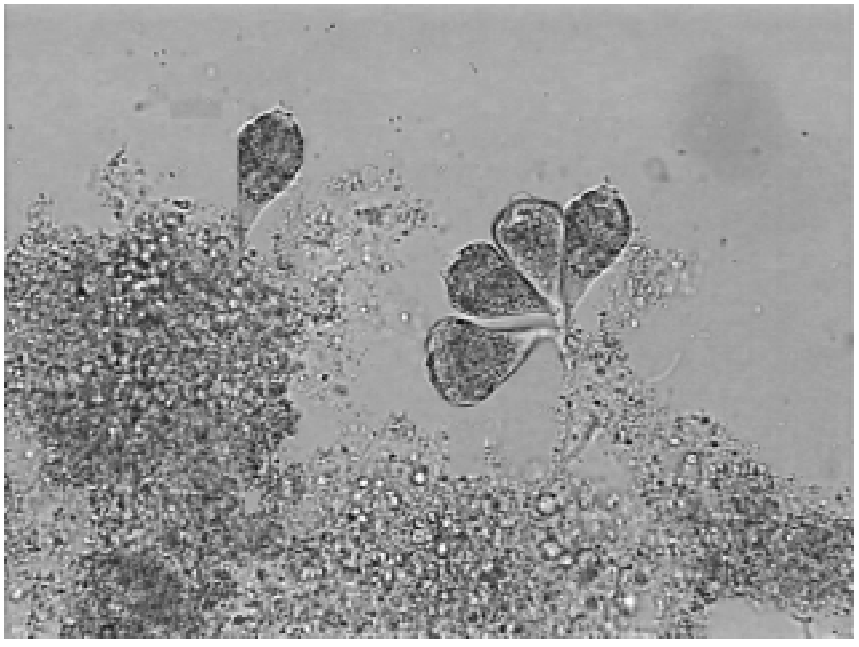

Fig. 3B. Microphotograph showing the appearance of attached ciliates (*Epistylis rotans*), floc-forming bacteria, amoeba and flagellates (seen as bright dots) after washing the biomass. Sample was taken 10 h after washing the biomass in experiment 1 (*i.e.*, experiment 3). Magnification x100.

crawling ciliates were still present (Table III). Moreover, floc-forming bacteria, flagellates and amoeba were also present (Fig. 2). Most interestingly, invertebrates that appeared in experiment 1 were not observed in experiment 2 (Table III). These results suggest that the presence of invertebrates in experiment 1 was associated with the presence of SMP at high concentration. Further we calculated the total number of microbial species (species richness) observed each day in order to gain insight into the diversity of the microbial community (Tables II and III). During the 16 days the species richness in experiment 1 decreased drastically to as low as 7 while that of experiment 2 remained relatively higher.

After day 16, we removed the SMP (as described in experiment 3, see Materials and Methods) that had accumulated during experiment 1 and monitored the behavior of the microbial community. Samples were taken for microscopic examinations after 10 and 24 h of starting the experiment. Before washing the biomass, the microbial community contained invertebrates (Table I and Fig. 3A). Attached ciliates, flagellates and floc-forming bacteria reappeared within 10 h and their presence was still evident even after 24 h (Figs. 3B and C). Most interestingly the microbial community in experiment 1 resembled that in experiment 2 (Fig. 3D) and the mixed liquor was yellowish-brown – a sign of badly managed sludge.

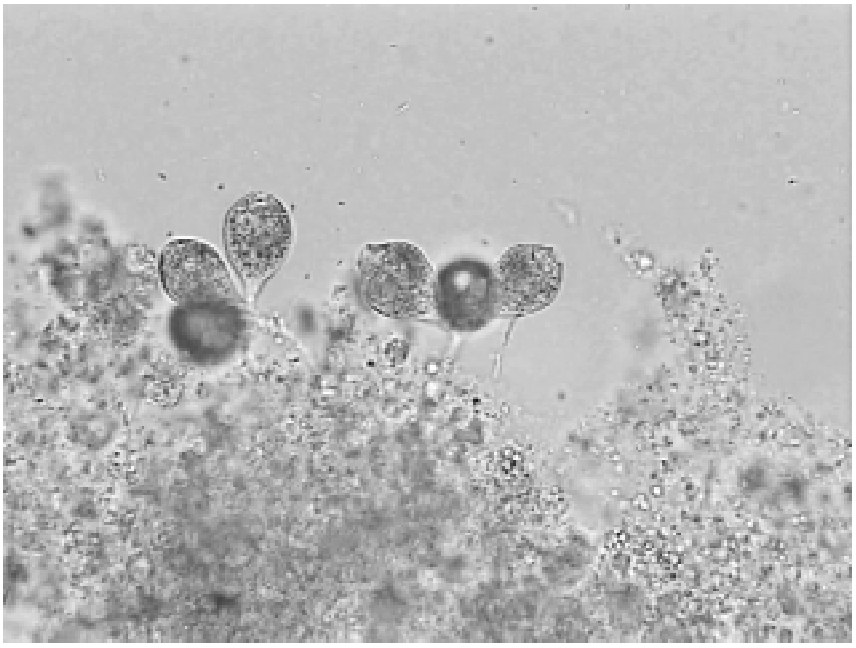

Fig. 3C. Microphotograph showing the appearance of attached ciliates (*Epistylis rotans*), floc-forming bacteria, amoeba and flagellates (seen as bright dots) after washing the biomass.

An unidentified microorganism was also observed (seen on the microphotograph as dark ball-like structures near the attached ciliates). The microorganism moved very fast in circular motions. Sample was taken 24 h after washing the biomass in experiment 1 (*i.e.*, experiment 3). Magnification ×100.

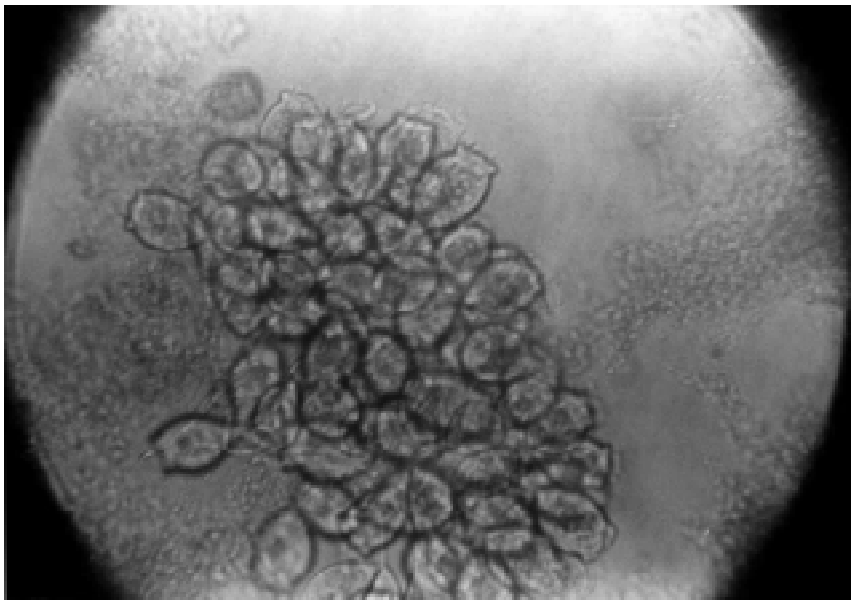

Fig. 3D. Microphotograph showing the presence of attached ciliates (colonies of *Epistylis*), flagellates (seen as bright dots on the photo), and floc-forming bacteria.

Sample was taken 24 h after washing the biomass in experiment 2 (*i.e.*, experiment 3). Magnification ×100.

Discussion

We observed changes in compositions of microbial communities during their development in the presence of high and reduced levels of SMP in wastewater. In this study, the purpose was not to detect each microorganism in the microbial community, but rather to determine the occurrences of microorganisms, particularly higher microorganisms, in the samples with increase in SRT, which implies increase in SMP concentration (Chipasa and Mędrzycka, 2004a; Namkung and Rittmann, 1986). Higher microorganisms (*i.e.*, ciliates, rotifers and nematodes) are used for the assessment of condition and performance of activated sludge because they have enough morphological detail to be reliably identified (Amaral *et al*., 2004; Curds and Cockburn, 1970; Da Motta *et al*., 2001). Other researchers have also used higher microorganisms as useful indicators of conditions of wastewater treatment processes (Amann *et al*., 1998, Madoni, 1994).

The literature shows that the composition of microbial communities in wastewater treatment systems changes with increase in SRT (Kucnerowicz and Verstraete, 1983; Madoni, 1994; Wanner, 1994). Results of studies on the colonization behavior of ciliates in activated and rotating contactor plants show that free-swimming ciliates dominated in the initial phases, whereas attached ciliates dominated at longer

Table III
Occurrence of microorganisms developing in the presence of reduced level of soluble microbial products

Microorganisms		* Occurrence of microorganisms developed in the presence of reduced level of soluble microbial products																
		Number of days																
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Attached ciliates	*Carchesium*	+	+	++	+++	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Epistylis	++++	++++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	+++	+++	+++	+++
	Opercularia	+	+	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++
	Vorticella	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	++	++	++	+++	+++	+++	+++
	Tocophrya	++	++	+	++	+	++	+	–	–	–	–	–	–	–	–	–	–
	Podophrya	++	++	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	Acineta	++	++	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–
Free-swimming and crawling ciliates	*Litonotus*	+++	+++	+++	++	+	+	+	+	+	+	+	–	+	+	+	+	+
	Aspidisca	++++	++++	++	+++	++	++	++	++	++	++	+	+	+	–	–	–	–
	Euplotes	++	++	+	+	+	++	++	+++	+++	+++	++	++	+++	+++	+++	++	++
	Chilodonella	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Colpidium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Paramecium	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Amphileptus	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+
	Trachelophyllum	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
	Prorodon teres	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Glaucoma	++	++	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–
	Tetrahymena	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Invertebrates	*Cephalodella*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
	Monostyla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Dicranophorus	++	++	+	+	+	+	+	+	++	++	+	+	+	+	+	+	+
	Habrotrocha	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Lecane	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Philodina	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Rotaria	++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Tardigrades	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Arachnoidea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Nematodes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total number of species		24	24	22	23	22	23	23	20	20	20	20	20	17	16	16	16	16

* Occurrence: common (++++), frequent (+++), occasional (++), rare (+), not observed (–).

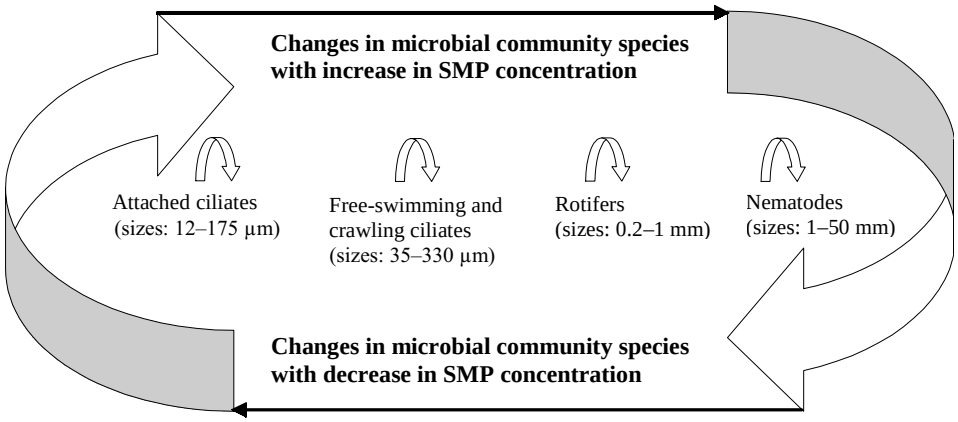

Fig. 4. Model of succession of microorganisms.
The model illustrates the observed changes in microbial community species with increase in SMP concentration. It shows the relative shifts from small-size to large-size microbial species and vice versa; this was confirmed in experiment 3. The inside curved arrows indicate that changes also occurred within a group of individuals of one species. Sizes of microorganisms shown were adapted from literature (APHA *et al.*, 1995; Berk and Gunderson, 1993).

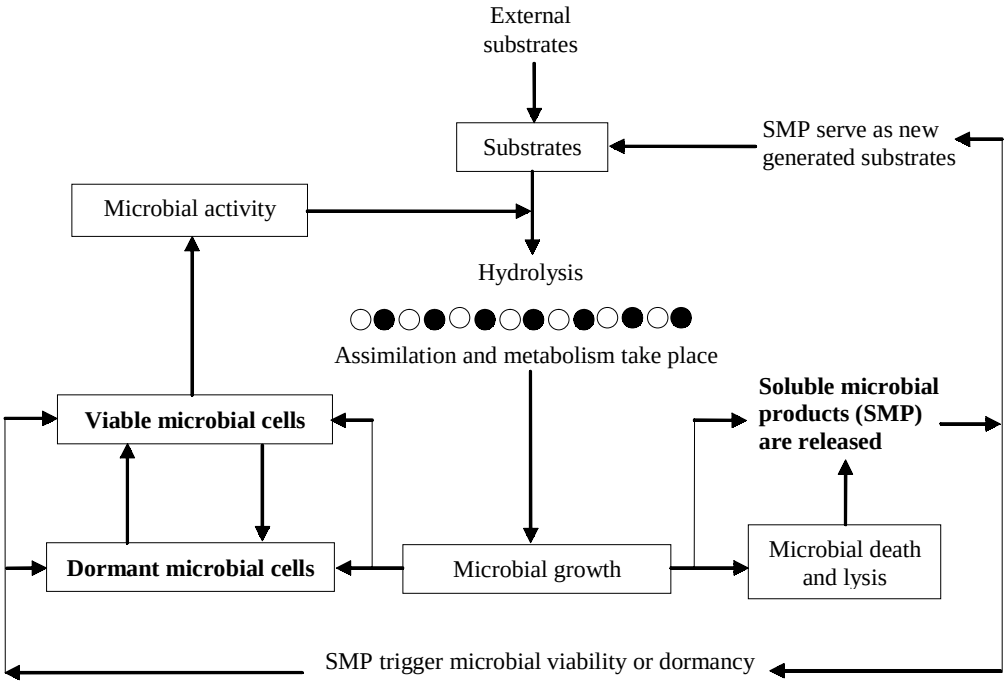

Fig. 5. Conceptual model of the influence of soluble microbial products on microbial communities.

SRT (Pauli *et al.*, 2001). These results agree with our results of experiment 2 (Table III) in which SMP content was low. The species richness decreased in both experiments. This was expected because the microbial communities in both experiments were exposed to SMP during each 24-h period of acclimation. These results show that the microbial community composition was more diverse in experiment 2 than in experiment 1. These results are analogous with the data in the literature. Firstly, the differences in species richness can be attributed to sludge age as reported by Ziembińska *et al.* (2007), who have shown that bacterial groups in younger sludge is characterized by a much higher diversity of the genotypes than bacterial groups in older sludge; sludge in experiment 1 was older than in experiment 2 which was washed and fed with fresh substrate daily. Secondly, Gray (1989) has reported that the greatest diversity and largest abundance of ciliates occur when the concentration of less biodegradable organic matter in wastewater is low; SMP are reported to be less biodegradable (Namour and Muller, 1998). We have therefore attributed the decrease in species richness to the influence of SMP on microorganisms, which was more pronounced in experiment 1 than in experiment 2. Recently, under the same experimental conditions as in

this study, we have reported that the accumulation of SMP consequently causes both irregular biomass accumulation and sCOD removal with increase in SRT (Chipasa and Mędrzycka, 2004a). Additionally, the observations reported in this study show that changes in types of microbial species occurred rapidly with increase in SRT (Table II). This confirms that SMP which were released into the mixed liquor played a significant role in regulating microbial community composition.

Model of succession of microorganisms. Further analysis of Table II shows that the changes in the microbial community composition occurred in a succession pattern with respect to increase in SMP content. As schematically shown (Fig. 4), we observed that the succession of types of microbial species was from attached ciliates to invertebrates that have much larger sizes (APHA *et al*., 1995; Berk and Gunderson, 1993). Changes in types of microbial species also occurred within groups of individuals of one species. For attached ciliates, for example, we observed that small ciliates of sizes 35–50 μm (*Acineta, Podophrya* and *Tocophrya*) disappeared after day 4, while the largest attached ciliates (*Vortecella*, 40–175 μm) disappeared after day 6 (Table II). Thereafter, no attached ciliates were observed in experiment 1. Succession patterns of free-swimming and crawling ciliates and invertebrates were similar to that of attached ciliates in which small individual species were succeeded by large ones (Table II).

To support these results, other researchers (Amann *et al*., 1998; Kucnerowicz and Verstraete, 1983; Madoni, 1994) have reported that at long SRT the number of higher microorganisms increases. Similarly, studies by Salvado (1994) show that small ciliates (scuticociliates) were succeeded by large ciliates (peritichida) with increase in SRT. Studies using flagellates also show that large flagellates (greater than 20 μm) succeed small ones with increase in SRT (Salvado, 1994). Explanations for the causes of microbial community shifts include competition for substrates, predation, and adaptation to new environmental conditions (Amann *et al*., 1998; Grady and Williams, 1975; Kucnerowicz and Verstraete, 1983; Ratsak *et al*., 1996; Wanner, 1994). Although these studies show that the growth of higher microorganisms and microbial morphological changes indicate long SRT, none of them have linked the growth of higher microorganisms to accumulation of SMP. Our microscopic observations reported in this study agree with our earlier conclusion that higher microorganisms proliferate at high SMP concentration (Chipasa and Mędrzycka, 2004), thus showing a link between the increase in SMP concentration and the growth of higher microorganisms. It is apparent that SMP mediates the changes in compositions of microbial species.

SMP control viability and dormancy of microorganisms. Since SMP are less biodegradable (Namour and Muller, 1998), the increase in their concentration in the bioreactors implied that organic substrates were the limiting factor (Chipasa and Mędrzycka, 2004). Under this condition, in accordance with the top-down effect, higher microorganisms proliferate because of their grazing pressure targeted at small-size microorganisms, *e.g.*, bacteria. In contrast, under resource-rich conditions, accelerated growth of small-size microbial species is observed and changes induced by increases in resource supply overwhelm losses due to grazing (Simek *et al*., 2003). This is exactly what we observed in this study. Most importantly, the interpretation of our results indicates that as the SMP concentration increases the microbial community shifts towards microorganisms of much bigger sizes, such as invertebrates. This interpretation is in agreement with the well-established ecological hypothesis of top-down effect (Jardillier *et al*., 2004; Muylaert *et al*., 2002).

Practically, in wastewater treatment plants, the appearance of rotifers and nematodes, which indicate long SRT, leads to taking measures to change the microbial community structure and function (Amann *et al.*, 1998). Certainly, this was the case in this study. The effect of washing the biomass (*i.e.*, removal of SMP that had accumulated during experiment 1) and supplying the bioreactor with fresh synthetic wastewater caused significant changes in the microbial community composition. Attached ciliates, flagellates and floc-forming bacteria reappeared within 10 h and their presence was still evident even after 24 h (Figs. 3B and C). Most interestingly the microbial community in experiment 3 resembled that in experiment 2 (Fig. 3D). These results suggest that the microbial community in experiment 3 recovered after removing the SMP that had accumulated. These results also confirm our model of succession of microorganisms (Fig. 4), showing that small-size microorganisms proliferate with decrease in SMP concentration. These results are in accordance with the bottom-up effect (*i.e.*, small-size microorganisms proliferate under resource-rich conditions) as discussed above.

In addition to possible bottom-up and top-down effects, our results suggest that SMP trigger viability and dormancy of microorganisms. Table II shows that invertebrates appeared at long SRT. Therefore, the appearance of invertebrates at high level of SMP shows that SMP stimulated their growth. Moreover, considering that attached ciliates disappeared by day 7 (Table II), their reappearance (which we have called a resurrection phenomenon of microorganisms) after washing the biomass showed further evidence that SMP do not only serve as inhibitors, stimulants and substrates (Barker and Stuckey, 1999; Laspidou

and Rittmann, 2002), but also trigger the viability and dormancy of microorganisms. This novel interpretation of the effect of SMP on microbial communities is in agreement with the results of Kirkwood *et al.* (2006), who studied the effect of cyanobacterial exudates on bacterial growth and removal of organic contaminants from wastewater. They found that exudates repressed as well as enhanced bacterial (*Pseudomonas* and *Ancylobacter* strains) degradation of organic contaminants, suggesting that exudates have dissimilar impacts on bacterial species. Other researchers (Casamatta and Wickstrom, 2000) have similarly reported that dominant members of bacterial communities could tolerate or benefit from microbial exudates. The absence of other bacterial species was found to be related to their intolerance to exudates. These findings show that microbial by-products can potentially alter microbial community composition in wastewater treatment systems. In this regard, our results also suggest that SMP are species-specific, *i.e.*, they are responsible for causing microbial species to be viable or dormant. To show this conclusion, a conceptual model is presented (Fig. 5). It illustrates the hypothesis that SMP are apparently the immediate cause of the changes in microbial community composition in activated sludge.

Considering the fact that protozoa are directly dependent on bacteria as their food (Ratsak *et al*., 1996; Hahn and Hofle, 2001), further research is needed to establish whether the link between accumulation of SMP and growth of protozoa is direct or indirect. To achieve this aim experiments should be designed to determine changes in protozoa communities in relation to changes in concentrations of SMP and bacteria.

Conclusion. What is in our opinion more exciting is that our study shows a general proof-of-concept – SMP influence microbial community compositions. Shift of small-size microorganisms to large ones was observed as one of the most significant influences. In summary, the conclusions of the study were as follows:

1. The increase in the SMP concentration was accompanied by rapid microbial community changes. It was microscopically confirmed that microbial species shifted from attached ciliates (12–175 μm) to free-swimming and crawling ciliates (35–330 μm) and then invertebrates, which included rotifers (0.2–1 mm) and nematodes (1–50 mm). Such rapid changes in microbial community composition were not observed in the experiment with low concentration of SMP. Therefore, the results suggest that the increase in SMP concentration caused the microbial community to shift towards microorganisms of much bigger sizes.

2. Analysis of these results showed that SMP do not only serve as inhibitors, stimulants and substrates, but also trigger the viability and dormancy of microorganisms. Hence, accumulation of SMP is one of the intrinsic regulatory mechanisms that control viability and dormancy of microbial communities in activated sludge. The suggested model can with time be used to examine the effect of microbial by-products on microbial community compositions in managed as well as natural ecosystems.

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