Aerobic granular sludge in an SBR-system treating wastewater rich in particulate matter

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Abstract Aerobic granular sludge was successfully cultivated in a lab-scale SBR-system treating malting wastewater with a high content of particulate organic matter (0.9 gTSS/L). At an organic loading rate (COD_{total}) of 3.4 kg/(m^3·d) an average removal efficiency of 50% in COD_{total} and 80% in $COD_{dissolved}$ was achieved. Fractionation of the COD by means of particle size showed that particles with a diameter less than 25–50 μm could be removed at 80% efficiency, whereas particles bigger than 50 μm were only removed at 40% efficiency. Tracer experiments revealed a dense sessile protozoa population covering the granules. The protozoa appeared to be responsible for primary particle uptake from the wastewater.
Keywords Aerobic granular sludge; malting wastewater; particulate organic matter; protozoa; SBR

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Introduction

The performance of a biological wastewater treatment unit is determined by the metabolism of the microorganisms in the system and by the efficiency of the solid/liquid separation at the last stage of a treatment process. Granular sludge can significantly improve both factors at the same time. High settling velocities of the microbial aggregates (Moy *et al.*, 2002) concur with a comparably higher and robust metabolism (Zhu and Wilderer, 2003). In the field of anaerobic wastewater treatment, granular sludge has been widely investigated and applied over the past decades and is strongly related to the UASB-technology (Seghezzo *et al.*, 1998). The formation of aerobic sludge granules was reported comparatively recently (Shin *et al.*, 1992; Morgenroth *et al.*, 1997). Up to now aerobic granules have successfully been cultivated in different experimental setups using synthetic wastewaters containing purely dissolved and readily degradable substrates of various compositions (e.g. Etterer and Wilderer, 2001; Beun *et al.*, 2002; Moy *et al.*, 2002; Tay *et al.*, 2002). No attention has been paid so far to the fate and the influence of particulate organic matter in the process of aerobic sludge granulation and in wastewater treatment processes using aerobic granular sludge. This aspect on the other hand is crucial for the application of aerobic granules to the treatment of real wastewaters. Major fractions of the pollutants are, in real wastewaters, present in the form of colloids or particles and need to be removed during the process of wastewater treatment. Previous studies on biofilm airlift suspension reactors (Roessink and Eikelboom, 1997) and on sequencing batch biofilm reactors (Arnz, 2001) indicate that biofilms have a significant potential for utilizing particulate organic matter as substrates for their metabolism. In both cases protozoa played an important role in the removal of particulate organic matter from the wastewater.

Within the present study, attempts were made to cultivate aerobic granular sludge in an SBR system treating malting wastewaters with a high content of particulate organic matter. Overall, the applicability of aerobic granules for the treatment of real wastewaters was demonstrated and the removal of particulate organic matter further investigated.

Material and methods

Reactor set-up and operation

The experiment was conducted in a lab-scale SBR with a total working volume of 12 L, an internal diameter of 20 cm and a filling height of 38 cm. The reactor was aerated through air bubble diffusors at a volumetric flow rate of 600–800 L/h. Temperature in the reactor was ambient (17°C ± 3°C). Total cycle duration was 8 h, with 6 min of fill, 120 min of anaerobic no mix, 345-348 min of aeration, 5-2 min of settling and 4 min of effluent withdrawal. Volumetric exchange ratio was set to 66%, giving a hydraulic retention time (HRT) of 0.5 days. Average COD_{total}-loading rate was 3.2 kg/(m^3·d). Activated sludge (V = 12 L) from a municipal wastewater treatment plant was used as inoculum. Biofilm growing on the reactor walls was removed from the reactor every fortnight while storing the reactor content separately. The wastewater used in the experiment was prepared as a concentrate by mixing barley dust together with tap water. Concentrated wastewater (V = 4 L) was then fed from a storage tank into the reactor at the beginning of the filling period and immediately diluted with tap water (V = 4 L) giving pollutant concentrations as indicated in Table 1.

Analytical procedures

Physico-chemical analysis. COD, N and P were measured spectrophotometrically (Dr. Lange test equipment); MLSS and BOD_5 were measured according to standard methods (German Standard Methods, 2000). Specific oxygen uptake rates (SOUR) were measured using oxygen electrodes (WTW) at a controlled temperature of 18.5°C ± 0.5°C. Settling properties of the sludge bed were characterized by means of a modified sludge volume measurement: sludge samples were put into a graduated cylinder (V = 0.5 L) and sludge volume was recorded every five minutes. Sludge volumes were subsequently normalized to the MLSS-content of the sample. For detailed investigations of the COD-removal four different COD-fractions were separated by means of particle size and settleability according to Roessink and Eikelboom, 1997. Measurement of the removal efficiency was conducted after complete granulation had occurred (week 21 of operation).

Tracer experiments and microscopy. Particle uptake was studied in a separate reactor (V = 1.0 L) with an extended cycle duration of 24 h. Fluorescent polystyrene beads (D_P = 1.0 μm) were used as tracer particles (Molecular Probes F8852 with hydrophobic and F8816 with hydrophilic surface). The beads were sonicated and suspended in filtered wastewater (0.45 μm) prior to feeding into the reactor. The concentration of beads in the reactor was determined by means of filter counts (pore size 0.1 μm) using epifluorescence microscopy. The spatial structure of particle uptake was studied using confocal laser scanning microscopy (Zeiss Axioplan LSM510) of intact granules after incubation with the tracer particles. Nucleic acid staining (Molecular probes Syto 60) was applied to the samples prior to microscopic investigation. For the investigation of the internal structure, granules from the same experiment were cryo-sectioned. The protozoa population covering the surface of granules was studied using phase contrast microscopy (Zeiss Axioplan).

Table 1 Average pollutant concentrations of the wastewater used throughout the experiment

COD_{total} [mg/L]	$COD_{dissolved}$ [mg/L]	TKN [mg/L]	NH_4-N [mg/L]	P_{total} [mg/L]	Settleable matter [mL/L]	TSS [g/L]
1,700	470	45	3	9	15	0.95

Results and discussion

Granule formation and granule structure

Single granules formed within the first week of operation. Granules evolved from a typical integrated structure of a filamentous backbone, particles and floc-forming microorganisms (Figure 1 left), finally growing into smooth and dense granules with only few filaments being present (Figure 1 right). Complete granulation of the biomass occurred within 21 weeks, after a transient period of 8 weeks, when a mixture of granules and flocs was present in the reactor. Altogether flocs originated from three sources: a high level of particulate matter (TSS = 0.95 g/L) in the influent, anaerobic growth in the storage tank and suspended microbial growth in the reactor.

Mature granules showed a high number and diverse population of protozoa growing on the surface of the aggregates (Figure 2). Amongst a vast majority of peritrichous ciliates several other species were found. The colonisation of aerobic granules by protozoa had so far only been reported in one case for granules grown on synthetic wastewaters (i.e. containing readily degradable and soluble substrates) (Zhu *et al.*, 2001). Also little attention had been paid to the role that protozoa play in the degradation of wastewater compounds in granular sludge reactors.

Reactor performance

Settling properties of the sludge bed. With regards to the design of a large-scale wastewater treatment unit that is operated with aerobic granular sludge, an adequate mathematical description of a settling granular sludge bed is vital. So far only static parameters (e.g. sludge volume index SVI and settling velocity of single granules) have been used for the characterisation of the settling properties. However, both methods do not include the dynamic

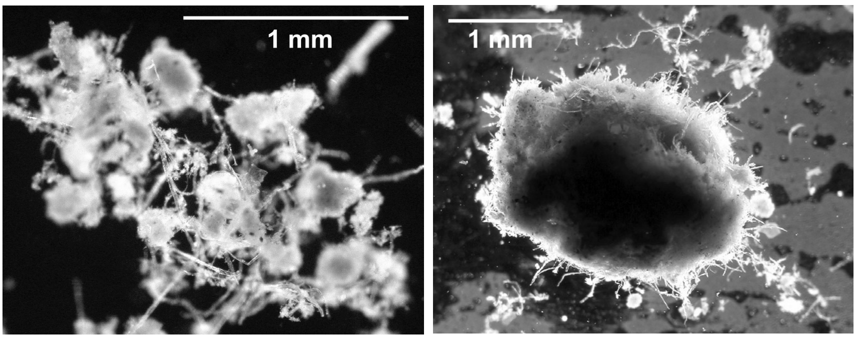

Figure 1 Evolution of aerobic sludge granules in the reactor from an originally flocculant sludge inoculum (left: first week of operation, right: fourth week of operation, dark field microscopy)

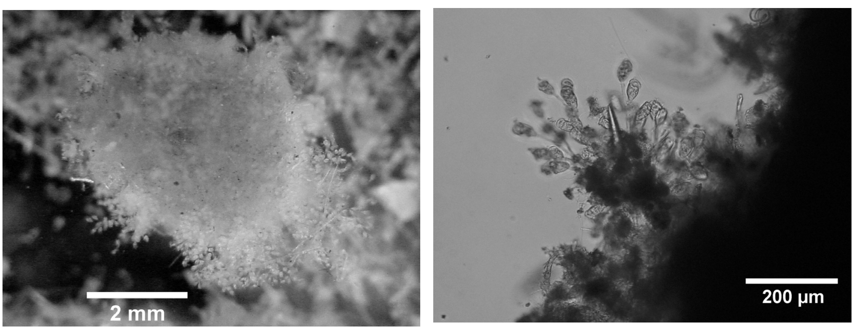

Figure 2 Mature granule (24th week of operation) covered with peritrichous ciliates (left), colony of *Epistylis sp.* (right) (photos: J. Fried)

interactions between the moving liquid and between individual sludge aggregates, which finally determine the settling rate of a sludge bed. One possible solution is to record the changes in SVI over a settling period of 30 minutes accompanying the reactor operation (Figure 3). A low SVI (t = 30 min) does not necessarily imply sludge granulation and vice versa. Nevertheless a granular sludge bed does consolidate much faster (i.e. the terminal SVI (t = 30 min) is already reached after 5 minutes of settling). In terms of SBR-operation this would enable higher operational efficiency through shorter settling phases. For continuous flow systems highly reduced volumes for secondary clarifiers could be designed.

COD-removal and specific oxygen uptake rate (SOUR) after complete granulation. At an organic loading rate (COD_{total}) of 3.4 kg/(m^3·d) and an influent particle concentration (TSS) of 0.9 g/L an average removal efficiency of 50% in COD_{total} and 80% in $COD_{dissolved}$ was achieved at a biomass concentration (MLSS) of 6-7 g/L in the reactor. The level of metabolic activity in terms of specific oxygen uptake rate (SOUR) was lower ($r_{O2,X}$ = 29.4 mgO_2/(gMLSS·h)) than reported in the literature for experiments with synthetic wastewaters, containing solely dissolved and readily degradable substrates (e.g. Liu and Tay, 2002, $r_{O2,X}$ = 96.3 mgO_2/(gMLSS·h)). This difference can be attributed to the composition of the wastewater that was used throughout this experiment: only a fraction of 30% of the COD_{total} in the influent was found to be available for biological degradation in terms of BOD_5. Furthermore a clear relation between particle size and removal efficiency could be determined through fractionation of the COD in the influent and effluent of the reactor according to Roessink and Eikelboom, 1997.

Settleable particles of larger size (D_P>50 µm) could be degraded at 43% efficiency, whereas the smaller fractions and dissolved COD could be degraded at more than 70% efficiency (Table 2). Removal efficiency was hence limited by a maximum "pore size" and restricted availability of larger particles for biological degradation. Table 2 gives a detailed overview of the removal capacities for the different particle size fractions.

Particle uptake The ability of aerobic granular sludge to remove particulate organic matter from the wastewater results from two different mechanisms. During initial granule formation and growth, particles are incorporated into the biofilm matrix of the granules.

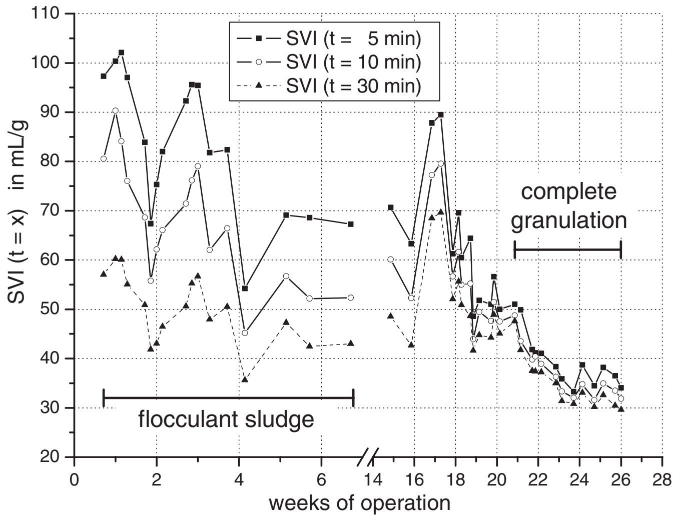

Figure 3 Development of the sludge volume index SVI at different settling times (t = 5, 10 and 30 min) throughout the experiment

Table 2 COD-removal efficiency in the granular sludge SBR as a function of particle size

	Total	Dissolved	D_P>25–50 μm	D_P<25–50 μm	1 μm>D_P>0.2 μm	D_P<0.2 μm
$COD_{influent}$ [mg/L]	1,700	325	1,550	65	120	250
$COD_{effluent}$ [mg/L]	875	65	875	18	2	52
Removal [%]	49	80	43	72	98	79

For the completely granulated sludge bed with mature granules, particulate matter is removed as a result of the presence and metabolic activity of a dense protozoa population covering the granules' surface. Incubation of granules together with fluorescent tracer particles (D_P = 1.0 μm, $t_{incubation}$ = 24 h) and subsequent cryosectioning showed that particles only attached to the outer, fluffy layer of the granules and did not penetrate the biofilm-corpus of the granules. Investigations of the spatial distribution of particles by means of confocal laser scanning (CLSM), epifluorescence and phase contrast microscopy after nucleic acid staining showed, furthermore, that particles are almost exclusively ingested by the protozoa growing on the granules' surface (Figure 4). Surface hydrophobicity of the particles did not influence the uptake kinetics. Scanning of regions more distant from the granule surface showed that no particles attached to the biofilm aggregate (granule). Protozoa were hence concluded to be the location of primary particle uptake.

Conclusions

- Aerobic granular sludge can successfully be cultivated in SBR-systems treating real wastewaters containing particulate matter.
- Sludge volume index is not an appropriate method to describe the settling properties of a granular sludge bed.
- The treatment efficiency (COD-removal) is strongly dependent on the particle size and increases with decreasing particle size.
- Protozoa play an important role in the removal of particulate matter from wastewaters in granular sludge bed reactors.

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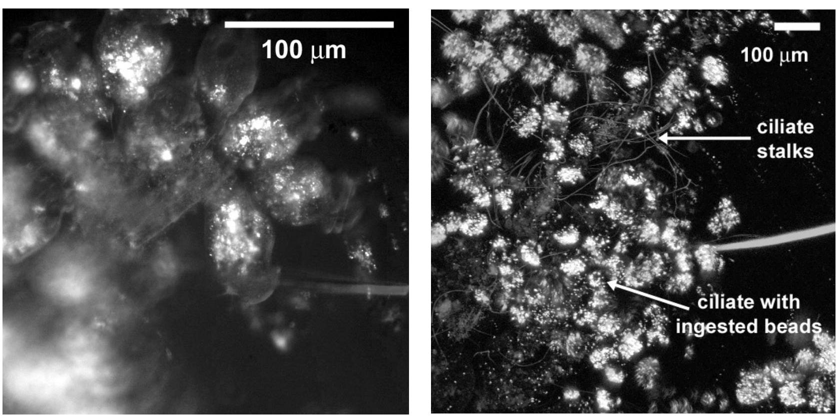

Figure 4 Peritrich ciliates covering a granule surface after incubation with nucleic acid staining (Syto 60) and ingestion of fluorescent particles (phase contrast-/epifluorescence-microphotograph (left); 3D-CLSM-projection, z-stack: 100 images, distance 10 μm (right))

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