Comparison of some characteristics of aerobic granules and sludge flocs from sequencing batch reactors

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Abstract Physical, chemical and biological characteristics were investigated for aerobic granules and sludge flocs from three laboratory-scale sequencing batch reactors (SBRs). One reactor was operated as normal SBR (N-SBR) and two reactors were operated as granular SBRs (G-SBR1 and G-SBR2). G-SBR1 was inoculated with activated sludge and G-SBR2 with granules from the municipal wastewater plant in Garching (Germany). The following major parameters and functions were measured and compared between the three reactors: morphology, settling velocity, specific gravity (SG), sludge volume index (SVI), specific oxygen uptake rate (SOUR), distribution of the volume fraction of extracellular polymeric substances (EPS) and bacteria, organic carbon and nitrogen removal. Compared with sludge flocs, granular sludge had excellent settling properties, good solid–liquid separation, high biomass concentration, simultaneous nitrification and denitrification. Aerobic granular sludge does not have a higher microbial activity and there are some problems including higher effluent suspended solids, lower ratio of VSS/SS and no nitrification at the beginning of cultivation. Measurement with CLSM and additional image analysis showed that EPS glycoconjugates build one main fraction inside the granules. The aerobic granules from G-SBR1 prove to be heavier, smaller and have a higher microbial activity compared with G-SBR2. Furthermore, the granules were more compact, with lower SVI and less filamentous bacteria.

Keywords Aerobic granules; biofilm; sequencing batch reactor; sludge flocs

Introduction

Aerobic granulation is an attractive biotechnology for wastewater treatment and has been commonly reported since the late 1990s (Morgenroth *et al.*, 1997; Beun *et al.*, 1999; Peng *et al.*, 1999). The researchers intended to demonstrate that granular sludge has high settling velocities leading to good solid–liquid separation, high biomass retention, high activity and an ability to withstand high loading rates (Tay *et al.*, 2001, 2004; Arrojo *et al.*, 2004; Schwarzenbeck *et al.*, 2004; Zheng *et al.*, 2005). A large number of parameters were investigated experimentally that represent characteristics of aerobic granules. These parameters include settling velocity, density, specific gravity (SG), sludge volume index (SVI), specific oxygen uptake rate (SOUR), extracellular polymeric substances (EPS), organic removal and heavy metal adsorption (Beun *et al.*, 1999; Etterer and Wilderer, 2001; McSwain *et al.*, 2004, 2005). However, little information can be found on direct comparative studies between characteristics of aerobic granule and sludge flocs under similar reactor conditions. Some conclusions were drawn only from the aerobic granular sludge system.

The purpose of this research is to compare physical, chemical and biological characteristics of aerobic granules and sludge flocs out of sequencing batch reactors (SBRs). The SBRs were operated in the same way.

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Materials and methods

Experimental setup

Three aerobic sequencing batch reactors were used for the experiments. The cylindrical reactors had a working volume of 4.2 L and a diameter of 9 cm. The influent entered through the top of the reactors, and the effluents were drawn at a volume of 2 L. The reactors operated with the same parameters, for example substrate type, influent time, aeration time, airflow rate and discharge time. The difference in reactor operation was the settling time: 30 minutes in the normal sequencing batch reactor (N-SBR); and 1 minute in the granular sequencing batch reactors (G-SBR1 and G-SBR2). The very short settling time of 1 minute for the G-SBR was applied to separate the granules from the flocs. Synthetic wastewater with 850–1100 mg/L COD was supplied through the influent. The seed sludge was taken from the aeration tank in the Garching Municipal Wastewater Treatment Plant near Munich, Germany. The N-SBR and G-SBR1 were inoculated with activated sludge, but G-SBR2 was inoculated with granules or small particles that could be sieved, with a size from 0.5 to 1.4 mm, in the activated sludge. The synthetic wastewater was composed as follows: CH_3 COONa 1,500 mg/L, NH_4Cl 400 mg/L, $K_2HPO_4 \cdot 3H_2O$ 21 mg/L, $FeSO_4 \cdot 7H_2O$ 10 mg/L, $MgSO_4 \cdot 7H_2O$ 12 mg/L, $CaCl_2 \cdot 2H_2O$ 14 mg/L and trace solution 1 mL/L. The composition of the trace solution was: H_3BO_3 100 mg/L, $CoCl_2 \cdot 6H_2O$ 100 mg/L, $CuSO_4 \cdot 5H_2O$ 30 mg/L, $FeCl_3 \cdot 6H_2O$ 1,000 mg/L, $MnCl_2 \cdot 2H_2O$ 110 mg/L, $Na_2Mo_7O_{24} \cdot 2H_2O$ 70 mg/L, $ZnSO_4 \cdot 7H_2O$ 100 mg/L, KI 30 mg/L, $NiCl_2$ 60 mg/L.

Table 1 displays the operational parameters for 4 hours per cycle. All reactors operated six cycles per day. The mean volumetric organic load was 4.2–5.5 kgCOD/m^3.d and the ammonium load was 225 gNH_4-N/m^3.d.

The airflow rate was 300 L/h, which results in an upflow air velocity of 1.3 cm/sec in each column. The temperature of operation was kept at approximately 17–19 °C. The sludge concentrations were 2.3–3.6 g/L, 6.2–10.7 g/L and 4.5–6.6 g/L in the N-SBR, G-SBR1 and G-SBR2, respectively. Excess sludge was removed from the N-SBR.

Analytical methods

Sludge volume index (SVI), mixed liquid suspended solid (MLSS), mixed liquid volatile suspended solid (MLVSS), specific oxygen uptake rate (SOUR) and specific gravity (SG) were measured according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). TOC and nitrogen were measured according to Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (DEV, 2004). COD and TP were measured using Dr. Lange test kits (Dr. Lange GmbH) following instructions for the colorimetric standard method. Granule size, distribution of diameters and roundness were analysed using Leica Digital optical Microscope and Image-Pro Plus. Settling velocity of flocs and single granules was measured in a cylinder (80 cm height) filled with tap water.

CLSM images were recorded using a TCS SP confocal laser scanning microscope (Leica, Germany). Biofilm bacteria were stained using the SYTO 60 nucleic acid stain (Molecular Probes, Eugene, OR, USA). The stock solution of SYTO 60 as supplied was used at a dilution of 1:1,000 in deionised water. Glycoconjugates of biofilms were stained with the *Aleuria aurantia* lectin (Vector, Burlingame, CA, USA) conjugated to Alexa488

Table 1 Operational parameters in one cycle

Parameter	Influent	Aeration	Settling	Discharge	Idle	Total time
N-SBR	30 min	150 min	30 min	6 min	53 min	240 min
G-SBR1, G-SBR2	30 min	150 min	1 min	6 min	24 min	240 min

(Molecular Probes) according to a screening of all commercially available lectins on the same type of biofilms (Staudt *et al.*, 2002). Detailed information on the methods can be found elsewhere (Neu and Lawrence, 2002, 2005; Staudt *et al.*, 2004).

Results and discussion

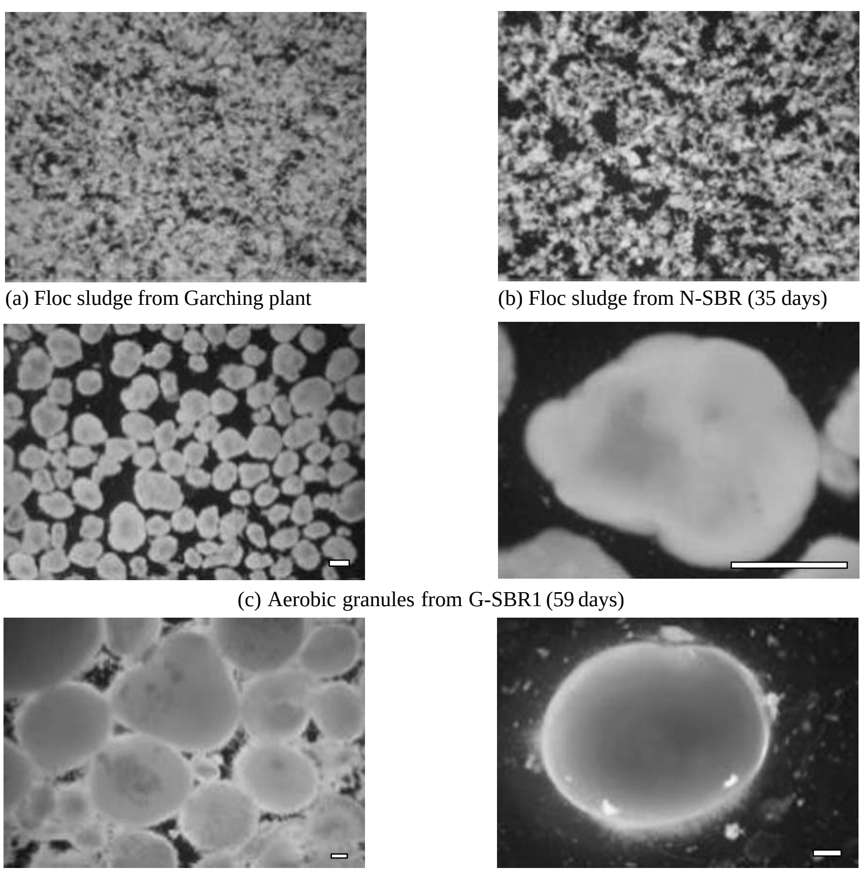
Morphology

The microscopic examination shown in Figure 1 presents different types of morphology that were found in the reactors. The inoculum from Garching municipal wastewater treatment plant was black-brown. The colour of flocs gradually became yellow-brown in N-SBR. Granules from G-SBR1 became yellow-brown and white-brown in G-SBR2. The maximum diameters of granules were 4.1 mm in G-SBR1 and 18.3 mm in G-SBR2.

The distribution of granule diameters is displayed in Figure 2. The average roundness of granules was 1.32 in G-SBR1 and 1.20 in G-SBR2, which means the granules in G-SBR2 are more round than the granules in G-SBR1 (see also Figure 1(c) and (d)).

Separation of sludge from treated water

An efficient separation of microbial sludge from treated water is one of the major criteria by which the wastewater treatment system is judged. Figure 3 displays the specific gravities for various concentrations of sludge from Garching wastewater treatment plant (G-plant), N-SBR, G-SBR1 and G-SBR2. The results show that granules from G-SBR1



(a) Floc sludge from Garching plant

(b) Floc sludge from N-SBR (35 days)

(c) Aerobic granules from G-SBR1 (59 days)

(d) Aerobic granules from G-SBR2 (65 days)

Figure 1 Microscopic examination of granules and sludge flocs (size of bar = 1 mm)

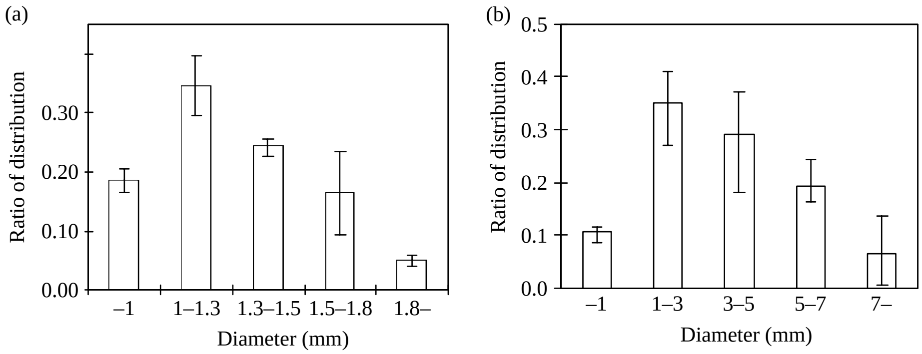


Figure 2 Distribution of granule diameter (after 60 days) in (a) G-SBR1 and (b) G-SBR2

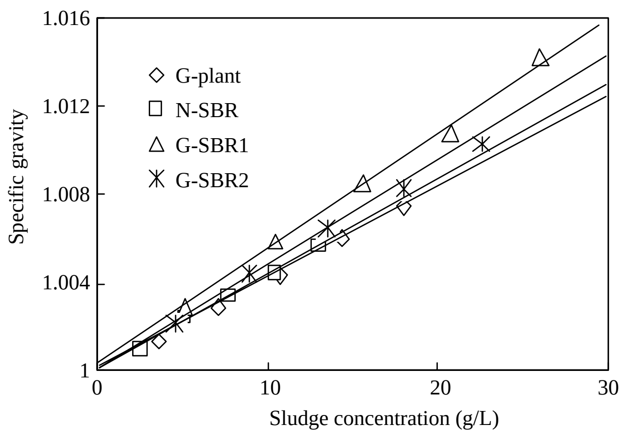


Figure 3 Specific gravity of granules and sludge flocs

are the heaviest followed by granules from G-SBR2, the flocs from the N-SBR and G-plant.

Sludge flocs from N-SBR were tested for the maximum floc particle settling velocity with wide variation from 4 to 51 m/h, while some floc particles hardly settled down. The settling velocity of the granules from G-SBR1 ranged from 37.4 to 89.7 m/h. Figure 4

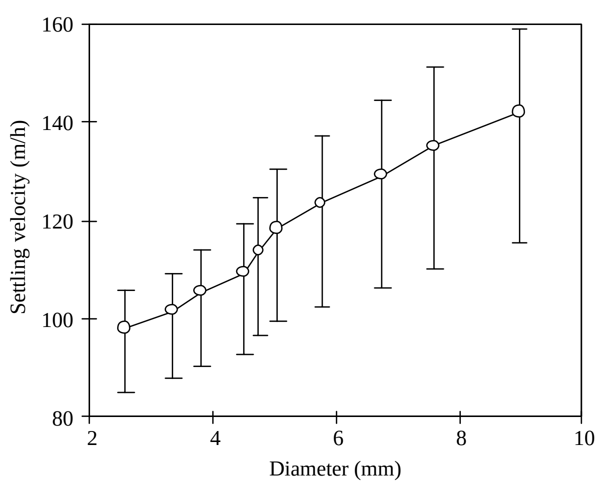


Figure 4 Settling velocity of granules from G-SBR2

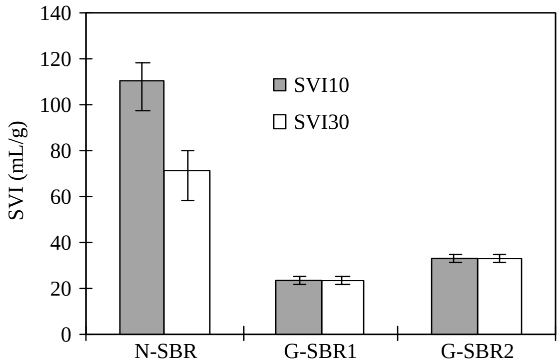


Figure 5 SVI of granules and sludge flocs

displays the settling velocity of granules from G-SBR2. According to the highest diameter in this reactor the granules had the highest settling velocities, which rose up to 140 m/h with increasing diameter.

Figure 5 displays the SVI_{10} (10 min settling) and SVI_{30} (30 min settling) of sludge flocs and granules from all three reactors. The graph apparently shows excellent settling ability of the granular sludge. It took less than 10 minutes to have completely separated most of the granular biomass from water. Figure 6 displays the effluent suspended solid of the reactors during the stable operation. The effluent SS from the granule reactors (G-SBR1 and 2) were high due to a short settling time. The highest concentration of suspended solids (SS) was found in the effluent of G-SBR1. This can be explained with the highest granular sludge concentration, which ranged from 8.1 to 10.7 g/L.

The results demonstrate that granular sludge had excellent solid–liquid separation due to density, large size, compactness and roundness. However, the very short settling time caused more SS in the effluent.

Activity of sludge

The microbial activity usually can be estimated by the specific oxygen uptake rate (SOUR). It is usually assumed that the SOUR of aerobic granular sludge is higher compared with normal activated sludge. Nevertheless, Figure 7 shows that the SOUR of sludge flocs from N-SBR after 60 days was similar to SOUR of granules from reactor G-SBR1. Both were higher than the SOUR of granules from G-SBR2.

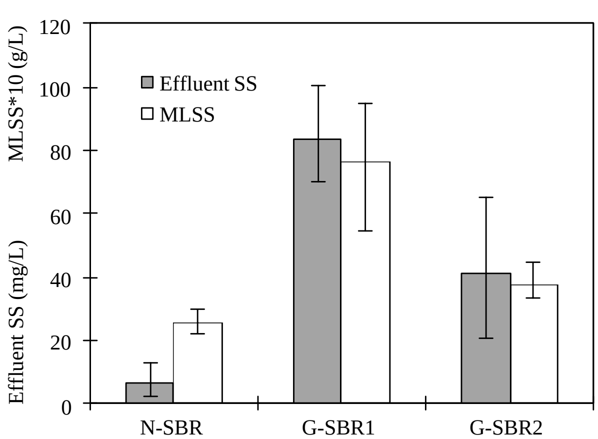


Figure 6 Effluent suspended solid of reactors

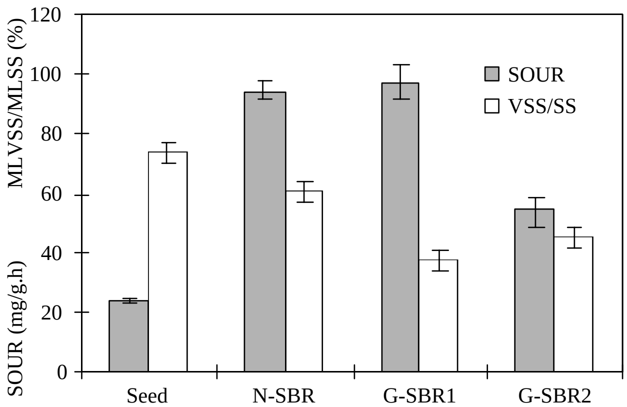


Figure 7 SOUR of granules and sludge flocs after 60 days of cultivation

The seed sludge from Garching municipal wastewater treatment plant had the lowest SOUR, but its sludge contained the highest ratio of VSS. The experimental results show an increasing inert inorganic volume fraction inside the granules compared with the inoculum material (seed) during the first 60 days. At 112 days (see Figure 8) of cultivation the ratio of VSS/SS in the granular sludge had increased to 80%, but the SOUR decreased.

One reason for the discrepancy between increasing organic biomass (measured as VSS) and decreasing SOUR of the granules may be the mean diameter of the granules, which is above 2 mm in G-SBR1 and above 3 mm in G-SBR2. Only the outer 200–250 μm and thereby only around about one half of the granules are supplied with oxygen.

COD and N removal

Additional to the batch experiments for specific oxygen uptake rate, COD removal was measured directly in the SBRs during aeration. Therefore, the sludge concentrations remained similar with 2,712, 2,884 and 2,898 mg/L in the N-SBR, G-SBR1 and G-SBR2, respectively. Figure 9 shows the time course of COD concentration in all three reactors. The highest COD removal rate was found in N-SBR, followed by G-SBR1 and G-SBR2. The COD removal rates measured were 36, 24 and 14.4 kgCOD/kgMLSS.d. These results correspond to the SOUR measurement and depend mainly on diffusion limitation inside the granules. It can be seen quite clearly that the flocs are more efficient than granules at

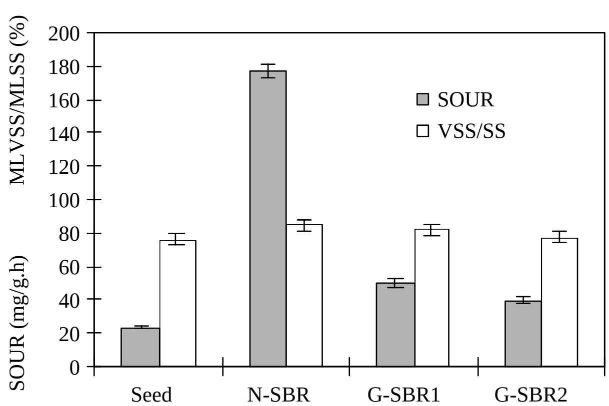


Figure 8 SOUR of granules and sludge flocs after 112 days of cultivation

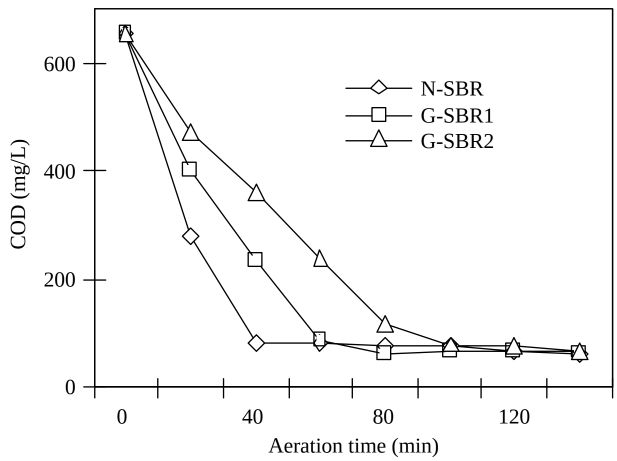


Figure 9 COD removal of granules and sludge flocs

similar biomass concentrations. However, higher sludge concentrations can be operated in the granule system. For example, there was approximately 10 g/L biomass in G-SBR1.

During the first 60 days of operation, a complete nitrification was only detected in N-SBR, but almost no nitrification was found in G-SBR1 and G-SBR2. After about 60 days nitrification and also simultaneous denitrification (40%) was established in G-SBR1 and G-SBR2 at high oxygen concentrations of 8 mg/L. Figure 10 shows the removal efficiency for all three reactors before 60 days of operation and after 112 days. The NH_4-N- load was around about 225 g/m^3.d.

Distribution of EPS and bacteria

Staining with fluorescent labelled lectin for EPS glycoconjugates and with Syto 60 for bacteria has been carried out to compare the distribution of these two components in flocs and granules. Figure 11(a) shows a CLSM image from both, flocs and a small granule from reactor N-SBR. In Figure 11(a), the granule (No. 1) consisted mainly of bacteria, and the penetration depth of the laser is less than 100 μm due to the high density. In comparison, for flocs (No. 2) the volumetric ratio of EPS/bacteria was 2:1. Figure 11(b) displays a compact granule from G-SBR1 with a diameter of approximately 500 μm. The granule is very compact having a ratio EPS/bacteria of 1:1. The inner part of a granule from G-SBR2 (Figure 11(c)) representing a mosaic scan (12 × 2) is dominated by filamentous bacteria. The CLSM image has been recorded after manual sectioning,

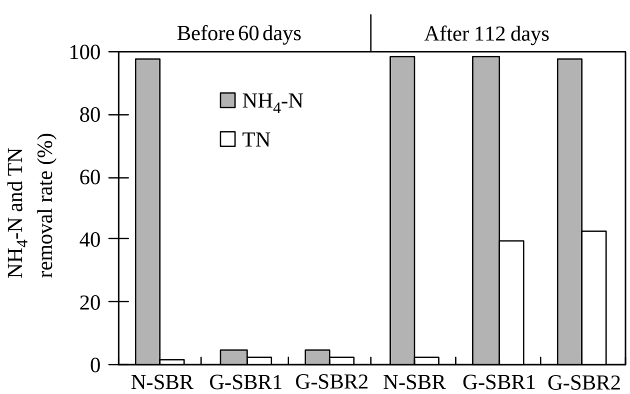


Figure 10 Nitrification and denitrification of granules and sludge flocs

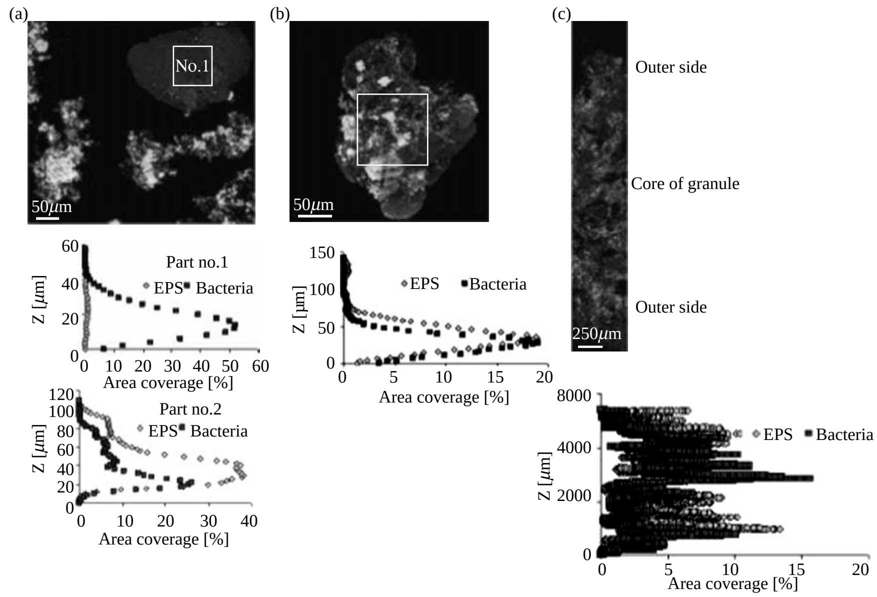


Figure 11 Distribution of extracellular polymeric substance (EPS glycoconjugates = light grey) and bacteria (Syto 60 = dark grey) in flocs and granules: (a) N-SBR, (b) G-SBR1 and (c) G-SBR2. Quantification in (a) and (b) was carried out in the selected areas indicated in the images

which is necessary to display the EPS/bacteria distribution over the entire diameter of the granule. The CLSM images in combination with the chemical data help to understand the structure and function of granules in comparison to floc systems.

Conclusions

Compared with sludge flocs, granular sludge had excellent characteristics, for example good settling properties, high biomass concentration, simultaneous nitrification and denitrification. The aerobic granular sludge did not have a higher microbial activity, which can be clearly shown with batch experiments and specific oxygen uptake rates. Furthermore, the granule reactors have problems with respect to higher effluent suspended solids. The inert inorganic fraction is also higher compared with flocs, which can be explained by starvation and inactivation processes inside the granules. The CLSM technique may help to throw more light on these processes. Therefore, more images have to be taken over a longer cultivation time.

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