ARCHIVESOFENVIRONMENTALPROTECTIONvol. 35no. 2pp. 41 - 522009

PL ISSN 0324-8461

© Copyright by Institute of Environmental Engineering of the Polish Academy of Sciences, Zabrze, Poland 2009

CHARACTERISTIC OF GRANULATED ACTIVATED SLUDGE FED WITH GLYCERIN FRACTION FROM BIODIESEL PRODUCTION

AGNIESZKA CYDZIK-KWIATKOWSKA^{1*}, ANDRZEJ BIAŁOWIEC¹, IRENA WOJNOWSKA-BARYŁA¹, LECH SMOCZYŃSKI²

¹University of Warmia and Mazury in Olsztyn, Department of Environmental Biotechnology ul. Słoneczna 45G, 10-957 Olsztyn-Kortowo, Poland ²University of Warmia and Mazury in Olsztyn, Department of Chemistry pl. Łódzki 4 10-957 Olsztyn-Kortowo, Poland ^{*}Corresponding author e-mail: agnieszka.cydzik@uwm.edu.pl

Keywords: Glycerin fraction from biodiesel production, granulated activated sludge, free settling test, waste treatment.

Abstract: In the presented research glycerin fraction from biodiesel industry was used for granulated aerobic activated sludge production in a typical sequencing batch reactor (h/d equal 2.1). After 7 weeks of operation, granulated activated sludge with SVI at the level of 40–50 cm³·g⁻¹ was obtained. At organic compounds load of 1.43 ± 0.1 mg COD·mg VSS⁻¹·d⁻¹, the efficiency of carbon removal was $94.14 \pm 2.7\%$ and most of the introduced COD was removed during the first 2–3 hours of aeration. The sieve analysis revealed that 60% (w/w) of biomass consisted of particles with a diameter in the range of 4–8 mm. A free settling test procedure proved that granules with a diameter between 2–4 mm were numerically most abundant in biomass (32.3%) and that the settling volume, mass and Reynolds number values significantly (p < 0.05) increased parallel with increasing granule diameter. Adverse tendency was observed for the mean effective, buoyant density of a granule in a liquid.

INTRODUCTION

Systems with immobilized biomass are widely used for treating wastewater because of a resistance to environmental factors and high concentration of pollutants. In recent years, attention was focused on a new form of immobilization that is biogranulation. This process involves cell-to-cell interactions resulting in formation of dense microbial consortia – granules. The main advantages of granulated activated sludge are very good settling ability, long biomass retention time and biomass concentration as well as ability to withstand high organic loading rate [15]. From engineering perspective employing technologies based on granulated activated sludge results in smaller dimensions of clarifiers, shorter standstill for settling and more time for biological removal of pollutants.

Most of the research on biogranulation is carried out in reactors with h/d ratio of 10 or more [5, 27, 28]. The literature data indicate that in order to obtain aerobic granules different organic substrates, like glucose, acetate [18], phenol [11], dairy effluents [22] or wastewater, were used as the carbon source [3, 9]. It seems especially advantageous to use organic wastes form different branches of economy for granulated activated sludge production.

Present trends in fuel market encourage the production of biodiesel. Biodiesel is the name of a clean burning alternative fuel, produced from domestic, renewable resources [8]. Its production can be carried out in both large factories and small installations for a farmer own use. It is made through a chemical process called transesterification whereby the glycerin is separated from the fat or vegetable oil. Unfortunately, the process leaves behind other byproducts than methyl esters (the chemical name for biodiesel) that is so called glycerin fraction. Glycerin fraction consists of glycerin (about 30–75%), methanol (5–20%), mono- and diglycerides, free fatty acids, phospholipids, water, soaps etc. – their percentage participation depends on the substrate and parameters of transesterification process [17].

In large factories a glycerin fraction is purified and can be further processed, in the case of production for farmers own use, however, chemical purification of contaminated glycerin is unprofitable and there is a risk that the glycerin fraction will be treated as waste. Because of a high load of organic compounds, contaminated glycerin may cause environmental pollution or disturb wastewater treatment plant functioning. To partially resolve this problem the glycerin fraction can be used as a carbon source for granulated activated sludge production. Granulated activated sludge is a promising technology for decentralized wastewater treatment [14] and can be used, for example, in household sewage treatment plants.

The aim of the presented research was to investigate possibilities of forming of aerobic granulated activated sludge in the reactor with h/d of 2.1 fed with glycerin fraction from biodiesel production. This research, according to our knowledge, is the first time when granulated activated sludge was obtained on the base of this substrate. In order to characterize the obtained biomass, free settling tests and wet sieving analysis were employed. Employment of a typical SBR reactor and easily available organic substrate enhances a utilitarian potential of presented technology of granulated activated sludge production.

MATERIALS AND METHODS

SBR operation

The experiment was carried out for 130 days. In the experiment a 8.0 dm³ sequencing batch reactor with a height of 36 cm and 17 cm diameter was employed (h/d = 2.1). Seed sludge was collected from a conventional municipal wastewater treatment plant in Olsztyn (Poland). The glycerin fraction (423 g COD·dm⁻³, oil and grease 77 g·dm⁻³, total nitrogen 0.98 g N·dm⁻³) were obtained from a farmer biodiesel installation in Lubomino (Poland). The temperature in the experiment room was ambient (varying from 18 to 21°C) and pH in reactor was kept between 7 and 8. The reactor was supplied with a constant air velocity of 8 dm³·min⁻¹, and wastewater exchange ratio of 75% was employed. The SBR operated in 12-hour cycle, with the following operating strategy: aeration (705 min), settling (5 min) decantation (5 min) and filling (5 min). During the filling period 4 dm³ of artificial wastewater and 2 dm³ of mixture of glycerin fraction and tap water were added to the reactor to make the final working volume of 8 dm³. The artificial wastewater was composed of NH₄Cl (76.1 mg·dm⁻³), Na₂HPO₄·12H₂O (46.0 mg·dm⁻³), NaCl (10.1 mg·dm⁻³), KCl (4.7 mg·dm⁻³), CaCl₂ (3.5 mg·dm⁻³), MgSO₄·7H₂O (16.7 mg·dm⁻³), NaHCO₃ (243.2 mg·dm⁻³), Na₂CO₃ (162.2 mg·dm⁻³), FeCl₃·6H₂O, MnSO₄·H₂O, ZnSO₄,

CuSO₄·5H₂O (0.2 mg·dm⁻³) [modified 7]. The glycerin fraction was added in amount that led to mean COD concentration in the reactor at the beginning of aeration phase of 0.522 \pm 0.11 g COD·dm⁻³.

Analytical measurements

Sampling was made at the influent and effluent of the reactor. All samples were filtered using 0.2 μ m micro-pore filter before being assayed. Oil and grease in glycerin fraction were measured using Soxhlet extractor and total nitrogen was measured by distillation method. Chemical oxygen demand, total suspended solids, volatile suspended solids and sludge volume index were measured according to Standard Methods [2]. On the 114 and 120 day of the experiment, samples were taken every hour during SBR cycle to describe COD and TSS concentration changes in the reactor.

Activated sludge characteristic

Respirometric measurements were performed in duplicate using OxiTop® System (WTW, Germany). Samples of granulated activated sludge were taken after 120 days of reactor operation at the end of SBR cycle. The biomass was washed in duplicate with phosphate buffer then with distilled water, and the TSS was measured. The procedure was conducted as previously described [4].

The particle size selection of granulated activated sludge was done according to commonly used particle size analysis of soil [21]. A wet sieving technique using Retsch AS 200 Sieve Machine was employed with the following sieve size classes: 0.25, 0.5, 1, 2, 4, and 8 mm. For sieving, 750 cm³ of granulated activated sludge was taken. The sieving process was supported by tap water (temperature 12°C) from the spray nozzle which was located above the uppermost sieve. The water left the sieve stack together with the last fraction through the outlet in the collector. Rinsing was carried out until the liquid leaving the sieve stack outlet was no longer turbid with solid particles. In this experiment sieving lasted for 5 minutes and an amplitude of vibration was 1.5 mm.

In order to examine physical properties of granulated activated sludge and separated granule classes the free settling test procedure described by [23] was used. Granulated activated sludge sample of 2 cm³ was taken from the reactor and placed in a column filled with tap water (temperature 12°C). A floc settling was photographed in the dark room. The settling flocs were illuminated with photo-flash-lamp controlled by an electronic system ensuring flashes every 3 seconds. The digital camera (Canon G5, resolution 5 Mpic.) shutter was kept open for 5 flashes for every photo. Four photos were taken during 15 to 20 min of settling, before the next sludge sample was introduced. In the experimental run 24 photos were taken. During the experiment 573 granules were examined. The equivalent diameter, granule radius and settling velocity of granules were measured using the software UTHSCSA Image Tool version 3.0. According to the Stoke's law the following physical parameters were determined: density of granules, effective, buoyant density of aggregate in liquid, granule volume, granule mass, Reynolds number, and fractal dimension of granule. For calculations it was provided that the granules were impermeable and spherical. Water density and viscosity values were presumed for 12°C and made 0.9995 g·cm⁻³ and 1.230·10⁻³ kg·m⁻¹·s⁻¹ respectively.

According to Stoke's law the granules density may be expressed as:

$$\rho_f = \rho_w + \frac{9V\eta}{2r^2g} \tag{1}$$

This is the real density of a granule containing aggregated microorganisms and water. The effective, buoyant density of an aggregate in liquid (excluding water) may be expressed as:

$$\rho_e = \rho_f - \rho_w \tag{2}$$

The granule volume was calculated on the basis of the equation for the volume of the sphere. The granule mass was calculated as:

$$m = V_f \cdot \rho_e \tag{3}$$

The Reynolds number of granules settling was determined on the basis of the following equation:

$$\operatorname{Re} = \frac{2 \cdot \mathcal{V} \cdot r \cdot \rho_{w}}{\eta} \tag{4}$$

An important parameter that characterizes a fractal object is the fractal dimension, which corresponds to the space-filling capacity of an object. The mass of a fractal object with fractal dimension ranging $1 \le D \le 3$ can be considered to be proportional to its size [29]. With known granule density, granule mass, and with known dependence for fractal objects:

$$m \alpha r^{D}$$
 (5)

provided that the granule is spherical, (m) may be expressed as:

$$m = \frac{4 \cdot \pi \cdot r^3}{3} \cdot \rho_e \tag{6}$$

SO

$$r^{D} = \frac{4 \cdot \pi \cdot r^{3}}{3} \cdot \rho_{e} \tag{7}$$

hence

and

$$\rho_e \alpha r^{D-3} \tag{8}$$

 $\log \rho_e \alpha \left(D - 3 \right) \cdot \log r \tag{9}$

(D-3) means the slope of the log-log line; therefore the fractal dimension may be obtained as:

$$D = 3 + \frac{\Delta \log \rho_e}{\Delta \log r} \tag{10}$$

Statistical analyses

The analysis of variance between mean values of estimated parameters of granules was carried out with the use of ANOVA test at the significance level of p < 0.05.

The normality of the distribution was confirmed by Szapiro-Wilk's test, whereas the hypothesis of the homogeneity of variances across the groups was verified on the basis of Levene's test. In the text after symbol \pm standard deviation was given.

RESULTS

In the presented experiment SVI value of the seed sludge was 103 cm³·g⁻¹. After a startup of the experiment this value gradually decreased parallel with the growth of granules

and after about 80 cycles (40 days) of reactor operation it stabilized at the level of $40-50 \text{ cm}^3 \cdot \text{g}^{-1}$ (Fig. 1A). Small granules with a diameter of about 2 mm were observed for the first time after 70 cycles, after 80 cycles of reactor operation a well shaped granulated activated sludge was obtained in the reactor (Fig. 1B).



Fig. 1. Granulated activated sludge fed with glycerin fraction A) sludge volume index changes during the experiment, B) mature granules after 7 weeks of reactor operation

Chemical analyses were started from cycle 50. During investigation period the mean cellular residence time (sludge age) averaged 2.2 ± 0.4 day. At mean organic compounds load of 1.43 ± 0.1 mg COD·mg VSS⁻¹·d⁻¹, the removal of COD during the experiment averaged $94.14 \pm 2.7\%$ (Fig. 2A) and chemical oxygen demand in the effluent did not exceed 124 mg COD·dm⁻³. COD concentration changes during the SBR cycle (mean values from cycles 114 and 120) are presented in Figure 2B. After wastewater addition, COD concentration in the reactor was 450 ± 19 mg O₂·dm⁻³, during the first 3 hours it rapidly decreased to about 40 mg COD·dm⁻³ and remained at this level to the end of SBR cycle. Biomass increased according to 0-order reaction (data not shown) and the mean growth



Fig. 2. COD concentration changes A) in the reactor from day 50 to the end of the experiment, B) during the SBR cycle (mean values are given with standard deviation bars)

yield of microorganisms was 0.41 ± 0.23 mg TSS·mg⁻¹ COD. Respirometric measurements showed that oxygen uptake for endogenous respiration was at the level of 11.4 ± 5.8 mg O₂·g VSS⁻¹·h⁻¹ and no oxygen depletion for nitrification was observed. The oxygen depletion for substrates oxidation was, however, high and averaged 69.5 ± 6.5 mg O₂·g VSS⁻¹·h⁻¹.

In order to characterize the obtained biomass, the sieve analysis and free settling tests were employed. The sieve analysis showed that more than 60% (w/w) of biomass consisted of particles with the diameter in the range of 4–8 mm. Granules with diameters 2–4 mm and > 8 mm had also significant contribution in total biomass – 10.7 and 18.4% (w/w), respectively (Fig. 3). On the basis of the cumulative curve of granules size distribution it was calculated that 80% (w/w) of all granules had diameter in the range from 2.1 to 9.8 mm.



Fig. 3. The granule size distribution in granulated activated sludge

The analysis of granulated activated sludge digital photos allowed for detailed characterization of granulated activated sludge as a whole and granules in particular size classes (Tab. 1). The granulated activated sludge was characterized by a mean settling velocity of $3.26 \pm 2.19 \text{ mm}\cdot\text{s}^{-1}$, effective, buoyant density of $3.86 \pm 5.64 \text{ mg}\cdot\text{cm}^{-3}$ and granule equivalent diameter of $2.71 \pm 2.14 \text{ mm}$. In general, the settling of granulated activated sludge had laminar character because Reynolds number value was below 2300. The ratio of settling velocity to granule diameter (V/R) equaled 1.79 and confirmed the good settling properties of granulated activated sludge. The mean real density of granules containing aggregated microorganisms and water was on the level of 1.0034 ± 0.0056 g·cm⁻³, and was higher than the density of water in the same temperature (12° C). The fractal dimension calculated for granulated activated sludge was 1.459, with r² of $\log(\rho_e)$ - $\log(r)$ equal 0.768 (Tab. 1.).

Parameters		Diameter range of granules [mm]						Total in
of granulated activated sludge		0.25-0.5	0.5–1.0	1.0-2.0	2.0-4.0	4.0-8.0	> 8.0	reactor
R	[mm]	0.37	0.69	1.45	2.90	5.47	9.66	2.71
		± 0.05	± 0.15	± 0.30	± 0.50	± 0.99	± 2.48	± 2.14
V	[mm·s ^{·1}]	0.75	1.99	4.17	2.86	4.13	5.47	3.26
		± 0.38	± 1.41	± 2.42	± 1.79	± 1.90	± 1.23	± 2.19
V/R	[S ⁻¹]	2.08	2.75	2.97	1.03	0.78	0.61	1.79
		± 1.27	± 1.63	± 1.73	± 0.74	± 0.40	± 0.22	± 1.53
Re	-	0.44	2.41	9.72	13.05	35.92	84.68	16.12
		± 0.21	± 2.14	± 6.18	± 7.87	± 18.08	± 30.47	± 19.48
ρ	[mg·cm ^{·3}]	13.66	9.08	5.01	0.88	0.35	0.16	3.86
		± 10.63	± 5.21	± 3.23	± 075.	± 0.22	± 0.10	± 5.64
ρ	[g·cm ⁻³]	1.0132	1.0086	1.0045	1.0004	0.9998	0.9997	1.0034
		± 0.0106	± 0.0052	± 0.0032	± 0.0018	± 0.0002	± 0.0001	± 0.0056
V _r	[cm ³]	0.0002	0.0016	0.0144	0.1109	0.7560	4.4971	0.3080
		± 0.0001	± 0.0011	± 0.0085	± 0.0551	± 0.4327	± 3.4726	± 0.9917
m	[mg]	0.003	0.014	0.058	0.078	0.216	0.508	0.097
		± 0.001	± 0.013	± 0.037	± 0.047	± 0.109	± 0.183	± 0.117
N	-	47	63	159	185	102	17	573

Tab. 1. The physical parameters of examined activated sludge (after symbol \pm standard deviation is given, ANOVA test proved significant (p < 0.05) differences between granules classes for all measured parameters)

Wet sieving procedure categorized granules in 6 size classes. Statistical analysis proved significant (p < 0.05) differences of all measured parameters between the classes. Granules with the diameter between 2–4 mm were numerically most abundant in biomass and consisted in 32.3% of all granules (Tab. 1). It was observed that V, V_p, m and Re values significantly (p < 0.05) increased parallel with increasing granules diameter. The granules with diameter below 2 mm, weighted less than 0.058 ± 0.037 mg while larger granules with diameter exceeding 2 mm had mass in the range from 0.078 ± 0.047 to 0.508 ± 0.183 mg. Adverse tendency was observed for the mean effective, buoyant density of an aggregate in a liquid (ρ_e) (Tab. 1). For small granules (0.25 < R < 2 mm) it was in range between 5.01 ± 3.23 and 13.66 ± 10.63 mg·cm⁻³, but larger granules with R higher than 2 mm had relatively small ρ_e ranging from 0.13 ± 0.1 to 0.88 ± 0.08 mg·cm⁻³.

An additional experiment proved that a twofold increase in glycerin fraction load resulted in disintegration of the granules in the reactor in given technological conditions. The obtained biomass was also stored for 4 weeks at 4°C in the effluent wastewater without oxygen and substrate supply. After 4 weeks most of the granules preserved their structural integrity.

DISCUSSION

The major selection pressures responsible for aerobic granulation are settling time and wastewater exchange ratio. On the basis of literature data in the presented experiment settling time of 5 minutes and wastewater exchange ratio of 75% were employed [16, 20]. The transition from flocculated sludge to sludge with low SVI (40–50 cm³·g⁻¹) typical

for granulated activated sludge [27, 28] proved that the chosen strategy was successful. Moreover, only granules with diameter between 0.25–0.5 mm had Re below 1, characteristic for activated sludge flocs [12]. Remaining granules classes had Re value higher than 1 and this fact additionally confirmed granulation of activated sludge.

In the presented research wet sieving technique and free settling tests were employed for granules characteristics. Because of their simplicity and low testing cost, the free-settling tests are the most widely described method in literature for measuring the flocs basic physical properties [6]. From practical point of view, free settling tests may be useful for technological description of granulated activated sludge properties. Providing that highly compacted flocs (granules) are impermeable, this method allows for determining settling velocity, mass, volume and fractal dimension of granules.

Reynolds number values pointed out that the sludge settling in the reactor had laminar character and SVI changes in time showed that settle ability improved as the granulation progressed. Presuming that granules are characterized by Re > 1 [12], 2.5% (w/w) of biomass in the reactor (a sum of masses of particles with diameter below 0.5) were flocs. Free settling tests results indicate that granules with Re > 1 had equivalent diameter higher than 0.5 mm and minimal settling velocity of about 2 mm·s⁻¹. On the basis of these results it can be concluded that in order to separate the small fraction of flocs from granules in employed reactor, sedimentation period should be shortened to about 1–1.5 minute, so that only particles with settling velocity equal or higher than 2 mm·s⁻¹ would be separated.

Settling velocity of microbiological aggregates depends on the geometric parameters of these particles as well as their density and porosity. Fractal dimension of an object is a quantitative measure of how the primary particles occupy the floc interior space. Li and Ganczarczyk [13] proved that aggregates generated in water and wastewater treatment processes exhibit a fractal dimension between 1.4 and 2.8. The values close to 1.4 are representative for flocs with smooth surface, while 2.8 is typical for very wrinkled and porous flocs [12]. The low value of fractal dimension indicates that granules obtained in the experiment were compact and relatively smooth in comparison with typical flocs. Our experimental data also point to a very important consequence of the fractal, self-similar nature of the aggregates, i.e., their density noticeable decrease with an increasing size.

A wet sieving technique can be used for activated sludge characteristic of both aerobic [26] and anaerobic granulated activated sludge [19]. Presuming that the smallest floc is made of two microcolonies (125 μ m) [25], we decided to use the smallest sieve with the holes of 250 um diameter. Usually microbial flocs formed in a conventional wastewater biological treatment are loose aggregates with undefined shape and a size from 0.05 to 0.2 mm [10]. The average diameter of aerobic granules varies in the range from 0.2 to 5 mm and is mainly due to a balance between growth and abrasive detachment due to strong hydrodynamic shear force in aerobic reactors [15]. In our experiment wet sieving proved that granules with diameter in the range 2-4 mm were numerically most abundant while 60% (w/w) of granulated activated sludge mass posed particles with a diameter in the range of 4-8 mm. Similar results were obtained by Wang et al. [28]. In research conducted in SBR fed with glucose as a carbon source at organic loading rate of 4.8 kg COD·m⁻³·d⁻¹ granules were dominant sludge forms with most of diameters about 6–9 mm. Authors explain that for a given organic loading rate and shearing force, the dynamic growth-decay equilibrium was maintained in the reactor. As a result, the granules existing in the reactor were matured ones with a larger size.

Despite the high organic compounds load the efficiency of carbon removal in the presented technology was high (94.14 \pm 2.7%) and most of the introduced COD was rapidly removed during the first 2–3 hours of aeration. It can be assumed that the length of the cycle could have been shortened without decrease in carbon removal efficiency. The respirometric measurements indicated that the rate of substrate oxidation by investigated granulated activated sludge was relatively high. For example, in research conducted by [1] in a plant treating mainly domestic wastewater, oxygen uptake rate varied between 26.3–38.9 mg O₂·g VSS⁻¹·h⁻¹ and was about two times lower in comparison with values obtained in the presented research. The observed high activity of microorganisms confirms a very good potential of granulated activated sludge in organic pollutants removal. The mean growth yield of microorganisms of 0.41 ± 0.23 mg TSS·mg⁻¹ COD was similar to values obtained by [26]. Authors reported that growth yield of microorganism in reactors differing in superficial air velocity changed from 0.48 to 0.33 MLSS·mg⁻¹ COD for an air velocity of 0.5 to 4 dm³·min⁻¹, respectively.

Literature data reveal that nearly 100% of aerobic and anaerobic granules are produced in column-type air or liquid up flow reactors, since a high ratio of reactor height to diameter seems to favor the formation of granular sludge [15]. It improves selection of granules by the difference in settling velocity [5] and ensures a longer circular flow trajectory which provides a more effective hydraulic attrition to microbial aggregates. The high shearing force can induce microorganisms to secrete more exopolysaccharides, which mediate cohesion and adhesion of cells and play a crucial role in maintaining the structural integrity of aerobic granules [26]. In the presented experiment granulated activated sludge was obtained in a traditional, not a column-type, SBR with h/d ratio of 2.1. This fact confirms that SBRs presently used in wastewater treatment plants can be adapted to granulated activated sludge technology. It is, however, necessary to stress that the drawback of this solution is a larger supply of air to the reactor (8 dm³·min⁻¹) in comparison with column reactors in which air supply at the level of 2.4–6 dm³·min⁻¹ is employed [11, 25, 26].

The fact that granules maintained their structural integrity despite the long storage implies that the obtained granulated activated sludge can be used when substrate availability changes with time, as it happens during biodiesel production. The fact that a 2-fold increase in glycerin fraction load resulted in disintegration of the granules can be explained by results obtained by Moy *et al.* [18]. Authors proved that after reaching organic loading rate of 9 g COD·dm⁻³·d⁻¹ (acetate addition) granules in the reactor disintegrated, and they concluded that for a given organic substrate granules formation can be successful only in a defined range of concentrations. It can be also assumed that collapsing of the granulation process after 2-fold increase in organic carbon load could have been caused by the fact that glycerin fraction has toxic influence on microorganisms caused by methanol and soaps presence.

CONCLUSIONS

It is possible to obtain aerobic granulated activated sludge feeding the traditional SBR reactor (h/d equal 2.1) with glycerin fraction from biodiesel production as a sole organic carbon source. The obtained granulated activated sludge was characterized by low SVI (40–50 cm³·g⁻¹), 60% (w/w) of granulated activated sludge constituted spherical aggre-

50 A. CYDZIK-KWIATKOWSKA, A. BIAŁOWIEC, I. WOJNOWSKA-BARYŁA, L. SMOCZYŃSKI

gates with diameter in the range of 4-8 mm. The effectiveness of COD removal averaged $94.14 \pm 2.7\%$ and most of the introduced COD was rapidly removed during the first 2-3 hours of aeration. On the basis of free settling test results it can be concluded that, for given technological parameters and reactor configuration in order to remove flocs from the granulated biomass, the time of settling should be adjusted so that only granules with settling velocity of 2 mm·s⁻¹ or higher are separated.

LIST OF ABBREVIATIONS

COD - chemical oxygen demand [mg COD·dm-3],

D – fractal dimension,

d – reactor diameter [cm],

g – the standard gravity $[m \cdot s^{-2}]$,

h – reactor height [cm],

m – mass of granule [mg],

N – number of analyzed granules,

% N – share of each granule fractions in total number of analyzed granules [%],

p - significance level,

R – equivalent diameter of granule [mm],

 r^2 – determination coefficient,

R_o – Reynolds number,

SBR – sequencing batch reactor,

SVI – sludge volume index [cm³·g⁻¹],

TSS – total suspended solids [mg·dm⁻³],

V – settling velocity $[mm \cdot s^{-1}]$,

 V_{f} – granule volume [cm³],

VSS – volatile suspended solids [mg·dm⁻³],

 η – liquid viscosity [kg·m⁻¹·s⁻¹],

 ρ_e – effective, buoyant density of an aggregate in liquid [mg·cm⁻³],

 $\rho_{\rm f}$ – density of granule [g·cm⁻³],

 ρ_{w} – density of the liquid [g·cm⁻³].

REFERENCES

- Andreottola G., L. Baldassarre, C. Collivignarelli, R. Pedrazzani, P. Principi, C. Sorini, G. Ziglio: A comparison among different methods for evaluating the biomass activity in activated sludge systems: preliminary results, Water Sci. Tech., 46, 413–417 (2002).
- [2] APHA: Standard methods for the examination of water and wastewater, 18th edition APHA, AWWA and WEF, Washington DC, USA 1992.
- [3] Arrojo B., A. Mosquera-Corral, J.M. Garrido, R.R. Méndez: Aerobic granulation with industrial wastewater in sequencing batch reactors, Water Res., 38, 3389–3399 (2004).
- Bernat K., I. Wojnowska-Baryla: Influence of VFA/TKN ratio in wastewater on the effectiveness of nitrification, Pol. J. Nat. Sci., 21, 741–753 (2006).
- [5] Beun J.J., A. Hendriks, M.C.M. van Loosdrecht, E. Morgenroth, P.A. Wilderer, J.J. Heijnen: Aerobic granulation in a sequencing batch reactor, Water Res., 10, 2283–2290 (1999).
- [6] Chu C.P., D.J. Lee: *Multiscale structures of biological flocs*, Chem. Eng. Sci., 59, 1875–1883 (2004).
- [7] Coehlo M.A.Z., C. Russo, O.Q.F. Araujo: Optimization of sequencing batch reactor for biological nitrogen removal, Water Res., 34, 2809–2817 (2000).
- [8] European Standard EN 14214:2003/AC:2007: Automotive fuels Fatty acid methyl esters (FAME) for

CHARACTERISTIC OF GRANULATED ACTIVATED SLUDGE FED WITH ...

diesel engines – Requirements and test methods.

- [9] Hailei W., Y. Guangli, L. Guosheng, P. Feng: A new way to cultivate aerobic granules in the process of papermaking wastewater treatment, Bioch. Eng. J., 28, 99–103 (2006).
- [10] Ivanov V., X.-II. Wang, S.T.-L. Tay, J.-H. Tay: Bioaugmentation and enhanced formation of microbiological granules used in aerobic wastewater treatment, Appl. Microbiol. Biotechnol., 70, 374–381 (2006).
- [11] Jiang H.L., J.H. Tay, S.T.L. Tay: Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol, Lett. Appl. Microbiol., 35, 439–445 (2002).
- [12] Lee D.J., G.W. Chen, Y.C Liao, C.C. Hsieh: On the free-settling test for estimating activated sludge floc density, Water Res., 30, 541–550 (1996).
- [13] Li D.H., J.J. Ganczarczyk: Fractal geometry of particle aggregates generated in water and wastewater treatment processes, Environ. Sci. Tech., 23, 1385–1389 (1989).
- [14] Li Z.H., T. Kuba, T. Ksuda: Aerobic granular shudge: a promising technology for decentralized wastewater treatment, Water Sci. Tech., 53, 79–85 (2006).
- [15] Liu Y., J.-H. Tay: State of the art of biogranulation technology for wastewater treatment, Biotechnol. Adv., 22, 533–563 (2004).
- [16] Liu Y., Z.-W. Wang, L. Qin, Y.-Q. Liu, J.-H. Tay: Selection pressure-driven aerobic granulation in sequencing batch reactor, Appl. Microbiol. Biotechnol., 67, 26–32 (2005).
- [17] Meher L.C., D. Vidya Sagar, S.N. Naik: *Technical aspects of biodiesel production by transesterification a review*, Renew. Sust. Energ. Rev. Renew. Sust. Energ. Rev., 10, 248–268 (2006).
- [18] Moy B.Y.-P., J.-H. Tay, S.-K. Toh, Y. Liu, S.T.-L. Tay: *High organic loading influences the physical char-acteristics of aerobic shudge granules*, Lett. Appl. Microbiol., 34, 407–412 (2002).
- [19] Pereboom J.H.F.: Size distribution model for methanogenic granules from full scale UASB and IC reactors, Water Sci. Tech., 30, 211–221 (1994).
- [20] Qin L., Y. Liu, J.-H. Tay: Effect of settling time on aerobic granulation in sequencing batch reactor, Bioch. Eng. J., 21, 47–52 (2004).
- [21] Recuwijk van L.P.: *Procedures for soil analysis*, 6th edn., International Soil Reference and Information Centre, FAO, Wageningen 2002.
- [22] Schwarzenbeck N., J.M. Borges, P.A. Wilderer: Treatment of diary effluents in an aerobic granular sludge sequencing batch reactor, Appl. Microbiol. Biotechnol., 66, 711–718 (2005).
- [23] Smoczyński L., R. Wardzyńska: Study on macroscopic aggregation of silica suspensions and sewage, J. Colloid Interfac. Sci., 183, 309–314 (1996).
- [24] Snidaro D., F. Zartarian, F. Jorand, J.-Y. Bottero, J.-C. Block, J. Manem: *Characterization of activated sludge flocs structure*, Water Sci. Tech., 36, 313–320 (1997).
- [25] Tay J.-H., Q.S. Liu, Y. Liu: Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor, J. Appl. Microbiol., 91, 168–175 (2001).
- [26] Tay J-H., Q.-S. Liu, Y. Liu: The effects of shear force on the formation, structure and metabolism of aerobic granules, Appl. Microbiol. Biotechnol., 57, 227–233 (2001).
- [27] Toh S.K., J.H. Tay, B.Y.P. Moy, V. Ivanov, S.T.L. Tay: Size-effect on the physical characteristics of the aerobic granule in a SBR, Appl. Microbiol. Biotechnol., 60, 687–695 (2003).
- [28] Wang Q., D. Guocheng, J. Chen: Aerobic granular sludge cultivated under the selective pressure as a driving force, Process Biochem., 39, 557–563 (2004).
- [29] Wu R.M., D.J. Lee, T.D. Waite, J. Guan: *Multilevel structure of sludge flocs*, J. Colloid Interfac. Sci., 252, 383–392 (2002).

Received: April 4, 2008; accepted: March 5, 2009.

CHARAKTERYSTYKA OSADU CZYNNEGO GRANULOWANEGO HODOWANEGO NA FRAKCJI GLICERYNOWEJ Z PRODUKCJI BIODIESLA

Osad czynny granulowany hodowano z wykorzystaniem frakcji glicerynowej powstalej przy produkcji biodiesla. Po 7 tygodniach hodowli w warunkach tlenowych w reaktorze SBR (h/d = 2,1) uzyskano granulowany osad czynny charakteryzujący się indeksem osadu na poziomie $40-50 \text{ cm}^3 \cdot \text{g}^{-1}$. W warunkach hodowli przy obciążeniu osadu czynnego ładunkiem zanieczyszczeń na poziomie $1,43 \pm 0,1$ g ChZT·g sm·d⁻¹, efektywność usuwania związków węglowych wyniosła 94,14 ± 2,7%. Analiza sitowa wykazała, że około 60% masy osadu czynnego stanowiły granule o średnicy 4–8 mm. Równolegle na podstawie testu swobodnego opadania wyznaczono ilościowy udział granul o różnych średnicach w osadzie czynnym granulowanym. Stwierdzono, że

51

52 A. CYDZIK-KWIATKOWSKA, A. BIALOWIEC, I. WOJNOWSKA-BARYLA, L. SMOCZYŃSKI

granule o średnicy 2–5 mm stanowiły najliczniejszą frakcję tj. około 32% wszystkich granul osadu czynnego. Prędkości opadania, masy oraz wartości liczby Reynoldsa dla granul znacząco wzrastały (p < 0.05) wraz ze wzrostem ich średnicy. Obserwowano, że wraz ze spadkiem średnicy granul następował wzrost ich średniej gęstości w cieczy.