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RESPIROMETRIC ACTIVITY OF ACTIVATED SLUDGE IN SEQUENCING BATCH REACTOR DEPENDING ON SUBSTRATE AND DISSOLVED OXYGEN CONCENTRATION

The effect of the food/microorganisms (*F*/*M*) ratio and dissolved oxygen (DO) concentration in sequencing batch reactors (SBRs) on the microbial activity has been examined. One of the SBRs was fed with synthetic wastewater containing inorganic carbon compounds (COD/N ratio 0.7 g COD/g N). To the other SBR, both inorganic and organic carbon was supplied leading to 6.9 g COD/g N. The *F*/*M* ratios in the reactors were 0.02 and 0.065 g COD/g cell COD, respectively. Under the above feeding conditions, two experimental series were carried out at DO concentration of 0.5 and 1.5 mg O_2/dm^3. The activity of the biomass was determined through respirometric tests based on the determination of oxygen uptake rate (OUR). Independently on operational parameters, nitrification was the process consuming the highest amount of oxygen. Both at the *F*/*M* ratio of 0.02 and 0.065 g COD/g cell COD, higher DO concentration resulted in increased carbonaceous and endogenous respiration.

1. INTRODUCTION

In biological wastewater treatment, the knowledge on activity of microorganisms is very important, since it delivers information about the conditions of the most crucial biochemical processes: biomass growth and substrate consumption. In order to determine the microbial activity, respirometric measurements are often carried out that allow one to estimate oxygen utilization due to substrate consumption. During aerobic processes, oxygen uptake rate (OUR) is commonly measured [1–3]. The oxygen uptake rate is the amount of oxygen consumed by the microorganisms per unit of time and unit of volume [4].

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The measurement of oxygen consumption rates by means of respirometry is widely used in various areas of wastewater treatment and activated sludge modelling. Respirometry was utilised to determine the nitrification kinetics [4], to assess the storage yield for various substrates [5], to assess the readily biodegradable COD of domestic, textile, dairy, meet processing, tannery and confectionery wastewater [6], to measure the readily biodegradable COD in the influent and the effluent of the industrial wastewater treatment plant [7]. Witzig et al. [8] considered the value of oxygen uptake rate to be equivalent to the overall metabolic activity of the sludge community, and measured the metabolic activity of the bacteria present in the membrane filtration sludge. Benes et al. [9] suggested that in order to predict and control the activated sludge process in wastewater treatment, not only substrates concentration in wastewater, but also respiration rates of activated sludge should be measured.

Three processes contribute to oxygen consumption: carbonaceous respiration, endogenous respiration and nitrification. Carbonaceous respiration rate is defined as the oxygen consumption rate when all individual substrates that can be oxidised by a heterogeneous microbial population are present in excess [10]. The endogenous respiration rate means the oxygen consumption rate in the absence of substrate and includes consumption for bacterial growth–decay cycle, maintenance energy production and protozoa respiration [10]. Nitrification activity can be monitored by measuring product formation rates or substrate (ammonia or oxygen) uptake rates [11]. Since ammonia oxidation accompanies high oxygen consumption (4.57 mg O_2 consumed per 1 mg of ammonia nitrogen oxidized), even a low nitrification rate results in measurable oxygen consumption [12]. It is necessary to distinguish the oxygen uptake for heterotrophic substrate oxidation and endogenous respiration from oxygen uptake for ammonia oxidation, particularly when a mixed activated sludge is used.

The aim of the study was to determine the effect of the food/microorganisms ratio (*F*/*M*) and dissolved oxygen concentration (DO) on the activity of activated sludge. The activity measurement was evaluated through respirometric tests and was based on the determination of oxygen uptake rate (OUR). Carbonaceous, endogenous respiration rates, and oxygen consumption for ammonia oxidation in activated sludge were determined.

2. EXPERIMENTAL

Experiment. The experiment was carried out under aerobic conditions in sequencing batch reactors (SBRs). The SBRs, with the working volume of 5 dm^3, were seeded with sludge from municipal wastewater treatment plant with simultaneous nitrification and denitrification. The lab-scale reactors were operated with a cycle time of 24 h at temperature of 20 ºC. Each cycle consisted of 15 minutes of feeding, 23 hours of aerobic period, followed by 30 minutes of settling and 15 minutes of decanting. After the

settling period, 2.5 dm^3 of supernatant was removed, resulting in a volumetric exchange rate (n) in both reactors of 0.5 d^{-1}. The reactors were constantly mixed (50 rpm). pH in the system was maintained at the level between 6.5 and 7.5. The reactors were equipped with controlled air supply system. Gas flow rate was controlled by a thermal mass flow controller (TMFC). The amount of air entering the sequencing batch reactors was automatically adjusted to stable set-point. The dissolved oxygen concentration in the reactors was measured online as a percentage of air saturation.

The synthetic wastewater was prepared according to Coelho et al. [13] (modified). Ammonium chloride and urea were the nitrogen sources in wastewater flowing into the reactors. To the SBR A only inorganic carbon compounds (carbonates and bicarbonates) were introduced with wastewater, while in the influent to the SBR B both inorganic and organic carbon compounds (acetate) were present. Sodium acetate was added to evaluate the carbonaceous respiration rate under non-limiting conditions. The average concentration of total Kjeldahl nitrogen (TKN) in the influent of both reactors was 63.1 ± 4.6 mg/dm^3, ammonia nitrogen 26.6 ± 5.6 mg/dm^3. The chemical oxygen demand (COD) in the influent averaged 45.9 ± 12.8 mg/dm^3 and 434.8 ± 63.4 mg/dm^3 for SBR A and SBR B, respectively. In both reactors two series of experiments were performed, differing in dissolved oxygen concentration (DO) in the reactors (Table 1). In SBR A, the sludge concentration was maintained at around 2.9 g TSS/dm^3, in SBR B – 3.5 g TSS/dm^3. The volatile suspended solids (VSS) in SBR A and SBR B accounted for 55% and 65% of total suspended solids (TSS), respectively.

Table 1

Plan of the experiment

Reactor	SBR A		SBR B	
Series	1	2	1	2
n (d^{-1})	0.5			
COD/N in the influent (mg COD/mg N)	0.7		6.9	
DO (mg O_2/dm^3)	0.5	1.5	0.5	1.5

Respirometric measurements. The adaptation period before every series lasted about 30 days and was considered complete when the range of changes of particular parameters of the effluent (COD, TKN, $N-NH_4^+$) within 7 days did not exceed 5–10%. The above mentioned parameters and activated sludge characteristics (TSS and VSS) were assayed according to the Polish Standards [14].

In every series, after biomass adaptation to the experimental conditions, the cultivation of activated sludge was carried out for about 2 weeks. During this time, mixed liquor samples were collected directly from the reactors at the end of the feeding period and used for the measurement of the oxygen uptake rates (OUR) of activated

sludge. In every series, the respirometric measurements were done in quadruplicate. The concentrations of oxygen used to calculate the OUR were the mean values of the concentrations obtained during four measurements.

OUR was measured using a closed respirometric unit OxiTop Control OC 110 (WTW). The respirometer comprised a stirred vessel with the volume of 0.5 dm^3. The dissolved oxygen concentrations in the samples were determined based on variations of partial pressure inside the measuring vessels. Carbon dioxide, forming during the microbial metabolism, was absorbed by NaOH placed in the tube above the liquid level. Since the amount of used O_2 is proportional to the amount of formed CO_2, removal of CO_2 from the gaseous phase causes the drop of the pressure in the measuring vessel. Pressure decline is automatically converted to the changes of oxygen concentration in the sample.

In order to estimate total oxygen uptake rate (OUR_{tot}) that consists of carbonaceous respiration rate (OUR_1) and the rate of oxygen uptake for nitrification (OUR_2), the mixture of wastewater and activated sludge was placed in a measuring vessel. Carbonaceous respiration rate (OUR_1) was determined in the mixture of wastewater, activated sludge and allylthiourea (ATU) serving as the inhibitor of nitrifying activity. To determine the endogenous respiration rate (OUR_3), a mixture of wastewater and activated sludge was centrifuged for 10 min (4000 rpm), rinsed three times with phosphate buffer and twice with distilled water to remove the residual COD. Activated sludge was placed into the measuring vessel and distilled water was added to maintain the same sample volume as in the previous measurements.

The rate of oxygen uptake for nitrification (OUR_2) was the result of subtraction:

$$OUR_2 = OUR_{tot} - OUR_1$$

To keep constant temperature of the systems at the level of 20 °C, vessels with activated sludge were placed in the thermostatic chamber for 5 days.

Methods of calculation. The oxygen uptake rates (OUR_1, OUR_2 and OUR_3) in terms of milligrams O_2 utilized per 1 dm^3 per hour for each operating conditions were determined by non-linear regression from the slope of time dependence of dissolved oxygen concentration, using the Statistica 7 software (StatSoft). The oxygen uptake rates were described by the first order kinetics and defined by the equation:

$$OUR = kL_0 \tag{1}$$

The solution for this could be fitted to the experimental data according to the equation:

$$L = L_0 \left(1 - e^{-kt}\right) \tag{2}$$

where: k is the reaction rate constant (h^{-1}), L_0 is the maximum concentration of used oxygen (mg/dm^3), L is the concentration of used oxygen after time t (mg/dm^3).

To determine the activity of microbial communities in activated sludge, specific oxygen uptake rates ($SOUR_1$, $SOUR_2$ and $SOUR_3$) were calculated according to Park and Lee [15]:

$$SOUR_{1-3} = \frac{OUR_{1-3}}{VSS} \quad \left[\text{mg } O_2/(\text{g VSS}\cdot\text{h})\right] \tag{3}$$

where OURs are the oxygen uptake rates (mg O_2/(dm^3·h)), and VSS is the volatile suspended solids concentration of activated sludge (g VSS/dm^3) for each operating conditions. $SOUR_1$, $SOUR_2$, $SOUR_3$ mean respectively specific oxygen uptake rates for carbonaceous respiration, nitrification and endogenous respiration.

The food/microorganisms ratio (*F*/*M*) was calculated as follows:

$$\frac{F}{M} = \frac{C_{\text{COD r}}}{C_{\text{COD cell}}} \quad \left[\text{g COD/g cell COD}\right] \tag{4}$$

where $C_{\text{COD r}}$ is the initial COD load in the reactor (g COD/d), and $C_{\text{COD cell}}$ is the COD load of the organic part of activated sludge in the reactor (g cell COD/d). $C_{\text{COD cell}}$ was calculated assuming that 1 g of VSS corresponds to 1.48 g of COD [16].

3. RESULTS AND DISCUSSION

In order to compare the activity of activated sludge through respirometry under various feeding and aerobic conditions, OUR curves have been constructed. The concentrations of uptaken oxygen (in mg O_2/dm^3), obtained from OxiTop system, in every measuring moment were divided by the time (in hours) and presented as OUR profiles.

Table 2

Specific oxygen uptake rates (SOURs)

Reactor	SBR A		SBR B	
Series	1	2	1	2
F/*M*, g COD/g cell COD	0.02	0.02	0.065	0.065
$SOUR_1$, mg O_2/g VSS·h	1.7	4.7	2.4	3.6
$SOUR_2$, mg O_2/g VSS·h	4.2	10.3	8.9	6.2
$SOUR_3$, mg O_2/g VSS·h	0.7	1.9	1.6	1.7

Figure 1 shows OUR curves obtained for the reactor A, under low COD concentration in the influent, reflected in the *F*/*M* ratio in activated sludge of 0.02 g COD/g cell COD (Table 2). The total area under the curves represents the total

amount of oxygen to be dissolved to obtain complete oxidation. The oxygen uptake rate for the oxidation of carbonaceous and endogenous substrates, as well as for ammonia oxidation was higher at the higher dissolved oxygen concentration (DO) in the reactor. Specific oxygen uptake rates for carbonaceous substrate oxidation, for nitrification and for endogenous respiration increased about 2.5 times as the DO changed from 0.5 to 1.5 mg O_2/dm^3 (Table 2). As regards endogenous respiration rate, it should be noticed that at DO of 0.5 mg O_2/dm^3, during the first 16 h of the measurement, the OUR_3 equalled zero, whereas at DO of 1.5 mg O_2/dm^3 only for 2 initial hours a similar result was obtained (Fig. 1). Under famine conditions activated sludge does not accumulate intracellular stored substances. At low organic loading cell, lysis takes place and cells may be the source of organic carbon.

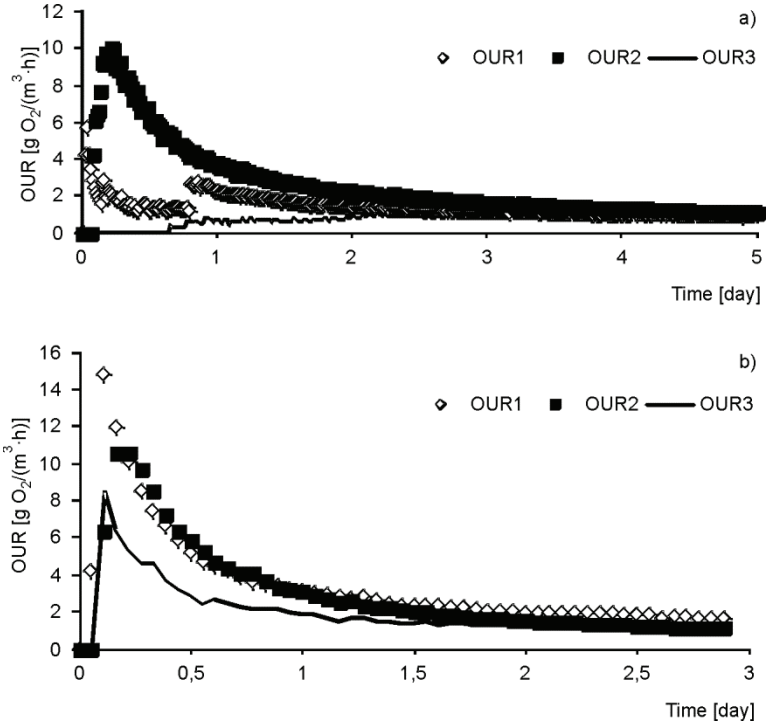

Fig. 1. OUR curves obtained for SBR A at dissolved oxygen concentration of (a) 0.5 mg O_2/dm^3 and (b) 1.5 mg O_2/dm^3

Before cell lysis, respirometric measurements may show no endogenous activity of activated sludge, in other words – oxygen uptake rate can equal zero. In our experiment, in SBR A, at the *F*/*M* ratio of 0.02 g COD/g cell COD, there were no conditions favouring accumulation of stored substances. At DO of 0.5 mg O_2/dm^3, the oxygen uptake rate for endogenous respiration (Fig. 1a, OUR profile) was zero during the

first 16 h of the measurement. After this time, cell lysis occurred and endogenous respiration started to increase. Higher DO concentration promotes cell lysis. For this reason, at DO of 1.5 mg O_2/dm^3, the oxygen uptake rate for endogenous respiration (Fig. 1b, OUR profile) was zero only during the first 2 h of the measurement.

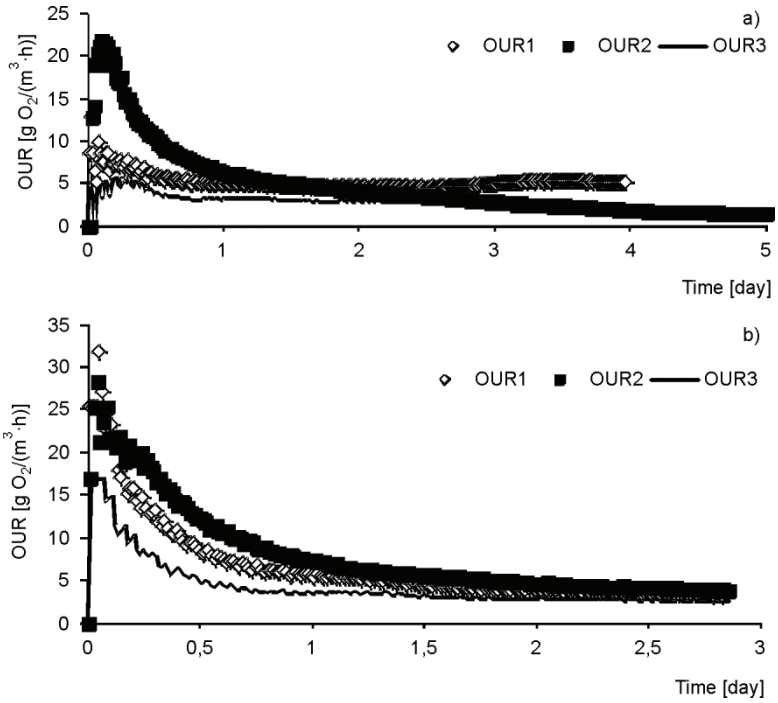

Fig. 2. OUR curves obtained for SBR B at dissolved oxygen concentration of (a) 0.5 mg O_2/dm^3 and (b) 1.5 mg O_2/dm^3

Figure 2 shows the oxygen uptake in the reactor B, i.e. under the conditions of increased concentration of organic compounds in the influent caused by addition of acetate into the feeding medium. The *F*/*M* ratio in activated sludge in this reactor reached 0.065 g COD/g cell COD. The addition of acetate led to higher oxygen uptake rates only at DO of 0.5 mg O_2/dm^3, comparing to reactor A (Table 2). At DO of 0.5 mg O_2/dm^3, growth of the *F*/*M* ratio from 0.02 to 0.065 g COD/g cell COD caused an increase of SOURs, the highest in the case of nitrification and endogenous respiration. At DO of 1.5 mg O_2/dm^3, the values of specific oxygen uptake rates for all the metabolic processes investigated declined along with an increase in the *F*/*M* ratio. However, Pollice et al. [10] observed that by doubling the volumetric loading rate (0.8–1.7 g COD/(dm^3·d)) both the endogenous and the maximum heterotrophic respiration rates triplicated in a lab-scale hollow fibre membrane reactor. Stachowiak et al. [17] in the SBR with activated sludge operated at DO concentration of 3 mg O_2/dm^3

obtained the specific oxygen uptake rates for nitrification of 1.13 mg O_2/(g VSS·h) and 1.46 mg O_2/(g VSS·h) at organic compounds concentration in the influent of 353 and 564 mg COD/dm^3, respectively.

In SBR B, an increase in DO concentration from 0.5 to 1.5 mg O_2/dm^3 caused a decline in the amount of oxygen taken for ammonia oxidation. It can be supposed that under these conditions the competition between autotrophic and heterotrophic microorganisms occurred. Better aerobic conditions may have caused development of heterotrophic bacteria with a higher growth rate and reduction of the number of autotrophic bacteria whose growth is slower [18]. In reactor B, in contrary to reactor A, no periods with zero oxygen uptake rate for endogenous respiration was observed (Fig. 2).

According to Yun-Chang and Bishop [19], an increase in oxygen concentration in the biofilm reactors caused only more rapid respiration of the biofilm and did not influence the effectiveness of organic compound removal from wastewater. Similar results have been obtained in our research. In the presence of acetate in the influent and at higher DO concentrations, the rate of specific oxygen uptake for the oxidation of organic compounds in wastewater was 1.5 times higher than at DO of 0.5 mg O_2/dm^3, whereas the effectiveness of COD removal was about 90% and 80%, respectively at DO of 0.5 mg O_2/dm^3 and 1.5 mg O_2/dm^3.

In every experimental series, nitrification was the process inducing the highest oxygen uptake. Similar results were obtained in the research of Ginestet et al. [20], where the nitrifying activated sludge from wastewater treatment plant was enriched and developed by repeated laboratory subculturing in mineral medium. The acetate-dependent OUR was very low (lower than 3 mg O_2/(h·g of protein)) compared to the ammonia-dependent respiration rates (352 mg O_2/(h·g of protein)), suggesting that the culture was highly enriched with autotrophic nitrifying microorganisms. It is also possible that the acetate-dependent OUR was due to nitrite oxidizers which are capable of growing mixotrophically.

Strotmann et al. [21] analysed the oxygen uptake rate for endogenous respiration depending on the *F*/*M* ratio. In the presence of acetate in the influent, an increase in the *F*/*M* ratio in the range of 0.01–0.16 g/g TSS caused a growth of OUR. In the research presented here, at DO concentration of 0.5 mg O_2/dm^3, endogenous respiration rate was higher under the conditions of higher *F*/*M* ratio.

4. CONCLUSIONS

The food/microorganisms ratio (*F*/*M*) and oxygen concentration in the SBRs influence the OUR of activated sludge. In the experiment, under every operating conditions, nitrification was the process consuming the highest amount of oxygen supplied to the reactor. According to OUR profiles, the oxygen uptake rate for endogenous

respiration remained at the lowest level. At the *F*/*M* ratio of 0.02 g COD/g cell COD, OUR for endogenous respiration equalled zero in the initial hours of measurement.

Generally, at the *F*/*M* ratio of 0.02 g COD/g cell COD, at DO of 1.5 mg O_2/dm^3 respirometric activity of activated sludge, expressed as SOUR, was higher than at the DO of 0.5 mg O_2/dm^3. Similar results were obtained at the *F*/*M* ratio of 0.065 g COD/g cell COD in case of carbonaceous and endogenous respiration.

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