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The Effect of Bioaugmentation with Archaea on the Oxygen Uptake Rate in a Sequencing Batch Reactor

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Abstract: The aim of this study was to evaluate the effect of bioaugmentation with Archaea domain organisms on the activated sludge (AS) expressed by the oxygen uptake rate (OUR) in a laboratory sequencing batch reactor (SBR). The influence of depletion of the external substrate in bioaugmented (SBR-A) and non-bioaugmented (SBR-B) activated sludge during aerobic stabilization was investigated. The experiment was divided into two steps. First, the OUR was measured in the standard conditions of biological treatment. Second, AS was only aerated in the absence of the substrate. It was observed that bioaugmentation with Archaea had an increasing effect on the endogenous and exogenous OUR of the sludge in both phases. In the first phase, the average endogenous OUR was 28.70 ± 2.75 and 21.63 ± 0.9 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in the SBR-A and SBR-B, respectively. Regarding the exogenous OUR, the average values were 95.55 ± 11.33 and 57.15 ± 24.56 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ for the SBR-A and SBR-B, respectively. Archaea enhancing its biological activity, expressed as the OUR, exert a stabilizing effect on this parameter of AS and ensure its lower sensitivity to changes in the process conditions, substrate supply disruption and prolonged aeration.

Keywords: bioaugmentation; Archaea domain organisms; oxygen uptake rate (OUR); aerobic processes; sequencing batch reactor (SBR)

1. Introduction

Currently, the increasing demand for control and process optimization in wastewater treatment plants (WWTPs) requires an advanced approach to the improvement of the system, e.g., by bioaugmentation. This method is defined as the introduction of a specific strain or a consortium of organisms to enhance the biological activity of a process factor [1,2]. It has effectively been used in several environmental aspects such as bioremediation of contaminated soils and groundwater treatment [3–6]. In WWTP, this method has been applied in both aerobic and anaerobic systems [7–9]. Bioaugmentation has been used to increase the population of nitrifiers and increase the tolerance of microorganism against various negative factors such as pH fluctuations, toxic agents, temperature changes, and shock loading [10–15]. Moreover, Van Limbergen et al. [16] indicated that bioaugmentation could improve degradation of refractory compounds as well as flocculation, which in turn affects the parameters of activated sludge (AS) floc and their composition [17,18]. It was also found that this method could support the start-up of new reactors [19,20]. In anaerobic systems, such a technique is involved in improvement of the process stability and biogas yields [21] as well as odor reduction [22,23].

Various organisms can be used in the bioaugmentation process, e.g., autotrophs, heterotrophs, facultative anaerobes and aerobes [2]. Archaea can also be applied for this purpose [24–28]. These

microorganisms are frequent constituents of AS [29–31]. However, their contribution to the total biomass is usually inconsiderable and most frequently does not exceed 8% of the total number of bacterial cells [32,33]. In classical bioreactors for removal of C, N, and P with AS, Archaea occur mainly due to the supply of supernatants formed during the sludge treatment process in fermentation chambers. Previous studies [25–27,34–36] have shown that Archaea are involved in many biochemical processes and, therefore, they can be used for removal of nutrients from various types of industrial and municipal wastewater. An important fact in this context seems to be that the Archaea domain microorganisms have been reported to play an important role in ammonia removal from wastewater [29,33]. They are useful in a removal of nitrogen compounds from wastewater both in the intermittent aeration system [24] and in the system with alternating anaerobic, anoxic, and aerobic conditions [25–27]. They also exert a beneficial effect on the stability of the process, ensuring its lower sensitivity to shock pollutant loading, and help reduce the organic carbon demand during the biological processes of removal of nitrogen compounds [28].

One of the parameters that can be used for characterization of AS is the oxygen uptake rate (OUR). It describes the respiration rate, i.e., the amount of oxygen per unit volume utilized per time unit by the available microorganisms [37]. OUR can be applied to control and optimize process conditions as well as identify potential instabilities of AS systems [38–41]. Furthermore, OUR has also been used to determine microbial activity and viability [42–44]. In AS stabilization, this measurement presents the degree of sludge stability. This parameter is related to the main biochemical processes of biomass growth/decay and substrate removal [45]. The exogenous oxygen uptake rate characterize the activity of heterotrophs and assessment of easily biodegradable substrate in wastewater [46]. However, the endogenous OUR measurement (absence of substrate in wastewater) indicates the consumption for bacterial growth–decay cycle, maintenance energy production and protozoa respiration [47].

The aim of this study was to determine the effect of bioaugmentation with Archaea on AS expressed by the OUR parameter, during the wastewater treatment process in a sequencing batch reactor (SBR). Moreover, the influence of external substrate depletion and aerobic stabilization in bioaugmented and non-bioaugmented AS systems was investigated.

2. Materials and Methods

The study was divided into two steps. First, labeled "feed on" (Step I), the oxygen uptake rate (OUR) (both endogenous and exogenous) was measured in standard stable operational conditions of biological treatment for 30 days of an experiment in SBRs bioaugmented and not bioaugmented with Archaea. In the second step, called "feed off" (Step II), AS was only aerated in the absence of the substrate (under conditions characterizing aerobic stabilization of AS). At this point, the supply of Archaea liquor into SBR was stopped. The second step lasted 30 days as well, counting from the end of the first, feed-on step. Both feed-on and feed-off steps were conducted at the temperature of 20 ± 0.1 °C.

2.1. Experiment in the Sequencing Batch Reactor

The experiment was carried out using two identical sequencing batch reactors (A, bioaugmented; and B, non-bioaugmented AS), each with an active volume of 8 dm^3 (Figure 1). At the beginning, SBRs were inoculated with AS from a municipal wastewater treatment plant (WWTP) in Lublin (southeastern Poland), a mechanical-biological plant, which employs a modified Bardenpho method, with a daily wastewater volume amounting to approximately 65,000 m^3.

The temperature in the SBR was maintained at 20 ± 0.1 °C by using a water bath with controlled and regulated temperature, because during collection of AS for laboratory SBR inoculation, the temperature within WWTP bioreactor was ca. 20 °C. During the experiment, pH (in AS) was also monitored—the mean value of this parameter was 8.1 ± 0.1. The seeded SBR A and B was characterized by mixed liquor suspended solids (MLSS) 3.19 $g \cdot dm^{-3}$ and mixed liquor volatile suspended solids

(MLVSS) 2.43 g·dm^{-3}. During experiment, values of MLSS were monitored, and the average values were 3.56 mg MLSS·dm^{-3} in SBR A and 3.75 mg MLSS·dm^{-3} in SBR B.

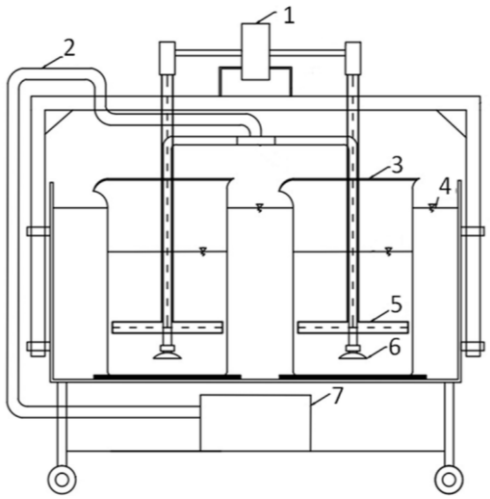

Figure 1. Scheme of laboratory sequencing batch reactors: 1, electric motor driving the mixing system; 2, distribution pipes for pressured air; 3, SBR-type bioreactor; 4, water bath with stabilized temperature; 5, low-speed blade stirrer; 6, membrane diffuser; 7, membrane supercharger supplying the aeration system with pressured air.

In the feed-on step, the SBRs were operated using a 12-h cycle. Each cycle included four distinct phases: Filling (30 min), reacting (stirring 120 min and aeration 420 min), sedimentation (90 min) and decantation (30 min) as well as an idle phase for removal of excessive AS and sampling of probes for analysis (30 min). During the aeration step, oxygen concentration was sustained at a level of approximately 2 mgO_2·dm^{-3}.

The two SBR-type bioreactors used in the experiment were operated under the same conditions; However, SBR-A was bioaugmented. It was fed with 2.5 dm^3 of pre-settled wastewater and 0.25 dm^3 of an Archaea-containing suspension used for bioaugmentation. The second reactor (SBR-B) was fed with the same wastewater volume, but the bioaugmentation liquor was replaced with an equal amount of distilled water.

The wastewater feed in the filling step was obtained at WWTP in Lublin. The presettled wastewater was taken (twice a week) from a primary sedimentation tank, then portioned into a container used for one feeding (2.5 dm^3 for each bioreactor), and kept in a refrigerator at 4 °C. About 1 h before the filling procedure, raw wastewater was transferred from the refrigerator into a temperature of 20 °C to avoid temperature fluctuation inside the SBRs. The main characteristics of pre-settled wastewater are presented in Table 1.

Table 1. Pre-settled wastewater composition (the mean value and standard deviation are given).

Parameter	Unit	Mean Value ± Standard Deviation
Chemical oxygen demand (COD)	mg·dm^{-3}	899 ± 81.0
Total suspended solids (TSS)	mg·dm^{-3}	297 ± 24.3
Total nitrogen (TN)	mg·dm^{-3}	105 ± 7.8
Ammonia nitrogen (N-NH_4^+)	mg·dm^{-3}	88.2 ± 2.2
Total phosphorus (TP)	mg·dm^{-3}	11.8 ± 0.7
pH	pH	7.8 ± 0.3

Archaea microorganisms used for bioaugmentation was prepared as liquor in a continuous mode throughout the experiment. The liquor preparation device operated according to the principle specified

below. A nylon pouch filled with a solid (powdery) substrate inducing incubation of Archaea provided by ArchaeaSolutions Inc (Evansville, IN, USA) was mounted inside the generator. The substrate was packed in a vinyl alcohol coating, which dissolved upon contact with dechlorinated tap water flowing through the generator. The release of an appropriate Archaea microbial load required a continuous flow of water through the generator at a flow rate established at a level of 0.5 $dm^3 \cdot min^{-1}$. After 30 days, the Archaea-containing pouch in the generator was replaced by a new one. The generator was linked to two serially-connected storage tanks. At the highest point of the second tank, an emergency spillway was mounted. The total volume of storage tanks was 320 dm^3. The suspension used during the experiment was sampled every 12 h immediately before supplying into the bioreactor during the filling phase. Analysis of the substrate identical to that used in this study and obtained in a similar way [25–27] with the PCR technique using a GeneMatrix Soil DNA Purification Kit (EUR_X, Gdańsk, Poland) showed that the prepared liquor contained Archaea microorganisms having the 16S rRNA gene and archaeal ammonia monooxygenase subunit A genes. The physical and chemical characteristics of the Archaea liquor used for bioaugmentation are presented in Table 2. Most experimental analyses were performed with Hach Lange UV–VIS DR 5000 (Hach, Loveland, CO, USA) using Hach analytical methods. The pH values were monitored by a multimeter HQ 40D Hach-Lange (Hach, Loveland, CO, USA). Total solids (TS), volatile solids (VS) and total suspended solids (TSS) were determined according to Polish standard methods.

Table 2. Characteristics of Archaea liquor used for bioaugmentation (the mean value and standard deviation are given).

Parameter	Unit	Mean Value ± Standard Deviation
Chemical oxygen demand (COD)	$mg \cdot dm^{-3}$	22 ± 1.0
Volatile fatty acids (VFA)	$mg \cdot dm^{-3}$	21 ± 1.0
Total solids (TS)	$mg \cdot g^{-1}$	0.47 ± 1.0
Volatile solids (VS)	$mg \cdot g^{-1}$	0.042 ± 1.0
Total suspended solids (TSS)	$mg \cdot dm^{-3}$	6 ± 1.0
Total nitrogen (TN)	$mg \cdot dm^{-3}$	75 ± 1.0
Ammonia nitrogen ($N\text{-}NH_4^+$)	$mg \cdot dm^{-3}$	0.4 ± 0.02
Total phosphorus (TP)	$mg \cdot dm^{-3}$	0.17 ± 0.03
Alkalinity	$mg \cdot dm^{-3}$	330 ± 0.03
pH	pH	7.16

2.2. Measurement of the Oxygen Uptake Rate (OUR)

To measure OUR, the AS volume of 0.9 dm^3 was sampled from both SBR-A and -B and then added to a respirometer with a stirring mechanism and dissolved oxygen probe (HQ 40D by Hach-Lange). The respirometer was placed in a thermostatic bath (20 ± 0.1 °C). Before the measurements, the AS was continuously aerated to obtain the initial dissolved oxygen concentration (DO_1) of 7–8 $mgO_2 \cdot dm^{-3}$, and then the aeration was stopped.

The dissolved oxygen concentrations (DO) were measured at 30-s intervals until they reached a value close to full depletion. The respiration rate was calculated from the slope, according to the following equation:

$$OUR = \frac{DO_1 - DO_2}{t_2 - t_1} \quad (1)$$

where $DO_{1,2}$ are initial and final dissolved oxygen concentration, respectively; and $t_2 - t_1$ is the time interval between the first and last DO measurement.

The OUR measurement was determined in two replications for two variants, exogenous (OUR_{exo}) and endogenous (OUR_{endo}). The wastewater from the primary sedimentation tank effluent was used as an external substrate during the OUR_{exo} measurements. The doses of 0.1 dm^3 were added at each measurement. The dose value was determined experimentally, as volume percentage of added

wastewater. No supplementation of the substrate was applied in the OUR_{endo} investigations. However, to ensure the same volume of suspension in the OUR_{endo} measurements, 0.1 dm^3 of dechlorinated tap water was added at each measurement.

The statistical analyses were conducted by means of R programming environment (v. 3.4.3.). Each comparative analysis of means was preceded with a test of significance of variance differences, which was performed using F-test. In the case of equal variance, Student's *t*-test was employed for two independent samples, whereas Welch test was applied when the values differed [48].

3. Results and Discussion

The results of the study are shown in Figures 2 and 3. The values of endogenous OUR (Figure 2) indicate the presence of the five-day-long adaptation period (Stage I) for AS transferred from a full-scale bioreactor to the laboratory-scale SBRs, in both Bioreactor A and B. This relatively short duration was related to the fact that only the scale and type of the bioreactor were changed, as the AS was transferred from a large-scale flow system to the laboratory-scale batch one. All process parameters as well as the wastewater subjected to the treatment remained unchanged. The next easily distinguishable stage of the feed-on step involved stable operation of the SBR bioreactors in standard conditions for 25 days (Stage II in Figure 2).

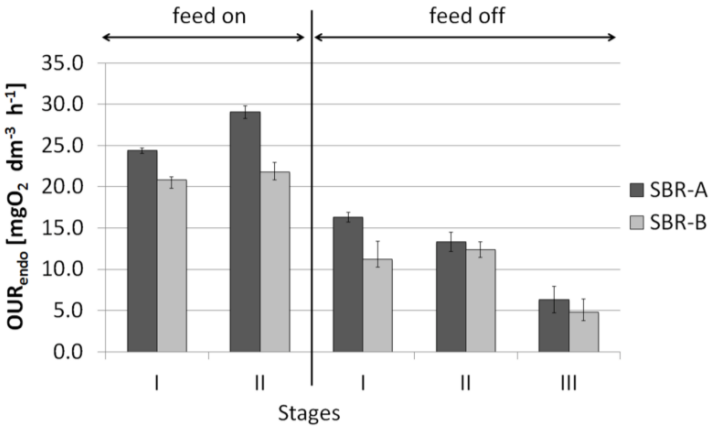

Figure 2. Endogenous oxygen uptake rate values during the experiment. SBR-A contained bioaugmented and SBR-B contained non-bioaugmented AS. Standard deviations are also given.

Inconsiderable changes in the average OUR_{endo} values were found during the Step I (feed-on step) in Bioreactor B. Regarding Bioreactor A, noticeable and statistically significant OUR_{endo} increases were observed in Stages I and II. This is caused by Archaea bioaugmentation, as bioreactor performance differs only in this aspect. It is also visible that the standard deviation of the OUR measurements was lower for Bioreactor A than for B at both stages.

In the Step II (feed-off step) can be distinguished Stage I (lasting 8 days), where the OUR_{endo} decline in SBR-A was substantially lower than in SBR-B in comparison with the feed-on step; Stage II (lasting 16 days), where the OUR_{endo} decreased in SBR-A (F test showed that variances at Stages I and II are different $p = 0.029$, Welche test applied for comparison of average values gave the results that they were statistically different $p = 2 \times 10^{-8}$) and increased slightly in SBR-B relative to the previous step (variances different with $p = 0.002$ and differences in average values are statistically insignificant $p = 0.17$); and Stage III (lasting six days), where the OUR_{endo} dropped significantly in both bioreactors in comparison to the previous step (for SBR-A test F showed no differences in variances $p = 0.126$ and T student test showed differences in average values $p = 3.306$, for SBR-B test F showed differences in variances $p = 0.030$ and Welche test showed differences in average values $p = 2.911 \times 10^{-5}$). In the feed-off step, the standard deviation of the OUR_{endo} measurements was lower for SBR-A only in Stage I and comparable to the level achieved for SBR-B in the other two stages.

While averaging the results of the feed-on and feed-off steps, it was found that, in the Step I (i.e., in substrate presence), the average endogenous oxygen uptake rate was 28.70 ± 2.75 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in bioaugmented SBR-A and 21.63 ± 0.9 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in non-bioaugmented SBR-B (Figure 2). In turn, in the Step II (when the absence of substrate and continuous aeration conditions occurred), the average endogenous oxygen uptake rate was 12.73 ± 3.93 and 10.56 ± 4.23 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in previously bioaugmented and non-bioaugmented AS, respectively (Figure 2).

Given the analysis of the OUR_{endo}, values in SBR-A and SBR-B were are not stable during the specified stages, which is reflected in the standard deviations of the results. The increase in the SBR-A of the OUR_{endo} value after the adaptation period in the bioaugmented system is considerable (for SBR-A, F test showed differences in variance $p = 0.030$ and Welche test shows differences in average results $p = 8.809 \times 10^{-13}$; for SBR-B, F test showed differences in variance $p = 0.012$ and Welche test showed differences in average results $p = 0.003$), which generally yields a higher standard deviation of the results calculated for the total feed-on step (28.70 ± 2.75 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$) and may indicate lesser stability of the system. However, the upward trend in the changes and the analysis of the individual stages allows concluding that bioaugmentation exerts a positive effect on the respiratory activity of AS.

The results of OUR_{exo} measurements (Figure 3) indicate that, just as for OUR_{endo}, there is a visible AS adaptation period in both laboratory-scale SBRs referred to as Stage I (five days), and a subsequent stable operation Stage II (25 days). The OUR_{exo} values achieved in SBR-B changed substantially in the feed-on step and were considerably lower in Stage II (differences in variance $p = 0.015$ and differences in average value $p = 5.206 \times 10^{-10}$). In SBR-A, they were also different in Stage I (differences in variance $p = 0.054$ and differences in average value $p = 2.737 \times 10^{-4}$), but the change was not as drastic as in SBR-B. The standard deviation of the measurement results for SBR-A and SBR-B in both these stages were not different (for I $p = 0.22$ and for II $p = 0.16$).

In the feed-off step, three distinct stages can be distinguished, similar to Figure 2. These involve Stage I (lasting eight days), where the OUR_{exo} in SBR-A declines to a level similar to that achieved for SBR- B; Stage II (lasting 16 days), where OUR_{exo} drops in SBR-A (variance not different $p = 0.46$ and different averages $p = 0.0002$) and in SBR-B in comparison to Stage I (variance not different $p = 0.29$ and different averages $p = 1.754 \times 10^{-11}$); and Stage III (lasting six days), where OUR_{exo} in both bioreactors falls distinctly relative to Stage II, in SBR-A (variance not different $p = 0.24$ and different averages $p = 7.842 \times 10^{-14}$) and in SBR-B (variance not different $p = 0.47$ and different averages $p = 0.001$).

While averaging the results of the feed-on and feed-off steps, it was found that, in the first step (in substrate presence), the average exogenous oxygen uptake rate value was 95.55 ± 11.33 and 57.15 ± 24.56 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in bioaugmented and non-bioaugmented AS, respectively (Figure 3).

For the second step (characterized by an absence of the substrate and continuous aeration), the average exogenous oxygen uptake rate was 29.74 ± 10.67 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in previously bioaugmented AS, while decreasing considerably to 19.82 ± 12.42 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in non-bioaugmented Bioreactor B (Figure 3).

The analysis of the OUR_{exo} values (Figure 3) in both stages of the experiment allows a conclusion that, similar to the OUR_{endo} results (Figure 2), SBR-A was characterized by a greater stability of the individual stages of both steps, which is reflected in the standard deviations exhibiting lower values.

For both OUR_{endo} and OUR_{exo}, two basic stages can be distinguished in the feed-on step and three stages in the feed-off step. At the beginning of the feed-on step, there is a ca. 5-day long adaptation period for AS transferred from a large-scale bioreactor to a laboratory-scale SBR, and the rest of the time (25 days) is a stable operation period. For both OUR_{endo} and OUR_{exo}, this parameter in the feed-on step is higher for the bioaugmented bioreactor. However, when the adaptation step is finished, the OUR_{exo} clearly declines in both SBR-A and -B, which is particularly evident for the non-bioaugmented bioreactor, where it falls by 1/3. In the feed-off step, the OUR_{endo} and OUR_{exo} are always higher for the bioaugmented Bioreactor A. Interestingly, in Stage II of the feed-off step in Bioreactor B, the OUR_{endo} increases in comparison to Stage I, which is not the case in Bioreactor A.

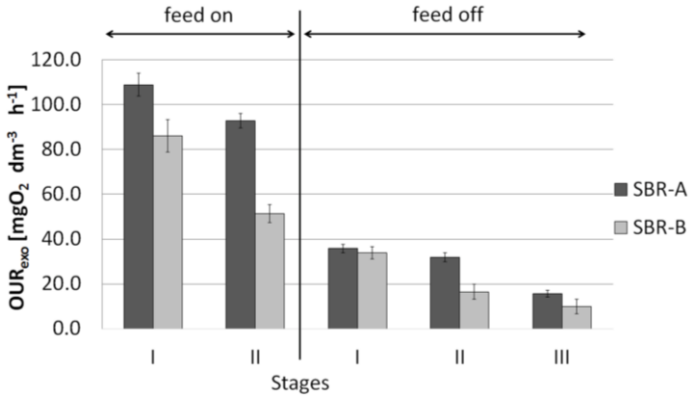

Figure 3. Exogenous oxygen uptake rate values during the experiment. SBR-A contained bioaugmented and SBR-B contained non-bioaugmented AS.

Similarly, the standard error of the measurements is usually lower for the bioreactor with the bioaugmented AS. Regarding OUR_{endo} in the feed-on step, it is 0.5 for the bioaugmented SBR-A and 0.16 for the non-bioaugmented SBR-B, while in the feed-off step these values are higher: 0.72 and 0.77, respectively. When OUR_{exo} is considered, the standard error in the feed-on step reaches 2.07 and 4.48 for SBR-A and -B, respectively. In the feed-off step, lower values are found: 1.95 for SBR-A and 2.27 for SBR-B.

Generalization and averaging of the results for the two feed-on and feed-off steps allows concluding that the maximum respiration rate was observed in the feed-on step of the experiment, and a decrease in both OUR_{exo} and OUR_{endo} occurred during the feed-off step. In this case, the average endogenous oxygen uptake rate was 12.73 ± 3.93 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in bioaugmented AS, and 10.56 ± 4.23 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in non-bioaugmented one. In the case of the exogenous oxygen uptake rate, the average values were 29.74 ± 10.67 and 19.82 ± 12.42 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ in bioaugmented and non-bioaugmented AS, respectively.

As background for OUR measurement and supplementary tools for checking the stability of processes in bioreactors during the feed on step the effectiveness of wastewater treatment in bioaugmented and non-bioaugmented SBRs was observed. Higher changeability of effectiveness was noticed in non-bioaugmented one however no significant differences were observed between level of treatment effectiveness in both of bioreactors. In addition, higher changeability in effectiveness of treatment was observed at beginning of experiment in both bioreactors which reflects the adaptation phase of AS for laboratory condition.

According to Henze et al. [49], the low values of the respiratory rate might be caused by oxygen stabilization of sludge. The typical OUR_{exo} values for AS range from 30.0 to 100 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ [50]. In the study by Puig et al. [51] similar data was obtained for SBR, i.e., the exogenous respiration rate ranged from 35 to 110 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$. The results presented in this paper are mostly consistent with those reported by others. However, the endogenous oxygen uptake rate exceeds the OUR_{endo} values given by Avcioglu et al. [52] that varied from 2.0 to 8.0 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ This suggests that AS used for the experiment had a good quality due to the presence of many microorganisms in the flock assemblages as well as a sufficient substrate [49].

The OUR_{exo} results in aerobically stabilized sludge (i.e., in feed-off step) were comparable to these presented by Cokgor et al. [53]. In their study, domestic sludge was aerobically digested at room temperature of 20 °C for 35 days. A maximum OUR value of ca. 40.0 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ was observed at the beginning of aerobic digestion. Then, it decreased to 18.0 and 21.0 $mgO_2 \cdot dm^{-3} \cdot h^{-1}$ after 17 and 30 days, respectively. Bernard and Gray [54] investigated aerobically digested domestic sludge at a temperature of 16.5–20 °C; they also observed a significant reduction of specific oxygen uptake rate ranging from 65.8% to 93.1% (after 35 days) (SOUR was expressed as milligram of oxygen

consumed per gram of volatile suspended solids (VSS) per hour and determined using equation SOUR = OUR/VSS).

Both endogenous and exogenous oxygen uptake rates were higher in the case of bioaugmented AS, which corresponded to the research carried out by Jun et al. [24] (SOUR was investigated) and also mentioned by Fredriksson et al. [33]. The authors suggested a symbiotic relationship between bacteria and Archaea. However, the difference between OUR values observed in the present study for non-bioaugmented and bioaugmented AS was higher in the case of AS (feed-on step) than in stabilized sludge (feed-off step). Based on these investigations, it can be supposed that the bioaugmentation-assisted process is considerably more stable, as evidenced by the lower standard deviation value in each particular stage of the feed-on and feed-off steps. Therefore, it can be assumed that Archaea have a stabilizing effect on AS and decrease its sensitivity to changes in the quality of supplied wastewater and to disruption of substrate supply. This supports the advisability of bioaugmentation of AS and confirms the findings concerning enhancement of bioreactor stability presented in the Introduction of this paper. On the other hand, the research indicates that Archaea-bioaugmented AS is characterized by higher activity (expressed by a higher OUR) at prolonged aeration and exhibits increased resistance to oxygen stabilization, which makes this type of stabilization less effective and therefore less cost-efficient.

Summarizing, it should be stressed that aspects of the novelty in the work is the description of the influence of bioaugmentation with Archaea on oxygen uptake rate in AS system in wide range of process stages (adaptation phase, stable operation and aerobic stabilization). Moreover, the study confirmed the increasing as well as stabilizing influence of Archaea addition on respiration activity of AS described by oxygen uptake rate.

4. Conclusions

In this study, the influence of bioaugmentation with Archaea on OUR of AS in a laboratory-scale SBR was investigated. Furthermore, the effect of absence of an external substrate in bioaugmented and non-bioaugmented AS during aerobic stabilization was evaluated. The conclusions are as follows:

(1) It was observed that bioaugmentation with Archaea had a positive effect on both the endogenous and exogenous oxygen uptake rate of AS. The values of the OUR_{endo} and OUR_{exo} in the bioaugmented SBR was higher than in not bioaugmented SBR during the standard performance of the SBR bioreactor operating under sufficient substrate availability. The feeding inhibition of AS together with continuous aeration resulted in gradual stabilization and aerobic digestion of the bioaugmented and not bioaugmented AS, however in presence of Archaea the mentioned process is slower.

(2) The results indicate an increase in the OUR value of bioaugmented AS in comparison with non-bioaugmented one in exactly the same process conditions and greater invariability of the OUR level in the individual stages of the experiment. Therefore, it can be stated that Archaea exert a stabilizing effect on OUR of AS (increase the system's resistance to external factors) and decrease its sensitivity both to changes in the quality of supplied wastewater and to disruption of substrate supply as well as prolonged aeration.

(3) Because OUR is only one of the possible parameters describing AS, future work should be conducted, for instance related to influence of Archaea bioaugmentation on biogene congested bioreactors performance, bioreactors working in high range of temperatures, but also to describe reactions of eukaryotic organisms present in AS on supplementation with Archaea.

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Conflicts of Interest: The authors have declared no conflict of interest.

Abbreviations

OUR	oxygen uptake rate
OUR_{exo}	exogenous oxygen uptake rate
OUR_{endo}	endogenous oxygen uptake rate
AS	activated sludge
SBR	sequencing batch reactor
SBR-A	bioreactor with bioaugmented activated sludge
SBR-B	non-bioreactor with bioaugmented activated sludge

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