



# Article Reconstruction of the Municipal Wastewater-Treatment Plant According to the Principles of Aerobic Granular Sludge Cultivation

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Abstract: The work presents the concept of aerobic granular sludge (AGS) and its potential for wastewater treatment. The work also evaluates the condition of the SBR (Sequencing Batch Reactor) type of municipal wastewater-treatment plant (WWTP) after its reconstruction into a system with AGS. The WWTP parameters achieved before and after reconstruction were compared. Operational measurements of the process during the individual phases of the treatment process showed a balanced concentration profile of the monitored parameters in the span of the semicontinuous cycle. Laboratory tests showed that the sludge from the WWTP has nitrification and denitrification rates comparable to the rates achieved for flocculent sludge, and it is also comparable to the nitrification and denitrification rates of AGS with size of granules below 400  $\mu$ m. Despite the fact that complete sludge granulation was not achieved, the results measured at the WWTP confirmed the advantages of the AGS concept. Neither anaerobic nor anoxic conditions were identified in the SBR during the individual phases of operation, yet high removal efficiencies of ammonia and nitrate nitrogen and orthophosphate phosphorus were achieved. The concentration of phosphorus was below 0.5 mg/L.

**Keywords:** anaerobic granulated sludge; denitrification; enhanced biological phosphorus removal; nitrification; sequencing batch reactor

# 1. Introduction

Aerobic sludge granulation is a relatively new technology developed for the biological treatment of wastewater. In the last two decades, it has become the subject of extensive research due to its multiple advantages over the activated sludge system. The aerobic granular sludge (AGS) process has evolved into an advanced technological solution for large wastewater-treatment plants (WWTPs) that treat both municipal and industrial wastewater. The use of AGS allows for the construction of more compact treatment plants with lower operational costs compared to conventional systems such as activated sludge (AS). Granular sludge and achieve significantly higher settling velocities [1]. Sludge volume indexes for granular biomass, determined after 5 min of sedimentation, are comparable to those for activated sludge, determined after 30 min of sedimentation [2]. Therefore, the separation of treated water is achieved much faster and without the need for external settling tanks. Compared to AS, AGS allows for the reduction of the necessary surface area of treatment tanks and decreases operational energy requirements.

The granules can contain several microbial layers. Different layers are composed of distinct bacterial species responsible for various ongoing processes such as nitrification,



Citation: Hutňan, M.; Jankovičová, B.; Jajcaiová, L.; Sammarah, M.; Kratochvíl, K.; Šoltýsová, N. Reconstruction of the Municipal Wastewater-Treatment Plant According to the Principles of Aerobic Granular Sludge Cultivation. *Processes* 2024, *12*, 1782. https:// doi.org/10.3390/pr12091782

Academic Editor: Andrea Petrella

Received: 24 July 2024 Revised: 14 August 2024 Accepted: 21 August 2024 Published: 23 August 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). denitrification, or phosphorus removal [3]. The presence of an aerobic outer layer and an anaerobic or anoxic core facilitates the coexistence of nitrifying organisms in the outer layers of the granules and denitrifying organisms in the center, along with poly-P bacteria capable of accumulating polyphosphates, as well as (facultative) anaerobic organisms towards the center of the granule. Due to this structure, AGS can simultaneously remove phosphorus, nitrogen, and organic pollution [4]. Table 1 compares selected properties of aerobic granular sludge and activated sludge.

	Aerob	Aerobic Sludge
Parameter –	arameter Activated/Suspended Granulated	Granulated
Shape	Irregular	Regular and spherical
Average size	<0.2 mm	0.2–5.0 mm
Sedimentation rate	Lower settling rates <10 m/h	Higher settling rates >10 m/h
SVI <sup>1</sup>	$SVI_5 ^2 > SVI_{30} ^3$	$SVI_5\approx SVI_{30}$
Degree of compactness and density	Low	High
Layered structure or coexistence of aerobic, anoxic and anaerobic microenvironment	Minimum options for anaerobic zones	Aerobic, anaerobic and anoxic zones predominate
EPS <sup>4</sup>	Lower content of EPS	High content of EPS
Tolerance to toxic compounds	Lower tolerance to toxic pollutants	Higher tolerance to toxic pollutants
Ability to withstand impact pollution	Weak	Good

Table 1. Comparison of selected properties of AGS and AS. Adjusted according to [5-8].

<sup>1</sup> SVI -Sludge Volume Index (g/L); <sup>2</sup> SVI<sub>5</sub>—SVI for 5 min sedimentation; <sup>3</sup> SVI<sub>30</sub>—SVI for 30 min sedimentation (standard SVI); <sup>4</sup> EPS—Extracellular Polymeric Substances.

In systems with granular sludge, vertical segregation of biomass can easily occur due to slight changes in the density and diameter of the particles. The microbial population at the bottom of the reactor may differ from the population present at the top, as the biomass at different levels of the sludge bed is exposed to varying conditions [9]. Various biological conversions occur in different layers or zones within the aerobic granules in a single reactor tank. The operation of the reactor with alternating aerobic and anaerobic periods in a sequential batch mode causes segregation of microorganisms in the granules due to the dissolved oxygen gradient throughout the depth [8].

Individual findings from studies focused on the aerobic biomass-granulation process are summarized in reference [10]. They indicate that the formation of microbial aggregates is a multi-step process with significant influence from hydrodynamic shear forces, and it can be divided into the following four steps:

Step 1: Physical movement leading to contact between bacteria. The forces acting in this step are hydrodynamic, diffusive, gravitational, and thermodynamic, such as Brownian motion and the mobility of microorganisms.

Step 2: Initiation of attractive forces to maintain multicellular contact between microorganisms. Van der Waals forces, attraction due to opposite charges, and thermodynamic forces are involved in this step. Filamentous microorganisms can significantly contribute to the formation of three-dimensional structures. In addition to these forces, chemical and biochemical processes also play an important role.

Step 3: Microbial forces cause the maturation of aggregated bacterial structures. This primarily involves the formation of extracellular polymers, such as exopolysaccharides.

Metabolic changes and genetic competencies facilitate and further strengthen interactions between cells, resulting in a high density of connected cells.

Step 4: Three-dimensional structures of microbial aggregates are stabilized by hydrodynamic shear forces. The resulting shape and size are determined by the force and manner of interaction between aggregates and hydrodynamic forces, microbial species, and substrate loading [10].

These facts were also confirmed in several later works, such as study [11].

Currently, several full-scale operational facilities are already in place abroad [4,12,13]. The implementation of this technology is relatively straightforward and has several advantages over conventional technologies (Table 1). The high biomass retention capacity of these systems and the possibility of cultivating granular biomass with stratified bacterial populations allow simultaneous processes for wastewater treatment to occur in one tank.

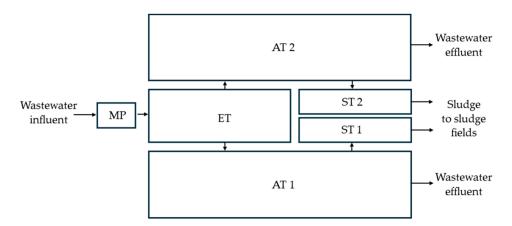
In works dealing with the granulation process and operation of full-scale WWTP, input–output relationships, AGS growth and microorganisms found in AGS are mostly studied. Very few works deal with measuring the concentration profile of individual pollution components during a semi-continuous cycle and the nitrifying and denitrifying activity of AGS.

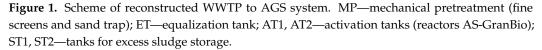
This work focuses on assessing the operation of the wastewater-treatment plant with capacity 3000 PE. This WWTP, originally designed as a Sequencing Batch Reactor (SBR) system with suspended activated sludge, with time segregated nitrification and denitrification was reconstructed to a WWTP with the concept of aerobic granular biomass, because it did not reach the required quality of treated water. The aim of this work was to conduct and evaluate operational measurements to confirm the suitability of the WWTP reconstruction, the progress of nitrification, denitrification, and enhanced biological phosphorus removal. Additionally, the nitrification and denitrification activity of aerobic granular biomass was assessed.

#### 2. Materials and Methods

#### 2.1. Technology of the Reconstructed WWTP

The reconstructed WWTP is a municipal wastewater-treatment plant for 3000 PE (population equivalent) with average wastewater flowrate 300  $\text{m}^3/\text{d}$ . The scheme of reconstructed WWTP is shown in Figure 1. The wastewater-treatment plant is a mechanical-biological WWTP that removes organic pollution and nutrients N and P from the wastewater. Nitrogen is removed through nitrification and simultaneous denitrification. Phosphorus is removed through the process of enhanced biological removal.





The core of the technological line is the biological stage, consisting of two activation tanks (AT1 and AT2), AS-GranBio reactors ( $2 \times 215 \text{ m}^3$ ), which are operated automatically

by a control system. In the activation tank, the process of aerobic biological treatment and cultivation of aerobic granular biomass takes place. Oxygen is supplied to the tank in the form of compressed air using a volumetric rotary blower. Each activation tank has a separate blower with air delivery through a fine-bubble aeration system at a volume of 234 m<sup>3</sup>/h. Wastewater flows into the WWTP through a divided sewer system and undergoes mechanical pre-treatment (MT) on fine screens and in a grit chamber and equalization tank (ET) with a volume of 100 m<sup>3</sup> before entering the biological stage. The treated water is discharged into the recipient after the sedimentation phase, while excess sludge is sent to sludge storage tanks (ST1 and ST2 2 × 35 m<sup>3</sup>) and subsequently dewatered on sludge fields.

The cycle of the original SBR technology consisted of the classic phases—aeration, denitrification, settling, sludge removal, and wastewater filling.

After the reconstruction, the phases of the individual cycles of the AGS system were as follows:

- Phase I—aeration;
- Phase II—intermittent aeration (the period of aeration interruption and aeration alternates several times);
- Phase III—sedimentation;
- Phase IV—filling wastewater into the lower part of the sludge bed while simultaneously discharging purified water (upper overflow into the drain)—fill-anddraw running;
- Phase V—sludge removal from the upper part of the sludge bed into the storage tanks.

The course of the individual phases of the semi-continuous cycle is shown in Figure 2. Arrows indicate the sampling points. The exact setting of the duration of each phase and the overall cycle depends on the WWTP technology, wastewater quality, and the minimization of output parameters. Sampling was conducted from the activation tank in accordance with the timing of the individual phases of the treatment process.



Figure 2. Individual phases of the semi-continuous cycle and sampling from the activation tank.

Samples in individual phases of semi-continuous cycle marked in Figure 2 were grab samples, meaning one-time samples, which were taken from the activation tank AT1. A sample of the incoming wastewater was taken from the equalization tank ET in the same way as the other samples. As mentioned above, these measurements were taken over a total in six days in the three weeks high spring period; three days at low load of WWTP and three at high load of WWTP. Samples taken from the WWTP during the start-up and trial operation were 24 h composite samples.

The semi-continuous cycle with these phases does not occur simultaneously in both activation tanks but is shifted in time to minimize the amounts of pollution discharged into the recipient.

## 2.2. Analyses

In the samples, parameters such as COD (chemical oxygen demand), N-NH<sub>4</sub> (ammonium nitrogen), N-NO<sub>3</sub> (nitrate nitrogen), P-PO<sub>4</sub> (phosphate phosphorus), and pH were analyzed. Analyses at the outlet of the WWTP during start-up and trial operation were made in unfiltered samples. Analyses of samples taken during the semi-continuous cycle were made on filtered samples. In selected samples, the dry matter of the sludge—suspended solids (SSs) and its loss on ignition—volatile suspended solids (VSSs) were also determined. The analyses were carried out using methodologies according to [14]. Spectrophotometric methods were used to determine the concentrations of COD, N-NH<sub>4</sub>, N-NO<sub>3</sub> and P-PO<sub>4</sub>. For COD, it was a modified semi-micro method with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and a silver catalyst. After heating for two hours at 150 °C in a closed cuvette and cooling, the absorbance was measured at a wavelength of 600 nm. The method with Nessler's reagent and Seignet's salt was used for the determination of N-NH<sub>4</sub>. Absorbance was measured at a wavelength of 425 nm. N-NO3 was determined using salicylic acid followed by absorbance measurement at a wavelength of 415 nm. The P-PO<sub>4</sub> concentration was measured using a mixed reagent composed of sulfuric acid, ammonium heptamolybdate, ascorbic acid and antimony potassium tartrate. Absorbance was measured at 690 nm. A DR3900 VIS spectrophotometer (Hach Lange, GmbH, Germany) was used to measure absorbance. The concentration of SS was measured after filtering the aggregated sludge on a membrane filter with 0.4  $\mu$ m pores and after drying to constant weight at 105 °C (laboratory oven UN55, Memmert, GmbH, Schwabach, Germany) and VSS as loss on ignition at 550 °C (laboratory muffle oven LMH, LAC, s.r.o., Zidlochovice, Czech Republic). The pH values were measured on a Hach HQ11D pH meter (Hach Lange, GmbH, Düsseldorf, Germany). During both the aeration phase and the intermittent aeration phase, the oxygen concentration in the tank was also measured (Oxymeter WTW Oxi 3250, with DO sensor CellOX<sup>®</sup> 325, WTW, Weilheim, Germany).

#### 2.3. Tests for Nitrification and Denitrification Activity

Kinetic tests were conducted under laboratory conditions with sludge taken from the monitored activation tank to determine its nitrification and denitrification activity.

One of the parameters characterizing nitrification is the specific nitrification rate ( $v_N$ ) measured in mg/(g·h). It is typically measured using a kinetic batch test and represents the change in nitrogen concentration oxidized by a unit mass of biomass per unit time. The change in nitrogen concentration ( $\Delta c_N$ ) is evaluated as a decrease in N-NH<sub>4</sub> concentration or an increase in N-NO<sub>3</sub> concentration. The specific nitrification rate is determined using the following equation:

$$v_N = \frac{\Delta c_N}{\Delta t \times X_b} \tag{1}$$

where  $\Delta t$  is the time during which the decrease in N-NH<sub>4</sub> or increase in N-NO<sub>3</sub> is linear (h), and  $X_b$  is the biomass/sludge dry matter concentration (g/L).

Denitrification activity is most characterized by the specific denitrification rate ( $v_D$ ) (mg/(g·h). This is the amount of N-NO<sub>3</sub> reduced per unit mass of biomass dry matter per time unit. It is measured using a kinetic batch test, during which the decrease in N-NO<sub>3</sub> concentration ( $\Delta c_N$ ) is measured. In kinetic tests, both endogenous and exogenous denitrification, or nitrate respiration, occur. Endogenous denitrification takes place without the presence of external organic carbon, with bacteria utilizing their internal (endogenous) sources. In exogenous denitrification, bacteria use an external organic substrate, which in our case was glucose. The specific denitrification rate is calculated using the same equation as for the nitrification rate, but  $v_D$  is used instead of  $v_N$ .

The initial concentration of N-NH<sub>4</sub> was 45 mg/L, and for N-NO<sub>3</sub> it was 43 mg/L. The initial COD concentration at the beginning of the denitrification tests was 500 mg/L.

The nitrification and denitrification activity tests were conducted in glass cylinders with a volume of 1 L. During the nitrification tests, the cylinders were aerated, and the dissolved oxygen concentration was maintained above 2 mg/L. During the denitrification tests, the cylinders were only mixed. Sampling of the sludge mixture during the tests was carried out at 30 min intervals for 3 h, followed by 60 min intervals until the test ended after 6 h. In the collected samples, the concentrations of N-NO<sub>3</sub> or N-NH<sub>4</sub> were determined. At the beginning of the test, the concentration of sludge dry matter (SS) was also determined.

#### 3. Results and Discussion

### 3.1. Status of the WWTP before Reconstruction

Average values of selected wastewater parameters at the influent and effluent of the WWTP are shown in Table 2. The parameters that the wastewater should reach at the

effluent of the WWTP are specified by the Regulation of the Government of the Slovak Republic no. 269/2010 for the size category up to 10,000 PE:

	р	m
COD	$\leq$ 120 mg/L	150 mg/L
$BOD_5$	$\leq$ 25 mg/L	45 mg/L
SS	$\leq$ 25 mg/L	50 mg/L
$N-NH_4$	$\leq 20 \text{ mg/L}$	40 mg/L
$N-NH_4$	$\leq$ 30 mg/L <sup>Z1</sup>	$40 \text{ mg/L}^{Z1}$

where

*p*—limit value of the respective indicator in a composite sample over a certain period; m—maximum limit value of the respective indicator in a qualified point sample;

Z1—values apply for the period during which the temperature of the wastewater at the outflow from the biological stage is lower than 12 °C. If the temperature is below 9 °C, this parameter is not observed.

**Table 2.** Average concentrations at the inflow and outflow of the WWTP before reconstruction (unfiltered samples).

Year	BOD <sub>5</sub> (mg/L)	COD (mg/L)	SS (mg/L)	N-NH <sub>4</sub> (mg/L)
		Influent		
2016	664	1246	643	100
2017	665	1246	1073	157
2018	629	1152	882	98
Average	653	1214	866	118
		Effluent		
2016	43	219	52	90
2017	274	608	229	94
2018	57	247	69	77
Average	125	358	116	87

From Table 2, it is evident that the wastewater-treatment plant (WWTP) before reconstruction did not meet the limits for most parameters set by the Government Regulation of the Slovak Republic No. 269/2010 for the size category up to 10,000 PE. Table 2 shows that in 2017 the BOD<sub>5</sub> and COD values at the WWTP inlet were similar to other years, only the SS and N-NH<sub>4</sub> values were higher. Despite this, the values of BOD<sub>5</sub> and COD at the outlet of the WWTP were significantly higher than in other years. These values document the emergency status of the WWTP. Therefore, in 2018, the reconstruction of the aeration system of the WWTP was carried out. The COD values decreased in the following year, but still did not meet the required parameters. This observation applies not only to the average values listed in Table 2 but also to most of the measurements conducted at the WWTP before the reconstruction. Organic Loading Rate (OLR) before reconstruction was 0.150 kg/(kg·d) BOD<sub>5</sub>, Sludge Volume Index (SVI) was in the range 100–200 mL/g and Solid Retention Time (SRT)—sludge age was about 15 d.

#### 3.2. Operational Measurements at the WWTP after Reconstruction

The course of the monitored parameters at the outlet of the reconstructed wastewatertreatment plant (WWTP) during the start-up and trial operation is shown in Figures 3 and 4. Figure 3 shows the values of COD (chemical oxygen demand) and suspended solids (sludge concentration), and Figure 4 shows the concentrations of ammonia nitrogen, nitrate nitrogen, and phosphate phosphorus.

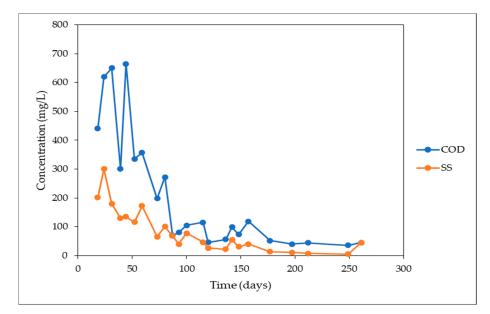
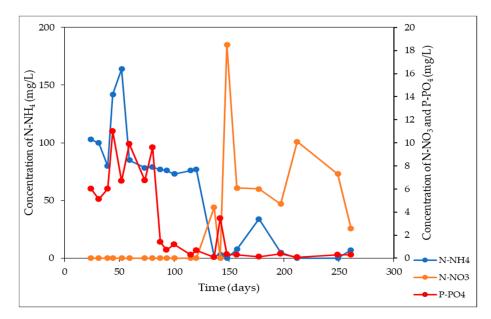


Figure 3. Course of effluent COD and SS concentration during start-up and trial operation of WWTP.



**Figure 4.** Course of effluent N-NH<sub>4</sub>, N-NO<sub>3</sub> and P-PO<sub>4</sub> concentration during start-up and trial operation of WWTP.

From these figures, it is evident that during the adaptation of the sludge to conditions suitable for the growth of aerobic granular biomass, all monitored parameters gradually decreased. The COD values declined, and with the improvement of AGS sedimentation properties, the concentrations of suspended solids also decreased (Figure 3).

Figure 4 clearly shows that enhanced biological phosphorus removal was the first process to start in the WWTP, and its concentrations gradually falling below 0.5 mg/L. Nearly 50 days later, nitrification began, as evidenced by the decrease of ammonia nitrogen and the increase in nitrate nitrogen concentration. However, if we compare the concentrations of ammonia nitrogen and nitrate nitrogen, we see that the nitrate nitrogen concentration is significantly lower, indicating that denitrification was also occurring and its efficiency was gradually increasing.

Measurements taken during the start-up and trial operation indicate that the WWTP reconstruction was successful. The outlet values of COD, suspended solids, and ammonia nitrogen (Figures 3 and 4, measurements taken from 24 h composite samples, unfiltered)

are significantly lower than the values before reconstruction, as listed in Table 2, and they also meet the legislative requirements mentioned above. The concentration of dissolved oxygen during start-up and test operation in the activation tanks did not fall below 3 mg/L.

OLR after reconstruction decreased to  $0.080 \text{ kg/(kg} \cdot d) \text{ BOD}_5$ , SVI steeply decreased to the range 80–120 mL/g and SRT—sludge age increase to more than 25 d. Decrease of OLR was caused by increase of average sludge concentration in biological stage from 3 g/L to 5 g/L. Improvement of sludge settling properties characterized by SVI was caused by aggregation of sludge.

In Figure 4, peaks in N-NH<sub>4</sub> (approx. 50th day) and N-NO<sub>3</sub> (approx. 150th day) concentrations are evident. These values and their subsequent reduction illustrate the optimization of the length of individual phases of the semi-continuous cycle and its total length. The total length of the cycle was set to 6 h after optimization, and the lengths of the individual phases are illustrated in Figure 2. The peak in the concentration of N-NH<sub>4</sub> on the 50th day corresponds to a still unstable treatment process, when at high concentrations of COD nitrification does not take place (Figure 3). Stable nitrification started only after about 75 days of operation of the WWTP, which is evident from the permanent decrease in N-NH<sub>4</sub> and increase in N-NO<sub>3</sub>. The peak of N-NO<sub>3</sub> at about day 150 illustrates complete nitrification, without denitrification. It can be assumed that the sludge has not yet been sufficiently aggregated, but the subsequent decrease in  $N-NO_3$ already indicates the start of denitrification and granulation of the activated sludge. This means that the sludge-granulation time in the WWTP was after the 150th day. The work [5] states that it is possible to grow granulated biomass even with the use of real wastewater, but the granulation time is longer than 50 days. With the use of synthetic substrates, such as volatile fatty acids, granulation can be significantly faster.

After the trial operation, we conducted operational measurements, during which we measured not only the output concentrations of the monitored parameters but also their values during the semi-continuous cycle. The aim of these measurements was to gather information about the operation of the SBR (Sequencing Batch Reactor) technology, which works with the concept of AGS.

We conducted operational measurements at the WWTP on two separate days, during which different quality inflows to the WWTP were expected due to the unregulated discharge of septic tank wastewater and household operations over the weekend. Long-term measurements showed higher inflow concentrations at the WWTP on Tuesdays compared to the relatively average inflows on Fridays. The values of selected average parameters of wastewater inflow to the activation tank are listed in Table 3. The average values were calculated from three Tuesdays (higher load, HL) and three Fridays (lower load, LL) measurements. These values confirm the assumption of poorer wastewater quality at the beginning of the week. The difference in quality is not too significant, as it is partially balanced by the homogenization tank.

**Table 3.** Average values of selected parameters at the WWTP inflow on the days of operational measurements.

BOD <sub>5</sub> (mg/L)	COD (mg/L)	SS (mg/L)	N-NH <sub>4</sub> (mg/L)	P-PO <sub>4</sub> (mg/L)
		Higher load		
$760\pm55.6$	$1~390\pm102.2$	$880 \pm 143.4$	$140\pm18.2$	$16\pm1.9$
		Lower load		
$530\pm53.4$	$1~050\pm82.3$	$640\pm94.1$	$95\pm11.4$	$10\pm1.2$

Sampling was conducted during the aeration phase, interrupted aeration, at the wastewater inflow, and at the outflow of the treated water, as shown in Figure 2. For all samples, COD, N-NH<sub>4</sub>, N-NO<sub>3</sub>, and P-PO<sub>4</sub> were determined, and in some samples, the concentration of dry suspended solids (SS) and its volatile suspended solids (VSS) were also measured. During the interrupted aeration phase, the concentration of dissolved oxygen in the tank was measured.

The results of the concentration monitoring of individual parameters are shown in Figure 5. In this figure, we can see that the only significant concentration profile during the semi-continuous cycle can be observed in the case of N-NH<sub>4</sub>, where its concentration at the inflow on higher load days was significantly higher than on the lower load days. However, the output concentrations of N-NH<sub>4</sub> at the WWTP outflow were not affected by this.

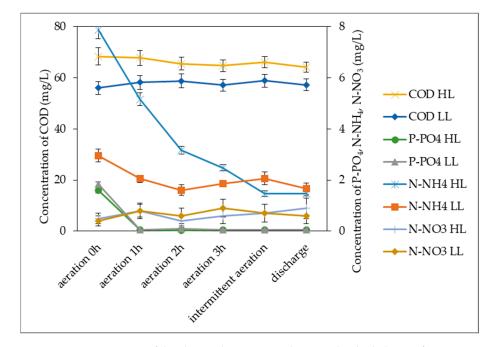


Figure 5. Concentrations of the observed parameters during individual phases of semi-continuous cycle.

For all parameters, significant removal efficiency was recorded on both monitored days. The COD-removal efficiency was around 95%, the total nitrogen-removal efficiency was over 97%, and the total phosphorus-removal efficiency was over 99%. These are interesting results since anoxic conditions (dissolved oxygen concentration below 0.5 mg/L) necessary for the denitrification of N-NO<sub>3</sub>, which is formed by the oxidation of N-NH<sub>4</sub>, were not observed during the individual phases of the semi-continuous cycle. Similarly, anaerobic conditions, necessary for enhanced biological phosphorus removal, were not observed. No precipitants (e.g., Fe<sup>3+</sup> or Al<sup>2+</sup> salts) that enable chemical phosphorus removal were used either.

The oxygen concentration during the continuous aeration phases did not fall below 3.8 mg/L, at which aerobic or oxic processes, oxidation of organic carbon, and nitrification occur. The rate of these processes becomes limited only at dissolved oxygen concentrations below 2 mg/L. Even during the intermittent aeration phase, the oxygen concentration did not drop below 2 mg/L (Figure 6).

From Figure 6, it is evident that the aim of the intermittent aeration phase is not to achieve anoxic conditions, but to create hydrodynamic conditions for the agglomeration of sludge. These conditions, together with the hydrodynamic conditions created by the inflow of wastewater into the lower part of the sludge layer after sedimentation, are the main forces that promote the formation of sludge agglomerates. The existence of these agglomerates can explain the processes of denitrification and enhanced biological phosphorus removal, even though anaerobic or anoxic conditions necessary for these processes were not observed in the semi-continuous cycle of the tank.

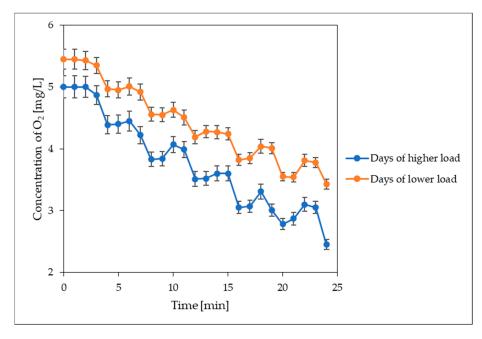
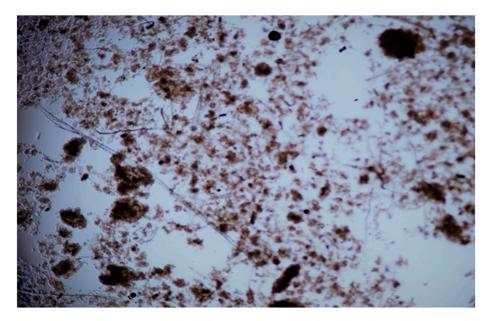


Figure 6. Trend of oxygen concentration in the phase of intermittent aeration.

The low concentrations of N-NO<sub>3</sub> and P-PO<sub>4</sub> at the tank outflow indicate the presence of an anoxic to anaerobic core within the sludge agglomerates, which allows these processes to occur at the micro-level through the segregation of anoxic or anaerobic zones within the agglomerates. Such conditions in the agglomerates are reported in many studies dealing with this issue [5–8]. The formation of sludge agglomerates is illustrated by the microscopic image shown in Figure 7. At 60× magnification, it is clear these agglomerates have formed, and even in smaller flocs, a denser core is evident.



**Figure 7.** Microscopic image of agglomerated sludge (magnification 60×).

Very similar microscopic images are presented in the work [15], which deals with the cultivation of nitrifying-denitrifying aerobic granular biomass.

The consistent COD concentration during different phases of the semi-continuous cycle, even at the beginning of aeration, is also somewhat surprising. Theoretically, it is possible that during the inflow of wastewater, in the initial phases of its contact with the granular biomass, the pollution accumulated in the sludge through adsorption on the

sludge surface and through the formation of storage substances inside the sludge cells. This is a mechanism particularly applied in biological wastewater-treatment systems with sludge regeneration [16]. It is clear from Figure 5 that the concentration of most monitored parameters hardly changes during individual phases and is the same as at the outlet of the WWTP. This confirms claims from the literature that SBR is often used for aerobic granulation of sludge for its fill-and-draw running and aerobic starvation stage. As a filling-and-draw reactor, the settling time of SBR is short and contributes to granulation. In addition, there are two independent stages of sufficient substrate activity and substrate starvation in SBR, in which aerobic starvation can represent for more than 75% of the total semi-continuous cycle [17].

Therefore, at the beginning of aeration and after two hours of aeration, we took a sample of AGS to determine SS and their VSS. In all three cases of measurements on both high and low load days, the concentration of suspended solids at the beginning of aeration was higher, with an average SS concentration of 4.58 g/L and VSS of 87.1%. After two hours of aeration, the concentration of SS was 3.67 g/L and VSS was 85.7%. This means that the idea of adsorption and accumulation of organic pollution in the sludge cannot be excluded. Of course, further measurements would be needed to confirm this. The content of VSS in the sludge was similar to that reported in [18], where in the full-scale SBR system the VSS content was 86%, as well as in the compared continuous AS system. In work [13], the authors report significantly lower values of VSS—56.3% for Anaerobic/Oxic technology, 57.2% for Oxidation ditch technology and 62.4% for SBR technology.

To determine the nitrification and denitrification activity, sludge samples were taken from the activation tank, for laboratory kinetic tests. The specific nitrification rate in the tested sludge and also the specific rate of endogenous and exogenous denitrification were determined according to the procedure outlined in the Material and Methods section. Sampling was carried out every 30 min for 6 h, and the progression of N-NH<sub>4</sub> and N-NO<sub>3</sub> concentrations was monitored. The results of the nitrification tests are shown in Figure 8.

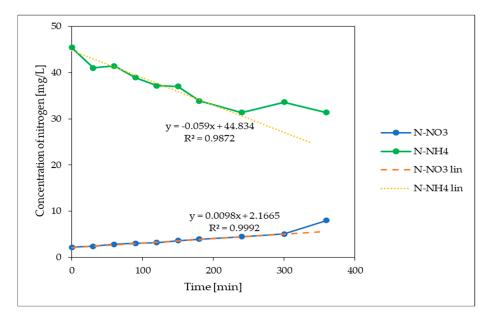


Figure 8. Progress of N-NH<sub>4</sub> and N-NO<sub>3</sub> during the nitrification test.

The nitrification rate can be determined from the rate of decrease in N-NH<sub>4</sub> or the increase in N-NO<sub>3</sub> concentration. Using linear regression, the slope of the linear part of the curves shown in Figure 5 was determined. The volumetric nitrification rate was found to be 0.059 mg/(L·min) N-NH<sub>4</sub> and 0.0098 mg/(L·min) N-NO<sub>3</sub>. These values clearly show that the nitrification rate determined from the decrease in ammonia nitrogen is significantly higher than that measured from the increase in nitrate nitrogen. Considering the concentra-

tion of agglomerated biomass during the test, the nitrification rate is  $2.22 \text{ mg/(g}\cdot\text{h}) \text{ N-NH}_4$  and  $0.365 \text{ mg/(g}\cdot\text{h}) \text{ N-NO}_3$ .

Theoretically, these rates should be comparable, but as indicated by the operational measurements at the WWTP, denitrification occurs even during the aeration phase due to microsegregation of anoxic conditions within the sludge aggregates. Therefore, it can be said with high probability that part of the nitrates formed was immediately denitrified during the tests, despite the dissolved oxygen concentration being 7.8 mg/L during the test. Therefore, the nitrification rate determined from the decrease in ammonia nitrogen concentration should be considered. This rate falls within the range of nitrification rates reported in the literature for suspended activated sludge [19–22]—see Table 4. Our achieved specific nitrification rate values are comparable to those reported for nitrifying-denitrifying AGS with enhanced biological phosphorus removal [15,23], as shown in Table 4. These values were measured for AGS cultivated under laboratory conditions, while in our case, it is real sludge. It is evident that the nitrification rates of pure nitrifying cultures grown on synthetic substrates are significantly higher.

$v_{ m N}$ (mg/g·h)	Conditions	Reference
	Aerobic suspended sludge	
1.5	at $2 \text{ mg } O_2/L$	[19]
1.2–1.4	at X <sub>b</sub> 8.6–14.4 g/L	[20]
1.506	at 1 mg O <sub>2</sub> /L	[01]
3.57	at 4.5 mg O <sub>2</sub> /L	[21]
1.802	at $C/N = 20$	[22]
	Aerobic granulated sludge	
31.25	Pure nitrifying culture	[22]
5.83	Nitri. and denitri. sludge with	[23]
5.65	biol. remov. of P	
29.8	Pure nitrifying culture	[24]
40.36	Pure nitrifying culture	[15]
6.86	Nitri. and denitri. sludge with	[15]
0.00	biol. remov. of P	
2.22	Nitri. and denitri. sludge with	In this study
2.22	biol. remov. of P	in this study

Table 4. Specific nitrification rate from the literature.

When determining the denitrification activity of AGS, the values of N-NO<sub>3</sub> concentrations were measured over time. Glucose was used as an external substrate. The course of the decrease in nitrate nitrogen without the addition of an external substrate (endogenous denitrification) and with the addition of an exogenous substrate (total denitrification) is shown in Figure 9. From the slopes of the linear parts of the denitrification curves, the denitrification rates of aerobic granular biomass were determined using linear regression. The volumetric rate of endogenous denitrification was 0.0095 mg/(L·min) N-NO<sub>3</sub> and the total denitrification rate was 0.0311 mg/(L·min). From these values, we calculated the specific endogenous rate of 0.27 mg/(g·h) and the total denitrification rate of 0.93 mg/(g·h) N-NO<sub>3</sub>. Comparing this with the values for suspended sludge listed in Table 5, we see that it is in the same range [25–27]. However, it should be noted that in our case, the sludge is in oxic conditions throughout the entire semi-continuous cycle. When we compare our measured total denitrification rate with the rates achieved by granular sludge listed in Table 5, it is again comparable [22,28,29]. Higher rates are achieved with granular sludge with larger granule sizes up to 400  $\mu$ m [28].

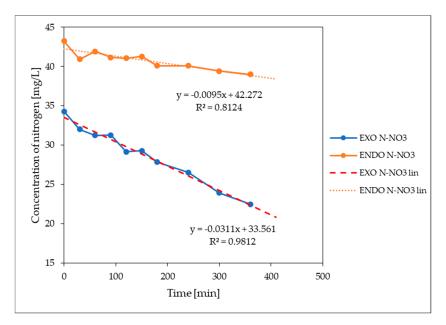


Figure 9. Progress of N-NO<sub>3</sub> during of denitrification test.

$v_{\mathrm{D}}$ (mg/g·h)	Conditions	Reference
	Aerobic suspended sludge	
0.80-0.90	7 °C	[25]
0.97-2.50	Membrane reactor	[26]
1.00-2.20	Pure volatile fatty acids	[27]
	Aerobic granulated sludge	
0.697-0.775	At C/N = 20-10	[22]
0.51	Small AGS diameter < 200 μm	
2.24	Medium AGS diameter 200-400 μm	[28]
5.48	Large AGS diameter > 400 μm	
2.93	$X_{b} 4 g/L$	[29]
0.93	SBR	In this study

Table 5. Specific denitrification rate from the literature.

From the measurements of nitrification and denitrification rates, it is evident that the granular activated sludge from the reconstructed WWTP does not exhibit exceptional parameters that might be expected given the concept of granular biomass. Its unique characteristic is the ability to form larger compact agglomerates, in which an anoxic to anaerobic core is created. This enables simultaneous denitrification and enhanced biological phosphorus removal even at dissolved oxygen concentrations above 3.8%.

# 4. Conclusions

The municipal wastewater-treatment plant with a capacity of 3000 PE did not meet the required parameters of treated wastewater according to current legislation for any of the monitored parameters before the reconstruction. The WWTP was reconstructed following the principles of cultivating aerobic granular sludge, utilizing only the existing volumes of individual structures. Measurements during the start-up and trial operation confirmed that the reconstructed WWTP achieves the required parameters for treated wastewater, even for a WWTP size of up to 100,000 PE.

When measuring the concentrations of monitored parameters during various phases of the semi-continuous cycle, it was found that a significant concentration profile was only observed for ammonia nitrogen, and only at higher loads. For other parameters, their concentration profile was balanced across different phases. The measurement of nitrification and denitrification rates indicates that the agglomerated biomass at the reconstructed WWTP achieves average values characteristic of both suspended activated sludge and aerobic granular sludge with granule sizes up to 400  $\mu$ m.

Changes in energy consumption after the reconstruction of the WWTP were not observed. However, significantly better parameters for treated wastewater were achieved with the same energy consumption.

If the conventional SBR technology had been used in the reconstruction of the WWTP and not the concept with aerobic granular biomass, the energy consumption would certainly be higher.

**Author Contributions:** Conceptualization, M.H. and K.K.; methodology, M.H., K.K. and M.S.; validation, M.H. and B.J.; formal analysis, B.J., M.S., L.J. and N.Š.; investigation, B.J., L.J., M.S. and N.Š.; resources, all authors.; data curation, M.H. and K.K.; writing—original draft preparation, M.H. and K.K.; writing—review and editing, M.H. and K.K.; visualization, B.J., L.J., M.S. and N.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are included within the article.

**Conflicts of Interest:** Author Karol Kratochvíl was employed by the company ASIO-SK s.r.o. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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