Toxic Effect of Ammonium Nitrogen on the Nitrification Process and Acclimatisation of Nitrifying Bacteria to High Concentrations of NH₄-N in Wastewater

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Abstract: The aim of the conducted research was to assess the effectiveness of the nitrification process, at different concentrations of ammonium nitrogen, in biologically treated wastewater in one of the largest municipal and industrial wastewater treatment plants in Poland. The studies also attempted to acclimate nitrifying bacteria to the limited concentration of ammonium nitrogen and determined the efficiency of nitrification under the influence of acclimated activated sludge in the biological wastewater treatment system. The obtained results indicate that the concentration of ammonium nitrogen above 60.00 mg·dm⁻³ inhibits nitrification, even after increasing the biomass of nitrifiers. The increase in the efficiency of the nitrification process in the tested system can be obtained by using the activated sludge inoculated with nitrifiers. For this purpose, nitrifiers should be preacclimated, at least for a period of time, allowing them to colonize the activated sludge. The acclimated activated sludge allows reducing the amount of ammonium nitrogen in treated sewage by approx. 35.0%. The process of stable nitrification in the biological treatment system was observed nine days after introducing the acclimated activated sludge into the aeration chamber.

Keywords: activated sludge; ammonium nitrogen; chemical sewage; nitrification inhibition; acclimatization; nitrifiers

1. Introduction

Nitrogen is the basic building element that regulates biological productivity in the aquatic environment. This element, like other biogeochemical elements, has its own circulation cycle. The main source of nitrogen in wastewater is proteins and other organic compounds, including urea. In the sewage system, as a result of hydrolysis and ammonification processes, part of the nitrogen present in the organic form (especially urea) is converted into the ammonium or ammonia form. The balance between ammonia (NH₃-N) and ammonium (NH₄-N) in wastewater is governed by pH [1]. Additionally, total ammonium nitrogen (TAN) represents the sum of these two forms. Therefore, in the inflow to the treatment plant, total nitrogen (TKN) is usually in the non-oxidized form. The major part of the non-oxidized forms of nitrogen is TAN, while the rest is bound in the form of various organic compounds [2,3].
In wastewater treatment plants that remove biogenic compounds in a biological way, the elimination of ammonium nitrogen is carried out through the processes of metabolic assimilation and dissimilation [4–12]. In the assimilation process, ammonium nitrogen is used to create new, activated sludge cells, which are then removed from the system in the form of biomass [13,14]. The ratio of metabolizable organic carbon to nitrogen (C:N) determines the rate of biomass growth, and thus the amount of ammonium nitrogen removed with excess activated sludge. It also depends on the availability and biodegradability of the wastewater organic carbon. According to the literature data, the most favourable C:N ratio ranges from 10:1 to 12:1 [15] or 17:1 [16] and enables the assimilation of about 20–30% of nitrogen [17–19]. A higher rate of biomass increase is observed in municipal wastewater due to the maintenance of the required proportions between biogenic elements. In the case of industrial wastewater, in which biogenic elements are present in unfavourable proportions or carbon is in a form that is not available for organisms, the rate of biomass increase is lower [20–24]. During metabolic dissimilation, ammonium nitrogen is transformed in the processes of nitrification and then denitrification [25–29]. In the process of nitrification, nitrogen changes from one mineral form (ammonium) to another (nitrite or nitrate). Therefore, this process does not contribute to the direct elimination of nitrogen from the system, but is only a process that determines the occurrence of the second, key dissimilation pathway—denitrification [30,31].

Nitrifying bacteria belong to the most sensitive group of microorganisms involved in the wastewater treatment process [32,33]. The rate of oxidation of ammonium nitrogen during nitrification is influenced by: dissolved oxygen concentration, pH, total alkalinity, ammonia concentration [34–36], C/N ratio [37–39], temperature and the presence of inhibitors [40–42]. Moreover, in order to prevent the removal of nitrifying bacteria from the system and to maintain a constant effect of NH$_4$-N oxidation, a sufficiently long age of the activated sludge and biomass reserve should be ensured [43–45].

The aim of the research was to evaluate the effectiveness of the nitrification process at high concentrations of ammonium nitrogen and to determine the limit concentration of NH$_4$-N inhibiting nitrification. Moreover, the study attempted to acclimatize nitrifying bacteria to the increased concentration of ammonium nitrogen and the effectiveness of NH$_4$-N elimination in the aeration chamber inoculated with acclimatized cultures of nitrifying bacteria in WWTP was checked.

2. Materials and Methods

The research was carried out in the Municipal and Industrial Sewage Treatment Plant in Oświęcim (50°02′17.1″ N 19°19′13.8″ E), one of the largest sewage treatment plants in Poland. The wastewater treatment plant treats industrial and municipal wastewater mixed in a 2:1 ratio. The technological design of the Municipal and Industrial Sewage Treatment Plant in Oświęcim assumed the dephosphatation process, without taking into account nitrification. Due to the above, the basic problem of the sewage treatment plant has become the need to obtain stable nitrification in order to reduce the TAN concentration in the treated sewage supplied to the Vistula River. This task turned out to be difficult, because, in the discussed system, the nitrification process is additionally limited by temperature drops and the presence of inhibitory substances contained in industrial wastewater, flowing from the nearby Chemical Production Plants. The diagram of the conducted experiments is shown in Figure 1.
2.1. Determination of the Limiting Concentration of Ammonium Nitrogen Which Inhibits Nitrification—Laboratory Tests

Industrial wastewater mixed with municipal wastewater in the ratio 2:1, with different concentrations of NH₄-N, was used for the research. Before starting the research, the activated sludge was centrifuged and then washed in an aqueous solution of sodium bicarbonate and ammonium sulphate. The activated sludge prepared in this way was diluted with distilled water in the ratio 1:10 and centrifuged again. Then, the activated sludge was suspended in the appropriate volume of tap water in order to obtain the concentration required for the tests. Before the tests, the TAN concentration in the wastewater was manually adjusted to the values of 140.00 mg·dm⁻³, 120.00 mg·dm⁻³, 100.00 mg·dm⁻³, 80.00 mg·dm⁻³, 70.00 mg·dm⁻³, 60.00 mg·dm⁻³, 50.00 mg·dm⁻³. These concentrations were the initial concentrations in the mixture of wastewater and activated sludge in each test series. In order to carry out the research, two experimental systems with activated sludge were established:
1. Static system with aeration
2. Dynamic system operating under continuous supply conditions based on wastewater flowing to biological treatment.

The basic parameters of both systems were maintained at a level similar to the values of the WWTP parameters (Table 1).

| Technological Parameter                  | Value     |
|-----------------------------------------|-----------|
| pH                                      | 7.0–7.6   |
| DO (mg O₂·dm⁻³)                         | 2–3       |
| Aeration time (h)                       | 4–5       |
| MLVSS (mg·dm⁻³)                         | 3500–4000 |
| Recirculation (% in relation to the incoming sewage) | 95         |
| Dry mass of activated sludge (%)        | 1.64      |
| COD (mg O₂·dm⁻³)                        | 251–393   |
| BOD₅ (mg O₂·dm⁻³)                      | 150–280   |

Under static conditions, a system consisting of fourteen conical flasks with a capacity of 500 cm³ was prepared (Table 2). An amount of 125 cm³ of active sludge with a biomass concentration (MLVSS) 3.748 g·dm⁻³ and 125 cm³ of sewage with the currently tested NH₄-N concentration were introduced into each of the flasks. The volume of the mixture in each of the flasks was 250 cm³. Additionally, the biomass of nitrifying bacteria was increased in seven flasks by adding nitrifying cultures with a biomass of 0.25 mg. The activated sludge was kept in suspension for 4 h by mixing and aerating. A controlled
method of supplying air with a constant capacity of 2 mg O₂·dm⁻³ was used. Then the samples were filtered and the concentration of NH₄-N, NO₂-N and NO₃-N was determined. The experiment was performed in triplicate.

**Table 2. Diagram of a static system.**

| Conical Flasks Content | Flask Number |
|------------------------|--------------|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | 125 125 125 125 125 125 125 125 125 125 125 125 125 125 |
| Activated sludge (cm³) | 125 125 125 125 125 125 125 125 125 125 125 125 125 125 |
| NH₄-N 140.00 mg·dm⁻³ | - - - - - - - - - - - - - - - - |
| NH₄-N 120.00 mg·dm⁻³ | - - - - - - - - - - - - - - - - |
| NH₄-N 100.00 mg·dm⁻³ | - - - - 125 125 - - - - - - - - - - |
| NH₄-N 80.00 mg·dm⁻³ | - - - - - - 125 125 - - - - - - - - - - |
| NH₄-N 70.00 mg·dm⁻³ | - - - - - - - - 125 125 - - - - - - - - - - |
| NH₄-N 60.00 mg·dm⁻³ | - - - - - - - - - - 125 125 - - - - - - - - - - |
| NH₄-N 50.00 mg·dm⁻³ | - - - - - - - - - - - - 125 125 - - - - - - - - - - |
| Nitrifiers (mg) | 0.25 - - 0.25 - 0.25 - 0.25 - 0.25 - 0.25 - 0.25 - 0.25 |
| Total volume (cm³) | 250 250 250 250 250 250 250 250 250 250 250 250 250 250 |

Under dynamic conditions (Figure 1), a system consisting of a glass bottle (3) with a capacity of 10 dm³ was used for the sewage flowing into the aeration chamber through a silicone hose connected to a peristaltic MICRODOSING PUMP TYPE 335 with a capacity of 20–40 cm³·min⁻¹. Another element of the system is an aeration chamber (1) with a capacity of 1 dm³ with a side outlet connected by a silicone hose with a peristaltic pump discharging the activated sludge with sewage to the secondary settling tank (4). The lower part of the chamber was equipped with a Schott G-1 filter, terminated with an outlet connected through a silicone hose with the pump aerating the active sludge with compressed air VEB ELMET TYPE Fp 09 (2). The vertical secondary settling tank was equipped with two outlets, a side discharge of the supernatant to the treated sewage tank (5) and a bottom one through which the activated sludge was recirculated to the aeration chamber with a silicone hose connected to the peristaltic pump. HRT was about 4 h.

Activated sludge with a volume of 500 cm³ and a biomass concentration (MLVSS) of 3.748 g·dm⁻³ was mixed with nitrifying bacteria (biomass of 1.00 mg) and sewage with a specific concentration of NH₄-N (in a 1:1 ratio), then placed in the chamber and aerated compressed air VEBAEMET TYPE Fp 09 pump (2). The concentration of NH₄-N in the system at the beginning of the experiment was 70.00 mg·dm⁻³. The mixture was alternately aerated with compressed air at four-hour intervals using a VEB ELMET TYPE Fp 09 pump (2) and sedimented. The duration of the experiment was 8 h. Samples were collected from the treated sewage tank each hour, filtered and the concentration of NH₄-N, NO₂-N and NO₃-N was determined.

### 2.2. Acclimatisation of Nitrifiers to the Limit Concentration of NH₄-N

Due to the removal of nitrifying bacteria from the system, an attempt was made to populate the activated sludge with nitrifying cultures. For this purpose, in the first series of tests (Figure 1) nitrifying bacteria (with a biomass of 3.00 mg for each dm³ of sludge) were added to 3 dm³ of activated sludge (with a biomass of 3.748 g·dm⁻³), the whole was mixed with an ammonium sulphate solution (buffered with sodium bicarbonate) in a ratio of 1:1 (to maintain the pH at about 7) and transferred to the aeration chamber (1). The concentration of NH₄-N in the system at the beginning of the experiment was 70.00 mg·dm⁻³. The mixture was alternately aerated with compressed air for four-hour intervals using a VEB ELMET TYPE Fp 09 pump (2) and sedimented. The concentration of dissolved oxygen in the aeration chamber was about 2 mg·dm⁻³. The experiment was carried out for 4 days. Every 24 h a small amount of supernatant was collected, filtered and the concentration of NH₄-N, NO₂-N and NO₃-N was determined. At the same time, after each test day, an activated sludge (MLVSS=3.879 g·dm⁻³) sample was collected from the system, with a volume of 500 cm³, which was transferred in the aeration chamber, mixed in a 1:1 ratio.
with the sewage with a NH$_4$-N concentration of 70.00 mg dm$^{-3}$ and used in dynamic tests (as described in Section 2.1.), dosing sewage (with the tested concentration of TAN) into the chamber in the amount of 20 cm$^3$·min$^{-1}$. The studies were conducted over eight hours. Every hour the concentration of NH$_4$-N, NO$_2$-N and NO$_3$-N was determined in the sample of treated sewage.

In the second series of tests, in order to increase the biomass of nitrifying bacteria in the activated sludge chamber, a breeding system was established consisting of two tanks with a capacity of 250 dm$^3$ each (Figure 1). Active sludge (MLVSS–3.879 g dm$^{-3}$) mixed with sewage in a 1:1 ratio was placed in both tanks (1), and then previously grown nitrifying bacteria with a biomass of 30.00 mg were introduced into each tank.

In order to enable the nitrifiers to colonize the activated sludge, both tanks were alternately aerated compressed air (2) with a constant efficiency at the level of 2 mg O$_2$·dm$^{-3}$ (4 h) and subjected to sedimentation (4 h) for a period of 4 days. Every 24 h, a sample was collected from each tank and the concentration of NH$_4$-N, NO$_2$-N and NO$_3$-N was determined after filtering.

After four days, when the concentration of TAN in both tanks decreased significantly, their content (400 dm$^3$) was introduced into the working aeration chamber (research in a working WWTP). The effectiveness of TAN removal by activated sludge enriched in nitrifying bacteria cultures was assessed based on the differences in the concentration of NH$_4$-N, NO$_2$-N and NO$_3$-N in the wastewater flowing into the aeration chamber and in the treated sewage. Samples were taken every 24 h and observations were made for 16 days.

2.3. Isolation of Cultures of Nitrifying Bacteria

Cultures of nitrosifying bacteria were isolated from the activated sludge from the Municipal Industrial Sewage Treatment Plant in Oświęcim using the serial dilution technique in order to minimize the amount of organic compounds and eliminate heterotrophic organisms. Then the isolates were grown on mineral liquid media, enriched with ammonia (culture of the first phase nitrifiers) and nitrite (culture of the second phase nitrifiers) [46].

A mineral medium was used for the isolation and cultivation of the first phase nitrifiers belonging to the genus *Nitrosomonas* [46]. For this purpose, the following was added to a small amount of distilled water:

K$_2$HPO$_4$—0.5 g  
(NH$_4$)$_2$SO$_4$—0.5 g  
phenol red—0.5 g  
MgSO$_4$·7H$_2$O—0.05 g  
CaCl$_2$·2H$_2$O—0.02 g  
NaCl—0.02 g  
Na$_2$MoO$_4$·2H$_2$O—2.4 µg  
microelements—1 cm$^3$

The microelements solution was prepared by adding a few drops of H$_2$SO$_4$ to a small amount of distilled water, and then:

ZnSO$_4$·7H$_2$O—1.1 g  
FeSO$_4$·7H$_2$O—0.5 g  
EDTA—0.25 g  
MnSO$_4$·7H$_2$O—0.154 g  
CuSO$_4$·5H$_2$O—0.04 g  
Co(NO$_3$)$_2$·6H$_2$O—0.025 g  
Na$_2$B$_4$O$_7$·10H$_2$O—0.018 g, the whole was made up of water to 100 cm$^3$.

Then, the medium was supplemented with distilled water to a volume of 1 dm$^3$ and sterilized in an autoclave for 15 min, at 121 °C, under a pressure of 1 atmosphere. The pH of the medium was 7.5.
A mineral medium was used for the isolation and cultivation of the second phase nitrifiers belonging to the genus *Nitrobacter* [46]. For this purpose, the following was added to a small amount of distilled water:

\[
\begin{align*}
\text{KHCO}_3 & : 1.5 \text{ g} \\
\text{KH}_2\text{PO}_4 & : 0.5 \text{ g} \\
\text{K}_2\text{HPO}_4 & : 0.5 \text{ g} \\
\text{KNO}_2 & : 0.3 \text{ g} \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & : 0.2 \text{ g} \\
\text{NaCl} & : 0.2 \text{ g} \\
\text{CaCl}_2 \cdot 2\text{H}_2\text{O} & : 0.01 \text{ g} \\
\text{FeSO}_4 \cdot 7\text{H}_2\text{O} & : 0.01 \text{ g}.
\end{align*}
\]

The prepared medium was supplemented with distilled water to the volume of 1 dm\(^3\). The medium was sterilized in an autoclave for 15 min at 121 °C and 1 atmosphere pressure. The pH of the medium was 7.5.

The nitrifying bacteria cultures were incubated for 14 days at 28 °C.

### 2.4. Analytical Methods

- **pH**—determined using a UF 100-01 UniFETTM pH meter.
- **DO**—was determined using a MYCOM/COM 141 S-1A00 ELMETRON oxygen meter. This probe consists of two electrodes, an electrolyte layer surrounding the electrodes and an oxygen-permeable membrane that separates the electrodes and electrolyte from the test sample. The determination consisted in measuring the intensity of the current as a result of the reduction in oxygen particles on the cathode. Dissolved oxygen concentration was measured continuously and the result was given in mg·dm\(^{-3}\).
- **MLVSS**—the concentration of activated sludge biomass was determined by the weight method. For this purpose, 250 cm\(^3\) of a well-mixed sample was filtered through a medium, balanced filter paper, and then the material was dried to constant weight at 105 °C. The amount of total suspended solids was calculated according to the formula:

\[
X = \frac{(m_2 - m_1) \cdot 1000}{V}
\]

Where:
- \(X\)—biomass concentration, mg·dm\(^{-3}\)
- \(m_1\)—weight of the weighing bottle and the dried filter, mg
- \(m_2\)—weight of the weighing bottle with filter and activated sludge, mg
- \(V\)—volume of the sample taken for testing, cm\(^3\)
- **COD**—determined by the bichromate method. For this purpose, the number of milligrams of potassium dichromate was determined, converted into milligrams of oxygen used for the oxidation of organic compounds and some inorganic compounds present in the analyzed wastewater. The oxidation process was carried out in sulfuric acid in the presence of silver sulfate as a catalyst. The results were calculated according to the formula:

\[
X = \left(\frac{V_2 - V_1}{V}\right) \cdot n \cdot 8 \cdot 1000 \cdot f
\]

Where:
- \(V\)—the volume of the wastewater sample, cm\(^3\)
- \(V_1\)—volume of ferrous ammonium sulphate solution used for titration of the test sample, cm\(^3\)
- \(V_2\)—the volume of the ferrous ammonium sulphate solution used for the titration of the control sample, cm\(^3\)
- \(n\)—normality of the ferrous ammonium sulphate solution
- 8—the conversion factor of the result into milligrams of oxygen
- \(f\)—factor of ferrous ammonium sulphate solution

- The concentration of NH\(_4\)-N, NO\(_2\)-N, NO\(_3\)-N—was determined by the colorimetric method on the CADAS 30S spectrophotometer by Dr Lange, using appropriate cu-
The following tests were used to determine the concentration of NH4-N: LCK-302 (with a measuring range of 47.00–130.00 mg.dm⁻³), LCK-303 (with a measuring range of 2.00–47.00 mg.dm⁻³) and LCK-304 (with a measuring range of 0.015–2.00 mg.dm⁻³). The concentration of NO2-N was determined based on the LCK-341 test (with a measuring range of 0.015–0.60 mg.dm⁻³). As in many test series, nitrite nitrogen was present in trace amounts and its concentration was beyond the detection range of the CADAS 30S spectrophotometer, on most Tables, this parameter was omitted. The LCK-339 tests (with a measuring range of 0.23–13.50 mg.dm⁻³) and LCK-340 (with a measuring range of 5.00–35.00 mg.dm⁻³) were used to determine the concentration of NO3-N.

- The specific rate of nitrification was determined on the basis of changes in NH4-N concentration, according to the formula:

\[
X = \frac{(NH_4-N)_{t_1} - (NH_4-N)_{t_2}}{MLVSS \cdot 4}
\]

where:
- \(X\)—specific rate of nitrification, mg·gh⁻¹
- \(NH_4-N\)—concentration of ammonium nitrogen, mg.dm⁻³
- \(t\)—time of the initial \((t_1)\) and final \((t_2)\) measurement, h
- \(MLVSS\)—concentration of activated sludge biomass used for research, g.dm⁻³.

### 2.5. Statistical Analysis

To determine the significance of differences between the concentration of NH₄-N, NO₂-N, and NO₃-N in the samples (depending on the variant of the experiment), a one-way ANOVA analysis was applied—Tukey test aimed to verify the differences in concentrations (significant differences for \(p < 0.05\)). The tests were performed in Statistica v. 13.1 (StatSoft, Inc., Tulsa, OK, USA).

### 3. Results

#### 3.1. Determination of the Limit Concentration of Ammonium Nitrogen

In the first series of tests, the limit concentration of TAN was determined in wastewater directed to biological treatment, above which the nitrification process is inhibited. The tests were carried out in static conditions, during a four-hour aeration time on a laboratory scale.

The results obtained in the first test series (Table 3) indicate a low efficiency of NH₄-N oxidation both in the samples containing cultures of nitrifying bacteria, with a biomass of 0.25 mg, and in the control samples, only when the concentration of ammonium nitrogen did not exceed 60.00 mg.dm⁻³ (Figure 2). Starting from the concentration of NH₄-N, amounting to 70.00 mg.dm⁻³, there was a clear inhibition of nitrification. In the samples containing TAN in the concentration of 140.00 mg.dm⁻³, 120.00 mg.dm⁻³ and 100.00 mg.dm⁻³, inhibition of both the first and the second phase of nitrification was observed. In this series of tests, the average specific nitrification rate of the control samples was 0.44, and of the samples with the addition of nitrifying bacteria—0.76. A slight loss of ammonium nitrogen in these samples (1.60 mg.dm⁻³ on average) could have occurred because of chemical oxidation, stripping, denitrification or incorporation into the biomass of activated sludge. Tests performed on wastewater, where the concentration of ammonium nitrogen was 70.00 mg.dm⁻³ and 80.00 mg.dm⁻³, respectively, indicate a greater sensitivity of the second phase nitrifiers to the toxic effect of this ion. This is evidenced by the accumulation of NO₂-N in the samples after four hours of aeration, with a slight increase in the concentration of NO₃-N, on average by approx. 1.00 mg.dm⁻³.
Influence of nitrifying bacteria cultures on the efficiency of NH₄-N oxidation—static conditions. Statistical analysis showed significant differences in concentration of NH₄-N between the samples containing cultures of nitrifying bacteria (with a biomass of 0.25 mg) and the control samples, only when the concentration of ammonium nitrogen was not exceeded 60.00 mg dm⁻³ (for 50 and 60 mg dm⁻³ of NH₄-N). When the concentration of ammonium nitrogen was exceeded 60.00 mg dm⁻³ (70–140 mg dm⁻³), there were no significant differences in concentration of NH₄-N between the samples containing cultures of nitrifying bacteria, and the control samples. The analysis showed significant differences in average concentration of NH₄-N in control samples and samples with cultures of nitrifying bacteria after 4 h of aeration, only when the concentration of ammonium nitrogen was not exceeded 60.00 mg dm⁻³.
Conducting tests under static conditions, it was found that the lowest concentration of NH₄-N, which is toxic to nitrifying bacteria, was 70.00 mg·dm⁻³. Therefore, using NH₄-N concentrations of 70.00 mg·dm⁻³, 60.00 mg·dm⁻³ and 50.00 mg·dm⁻³, respectively, in the second series the model tests were continued in the activated sludge chamber under dynamic conditions. For this purpose, active sludge mixed with sewage, with the currently tested concentration of NH₄-N, was introduced into the aeration chamber, nitrifying bacteria with a biomass of 1.00 mg were added and sewage was dosed in the amount of 20 cm³·min⁻¹. for a period of eight hours.

In the case of wastewater in which the concentration of NH₄-N was 70.00 mg·dm⁻³, the toxic effect of TAN on the nitrifying bacteria of the second phase was clearly visible (Table 4). During the eight-hour test cycle, the NO₃-N concentration increased only by approx. 1.00 mg·dm⁻³, which confirms the results obtained in static tests. In the case of the first phase of nitrification, a slight and constant loss of NH₄-N, were observed (62.76 mg·dm⁻³), in the fourth hour of the experiment. In the fifth hour of the tests, the concentration of ammonium nitrogen increased to 64.24 mg·dm⁻³ and remained more or less than this until the end of the tests (up to the eighth hour). The specific nitrification rate was 0.28. However, there were no significant differences in the concentration of NH₄-N and NO₃-N during the eight-hour test cycle (Tukey test, p > 0.05).

Table 4. The effect of ammonium nitrogen at a concentration of 70 mg·dm⁻³ on the activity of nitrifying bacteria—dynamic conditions.

| Experiment Time (h) | NH₄-N | Removal NH₄-N (%) | NO₃-N | Increase in NO₃-N Production (mg·dm⁻³) |
|---------------------|-------|------------------|-------|--------------------------------------|
| 0                   | 70.00a * | - | 0.00a | -                                    |
| 1                   | 68.72a  | 1.83 | 0.01a | -                                    |
| 2                   | 65.24a  | 6.80 | 0.12a | 0.11                                 |
| 3                   | 64.12a  | 8.40 | 0.06a | 0.50                                 |
| 4                   | 62.76a  | 10.34 | 1.02a | 1.01                                 |
| 5                   | 64.24a  | 8.23 | 1.01a | 1.00                                 |
| 6                   | 65.18a  | 6.89 | 0.93a | 0.92                                 |
| 7                   | 67.23a  | 3.96 | 1.11a | 1.10                                 |
| 8                   | 65.74a  | 6.09 | 1.13a | 1.12                                 |

MLVSS—3.748 g·dm⁻³; * averages marked with the same letters are not significantly different by Tukey test (α = 0.05) (one-way ANOVA, taking into account the time of the experiment).

In the case of tests on wastewater containing TAN at a concentration of 60.00 mg·dm⁻³, in the first hour of the experiment, a clear loss of NH₄-N by 16.88 mg·dm⁻³ was observed (Table 5). In the following hours of the experiment, the concentration of ammonium nitrogen fluctuated slightly, reaching a value equal to 46.11 mg·dm⁻³ (at eight hours), i.e., close to the concentration of NH₄-N in the control test, after four hours of static aeration (45.19 mg·dm⁻³, Table 3). The concentration of NH₄-N 60.00 mg·dm⁻³ did not inhibit the second phase of nitrification. The content of nitrate nitrogen from the first to the eighth hour of the experiment was on the average level of about 11.00 mg·dm⁻³, which was not reflected in the static tests, in which after four hours of aeration, the concentration of this ion in the control flask was only 4.29 mg·dm⁻³, and in the flask with the addition of nitrifying bacteria—9.36 mg·dm⁻³ (Table 3). The specific nitrification rate was 0.93. In this case, the analysis showed significant differences in the concentration of NH₄-N and NO₃-N during the eight-hour test cycle (Tukey test, p < 0.05).
Table 5. The effect of ammonium nitrogen at a concentration of 60 mg·dm\(^{-3}\) on the activity of nitrifying bacteria—dynamic conditions.

| Experiment Time (h) | Concentration (mg dm\(^{-3}\)) 1 | Removal NH\(_4\)-N (%) | NO\(_3\)-N Increase in NO\(_3\)-N Production (mg dm\(^{-3}\)) |
|---------------------|----------------------------------|------------------------|--------------------------------------------------|
| 0                   | 60.00b *                          | -                      | 0.00a                                            |
| 1                   | 43.12a                           | 28.13                  | 9.48b                                            |
| 2                   | 48.16a                           | 19.73                  | 10.90b                                           | 1.42 |
| 3                   | 52.64ab                          | 12.27                  | 11.92b                                           | 2.44 |
| 4                   | 49.46a                           | 17.57                  | 12.63b                                           | 3.15 |
| 5                   | 47.20a                           | 21.33                  | 11.51b                                           | 2.03 |
| 6                   | 45.13a                           | 24.78                  | 12.14b                                           | 2.66 |
| 7                   | 47.23a                           | 21.28                  | 10.09b                                           | 0.61 |
| 8                   | 46.11a                           | 23.15                  | 11.29b                                           | 1.81 |

| Experiment Time (h) | Concentration (mg dm\(^{-3}\)) 1 | Removal NH\(_4\)-N (%) | NO\(_3\)-N Increase in NO\(_3\)-N Production (mg dm\(^{-3}\)) |
|---------------------|----------------------------------|------------------------|--------------------------------------------------|
| 0                   | 50.00b *                          | -                      | 0.00a                                            |
| 1                   | 29.83a                           | 40.34                  | 9.48b                                            |
| 2                   | 36.94a                           | 26.12                  | 12.93b                                           | 3.28 |
| 3                   | 39.12ab                          | 21.76                  | 14.06b                                           | 4.41 |
| 4                   | 35.79a                           | 28.42                  | 12.11b                                           | 2.46 |
| 5                   | 26.11a                           | 47.78                  | 13.62b                                           | 3.97 |
| 6                   | 29.16a                           | 41.68                  | 15.91b                                           | 6.26 |
| 7                   | 28.14a                           | 43.74                  | 14.26b                                           | 4.61 |
| 8                   | 28.95a                           | 42.10                  | 15.08b                                           | 5.43 |

1 MLVSS—3.748 g·dm\(^{-3}\); * averages marked with the same letters are not significantly different by Tukey test (\(\alpha = 0.05\)) (one-way ANOVA, taking into account the time of the experiment).

Similar results were obtained when examining the sewage in which the concentration of ammonium nitrogen was 50.00 mg·dm\(^{-3}\) (Table 6). In the first hour of the experiment, the concentration of NH\(_4\)-N decreased by 20.17 mg·dm\(^{-3}\), and then it fluctuated, reaching in the eighth hour the value equal to 28.95 mg·dm\(^{-3}\). In the case of nitrate nitrogen, in the first hour of the tests, the concentration of this ion increased to 9.65 mg·dm\(^{-3}\), and in the eighth hour to 15.08 mg·dm\(^{-3}\). The specific nitrification rate was 1.41. Thus, both the concentration of NH\(_4\)-N and NO\(_3\)-N after eight hours of aeration under dynamic conditions confirm the results obtained in control tests, during static tests (Table 3). The statistical analysis showed significant differences in the concentration of NH\(_4\)-N and NO\(_3\)-N during the eight-hour test cycle (Tukey test, \(p < 0.05\)).

Table 6. The effect of ammonium nitrogen at a concentration of 50 mg·dm\(^{-3}\) on the activity of nitrifying bacteria—dynamic conditions.

| Experiment Time (h) | Concentration (mg dm\(^{-3}\)) 1 | Removal NH\(_4\)-N (%) | NO\(_3\)-N Increase in NO\(_3\)-N Production (mg dm\(^{-3}\)) |
|---------------------|----------------------------------|------------------------|--------------------------------------------------|
| 0                   | 50.00b *                          | -                      | 0.00a                                            |
| 1                   | 29.83a                           | 40.34                  | 9.65b                                            |
| 2                   | 36.94a                           | 26.12                  | 12.93b                                           | 3.28 |
| 3                   | 39.12ab                          | 21.76                  | 14.06b                                           | 4.41 |
| 4                   | 35.79a                           | 28.42                  | 12.11b                                           | 2.46 |
| 5                   | 26.11a                           | 47.78                  | 13.62b                                           | 3.97 |
| 6                   | 29.16a                           | 41.68                  | 15.91b                                           | 6.26 |
| 7                   | 28.14a                           | 43.74                  | 14.26b                                           | 4.61 |
| 8                   | 28.95a                           | 42.10                  | 15.08b                                           | 5.43 |

1 MLVSS—3.748 g·dm\(^{-3}\); * averages marked with the same letters are not significantly different by Tukey test (\(\alpha = 0.05\)) (one-way ANOVA, taking into account the time of the experiment).

The obtained results indicate that the cultures of nitrifying bacteria added to the samples did not manage to colonize the activated sludge flocs and were removed from the system together with the treated sewage.

3.2. Acclimatisation of Nitrifying Bacteria to the Limit Concentration of Ammonium Nitrogen

Due to the removal of nitrifying bacteria from the system, an attempt was made to adapt the activated sludge by inoculating with nitrifying cultures. For this purpose, the research was conducted in two parallel series.

In the first research series, activated sludge, to which nitrifier cultures with a biomass of 3.00 mg were added for each dm\(^3\) of activated sludge, were alternately aerated (4 h) and
sedinmented (4 h) for four days. In order to prevent the removal of nitrifying bacteria from the system, the tests were carried out in static conditions, without dosing and discharging sewage. The results obtained in the first series of tests (Figure 3) showed that over time there was a constant significant decrease in the content of TAN, the initial concentration of which in the sample was 70.00 mg dm$^{-3}$, while there was a constant significant increase in nitrate concentration (Tukey test, $p < 0.05$). Already after the first day of testing in the system, a significant decrease in the concentration of NH$_4$-N (from 70.00 mg dm$^{-3}$ to 49.46 mg dm$^{-3}$) was observed, amounting to 20.54 mg dm$^{-3}$, with a simultaneous increase in the concentration of NO$_3$-N from 0.00 mg dm$^{-3}$ to 6.72 mg dm$^{-3}$. In the last monitoring day (after 96 h), the concentration of TAN decreased by 63.98 mg dm$^{-3}$, reaching the value of 6.02 mg dm$^{-3}$, with an increase in the concentration of NO$_3$-N to 35.22 mg dm$^{-3}$. The specific nitrification rate was 4.23. As on the fourth day of the experiment the NH$_4$-N concentration dropped to 6.02 mg dm$^{-3}$, the tests under static conditions were discontinued due to an insufficient amount of substrate necessary for the further course of nitrification.

Parallel to the static tests, an experiment was conducted under dynamic conditions to determine the effect of the time of settling of activated sludge by nitrifier cultures on the efficiency of TAN oxidation. Therefore, every 24 h, a sample of acclimated activated sludge was collected, which was used in studies taking into account the constant inflow of sewage (with the concentration of NH$_4$-N equal to 70.00 mg dm$^{-3}$) to the system. At the same time, under the same conditions, control tests were carried out in which activated sludge was added to the system and, immediately before the start of aeration, nitrifier cultures with a biomass of 3.00 mg for each dm$^3$ of activated sludge (Figure 4). The specific nitrification rate was 0.23. There were no statistically significant differences in concentration of NH$_4$-N and NO$_3$-N during eight-hour control tests under dynamic conditions (Tukey test, $p > 0.05$).

The obtained results (Figure 5a–d) indicate that even a 24-h time of settling the activated sludge by nitrifying bacteria increases the efficiency of the nitrification process (Figure 5a; Tukey test, $p < 0.05$). The specific nitrification rate was 0.70.
Figure 4. Removal of TAN at a concentration of 70.00 mg dm\(^{-3}\) by the activated sludge method—control tests under dynamic conditions; *averages marked with the same letters are not significantly different by Tukey test (α = 0.05) (one-way ANOVA, separately for concentration of NH\(_4\)-N and NO\(_3\)-N).

Figure 5. Influence of the (a) 24-h, (b) 48-h, (c) 72-h, (d) 96-h time of settling of activated sludge by nitrifying bacteria on the increase in the efficiency of the nitrification process—dynamic conditions; *averages marked with the same letters are not significantly different by Tukey test (α = 0.05) (one-way ANOVA, separately for concentration of NH\(_4\)-N and NO\(_3\)-N).

The studies conducted on activated sludge, in which nitrifying bacteria were colonized for 48 h, indicate a further gradual increase in the efficiency of the nitrification process (Figure 5b; Tukey test, p < 0.05). The concentration of TAN gradually decreased during the eight hours of the experiment, reaching its final value equal to 46.12 mg dm\(^{-3}\). The loss
of NH$_4$-$N$ in the sample amounted to 23.88 mg·dm$^{-3}$, so compared to the control sample it was greater by 19.40 mg·dm$^{-3}$. The specific nitrification rate was 1.58. Additionally, in this case, the concentration of NO$_3$-$N$ increased, reaching the value of 7.78 mg·dm$^{-3}$ in the eighth hour of the experiment.

In the case of tests with the use of activated sludge, in which the time of colonization by nitrifying bacteria was extended to 72 h, the concentration of NH$_4$-$N$ decreased by 38.79 mg·dm$^{-3}$, reaching a value of 31.21 mg·dm$^{-3}$ after eight hours of experiment (Figure 5c; Tukey test, $p < 0.05$). The loss of NH$_4$-$N$ in the system, compared to the control sample, increased by 34.31 mg·dm$^{-3}$. The specific nitrification rate was 2.56. The studies also showed a much more effective functioning of the second phase nitrifying bacteria, as evidenced by the increase in the concentration of N-NO$_3$ from 0.00 mg·dm$^{-3}$ to 10.98 mg·dm$^{-3}$.

The most favourable results were obtained in studies with the activated sludge colonized with nitrifiers for 96 h. In this case, the concentration of NH$_4$-$N$ in the eighth hour of the experiment decreased by 41.99 mg·dm$^{-3}$, reaching the final value of 28.01 mg·dm$^{-3}$ (Figure 5d; Tukey test, $p < 0.05$). The specific nitrification rate was 2.77. Compared to the control sample, the loss of NH$_4$-$N$ in the system increased by 37.51 mg·dm$^{-3}$. It should be noted that the stable nitrification process was achieved already in the fifth hour of the experiment. From the fifth to the eighth hour of aeration, the NH$_4$-$N$ concentration was around 28.00 mg·dm$^{-3}$, while the NO$_3$-$N$ content was around 12.00 mg·dm$^{-3}$.

In the second series of studies, the effectiveness of the nitrification process on the macro scale in the working aeration chamber was assessed under the influence of the increased biomass of nitrifiers in the activated sludge. The research was conducted in two stages.

In the first stage, the activated sludge (MLVSS-3.879 g·dm$^{-3}$) was colonized with cultures of nitrifying bacteria. The research was carried out in a model system under static conditions, acclimating the sludge until the maximum degree of NH$_4$-$N$ oxidation was achieved in the wastewater directed for biological treatment. During the period of the research, the average concentration of TAN in the sewage flowing into the biological treatment system was approx. 30.00 mg·dm$^{-3}$, the concentration of NH$_4$-$N$ in the model system was adjusted to 35.00 mg·dm$^{-3}$ (Figure 6). The loss of NH$_4$-$N$ on the first day of the experiment was only 3.55 mg·dm$^{-3}$ (Tukey test, $p > 0.05$). However, after a period of sludge acclimatisation, were observed the decrease in TAN concentration and at the final of the monitored day, the effluent was with 2.13 mg·dm$^{-3}$. The specific nitrification rate was 2.13. Concomitantly, the concentration of NO$_3$-$N$ increased by 31.28 mg·dm$^{-3}$ (Tukey test, $p < 0.05$). These studies confirmed the results obtained in laboratory conditions (Figure 3), disregarding the fact that the biomass used to colonize activated sludge flocs was shown 10 times lower.

In the second stage, the activated sludge (MLVSS-3.879 g·dm$^{-3}$), acclimated in model studies, was used to carry out the relevant research in the WWTP. For this purpose, the activated sludge previously inhabited by nitrifiers was transferred to one aeration chamber and for the next 16 days the effectiveness of the nitrification process in the system was assessed (Table 7 and Figure 7). On the first day from the inoculation of the chamber with the acclimated-activated sludge, the concentration of ammonium nitrogen significantly decreased by 7.86 mg·dm$^{-3}$, while the concentration of NO$_3$-$N$ significantly increased by 7.73 mg·dm$^{-3}$ (Tukey test, $p < 0.05$). The difference in the concentration of both forms of nitrogen proves that TAN was completely oxidized by the nitrification process, and not removed as a result of the increase in biomass. On the second and third days, TAN and nitrate nitrogen were lost (Tukey test, $p < 0.05$). This removal could be attached by the simultaneous consortium with heterotrophic bacteria (denitrification process). Then, in the following days, a successive significant decrease in the concentration of NH$_4$-$N$ and a significant increase in the concentration of NO$_3$-$N$ were observed. Nine days after inoculation of the chamber with the acclimated active sludge, a stable nitrification process was achieved, with an average concentration of NH$_4$-$N$ in the treated sewage amounting to
approx. 0.72 mg·dm⁻³ and an average concentration of NO₃-N of approx. 10.24 mg·dm⁻³. The specific nitrification rate was 1.94.

![Figure 6](image)

**Figure 6.** Settlement of activated sludge by nitrifying bacteria; * averages marked with the same letters are not significantly different by Tukey test (α = 0.05) (one-way ANOVA, separately for concentration of NH₄-N and NO₃-N).

| Table 7. The differences in average concentration of NH₄-N and NO₃-N (mg·dm⁻³) in the Municipal and Industrial Sewage Treatment Plant in Oświęcim. |
|---|---|---|---|---|
| Experiment Time (Days) | Wastewater Temperature (°C) | Concentration (mg·dm⁻³) Inflowing Sewage | Concentration (mg·dm⁻³) Treated Sewage |
| | | NH₄-N | NH₄-N | NO₃-N |
| 0 | 14.3 | 32.30 | 12.30e* | 7.57a |
| 1 | 15.0 | 32.30 | 4.44d | 15.30d |
| 2 | 14.7 | 32.30 | 3.33c | 7.73a |
| 3 | 14.0 | 34.20 | 3.15bc | 6.70a |
| 4 | 11.9 | 34.20 | 3.27bc | 6.98a |
| 5 | 12.0 | 34.20 | 2.54b | 8.65ab |
| 6 | 11.0 | 27.70 | 2.16b | 8.99b |
| 7 | 11.1 | 27.70 | 3.31c | 7.21a |
| 8 | 11.2 | 37.50 | 2.04b | 9.82b |
| 9 | 11.1 | 37.50 | 1.08a | 9.94b |
| 10 | 11.2 | 37.50 | 1.01a | 9.99b |
| 11 | 11.2 | 31.60 | 0.93a | 10.01b |
| 12 | 11.8 | 31.60 | 0.61a | 10.00b |
| 13 | 10.5 | 31.60 | 0.92a | 9.48b |
| 14 | 10.6 | 28.80 | 0.32a | 11.30 |
| 15 | 10.3 | 28.80 | 0.64a | 10.60c |
| 16 | 10.9 | 28.80 | 0.28a | 10.60bc |

*averages marked with the same letters are not significantly different by Tukey test (α = 0.05) (one-way ANOVA, taking into account the time of the experiment).
In parallel, tests were carried out in a control chamber where no activated sludge with nitrifiers was added. The comparison among the introduction of the acclimated-activated sludge into the aeration chamber with the control (Table 8 and Figure 8) showed the significant difference in the decrease TAN in the treated sewage, with a simultaneous increase in the concentration of nitrate nitrogen (Tukey test, \( p < 0.05 \)). The tests in the control chamber were carried out in similar conditions, i.e., with the average concentration of NH\(_4\)-N in the incoming sewage, amounting to 30.00 mg dm\(^{-3}\), biomass concentration 3.871 g dm\(^{-3}\) and with the average sewage temperature of 12.5 °C. The specific nitrification rate was much lower (0.6).

Table 8. The differences in average concentration of NH\(_4\)-N and NO\(_3\)-N (mg dm\(^{-3}\)) in the biological wastewater treatment system in the control chamber.

| Experiment Time (days) | Wastewater Temperature (°C) | Concentration (mg dm\(^{-3}\)) Inflowing Sewage | Concentration (mg dm\(^{-3}\)) Treated Sewage |
|------------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|
|                        |                             | NH\(_4\)-N | NH\(_4\)-N | NO\(_3\)-N |
| 0                      | 12.0                        | 28.90      | 19.20c *    | 0.74a      |
| 2                      | 12.6                        | 29.60      | 22.70d      | 1.07a      |
| 4                      | 13.6                        | 29.20      | 25.50e      | 1.81b      |
| 6                      | 13.8                        | 28.80      | 24.10e      | 4.76c      |
| 8                      | 10.3                        | 41.60      | 24.10e      | 2.08b      |
| 10                     | 10.4                        | 28.80      | 22.50d      | 2.77b      |
| 12                     | 11.6                        | 26.30      | 15.20b      | 4.17c      |
| 14                     | 11.6                        | 23.90      | 18.90c      | 1.57a      |
| 16                     | 12.9                        | 33.80      | 12.74a      | 2.53b      |

*averages marked with the same letters are not significantly different by Tukey test (\( \alpha = 0.05 \)) (one-way ANOVA, taking into account the time of the experiment).
concentration in inhibited specific tation wastewater the period of aeration, the concentration of ammonium nitrogen in the control flask without supported by nitrifiers added to the sample, but with time their successive removal from that observed in static conditions after four hours of aeration, using the same concentrations further studies determined the effect of increased biomass of nitrifying bacteria on the efficiency of the nitrification process, or even its complete absence, due to the low efficiency of the nitrification process. Due to this fact, supported the process of ammonia oxidation, however, they were equally sensitive to high concentrations of TAN on the nitrification process. According to the literature, nitrifying bacteria are more sensitive to the influence of NH$_4$-N than nitrifiers of the first phase belonging to the genus of Nitrosonomas [49]. In samples where the NH$_4$-N concentration was 100.00 mg·dm$^{-3}$, 120.00 mg·dm$^{-3}$ and 140 mg·dm$^{-3}$, complete inhibition of nitrification was observed, both in the first and second phases. 

Due to the low efficiency of the nitrification process, or even its complete absence, cultures of previously isolated nitrifying bacteria were added to the systems. The obtained results confirmed that the NH$_4$-N concentration exceeding the value of 60.00 mg·dm$^{-3}$, inhibits the nitrification process. Cultures of nitrifying bacteria, added to the samples, supported the process of ammonia oxidation, however, they were equally sensitive to high NH$_4$-N concentrations as the nitrifiers present in the activated sludge. Due to this fact, further studies determined the effect of increased biomass of nitrifying bacteria on the efficiency of the ammonium nitrogen oxidation process in wastewater with a concentration of 50.00 mg·dm$^{-3}$, 60.00 mg·dm$^{-3}$ and 70.00 mg·dm$^{-3}$ in a dynamic system. The efficiency of the nitrification process after eight hours of experiment, in all three systems, was similar to that observed in static conditions after four hours of aeration, using the same concentrations of ammonium nitrogen and not increasing the biomass of nitrifiers in the activated sludge. The obtained results suggest that in the first hours of the tests, the nitrification process was supported by nitrifiers added to the sample, but with time their successive removal from the system along with the treated sewage took place. In the following hours of the study, only the reduced activity of nitrifying bacteria present in the activated sludge was visible. These tests confirm the results obtained in static conditions, during which, after a four-hour period of aeration, the concentration of ammonium nitrogen in the control flask without the addition of nitrifiers was 65.50 mg·dm$^{-3}$. Under dynamic conditions, a similar value was obtained after eight hours (65.74 mg·dm$^{-3}$), ie after removing the nitrifiers added to the system. In the static system with the addition of nitrifiers, after four hours of aeration,
the NH$_4$-N concentration decreased to the value of 62.30 mg dm$^{-3}$. In the dynamic tests, a similar result was obtained in the fourth hour of the experiment—62.76 mg dm$^{-3}$. This fact suggests a tendency to remove from the system nitrifying bacteria that did not manage to colonize the activated sludge during the eight hours of the experiment.

The NH$_4$-N concentration of 60.00 mg dm$^{-3}$ showed no toxic effect on the nitrifying bacteria of the second phase. The content of nitrate nitrogen from the first to the eighth hour of the experiment was on average at the level of about 11.00 mg dm$^{-3}$, which was not reflected in the static tests, where after four hours of aeration, the NO$_3$-N concentration in the control sample was only 4.29 mg dm$^{-3}$, and in the sample with the addition of nitrifying bacteria—9.36 mg dm$^{-3}$.

The results obtained in dynamic tests indicate that the cultures of nitrifying bacteria added to the system were successively removed with the treated sewage over time, which corresponds to the data found in the literature [11,23,50].

The research conducted on the acclimated activated sludge shows that even a 24-h time of settling the activated sludge by nitrifying bacteria prevents the removal of these microorganisms from the system, and thus increases the efficiency of the nitrification process. The best results were obtained on the fourth and last day of acclimatisation, when the NH$_4$-N concentration added to the sample was reduced by 91.4%. The obtained results were satisfactory, so it was decided to conduct similar tests in the WWTP. Additionally, in this case, the four-day acclimatisation time of the activated sludge allowed the oxidation of ammonium nitrogen to a degree equal to 93.9%. Skoyles et al. [49] obtained a similarly high degree of TAN oxidation in industrial wastewater with a four-day aeration time.

The activated sludge, acclimated under static conditions, was then transferred to the aeration chamber in the WWTP, and nine days after inoculation, a stable nitrification process was achieved. The degree of ammonium nitrogen oxidation increased from approx. 62.0% (before the chamber inoculation) to approx. 97.0%, with the average sewage temperature of approx. 12 °C. It should be emphasized that in the previous years, the limit temperature for the nitrification process in the system was 13 °C. Similar results were obtained by Li et al. [49], in which the biological wastewater treatment system supported the nitrification process with acclimated nitrifying bacteria, allowing shortening the sludge age from 13–18 days to 7–10 days at a temperature of 10 °C.

Proper operation of mechanical and biological wastewater treatment plants from the chemical industry is a difficult matter, especially in the case of plants that receive different wastewater. In such plants, the amount and composition of wastewater and the concentration of the organic pollutant are constantly fluctuating [51,52]. Meanwhile, the biocenosis of activated sludge is reluctant to acclimate to specific industrial substances [53]. There are many wastewater treatment plants that are unable to eliminate toxic substances and to reduce the concentration of incoming organic pollutants by wastewater pretreatment by the plants producing them. Additionally, low temperatures limit the course of many biochemical processes, the most sensitive of which is the nitrification process [54]. An example of such a treatment plant is the Municipal and Industrial Wastewater Treatment Plant in Oświęcim, where the only real chance of achieving stable nitrification after the occurrence of disturbances is the use of inoculation with cultures of nitrifying bacteria.

5. Conclusions

1. The limit concentration of ammonium nitrogen at which the nitrification process is inhibited at the Municipal and Industrial Sewage Treatment Plant in Oświęcim is 70 mg dm$^{-3}$.
2. Ammonium nitrogen at the concentration of 100 mg dm$^{-3}$, 120 mg dm$^{-3}$ and 140 mg dm$^{-3}$ inhibits both the first and the second phase of nitrification in the tested system.
3. The cultures of nitrifying bacteria added to the dynamic system do not colonize activated sludge during the 8-h aeration time and are removed from the system together with the treated sewage.
4. In the WWTP, the inoculation of the aeration chamber with cultures of nitrifying bacteria gives effect only after the previous four-day acclimatisation of the activated sludge and significantly shortens the waiting time for the effective process of NH₄⁻N oxidation during periods of nitrification disappearance.

5. Acclimated activated sludge, added to the aeration chamber in the form of inoculum in periods unfavourable for nitrifiers, allows increasing the degree of TAN oxidation in treated sewage by approx. 35.0%.

6. The nitrification process was achieved with the tested strategies, which is good in the WWTP point-of-view. With such is possible to apply the subsequent denitrification process.

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