Increase of nitrification efficiency at waste water treatment by implementation of bioaugmentation process

Y. V. Mikhailova, M. V. Kevbrina, V. A. Grachev, Y. A. Nikolaev and V. G. Aseev

Abstract

Low content of ammonia in the treated water is one of the most important indicators of the efficiency of biological wastewater treatment. Oxidation of ammonium to nitrate (nitrification) is carried out by nitrifying bacteria, which have low growth rates and are very sensitive to certain unfavorable technological factors, such as low oxygen concentration and toxicants. To stabilize the number of nitrifying bacteria, increasing their activity in bio-reactors with activated sludge and, therefore, to achieve stable and efficient removal of nitrogen compounds, various techniques are used, one of which is bioaugmentation technology. Bioaugmentation implies addition of the necessary microorganisms or creation of the conditions favoring their development in order to increase the specific activity of biological systems, such as activated sludge. In the Engineering and Technological Centre of JSC Mosvodokanal for the first time in world practice, we studied the efficiency of nitrification in a plant operating under the scheme of the University of Cape Town process, in combination with an additional bioaugmentation reactor. Activated sludge in the reactor was enriched with nitrifying bacteria. At higher ammonium loads, which were modeled by adding the liquid phase of digested sludge, the main line did not remove ammonium to the maximum permissible concentration for fishery water bodies. The use of a bioaugmentation reactor resulted in ammonium concentration decrease from 40–50 to 0.4 mg N-NH₄/l. This approach increased the stability of the activated sludge nitrifying bacteria to toxicants (thiourea).

Key words: bioaugmentation, inhibition, nitrification, thiourea

INTRODUCTION

An important indicator of the quality of biological wastewater treatment is low ammonium content in the purified water, which results from a stable activity of nitrification. At many of the major wastewater treatment plants precipitates undergo the phase of anaerobic digestion. The content of ammonium nitrogen in the water of the sludge treatment facilities, which are usually directed in headworks, is high (up to 1,000 mg N/l), which leads to elevated ammonium levels in the purified wastewater (up to 50% of the ammonium load of the municipal wastewater). The process of nitrification is known to be sensitive to the presence of various toxicants, which often occur in wastewater treatment plants and leads to the disruption of the process. Stabilization of the nitrification process is a very important problem.

These issues can be successfully solved using the bioaugmentation technology. Bioaugmentation was initially understood as the introduction of a group of certain microbial strains into the environment in order to achieve its original undisturbed status. In a broader sense and in relation to technological biological systems, the term bioaugmentation is used for enrichment of a natural or
man-made object with certain microorganisms (nitrifying bacteria in the case of wastewater treatment) by their introduction into the system or growth (Neethling et al. 1998; Khan et al. 2007). In addition to improved nitrogen removal (Plaza et al. 2001; Stephen et al. 2006; Parker & Wanner 2007b), bioaugmentation may have other advantages, such as improved sludge flocculation, improved removal of suspended solids, and removal of toxic pollutants (Cardinal et al. 1992; Kos 1998; Stephen et al. 2006). There is evidence that bioaugmentation makes it possible not only to ensure the continuity of the treatment process, decreasing the load on the constructions, but also to increase the resistance of activated sludge to the effects of toxic compounds (Cardinal et al. 1992).

There are several approaches for the implementation of this technology (Hellinga et al. 1998; Mendoza-Espinosa & Stephenson 1999; Berends et al. 2005; Krhutkova et al. 2006; Parker & Wanner 2007a). Originally, external sources of microbial cultures, purchased as commercial products, were used (Parker & Wanner 2007a). However, this approach is inefficient, since acquired cultures may lose activity when stored or subjected to new conditions of the reactor, and the cost of the required cell mass may be too high for regular use. An alternative and more rational scheme of enrichment of activated sludge with the necessary microorganisms is to create conditions for development of the necessary organisms in the sludge during the technological process (Hellinga et al. 1998; Mendoza-Espinosa & Stephenson 1999; Berends et al. 2005; Krhutkova et al. 2006). This approach (enrichment of activated sludge with nitrifying bacteria by growing them in the operating reactor) was used in the present work. The aim of the study was to determine the effect of fortiﬁer reactor (bioaugmenter) on the efficiency of nitrification in the presence of high concentrations of ammonium (i.e. beyond the capacity of the bioreactor) and stability of the process of nitrification in the presence of a toxicant (thiourea).

**METHODS**

The efficiency of nitrification, the overall quality of treatment, and the effect of the augmenter reactor was studied on a setup operating according to two schemes: (1) the classical University of Cape Town (UCT) scheme (Figure 1) and (2) UCT supplemented with an aerated reactor (bioaugmenter) in the return activated sludge line (RAS) (Figure 2). The traditional scheme of Cape Town University was chosen as a baseline for the study because of the fact that wastewater in Moscow (as in most Russian cities) has low concentration of nutrients (due to high rates of water consumption). It is characterized by low ratios of biological oxygen demand (BOD) to the concentrations of nitrogen and phosphorus. The UCT technological scheme was designed speciﬁcally for such water. In accordance with the scheme, aerobic, anaerobic, and anoxic zones were organized in the reactor (Figure 1). Recycles of the mixed sludge liquor, nitrate, and return sludge were used. Into the reactor, operating according to the UCT technology, clarified wastewater of old Kuryanovo wastewater treatment plant was fed. The reactor was designed in such a way that the major parameters of treated water were in agreement with the maximum permissible concentration (MPC) for ﬁshery water bodies.

**Figure 1** | Traditional Cape Town University scheme. *RAS – return activated sludge, **WAS – waste activated sludge.
The stages of study were: (1) operation under the conditions according to the UCT scheme, treatment of usual wastewater; (2) operation according to the UCT technology, but with a 50% increase of ammonium load by mixing wastewater with the liquid phase (reject water) of fermented sludge thickener (6% of total flow rate); and (3) UCT operation with the bioaugmentation reactor with reject water supplied to the bioaugmentation reactor. Alongside with the study of the bioreactor operation, a study of activated sludge with regard to its stability in the presence of thiourea was carried out. The effect of thiourea was assessed by its impact on the respiration of nitrifying bacteria and on the rate of nitrification. The respiration rates of the microbial population were determined by measuring oxygen uptake rates by the aerobic biomass using a ‘Bioaktiv’ setup (Głodniok 2011). The experiments for calculation of the rates of nitrification, denitrification, and de-phosphatation were carried out at the same time (Bortone et al. 1992; Janssen et al. 2002).

RESULTS

Technological properties of the installation

During the first phase of the study, when the setup worked in accordance with the calculated load, nitrification was efficient, the average content of N-NH4 in treated water did not exceed 0.2 mg/l (Table 1).

Table 1 | Contents of the main pollutants in the clarified and treated water at different stages of the research in the absence of thiourea

<table>
<thead>
<tr>
<th>Water indicator mg/l investigation phase (applied scheme)</th>
<th>COD</th>
<th>BOD5</th>
<th>SS</th>
<th>N-NH4</th>
<th>N-NO2</th>
<th>N-NO3</th>
<th>P-PO4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requirements, according to MPC for fishery water bodies</td>
<td>30</td>
<td>2.0–3.0</td>
<td>10</td>
<td>0.39</td>
<td>0.02</td>
<td>9.1</td>
<td>0.2</td>
</tr>
<tr>
<td>(1.1) Traditional UCT scheme</td>
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</tr>
<tr>
<td>Clarified water</td>
<td>349.7</td>
<td>124.3</td>
<td>235.1</td>
<td>26.2</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated water</td>
<td>40.0</td>
<td>4.1</td>
<td>8.7</td>
<td>0.1</td>
<td>0.05</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>(1.2) Traditional UCT scheme with wastewater from digested sludge 5%</td>
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<td></td>
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<tr>
<td>Clarified water</td>
<td>271.5</td>
<td>130</td>
<td>158.9</td>
<td>42.3</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated water</td>
<td>31.5</td>
<td>3</td>
<td>11.1</td>
<td>8.7</td>
<td>0.5</td>
<td>14.3</td>
<td>0.4</td>
</tr>
<tr>
<td>(2.1) Modified UCT scheme</td>
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</tr>
<tr>
<td>Clarified water</td>
<td>312.5</td>
<td>114.2</td>
<td>169</td>
<td>32.9</td>
<td>2.50</td>
<td></td>
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<tr>
<td>Treated water</td>
<td>33</td>
<td>3.7</td>
<td>7.9</td>
<td>0.2</td>
<td>0.03</td>
<td>14.2</td>
<td>0.6</td>
</tr>
<tr>
<td>(2.2) Modified UCT scheme with wastewater from digested sludge 5%</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Clarified water</td>
<td>318</td>
<td>110</td>
<td>170.7</td>
<td>46.1</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated water</td>
<td>37.6</td>
<td>3.6</td>
<td>11.5</td>
<td>0.3</td>
<td>0.05</td>
<td>23.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>
and Figure 3). Addition of reject water to the treated water caused a sharp decrease in the efficiency of nitrification – content of N-NH₄ in the treated water increased to 10 mg/l. To improve nitrification, a bioaugmenter reactor was introduced into the RAS line (Figure 2). Within 2 days nitrification efficiency was restored to the former high level. Content of ammonium nitrogen in the treated water did not exceed 0.5 mg/l (Table 1 and Figure 3).

![Figure 3](https://iwaponline.com/wpt/article-pdf/9/4/551/382406/551.pdf)

**Figure 3** | Dynamics of content of ammonium nitrogen in the clarified (1) and treated (2) water. Arrows indicate the onset of wastewater addition (3) and of the bioaugmentation reactor (4).

Nitrate content in treated water in the setup with the modified UCT scheme exceeded MPC for fishery water bodies, due to the fact that the increased ammonium load exceeded the design capacity of the denitrifying reactor. However, industrial application of bioaugmentation technology appears to be promising, since the total environmental payments for discharge of nitrogen pollution in this case will be much lower compared with the excess discharge of ammonium nitrogen without the bioaugmenter.

High efficiency of the technology applied was due to the fact that ammonium was efficiently oxidized and the biomass of denitrifying bacteria increased in the bioaugmentation reactor under conditions of short-term aeration (30 minutes), high concentration of activated sludge (12 g/l), and suppressed activity of heterotrophic bacteria due to the lack of substrate for them – available organic substances.

**Effect of the toxicant on nitrification**

Effect of thiourea on nitrification activity of the sludge was assessed as its effect on the respiratory activity of the nitrifying bacteria in activated sludge samples (Figure 4) and as quality of ammonium removal from water under conditions of acute or chronic poisoning of the sludge (Table 2).

The data presented on Figure 4 indicate that nitrifying bacteria from the activated sludge formed in the presence of a bioaugmentation reactor were somewhat more sensitive to thiourea. The ‘half-inhibitory’ concentration, at which the rate of the process decreased twofold, was 0.2 and 0.3 mg/l for sludge grown with or without the augmenter reactor, respectively. Introduction of 0.5 mg/l thiourea inhibited the activity of nitrifying microorganisms by 95% (Figure 4). At a concentration of 0.8 mg/l, the respiration was almost completely absent, since the experimentally determined respiration rate (1–5% of the initial value), was due to heterotrophic bacteria present in the sludge, rather than to nitrifying bacteria.

While one-time introduction of thiourea into the purified wastewater at a concentration of 0.8 mg/l (acute poisoning), which completely suppressed the respiration of nitrifying bacteria in the laboratory setup, led to a decrease in the efficiency of ammonium removal from wastewater (Table 2), the
The inhibitory effect was somewhat less pronounced in the presence of the bioaugmentation reactor (44 and 20% in the control variant and in the variant with enrichment reactor, respectively). After 3 days the nitrification activity was fully restored in both versions.

Daily addition of thiourea for 5 days at its concentration in the incoming water 0.6 mg/l (chronic poisoning) caused a decrease in the efficiency of nitrification by 36–41 and 16–23% in the control experiment and in the presence of the bioaugmenter reactor, respectively, i.e. activated sludge was more resistant to the toxicant in the presence of the enrichment reactor. After cessation of toxicant supply, efficiency of nitrification recovered after 2 weeks.

Interestingly, the effect of the bioaugmenter reactor on the sensitivity of nitrifying bacteria thiourea determined by different methods was different. Short-term measurements of the respiration of nitrifying bacteria revealed that the presence of the enrichment reactor increased their sensitivity to the toxicant (Figure 4), whereas under conditions of chronic toxicity, the sensitivity was decreased (Table 2). This was obviously due to the activity of bacterial protective mechanisms for adaptation to the toxicant, which required longer than the time of a laboratory experiment (15 minutes). Protective mechanisms were not able to develop during short-term laboratory acute poisoning respiration tests, while higher content of nitrifying bacteria possibly made them more exposed to toxicant.

Addition of the toxicant also affected the activity of phosphate-accumulating bacteria: in the experiment, there was a decline in the quality of phosphorus removal from wastewater.

**DISCUSSION**

A number of examples of industrial application of bioaugmentation for wastewater treatment are known. Using inoculum from an external reactor with activated sludge, working on the circulating water is realized in the solids retention time or In-Nitri processes. In this technology, the flow from the line of dewatering of digested sludge feeds an additional nitrifying reactor, and this sludge is supplied to the head main structures (Riska et al. 2004). In the TF/PAS technology, squamous biofilm of a trickling biofilter is fed into the main bioreactor-aeration tank, thereby increasing the number of nitrifying bacteria (Parker 2007). The process Main stream AUtotrophic Recycle Enabling Enhanced N-removal (MAUREEN) includes an additional bioreactor in the line of return flows that implements both nitrification and denitrification (Constantine et al. 2005). The shortcoming of the above schemes is that the nitrifying bacteria added to the main aeration tank were grown in different conditions and...
may have problems developing in a new environment. The so-called \textit{in situ} bioaugmentation, in which conditions for growth of nitrifying bacteria are established in the activated sludge of the primary fermentor, is a more advanced technology. The process of bioaugmentation regeneration (BAR) was developed and tested simultaneously and independently in the Czech Republic and the United States (Parker & Wanner 2007b). According to this scheme, a flow with a high concentration of ammonium is introduced into the regeneration zone of the bioreactor (the zone is located directly in the primary fermentor, rather than in an additional external one). The BAR technology was successfully tested, introduced, and studied in the Czech Republic at the treatment facilities of Central Prague, Mlada Boleslav, and Usti nad Labem. Currently, BAR-based technologies are the most common among adopted technologies for bioaugmentation. The AT3 process is similar to BAR, the main difference being that a smaller portion of RAS is supplied to the bioaugmentation reactor (regenerator). The technological scheme of the Biological Augmentation Batch Enhanced process (BABE) includes a reactor into which return flows from the line of sludge dewatering and some of the RAS are supplied. The main difference from the AT3 technology is the presence of the stage of deposition (of RAS) in the bioaugmentation reactor (Berends \textit{et al}. 2003). The sludge age sufficient for development of nitrifying bacteria is achieved in the reactor, and growth of these organisms occurs.

In these technologies, water and sludge from the augmentation reactor was fed to the reactors of the main line, operating according to various technologies, apart from UCT. Given the characteristic feature of the Russian municipal wastewater with low BOD concentrations and the almost complete removal of readily available organic matter from the flow of RAS, organization of denitrification is very important. That is why the UCT scheme was chosen, with the flow from the enrichment reactor directed into the denitrification zone. Our data on high efficiency of the introduction of a bioaugmentation reactor for stabilization of nitrification process confirmed the correctness of our choice.

While the obtained data are consistent with the literature data, the introduction of a reactor for enrichment of denitrifying bacteria into the UCT technological scheme has not been previously reported.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Water indicator mg/l & Investigation phase (the applied scheme) & COD & BOD$_5$ & SS & N-NH$_4$ & N-NO$_2$ & P-PO$_4$ \\
\hline
Requirements, according to MPC for fishery water bodies & & 30 & 2.0-5.0 & 10 & 0.39 & 0.02 & 9.1 & 0.2 \\
(1.1.1) Traditional UCT scheme with thiourea addition & Acute poisoning (0.8 mg/l) & & & & & & & \\
Clarified water & & 382 & 150 & 212.5 & 23.5 & & 2.4 \\
Treated water & & 68 & 3 & 8.4 & 10.3 & 0.1 & 8.3 & 1.2 \\
(2.1.1) Modified UCT scheme with thiourea addition & Acute poisoning (0.8 mg/l) & & & & & & & \\
Clarified water & & 322 & 130 & 212.75 & 36.4 & & 4.2 \\
Treated water & & 77 & 4 & 3.4 & 7.5 & 1.3 & 10.85 & 2.7 \\
(1.1.2) Traditional UCT scheme with thiourea addition & chronic poisoning (0.6 mg/l) & & & & & & & \\
Clarified water & & 296 & 125 & 228.8 & 30.1 & & 1.4 \\
Treated water & & 66 & 3 & 5.6 & 10.8 & 0.1 & 9.15 & 0.32 \\
(2.1.2) Modified UCT scheme with thiourea addition & chronic poisoning (0.6 mg/l) & & & & & & & \\
Clarified water & & 217.17 & 110 & 97.18 & 34.9 & & 3.7 \\
Treated water & & 65 & 3 & 6.44 & 5.72 & 1.27 & 9.4 & 1.6 \\
\hline
\end{tabular}
\caption{Contents of the main pollutants in the clarified and treated water at different stages of the research in the presence of thiourea}
\end{table}
CONCLUSIONS

(1) A new technology was successfully tested, including the introduction of a reactor for enrichment of nitrifying bacteria into the University of Cape Town technology.

(2) Introduction of the stage for enrichment stage of activated sludge with nitrifying microorganisms improved the efficiency of nitrification and ensured the stability of the ammonium oxidation under conditions of high ammonium load, exceeding the design capacity of the bioreactor 1.5 times.

(3) Bioaugmentation has a positive effect on the resistance of the nitrification process in wastewater to the toxicant (thiourea).

REFERENCES

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