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# ОЧИСТКА СТОЧНОЙ ВОДЫ ОТ НИТРОСОЕДИНЕНИЙ АРОМАТИЧЕСКОГО РЯДА В РЕАКТОРЕ БИОПЛЕНКИ С АНАЭРОБНОЙ- АНОКСИЧЕСКОЙ- ОКСИЧЕСКОЙ ЗОНАМИ И ДВИЖУЩИМСЯ СЛОЕМ (A2O-MBBR)

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***2,4,6-Тринитротолуол (ТНТ) широко используется в производстве взрывчатых веществ. Поскольку он токсичен и мутагенен для людей и животных, необходима очистка сточных вод от TNT. Были собраны сточные воды, содержащие ТНТ, из четырех заводов во Вьетнаме, производящих промышленные взрывчатые вещества. Образцы имели разные характеристики и широко варьировались, особенно концентрации ТНТ, в зависимости от технологии и метода управления производством на каждом заводе. Конкретно, концентрация ТНТ варьировалась от 25 до 128 мг/л, химическая потребность в кислороде (ХПК) варьировалась от 128 до 650 мг/л, БПК – от 28 до 67 мг/л, $NH_4^+$ – от 23 до 325 мг/л, ТР – от 0,13 до 0,38, а pH – от 6,5 до 8,2. Во Вьетнаме удаление TNT проводят путем адсорбции на гранулированном активированном угле и дальше сжигания. Этот метод является дорогостоящим и создает вторичное загрязнение. Чтобы преодолеть эти недостатки, в этом исследовании была предложена комбинация реактора биопленки с анаэробно-аноксическо-оксическими зонами и движущимся слоем (A2O-MBBR) для очистки сточных вод ТНТ. Реакционные резервуары системы A2O-MBBR были дополнены активными илами от станции очистки сточных вод в химической и электротехнической компании 15, содержащей биологические препараты нитрифицирующих и денитрифицирующих бактерий. Результаты показали, что в системе A2O-MBBR в течение 30 дней с 24-часовым сроком хранения при начальных условиях: ХПК: 200-250 мг/л, ТВЧ: 1800-3000 мг/л, свободный кислород: 5 мг/л, эффективность удаления ХПК достиглась приблизительно 70-83%, концентрация ХПК в отходящем потоке составляла примерно 50-75 mg/l; эффективность удаления ТНТ – 91-99,7%, а концентрация ТНТ на выходе составляла примерно 0,5-2 мг/л и эффективность удаления аммония составляла приблизительно 42,8-66%, выходная концентрация – от 15 до 17 мг/л.***

**Ключевые слова:** очистка сточных вод, нитросоединения ароматического ряда, реактор биопленки с анаэробно-аноксическо- оксическими зонами и движущимся слоем (A2O-MBBR)

# TREATMENT OF WASTEWATER CONTAINING AROMATIC NITRO COMPOUNDS USING THE A2O-MBBR METHOD

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***2,4,6-Trinitrotoluene (TNT) is widely used in explosives production. Since it is toxic and mutagenic to humans and animals, decontamination of TNT wastewater is necessary. Wastewater, containing TNT, from four factories in Vietnam producing industrial explosives were collected. The samples had different characteristics and varied widely, especially in terms of TNT concentration, depending on the technology and production management method at each plant. The TNT concentration ranged from 25 to 128 mg/l, chemical oxygen demand (COD) ranged from 128 to 650 mg/l, BOD5 ranged from 28 to 67 mg/l, $NH_4^+$ranged from 23 to 325 mg/l, T-P ranged from 0.13 to 0.38, and pH ranged from 6.5 to 8.2. In Vietnam, removal of TNT is carried out using granular activated carbon adsorption and an incineration method. This method is expensive and generates secondary pollution. To overcome these drawbacks, the combination of an anaerobic-anoxic-oxic and moving bed biofilm reactor (A2O-MBBR) for treating TNT wastewater was investigated in this study. Reaction tanks of the A2O-MBBR system were supplemented with activated sludges, from a wastewater treatment station in Chemical and Electrical Engineering Company 15, containing biological preparations of nitrifying and denitrifying bacteria. The results showed that after 30 days under operating conditions of COD of 200-250 mg/l, MSLL of 1800-3000 mg/l, and DO of 5 mg/l, COD removal efficiency reached approximately 70-83%, the TNT concentration decreased by 91-99.7% (to approximately 0.5-2 mg/l), and the ammonium concentration fell to 15-17 mg/l (reaching 42.8–66% removal efficiency after 24 h of treatment).***

**Key words:** TNT treatment, A2O-MBBR method, wastewater treatment

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Нхан Ву Зуй, Ту Нгуен Ван, Нхан Нгуен Тхи, Хыонг Ле Май, Царев Ю.В., Хыонг Ле Тхи Май Очистка сточной воды от нитросоединений ароматического ряда в реакторе биопленки с анаэробной- аноксической- оксической зонами и движущимся слоем (A2O-MBBR). *Изв. вузов. Химия и хим. технология*. 2018. Т. 61. Вып. 9-10. С. 113–119

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## INTRODUCTION

2,4,6-Trinitrotoluene (TNT) is widely used in the weapons industry as a material for explosives production and is important for the national economy. Since it is toxic and mutagenic to humans and animals, even at low concentrations, decontamination of TNT wastewater is necessary. In addition, TNT and its derivatives can persist and accumulate over the long-term in nature and are difficult to biodegrade [1-3]. Therefore, biological methods combined with other pre-treatment methods, that are based on common microbiological methods, enzymes methods, or plant methods, have been developed for the treatment of TNT-contaminated wastewater and soil water [1-10, 20-22]. Previous studies on TNT biodegradation used microorganisms, such as immobilized white rot fungi (*Phanerochaete chrysosporium*), for the treatment of TNT wastewater; biological aerated filters (BAF), anaerobic filters (AF), and anaerobic-anoxic-oxic moving bed biological reactors (A2O-MBBR) are commonly applied to wastewater treatment containing TNT in the next stage [1-5] after the wastewater has been pre-treated by physical or chemical methods.

Recently, TNT wastewater in Vietnam has been treated via granular activated carbon adsorption followed by incineration. This method is expensive and generates secondary pollution during treatment. Therefore, recent studies have focused on biotreatment, which is both highly efficient and cost-effective.

In recent years, researchers have focused on new techniques: A2O methods (anaerobic-anoxic-oxic) [10-13] combined with biological membranes, moving bed biological reactors (MBBR) [14-19]. Compared with conventional methods, A2O can be applied to certain types of difficult-to-treat industrial wastewater, such as coal gasification wastewater, textile wastewater, and, especially, wastewater with high COD concentrations or persistent organic substances. Treatment efficiency is high in these cases, and high COD removal can be achieved.

For this treatment method, the phosphorus, ammonia, and nitrogen treatment efficiencies are stable above 80%. Additionally, nitrate and nitrite metabolizing microorganism systems have richness and high density. In an aerobic tank, good bacteria for treatment density are towering varieties, such as ammonium oxidizing bacteria, nitrite oxidation bacteria, and heterotrophic bacteria. The A2O method does not require additional nutrients, especially phosphorus. Phosphorous, which is decomposed and discharged in the aeration tank, can be collected and accumulated as a nutrition source for the anoxic and aerobic tank.

In this study, the A2O-MBBR method was used to treat wastewater from an explosives production plant, which contained TNT and $NH_4NO_3$.We evaluated this technique as an effective technological solution with reasonable construction costs and a simple operating process.

## EXPERIMENTS

### Wastewater sample, chemicals, and equipment

*TNT contaminated wastewater sample*

Wastewater samples were collected directly from four factories in Vietnam: Chemical Company 21, Chemical and Mechanical Engineering Company 13, Electrical Engineering and Explosives Company 31, and Chemical and Electrical Engineering Company 15. Samples were collected at different times, and each sample had different concentrations of TNT. The wastewater samples at Chemical Company 21, Chemical and Mechanical Engineering Company 13, Electrical Engineering and Explosives Company 31, and Chemical and Electrical Engineering Company 15 were labelled F21, F13, F31, and F15, respectively. The characteristics of the TNT contaminated water samples are shown in Table 1.

***Table 1***

**Composition of TNT and $NH_4NO_3$ contaminated wastewater**

***Таблица 1.*** **Состав сточной воды, содержащей соединения азота в органической и неорганической форме**

| Factory | COD (mg/l) | $BOD_5$ (mg/l) | TNT (mg/l) | $NH_4$ (mg/l) | pH | T-P (mg/l) | Colour |
|---|---|---|---|---|---|---|---|
| F13, Enterprise A6 | 155-210 | 30-56 | 25-56 | 23-45 | 6.5-7.6 | 0.25 | Dark brown, red |
| F21, Enterprise 4 | 250-270 | 42-67 | 32-128 | 35-42 | 7.9-8.2 | 0.13 | Red-brown, yellow |
| F31, Enterprise 2 | 165-207 | 37.5-52 | 35-94 | 42-57 | 6.8-8.2 | - | Dark brown, red |
| F31, Enterprise 3 | 148-243 | 37-58 | 27-105 | 32-47 | 6.8-7.8 | - | Red-brown, yellow |
| F15, Plant AD1 | 128-310 | 28.3-35 | 43-92 | 68-79 | 7.2 | 0.38 | Red-brown, yellow |
| F15, Plantemulsion | 212-650 | 47-54 | - | 150-325 | 7.2-7.8 | - | Milky-white |

*Microorganism*

The bacterial strains used in these experiments were isolated from the activated sludge samples obtained from Chemical Company F21, Chemical and Mechanical Engineering Company F13, Electrical Engineering and Explosives Company F31, and Chemical and Electrical Engineering Company F15.

*Chemicals*

The chemicals commonly used in environmental engineering for the analysis of COD include total phosphorus (T-P), total ammonium ($NH_4NO_3$), glucose, $(NH_4)_2SO_4$, HCl, $H_2SO_4$, $Ag_2SO_4$, $HgSO_4$, and phenol (pure analytical grade).

**Equipment and research methods**

The mixed liquor suspended solids (MLSS), chemical oxygen demand (COD), biological oxygen demand (BOD), $NH_3$-N, T-P, and nitrite concentrations were measured according to standard methods [17]. Effluent turbidity was monitored with a digital direct-reading turbidimeter (model 965-10, Orbeco-Hellige, USA). The demand oxygen (DO) and pH of the suspensions in each reactor was determined using a dissolved oxygen meter (model HI9146-04, Hana Instruments) and a pH meter (model HI2002, Hana Instruments), respectively.

TNT concentration was analysed using voltammetry methods (797 VA Computrace Metrohm, Metrohm AG, Switzerland) and high-performance liquid chromatography (HPLC, Agilent 1100 liquid chromatography, Agilent Corporation, USA) [12]. We used working electrodes (solid rotating disk-modified glassy carbon electrode (DMF)), a counter electrode (Pt), and a reference electrode (Ag/AgCl/KCl 3M) for electrochemical measurements.

Operating conditions:

Electrolyte solution was 0.5 M NaCl with a standard voltage of 0.1 V. The end voltage was 1.0 V, and the electrolyte voltage was 0 V. The solution time was 60 s, and the waiting time was 15 s. The step voltage was 0,05 V, and the scanning rate was 0.0248 m/s.

The operating conditions

Electrolyte solution: NaCl 0.5 M

| | |
|---|---|
| Standard voltage: 0.1 V | Waiting time: 15 s |
| End voltage 1.0 V | Step voltage: 0.05 V |
| Electrolyte voltage: 0 V | Scanning rate: 0.0248 m/s |
| Time solution: 60 s | |

The HPLC had a Hypersil column $C18^+$ (200 x 4 mm). The proportion of the mobile phase was MeOH: $H_2O$ = 70: 30 (V/V), and the flow rate was 0.8 ml/min. The pressure was 280 bar. The procedure was stable after setting the necessary parameters, pumping, washing the columns, running the baseline, and waiting for 30–45 min.

We placed 5 ml of the analyte in the sample chamber using a micropipette; the machine automatically recorded retention time (RT), pic heights, electrical properties, and the percentage (%) of each component in the mixture.

## RESULTS AND DISCUSSION

**Set-up and start of the A2O-MBBR system**

The A2O-MBBR system was designed with a volume of 9 l for each reactor, and various methods were used to facilitate selection of the hour retention time (HRT) (Fig. 1). The domesticated activated sludge from the wastewater treatment plant AD1of the Chemical and Electrical Engineering Company 15 factory was supplemented with biological preparations containing nitrifying and denitrifying bacteria.

Activated sludge preparation: In the aeration tank, the DO level was maintained at approximately 5-8 mg/l and 1-2 mg DO/l in the anoxic reactor with a stirring speed at 120 rounds/minute; a moving bed biofilm reactor (MBBR) had a protected surface area 485 $m^2/m^3$; biofilms were added to the anaerobic reactor: anoxic reactor: aerobic reactor at 60%: 30%: 15% v/v.

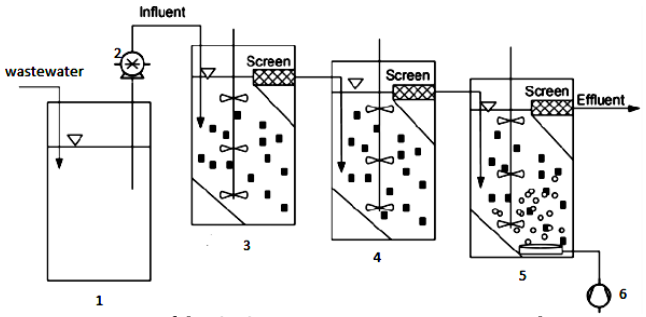


Fig. 1. Diagram of the A2O-MBBR system: 1 – storage tank; 2 – peristaltic pump; 3 – anaerobic reactor; 4 – anoxic reactor; 5 – aerobic reactor; 6 – air compressor

Рис. 1. Схема лабораторной установки очистки сточной воды в МБР-реакторе: 1 – напорная емкость; 2 – перистальтический насос; 3 – анаэробный реактор; 4 – бескислородный реактор; 5 – аэробный реактор; 6 – воздушный компрессор

We used artificial wastewater and conducted the reaction for 30 days; operating parameters of the processor system are shown in Table 2.

***Table 2***

**Operating parameters for the A2O-MBBR system**

***Таблица 2.* Параметры процесса очистки сточной воды в МБР-реакторе**

| COD (mg/l) | MLSS(mg/l) | pH | DO (mg/l) | HRT (h) |
|---|---|---|---|---|
| 200-250 | 2400-3000 | 6.5-7.2 | 5-7 | 24 |

**Efficiency of TNT treatment by the A2O-MBBR system**

The system was operated for 30 days. At the early stage of system operation, the concentration of TNT in the wastewater was 3 mg/l to 8 mg/l, and it was approximately 2.2 mg/l in the effluent of the oxic reactor. Over the first 10 days, TNT in the waste water increased from 8 mg/l to 23 mg/l, but the TNT in the aerobic reactor effluent was below 2 mg/l (Fig. 2, 3).

Initially, the A2O-MBBR treatment system had a TNT concentration of 3 mg/l and it was capable of stable operation up to a TNT concentration of 23 mg/l. At the highest TNT concentration, the treatment efficiency was quite clear. After 24 h of operation, the TNT removal efficiency improved from 91.3 to 99.7%, and the concentration of TNT after treatment reached approximately 0.5-2 mg/l. The TNT concentration in the anaerobic, anoxic, and oxic reactors of the A2O system and the effluent are shown in Fig 5.

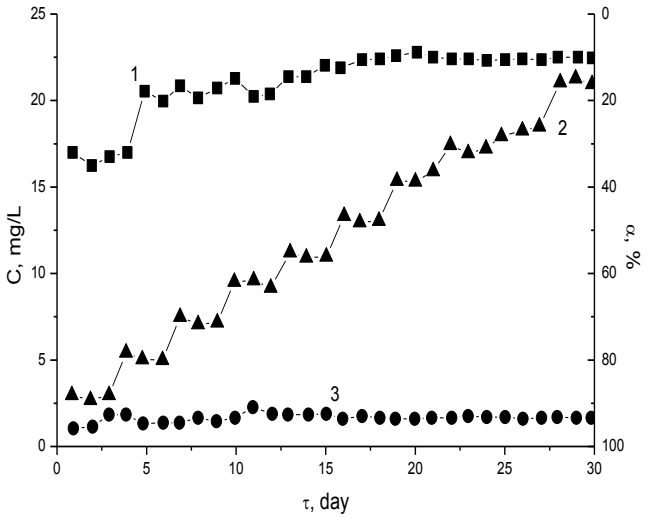


Fig. 2. The dependence of concentration and purification degree of wastewater in MBBR system: 1 – TNT concentration at reactor inlet, 2 – TNT concentration at reactor outlet, 3 – purification degree from TNT

Рис. 2. Зависимость концентрации и степени очистки сточной воды от времени в МБР-реакторе: 1 – концентрация TNT на входе в реактор; 2 – концентрация TNT на входе в реактор; 3 – степень очистки от TNT

The results showed that the TNT removal efficiency of the MBBR system can be described as:

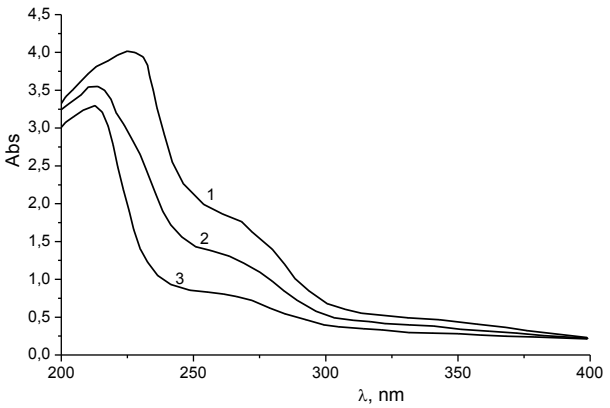


Fig. 3. Changes in absorbence spectra in the A2O-MBBR system: 1 – anaerobic zone; 2 – anoxic zone; 3 – oxic zone

Рис. 3. Изменение спектров поглощения в системе A2O-MBBR: 1 – анаэробная зона; 2 – бескислородная зона; 3 – аэробная зона

initially, the treatment system had a TNT concentration of 3 mg/l, and it was capable of stable operation up to a TNT concentration of 23 mg/l. At this concentration, the treatment efficiency was quite clear. After 24 h of operation, the TNT removal efficiency increased from 91.3 to 99.7%, and the concentration of TNT after treatment was 0.5-2 mg/l (Fig. 4).

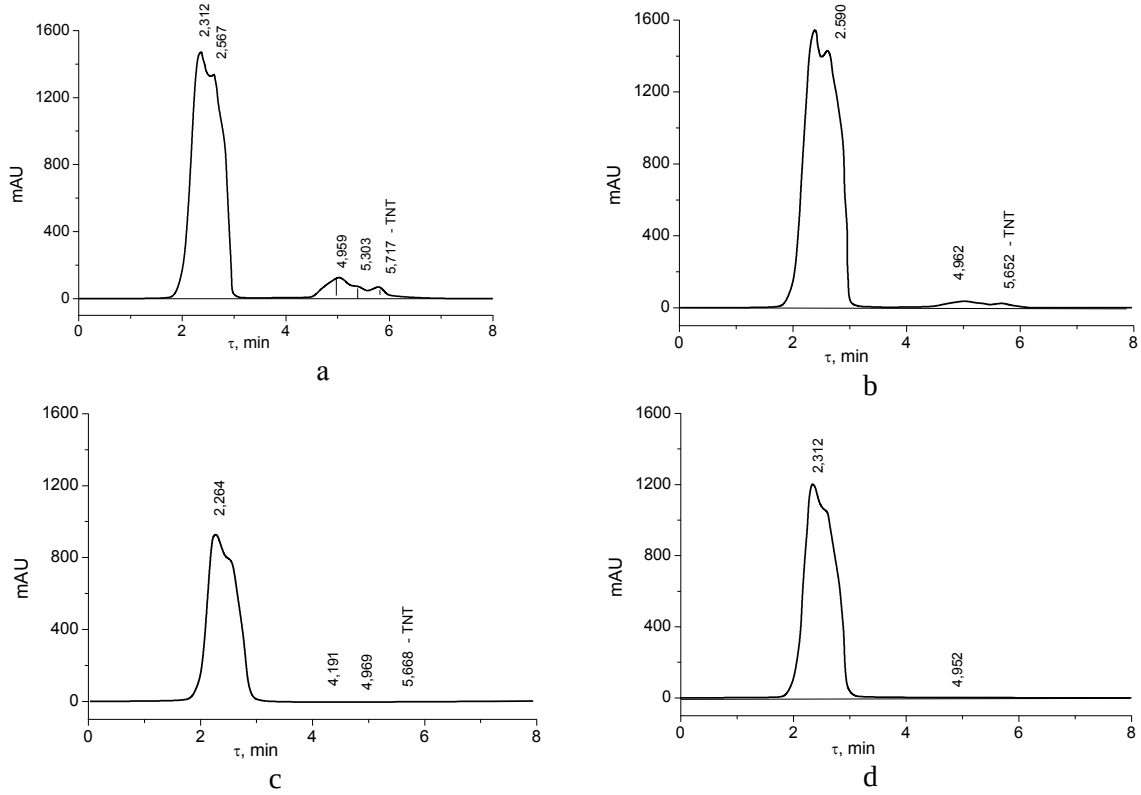


Fig. 4. HPLC spectrum of TNT in the wastewater samples: a – concentrations of TNT before treatment; b – concentrations of TNT after anaerobic treatment; c – concentrations of TNT after anoxic treatment; d – concentrations of TNT after aerobic treatment

Рис. 4. ВЭЖХ спектр сточной воды, содержащей ароматические нитросоединения: a – до реактора; b – после очистки в анаэробной зоне реактора; c – после очистки в бескислородной зоне реактора; d – после очистки в аэробной зоне реактора

The results of this study were consistent with those of previous studies [1-5], which showed that bacteria tended to reduce nitro groups. Zhouyou Wang [1, 2] investigated the biodegradation of TNT in an immobilized microorganism-biological filter system where TNT was reduced to 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT), and 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT). In another study, TNT was reduced to TAT by a mixed culture acclimated and maintained on crude oil-containing media [5].

From the results of their study, Mario Kroger [4] proposed a mineralization of TNT biodegradation pathway as show in Fig.5 (but most of the published studies on biological TNT treatment only reduced TNT to ADNT and DANT metabolites).

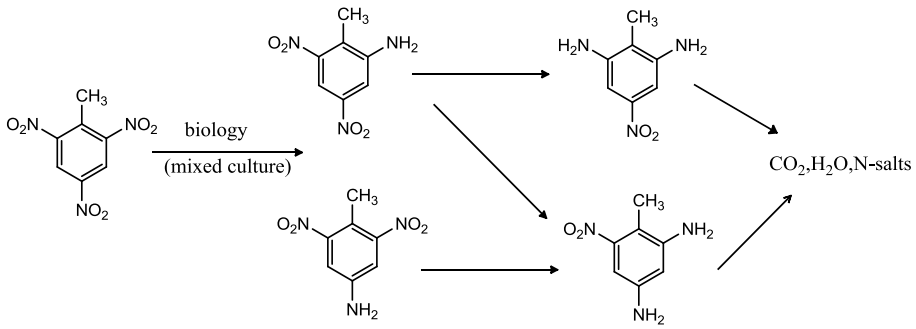


Fig. 5. Proposed mineralization of the TNT biodegradation pathway

Рис. 5. Предполагаемый механизм биодеградации ароматических нитросоединений

### COD removal efficiency

The A2O-MBBR system operated for 30 days with a HRT of approximately 24 h, and the COD concentration was reduced from 220-270 mg/l to 50-75 mg/l; COD removal efficiency reached 72.2-77.2%, as shown in Fig. 6.

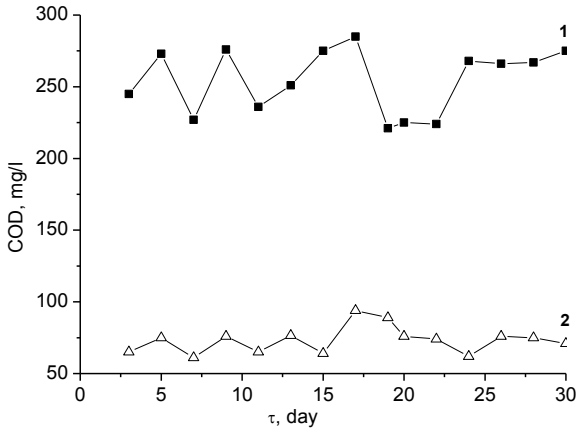


Fig. 6. COD removal efficiency: 1 – before treatment; 2 – after treatment

Рис. 6. Зависимость ХПК от времени: 1 – перед очисткой; 2 – после очистки

### Ammonium removal efficiency

Ammonium removal efficiency after 30 days of A2O-MBBR system operation is shown in Fig. 7.

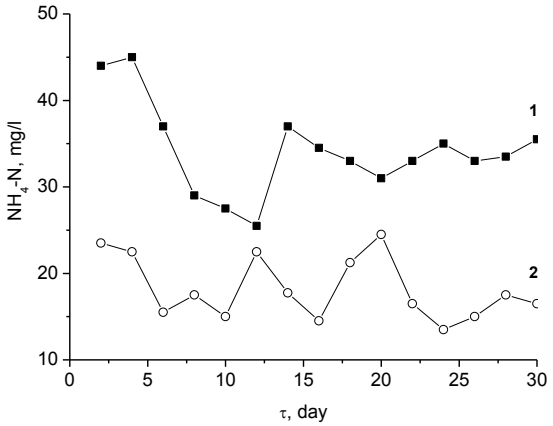


Fig. 7. Ammonium removal efficiency: 1 – before treatment; 2 – after treatment

Рис. 7. Зависимость содержания ионов аммония от времени: 1 – перед очисткой; 2 – после очистки

As shown in Fig. 7, in the first 15 days of operation, the concentration of ammonium in the effluent fluctuated from 28 to 45 mg/l; from 16 to 30 days, the concentration of ammonium in the influent was stable at approximately 35-37 mg/l. After 30 days, the ammonium removal efficiency reached 42.8 to 66%, and the concentration of $NH_4^+$ reduced to 15-17 mg/l.

## CONCLUSION

In this study, an A2O-MBBR system was established and operated for TNT wastewater treatment with a volume of 9 l for each reactor. Domestic activated sludge from Chemical and Electrical Engineer-

ing Company 15 was used, and it was supplemented with nitrifying bacteria and denitrifying bacteria from wastewater treatment biological products with high ammonium levels. We evaluated the effectiveness of COD, TNT, and ammonium removal from the wastewater.

We operated the A2O-MBBR system for 30 days at a 24-hour retention time COD of 200-250 mg/l, MSLL of 1800-3000 mg/l, and DO of 5 mg/l. COD removal efficiency was approximately 70-83%; TNT removal efficiency was approximately 91-99.7% and the concentration of TNT in the effluent was approximately 0.5-2 mg/l. Ammonium removal efficiency was approximately 42.8-66%, for initial concentrations of 15 to 17 mg/l.

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