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Modeling of Anammox Process with the BioWin Software Suite

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Abstract—Mathematical modeling of the biotechnology for the removal of ammonium nitrogen from wastewater based on the anammox process was performed with the specialized BioWin software suite (EnviroSim Associates Ltd., Canada). Nitrogen removal by means of the transformation of ammonium nitrogen to molecular nitrogen was conducted in a continuous stirred bioreactor carrying both suspended and immobilized activated sludge. Both basic values of the kinetic and stoichiometric coefficients are incorporated in the BioWin software, and those that changed based on the results of the experimental studies were used for the calculations. The optimal temperature and dissolved oxygen concentration revealed by mathematical modeling were 35°C and 0.14 mg/L. The results obtained from calculations were similar to those obtained in the experiments. The calculated and experimental concentrations of ammonium, nitrites, and nitrates in the treated water were similar and comprised 10.7 and 11.7% of the initial concentration entering the bioreactor, respectively. The selected mathematical model possessed a high predictive ability for the calculation of biotechnologies based on the anammox process

Keywords: wastewater treatment, anaerobic ammonium oxidation, anammox, mathematical modeling, BioWin

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INTRODUCTION

At the present, nitri–denitrification biotechnology based on the oxidation of ammonium to nitrate by nitrifying bacteria under aerobic conditions, followed by the reduction of nitrate to gaseous nitrogen by denitrifiers under anaerobic conditions, is the traditional method of nitrogen removal from wastewater. The discovery of anammox, the process of anoxic chemolithoautotrophic oxidation of ammonium with nitrite to molecular nitrogen, radically improved the potential for nitrogen removal from wastewater. The anammox process is highly efficient (90% nitrogen removal) and economical, since it requires 60% less oxygen than the tradition method, produces less excess biomass, and does not require organic matter. Thus, the anammox process makes it possible to decrease the cost of nitrogen removal by two to three times [1].

Despite the obvious technological and economic attractiveness of this novel biotechnology, its commercialization around the world has begun only in recent years. The low growth rate of anammox bacteria is among the most important difficulties in the scaling of anammox technologies; they are sensitive to a wide range of external factors, impeding their cultivation and the study of their physiological properties [2]. Difficulties in the implementation of anammox technologies are also caused by the fact that the processes involving anammox bacteria are in fact carried out by microbial communities rather than by pure cultures. The composition of each community is defined by the composition of the specific wastewater and the technological parameters of biological treatment. The coexistence of anammox bacteria and ammonium-oxidizing bacteria (**AOB**) is a common occurrence. AOB protect anammox bacteria, which are sensitive to oxygen, during the aerobic cycles of bioreactor operation and supply them with nitrite. The competition for space and nitrite occurs between anammox bacteria, denitrifiers, and nitrite-oxidizing bacteria (**NOB**) [3].

At the present, the anammox process is used to treat municipal and industrial wastewater on an industrial scale in Europe, the United States, China, and Japan [4–6]. In Russia, the anammox process has not yet been commercialized for the treatment of wastewater and return flows from sludge treatment facilities. However, in recent years, JSC Mosvodokanal, the largest Russian company operating wastewater treatment systems in Moscow, has started intensive experimental studies of the process [7–9].

To study potential of the anammox process and estimate its prospects for biotechnology, both physical and mathematical modeling is required. The mathe-

Table 1. Kinetic growth parameters of anammox bacteria

Parameter	Value
*μ_{max}, 1/day	0.2 (base value 0.1)
**K*s* NO_2^-, mg N/L	0.1 (base value 1)
**K*s* NH_4^+, mg N/L	0.1 (base value 2)
Aerobic rate of biomass decay, 1/day	0.019
Anoxic/ Aerobic rate of biomass decay, 1/day	0.0095
***Ki NO_2^-, mg N/L	1000
****Nitrite sensitivity constant, L/(d mg N)	0 (base value 0.016)

* μ_{max}—maximum growth rate (increased up to values obtained by Lotti and coauthors [15]).

** K*s* NO_2^-, K*s* NH_4^+—half-saturation constants for nitrite and ammonium, respectively.

*** Ki NO_2^-—inhibition constant for nitrite.

**** The resistance of the microbial consortium used in the present work to nitrite was higher than that demonstrated in the literature.

matical model should most adequately simulate the behavior of the microbial consortia responsible for the transformation of nitrogen compounds. There are a number of software suites used in modeling of the microbiological processes involved in wastewater treatment (BioWin, GPS-X, STOAT, SIMBA, etc.) [10]. In the present study, we used the BioWin software suite (EnviroSim Associates Ltd., Canada), which allows mathematical simulation of anammox bacterial activity.

The goal of the present work was to estimate the possibility use of the BioWin software suite for predictive calculations of technological modes of nitritation/anammox technology for the biological removal of nitrogen from wastewaters by means of a comparison of the results of mathematical modeling and experimental data.

MATERIALS AND METHODS

The biotechnology for the removal of nitrogen compounds from wastewater by nitritation/ anammox processes was simulated with the BioWin software suite (version 2007, EnviroSim Associates Ltd., Canada) [11], which is based on the widely used activated sludge model (ASM) developed by an international scientific group, the International Water Association (IWA) [12–14]. BioWin software uses the Activated Sludge/Anaerobic Digestion model (ASDM), which includes 50 state variables combined in 60 equations as a base model. The base model describes the main aerobic and anaerobic microbial processes (substrates utilization, biomass production and decay, metabolites production) occurring during biological wastewater treatment with activated sludge, as well as the reaction of chemical sedimentation, gas-liquid mass transfer, etc. We considered the following stages of biological nitrogen transformation: (1) nitrification to nitrite by ammonium-oxidizing bacteria (AOB); (2) nitrification to nitrate by nitrite-oxidizing bacteria (**NOB**); 3) denitrification; (4) nitrogen removal by autotrophic anammox bacteria; (5) nitrogen utilization for biosynthesis; (6) ammonification of dead bacterial biomass and organic compounds. The stationary values of the biomass of AOB, NOB, and anammox bacteria developing in the bioreactor, as well as the stationary concentrations of the main forms of dissolved nitrogen, total phosphorous content, and biologically degradable organic material in treated water (expressed as biological oxygen demand (**BOD**)) were calculated with the BioWin software. The base values of the kinetic and stoichiometric parameters of the microbial and chemical processes included in the BioWin software suite were used for the calculations. Several kinetic parameters that were changed based on experimental data obtained in recent years were exceptions (Table 1) [15, 16].

The process of nitrogen removal from the filtrate of digested sludge from the decanter of an anaerobic digester of Kuryanovo wastewater treatment plant (Moscow, Russia) was simulated. The filtrate of the digested sludge is an intermediate technological product that enters at the start of biological wastewater treatment and is characterized by a high concentration of ammonium nitrogen (650–770 mg/L of N-NH_4). It substantially determines the final ammonium concentration in the treated wastewater. Experimental biological treatment of the filtrate was performed in a laboratory stirred bioreactor (Fig. 1). The filtrate from the decanters entered the receiving tank (E-1) and then was pumped into a bioreactor (E-2) equipped with a polyethylene carrier for immobilization of the biomass of activated sludge. Part of the biomass of the activated sludge was constantly present in the reactor as a free-floating fraction. The dissolved oxygen concentration was measured with an oxygen sensor and regulated by means of air supply to the bioreactor by an air compressor (C). The temperature in the reactor

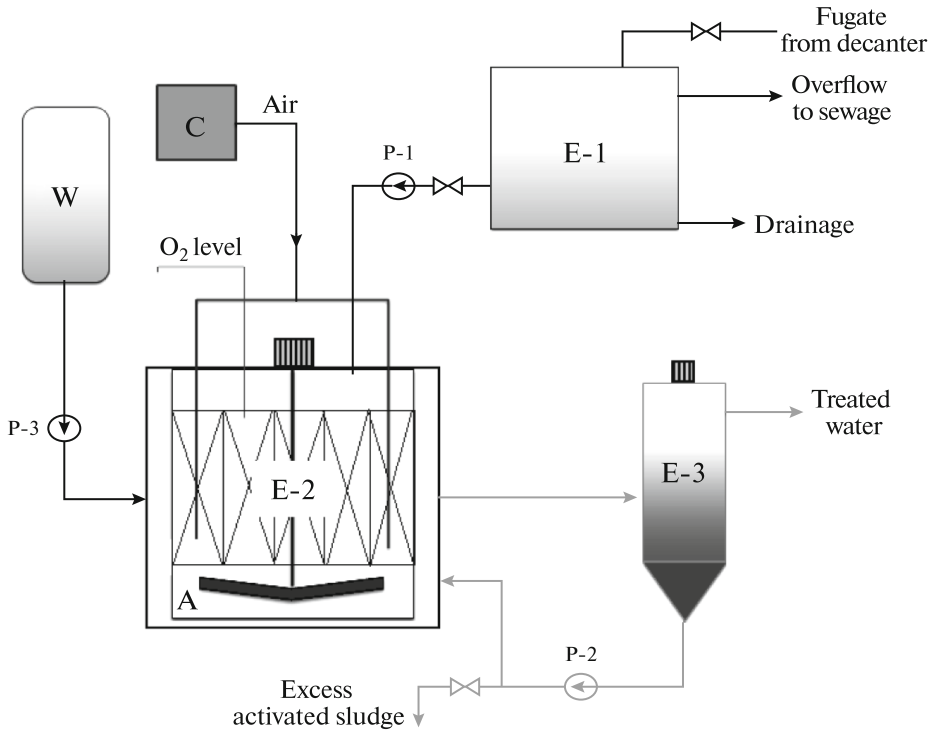

Fig. 1. Flowsheet of laboratory continuous stirred setup. E-1—receiving tank for the filtrate; E-2—bioreactor; E-3—secondary settling tank; C—air compressor; P—pump; A—aerators; W—water heater.

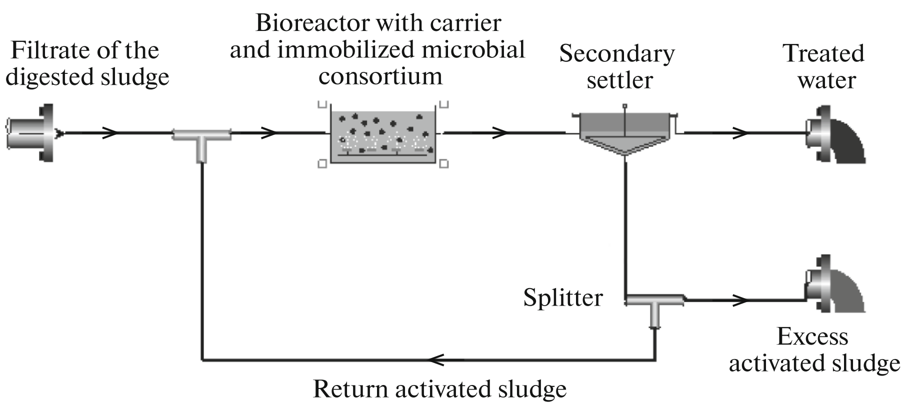

Fig. 2. Configuration of the process used for the simulation.

was regulated by heat-exchange between the water jacket and working volume of the reactor. Additional heating was provided by a water heater (W). The sludge mixture from the reactor was transferred to a secondary settling tank (E-3) to precipitate activated sludge and return its part to the bioreactor. The treated water was transferred to the overflow. The following parameters of laboratory setup were used: bioreactor volume—100 L, wastewater flow rate—180 L/day, volume of the bioreactor occupied by the carrier—75%.

A simplified flowsheet of the process included the wastewater source, bioreactor with the carrier, secondary settler, splitter, output of treated wastewater, and output of excess sludge (Fig. 2). The following are the average parameters of the filtrate of digested sludge from Kuryanovo wastewater treatment plant fed in the laboratory setup that were taken as base parameters of the medium entering the reactor used in the model: ammonium nitrogen concentration—600 mg/L; biological oxygen demand—180 mg/L; chemical oxygen demand (COD)—800 mg/L; suspended solids—130 mg/L; phosphate phosphorus—10 mg/L; pH—7.0. The data on the composition of the medium entering the reactor and the ratio between separate

Table 2. Model composition the filtrate of the digested sludge

Parameter	Value
Demand, L/day	180
Total COD, mg COD/L	800
Total Kjeldahl nitrogen, mg/L	660
Total phosphorous, mg P/L	10
Nitrate N, mg/L	0
pH	7.3
Suspended inorganic matter, mg/L	45
Ca^{2+}, mg/L	80
Mg^{2+}, mg/L	15
Dissolved oxigen, mg/L	0

components used in the BioWin model are shown in Tables 2 and 3. The numerical values not measured analytically were calculated based on long-term monitoring and experimental studies on the composition of the filtrate produced on Moscow wastewater treatment plants performed by JSC Mosvodokanal. The concentrations of the biomass and nitrogen compounds in the treated wastewater were measured by standard methods [17].

RESULTS AND DISSCUSSION

The results of mathematical modeling demonstrated that the quantitative ratio of biomass of the main groups of nitrogen-converting microorganisms (AOB, NOB, and anammox bacteria) and the quality of treatment of the wastewater entering the reactor sufficiently depended on the oxygen and temperature mode of the bioreactor.

Modeling of oxygen mode effect. The effects of oxygen mode were studied at concentrations ranging from 0.1 to 1.0 mg/L at a fixed temperature (35°C). The AOB density significantly increased when the oxygen concentration increased from 0.1 to 0.15 mg/L and then remained high (the COD was 226.6–294.4 mg/L). In contrast to AOB, the NOB density was low and changed insignificantly (the COD was 0.3–2.2 mg/L) at low oxygen concentrations (0.1–0.4 mg/L). The NOB biomass dramatically increased (the COD increased from 2.2 to 175.5 mg/L) at oxygen concentrations above 0.4 mg/L. Anammox bacteria accumulated in the bioreactor at oxygen concentrations of 0.13 to 0.4 mg/L and their biomass was maximal (69–78 mg COD /L) within a narrow range of oxygen concentrations (0.13–0.14 mg/L) (Table 4). Thus, the ratio of AOB, NOB, and anammox bacteria was optimal for the nitritation/anammox process within a range of oxygen concentrations of 0.13–0.14 mg/L. Within the same range of oxygen concentrations, removal of the main nitrogen compounds from the filtrate was the most qualitative, i.e., the ammonium concentration rapidly decreased, whereas the content of nitrites and nitrates increased insignificantly (Table 5). The total content of ammonium, nitrite, and nitrate nitrogen in the bioreactor (in the treated water) was ten times lower than that in the entering water (Fig. 3). A decrease or increase of the oxygen concentration resulted in an increase in the residual concentrations of ammonium and products of its oxidation.

Table 3. Ratios of separate components of the filtrate

Component	Value
Fbs—easily biodegradable organic matter, g COD/g total COD	0.22
Fac—acetate, g COD/g easily biodegradable organic matter COD	0.15
Fxsp—noncolloidal slowly degradable organic matter COD, g COD/g slowly degradable organic matter COD	0.75
Fus—nondegradable soluble COD, g COD/g total COD	0.12
Fup—nondegradable insoluble COD, g COD/g total COD	0.65
Fna—ammonium, g N-NH_4/g Total Kjeldahl nitrogen	0.9
Fnox—insoluble inorganic nitrogen, g N/g organic N	0.5
Fnus—nitrogen of soluble non-biodegradable matter, g N/g Total Kjeldahl nitrogen	0.02
FupN—N: COD ratio of nonbiodegradable insoluble COD fraction, g N/g COD	0.035
Fpo4—phosphates, g P-PO_4/g total P	0.5
Fup—P: COD ratio of nonbiodegradable insoluble COD fraction, g P/g COD	0.0011
FZbh—nonpolyphosphate phosphorus of heterotrophic microorganisms, g COD/g total COD	0.0001
FZbm—anoxic methanol-utilizing microorganisms, g COD/g total COD	0.0001
FZaob—ammonium-oxidizing bacteria, g COD/g total COD	0.0001
FZnob—nitrite-oxidizing bacteria, g COD/g total COD	0.0001
FZamob—anammox bacteria, g COD/g total COD	0.0001

Table 4. Results of modeling of oxygen effect on the amount of biomass of main groups of microorganisms involved in nitrogen removal

O_2 concentration, mg/L	AOB, mg COD/L	NOB, mg COD/L	Anammox bacteria, mg COD/L
0.1	2.51	0.29	0.40
0.13	170.75	0.39	68.86
0.14	186.76	0.48	78.17
0.15	213.98	0.78	42.98
0.17	226.56	0.95	37.46
0.19	229.47	0.99	34.89
0.25	235.91	1.15	29.22
0.40	245.46	2.20	21.34
0.60	296.34	175.45	1.83
1.00	294.40	180.61	0.73

Table 5. Results of modeling of the effect of oxygen mode on the composition of the treated water

O_2 concentration, mg/L	Ammonium, mg N/L	Nitrites, mg N/L	Nitrates, mg N/L
0.1	609.50	0.03	0.00
0.13	61.10	8.21	43.31
0.14	12.37	8.97	49.38
0.15	8.25	13.96	101.87
0.17	7.27	36.84	119.95
0.19	7.05	59.98	116.06
0.25	6.51	116.85	105.19
0.40	5.55	207.78	88.57
0.60	0.69	1.12	568.88
1.00	0.55	0.18	597.00

Similar results were obtained previously with the AQUASIM software for modeling of the nitritation/anammox process in bioreactors [18, 19]. Ni and coauthors applied the AQUASIM V2.1 model to simulate a two-stage process that includes stages of nitrification/denitrification and the anammox process. The reliability of the model was estimated by means of comparison of the simulation results with experimental data obtained from two independent Sequencing Batch Reactors (SBRs) with free-floating biomass performing the nitritation/anammox process at different aeration modes (continuous and periodic aera-

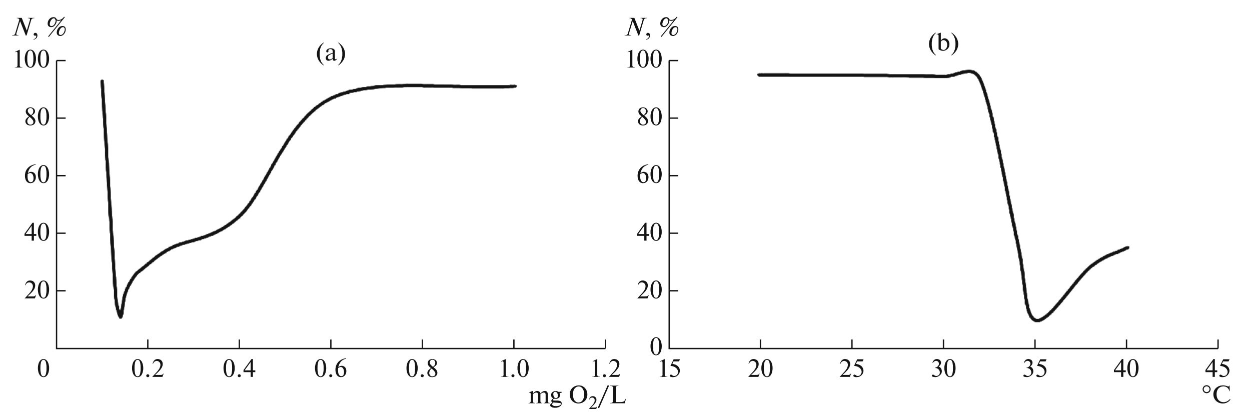

Fig. 3. Effect of oxygen at 35°C (a) and temperature (b) on the total content of ammonium, nitrites, and nitrates in the treated water (% of initial nitrogen concentration in the entering water).

Table 6. Results of modeling of the effect of temperature on the growth of the main groups of microorganisms involved in nitrogen removal

Temperature, °C	AOB, mg COD/L	NOB, mg COD/L	Anammox bacteria, mg COD/L
20	0.55	0.31	0.38
25	0.76	0.30	0.38
30	1.66	0.30	0.39
32	4.35	0.30	0.45
34	120.92	0.34	43.14
35	186.76	0.48	78.17
38	232.00	0.95	35.20
40	238.79	0.99	30.52

Table 7. Results of modeling of the effect of temperature on the composition of treated water

Temperature, °C	Ammonium, mg N/L	Nitrites, mg N/L	Nitrates, mg N/L
20	616.67	0.00	0.00
25	615.59	0.01	0.00
30	612.72	0.02	0.00
32	605.80	0.07	0.01
34	214.88	6.08	26.96
35	12.37	8.97	49.38
38	6.56	62.61	121.53
40	5.84	111.65	115.32

tion). It was shown that nitritation and anammox processes occurred simultaneously under conditions of continuous aeration at an oxygen concentration of 0.15–0.3 mg/L. At higher oxygen concentrations, NOB displaced AOB and anammox bacteria competing for oxygen and nitrite [18]. Using two novel 1D multispecies models based on AQUASIM, Hubaux and coauthors demonstrated that the dissolved oxygen concentration has the same effect on the nitritation/anammox process in reactors with granulated and free-floating biomass [19]. According to the results, oxygen concentrations <0.1 mg/L limited AOB activity, whereas oxygen concentrations >0.5 mg/L inhibited anammox activity and induced NOB growth [19]. However, under the actual operating conditions of industrial reactors, the optimal range of oxygen concentrations for nitritation/anammox process is significantly wider and varies from 0.05 to 1.5 mg/L depending on the reactor configuration [6].

Modeling of temperature effect. The effect of temperature was studied within the range of 20 to 40°C at a fixed oxygen concentration. The concentration of the biomass of the main groups of nitrogen-converting microorganisms reached values sufficient to perform removal of nitrogen at temperatures above 34°C (Tables 6 and 7). The optimal temperature for nitrogen removal providing maximal biomass of anammox bacteria was 35°C. Under these conditions, the total content of ammonium, nitrite, and nitrate nitrogen in the treated water was ten times lower than that in the entering water (Fig. 3). The AOB content was decreased at lower temperatures (Table 6), whereas the residual ammonium concentration increased. The concentrations of nitrites and nitrates rapidly increased at higher temperatures (Table 7).

Comparison of calculated and experimental parameters of laboratory setup. The stationary solutions of operating parameters of the reactor at different concentrations of dissolved oxygen and temperature were obtained from the simulation. The mode at dissolved oxygen concertation of 0.14 mg/L and temperature of 35°C was close to optimal.

The simulated optimal temperatures and dissolved oxygen concentrations, as well as parameters of nitrogen compounds removal, were similar to those obtained during experiments on the laboratory setup (Table 8). The calculated and experimental total contents of ammonium, nitrite, and nitrate nitrogen in the treated waters were 10.7 and 11.7% of those in the entering water.

Comparison of the results of mathematical modeling and experimental data demonstrated a high predictive ability of mathematical modeling of anammox process. The efficiency of modeling of biotechnologies based on activated sludge has been thus far confirmed by examples of mathematical modeling of processes based on the activity of heterotrophic, nitrifying, and denitrifying microorganisms [13]. The reliability of the

Table 8. Calculated and experimental parameters of laboratory anammox setup

Parameter	Calculated parameters	Experimental parameters
Density of total biomass of the activated sludge in the bioreactor, g/L	6.3	5.7
Optimum range of oxygen concentrations, mg/L	0.14	0.1–0.8
Optimal temperature range, °C	35	30–37
Nitrogen concentration in the treated water, mg/L*		
$N\text{-}NH_4^+$	1.9	4.5
$N\text{-}NO_2^-$	1.4	2.9
$N\text{-}NO_3^-$	7.5	4.2
Total N ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$)	10.7	11.7

* % of initial nitrogen concentration entering in the bioreactor.

modeling of behavior of anammox bacteria under these conditions has been confirmed in a limited number of works in recent years [18–20]. It can be explained by the facts that the physiology of anammox bacteria is poorly studied and anammox technologies are complex for modeling.

The algorithms and simulation constants obtained in the present study can be used to calculate bioreactor operating modes at subsequent stages of the work. It is planned to simulate a pilot bioreactor with a capacity of 20 m^3/day, as well as an industrial bioreactor with a capacity of 18000 m^3/day.

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