JOURNAL OF ENVIRONMENTAL ENGINEERING
AND LANDSCAPE MANAGEMENT
2009
17(2): 114–120

MODELLING OF CHLORIDE INFLUENCE UPON ACTIVATED SLUDGE COMMUNITY GROWTH

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Submitted 29 May 2008; accepted 16 Jan. 2009

Abstract. Growth kinetics, i.e. the relationship between specific growth rate and the concentration of a substrate, is one of the basic tools in the modelling of activated sludge community growth. The above-described conventional growth kinetics derived from single-substrate-controlled laboratory experiments have invariably been used for describing both growth and substrate utilization in wastewater treatment. After technological processes various mineral substances, such as chlorides, get into water bodies. These substances are not removed from wastewater by the biological treatment method. The purpose of this study is to determine the concentrations of chlorides, investigate the influence of enzyme preparations upon wastewater treatment quality during biological process modelling. Kinetics of microbial growth was designed using the model of Monad.

To analyse the chloride influence on the biooxidation process a series of laboratory tests was carried out: biochemical oxygen consumption (BOD), activated sludge concentration and other indexes. It is found experimentally that a 400 mg/l concentration of chlorides disarranges the activity of microorganisms and activated sludge is no longer suitable for biological treatment. Also, we investigated the effect of enzyme preparation on effluence on sewage treatment. As chloride concentrations are increased in a tank without an enzyme, bacterial spores contained in the enzyme preparation produce a renewing effect upon active sludge.

Keywords: wastewater, aerobic treatment, activated sludge, chlorides, enzymes, mathematical modelling.

1. Introduction

Growth kinetics, i.e. the relationship between specific growth rate and the concentration of a substrate, is one of the basic tools in the modeling of activated sludge community growth.

The problems are mainly due to the analytical difficulty in measuring substrates at growth-controlling concentrations and the fact that during a kinetic experiment, particularly in batch systems, microorganisms alter their kinetic properties because of adaptation to the changing environment.

The above-described conventional growth kinetics derived from single-substrate-controlled laboratory experiments have invariably been used for describing both growth and substrate utilization in ecosystems. However, in nature, microbial cells are exposed to a wide spectrum of potential substrates, many of which they utilize simultaneously (in particular carbon sources). The kinetic data available to date for growth of pure cultures in carbon-controlled continuous culture with defined mixtures of two or more carbon sources (including pollutants) clearly demonstrate that simultaneous utilization results in lowered residual steady-state concentrations of all substrates. This should result in a competitive advantage of a cell capable of mixed-substrate growth because it can grow much faster at low substrate concentrations than one would expect from single-substrate kinetics. Additionally, the relevance of the kinetic principles obtained from defined culture systems with single, mixed, or multicomponent substrates to the kinetics of pollutant degradation as it occurs in the presence of alternative carbon sources in complex environmental systems is discussed (Kovárová and Egli 1998).

Microbial growth kinetics, i.e. the relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (s), is an indispensable tool in all fields of microbiology, be it physiology, genetics, ecology or biotechnology.

In contrast, considerable attention has been paid to the modelling aspects of both growth and substrate removal (biodegradation) kinetics (Battersby 1990; Esener *et al*. 1983; Nielsen and Villadsen 1992).

Although some of these authors dealing with microbial growth kinetics started to emphasize the ecological point of view, they almost totally neglected the facts that in nature microorganisms grow mostly with mixtures of substrates, that growth may not be controlled by only a single nutrient but by two or more nutrients simultaneously (Rutgers *et al*. 1990), and that kinetic properties of a cell might change due to adaptation (Kovárová 1996).

ISSN 1648–6897 print / ISSN 1822-4199 online
DOI: 10.3846/1648-6897.2009.17.114-120

The kinetics of biodegradation of specific compounds has been investigated in complex systems consisting of undefined mixtures of cultures and substrates, e.g. in natural and technical environments directly or in laboratory microcosms (Hurst *et al*. 1997; Panikov 1995). Such data are preferentially used to model processes in wastewater treatment plants or environmental compartments (Gujer *et al*. 1995; Henze *et al*. 1995).

Defined laboratory studies with mixed substrates and pure and mixed cultures performed in continuous culture is one of the most appropriate experimental approaches to understanding the kinetic and physiological behavior of microorganisms in their natural environment (Egli 1995; Morita 1993).

Typically, specific rates of growth were measured at different substrate concentrations which, in turn, were estimated either by calculation from the biomass produced and a growth yield factor or simply by calculation from known dilution factors (Koch and Wang 1982).

Although, the Monod equation is mathematically analogous to the formula that was proposed by Michaelis and Menten to describe enzyme kinetics, the meaning of the two parameters K_s and K_m is quite different. Monod had already stressed (1949) that there is no relationship between the K_s and the Michaelis-Menten constant K_m. In contrast to Michaelis-Menten kinetics, which is used to describe a process catalyzed by a single enzyme, Monod kinetics describes processes (both growth and growth-linked biodegradation) of a more complex nature in which many enzyme systems are involved.

However, biochemical wastewater treatment processes are very complex and depend on a number of factors, including the chemical composition and concentration of organic matter in wastewater, water temperature and pH, and the content of toxic substances in the water (Grady *et al.* 1999).

It is noted that the concentrations of wastewater delivered to treatment facilities have increased lately. After technological processes various mineral substances, such as chlorides and sulphates, get into water bodies. These substances are not removed from wastewater by the biological treatment method. Chlorides get into water both with domestic and industrial wastewater because chlorine and chlorine compounds are used for rendering wastewater harmless (Eckenfelder 1989). Chlorine compounds are used to destroy pathogenic microorganisms, to remove odours in slaughterhouses and fish-processing enterprises, to salt foodstuffs in food industries, etc. Microorganisms are sensitive to changes in osmotic pressure in the medium. Larger amounts of mineral salts (KCl, NaCl) evoke plasmolysis inside the cells of microorganisms as a result of which microorganisms are destroyed.

Many of the environmentally relevant aspects in growth kinetics are still waiting to be discovered, established and exploited. The main aim of this work is to analyse the effect of chlorides on biochemical oxidation process and activated sludge community growth, to investigate the influence of the enzyme preparation upon water quality.

2. Methods

2.1. Procedures of lab-scale experiments

A model consisting of two tanks with a capacity of 5 litres each was used for the experiment. The working volume of each tank was 3 litres. Using air supplied by micro-compressors, activated sludge is constantly mixed with water and maintained in a suspended state. The operating conditions selected were similar to those of an aeration tank of the operation period in which aeration and mixing of activated sludge takes place 20 hours per day, then aeration is switched off for 4 hours leaving water to settle (Skaisgiriene *et al.* 2004).

During the adaptation period (3–4 days), the microorganisms in the sludge adapt to the medium. The substrate used for the adaptation stage and the experiment (Aravinthan *et al.* 2001) consisted of: glucose – 150 mg/l, yeast suspension – 150 mg/l, CH_3COONa – 150 mg/l, NH_4Cl – 90 mg/l, KH_2PO_4 – 42 mg/l, K_2HPO_4 – 42 mg/l, KCl – 60 mg/l, NaCl – 30 mg/l, $MgSO_4$ – 18 mg/l, $NaHCO_3$ – 720 mg/l.

Additionally, the following pollutants were introduced: KCl (chloride concentration of 200 mg/l and 400 mg/l). The enzyme preparation (proteases and oxygenases) was added into the second tank (1.2 ml/l). After 20 hours aeration was switched off and samples taken upon settlement of activated sludge.

2.2. Parameters measured

In batch-culture experiments, either the consumption of the growth-controlling substrate or the increase in biomass concentration was monitored as a function of time.

In order to analyse the wastewater treatment effects during the experiments there were tested the indicators, such as BOD, dissolved oxygen, total and volatile suspended solids and sludge physical characteristics. Sludge index and sludge concentration were observed in the course of the experiment. Indicator microorganisms were also observed through a microscope.

The amount of oxygen dissolved in water was determined by the Winkler's method (Environment ... 1994). The essence of the biochemical oxygen consumption (BOD_5) method (LAND 47-1:2002, LAND 47-2:2002) consists in the analysis of a water sample after shaking it and keeping at 20 ºC for five days in a dark place in filled and sealed bottles. The concentration of dissolved oxygen is measured before and after the incubation. Oxygen consumption per litre of a sample is calculated.

2.3. Model formation (equations, model calculation)

Some models for environmental transfer processes are used: Gausian air dispersion exponential law (Vaitiekūnas, Banaitytė 2007) and for the solid particle transfer process – Algebraic Slip Method (Baltrenas *et al.*2008), i.e. a full-cale three-dimensional mathematical model.

The dynamics of any population can be described by Maltus equation, that separates solution, defines population exponential growth law. More correspondent real popula-

tion dynamics conditions are proposed by P. F. Ferchiulst population dynamics logistics differential equation:

$$\frac{dN}{dt} = \lambda N(t)\big(K - N(t)\big), \quad (1)$$

where *N(t)* – any population individual number at time moment *t*.

Kinetics of microbial growth was designed using the model of Monad (Casey 1997):

$$\frac{dX}{dt} = \mu X - k_d X\ , \quad (2)$$

where X – concentration of microorganisms; μ – coefficient of growing; k_d –coefficient of degradation.

In the case of culture with food limit the specific growth coefficient is:

$$\mu = \mu_{\max}\left[S/(K_s + S)\right], \quad (3)$$

where μ_{max} – maximal specific growth coefficient, K_s – saturation constant, S – substratum concentration. Active sludge change and substratum degradation is related by the equation:

$$\frac{dX}{dt} = -Y\frac{dS}{dt}, \quad (4)$$

where Y – output coefficient.

The kinetics of the microorganism growth is closely related to the decrease in the substrate concentration (biodegradation). A system of equations has been used for the modelling of the substrate biodegradation (Casey 1997):

$$\begin{cases} \dfrac{dX}{dt} = \mu X - k_d X, \\ -\dfrac{dS}{dt} = \dfrac{\mu_{\max} XS}{Y(K_S + S)}, \end{cases} \quad (5)$$

where the rate of the substrate biodegradation depends upon the mass balance of the active sludge microorganism growth.

Numerical modelling. Describing transfer processes any differential equations, often are obtainable more complicated differential equations, which don't have analytical solution. One of mostly used approximate differential equation solution methods is Runge-Kutto solution method, applicable for differential equations $y' = f(x, y)$ with initial conditions $y(0) = y_0$.

3. Results and discussion

3.1. Influence of chlorides upon biological wastewater treatment

The biological treatment of wastewater is based on the biochemical oxidation of substances. Biological oxidation is enzyme oxidation of cell substrates with the main final products being CO_2, H_2O, and urea. It differs from oxidation taking place in inanimate nature in that 1) it is a gradual oxidation of substrates and the released energy is stored in macro energetic compounds and specific chemical bonds; 2) enzymes are its catalysts, and 3) energy is released as hydrogen is oxidised to produce water. The larger the number of nutritional links in the system, the higher the energy consumption.

Organic matter dissolved in water accounts for the largest part of pollutants. In the course of the experiment with KCl the effect of chloride ions upon the biochemical oxidation processes and splitting of organic pollutants was studied. The influence of KCl over metabolism in the active sludge system was also determined. Three parallel investigations were conducted: 1) KCl without pollution, 2) 200 mg/l Cl^-, 3) 400 mg/l Cl^-. A daily concentration of Cl^- that does an irreparable damage to active sludge was established. Where the concentration of chlorides in wastewater reaches 700 mg/l, the microorganisms of active sludge are destroyed and sludge flakes rise to the surface of water instead of settling after aeration is switched off. Such an active sludge is not suitable for the biological treatment of wastewater.

It is noted during the experiment that the higher chloride concentration in wastewater, the stronger a positive effect of enzymes. Fig. 1 shows the dependence of biochemical oxygen demand upon the concentration of chlorides after the biological treatment of wastewater.

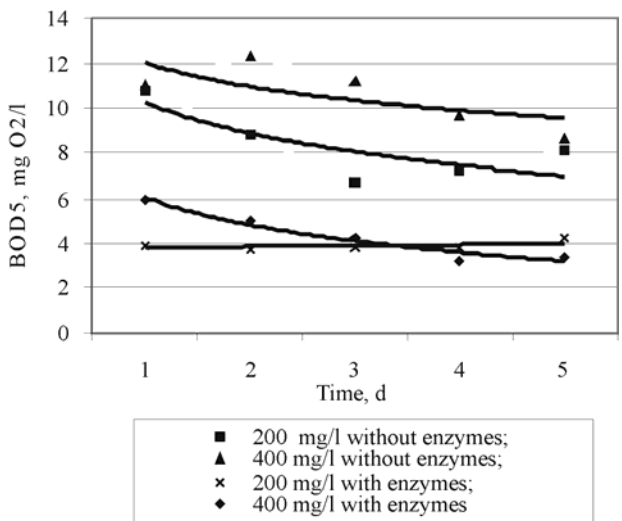

Fig. 1. Dependence of biochemical oxygen demand upon chloride concentration

As the chloride concentration varies from 0 to 400 mg/l, the dependence is described by a second order parabola. As the concentration of chlorides was increased to 400 mg/l, BOD remained stable throughout the experiment in the tank with an enzyme preparation. This is because the enzyme preparation used contained bacterial spores that enable continuous renewal of active sludge. In the tank without an enzyme preparation the concentration of BOD increased with increase in chloride concentration and was higher by almost 20 mg/l than that in the tank with the preparation. Chlorides are characterised by an antiseptic effect. Where the concentration of Cl^- in wastewater reaches 400 mg/l, a large part of active sludge microorganisms, including microorganisms participating in the BOD process, are destroyed.

As a result of a slow hydrolysis of particulate organic matter, the growth of heterotrophic microorganisms in most ecosystems is controlled by the availability of carbon and energy substrates (reviewed in references (Harms and Bosma 1997). Note that evidence of the removal of "solubilized" and bioavailable substrates is not a limiting factor in the activated-sludge process.

Without substrates, which limit mikroorganism growth speed, the factor is other agent that proceeds negative influence on active sludge.

While a high concentration of chlorides (400 mg/l) that get into the tank with wastewater reduced active sludge concentration more than twice (from 2.45 g/l to 1.05 g/l) in five days, the use of an enzyme preparation resulted in a very slight change in active sludge concentration (from 2.21 g/l to 1.80 g/l) (Fig. 2).

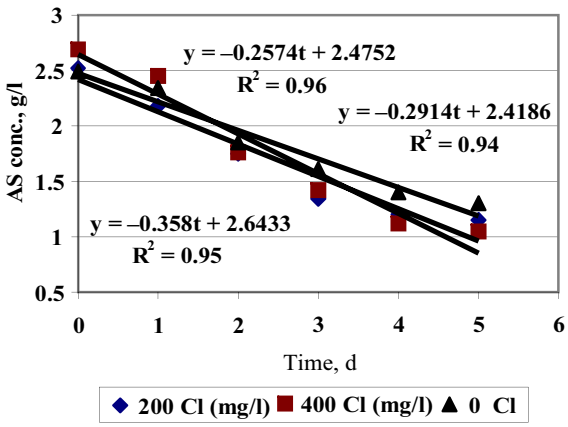

Fig. 2. Dependence of activated sludge (AS) concentration dynamics upon chloride concentration without enzymes

Osmotic pressure increases with increase of chloride concentrations. Different concentration of solutions of the medium and the cell cytoplasm is the main driver of osmotic absorption of nutrients. The higher the concentration of chlorides in the medium, the larger the amount of water diffused from the cells to the medium; the cytoplasm shrinks, wrinkles and comes off the membrane and wall, as a result of which nutrients do not reach the cells. Cells are destroyed and the concentration of active sludge lowers.

Experimental investigation was carried out introducing ferment preparation into a biologic refinement system. Using ferment preparation active sludge concentration changes fractionally, i.e. from 2.21 g/l till 1.80 g/l. Linear dependence under different chloride concentration was given. The given parameter R^2 was 0.97 and 0.92, accordingly (Fig. 3).

Using ferment preparation and given chloride concentration, as the values of coefficient k_d show, active sludge concentration decreases slowly. Given major chlorides concentrations increases osmosic pressure. Main osmotic food substance suck drive power is different trophic medium and cells cytoplasm soak concentration.

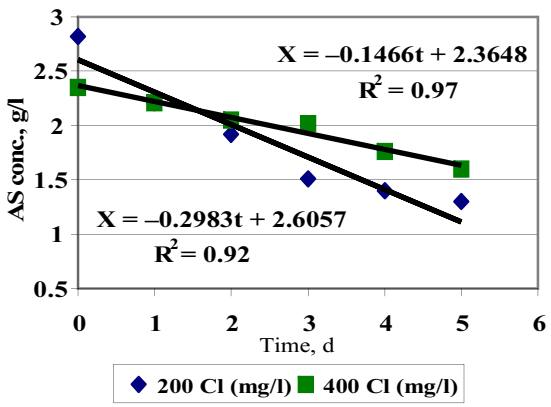

Fig. 3. Dependence of activated sludge concentration dynamics upon chloride concentration with enzymes

Bacterial spores contained in an enzyme preparation produce a renewing effect upon active sludge. The larger the amount of microorganisms, the higher the enzyme release levels; thus defence against the negative effect of chlorides is improved. As the amount of microorganisms (protozoa) in the system increases, i.e. the number of nutritional links is increased, the growth of biomass is reduced and more energy is consumed.

Metabolism is an essential sign of life. Each reaction of changes in substances causes an energy change in a system. An organism is an open system with a permanent equilibrium of chemical and energy exchange with the environment – metabolism. Metabolism as an entirety of complex enzyme reactions provides a basis for the functioning of a living system. Substances that get into an organism are affected by protein biocatalysts (enzymes) and non-protein parts of an active enzyme (coenzymes). The effect of enzymes and coenzymes determines the course of reactions. Under the influence of enzymes and coenzymes the initial amount of energy necessary for the processing of substrate is reduced and further transformation of substrate is regulated. The effect of enzymes helps to maintain an equilibrium between primary substances and the final products of their processing.

3.2. Modelling of activated sludge community growth and substrate biodegradation

J. Monad degradation ratio has been introduced into the model of growth of bacterial cultures (Eq. 2).

Increased chloride concentrations, in this case, appreciates the degradation coefficient k_d. Eq. (2) gives the active sludge degradation coefficient k_d, and it is seen that it depends upon chloride concentration when substratum chloride concentration is 200 mg/l, k_d is –0.348 and when 400 mg/l, k_d is –0.417, accordingly. As it is seen from the given results, less active sludge biocenosis degradation is given under less chloride concentrations in substratum. Active sludge degradation coefficient, using ferment preparation, is the following: when substratum chloride concentration is 200 mg/l, k_d is 0.1466, and at 400 mg/l, k_d is 0.2983, accordingly.

The solution of differential equation Eq. (2) is:

$$X = \frac{e^{\mu t} C}{e^{k_d t}} = C \cdot e^{(\mu - k_d)t}, \quad (6)$$

where constant C is deduced from the initial condition, when $t = 0$, $X = X_0$; X_0 is initial concentration of active sludge. We get $C = X_0$. Then we get solution of Eq. (2) which describes the dynamic of active sludge:

$$X = X_0 \cdot e^{(\mu - k_d)t}. \quad (7)$$

Upon solving the differential equation and adding the k_d (7) dependence upon polluting substances the following analytical solution of the equation has been obtained:

$$X = X_0 \cdot e^{[0,071-(0,4075c^2 - 0,085c - 0,2574)]t}, \quad (8)$$

then $0 \le c \le 0.4$ g/l,
where X_0 – initial concentration of activated sludge, g/l; c – chloride concentration, g/l.

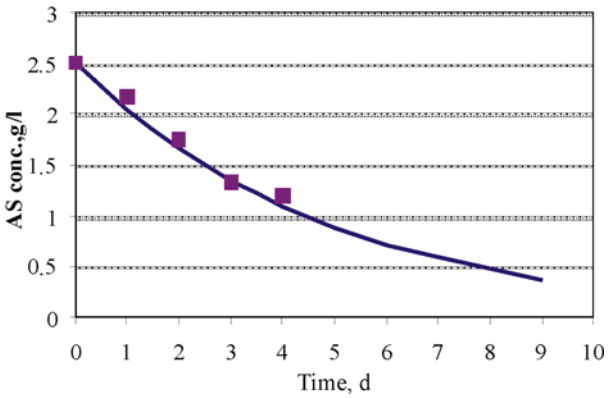

Fig. 4. Dynamics of activated sludge concentration: points – results of experiments, curve –solution of Eq. (4)

Changing chloride concentration, it is possible to model active sludge biocenosis dynamics. The dynamics model is presented in Fig. 4.

During experiment initial active sludge concentration was X_0 = 2.49 g/l, specific active sludge coefficient $\mu = 0.083$, maximal specific growth coefficient is taken from literature source – $\mu_{max} = 0.25$, substratum concentration was retained constant, S = 200 mg/l, and degradation coefficient in this case depends on chloride concentration in the feet medium.

Fig. 4 shows a model of the decrease in the active sludge concentration in time marked by a line, while the concentrations obtained on certain days of the experiment are marked by dots. The points of the modelled curve nearly coincide with the results of the experiment, therefore, one may state that this model is suitable for modelling the dynamics of the active sludge population at different chloride concentrations.

The kinetics of microorganism growth is closely related to the decrease in the substrate concentration (S) (biodegradation). A system of equations (Eq. 5) has been used for the modelling of the substrate biodegradation. The rate of the substrate biodegradation depends upon the mass balance of the active sludge microorganism growth. A solution of the equation system has been found upon solving it by the Runge-Kutta numerical method. Also, from the results of the experiment, it is assumed that dynamic of substrate biodegradation adheres to the law of exponent:

$$S = S_0 \cdot e^{-\alpha t}. \quad (9)$$

From the experimental results we get $\alpha \approx -(2.9 \div 3.2)$. Two curves representing the substrate dynamics are compared (Fig. 5).

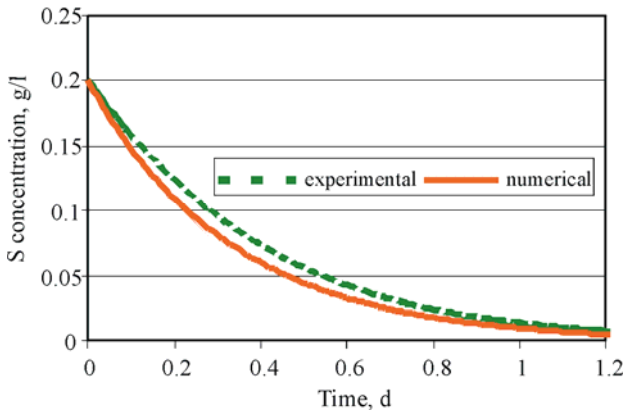

Fig. 5. Dynamics of substrate (S) biodegradation

As it is seen, the curves are similar in character and one may assume that the description of the substrate biodegradation provided by both methods is sufficiently accurate.

4. Conclusions

1. During the experiments pollutant concentrations influencing the biological treatment process were established: chloride concentrations of 400 mg/l and higher disrupt the activity of microorganisms, and activated sludge is no longer suitable for biological treatment.

2. While a high concentration of chlorides (400 mg/l) that get into a tank with wastewater reduces active sludge concentration more than twice (from 2.45 g/l to 1.05 g/l) in five days, the use of an enzyme preparation results in a very slight change in active sludge concentration (from 2.21 g/l to 1.80 g/l). Bacterial spores contained in the enzyme preparation produce a renewing effect upon active sludge.

3. Modelling of the dynamics of the active sludge biocenosis in the aerobic sewage treatment process and of the substrate biodegradation was carried out: the dynamics of the active sludge concentration was modelled on the basis of chloride concentrations, and a solution of the differential equation identifying biodegradation ratios was obtained.

4. A substrate biodegradation model, including a description of dynamics of the active sludge concentration and of the system of equations for the substrate biodegradation, has been formulated.

5. It is recommended that the active sludge dynamics model and the substrate biodegradation models, which were refined using the results of the experiments, are used for sewage treatment computations in practice.

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CHLORIDŲ ĮTAKOS VEIKLIOJO DUMBLO BIOCENOZĖS DINAMIKAI MODELIAVIMAS

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Santrauka

Mikroorganizmų augimo dinamika, t. y. priklausomybė tarp jų savitojo augimo spartos ir substrato koncentracijos, yra vienas iš svarbiausių veiksnių modeliuojant veikliojo dumblo kinetiką. Laboratorinėmis sąlygomis buvo atlikti eksperimentiniai nuotekų valymo tyrimai kompleksiškai įdėjus į substratą ir chloridų kaip papildomos užtaršos bei fermentinio preparato. Rezultatams palyginti buvo atlikti tyrimai ir be fermentinio preparato.

Po įvairių technologinių procesų į valymo įrenginius patenka tokios mineralinės medžiagos kaip chloridai. Šios medžiagos biologiniu valymo būdu iš nuotekų nepašalinamos. Darbe nagrinėjama, kokios chloridų koncentracijos turi įtakos biologinio nuotekų valymo kokybei, veikliojo dumblo augimo dinamikai bei substrato biodegradacijai. Eksperimentų metu nustatytos tiriamų teršalų koncentracijos, turėjusios įtakos biologiniam valymo procesui: 400 mg Cl^-/l ir didesnės sutrikdo mikroorganizmų veiklą, veiklusis dumblas biologiniam nuotekų valymui tampa netinkamas.

Darbe modeliuojama veikliojo dumblo mikroorganizmų biocenozės dinamika, atliekant aerobinį nuotekų valymą. Nagrinėtas atvejis, kai nuotekų užtarša yra didesnė chloridų koncentracija. Veikliojo dumblo biocenozės dinamikai modeliuoti pasirinktas Monodo modelis. Atlikus eksperimentą nustatyti degradacijos koeficientai k_d. Gautas Monodo diferencialinės

lygties sprendinys, nusakantis veikliojo dumblo dinamiką. Skaitiniais metodais išspręsta veikliojo dumblo augimą bei substrato biodegradaciją nusakanti diferencialinių lygčių sistema.
Įvertinus eksperimentų rezultatus nustatyti veikliojo dumblo sistemos degradacijos koeficientai įdėjus fermentinio preparato. Rezultatai parodė, kad fermentinis preparatas teigiamai veikia mikroorganizmų augimą t. y. veikliojo dumblo koncentracija mažėja lėčiau.

Reikšminiai žodžiai: nuotekos, aerobinis valymas, veiklusis dumblas, chloridai, fermentai, matematinis modeliavimas.

МОДЕЛИРОВАНИЕ ВЛИЯНИЯ ХЛОРИДОВ НА ДИНАМИКУ БИОЦЕНОЗА АКТИВНОГО ИЛА

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Р е з ю м е

Кинетика биомассы микроорганизмов, т. е. зависимость между концентрацией активного ила и концентрацией субстрата, один из важнейших аспектов в моделировании динамики активного ила. В лабораторных условиях были проведены экспериментальные исследования сточных вод в комплексе с добавлением в субстрат хлоридов как дополнительного загрязнения и ферментного препарата. Для сравнения результатов были проведены параллельные эксперименты без ферментного препарата. В результате технологических процессов в очистные сооружения попадают разные минеральные вещества, как, например, хлориды. Эти вещества методом биологической очистки не удаляются. В настоящей работе были исследованы концентрации хлоридов, влияющие на качество биологической очистки сточных вод, качество и кинетику активного ила и биодеградацию субстрата. Для определения влияния хлоридов на биологические процессы были проведены лабораторные исследования по определению биохимического потребления кислорода (БПК), концентрации активного ила и др. В результате эксперимента было установлено, что концентрация хлоридов, составляющая 400 мг/л и больше, поражает работу микроорганизмов, активный ил становится непригодным для биологической очистки сточных вод. Также исследовалось влияние ферментного препарата на качество очистки. Моделируя кинетику динамики активного ила, получено решение дифференциального уравнения, в котором идентифицированы коэффициенты деградации ила k_d. В результате эксперимента определены коэффициенты деградации на систему активного ила в резервуаре с ферментным препаратом, т. е. концентрация активного ила уменьшается медленнее. Можно сделать вывод, что ферментный препарат эффективно влияет на концентрацию микроорганизмов.

Ключевые слова: сточные воды, аэробная очистка, активный ил, хлориды, ферменты, математическое моделирование.

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