

## SOME PECULIARITIES BIOFILM FORMING AT WASTEWATER TREATMENT IN BIOREACTORS

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*In article the improvement model of forming the biofilm structure taking into account on the heterogeneity along its thickness with steady and changeable porosity in the constant and transient conditions at the aerobic wastewater treatment and taking into account on the influence of different hydrodynamical factors on its operation, availability of the other substances in particular inert biomass and polymeric ones is proposed. To a great extent the attention was allotted on investigation of the influence of the biofilm heterogeneity on the mass transport processes. Besides the analysis of the main factors the correlations for building of the mathematical model of the wastewater treatment with using of biofilms was carried out. It was shown that for the practical calculations of wastewater treatment the one-dimensional model of homogeneous biofilm is proposed taking into account on the obtained valuations and limitation of its using. Comparisons the calculation results with experimental data were shown that such homogeneous model in many cases has a good agreement with experimental ones. The some recommendations relative determination of the form of the biofilms surface are proposed. It can to say that the effectiveness of biological methods of removing pollutants of various origins in particular organic compounds (OC) and nitrogen (N) in various bioreactors may be improved by means of the arrangement on the surface of the additional loading a fixed biocenosis with a high concentration of microorganisms (biofilm).*

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### Introduction

In the last years in Ukraine sharply worse the ecological situation of the water media. Intensive loading on it connected with non-control pollution of the different origin in the river net, reservoirs and aquifers substantially decreased the quality of the drinking waters and in some regions of the state the deficit of the water resources which are suitable for water supply of population have a place. On the other hand the war with Russia created the additional technogenical loading on the all ecosystem of our state in whole. Therefore there is the necessity in construction and implementation of the modern technologies for wastewater treatment on the objects of the different profile that to help improve the ecology in Ukraine in post-war time. One of the method for improving of the such situation with the wastewaters which are polluted water media are treatment ones. Now most effective among them are methods of biological cleaning with using of the bioreactors of the different construction.

Actuality of problems of wastewater treatment is without any doubt today. Many scientific works and practical recommendations are devoted by the different aspects of solution the considered tasks, for example, ones (Beyenal et al., 2005; Henze et al., 2008; Kelareva et al., 2012; Oliynyk et al., 2016; 2023; Violi et al., 2002; Wanner et al., 2006 and etc.).

The analysis of the influence of some hydrodynamical processes and biochemical transformation on the characteristic and the structural peculiarities of the biofilm at the wastewater treatment in bioreactors of different constructions is carried out. The different types of biofilm and main its parameters which characterized its structure and exploitation possibility are considered.

### **Aim of research**

Substantiation and development of the main relations for creating of the mathematical models of forming of the biofilm in detail in particular its thickness with constant and changed porosity at the steady and transient conditions for more effective wastewater treatment from contaminants in bioreactor of the different constructions.

### **Materials and Methods**

The investigations were carried out with using of the analytical, numerical methods and experimental data and with a wide using of the personal computers.

### **Results and Discussion**

In real conditions as a rule the treatment of wastewaters in bioreactors with suspended biocenosis and the heterogeneous biofilm is considered. Under these conditions for such biofilm the well-known equation that describes the disposal of organic pollutants with a single limiting substrate in steady-state conditions is used (Beyenal et al., 2005; Henze et al., 2008; Kelareva et al., 2012):

$$D_L(z) \frac{d^2 L}{dz^2} + D_L \frac{dL}{dz} - \frac{\mu_{max} X_L}{Y} \cdot \frac{L}{K_{mL} + L} = 0 \quad (1)$$

where-  $L = L(r,t)$ - concentration of the liquid contaminants in biofilm,  $\text{kg}/\text{m}^3$ ;  $X_L$  – concentration of microorganisms in biofilm (average density of biofilm),  $\text{kg}/\text{m}^3$ ;  $Y$ - mass of unused norm of oxygen on the unit of mass of used substrate,  $\text{kg}/\text{m}^3$ ;  $\mu_{max}$  – maximal velocity of utilization,  $1/\text{c}$ ;  $K_{mL}$  – concentration of contaminants when the transformation velocity is 50% from maximal,  $\text{kg}/\text{m}^3$ ;  $D_L$ - coefficient of effective diffusion,  $\text{m}^2/\text{c}$ ;  $z$  – vertical coordinate.

It is a balance equation relative the speed of substrate utilization under the conditions of nonlinear Monod reaction kinetics.

In the proposed mathematical model of the stratified biofilm according to the conducted analysis the following is accepted (Henze et al., 2008; Kelareva et al., 2012; Oliynyk et al., 2023):

1) The relationship between the effective diffusion coefficient  $D_L$  and biofilm density  $X_L$

$$\frac{D_L}{D_w} = 1 - \frac{0.43 \cdot X_L^{0.92}}{11.19 + 0.27 \cdot X_L^{0.99}} \quad (2)$$

where  $D_w$  – diffusion coefficient in the pure water,  $\text{cm}^2/\text{c}$ .

For this case minimum concentration of the substrate at which there is no stable biofilm and below of which the flow of substrate equal zero can be determined by the next formula:

$$S_{\min} = \frac{k_d K_s}{(\mu_{\max} - k_d)}, \quad (3)$$

where  $\mu_{\max}, k_d, K_s$  - are the known parameters of the model, relatively maximal velocity of utilization, concentration of contaminated water and concentration of substrate in biofilm.

At realization of the proposed model the following results were obtained when determining the thickness of the biofilm  $L_f$  at  $X_f$ . (Table 1):

**Table 1. Value of biofilm thickness  $L_f = f(X_f, S_0)$  according to (Beyenal et al., 1994, 2005)**

$X_f$ mg/cm <sup>3</sup>	$S_0 = 0,02$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm	$S_0 = 0,04$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm	$S_0 = 0,06$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm	$S_0 = 0,08$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm	$S_0 = 0,10$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm	$S_0 = 0,01$ m <sup>2</sup> /cm <sup>3</sup> $L_f$ , cm
5	0.055	0.068	0.072	0.078	0.083	0.045
15	0.028	0.034	0.038	0.041	0.043	0.022
25	0.018	0.023	0.025	0.030	0.028	0.014
50	0.009	0.013	0.014	0.016	0.017	0.010

Beside in this work the results of flow calculations the flow  $I$  (mg/cm<sup>2</sup>·c) at different substrate concentrations in the reactor  $S_0$  and biomass concentrations  $X_f$  are given also.

As apparently with examples in general the processes of extracting the substrate depend on the concentration of microorganisms in the biofilm. However at significant concentrations  $X_f$  there is some decreasing of the substrate in the one. It may be explained that according equation (2) a significant decreasing of diffusion coefficient have a place and substrate extraction processes are more controlled by mass transfer ones and have greater influence in comparison with the concentration  $X_f$ .

For example on Figure 1 shows the change in internal porosity  $\varepsilon$  during biofilm growth respectively for cases A and B with high concentrations ( $L=300\text{gBOD}/\text{m}^3, N=30\text{gN}/\text{m}^3$ ) and small ones ( $L=30\text{gBOD}/\text{m}^3, N=30\text{gN}/\text{m}^3$ ) at high and low ratios OC/N. At the same time the change in internal porosity in the biofilm during its growth depends on significantly on the distribution of biomass namely the location of undissolved components. The change in internal porosity (its decrease) during the growth of the biofilm in both cases depends on the gradient of internal porosity along the length of the biofilm and increases with time.

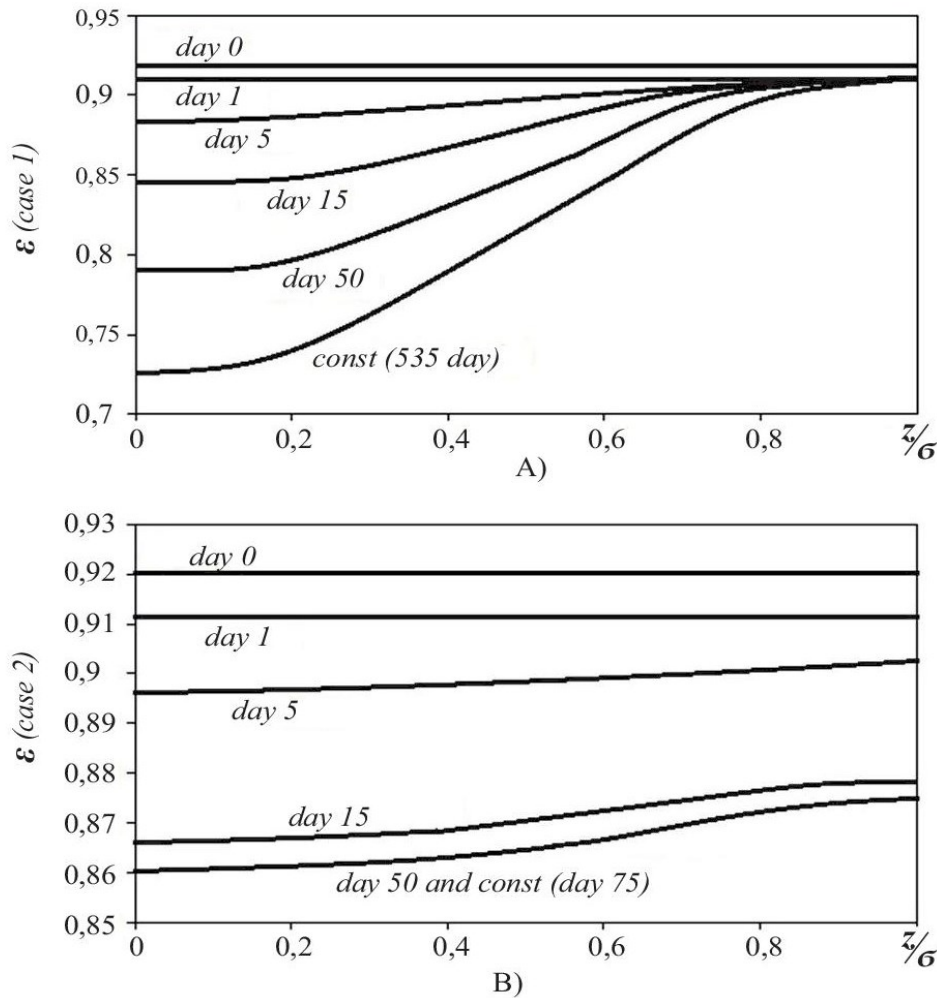
At solution equation (1) a number of requisites were adopted in particular that the substrate is transported by diffusion and are used by microorganisms according to the well-known Monod kinetic equation. The effective diffusion and density of the biofilm can be calculated at the boundary of each layer of stratified biofilm, the process in the biofilm can be considered in stationary conditions, the substrate is transferred in a perpendicular direction and the biofilm is formed on an impermeable and inactive surface. We also assume that pollution is transported to the lower surface in the direction  $Z$  only by diffusion. The solutions of equations (1) in which the concentration values  $X_{f_c}$  and the coefficient the effective diffusion value  $D_{f_z}$  are taken into account on according to the given dependencies is proposed in the next work (Beyenal et al., 2005).

When forming a homogeneous biofilm the following is adopted:

1) a homogeneous biofilm has an effective diffusion coefficient that is equal to the average value of the effective diffusion coefficient for a homogeneous stratified biofilm.

2) a homogeneous biofilm has a value of the effective diffusion coefficient which is equal the value one near the lower surface of a stratified biofilm.

3) these coefficients are assumed to be the same near the upper surface.



**Figure 1. Changes in internal porosity during biofilm growth (A) – a big ratio OC/N (case1) and (B) -small ratio OC/N (case 2),  $\sigma$  – thickness of biofilm**

In the theoretical investigations it is accepted that the biofilm consists of two parts namely the liquid phase in which dissolved substances are transferred by molecular diffusion and the solid part. Concentration of undissolved substances and the density of the biofilm can be expressed by the following equation (Beynal et al., 2005; Henze et al., 2008; Ebert et al., 2000; Ellenberg et al., 2007):

$$\varepsilon = \varepsilon_p = 1 - \sum_1^{n_x} \frac{X_i}{\rho_i} \quad (4)$$

$\varepsilon$  - coefficient of internal porosity of the biofilm.

$$\varepsilon_p + 1 - \sum_{i=1}^{n_x} \varepsilon_{Ti} = 1, \quad \varepsilon_{Ti} = \frac{X_i}{\rho_i} = \frac{M_{Fi}}{V_F} \cdot \frac{1}{\rho_i}, \quad (5)$$

$\varepsilon_p$  - relative volume of the liquid fraction,  $\varepsilon_{Ti}$  - relative volume of solids in undissolved form,  $n_i$  - amount of solid components,  $\rho_i$  - density of the component (mass per unit volume of the component).

The concentration of each component  $X_i$  is obtained as:

$$X_i = \frac{M_{Fi}}{V_F}, \quad (6)$$

where  $M_{Fi}$  - is the mass of the component,  $V_F$  - is the total volume of the biofilm that is the volume of the liquid and solid phase in whole.

The density of each component  $\rho_i$  (mass per unit volume of a particle of a given component) is related to the density of the biofilm  $\rho_m$  by the next equation:

$$\rho_m = \sum_{i=1}^{n_x} \varepsilon_{Ti} \rho_i = \frac{\sum M_{Fi}}{V_F}, \quad (7)$$

If the biomass in the biofilm is homogeneous then we have:

$$\rho_i = const = \rho_m (1 - \varepsilon_p) \quad (8)$$

Note that in general the solution of the problems related to the biological treatment of wastewater from various contaminants by biofilm depends on the accepted model of the biofilm structure.

Based on the analysis of existing literary sources as well as the obtained research results a next general conclusion was made that when developing and using biofilm models it is enough to restrict the consideration and study of the one-dimensional models with flat surfaces. At the same time that to take into account on the curvature of the surface we may to use a correction parameter  $A_\delta$  the value of which for various geometric shapes is given below. When using biofilms with flat surfaces it is necessary to pay attention to the fact that not the entire thickness of the biofilm takes part in biofilm models but only its active part. The active thickness of the biofilm under the conditions of extraction of various substrates for example organic pollutants and nitrogen consists of different bacteria and therefore the processes that take place in the biofilm in particular its growth will depend on the competition between them since both types of bacteria are using the oxygen for their nutrition.

Initially at the formation of a heterogeneous biofilm structure which consists of the autotrophs, heterotrophs and inert biomass in the absence of oxygen limitation the both types of bacteria can grow. At the same time the rapid growth of heterotrophic microorganisms over the entire thickness of the biofilm prevails. After approximately 15 days the thickness of the biofilm increases, slowly growing autotrophic microorganisms begin to dominate in its thickness while the heterotrophic microorganisms are accumulated slowly near its surface. At 19.5 days detachment (dying) occurs after which more autotrophic biomass remains. It should be noted that detachment generally occurs almost during the entire time of biofilm formation however during the main period the detachment rate will be lower than the rate of biomass formation and therefore the biofilm grows. The formation of biofilm is considered in more detail in the works (Wanner et al., 2006; Wasche et al., 2002; Tsuno et al., 2002).

In the joint treatment of wastewater of different substrates in particular organic (OC) and nitrogen (N) that to ensure the processes of their extraction by heterotrophic and autotrophic bacteria which are located in different layers of the biofilm oxygen is needed. At this there is significant competition between the specified groups of bacteria for oxygen is observed and therefore the processes of extraction of OC and N which occur in different layers of the biofilm may be limited by a lack of oxygen. In previous studies the conditions and criteria were defined under which oxygen will limit the process of separate extraction of OC and N in the biofilm due to its amount of

penetration in the biofilm (Ellenber et al., 2007; Wasche et al., 2002; Oliynyk et al., 2023). The the rapid growth of heterotrophic bacteria leads to a slight slow growth of autotrophic bacteria and that heterotrophic growth and extraction of organic matter (COD) is limited by autotrophic growth and that autotrophic growth and oxidation of ammonium are associated with a lack of oxygen which is taken by heterotrophic bacteria through the layer in which it passes and that the heterotrophs and autotrophs can exist only in the case when the concentration of organic matter (COD) in the reactor is less than 30 mg/l. That is at a concentration of more than 30 mg/l below the heterotrophic layer there will be no oxygen for the autotrophic layer. Thus we may to conclude that the existence of heterotrophic and autotrophic bacteria is possible only when the oxidation of organic matter is limited by the value of COD and not by oxygen. Analysis of existing studies on the combined purification of OC and N shows that an approximate critical values of COD was obtained for different reactors operation (Henze et al., 2008; Kelareva et al., 2012; Oliynyk et al., 2023).

Besides it was established that at the beginning of wastewater treatment there is a significant growth of the biofilm which then intensively decreases and this also leads to significant changes in the distribution of biomass. The formation of the thickness of the biofilm over time at large and low ratio OC/N occurs within about 10 days. When the ratio OC/N is large the growth of the biofilm occurs mainly due to the removal of organic matter by heterotrophs and the removal of nitrogen (ammonium) by autotrophs is almost absent. At low ratios OC/N a significant nitrification occurs along with the growth of biofilm and nitrogen removal began almost after complete removal of OC.

At the changes of the internal density the formation of activity of the biofilm changes too. The growth of the biofilm depends on significantly on the distribution of biomass that is on the location of individual components in it.

It was established that the formation of the active part of the biofilm is influenced by the processes of formation the inert sediment and extracellular polymeric substances in the composition of the biofilm which occupy a significant additional volume during growth biofilm and which generally influence on the parameters of the treatment process.

Based on the analysis of the results of theoretical and experimental studies a stratified one-dimensional model of the biofilm structure with the dominance of the location of heterotrophic bacteria on the surface of the biofilm and the growth of nitrifiers in the depth of the biofilm in the lower layers can be used for the study of the combined extraction of OC and N.

To assess the impact of biofilm stratification the additional biofilm modeling with a homogeneous distribution of biomass across the biofilm thickness was also investigated. In the case of homogeneous biofilm the relative amount of heterotrophs, autotrophs and inert biomass changes over time as a result of overall growth. In such way the homogeneous distribution of biomass reduces the advantages of heterotrophs which no longer dominate on the surface of the biofilm.

It was established that during the treatment of wastewater of dissolved pollutants of various origins the upper surface of the biofilm is formed mainly curvilinear and of various wavy shapes. Some research results are given in (Wanner et al., 2006).

Below the some recommendations for taking into account on the different forms of the biofilm surface starting from a flat surface to biofilms of the "mushroom" type with significant channels and pores are presented.

On the basis of conducting research a dimensionless coefficient is proposed (Wanner et al., 2006; Wasche et al., 2002):

$$G = \frac{L_f^2 \mu_{\max} X_p}{DS_b}, \quad (9)$$

where  $X_p$  – the concentration of bacteria in the biofilm,  $S_b$  – the concentration of the substrate with a limited growth rate in the main liquid,  $D$  – diffusion coefficient,  $L_f$  – biofilm thickness,  $\mu_{\max}$  – known model parameter, which is determined above in article.

In an result of the carried out analysis with using experimental data it was elucidated that for biofilm which has a dense solid matrix a flat surface parameter will be  $G < 5$ . When  $G > 10$  then we have a complex structural surface of a biofilm of the "mushroom" type - different clusters and channels and in this case the complex structure of the biofilm should be taken into account on when making calculations. Thus the attempt was made to take into account on the non-flat geometry of the biofilm surface by introducing a correction factor  $A_e$  that depends on the geometric structure of the biofilm and will be greater than a flat biofilm which has  $A_e=1.0$  (Wanner et al.,2006).In particular the values of the parameter  $A_e$  for 13 studied structures of biofilm surfaces at comparing with the case of a completely flat surface were investigated and show that the parameter  $A_e$  varied within the range  $A_e=1.0-2.32$ .

Thus the parameter  $A_e$  can be considered as a measure of biofilm heterogeneity. The more irregular heterogeneous structure the greater the value of  $A_e$  which deviates from the value obtained for a flat biofilm ( $A_e=1$ ). When  $G \approx 10$  as already noted  $A_e \approx 2,25-2,35$ . To our mind the study of the problem of the shape of the surface of the biofilm and its influence on the flow of substrates of pollutants entering into the biofilm should be continued.

In the studies carried out during the extraction of pollutants by biofilms formed in various bioreactors the concentration profiles in biofilms were calculated under the condition that the concentration of pollutants on the surface of the biofilm  $L_\delta$  was assumed equal to the concentration of pollutants on the surface of the liquid  $L_e$ . At the same time the hydrodynamics of the flow and the concentration of  $L_e$  in different bioreactors was different and was not taken into account on. However even with complete mixing in bioreactors the formation of a boundary layer (liquid film) is observed and the processes of mass transport of pollutants into the biofilm must be taken into account on in the biofilm models. In some works deal with the formation of the boundary layer and the need to take it into account on the value of the flow of pollutants entering to biofilm through its surface is proposed in the next form (Beynal et al.,2005; Henze et al.,2008):

$$I_{L_F} = -D_F \cdot \frac{dL_F}{dz} \quad \text{at } z = 0, \quad (10)$$

which shows that mass transfer in the boundary layer occurs due to diffusion,  $D_F$ . For case the extraction of pollutants in the bioreactor aerotank-mixer the general equation will have the form:

$$Q(L_0 - L_a) - I_{L_F} A_F - r_b V_b = 0 \quad (11)$$

where  $A_F$  - the surface area of the biofilm,  $V_b$  - the volume of liquid in the aeration tank,  $r_b$  - removal of pollutants due to the floating biocenosis,  $L_0$ - concentration of substrate on exit,  $L_a$ - concentration of substrate in entering liquid (Henze et al. 2008). Assuming that

$$r_b V_b \ll I_{L_F} A_F, \quad (12)$$

then for the definition  $I_{LF}$  we obtain

$$I_{LF} = \frac{QL_0}{A_F} - \frac{QL_a}{A_F}. \quad (13)$$

In the future the equation (15) in models and calculations will be used in the next form:

$$I_{LF} = -D_F \frac{L_b - L_\delta}{\delta_p}, \quad (14)$$

where  $\delta_p$  - thickness of the liquid film.

For practical calculations the next equations are used to determine the flow  $I_{LF}$ :

$$I_{LF} = K_{\delta p} (L_b - L_\delta) = \frac{1}{R_{\delta p}} (L_b - L_\delta), \quad (15)$$

where  $K_{\delta p}$  - transfer coefficient,  $R_{\delta p}$  - external resistance which is determined as (Henze et al., 2008):

$$K_{\delta p} = \frac{D_p}{\delta_p}, \quad K_{\delta p} = \frac{1}{R_{\delta p}}; \quad R_{\delta p} = \frac{\delta_p}{D_p}. \quad (16)$$

We can see that the parameters  $K_{\delta p}$  i  $R_{\delta p}$  depend on the thickness of the liquid film (boundary layer)  $\delta_p$  and the diffusion coefficient in the liquid phase  $D_p$ .

Thus according to (16) the main difficulty in the calculation  $K_{\delta p}$  i  $R_{\delta p}$  lies in determining the thickness of boundary layer and values of the parameter  $D_p$  which for various contaminants is given in the literature in particular (Henze et al., 2002, 2008).

In some cases the shape of the loading elements on which the biofilm is formed has the spherical one and the resistance  $R_{\delta p}$  can determine using known hydrodynamic dimensionless criteria the Sherwood number:

$$Sh = \frac{d_L}{R_{\delta p} D_p}, \quad (17)$$

here  $d_L$  - characteristic length (for example, diameter):

$$Sh = A + B Re^m Sc^n \quad (18)$$

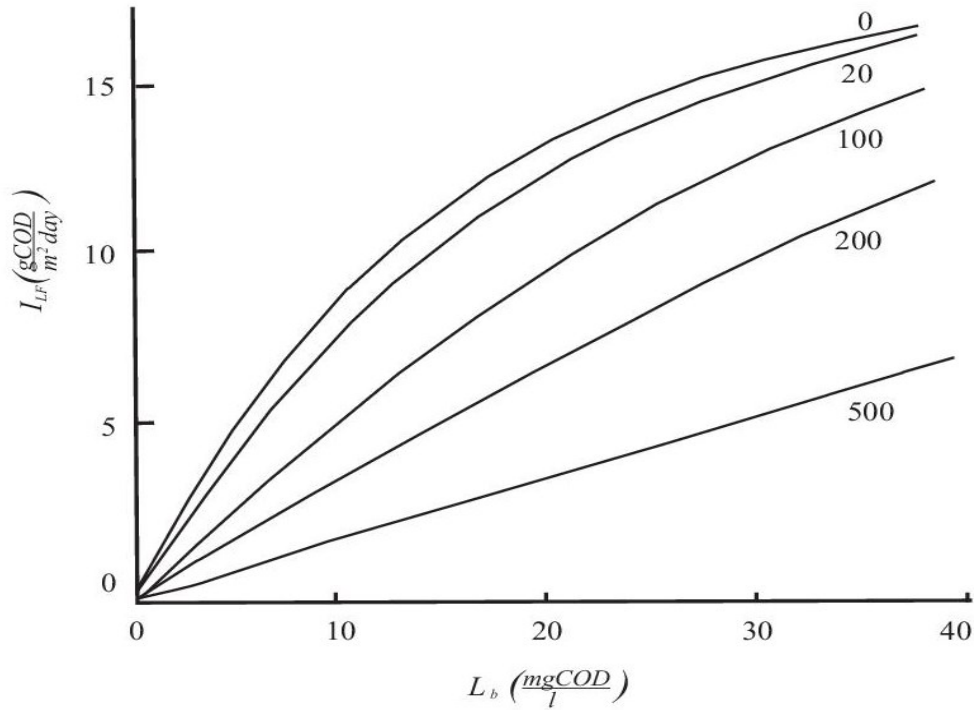
Note that the parameters  $A$ ,  $B$ ,  $m$ , depend on the geometry of the base that supports the biofilm and as a rule are justified only in individual cases based on the use of experimental data.

On the basis of equation (18) the next dependence is proposed for determining the coefficient  $K_{\delta p}$  under conditions of spherical loading:

$$K_{\delta p} = \frac{1,09}{\varepsilon} Re^{1/3} Sc^{1/3} \frac{D_p}{d}, \quad (19)$$

where  $Sc = \frac{\nu}{D}$  - Schmidt number,  $Re = \frac{Vd_L}{\nu}$  - Reynolds number,  $\nu$  - kinematic viscosity, m<sup>2</sup>/c,  $d_L$  - the diameter of the bioparticle,  $\varepsilon$  - porosity.

In Figure 2 as an example of impact the assessment  $\delta_p$  a graph  $I_{LF} = f(L_b, \delta_p)$  is given for the different cases of  $\delta_p$  according to Monod kinetic in a bioreactor-mixer at  $L = const$ . Thus with a significant thickness of the boundary layer  $\delta_p = 500\mu\text{m}$  the flow of OC decreases by almost 70%.



**Figure 2. Graph of dependence of flow  $I_{LF} = \int L_b \delta_p$  according to Monod kinetic**

Therefore for efficiency of the bioreactor operation the thickness of the boundary layer will be in the limits of 100-200  $\mu\text{m}$ .

Relative growth rate  $\mu^*$  is determined as the ratio of the specific growth rate  $\mu_{LF}$  to the maximum growth rate  $\mu_{\max}$ . For heterotrophic it is accepted  $\mu_{\max} = 5.5 \text{ day}^{-1}$ :

$$\mu^* = \frac{\mu_{LF}}{\mu_{\max}} \quad (20)$$

This coefficient increases with an increasing of in the substrate load and the Re number. The greatest effect is observed when the load increases at low Re (less than 2000). The thickness and surface structure of the biofilm depends on the load and hydrodynamic conditions too. A biofilm with a homogeneous smooth surface has a place at high tangential shear and low loading. Such biofilm has the largest number  $Sh$  and it decreases with increasing roughness of the biofilm surface. (Oliynyk et al., 2016). Beside it was concluded that not only diffusion but also convective transport of the substrate is observed in the boundary layer and the mass transfer through a boundary layer of biofilm is greater than through a surface without a biofilm.

## Conclusions

Carried out studies allows to do the next conclusions: for practical calculations of wastewater treatment the one-dimensional model of homogeneous biofilm is proposed taking into account on the obtained valuations and limitation of its using. Comparisons the calculation results with experimental data were shown that such homogeneous model in many cases has a good agreement with experimental ones. The some recommendations are proposed relative determination of the surface form of biofilms.

In general the analysis of theoretical studies showed that the extraction of the substrate mainly occurs in the initial part of the biofilm which is approximately 20-25% of the total thickness of the biofilm  $\delta$ .

It can be said that the effectiveness of biological methods of removing pollutants of various origins in particular organic compounds (OC) and nitrogen (N) in various bioreactors may be improved by means of the arrangement on the surface of the additional loading a fixed biocenosis with a high concentration of microorganisms (biofilm). However the accepted homogenous model of a biofilm with uniform thickness requires further improvement relative forming its structure in particular taking into account on the heterogeneity of its thickness, the influence of various hydrodynamic factors on the functioning of the biofilm, the presence of other inactive substances in particular inert biomass and polymeric substances, etc.

### **Conflict of interest**

The authors state no conflict of interest.

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