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Original Research

Experimental Determination of Kinetic Parameters for Heterotrophic Microorganisms in Biofilm under Petrochemical Wastewater Conditions

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Abstract

This paper presents the methods and results of Monod kinetic constant determination for heterotrophic bacterial growth in an aerated submerged fixed-bed biofilm reactor (ASFBBR) treating petrochemical wastewater. The methods originally developed for activated sludge are successfully adapted to biofilm kinetics characterization. The results are discussed and compared with the values given by other authors. The purpose of this calibration is to develop a mathematical model for a novel approach toward biofilm system dimensioning.

Keywords: biofilm, kinetics, petrochemical wastewater treatment

Introduction

The main purpose of the studies presented in this article was to determine the kinetic parameters for biofilm heterotrophic bacteria treating petrochemical wastewater using methods that could be easily implemented even in a simple water and wastewater laboratory. In order to achieve this goal, methods originally developed for activated sludge were successfully adapted to biofilm kinetics characterization. A novel approach toward respirometric determination of heterotrophic maximum growth rate ($\mu_{max,H}$) allowed us to simultaneously estimate half saturation coefficient (K_S).

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Biofilm reactors might be a good alternative to commonly applied activated sludge systems, particularly in the area of industrial wastewater treatment [1-6]. A new opportunity for faster development of this technology results from development of mathematical models. Their application could improve the understanding and designing of such types of bioreactors [1-3, 7-11]. Mathematical models can be applied for practical purposes after choosing the proper model structure and determining the values of model parameters [3, 11]. The kinetic parameters (kinetic constants) of heterotrophic microorganism growth are the most important part of every existing mathematical model of biological wastewater treatment.

Determination of the parameters of a model can be conducted using the following procedures:

- calibration and verification - upon collecting a series of data for different loadings of the treatment plant, the

parameters of the model are changed in order to achieve the best fit to the effluent data,
- parameter estimation with advanced statistical procedures,
- model parameter determination during dedicated laboratory or on-line experiments consistent with applied models.

The experimental approach enables the researcher not only to quantify model parameters accordingly to the concept used in the model, but also to increase the reliability of results [12]. This method of parameter determination also allows avoidance of the uncertainty that another set of parameters estimated via the calibration/verification approach would not have achieved a similar fit of the model to experimental results [3]. Moreover, kinetic parameter values often depend upon local conditions of the wastewater treatment plant and may vary from system to system. This is especially important for studies on industrial wastewater treatment, i.e. petrochemical discharge, because variable characteristics of such wastewater may cause changes in the biocenosis of heterotrophic bacteria in the treatment plant and, as a consequence, the values of kinetic parameters may change. Therefore, determination of these parameters as a purpose of dedicated research work is justified both because of its practical potential (model application to practical purposes such as bioreactor dimensioning) and gaining knowledge on biofilms growing in a special environment (in this case, in a petrochemical wastewater treatment plant) [11]. This kind of research is also necessary due to lack of available data on kinetic constant values determined for heterotrophs that form biofilms in petrochemical wastewater.

In this paper, the methods and results of the kinetic constant determination of heterotrophic microorganisms in biofilm are presented. The data obtained is compared to values determined for activated sludge and biofilms by other authors.

Materials and Methods

Biofilm and Wastewater

The biofilm used in this study originated from a pilot-scale aerated submerged fixed-bed biofilm reactor (ASFB-BR) located in a plant treating petrochemical wastewater from the "Glimar" oil refinery in Gorlice, Poland. The reactor served as a test unit for post-treatment of mechanically and chemically treated wastewater (oil-water separators API and CPI, dissolved air flotation DAF with coagulation and floculation processes). Prior to the experiments, the biofilm sample was ground into a size comparable to activated sludge flocks using a 0.45 mm sieve to eliminate diffusion constraints to process kinetics. Just before testing, the aerated biofilm sample was rinsed three times with a solution prepared according to Park et al. [16] (2 liters contained: 17 mg KH_2PO_4, 43.5 mg K_2HPO_4, 66.8 mg $NaHPO_4 \cdot 7H_2O$, 3.4 mg NH_4Cl, 45 mg $MgSO_4$, 55 mg $CaCl_2$, 0.5 mg $FeCl_3 \cdot 6H_2O$) in order to remove residual impurities (substrates) adsorbed by biofilm particles. In the case of decay rate determination, the crumbled biofilm was transferred into a 1L beaker and aerated for 24 hours to achieve the endogenous respiration state of heterotrophic bacteria.

Wastewater, used as a substrate, was sampled from the outlet of an oil-water separator unit or DAF unit on the "Glimar" WWTP and filtered through a 0.45 μm filter before use. As the wastewater was coagulated via a full-scale process before filtration in the laboratory it contained mostly soluble organic compounds (soluble fraction of COD). The concentration of the biodegradable soluble COD of the wastewater was estimated by multiplying the measured COD of the wastewater filtered through 0.45 μm by the value of the biodegradable fraction of COD ($f_B = 0.4$), estimated in separate experiments. In all calculations, the active fraction of heterotrophic biomass was expressed in a COD unit. The value of active heterotroph concentration was obtained in the earlier respirometric determination of the active fraction coefficient ($f_{A,H}$). It was assumed that this value is constant for the particular biofilm growing in steady-state conditions in the pilot-scale biofilm bioreactor.

OUR Measurement Equipment (Respirometer)

All measurements of the oxygen uptake rate (OUR) were conducted using an analytical set consisting of:
- measurement reactor ("2" Fig. 1) – 280 ml flask equipped with magnetic stirrer, placed in a glass beaker filled with water and ice to stabilize the temperature in the reactor during measurement at about 20ºC,
- electrochemical dissolved oxygen measurement unit ("3" Fig. 1.) consisting of Oximeter 1900, WTW: Oxi 300 (WTW); Oxi-Stirrer 300 D201; OxiCal – Auto OxiCal-SL; TriOximatic 300 – the dissolved oxygen probe ("1" Fig. 1) was placed inside reactor ("2").

To prevent air getting into the reactor, the electrode has a rubber o-ring fitted in the flask neck, and the reactor ("2") was completely filled with no air bubbles inside.

In order to measure OUR, a 280 ml sludge sample was taken from an aerated batch reactor (10 L glass bottle with an air diffuser and mechanical mixer) and transferred into the measurement reactor ("2"). 6 mg of allyl-thiourea was then added to inhibit nitrification. Upon insertion of the dissolved oxygen probe, the reactor was placed in a beaker

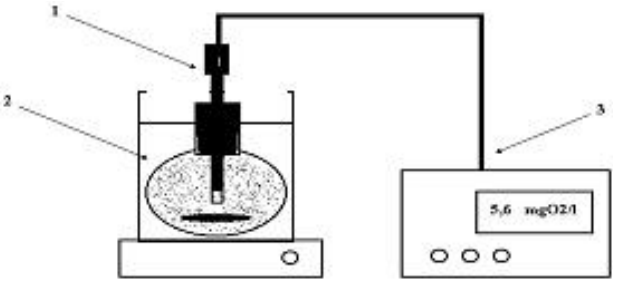

Fig. 1. Oxygen uptake rate measurement system (1 – dissolved oxygen probe, 2 – measurment reactor, 3 – electrochemical dissolved oxygen measurment unit).

filled with water (temperature 20ºC) and placed on the magnetic stirrer. The stirring speed was fixed at 500 rpm. The first reading of DO concentration was taken after 10 minutes, which was considered long enough to stabilize the measurement system.

Analytical Methods

All the analytical measurements to characterize wastewater were done according to Polish standard methods. Determinations included the dissolved COD value, total suspended solids concentration and pH value.

Determination of Decay Coefficient (b_H) and Active Fraction of Heterotrophic Biomass in Biofilm ($f_{A,H}$)

The method used was described by Grady [1] and originally applied to an estimation of kinetic parameters for activated sludge heterotrophic growth. The principle of this method is measurement of the OUR change over time during prolonged biomass aeration without any external substrate. The oxygen uptake is then a result of biomass endogenous respiration (decay) only and decreases with time as the number of living (metabolizing) bacteria cells diminishes. This change is directly proportional to the decay rate of microorganisms (b_H). The formula for the oxygen uptake rate as a function of time is defined as follows [1]:

$$\ln OUR = \ln\left[(0.8 \cdot b_H) \cdot X_{T,t_0} \cdot f_{A,H}\right] - b_H \cdot t \qquad (1)$$

...assuming $f_D = 0.2$ and $X_{H,t0} = X_{T,t0} \cdot f_{A,H}$. The value of decay constant (b_H) could be easily determined using the linear regression technique as a slope of the curve obtained by plotting ln (OUR) versus aeration time. An f_D value of 0.2, applied in the calculations (fraction of biomass leading to debris), was taken from literature, as it does not vary significantly from system to system [1]. The value of the active fraction of biomass ($f_{A,H}$) might next be estimated using the relation (2):

$$f_{A,H} = \frac{OUR_{t0}}{(0.8 \cdot b_H) \cdot X_{T,t0} \cdot 1.12} \qquad (2)$$

$X_{T,t0}$ was determined gravimetrically as a TSS concentration at the beginning of the experiment. The conversion factor (1.12) for recalculation of the total suspended solids mass concentration to the COD unit was determined in a separate experiment.

Determination of Heterotroph Yield Constant (Y_H) in the Batch Test

The principle of the method is to measure the real growth of heterotrophs in a batch reactor (taking the decaying process into consideration using the previously determined b_H value) in a given time period (Δt) with relation to the amount of wastewater soluble COD utilized at the same time. The biomass growth was determined directly as a difference between the total dry mass of suspended solids at the beginning and at the end of the experiment. Next, using the previously estimated value of active heterotroph fraction in the biofilm and a COD/dry mass conversion factor, the biomass concentration was calculated as biomass COD.

The source of organic carbon for this experiment was petrochemical wastewater filtered through a 0.45 μm filter. The final concentration of biomass in the batch reactor (10L glass bottle with air diffuser and mechanical mixer) at the beginning of the experiment was 20 to 50 mg COD/L, which allowed for a high organic loading rate of active biomass (3-4 g wastewater COD/g biomass COD) and gravimetric measurements of concentration changes of the suspended solids over time. A portion of about 50 mL of BIOACTIVATOR nutrient supplement (Bioindustries Ltd. Dublin) was added to the batch reactor as a source of nitrates and phosphates. The batch reactor content was mixed and aerated for 24 hours. The value of the real growth of the active heterotrophic biomass was estimated from the equation:

$$\Delta X_H = \left(X_{H,t24} - X_{H,t0}\right) + \left[\frac{\left(X_{H,t24} + X_{H,t0}\right)}{2} \cdot b_H\right]\left[\frac{mgCOD}{L}\right] \qquad (3)$$

The amount of utilized COD during measurements was determined as a difference of the soluble COD in the reactor (measured in a filtered sample):

$$\Delta S = S_{S,t0} - S_{S,t24}\left[\frac{mgCOD}{L}\right] \qquad (4)$$

The value of the real yield constant of heterotrophs was determined as:

$$Y_H = \frac{\Delta X_H}{\Delta S}\left[\frac{mgCOD}{mgCOD}\right] \qquad (5)$$

Simultaneous Determination of the Half Saturation Coefficient (K_S) and Maximum Growth Rate ($\mu_{max,H}$) of Heterotrophic Bacteria by the Respirometric Method

Determination of the heterotrophic maximum growth rate was based on the respirometric methods presented by Grady [1] and Almeida and Butler [12]. This determination relies upon the OUR measurement at set time intervals in an aerated batch test reactor. The starting conditions must assure a high biomass loading rate (S_S/X_H), more than 0.5 g COD/g biomass COD [15]. In such conditions, it is possible to achieve an 8 to 16-hour increase of OUR, resulting from heterotrophic biomass growth on a readily biodegradable substrate. The aeration capacity has to be high enough (DO 4-6 mgO_2/L) to prevent DO limitation of the growth rate. The wastewater and biofilm used during

the experiment were sampled and prepared before the determinations as described in the previous sections. The method described above was modified in a way that allowed us to simultaneously estimate also half saturation coefficient. It was possible by estimating values of active heterotrophs and biodegradable substrate concentrations in the batch reactor during respirometric experiments. Fig. 3 presents a typical respirogram obtained during such ($\mu_{max,H}$) and (K_s) determination. By knowing the initial active biomass concentration X_{Ht0} and initial soluble biodegradable substrate concentration S_S, it is possible to calculate the initial growth rate of heterotrophs using the equation (8) [1]. X_{Ht0} was determined directly as the total dry mass of suspended solids and then by using the previously estimated value of the active heterotroph fraction in the biofilm and a COD/dry mass conversion factor, calculated as biomass COD. S_S was determined as the COD value of the wastewater taken from the batch reactor and filtered through a 0.45 μm filter, multiplied beforehand by the estimated value of the biodegradable fraction of COD in the wastewater (f_B = 0.4).

$$OUR = \left(\frac{1-Y_H}{Y_H}\right)\cdot\mu_H\cdot X_H + (1-f_D)\cdot b_H\cdot X_H \quad (6)$$

Due to the fact that the values of b_H and f_D presented in literature are usually much smaller than μ_{max} (see Tables 4 and 5), they do not significantly influence μ_H estimation, and the above equation can be simplified as follows:

$$OUR = \left(\frac{1-Y_H}{Y_H}\right)\cdot\mu_H\cdot X_H \quad (7)$$

Therefore, we can express the heterotrophic growth rate with the formula:

$$\mu_H = SOUR\cdot\left(\frac{Y_H}{1-Y_H}\right) \quad (8)$$

...where SOUR is the specific oxygen uptake rate [mgO_2/mgCOD biomass·h] at starting time:

$$SOUR = \frac{OUR}{X_H} \quad (9)$$

The increase of biomass concentration ΔX_H and the amount of the utilized substrate ΔS in the time interval Δt can be calculated using the OUR value and the previously determined coefficients:

$$\Delta X_H = \Delta S\cdot Y_H - b_H\cdot X_{Ht0}\cdot\Delta t \quad (10)$$

$$\Delta S = \frac{1}{(1-Y_H)}\cdot(OUR\cdot\Delta t - 0.8\cdot b_H\cdot X_H\cdot\Delta t) \quad (11)$$

The value 0.8 relates to the death biomass fraction that is oxidized. The death biomass residue (coefficient f_D=0.2) cannot be utilized and becomes part of the inert suspended solids.

Thus, it is possible to find biomass and substrate concentrations after Δt time:

$$X_{H,ti} = X_{H,t0} + \Delta X_H \quad (12)$$

$$S_{S,ti} = S_{S,t0} - \Delta S \quad (13)$$

The whole procedure was then repeated for the next time interval Δt, beginning with the OUR measurement. The OUR value used for determination of the utilized substrate ΔS was the mean value of two OUR measurements (conducted at the beginning and at the end of the given time interval Δt). The procedure was reiterated until the biomass entered the lag phase of growth.

By plotting the obtained μ_H values against the respective S_S values for the whole test and using a nonlinear parameter estimation technique to fit the Monod kinetics equation, it is possible to determine the values of the half saturation coefficient (K_s) and maximum growth rate ($\mu_{max,H}$).

Results

All conducted determinations were repeated at least three times in order to calculate the mean value and relative standard deviation (RSD) of each estimated parameter. The obtained results were characterized by good repeatability – the RSD value was less than 10% for all estimated parameters, except for K_S, whose RSD of the mean value was 22%.

The results of kinetic parameter determination of heterotrophic bacteria grow in biofilm obtained using the methods described in the previous section are presented in Tables 1-3.

Table 1. Results of determination of the decay coefficient (b_H) and active fraction ($f_{A,H}$) of heterotrophic biomass in biofilm. Correlation coefficients (R) are given in brackets.

Run	b_H [d^{-1}] (R)	$f_{A,H}$
1	0.184 (0.87)	0.47
2	0.188 (0.97)	0.48
3	0.171 (0.99)	0.45
4	0.188 (0.80)	0.45
Mean	**0.180**	**0.46**
SD	**0.008**	**0.015**
RSD %	**4.4**	**3.2**

SD – standard deviation, RSD – relative standard deviation.

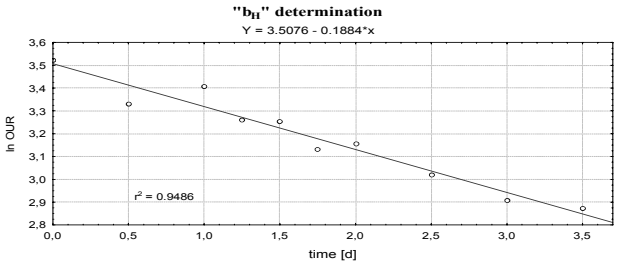

Fig. 2. Example of the plot of ln OUR versus time (t) during determination of the decay coefficient and active fraction of heterotrophic biomass. 2nd run.

Table 2. Results of yield constant (YH) determination for heterotrophic biomass in biofilm.

Run	Y_H [$mgCOD_{biomass}/mgCOD$]
1	0.58
2	0.57
3	0.60
Mean	**0.58**
SD	**0.015**
RSD %	**2.6**

Table 3. Results of the half saturation coefficient (K_S) and maximum growth rate of heterotroph ($\mu_{max,H}$) determination.

Run	$(S_S/X_{HA})t_0$	$\mu_{max,H}$	K_S	Correlation coefficient
	[$mgCOD_{biomass}$/ mg COD]	[d^{-1}]	[mgCOD/L]	R
1	1.51	6.2	6.80	0.85
2	4.60	6.8	11.20	0.71
3	3.96	5.5	9.80	0.82
4	4.96	5.8	10.99	0.90
5	5.00	5.7	6.80	0.92
6	3.90	6.6	10.96	0.88
Mean	**3.99**	**6.1**	**9.4**	
SD	**1.30**	**0.58**	**2.09**	
RSD %	**32.7**	**9.5**	**22.2**	

Example of the calculation used for determination of the decay coefficient and active fraction of heterotrophic biomass in biofilm.

$\ln OUR = 3.51 - 0.188 \cdot t \qquad (R = 0.97)$

$b_H = 0.188\ [d^{-1}]$

$f_{A,H} = (33.448/((0.188 \cdot 0.8 \cdot 409 \cdot 1.12)) = 0.48$

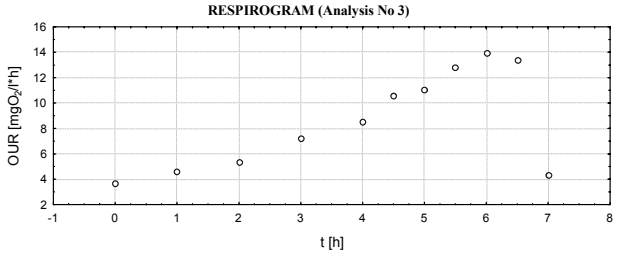

Fig. 3. Example of respirogram during estimation of K_S and μ_{max} (Run 3).

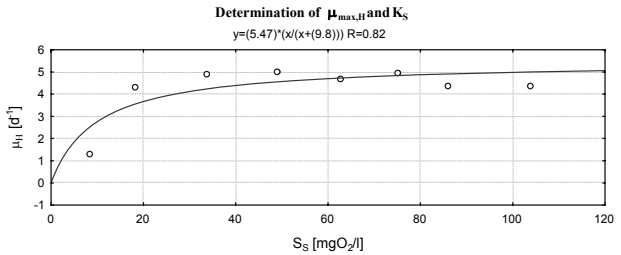

Fig. 4. Example of the plot presenting the relation between the calculated values of heterotrophic growth rate and soluble substrate concentration. The line illustrates the Monod type curve fit to the experimental data. The estimated value of the half saturation coefficient (K_S) is 9.8 mgCOD/L, and the maximum growth rate ($\mu_{max,H}$) is 5.5 d^{-1}.

Discussion

The purpose of the research presented in this article was to determine kinetic coefficients for heterotrophic bacteria that form biofilms in the environment of petrochemical wastewater. This goal was fully achieved during the conducted studies and analysis. The methods presented in this article can be conducted in every wastewater laboratory with the use of simple equipment. Both the respirometric methods chosen to determine the heterotrophic growth rate ($\mu_{max,H}$), decay coefficient (b_H) and half saturation coefficient (K_S) and the method for heterotrophic yield (Y_H) determination based directly on COD and the total suspended solids measurements during batch tests proved their suitability to this purpose. Methods for determination of heterotrophic yield, maximum heterotrophic growth rate, decay coefficient and half saturation coefficient for bacteria in biofilms were developed on the basis of the methods originally used for activated sludge. Because of this, the sampled biofilm was ground into a size comparable to activated sludge flocks in order to eliminate diffusion constrains to process kinetics. Carrion et al. [25] presents an alternative approach toward respiration rate measurement in biofilm reactors with the use of a batch reactor packed with Rashig rings on which the biofilm was cultured. This method ensures the same hydraulic conditions inside the reactor as in the full- or pilot-scale and conserves the substrates transport limitations into the biofilm which could be an important factor affecting the measurement of the respiration rate.

Table 4. Values of kinetic parameters determined at neutral pH and a temperature of 20ºC for activated sludge and municipal wastewater.

	Kinetic parameter			
	$\mu_{max,H}$ (d^{-1})	K_S (gCOD/m^3)	b_H (d^{-1})	Y_H (gCOD/gCOD)
ASM1 Henze et al., [16]	3.0 – 6.0	20.0	0.2 – 0.62	0.67
Sollfrank and Gujer [18]	1.5	5	0.24	0.64
Kappeler and Gujer [17]	1 – 8	2.5 – 4.0	—	---
ASM2 Henze et al., [19]	3.0 – 6.0	—	0.2 – 0.4	0.63
Bjerre [20]	6.8	1	—	0.55
Hvitved-Jacobsen et al. [22]	3.25	1	—	0.55
Almeida and Butler [12]	6.3	—	—	0.57
Sin and Vanrolleghem [29]	2	0.5	—	---
Karahan and Dogruel [28]*	2	12	0.10	0.68
Ni and Yu [30]	1.68	11.38	0.34	0.58
This study	6.1	9.4	0.18	0.58

* parameters estimated for tannery wastewater.

Relatively good precision (repeatability) and uncomplicated, relatively fast procedures with the use of basic laboratory equipment are the advantages of the presented methods. On the other hand, the most significant disadvantages were lack of automation (all OUR measurements were performed manually, the time interval between two measurements was relatively long, and the analysis was laborious) and the process *a priori* knowledge of some parameters. Therefore, evaluation of the parameters had to be performed in a particular order, because the values of some were needed before others could be evaluated. This was a potential source of errors (uncertainty) of the method due to error propagation from measurement of one parameter to the measurement of the other parameters.

As mentioned earlier, OUR measurement intervals during respirometric experiments were significantly longer in comparison to the methods described by other researchers [12, 17, 26]. Relatively low OUR measuring frequency (with a time interval of approx. one hour) during the phase of analysis when OUR promptly decreases due to the declining concentration of substrates in the batch reactor (the curve slope on the respirogram) influences the correlation of the obtained data with the Monod type curve using the nonlinear regression technique. This was a possible cause for the lesser precision and accuracy of K_S value determination. Another source of respirometric method errors was limited control of temperature in the batch and measuring reactor. As was proven by other researchers, temperature influences the μ_{max} and b_H values [17]. The problems could be avoided using a fully automated respirometer with thermostated reactors and a high frequency of OUR measurement during kinetic parameter determination. Examples of such a respirometer are described in literature [12, 17, 26]. Despite the differences between OUR measurement systems used by other authors and presented in this article, the values of oxygen uptake rates and curve shapes in the respirograms obtained during b_H and μ_{max}, K_S determinations are comparable [12, 17, 27].

Another common problem with kinetic parameter determination is that there is no possibility to validate the methods, because there are no reference materials available (standard reference: activated sludge, biofilm or wastewater) that would allow us to examine, for example, the accuracy of the methods or calibrate them before quantitative determinations. The validity of results can be evaluated only by comparing them with the results obtained by other researchers or by checking the plausibility of the parameter values obtained during research by experiments (simulations with mathematical models followed by verification of the obtained results) or by some theoretical considerations [7, 32, 33]. This is a general concern of researchers who cope with kinetic parameter determinations [1, 14, 15]. In fact, the kinetic parameters obtained during the described study have been successfully applied to calibration of a biofilm mathematical model and were verified during simulations (which is the subject of a separate article).

The determined values for heterotrophic yield, maximum heterotrophic growth rate, decay coefficient and half saturation coefficient are presented in Tables 1, 2 and 3. Comparison of the average values of these parameters obtained for petrochemical wastewater and adapted biofilm to those determined by other authors for municipal wastewater (see Table 4 and 5 – there is a lack of suitable data for petrochemical wastewater in literature) [7, 12, 16-23, 28-31] shows that there are no significant differences. This also confirms the validity of the presented methods. Only the half saturation coefficient (K_s) value is larger, although it is in the range of the values presented by Henze et al.(1987) and almost the same as those presented by Horn and Hampel (1997), Karahan and Dogruel (2008) and Ni

Table 5. Values of kinetic parameters recommended for application of biofilm mathematical models.

	Ritmann and McCarty [21]	Horn and Hempel [23]	Wanner et al. [7]	Alpkvist et al. [31]	This study
$\mu_{max,H}$ (d^{-1})	—	5.50	6.00	4.707	6.1
K_S ($gCOD/m^3$)	3.900	10.00	4.00	4	9.4
b_H (d^{-1})	0.205	0.03	0.32	0.08	0.18
Y_H (gCOD/gCOD)	—	0.90	0.63	0.206	0.58

Table 6. Order of kinetic parameter estimation during experiments.

Symbol	Name	Prior information needed
f_B	fraction of biodegradable substrate in total soluble substrate in wastewater	
f_D	fraction of biomass leading to debris	
S_T	total soluble substrate	
S_S	biodegradable soluble organic substrate	f_B
X_T	total suspended solid concentration	
b_H	decay coefficient (endogenous respiration)	
$f_{A,H}$	fraction of active heterotrophs in total suspended solids	X_T, b_H, f_D
X_H	active heterotrophs concentration	$f_{A,H}$
Y_H	Yield coefficient	X_H, b_H, S_T
K_S	half saturation coefficient	Y_H, X_H, b_H, S_S
$\mu_{max,H}$	heterotrophic maximum growth rate	Y_H, X_H, b_H, S_S

and Han-Qing Yu (2008) [16, 23, 28, 30]. The most probable cause of this, which was described earlier in this section, was the applied procedure for this parameter determination with a low OUR measuring frequency during batch tests. Another explanation of such results could be the fact that the biodegradable substrate in the investigated wastewater was not a single readily biodegradable compound but a mixture of both readily biodegradable and unbiodegradable organic compounds (filtered petrochemical wastewaters). In such a case, what emphasizes that the Grady [1] K_S value obtained will be larger than that associated with a single compound. Moreover, some researchers observed that the K_S value is higher in the case of high soluble substrate concentration in the reactor and significantly decreases when the bacteria are cultured under a low concentration of the soluble substrate [15]. K_S and μ_{max} values in the presented studies were determined under high initial S_S/X_H values in the batch reactor (Table 3) and, therefore, they might be recognized as "intrinsic parameters" [15]. The obtained results indicate that no inhibitions occurred during performance of the experiments because the μ_{max} values do not vary significantly at different S_S/X_H values. In the case of inhibition, higher S_S/X_H values would cause a decrease in the determined μ_{max} values [24].

As mentioned earlier, evaluation of the parameters proceeded in a particular order. Table 6 summarizes the order of the kinetic parameter determination presented in this article. This procedure allowed for the expansion of the applicability of the heterotrophic growth rate method [1, 12] by estimating the soluble biodegradable substrate and heterotroph concentration values over the time of the experiment on the basis of well known kinetic equations and with the use of previously estimated values of the biodegradable fraction of COD in the wastewater (f_B = 0.4) and the active fraction of heterotrophs in the biofilm ($f_{A,H}$ = 0.46), which makes it possible to determine not only the value of maximum growth rate, but also the half saturation coefficient.

Conclusion

The experimental procedures presented in this paper for determination of heterotrophic yield, decay coefficient, maximum growth rate, half saturation coefficient and active fraction of heterotrophs, originally developed for suspended biomass, were successfully adapted for biofilm characterization. Kinetic constant values obtained for heterotrophs growing in the biofilm of a pilot scale bioreactor located in a petrochemical wastewater treatment system do not differ

significantly from those presented by other authors for suspended biomass and those recommended for use in biofilm models. The estimated values of kinetic constants might be applied to the calibration of biofilm mathematical models.

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Nomenclature

Symbol	Definition	Unit
T	current time	h
t_i	time at any given moment "i"	h
t_0	start point of measurement	
Δt	time period	h
C_{VSS}	concentration of dry mass of total suspended solids	g_{VSS}/m^3
X	concentration of the particulate material	$gCOD/m^3$
X_T	concentration of total suspended solids	$gCOD/m^3$
$X_{T,ti}$	concentration of total suspended solids at any time "t_i"	$gCOD/m^3$
X_H	concentration of active heterotrophic bacteria	$gCOD/m^3$
$X_{H,ti}$	concentration of active heterotrophic bacteria at any time "t_i"	$gCOD/m^3$
X_I	concentration of inert suspended solids	$gCOD/m^3$
$X_{I,ti}$	concentration of inert suspended solids at any time "t_i"	$gCOD/m^3$
Y_H	yield of heterotrophic biomass produced on substrate utilized	gCOD/gCOD
μ_H	growth rate for heterotrophs	d^{-1}
$\mu_{max,H}$	maximum specific growth rate for heterotrophic biomass	d^{-1}
b_H	decay coefficient for heterotrophic biomass (endogenous respiration rate)	d^{-1}
S_T	total soluble substrate concentration	$gCOD/m^3$
S_S	soluble biodegradable substrate concentration	$gCOD/m^3$
$S_{S,ti}$	soluble organic substrate concentration at any time "t_i"	$gCOD/m^3$
SO	dissolved oxygen concentration	gO_2/m^3
OUR	oxygen uptake rate	$gO_2/m^3 \cdot d$
SOUR	specific oxygen uptake rate	$gO_2/gO_2 \cdot m^3 \cdot d$
$f_{A,H}$	fraction of active heterotrophic biomass	
f_D	fraction of biomass leading to debris	
f_B	fraction of biodegradable substrate in total soluble substrate	
ΔS	change of substrate concentration	$gCOD/m^3$
ΔS_O	change of oxygen concentration	$gCOD/m^3$
ΔX_H	change of active heterotrophic bacteria concentration	$gCOD/m^3$
S_S/X_H	organic loading of biomass	gCOD/gCOD
K_S	half saturation coefficient for organic substrate	$gCOD/m^3$

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