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# Treatment of Tobacco Dust Leachate by Activated Sludge – Evaluation of Biokinetic Parameters

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Treatment of tobacco dust leachate represents an important problem because of high concentrations of organic compounds. Experiments were carried out in a batch conditions at initial concentrations of activated sludge of 3.1 g dm<sup>-3</sup> (Exp.1) and 6.0 g dm<sup>-3</sup> (Exp.2) and different initial concentrations of organic matter in leachate, expressed as COD concentrations, which were in range from 500 to 5000 mg dm<sup>-3</sup>. Efficiency of biodegradation process was approximately 89.4 % of COD removal. The kinetic parameters, maximum specific growth rate ( $\mu_{max}$ ), substrate saturation constant ( $K_s$ ) and overall yield coefficient (Y), during experiments were found to be 0.088 h<sup>-1</sup>, 4241 mg dm<sup>-3</sup>, and 0.400 mg mg<sup>-1</sup> for Exp. 1; and 0.052 h<sup>-1</sup>, 3168 mg dm<sup>-3</sup>, and 0.257 mg mg<sup>-1</sup> for Exp. 2, respectively. Monod model gives very good fits to experimental data, accompanied by a high regression coefficient (R<sup>2</sup>).

*Key words:* Tobacco dust, leachate, activated sludge, biodegradation

# Introduction

Increasingly affluent lifestyles, continuing industrial and commercial growth in many countries around the world in the past decade has been accompanied by rapid increases in both the municipal and industrial solid waste. The classification of industrial wastes of organic origin includes tobacco wastes, which are generated during different processes of the tobacco and cigarette production cycle.<sup>1,2</sup>

Tobacco waste and dust appear in different steps of tobacco processing after harvest as well as in the course of manufacturing of some tobacco products such as cigarettes. The consequence of increase manufacture of tobacco products is raising level of various tobacco wastes. These waste products are resold, recirculated, compacted, or put in landfills.<sup>3,4</sup> The biggest problem concerning these wastes is the presence of toxic compounds. Investigations carried out showed that the most important sources of these toxic contaminants are nicotine, flavouring chemicals containing glycogen and alcohol, absorbable organic halogens (AOX), and pesticides from tobacco leaves.<sup>2,5,6</sup> The nicotine contains high concentration of toxic compounds, and the powdery structure which cannot be recycled. Nicotine is highly soluble in water; therefore the toxic compound, nicotine can be transferred from the solid phase to an aqueous solution through efficient percolation. It may also be leached from the wastes and may permeate into ground waters and surface waters.<sup>1,7–9</sup>

The main characteristic of tobacco solid wastes and leachate, beside their toxicity is their low moisture content. Due to of this problem it is desirable to apply biological or thermal process during the treatment of these wastes. Biological treatment is flexible, reliable, and high cost-effective, which is commonly used for treatment of tobacco solid wastes and leachate.<sup>1,6–12</sup>

Legal regulation dictates that wastewater and leachate must be treated before it can be discharged.

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Biological treatment methods are extensively applied for the treatment of different type of wastewater and leachate.<sup>1</sup> The main purpose of this process is a removal of organic pollution from wastewater by microorganisms that are in activated sludge. Activated sludge process has been widely applied to treat wastewater and leachate due to its advantages including low running cost and high degradation efficiency.<sup>1,9–11</sup>

This study aims to investigate the aerobic biodegradation of organic pollutants of leachate from tobacco dust in batch bioreactor, and evaluate biokinetic parameters using the Monod model.

# **Experimental**

The activated sludge sample was taken from the Wastewater Treatment Plant (WWTP) in Zagreb, ZOV, Croatia. The sludge sample from WWTP is collected from aeration tank, centrifuged (Sigma 3K15, Germany) at RCF =  $5411 \times g$  for 10 min and 0 °C. Initial concentrations of activated sludge were 3.1 and 6.0 g dm<sup>-3</sup>. The leachate used in the research was prepared from tobacco dust, Hrvatski duhani d.d., Virovitica, Croatia according to European standard of EN 12457-4:2002.<sup>13</sup> The starting concentration of prepared leachate was 34430 mg COD dm<sup>-3</sup>. For the set of experiments, the leachate sample was diluted with tap water to initial concentrations of 500, 1000, 1500, 3000, 5000 mg dm<sup>-3</sup>, and marked as S1-S5, respectively.

Batch biodegradation experiments were conducted in 500 cm<sup>3</sup> conical flasks using 250 cm<sup>3</sup> of diluted leachate and inoculated with 7.5 g (Exp. 1) and 15 g (Exp. 2) of the centrifuged activated sludge, what corresponds to initial concentrations of activated sludge of 3.1 g dm<sup>-3</sup> (Exp.1) and 6.0 g dm<sup>-3</sup> (Exp.2). The first set of experiments was carried out for 96 hours. Samples were analyzed every 12 hours. The second set of experiments was carried out within the first 12 hours. Samples were taken every hour for determination of chemical oxygen demand (COD), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MVLSS), pH value and dissolved oxygen (DO), and for microscopic investigation. All experiments were performed at 25±2°C and were maintained in aerobic conditions agitated on a rotary shaker at 2.7  $s^{-1}$ 

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MVLSS) were determined gravimetrically and the chemical oxygen demand (COD) was determined by means of the dichromate method using Standard Methods (APHA, 1998)<sup>14</sup>. Dissolved oxygen (DO) and pH values were measured with an oxygen meter and pH meter (WTW Multi 340i, Germany). All determinations were averages of duplicate samples.

The morphology of the activated sludge was investigated by light microscopy. Samples of activated sludge were monitored under a light microscope (Olympus BX50, Olympus Optical Co. Ltd., Japan, with Olympus DP 10 camera). A drop of mixed liquor was carefully deposited on a glass slide and covered with a cover slip before being observed through the microscope.

# **Results and discussion**

## Pollutant removal performances

Chemical oxygen demand (COD) value is used to evaluate the organic strength of leachate. A biodegradation was conducted for leachate originated from tobacco dust. This leachate sample contained very high COD, 34430 mg dm<sup>-3</sup>. Therefore it was diluted to initial concentrations of 500, 1000, 1500, 3000, 5000 mg dm<sup>-3</sup> marked by from S1 to S5, respectively. The total removal reached in every experiment is calculated and shown in Fig. 1.



Fig. 1 – Efficiency of biodegradation of leachate from tobacco dust at different starting concentrations of substrate for Exp. 1 (□) and Exp. 2 (■)

For the series varying initial substrate concentration, values of COD concentration decreased continuously with reaction time (Fig. 1). Values of COD removal efficiency for 96 hour varied from  $83.8\pm2.3$  % to  $95.9\pm1.8$  % for Exp. 1 and from  $80.0\pm0.7$  % to  $90.8\pm2.7$  % for Exp. 2. These results demonstrated that the leachate was biologically treatable. Similar values were obtained for biological treatment of tobacco industry wastewater with effectiveness of around 84.0 %.<sup>9,15</sup> Results show

that efficiency COD removal in leachate is the best in first 12 hours. Fig. 1 shows that in that period more than 61 % of COD value for both experiments was removed. In both experiments an average COD reduction were  $65.6\pm2.3$  % for Exp. 1 and  $74.3\pm2.2$ % for Exp. 2. It could be seen that efficiency of biodegradation was gradually increased for samples with highest initial concentration for Exp. 1 and Exp. 2, respectively. The additional increase in efficiency after the first 12 hours up to 96 hours was an additional 25.4 % for Exp.1 and 13.6 % for Exp.2.

Since the largest efficiency was recorded in the first 12 hours, in the following the results are presented for a period of 12 hours with COD removal up to 79.8 % for both experiments.

## Response of the control variables

In biological treatment process, use of activated sludge is an effective way of treating leachate with high concentrations of organic compounds. In a biological treatment process, sludge concentration is an important factor to ensure biological treatment ability. A sufficient sludge concentration will ensure good performance in pollutant removal and better effluent quality.12 The mixed liquor suspended solids (MLSS) present a mixture of activated sludge and suspend solids from leachate. It should be noted that the MLSS consists of organic and inorganic matter, as well as microorganisms, and other inert suspended matter. Therefore, the MLSS is an indirect measure of total biomass.<sup>10</sup> Initial MLSS concentrations were 3.1 and 6.0 g dm<sup>-3</sup> for conducted experiments marked as Exp. 1 and Exp. 2, respectively.

For both experiments in the first 12 hours concentration of MLSS was lightly and gradually increased. By increase of initial concentrations of substrate from S1 to S5, values of MLSS increased up to 26.13 % for Exp. 1. In the Exp. 2, values of concentrations of MLSS show significantly smaller increase with respect to starting concentrations of  $6.0 \text{ g} \text{ dm}^{-3}$  and increased up by only 6.98 %.

The mixed liquor volatile suspended solid (MLVSS) represents the amount of organic or volatile suspended solids in sample of MLSS. This volatile portion is used as a measure or indication of the presence of microorganisms.<sup>16</sup> The presence of microorganisms plays an important role in the process of biodegradation. Therefore, the ratio of MLVSS/MLSS can be used to determine whether there are enough microorganisms present for biodegradation. Variations in ratio of MLVSS/MLSS indicate a change in amount of biomass share.<sup>12</sup>

Tables 1 and 2 show that the values obtained for ratio of MLVSS/MLSS were almost constant and ranged in limits from 0.714 to 0.732 for Exp. 1,

Table 1 – Results of the control variables obtained in the Exp. 1

	-		
Exp. 1	MLVSS/MLSS (-)	рН (-)	DO (mg dm <sup>-3</sup> )
S1	$0.714 \pm 0.03$	$8.15\pm0.16$	5.91 ± 1.08
S2	$0.716 \pm 0.02$	$7.99\pm0.12$	$4.36\pm1.16$
S3	$0.719 \pm 0.02$	$7.94\pm0.10$	$3.62\pm0.92$
S4	$0.726 \pm 0.02$	$7.85\pm0.10$	$1.60\pm0.90$
S5	$0.732\pm0.03$	$7.76\pm0.13$	$0.57\pm0.25$

Table 2 – Results of the control variables obtained in the Exp. 2

	1		
Exp. 2	MLVSS/MLSS (-)	рН (-)	DO (mg dm <sup>-3</sup> )
S1	$0.717 \pm 0.02$	$7.92 \pm 0.19$	4.27 ± 1.30
S2	$0.719 \pm 0.01$	$7.89\pm0.19$	$3.88 \pm 1.22$
S3	$0.723 \pm 0.01$	$7.85\pm0.18$	$2.88\pm1.34$
S4	$0.748\pm0.02$	$7.77\pm0.21$	$1.05~\pm~1.01$
S5	$0.725 \pm 0.03$	$7.69\pm0.24$	$0.31 \pm 0.02$

and from 0.717 to 0.725 for Exp. 2. These results show that there were no significant changes in the amount of viable sludge. The environmental factor that influences rates and limits microbial growth and thus the process of biodegradation is the pH value. The pH value lightly decreased by increasing initial concentration of leachate and ranged in optimal limits 7.76 - 8.15 and 7.69 - 7.92 for Exp. 1 and Exp. 2, respectively. These values correspond to the biological activity of sludge.<sup>16</sup>

In aerobic bioprocesses, the control of the dissolved oxygen level plays an important role. The dissolved oxygen concentration in the activated sludge process should be sufficiently high to supply enough oxygen to microorganisms in the sludge in order for the organic matter to be degraded by them.<sup>17</sup> Average values of DO concentration (Tables 1 and 2) decreased by increasing initial concentration of leachate. We can see that values of the average concentration of dissolved oxygen are significantly low at higher concentration of leachate in both conducted experiments. It is due to the fact that at higher initial concentration of leachate a lot more oxygen is necessary for substrate degradation.<sup>18</sup>

#### **Biokinetic analysis**

Microbial degradation is generally defined as the biologically catalyzed reduction in chemical complexity. In the natural environment, conditions for biodegradation are very complex and the rate and extent of biodegradation depend on the chemical, physical and biological factors that may be different for different ecosystems. Although microbial processes are very complex, individual process incidences or groups of these incidences can be represented by model.<sup>18–20</sup>

Most analyses with regards to biokinetic in wastewater treatment processes were done on the assumption that the reaction follows Monod kinetics or first-order reactions. This model defines the functional dependence of the specific growth rate and biomass concentration, equation (1):<sup>16–19</sup>

$$\mu = \mu_{\max} \cdot S / (K_s + S) \tag{1}$$

where in  $\mu$  (h<sup>-1</sup>) is the specific growth rate,  $\mu_{\text{max}}$  (h<sup>-1</sup>) is maximum specific growth rate, *S* (mg COD dm<sup>-3</sup>) is the limiting substrate concentration and  $K_{\text{s}}$  (mg dm<sup>-3</sup>) is substrate saturation constant (i.e. substrate concentration at half  $\mu_{\text{max}}$ ).

Microbial growth occurs as a consequence of the process of biodegradation by removing substrates as described by equations (2) and (3):

$$r_x = dX_v / dt = \mu X_v \tag{2}$$

$$r_s = dS / dt = q_s X_v \tag{3}$$

where  $r_s$  is substrate degradation rate in mg COD dm<sup>-3</sup> h<sup>-1</sup>,  $r_x$  is biomass growth rate in mg MLVSS dm<sup>-3</sup> h<sup>-1</sup>,  $X_v$  is biomass concentration in mg MLVSS dm<sup>-3</sup>,  $q_s$  is specific substrate degradation rate in mg COD (mg MLVSS)<sup>-1</sup> h<sup>-1</sup> and t is time in h.

The concentration of the substrate is usually reduced by growth of microorganisms, therefore  $r_s$  is well described by equation (4):

$$r_s = \mu_{\max} X_v S / Y (K_s + S) \tag{4}$$

where Y is an overall yield coefficient in mg MLVSS (mg COD)<sup>-1</sup>.

Real biomass yield per substrate,  $Y_{x/s}$ , and specific substrate degradation rate,  $q_s$  can be calculated directly from experimental data from the following equations (5) and (6):

$$Y_{x/s} = (X_v - X_{v0}) / (S_0 - S_i)$$
(5)

$$q_s = (S_0 - S_i) / (t_0 - t_i) / (X_v - X_{v0})$$
(6)

According to this procedure, Tables 3 and 4 show values of kinetic results in the aerobic degradation process and biokinetic parameters evaluated by kinetic model. Model parameters were estimated by non-linear regression analysis using least – squares method implemented in MS Excel software. Numerical values of model parameters were obtained by fitting the model to experimental data using MS Solver software. The optimization method was conducted according to GRG2 (Generalized Reduced Gradient) which is integral part of MS Solver software. Differential equations of the model are solved numerically by Runge Kutta 4 algorithm. A set of optimal parameters of the model were used for the process simulations. The residual error was defined as the sum of squares of the differences between the experimental and calculated data.

Table 3 shows the values obtained for parameters ( $Y_{x/s}$  and  $q_s$ ) in the experiment according to eq. (5) and eq. (6). The growth yield coefficient,  $Y_{x/s}$ , is defined as increase in biomass which results from the utilization of amount of substrate. Mean values obtained for growth yield coefficient,  $Y_{x/s}$ , in mg MLVSS (mg COD)<sup>-1</sup> are in the range from 0.355 to 0.393 mg mg<sup>-1</sup> and from 0.189 to 0.242 mg mg<sup>-1</sup> in conducted experiments marked as Exp. 1 and Exp. 2. These values show good match with expected values for the activated sludge process. Data obtained for specific substrate degradation rates,  $q_s$ , in mg COD (mg MLVSS)<sup>-1</sup> h<sup>-1</sup> are very close with other values proposed in the literature for the aerobic biodegradation of different wastewater.<sup>20</sup>

Table 3 – Mean values of kinetic results in the aerobic degradation process

	0	1		
S <sub>0</sub> (mg dm <sup>-3</sup> )	Exp. 1		Exp. 2	
	$\begin{array}{c} Y_{\rm x/s} \\ ({\rm mg \ mg^{-1}}) \end{array}$	$q_{\rm s} \ ({\rm mg \ mg^{-1} \ h^{-1}})$	$\begin{array}{c} Y_{\rm x/s} \\ ({\rm mg \ mg^{-1}}) \end{array}$	$q_{\rm s} \ ({\rm mg \ mg^{-1} \ h^{-1}})$
500	0.355±0.12	$0.027 \pm 0.04$	0.189±0.06	$0.022 \pm 0.02$
1000	0.369±0.07	$0.074 \pm 0.06$	0.193±0.06	$0.048 \pm 0.04$
1500	0.391±0.04	$0.094{\pm}0.07$	0.213±0.08	$0.065 \pm 0.06$
3000	0.389±0.02	$0.144 \pm 0.04$	0.235±0.10	0.106±0.10
5000	0.393±0.07	0.217±0.09	0.242±0.09	0.120±0.20

Table 4 - Biokinetic parameters

Biokinetic parameters	Exp. 1	Exp. 2
$\mu_{\rm max}~({\rm h}^{-1})$	0.088	0.052
$K_{\rm s} \ ({\rm mg} \ {\rm dm}^{-3})$	4241	3168
$Y (mg mg^{-1})$	0.400	0.257

Applying a non-linear regression analysis to the experimental results were estimated biokinetic parameters according to eq. (4). Table 4 shows the values of obtained results. Comparison of model and experimental results are shown in Figs. 2 and 3 for Exp. 1 and Exp. 2. A good arrangement of the experimental points around a straight line confirms the agreement with the proposed model.



Fig. 2 – Influence of initial COD on substrate degradation rate for Exp. 1, (■) experimental data, and (—) model



Fig. 3 – Influence of initial COD on substrate degradation rate for Exp. 2, (■) experimental data, and (—) model

Fig. 2 shows fit of calculated values obtained from model and experimental results for Exp. 1. As can be seen substrate degradation rate,  $r_s$  (mg COD dm<sup>-3</sup> h<sup>-1</sup>), is increased when initial substrate concentration also increases, as could be expected.

Also, Fig. 3 shows fit of calculated values obtained from model and experimental results for Exp. 2. We can see that Exp. 1 shows insignificantly better match to experimental data than Exp. 2. These results demonstrated that the applied Monod model describes well the dynamics of the process accompanied by a high regression coefficient ( $R^2 = 0.9704$  for Exp. 1, and  $R^2 = 0.9478$  for Exp. 2).

#### **Microscopical examination**

MLSS were regularly monitored to adjust the sludge concentration to a preset level. In order to



Fig. 4 – Microphotographs of (a) activated sludge flocs and (b) protozoa in batch conditions for Exp. 1 and Exp. 2,  $P = 100 \times$ 

describe the morphology of the activated sludge, microscopic investigation was conducted. The morphological properties of sludge were characterized by flocs.<sup>21,22</sup> Flocs present in water play an important part in all issues related to water quality and treatment. Small sludge flocs can provide a favourable environment for enhancement of mass transfer, thus enabling the system to show a higher organic removal rate.<sup>22</sup> The biological component of the activated sludge system is comprised of microorganisms. The diversity of the microbial community in activated sludge is very large. Protozoa are a useful biological indicator of the condition of the activated sludge. The indicator of a well-operated, stable activated-sludge system is the existence of large numbers of highly evolved protozoa in the biological mass.16,23

The results of microscopic examination are shown in Fig. 4. The flocs of the process shown in Fig.4a are characterized by regular formed flocs which enable producing a high quality effluent.<sup>18</sup> These microscopic images show some species of protozoa which were present over the course of the experiment (Fig.4b). Presence of protozoa populations provides evaluation of sludge quality and biodegradation ability. Protozoa is a significant predator of bacteria which plays a key role in the degradation processes. Therefore we can say that the presence of protozoa in activated sludge indicates a good quality of activated sludge, as well as the good performance of the process of biodegradation.<sup>10,24</sup>

# Conclusions

The obtained results have shown efficiency of biodegradation of organic pollutants of tobacco dust leachate under batch conditions. Average COD removal efficiencies were  $65.6\pm2.9$  % and  $91.0\pm3.2$ % for Exp. 1 and 74.3±2.2 % and 87.8±2.4 % for Exp. 2, for 12 h and 96 h, respectively. The ratio of MLVSS/MLSS and pH value were almost constant and ranged in optimal limits during the whole experimental period. Values of DO were in the optimal range for biodegradation processes. Mean value of  $Y_{x/s}$  was approximately 0.379 mg mg<sup>-1</sup>. The  $q_s$  was between 0.022 and 0.217 mg mg<sup>-1</sup> h<sup>-1</sup>. By investigating the kinetics of leachate biodegradation the kinetic parameters,  $\mu_{\text{max}}$ ,  $K_{\text{s}}$  and Y were found to be 0.088  $h^{-1}$ , 4241 mg dm<sup>-3</sup>, and 0.400 mg mg<sup>-1</sup> for Exp. 1, and 0.052  $h^{-1}$ , 3168 mg dm<sup>-3</sup>, and  $0.257 \text{ mg mg}^{-1}$  for Exp. 2, respectively. Good fits of experimental data to Monod model, accompanied by a high regression coefficient  $(R^2)$ , indicates that the model describes the investigated system well.

## Nomenclature

COD	- chemical oxygen demand	mg dm <sup>-3</sup>
DO	<ul> <li>dissolved oxygen concentration</li> </ul>	mg dm <sup>-3</sup>
$K_{\rm s}$	- substrate saturation constant	mg dm <sup>-3</sup>
MLSS	- mixed liquor suspended solids	g dm <sup>-3</sup>
MLVSS	<ul> <li>mixed liquor volatile suspended solids</li> </ul>	g dm <sup>-3</sup>
$q_{\rm s}$	<ul> <li>specific substrate degradation rate</li> </ul>	mg mg <sup>-1</sup> h <sup>-1</sup>
RCF	- relative centrifugal force	× g
$r_{\rm s}$	- substrate degradation rate	mg dm <sup>-3</sup> h <sup>-1</sup>
r <sub>x</sub>	- biomass growth rate	$g dm^{-3} h^{-1}$
S	- substrate concentration	mg dm <sup>-3</sup>
t	– time	h
$X_{\rm v}$	- biomass concentration	mg dm <sup>-3</sup>
Y	- overall yield coefficient	mg mg <sup>-1</sup>
$Y_{\rm x/s}$	- growth yield coefficient	g g <sup>-1</sup>
μ	<ul> <li>specific growth rate of biomass</li> </ul>	$h^{-1}$
$\mu_{\rm max}$	<ul> <li>maximum specific growth rate of biomass</li> </ul>	$h^{-1}$

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#### References

- 1. Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F., Moulin, P., J., Hazard. Mater. **150** (2008) 468.
- Piotrowska-Cyplik, A., Olejnik, A., Cyplik, Z., Dach, J., Czarnecki, Z., Bioresour. Technol. 100 (2009) 5037.
- 3. Novotny, T. E., Zhao, F., Tobacco Control 8 (1999) 75.
- US National Industrial Pollution Control Council, The tobacco industry and pollution, sub-council report. Washington DC, US Government Printing Office (Publication No 483-135/6.), 1972.
- 5. Sponza, D. T., Water Air Soil Poll. 134 (2002) 137.
- Briški, F., Horgas, N., Vuković, M., Gomzi, Z., Clean Technol. Environ. Pol. 5 (2003) 295.
- Tang, H. Z., Wang, S. N., Ma, L. Y., Meng, X. Z., Deng, Z. X., Zhang, D. K., Ma, C. Q., Xu, P., Appl. Environ. Microbiol. 74 (2008) 1567.
- Wang, S. N., Liu, Z., Tang, H. Z., Meng, J., Xu, P., Ma, C. Q., Environ. Sci. Technol. 39 (2005) 6877.
- Wang, M., Yang, G., Min, H., Lv, Z., Jia, X., Water Res. 43 (2009) 4187.
- 10. Vuković, M., Briški, F., Matošić, M., Mijatović, I., Chem. Eng. Technol. **29** (2006) 357.
- 11. Kargi, F., Pamukoglu, Y., Enzyme Microb. Tech. 33 (2003) 588.
- 12. Huang, X., Gui, P., Qian, Y., Process Biochem. 36 (2001) 1001.
- EN 12457-4:2002, Characterization of waste Leaching; Compliance test for leaching of granular waste materials and sludges – Part 4: One-stage batch test at a liquid to solids ratio of 10 l/kg for materials with particle size below 10 (without or with size reduction), European Committee for Standardization.
- 14. APHA (1998), Standard methods for examination of water and wastewater, 20th Edition. APHA, Washington DC, USA.
- 15. Wei, Y., Ji, M., Li, R., Qin, F., Waste Manage. 32 (2012) 448.
- 16. *Bitton, G.*, Wastewater Microbiology, pp 213–257, John Wiley & Sons, New York, 2005.
- 17. Holenda, B., Domokos, E., Rédey, A., Fazakas, J., Comput. Chem. Eng. **32** (2008) 1270.
- Casey, T. J., Unit Treatment Processes in Water and Waste Engineering, pp 141–257, John Wiley & Sons, New York, 1996.
- 19. Nakhla, G., Liu, V., Bassi, A., Biores. Technol. 97 (2006) 131.
- Beltran, J., Gonzalez, T., Garcia, J., J. Hazard. Mater. 154 (2008) 839.
- 21. Liwarska-Bizukojc, E., Bizukojc, M., Process Biochem. 40 (2005) 2067.
- 22. Wu, J., Jiang X., Wheatley, A., Desalination 249 (2009) 969.
- Papadimitriou, C. A., Papatheodoulou, A., Takavakoglou, V., Zdragas, A., Samaras, P., Sakellaropoulos, G. P., Lazaridou, M., Zalidis, G., Desalination 250 (2010) 378.
- 24. Yang, S. F., Li, X. Y., Yu, H. Q., Process Biochem. 43 (2008) 8.