Biodegradation Kinetics of Tobacco-waste Leachate by Activated Sludge in a Sequencing Batch Reactor (SBR)

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Treatment of wastes and leachate evolved in landfills is today an imperative due to rigorous environmental protection legislation. In this work, biodegradation of the organic fraction in tobacco waste leachate was studied. Experiments were carried out in a sequencing batch reactor at initial concentrations of activated sludge of 3.03 and 5.95 g L^{-1} and different initial concentrations of organic matter in leachate, expressed as COD concentrations, ranging from 0.5 to 3.0 g L^{-1}. The results of the experiments showed that activated sludge possessed a strong ability to degrade organic matter in leachate. Efficiency of the biodegradation process was approximately 82.6 %. A simple Monod equation was selected to describe the kinetics of leachate biodegradation. The kinetic parameters Y, k_d, μ_{max} and K_s during experiments E1 and E2 were found to be 0.25 g g^{-1}, 0.005 d^{-1}, 0.39 d^{-1} and 5.45 g L^{-1}, and 0.23 g g^{-1}, 0.003 d^{-1}, 0.44 d^{-1} and 5.63 g L^{-1}, respectively.

Key words:
Leachate, tobacco waste, biodegradation process, kinetics, activated sludge

Introduction

During various processing and production cycles, the tobacco industry generates large amounts of organic waste. Estimates show that about 3 million tons of tobacco waste is produced globally every year. The composition of tobacco waste depends on the point of cigarette production system in which it is generated. It may contain dust of various particle size, and entire or parts of tobacco leaves. Tobacco waste represents a significant problem for the environment, and contains toxic substances, primarily nicotine.[1,2] The European Union classifies it as toxic and hazardous waste, since the nicotine concentration in these wastes exceeds 500 mg kg^{-1} of dry matter.[3] Nicotine is the primary alkaloid in tobacco. It is soluble in water and can easily end up in groundwater after being leached from the solid waste, which is especially harmful to the environment and human health.[3,4]

There are various possible waste-treatment technologies as composting, incineration or landfilling. The release from a landfill consists mainly of methane gas and leachate, which has became the subject of interest as a strongly polluted wastewater. In the EU countries, the problem of leachate treatment has existed for some time now, but a universal solution has not been found.[5–8] Leachates contain high concentrations of various organic, inorganic, and toxic compounds, which may endanger the surrounding soil, ground and surface waters.[5] Leachate can be treated through biological, chemical, and physical processes or a combination of the three, in order to increase treatment efficiency and meet the stringent regulatory requirements.[7,8]

Toxicity tests conducted using various bacteria (*Vibrio fischeri, Photobacterium phosphoreum*, *Vibrio harveyi* and *Pseudomonas fluorescens*) and higher organisms (*Daphnia similes*, *Artemia salina* and *Brachydanio rerio*) show that leachates pose a potential hazard and that effective treatment is required to meet the standards for releasing the effluents into natural recipients.[5,6]

Biological wastewater treatment has been utilized globally for decades. There are many different processes that vary depending on the origin of the wastewater, i.e. the existing contamination.[1,8,9] Still, one of the most frequently used processes is biological wastewater treatment using activated sludge process.[10] This process is so widely used because of its effectiveness in treatment of either municipal and industrial wastewater, or leachates.[11,12] The activated sludge process is a complex biological system in which organic matter is removed from the wastewater by aerobic microorganisms. The microorganisms are incorporated within or on floc of activated sludge, and aeration of wastewater suspension ensures the exchange of products of metabolism and substrates, as well as the oxygen required to maintain aerobic respiration and catabolitic reactions.[13,14]

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Sequencing batch reactor (SBR) technology has become an attractive option for tobacco waste leachate treatment. The higher process flexibility of SBR is particularly important when considering landfill leachate treatment, which has a high degree of variability in quality and quantity. The SBR system seems suitable for treating the leachate, which volumes and concentrations may vary considerably with time.[1,5,11] The characteristics of the leachate can be represented by chemical oxygen demand (COD), total organic carbon (TOC), biochemical oxygen demand (BOD), which provide a prerequisite insight into the prediction of future trends of leachate quality and the design and operation of leachate treatment facilities.[5,7]

The substrate concentration surrounding the microorganisms within the microbial ecosystem is important for the determination of kinetic parameters. The substrate, which is the source of carbon and energy, and a limiting factor for the growth of microbial biomass during biodegradation processes, may be expressed as BOD and/or COD. Several kinetic models have been developed to describe biological degradation of organic matter in wastewater. The relationship between microbial growth and substrate degradation has been described successfully using Monod kinetics. The models are used to gain insight into the applicability and restrictions of treatment processes. Mathematical models are very important for further development, design and management of wastewater treatment processes.[6,7,14,15]

The application of stringent environmental regulations and higher control of contaminated leachate flows[16] have brought on unavoidable changes in landfill design, planning and operation. This has resulted with the research of selective, reliable and durable solutions for the treatment of contaminated leachate. Leachate treatment using activated sludge is a technically and economically efficient and warranted process. The COD value in the leachate and the tobacco-industry wastewater is estimated at 1.0 to 70.9 g L^{-1}.[1,5–7,9]

The aim of this work was to study the biodegradation of laboratory-provided leachate (LPL) inoculated with municipal activated sludge in SBR, investigate the substrate biodegradation rate and evaluate the biokinetic parameters using the Monod model.

Materials and methods

Materials

Activated sludge used for leachate inoculation in the reactor was obtained from the Wastewater Treatment Plant in Zagreb, ZOV, Croatia. It was washed three times and then settled. The initial concentrations of activated sludge, expressed as biomass dry mass (MLSS), were X_1 = 3.03 g L^{-1} and X_2 = 5.95 g L^{-1} while the volatile solids (MVLSS), were X_{v1} = 2.09 g L^{-1} and X_{v2} = 4.12 g L^{-1}.

The laboratory-provided leachate (LPL) used in the research was prepared from tobacco waste, TDR d.d., Rovinj, Croatia according to European standard of EN 12457-4:2002.[17] The substrate concentration, representing the organic loading rate of leachate, is expressed using the COD value. The initial COD and toxicity of LPL were determined after filtration of the sample through a 0.45 μm membrane. The initial COD of the resultant LPL was S = 16.03 g L^{-1}. The LPL was tested for toxicity using bioluminescent bacteria *Vibrio fischeri*. Bioluminescence inhibition was measured on Lumistox 300 (Dr Lange GmbH, Germany) after 30 min of incubation, using the standard method (EN ISO 11348-3, 1998).[18] The method is based on the measurement of *V. fischeri* light emissions. The bacterial cells were exposed to a series of variously diluted leachate. Light emission was determined by comparing the response given by a saline control solution. The concentrations of sample (% v/v) that reduced light emission to 50 % in relation to the control (EC_{50}) were calculated by using LumiSoft data acquisition software. Toxicity Impact Index (TII_{50}) is calculated based on EC_{50} value and is defined by the expression $TII_{50} = (EC_{50})^{-1} \cdot 100$. For the set of experiments, initial concentrations of 0.5, 1.0, 1.5, 2.3, and 3.0 g L^{-1} were prepared from resultant LPL, and marked as S1-S5. Experiments E1 and E2 were also defined for initial activated sludge concentrations X_1 and X_2.

Experimental set-up

Laboratory-scale reactor Armfield W11, Armfield Limited, UK, with a working volume of 7.0 L was operated in a sequencing mode. Fine air bubbles for aeration were introduced through an air diffuser at the bottom of reactor with an air flow rate of 1.5 L min^{-1}. A peristaltic pump was used to feed LPL directly into the SBR, as well as to remove the treated effluent. The leachate in the reactor was kept at 23±2 °C. The reactor was operated at a hydraulic retention time (HRT) of 48 h and there were 3 cycles. Each cycle lasted 48 h: the filling in lasted 0.5 h, the reaction took place in 46 h, the settling lasted 1.0 h and the withdrawal lasted 0.5 h. During the feeding of leachate, the system had to be fully aerated. The aeration was then continued for another 46 h. Aeration was then shut down for 1 h (settle step). After the activated sludge was fully settled, the supernatant had to be removed within 0.5 h (draw step: decant). After that, fresh leachate

was filled into the reactor to the final volume of 7 L and the above operation program was repeated. The exchange factor (added volume/total volume) was 0.65. Solid retention time (SRT) was 6 days.

The biodegradation was performed with LPL concentrations of S1 – S5, and of X_1 and X_2. During the experiments, the leachate and activated sludge samples were taken from the reactor in 12-hour intervals and then analyzed for MLSS, MVLSS, and for COD in accordance with standard methods.[19] The concentration of dissolved oxygen and pH-value were measured directly in bulk liquid of reactor with DO-electrode and pH meter (WTW Multi 340i, Germany). The average sample taken daily from the reactor was 100 mL. The activated sludge sample was examined daily under a light microscope (Olympus BX50, Olympus Optical Co. Ltd., Japan) equipped with a microphotography system (Olympus DP 10 camera) and the associated software to measure the size of activated sludge flocs (Olympus DP-Soft).

Biodegradation kinetics

Microbial degradation is generally defined as biological oxidation of organic matter. In natural environments, biodegradation conditions are very complex, and the rate and degree of biodegradation depends on chemical, physical, and biological factors that can differ from one ecosystem to another. Though microbial processes are very complex, some events or groups of events can be presented using a model. The most widely accepted of these is the Monod equation. This equation assumes that the rate of biomass production is limited by the rate of enzyme reactions involving utilization of the substrate compound that is in shortest supply relative to its need.[14,20] Eq. (1) shows this relationship.

$$\mu = \mu_{\max} \cdot \frac{S}{K_s + S} \tag{1}$$

where μ represents specific growth rate, d^{-1}; $\mu_{\max}$ represents the maximum specific growth rate, d^{-1}; S represents substrate concentration, g L^{-1}; K_s is the substrate saturation constant, g L^{-1}, defined as the concentration of substrate at half the maximum specific growth rate. The biodegradation process results in microbial growth with the removal of substrate.

Substrate concentration decreases with the growth of microorganisms. Therefore, the following equation can be developed:

$$r_s = \frac{1}{Y}\frac{\mu_{\max} X_v S}{K_s + S} \tag{2}$$

where r_s is substrate consumption rate, g L^{-1} d^{-1}; Y is the growth yield coefficient, g g^{-1}; X_v is biomass concentration, g L^{-1}. The carbon and energy source, as measured COD is usually considered the growth-limiting substrate in biological wastewater treatment processes.

Microbial growth rate can be described by eq. (3):

$$r_x = \frac{\mu_{\max} X_v S}{K_s + S} - k_d X_v \tag{3}$$

where r_x is biomass growth rate, g L^{-1} d^{-1}; and k_d is the decay coefficient, d^{-1}. Cell concentration reduction is known as endogenous respiration stage.[21,22]

Specific growth rate, specific substrate consumption rate, and the real biomass yield from substrate $Y_{x/s}$ can be calculated directly from the experimental data using the following eqs. (4)–(6):

$$\mu = \frac{\ln(X_{vi}/X_{v0})}{(t_i - t_0)} \tag{4}$$

$$q_s = \frac{(S_0 - S_i)/(t_0 - t_i)}{X_{vi} - X_{v0}} \tag{5}$$

$$Y_{x/s} = \frac{X_{vi} - X_{v0}}{(S_0 - S_i)} \tag{6}$$

where q_s is the specific substrate consumption rate, g g^{-1}d^{-1}.

Specific biomass growth rate (μ) is directly related to the specific substrate consumption rate (q_s):

$$\mu = Yq_s - k_d \tag{7}$$

For the batch test, the reactor is filled with reactant(s) to create the desired reaction conditions within the system. Samples are then analyzed during the reaction time, and the concentration is recorded over time.

Parameters of the model are calculated with certain criteria for the correlation of experimental data with the values obtained with the use of the model. The generally accepted criterion is the mean square deviation (SD) defined as:

$$SD = \frac{1}{n}\sqrt{\sum_{1}^{n}(y_e - y_t)^2} \tag{8}$$

where y_e and y_t represent experimental and theoretically calculated values of a dependent variable y, and n is the number of experimental point.

Data analysis

Model parameters were analyzed using linear and non-linear regression. Linear regression was

evaluated using the least squares method implemented in MS Excel software. Numeric values of model parameters were obtained through comparison of model and experimental results, using MS Solver[23] software that conducts non-linear regression and optimization method using Generalized Reduced Gradient (GRG2). Differential equations of the model were solved numerically by Runge Kutta 4 algorithm. A set of optimal model parameters was used in simulations that were then compared with experimental results.

Results and discussion

Toxicity of LPL and efficiency of its biodegradation

Before setting up the biodegradation experiment, a toxicity test was conducted on the leachate using *V. fischeri* bacteria, a standard and recognized method for assessing ecotoxicity of industrial wastewaters, communal wastewaters and landfill leachates to the natural water quality.[24] Toxicity Impact Index (TII_{50}) is related to the amount of unknown compound in the sample and directly is proportional to toxicity. It is expressed as a percentage and enables comparison of toxic impact of various types of wastewater to natural waters.[25] Based on the analysis, the acute toxicity of leachate EC_{50} was 1.6 g L^{-1} and TII_{50} = 9.99, indicating its toxicity. This result shows that the leachate should not be released into the environment without prior treatment. According to the literature[26–29] several processes such as coagulation–flocculation process, adsorption to activated carbon, membrane process and advanced oxidation processes (AOPs) showed efficiency in reducing toxicity of leachates and industrial wastewaters. These chemical-physical pre-treatments are proposed before biological treatment. However, in order to meet stringent quality standards for direct discharge of leachate into the surface water or public sewage system,[16] development of an integrated treatment process is required.[4,5,8]

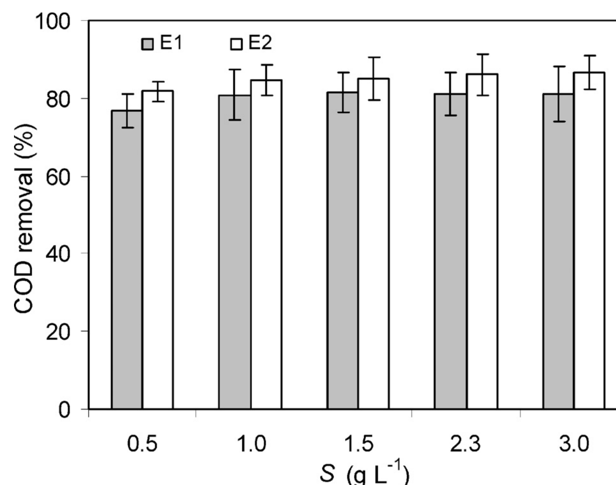

Fig. 1 – *Efficiency of leachate biodegradation in SBR reactor*

The efficiency of the biodegradation process in SBR during experiments E1 and E2 with initial substrate concentrations of 0.5 – 3.0 g L^{-1} (S1–S5) is shown in Fig. 1. Substrate concentrations applied in this research corresponded to those published in literature.[1,4,11,30] At lower initial activated sludge concentration, the average effectiveness of the biodegradation process was 80.3 % (0.32 g L^{-1}), while at higher concentration, the biodegradation of leachate proved more effective and ranged between 81.8 % and 86.7 %. Biodegradation of leachate at all initial substrate concentrations in E2 was 5.4 % higher than in E1. Similar values were obtained for biological treatment of tobacco-industry wastewater with effectiveness of around 84.0 %.[1,12]

LPL biodegradation kinetics

An understanding of the kinetics of biodegradation is very important for the prediction of future trends of leachate quality, and the overall design and operation of leachate management facilities. Obtaining kinetic data is one of the first steps necessary when attempting to model the biodegradation process. Tables 1 and 2 summarize the ranges of the pH-values, concentrations of DO, values of

Table 1 – *Experimental and kinetic results obtained from biodegradation process of leachate in experiment E1*

E1	pH (–)	DO (mg L^{-1})	X_v/X	μ (d^{-1})	q_s (g g^{-1} d^{-1})	$Y_{x/s}$ (g g^{-1})
S1	7.81±0.14	7.86±0.29	0.69±0.03	0.03±0.006	0.12±0.02	0.26±0.10
S2	7.90±0.17	7.16±0.64	0.71±0.01	0.06±0.014	0.25±0.05	0.27±0.11
S3	7.95±0.15	5.83±1.05	0.71±0.04	0.09±0.018	0.38±0.07	0.29±0.04
S4	7.98±0.21	5.75±0.92	0.72±0.02	0.13±0.035	0.53±0.11	0.29±0.13
S5	7.92±0.19	5.48±1.35	0.73±0.03	0.15±0.025	0.59±0.09	0.28±0.08

Table 2 – *Experimental and kinetic results obtained from biodegradation process of leachate in experiment E2*

E2	pH (–)	DO (mg L^{-1})	X_v/X	μ (d^{-1})	q_s (g g^{-1} d^{-1})	$Y_{x/s}$ (g g^{-1})
S1	7.61±0.10	6.93±0.34	0.69±0.03	0.01±0.002	0.05±0.01	0.22±0.03
S2	7.72±0.10	5.68±0.79	0.70±0.03	0.03±0.004	0.15±0.04	0.24±0.04
S3	7.75±0.15	4.91±0.66	0.70±0.06	0.04±0.009	0.20±0.06	0.27±0.06
S4	7.96±0.18	4.52±1.20	0.71±0.04	0.06±0.007	0.28±0.05	0.28±0.04
S5	8.02±0.09	4.16±1.36	0.72±0.02	0.08±0.006	0.37±0.10	0.27±0.05

ratio MLVSS/MLSS, specific growth rate, specific substrate degradation rate and growth yield for tobacco-waste leachate biodegradation in a batch reactor and their mean square deviations.

The environmental factor effecting and inhibiting microbial growth is the pH value, i.e. acid or base conditions of liquid waste streams. Biological treatment of wastewater or leachates occurs generally at neutral pH because the optimum pH for growth of bacteria is around 7.[30] The mean values of pH measured during experiments E1 and E2 were in the range of 7.81 to 7.98, and 7.61 to 8.02, respectively (Tables 1 and 2), which corresponds to the published data.[5] The value of pH is an important factor in tobacco-waste leachate biotreatment. The mean square deviations were approximately the same and satisfactory. The small increase in pH can be attributed to the release of ammonium nitrogen during biodegradation.[6,8] The average change in dissolved oxygen concentration during biodegradation was 6.42±0.85 mg L^{-1} during E1 and 5.24±0.87 mg L^{-1} during E2, for all concentrations. During experiments S1–S5, the mean value of DO decreased proportionally to the increase in substrate concentration (Tables 1 and 2). The concentration of oxygen decreases during biodegradation of organic matter, so at higher organic loading rate more oxygen is consumed and vice versa. In aerobic biological processes, the available amount of oxygen for microbial growth is often the most critical parameter limiting the efficiency of the process.[8,30] In summary, the highest dissolved oxygen consumption was detected in the experiment using substrate S5 with 3.0 g COD L^{-1}. In other words, less oxygen is consumed at lower substrate concentrations. When comparing the two experiments, average oxygen consumption was 18.4 % higher in E2 than in E1. Tables 1 and 2 clearly show that ratio X_v/X remained nearly constant during the experiments. These values were within the 0.69–0.73 range, showing that the cells of microbial biomass in mixed liquor suspended solids were viable and in good condition during both experiments. The mean values resulted in satisfactory mean square deviations. The ratio X_v/X indicates biomass concentration within activated sludge, and a change in the ratio would therefore also indicate a change of microbial diversity or decay.[22] The average biomass yield during experiment E1 was up to 1.6 % higher than during E2. This difference is because more substrate was available for initial lower biomass concentration in E1 than in E2, and cells more rapidly grew and divided producing new biomass. Specific growth rate μ was calculated directly from experimental data based on biomass concentration change (X_v) in time, using eq. (4). The mean value of μ increases proportionally with the increase of initial substrate concentration. Higher μ values were obtained during E1, ranging from 0.03 to 0.15 d^{-1}, while the values obtained during E2 ranged from 0.01 to 0.08 d^{-1}. It means that specific growth rate is in direct correlation with the substrate availability for growth of new cells, and the average μ in E1 was 0.04 d^{-1} higher than in E2. Mean specific substrate consumption rate q_s, calculated using eq. (5), represents the rate at which the substrate is consumed in relation to biomass growth. The q_s in both experiments (E1 and E2) increased from the initial substrate concentrations of S1 to S5 from 0.12 to 0.59 g g^{-1} d^{-1}, and from 0.05 to 0.37 g g^{-1} d^{-1} respectively. Standard deviation for the experiments was up to 0.11 for E1, and up to 0.10 for E2. The range of μ and q_s as shown by Tables 1 and 2, fits relatively well with previously published values.[31,32] Real yield coefficient, $Y_{x/s}$ was calculated based on experimental results and using eq. (6). Average $Y_{x/s}$ for E1 and E2 was 0.28±0.09 g g^{-1} and 0.26±0.04 g g^{-1}, which shows that $Y_{x/s}$ during E1 increased 7.9 % in relation to E2. Similarly, it was reported that experiments with activated sludge in aerobic conditions resulted in $Y_{x/s}$ from 0.25 to 0.40.[31]

Growth yield coefficient Y is one of the most important parameters used in biological kinetics models. It represents biomass concentration produced by unit of removed substrate. Endogenous respiration rate k_d is the biomass decay rate. The dependency of consumed organic substrate to the production of microorganism cells is shown by eq. (7).

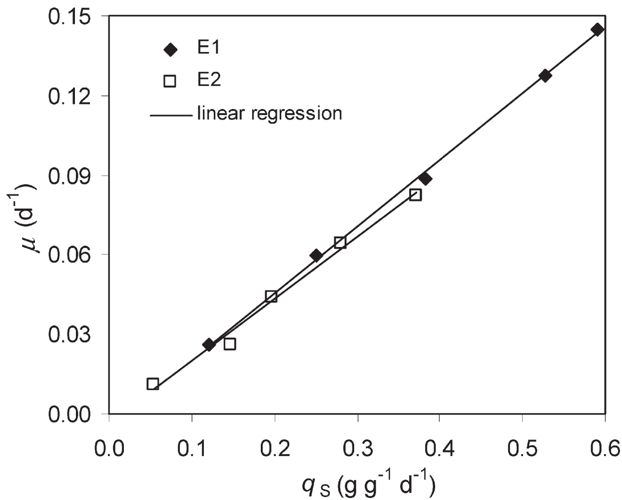

Fig. 2 – *Dependence of specific growth rates on specific substrate consumption rate for estimation of Y and k_d in E1, R^2 = 0.9986 and E2, R^2 = 0.9896*

Linear regression of μ and q_s dependency,[32] based on eqs. (4) and (5), produces Y and k_d parameters from the slope and intercept of plot, respectively (Fig. 2, Tables 1 and 2) with high R^2 value. Values of Y and k_d (Table 3) correspond to the published value ranges for activated sludge biodegradation processes.[31]

The biokinetic parameter optimization method was used to obtain μ_{max} and K_s values. A model (eqs. 2 and 3) with parameter values presented in Table 3 was used to simulate leachate biodegradation process in a batch reactor. Figs. 3 and 4 show a comparison of experimental results and those of the model. Value μ_{max} of 0.44 d^{-1} corresponds to the value obtained using the same initial sludge concentration in E2.[32] Other obtained values of biokinetic parameters also fall within the expected range for biodegradation process in similar conditions, such as for high strength food processing wastewater, landfill leachate and pharmaceutical wastewaters.[20,32–34] A comparison of kinetic parameters obtained for this study with those reported in other studies is shown in Table 3. Activated sludge has been used in referred studies.

Table 3 – *Comparison of biokinetic parameters values*

μ_{max} (d^{-1})	K_s (g L^{-1})	Y (g g^{-1})	k_d (d^{-1})	Reference
0.39	5.45	0.25	0.005	This study (E1)
0.44	5.63	0.23	0.003	This study (E2)
0.87	6.70	0.50		20
0.44	0.14			28
0.21	11.00	0.28	0.019	29
0.77	2.98	0.48	0.045	30

Fig. 3 shows the substrate consumption rate during biodegradation process in relation to initial substrate concentration. R^2 values obtained during E1 and E2 were 0.9944 and 0.9976, respectively. Comparing the results of both experiments, it can be determined that the model (eq. 2, Table 3) with the same initial concentration range as in the conducted experiments offers a good description of process dynamics with high R^2 values.

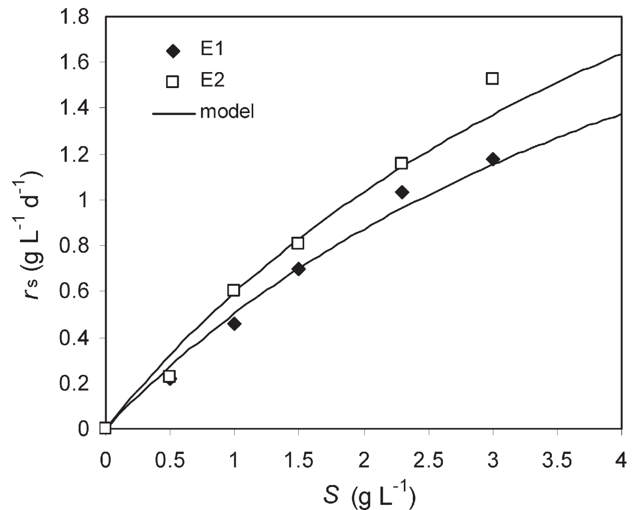

Fig. 3 – *Influence of initial substrate concentrations on substrate degradation rate. Comparison of experimental results and model.*

Biomass growth rate dependency of initial substrate concentration during biodegradation process is shown in Fig. 4, with R^2 = 0.9917 obtained for E1 and R^2 = 0.9987 obtained for E2. R^2 shows a good correlation between the model (eq. 3, Table 3) and values of both experiments. It is evident that the se-

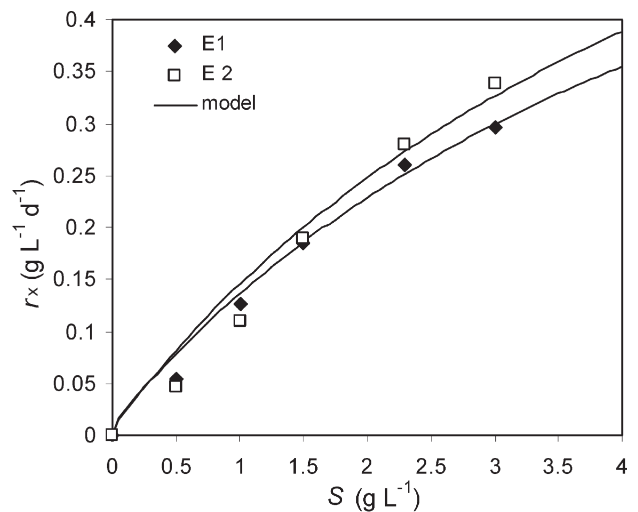

Fig. 4 – *Influence of initial substrate concentrations on biomass growth rate. Comparison of experimental results and model.*

lected model offers an accurate description of process dynamics.

During microscopic analysis, the morphology of activated sludge was studied using brightfield microscope. The analysis was conducted to determine floc characteristics, size, and shape in order to gain insight into the changes that occur in activated sludge during tobacco-waste leachate treatment.[15,30] Fig. 5 shows microphotographs of activated sludge flocs on the second day of S3 substrate biodegradation process with initial activated sludge concentrations of X_1 = 3.03 g L^{-1} (Fig. 5a) and X_2 = 5.95 g L^{-1} (Fig. 5b). During both experiments, the activated sludge flocs were relatively firm, compact, and rounded, which contributed to the reduction of COD value in the leachate. The transparent and loose flocs indicate new biomass developed during the biodegradation process in the reactor.[35]

Floc structure, like size and morphology, plays an important role in determining the efficiency and economics of the activated sludge process. The changes in morphology of flocs are connected with different phenomena: growth, flocculation and deflocculation of flocs, and starvation.[30,36] Formations of flocs of activated sludge occur when microorganisms present in the leachate are bound to each other, and the change in their count directly influences the change in flocs size. At the start of the experiments, the flocs were larger (Table 4). In both experiments, the mean floc size was reduced by 37.26 μm in average. After day two (Fig. 5), minimum floc size in E1 and E2 was approximately the same at 58.49 μm, and 54.70 μm, respectively (Table 4). Maximum floc sizes stabilized on the second day and reduced by 38.60 and 32.47 μm from the first day, as seen in mean floc sizes of 31.91 μm and 26.28 μm. Low biomass yield resulted in the stabilization of floc size (Fig. 5), as seen from SD values (Table 4) showing lower deviations. At the same time, the compact structure of activated sludge results in shorter settling time, good effluent quality at the outflow from the reactor,[36] and the process generates no excess activated sludge as do conventional wastewater treatment processes.[30] Changes in floc size may also be the effect of the different stirring in the SBR relative to the WWTP.

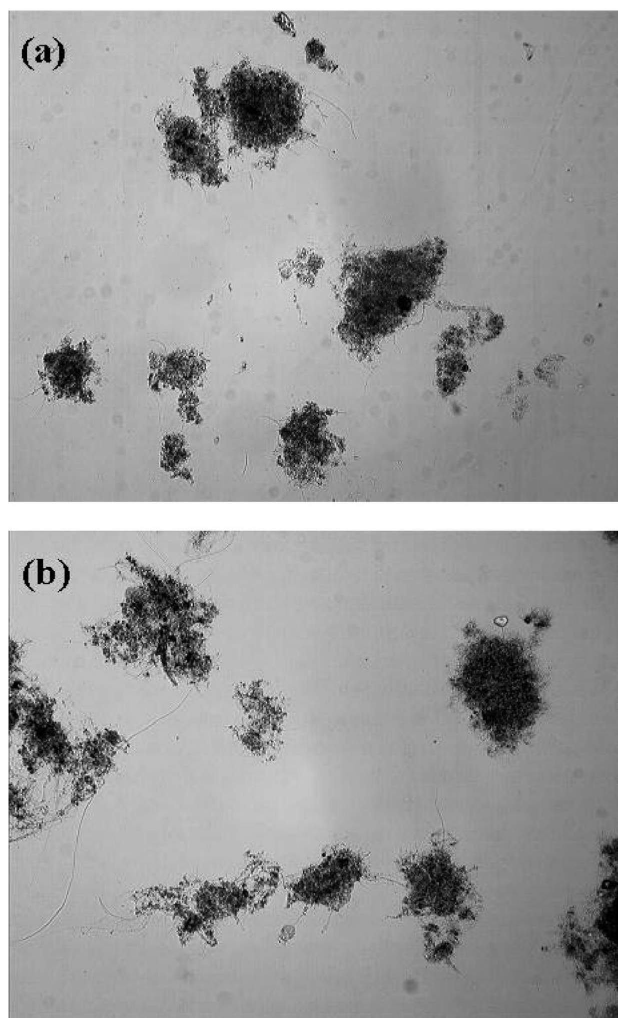

Fig. 5 – *Microphotographs of activated sludge flocs second day in batch reactor, S = 1.5 g L^{-1}, for experiments (a) E1 and (b) E2, P = 100×*

Table 4 – *Floc size of activated sludge in a batch reactor, S = 1.5 g L^{-1}*

Exp.	t (d)	Mean size (μm)	Minimum (μm)	Maximum (μm)	SD
E1	0	279.37	111.78	429.66	83.40
	1	223.26	72.31	371.60	76.07
	2	191.35	58.49	333.00	68.00
E2	0	325.32	152.52	587.87	136.48
	1	290.60	89.21	486.51	107.54
	2	264.32	54.70	454.04	90.70

The flocculation–deflocculation of activated sludge flocs is a highly dynamic process, depending on the microbial community structure, environmental and operating conditions in sequencing batch reactor. This type of reactor is increasingly recognized as a persuasive option in municipal and industrial wastewaters and leachate treatments.[5,36]

Conclusions

Treatment of heavily loaded wastewaters and leachates by activated sludge is very promising and of great interest from an environmental point of view. Toxicity tests showed that tobacco-waste leachate is harmful for aquatic environments when

discharged without treatment. Therefore, the biodegradation of tobacco-waste leachate by activated sludge in SBR was investigated in this study. The efficiency of COD removal through biodegradation ranged from 76.80 % to 86.57 %, indicating that the activated sludge was resistant to potentially toxic substrate. By investigating the kinetics of substrate utilization and biomass growth, the kinetic parameters Y, k_d, μ_{max} and K_s were in the range of 0.25 g g^{-1}, 0.005 d^{-1}, 0.39 d^{-1} and 5.45 g L^{-1} for experiment E1, and 0.23 g g^{-1}, 0.003 d^{-1}, 0.44 d^{-1} and 5.63 g L^{-1} for experiment E2, respectively. SBR with activated sludge was an efficient, reliable and stable process for LPL treatment. Therefore, these results can be used to create guidelines for LPL biodegradation.

ACKNOWLEDGMENTS

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List of abbreviations and symbols

COD – chemical oxygen demand, g L^{-1}
DO – oxygen concentration, mg L^{-1}
EC – effective concentration, g L^{-1}
k_d – decay constant, d^{-1}
K_s – substrate saturation constant, g L^{-1}
q_s – specific substrate degradation rate, g g^{-1} d^{-1}
r_s – substrate consumption rate, g L^{-1} d^{-1}
r_x – biomass growth rate, g L^{-1} d^{-1}
S – substrate concentration, g L^{-1}
t – time, d
X – activated sludge concentration, g L^{-1}
X_v – biomass concentration, g L^{-1}
$Y_{x/s}$ – overall yield coefficient, g g^{-1}
Y – growth yield, g g^{-1}
μ – specific growth rate, d^{-1}
μ_{max} – maximum growth rate, d^{-1}

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