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KINETICS OF AEROBIC TREATMENT OF TWO-PHASE OLIVE-MILL WASTE BY ACTIVED SLUDGE IN SEQUENCING BATCH REACTOR

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Olive mill waste has a very high organic load, high amount of toxicity/phytotoxicity-associated compounds, low pH value and high electrical conductivity. In this study, the removal of COD (chemical oxygen demand) and total phenols from extracted wastewater (EW) from two-phase olive-mill waste (TPOMW) in sequencing batch reactor (SBR) was studied. The experiments were carried out at 23 °C with the initial COD concentration of 5 g L^{-1} and 10 g L^{-1} at different initial concentrations of activated sludge (3 and 6 g L^{-1}). The obtained results showed that activated sludge possessed a strong ability to degraded organic matter and total phenols. The COD and phenols removal efficiency was between 80 - 90 %. Toxicity Impact Index (TII_{50}) was 312.5 which indicated that this wastewater is extremely toxic. Four different kinetic models were select to describe the kinetics of TPOMW biodegradation. The lowest deviation and the best fit with experimental data were achieved using Endo-Haldane model.
Key words: activated sludge, kinetic analysis, olive mill wastewater, SBR.

Kinetika biorazgradnje otpadne vode iz proizvodnje maslinovog ulja aktivnim muljem u sekvencijalnom šaržnom reaktoru. Otpad nastao proizvodnjom maslinovog ulja visoko je organski opterećen, toksičan te ima nizak pH i visoku električnu vodljivost. U ovom radu provedeno je istraživanje uklanjanja organskog opterećenja izraženog kao KPK vrijednost i ukupnih fenola iz otpadne vode nastale dvostupanjskim procesom proizvodnje maslinovog ulja u SBR reaktoru. Eksperimenti su provedeni pri 23 °C pri početnim koncentracijama organskog opterećenja 5 i 10 g L^{-1} i različitim početnim koncentracijama aktivnog mulja 3 i 6 g L^{-1}. Dobiveni rezultati ukazuju da aktivni mulj dobro razgrađuje organsku tvar i fenole. Postotak uklonjenih organskih tvari izražene kao KPK vrijednost i fenola iznosio je između 80 - 90 %. Indeks toksičnosti otpadne vode iznosio je (TII_{50}) 312.5 što ukazuje na ekstremno toksičnu otpadnu vodu. Četiri različita kinetička modela primijenila su se za opis kinetike biorazgradnje TPOMW. Najmanje odstupanje i najbolje slaganje s eksperimentalnim rezultatima dobiveno je primjenom Endo-Haldane modela.
Ključne riječi: aktivni mulj, kinetička analiza, otpadna voda iz proizvodnje maslinovog ulja, SBR.

INTRODUCTION

Olive oil industry is traditionally a primary alimentary sector for Mediterranean Countries (Spain, Italy, Greece, Turkey, Syria, Tunisia and Croatia) and it generates a huge amount of wastewater and other wastes [1, 2]. The olive mill wastes have a great impact on land (soil microbial population) and water environments because of their high phytotoxicity [1, 3]. Olive cake which is developed during the process of production of olive oil contains about 60 % of water and cannot be disposed on the ground.

The characteristics of olive mill wastewater (OMWW) and other wastes are variable, depends on factors such as olive variety (type and maturity), method of extraction, region of origin, soil type, climatic conditions and associated cultivation/processing methods [4, 5]. Also, the olive mill wastes contain high concentrations of recalcitrant compounds

such as lignin, phenolic compounds (tannins) which give it a characteristic dark color [5, 6]. Because of toxicity and high organic load, treatment of OMWW will improve the quality of the wastewater. Several treatment options have been investigated, including physical (dilution, evaporation, sedimentation, filtration and centrifugation), chemical and biological processes (aerobic treatment, composting, anaerobic digestion) as well as combinations of them [5]. Aerobic and anaerobic continuous and discontinuous treatments as well as SBR system are usually exploited to remove organic matter from OMWW. The sequencing batch reactor (SBR) is an example of activated sludge system with fills and draw basis. The SBR has gained great attention as its performance in treating wastewater is more superior than continuous flow activated sludge process and the removal capacity of toxic pollutant is higher than in continuous system [7]. The operation of SBR can be adjusted flexibly to achieve desired treatment performance. The selection of operating strategy is dependent on wastewater composition, biodegradability and the concentration of toxic organic matter [7]. Phenol removal, as major toxic component, is an important step in biodegradation of OMWW and TPOMW. The maximum allowed concentration of phenols in wastewater is 10 mg L^{-1} (Croatian regulation). When the phenol content in the wastewater exceeds 1000 mg L^{-1}, the recovery scheme becomes an attractive proposition, because the pure cultures of microorganisms in the biological treatment cannot survive in high concentrations of phenol as an inhibitory substrate, e.g. more than 3000 mg L^{-1} [8, 9]. The purpose of this study was to investigate biodegradation of extracted wastewater from TPOMW by activated sludge in SBR, the substrate biodegradation rate and to evaluate the kinetic parameters of each model such as Monod, modified Monod, Haldane and Endo-Haldane model.

MATERIALS AND METHODS

Extracted wastewater from TPOMW

The extracted wastewater (EW) from two phase olive mill waste (TPOMW) used in this study was obtained from olive oil manufacturer Agrolaguna d.o.o, Poreč, Croatia. Chemical analyses were performed after filtration of the sample (membrane pore size 0.45 μm, Sartorious, Germany). The main physical and chemical characteristics of EW from TPOMW are presented in Table 1. The organic load was expressed as COD (chemical oxygen demand) and it was determined spectrophotometrically (Spectrophotometer DR/2400, Hach at USA) at λ = 670 nm [10]. Total phenols were determined spectrophotometrycally using the 4-Aminoantipyrin at λ = 500 nm [10]. The N content was determined according to the Kjeldahl method [11]. The toxicity of TPOMW was determined 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 [12] and EC_{50} value was calculated by using LumiSoft data acquisition software. Toxicity Impact Index (TII_{50}) is calculated based on EC_{50} value according to following equation (1) [12]:

$$TII_{50} = \frac{1}{EC_{50}} \qquad (1)$$

Activated sludge

The activated sludge used in the experiments was collected from a Wastewater Treatment Plant in Zagreb, ZOV, Croatia. It was washed in the aerobic reactor three times and then settled. The initial concentration of activated sludge, expressed as biomass dry mass (MLSS) was X = 8.27 g L^{-1} while the volatile solids (MLVSS) and non volatile solids (MLNVSS) were X_v=7.07 g L^{-1} and X_{nv} = 1.2 g L^{-1}, respectively.

Experimental set-up

The experiments were carried out in the laboratory-scale reactor Armfield W11, Armfield Limited, UK. The working volume of the reactor was 5 L and it was operated in a sequencing mode. The flow rate was 1.5 L min^{-1}. The air diffusers were at the bottom of reactor. A peristaltic pump was used to feed EW from TPOMW directly into the SBR, as well as to remove effluent.

The wastewater in the reactor was kept at t = 23 °C. The activated sludge was adapted to extracted wastewater from TPOMW where the initial COD concentration was 5 and 10 g O_2 L^{-1}. The sequencing batch reactor (SBR) was operated at a hydraulic retention time (HTR) of 5 and 7 days. There were 4 cycles with fill, react, settle and draw in the ratio of 1:117.5:1:0.5 and 1:165.5:1:0.5 for a cycle time of 5 and 7 days, respectively. After draw step, the fresh EW from TPOMW was filled into the reactor and above operation was repeated. The biodegradation experiments were performed at different initial COD concentrations of EW from TPOMW (5 and 10 g L^{-1}) and different initial concentrations of activated sludge, Table 2. During the experiments, every 24 hours the sample (activated sludge + wastewater) was filtrated and analyzed for MLSS, MLVSS, COD, phenols, NH_4^+, NO_2^-, NO_3^- in accordance with standard methods [10]. The concentrations of dissolved oxygen, conductivity and pH value were measured directly in bulk liquid in reactor with DO, EC electrode and pH meter (WTW Multi 340i, Germany).

Modelling the growth kinetics of the mixed culture

The modelling was conducted using the assumption that COD (S) represents the rate limiting substrate and MLVSS (X) represents the amount of the active biomass. In the exponential growth phase, the rate of production of biomass is well described by a first order kinetic equation (1):

$$r_X = \frac{dX}{dt} = \mu \cdot X \qquad (1)$$

where r_x is biomass growth rate (g L^{-1} h^{-1}) and μ is the specific growth rate of biomass. Simultaneously to the production of the cells, the substrate (S) is degraded, and its rate is also proportional to the mass of cells present, according to the expression, equation (2):

$$r_S = -\frac{dS}{dt} = \frac{\mu \cdot X}{Y} \qquad (2)$$

where r_s is substrate degradation rate (g L^{-1} h^{-1}) and Y is the growth yield coefficient (g g^{-1}). The literature provides several expressions which relate the specific rates to the substrate concentration, for example Monod, Contois, Haldane models.

The equations (3) represent the Monod equation, which is based on Michaelis-Menton enzyme kinetics, shows relationship between specific growth rate of biomass and limiting nutrient (substrate):

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \qquad (3)$$

where μ is the specific growth rate (h^{-1}), μ_{max} is maximum specific growth rate (h^{-1}) and K_s is Monod saturation constant (g L^{-1}) (i.e. substrate concentration at half μ_{max}). Also, K_s values show the affinity of microorganisms to substrate, but if the substrate is inhibitory it is not possible to observe an actual μ_{max}. Thus, K_s take on a hypothetical meaning [9]. Monod equation becomes unsatisfactory for explaining inhibitory growth of microorganism at higher substrate concentrations [13]. Further, this expression only holds for the exponential growth phase. For the whole growth cycle of microorganisms, the death phase must be also take into account, when the decline in the cell number takes place [14]. The decay coefficient correspondents to endogenous metabolism which involve reactions in cells that consume cell substances [15]:

$$\mu = \frac{\mu_{max} \cdot S}{K_S \cdot S} - k_d \qquad (4)$$

A modified Monod model is the Haldane equation (eq. 5) which incorporates the inhibitory effect of toxic substrates [16]:

$$\mu = \frac{\mu_{max} \cdot S}{\left[K_s + S + \left(\frac{S^2}{K_i}\right)\right]} \qquad (5)$$

The inhibition constant (K_i) indicates the concentration up to which cultures can tolerate shock loads [17]. A higher K_i value implies that the culture is less sensitive to substrate inhibition [18]. This value is particularly important for subsequent applications since it defines a concentration threshold that should not be exceeded. Some authors proposed that decline in cell population, i.e. biomass decay (k_d), after the complete consumption of substrate should be taken into account, eq. (6) [15]:

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S + \left(\frac{S^2}{K_i}\right)} - k_d \qquad (6)$$

This is Endo-Haldane model, which is frequently used because of its ability to account for the effect of inhibition at high concentration and of cell death and/or maintenance metabolism at low concentration. The biomass yield from substrate, $Y_{X/S}$, can be calculated using the following equation (7):

$$Y_{X/S} = \frac{X_i - X_0}{S_0 - S_i} \qquad (7)$$

RESULTS AND DISCUSSION

Physiochemical characterization of extracted wastewater

The determinations carried out for EW from TPOMW were summarized in Table 1. As shown in Table 1, EW is characterized by an acidic pH, high electrical conductivity, high BOD and COD concentrations, high toxicity and red black colour of water.

Table 1. Physical and chemical characteristics of EW from TPOMW
Tablica 1. Fizikalno-kemijska karakterizacija TPOMW

Parameter	Values in this experiment	Range acording to literature (Dermeche et al., 2013; Morillo et al., 2009
Dry matter (%)	37.2	28.6 – 50.4
Volatile solids (%)	96.8	60.3 – 98.5
pH	4.9	4.9 – 6.8
Electrical conductivity (mS/cm)	13.8	1.2 – 5.2
Total nitrogen (%)	0.8	0.2 – 1.8
C/N	59.9	29.3 – 59.7
Total phenols (mg L^{-1})	1000	
COD (g L^{-1})	105 – 121	30 – 320
BOD (g L^{-1})	90 – 95	35 – 132
EC_{50} (%)	0.3	

Acute toxicity of extracted wastewater

The marine luminescent bacterium *Vibrio fischeri* is one of the most widely used organism in toxicity analysis, and has been used to facilitate easy, quick and reliable toxicity measurements of chemicals and wastewater [19]. In this work, before the biodegradation experiments were set up, a toxicity test was conducted on extracted wastewater from TPOMW using *Vibrio fischeri* bacteria. *Vibrio fischeri* inhibition test is the most sensitive test, cost effective, easy to operate and requires only 5-30 min for toxicity prediction [20]. 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 wastewaters to natural waters [21]. The acute toxicity of extracted wastewater from TPOMW was EC_{50} = 0.32 % and TII_{50} = 312.5. Based on literature [19, 20, 21] the samples with toxicity units (reaction time of 15 minutes) equal to or greater than 10 (EC_{50} □ 10.0%;TII_{50} □ 10.0) should be considered toxic when *Vibrio fischeri* was used. So, before application of TPOMW on soils and crops as a fertilizer the pretreatment is necessary.

Biodegradation of extracted wastewater from TPOMW

Efficient treatment of TPOMW is a difficult task, as they contain many phenolic toxic and other inorganic and organic compounds which are given in Table 1.

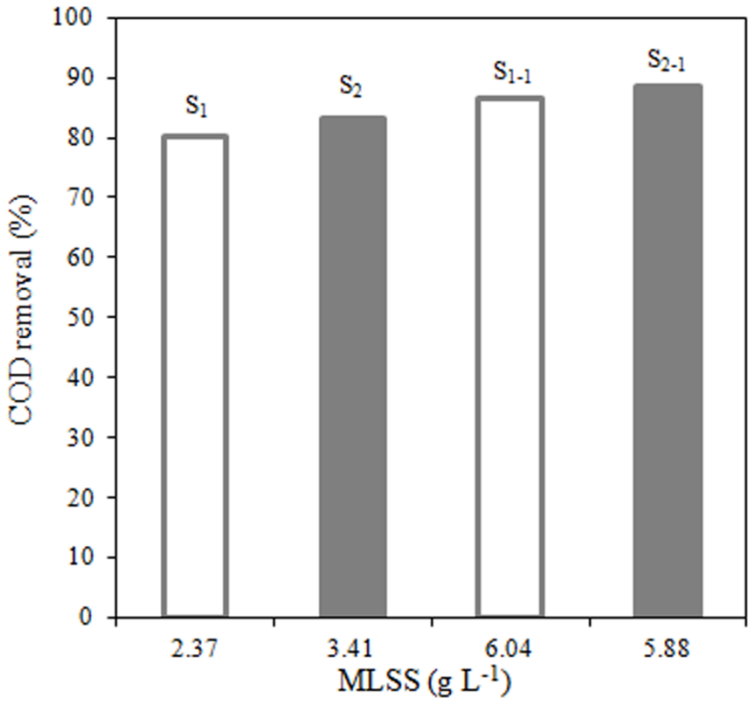


Figure 1. The removal percentage of organic matter from extracted wastewater from TPOMW in SBR reactor (S_1 and S_{1-1} (COD = 5 g L^{-1}, MLSS = 2.37 and 6.04 g L^{-1}); S_2 and S_{2-1} (COD = 10 g L^{-1}, MLSS = 3.41 and 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) during 5 days
Slika 1. Postotak uklonjenih organskih tvari iz otpadne vode maslina TPOMW u SBR reaktoru (S_1 and S_{1-1} (KPK = 5 g L^{-1}, MLSS = 2.37 and 6.04 g L^{-1}); S_2 and S_{2-1} (KPK = 10 g L^{-1}, MLSS = 3.41 and 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) tijekom 5 dana

Fig. 1. shows efficiency of the biodegradation of EW from TPOMW by activated sludge in SBR in experiments S_1, S_{1-1}, S_2 and S_{2-1}. It can be seen that at lower concentrations of activated sludge in experiments S_1 and S_2 the effectiveness of biodegradation process was lower than at higher concentrations of activated sludge, experiments S_{1-1} and S_{2-1}. Also, the biodegradation rate increased with increasing the initial COD concentrations because more organic matter was available for microorganisms, Fig. 2. On the contrary, as the concentration of phenol increased the biodegradation rate decreased because of toxic effect of phenol on microorganisms, Fig. 2. The concentrations of dissolved oxygen (DO, mg L^{-1}) during the first three days of the experiments were low due to the intensive biodegradation process. Microorganisms degraded organic matter present in wastewater, energy was released and more oxygen was consumed, Fig. 3. At the end of the experiments the concentration of dissolved oxygen increased as the COD concentration decreased, which means that the process of biodegradation of EW from TPOMW was nearing the end. In

experiments S_2 and S_{2-1} the concentration of dissolved oxygen started to increase at day 3 and at day 5, respectively. The concentration of biomass in experiment S_{2-1} was higher than in experiment S_2, which means that more active microorganisms were available to metabolize organic matter and consequently more oxygen with the time was consumed.

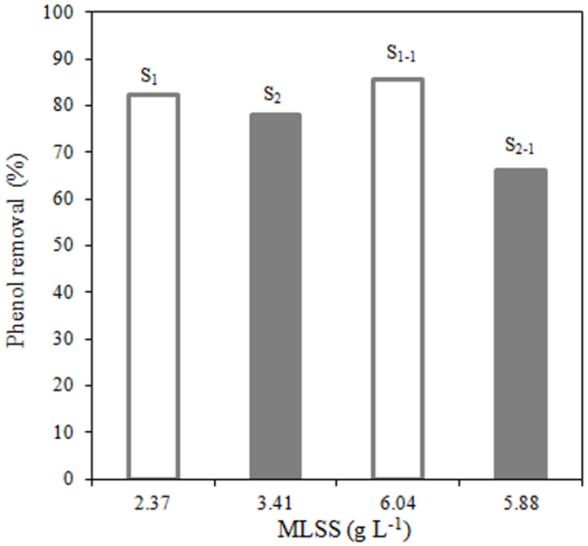


Figure 2. The removal percentage of phenols from extracted wastewater from TPOMW in SBR reactor (S_1 and S_{1-1} (COD = 5 g L^{-1}, MLSS = 2.37 and 6.04 g L^{-1}); S_2 and S_{2-1} (COD = 10 g L^{-1}, MLSS = 3.41 and 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) during 5 days

Slika 2. Postotak uklonjenih fenola iz otpadne vode maslina TPOMW u SBR reaktoru (S_1 and S_{1-1} (KPK = 5 g L^{-1}, MLSS = 2.37 and 6.04 g L^{-1}); S_2 and S_{2-1} (KPK = 10 g L^{-1}, MLSS = 3.41 and 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) tijekom 5 dana

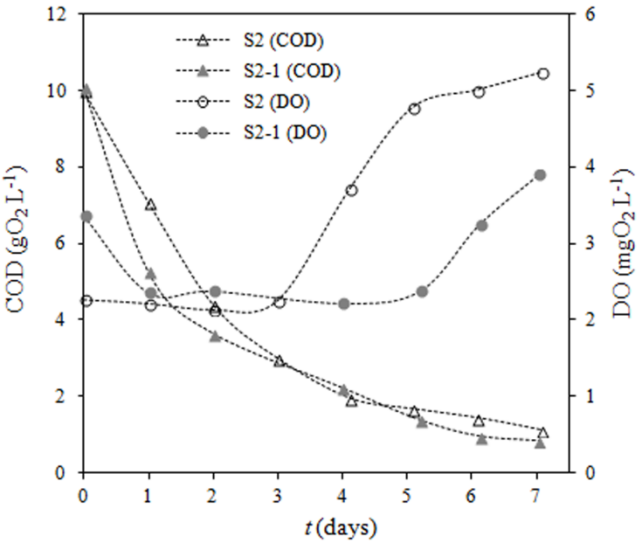


Fig. 3. COD reduction of extracted wastewater from TPOMW during 7 days in SBR reactor and influence on changes of DO (S_2 (COD = 10 g L^{-1}, MLSS = 3.41 g L^{-1}); S_{2-1} (COD = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1})

Slika 3. Promjena organskog opterećenja izraženog kao KPK vrijednost u TPOMW tijekom 7 dana u SBR reaktoru i utjecaj na promjenu otopljenog kisika (S_2 (KPK = 10 g L^{-1}, MLSS = 3.41 g L^{-1}); S_{2-1} (KPK = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1})

Influence of pH value on biodegradation of phenols, on colour of wastewater and on formation of ammonia ions

Biological removal of phenol, mostly based on activated sludge-type system, is an economical and well-suited detoxification technology that leads to complete conversion of this toxic compound to innocuous products (CO_2 and H_2O). The major problem of this process is sensitivity to high phenol load, because at certain concentrations phenol is an inhibitory substrate even for the bacterial species which are able to use it as an energy and carbon source [22, 23]. Numerous environmental factors influence the growth of microorganisms. Phenol degradation seems to be determined by some environmental factors such as temperature and pH value [9, 22]. The pH value can be an indicator of the phenol degradation and one of the factors significant in the success of the biological treatment. According to Ucun et al. [9], it was found that pH value affects the surface charge of cells of the activated sludge biomass.

Thus, electrostatic attraction between phenol and activated sludge biomass would be impacted. From Fig. 4. it can be seen that removal of phenols increased with increase of pH value. In this work, the most favourable pH value to achieve maximum rate of phenol degradation was between 7 and 8, which is in agreement with published data [9, 22, 24, 25]. According to that it seems that the best pH range for the phenol degradation does exist. It is possible that enzymes for phenol degradation have their optimum enzymatic activities at an optimum pH [22]. Furthermore, Fig. 4. shows that in experiment $S_{2\text{-}1}$ the pH value at day 3 dropped. The drop of pH value during the consumption of phenol can be attributed to the production of organic acids from intermediates, which are formed in the degradation of phenol through the meta cleavage by mixed culture [9]. The pH value influenced to the colour of the extracted wastewater from TPOMW. The dark colour increases considerably at pH > 8 (results not shown). Tannins and anthocyanins, among other pigments, are responsible of the OMWW and TPOMW colour. These pigments are pH sensitive and change colours with changing acidity levels. At acidic pH the pigments display a red coloration and at basic conditions the colour becomes black-purple and darker [26, 27].

Fig. 5. shows a formation of ammonia ions (NH_4^+), nitrite ions (NO_2^-) and nitrate ions (NO_3^-) during biodegradation of EW from TPOMW. It can be seen that the concentration of ammonia ions at the beginning of the process was low, as the pH value increased to alkaline range, the concentration of NH_4^+ increased due to the decomposition of organic nitrogen that liberates ammonium. The concentration of NO_2^- was detected in trace only and the concentration remained unchanged over the 7 days of the experiment, which is a clear indicator that no anaerobic conditions occurred during biodegradation process [28]. The concentration of NO_3^- at the beginning of biodegradation process decreased and remained unchanged till the end.

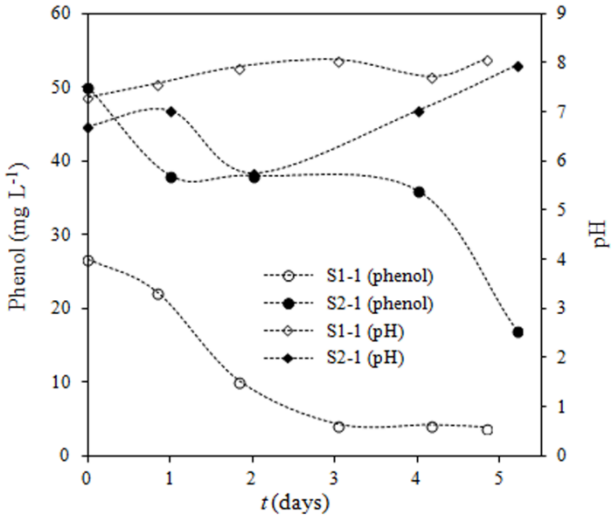


Fig. 4. Effect on pH value on removal of phenols from extracted wastewater from TPOMW in SBR reactor ($S_{1\text{-}1}$ (COD = 5 g L^{-1}, MLSS = 6.04 g L^{-1}); $S_{2\text{-}1}$ (COD = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) during 5 days

Slika 4. Utjecaj pH vrijednosti na uklanjanje fenola iz TPOMW u SBR reaktoru ($S_{1\text{-}1}$ (KPK = 5 g L^{-1}, MLSS = 6.04 g L^{-1}); $S_{2\text{-}1}$ (KPK = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) tijekom 5 dana

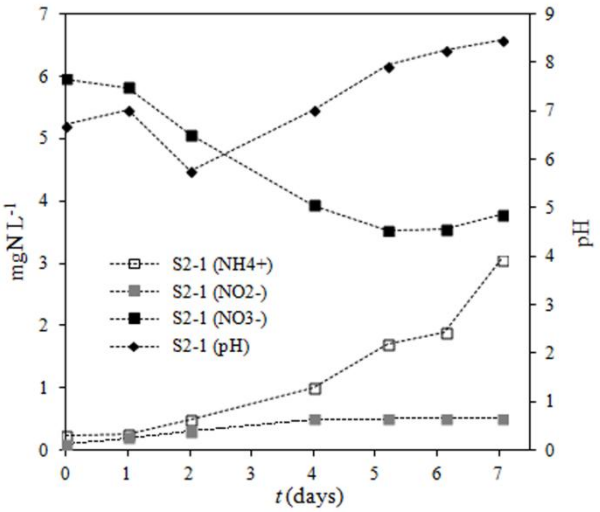


Figure 5. Influence of pH value on formation of NH_4^+ ions ($S_{2\text{-}1}$ (COD = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) during 7 days

Slika 5. Utjecaj pH vrijednosti na stvaranje NH_4^+ iona ($S_{2\text{-}1}$ (KPK = 10 g L^{-1}, MLSS = 5.88 g L^{-1}), t = 23 °C, Q = 1.5 L min^{-1}) tijekom 7 dana

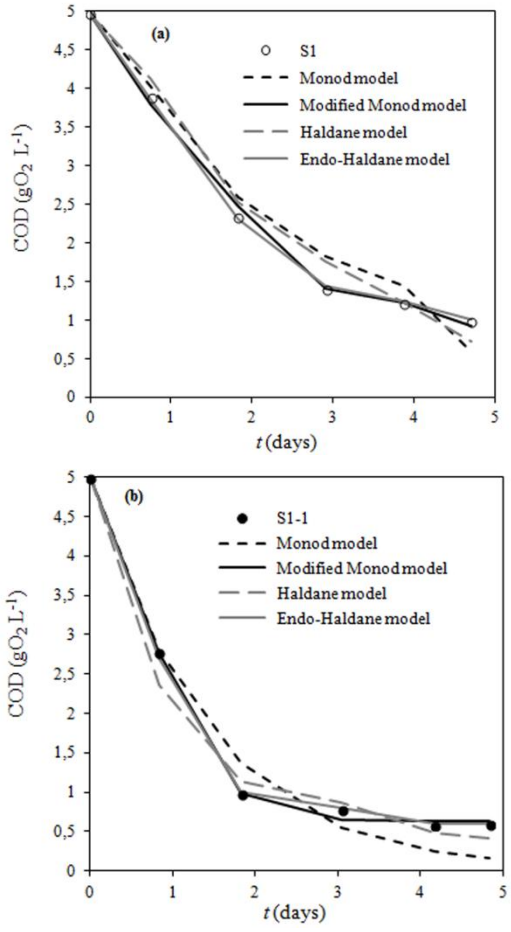
Determination of growth kinetic parameters

The extracted wastewater from TPOMW was degraded by aerobic microorganisms, in a series of experiments where the initial substrate concentration, COD_0, was 5 and 10 g O_2 L^{-1}, and the initial biomass concentration 2.37 and 6.04 g L^{-1}, according to values in Table 2.

Table 2. Initial concentrations of EW from TPOMW and activated sludge
Tablica 2. Početne koncentracije organskog opterećenja TPOMW i aktivnog mulja

Experiment	COD (g L^{-1})	MLSS (g L^{-1})	MLVSS (g L^{-1})	MLNVSS (g L^{-1})
S_1	5	2.37	1.69	0.68
S_{1-1}	5	6.04	4.52	1.52
S_2	10	3.41	2.94	0.47
S_{2-1}	10	5.88	4.56	1.32

The kinetic analyses were performed using four different models, Monod model, modified Monod model with endogenous metabolism, Haldane model and Endo-Haldane model which consider effects of inhibition and biomass decay. In Fig. 6 (a) – (d) is shown the comparison of experimental results and different models for the experiments S_1, S_{1-1}, S_2 and S_{2-1}.



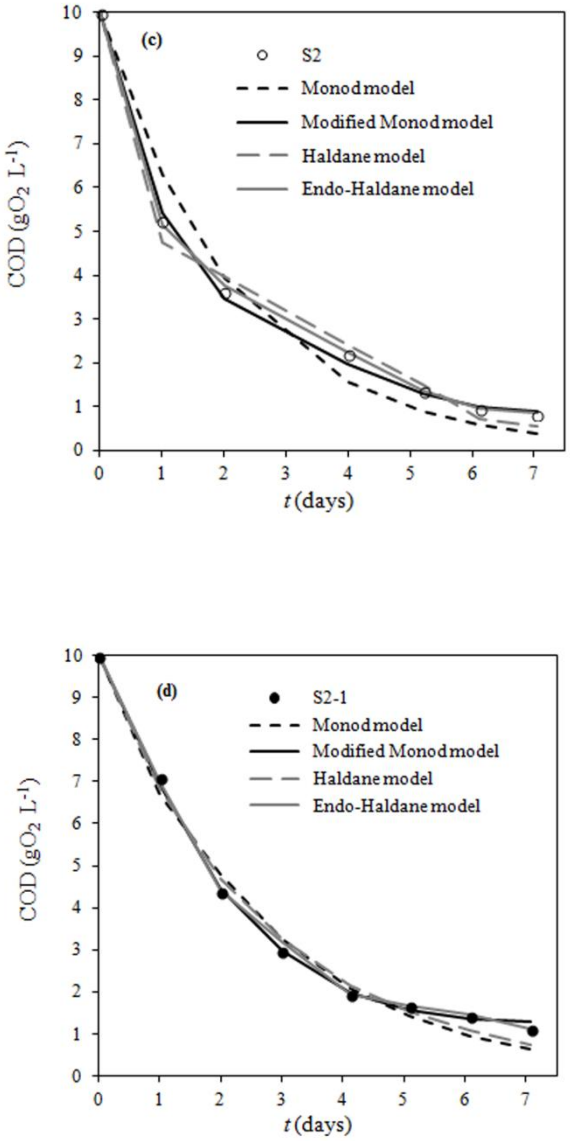


Figure 6. COD removal from extracted wastewater from TPOMW by activated sludge: (a) S_1, (b) $S_{1\text{-}1}$, (c) S_2 and (d) $S_{2\text{-}1}$ in SBR reactor and comparison of experimental data with values obtained using Monod model, modified Monod model, Haldane model and Endo-Haldane model
Slika 6. Uklanjanje organskog opterećenja iz TPOMW aktivnim muljem: (a) S_1, (b) $S_{1\text{-}1}$, (c) S_2 i (d) $S_{2\text{-}1}$ u SBR reaktoru i usporedba eksperimentalnih rezultata s vrijednostima dobivenih primjenom Monod model, modificirani Monod model, Haldane model i Endo-Haldane model

The estimated kinetic parameters are shown in Table 3 (a) – (d). To fit the data and estimate the values of kinetic constants of the equations 3 - 6, *Scientist* 5.0 computer program module, based on non-linear least square technique was used. From Fig. 6. and Table 3 it can be seen that modified Monod model and Endo-Haldane model describe the process of degradation of organic matter from extracted wastewater from TPOMW better than Monod model and Haldan model. These models take into account endogenous respiration and substrate inhibition terms. The presence of the microbial endogenous respiration allows the model to predict constant substrate concentration attained at

the end of the process, whereas classical Monod model which does not have this term, predict zero concentration at the end of the process [15]. From Fig. 6. (a) - (d) evident is the decrease of the amount of organic matter. Also, the activity of aerobic microorganisms decrease too, because a particular cell population becomes food for the healthier one what causes the change in microbial community and inhibition of process [15].

Table 3. Evaluated kinetic parameters for (a) Monod model, (b) Modified Monod model, (c) Haldane model and (d) Endo-Haldane model
Tablica 3. Procijenjeni kinetički parametri za (a) Monod model, (b) Modified Monod model, (c) Haldane model and (d) Endo-Haldane model

(a) Monod model

Parameters	Experiment			
	S_1	$S_{1\text{-}1}$	S_2	$S_{2\text{-}1}$
μ_{max} (h^{-1})	0.15	0.26	0.29	0.36
K_s (g L^{-1})	0.084	0.063	0.071	0.057
K_i (g L^{-1})	-	-	-	-
k_d (h^{-1})	-	-	-	-
SD × 10^2 (-)	1.157	1.180	2.105	1.213

(b) Modified Monod model

Parameters	Experiment			
	S_1	$S_{1\text{-}1}$	S_2	$S_{2\text{-}1}$
μ_{max} (h^{-1})	0.34	0.44	0.56	0.57
K_s (g L^{-1})	0.021	0.027	0.039	0.033
K_i (g L^{-1})	-	-	-	-
k_d (h^{-1})	0.0019	0.0019	0.0021	0.0021
SD × 10^2 (-)	0.308	0.241	0.516	0.370

(c) Haldane model

Parameters	Experiment			
	S_1	S_{1-1}	S_2	S_{2-1}
μ_{max} (h^{-1})	0.16	0.47	0.78	0.73
K_s (g L^{-1})	0.062	0.061	0.036	0.043
K_i (g L^{-1})	0.944	1.196	1.345	1.345
k_d (h^{-1})	-	-	-	-
SD × 10^2 (-)	0.897	0.826	1.075	0.928

(d) Endo-Haldane model

Parameters	Experiment			
	S_1	S_{1-1}	S_2	S_{2-1}
μ_{max} (h^{-1})	0.49	0.57	0.91	0.88
K_s (g L^{-1})	0.034	0.039	0.049	0.058
K_i (g L^{-1})	0.882	0.789	1.102	1.136
k_d (h^{-1})	0.0021	0.0021	0.0025	0.0025
SD × 10^2 (-)	0.095	0.148	0.256	0.272

A comparison of the values obtained for kinetic constants for activated sludge in this study with those reported in other studies is shown in Table 4.

Table 4. Kinetics constants for biodegradation of organic matter in different studies
Tablica 4. Kinetičke konstante biorazgradnje organske tvari u različitim literaturnim izvorima

μ_{max} (h^{-1})	K_s (mg L^{-1})	K_i (mg L^{-1})	Reference
0.309	74.60	648.10	Bajaj et al. (2009)
0.308	44.92	525.00	Saravanan et al. (2008)
0.400	20.00	200.00	Ben - Youssef et al. (2011)
0.119	11.13	250.88	Ucun et al. (2010)
0.746	11.08	410.00	Kumaran and Paruchuri (1997)
0.381	57.35	1503.00	Lallai and Mura (1989)
0.730	43.00	1345.00	This work

Values are all within the range reported in the literature. The value of μ_{max} in present study was higher among the reported studies because there are many factors which influence the growth of microorganisms in a given environment. Competition for the common substrate is one of the possible reasons for a lower growth rate in other literature while non-competitive inhibition by secondary inhibitors is another reason [29]. Also different working conditions like temperature, pH value, real or model wastewater can influence on growth rate. Further, in this work the specific growth rate increased as the concentration of substrate increased, Table 3, but μ_{max} also tends to decrease due to the inhibitory effect of substrate as a concentration increased [29]. The values of K_s and k_d were in the ranges reported in other literatures [9, 14, 30].

The inhibition constant in this study was high and show that inhibition effect can be observed at relatively high substrate concentration. Growth yield coefficient Y is one of the most important parameters used in biological kinetics models. The growth yield was for experiments S_1 and S_2 0.14 and 0.15 g g^{-1}, respectively, and for experiments $S_{1\text{-}1}$ and $S_{2\text{-}1}$ was 0.19 g g^{-1}, respectively. It is evident that growth yield slightly increased as the concentration of biomass increased from 3 to 6 g L^{-1}. Further, growth yield was constant as the concentration of substrate increased which is similar with results reported by Hao et al. [31]. This phenomenon is based on the fact that the percentage of the total substrate carbon converted to energy for cell growth and maintenance is increased as the specific growth rate decrease, when the inhibition effect of phenol becomes predominant. More energy is required to overcome the effect of substrate inhibition during the degradation of phenol. While the percentage of the total substrate carbon assimilated into biomass decreases as specific growth rate decreased. Thus substrate inhibition is known not only to reduce the specific growth rate, but also to reduce the yield coefficient [32].

CONCLUSION

This study demonstrates that activated sludge could be successfully applied in a SBR system for biodegradation of EW from TPOMW. The sludge was able to degrade initial COD concentrations of 5 and 10 g L^{-1}, respectively. The kinetic analysis of biodegradation of organic matter presents in extracted wastewater from TPOMW was explained by using four different mathematical models. Between the four models, Endo-Haldane model gave a better fit to the experimental data. The kinetics constants evaluated using the models showed good tolerance and growth of mixed culture.

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