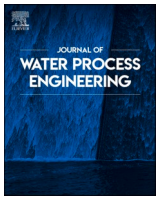
Journal of Water Process Engineering 55 (2023) 104077

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe



Comparison between batch aerobic biodegradation of paracetamol and glucose with open mixed microbial cultures: Experimental data and kinetic modelling

Bishnu Bhattarai, Davide Dionisi *, Waheed Afzal

Materials and Chemical Engineering Group, UK
School of Engineering, University of Aberdeen, Aberdeen, AB24 3UE, UK

ARTICLE INFO

Keywords:
Biological processes
Biodegradation
Wastewater treatment
Glucose
Paracetamol
Kinetic modelling

ABSTRACT

Biodegradation of man-made chemicals (xenobiotics) in biological wastewater treatment processes is essential to avoid the accumulation of these substances with negative effects on the environment and on human health. This study investigated the aerobic biodegradation of paracetamol as sole carbon and energy source and compared it to a model biogenic substrate (glucose). Batch experiments with unacclimated open mixed cultures from soil were carried out with glucose or paracetamol in a range of concentrations (100–2000 mg/L), measuring the rate of substrate removal, the acclimation time, the oxygen consumption and the COD (chemical oxygen demand) removal. The experimental data were used for kinetic modelling, using several different models based on the Monod kinetics with modifications for inhibition or for acclimation. Paracetamol was biodegraded as sole carbon and energy source at all concentrations (90–97 % COD removal), with slower degradation rate and longer acclimation time than for glucose. From the COD balance, a slightly higher production of microorganisms per unit of substrate COD was calculated for paracetamol than for glucose. The kinetic modelling of the experimental data achieved a good fit with the simple Monod model, but with different parameters for each substrate concentration. Combined fitting of all the experiments with the same set of parameters was attempted, and overall the best fitting was observed with a new model that includes an empirical time constant for acclimation. Compared to glucose, microbial growth on paracetamol was characterised by lower maximum specific growth rate, slightly higher growth yield and higher time constant for acclimation.

1. Introduction

Presence of xenobiotics in wastewaters, increasingly caused by urbanisation, population growth and industrialisation, is a current global environmental challenge. Xenobiotics are human-made chemicals that do not occur naturally and are usually synthesised for industrial or agricultural purposes [1]. They include a vast and expanding array of substances, e.g., pharmaceuticals, personal care products, steroid hormones, pesticides and industrial chemicals. Xenobiotics from different sources cause concerns due to their potential toxic effects and the possibility of accumulation in the environment [2]. The release of xenobiotics in the environment can result in long-term adverse effects on aquatic flora, fauna and on humans, even when present at low concentrations [3].

Xenobiotics are usually present in municipal wastewater and in some industrial wastewater [4]. In order to minimise the accumulation of xenobiotics in the environment, it is desirable to remove them as much as possible from wastewater before the treated wastewater is discharged. Some advanced techniques, such as advanced oxidation process, adsorption, and membrane separation, are known to remove many xenobiotics. However, these techniques have several disadvantages: either they do not modify the chemical nature of the xenobiotic substance but simply transfer it into another phase (adsorption and membrane processes) or they oxidise xenobiotics with usually large energy and chemical costs (advanced oxidation). Compared to chemical-physical technologies, aerobic biodegradation processes offer some advantages because they can remove biodegradable xenobiotics by converting them into harmless end products (CO_2 and H_2O) and are relatively cheaper to operate. Aerobic wastewater treatment processes, e.g. the activated sludge process, consist of open (undefined) mixed

* Corresponding author.
E-mail address: davidedionisi@abdn.ac.uk (D. Dionisi).

https://doi.org/10.1016/j.jwpe.2023.104077
Received 4 May 2023; Received in revised form 17 June 2023; Accepted 20 July 2023
Available online 28 July 2023

microbial communities and they are only effective if the microorganisms are able to metabolise the carbon sources in the wastewater.

Although many studies have investigated the biodegradation of xenobiotics by pure or open mixed cultures and significant advances have been made in the understanding of metabolic pathways [5], an analysis of the literature [4] shows that there are several limitations in our knowledge of the biodegradation of xenobiotics in biological wastewater treatment processes:

- Limited number of studies (e.g. [6–8]) with open mixed cultures and xenobiotics as sole carbon sources. In most published studies with open mixed cultures removing xenobiotics [9–11], xenobiotics represented only a minor fraction of the total organic matter. Although these studies are representative of actual conditions in biological wastewater treatment processes, they do not allow to establish whether xenobiotics are removed as primary carbon sources and are used for microbial growth or they are just removed by cometabolism while the microorganisms grow on biogenic carbon sources [4].
- Lack of quantitative data on the oxygen consumption and production of microorganisms associated with microbial growth on xenobiotics. Substrate removal, oxygen consumption and microorganisms production are key variables that define the performance and sustainability of biological wastewater treatment processes; however, this data is usually not available for the biodegradation of xenobiotics. Measurement of oxygen consumption and microorganism production is only possible when xenobiotics are used as sole carbon sources. An example of measurement of cell density for growth on pentachlorophenol was reported by Stanlake and Finn [12].
- Lack of kinetic models for the growth, substrate degradation and oxygen consumption for open mixed cultures degrading xenobiotics as sole carbon sources. Kinetic models are useful for the design and optimisation of biological wastewater treatment processes. However, only few studies, usually done with pure cultures, have quantified the kinetic parameters of microorganisms growing on xenobiotics [13,14]. An example of a kinetic study of xenobiotic biodegradation by open mixed cultures is the one by Tomei and Annesini [15].

Considering these limitations in state of the art, the main aims and novelties of this study are the following:

- To investigate the removal of xenobiotics as sole carbon sources by open mixed cultures, to determine the ability of these cultures to grow on xenobiotics as primary carbon sources.
- To quantify the rate of xenobiotic biodegradation and microorganism acclimation, and the oxygen consumption and production of microorganisms associated with the biodegradation.
- To develop a kinetic model for xenobiotic biodegradation, able to describe quantitively the processes of microbial growth, substrate removal and oxygen consumption.
- To compare the biodegradation of xenobiotic and biogenic substrates.

This study used paracetamol as model xenobiotic and glucose as model biogenic substrates, in a range of initial concentrations. Overall, this study contributes to improving our understanding of biodegradation of xenobiotics with open mixed cultures.

2. Materials and methods

2.1. Wastewaters, chemicals and inoculum

This study used two different synthetic wastewaters, one with glucose and another with paracetamol as the only carbon source. Glucose and paracetamol were purchased from Sigma-Aldrich with over 98 % purity (glucose product code: A0384062 and paracetamol product code: A0437381). The concentration of glucose and paracetamol were 100–2000 mg/L; in both cases, nutrients and micronutrients (prepared with deionised water) were added to the wastewater: KH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), NaCl (0.2 g/L), NH_4Cl (0.2 g/L), $MgSO_4.2H_2O$ (0.2 g/L), $CaCl_2.2H_2O$ (0.0225 g/L) and CH_4N_2S (0.02 g/L) and micronutrients $CuSO_4.H_2O$ (0.03 mg/L), $MnSO_4.H2O$ (0.13 mg/L), $ZnCl_2$ (0.23 mg/L), $CoCl.6H_2O$ (0.42 mg/L), $NaMoO_4.2H_2O$ (0.15 mg/L) and $AlCl_3.6H_2O$ (0.05 mg/L). The inoculum was soil from the Craibstone farm in Aberdeenshire, UK, with a VSS concentration of 0.11gVSS/g soil. The soil was homogenised, sieved (150 μm porosity), and then stored in a plastic container in a fridge (0–2 °C) before inoculation. Soil from the same site was used as an open mixed culture inoculum in earlier studies (e.g. [7,16]) and its microbial community was analysed by Bartram et al. [17].

2.2. Reactors set-up and experimental procedure

The bioreactors were glass vessels with a working volume of 250 mL. The bioreactors were stirred at 350 rpm using a 5 mm PTFE coated magnetic bar with a raised ring in the middle. VELP Scientific multimagnetic stirrer with 15 stirring positions was used for agitation. The runs were carried out at room temperature (23–25 °C).

The bioreactors were operated in batch and duplicate runs were done for each substrate concentration (glucose and paracetamol at 100, 200, 500, 1000 and 2000 mg/L). One experiment, also done in duplicate, was set up as a control, without substrate but with the soil and with the mineral solution. For all experiments, 1 g/L of wet soil was used. After addition of soil and the mineral solution, the experiments were started with the addition of the substrate (except for the control experiments) at the desired concentration. The dissolved oxygen concentration was measured every 1 h. Error bars represent the standard deviation.

2.3. Analytical methods

Hach HQ40d oxygen meter coupled with a Hach probe was used to measure dissolved oxygen concentration. Whatman 1822-047 Grade GF/F glass fibre filter paper of 0.8 μm pore size was used to filter the sample. Soluble COD was measured before and after filtration using COD Cell Test kits (Merck), according to the manufacturer's instructions. VSS measurements were carried out according to the previously described procedure [16]. The bioreactor's oxygen mass transfer coefficient (k_La), used in the mathematical model, was measured with the dynamic oxygen absorption method as described in the literature [18,19].

2.4. Data analysis: acclimation time, biodegradation rate and COD balance

The acclimation time was defined arbitrarily as the time required for the oxygen concentration to drop by 2 mg/L and was measured from the dissolved oxygen concentration in each experiment. The biodegradation rate was calculated as the initial substrate concentration divided by the time elapsed from the start of the experiment to the start of the increase in dissolved oxygen concentration, which was taken as indication of complete substrate removal [20].

The COD balance was used to calculate the fraction of the initial substrate COD that was left as residual at the end of each experiment or was converted to microorganisms or was oxidised. The equation used for the COD balance was: [18].

$$S_0 = S + \Delta X + (-\Delta O_2)$$

In this equation, all terms are in COD units. S_0 represents the initial concentration of substrate at the start of the experiment, S the final soluble COD, ΔX represents the produced microorganisms and $(-\Delta O_2)$ the oxygen consumed at the end of the experiment. In using this equation, the terms S_0 and S were measured as soluble COD at the start and end of the test, $(-\Delta O_2)$ was calculated by integrating the oxygen consumption over the length of the experiments, ΔX was calculated as the

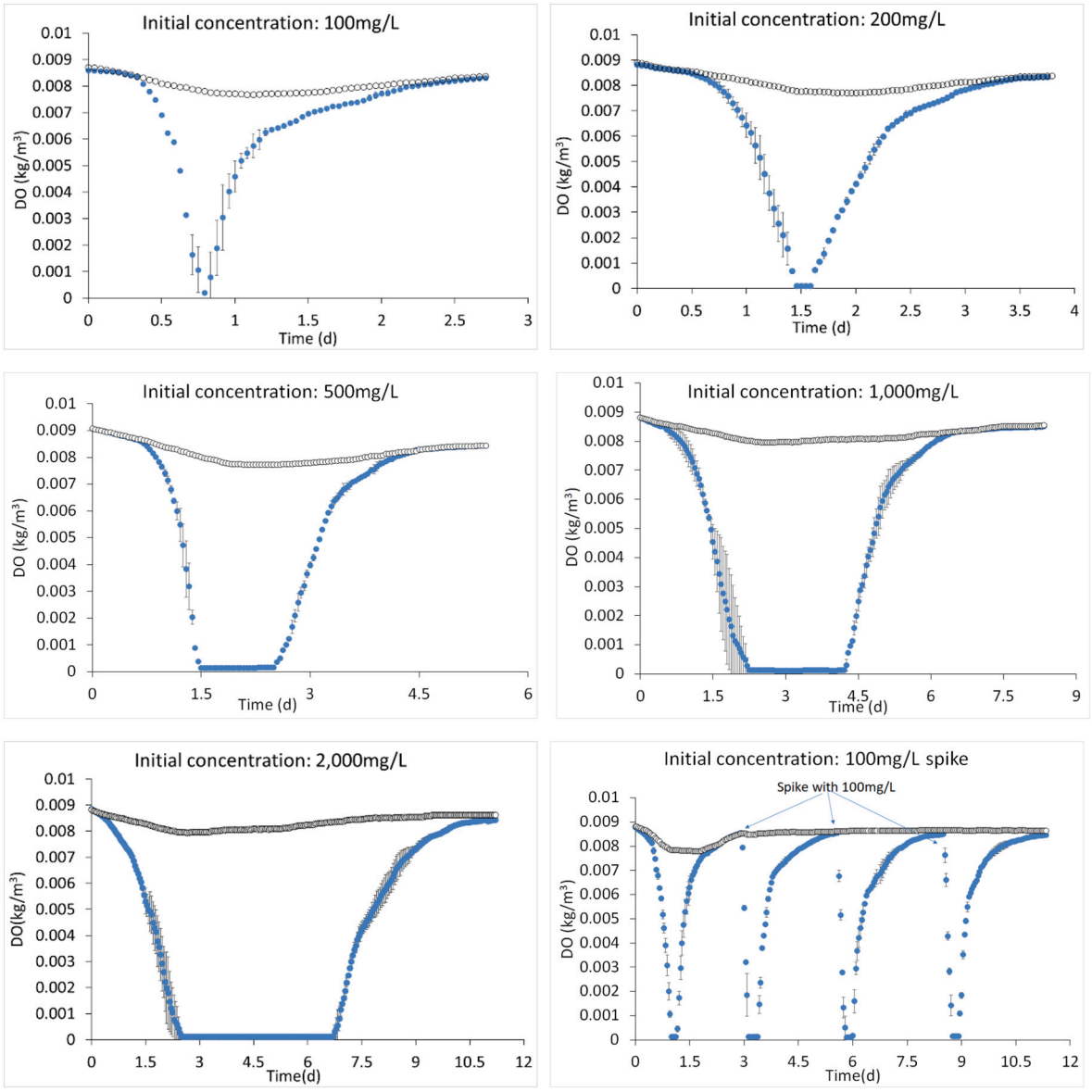


Fig. 1. Glucose: experimental data of dissolved oxygen (DO) vs time. Blue points: experiments with the substrate; white points: control experiments. The error bars indicate the standard deviation of the two duplicate experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

difference. The ratios $\frac{S}{S_0}, \frac{\Delta X}{S_0}, \frac{(-\Delta O_2)}{S_0}$ were used to calculate the fraction of the initial substrate that respectively was left as residual, was converted into microorganisms or was oxidised.

2.5. Mathematical model and parameter fitting

Several mathematical models were developed. All the models were based on the rate equations for biomass growth, endogenous metabolism and substrate removal shown below. In the kinetic model, the concentration of microorganisms (X) was expressed as their dry weight, while the concentration of substrate (S, glucose or paracetamol) was expressed in COD units. The use of COD in the mathematical model was due to the need of using the COD balance for the differential equation representing the mass balance for oxygen during the batch tests (Eq. (10) shown later).

$$\text{Biomass growth: } r_X\left(\frac{kg}{m^3 \cdot d}\right) = \mu X \tag{1}$$

$$\text{Endogenous metabolism: } r_{end}\left(\frac{kg}{m^3 \cdot d}\right) = -bX \tag{2}$$

$$\text{Substrate removal: } r_S\left(\frac{kgCOD}{m^3 \cdot \text{d}}\right) = -\frac{r_X}{Y_{X/S}} \tag{3}$$

In these equations, μ (d^{-1}) is the specific growth rate, X (kg/m^3) is the biomass concentration, b (d^{-1}) is the parameter for endogenous metabolism, and $Y_{X/S}$ (kg/kg_{COD}) is the growth yield.

The models were different in the equation used for the specific growth rate μ. Model A used the simple Monod equation $\left(\mu = \frac{\mu_{max} S}{K_S + S}\right)$, where S is the substrate concentration, expressed in $kgCOD/m^3$ for all models. Model B included substrate inhibition according to the Haldane's empirical model $\left(\frac{S}{K_S + S + \frac{S^2}{K_I}}\right)$ [21]. Models C and D were new models which accounted for acclimation using an empirical term $(1 - e^{-\frac{t}{\tau}})$ with the parameter τ to account for the time required for the microorganisms to completely acclimate to the substrate and therefore to

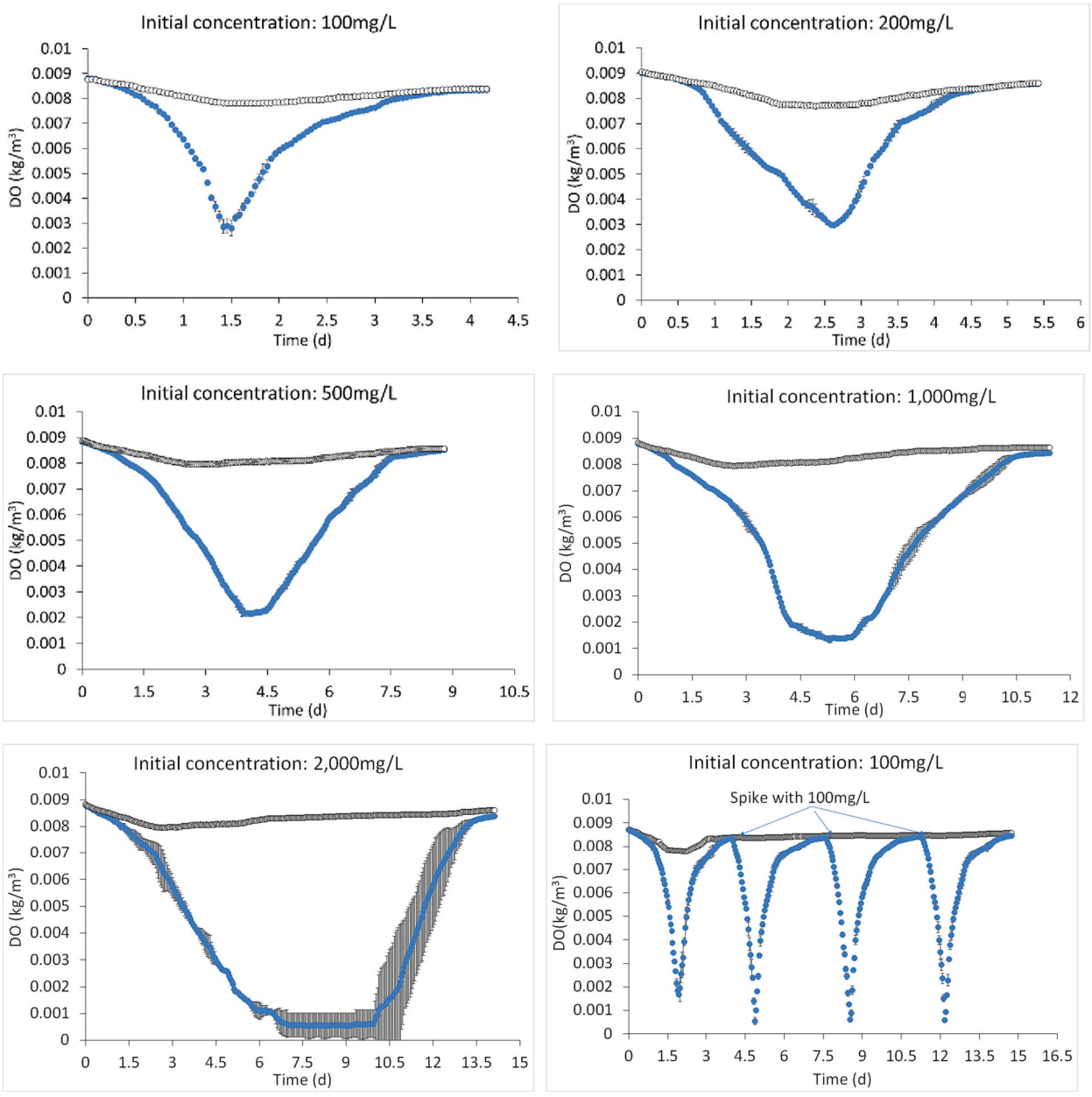


Fig. 2. Paracetamol: experimental data of dissolved oxygen (DO) vs time. Blue points: experiments with the substrate; white points: control experiments. The error bars indicate the standard deviation of the two duplicate experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reach their maximum growth rate μ_{max}. In Model C, τ was independent on the initial substrate concentration, while in Model D it was dependent on it (C_0, expressed in kg/m^3) according to the empirical equation $\tau = \tau_0 C_0^{\alpha}$. Therefore, in Model D the acclimation term took the form: $\left(1 - e^{-\frac{t}{\tau_0 C_0^{\alpha}}}\right)$. In all models, a term to account for oxygen limitation was added; this was important to simulate the experiments where very low oxygen concentration was reached. The oxygen limitation term took the form of: $\frac{C_{O2}}{K_{O2}+C_{O2}}$ where C_{O2} is the dissolved oxygen concentration, and K_{O2} is an empirical parameter. The variable μ was expressed, therefore by the following equations in models A-D:

$$\text{Model A: } \mu = \frac{\mu_{max} S}{K_S + S} \frac{C_{O2}}{K_{O2} + C_{O2}} \tag{4}$$

$$\text{Model B: } \mu = \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_I}} \frac{C_{O2}}{K_{O2} + C_{O2}} \tag{5}$$

$$\text{Model C: } \mu = \frac{\mu_{max}\left(1 - e^{-\frac{t}{\tau}}\right) S}{K_S + S} \frac{C_{O2}}{K_{O2} + C_{O2}} \tag{6}$$

$$\text{Model D: } \mu = \frac{\mu_{max}\left(1 - e^{-\frac{t}{\tau_0 C_0^{\alpha}}}\right) S}{K_S + S} \frac{C_{O2}}{K_{O2} + C_{O2}} \tag{7}$$

The parameters of models A-D were adjusted to fit the experimental data. In the parameter fitting the following approach was followed. The parameters K_S and K_{O2} were held fixed in all cases to the values of 0.005 kgCOD/m^3 and 0.0002 kg/m^3 respectively. These values are within the typical ranges reported in the literature for these parameters, which have relatively minimum effect on the model results and therefore could

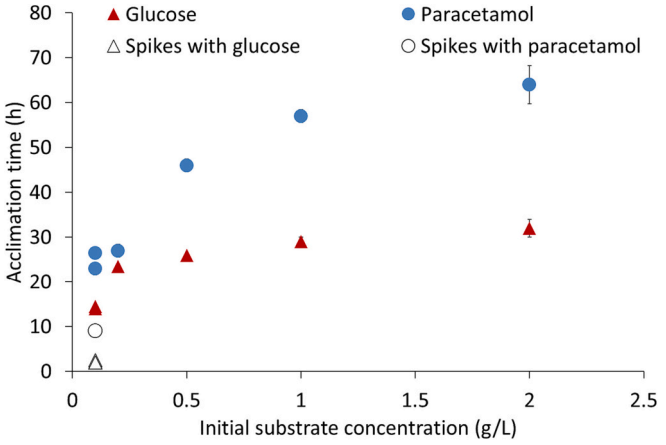


Fig. 3. Effect of initial glucose and paracetamol concentration on the acclimation time. For the experiments with multiple substrate spikes, each data point represents the acclimation time for each spike. Data points overlap when the acclimation time is the same.

not be reliably estimated based on the experimental data. The kinetic parameters that were adjusted to fit the experimental data were: μ_{max}, $Y_{X/S}$ and b for all models, K_I (for Model B), τ (for Model C), τ_0 and α (for Model D). In addition, the initial concentration of microorganisms able to metabolise the substrate, X_0, was also a parameter adjusted to fit the experimental data. Initially, each experiment was modelled individually with Model A, obtaining in different general parameters in each case. Then a combined fitting of all experiments for the same substrate with the same set of parameters was attempted with models A-D. For the individual fitting, the average values of the parameters and the upper/lower confidence intervals were calculated using Microsoft Excel.

The rate Eqs. (4)–(7) were used in the mass balances of biomass, substrate and dissolved oxygen Eqs. (8)–(10):

$$\text{Biomass balance: } \frac{dX}{dt}\left(\frac{kg}{m^3 \cdot d}\right) = r_X + r_{end} \tag{8}$$

$$\text{Substrate balance: } \frac{dS}{dt}\left(\frac{kgCOD}{m^3 \cdot d}\right) = r_S \tag{9}$$

$$\text{Oxygen balance: } \frac{dC_{O2}}{dt}\left(\frac{kg}{m^3 \cdot d}\right) = 1.42(r_X + r_{end}) + r_S + k_L\alpha\left(C^*_{O2} - C_{O2}\right) \tag{10}$$

$C^*_{O2}$is the saturation concentration of dissolved oxygen, and C_{O2} is the concentration of oxygen under working conditions. k_La is the mass transfer coefficient, measured under the experimental conditions as described in Section 2.3. C^*_{O2} was assumed to be equal to the dissolved oxygen concentration measured in the control experiments and was therefore time dependent.

Model simulations and parameter fitting were done by solving Eqs. (8)–(10) and by finding the parameter values that minimised the objective function $\sum(C_{O2exp} - C_{O2sim})^2$ were C_{O2exp} and C_{O2sim} represent the experimental and simulated, respectively, oxygen concentrations at the same time interval during the experiments. The simulated oxygen concentration was obtained as solution of the differential Eq. (10). The $\sum$ symbol indicates that the objective function was obtained as the sum considering all experimental data points with a given substrate. The system of Eqs. (8)–(10) was solved numerically in Microsoft Excel using the finite difference method with a Δt (time interval) of 0.0001 d. Parameter optimisation was done with the Excel add in Solver using the GRG nonlinear optimisation method.

3. Results and discussion

3.1. Experimental study

Figs. 1 and 2 show the dissolved oxygen profiles in experiments with glucose and paracetamol at different initial concentrations. In all cases, biodegradation of the substrate was observed, as indicated by the decrease in oxygen concentration below the levels observed for the control. This indicates that the decrease in oxygen concentration resulted from the microbial metabolic activity due to the substrate added and not to endogenous metabolism or to the consumption of other carbon sources that might have been present in the inoculum.

The point at which oxygen concentration starts increasing again indicates a much slower oxygen consumption by the microorganisms, which is usually associated with the complete removal of the primary carbon source. The profiles for glucose and paracetamol are

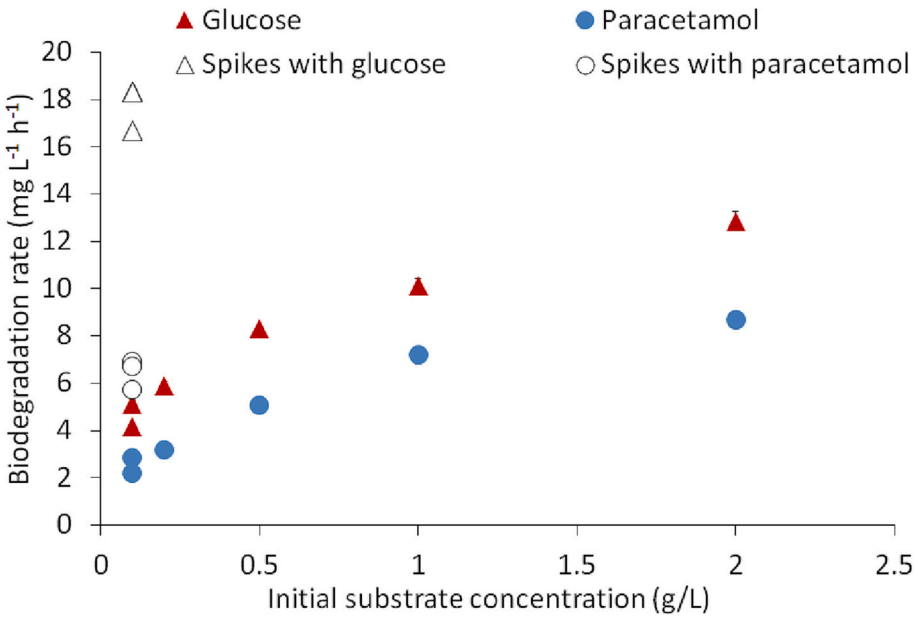


Fig. 4. Effect of initial glucose and paracetamol concentration on the biodegradation rate. For the experiments with multiple substrate spikes, each data point represents the biodegradation rate for each spike. Data points overlap when the rate is the same.

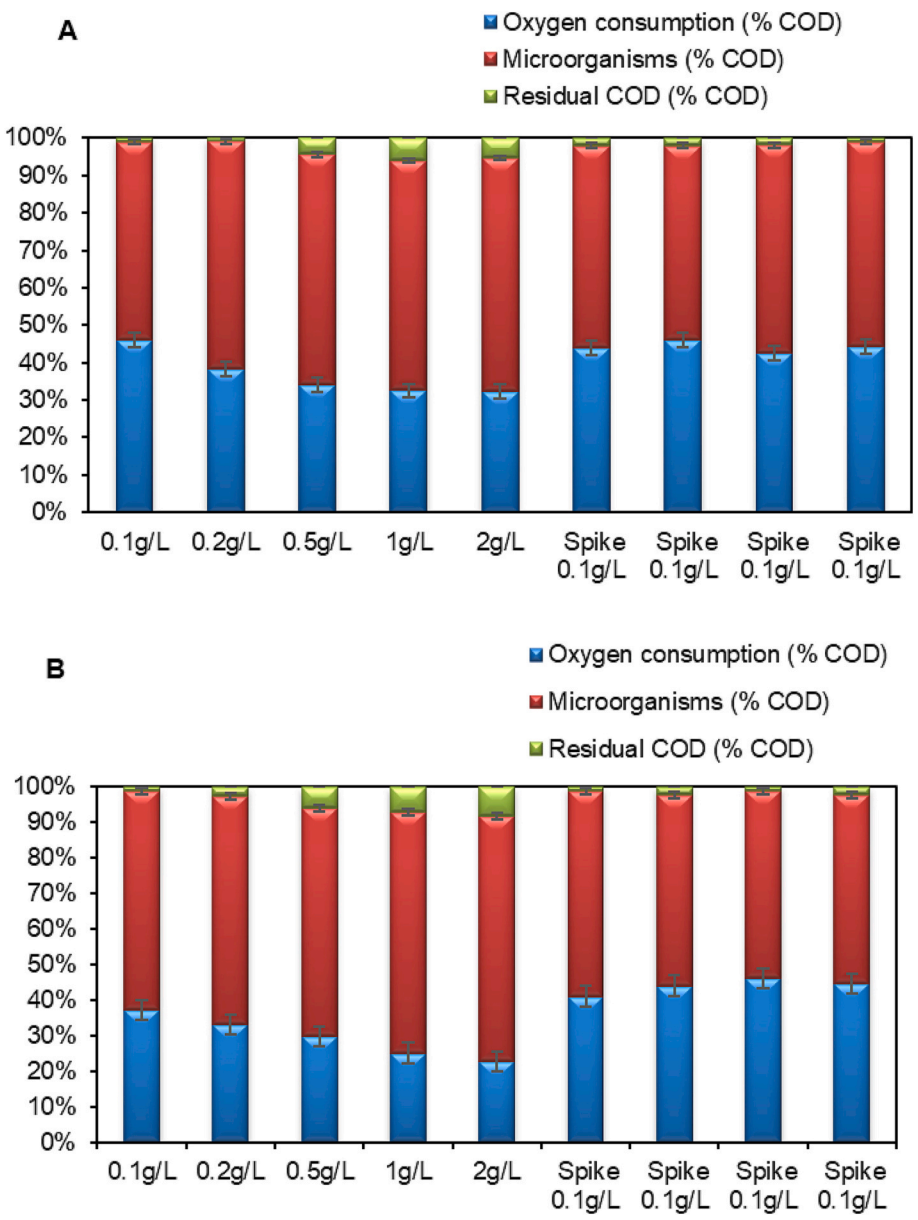


Fig. 5. COD balance: Oxygen consumption, amount of microorganisms produced and residual COD of various concentrations of glucose (A) and paracetamol (B).

qualitatively similar. However, for paracetamol, oxygen drops less than for glucose. For example, in the experiment with 100 mg/L of carbon source, the oxygen concentration drops to a minimum of 0.0 and to 2.2 mg/L for glucose and paracetamol, respectively. The lower drop in oxygen concentration for paracetamol indicates a slower oxygen consumption rate with this substrate. The slower oxygen consumption rate with paracetamol indicates that the microorganisms used in this study biodegraded paracetamol more slowly than glucose. This was quantified later in this study by measuring the substrate removal rate (below in this section) and the maximum specific growth rate (Section 3.2 Mathematical model). The experiments with multiple spikes were carried out to investigate the biodegradation of the substrates after microorganisms had already completely removed the first addition and were therefore acclimated. These experiments indicated a faster removal rate for both substrates than under the initial conditions. This indicates complete acclimation of the microorganisms to the carbon source after the first substrate addition and that, once microorganisms are acclimated to these substrates, they are able to remove them without any lag phase. For both glucose and paracetamol, the oxygen concentration remained at low level for longer times in the experiments at higher initial concentrations. This was due to the longer time required for microorganisms to completely remove higher initial concentrations of the substrates.

Fig. 3 shows the acclimation time for both substrates as a function of the initial concentration. The acclimation time is always longer for paracetamol than for glucose. For both substrates, the acclimation time increases as the initial substrate concentration increases; however, the effect of the substrate concentration is more evident for paracetamol than for glucose. Indeed, for glucose, there is only a modest increase in the acclimation time in the 200–2000 mg/L concentration range. Note that the definition used in this study for the acclimation time is based on the initial drop in oxygen concentration, which is not expected to be affected by increasing substrate concentrations unless higher substrate concentrations become inhibiting or affect the rate at which microorganisms acclimate to the substrate. Fig. 3 also shows that the acclimation time is greatly reduced after the first substrate addition in the experiments with multiple substrate spikes at the same 100 mg/L concentration.

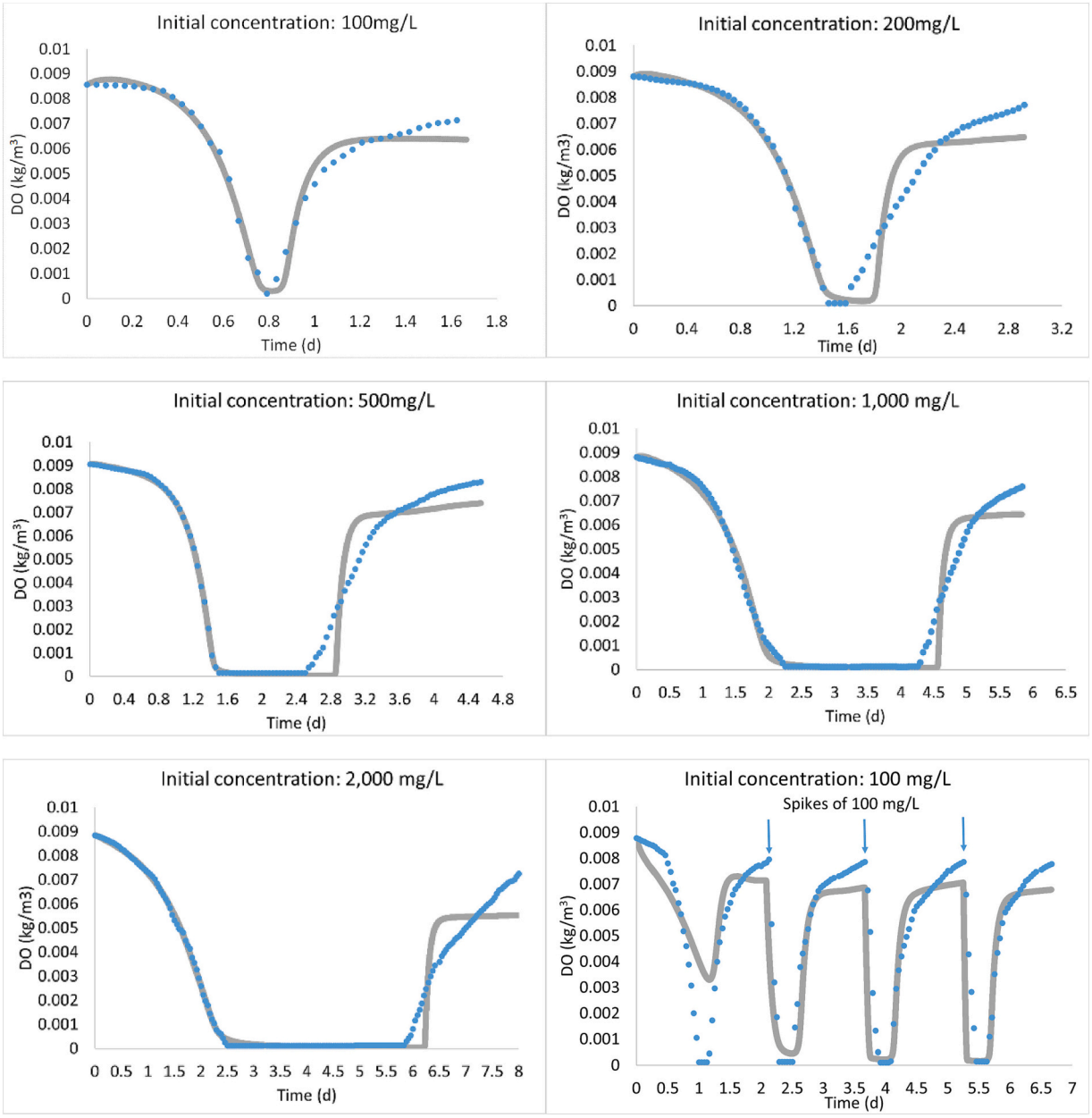


Fig. 6. Experimental data and model simulations for glucose. Individual fitting of each experiment. Comparison between experimental data (blue points) and model simulation (grey line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4 shows the biodegradation rate of glucose and paracetamol. As expected, the biodegradation rate for both substrates increased with the substrate concentration. The biodegradation rate was higher for glucose than for paracetamol. For both glucose and paracetamol, the biodegradation rate increased considerably after multiple spikes of the substrate.

Fig. 5 shows the COD balance, i.e., the fraction of the initial COD of the substrates glucose (A) and paracetamol (B) that ended up as oxygen consumed, microorganisms and residual COD in the liquid phase by the end of the experiments. Fig. 5 shows almost complete removal of the COD for both carbon sources in all experiments. For the highest tested concentrations (1000 and 2000 mg/L), the COD removal was slightly lower than for the lower concentrations, but it was still higher than 90 %. The contribution of oxygen consumption to the COD balance was slightly lower for paracetamol than for glucose. In general, the contribution of oxygen consumption tended to decrease as the initial concentration of the substrate increased. The largest contribution to the COD balance was the production of microorganisms, for both glucose and paracetamol.

To the best of our knowledge, most of the studies published on paracetamol degradation focused on pure strains in batch reactors. Apart from our earlier study [7], there is no evidence in the literature that paracetamol can be degraded by open mixed culture as a sole carbon source. In addition, there is virtually no evidence in the literature about which fraction of the biodegraded paracetamol is used for microbial growth, as we investigated in this study. Zhang et al. [22] stated that three pure bacterial cultures (*Stenotrophomonas sp* and two belonging to *Pseudomonas sp*) were able to degrade paracetamol as sole carbon, energy and nitrogen source with higher removal of paracetamol by the consortium of the three cultures than by the individual strains. For an initial paracetamol concentration of 400 mg/L, the consortium achieved a paracetamol removal (measured as TOC, total organic carbon) of 97 %, vs the 65, 85 and 86 % removal of the three individual strains. The consortium also performed better than the individual strains in removing higher concentrations of paracetamol, up to 4000 mg/L.

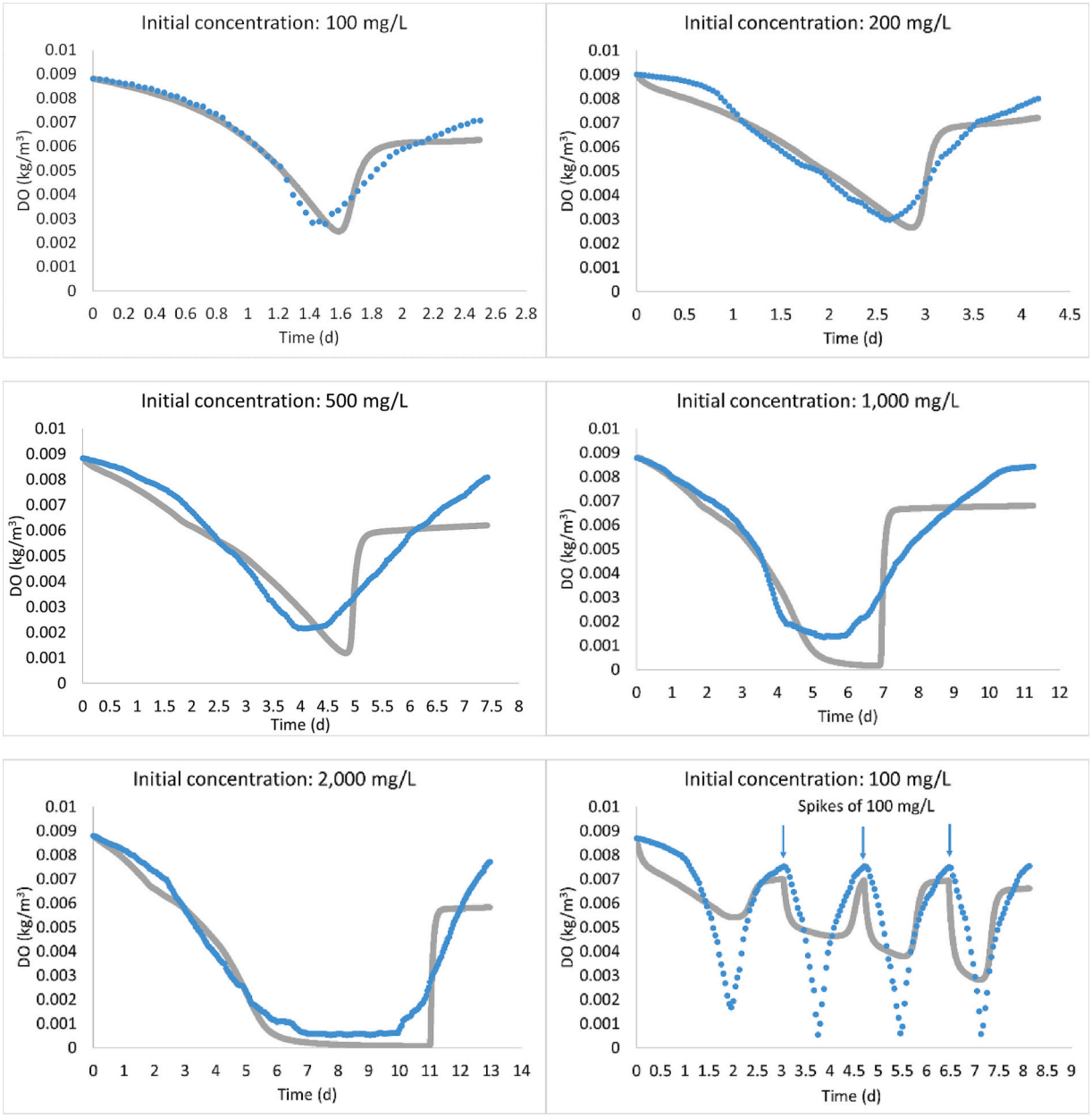


Fig. 7. Experimental data and model simulations for paracetamol. Individual fitting of each experiment. Comparison between experimental data (blue points) and model simulation (grey line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Kinetic parameters and objective functions for the Monod model (model A) with individual fitting for each experiment. Units: μ_{max} and b d^{-1}; $Y_{X/S}$ kg/kg_{COD}; X_0, substrate and oxygen concentration kg/m^3. (Obj Func = Objective function).

		Glucose					Paracetamol				
		μ_{max}	$Y_{X/S}$	b	X_0	Obj Func	μ_{max}	$Y_{X/S}$	b	X_0	Obj Func
Init. subs. Conc.	100	7.23	0.48	0.41	$3.1{\cdot}10^{-4}$	$6.47{\cdot}10^{-6}$	2.23	0.55	0.16	$4.4{\cdot}10^{-3}$	$1.03{\cdot}10^{-5}$
	200	3.69	0.46	0.15	$4.4{\cdot}10^{-4}$	$9.21{\cdot}10^{-5}$	0.95	0.55	0.04	$1.7{\cdot}10^{-2}$	$2.73{\cdot}10^{-5}$
	500	4.96	0.48	0.034	$5.1{\cdot}10^{-5}$	$5.21{\cdot}10^{-5}$	0.68	0.60	0.045	$2.9{\cdot}10^{-3}$	$1.35{\cdot}10^{-4}$
	1000	2.15	0.50	0.038	$1.8{\cdot}10^{-3}$	$4.51{\cdot}10^{-5}$	0.76	0.57	0.017	$1.3{\cdot}10^{-2}$	$3.29{\cdot}10^{-4}$
	2000	1.57	0.55	0.025	$6.3{\cdot}10^{-3}$	$5.99{\cdot}10^{-5}$	0.61	0.56	0.013	$2.2{\cdot}10^{-2}$	$1.91{\cdot}10^{-4}$
	Spike	1.94	0.40	0.14	$6.5{\cdot}10^{-3}$	$2.72{\cdot}10^{-4}$	0.37	0.55	0.045	$8.9{\cdot}10^{-2}$	$4.08{\cdot}10^{-4}$
Total objective function						$5.28{\cdot}10^{-4}$					$1.10{\cdot}10^{-3}$
Average value of the parameters		3.59	0.48	0.13	0.0026		0.93	0.56	0.05	0.029	
Upper 90 % confidence value		5.24	0.52	0.24	0.005		1.43	0.58	0.094	0.052	
Lower 90 % confidence value		1.59	0.43	0.00	0.000		0.33	0.55	0.004	0.0013	

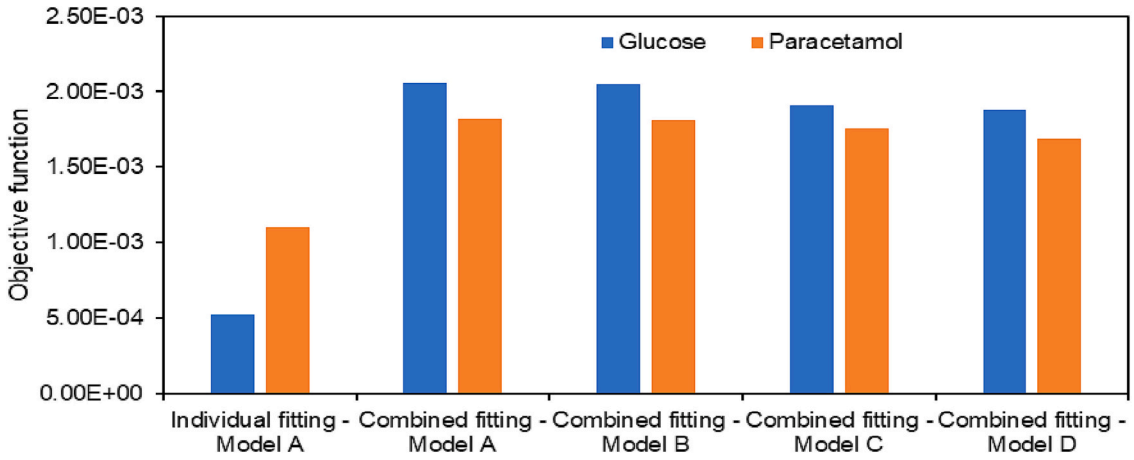


Fig. 8. Objective function for the different models and modelling approaches.

Overall, the extent of paracetamol biodegradation by the consortium of pure cultures by Zhang et al. [22] was in line with the paracetamol biodegradation observed in this study with open mixed cultures, which contributes to reinforcing the evidence of better biodegradation of recalcitrant compounds by mixed cultures than by pure cultures, as already observed by Zhang et al. themselves and by other authors [23–25]. Zur et al. [26] investigated paracetamol degradation by several strains isolated from activated sludge, obtaining the best results with *Pseudomonas moorei KB4* which removed up to 50 mg/L of paracetamol. In the same study, the authors also observed that the activated sludge was able to remove after acclimation up to 100 mg/L of paracetamol in 24 h, but observed no microbial growth on this substrate. While the paracetamol biodegradation rate observed by Zur et al. [26] with 100 mg/L was in the same order of magnitude of what we observed in this study, differently from the authors we showed the ability of open mixed cultures to grow on this substrate. Chopra and Kumar [13] isolated from activated sludge five strains able to metabolise paracetamol as sole carbon and energy source. All the isolated strains were able to remove paracetamol at initial concentrations 100–2000 mg/L, although usually not completely in the time length of their experiments (20 d). Similarly to the other studies cited above, Chopra and Kumar also observed better paracetamol removal by the microbial consortium than by the individual strains. In studies with open mixed cultures under conditions typical of biological wastewater treatment processes, high removal of paracetamol was observed in several studies [27–29], however in these studies paracetamol and other pharmaceuticals were only added at μg/L concentration while most of the COD consisted of biogenic carbon sources. Therefore, these literature studies, differently from our study, did not indicate whether paracetamol was being degraded for metabolism or co-metabolism.

Our study shows that the acclimation time for glucose is faster than for paracetamol. This can be explained considering that acclimation is affected by the substrate's chemical nature, physical state, and concentrations. To the best of our knowledge, there is no quantitative investigation reported in the literature about the effect of substrate concentration on the acclimation time. Our study indicates that higher substrate concentrations can delay the acclimation of the microorganisms and that this effect is more important for paracetamol than for glucose. In an earlier study by our group [30] we observed that for open mixed culture the nature of the substrates and their concentrations are more important in determining the acclimation time than the type of mixed culture (inoculum from soil vs anaerobic digester were compared) and measured longer acclimation time for C_{12}-C_{20} hydrocarbons than for more readily biodegradable substrates like glucose, ethanol and acetic acid. Comparing the acclimation times measured in this study with those in our earlier study, which were measured at a fixed substrate concentration (0.5 g/L), this study extends the range of substrates and concentrations investigated: for glucose, the acclimation time was 1 d in [30] while in this study it was 12 h for the lowest concentration (100 mg/L) and increased up to 28 h for the highest concentration (2 g/L); paracetamol was not investigated in [30], but the values measured in the present study (22–60 h) are not too different from those reported in that study for C_{12}-C_{20} hydrocarbons (2–4 days).

3.2. Mathematical model

The first step in the kinetic modelling of the experimental data was the individual fitting (i.e., with parameters optimised separately for each experiment) of each experiment with the Monod model (Model A). Figs. 6 and 7 show the comparison of the model and the experimental data for each experiment for glucose and paracetamol, respectively. Table 1 shows the optimum parameter values calculated for each experiment. Generally, the model is able to describe the experimental data well, although, especially for paracetamol, the fitting is less good for the experiments with multiple spikes. This is probably due to the higher complexity of the microbial response in these experiments compared with the experiments when the substrate was only fed once. Generally, in most cases, the fitting is worse in the final part of the experiments when the substrate is completely removed and the oxygen concentration increases. In this final part of the experiments, the model generally predicts a faster increase in oxygen concentration than it is experimentally observed. This may be due to the difficulty in simulating the complex phenomenon of endogenous metabolism, which is responsible for oxygen consumption when the carbon source is completely removed. Table 1 shows that the optimum values of the model parameters were different for each experiment. Generally, the μ_{max} values were higher for glucose than for paracetamol. The average μ_{max} value was 3.59 d^{-1} for glucose and 0.93 d^{-1} for paracetamol. Although the standard deviation of these values is considerable, these values are different with a degree of confidence of at least 90 %. The average $Y_{X/S}$ value was higher for paracetamol (0.56 kg/kg_{COD}) than for glucose (0.48 kg/kg_{COD}) and these values were different with a degree of confidence of 90 %. The values of b and X_0 were very variable for the different experiments and their average values for glucose and paracetamol were not found to be significantly different.

The second step in the kinetic modelling was the attempt to combined modelling, i.e., all the experiments for each substrate with the same set of parameters. This was attempted with various versions of the Monod model: without modifications (Model A), modified for inhibition (Model B), for acclimation (Model C) and for acclimation dependent on the initial substrate concentration (Model D). Fig. 8 shows the objective function for the different models for combined fitting, compared with

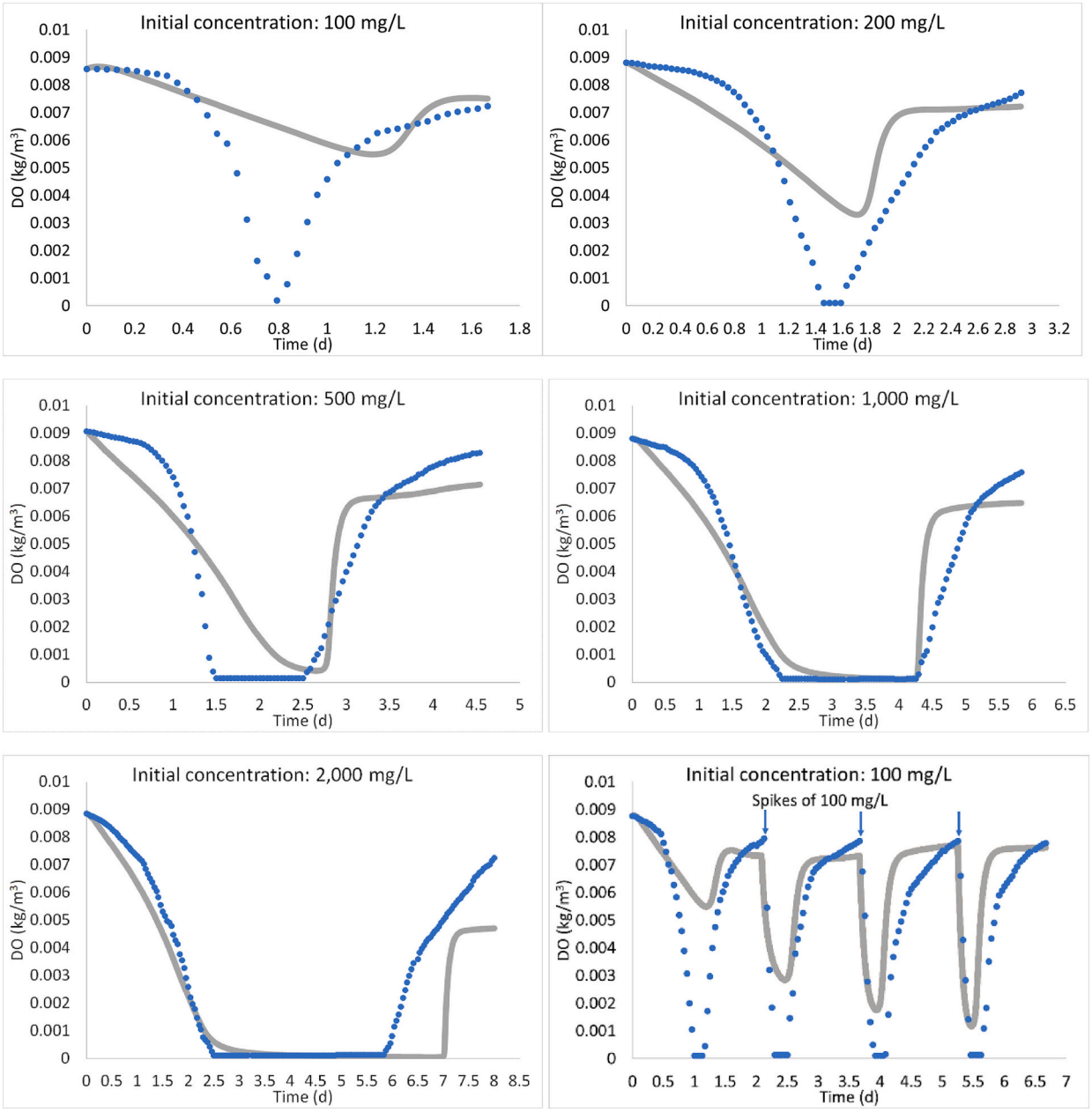


Fig. 9. Combined modelling with Model D for glucose. Comparison between experimental data (blue points) and model simulation (grey line). Best fit parameters in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the individual fitting with the Monod model. Overall, in all cases, the fitting was considerably worse than for the individual fitting with the simple Monod model. This indicates that probably the experiments were affected by daily variations in biomass response, which could be better accounted for by using a simple model with different values of the kinetic parameters, rather than by a more complex model with the same set of parameters. Model D gave a marginally better combined fitting than the other models. The comparison of experimental data and model simulations for Model D is shown in Fig. 9 and 10, and the parameters that gave the best fitting are reported in Table 2. Generally, for both glucose and paracetamol, the experiments with initial concentrations 200, 500, 1000 and 2000 mg/L are described quite well, while there are major discrepancies in the model description of the experiments with 100 mg/L with only addition and with multiple spikes. As stated above, these discrepancies between the model and the experimental data are probably due to daily variations in the microbial response and in the more complex response when microorganisms are subject to multiple substrate spikes. Considering the parameters of Model D which gave the best fitting, shown in Table 2, it is confirmed that the removal of paracetamol is characterised by lower μ_{max} (0.43 vs 0.99 d^{-1}) and slightly higher $Y_{X/S}$ (0.57 vs 0.53 kg/kg_{COD}) than the removal of glucose, as observed earlier for the simple Monod model with individual fitting. As expected, the best-fit values of μ_{max} are different, for both glucose and paracetamol, in the model with acclimation compared with the simple Monod model used in the individual fitting, however, what matters more is the confirmation of the general evidence of a significantly lower μ_{max} for paracetamol than for glucose. It is also noteworthy that, according to the best-fit parameter values in Model D, the effect of the initial concentration of the substrate on the acclimation time is more important for paracetamol than for glucose. This is shown by the higher value for the parameter α for paracetamol (0.31) than for glucose (0.14) and qualitatively matches the trends of the acclimation time observed in

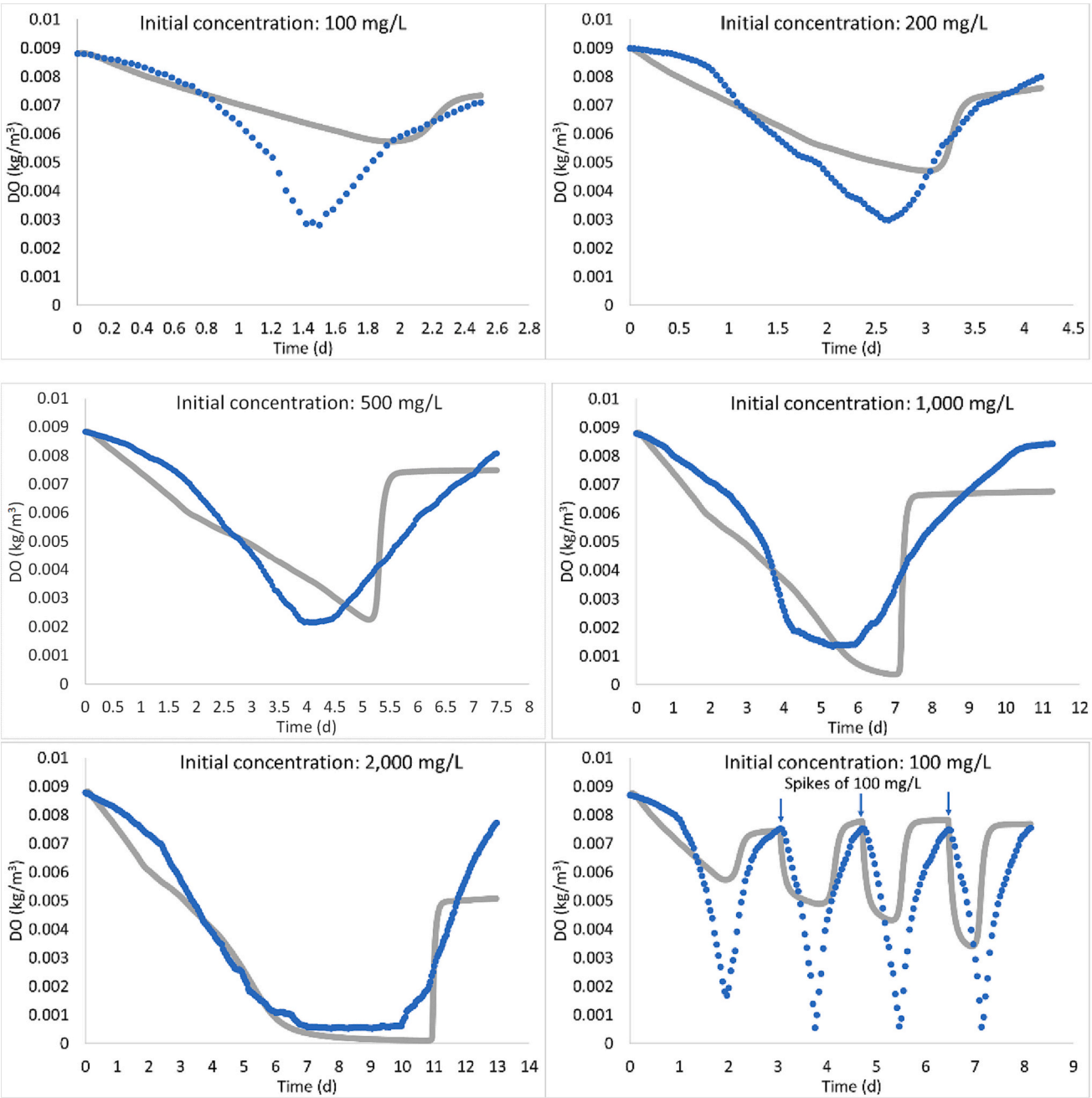


Fig. 10. Combined modelling with Model D for paracetamol. Comparison between experimental data (blue points) and model simulation (grey line). Best fit parameters in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Kinetic parameters obtained in the combined fitting with model D. Same units as in Table 1 and τ_0 d^{-1}, α adimensional.

Substrate	μ_{max}	$Y_{X/S}$	b	X_0	τ_0	α	Obj. func.
Glucose	0.99	0.53	0.032	0.071	0.98	0.14	1.88×10^{-3}
Paracetamol	0.43	0.57	0.016	0.11	0.89	0.31	1.69×10^{-3}

Fig. 3.

In the literature, while there are many studies on the kinetic parameters of microbial growth for glucose or other readily biodegradable substrates for pure or mixed cultures, to the best of our knowledge there are only very few published studies on the kinetic parameters for paracetamol and they usually refer to pure cultures [13,14]. Table 3 lists

Table 3
Selected values of the kinetic parameters μ_{max} and $Y_{X/S}$ in literature studies with open mixed cultures compared with the values found in this study. [a] individual fitting with simple Monod model; [b] combined fitting with Model D.

Substrate	μ_{max} (d^{-1})	$Y_{X/S}$ (kg/kgCOD)	References
Glucose	5.5–23	0.52–0.66	[31]
Glucose	6.2	–	[32]
Glucose	1.57–7.23	0.48–0.55	This study[a]
Glucose	0.99	0.53	This study[b]
Paracetamol	–	0.51	[7]
Paracetamol	0.37–2.23	0.55–0.60	This study[a]
Paracetamol	0.43	0.57	This study[b]

the μ_{max} and $Y_{X/S}$ values from selected literature studies with open mixed cultures. For glucose, the literature values for μ_{max} vary in a wide range, depending on the type of culture and on the process conditions, while the $Y_{X/S}$ values are generally less variable. In general the values for μ_{max} calculated in this study for glucose are within the ranges reported in the literature, although at the lower end. The values for $Y_{X/S}$ are within the literature range. To the best of our knowledge, there is no published study reporting the maximum growth rate on paracetamol for open mixed cultures. The only literature report of $Y_{X/S}$ values for paracetamol is the earlier study by this research group, where a $Y_{X/S}$ value of 0.51 kg/kgCOD, in good agreement with this study, was calculated from SBR studies.

4. Conclusions

Paracetamol can be metabolised by open mixed cultures as sole carbon and energy source, at all concentrations investigated in this study (100–2000 mg/L). The metabolization of paracetamol was almost complete in all cases, with over 90 % removal of the soluble COD in all cases and, based on the COD balance, conversion to microorganisms was the main removal mechanism. However, paracetamol biodegradation was slower and with a longer acclimation time than glucose biodegradation by the same microorganisms. The maximum growth rate was lower for paracetamol than for glucose while the growth yield for paracetamol was slightly higher.

Overall, we believe that the results in this paper contribute to a better and more quantitative understanding of biodegradation of xenobiotics in biological wastewater treatment processes. After due verification with other systems, substrates and microorganisms, the mathematical model developed in this study can be incorporated in existing models of biological wastewater treatment processes, leading to an improved design and to performance optimisation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] T. Stefanac, D. Grgas, T. Landeka Dragicevic, Xenobiotics—division and methods of detection: a review, J. Xenob. 11 (4) (2021) 130–141, https://doi.org/10.3390/jox11040009.

[2] J. Zur, A. Pinski, A. Marchlewicz, K. Hupert-Kocurek, D. Wojcieszyńska, U. Guzik, Organic micropollutants paracetamol and ibuprofen—toxicity, biodegradation, and genetic background of their utilization by bacteria, Environ. Sci. Pollut. Res. 25 (2018) 21498–21524, https://doi.org/10.1007/s11356-018-2517-x.

[3] O. Rozas, C. Vidal, C. Baeza, W.F. Jardim, A. Rossner, H.D. Mansilla, Organic micropollutants (OMPs) in natural waters: oxidation by UV/H2O2 treatment and toxicity assessment, Water Res. 98 (2016) 109–118, https://doi.org/10.1016/j.watres.2016.03.069.

[4] D. Dionisi, Potential and limits of biodegradation processes for the removal of organic xenobiotics from wastewaters, ChemBioEng Rev. 1 (2) (2014) 67–82, https://doi.org/10.1002/cben.201300008.

[5] D.T. Marghade, A.D. Chahande, M.S. Tiwari, P.D. Patil, Microbial Degradation of Xenobiotic Compounds, Recent Advances in Microbial Degradation, Springer Singapore, 2021, pp. 173–217, https://doi.org/10.1007/978-981-16-0518-5_7.

[6] C. Seignez, V. Mottier, C. Pulgarin, N. Adler, P. Peringer, Biodegradation of xenobiotics in a fixed bed reactor, Environ. Prog. 12 (4) (1993) 306–311, https://doi.org/10.1002/ep.670120412.

[7] D. Dionisi, C.C. Etteh, Effect of process conditions on the aerobic biodegradation of phenol and paracetamol by open mixed microbial cultures, J. Environ. Chem. Eng. 7 (5) (2019), 103282, https://doi.org/10.1016/j.jece.2019.103282.

[8] M.C. Tomei, M.C. Annesini, Biodegradation of phenolic mixtures in a sequencing batch reactor, Environ. Sci. Pollut. Res. 15 (3) (2008) 188–195, https://doi.org/10.1065/espr2007.12.470.

[9] A. Joss, S. Zabczynski, A. Gobel, B. Hoffmann, D. Loffler, C.S. McArdell, T. A. Ternes, A. Thomsen, H. Siegrist, Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme, Water Res. 40 (8) (2006) 1686–1696, https://doi.org/10.1016/j.watres.2006.02.014.

[10] M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants, Water Res. 39 (19) (2005) 4797–4807, https://doi.org/10.1016/j.watres.2005.09.015.

[11] T. Alvarino, S. Suarez, J.M. Lema, F. Omil, Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors, J. Hazard. Mater. 278 (2014) 506–513, https://doi.org/10.1016/j.jhazmat.2014.06.031.

[12] G.J. Stanlake, R.K. Finn, Isolation and characterisation of a pentachlorophenol-degrading bacterium, Appl. Environ. Microbiol. 44 (6) (1982) 1421–1427, https://doi.org/10.1128/aem.44.6.1421-1427.1982.

[13] S. Chopra, D. Kumar, Biodegradation and kinetic analysis of acetaminophen with co-culture of bacterial strains isolated from sewage wastewater, Curr. Microbiol. 77 (10) (2020) 3147–3157, https://doi.org/10.1007/s00284-020-02137-6.

[14] S. Chopra, D. Kumar, Characterisation, optimisation and kinetics study of acetaminophen degradation by *bacillus drentensis* strain S1 and waste water degradation analysis, Bioresour. Bioprocess. 7 (1) (2020) 1–18, https://doi.org/10.1186/s40643-020-0297-x.

[15] M.C. Tomei, M.C. Annesini, Biodegradation of phenolic mixtures in a sequencing batch reactor, Environ. Sci. Pollut. Res. 15 (3) (2008) 188–195, https://doi.org/10.1065/espr2007.12.470.

[16] D. Dionisi, A.A. Rasheed, Maximisation of the organic load rate and minimisation of oxygen consumption in aerobic biological wastewater treatment processes by manipulation of the hydraulic and solids residence time, J. Water Process. Eng. 22 (2018) 138–146, https://doi.org/10.1016/j.jwpe.2018.02.002.

[17] A.K. Bartram, X. Jiang, M.D. Lynch, A.P. Masella, G.W. Nicol, J. Dushoff, J. D. Neufeld, Exploring links between pH and bacterial community composition in soils from the Craibstone experimental farm, FEMS Microbiol. Ecol. 87 (2) (2014) 403–415, https://doi.org/10.1111/1574-6941.12231.

[18] D. Dionisi, Biological wastewater treatment processes: mass and heat balances, CRC press, Boca Raton FL USA, 2017. https://doi.org/10.1201/9781315163345.

[19] A. Benedek, W.J. Heideger, Effect of additives on mass transfer in turbine aeration, Biotechnol. Bioeng. 13 (5) (1971) 663–684, https://doi.org/10.1002/bit.260130507.

[20] J. Suschka, E. Ferreira, Activated sludge respirometric measurements, Water Res. 20 (2) (1986) 137–144, https://doi.org/10.1016/0043-1354(86)90003-5.

[21] R. Sathya, M. Rasi, L. Rajendran, Nonlinear analysis of Haldane kinetic model in phenol degradation in batch operations, Kinet. Catal. 56 (2015) 141–146, https://doi.org/10.1134/S0023158415020111.

[22] L. Zhang, J. Hu, R. Zhu, Q. Zhou, J. Chen, Degradation of paracetamol by pure bacterial cultures and their microbial consortium, Appl. Microbiol. Biotechnol. 97 (8) (2013) 3687–3698, https://doi.org/10.1007/s00253-012-4170-5.

[23] Y.M. Kim, C.K. Ahn, S.H. Woo, G.Y. Jung, J.M. Park, Synergic degradation of phenanthrene by consortia of newly isolated bacterial strains, J. Biotechnol. 144 (2009) 293–298, https://doi.org/10.1016/j.jbiotec.2009.09.021.

[24] T. Joshi, L. Iyenga, K. Singh, S., Garg isolation, identification and application of novel bacterial consortium TJ-1 for the decolourisation of structurally different azo dyes, Bioresour. Technol. 99 (2008) 7115–7121, https://doi.org/10.1016/j.biortech.2007.12.074.

[25] M.G. Carvalho, C.C.T. Alves, M.I.M. Ferreira, P. De Marco, P.M.L. Castro, Isolation and initial characterisation of a bacterial consortium able to mineralise fluorobenzene, Appl. Environ. Microbiol. 68 (1) (2002) 102–105, https://doi.org/10.1128/AEM.68.1.102-105.2002.

[26] J. Zur, D. Wojcieszyńska, K. Hupert-Kocurek, A. Marchlewicz, U. Guzik, Paracetamol–toxicity and microbial utilisation. Pseudomonas moorei KB4 as a case study for exploring degradation pathway, Chemosph. 206 (2018) 192–202, https://doi.org/10.1016/j.chemosphere.2018.04.179.

[27] H. Fan, J. Li, L. Zhang, L. Feng, Contribution of sludge adsorption and biodegradation to the removal of five pharmaceuticals in a submerged membrane bioreactor, Biochem. Eng. J. 88 (2014) 101–107, https://doi.org/10.1016/j.bej.2014.04.008.

[28] J.L. Tambosi, R.F. de Sena, M. Favier, W. Gebhardt, H.J. José, H.F. Schröder, R.D.F. P.M. Moreira, Removal of pharmaceutical compounds in membrane bioreactors (MBR) applying submerged membranes, Desal. 261 (1–2) (2010) 148–156, https://doi.org/10.1016/j.desal.2010.05.014.

[29] F.P. Shariati, M.R. Mehrnia, B.M. Salmasi, M. Heran, C. Wisniewski, M. H. Sarrafzadeh, Membrane bioreactor for treatment of pharmaceutical wastewater containing acetaminophen, Desal. 250 (2) (2010) 798–800, https://doi.org/10.1016/j.desal.2008.11.044.

[30] C. Uzukwu, D. Dionisi, Comparison of the biodegradation of n-alkanes and readily biodegradable substrates using open mixed culture under aerobic, anoxic and anaerobic conditions, Int. J. Environ. Bioremediat. Biodegrad. (2017) 65–76, https://doi.org/10.12691/ijebb-5-2-5.

[31] S.Y. Chiu, L.T. Fan, I.C. Kao, L.E. Erickson, Kinetic behavior of mixed populations of activated sludge, Biotechnol. Bioeng. 14 (2) (1972) 179–199, https://doi.org/10.1002/bit.260140204.

[32] L. Nunez, N. Barros, I. Barja, A kinetic analysis of the degradation of glucose by soil microorganisms studied by microcalorimetry, Thermochim. Acta 237 (1) (1994) 73–81, https://doi.org/10.1016/0040-6031(94)85185-9.