Propionate addition enhances the biodegradation of the xenobiotic herbicide propanil and its metabolite

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HIGHLIGHTS

► Enhanced degradation rates of propanil and DCA achieved with propionate addition.
► Co-metabolism of the xenobiotics with propionate was not a significant mechanism.
► Metabolic model was developed for biodegradation of the herbicide and its metabolite.
► Higher metabolic efficiency of the culture led to the stimulated degradation rates.

ABSTRACT

This study investigated ways of stimulating the biodegradation rates of the commonly applied herbicide, 3,4-dichloropropionanilide (propanil), and its metabolite, 3,4-dichloroaniline (DCA), as well as the growth rate of propanil- and DCA-degrading organisms in a mixed culture. Propionate, the other metabolite of propanil, stimulated the specific degradation rates of both propanil and DCA after a brief acclimation period. A metabolic model developed to characterise the metabolism of propanil and DCA biodegradation showed that the efficiency of oxidative phosphorylation (i.e. P/O ratio), which measures the metabolic efficiency, increased over time by 6- to 10-fold. This increase was accompanied by a 5- to 10-fold increase in the propanil and DCA biodegradation degradation rates. The biodegradation rates of the culture were unaffected when using an irrigation water matrix (Tejo river, Portugal), highlighting the utility of the culture for bioaugmentation purposes.

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1. Introduction

3,4-Dichloropropionanilide (propanil) is a herbicide that is applied worldwide in rice paddies. Propanil is primarily transformed in nature into propionate (readily biodegradable), and 3,4-dichloroaniline (DCA), which is very difficult to biodegrade. Both propanil and DCA can attack the nervous and immune systems and cause methemoglobinemia (Salazar et al., 2008). Therefore, these compounds should be removed from irrigation waters to avoid contaminating aquatic systems, soils and drinking water supplies. Propanil and DCA have been found in irrigation waters at levels that were several orders of magnitude above the maximum allowable discharge limit for pesticides of $0.1\ \mu g\ L^{-1}$ (Primel et al., 2007; Pesticides Framework Directive, 2009/128/EC).

In an earlier study, a suitable mixed culture for the degradation of both propanil and DCA in a sequencing batch reactor (SBR) was established for the purpose of treating irrigation waters through bioaugmentation (Carvalho et al., 2010). When a single feed at high initial propanil concentrations was replaced by step-feeding at lower initial propanil concentrations, the rate of propanil and DCA biodegradation increased after an acclimatisation period and DCA inhibition was not observed.

Despite the faster kinetics achieved with step-feeding, the overall removal of propanil and DCA by the culture was still limited by a very low biomass growth rate. This implies that a very long enrichment phase would be needed in order to cultivate the biomass and use it for bioaugmentation if propanil was used as the only carbon source. For this reason, in the present study, supplementary propionate was added, since the culture had already been demonstrated to degrade this readily biodegradable substrate generated through the primary degradation of propanil (Carvalho et al., 2010). Furthermore, the effect of propionate on the metabolism of the enriched

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culture was investigated through a metabolic model that was developed based on the theoretical biochemical pathways involved in propanil and DCA biodegradation. The model allows comparison of the metabolic efficiency (i.e. changes in the efficiency of oxidative phosphorylation, or P/O ratio) of the culture with and without propionate addition in order to better understand the metabolism of the culture during the acclimatisation period. To the best of our knowledge, this is the first report where a metabolic model for xenobiotic biodegradation has been developed and applied. Finally, the kinetic rates achieved by the culture in mineral media were compared to those obtained using an irrigation water matrix from the Tejo river (Portugal).

2. Methods

2.1. Bioreactor operation

A sequencing batch reactor (SBR) with a working volume of 0.5 L was operated with a step-feeding strategy as detailed by Carvalho et al. (2010), with an initial concentration of 0.15 mM propanil in the reactor, which was fed twice per day. The inoculum of the SBR was the mixed microbial culture obtained from the study of Carvalho et al. (2010), fed with propanil as the sole carbon source. The mineral media used for enrichments and reactor feeding was similar to medium B described by Barreiros et al. (2003), and consisted of (mg L^{-1}): 1162 KH$_2$PO$_4$, 6609 Na$_2$HPO$_4$·12H$_2$O, 29.4 CaCl$_2$·2H$_2$O, 441.9 NaCl, 135.9 MgCl$_2$·6H$_2$O, 1.03 FeCl$_2$·4H$_2$O, 0.07 ZnCl$_2$, 0.1 MnCl$_2$·4H$_2$O, 0.062 H$_3$BO$_3$, 0.19 CoCl$_2$·6H$_2$O, 0.017 CuCl$_2$·2H$_2$O, 0.024 NiCl$_2$·6H$_2$O, 0.036 NaMoO$_4$·2H$_2$O, 528.4 (NH$_4$)$_2$-SO$_4$ and 1.3 μL of 25% HCl.

The 24-h SBR cycle consisted of an 11-h aerobic phase after the first feeding period, followed by a 12-h aerobic phase after the second feed (an initial concentration of 0.15 mM propanil in the reactor was present after both feeds) and finally a 1-h settling/decanting period (Carvalho et al., 2010). The hydraulic retention time was 48-h, and the sludge retention time was approximately infinite (i.e. no sludge was wasted except during sampling and on Days 0 and 170, which corresponded to the sudden decreases in biomass concentration shown in Fig. 1). The pH of the SBR was controlled at 7.2 ± 0.1 and the temperature was maintained at 28 ± 1 °C.

The SBR was operated in this configuration for 357 days before the addition of 1.5 mM of propionate (Day 0). This addition of propionate to each feeding phase (i.e. twice per day) continued for 170 d. On day 171, the addition of propionate was terminated and the culture was fed only with propanil and the mineral media described above until Day 267. A timeline of these key events and the batch tests performed in this study is illustrated in Fig. 1.

Previous abiotic control tests showed that neither the propanil nor the DCA were removed via photodegradation, adsorption to biomass, or volatilisation (Carvalho et al., 2010). Further, the accumulation of other metabolites was not observed, suggesting that propanil, DCA and propionate were completely biodegraded.

2.2. SBR monitoring and parallel batch tests

The SBR was monitored through a series of cycle studies, where propanil, DCA, propionate, and biomass concentration were sampled along the parent SBR cycle. On Day 29, propanil was fed at a level of 0.59 mM and propionate was fed at 5.9 mM once per day. Batch tests were carried out in several parallel, aerated Erlenmeyer flasks on Days 147, 221 and 267. On Day 147, the sludge was divided into four equal parts to test propanil concentrations of 0.075, 0.15, 0.30 and 0.59 mM, where the propionate concentration was 10 times the molar concentration of the propanil in each case. On Day 221, the sludge was split into four parts to test feeds of 0.15 mM propanil, 0.15 mM propanil + 1.5 mM propionate, 0.15 mM DCA and 0.15 mM DCA + 1.5 mM propionate. On Day 267, the sludge was divided into four parts to test the effect of water from the Tejo river (Portugal) with 0.15 mM propanil, 0.15 mM DCA, 0.15 mM linuron and 0.15 mM diuron. Linuron and diuron are two other herbicides where DCA is also a commonly reported metabolite, but they were not degraded after two weeks of exposure to the mixed culture. It should be noted that tests carried out in the Erlenmeyer flasks were found to demonstrate similar kinetic behaviour as compared to parallel tests carried out in the parent bioreactor, thus confirming the representivity of the data obtained from each system (data not shown).

2.3. Analytical procedures

Propanil and DCA were analysed by HPLC with diode array detection (DAD) using a reverse-phase column, while propionate was analysed by HPLC with a UV detector and an Aminex HPX-87H column (Carvalho et al., 2010). The biomass concentration was measured through the optical density (OD) at 610 nm, where the OD was related to the total suspended solids (TSS) and volatile suspended solids (VSS) through calibration curves (Carvalho et al., 2010). TSS and VSS were measured according to standard methods (APHA et al., 1995).

3. Results and discussion

3.1. Influence of propionate addition on biomass growth and propanil degradation

The SBR was operated for approximately 1 year in fed-batch feeding mode prior to the addition of propionate. Fig. 2 shows that during that time, the biomass concentration in the reactor increased from 0.09 to 0.26 g L^{-1} over the course of approximately 300 d (Days −357 to −75), despite the fact that no sludge was wasted from the SBR. This extremely low net growth is not compatible with the development of biomass for bioaugmentation purposes. Further, it had been established that the addition of higher amounts of propanil led to substrate inhibition by DCA and propanil (Carvalho et al., 2010), thus adding more propanil was not con-
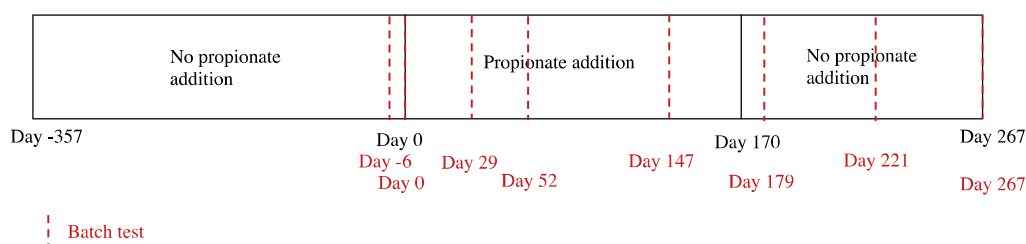

Fig. 1. Timeline of experimental study performed using the mixed culture. Sludge wastage was performed on the transition periods between propionate addition and no propionate addition (i.e. Day 0 and Day 170).
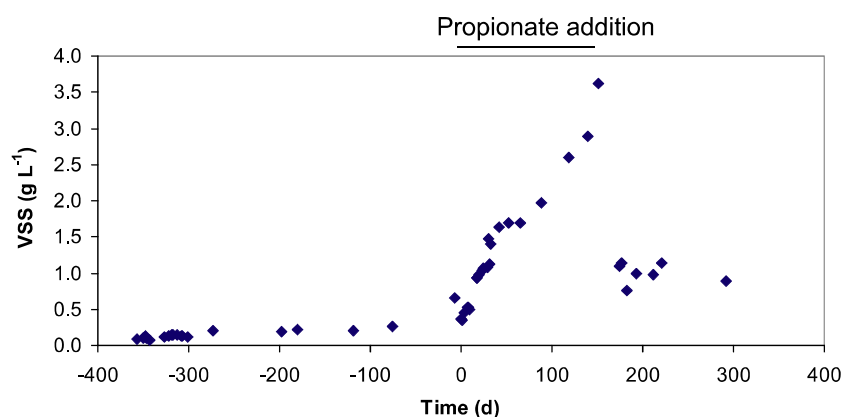
Fig. 2. Biomass growth before, during and after propionate addition.

Table 1
Biomass yield coefficients ($Y_{x/s}$) and propionate uptake rate in the presence and absence of propanil.

Day	$Y_{x/s}$ (C-mol C-mol^{-1})	Propionate uptake rate	
		Presence of propanil	Absence of propanil
		mmol gVSS^{-1} d^{-1}	mmol gVSS^{-1} d^{-1}
−6	0.09	–	–
0	0.20	3.4	9.8
29	0.39	–	42.9
52	0.58	41.9	–
147	0.62	71.6	–
221	0.53	8.3	12.7

sidered to be a viable option to increase the biomass growth rate. For this reason, propionate, the other main metabolite of propanil degradation, was added to the media. After the addition of propionate, the net specific biomass growth rate increased by a factor of 3.1 (0.031–0.094 d^{-1}), which was not surprising considering that 3.9 times more total carbon was being fed each cycle (1.35 C-mM propanil as compared to 3.94 C-mM of propionate). Furthermore, the addition of propionate led to a substantial increase in the net biomass growth per C-mol of substrate added (Table 1).

Interestingly, Fig. 3 shows that the addition of propionate to the culture not only increased the biomass concentration, but also the specific removal rates of both propanil and DCA by 10- and 5-fold, respectively, after 29 days of propionate feeding. While propionate can be readily consumed by many organisms besides propanil and DCA degraders, it is noteworthy that the specific xenobiotic degradation rates increased, suggesting that the selectivity of the culture

towards propanil and DCA removal was maintained during this time period. However, after a prolonged period of propionate addition (>52 d), the specific rates of propanil and DCA degradation decreased to levels below the starting point before propionate addition (Day 147, Fig. 3). This effect was most likely due to growth of non-propanil or DCA degraders. Nevertheless, the volumetric propanil and DCA removal rates were 4.7 and 1.9 times higher at Day 147 than before propionate addition, which was due to the increase in biomass concentration caused by the propionate feeding (Fig. 2).

At this point, in order to increase the selectivity of the culture once again, propionate addition was terminated on Day 170 (while sludge was simultaneously wasted on this day in order to achieve a biomass concentration of ~1 g L^{-1} in the reactor). This operation led to an immediate increase in propanil and DCA specific degradation rates (Day 179, Fig. 3), where DCA degradation required a longer time to recover as compared to that of propanil (Day 221, Fig. 3). The biomass concentration stabilised at ~1 g L^{-1} after propionate addition ceased (Fig. 2).

3.2. Effect of propionate addition on the metabolism of the mixed culture

The reason for the increase in specific propanil and DCA degradation rates after propionate addition was initially unclear, and of great interest since it can be used as a promoter of propanil and DCA degradation. Indeed, Fig. 4a and b show that during the first day and 29th day following propionate addition, propionate was taken up very slowly in the fed-batch reactor until propanil was fully consumed. Since the propionate degradation rate
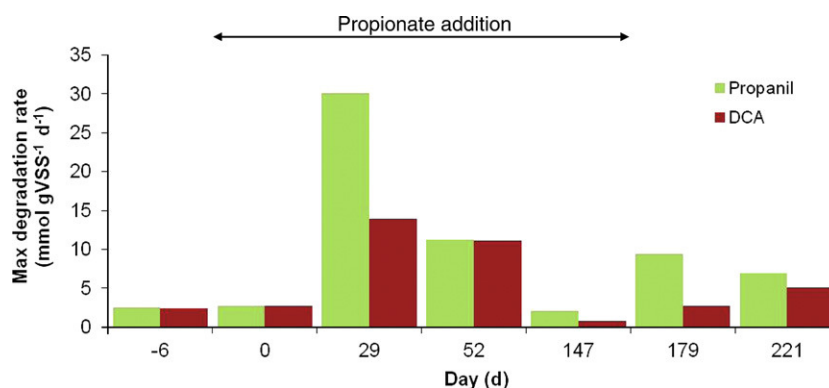

Fig. 3. Propanil and DCA degradation rates before during and after propionate addition.
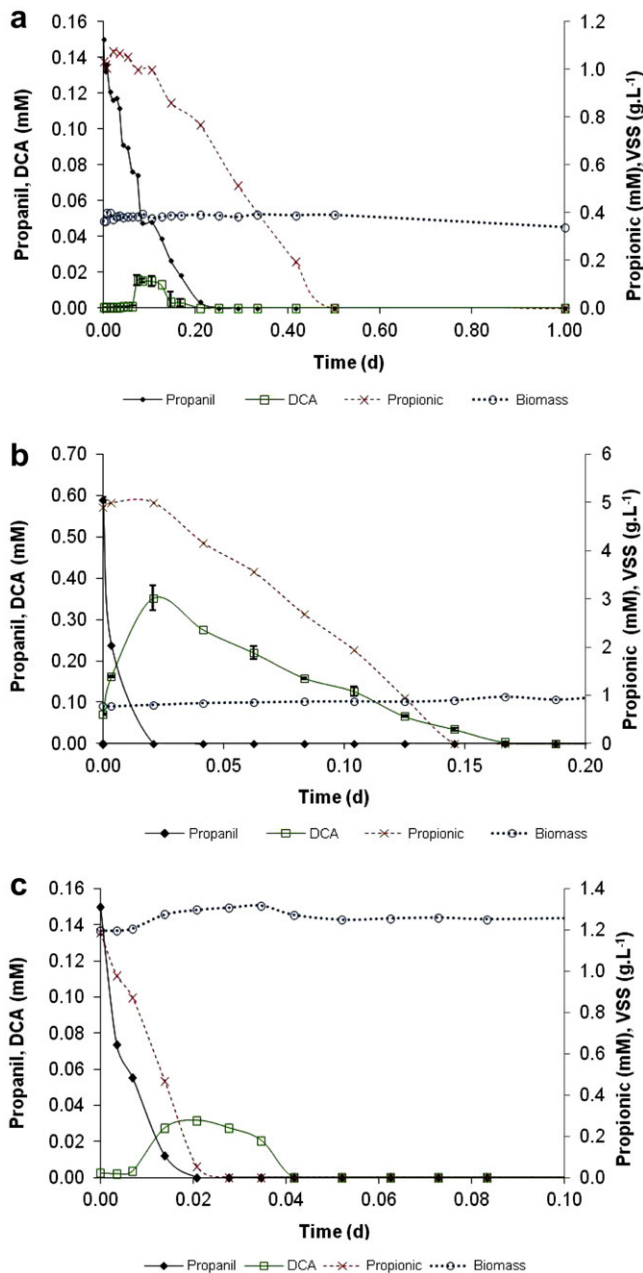
Fig. 4. Propanil, DCA and propionate degradation and biomass growth on (a) Day 0 ($S_{prop,ini}$ = 0.15 mM), (b) Day 29 ($S_{prop,ini}$ = 0.59 mM) and (c) Day 52 ($S_{prop,ini}$ = 0.15 - mM) after propionate addition.

was influenced by the presence or absence of propanil (see Table 1 for a comparison), propionate was actually a competitor substrate to propanil, suggesting that the specific degradation rate of propanil would be slower in the presence of propionate as compared to when propionate was not fed. Nevertheless, this was not the effect observed, as the specific degradation rate of propanil was significantly higher after propionate feeding commenced (30.0 mmol gVSS^{-1} d^{-1} on Day 29 as compared to 2.5 mmol gVSS^{-1} d^{-1} on Day −6). Fig. 4c shows that after Day 52 the culture started to take up propionate concurrently with propanil, likely due to the development of a microbial community that was less specialised for propanil and DCA. Further supporting this hypothesis is the fact that the propionate uptake rate continually increased during the acclimatisation period (Table 1). Interestingly, after propionate feeding was stopped on Day 170, not

only did the propanil and DCA degradation rates increase once again, but in a batch test fed with propionate on Day 221, the propionate uptake rate again increased after propanil depletion, similarly to what was observed on Days 0 and 29 (Table 1). This finding suggests that the culture at least partially recovered its selectivity towards propanil and DCA degradation.

These results prompted an investigation into the understanding of the propanil degrading metabolism in order to determine why the specific degradation rates initially increased in the presence of propionate. One possibility is co-metabolism of propionate with propanil, which is a known mechanism that increases degradation kinetics (Xu et al., 2011). However, due to the fact that the immediate effect of propionate on the propanil degradation rate was negligible (see Day 0, Fig. 3), coupled with the fact that propionate uptake tended to succeed propanil uptake instead of both substrates being removed simultaneously, it was considered unlikely that co-metabolism was the effect that triggered the stimulus in propanil and DCA degradation rates. Therefore, further analysis was performed through building a metabolic model of the process, in order to determine the effect of propionate on propanil/DCA metabolism.

3.3. Metabolic model description

A description of the metabolic model developed in this study is shown in Fig. 5, while the individual reactions of each metabolic step are shown in Table 2. A description of each reaction is as follows: in reaction 1 (R1), one mol of ATP is needed per mol of propanil to generate propionyl-CoA, since transfer of the coenzyme with its associated energy bond requires ATP (Zeng et al., 2002). Additional ATP is required (parameter 'z') for active transport of propanil across the cell membrane. Since previous studies have suggested that individual organisms display distinct preferences for propanil or DCA degradation (Carvalho et al., 2010), and mixed microbial consortium members tend to have interactions that include the exchange of metabolites, it was assumed that both propanil and DCA needed to be transported across bacterial cell membranes. Thus, one mol of ATP per mol of compound (propanil, DCA), or two ATP moles in total would be required for aerobic transport across the cell membrane (van Aalast van Leeuwen et al., 1997). For these reasons, this unknown energy (z) was considered to be 2/9 mol ATP C-mol^{-1} propanil (i.e. propanil has 9 C atoms). However, the possibility of a higher amount of energy being required was also investigated (see below).

When considering propionate uptake across the cell membrane in reaction 2 (R2), previous findings have shown that propionate requires similar energetic requirements as acetate for active transport across the cell membrane (Oehmen et al., 2005), thus 2 mol of ATP per mol of propionate was considered in this model (van Aalast van Leeuwen et al., 1997). In reaction 3 (R3), DCA degradation proceeds along a modified *ortho* cleavage pathway, as proposed by You and Bartha (1982), where the products of this degradation are acetyl-coA and succinate. The propionyl-coA that is generated in R1 and R2 is degraded through succinate (R4) and then onwards to the TCA cycle where it is combined with acetyl-CoA produced from DCA degradation (R5) (Gottschalk, 1986). Catabolism (R6) and biomass (X) growth occur from acetyl-coA (R7), where the standard biomass formula ($CH_{1.8}O_{0.5}N_{0.2}$) is considered (Roels, 1980). Finally, oxidative phosphorylation (R8) provides energy through cell respiration, where δ represents the efficiency of the reaction (i.e. P/O ratio).

Given the above set of reactions, these equations can be related to the observable conversion rates, whereby the measurable components that are produced or consumed in each individual metabolic reaction are united through material balancing as follows:
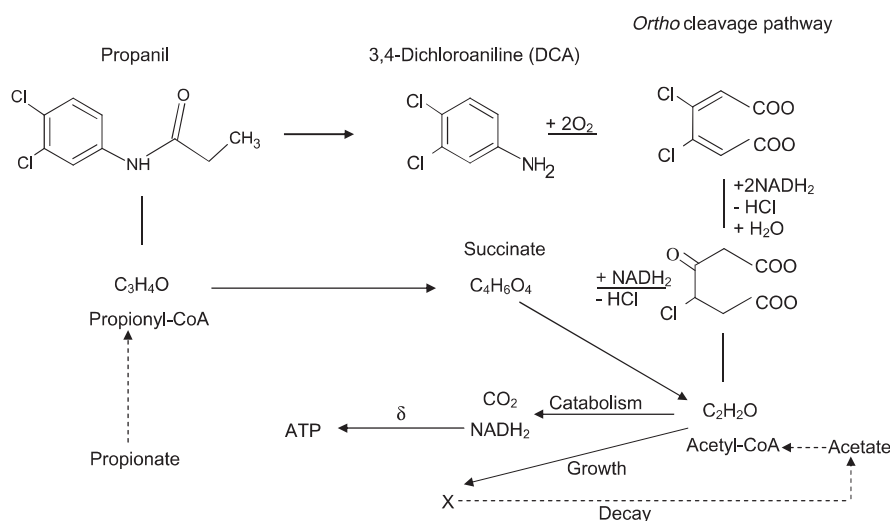
Fig. 5. Proposed metabolism of propanil, DCA and propionate degradation in the presence and absence of propionate addition.

Table 2
Metabolic model reactions.

R1: Propanil uptake	$CHCl_{\frac{2}{9}}N_{\frac{1}{9}}O_{\frac{1}{9}} + (\frac{1}{9}+Z)ATP \rightarrow \frac{2}{3}CH_{\frac{5}{6}}Cl_{\frac{1}{3}}N_{\frac{1}{6}} + \frac{1}{3}CH_{\frac{4}{3}}O_{\frac{1}{3}}$
R2: Propionate uptake	$CH_2O_2 + \frac{2}{3}ATP \rightarrow CH_{\frac{4}{3}}O_{\frac{1}{3}} + \frac{1}{3}H_2O$
R3: DCA degradation	$CH_{\frac{5}{6}}Cl_{\frac{1}{3}}N_{\frac{1}{6}} + \frac{1}{3O_2+\frac{1}{2}}NADH_2 + \frac{1}{6}H_2O \rightarrow \frac{1}{3}CHO_{\frac{1}{2}} + \frac{2}{3}CH_3O + \frac{1}{6}NH_3 + \frac{1}{3} + \frac{1}{3}HCl$
R4: Propionyl-coa to succinate	$CH_{\frac{4}{3}}O_{\frac{1}{3}} + \frac{1}{3}CO_2 + \frac{1}{3}H_2O \rightarrow \frac{4}{3}CH_{\frac{3}{2}}O + \frac{1}{3}ATP$
R5: Succinate to acetyl-coa	$2CH_{\frac{3}{2}}O + \frac{1}{2}ATP + \frac{1}{2}ATP + \frac{1}{2}H_2O \rightarrow \frac{4}{3}CH_{\frac{3}{2}} + \frac{1}{3}ATP$
R6: Acetyl-coa catabolism	$CHO_{\frac{1}{2}}O + \frac{3}{2}H_2O \rightarrow 2NADH_2 + \frac{1}{2}ATP$
R7: Biomass growth	$1.27CHO_{\frac{1}{2}} + 0.2NH_3 + \left(1.7 + \frac{m_{ATP}}{\mu}\right)ATP + 0.405H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 0.27CO_2 + 0.44NADH_2$
R8: Oxidative phosphorylation	$NADH_2 + \frac{1}{2}O_2 \rightarrow \delta ATP + H_2)$

Where z is the ATP needed for propanil transport across the cell membrane, m_{ATP} is the ATP required for cell maintenance processes, μ is the maximum biomass growth rate and δ is the efficiency of oxidative phosphorylation.

$r_{propanil} = -r_1$
$r_{propionate} = -r_2$
$r_{DCA} = 2/3r_1 - r_3$
$r_{propionyl\text{-}coa} = 1/3r_1 + r_2 - r_4$
$r_{succinyl\text{-}coa} = 2/3r_3 + 4/3r_4 - 2r_5$
$r_{acetyl\text{-}coa} = 1/3r_3 + r_5 - r_6 - 1.27r_7$
$r_x = r_7$
$r_{NADH} = -1/2r_3 + 3/2r_5 + 2r_6 + 0.44r_7 - r_8$
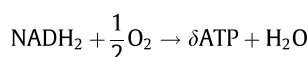$r_{ATP} = -(1/9+z)r_1 - 2/3r_2 + 1/3r_4 - 1/2r_5 + 1/2r_6 - 1.7r_7 - m_{ATP} + \delta r_8$
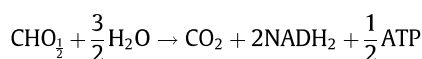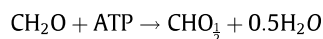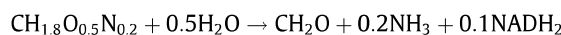
Assuming steady-state conditions, i.e. no net accumulation of intermediates DCA, propionyl–CoA, succinyl-CoA, and acetyl-CoA, nor of reducing equivalents ($NADH_2$) and energy (ATP), then r_{DCA} = $r_{propionyl\text{-}coa}$ = $r_{succinyl\text{-}coa}$ = $r_{acetyl\text{-}coa}$ = r_{NADH} = r_{ATP} = 0.

Solving for the biomass growth rate, the following relationship can be found

$$r_x = \frac{(\frac{5}{3}\delta + \frac{1}{9} - z)r_{propanil} + (\frac{7}{3}\delta - \frac{1}{3})r_{propionate} - m_{ATP}}{2.1\delta + 2.335}$$

Furthermore, it can be assumed that the culture obtains all of its energy (ATP) for cell maintenance through the decay of active biomass, which is subsequently hydrolysed to soluble substrate

through the death-regeneration concept (see van Loosdrecht and Henze, 1999 for more detail). Biomass decay can be expressed similarly to the degradation and metabolism of external substrates as follows:

$$CH_{1.8}O_{0.5}N_{0.2} + 0.5H_2O \rightarrow CH_2O + 0.2NH_3 + 0.1NADH_2$$

$$CH_2O + ATP \rightarrow CHO_{\frac{1}{2}} + 0.5H_2O$$

$$CHO_{\frac{1}{2}} + \frac{3}{2}H_2O \rightarrow CO_2 + 2NADH_2 + \frac{1}{2}ATP$$

$$NADH_2 + \frac{1}{2}O_2 \rightarrow \delta ATP + H_2O$$

Since acetate, acetyl-coa and $NADH_2$ do not accumulate, r_{CH_2O} = $r_{CHO_{\frac{1}{2}}}$ = r_{NADH_2} = 0, where it is then derived that:

$$m_{ATP} = -2.1\delta r_{xb} + 0.5r_{xb} \qquad (eq.9)$$

where r_{xb} represents the biomass decay rate. Combining together this system of equations, we find that the biomass growth rate is solved as follows:

Table 3
Change in P/O ratio and propanil and DCA degradation rates over time, before (<Day 0), during (Day 0 to Day 147) and after (>Day 170) propionate addition.

Day	P/O ratio		Propanil deg rate mmol gVSS^{-1} d^{-1}	DCA deg rate mmol gVSS^{-1} d^{-1}
	$z = 2/9$ mol ATP mol NADH$_2$$^{-1}$	$z = 5/9$ mol ATP mol NADH$_2$$^{-1}$		
−6	0.21	0.43	2.5	2.4
0	0.32	0.46	2.8	2.8
29	0.67	0.72	30.0	13.9
52	1.93	2.10	11.2	11.1
147	1.79	1.82	2.1	0.8
221	1.79	2.06	7.0	5.1

$$r_x = \frac{(\frac{5}{3}\delta + \frac{1}{9} - z)r_{\text{propanil}} + (\frac{7}{3}\delta - \frac{1}{3})r_{\text{propionate}} + (2.1\delta - 0.5)\ r_{\text{xb}}}{2.1\delta + 2.335}$$

(eq.10)

Solving instead for δ, this parameter can be calculated from the propanil and propionate uptake rates, the biomass growth and the biomass decay rates:

$$\delta = \frac{2.335 r_x + \frac{1}{3}r_{\text{propionate}} + (-\frac{1}{9} + z)r_{\text{propanil}} + 0.5 r_{\text{xb}}}{\frac{5}{3}r_{propanil} + \frac{7}{3}r_{\text{propionate}} - 2.1 r_x + 2.1 r_{\text{xb}}}$$

3.4. Calculation of the P/O ratio for the propanil degrading culture

In Table 3, the P/O ratio (δ) was calculated over time using Eq. (10) to estimate the efficiency of the culture and the effect that acclimatization time to propionate has on the culture. Since the model describes both propanil/DCA metabolisms as well as propionate metabolism, all substrates present in the system are accounted for and the P/O ratio must therefore be viewed as the overall metabolic efficiency. The P/O ratio gradually increased from 0.21 prior to propionate addition to 1.7–2.0 after 52 days of feeding with propionate. This suggests that the metabolism of the culture became more efficient over time, and that propionate was responsible for this change in efficiency. However, there are two important factors influencing the specific xenobiotic degradation kinetics: the selectivity of the microbial culture as well as the metabolic efficiency. During the first 52 days of propionate feeding, the increase in propanil and DCA degradation rates was coupled with an increase in P/O ratio, suggesting that the selectivity of the culture towards propanil and DCA removal was maintained but the metabolic efficiency of the cells increased. After Day 52, while the P/O ratio remained approximately constant, the specific propanil and DCA degradation rates were lower (Day 147, Fig. 3), likely

due to decreased selectivity of the culture rather than a change in metabolic efficiency. Finally, after propionate addition was halted, the P/O ratio remained constant at its highest level, and propanil and DCA degradation rates went up again, suggesting that the efficiency of the culture was maintained even after propionate addition ceased. Thus, it is possible to maintain this high overall metabolic efficiency even after the readily biodegradable substrate is no longer added. It is expected that the metabolic efficiency of the easily degradable substrate (i.e. propionate) is higher than that for xenobiotic compounds; however, if this high efficiency can be achieved simultaneously to a selective culture for xenobiotic removal, higher specific xenobiotic degradation rates can also be expected.

The results in the P/O ratio (δ) from this study are consistent with those of Muller and Babel (1994), who found a P/O ratio <1 for the chlorinated phenolic herbicide derivative 2,4-dichlorophenoxyacetic acid (2,4-D). These low P/O ratios may be explained by the fact that chlorine atoms reduce the energy content of organic substances, since their electrons are not available for cell respiration. In contrast, a P/O ratio of 2 was estimated to be the theoretical maximum from propionate feeding (Jiang et al., 2011), and the P/O ratio approaches this level after prolonged feeding with propionate, which dominated the metabolism after it was introduced.

While the P/O ratio is known to vary with m_{ATP} (Lopez-Vazquez et al., 2009), the m_{ATP} coefficient was estimated based on the decay rate before (Day −6) and after propionate addition (Day 52) and found to fluctuate by less than 6%, (0.0747 C-mol C-mol^{-1} d^{-1} and 0.0701 C-mol C-mol^{-1} d^{-1}, respectively). Since m_{ATP} is dependent on the decay rate as shown in Eq. (9), the differences in P/O ratio are uncoupled from m_{ATP} and reveal the metabolic efficiency of the culture.

The differences in propanil and DCA degradation rates before and after propionate addition and the higher P/O ratio suggested that propionate provided the cells with an additional energy source, thus driving their propanil/DCA removal capacity. Nevertheless, initially, propionate is only taken up very slowly until after propanil is consumed (Fig. 4a and b), thus co-metabolism of propanil/DCA with propionate is unlikely to be a significant factor. These results are consistent with those of Egli (2010), who suggested that the availability of alternative carbon/energy sources does not inhibit but does support the induction of catabolic enzymes, supplying energy and building blocks for the synthesis of proteins. The present study also showed that the energetic efficiency of the cells increased over the long-term after propionate addition, suggesting that, at least in the present case, this effect is gradual and builds up proteins and energy over time, rather than due to a short-term addition of propionate, which had little
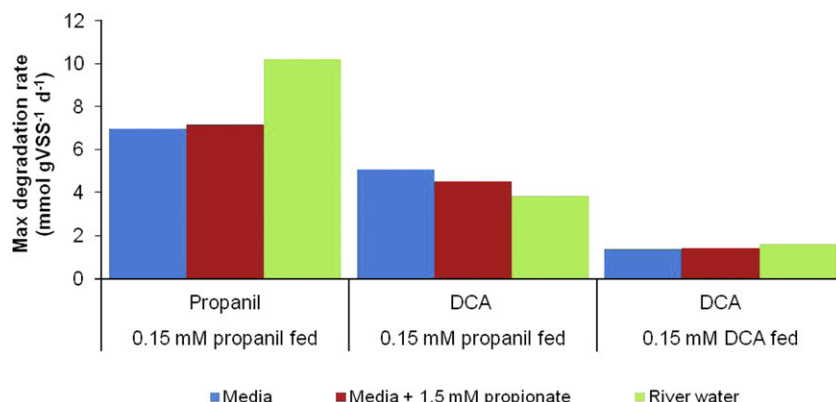

Fig. 6. Propanil and DCA degradation rates from propanil or DCA feeding to river water, and with and without propionate addition in the media.

immediate effect on propanil and DCA degradation rates by the culture (Day 0, Fig. 3). Also in accordance with the results of this study, Chong et al. (2012) found that biogenic substrates increase the ATP content of activated sludge, thereby improving the degradation efficiency of a xenobiotic, 2,4-dichlorophenoxyacetic acid.

Furthermore, changing the z parameter was performed in order to evaluate its sensitivity, given the uncertainty surrounding this parameter. It can be inferred from Fig. 4a and b that the energy required for transport of propanil across the cell membrane is lower than that of propionate because the culture tended to take up propanil first, while propionate was mostly consumed afterwards. This implies that the constraint of z is $\leqslant 5/9$ ATP C-mol^{-1}, since this is the level of energy needed in order for the uptake of both substrates to be equivalent energetically. It was found that the same trend in P/O ratio can be observed (Table 3), and after propionate addition, the P/O ratio was relatively insensitive to different transport energy fluxes. While $z = 2/9$ was considered a more reasonable estimation for this unknown transport energy flux, as explained above, a higher transport energy requirement would not affect the conclusions made in this study.

3.5. Effect of the water matrix on propanil/DCA degrading rates

After propionate addition to the SBR had ceased for >50 d, a separate set of tests was performed to determine if the addition of propionate had any immediate effect on the degradation rates and if the use of an irrigation water matrix had any impact on degradation rates. Since river water is commonly used for irrigation (Steen and Collette, 1989; Pothuluri et al., 1991; Correa and Steen, 1995), water from the Tejo river in Portugal was used for the experiment. The results are shown in Fig. 6 and reveal that propionate had no immediate impact on degradation rates, which is similar to the effect noted in Fig. 3 during the first cycle of propionate addition. This result again suggests that the culture did not take up propanil through co-metabolism with propionate, and it is noteworthy that the effect can be shown not only for propanil and DCA degradation from propanil feeding, but also DCA degradation directly from DCA feeding.

Furthermore, it was observed that the water matrix increased the propanil degradation rate, while the DCA degradation rates remained essentially constant as compared to the case of using mineral media. This finding suggests that the water matrix is unlikely to have a significantly negative impact on the bioremediation process when an enriched culture such as the one used in this study is employed during bioaugmentation. This result supports the utility of the culture for practical application. The fact that the culture developed in this study removes propanil and DCA at a similar efficiency in mineral media and irrigation water matrices is in good agreement with the work of Correia et al. (2006), who also found similar degradation efficiency between the two matrices for their culture when applied to degrade the herbicide molinate.

4. Conclusions

The addition of a simple degradable substrate (propionate) not only stimulated the biomass growth rate of a xenobiotic-degrading culture, but increased the specific propanil/DCA degradation rates. Co-metabolism of both propanil and propionate was unlikely to be the mechanism responsible for the increased degradation rates. Based on the development of a metabolic model to describe the biodegradation of propanil and DCA, the metabolic efficiency of the culture increased due to propionate addition, which was likely

responsible for the initially higher degradation rates. Adequate control of propionate feeding can stimulate culture selection and propanil/DCA degradation from irrigation waters.

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