Strategies for the design and operation of enzymatic reactors for the degradation of highly and poorly soluble recalcitrant compounds

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Abstract

The presence of recalcitrant compounds in both wastewaters and soils is an important environmental problem. The oxidative enzymes from white-rot fungi have been successfully utilised for the *in vitro* degradation of xenobiotics, such as the azo dye Orange II and the polycyclic aromatic hydrocarbon anthracene (compounds with high and low solubilities, respectively). Two different reactor configurations are proposed: i) an enzymatic membrane reactor for the treatment of soluble compounds consisting of a continuous stirred tank reactor coupled to an ultrafiltration membrane to facilitate the retention and recycling of enzyme; ii) a two-phase enzymatic reactor for the degradation of poorly soluble compounds consisting of an immiscible solvent, which contains the contaminant at high concentrations, and the aqueous phase containing the enzyme and cofactors involved in the catalytic cycle. In this paper, factors affecting the conception, design and operation of both systems are discussed and the experimental results concerning the efficiency and stability of the processes are presented.

Keywords: Enzymatic membrane reactors, two-phase partitioning bioreactors, recalcitrant compounds, anthracene, Orange II, manganese peroxidase.

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Features of enzymatic reactors

Numerous advantages arise from the use of enzymes against microorganisms for environmental purposes. The advantages are as follows: i) enzymes can be active under a wider variety of conditions such as pH, ionic strength or temperature; ii) higher pollutant concentrations can be maintained in enzymatic reactors with no inhibitory problems; iii) operational times are reduced with no lag period due to microbial growth; iv) media composition is simpler and enzymatic requirements are low provided that the enzyme can be reused; v) control of the process is easier; and vi) no sludge is produced. On the contrary, the cost of enzyme production and its sensitivity to changes in the environmental conditions are the main weaknesses that have to be taken into account when favouring efficiency of the enzymatic process.

In the present work, we studied the feasibility of using enzymatic reactors for the degradation of soluble and poorly soluble recalcitrant contaminants. Some typical examples of soluble compounds are nitroaromatic explosives, phenols, dyes, and among them, the azo dye Orange II, a recalcitrant compound commonly present in industrial wastewaters. Typical examples of low-soluble contaminants are polycyclic aromatic hydrocarbons (PAHs), pesticides and polychlorinated biphenyls (PCBs). Their high hydrophobicity makes these compounds environmentally persistent. Anthracene, a tricyclic PAH, was selected as a model compound due to its low solubility.

Factors to consider for the operation of enzymatic reactors

Selection of the enzyme

The enzyme used as a catalyst for the degradation of compounds with recalcitrant character should exhibit high oxidation and ionisation potential and non-specific activity, thereby providing the capability to degrade a broad range of compounds such as those present in contaminated effluents or soils. Because the enzyme-substrate interaction may be confined by the large size of the enzyme, the use of diffusible enzymes or related mediators is desirable. Moreover, extracellular enzymes are preferred since their production is easier and cheaper. All these characteristics are fulfilled by the ligninolytic enzyme referred to as manganese peroxidase (MnP).

Catalytic cycle of the enzyme

The first step required for successful application is a deep knowledge of enzyme characteristics, particularly the cofactors and cosubstrates that are involved in the catalytic cycle. MnP, an extracellular enzyme, was first discovered in *Phanerochaete chrysosporium* and is produced by a number of white-rot fungi (Tien and Kirk 1988). MnP catalytic cycle is similar to that of other peroxidases and involves a 2-electron oxidation (Figure 1). The initial oxidation of MnP by H_2O_2 produces an intermediate compound that promotes the oxidation of Mn^{+2} to Mn^{+3}. Mn^{+3} is stabilised by organic acids and the Mn^{+3}-organic acid complex acts as a strong diffusing oxidiser (oxidation potential: 1.54 V) (Kuan and Tien 1993). Excess H_2O_2 promotes enzyme inactivation. The value of this enzyme is supported by its ability to degrade a variety of complex compounds (Kuan et al. 1993; Martínez 2002).

[Insert Figure 1 about here]

The factors affecting the catalytic cycle are related to the presence of different cosubstrates and cofactors as well as environmental reaction conditions (Wariishi et al. 1988). These decisions affect not only the stoichiometry of the reaction, but also the kinetics and stability of the enzyme. Though the stoichiometry describes the minimum requirements of the cofactors, the proper selection of their concentrations and operational variables may accelerate reaction kinetics and reduce enzymatic consumption. Two main goals have to be achieved: i) adequate conversion, which may be defined by environmental restrictions; and ii) high efficiency, defined as the amount of substrate degraded per unit of enzyme consumed during the reaction.

H_2O_2 is directly involved both in the activation of the catalytic cycle at balanced concentrations and the deactivation of the enzyme at high concentrations (Timofeevski et al. 1998). A low concentration of H_2O_2 would imply kinetic or even stoichiometric limitations while the enzymatic activity would be protected towards inactivation (Mielgo et al. 2003a). On the other hand, organic acids present a similar crossed effect: a high concentration favours chelation of Mn^{3+} although it may affect the stability of the enzyme (Hofrichter et al. 1998), likely due to the endogenous formation of H_2O_2 during decarboxylation (Schlosser and Hofer 2002; Van Aken and Agathos 2002).

Operational parameters

With respect to the operational parameters affecting the continuous process, the hydraulic retention time (HRT), loading rate and environmental parameters (temperature, pH) were the most significant. With HRT, we should be aware of the desired conversion (degradation) of pollutant and kinetics, which is directly affected by the pollutant concentration. Thus, a higher loading rate could lead to a faster but a less efficient process. Increasing temperature could favour, in some cases, the reaction rate, but it may also increase enzyme deactivation. An appropriate temperature must be selected when trying to find a compromise between both aspects, as well as when considering economical factors. Operational pH must also be controlled at values near the optimal range. In the case of MnP, pHs lower than 4 and higher than 6 should be avoided.

Modelling and control of enzymatic reactors

When changes in the influent flow or the pollutant concentration occur, one of the main aspects to consider for proper continuous operation of an enzymatic reactor is the system dynamics. The conception, design and operation of an efficient control system require a deep knowledge of the kinetic model, which depends on the enzyme. In the case of MnP, the substrate to be oxidised (pollutant) is not the primary substrate for the enzyme (H_2O_2), although the degradation rate is greatly influenced by the concentration of the substrate and, thus, this parameter must be considered as a variable (López et al. 2006). Furthermore, the study of reactor behaviour in unsteady-state conditions will be indicative of system stability as it depicts system resistance against alterations.

Retention of the enzyme

To minimise losses, the retention of enzyme in the reactor is a main factor when dealing with the design and operation of an enzymatic reactor. When the pollutant is highly soluble in water, the recovery of the enzyme from the effluent requires an additional system, whereas for low solubility compounds, the enzyme is trapped onto the aqueous phase. Below, we discuss different strategies depending on the solubility of the pollutants.

Reactors treating highly soluble compounds. The configurations of enzymatic reactors treating soluble compounds can be classified according to the manner in which the enzyme is retained: i) immobilised onto a support, forming bigger structures that can be retained due to their size or ii) free in solution, being retained by a membrane.

Immobilisation of the enzyme onto a support is usually complex and expensive, and increases processing costs. To improve the economical feasibility of immobilised enzyme reactors, a number of requirements should be met: the specific activity of the derivative (units of enzyme per g of support) should be as high as possible; the support or membrane could be applied with a secondary function, such as the separation of substrates or products; and the support should have good mechanical resistance and minimum interaction with the substrates or products. Previous studies have determined a support based on agarose activated with glutaraldehyde groups as suitable for the immobilisation of MnP (Mielgo et al. 2003b). The immobilised enzyme was applied for the degradation of the dye Orange II in a continuous stirred tank reactor. However, there were some operational difficulties in this configuration with the free enzyme: high activity loss during the immobilisation process, lower reaction rates, more complex control, adsorption of the dye onto the support and, mainly, the ability to replace the deactivated immobilised enzyme with fresh enzyme.

The second option corresponds to a continuous stirred tank reactor where the soluble enzyme is retained by means of an ultrafiltration membrane coupled to the reactor (Figure 2A) (López et al. 2004a). The main advantages of this configuration are: i) operation with free enzyme, avoiding limitations of mass transfer and, consequently, low kinetic rates; ii) retention of non-biodegradable molecules with high molecular weights; iii) ability of the products of degradation to cross the membrane, being discharged in the effluent; and iv) easy operation.

Reactors treating poorly-soluble compounds. The *in vitro* degradation of poorly soluble compounds is limited by their enzyme availability. The addition of miscible cosolvents is a good approach to increase the solubility by several orders of magnitude. Eibes et al. (2005) established a system for the degradation of anthracene by MnP in a medium containing a mixture of acetone:water (36% v:v). In the mentioned work, 5 mg L^{-1} of anthracene were degraded after 6 h of operation under optimal conditions. However, this system presented a number of limitations: i) the concentration of anthracene in the medium was limited by the amount of cosolvent; ii) higher amounts of solvent could lead to a higher inactivation of the enzyme; iii) the enzyme could not be recycled; and iv) the reuse of the solvent would require a separation process.

To overcome these limitations, a completely different option, a two-phase partitioning bioreactor (TPPB), was considered (Figure 2B). In TPPBs, a solvent immiscible in the water phase with an appropriate partition coefficient is added. The substrate is almost completely dissolved in the organic phase, from which it diffuses to restore equilibrium, as the enzyme degrades the pollutant in the aqueous phase (Vrionis et al. 2002). TPPBs have been successfully used for the biological treatment of toxic and recalcitrant pollutants, such as polycyclic aromatic hydrocarbons. Janikowski et al. (2002) performed the degradation of anthracene and other PAHs in biphasic reactors in cultures of *Sphingomonas aromaticivorans* and dodecane as the organic phase.

[Insert Figure 2 about here]

The selection of the appropriate solvent is critical for the design of TPPBs because it greatly influences mass transfer and consequently degradation rates. The selected solvent should be inexpensive, readily available, and exhibit suitable physical and chemical properties (be immiscible, non-volatile, etc.) (Déziel et al. 1999; MacLeod and Daugulis 2003; Marcoux et al. 2000; Villemur et al. 2000). Furthermore, when dealing with enzymatic reactors, the possible interaction between the solvent and enzyme is critical. It is important that the solvent is not a substrate of the enzyme (MacLeod and Daugulis 2003) and its effect on enzymatic activity is as low as possible (Ross et al. 2000). The partition coefficient should enable the system to achieve the highest possible concentration of substrate in the aqueous phase. It has been stated that solvents with high partition coefficients can sequester the target compound, thus limiting its biodegradation rate (Efroymson and Alexander 1995).

The substrate transfer rate from the water-immiscible to the aqueous phase is another essential factor and has to be enhanced so as not to limit the overall degradation rate. Mass transfer is favoured by an increased surface area for partitioning, therefore, the rate of biodegradation in a TPPB is governed by the size of the interface between the two liquid phases (Ascón-Cabrera and Lebeault 1995; Köhler et al. 1994). The interfacial area is defined by equation 1:

$$a = \frac{6 \cdot \varphi}{d_{sm}} \tag{1}$$

where φ is the proportion of the organic phase in the reactor and d_{sm} is the Sauter mean diameter of the solvent drops. Therefore, increasing the proportion of organic solvent or decreasing the diameter of the drops by increasing the agitation speed would augment the interfacial area.

In vitro degradation of Orange II in an enzymatic membrane reactor

The operation of a continuous enzymatic membrane reactor for the degradation of soluble compounds must consider the following steps: i) optimisation of the parameters involved in the catalytic cycle of the enzyme; ii) optimisation of the operational parameters in the enzymatic membrane reactor and iii) development of a control system.

Optimisation of the parameters involved in the catalytic cycle

The study of the effect of different variables involved in the MnP catalytic cycle on the degradation of the azo dye, Orange II, was performed in discontinuous experiments (Mielgo et al. 2003a). The authors studied the effect of the type and concentration of organic acid and concentrations of Mn^{2+}, H_2O_2 and MnP, as well as pH and temperature on the extent of degradation (Table I). The most remarkable results were observed with the addition of H_2O_2: a continuous pumping of H_2O_2 with a rate equal to the rate of H_2O_2 consumption in the enzymatic reaction (34 µmol H_2O_2 L^{-1} min^{-1} (Moreira et al. 2001)), improved degradation with a very low enzymatic deactivation. Once all the parameters and conditions were optimised, an initial Orange II concentration of 100 mg L^{-1} was degraded up to 90% after 10 min with minimum consumption of enzyme (Mielgo et al. 2003a).

[Insert Table I about here]

Optimisation of the operational parameters of the enzymatic membrane reactor

An enzymatic membrane reactor configuration was selected for the continuous degradation of an effluent containing 100 mg L^{-1} of the soluble dye Orange II (López et al. 2004a). Efforts were focused on determining the effect of operational parameters on the efficiency of the process.

Two strategies were considered to replace the enzyme consumed during the reaction: i) the stepwise addition of enzyme when the activity reached levels below 100 U L^{-1}, which is considered the minimum level the enzyme needs to degrade the dye (Mielgo et al. 2003a); ii) the continuous pumping of the enzymatic crude into the vessel trying to maintain a constant level of activated enzyme into the reactor (López et al. 2004a). The results were compared to the experiment with a single addition of enzyme at the beginning of the assay. Experiments E1 in Figure 3 show that the continuous addition of MnP increased the efficiency to 2.58 mg of Orange II degraded per U.

[Insert Table II about here]

[Insert Figure 3 about here]

Experiments E2 in Figure 3 show that 225 U L^{-1} of enzyme enhanced the degradation compared to 125 U L^{-1} with no superior consumption of enzyme, as the enzyme requirement was higher at the beginning of the reaction, but the loss per hour was similar. An addition of 50 μmol H_2O_2 L^{-1} min^{-1}, selected from discontinuous assays, favoured the kinetics of the process and enabled to work at low HRT (20 min) resulting in an Orange II loading rate (OLR) of 0.3 g L^{-1} h^{-1}. However, it promoted an activity loss greater than 30 U of MnP L^{-1} h^{-1}. To decrease the magnitude of enzyme loss, the H_2O_2 addition rate would have to be decreased and HRT must be increased to maintain a high conversion. The experiments E3 depicted in Figure 3 performed at a HRT of 45 min and 15 μmol H_2O_2 L^{-1} min^{-1} achieved degradation near 90% and increased efficiency to 12.2 mg U^{-1}. Finally, some experiments were performed to determine the best HRT required to achieve significant decolourisation. Although a HRT of 90 min resulted in higher decolourisation, the loading rate decreased to 0.067 g Orange II L^{-1} h^{-1}, thus promoting decreases in efficiency. Hence, a HRT of 60 min was adequate for the continuous treatment of Orange II at these conditions.

Control systems

The acquired experience of the Orange II degradation process led us to consider two different control systems: i) a feed-back system based on the measurement of dissolved oxygen (DO) in the reaction vessel or ii) a feed-forward system based on a dynamic model including kinetics and hydraulics.

Monitoring the DO concentration during the different decolourisation assays enabled us to assess the influence of a variety of operational variables. The DO concentration was modified when we changed the H_2O_2 addition rate. The constant DO concentration attained in the different steady states depended on the OLR applied

(López et al. 2004a). This suggests that DO is parameter indicative of the behaviour of the reaction, as it is generated in the reaction between H_2O_2 and Mn^{3+} (Martínez et al. 1996) and consumed during the degradation of Orange II (López et al. 2004b). A high level of DO indicates an excess of H_2O_2 concentration with respect to the stoichiometry of the enzymatic reaction.

The Orange II concentration and H_2O_2 addition rate were considered the variables essential to defining the kinetic model (López et al. 2006). The resulting equation followed a Michaelis-Menten model with respect to the Orange II concentration and a first-order linear dependence with respect to the H_2O_2 addition rate (equation 2).

$$r = \frac{r_m S}{K_M + S}\left(K_{H_2O_2} + Q_{H_2O_2}\right) = \frac{0.33S}{58.2 + S}(2.4 + Q_{H_2O_2}) \qquad (2)$$

The kinetics were combined with the hydraulics of the system and the resulting dynamic model was successfully validated by comparing experimental data with model predictions in different assays in steady-state conditions (Figure 4A).

Finally, an unsteady-state continuous process was performed to assess system stability and verify the control systems based on both the DO concentration and dynamic model (Figure 4B). The initial conditions were similar to previous assays; once steady-state was reached, some perturbations of Orange II concentration, HRT, H_2O_2 addition rate or temperature were performed for 1 h periods. The system recovered the steady state within 3 h after the end of each alteration. DO followed the same behaviour determined in previous experiments. This verified the fact that it could be used as a good control parameter, as it helps the reactor to be maintained for a prolonged period. The dynamic model predicted the Orange II concentration in the effluent, not only during the steady states, but also when deviations in stationary conditions occurred.

[Insert Figure 4 about here]

In vitro degradation of anthracene in TPPBs

Optimisation of the operation of TPPBs must address the following: i) selection of an appropriate solvent; ii) study of the parameters involved in the catalytic cycle of the enzyme; iii) enhancement of the mass transfer of the substrate from the organic phase; and iv) model and control of the process.

Selection of the solvent

Several solvents including mineral and vegetable oils, alcohols, alkanes, ketones and esters were considered due to their high boiling points, low water solubility, low cost, lack of toxicity and commercial availability. The partition coefficients (K_{sw}) were evaluated for each solvent (Table III). The values of log K_{SW} obtained ranged from 3.7 (silicone oil) to 5.2 (undecanone). We avoided the highest values of K_{SW} and selected two solvents for further study: silicone oil, with the minimum log K_{SW} of 3.7, and dodecane, with an intermediate log K_{SW} value of 4.5.

[Insert Table III about here]

The second factor in the selection of the solvent was its interaction with the enzyme. The enzyme was subjected to different interfacial areas modifying the agitation rate (Figure 5). Under similar agitation rates, silicone oil formed higher interfacial areas due to lower interfacial tensions (20 and 53 mN m^{-1} for silicone oil and dodecane, respectively). As a result, even at higher interfacial areas, enzyme inactivation in silicone oil was lower.

[Insert Figure 5 about here]

Both factors, partition coefficient and enzyme inactivation, were more favourable in the case of silicone oil. Consequently, silicone oil was selected for subsequent experiments.

Optimisation of the parameters involved in the catalytic cycle

As described for the degradation of Orange II, the main factors involved in the catalytic cycle of MnP were evaluated for the degradation of anthracene (Eibes et al. 2006a). Figure 6 describes the efficiency, in terms of anthracene degraded per unit of enzyme consumed, for experiments at different hydrogen peroxide addition rates, malonate concentrations and pH control.

[Insert Figure 6 about here]

The highest efficiencies were obtained at H_2O_2 addition rates of 1 and 5 µmol L^{-1} min^{-1}. The efficiency decreased with higher addition rates due to enzyme inactivation as a result of excess H_2O_2. The concentration of the organic acid had a similar effect: higher concentrations resulted in greater enzyme inactivation and lower efficiency. Finally, control of pH was evaluated because an increase in pH was observed along the reaction reaching values closer to 8 after 70 h of operation. Although the pH was initially maintained at 4.5 by the addition of HCl, addition of malonic acid to control the pH was considered as the concentration of sodium malonate was decreased in the reactor. The efficiency of the experiment with 10 mM of sodium malonate was then increased 1.9 fold relative to the experiment with no pH control.

Optimisation of the mass transfer coefficients

Enhancement of substrate transfer rate was conducted by increasing the volume of silicone oil and the agitation speed. Both factors favoured diffusion from the organic phase because they increased the interfacial area, but also affected enzymatic activity. A

2^2 experimental design was considered to optimise the system efficiency. The ranges evaluated were 200-300 rpm and 10-30% of silicone oil (v:v) and the response surface corresponding to efficiency is represented by equation 3 (Eibes et al. 2006a):

$$EF = 0.152 + 0.026 \cdot AG + 0.054 \cdot SO + 0.026 \cdot AG \cdot SO \qquad (3)$$

where EF denotes system efficiency, AG denotes the agitation rate and SO denotes the fraction of silicone oil (both factors are normalised). The equation shows that increasing both the volume fraction of silicone oil and the agitation speed led to higher efficiencies with a maximum occurring at 300 rpm and 30% silicone oil.

Model of the process

The equation describing substrate concentration in the aqueous phase (S_w) is given by:

$$\ln(S^* - S_W) = \ln S^* - k_L a \cdot t \qquad (4)$$

where S* is the equilibrium concentration of the substrate in the aqueous phase and k_La is the mass transfer coefficient. The determination of substrate concentration in the aqueous phase enabled us to find the mass transfer coefficient for each condition of agitation speed and volume fraction of solvent (Table IV). The data show great increases in the values of k_La especially in a short range of agitation speed (200-250 rpm). This effect was more pronounced when low volumes of silicone oil were present. These results agree with the conclusions obtained above, which suggest an optimised mass transfer rate of 300 rpm and 30% silicone oil. The values of k_La were fitted to a surface and thus related to the agitation and volume of solvent through an empiric correlation.

[Insert Table IV about here]

The equation, which describes the behaviour of anthracene in the organic phase (S_S) considering the mass transfer and the degradation kinetics (as a pseudo-first order (Eibes et al. 2006b)), is shown in equation 5:

$$\ln S_S = \ln S_{S0} - \frac{k_L a}{k_{sw}} \cdot \left(\frac{k_{cat}}{k_L a + k_{cat}} \right) \cdot \frac{V_w}{V_S} \cdot t \tag{5}$$

The partition coefficient of anthracene in silicone oil (k_{sw}) had been previously determined (k_{sw}=5012, Table III) and the mass transfer coefficient ($k_L a$) was correlated with the operational parameters, as described above. Finally the kinetic constant k_{cat} was determined from batch experiments. The model was validated by comparing experimental data with model predictions in experiments at different agitation speeds and volumes of silicone oil (Figure 7). As presented previously, the highest degradation rate was obtained at 300 rpm and 30% silicone oil (v:v), oxidising 90% of anthracene present in the organic phase after 56 h.

Conclusions and perspectives

Enzymatic membrane reactors are a promising technology, as they are easy to operate and control. Degradation processes can be conducted continuously for more than 20 days without membrane cleaning or replacement. Furthermore, enzymatic reactors are versatile since there are a wide variety of commercially available membrane shapes, materials and modules. The selection process must primarily consider the characteristics of the effluent and the enzymatic molecular weight.

The use of a two-phase partitioning bioreactor, which utilises a second immiscible phase for enzymatic degradation of poorly-soluble compounds, is advantageous because of simpler operation due to easy recovery of the solvent depleted in substrate and its reuse in subsequent experiments. Although a priori mass transfer could be considered a limitation of this system, the selection of the appropriate solvent, as well as the study of the conditions resulting in the maximum efficiency enables us to obtain unprecedented degradation rates in enzymatic reactors.

The challenges for those processes are now focused on the development and implementation of a control system and further scale-up for application on an industrial scale.

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Table I. Optimisation of reaction conditions for the discontinuous degradation of Orange II by MnP. V=25 mL; initial Orange II concentration=100 mg L^{-1}.

Parameter	Range	Optimal value
Organic acid	Oxal-Malon-Acet	Acetate
[acid]	1-50 mM	1 mM
$[Mn^{+2}]$	0-1000 μM	33 μM
H_2O_2 addition	FB-Continuous	Continuous
$[H_2O_2]$	50-200 μmol L^{-1} min^{-1}	50 μmol L^{-1} min^{-1}
[MnP]	50-200 U L^{-1}	200 U L^{-1}
pH	4.5-8	4.5
T	20-30ºC	30ºC

Table II. Experimental conditions of assays E1-E4 in Figure 3.

Experiment	MnP ($U\ L^{-1}$)	H_2O_2 addition rate ($\mu mol\ L^{-1}\ min^{-1}$)	Organic acid	HRT (min)
E1	-	50	Acetic	60
E2	-	50	Acetic	20
E3	200	-	Acetic	45
E4	200	15	Oxalic	-

Table III. Log K_{SW} of 15 different solvents.

Solvent	log K_{sw}	Solvent	log K_{sw}
Silicone oil	3.7	Triacetin	4.8
Paraffin oil	4.3	Olive oil	4.9
Sunflower oil	4.3	Corn oil	4.9
Oleic alcohol	4.4	Ethyl acetate	5.0
Decanol	4.4	Biodiesel	5.0
n-Hexadecane	4.5	Marc olive oil	5.0
Dodecane	4.5	Undecanone	5.2
Engine oil	4.6		

Table IV. Values of k_La obtained for experiments at different agitation rates and volumes of silicone oil.

Agitation speed (rpm)	k_La (min^{-1})		
	10% silicone oil	20% silicone oil	30% silicone oil
50	0.01	0.02	0.12
150	0.10	0.36	0.36
200	0.27	0.30	0.68
250	2.99	2.26	3.14
350	3.29	3.30	3.30

CAPTIONS TO FIGURES

Figure 1. Scheme of the catalytic cycle of MnP.

Figure 2. Schematic diagrams of: (A) an enzymatic membrane reactor; (B) an enzymatic biphasic reactor.

Figure 3. Comparative parameters of different strategies for the degradation of Orange II in an enzymatic membrane reactor: percentage of decolourisation (white bars) and efficiency (grey bars). Concentrations in the influent: Orange II 100 mg L^{-1}, malonate 1 mM, Mn^{2+} 33 μM. Experimental conditions are summarised in Table II.

Figure 4. Experimental (○) and simulated data (—) of Orange II concentration in continuous assays. Initial conditions: Orange II 100 mg L^{-1}; malonate 1 mM; Mn^{2+} 33 μM; MnP 200 U L^{-1}; HRT 60 min; H_2O_2 addition rate 15 μmol L^{-1} min^{-1}; pH 4.5. (A) Steady-state operation at different Orange II concentrations in the influent: (a) 100 mg L^{-1}; (b) 150 mg L^{-1}; (c) 200 mg L^{-1}; (d) 250 mg L^{-1}; (e) 300 mg L^{-1} and (f) 100 mg L^{-1}. (B) Non-steady state operation with 1-h overloads: (1) Orange II 200 mg L^{-1}; (2) HRT 30 min; (3) H_2O_2 7.5 μmol L^{-1} min^{-1}; (4) H_2O_2 30 μmol L^{-1} min^{-1}; (5) T 20ºC; (6) T 30ºC; (--) dissolved oxygen.

Figure 5. Effect of agitation (—) on MnP activity in media with dodecane (○) or silicone oil (□).

Figure 6. Degradation of anthracene (grey bars) and different efficiency values (white bars) obtained for each condition of hydrogen peroxide addition (E1), initial malonate concentration with no pH control (E2) and initial malonate concentration with pH control via the addition of HCl (*) or malonic acid (E3).

Figure 7. Experimental and fitted data of assays at different agitation rates and volumes of solvent: 200 rpm - 10% (□), 250 rpm - 20% (○), 300 rpm - 30% (△).

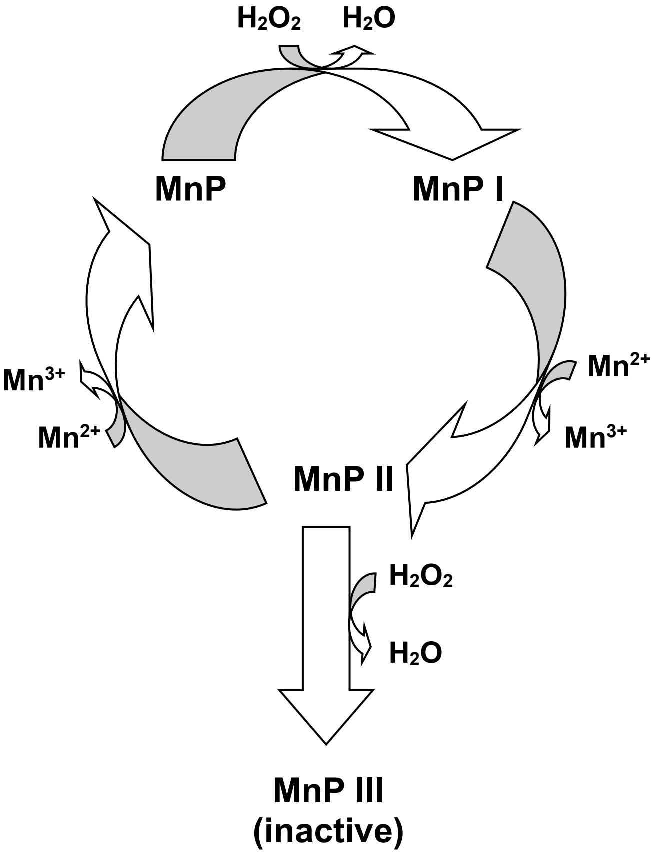

H_2O_2
H_2O
MnP
MnP I
Mn^{3+}
Mn^{2+}
Mn^{2+}
Mn^{3+}
MnP II
H_2O_2
H_2O
MnP III
(inactive)

Figure 1

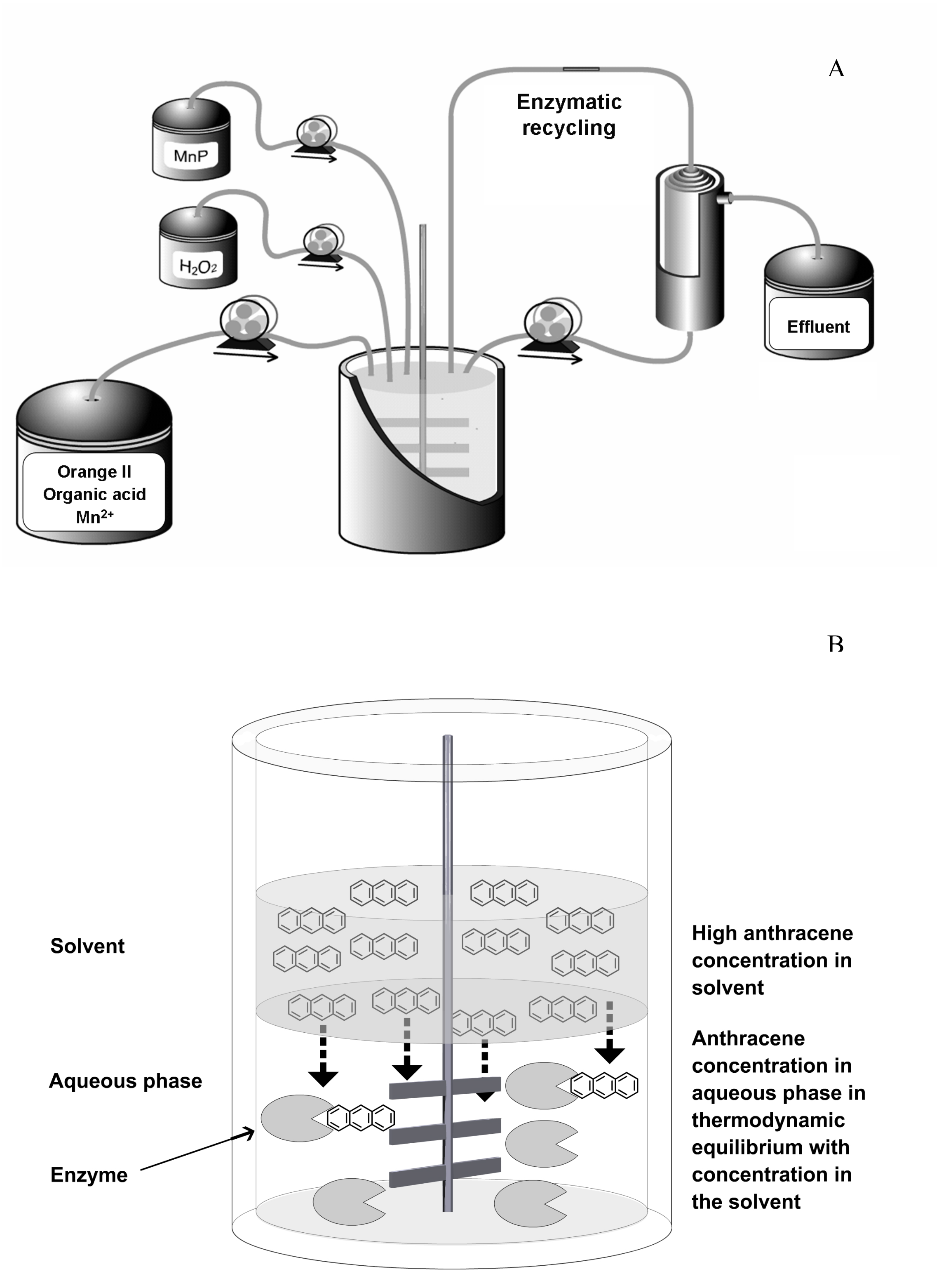

A
MnP
H_2O_2
Enzymatic recycling
Effluent
Orange II
Organic acid
Mn^{2+}
B
Solvent
High anthracene concentration in solvent
Anthracene concentration in aqueous phase in thermodynamic equilibrium with concentration in the solvent
Aqueous phase
Enzyme

Figure 2

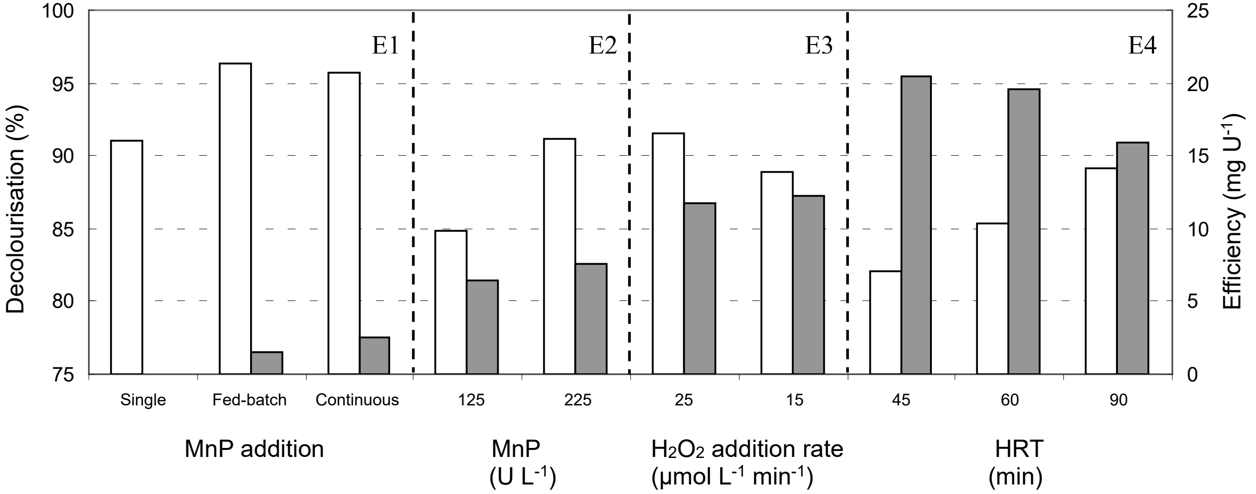
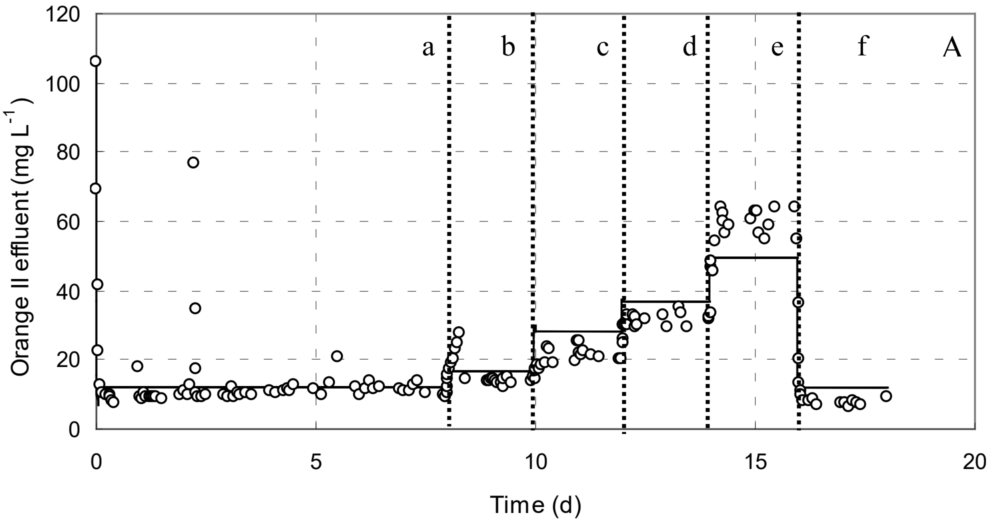

100
95
90
85
80
75
Decolourisation (%)
25
20
15
10
5
0
Efficiency (mg U^{-1})
E1
E2
E3
E4
Single
Fed-batch
Continuous
125
225
25
15
45
60
90
MnP addition
MnP (U L^{-1})
H_2O_2 addition rate (µmol L^{-1} min^{-1})
HRT (min)

Figure 3


Orange II effluent (mg L^{-1})
0
20
40
60
80
100
120
Time (d)
0
5
10
15
20
a
b
c
d
e
f
A

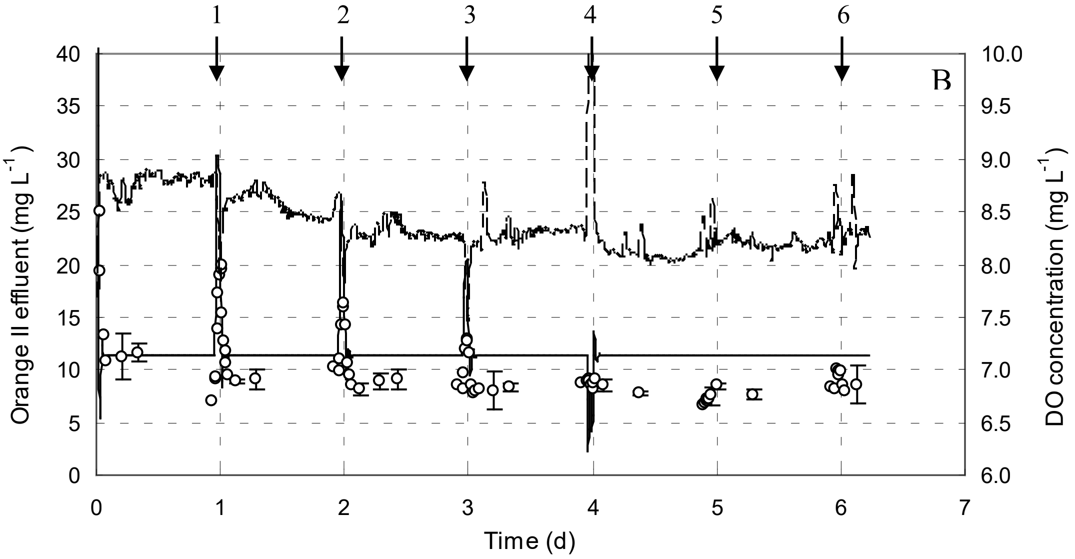

1
2
3
4
5
6
B
Orange II effluent (mg L^{-1})
0
5
10
15
20
25
30
35
40
DO concentration (mg L^{-1})
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
Time (d)
0
1
2
3
4
5
6
7

Figure 4

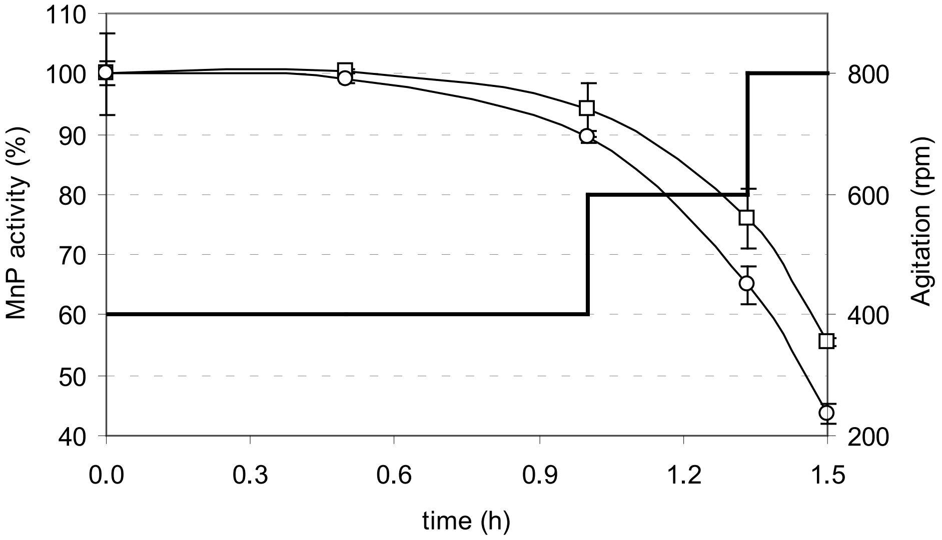

110
100
90
80
70
60
50
40
MnP activity (%)
0.0
0.3
0.6
0.9
1.2
1.5
time (h)
800
600
400
200
Agitation (rpm)

Figure 5

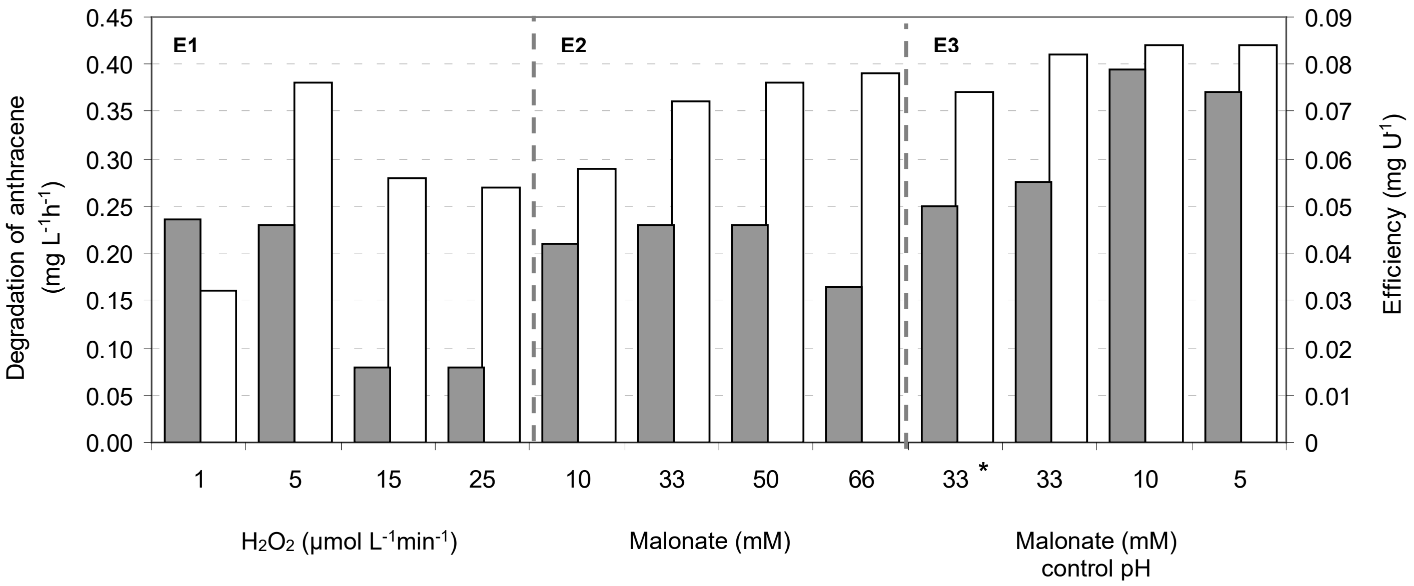

E1
E2
E3
Degradation of anthracene (mg L^{-1}h^{-1})
Efficiency (mg U^{-1})
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1
5
15
25
10
33
50
66
33 *
33
10
5
H$_2$O$_2$ (µmol L^{-1}min^{-1})
Malonate (mM)
Malonate (mM)
control pH

Figure 6

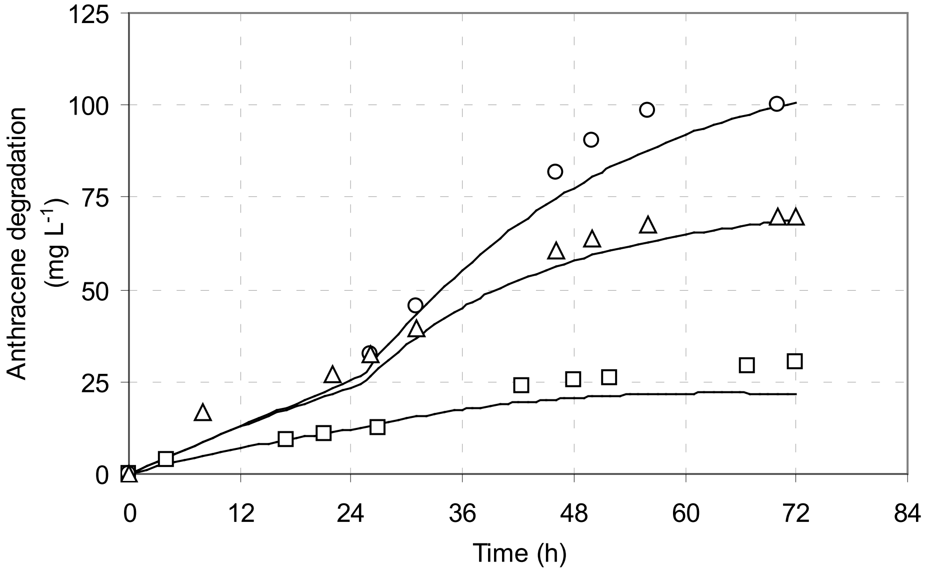

Anthracene degradation (mg L^{-1})
0
25
50
75
100
125
0
12
24
36
48
60
72
84
Time (h)

Figure 7