ARTICLE

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The Use of an Oil Absorber as a Strategy to Overcome Starvation Periods in Degrading 1,2-Dichloroethane in Waste Gas

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ABSTRACT: This work investigates the use of an oil absorber as an operational strategy in vapor phase bioreactors exposed to starvation periods, during the treatment of inhibitory pollutants. After being exposed to 1,2-dichloroethane (DCE) starvation periods, the response and stability of a combined oil-absorber-bioscrubber (OAB) system was compared to that of a bioscrubber only (BO) system. In the BO system, after a 5.2 days starvation period, the DCE removal efficiency was reduced to 12%, and 6 days were needed to recover the initial removal efficiency when the DCE feed resumed. The total organic discharged (TOD_{DCE}) was 16,500 g_{DCE} $m^{-3}_{bioscrubber}$ after the DCE starvation. Biomass analysis performed using fluorescence in situ hybridisation (FISH) showed that the microbial activity was significantly reduced during the starvation period and that 5 days were needed to recover the initial activity, after the re-introduction of DCE. In contrast, the performance of the OAB system was stable during 5.2 days of DCE starvation. The DCE removal efficiency was not affected when the DCE feed resumed and the TOD_{DCE} was significantly reduced to 2,850 g_{DCE} $m^{-3}_{bioscrubber}$. During starvation, the activity of the microbial culture in the OAB system showed a substantially lower decrease than in the BO system and recovered almost immediately the initial activity after the re-introduction of DCE. Additionally, a mathematical model describing the performance of the OAB system was developed. The results of this study show that the OAB system can effectively sustain the biological treatment of waste gas during starvation periods of inhibitory pollutants.

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KEYWORDS: absorber; bioscrubber; starvation period; 1,2-dichloroethane; mathematical model; fluorescence in situ hybridisation

Introduction

Biological treatment plants operated for the removal of pollutants from waste streams are often exposed to periods of no chemical load (starvation periods). The length of such periods can range from a few hours (due to facility operation for a fraction of the day) to a few weeks or months (shut down for factory retooling, equipment maintenance, holiday breaks, process changes) and can negatively affect the elimination capacity of biological processes. Various studies have previously reported the negative effects of starvation periods on microbial activity (Buitron and Capdeville, 1995; Buitron et al., 1992, 1993; Pacheco et al., 2003), cell viability (Buitron et al., 1994), biomass yield (Neubauer et al., 1995), endogenous respiration rate, ATP content and biomass level (Konopka et al., 2002). The above suggest that starvation periods may require microorganisms to re-acclimate to differences in loading when the chemical load is re-applied (Martin and Loehr, 1996).

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Re-acclimation periods can be either very short or very long depending on the nature of the pollutants, operating conditions and composition of the microbial population (Moe and Qi, 2004). Therefore, a broad range of different system responses have been reported. Some systems are robust and have the ability to survive after long starvation periods, ranging from a few months to a year, even with minimal lag phase (Carvalho et al., 2001; Ferreira Jorge, 2000; Hekmat et al., 2004; Pacheco et al., 2003). Other systems are able to respond to shorter load interruptions (1–2 weeks), within the first few hours after substrate re-introduction (Cox and Deshusses, 2002; Marek et al., 2000; Wani et al., 1998, 2000), while some systems need 3–10 days for full recovery (Lackey et al., 1998; Moe and Qi, 2004). Although cyclical starvation periods caused by shift work operations may result in an improvement of the overall elimination capacity, after the re-introduction of the feed 6–7 h bioreactor response time might be required (Fitch et al., 2002; Hekmat et al., 1997). The studies presented above indicate that a robust technology is needed to overcome the negative effects of starvation periods.

Minimising or eliminating re-acclimation periods could be achieved by either (i) the artificial addition of the pollutant during shut down of the feed or (ii) the installation of an absorption or adsorption unit prior to the bioreactor to release the pollutant slowly during starvation. Ferreira Jorge and Livingston (2000) maintained a high concentration of active bacteria during starvation, with the introduction of a ''maintenance feed'' consisting of 2% of the total carbon fed at steady state, decreasing significantly the organic mass discharged after substrate re-introduction. Granular activated carbon (GAC) has been applied both to control fluctuating pollutant concentrations prior to a bioreactor (Weber and Hartmans, 1995) or to supply the pollutant to immobilised cells during starvation periods (Carvalho et al., 2001). However, GAC systems can be severely affected by high moisture contents of waste gas, or slow rates of desorption (Al-Rayes et al., 2001). Recently, the potential of cost effective and environmentally friendly organic absorbents was exploited for the control of inhibitory concentrations of pollutants and starvation periods in bioscrubber systems (Koutinas et al., 2006; Nielsen et al., 2005; Oliveira and Livingston, 2003). Such systems were able to successfully control sudden fluctuations in the pollutant load; however, their application during starvation periods has not yet been extensively tested.

The present work investigates the potential of an oil absorber placed upstream of a bioscrubber to stabilise the biological treatment of Dichloroethane (DCE) containing waste gas during starvation periods. The stability of the oil-absorber-bioscrubber (OAB) and the bioscrubber only (BO) systems were compared under DCE starvation periods and a mathematical model was used to describe the operation of the two systems. In parallel, the microbial culture dynamics were monitored using fluorescence in situ hybridisation (FISH).

Materials and Methods

Cultivation of Microorganism

Subcultures of *Xanthobacter autotrophicus* GJ10 (ATCC no. 43050) used for bioscrubber inoculation, were grown under mineral medium and conditions described by Koutinas et al. (2006).

Experimental Set-Up

The experimental set up is shown in Figure 1. The total flow rate of air influent to the system was 0.3 L min^{-1}, giving a volumetric gas flow rate per bioscrubber volume of 0.21 min^{-1}. The DCE concentration in the inlet gas stream was controlled as described by Oliveira and Livingston (2003). Gas Direction I was followed when the oil absorber was not used and the waste gas was introduced directly to the bioscrubber. The total volume of the bioscrubber was 1.8 L (SGI ''30/SET002'', SGE, Toulouse, France) and it was operated with 1.4 L working volume. A Watson Marlow 502S (Watson-Marlow Bredel Products, Northampton, UK) peristaltic pump was used to feed the bioscrubber with mineral medium continuously at a dilution rate of 0.049 h^{-1}. The dissolved oxygen concentration was monitored via an Ingold oxygen probe (Mettler Toledo Ltd., Leicester, UK), pH was controlled at 7 ± 0.05 with the addition of 2 M H_2SO_4 or 2 M NaOH and temperature was maintained constant at 30°C. A stainless steel sparger was used to supply the inlet gas stream to the bioscrubber and two impellers rotating at 1,000 rpm were used for gas dispersion. The oil absorber was a glass column (50 cm height, 5 cm i.d.) divided into a packed section with 27 cm high bed of pall rings, and an oil reservoir. The gas stream inlet was between the two sections, at 10 cm column height and the two streams (gas and oil) flowed in counter-current mode. The oil (Pure Sunflower Oil, Tesco Stores Ltd., Dundee, UK) was recirculated through the column at a flow rate of 1 L min^{-1} via a gear pump (model 130-000-110, Cole-Parmer Instrument Company Ltd., London, UK). A temperature controller was employed to maintain the column temperature constant at 26°C, utilising a thermo-couple and a heating coil.

Chemicals

All chemicals used were obtained from Merck (Lutterworth, UK) and were of ANALAR grade. DCE was obtained from Sigma (Gillingham, UK) 99% pure.

Analysis

Carbon dioxide, biomass and DCE concentrations in the gaseous and aqueous samples, as well as the bacterial

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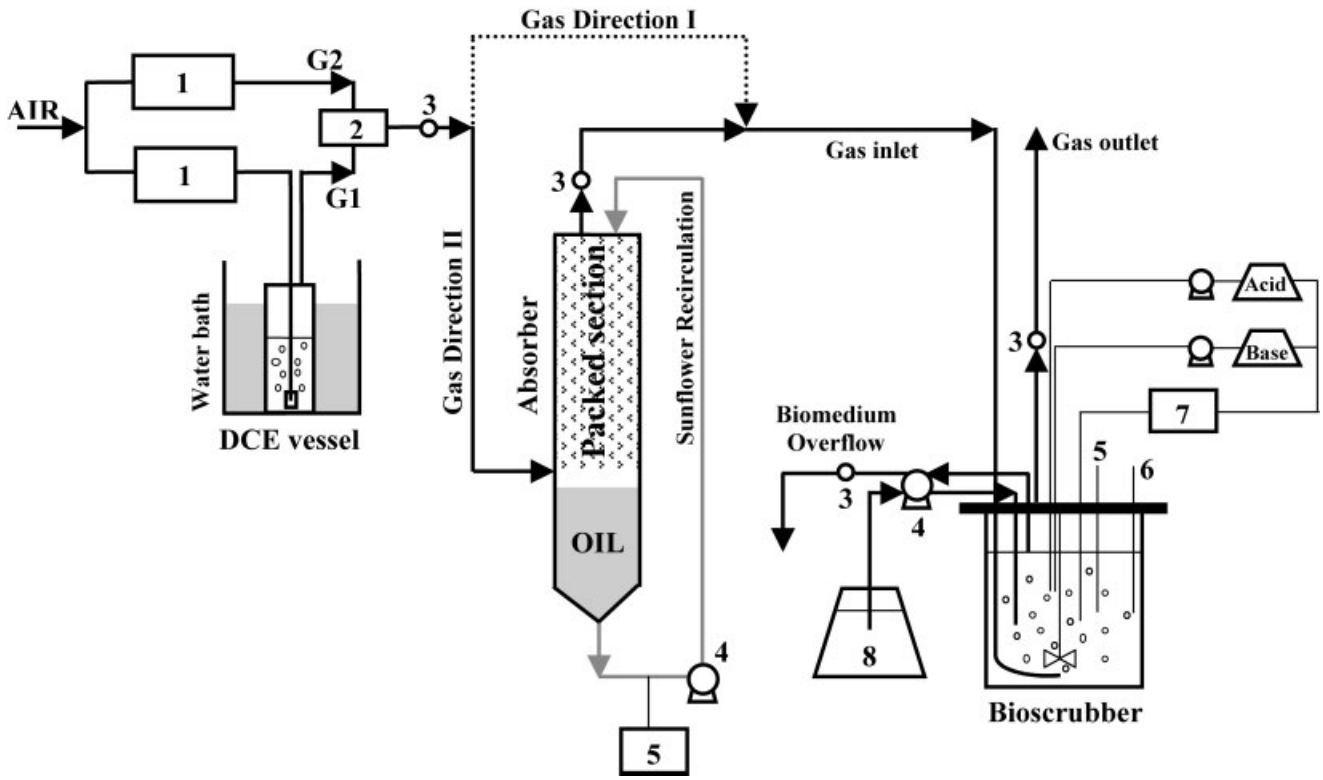

Figure 1. Experimental set-up. Gas direction I was followed when the waste-gas was introduced directly to the bioscrubber, bypassing the absorber. In gas direction II the waste-gas passed through the absorber and was fed to the bioscrubber. (1) Mass flow controller; (2) mixing vessel; (3) sampling port; (4) pump; (5) temperature controller; (6) dissolved oxygen meter; (7) pH controller; (8) mineral medium; G1, G2: gas streams.

population analysis were determined as previously described (Koutinas et al., 2006).

Estimation of Model Parameters

Henry's law coefficient for DCE (H_{DCE}) was measured experimentally. Water samples containing DCE concentrations ranging between 0 and 4 $g_{DCE}\,L^{-1}$ were prepared. One millilitre of each sample was placed in 2 ml Teflon coated closed vials, vortexed for 1 min and left at room temperature overnight. After equilibrium was established between the two phases, the headspace DCE concentration was measured by Gas Chromatography to obtain H_{DCE}.

The volumetric mass transfer coefficient $(K_La)_{DCE}$ was estimated from Equation 5 (presented below). The rest of the parameters used in Equation 5 for estimation of $(K_La)_{DCE}$ were measured experimentally during steady state operation of the bioscrubber.

Bacterial Population Analysis

The determination of the total number of cells, active cells, viable cells and *X. autotrophicus* strain GJ10 used for the analysis of the bioscrubber microbial dynamics was performed as previously described (Koutinas et al., 2006).

Mathematical Modelling

A mathematical model based on unstructured biological kinetics was developed for the system based on Chang's model (Chang et al., 2005). The following assumptions were made:

Oil Absorber

- The partition coefficient for DCE between sunflower oil and air is temperature dependent;
- the liquid and gas phases in the oil absorber are perfectly mixed;
- no volume change occurs upon absorption.

Bioscrubber

- Unstructured model, therefore there is no division between different types and ages of cells;
- the liquid phase in the reactor is well mixed;

- biomass can grow in suspension and as a biofilm on the walls of the reactor;
- the biofilm is considered as a uniform, homogenous and non-porous solid;
- the concentration of cells in the biofilm (X_f) is uniform.
- the biofilm occupies a small fraction of the bioreactor volume ($V_v \sim V_b$);
- the mineral medium fed to the reactor is sterile;
- Ferreira Jorge and Livingston (1999) showed that Luong (1986) kinetics describe the microbial kinetics for *X. autotrophicus* strain GJ10 growing on DCE.

The kinetics of the wall-attached biomass is assumed to be growth-limited both by DCE and oxygen. Thus, the specific growth rate in the biofilm is described by the following equation:

$$\mu_f = \frac{\mu_{max} S_f}{K_s + S_f}\left(1 - \frac{S_f}{S_m}\right)\frac{C_f}{C_f + K_{O_2}} \quad (1)$$

- During the experimental runs, no oxygen limitation was observed for the cells in suspension. Therefore, in order to simplify the mathematical model, DCE is considered as the only source of carbon and energy and as the only growth-limiting substrate for the cells in suspension;
- the biomass yield coefficients for DCE and oxygen are assumed to be constant.

The OAB can be described by the following system of equations. The DCE mass balance in the gas stream and the accumulation of DCE in the sunflower oil contained in the oil absorber unit are expressed by Equations 2 and 3, respectively. Also, the sunflower oil partition coefficient for DCE (Eq. 4) is temperature dependent as described previously (Koutinas et al., 2006).

$$G(C_{a,in} - C_{a,out}) = (K_L a)_{oil}(C_{a,out} P_{sun,oil} - C_{sun,oil}) V_{oil} \quad (2)$$

$$(K_L a)_{oil}(C_{a,out} P_{sun,oil} - C_{sun,oil}) = \frac{dC_{sun,oil}}{dt} \quad (3)$$

$$P_{sun,oil} = -12.1T + 1049.6 \quad (4)$$

For the bioscrubber unit of the OAB system, the DCE mass balance in the gas stream is expressed by Equation 5, while the DCE and the biomass balances are expressed by Equations 6 and 7 respectively. The third term on the right hand side of Equation 6 accounts for the DCE consumption in the biofilm. The last term of Equation 7 expresses the rate of biomass detachment from the biofilm. The microbial kinetics for *X. autotrophicus* strain GJ10 growing on DCE follow Luong kinetics (Luong, 1986) and include an inhibitory effect with increasing DCE concentrations up to 1,080 $g_{DCE}\ m^{-3}$ (Eq. 8).

$$G(C_{a,out} - C_{b,out}) = (K_L a)_{DCE}\left(\frac{C_{b,out}}{H_{DCE}} - S_b\right) V_b \quad (5)$$

$$(K_L a)_{DCE}\left(\frac{C_{b,out}}{H_{DCE}} - S_b\right) V_b = M S_b + \frac{\mu_b}{Y_{DCE}} X_b V_b + k_f A (S_b - S_f|_{z=L_f}) + \frac{dS_b}{dt} V_b \quad (6)$$

$$\frac{dX_b}{dt} V_b = (\mu_b - b_d) X_b V_b - M X_b + A b_s L_f X_f \quad (7)$$

$$\mu_b = \frac{\mu_{max} S_b}{K_s + S_b} + \left(1 - \frac{S_b}{S_m}\right) \quad (8)$$

The variations in DCE and oxygen concentrations in the biofilm are described by Equations 9 and 11 which are subjected to the boundary conditions given by Equations 10 and 12. At $z = 0$, the consumption of DCE and oxygen is not permitted due to the non-porous solid (interface between the walls of the reactor and the biofilm).

$$\frac{dS_f}{dt} = D_f \frac{d^2 S_f}{dZ^2} - \mu_f \frac{X_f}{Y_{DCE}}; \quad 0 < z < L_f \quad (9)$$

$$\frac{dS_f}{dz} = 0, \text{ at } z = 0; \quad D_f \frac{dS_f}{dz} = k_f (S_b - S_f), \text{ at } z = L_f \quad (10)$$

$$\frac{dC_f}{dt} = D_{of} \frac{d^2 C_f}{dz^2} - \mu_f \frac{X_f}{Y_{O_2}}; \quad 0 < z < L_f \quad (11)$$

$$\frac{dC_f}{dz} = 0, \text{ at } z = 0 \quad D_{of} \frac{dC_f}{dz} = k_{of} (C_B - C_f), \text{ at } z = L_f \quad (12)$$

Cells attached to the biofilm grow due to the utilisation of the substrate, can get inactive and can be removed as a result of the biomedium in motion. Taking into account the above, Equation 13 can describe the biomass balance in the biofilm, as well as the biofilm thickness over time.

$$\frac{dL_f}{dt} = \int_0^{L_f} [\mu_f - b_d - b_s] dz \quad (13)$$

Equations 1–13 were solved using gPROMS (Process Systems Enterprise, London, UK). The parameters used in the model are presented in Table I. The input values of the bioscrubber inlet DCE gas concentration ($C_{a,out}$) for System I operation and the oil absorber inlet DCE gas concentration

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Table I. Values of parameters used in the model.

Parameter	Value	Reference
A	11.04 dm^2	Present study
b_d	2.14×10^{-7} s^{-1}	Lin and Lee (2001)
b_s	3.322×10^{-6} s^{-1}	Fouad and Bhargava (2005)
C_B	2.5 mg L^{-1}	Present study
D_f	6.66×10^{-7} dm^2 s^{-1}	Freitas dos Santos and Livingston (1995)
D_{of}	1.54×10^{-7} dm^2 s^{-1}	Freitas dos Santos and Livingston (1995)
G	0.005 L s^{-1}	Present study
H_{DCE}	0.0562 [−]	Present study
K_s	7.8 mg L^{-1}	Ferreira Jorge (2000)
k_f	2.2×10^{-4} dm s^{-1}	Zhang et al. (1998)
k_{of}	9.4×10^{-4} dm s^{-1}	Nicolella et al. (2000)
$(K_La)_{DCE}$	7.486×10^{-4} s^{-1}	Present study
$(K_La)_{oil}$	5×10^{-5} s^{-1}	Koutinas et al. (2006)
K_{O_2}	0.01 mg L^{-1}	Freitas dos Santos and Livingston (1995)
M	1.906×10^{-1} L s^{-1}	Present study
S_m	1,080 mg L^{-1}	Ferreira Jorge (2000)
V_b	1.4 L	Present study
V_{oil}	0.56 L	Present study
X_f	5.7×10^4 mg L^{-1}	Zhang et al. (1998)
Y_{DCE}	0.23 g_{biom} g_{DCE}^{-1}	Ferreira Jorge (2000)
Y_{O_2}	0.304 g_{biom} g_{O2}^{-1}	Freitas dos Santos and Livingston (1995)
μ_{max}	4.167×10^{-5} s^{-1}	Ferreira Jorge (2000)

($C_{a,in}$) for System II operation, were estimated according to the experimental loading introduced to the system each time. The biofilm shear loss coefficient (b_s) and the coefficient for cell death (b_d) were derived from the literature for the detachment and decay of sludge cells from a biofilm. The mass transfer coefficients for DCE and oxygen in the biofilm (k_f, k_{of}) were also taken from the literature. k_f was obtained for the transport of trichloroethylene in a GJ10 biofilm and k_{of} for the transport of oxygen in a mixed microbial culture biofilm degrading monochloro-benzene. Finally, the dissolved oxygen concentration in the bulk biological liquid (C_B) was measured experimentally and it was observed that did not vary significantly with time during the experimental runs. Therefore, the experimental value monitored was used for the simulations.

Results and Discussion

System I: Bioscrubber Only (BO)

For System I experiments the oil absorber was not connected and the DCE contaminated gas stream was fed directly to the bioscrubber. During the interruption of the DCE feed, air was introduced into the bioscrubber at the same flow rate as before. The other settings of the system (dilution rate, bioscrubber temperature control, pH control, stirring speed) were maintained constant.

Starvation Period I.1

DCE was fed continuously to the bioscrubber at an average DCE loading rate of 129 g m^{-3} h^{-1}. When constant DCE removal efficiency was observed, given by DCE outlet loading rate of 4 g m^{-3} h^{-1} and no DCE detected in the biomedium, the feeding of DCE was stopped for a period of 2 days, and then subsequently returned to an average loading rate of 142 g m^{-3} h^{-1} (Fig. 2A). After the re-introduction of DCE, the values of the DCE outlet loading rate and the biomedium DCE concentration increased significantly. These parameters increased from 4 g m^{-3} h^{-1} and 0 g m^{-3} before the starvation period, to maximum values of 45 g m^{-3} h^{-1} and 45 g m^{-3} respectively, 1 h after the DCE feed was resumed (Fig. 2A and C). Thus, the 2 days starvation period resulted in reduction of the DCE removal efficiency from an average value of 97% before the starvation, to a minimum of 62%, 2.5 h after the substrate was re-introduced. However, the DCE removal efficiency increased to values higher than 78%, 8.5 h after the re-introduction of DCE. The increase of the bioscrubber DCE outlet load after the starvation period resulted in $TOD_{DCE} \sim 1900$ g_{DCE} m_{biosc}^{-3}. Total organic discharged (TOD_{DCE}) was measured starting at the end of the starvation and finishing at the end of the monitoring of the experiment (time range 48–125.5 h, Fig. 2A and C).

The production of carbon dioxide followed the same trend as the DCE inlet loading rate to the system. When the DCE feed was stopped, the carbon dioxide concentration decreased from an average value of 0.52% v/v to 0.11% v/v after 2.25 h. However, the carbon dioxide concentration recovered to values close to the initial concentration (before the starvation period) at least 6 h after DCE was re-introduced. This fact indicates that after the 2 days of DCE starvation, a relatively short re-acclimation period of 6 h was needed for the system to readapt to the DCE loading. Finally, the suspended biomass concentration was significantly reduced from 4038 g m^{-3} before the starvation to a minimum of 1354 g m^{-3} due to wash out during the starvation period.

Starvation Period I.2

The BO configuration was also challenged with a longer DCE starvation period and at higher DCE loadings. Initially the bioscrubber was fed with an average DCE loading rate of 198 g m^{-3} h^{-1} until constant DCE removal efficiency 76% was observed. Under steady state conditions, the DCE feed was stopped for a period of 5.2 days and was re-introduced at an average loading rate of 277 g m^{-3} h^{-1}. The results presented in Figure 2B and D indicate that a longer starvation period combined with increased loading rate (in comparison to Starvation Period I.1) may cause a series of unfavourable effects on the system, especially when inhibitory substrates are fed. When the DCE feed was resumed, the DCE outlet loading rate and the biomedium DCE concentration average values increased from 47 g m^{-3} h^{-1}

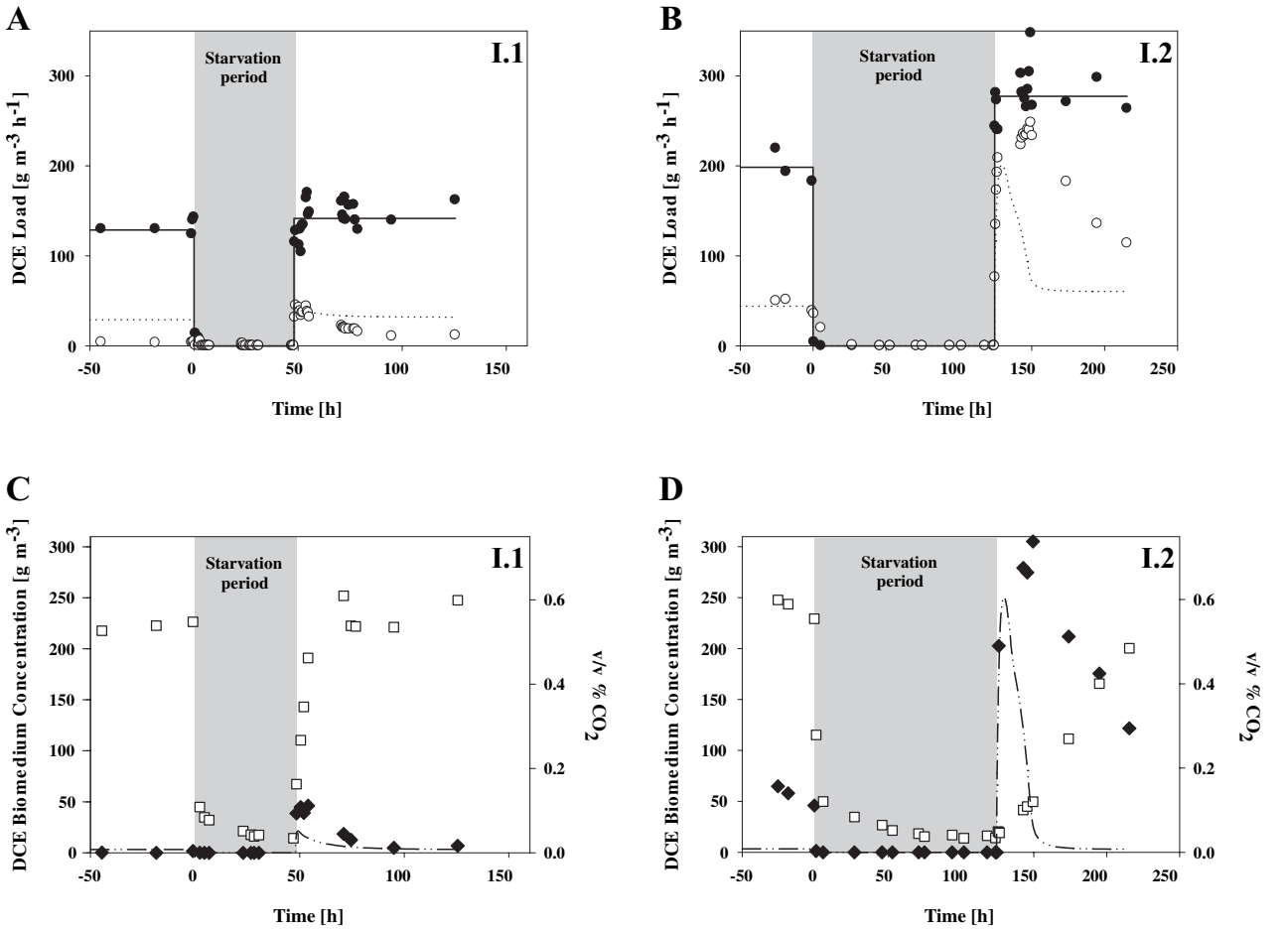

Figure 2. Evolution of DCE inlet loads, outlet loads, biomedium DCE concentration and outlet carbon dioxide (% v/v) during the DCE starvation periods applied to System **I**. Figures are given as loadings per $m^3_{bioscrubber}$. **A** and **C**: Starvation I.1 (2 days). **B** and **D**: Starvation I.2 (5.2 days). (●), Bioscrubber DCE inlet load—experimental; (○) bioscrubber DCE outlet load—experimental; (◆), biomedium DCE concentration—experimental; (□), outlet carbon dioxide% v/v—experimental; (———), bioscrubber DCE inlet load—imposed; (·····), bioscrubber DCE outlet load—predicted; (— ··), biomedium DCE concentration—predicted.

and 56 g m^{-3} before the starvation period, to maximum values of 248 g m^{-3} h^{-1} and 305 g m^{-3} respectively, 25 h after the re-introduction of DCE. Thus, the 76% average DCE removal efficiency observed before the starvation period, dropped severely during the first few hours following the restart of the DCE feed to 12% (22 h after DCE re-introduction). After the first 22 h the DCE removal efficiency started increasing again, though it reached the initial efficiency (>70%) 6 days after the DCE feed was re-introduced. TOD_{DCE} measured between time 124.5 and 215 h (Fig. 2B and D) was ~16,500 g_{DCE} m^{-3}_{biosc}, signifying that a considerably higher amount of DCE was not treated as a result of the 5.2 days starvation period.

In the beginning of the starvation period, the carbon dioxide concentration decreased rapidly from an average value of 0.58% v/v to 0.12% v/v within 5.5 h. After 2 days of DCE starvation (Experiment I.1) the time period needed for the culture to produce the initial carbon dioxide concentration was only 6 h, while after 5.2 days of starvation the time period needed for the carbon dioxide concentration to increase to 0.48% v/v was 4 days. During the starvation period the suspended and the wall attached biomass decreased over time. Thus, at the end of the starvation period it was observed that the biofilm on the bioscrubber walls was significantly reduced (only traces could be detected), while the suspended biomass concentration was reduced from 2437 g m^{-3} before the starvation to 81 g m^{-3}. However, due to the biofilm remaining on the walls after the DCE starvation, the system was able to recover to its initial performance. Nevertheless, DCE removal was very poor for the first 5 days after the starvation, having a possible negative impact on the environment.

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System II: Oil-Absorber-Bioscrubber (OAB)

The oil absorber was placed upstream of the bioscrubber in order to provide DCE to the microbial culture during starvation periods, due to the desorption of DCE from sunflower oil (Fig. 1). As in System I experiments, during the interruption of the DCE feed, air was introduced into the oil absorber at the same flow rate as before, while the other settings of the system were maintained constant.

Starvation Period II.1

The oil absorber was fed with average DCE inlet load of 160 g m^{-3} h^{-1}, resulting in an average bioscrubber inlet load of 148 g m^{-3} h^{-1} (Fig. 3A). The average values of parameters such as DCE removal efficiency, carbon dioxide concentration and biomedium DCE concentration were 90%, 0.53% v/v and 7 g m^{-3} respectively, showing that similar conditions to Starvation Period I.1 were achieved. At time 0 (Fig. 3A and C) the DCE feed to the system was stopped for 2 days. Although during the starvation period there was no DCE present in the process inlet, the absorbed DCE into the sunflower oil was desorbed over time and supplied to the bioscrubber. Thus, the DCE inlet load to the bioscrubber decreased over time to reach a minimum of 28 g m^{-3} h^{-1} after 2 days. The system continued to remove the DCE fed to the bioscrubber during the starvation with average removal efficiency 93% and as shown at Figure 3C the carbon dioxide concentration decreased proportionally to the decrease in the DCE inlet load to the bioscrubber. Therefore, at time 48 h (Fig. 3A) the DCE inlet load to the bioscrubber was only 20% of the average inlet load before the starvation and respectively the percentage v/v carbon dioxide concentration reached 26% of the initial average value. The suspended biomass concentration decreased from 2,442 g m^{-3} before the starvation to 742 g m^{-3} at the end of the starvation.

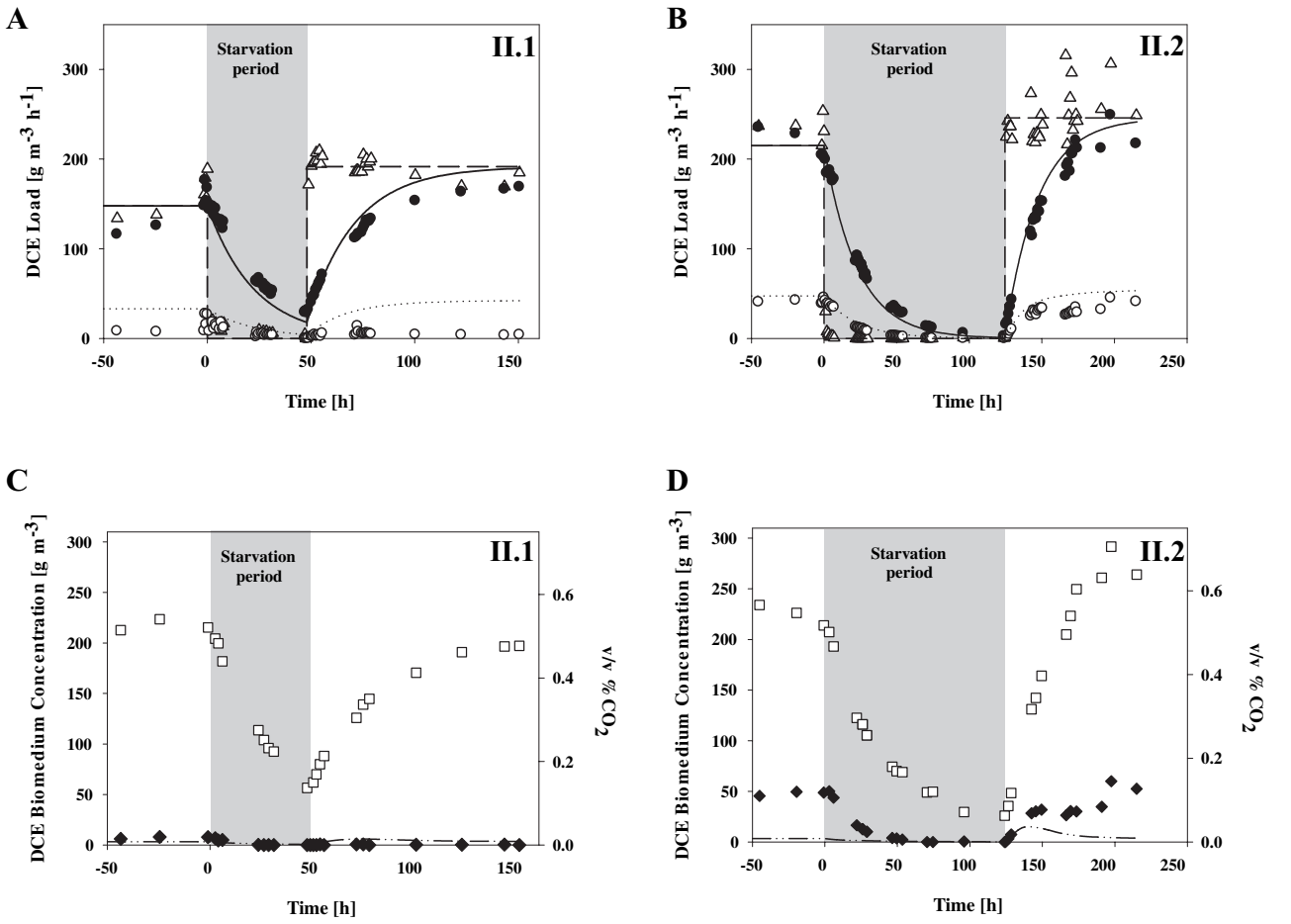

Figure 3. Evolution of DCE inlet loads, outlet loads, biomedium DCE concentration and outlet carbon dioxide (% v/v) during the DCE starvation periods applied to System **II**. Figures are given as loadings per $m^3_{bioscrubber}$. **A** and **C**: Starvation II.1 (2 days). **B** and **D**: Starvation II.2 (5.2 days). (△), Absorber DCE inlet load—experimental; (●), bioscrubber DCE inlet load—experimental; (○), bioscrubber DCE outlet load—experimental; (◆), biomedium DCE concentration—experimental; (□), outlet carbon dioxide% v/v—experimental; (– – –), absorber DCE inlet load—imposed; (———), bioscrubber DCE inlet load—predicted; (· · · · · · · · ·), bioscrubber DCE outlet load—predicted; (— · ·), biomedium DCE concentration—predicted.

At time 48 h, the DCE feed was resumed and an average loading of 191 g m^{-3} h^{-1} was introduced to the system. Due to the absorption of DCE into the oil, the DCE inlet load to the bioscrubber increased slowly over time. Therefore, the absorption process allowed sufficient time for the reduced (due to the starvation) biomass content to grow both in suspension and on the walls, showing the beneficial effects of the oil absorber comparing to the BO configuration. The TOD_{DCE} was significantly less than in Starvation Period I.1 reaching ~300 g_{DCE} m^{-3}_{biosc} calculated for the same time period (time range 48–125.5 h, Fig. 3A and C). Obviously, acclimation of the microbial culture after the DCE re-introduction was not needed in this case as the DCE removal efficiency remained higher than 88% and the carbon dioxide concentration increased proportionally to the increase of the bioscrubber DCE inlet load. Furthermore, the biomedium DCE concentration was always 0 g m^{-3} since the DCE feed was resumed.

Starvation Period II.2

A similar starvation period to Starvation Period I.2 was also applied in the OAB system. The oil absorber was fed with average DCE inlet load of 235 g m^{-3} h^{-1} which resulted in an average DCE bioscrubber inlet load of 214 g m^{-3} h^{-1} (Fig. 3B). The DCE removal efficiency of the bioscrubber was 81%, the bioscrubber DCE outlet load 41 g m^{-3} h^{-1}, the carbon dioxide concentration 0.54% v/v and the biomedium DCE concentration 48 g m^{-3}. Under steady state conditions, the DCE feed to the system was stopped for a period of 5.2 days. During the starvation period, DCE was still supplied to the bioscrubber due to the desorption of DCE from sunflower oil. However, the bioscrubber DCE inlet load was reduced over time and reached a minimum value of 2 g m^{-3} h^{-1} at the end of the starvation period. The bioscrubber DCE outlet load and the biomedium DCE concentration were reduced to 0 g m^{-3} h^{-1} and 0 g m^{-3} minimum values. Furthermore, the carbon dioxide concentration decreased proportionally to the decrease of the DCE inlet load to the bioscrubber (Fig. 3D).

After 5.2 days, the substrate feed to the system was resumed to an average DCE inlet loading of 246 g m^{-3} h^{-1}. Once again, due to the absorption of DCE into the oil, the DCE inlet load to the bioscrubber increased slowly over time. The bioscrubber DCE inlet load increased to higher values than 200 g m^{-3} h^{-1} after 46 h, thus giving enough time to the biological process to recover completely. For the whole duration of this experiment the DCE removal efficiency was always higher than 77% and the bioscrubber DCE outlet load did not reach higher values than 45 g m^{-3} h^{-1} at any time. The TOD_{DCE} discharged untreated after the re-introduction of DCE was considerably lower than in Starvation Period I.2 ~2,850 g_{DCE} m^{-3}_{biosc} versus 16,500 g_{DCE} m^{-3}_{biosc} (time range 124.5–215 h, Figs. 2B and D, 3B and D respectively). The suspended biomass concentration was reduced from 2432 g m^{-3} before the starvation to 447 g m^{-3} at the end of the starvation. Thus, the microbial culture was maintained in the bioscrubber at higher concentration than in Starvation Period I.2 due to the continuous supply of DCE to the bioscrubber during the starvation. The carbon dioxide concentration increased proportionally to the increase of the DCE inlet load to the bioscrubber. Therefore, there was no delay monitored for the bioconversion of DCE to carbon dioxide, due to re-acclimation of the culture to DCE when the substrate feed was resumed.

Mathematical Modelling Results

The mathematical model can effectively describe the operation of the oil absorber. As shown in Figure 3A and B the bioscrubber DCE inlet load is calculated by the model successfully during the starvation and the substrate re-introduction periods. The model predictions generally follow the trends for the bioscrubber DCE outlet load and the biomedium DCE concentration in the experiments. However, there is a discrepancy observed between the model calculated and the experimental values, for the bioscrubber DCE outlet load and the biomedium DCE concentration in the experiments.

The discrepancy described above is more obvious during the substrate re-introduction for Starvation Period I.2. During this period the model gives maximum values for the bioscrubber DCE outlet load and the DCE biomedium concentration of 201 g m^{-3} h^{-1} and 251 g m^{-3} respectively (Fig. 2B and D). Also, the maximum values of these parameters were reduced to the same level as at steady state after approximately 1 days. On the other hand, the experimentally measured maximum values of the bioscrubber DCE outlet load and the DCE biomedium concentration were 248 g m^{-3} h^{-1} and 305 g m^{-3} respectively. Furthermore, the values mentioned above were reduced to the steady state values after 6 days. Therefore, in practice the system required much longer time period (6 days) to recover to the steady state performance than the time period calculated by the model (1 day). The inconsistency between the model calculated and the experimental values can be attributed to the fact that the reduction in the activity of the microbial culture during the starvation period is not taken into account by the model. Therefore, during the substrate re-introduction of Starvation Period I.1, the values calculated by the model are closer to the experimental values than for the same period of Starvation Period I.2, because the cells are expected to be more active after a shorter period of starvation. Furthermore, in the OAB system the calculations of the model for the bioscrubber DCE outlet load after the DCE starvation are also in agreement with the experimental values, because due to the effect of the oil absorber the microbial culture is expected to be more active than in the BO system even after a 5.2 days starvation period (Fig. 3).

In an attempt to get a better insight into the importance of different process parameters for the modelling results, we

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used the model to address the following questions: (i) Is the biofilm formation important for the results of the model? and (ii) If the biofilm formation is important, are the coefficients for cell death (b_d) and the biofilm shear loss coefficient (b_s) values important for the model calculations? It should be noted at this point that the values of these two parameters were not derived for the specific microbial culture used in this work. Figure 4 shows that when the biofilm is not taken into account by the model, there is a significant difference in the model calculations for the bioscrubber DCE outlet load. This fact is clear in Starvation Periods I.1, I.2 and II.2 where the model without the biofilm predicts much higher bioscrubber DCE outlet loads than when the biofilm is taken into account. Thus, the biofilm formation is important for both systems (BO and OAB) and should be included in the model. In order to estimate whether the calculations of the model are sensitive to the values of the coefficient for cell death (b_d) and the biofilm shear loss coefficient (b_s), simulations of the model were run with increasing b_d and b_s values ($b_d \times 2$, $b_d \times 3$, $b_s \times 2$, $b_s \times 3$). When the value of the coefficient for cell death was increased by a factor of two and three, there was no significant change in the calculations of the model for the bioscrubber DCE outlet load than when its actual value was used. However, Figure 4 shows that with increasing b_s value the bioscrubber DCE outlet load increases significantly after the starvation period. Thus, the model calculations are not very sensitive to the value of the coefficient for cell death, but on the other hand the value of the biofilm shear loss coefficient is very important.

Analysis of the Bioscrubber Microbial Dynamics Using FISH

Classical microbiology states that microbial populations are homogenous with respect to their physiological states. In continuous processes and especially when substrate concentration is unstable, this is not always the case. Fernandez et al. (1999) showed that a highly dynamic community can maintain a stable ecosystem function for years. Particularly when the microbial culture is under stress conditions, a dynamic community can be formed due to the development of other competing degrading species (Carvalho et al., 2001).

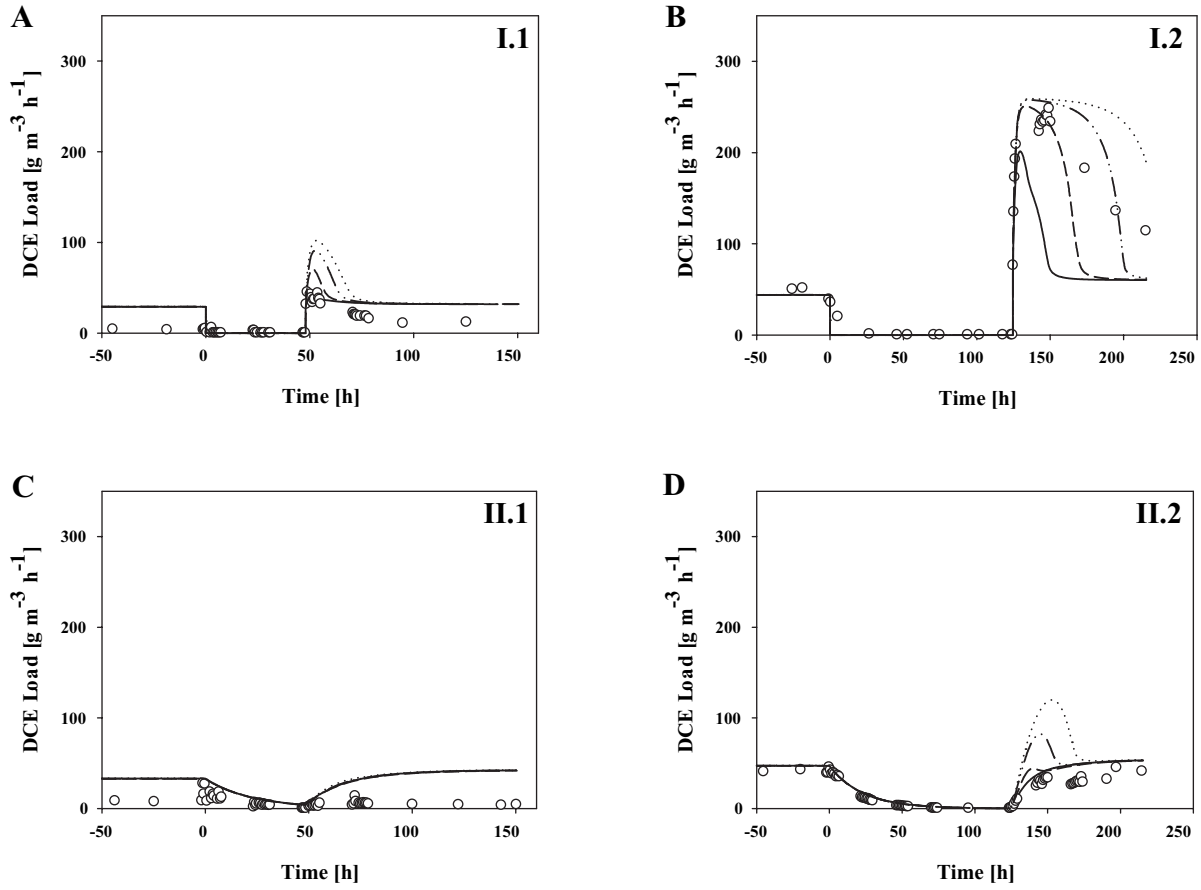

Figure 4. The effect of the biofilm formation and the biofilm shear loss coefficient value (b_s) in the model calculations. Figures are given as loadings per $m^3_{bioscrubber}$. **A** and **B**: Starvations **I.1**–**I.2**. **C** and **D**: Starvations **II.1**–**II.2**; (○), bioscrubber DCE outlet load—experimental; (———), bioscrubber DCE outlet load ($b_s \times 1$)—predicted; (– – –), bioscrubber DCE outlet load ($b_s \times 2$)—predicted; (— · ·), bioscrubber DCE outlet load ($b_s \times 3$)—predicted; (· · · · · · · · ·), bioscrubber DCE outlet load (without biofilm)—predicted.

The physiological responses of the culture to starvation periods can be very different to that of steady state (da Silva et al., 2005) and because microbial species have different decay rates, starvation periods can affect the relative abundance of species in the community (Moe and Qi, 2004). In contrast, Smith et al. (2003) observed that the bacterial community can be stable during normal operation, shut down and start-up of the bioreactor. The different responses of the microbial culture reported during steady state operation or disturbances in the substrate feed indicate that it would be very interesting to relate the functional stability of treatment processes to the degrading strain behaviour during dynamic conditions.

The relationship between the operational stability of the two systems (BO and OAB) and the behaviour of the microbial population was monitored during the starvation experiments. Figure 5 displays the evolution of active cells (% of active cells = 100% × EUB stained cells/DAPI stained cells) and strain GJ10 cells (% of GJ10 = 100% × GJ10 cells/EUB stained cells) for both systems tested.

During steady state operation prior to the 5.2 days starvation periods (Starvation Periods I.2 and II.2), similar performance of the microbial culture was observed for both systems. In the BO system, the active cells in the suspended biomass were 68% of the total number of cells detected, and only 3% of the active cells were strain GJ10 (Fig. 5A). Correspondingly, in the OAB system the active cells were 64% of the total number of cells and strain GJ10 was 5% of the active cells (Fig. 5B). However, the response of the microbial culture during the starvation period and after the DCE re-introduction was different for the two systems, in each case following the same trend as the functional stability. This fact is evident in the BO system due to the immediate decrease of the percentage of active cells to 22% after the first 4 days since the interruption of the DCE feed. The activity of the microbial culture recovered slowly after the DCE re-introduction, failing to re-establish the initial activity within the first 5 days (52% at day 10, Fig. 5A). The evolution of the microbial culture activity during and after the DCE starvation is in agreement with the performance of the BO system, which showed poor DCE removal for the first 5 days after the re-introduction of DCE. On the other hand, in the OAB system the decrease of the percentage of active cells was lower than in the BO system during the starvation (39% at day 3, Fig. 5B). Additionally, the recovery of the percentage of active cells to the initial level (>60% active cells) was rapid, achieved approximately 3 h after the re-introduction of DCE. The above was attributed to the constant supply of DCE to the bioscrubber due to the oil absorber that not only maintained higher biomass concentration but also sustained the activity of the culture at higher levels than in the BO configuration. Thus, due to the oil absorber the OAB system was able to rapidly achieve high DCE removal and low TOD_{DCE}, while the BO system had to re-acclimate to the substrate re-introduction for several days, leading to high TOD_{DCE}.

During the experiments described previously, there was thick biofilm formed on the walls of the bioscrubber. However, during FISH analysis samples were taken only from the suspended culture. In Figure 5A and B it is evident that before and after the starvation period in both systems the percentage of GJ10 within the active cells was very low (3–5%). Interestingly, during the DCE starvation the percentage of GJ10 increased, reaching a maximum of 21% in the BO system 4 days after the interruption of the DCE feed and a maximum of 15% in the OAB system at the end of the starvation. Consequently, the percentage of GJ10 decreased to the initial level in both systems after the starvation. The variation in the percentage of GJ10 during the starvation indicates that dynamic changes occurred in the microbial community composition. The dynamic communities formed in the two systems may comprise either DCE degraders other than GJ10, or other secondary microorganisms that evolved in the culture. It has been previously stated that especially in systems where biofilms are formed, the microbial culture is always subject to interactions such as symbiosis, competition for space or substrates and dynamic communities may occur (Carvalho et al., 2001). Also, Metris et al. (2001) reported that the total biomass comprises not only pollutant degraders but other microorganisms as well, defined as "secondary biomass." Therefore, the increase in the percentage of GJ10 when subject to DCE starvation in both systems can be due to the different decay rates that different microbial species have within a dynamic community. The influence of starvation periods on the relative abundance of cells has been previously shown (Moe and Qi, 2004). The percentage of GJ10 might increase during the starvation due to slower decay than the rest of the species in the culture. Also, the low percentage of GJ10 in the suspended culture indicates that GJ10 might have been growing mainly in the biofilm. Thus, the biofilm sloughing off into the suspension during the starvation period might be another explanation for the increase of the percentage of GJ10 in the suspended biomass during this period. This suggests that in suspended growth bioreactors with extensive wall growth, biomass analysis of the biofilm is also necessary in order to have a better understanding of the microbial dynamics and the different physiological states of the microbial culture in the system.

The low percentage of GJ10 as well as the evolution of the percentage of the active cells within the community is in fair agreement with past studies in similar systems. Hekmat et al. (2004) observed that during steady state operation of a trickle bed bioreactor, only 8.8% of all cells were specific degraders, and that 30% of the cells were inactive. Also, during a starvation period nearly 60% of all cells were inactive and after the starvation the fractions of active cells increased within several days showing the same profile as in this study for the BO system. The fact that GJ10 was a small fraction (less than 20%) of the active cells in both systems contradicts the model assumption that all the cells in the bioscrubber grow based on the kinetics of GJ10. The rest of the active biomass in the bioscrubber might be satellite

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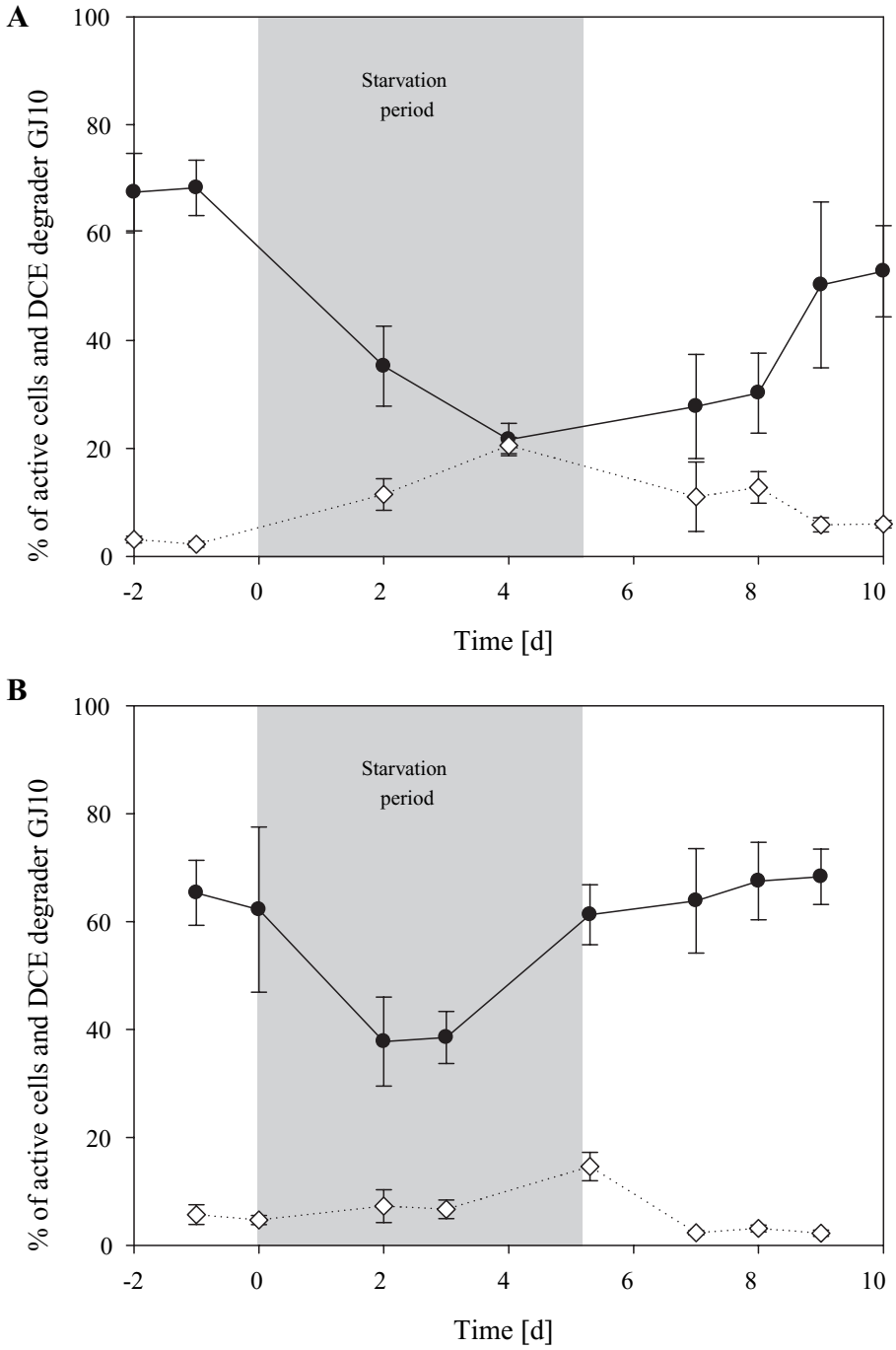

Figure 5. Evolution of active cells and *Xanthobacter autotrophicus* strain GJ10 cells in the BO and OAB configurations. **A**: Starvation I.2 (5.2 days). **B**: Starvation II.2 (5.2 days); (—●—), % of active cells = 100% × EUB stained cells/DAPI stained cells; (····◇····), % of GJ10 = 100% × GJ10 cells/EUB stained cells.

strains involved in the degradation of intermediates produced during DCE degradation or polysaccharides excreted from GJ10 (Ferreira Jorge, 2000). Therefore, their growth rate could be closely related to the GJ10 growth rate. However, we believe that GJ10 is the primary DCE degrader due to the very specific degradation pathway (Janssen et al., 1994).

The viability of cells was also monitored during the starvation periods (Fig. 6). In the BO system, the percentage of viable cells at steady state before the starvation period was between 35 and 45%. However, due to the lack of substrate when the DCE feed was stopped the percentage of viable cells decreased significantly and reached a minimum value of 6% (day 4, Fig. 6). After the re-introduction of DCE, the

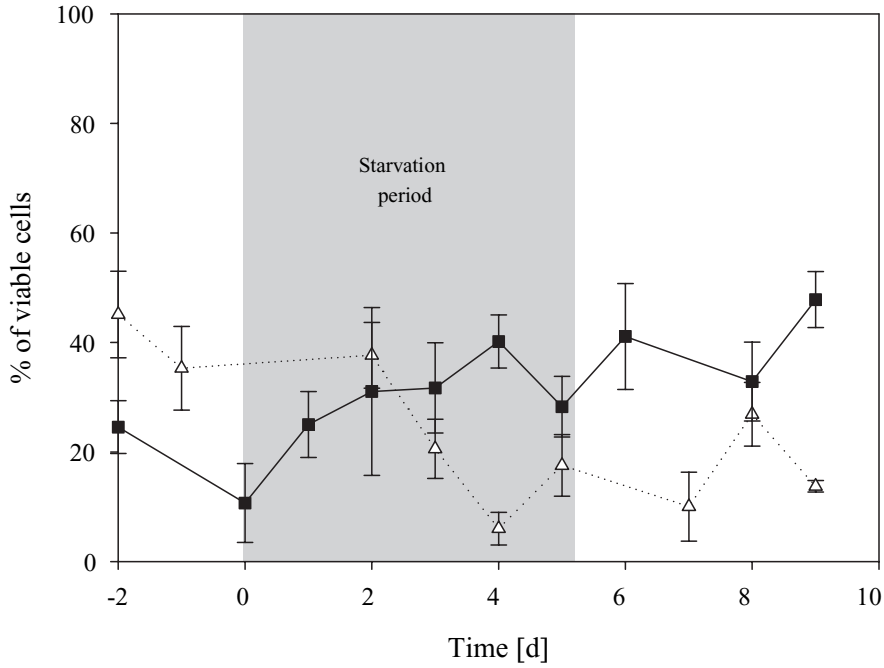

Figure 6. Evolution of cell viability in the BO and OAB configurations. Percentage of viable cells = 100% × (DAPI stained cells − PI stained cells)/DAPI stained cells; (·····△·····), evolution of viable cells in the BO system (Starvation period I.2); (—■—), evolution of viable cells in the OAB system (Starvation period II.2).

percentage of viable cells did not increase significantly and was lower than 27% for the next 4 days. In contrast, when the oil absorber was placed upstream of the bioscrubber, the evolution of the percentage of viable cells over time was different. Although during steady state conditions the percentage of viable cells was significantly lower (11–24%), during the starvation period viable cells increased over time reaching a maximum of 40%, 4 days after the DCE feed was stopped. After the re-introduction of DCE the percentage of viable cells did not change significantly.

In the BO system, the viability of the microbial culture remained at low levels even after the DCE re-introduction, a fact that is in agreement with the low DCE removal performance during this period. When the oil absorber was used, the low percentage of viable cells at steady state did not deteriorate—to the contrary it increased during the starvation period. Thus, it is possible that before the starvation period the DCE loading was too high and inhibited the bacterial growth. Therefore, the reduction of the bioscrubber DCE inlet loading during starvation was beneficial for the microbial culture to recover the viability of cells at higher values.

Conclusions

Overall the performance of the OAB system was much more stable than the BO system during DCE starvation periods. The biomass concentration and the activity of cells were retained at higher values during starvation periods in the OAB system. Therefore, when DCE was re-introduced, due to the effect of the oil absorber the microbial activity recovered immediately to the initial level, significantly reducing the TOD_{DCE}. The stable performance of the OAB system for long starvation periods, or starvation periods with different duration, shows the improvement of the process with the introduction of the oil absorber. The experiments presented above also show that if the absorbent oil has to be discharged periodically, the pollutant retained can be treated in the bioreactor by passing a non-contaminated air stream through the absorber column. In contrast, Weber and Hartmans (1995) have reported that the desorption of the pollutant from GAC is a difficult and slow process, emphasising the advantage of the oil absorber over GAC applications to control fluctuating pollutant concentrations prior to the bioreactor. This study also showed that although wall growth is usually neglected when modelling suspended growth bioreactors, the wall attached biomass can make a significant difference in case of dynamic scenarios of treatment.

Nomenclature

A	surface area available for biofilm attachment and growth [dm^2]

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b_d	coefficient for cell death [s^{-1}]
b_s	biofilm shear loss coefficient [s^{-1}]
$C_{a,in}$	oil absorber inlet DCE gas phase concentration [mg L^{-1}]
$C_{a,out}$	oil absorber outlet (or bioscrubber inlet) DCE gas phase concentration [mg L^{-1}]
C_B	dissolved oxygen concentration in bulk biological liquid [mg L^{-1}]
$C_{b,out}$	bioscrubber outlet DCE gas phase concentration [mg L^{-1}]
C_f	dissolved oxygen concentration in the biofilm [mg L^{-1}]
$C_{sun,oil}$	DCE concentration in sunflower oil [mg L^{-1}]
D_f	diffusion coefficient of DCE in the biofilm [dm^2 s^{-1}]
D_{of}	diffusion coefficient of oxygen in the biofilm [dm^2 s^{-1}]
G	gas phase flow rate [L s^{-1}]
H_{DCE}	DCE Henry's law coefficient based on concentration ratio [−]
k_f	mass transfer coefficient for DCE in the biofilm [dm s^{-1}]
k_{of}	mass transfer coefficient for oxygen in the biofilm [dm s^{-1}]
$(K_La)_{DCE}$	DCE volumetric mass transfer coefficient [s^{-1}]
$(K_La)_{oil}$	volumetric mass transfer coefficient in the oil absorber [s^{-1}]
K_{O_2}	Monod rate constant for oxygen [mg L^{-1}]
K_s	substrate saturation constant [mg L^{-1}]
L_f	biofilm thickness [dm]
M	mineral medium flow rate [L s^{-1}]
$P_{sun,oil}$	partition coefficient for DCE between sunflower oil and air [−]
S_b	DCE concentration in the biomedium [mg L^{-1}]
S_f	DCE concentration in the biofilm [mg L^{-1}]
S_m	DCE biomedium concentration for complete inhibition [mg L^{-1}]
T	oil absorber temperature [°C]
t	time [s]
V_b	bioscrubber volume [L]
V_{oil}	volume of sunflower oil [L]
V_v	volume of void space in the bioscrubber [L]
X_b	suspended biomass concentration [mg L^{-1}]
X_f	cell concentration in the biofilm [mg L^{-1}]
Y_{DCE}	yield coefficient for biomass on DCE [$g_{biomass}$ g_{DCE}^{-1}]
Y_{O_2}	yield coefficient for biomass on oxygen [$g_{biomass}$ g_{O2}^{-1}]
z	distance in the biofilm for the solid-biofilm interface [dm]

Greek Letters

μ_b	specific growth rate for cells in suspension [s^{-1}]
μ_f	specific growth rate for cells in the biofilm [s^{-1}]
μ_{max}	maximum specific growth rate [s^{-1}]

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