Application of Different Processes for the Biodegradation of 1,3-dichloro-2-propanol by the Bacterium *Pseudomonas putida* DSM 437

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1,3-Dichloro-2-propanol (1,3-DCP), is a highly toxic compound used in many industrial processes. Biodegradation of 1,3-DCP, by the bacterial strain *Pseudomonas putida* DSM 347, was studied applying three different processes. A number of combinations, with respect to glucose and 1,3-DCP concentration were examined during batch process. When the initial concentration of 1,3-DCP was 600 mg L^{-1} in the presence of 400 mg L^{-1} glucose, the biodegradation degree and rate were 10.8 % and 0.68 mg L^{-1} h^{-1} respectively. 1,3-DCP biodegradation by the resting cells of *P. putida* DSM 347 was tested at mass concentrations from γ = 200 to 1 000 mg L^{-1} using biomass concentration of 5 g dry cell mass L^{-1}. Biodegradation of 1,3-DCP ranged from 84 to 90 %, initial biodegradation rates ranged from r = 2.36 to 10.55 mg L^{-1} h^{-1}, while dependence of both parameters from the initial concentration of halohydrin was observed. A system of two Continuous Stirred Tank Reactors (CSTRs) in series was developed for the biodegradation of a highly toxic stream of 1,3-DCP (2000 mg L^{-1}). The overall biodegradation degree of the system was 68 %, while biodegradation rates of the first and second bioreactor were r = 2.88 and 5.21 mg L^{-1} h^{-1} respectively.

Key words:
Pseudomonas putida, 1,3-dichloro-2-propanol, biodegradation, batch process, resting cells, continuous stirred tank reactors

Introduction

Epichlorohydrin (1-chloro-2,3-epoxypropane, ECH) and its precursors 1,3-dichloro-2-propanol (1,3-DCP), 2,3-dichloro-1-propanol (2,3-DCP), and 3-chloro-1,2-propanediol (3-CPD) are halohydrins used widely as solvents and as starting materials for resins, polymers, agrochemicals and pharmaceuticals.[1,2] Especially, polyamidoamine-epichlorohydrin (PAE) resins were the first commercially important thermosetting products for use in neutral-to-alkaline furnishes for the manufacture of wet-strength paper.[3,4]

1,3-Dichloro-2-propanol is hepatotoxic and embryotoxic in mammals including humans[5–8] and also a carcinogenic and mutagenic compound.[6] Due to its toxicity, it is considered significantly hazardous to human health and the environment.[9,10] 1,3-Dichloro-2-propanol can enter natural waters as a consequence of improper waste and drinking water treatment and chlorination.[10] It has been located to a commercial acid – Hydrolyzed Vegetable Proteins (a-HVPs), a widely used ingredient of savoury foods, such as soy sauce. It is formed as a contaminant, by chlorination of the precursor glycerol existing in the raw material, during hydrolysis in the presence of hydrochloric acid.[10,11] The Environmental Protection Agency Priority Chemical List[12] (available at http:/www.epa.gov/iriswebp/iris/index.html) showed that the overall score (as the sum of the persistence, bioaccumulation and toxicity scores for human health risk potential added to the corresponding scores for ecological risk) for 1,3-DCP and epichlorohydrin were 11 out of 18 each. Both 1,3-DCP and epichlorohydrin have a high risk factor for animal and human toxicity with regards to environment. According to EU Directive 91/155/EEC all formulations containing more than 0.1 % of 1,3-DCP have to be labelled as toxic and carcinogenic.

Microorganisms have evolved a diverse potential to transform and degrade halogenated organic

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compounds.[13–15] The key reaction during microbial degradation of halogenated compounds is the actual dehalogenation. During this step, the halogen substituent, which is usually responsible for the toxic and xenobiotic character of the compound, is replaced – often by hydrogen or a hydroxyl group. Halogen removal reduces both recalcitrance to biodegradation and the risk of forming toxic intermediates during subsequent metabolic steps.[15] Bacterial strains capable of utilizing 1,3-DCP and related compounds have been reported[2,16–18] and fewer capable of degrading 2,3-DCP[1] have been isolated from soil samples.

It is well known that the choice of the appropriate process can be of great importance for the performance of any bioprocess. The choice is made based on the particular characteristics and the advantages of each fermentation technology.[19–22] The stirred continuous methods, for example, have the advantage of growing at final conditions, which can be important in the case of substrate inhibition. The joining together of two or more CSTRs in series produces a multi-stage process in which conditions such as pH, temperature and medium composition can be varied in each reactor. This is advantageous if reactor conditions required for growth are different from those for product synthesis or biodegradation purposes. On the other hand, batch methods are more genetically stable and have lower risk of contamination.[19]

In this work, three different processes for the biodegradation of 1,3-DCP by the bacterium *P. putida* DSM 437 were investigated. The processes were: (a) direct batch degradation using 1,3-DCP and glucose as carbon and energy sources, (b) biodegradation by the resting cells of the bacterium and (c) a system of two continuous stirred tank reactors (CSTRs) in series. The main objective of this study was to increase the concentration of 1,3-DCP which could be detoxified by *P. putida* DSM 437.

Materials and methods

Microorganism

Pseudomonas putida DSM 437 was obtained from DSMZ (Deutsche Sammlung von Microorganismen und Zellkulture GmbH), Germany and used throughout this work. Stock cultures were maintained on glycerol at –75 °C.

Growth media and conditions

The bacterium was grown in a mineral medium containing (g L^{-1}): Na_2HPO_4, 2.4; KH_2PO_4, 1.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.5 and 10 mL of SL-4 solution (composition of the SL-4 solution is available at www.dsmz.de). The pH of the medium was adjusted to 6.9. Following heat sterilization (121 °C, 20 min), glucose (γ_S = 5.0 g·L^{-1}) was supplemented the medium. The inoculum was prepared by transferring cell suspension from the stock culture to 250-mL Erlenmeyer flasks containing 50 mL of the above medium. The cells were grown at 30 °C for 24 h on an orbital shaker (n = 250 min^{-1}), and served as a preculture for batch experiments as well as for the bioreactors.

Batch degradation of 1,3-DCP was studied in the presence of glucose. Batch degradation of 1,3-DCP was carried out in 250-mL Erlenmeyer flasks containing 50 mL of the mineral medium supplemented with different amounts of 1,3-DCP and glucose. Following heat sterilization (121 °C, 20 min) 1,3-DCP and glucose were supplemented the mineral medium. The Erlenmeyer flasks were inoculated with φ = 10 % of the above mentioned preculture. Growth was allowed to proceed at 30 °C on an orbital shaker (n = 250 min^{-1}). All experiments were performed in duplicate.

Biomass for 1,3-DCP biodegradation by resting cells was prepared as described previously.[23]

1,3-DCP biodegradation by the resting cells of *P. putida* DSM 437

Cells were harvested at late exponential phase, by centrifugation (t = 15 min, a_c = 10 000 g, T = 5 °C), washed with 0.02 mol L^{-1} phosphate buffer pH 7.0 and resuspended in the same buffer in order to achieve a final biomass concentration of 5 g L^{-1} DM. Stock solution of 1,3-DCP was prepared by diluting the above-mentioned compound in deionized water. Different amounts of the stock solution were added in 25-mL Erlenmeyer flasks containing the above-mentioned cell suspension and placed on an orbital shaker (T = 30 °C, n = 100 min^{-1}). Samples were withdrawn periodically and after centrifugation (a_c = 5 000 · g, t = 15 min) the supernatant was used for the determination of the remaining 1,3-DCP. In order to determine if the removal of 1,3-DCP is due to adsorption to biomass, two different approaches were applied: (a) the remaining biomass after centrifugation of the samples was extracted applying the method of Mueller & Fischer[24] and (b) cells were deactivated by boiling and after addition of the appropriate substrate were placed on the orbital shaker for incubation. Samples were withdrawn periodically and after centrifugation (a_c = 5 000 · g, t = 15 min) the supernatant was used for the determination of the remaining 1,3-DCP. All experiments were conducted in duplicate.

Continuous stirred tank reactors in series

A system of two continuous stirred tank reactors in series was used in this study. The experimental apparatus is presented in Fig. 1. The feed in the first bioreactor was a mixture of glucose and 1,3-DCP in order to achieve high cell mass concentration and at the same time cells adapted to 1,3-DCP. In the second bioreactor the cells are exposed to a high mass concentration of 1,3-DCP in the absence of glucose. Air was supplied at 0.8 vvm, while temperature and agitation were kept constant at 30 ± 1 °C and n = 220 rpm respectively. Details of the quantities applied to the specific system are presented in Fig. 1. Samples (from four different sampling points, Fig. 1) were withdrawn periodically for biomass estimation and after centrifugation (a_c = 5 000 · g, t = 15 min) for the determination of the remaining glucose and 1,3-DCP as described below.

It should be mentioned that in order to determine if 1,3-DCP could be removed due to aeration, blank runs of the system were performed. Samples were withdrawn from the four different sampling points for the determination of 1,3-DCP.

Biomass estimation

Cell growth was monitored by measuring the optical density of culture samples using a spectrophotometer (Hitachi UV 1100, Japan) at λ = 600 nm. The biomass concentration was calculated from optical density measurements using a standard curve prepared with *P. putida* cells grown on glucose and expressed as dry mass (γ_x/g L^{-1} DM). All measurements were performed in triplicate.

Glucose determination

Glucose was determined using a commercial enzymic kit obtained from Biosis S.A. (Athens, Greece). The method exploits the action of glucose oxidase to convert glucose to gluconic acid and hydrogen peroxide, which is further modified by peroxidase to form a coloured product. All measurements were performed in triplicate.

1,3-dichloro-2-propanol determination

Prior to determination, 1,3-DCP was extracted three times from the samples with ethyl acetate according to Mueller & Fischer.[24]

1,3-DCP was determined with a Shimadzu gas chromatograph GC-17A equipped with an electron capture detector (ECD-17, Shimadzu). A split/splitless injection port was used in the split mode (1:25). An auxiliary makeup of nitrogen was used. The column was 60 m · 0.25 mm · 0.25 μm

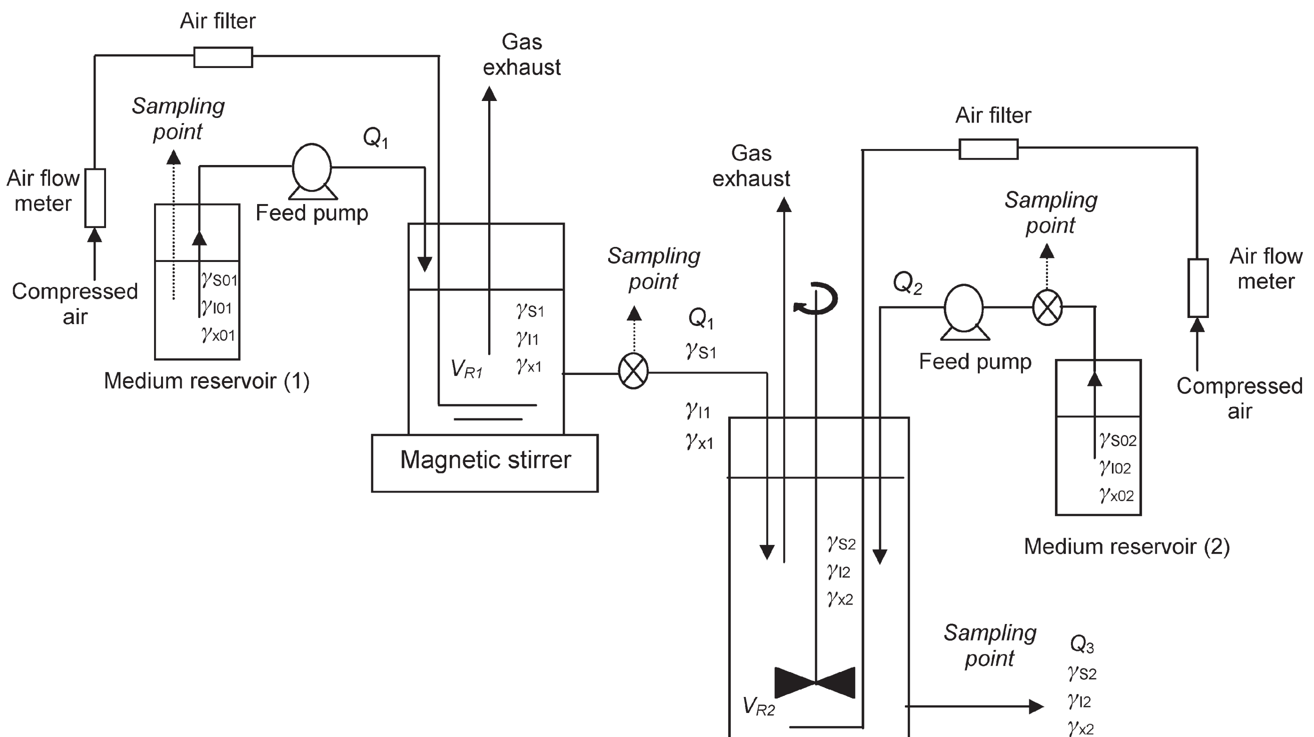

Fig. 1 – *Schematics of continuous stirred tank Reactors in series. Parameters applied to the specific system were: (a) first bioreactor volume V_{R1} = 431 ml, flow rate Q_1 = 8 ml h^{-1}, the feed of the first bioreactor was sterile (therefore γ_{x01} = 0) and contains glucose and 1,3-DCP at mass concentrations of γ_{S01} = 25 g l^{-1} and γ_{I01} = 500 mg/l respectively (b) second bioreactor volume V_{R2} =1700 ml, additional flow rate Q_2 = 5.4 ml h^{-1}, total flow rate of the second bioreactor Q_3 = Q_1 + Q_2 = 13.4 ml h^{-1}, the additional feed of the second bioreactor was sterile (γ_{x02} = 0) in absence of glucose (γ_{S02} = 0) and contained 1,3-DCP at concentration of γ_{I02} = 2000 mg l^{-1}.*

Equity™–1 from Supelco. Helium was used as carrier gas at a column flow rate of Q = 1 mL min^{-1}. Injector and detector temperatures were set at 280 °C and 300 °C respectively. The oven was initially set at 90 °C for 2 min, ramped at T = 5 °C min^{-1} to 150 °C for 1 min and finally ramped at T = 25 °C min^{-1} to 240 °C for 1 min. The retention time for 1,3-DCP was 8.3 min, under the above-mentioned conditions. 1,3-DCP and 3-CPD mass concentrations were calculated using the appropriate standard curves. 1,3-Dichloro-2-propanol determinations were performed in triplicate.

Results and Discussion

Batch degradation of 1,3-dichloro-2-propanol

The ability of *P. putida* DSM 437 cells to degrade 1,3-DCP in batch culture was investigated. A conventional carbon source (glucose) supplemented the growth medium in the presence of 1,3-DCP. A number of combinations, with respect to glucose and 1,3-DCP concentration were examined, namely (1,3-DCP + glucose) (mg L^{-1}), (200 + 800) (mg L^{-1}), (600 + 400) (mg L^{-1}), (1000 + 0.0) (mg L^{-1}), in order to find a condition in which enhanced rates of 1,3-DCP degradation could be achieved. All cultures were inoculated with γ_x = 0.03 g L^{-1} DM of the preculture. The time course of 1,3-DCP batch degradation in the presence of glucose, is presented in Fig. 2. Glucose was consumed in the first 24 h. Increasing 1,3-DCP concentration resulted in decrease on microbial growth. Furthermore, a decrease in biomass was observed after 48 h of cultivation, in all combinations tested (Fig. 1). Maximum biomass production (γ_x = 0.38 g L^{-1} DM) was associated with the highest glucose mass concentration applied (0.8 g L^{-1}). The highest rate of 1,3-DCP degradation (r = 0.68 mg L^{-1} h^{-1}) was observed at γ = 600 and 400 mg L^{-1} initial 1,3-DCP and glucose mass concentrations, respectively (Table 1).

Biomass seems to affect 1,3-DCP degradation. In the absence of glucose biomass production was approximately γ_x = 0.06 g L^{-1} DM while the biodegradation degree was estimated at 5.9 %. When biomass concentration reached γ_x = 0.38 g L^{-1} DM (maximum biomass production) the biodegradation degree increased approximately two times (Table 1).

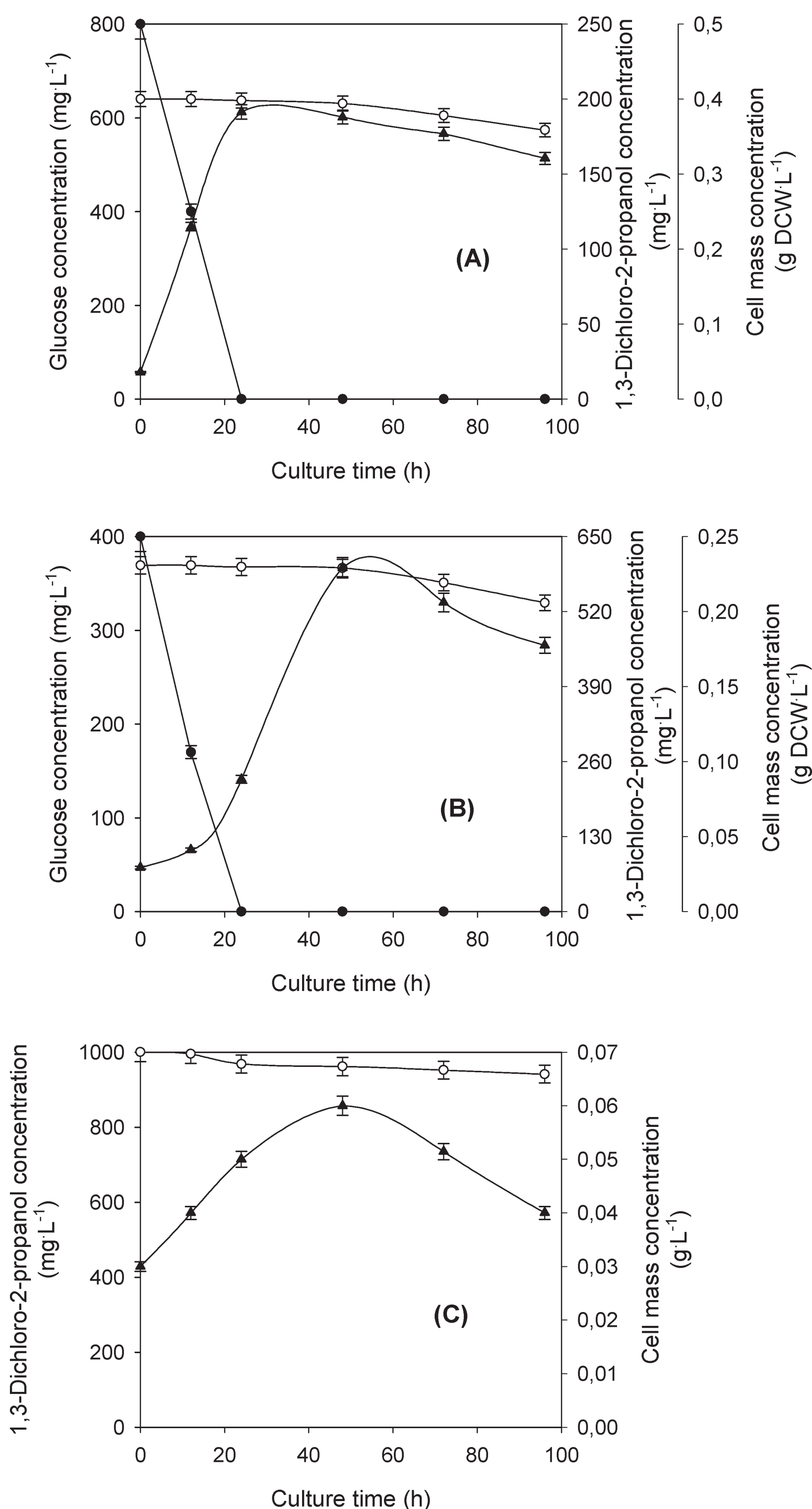

Fig. 2 – *Direct batch biodegradation of 1,3-DCP in the presence of glucose by P. putida DSM 437. The concentrations of 1,3-DCP + glucose were: (A) 200 + 800 (mg L^{-1}), (B) 600 + 400 (mg L^{-1}) and (C) 1000 + 0 (mg L^{-1}). Symbols: (●) glucose, (○) 1,3-DCP, (▲) cell mass.*

Table 1 – *Direct batch biodegradation of 1,3-DCP by the bacterium P. putida DSM 437 in the presence of glucose*

Conditions	Maximum cell dry mass concentration	Biodegradation degree	Biodegradation rate
	γ_x/g L^{-1} DM	?/%	r/mg L^{-1} h^{-1}
A	0.38 ± 0.01	10.3 ± 0.3	0.22 ± 0.01
B	0.23 ± 0.01	10.8 ± 0.3	0.68 ± 0.02
C	0.060 ± 0.002	5.9 ± 0.1	0.62 ± 0.02

A: 200 mg L^{-1} 1,3-DCP plus 800 mg L^{-1} glucose, B: 600 mg L^{-1} 1,3-DCP plus 400 mg L^{-1} glucose and C: 1000 mg L^{-1} 1,3-DCP plus 0 mg L^{-1} glucose

Values are the mean of three determinations and the standard deviation was below 3 % in all cases.

The addition of conventional carbon sources, such as glucose, yeast extract, and sodium glutamate has been considered a promising method to increase cell tolerance towards substrate inhibition.[25] The result of this addition is improved degradation of several aromatic compounds which can be attributed to the build-up of increased cell mass.[26] Additional methods, which have been proposed to overcome substrate inhibition, include genetically modified microorganisms,[27] immobilization of cells[28,29] and gradual adaptation of cells to higher concentrations of the toxic compound.[30] The method selected in this study to confront the inhibitory effect of 1,3-DCP on microbial growth, was the addition of a conventional carbon source (glucose). Furthermore, the presence of 1,3-DCP in the medium induces haloalcohol dehalogenases, which are the key enzymes for the dehalogenation of the above-mentioned compound.[2] It should be mentioned that properly acclimatized cells of *P. putida* DSM 437 exhibited remarkable ability to withstand and degrade phenol.[31]

Fauzi et al.[2] isolated bacteria (*Agrobacterium* sp.) from soil and studied the degradation of 1,3-DCP at various concentrations. They reported that the specific growth rate started to decrease at concentrations above 360 mg L^{-1} 1,3-DCP, which indicates inhibition of growth at higher concentrations.

Bastos et al.,[32] investigated the effect of 1,3-DCP concentration on growth, of an enriched microbial consortium. The concentrations tested, ranged from γ = 20 to 80 mg L^{-1}. Microbial growth as well as 1,3-DCP biodegradation was observed in all cases, but the authors reported, that increasing 1,3-DCP concentration resulted in a decrease in the percentage of 1,3-DCP degraded. The 1,3-DCP concentration applied to already acclimatized consortia were much lower than that used in this study. Furthermore, the same authors reported that 1,3-DCP degradation rate in batch cultures was found r = 4 mg L^{-1} d^{-1} (equivalent to 0.17 mg L^{-1} h^{-1}), before acclimatization, which is lower than those reported in this study.

Van de Wijngaard et al.,[18] reported that *Agrobacterium radiobacter* strain AD1 could degrade c = 4 mmol L^{-1} (approximately 516 mg L^{-1}) of 1,3-DCP completely in 6 days (degradation rate r = 86 mg L^{-1} d^{-1}, equivalent to 3.58 mg L^{-1} h^{-1}) but the ability of another bacterium, *Arthrobacter* sp. strain AD2, was lower than that of AD1. Yonetani *et al.*,[33] isolated a bacterial strain from soil samples obtained from a chemical industry which uses 1,3-DCP, identified it as *Arthrobacter* sp. strain PY1 and reported that it had the ability to degrade 1000 mg L^{-1} 1,3-DCP in 7 days.

1,3-dichloro-2-propanol biodegradation by resting cells of *P. putida* DSM 437

The ability of *P. putida* DSM 437 resting cells (*i.e.* non-growing bacteria) to degrade 1,3-DCP was investigated. 1,3-DCP was tested at the following concentrations: γ_I = 1000, 800, 600, 400 and 200 mg L^{-1} while the biomass concentration was γ_x = 5 g L^{-1} DM. The time course of 1,3-DCP degradation by resting cells is presented in Fig. 3A. The degree of 1,3-DCP biodegradation ranged from 84 to 90 % depending on its initial concentration (Fig. 3B). Maximum biodegradation degrees were achieved at 96 hours of incubation. Initial rates of 1,3-DCP biodegradation by the resting cells ranged from r = 2.36 to 10.55 mg L^{-1} h^{-1} (Table 2). Increasing initial

Table 2 – *Initial biodegradation rates (I. B. R.$_i$, i denotes the concentrations of 1,3-DCP) of 1,3-DCP, by the resting cells of P. putida DSM 437 (biomass concentration 5 g L^{-1} DM)*

	Standard error P		
I.B.R$_{200}$	2.36	0.06	< 0.0001
R^2 = 0.99, Standard error of estimate = 3.99			
I.B.R$_{400}$	4.77	0.16	< 0.0001
R^2 = 0.99, Standard error of estimate = 12.38			
I.B.R$_{600}$	7.17	0.24	< 0.0001
R^2 = 0.99, Standard error of estimate = 16.85			
I.B.R$_{800}$	8.53	0.20	<0.0001
R^2 = 0.99, Standard error of estimate = 14.42			
I.B.R$_{1000}$	10.55	0.34	< 0.0001
R^2 = 0.99, Standard error of estimate = 20.22			

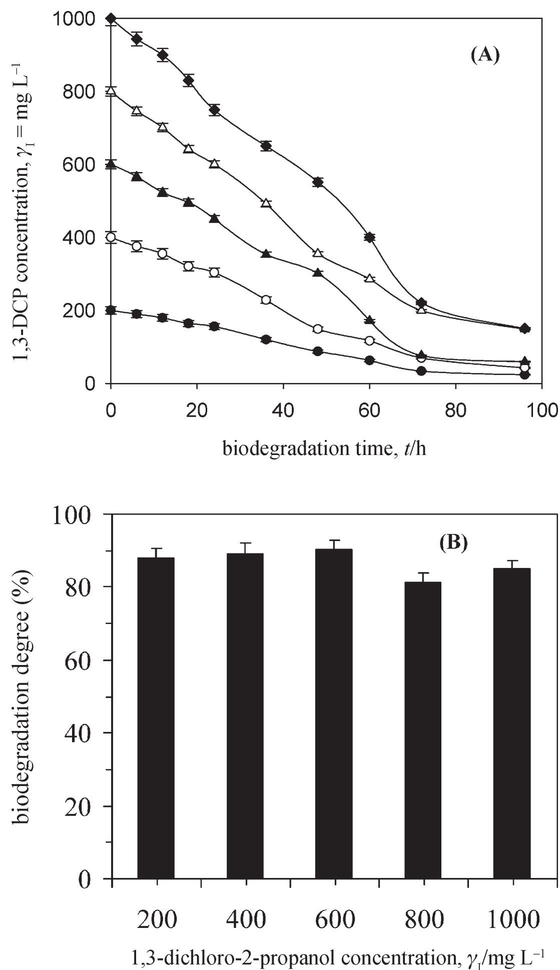

Fig. 3 – *(A) Time course of 1,3-DCP biodegradation and (B) 1,3-DCP biodegradation degree, by the resting cells of P. putida DSM 437 (biomass concentration γ_x = 5 g L^{-1} DM)*

concentration of 1,3-DCP resulted in higher biodegradation rates. 1,3-DCP biodegradation by the resting cells of *P. putida* DSM 437 follows a first-order kinetics.

$$r = k \cdot \gamma_I \tag{1}$$

where

r – biodegradation rate of 1,3-DCP, mg L^{-1} h^{-1}

γ_I – initial concentration of 1,3-DCP, mg L^{-1}

The rate coefficient (k) was found 0.0109 (h^{-1}) (R^2 = 0.99, standard error = 0.0003, P < 0.0001) (Figure 4). No adsorption of 1,3-DCP to biomass was observed applying the methods described under Materials and Methods. *Fauzi* et al.,[2] studied the degradation of low mass concentrations of 1,3-DCP and related halohydrins by resting cells of soil bacteria, which dehalogenated 1,3-DCP at all concentrations tested (from 1 mg L^{-1} to 10 mg L^{-1}) at a similar rate (approximately 0.5 mg L^{-1} h^{-1}). Furthermore, they found that the value of k for 1,3-DCP was 1.13 h^{-1}.

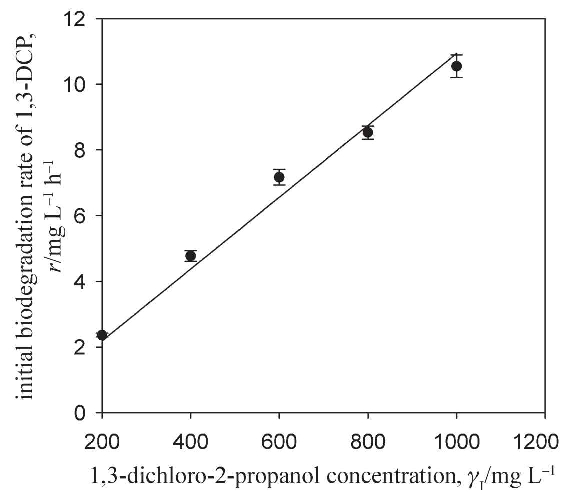

Fig. 4 – *Determination of rate coeffient (k) for 1,3-DCP biodegradation by the resting cells of P. putida DSM 437*

Continuous stirred tank reactors in series for the biodegradation of 1,3-dichloro-2-propanol

The joining together of two or more CSTRs in series produces a multi-stage process in which conditions can be varied in each reactor. This is advantageous if reactor conditions required for growth are different from those for product synthesis or biodegradation purposes. As mentioned previously, biomass is a critical parameter in 1,3-DCP degradation. In order to achieve high biomass concentration and at the same time adaptation of the cells to 1,3-DCP, the feed in the first bioreactor was a mixture of glucose (γ_{S01} = 25 g·L^{-1}) and 1,3-DCP (γ_{I01} = 500 mg L^{-1}). Dilution rate ($D_1 = Q_1/V_1$) in the first bioreactor was set at 0.01855 h^{-1} which corresponds to a residence time ($\tau = 1/D_1$) of 55.1 h. The remaining glucose in the stream entering the second bioreactor was γ_{S1} = 10.6 g L^{-1}, 1,3-DCP was γ_{I1} = 356 mg L^{-1}and cell mass was γ_x = 4.5 g L^{-1} DM. Furthermore, the second bioreactor was fed with a stream of high 1,3-DCP mass concentration (γ_{I02} = 2000 mg L^{-1}), in the absence of glucose (γ_{S02} = 0). Dilution rate ($D_1 = Q_1/V_1$) in the second bioreactor was 0.00787 h^{-1} (or τ_2 = 126.9 h). Steady state was achieved after approximately 350 h. At steady state the stream exiting the second bioreactor contained 1,3-DCP at a concentration of γ_{I2} = 353 mg L^{-1} while biomass concentration reached γ_{x2} = 7.5 g L^{-1} DM. Biomass concentration in the second bioreactor was significantly increased compared to the first bioreactor. Both substrates (1,3-DCP and glucose entering from the outlet flow of the first bioreactor) contribute to biomass concentration increment. The actual glucose concentration in the second bioreactor is $\gamma_{Sbioreactor}$ = 6.3 g L^{-1} (calculated according to eq. 2 below). A summary of the performance of the above

Table 3 – *Summary of the performance of CSTRs in series*

	Inlet flow to the 1st CSTR, Q_1 = 8 ml h^{-1}	Outlet flow from the 1st CSTR and inlet flow to the 2nd CSTR, Q_1 = 8 ml h^{-1}	Additional flow to the 2nd CSTR, Q_2 = 5.4 ml h^{-1}	Outlet flow from the 2nd CSTR, Q_3 = 13.4 ml h^{-1}
Glucose concentration, γ_I/g L^{-1}	25 ± 0.5	10.6 ± 0.2	0	0
1,3-DCP concentration, γ_I/mg L^{-1}	500 ± 7.5	356 ± 5.3	2000 ± 30	353 ± 5.3
Biomass concentration, γ_x = g L^{-1} DM	0	4.5 ± 0.1	0	7.5 ± 0.1

Values are the mean of three determinations and the standard deviation was below 3 % in all cases.

described system is presented in Table 3. No loss of 1,3-DCP was observed due to aeration. Concentrations, presented in Table 4 for the second bioreactor and the overall system, were calculated according to eq. 2 and 3 respectively.

$$\gamma_{I_{bioreactor2}} = \frac{Q_1 \cdot \gamma_{I_1} + Q_2 \cdot \gamma_{I_{02}}}{Q_1 + Q_2} \quad (2)$$

Where $I_{bioreactor2}$ is the actual 1,3-DCP concentration in the 2nd bioreactor

Q_1 – is the flow rate of the 1rst bioreactor

γ_{I1} – is 1,3-DCP concentration in the outlet of the 1rst bioreactor

Q_2 – is the additional flow rate of the 2nd bioreactor

γ_{I02} – is 1,3-DCP concentration in the additional inlet flow of the 2nd bioreactor

$$\gamma_{I_{overall}} = \frac{Q_1 \cdot \gamma_{I_{01}} + Q_2 \cdot \gamma_{I_{02}}}{Q_1 + Q_2} \quad (3)$$

Table 4 – *Performances of the processes tested for the biodegradation of 1,3-DCP by the bacterium P. putida DSM 437*

Process	Concentration of 1,3-DCP, γ_I/mg L^{-1}	Degradation degree, %	Degradation rate, r/mg L^{-1}·h^{-1}
Direct batch degradation	600 ± 15	10.8 ± 0.3	0.68 ± 0.02
	1000 ± 25	5.9 ± 0.1	0.62 ± 0.02
Resting cells biodegradation	1000 ± 30	84.9 ± 2.5	8.9 ± 0.3
CSTRs in series			
1rst Bioreactor	500 ± 7.5	28.8 ± 0.4	2.61 ± 0.04
2nd Bioreactor	1018 ± 15.3*	65.3 ± 1.0	5.24 ± 0.08
Overall	1100 ± 16.5 **	68.0 ± 1.0	–

*Calculated according to equation 2.

**Calculated according to equation 3.

Values are the mean of three determinations and the standard deviation was below 3 % in all cases.

where $\gamma_{Ioverall}$ is actual 1,3-DCP mass concentration of the overall system

Q_1 – is the flow rate of the 1rst bioreactor

γ_{I2} – is 1,3-DCP concentration in the inlet of the 1rst bioreactor

Q_2 – is the additional flow rate of the 2nd bioreactor

γ_{I02} – is 1,3-DCP concentration in the additional inlet flow of the 2nd bioreactor

Biodegradation degrees of the first and second bioreactor were found 28.8 and 65.3 % respectively, while the overall process resulted in 68 % 1,3-DCP biodegradation. As for the biodegradation rates of the 1rst and 2nd bioreactor, they were found to be 2.88 and 5.21 mg L^{-1}·h^{-1} respectively (Table 4). The continuous system described by *Bastos* et al.,[32] for the enrichment of a microbial consortium, was fed with 50 mg L^{-1} 1,3-DCP, achieved steady state after approximately 40 days, while the 1,3-DCP degradation rate was r = 79 mg L^{-1} d^{-1} (corresponding to r = 3.29 mg L^{-1} h^{-1}). This value is lower than that reported for the continuous system in this study.

Comparison of the three processes

A summary of the performance of the three processes investigated is presented in Table 4. As mentioned earlier, the choice of the appropriate process is of great importance. Different processes exhibited different characteristics. The stirred continuous methods have the advantage of growing at final conditions, which can be important in the case of substrate inhibition, while batch methods are more genetically stable and have lower risk of contamination.[19–22]

The highest biodegradation degree (10.8 %) in batch process was observed when the initial mass concentration of 1,3-DCP was 600 mg L^{-1} in the presence of γ_S =400 mg L^{-1} glucose, while the corresponding biodegradation rate was r = 0.68 mg L^{-1} h^{-1}. From the batch process it can be concluded that

biomass plays a significant role in 1,3-DCP biodegradation. The use of glucose, a readily metabolizable carbon source, enhances cell mass concentration but probably inhibits the utilization of 1,3-DCP. *Yonetani* et al.,[33] reported that an addition of 2 % peptone in the culture medium in the presence of 1000 mg L^{-1} 1,3-DCP resulted in complete degradation of 1,3-DCP within 7 days similarly to cultivation without peptone, while an addition of c = 50 mmol l^{-1} glucose caused an inhibitory effect in *Arthrobacter* sp. PY1 degrading activity. On the other hand, the presence of 1,3-DCP induces haloalcohol dehalogenases, which are the key enzymes for the dehalogenation of the above-mentioned compound.[2] Batch degradation process could perform better through gradual acclimatization of *P. putida* cells to higher 1,3-DCP concentrations. The development of acclimatized cells and the use of glucose as an added growth substrate were applied to phenol biodegradation by the same bacterial strain. The process resulted in complete removal of 1200 mg L^{-1} phenol.[31] Gradual adaptation to higher 1,3-DCP mass concentrations was proposed by *Yonetani* et al.,[33] who reported that 1,3-DCP mass concentrations up to 4.0 g L^{-1} were completely biodegraded by *Arthrobacter* sp. strain PY1 in 7 days after proper acclimatization.

Cells for the resting cells biodegradation process, grown on a medium contained both glucose and 1,3-DCP, and were harvested at late exponential phase and used in biodegradation studies as described under Materials and methods. The resting cells biodegradation process resulted in high biodegradation degrees as well as high biodegradation rates. At 1.0 g L^{-1} the biodegradation degree was found to be 85 %, while the respected biodegradation rate was r = 8.9 mg L^{-1} h^{-1}, approximately 13 times higher compared to the batch process. The reuse of resting cells probably depends on the stability of the enzymic system responsible for 1,3-DCP biodegradation. The conversion of 1,3-DCP, 3-CPD and other halohydrins by *Pseudomonas* sp. AD1 and *Arthrobacter* sp. AD2 have been proposed to proceed *via* intramolecular substitution.[34,35] During the early steps of 1,3-DCP degradation, a halohydrin hydrogen–halide lyase (haloalcohol dehalogenase) catalyzes an intramolecular substitution reaction yielding the corresponding epoxide (epichlorohydrin) which is converted to 3-chloro-1,2-propanediol by the action of an epoxide hydrolase. A halohydrin hydrogen-halide lyase catalyzes the conversion of 3-chloro-1,2-propanediol to glycidol, which is converted to glycerol by the action of an epoxide hydrolase.[14]

The system of the two continuous stirred tank reactors in series was an effort to combine all the parameters affecting 1,3-DCP biodegradation, namely high biomass concentration in absence of glucose (increased bio-utilization of 1,3-DCP) and cells adapted to toxic environment. The overall biodegradation degree of the particular system was found to be 68 %, while the biodegradation rate of the second bioreactor was r = 5.21 mg $L^{-1} \cdot h^{-1}$. Even though biomass concentration in the second bioreactor is higher (γ_x = 7.5 g L^{-1} DM) compared to the resting cells process (γ_x = 5 g L^{-1} DM), the performance of the CSTRs in series system is lower (Table 4). This could be attributed to the presence of glucose which enters the second bioreactor.[33] The CSTRs in series is quite a flexible system and has the ability to manipulate highly contaminated streams.

In this study, an effort was made to increase the ability of a *P. putida* strain to degrade highly contaminated streams of 1,3-DCP, applying the main tools of bioprocess engineering, in contrast to the main trend of biotechnology, the genetically engineered microorganisms (GEMs), which aside from the environmental considerations that they have raised, are not genetically steady at scale up and scale down processes.[36–40]

Although several bacteria have been reported to have 1,3-DCP degrading activity, information on their ability is insufficient. On the other hand, enzymes responsible for the halogen removal from xenobiotics (dehalogenases) have been extensively studied in view of their detoxifying properties.[14,41] Furthermore, the observation that the dehalogenation of chiral halohydrins can proceed with high enantioselectivity has attracted broad interest in these enzymes.[17,42–46]

Conclusions

The ability of *P. putida* DSM 437 to degrade 1,3-DCP was investigated applying three different processes. The type of process for the biodegradation of a persistent xenobiotic is of great importance. The direct batch degradation using 1,3-DCP (600 mg L^{-1}) and glucose (400 mg L^{-1}) as carbon and energy sources resulted in low biodegradation degree and degradation rate 10.8 % and 0.68 mg L^{-1} h^{-1} respectively, which could be attributed to low cell mass concentration. Biodegradation of 1000 mg L^{-1} 1,3-DCP by the resting cells of *P. putida* DSM 437, resulted in biodegradation degree of 85 %, and biodegradation rate of 8.9 mg L^{-1} h^{-1}. Finally, the system of two continuous stirred tank reactors (CSTRs) in series performed very well. The additional stream entering the second bioreactor contained only 1,3-DCP at high concentration (γ_I = 2000 mg L^{-1}). The overall biodegradation degree of the particular system was 68 %, while the biodegra-

dation rate of the second bioreactor was 5.21 mg L^{-1} h^{-1}. The system of two CSTRs in series is a successful effort in combining all the important parameters affecting 1,3-DCP biodegradation. Also, the system is very promising in manipulating streams with higher 1,3-DCP mass concentration and worth further investigation.

Nomenclature

a_c – acceleration, m s^{-2}
c – concentration, mmol L^{-1}
r – biodegradation rate, mg L^{-1} h^{-1}
D – dilution rate, h^{-1}
τ – residence time, h
k – rate coefficient, h^{-1}
n – stirring speed, min^{-1}
Q – volume flow rate, mL h^{-1}
T – temperature, °C
t – time, h, d
γ – mass concentration, mg L^{-1}, g L^{-1}
γ_I – mass concentration of DCP, mg L^{-1}
γ_S – mass concentration of glucose, mg L^{-1}
γ_x – cell dry mass concentration, mg L^{-1} DM
φ – volume fraction, %

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