ADAPTATION OF AN INOCULUM TO 2,4,6-TRICHLOROPHENOL BIODEGRADATION IN AN ACTIVATED-SLUDGE BIOREACTOR

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ABSTRACT

The aim of this work is to study the acclimation of an activated sludge fed at a constant COD volumetric load, to a recalcitrant substrate: the 2,4,6-TCP. Phenol was used as an inductive and energy-supplier co-substrate and was progressively replaced by TCP. A loss in primary biodegradation activity until 60 and then 94% was the result of two events: A shock load due to the first TCP addition that had represented 5% of the COD volumetric load and a second shock overload achieved with a 100% TCP inlet. This last overload stage was characterized by the reduction of dehalogenation and mineralization deficits. On the other hand, the progressive increase of TCP generated a loss in dehalogenation and mineralization activities while TCP removal rate remained high. A load of 11.3 $mgCOD.g^{-1}VSS.h^{-1}$ of TCP was achieved without exceeding 17% of mineralization deficit and no significant dehalogenation deficit.

KEYWORDS

Biodegradation, biomass acclimation, chlorophenol, dehalogenation, mineralization

INTRODUCTION

The chemical modification of a molecule so as to build a xenobiotic compounds, generates changes of the behavior of such molecule as far as biodegradability and ecotoxicity are concerned. Phenol that exhibits recalcitrant properties and inhibitory effects on many micro-organisms has been selected as a reference molecule. However, phenol biodegradation occurs and can support the growth of an adapted biomass as sole carbon and energy source (Lallai and Mura, 1989 ; Menke and Rehm, 1992). Phenol gives rise to a line of xenobiotic compounds, out of which, chlorophenols are widely used as antiseptics and biocides. This line, including 2,4,6-TCP, brings together 4 of the 129 compounds classified as priority pollutants by the US EPA (Keith and Telliard, 1979). This paper deals with the acclimation of an activated-sludge bioreactor to 2,4,6-TCP, one of the most recalcitrant chlorophenol. We will introduce the different adaptation phases undergone by the biomass during the progressive modification of its feeding from phenol to 2,4,6-TCP while maintaining a constant volumetric COD loading rate.

MATERIAL AND METHODS

This study was carried out using a bioreactor inoculated with 1 $gVSS.L^{-1}$ of an activated sludge obtained from a wastewater treatment municipal plant. The bioreactor's working volume was of 9 L and it was equipped with a 1.6 L settling unit. The reactor was operated at a 1 day Hydraulic Retention Time with a 11.3 $mgCOD.L^{-1}.h^{-1}$ volumetric load ($\dot{C}_{COD}$).The feeding medium was made up of 20 $mg.L^{-1}$ $MgSO_4$, 234.3 $mg.L^{-1}$ Na_2HPO_4 and 115.6 $mg.L^{-1}$ KH_2PO_4. The carbon source was variable with a progressive change from 100% Phenol to 100% 2,4,6-TCP through 10 successive stages labeled « a » to « j » (table 1). The C/N ratio was adjusted to 5 with $(NH_4)_2SO_4$. Determinations of COD and Solids were achieved as described by the French standards NF-T90-101 and NF-T90-029 respectively (AFNOR, 1978a,b). Samples were filtered successively through a cellulose filter and a 0.45 µm cellulose nitrate filter for the analysis of phenol and 2,4,6-TCP. The filtered samples were injected into a C18 SHANON Spherisorb

ODS2 HPLC column equipped with a UV detector at 270 nm. The mobile phase consisted of methanol-acetic acid 1% (70:30 v/v) at a flow rate of 0.9 ml.min^{-1}. Chloride measurements were performed using an ion analyzer (Orion Research, Inc., Model 290A) with a chloride electrode (Ingold, Model 94-17B). AOX were analyzed following ISO 9562 standard method with a coulometric analyser Ströhlein 7020 Cl equipped with an adsorption unit Ströhlein SAE II.

RESULTS AND DISCUSSION

The biomass acclimation to phenol was performed during the first stage « a » (Table 1). It revealed a phenol specific residual flow of 27 µmol.g^{-1}VSS.h^{-1} in the outlet providing a removal efficiency of 53.5% (Figure 1A). The COD specific outlet flow $\dot{B}_{COD}$ indicated the same removal efficiency of 53.7%. Nevertheless, we observed a gap between COD removal efficiency which remained at 66% after 6 days and at 91% after 17 days and the complete elimination of phenol achieved throughout the same period (Figures 1A and 1B). The difference between the measured COD and the theoretical COD calculated from the residual phenol in the outlet (and 2,4,6-TCP) (Figure 1B), expressed a mineralization deficit of 33% on day 6, decreasing progressively to 2.7% on day 17. This difference was probably due to the fact that part of the phenol feed did not go through complete mineralization (Lallai and Mura, 1988 ; Menke and Rehm, 1992) providing intermediary metabolites in the reactor effluent (Buitron et al, 1998).

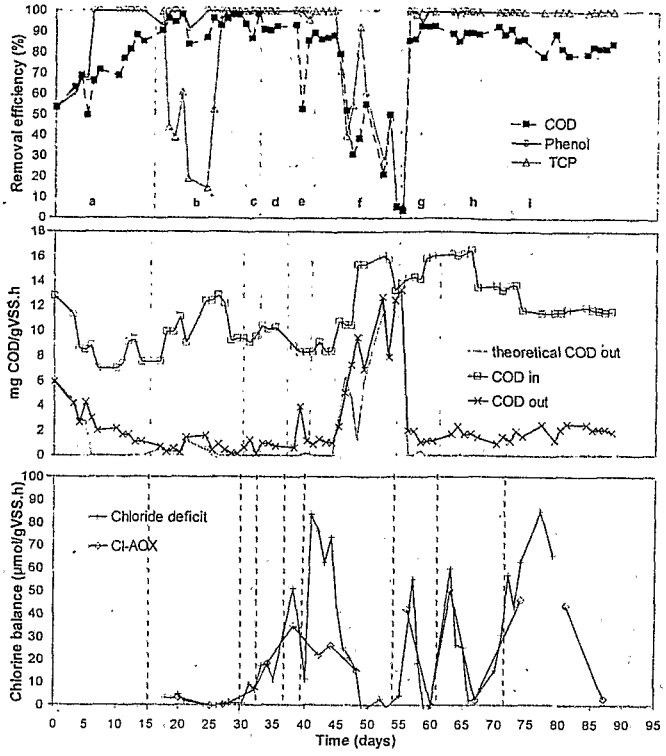
Table 1: Divisions of the acclimation stages of the activated-sludge inoculum to phenol and then to 2,4,6-TCP.

Period (days)	Stage	HRT (day)	COD loading rate $\dot{C}_{COD}$ (mgCOD.L^{-1}.h^{-1})	Feed composition (Percent of $\dot{C}_{COD}$)		Biomass concentration (gVSS.L^{-1})
				Phenol	2,4,6-TCP	
0-16	a	1	10.0	100	0	1.26
17-30	b	1	11.0	95	5	0.93
31-32	c	1	11.3	90	10	1.21
33-37	d	1	11.4	80	20	1.11
38-40	e	1	11.5	50	50	1.36
41-55	f	1	11.4	0	100	0.73
56-62	g	1	11.3	50	50	0.79
63-69	h	1	11.5	30	70	0.71
70-73	i	1	11.3	20	80	0.84
74-88	j	1	11.4	0	100	0.98

Only 5 days after reactor starting up, a biomass loss occurred until day 26. By that time 26.3% of the initial sludge VSS had been lost (Table 1). This loss was related to a biomass washing out, resulting from the elimination of those micro-organisms that could not grow on phenol as sole carbon and energy source or that had lost their ability to settle. It is worth to emphasize that depending on the criterion chosen the lag time for phenol acclimation would be different. It would be of 6 days based on phenol removal (primary biodegradation efficiency) or 26 days based on COD removal (mineralization efficiency).

Transitory effects related to the first intake of 2,4,6-TCP

During the first 9 days of the "b" stage, a residual concentration of 2,4,6-TCP was observed in the reactor outlet, which indicated a removal efficiency loss of 85.4% on day 24 (Figure 1A). The appearance of a chloride-ion deficit (difference between the measured chloride ions and the theoretical liberation of chlorine calculated from the 2,4,6-TCP balance) of 100% was detected during the 4 first days (Figure 1C). This was confirmed by the detection of an intermediate chlorine reservoir $\left(\dot{C}l_{INTERMEDIATE} = \dot{C}l_{AOX} - \dot{C}l_{TCP}\right)$ of 4.7 µmol.g^{-1}VSS.h^{-1}. From days 21 to 27, the chloride-ion deficit disappeared while the outlet intermediate chlorine reached 0.08 µmol.g^{-1}VSS.h^{-1} on day 26 (Figure 1C).



Figures 1: Monitoring of bioreactor along 87 days: (A) removal efficiency, (B) inlet and outlet COD flow and theoretical outlet flow, (C) chlorine balance and intermediate chlorine reservoir.

Adaptation to 2,4,6-TCP was a multi-step phenomenon: From days 17 to 20, the adaptation to the primary biodegradation of TCP generated very little effect on phenol and COD removals (Figure 1A). From days 20 to 27, the dehalogenation activity started, allowing chloride-ion deficit to disappear (Figure 1C). Phenol brought the energy that had been used by the biomass, so that we can consider that the observed phases were not energy-dependent but required a biomass adaptation implementing the selection of competent flora or of an enzymatic induction phase.

Increasing of the 2,4,6-TCP load

The objective of these rapid feeding series was to increase the proportion of TCP in the inlet in such a way that the degradation capacities of the biomass were enhanced. The sequence of these phases resulted in the occurrence of outlet intermediate chlorinated compounds (chloride-ion deficit) which increased progressively along the phases « c » and « d » and remained stable to a flow of 34.7 $\mu mol.g^{-1}VSS.h^{-1}$ on day 38 (Figure 1C). These phases (c, d, e) of TCP increase also resulted in a biomass production until 1.4 $gVSS.L^{-1}$ (table 1). The transition to a 2,4,6-TCP 100% feeding was characterized at the beginning of stage « f » by the occurrence of an intermediate chlorinated compound flow of 25 $\mu mol.g^{-1}VSS.h^{-1}$ (Figure 1C). This step resulted in a decrease of COD removal to 61.5% (day 48) (Figures 1A and B) and in the occurrence of TCP in the outlet stream. The first period of this stage (days 41 to 48) was characterized by maximum dehalogenation and mineralization deficits of 58% (Figure 1C). It

corresponded essentially to an adaptation period of the TCP primary biodegradation activity (Figure 1A). The second stage (days 48 to 55) concerned the development of a substrate inhibitory activity of the biodegradation resulting in a removal efficiency of only 6.2% on day 55 which could depend on lack of maintenance energy or on a toxic shock on some phenol-users micro-organisms. The dehalogenation activity benefited from the slowing down of biodegradation activity to restore the balance $-r_{TCP}/r_{Cl}$. We emphasized that the dehalogenation deficit did not appear while the removal inhibitory shock was in progress and on the contrary would rather proceed from an adaptation stage to a transient progressive increase of the biodegradation activity. The return to a feeding composed with 50% of phenol and 50% of TCP stopped the spiral of the shock load providing energy through phenol. Along this stage from « g » to « j », TCP removal efficiency remained stable at 100% (Figure 1A). This phenomenon demonstrated the possibility to reduce the incidence of this threshold thanks to a perfect management of the adaptation phase. The removal efficiency of COD remained around 78 to 84% during these stages (Figures 1A and B). This equilibrated system resulted in the occurrence of a mineralization deficit of only 17%.

CONCLUSION

The progressive adaptation of a biomass to chlorophenol biodegradation resulted in an interaction between biodegradation, dehalogenation and mineralization activities. The dehalogenation activity presented a quick adaptation despite some delay during transition phases, when the load was increasing. The mineralization showed a quick and stable adaptation but there always remained a delay compared to primary biodegradation activity resulting in a residual COD deficit of about 15%. When the unbalance was too important or when the chlorophenol load was still raising up, a shock load related to following phenomena was observed: Primary biodegradation was subjected to inhibition related to substrate which allowed an important slowing down of biodegradation activity. This phase had no consequences on dehalogenation and mineralization activities so that the complete disappearance of chloride-ion deficit was achieved. During the different stages, biomass washing out was often observed in some of the critical phases. Those concerned the elimination of micro-organisms that could not use recalcitrant substrates or that were sensitive to these inhibitory substrates (Liu et al., 1982 ; Blum and Speece, 1991).

REFERENCES

AFNOR, (1978a). Détermination de la demande chimique en oxygène (DCO). *Normalisation française* NF-T 90-101.

AFNOR, (1978b). Détermination des matières en suspension. *Normalisation française* NF-T 90-105.

Buitrón G., González A. and López-Marín L. M. (1998). Biodegradation of phenolic compounds by an acclimated activated sludge and isolated bacteria. *Wat. Sci. Technol.*, **37**(4-5), 371-378.

Blum D. J. W. and Speece R. E. (1991). A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. *J. Water Pollut. Control Fed.*, **63**(3), 198-207.

Keith L. H. and Telliard W. A. (1979). Priority pollutants. I- a perspective view. Special report. *Environ. Sci. Technol.*, **13**(4), 416-423.

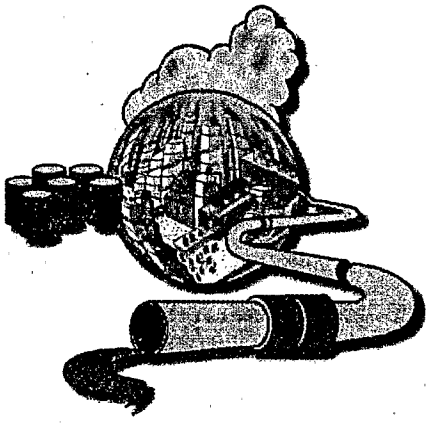
Lallai A. and Mura G. (1989). pH variation during phenol biodegradation in mixed cultures of microorganisms. *Wat. Res.*, **23**(11), 1335-1338.

Lallai A. Mura G. Miliddi R. and Mastinu C. (1988). Effect of pH on growth of mixed cultures in batch reactor. *Biotechnol. Bioeng.*, **31**, 130-134.

Liu D., Thomson K. and Kaiser K. L. E. (1982). Quantitative structure – toxicity relationship of halogenated phenols on bacteria. *Bull. Environ. Contam. Toxicol.*, **29**, 130-136.

Menke B. and Rehm H.J. (1992). Degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes sp. A72. Appl Microbiol Biotechnol*, **37**, 655-661.

PROCEEDINGS



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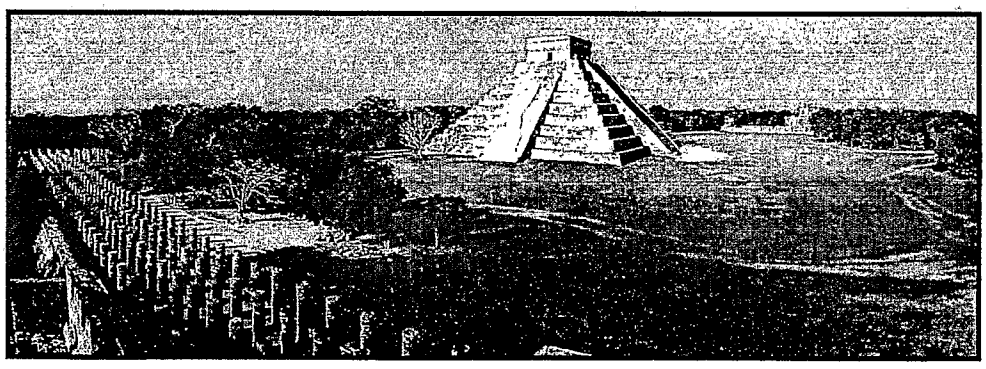

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