Wat. Sci. Tech. Vol. 26, No. 9-11, pp. 2049-2052, 1992.
Printed in Great Britain.

0273–1223/92 $15·00

MICROBIAL ACTIVITY EVOLUTION DURING THE ACCLIMATION OF A MIXED CULTURE TO PHENOL: USE OF CO_2 EVOLUTION RATE AS INDICATOR

G. Buitrón, A. Koefoed and B. Capdeville

Unité de Recherche Traitement Biologique des Eaux, Département Génie des Procédés Industriels, Institut National des Sciences Appliquées, Complexe Scientifique de Rangueil, 31077 Toulouse, France

ABSTRACT

The microbial activity during the aerobic acclimation of activated sludge to phenol was studied. Carbon dioxide evolution rate (CER), measured in a sequencing batch reactor coupled to an infra-red system, was utilized as the activity control parameter. It was found that CER is representative of the microbial metabolism. Moreover, it was observed that starvation periods during acclimation had a negative effect on biodegradation rate.

KEYWORDS

Acclimation, microbial activity, CO_2 evolution rate, phenol, biodegradation, sequencing batch reactor, xenobiotics

INTRODUCTION

The biodegradation or the persistence of an organic chemical in the environment may be due to a large number of factors. An important consideration when persistence is found is whether that persistence is due to recalcitrance or to unsatisfactory environmental conditions. If the second case is present, an evaluation must be made for altering or controlling the limiting conditions in such a way as to allow biodegradation to occur (Grady, 1985).

Acclimation of microorganisms to the organic chemicals is an important phase of the biodegradation process, especially when hazardous wastes from landfills or chemical dumps have to be degraded. The duration of this phase, when mixed cultures are exposed to new or unusual chemicals, ranges from a few hours to several weeks or months and it depends on the quantity and quality of the inoculum utilized (Ventullo and Larson, 1986; Aelion *et al.*, 1989). Different phenomena have been suggested to account for lag phases. Thus, a microbial selection and physiological transformations in the metabolic system occur. This involves changes in enzymes regulation and production, and may implicate alterations in cell size and composition, as well as in the genetic characteristics (Kim and Maier, 1986).

In this work we studied, during the acclimation phase, the activity evolution of a microbial ecosystem exposed to phenol in aerobic conditions. The microbial activity was evaluated by means of the carbon dioxide evolution rate in gas phase (CER) using a sequencing batch reactor (SBR) coupled to an infra-red system (IRS).

EXPERIMENTAL PROCEDURES

Sequencing Batch Reactor System

Activated sludge was utilized as inoculum for the acclimation studies. It was taken from the municipal wastewater treatment plant in Toulouse City. Phenol was the sole source of carbon and energy. The mineral salts medium used was as proposed by the French norm (NF T 90-312, 1985). The pH of the mixed liquor was buffered and, throughout fermentation, it varied from 7.5 to 7 .

The experimental assembly is illustrated in figure 1. The system consists of a batch reactor with a working volume of 5 litres, equipped with a dissolved oxygen meter, a pH meter and an IRS (330 Gas analyser, Automated Custom Systems, Inc.). The output signal of these instruments is continuously recorded. The reactor is equipped with a water jacket in order to ensure constant temperature (25 ± 0.2 °C). An air pump continuously introduces constant air flow through the reactor. This flow is passed to the IRS where CO_2 is analysed and CER is computed. Specific carbon dioxide evolution rate (SCER) was calculated by dividing CER by mixed liquor suspended solids (MLSS). Acclimation of biomass was made by increasing the phenol concentration, stepwise, from 50 to 700 mg/l. Sludge age was 9 days and concentration of MLSS varied from 400 to 1600 mg/l.

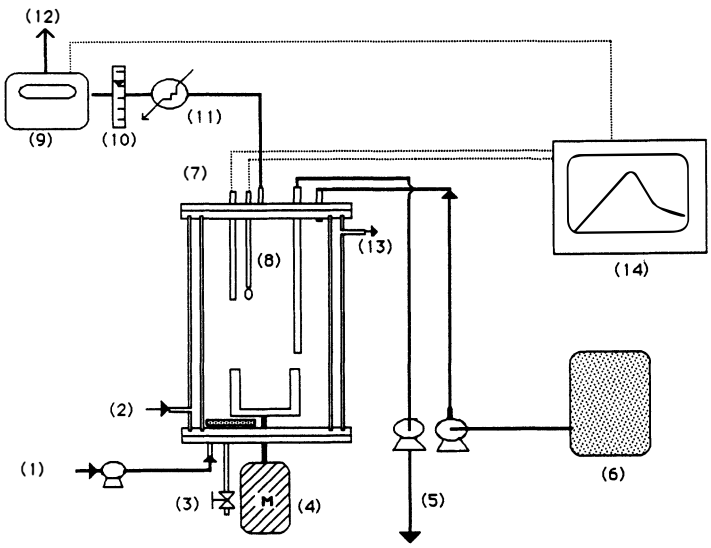


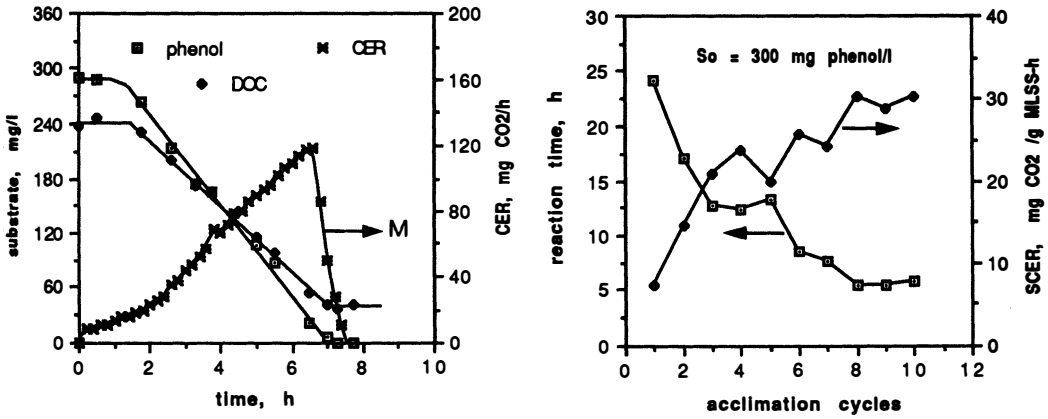
Fig. 1. System utilized for the acclimation studies.1. air pump; 2. water jacket in; 3. sample point; 4. mixing motor; 5. mixed liquor and supernatant purge; 6. phenol and mineral salts' tank; 7. dissolved oxygen meter; 8. pH meter; 9. infra-red system; 10. flow meter; 11. water condenser; 12. air out; 13. water jacket out and 14. recorder

Analytical Procedure.

Phenol concentration (4-aminoantipyrine method), dissolved organic carbon, DOC (Dohrmann DC-180 carbon analyser) and suspended solids were determined in liquid phase (Standard Methods, 1981).

RESULTS AND DISCUSSION

Figure 2 shows a typical set of results obtained during acclimation. Phenol biodegradation was superior to 99% and DOC elimination was around 85% for all the concentrations studied. It is interesting to note, in figure 2a, that at the time when almost all phenol is removed, the CER reaches its maximal value. This can be attributed to the portion of substrate that has been mineralized. After that, the CER decreases and the microbial metabolism slows down. So, it is clear that CER is a representative parameter of the biodegradation rate and can be utilized as a microbial activity indicator.



(a) (b)

Fig. 2. Microbial activity during phenol degradation, So/Xo= 0.45 mg phenol/mg MLSS. a) CER, phenol concentration and dissolved organic carbon as function of time; b) SCER and time necessary to reach maximal CER (or time necessary to degrade 99% of phenol) as function of acclimation cycles

In order to maintain the maximal activity, a new cycle of the SBR was initiated at point M (figure 2a). A preselected volume of mixed liquor was purged, mixing and aeration were stopped and the activated sludge was allowed to settle for 30 min. After settling, the supernatant was syphoned off. Phenol and mineral salts medium were added and the volume adjusted with tap water. Figure 2b shows the activity evolution as SCER and the time required to reach the maximal CER during acclimation cycles.

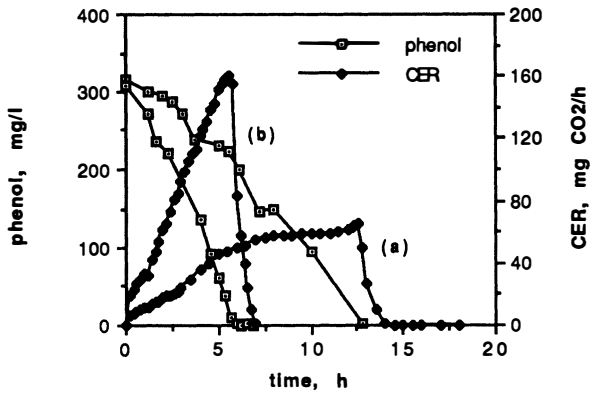


Fig. 3. Influence of starvation period on microbial activity. So/Xo = 0.45 mg phenol/mg MLSS. (a) after and (b) before starvation period

Once the microorganisms were acclimated to phenol, the influence of starvation period on activity was studied. Thus, a set of 8 cycles of the SBR was carried out, but they were stopped after 24-28 hours instead of at the point M in figure 2a. Results are shown in figure 3. As it can be seen, there is a loss of activity after starvation. Phenol biodegradation was achieved in 13 hours instead of 5.5 hours before starvation period and CER decreased from 160 to 65 mgCO_2/h. Similar results were obtained by Arbuckle and Kennedy (1989), who found that activated sludge acclimated to degrade phenolic compounds lost its ability when these compounds were absent for several days. Therefore, in order to maintain the maximal activity during acclimation, and to develop microorganisms capable of degrading highly concentrated xenobiotics, starvation periods must be avoided. An on-line computer system, with the SCER as the activity control parameter, could be utilized.

CONCLUSION

The microbial capability to degrade organic compounds depends on the adaptation of such microorganisms to the new environment. Thus, throughout acclimation all conditions needed to satisfy microbial requirements must be present in order to maintain their maximal activity. In this study the utility of CER as an indicator of the biomass activity in the acclimation period was demonstrated. It was found that microbial starvation during acclimation has a negative effect on subsequent substrate biodegradation rate.

ACKNOWLEDGEMENT

The research was partially supported by Groupement de Recherches de Lacq, Service Environnement, Groupe ELF Aquitaine.

REFERENCES

Aelion, C.M., Dobbins, D.C. and Pfaender, F.K. (1989). Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: influence of substrate concentration and preexposure. *Environ. Toxicol. Chem.*, 8, 75-86.

AFNOR (1985). Evaluation en milieu aqueux de la biodégradabilité aérobie "ultime" des produit organiques solubles. *Norme Française.* NF T 90-312

APHA (1981). *Standard methods for the examination of water and wastewater*, 15th edition. American Public Health Association, New York.

Arbuckle, W.B. and Kennedy M.S. (1989). Activated sludge response to a parachlorophenol transient. *J. Wat. Pollut. Control Fed.*, 61, 476-480.

Grady, C.P.L. Jr, (1985). Biodegradation: Its measurement and microbiological basis. *Biotechnol. Bioeng.*, 27, 660-674.

Kim, Ch.J. and Maier, W.J. (1986). Acclimation and biodegradation of chlorinated organic compounds in the presence of alternate substrates. *J. Wat. Pollut. Control Fed.*, 58, 157-164.

Ventullo, R.M. and Larson, R.J. (1986). Adaptation of aquatic microbial communities to quaternary ammonium compounds. *Appl. Environ. Microbiol.*, 51, 356-361.