

# Aerobic biodegradation of 3- chlorophenol in a Sequencing Batch Reactor. Effect of cometabolism.

A. Chiavola\*, R. Baciocchi\*\*, R. L. Irvine \*\*\*, R. Gavasci\*\*, P. Sirini\*\*\*\*,

\*Department of Hydraulics, Transportation and Roads, University of Rome “La Sapienza”, Via Eudossiana 18, 00184 Rome, Italy, (E-mail: [Agostina.Chiavola@uniroma1.it](mailto:Agostina.Chiavola@uniroma1.it))

\*\*Department of Civil Engineering, University of Rome “Tor Vergata”, Via del Politecnico, 1, 00133 Rome, Italy, (E-mail: [baciocchi@stc.uniroma2.it](mailto:baciocchi@stc.uniroma2.it))

\*\*\*Department of Civil Engineering and Geological Science, University of Notre Dame, Notre Dame, Indiana 46556 USA, (E-mail: [Robert.L.Irvine.1@nd.edu](mailto:Robert.L.Irvine.1@nd.edu))

\*\*\*\* Department of Civil and Environmental Engineering, University of Florence, Via S. Marta, 3 Florence, Italy, (E-mail: [p.sirini@tiscalinet.it](mailto:p.sirini@tiscalinet.it))

## Abstract

The aim of the present study was to investigate how phenol modifies, through cometabolism, the biodegrading capability of 3-chlorophenol (3-CP) in a Sequencing Batch Reactor (SBR) seeded with a mixed culture obtained from a domestic sewage treatment plant. Two laboratory scale SBRs, one fed 3-CP only and the other fed 3-CP and phenol in the same concentration, were seeded with the partially acclimated biomass. The removal capability in both reactors was measured for progressive increases in the feed organic loading. Cometabolism enhanced biodegradation of 3-CP by reducing both the initial lag period and the time required for the complete removal. 700 mg/L 3-CP was demonstrated to be the highest concentration, which could be completely degraded during the active phase (fill plus react) either in the presence or absence of phenol as the growth substrate even though the lag period was shorter when phenol was present. The operating strategy required modification for the complete removal of 800 mg/L 3-CP. An increase in the phenol to 3-CP ratio did, however, improve 3-CP degradation rate.

## Keywords

3-chlorophenol; Cometabolism; Competition; Inhibition; Phenol; Sequencing Batch Reactor

## INTRODUCTION

While biological processes for the decontamination of water and wastewater is often more difficult when several organic compounds with different biodegradability rates are present, the interaction among such compounds can sometimes enhance the biodegradation rate of potentially toxic and biorefractory compounds. Many chemicals, some of which are persistent, are degraded co-metabolically by microorganisms unable to use them as a source of energy or essential nutrients (Alexander, 1994). Therefore, such microorganisms need one substrate to support growth while another substrate, the cometabolite, is converted to an organic product that often accumulates. The more accepted explanation to this phenomenon is linked to the fact that many enzymes act on several structurally related substrates. Consequently, non growth substrate that have a similar structure to that of the growth one could bind to enzymes and be transformed due to nonspecific activity of the existing enzymes and cofactors. However, cometabolic transformation kinetics usually are difficult to interpret and depend upon many factors. Chlorophenols belong to one class of compounds, which are subject to cometabolism. (Kim and Hao, 1999; Hao *et al.*, 2002).

The present study shows the results of an experimental work aimed at investigating how cometabolism affects the aerobic biodegradation of 3-chlorophenol (3-CP) in a Sequencing Batch Reactor (SBR). 3-CP has been selected as the target compound being representative of chlorophenols, a class of organic pollutants widespread distributed and persistent in the natural environment (Häggblom and Valo, 1995).

Phenol, being a structurally analog compound, was chosen as growth substrate and a mixed culture from a treatment plant for domestic sewage was used as seed. An SBR (Irvine and Ketchum, 1989) was selected since it has been successfully applied to the degradation of a wide range of organic compounds, many of which are biorefractory and toxic to microorganisms (Buitron *et al.*, 2001). Some experiences have been reported on the application of SBR to chlorophenol degradation (Basu and Oleszkiewicz, 1995; Yoong *et al.*, 2000); however, few studies are available on 3-CP removal by mixed culture in a SBR (Chiavola, *in press*).

## MATERIALS AND METHOD

### Acclimation tests

Activated sludge from the oxidation tank of a full-scale continuous flow treatment plant for domestic sewage was used as seed. Prior to be transferred into SBRs, it was progressively adapted to phenolic compounds through several acclimation tests. The acclimation tests were performed in 1 L Erlenmeyer flasks, maintained in agitation through a magnetic stirrer. Air was supplied through a porous stone located on the bottom of the flask. Water volatilisation was always taken into account before sampling. In each test, feed solution was added to the reactor at the beginning either in presence or absence of phenol as growth substrate, and then the residual concentration along with mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration periodically measured until the complete removal was observed. Biomass concentration was maintained around 1,5 gVSS/L throughout the study period.

### Sequencing Batch Reactors (SBRs)

The aerobic biodegradability studies of 3-CP alone and in cometabolism with phenol were conducted in two identical laboratory scale SBRs. The reactors were inoculated with the activated sludge from the acclimation tests. Each reactor had an operating volume of 3 L and was constructed of Erlenmeyer flask. The reactors were mounted on magnetic stirrer to maintain complete mixing during fill and react phases and were operated in parallel. Air was supplied during fill and react through a porous stone located at the base of the cylinders and connected to a compressor: the dissolved oxygen (DO) level in the mixed liquor was always maintained greater than 2 mg/L. Filling, mixing, aeration and drawing were controlled by time-switches. At the end of the settle phase 750 mL of effluent were decanted while the same volume of influent was added at the beginning of each cycle. The influent flow rate and the effluent were delivered through peristaltic pumps. On the two parallel reactors, SBR1 received no supplemental substrate other than 3-CP, whereas reactor SBR2 received 3-CP and phenol usually in the same concentration as SBR1. Particularly, the feed concentration of 3-CP and phenol were initially selected so as to obtain the same value inside the reactor at the end of the fill (assuming no degradation) as used in the acclimation tests, i.e., 200 mg/L so as to reach 50 mg/L (due to the dilution ratio, i.e., total volume,  $V_T$ , to influent volume,  $V_I$ , of 4).

The operating strategy, and particularly the length of the cycle, was initially adjusted based upon the degradation rate observed, in order to accomplish progressive selection and enrichment of microorganisms able to degrade the target compounds and avoid any accumulation of the compounds in the reactors which would have caused biomass inhibition. When the feed removal was shown to occur in less than one day, then the reactors started to be operated on 3 daily cycles, each one lasting 8 hours and consisting of 0.50 h fill, 6.50 h react, 0.67 h settle, 0.25 h draw and 0.08 idle. Then, the operating cycle was maintained constant and the feed concentration was progressively increased up to the maximum value of 800 mg/L of 3-CP only (in SBR1) and 800 mg/L of 3-CP and 800 mg/L phenol (in SBR2). The nominal hydraulic retention time at regime was 32 h.

## Feed preparation

The feeds to SBR1 and SBR2 were made by diluting in tap water known amounts of 3-CP and 3-CP and phenol respectively, so as to obtain the target concentration; nitrogen (in the form of  $\text{NH}_4\text{NO}_3$ ) and phosphorus (in the form of  $\text{KH}_2\text{PO}_4$ ) were also added to provide the microorganisms with nutrient elements, based upon a COD/N/P ratios equal to 100/15/4.

## Chemical analyses

Samples from the reactors were filtered on 1.2  $\mu\text{m}$  pore size filters to remove solids: supernatant was immediately analyzed for 3-CP and phenol concentrations while solids used to calculate MLSS and MLVSS by using the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). 3-CP and phenol concentrations were measured after filtration and/or centrifugation for 30 minutes at 4000 rpm, by extraction with Solid Phase Micro-Extraction (SPME) using polyacrilate fiber (Supelco), followed by Gas Chromatography/Flame Ionization Detector analysis.

pH was measured directly in each reactor by using a pH meter (HI8314, Hanna Instruments). Oxygen Uptake Rate (OUR) measurements were conducted by an automatic DO/OUR meter (UCT Chemical Engineering High Tech Micro Systems).

## Track studies

Track studies or time profiles were carried out within typical operating cycle at each feed increasing step, in order to determine the removal patterns and kinetic parameters. They were always performed in parallel in SBR1 and SBR2, in order to investigate the effects of cometabolism on 3-CP biodegradation rate. Samples of mixed liquor were collected at regular intervals during the fill and the react phases, filtered and then analyzed for 3-CP alone (SBR1) and 3-CP and phenol (SBR2). Besides, MLSS and MLVSS concentrations, pH and OURs were also measured. Supernatant of the previous cycles was analyzed for each track study.

## Abiotic processes

Contribution of abiotic processes such as volatilisation, photodegradation and adsorption on sludge flocs to the degradation observed was studied. To this purpose, test of volatilisation and photodegradation were performed under similar operating conditions as employed in the acclimation tests and SBRs, but with the absence of biomass in wastewater. In addition, in the tests with phenol, being a more biodegradable substrate than 3-CP, the addition of the toxic substance  $\text{HgCl}_2$  was needed to prevent biomass growth. Besides, in the photodegradation tests one flask was covered with isolating material to avoid photosynthesis phenomena. Under such conditions any loss of phenol/3-CP would be attributed to air stripping from aeration. The duration of the tests was about 10 days, during which time periodical analyses of 3-CP/phenol concentrations, pH and DO were carried out. Adsorption of the target compounds on the activated sludge flocs was measured on samples after extraction with Soxhlet following the Environmental Protection Agency (EPA) method 8041.

## RESULTS AND DISCUSSION

### SBRs

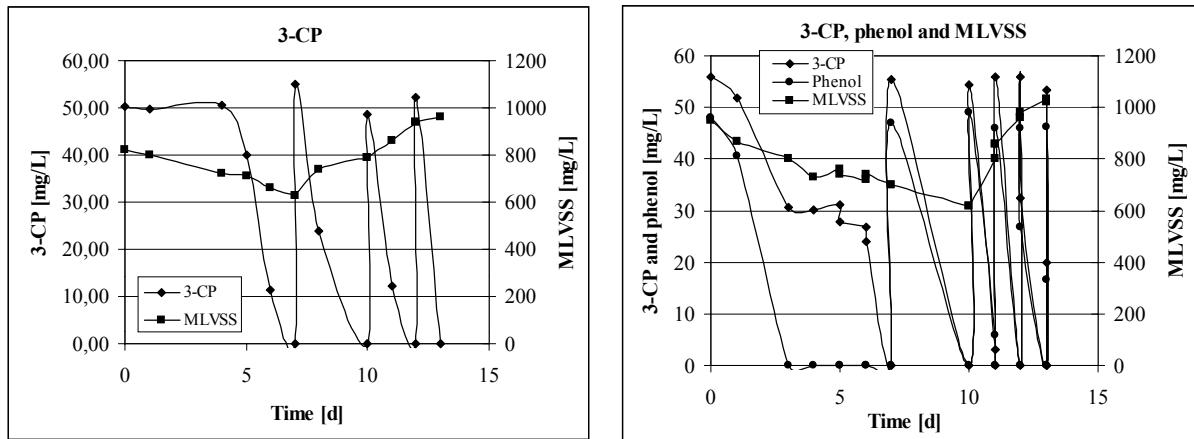
Contribution of volatilisation, photodegradation and adsorption on sludge flocs to degradation was experimentally demonstrated to be negligible for both compounds.

Figure 1 shows 3-CP concentration versus time in SBR1 in the first experimental phase during which time the feed concentration was maintained constant and equal to 200 mg/L 3-CP in SBR1 and 200 mg/L 3-CP and 200 mg/L phenol in SBR2, and the length of the cycle progressively reduced. It can be observed that the first feed took about 6 days to be completely degraded, with an initial lag period of about 4 days, followed by a rapid consumption. This pattern is in accordance

with 3-CP profiles observed in the acclimation tests (not here reported) and in previous studies (Chiavola, *in press*). After the first cycle, the following feeds took progressively less time to be degraded due to the adaptation of the biomass to 3-CP. MLVSS concentration profile, shown in the same Figure, confirmed such a pattern.

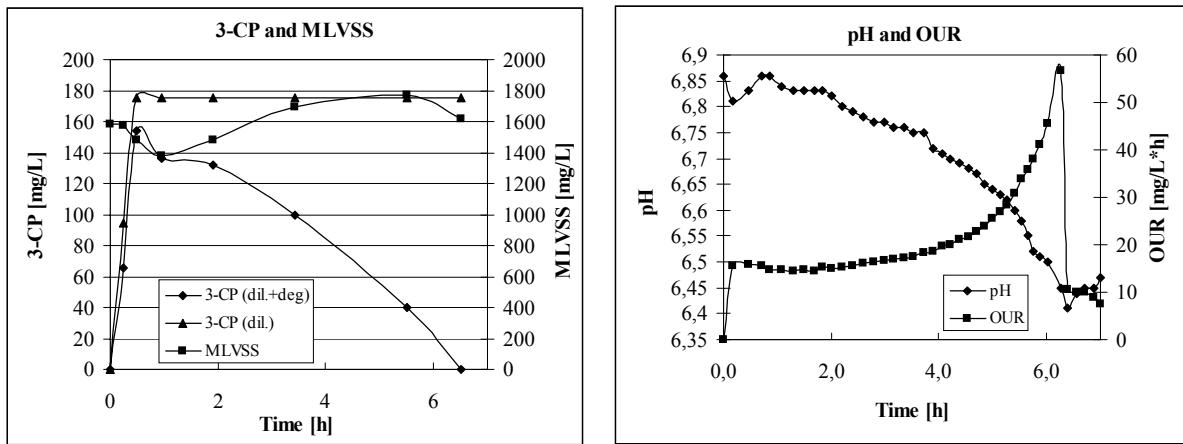
Figure 2 shows 3-CP, phenol and MLVSS concentration versus time in SBR2 during the same experimental period. Again, progressive biomass adaptation due to selection and enrichment of microorganisms able to degrade both compounds, led to faster degradation rates; therefore, the length of the cycle could be reduced to 8 h. However, a different pattern for 3-CP degradation was observed in SBR2 as compared to SBR1. Particularly, 3-CP removal commenced at the beginning of the cycle, instead of lagging initially for few days as in SBR1, and continued until phenol was removed. Minimal 3-CP removal occurred after phenol removal until the 6<sup>th</sup> day, when the degradation resumed at a rate greater than that observed during the first period. Such a pattern can be explained by the non-specificity of the enzymes of phenol degradation, which are also responsible for 3-CP removal, a structural analog of phenol. However, as soon as the growth substrate (i.e., phenol) was depleted, cometabolism ceased. Resumption of 3-CP removal probably resulted from the enrichment of another member of the consortium rather than one of the phenol degraders developing a different metabolic pathway. Comparison of Figure 2 with Figure 1 shows that cometabolism in presence of growth and non-growth substrate at similar concentrations did not induce a reduction of the time required for compete degradation to occur; however, overall the length of the cycle could be shortened to 8 h within 13 days in SBR2 against 20 days as in SBR1, presumably because of the cometabolic removal of 3-CP by the phenol degraders and the direct removal of 3-CP by the 3-CP degraders.

Once the operating strategy reached three 8 h cycles per day, the feed concentration was increased progressively until 700 mg/L 3-CP was reached in SBR1 and 700 mg/L 3-CP and 700 mg/L phenol was reached in SBR2. Higher concentrations were also tested but could not be completely degraded within the cycle and caused inhibition. Therefore, the feed was fixed to 600 mg/L 3-CP in SBR1 and 600 mg/L 3-CP and 600 mg/L phenol in SBR2 so as to ensure a margin of safety for complete degradation, and to maintain 3 continuous cycles. Then, track studies were carried out within typical operating cycles for monitoring removal pattern in presence and absence of phenol, and determining related kinetic parameters even at higher organic loadings.



**Figures 1 and 2.** MLVSS and 3-CP degradation alone and in presence of phenol in SBRs

Figure 3 shows 3-CP (due either to dilution only or dilution and degradation) and MLVSS concentration profiles versus time during a track study performed at 700 mg/L 3-CP only using microorganisms from SBR1. It can be noted low degradation during fill period (profiles due to dilution only and dilution and degradation are quite similar), followed by a lag period lasting up to the 2<sup>nd</sup> hour; then a continuous degradation occurred until the substrate was completely depleted.



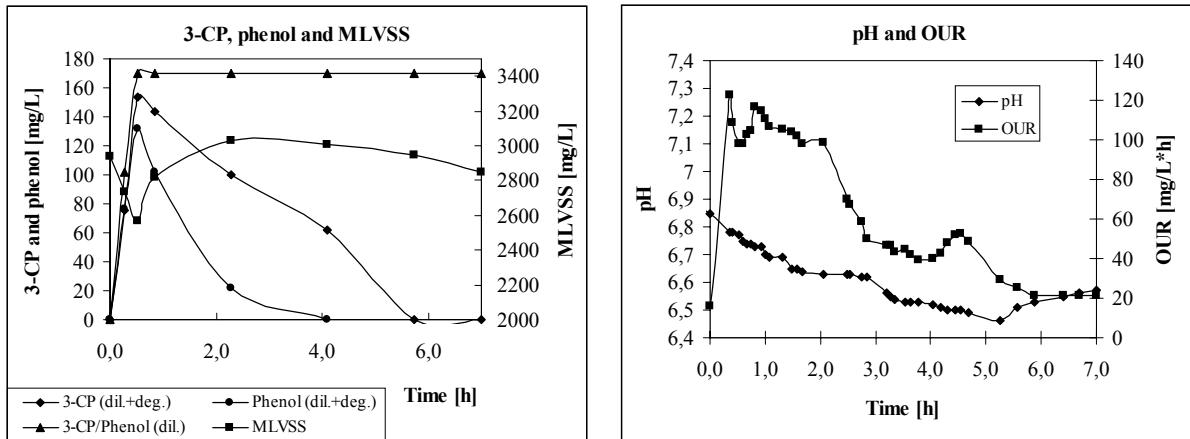
**Figures 3 and 4.** 3-CP, MLVSS, pH and OUR in track study for SBR1 at 700 mg/L 3-CP

Such a pattern is in agreement with that observed during the acclimation period either in batch tests or in the SBRs. During the fill time the 3-CP increased to about 160 mg/L inside the reactor. Any inhibition resulting from this elevated 3-CP level was overcome by the microorganisms in the last 4-5 hours of the react time during which time almost linear degradation was observed. The inhibition was confirmed by the OUR profile for the same track study, shown in Figure 4 along with pH values.

OUR profile, after an initial rapid increase at the commencement of the feed cycle, was constant during the first 2 hours, then it slightly increased reaching the highest value (about 60 mg/L/min) around the 6<sup>th</sup> hour when 3-CP concentration was about 40 mg/L. Such a profile is likely to be attributed to the progressive decrease of the biomass inhibiting effect occurring along with the reduction of 3-CP concentration within the reactor. Therefore, the OUR peak should be explained with the attainment of a non-inhibiting value which brought about the highest microbiological activity. The pH decreased after the 2<sup>nd</sup> hour and confirmed that degradation was taking place with the consequent release of chloride and hydrogen atoms.

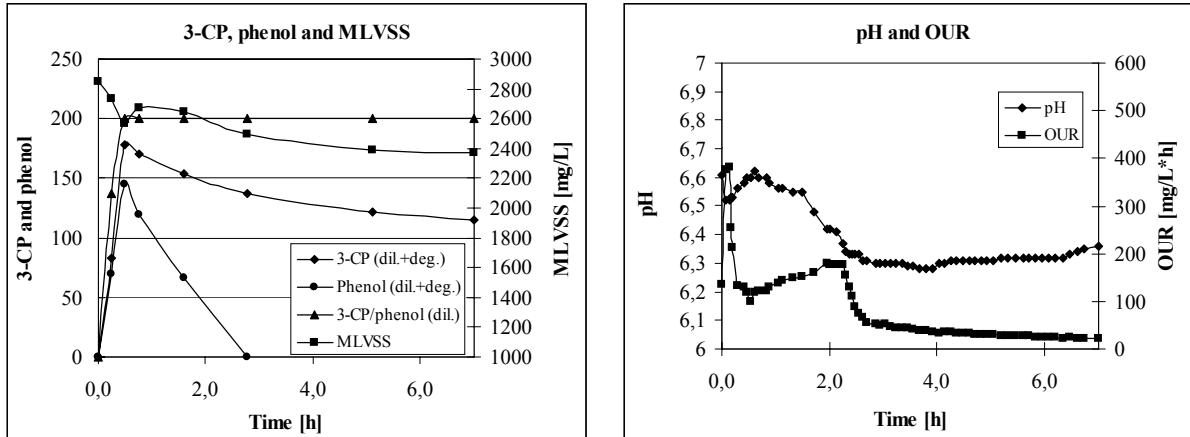
Figure 5 shows 3-CP, phenol and MLVSS concentration versus time in the track study for SBR2 carried out at 700 mg/L 3-CP and 700 mg/L phenol. 3-CP degradation started at the beginning of the cycle, as opposed to that observed for the consortium grown on 3-CP only, and continued until completion, even after phenol depletion. 3-CP complete removal also occurred at a faster rate than when phenol was present. However, it must be taken into account that although the overall organic loading factor in terms of mass of chemical oxygen demand (COD) applied per unit mass of MLVSS present per day was higher in SBR2 than in the previous track study for SBR1, the organic loading for 3-CP COD only was lower for SBR2, due to higher biomass concentration. As shown during acclimation in SBRs, cometabolism allowed continuous degradation of 3-CP during fill and react phases, however, in this case some microorganisms of the mixed culture showed to have developed capability of using 3-CP as growth substrate, since 3-CP degradation continued even after phenol disappearance.

The OUR profile and pH are shown in Figure 6. As can be seen, the OUR increased at the beginning of the feed cycle and remained relatively constant until virtually all of the phenol was consumed. However, competition-inhibition between phenol and 3-CP was also operative in the metabolic pathway of 3-CP and phenol degradation (Kim and Hao, 1999). The maximum specific 3-CP utilization rate was achieved after about 4.5 h, i.e., when phenol concentration was removed and 3-CP reached non-inhibitory concentrations. An almost linear correlation between OUR and the sum of phenol and 3-CP values was calculated (Yoong *et al.*, 2000).



**Figures 5 and 6.** MLVSS, 3-CP, phenol, pH and OUR in track study for SBR2 at 700 mg/L 3-CP and 700 mg/L phenol

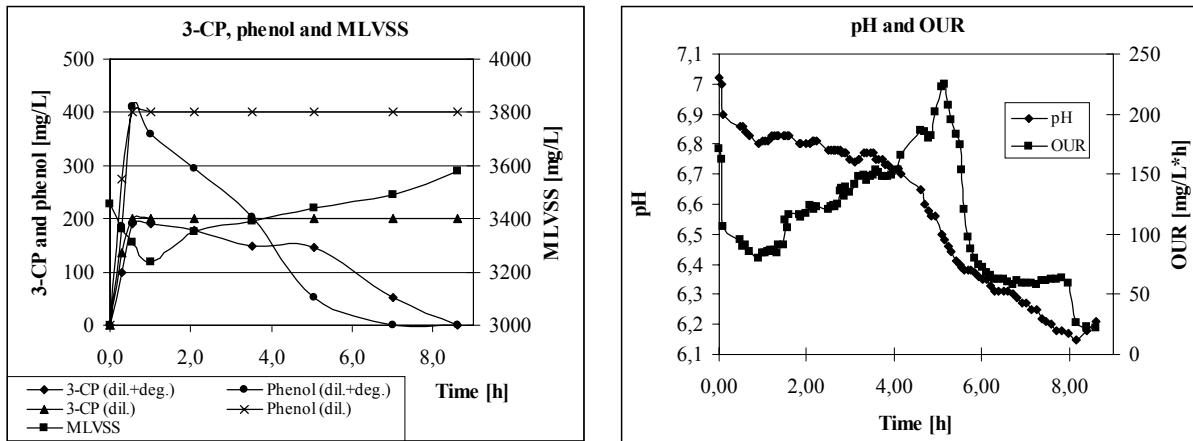
Figure 7 shows phenol and 3-CP (due to either only dilution or dilution and degradation) and MLVSS concentration versus time during track study performed at 800 mg/L phenol and 800 mg/L 3-CP. This 3-CP organic loading was the highest level that could be applied to the SBRs for the specific operating conditions tested. In fact, even though 3-CP was degraded along with phenol from the start of the cycle, the rate of phenol degradation was decreased appreciably, presumably because of inhibition induced by some combination of the elevated phenol concentrations reached and the low pH, leading to accumulation of 3-CP within the reactor. The OUR profile (see Figure 8) supports the notion of phenol inhibition. The OUR has a maximum value during fill when the phenol concentration is increasing from non inhibitory concentrations to much higher inhibitory concentrations when it quickly decreases to reduced rates. The OUR then steadily increases until the phenol disappears when it decreases to a background rate supported by the slow removal of 3-CP.



**Figures 7 and 8.** MLVSS, 3-CP, phenol, pH and OUR in track study for SBR2 at 800 mg/L 3-CP and 800 mg/L phenol.

The incomplete degradation of 3-CP was probably the result of the low pH that was reached before cometabolism by the phenol degraders could reduce 3-CP to background levels. Therefore, a new test was carried out by doubling phenol concentration in the feed, while 3-CP concentration was maintained equal to 800 mg/L. As can be seen from Figures 9 and 10, the additional phenol degradation removed more 3-CP by cometabolism (compare with Figure 7) and suppressed the reduction in pH. As a result, 3-CP was removed even though the benefit of the increased ratio of

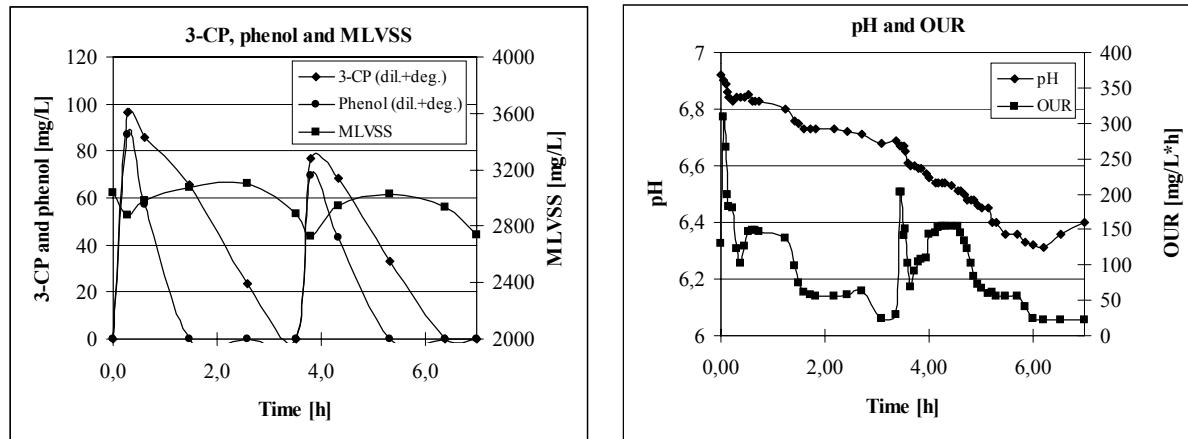
phenol to 3-CP was not optimal. The OUR profile (see Figure 10) showed a second peak as phenol concentration approached zero.



**Figures 9 and 10.** MLVSS, 3-CP, phenol, pH and OUR in track study at 800 mg/L 3-CP and 1600 mg/L phenol.

It can be concluded that under such 3-CP loading factor, either the ratio of the growth to non-growth substrate must be further implemented or the operating strategy be changed (e.g., the length of the react phase prolonged) in order to achieve complete 3-CP removal.

A final track study was carried out by exploiting typical flexibility of SBR: fractionation of feed flow rate into two consecutive steps within one cycle was introduced, while the overall feed concentration was maintained constant, i.e., 800 mg/L 3-CP and 800 mg/L phenol. Figure 11 shows 3-CP and phenol degradation (due either to dilution only and to dilution and degradation), along with MLVSS concentration profile versus time. A shorter time was required in the second step for complete 3-CP removal to occur, owing to higher dilution ratio ( $V_T/V_I$ ). Anyway, in both steps, complete degradation of both substrates was achieved within the react phase. OUR profile (see Figure 12) of each step was similar to that measured for one feed step only (see Figure 6), with the achievement of the maximum value correspondingly to a non inhibiting 3-CP concentration, highest MLVSS concentration and decreasing pH value. It is also useful to compare these results with those obtained in a track study with 400 mg/L 3-CP and 400 mg/L phenol (not here reported) in the feed, since the same substrate concentration inside the reactor at the end of the fill phase was established. The different operating strategy adopted, and particularly the longer fill phase used in the second case, determined 3-CP complete removal occurring within 2.8 and 1.3 hours, respectively.



**Figures 11 and 12.** MLVSS, 3-CP, phenol, pH and OUR in track study at 800 mg/L 3-CP and 800 mg/L phenol with two feed steps.

## CONCLUSIONS

The present study showed that cometabolism of similar concentrations of phenol and 3-CP, being used as growth and non growth substrate respectively, can enhance biodegradation of chlorophenol by a mixed culture, at least by reducing the length of the initial lag period and the time required for the complete removal to occur. Removal of increasing 3-CP concentrations can be accomplished by adjusting the ratio of phenol to 3-CP or by changing the operating strategy. However, competition-inhibition between 3-CP and phenol likely affects cometabolic kinetics, particularly at higher phenol to 3-CP ratio. Therefore, the best ratio must be selected based upon a compromise between the need of high enough reductant supply from phenol degradation and the risk of inhibition or low pH on 3-CP removal. The results obtained show also that even though 3-CP removal is triggered by the presence of phenol, it continues after phenol depletion, provided that the mixed culture is properly acclimated.

Due to the operating strategy selected, initial inhibition to microorganisms by the establishment of high 3-CP concentration in the reactors was observed, particularly displayed by the OUR profile: Such a condition could be avoided or at least reduced not only by adjusting phenol to 3-CP ratio, but also by modifying the fill strategy even maintaining the same organic loading factor (e.g by prolonging or by fractionating the feed phase). Therefore, SBR still remains the most suitable reactor configuration for the degradation of toxic and biorefractory compounds. In fact, its high flexibility allows to easily adjust the operating cycle with the aim of establishing and maintaining proper environment conditions for selection, enrichment and sustainment within a mixed culture of the microorganisms being specific for the degradation of the target compound. Besides, constant and high efficiency can be ensured even in spite of increasing or varying influent organic loading.

Finally, the 3-CP degradation patterns here observed are in agreement with those determined by the same authors in a previous study (Chiavola, *in press*): however in that case the initial acclimation was much longer. Such a difference could be explained by the different physiology of the mixed culture used in the two cases, even though it was collected at the same treatment plant. Besides, lower external temperature could also have had some effect on the biomass performance. Therefore, a complete microbiological study on microorganisms physiology would be an useful tool to better interpret the phenomenon observed.

## REFERENCES

Alexander M. (1994). *Biodegradation and bioremediation*. Academic Press, San Diego, CA.

Basu S. K. and Oleszkiewicz J. A. (1995) Factors affecting biodegradation of 2-chlorophenol in sequencing batch reactors. *Environ. Tech.* **16**, 1135-1143.

Buitron G., Soto G., Vite G. and Moreno J. (2001) Strategies to enhance the biodegradation of toxic compounds using discontinuous processes. *Wat. Sci. Tech.*, **43**(3), 283-290.

Häggblom M. M. and Valo R. J. (1995) Bioremediation of chlorophenol wastes. In *Microbial Transformation and Degradation of Toxic Organic Chemicals*, Wiley-Liss, Inc., pp. 389-434.

Hao O. J., Kim H. M., Seagren A. E. and Kim H. (2002) Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species. *Chemosphere*, **46**, 797-807.

Irvine R. L. and Ketchum Jr. L. H. (1989) Sequencing Batch Reactors for biological wastewater treatment. *CRC Critical Reviews in Environmental Control*, **8**(4), 255-294.

Yoong E. T., Lant P. A. and Greenfield P. F. (2000) *In situ* respirometry in an SBR treating wastewater with high phenol concentrations. *Wat. Res.*, **34**(1), 239-245.

Kim H. M. and Hao O. (1999) Cometabolic degradation of chlorophenols by *Acinetobacter* species. *Wat. Res.*, **33**(2), 562-574.

*Standard Methods for the Examination of Water and Wastewater* (1995). 19<sup>th</sup> edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.