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Biodegradation of 3-Chlorophenol in a Sequencing Batch Reactor

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ABSTRACT

The present paper shows the results obtained through a study on the biodegradation of 3-chlorophenol (3-CP) in a Sequencing Batch Reactor (SBR). To such a purpose a lab-scale SBR was fed a synthetic wastewater containing 3-CP and nutrients (nitrogen and phosphorus) diluted in tap water. The operating strategy, in terms of both the duration of either the cycle or the react phase, was changed throughout the experimental activity in order to find out the optimal one allowing to ensure constant and high removal efficiency despite the increasing 3-chlorophenol concentration in the feed. Biomass collected from a full-scale continuous flow activated sludge facility treating domestic wastewater was used as seed, after being acclimated to 3-CP by means of several batch tests. The results showed that a periodically operated activated sludge system can be successfully used for the biodegradation of chlorophenol compounds, after the needed members of the microbiological consortium are selected and enriched.

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Key Words: Acclimation; 3-Chlorophenol; Oxygen uptake rate; Selection; Sequencing batch reactor.

INTRODUCTION

Chlorophenols are among the most ubiquitous chemicals found in the environment and represent a major class of environmental pollutants. They are widely used in both industrial and agricultural processes (e.g. pulp bleaching, insecticides, fungicides, herbicides, wood preservatives); they are also formed during chlorination of potable water containing humic substances, and during combustion of organic material in the presence of chlorine. Due to their persistence, chlorophenols are frequently found in both surface and ground water, soil, sediments, and even the atmosphere. Chemical-physical characteristics of chlorophenols and thus their fate in the natural environment depend upon the number and the position of chlorine atoms, and their state (ionic or nonionic form). Temperature, the presence of oxygen and inorganic and organic compounds may also have some influence on the chemical-physical characteristics.[1]

Chlorophenols can be removed from wastewater by means of chemical-physical treatments such as adsorption on activated carbon, advanced oxidation and extraction with solvents, or by biological processes.[2–4] However, as do many of the halo-substituted aromatic compounds, chlorophenols show toxic effects and are difficult to biologically degrade. Nevertheless, microorganisms, which have developed the capability of utilizing them as carbon and energy source, can be isolated. The biodegradation takes place either aerobically or anaerobically; in either case the chlorophenols can be used as the sole source of carbon and energy or as a co-metabolite.[5–9]

SBR is a discontinuous process that have been widely applied to the treatment of many kinds of wastewater.[10] Due to its high flexibility, it allows to sustain wide variations of the influent organic loads without affecting the performance efficiency. This capability has been exploited to treat water contaminated by biorefractory and potentially toxic compounds.[11,12]

The present paper shows the results obtained from the study of the aerobic biodegradation of 3-CP in a Sequencing Batch Reactor (SBR). Such a compound has been selected as representative of the chlorophenol class; it can be produced by chlorination of wastewater containing phenols or by degradation of more chlorinated compounds.

Firstly, the experimental activity investigated the suitability of employing a seed, being readily available such as that one collected from a continuous flow treatment plant for domestic wastewater, for the removal of bio-refractory compounds, such as 3-CP. Secondly, after the seed have been acclimated, the 3-CP removal efficiency of the same biomass in a lab-scale SBR was searched.

METHODS

A synthetic wastewater containing 60 mg/L 3-CP (corresponding to about 96 mg/L Chemical Oxygen Demand, COD), 15 mg N/L and 6 mg P/L in the form

of NH_4NO_3 and KH_2PO_4 respectively, diluted in tap water, was used as feed for the acclimation tests. The biomass had an initial concentration of 1500 mg/L Mixed Liquor Suspended Solids (MLSS) and a negligible soluble COD.

Two different acclimation tests were performed. In the first, the seed was used without any prior treatment. In the second, the biomass underwent a 24 h aeration period, prior to the test, in order to take it into the endogenous state in order to stimulate removal capability.

A third test was also carried out in which the synthetic wastewater was added to the influent to the full-scale treatment plant, having 430 mg COD/L and 120 mg MLSS/L, with no external seed. This was aimed at investigating if the addition of a seed (i.e., other than microorganisms present as contaminants in feed constituents) would lead to a shorter acclimation period.

In all the tests, the addition of the synthetic feed was made only once at the beginning and then the 3-CP concentration was periodically measured on samplings of mixed liquor. MLSS and Mixed Liquor Volatile Suspended Solids (MLVSS) were also periodically determined.

When the biomass of the first 2 tests above described showed to be acclimated, then it was used in the second phase of the experimental activity aimed at studying the degradation of 3-CP in a lab-scale SBR. A plexiglass-made cylinder having 8 L total volume served as the reactor. Three peristaltic pumps were used for the influent addition, effluent draw and sludge wasting, respectively. A mechanical mixer and a porous stone located close to the bottom of the reactor and connected to an external compressor were used to establish the aerobic conditions during the react phase. The oxygen concentration in the mixed liquor was always kept above 2 mg O_2/L. The duration of aeration, mixing, feed, draw, and wasting was controlled by a timer. The optimal operating strategy was determined by changing the time for each phase and the ratio between the fill volume and the total volume (i.e., the hydraulic residence time) until an efficient and stable removal was obtained. In the first experimental period, an SBR of 5 L total volume, 1.5 L influent and effluent in each cycle, was operated with the strategy shown in Table 1. The second experimental period utilized a 1 L total volume SBR, 0.25 L influent and effluent in each cycle, operated with the strategies shown in Table 2.

Mass loading of 3-CP was progressively increased, starting with a feed concentration of 167–200 mg/L 3-CP (corresponding to a theoretical concentration of 50–60 mg/L inside the SBR at the end of the fill period), up to 300 mg/L, in order

Table 1. Operating dtrategy during the 1st experimental period of the SBR.

Phase	24 h cycle Time (h)
Aerated and mixed feed	1
Aerated and mixed react	21
Settle	1.25
Draw	0.5
Idle	0.25

Table 2. Operating strategy during the 2nd experimental period of the SBR.

Phase	24 h cycle time (h)	8 h cycle time (h)	6 h cycle time (h)
Aerated and mixed feed	1	0.17	0.17
Aerated and mixed react	21	7	5
Settle	1.25	0.67	0.67
Draw	0.5	0.08	0.08
Idle	0.25	0.08	0.08

to find out the maximum concentration which could be tolerated for each operating strategy selected. Performance was monitored by measuring 3-CP concentration in the effluent collected at the end of each cycle.

The biomass activity was evaluated by means of Oxygen Uptake Rate (OUR) measurements.[13]

pH was also recorded throughout the cycle by means of a standard probe. MLSS and MLVSS concentration were determined twice a week by following Standard Methods for the Examination of Water and Wastewater.[14]

During each experimental period, kinetic studies were carried out by collecting samples of mixed liquor at 1 h-time intervals, in order to evaluate the specific removal rate. On such samples, MLSS, Mixed Liquor Volatile Suspended Solids (MLVSS) and 3-CP concentrations were determined.

3-CP concentration was measured after filtration and/or centrifugation for 30 min at 4000 rpm, by extraction with Solid Phase Micro-Extraction (SPME) using polyacrilate fiber (Supelco), followed by Gas Chromatography/Flame Ionization Detector analysis.

Both volatilization of 3-CP and its adsorption to flocs were negligible.

RESULTS AND DISCUSSION

The first experimental phase used batch reactors to acclimate the biomass to both 3-CP and new operating conditions. The results obtained in such a phase expressed in terms of 3-CP time-profiles are shown in Fig. 1 for the three types of seeds used. ''Seed 1'' was added to the batch reactor without prior aeration. ''Seed 2'' was added to the batch reactor after being aerated for 24 h prior to the test. ''Seed 3'' was the raw influent with no external seed added. F1

MLVSS time-profiles for the same tests are reported in Fig. 2. F2

All the tests performed are characterized by an initial lag-phase with no or very low degradation, followed by a rapid consumption. Particularly, in ''seed 1'' and ''2'' tests the lag phase lasted about 5 days. This was followed by a slow reduction of 3-CP concentration until the 20th day, when a rapid and complete removal took place over the last 8 days.

These results are confirmed by the biomass time-profiles: a continuous reduction of the MLSS and MLVSS concentrations occurred in both tests 1 and 2 during the first 10 days, when they reached a constant value. pH (not here reported) increased

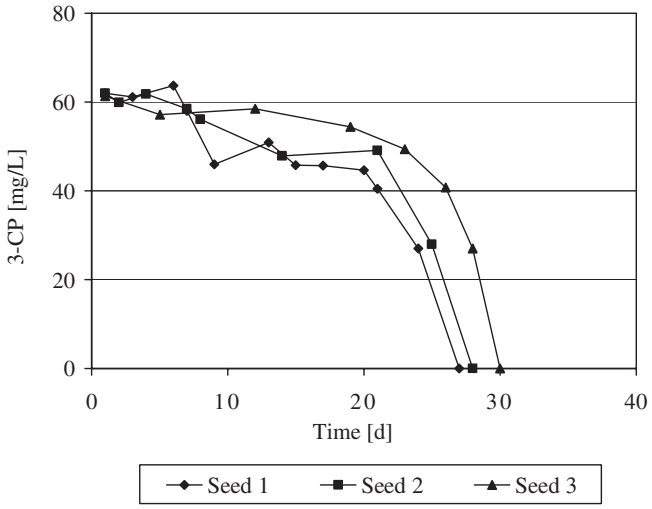

Figure 1. 3-CP time-profiles during acclimation tests.

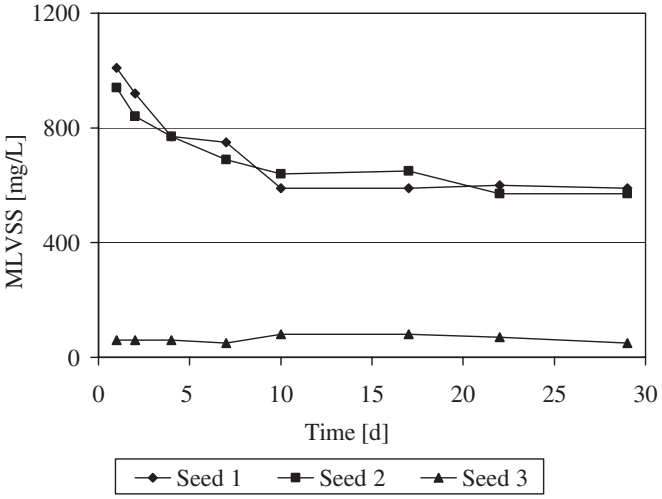

Figure 2. MLVSS time-profiles during acclimation tests.

up to the 15th day, then it decreased because of the chloride produced during 3-CP destruction.

In "seed 3" test, the lower initial biomass concentration led to a slightly longer lag-phase; however, complete removal of 3-CP took place within 30 days even though the MLSS concentration remained below 100 mg/L throughout the test period. Indeed, the yield of biomass from the low initial concentration of 3-CP present in the system would not be expected to be much more than 20 mg/L (as MLSS).

The second experimental phase was dedicated to the investigation of the best performance of the acclimated biomass from "seed 1" and "2" tests in a lab-scale

SBR, by means of both periodical analysis of the effluent and the determination of the removal kinetics within typical operating cycles. Figure 3 shows 3-CP concentration time-profiles measured in the influent and the effluent in the first period during which a 5 L total volume and 24 h cycle was used. MLSS and MLVSS concentrations in the same period are reported in Fig. 4.

It can be observed that the acclimated biomass performed complete removal of 3-chlorophenol during the first 20 days when the feed concentration was kept

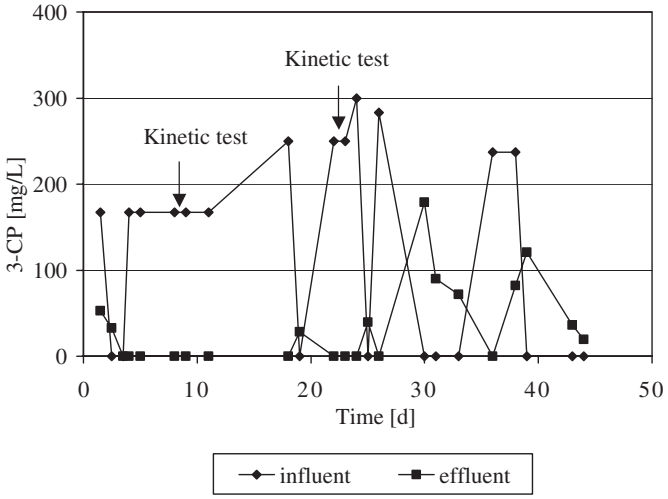

Figure 3. 3-CP time-profile for SBR during the 1st period ($V = 5$ L; 24 h cycle).

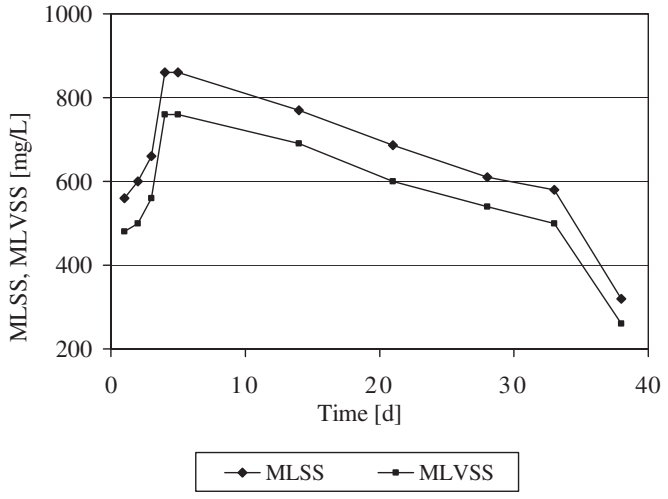

Figure 4. MLSS and MLVSS time-profiles for SBR during the 1st period ($V = 5$L; 24 h cycle).

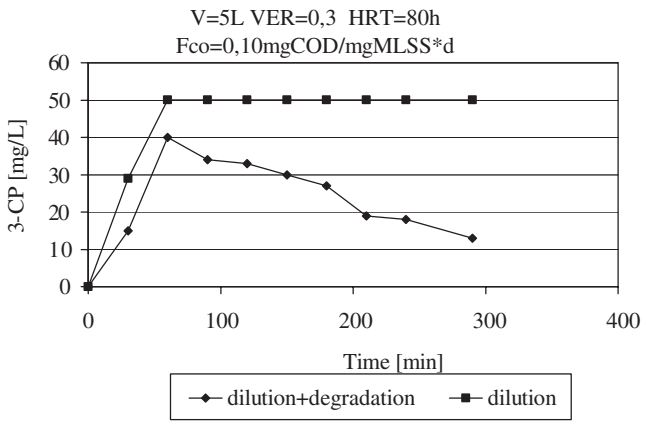

Figure 5. 3-CP degradation of the kinetic tests in the SBR during the 1st period.

constant and equal to about 167 mg/L. Then, the increased organic loading along with low external temperature (about 15°C) slowed the metabolic activity, thus leading to a discontinuous efficiency and a periodic accumulation of 3-CP within the reactor. In order to avoid further biomass inhibition, the feed was stopped several times thus allowing microorganisms to remove the residual loading.

Such results are also confirmed by the MLVSS time-profile: after an initial increase in the MLVSS for the first 10 days, a continued decrease in MLVSS was observed. This fact is also related to the excessive length of the cycle (24 h), which makes the endogenous conditions take place after about 6 h as is shown by the kinetic tests. Indeed, Fig. 5, representing the results of the kinetic test performed **F5** on the 10th day, shows that 3-CP concentration was reduced to about 10 mg/L within the first 5 h.

Owing to the low biomass concentration, the total volume was reduced to 1 L along with the 3-CP concentration of the feed, in order to reduce the organic loading and allow microorganisms to overcome the inhibition. Figure 6 shows 3-CP concen- **F6** tration time-profiles measured in the influent and effluent, and the kinetic tests performed during this second period. MLSS and MLVSS concentrations for the same period are reported in Fig. 7. **F7**

It can be observed that the effluent concentration of 3-CP was always below detectable limits, thus showing a rapid recovery of the biomass capability of degrading the selected compound, despite the increasing organic load. However, due to the excessive length of the cycle, MLVSS decreased continuously. As the cycle was reduced to 6 h, the MLVSS started to increase but the 3-CP concentration in the effluent also increased.

Figures 8–10 show the results obtained by one of the kinetic tests performed, in **F8–F10** terms of 3-CP removal, OUR and pH profiles, respectively.

The complete removal of 3-CP occurred within the first 3 h. During the same period, pH decreased due to chloride production and the concomitant association of H^+ to serve as the counter ion for the chloride ion. As far as the OUR measurement

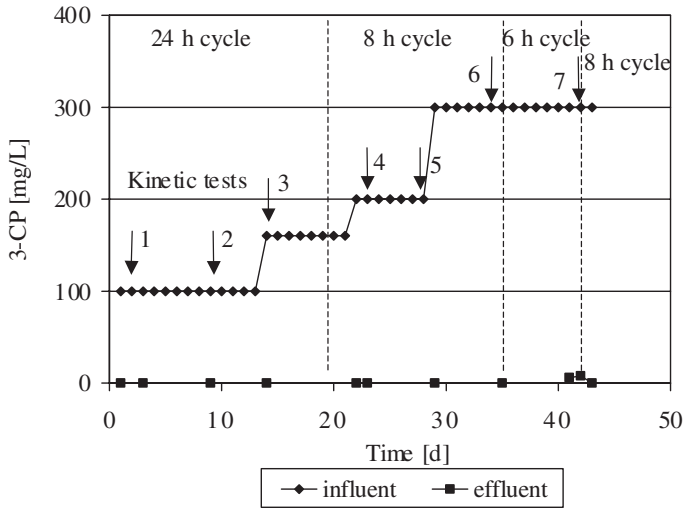

Figure 6. 3-CP time-profile for SBR during the 2nd period ($V = 1$ L).

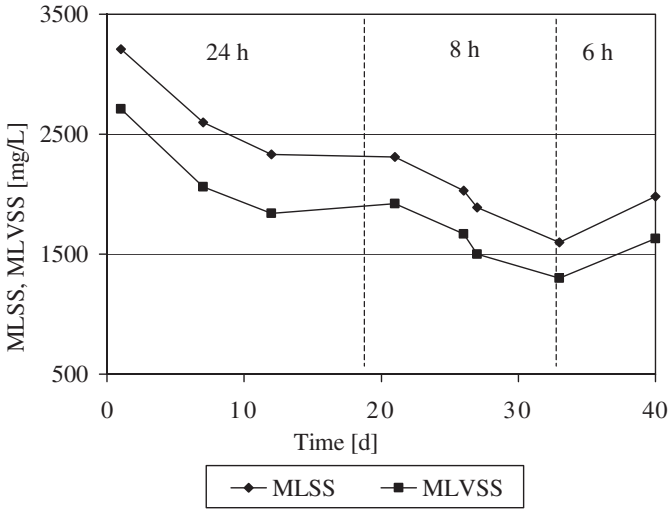

Figure 7. MLSS and MLVSS time-profiles for SBR during the 2nd period ($V = 1$ L).

is concerned, it increased along with the 3-CP removal because of biomass production; as soon as degradation stopped, the oxygen consumption became very low and the endogenous respiration took place.

It is also possible to compare these results with those obtained by the kinetic test represented in Fig. 5, since they were both performed under the same loading conditions (i.e., $F_{co} = 0.10$ mg COD/mg MLSS*d). It can be said that selection and

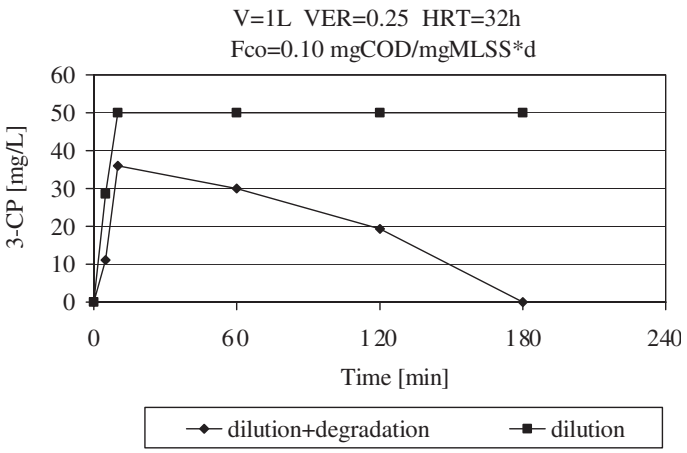

Figure 8. 3-CP degradation of the kinetic test in the SBR during the 2nd period.

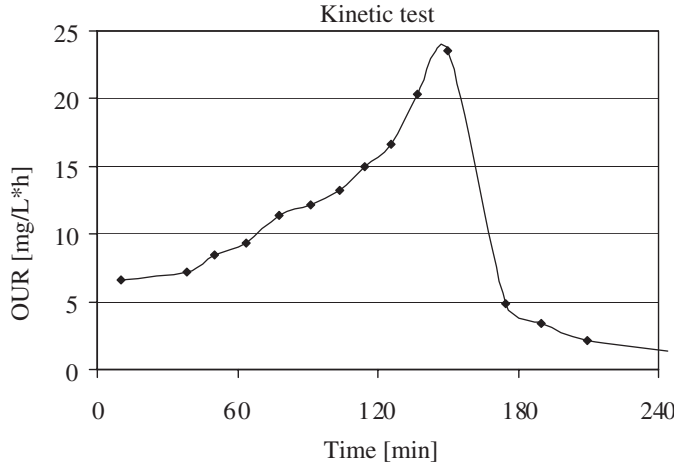

Figure 9. OUR time-profile of the kinetic test in the SBR during the 2nd period.

adaptation of the biomass towards the 3-CP removal progressively occurred since the removal rate is now much higher.

Table 3 shows the results obtained by the SBR through all the kinetic tests performed during the 2nd period. A strict relationship among the hydraulic residence time (HRT), the organic loading factor (F_{co}) and the specific 3-CP removal rate (R_{cp}) can be observed. Particularly, R_{cp} always improves with increasing F_{co} and decreasing HRT, except for the last test performed within a 6 h operating cycle probably being too short to allow the complete degradation of 300 mg/L of 3-CP.

A linear relationship was found between R_{cp} and F_{co}, determined without taking into account the data from the last kinetic test ($R^2 = 0.9162$).

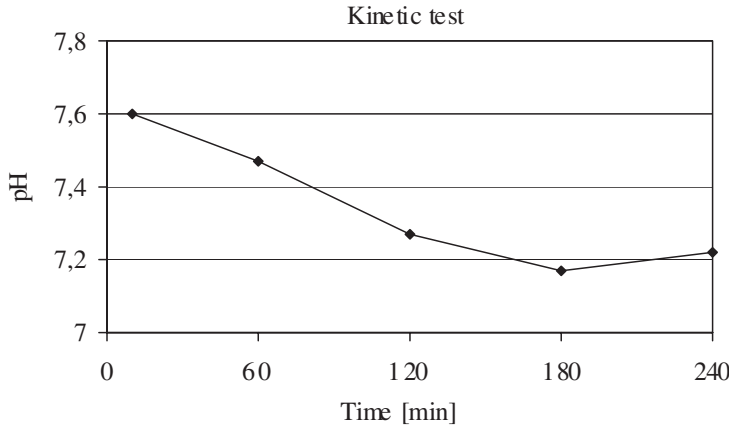

Figure 10. pH time-profile of the kinetic test in the SBR during the 2nd period.

Table 3. Results from the kinetic tests performed in the SBR during the 2nd period.

Test	F_{co} (mg COD/mg MLSS*d)	R_{cp} (mg 3-CP/g MLSS* h)	HRT (d)	3-CP infl. (mg/L)	MLSS (mg/L)	Cycle time (h)
1	0.038	0.71	4	100	3,200	24
2	0.046	0.80	4	100	2,600	24
3	0.082	1.40	4	160	2,330	24
4	0.10	5.50	1.3	200	2,310	8
5	0.13	6.35	1.3	200	1,890	8
6	0.23	10.15	1.3	300	1,600	8
7	0.18	5.7	1	300	1,980	6

CONCLUSIONS

In this study three methods of selection and enrichment were investigated, and the SBR cycle was optimized for an increasing organic load of 3-CP to the system. Preliminary results clearly demonstrated that a periodically operated activated sludge system can be successfully used for the aerobic biodegradation of 3-CP after the needed members of the microbial consortia are selected and enriched. The SBR confirmed to be effective in the degradation of recalcitrant and potentially toxic compounds provided that the optimal operating strategy is selected. Furthermore, the high flexibility of the SBR allows the operating strategy to be adjusted for varying organic loads in order to ensure high degradation efficiency.

Further studies will investigate if the biomass, previously acclimated to a mono-chlorophenol such as 3-CP, developed the capability of removing more highly chlorinated compounds, which are known to be more refractory to biological treatments. Particularly, the same biomass will be fed with increasing concentrations f 3,5-dichlorophenol (3,5-DCP), and its removal capability will be measured.

A shorter acclimation period to 3,5-DCP is expected to occur by means of this strategy, compared to the direct addition of the same compound to the raw seed.

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