Hybrid reactor performance in pentachlorophenol (pcp) removal by anaerobic granules

M. de Almeida Prado Montenegro1**, E. de Mattos Moraes1, H. Moreira Soares2 and R. Filomena Vazoller1
1 Department of Hydraulic & Sanitation, Sào Carlos School of Engineering, University of São Paulo – USP. Av. Tabalhador Sào-carlense, 400; CEP13566-590, Sào Carlos, SP, Brazil
2 Department of Food and Chemical Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil
** Corresponding author: E-mail: marthapm@enq.ufsc.br. Fax: (55-16) 273-9550.

Abstract The present research aimed at evaluating pentachlorophenol (PCP) degradation in a hybrid reactor supplied with a mixture of fatty acids (propionic, butyric, acetic and lactic) and methanol. The performance of the reactor is remarkably stable and efficient during PCP additions at range of 2.0 to 21.0 mg/L. The reduction of chemical oxygen demand (COD) was around 97% and methane was found to be 86% in the biogas production. The efficiency of volatile fatty acids breakdown was 93%, 64% and 74% respectively for butyric, propionic and acetic. PCP total removal of more than 99% was reached by granular sludge activities formed during 21 months of reactor operation. Methanogenic microorganisms predominance was noticed with 105 to 106 cells/mL during enumeration on methanol or lactate added to sulfate culture media. The removal rate was 1.07 mg PCP · g−1 VS · d−1 during the highest PCP concentration addition.

Keywords Anaerobic granules; methanogenic cells; chlorinated compounds; hybrid reactor; pentachlorophenol

Introduction
Pentachlorophenol (PCP), a highly toxic and environmentally persistent compound, was widely used as a biocide, especially for wood preservation. Chlorinated phenols are common contaminants in water and soil, and are also frequently present in effluents from paper mills that use traditional bleaching processes (Swoboda-Colberg, 1995). Though PCP has been shown to resist biodegradation, several pathways for microbial degradation of PCP have been identified, as reductive dehalogenation in anaerobic conditions (Mohn and Tiedje, 1992).

PCP removal from different wastes by anaerobic treatment has been studied due to its easy degradation in environments with low redox potential. PCP dechlorination and anaerobic degradation have been observed involving microbial consortia or co-cultures, by Mikesell and Boyd (1986), and pure cultures, such as dehalogenating sulfate reducer Desulfomonile tiedjei, by Mohn and Kennedy (1992). Nevertheless, few anaerobic microbial cultures have been isolated so far.

Under anaerobic conditions, PCP degradation proceeds at good efficiency in continuous systems with granular sludge (Wu et al., 1993; Hendriksen and Ahring, 1993) and also in batch reactors (Kennes et al., 1996). The research of Wu et al. (1993) showed that granular sludge was able to remove PCP of more than 99% in an upflow anaerobic sludge blanket reactor (UASB) fed with synthetic effluents containing 40 to 60 mg/L of PCP. Similar results were obtained by Hendriksen and Ahring (1993), during comparison of fixed-film reactor and UASB for PCP removal under glucose supplementation; Juteau et al. (1995), when observed that PCP was dechlorinated by methanogenic consortia in the presence of glucose, formate and yeast extract in UASB reactors; Duff et al. (1995), working with...
phenol as an additional source of carbon, with PCP reduction to less chlorinated phenols in UASB reactors; Damjanovic (1997), using different sources of microbial consortia obtained PCP degradation in a range of 0.2 to 8.0 mg/L in a horizontal-flow anaerobic immobilized sludge reactor (HAIS) fed with fatty acids and alcohol as organic sources at 3.0 g/L of total COD.

The present study evaluated the PCP removal in a hybrid reactor (Craveiro, 1994) amended with a range of 2.0 to 21.0 mg/L of PCP and fatty acids such as acetic, propionic, butyric, lactic and the alcohol methanol. The total COD concentration was 7.0 g/L. The reactor was seeded with a flocculent anaerobic sludge, collected from an UASB system operating with semi-kraft paper industry effluent and was buffered with NaHCO₃.

Materials and methods
Reactor design
According to Craveiro (1994), hybrid reactors combine the advantages of upflow sludge blanket reactors (UASB) and anaerobic filters, decreasing deficiencies of both. The volume of the hybrid reactor used in this research was one litre (7.3 cm diameter, 25 cm length) with two distinct compartments, where the upper part was the anaerobic filter, represented 30% of the total reactor’s volume (Figure 1). The filter was filled with 1.0 cm³ of polyurethane foam cubes. The reactor temperature was kept at 32°C–37°C by warmed water pumped around the system through a plastic coil. To measure biogas production, a 600 mL floating-dome gas meter was installed in the reactor. The gas meter contained a sodium sulfate solution (200 g/L) in order to avoid the absorption of carbon dioxide by the culture medium.

Inocula and experimental conditions
The inoculum made up with a sludge from one UASB system fed with semi-kraft paper industry effluent and was monitored by specific methanogenic activity tests (SMA). The start up and the steady-state conditions were established after 3 months with a gradual nutrient supply increase. The initial HRT was 5.1 d, and 1.3 d at the end of the operation with flow of 800 mL/d. The organic loading was 4.96 g/L of COD at the beginning and it

Figure 1 Schematic diagram of laboratory-scale hybrid reactor (HR) system. The lower darkened portion of the reactor represents granules, and the upper portion (4) represents anaerobic filter. 1, influent; 2, feeding pump; 3, sampling port; 5, effluent exit; 6, effluent collecting flask; 7, warmed water reservoir; 8, solenoid valve; 9, gas meter
was increased to 6.88 g/L after the first month by the lactate addition. This value was main-
tained during all the experiment. The reactor was supplied with 20 mM each of acetic and
lactic acids, 10 mM each of propionic and butyric acids plus 20 mM of methanol, a mineral
solution (Soares and Hirata, 1997) was added as well. The acids were added as their sodium
salts. The process was buffered by using 0.05% of NaHCO₃ (pH 7.0) in the medium and
0.1 mg/L of Na₂S was used to improve the anaerobic conditions. 0.02% of yeast extract as
also amended for vitamins supply as indicated by Vazoller (1997). PCP additions started
after 21 months of reactor operation and the concentration range was 2.0 to 21.0 mg/L. The
PCP feeding concentrations were carefully increased, depending on the stability of the
process. The total period of PCP additions was almost 4 months.

Monitoring and control
The reactor was monitored by: COD and volatile solid measurements according to
Standard Methods (1995); volume of methane was measured in a gas meter with a Na₂SO₄
solution to avoid carbon dioxide absorption; methane determined by gas chromatography
with Porapaq N 3/16 packed column, thermal conductivity detector and helium as a carrier
gas, the oven, injector and detector temperatures at 60°C, 70°C and 100°C, respectively;
volatile fatty acids measured according Moraes (1999) by gas chromatography with capil-
lary column 30 m × 0.25 mm × 0.25 µm – Nukol™, flame ionization detector and nitrogen
as a carrier gas, the oven, injector and detector temperatures at 80°C–150°C, 220°C and
250°C, respectively; chlorophenols analysis according to Damianovic (1997); quantitative
microorganism evaluation using serial decimal dilutions for methanogenic and acetogenic
groups according Vazoller (1997); microscopic examinations under scanning microscopy
using a digital scanning microscope DSM 960 Zeiss and DSM Image Transfer U1.7 93
Zeiss and under phase contrast and fluorescence OLYMPUS BHT-2 microscope; sulfide
measurements according to Standard Methods (1995) were used to verify SRB growth dur-
ing microorganism enumeration.

Results and discussion
The sludge inoculated to the hybrid reactor yielded SMA mean values of 1.43 g
COD-CH₄•g⁻¹ VS•d⁻¹. The SMA tests were carried out with a mixed acid solution (acetic,
propionic and butyric acids) at a total concentration of 5 g/L and 1:1 ratio of VFA/VS. PCP
additions started after 21 months under steady-state conditions of the reactor, which mean
reduction of total COD in the effluent around 93.4% at pH range of 6.8 to 7.5, biogas pro-
duction up to 1.22 L/d with 84% of methane content and biogas conversion factor of 0.27
L•g⁻¹. The removal efficiency of fatty acids could be considered almost 100% and a granu-
lar sludge bed was developed with 9.0 cm in height, which represented 36% of the total
reactor volume. In these conditions, the reactor began to receive PCP compound, and the
concentration was increased from time to time during 4 months, according the stability of
the process (see Figure 4). Table 1 showed the mean values of parameters measured to eval-
uate the reactor performance under PCP additions. The volatile solid content in the system
was 0.051 g VS/g of sludge, and the sludge bed was 10.5 cm high.

Microscopic examinations before the addition of PCP showed a remarkable observa-
tion. The granules grown up to 3 mm in diameter presented cell morphologies close to the
genera Methanosarcina and Methanosaeta (formerly nominated Methanothrix), and two
colors, very dark and slightly gray were also noticed. The color difference seemed to
depend on the predominance with one or another genus inside the granules, in dark granules
Methanosaeta genus prevailed. Figures 2a and 2b highlight the morphologies pointed out
for the granular samples removed from the hybrid reactor. Authors such as Florêncio
(1994) have observed a microorganism selection in the presence of specific substrate. The
The study was aimed to verify the behavior of UASB reactor supplied with methanol as the only substrate as 5.0 g COD L⁻¹. The author found in methanol concentration greater than those used in our experiment, the unique presence of *Methanosarcina*-like cell morphology, which is close to the sarcina cellular arrangements observed in Figure 2(b). Vibrio morphology was verified in abundance during lactic acid addition, and it could be related to sulfate reducers' organisms. The cell number of microorganisms cultivated, at 35°C, on lactate plus sulfate medium was 10⁶ cells/mL, confirmed by sulfide determinations. The methane gas in the atmosphere of culture media during cell enumeration showed 10⁵ cells/mL in methanol and 10⁶ cells/mL in lactate plus sulfate.

The PCP additions did not significantly affect the reactor. As observed in Table 1, the reactor performance was similar to the period without additions that correspond to the values of 93.4% of COD removal, 84% of methane in the biogas and 0.270 L/g of biogas conversion factor calculated as an average of 21 months of sampling and determinations. After the first PCP concentration added (2.0 mg/L) and 8 days later, the efficiency of COD removal slightly decreased (Figure 3). However, was recovered in good conditions and remained close to 97% until 15.6 mg/L of PCP addition. The anaerobic process seemed to be affected with PCP concentrations over 15.6 mg/L confirmed by the decrease of COD removal and biogas conversion factor values. Therefore, the methane content in the biogas was almost the same throughout the process, approximately 85% (Table 1).

The mean value of biogas conversion factor in the reactor before the addition of PCP, 0.27 L of biogas /g of COD added decreased until 0.17 L/g when 2.0 mg/L and 4.0 mg/L of PCP were added. Despite a slight increase during feeding with 6.0 to 12.8 mg/L of PCP, this value was not reached again in the process. In the concentrations of 15.6 and 21.0 mg/L of PCP the biogas conversion factor decreased, that seemed to affect the process overall (Table 1).

The pattern of volatile fatty acids during the anaerobic degradation under PCP additions reflected the fact that propionic acid removal from the bulk fermentation was marked, as can be seen in Figure 4. After 15.6 mg/L of PCP addition propionic acid began a descent and remained so at a PCP concentration of 21.0 mg/L. The acetic acid decrease also proceeded in a similar way, nevertheless its removal was greater than that for propionic at PCP concentration of 15.6 mg/L. Under experiment conditions, butyric acid was found to be the least affected by PCP addition. A removal efficiency decrease to 88% was only observed with the addition of 15.6 mg/L of PCP, and an efficiency of 99% was recovered after 4 days. The intake average concentrations during the PCP addition of acetic, propionic and butyric acids were, respectively, 1237, 738 and 904 mg/L.

The high PCP removal performance was over 96% during almost the total experimental
Table 1 Values of COD removal efficiency, methane content in biogas, and conversion factors of COD to biogas according PCP concentrations added

<table>
<thead>
<tr>
<th>PCP concentration (mg/L)</th>
<th>Efficiency of COD removal (%)</th>
<th>Methane content in biogas (%)</th>
<th>Biogas conversion factor (L/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>93.4</td>
<td>84.1</td>
<td>0.270</td>
</tr>
<tr>
<td>2.0</td>
<td>88.1</td>
<td>84.0</td>
<td>0.172</td>
</tr>
<tr>
<td>4.0</td>
<td>91.6</td>
<td>87.6</td>
<td>0.169</td>
</tr>
<tr>
<td>6.0</td>
<td>96.7</td>
<td>87.2</td>
<td>0.249</td>
</tr>
<tr>
<td>8.0</td>
<td>96.1</td>
<td>83.4</td>
<td>0.229</td>
</tr>
<tr>
<td>10.0</td>
<td>96.8</td>
<td>87.1</td>
<td>0.236</td>
</tr>
<tr>
<td>12.8</td>
<td>97.4</td>
<td>84.2</td>
<td>0.227</td>
</tr>
<tr>
<td>15.6</td>
<td>94.3</td>
<td>85.0</td>
<td>0.176</td>
</tr>
<tr>
<td>21.0</td>
<td>92.4</td>
<td>82.8</td>
<td>0.207</td>
</tr>
</tbody>
</table>

(-) No addition of PCP

period (Figure 5) and intermediates such as 2,4-dichlorophenol (DCP), 2,6-DCP, 2,3-DCP, 2,3,4-trichlorophenol (TCP), 2,3,6-TCP and 2,4,6-TCP were not verified. Figure 4 presented the behavior of PCP values measured during 110 days of additions.

Probably due to the reactor’s good stability the PCP addition did not disturb the overall performance of the anaerobic process. These former conditions favored formation of a microbial consortia in the granules that was able to grow in the presence of the organochlorine and its intermediates. The presence of methane archaea genera was confirmed by cellular enumeration as well as by SEM and fluorescence microscopic examinations after PCP additions (Figure 6). In methanol culture medium the value of $10^5$ cells/mL was determined for methanogenic microorganisms and remained unaltered after PCP feeding. The same behavior was observed for lactic acid plus sulfate culture medium for cellular
enumeration and methane measurements when 10^6 cells/mL were counted. Nevertheless, SRB group enumeration decreased from 10^6 to 10^4 cells/mL at the latest period of PCP addition. In these experiments, the number of methanogenic cells did not change with time and the methanogenic predominant morphologies did not modify at the highest PCP concentration.

The results on the removal of PCP by the anaerobic microbial consortia and specially the answer that methanogenic cells were not affected by the presence of the organochlorine highlighted the possible implication of these cells to the degradation of PCP. This hypothesis is strengthened by Tsuno et al. (1996), who concluded that 60% of the PCP added to an expanded bed anaerobic reactor, inoculated with acetate, was transformed into methane and carbon dioxide. In this sense, Kennes et al. (1996) demonstrated the important role of methanogenic microorganisms in PCP mineralization due to the presence of a specific inhibitor in the medium. In this case, methane production was prevented and dechlorination was not observed.

On the basis of the results obtained, syntrophic relations involved methanogenic microorganisms, at least must have occurred. Tests with granules from the hybrid reactor with PCP, carried out in batch and similar nutritional conditions, showed that a syntrophic relation between the microorganisms in the sludge present us in the continuous reactor was confirmed.

For the light colored granules, methane production was high only in the cultures with methanol and acetate. However, with sulfate present, lactate, propionate and butyrate produced much methane even in the presence of sulfide, observed in the culture with propionate. The same occurred in the culture with lactate for the dark granules: methane production was low without sulfate but increased with its presence, even when sulfide was
also present. This reveals an important syntrophic relation between methanogenic microorganisms in the hybrid reactor sludge under the PCP degradation in such experiments.

Therefore, the hybrid reactor good performance during PCP additions can be related at specific microbial consortia developed from the carbon sources used in this study, before and during PCP addition. Moreover, cosubstrates must be supplied since PCP cannot be used as the sole carbon source to develop anaerobic granules (Wu et al., 1993). On the other hand, the propionic acid behavior under PCP addition and the SRB decrease, verified at the highest PCP concentration, indicate an interference with the sulfate reducers’ specific metabolism.

The system’s performance can also be due to the low PCP/VS ratio, not much commented on research involving chlorinated organic compounds. Wu et al. (1995) obtained a removal rate of 14.6 mg PCP·g⁻¹·VS·d⁻¹. Our results showed PCP concentration of 21.0 mg/L and a removal rate of 1.07 mg PCP·g⁻¹·VS·d⁻¹, considering 0.034 g VS/g of sludge. The system’s VS concentration was high enough to not be affected by the PCP concentration studied. Using a continuous system with a specific rate of PCP application of 0.002 mg PCP·g⁻¹·VS, Damianovic (1997) obtained a COD removal rate near 98%. Inversely, COD/PCP relation in the present study was high enough to allow PCP removal under the conditions involved. In the highest PCP concentration, this relation was 0.33 g COD/mg PCP (327.6 g/g). Wu et al. (1995) indicated the possibility of treating effluents containing PCP with COD concentrations above 1.2 g/L, pointing out the need to establish the lower limit for the organic loading rate in order to maintain active dechlorination.

Conclusions

The potential for degradation of PCP under anaerobic conditions was certified in the hybrid reactor supplied with a mixture of fatty acids (acetic, propionic, butyric and lactic) and alcohol methanol. Granular sludge was formerly formed before PCP additions and two kinds of methanogenic-like genera were selected, Methanosarcina and Methanosaeta-like cells. The homeostasis process which maintained during 21 months previously the organochlorine feeding, may be responsible for the results obtained, where biogas conversion factor presented a slight decrease with the increase of PCP concentrations and methane metabolism seemed to be less affected. Moreover, the hybrid reactor configuration facilitated a good sludge retention and avoided the solids losses in the whole process.

It is possible to affirm that the gradual introduction of PCP to the reactor fed with low concentrations (2.0 to 4.0 mg/L) was an adequate procedure for the granular sludge adaptation to the compound. Since better conditions were restored during this PCP feeding until 15.6 mg/L, the adapted sludge avoiding the compound toxicity in this period. The high concentrations of PCP studied should be defined as a limit for the system to be disturbed and need an appropriate period for the sludge to adapt to them under the present operational conditions.

Despite the slight efficiency decline with PCP concentrations above 15.6 mg/L, the reactor’s performance between 2.0 and 21.0 mg/L of PCP can be related to such microbial types selected during 21-month operation without organochlorine. Enumeration of methanogenic cell showed that these microorganisms were not affected by PCP addition. However, SRB cells showed marked decrease in the presence of PCP.

The system’s efficiency can also be explained by the low PCP/VS ratio (1.07 mg PCP/g VS·d) in the highest concentration of PCP added (21.0 mg/L).

Acknowledgements

To Prof. Paulo Belli Filho, Environmental Engineering Department of the Federal University of Santa Catarina, for the support to chromatographic analysis; Dr. Nelson José...
Heraldo Gallo, São Carlos Physics Institute of the University of São Paulo, for the SEM images and Brazilian National Council of Research (CNPq) for the grant that supported this research.

References


