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Rapid restoration of methanogenesis in an acidified UASB reactor treating 2,4,6-trichlorophenol (TCP)

María Consuelo Díaz-Báez [a], Juan Daniel Valderrama-Rincon [b,*]

[a] Department of Civil and Environmental Engineering, National University of Colombia, Bogotá 111321142, Colombia
[b] Department of Environmental Engineering, Antonio Nariño University, Bogotá 111321142, Colombia

HIGHLIGHTS

- 4-chlorophenol is the most recalcitrant byproduct during TCP degradation.
- TCP was not detected at the UASB reactor effluent except when it was acidified.
- Total restoration of the UASB bioreactor was achieved by neutralization with NaOH.

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ABSTRACT

Anaerobic bioreactors are often used for removal of xenobiotic and highly toxic pollutants from wastewater. Most of the time, the pollutant is so toxic that the stability of the reactor becomes compromised. It is well known that methanogens are one of the most sensitive organisms in the anaerobic consortia and hence the stability of the reactors is highly dependant on methanogenesis. Unfortunately few studies have focused on recovering the methanogenic activity once it has been inhibited by highly toxic pollutants. Here we establish a quick recovery strategy for neutralization of an acidified UASB reactor after failure by intoxication with an excess of TCP in the influent. Once the reactor returned to pH values compatible with methanogenesis, biogas production was re-started after one day and the system was re-acclimated to TCP. Successful removal of TCP from synthetic wastewater was shown for concentrations up to 70 mg/L after restoration.

1. Introduction

Chlorophenols (CPs) are toxic molecules widely distributed in the environment due to its extensive industrial use and resistance to microbial degradation [1,2]. One of the most toxic CPs is 2,4,6-trichlorophenol (TCP), an anti-mildew agent, byproduct of the paper bleaching process, and precursor of fungicides, herbicides, glue, wood preservatives, and leather preservatives [1,3,4]. TCP tends to accumulate in the lipid tissues of several organisms, is suspected to be mutagenic or co-mutagenic [5], and has been associated with cancer in animals, producing lymphomas and leukemia after consuming contaminated water or food [6]. For these reasons TCP has been included in the European Economic Community directive 76/464/CEE on dangerous substances discharged into the aquatic environment, and the US EPA list of priority pollutants [7].

Being a highly dangerous compound, the interest on removal of TCP from contaminated wastewaters has been increasing during the last two decades. Due to its low Henry's constant [8], TCP is minimally removed by air stripping, so chemical or biological approaches are necessary. Among biological approaches, the most commonly evaluated are photobiodegradation [3], aerobic biodegradation [1,9], anaerobic biodegradation [2], [10,11], hybrid systems [12], and biofilm reactors [13]. However, as highly chlorinated phenols are usually recalcitrant to microbial degradation [5], the behavior of the bioreactors used for its treatment is unstable and microorganisms in those bioreactors get inhibited by toxicity.

Toxicity of CPs increases with the number of chlorine atoms attached to the aromatic ring [8]. Several studies suggest that microbial dehalogenation of highly chlorinated CPs happens preferably under anaerobic conditions [5,14], in both acclimated and unacclimated cultures [5]. The evidence to date suggests that reductive dehalogenation is the most common dechlorination mechanism [15]. Among the bacterial species involved in this process are *Desulfomonile* spp., *Anaeromyxobacter dehalo-*

* Corresponding author.
E-mail address: juan.d.valderrama@gmail.com (J.D. Valderrama-Rincon).

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genans, *Desulfovibrio dechloroacetivorans*, *Dehalococcoides* spp. and *Desulfitobacterium* spp. [2]. Unfortunately, full-scale implementation of a technology based on this process has been hampered by poor operational stability of stressed anaerobic reactors [16].

In general, anaerobic digestion is highly efficient, cost effective, yields low sludge quantities, and produces methane [17]; but its difficult operation have restrained a wider commercialization of the technology [18]. For instance, toxic compounds like TCP, can inhibit methanogenesis, resulting in acidification and destabilization of the anaerobic reactor [15]. After interruption of methanogenesis, acidogenic bacteria cause the accumulation of volatile fatty acids (VFAs) in the reactor, lowering the pH, and further increasing the burden over methanogens [18]. Because of this, disruption of the balance between acidogenic and methanogenic organisms is the most common cause for anaerobic reactors failure [18,19].

The use of upflow anaerobic sludge blanket (UASB) reactors can help to overcome these unstability problems, thanks to the formation of granular sludge. It has been established that immobilization of microorganisms confers resistance to microbial consortia against toxic pollutants [18]. When analyzing the granular sludge, several layers containing different immobilized microbial populations can be identified. Organisms which are less sensitive to a given inhibitor tend to localize in the outer layer protecting the microflora in the inner layers [20]. Despite this inherent advantage, UASB reactors can still become unstable when an excess of the inhibitor is fed to the process. In such cases, the inhibitor cannot be contained by the outer layers and eventually reaches the inner layers, where methanogens are usually located [21].

As a result of destabilization and acidification, the reactor suffers a microbial population shift, usually associated to inhibition of methanogens which is one of the most sensitive groups of microorganisms [22]. Taking that into account, methane production is an important parameter for assessment of anaerobic reactors stability. A drop in the methane production rate suggest inhibition, while ceasing of methane production indicates a toxic effect [22]. Controlling influent alkalinity and pH can help with restoration of steady state conditions in the reactor, except when the toxic effects are so severe that methanogenesis cannot be reconstituted [23].

In this study we acclimated a continuous UASB reactor, seeded with granular sludge, to increasing concentrations of TCP diluted in a synthetic wastewater. After reaching a TCP concentration of 80 mg/L in the influent, methane production ceased and pH and alkalinity dropped quickly. Interestingly, methanogenesis was quickly recovered by stopping TCP feeding, washing the anaerobic granules with a NaOH solution, and increasing alkalinity in the influent using $NaHCO_3$. This suggests that methanogens did not die because of the TCP and a second acclimation process was possible, reaching a concentration of 70 mgTCP/L in the influent.

2. Materials and methods

2.1. Granular sludge and UASB reactor

The granular sludge used for all TCP dehalogenation experiments was obtained from a UASB reactor treating wastewater from a brewing company (Barranquilla, Colombia). It contained 86420 mg/L of total suspended solids (TSS) and 75187 mg/L of volatile suspended solids (VSS). The mean particle size was 1.2 mm with a sludge volumetric index (SVI) of 4.41 mg/L. The sludge was inoculated (500 mL) into a plexiglass lab-scale UASB reactor (diameter: 6 cm, height: 60 cm) and maintained at 35 °C ± 1 °C by using an external heating circuit. The reactor was continuously fed from a 5 L tank that was replaced (refilled) every time it was depleted.

2.2. Synthetic waste water

Meat extract (1.95 g/L) and glycerol (0.2 g/L) were used as carbon source for the synthetic waste water (SWW), adjusting a chemical oxygen demand (COD) of 3000 mgCOD/L. The SWW was supplemented with a minimum salt media constituted by: ammonium chloride 0.36 g/L, sodium chloride 0.05 g/L, calcium chloride 0.024 g/L, dibasic potassium phosphate 0.03 g/L, and hepta-hydrated magnesium sulfate 0.0075 g/L. Trace elements were added from 1 mL of an acid solution (HCl 1800 mg/L, H_3BO_3 60 mg/L, $MnCl_2$ 61.2 mg/L, $FeCl_2$ 943.5 mg/L, $CoCl_2$ 64.5 mg/L, $NiCl_2$ 13 mg/L, $ZnCl_2$ 68 mg/L) and 1 mL of a basic solution (NaOH 400 mg/L, Na_2SeO_3 17 mg/L, Na_2WO_4 30 mg/L, Na_2MO_4 20.5 mg/L) per liter of SWW. $NaHCO_3$ was used as buffering agent in concentrations ranging from 500 mg/L to 3000 mg/L. Na_2S was employed as reducing agent and resarzurin as dissolved oxygen (DO) indicator.

2.3. Dehalogenation assays in enrichment media

Preliminary dehalogenation assays were performed following the method recommended by Arbely and Ronen [24]. Experiments were set up by triplicate in 30 mL vials filled at a third of their total capacity with a mixture of liquid medium and granular sludge (2.7 gTSS/L and 2.4 gVSS/L). The liquid medium was prepared using sodium piruvate as carbon source, keeping a piruvate:TSS mass ratio of 2:1. TCP (50 mg/L) was injected to each vial and a total of 30 vials were incubated during 1 week at 35 °C. Five vials were autoclaved as negative control.

2.4. Analytical methods

Methane production was measured by displacement of a 2.5% NaOH solution [25]. VFAs were measured by colorimetry [26] using a Varian DMS-100S spectrophotometer. pH was determined using an InoLab potenciometer. Methods from the American Water Works Association were used for determination of alkalinity (2320), TSS (2540B), VSS (2540E), and COD (5220) [27]. Granular sludge characterization (settling velocity, sludge volumetric index and particle size) was based on methods reported by Diaz-Baez [28]. TCP dehalogenenation byproducts were measured using a Waters 510 HPLC coupled to a Waters 486 UV detector (wave length of 290 nm). Byproducts separation was achieved using a Merck C-18 LiChroCART 250-4 cartridge at 30 °C and a mobile phase comprised of a mixture of acetic acid 1% in methanol (75% v/v) and ammonium acetate (1.34 g/L) in methanol with 1% acetic acid (25% v/v).

3. Results

3.1. Dehalogenation assays in enrichment media

Prior to acclimatation of the UASB reactor, the dehalogenation capacity of the granular sludge was tested using sealed 30 mL vials. TCP was completely removed after 18 days and 3 partially dehalogenated byproducts were detected in the liquid medium (Fig. 1). The product of the first dehalogenation step, 2,4-dichlorophenol (2,4-DCP), accumulated during the first 10 days and then started to be replaced by monochlorinated compounds: 2-chlorophenol (2-CP) and 4-chlorophenol (4-CP), being 4-CP the most prevalent. Phenol was not detected. At day 18, stoichiometrical analysis equated the sum of 2-CP, 4-CP and 2,4-DCP to the initial amount of TCP, which suggests that the aromatic ring was not mineralized. Negative controls showed no abiotic degradation of 2,4,6-TCP by autoclaved granular sludge, as expected [4].

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