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Biodegradability and toxicity of styrene in the anaerobic digestion process

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Abstract

Start-up and operation of an Upflow Anaerobic Sludge Blanket (UASB) reactor fed with an industrial effluent from a polymer synthesis plant containing 6 mg styrene l^{-1} was unstable. In batch assays with 200 mg styrene l^{-1}, 74% of styrene was degraded at a rate of 7 ml methane g^{-1} volatile suspended solids.day, without a lag phase. The toxicity limit (IC_{50}) of styrene was 1.4 mM for the acetoclastic activity, 0.45 and 1.6 mM for the methanogenic activity in the presence of 30 mM of propionate and ethanol respectively. Instability of UASB operation was attributed to other compounds such as acrylates or detergents present in the industrial effluent.

Introduction

The accumulation of toxic compounds in the environment has increased with industrial production of pesticides, pigments, derivates of the paper, plastics and polymers. Styrene has greatly increased its participation in the market of synthetic products. As an example, its estimated United States production in 1999 was 5.5×10^9 tons (Chemical Market Associates Inc. 1999). Styrene is mainly used as synthetic monomer for plastics production of electronic and domestic hardware and in the manufacture of reinforced plastics. Its structure is presented in Figure 1.

Short-term exposure to styrene leads to mucous membrane and eye irritations, whereas long-term exposure affects the central nervous system, increasing the risk of leukemia and lymphoma. It is pointed out as a possible human carcinogen compound (Environmental Protection Agency 2000). Due to its high volatility, styrene emissions to the atmosphere have steadily increased during the last years, leading to the development of gas treatment processes (Pol *et al.* 1998). However, in spite of its high volatility, a fraction of styrene remains in the liquid phase of industrial effluents, being potentially toxic to the biomass used in biological treatment processes.

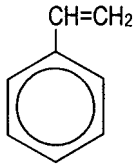

Fig. 1. Chemical structure of styrene.

Effluents from chemical industry present, in general, unfavorable environmental characteristics for the growth of microorganisms such as extreme pH, high temperatures and presence of toxic compounds. It is the case, for example, of effluents from plastic and pharmaceutical industry. Aerobic treatment systems have been traditionally used for this kind of effluent, being the anaerobic technology not so widely applied due to the lack of knowledge regarding the effects of some toxics on the anaerobic consortia. There are only a few publications reporting the use of anaerobic treatment with effluents of this type of industry (Araya *et al.* 1999, Henry *et al.* 1996). Araya *et al.* (1999) reported that an effluent from a polymer synthesis plant was efficiently treated in a Upflow Anaerobic Sludge Blanket (UASB) reactor after a gradual acclimatization to the effluent. In the present work, the influence of a start-up period without acclimatization was eval-

Table 1. Characteristics of the industrial effluent.

COD (mg l^{-1})	2000
BOD5 (mg l^{-1})	150
pH	6.5–8.5
VSS (g l^{-1})	<0.04
Colour	Transparent
Alkalinity (mg $CaCO_3$ l^{-1})	25–50
Styrene (mg l^{-1})	6
Other known compounds	Acrylates
	Detergents
	Silicates
	Ferric chloride

COD – Chemical oxygen demand.
BOD5 – Biochemical oxygen demand.
VSS – Volatile suspended solids.

uated for the same type of effluent in a UASB reactor. As styrene was the main aromatic compound present in the effluent, the anaerobic biodegradability and toxicity of this compound was studied in batch assays, evaluating its effect on some key bacterial groups of the anaerobic consortium, such as acetoclastic and syntrophic bacteria.

Material and methods

Continuous experiment

A 4-liter Upflow Anaerobic Sludge Blanket (UASB) reactor was operated for 58 days. The granular sludge used in this experiment was obtained from a pilot plant operating successfully in a brewery in Santiago, Chile. Routine reactor performance was evaluated by daily measuring the influent and effluent chemical oxygen demand (COD), the influent flow rate and the pH. In the first 12 days a synthetic lactose-based effluent was fed into the UASB. An effluent from a polymer synthesis plant was fed afterwards (Table 1). This effluent had no chlorinated compounds and the levels of ammonia and NO_x were very low (<2 mg l^{-1}). The pH and alkalinity were adjusted to 7.0 ± 0.2 and 2500 mg $CaCO_3$ l^{-1}, respectively. Apart from styrene, acrylates and detergents were also present in the effluent.

Table 2 summarizes the operating conditions applied to the reactor.

The inoculum and the sludge collected at the end of the experiment were characterized in terms of methanogenic activity according to the method described by Soto *et al.* (1992). Bottles with the sludge and a mixture of acetate, propionate and butyrate were

Table 2. Operating conditions applied in the UASB reactor.

Time (days)	HRT (h)	COD (mg l^{-1})	Type of effluent	Styrene (mg l^{-1})
1–5	60	800	Synthetic[a]	–
5–10	26	800	Synthetic[a]	–
10–12	26	1000	Synthetic[a]	–
12–24	26	1000	Industrial	3.0
24–44	17	1500	Industrial	4.5
44–53	17	2000	Industrial	6.0
53–58	17	1000	Industrial	3.0

[a]Lactose based. HRT – Hydraulic retention time.

connected to a Mariotte-bottle system, in order to measure the amount of methane produced by biomass activity.

Batch experiments

Methanogenic activity, toxicity and biodegradability batch tests were performed using a pressure transducer technique (Colleran & Pistilli 1994, Coates *et al.* 1996). The methanogenic activity test involved the monitoring of the pressure increase developed in sealed vials fed with non-gaseous substrates (30 mM of acetate, propionate or ethanol) or pressure decrease in vials previously pressurized at 1 bar with gaseous substrates (H_2/CO_2, 80:20 v/v). Methanogenic toxicity tests were performed by adding increasing styrene concentrations and 30 mM acetate, propionate or ethanol to the sludge. The fifty percent inhibition concentration (IC_{50}) was defined as the styrene concentration that caused a 50% relative activity loss. Biodegradability tests were performed by adding increasing styrene concentrations to the sludge and by measuring the initial rate of methane production.

The hand-held pressure transducer was capable of measuring a pressure increase or decrease of two bar (0 to $\pm$ 202.6 kPa) over a range of -200 to $+200$ mV, with a minimum detectable variation of 0.005 bar. The basal medium used in the batch experiments, made up with demineralized water, was composed of cysteine-HCl (0.5 g l^{-1}) and $NaHCO_3$ (3 g l^{-1}) and was prepared under strict anaerobic conditions. All batch experiments were performed in triplicate assays. Methane production was corrected for standard temperature and pressure (STP) conditions.

The granular sludge used in these experiments was obtained from a UASB treating a brewery effluent located in Oporto, Portugal. Table 3 summarizes

Table 3. Characteristics of biomass used in the biodegradability and toxicity batch experiments (± standard deviation).

Sedimentation rate (m h^{-1})	20.4 ± 1.6
VSS (g l^{-1})	65.4 ± 2.8
Methanogenic activity in presence of: ($mlCH_{4(STP)}$ $gVSS^{-1}$ d^{-1})	
Acetate	118 ± 16
Propionate	284 ± 17
Ethanol	417 ± 42
H_2/CO_2	246 ± 18

STP – Standard temperature and pressure conditions.

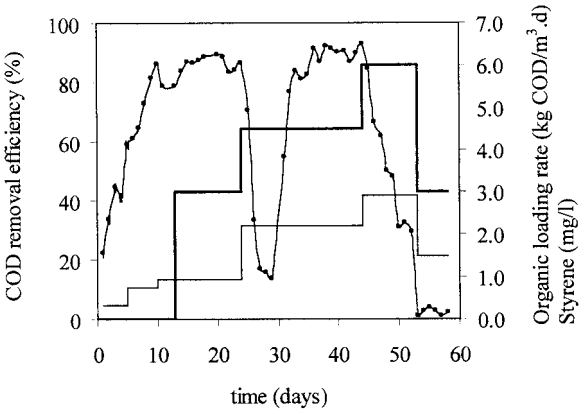

Fig. 2. Time course of the applied organic loading rate (—), styrene concentration (▬) and removal efficiency (—●—) during the continuous experiment in a upflow anaerobic sludge blanket (UASB) reactor fed with the industrial effluent from the polymer synthesis plant.

sludge characteristics, including the methanogenic activity with acetate, H_2/CO_2, propionate and ethanol as substrates.

Results and discussion

Continuous experiment

During the start-up the COD removal efficiency achieved 90% in the first 8 days (Figure 2). After replacing the synthetic by the industrial effluent, on day 12, performance was very instable even for organic loading rates as low as 2.2 kg COD m^{-3} d^{-1}. Attempts were made to control excessive acidification by increasing alkalinity from 2500 to 3000 mg $CaCO_3$ l^{-1} on day 27 and from 3000 to 4000 mg $CaCO_3$ l^{-1} on day 48. However, at this moment, the pH dropped to 6.2 and, in spite of the decrease in the organic loading rate to 1.48 kg COD m^{-3} d^{-1}, there was no evidence of performance recovery.

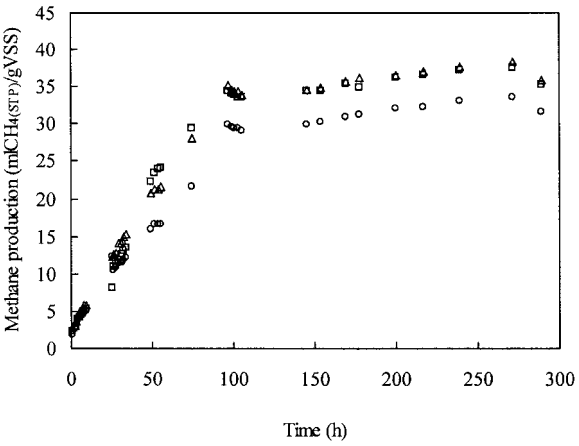

Fig. 3. Specific methane production from 200 mg styrene l^{-1} as the sole carbon source in three separate anaerobic batch assays.

Table 4. Characterization of the granular sludge at the beginning and at the end of the continuous experiment with the UASB reactor.

Property	Inoculum	End of operation
Sedimentation rate (m h^{-1})	23 ± 2	16 ± 2
VSS (g l^{-1})	28 ± 3	22 ± 2
Methanogenic activity ($mlCH_{4(STP)}$ ($gVSS^{-1}$ d^{-1})	182 ± 20	76 ± 10

STP – Standard temperature and pressure conditions.

This result suggests that this type of effluent is problematic for anaerobic treatment with no acclimatized sludge. In a previous work with the same effluent the start up was made by gradually shifting the synthetic lactose based effluent by the industrial effluent (Araya *et al.* 1999). Apparently that was a better start up strategy that resulted in removal efficiency around 75% at an organic loading rate of 4 kg COD m^{-3} d^{-1}, 120 days after starting the continuous operation. Although styrene was the sole aromatic compound in the effluent, the ratio between COD and BOD suggests a low aerobic biodegradability. However, under anaerobic conditions 62% of biodegradability was attained in batch assays with this effluent (Araya *et al.* 1999).

The different periods of acidification indicate that acetogenic or acetoclastic bacteria were affected and this led to volatile fatty acids accumulation. After 58 days of operation, the settling rate and the methanogenic activity of the granular sludge decreased significantly (Table 4), indicating that in the present work, operating conditions were more drastic

Table 5. Effect of increasing styrene concentration on the methanogenic activity with acetate, propionate and butyrate as substrates (± standard deviation). Toxicity limits (IC_{50}) for methanogenic acetoclastic propionate and ethanol degrading syntrophic bacteria.

Styrene (mM)	Specific methanogenic activity in the presence of: (ml $CH_{4(STP)}$ $gVSS^{-1}$ d^{-1})		
	Acetate	Propionate	Ethanol
0 (Control)	118 ± 16	284 ± 17	417 ± 42
0.05	107 ± 16	222 ± 68	381 ± 19
0.1	90 ± 20	175 ± 46	322 ± 25
0.5	83 ± 15	167 ± 24	332 ± 38
1	78 ± 19	123 ± 34	363 ± 34
1.5	53 ± 20	115 ± 38	339 ± 53
2.0	10 ± 7	118 ± 11	73 ± 17
IC_{50}(mM)	1.4 ± 0.2	0.45 ± 0.2	1.6 ± 0.2

STP – Standard temperature and pressure conditions.

to the methanogenic populations than previously reported, where only 2.5% of the initial methanogenic activity was lost in the subsequent 120 days of operation with a similar effluent (Araya *et al.* 1999).

Biodegradability and toxicity of styrene in batch experiments

Seventy-four % of methanization was obtained in a batch assay with styrene as the sole carbon source at 200 mg l^{-1} after 180 h and no lag-phase was detected (Figure 3). It was concluded that the non-acclimatized biomass was able to degrade styrene, although the initial methane production rate was low – 7 ml methane ($gVSS\ d^{-1}$).

The methanogenic toxicity tests of styrene for the acetoclastic bacteria and for the propionate and ethanol degrading syntrophic bacteria indicate that styrene exerts a differentiated toxicity to the different trophic groups under study (Table 5).

Styrene presents a relative greater toxicity than other compounds of similar structure. Speece (1996) reported values for acetoclastic bacteria of 15.3, 6.3 and 2.4 mM for benzene, toluene and xylene, respectively. Although acetoclastic bacteria are, in general, recognized as the most sensitive trophic group of the anaerobic consortium (Speece 1996), the styrene exerted a more acute toxicity to the propionate consuming syntrophic bacteria than to the acetoclastic bacteria. A similar result was referred to by Sierra-Alvarez *et al.* (1988) for phenol compounds and by Lin *et al.* (1992) for cromium and zinc.

The obtained results should be interpreted carefully, because conversion of propionate and ethanol to CH_4 and CO_2 requires the combined action of syntrophic, acetoclastic and hydrogenophilic bacteria, according to the following equations (example for ethanol):

$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$ (syntrophic),
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ (acetoclastic),
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ (hydrogenotrophic).

The combined reaction is

$$2CH_3CH_2OH \rightarrow 3CH_4 + CO_2 + H_2O.$$

The styrene concentration (1.6 mM) that inhibits in 50% of the methanogenic activity with ethanol as substrate was close to the concentration needed (1.4 mM) to achieve 50% inhibition of acetoclastic activity. Since conversion of ethanol requires the activity of both syntrophic and acetoclastic bacteria it is not clear if the toxicity measured in the ethanol experiment was mediated by syntrophs, acetoclasts or both groups of bacteria. It is assumed that, although not determined in the present work, the styrene toxicity to the hydrogenophilic bacteria is much less important than to the acetoclastic bacteria as reported for many other xenobiotic compounds (Colleran & Pistilli 1994). In the case of propionate degrading bacteria, however, the toxicity limit was significantly lower than the one measured for the acetoclasts, evidencing the more toxic effect of this compound to the propionate degrading than to the acetoclastic bacteria.

When treating styrene-based effluents from the plastic industry, specially if this compound is present in concentrations close to the toxicity limits, reactor design should be appropriate in order to protect syntrophic bacteria. A possible way might be the use of staged systems where acidogenic, syntrophic and methanogenic populations can be segregated along the reactor (Alves *et al.* 1998).

The comparison between results from continuous and batch experiments led to the conclusion that although operation was unstable when feeding the industrial effluent from the polymer synthesis plant, probably it was not due to the presence of styrene, which was fed in a concentration of 6 mg l^{-1}, far below the toxicity limit calculated for propionate consuming bacteria. The presence of acrylates and detergents can, in this case, be responsible for the unstable

operation of the UASB reactor (Demirer & Speece 1998).

Conclusions

Start-up and operation of a UASB reactor fed with an industrial effluent from a polymer synthesis plant containing 6 mg styrene l^{-1} was unstable at organic loading rates lower than 3 kg COD m^{-3} d^{-1} with non-acclimatized sludge. However, in batch assays, non-acclimatized granular anaerobic sludge was able to utilize styrene as a sole carbon source, achieving 74% of methanization for a concentration of 200 mg l^{-1} without lag-phase, although the methane production rate was only 7 ml $CH_{4(STP)}$ $gVSS^{-1}$ d^{-1}. Acetoclastic bacteria, propionate- and ethanol-degrading syntrophic bacteria presented a differentiated sensitivity to the presence of styrene. The toxicity limit (IC_{50}) of styrene was 1.4 mM for the acetoclastic activity, 0.45 and 1.6 mM for the methanogenic activity in the presence of propionate and ethanol, respectively. It was concluded that instability during the operation of an UASB reactor fed with the industrial effluent was not due to the presence of styrene, but probably to the presence of other known compounds such as acrylates or detergents.

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