



INFLUENCE OF FLOW REGIME ON THE CONCENTRATION OF CYANIDE PRODUCING ANAEROBIC PROCESS INHIBITION

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ABSTRACT

A study was conducted to analyze the effects of cyanide on methanogenic bacterial populations in both continuous and batch reactors. The appropriate toxicity assays were divided into two parts, i.e. 1) batch assays, and 2) continuous flow assays, using a 6.5-litre up-stream anaerobic sludge blanket reactor (UASB). A synthetic substrate was used as feed for both assays using 99% sucrose as a carbon source, and potassium cyanide (KCN) as a free cyanide source. In the first part, the treatments with the lowest and highest percentages of Specific Methanogenic Activity (SMA %) were 3 and 10 mg CN/l. A 0.96 mg/l concentration of cyanide caused methanogenic activity inhibition by 50% during the exposure stage. During the recovery stage, Methanogenic Activity of treatments had a considerable increase compared to the results from direct cyanide exposure, except for treatments of 7 and 10 mg CN/l. The concentration of cyanide that inhibits Specific Methanogenic Activity by 50% was 6.1 mg CN/l during this stage. In the second part, the reactor was continuously fed with a synthetic substrate (COD= 1500 mg/l). After the anaerobic sludge became acclimated, cyanide concentrations from 0.5 and 130 mg CN/l were progressively added. The hydraulic retention time remained constant at 12 hours throughout the entire experiment. Results indicated that while partial inhibition occurred for concentrations of 3, 20 and 100 mg CN/l, severe inhibition occurred for 130 mg CN/l. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved.

KEYWORDS

Anaerobic sludge; cyanide toxicity; methanogenic activity; methanogenic inhibition.

INTRODUCTION

Toxicity assays should be concurrent with anaerobic biodegradability assays of effluents under analysis. Toxicity assays are conducted as a tool in determining the viability of anaerobic processes in the presence of toxic substances. Considering that a toxic agent may occur either as a batch or a continuous flow in industrial effluents, assays should therefore be conducted in these two ways.

Wastewater from the process of extracting sour cassava starch is susceptible to degradation (Oviedo, 1995) using different anaerobic treatment systems, c.g., sludge blanket and up-stream flow systems (Castrillon *et al.*, 1994) and separate phase systems (Arroyave, 1996). The presence of toxic compounds in wastewater requires the following considerations to be taken into account: 1) calculating the concentration of the toxic

agent causing inhibition; 2) determining whether the response to the toxic agent is reversible or not; and 3) estimating the acclimation potential of different methanogenic microorganism groups involved in the process.

In the specific case of effluents from the cassava starch extraction process, the presence of cyanide is due to the existence of cyanogenic compounds in the cassava. Linamarina accounts for 93% to 97% of cyanogenic glucosides in cassava (Cuzin, 1992). Cyanhydrous acid is highly toxic; it interferes with enzymes that play a major role in the processes of life. The action of cyanide on enzymatic processes of methanogenic bacteria is specifically focused on the biodegradation of acetate by acetoclastic bacteria.

The toxicity assay allows determination of the percentage of methanogenic activity that may be lost because of the presence of inhibiting compounds. In discontinuous flow systems, toxicity is determined by comparing the activity of the control sample; i.e., sludge fed only with substrate with the sludge activity fed with the same substrate plus the toxic compound. In the continuous flow assay, cyanide is increasingly added as the reactor stabilizes after an increase of the toxic agent concentration. Inhibition is determined based on methanogenic activity in the sludge after it has been in contact with the toxic agent (Rojas *et al.*, 1987).

MATERIALS AND METHODS

Toxicity assays

Each test in the **batch assays** included: a blank and several treatments, whose toxic concentrations were 0, 1, 3, 5, 7 and 10 mg/l of CN^- added as KCN, exposure phase; in the recovery phase, the biodegradability and toxic substrate was removed and then the sludge was re-exposed to synthetic substrate without cyanide. The **continuous flow assays** had the following CN^- test concentrations: 0, 0.25, 1, 2, 3, 5, 10, 20, 30, 55, 70, 100 and 130 mg CN^-/l .

Experimental units

0.5-litre serum bottles were used for the batch assays (Figure 1A shows the assay set-up). Methodology proposed by Field (1987) was followed. Continuous flow assays were performed in a 6.5-litre useful volume reactor, keeping a HRT of 12 hours. Figure 1B shows this set-up. In both cases the temperature was controlled at $30^\circ\text{C} \pm 5^\circ\text{C}$.

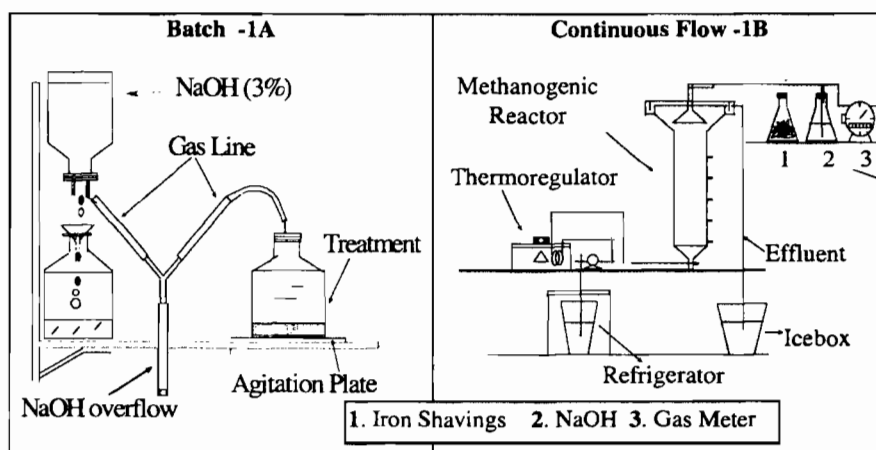


Figure 1. Schematics of set-ups used for toxicity assays.

Inoculum characterization

Sludge from the wastewater treatment plant at *Matadero de Carnes y Derivados de Occidente* (a slaughterhouse located on the road to Candelaria in the Northeast of Santiago de Cali, Colombia) was used as inoculum, (Tabla 1). Characterization of this sludge included the determination of Volatile Suspended Solids (V.S.S), Specific Methanogenic Activity, Stability and Sedimentability (Field, 1987; Uterlynde, 1987).

Table 1. Inoculum characterization results

Parameter	Reading	Units of Measurement
Volatile Suspended Solids	0.1021	g VSS/ g of humid sludge
Methanogenic Activity 1	0.40	g COD-CH ₄ /g VSS day
Methanogenic Activity 2	0.41	g COD-CH ₄ /g VSS day
Stability	23.05	ml CH ₄ / g VSS
Sedimentability	34.00	m/h

Analyses performed

pH, Chemical Oxygen Demand (COD micro) and cyanide analyses were performed according to the AWWA, WPCF and APHA "Standard Methods" (1985). Cyanide was also determined by colorimetry using Merck's KIT SPECTROQUANT 14800. Volatile Fatty Acid determinations were made using High Performance Liquid Chromatography (HPLC), (Column: AMINEX HPX 87H, Detector: Merck-Hitachi R1-71). Differential Refractometer (Solvent: 0.005 M H₂SO₄). Gas chromatography was also used for analyzing Volatile Fatty Acids using a flame ionization detector and FFAP as stationary phase. Sugar determination was made using the modified total sugar method with Anthrone (Hassid, 1964).

RESULTS: ANALYSIS AND DISCUSSION

Toxicity assays

Batch Assays. A potassium cyanide salt was used as cyanide source in toxicity assays with free cyanide. As shown in Table 2 and Figure 2A, inhibition occurs from 1 mg CN⁻/l. Low methane production was constant throughout the development of the exposure stage. Treatments with 1 and 3 mg CN⁻/l four days from the beginning of the assay, but especially the treatment with 3 mg CN⁻/l, showed higher Specific Methanogenic Activity than the rest of the treatments with cyanide. For the first 20 hours following the beginning of the exposure stage, the treatments exhibited a delay or latency period in which the bacterial population adjusts to the new substrate and begins to induce enzymes with substrate degrading capability. This period was readily overcome by the control sample. No conspicuous production of methane was observed for treatments with 7 and 10 mg/l during the first 100 hours in which the production was monitored.

Table 2. Cyanide inhibition percentage (added as KCN)

Cyanide mg/l	Exposure phase			Recovery phase		
	% SMA	I%	Remaining CN ⁻	Final pH	% SMA	Final pH
0	100.00	0.00	—	6.85	100.00	6.68
1	48.04	51.96	—	6.96	80.85	6.98
3	52.34	47.66	—	7.01	104.50	7.12
5	46.21	53.79	1.50	5.89	70.81	6.60
7	29.97	70.03	4.75	6.02	32.62	5.98
10	25.03	74.97	7.36	6.12	20.29	5.94

Figure 2B illustrates the recovery phase in which a measurement is made of the capacity of the methanogenic microflora to recover activity after being in contact with a toxic substance. In this phase methane production of the reactor with 3 mg CN⁻/l shows higher activity than that of the control sample.

Except for treatments with 7 and 10 mg CN⁻/l, recovery capacity of all treatments is indicated by their Specific Methanogenic Activities (SMA %), in Table 2.

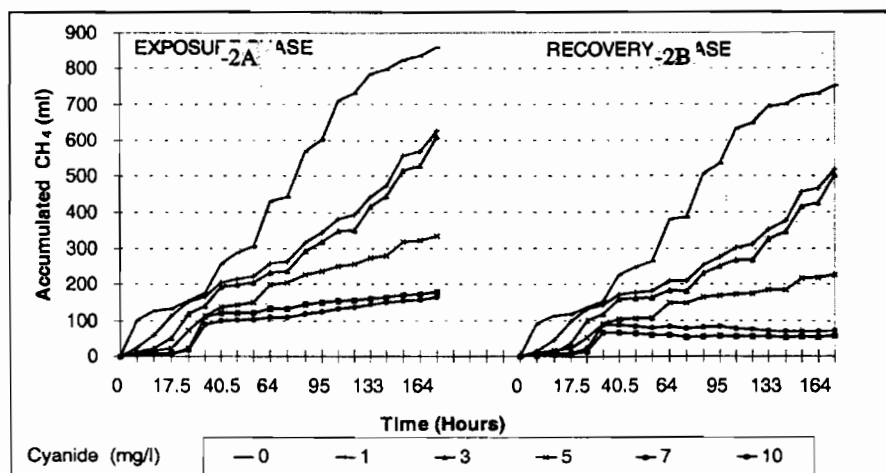


Figure 2. Cyanide effects on methane production. salt: K CN.

All cyanide addition treatments showed a similar behavior with respect to the appearance of VFAs. During the exposure phase, while acetate was the compound with the highest accumulation, propionate and butyrate were present in low concentrations. Figure 3 shows the behavior of VFAs during the exposure and the recovery phase for the treatment with 10 mg/l of CN⁻. In the recovery phase, butyrate accumulated in much greater concentrations than those reached in the first phase.

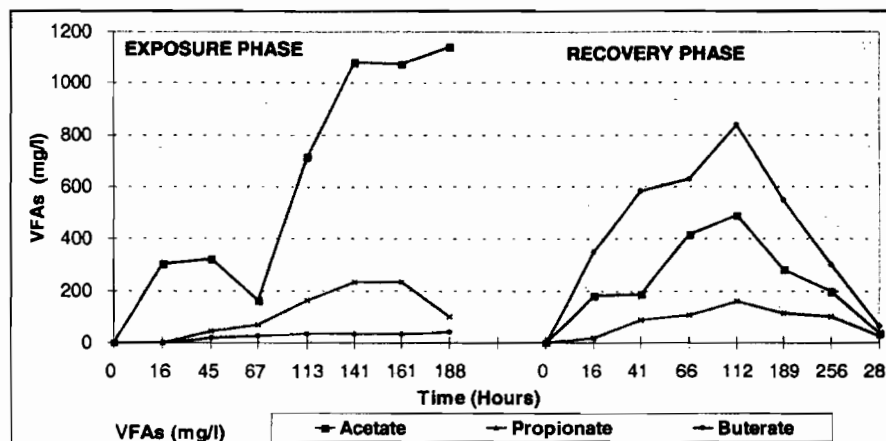


Figure 3. Variation of VFAs during the exposure and recovery phases for 10 mg CN⁻/l.

In the assays to test free cyanide (added as potassium cyanide), the cyanide concentration that inhibits 50% of Methanogenic Activity was extrapolated from the Specific Methanogenic Activity % graph. The above concentration was calculated in the exposure phase as 0.96 mg/l of CN⁻. As can be observed, Methanogenic Activity is approximately six times greater (Figure 4) upon removal of the toxic agent.

Continuous flow assays

Cyanide concentrations of 0.5, 1, 3, 10, 20, 30, 55, 70, 100, 130 mg/l were tested in the continuous flow reactor. There was a gradual decrease of methane production until the bacterial population adapted to cyanide and began to generate enzymes capable of degrading the cyanide (Figure 5).

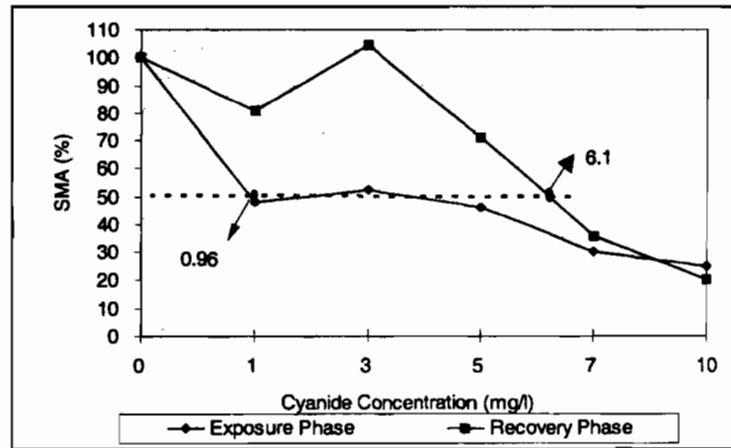


Figure 4. Activity percentages during the exposure and recovery phases.

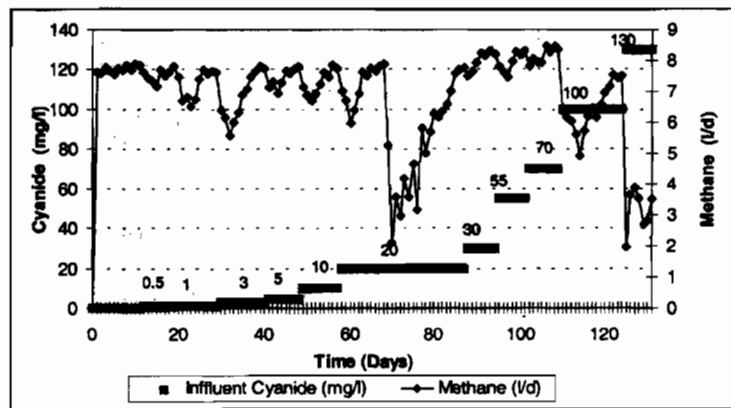


Figure 5. Cyanide concentration and methane production ratio.

Minimum values of methane production occurred for CN^- concentrations of 3 and 20 mg/l with readings of 5.59 and 5.96 l CH_4 /day, respectively. These low methane production readings probably occurred because the sludge was not adapted to a high increase of cyanide from one phase to another. For example: CN^- concentration went from 1 - 3 mg/l to 10 - 20 mg/l.

For CN^- concentrations of 30, 55, and 70 mg/l, the difference in methane production with respect to the blank was minimal. This result is due to both an increase of the biological reaction rate and the growth of microflora better adapted to cyanide. When a 0.86 g VSS/g Humid Sludge (HS) and 70 mg/l of CN^- ratio is achieved, any increase of the toxic concentration causes a decrease of the biomass, (Figure 6) and the biological activity rate of methanogenic bacteria (Table 3). Inhibition is stronger in CN^- concentrations of 100 mg/l, but specially of 130 mg/l when severe inhibition occurs.

Table 3. Cyanide inhibition percentages (added as KCN)

Cyanide (mg/l)	SMA	SMA (%)	Inhibition(%)
0	0.57	100.0	0.0
55	0.77	135.1	-35.1
70	0.89	154.6	-54.6
100	0.23	39.7	60.3
130	0.57	9.9	90.0

The sludge was tested for specific methanogenic activity after being exposed to a determined cyanide concentration. The sludge sample was taken from the reactor's sampling point No. 4 on the third day after the cyanide concentration was added. Table 3 shows the results obtained for CN^- concentrations of 0, 55, 70, 100 and 130 mg/l. These results show that methanogenic activity for CN^- concentrations of 55 and 70 mg/l is higher than the activity of the control sample. On the other hand, CN^- concentrations of 100 mg/l and 130 mg/l had lower SMAs than those of the control sample showing a slow recovery at a CN^- concentration of 100 mg/l and a very slow recovery at 130 mg/l.

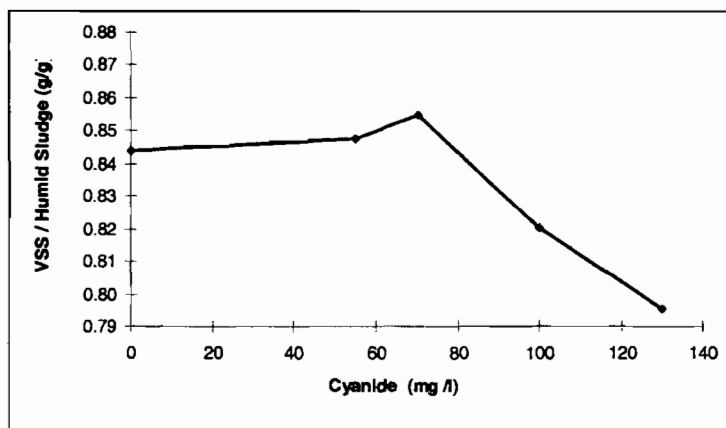


Figure 6. Volatile suspended solids vs. cyanide concentration.

Figure 7 was used for calculating the concentration of free cyanide (added as potassium cyanide) that inhibits 50% of the specific methanogenic activity. This concentration was 90.5 mg/l of CN^- .

Figure 8 shows the high capability of microorganisms to adapt to cyanide because it is a reversible inhibitor. When a given concentration of cyanide is initially added, it maintains high values in the effluent, but decreases in the effluent as the phase evolves, except for the CN^- concentration of 130 mg/l which always had high cyanide levels in the effluent.

During the phases with the highest inhibition of methane production, CN^- removal was lower. Therefore, methanogenic microflora is inhibited when there is a low CN^- removal (Yang and Speece, 1981).

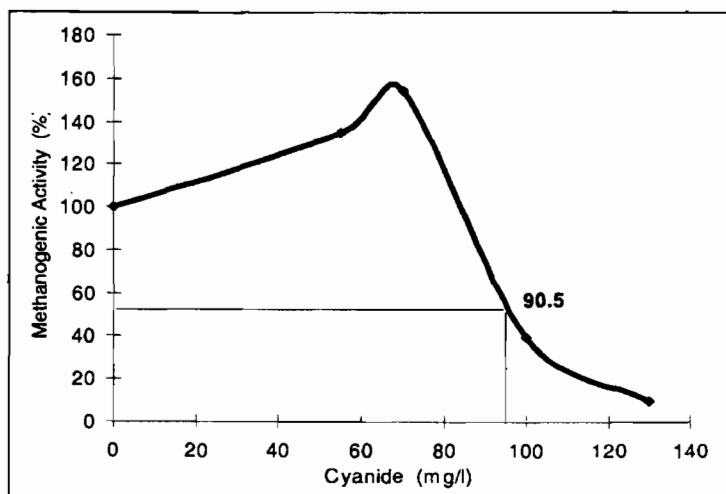


Figure 7. Specific methanogenic activity (SMAs) vs. cyanide concentration.

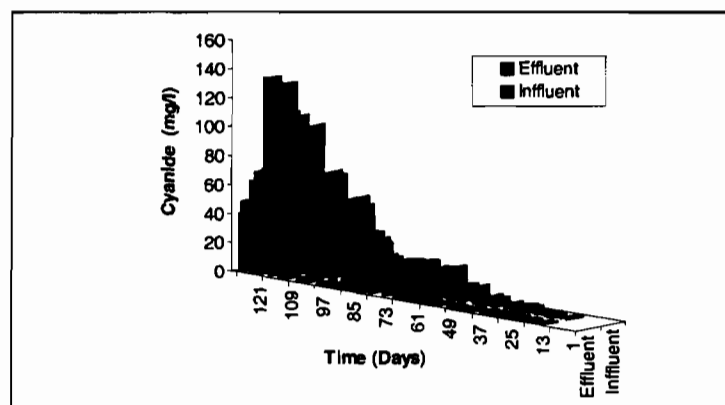
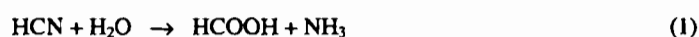


Figure 8. Variation of cyanide concentration in the influent vs. effluent as a function of time.

Knowledge of cyanide biodegradation serves as a good tool to indicate the inhibiting capacity of cyanide in anaerobic processes. According to Fallon (1992), biodegradation of cyanide in methanogenic media occurs hydrolytically when enzymes break the CN triple bond of HCN which destroys the cyano unit, thus eliminating the possibility of increasing reactivity.



Hydrolysis produces formic acid and ammonia which are considerably less toxic than cyanide. Under anaerobic conditions, formic acid turns into bicarbonate. Cyanide degradation by hydrolysis requires special conditions of pH, temperature, and metal salt concentration. This was demonstrated with this research by analyzing ammonia at the end of each phase. Figure 9 shows that as the concentration of cyanide increases, the amount of ammonia in the effluent also increases. This shows the adaptation of microflora to these cyanide concentrations, except to 130 mg/l which causes the ammonia concentration to decrease suddenly, thus showing the inhibiting capacity of this concentration on the biomass. This also explains the process failure observed in the accumulation of VFAs and low methane production.

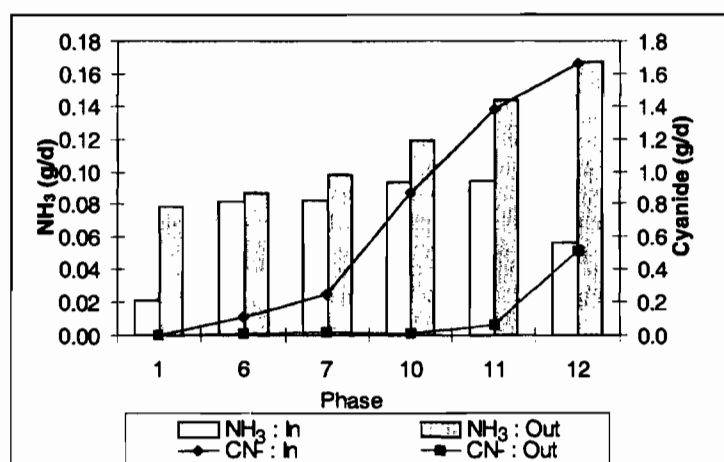


Figure 9. Variation of NH_3 and CN^- concentrations in both influent and effluent.

In an anaerobic reactor where complex substrates are fermented and produce volatile fatty acids, HCO_3^- and CO_2 act as the main pH regulators of the system. Total alkalinity in the effluent shows a trend to increase as the toxicity assay evolves; it initially had a value of 1,100 mg CaCO_3/l in the first phases, but at the end of

the assay it was 1,400 mg CaCO_3/l . For concentrations with accumulation of VFAs, the buffer index value was greater or equal to 0.35, specially at a CN^- concentration of 130 mg/l at which the values were between 0.40 and 0.46, thus showing the severity of inhibition at this concentration. Volatile fatty acids showed a trend to increase with cyanide concentrations with methanogenic inhibition. At these cyanide concentrations, VFAs values are higher than 3 meq/l, but VFAs concentration decreases over time, except at CN^- concentrations of 100 and 130 mg/l at which VFAs rise to 13 meq/l (Figure 10).

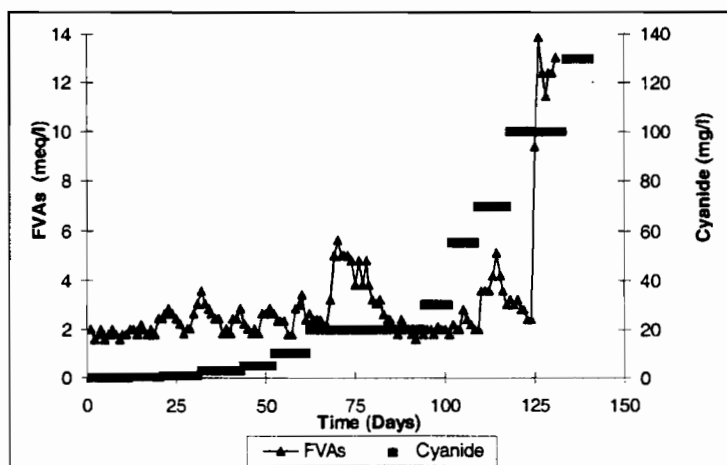


Figure 10. VFA variation vs. cyanide addition as a function of time.

During the progressive addition of cyanide, a steady increase of the COD was observed. This is probably because of the effect of toxicity on the kinetics of the anaerobic treatment (Speece *et al.*, 1986). Cyanide concentrations added in terms of COD removal efficiencies showed three trends (0.5, 1, 3, 5, 10, and 20 mg/l of CN^-); COD removal decreases at the beginning of each phase and increases over time.

This shows the adaptation of bacteria and the recovery of the reactor. The second trend occurs at CN^- concentrations of 30, 55, and 70 mg/l for which COD removal is high, showing that anaerobic microflora has a strong adaptation. The third trend occurs at CN^- concentrations of 20, 100 and 130 mg/l, at which COD removal decreases and recovery is either slow or zero, in particular at a CN^- concentration of 130 mg/l. This confirms the strong inhibition of cyanide on methanogenic bacteria at high concentrations.

There is a slim difference between the raw and filtered effluent COD, which indicates that the effluent's COD is quite soluble (85%), and sludge loss for an HRT of 12 hours is therefore small. Only during those days when a strong methanogenic inhibition occurs, is sludge observed in the effluent. Nevertheless, this sludge loss is not critical because it occurs gradually. COD removal varied a lot especially after adding CN^- , which causes a significant reduction of biogas production.

CONCLUSIONS

The results of this study indicate different responses depending on the flow regime applied to the system. There is no question that feeding a toxic compound has a lesser inhibition of methanogenic activity of sludge when it is fed on a continuous flow basis, because microorganisms can adapt to certain kinds of toxic agents, but they easily lose their adaptation capability over a short period of time. Microorganisms induce enzymes capable of breaking the triple CN^- bond, releasing ammonia and formic acid, which increase the buffer capacity of the system.

Decrease of methane production is small for certain CN^- concentrations in both assays, and there is a larger production of methane in the recovery phase than that of the blank. In batch assays, this occurs at a CN^- concentration of 3 mg/l, and in continuous flow assays at CN^- concentrations of 30, 55 and 70 mg/l. These

results are due to both an increase of the biological reaction rate and the growth of microflora with a better adaptation to cyanide.

It is worth emphasizing the importance of conducting these kinds of assays as part of treatability studies of industrial wastewater. In these kinds of effluents, a toxic substance for methanogenic bacteria may occur as batch or continuously. Thus, any testing for this parameter should be conducted according to the way the toxic agent comes to the wastewater, because anaerobic microorganisms respond differently in each case.

ACKNOWLEDGEMENT

This study was conducted with funding from the European Union as part of a macro research program to optimize the production process of cassava starch for small and medium industrial farmers in Latin America.

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Rojas Ch. O., Alazard Didier, Aponte R.L., Hidrobo L.F. (1999)

Influence of flow regime on the concentration of cyanide
producing anaerobic process inhibition

Water Science and Technology, 40 (8), 177-185

ISBN 0-08-043677-3