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Solubilization and Fermentation in a Modified VFA-Potential Method

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ABSTRACT

The VFA-potential method is a fermentation technique for estimating the total volatile fatty acids (VFA) availability in wastewater. The VFA-potential was conceived as a measurement of the readily fermentable COD, because fermentation utilizes this COD as a substrate. However, anaerobic hydrolysis can also be a potential source of fermentable matter. In this work, the VFA-potential method has been modified and simplified to avoid nitrogen gas usage. In the modified method, several conventional BOD flasks are completely filled with wastewater and subsequently opened at scheduled times. This procedure keeps oxidation-reduction potential (ORP) at very low levels while reducing the laboratory equipment required. COD fractionation of initial wastewater was carried out. Analysis of total suspended solids (TSS), soluble phosphate, ammonia nitrogen, total and soluble COD, and individual VFA were obtained throughout the VFA-potential test, to evaluate hydrolysis and fermentation. The mean ratio between VFA-potential and the initial total COD was 0.23 mg VFA-COD/mg COD. The VFA to phosphate ratio was in the range of 14.6–22 mg VFA-COD/mg P, which is in accordance with the recommended value for biological phosphorus removal (20 mg/mg). A strong hydrolysis of solids was detected throughout the degradation of TSS and soluble COD. The observed behavior of TSS, soluble COD, and VFA is compatible with a partial utilization of hydrolyzed COD for fermentation. Phosphorus and nitrogen solubilization are described, as well as pH and alkalinity behavior, throughout the test.

Key words: volatile fatty acids; VFA potential; acidogenic fermentation; readily biodegradable COD; COD fractionation.

INTRODUCTION

DOLD *ET AL.* (1980) established that the influent wastewater contained two biodegradable COD fractions—the soluble readily biodegradable fraction (RBCOD), and the particulate slowly biodegradable fraction (SBCOD). The readily biodegradable fraction is immediately utilized by micro-organisms, while the slowly biodegrad-

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able fraction must be broken down, for example, by hydrolysis with extracellular enzymes, into simpler forms before micro-organisms can utilize it (Henze *et al.*, 1995).

Volatile fatty acids uptake during anaerobic stages has been characterized as a key step in enhanced biological phosphorus removal (EBPR). Low-strength wastewaters are frequently unable to supply enough VFA for anaerobic phosphorus release. The amount of VFA present in primary settled municipal wastewater is a small fraction of the total COD, approximately 2 to 10%. However, there is at least an additional 10–20% RBCOD that can usually be fermented into VFA and other fermentation products. On the other hand, anaerobic hydrolysis of SBCOD can produce fermentable matter that can be further fermented to VFA (Henze *et al.*, 1995).

Some parameters—or their ratios to phosphorus—have been used to characterize the suitability of a wastewater for EBPR, namely COD, BOD, RBCOD, and VFA concentration. A more specific way of measuring the suitability of a wastewater for biological phosphorus removal is to apply the VFA-potential method (Lie and Welander, 1997). This is a method for estimating the availability of VFA in a wastewater, including both free VFA and VFA that can be released by fermentation of the readily fermentable matter. Slowly biodegradable COD was not considered a source of readily fermentable matter in the VFA-potential method. In this method, a wastewater sample is stored in a sealed bottle for spontaneous fermentation by the micro-organisms present in the wastewater.

In the original method, oxygen is previously purged from the wastewater and the headspace of the bottle with nitrogen gas. After sealing with a butyl rubber stopper, wastewater samples are drawn at predetermined times with a syringe through the stopper. Afterwards, the samples are filtered through 0.45-μm filters and then kept frozen until VFA analysis.

The effect of several parameters on the VFA-potential test was studied by Martin *et al.* (2002), including the effect of sample freezing, inhibitors, stirring, pH alkalinity, and temperature. A set of optimized operating conditions was proposed in this study. Curto *et al.* (2002) compared the VFA potential and the readily biodegradable COD as methods for determining the substrate available for enhanced biological phosphorus removal. Also, a clear correlation was found between the VFA potential and the performance of three EBPR treatment plants.

In this work, the original VFA-potential method (Lie and Welander, 1997) has been modified to avoid the need for nitrogen usage. Besides, stirring has been applied to the samples after fermentation, to ensure a homogeneous VFA concentration. COD fractionation has been applied to wastewater before fermentation, to compare VFA formation to initial total COD fractions. The modified method has been applied to an urban wastewater fed to two bench-scale EBPR plants. Evolution of VFA, total and soluble COD, TSS, ORP, as well as the solubilization of nitrogen and phosphorus have been studied.

MATERIALS AND METHODS

Wastewater sampling and analysis

Daytime composite samples were taken from a nearby street sewer in the residential area around the university laboratory. Samples were analyzed for TSS, volatile suspended solids (VSS), total and soluble COD, COD fractionation, VFA, VFA-potential, dissolved oxygen (DO), soluble phosphate (PO_4-P), ammonia nitrogen, pH, and alkalinity. Standard methods were applied (APHA-AWWA-WPCF, 1995), except for VFA determination and COD fractionation. These parameters were determined as described below.

COD fractionation

Total COD (COD) was determined according to the APHA-AWWA-WPCF (1995) standard methods. Total biodegradable COD (TBCOD) was determined by the Park *et al.* (1997) procedure. This method is based on the total biodegradable oxygen demand, computed from the total biological oxidation of wastewater in a 24-h batch test. The details of the TBCOD procedure and calculation are given in Park *et al.* (1997).

Readily biodegradable COD (RBCOD) was determined by the Mamais *et al.* (1993) method. In this method, a 24-h fill-and-draw biological reactor is fed with the wastewater. Influent and effluent are flocculated with zinc sulphate for determining truly soluble COD (CODsol). RBCOD is computed as the difference between influent and effluent CODsol.

The total unbiodegradable COD (TUCOD) was computed as the difference between COD and TBCOD. Soluble unbiodegradable COD (SUCOD) was estimated as the effluent CODsol, as in Mamais *et al.* (1993). Finally, particulate unbiodegradable COD (PUCOD) was computed as the difference between TUCOD and SUCOD.

VFA potential

For each determination, 9 to 11 300-mL BOD glass flasks were completely filled with a given wastewater. The flasks were capped with ground-glass stoppers, avoiding bubble formation, and stored in darkness at 20°C for fermentation. No stirring was applied during fermentation. The flasks were successively opened for analysis at predetermined times to evaluate the kinetics

Table 1. Wastewater parameters before the VFA-potential test.

Parameter	*Experiment No. 1*	*Experiment No. 2*
TSS, mg/L	300	375
VSS, mg/L	270	320
COD, mg/L	396	616
VFA, mg COD/L	2.6	16
DO, mg/L	<0.05	<0.05
TKN, mg N/L	—	49
NH_4-N, mg N/L	26	40
P, mg/L	7.5	9.2
PO_4-P, mg/L	5.9	6.8
pH	8.11	8.47
Alkalinity (mg $CaCO_3$/L)	271	388

of VFA formation. The maximum time considered was 168 h. This technique differs from the original method (Lie and Welander, 1997) in that several flasks are used—instead of one—and that no nitrogen gas is required.

The contents of each flask were stirred, and subsequently analyzed for soluble COD and VFA. Changes of TSS and total COD were also determined in one of the VFA-potential tests. A flask was set apart in one of the tests to monitor the ORP (Ag/AgCl). Samples for soluble COD, VFA, soluble phosphate, ammonia nitrogen, pH, and alkalinity were filtered through 0.45-μm membrane filters. For VFA determination, filtered samples were kept frozen until analyzed. Volatile fatty acids were analyzed by an external team, using solid-phase microextraction (SPME) and high-resolution gas chromatography coupled with mass spectrometry (HRGC/MS). The method has been described elsewhere (Ábalos *et al.* 2000).

RESULTS AND DISCUSSION

Tables 1 and 2 display the wastewater parameters determined before the VFA-potential test. Figure 1 represents the changes of total and soluble COD and VFA throughout experiments No. 1 and No. 2. TSS were also represented for experiment No. 2. In the first experiment, the sample presented a very low initial VFA concentration (2.6 mg VFA-COD/L), as well as a low initial RBCOD (38 mg/L). VFA concentration showed a consistent increase with time—up to 86 mg VFA-COD/L—without reaching a steady value. This means that the maximum time allowed for the test duration should be extended. Soluble COD increased consistently with time, from 92 to 230 mg/L, while particulate COD decreased from 304 to 131 mg/L. This indicates that strong anaerobic hydrolysis occurred under the prevailing conditions during the test, as pointed by Curto *et al.* (2002). Moreover, VFA concentration reached a value (86 mg VFA-COD/L) much higher than the initial RBCOD (38 mg/L). This can be interpreted as a mismatch between aerobically determined RBCOD and anaerobic readily fermentable matter (Lie and Welander, 1997). However, it is also compatible with a partial utilization of hydrolyzed COD as a substrate for fermentation.

Figure 2 shows the evolution of ORP with time in Experiment No. 1. Very low ORP potential prevailed dur-

Table 2. COD fractionation before the VFA-potential test.

	Total (mg/L)			*Biodegradable (mg/L)*		*Unbiodegradable (mg/L)*	
	COD	*TBCOD*	*TUCOD*	*RBCOD*	*SBCOD*	*SUCOD*	*PUCOD*
Experiment No. 1	396	319	77	38	281	52	25
Experiment No. 2	616	502	114	140	362	60	54

COD: total COD; TBCOD: total biodegradable COD; TUCOD: total unbiodegradable COD; RBCOD: soluble readily biodegradable COD; SBCOD: particulate slowly biodegradable COD; SUCOD: soluble unbiodegradable COD; PUCOD: particulate unbiodegradable COD.

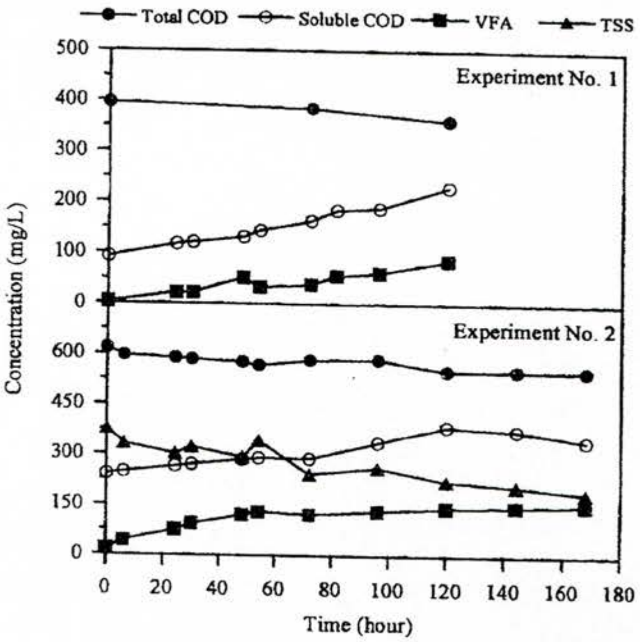

Figure 1. Evolution of COD, VFA, and TSS in both experiments.

ing test evolution. ORP decreased from +117 to −412 mV in 6 h, and remained well below this value for the rest of the test. This indicates that purging with nitrogen is not required for obtaining appropriate conditions for acidogenic fermentation. If initial DO is low—as it usually is in urban wastewater—and test flasks are completely filled, no oxygen purging is needed. The use of one flask for each sampling time allows for higher volume samples to be used. Because anaerobic conditions do not have to be kept beyond the time scheduled for each flask, flasks can be opened for sampling. Butyl rubber stoppers and syringes were not required. Conventional BOD flasks with ground-glass stoppers can be used.

A second experiment was performed, using another wastewater sample, for a total time of 168 h. TSS and soluble COD curves indicated strong hydrolysis throughout the experiment (Fig. 1). Again, the VFA-potential (150 mg VFA-COD/L) was much higher than the initial VFA concentration. VFA-potential was also greater than in Experiment No. 1. VFA concentration showed a rapid increase during the first 54 h (from 16 to 128 mg VFA-COD/L) and a slower increase for the next 114 h (from 128 to 150 mg VFA-COD/L). The slope change coincides with the VFA concentration (128 mg VFA-COD/L) approaching the RBCOD value. This slope change can be interpreted as a decrease in the availability of readily

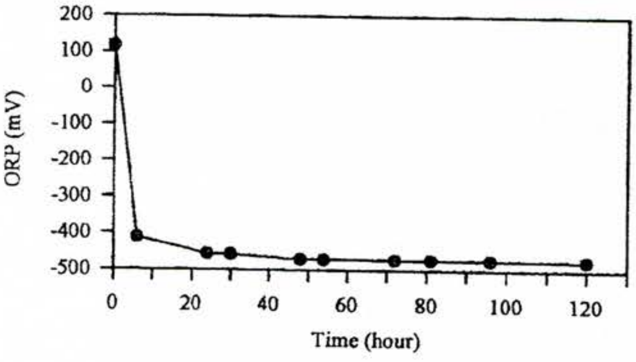

Figure 2. Evolution of ORP in Experiment No. 1.

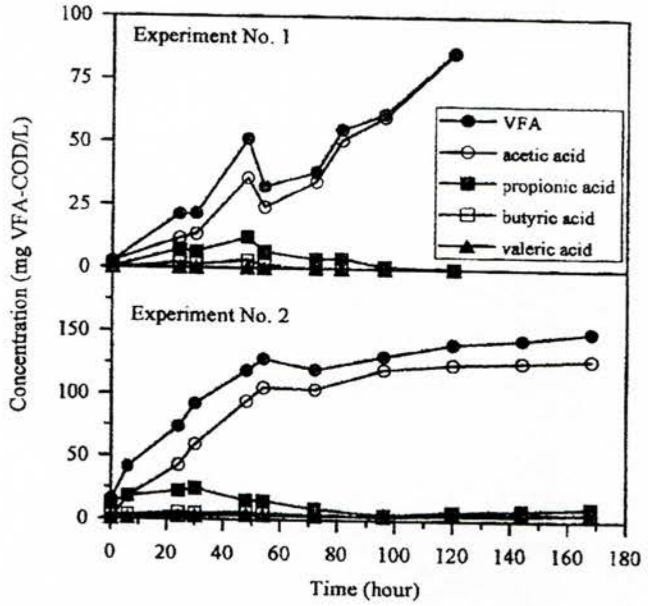

Figure 3. Specific VFA production in each experiment.

fermentable COD. The second slow stage of VFA generation can be interpreted as a slow utilization of the hydrolyzed COD.

The VFA-potential represents 22% of the initial total COD in the first experiment and 24% in the second experiment. These values are comparable to previously reported data (Lie and Welander, 1997). On the other hand, the VFA-potential to soluble phosphate ratios were 14.6 and 22 mg VFA-COD/mg soluble P, for the first and second experiment, respectively. These values are in accordance with data reported for an efficient biological phosphorus removal (20 mg/mg) (Abu-ghararah and Randall, 1991).

Finally, Fig. 3 shows the evolution of specific volatile fatty acids in both VFA-potential tests. Fermentation begins immediately and VFA concentration increases in the first stage of the experiment (approximately during the first 50 h). until reaching a value corresponding to the RBCOD of the wastewater. Afterwards, a momentary stabilization—or even a small decrease—of the VFA concentration takes place. This phenomenon can be related to exhaustion of initial RBCOD of wastewater. This can indicate that the initially present RBCOD is first depleted, rather than the COD being solubilized throughout the test. After 10 or 20 h, the VFA production—especially acetic acid—starts again, probably at the expense of solubilized COD. In this second stage, VFA production follows a different pattern in both experiments. In Experiment No. 1, the increase of VFA concentration was quick and basically due to the presence of acetic acid. In Experiment No. 2, the production of acetic acid, as well as of other VFA, was slow and reached low concentrations. In both experiments, acetic acid was the dominant fermentation product. Nevertheless, significant quantities of VFA were produced in the first stages which were later consumed.

In conclusion, the levels of VFA achieved by the modified method in both VFA-potential tests (86 and 150 mg VFA-COD/L) indicate that the wastewater analyzed had a potential of VFA production by fermentation of both the RBCOD and a fraction of the SBCOD.

Figure 4 shows the PO_4-P and NH_4-N evolution throughout both experiments. Some nitrogen and phosphorus solubilization occurred in both VFA-potential tests. Maximum solubilization for both parameters was observed in the sample with the highest TSS. Table 3 shows the solubilization of phosphate and ammonia nitrogen associated with influent COD and VFA production. The difference between final and initial PO_4-P concentration was small (1.3 mg/L in average), so its statistical significance was studied. In Fig. 4, the PO_4-P change with time is a steadily growing function, which can be fairly approximated by a line. The slope of the regression line is an indicator of the PO_4-P solubilization trend throughout the test. In Experiment No. 1, the slope of the regression line was 0.19 mg/(L·day), with a 95% confidence interval of 0.13–0.25 mg/(L·day). In Experiment No. 2, the slope was 0.21 mg/(L·day) with a 95% confidence interval of 0.17–0.25 mg/(L·day). In both cases, the 95% confidence interval was far from zero, in-

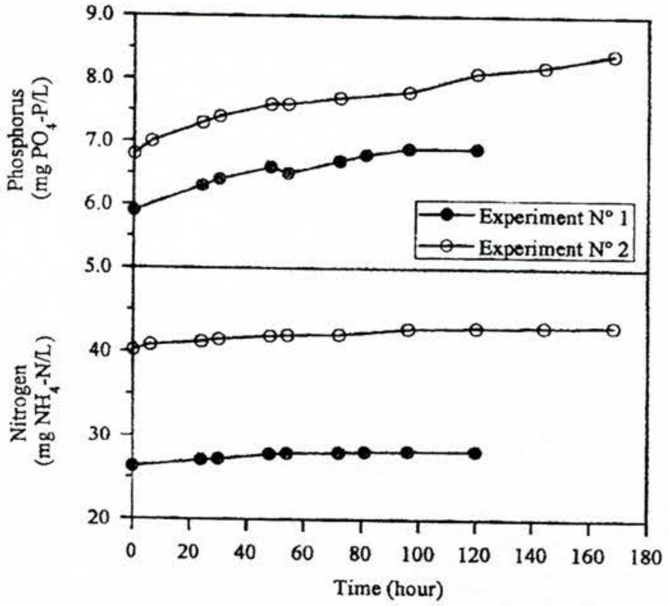

Figure 4. PO_4-P and NH_4-N evolution in each experiment.

dicating a significant PO_4-P growing trend, at the 0.05 significance level.

The mean ratio between phosphate released and COD was 2.6 mg PO_4-P/g COD. The amount of phosphate released in both experiments, in terms of the amount of VFA produced, was 12.0 mg PO_4-P/g VFA-COD on average. This solubilization can be attributed to the reduction of ferric phosphate (insoluble) to ferrous sulphate (soluble), under the reducing conditions of the test, as stated by Christensson (1997) on PO_4-P solubilization in prefermenters. Hydrolysis of polyphosphates from detergent formulations could also occur during the test.

For the N solubilization (2.6 mg/L in average), the slopes were 0.37 mg/(L·day), with a 95% confidence interval of 0.22–0.53 mg/(L·day), and 0.21 mg/(L·day) with a 95% confidence interval of 0.17–0.24 mg/(L·day). These 95% confidence intervals do not include zero, indicating a significant N solubilization trend at the 0.05 significance level. The ratios of ammonia nitrogen to initial COD were similar in both experiments. Regarding the VFA-potential, the mean ratio was 23.5 mg NH_4-N/g VFA-COD. As observed, nitrogen solubilization was higher than phosphorus solubilization in both experiments. This has been reported in other investigations associated with primary sludge fermentation, and is attributed to particulate nitrogenous materials (nitrogenated COD) that are easily biodegraded during the acidogenic phase of anaerobic fermentation (Eastman and Ferguson, 1981).

The changes of pH for both VFA-potential tests is shown in Fig. 5. In these tests, pH decreased in the initial stages, associated with a greater VFA production. Several authors have reported the influence of VFA production on pH (Eastman and Ferguson, 1981, Christens-

Table 3. Solubilization of phosphate and ammonia nitrogen observed in each experiment.

Parameter	*Experiment No. 1*	*Experiment No. 2*
Increase in phosphate (mg P/L)	1.0	1.6
Phosphate/initial COD (mg P/g COD)	2.5	2.6
Phosphate/VFA produced (mg P/g VFA-COD)	12	12
Increase in ammonium (mg NH_4-N/L)	2.0	3.1
NH_4-N/initial COD (mg NH_4-N/g COD)	5.1	5.0
NH_4-N/VFA produced (mg NH_4-N/g VFA-COD)	24	23

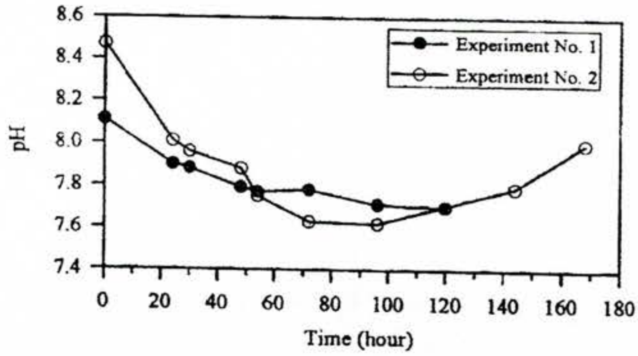

Figure 5. Evolution of pH in each experiment.

son, 1997, Lie and Welander, 1997). These authors associate the decrease of pH with a production of VFA. The behavior of pH in the second stage of the tests was different. In Experiment No. 2, which presented a very low production of VFA during the second stage, pH recovered considerably. This can be attributed to an increase of alkalinity, without a parallel production of VFA. In Experiment No. 1, which showed an appreciable production of VFA during the second stage, pH did not recovered, and continued decreasing during this stage.

Despite the variations in the reported pH values, the wastewater tested showed a remarkable buffering capacity, because pH remained within the range 7.5–8.6. This can be attributed to the considerable alkalinity of the wastewater and its parallel production. This is in accordance with the Martin *et al.* (2002) study, where wastewater showed a self-regulating capacity, and pH regulation at 6.5 or 7.5 did not affected the test.

Alkalinity consistently increased throughout both experiments. The increase was higher during the second experiment (31%), while the first experiment only showed an 18% increase. The evolution of alkalinity in potential tests can be associated with mechanisms like ammonification of organic nitrogen, which causes an alkalinity production at a ratio of 3.57 mg $CaCO_3$/mg N (Araújo *et al.*, 1998). Another mechanism for alkalinity increase can be the production of bicarbonate from evolved CO_2 reacting with calcium carbonate of the sample (Wetzel, 1983).

CONCLUSIONS

The original method (Lie and Welander, 1997) for measuring VFA-potential has been modified and simplified, eliminating the need for nitrogen gas. The modified method allows for strong anaerobic conditions to take place while simplifies the laboratory equipment required. Because anaerobic conditions do not have to be kept beyond the time scheduled for sampling, flasks can be opened for sampling. Greater volumes can easily be used for analysis, and no syringes have to be used for sampling through a rubber stopper. The modified method has provided an estimation of the VFA availability for EBPR. The ratios obtained, using the modified method, between VFA-potential and the concentrations of COD and soluble phosphate were 0.22–0.24 mg VFA-COD/mg COD, and 14.6–22 mg VFA-COD/mg PO_4-P, respectively. These values are comparable to those obtained in other wastewaters by the original method. A strong anaerobic hydrolysis was detected throughout the VFA-potential test, as a function of TSS and soluble COD. The VFA-potential was higher than the initial RBCOD, which is compatible with a partial utilization of hydrolyzed COD as a substrate for fermentation. Analysis of TSS and COD fractionation in the VFA-potential test can help in evaluating the contribution of both RBCOD and SBCOD to VFA formation. In both VFA-potential tests, the solubilization of phosphorus and nitrogen was observed. Both parameters increased gradually with time. Likewise, pH decreased in the stages with a strong liberation of VFA, while it remained constant or even increased when the liberation of VFA decreased or stopped. Alkalinity increased throughout the two experiments. More experimentation is required to achieve definitive conclusions.

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