

APÉNDICE C

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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 recommended value for biological phosphorus removal (20 mg/mg). A strong hydrolysis of solids was detected throughout the evolution 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 of 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 for-

mation 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 ($\text{PO}_4\text{-P}$), ammonia nitrogen, pH, and alkalinity. Standard methods were applied (APHA, 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 (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 observe the kinetics

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

<i>Parameter</i>	<i>Experiment No. 1</i>	<i>Experiment No. 2</i>
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. Evolution 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- μ m 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 repre-

sents 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 reduced from 304 to 131 mg/L. This fact 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 fact 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.

	<i>Total (mg/L)</i>			<i>Biodegradable (mg/L)</i>		<i>Unbiodegradable (mg/L)</i>	
	<i>COD</i>	<i>TBCOD</i>	<i>TUCOD</i>	<i>RBCOD</i>	<i>SBCOD</i>	<i>SUCOD</i>	<i>PUCOD</i>
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.

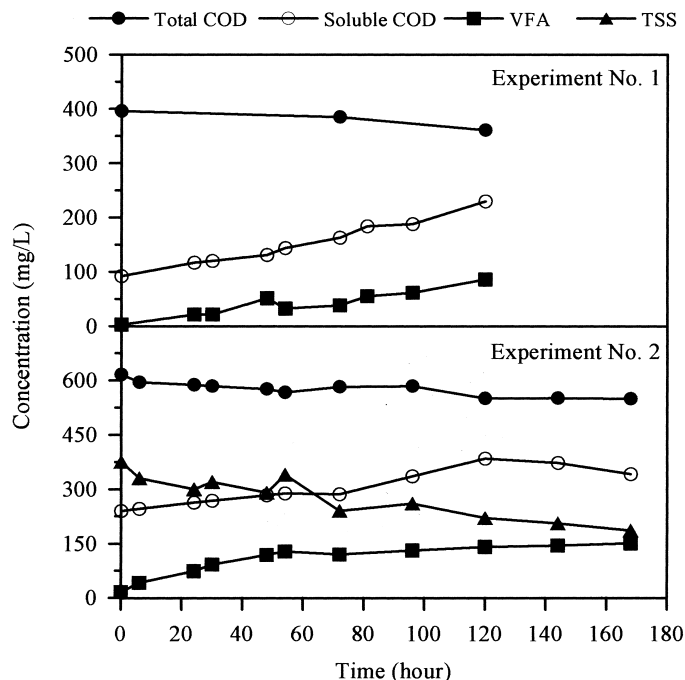


Figure 1. Evolution of COD, VFA, and TSS in both experiments.

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 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

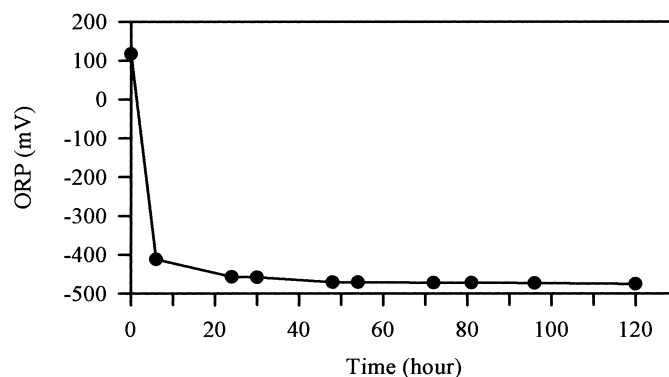


Figure 2. Evolution of ORP in Experiment No. 1.

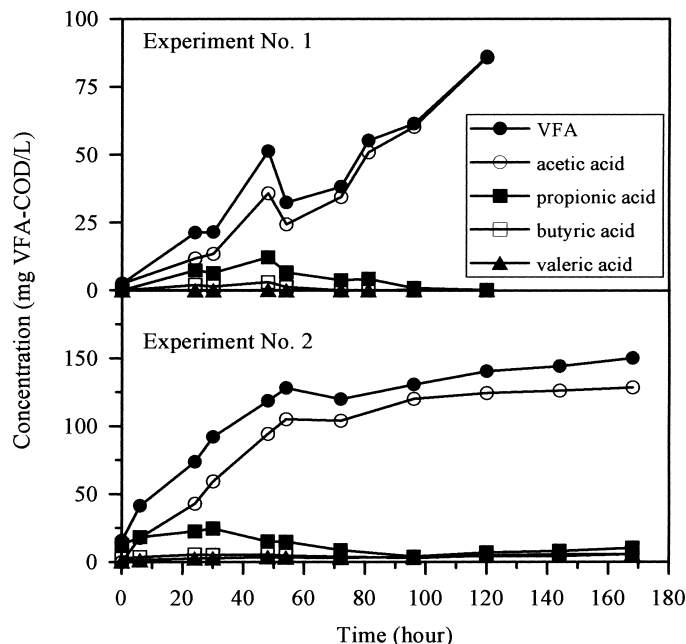


Figure 3. Specific VFA production in each experiment.

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-gharah 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 $\text{PO}_4\text{-P}$ and $\text{NH}_4\text{-N}$ 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 $\text{PO}_4\text{-P}$ concentration was small (1.3 mg/L in average), so its statistical significance was studied. In Fig. 4, the $\text{PO}_4\text{-P}$ change with time is a steadily growing function, which can be fairly approximated by a line. The slope of regression line is an indicator of the $\text{PO}_4\text{-P}$ solubilization trend throughout the test. In Experiment No. 1, the slope of the regression line was 0.19 mg/(L·d), 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-

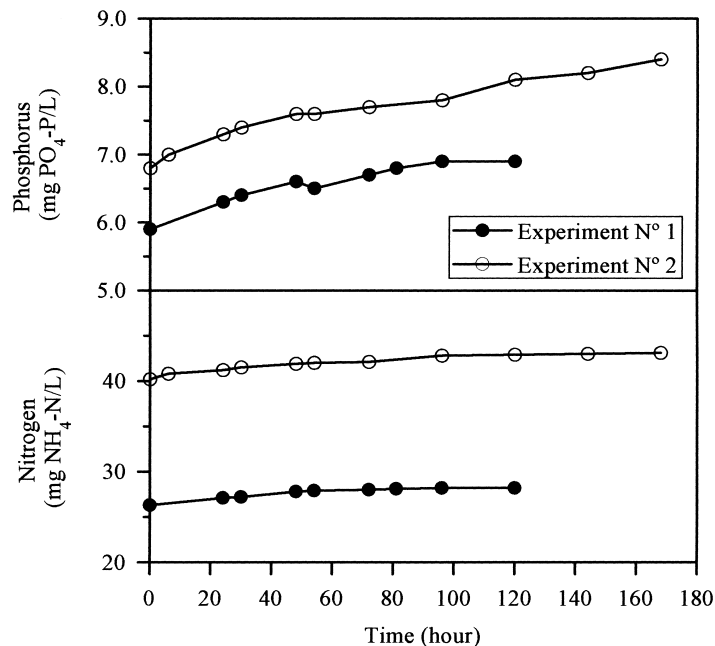


Figure 4. PO₄-P and NH₄-N evolution in each experiment.

dicating a significant PO₄-P growing trend, at the 0.05 significance level.

The mean ratio between phosphate released and COD was 2.6 mg PO₄-P/g COD. The amount of phosphate released in both experiments, in terms of the amount of VFA produced, was 12.0 mg PO₄-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 PO₄-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 embrace zero, in-

dicating 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 NH₄-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 change 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

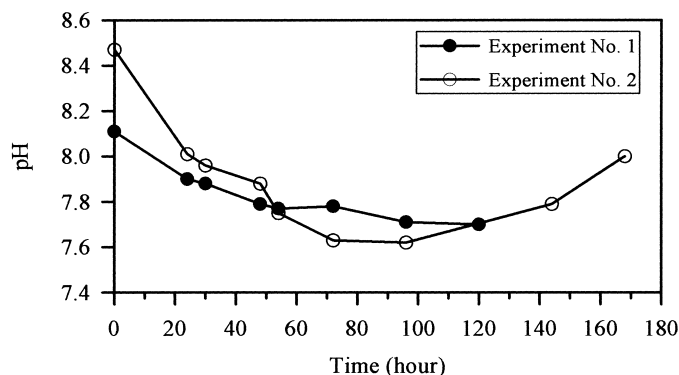


Figure 5. Evolution of pH in each experiment.

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 recover, 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 CaCO₃/mg N (Araújo *et al.*, 1998). Another mechanism for alkalinity increase can be the production of bicarbonate from evolved CO₂ 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 taken for analysis, because 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 PO₄-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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REFERENCES

- ABALOS, M., BAYONA, J.M., and PAWLISZYN, J. (2000). Development of a headspace solid-phase microextraction procedure for the determination of free volatile fatty acids in waste waters. *J. Chromatogr. A* **873**, 107–115.
- ABU-GHARARAH, Z.H., and RANDELL, C.W. (1991). The effect of organic compounds on biological phosphorus removal. *Water Sci. Technol.* **23**, 585–594.
- APHA-AWWA-WPCF (1995). *Standard Methods for the Examination of Water and Wastewater*, 19th ed. Washington, DC: American Public Health Association.
- ARAÚJO, L.S., CATUNDA, P.F.C., and VAN HAANDEL, A.C. (1998). Biological sludge stabilisation Part 2: Influence of the composition of waste activated sludge on anaerobic stabilisation. *Water SA* **24**, 231–236.
- CHRISTENSSON, M. (1997). Enhanced biological phosphorus removal. Carbon source, nitrate as electron acceptor, and characterisation of the sludge community. Ph.D. Thesis, Department of Biotechnology, Lund University, Sweden.
- CURTO, P.R., PEARCE, P., and PARSON, S.A. (2002). Determining the potential for enhanced phosphorus removal based on wastewater characteristics. 75th annual conference WEFTEC'02, Municipal Wastewater Treatment Process Symposium: Biological phosphorus removal, October 2, Chicago.
- DOLD, P.L., EKAMA, G.A. and MARAIS, G.v.R. (1980). A general model for the activated sludge process. *Prog. Water Technol.* **12**, 47–77.
- EASTMAN, J.A., and FERGUSON, J.F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. Water Pollut. Control Fed.* **53**, 352–366.
- HENZE, M., GUJER, W., MINO, T., MATSUO, T., WENTZEL, M.C., and MARAIS, G.v.R. (1995). *Activated Sludge Model No. 2*. Scientific and Technical Report No. 3. London: IAWQ.
- LIE, E., and WELANDER, T. (1997). A method for determination of the readily fermentable organic fraction in municipal wastewater. *Water Res.* **31**, 1269–1274.
- MAMAIS, D., JENKINS, D., and PITT, P. (1993). A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Res.* **27**, 195–197.
- MARTIN RUEL, S., COMEAU, Y., HÉDUI, A., DERONZIER, G., GINESTET, P., and AUDIC, J.M. (2002). Operating conditions for the determination of the biochemical acidogenic potential of wastewater. *Water Res.* **36**, 2337–2341.
- PARK, J.K., WANG, J. and NOVOTNY, G. (1997). Wastewater characterisation for evaluation of biological phosphorus removal. WDNr Research Report 174, Wisconsin Dept. of Natural Resources, Wisconsin–Madison, (<http://www.dnr.state.wi.us/org/water/wm/ww/biophos/abstract.htm>).
- WETZEL, R.G. (1983). *Limnology*, 2nd ed. Philadelphia: Saunders College Publishing.

M. G. Barajas (*) J. Knobelsdorf (*) A. Escalas (**) R. Mujeriego (*)	HYDROLYSIS AND FERMENTATION IN A MODIFIED VFA-POTENTIAL TEST
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ABSTRACT

The VFA-potential test is a fermentation technique for estimating the total VFA availability in wastewater. This test is applicable for evaluating the maximum anaerobic VFA-uptake in enhanced biological phosphorus removal. The VFA-potential was conceived as a measurement of the readily-fermentable COD, since fermentation utilises readily-fermentable COD as substrate. However, anaerobic hydrolysis can be a potential source of fermentable matter. In this work, the VFA-potential test has been modified and applied to municipal wastewater samples. The VFA-potential test has been simplified in order to avoid nitrogen gas usage. COD fractionation of initial wastewater, and analysis of total suspended solids, total and soluble COD, and individual VFA have been applied throughout the VFA-test, in order to evaluate hydrolysis and fermentation. A strong hydrolysis of solids has been detected through the evolution of total suspended solids and soluble COD. The behaviour of total suspended solids, soluble COD, and VFA is compatible with a partial utilisation of hydrolysed COD, though more experimentation is required for definitive conclusions.

KEYWORDS

COD fractionation; enhanced biological phosphorus removal; fermentation; readily biodegradable; VFA potential; Volatile fatty acids.

INTRODUCTION

Volatile fatty acid (VFA) uptake during anaerobic stages has been characterised as a key step in enhanced biological phosphorus removal (EBPR). Moreover, the availability of VFA to phosphorus-accumulating organisms (PAO) has proved to be a determining factor in anaerobic phosphorus release. In a full-scale plant VFAs are expected to be supplied by influent wastewater, since external VFA addition is usually avoided. Low-strength wastewaters are usually unable to supply enough VFA for anaerobic phosphorus release. Some parameters –or their ratios to phosphorus– have been used to characterise the suitability of a wastewater for EBPR, namely: chemical oxygen demand (COD), biochemical oxygen demand (BOD), readily biodegradable COD (RBCOD), VFA concentration, and VFA potential.

Fermentable matter can be converted to VFA in anaerobic stages. According to Activated Sludge Model No. 2 (Henze *et al.*, 1991), anaerobic hydrolysis of particulate, slowly-biodegradable COD can produce fermentable matter, that can be further fermented to VFAs. The contribution of this reaction pathway to VFA formation is still under discussion (Ekama and Wentzel, 1999).

Lie and Welander (1997) developed the VFA-potential 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 (SBCOD) was not considered to be a source of

readily fermentable matter in the VFA-potential test. In this method, a wastewater sample is stored in a sealed bottle for spontaneous fermentation by the microorganisms present in the wastewater.

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. Then the samples are filtered through 0.45 µm filters, and kept frozen until VFA analysis.

In this work, the original VFA potential method (Lie and Welander, 1997) has been modified in order to avoid the need for nitrogen usage. Stirring has been applied to the samples after fermentation, in order to ensure a homogeneous VFA concentration. COD fractionation has been applied to wastewater before fermentation, in order to compare VFA formation to initial COD fractions. The modified method has been applied to a urban wastewater fed to two bench-scale EBPR plants. Evolution of VFA, total and soluble COD, total suspended solids (TSS), and oxidation-reduction potential (ORP) has been studied.

MATERIALS AND METHODS

Wastewater sampling and previous analysis

Daytime composite samples were taken from a nearby street sewer in the residential area around the university laboratory. Samples were analysed for TSS, volatile suspended solids (VSS), COD, VFA, dissolved oxygen (DO), pH, alkalinity, COD fractionation, and VFA potential. Wastewater COD fractionation was determined according to the method by Park *et al.* (1997). In this method, readily-biodegradable COD is determined according to Mamais *et al.* (1993).

VFA potential

For each determination, nine to eleven 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, in order to observe the kinetics of VFA formation. The maximum time considered was 168 hours. 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 analysed for soluble COD, and VFA. TSS, and total COD were also determined in one of the VFA-potential tests. A flask was set apart in one of the tests in order to monitor the ORP. Samples for soluble COD, VFA, pH, and alkalinity were filtered through 0.45 µm membrane filters. Filtered samples for VFA analysis were kept frozen until analysed. Volatile fatty acids were analysed 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 (Abalos *et al.*).

RESULTS AND DISCUSSION

Table 1 and Table 2 display the wastewater parameters determined before the VFA-potential test. The samples show different compositions. Figure 1 shows the evolution of total and soluble COD, and VFA throughout the test No. 1. This sample had a very low initial VFA concentration (2.6 mg COD/l) as well as a low initial RBCOD (38 mg/l). VFA concentration shows a consistent increase with time –up to 86 mg COD/l– without reaching a steady value. This means the maximum time allowed for the test should be extended. Soluble COD grew consistently with time, from 92 to 230 mg/l, while insoluble COD fell from 304 to 115 mg/l. This is an indication that strong anaerobic hydrolysis occur under the test conditions. Moreover, VFA concentration reached a value (86 mg/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, as pointed by Lie and Welander (1997). However, it is also compatible with a partial utilisation of hydrolysed COD as a substrate for fermentation.

Table 1. Wastewater parameters before the VFA test

	TSS [mg/l]	VSS [mg/l]	COD [mg/l]	VFA [mg COD/l]	DO [mg/l]	pH	Alkalinity [mg CaCO ₃ /l]
Sample no. 1	300	270	396	2.6	< 0.05	8.11	271
Sample no. 2	375	320	616	16	< 0.05	8.47	388

Table 2. COD fractionation before the VFA test

	Total biodegradable and unbiodegradable			Biodegradable		Unbiodegradable	
	TCOD [mg/l]	TBCOD [mg/l]	TUCOD [mg/l]	RBCOD [mg/l]	SBCOD [mg/l]	USCOD [mg/l]	UPCOD [mg/l]
Sample no. 1	396	319	77	38	281	52	25
Sample no. 2	616	502	114	140	362	60	54

TCOD: total COD

TBCOD: total biodegradable COD

TUCOD: total unbiodegradable COD

RBCOD: soluble readily biodegradable COD

SBCOD: particulate slowly biodegradable COD

USCOD: unbiodegradable soluble COD

UPCOD: unbiodegradable particulate COD

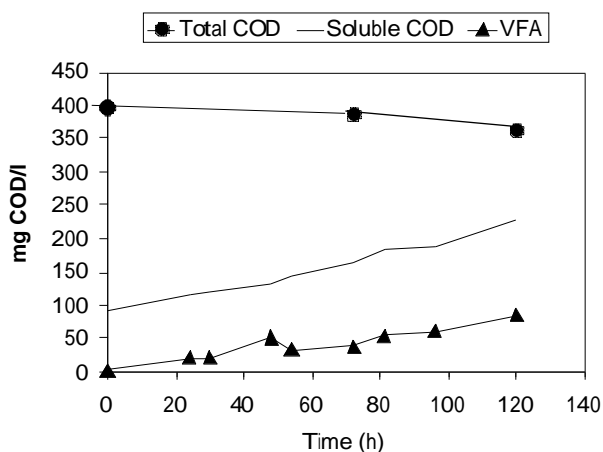


Figure 1. COD and VFA in test No. 1.

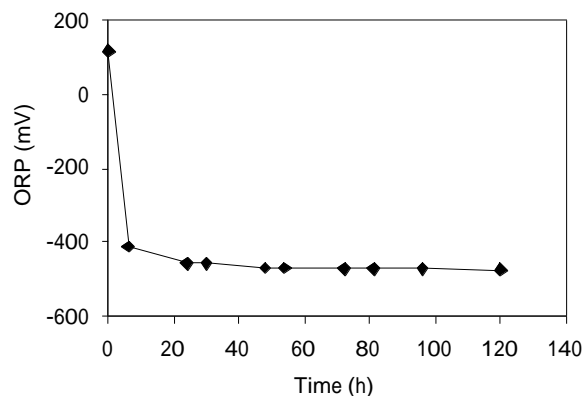


Figure 2. ORP in test No. 1.

Figure 2 shows the evolution of ORP with time. Very low ORP potential prevailed during most of the test. ORP fell from +117 to -412 mV in six hours, and remained well below this value for the rest of the test. This confirms that purging with nitrogen is not required if initial DO is low and no headspace is allowed in the flask. A second test was run, using another wastewater sample (Figure 3), for a total time of 168 h. TSS were determined throughout the test. Figure 3 shows the evolution of total and soluble COD, TSS, and VFA for this sample. The VFA potential (150 mg/l) was, again, much higher than the initial VFA concentration. VFA potential was also greater than in test No. 1. TSS and soluble COD curves indicate strong hydrolysis throughout the test. VFA concentration shows a rapid growth during the first 54 hours (from 16 to 128 mg COD/l) and a slower growth for the next 114 hours (from 128 to 150 mg COD/l). The slope change coincides with the VFA concentration (128 mg/L) approaching the RBCOD (140 mg/l). This change in the slope can be interpreted as a decrease in the availability of readily fermentable COD. The second, slow stage of VFA generation can be interpreted as a slow utilisation of the hydrolysed COD. Figure 4 shows the evolution of specific volatile fatty

acids during test No. 2. The slopes of the VFA curves at the end of the test –especially the increase of the intermediate propionic acid after 100 hours– indicate that fermentation has not been completed at the end of the test. At that point, VFA concentration is greater than initial RBCOD, which would indicate a utilisation of soluble COD from anaerobic hydrolysis.

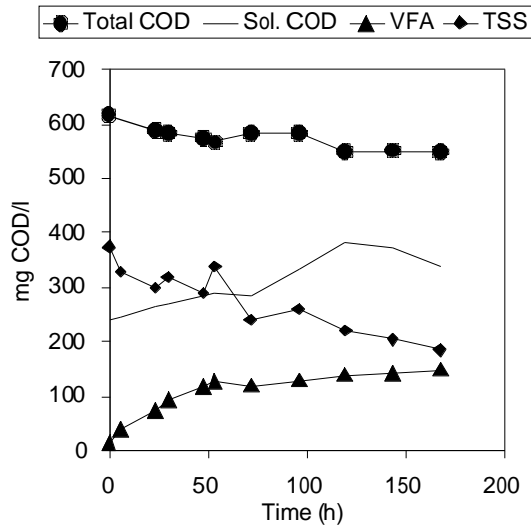


Figure 3. COD, VFA and TSS in test No. 2.

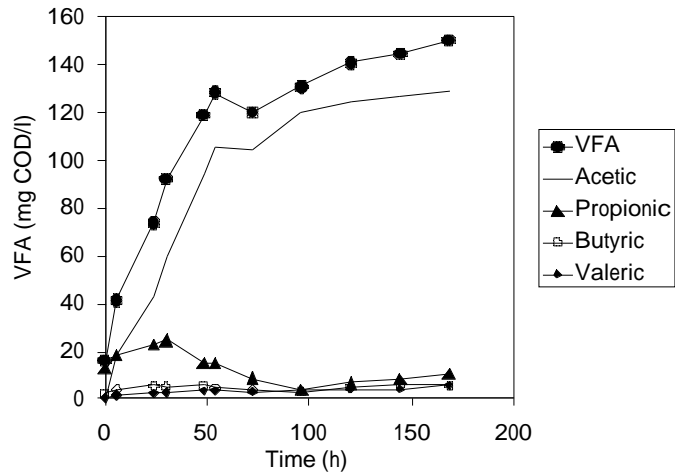


Figure 4. Specific VFA in test No. 2.

CONCLUSIONS

The Lie and Welander (1997) method for VFA-potential has been simplified, eliminating the need for nitrogen gas. The modified test has provided an estimation of the VFA availability for EBPR. A strong anaerobic hydrolysis has been detected throughout the VFA-potential test, through the evolution of TSS and soluble COD. The VFA potential has been greater than initial RBCOD, which is compatible with a partial utilisation of hydrolysed 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. More experimentation is required to achieve definitive conclusions.

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REFERENCES

- Abalos, M., Pawliszyn, J., and J.M. Bayona. Development of a Headspace Solid-Phase Microextraction Procedure for the Determination of Free Volatile Fatty Acids in Wastewaters. *Journal of Chromatography (A)*, in press.
- Ekama, G.A. and Mark C. Wentzel (1999). Difficulties and developments in biological nutrient removal technology and modelling. *Wat. Sci. Tech.* **39**(6), 1-11.
- Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C., and Marais, G. v. R. (1991). *Activated Sludge Model No. 2*, IAWQ Scientific and Technical Report No. 3, IAWQ, London. 32 pp.
- Mamais, D., Jenkins, D., and P. Pitt (1993). A rapid physical-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Wat. Res.*, **27**(1) 195-197.
- Lie, E. And Welander, T. (1997). A Method for Determination of the Readily Fermentable Organic Fraction in Municipal Wastewater. *Wat. Res.*, **31**(6), 1269-1274.
- Park, J.K., Wang, J., and Novotny, G. (1997). *Wastewater characterization for evaluation of biological phosphorus removal*. Wisconsin Department of Natural Resources. Research Report 174. 29 pp.

Hydrolysis and Fermentation in a Modified VFA-Potential Test

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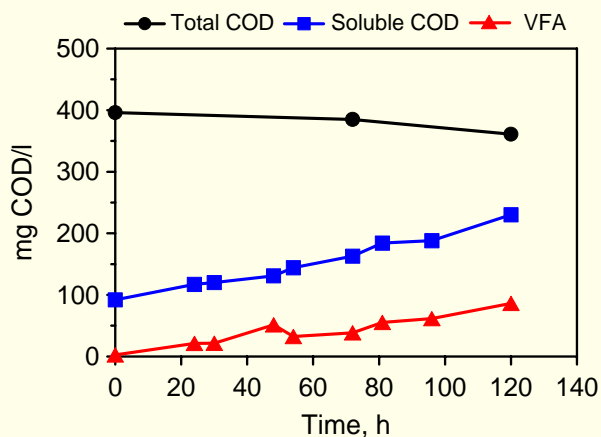
Lie and Welander (1997) developed the VFA-potential method, a fermentation technique for estimating the total maximum amount of volatile fatty acids (VFA) that can be available for the bio-P bacteria in the anaerobic stage of an enhanced biological phosphorus removal process. In this work, the original method has been modified in order to avoid nitrogen usage, and applied to urban wastewater. TSS and soluble COD evolution has indicated a strong hydrolysis throughout the test. VFA potential has been greater than initial readily biodegradable COD (RBCOD). This can be interpreted as a mismatch between aerobically determined RBCOD and anaerobic readily-fermentable matter, as pointed by Lie and Welander (1997). However, it is also compatible with a partial utilisation of hydrolysed COD as a substrate for fermentation.

Wastewater parameters

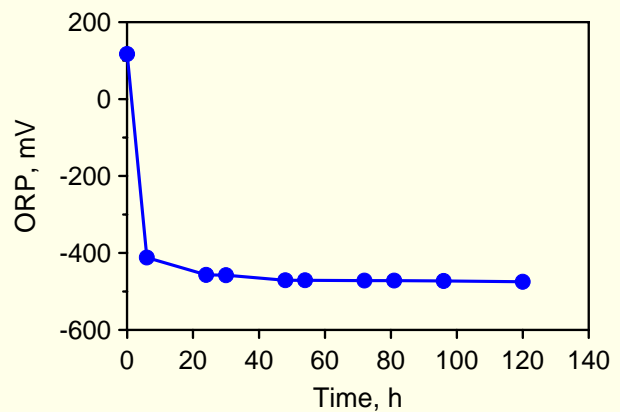
Determined before the VFA-potential test. The samples show different compositions.

	TSS [mg/l]	TCOD [mg/l]	VFA [mgCOD/l]	RBCOD [mg/l]	SBCOD [mg/l]
Sample N° 1	300	396	2.6	38	281
Sample N° 2	375	616	16	140	362

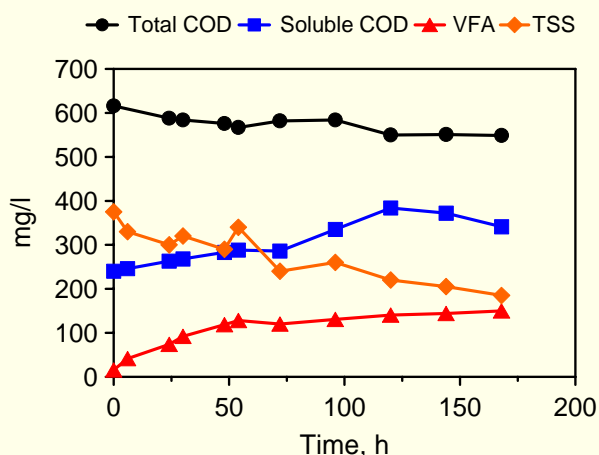
SBCOD: particulate slowly biodegradable COD



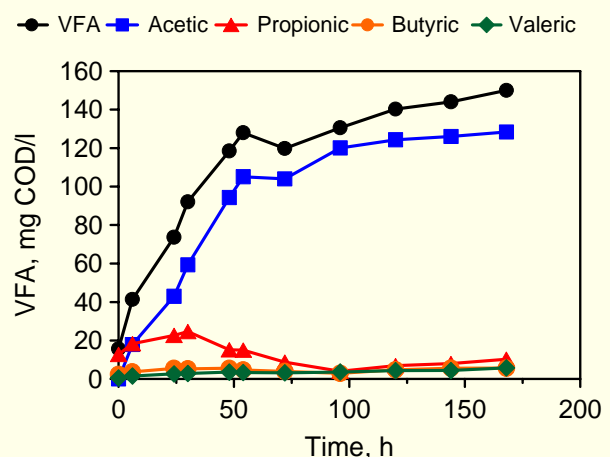
Total and soluble COD, and VFA throughout test N° 1. VFA concentration consistently increased without reaching a steady value.



Oxidation-reduction potential (ORP). Very low ORP prevailed. Purging with nitrogen is not required.



Total and soluble COD, TSS and VFA throughout test N° 2. TSS and soluble COD indicate strong hydrolysis.



Evolution of specific volatile fatty acids during test N° 2.