Biochemical acidogenic potential in domestic wastewaters: effect of sampling and storage to characterize daily average composite samples

J. -M. Choubert, D. Granger, C. Bourdon and A. Héduit

ABSTRACT

To estimate the expectable enhanced biological phosphorus removal value of a wastewater, the concentration of volatile fatty acids (VFAs) and the biochemical acidogenic potential (BAP) are generally determined on grab samples of wastewater, as these variables are prone to rapid change after sampling. However, such sampling technique do not take into account the variations of these parameters during the day. This work has evaluated the changes of VFAs and BAP occurring during sampling and storage in an automatic sampler over 24 h. The consequences of waterfall oxygen input during sampling, and changes during storage (fermentation and sulfate-reducing process) were studied. The results for two wastewaters showed that the sampling technique used for daily flow proportional composite samples provided a correct estimation of VFAs, and underestimated BAP by up to 25%. For hourly-average composite samples of wastewaters, significant modifications of the concentrations of these two parameters were recorded around the daily average values.

Key words | Biochemical Acidogenic Potential (BAP), COD fractionation; municipal wastewater, volatile fatty acids (VFAs), wastewater storage

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INTRODUCTION

The key parameter to maximize the storage of phosphates by enhanced biological phosphorus removal (EBPR) is the amount of available volatile fatty acids (VFAs) accessible in the anaerobic tank (Marais *et al*. 1983). VFAs can either be present in the wastewater as it enters the treatment plant, or be produced from fermentable organic matter (Nicholls *et al*. 1985). The total VFAs that can be produced by a wastewater is called the biochemical acidogenic potential (BAP, Shelton & Tiedje 1984) or the VFA potential (Jönsson *et al*. 1996). Besides the VFAs initially present, the BAP is composed of two fermentable fractions, S_F, with a high hydrolysis rate and X_F, with a low hydrolysis rate (Henze *et al*. 1999). Their fermentation into VFAs is measured with a 15-day laboratory test consisting of successive sampling in the same flask under anaerobic conditions with inhibitors of methanogens and sulfate-reducing bacteria (Martin-Ruel *et al*. 2002b). Modified methods for the test have recently been suggested: these include the use of separate flasks opened successively to keep the oxidation reduction potential (ORP) at low levels (Barajas *et al*. 2003), and the addition of a sealing biomass (Colmenarejo *et al*. 2004; de Lucas *et al*. 2007).

To limit changes when sampling and storing with an automatic refrigerated sampling machine, despite the low temperature, the BAP test is mainly carried out on grab samples of wastewater (Martin-Ruel *et al*. 2002b). For flow proportional composite samples, a freezing stage is recommended (Lie & Welander 1997). However, this method requires specific equipment that rules out systematic application by practitioners. Thus little information is available on the VFAs and BAP in 24 h flow proportional composite samples of raw wastewater. Also, the BAP:COD ratio measured on grab samples is often applied to the COD of the 24 h composite sample. However, if the ratio changes over time, taking an overestimated BAP result can potentially lead to poor plant design, as it is used to predict the phosphate concentration in treated effluents (Lie *et al*. 1997;

Wentzel *et al.* 1999; Mulkerrins *et al.* 2004) or to assess the suitability of a wastewater for EBPR (Abu-ghararah & Randall 1991; Martin-Ruel *et al.* 2005).

This study was conducted to determine the changes of the concentrations in VFAs and BAP, during the sampling and storage of wastewater occurring in a typical automatic refrigerated sampler (flow proportional composite sample at full-scale plant). In addition, the error made in using a 24 h flow proportional composite sample of wastewater (due to VFA and BAP change), was determined for two types of wastewater. The range of VFA content and BAP values measured in hourly average composite samples were then compared with the values of the daily average composite sample.

MATERIALS AND METHODS

Measurement techniques

BAP tests were applied to wastewaters kept at 20°C in the dark for 15 days (Martin-Ruel *et al.* 2002b). 250 mL sealed serum bottles equipped with magnetic stirrers (300 rpm) were used. Chemicals were added for the inhibition of methanogenesis (bromoethane sulfonate (BES), 2 mM) and sulfate-reducing processes (molybdate and barium ($BaCl_2$), 1 mM) as proposed by Martin-Ruel *et al.* (2002b) with little impact on the VFA production.

Successive sampling in the flasks was carried out to determine VFA production. The maximum concentration of VFAs produced (at t_F) was used to determine the BAP (see example in Figure 1).

The VFAs (acetic, propionic and butyric acids) were analyzed with a ThermoQuest gas chromatograph (Trace 2000) equipped with a polyethylene glycol polymer column (DB-FFAP, length 30 m, diameter 0.25 mm) and a flame ionization detector. Isobutyric acid was used as an internal
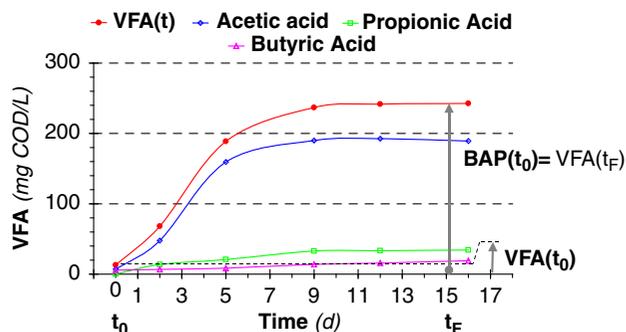
standard for calibration. The analyses were conducted on 2 mL vials filled with the sample, after pretreatment consisting of filtration (through 0.45 μm membrane filters), acidification with orthophosphoric acid (pH < 2), and storage at 4°C. The conversion factors presented in Table 1 were used to transform the VFA concentration measured by gas chromatography into a COD equivalent (Barajas *et al.* 2002; Martin-Ruel *et al.* 2002b).

Study of the impact of sampling, cooling and storing

Two series of laboratory experiments were carried out on grab samples of wastewater to quantify the changes in VFAs and BAP during the sampling, cooling and storing periods to mimic those occurring in an automatic sampling machine.

Inhibitors during a 2 h cooling period (Series 1)

The effects of adding inhibitors (BES and $BaCl_2$) were studied over a period of cooling that represented the first 2 h of storage in an automatic sampling machine.

Eleven 1-litre bottles placed in a thermoregulated bath were filled with fresh wastewater from a grab sample. Bromoethane sulfonate (1 mM) and $BaCl_2$ (0.2 mM) were added to five samples. No addition was made to the other five samples. pH, ORP and temperature was monitored in the reference bottle. The temperature was progressively lowered from 16°C to 4°C in 2 h, like in an automatic sampler. The VFAs and BAP were measured every 0.5 h, up to 2 h of exposure time.

Oxygen input during sampling, and changes during storage (Series 2)

As a waterfall of approximately 30 cm usually occurs between the sample discharge pipe and the bottom of the

Figure 1 | Example of VFAs produced during a BAP test.

Table 1 | Conversion factor for VFA COD equivalents

VFA type	Molar weight (g/mol)	Oxygen for aerobic degradation (g O_2/mol VFA)	Conversion rate (g COD/g VFA)
Acetic acid (CH_3COOH)	60	64	1.067
Propionic acid (C_2H_5COOH)	74	112	1.513
Butyric acid (C_3H_7COOH)	87	160	1.839

bottles of the sampling machine, it was decided to evaluate the influence of this effect at laboratory scale. No inhibitors (BES, $BaCl_2$) were added as the temperature was lowered to 4°C within 2 h of storage, as this would inhibit methanogenesis and sulfate-reducing processes (Hansson & Molin 1981). To verify this assumption, the changes in VFAs and BAP were measured.

Seventeen 1-litre bottles placed in a thermoregulated bath were filled with fresh wastewater (grab sample):

- 8 bottles were filled with a 30 cm wastewater fall similar to that observed in an automatic sampler (see Figure 2);
- 8 bottles were filled manually with a limited oxygen input through a pipe carrying the influent to the bottom of the container;
- 1 bottle to monitor pH, oxidation reduction potential (ORP) and temperature.

The temperature of the bottles was progressively lowered from 16°C to 4°C in 2 h, and maintained at 4°C for 24 h. VFAs and BAP were measured after 0, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and 28 h exposure.

Evaluation of the daily average composite sample technique

To quantify the error introduced by the sampling technique usually used by practitioners for flow proportional composite sampling when monitoring a WWTP (using an automatic sampler discharging into a single container), two automatic cooling samplers (ISCO) were run in parallel for a period of 24 h. Sampler 1 (S1) was equipped with a single container (diameter 30 cm, maximum height 50 cm) with a feed pipe carrying the wastewater to the bottom of the container to limit the oxygen input into the sampled wastewater.
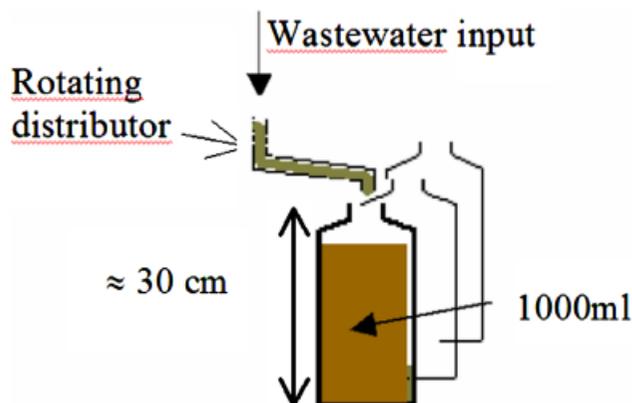
Sampler 2 (S2) used 24 separate 1-litre bottles with a rotating distributor (discharge pipe) delivering wastewater with a 30 cm waterfall. 90 mL of liquid was pumped every 6 min., and the bottle change occurred every hour. The liquid in the bottles was mixed to mimic a situation of almost constant inflow rate.

After the sampling period of 24 h had elapsed, VFAs and BAP were determined on the samples. One sample for S1 and hourly samples for S2 (separate 1-litre bottles). The measured values [$VFA_{obs}(N)$ and $BAP_{obs}(N)$] of each bottle of S2 were used to evaluate the theoretical actual values [$VFA_{act}(N)$ and $BAP_{act}(N)$] on the hourly average influent of sampler 2. The theoretical actual values for the daily average composite sample were calculated by the method presented in the appendix, taking into account the changes during sampling, cooling and storing. The observed and corrected S2 values were then compared with the observed S1 value.

The wastewaters (WWs) of two different municipal treatment plants (WWTPs) were studied (Table 2). The facilities were selected for their different influent characteristics. 24 h composite and hourly composite samples of raw wastewater were taken during dry weather conditions.

RESULTS AND DISCUSSION

Impact of inhibitors during the cooling period (Series 1)

The results for VFAs and BAP obtained with and without addition of chemicals (inhibitors $BES + BaCl_2$), to a grab sample of wastewater, are presented in Figure 3 during cooling from 16°C to 4°C, over a period higher than 2 h (five samples).

During the 2 h cooling period, with and without addition of inhibitors, the values were almost constant: VFAs were

Figure 2 | Diagram of 1 L bottles (with 30-cm water fall) in an automatic sampler.

Table 2 | Characteristics of the two raw influents studied

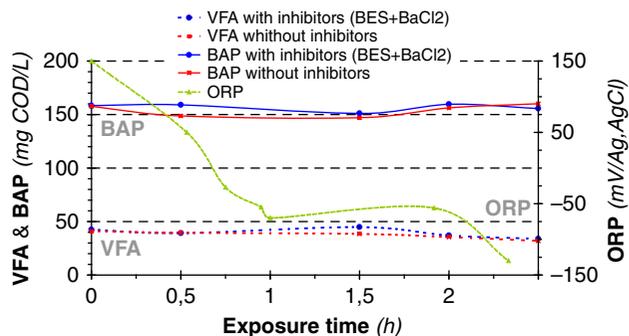| Facility (design treatment capacity)	Sewer and effluent types	COD range (mg/L)
WW1 (8 000 P.E.)	Separate, Domestic	400–700
WW2 (130 000 P.E.)	Combined, Domestic with <30% industrial discharge [beverage industry release 6 h/d]	400–1400

P.E.: population equivalent

Figure 3 | VFAs, BAP and ORP when cooling from 16 to 4°C for a 2 h exposure time, with and without inhibitors of methanogenesis and sulphate-reducing processes (Series 1).

around 38 ± 4 mg COD/L and those of BAP around 155 ± 4 mg COD/L. No degradation of VFAs or BAP was measured during the exposure time. A typical dissolved oxygen concentration in a freshly collected sample of wastewater is between 2 and 5 mg O_2/L (Bjerre et al. 1998). The ORP was higher than -200 mV/Ag-AgCl, so anaerobic conditions were not reached. Thus the sulfate-reducing process, and the acidogenic or methanogenic fermentation did not occur (Randall et al. 1992), even without inhibitors of methanogenesis (bromoethane sulfonate) and sulfate-reducing processes (molybdate and barium).

Changes during sampling and storage (Series 2)

Figure 4 presents the concentrations of the VFAs and BAP observed with and without a 30 cm waterfall (oxygen input) during the sampling of a grab sample. A 2 h period was first applied to confirm the effect of the temperature fall from 16°C to 4°C (no inhibitors added). A 24 h period was
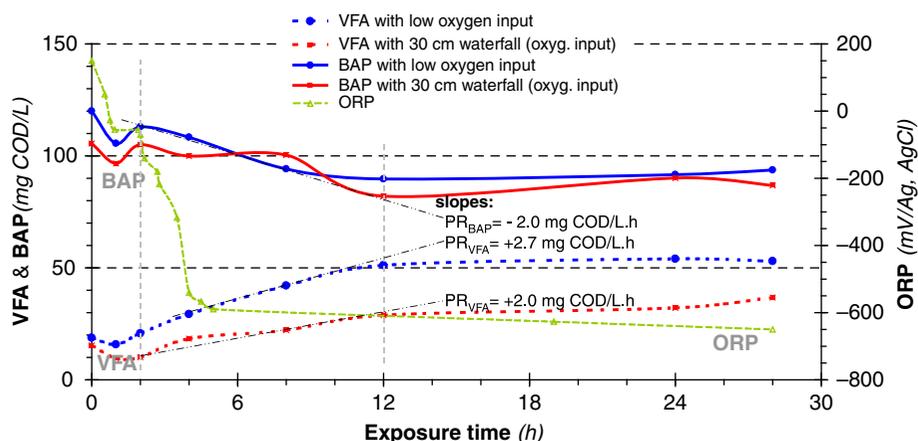
then applied at constant temperature of 4°C to study the changes during storage.

Oxygen input

After 1 h and until the end of the second hour of exposure, the mean VFA concentration in the 30 cm waterfall series bottles was lower than in the low oxygen input series bottles. The difference of around 10 mg COD/L could correspond to a higher consumption of the VFAs by aerobic heterotrophic bacteria due to the oxygen input. The difference of 10 mg COD/L was not observed for the VFA at the start time ($t = 0$), but was observed for the BAP, probably owing to the elapsed time necessary to start the BAP test.

During the 2 h long period (cooling), a mean BAP of 102 ± 5 mg COD/L was obtained in the bottles filled with the 30 cm waterfall, whereas 113 ± 7 mg COD/L was recorded in the other bottles. The difference (approx. 10 mg COD/L) gives an estimate of the oxygen transferred to the liquid phase by the 30 cm waterfall, and is limited by the dissolved oxygen at saturation value.

24 h storage period

The ORP reached -200 mV/Ag-AgCl after 3 h, indicating that it took this long for anaerobic conditions to be reached. An increase in the VFA concentration was observed from the third to the twelfth hour of storage despite the temperature being maintained at 4°C: VFAs increased by 20 mg COD/L with the waterfall and by 27 mg COD/L without the waterfall. The fermentation of part of the fermentable fraction of COD (S_F) into VFAs is the most likely explanation for this observation. The increase then stopped. During the

Figure 4 | VFA, BAP and ORP, with and without oxygen input, during cooling (2 h) and storing (24 h) (Series 2).

same period of time, a decrease of 20 mg COD/L in the BAP concentration was observed at 4°C, which then stopped. The conversion of sulfate into H_2S (reduction) is the most likely explanation for the decrease in BAP concentration (Martin-Ruel et al. 2002a; Krier 2004), even at 4°C, and the changes are likely to depend on the initial concentration of sulfate. Unfortunately, the concentration of sulfate was not measured.

From the thirteenth to the twenty-fourth hour, the VFA and BAP concentrations could be considered as stable, as only non-significant changes in the concentrations were recorded.

A net volumetric VFA production rate (noted PR_{VFA}) of 2.0 and 2.7 mg $COD_{produced}$/L.h were observed between the third and twelfth hour respectively for the samples with low oxygen input and for those with the 30 cm waterfall bottles. These values, obtained at 4°C with partial settling of suspended solids, are close to the production rates observed at 20°C with settled wastewater (Martin-Ruel et al. 2005) during the first 24 h of a BAP test.

Influence of the 24 h composite sampling technique

The automatic sampling technique using a single sample container and a limited oxygen input (sampler S1) was evaluated. To evaluate this technique, the value obtained from the experiments conducted with 24 bottles and a 30 cm waterfall (sampler S2) were selected, according to the information summed-up in the method section. The detailed information used for the calculations is given in the appendix section, taking into account the changes during sampling, cooling and storing periods.

Table 3 presents the daily average concentrations for VFAs and BAP for two wastewaters (WW1 and WW2): observed values for S1, observed and corrected values for S2. Absolute and relative differences between the methods are also presented ($S2_{corr}$-$S2_{obs}$, $S2_{obs}$-$S1_{obs}$ and $S2_{corr}$-$S1_{obs}$

in mg COD/L; ($S2_{obs}$-$S2_{corr}$)/$S2_{obs}$, $S2_{obs}$-$S1_{obs}$)/$S2_{obs}$ and ($S2_{cor}$-$S1_{obs}$)/$S2_{corr}$ in %).

Observed S2 vs. observed S1

Comparing the observed values drawn from samplers S1 and S2, differences of $+6$ mg and $+1$ mg COD/L (13% and 1% respectively) were observed for WW1 and WW2 (Table 3). For the BAP, the differences were $+15$ mg and -24 mg COD/L, i.e. $+11\%$ and -10% of the initial BAP, respectively. This greater magnitude of the difference was previously suggested by Lie & Welander (1998), who recommended applying a freezing stage to the samples.

Observed S2 vs. corrected S2 (S2 correction effects)

Corrected vs. observed VFA and BAP concentrations obtained from 24 h composite sample of the 24 bottles (S2) are presented in Table 3. A net VFA difference of -6 mg COD/L and a net BAP difference of $+24$ mg COD/L for both wastewaters was observed. Relative differences of -13% and -6% were calculated for VFA, with 17% and 9% for BAP.

Corrected S2 vs. observed S1

The VFA observed for S1 were 0 and -5 mg COD/L lower than those observed for corrected S2 (a negligible difference of -1% and -4%, respectively). The BAP value observed for S1 was $+39$ mg/L and -1 mg COD/L lower than that observed for the corrected S2 (24% and 0 respectively). The results show a negligible difference for three results out of four. The greater difference observed for the BAP of WW1 can be explained by an underestimation of the BAP measurement. The use of inhibitors of methanogenesis and sulfate-reducing processes should therefore be more systematically employed when sampling.

Table 3 | Observed and corrected VFA and BAP concentrations (daily average samples) with two sampling technique (S1 single container, and S2 with 24 bottles + waterfall) for two wastewaters

		Sampler 1 (S1)	Sampler 2 (S2)		Obs. S2 vs. Obs. S1	Obs. S2 vs. Corr. S2	Obs. S1 vs. Corr. S2
		Observed mg/L	Observed mg/L	Corrected mg/L	Difference mg/L (%)	Difference mg/L (%)	Difference mg/L (%)
VFA	WW1	41	47	41	+6 (13%)	−6 (−13%)	0 (−1%)
	WW2	106	107	101	+1 (1%)	−6 (−6%)	−5 (−4%)
BAP	WW1	126	140	164	+15 (11%)	+24 (17%)	+39 (24%)
	WW2	272	248	271	−24 (−10%)	+23 (9%)	−1 (0%)

VFA and BAP: morning sample vs. other hourly and daily average concentrations

For the daily average composite samples of WW1 and WW2, a BAP of 164 mg COD/L (25% VFA with 41 mg COD/L) and 271 mg COD/L (37% VFA with 95 mg COD/L) were obtained, respectively (Table 3). The values for WW1 match the literature values available for grab samples: VFA concentrations are mainly below 60 mg COD/L (20 values) and BAP between 60 mg/L and 240 mg COD/L (11 values) (Abu-ghararah & Randall (1991); Carlsson et al. (1996); Jönsson et al. (1996) ; Lie & Welander (1997); Barajas et al. (2002); Martin-Ruel et al. (2002a, b); Pasztor et al. 2009); Tchobanoglous et al. 2005). For WW2, the values are in line with studies reporting characterization of wastewater sustaining a high biological phosphorus removal (Vale et al. 2005; Lim et al. 2008).

The corrected hourly and daily average concentrations of VFA and BAP are presented in Figure 5 for WW1 and WW2.

For the hourly composite samples of WW1 taken between 11:00 am and 2:00 pm, a BAP value of 350 mg/L was observed (max:mean ratio of 2.5) with 30% VFA (80–90 mg COD/L),

close to the upper range reported in the literature. During the rest of the day (except for the 2:00 am grab sample), the BAP concentrations were in the range 100–150 mg COD/L, with 20% VFA during the afternoon/evening, and 40–50% at night and in the morning (20–50 mg COD/L). For the hourly composite samples of WW2 taken between 11:00 am and 11:00 pm, the BAP values were in the range 250–570 mg COD/L, and in the range 100–200 mg COD/L during the rest of the day (max:mean ratio of 3.0), due to the beverage industry effluent. The proportion of VFAs was about 50–55% for the first period, which are high values compared with those usually measured for conventional domestic wastewater. A proportion of about 20–50% was measured in the second period, which is a more commonly found value.

Hence grab samples taken during the period of peak loading contained a much higher VFA and BAP concentration than grab samples taken at another time or the 24 h composite sample (daily average). This was true both for the wastewater from a conventional municipal facility and for wastewater including a biodegradable trade component.

The use of a sample taken once, in a narrow time span, e.g. 11:00 am to 2:00 pm (peak load), gives values
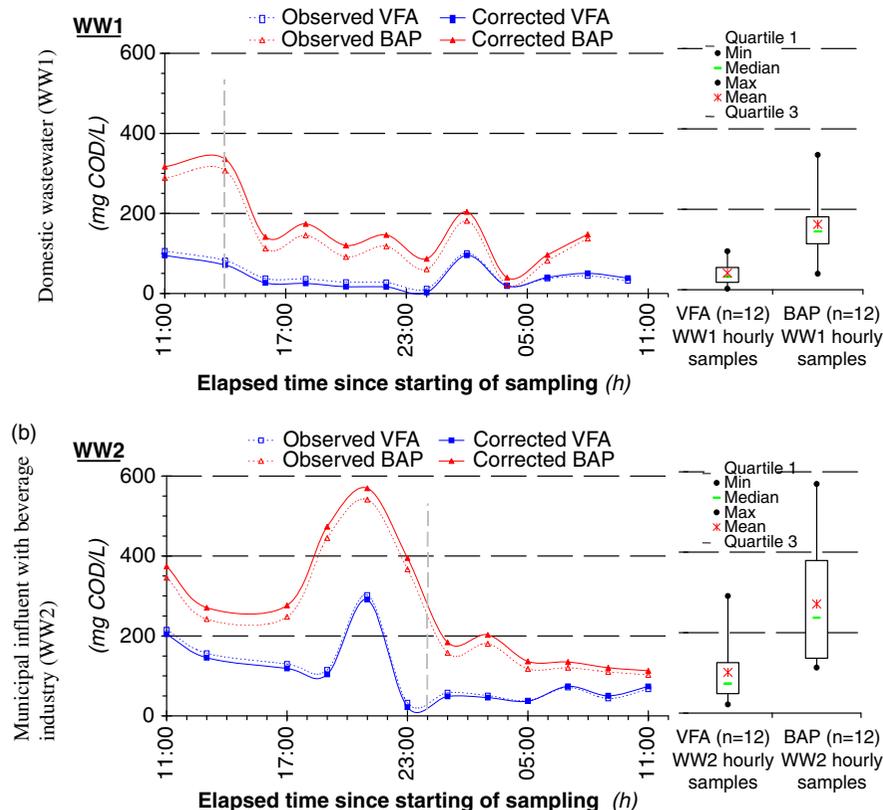

Figure 5 | VFAs and BAP at hourly samples and daily average sample for WW1 (domestic influent) and WW2 (municipal + beverage industry influent) during the time elapsed since start of sampling.

significantly different from those recorded at other times, and also from the daily average value. Also, the ratio COD:BAP, about 6 for a daily average composite sample, takes a value of 3 at peak load. Applying this last value to the daily average COD value causes misjudgment of the phosphorus removal capacity through EBPR. It overestimates the BAP and VFA results and so can lead to poor plant design. Hence although there are some changes to VFAs and BAP when making a daily average composite sample (automatic refrigerated sampling machine), this technique provides some values that are lower, taking into account hourly variations, and thereby making a better estimate of phosphorus removal by EBPR.

CONCLUSIONS

Different experiments were conducted to study changes of the VFAs and BAP concentrations of discrete samples occurring in an automatic refrigerated sampling machine. A 30 cm waterfall caused a decrease in the VFAs and BAP concentration by up to 10 mg COD/L (equivalent to the saturation concentration of dissolved oxygen). During the first 2 h of cooling/storing, no modification occurred even without chemicals for the inhibition of methanogenesis (bromoethane sulfonate) and sulfate-reducing processes (molybdate and barium). After 3 h of storage, there was a fall in the BAP concentration due to fermentation, converting substrate into VFAs (soluble COD). Sulfate-reducing processes occur even at 4°C, with changes depending on the initial concentration of sulfate. Hence the use of inhibitors of methanogenesis and sulfate-reducing processes should be more systematically employed for daily average composite sampling.

For the two wastewaters tested with daily average flow proportional composite sampling, the results were underestimated by less than 5% for VFAs (maximum error −5 mg COD/L). The BAP results were underestimated by less than 25% for domestic influent and by less than 2% for municipal + beverage influent.

The BAP values measured on samples taken in the morning (during peak load, at 11:00 am) were double those measured on the average composite sample. It is thus preferable to take several grab samples or an average flow-proportional composite sample to evaluate the suitability of a wastewater for biological phosphorus removal.

APPENDIX

VFA and BAP analyzed from samples contained in 1-litre separate bottles (sampler 2) were used to evaluate the values that would be obtained if no modification had occured in the automatic sampling machine comprising of a single container (sampler 1). These results were obtained from the results of lab-scale tests (series 1 and 2) that consisted of measuring the effects of the oxygen input by a waterfall (noted $VFA\_DO_{decrease}$ and $BAP\_DO_{decrease}$), and the transformation during cooling and storage (noted PR_{VFA} and PR_{BAP}).

For bottle number N filled with a wastewater sampled and cooled for a delay of $\Delta t(N)$, the theoretical concentrations of VFA and BAP were calculated as shown in Figure 6.

The theoretical concentration of VFA [noted $VFA_{act}(N)$] was determined with Equation (1) applied to the observed concentration [$VFA_{obs}(N)$], with two parameters obtained with series n°2 once the storage delay was greater than 4 hours: VFA decrease due to wastewater fall ($VFA\_DO_{decrease}$) and a production rate by methanogenesis [PR_{VFA}].
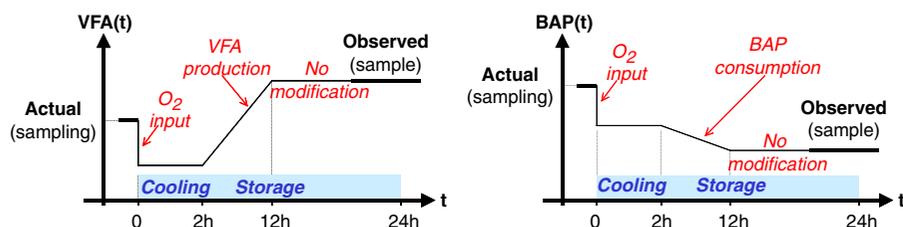

Figure 6 | Schematic modification of VFA and BAP vs. time during sampling, cooling and storage.

$$\text{VFAact}(N) = \begin{cases} \text{if } \Delta t(N) \leq 2h, & \text{VFAobs}(N) + \text{VFADOdecrease} \\ \text{if } 3h \leq \Delta t(N) \leq 12h, & \text{VFAobs}(N) + \text{VFADOdecrease} - [\Delta t(N) - 3h]\text{PRVFA} \\ \text{if } \Delta t(N) \geq 13h, & \text{VFAobs}(N) + \text{VFADOdecrease} - 20 \end{cases} \tag{1}$$

Equation (2) was applied to obtain the theoretical concentration of BAP [$\text{BAP}_{act}(N)$] from the observed values [$\text{BAP}_{obs}(N)$], a BAP decrease due to the wastewater fall [$\text{BAP\_DO}_{decrease}$] and a consumption rate [PR_{BAP}].

$$\text{BAPact}(N) = \begin{cases} \text{if } \Delta t(N) \leq 2h, & \text{BAPobs}(N) + \text{BAPDOdecrease} \\ \text{if } 3h \leq \Delta t(N) \leq 12h, & \text{BAPobs}(N) + \text{BAPDOdecrease} + [\Delta t(N) - 3h] * \text{PRBAP} \\ \text{if } \Delta t(N) \geq 13h, & \text{BAPobs}(N) + \text{BAPDOdecrease} + 20 \end{cases} \tag{2}$$

with: $\text{VFA\_DO}_{decrease} = \text{BAP\_DO}_{decrease} = -10$ mg COD/L
$\text{PR}_{VFA} = -\text{PR}_{BAP} = 2.0$ mg COD/L.h

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