Journal of Environmental Sciences 26 (2014) 926–933

Available online at www.sciencedirect.com

Journal of Environmental Sciences

www.jesc.ac.cn

Determining short chain fatty acids in sewage sludge hydrolysate: A comparison of three analytical methods and investigation of sample storage effects

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ARTICLE INFO

Article history:
Received 19 June 2013
revised 22 October 2013
accepted 29 October 2013

Keywords:
five pH point titration
gas chromatography
spectrophotometry
short-chain fatty acids
sludge hydrolysis
sample storage

DOI: 10.1016/S1001-0742(13)60516-1

ABSTRACT

In anaerobic digestion, the production of short-chain fatty acids (SCFAs) can be beneficial or harmful to the overall process, depending on the concentration of accumulated acids. Therefore, the accurate determination of the SCFA concentration in both fresh and stored sludge hydrolysates is important. To select a suitable method for monitoring SCFAs during the anaerobic digestion of sewage sludge, the accuracy of three available analytical methods, including 5 pH point acid titration (TITRA5), gas chromatography (GC), and spectrophotometry, were compared in the present study. The results revealed that TITRA5 and GC displayed better agreement in the achieved measurements and higher precision and accuracy than the spectrophotometric assay, as supported by the application of different statistical models. TITRA5 excelled in titrating unfiltered hydrolysate while simultaneously measuring the alkalinity, whereas the GC method provided detailed information on the contribution of different fatty acids to the total acidity. In contrast, the spectrophotometric assay suffered from many forms of interference, depending on the sample's matrix. SCFA production followed the pattern of enzymatic reactions and fitted the Michaelis-Menten model. In addition to promoting TITRA5 as an accurate and robust analytical tool for routine SCFA analyses, this comparative study also demonstrated the possibility of storing hydrolysate samples at different temperatures and durations without altering the SCFA measurements.

Introduction

The metabolism of short-chain or volatile fatty acids (SCFAs or VFAs) follows complex mechanisms involving multiple agents. For instance, in the large intestine of humans, fatty acids are produced via anaerobic fermentation of undigested biopolymers (Macfarlane and Macfarlane, 2012). The process is mediated by over 500 bacterial species that co-exist in the colonic flora (Mai and Morris, 2004) and has benign effects on colonic health (Wong et al., 2006). The principles of the *in vivo* production and use of SCFAs have been extended to several *ex vivo* applications, including wastewater treatment and biomethane production.

The utility of SCFAs in wastewater treatment has been manifested through their contribution to the biological removal of nitrogen and phosphorus by serving as a carbon source for denitrifying bacteria (Elefsiniotis et al., 2004) and phosphorus accumulating organisms (PAOs) (Horgan et al., 2010), respectively. SCFAs have also been proven to play a crucial role in biomethane production, a process that takes place through four well defined stages of anaerobic digestion, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During acidogenesis, fermenting bacteria make use of soluble organic substances generated

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upon hydrolysis and convert them into SCFAs, lactate and hydrogen (Yuan et al., 2011). The produced SCFAs are then consumed by acetogenic bacteria and converted into acetic acid, a substrate of preference for acetoclastic methanogens (Appels et al., 2008). Several studies showed that the SCFA concentration could be used as a process indicator to measure the performance of anaerobic systems (Ahring et al., 1995; Chen et al., 2008; Wang et al., 2009). Moderate to significantly high amounts (0.73 to 12 g/L acetic acid equivalent) of SCFAs can stress acetogenic and methanogenic microorganisms and lead to low methane yields or even to the termination of the entire process. Therefore, due to the cardinal and decisive role of SCFAs in the aforementioned operations, a range of analytical tools has been used to determine the quantity of SCFAs in sludges (Morgan-Sagastume et al., 2011; Robert-Peillard et al., 2012) wastewaters (Robert-Peillard et al., 2009; Banel et al., 2012) and other environmental samples (Feng et al., 2008; Bilgili et al., 2012).

In the present study, three commonly used techniques were adopted and were compared to quantify SCFAs produced during the pilot scale anaerobic sludge hydrolysis situated on a full scale wastewater treatment plant (WWTP). The first technique consisted of a 5 pH point acid titration (TITRA5), which has been recently investigated by Hey et al. (2013) to monitor the SCFA content and alkalinity in wastewater subjected to in-line, full scale, primary sludge hydrolysis. Gas chromatography (GC) is another technique that has been used in numerous reports to measure fatty acids in diverse samples (Elefsiniotis et al., 2004; Banel et al., 2011; Krzeminski et al., 2012). The third analytical tool was a spectrophotometric method based on a commercial kit. The procedure consisted of an esterification step, in which fatty acids react with a diol at high temperatures, followed by hydroxamic acid and iron hydroxamate complex formation, which can be detected spectrophotometrically at 500 nm.

The three methods were also compared to assess the effects of sample storage on SCFA recovery in anaerobically digested sludge.

1 Materials and methods

1.1 Pilot scale mixed sludge hydrolysis and sampling

The hydrolysis experiment was performed over 10 days at the Källby WWTP in Lund, Sweden. A mixture of primary, waste-activated and post-precipitated ($FeCl_3$) sludge, in a mass ratio of 60:30:10 ($m/m/m$), was pumped directly from the bottom of the hopper into a 2.5 m^3 cylindrical stainless steel tank placed above the primary settler. The pilot plant was equipped with a stirrer (0.25 kW, 1380 r/min), luminescent dissolved oxygen meter (0–20 mg O_2/L, LDO™) and suspended solids meter (0–150 g/L, SOLITAX SC) for the continuous monitoring of the dissolved oxygen and suspended solid concentration. Both sensors were connected to a probe module (SC1000) and were monitored online. All of the sensors and probe module were obtained from Hach Lange, Düsseldorf, Germany. Grab samples were taken from the hydrolysis tank at 24 hours interval for analyses.

1.2 Short chain fatty acid determination

The hydrolysate samples for SCFA determination were first centrifuged for 10 min at a relative centrifugal force (RCF) of 2590 $\times g$ (Unicen 21; Orto Alresa, Spain) and were vacuum filtered through a 1.6 μm micro-glass fibre paper on a 55 g/m^2 weight basis (Munktell MGA grade, Munktell & Flitrak, Germany). Samples for titration were diluted 10 times using deionised water (Elix 10, Millipore, France).

1.2.1 Five pH point titration procedure

Five pH point acid titration (TITRA5) was performed using a fully automated titration system (TitroLine alpha 50 plus) connected to a sample changer (TW alpha plus) equipped with magnetic stirring and a pH electrode (Blue Line pH 14), which were obtained from SI Analytics, Mainz, Germany. Titrisoft software (Version 2.6) was used to control the titration procedure, configure the titration curves and export the corresponding data. The pH electrode was calibrated using three standard buffer solutions at pH values of 4.01, 7.00 and 9.23 (Hamilton Duracal Buffer, Switzerland). The calculated calibration slopes were always $\geqslant$ 0.99. The total dissolved solid content (TDS, mg/L), specific electrical conductivity (EC, mS/m) and temperature (°C) of 100 mL samples were measured with a handheld conductivity meter (EC 300, VWR International) prior to the titration procedure. The titrant (0.1 mol/L HCl, Scharlab S.L., Barcelona, Spain) was added in 5 μL increments during the titration procedure, as described by Buchauer (1998). The recorded titrant volumes of the pre-selected pH points (6.7, 5.9, 5.2 and 4.3) and initial pH of the sample, TDS, temperature, ammonium nitrogen (NH_4^+-N, Hach Lange LCK 303, Germany) and phosphate phosphorus (PO_4^{3-}-P) (ISO 6878, 2004) values were entered into a specific calculation matrix (TT 57-92) to simultaneously calculate the SCFAs (mg/L as CH_3COOH) and the carbonate system alkalinity with H_2CO_3 as the reference species (H_2CO_3* alkalinity in mg/L as $CaCO_3$, where H_2CO_3* accounts for H_2CO_3 and dissolved CO_2) (Moosbrugger et al., 1992).

1.2.2 Spectrophotometric measurements

The spectrophotometric determination of SCFAs was carried out using a commercial kit (LCK 365) and spectrophotometer (DR 2800) both from Hach Lange, Düsseldorf, Germany. A filtered sample volume of 0.4 mL was used in the experiment, according to the cuvette