Bioresource Technology 190 (2015) 289–298

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation

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HIGHLIGHTS

- pH 11 gave highest solubilization but the optimal VFA accumulation was at pH 8.
- Low biological activity and high MW substance production dissuaded use of pH > 8.
- Different acidogens were enriched according to the operating pH.
- Enrichment of specific genera at pH 8 maximized VFA accumulation.

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 20 April 2015
Accepted 24 April 2015
Available online 29 April 2015

Keywords:
Sludge fermentation
pH
Volatile fatty acids (VFA)
Size exclusion chromatography (SEC)
454 pyrosequencing

ABSTRACT

Sludge fermentation between pH 4 and 11 was investigated to generate volatile fatty acids (VFA). Despite the highest sludge solubilization of 25.9% at pH 11, VFA accumulation was optimized at pH 8 (12.5% out of 13.1% sludge solubilization). 454 pyrosequencing identified wide diversity of acidogens in bioreactors operated at the various pHs, with *Tissierella*, *Petrimonas*, *Proteiniphilum*, *Levilinea*, *Proteiniborus* and *Sedimentibacter* enriched and contributing to the enhanced fermentation at pH 8. Hydrolytic enzymatic assays determined abiotic effect to be the leading cause for improved solubilization under high alkaline condition but the environmental stress at pH 9 and above might lead to disrupt biological activities and eventually VFA production. Furthermore, molecular weight (MW) characterization of the soluble fractions found large MW aromatic substances at pH 9 and above, that is normally associated with poor biodegradability, making them disadvantageous for subsequent bioprocesses. The findings provided information to better understand and control sludge fermentation.

1. Introduction

Sludge treatment and its disposal remain one of the biggest challenges in the wastewater treatment industry, and could account for up to 50% of a wastewater treatment plant's operating cost (Appels et al., 2008). Municipal sludge is often a mixture of primary and secondary (waste activated sludge, WAS) sludges at many wastewater treatment plants. Microbial fermentation could be utilized to reduce the organic content, produce volatile fatty acids (VFA) and subsequently methane. The VFA can be used as carbon source for biological nutrient removal (BNR) processes or biopolymer production (Jiang et al., 2009; Li et al., 2011), while methane is a source of renewable energy (Appels et al., 2008).

Recently, extensive studies had assessed the impact of pH to improve waste activated sludge (WAS) fermentation in batch configuration between pH 4 and 12 at 21 °C (Chen et al., 2007), 28 °C (Jie et al., 2014), 35 °C and 55 °C (Zhang et al., 2009, 2010). According to these studies, alkaline condition was consistently reported to be more efficient than acidic condition for sludge solubilization, and the highest solubilization was found at the highest

Abbreviations: AD, anaerobic digestion; BNR, biological nutrient removal; COD, chemical oxygen demand; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acids; EPS, extracellular polymeric substances; FISH, fluorescence in situ hybridization; MW, molecular weight; qPCR, quantitative polymerase chain reaction; rRNA, ribosomal ribonucleic acids; RI, refractive index; SEC, size exclusion chromatography; SRT, solids retention time; TS, total solids; TSS, total suspended solids; UV, ultraviolet; VFA, volatile fatty acids; VS, volatiles solids; VSS, volatile suspended solids; WAS, waste activated sludge.

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http://dx.doi.org/10.1016/j.biortech.2015.04.087
0960-8524/

pH value tested. VFA accumulation was found to reach maximum at pH 10 at 21 °C and 28 °C after 8–10 days (Chen et al., 2007; Jie et al., 2014). At higher temperature, Zhang et al. (2009) reported that sludge fermentation at 35 °C yielded the highest VFA accumulation at pH 9 after 5 days and even higher VFA concentrations at 55 °C at pH 8 after 9 days.

Furthermore, Zhang et al. (2009) also found that the VFA accumulation did not increase despite of higher solubilization observed at pH 10 and above at mesophilic (35 °C) temperature under batch configuration treating WAS. Yu et al. (2008a) found the abiotic effect was the leading cause of the enhanced sludge solubilization at pH 10. Too high pH value could actually impair the biological microbial fermentation activities as acidogens generally functioned from pH 4 to 8.5 (Appels et al., 2008). However, Yuan et al. (2006) established that some degree of biological activities was still retained at pH 10. It is thus necessary to understand the extent of biotic activities during sludge fermentation in milder acidic and alkaline conditions (between pH 4 and 10). This would improve the understanding of biotic activities contribution to sludge solubilization and VFA production, which may potentially reduce chemical usage during sludge fermentation. Besides microbial activity, pH 2.5, 9.8 and 11.8 had been found to influence the molecular weight (MW) distributions of solubilized organic matters released from the sludge, which consequently affected its biodegradability (Eskicioglu et al., 2006; Xie et al., 2014). Similarly, the MW distributions of the soluble fractions between pH 4 and 11 have not been evaluated. Understanding the sludge solubilization products and its solubilization mechanism at different pHs could help to better control and optimize the sludge fermentation process.

In addition, production of VFA is driven by the concerted effort of various acidogenic microorganisms. Investigation of the microbial community structures at different pHs during WAS fermentation have been reported previously. The studies had been performed with batch configuration between pH 4 and 12 at 28 °C using denaturing gradient gel electrophoresis (Jie et al., 2014) and semi-continuous configuration at pH 8 (55 °C), 9 (35 °C) and 10 (21 °C) using clone library and fluorescence in situ hybridization (FISH) (Zhang et al., 2010). However, the results could not yet establish a proper link between the roles of microbial populations at different pHs to the performance of sludge fermentation. Correlation of the identified microorganisms with their possible functions during sludge fermentation at different pHs would provide valuable insights into the underlying biological reactions. This study used 454 pyrosequencing to gather more sequencing reads than would have been possible with DGGE or other cloning related methods. This way, a more comprehensive representation of the microbial community could be achieved.

The feed for this study would be the mixture of primary and secondary sludges, commonly found at many wastewater treatment plants. This study aims to evaluate the characteristics of the soluble organic matter during semi-continuous mixed sludge fermentation process under mesophilic (35 °C) condition and 3 days solids retention time (SRT) at different pHs. Particular attention is given on the organic compositions and the MW distributions. Mesophilic condition is operated in this study so that the process could be readily applied to existing mesophilic anaerobic digesters in the tropical climate. Batch experiments had also demonstrated that the bulk of sludge acidogenesis of waste activated sludge at 35 °C was achieved within 3 to 4 days (Zhang et al., 2009, 2010). Hence, SRT of 3 days is chosen for the sludge fermentation in this study. The microbial community acclimated at different pHs will also be characterized to give microbiological insight to the sludge fermentation process.

Table 1
The pH variations and chemical dosage of the bioreactors.

Target pH of bioreactor	pH variations	Chemical dosage
No control	5.8–6.3	–
pH 4	3.9–4.3	0.05 ± 0.01 g HCl g TS feed^{-1}
pH 5	4.9–5.1	0.01 ± 0.00 g HCl g TS feed^{-1}
pH 6	5.8–6.2	0.01 ± 0.00 g NaOH g TS feed^{-1}
pH 7	6.5–7.2	0.03 ± 0.02 g NaOH g TS feed^{-1}
pH 8	7.1–8.2	0.07 ± 0.02 g NaOH g TS feed^{-1}
pH 9	8.2–9.2	0.1 ± 0.02 g NaOH g TS feed^{-1}
pH 10	9.2–10.2	0.14 ± 0.02 g NaOH g TS feed^{-1}
pH 11	10.2–11.2	0.17 ± 0.02 g NaOH g TS feed^{-1}

2. Methods

2.1. Bioreactor operation

Semi-continuous bioreactors were operated in 1 L glass bottles with 0.9 L working volume for 106 days. 0.3 L of sludge was withdrawn from each reactor as they were mixed and replaced with 0.3 L of feed sludge daily to make SRT of 3 days. Bioreactors were incubated on a temperature controlled shaker at 35 °C and 120 rpm. One bioreactor was operated without pH adjustment and served as control, while eight bioreactors were controlled at pH 4, 5, 6, 7, 8, 9, 10 and 11 using 5 M hydrochloric acid or 5 M sodium hydroxide. The pH adjustments were done throughout the experiment, twice every day with 12 h intervals. However, a fixed pH value could not be maintained. The pH variations and chemical dosage are presented in Table 1. The bioreactors were initially seeded with anaerobic sludge treating sewage sludge from a local full-scale wastewater treatment plant and purged with nitrogen to create the anaerobic condition. The feed sludge is sewage sludge, composed of mixed primary and secondary sludge from the same plant. The feed sludge characteristics were 25.4 ± 3.0 g TS L^{-1}; 20.3 ± 2.5 g VS L^{-1}; 37,200 ± 2,800 mg tCOD L^{-1}; 2,000 ± 400 mg sCOD L^{-1}; and pH 5.8–6.0.

2.2. Sludge fractioning protocol

Sludge fractioning protocol was performed to extract the extracellular polymeric substances (EPS), namely the pellet, tightly-bound EPS, loosely-bound EPS and slime fractions as described by Yu et al. (2008b) for enzymatic activity measurements. The tightly-bound EPS, loosely-bound EPS and slime fractions were then filtered through 0.45 μm nylon filters to remove particulates. All EPS fractions were resuspended in phosphate buffered saline solutions, pH adjusted according to the pH of their respective bioreactors. All EPS fractions were subsequently stored at 4 °C before further use.

2.3. Analytical methods

Chemical oxygen demand (COD), totals solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS) were measured in accordance with the standard methods (APHA et al., 2005). Soluble fraction of sludge sample to measure soluble COD (sCOD), soluble carbohydrates and soluble proteins were prepared by centrifugation at 12,000×*g* for 5 min and the supernatant was filtered through 0.45 μm nylon filter. Soluble protein and carbohydrate concentrations were measured using Lowry–Folin and phenol–sulfuric acid methods, respectively (Feng et al., 2009). The COD conversion factors for carbohydrate (glucose) was 1.07 mg COD mg glucose^{-1} and protein (bovine serum albumin, BSA) was 1.5 mg COD mg BSA^{-1} (Feng et al., 2009). VFA concentrations and biogas compositions were