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Effect of ozonation on anaerobic digestion sludge activity and viability

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HIGHLIGHTS

- Ozonation initially and temporarily reduced viability and methanogenic activity.
- Following the lag phase, ozonation enhanced methane production.
- An optimal ozone dose of 86 mg O_3/g COD increased the methane yield up to 52%.

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ABSTRACT

The effect of ozonation of anaerobic digested sludge on methane production was studied as a means of increasing the capacity of municipal anaerobic digesters. Ozone doses ranging from 0 to 192 mg O_3/g sludge COD were evaluated in batch tests with a bench scale ozonation unit. Ozonation initially, and temporarily, reduced biomass viability and acetoclastic methanogenic activity, resulting in an initial lag phase ranging from 0.8 to 10 days. Following this lag phase, ozonation enhanced methane production with an optimal methane yield attained at 86 mg O_3/g COD. Under these conditions, the yield of methane and the rate of its formation were 52% and 95% higher, respectively, than those factors measured without ozonation. A required optimal ozone dose could be feasible to improve the anaerobic digestion performance by increasing the methane production potential with a minimum impact on microbial activity; thus, an optimal ozone dose would enable an increase in the capacity of anaerobic digesters.

1. Introduction

Anaerobic digestion (AD) of primary and secondary sludge is commonly used for sludge reduction, stabilization and energy recovery at municipal water resource recovery facilities (WRRFs) (Appels et al., 2008). Sludge consists of a polymeric network of organic and inorganic compounds; however, its actual composition depends on the source of the sludge (Sheng et al., 2010). The presence of these chemicals, including extracellular polymeric substances (EPS), e.g., polysaccharides, proteins, and lipids, strongly influence the hydrolysis of sludge during anaerobic digestion (Sheng et al., 2010). The hydrolysis of sludge requires long hydraulic retention times (20–30 days), leading to moderate degradation efficiencies (30–50%) and translating into large volume digesters and high capital expenditures (Foladori et al., 2010a).

Usually, the main factor limiting anaerobic digestion is the hydrolysis of particulate matter. Improving anaerobic digestion through enhancing rate-limiting hydrolysis can increase degradability leading to improve anaerobic digestion performance (Appels et al., 2008). A variety of treatment techniques have been studied to enhance sludge hydrolysis by using thermal, chemical, mechanical and other biological processes (Appels et al., 2008). One of the preferred treatments is ozonation, which permits sludge reduction and is effective in enhancing methane production via the oxidation and solubilization of sludge (Weemaes et al., 2000). Ozonation of activated sludge prior to anaerobic digestion (pre-ozonation) effectively enhances its anaerobic biodegradability, but ozonation is not effective with primary sludge (Carrère et al., 2010). Alternatively, the ozonation of digested sludge in the recirculation loop of

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the anaerobic digester (post-ozonation) has been shown to produce a significant increase in methane production (Battimelli et al., 2003).

Past studies on the effect of ozone have mainly focused on activated sludge but limited information about the effect of ozonation on anaerobic digested sludge is available. The effect of ozonation differs due to the nature and composition of different sludge samples. The evaluation of the biological response of anaerobic digested sludge to ozonation by monitoring the microbial cell integrity, the metabolism (key enzyme), the acetoclastic methane activity and the production of intracellular reactive oxygen species (ROS) has not been reported. In addition, the changes in the distribution pattern of proteins and polysaccharides among different sludge layers (soluble EPS, bound EPS, pellet) will provide an original and valuable information to understand the potential mechanisms for improving anaerobic biodegradability through ozonation. A better understanding of the mechanisms of sludge ozonation and its impact on methane production and biological responses will allow for better operational control and design of an anaerobic digestion process integrated with post-ozonation.

The objective of this study was to evaluate the effect of ozonation on the methane production of anaerobic digested sludge, including the mechanisms involved in this process. The specific objectives were to evaluate the impact of ozonation on the methane yield and methane production rate in batch tests, and to evaluate the microbial response of ozonated sludge for various ozone dosages.

2. Material and method

2.1. Sludge ozonation

Anaerobic digested sludge was obtained from the Repentigny WRRF (Quebec), which treats 25 000 m^3/d using a chemically enhanced primary treatment (CEPT) process and stabilizes the sludge in a completely mixed mesophilic (35 °C) anaerobic digester with a hydraulic retention time of 19 days. The collected sludge was passed through a 5 mm sieve to remove large debris and was then stored at 4 °C until further use.

Ozone was generated by a pure oxygen ozone generator (Peak 2X, Pinnacle, USA). Ozonation of digested sludge was performed in a batch reactor. The gas flow rate was 6 L STP/min with an ozone mass concentration of approximately 12% by weight. The transferred ozone dose (mg/L) was calculated from the difference between the mass of ozone transferred (mass fed to the reactor minus the mass in the off gas) divided by the volume of sludge. Ozone dosages were normalized as mg O_3/mg COD by dividing the transferred ozone dosage by the initial total COD content of the sample.

Sludge ozonation was conducted on 2.2-L volumes of digested sludge fed into a 3.8 L column and operated at room temperature. Using a peristaltic pump operating at a flowrate of 6 L/min, the sludge was recirculated through a Venturi (484X, Mazzei, USA) into which ozone was injected continuously. Higher ozone dosages required longer recirculation time. The contact time ranged from 0.0 to 6.1 min for ozone doses between 0 and 192 mg O_3/g COD. Sludge samples were periodically collected during the operation of the ozonation system. Additionally, a control was prepared to evaluate the effect of treatment without ozone injection.

2.2. Analytical methods

2.2.1. Ozone measurements

The inlet ozone concentration was measured using an ultraviolet ozone meter (BMT 964, BMT Messtechnik GmbH, Germany) while ozone in the off gas was measured using the standard KI method (Rakness, 2005). Dissolved ozone was not measured; it was considered negligible as it was never detected during preliminary tests.

2.2.2. EPS extraction and quantification

EPS were extracted from the control and ozonated samples based on the method of EPS extraction of Liu and Fang (2002) and Yu et al. (2008). First, 15 mL of the sample was centrifuged at 2000 g for 15 min at 4 °C. The supernatant was collected and filtered (S-Pak 0.45 μm filter, Millipore, USA) to measure soluble EPS. The sludge pellet was re-suspended to its original volume using a phosphate buffer saline (PBS) solution supplemented with 90 μL of formaldehyde (36.5% v/v), then incubated at 4 °C for 1 h under agitation. The suspension was centrifuged at 5000 g for 15 min at 4 °C and the supernatant was collected and filtered (0.45 μm) for measuring the loosely bound EPS (LB-EPS). The remaining sludge pellet was re-suspended with a PBS solution to its original volume and incubated for 3 h at 4 °C after the addition of 6 mL of a 1 M NaOH solution. The suspension was then centrifuged at 12 000 g for 15 min at 4 °C, the decanted supernatant contained the tightly bound EPS fraction (TB-EPS). The residual sludge pellet was re-suspended with a PBS solution to its original volume (pellet fraction).

Proteins and polysaccharides were then measured in the samples before extraction and in soluble EPS, LB-EPS, TB-EPS and pellet fraction. The protein content in the samples was determined using the bicinchoninic acid (BAC) method (Pierce© BCA Protein Assay Kit, Thermo Scientific, USA) with bovine serum albumin (BSA) as the standard. The polysaccharide content of the extracts was analyzed using the phenol-sulfuric acid method with glucose as a standard. Proteins and polysaccharides were measured using a microplate reader (Synergy-HT, BioTek, USA). Excitation-emission matrix (EEM) fluorescence spectra were obtained from the extracts using luminescence spectrometry (RF-5301pc, Shimadzu, Japan). Samples for EEM analysis were diluted to a final COD of 30 mg COD/L with Milli-Q water. The EEM spectra were collected with the scanning emission spectra (Em) from 220 to 550 nm at 1 nm intervals by varying the excitation wavelengths (Ex) from 220 to 400 nm at 10 nm sampling intervals. Excitation and emission slits were set to 5 nm.

2.2.3. Biochemical methane potential

Methane yield and acetoclastic activity were evaluated by measuring the biochemical methane potential (BMP) in 160 mL serological bottles incubated at 35 °C based on Saha et al. (2011). A gas manometer (DG25, Ashcroft, USA) was used to measure the biogas production and the methane gas content was quantified with a gas chromatograph (GC-456, Bruker, USA) equipped with a thermal conductivity detector (150 °C). The modified Gompertz model was applied to the cumulative methane production data to determine the maximum methane production rate in the samples (Lay et al., 1996). Methane yield was evaluated without substrate addition, and the acetoclastic activity test was fed with a sodium acetate solution. The methane production was evaluated at the standard temperature and pressure (STP) of 0 °C and 1 atm.

2.2.4. Characterization of biological response

Bacterial viability of anaerobic sludge was evaluated using the Live/Dead *Baclight* bacterial viability kit (Molecular Probes, Invitrogen, Kit L13152) and the microplate reader (Synergy-HT, BioTek, USA) using the modified protocol of Chen et al. (2012). The fluorescence intensity of the stained bacterial suspensions (F_{cell}) was determined at an excitation of 488 nm and detection at 635 nm (red) and 530 nm (green), for red-fluorescent nucleic acid stain