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High frequency ultrasound pretreatment for sludge anaerobic digestion: Effect on floc structure and microbial population

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ABSTRACT

In this work the potential of high frequency ultrasounds as pretreatment for sludge anaerobic digestion has been assessed. Irradiation with 200 kHz ultrasounds was efficient in disintegrating the floc structure increasing the available fraction of soluble organic matter (up to seven times at 25,000 kJ/kg TS). Batch anaerobic digestion tests were carried out on lab-scale reactors fed either with untreated or disintegrated sludge inoculated with anaerobic sludge, at different feed/inoculum ratio (F/I = 0.5 and 1). Degradation of particulate matter, biogas production and related microbial community composition (estimated by fluorescence in situ hybridization, FISH) were investigated. Sludge ultrasounds pretreatment led to an overall improvement of the digestion performances, with a maximum biogas gain of 40% at F/I = 0.5. FISH showed a key-role of *Methanosarcina* spp. in the main reactions of biogas synthesis.

1. Introduction

The very fast increase in sludge production, mainly due to the implementation of the European Council Directive 91/271/EEC concerning urban waste-water treatment requests new strategies in sludge management to cope with this critical issue in an economically and environmentally fully acceptable manner. The challenge in the coming years will be both to sustain agricultural use of good quality sludge, and to reduce as much as possible disposal of polluted sludge especially in landfill (Braguglia et al., 2011).

Due to environmental and economical constraints, there is a need for affordable and sustainable technologies for sludge treatment and disposal. The anaerobic digestion of sludge is an efficient and sustainable technology to stabilize sludge by means of mass reduction, odor removal, pathogen reduction, and in particular, energy recovery in the form of biogas (Pilli et al., 2011). For these reasons anaerobic digestion plays an important role for its abilities to produce energy, thereby also reducing the amount of final sludge to be disposed of, optimizing the costs of a wastewater treatment plant (Appels et al., 2008).

Anaerobic degradation of viable, biological solids like waste activated sludge (WAS) requires the hydrolysis of particulate matter to soluble substrates, rate-limiting step of the whole process (Pavlostathis and Giraldo-Gomez, 1991). For this reason, in order to accelerate the low rate of biodegradation and to enhance the digestibility of WAS, a mechanical pretreatment could improve the disintegration of biomass and cell walls, facilitating the release of intracellular matter in the aqueous phase.

Low frequency ultrasounds (20–40 kHz) have been widely investigated on laboratory, pilot and full-scale level as sludge digestion pretreatment (Kim et al., 2003; Grönroos et al., 2005; Braguglia et al., 2006; Khanal et al., 2007; Nickel and Neis, 2007; Show et al., 2007; Feng et al., 2009). In addition, considerable interest in the last years was given to the application of high frequency ultrasounds as oxidative destructive process for the treatment of hazardous contaminants in water and wastewater whereas no application has been investigated directly for sludge decontamination. Tiehm et al. (2001) showed that the efficacy in sludge disintegration decreased by increasing ultrasounds frequency up to 3000 kHz, and Gallipoli (2010) observed a significant sludge disintegration applying 200 kHz ultrasounds, besides the sonochemical degradation of surfactants directly in sludge.

Anaerobic digestion of organic matter occurs by the sequential co-operative action of a number of different bacterial trophic groups. These microorganisms cooperate sequentially in order to achieve degradation of a variety of polymeric and monomeric substrates (O'Flaherty et al., 2006). Thus, the performance of an anaerobic digestion process is primarily linked to the structure of the microbial community present in the system (Narihiro and Sekiguchi, 2007; Demirel and Scherer, 2008). A deeper knowledge of the identity and function of the microbial components would therefore allow to better control the biological processes.

Several investigations were carried out to estimate the activity and behavior of the microbial communities in anaerobic reactors operating with different substrates (McMahon et al., 2004; Roest,

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2007; Lee et al., 2009; Nettmann et al., 2010; Ike et al., 2010), but only few studies (Hwang et al., 2008; Kobayashi et al., 2009; Kim et al., 2010; Shin et al., 2010) focused on the characterization of microbial communities (especially Archaea) during the anaerobic digestion of sewage sludge. This aspect is very important because the methanogenic community of the anaerobic sludge could strongly affect the evolution of the digester operation, especially in terms of process stability and energy production.

Besides, the operational and environmental parameters of the anaerobic digestion obviously affect the behavior, performance and eventually the fate of the microbial community in anaerobic digesters. Furthermore, the nature and influence of the inoculum should also be accounted for as well (Demirel and Scherer, 2008; Tomei et al., 2008).

Objective of this study was to evaluate the dynamics of the microbial population, especially methanogenic Archaea, during batch anaerobic digestion of either untreated or sonicated waste activated sludge, at different food/inoculum ratios. The performance of the anaerobic digestion process was assessed by volatile solids degradation and biogas production, too. The potential efficiency of the new 200 kHz sonication was investigated in terms of floc disintegration, COD, proteins and carbohydrates release.

2. Methods

2.1. Sludge

Waste activated sludge (WAS) samples was obtained from the municipal "Roma-Nord" wastewater treatment plant, one of the four wastewater treatment plants serving the city of Rome characterized by an organic load of about 700,000 p.e., high sludge age (20 days) and a COD average value of incoming sewage of 200 mg/L. The activated sludge was sampled from recycle stream before thickener. The anaerobic inoculum was sampled from the full scale digester of the plant fed with primary and secondary sludge.

2.2. Chemical and structural WAS characterization

2.2.1. Chemical analysis

Total and volatile solids (TS and VS) were determined according to standard methods (APHA, 1998).

The soluble phase was filtrated through 0.45 μm pore size membrane filters after removal of the particulate sludge matter by centrifugation for 10 min at 6000 rpm. Soluble COD (Chemical Oxygen Demand), measured in duplicates, was determined by photometric determination of chromate consumption by the organic compounds, subsequent to digestion in concentrated sulphuric acid solution for 2 h at 148 °C by means of COD Cell Test by Spectroquant Merck (EPA method 410.4).

For the EPS (extracellular polymeric substances) content, sludge aliquots were filtered through glass filters with 1.2 μm pores (GF/C Whatman); the filtrate was used for protein and carbohydrates determination. Protein content was calculated by means of the modified Lowry Kit (Sigma–Aldrich) for protein determination (Ras et al., 2008).

Carbohydrates determination is based on a modified Dubois method (Dubois et al., 1956), using fructose as standard. Concentrated H_2SO_4 (1 mL) was added to the sample (200 μL) in a glass tube. The tubes were mixed by vortexing 20 s, then allowed to reach room temperature. Besides, 200 μL of phenol solution (5%) was added, and the tubes are vortexing again. The tubes were kept 3 h in the dark at room temperature. The absorbance of standards and samples was determined spectrophotometrically at 480 nm (Perkin Elmer Lambda Bio 20).

2.2.2. Floc structure

The floc dimensional analysis of untreated and sonicated WAS was performed by contrast phase microscopy examination at 100× (Zeiss Axioskop). Average floc size was estimated from at least 100 images randomly taken on at least triplicate samples. Images were processed with IMAGEJ software (version1.37v, Wayne Rasband, National Institute of Health, Bethesda, MD, USA, available in the public domain at http://rsb.info.nih.gov/ij/index.html), by measuring the major axis of flocs. Floc size distribution was referred to five different intervals from 0 to 1500 μm (0–50, 50–150, 150–500, 500–1000, 1000–1500).

2.3. Ultrasound pretreatment

Sonication was performed for 10 and 40 min on 300 mL of WAS. The ultrasound apparatus is constituted of an amplifier T&C Power Conversion and a sonoreactor Elac Nautik USW 51-051, operating at 200 kHz and at an average power of 90–100 W. The transducer is made 48 up of a piezo-ceramic element with an active area of approx. 25 cm^2. The glass sonication cell is covered and water jacketed for cooling sonicated sludge mixture. The effect of pretreatment on sludge flocs was investigated by applying ultrasounds at specific energy in the range 9500–45,000 kJ/kg TS, whereas the batch digestion tests were carried out on low-energy (∼25,000 kJ/kg TS) sonicated sludge. The specific energy input is a function of ultrasonic power, ultrasonic duration, and volume of sonicated sludge and TS concentration, and can be calculated following the equation reported elsewhere (Braguglia et al., 2011).

The degree of disintegration (DD_{COD}) is calculated as the ratio between COD increase due to sonication and the total COD as reported elsewhere (Braguglia et al., 2006).

2.4. Anaerobic digestion batch tests

Experiments were carried out on bench scale anaerobic reactors of 0.4 L (working volume) that were operated in batch mode, immersed in a temperature controlled, agitated water bath at 37 °C. The reactors were fed with a mixture of WAS, either untreated or sonicated, and raw anaerobic inoculum, at different ratio. Food/inoculum (*F/I*) ratio was calculated as follows:

$$F/I = [V_{sludge} \cdot VS_{sludge}]/[(V_{tot} - V_{sludge}) \cdot VS_{inoculum}]$$

The digestion period was 20 days, and the pH variation during this period was in the range 6.9–7.4.

The produced biogas was collected in calibrated 1-L eudiometer tube placed on the digestion bottle via a ground-glass connection (Fig. 1). The tube has a glass hose-coupling from which a sufficiently long hose connection leads to a leveling flask. The upper end of the eudiometer tube is fitted with a conical stopcock for adjusting the zero point. The liquid contained in the tube and in the leveling flask was NaCl at pH 3 to avoid CO_2 losses by carbonate formation. The biogas was read daily. At regular time intervals (after 2, 6 and 20 digestion days) one batch reactor containing untreated and another one containing sonicated sludge were stopped, opened and the sludge were analyzed.

The pretreated sludge used as feed for the anaerobic digestion tests was sonicated with a specific energy of ∼25,000 kJ/kg TS, corresponding to a DD_{COD} of about 6.5%.

Blank experiments carried out with the reactors fed with the sole inoculum showed that, as expected, the particulate matter removal was lower than in the tests at $F/I > 0$ and the final removal