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Research article

Enhancing biomethanation from dairy waste activated biomass using a novel EGTA mediated microwave disintegration

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ABSTRACT

A novel approach to explore the impact of calcium specific chelant - Ethylene glycol tetra acetic acid (EGTA) on deflocculation followed by biomass disintegration using microwave (MW) was investigated. In the first phase of the study, the EGTA dosage of 0.012 g/g suspended solids (SS) was found to be optimal for disassociating the biomass. Subsequent disintegration of biomass in microwave (EGTA-MW) yielded a biomass lysis and solids reduction of about 39.7% and 30.5%. EGTA-MW disintegration reduces the amount of specific energy required to disintegrate the biomass from 18,900 kJ/kg TS to 13,500 kJ/kg TS, when compared to control. The impact of EGTA-MW disintegration on anaerobic digestion was also evident from its methane yield (235.3 mL/g VS) which was 36.2% higher than control. An economic assessment of this study provides a net profit of 8.48 €/ton in EGTA-MW and highly endorsed for biomass disintegration.

1. Introduction

India is the leading milk manufacturer in the world by a best margin and accounting for more than 17% of the world's total milk production. India's processed dairy segment has grown due to increased demand for more diversified dairy and dairy products. However, waste activated biomass produced from dairy industries is estimated to be 5–25% of total volume of treated effluent with high proportion of biodegradable organic matter (Dhar et al., 2012). The appropriate treatment and disposal of leftover biomass demands high expenses which consequently have an impact on the general economy. Anaerobic degradation (AD) is applauded as the established biomass stabilization technique, giving the benefits of volume reduction together with the production of eco - friendly energy rich gaseous product by the way of biomethane. Among the range of steps in AD process, hydrolysis is recognized as the rate - constraining step and extended time of the overall process, as the microbial cell membrane and the exopolysaccharides (EPS) offered physical and chemical hindrances to direct anaerobic degradation. Hence, proper disintegration is mandatory for disrupting the network and to accelerate the biomass degradability. This can be intensified with the help of various biomass disintegration techniques, to hasten the slow and rate - deciding phase of hydrolysis and to enhance the biomethane production. Conversely, economical limitations of those techniques have restricted their scale-up and lab-to-field application. As to other techniques, microwave (MW) disintegration found many uses in the field of industries; in the discipline of environmental engineering; waste processing was a subject that attracted quite a lot of interests (Jones et al., 2002). MW disintegration is becoming upscale due to its remarkable combination of thermal and athermal effects. The thermal effect is related to rupturing of microbial cells with promising release of organic matter due to the internal heating and dissolution of the endocellular bound water (Uquichea et al., 2008). In addition, the thermal effect happens as soon as the hydrogen bonds of cell wall molecules dislocate by the microwave electric field, which subsequently alters the structure (Rougier et al., 2014).

Further, only a few reviews had discussed on improving the effectivity of MW energy through deflocculation, prior to MW disintegration. The presence of EPS can deed as a barrier for the enzymes entrapped on biomass matrix, which restrict the liberation of organic substances into the matrix and also lessen the surface area for the succeeding disintegration. This in turn limits the accessibility of organic compounds to methanogenic microbes during anaerobic degradation. Current research studies suggest that, cations are significant which provides

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binding between negatively charged endobiopolymers to microbial flocs. The nature of mono-, di- or trivalent cations decides the biomass degradability and the high-quality choice to digest the waste activated biomass (Keiding and Nielsen, 1997). The binding ions upholding the joining of matrix are easily removed with the support of the cation binding agents, thereby liberating increased volume of proteins, carbohydrates and soluble organics, which could be doubtlessly used in anaerobic digestion. When the divalent cations are in comparison, it can be cited that Ca^{2+} hooked up better bonding with negatively charged sites than Mg^{2+}, because Ca^{2+} reduces radius of hydration (0.96 nm for calcium; 1.08 nm for magnesium) that may be effortlessly lost during bond formation. Thus it may be concluded that, Ca^{2+} is an excellent flocculator amongst the cations (Keiding and Nielsen, 1997). The Ca^{2+} associated with the exopolymers form alginate-like gel structure, which is taken into consideration to be the backbone of cell structure. It can be effectively removed by using calcium specific chelating agent EGTA (Bruus et al., 1992). The addition of EGTA cause decrease in the biomass pH, which in turn influences the release of Ca^{2+} as described by Bruus et al. (1992). As a result, the endobiopolymers and other organic materials adsorbed on the biomass matrix got liberated to enhance the succeeding MW disintegration. To the best of our knowledge, researches about the impact of EGTA on deflocculation prior to microwave disintegration were seldom mentioned. Many researchers have used EDTA for deflocculation process. (Jachlewski et al., 2015; Kavitha et al., 2013; Merrylin et al., 2013). So far, deflocculation using EGTA was not studied with the combination of microwave, specifically in the dairy waste activated sludge. Though EDTA was cheaper than EGTA, the concentration of EDTA suggested by researchers for deflocculation is high. For example, Kavitha et al. (2013) and Merrylin et al. (2013) have used relatively very high concentration of about 0.2 g/g SS and 0.4% of EDTA which may lead to environmental and ecological problems. In addition, the researchers have achieved only 17.33 and 25% of COD solubilization through EDTA mediated sludge disintegration. Therefore, with the aim to achieve higher deflocculation at very low dosage of EGTA and to attain better solubilization in excess of 30%, in the present study, it was planned to deflocculate the sludge with EGTA. Hence, this article provides an extended study on the influence of deflocculation by means of a calcium specific chelant EGTA, prior to microwave disintegration, on the way to increase the energy in a profitable way and to lessen the energy necessity for the subsequent method.

The major goals of the current work includes, (1) to disrupts the biomass flocs, with a calcium specific chelating agent (EGTA) (2) to delve into the effect of EGTA mediated MW on biomass disintegration (3) to investigate the disintegration efficiency on subsequent methane production potential (4) to scrutinize the feasibility of implementing EGTA mediated MW disintegration process to demonstrate their economic value at large scale.

2. Methods

2.1. Substrate and characterization

Dairy waste activated biomass was collected from the secondary sedimentation tank of Aavin Dairy industry located in Madurai, Tamilnadu, India. Samples were collected and stored at 4 °C. Before the commencement of experimentation, physico-chemical parameters of the substrate were characterized. Table 1 summarizes the initial characteristics of the raw substrate. To minimize random errors, every experiment was tripled and the results were averaged (Table 1. Characteristic of Substrate and Rumen Inoculum).

2.2. Deflocculation using calcium specific chelating agent - EGTA

Deflocculation of EPS matrix was performed with 500 mL of sludge taken in 1 L conical flask, with various distinct dosages of EGTA (0.001–0.03 g/g SS) at 10 min for effective disassociation of EPS matrix. A preliminary work was executed by varying the reaction time 0–60 min (Data not shown). Based upon that, 10 min reaction time was considered to be optimal for efficacious disassociation of EPS matrix. Two numbers of replicates were used. The error bar represents the SD.

Table 1
Characteristic of substrate and rumen inoculum.

Initial Characteristics	Substrate	Inoculum
Total solids (mg/L)	22,000 ± 300	19,500 ± 200
Total COD (mg/L)	24,000 ± 400	14,700 ± 300
Soluble COD (mg/L)	400 ± 10	370 ± 15
Suspended solids (mg/L)	20,000 ± 200	11,750 ± 350
Volatile solids (mg/L)	16,150 ± 300	9400 ± 250
Soluble protein (mg/L)	41.3 ± 0.5	130 ± 20
Soluble carbohydrate (mg/L)	5.2 ± 0.1	55 ± 10
pH	7.15	7.12

2.3. MW disintegration

A commercial IFB microwave oven (M-30SC2, F-2450 MHz and P-900 W, Manufacturer- Ge India Industrial Pvt Ltd) was used to expose the irradiation for biomass disintegration. In each trial, a thoroughly mixed sample volume of 500 mL in a polytetrafluoroethylene vessel was placed in the microwave at varying power inputs of 30–800 W and the contact time was varied from 1 to 30 min. The experimental temperature was observed from 20 to 120 °C during MW disintegration and the calculation of specific energy was enumerated rendering to the earlier study specified by Parvathy et al. (2016). The physico-chemical parameters were analyzed at different time intervals once the samples had been collected.

2.4. Biomethane Potential (BMP) assay

BMP experimentation was carried out to estimate the efficacy of disintegration techniques by means of methane generation and was performed using the method defined by Uma Rani et al. (2012a). The substrates were labeled as S1 (Control - untreated biomass), S2 (MW) and S3 (EGTA-MW). The rumen microorganisms of livestock dung were used as an inoculum, which have an excessive degradation efficiency and conversion rate. Table 1 depicts the characteristics of the inoculum. The substrates and inoculum have been introduced collectively within the reactors in the ratio of 3:1 (180 mL: 60 mL). These had been protected hermetic and in the end, purged to ensure strict anaerobiotic conditions for the better growth of anaerobic microbes (Uma Rani et al., 2013b). These experiments were executed with duplicate samples and kept incubated (37 °C; 150 rpm) in a digital orbital shaker (IKA KS 130). The biogas produced was quantified through placing a needle at the septum. The displacement of syringe plunger due to gas pressure in the reactor turned into measured, to record the quantity of biogas generated. Increase in biodegradability was evaluated in line with the quantity of biogas generation by biomass samples (MW and EGTA-MW). To check out the methane composition of biogas, thermo gas chromatograph (GC) geared up with the use of thermal conductivity detector and porapack Q column was used (G1311C, 1260, Baroda). Modified Gompertz model was used to assess the methane fraction for the control. Exponential model and logistic model were used for phase separated methane generation of MW and EGTA-MW samples. The percentage increase of methane was computed based on experimental and control value of methane generation.

2.5. Analytical methods

Volatile Solids (VS), Total Solids (TS), Suspended Solids (SS) Soluble Chemical Oxygen Demand (SCOD) and Total Chemical Oxygen Demand