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Enhancement of anaerobic degradation of sludge biomass through surfactant-assisted bacterial hydrolysis

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ABSTRACT

In the present paper, the study focuses on the effects of sodium dodecyl sulfate (SDS) surfactant on the release of extracellular polymeric substance (EPS) followed by pretreatment with a thermophilic protease-secreting bacterial strain on WAS (waste activated sludge). This in turn enhanced the subsequent anaerobic biodegradability. The extracellular polymeric substances were released using SDS (0.03 g/g SS of dosage) to stimulate the bacterial pretreatment. The thermophilic bacterial pretreatment results indicated that deflocculated (EPS released with SDS and pretreated with bacteria) sludge showed higher Suspended Solids (SS) reduction of about 27% and Chemical Oxygen Demand (COD) solubilization of about 24%, whereas flocculated (pretreated with bacteria alone) showed SS reduction of about 18% and COD solubilization of about 16%. The biogas production potential of deflocculated, flocculated, and raw (untreated) samples was found to be 2.5211 L/(gVS), 1.7677 L/(gVS), and 0.6140 L/(gVS), respectively. As a result, the EPS release followed by disintegration of sludge by bacteria enhanced the biogas production.

1. Introduction

The dairy industry is a large-scale food production industry. Wastewater generated by the dairy industry is categorized as raw waste and waste activated sludge (WAS), which has to be treated while considering various parameters. Increased amount of WAS has become a serious environmental issue. Anaerobic degradation is one of the most frequently used stabilization techniques in sludge treatment and known for its energy reserve in the form of biogas (Uma Rani et al., 2012). Anaerobic biodegradability assay test is used to measure the potential of biogas production. Waste activated sludge needs additional processing for disposal due to rate-limiting cell lysis. This is because the cell walls and the membranes of prokaryotic organisms are composed of complex organic materials, such as peptidoglycan and teichoic acid, which are recalcitrant to biodegradation. Sludge reduction has been proved to be efficient to circumvent this problem. To improve the digestion efficiency and to increase the biogas production, different disintegration methods have been used. Pretreatment methods include physical, chemical, and biological treatment. Biological treatment includes enzymatic treatment and microbial treatment. The enzymatic treatment has one disadvantage, that is, the commercial enzymes are very expensive (Erikkson et al., 2002). In this research work, a thermophilic aerobic protease-secreting bacterial strain, *Bacillus licheniformis*, is used for sludge solubilization because it is immensely effectual for the rapid degradation of sludge (Juteau, 2006).

Extracellular polymeric substance (EPS) plays a significant role in the flocculation by interrelating with the organic part (Garnier et al., 2005). Therefore, release of EPS prior to

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disintegration enhances the speed and extent of liquefaction (Merrylin et al., 2014; Poornima et al., 2014; Kavitha et al., 2015a; Kavitha et al., 2015b; Sowmya et al., 2015; Ebenezer et al., 2015). Based on this, in the present study, EPS was released by the surfactant, SDS. Among many surfactants, the anionic SDS is proved to be an effective surfactant in solubilizing EPS. It disrupts non-covalent bonds within and between EPS; thus denaturing them, resulting in the loss of their native conformation and function. The SDS is easily biodegradable both under aerobic and anaerobic conditions. It is the major anionic surfactant widely used in industrial and bioprocessing formulations due to its excellent dispersive, wetting, and emulsifying properties and relatively low cost when compared to other synthetic surfactants (Ying, 2006; Yeldho et al., 2011). The immobilization diminishes the enzyme action on the matrix, thereby diminishing the effectiveness of biopretreatment. The destabilization of floc structure would stimulate efficiency of WAS pretreatment by solubilizing the interrelating solids; hence, it is planned to release EPS from the sludge before pretreatment. By removing the EPS with SDS, the biological polymers that are immobilized in matrix got liquefied and then dissolved into the liquid part as the surfactant has the trait of liquefaction (Myers, 2006). Surfactant has the ability to liquefy a larger amount of soluble organics since it has the feature of solubilization (Li and Yang, 2007) and therefore hasten the liquefaction rate of solid substances into the liquid phase, which will be able to weaken the immobilization of floc matrix, set free the trapped enzyme (within the floc matrix and on the cell surface), and also release more organics from floc matrix. Then these organics were consumed by the inoculated bacteria for their growth.

As an outcome of releasing EPS, the immobilized organics in the flocs matrix are set free and consumed as substrates by the bacteria, thereby enhancing the pretreatment. As to our knowledge, surfactant-stimulated thermophilic bacterial pretreatment has not been reported so far. In the present study, the novel insight of surfactant-stimulated thermophilic bacterial pretreatment has been assessed and the results are reported in the present paper.

The core objectives of this work are to (1) improve WAS hydrolysis with an appropriate surfactant (SDS), which competently releases EPS without cleavage of cells and efficiently accelerates sludge enzyme action at a minimal dosage, (2) assess the potential of EPS release on biopretreatment, (3) assess the kinetic study of disintegration, and (4) evaluate the enhancement of degradability and biogas production of deflocculated sludge through bacterial disintegration.

2. Materials and methods

2.1. A collection of the sample

The waste activated sludge was collected from a dairy plant at Tirunelveli in Tamil Nadu (India). Samples were stored at 4 °C. The initial characteristics of the raw sludge were summarized as follows: pH–6.7 ± 0.5; Total solids (TS)—9160 ± 120 (mg/L); Suspended Solids (SS)—6600 ± 95 (mg/L); Total Dissolved Solids (TDS)—2560 ± 35 (mg/L); Volatile Suspended Solids (VSS)—4220 ± 55 (mg/L); Total Chemical Oxygen Demand (TCOD)—10,000 ± 130 (mg/L); and Soluble Chemical Oxygen Demand (SCOD)—100 ± 10 (mg/L)

2.2. Bacterial strain

A thermophilic protease-secreting bacterial strain isolated and identified as *B. licheniformis* in the previous work (Merrylin et al., 2013) was used for the present study. The essential growth factors (pH, temperature, incubation time) for the bacterial strain were found to be 55 °C, 6.5, and 24 h, respectively. The strain was cultured in a 1-L conical flask containing 500 mL of nutrient medium at pH 6.5 and 55 °C and was kept in an orbital shaker at 150 rpm for 24 h. Then the culture was collected at 24 h and employed for pretreatment.

2.3. Optimization of bacterial concentration for cell disintegration

Optimization of bacterial concentration for cell disintegration was carried out in 250-mL conical flasks containing 100 mL of sludge. Different concentration (1–5 g dry cell weight/L of nutrient medium) of protease-secreting strain was added to all flasks and incubated at 55 °C for 24 h. Reduction of SS and solubilization of COD was examined to obtain the optimal concentration of bacteria for biomass disintegration. The COD solubilization was calculated using the following equation (Khac-Uan et al., 2012):

$$a = \frac{(\mathrm{SCOD}_p - \mathrm{SCOD}_i)}{\mathrm{TCOD}_i - \mathrm{SCOD}_i} \quad (1)$$

where, α solubilization efficiency (%),
SCOD_p SCOD concentration of the sludge after disintegration (mg/L),
SCOD_i SCOD concentration of the sludge before disintegration (mg/L),
TCOD_i TCOD concentration of the sludge before disintegration (mg/L).

2.4. Release of EPS experiment

EPS release experiment was carried out with 100 mL of sludge in various conical flasks. In each flask, different concentrations of SDS from 0.005 to 0.1 g/g SS were added. The contents were mixed using a mechanical shaker for 1 h. Then the samples were centrifuged at 10,000 × *g* for 20 min. The liquid part was used for further analysis.

2.5. Pretreatment by bacterial strain

Pretreatment was performed with 100 mL of deflocculated sludge (EPS released) and inoculated with bacterial strains in a conical flask. Similarly, 100 mL of control (raw, untreated sludge) and flocculated (bacterially pretreated alone) sludges were taken in two separate conical flasks with the aim to study the competence of deflocculation in subsequent bacterial disintegration. All the flasks were incubated at 55 °C for 24 h at 150 rpm.

2.6. Anaerobic biodegradability assay

Anaerobic biodegradability assay was carried out in three 300-mL serum bottles (1, 2, and 3) to compare the biogas generation effectiveness of deflocculated, flocculated, and raw sludge. The experiments were carried out as per the previous work (Kavitha et al., 2014a; Gayathri et al., 2015). The