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Sodium thiosulphate induced immobilized bacterial disintegration of sludge: An energy efficient and cost effective platform for sludge management and biomethanation

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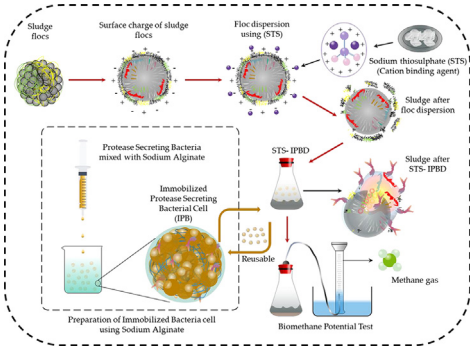
GRAPHICAL ABSTRACT



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ABSTRACT

The present study aimed to gain better insights into profitable biomethanation through sodium thiosulphate induced immobilized protease secreting bacterial disintegration (STS-IPBD) of sludge. STS disperse the flocs at 0.08 g/g SS of dosage and assists the subsequent bacterial disintegration. Immobilization of bacteria increases the hydrolytic activity of cells towards effective liquefaction of sludge. A higher liquefaction of 22% was accomplished for STS-IPBD when compared to immobilized protease secreting bacterial disintegration (IPBD alone). The kinetic parameters of Line Weaver Burk plot analysis revealed a maximal specific growth rate (μmax) of 0.320 h^{-1} for immobilized cells when compared to suspended free cells showing the benefit of immobilization. Floc dispersion and immobilization of bacteria imparts a major role in biomethanation as the methane generation (0.32 gCOD/g COD) was higher in STS-IPBD sample. The cost analysis showed that STS – IPBD was a feasible process with net profit of 2.6 USD/Ton of sludge.

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1. Introduction

The global energy requirement is anticipated to be increase to 53% in 2035 (IEO, 2011). Energy safety and ecological sustainability are the main embryonic concerns in the globe which can only be managed via the divergence in the energy sources and green fuels. In this perspective, anaerobic digestion (AD) of waste activated sludge (WAS) biomass is the possible solution to solve these issues. WAS can be utilized as a profitable renewable energy sources due to its high organic content which can be converted into clean energy rich biogas containing 65% of methane that can be utilized as heat or electrical energy of biofuel (Mottet et al., 2010). However, biodegradable potential (hydrolysis) of AD process is limited due to the complex nature and recalcitrant substances of sludge biomass (Ushani et al., 2017a,b). The biodegradability of WAS can be accelerated by sludge disintegration methods such as chemical, mechanical, physical, biological and combinative disintegration prior to AD (Rajesh Banu et al., 2012; Kavitha et al., 2016; Eswari et al., 2017; Ushani et al., 2017a; Yukesh Kannah et al., 2017a). Among various pretreatment processes, an environmental friendly disintegration of sludge is the biological method (Rajesh Banu et al., 2017). Biological disintegration has many benefits such as toxic less, environmental compatible and better liquefaction. Thus, biological disintegration of sludge is found to be an efficient disintegration process for enhanced AD.

In biological sludge disintegration, the existing time of inoculated microbes in the medium is identified to be ineffectual (Ushani et al., 2017a) due to the prevalence of toxic substance, and metal ion. In order to extend the existence period of inoculated microbes, immobilization of bacteria is essential in biological disintegration of WAS. Through immobilization, certain bacteria can be selected and immobilized in the carrier to proliferate its strength so that the immobilized microbes is healthier in solubilizing organic compounds (Paliwal et al., 2015) of sludge. Moreover, immobilized cells can be reutilized several times without significant loss of their actions and their enzyme secreting potential. Thus, immobilization of bacterial cells is a beneficial and profitable technique.

In WAS, an Extracellular Polymeric Substances (EPS) layer that are formed around the sludge biomass adheres together to form flocs. This layer keeps the floc firm and it increases its mechanical strength. By removing the EPS, flocs are dispersed and as a result the surface area for the subsequent bacterial disintegration can be enhanced. Several methods are employed for floc dispersion. The chemical methods involves use of chemicals such as surfactants- dioctyl sodium sulfosuccinate (DOSS) – (Ushani et al., 2016, 2017b), alkali -potassium hydroxide- (Rajesh Banu et al., 2017), acids -citric acid- (Gayathri et al., 2015). In this proposed work, an attempt has been taken to facilitate the surface area for bacterial disintegration and to enhance the methane generation. The main objectives of the present study were: (1) to assess the effective role of sodium thiosulphate on floc dispersion with minimal biomass stress (2) to immobilize the bacterial cells for effective catalytic and hydrolytic activity (3) to evaluate the effect of floc dispersion on subsequent bacterial disintegration (4) to assess the effect of floc dispersion followed by bacterial disintegration on energy efficient methane recovery (5) to evaluate the economic feasibility of implementing the proposed disintegration on large scale.

2. Materials and methods

2.1. Sludge collection and characterization

In the present work, sludge was collected from waste water treatment plant in Kerala. The sludge was characterized as: pH – 6.8, Total Chemical oxygen demand – 10,100 mg/L, Soluble chemical oxygen demand −110 mg/L, Suspended solids – 7000 mg/L, Total solids – 12,000 mg/L.

2.2. Bacterial strains

The bacteria used for disintegration was isolated, screened for hydrolytic enzyme , such as protease and identified as *Bacillus cereus* in the previous work were used in the present study (Ushani et al., 2016).

2.3. Immobilization of bacteria

Three grams of sodium alginate was dissolved in 100 mL of growth media so that the final solution weighs 3% of alginate. About 250 mg of wet bacterial cells were completely suspended in the alginate solution. The bacteria-alginate mixture was dropped from a height of 20 cm into 1000 mL of crosslinking solution. The crosslinking solution is prepared by adding an additional 0.05 M of $CaCl_2$ to the growth media. The calcium crosslinking solution is agitated using a magnetic stirrer. Beads were formed at room temperature as soon as the sodium alginate drops come in direct contact with the calcium solution. The beads were harden for 1–2 h and washed with a fresh calcium crosslinking solution.

2.4. Floc dispersion by sodium thiosulphate

The optimization of sodium thiosulphate (STS) was performed in ten 250 mL conical flasks containing 100 mL of sludge. Each conical flask contained STS concentration ranging from 0.01 to 0.18 g/g SS. These samples were placed in an orbital shaker for 1 h for adequate mixing. Later, the sludge was centrifuged at 10,000 rpm for 5 min. The pellets were discarded and the supernatant was analysed.

2.5. Bacterial disintegration

During bacterial disintegration, 250 mg of immobilized wet bacterial cells were inoculated into conical flask containing 500 mL of floc dispersed sludge. These flasks were incubated at 35 °C for 36 h at 150 rpm. Similarly, parallel experiments were conducted with immobilized protease secreting bacterially disintegrated sludge (raw sludge + 250 mg of immobilized wet bacterial cells) and control (raw sludge) sludge samples to assess the efficiency of floc dispersion.

2.6. Assessment of growth dynamics of inoculated immobilized and suspended free cells

To assess the efficiency of immobilization on increased growth, hydrolytic and catalytic activity of inoculated bacterial strain, an experiment was done with immobilized and suspended free cells with interest focussed on specific growth rate, intracellular enzyme activity and soluble organic matter (SOM) release. The growth dynamics experiment was done as per the procedure detailed by Kavitha et al. (2015). In case of immobilized cells, the cell beads were dissolved in water and subjected to further analysis.

2.7. Methane potential assay

In methane potential assay, the substrates used were raw (untreated), immobilized protease secreting bacterially disintegrated (IPBD) and sodium thiosulphate induced immobilized protease secreting bacterially disintegrated (STS-IPBD) sludges. The sludges that were disintegrated or pretreated as mentioned in Section 2.5 were used as substrates. The experiments were done at 35 °C in three 300 mL capacity reactors. Bovine rumen fluid was used as inoculum. An inoculum to substrate ratio of 3:1 was used. The reactors were purged with N_2 to maintain the anaerobic environment. Later, the reactor was sealed with a rubber septum to make it air tight, and then placed on a shaker (150–200 rpm). The biogas was quantified through needle insertion. Syringe plunger was displaced by pressure inside the reactors and volume was noted. The methane content was estimated with a gas chromatograph. The methane content was modelled through nonlinear regression with first order kinetics.