



# Achieving profitable biological sludge disintegration through phase separation and predicting its anaerobic biodegradability by non linear regression model



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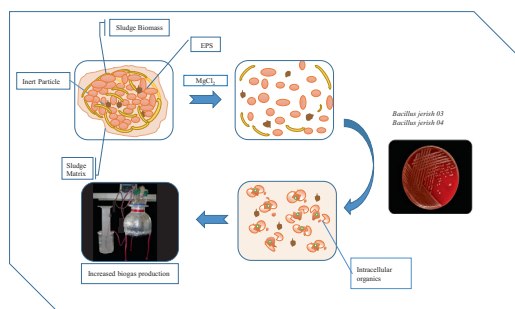
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## HIGHLIGHTS

- MgCl<sub>2</sub> induced bacterial pretreatment is a profitable disintegration process.
- The disintegration was proficient with solubilization of about 21.4%.
- The kinetic analyses reveal that floc disrupted sludge showed faster rate 0.005 h<sup>-1</sup>.
- Biodegradability was comparatively higher in this effectual disintegration.
- Floc disrupted sludge shows higher net profit of about 27.3 USD.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The present study investigates the influence of phase separated disintegration on waste activated sludge (WAS) biodegradability in a profitable manner. An effort has been taken in this study to disintegrate WAS in an economical way by deflocculating the WAS with 0.04 g/g SS of MgCl<sub>2</sub>. The bacterial cell disruption was proficient at 42 h, where floc disrupted-bacterially pretreated sludge showed prominent solubilization of about 21.4% than the bacterially pretreated sludge (10.5%). The kinetic analysis of disintegration (suspended solids (SS) reduction and particulate chemical oxygen demand (PCOD) reduction) for control, bacterially disintegrated, floc disrupted-bacterially disintegrated sludges was observed to be first order with rate constants 0.001, 0.003, 0.005 and 0.001, 0.002, 0.005 (h<sup>-1</sup>), respectively. The proposed method articulates the effectiveness of sludge disintegration in terms of VFA production of about 640 mg/L in anaerobic fermentation experiment. The model (exponential rise to maximum) explaining the Biochemical methane potential (BMP) assay was implemented in Matlab. 12v. The parameter estimation analysis affords an estimate of parameter uncertainty and correlation, and is obviously explains that floc disrupted-bacterially pretreated sludge showed higher biodegradability of about 0.22 (g COD/g COD) than the others. Cost estimation of the present study revealed net profit of about 27.3 USD in floc disrupted-bacterially pretreated sludge.

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## 1. Introduction

The activated sludge process is the most frequently used technique in waste water treatment plants (WWTPs). On the other hand

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the surplus sludge generated by this technique creates problems with environmental pollution [1]. To reduce and stabilize excess sludge, anaerobic and aerobic digestions are the mostly employed processes for treating these organic wastes. Among these two, anaerobic digestion (AD) has been extensively applied as a promising technique for treating wastes, since it converts organic waste into biogas in the form of methane, which is a renewable energy source [2]. However, it is difficult to overcome the rate limiting step (hydrolysis) of AD [3] as waste activated sludge (WAS) is mainly composed of microbial cells entangled in extracellular polymeric substance (EPS), which is a brawny structure against hydrolytic enzyme [4]. On this basis, a number of disintegration approaches such as ultrasonic, thermal, chemical and biological have been developed to bypass the hydrolytic phase of AD and to increase the biogas production [5]. Among them, biological pretreatment is a most promising technique because of ease operation, maintenance and ecofriendliness [6]. EPS are microbial products located on or outside the cell surface and are formed by a complex mixture of proteins, carbohydrates, lipids, DNA, and humic acid substances [7,8]. These biological substances play a significant role in flocculation [9]. Hence it is indispensable to release the EPS (deflocculate the floc matrix) before disintegration to extent the speed of degradability. On the basis of above mentioned reports, the EPS was removed with the chemical,  $MgCl_2$ . As a result, the particulate biopolymers adsorbed on sludge matrix got released. The extracellular enzymes entrapped on the sludge matrix which further enhances hydrolysis (bacterial pretreatment) of WAS. Hitherto, reports regarding the removal of EPS with  $MgCl_2$  followed by subsequent bacterial pretreatment have been barely reported. By this means, the key objectives of the present study is (1) to evaluate the impact of EPS removal on two phase disintegration (EPS removal followed by bacterial pretreatment) (2) to augment the action of extracellular enzymes on sludge matrix through floc disruption (3) to assess anaerobic biodegradability of pretreated sludge by non linear regression and evaluation of cost.

## 2. Materials and methods

### 2.1. Collection of sample and characterization

The sludge employed in the present study was sampled from municipal WWTP in Kerala. The characteristics of sample was characterized as: pH = 6.6, Total COD = 10,100 mg/L, SCOD = 100 mg/L, Suspended solids = 7000 mg/L, Total solids = 12,500 mg/L.

### 2.2. Bacterial cultures employed

The bacterial cultures employed in the present study were isolated and identified and screened for enzymes protease and amylase in our previous works (Lakshmi et al. [6]; Kavitha et al. [7]). The bacterial culture hold two strains (*Bacillus jerish* 03 Accession number KC597266 and *Bacillus jerish* 04 Accession number KC597267). The optimal temperature, pH, and incubation time for these cultures were 40 °C, 6.5, and 42 h, respectively. These novel strains increase the usage of substrates by the combined action of enzymes protease and amylase and enhance the sludge solubilization. As WAS contains protein and carbohydrates as the main components, these mixed cultures initiates higher enzyme secretion which may perhaps improve the liquefaction of WAS further. These colonies were appeared as white in color, fibrous in shape with an uneven margin, increased elevation, crumpled surface and were translucent.

### 2.3. Cell dose determination for sludge disintegration

The cell dose determination experiment was carried out with 100 mL of WAS in five conical flasks of working volume 250 mL.

The conical flasks were inoculated with different dosages (1–5 g dry cell weight/L) of bacterial strains. The flasks were incubated at 40 °C for 42 h at 100 rpm. SS reduction and COD solubilization were analyzed to obtain the optimal bacterial dosage for WAS disintegration.

### 2.4. Floc disruption of WAS by $MgCl_2$

This experiment was carried out with 100 mL of WAS in eleven conical flasks of working volume 250 mL. The conical flasks were added with different concentration of  $MgCl_2$  begins from (0.003 to 0.08 g/g SS). Then the conical flasks were kept in the orbital shaker for 2 h at 100 rpm for complete mixing at a temperature of 30 °C. Then the samples were spin at 10,000g for 15 min. Watery portion was filtered to obtain the EPS.

### 2.5. Pretreatment of WAS with Bacterial strains

In Pretreatment experiment, 100 mL of floc disrupted (EPS removed) WAS was added with 2 g/L (dry cell weight) of bacterial strains in a conical flask. The cell dose was determined as per the previous work Kavitha et al. [7]. The contents were mixed with slow speed stirrer at 100 rpm for 42 h. Additionally, 100 mL of control and bacterially pretreated alone samples were taken in two conical flasks to evaluate the competence of floc disruption.

### 2.6. Analysis of growth increment of inoculated bacteria

Growth dynamics of inoculated bacteria was done as per the procedure described by Kavitha et al. [10].

### 2.7. Hydrolysis and acidification of WAS

Anaerobic fermentation experiments were performed in 300 mL serum bottles. The duration of experiment was three days. Substrate and inoculum were taken in the ratio of 9:1 in the bottles. The inoculum used was anaerobically digested sludge. The substrates used were control (raw sludge), bacterially disintegrated sludge and floc disrupted-bacterially disintegrated sludge. The activity of methanogens in the bottles was eliminated by treating the sludge at 102 °C for 30 min. The heated samples were cooled to room temperature and were added with 50 mM BESA (2-Bromoethane sulfonic acid). Then, the bottles were purged with nitrogen and placed in a shaker at 120 rpm for 72 h at 35 °C.

### 2.8. Biochemical methane potential assay

Anaerobic biodegradability assay was done as per the procedure described by Uma et al. [11]. The inoculum and substrates were taken in the ratio 3:1 according to the Standard German procedure [12] in 500 mL bottles. The inoculum used was Bovine rumen fluid. The substrates used were control (raw sludge), bacterially disintegrated sludge and floc disrupted-bacterially disintegrated sludge. The pH of the pretreated samples were adjusted to be in the range of 7.5–8 since there was a marginal drop in pH after pretreatment due to solubilization and release of intracellular materials. The methane content in the biogas was analyzed using a Baroda gas chromatograph (GC) equipped with a thermal conductivity detector and porapack Q column with hydrogen as carrier gas at a flow rate of 40 mL/min. The following first order kinetic model was employed to study the cumulative methane production:

$$Y_{(t)} = Y_{(fd)} \cdot (1 - \exp(-k_{hyd}t)) \quad (1)$$

where  $Y_{(t)}$  is the cumulative methane yield at digestion time  $t$  days (g COD/g COD added) [13],  $Y_{(fd)}$  is methane potential of the

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