Bioresource Technology 169 (2014) 700–706

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Improving the amenability of municipal waste activated sludge for biological pretreatment by phase-separated sludge disintegration method

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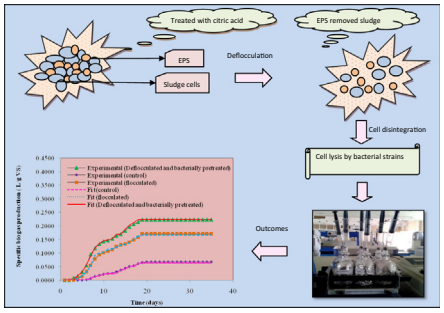
HIGHLIGHTS

- 0.05 g/g SS of citric acid dosage disrupt flocs & enhances sludge enzyme activity.
- Suspended solids reduction was superior in deflocculated sludge of about 16.2%.
- Kinetic parameters show that rate was 4 times higher in deflocculated sludge.
- Citric acid mediated bacterial pretreatment enhances biogas production potential.
- Biogas production was higher in deflocculated sludge than the control.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 15 July 2014
Accepted 16 July 2014
Available online 23 July 2014

Keywords:
Extracellular polymeric substance
Citric acid
Phase separated disintegration
Anaerobic biodegradability
Waste activated sludge

ABSTRACT

The significance of citric acid, a cation binding agent, was investigated for the exclusion of extracellular polymeric substance (EPS) from waste activated sludge (WAS) and anaerobic biodegradability following enzymatic bacterial pretreatment. EPS was removed with 0.05 g/g SS of citric acid. The results of pretreatment found that the suspended solids reduction and chemical oxygen demand solubilisation were 21.4% and 16.2% for deflocculated-bacterially pretreated sludge, 14.28% and 10.0% for flocculated sludge (without EPS removal and bacterially pretreated) and 8.5% and 6.5% for control sludge (raw sludge), respectively. Further assessing anaerobic biodegradability, the biogas yield potential of deflocculated and bacterially pretreated, flocculated, and control sludges were found to be 0.455 L/(g VS), 0.343 L/(g VS), and 0.209 L/(g VS), respectively. Thus, phase-separated disintegration enhanced anaerobic biodegradability efficiently.

1. Introduction

Activated sludge method is the most commonly utilised practice in urban wastewater treatment plants (WWTPs). A noteworthy quantity of waste activated sludge (WAS) is produced by this process, which is a pretence to trouble with environmental contamination (Burger and Parker, 2013), and should undergo treatment processes prior to final disposal. Disposal expense accounts for approximately 40–60% of all WWTP functioning outflow (Uma et al., 2012).

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http://dx.doi.org/10.1016/j.biortech.2014.07.065
0960-8524/

Anaerobic and aerobic digestions are widely employed in most WWTPs to stabilize excessive sludge generation. Anaerobic degradability has been used as a proficient treatment, changing organic waste into biogas, a renewable energy resource. Renewable energy plays a vital role in decreasing greenhouse gases (Jagadish et al., 2012). An anaerobic biodegradability assay is a cost-effective method for critical evaluation of anaerobic digestibility and possible biogas production among various substrates (Thaniya and Narumol, 2012; Kavitha et al., 2014).

To advance anaerobic digestion competence, mechanical, chemical, thermal and biological disintegration techniques have been applied as pretreatment. These processes rupture cell walls of biomass and release intensely accessible organic materials from the internal part of cells (Appels et al., 2008). Compared to other pretreatment methods, biological pretreatment can effectively solubilise particulate organic matter in the sludge in an eco-friendly way. Enzymatic pretreatment has advantages, such as higher yield, low energy requirements, and mild operational conditions. However, commercial enzymes are costly. Therefore, adding lytic bacteria may serve as a suitable alternative.

Extracellular polymeric substances (EPS) of natural origin are ever-present in surplus sludge and were primary components of flocs (Monique et al., 2008). The build-up of EPS signifies up to 80% of activated sludge mass and plays a crucial role in flocculation due to notable content of complex molecules and cations (Yu et al., 2009). EPS diminishes the connection between the sludge enzyme and substrate and reduces the substrate dissemination competency in the floc matrix (Jang et al., 2014). Therefore, it is crucial to remove EPS to augment ensuing bacterial pretreatment.

In this study, citric acid was used to remove cations that bind with EPS to enhance subsequent bacterial pretreatment by exposing immobilized enzymes to substrates that further enhance sludge degradability. The core objectives were: (1) to remove EPS with citric acid for enhancing enzyme secreting bacterial pretreatment (2) to examine growth dynamics and kinetics of inoculated bacterial strain and (3) to examine the competence of bacterial pretreatment in further anaerobic degradability studies and biogas production.

2. Methods

2.1. Sludge sampling

Waste activated sludge was sampled from a secondary clarifier of a sewage wastewater treatment plant in Trivandrum, Kerala. Preliminary characteristics of WAS were: pH = 6.7, TCOD = 10,200 mg/L, SCOD = 110 mg/L, SS = 6900 mg/L, VS = 5400 mg/L.

2.2. Bacteria

The bacterial culture contained two strains (*Bacillus jerish 03* Accession number KC597266 and *Bacillus jerish 04* Accession number KC597267) that were isolated from municipal WAS and previously identified (Veera Lakshmi et al., 2014). The most favourable temperature, pH and time for augmentation of bacterial strains were 40 °C, 6.5 and 42 h, respectively. The strains were mass-cultivated in a 1-L jar fermentor with 500 mL of nutrient broth with an agitation speed of 150 rpm for 42 h. Cells were collected in the exponential phase (42 h) and used for pretreatment.

2.3. Removal of EPS with citric acid

Citric acid dosage selection was performed in nine 250-mL conical flasks containing 100 mL of sludge and citric acid with 0.01–0.09 g/g SS concentration. Mixtures were incubated for 3 h with constant agitation to ensure proper mixing (Merrylin et al., 2014) in a shaker at 150 rpm. Samples were centrifuged at 10,000×*g* for 15 min, and the liquid was analysed for soluble EPS.

2.4. Phase separated disintegration

A 100 mL portion of deflocculated (EPS removed) sludge was inoculated with 2 g dry cell weight/L of bacterial inoculum in a 250-mL conical flask and incubated for 42 h at 40 °C and 120 rpm. Two flasks, one control and one without EPS removal inoculated with bacteria alone (flocculated), were sustained to compare the effectiveness of EPS removal.

2.5. Growth dynamics of inoculated bacterial strains

A 250-mL conical flask with 100 mL of sludge was taken. The sludge was washed 10 times with sterile distilled water, then inoculated with bacterial strains. Cells were inoculated at a concentration of 69×10^7 CFU/mL. Growth of the inoculated strains was monitored at regular time intervals using the viable plate count technique by plating the mixed liquor on a selective medium (Mannitol salt agar medium). Similarly, 100 mL of sludge without bacterial inoculation was maintained as a control, and the colonies were quantified by plating on Mannitol salt agar medium to insure comparison with the growth of inoculated strains.

2.6. Anaerobic biodegradability assay

A batch assay was performed at 35 °C in three reactors (A, B, and C) to study biogas production efficiency of deflocculated-bacterially pretreated, flocculated and bacterial inoculated and raw sludge. In reactor A, 50 mL of deflocculated bacterially pretreated sludge was mixed with 150 mL of bovine rumen juice and fed into a 300-mL reactor. In reactor B, 50 mL of flocculated sludge (treated with bacteria alone) was mixed with 150 mL of bovine rumen fluid and fed into a 300-mL reactor. Reactor C was run as a control, in which 50 mL of raw sludge was mixed with 150 mL of inoculum (bovine rumen fluid) and fed into a 300-mL reactor. As bovine rumen fluid seeded in a reactor as inoculums produces noteworthy results on increasing the biogas production rate (Budiyono et al., 2010). Following the addition of substrates and inoculum, the head space above the sample in reactors was purged with N gas to eliminate oxygen from the system to insure anaerobic conditions. Mixing was performed daily using an orbital shaker (Digital IKA KS 130) at 150 – 200 rpm. Biogas was measured by inserting a needle with syringe into the septum. Gas pressure in the reactor was allowed to displace the syringe plunger, and the displaced volume was recorded. A modified Gompertz equation was used to study cumulative biogas generation and the kinetics of biogas production.

$$B_t = B * \exp[-\exp[R_b/B * \exp(\lambda - t) + 1]] \quad (1)$$

where B_t is cumulative biogas produced (mL) at time (t), B is biogas production potential (L/(g VS)), R_b is the maximum biogas production rate (L/(g VS .d)), and λ is lag phase (days), which is the minimum time taken to produce biogas. The constants B, R_b, and λ were determined by non-linear regression method with the help of Polymath software (Version 6, Shareware company).

2.7. Analytical methods

SS, Total COD, VS, Soluble COD were calculated according to standard methods from the American Public Health Association (APHA, 2005). Protein, carbohydrate and DNA were measured according to methods described by Merrylin et al., 2013. The extent of extracted EPS was calculated by totting up of protein and