Renewable Energy 98 (2016) 148–152

Contents lists available at ScienceDirect

Renewable Energy

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

Community dynamics and significance of anaerobic protozoa during biomethanation of lignocellulosic waste

Priya Prabhakaran, Arya Bhasi, Shabeer Ali, Nimi Narayanan, Manilal Vattackatt Balakrishnan, Krishnakumar Bhaskaran*

Environmental Technology, CSIR-National Institute for Interdisciplinary Science &Technology, Industrial Estate (PO), Thiruvananthapuram, 695 019, India

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form
8 March 2016
Accepted 25 March 2016
Available online 1 April 2016

Keywords:
Anaerobic digestion
Cellulase
Ciliate
Flagellate
Lignocellulose
Pectinase

ABSTRACT

The diversity and community dynamics of anaerobic protozoa and their functional role during anaerobic digestion of a typical lignocellulose biomass in a lab scale leach bed coupled UASB reactor is reported in this study. The functional role played by different protozoa during various stages of methanogenesis was analyzed through linear regression analysis of individual protozoon counts with major hydrolytic enzyme activities, volatile fatty acid levels and biogas production. The protozoa community in the digester was represented by ciliates (*Metopus*, *Cyclidium* and *Colpoda*) and flagellates (*Rhyncomonas, Menoidium* and *Bodo*). Regression analysis revealed the relationship between total protozoa counts with the activity of cellulase ($R^2 = 0.71$) pectinase ($R^2 = 0.50$) amylase ($R^2 = 0.53$) and xylanase ($R^2 = 0.34$), total volatile fatty acid levels ($R^2 = 0.86$) and biogas production ($R^2 = 0.78$) in the digester. Moreover, it was found that both volatile fatty acid and biogas production is correlated with ciliate and flagellate populations. This study underlines the importance of both ciliates and flagellates in the anaerobic digestion process and, more specifically, the contribution by individual protozoa on hydrolysis, which is the rate limiting stage in anaerobic digestion.

1. Introduction

Anaerobic digestion of lignocellulosic biomass is a complex microbial process where a series of metabolically interacting microorganisms convert organic matter into methane, carbon dioxide, reduced nitrogen and sulphur compounds. The sequential steps involved in the digestion process are hydrolysis, acidogenesis, acetogenesis and methanogenesis. Among these hydrolysis is often identified as the rate limiting step, where complex organic matter is broken down to simpler organics by hydrolytic enzymes [1,2]. Previous studies on the microbial ecology of anaerobic digestion have focused mainly on the diversity and involvement of bacteria and archaea, inorganic break down and/or methane production [3]. But, in addition to bacteria and archaea, higher trophic organisms such as protozoa and micro-metazoa like rotifers and nematodes are also common inhabitants of anaerobic environments including engineered treatment systems. However, compared to aerobic treatment systems, these higher trophic level organisms have not received much attention in anaerobic treatment systems. Studies on the diversity, population dynamics and ecological niches occupied by protozoa in anaerobic environments are mainly confined to rumen and other anaerobic natural environments [4–6]. Limited studies have addressed protozoa in anaerobic bioreactors for wastewater treatment [7–11]. With this background, the present study focuses on the diversity, population dynamics and role of protozoa during anaerobic digestion of a lignocellulose feedstock (Water Hyacinth biomass) in a bioprocess unit. The results of the present study specifically the roles of different protozoa on the hydrolysis, acidogenesis and methanogenesis stages of lignocellulosic biomass.

2. Materials and methods

2.1. Lignocellulosic waste and anaerobic digestion

The lignocellulose waste biomass used in this study was water hyacinth (*Eichhornia* sp.) collected from a local eutrophic lake in Thiruvananthapuram district (India). A two stage process unit consisting of a 20 L capacity plastic Anaerobic Leach Bed Reactor (ALBR) coupled with an 8.5 L capacity glass Upflow Anaerobic

* Corresponding author.
E-mail address: krishna@niist.res.in (K. Bhaskaran).

http://dx.doi.org/10.1016/j.renene.2016.03.085
0960-1481/

Sludge Blanket (UASB) reactor was used for the digestion (Fig. 1). The bioreactor was maintained at room temperature (33±3 °C) and neutral pH conditions. At start up, two kg (wet weight) of mechanically crushed whole plant material was mixed with 2 L of anaerobic reactor liquor (as inoculum) and loaded into the first stage ALBR. Mixing of the contents in ALBR was done manually twice a day. Subsequent to digestion, leachate produced in ALBR was pumped into the UASB reactor using a peristaltic pump (Watson-Marlow-505 S). The UASB effluent was then recycled back to the digester. The duration for startup digestion of the biomass was 10 days and during this period no biogas was produced. The biogas produced in the system was measured with a wet gas low meter (INSRF-IRI101). The composition of biogas was not analyzed directly during this study, but the methane fraction was estimated by removing CO_2 using a caustic scrubber in the biogas line and comparing results. Using standard methods, regular analysis of parameters such as volatile fatty acids (VFA), chemical oxygen demand (COD) and alkalinity was performed to assess the reactor performance [12,13].

2.2. Analysis of hydrolytic enzyme activity in the ALBR

Quantitative analysis of cellulase, xylanase, pectinase and amylase in the ALBR was performed by estimating the amount of reducing sugar released from different substrates by the action of corresponding enzymes. ALBR leachate was centrifuged at 2862*g* for20 min (Hermle Z 83K). One ml of the filtered (0.2 μm filter) supernatant was used as the test sample for enzyme assay. The reducing sugar was estimated by 3,5-dinitrosalicylicacid (DNS) method [14]. One percentage solution of carboxy methyl cellulose (for cellulase assay), xylan (for xylanase assay), pectin (for pectinase assay) and starch (for amylase assay) were used as substrates. Standard curve for cellulase, xylanase, pectinase and amylase activity were plotted by taking 0.2–1.0 ml of 1.0 mg/ml stock solution of glucose, xylose, galacturonic acid and maltose respectively as substrates and the assay was done as mentioned above.

In order to localize the hydrolytic enzymes in the ALBR, the activity of one of the enzymes, cellulase was analyzed separately in bulk liquid (in suspension) as well as bound to flocks. After uniform mixing, approximately 100 ml sludge was withdrawn from the reactor. To estimate cell free cellulase, 10 ml sludge was centrifuged and the supernatant was used for direct enzyme assay. For estimating flock bound cellulase, 10 ml sludge was centrifuged and the pellet was resuspended in10 ml phosphate buffered saline (pH-7.0). The suspension was sonicated (Ultra-Turrax T25) for one minute, centrifuged again and the supernatant obtained was used for cellulase assay.

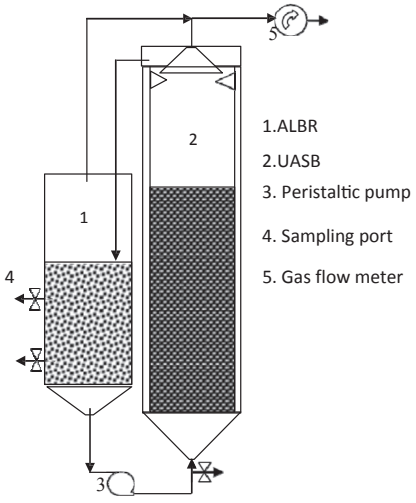

Fig. 1. Schematic representation of the two stage anaerobic bioreactor system: Anaerobic Leach Bed Reactor (ALBR) coupled with Upflow Anaerobic Sludge Blanket (UASB).

2.3. Protozoa diversity & population dynamics

The protozoa diversity and population dynamics were followed through periodic analysis of sludge samples from ALBR during one digestion cycle. The identification of different protozoa present in the sludge was done through microscopic observation (Leica DM 2500) under live, fixed and stained conditions according to the guidelines summarized by Patterson [15] and Foisner and Berger [16]. To arrest motility for detailed morphological observation, the protozoa was fixed in Schaudinn's fixative (Saturated $HgCl_2$ in 0.9% saline, 60 ml; ethanol, 30 ml and acetic acid, 10 ml) [17]. Staining was done with 1% Lugol'siodine to identify ciliates and flagellates [15]. Cell counting was done on a Neubaur counting slide on one ml of fixed reactor sample, diluted four times with distilled water. Each count was repeated three times and the average number was accounted with variations.

2.4. Statistical analysis of the data

The relation between protozoa dynamics and enzyme activity was assessed by regression analysis of total and individual protozoa counts, differential enzyme activity and concentration of VFA in the digester as well as biogas produced in the UASB reactor. The analysis was repeated for three different batch cycles and the average values were determined. The counting of the protozoa and analysis of other parameters were done on the same day to establish the relationship between them.

3. Results and discussion

3.1. Lignocellulose biomass & anaerobic digestion

The lignocellulose biomass (Water hyacinth) was digested in a two stage anaerobic process unit to minimize the direct inhibition of hydrolytic products (organic acids) on methanogens. The pH was not controlled at any stage of the process. A number of previous studies on the anaerobic digestion of organic wastes containing lignocelluloses reported two stages or multistage process without pH control as more effective than single stage systems [18,19]. During the digestion process a total of 36.5 L biogas was produced from 2.5 kg biomass (wet wt.) in 10 days, equivalent to 389 ± 20 ml methane/gram volatile solids (VS). This was comparable with 300–440 ml biogas/g VS reported in earlier studies [18,20]. Kivaisi and Mtila have reported around 440 ml biogas per gram VS from water hyacinth in 21 days [18]. A slightly lower biogas yield (300 ml/g VS) was reported from water hyacinth with poultry litter in 30 days [20].

3.2. Activity of hydrolytic enzymes in the ALBR and UASB reactor

Cellulase, xylanase, pectinase and amylase were the major hydrolytic enzymes identified and monitored in both reactors units (ALBR and UASB) in this study. Between the two reactors, the enzyme activity was more or less uniform with values for cellulase – 25.3 ± 2.4, Xylanase – 20.7 ± 1.85, Pectinase – 22.23 ± 3.68 and Amylase 9.34 ± 0.73 (all U/ml). The more or less uniform enzyme activity between the two reactors could be due to continuous circulation of liquid between ALBR and UASB reactors providing mixing up of enzymes through both systems. Compared