International Research Journal of Natural and Applied Sciences

ISSN: (2349-4077)

Impact Factor 5.46Volume 5, Issue 5, May 2018

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Influence of protozoa on biodegradation of Ocimum sanctum (Holy basil)

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ABSTRACT

This study was to investigate influence of protozoa on biodegradation of Ocimum sanctum (Holy basil). It was done by specifically controlling the growth of protozoa in batch anaerobic reactors. The reactor effluent samples were routinely analyzed at 5 days interval for total solids (TS), Chlorides, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Nitrates, Phosphates according to the standard procedures. The study has demonstrated a non-stimulatory effect on anaerobic bacterial community activity by anaerobic protozoan as measured by nutrient reduction.

Keywords

Protozoa, biodegradation, Holy Basil, anaerobic treatment

1. Introduction

With increasing demand for energy and cost-effective environmental protection, anaerobic digestion biotechnology has become focus of worldwide attention (Patel and Madamwar, 1998). In the anaerobic process organic carbon is converted by subsequent oxidations and reductions to its most oxidized state (CO_2), and to its most reduced state (CH_4). A wide range of microorganisms catalyze the process in the absence of oxygen (McInerney et al., 1980).

The significance of protozoa in anaerobic environments has been less studied with respect to their role in biodegradation except in the rumen ecosystem where protozoa is said to enhance the degradation of organic material by direct uptake (Williams 1991; Santra and Karim, 2002). Finlay and Fenchel (1991) have reported that grazing and flocculation activity of anaerobic ciliates lead to an overall stimulation of anaerobic activity and to an increased turnover rate in wet anaerobic landfill sites. Giancarlo *et al* (1998) studied effect of the introduction of a single anaerobic ciliate *Metopuspalaeformis*which resulted in a reduction of bacterial abundance but an increase in the rate of CH_4 and sulfide production. In order to understand the anaerobic degradation, a detailed study on the growth of anaerobic protozoa was studied by Priya*et al* (2008). It was done specifically controlling and monitoring growth of protozoa versus biogas production in continuous stirred anaerobic reactors and batch anaerobic reactors with oleate, a synthetic substrate. The objective of the present study was to investigate whether anaerobic, free-living bacterivorous protozoa influence the activity of anaerobic degradation of Holy Basil under

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laboratory conditions.

2. Materials andMethods

Study Area

Simhachalam temple is situated in the city of Visakhapatnam in Andhra Pradesh resembles the rich cultural heritage of Visakhapatnam. Its geographical coordinates are 17° 45' 0" North, 83° 10' 0" East. One of the most exquisitely sculpted shrines of Andhra Pradesh, Simhachalam temple is situated 16 km from Visakhapatnam among thickly wooded hills.

Basil (Ocimum sanctum):

Ocimum sanctum (also known as Ocimumtenuiflorum, tulsi) is an aromatic plant in the family Lamiaceae. (Warrier, P K, 1995). It is cultivated for religious and medicinal purposes, and for its essential oil. It has an important role within the Vaishnavite tradition of Hinduism, in which devotees perform worship involving Tulsi plants or leaves.

Collection and Preparation of Material:

The leaf waste was collected from Sanctum sanctorum (garbhagudi) of the famous Simhachalam temple, Visakhapatnam. The leaves were washed to remove the dirt. It was first dried in shade. The substrates were then blended to make a paste of the substrate. In each digester bottle, 1500 ml of the slurry(6% TS w/v) of the substrate, with 10% (v/v) of the active innoculum (cow dung) was added.

Experimental Set Up:

The set up (Figure 1) comprises of three graduated glass bottles of 2.5 L capacity and 1.5 L working volume, fitted with rubber caps connected to thermometers and pH electrodes. The whole reactor was made airtight. The bottles were connected with pipes for taking away gas from the digester to a gas collector to a collection bottle. Biogas produced in the digester was measured once a day by reading the level of saline water displaced by gas pressure (Singh et al., 2007). The contents of the digester were mixed once a day by shaking them manually for 5 minutes. As a direct method for contribution of protozoa to biogas generation and nutrient removal, experimental set up was made with (Reactor 1) and without protozoa (Reactor 2). Reactors without protozoa were obtained by adding cycloheximide, which inhibits eukaryotic protein synthesis (Priya et al, 2008). The reactor effluent samples were routinely analyzed at 5 days interval for total solids (TS), Chlorides, Alkalinity, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Nitrates, Phosphates according to the standard procedures (APHA, 1998)

3. Results andDiscussion

Temperature is the most important variable in controlling the rate of microbial metabolism in anaerobic environments. The biodegradation rates can be so slow that the achievable biogas potential appears to be lower than at optimal conditions. The range of temperature was between 27 ^{0}C to 32 ^{0}C in reactor 1 and reactor 2. As the temperature is below the optimum temperature, the biogas potential was lower. The lower potential was attributed to unstable fermentation rather than decreased substrate availability at that temperature (Angelidaki and Sanders, 2004). pH influences the activity of the hydrolytic enzymes and the microorganisms which are active within

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certain, usually narrow pH ranges (Angelidaki and Ahring, 1994; Angelidaki and Sanders, 2004). The pH tested was maintained in both with and without protozoa reactors between 6-7 range which is optimum for biogas production. The biogas was determined by liquid displacement method (Raju and Ramalinghaiah, 1997). The gas production was produced after 5 days. The low yield of biogas produced can be traced back to the presence of more cellulose material (Ojolo et al., 2007) (figure1).

The range of total solids was between 32000 mg/l to 8000 mg/l. A maximum reduction of TS solids upto 25% was observed after 10 days of retention time in reactor 1 and 40% in reactor 2 (figure 2). A maximum of 51.85% of reduction was observedafter30daysinreactor1and61.72%after20days in reactor 2. It has been shown that identifiers are able to outgrow the methanogens. This is due to the higher energy gained by nitrate reduction compared to methanogenesis. Therefore, the presence high concentrations of nitrate will result in determination of low methane potentials of the wastes. In pollution monitoring activities for water bodies, BOD is a commonly determined variable and is an important water quality indicator and descriptor of effluent content (Mcgloneetal, 2000). The BOD decreased continuously and attained a maximum reduction (81%) on 30 days of retention time in the reactor1 and 82% in reactor 2. (figure 4). The most common single parameter used to describe the concentration of waste or wastewater is the chemical oxygen demand (Angelidaki and Sanders, 2004). There was a continuous decease of COD in both the reactors and a maximum reduction of 75% was observed in both reactor 1 and reactor 2. (figure 3). The decrease of COD may be due to product inhibition (Bhattacharya et al.,2008).

4. Conclusion

This is the first report on protozoan effect on terminal decomposition in natural anaerobic system with mixed consortium. This study has demonstrated a non-stimulatory effect of anaerobic protozoans on anaerobic bacterial community as measured by nutrient reduction and biogas production. Further studies on the microbial consortium will give a deeper knowledge of thework.

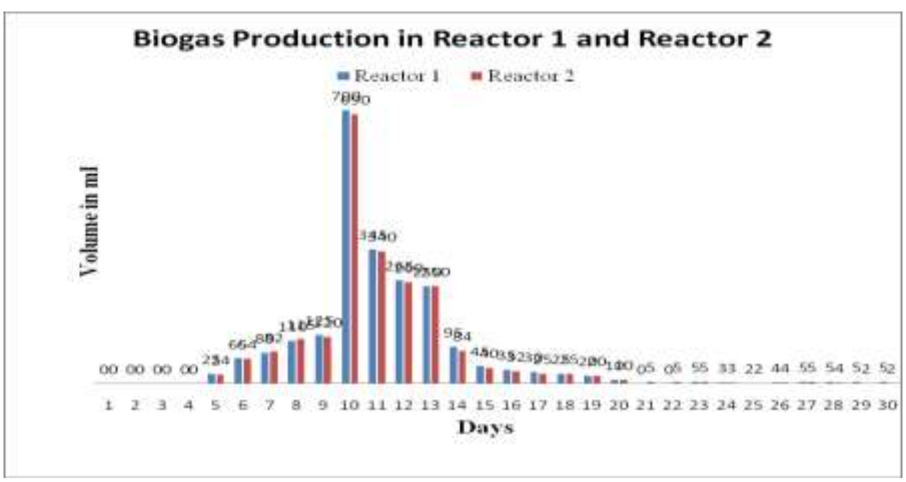

Figure 1: Biogas production in reactor 1 and reactor2

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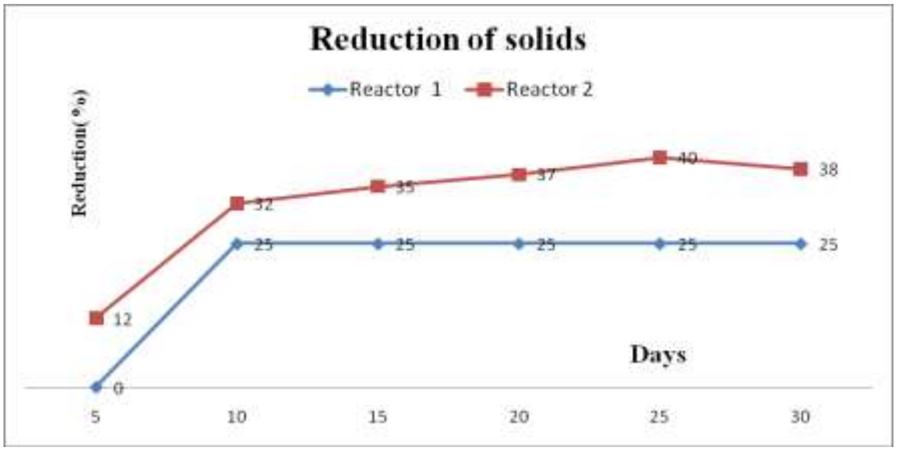

Figure 2: Percentage reduction of solids in Reactor 1 and Reactor 2

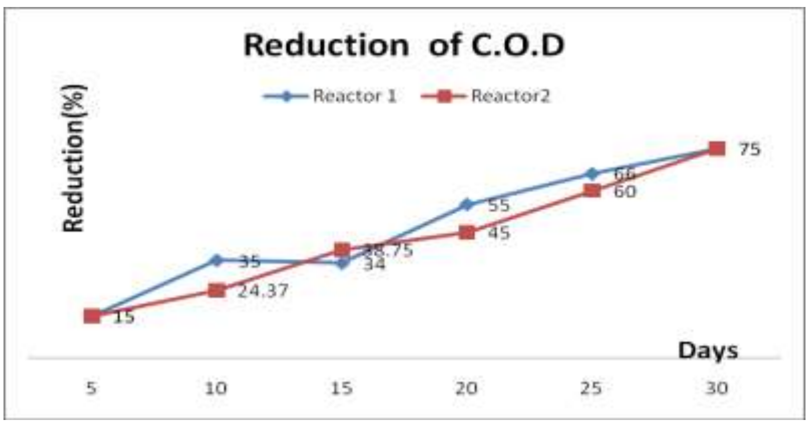

Figure 3: Percentage reduction of C.O.D in Reactor 1 and Reactor2

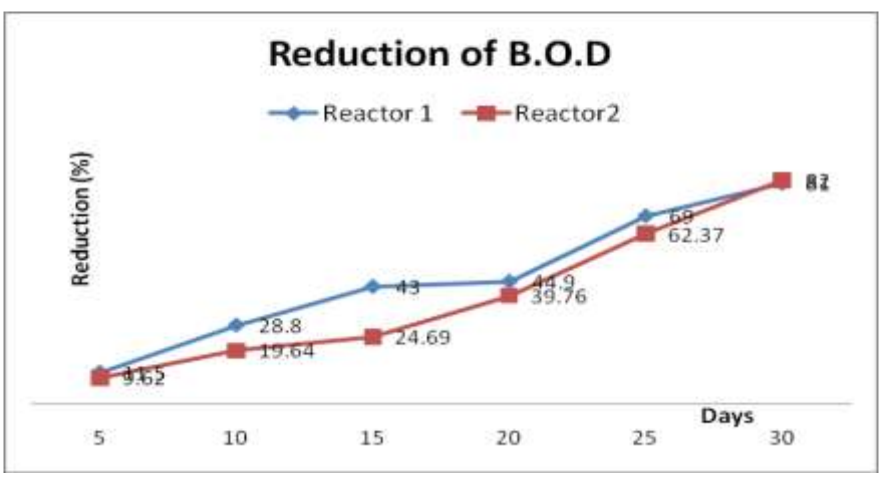

Figure 4: Percentage reduction of B.O.D in Reactor 1 and Reactor2

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