# Anaerobic Co-Digestion of Duckweed (*Lemna Gibba*) and Waste Activated Sludge

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**Abstract**
In this study duckweed *(Lemna gibba)* and waste activated sludge (WAS) were co-digested in different proportions with acclimatized anaerobic granular sludge (AAGS) in mesophilic conditions. The aim was to evaluate if the co-digestion could lead to an increased efficiency of methane production compared to digestion of waste activated sludge alone. Results indicate that co-digestion with both duckweed and WAS, in certain proportions i.e. T4, increased the methane generation compared with digestion at other proportions. The methane generation yield in treatment setup T4 was significantly higher than the calculated in many of the proportion. The Gompertz model fits well on the experimental data of the treatment setup T4. The values of correlation coefficient were achieved relatively higher ($R^2 \geq 0.99$). The trend was that of without pre-treatment, co-digestion of duckweed and WAS did not give the same synergy.

**Keywords**
Biogas, Aquatic weed; Biomass; Waste activated sludge

## INTRODUCTION

Anaerobic digestion is considered as one of the prospective alternative to recover energy and other valuable resources from organic biomass but it has few operational constraints such as slow hydrolysis and lower biogas yield. These process limitations can be overcome by pre-treating the feedstock before anaerobic digestion and or anaerobic co-digestion. Aquatic weeds are ubiquitous and grow in different environmental and climatic conditions and acts as a reservoir of both energy and nutrients (Koyama et al., 2014). Although co-digestion of different substrate has been studies significantly but still, the process lacks in co-digestion of duckweed with a waste activated sludge and required more exploration under specific species with variety of sewage sludge. The present study was designed to investigate the potential of indigenous duckweed species *L. gibba* as a co-substrate in anaerobic digestion with waste activated sludge (WAS) in various proportions to investigate the methane generation potential. Therefore, the primary aim of this study was to investigate the feasibility of addition of duckweed with waste activated sludge (WAS) as a co-substrate for anaerobic co-digestion and its subsequent effect on bio-methane generation.

## MATERIALS AND METHODS

### Reactor Configuration and Experimental Protocol

The present study was investigated in batch reactors of 500 mL capacity reagent bottles. All experiments were carried out in triplicates. Batch experiments were performed in water bath shaker at 30$^{o}$C. The duckweed biomass was thermally pre-treated using autoclave to get solubilised form of organic matter to accelerate the hydrolysis process. Batch studies of varied combination of pre-treated duckweed plant biomass with constant volume of anaerobic inoculum (AAGS - 100 mL) and waste activated sludge (WAS - 22.5 mL) were carried out. Typical five different compositions with varied ratios of substrate, inoculum and waste activated sludge were as follows:
T1 - 22.5 mL pre-treated *L. gibba* (5% total volume) + 100 mL AAGS + 22.5 mL WAS
T2 – 45.0 mL pre-treated *L. gibba* (10% total volume) + 100 mL AAGS + 22.5 mL WAS
T3 - 67.5 mL pre-treated *L. gibba* (15% total volume) + 100 mL AAGS + 22.5 mL WAS

T4 – 90.0 mL pre-treated *L. gibba* (20% total volume) + 100 ml AAGS + 22.5 mL WAS
T5 – 90.0 mL without pre-treatment *L. gibba* (20% total volume) + 100 mL AAGS + 22.5 mL WAS

**Substrate Collection**

Two different substrates, duckweed *Lemna gibba* and WAS were used for the anaerobic co-digestion study. The duckweed *L. gibba* (whole plants) was freshly harvested from a pond situated near Doon University campus (30° 16′ N, 78° 02′ E), in Dehradun, India. The plant was brought to the laboratory in a sampling bucket and rinsed with tap water followed by distilled water. The cleaned plant was spread over bloating papers to absorb excess water droplets. The plant was shredded in a mixer to reduce its particle size to easily available for microbes. Waste activated sludge was brought from the excess sludge line of the Sequencing Batch reactor (SBR) ~ 27 MLD capacity from a sewage treatment plant (STP) situated at Haridwar (India).Acclimatized anaerobic granular sludge (AAGS) was used as inoculum in the present study which was obtained from anaerobic digester of an activated sludge process (ASP) capacity~18 MLD, STP, Haridwar, India.

## RESULTS AND DISCUSSION

**Performance Evaluation under Different Treatment Setup**

Table 1 summarizes the various physico-chemical parameters obtained from different batch studies. As it is shown, there is a significant variation in the composition of feed mixtures, which is due to the variability in the composition of the samples of the different substrates taken over the experimental period. The initial content of TS and VS of treatment T1 to T5 ranged between 167.10±0.85 to 290.46±1.19 g/Kg and 98.83±0.60 to 138.53±0.60 g/Kg reduced to 8.77 ± 0.05 to 22.83 ± 0.25 g/Kg and 0.14 ± 0.01 to 5.60 ± 0.07 g/Kg. Results revealed that the overall reduction occurs in TS and VS concentration ranged 94-98% in all treatment setups. Similar trend for the removal of SCOD was observed in all treatment setups; however, SCOD removal was more in treatment setup T4. The VFA concentration was observed in sufficient range as reported in previous studies. No adverse affect on lowering of alkalinity observed. The C/N ratio varied between values of 16 and 18. Although most researchers recommend operating within a C/N ratio range of 20–30 for anaerobic bacterial growth in anaerobic digestion systems (Li et al., 2011), the optimal C/N ratio varies with the type of feedstock to be digested. For example, Romano and Zhang (2008) recommended maintaining the C/N ratio at 15 for the co-digestion of onion juice and digested sludge.

**Table 1: Performance evaluation in terms of physico-chemical parameters under different treatment-setup**

| Treatment | Duration (Day) | TS (g/kg) | VS (g/kg) | SCOD (g/kg) | VFA (mg/L) |
|---|---|---|---|---|---|
| 1 | At start | 250.8 ± 2.52 | 126.3 ± 1.15 | 188.01±21.63 | 501.3 ± 0.57 |
| | At end | 9.93 ± 0.20 | 3.66 ± 0.15 | 3.66±3.51 | 396.00 ± 4.00 |
| 2 | At start | 260.43 ± 0.94 | 130.10 ± 0.40 | 194.50±1.81 | 512.00 ± 2.00 |
| | At end | 22.83 ± 0.25 | 1.23 ± 0.11 | 1.94±0.01 | 388.33 ± 1.12 |
| 3 | At start | 279.20 ± 1.47 | 133.79 ± 1.11 | 209.95±0.95 | 546.33 ± 3.05 |
| | At end | 6.23 ± 0.05 | 0.48 ± 0.04 | 0.71±0.03 | 498.33 ± 1.52 |
| 4 | At start | 290.46 ± 1.19 | 138.53 ± 0.60 | 208.18±0.41 | 566.33 ± 2.08 |
| | At end | 8.77 ± 0.05 | 0.14 ± 0.01 | 0.22±0.00 | 559.67 ± 1.25 |
| 5 | At start | 167.10 ± 0.85 | 98.83 ± 0.60 | 148.48±0.45 | 245.80 ± 1.62 |
| | At end | 14.23 ± 0.32 | 5.60 ± 0.07 | 8.42±0.08 | 284.66 ± 4.21 |

**Variation in VSS, VFA, SCOD and Methane Production**

Fig. 1 shows temporal variation in SCOD, VSS, VFA and methane production from different

experimental set-ups supplemented with variable duckweed biomass. It was found that the SCOD and VSS decrease exponentially with time in all cases. In case of VFA, after initially accumulation from 5$^{th}$ to 10$^{th}$ day, a continuous reduction in VFA was observed. The maximum value of VFA of 1800 mg/L in treatment set-up T4 was found and remain 600 mg/L at the end of the test run. The methane gas generation during maximum VFA in this treatment set-up was less but continues to rise until the experiment ends (Fig.1). The initial low production of the methane might be due to acclimatization of the methanogens to the environment. Similar pattern of VFA and methane gas generation was observed in other treatment set-ups.

Methane generation in the anaerobic digestion is closely related to the typical composition of the feed. Results indicate one day lag phase in all experimental set-ups, then a progressive trend was witnessed for methane generation. The maximum methane yield was observed in experimental set-up T3 followed by T2>T1>T4>T5 (Fig. 2). The methane production was observed 12 to 123% in pre-treated duckweed indicates clearly the solubility of organic matter is higher compared to methane production, however, contradictory results were reported in literature. Chen and Oswald (1998) observed increased algal methane fermentation up to 33% at 100$^{o}$C for 8h, while de Schamphelaire and Verstraete (2009) did not detect any effect when pre-treating a mixture of microalgae at 80$^{o}$C for 2.5 h. Thus, the methane production greatly varies with factors such as inoculum, VS, VFA, and temperature. The methane production was a result of acidogenic/ acetogenic activity of associated microbial communities. It was mainly depends on the inoculum activity and adoptability of the inoculum towards substrate (Dhamodharan et al., 2015). Loss in volatile solid was related to transition process (hydrolysis and fermentation) of biomass to biogas. The transitional process also pre-dominates the formation of volatile fatty acids (acetic acid, propionic acid, butyric acid). The VFA and SCOD production/degradation was closely related to changes in pH, alkalinity, and the activity of methanogens. The relative degradation of solids and release of volatile fatty acids helps to maintain relevant pH range for methanogenic microorganisms and acid-forming bacteria activity (Angelidaki and Sanders, 2004). Results inferred that the pre-treatment mechanism along with different substrate to inoculum ratio will helps to optimize the methane production.

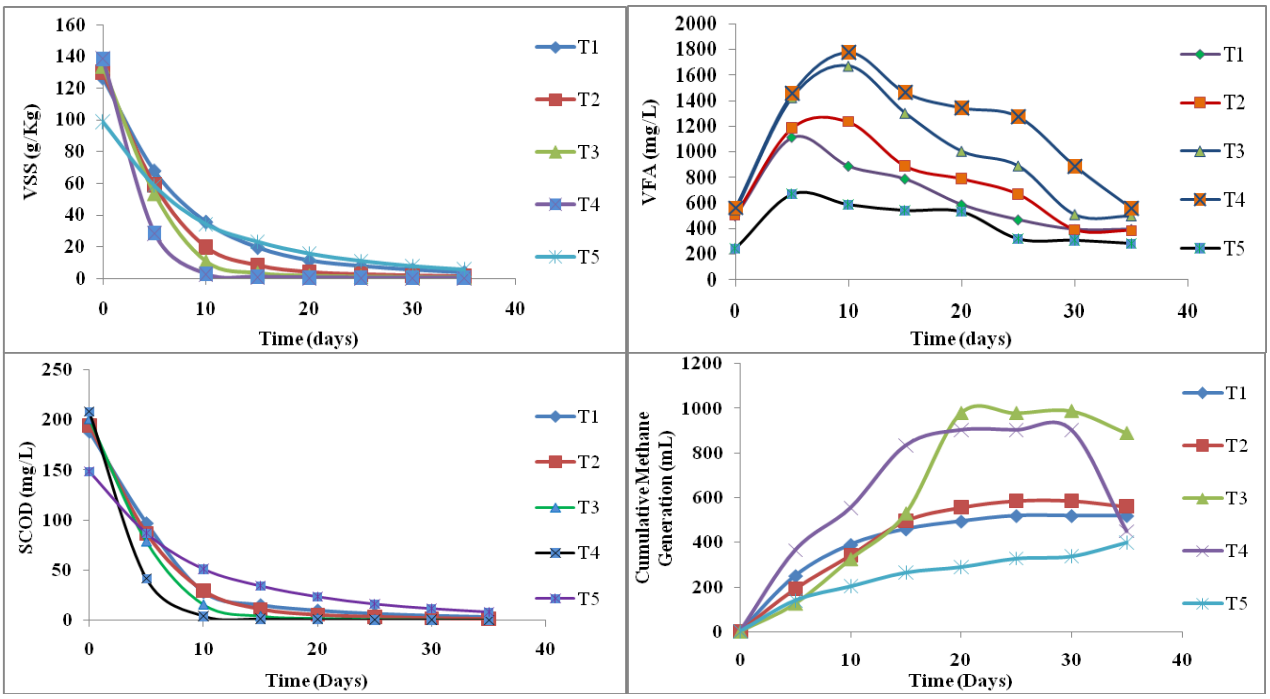


**Figure 1. Temporal variation of VSS, VFA, SCOD and methane production in different duckweed inoculum reactor**

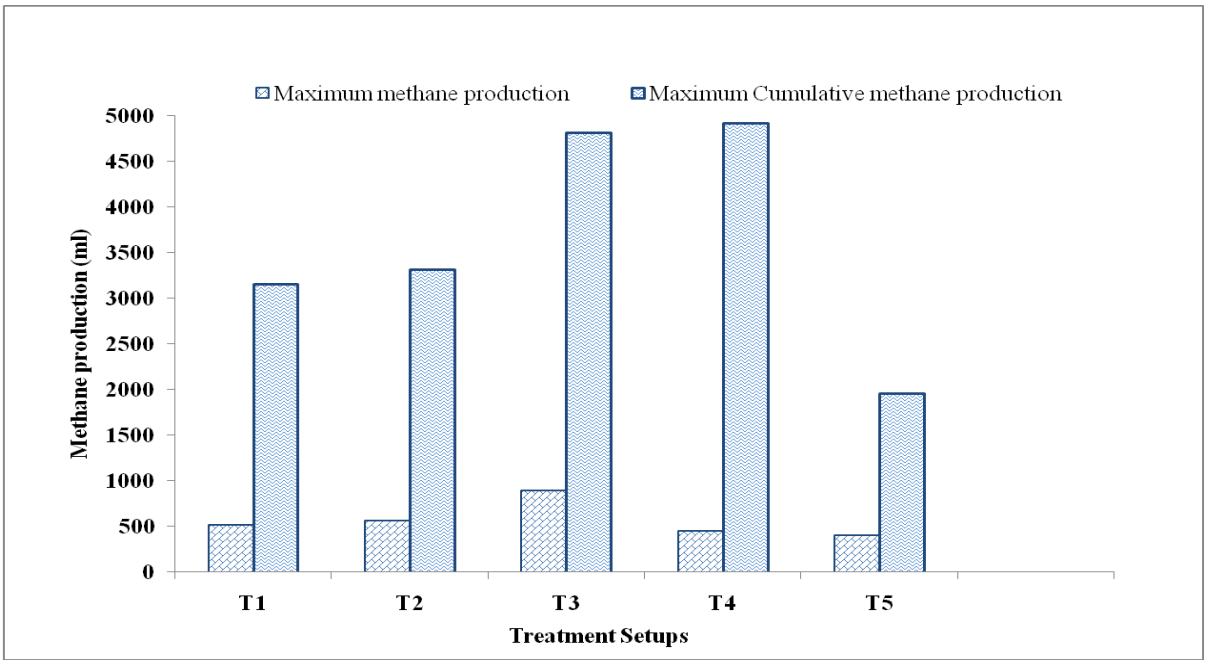


**Figure 2. Maximum methane & cumulative methane production in different batch reactors**

## CONCLUSIONS

The applicability of duckweed biomass in anaerobic co-digestion process revealed it suitability as a substrate. The maximum value of methane yield (140.5 ml/d) was achieved for the co-digestion of 20% duckweed biomass, 5% of WAS and 10% of AAS for treatment setup T4. The variation of duckweed substrate in different treatment set-ups interlinked to the methane yield. It can be concluded that co-digestion of duckweed with WAS and AAS should be a better approach in order to get higher gas yield.

## ACKNOWLEDGEMENT

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