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Resoluteness of biogas potential of Cr, Pb & Cd accumulated green algae *Spirogyra* sp. and *Oscillatoria* sp.

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Abstract: Water pollution due to heavy metals are spreading world-wide along with industrial progress. In this research work cultivation of *Spirogyra* sp. & *Oscillatoria* sp. were done and wastewater treatment of heavy metal such as Cr, Cd & Pb was accumulated by algae. After treatment of algae *Spirogyra* sp. & *Oscillatoria* sp. were decomposed and to prepare compost. The production of biogas from algal biomass becomes economically feasible and cheaper. This present study revealed that the nutrient content of natural compost was recorded about C- 38.5%, N- 3.31%, P- 1.02%, & K- 3.42%, heavy metal content of Cd- BDL, Pb- 0.21ppm & Cr- 0.01ppm and biogas value 19.19 l/kg. The maximum value of biogas was recorded at 30 ppm concentration (27.95 l/kg) of algal compost as compare to natural compost (19.19 l/kg) in *Spirogyra* sp. The experimental study concluded that the selected algae showed better biogas production as compared to natural. Whereas out of selected two algae, *Spirogyra* sp. gave better outcome as compare to *Oscillatoria* sp. Thus, Study suggested that algal biomass constitute a promising, efficient, cheap, decayed material for making compost and biogas production.

Key words: Biogas; GC- MS; ICP; Cd; Pb; Cr; C- N- P- K; *Spirogyra sp.*; *Oscillatoria sp.*

Introduction

Bioremediation of heavy metals are one of the most promising technology involved in environmental pollution control. Bioremediation is economical way to reduce environmental toxins using indigenous or introduced microbes that naturally degrade contaminants. Bioremediation is a natural process and can be used at much lower cost than other technologies, so bioremediation is the most efficient treatment.

Microalgae are a potentially important biomass for wastewater treatment Algae are the ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological properties. In these study, the multicellular filamentous algae used that was well known for its nutrient content. The composting is a controlled aerobic (oxygen-using) biological decomposition of moist organic carbon solid matter to produce a soil conditioner. In this compost microorganisms require digestible carbon as an energy source for growth and nitrogen (N) for protein synthesis to build cell walls. The accumulated algae biomass used to prepare the compost for improvement in agriculture field and also the production of biogas that was used in house. Anaerobic digestion is a common process for the treatment of a variety of organic wastes and biogas production. The macro and microalgae are suitable renewable substrates for the anaerobic digestion process.

Some heavy metals like copper, cadmium, zinc and aluminium accumulated by algae like *Chlorella vulgaris, Oscillatoria bornettia, Phacus curvicauda* and *Euglena acus* for four weeks in the laboratory (Nayana Brahmbhatt *et al.,* 2012). The accumulation of heavy metal from aqueous solution capable for especially of metal concentration below 50 mg/l. The metal- accumulation in cell binding capacities in few biological materials like marine algae, fungi and yeasts have been identified to be very high. He was proved that these micro- organisms can accumulate a wide range of metal species (Lu *et al.,* 1995). In different concentration of heavy metal at different intervals in *Spirogyra* to accumulate minimum and maximum amount of lead (Pb) 20.654 mg/l after 7 days at 2 ppm and 34.916 mg/l after 21 days at 30 ppm respectively (Dr. N. H. Brahmbhatt *et al.,* 2012).

Different paramerers like growth promoting regulators, antibacterial, vitamins, polymers, amino acids and polypeptides especially exopolysaccharides excrete in blue-green algae, which revealed growth promoter microorganisms and enzymes activities increased (De-Caire *et al.,* 2000; De- Mule *et al.,* 1999). Phosphate content was enhanced in coffee husk with comparison

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of same untreated material in composting (Sathianarayanan, 2008). The accumulation of chromium (Cr) by *Pithophora* sp. and their effect on biochemical parameters like Chl-a, Chl-b, Total- Chl, enzymes like peroxidase & catalase, protein, sugar, proline and after experiment very fruitful results concluded (Nayana Brahmbhatt, 2012). The use of *Pithophora* algae to accumulate heavy metal, as compost supplier, especially nitrogen, potassium, phosphorus nutrients (Dr. N.H. Brahmbhatt *et al.,* 2015).

Material and Methods

To culture algal sp.

The algal species of *Spirogyra* sp. & *Oscillatoria* sp. were used in this experiments, filamentous algae was cultured under different condition like *Spirogyra* sp. in pH 7.5 under control condition at 15±1^{0}C, 16/8 hr light dark cycle and *Oscillatoria* sp. in pH 8-9 to obtain their healthy growth at 25±2 ^{0}C, continuous light illumination respectively by Pringsheim (1946) and Allen & Arnon (1955) method in laboratory.

Preparation of compost

The effluent treatment was carried out in 2ppm, 5ppm, 10ppm, 20ppm, 30 ppm concentration; from the study algae accumulates heavy metal such as Cr, Cd & Pb. These accumulated algae samples were taken after treatment to prepare compost. The fresh algae compost was taken as control and compost without algae was prepared for comparison. The algae were mixed with dry and green leaves, vegetables waste, weeds and were chopped into 2-3-inch size. The slurry of cattle dung mixed with chopped algae and organic waste. All this mixture is buried in pits of size 1m × 1m ×1m with sloping sides. Close the pit with waste fodder and then heap the soil till it gets convex shape. The compost was ready after 6 to 8 weeks.

Determination of compost nutrient content

The nutrient contents - C, N, P, K of the compost were estimated by standard practice. Organic carbon and nitrogen content were determining by colorimetric method and Kjeldahl method respectively. Phosphorus content was estimated by Olsun process. Whereas, potassium was measured by Flame Photometer technique.

Measurement of heavy metals

To determine heavy metals like cadmium (Cd), chromium (Cr) and lead (Pb) contents of compost. Samples were digested with $HClO_4$: HNO_3 (1:4 V/V). After digestion samples were diluted with double distilled water. The various concentrations of metals were measured by using ICP-OES, Perkin Elmer Corporation (ICP optima 3300RL).

Preparation of biogas production

In the laboratory experiment was conducted using 1000ml aspirator bottles. The prepared fresh algae compost, five different concentration such as 2ppm, 5ppm, 10ppm, 20ppm & 30ppm accumulated algal compost and without algal compost was filled in each bottle as per the different treatment. Seven such bottles were prepared as per the treatments explained earlier. All the bottles were closed with one holed rubber cock and sealed with melted wax on the side to prevent any leakage of gas.

Measurement of gas output

The gas was collected by the downward displacement of water in the gas collecting jar which is graduated after 6-7 days of feeling the material and continued for a period of 30 days of retention time. The biogas produced by compost was estimated by using GC-MS, Perkin Elmer at SICART. In GC analysis, sample was injected at 80 ^{O}C temperature (oven temperature – 60 ^{O}C for 5 min and detector temperature- 250 ^{O}C), Silica gel SS column (2-meter length) and Nitrogen gas used as a mobile phase.

Results

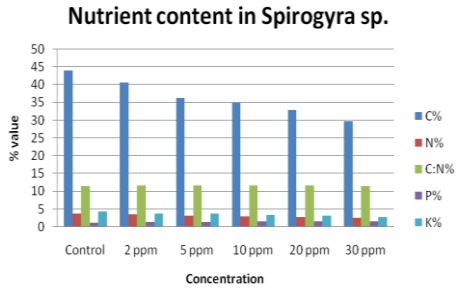
The representing data taken after the duration (days) for completion of fresh algae, different concentration accumulated algae and without algae compost. The elemental analysis of compost without algae shows organic carbon (38.5%), nitrogen (3.31%), phosphorus (1.02%) and potassium (3.42%) and heavy metal content like Cd (BDL), Pb (0.21 ml/mg) & Cr (0.01 ml/mg) and the biogas content was found 19.19 l/kg.

As presented in graph- 1 & 2, heavy metal treated algae of *Spirogyra* sp. and *Oscillatoria* sp. compost highest value of nutrients were found at 2ppm concentration. The organic carbon percentage was significantly affected by heavy metals accumulated algae. The organic carbon content were decreased with increases metal accumulation however C:N ratio remains unaffected. However, the concentration of heavy metals accumulation was increased the nutrients value decreases except for % P. In the *Spirogyra* sp. algal compost, at 2ppm concentration the nutrients values found were organic carbon (44%), nitrogen (3.79%), C:N ratio (11.6%) and at 30ppm concentration the value of nutrients were found organic carbon (29.7%), nitrogen (2.56%), C:N ratio (11.6%). In *Oscillatoria* sp. algal compost, at 30ppm heavy metal treated algal compost, the nutrient contents found were organic carbon (48.4%), nitrogen (4.17%), C: N ratio (11.6%). In fresh *Oscillatoria* sp. algal compost, organic carbon (58.3%), nitrogen (5.02%) was found highest as compare to fresh *Spirogyra* sp. algal compost.

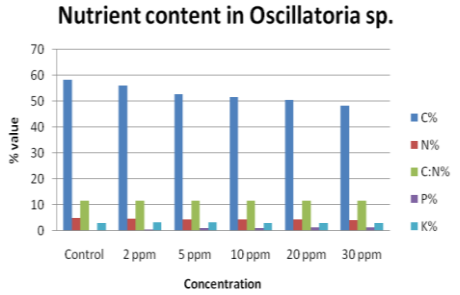
As presented in graph-3, The lowest biogas yield at 2ppm in fresh *Spirogyra* sp. algal compost and in fresh *Oscillatoria* sp. (18.93 l/kg) and (15.24 l/kg) respectively. The highest biogas yield was found at 30ppm for both the species. 25.85 l/kg and 21.71 l/kg biogas production was reported in fresh algal compost from *Spirogyra* sp. and *Oscillatoria* sp. respectively. The biogas yield was shows increasing trend with increased metal concentration.

The Cd & Pb was BDL and Cr was estimated 0.06 ml/mg at 30ppm in *Spirogyra* sp. (graph-4). At the same concentration Pb content was found to BDL however the highest Cd content (0.05ml/mg) and Cr content (0.09ml/mg) was found in *Oscillatoria* sp. The Cr content shows decreasing trend with increased metal concentration. In both fresh algal compost, Pb content observed highest that was 0.98ml/mg and 0.96ml/mg in *Spirogyra* sp. and *Oscillatoria* sp. respectively.

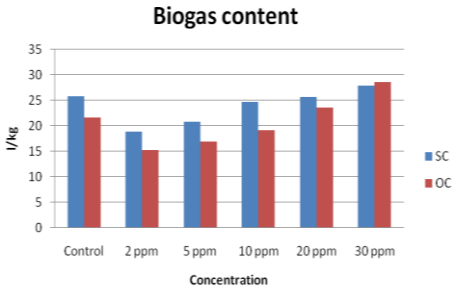
Analysis of biogas was measured by GC-MS. Figure-1 and figure-3 represents the chromatogram of *Spirogyra* sp. and *Oscillatoria* sp. which was analyzed in MS and the molecular weight 44 and 16 respectively for CO_2 and CH_4 molecules which indicates the presence of biogas components.



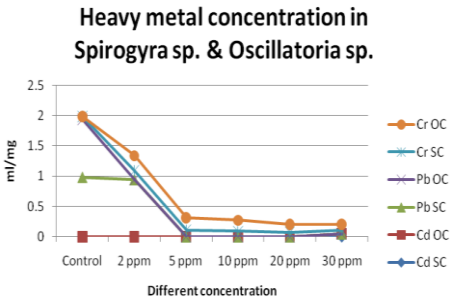
Graph 1: Nutrient content of accumulated *Spirogyra* sp. algal compost in different concentration of heavy metals like Cr, Pb, Cd



Graph 2: Nutrient content of accumulated *Oscillatoria* sp. algal compost in different concentration of heavy metals like Cr, Pb, Cd



Graph 3: Biogas production (l/kg) from accumulated algae compost of *Spirogyra* sp. and *Oscillatoria* sp. in different concentration of heavy metals like Cr, Pb, Cd



Graph 4: Heavy metal concentration in fresh and accumulated algae compost from *Spirogyra* sp. and *Oscillatoria* sp.

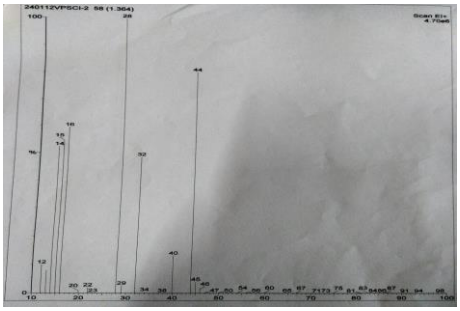


Figure 1: MS of biogas from accumulated *Spirogyra* sp.

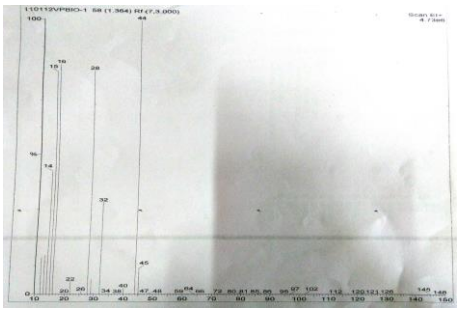


Figure 2: MS of biogas from accumulated *Oscillatoria* sp.

Discussion

The algal strains can lead to faster conversion in biomass to methane. Some strains possess no cell walls and some cell walls protein- based without cellulose or hemicellulose that make more easily degradable (Mussgnug *et al.,* 2010). The storage microalgae can have a significant effect on the methane yield (Gruber – Brunhumer *et al.,* 2015). In addition to resistance to bio-remediation, methane production can reduce when algal biomass was a low ratio of carbon-nitrogen ratio (C:N) (Yen and Brune, 2007). In this present investigation, the wastewater containing heavy metals such as Cr, Pb, Cd were treated to lessen heavy metal contents by algae of *Spirogyra* sp. and *Oscillatoria* sp. The compost study reveal that the nutrients content such as organic carbon, nitrogen, phosphorus and potassium were recorded maximum at 2ppm and minimum in 30ppm concentration of heavy metal. The algal sludge prepared by anaerobic digestion results a potential source of nitrogen and phosphorus as a fertilizer (Mulbry *et al.,* 2005; Yen and Brune, 2007). The concentration of the

plant nutrients like nitrogen, phosphorus and potassium were low (0.96, 0.75, 0.33% respectively) in compost of municipal waste compared with manure of farmyard (2.5, 1.60 and 1.70% respectively) (Hann, S.D., 1981). The cattle dung contains higher number of methanogenic bacteria and high biogas production by adding of chopped weed residue & cow dung razing and results was found C/N ratio between 25-30 (Hills *et al.,* 1981 & 1979; Thakur *et al.,* 2000).

Several projects were done the aim to cultivate microalgae by application of CO2 from flue gas (Van Iersel and Flammini, 2010). The methane yield strongly depends on the species and culture condition (Sialve *et al.,* 2009). The methane production was increased by *Nannochloropsis salina* that thermal pre-treatment, prior to anaerobic digestion (Schwede *et al.,* 2013). Biogas production was recorded from algal biomass may be less than the theoretical predictions because resistant algae cell walls may limit accessibility to intracellular components (Angelidaki and Sanders, 2004; Sialve *et al.,* 2009). Different technology such as in activated and primary sludge treatment have been applied to pre-treated biomass of algae successfully to increase the methane yield (Carrere *et al.,* 2010). In the present study, these heavy metals treated algal compost used for the production of biogas in the laboratory, it was found that the maximum biogas yield was recorded at 30ppm concentration of heavy metals. The yield of methane of doubled when wastewater sludge in equal masses and biomass of *Spirulina* were co-digested (Samson *et al.,* 1983). Similarly, when added waste paper (50% w/w) to aquacultural microalgal sludge to adjust the C:N ratio (20-25:1), in turn, the methane production was doubled rate from 0.6 L/L day to 1.2 L/L day at 35°C & after 10 days with a hydraulic retention time (Thakur *et al.,* 2000, Yen, 2004). The moisture content was increased (1:1 liquid: solid ratio) and content of nitrogen was decreased, which may due to the microbial activity was observed high and more loss of nitrogen by denitrification process and decomposition rate of compost. C:N ratio was reduced due to applying both algal species either alone or mixer of algae together at 10% concentration of compost under 1:2 (liquid: solid), compared with non-treated compost under the same moisture content, which was recorded in *Spirulina maxima* (11.69%), *Nostoc muscorum* (11.65) and mixed culture (11.70) (Manal , 2011). The addition of waste with high strength & carbon may balance the nitrogen content of high in nature of the waste-grown algae, with significant increases in production of biogas rate occurring into the tests with multiple weeks. Their study concluded that possible to digest algae in anaerobic digesters issues, and benefits, of implementing algae co-digestion at full-scale wastewater treatment plant (Michael Salerno, 2009). Qualitative analysis of the biogas revealed that it was composed of 62.6% v/v methane using different microalgae species (Vergara-Fernandes *et al.,* 2008; Mussgnug *et al.,* 2010). The microalgae used to the biogas upgrade biogas and found that the CO_2 content in biogas can be greatly reduced (by up to 97%) using *Chlorella* sp., but the photosynthetic oxygen (approximately 20%) made the gas mixture potentially (Mann *et al.,* 2009).

Conclusion

The *Spirogyra* sp. and *Oscillatoria* sp. can be utilized for accumulation of heavy metal from wastewater through compost. The project study was focused to identify the essential information required for full-scale implementation of wastewater treatment plants, including the methane (CH_4) yield. The results of anaerobic digestion of filamentous algae biomass shows that plays an important role of substrate composition in biogas production.

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Abbreviations

Cd- Cadmium
Pb- Lead
Cr- Chromium
ml/mg- mililitre/miligramm
l/kg- litre/kilogramm
GC-Gas Chromatography
MS-Mass Spectroscopy
ICP-OES- Inductively Coupled Plasma- Optical Emission Spectrometer
C- Organic Carbon
N- Nitrogen
P- Phosphorus
K- Potassium
ppm- Parts per millions
BDL- Below Detection Limit

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