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Biogas and biofertilizer production of marine macroalgae: An effective anaerobic digestion of *Ulva* sp.

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ABSTRACT

Nowadays the increasing prices of petroleum-based fuels have persistently focused on the development of alternative energy sources, whereas the solid residue can be used as an organic fertilizer which can be one of the solutions from depending on conventional synthetic fertilizer. The research work mainly focused on biomethanation and development of biofertilizer from the seaweed *Ulva* sp. The *Ulva* sp. mixed with organic matter (cow dung) has been used in different concentrations for biogas production and the methane was theoretically computed that 1.528 kg/m^3 volatile solids (VS). The ratio 3:1 proportion obtained was 574 ± 26 ml^{-g} VS biogas production than the control and 1:1, 2:1 ratio. The cumulative biogas was quantitatively analyzed by gas chromatography for the proportion of methane (CH_4) and ranged from 72% in *Ulva* sp.: cow dung 3:1. The solid residue was used as an organic fertilizer for the growth of *Vigna radiata*. The results revealed that the physicochemical parameters, pigments and nitrogen, phosphorus and potassium (NPK) analysis were increased in the 3:1 ratio when compared to the others. The *Ulva* sp. was used in eco-friendly and zero waste management process. From the seaweed *Ulva* sp. to reduce the sustainable and clean environment; to improve the agricultural crop production and also enhances the soil fertility.

1. Introduction

The Gulf of Mannar coastal region of India bears luxuriant growth of seaweeds. The south shore harbours around the 200 species of seaweeds. Their growth in coastal waters resemble like grass in large regions, stretching out and spreading to more than meters. Seaweeds grow bounteously along the Indian coastline especially in rough shore areas (Kumar and Sahoo, 2011). Around 841 taxa of marine algae were found in both between tidal and profound water areas Indian coast (Oza and Zaidi, 2001).

Seaweeds are mostly moored to the ocean depths or other stable structures by root like "holdfasts," which helps play out the individual capacity of connection but unlike the roots of higher plants, seaweeds do not extract supplements from their host (Wyles et al., 2014). A particular seaweed's arrangement in one of these groups is resolved first by its photosynthetic colorants, at the end its regenerative mode, at the point its micro and macro morphologies and finally by its phycopolymers (Ryan Drum, 2018). *Ulva* species is thin flat green algae growing from a small discoid holdfast that may reach 18 cm or more in length, however for the most part substantially less and up to 30 cm across. Thalli are one cell thick, soft and translucent (Guiry and Guiry, 2017).

Today, in quick-paced society, the rising number of world population brings about a massive energy consumption described by the reduction of oil assets which are thought to be the principle prerequisites for using sustainable energies. The utilization of biomass as an economical source for energy production represents to in this way a promising alternative for the substitution, at any rate partially, of fossil fuels consumption (Yahmed et al., 2017).

Diverse strategies for biomass treatment were utilized for extraction of conceivable inhibitors and residual biomass was assessed for methane yields. In the previous investigation also includes strategizing the treatment process for proficient extraction of individual components of biomass with that goal that every fraction offers value-addition and thus supports bio-based economy approach. The investigating strategies are enhanced biogas production from *U. lactuca* (Mhatre and Gupta, 2018). However, a significant portion of the *Ulva* biomass remains unutilized, making biological waste (Briand and Morand, 1997; Morand et al., 2006). It is a rich source of proteins (16–30%) and carbohydrates

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(60–65%) consisting of high-value sulfated polymer ulvan along with cellulose and hemicellulose and 4–5% lipids (Lahaye and Robic, 2007; Msuya and Neori, 2008). The most energy efficient route to acquiring biofuel from macroalgae is employing anaerobic digestion (AD) to biogas (Hughes et al., 2012). The production of biogas through AD provides substantial benefits over different types of bioenergy production. It has been considered one of the most energy-efficient and environmentally beneficial innovations for bioenergy production (Olabi, 2012; Karray et al., 2017). In a natural habitat, complex organic substrates require hydrolysis, acidogenesis, acetogenesis and methanogenesis for completing AD reactions.

Biogas, the end-product of the AD, comprise major methane substance of 60–70% and can be promptly used for heat/electricity generation (Kim et al., 2014). The conversion of marine biomass to methane is feasible; involving few obstacles that need to be tackled carefully. Efficient cultivation, harvesting and conversion technologies are privilege for effective and efficient use of macroalgae to its maximum capacity. In previous studies, the methane yield was recorded at 68% increasingly when contrasted with untreated biomass, with a final yield of 255 ml CH_4 g^{-1} VS (Montingelli et al., 2016). The exploit of marine biomass as an alternative feedstock for biogas invention could also reduce the environmental nuisance and therefore the waste sludge can be used as bio-manure.

Use of various inorganic fertilizers, pesticides, bug sprays has harmed the soil ecosystem widely. This kind of practice makes the soil environment unsuitable for crop development in the future (Uthirapandi et al., 2018). The suitability of *U. lactuca* as biofertilizer or biostimulant has not yet been examined adequately and there are no examinations on the impacts of sulfuric acid on *U. lactuca* to produce seaweed extract (Castellanos-Barriga et al., 2017). The utilization of algal biomass as manure for the improvement of soil quality for agricultural crop production has been intensively studied in recent existences (Wosnitza and Barrantes, 2006; Cole et al., 2016). The most important advantage is that the algal biomass is a readily biodegradable material and has a high content of organic matter and other macronutrients such as nitrogen, phosphorus, potassium and calcium (N, P, K & Ca) etc. which makes them useful for the improving the quality of soil (Tabarsa et al., 2012).

The green macroalgae *Ulva* sp. has great abundant of potentially important species. In Tamil Nadu, India and some of the countries, the suitability of *Ulva* sp. as used as biomethanation and bioconversion has not yet been inspected sufficiently and there are no studies on the end product used as organic fertilizer. However, the combinations of cow manure and *Ulva* sp. has produced more methane which is used for household and commercial activities. The bioconversion of organic manure enhances the soil fertility and improves the plant growth. This research work was used to initiate with large scale biogas production and increase the agricultural crop yield in future. One of the major important roles in the *Ulva* sp. used to zero waste management without any effects of environment and soil. The present study aims at exploring strategies for enhanced biogas production from *Ulva* sp. various approaches for biomass treatment were employed for extraction of biogas, the gas was intern analyzed by gas chromatography and residual biomass was evaluated for the growth of *Vigna radiata*.

2. Materials and methods

2.1. Sample collections

Seaweed samples were collected from Manapad (8°22′39″N; 78°3′8″E), Colachel (8.1786°N; 77.2560°E) and Muttom (8.13°N; 77.32°E) area situated in Southeast shoreline of Tamil Nadu, India. The samples were collected and washed completely using seawater to eliminate epiphytes and sand and kept in sterile polythene covers, placed in a temperature controlled icebox, transported to the laboratory for further study. Then the samples were washed completely with tap water to expel the salts and foreign particles and then identified based on macroalgae fact-sheet prepared by Moroney et al. (2017) and seaweeds field manual (Dhargalkar and Verlecar, 2009).

2.2. The biochemical portrayal of seaweed

As preparatory to biochemical tests, the seaweed was dried by filter paper to remove excess water and shade dry at room temperature for a week and incubated in hot air oven at 50 °C for overnight, powdered and kept in impermeable plastic container at room temperature for further biochemical examination of protein (Lowry et al., 1951), sugar (Dubois et al., 1956), lipid (Folch et al., 1957), ash (Marsham et al., 2007) and moisture (Feldsine et al., 2002) which are the significant substrates of methane.

2.3. Theoretical estimation of methane production

The methane yield was theoretically measured by the method according to Angelidaki and Sanders (2004); Sialve et al. (2009); Mhatre and Gupta (2018).

$$\text{CH4 kg/m3 VS} = (0.496X) + (1.014Y) + (0.415Z) \quad (1)$$

where X=protein fraction of volatile solids,
y=the lipid fraction of volatile solids,
Z= the carbohydrate fraction of volatile

2.4. Biomethanation process

The fresh *Ulva* sp. was chopped into a few pieces and grinded to obtain thick paste. The *Ulva* sp. was blended with an organic matter (cow dung) into various proportions (1:1, 2:1 and 3:1). *Ulva* sp. sample without blending with organic matter and cow dung without the presence of *Ulva* sp. were used as controls at neutral pH (6.8 ± 7.3) at lab scale level and kept at 37 °C. Gas was collected from each exploratory setup; the quantitative and qualitative investigation was done periodically up to the 60-day interval. The quantitative test was accomplished by down displacement technique and the qualitative test was accomplished by gas chromatography. Data has conversed mean (±) SD of the examinations ($n = 2$) using statistical tools origin 2017 were calculated in excel sheet (Hansen et al., 2004; Mhatre and Gupta, 2018).

2.4.1. Batch experiments

The substrate was loaded with the 2.5 L of darker serum bottles and kept in the dark room without light power 35 °C, pH 7.5 ± 0.2. In order to assess CH_4 generation from marine biomass, Tedlar® Gas Inspecting packs with Thermogreen® LB-2 septa (600 ml) were fixed with the bottles. Tests were conducted in duplicate with two controls (*Ulva* sp. alone and cow dung alone) for total eight tests and the final volume up to 2L. The bottles were then purged with nitrogen (N_2) for 5 min to create anaerobic conditions and capped with elastic sleeve plugs, incubated at 35 °C, and agitated at thrice a day for fermentation. The test reactors were worked for 60 days (Gurung et al., 2012).

2.4.2. Biogas separation

Biogas production was estimated following 60 days incubated using the technique developed by (Owen et al., 1979). The methane concentration from the headspace of the serum bottles was resolved by using a Gas Chromatography (Chemito 7610 series) furnished with a thermal conductivity detector (GC-TCD) and Poropak Q column used for this study. The temperature of the column, the injection port and the sensor were 60 °C and 90 °C respectively. The carrier gas was used to nitrogen at a flow rate of 30 ml^{-1} min. Gas samples (500 µl) in the headspace were collected using a pressure-lock gas syringe and injected into the injection port A. The outcomes were deciphered using the software clarity Lite accessible with the GC. The GC was calibrated by injecting 500 µl of a standard gas mixture containing carbon dioxide, methane and hydrogen (CO_2, CH_4 and H_2).

2.5. Utilization of solid residue as a bio-manure for Vigna radiata growth

2.5.1. Assortment of harvest plant

The harvested mung bean *V. radiata* belonging to family Leguminoseae were chosen for growth study and the seeds were purchased from farming office, Tiruchirappalli, Tamil Nadu. The seeds were monitored for ostensible disease, with uniform size, shading, weight and finally stored in a metal tin holder Basra et al. (2005); Larreta et al. (2008); Castellanos-Barriga et al. (2017).

2.5.2. Germination test

Preceding the analysis, the seeds were sterilized at 70% ethanol for 5–10 min and washed with sterilized distilled water. The seeds were placed on filter paper over the petriplate. The plates were kept at room temperature for 12 h. Thirty seeds were maintained on each plate with two sub duplicates and the germination level was calculated followed by Hernández-Herrera et al. (2014); Castellanos-Barriga et al. (2017); Uthirapandi et al. (2018).

2.5.3. Preparation of bio-manure

This experiment was carried out in six set of pot treatments under the greenhouse. The treatment setup was followed by Table 4. The control was used as soil without any amendments. The seeds were sown according to Sivasankari et al. (2006). This investigation was carried out by 50 days. The nutrient analysis and the germination study were attained simultaneously.

2.5.4. Morphological parameters

During the experiment period, the plants were uprooted after every seven days of growth intervals and the vegetative parameters, pigment investigation of the plant such as root and shoot length, fresh weight, dry weight, chlorophylls and carotenoids were estimated. The root and shoot length were estimated from the collar region to tip. The uprooted plants were washed then the fresh weight was measured by electronic adjust. At that point, the plants were dried in a hot air oven at 50 °C for 12 h and dry weight was recorded in mg/g according to Rama Rao (1990); Kumar et al. (2012); Castellanos-Barriga et al. (2017).

2.6. Photosynthetic pigment analysis

2.6.1. Chlorophyll content

The fresh leaves were handpicked, washed with tap water and grind with mortar and pestle. About 500 mg homogenized sample was blended with of 80% acetone (20 ml) followed by centrifugation (5000 rpm) for 5 min and the supernatant was transferred into the new disinfected centrifuge tube. The above procedure was repeated to obtain colourless residue. The total volume of the solution was made up to 25 ml with 80% acetone. The absorbance of the solution was read at 645 and 663 nm against the solvent (80% acetone) blank. Pigment concentration was calculated using equations (2)–(4) (Arnon, 1949; Ramya et al., 2015).

$$\text{Chlorophyll} - \text{a} = 12.7\,(\text{A }663) - 2.69\,(\text{A }645) \times \frac{V}{1000 \times W}\text{ mg of fresh weight} \quad (2)$$

$$\text{Chlorophyll} - \text{b} = 22.9\,(\text{A }645) - 4.68\,(\text{A }663)\,\frac{V}{1000 \times W}\text{ mg of fresh weight} \quad (3)$$

$$\text{Total chlorophyll} = 20.2\,(\text{A }645) + 8.02\,(\text{A }663) \times \frac{V}{1000 \times W}\text{ mg of fresh weight} \quad (4)$$

where A = absorbance at specific wavelengths.

V = final volume of chlorophyll extract in 80% acetone.
W = fresh weight of tissue extracted.

2.6.2. Carotenoid content

Ten ml of acetone, 20 ml of hexane and 0.001 g of magnesium carbonate were added to homogenized leaves (50 mg) and blended for 5 min using a vortex. The supernatant was transferred into a new tube. The pellet was washed twice with 10 ml of acetone and 10 ml of hexane. Fifty ml of distilled water was added and the upper layer was transferred into the new sterile tube. Three ml of acetone was added and diluted to 3 ml with hexane. Read the absorbance of the solution at 436 nm against the solvent (acetone and hexane) as a blank (Wellburn and Lichtenthaler, 1984).

2.7. NPK analysis

The nutrient analysis of nitrogen, phosphorus and potassium were analyzed by peroxymono sulphuric acid digestion, using a spectrophotometer. Five gram of homogenized *V. radiata* wet samples were digested with concentrated sulfuric acid at 440 °C for five hrs and treated with 30% hydrogen peroxide solution. After cooling, the total nitrogen content in the samples was read spectrophotometrically at 485 nm. Phosphorus content was determined by dissolving 5 g of homogenized sample in the mixture of acids (hydrochloric acid, nitric acid and perchloric acid) at proportion of 10:5:1, respectively at 300 °C using digital flame photometer (Humphries, 1956; Zeidan, 2007; Ramya et al., 2015). Potassium content was determined by dissolving 5 g of homogenized sample in 0.5 ml of 0.387M ammonium molybdate and 3 ml of 0.25N H_2SO_4 and aliquots of disodium hydrogen phosphate was added. One ml of sodium sulphide solution was added to the above sample. The tubes were blended and incubated at room temperature for 20 min and the absorbance was dignified at 715 nm in UV-Vis spectrophotometer against water.

2.8. Statistical analysis

The statistical examination of the records was executed as per the method described by Snedecor and William (1989). Mean ± (SD) and One-way analysis of variance (ANOVA) were analyzed. Significant modifications amid the diverse treatments were resolute using Duncan multiple range test at 0.05 level of probability. Statistical tools Origin (Ver. 8.0) were calculated in excel sheet (Duncan, 1970).

3. Results and discussion

3.1. Proximate composition analysis of dried Ulva sp

Based on the biochemical outcomes, the protein (12.02 ± 2.5), carbohydrate (44.3 ± 1.01), lipid (2.6 ± 0.21), ash (31 ± 5.2), moisture (11 ± 2.3) and pigments such as (chl – *a* (0.89 ± 0.13), chl – *b* (0.75 ± 0.04), total chl (1.64 ± 0.14) and carotenoids (3.62 ± 0.13). The values are not significantly different ($P > 0.05$) appeared in Table 2. The results revealed that; the *Ulva* sp. can be a potential of methane formation, which contains a higher measure of starch which can be effectively depolymerized. These results are in concurrence with (Jard et al., 2013) study that depicted *Ulva lactuca* as a substrate affluent on volatile solids, carbohydrates and proteins and resembles a virtuous contender for biogas engenderment. Virtually, affording to the total solids (TS) of *U. lactuca* amassed in November,

Table 1
Comparative table on the production of methane from macroalgal species.

Groups	Macroalgae	Methane Yield (mL. g^{-1} VS)	Reference
Red	*Ascophyllum*	110[a]	Hanssen et al. (1987); Barbot et al. (2016)
	Gracilaria	280–400	Singh and Gu, 2010; Parmar et al., 2011; Barbot et al., 2016)
	Gracilaria vermiculophylla	255	(Maia et al., 2016; Silwer, 2018)
	Gigartina spp.	266	Maia et al. (2016)
	Gracilaria verrucose	144	(Jard et al., 2013; Song et al., 2015)
	Palmaria palmate	279	Jard et al. (2013)
	Gracilaria tikvahiae	220	Habig et al. (1984)
Brown	*Laminaria ochroleuca*	472	(Maia et al., 2016; Silwer, 2018)
	Laminaria sp.	180–300	(Reith et al., 2005), (Vanegas and Bartlett, 2013)
	Macrocystis pyrifera	180–430	Chynoweth (1987); Dębowski et al. (2013)
	Sargassum	120–190	Bird et al. (1990)
	Saccharina latissima	425	Maia et al. (2016)
	Sargassum spp.	260–380	(Roesijadi et al., 2010; Song et al., 2015)
	Saccharina japonica	204–214	(Gurung et al., 2012; Lee et al., 2010)
	Saccharina digitata	219	Adams et al. (2011)
	Undaria pinnatifida	283	Jard et al. (2013)
	Saccorhiza polyschides	232	Jard et al. (2013)
	Saccharina hyperborea	260	Hinks et al. (2013)
	Nizimuddinia zanardini	143	(Yazdani et al., 2015; Maneein et al., 2018)
	Fucus vesiculosus	146.9	Romagnoli et al. (2017)
	Pelvetia canaliculata	340	Rodriguez et al. (2018)
Green	*Ulva lactuca*	270–480	(Briand and Morand, 1997; Reith et al., 2005; Barbot et al., 2016)
	Ulva sp.	308	(Maia et al., 2016; Silwer, 2018)
	Chaetomorpha linum	166–195	(Nielsen and Heiske, 2011; Song et al., 2015)
	Codium tomentosum	158	Jard et al. (2013)
	Ulva sp.	284–330	(Habig et al., 1984; Gurung et al., 2012)
	U. rigida	–	(Karray et al., 2015; Maneein et al., 2018)
	U. rigida	150	Karray et al. (2017)
	Ulva sp + *Cladophora* sp. + *Chaetomorpha* sp.	480	Sarı and Tuzen (2008)
	Manure	262 ± 20 [b]	(Habig et al., 1984; Nikolaisen et al., 2011)
	80% manure: 20% *Ulva*	259 ± 8 (total); 247 ± 25 (*Ulva*)	Nikolaisen et al. (2011)
	60% manure: 40% *Ulva*	238 ± 23 (total); 202 ± 20 (*Ulva*)	Nikolaisen et al. (2011)
	50% manure: 50% *Ulva*	206 ± 11 (total); 150 ± 21 (*Ulva*)	Nikolaisen et al. (2011)
	Ulva sp.: cow dung (3:1)	386 ± 26	Present study

[a] The values represented methane yield (mL. g^{-1}VS) volatile solids.
[b] The values denotes mean ± SD (standard error).

Table 2
Proximate composition of *Ulva* sp.

Proximate composition (*Ulva* sp.)	Mean ± SD
Protein (%) [a]	12.02 ± 2.5
Carbohydrate (%)	44.3 ± 1.01
Lipid (%)	2.6 ± 0.21
Moisture (%)	11 ± 2.3
Ash (%)	31 ± 5.2
Chl – *a* (mg/g) [b]	0.89 ± 0.13
Chl – *b* (mg/g)	0.75 ± 0.04
Total chlorophyll (mg/g)	1.64 ± 0.14
Carotenoids (mg/g)	3.62 ± 0.13

[a] The *Ulva* sp. has high carbohydrate content for the biomethanation process and calculated by percentage.
[b] The pigment content also good in *Ulva* sp. for photosynthesis of the plant growth and measured by mg/g.

from the coastal area of Northern Chile, comprise of 27% protein, 0.3% lipids and 62% carbohydrates (Ortiz et al., 2006). The researchers discussed about the *U. lactuca* as affluent source of carbohydrates (55–60%) made out of various monomeric sugars like rhamnose, glucose, glucuronic acid, xylose and arabinose which makes it a potential substrate for biomethane engenderment (Briand and Morand, 1997; Pengzhan et al., 2003; Zhang et al., 2008).

3.2. Theoretical estimation of methane production

Theoretical evaluation of methane production based on the biochemical parameters of protein, lipid and carbohydrate from depolymerized *Ulva* sp. The methane yield was theoretically calculated by equ. 1. As by the theoretical estimation, methane production would be approximately 1.528 kg/m^3 VS from the *Ulva* sp. Mhatre and Gupta (2018) reported their findings, the highest methane yield of 408 ± 20.02 ml CH_4 g^{-1} VS (70.93% of hypothetical) was perceived in sap and ulvan removed filtrate (batch VI) trailed by 323 ± 21.71 ml CH_4 g^{-1} VS (57.50% of hypothetical) in sap, ulvan and protein evacuated residue (batch VII). Their investigation limpidly demonstrates the effective utilization of successive treatment in contrast to the individual treatment for abstraction of inhibitory components and subsequently enhanced biomass agreeability.

3.3. Batch experiment tests

In the biomethanation process, the down displacement due to gas production by control (cow dung) was higher (400 ml) per day than the seaweed. In *Ulva* sp.: cow dung mixture, the 3:1 proportion obtained higher (600 ml/day) gas production compared to the 1:1, 2:1 and control. Cumulative biogas yield after 60 days was highest for *Ulva* sp.: cow dung 3:1 (574 ± 26 mL g^{-1} VS) and lowest for *Ulva* sp.: cow dung 1:1 (195 ± 32 mL/g VS) while using control (356 ± 19 mL g^{-1} VS) and *Ulva* sp.: cow dung 2:1 in Fig. 1. The C/N ratio was analyzed in the cow dung and green seaweed *Ulva* sp. The ratio varied from 11.2% to 24.8% respectively. The co-digested cow dung and seaweed mixture of C/N ratio resulted higher than the initial cow dung and seaweed. The different ratio was employed such as *Ulva* sp. + Cow dung (1:1), *Ulva* sp. + Cow dung (2:1) and *Ulva* sp. + Cow dung (3:1) and the ratio is 18.4, 21.2 and 24.8 showed as Table 3. The C/N was increased when the seaweed was mixed with cow dung and our findings were supported

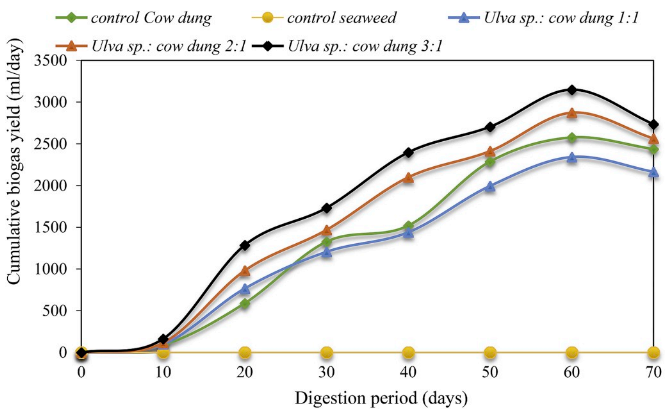

Fig. 1. Cumulative biogas production by various experimental setups using *Ulva* sp. mixed with cow dung. Values correspond to means of two replicates of independent values ± standard deviations (error bars). All values are significantly different ($p < 0.001$).

Table 3
Composition of C/N ratio from co-digested cow dung and green seaweed *Ulva* sp.

S. No	Biomass	C/N Ratio (%)
1	Cow dung	15.4
2	*Ulva* sp.	11.2
3	*Ulva* sp. + Cow dung (1:1)	18.4
4	*Ulva* sp. + Cow dung (2:1)	21.2
5	*Ulva* sp. + Cow dung (3:1)	24.8

The C: N ratio was analyzed at cow manure, *Ulva* sp and different ratios. The result denotes as percentage.

Table 4
Preparation of bio-manure by different treatment.

Treatments	Combinations (9:1 proportion)
Treatment 1	Control – soil
Treatment 2	Soil: *Ulva* sp.: cow dung (3:1 ratio)
Treatment 3	Soil: chemical fertilizer (CMF)
Treatment 4	Soil: seaweed powder (SP)
Treatment 5	Soil: farmyard manure (FYM)
Treatment 6	Soil: seaweed liquid fertilizer (SLF)

The treatment 2 combinations gave the best results compared to other treatment.

by (Nikolaisen et al., 2011). They stated that the, mixing of cattle manure with *Ulva lactuca* with the different ratio for producing biogas and also they concluded that the increasing *Ulva lactuca* biomass and reducing manure concentration is significantly increasing COD/N ratio. Similarly, McKennedy and Sherlock (2015) have their research findings the C:N ratios for *Ulva* varied from 8.72 to 30.71 and the methane produced varied from 220 to 330 Liters per Kilogram (L Kg^{-1}) with increasing C:N ratios. Habig et al. (1984) reported that a C:N ratio greater than 20 for *Sargassum* which did not lead to very successful methane production (150 L CH_4 Kg^{-1} VS) when compared to *Gracilaria* (190 L CH_4 Kg^{-1} VS) and *Ulva* (230 L CH_4 Kg^{-1} VS) from their work. Anjaneyulu et al. (1989) found that *Sargassum tenerrimum* to have a C:N ratio of 18:1 which produced methane levels at 132 L Kg^{-1}TS. McKennedy and Sherlock (2015) suggested that the effect of C:N ratio is dependent on the different algal species. Similarly (Gurung et al., 2012) pronounce the biochemical methane production from seaweed, brown algae, green algae and fish viscera as substrates. The authors reported to methane yield from their research findings represent as Table 1. Aggregate CH_4 methane yields were observed using green and brown algae, following 60 days of digestion. The CH_4 substance of the biogas was approximately 70% for brown and green algae. Lower CH_4 yields were observed using fish viscera and seaweed. Numerous scientists have endeavored to increase the bioenergy potential of *Ulva lactuca* biomass using several of pre-processing steps; synthetic as well as biological. Bruhn et al. (2011) discussed their findings washing, chopping, maceration and heat treatments (110 °C and 130 °C) for expanding the bioenergy potential of *Ulva lactuca*. However, the most extreme methane acquired was 271 mL g^{-1} VS, which is similar to cattle manure based methane production. The biomethanation of fresh water hyacinth, dry water hyacinth, poultry litter, cow manure and primary sludge were experimented using 250 ml bio-digesters with 7% total solids and 60 days retention time. The digester fed with poultry litter produced the highest biogas of 0.39 lit/g volatile solids (VS) followed by the digester fed with primary sludge (0.28 lit/g VS) (Patil et al., 2011).

3.3.1. Biogas composition

The percentage of gas composition determined the H_2, CH_4 and CO_2 concentration in biogas was an illustration in Fig. 2a and cumulative CH_4 ranged from 72% for *Ulva* sp.: cow dung 3:1 and 61% for *Ulva* sp.: cow dung1:1 ratio represented in Fig. 2b. In general, it takes several days to decay undissolved compounds like cellulose, proteins or fats into monomers through the hydrolysis of soluble carbohydrates happens within a few hours of digestion time. Mhatre and Gupta (2018) revealed the findings on yields of methane and potential of untreated and treated biomass residues were assessed in batch studies. All the batches were routinely observed for methane produced on regular basis, whereas the overall yields and substrate utilization were computed toward the end of each batch. Even though that biogas production commenced at 24 h in all the batches, the methane composition in each batch varied. The untreated *U. lactuca* biomass generated 210.08 ± 6.15 ml CH_4 g^{-1} VS which was similar to earlier reported yields (Habig et al., 1984; Briand and Morand, 1997; Morand et al.,

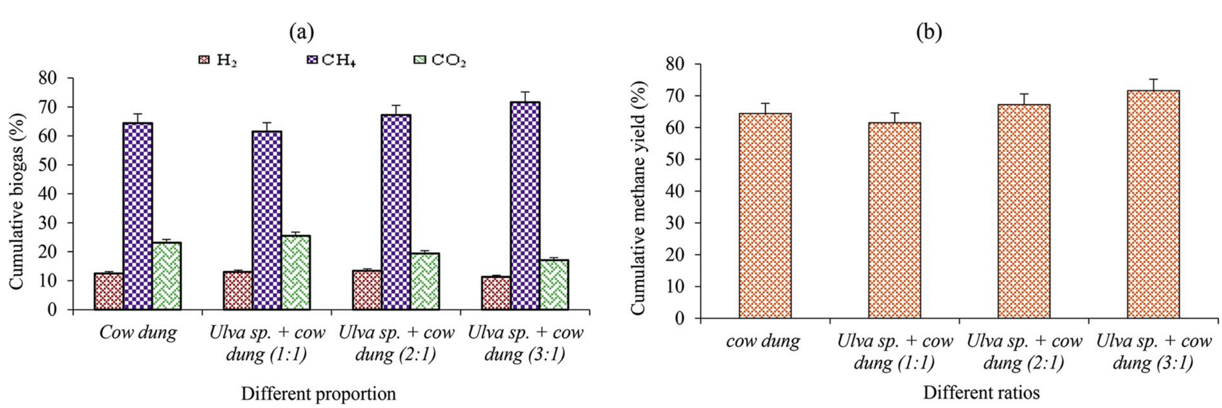

Fig. 2. Percentages in the cumulative biogas and methane yield from *Ulva* sp.: cow dung 1:1, 2:1 and 3:1.

2006; Bruhn et al., 2011; Nielsen and Heiske, 2011; Costa et al., 2012). Patil et al. (2011) reported that the biogas produced from water hyacinth (*Eichhornia crassipes*) as a substrate. The sludge can be used as a fertilizer from the biogas process, because the substrate has all the nutrients for the plant growth. In the present work biomethanation of fresh water hyacinth with different amount of water was carried out in the mesophilic temperature range of 30 °C–37 °C for 60 days. Fermentation slurry of water hyacinth to water produced maximum biogas yield of 0.245L/g VS at 1:4 ratio (FWH-1:4) followed by FWH-1:5 and FWH-1:3. Anantharaj and Venkatesalu (2002) reported that the seaweed applications are so many like to improve the soil conditioners and green manure composts, which is presence of high level potassium salts, micronutrients and plant growth elements.

3.4. Analysis of seaweed fertilizer

3.4.1. Seed germination

In the seed germination process, the plates were observed with germinated seeds and calculated by the percentage of *V. radiata* was varied from 98 to 99%.

3.4.2. Effect of different fertilizers on V. radiata

In this experiment, the results revealed that the most significant shoot length (9.8 ± 0.5 cm) was seen in *Ulva* sp.: cow dung (3:1) treatment and least (0.1 ± 0.02 cm) were recorded in control (Fig. 3a). Bai et al. (2007, 2013) reported that the foliar application of extracts from seaweeds on field crops obtained shoots and roots longer by 35% and 22%, in comparison with the control. The highest root length (6.9 ± 1.5 cm) was observed in *Ulva* sp.: cow manure (3:1) treatment and minimum value attained (0.1 ± 0.05 cm) were in control (Fig. 3b). This study was in accordance with the study of (Gandhiyappan and Perumal, 2001). The highest fresh weight (1.61 ± 0.34 g) was recorded at *Ulva* sp: cow dung (3:1) compound and least value (0.10 ± 0.5 g) were seen in SP compound (Fig. 3c). The maximum dry weight (4.0 ± 1.5 g) was found at *Ulva* sp.: cow dung fertilizer (3:1) compound and the minimum value (0.02 ± 0.01 g) were seen at control and SP mixes (Fig. 3d).

3.4.3. Pigment analysis of V. radiata

Photosynthetic pigment chl –*a* was enhanced by the treatment of CMF, SP, SLF, cow dung, *Ulva* sp.: cow dung (3:1) and control. The highest chl – *a* pigment content (19.77 ± 3.0 mg/g) was found at *Ulva* sp.: cow dung (3:1) and the lowest value (3.97 ± 0.04 mg/g) was recorded in control (Fig. 4a). The maximum chl – *b* content (9.14 ± 3.0 mg/g) was observed at *Ulva* sp.: cow dung (3:1) and the lowest value (0.97 ± 0.02 mg/g) was recorded in control (Fig. 4b). The highest total chlorophyll value (24.74 ± 5.0 mg/g) was obtained from *Ulva* sp.: cow dung (3:1) than the other compounds. The lowest value (5.03 ± 0.05 mg/g) was found at control (Fig. 4c).

The maximum carotenoids content value (2.01 ± 1.01 mg/g) was recorded at *Ulva* sp.: cow dung (3:1) than the other compounds and the minimum amount (0.473 ± 0.01 mg/g) was recorded at CMF (Fig. 4d). The seaweed extract applied as foliar spray enhanced the leaf chl level in plants (Blunden et al., 1996). The lot of researches have attentive on different seaweed fertilizer treated with plants in wide range. In particular, the seaweed *Brassica nigra* were found to be effective concentration of extract (Kalidass et al., 2010), *Abelmoschus esculentus* (Sasikumar et al., 2011), *Lycopersicon esculentum* (Zodape et al., 2011), *V. radiata* (Bai et al., 2013; Parthiban et al., 2013), *V. mungo* (Kalaivanan et al., 2012), *Solanum lycopersicum* (Hernández-Herrera et al., 2014). In the experimental setup, the plant pigment gradually increased with increasing seaweed compositions. It is the evidence that seaweed is promoting the pigments content in the plant.

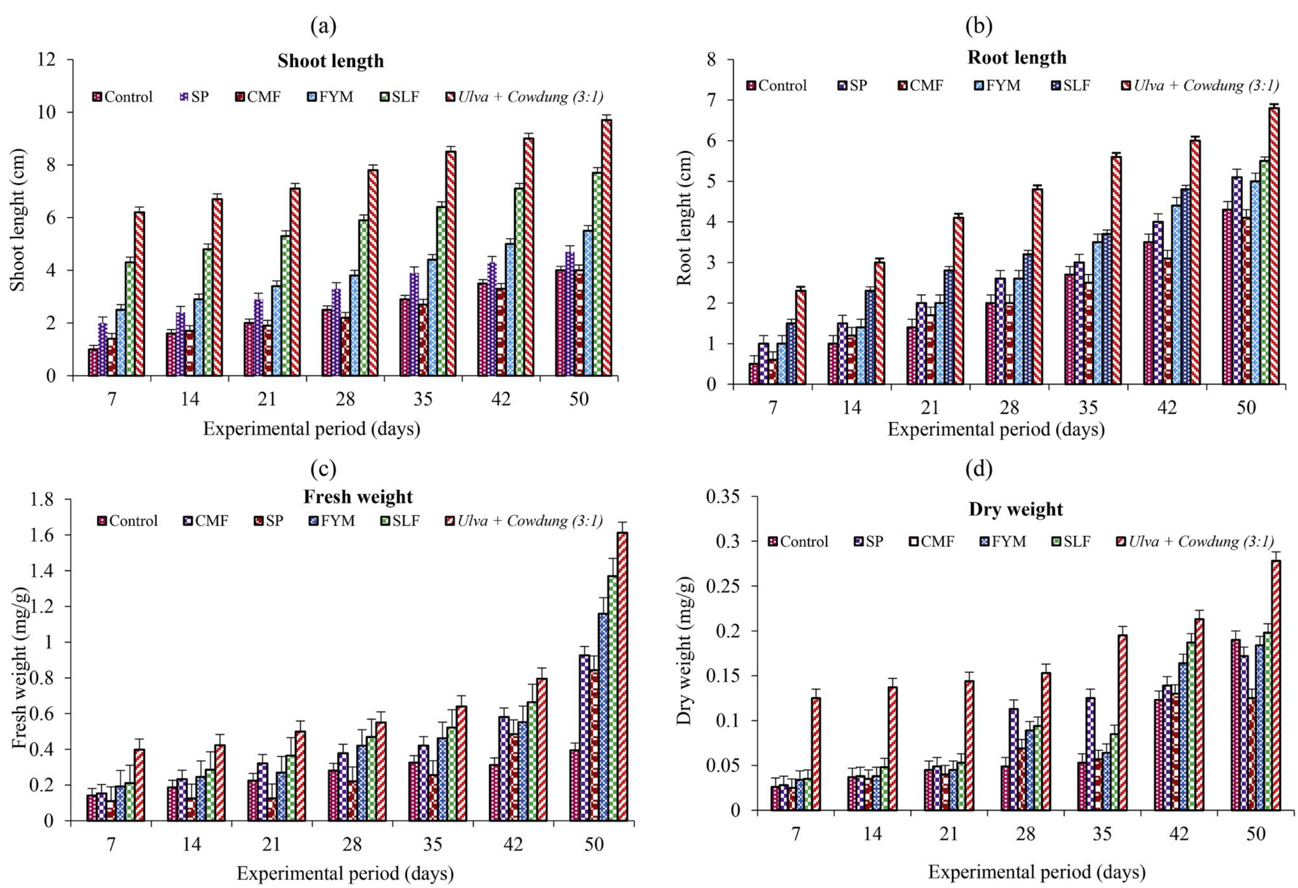

Fig. 3. Physicochemical parameters on *Vigna radiata*.

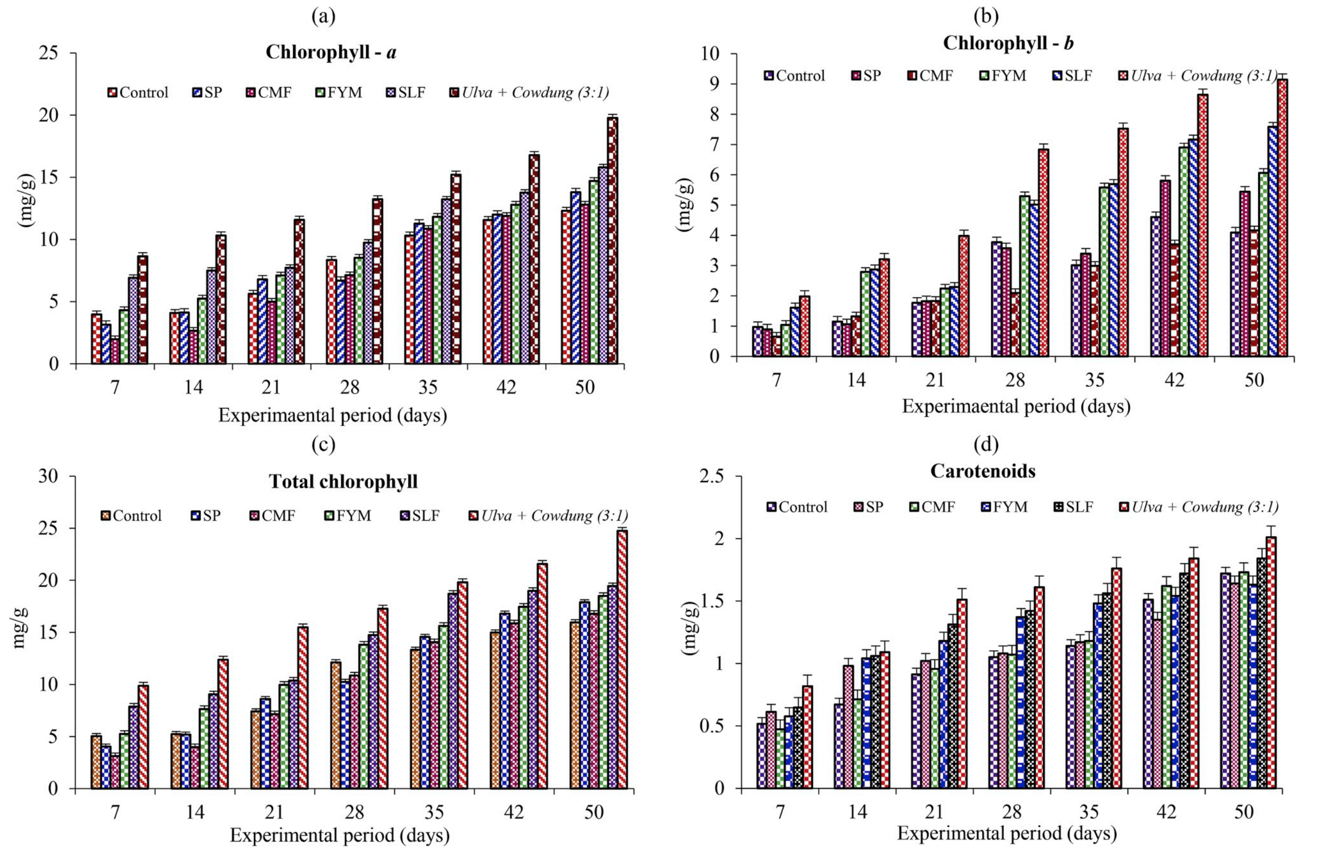

Fig. 4. Pigment analysis in *V. radiata* under different fertilizers treatment.

3.5. NPK analysis

The nutrient composition was analyzed for wet and dry samples of *Ulva* sp. the N remain same, whereas P was higher and K was lower in the wet as compared to the dry sample. In NPK analysis, *Ulva* sp. wet sample has obtained the higher phosphorus content (2.42 ± 0.5 mg/g) than the dry sample followed by *V. radiata* maximum nutrient value (3.12 ± 0.5 mg/g) when paralleled to the control. The nutrient content was increased when the *V. radiata* was treated with *Ulva* sp. showed in Fig. 5a. Jothinayagi and Anbazhagan (2009) worked in *V. radiata* the nutrients NPK were increased (3.46 ± 3.27 mg/g) than the control.

The NPK analysis was analyzed before and after treatment at soil testing laboratory, Tiruchirappalli. In this study, the potassium was increased *Ulva* sp.: cow dung 3:1 followed by seaweed liquid fertilizer (Fig. 5b). The NPK nutrients induced the plant growth and hormones level. These nutrients are mainly responsible for photosynthesis and growth. The growth parameters increased at a lower concentration when may presence of N, P, K nutrients higher levels in the seaweed extract of *Caulerpa scalpelliformis* (Kumar and Sahoo, 2011).

The biochemical constituents of *V. radiata* was effective solid waste fertilizer prepared from *S. wightii* (Sivasankari et al., 2006) and increased the pigment, protein, amino acid, total sugar content, catalase, peroxidase and polyphenol oxidase when using the lowest (10%) concentration. The results are reported in accordance (Nedumaran and Perumal, 2009), indicating the relation of plant hormones contained in seaweeds with the germination energy of plant seeds subjected to their action. The lower doses of seaweed compost waste significantly increase the growth (10%) concentration as compared to the control than

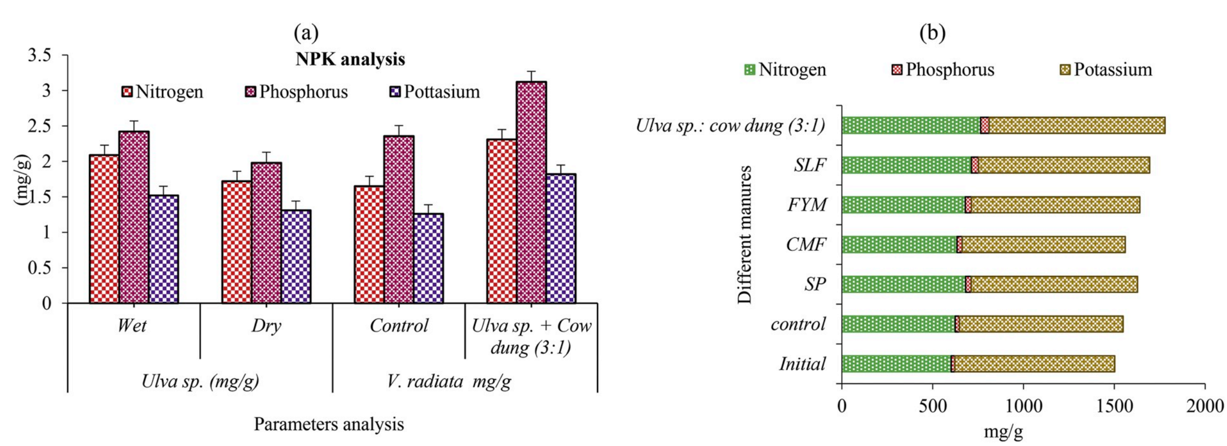

Fig. 5. NPK analysis from plants and soil.

the high doses (Jothinayagi and Anbazhagan, 2009). This approach would improve and the advantage of energy conversion from the end biomass of various biofuels.

4. Conclusion

In this research work, the *Ulva* sp. was showing to analysis including biogas production and biofertilizers. In the biomethanation process, it produced more methane in combination with cow dung and reported that *Ulva* sp. was present in rich source of methane. In a bio manure study also, the best results were observed with *Ulva* sp. It is suggested that *Ulva* sp. compounds are to improve sustainable crop growth and yield and can be used as a substitute for synthetic fertilizers. It is concluded that *Ulva* sp. is effectively used as an eco-friendly approach to biogas production and organic farming. Macroalgae are the new biomass resource for the next decades and production of biodiesel. Under this situation, biofertilizers may work as a good inducer for sustainability in agricultural production coupled with the maintenance of soil health.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101035.

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