

## Study on extraction of biofuel from selected algae species

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**Abstract:** The rising level of pollution and depleting fossil fuels has compelled every nation to seek a clean and abundant fuel source. Thus the demand for clean energy increases concerning the development of industrialization. With a clean, abundant fuel like algae biofuel, this demand can be counteracted. The main motivation behind this research is that a person as simple as a farmer can be profited along with this fuel preparation. This algae biofuel will be one of the key tools to attain sustainable development in the Energy and environment sectors. As algae species are so vast and almost present in every habitat, algae can be used as biomass for biofuel preparation and their nutritional values and ability to perform photosynthesis must be mentioned here. Being a tropical country, India has a great potential to indulge in algae biofuel generation to meet the energy demands. Among the most commonly available and known species of algae, three species were selected for this study. They are Spirogyra sp, Chlorella sp, and Spirulina sp. The process used here is anaerobic digestion and pyrolysis for the extraction of fuel content from the biomass. Among the cultures, chlorella seems to have the best potential for biodiesel production both in terms of nutrients and maintenance & yield and spirogyra for biogas production. Thus the mass culture of chlorella can be done by any person and biomass yield can be matched with the demand for biodiesel production. Although spirogyra undergoes anaerobic digestion and yields biogas, the yield rate was not up to the level of conventional biomass sources for biogas production. Biomasses termed as waste like agricultural, cattle dung and other putrescible wastes are most suitable for biogas production because as it a resource from waste i.e. free from culturing, maintenance, expenses, etc

**Keywords:** Energy, Biofuel, Biomass, Algae, Algae Biofuel, Pyrolysis, Anaerobic digestion, Spirogyra, Spirulina, Chlorella

### 1. Introduction

Energy availability is directly responsible for development and prosperity across the Earth. Facing the growing demand with the consideration of environment-friendly and socio-economic-friendly factors is a great deal today. In this time of great demand around the planet with unstable sources, we are pushed to a situation to adopt with a new source. There will be no development without energy, and all the technological developments would be useless. Thus it also affects the quality of life and other factors all around the world. Even in the case of well-developed countries, the HDI is high because of the sufficient energy sources and supplies. And with the case of developing and under-developing countries energy plays a vital part.

Almost around 86% of global energy is obtained using fossil fuels through oil, coal, and gas with global usage rates of around 32%, 30%, 24% respectively. . Even with the increased renewable energy sources like solar energy, wind energy, etc. the demand for fossil fuels remains inevitable. The extraction of energy from renewable energy sources must be increased to nullify the existing demand. This will be practically hard and has its demerits. Thus to balance the missing part a new alternative source of energy is required. The major issue with fossil fuels is due to their nature of heavy pollution and contamination in both extraction and usage phases. Moreover, these sources aren't going to last much longer. And the stage of depletion is nearing by time.

Rising concern for climate change and the negative impacts due to the high emission of greenhouse gasses has taken a great toll on the health of the environment. Thus alternate fuels with less polluting characteristic are much needed one of such sources are biofuels. These fossil fuels are primarily used for Industrial purposes and Transportation. Thus altering these with clean biofuels can be the savior of the environment from air pollution.

Biofuels are of primarily three types they are 1st, 2nd, and 3rd generation biofuels. The first generation involves using food substances for biofuel production, this generation of biofuel has a big disadvantage as the existence of food scarcity is a major issue going on. The second-generation biofuels are not generated from food substances but require the same resources for growing biomass like food crops. The third generation has many possibilities as it does not affect food production but they lack the economic feasibility to process. The currently developed genetically modified biomass seems to have promising characters for biofuel production in terms of cultivation, yield, processing, efficiency, etc.

The kingdom of algae is so vast that it has more diversity than any other group of organisms. Algae range from being unicellular (Diatoms) to multicellular organisms. Most of the algae species contain chlorophyll thus leading them to produce their own food and staying as autotrophs. Algae can grow at an exponential rate ranging from various habitats. It includes pure water, saline water, contaminated water, etc. It is to be noted that algae do not

require terrestrial land for growing and unlike food crops algae can be grown with contaminated water also. The main parameters for algae are sunlight, air contact, and a suitable medium.

Algae is a great player in the field of biofuel production due to its nature of nutrients, capability to perform photosynthesis, easily cultivatable, available in numerous forms. But it is also a great supplement of nutrients for human and animal consumption as it is rich in proteins unlike in any other sources, essential amino acids and it contains a high amount of simple and complex carbohydrates besides having an extensive fatty acid profile, including Omega 3 and Omega 6 it is termed to be a superfood.

Algae have a high potential for biofuel production and are classified under third-generation biofuels. Algae can produce 70- 100 dry tons of biomass per ha whereas terrestrial plants contribute around at only 12-25 dry tons per ha. Algae cultivation has a greater advantage as it can be cultivated in wastelands and wastewater bodies that can be effectively used for biofuel production. The bioremediation property of algae makes it a wonderful source in this case. Algae have the potential to yield up to 10000 gallons of oil per annum per acre. Table 1 shows the oil yield rate for every crop.

**Table 1** Crops and their oil yield rate <sup>[2]</sup>

Crop	Oil yield gallon/acre
Corn	18
Cotton	35
Soya bean	48
Mustard seed	61
Sunflower	102
Rapeseed/Canola	127
Jatropha	202
Oil palm	635
Algae - 10g/m <sup>2</sup> /day at 15% Triglycerides	1,200
Algae - 50g/m <sup>2</sup> /day at 50% Triglycerides	10,000

**2. Materials and methodology**

The main raw material required here is the bulk biomass of the selected algae species for the production of biofuel. The process begins with the selection of the desirable species, collection of mother culture followed by mass culturing in a culture medium. Later followed by the methodologies adopted for the synthesis of biofuel from the biomass which includes the process of pyrolysis and anaerobic digestion for gasification. The growth of Algal biomass involves the collection of mother culture or sample, Set up of culture medium, introducing the culture to the medium which leads to the growth of culture medium, and later the biomass can be harvested.

The sources of live algal species of spirogyra and chlorella are collected from a small water body which is refilled with the drains from the surrounding agricultural land. The water body is located in the small village of ‘Aandakothampalayam’ in the municipality of ‘Nanjai Uthukuli’ municipality. The coordinates of the location of the waterbody are 11.256355,77.7544197. (Dropped pin Near Erode, Tamil Nadu <https://maps.app.goo.gl/ajcKKKyKfsUeHTBd6> ). The mother culture of spirulina was brought from a spirulina farm.

The Culture medium was set up using two irrigation tanks which are drained and cleaned and later filled with bore well water. Nutrients were added to the water to support the algal growth of spirogyra and Chlorella. In the case of spirulina due to its sensitive nature, the medium was made in an isolated tank. Once the algal samples were introduced to the medium, it was left out for growing. The temperature was noted regularly in all the mediums. Periodical addition of nutrients was done besides checking of the other parameters like availability of sunlight, contamination, etc. and maintenance of the medium was checked regularly.



Fig A



Fig B



Fig C

**Fig A & B** – Harvesting the cultured spirogyra.

**Fig B** – Harvesting the Spirulina culture.

The yields were collected for every batch between 15 – 20 days. The biomass was collected delicately in a plastic container. After yield collection, the biomass collected will be cleared from impurities like leaves, twigs, etc. The biomass will be laid on an inert sheet-like tarpaulin under shade till the water in the biomass escapes.

The extraction of biofuel from the biomass is done via two processes pyrolysis (thermochemical degradation) and Gasification (Anaerobic digestion). Biofuels can be of biodiesel and biogas. Pyrolysis



**Fig D** – Pyrolysis under process.

is defined as the thermal degradation of biomass by application of heat in the absence of oxygen, which results in the production of multiple products: bio-oils [6] [7], solid residues [7], etc. The pyrolysis setup consists of a masonry furnace with a pyrolysis chamber mounted on it, a condensation chamber made with copper tubes and water as a coolant, an oil collection chamber, followed by a bubble filter which traps the escaped oil, and a gas collection tube if required.



**Fig E** – Pyrolysis setup.



**Fig F & G** – Smoke point and Oil yield.

Anaerobic digestion is the process of degradation using anaerobic microorganisms which ends in the generation of biogas and degraded materials. The main parameters which affect anaerobic digestion are pH, Temperature, C: N ratio, Loading rate, Pressure rate, etc. Since the algal biomass was rich in Nitrogen, the ratio must be balanced by adding a suitable buffer (Cow dung). The biomass was well mixed with the buffer which also acts as seeding material in a 500ml bottle with an attached outlet via an airtight cork. The biogas produced is collected via the inverted gas collection method. Later the biogas is immersed in a 2M NaOH solution. This process is done to estimate the amount of CO<sub>2</sub> present in the biogas. The volume of CO<sub>2</sub> is replaced by the NaOH solution. Thus the approximate values of CO<sub>2</sub> present in the biogas is found.



**Fig H** – Three samples under anaerobic digestion.



**Fig I** – Inverted gas collection.



Fig J – The collection vessel shows the gas collected with their quantities.

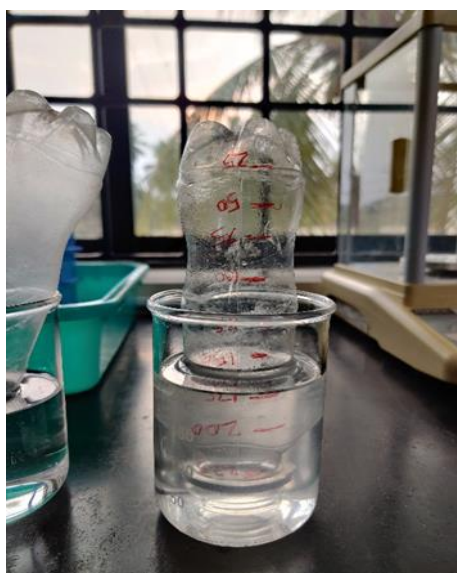


Fig K – The collection vessel is immersed in 2M NaOH solution for estimating the quantity of CO<sub>2</sub>.

### 3. Results

#### 3.1 Growth parameters

The growth parameters of the algal biomass are given in Tables 2, 3, and 4 respectively. It shows the nature, characteristics, and parameters required for the growth and yield of algal biomass.

Table 2 Growth parameters of spirogyra

S.No	Parameter	Values
1	Nutrients	NPK
2	Mode	Added a scoop of fertilizer for every 10 days
3	Temperature	25-28 °C
4	pH	7.5
5	Buffer	NIL
6	Mode of medium	Irrigation water tank
7	Dimensions	25 * 25 feet
8	Surface area	625 ft <sup>2</sup>
9	Volume of the medium	3125 ft <sup>3</sup> / 88.49 m <sup>3</sup> / 88490 Lts
10	Yield rate	20 Kg
11	Time interval	28 Days
12	Yield Ratio (yield rate / Time/ surface area)	0.00112 kg/day/ ft <sup>2</sup>

**Table 3** Growth parameters of chlorella.

S.No	Parameter	Values
1	Nutrients	NPK
2	Mode	Added a scoop of fertilizer for every 10 days
3	Temperature	25-28 °C
4	pH	7.5
5	Buffer	NIL
6	Mode of medium	Irrigation water tank
7	Dimensions	25 * 25 feet
8	Surface area	625 ft <sup>2</sup>
9	Volume of the medium	3125 ft <sup>3</sup> / 88.49 m <sup>3</sup> / 88490 Lts
10	Yield rate	28 Kg (with water content)
11	Time interval	28 Days
12	Yield Ratio (yield rate / Time/ surface area)	0.0016 kg/day/ ft <sup>2</sup>

**Table 4** Growth parameters of spirulina.

S.No	Parameter	Values
1	Nutrients	Calcium chloride, Urea, Sodium bicarbonate, Ferrous sulfate, and magnesium sulfate.
2	Mode	Supplements readily available as a powder.
3	Temperature	25 – 28 °C
4	pH	8
5	Buffer	Sodium bicarbonate
6	Mode of medium	Water tank and 20L water Can
7	Dimensions	Tank – 5*3 ft.
8	Surface area	15 ft <sup>2</sup>
9	Volume of the medium	45 ft <sup>3</sup> / 1.274 m <sup>3</sup> / 127 Lts
10	Yield rate	7 kg
11	Time interval	28 days
12	Yield Ratio (yield rate / Time/ surface area)	0.016

### 3.2 Macronutrients

The macronutrients show the primary nutrients like Lipids, Protein, Carbohydrate, Etc. amounts present in the biomass.

**Table 5** Macronutrients in spirogyra. [4]

S.No	Parameters	Concentration (mg/100g)
1	Carbohydrate	0.5357 mg
2	Starch	0.48159 mg
3	Protein	0.1811 mg
4	Amino Acid	0.0254 mg

**Table 6** Macronutrients in chlorella. [3]

S.No	Parameters	Concentration (g/100g)
1	Protein	58.4 g
2	Fat	9.3 g
3	Carbohydrate	23.2 g
4	Fiber	0.3 g
5	Ash	4.2 g
6	Moisture	4.6 g
7	Calories	411 Cal

**Table 7** Macronutrients in spirulina. [1]

S.No	Parameters	Concentration (g/100g)
1	Calories	373
2	Total fat	4.3
3	Saturated fat	1.95
4	Polyunsaturated fat	1.93
5	Monounsaturated fat	0.26
6	Cholesterol	< 0.1
7	Total carbohydrate	17.8
8	Dietary fiber	7.7
9	Sugars	1.3
10	Lactose	< 0.1
11	Total amino acids (Protein)	60.03

### 3.3 Pyrolysis

Pyrolysis is the process of heating the biomass at more than 300 degrees C in absence of oxygen. The grown biomass which will be extracted is prepared for the pyrolysis process. This process will yield oil and gas content from the biomass employing thermochemical decomposition

**Table 8** Spirulina Biomass parameters

S.No	Parameters	Observation
1	Wet weight	20 g
2	Dry weight	2.84 g
3	Water content	85.8 %
4	Volume occupied	0.93 gcm <sup>-3</sup> (or) 0.93 gml <sup>-3</sup>
5	Time for drying	30 Days
6	Batch no	1
7	Biomass used	20 g

**Table 9** Spirulina pyrolysis end products

S.No	Parameters	Observation
1	Whole crude	4 g
2	Gas	4 g
3	Residue	12 g
4	Moisture content	78.94 %
5	Bio crude	21.05 %

**Table 10** Spirulina reaction timings

S.No	Parameters	Observation
1	Initial	0
2	Smoke point	2 mins
3	Yield point	4 mins
4	Reduction point	19 mins
5	Endpoint	31 mins
6	Temp	450 – 550 °C
7	Environmental temp	35 °C

**Table 11** Spirogyra Biomass parameters

S.No	Parameters	Observation
1	Wet weight	20 g
2	Dry weight	1.6 g
3	Water content	92 %
4	Volume occupied	1.35 gcm <sup>-3</sup> (or) 1.35 gml <sup>-3</sup>
5	Time for drying	30 Days
6	Batch no	2
7	Biomass used	20 g

**Table 12** Spirogyra pyrolysis end products

S.No	Parameters	Observation
1	Whole crude	0.3 g
2	Gas	1.7 gm
3	Residue	18 g
4	Moisture content	100 % (Special observation)
5	Bio crude	0 %

**Table 13** Spirogyra reaction timings

S.No	Parameters	Observation
1	Initial	0
2	Smoke point	5 mins
3	Yield point	14 mins
4	Reduction point	33 mins
5	Endpoint	43 mins
6	Temp	450 – 550 °C
7	Environmental Temp	35 °C

**Table 14** Chlorella Biomass parameters

S.No	Parameters	Observation
1	Wet weight	20 g
2	Dry weight	2.17 g
3	Water content	89.15 %
4	Volume occupied	1.25 gcm <sup>-3</sup> (or) 1.25 gml <sup>-3</sup>
5	Time for drying	30 Days
6	Batch no	3
7	Biomass used	20 g

**Table 15** Chlorella pyrolysis end products

S.No	Parameters	Observation
1	Whole crude	6 g
2	Gas	4 g
3	Residue	10 g
4	Moisture content	62.5%
5	Bio crude	37.5 %

**Table 16** Chlorella reaction timings

S.No	Parameters	Observation
1	Initial	0
2	Smoke point	2 mins
3	Yield point	4 mins
4	Reduction point	27 mins
5	Endpoint	44 mins
6	Temp	450 – 550 °C
7	Environmental Temp	35 °C

### 3.4 Anaerobic digestion (biogas)

Biogas is extracted using anaerobic digestion. The process is carried out by maintaining the parameters of pH, C: N ratio, loading rate, and by adding a suitable seeding material. Here cow dung acts as a dual agent by stabilizing the C: N ratio and as seeding material.

**Table 17** Revised C: N ratio

S.No		Spirulina	Spirogyra	Chlorella
1	C:N ratio	4.56 : 1 <sup>[17]</sup>	20.68 : 1 <sup>[18]</sup>	5.38 : 1 <sup>[20]</sup>
2	Buffer	Cow dung <sup>[19]</sup>	Cow dung <sup>[19]</sup>	Cow dung <sup>[19]</sup>



		(C:N is 25:1)	(C:N is 25:1)	(C:N is 25:1)
3	Algae: Buffer Ratio	1 : 5	1 : 1	1 : 5
4	Optimum C:N ratio	20 – 35	20 - 35	20 - 35
5	Revised C:N ratio	21.6	22.84	21.73
6	Algae + Buffer (Total 50 g)	8.33 g + 41.67 g	25 g + 25g	8.33 g + 41.67 g
7	pH	7	7	7
8	Water added	175 ml	175 ml	175 ml
9	Total gas collected	40 ml	410 ml	80 ml

Table 18 Biogas production rate

Biomass No. of days	Spirulina		Spirogyra		Chlorella	
	Daily volume of gas (ml)	Cumulative Volume of gas (ml)	Daily volume of gas (ml)	Cumulative Volume of gas (ml)	Daily volume of gas (ml)	Cumulative Volume of gas (ml)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	5	5	0	0	10	10
8	10	15	10	10	5	15
9	10	25	10	20	15	30
10	15	40	30	50	10	40
11	0	40	25	75	0	40
12	0	40	35	110	0	40
13	0	40	45	155	0	45
14	0	40	45	200	0	50
15	0	40	20	220	0	55
16	0	40	15	235	0	63
17	0	40	10	245	0	70
18	0	40	15	260	0	70
19	0	40	8	268	0	75
20	0	40	42	310	0	80
21	0	40	28	338	0	80
22	0	40	37	375	0	80
23	0	40	23	398	0	80
24	0	40	12	410	0	80
25	0	40	12	410	0	80

Table 19 CO2 estimation

S.No	Biomass	Water Uplifted (ml)	Total gas collected (ml)	% of CO <sub>2</sub>
1	Spirulina	6	40	15
2	Spirogyra	102	410	25
3	Chlorella	16	80	20

4. Discussion

The maintenance of chlorella and spirogyra were the easiest among the cultures with culture collection, growing, maintenance, yield collection. It was simple as well with a less sensitive nature and complications.

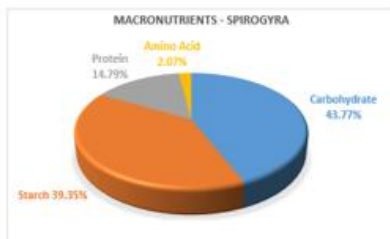


Fig L

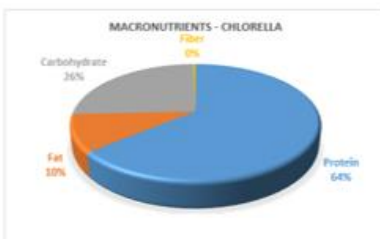


Fig M

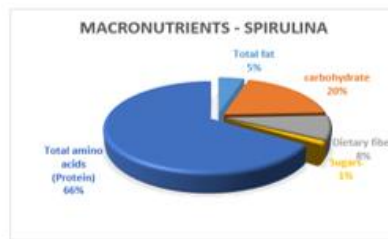


Fig N

Fig L, M, N – Shows the macronutrients in spirogyra, Chlorella and spirulina respectively.



Fig O – Visual results of all three batches.

From the comparison of macronutrients and yields, it is clear that the algal value with the highest lipid content yields the maximum. And for the production of biodiesel chlorella is a promising one followed by spirulina and spirogyra.

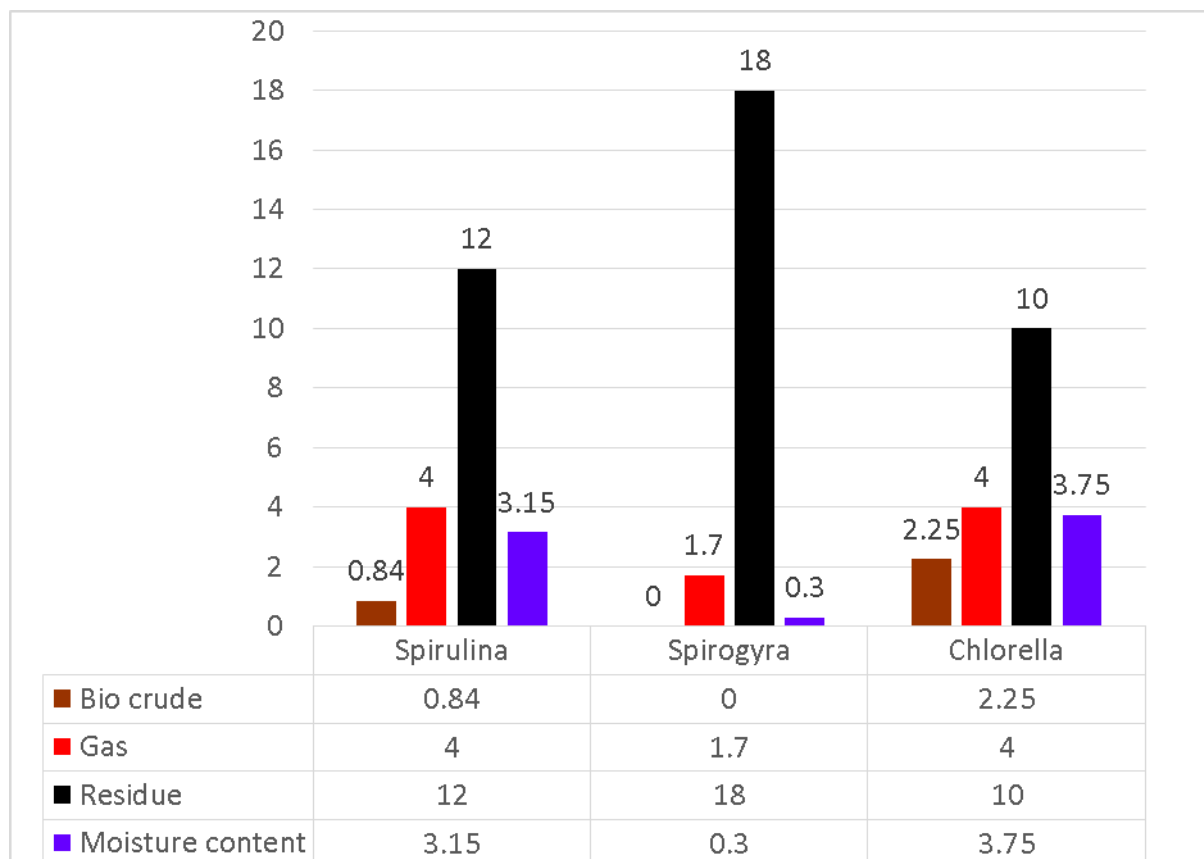


Fig P - Data comparison of pyrolysis results in grams.

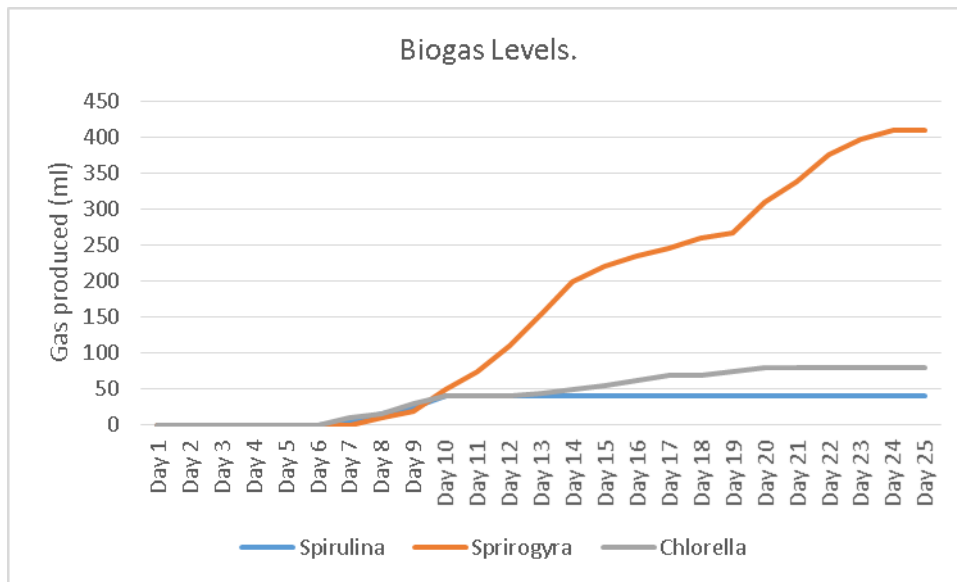


Fig Q – Total biogas produced by the specimens concerning time.

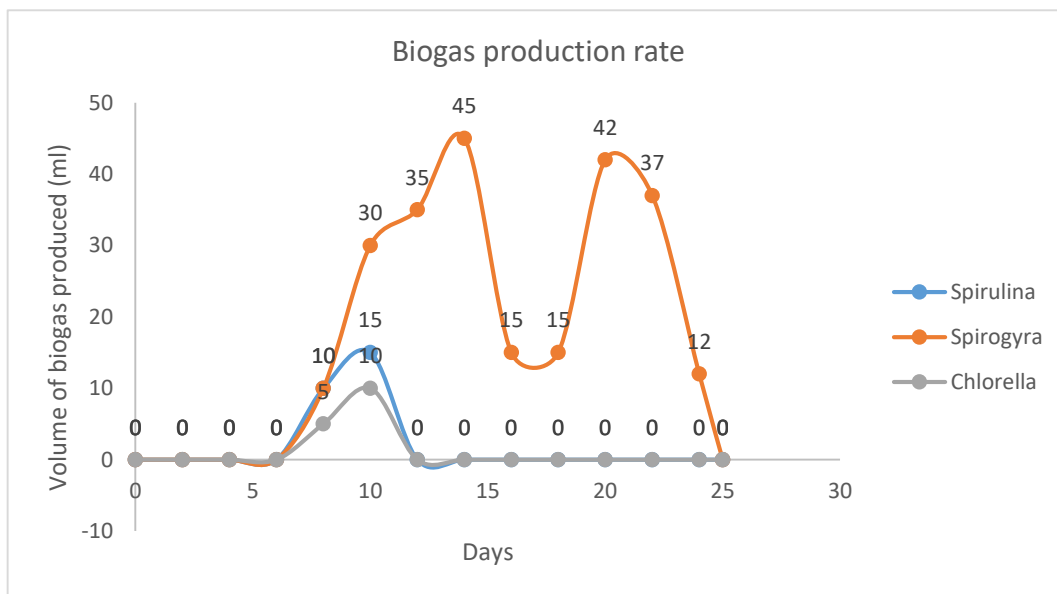


Fig R – Biogas production rate of each species of algae.

From Fig Q and R, it is clear that Spirogyra has the highest biogas yield and spike rates followed by chlorella and spirulina. The lower the yield the faster the reaction starts. The CO<sub>2</sub> level is around 25% in spirogyra yet it has the highest yield of biogas. So the methane levels are highest in spirulina followed by Chlorella and spirogyra. Thus spirogyra is the most suitable species for biogas production.

**5. Conclusion**

Chlorella is the best species among the three for producing biodiesel due to its lipid content, growth, maintenance, etc. as well as its easy cultivable nature, followed by spirulina. No oil was extracted from spirogyra. Spirogyra is the best species for biogas production due to its C: N ratio and volume of gas emitted. The least satisfactory was spirulina due to its poor yield rate. And the performance of chlorella was intermediate.

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