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Effects Of Nitrogen Concentrations On The Biomass, Lipid And Biodiesel Production Potentials Of Spirogyra Specie

[1] Salisu, A., [1]Umar, B., [2]Appah, J., [3]Aina, V. O., [4]Tanimu, Y*., and [5]Yahaya, U.

[1] Department of Biology, Federal College of Education (Technical), Gusau, Zamfara State, Nigeria.
[2]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.
[3]Department of Biochemistry, Kaduna State University, Kaduna, Nigeria.
[4]Department of Botany, Ahmadu Bello University, Zaria, Nigeria.
[5] Federal College of Forest Resources Management, Northeast, Maiduguri, Nigeria.

Abstract
The international dependence on non-renewable power supply including fossil fuels has led the sector toward power insecurity. This has necessitated the worldwide attempt to reduce the dependence on fossil fuels and broaden an economically feasible and scalable alternative fuel supply so that it will additionally lessen huge emissions of CO_2 within side the surroundings which in flip will result in discount environmental pollution. Biofuels have the potentials to offer a feasible opportunity to fossil fuels and can lessen overall CO_2 emissions. Algae appear like a rising supply of biomass for biodiesel that has the capacity to absolutely displace fossil fuel. Biodiesel has gain sufficient interest in latest years because of its green nature, non-poisonous characteristics, biodegradability and decrease net carbon cycle as compared to standard diesel fuel. In this research, Spirogyra sp. was collected from Tubu river, a tributary of River Kaduna, as a source of biomass feedstock for biodiesel production. Spirogyra sp. turned into cultured in various nitrogen (2.94×10^3 (100%), 2.20×10^3 (75%), 1.47×10^3 (50%), 0.73×10^4 (25%), and 0.000 (0%)) concentrations in Bold's Basal Media (BBM) in a laboratory test for 21 days at 25-28°C ambient temperature. Oil from Spirogyra sp. become extracted in soxhlet extractor using n-hexane as solvent and transformed into biodiesel through transesterification reaction. The highest biomass growth, oil, lipid content and biodiesel production were observed in treatment with 75% (2.20×10^3) Spirogyra sp. has a big capability for biodiesel production.
Keywords: Alternative fuel supply, Spirogyra sp., Bold's Basal Media, Transesterification, Biodiesel
*Corresponding author. Tel.: +2348066010120; E-mail address: yahuzatanimu@gmail.com

Introduction

Currently, the world is witnessing a growth in demand of energy at the side of the improvement of the arena's financial system through development, growth and modernization. With power charges accomplishing ancient highs, biodiesel as an opportunity gasoline is increasingly attracting interest. Currently, biodiesel is crafted from quite a few feedstocks, along with oils seed crops, waste cooking oils, and animal fat; however, the constrained deliver of those feeds inventory delays the additional growth of biodiesel invention. Algae have long been identified as probably accurate reasserts for

biofuels (biodiesel, bioethanol and biogas) manufacturing due to their excessive oil content material and fast biomass production (Ozkurt, 2009). Alternative fuel sources like bioethanol and biodiesel may be generated from non-fossil sources to fight the threats of worldwide warming, excessive value of fossil gasoline and emission of greenhouse gases caused by fossil fuels. Thus, biofuel like biodiesel and bioethanol gives several advantages in phrases of environmental protection, financial improvement and country wide security (Arthe et. al., 2008).

Biodiesel is numerous ester-primarily based oxygenated fuels derived from normal renewable organic bases such as algae biomass. Algae range from lesser, sole celled organisms to multicellular organisms that are mainly marine and microscopic. Algae are typically discovered in damp locations or our bodies of water and therefore are collective in native as well as aquatic environs. Like plants, algae require ordinarily 3 additives to develop: daylight, carbon dioxide and water that are transformed to algal biomass (Ozkurt, 2009). Photosynthesis is a vital biochemical procedure wherein plants, algae and a few microorganisms convert the strength from daylight into chemical energy. Algae shop this strength within side the shape of oils, carbohydrates, and proteins (Ozkurt, 2009).

Algae biomass is classified into four essential classes: Diatoms (Bacillariophyceae), inexperienced algae (Chlorophyceae), blue – inexperienced algae (Cyanophyceae) and golden algae (Chrysophyceae). Algae are regarded to growth in range swiftly to shape algal blooms (Demirbas, 2009). Like higher plants, they produce and store lipids in the form of Triacylgycerol (TAGS). Many species showcase speedy boom and excessive productiveness. Many microalgal species may be manipulated to build up giant portions of lipids, frequently more than 60% in their dry biomass (Sheehan et. al., 1998). Ordinary, the lipid matters typically ranges from 10 to 30% of dry weight. Depending at the precise algae species and their cultivation situations, however, algal lipid manufacturing might also additionally ranges extensively from 2 to 75% (Mata et. al*.,* 2010). In a few excessive cases, it may attain 70%–90% of dry weight (Li et. al., 2008). Macroalgae display a whole lot of promise as a feed inventory for biodiesel manufacturing (Chisti, 2007). Spirogyra (commonly known as water silk, mermaid's tresses, and blanket weed) is a genus of filamentous chlorophyte green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is normally established in fresh water areas, and there are additional 400 species of *Spirogyra* in the world (John and Brook*,* 2002) *Spirogyra* measures almost 10 to 100µm in width and can raise to numerous centimeters in span (Mata *et. al.,* 2010).

Spirogyra sp. is a common filamentous macroalgae determined freely in open ponds, river and different clean water habitats (Lee, 2008) in international distribution (Kim et. al., 2004). Since it generally paperwork free-floating loads that anchor to the substratum (Lee, 2008), it can cause issues in certain human activities such as inclusive of pumping impairment for drip irrigation in farm ponds (Bonachela et al., 2013) or hampering conventional fishing in lakes (Onyema and Emmanuel, (2009). In the case of Andalusian farm ponds; *S. africana* is one of the maximum not unusual places developing macroalgae, which may also bureaucracy mono algal mats all through spring months (Lee, 2008). Spirogyra is the maximum common inexperienced algae, to be had abundantly in springs, ponds and brackish water. Spirogyra has an easy cell hair like structure, and has unbranched cylindrical fibers, which is ready 1/10 mm in diameter and few centimeter lengthy. The cell wall consists of two major layers namely, the internal layer which is made of cellulose, and the outer layer is made of the pectone in nature. It produces lipids, carbohydrate and proteins that may be applied for the manufacture of biodiesel or bioethanol (Meng et. al., 2009).

Consequently, this study was conducted to isolate, propagate and evaluate lipid productivity for biodiesel production from *Spirogyra species* (macroalgae) cultivated in an indoor condition under nitrogen limitation.

Materials and methods

Sample Collection

The samples of *Spirogyra* sp*.* filaments were collected from Tubu River; a tributary of River Kaduna using a sterile container. The algal

sample were rinsed in pond and river water to remove sediments and immediately taken to hydrobiology laboratory, in the Department of Biology, Ahmadu Bello University, Zaria, for isolation and identification using an electrical compound camera microscope (Prescott, 1975).

Sample Identification

The sample of *Spirogyra sp.* were taxonomically identified according Prescott, (2007), by preparing thin smear of the sample on a glass slide with a few drops of water and covered with a cover slip and then viewed under compound microscope under x40 objective lens. The colony morphology, appearance and cellular structures observed were compared with those in a phycological atlas to identify *Spirogyra sp.* (macroalgae) from the algae sample collected, according to the morphological characterization given by Prescott (2007) and Zarina et al., (2009). Isolation was then done using serial dilution to obtain pure culture of algae to be used for inoculation.

Culture of Spirogyra

Bold's Basal media (BBM) was prepared and sterilized in an autoclave at 121°C for 15 minutes. Few strands were inoculated and incubated to obtain pure culture of isolated *Spirogyra sp.* 25 grams of *Spirogyra sp.* was then inoculated and cultured in three different 500-mLs Erlenmeyer flasks each containing 330mLs BBM with concentrations of nitrogen comprising 2.94×10^{-3} (100%), 2.20×10^{-3} (75%), 1.47×10^{-3} (50%), 0.73×10^{-4} (25%), and 0.000 (0%) respectively, and then incubated at 20°C in an orbital shaker set to 150 rpm. The conical flasks were covered with cotton wool to allow diffusion of air but prevent contamination. The cultures were then incubated and kept inside the laboratory and exposed at room temperature (24-28°C) and 12-12 hour light-dark photoperiod for three weeks (Abirhire and Kadiri 2012). The illumination was provided by 40 W Cool White Plus fluorescent lights at 110-150 µmol s^{-1} m^{-2}. The light provided by the 40 W Cool White Plus fluorescent bulbs was measured with an LI-250A light meter and Quantum Q40477 sensor (Li-Cor Biosciences, Lincoln, NE, USA). The BBM Media pH was maintained within alkaline level (8.0pH) and measured using pH meter after two days interval to ensure maximum productivity of algal species (Qu et. al., 2008).

Algal Biomass Harvest

Positively charged Pellets of alum and ferric chloride (inorganic) or cationic starch (organic) flocculants were added to the *Spirogyra sp.* samples to induce flocculation or aggregation of negatively charged macroalgal particles to aggregate with each other to form flocks of the biomass at the end of the experiment. The flocks of the biomass were settled as large clumps at the bottom of the conical flask. The top clear liquid was decanted off and the biomass was harvested by filtering through a GFC filter and the final biomass was obtained (Poelman et al., 1997).

Estimation of Algal Growth Biomass

The algal biomass are estimated using dry weight measurement method, by filtering harvested wet algal biomass in a funnel with a filter paper and then measured using a metric weighing balance. The actual weight (gm) of algal mass was taken and recorded after subtracting the weight of a filter paper (Haesman et. al., 2000).

Algal Oil Extraction

Extraction of lipids from wet biomass was performed according to the procedure of Sharif et. al*.,* (2008). Oil extraction from algae species are carried out by Soxhlet extractor method for 16 hours. In this process, *Spirogyra* sp*.* samples were fully dried in hot air oven at 70^0C for 8 hours for releasing water. The dried macroalgae were then grinded to powder form with motor and pestle as much as possible. The powders (10 grams) of five different biomasses of *Spirogyra sp.* grown under different nitrogen concentrations were mixed with n-hexane to extract oil in the thimble of soxhlet. In bottom flask, 400 ml n-hexane solvent was used to extract the oil from five different dried algal biomasses at 70°C for 16- 24 hours. The extracted oil in solvent was detached by rotary vacuum distillation.

Results

Measurement of Spirogyra sp. biomass produced from BBM

Table 1 shows the measurement of *Spirogyra sp.* biomass produced from BBM. Highest wet and dry biomass (g) of 55.1 g and 45.1g respectively were produced at 75% $NaNO_3$ concentration while the lowest wet and dry biomass (g) of 31.0g and 21.0g respectively were obtained from control experiment (0% $NaNO_3$). Highest dry biomass (81.9%) was achieved at 75% $NaNO_3$concentration while the lowest % of 67.7 was achieved at control experiment (0% $NaNO_3$).

Table 1: Measurement of *Spirogyra sp.* biomass produced from BBM

% $NaNO_3$ Conc.	Inoculated Sample (g)	Wet biomass (g)	Dry biomass (g)	% dry biomass produced (g)
100	10	$43.9^b \pm 0.01$	$33.5^b \pm 0.03$	76.3
75	10	$55.1^a \pm 0.03$	$45.1^a \pm 0.01$	81.9
50	10	$41.6^c \pm 0.02$	$31.6^c \pm 0.02$	76.0
25	10	$35.0^d \pm 0.02$	$25.0^d \pm 0.04$	71.4
0	10	$31.0^e \pm 0.01$	$21.0^e \pm 0.01$	67.7

Note: a, b, c, d, e means within a raw with different superscripts are significantly different (P>0.05)

Values are means ± standard deviation of three replicate

Measurement of oil and biodiesel yield from Spirogyra sp. biomass

Table 2 shows the measurement of oil and biodiesel yield from *Spirogyra sp.* biomass. Highest oil and biodiesel yield of 9.5mls and 6.8mls respectively were obtained from 75% $NaNO_3$ concentration while the lowest oil and biodiesel yield of 3.0ml and 1.8ml respectively were obtained from control experiment (0% $NaNO_3$). Highest % lipid content (95%) and biodiesel produced (68%) were obtained from 75% $NaNO_3$ concentration while the lowest % lipid content (30%) and biodiesel produced (18%) were obtained from control experiment (0% $NaNO_3$).

Table 2: Mean (±SE) oil and biodiesel yield from *Spirogyra sp.* biomass

% $NaNO_3$ Conc.	Oil yield per 10g biomass (ml)	Biodiesel yield per 10g biomass (ml)	% lipid content	% biodiesel produced
100	$4.00^d \pm 0.01$	$2.00^d \pm 0.02$	40	20
75	$9.50^a \pm 0.04$	$6.80^a \pm 0.01$	95	68
50	$7.50^b \pm 0.01$	$4.80^b \pm 0.01$	75	48
25	$5.50^c \pm 0.02$	$4.00^c \pm 0.02$	55	40
0	$3.00^e \pm 0.03$	$1.80^e \pm 0.03$	30	18

Note: Within the column a>b> c> d > e (P>0.05). Means within the column with the same superscripts are not significantly different.

Discussion

From the end result of this examine, it may be discovered that Spirogyra sp. filaments have followed and acclimatized the Bold's Basal media (BBM) additives, physiological parameters and environmental situations. This is likewise in

settlement with the findings of *Negoro* et. al., (1991) which said that top-rated boom of Spirogyra sp. filaments after 21 days of incubation turned into because of the truth that Spirogyra sp. filaments develop nicely within side the additives that had been furnished within side the media. A non-stop propagation and cultivation of Spirogyra sp. is wanted at 75% $NaNO_3$ awareness for max lipids accumulation and Fatty Acid Methyl Esters (FAMEs) productivity. The end result of this look at additionally confirmed that, macroalgae may be harvested optimally to have a stabile lipid manufacturing with the aid of using flocculation method. This is consistent with end result acquired through Abu et. al., 2012) which mentioned that algal oil extracted from 10g of algal biomass from 5 extraordinary macroalgal species become performed via soxhlex method, and the oil received turned into biodiesel of 9.5mls below maintained parameters of NaOH 0.3 (wt %), 2mls of Methanol, response time 30 minutes, RPM 150, temperature at 60^oC and pH of 8. This is likewise much like the report of Abu et. al., 2012) which recorded that 92% most biodiesel manufacturing from 5 exceptional macroalgal species.

Moreover, the fatty acid methyl ester (FAME) received from the transesterification of macroalgal oil derived from Spirogyra sp. at optimized indoor situations have met the specification of European Nations (EN) 14214 which specifies a minimal content material of 96.5%. This arises because of the presence of saturated and unsaturated fatty acids within the Spirogyra sp. oil, which can be without difficulty at risk of transesterification.

Conclusion

From the present study, it can be concluded that *Spirogyra sp.* could be utilized for green fuel/ biodiesel production as they are considered renewable energy source for biodiesel production even though it contain lower lipid content than microalgae. Thus, *Spirogyra sp.* can be used as feedstock of renewable source of energy to replace the existing energy sources of fossil fuel and also reduce pollution.

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References

Abirhire, O. and Kadiri, M. O. (2013). Growth and biodiesel production from the blue-green alga *Microcystis aeruginosa Kutz. Afri. J. Env. Poll. Health 10: 33-39.*

Abu, S. A., Sarfaraj Khan, SininHamdan, M. R., Rhaman, M. S. and Mohd, A. M. (2012). Biodiesel production from macro algae as a green fuel for diesel engine. EnCon 5th Engineering Conference, "Engineering Towards Change - Empowering Green Solutions", Kuching: Sarawak.

Arthe, E., Alamu, O. J., Akintola, T. A. Enweremandu, C. C. and Adeleke E. (2008). Characterization of palm-kernel oil biodiesel produced through NaOH catalyzed transesterification process*. Scientific Research and Essay* Vol. 3(7). 308 - 311.

Bonachela, S. (2013). Pond management and water quality for drip irrigation in Mediterranean intensive horticultural systems: multifunctional pond management using submerged aquatic plants. *Irrig. Sci.*, 31, 769-780.

Chisti, Y. (2007). "Biodiesel from microalgae." Biotech. Advan. 25 (3): 294-306.

Demirbas, A. H. (2009). Production of biodiesel from algal oils. *Ener. sources. 31:163-168.*

Haesman, M., Diemar, J., O., Soushames, W and Foulkes, T. L. (2000). Development of extended shelf – life microalgae concentrate diets harvested by centrifugation for bivalve moluscs a summary. *Aquacult Res. 31: 637 - 659.*

John, W. B. A. and Brook, A.J. (2002). *The Freshwater Algal Flora of the British Isles.* Cambridge University Press, Cambridge.

Kim, J. H., Kim, Y. H. and LeeI. K. (2004). Morphotaxonomy of the genus *Spirogyra*(Zygnemataceae, Chlorophyta) in Korea. *Algae*, *19, 91-105*

Lee, R. E. (2008). *Phycology*. 4th ed. Cambridge, UK: Cambridge University Press.

Li, Q., Du, W. and Liu, D. (2008). Perspectives of microbial oils for biodiesel production. *Appl.Microbiol. Biotech.*; *80*: 749 –756

Mata, T. M., Martins, A. A., Caetano, N. S. (2010). Microalgae for biodiesel production and Other applications: A review. Renewable and Sustainable Energy Reviews; 14: 217 - 232.

Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q. and Xian, M. (2009). Biodiesel production from oleaginous microorganisms. *Renewable Ener., 34: 1–5.*

Negoro, M., Shioji, M., Miura, Y. (1991). Growth of microalgae in high CO_2 gas and effects on SO_X and NO_X . J. Appl. Biochem. Biotech. 28 (29), 877- 886.

Onyema I.C. and Emmanuel B. E. (2009). *Spirogyra africana* (Fritsch) Czurda bloom and ssociated fishing impairment in a tropical freshwater lagoon. *J. of Ecology,* 58, 18-26.

Ozkurt, I. (2009). Qualifying of safflower and algae for energy. *Ener. educ. Sci. techno., A 23: 14–- 151.*

Poelman, E., Depauw, N., and Jeurissen, B. (1997). Potential of electrolytic flocculation for recovery of microalgae. *Resour. conserve. Recyc.19: 1- 10.*

Prescott, G. W. (1975). How to know the freshwater algae; Iowa, USA: C. Brown Company Publishers, p. 348.

Prescott, G. W. (2007). Algae of western great lakes Area, 9th edition; New York: John Wiley and sons.

Qu, C. B., Wu, Z. Y. and Shi, X. M. (2008). "Phosphate Assimilation by Chlorella and adjustment of phosphate concentration in basal medium for its cultivation. *Biotech. Letters*, Vol. 30: No. 10, 1735-1740.

Sheehan, J. T and Dunahay, J. B. (1998). "A Look Back at the U.S. Department of Energy's Aquatic Species Program — Biodiesel from Algae", Technical Report, National Renewable Energy Laboratory.

Zarina, A., Hasan, M.U., and Shameel, M., (2009). Diversity of fresh water green microalgae in the Punjab and neighboring areas of Pakistan Pak. *J. of Bot.; 41(1):* 277 – 291.