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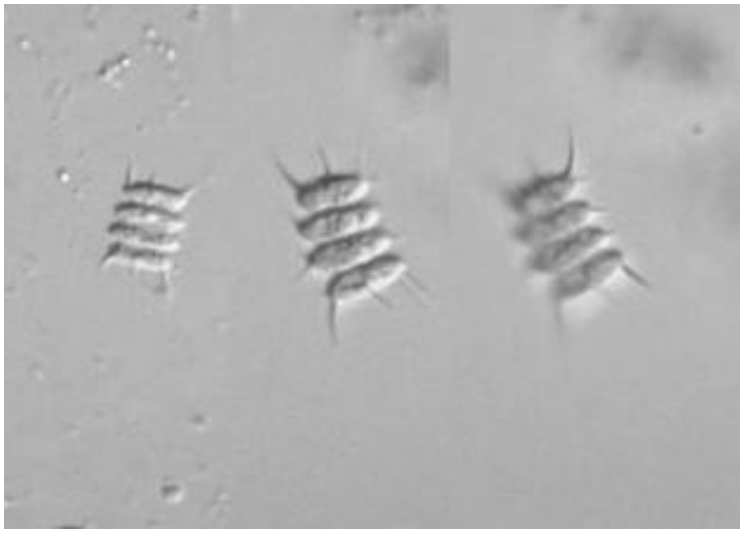

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RESEARCH ARTICLE

LIPID PRODUCTIVITY FROM MICROALGAE FOR THE PRODUCTION OF BIODIESEL

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ABSTRACT

Energy is essential and vital for development, and the global economy literally runs on energy. Continuous use of petroleum sources is not favorable as it is obtained from non renewable sources like coal .Thus the production of sustainable energy is the major necessity in this globally competent world. So bio diesel stands out as the best source for energy. The second generation bio fuel produced from feed stock such as micro algae produce the long term solution for the increasing fuel demand. Micro algae have the ability to produce biodiesel due to transesterification of lipids. There is also an added advantage as microalgae have the ability to mitigate CO_2 emission and produce oil with a high productivity; they can be used for the wastewater or daily effluent treatment. The present study involves in finding out the micro algal strain which produce high lipid content, best media selection which produces high lipid, optimization of best media using different water sources and nitrogen concentrations

INTRODUCTION

The global economy literally runs on energy. An economic growth combined with a rising population has led to a steady increase in the global energy demands. If the governments around the world stick to current policies, the world will need almost 60% more energy by 2030 [1]. Transportation is one of the fastest growing sectors using maximum fuels [2] as the global warming is increasing because of the fossil fuels society started to look towards a new fuel source as a replacement for fossil fuels. Scientists started focusing on biofuels as a replacement energy source which is produced by Photosynthetic organisms like Algae and Plants. These organisms convert CO_2 and Sunlight into biomass and Oxygen. This biomass of algae contains lipids which are used for the production of biodiesel. It is a domestic, Renewable source of energy, and Eco friendly.

The production of biofuels in large scale consumes large amounts of farmlands which drive the food prices high. As the technology is developing supply of biofuels is about 30% of global demand. Presently bioethanol and biodiesel are produced in industrial scale as a replacement for petroleum products to the engines compatibility. These are called as first generation biofuels and are produced from food crops like sugarcane, maize, sorghum and wheat. As these first generation biofuels are using the food crops and are affecting the food chain the second generation of biofuel production has started from non-food feed stocks. These non-food feed stock are extracted from microalgae and lingo-cellulose biomass, rice straw and some microbial sources. Algal basic requirements are sun light, CO_2 and water. It can flourish in non-arable lands and even in waste water.

Fig 1 Scenedusmus abundans

Strain: Scenedesmus abundans

Scenedesmus abundans is a type of species found mosty in lake water. This strain is isolated from Ramanthapur Lake of Hyderabad.

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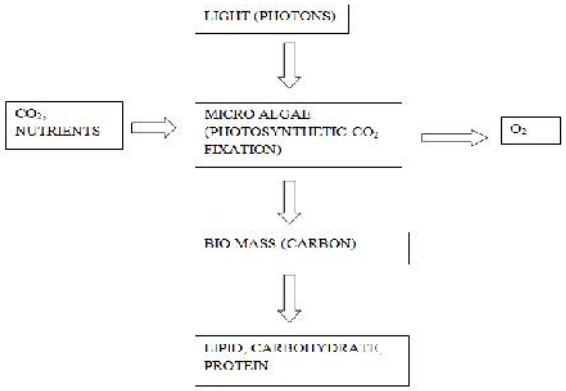
***Classification**:*
Empire Eukaryota
Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Scenedesmaceae
Subfamily Scenedesmoidea
Genus Scenedesmus

Production of Lipids from Microalgae

The Biodiesel production is based on the quantity of lipids produced during its growth. Microalgae are autotrophic organisms and as such they do photosynthesis which allows them to create their own food. It is during photosynthesis that microalgae grow its biomass and it is composed of carbohydrates, proteins and lipids. The main importance in lipid production is that they can be converted to biofuels by transesterification.



Synthesis of lipids and carbohydrates by microalgae

MATERIALS AND METHODS

Selection and procurement of microorganisms

Scenedesmus abundans is selected for analysis. Scenedesmus abundans was isolated from Ramantapur lake water.

Growth

The organism was first grown in BG11 media. The composition is as follows:

SL.NO	CHEMICAL	WEIGHT(gm/L)
1	$NaNO_3$	1.5
2	$MgSO_47H_2O$	0.075
3	Citric acid	0.006
4	H_3BO_3	0.00286
5	$ZnSO_4.7H_2O$	0.00022
6	$CuSO_4.5H_2O$	0.000018
7	$(NH_4)_6Mo_7o_{24}4H_2O$	0.003
8	K_2HPO_4	0.04
9	$CaCl_2.2H_2O$	0.036
10	Na_2CO_3	0.02
11	$MnCl_24H_2O$	0.00181
12	$Na_2MoO_42H_2O$	0.0003
13	$Co(NO_3)_{26}H_2O$	0.00005
14	Na_2EDTA	0.00001

All these chemicals were weighed in conical flask (one liter) in which 500mL of distilled water was added priorly and then the volume was made to 1litre using distilled water. The media pH was then checked and was adjusted to 7.1 and the media was kept for autoclaving at 121°C 15 lbs pressure for 15 minutes. After autoclaving the media was allowed to cool down and then the medium was inoculated with 10% algal strain in laminar air hood for sterile conditions. Proper aeration and light source was provided for the growth of the culture.

Different Growth Mediums For Micro Algae Growth

Different growth mediums were prepared and were inoculated with the strains Scenedesmus abundans was provided with proper physical conditions such as aeration, temperature, pH and light source. Three mediums were considered and the experiment was performed. They are BG11, BBM, FOGG'S medium. The compositions of the medium are as follows BG11 MEDIUM was prepared as mentioned above by adding all the chemicals weighed and The pH was checked and was set to 7.1. The medium volume was made up to 1L. and was kept for autoclave at 121°C 15 lbs pressure for 15 minutes.

Bold Basal Medium (BBM)

Stock Solutions	WEIGHT per Litre distilled water (dH_2O)	
1. NaNO3		0.25 g
2. CaCl2.2H2O		0.025g
3. MgSO4.7H2O		0.075g
4. K2HPO4		0.075g
5. KH2PO4		0.175g
6. NaCl		0.025g
7. EDTA	50 g	1ml of this stock solution was added to the medium
KOH	31.0g	
8. FeSO4.7H2O		0.00498 g
H2SO4		1.0 Ml
9. H3BO3		0.01142 g
10. Micronutrients	$g.L^{-1}$	
ZnSO4.7H2O	8.82 g	
MnCl2.4H2O	1.44 g	This stock is made for 1L and 1ml of this has to be added to the medium.
MoO3	0.71 g	
CuSO4.5H2O	1.57 g	
Co(NO3)2.6H2O	0.94 g	

The medium was prepared for required volume and then was autoclaved for proper sterility.

Sl.no	Chemical	Weight per one litre medium
1	MgSO4.7H2O	0.2
2	K2HPO4	0.2
3	Macronutrient solution	
	CaCl2.H2O	0.1 g
	Fe-EDTA solution	5.0 ml
	Distilled water	1.0 L
	Agar(Difco)	12.0 g
4	Micronutrient solution	
	H3BO3	286.0 mg
	MnCl2.4H2O	181.0 mg
	ZnSO4.7H2O	22.0 mg
	Na2MoO4.2H2O	39.0 mg
	CuSO4.5H2O	8.0 mg
	Distilled water	100.0 ml
5	Fe-EDTA solution	In hot water dissolve 745.0 mg of Na2EDTA and then add 557.0 mg of FeSO4.7H2O. Boil the solution for few minutes and make the volume to 100.0 ml

All these chemicals were weighed in conical flask (one litre) in which 500mL of distilled water was added priorly and then the volume was made to 1litre using distilled water. The media pH was then checked and was adjusted to 7.1 and the media was kept for autoclaving at 121°C 15 lbs pressure for 15 minutes. After autoclaving the media was allowed to cool down and then the medium was inoculated with 10% algal strain in laminar air hood for sterile conditions. Proper aeration and light source was provided for the growth of the culture.

Bligh And Dyer Method (Lipid Analysis)

- Cells were harvested by centrifugation at 10000RPM for 10 minutes at 4^0C. Cells were washed once with distilled water and re-centrifuged.
- Pellet was then subjected to wet weighed estimation and then dried in over for 2hours at 80^0C.
- For one gram of algal biomass 2mL of methanol and 1 mL oh chloroform was added and kept for 18hours at 25^0c.
- The mixture was agitated in vortex for 2 minutes. 1ml of chloroform was again added and the mixture was shaken vigorously for 1 minute.
- After that 1ml of distilled water was added and then mixture was mixed in vortex again for 2 minutes.
- The layers were separated by centrifugation for 10 minutes at 2000RPM.
- The lower layer was separated and then procedure was again repeated with pellet. The two supernatants collected were allowed to stand for two hours.
- Lower organic layer with the lipids was transferred to a clean pre weighed vial.
- Evaporation was carried out in hot air oven at 80^0c for 50 minutes.
- The weight of vial was again recorded (w_2). Lipid content was calculated by subtracting w1 from w_2 and was expressed as % dry cell weight.

%dry cell weight = $[(w_1-w_2)100]/w_1$

RESULTS

Algal cells were grown in different media such as Bold Basal Medium (bbm), Bg11 and FOGG's medium. The best medium for lipid productivity was found is Bold Basal Medium and it has high lipid content after 15days.

LIPID	DAY 5	DAY 15	DAY 25
BBM	10.3	14.92	10.586
Bg11	9.902	12.02	9.86
FOGG'S	7.8	10.42	8.60

Lipid productivity variation by change in $NaNO_3$ Concentration (%)

Different concentrations (%) of sodium nitrate was taken in one litre of medium with 10% inoculum and was grown to find out the optimum medium for highest lipid productivity was high when 0.05% of $NaNO_3$ was added to the Bold Basal Media.

Concentration	% LIPID
0.05	12.33
0.125	10.085
0.25	9.588
0.5	7.66

Lipid productivity variation by change in urea concentration (%)

To optimize the media sodium nitrate was replaced by urea and different concentrations of urea was added to find the optimum content of urea for higher lipid productivity and found that the lipid production was high when 0.05% of urea is added to Bold Basal Media.

Concentration	Lipid content
0.05	14.86
0.125	12.05
0.25	10.14
0.5	8.75

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