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GROWTH OF SPIRULINA AND ITS PROTEIN ANALYSIS

Engineering

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ABSTRACT

Day by day disposal of waste water from industry is becoming a great challenge. Waste water released from food industries generally consists of vital nutrients which can be further utilized. For this work we have chosen synthetic water consisting of food waste for experimental runs. Beet and potato are selected as substrates for algal growth and culture selected was Spirulina platensis. Effect of different growth medium, temperature and sunlight were studied in this work. The maximum biomass of Spirulina found to be in beetroot medium was 4.205 g/lit and in potato medium was 3.347 g/lit kept in natural sunlight. Protein analysis of these two samples was also conducted to estimate their protein content.

KEYWORDS

Spirulina platensis, potato and beetroot as substrates, protein analysis.

I. INTRODUCTION

Spirulina is a multicellular and filamentous cyanobacteria or bluegreen algae. It is a large-size microscopic, multicellular alga which grows in shallow ponds of high salinity and alkalinity in tropical countries. Spirulina has achieved considerable popularity in the health sector as an ecologically sound, nutrient-rich, dietary supplement. Its interest in food security is because lower land and water needs to produce protein and energy than those required for livestock,It develops and grows in water, can be harvested and processed easily. It has very high contents of both macro and micronutrients, essential amino-acids, proteins, lipids, vitamins, minerals and anti-oxidants. Spirulina is considered as a complete food supplement to fight against malnutritional deficiencies in developing countries. Spirulina is deemed safe for human consumption as evident by its long history of food use and latest scientific findings. [1][4]

In recent years, Spirulina has gathered enormous attention from research fraternity as well as industries as a flourishing source of nutraceutical and pharmaceuticals.

Spirulina is one of the richest protein sources ever found, the protein also contains all essential amino acids in a proportion comparable to other conventional protein foods and satisfies the required composition and as a food source also supplies a considerable amount of fat, carbohydrate, vitamins and proteins. Spirulina has between 55 and 70% protein (more than beef, chicken, and soybeans), 8 essential and 10 non-essential amino acids, as well as high levels of gamma-linolenic acid (GLA), beta-carotene, linoleic acid, arachidonic acid, vitamin B12, iron, calcium, phosphorus, nucleic acids RNA & DNA, chlorophyll, and phycocyanin, a pigment-protein complex that is found only in blue-green algae.[5]

The aim of our project was to successfully grow spirulina in a nutrient medium which contained waste mater from industrial sources. Thus it was necessary to select a nutritive media which would be suitable for the Indian climate and provide a suitable source of carbon i.e a substrate, generally found in the waste from food industries. Through literature survey, various nutrient mediums have been used for the growth of Spirulina.

II. MATERIAL AND METHOD

It is very essential that the nutrient medium must contain high alkalinity and high levels of carbonate and bicarbonate to maintain its pH levels.

Thus, the suitable mediums used for the growth of Spirulina in this project were Zarrouks, BG11 and a medium prepared according to instructions provided by NCL (NCL Medium).

1. Zarrouk Medium:

The spirulina culture was inoculated in small transparent container containing the nutrient medium according to the composition given in Fig 1. at room temperature around 28OC and pH around 8.5 -9. [5]

Sr. No.	Ingredients	Amount(g/l)
1	$NaHCO_3$	16.8
2	$NaNO_3$	2.5
3	NaCl	1
4	K_2SO_4	1
5	K_2HPO_4	0.5
6	$MgSO_4.7H_2O$	0.2
7	$FeSO_4.7H_2O$	0.01
8	$CaCL_2.2H_2O$	0.04
9	EDTA	0.08
10	Distilled Water	1000 mL

Fig1.Composition of Zarrouk medium

2. BG11 Medium:

The spirulina culture was inoculated in small transparent container containing the nutrient medium according to the composition given in Fig 2 . at room temperature around 280C and pH around 9. [5]

Sr. No.	Ingredients	Amount(g/l)
1	Citric Acid	0.006
2	$NaNO_3$	1.5
3	Na_2CO_3	0.001
4	K_2HPO_4	0.04
5	$MgSO_4.7H_2O$	0.075
6	$FeSO_4.7H_2O$	0.006
7	$CaCL_2.2H_2O$	0.04
8	EDTA	0.02
9	Distilled Water	1000 mL

Fig2.Composition of BG11 medium

3. NCL Medium :

The spirulina culture (50 mg/l) was inoculated in various flasks in batches for experiments in sunlight as well as incubator (artificial light). It was observed in the previous experiments that growth of Spirulina was unsatisfactory, thus in this medium substrates like potato and beet were added to improve the growth of culture.

Sr. No.	Ingredients	Amount(g/l)
1	Sodium Bicarbonate ($NaHCO_3$)	8
2	Sodium Chloride (NaCl)	5
3	Urea ($CO(NH_2)_2$)	0.2
4	Dipotassium Hydrogenphosphate (K_2HPO_4)	0.5
5	Phosphoric Acid (H_3PO_4)	0.052 ml
6	Ferrous Sulphate ($FeSO_4.7H_2O$)	0.05
7	Beetroot or potato pieces	3
8	Distilled Water	1000 mL

Fig3.Composition of NCL medium

III. EXPERIMENTATION

1. Nutrient media and Cultivation:

In this project the blue green algae Spirulina platensis was grown in various nutrient media, at lab scale. The culture of Spirulina was obtained from CSIR-NCL. The culture media prepared for the growth of Spirulina were Zarrouk , BG11, NCL. The water used was distilled water and the pH of the nutrient medium was maintained at 8 to 10 depending on the medium used. Beetroot and Potato pieces were also added to the standard NCL culture medium as a substrate, to enhance the growth of Spirulina. All reagents used were of analytical grade.

The cultures were grown in glass jars (height 8cm and inner-diameter 5.5cm), which were sterilized in an autoclave, with 150 ml of nutrient medium. A very small amount (approx 5mg/l)of Spirulina from CSIR-NCL culture was added to the nutrient medium as an inoculum. The cultures were grown in natural sunlight as well as in an incubator. The temperature of the surroundings was around 30oC and the temperature in the incubator was maintaine at 35 OC. The cultures kept in sunlight were stirred every 4 hours to prevent the formation of clumps and promote the efficient mass transfer of Carbon-Di-oxide and Oxygen. The cultures kept in the incubator were constantly stirred at 80 rpm.

3. Determination of biomass concentration:

In order to monitor the growth cycle and determine the biomass concentration of the NCL + Beetroot and NCL+ Potato cultures, the Optical density method was used. In this method the biomass concentration was determined with the help of their optical density values. First the optical densities of various concentrations of dried spirulina powder suspended in a fresh nutrient medium were found using a UV-Visible spectrophotometer. A calibration curve of Concentration vs Optical Density was then plotted . The values for the calibration curve were:

A. For Calibration curve values for NCL + Beetroot culture:

Table 1:Calibration curve data for various biomass concentration for NCL+Beet

Biomass concentration (g/l)	Optical density
0	0.287
5	3.088
2.50	1.42
2	1.204
1.5	0.970
1.00	0.680

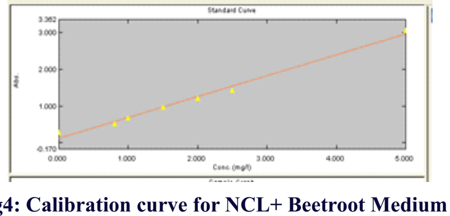

Fig4: Calibration curve for NCL+ Beetroot Medium

B. Calibration curve values for NCL+ Potato culture:

Table 2:Calibration curve data for various biomass concentration for NCL+Potato

Biomass concentration (g/l)	Optical density
5	4.000
2	2.552
1	1.434
0.8	1.240
0.6	0.989
0.4	0.621
0.2	0.430

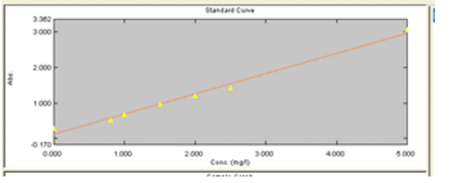

Fig5: Calibration curve for NCL+ Potato Medium

Samples were extracted every three days from the grown cultures of Spirulina and were analyzed using a UV-Visible spectrophotometer. Their biomass concentration was determined with the help of the calibration curve.

IV. RESULTS AND DISCUSSIONS

1. Zarrouck Medium:

After inoculation, the culture media was exposed to sunlight for the cultivation of algae for approximately 20 days. During the period it was observed that the growth was not as expected and the appearance of the species was dark brown colour as depicted in Fig which could be due to deficiency of nitrogen or inappropriate atmospheric conditions.

Fig6:Culture Growth in Zarrouk Medium

2. BG11 Medium:

After inoculation, the culture media was exposed to sunlight for the cultivation of algae for approximately 20 days. During the period it was observed that the growth was negligible.

Fig7:Culture Growth in BG11 Medium

3. Comparison of biomass concentrations of NCL + Beetroot Medium Kept in sunlight and incubator:

Table2:Biomass Concentration for growth of culture in sunlight and incubator in NCL+Potato Medium

NCL+ Beetroot Spirulina culture					
Kept in Sunlight			Kept in Incubator		
time	Concentration	O.D	time	Concentration	O.D
5	1.759	0.287	5	1.985	1.431
10	2.411	1.495	10	4.546	
15	2.719	1.67	15	4.595	
20	2.807	1.946			
25	4.205	2.515			
30	4.02	2.201			

The culture kept in the incubator was found to decay after 15 days.

4. Comparison of biomass concentrations of NCL + Potato Medium Kept in sunlight and incubator:

Table3:Biomass Concentration for growth of culture in sunlight and incubator in NCL+Potato Medium

NCL+Potato Spirulina culture					
Kept in Sunlight			Kept in Incubator		
time (days)	Concentration	O.D	time	Concentration	O.D
5	1.866	1.927	5	1.189	1.431
10	1.91	1.959			
15	2.181	2.157			
20	3.347	3.01			

The culture kept in the incubator was found to decay after 5 days

5. Determination of growth rate:

5.1 NCL+Beetroot Medium grown in Sunlight:

Experimental data shows the biomass concentration of micro-algae at various time intervals shown in Table 2. The Ln(Biomass concentration) vs. time follows S shaped curve indicating all the phases of growth stage shown in Fig. 8.

Table 4: Values for plotting Ln(conc) vs time for NCL+Beet Medium

conc (g/l)	time	od	ln Conc
0.05	0	0.287	-1.301029996
1.759	5	1.495	0.245265839
2.411	10	1.67	0.38219721
2.719	15	1.946	0.434409208
2.807	20	2.515	0.448242413
4.205	25	2.201	0.623766
4.02	30		0.604226053

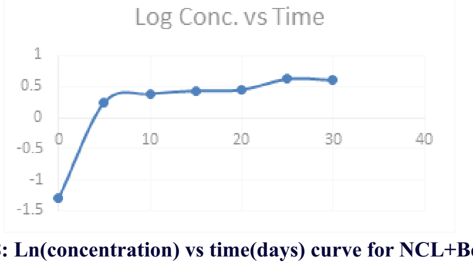

Fig 8: Ln(concentration) vs time(days) curve for NCL+Beet medium

5.1 NCL+Potato Medium grown in Sunlight:

Experimental data shows the biomass concentration of micro-algae at various time intervals shown in Table 3. The Ln(Biomass concentration) vs. time follows S shaped curve indicating all the phases of growth stage shown in Fig. 3.

Table 4: Values for plotting Ln(conc) vs time for NCL+Potato Medium

conc (g/l)	time	od	ln Conc
0.05	0	1.134	-1.301029996
1.866	5	1.495	0.270911639
1.91	10	1.67	0.281033367
2.181	15	1.946	0.338655666
3.347	20	2.515	0.524655712

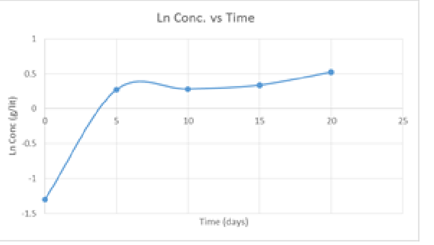

Fig 9: Ln(concentration) vs time(days) curve for NCL+Potato medium

6. Determination of Specific Growth Constant (μ) :

After the graphs of growth curves have been plotted, the next step is to calculate the specific growth constant (μ) of the exponential growth phase.[6]

From Fig 9. , the exponential part of the curve will now be a straight line which is depicted by linear trend line in the graph.

By choosing the two points between the exponential phase, μ can be calculated using the formula :

$$\mu = \mathrm{Log}\,(N_1) - \mathrm{Log}\,(N_0) \,/\, (t_1 - t_0)$$

The μ(exponential) for beet medium was : 0.0152 /day

The μ(exponential) for potato medium was : 0.0265 /day

7. Determination of Growth Rate:

In order to estimate the growth rate of the algae, the below given equation is used :[1]

$$\mu = \frac{\mu \max I}{I + K_i}$$

where,

μ=Growth Rate (day-1)

μ max = Maximum Growth Rate (day-1)

I = 2000

Ki = 10.2 = Half-Life Constant

The μ(max) for beet medium was : 0.01528 /day

The μ(max) for potato medium was : 0.02667 /day

8. Determination of Doubling Time 'G' :

While the specific growth constant represents a measure of the ability of the organism to grow under a given set of environmental conditions, doubling times are more easily understood or meaningful. The doubling time is simply the time required for the cells to divide. A large doubling time value means slow growth, while a small doubling time value means rapid growth.[1]

The doubling time for this batch study is found by the formula

G = (0.301/ μ) ; where, μ : Specific growth rate constant.

The doubling time for beet medium was : 20 days

The doubling time for potato medium was : 11 days

9. Estimation of Maximum Yield (Ymax) :

Factors such as depletion of nutrients, production of toxic waste products or simply overcrowding will lead to a decrease in the rate of growth of the population of algae in the culture. At some point a maximum yield (Ymax) will be attained. This is the time in the growth of the culture when the number of cells dying is equal to the number of cells being produced, therefore , the total cell number does not change.

The maximum yield for beet medium was : 4.205 g/lit

The maximum yield for potato medium was : 3.347 g/lit

10. Protein Analysis:

The protein yield for beet medium was: 5.8%

The protein yield for potato medium was: 5.75%

V. CONCLUSION

1. From the results obtained we observed that Zarrouk Medium and BG11 Medium were not suitable of Growth of Spirulina.
2. The biomass concentration curves obtained for NCL + Beetroot Medium and NCL + Potato medium were very close to the ideal curves obtained for algal concentrations in literature.
3. The growth of culture in sunlight is higher as compared to Incubator.
4. The maximum yield obtained at the end of the cycle is higher in NCL+Beet medium but doubling time is higher as Compared to NCL+potato medium.
5. After the protein analysis, it was observed that the protein Content is same for both media.

VI. ACKNOWLEDGMENT

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VII. REFERENCES

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