GROWTH KINETICS OF CHLORELLA VULGARIS AND BOTRYOCOCCUS BRAUNII BASED ON PRESENCE OF SODIUM BICARBONATE IN MEDIUM AS PARAMETER

Ojasvi bhatnagar[1], Rajinikanth[2], Archana Rao P[3]
[1,2,3]Department of Chemical engineering, BVRIT Narsapur
tanuanjul@gmail.com, rajnivgks@gmail.com, archana.r@bvrit.ac.in

Abstract— Growth studies were conducted on microalgae species Chlorella Vulgaris and BotryococcusBraunii in a batch mode. In the present study the effect of sodium bicarbonate salt NaHCO3 in the culture medium on growth rate of the two selected microalgae species was investigated. For this purpose the growth kinetics of the aforementioned two species under varying concentrations of sodium bicarbonate salt in the medium (0ppm, 15ppm, and 70ppm) was investigated. Growth was conducted in batch mode for 7 days with temperature, pH, and light intensity maintained constant. Specific growth rate, doubling time and dry weight of biomass obtained were calculated. Maximum specific growth rate showed by Chlorella vulgaris was observed at 70ppm bicarbonate, while BotryococcusBraunii showed best specific growth rate at 15ppm bicarbonate.

Keywords: microalgae, bicarbonate, growth kinetics, batch culture

I. INTRODUCTION

Introduction:

Microalgae are unicellular, photosynthetic microorganisms, they have minimal nutrient requirements and are being used as source materials for a variety of products such as protein rich nutritional supplements, pharmaceutical chemicals and pigments (used in food and cosmetics). Microalgae can fix carbon dioxide from different sources which can be categorized as (i) carbon dioxide from atmosphere, (ii) carbon dioxide from industrial flue gases, and (iii) fixed carbon dioxide in the form ofsoluble carbonates ($NaHCO_3/Na_2CO_3$). The main environmental factors influencing microalgae growth and chemical composition are light, nutrients, temperature and pH and carbon dioxide. Carbon source is an essential factor for microalgae growth.

In this study, growth rate of microalgae in 250 ml conical flasks in batch operation with varying concentration of sodium bicarbonate (15ppm, and 70ppm) in BG 11 medium was investigated. Cultivation of microalgae was carried out for 7 days and biomass concentration was estimated using spectrophotometer. Monod model is assumed to be applicable. The Monod equation is a mathematical model for the growth of microorganisms. It is named for Jacques Monod who proposed using an equation of this form to relate microbial growth rates in an aqueous environment to the concentration of a limiting nutrient. The Monod equation has the same form as the Michaelis–Menten equation, but differs in that it is empirical while the latter is based on theoretical considerations.

The Monod equation is:

$$\mu = \mu_{max}\frac{S}{K_s + S}$$

where:

- μ is the specific growth rate of the microorganisms
- μ_{max} is the maximum specific growth rate of the microorganisms
- S is the concentration of the limiting substrate for growth
- K_s is the "half-velocity constant"—the value of *S* when $\mu/\mu_{max} = 0.5$

μ_{max} and K_s are empirical coefficients to the Monod equation. They will differ between species and based on the ambient environmental conditions

II. PROCEDURE

Sterilization:

Before carrying out the experimental analysis first and foremost thing done was sterilization of all the experimental instruments to minimize the contamination of fungus,germs and other viruses.

Various sterilization techniques which we used were :

- Hot air gas oven sterilization
- U.V sterilization
- Autoclaving

Hot air oven was used for sterilizing all the glass wares at 160˚C for 2 hours and then other metallic instruments like spatula, metal loops etc were sterilized in laminar flow chamber under U.V light for 2 hours.

Autoclave was used for sterilizing the medium at 120˚C at 15 psi pressure for 20 minutes. After sterilizing all the instruments medium was prepared.

Types of Algae :

We have taken two types of algae species for our experiment. They are

- Chlorella vulgaris
- Botryococcus Braunii

Medium preparation:

Below is the composition of BG11 medium which we used in our experimentation

Stock 1:

Na_2MG EDTA	0.025 g
Ferric ammonium citrate	0.15 g
Citric acid . $1H_2O$	0.15 g
$CaCl_2$. $2H_2O$	0.9 g

Stock 2:

$MgSO_4$. $7H_2O$	1.875 g

Stock 3:

K_2HPO_4	0.7625 g

Stock 5:

H_3BO_3	0.715 g
$MnCl_2$. $4H_2O$	0.4525 g
$ZnSO_4$. $7H_2O$	0.0555 g
$CuSO_4$. $5H_2O$	0.0198 g
$COCl_2$. $6H_2O$	0.0125 g
$NaMoO_4$. $2H_2O$	0.0978 g

For Basic Medium We have to combine All the Stock solutions In a specified composition.

Stock Solution	**Per Liter of medium**
Stock 1	10 ml
Stock 2	10 ml
Stock 3	10 ml
Stock 5	1.0 ml

Algae Sub culturing:

Dried cells of algae are kept in 10ml of medium in a test tube for two-three days. Agar medium is prepared by adding 1g of Agar powder in 250 ml of medium. Agar plates are prepared. Cells from the test tube are streaked on the agar plate and left to grow for 3-4 days. Pure colonies are taken from the agar plate and transferred to a test tube containing 20 ml of medium and left to grow.

Algae Inoculation:

After two weeks of isolation the next step was algae inoculation, the pure cells of algae which grew in the petridishes were taken out with the sterilized metal loops and inoculated in the broth in the test tube. The broth was kept under the light for two days for algae to grow and then it was kept inside the refrigerator for storing so as to keep the species from getting contaminated.

Algae Cultivation:

After inoculation, coming to our experimental setup we prepared two liters of medium and poured 250 ml of it in each 4 flasks under different light of 60 W and 20 W and the sodium bicarbonate concentration is 15 PPM and 70 PPM respectively.

Spectrometric Analysis:

At first the blank medium was prepared as the reference to calculate the absorbance of algae i.e. absorbance will tell us how much the algae has grown every day, the growth is directly proportional to the absorbance. 1ml of blank was taken in 10 ml of conditioning reagent, similarly every time 1ml of algae was taken in 10 ml of conditioning reagent to check their absorbance separately for 16 times. This was then poured into two different cuvettes one with the blank and other with one of the species in it.

III. Results And Discussions

Time (day)	Absorbance
1	.00615
2	.0079
3	.0103
4	.0134
5	.0174
6	.022
7	.029

Table 1: Absorbance of Botryococcus Braunii

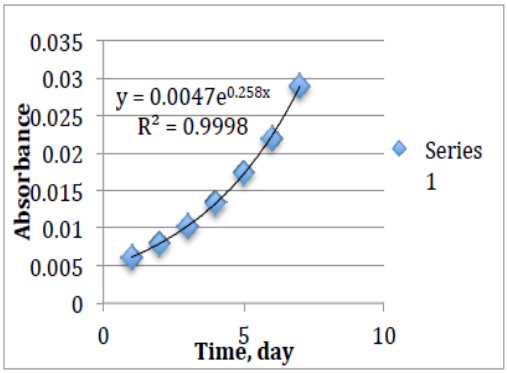


Fig1: exponential regression for BB

From exponential regression equation, we have,

$Y=0.0047e^{0.258x}$

Let specific growth rate,

$\mu = dY/dx = 0.0047*0.258e^{0.258x}$

When x=1 (for first day)

$\mu = 0.001569\ day^{-1}$

Similarly specific growth rate and doubling times are evaluated for the remaining days and was plotted against time as shown in the fig 2.

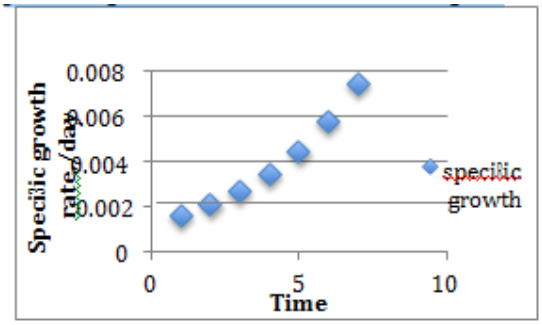
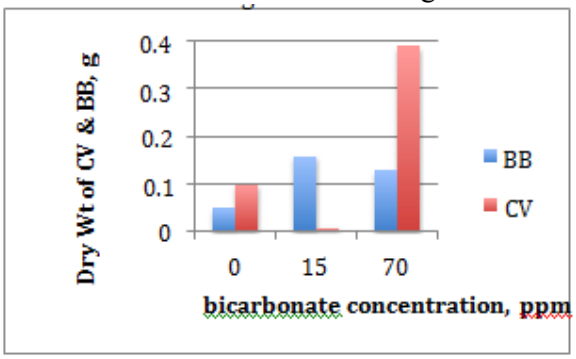


Fig 2: Specific growth rate of Botryococcus Brauni

Biomass produced with and without the presence of sodium bicarbonate in the medium can be seen from the table 3 and figure 3.



Sodium bicarbonate concentration	Biomass produced, g	
	BB	CV
0 ppm	0.051	.099
15 ppm	.16	.01
70 ppm	.13	.39

Fig 3, table 3: biomass produced for various bicarbonate concentrations

For chlorella vulgaris

CV1: $Y= 0.017e^{0.331x}$

$\mu=0.017*0.331^{0.331x}$

Table: 5.1 CV1

Days	specific growth rate (μ)g/ml.day
1	0.0078
2	0.0109
3	0.0152
4	0.0211
5	0.0294
6	0.041

7	0.0571
8	0.0795
9	0.1106

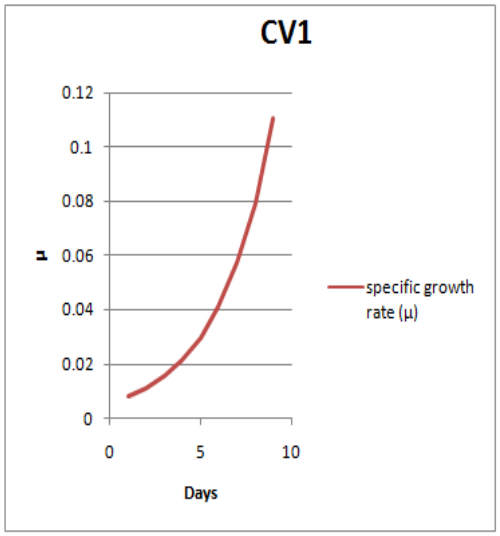


Fig: 5.2

CV2: $Y= 0.031e^{0.178x}$

$\mu=0.031*0.178^{0.178x}$

Table: 5.3 CV2

Days	specific growth rate (μ)g/ml.day
1	0.0065
2	0.0078
3	0.0094
4	0.0112
5	0.0134
6	0.0161
7	0.0192
8	0.0229
9	0.0229

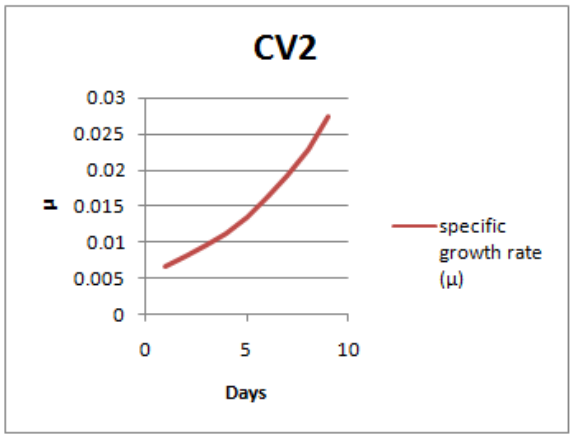


Fig: 5.4

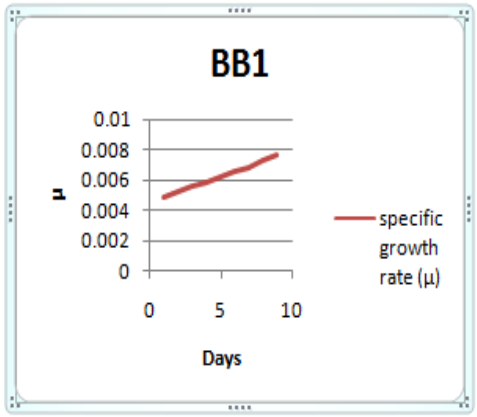
For Botryococus Braunii:

BB1: $Y= 0.086e^{0.055x}$

$\mu=0.086*0.055e^{0.055x}$

Table: 5.5

Days	specific growth rate (μ)g/ml.day
1	0.0049
2	0.0053
3	0.0056
4	0.0059
5	0.0062
6	0.0066
7	0.0069
8	0.0073
9	0.0077



For BB2: $Y=0.05\ e0.121^{x}$

$\mu=0.05*0.121e^{0.121x}$

Days	specific growth rate (μ)g/ml.day
1	0.0068
2	0.0077
3	0.0087
4	0.0098
5	0.0111
6	0.0125
7	0.0141
8	0.0159
9	0.0179

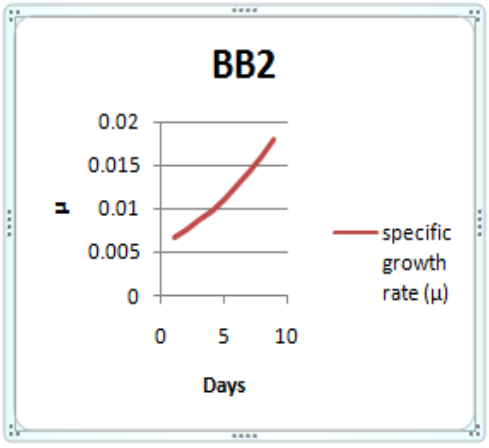


Fig:5.8

Conclusions:

In the present study we set out to investigate the effect of sodium bicarbonate in the medium c/ on the growth rate of microalgae Chlorella Vulgaris (CV) and Botryococcus Braunii(BB). As sodium bicarbonate concentration increased the specific growth rate of BB has increased and reached maximum at 15 ppm, for which the biomass produced is 0.16g. For CV the growth rate decreased when sodium biocarbonate concentration was increased to 15ppm and then increased for 75ppm, for which biomass produced is 0.39g.

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