Design of Photo Bioreactor for Lipid Production-A Systematic Study

Rajesh Ghosh[1*], Roshan Makam[2], Debasish Mahato[3]
[1]Assistant Professor, Chemical Engineering, Dept. Calcutta Institute of Technology, Uluberia, Howrah -711316,India
Email-rajesh_laltu@yahoo.co.in
[2]Professor, PES Institute of Technology, Bangalore, Karnataka, Banashankari 3rd, 560085,India
Email-mvrmakam@gmail.com
[3]Chemical Engineering Dept, Calcutta Institute of Technology, Uluberia, Howrah -711316,India
Email-abdebasish18@gmail.com

Abstract: With the depletion of fossil fuels and with the uncertainty in its supply from unstable regions of world, there is a need of transition to renewable energy sources. In this context, biodiesel production from microalgae could be a viable option. In the present work two approaches were considered to produce maximum amount of lipids from microalgae, the raw materials for biodiesel, based on field works. The first approach is to select the microalgae having characteristics of higher lipid content. The second approach is to modify the microalgae through selective metabolic pathway using nutrients and metabolic conditions to produce more lipids from modified microalgae. The goal in this study resorts to the first approach along with the conceptual design of a hollow-fibre photo-bioreactor. Hollow Fibre Photo -Bioreactors (HFPB) are primarily used for cell culture. Since the microalgae are plant cells and their size falls with animal cells, it warrants that the HFPB can be employed for microalgal culture. Several tested models were looked at for mathematical modelling purposes and finally techno-economic analysis was performed to check the feasibility and viability of the microalgal process. Using the first approach, macronutrients such as Potassium Nitrate, Magnesium Sulphate and environmental parameters such as pH and light intensity were studied. It was found that Light Intensity of 2554 lux, concentration of Potassium Nitrate 397 mg/L, concentration of Magnesium Sulphate ($MgSO_4$) 292mg/l and pH 9.2 respectively were found to be the optimal parameters for a 22.7 g/L of biomass production matching the mathematical model. Then a conceptual design was undertaken to design HFPB. The shell volume of 59 L and lumen volume of 28 L was considered to be ideal for the production of algal biomass based on the shelf availability of a small HFPB. A techno-economic analysis was carried out to check the feasibility and viability of a continuous algal process. Based on the analysis it was found that the dry biomass should be 35 g/L with a 30% lipid content giving an ROI of 34.2% with a breakeven of 12 years at an interest rate of 12% PA. Although this research yielded a viable process, but its commercial exploitation needs further research into optimization of another macro/micro nutrients.

Keywords: Photo bio reactor, mathematical modelling, micro algae

1. INTRODUCTION

Renewable energy sources are getting recognition because of the decreasing supply of conventional fuels due to its depleting reserve in earth and increase in concentration of greenhouse gases (GHG) in the environment. Biofuels are practically a very suitable alternative aid with the fact that they can be obtained from bio organics and algal sources which is being renewable. Biofuels based on algae have tremendous potential to bring a revolution in the energy sector and also provide a solution to the climatic aversions resulting from the use of conventional fossil fuels. [1][2] **Algae** encompass different groups of living organisms. They are photosynthetic, heterotrophic, mostly unicellular organisms, aquatic microorganisms, which has chain wise or individual existence. Microalgae are microscopic algae, found mainly in freshwater and some marine systems. Species wise, their size range from around a few micrometers (µm) to a few hundred micrometers (µm). They are unicellular or multicellular, photosynthetic, prokaryotic like Cyanobacteria or eukaryotic-like green algae are capable of living in rough conditions owing to unicellular or simple multi

cellular structure and are driven by sunlight and possess the inherent ability to aid conversion of carbon dioxide into biofuels, food items, high energy stocks etc. They are also useful for bioremediation applications in wastewater. Microalgae are remarkably efficient biological substance capable of taking waste in the form of carbon dioxide and converting them into natural oil. Microalgae have been found to have incredible production levels compared to other oilseed crops like Soybeans. Considering the burgeoning requirement of energy globally, the use of cyanobacteria for sustainable energy production has gained importance because they use dissolved carbon dioxide to achieve carbon-neutral production. Since maximizing biomass productivity is the goal it has been known that optimum culturing conditions are of varying proportion for cell, which becomes a concern for biomass productivity. The above study addresses the needs of the national requirement of a renewable energy source [3].

2. Literature Survey

Brown et al. in 1969 conducted the hydrocarbon content analysis and devised a relationship to throw light upon the physiological state in the green alga Botryococcus braunii and proceed to a conclusion and thereby postulated that they contain a complex mixture of hydrocarbons having the general formula C_nH_{2n-2} and C_nH_{2n-4}[4].

C Largeau et al. in 1980 conducted accumulation sites and hydrocarbons composition in Botryococcus braunii *and came to the conclusion that* Raman spectrometry and electron microscopy further given in the hydrocarbon-rich alga Botryococcus braunii[5].

L V Wake et al (1981) conducted experiments on nature and hydrocarbon content of blooms of the alga Botryococcus braunii found in Australian freshwater lakes and found that under Australian conditions blooms of Botryococcus braunii were found to have hydrocarbon oil contents between 27 and 40 per cent of the dry mass [6].

Morphology and Characteristics of *Botyrococcus braunii* KUTZING NIES 2199

Botryococcus braunii (Bb) is a green, pyramid shaped planktonic microalga that is of potentially great importance in the field of biotechnology. Colonies held together by a lipid bio film matrix can be found in temperate or tropical oligotrophic lakes and estuaries, and will bloom in the presence of elevated levels of dissolved inorganic phosphorus. The species is notable for its ability to produce high amounts of hydrocarbons, especially oils in the form of Tri-terpenes, those are typically around 30-40 percent of their dry weight. Compared to other green algae species, ***Botyrococcus braunii*** has a relatively thick cell wall that is accumulated from previous cellular divisions; making extraction of cytoplasmic components rather difficult. Fortunately, much of the useful hydrocarbon oil is outside of the cell.

Optimal growth environment: *Botryococcus braunii*(*B. braunii*) has been shown to grow best at a temperature of 23°C, a light intensity of 60 W/M², with a light period of 12 hours per day, and a salinity of 0.15 Molar NaCl. However, this was the results of testing with one strain, and others certainly vary to some degree. In the laboratory, *B*. braunii is commonly grown in cultures of Chu 13 medium .P.Mertzer and Largeau in 2004 explained the morphological characteristics of *B. braunii* Colonies of *B. braunii* under microscope observation exhibit a typical morphology characterised by botryoid organisation of individual pyriform-shaped cells held together by a refringent matrix containing lipids. Oil droplets can be excreted from the matrix by the pressure of a coverglass. Ultrastructural studies reveal that the matrix surrounding the basal part of the cells consists of outer walls originating from successive cellular divisions stored in these outer walls (Largeau et al. 1980)[7]. However, there exists an important morphological heterogeneity within algae examined after water-sampling from the lakes and cultivation of strains in the laboratory. The most striking variations concern the size and shape of cells, which can be more or less embedded in the matrix, and the presence (or not) of refringent threads linking clusters of cells, thus leading to the formation of very large colonies. On the basis of such morphological differences, but ignoring chemical analyses, Komárek and Marvan (1992) [8] proposed the existence of at least 13 species in Botryococcus.

However, Plain et al. (1993) noted that, in each chemical race and for the same strain, some of these features could vary in relation to age and culture conditions [9]. Recently, 18S rRNA sequences of four strains of B. braunii belonging to the three chemical races established that these strains formed a monophyletic group (Senousy 2003; Senousy et al. 2004) [10]. Now, whether the numerous strains of B. braunii belong to a single species, to three species in connection with the nature of the synthesised hydrocarbons, or to several sub-species is still under debate. The SEM and inverted microscope photos of *Botyrococcus braunii* KUTZING NIES 2199overCladophora sp. shows the symbiotic growth of *Botyrococcus braunii* KUTZING NIES 2199 with Cladophorasp.The inverted microscope photo with 40 X magnification the dark oval portions represent the pyriform cells of *Botyrococcus braunii* KUTZINGG NIES 2199 and the hair like filamentous fibres depict Cladophorasp.In figure 1.5 the SEM photo with 2000 X clearly depicts the pyriform cells of *Botyrococcus braunii* KUTZING NIES 2199 growing symbiotically with hair like filaments of Cladophora sp.

3. Materials and Methods

Chemicals used: KNO_3,$K_2HPO_4$$CaCl_2$ dihydrate,$MgSO_4$heptahydrate,Ferric Citrate,Citricacid,$CoCl_2$$H_3BO_3$,$MnCl_2$tetrahydrate,$ZnSO_4$heptahydrate,CuSO4pentahydrate,0.072 N $H_2SO_4$$Na_2$EDTA,Ammonium Molybdate, $NaHCO_3$Boric Acid, $CuSO_4$$NaNO_3$ $MgSO_3$, KCl, KI, Cholesterol, $FeCl_3$, Zinc Acetate

Equipment's used: Autoclave, Analytical balance, Centrifuge, Hot air oven, Incubator-Shaker, Laminar flow chamber, Magnetic stirrer cum hot plate, Microscope, Refrigerator

The pellet was dried in an oven for 105 ^{0}C for an hour. The dried pellet (1.791 g) was homogenized with a mortar and pestle for 10 minutes with 40 ml Methanol and 20 ml Chloroform. Then 20 ml of Chloroform and 20 ml Water was added to the mixture and homogenized for another 10 minutes. The mixture was filtered through a 150 mm Whatman filter paper (Dassel, Germany) using a glass funnel and the solvent was collected in a cylinder. A watch glass was used to cover the funnel to prevent any solvent loss during filtration. The solvent was then allowed to settle in a separating funnel for 15 minutes. The dark Modified Bligh and Dyer Method: The sample room was centrifuged at 100 rpm for 20 minutes and the supernatant discarded. green biomass rich chloroform layer at the bottom was transferred to the original flask and then 20 ml chloroform was added and homogenized for another 5 minutes. The sample was filtered again and the clear methanol/water layer was discarded. The chloroform layer was then evaporated of the solvent under an infrared lamp at 55-60 ^{0}C and the lipid was estimated gravimetrically.

Soxhlet Method: Thesoxhlet flasks were dried in the oven at 105ºC for an hour and then cooled in the desiccators for 30min and tarred with and without the stopper. 12 gram of dried biomass sample was accurately weighed and transferred to the extraction tube. The sample was covered with cotton wool in the extraction tube and placed in the soxhlet apparatus with a tarred flask. Approx. 100 ml of chloroform was added to the soxhlet flask. The sample was extracted over night with a condensation rate of 2-3 drops per second. The soxhlet flask was weighed with glass stopper again. The chloroform was distilled off; the flask was dried in the oven for 2 hours at 105±1ºC. The soxhlet flask was cooled in the desiccators and weighed. The two reaction tubes were subjected to methylation procedure.

4. Results and Discussion

The experimental results are presented in tabular form and in photographs. From the results found ,mathematical model is used in designing photo bio reactor

Table 1.Growth Curve and Specific Growth Rate:

Time (Days)	Biomass Dry weight (g)	Biomass (g/L)
0	0.000	0
7	0.001	0.009
11	0.002	0.016
24	0.002	0.020
33	0.003	0.026
45	0.006	0.065
62	0.015	0.151

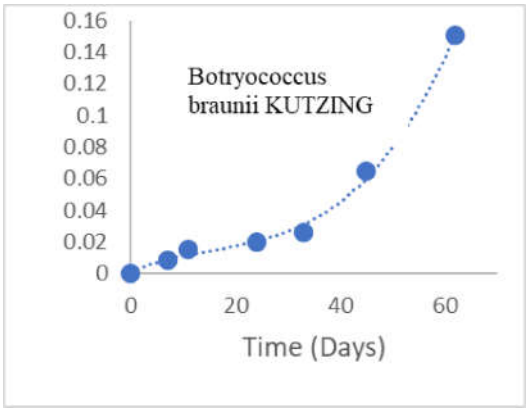


Fig. no -1. Shows a plot of Biomass versus time and hence shows the growth curve for *Botryococcusbraunii.*

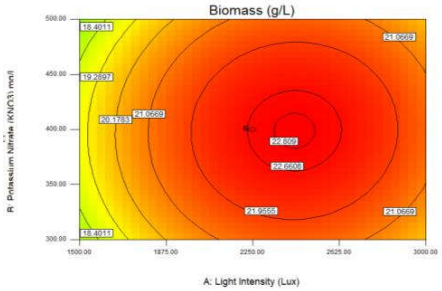


Fig. no-2. Isoresponse contour plots showing the effect of Potassium Nitrate and Light Intensity and interactive effect on Biomass

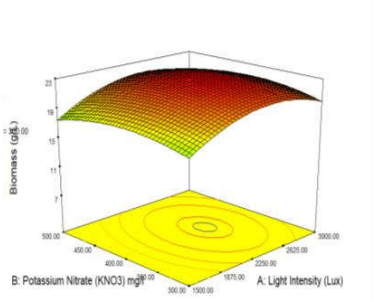


Fig.no-3. Response surface plot showing the effect of Potassium Nitrate and Light Intensity and interactive effect on Biomass

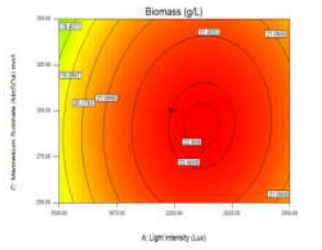


Fig.no-4. Isoresponse contour plots showing the effect of Magnesium Sulphate and Light Intensity and interactive effect on Biomass

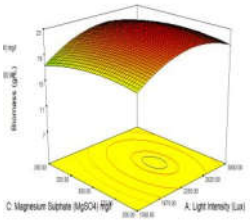


Fig.no-5. Response surface plot showing the effect of Magnesium Sulphate and Light Intensity and interactive effect on Biomass

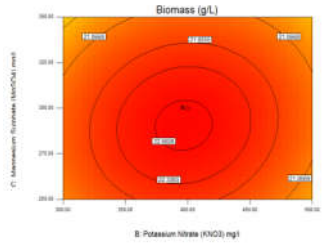


Fig. no- 6. Isoresponse contour plots showing the effect of Magnesium Sulphate and Potassium Nitrate and interactive effect on Biomass

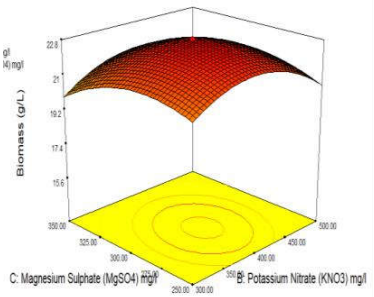


Fig. no-7. Response surface plot showing the effect of Magnesium Sulphate and Potassium Nitrate and interactive effect on Biomass

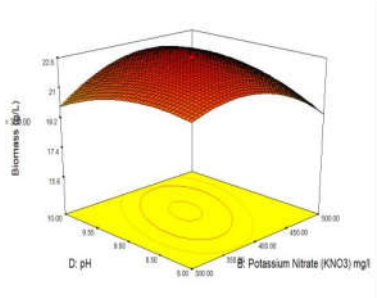


Fig. no- 8. Response surface plot showing the effect of $P^H$and Potassium Nitrate and interactive effect on Biomass

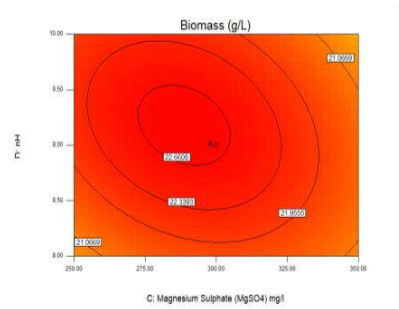
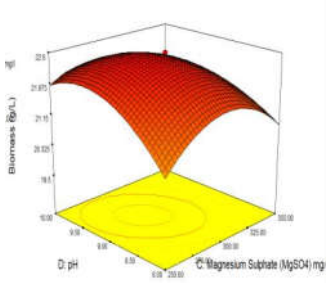


Fig. no-9. Isoresponse contour plots showing the effect of P^H and Magnesium sulphate and interactive effect on Biomass



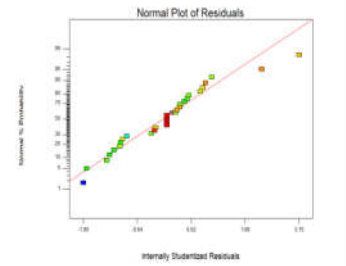


Fig. no-10. Response surface plot showing the effect of $P^H$and Magnesium Sulphate and interactive effect on Biomass

Table 2. The response from the second order polynomial equation and the experimental data of Botryococcus brauni

Sl. No.	Light Intensity (Lux)	Potassium Nitrate (KNO_3) mg/l	Magnesium Sulphate ($MgSO_4$) mg/l	pH	Biomass (g/L)	
					Experimental	Predicted
1	1500	300	250	8	17.1	17.5625075
2	3000	300	250	8	15.6	18.31253
3	1500	500	250	8	15.9	15.2125075
4	3000	500	250	8	16.8	16.26253
5	1500	300	350	8	17.4	17.0125075
6	3000	300	350	8	16.5	18.51253
7	1500	500	350	8	16.2	15.4125075
8	3000	500	350	8	15.3	17.21253
9	1500	300	250	10	16.8	15.9625075
10	3000	300	250	10	17.4	18.96253
11	1500	500	250	10	18.3	17.0625075
12	3000	500	250	10	18.9	20.36253
13	1500	300	350	10	12.3	13.6125075
14	3000	300	350	10	15.6	17.36253
15	1500	500	350	10	17.1	15.4625075
16	3000	500	350	10	19.2	19.51253
17	750	400	300	9	7.5	10.3250018
18	3750	400	300	9	19.8	15.1250468
19	2250	200	300	9	20.7	17.3250168
20	2250	600	300	9	15.6	17.1250168
21	2250	400	200	9	20.1	19.5750168
22	2250	400	400	9	19.5	18.1750168
23	2250	400	300	7	20.7	19.2750168
24	2250	400	300	11	20.4	19.9750168
25	2250	400	300	9	22.2	22.7000168
26	2250	400	300	9	22.8	22.7000168
27	2250	400	300	9	22.8	22.7000168
28	2250	400	300	9	22.8	22.7000168
29	2250	400	300	9	22.8	22.7000168
30	2250	400	300	9	22.8	22.7000168

Table 3. Experimental range and levels of Light Intensity, Potassium Nitrate, Magnesium Sulphate and pH in Central co mposite design (CCD) on the biomass.

Variable	Parameter	Level				
		-α	-1	0	+1	+α
X_1	Light Intensity (Lux)	750	1500	2250	3000	3750
X_2	Potassium Nitrate (KNO_3) mg/l	200	300	400	500	600
X_3	Magnesium Sulphate ($MgSO_4$) mg/l	200	250	300	350	400
X_4	pH	7	8	9	10	11

4.1 Conceptual Design of Photobioreactor

Based on the production of optimal biomass, the lumen fibre diameter, the shell diameter, the number of fibres, the length of the lumen fibre and the shell length were calculated based on the technical specifications of a hollow fibre reactor normally used for cell culture from Celab, Germany. An aspect ratio of L/D equal to 5 was chosen, the diameter of the housing was fixed and the volume of the housing was calculated. The outer diameter and the inside diameter of the lumen fibre was taken as 280 □m and 200 □m respectively. The active length of the fibre was 0.24 m. The number of fibres for the representative hollow fibre reactor was 12500. The ICS volume was 0.1 L and the ECS volume was 0.214 L with a combined ICS and ECS volume of 0.315 L. Based on these representative dimensions and a scale factor of 275 was chosen which was based on the volume of the bioreactor required. Using the scale factor of 275 all other parameters were calculated or determined.

The conventional approach to design and modelling of hollow fibre photo- bio reactors has its own pros and cons. The disadvantage is being that of practical limitations preventing from designing an ideal hollow fibre photo- bio reactor. Hence, in this study based on the availability of a hollow fibre photo- bio reactor, design calculations were carried out to determine the dimensions, number of fibres, length and diameter of the shell and that of the lumen fibre.The hollow fibre photo- bio reactor normally used for cell culture that was availbale of the shelf type was chosen as the basis and standard for designing the hollow fibre photo- bio reactor for the purpose of growing alga. The dimensions of the standard hollow fibre photo- bio reactor are given.Using a scale factor of 275, the shell volume (ECS) and the lumen volume (ICS) were determined to 59L and 28L respectively. Based on the ratio of the (ECS + ICS) volume and that of the housing volume, the housing volume was designed correspondingly. Based on the aspect ratio of Length/Diameter , the diameter of the housing and the active fibre length was determined or calculated. The same MWCO,OD,ID of the fibres were chosen for bot the standard and the designed hollow fibre photo- bio reactor. The Number of Fibres were finally determined based on the ICS volume.Although the number of fibres , ID and OD of the fibres have been chosen to be constant, based on the permeability of these fibres one can then determine them for this particular scaled hollow fibre photo- bio reactors.

4.1 .1 Sample calculation to solve the polynomial equation Y_1(Quadratic Equation)

$$Y_1 = 22.70 + 1.20A - 0.050B - 0.35C + 0.17D + 0.075AB + 0.19AC + 0.56AD + 0.19BC + 0.86BD - 0.45CD \ -2.49A^2 - 1.37B^2 - 0.96C^2 - 0.77D^2$$

NOTE: $x_1 = A$ $x_2 = B$ $x_3 = C$ $x_4 = D$

Partially differentiating the above equation with respect to x_1, x_2, x_3 and x_4 respectively

$$\frac{dY}{dA} = 1.20 - 4.98A + 0.075B + 0.19C + 0.56D$$

$$\frac{dY}{dB} = -0.050 + 0.075A - 2.74B + 0.19C + 0.86D$$

$$\frac{dY}{dC} = -0.35 + 0.19A + 0.19B - 1.92C - 0.45D$$

$\frac{dY}{dD}$ = 0.17 + 0.56A + 0.86B – 0.45C - 1.54D

MATLAB program for evaluating values of x_1, x_2, x_3 and x_4.

By writing the coefficients of x_1, x_2, x_3 and $x_4$of above equations in matrix form

Input

a = [1.20 – 4.98 + 0.075 + 0.19 + 0.56 ; -0.050 + 0.075 – 2.74 + 0.19 + 0.86;
-0.3 + 0.19 + 0.19 – 1.92– 0.45 ; 0.17 + 0.56 + 0.86 – 0.45 - 1.54]

b= [-1.20 ; 0.050 ; 0.35 ; - 0.17]

x= inv (a) * b

Output

x= [0.406 ; -0.030 ; -0.149 ; 0.183]

Hence coded values of Light Intensity; Potassium Nitrate; Magnesium Sulphate are,

x_1= 0.406 x_2 = -0.030 x_3 = -0.149 x_4= 0.183

These can be decoded as follows:

x_1= ($2X_1$ – (3000 + 1500)) / (3000-1500)

Therefore, Light Intensity (Lux)

X_1 = (x_1(3000-1500) + (3000+1500)) / 2 Substituting, x_1 =0.406 in this equation

We get, **X_1 = 2554Lux**

x_2= ($2X_2$ – (500+300)) /(500-300)

Therefore, Potassium Nitrate (KNO3) mg/l

X_2 = (x_2(500-300) + (500+300)) / 2 Substituting, x_2 =-0.030 in this equation

We get, **X_2 = 397mg/l**

x_3= ($2X_3$ – (350+250)) /(350-250)

Therefore, Magnesium Sulphate (MgSO4) mg/l

X_3 = (x_3(350-250) + (350+250)) / 2 Substituting, x_3 = -0.149 in this equation

We get, **X_3 = 292mg/l**

X_4= ($2X_4$ – (10+8)) /(10-8)

Therefore, pH

X_4= (x_4(10-8) + (10+8)) / 2 Substituting, x_4= 0.183 in this equation

We get, **X_4 = 9.2**

Biomass (Y_1) = 22.7 g/L

Table 4.Comparisons of Design and Cost Analysis with Literature

Sl. No.	Description of Photo Bioreactor Design parameters considered	Cost Analysis	References
1	Tetraselmissuecica in a 1-ha plant made of "Green Wall Panel-II" (GWP®-II) photobioreactors. Turn over 54 tonnes per hectare annually. The plant consists of eight 1250-m2GWP®-II modules (GWP_1 toGWP_8) served by fourmain pipelines	The total cost of the microalga - GWP®-II photobioreactors is estimated to be 1,661,777 (€), which is nearly Rs.13,02,56,728.37 (13 Crores)	M. R. Tredici, *et al.* (2016), Algal Research 19, 253-263. [11]
2	Design of PBRs for Photocatalysis: • Tubular PBR-Simple; large illumination surface area • Plastic bag PBR • Flat-panel airlift PBR	• high capital and operating costs • Low capital cost in the short term • Low power consumption and shear stress; easy temperature control; low operating cost	Q. Huang, *et al.* (2017), Engineering 3, 318–329. [12]
3	A combination of attached and suspended microalgae cultivation patterns, i.e., a suspended-solid photobioreactor (ssPBR), has been proposed for the potential ofeconomical biomass productionwith an annual production of 3600 t microalgae biomass	profit of 146 K dollars/ (Rs. 10396952.00) with an annual production of 3600 t microalgae biomass	L.-L. Zhuang, *et al.* (2019), Algal Research 39, 101463. [13]
4	Hollow-Fibre Photo-bioreactor for Biomass and Lipid Production. Material (Housing)-Polycarbonate Material (Fibres)- Poly-sulfone	Rs. 18,31,58,896 Rs. 2,81,58,896	Present Work (PW)

5. CONCLUSION

Effect of four parameters viz., Potassium Nitrate, Magnesium Sulphate, pH and light intensity on the growth of Botryococcus barunii(B. barunii) microalgae were studied. Medium and environmental parameters were optimized using response surface methodology .A mathematical model was developed for producing maximal amount of microalgae biomass. Further, maximal lipids were extracted from the microalge.The hollow fibre photo-bioreactor (HFPB) was conceptually designed based on the available technical information of the reactor and a scaled version of the hollow fibre photo-bioreactor was established. However, physical design could not be carried out due to technical and cost constraints. Different models were studied for different microalgae. Economic and feasibility studies were carried out towards scale-up of biomass and lipid production using the hollow-fibre

photo-bioreactor.A continuous process was considered to be suitable for viability and feasibility oftheprocess.Both return on investment and breakeven were calculated and was found to be a viable process. However, this needs to be further validated with a real physical process to be setup.

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