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Research Article

Process Development Engineering of *Botryococcus braunii* Cultivation: Designing the Economical Nutrient Medium for Commercial Cultivation of Potential Hydrocarbon-rich *Botryococcus braunii*

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Abstract

Botryococcus braunii is a hydrocarbon rich alga which has the potential to produce good quality fungible fuel i.e. biodiesel having high calorific value. As it contains lipids and hydrocarbons having branched carbon chains, the biodiesel produced from it may have better fuel characteristics such as cloud and pour point and oxidative stability. The major bottleneck in the production of biodiesel and other value-added chemicals, products and fuels from algae is the cost of nutrient medium used for the growth of algae. An economical nutrient medium containing very few essential nutrients in considerably reduced concentrations, 0.2 g/L $NaNO_3$, 0.028 g/L single super phosphate, 0.12 g/L $MgSO_4.7H_2O$ and 0.05 g/L muriate of potash was designed and formulated by reducing the amount and number of components of Chu-13 medium and substituting some of these with economical commercial grade chemical fertilizers. This devised nutrient medium with almost 87% reduction in cost can be effectively exploited for the commercial cultivation of the alga for the production of biomass. The co-cultivation of cyanobacteria along with *Botryococcus braunii* for supplying the nitrogen required in the media would also further help in economizing the cost of the media. Algal biomass produced can be effectively utilized for the production of fungible fuels such as oil and hydrocarbons. Thus, present studies would help in the reduction of the cost of the process of production of biodiesel having higher calorific value from the *Botryococcus braunii* in future algal biorefineries.

Keywords: *Botryococcus braunii*; Economic Medium; Single Super Phosphate; Muriate of Potash

Introduction

Microalgae have higher photosynthetic efficiencies with swift replicating capabilities and these have several applications for the production of food, chemicals, biofuels, pigments, nutrients etc. Microalgae are already being exploited commercially by industries for the production of some of these value-added products even today. Microalgae have emerged as the potential carbon neutral candidate of biodiesel production as it can supplement global transportation needs [1,2] unlike the oil crops which can substitute only about 0.3% of the current transportation fuels demand [3,4]. Presently more than 100 MTOE of biofuels are produced and this figure may reach 300 MTOE by 2030. The global energy demand is increasing [5].

Most of the biofuels are produced presently from land biomass leading to land crisis and food versus fuel controversies. However, plenty of algae are available. So far algae have been commercially exploited for the production of food and specialty chemicals mainly. However, the potential of algae has been realized lately for the production of biofuels [2,6]. These do not require much land and have shorter production cycles and at the same time these lead to the utilization of CO_2 for arresting the global warming and consequently reducing the climate change effect [7]. *Botryococcus braunii* (*B. braunii*) can lead to the production of extracellular hydrocarbons, especially botryococcenes and squalene and exopolysaccharides which can be used as scaffold for polyester production and has a good potential for the production of nanoparticles which can be exploited for the production of nanomaterials and nanocomposites [8-10].

Concept of algal biorefineries

In fact, besides biofuels the microalgae such as *Botryococcus braunii* can also produce single cell proteins, carotenoids, omega-3-fatty acids, polysaccharides, vitamins, nutraceuticals, drugs, anticancer, anti-oxidants, etc. compounds of great commercial interest [11,12].

It was suggested that the concept of biorefinery is being created to replace the petroleum refineries by producing the fuels and a broad spectrum of chemicals and products sustainably from the downstream processing of biomass which are marketable and thus can also replace those which are presently being produced from the downstream processing of crude oils in petroleum refineries [13]. It has been reported that in an integrated biorefinery high value low volume products would become an economic leveller for the production of low value high volume transportation biofuels. Additionally, it has also reported on the concepts of biorefineries mainly based on algal biofuels and described how these can be actually set up for the successful operations [14,15]. In fact, integrated biorefineries are those where several sustainable biomass conversion technologies are employed in one refinery to produce value added biofuels, chemicals, products including food, feed, materials etc. products.

Sudhukhan., *et al.* (2014) have reported that algae produce a number of products such as, proteins, carbohydrates, nucleic acid, amino acids, vitamins, antioxidants, and carotenes and other pigments, besides lipids and hydrocarbons. The proportion of these compounds varies depending upon the microalgae species and the nutritional conditions available during algal cultivation [16].

Anbuchezhian., *et al.* (2015) have described that the algae are able to synthesise a large number of antioxidants including carotenoids, tocopherol, ascorbic acid, chlorophyll and derivatives and rubisco white protein, phlorotannins, polyphenols, different amino acids, vitamins, anticancer drugs, proteins, fibres, omega 3 fatty acids and other polyunsaturated acids [17].

Botryococcus braunii is a potential candidate for establishing algal biorefineries. It is possible to improve the production economics of *Botryococcus braunii* by selection or development of suitable strain. This would be further boosted by the designing and engineering an advanced culture medium using an economical nutrient media.

Present authors have also explored the potential of the use of *B. braunii* in biological photovoltaics i.e., microbial fuel cells [18,19]. Microalgae would also play a significant role in the development of future biodegradable biological solar cells [20]. Talukdar., *et al.* (2016) have reported the potential of *Botryococcus braunii* (*B. braunii*) as a source of biodiesel having higher calorific value with 57% lipids and 53% hydrocarbons after 58 and 28 days of cultivation [21]. Present authors have also reported the great potential of *B. braunii* as a source of fungible biofuels including bio-jet oil and green diesel fuels [22]. Currently, the cost of production of algal biodiesel is higher in comparison to that of petro-diesel because of the requirement of the use of costly nutrient media. However, this can be minimized by reducing the cost of nutrient media used for production at various levels and one of the main being cultivation of algae. Gautam., *et al.* (2015a) has reviewed the production of biofuels from microalgae and macroalgae [6]. The major bottleneck in the commercialization of microalgae is that a nutrient medium is required for the growth of algae which contains costly chemicals. Therefore, there is a need to make the nutrient medium economical in order to reduce the production cost of algal biomass for commercial production of algal biodiesel and various other valuable products. In fact, there is a need for process intensification studies as there exists an ample scope of not only reducing the concentration of certain chemicals but also the costly chemicals used can be replaced by the use of inexpensive and easily available chemicals. Not much work on the reduction of the cost of media used for the algae producing biodiesel seems to have been reported. Tasic., *et al.* (2016) have reviewed the research work on the production of biodiesel from *B. braunii* for industrial commercialization of biodiesel production. These authors have suggested the use of wastewaters and flue gases. These authors have recommended that there is a need to design cultivation systems for the optimal growth of *B. braunii* [23].

Earlier research work from the author's laboratories was also directed towards modifying and economizing the nutrient media by using wastes and different readily available cost-effective carbon substrates [24,25]. The use of organic wastes and different carbon substrates for improving the growth of algae for the production of biodiesel. Use of sea water and industrial wastewater for improving the growth of algae for the production of biodiesel has also been reported by the present authors earlier [18,26]. Zhou., *et al.* (2014) have also reported the use of nitrogen, potassium and phosphorus constituents extractable from the pig urine source for the growth of *B. braunii* for biofuel production [27]. Authors feel that in order to avoid contaminations the issue of the requirement of cleaner nutrients for the clean algal growth without much contamination would also be important. Therefore, attempts must be made to reduce or eliminate the use of nutrients in the media used for algal growth.

Fuel characteristics of biodiesel

Manchanda., *et al.* (2017) have reported on the fuel characteristics of green diesel and biodiesel including those of algal biodiesel [22]. In fact, the fuel characteristics of biodiesel are also important for its use in engines. *B. braunii*, a colonial green alga contains not only lipids (i.e. long hydrocarbon chain linked glycerides) but also long chain hydrocarbons. In fact, *B. braunii* and *Aurantiochytrium* sp. are known to produce lipids and hydrocarbons having branched carbon chains (BCC). Out of these the *B. braunii* can give better yield of biodiesel. In fact, biodiesel containing BCC has good cold flow property and higher oxidative stability. In fact, UPLC-MS analysis of lipids of two typical algal species has been reported by Sharma., *et al.* (2015) showing the presence of branched chain lipids [28]. Therefore, biodiesel produced from *B. braunii* containing branched chain hydrocarbons and lipids can be blended with other fungible fuels such as, oil-seed produced biodiesel, green diesel or petro-diesel to improve their properties. Therefore, it has become exclusive focus of research for the production of biodiesel as well as green diesel in some laboratories [22]. The presence of hydrocarbons would boost the quality of biodiesel produced, besides enhancing the calorific value of the same.

Some bacteria (including halophiles), fungi are also known to produce biodiesel having BCC [29]. However, though these bacteria and fungi may have higher growth rates, but these require sugars and carbohydrates for their growth. Recently, Singh and Choudhury, 2018 have reported the production of biodiesel having BCC esters and hydrocarbons from halophiles [30]. Since *B. braunii* had shown good growth using sea water [26], it may be worth studying co-culturing of this algae with halophiles using economic media.

Several bacterial species also contain branched carbon chain fatty acids and hydrocarbons, e.g. *Arthrobacter* sp., *Desulfovibrio desulfuricans*, *Clostridium pasteuriarum* and *Lentibacillus* sp. [31-33]. The addition of branched chain methyl ester in biodiesel can reduce the cloud and pour points and improve the oxidation stability as well [34,35]. Since, biodiesel produced from using *B. braunii* also contains BCC. Therefore, it may also have desired reduction of cloud and pour point and better oxidative stability.

Economizing the cell growth media

As described before, the major issue in the commercialization of algal biodiesel is the higher cost of the production of algal biomass. Present study targets the formulation of economical medium using low-cost chemicals or commercially used fertilizers in substantially reduced concentrations in comparison to the standardized or optimum Chu-13 medium for the cultivation of algae. Studies were conducted using two strains of *B. braunii.* Presently designed and devised economical medium can be effectively used for the commercial cultivation of algae for the production of not only biodiesel but also various other fuels and valuable products after its successful trial at larger scales. The parallel studies in the author's laboratory have also shown that co-cultivation of cyanobacteria with *B. braunii.* The synergistic interaction of *B. braunii–N. muscorum* showed the enhancement of 50% fixation of nitrogen which had also led to the 38% enhancement in the biomass production and an increase of 27 % in the production of lipids [36].

However, there is still a further scope of economizing the media costs. Therefore, presently the studies were extended to economize the media for the growth of *B. braunii* under proper optimal conditions for producing fungible biodiesel fuel as a part of process engineering studies.

Materials and Methods

Test organisms and growth conditions

One of the two strains of *B. braunii* was isolated from Udaisagar Lake, Udaipur, Rajasthan (24.60° N, 73.67° E) in India and the other from Loktak Lake, Manipur (24.55° N, 93.78° E) in India by serial dilution method followed by plating on solidified Chu-13 nutrient medium [37]. The individual colonies were isolated and inoculated into liquid medium. Cultures were incubated at 27 ± 1ºC under 1.2 ± 0.2 klux light intensity with 16:8 hrs light:dark cycle.

Extraction and estimation of total chlorophyll

For measurement of growth, cultures were harvested and total chlorophyll was estimated at different time intervals in 90% methanol using Lichtenthaler equations [38].

Results and Discussion

The process economics of biorefineries demands the reduction of inputs into the growth of algae by deigning and devising an economical media. The best medium for the cultivation of the algal strains was found to be Chu-13 medium. In fact, previously, authors conducted preliminary studies on the formulation of economical medium for the growth of *B. braunii* by using a single strain. Though these studies were conducted under its incomplete growth conditions still these showed that there was a scope of further studies on optimizing the complete conditions for the algal growth and production of lipids and hydrocarbon. In fact, cell growth still continued making it important to optimize the algal conditions i.e. the final growth stage [39].

Process engineering of algal cell growth media

Photosynthesis rate of fixing CO_2 in algae is higher than that of land plants as the carbon concentrating mechanism (CCM) in algae is more efficient in fixing CO_2 under limiting carbon and nutrient conditions in water [7,40]. The CCM is more efficient than C_3, C_4 and Crassulacean acid mechanisms in regulating rubisco and carbonic anhydrase enzymes. Therefore, algal cell growth can occur even in the minimum nutrient media due to carbon fluxes at lower concentrations of CO_2 in pyrenoid and outside the cells which at times can even lead to algal blooms as well [7]. The CCM in algal systems promotes the forward reaction of photosynthesis requiring minimum of nutrients even by concentrating the CO_2 mainly to increase its proportion in pyrenide. In fact, process intensification studies on the cell growth kinetics of cultivation of *B. braunii* revealed that the optimum time of the algal growth was 21 days. The process of cell growth was mass transfer controlled. Thus, this dictated that there was a need of extending further process development engineering studies on the process intensification in the light of newly established process conditions. Therefore, the detailed studies were undertaken on economizing the nutrient media components under optimum conditions. To further establish the studies one more strain of *B. braunii* was also used in these studies.

Process intensification studies of economizing nutrient media for enhanced algal growth

The issue of reducing the cost of media is crucial for the commercialization of the process of biodiesel production from *B. braunii.* Tasic., *et al.* (2016) have also reported on the grave need of designing the cultivation system under optimal conditions for growing the *B. braunii* for establishing the industrial commercial process of biodiesel production [23]. Therefore, it was decided presently to extend process development studies on designing, devising and developing a low-cost media for the cultivation of *B. braunii* for the production of biofuels, bioenergy and other value-added products. Two different strains of *B. braunii* were used in the process intensification studies on the optimization of time for the production of lipids and hydrocarbons from *B. braunii.* It was found that the best time of harvesting for both the isolated strains of *B. braunii* in Chu-13 medium with respect to the three parameters (growth, hydrocarbon and oil production) was 21 days [41]. Talukdar., *et al.* (2016) have also reported that the production of hydrocarbons and lipids from this alga is completed after more than 20 days [21].

The studies were carried out on the reduction of the cost of the Chu-13 medium with respect to the three parameters (growth, hydrocarbon and oil production) as mentioned earlier in the text. Agrawal and Krishna, 2016 have reported on the standardization of conditions for the growth of *B. braunii*, which may be used for growing this alga [42]. These authors used the soil extracts as well in the media in their studies. Present authors have given a due consideration on the use of type, quality and quantity of each media constituent without compromising on possibility of contamination in the media and also the yield of production of lipids and hydrocarbons and the biodiesel. The minimization of nutrients would also lead to their cross interaction and produce limited stresses. The stresses of nutrients also result in the enhancement in the production of biofuels from algae [41,43,44]. Cheaper and ready availability of chemicals is also important. Presently interesting results were obtained which showed a great scope of economizing the media used for cultivating the *B. braunii* at larger commercial scales in future.

Substitution of costly KNO_3 by inexpensive nitrogen

Firstly, the studies were undertaken to reduce the cost of using KNO_3 in the Chu-13 medium. The amount of KNO_3, the major source of nitrogen, in Chu-13 medium was reduced and it was observed that reduction in its amount to 60% of the control value (control is 100% with 0.4 g/L) resulted in almost similar growth as obtained with the optimum value of KNO_3. Thus, in order to make the nutrient medium economic, the amount of KNO_3 could be effectively reduced to 60% of the control value as shown in figure 1.

Figure 1: Growth of the two strains of *B. braunii* in Chu-13 medium with different concentrations of KNO_3 (Control being 100% which is 0.4 g/L) on 21st day.

Further studies were extended to replace KNO_3 with an economic source of nitrogen. Thus, different sources of nitrogen, such as $NaNO_3$, $Ca(NO_3)_2$ and urea were used and their effect on growth, was analyzed and compared to that observed when the costly KNO_3 was used. Growth with $NaNO_3$ was found to be comparable to that observed using KNO_3 as shown in figure 2.

Figure 2: Growth of the *B. braunii* strains in Chu-13 medium with different sources of N (with equal concentration of nitrogen as found in Chu-13 medium as KNO_3 i.e. 0.4 g/L KNO_3 and 0.34 g/L $NaNO_3$) on 21st day.

Thus, KNO_3 in Chu-13 medium could be successfully substituted by $NaNO_3$. It was also noticed that up to 60% concentration of nitrogen as $NaNO_3$ (0.2 g/L $NaNO_3$) in the absence of KNO_3 supported almost similar growth as observed with the control concentration of KNO_3 (Figure 3). Therefore, KNO_3 in Chu-13 medium was substituted by 0.2 g/L $NaNO_3$.

Figure 3: Growth of the *B. braunii* strains in Chu-13 medium with different concentrations of $NaNO_3$ (without any KNO_3) on 21st day.

Reduction in the amount of costly phosphorus based components in the medium

Further, the amount of K_2HPO_4 in Chu-13 medium was varied and it was found that with up to 40% amount of K_2HPO_4 (40% being 0.032 g/L) the growth of the algal strains was closer to that obtained with control concentration (0.08 g/L) as shown in figure 4. Consequently, the amount of K_2HPO_4 could be effectively lowered to 40% of the control value.

Figure 4: Growth of the *B. braunii* strains in Chu-13 medium with different concentrations of K_2HPO_4 (Control is 100% with 0.08 g/L K_2HPO_4) on 21st day.

Studies were further extended to replace the use of costly K_2HPO_4 by other phosphorus based compounds. Different sources of another important nutrient, phosphorus, were thus used and their effect on growth was compared to that observed by using K_2HPO_4. Growth of both the algal strains with $Ca(H_2PO_4)_2$ was found to be comparable to that observed by using K_2HPO_4 as shown in figure 5.

Figure 5: Growth of the *B. braunii* strains in Chu-13 medium with different sources of P [(with equal concentration of phosphorus as found in Chu-13 medium as K_2HPO_4 i.e. 0.08 g/L K2HPO$_4$, 0.07 g/L Ca(H$_2$PO4)$_2$] on 21st day.

Additionally, the growth of the algal strains in Chu-13 medium with up to 40% concentration of phosphorus in the form of $Ca(H_2PO_4)_2$ (40% being 0.028 g/L) in the absence of K_2HPO_4 was almost similar to that obtained with control amount of K_2HPO_4 in Chu-13 medium (Figure 6). Therefore, K_2HPO_4 in Chu-13 medium (0.08 g/L) was replaced by 0.028 g/L of $Ca(H_2PO_4)_2$ which is the main component of single superphosphate (SSP) which is a commonly used low-cost fertilizer. Thus, SSP can be utilized as a cheaper source of phosphorus in the nutrient medium and this is also readily available.

Figure 5: Growth of the *B. braunii* strains in Chu-13 medium with different concentrations of $Ca(H_2PO_4)_2$ (without any K2HPO$_4$) on 21st day.

Reduction in the amount of $MgSO_4$ used in the medium

Further, the amount of $MgSO_4$ was varied in Chu-13 medium and reducing the amount of $MgSO_4$ to 60% of the control value (control being 0.2 g/L) was found to support almost the similar growth of algal strains as observed with Chu-13 medium as shown in figure 7. Thus, the amount of the $MgSO_4$ was reduced to 60% (0.12 g/L).

Figure 7: Growth of the B. braunii strains in Chu-13 medium with different concentrations of $MgSO_4$ (Control is 100% with 0.2 g/L $MgSO_4.7H_2O$) on 21st day.

Supplementation of source of potassium in the devised economic medium:

As both the sources of potassium in Chu-13 medium, K_2HPO_4 and KNO_3, were substituted by $Ca(H_2PO_4)_2$ (0.028 g/L) and $NaNO_3$, (0.2 g/L), respectively, thus, another source of potassium was desirable for the growth of algal strains. Component of muriate of potash (MP), an economical and commercially used fertilizer, KCl was used and 0.05 g/L of KCl was observed to be the optimum amount for the growth of algal strains (Figure 8). Thus, MP was used as a source of potassium in the formulated economic medium.

Figure 8: Growth of *B. braunii* strains in Chu-13 medium (Control) and modified Chu-13 medium.

Thus, the devised economic medium had the composition 0.2 g/L $NaNO_3$, 0.028 g/L single super phosphate, 0.12 g/L $MgSO_4.7H_2O$ and 0.05 g/L MP.

Extent of reduction in the cost of Chu-13 nutrient medium

Reduction in the cost of the Chu-13 medium in terms of US $ and Indian rupees (INR) is shown in table 1. It can be seen that there could be a considerable reduction in the cost of the medium, firstly by avoiding the use of costly chemicals such as $CaCl_2$, K_2HPO_4, KNO_3, ferric citrate, citric acid and micronutrients. Secondly, as desirable before the concentration of other chemicals, such as, KNO_3, K_2HPO_4, SSP, $MgSO_4$ and MP can also be reduced alternately. Therefore, overall, there would be a saving of almost 87% in the cost of nutrient medium by using presently designed and devised Chu-13 nutrient medium for growing hydrocarbon rich *B. braunii* algae for the production of good quality hydrocarbon rich biodiesel having high calorific value.

S. No.	Nutrient	Approx. Rate (Rs./Kg)	Approximately Rate ($/Kg)	Chu-13 medium			Economic medium		
				Conc. (g/L)	Approx. Cost (Rs./KL)	Approx. Cost ($/KL)	Conc. (g/L)	Approximately Cost (Rs./KL)	Approximately Cost ($/KL)
1.	KNO_3	888.00	12.77	0.400	355.2	5.11	-	-	
2.	$NaNO_3$	626.00	9.00	-	-		0.200	125.20	1.80
3.	K_2HPO_4	1280.00	18.40	0.080	102.4	1.47	-	-	
4.	SSP	8.40	0.12	-	-		0.028	0.23	0.0033
5.	$MgSO_4.7H_2O$	410.00	5.89	0.200	82.0	1.18	0.120	49.20	0.71
6.	$CaCl_2.2H_2O$	580.00	8.34	0.107	62.06	0.89	-	-	
7.	Ferric citrate	1646.00	23.66	0.020	32.92	0.47	-	-	
8.	Citric acid	872.00	12.54	0.100	87.2	1.25	-	-	
9.	MP	14.8	0.21	-	-		0.050	0.74	0.011
10.	Micro Nutrients				514.17	7.39	-	-	-
	$CoCl_2.2H_2O$	25,100	360.84	0.020000	502.00	7.22			
	H_3BO_3	998.00	14.35	0.005720	5.70	0.082			
	$MnCl_2.4H_2O$	1,270.00	18.26	0.003670	4.66	0.067			
	$ZnSO_4.7H_2O$	666.00	9.57	0.000440	0.29	0.0042			
	$CuSO_4.5H_2O$	1,540.00	22.14	0.000160	0.25	0.0036			
	Na_2MoO_4	15,110.00	217.22	0.000084	1.27	0.018			
	Total				1235.95	17.77		175.37	2.52

Table 1: Comparison of composition and cost of Chu-13 and economic nutrient medium devised in the present study for the cultivation of the two strains of *Botryococcus braunii*.

SSP- Single Super Phosphate
MP- Muriate of Potash
Rs= INR
1$=69.56 INR.

The preliminary studies on genomics and proteomics on *B. braunii* algae did not yield expected results on the reduction of the cost of media. Mixotrophic studies [36] on interactomics and secretomics showed some encouraging results however the details of these studies are beyond the scope of discussion here.

Scope of integration of studies with green algal and cyanobacterial interaction

In fact, these studies open a wide scope of integrating these with the parallel studies [36] in the laboratory on the synergistic co-cultivation of *B. braunii and cyanobacteria N. muscorum* where the cyanobacteria had fixed 50% more nitrogen for donation to the green algae *B. braunii.* This co-cultivation has not only helped in reducing and avoiding the cost of using nitrogenous and other constituents in the medium. In fact, this had also led to the enhancement in the growth of *B. braunii* while increasing the production of biodiesel producing lipids also. The studies on interactomics, secretomics, metabolomics including quorum sensing and quorum quenching have been reported. Here the successful utilization of nutrients from the blue green algae by green algae can help in economizing the media where the use of animal or human urine may also help further [36]. Of course, a due care may be required to avoid the possibility of any contaminations. *B. braunii* associated bacteria or fungi, especially those which also produce lipids and hydrocarbons may also be grown and studies may be extended to standardize the cultivation conditions by using economical media in future. Therefore, there is still a further scope of economizing the media costs by using photobiological or other algal reactors. These studies in combination with the present studies would also further reduce the cost of the nutrient media used for the growth of *B. braunii.*

Production of biofuels and other value-added products from *B. braunii* in biorefineries

Figure 9 shows the flow scheme of the process of producing biodiesel, bio-jet oil, green diesel, bioethanol, hydrothermal liquids, biogas, bio-CNG etc. along with other value-added chemicals, pharmaceutical, products etc. in future algal biorefineries. The use of microalgae may also be made in the clean power generation using microalgal fuel cells and biodegradable biological solar cells. The use of flue gas derived CO_2 may be made in the extraction of hydrocarbons under subcritical conditions. The use of CO_2 may also be made for the growth of *B. braunii*. The harvested algae may also be used in microbial fuel cells using wastewaters helping the wastewater treatments [6,18,19,25,36].

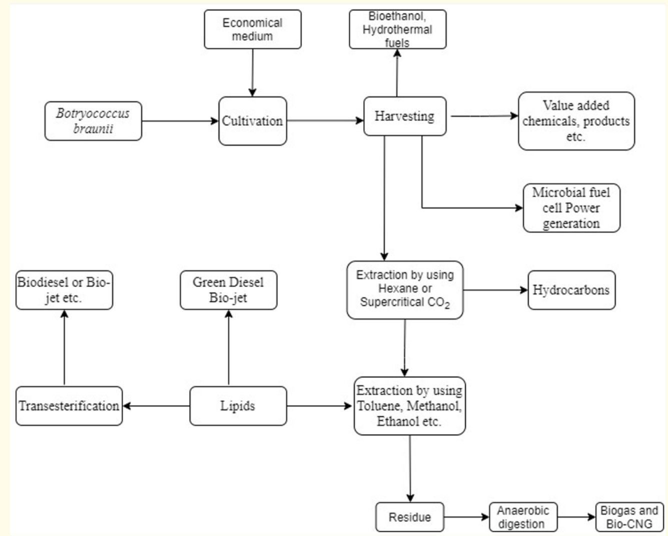

Figure 9: Flow scheme of the production of biofuels and value added chemicals, pharmaceuticals, foods, chemicals, pigments etc. from *Botryococcus braunii* in algal biorefineries using economical medium.

Scope of future studies

There are several bacteria and fungi which can also produce biofuels. This has been reviewed by [45]. There is a possibility of co-metabolizing or co-culturing these microbes with *B. braunii*, besides of course the associated microbes of this algae for the enhanced biofuel production in future.

Growth kinetics of *B. braunii* showed the process of algal biomass production by using optimized cost-effective nutrient media was controlled by material and mass transfer. Here cross interactions of media and CO_2 are important in the presence of rubisco and carbonic anhydrase enzymes. The process engineering studies on the design of scaled up photobiological reactor may be undertaken for developing advanced cost-effective cultivation systems by selecting suitable *B. braunii* strains [46]. In fact in the process engineering of setting up and design of photobiological reactors besides the cell growth and growth kinetics, light, mixing, material transfer, exploiting the carbon concentrating mechanism of CO_2 and carbon flux at different environments [7] are important. Further studies in these directions are also warranted. There is a need to extend further research work on developing the concept of setting up biorefineries and establishing bioeconomy and formulate the policies and planning accordibgly [47-51] to reduce the CO_2 concentration in the atmosphere and sageguard the climate change.

Conclusions

B. braunii has the potential to produce lipids and hydrocarbons having branched carbon chain (BCC). The biodiesel containing BCC esters and hydrocarbons would have better cold flow properties and oxidative stability. Thus, biodiesel produced from *B. braunii* would have better fuel properties.

The higher cost of nutrient medium used for the cultivation of *B. braunii* is the major obstacle in the commercial production of biomass. Thus, in the present studies an economic nutrient medium has been designed for the cultivation of *B. braunii* biomass for the studies on the possible commercial scale production of biodiesel and bioenergy later.

Interestingly, both the *B. braunii* algal strains grown by using the presently designed modified nutrient medium were found to give good yield of biodiesel. Therefore, the presently designed and modified medium may be used for growing *B. braunii* for the production of biodiesel. There is also a scope of further improving the growth of *B. braunii* by using organic wastes such as animal urine, cow dung slurry, poultry chicken waste, food and other industrial wastes etc. along with this designed medium. This would also further reduce the cost of biodiesel production from algae in future algal biorefineries. This may lead to the establishment of bioeconomy in future. There is also a wide scope of integrating the synergistic use of cyanobacteria such as *N. muscorum* with the *B. braunii* for eliminating the cost of nitrogenous constituents in the media. This would also lead to the enhancement in the biomass growth while at the same time this would also result in an increase in the production of biodiesel producing lipids. The use of animal i.e. pig/swine or human urine would also provide nutrients along with waste utilization as has also been advocated by Gautam *et al.* and present authors earlier. There is still a wide scope of further studies in this direction as algal biorefineries would solve not only the problem of food versus fuel controversies but also a wide range of different useful chemicals, nutrients, vitamins, drugs etc. can be produced from algae to increase the interest in their establishing these commercially in future. Present studies open the avenues and directions for further studies on the scale up of algal bioreactors and the possibility of further developing and using economical media which may lead to economizing the process of algal cultivation in commercial reactors. These conditions may be employed in future studies for setting up algal biorefineries and for establishing bioeconomy.

Conflict of Interest

The authors declare that they have no conflict of interest.

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