NUTRIENT INFLUENCE AND EXTRACTION OPTIMIZATION IN MICROALGAE FOR ENHANCED BIOFUEL AND BIOMATERIAL PRODUCTION

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ABSTRACT

This study explores the effects of the optimization of nutrients specifically nitrate and phosphate on the aspects of growth and lipid productivity on microalgae with an aim of supporting development of biofuels through sustainable production. Different cultures of algae were grown with differing nutrient conditions and their lipid content was determined based on the sulfo-phospho-vanillin (SPV) method. Three well-known methods of lipid extraction, i.e., Folch extraction, Bligh and Dyer extraction, and ultra-sonic assisted extraction (UAE), were compared in terms of their critical element of efficacy. Among them, UAE resulted as the best with a lipid recovery of 49.8%.The lipids were then transformed into biodiesel by means of acid-catalyzed transesterification. GC-MS analysis of the product revealed that biodiesel was produced successfully and contains such fatty cids methyl esters as tridecanoic, pentadecanoic, linolenic acid (C18:3), and eicosadienoic acid (C20:2). These findings demonstrate why microalgal lipids would make ideal biodiesel feedstock.Carbohydrates were also extracted using acid hydrolysis, although in a more pH-sensitive manner than lipids. Analysis revealed that carbohydrates formed about 63 % of the dry biomass. This large portion of carbohydrates could be used in the potential production of a bioethanol fermentation or other biopolymers, which would only increase the profitability of the overall process. Through intelligent nutrient-supplying solutions and efficient extraction techniques, biodiesel can be produced and at the same time value added co-products such as bioethanol and biomaterial can be generated. This integrated system not only ensures maximum utilization of resources, but also is in line with the concept of a circular bioeconomy, providing a sustainable solution to the global energy demands through environmental protection.

Keywords: *Microalgae, Biodiesel, Lipid Extraction, Carbohydrate, Biofuel*

1. INTRODUCTION

The growing global energy consumption, the issues of climate change, the emission of greenhouse gases, and the problem of energy security on a long-term basis have made the search of renewable and sustainable alternatives to the fossil fuel more acute. In this regard, biomass-based biofuels have attracted a lot of interest as energy sources which are carbon-neutral and can be included in existing energy infrastructure with only minor changes. Biofuels are in various forms, which are solid biochar, liquid bioethanol and biodiesel, gaseous biogas and biohydrogen and they have been widely explored as alternative fuel to petroleum products (Oh et al., 2018; *Demirbas, 2009)*. Among them, biodiesel has been extensively researched on non-edible feedstocks which include jatropha, karanja, mahua and polanga but high cost of raw materials and processing may render biodiesel almost twice as expensive as conventional diesel, thus the issue of biodiesel large scale commercial competitiveness is hampered (Bellou et al., 2014; Demirbas, 2008). A more common classification of biofuel research by feedstock type is the generation of biofuels with food crops like corn as a primary feedstock, with soybean, wheat, and sugarcane as alternative feedstocks, by which these foods have fuel potential, which in turn made food-versus-fuel a debatable and even ethical and economic issue, depending on the land under cultivation sometimes in the name of food production and biofuel generation. To overcome these issues, second-generation biofuels made of non-food lignocellulosic biomass, such as agricultural residues and specifically energy crops, such as switchgrass and jatropha, were invented(Zhu, 2015). Even though these feedstocks do not directly compete with food production, their productivity is highly contingent on the context of the environment like climate variability, soil fertility, water and nutrient balance casting doubts on their sustainability and reliability in the large scale(Carroll & Somerville, 2009; Lee et al., 2019). These restrictions have further forced the research focus into 3rd-generation biofuels and micro algae are becoming one of the most promising platforms in terms of sustainable bioenergy generation(Misra et al., 2016). The growth of microalgae can be done in marginal areas, salty soils or wastewater systems, thus, not competing with arable land and freshwater resources(Chisti, 2008; Thanigaivel et al., 2022)[9][10], and they have high growth rates, are highly photosynthetically active, and have the potential to accumulate large amounts of lipid content, making them desirable biodiesel feed stocks (Chokshi et al., 2015). Moreover, their high taxonomic diversification allows the use of strains with preferred characteristics, including fast biomass growth rate, high lipid productivity, environmental pressure resistance, and lipid accumulation is increased by controlling factors including nutrient limitation, light regulation, and temperature. Besides the production of biofuels, microalgae also have a large potential in the integrated biorefinery processing, through which some valuable co-products, including proteins and pigments, can be extracted to enhance the overall economic viability(Brennan & Owende, 2010; Chisti, 2008; Chokshi et al., 2015). In spite of these benefits, commercialization is still hindered by major technical and economic obstacles such as high cost of cultivation, energy-intensive harvesting and dewatering procedure, and problems of extracting lipids in free-standing microalgal cell walls; although some methods like solvent extraction, ultrasonic, electroporation, and supercritical CO_2 extraction are promising, they are still expensive, energy-demanding (Arumugam et al., 2011; Ranjith Kumar et al., 2015)*;(*Carroll & Somerville, 2009)[6]. In turn, modern studies are starting to pay more attention to the optimization of cultivation and nutrient use plans, the increase in the extraction rate, and the creation of combined systems that combine the production of algal biofuels and the capture of carbon, as well as water purification to enhance scalability and sustainability.

2. METHODOLOGY

2.1 Microalgal Sample

Samples of freshwater were taken in two ecologically different places, which were then cultured (100 mL) independently in the BG-11 medium in photosynthetic glass tubes (1 L) and their growth conditions were kept in the laboratory under regulated environmental parameters. An optimised regime was created based on aeration, temperature, and illumination that would encourage maximum growth. The algal consortia that ensued were called the Green Consortium and the Brown Consortium and were morphologically characterised through light microscopy as per standard taxonomic keys. As shown in Fig.1(a), Brown Consortium included Euglena sanguina, Navicula spp., and Haematococcus sp., and Green Consortium included Chlorella spp., Scenedesmus spp., Chlorecoccum spp., Oocystis sp., Selenastrum sp., Chlamydomonas sp. and Microcystis spp.

2.2 Media Composition & Experimental Setup

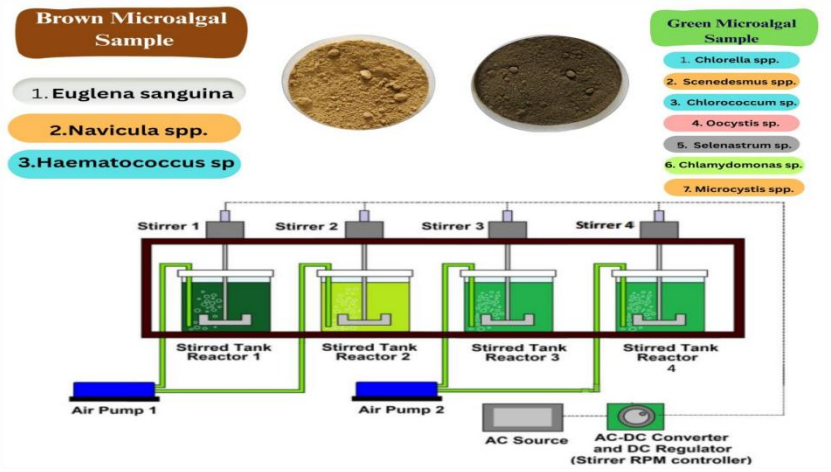

Fig.1 (a) Species of green & Brown microalgal sample. (b) Experimental setup

In the given current experimental study, the Bg-11 medium has been used as its nutrient source. In case of 1000- ml preparation, the solution includes $NaNO_3$ (1.5g), K_2HPO_4 (0.03g), $MgSO_4$.$7H_2O$ (0.075 g), $CaCl_2.2H_2O$ (0.036 g), citric acid (0.006 g), ferric ammonium citrate (0.006g), Na_2CO_3 (0.02 g), and EDTA (0.001 g). The four stirred tank reactors are used per batch and are run over a period of 15-20 days. Regulated nitrogen sources are added and aeration is maintained by use of an air pump along with mixing by use of stirrers. The stirring regulator is controlled via the AC-DC converter and a DC regulator, thus creating the optimal conditions of culture to stimulate algal growth as shown in Fig.1(b).

2.3 Biomass Quantification

An identified amount of sample is filtered using pre-weighted filter paper followed by total evaporation of moisture content in an oven at optimum temperatures (60° C) at 30 minutes and allowed to absorb moisture overnight. The dry biomass weight is obtained by deducting the weight of clean filter paper with the weight of the paper with adsorbed biomass. The resultant sample is itself diluted in waves of 5/4, 5/3, 5/2 and 5 and respective dry weights are estimated by dividing measured weight/dilution factor. Concentration of biomass is calculated as the ratio of the dry weight to the volume of the culture media taken out.The construction of a calibration curve is carried out on the basis of absorbance at 680 nm.

2.4 NO_3^- Measurement Procedure

The determination of $NaNO_3$ concentrations of algal cultures was made in standard solutions (300-1200 mg/L) in BG-11 medium supplemented with 1.5 g/L $NaNO_3$ and a $NaNO_3$-blank. 0.8 mL of 5% salicylic acid in concentrated H_2SO_4 is added to each 10 mL sample and incubated for 20 minutes, then neutralized with 19 mL 2N NaOH, after which the absorbance at 410 nm could be measured; concentrations were calculated using a standard calibration curve.

2.5 PO_4^{3-} Measurement Procedure

The standard phosphate solutions were prepared (2-12 ppm) by dilution of the stock solution in phosphate-free medium. The combined reagent was made fresh and added 2 mL to each standard containing 10 mL of 2.5M H_2SO_4, 3 mL of ammonium molybdate, 1 mL antimony tartrate and 6 mL of ascorbic acid. Following the development of color that took 30 min, the absorbance was recorded at 710 nm and a calibration curve constructed. Similar treatment happened to algal samples and the value of phosphate was determined based on slope of the calibration curve.

2.6 Lipid Quantification

As shown in fig.2(a),The algae were dried up in the sun followed by weighing of the biomass. Afterwards a unit of dried cell/mL of sulfuric acid was added and this sample was heated further to 100° C and later cooled. To give a working solution, stock acid solution (1 mg/mL) was diluted to 0.05-0.1 mg/mL. In its turn, 1.8 mL of the solution was heated to 90° C and left there in a period of 10 minutes after which it was cooled then added to 5 mL of a vanillin-phosphoric acid reagent. Absorbance was read at 530 nm after 45 min of standing in the dark(Mishra et al., 2014).

2.7 Lipid Extraction by Different Method

As shown in fig.2(b), A 0.1g of dried microalgae biomass is blended to a fine powder and chloroform and methanol are added with 2:1 v/v ratio (Folch method: 2:1, Bligh & Dyer method: 1:2). The sonication of the solution is done in the process of ultrasound-assisted extraction and vortexes to mix the solutions of Folch and Bligh and Dyer method, which takes 10 minutes. Subsequently, 0.9% NaCl is added to a final volume of 9 mL to make it separate into phases. The sample is next centrifuged 3000 rpm in 10 minutes and the lower chloroform layer taken with the help of a pipette, which has the lipids in it. The extracted lipids are obtained by letting the sample stand at room temperature without any disturbance and the chloroform evaporates naturally within a day.

2.8 Carbohydrate Extraction

As shown in fig. 2(d), A 5g weight of dry microalgae sample biomass was decarbonated by using 1 mL 3% sulfuric acid. The mixture was autoclaved in 120°C and 1.2 bar 30 min and subsequently this solution was stirred with 4 mL of distilled water. Supernatant was collected and the entire volume was diluted by 1:1000 subsequent to centrifugation. The quantity of sugar is obtained by the modified Anthrone where 2mL of the diluted supernatant was pipetted into 50 µL of 80% phenol and 5mL of 95% sulfuric acid. The sample was stored at room temperature and the reading was taken at 490 nm as a result.(Silva et al., 2023)

2.9 Transesterification of Lipid

As shown in fig.2(c), The extract was transesterified with some amount of sulfuric acid (H_2SO_4) mixed with methanol and heated at a temperature of 60° C within the range of 1- 2 h. The resultant reaction was cooled to ambient temperatures and introduced to hexane to bring about phase separation The crude biodiesel-rich hexane layer was spun down and the layer collected subsequently shipped to GC-MS to continue analysis(Tran et al., 2013).

2.10 Kinetic Parameters

Calculation of the specific growth rate is important to understand the growth condition and its interaction with the environment of the microalgae. The calculation of biomass productivity in the microalgae is estimated by the dry biomass produced per unit volume of the culture per unit of time.

Whereas, lipid productivity is the product of the microalgal biomass productivity rate and the lipid content. Microalgae of higher lipid productivities are desired for biofuel production (Chisti et al. 2007). The Specific growth rate, Biomass productivity are determined by using the following equations:

Specific Growth Rate = $(ln(X/X_0))/\Delta t$ (1)

Biomass Productivity = $(X-X_0)/\Delta t$ (2)

Where X = concentration of biomass at the end of the batch run (g/L), X_0 = concentration of biomass at the beginning of the batch run (g/L), t = duration of the batch run

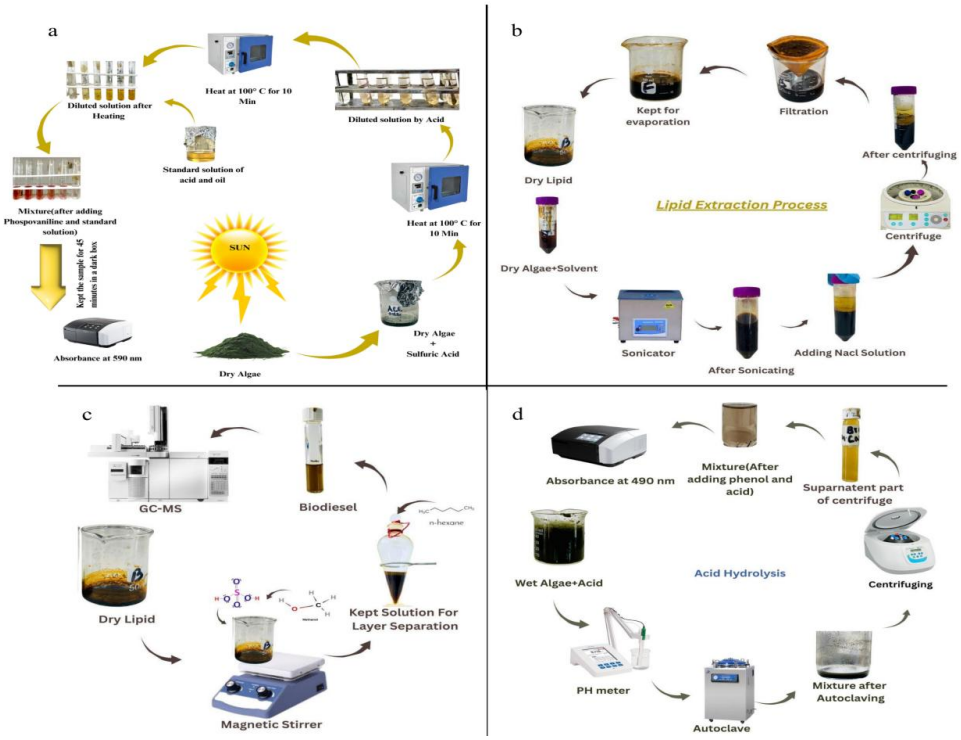

Fig.2 (a) Lipid Quantification by SPV Method (b) Lipid Extraction by Ultrasound assisted (c)Transesterification of Lipid to Biodiesel. (d) Carbohydrate Extracion by Acid Hydrolysis.

3. RESULTS

3.1 Growth & Biomass Productivity

The effects of nitrogen supply on microalgal growth were determined through experiments in which 4 sets of nitrate concentrations (0.6, 0.8, 1.0, and 1.2 g/L) were used in parallel, regulated photobioreactors. These findings denoted that the supply of nitrogen has a high impact on the algal physiology and metabolism, as the growth patterns and biomass yields had distinct differences.

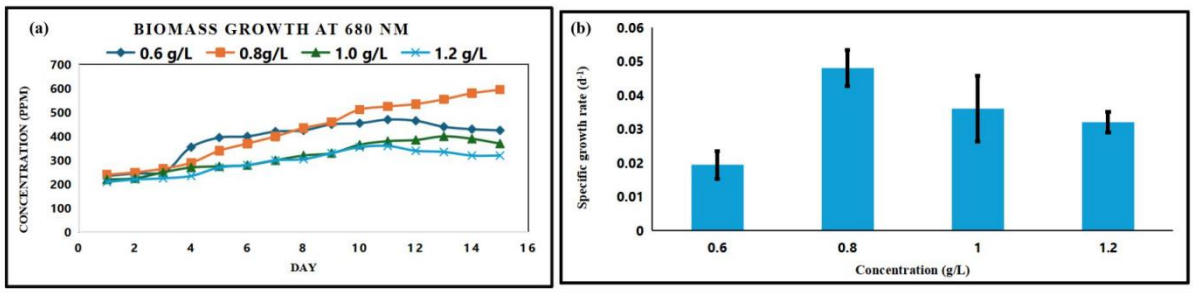

Fig.3 (a) Biomass growth profile at 680 nm over 15 days (b) Specific growth rate at different concentrations (g/L)

Kinetic studies and biomass growth dynamics have shown that the concentration of nitrate (NO_3^-) is a determinant in controlling microalgal growth. Of the tested treatments, the concentration of 0.8 gL^{-1} of sodium nitrate yielded the largest terminal concentration of biomass (~600 PPM) after 15 days as shown in fig.3(a).This regime also recorded the most specific growth rate (0.048±0.007 day^{-1}) and biomass productivity (0.04000 $gL^{-1}day^{-1}$) implying that it provided optimal nitrogen supply that enhanced balanced metabolic activities, continued cell division and higher photosynthetic efficiency. The 0.6 gL^{-1}dose exhibited significantly high early growth and peaked at approximately day 6 and then entered into a plateau phase which is due to the depletion of nitrogen. The specific growth rate was similar to the one observed at 0.8 gL^{-1}, but the biomass productivity (0.03373 g L^{-1} day^{-1}), indicated continued nutrient deficiency during prolonged growth. The high concentration levels of sodium nitrate(1.0 gL-1and 1.2 gL-1) showed inhibition of growth. Modest biomass (~400 PPM) was achieved at 1.0 gL^{-1}, with slower productivity (0.02613 $gL^{-1}day^{-1}$); at 1.2 gL^{-1}, the lowest yield of ~300 PPM and lowest productivity of 0.01600 $gL^{-1}day^{-1}$ was achieved which may be attributed to nitrogen oversaturation stress or osmotic imbalance. Overall, the findings indicate that the curve is bell-shaped, and the concentration of 0.8 gL^{-1} NO_3^- was determined as the most suitable in terms of microalgal growth and biomass productivity when the current set of the conditions is considered.

Table 1: Growth rate & Biomass productivity rate at different NO_3^- concentrations.

Concentration (g/L)(NO_3^-)	**Growth Rate (Day^{-1})**	**Biomass Productivity (g/l/day)**
0.6	0.016±0.009	0.03373
0.8	0.048±0.007	0.04000
1.0	0.037±0.017	0.02613
1.2	0.0311±0.02	0.01600

3.2 Nutrients Effect on Algal Growth

Fig.5(a,b) shows variations of NO_3^- and PO_4^{3-} concentrations over a period of 15 days of cultivation. It is worth noting that nitrate showed a steady decline in all treatments indicating active nutrient acquisition by the algae. The highest rate of reduction was found in the 0.8 gL-1 treatment that is consistent with its better growth performance. In comparison, phosphate was comparatively constant in all the treatments during the initial phases. A significant decrease in PO_4^{3-} was noted at around day 7, which is when the algae were at their maximum growth and metabolic activity and provides evidence of increased phosphate requirements in vital activities like ATP production and nucleic acid synthesis. Despite partial recovery of PO_4^{3-} levels after this phase, the recovery can be attributed to recycling of nutrients due to cell lysis or decreased uptake as the growth rates slowed. All these findings point to the fact that the consumption of nutrients in the 0.8 gL-1 NO_3^- treatment was the most efficient, thus facilitating the best biomass production.

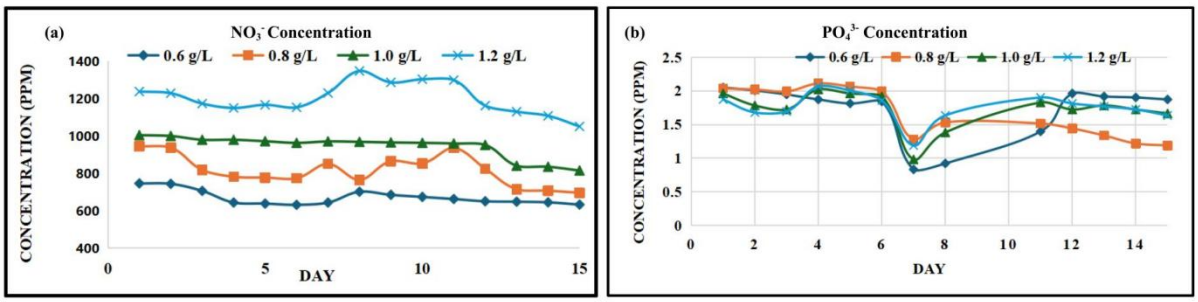

Fig.5 (a)Evolution of NO_3^- Concentration Over Time. (b) Evolution of PO_4^{3-} Concentration Over Time.

3.3 Lipid Extraction by different methods

This current paper critically investigated the lipid extraction efficacies with three approaches namely; Ultrasound-Assisted Extraction, Bligh & Dyer method, and Folch method, which acted on the microalgal biomass samples of green and brown consortia. The relative analysis of the lipid extraction showed that there were differences in the recovery efficiency of the methods studied. UAE yielded the best (49.85±0.27) in the green algal consortium, followed by Folch (49.15±0.27) and Bligh and Dyer (48.66±0.21). UAE also performed better in the brown algal consortium where solvent penetration is limited by the presence of bulk polysaccharide cell walls with a UAE of 37.95 ± 0.25% against Folch (37.24 ± 0.49%) and Bligh & Dyer (33.68 ± 0.24%). These data support the similarity of the order of efficiency: UAE > Folch > Bligh and Dyer, and UAE performed better in both green and brown algal communities.

Similar extraction patterns have been observed with Chlorella vulgaris, in which ultrasound-assisted Bligh and Dyer extraction has obtained lipid recoveries as high as 52.5 per cent (w/w), which is far higher than solvent-only extraction methods, including Folch (19.69 ± 0.67 per cent) and Bligh and Dyer (15.46 ± 0.24 per cent) (Araujo et al., 2013; Munir, 2016). The amplified performance of UAE can be explained by the fact that cavitation by ultrasound stimulates effective disruptions of the cells, as well as enables better solvent penetration into the intracellular lipids. Taken together, these results suggest that UAE offers better recovery of lipids, better reproducibility, and better performance with a variety of algal taxa with important implications of biofuel and nutraceutical industry.

Table 2: Percentage of Lipid Extraction by different methods

Algae	**Extraction Method**	**Lipid Content (%)**	**Efficiency (%)**
Brown	Folch	23.28	37.24±0.49
Brown	Bligh & Dyer		33.68±0.24
Brown	Ultrasound Assisted		37.95±0.25
Green	Folch	26.85	49.15±0.27
Green	Bligh & Dyer		48.66±0.21
Green	Ultrasound Assisted		49.85±0.27

Within the brown consortium, the extraction of lipids using the Ultrasound-Assisted Extraction method was 37.95±0.25 %, using the Folch technique was 37.24±0.49 %, and the Bligh & Dyer technique was 33.68±0.24 %. Such relatively lower efficiencies are likely to be attributed to the existence of complicated polysaccharide-packed cell walls in brown algae namely alginates and fucoidans, which tend to inhibit further mechanical and solvent degradation. However, Ultrasound-assisted extraction reached the highest lipid recoveries in both consortia, suggesting that it may be effective on the large diversity of microalgae species.

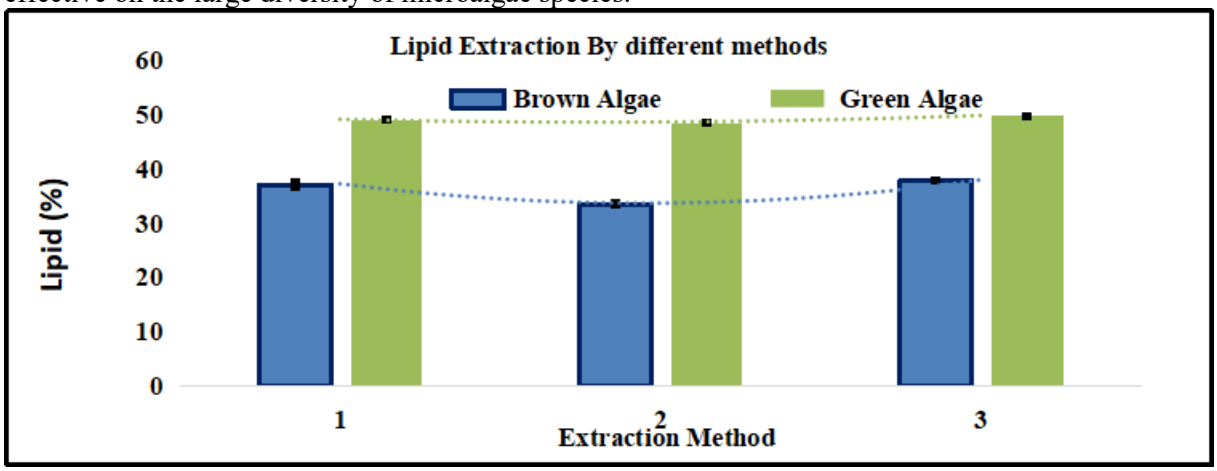

Fig.6 Lipid extraction (%) from brown and green algae using different methods

3.4 Transesterification of Lipid to Biodiesel

3.4.1 Characterization of Green Algae

As shown in fig.7(a) and table 3 ,GC-MS chromatography of green algae biodiesel has revealed the existence of a few of the major fatty-acid methyl ethers (FAMEs) in it, which supports the quality of the fuel in the future. Methyl nonanoate (C9:0), methyl caprate (C10:0), methyl undecanoate (C11:0), methyl laurate (C12:0), methyl tridecanoate (C13:0), methyl pentadecanoate (C15:0), methyl palmitate (C16:0) and methyl oleate (C18:1) were the main ingredients respectively, with branched isomers including iso-methyl laurate and iso-methyl Pentadecanoate.These FAMES are particularly important in terms of biodiesel use, which possess optimal ignition-quality properties as well as desirable oxidative stability and viscosity/cold-flow characteristics.

Table 3: Compound Analysis of Biodiesel Components from Green algae

Retention Time (min)	Compound
9.642	C9:0 (Methyl Nonanoate)
10.798	C10:0 (Methyl Caprate)
11.823	C11:0 (Methyl Undecanoate)
12.767	C12:0 iso (Methyl iso-Laurate)
13.645	C13:0 (Methyl Tridecanoate)
14.474	C15:0 (Methyl Pentadecanoate)
15.243	iso-C15:0 (Methyl iso-Pentadecanoate)
15.993	C16:0 (Methyl Palmitate)
17.2	C18:1 (Methyl Oleate)
18.484	C18:1-oxo (Methyl 10-oxo-octadecenoate)

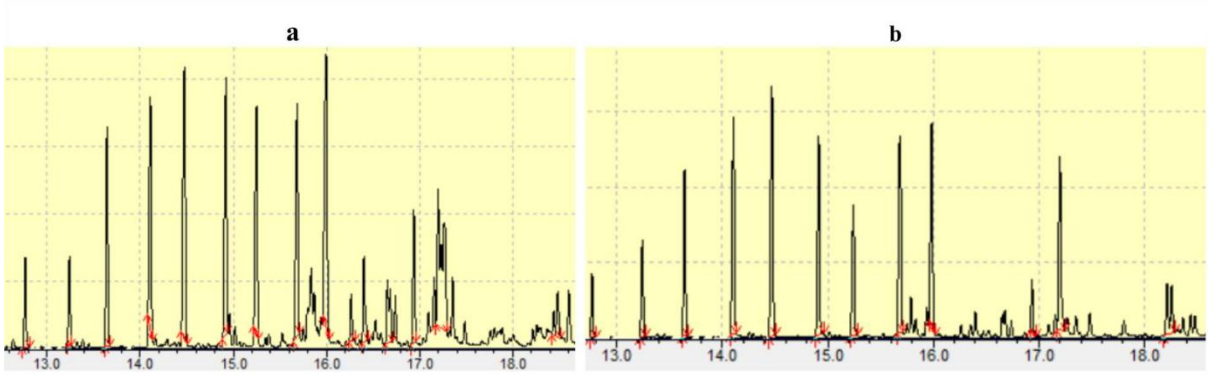

Fig.7 (a).GC-MS chromatogram of biodiesel from green microalgae.(b) GC-MS chromatogram of biodiesel from brown microalgae.

The analysis and a subsequent comparison to the GC-MS profile of Chromulina freiburgensis also supports the reveality of green algae biodiesel feedstock discussed in the present paper. The two datasets revealed a strong enrichment of mid-chain and long chain saturated and monosaturated FAME such as C11:0, C12:0, C13:0, C15:0, C16:0 and C18:1(Mondloch, n.d.) .
Palmitic acid is a saturated fatty acid that is known to have high oxidative stability and high cetane numbers- a feature that can be described as the foundation of good engine performance. These are in agreement with Sharmin et al. (2016), who also identified the presence of palmitic acid (C16:0) as a major fatty acid in the lipid extract of *Skeletonema costatum*.(Sharmin et al., 2016).It is especially interesting to find the presence of methyl oleate (C18:1) which is widely tested in terms of combustion and exhaust characteristics in engines that run cars, and the list of characteristics that it brings include high cetane numbers, that is, the ability to burn efficiently(Jiaqiang et al., 2016).

3.4.2 Characterization of Brown Algae

As shown in fig.7(b) and table 4. Biodiesel produced by brown algae using GC-MS demonstrated a profile that was very similar to the regular profile of FAME (produced by Chromulina freiburgensis). One of the compounds that were found in both biodiesel samples is the Methyl Tridecanoate (C13:0) which is a medium chain methyl ester fatty acid, however, it is very important in increasing the ignition quality of the biodiesel as well as the oxidative stability of the biodiesel. The presence of this compound argues its importance in brown algae in addition to Chromulina biodiesel, which is important in biofuel production. Also, Dichloroacetic Acid, Tridec-2-ynyl Ester (C15:0) and Valeric Acid, Undec-2-enyl Ester (C16:0) was observed on both samples and this proves further that both biodiesel compositions are similar. The compounds have positive contribution to properties of biodiesel, including fuel stability and efficiency in combustion. Additionally, other such compounds as 9,12,15-Octadecatrienoic Acid, Methyl Ester (C18:3) and 5,8,11,14-Eicosatetraenoic Acid, Methyl Ester (C20:4) were identified, which increase the oxidative stability and low-temperature flow of biodiesel(Mondloch, n.d.).

Such a comparison of the brown algae biodiesel to the reference biodiesel profile highlights the potential of brown algae as a renewable source of biodiesel production. The high abundance of major FAMEs in both types of biodiesel indicates that brown algae may be a possible and dependable alternative to generate biofuels of high quality and this makes it viable to use brown algae in producing biodiesel on large scale.

Table 4: Compound Analysis of Biodiesel Components from Brown algae

Retention Time (min)	Compound
13.240	C13:0 (Methyl Tridecanoate)
15.680	C15:0 (Dichloroacetic acid, tridec-2-ynyl ester)
16.937	C16:0 (Valeric acid, undec-2-enyl ester)
18.220	C18:3 (9,12,15-Octadecatrienoic acid, methyl ester (2.2.2))
18.437	C20:4 (5,8,11,14-Eicosatetraenoic acid, methyl ester (all-Z))
18.437	C20:4 (cis-11,14-Eicosadienoic acid, methyl ester)

3.5 Carbohydrate extraction by Acid Hydrolysis

The extraction of carbohydrates was followed using acid hydrolysis by utilising two different groups of microalgae, the green algae (Chlorella, Scenedesmus, and Chlamydomonas) and the brown algae (Euglena sanguina, Navicula spp., and Haematococcus sp.).To examine the extraction of carbohydrates of green and brown microalgae, 1 mL of 3 % sulfuric acid was used at each pH (3.15 in the case of the green sample and 2.15 with the brown). The green microalgae sample showed that 51.00±0.1804 % of the extractable fraction is composed of carbohydrates, whereas under the same conditions of exposure to acid, the brown microalgae sample had 45.10±0.200 % carbohydrates as extractable fraction as shown table 5.

Table 5: Carbohydrate content from Brown and Green Microalgae.

pH	Algae	Carbohydrate Content(%)	sulfuric acid(3 %) (ml)
3.15	Green	51.00±0.1804	1 ml
2.15	Brown	45.10±0.200	

These findings suggest that the increased carbohydrate content evident in the extract of the green microalgal cell indicates variations in the architecture and biochemical forms of the cells walls. The cell walls of green microalgae are relatively easily digested and acid-labile and accelerate the soluble sugar release by hydrolysis. Brown microalgae on the other hand are likely to produce more complex or recalcitrant cells and as such tend to have higher lipid or pigment concentrations and cells may dilute carbohydrate recovery in acid by sulfuric acid. Therefore, the green microalgae showed better recovery of carbohydrates when compared with similar treatment in acidic condition, which explains their viability and potential use as low-concentration of sulfuric acid hydrolysis bio-based resources in the production of sugars.

3.6 pH Influence on Carbohydrate Content

This current study has shown beyond reasonable doubt that the extraction of carbohydrates in brown microalgae is highly controlled by pH with the highest recovery recorded in highly acidic environments. The highest recovery in the experimental range was 45.10 ± 0.200% at pH 2.15, as in Table 6 and Fig. 8, and this was the optimum yield. The yield of carbohydrates increased gradually as the pH increased and reached 32.28 ± 0.2532% at pH 5.

Table 6: Carbohydrate percentage extracted from Brown Microalgae at different PH.

pH	Carbohydrate (Green) (%)	Carbohydrate (Brown)(%)
2.15	62.94±2.1385	45.10±0.200
3	55.86±1.51	32.57±0.197
3.5	51.00±0.1804	36.70±0.1301
4	43.19±0.3055	33.46±0.2157
4.5	38.77±0.2307	34.05±0.2715
5	34.93±0.2043	32.28±0.2532

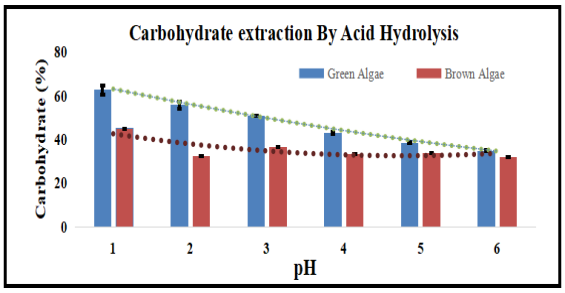

Fig. 8 Carbohydrate extraction (%) from algal biomass by acid hydrolysis at different pH

These repetitive negative patterns confirm the primary part of acid hydrolysis in destabilising the recalcitrant algal cell wall matrix and in increasing the release of intracellular polysaccharide. These findings are not new as the current body of literature suggests that acidic conditions favour the release of carbohydrates as they break down structurally recalcitrant biopolymers such as cellulose and hemicellulose.Studies found that *Chlorella vulgaris* yields approximately 27% carbohydrate content under the same acidic conditions (Vahabisani et al., 2015).Significantly, The current study confirms the presence of approximately 45.10±0.20 % carbohydrate in brown microalgae, significantly higher than in C. vulgaris, thus indicating the higher susceptibility of brown algal cell walls to disruptions under acidic conditions.Such increased susceptibility can be plausibly explained by natural structural variations in cell wall architecture in algal lineages. Brown microalgae include cell walls rich in alginates, sulfated polysaccharides, and cellulose fibrils, which are relatively less resistant to acid hydrolysis than the hard, stiff cell walls of green microalgae. These compositional differences warrant a mechanistic explanation to the higher yields obtained and resoundingly place brown microalgae in the limelight as viable options in a carbohydrate recovery strategy using acid hydrolysis. Whereas the literature has generally focused on the possible disadvantages of extreme acidic pretreatment, such as degradation of sugars and corrosion of equipment, the current research has shown that acid hydrolysis using pH 2.15 could lead to considerable carbohydrate release without the need to incur high losses in the form of corrosion. Therefore, the findings are not only in line with previously established results but also expand on them by showing that brown microalgae obtained close to twice the carbohydrate output of green microalgae under similar acidic conditions. This article therefore gives strong support of the bi-directional use of brown microalgae in biomass valorization models based on acidic hydrolysis.

4. CONCLUSION

The current paper propels the microalgal biofuel research by incorporating the nutrient optimization, extraction improvement and downstream valorization in a single experimental platform. In particular, the influence of nitrate concentration on the kinetics of microalgae growth and biomass productivity in the controlled conditions of photobioreactors was studied with four discrete concentrations of nitrate (0.6, 0.8, 1.0 and 1.2 gL^{-1}). A steady state level of 0.8 gL^{-1} was determined, which produced the best specific growth rate of 0.048 day -1 and a productivity of 0.040 g L^{-1} day^{-1} of biomass, which showed the significance of optimum nitrogen content to ensure continuous metabolism and biomass growth. In addition to the optimization of growth, the lipid recovery was compared between the traditional solvent extraction method and ultrasound-assisted extraction (UAE) and UAE persistently

yielded more lipids implying a greater cell disruption and better accessibility to solvents. It was possible to convert the extracted lipids into biodiesel through base-catalyzed transesterification, resulting in fuel which met acceptable standards of renewable energy quality, indicating the technical feasibility of the transformation route. Moreover, the remaining defatted biomass was then acid hydrolysised with the highest level of fermentable sugar liberation being attained at pH 2.15, and thus its potential use as a bioethanol source was facilitated and the concept of a cascading biorefinery further justified. The study provides engineering insight although the results are known biological patterns, as the optimisation of nutrients is associated with both efficiency of extraction and recovery of multi-products in a single process chain. However, scaled-up photobioreactor-related challenges such as attenuation of light, mass transfer, inefficiency in nutrient delivery, and the intensity of energy in the UAE are still a serious impediment. The solution to these limitations lies in the optimization of reactor design and integration of renewable energy to enable the laboratory level of performance to be converted into the commercially viable microalgal biofuel systems.

5. ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from SUST research center (Project ID:AS/2024/1/08), Shahjalal University of Science and Technology, Sylhet, Bangladesh.

6. REFERENCES

Araujo, G. S., Matos, L. J., Fernandes, J. O., Cartaxo, S. J., Gonçalves, L. R., Fernandes, F. A., & Farias, W. R. (2013). Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method. *Ultrasonics Sonochemistry*, *20*(1), 95–98.

Arumugam, M., Agarwal, A., Arya, M., & Ahmed, Z. (2011). Influence of organic waste and inorganic nitrogen source on biomass productivity of Scenedesmus and Chlorococcum sp. *Journal Homepage: Www. IJEE. IEEFoundation. Org*, *2*(6), 1125–1132.

Bellou, S., Baeshen, M. N., Elazzazy, A. M., Aggeli, D., Sayegh, F., & Aggelis, G. (2014). Microalgal lipids biochemistry and biotechnological perspectives. *Biotechnology Advances*, *32*(8), 1476–1493.

Brennan, L., & Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, *14*(2), 557–577.

Carroll, A., & Somerville, C. (2009). Cellulosic biofuels. *Annual Review of Plant Biology*, *60*(1), 165–182.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, *26*(3), 126–131.

Chokshi, K., Pancha, I., Trivedi, K., George, B., Maurya, R., Ghosh, A., & Mishra, S. (2015). Biofuel potential of the newly isolated microalgae Acutodesmus dimorphus under temperature induced oxidative stress conditions. *Bioresource Technology*, *180*, 162–171.

Demirbas, A. (2008). Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Conversion and Management*, *49*(8), 2106–2116.

Demirbas, A. (2009). Political, economic and environmental impacts of biofuels: A review. *Applied Energy*, *86*, S108–S117.

Jiaqiang, E., Liu, T., Yang, W., Li, J., Gong, J., & Deng, Y. (2016). Effects of fatty acid methyl esters proportion on combustion and emission characteristics of a biodiesel fueled diesel engine. *Energy Conversion and Management*, *117*, 410–419.

Lee, S. Y., Sankaran, R., Chew, K. W., Tan, C. H., Krishnamoorthy, R., Chu, D.-T., & Show, P.-L. (2019). Waste to bioenergy: A review on the recent conversion technologies. *Bmc Energy*, *1*(1), 1–22.

Mishra, S. K., Suh, W. I., Farooq, W., Moon, M., Shrivastav, A., Park, M. S., & Yang, J.-W. (2014). Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric

method. *Bioresource Technology*, *155*, 330–333. https://doi.org/10.1016/j.biortech.2013.12.077

Misra, N., Panda, P., Parida, B., & Mishra, B. (2016). Way forward to achieve sustainable and cost-effective biofuel production from microalgae: A review. *International Journal of Environmental Science and Technology*, *13*, 2735–2756.

Mondloch, A. (n.d.). *ANALYSIS OF LIPIDS FROM CHROMULINA FREIBURGENSIS FOR THEIR POTENTIAL USE AS BIOFUELS*.

Munir, N. (2016). Screening of lipid contents of microalgae by optimization of oil extraction processes. *Pakistan Journal of Science*, *68*(1).

Oh, Y.-K., Hwang, K.-R., Kim, C., Kim, J. R., & Lee, J.-S. (2018). Recent developments and key barriers to advanced biofuels: A short review. *Bioresource Technology*, *257*, 320–333.

Ranjith Kumar, R., Hanumantha Rao, P., & Arumugam, M. (2015). Lipid extraction methods from microalgae: A comprehensive review. *Frontiers in Energy Research*, *2*, 61.

Sharmin, T., Monirul Hasan, C. Md., Aftabuddin, S., Rahman, Md. A., & Khan, M. (2016). Growth, Fatty Acid, and Lipid Composition of Marine Microalgae *Skeletonema costatum* Available in Bangladesh Coast: Consideration as Biodiesel Feedstock. *Journal of Marine Biology*, *2016*, 1–8. https://doi.org/10.1155/2016/6832847

Silva, G. D. N., Cerqueira, K. S., Silva, K. M., Rodrigues, J. R. D. S., & Souza, R. R. D. (2023). Extraction of total carbohydrates by solvents from the microalgae Chlorella vulgaris and analysis of the influence of NaNO3 and photoperiods on microalgae cell growth. *Brazilian Journal of Development*, *9*(1), 2473–2487. https://doi.org/10.34117/bjdv9n1-169

Thanigaivel, S., Priya, A., Dutta, K., Rajendran, S., & Vasseghian, Y. (2022). Engineering strategies and opportunities of next generation biofuel from microalgae: A perspective review on the potential bioenergy feedstock. *Fuel*, *312*, 122827.

Tran, H.-L., Ryu, Y.-J., Seong, D. H., Lim, S.-M., & Lee, C.-G. (2013). An effective acid catalyst for biodiesel production from impure raw feedstocks. *Biotechnology and Bioprocess Engineering*, *18*(2), 242–247. https://doi.org/10.1007/s12257-012-0674-1

Vahabisani, A., Tavakoli, O., & Karbassi, A. (2015). The influence of acid Hydrolysis on carbohydrate extraction from biomass of microalgae (C-Vulgaris). *The Influence of Acid Hydrolysis on Carbohydrate Extraction from Biomass of Microalgae (C-Vulgaris), February 2016*, 2–6.

Zhu, L. (2015). Microalgal culture strategies for biofuel production: A review. *Biofuels, Bioproducts and Biorefining*, *9*(6), 801–814.