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A Method to Utilize Waste Nutrient Sources in Aqueous Extracts for Enhancement of Biomass and Lipid Content in Potential Green Algal Species for Biodiesel Production

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Abstract

In the present study, ten waste nutrient sources namely potato peels, banana peels, cow dung, press mud, molasses, bagasse hydrolysate, glycerol, succinate, cheese whey and cyanobacterial spent media extract were used for the growth of five potential green algal species namely *C. reinhardtii, S. obliquus, C. vulgaris, C. minutissima* and *B. braunii*. The nutrient extracts were prepared so as to not interfere with algal biomass harvesting. The effect of waste nutrients was investigated especially on the biomass and lipid accumulation in algae. The lipids so produced were quantified gravimetrically and analyzed using GC-MS and proton NMR. As a result, all the microalgal species showed enhancement in their growth and lipid production with most of the waste nutrients. *C. reinhardtii* showed maximum increase in lipid production with potato peels (89%) and banana peels (90%). However, a maximum of 130% increase in biomass content was observed in *C. vulgaris* with glycerol supplementation. ^{1}H-NMR and GC-MS studies showed that carbon supplementation leads to reduction in the aromaticity of lipids and increase in the C16 and C18 fatty acids fractions, respectively rendering it more suitable for biodiesel production.

Keywords: Proton NMR; Lipid; Biodiesel; Gas chromatography; Waste carbon source

Introduction

The ever increasing world population and anthropogenic energy requirements, has necessitated the need for the scientific community to look out for novel/alternate sources of energy. Alternate fuels especially biodiesel is now known as a promising option to support our ever growing energy needs. Biodiesel was earlier produced from plant oil seeds such as *Jatropha*, Pongamia, *Madhuca*, Sunflower, etc. [1,2], however, microalgae are presently studied widely as the next generation source of biofuels. Microalgae are miniature plants which photosynthesize and utilize atmospheric carbon dioxide and sunlight for their growth and multiplication. Microalgae produce lipids in substantial amounts which may be extracted and used for biodiesel production [3]. Microalgae may be grown mixotrophically/heterotrophically i.e., under the effect of carbon sources to accumulate higher amount of lipids. Certain wastes contain valuable carbohydrates which could act as a good carbon-rich source for the growth of algae especially enhancing lipid contents in the same. The wastes after recovery of carbohydrates may be disposed of either in incinerators or landfills.

Different types of waste carbon sources have been used to enhance biomass growth and lipid accumulation in microalgae. Some readily available chemical compounds such as glucose, acetate and glycerol have shown to expedite growth and lipid accumulation in *Chlorella protothecoides* [4-6] and *Phaeodactylum tricornutum* [7]. Liu et al. [8,9] used various carbon sources to study *Chlorella zofingiensis* as a feedstock for biodiesel production and found glucose as one of the best source. Heterotrophic growth of algae also led to 900% increase in lipid production in case of *Chlorella zofingiensis* with glucose as organic carbon source. Corn starch hydrolysate has been used for enhancement of lipid content by 20% in *C. protothecoides* [10]. Molasses has also been used for the enhancement of biomass productivity in a prokaryotic alga, *Spirulina platensis* [11]. Press mud extract and cane molasses were used to enhance the lipid content in *C. minutissima* [12]. Liang et al. [13] studied the effect of glycerol and glucose on the growth and lipid content of *C. vulgaris* which resulted in high increases in biomass production. *B. braunii* also showed enhancement in the lipid content with different sugars such as glucose, mannose, fructose [14]. Various inexpensive and low cost carbon feed stocks from waste have also been used for algal growth which has been extensively reviewed by Subramanian et al. [3,15]. Some other nutrient sources such as sodiumthiosulphate [16], swine waste water [17] and iron [18] have been used for the enhancement of growth and lipid content in various green algal species such as *Scenedesmus* and *Chlorella*. Sugars have also been converted to oil in *C. vulgaris* by using a photosynthetic fermentation model which resulted in improvement of growth and lipid content [3,19]. Further, Taylor et al. [20] used the ethyl acetate extract of two algal species *Skeletonema marinoi* and *Dunaliella salina* for enhancement of growth of *Nannochloropsis oculata*.

In the present study, ten waste nutrient sources namely Potato Peels (PP), banana peels (BP), cow dung (CD), Press Mud (PM), Molasses (MO), Bagasse Hydrolysate (BH), Glycerol (GL), Succinate (SU), Cheese Whey (CW) and Cyanobacterial spent media extract (CY) have been used for the growth of five green algal species namely *C. reinhardtii, S. obliquus, C. vulgaris, C. minutissima* and *B. braunii*. All the wastes used as nutrient sources for algal growth except CY are produced in large amounts from various industries such as sugar distilleries, biodiesel industry, pharmaceutical industry and food industry. The motivation behind this study was to work out a low cost process to economize the production of biodiesel from microalgae at

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a commercial level. The main contribution of the study was to come up with a method that allows for utilization of the plethora of waste nutrients in the form aqueous extracts. The process is useful as it facilitates easy harvesting of algal biomass. The effect of these waste nutrient sources was studied on chlorophyll, biomass, lipid, protein and carbohydrate contents. The analysis of the extracted lipids was done proton nuclear magnetic Resonance Spectroscopy (^{1}H-NMR). The fatty acid composition of these lipids was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods

Strains and culture media

S. obliquus 276-1 and *C. vulgaris* 211-12 strains were procured from EPSAG culture collection, maintained by the University of Goettingen, Germany. *B. braunii* UTEX LB572 strain was procured from University of Texas, Texas, USA. *Chlorella minutissima* were procured from Blue Green Algae Culture Collection at Indian Agricultural Research Institute (IARI), New Delhi, India. *Chlamydomonas reinhardtii* strain was kindly donated by Dr. Laurent Cournac, FEA, France. The algal cultures except *C. reinhardtii* were maintained at pH 8 in Basal Medium (ES medium) *C. reinhardtii* was maintained in mineral medium at pH 7.2. The cultures were grown under 2.5-2.7 K Lux of light intensity with 16:8 light and dark regimes at 28°C temperature in a bioreactor. 10% inoculum of exponentially growing cells was used to inoculate the media and the cultures were grown with continuous shaking at 80-100 rpm. *S. obliquus* was cultivated for 12 days and *C. reinhardtii, C. vulgaris* and *C. minutissima* was cultivated for 15 days under shaking conditions. *B. braunii* was cultivated for 24-25 days using manual stirring twice a day. All experiments were conducted on three independent replicate flask cultures.

Preparation of nutrient aqueous extracts

Molasses and press mud were obtained from Indian Distiller's Association, New Delhi. Bananas and potatoes were bought from local vendors in Delhi. Succinate (w/v), molasses (v/v) and glycerol (v/v) were added directly at the concentration of 1%. Banana peels (w/v), potato peels (w/v), press mud (w/v) and cow dung (w/v) extracts were prepared in distilled water. Bananas and potatoes were washed with distilled water and the surface was pat dried using a tissue paper. The bananas and potatoes were peeled off. Each of the peels was homogenized with small amount of distilled water using a grinder. The homogenized biomass was passed through whatman's filter paper to obtain the filtrate. The extract was prepared such that the resultant concentration of the extracts was 0.1 g /mL. The extracts were autoclaved and used immediately for growth of algae. 10 mL of each of the extracts prepared was added to 100 mL of the algal nutrient medium. The press mud and cow dung extracts were prepared in a similar manner and autoclaved and stored at 4°C. Cheese whey was used as such in the nutrient medium at the concentration of 1% (v/v). Bagasse hydrolysate was prepared by grinding 20 g of oven dried bagasse and mixing it with 40 mL of 1% sulphuric acid and was allowed to soak overnight. 100 mL of freshly prepared distilled water was added to it and was heated to 121°C for one hour in an autoclave [21]. The solution was passed through Whatman's No. 1 filter paper. The filtrate was neutralized with 1N sodium hydroxide solution. The volume of the solution was made up to 200 mL with autoclaved distilled water and the solution was immediately used for supplementation of culture media for algal growth. Cyanobacterial spent media extract was prepared by adding equal volume of ethyl acetate to an exponentially growing 100 mL culture of *A. variabilis*. The mixture of culture and ethyl acetate was shaken vigorously for 30 sec and allowed to settle in a separating funnel to separate the organic layer. The mixture was transferred to a separating funnel and the ethyl acetate (EtAc) layer was separated. The culture was extracted thrice with fresh ethyl acetate and the EtAc extract was pooled each time. Anhydrous sodium sulphate was used to remove traces of moisture from the EtAc extract. The resultant ethyl acetate was evaporated to 1 mL and added at the concentration of 0.1% in 100 mL of nutrient media for algal growth.

Ultimate/CHNSO analysis of waste nutrients

VarioEL cube CHNSO analyzer sold by Elementar Analysensysteme GmbH was used for the ultimate analysis. Oxygen percentage was calculated by difference.

Preparation of cultures with nutritional supplements/sources

The waste nutrient supplemented cultures were prepared by adding 1% (v/v) of the different extracts prepared as described earlier to the nutrient culture media. The control media contained only nutrient medium without any additional nutrient. All the cultures were inoculated with 10% of exponentially growing cells of respective algal species. The cells were harvested in the mid exponential phase for the estimation of chlorophyll content, carotenoid content, carbohydrate, protein, biomass and lipid contents. All the cultures were cultivated in triplicates and the estimations were carried out on three independent biological replicates and are presented as average of the triplicates ± standard deviation.

Chlorophyll estimation

Chlorophyll content was used as a measure of growth. 2 mL culture pellet was suspended in equal volume of methanol and incubated in a water bath set at 65-70°C for 20-30 minutes [22]. The absorbance was recorded at 650 and 665 nm using a UV-VIS spectrophotometer (Elico).

Carotenoid estimation

Cell pellet from 5 mL cell suspension was resuspended in equal volume of 85% of acetone. The suspension was vortexed and kept at 4°C for 2-3 days. The suspension was centrifuged and the absorbance of the supernatant was recorded at the wavelength of 480 nm. The carotenoids were extracted in the acetone and the absorbance was recorded at 480 nm [23].

Biomass estimation

200 mL of culture was centrifuged in a centrifuge tube and the supernatant was discarded. The pellet was washed once with distilled water and the resultant pellet was lyophilized for 24 h. The dried pellet was weighed immediately and stored in deep freezer (Dairei Europe) at -70°C. The pellet was used for lipid extraction.

Lipid extraction

The algal pellet after the estimation of total biomass was crushed in a mortar and pestle. The biomass was homogenized in hexane for 15 min [26]. The homogenized biomass with the solvent was centrifuged in a falcon tube and the supernatant was collected in glass vials. The biomass was extracted in hexane thrice and all the supernatant was pooled in the glass vial. The hexane extracted biomass was further extracted with C-M (2:1) by homogenizing it in mortar and pestle [27]. The homogenized biomass with solvent was centrifuged and the supernatant was collected in another glass vial. Both the hexane and C-M fractions are filtered using Whatman's filter paper No.1. The

solvent in filtrate is evaporated using rotatory vacuum evaporator in a round bottom flask. The oil is then transferred to preweighed vials using a small amount of solvent. The lipid sample is further dried in oven set at 50°C. The hexane and C-M fractions were transferred to preweighed glass vials. The oil samples were completely dried in dessicator and weighed to obtain the weight of the oil fractions in grams. The hexane and C-M fractions were mixed to obtain the total lipid extract that was used for the ^{1}H-NMR analysis [28] and transesterification for GC-MS analysis studies [12].

Total soluble sugar estimation

Phenol-H_2SO_4 method was used for determining the sugar content of the biomass [24]. 1 mL of culture sample was used for estimation of total soluble sugars. Blank was prepared by taking 1 mL of distilled water in the tube instead of the algal sample. The absorbance of all tubes was recorded at 488 nm and total sugars in the sample were quantified using a glucose standard curve.

Total soluble protein estimation

The total soluble protein was determined using the modified Folin's method [25]. 0.5 mL algal cell suspension was used for estimation of total soluble protein. The intensity of blue color was read at 650 nm using spectrophotometer against the blank. Bovine Serum Albumin (BSA) was used to prepare protein standard curve.

Gas Chromatography-Mass Spectroscopy (GC-MS) of transesterified oil samples

For gas chromatography analysis, 10 mg of total lipid extract was derivatized by using 25 mL of 2% sulphuric acid solution prepared in methanol. Fatty Acid Methyl Esters (FAME) were extracted EtAc [11]. FAME were analyzed using GC (Shimadzu GC-2010) equipped with FID using SP™ – 2560 capillary column (100 m × 0.25 mm 1D 0.20 µm film). The temperature programming was adjusted to the following temperature cycles. The temperature programming was adjusted to 140°C (hold time 5 min) to 240°C (hold time 20 min) at a rate of 4°C/min. The fragmentation patterns were compared with standard for each peak for identification of FAMEs.

Results and Discussion

Analysis of waste nutrient sources

The waste nutrient sources used for the study were added in the concentration of 1% in the culture medium. However, in case of molasses and cow dung extract, the amount of the waste nutrient was further reduced so that it does not have an inhibitory effect on the growth of the algae. Glycerol, press mud, molasses and succinate are waste products produced from various industries. However, for the present study glycerol and succinate were used in the pure form.

Total soluble sugar and soluble protein analysis

The protein and carbohydrate content of different waste nutrient sources was determined to understand the amount of usable protein and carbohydrates that may be available for the algal growth as shown in Table 1. Carbohydrates (soluble sugars) were maximum in case of banana peel (5.4%) followed by molasses (3.5%) and potato peels (2.6%). The soluble sugar content in case of bagasse was found to be 0.6%. The soluble sugar content in cow dung, press mud and potato peels was found to be 1.1%, 0.1% and 2.6%, respectively. The soluble protein content was found to be maximum in case of molasses (33.4%) followed by cow dung (9.5%) and banana peels (7.3%). In case of bagasse and potato peels, the soluble proteins were found to be 2.7% and 3.9%, respectively. In case of cheese whey and press mud, the total soluble protein was found to be 0.6% and 1.4%, respectively.

Ultimate/CHNSO analysis

The ultimate analysis of the waste nutrient sources used for the present study showed the presence of carbon, nitrogen, sulphur, hydrogen and oxygen elements as shown in Table 2. Maximum amount of carbon was present in bagasse (44%) followed by cow dung (40.5%). For the preparation of potato peel and banana peel extracts, fresh peels were used. However, the ultimate analysis for both fresh and dry peels was carried out to understand the difference in the elemental composition. The carbon content was 2% and 6% in case of fresh banana and potato peels. However, in case of dry peels the carbon content was 38% and 38% in banana and potato peels, respectively.

The carbon content in case of press mud and molasses was almost similar i.e., 35% and 34%, respectively. Cheese whey contained about 24% of carbon. Nitrogen content was maximum in press mud (3%) followed by molasses (2%). In cow dung, the nitrogen content was found to around 2%. In case of potato peel and bagasse, the nitrogen content was found to be 0.4% and 0.3%, respectively. Cheese whey contained negligible amount of nitrogen. Sulphur content was negligible in many of the nutrients except cow dung, press mud and molasses that contained 0.1%, 0.2% and 0.4% sulphur, respectively.

GC-MS analysis of cyanobacterial (*A. variabilis*) spent media extract

The cyanobacterial spent media extract was analyzed using gas chromatography-mass spectrometry. The GC-MS analysis identified the presence different types of compounds in the extract. The elution of different compounds started after nine minute till about 40 min when the entire sample had eluted. The results of the GC-MS analysis are given in Table 3. It was seen from the analysis results that about 36% of alkenes in the form of three compounds was present in the extract. Nine alcoholic compounds were present which amounted to 21% of

Waste Nutrient Sources	Soluble sugars (%)	Soluble proteins (%)
Potato Peels*	2.6	3.9
Banana Peels*	5.4	7.3
Cheese Whey	0.6	1.4
Cow dung*	1.1	9.5
Molasses	3.5	33.4
Press Mud*	0.1	0.6
Bagasse*	0.6	2.7
*On dry weight basis		

Table 1: Total soluble sugar and total soluble protein content of the extracts of the waste nutrient sources.

Nutrient sources	N (%)	C (%)	S (%)	H (%)	O (%)*	C/N ratio	C/H ratio
Banana Peel (dry)	1.4	37.8	0.2	5.8	54.8	26.0	6.4
Banana peel (fresh)	0.08	2.30	0	7.5	90.1	28.0	0.3
Bagasse	0.3	44.1	0	6.2	49.4	136.6	7.1
Cow dung	1.6	40.5	0.1	5.8	49.5	24.7	6.9
Press mud	2.9	34.5	0.2	5.1	57.6	11.6	6.7
Molasses	1.8	34.4	0.4	6.3	42.9	18.6	5.4
Potato Peel (dry)	2.2	38.2	0	6.2	53.4	17.1	6.0
Potato Peel (fresh)	0.37	6.3	0	9.8	83.4	16.6	0.6
Cheese whey	0.01	24.1	0	2.1	73.7	2410.5	0.2
*By difference							

Table 2: Ultimate analysis of the waste nutrient sources.

the total extract. Esters were present in the form of seven compounds which amounted to total of 18%. About 9.91 percent of nine different acidic compounds were present. One compounds each of carbazone, pyridine, furan, aldehyde and amine was present. Four ketones and three amide compounds were present; however, their total percentage was less than one percent. Thirteen unknown compounds were present in the extract which did not match with any compound from the NIST or WILEY library used by the GC-MS instrument.

Effect of different waste nutrient sources on growth and lipid production

Different types of nutrient sources had varying effects on the chlorophyll, protein, carbohydrates, biomass and lipid production of different algal species.

Effect of waste nutrient sources on chlorophyll content

Control samples of *C. reinhardtii* produced 14.6 mg/L of chlorophyll. The chlorophyll content in case of *C. reinhardtii* on supplementation with waste nutrient sources was maximum with succinate with 33.5 mg/L followed by 29.6 mg/L with glycerol (Figure 1). Chlorophyll content was 13.2 mg/L that was the least with cow dung waste nutrient. In case of *S. obliquus*, the algal sample under control conditions contained 19.1 mg/L of chlorophyll (Figure 2). In all waste nutrient supplemented cultures, the chlorophyll content was lower than the control sample except with banana peels and potato peels. The chlorophyll content was minimum in case of succinate supplemented cultures. The control samples of *C. vulgaris* contained 7.2 mg/L of chlorophyll. Out of the waste nutrient supplemented cultures cheese whey supplemented cultures produced 28 mg/L of chlorophyll followed by molasses supplemented culture that contained 22.4 mg/L of chlorophyll (Figure 3). The least amount of chlorophyll out of all the waste nutrient supplemented cultures in case of *C. vulgaris* was produced with bagasse hydrolysate. In case of *C. minutissima*, the control sample contained 4.6 mg/L of chlorophyll. *C. minutissima* culture supplemented with cheese whey produced 20 mg/L that was maximum followed by 14.2 mg/L of chlorophyll with molasses (Figure 4). The control samples of *B. braunii* contained 9.6 mg/L of chlorophyll. The maximum amount of chlorophyll was produced with bagasse hydrolysate (10.9 mg/L) followed by cheese whey supplemented culture (10.3 mg/L) (Figure 5).

Effect of waste nutrient sources on carotenoid content

The carotenoid content in the control samples of *C. reinhardtii* was 4.5 mg/L. The maximum amount carotenoid out of the waste supplemented cultures was 11.4 mg/L that was produced with succinate. The least amount of carotenoid was 3.6 mg/L that was produced with potato peels in case of *C. reinhardtii*. In case of *S. obliquus*, the carotenoid content was 3.3 mg/L in the control samples. The maximum amount of carotenoid content was produced with banana peels (3.6 mg/L) and the minimum amount of carotenoids was produced in press mud supplemented cultures of *S. obliquus*. In case of *C. vulgaris*, the control samples produced 2.3 mg/L of carotenoids. The waste nutrient supplemented cultures produced maximum carotenoids with molasses (6 mg/L) followed by banana peels (3.6 mg/L). The minimum amount of carotenoids was produced with press mud (2.1 mg/L). In case of *C. minutissima*, the control sample contains 1.77 mg/L of carotenoids. Out of the waste supplemented cultures, a maximum of 7.08 mg/L of carotenoids were produced with cheese whey followed by molasses (4.1 mg/L). *B. braunii* control samples contain 2.9 mg/L of carotenoids. The maximum amount of carotenoids was produced in cyanobacterial spent media extract supplemented cultures followed by potato peels

Retention time (mins)	Concentration (%)	Name
9.908	9.88	1-nonadecene
14.813	19.99	1-docosene
17.807	0.54	hexadecanoic acid, methyl ester
18.997	1.95	phenol, 2,4-bis(1,1-dimethylethyl)-
19.549	15.13	1-docosanol
20.169	0.21	diethyl phthalate
21.069	0.37	cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-2-propenyl)-, cis-
22.062	0.26	2,5-pyrrolidinedione, 3-ethyl-3-methyl-
22.408	0.86	octadecanoic acid, methyl ester
22.599	0.68	dodecanoic acid
23.958	15.84	phthalic acid, butyl undecyl ester
24.501	0.15	2-cyclopenten-1-one, 2-methyl-
24.75	0.28	n-hexadecanoic acid
25.321	0.58	1-heptadecanol
25.800	0.40	hexadecanoic acid, phenylmethyl ester
26.082	0.46	2,2-dimethyl-n-phenethylpropionamide
26.304	0.65	1-eicosanol
26.854	0.56	tetradecanoic acid
26.972	0.87	1,2-benzenedicarboxylic acid, dibutyl ester
27.192	1.04	2-oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-
27.455	0.17	ethanone, 2-imino-1,2-diphenyl-, semicarbazone
27.675	0.08	1-[2-diethylaminoethyl]-2,3,4,5,6,7-hexahydro-4-oxo-1h-cyclopenta[b] pyridine
27.997	5.84	1-tricosene
28.868	0.37	pentadecanoic acid
29.445	0.23	o o'-biphenol, 4,4',6,6'-tetra-t-butyl-
29.798	0.20	(3as,9as,9br)-6,6,9aa-trimethyl-trans-perhydronaphtho[2,1-b]furan
30.194	0.04	2,6-octadienal, 3,7-dimethyl-
30.408	0.93	1-eicosanol
30.658	0.09	(1,3-dioxo-1,3-dihydro-2h-isoindol-2-yl) methyl 3-methoxybenzoate
30.854	10.52	n-hexadecanoic acid
31.192	0.06	2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester
31.463	0.17	2-hydroxy-cyclopentadecanone,
31.919	1.86	n-hentriacontanol-1
32.448	0.07	nonadecanamide
33.115	0.11	hexadecanoic acid
33.786	0.33	9-octadecenamide
34.801	0.10	4-methyl-cyclopentadecanone
35.414	0.45	2,5,5,8a-tetramethyldecahydro-2-naphthalenol
36.024	4.13	octadecanoic acid
36.811	2.55	octadec-9-enoic acid
37.378	0.23	2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester
38.194	0.40	di-n-octyl phthalate
38.275	0.10	bis(trifluoromethyl)ethylborane ,dimethylamine(n-b)
38.525	0.12	9,12-octadecadien-1-ol

Table 3: Compounds identified by GC-MS analysis from the cyanobacterial (*A. variabilis*) spent medium extract.

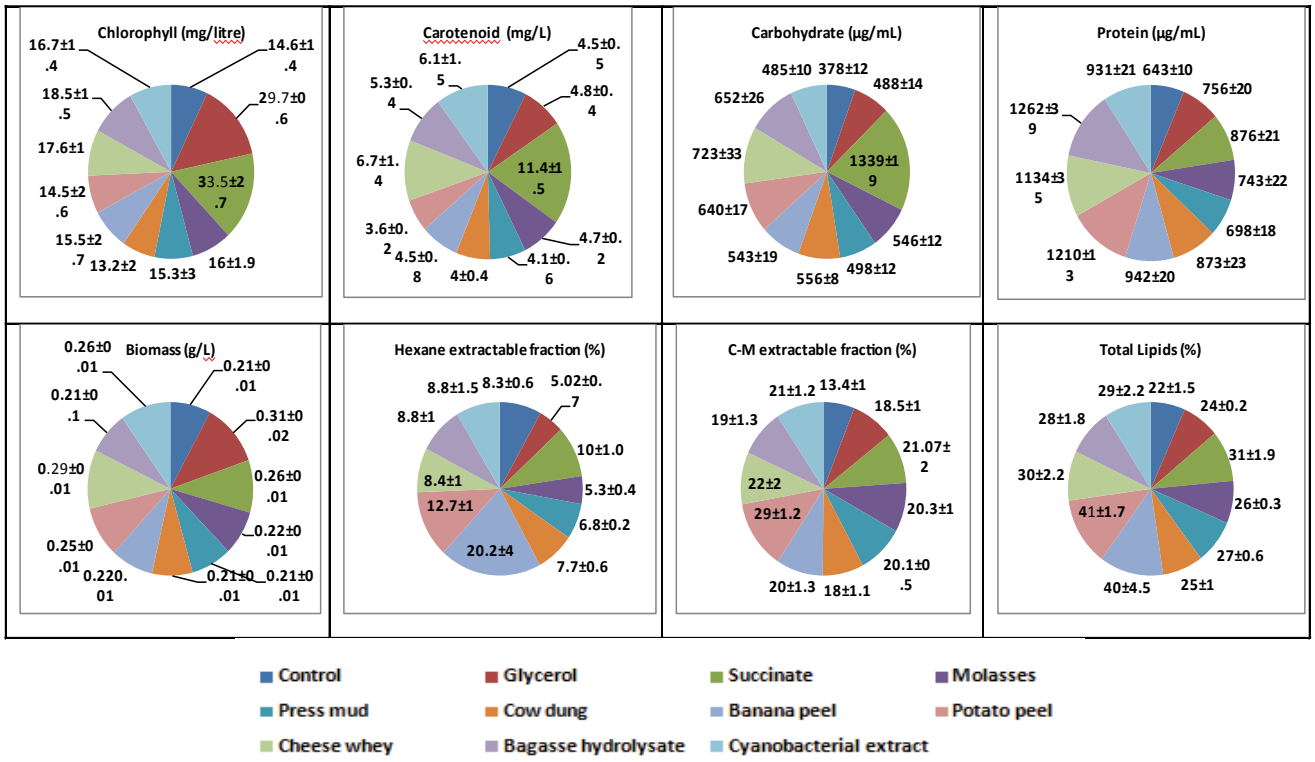

Figure 1: Effect of waste nutrient sources on the chlorophyll, carotenoid, carbohydrate, protein, biomass and lipid content in *C. reinhardtii.* All the estimations were performed in triplicates and are shown as average ± standard deviation.

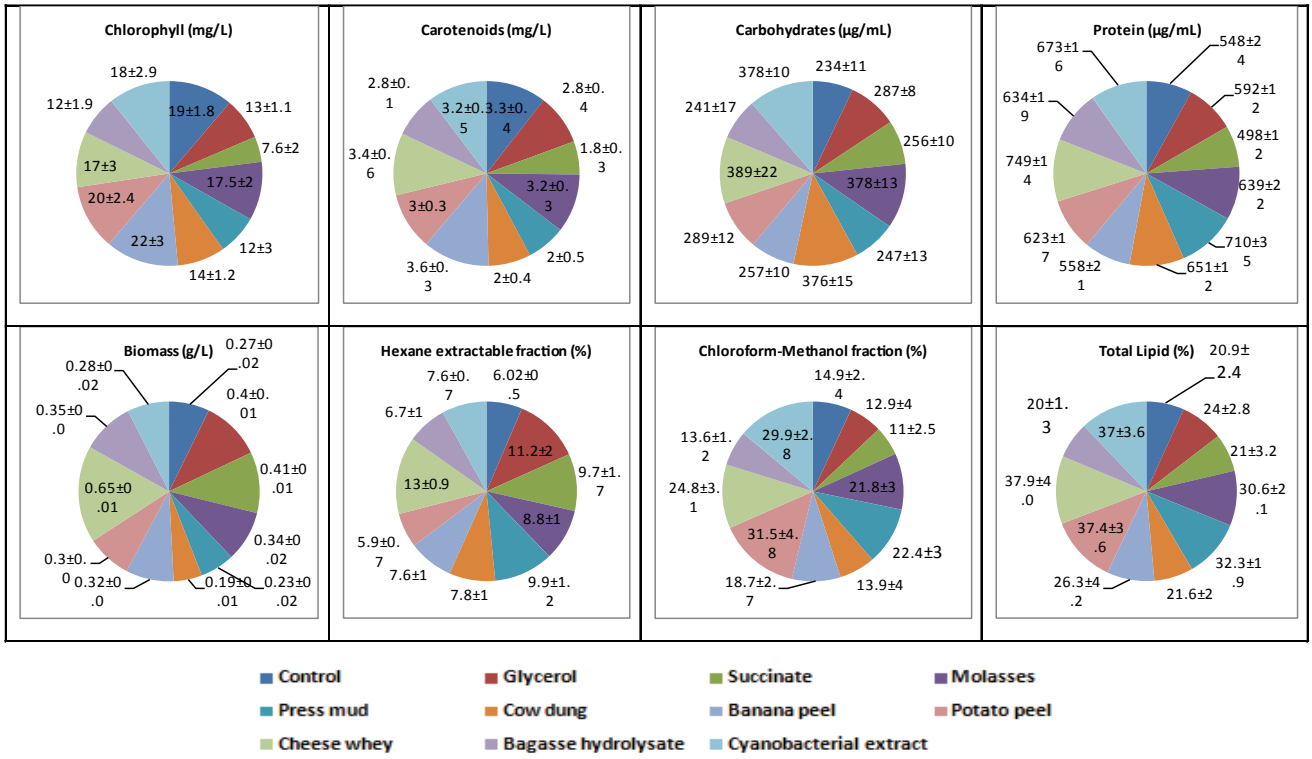

Figure 2: Effect of waste nutrient sources on the chlorophyll, carotenoid, carbohydrate, protein, biomass and lipid content in *Scenedesmus obliquus.* All the estimations were performed in triplicates and are shown as average ± standard deviation.

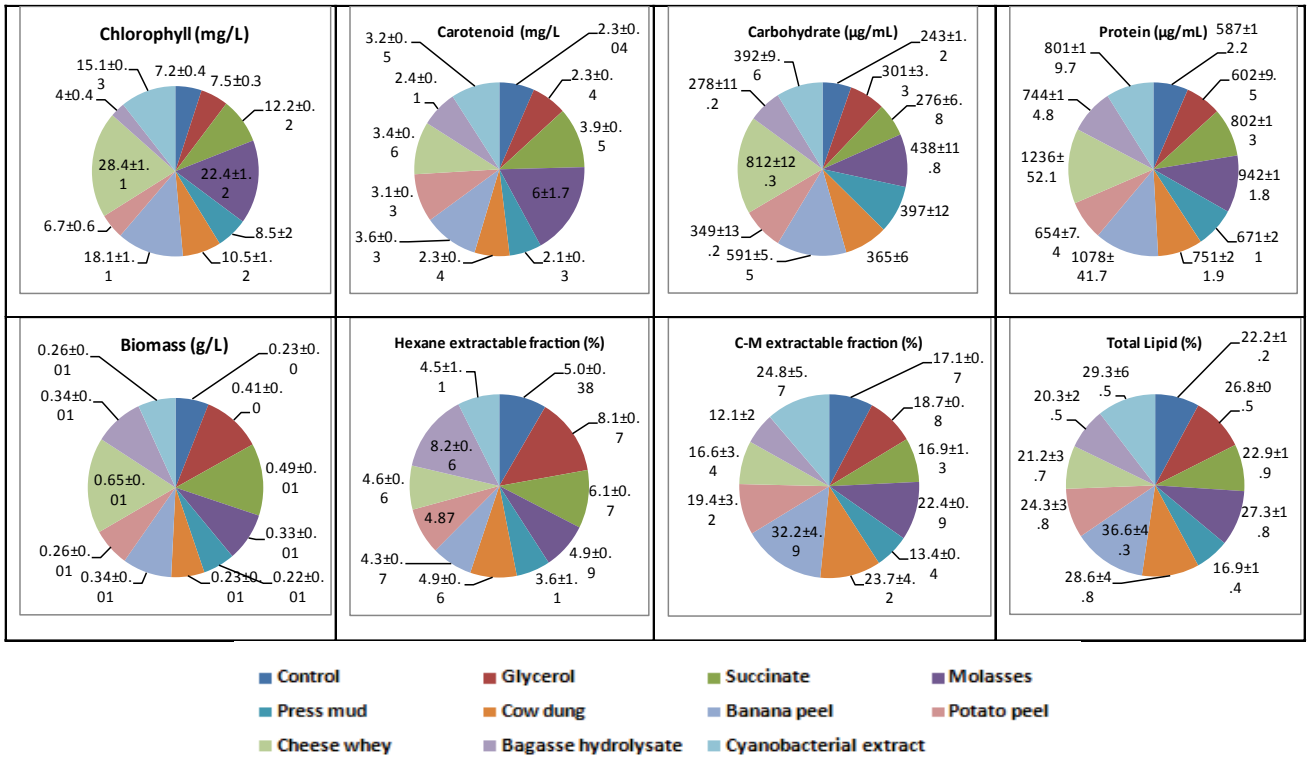

Figure 3: Effect of waste nutrient sources on the chlorophyll, carotenoid, carbohydrate, protein, biomass and lipid content in *Chlorella vulgaris*. All the estimations were performed in triplicates and are shown as average ± standard deviation.

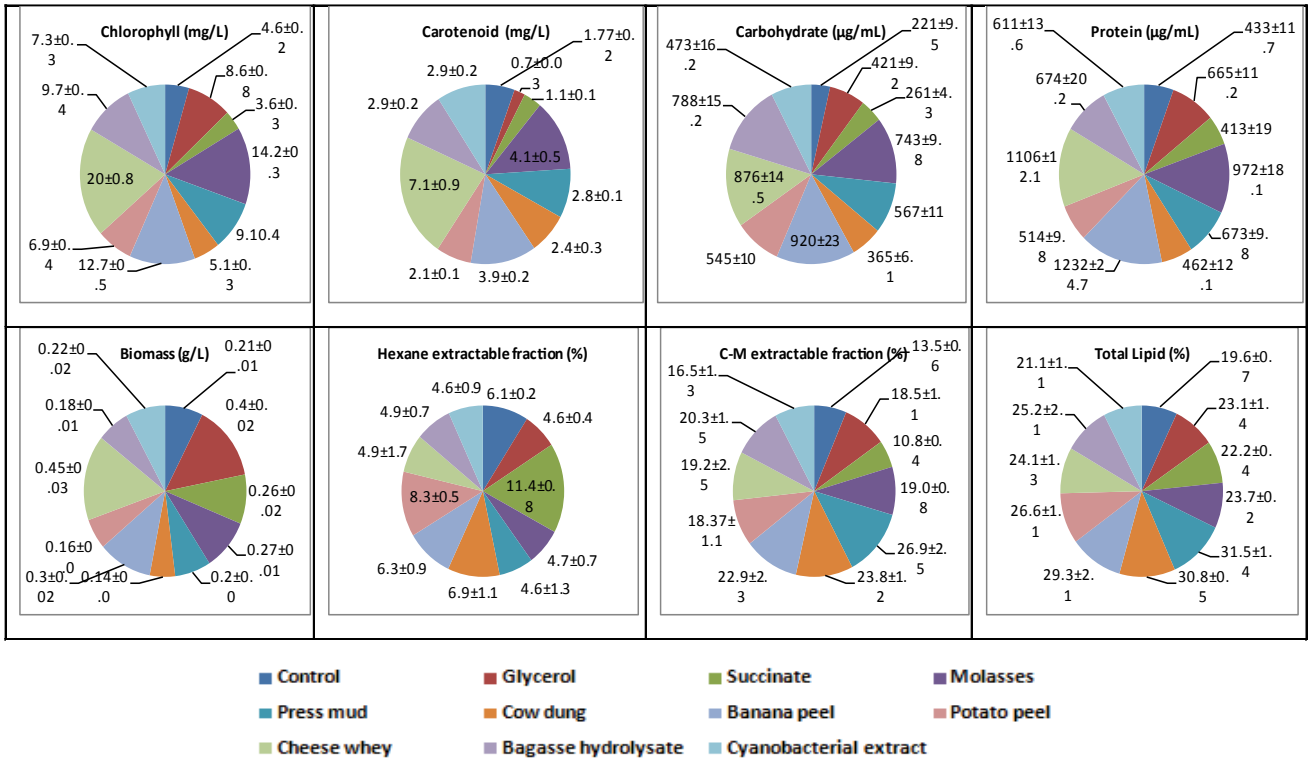

Figure 4: Effect of waste nutrient sources on the chlorophyll, carotenoid, carbohydrate, protein, biomass and lipid content in *Chlorella minutissima*. All the estimations were performed in triplicates and are shown as average ± standard deviation.

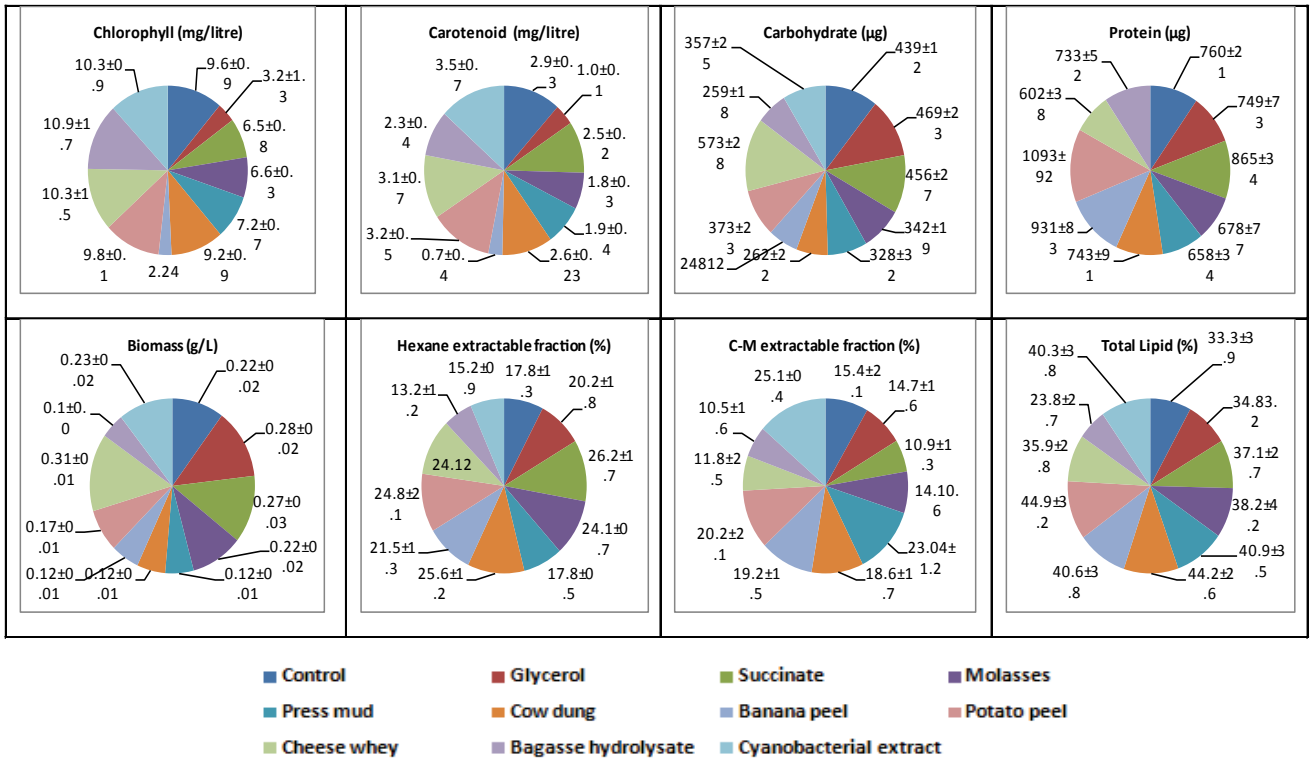

Figure 5: Effect of waste nutrient sources on the chlorophyll, carotenoid, carbohydrate, protein, biomass and lipid content in *Botryococcus braunii*. All the estimations were performed in triplicates and are shown as average ± standard deviation.

that produced 3.2 mg/L of carotenoids. The least amount of carotenoids was produced with banana peels supplemented cultures that produced 0.7 mg/L of carotenoids.

Effect of waste nutrient sources on carbohydrate (soluble sugar) content of algal species

Carbohydrate content was found to be 378 µg/mL in the control cultures of *C. reinhardtii*. Out of the waste nutrient supplemented cultures, succinate supplementation yielded maximum amount carbohydrates i.e., 1339 µg/mL. In case of *S. obliquus*, control cultures contain 234 µg/mL of carbohydrates. Waste nutrient supplementation yielded 389 µg/mL of carbohydrates with banana peels followed by 378 µg/mL of carbohydrates with molasses. Control culture of *C. vulgaris* yielded 243 µg/mL of carbohydrates. Waste nutrient supplementation yielded a maximum of 812 µg/mL of carbohydrates with cheese whey followed by 591 µg/mL of carbohydrates with banana peels. In case of *C. minutissima*, control cultures yielded 221 µg/mL of carbohydrates. Waste nutrient supplementation yielded a maximum of 920 µg/mL of carbohydrates with banana peels followed by 876 µg/mL of carbohydrates with cheese whey. Control cultures of *B. braunii* yielded 439 µg/mL of carbohydrates. Waste supplementation yielded 573 µg/mL of carbohydrates with cheese whey followed by 469 µg/mL of carbohydrates with glycerol.

Effect of waste nutrient sources on total soluble protein content

C. reinhardtii control culture contained 643 µg/mL of soluble proteins. Waste supplementation yielded 1262 µg/mL of soluble proteins with bagasse hydrolysate followed by 1210 µg/mL of soluble proteins with potato peels. The control cultures of *S. obliquus* contained 548 µg/mL of soluble proteins. Waste supplementation with cheese whey yielded 749 µg/mL of soluble proteins followed by 710 µg/mL of soluble protein with press mud. *C. vulgaris* control culture contained 587 µg/mL of soluble proteins. Waste supplementation with cheese whey yielded 1236 µg/mL of soluble proteins followed by 1078 µg/mL with banana peels. *C. minutissima* control culture contained 433 µg/mL of soluble protein. Waste supplementation with banana peels yielded a maximum of 1232 µg/mL soluble proteins followed by 1106 µg/mL soluble proteins with cheese whey. *B. braunii* control cultures yielded 760 µg/mL of total soluble proteins. Waste supplementation yielded a maximum of 1093 µg/mL of soluble proteins with potato peels followed by 931 µg/mL of soluble proteins produced with banana peels.

Effect of waste nutrient sources on biomass content

Control cultures of *C. reinhardtii* contained 0.21 g/L of biomass. Waste supplementation led to increase in the biomass production with glycerol that produced 0.31 g/L of biomass that was 47.6% more than that in the control sample. This was followed by cheese whey supplemented culture that produced 0.29 g/L of biomass that was 38% more than that of the control sample. *S. obliquus* control sample produced 0.27 g/L of biomass. Out of all the waste nutrient supplemented cultures, cheese whey produced 0.65 g/L of biomass that was 140.7% more as compared to that of the control sample. This was followed by succinate supplemented cultures which produced 0.41 g/L of biomass that was 51.8% more as compared to that of the control sample. In *C. vulgaris*, the control sample produced 0.23 g/L of biomass. Succinate supplemented cultures produced a maximum of 0.49 g/L of biomass that was 113% more as compared to that of control samples. This was followed by 0.41 g/L with glycerol that was 78.3% more as compared to that in the control cultures. In *C. minutissima*,

control cultures produced 0.21 g/L of biomass. A maximum of 0.45 g/L of biomass was produced with cheese whey supplemented cultures that was 114.3% more as compared to that of control samples. This was followed by 0.4 g/L with molasses supplementation in the nutrient media that was 90.5% more than that of control culture. *B. braunii* control culture produced 0.22 g/L of biomass. Waste supplemented cultures with cheese whey produced a maximum of 0.31 g/L of biomass that was 40.90% more than that of control culture. This was followed by glycerol supplemented culture that produced 0.28 g/L of biomass that was 27.27% more than that of control culture.

Effect of waste nutrient sources on lipid content

In case of control sample of *C. reinhardtii*, the content of hexane extractable, C-M extractables and total lipid content was 8.25%, 13.42% and 21.68%, respectively. Banana peel extract and potato peel extract supplementation led a maximum hexane extractable content of 20.17% and 12.66%. Maximum total lipid content of 40.34% and 41.17% was also produced with the two waste nutrients banana peel and potato peel extracts. The C-M content was maximum in case of potato peel extract (28.5%) followed by cheese whey (21.6%). Control samples of *S. obliquus*, produced 6%, 14.9% and 20.2% of hexane extractable content, C-M extractable content and total lipid content respectively. Maximum amount of hexane extractables were obtained with potato peels (31.51%) and cyanobacterial spent media extract (29.9%) in case of *S. obliquus*. Maximum total lipid content was obtained in case of cheese whey (37.9%), potato peels (37.4%) and cyanobacterial spent media extract (37.4%). The C-M extractable was highest with cheese whey (31.5%) and potato peels (29.9%). Control samples of *C. vulgaris* produced 5.04%, 17.1% and 22.2% of hexane extractables, C-M extractables and total lipid content, respectively. The hexane extractable content was found to be maximum with bagasse hydrolysate (8.2%) and glycerol (8.1%). The total lipid content was found to be maximum with banana peel extract (36.5%) and cyanobacterial spent media extract (29.3%). The C-M extractable content in case of *C. vulgaris* was maximum with banana peels (32.2%) and cyanobacterial spent media extract (24.83%). In case of *C. minutissima*, the control samples produced 6.1%, 13.5% and 19.6% of hexane extractables, C-M extractables and total lipid content, respectively. The maximum hexane extractable content was produced with succinate (11.4%) and potato peels (8.3%). The maximum total lipid content was produced with press mud (31.5%) and cow dung (30.8%). The C-M extractable content was also highest with press mud (26.9%) and cow dung (23.8%). In the *B. braunii* control cultures, the hexane extractable content, C-M extractable content and total lipid content was found to be 17.8%, 15.4% and 33.3%, respectively. Maximum hexane extractable content was produced with cow dung (25.6%) and potato peels (24.8%). The total lipid extract was found to be maximum with potato peel extract (45%) and cow dung extract (44%) supplementation. The C-M extractable content was found to be maximum with cyanobacterial spent media extract (25%) and press mud extract (23%) supplementation in case of *B. braunii*.

Effect of waste nutrients on fatty acid composition

C. reinhardtii control cultures produced about 10.7% of C16:0 fatty acid fraction. C18:1 and C18:3 fractions in the control culture were absent however, the amount of C18:0 and C18:2 were 3.2% and 8.3%, respectively, in the control culture of *C. reinhardtii*. Out of the waste nutrient supplemented cultures, maximum amount of C16:0 was present in cow dung (60.1%) supplemented cultures followed by banana peel (56.7%) and potato peels (55.9%). C18:0 fraction was maximum in press mud (5.8%) supplemented cultures. C18:0 fraction was absent in glycerol and bagasse hydrolysate supplemented cultures. Myristic acid was found to be absent in all the cultures of *C. reinhardtii* except with bagasse hydrolysate as shown in Table 4.

In case of *S. obliquus*, the fatty acid composition of control samples comprised of C8 to C22 carbon numbers as shown in Table 5. The percentage of C16 fatty acid was 8% and that of C18 fatty acids was 5.8%. Out of all the waste nutrient supplemented cultures, C16:0 fraction was maximum with glycerol (30.9%) followed by cheese whey supplemented culture (13.9%).

The C18:0 fraction was maximum with glycerol (9.2%) followed by press mud (8%) and cheese whey (6%) supplemented culture. Myristic acid i.e., C14:0 was maximum with potato peel extract (11.5%) followed by cyanobacterial spent media extract (6.5%). In *C. vulgaris*, the control samples consisted of 11.6% of C16 fraction and 5.1% of C18 fraction as shown in Table 6. Out of all the waste nutrient supplemented cultures, C16:0 fraction was maximum in case of cheese whey (34.2%) supplemented cultures followed by glycerol (21.2%) and potato peel (17.2%) supplemented cultures. The C18:0 fraction was maximum in case of cheese whey (9.1%) supplemented cultures followed by glycerol (5.9%) supplemented cultures. Myristic acid was highest in *C. vulgaris* culture grown with glycerol (8.1%) in the nutrient medium. In control sample of *C. minutissima*, the C16:0 fraction was 10.04% in amount. The C16:1 fraction was 7.55% in amount (Table 7). The control samples consisted of 3.36% of C10:0 fraction and 2.6% and 4.9% of C18:1 and C18:2 fractions, respectively. Out of all the waste nutrient supplemented cultures, C16 fraction was highest with molasses i.e., 62.7% followed by cow dung (26.2%) and cheese whey (24.9%) supplemented culture. The C18 fraction was produced in maximum amount in *C. minutissima* culture supplemented by succinate (6%) in the nutrient medium. C14:0 fatty acid was mostly absent in the supplemented cultures except for press mud, succinate and cow dung supplemented cultures in which it was present in negligible amounts. *B. braunii* control cultures produced 7.7% of C16:0 and 3.5% of C18:0 fatty acid fraction (Table 8). Total amount of C16 and C18 fatty acids were 17.6% and 16.2%, respectively.

The waste nutrient supplemented cultures produced maximum amount of C16:0 fatty acid with press mud (38.1%) and followed by banana peels (24.4%). The C18:0 fraction was produced in maximum amount with potato peel extract supplemented cultures i.e., 11.3%. C18:0 fraction was absent in cow dung, succinate and bagasse hydrolysate supplemented cultures.

Proton NMR analysis

The Proton Nuclear Magnetic Resonance Spectral Analysis of the total lipid extract was carried out for the total lipids extracted from the control and waste nutrient supplemented samples of five algal species used for the present study. The assignment of protons for different chemical shifts is as given in Table 9.

In *C. reinhardtii*, the control sample 97.7% of aliphatics, however, only in case of potato peels extracts supplemented cultures the percentage of aliphatics was more than that in the control sample i.e., 99.3% as shown in Table 10. The aromatics were not present in the control samples and in glycerol, molasses, press mud, bagasse hydrolysate, potato peels and cyanobacterial spent media extract supplemented cultures. The olefinic were 2.2% in amount out of the total lipid extract. The olefins were further reduced with potato peel extract and cyanobacterial spent media extract supplementation. In *S. obliquus*, the control sample contains 0.99% aromatic protons and 4.13% of olefinic protons. The aliphatic protons were 95.9% in total lipids of *S. obliquus* control sample. Out of all the waste nutrient supplemented cultures of *S. obliquus,* succinate supplementation led

Fatty acid	Percentage of fatty acids in different cultures (%)										
	Control	PP	BP	CW	PM	MO	CD	SU	GL	BH	CY
C8:0	15.2	2.9	3.6	12.7	5.1	7.6	3.7	9.4	14.2	7.1	5.7
C10:0	0.2	-	0.9	-	-	-	-	0.6	-	1.1	1.4
C11:0	15.1	3.9	5.4	13.5	7.1	9.4	4.8	11.4	16.1	9.4	7.8
C12:0	-	0.6	2.1	-	1.9	-	-	1.4	-	1.5	2.1
C13:0	13.9	6.2	5.4	15.4	10.4	11.9	5.4	12.2	14.8	11.1	9.3
C14:0	-	-	-	-	-	-	-	-	-	1.2	-
C14:1	-	-	-	-	-	1.4	-	-	11.2	1.1	1.1
C15:0	11.6	5.1	3.8	13.7	11.0	11.3	4.3	8.1	-	11.2	6.5
C15:1	-	-	2.1	-	-	-	-	-	7.5	-	-
C16:0	10.7	55.9	56.7	12.4	4.1	5.5	60.1	8.5	9.6	12.8	25.7
C16:1	3.6	4.8	2.5	-	5.8	8.7	2.9	9.3	-	-	6.2
C17:0	-	-	-	1.9	-	-	-	-	-	4.8	-
C17:1	-	-	-	-	-	-	-	-	0.8	-	-
C18:0	3.2	2.1	2.2	1.2	5.8	2.3	1.1	1.2	-	-	1.9
C18:1	-	1.7	-	0.5	5.4	4.1	3.2	8.6	6.2	4.3	2.8
C18:2	8.3	3.6	2.0	6.2	8.5	3.1	1.2	1.3	-	-	2.3
C20:0	-	0.9	-	1.8	2.3	-	-	-	0.8	-	2.1
C18:3	-	-	1.8	0.2	3.9	9.3	2.5	6.3	-	7.5	4.4
C20:1	-	-	1.3	-	0.7	-	-	-	4.9	-	-
C21:0	6.8	1.8	-	4.5	3.2	2.1	1.4	2.6	-	3.1	2.3
C20:2	-	-	-	-	-	-	-	-	0.6	-	-
C22:0	-	-	-	0.6	0.9	-	-	-	-	-	-
C22:1	-	-	-	-	-	-	-	-	-	-	6.2
C20:3	1.5	2.2	-	2.5	6.7	8.2	1.2	6.9	3.14	5.6	1.5
C23:0	4.2	1.2	1.6	2.6	2.6	2.1	0.8	2.1	-	1.1	-
C22:2	-	-	-	0.6	1.1	-	-	-	-	1.4	-
C24:0	-	-	-	2.1	1.2	1.7	-	-	-	-	-
C20:5	-	-	0.6	0.6	1.0	-	-	1.4	2.1	-	1.2
C22:5	-	-	-	1.3	-	-	-	-	-	-	-
C22:6	6.7	-	-	0.7	0.8	0.7	0.4	0.3	0.7	1.1	0.7

Table 4: Fatty acid composition of control and waste nutrient supplemented cultures of *C. reinhardtii*.

Fatty acid	Percentage of fatty acids in different cultures (%)										
	Control	PP	BP	CW	PM	MO	CD	SU	GL	BH	CY
C8:0	3.0	2.0	4.9	4.4	-	5.04	6.6	-	-	0.3	5.8
C10:0	1.7	3.0	1.9	1.8	1.5	2.8	4.6	-	1.5	0.3	3.2
C11:0	6.9	4.8	8.7	8.8	1.5	10.3	11.9	1.4	-	1.1	10.8
C12:0	7.0	2.3	1.4	1.3	3.3	2.1	2.5	1.3	2.4	2.3	-
C13:0	7.4	5.7	7.4	8.3	-	-	11.2	2.4	-	0.8	9.3
C14:0	5.2	11.5	5.1	0.7	1.5	0.2	-	-	-	1.3	6.5
C14:1	-	0.9	-	-	-	-	-	-	-	0.7	-
C15:0	4.7	3.3	-	6	-	6.4	8.5	1.6	-	0.9	-
C15:1	-	-	-	-	-	-	-	-	-	21.4	-
C16:0	11.8	8.3	9.4	13.9	10.5	8.9	3.2	6.6	30.9	1.1	3.1
C16:1	3.5	1.3	-	4.3	2.7	0.3	-	2.8	-	0.9	-
C17:0	1.6	3.3	3.6	1.5	3.6	5.1	7.6	1.4	-	2.6	5.2
C17:1	-	1	-	-	-	1.2	-	5.7	-	9.0	-
C18:0	3.1	3.0	2.6	6.0	8	2.2	2.4	4.4	9.2	2.1	1.4
C18:1	2.5	2.4	2.4	6.7	1.9	2.7	4.1	21.4	7.1	5.8	-
C18:2	2.2	4.3	2.9	5.3	-	5.1	3.5	4.8	-	-	3.7
C20:0	2.0	-	-	0.7	-	-	1.5	8.0	-	4.1	1.8
C18:3	-	2.2	2.8	2.2	3.4	2.7	-	2.0	5.9	-	2.2
C20:1	-	0.4	-	-	3.3	2.4	-	6.7	-	-	-
C21:0		0.9	1.6	-	2.6	-	2.2	-	-	-	-
C20:2	3.8	-	-	2.1	-	-	-	-	-	1.3	1.0

C22:0	-	0.4	2.7	-	-	-	-	5.5	-	-	-
C22:1	0.5	2.1	-	-	3.2	4.6	-	-	-	-	4.1
C20:3	3.7	2.1	-	1.3	-	-	11.6	6.7	-	1.1	0.9
C23:0	3.9	-	-	-	2.4	2.3	1.6	-	-	1.0	2.6
C22:2	-	1.3	1.3	1.9	-	-	-	2.8	-	0.4	-
C24:0	3.9	2	-	0.2	-	2.6	-	1.1	-	0.3	9.0
C20:5	1.2	-	-	-	-	-	0.8	-	8.1	-	-
C22:5	0.7	-	-	-	-	-	-	-	-	-	-
C22:6	11.5	11	4.3	9.5	34.4	12.3	12.5	10	24.1	24.6	5.7

Table 5: Fatty acid composition of control and waste nutrient supplemented cultures of *S. obliquus*.

Fatty acid	Percentage of fatty acids in different cultures (%)										
	Control	PP	BP	CW	PM	MO	CD	SU	GL	BH	CY
C8:0	1.8	4.6	7.2	1.2	6.6	5.2	1.2	4.8	3.9	4.9	4.9
C10:0	41.6	6.4	6.5	6.3	5.6	5.9	7.2	3.8	2.0	5.7	-
C11:0	6.1	9.1	11.5	1.4	11.1	7.9	3.4	32.2	5.9	10.2	7.1
C12:0	2.0	3.5	3.5	4.3	-	3.6	7.8	2.1	-	0.3	-
C13:0	6.2	5.7	9.6	1.3	4.4	5.9	2.1	6.3	7.0	3.5	7.1
C14:0	0.1	0.5	-	3.8	-	-	-	0.3	8.1	0.3	-
C14:1	0.8	-	1.0	1.3	1.6	-	-	0.9	-	3.7	2.9
C15:0	4.2	0.2	4.5	-	1.2	3.3	-	4.5	-	1.2	-
C15:1	-	-	-	-	-	-	-	-	4.2	0.8	11.9
C16:0	7.0	17.2	7.6	34.2	11.9	10.8	9.4	6.8	21.2	10.1	2.1
C16:1	4.6	2.1	0.8	3.4	2.2	7.5	2.6	0.4	14.7	2.7	13.2
C17:0	0.2	-	-	1.3	2.6	3.5	1.0	-	2.6	1.6	-
C17:1	-	-	-	-	0.5	-	0.6	0.5	-	2.4	-
C18:0	-	3.7	4.5	9.1	1.3	-	2.6	2.9	5.9	-	2.3
C18:1	2.0	9.0	4.2	-	10.8	7.6	-	8.8	0.9	11.8	-
C18:2	3.1	8.2	4.5	-	2.8	4.5	3.3	4.0	3.8	2.2	13.9
C20:0	4.3	-	-	1.33	-	-	-	-	1.2	-	-
C18:3	-	8.0	-	-	-	7.2	-	4.2	-	2.2	7.6
C20:1	2.7	-	-	1.5	2.8	1.4	2.9	-	-	-	4.0
C21:0	1.1	-	1.2	-	-	-	-	-	1.2	0.3	-
C20:2	-	-	10.8	-	-	-	-	-	2.7	-	-
C22:0	-	-	-	1.1	3.8	1.2	3.3	-	-	1.1	1.3
C22:1	-	15.3	0.7	-	-	-	-	-	-	0.4	-
C20:3	4.0	-	-	1.6	-	7.6	7.7	6.9	2.4	-	2.8
C23:0	0.7	-	-	1.5	-	-	-	0.5	1.8	0.5	-
C22:2	-	-	-	-	-	-	4.4	-	-	-	2.5
C24:0	-	-	-	-	-	-	1.3	-	-	-	1.7
C20:5	-	-	0.3	-	-	-	-	-	-	-	-
C22:5	-	-	-	-	-	-	-	-	-	-	-
C22:6	-	0.1	0.8	15.9	10.5	4.2	33.6	0.9	-	9.3	1.6

Table 6: Fatty acid composition of control and waste nutrient supplemented cultures of *C. vulgaris*.

to decrease in the amount of aromatic protons as shown in Table 10. The olefinic protons showed decrease with bagasse hydrolysate and potato peel extract supplementation. The aliphatic protons show slight decrease in the waste nutrient supplemented cultures as compared to that of control samples in *S. obliquus*. In *C. vulgaris*, the control sample of total lipid extract contained 1.1% of aromatics which were completely absent in press mud and bagasse hydrolysate supplemented cultures of *C. vulgaris*.

The olefinic compounds were 5.2% in the control sample of *C. vulgaris* which were reduced in amount in press mud, bagasse hydrolysate and cheese whey supplemented cultures. The aliphatic compounds in the control samples of *C. vulgaris* were 92.5% which increased to 96.6% in the bagasse hydrolysate supplemented cultures. In the control samples of *C. minutissima,* 0.7% of aromatic protons were present. These protons were further decreased in the molasses and cow dung supplemented cultures. The olefinic protons in the control samples of *C. minutissima* were 0.4%, however, in cow dung supplemented cultures olefinic protons were 0.3%. The aliphatic protons were 98.8% in control sample of *C. minutissima* which was maximum among all the cultures of *C. minutissima*.

Since biodiesel and biogasoline are used as mixtures of probably more than 1000-10,000 different organic compounds therefore it is also important to carry out the functional group / class analysis of the mixed fuel or mixed feedstock from the fuel. In fact, GC-MS studies reveal the individual components present in the lipids.

Fatty acid	Percentage of fatty acids in different cultures (%)										
	Control	PP	BP	CW	PM	MO	CD	SU	GL	BH	CY
C8:0	3.95	8.5	12.2	3.0	5.2	2.6	4.0	5.0	11.6	2.4	8.0
C10:0	2.2	3.3	5.2	1.6	3.5	-	3.0	22.2	4.0	1.4	2.7
C11:0	17.9	14.0	10.4	10.3	6.9	3.6	6.4	4.8	21.1	3.5	13.5
C12:0	-	3.1	2.6	1.8	2.5	-	2.3	1.7	1.9	2.9	2.1
C13:0	9.43	12.1	8.8	7.8	6.3	3.3	5.2	6.7	6.5	8.0	13.9
C14:0	-	-	-	-	0.3	-	1.2	0.8	-	-	-
C14:1	1.11	-	1.1	-	0.7	-	-	0.7	3.6	1.4	10.1
C15:0	6.67	8.3	4.5	7.2	3.4	0.6	3.3	3.9	-	8.0	-
C15:1	1.15	-	-	2.1	-	4.1	-	0.2	8.1	-	-
C16:0	10.04	16.6	5.8	24.9	14.2	62.7	26.2	13.1	11.7	5.3	11.3
C16:1	7.55	1.8	14.5	5.3	17.3	3.9	3.8	13.1	-	22.9	5.2
C17:0	-	1.3	-	-	-	-	-		-	2.8	0.9
C17:1	-	-	-	-	-	-	-		3.5	-	0.2
C18:0	3.36	3.5	3.0	3.5	2.2	0.6	4.3	6.0	2.6	-	0.7
C18:1	2.6	-	-	1.9	7.8	0.4	1.3		-	-	5.3
C18:2	4.93	0.6	3.3	2.2	9.6	6.8	2.8	2.7	1.0	3.0	0.4
C20:0	1.86	-	0.3	4.2	-	0.5	1.4		1.8	-	0.2
C18:3	1.66	-	1.4	1.2	1.8	-	-	1.0	-	2.1	1.7
C20:1	-	-	-	-	-	-	-	1.3	1.8	-	-
C21:0	5.01	-	1.1	2.4	-	1.9	0.8	0.3	-	1.7	3.4
C20:2	-	-	-	-	-	-	-		-	-	-
C22:0	-	-	-	-	-	0.3	0.6	2.6	-	0.4	-
C22:1	-	-	-	-	-	-	-		-	-	-
C20:3	4.04	5.1	5.1	4.0	7.5	1.0	0.7	0.4	5.9	13.4	4.7
C23:0	1.43	0.7	0.7	1.7	0.7	1.4	1.3	0.3	0.9	0.2	2.0
C22:2	-	0.4	0.4	-	0.5	0.5	-	0.4	-	0.6	-
C24:0	-	1	1	-	-	0.9	0.4	0.5	1.0	0.2	-
C20:5	-	-	-	1.0	0.6	-	-	0.1	0.6	0.8	1.4
C22:5	-	-	-	-	-	-	-	-	-	-	-
C22:6	1.1	1.6	1.6	1.9	0.7	1.1	13.4	-	2.1	0.9	1.2

Table 7: Fatty acid composition of control and waste nutrient supplemented cultures of *C. minutissima*.

Fatty acid	Percentage of fatty acids in different cultures (%)										
	Control	PP	BP	CW	PM	MO	CD	SU	GL	BH	CY
C8:0	7.1	5.8	5.8	6.5	3.6	9.3	9.0	5.2	4.9	7.9	8.3
C10:0	-	-	1.6	4.3	-	0.8	-	-	1.0	-	-
C11:0	-	7.1	11.5	6	5.2	11.2	11.2	6.8	6.4	10.2	10.2
C12:0	-	1.1	1.2	1.1	-	-	1.1	-	2.0	1.1	-
C13:0	10.5	10.2	9	8.5	8.8	12.2	13.4	11.1	7.2	13.3	11.2
C14:0	-	-	0.4	0.2	-	-	1.2	-	0.3	-	-
C14:1	-	-	0.6	2.3	-	-	-	-	-	-	-
C15:0	11.6	9.9	0.2	7.8	8.5	12.3	12.7	11.1	6.9	13.7	13.2
C15:1	-	-	7.7	-	-	-	-	-	2.9	-	-
C16:0	7.7	4.3	24.4	-	38.1	2.4	1.2	14.5	22.0	1.5	1.3
C16:1	9.9	8.3	6.0	23.1	6.8	10.0	9.9	5.9	5.4	10.9	11.4
C17:0	-	5.5	1.1	5.0	-	-	4.2	1.2	-	1.2	-
C17:1	-	-	-	1.3	-	-	-	-	-	-	-
C18:0	3.6	11.3	0.6	4.6	3.1	4.5	-	-	3.3	-	2.1
C18:1	9.1	-	6.9	6.0	3.1	5.4	2.9	10.0	14.9	4.2	3.5
C18:2	3.5	8.7	3.7	3.1	0.8	5.6	8.1	-	6.2	5.7	6.4
C20:0	4.0	2.9	2.4	2.2	2.8	-	-	-	-	-	-
C18:3	-	-	1.2	2.9	3.3	-	1.5	1.5	1.3	1.4	4.1
C20:1	-	-	-	0.2	0.1	0.3	-	1.1	0.6	0.4	-
C21:0	3.8	3.6	2.5	-	1.8	4.6	4.1	4.0	2.1	4.4	4.4
C20:2	-	-	-	0.8	0.5	0.6	-	2.2	0.4	0.3	-
C22:0	-	-	0.8	1.1	0.4	-	-	-	-	-	-

C22:1	-	3.4	1.1	0.7	1.7	1.1	-	-	-	-	-
C20:3	6.6	1.5	1.6	1.4	1.3	1.2	4.0	-	4.1	5.0	1.8
C23:0	2.1	1.1	-	1.4	0.8	2.6	2.3	3.4	0.7	-	2.9
C22:2	-	-	-	1.0	0.3	0.3	-	1.5	0.6	-	1
C24:0	1.7	-	-	-	0.7	1.9	-	-	0.9	-	-
C20:5	1.1	1.2	1.0	-	0.3	-	1.7	1.8	-	1.6	1.8
C22:5	-	-	-	-	-	-	-	-	-	-	-
C22:6	1.7	1.2	-	0.4	0.9	0.9	1.7	0.8	1.2	1.1	1.2

Table 8: Fatty acid composition of control and waste nutrient supplemented cultures of *B. braunii*.

Chemical Shift	Types of protons
9.0-6.0	Aromatic
4.6-6.6	Olefinic
4.0-0.5	Aliphatic
4.0-2.0	CH_3, CH_2 and CH protons α to aromatic range
2.0-1.0	CH_2 and CH protons of alkyl chains or further to the ring and CH_3 protons β to the ring
1.0-0.5	CH_3 protons of alkyl chains ϒ or further from aromatic ring or CH_3 of saturated compounds

Table 9: Chemical shifts and their assignment for types of protons.

Chemical Shift	% age of protons										
	Control	GL	SU	MO	PM	CD	BH	PP	BP	CW	CY
C. reinhardtii											
9.0-6.0	-	-	3.9	-	-	2.9	-	-	11.8	1.9	-
4.6-6.6	2.2	2.7	4.5	6.3	4.02	3.9	3.5	0.66	2.4	2.8	0.52
4.0-0.5	97.7	94.7	91.6	93.6	95.6	76.3	60.6	99.3	85.8	88.2	99.6
4.0-2.0	8.2	3.2	18.9	15.8	13.2	18.2	16.1	0.56	10.8	8.5	0.69
2.0-1.0	81.7	73.9	52.9	91.4	75.5	58.2	69.3	94.1	57.8	72.9	98.79
1.0-0.5	7.9	11.8	19.3	10.7	7.3	12.7	23.2	4.69	17.3	6.9	-
S. obliquus											
9.0-6.0	0.99	0.9	0.09	1.31	4.9	3.28	1.28	1.03	1.37	1.44	2.14
4.6-6.6	4.13	4.4	4.23	4.09	7.2	3.59	2.86	2.73	3.70	3.37	4.23
4.0-0.5	95.86	95.6	93.3	93.8	87.2	91.8	92.0	92.2	86.5	93.2	93.2
4.0-2.0	19.13	9.9	13.7	10.8	10.7	10.2	11.5	11.3	34.3	10.2	10.2
2.0-1.0	54.30	74.4	64.8	64.7	67.6	67.7	42.2	72.3	39.9	80.8	67.4
1.0-0.5	22.42	11.2	14.6	18.1	8.9	13.8	12.4	11.9	12.2	-	15.2
C. vulgaris											
9.0-6.0	1.14	1.87	0.79	1.47	-	1.25	-	2.55	2.49	1.05	1.63
4.6-6.6	5.15	7.62	6.31	6.58	1.72	5.75	2.29	3.52	4.02	2.29	3.95
4.0-0.5	92.5	89.0	90.8	88.7	68.39	91.12	96.6	91.9	91.7	82.10	93.2
4.0-2.0	6.87	11.05	10.3	16.9	7.75	14.66	8.75	12.81	11.02	13.20	5.97
2.0-1.0	70.48	68.02	67.5	60.63	77.01	64.81	68.54	66.33	66.81	23.08	76
1.0-0.5	16.33	9.73	12.8	12.07	12.35	11.64	19.37	10.42	13.91	58.16	11.29
C. minutissima											
9.0-6.0	0.7	0.43	2.3	0.4	3.4	0.5	0.55	0.53	4.3	5.6	0.5
4.6-6.6	0.4	2.3	1.5	0.9	3.2	0.3	0.73	0.68	4.2	0.5	0.7
4.0-0.5	98.8	93.2	95.4	95.8	93.3	99.2	98.7	98.7	91.4	93.8	98.7
4.0-2.0	12.8	7.0	10.0	2.5	14.8	2.6	2.3	3.6	22.2	2.6	5.9
2.0-1.0	84.9	71.6	74.3	85.6	70.2	65.1	90.3	89.1	58.4	71.8	85.4
1.0-0.5	10.4	14.6	10.9	7.6	8.3	7.2	6.1	8.1	10.8	17.6	7.3
B. braunii											
9.0-6.0	0.05	-	2.12	2.89	3.38	0.43	2.89	-	0.93	-	7.52
4.6-6.6	1.61	0.02	3.47	2.75	1.96	0.3	-	3.68	0.51	2.14	4.03
4.0-0.5	98.3	96.3	94.4	94.3	93.5	99.2	97.1	96.3	98.5	97.8	88.4
4.0-2.0	5.22	0.12	8.71	4.2	15.08	2.3	3.05	4.9	1.7	6.71	10.3
2.0-1.0	82.73	73.12	85.7	75.12	68.62	86.5	86.03	91.4	92.44	78.5	7.08
1.0-0.5	10.4	10.9	8.67	15.05	9.83	10.5	8.03	10.4	4.41	12.6	11.06

Table 10: Proton NMR analysis of total lipid extracts of the algal species under the effect of different waste nutrient sources.

However, FTIR and NMR spectral analysis would help in understanding the functional group/class analysis of lipid mixtures. Lipid extracts are processed through transesterification for production of biodiesel. Therefore, functional group/ class analysis will help in understanding the characteristics of lipid mixtures. In the control samples of *B. braunii*, the aromatic compounds were 0.05% in amount which became completely absent with glycerol, potato peels and cheese whey supplementation in the nutrient medium of *B. braunii*. The olefinic compounds were 1.6% in the control sample of *B. braunii* which were highly reduced or absent with glycerol, cow dung, bagasse hydrolysate and banana peels supplementation. The aliphatic compounds in the control sample were 98.3%. Cow dung supplementation increased the aliphatic compounds to 99.2% in the *B. braunii*.

Conclusion

It was concluded from the present studies that aqueous extracts of various waste nutrients could be efficiently utilized by various algae. The method adopted allowed for easy harvesting of algal biomass as the final culture remained in the form of slurry. *C. reinhardtii* and *C. minutissima* showed enhancement in total lipid content with most of the nutrient sources used. *C. reinhardtii* showed maximum increase in lipid production with potato peels (89%) and banana peels (90%) supplementation. Maximum biomass production in *C. reinhardtii* was observed with succinate (47%) and cheese whey (38%) supplementation. It is required that a good quality biodiesel should have lower aromatic and olefinic content and higher aliphatic content. Carbon supplementation led to reduction in the aromaticity as seen from the proton NMR studies. However, *C. reinhardtii* was found to contain 11.8% of aromatic protons with banana peels unlike the control sample which was found to be free of it. *C. reinhardtii* was found to be the best alga out of all the five algal species which showed the maximum improvement in lipid content. Out of the ten waste nutrients studied, mainly potato peels, banana peels and cheese whey showed maximum enhancement in biomass and lipid production with most of the algal species. The GC-MS analysis revealed *C. reinhardtii* to be most suitable for biodiesel production as it produced 60.1% and 55.9% of C16:0 fatty acid fractions with cow dung and potato peels. In fact, present studies were aimed to study the effect of carbon and protein contents of the wastes, algal growth and lipid production.

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