Bioprospecting of Indigenous Microalgae to Evaluate Their Potential for Bioenergy and Wastewater Treatment

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Abstract

In this study we investigated the phycoremediation ability of indigenous microalgal strains using synthetic wastewater along with the production of high-value biomass. Two algal strains were collected, cultured, purified, and later named as named as BERC3 and BERC4 after morphologically and genetically identification. Nutrient concentrations, pH, and light intensity were optimized for maximum growth and metabolite content. Harvested biomass was characterized to check the impact of wastewater on the biomass productivity and metabolite content (lipids, carbohydrates, proteins, pigments) of selected strains. Results shows that both strains produced maximum biomass in low-light (150µmol m^{-2} s^{-1}) at neutral to slightly alkaline pH. Both the strains performed very well in MGB11 (synthetic wastewater), indicated that these strains are suitable for growth in wastewater as those conditions favor biomass and metabolite production.

Introduction

Microalgae-based bioremediation is the most secure, promising, and most effective choice to supplant ordinary treatment strategies due to its immense accessibility and higher supplement utilization capacity (Fazal et al., 2018). The main concern of wastewater treatment is to remove nitrogen and phosphorus which come from different sources (Nayak, Karemore, & Sen, 2016). Microalgae remove contaminants, as well as their biomass, has many beneficial aspects such as bioenergy production and biodiesel production (Mujtaba, Rizwan, & Lee, 2017). Microalgae have a wide range of industrially important characteristics such as high growth rate, ability to grow in standard media and freshwater as well as in wastewater(Lam, Lee, & Mohamed, 2012). Availability of nutrients influences metabolite production limitation in nutrients specifically nitrogen affect lipid production. (Geada, Vasconcelos, Vicente, & Fernandes, 2017).

Many researchers are trying to extract useful products from microalgae (Cuellar-Bermudez et al., 2015). Extraction methods are separate for each molecule that is the loss of all other useful molecules (Chew et al., 2017). Culturing of microalgae in wastewater that containing nitrogen 42.3 mgL^{-1} and phosphorus 35.4 mgL^{-1} and observed that monoculture biomass was 0.58gL^{-1} and in mixed culture was 0.45 mgL^{-1}(Komolafe et al., 2014). It's reported that 61 percent of phosphate and 82 percent nitrogen wereremoved.

Several factors affect the efficiency of nutrient removal at large-scale such as nutrient composition, have metals, flow rate, microbial interaction, suspended solids, pH, light, temperature, etc. (Zhu et al., 2014) cultured *Chlorella sp.* at lab-scale and large-scale to compare the nutrient removal efficiency and biomass productivity of *Chlorella sp.* Some species of bacteria are beneficial for wastewater treatment which can be co-cultured with the microalgae (Wang, Liu, Zhao, Wei, & Sun, 2016). Pakistan is a farming country, and almost 70% of Pakistanis rely on agriculture (Dordio & Carvalho, 2013). Several studies have shown the treatment of agriculture wastewater using microalgae. Wastewater that contains animal manure has shown to be a suitable media for microalgal cultivation (Cai, Park, & Li, 2013).

The present study focuses on renewable and eco-friendly wastewater treatment methods. Microalgae can help in this regard, as they exhibit excellent nutrient uptake potential. So, we hypothesize the use of indigenous microalgae for primary wastewater treatment. The biomass produced in this way will be rich in valuable metabolites (due to cultivation in nutrient-rich conditions). The plan of work includes isolation of potential native microalgal strains, identification, cultivation in suitable media, optimization of abiotic factors, and extraction of metabolites (carbohydrates, lipids, proteins, and pigments).

Methodology

Sample collection and maintenance

Water samples were collected from a pond receiving wastewater located nearby the human settlement before 8 km of Gogera 580 ft Elevation (Okara District) and from the wastewater Gogera 640 ft Elevation. Sterilized plastic bottles were used for samples and were transported to the lab on the same day. The wastewater from the tubes was mixed with BG11 growth media (Table 2) and put under light (~ 120 µmoles m^{-2} s^{-1}) with a Light: Dark period of 12 h, at room temperature to enrich the cultures for seven days.

Isolation and purification of the dominating microalgal strains

The serial dilution and streak plate method were performed several times until the cultures became pure. While the purity of the cultures was observed under a compound microscope. Every time, 100 µl of the microalgal samples from each dilution left in the growth chamber for 3–5 days were poured BG11-agar plate. Morphologically different microalgal filaments were inoculated in BG11 as growth media and cultivated for fourteen days and after fourteen days of cultivation, the purity of the sample was checked through microscopy. After purification strains were named as BERC3 and BERC4.

Molecular marker-assisted identification

Sequencing of the marker genes encoding 18S rRNA, 23S rRNA, and/or 16S rRNA is the most efficient way to identify the microalgae. For cyanobacterial identification 16S rRNA, gene sequencing is commonly used while eukaryotic microalgae are identified by 18S rRNA gene sequencing.

Genomic DNA extraction and qualification and quantification

CTAB method was used to extract the genomic DNA of both strains (Porebski, Bailey, & Baum, 1997). CTAB and 0.2% mercaptoethanol were mixed and placed in a water bath set at 65°C. Fresh microalgal biomass was collected from a 10–12 days culture and grind with pre-warm CTAB + mercaptoethanol mixture to cell lysis and carbohydrate breakdown. Agarose gel electrophoresis was performed to check to

quality of the DNA and quantity was measured using a NanoDrop spectrophotometer. The collected DNA was stored at 4°C for further experiments.

Sequencing of the marker gene

Cyanobacterial-specific primers were used to amplify the 23S rRNA and 16S rRNA genes (Table S1) using the PCR master mix (Thermoscientific K1231). Genomic DNA of filamentous microalgal strains was used as a template to amplify 23S rRNA and 16S rRNA genes. The PCR profile was set as the step of denaturation at 95°C for 5 minutes followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 57°Cfor 1 minute, extension at 72°C for 2 min followed by a period of final extension for at least 10 min before a4°C hold. PCR profile used to amplify the 16S rRNA gene was also the same as 23S rRNA except for the annealing temperature that was 60°C. The PCR amplicons were directly ligated to pJET 1.2 vectors using the commercial kit (Thermo Scientific) by following the manufacturer's instructions. The recombinant vectors were transformed into DH5α strain of *E. coli* using the heat shock method. Antibiotic-resistant transformants were selected on LB + ampicillin plates. Colony PCR was performed to confirm the cloning of the desired fragment. After the confirmation of the colony, the plasmid extraction was performed using GeneJET Plasmid Miniprep Kit (K0502) and purified plasmids were sent for sequencing to Macrogen (Korea) for sequencing *(*Nelissen, Wilmotte, Neefs, & De Wachter, 1994*).*

Optimization of abiotic factors for better growth

All of media conditions including pH, and light were optimized to enhance the production of biomass and the content of metabolites. To ensure reliability and reproducibility, all the experiments were performed in triplicates. Recipes of the standard media used for algal growth aregiven in the supplementary table (Table S2).

Biomass characterization

Microalgae can accumulate different important metabolites such as carbohydrates, lipids, proteins, pigments, etc. Industrial and biotechnological applications can decide on the base abundant metabolites produced by microalgae. Therefore, different biochemical analyses were performed for growth and metabolites determination.

Biomass production and specific growth rate

The biomass productivity of pure microalgal strains was measured gravimetrically. The production of the biomass was measured by utilizing the given formula.

$$\mathrm{Biomassproduction} = \frac{\mathrm{F.M} - \mathrm{I.M}}{\mathrm{TotalVolume}}$$

Where F.M = final mass and I.M = initial mass.

The productivity of biomass was calculated withhelp of given formula;

$$\text{Productivity} = \frac{\text{Biomassproduced(grams)}}{\text{Time(days)}}$$

The specific growth rate was measured by using the given equation;

$\mu = \ln (N_y/N_x)/(t_y-t_x)$

Doubling time was calculated by using the equation:

$$\text{DoubingTime (Td)} = \frac{\mu(\text{ty} - \text{tx})}{\log 2 \left(\frac{\text{Ny}}{\text{Nx}}\right)}$$

Where, μ = specific growth, Nx = the mass at the first day and Ny = mass at the final day at the start and end of the growth, and Tx = initial time and Ty = final time.(Gill et al., 2016).

Carbohydrate estimation

Carbohydrates of both strains were measure using strong acid hydrolysis. Microalgal biomass was retained in the oven at 65°C overnight and then the 20mg of dry mass was transferred to glass test tubes in duplicates. All the sugars were released from the cell using concentrated H_2SO_4 (95–98%). Later, the acid was diluted to 4% by the addition of 17 mL distilled water. 1mL aliquot was subjected to centrifuge at maximum speed for 5 minutes to precipitate any suspended particles. Then 0.5mL supernatant was mixed with 0.25 mL phenol (5%) and was left for 3 min for reaction completion coupled with thorough efficientmixing. After that, concentrated 1.25 mL sulfuric acid was added and continuously stirred the reaction mixture for 15 minutes at room temperature. The brown color indicated the presence of sugar. Wavelength of 490nm was used to determine the absorbance and compared with the standard curve (Fig. 1) to estimate total sugar (Masuko et al., 2005).

Lipid analysis

Modified Bligh and Dyer method was used to measure the total lipids by utilizing the mixture solvent chloroform: methanol (2: 1). Pre-measured 100 mg of lyophilized algae powder was mixed with a solvent of 10 ml at 65 ° C for at least 1 hour. The mixture was first centrifuged, then the supernatant was collected, and then the remaining biomass was extracted again.All supernatants were mixed and the final volume ratio of 1:1:0.9 (chloroform: methanol: water) was made by adding 1% solution of NaCl and methanol. The chloroform phase was carefully transferred to the vial and dried at constant weight after centrifugation. Finally, we obtained the total lipids and calculated the contents as a percentage of the microalgae dry weight.

Lipid content (%) = $(W_2-W_1)/W_d \times 100\%$

Where W_1 = empty falcon tube weight, W_2 = weight of oil containing falcon tube and W_d= dry mass of microalgae.

Fatty Acids Methyl Esters analysis

Samples were first prepared for FAME analysis composition in triplicate. GC/MS analysis was carried out in collaboration with the International Centre of Biological and Chemical Sciences, University of Karachi, Karachi, Pakistan. Agilent 7000A Triple Quadruple Mass Spectro Photometer Pairs Equipped with Gas Chromatograph (Agilent 7890) Automopler. Agilent 7000A Triple Quadruple Mass Spectro Photometer Pairs Equipped with Gas Chromatograph (Agilent 7890) Automopler. The GC column used was combined with a silica capillary column (Agent 190905-433, 30 m × 250µm ID, film thickness 0.25 µm).The pressure of the carrier gas (Helium) was 7.0699 psi at the initial temperature of the oven with a flow rate of 64 ml / min.The split mode was selected for all standards and sample injection (split/column flow ratio 60:1). 250˚Cwas the injector temperature;50^{o}Cwas the oven temperature, rose to 220^{o}C at the rate of 14^{o}C per min with a 34 mintotal run time. The mass spectrophotometer was operated on electron impact mode (EI) at 70 eV in the scan range of 50–560 *m/z*. The transfer line and the ion source temperature were set to a value of 320^{o}C and 280^{o}C, respectively. The volume of the sample injection was 1.0 µL.

Hunter software (Agilent) was widely used to process data. Algal Oil's authentic identificationwas performed by comparison with retention times of mass spectra and standards obtained compared with those available in the Wiley and NIST libraries (Wiley Registry TM, 8th Edition Mass spectral library, and the NIST 08 Mass spectral library (NIST/EPA/NIH) 2008 version) with a match acceptance criterion above a key element of80%. In an MRM mode, the same GC setting was used, with collision energy of 30 eV and a solvent delay of 5 minutes. 6.5 cycles.s-1 was the scan rate.Examining the effects of wastewater cultivation on metabolite content., all the experiments were conducted using synthetic wastewater as growth media, whereas strain cultured in standard growth media (BG11/BBM) will serve as a control.

Protein estimation

The protein extraction was performed using alkali (T. Rausch, Hydrobiologia 1981). 2 mL aliquots of the sample were subjected to centrifugefor 5 min at maximum speed. The supernatant was removed and added 1mL 0.5N NaOH to the pellet. The mixture was then kept at 80 C for 10 minutes, stirring occasionally. The mixture was placed at room temperature in order to cool down and again centrifuged at maximum speed for 5 min. The supernatant was moved to a new test tube. All this procedure was repeated three times while at final repeat the mixture was warm at 100°C for 10 min for complete residual protein extraction. All the extractions were pooled and mixed gently before further analyses.

A rapid and sensitive microbiuret method was used to estimate extracted protein contents.2mL of extract and 1mL of reagent (30% NaOH with Copper Sulfate) at 310nm in a quartz cuvette. For blanking the spectrophotometer, 2mL of dH_2O (without protein) and 1mL of reagent (30% NaOH with Copper Sulfate) was used.The OD was measured at 310nm and a graph was made using GraphPad Prism. The good thing about this method was that it's not affected by high concentration of DNA. Bovine serum albumin(BSA) from Sigma–Aldrich was used as the standard for calibration (Fig. 2).

Pigment extraction and estimation:

Microalgal biomass contains many important pigments such as Chlorophylls, carotenoids, phycobilisomes. An absorbance method was used to extract pigments. 10mg of the oven-dried algal sample was resuspended in 5mL ethanol (95% v/v) kept at 4°C for 12 h.Centrifuged the mixture at 4000×*g*for at least 10 minutes. The supernatant was transferred to another tube optical density was measured at the wavelength of 665nm, 649nm and 470nm using a UV–Vis spectrophotometer as described previously(Nelson et al., 1987).

The chlorophyll a, and b and total carotenoids were calculated by using following formulae;

$Ch\text{-}a = 13.36A_{664} - 5.19\ A_{649}$

$Ch\text{-}b = 27.43A_{649} - 8.12\ A_{664}$

$C\ x + c = (1000A_{470} - 2.13Ch\text{-}a\text{-} - 97.63Ch\text{-}b)/209$

A is the Absorbance, Ch-a shows Chlorophyll a, Ch-b shows Chlorophyll b, Cx + c shows Carotenoids (Sumanta, Haque, Nishika, & Suprakash, 2014).

For phycobilin estimation (Zimba, 2012), 200mg of oven-dried powdered sample was taken and mixed with 200µL of 0.1M sodium phosphate buffer by vertexing for 30 min at normal temperature. The mixture was centrifuged at 6000rpm for at least 10–30 minutes and at the temperature of 4°C, the supernatant was transferred to another tube to measure the absorbance at λ498.5nm, λ614nm and λ651nm by using a spectrophotometer. Allophycocyanin, phycocyanin, and phycoerythrin were calculated by using formulae;

$Allophycocyanin = 181.3(A_{651})\text{-}22.3(A_{614})$

$Phycocyanin = 151.1(A_{614})\text{-}99.1(A_{651})$

$Phycoerythrin = 155.8(A_{498.5})\text{-}40(A_{614})\text{-}10.5(A_{651})$

All the experiments were performed in triplicate, and data was analyzed with GraphPad Prism 6.0.

Wastewater treatment evaluation

To check the wastewater efficiency as an alternative media, both strains BERC3, BERC4 inoculated in autoclaved municipal wastewater and synthetic wastewater (Table S3) and grown for two weeks under optimized conditions. Biomass characterization was performed as mentioned previously in the collected sample.

Results

Culture enrichment

The samples were subjected to enrich for the high number of cells. The solid green color was the signal of enriched samples. The enriched culture was shifted to a growth chamber at 12h light/dark period. After 14 days of cultivation, the dominant strains were a filamentous strain in both of the wastewater samples which are shown in Fig. 3.

Isolation and purification

The variety of microbes was present in collected wastewater. The microalgal culture was spread on agar media. BG11 growth media was used for the culturing of microalgae. Morphology based identification was performed (Fig. 4) both the strains were unbranched filamentous named as BERC-3 and BERC-4.

Morphological identification

Morphological identification was performed through microscopy.During microscopy, Fig. 5 shows both of the strains appeared as unbranched filaments. When the microscopy of the strains compared with literature, both of the strains resembled cyanobacterial group.

Molecular identification

After the extraction of genomic DNA, amplification of the marker genes encoding 23S rRNA, and/or 16S rRNA was performed. Where amplicon of fragment size of 500bp and 632 bpon agarose gelwere observed of 23S rRNA and 16S rRNA bands respectively (Fig. 6).

Optimization of abiotic factors

Two standard growth media were used for maximum biomass production i.e. BG11 and BBM media. Both the strains had a maximum growth rate in BG11 media. BERC-3 produced 0.34 gL^{-1}biomass in BBM and 0.55 gL^{-1} in BG11 and BERC-4 produced biomass was 0.45 gL^{-1} in BG11 and 0.29 gL^{-1} in BBM. Biomass productivity, specific growth rate and doubling time are shown in Table 1.

It was observed that BERC3 performed the best at pH 8 and BERC4 at pH 9 in the selected media. Biomass production of BERC3 and BERC4 was 1.96 $gL^{-1}$and 2.2 gL^{-1}respectively at optimum pH, on a fresh mass basis. Moreover, a variation in pH was observed in both strains in the batch culture of 14 days. It was observed that the pH values of both strains shown an increasing trend (Figs. 7 and 8).

Two type of light intensities were used to observe the effect of light on biomass production. High biomass production was observed in BG11 and MBG11 when light intensity was low (150µmol m^{-2} s^{-1}) while biomass production in MWW was higher at high-light intensity (300µmol m^{-2} s^{-1}). Both strains produced high biomass in MBG11 at the low-light intensity and in MWW at high light intensity (Table 2).

Pollutant removal efficiency of algal strains

To evaluate the nutrient removal efficiency, both strains were cultured in different growth media i.e. BG11, MBG11, and MWW, and biomass production and pH variations were observed. The data are shown in Figs. 9 and 10. Maximum growth (1.65 gL^{-1} dry weight) of BERC-3 was observed in MBG11that showed the high efficiency of nutrient removal. The same trend was observed in BERC-4 with a 1.55 gL^{-1}biomass production. Although growth was not consideredin MWW but the strain turned yellow to green within 12-days that reflected the higher rate of nutrient removal that led to the nutrient deficiency after 10-days.

Biomass production

For further biochemical analysis, biomass was produced at optimum conditions i.e. pH 8 for BERC3 and pH 9 for BERC4, using MBG11 growth media, 150 $\mu molm^{-2}s^{-1}$ light intensity using a 14-day batch culture. The data are shown in Figs. 11 and 12. BERC3 produced 0.65 gL^{-1} in MBG11 and 0.44 gL^{-1} in BG11. BERC4 produced 0.45gL^{-1} in MBG11 and 0.30gL^{-1} in BG11.

Impact of growth media on lipids production

According to metabolites production, BERC3 and BERC4 have a potential for lipids biosynthesis, as theirlipids contents were 39% in BERC3 and 43% in BERC4. Results in this study reveal that both of the strains shown high lipids biosynthesis in MBG11. BERC3 produced 21.5 mg/g in MBG11 and 14.5 mg/g in BG11. BERC4 produced 25.5 mg/g in MBG11 and 19 mg/gin BG11(Fig. 13).The high lipids production obtained in this study is also among the highest to be reported in the literature.Reported *Synechococcus sp. HS01* produced 15.4mg/g lipids.*Dunaliella sp.* produced 18.31 mg/g lipids.

Impact of media on FAME profile

BERC3 is considered a promising candidate for biodiesel production because the C16:0 was observed at a high level in MBG11. But the opposite trend was observed in BERC4, For C16:0a high level was observed in BG11 as compared to MBG11. BERC3 produced 28.591 mg/g Hexadecanoic acid, methyl ester in MBG11 and 25.748 mg/g in BG11. BERC4 produced 27.055 mg/g Hexadecanoic acid, methyl ester in MBG11, and 31.901 mg/g in BG11(Fig. 14).

Impact of media on carbohydrates biosynthesis

The biomass carbohydrate content depends on the microalgal species and composition of nutrient media. The production of microalgal carbohydrates was significantly improved in BG11 when optimized cultivation conditions are applied.A carbohydrate content BERC3 was 7.9mg/g in BG11 and 6.8mg/g in MBG11 and BERC4 was 7.7mg/g in BG11 and 6.5 mg/g in MBG11 (Fig. 15). The microalgal carbohydrates contents observed in this study were lower than reported *Synechococcus elongatesPCC 11801*(9.87mg/g) (Hassan, Williams, & Jaiswal, 2018).

Impact of media on protein biosynthesis

The amount and content of protein in microalgae varies greatly from one species to another and culture media with adequate available nitrogen, carbon fixated in the photosynthesis process is being used for the synthesis of protein. No significant difference was observed between BG11 and MBG11in this study. Both strains produced almost an equal amount of protein contents (5.5 mg/g) (Fig. 16). These proteins contents were lower than reported *Spirolina* (8mg/g) asboth strains accumulated high phycobilin (Teuling, Schrama, Gruppen, & Wierenga, 2019).

Impact of media on pigments biosynthesis

Total pigment accumulation by BERC3 and BERC4 was affected by the type of medium used. The MBG11 medium is richer in nutrients and yielded more pigments as compared to the BG11. Both strains produced different photosynthetic pigments such as phycobilins (phycocyanin, allophycocyanin,and phycoerythrin) and chlorophyll (chlorophyll A, chlorophyll B, and chlorophyll C). Among all different pigments, phycocyanin was accumulated in high concentrations. BERC3 produced 57.3 mg/g phycocyanin in BG11 and 80 mg/g in MBG11. BERC4 produced 57.3mg/g phycocyanin in BG11 and 79 mg/g in MBG11 which is remarkably higher than producedby reported Arthrospira platensis (30 mg/g) (Eriksen, 2018). Regarding the culture media, the MBG11 medium favored theaccumulation phycobilins, while the BG11 mediumfavored the production of chlorophyll in BERC3 (Figs. 17 and 18).

Discussion

Applied phycology has recently become one of the top research areas due to diverse applications of microalgae. Keeping in view the immense potential of microalgae for industrial, environmental, and pharmaceutical applications of microalgae, the current study was aimed at the investigation of indigenous microalgae for the same. Samples were collected from different areas of Punjab, Pakistan. Isolation and purification performed of newly isolated strains which were named BERC3 and BERC4. Based on the partial sequence of the 23S rRNA gene, the BERC3 strain showed a close relationship with the *Synechococcuselongatus*. While the cyanobacterial specific primers were used to amplify thegenesequencesfor both strainsof 16S rRNA, for which the results are awaited. For maximum biomass production, abiotic factors were optimized, and biomass was subjected to basic characterization to explore the future applications of each strain.

Both of the strains performed best in BG11 media, BERC3 produced 0.41gL^{-1} dry biomass and BERC4 produced 0.55gL^{-1} dry biomass in BG11 that is remarkable higher than other reported strains. pH and light intensity are important factors that affect biomass production, nutrient removal efficiency and metabolites contents of microalgae. Impact of pH and light intensity varies from species to species such as *Synechococcus elongatesPCC 11801* produced 1.8 gL^{-1} at pH 8 and highlightintensity (300 µmol m-2 s-1) while, in this study BERC3 produced 2.15g gL^{-1} dry biomass at pH 8 and BERC4 produced 2.21 gL^{-1} at pH 9 at lowlight intensity (150 µmol m-2 s-1) that reflects low saturation point and enhanced the

importance of both the strains(Qiu, Gao, Lopez, & Ogden, 2017; Sakarika & Kornaros, 2016). The pH not only affects biomass production but also influences the nutrient removal efficiency. Both strains showed good nutrient removal efficiency. Dry biomass of BERC3 was 1.53gL^{-1} and BERC4 biomass production 1.58gL^{1}that indicated the high nutrient removal efficiency in synthetic wastewater (MBG11). Although biomass production in MMW was low, the yellowish color indicated high nutrient removal efficiency; it turned within 12 days of the experiment. The growth rate of BERC3 was 0.32 and of BERC4 was 0.31 (de Oliveira Corrêa, Duarte, & Noseda, 2019; Rashid, Nayak, Lee, & Chang, 2019; Roselet, Vandamme, Muylaert, & Abreu, 2019).

Microalgae biomass mainly consists of lipid, carbohydrate, proteins and pigments. Media composition played important roles in lipid accumulation of BERC3 and BERC4. It was interesting to note that 39% of lipids were observed in MBG11 as compare to BG11 (35%) in BERC3. Similarly, high lipid contents (43%) of BERC4 were observed in MBG11 as compare to BG11 (40%). The highest lipid production in this study is also the highest ever reported in the literature. Reported *Synechococcus sp. HS01* produced 15.4mg/g lipids. *Dunaliella sp.* produced 18.31 mg/g lipids. Fatty acids containing 16–18 carbon atoms are considered suitable for making biodiesel.The main fatty acids in BERC3 and BERC4 were C14:0(tetradecanoic acid), C15:0(methyl-tetradecanoic acid), C15:0(pentadecanoicacid), C16:0(hexadecanoic acid) and C18:2(octadecadienoic acid). Among those,C18:2 (octadecadienoic acid) and C16:0 (hexadecanoic acid) were the two most dominant fatty acids. Higher Hexadecanoic acid, methyl ester was observed in this study as compared to reported microalgal species such as *Synechococcus sp. HS01* produced 23 mg/g and *Pseudanabaena sp. SK 01* produced 21mg/g Hexadecanoic acid, methyl ester (Modiri et al., 2015).

The production of microalgal carbohydrates was significantly improved in BG11 when optimized cultivation conditions are applied. Carbohydrate contents BERC3 was 7.9 mg/g in BG11 and 6.8 mg/g in MBG11 and BERC4 was 7.7 mg/g in BG11 and 6.5 mg/g in MBG11. The microalgal carbohydrates contents observed in this study were lower than reported *SynechococcuselongatesPCC 11801* (9.87mg/g)(Hassan et al., 2018). Total pigment accumulation by BERC3 and BERC4 was affected by the type of medium used. There was no significant difference was observed in proteins biosynthesis but the MBG11 medium is richer in nutrients and yielded more pigments as compared to the BG11. Among all different pigments, phycocyanin was accumulated in high concentrations. BERC3 produced 57.3mg/g phycocyanin in BG11 and 80mg/g in MBG11. BERC4 produced 57.3mg/g phycocyanin in BG11 and 79mg/g in MBG11 which is remarkably higher than produced by reported Arthrospira platensis (30mg/g) (Eriksen, 2018).

It was observed that abiotic factors influence the efficiency of locally isolated strains. But at optimized condition, these strains performed best in biomass production, wastewater treatment and are eco-friendly. In the future, isolation of more potential native microalgal strains, and the development of cost-effective and eco-friendly microalgal-based technology for wastewater treatment, and a more detailed study has to be performed to understand the molecular mechanisms.

Declarations

Authors Contributions

The manuscript was reviewed and approved for publication by all authors. FUK and MS conceived and designed the research. TS and WS performed the research work. FUK and WS analyzed the data. TS and WS wrote the paper. NU, AS and FUK reviewed and revised the paper. FUK and MScorrected the English language for the paper.

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Ethical approval

The manuscript was reviewed and ethical approved for publication by all authors.

Consent to participate

The manuscript was reviewed and consents to participate by all authors.

Consent to publish

The manuscript was reviewed and consents to publish by all authors.

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1; Biomass production, biomass productive, specific growth rate and doubling time of BERC3 and BERC4

Strain	Media	Biomass production gL^{-1} (Dry weight)	Biomass Productivity $gL^{-1}d^{-1}$	Specific Growth Rate d^{-1}	Doubling Time H
BERC3	BBM	0.34	0.37	0.22	0.49
	BG11	0.55	0.45	0.23	0.43
BERC4	BBM	0.29	0.43	0.23	0.44
	BG11	0.45	0.51	0.24	0.39

Table 2; Comparison of biomass production at low and high light intensities.

Strain	Light intensity	Media	Biomass production gL^{-1} (Dry weight)	Biomass Productivity $gL^{-1}d^{-1}$	Specific Growth Rate d^{-1}	Doubling Time H
BERC3	Low	BG11	1.44	0.98	0.28	0.25
		MBG11	1.88	1.27	0.30	0.20
		MWW	1.30	0.91	0.28	0.26
	High	BG11	0.72	0.47	0.23	0.42
		MBG11	0.54	0.38	0.22	0.48
		MWW	0.71	0.54	0.24	0.38
BERC4	Low	BG11	1.65	1.25	0.30	0.21
		MBG11	2.15	1.52	0.32	0.18
		MWW	2.00	1.34	0.31	0.20
	High	BG11	0.33	0.46	0.23	0.42
		MBG11	0.55	0.37	0.22	0.49
		MWW	0.12	0.70	0.26	0.32

Table 3; FAME profile of BERC3 and BERC4

RT (min)	Compound	FA chain length	BERC3		BERC4	
			BG11	MBG11	BG11	MBG11
19.59	Tetradecanoic acid, methyl ester	C14:0	4.29	5.45	6.50	5.17
20.44	l 9-Methyl-tetradecanoic acid, methyl ester	C15:0	3.72	3.54	2.93	3.85
20.53	4,8,12-Trimethyl-tridecanoic acid, , methyl ester	C16:0	-	-	1.22	1.36
20.93	Pentadecanoic acid, methyl ester	C15:0	1.11	1.57	2.14	1.42
21.83	7,10-Hexadecadienoic acid, methyl ester	C16:2	49.88	51.30	-	50.40
21.90	9-Hexadecenoic acid, methyl ester	C16:1	34.22	37.55	32.87	36.57
21.95	11-Hexadecenoic acid, methyl ester	C16:1	5.49	5.39	6.26	6.47
22.22	Hexadecanoic acid, methyl ester	C16:0	100	100	100	100
23.14	9-Heptadecenoic acid, methyl ester	C17:1	4.47	3.84	5.22	3.81
23.41	5,9,13-Trimethyl-tetradecanoic acid, methyl ester	C17:0	1.68	1.67	2.17	1.73
24.23	9,12-Octadecadienoic acid , methyl ester	C18:2	78.15	85.25	76.76	84.84
24.30	9-Octadecenoic acid, methyl ester	C18:1	53.67	59.48	65.30	63.20
24.36	11-Octadecenoic acid, methyl ester	C18:1	6.86	6.22	7.36	6.97
24.57	Octadecanoic acid, methyl ester	C18:0	16.14	15.39	19.39	16.15

Figures

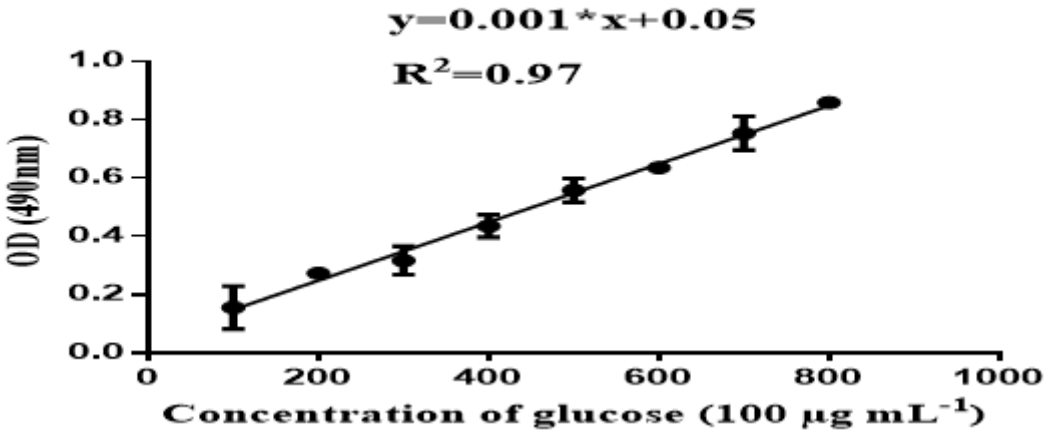

Figure 1

Standard curve of glucose concentration

Standard curve was drawn according to standard concentration of glucose. X-axis represents concentration of glucose (µg mL^{-1}) and Y-axis represents OD at λ_{490}.

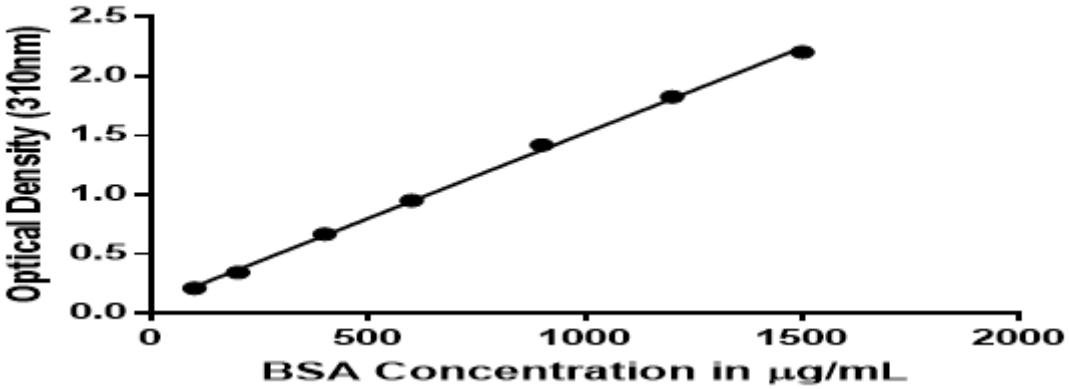

Figure 2

Standard curve for protein concentration

Figure 3

Culture enrichment and growth of algal strains.

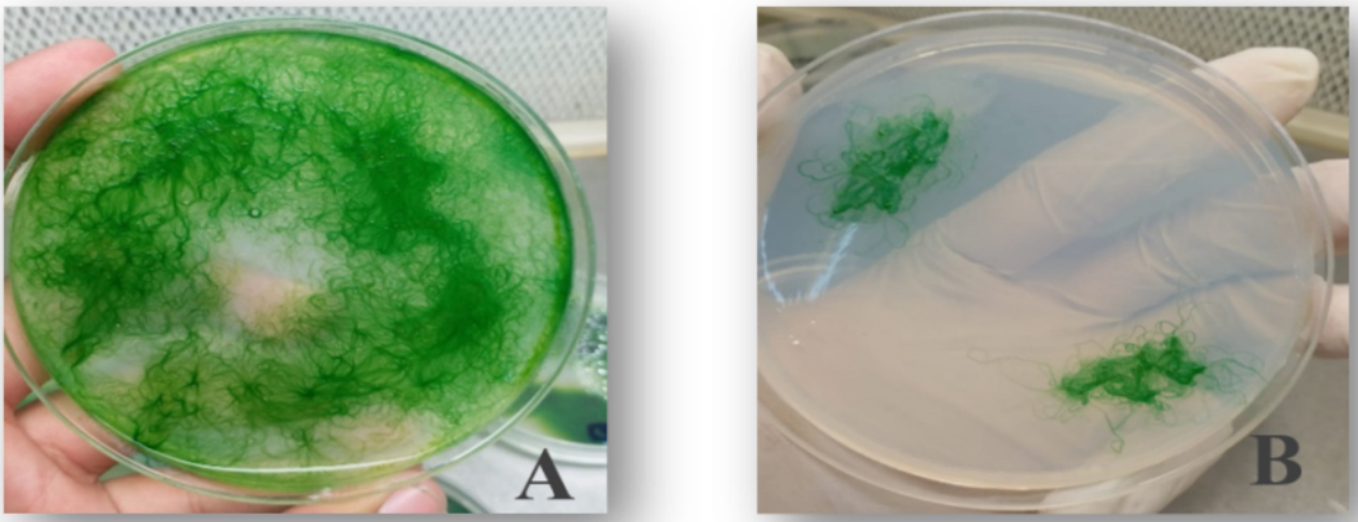

Figure 4

Isolation of microalgal strains; BERC3 (A), and BERC4 (B) through dilution plating method

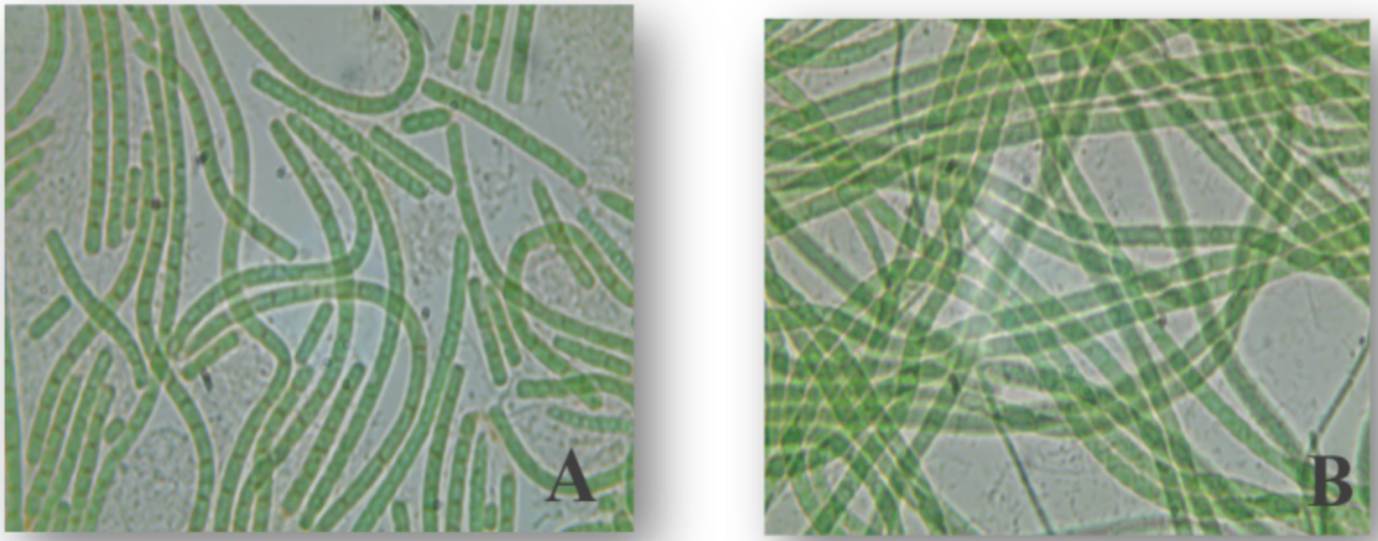

Figure 5

A) Morphology of BERC3, (B) morphology of BERC4

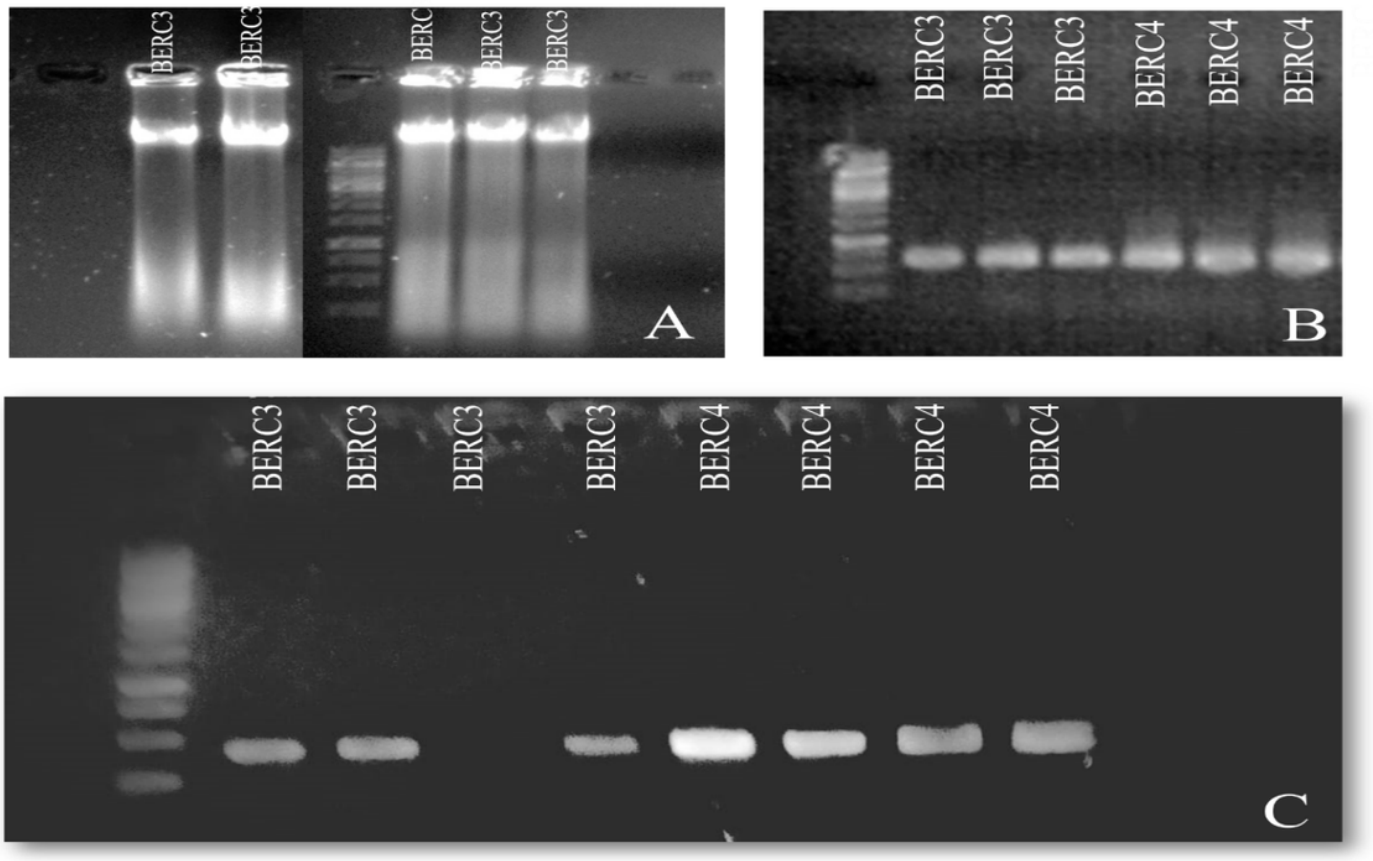

Figure 6

(A) Shows the genomic DNA of both strains BERC3 and BERC4. (B) Shows 16S rRNA gene, and (C) showed the 23S rRNA gene

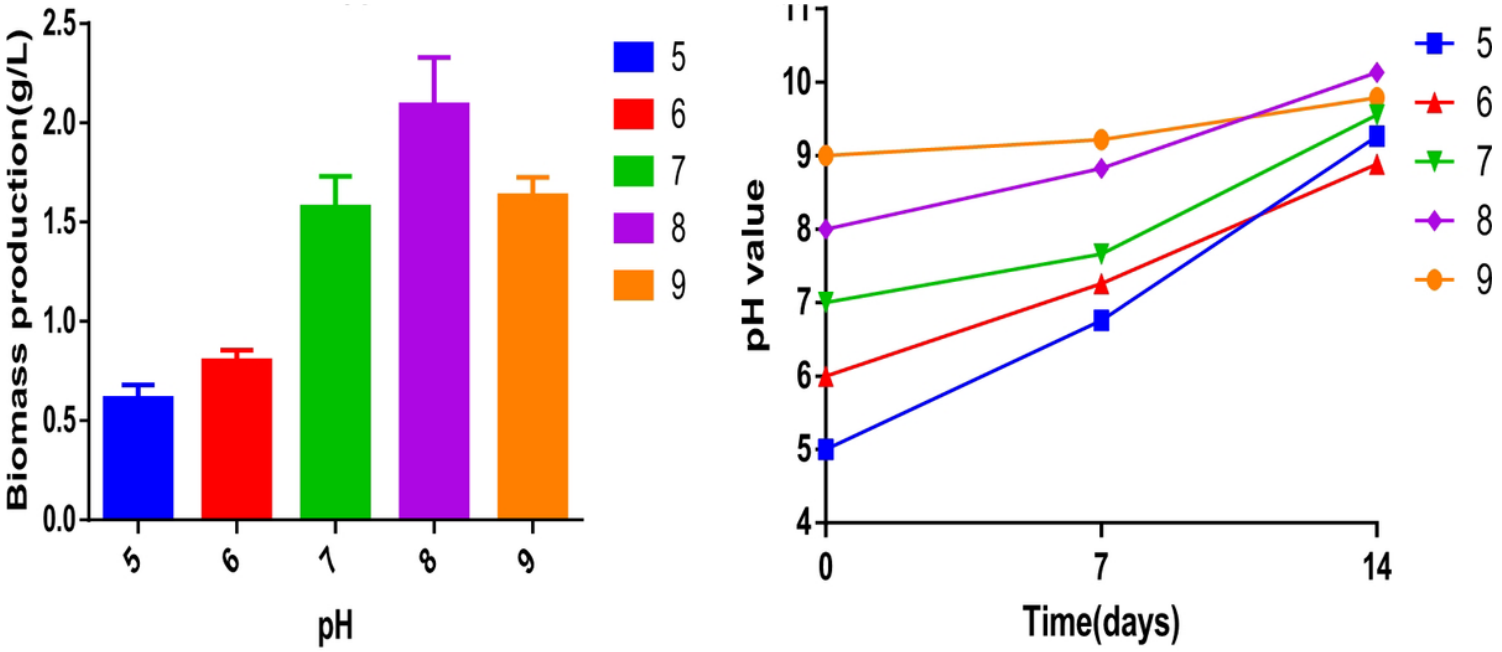

Figure 7

Biomass production at different pH values (A) and pH variation (B) of BERC3.

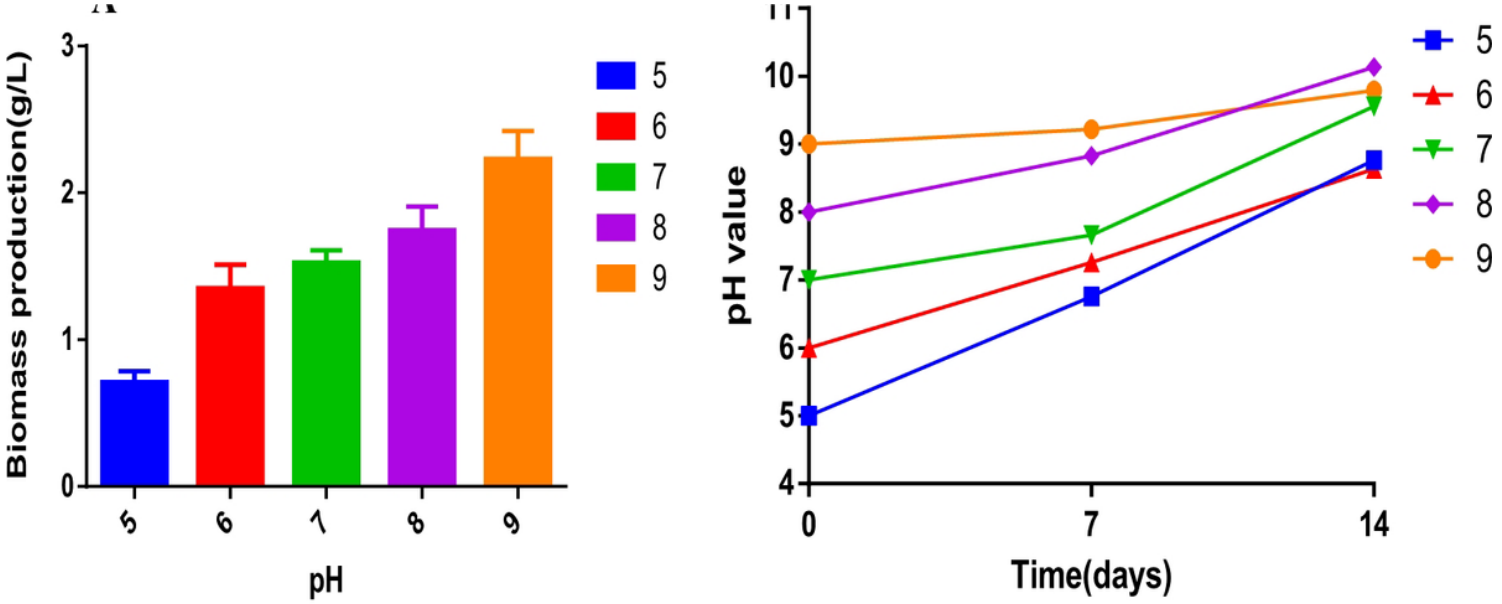

Figure 8

Biomass production at different pH values (A) and pH variation (B) of BERC4

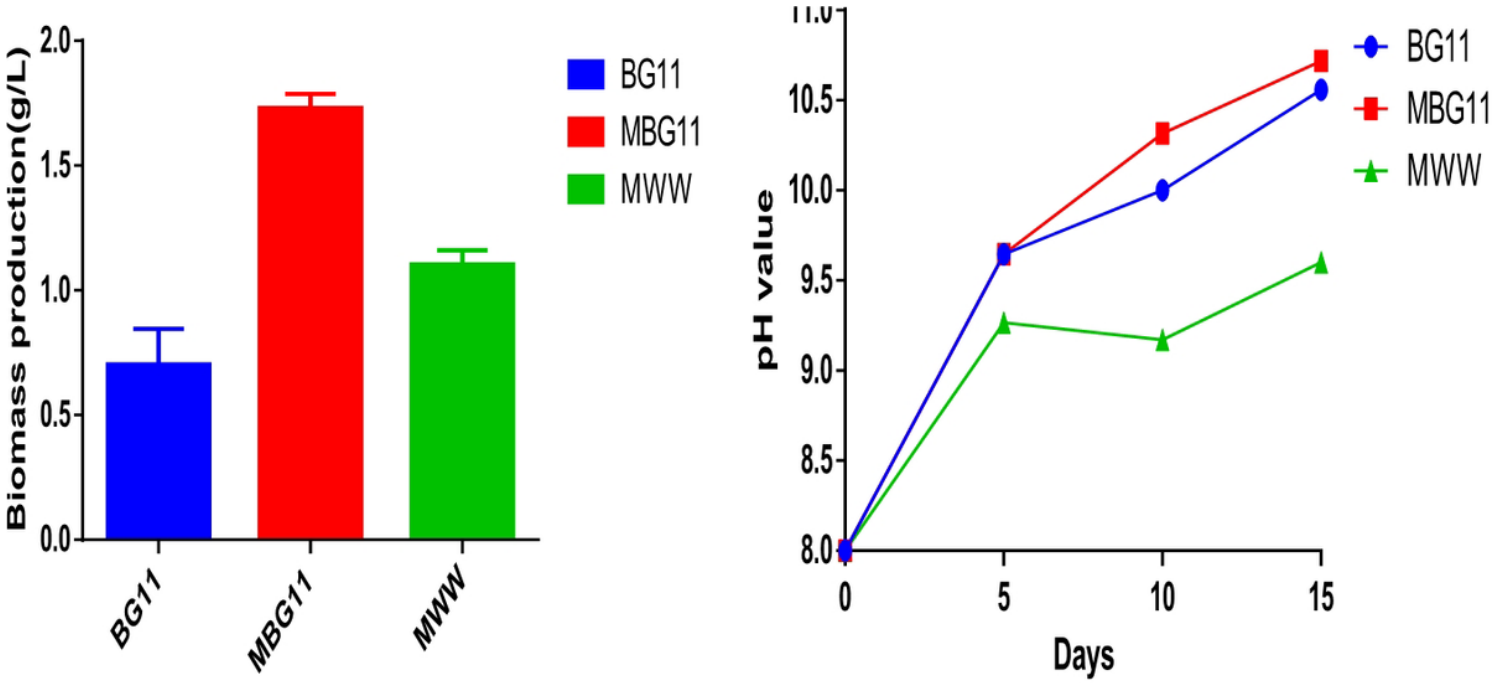

Figure 9

Nutrient removal efficiency of BERC3 (A) and pH variation (B) based on biomass production

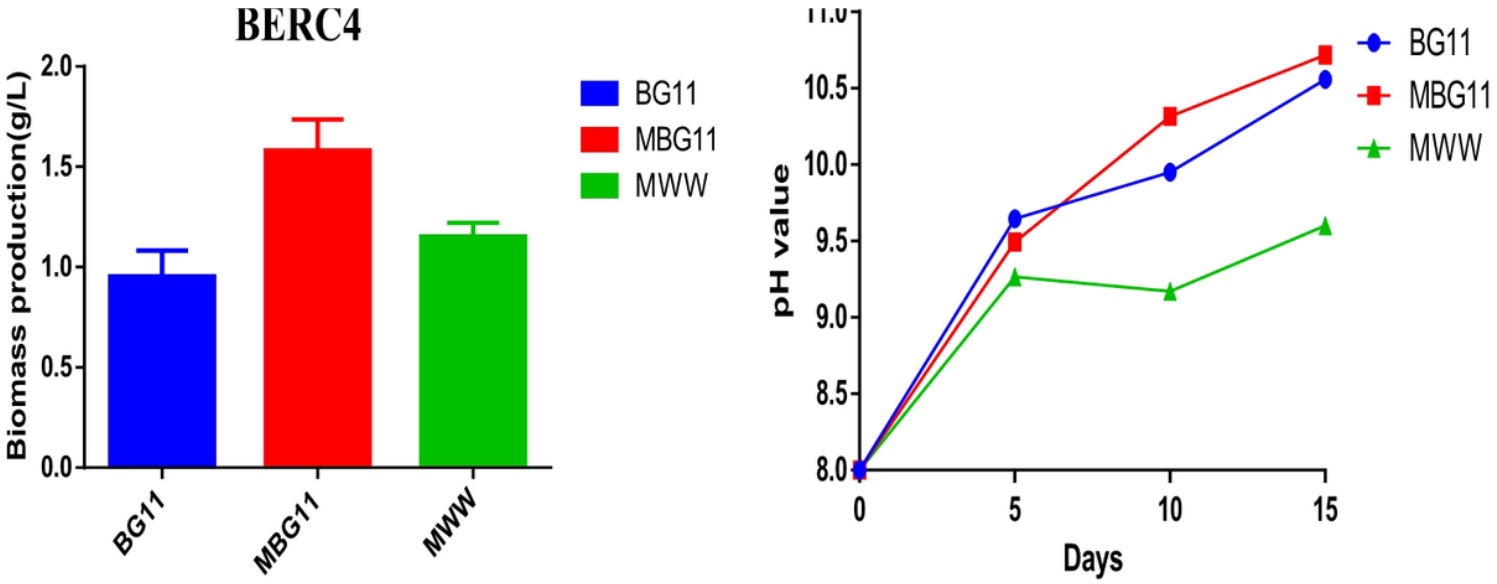

Figure 10

Nutrient removal efficiency of BERC4 (A) and pH variation (B) based on biomass production

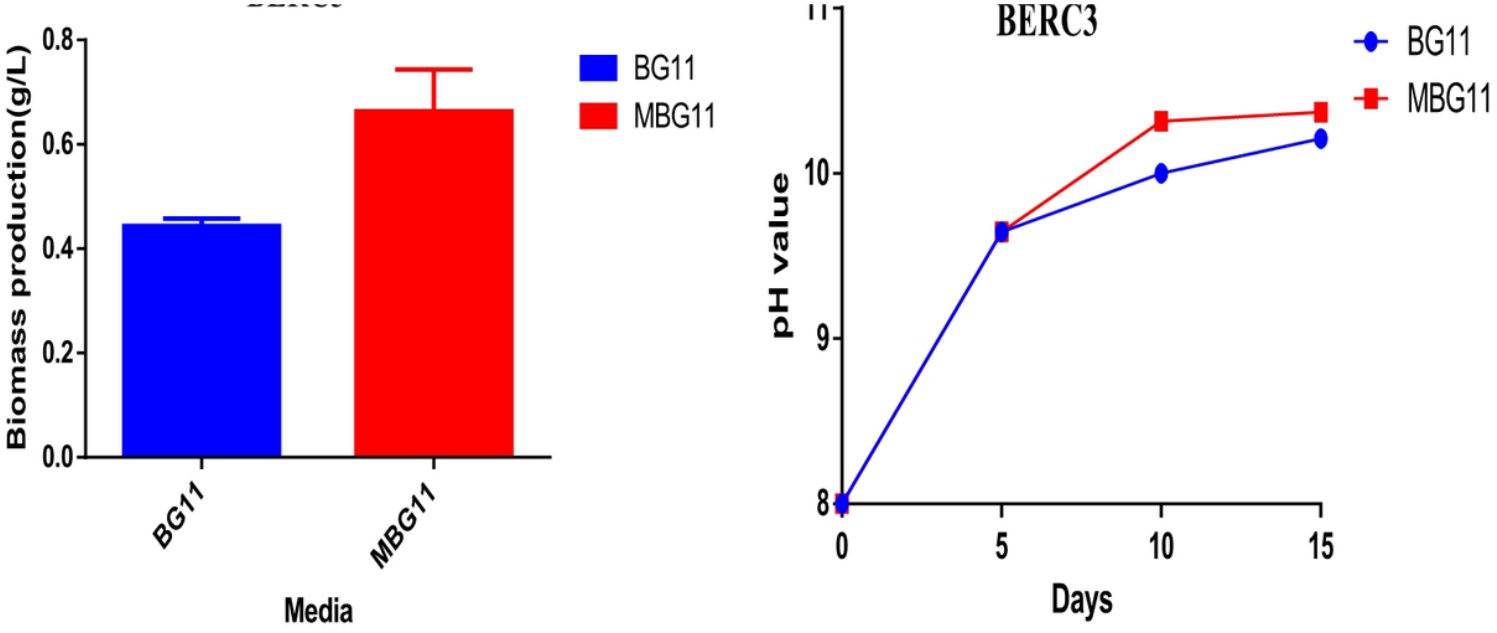

Figure 11

(A) Biomass production of BERC3 and BERC4. (B) pH variation.

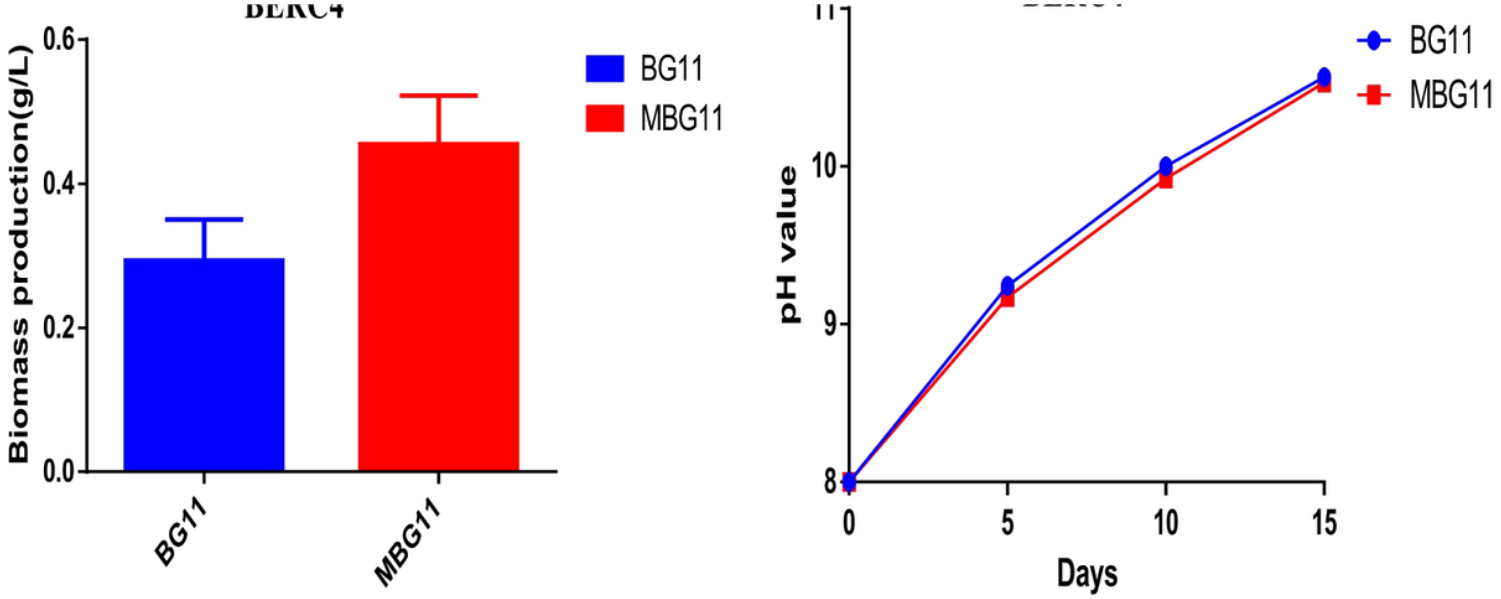

Figure 12

Biomass production of BERC4 at optimum conditions (A) and pH variation (B).

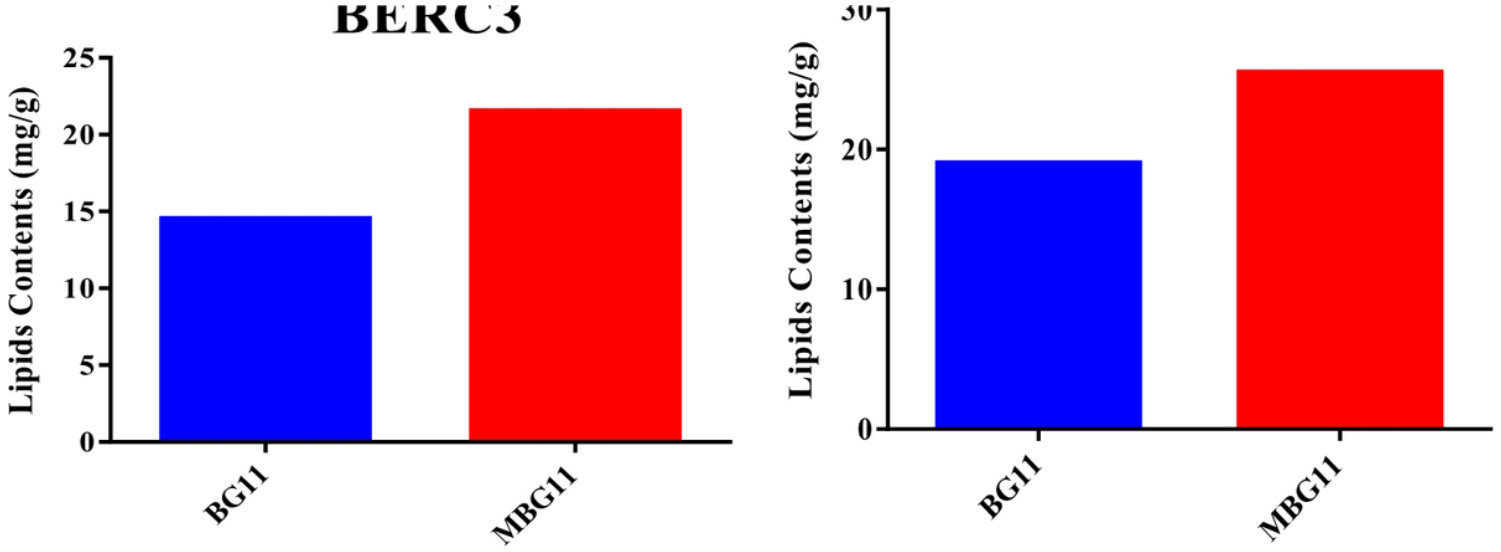

Figure 13

Lipids biosynthesis of BERC3 and BERC4

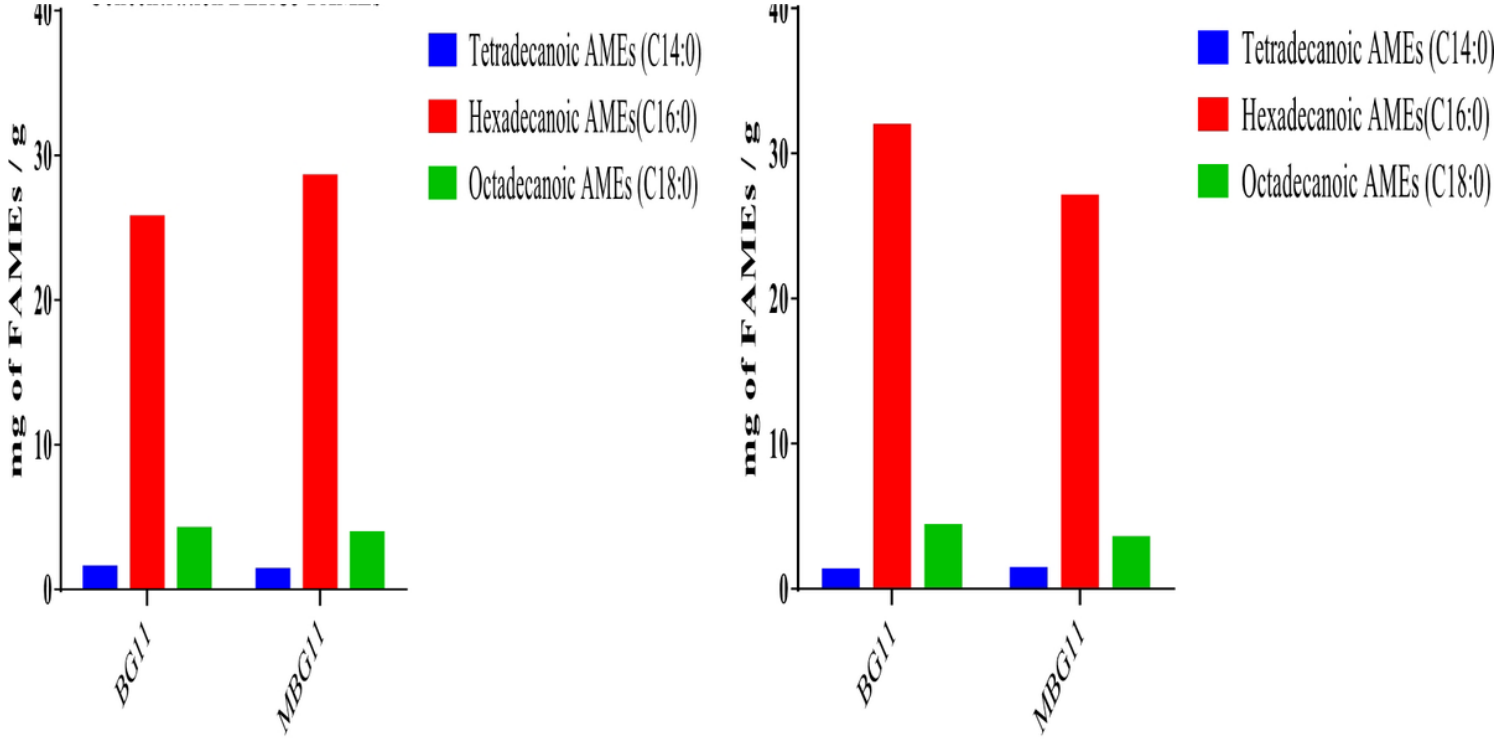

Figure 14

Impact of media on FAMEs profile

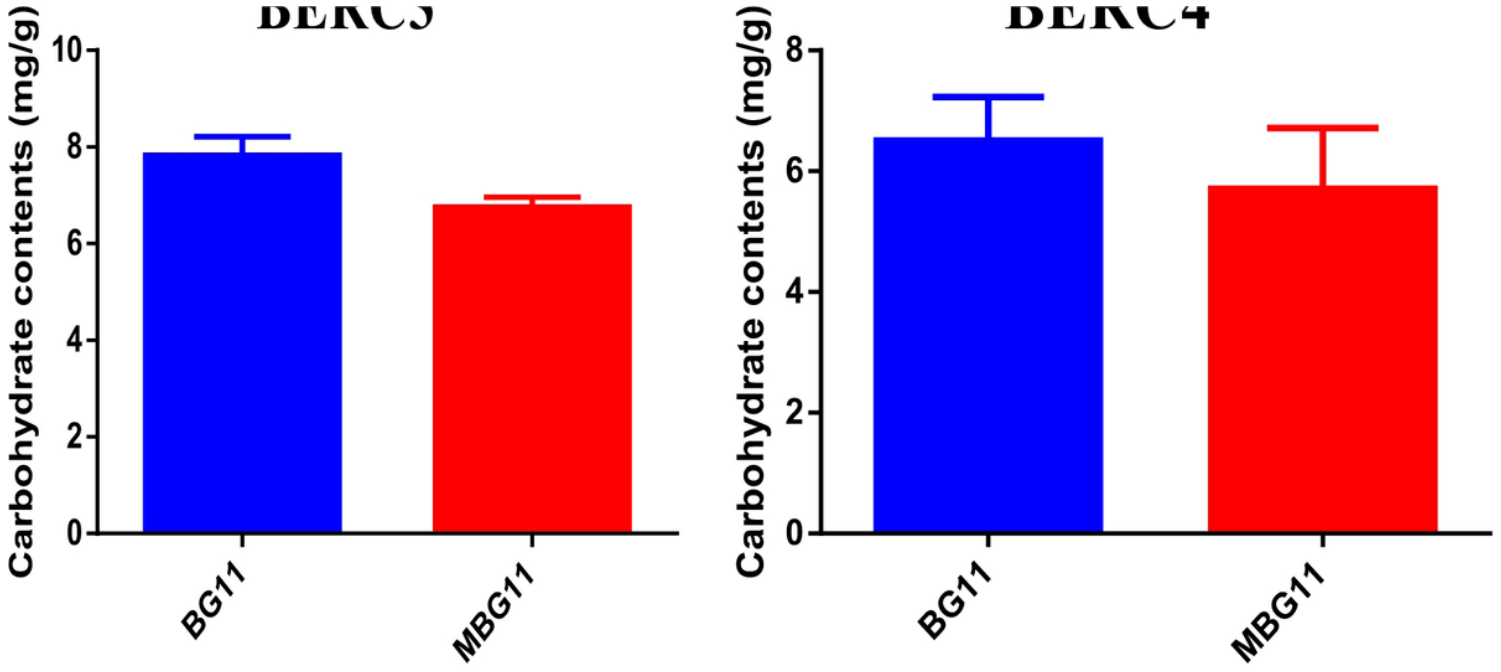

Figure 15

Impact of media on carbohydrates production

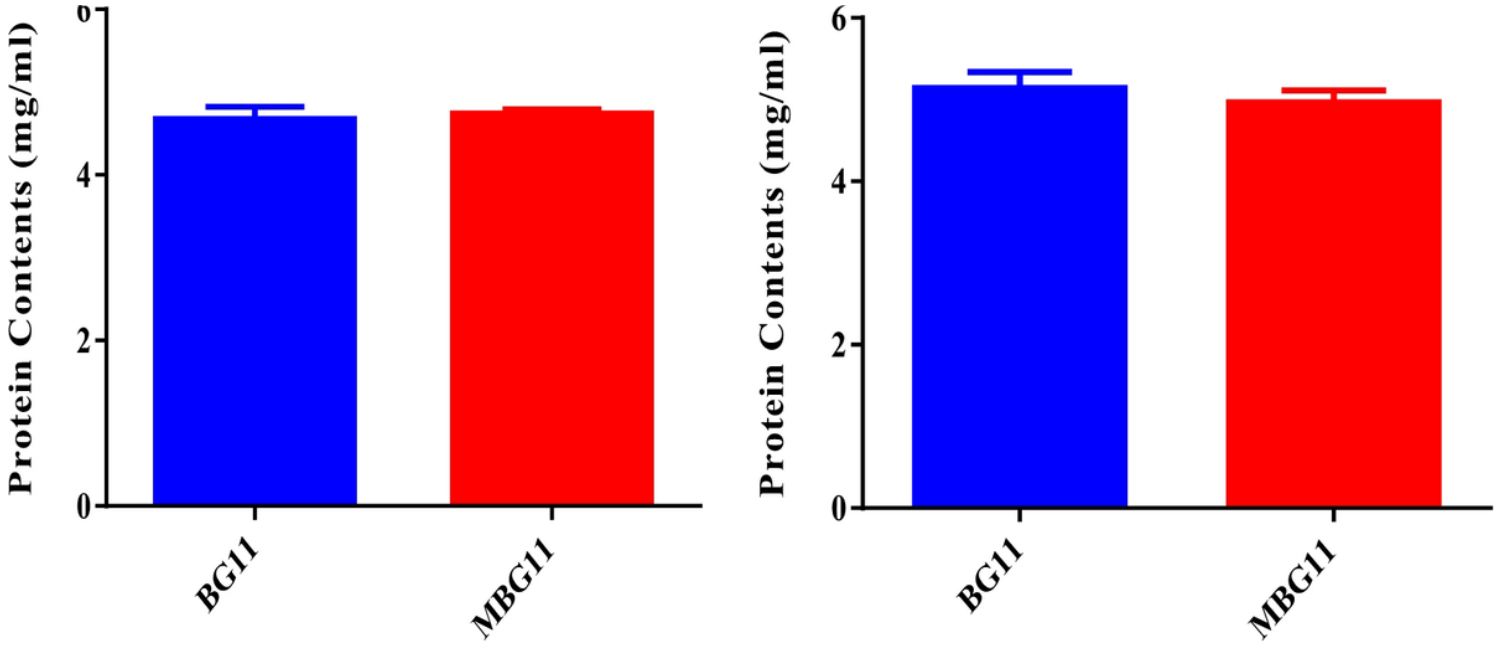

Figure 16

Impact of media on protein biosynthesis

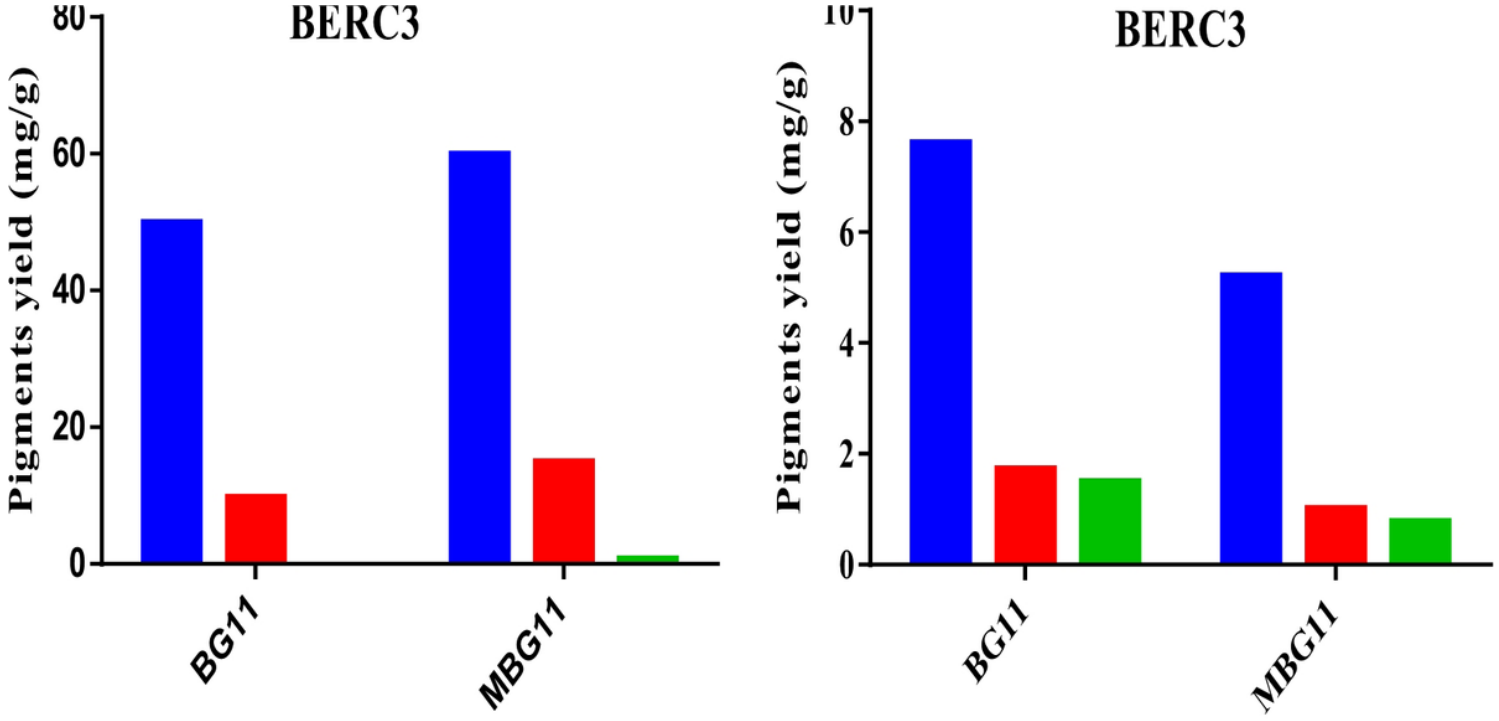

Figure 17

BERC3 (A) impact of media on phycobilins biosynthesis, BERC3 (B) impact of media on chlorophyll biosynthesis

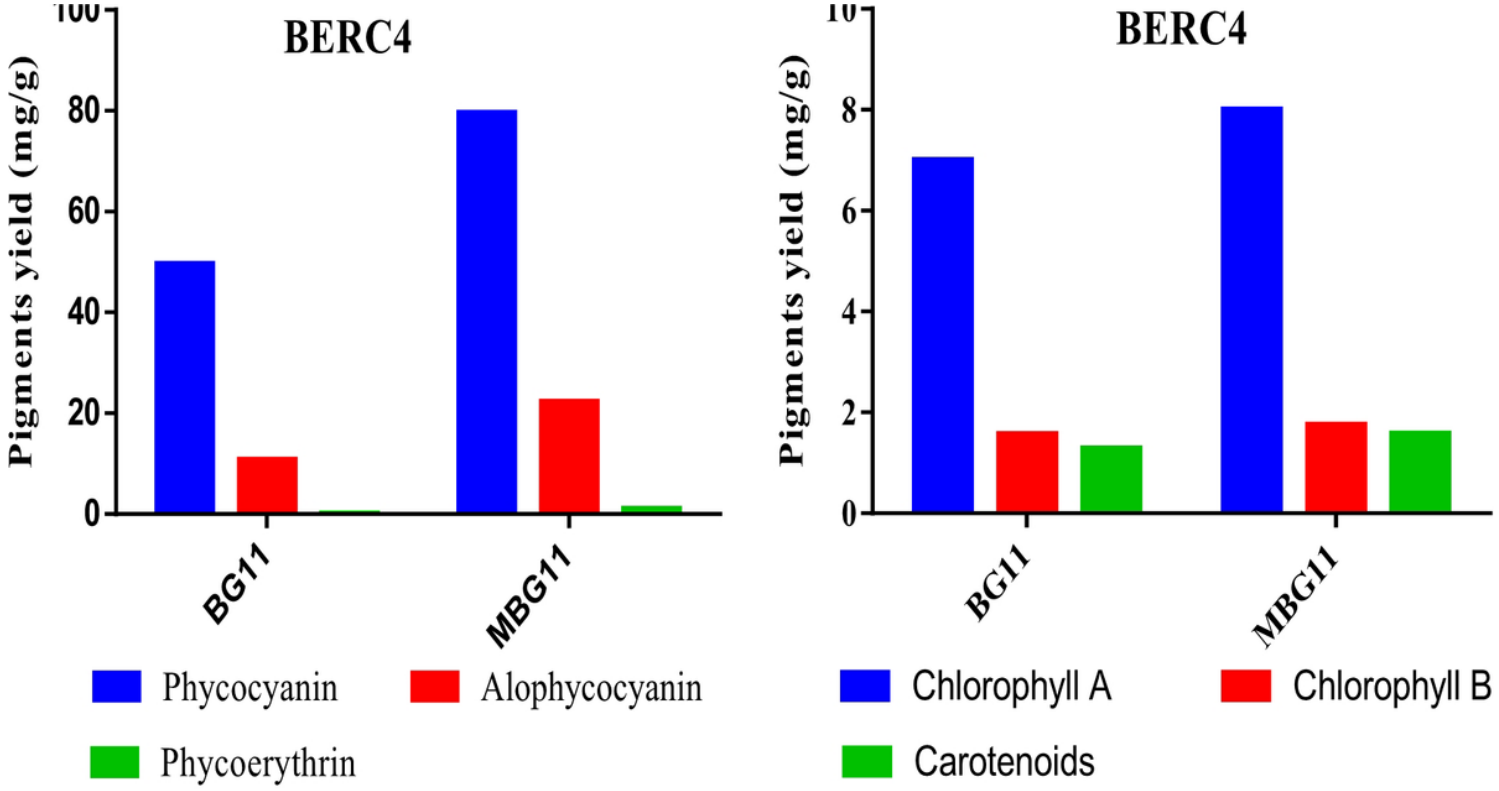

Figure 18

BERC4 (A) impact of media on phycobilinsbiosynthesis , BERC4 (B) impact of media on chlorophyll biosynthesis

Supplementary Files

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- SupplementaryTables.docx