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Research article

High carbohydrate polymer biosynthesis and CO_2 biofixation by indigenous acidophilic *Coccomyxa dispar* under elevated conditions system

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Abstract

Importance of the work: Integrating microalgal cultivation with CO_2 capture technology can improve microalgae-biorefinery process, yet, this approach is limited by the excessive supply of CO_2 (acidic) in the microalgal cultivation system.

Objectives: To isolate and screen acidophilic microalgae that could exhibit the highest growth and carbohydrate productivity under extremophile conditions

Materials & Methods: In this study, a robust strain is selected and identified. The microalgal growth, carbohydrate content and productivity of a newly isolated strain was evaluated under different extreme cultivation conditions. With the optimum condition determined at the previous stage, further up-scaling of the strain in 20 L photobioreactor under indoor and outdoor condition was compared. The CO_2 biofixation for the strain under both conditions were also evaluated.

Results: The strain was identified as *Coccomyxa dispar*. Results showed that the acidic conditions of pH 3.00, supplementation with 15% (v/v) CO_2 concentration, light intensity of 5,400 lux, and temperature of 30°C are the most suitable conditions for indoor cultivation of the selected strain. Further cultivation of the *C. dispar* in a 20 L photobioreactor showed that the growth and carbohydrate productivity for outdoor cultivation were 1.25 fold and 2.12 fold higher than for the indoor cultivation system. The CO_2 biofixation by *C. dispar* of 0.181 ± 0.002 g/L.d was obtained from cultivation under outdoor conditions. The monomer sugars found in *C. dispar* biomass are glucose, galactose and L-arabinose.

Main finding: This study showed that extremophile *C. dispar* PUSM1_2 is a robust strain that can be a great candidate for alternative bioresource based on commercial dual CO_2 capture and fine chemical products from a microalgal-carbohydrate-based product via bioconversion.

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Introduction

Energy and fine chemicals produced from petroleum-based feedstock via the conventional chemical process have reduced reserved crude oil and raised awareness of environmental issues (Nesbitt, 2020). Consequently, efforts have been made to identify a new natural resource for bio-based products as alternative feedstock. Among investigated bioresources, microalgal biomass is considered one of the renewable feedstocks that can be used to overcome this problem because microalgae that can utilize CO_2 for photosynthesis, with biomass production being adopted in integrated dual CO_2 removal and bioproduct biorefinery processes (Kassim and Meng, 2017; Bhatia et al., 2021). Microalgae biomass contains lipid, protein and carbohydrate that can be further converted into various products such as biofuel, bio-solvent and animal feed and so has great potential as a bioresource for a biorefinery system (Harun et al., 2010).

To date, integrated dual CO_2 removal and bioremediation for microalgal biomass is believed to be a potential approach to ensure the feasibility of microalgal biomass production. Mobin et al. (2019) reported that the prospect of integrating CO_2 from flue gas using a microalgal biorefinery process could reduce the microalgae operating cost by up to 20%. The introduction of CO_2 from the atmosphere or flue gas industry and nutrients from wastewater are good resources to promote microalgal growth, as the presence of additional high levels of nutrients from industrial waste could subsequently reduce the operating and production costs for microalgal cultivation.

However, the continuous introduction of CO_2 from flue gas for microalgae could acidify the medium (Morales et al., 2017). Generally, the introduced CO_2 molecules will react with water molecules forming carbonic acid (H_2CO_3). Then, the molecules would dissociate into HCO_3^- and H_2^+. The HCO_3^- generated will further dissociate into CO_2 or CO_3^{2-}, resulting in changes in the acidic medium (Hassan et al., 2012). It was reported by Tang et al. (2011) that *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 exhibited the lowest cell growth rate and biomass production cultivated under high CO_2 concentration due to the acidified cultivation medium. Adopting a robust microalgal strain that can tolerate extreme conditions, such as low pH and high CO_2 concentration, is believed could be beneficial to overcome this limitation (Tan et al., 2020). In addition, the cultivation of microalgae under these conditions could be beneficial in reducing contamination from foreign microbes during cultivation because not many microbes can sustain such elevated conditions.

In addition, the efficiency of microalgal growth and adaptation under extreme conditions could be influenced by other abiotic factors, such as temperature and light intensity. (Chiranjeevi and Mohan, 2016). Most studies have reported that changes in cultivation parameters could significantly affect the accumulation of the main biochemical compounds in microalgae, such as carbohydrates and lipids (Chiranjeevi and Mohan, 2016; Yang et al., 2018). For example, Markou et al. (2012) showed that light intensities and environmental temperature would significantly affect the growth and carbohydrate productivities of microalgae.

To date, there is limited information available on the characteristics of acidophilic microalgae under extreme conditions, especially regarding growth and carbohydrate productivity. The main objective of this study was to isolate and screen acidophilic microalgae that could exhibit the highest growth and carbohydrate productivity under extremophile conditions. At the same time, the influence was investigated of the cultivation conditions, such as pH, light intensity, CO_2 concentration and temperature on the microalgal growth rate and carbohydrate biosynthesis. At the end of this study, carbohydrate profiling and CO_2 biofixation of selected microalgae were calculated and tested using a 20 L PBR under indoor and outdoor conditions.

Material and Methods

Collection of microalgal sample

To isolate acidophilic microalgae, three water samples were collected from an abandoned mining site at Frog Hill Tasek Gelugor Penang, Malaysia (5.4354°N, 100.4847°E). The collected samples were put into sterile universal bottles and placed in an insulated box containing ice before being transported to the laboratory for isolation. The pH of the ponds was measured and recorded using a portable pH meter (Oakton pHTestr® 30; Singapore). The pH values were taken in triplicate and the average value was calculated and recorded (Table 1).

Table 1 The cell dry weight, carbohydrate content and productivity of *Coccomyxa dispar* strains

Strain	pH	Cell dry weight (g/L)	Carbohydrate content (%)	Carbohydrate productivity (mg/L.d)
PUSM1_1	4.35	0.43±0.03	14.61±0.01	3.71±0.01
PUSM1_2		1.71±0.24	37.68±0.04	50.10±0.02
PUSM1_3		0.67±0.00	25.86±0.01	10.53±0.02
PUSM1_4		0.60±0.03	39.00±0.03	13.93±0.02
PUSM1_5		0.40±0.00	41.87±0.05	10.47±0.05
PUSM1_6		0.94±0.20	16.18±0.01	9.75±0.01
PUSM2_1	3.88	0.24±0.00	35.08±0.06	4.91±0.01
PUSM2_2		0.50±0.08	28.35±0.02	0.87±0.02
PUSM2_3		0.27±0.00	30.00±0.02	4.17±0.00
PUSM2_4		0.61±0.01	11.86±0.04	4.65±0.00
PUSM3_1	5.93	0.47±0.02	11.23±0.01	2.97±0.01
PUSM3_2		0.89±0.00	23.18±0.04	13.08±0.01

Enrichment and isolation process

For the enrichment isolation process, an aliquot (10 mL) of each water sample was added to 90 mL of acidic bold basal medium (BBM) and the pH of the BBM was adjusted to pH 3.00 using 1M of hydrochloric acid (HCl). Then. the cultures were cultivated under extremophile conditions with pH 3.00 and 15% CO_2 concentration under a controlled temperature at 30 ± 2°C and illuminated with a photon intensity of 1,500 lux within photoperiod cycles of 12 hr light: 12 hr darkness for 15 d. The BBM was used throughout the process. In this stage, the BBM used throughout the study consisted of 25 g/L sodium nitrate ($NaNO_3$), 7.5 g/L magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), 2.5g/L sodium chloride (NaCl), 7.5 g/L dipotassium phosphate (K_2HPO_4), 17.5 g/L potassium dihydrogen phosphate (KH_2PO_4), 2.5 g/L calcium chloride dehydrate ($CaCl_2.2H_2O$), 8.82 g/L zinc sulfate heptahydrate ($ZnSO4.7H_2O$), 1.44 g/L manganese chloride tetrahydrate ($MnCl_2.4H_2O$), 0.71 g/L molybdenum trioxide (MoO_3), 1.57 g/L copper sulfate pentahydrate ($CuSO_4.5H_2O$), 0.49 g/L cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$), 11.42 g/L boric acid (H_3BO_3), 50 g/L ethylenediaminetetraacetic acid (EDTA), 31 g/L potassium hydroxide (KOH), 4.98 g/L iron sulfate heptahydrate ($FeSO_4.7H_2O$) and 1 mL sulfuric acid (H_2SO_4). The generic antibiotics, such as 100 ug/mL of ampicillin and 25 ug/mL of streptomycin, with fungicides, such as 20 ug/mL for both Carben-50® and Benocide®, were added to the enrichment cultivation liquid medium to avoid bacterial and fungal contamination.

After the incubation period, a series of purifications were performed using an agar plating technique, the serial dilution method followed by centrifugation and a rinsing treatment to purify the most tolerant isolates under extremophile conditions, as described by Chik et al. (2017), Izadpanah et al. (2018) and Haoujar et al. (2020). The coding of the isolated microalgal strains was recorded based on pond 1 (PUSM_1), pond 2 (PUSM_2), and pond 3 (PUSM_3). Then, the selected microalgal strains underwent further screening by observing their growth performance and calculating the cell dry weight. Finally, the most tolerant strain was selected and subjected to further analysis.

Morphological characterization and identification

Primary morphological identification and characterization of the selected purified microalgal strain was conducted by observing the bore cell size, structure and form. Identification of the selected microalga was performed using 18sRNA, for which the microalgal deoxyribonucleic acid (DNA) was extracted using a Plant DNA Extraction Kit (Brand Vivantis; KL, Malaysia) according to the manufacturer's protocol. Then, the extracted DNA was amplified on a highly conserved region (rDNA) with a length of 750 base pairs using polymerase chain reaction (PCR) with a pair of primers EUF 5'-GTC AGA GGT GAA ATT CTT GGA TTT A-3' and EUR 5'-AGG GCA GGG ACG TAA TCA ACG-3' (Gross et al., 2001). The amplification was performed for 35 cycles in a thermocycler with a Hot Start for 5 mins at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 60°C and a final extension for 1 min at 72°C.

Then, the retrieved full sequence was further compared with the known sequences in the NCBI Blast database (Chik et al., 2017). Subsequently, the top eight hits from the blast results with 98% to 99% similarity were selected and subjected to multiple sequence alignments using the Cluster X2.1 software (Sen et al., 2017). Then, the aligned sequence generated was further used to construct the phylogenetic tree using a neighbor-joining method with a 1,000 bootstrap value and Kimura's two-parameter model implemented within the Mega-X software (Sen et al., 2017).

Effect of cultivation parameters

The effect of the cultivation conditions on microalgal growth, carbohydrate content and productivity were investigated for different cultivation conditions, such as initial pH value (pH 3–11); light intensity (0–12,000 lux); temperature (20–45°C) and CO_2 concentration (0.04–25%, volume per volume, v/v) (Kassim and Meng, 2017; Barten et al., 2020). The selection of these ranges took into account the average climatic conditions in Malaysia, as described previously (Tan et al., 2020). The determination of the suitable conditions to achieve the highest microalgal biomass production and carbohydrate productivity was performed using the one-factor one-time test. This test accounts for merely one factor changing at a time, while other factors are kept fixed.

In this analysis, 10% (v/v) of active seed cultures was added into the cultivation medium and cultivated for 15 d (Kassim et al., 2021). Then, the cell growth was measured and expressed as a cell biomass concentration. The cell biomass concentration was determined by measuring the culture's optical density (OD) at 680 nm (Hadiyanto et al., 2014). Equation 1 describes the relationship between biomass concentration and OD_{680} (Fig. S1):

$$\text{Cell dry weight (g/L)} = X_{C.\ dispar} = \frac{OD_{680} + 0.0069}{0.574} \quad (1)$$

where $X_{C.\ dispar}$ is cell dry weight of *C. dispar*

Subsequently, the harvested biomass was used to determine the carbohydrate content and productivity analysis. The details of the carbohydrate analysis are presented in section 2.6. Each of the experimental readings was taken in triplicate.

Indoor and outdoor cultivation in 20 L photobioreactor system

The feasibility of the selected microalgal strain to grow at a large scale was conducted using a 20 L PBR. Approximately 10% (v/v) of microalgal culture with OD of 1.00 was added to 14.6 L of BBM in the 20 L fabricated PBR system. Cell growth, carbohydrate content and carbohydrate productivity were monitored for 15 d of cultivation. The purity of the microalga was checked frequently under 100× magnification using an oil immersion light microscope (Olympus CX41; Tyo, Japan). The schematic diagram of the experiment was set-up for a large-scale microalgae cultivation system, as shown in Fig. S2.

CO_2 biofixation by microalga

Microalgae are a type of photoautotrophic microorganism that have the potential to bio-sequestrate atmospheric CO_2. Generally, microalgal carbon biofixation is calculated by considering the ratio between the moles of CO_2 and the moles of a typical molecular formula of biomass (Suryata et al., 2010). Hence, the formula of carbon dioxide (CO_2) bio-fixation used for this study can be derived using Equation 2:

$$\underset{\text{Carbon dioxide}}{4CO_2} + \text{nutrient} + H_2O + \text{light} \longrightarrow \underset{\text{Microalgae Biomass}}{4\ CO_{0.48}H_{1.83}N_{0.11}P_{0.01}} + 3\tfrac{1}{2}\ O_2 \quad (2)$$

The CO_2 bio-fixation rate constant K can be defined using Equation 2

$$K = \frac{M_{CO2}}{M_{biomass}} = 44/23.2 = 1.89 \quad (3)$$

where M_{CO2} = 44gr/mol is the molecular biomass of carbon dioxide and $M_{biomass}$ = 23.2 gr/mol is the microalgal biomass. Hence, the total biofixation of microalgae is given by K × microalgal biomass productivity.

Carbohydrate content and productivity analysis

The carbohydrate analysis was done according to the phenol-sulfuric acid method described by Kassim et al. (2014). Briefly, 0.5 g of microalgal sample was weighed and dissolved in 2.5 mL of 2.5 N hydrochloric acid (HCl) and incubated in a hot water bath at 90°C for 3 hr. Afterward, the mixture was neutralized by adding sodium carbonate (Na_2CO_3) until the effervescence ceased and then further diluted to 50 mL with distilled water. Subsequently, the mixture was centrifuged at 4,500 revolutions per minute for 15 min. After centrifugation, 0.2 mL of sugar obtained from the supernatant was transferred to a test tube and diluted to 1.0 mL with distilled water. Then, 1.0 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid were added to the solution. Finally, the mixture was incubated in a water bath at 30°C for 30 min. The sample was measured using an ultraviolet-spectrophotometer at 485 nm (Hach DR 2800; Malente, Germany). All analyses were carried out in triplicate. The carbohydrate content and productivity were calculated using Equations 4 and 5, respectively, with glucose used as a standard for this analysis:

$$\text{Carbohydrate content, CW (\%)} = \frac{C}{V} \text{ x M} \quad (4)$$

$$\text{Carbohydrate productivity} = CP = BP \times CW \quad (5)$$

where C is the carbohydrate content (measured in milligrams per milliliter), V is the volume of the supernatant (in milliliters), M is the total volume (in milliliters) of the microalgal sample solution and BP is the microalgal biomass productivity (in milligrams per liter day).

Carbohydrate profiling

The carbohydrate profiling was done using two-step sulfuric acid hydrolysis (Van Wychen and Laurens, 2013). First, 25 ± 2.5 mg of dried microalgal biomass was weighed and added with 250 uL of 72% (weight per weight, w/w) sulfuric acid. Then, the mixture was vortexed carefully to ensure thorough mixing between the acid and the biomass. Next, the mixture was placed in a water bath at 30 ± 3°C for 1 hr. Later, the mixture was diluted to 4% (w/w) by adding 7 mL of 18.2 Mega Ohm water. Finally, the sample was autoclaved for 1 hr at 121°C using the slow setting mode. After 1 hr, the autoclave sample was neutralized using calcium carbonate and filtered using a 0.22 um filter into a vial before high-performance liquid chromatography (HPLC) analysis using a Rezex RCM-Monosaccharide Ca+2 (8%) column (Phenomenax; CA, USA) with dimensions of 300×7.8 mm and a Shimadzu RID-10A detector (Shimadzu; Kyoto, Japan). The mobile phase consisted of 100% degassed deionized water. The sample injection volume was set to 20 µL. The pump flow rate was set at 0.6 mL/min with 20 min per sample running time. The oven temperature was set at 40°C while the detector temperature was set at 70°C. Monomer sugars (glucose, galactose and L-arabinose) were used as the standard.

Statistical analysis

The statistical analysis of the data was conducted using one-way analysis of variance. Then mean comparisons was performed using One-Way ANOVA from Statistical Package for the Social Sciences (SPSS) software version 24. The results were considered significant at $p < 0.05$.

Result and Discussion

Isolation and identification

The pH of all the water samples was acidic, between 3.8 to 5.9 (Table 1), indicating that the pH of the surrounding mining area was acidic, which could have been contributed to by the chemical reaction between metals and water.

In total, 12 acidophilic microalgae were successfully purified from the enrichment process (Table 1). A further screening process was conducted by monitoring the growth of all isolates under acidic conditions. From this process, strain PUSM1_2 exhibited the highest cell dry weight of 1.71 ± 0.24 g/L followed by strains PUSM1_6 and PUSM3_2 with 0.94 ± 0.20 g/L and 0.89 ± 0.00 g/L, respectively (Fig. S3). Thus, isolate PUSM1_2 exhibited a high tolerance to a low pH and high CO_2 concentration. In contrast, the other strains showed slow growth rates under these extreme conditions. Therefore, the achievement of high growth rates by PUSM1_2 strains under acidic conditions could have been due to its origin from an acidic mining site (Tan et al., 2020).

The capability of the microalgae to accumulate carbohydrates was also evaluated in this study and the results are presented in Table 1. The study showed that PUSM1_2 exhibited remarkable differences in carbohydrate productivity of up to 50.10 ± 0.02 mg/L.d. In comparison, the lowest carbohydrate productivity of the microalgae was by the isolate PUSM2_2 (0.87 ± 0.02 mg/L.d). There was a distinct 57.59 fold difference in carbohydrate productivity for both strains. Although PUSM1_5 had a high carbohydrate content, this strain showed a very slow growth resulting in low carbohydrate productivity. Hence, PUSM1_2 was selected for subsequent investigation.

Identification of selected microalga

The selected PUSM1_2 was characterized using morphological and molecular approaches. Microscopic characterization under a light microscope showed that PUSM1_2 was a unicellular green alga with a relatively small size (6–14 µm × 3–6 µm), with an absence of pyrenoid. This alga is irregular elliptical in shape and consists of a cup-shaped chloroplast which makes up about half of the cell volume (Fig. 1A). The identity of PUSM1_2 was further confirmed using molecular phylogenetic analysis based on amplifying and sequencing its 18 rRNA genes. According to Fig. 1B,

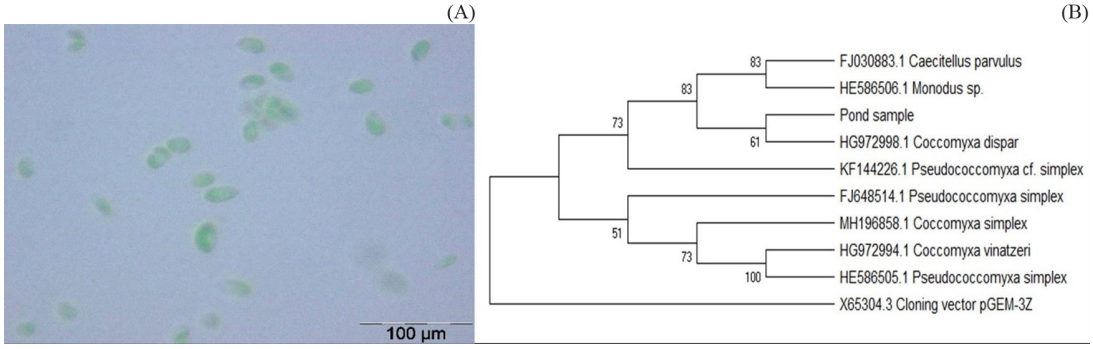

Fig. 1 Identification of PUSM1_2 isolated from mining site area: (A) morphological identification of *Coccomyxa dispar* under 100× magnification oil immersion light microscope; (B) bootstrapped neighbor-joining tree of isolated PUSM1_2 from mining site water body

the isolated PUSM1_2 was closely related to *C. dispar* with 99% similarity. Based on the morphological features and the molecular analysis performed this novel, isolated, acidophilic PUSM1_2 was named *C. dispar*, a unicellular genus belonging to the class Trebouxiophyceae. Bioprospective analysis of various biotechnology applications from this class of microalgae has been reported in multiple studies. For example, a study on *Coccomyxa* sp. KGU-D001 isolated from a dry aerial environment showed that higher biomolecular metabolites, such as the lipid content, contained 27% fatty acid that cultivated under stress conditions had great potential to be used as biodiesel feedstock (Abe et al., 2014). Another study by Triphati et al. (2021) investigating lipid accumulation in *Coccomyxa* sp. IITRSTKM4 under nitrogen-limited conditions indicated that the strain contained a high lipid content with C16–C18 fatty acid, which can potentially be converted into biodiesel. While there have been many investigations on the accumulation of lipids in *Cocomyxa* sp. for biodiesel, there is less information on the potential of microalgal carbohydrate for other bioproducts.

Effect of cultivation conditions

The cultivation parameters (pH, temperature, light intensity and CO_2 concentration) that could influence the growth and carbohydrate productivity in selected microalgae were determined using laboratory-scale experiments.

Effect of pH

Fig. 2 shows the growth profile, carbohydrate content and productivity of *C. dispar* cultivated at different initial pH values (3–11). The study showed that indigenous *C. dispar* grew well and achieved the highest biomass production of 1.81 ± 0.10 g/L under the initial pH value of 3.00. Cultivation of *C. dispar* at pH 5–9 only had biomass production levels of 1.63 ± 0.09 g/L, 1.17 ± 0.02 g/L and 0.79±0.04 g/L respectively. However, a further increase of the initial pH value to alkaline conditions up to pH 11.00 reduced the microalgal growth and biomass production by up to 85.64% compared to those at pH 3. In addition, the maximum carbohydrate content and productivity in the *C. dispar* biomass of 57.86 ± 0.58% and 49.53 ± 0.58 mg/L.d, respectively, were achieved for cultivation at pH 5. Lower carbohydrate contents and productivity levels were observed for cultivation at levels higher than pH 5.

Many studies have evaluated the influence of pH on microalgal growth and biochemical accumulation (Khalil et al., 2010; Yu et al., 2015; Khan et al., 2018). Based on those studies, microalgae can be categorized into acidophiles, neutrophiles or alkalophiles. According to the present study, the isolated *C. dispar* showed maximum growth under acidic conditions, indicating that this strain can be characterized as acidophilic. The present results were in agreement with investigations on the growth of other *Coccomyxa* species. According to Fuentes et al. (2016), the growth of *Coccomyxa onubensis* exhibited high biomass at pH levels in the range 2.5–4.5. Another study of *Coccomyxa melkoniannii* isolated from a contaminated river reported it could grow well in medium at pH 4–6.8 (Soru et al., 2019). The ability of microalgae to thrive under extreme conditions could also be attributed to the origin of the strain. The study by Eibl et al. (2014) reported that the maximum growth of the microalga Lih29 isolated from an abandoned lignite mine in Northern Ontario, Canada, was achieved for cultivation under pH levels having which a pH as the water body.

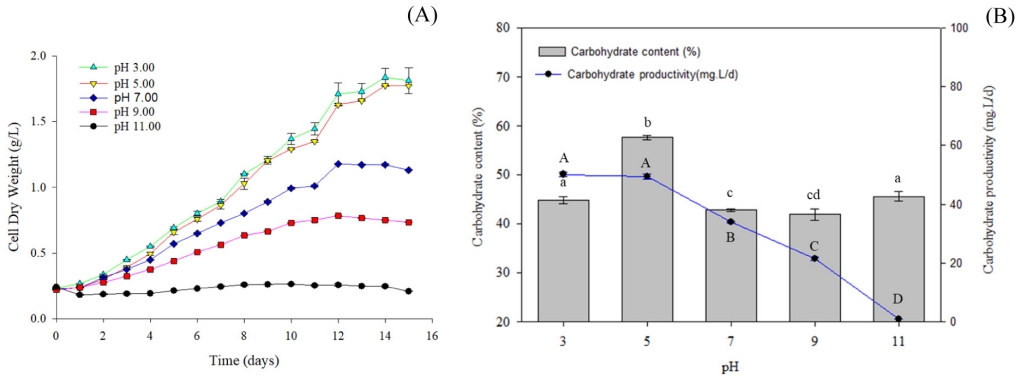

Fig. 2 Growth of *Coccomyxa dispar* cultivated at different pH: (A) growth profiles; (B) carbohydrate content and productivity, where different lowercase or uppercase letters indicate significant ($p < 0.05$) differences of carbohydrate contents or carbohydrate productivity, respectively

Effect of light intensity

Fig. 3 shows the growth profile and carbohydrate productivity for *C. dispar* cultivated at different light intensities. The maximum cell biomass production for *C. dispar* was observed for cultivation at 1.86 ± 0.10 g/L when the cultivation was illuminated under a light intensity of 5,400 lux. Notably, microalgal cultivation beyond this value reduced biomass production up to 5.10 fold. Although high growth was observed for cultivation at 5,400 lux, different results were obtained for the carbohydrate content in the *C. dispar*. The maximum carbohydrate content of 52.55 ± 1.86% was achieved when the cultivation was exposed to 1,600 lux, suggesting that the carbohydrate content of *C. dispar* was not directly correlated with growth (Fig. 5B). The lowest carbohydrate content was observed for the cultivation exposed to a low light intensity.

The study revealed that light intensity significantly affected growth and carbohydrate biosynthesis in *C. dispar*. This information agreed with another study that indicated the growth of polyculture microalgae increased up to 7.6% with increased light intensity from 740 lux to 74000 lux (Iasimone et al., 2018). Nzayisenga et al. (2020), found that the microalga *Desmodesmus* sp. Had higher biomass production up to 1.10 g/L when the culture was illuminated at 22,200 lux compared to 0.4 g/L when illuminated at 15,000 lux. Wahidin et al. (2013) showed that light could stimulate the photo-oxidation process in the cell microalgal metabolism and contribute to its growth until the saturated limit is reached. Generally, light is required for photosynthesis and the accumulation of biomass and carbohydrate. Most studies reported that the increased cell biomass production was directly proportional to the light intensity due to the illuminated light required by microalgae synthesizing cell protoplasm for growth. However, over exposure of microalgae to a high light intensity beyond it optimum value and saturation limit could negatively affect the growth and disrupt the cell.

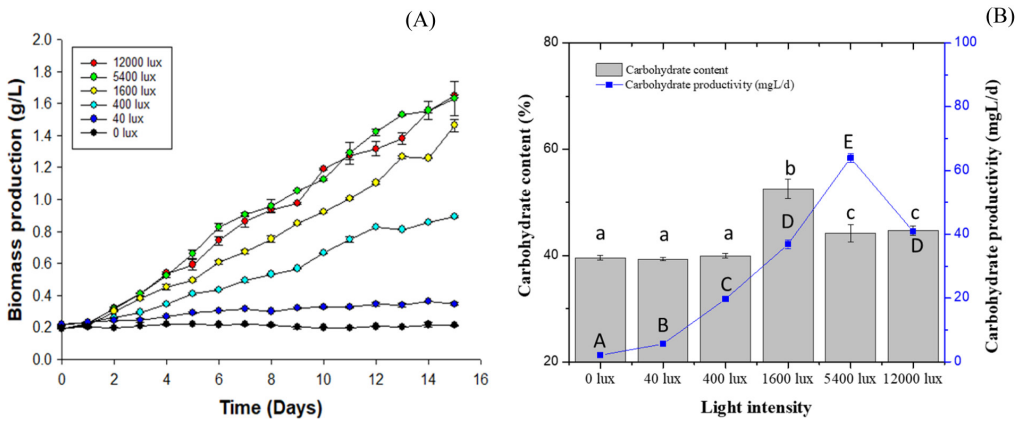

Fig. 3 Growth of *Coccomyxa dispar* cultivated at different light intensities: (A) growth profiles; (B) carbohydrate content and productivity where different lowercase or uppercase letters indicate significant ($p < 0.05$) differences of carbohydrate contents or carbohydrate productivity, respectively

Effect of temperature

The influence of cultivation parameters on *C. dispar* was further investigated by growing the microalga at different temperatures (20–45°C). The maximum *C. dispar* biomass of 1.93 ± 0.10 g/L was achieved for cultivation conducted at 30°C (Fig. 4). Cultivation of *C. dispar* at 20°C and 25°C produced biomass of 1.49 ± 0.13 g/L and 1.88 ± 0.03 g/L, respectively. Very low biomass production of 0.167 ± 0.002 g/L was observed for cultivation at 40°C. A further increase in the cultivation temperature (35–45°C), decreased the microalgal biomass production by up to 53.08%. The temperature relationship with carbohydrate production in *C. dispar* was also investigated and is illustrated in Fig. 4B. This study indicated that the values for the maximum carbohydrate content and productivity of 51.94 ± 1.1% and 66.64 ± 1.42 mg/L.d, respectively, were achieved for cultivation at 30°C. Further cultivation of the microalga at a higher temperature beyond this value could negatively affect the carbohydrate content.

The present study indicated that the cultivation temperature could significantly affect the growth performance of *C. dispar*. Generally, the optimal temperatures for microalgae are in the range 18–37°C (Thangavel et al., 2018), with temperatures lower or higher than the optimum temperature potentially slowing the growth and being lethal to most microalgal species (Singh and Singh, 2015). High temperatures led to the reduction in photosynthetic and enzyme activity in microalgae and subsequently reduced their microalgal growth rates over the cultivation period (Subhash et al., 2014). Several studies have been conducted to evaluate the effect of temperature on the microalgal growth of *Chlorella*, *Nannochloropsis*, *Chaetoceros* and *Scenedesmus* sp., indicating that this parameter is mostly species-dependent (Hodaifa et al., 2010; Chowdury et al., 2020; Peng et al., 2020).

Several factors could cause a reduction in the microalgal-carbohydrate content with increased temperature. A common reason was that temperatures could affect the outer membrane of microalgae that would then facilitate the nutrient uptake rate from the environment and affect microalgal cell carbohydrate productivity (Markou et al., 2012). In addition, cultivation beyond optimum temperature conditions would disrupt the cell enzymes in carbohydrate metabolism pathways (Khan et al., 2018).

Effect of CO_2

Another main factor influencing microalgal growth performance is the concentration of carbon dioxide (CO_2) during the cultivation process. Fig. 5 shows the growth profile and carbohydrate production of *C. dispar* cultivated using a CO_2 range between 0.04% and 25% (v/v). Cultivation of the microalga supplied with different CO_2 concentrations did not significantly influence the carbohydrate content in *C. dispar*. The study showed that the maximum *C. dispar* biomass concentration of 1.92 ± 0.10 g/L was obtained for cultivation using 15% (v/v) CO_2 concentration. *C. dispar* grown above 15% (v/v) produce a low amount of microalgal biomass.

Cultivation of *C. dispar* supplied with different CO_2 concentrations resulted in minor carbohydrate content changes but had significant impact on carbohydrate productivity. The carbohydrate content for *C. dispar* for cultivation supplemented with 15% (v/v) was slightly greater than those cultivated using air and 5% (v/v) CO_2. The highest values for carbohydrate content and productivity of 42.30 ± 1.00% and 66.27 ± 1.284 mg/L.d were achieved for cultivation supplemented with 15% CO_2. Cultivation of *C. dispar* supplemented with 5%, 10% or 25% produced 41.02 ± 0.319%, 40.54 ± 0.845% and 40.38 ± 0.426%, respectively.

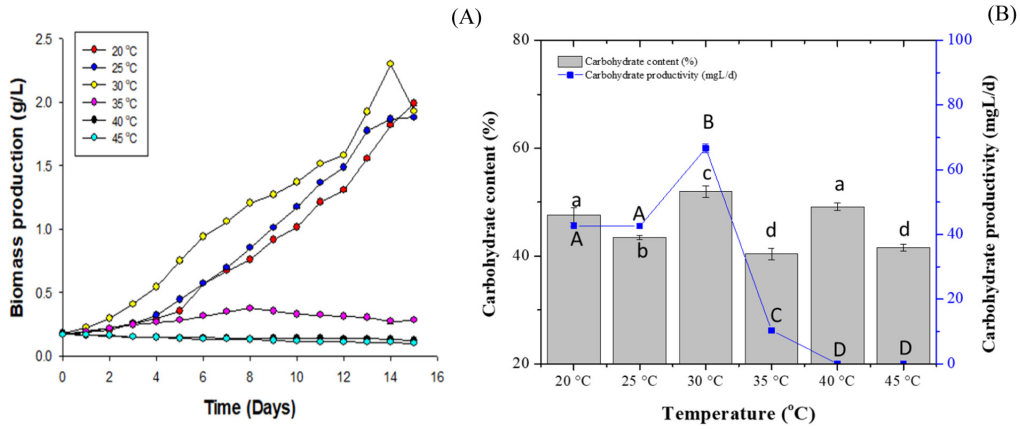

Fig. 4 Growth of *Coccomyxa dispar* cultivated at different temperature values: (A) growth profiles; (B) carbohydrate content and productivity where different lowercase or uppercase letters indicate significant ($p < 0.05$) differences of carbohydrate contents or carbohydrate productivity, respectively

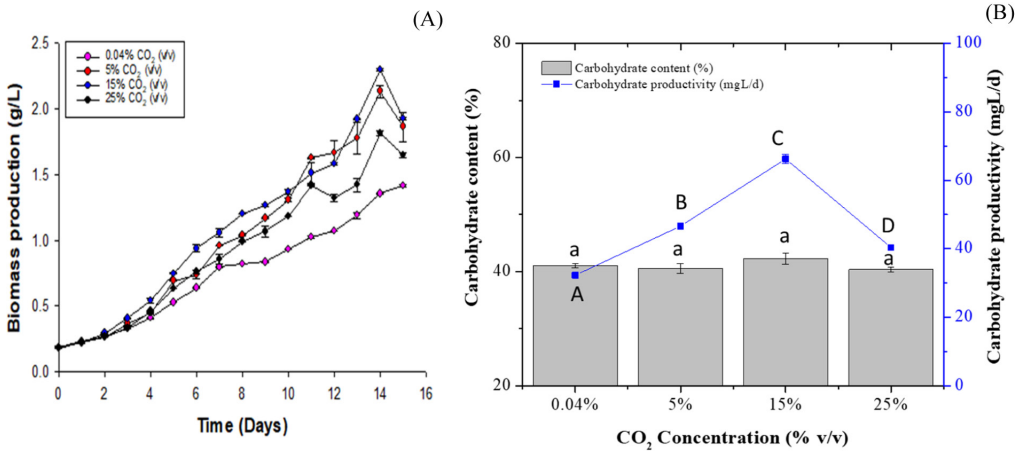

Fig. 5 Growth of *Coccomyxa dispar* supplemented with different CO_2 concentrations: (A) growth profiles (B) carbohydrate content and productivity where different lowercase or uppercase letters indicate significant ($p < 0.05$) differences of carbohydrate contents or carbohydrate productivity, respectively

The current study indicated that the isolated *C. dispar* had high tolerance to CO_2, suggesting that this strain has great potential to be used in many industrial applications. Numerous studies have been conducted on various microalgae, such as *Spirulina platensis*, *Scenedesmus bajacalifornicus*, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Patil and Kaliwal, 2017; Almomani et al., 2019). Generally, the influence of CO_2 on microalgal growth is species-dependent. For example, Wong et al. (2017) reported the most suitable CO_2 concentration to enhance *Chlorella* sp growth was 10% CO_2. In contrast, Kassim and Meng (2017) showed that *Chlorella* sp. and *Tetraselmis suecica* achieved their highest biomass concentrations when their cultures were supplied with CO_2 concentrations of 5% and 15%, respectively.

Notably, the present findings contradict other studies on the influence of CO_2 on the carbohydrate content. Most of studies on different microalgae, such as *Chlorella* sp., *Tetraselmis suecica* and *Scenedesmus dispar* indicated that a further increase of CO_2 concentration would enhance the accumulation of carbohydrates in microalgal cells (Tang et al., 2011). However, there was no significant difference in carbohydrate content observed for this new strain in the present study, suggesting that this newly isolated acidophilic *C. dispar* had high resistance toward high CO_2 concentration levels and can potentially be useful for extreme conditions without altering the carbohydrate content in the cell.

Indoor and outdoor cultivation

The suitability of *C. dispar* for large-scale and industrial applications was further evaluated in indoor and outdoor 20 L PBR systems. The indoor cultivation was performed under controlled conditions maintaining pH 3.00, 5,400 lux, 30°C and 15% (v/v) CO_2, whereas the outdoor cultivation was performed under uncontrolled light intensity and temperature, with pH 3.00 and supplied with 15% (v/v) CO_2.

The results showed that *C. dispar* under outdoor conditions had 25.68% higher biomass production than for the indoor conditions. The *C. dispar* biomass amounts obtained from outdoor and indoor cultivation experiments during the early exponential phase were 1.620 ± 0.001 g/L and 1.289 ± 0.012 g/L, respectively (Table 2). The higher biomass obtained from the outdoor cultivation could be attributed to the favorable average daily temperature and light intensity (30.38°C and 3,500 lux, respectively).

Table 2 The maximum biomass production, carbohydrate content and carbohydrate productivity under different environmental conditions

Environmental condition	Maximum biomass production (g/L)	Carbohydrate content (%)	Carbohydrate productivity (mg/L.d)	Maximum CO_2 biofixation rate (g/L.d)
Indoor	1.289±0.012[a]	28.48±0.480[a]	21.99±0.366[a]	0.153±0.001[a]
Outdoor	1.620±0.001[b]	48.74±0.779[b]	46.58±0.745[b]	0.181±0.002[b]

Mean±SD in the same column superscripted with different lowercase letters are significant ($p < 0.05$) different

The CO_2 biofixation by *C. dispar* under outdoor and indoor cultivation was also calculated according to Equations 2 and 3. The present study indicated that the CO_2 biofixation rates of *C. dispar* were better for the outdoor conditions than for indoor cultivation. Table 2 shows that the maximum value of the CO_2 biofixation rate by *C. dispar* (0.181 ± 0.002 mg/L.d) was achieved with outdoor cultivation, which was 18.30% higher than for indoor cultivation. The higher CO_2 fixation from outdoor cultivation could have been due to the different light intensities under the differing cultivation conditions. This finding was also supported by Sarker and Salam (2019) who showed that cultivation of microalgae under outdoor conditions had sufficient light transmission efficiency for microalgae compared to indoor cultivation and this resulted in high growth rates. Subsequently, this condition led to increased CO_2 biofixation rates by the cultivated microalgae. Another study on CO_2 biofixation by *Chlorella vulgaris* ESP-31 found that this strain could undergo CO_2 biofixation up to 0.211 ± 0.020 g/L.d when cultivated outdoors (Chou et al., 2019).

A similar observation was observed for the carbohydrate content and productivity. A higher carbohydrate content was achieved for *C. dispar* cultivated under outdoor conditions (Table 2). The outdoor cultivation of *C. dispar* for carbohydrate biosynthesis was 2.12 fold higher than for indoor cultivation. Another investigation reported a similar phenomenon for different microalgal strains (Huo et al., 2017; Lopes et al., 2020). For example, cultivation of *Scenedesmus rubescens* gave better results outdoors in terms of carbohydrate accumulation (Dahlin et al., 2018). Another study on temperature-tolerant *Chlorella* F&M-M49 cultivated using a Green Wall Panel PBR under outdoor conditions resulted in better growth and higher carbohydrate productivity of 190 mg/ L.d (Guccione et al., 2014).

The carbohydrate profile and monomer sugar produced by *C. dispar* were compared for growth in outdoor and indoor cultivation systems (Fig. 6). The results showed that glucose was the most abundant sugar in the *C. dispar* under outdoor and indoor conditions (0.436 ± 0.010 g/L and 0.408 ± 0.017 g/L, respectively), corresponding to 52.38% and 55.67%, respectively. In comparison, there was less *L-arabinose* in the microalgal biomass for both cultivation conditions. In addition, the study found that the glucose and galactose sugars were significantly different (6.26% and 20.77%, respectively) when for cultivation indoor compared to outdoors. The changes in the monomer sugars in *C. dispar* might have been related to the wide fluctuation in the outdoor cultivation conditions. During the cultivation period, there were clear fluctuations in the light intensity and temperature up to 11,720 lux and 41.68°C, which could have stimulated stress conditions toward *C. dispar*. Therefore, the monomer sugars in the microalga acted as a driving force to convert into macromolecules of carbohydrates, which can act as major structural features of the cell wall to counteract the effect of the stress conditions (Arad and Levy-Ontman, 2010).

Conclusion

Acid-tolerant *Coccomyxa dispar* with high CO_2 tolerance was successfully isolated from an abandoned mining area. The study showed that cultivation parameters (pH, temperature, light intensity and CO_2 concentration) significantly affected both growth and the carbohydrate content. The maximum amounts of biomass production and carbohydrate productivity of 2.299 ± 0.009 g/L and 66.27 ± 1.284 mg/L.d, respectively, were obtained for cultivation at pH 3.00, 5,400 lux, 30°C and 15% CO_2 (v/v). This study indicated that the strain had better growth and CO_2 biofixation from outdoor cultivation, suggesting that this approach is more suitable for mass biomass production. Furthermore, monomer sugars (glucose, galactose and L-arabinose) were among the dominant sugars available in the *C. dispar* biomass, which can

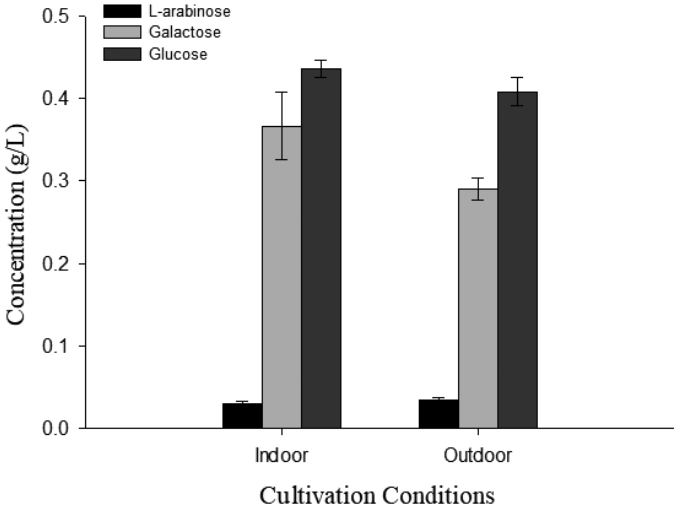

Fig. 6 The monomer sugars concentration presented in the microalgae biomass cultivated under indoor and outdoor conditions.

Table 3 Monomer sugars concentration present in microalgal biomass cultivated under indoor and outdoor conditions

Environmental conditions	L-arabinose (g/L)	Galactose (g/L)	Glucose (g/L)
Indoor	0.029±0.003[a]	0.366±0.041[a]	0.436±0.010[a]
Outdoor	0.034±0.003[a]	0.290±0.013[b]	0.408±0.017[b]

Mean±SD in the same column superscripted with different lowercase letters are significant ($p < 0.05$) different

be potentially converted into bioproducts such as bioethanol, biobutanol and biosolvent. The present study indicated that the newly isolated, acid-tolerant *C. dispar* is a potential candidate to be developed as an alternative bioresource for commercial microbial biofuel and chemicals.

Conflict of Interest

The authors declare that there are no conflicts of interest

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References

Abe, K., Ishiwatari, T., Wakamatsu, M., Aburai, N. 2014. Fatty acid content and profile of the aerial microalgae *Cocomyxa* sp. isolated from dry environments. Appl. Biochem. Biotechnol. 174: 1724–1735. doi.org/10.1007/s12010-014-1181-y

Almomani, F., Ketife, A.A., Judd, S., Shurair, M., Bhosale, R.R., Znad, H., Tawalbeh, M. 2019. Impact of CO_2 concentration and ambient conditions on microalgal growth and nutrient removal from wastewater by a photobioreactor. Sci. Total Environ. 662: 662–671. doi.org/10.1016/j.scitotenv.2019.01.144

Arad, S., Levy-Ontman, O. 2010. Red microalgal cell-wall polysaccharides: Biotechnological aspects. Curr. Opin. Biotechnol. 21: 358–364. doi.org/10.1016/j.copbio.2010.02.008

Barten, R.J.P., Wijffels, R.H., Barbosa, M.J. 2020. Bioprospecting and characterisation of temperature tolerant microalgae from Bonaire. Algal Res. 50: 102008. doi.org/10.1016/j.algal.2020.102008

Bhatia, S.K., Mehariya, S., Bhatia, R.K., et al. 2021. Wastewater based microalgal biorefinery for bioenergy production: Progress and challenges. Sci. Total Environ. 751: 141599. doi.org/10.1016/j.scitotenv.2020.141599

Chik, M.N., Yahya, L.Y., Kamarudin. K.F., Tao, D.G., Takriff, M.S. 2017. Isolation, purification and identification of microalgae from coal-fired power plant environment. Malaysian J. Anal. Sci. 21: 460–469. doi.org/10.17576/mjas-2017-2102-22

Chiranjeevi, P., Mohan, S.V. 2016. Optimizing the critical factors for lipid productivity during stress phased heterotrophic microalgae cultivation. Front. Energy Res. 4: 26. doi.org/10.3389/fenrg.2016.00026

Chou, H.H., Su, H.Y., Song, X.D., Chow, T.J., Chen, C.Y., Chang, J.S., Tse-Min, L., 2019. Isolation and characterization of *Chlorella* sp. mutants with enhanced thermo- and CO_2 tolerances for CO_2 sequestration and utilization of flue gases. Biotechnol. Biofuels 12: 251. doi.org/10.1186/s13068-019-1590-9

Chowdury, K.H., Nahar, N., Deb, U.K. 2020. The growth factors involved in microalgae cultivation for biofuel production: A review. Comput. Water Eng. Environ. Eng. 9: 185–215. doi.org/10.4236/cweee.2020.94012

Dahlin, L.R., Van Wychen, S., Gerken, H.G., McGowen, J., Pienkos, P.T., Posewitz, M.C., Guarnieri, M.T. 2018. Down-selection and outdoor evaluation of novel, halotolerant algal strains for winter cultivation. Front Plant Sci. 9: 1513–1513. doi.org/10.3389/fpls.2018.01513

Eibl, J.K., Corcoran, J.D., Senhorinho, G.N.A., et al. 2014. Bioprospecting for acidophilic lipid-rich green microalgae isolated from abandoned mine site water bodies. AMB Expr. 4: 7. doi.org/10.1186/2191-0855-4-7

Fuentes, J.L., Huss, V.A.R., Montero, Z., Torronteras, R., Cuaresma, M., Garbayo, I., Vilchez, C. 2016. Phylogenetic characterization and morphological and physiological aspects of a novel acidotolerant and halotolerant microalga *Coccomyxa onubensis* sp. nov. (Chlorophyta, Trebouxiophyceae). J. Appl. Phycol. 28: 3269–3279. doi.org/10.1007/s10811-016-0887-3

Gross, W., Heilmann, I., Lenze, D., Schnarrenberger, C. 2001. Biogeography of the Cyanidiaceae (Rhodophyta) based on 18S ribosomal RNA sequence data. Eur. J. Phycol. 36: 275–280. doi.org/10.1080/09670260110001735428

Guccione, A., Biondi, N., Sampietro, G., Rodolfi, L., Bassi, N., Tredici, M.R. 2014. *Chlorella* for protein and biofuels: From strain selection to outdoor cultivation in a Green Wall Panel photobioreactor. Biotechnol. Biofuels 7: 84. doi.org/10.1186/1754-6834-7-84

Hadiyanto, H., Soetrisnanto, D., Christwardana, M. 2014. Phytoremediation of palm oil mill effluent using *Pistia stratiotes* plant and algae *Spirulina* sp. for biomass production. Int. J. Eng. 27: 1809–1814. doi.org/10.5829/idosi.ije.2014.27.12c.02

Haoujar, I., Cacciola, F., Manchado, M., et al. 2020. Isolation of microalgae from Mediterranean seawater and production of lipids in the cultivated species. Foods 9: 1601. doi.org/10.3390/foods9111601

Harun, R., Singh, M., Forde, G.M., Danquah, M.K. 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sust. Energ. Rev. 14: 1037–1047. doi.org/10.1016/j.rser.2009.11.004

Hassan, S.H., Hameed, M.S.A., Hammouda, O.E., Ghazal, F.M., Hamed, S.M. 2012. Effect of different growth conditions on certain biochemical parameters of different cyanobacterial strains. Malays. J. Microbiol. 8: 266–272.

Hodaifa, G., Martínez, M.E., Sánchez, S. 2010. Influence of temperature on growth of *Scenedesmus obliquus* in diluted olive mill wastewater as culture medium. Eng. Life Sci. 10: 257–264. doi.org/10.1002/elsc.201000005

Huo, S., Shang, C., Wang, Z., Zhou, W., Cui, F., Zhu, F., Yuan, Z., Dong, R. 2017. Outdoor growth characterization of an unknown microalga screened from contaminated *Chlorella* culture. Biomed Res. Int. 2017: 5681617. doi.org/10.1155/2017/5681617

Iasimone, F., Panico, A., De Felice, V., Fantasma, F., Iorizzi, M., Pirozzi, F. 2018. Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics. J. Environ. Manage. 223: 1078–1085. doi.org/10.1016/j.jenvman.2018.07.024

Izadpanah, M., Gheshlaghi, R., Mahdavi, M.A., Elkamel, A. 2018. Effect of light spectrum on isolation of microalgae from urban wastewater and growth characteristics of subsequent cultivation of the isolated species. Algal Res. 29: 154–158. doi.org/10.1016/j.algal.2017.11.029

Kassim, M.A., Kirtania, K., De La Cruz, D., Cura, N., Srivatsa, S.C., Bhattacharya, S. 2014. Thermogravimetric analysis and kinetic characterization of lipid-extracted *Tetraselmis suecica* and *Chlorella* sp. Algal Res. 6: 39–45. doi.org/10.1016/j.algal.2014.08.010

Kassim, M.A., Meng, T.K. 2017. Carbon dioxide (CO_2) biofixation by microalgae and its potential for biorefinery and biofuel production. Sci. Total Environ. 584–585: 1121–1129. doi.org/10.1016/j.scitotenv.2017.01.172

Kassim, M.A., Ramli, S.H., Meng, T.K. 2021. Analysis of microalgal growth kinetic model and carbohydrate biosynthesis cultivated using agro-industrial waste residuals as carbon source. Prep. Biochem. Biotechnol. 1–11. doi.org/10.1080/10826068.2021.1969576

Khalil, Z.I., Asker, M.M.S., El-Sayed, S., Kobbia, I.A. 2010. Effect of pH on growth and biochemical responses of *Dunaliella bardawil* and *Chlorella ellipsoidea*. World J. Microbiol. Biotechnol. 26: 1225–1231. doi.org/10.1007/s11274-009-0292-z

Khan, M.I., Shin, J.H., Kim, J.D. 2018. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb. Cell Fact. 17: 36. doi.org/10.1186/s12934-018-0879-x

Lopes, A.P., Santos, F.M., Silva, T.F.C.V., Vilar, V.J.P., Pires, J.C.M. 2020. Outdoor cultivation of the microalga *Chlorella vulgaris* in a new photobioreactor configuration: The effect of ultraviolet and visible radiation. Energies 13: 1962. doi.org/10.3390/en13081962

Markou, G., Angelidaki, I., Georgakakis, D. 2012. Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Appl. Microbiol. Biotechnol. 96: 631–645. doi.org/10.1007/s00253-012-4398-0

Mobin, S.M.A., Chowdhury, H., Alam, F. 2019. Commercially important bioproducts from microalgae and their current applications – A review. Energy Procedia 160: 752–760. doi.org/10.1016/j.egypro.2019.02.183

Morales, M., Sánchez, L., Revah, S. 2017. The impact of environmental factors on carbon dioxide fixation by microalgae. FEMS Microbiol. Lett. 365. doi.org/10.1093/femsle/fnx262

Nesbitt, E. R. 2020. Using waste carbon feedstocks to produce chemicals. Ind. Biotechnol. 16: 1-17. doi.org/10.1089/ind.2020.29217.ern

Nzayisenga, J.C., Farge, X., Groll, S.L., Sellstedt, A. 2020. Effects of light intensity on growth and lipid production in microalgae grown in wastewater. Biotechnol. Biofuels. 13: 4. doi.org/10.1186/s13068-019-1646-x

Patil, L., Kaliwal, B. 2017. Effect of CO_2 concentration on growth and biochemical composition of newly isolated indigenous microalga *Scenedesmus bajacalifornicus* BBKLP-07. Appl. Biochem. Biotechnol. 182: 335–348. doi.org/10.1007/s12010-016-2330-2

Peng, X., Meng, F., Wang, Y., Yi, X., Cui, H. 2020. Effect of pH, temperature, and CO_2 concentration on growth and lipid accumulation of *Nannochloropsis* sp. MASCC 11. J. Ocean Univ. China 19: 1183–1192. doi.org/10.1007/s11802-020-4302-y

Sarker, N.K., Salam, P.A. 2019. Indoor and outdoor cultivation of *Chlorella vulgaris* and its application in wastewater treatment in a tropical city—Bangkok, Thailand. SN Appl. Sci. 1: 1645. doi.org/10.1007/s42452-019-1704-9

Sen, G., Zihao, Y., Li, N., Zhang, J., Liu, Z. 2017. Determining sequence identities: BLAST, phylogenetic analysis, and syntenic analyses. In: Liu, Z. (Ed.). Bioinformatics in Aquaculture. Wiley–Blackwell, Hoboken, NJ, USA, pp. 30–42. https://doi.org/10.1002/9781118782392.CH2

Singh, S.P., Singh, P. 2015. Effect of temperature and light on the growth of algae species: A review. Renew. Sust. Energ. Rev. 50: 431–444. doi.org/10.1016/j.rser.2015.05.024

Soru, S., Malavasi, V., Caboni, P., Concas, A., Cao, G. 2019. Behavior of the extremophile green alga *Coccomyxa melkonianii* SCCA 048 in terms of lipids production and morphology at different pH values. Extremophiles 23: 79–89. doi.org/10.1007/s00792-018-1062-3

Subhash, G.V., Rohit, M.V., Devi, M.P., Swamy, Y.V., Mohan, S.V. 2014. Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. Bioresour. Technol. 169: 789–793. doi.org/10.1016/j.biortech.2014.07.019

Suryata, I., Svavarsson, H.G., Einarsson, S. 2010. Geothermal CO_2 Bio-mitigation techniques by utilizing microalgae at the blue lagoon, Iceland. In: Proceedings of Thirty-Fourth Workshop on Geothermal Reservoir Engineering. Stanford, CA, USA, pp. 1-8.

Tan, K.M., Kassim, M.A., Ng, Z.J., Lalung, J. 2020. Isolation and characterization of novel acidophilic microalgae from abandoned mining site area for carbohydrate biosynthesis and its kinetic growth study in photobioreactor. In: Proceedings of IOP Conference Series: Materials Science and Engineering. Lumut, Perak, Malaysia, pp. 012011–012021. doi:10.1088/1757-899X/716/1/012011

Tang, D., Han, W., Li, P., Miao, X., Zhong, J. 2011. CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. Bioresour. Technol. 102: 3071–3076. doi.org/10.1016/j.biortech.2010.10.047

Thangavel, K., Krishnan, P.R., Nagaiah, S., Kuppusamy, S., Chinnasamy, S., Rajadorai, J.S., Olaganathan, N.G., Dananjeyan, B. 2018. Growth and metabolic characteristics of oleaginous microalgal isolates from Nilgiri biosphere Reserve of India. BMC Microbiol. 18: 1. doi.org/10.1186/s12866-017-1144-x

Triphati, S., Choudhary, S., Poluri, K.M. 2021. Insights into lipid accumulation features of *Coccomyxa* sp. IITRSTKM4 under nutrient limitation regimes. Environ. Technol. Innov. 24: 101786. doi.org/10.1016/j.eti.2021.101786

Van Wychen, S., Laurens, L.M.L. 2013. Determination of total carbohydrates in algal biomass: Laboratory analytical procedure (LAP). National Renewable Energy Laboratory. Washington, DC, USA. https://www.nrel.gov/docs/fy16osti/60957.pdf, 18 January 2022.

Wahidin, S., Idris, A., Shaleh, S.R.M. 2013. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. Bioresour. Technol. 129: 7–11. doi.org/10.1016/j.biortech.2012.11.032

Wong, Y., Ho, K., Lai, P., Leung C., Ho, Y., Lee, O., Yung, K., Leung, H. 2017. A study on algal growth behavior under different sparging period of CO_2 supplementation. In: Proceedings of The 1st International Conference on Beneficial Uses of Algal Biomass. Hong Kong, China, pp. 19–28.

Yang, L., Chen, J., Qin, S., et al. 2018. Growth and lipid accumulation by different nutrients in the microalga *Chlamydomonas reinhardtii*. Biotechnol. Biofuels 11: 40. doi.org/10.1186/s13068-018-1041-z

Yu, X., Chen, L., Zhang, W. 2015. Chemicals to enhance microalgal growth and accumulation of high-value bioproducts. Front. Microbiol. 6: 1–10. https://doi.org/10.3389/FMICB.2015.00056/BIBTEX