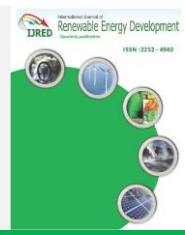




Contents list available at IJRED website

International Journal of Renewable Energy Development

Journal homepage: <https://ijred.undip.ac.id>



Research Article

Study on the Potential for Biodiesel Production of Microalgal Consortia from Brackish Water Environment in Rayong Province, Thailand

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Abstract. Microalgae are photoautotrophic microorganisms that can be grown in a wide variety of water environments. They are the most promising biodiesel source, with the potential to replace fossil diesel. In this study, microalgae samples were collected from the brackish water environment of three locations in Rayong province, Thailand including Phra Chedi Klang Nam (PKC), Noen Kho Canal (NKC), and Raksamae Bridge (RSM), and induced to form multi-algae communities or microalgal consortia (MC). All consortia were cultured and analyzed for their ability to produce biomass and lipid. The result was found that the biomass concentration of MC-RSM was 0.65 ± 0.05 mg.L⁻¹, which is higher than 1.2 and 1.5-times of MC-PCK and MC-NKC, respectively. The most common microalgae species found under all cultures were green algae (*Chlorophyta*) and diatom (*Bacillariophyta*), and the dominant species was the green algae, *Chlorella* sp. The lipid content of all samples ranged from 28.07 ± 0.60 to $33.21 \pm 0.79\%$ of dry weight, and the highest value was noticed in the MC-RSM sample. The fatty acid composition of fatty acid methyl ester (FAME) was also evaluated as feasibility for biodiesel production. FAME profiles of each sample showed high amounts of saturated fatty acids (SFAs) ranging from 67.82%-71.31% of total fatty acids. The majority of the SFAs in all were palmitic acid (C16:0) followed by myristic acid (C14:0, and stearic acid (C18:0). Therefore, all microalgal consortia showed great fatty acid profiles and these have the potential for use as feedstock for biodiesel production.

Keywords: Microalgal consortia; brackish water; lipid; FAME; biodiesel production



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Received: 2nd April 2022; Revised: 19th June 2022; Accepted: 22nd July 2022; Available online: : 28th July 2022

1. Introduction

In recent years, the exponential increase in industrialization, population, and urbanization has resulted in a worldwide energy crisis and concerns about reliance on non-renewable energy sources. So, biofuel such as biodiesel has received considerable attention because it is renewable energy and nontoxic fuel (Mohammady 2011; Khan *et al.* 2021). Microalgae are the most promising biodiesel source, with the potential to replace fossil diesel. Microalgae are photoautotrophic microorganisms that include prokaryotic and eukaryotic species. They have (1) higher bio-oil productivity than other forms of oil crops; (2) higher photosynthetic efficiency than terrestrial plants; (3) potentiality to grow in a wide range of environments like brackish or saline water, and wastewater from various sources including urban, agricultural, and industrial effluents; and (4) potentiality to utilize industrial exhaust gasses as a carbon source (Chisti 2007; Chankhong *et al.* 2018; Fuad *et al.* 2021).

Bio-oil or lipid from microalgae can be converted to biodiesel through a chemical reaction known as "transesterification" which involves the mixture of

triacylglycerol (TAG), alcohol, and the catalytic process (Veillette *et al.* 2012). Many microalgae species have favorable characteristics for biodiesel production, such as *Acutodesmus* sp., *Botryococcus braunii*, *Chlorella* sp., *Dunaliella* sp., *Nitzschia* sp., and *Spirulina* sp. The production level of lipid content in microalgae species can reach up to 75% and lipid levels between 20 and 50% are quite common (Mata *et al.* 2010; Cobos *et al.* 2017). Fatty acids in the microalgal lipids, including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), are similar to those found in plant oils (Janta *et al.* 2013; Thao *et al.* 2017). Moreover, microalgal biodiesel has properties similar to those of petroleum diesel. These include viscosity, density, flash point, CN, and heating value (Tayari *et al.* 2020).

Because of their great productivity with biomass and lipid content, microalgae have been considered as the source of third-generation biofuels. Thus, many researchers have been focused on identifying species with the highest potential for biofuel production. Although several research has found microalgae species or strains with high biomass or lipid yields, the development of large-scale algae biofuels has been limited by environmental

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disturbances, disease, and non-focal species contamination (Godwin *et al.* 2017). However, recent research has suggested that multi-algae species culture (polycultures or consortia) could be effective for developing algal biofuel systems if communities could be adapted to fit specific needs. In addition, a more diverse system produces more biomass and is more stable in fluctuating environments (Podkuiko *et al.* 2020). Previous studies have reported the use of microalgal consortia in wastewater treatment and biofuel production. Hena *et al.* (2015) found that the algal consortia culture dominated by *Chlorella* and *Scenedesmus* strains significantly reduced 98% of nutrients from treated wastewater and produced maximum biomass and lipid when compared with monoculture. Two microalgal consortia (MAC1 and MAC2) showed good growth in wastewater with 75% dilution and produced lipid content in the range of 25-31% of dry weight (Sharma *et al.* 2020). Omirou *et al.* (2018) have reported that five algal consortia cultures collected from coastal areas of Cyprus showed higher biomass and lipid productivity than those of monoculture. In a previous investigation by Boonma *et al.* (2019), the microalgal consortia cultured with CO₂ aeration had a higher growth rate and lipid content than that with ambient air. The dominant algal species in the culture were *Acutodesmus dimorphus* and *Chlorella vulgaris*.

Nevertheless, there has been very little research focused on the selection and cultivation of microalgal consortia for biodiesel production in Thailand, specifically in Rayong province. Thus, the present study focused on consortia culture. The potential of brackish water microalgal consortia (MC) as biodiesel feedstock was investigated. The native MC samples were collected from three different brackish water environments in Rayong province, Thailand, and cultured in a modified Guillard's F/2 medium with 10% (v/v) CO₂ supplementation. The growth and lipid performances of three MC samples were determined. In addition, the dominant microalgae species and fatty acid composition of FAME of all consortia were evaluated.

2. Materials and Methods

2.1 Samples collection

The brackish water samples were collected from three different locations in Rayong province, Thailand including Phra Chedi Klang Nam (PKC) (12°39'58.0"N, 101°14'30.6"E), Noen Kho Canal (NKC) (12°42'38.7"N, 101°38'49.1"E) and Raksamae Bridge (RSM) (12°43'06.3"N, 101°39'22.8"E) (Fig 1). These areas were selected due to they are located near the estuary area and mangrove ecosystem with high biological diversity. Moreover, the surrounding areas of those have different land uses, which were, ecotourism areas (PKC and RSM) and aquaculture (NKC). These various backgrounds were assumed to have a high influence on water quality and microalgae biodiversity in brackish water. Some physicochemical parameters of the water samples were determined using the portable multiparameter meter (HandyLab 680, SI Analytics). A 20 µm-mesh size plankton net was used to collect microalgal consortia samples. A 100 mL of each water sample was maintained in a sterile bottle and transported to the laboratory within 24 hours of collection.

2.2 Microalgal consortia cultivation

The microalgal sample (4 mL) of each water resource was enriched with 16 mL of modified Guillard's F/2 medium (Shah *et al.*, 2003) and incubated at ambient temperature (around 28-32°C), aeration with 10% (v/v) CO₂ under continuous illumination with white LED lamps (3400 lux) for 7 days. Next, the microalgal consortia samples were cultivated in a 500 mL laboratory glass bottle containing 300 mL of modified Guillard's F/2 medium with the initial cell density of 0.05 (optical density, OD at 680 nm) for 10 days at the described conditions. All treatments were conducted in triplicate.

2.3 Microalgae growth determination

Microalgae growth was monitored by measuring optical density (OD) every two days using a GENESYS 20 spectrophotometer at 680 nm (OD₆₈₀).

The biomass concentration of microalgal consortia was determined on the initial and final days of each cultivation using a modified method by Yoo *et al.* (2010). A 10 mL of microalgal suspension was collected, filtered through pre-weighed 0.45 µm filter paper using vacuum filtration equipment. After being washed with distilled water, the filters were dried at 60°C for 48 h. The algal biomass concentration was calculated in terms of dry weight (g.L⁻¹), which was determined gravimetrically.

The biomass productivity (P, g.L⁻¹.d⁻¹) of all treatments was calculated according to the following Eq. (1) (Tang *et al.* 2011):

$$P = (X_1 - X_0) / (t_1 - t_0) \quad (1)$$

where: X₁ and X₀ are the biomass concentration at the final and initial days of the cultivation period, and t₁ - t₀ represent the period of the growth phase

2.4 Microscopic observation

The populations of microalgal consortia were examined on the initial and final days of each cultivation using a light microscope (Nikon Eclipse E200). Three hundred cells were counted, and the total numbers of each species were calculated in terms of the percentage of the total microalgae population.

2.5 Lipid extraction

The lipid content of microalgal consortia was extracted using a procedure adapted from Bligh and Dyer (1959). In brief, 15 mL chloroform:methanol (2:1, v/v) was added to 0.5 g of dry algal powder. The extraction process was performed using an ultrasonic device Model Vibra-Cell VCX130PB (130W, 20 kHz) at 30% amplitude for 10 min. After that, the solvent layer was separated by centrifugation at 5000 rpm for 10 min, and the extracts were evaporated to dryness in a fume hood. Lipid contents were measured gravimetrically.

The lipid productivity (g.L⁻¹.d⁻¹) of all treatments was calculated using the following Eq. (2) (Yadavalli *et al.* 2012):

$$\text{Lipid productivity} = \text{Biomass productivity (g.L}^{-1}\text{.d}^{-1}\text{)} \times \text{lipid content (\%)} \quad (2)$$

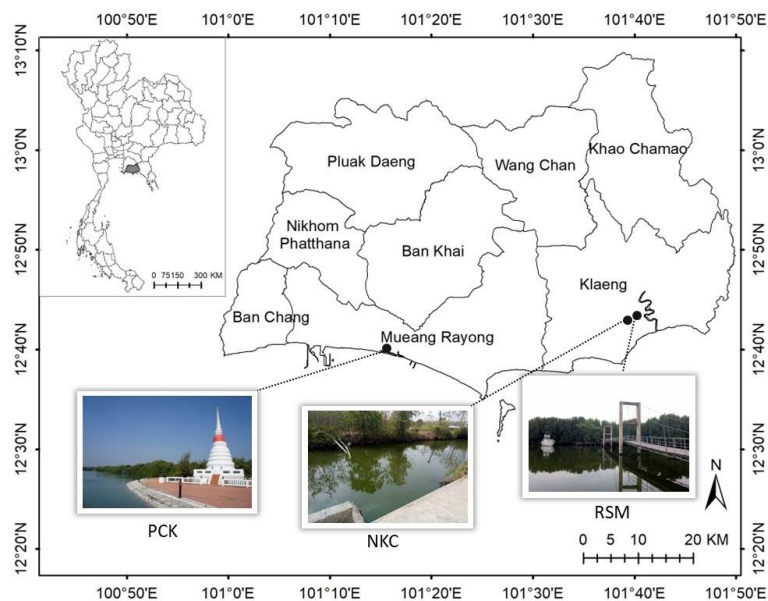


Fig 1. Sampling location of microalgal consortia from the brackish-water environment in Rayong, Thailand

2.5 FAME analysis

Fatty acid methyl esters (FAME) were prepared by transesterification using a modified method by Slover & Lanza (1979). Methanol and sodium hydroxide were used for the transesterification process. FAMEs were analyzed using gas chromatography (Agilent 7890a) with a flame ionization detector and using a 100 m × 0.25 mm × 0.2 μm CP-Sil 88 column (Agilent Technologies). The conditions for GC were: injector temperature 240 °C, detector temperature 250 °C, 1 uL injection with 50:1 split ratio, column temperature gradient was 70 °C for 1 min, followed by an increase to 175 °C at the rate of 13 °C min⁻¹, and then to 215 °C at the rate of 4 °C min⁻¹, and finally held at 240 °C for 12 min. Helium was used as carrier gas at flow rates of 0.38 mL.min⁻¹.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS version 26.0 for Windows. A one-way analysis of variance (ANOVA) and the HSD Tukey test were used to analyze the optical density, biomass, and lipid content data for all consortia samples.

3. Results and Discussions

3.1 Water sample collection and analysis

Microalgae are ubiquitous organisms that can be found in all existing earth ecosystems, both aquatic and terrestrial, and represent a diverse range of species that live in a variety of environments (Mahmoud *et al.* 2015). Some water quality parameters of three different water resources are presented in Table 1. It was found that pH and temperature range showed 7.34-7.74 and 29.20-29.9°C, respectively. Temperature and pH values in each station show the suitable value for the growth of phytoplankton, according to Rai & Rajashekhar (2014), who reported that the temperature and pH values in the range of 20-30°C and 7.5-8.4, respectively, are appropriate for phytoplankton development. The result of salinity measurement at the three water resources showed a value

range including 18.20 g.L⁻¹, 22.80 g.L⁻¹, and 23.40 g.L⁻¹. This is because those resources are located close to the estuary area, where the saline (marine) and fresh (riverine) waters mix (Telesh & Khlebovich 2020). Moreover, DO measurement results ranged between 5.59-8.31 mg.L⁻¹. These values are still within the threshold of the ASEAN Marine Water Quality Criteria for Aquatic Life Protection (ASEAN Secretariat 2008), and Surface Water Quality Standard in Thailand (Pollution control department 2000, with a dissolved oxygen content of >4 mg.L⁻¹. Electrical conductivity (EC) is a measure of the concentration of ions that are capable of conducting electrical currents. Thus, EC is used to estimate the salinity and total dissolved solids (TDS) of the water (Rusydi 2018). In general, the EC of a brackish water environment was observed in the range of 1-80 ms.cm⁻¹ (Nthunya *et al.* 2018). The EC values obtained in this research ranged from 29.20 to 38.90 ms.cm⁻¹, with the lowest value recorded at NKC and the highest at PCK, respectively.

3.2 Microalgae growth performance

Growth determination by optical density (OD) method is well-known to be used in the microorganism community since it indicates measuring the suspended biomass present in the liquid sample (Kumar *et al.* 2018). The growth curves of the MC, collected from three different water resources, were demonstrated in Fig. 2.

Table 1
Physicochemical parameters of the water sample at various locations

Parameters	PCK	NKC	RSM
pH	7.74	7.64	7.34
Water temperature (°C)	29.20	29.9	29.60
Salinity (g.L ⁻¹)	23.40	18.20	22.80
Dissolved oxygen, DO (mg.L ⁻¹)	6.0	8.31	5.59
Electrical conductivity, EC (ms.cm ⁻¹)	38.90	29.20	36.37

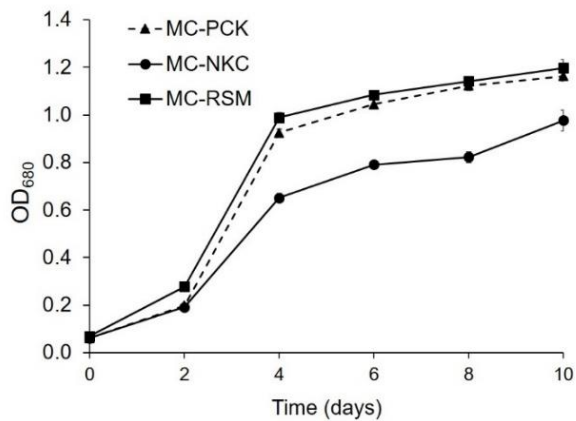


Fig 2. Growth curve of MC from different water resources.

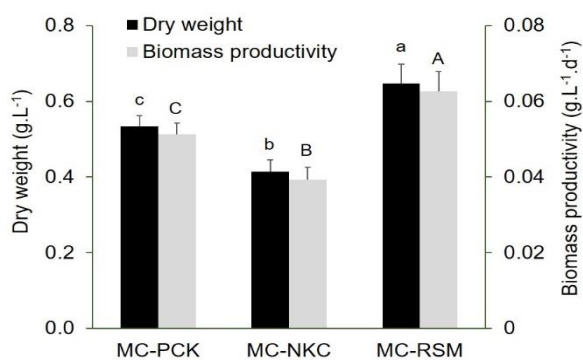


Fig 3. Growth performance of MC from different water resources. Different letters indicate statistical difference ($p < 0.05$).

All cultures showed three stages of growth are including lag phase (day 0-2), exponential phase (day 2-4), and stationary phase (day 4-10). The maximum OD values observed in the MC-PCK, MC-NKC, and MC-RSM were 1.16 ± 0.02^a , 0.98 ± 0.04^b , and 1.20 ± 0.04^a , respectively. The

OD value was not significantly different between the MC-PCK and MC-RSM ($p > 0.05$).

The growth characteristics of each consortia are shown in Fig. 3. The biomass concentration, measured in dry weight (mg.L^{-1}) was found between 0.45-0.65 mg.L^{-1} . MC-RSM had a significantly higher dry weight ($0.65 \pm 0.05^a \text{ mg.L}^{-1}$) compared to both MC-PCK ($0.53 \pm 0.03^b \text{ mg.L}^{-1}$) and MC-NKC ($0.45 \pm 0.03^c \text{ mg.L}^{-1}$) ($p < 0.05$).

Biomass productivity, ranging 0.039-0.063 $\text{g.L}^{-1}.\text{d}^{-1}$, was found significantly different among the consortia ($p < 0.05$). MC-RSM ($0.063 \pm 0.005^A \text{ g.L}^{-1}.\text{d}^{-1}$) had higher biomass productivity, compared to both MC-PCK ($0.051 \pm 0.003^B \text{ g.L}^{-1}.\text{d}^{-1}$) and MC-NKC ($0.039 \pm 0.003^C \text{ g.L}^{-1}.\text{d}^{-1}$). Qin *et al.* (2016) and Omirou *et al.* (2018) reported that the biomass productivity of algal consortia was ranged from 0.056 to 0.2 $\text{g.L}^{-1}.\text{d}^{-1}$. Hena *et al.* (2015) found that biomass production of microalgal consortia cultivated in treated wastewater was 1.3-fold higher as compared to monoculture. Mixed algae species culture (polyculture or consortia) has some advantages over monoculture. Multi-algae communities may reduce temporal variability in productivity through environmental fluctuations by increasing the possibility that a productive species will be present under prevalent conditions. Also, species-rich communities may be less sensitive to the impacts of invasion by other species or pathogens than monocultures (Shurin *et al.* 2013).

3.3 Microalgae populations

The populations of three MC cultures were examined on the initial and final days of the cultivation period, observed microalgae are shown in Fig. 4. The most common microalgae species found under all cultures were green algae (*Chlorophyta*) and diatom (*Bacillariophyta*). The observed microalgae species of green algae were *Acutodesmus* sp., *Chlorella* sp., *Coelastrum* sp., *Desmodesmus* sp., *Monoraphidium* sp.1, *Monoraphidium* sp.2, *Scenedesmus* sp., and *Selenastrum* sp. while that of diatom were *Cyclotella* sp., *Melosira* sp., *Nitzschia* sp.1, and *Nitzschia* sp.2.

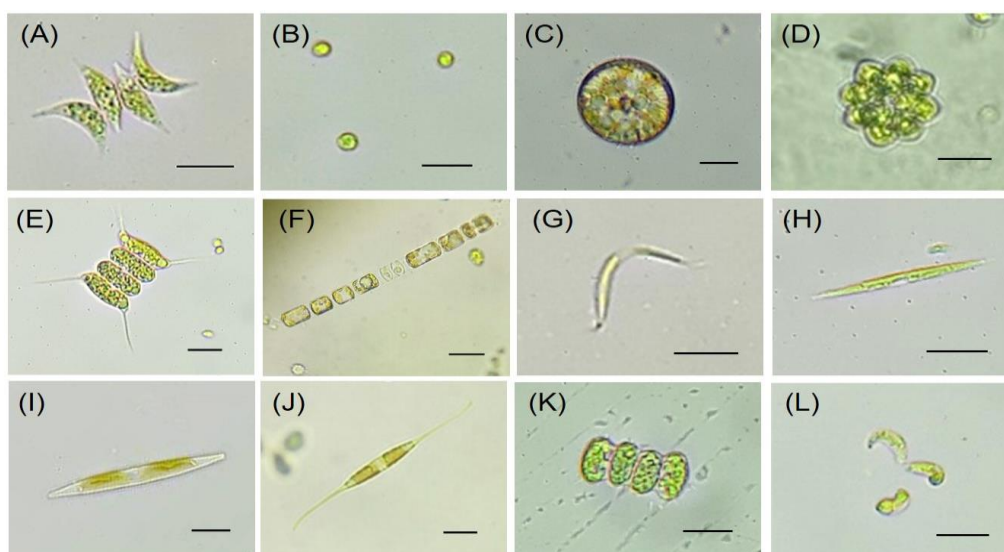


Fig 4. Dominant species of algal community found in different MC cultures during the cultivation process. (A)*Acutodesmus* sp. (B)*Chlorella* sp. (C)*Coelastrum* sp.(D)*Cyclotella* sp. (E)*Desmodesmus* sp. (F)*Melosira* sp. (G)*Monoraphidium* sp.1 (H)*Monoraphidium* sp.2 (I)*Nitzschia* sp.1 (J) *Nitzschia* sp.2 (K)*Scenedesmus* sp. (L)*Selenastrum* sp.; scale bar = 10 μm .

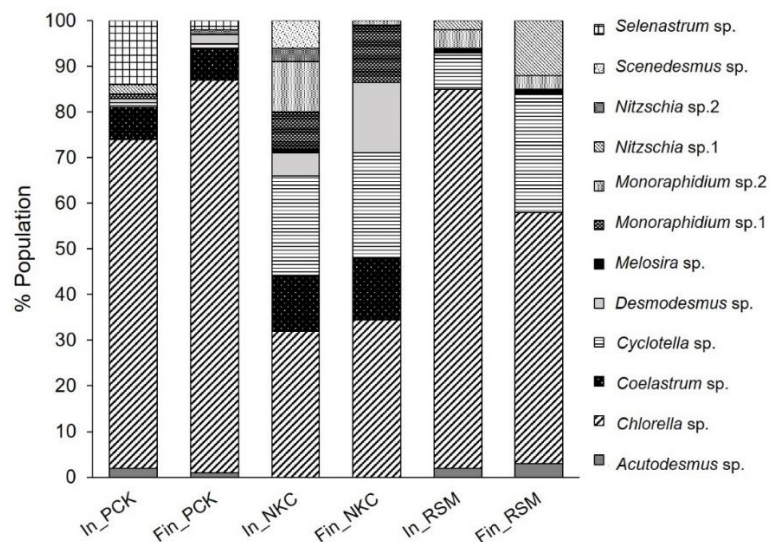


Fig. 5. % Population of algae species of the three different MC cultured at the initial and final days of the cultivation process. In = initial day, Fin = Final day

The percentage of the population of dominant microalgae species under all cultures was also estimated (Fig 5). It was found that the genus *Chlorella* and *Cyclotella* are the genera found in all cultures while the other genera are found only in some cultures. After 10 days of cultivation with 10% CO₂ aeration, the abundance of *Chlorella* sp. in MC-PCK, MC-NKC, and MC-RSM cultures was 85%, 35%, and 55% of the total population, respectively. The results indicated that *Chlorella* sp. was the dominant eukaryotic microalgae species in all mixed algae cultures during the operation period. A similar result was found in an algae consortium cultivated in treated wastewater with 10% CO₂ supplementation. Total *Chlorella* strains including *Chlorella* sp., *Chlorella vulgaris*, and *Chlorella sorokiniana* were the predominant strains during the operation period (Hena *et al.* 2015). It is well reported in the literature that *Chlorella* species can be grown in a wide range of environments like brackish or saline water, and various wastewaters (Wang *et al.* 2010; Mahmoud *et al.* 2015; and Ajala & Alexander 2020).

3.4 Lipid production and fatty acid composition

Lipid content and lipid productivity of three MC cultures were determined and shown in Fig. 6. Among all cultures, the MC-RSM showed the highest lipid content followed by MC- MC-PCK and MC-NKC. Lipid content of the three cultures were 33.21 ± 0.79^a %, 31.33 ± 0.58^b %, and 28.07 ± 0.60^c %, respectively. Schwenk *et al.* (2013) and Mahmoud *et al.* (2015) recorded that the lipid content of brackish water microalgae ranged from 3 to 40%. Lipid productivity of all samples ranged from 0.011 ± 0.001 to 0.021 ± 0.002 g.L⁻¹.d⁻¹, and the highest value was noticed in the MC-RSM culture. Accumulating high lipid in the MC-RSM might be because of the dominant lipid-rich species including *Chlorella* sp., *Cyclotella* sp., and *Nitzschia* sp., which lead the consortium to provide the maximum lipid content (Zhang *et al.* 2014; d'Ippolito *et al.* 2015; Demirel *et al.* 2017). Woertz *et al.* (2009) reported that the lipid productivity of polyculture of *Chlorella* sp., *Microactinium*

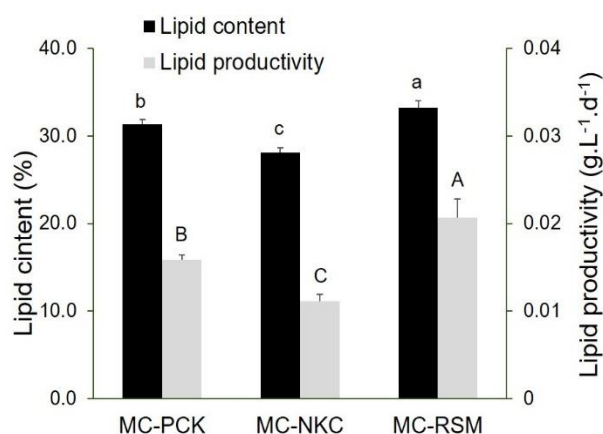
sp., and *Actinastrum* sp., cultivated with municipal wastewater were observed in the range of 0.009-0.024 g.L⁻¹.d⁻¹. Lipid productivities of three *Chlorella* strains, *C. vulgaris* UTEX 395, *C. vulgaris* UTEX 26, and *C. sorokiniana* UTEX 1230, were found between 0.01-0.033 g.L⁻¹.d⁻¹ (Arora & Philippidis 2021). Cao *et al.* (2022) showed that at nitrogen concentration of 117.65 mM, the highest productivities of *Nitzschia* sp. was 0.019 g.L⁻¹.d⁻¹.

To determine the fatty acid composition of biodiesel, the algal lipid was converted into FAME by the transesterification process. Table 2 presents the fatty acid composition of lipids extracted from three different MC samples. The three groups of fatty acids including SFAs, MUFAs, and PUFAs were found, and the length of fatty acid chains ranged from C14:0 to C24:0. This might be because different microalgae species in consortia respond to various conditions by generating different fatty acids or changing their fatty acid composition (Demirel *et al.* 2017). The fatty acid profile of both the MC-NKC and MC-RSM showed the dominance of SFAs followed by MUFAs and PUFAs, while the MC-PCK showed the dominance of SFAs followed by PUFAs and MUFAs. The majority of the SFAs were myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), which was similar to the results of Doung *et al.* (2015). Cetane number is one of the desirable properties of biodiesel, it is the ability of a fuel to ignite quickly after injection. The higher cetane number leads to better combustion of fuel with fewer emissions. Biodiesel fuels containing significant amounts of SFAs such as palmitic acid provide higher cetane number and oxidative stability (Mohammady, 2011; Sharma *et al.* 2020). Lamaisri *et al.* (2015) reported that cetane number and cold flow properties of biodiesel from palm oil had a positive correlation with saturated fatty acids such as myristic, palmitic, and stearic acids. In the recent study, SFAs of three consortia were observed at 67.82%-71.31% of total fatty acids. This result indicated that microalgal consortia culture under photoautotrophic conditions has potential for biodiesel production.

Table 2.

Fatty acid composition of the three different MC samples (% of total FAME)

Fatty acids	MC-PCK	MC-NKC	MC-RSM
Myristic (C14:0)	7.37	9.75	23.46
Pentadecanoic (C15:0)	2.42	1.30	0.85
Palmitic (C16:0)	54.32	52.66	42.30
Palmitoleic (C16:1)	5.27	23.17	21.83
Stearic (C18:0)	4.68	3.54	2.96
Oleic (C18:1)	6.70	3.55	0.84
Linoleic (C18:2)	7.66	4.86	7.14
Linolenic (C18:3)	0.45	-	-
Eicosadienoic (C20:2)	8.61	0.60	-
Heneicosanoic (C21:0)	2.52	0.57	-
Tetracosanoic (C24:0)	-	-	0.62
SFAs ^a	71.31	67.82	70.19
MUFAs ^b	11.97	26.72	22.67
PUFAs ^c	16.72	5.46	7.14

^aSaturated fatty acids^bMonounsaturated fatty acids^cPolyunsaturated fatty acids**Fig. 6.** Lipid production potential of the MC from different water resources. Different letters indicate statistical difference ($p < 0.05$)

6. Conclusion

This study demonstrated that brackish water microalgae can be found in a wide range of water qualities that have different values of physical and chemical parameters. After cultivation in a modified Guillard's with 10% (v/v) CO₂ supplementation, the growth performance of MC-RSM was higher 1.2 and 1.5-times than MC-PCK and MC-NKC, respectively. The green algae *Chlorella* sp. was the dominant algal species in all MC cultures. The maximum lipid concentration and productivity were achieved by MC-RSM. The SFAs of all MC samples were observed at 67.82%-71.31% of total fatty acids and the major fatty acid was palmitic acid. This research suggested that the native microalgal consortia from brackish water environments in Rayong province have the potential to be used as a model of multi-algae culture for large-scale biodiesel production in the country.

Acknowledgments

This research was supported by the Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Rayong Campus, Rayong, Thailand.

Author Contributions: S.U.: Conceptualization, methodology, formal analysis, writing—original draft, S.U.; supervision, resources, project administration, S.U.; writing—review and editing, project administration, validation. A.C.; writing—review and editing, project administration, validation. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by King Mongkut's University of Technology North Bangkok, Contact no. KMUTNB-61-NEW-007.

Conflicts of Interest: The authors declare no conflict of interest.

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