

Tackling Carbon Emission with Nature: Effectiveness of Indigenous Microalgae Mixed Culture

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Abstract. Marine microalgae species was isolated and identified from its native condition of sea water discharge canals at the TNB Janamanjung Sdn Bhd's coal-fired power station. The species was expected to be a robust one based on the prevalent harsh upstream conditions and processes the survived ones endured. The isolation involves streaking-plating method as well as serial dilution and liquid media culture propagation. Morphological and molecular identification were both carried out before satisfactory identification of *Chlorella sp.* be made. This species was later mixed with marine *Isochrysis sp.* with varying volumetric proportions and cultured in five separate air-lift bubbling column photobioreactors, as part of the experiment to determine which optimum volumetric culture ratio is best to fix CO₂ from the power plant. Results indicate that a more dominant ratio of *Chlorella sp.* 75% and *Isochrysis sp.* 25% by volume provide gives a better growth profile and indicates a better CO₂ fixation rates.

1 Introduction

Any utility power company in a developing country is on constant pressure to deliver electricity to consumers reliably, safely and affordably, amidst the ever-increasing power demand and the current carbon-constraint world. Nowadays, curbing CO₂ emissions has been a new agenda, internationally and nationally - as in our Prime Minister's pledge to reduce 40% carbon intensity, from 2005 level [1]. Utilizing renewable energy and embarking on energy efficiency are some of the major approaches in curbing CO₂ emissions, and they have been promoted and implemented in Malaysia with some level of success. Of late, tackling the issue at the point source through Carbon Capture, Utilization and Storage Technologies (CCUS) is getting its foot in Malaysia. Being part of it, many literatures and works have identified phototropic microalgae as an alternative or complementary method among available CCS technologies [1]. The use of its natural, more efficient photosynthesis process, and the potential of valuable downstream products it can generate, make algal bio-fixation process eligible and attractive to be a more sustainable method in curbing CO₂ emissions [3]. Using this biological method, the CO₂ is fixed bio-chemically into C_x-H_y-O_z compound in product of photosynthesis reaction originating from CO₂, water (H₂O) and photon energy from the sun as the reactants. By mass ratio, approximately 2 tonnes of CO₂ can be fixed by 1 ton of microalgae [5]. The baseline parameter

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of utmost concern is the CO₂ fixation rate by microalgae species, often expressed as grams of CO₂ fixed per culture volume per culture duration (e.g. g_{CO2}/(L.day)).

However, there are few issues and challenges regarding algae-based fixation and one of them is low productivity. Productivity of microalgae needs to be enhanced in order to portray a respected amount of CO₂ removal capability. One of the means to improve productivity is by using microalgae consortia, as it was reported to have better growth rate as it complement the metabolic activities of one another to grow and reproduce [6]. The growth characteristics, biochemical compositions, nutritional compositions and other growth factors of mixed culture can also be substantially different from those of monocultures [7]. Besides that, a mixed culture composed of various similarly productive strains with various optimal growing conditions would be less susceptible to environmental shifts than a monoculture [8]. Thus quests on finding a robust and high fixation rate microalgae species has been one of research focuses in this biological endeavour. This work expresses some results out of the effort in identifying robust indigenous microalgae consortia from the 3 x 700 MW coal-fired Sultan Azlan Shah Power Station in Manjung, Sitiawan, Perak Darul Ridzuan (TNBJ).

2 Materials and Methods

2.1 Species sampling and isolation of microalgae species

Samples were collected from the sea water outfall at Stesen Janakuasa Sultan Azlan Shah, Manjung Perak, west coast of Malaysia Peninsular, 4° 9' 31.158"S latitude and 100° 38' 30.3396" E longitude. This coal-fired power plant is among the major power generation plant in Malaysia, having an installed capacity of 3 units of 700 MW. This power station has been equipped with the state-of-the-art emission mitigation equipment to meet the stringent World Bank's emission standard for NO_x, SO₂ and particulate matters [9]. The outfall is a discharge point of spent sea water used in the power generation process in the upstream. Among processes undergone by the sea water at this point include high velocity spray, disinfection with hypochlorite, water-spray as part of flue gas desulphurisation and aerated (air bubbled). Thus, any microalgae species that survive at this point shall experience varying degrees of hydrodynamic stresses, disinfection and temperature difference based on the process described above, and thus any ones which found dominant in the outfall shall be the ones who are robust enough to dwell further in bioreactors. The samples were cultured using both F/2 and TNBR medium. The samples were maintained in 50 mL centrifuge tube, at room temperature at 28°C and kept with continuous light exposure at 12,000 lux by 50 W fluorescent daylight type lamps. Observation of microalgae growth in the liquid medium were continued with plating method by using respective medium by using 1.25% agar plate. In the parallel of liquid and 1.25% agar cultivation, the growth of microalgae were measured by biomass accumulation, cell density and amounts of colonies on the plate respectively.

2.2 Carbon fixation experiment

Optical densities were measured daily using HACH's 2700 Spectrophotometer at 560 nm wavelength. Algal biomass were extracted daily using vacuum filtration pump and its dry weight measured and recorded. The carbon fixation calculation was adopted by taking into considerations of the ratio between the moles of CO₂ and the moles of typical molecular formula of biomass [11]. By taking into considerations of a balanced photosynthesis formula as in Equation (1) below:



The ratio of molecular weight of CO₂ and biomass is 1.882, which upon various elemental analysis performed on microalgae biomass, the ratio ranges from 1.81 to 2.37 [11].

2.3 Morphological identification

Growing microalgae species were observed and morphological screening was done by comparing to the online catalogue as per University of Texas (UTEX), Culture Collection of Microalgae and The Culture Collection of Algae at Goettingen University (international acronym SAG). Morphological identification was done by determining the characteristic of isolated microalgae, which include size, availability of membrane wall and flagella and the shape of chloroplast.

2.4 Molecular identification

Single/pure strain of microalgae obtained in isolation process was used in molecular identification. Genomic DNA was extracted from the wet biomass by using liquid nitrogen and was further purified using Promega Wizard Genomic DNA Purification Kit. 18s rRNA genes Polymerase Chain Reaction (PCR) amplified using a primer designed by FirstBase Sdn Bhd. The sequenced products were compared with the National Center for Biotechnology Information (NCBI) data bank using the BLAST Program. (NCBI BLAST, USA). Top ten similar results were achieved and compared for the similarity in the percentage value.

2.5 Mixed culture growth profile experiment

Healthy culture of microalgae species isolated and identified earlier were later propagated through series of volume scaling-up before being tested in five 250-liter air-lift bubbling column photobioreactors located at TNBJ. Each of the reactors were cultured with varying volumetric ratio composition between the new species and *Isochrysis sp.* *Isochrysis sp.* was isolated from the open sea off-coast of the same power plant and its selection in this work to form a consortium of species was based on previous works [12]. Table 1 below lists species volumetric ratio distribution across each of photobioreactor columns in use.

Table 1. Species volumetric ratio distribution.

Column Label	Volume New Species	Volume <i>Isochrysis sp.</i>
2	25%	75%
3	50%	50%
4	0%	100%
5	100%	0%
6	75%	25%

Each of the columns is bubbled using actual flue gas from a unit of power plant's flue gas duct. Fine bubbles were made possible by BLOWTAC/Disc-170 diffusers. The flue gas was extracted from a sampling point using PermaPure Baldwin's (USA) stainless steel flue gas probe system, through KNF's (Germany) vacuum pump. The flowrate was maintained at 1 liter per minute using Dwyer's rotameters, while pH was monitored using ThermoScientific's (USA) pH sensors. In order to avoid excessive acidic culture condition, the admission of flue gas was controlled using actuator valves of which their operation (open or close) were dictated by miliampere signals from pH transmitters. The flue gas composition varied throughout the experiment duration, in relation to varying coal used and regimes of plant's operation. Average composition of carbon dioxide (CO₂) content in the flue gas was between 4 – 6 vol. % with maximum of 8 vol. %, alongside other major gas components e.g. carbon monoxide (CO), nitrogen oxides (NO_x), sulfur dioxide (SO₂), etc. Daily growth monitoring was done by reading the optical density of 25 mL sample using HACH's D4000 Spectrophotometer at 560 nm wavelength and the readings were plotted.

3 Results and Discussions

3.1 Morphological and molecular identification

From microscope observation, the size of the purified microalgae ranges around 4.24 ~10 μm with the area around 56.48 μm^2 . Notable characteristic that can be observed was round or ellipsoidal shape with transparent outer membrane, with thin layer of membrane without any extension of flagella. By these observations, the suspected species was *Chlorella sp.* By comparison with *Chlorella sp.* from UTEX. *Chlorella* is blue-green algae species which belongs to phylum *Chlorophyta* division. Report from Fott and Novakova (1969) showed the versatility of *Chlorella* species to adapt to marine environment, despite its abundance in freshwater environment. Other results from NCBI also showed that several microalgae which were isolated from South China Sea were confirmed to be *Chlorella* species. Next confirmation step requires molecular level analysis.

In Table 2 depicted below, top ten hits blast result were observed and the list suggests that seven out of ten confirm up to 99% that an isolated, purified and sequenced microalgae is *Chlorella sp.* Thus, from molecular result plus with similar characteristic by morphological identification, it was confirmed that isolated microalgae were *Chlorella sp.*

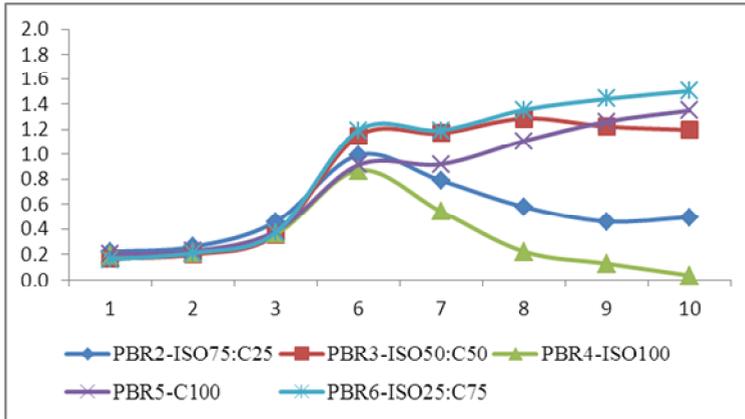
Table 2. Top 10 Hits blast results against NCBI nucleotide collection (nr/nt) database excluding bacteria.

Description	Identification	Accession
<i>Chlorella sp.</i> KAS001 gene for 18s rRNA, partial sequence	100%	AB176663.1
<i>Chlorella sp.</i> KAS002 gene for 18s rRNA, partial sequence	100%	AB176666.1
<i>Chlorella sp.</i> SAG211-18 8s rRNA gene	99%	X73992.1
<i>Chlorella sp.</i> MDL5-18 18S ribosomal RNA gene, partial sequence	99%	AY197632.1
<i>Chlorella sp.</i> KAS005 gene for 18s rRNA, partial sequence	99%	AB176664.1
<i>Chlorella sp.</i> KMMCC C-137 gene for 18S rRNA gene, partial sequence	99%	GQ122349.1
<i>Chlorella sp.</i> MBIC10088 gene for 18S rRNA, partial sequence	99%	AB058308.1
<i>Dictyosphaerium sp.</i> NIES 453 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,5.8S ribosomal RNA gene, and internal transcribed spacer 2	99%	GQ487248.1
<i>Dictyosphaerium sp.</i> CCAP 222/4 ribosomal RNA gene, partial sequence; internal transcribed spacer 1,5.8S ribosomal RNA gene, and internal transcribed spacer 2	99%	GQ487246.1
Kalenjinia gelatinosa strain CCAP 222/8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,5.8S ribosomal RNA gene, and internal transcribed spacer 2	99%	GQ477061.1

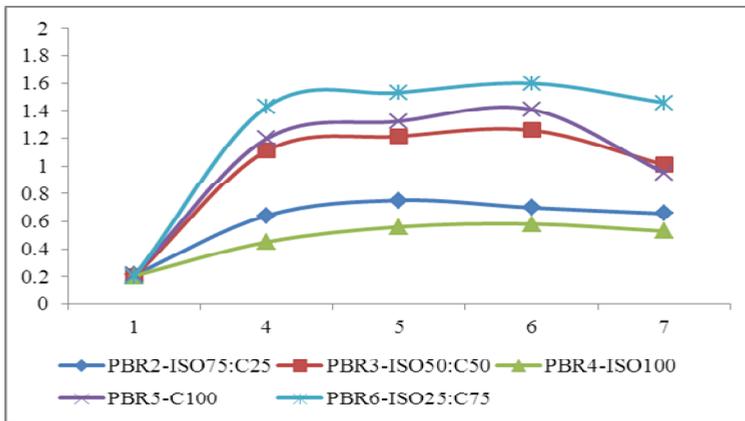
3.2 Growth profile of mixed culture

In discussing this, reference is made to the growth profile by optical density measurements of mixed culture between *Isochrysis sp.* and *Chlorella sp.* as depicted in Figure 1(a) for the first run and Figure 1(b) for the second run. General growth profiles for both runs are observed to be in accordance to the typical growth profile of microalgae, with distinct lag, exponential and decay phases especially for the first run. From Figure 1(a), the best growth curve – having the highest and steadiest optical density - occurs in column no. 6 where the mixture of *Isochrysis* 25% and *Chlorella* 75% by volume reside. The trend, in descending order then followed by *Isochrysis* 50% and *Chlorella* 50%, *Isochrysis* 0% and *Chlorella* 100%, *Isochrysis* 75% and *Chlorella* 25% and finally *Isochrysis* 100% and *Chlorella* 0%. Similar trend was also observed in the second run where *Isochrysis* 25% and *Chlorella* 75% tops the chart. The species in the second run also seemed to be more acclimatized to the experiment conditions where no lag phases were observed in early culture stage, steeper exponential phase and longer period of stationary phase. However, the second and third place here was interchanged with that from the second run, which warrants for a more finer volumetric ratio around that region to be

performed in the future. It can be concluded that better growth and fixation rates occur in cultures where there is higher proportion of consortia. The presence of *Isochrysis sp.*, together with the consortia, acting as symbiont and complements the metabolic activities of one another to grow and reproduce. The volumetric ratio of *Isochrysis* 25% and consortia 75% gives the best growth and fixation rates, now determined to be 0.965 g.CO₂/L.day, a 34% improvement from our previous effort.



(a)



(b)

Figure 1. Optical density for mixed culture (a) first run, (b) second run.

4 Conclusions

The observations obtained from the above suggest two important findings – *Chlorella sp.* isolated from the power plant’s outfall is a robust species and meet hypothesis made earlier based on the environmental condition and upstream processes they have endured. Its robustness is evidenced by the better growth profile observed in the columns where its volume is more dominant. Second interesting finding lies on the observed dependency of *Chlorella sp.* on *Isochrysis sp.* as illustrated by better growth profile on mixed culture against mono-culture. This suggests that *Isochrysis sp.* can act as a symbiont and complements *Chlorella sp.* A better data to support this can be made available through population dynamics study to be performed later.

Thus, this work has demonstrated the basic findings on the importance of searching an indigenous species in the locality where the CO₂ that is going to be fixed originates. *Chlorella’s* acclimatization

to the surrounding level of CO₂ and other flue gas components and its readiness to co-exist with other species has made it a good mixed-culture species for biological fixation at this power plant.

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