# SCANNING OF MICROALGAE SPECIES FOR BIOLOGICAL $CO_2$ FIXATION AT A MALAYSIAN COASTAL COAL-FIRED POWER STATION

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***Abstract***: Biological fixation of $CO_2$ has been identified as one of the emerging technologies under the Carbon Capture and Storage (CCS) program. This natural means of capturing emitted CO2 through photosynthetic microalgae while producing some value-added byproducts can be regarded to be economically viable and more sustainable. This work takes an early initiative in this regard by performing a scanning task for available marine microalgae species in the vicinity of a coastal coal-fired Janamanjung Power Plant along the Straits of Malacca. Samples from three locations in the vicinity of the station were collected using 35 □m mesh plankton net and their physical and chemical properties were compiled. Afterward, the samples were subjected to meticulous chemical enrichment employing suitable microbiological techniques and medium for their propagation. Identifications of survived species were carried out by careful observations and comparisons of the specimens' morphologies under microscope. Species names tagging were done in accordance to established taxonomic classification. Dominant microalgae species appears to be of cyanophyceae algae consisting of *Trichodesmium thiebautie*, *Nannochloropsis sp.*, *Tetraselmis sp.*, *Chlorella sp.* and *Isochrysis sp.*, forming in between 57 % - 75 % of mean population. Of these five species, *Isochrysis sp.*, has been found to be the most dominant species, being 40% - 50 % of population count. At least four of these identified species are renowned to be commonly cited in literatures of having satisfactory and good qualities on biomass yield, lipid content, antioxidant properties and nutritional values.

***Keywords: $CO_2$ fixation; Global warming; microalgae***

## INTRODUCTION

Among the early definition given to the word *'sustainable development'* is as given by the United Nations World Commission on Environment and Development (WCED) on March 20, 1987, that, *"... development that meets present needs without compromising the ability of future generations to meet their needs ... "*. At the 2005 World Summit, the meaning is described again by having three main pillars that must be met; social, economic and environment, as depicted in Figure 1.

In the case of power utility companies, sustainable energy should become an agenda of focus, especially during planning stage. One possible way to initiate this is through an assessment of several sustainability indicators as discussed in [2]. In it, there are seven

sustainability indicators to be assessed, which are (i) price of electricity, (ii) carbon dioxide ($CO_2$) emission, (iii) efficiency, (iv)availability, (v) land use, (vi) water use and (vi) social impact. Looking closer, each of them represents elaborations of the three pillars aforementioned.

As for $CO_2$ emission, the United States Energy Information Administration (EIA) has forecasted about 57% increase in energy demand from 2004 to 2030. Some 85% of this demand was predicted to be fulfilled through power generation using fossil fuel [3]. Coal, being abundant and cheap fuel source, coupled with its proven and matured technology remains to be dominant energy source to be utilized. Nevertheless, in a gesture to improve sustainability of fossil-fuel-based power generation, many noble efforts have been carried out internationally, of which one notable is the Carbon Capture and Storage (CCS) demonstrations and R&D programs. As of 2010, there are more than 240 active CCS projects worldwide [4].

These CCS initiative include separating, capturing, delivering and storing, or its combination thereof, of emitted $CO_2$ from utilities or industries. Some common technologies include gasification, oxy-fuel combustion and amine-based absorption, for pre-combustion, during combustion and post-combustion stages respectively. There are still much to be achieved however, for these technologies to be satisfactorily mature and well adopted, as issues like material degradation, loss of efficiency, larger plant footprint, high capital cost and high auxiliary power are still quoted.

Another candidate for CCS is through biological fixation, by the virtue of natural photosynthesis process by autotrophic microalgae. Essentially the process converts (fixes) $CO_2$ (with the help of photon energy) into $O_2$ and organic matter in the form of $C_x$-$H_y$-$O_z$ chain. This organic matter can offer multitudes of value-added downstream products, which, with appropriate additional processes, can be converted into biomass, biofuel, nutrional diets, aquaculture food and fertilizers, to name a few [5], [6]. Thus, this biological mitigation technique can be regarded to be economically viable and environmentally sustainable in the long term. In Malaysia, among active commercial activities dealt with microalgae are through health food supplements and aquaculture diets production. Use of microalgae for other end of products and purpose, like biofuel, fertilizers and waste-water treatment are still in early stage of development, being mostly pursued in the laboratories or small scale demonstration projects. The same also goes for making microalgae as the biological $CO_2$ fixation agent from power plant's flue gas.

This work sets an early effort in biological $CO_2$ fixation from a coal-fired power plant. The objective is to scan for dominant native marine microalgae species available in the vicinity of such a power station. Use of locally available microalgae species can facilitate and ease-out the in-situ biological $CO_2$ fixation process, due to a readily available nutrients and conducive environment for optimum growth of the species.

## METHODOLOGY

### Sampling Location

The sampling of microalgae species was done at the offshore of Janamanjung Power Plant. This is a 3 x 700 MW coal-fired power plant located in the district of Manjung, Perak, along the Straits of Malacca, west coast of Malaysia. Three locations of samplings were identified in the vicinity of this power plant, as tabulated in Table 1 and mapped in Figure 2 below.

Site 1 was chosen in due regards for lesser human activities in the area. The spot is within a bay of Teluk Rubiah where a resort was once operated. Site 3 on the other hand was chosen in anticipation of richer samples derived from rich river discharges. All the sites' depth were also checked to be at least 10 m deep, based on a bathymetry chart around the power station.

### Sampling Method

Samples were taken by dip net method using plankton net of 35 □m mesh size, 25 cm mouth diameter and about 1.5 m long. The net was submerged about 1.5 m below the surface of water, using a rope from a stationary boat. The net was then pulled up vertically using rope and pulley assembly, as depicted in Figure 3. The net was later sprayed with in-situ sea water, before the liquid was collected by a sampling bottle attached at the end of the net.

Physical properties like luminance, temperature, pH and dissolved $O_2$ were measured at each site and samples, using lux meter and EUTECH's portable multimeter model CyberScan PCD 650. Samples were kept in 500 mL plastic bottles, labeled and deposited in a cool-box during transportation to laboratory. At the laboratory, phosphate content ($PO_4^{3-}$) was later determined using spectrophotometer.

### Collection and Isolation of Microalgae

The collected sample was first enriched with Conway media as the broad spectrum medium right after collection to allow the entire algae population to

flourish. The growing culture was then introduced to a tolerable level of antibiotics to eliminate the bacteria and other contaminants. Air was bubbled through the culture with continuous light supply. After 3-4 days, a narrow range spectrum media was introduced to provide a conducive environment for the dominant species to survive. Small volumes (15 mL) samples from the enriched cultures were then centrifuged at 3000 rpm for 15 min. Supernatant was removed, and cells are re-suspended in fresh medium. Centrifugation process was repeated for few times to expel most of the microorganisms presented in algal sample and the cells were then streaked on to agar plates. Microalgae will be allowed to streak through loop in plates in axenic condition and to be kept for at least seven days to grow microalgae. Repeated streak-platings were carried out to peak up single colony from earlier streaked plates and to make free from bacteria. From last streaked plates, the single colonies were picked up by loop and allowed to grow in tubes and vials. Before putting in the tubes and vials, the single cell growth and purity of single species was confirmed after observing under microscope. Identification of species was done by visual inspection of the morphologies observed under microscope.

**Medium and cultivation conditions**

f/2 medium [7] was prepared and autoclaved under 121 $^{o}$C for 15 min. 50 mL of microalgae was poured into a 250 mL flask containing sterilized artificial seawater and fresh culture medium. All operation was conducted in the biohazard laminar flow to minimize contamination. Artificial sea water and f/2 medium was prepared in accordance to the composition and preparation as tabulated in Table 2 and Table 3 below.

The microalgae species was aerated with continuous air bubbling and 24 hours of illumination at room temperature. The species was subcultured by transferring the inoculums to larger flask upto 1 L.

**Analysis of parameters monitoring**

Three parameters were monitored on each culture which is cell count, chlorophyll A and phaeophytin analysis to study the growth rate of the species.

*Cell count cell density measurement*

Cell counting was performed with a Neubauer improved haemacytometer set from Hirschmann ® Laborgerate. One drop of microalgae sample was transferred to the haemacytometer for cell counting. The number of cells were counted under the inverted microscope.

**Chlorophyll-A and Phaeophytin Determination**

Chlorophyll-A and phaeophytin were analyzed by spectrophotometric analysis. 10 mL of sample species was filtered with milipore size membrane filter paper attached to filter milipore titration units and connected to a vacuum pump. Dried filter extract were folded and placed in test tubes containing 15 mL of acetone 90% and was left to be degraded for some times. The samples were then transferred into cuvette to measure the absorbance using spectrophotometer at 664 nm wavelength. The cuvette was retrieved and 1-2 drops of hydrochloric acid (HCl) was added and the reading was taken once again using spectrophotometer on the same wavelength. The chlorophyll-A content and phaeophytin were determined according to Eqn. 1 and Eqn. 2 as follows:

$$\textit{Chlorophyll-A (mg/L)} = (A_b - A_a) * 2.43 * 10.48 * V/L \quad (1)$$

$$\textit{Phaeophytin (mg/L)} = [A_b - 2.43 (A_b - A_a)] * 10.48 * 1.7 * V \quad (2)$$

Where $A_b$ is the optical density readings before addition of HCl, $A_a$ is the optical density readings after addition of HCl, V is the volume (ml) acetone (90%) used (15mL) and L is the width (cm) of cuvette (1cm).

**RESULTS AND DISCUSSION**

**Physical and Chemical Properties of Samples**

The samples from Site 1 and Site 2 were observed to be as clear water, as opposed to the sample from Site 3 where the liquid is murky and suspended solids were observed. Summary of average physical and chemical properties is as tabulated in Table 4.

Due to the local tide table, the sampling at Site 3 was done first early in the morning. The sampling was continued afterwards for Site 2 and finally for Site 1. This explains the lowest luminance for Site 3 and the gradual increase of temperatures from Site 3 to Site 1. The pH was almost neutral or slightly basic for all the sites. On the other hand lower dissolved $O_2$ at Site 3 might suggest increasing population of autotrophic microalgae that managed to reverse the photosynthesis process and produced dissolved $CO_2$, creating a weak carbonic acid. This can be ascertained by observing that the pH value is the lowest at Site 3. On phosphate level, it was obvious to note that sample at Site 3 contains much higher phosphate due to its location at the Perak river mouth that spews rich surface run-offs containing nutrients, among others, that could be the ideal place for microalgae species population.

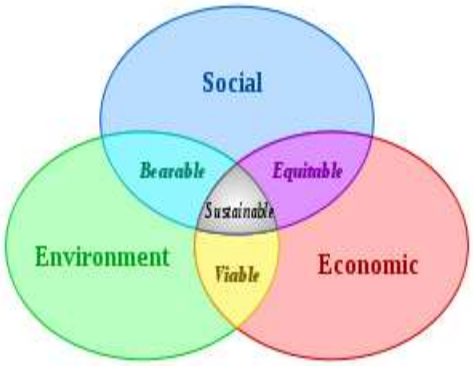


**Figure 1: The Pillars for Sustainable Development**

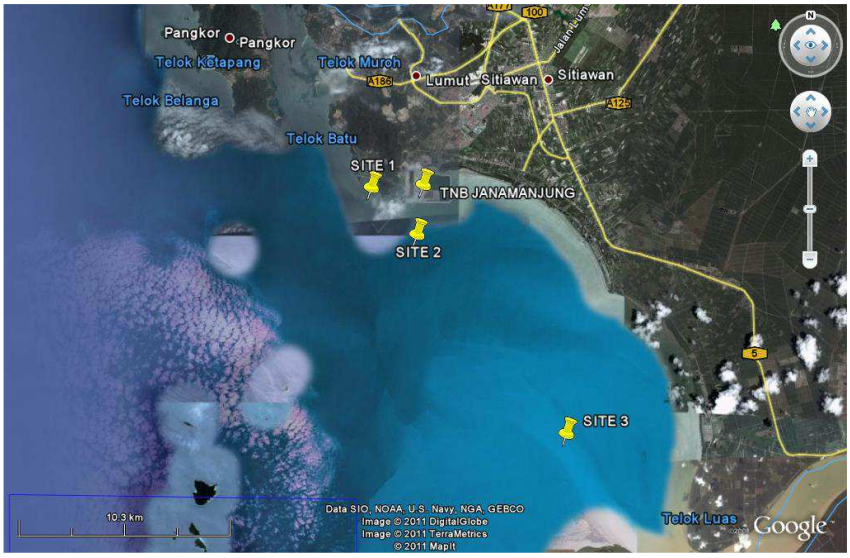


**Figure 2. Spatial map of sampling sites**

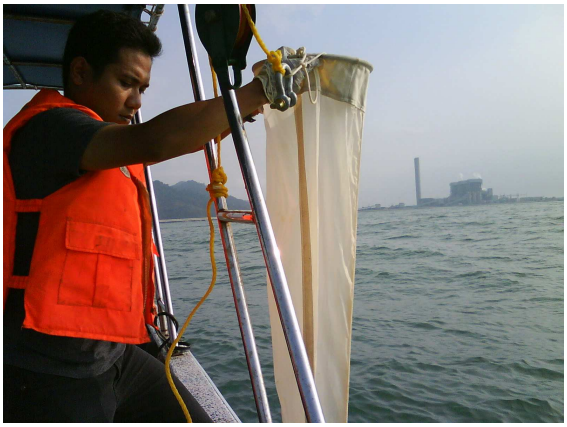

**Figure 3. Taking sample using plankton net. In the background shown the coal-fired power station**

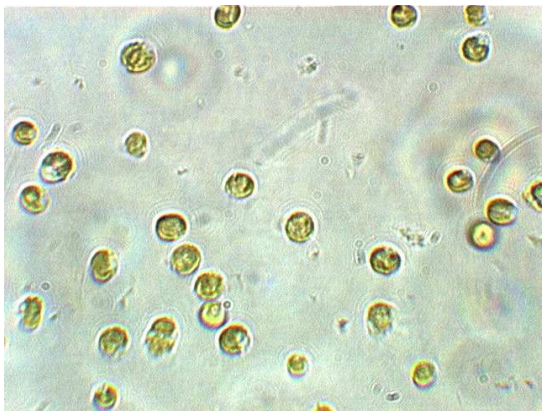

**Figure 4. Typical *Isochrysis sp.* appearance**

**Table 1. Particulars of the sampling sites**

| Site ID | GPS Coordinate | Approximate Distance from Power Plant | Remarks |
|---|---|---|---|
| Site 1 | N 4$^o$ 9.435';<br>E 100$^o$ 37.079' | 2.5 km | Near lighthouse @ Teluk Rubiah |
| Site 2 | N 4$^o$ 8.195';<br>E 100$^o$ 38.103' | 2.4 km | Near coal jetty |
| Site 3 | N 4$^o$ 2.675';<br>E 100$^o$ 41.859' | 14 km | At mouth of Perak river |

**Table 2. Artificial Sea Water Composition**

| Contents | Concentrations [g/L] |
|---|---|
| NaCl | 24.32 |
| $MgCl_2$ | 5.14 |
| $CaCl_2$ | 1.14 |
| KCl | 0.69 |
| $NaHCO_3$ | 0.2 |
| KBr | 0.1 |
| $H_3BO_3$ | 0.027 |
| $SrCl_2$ | 0.026 |
| $NH_4Cl$ | 0.0064 |
| NaF | 0.003 |
| $NaSiO_3$ | 0.002 |
| $FePO_4$ | 0.001 |

**Table 3. f/2 Medium Composition**

| **Contents** | **Concentrations [g/L]** |
|---|---|
| $NaNO_3$ | 75.0 |
| $NaH_2PO_4$ | 5.0 |
| $Na_2EDTA$ | 4.36 |
| $FeCl_3.6H_2O$ | 3.15 |
| Trace metal stock solution | 1.0 mL/L |
| Vitamin stock solution | 0.5 mL/L |

**Table 4. Summary of Physical and Chemical Properties of the Samples**

| **Properties** | **Site 1** | **Site 2** | **Site 3** |
|---|---|---|---|
| Luminance [klux] | 26.6 | 31.25 | 8.05 |
| Temperature [$^oC$] | 30.5 | 29.6 | 29.0 |
| pH | 7.97 | 8.36 | 7.50 |
| Dissolved $O_2$ [%] | 89.85 | 92.3 | 83.3 |
| Phosphate [mg/L] | 2.05 | 2.50 | > 4 |

**Table 5. Microalgae Distribution[1], expressed as the mean percentage of community, Chlorophyll A and Phaeophytin Values**

| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| **A. Diatom** | **19.4** | **18.1** | **13.6** |
| 1. Rhizosoleniaceae | 8.4 | 9.3 | 2 |
| 2. Cheatoceraceae | 6.2 | 5.1 | 2 |
| 3. Bacteriastraceae | 0.1 | 1.2 | 1 |
| 4. Nitzschiaceae | 0.1 | 0.1 | 1 |
| 5. Coscinodiscaceae | 1.1 | 1.1 | 1 |
| 6. Naviculaceae | - | Tr | - |
| 7. Surirellaceae | Tr | Tr | - |
| 8. Thalassiosiraceae | - | Tr | 0.5 |
| 9. Biddulphiaceae | 0.2 | 0.3 | 0.7 |
| 10. Asterionellaceae | 0.3 | 0.2 | 1.3 |
| 11. Dictyplaceae | 0.4 | 0.5 | 1.3 |
| 12. Eucanpiaceae | 0.4 | 0.3 | 0.4 |
| 13. Fragilariaceae | 0.1 | - | 1.3 |
| 14. Hemialceae | 0.6 | - | - |
| 15. Lauderiaceae | 0.7 | - | 1.1 |
| 16. Pleurosigmaceae | 0.1 | - | - |
| 17. Skeletonemaceae | 0.4 | - | - |
| 18. Thallasionemaceae | 0.3 | - | Tr |
| **B. Cyanophyceae (Blue-green)** | **72** | **57** | **75** |
| 1. Trichodesmium thiebautie | 30 | 1 | 15 |
| 2. Nannochloropsis sp | 1 | 5 | 5 |
| 3. Tetraselmis sp. | 0.5 | 0.5 | 5 |
| 4. Chlorella sp. | Tr | Tr | - |
| 5. Isochrysis sp. | 40.5 | 50.5 | 50 |
| **C. Dinoflagellate** | **2.5** | **Tr** | **5.7** |

| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| 1. Peridinium sp. | 0.5 | Tr | 3.1 |
| 2. Ceratium sp. | 0.5 | Tr | 0.7 |
| 3. Dinophysis sp. | 0.5 | Tr | 0.5 |
| 4. Protoperidinium sp. | 0.5 | Tr | 0.7 |
| 5. Gaunyaulax sp. | 0.5 | Tr | 0.7 |
| **D. Ciliophora sp.** | **4.2** | **5** | **4.1** |
| 1. Thintinnopsis sp. | 2.8 | 4 | 1 |
| 2. Favella sp. | 1.2 | 1 | 1 |
| 3. Codonellopsis sp. | 0.2 | Tr | 1.1 |
| 4. Epiplocylis sp. | - | Tr | 1 |
| **Total density ( x $10^4$ cells/L )** | **5.4** | **6.8** | **64.3** |
| **Chlorophyll A [mg/$m^3$]** | **0.2** | **0.3** | **0.61** |
| **Phaeophytin [mg/$m^3$]** | **0.1** | **0.1** | **0.60** |

[1] Values are means of duplicate or triplicate analysed. Standard deviations are omitted for clarity, were normally <5% (Tr - trace amount, less than 0.05%)

**Dominant Microalgae Species**

The dominant microalgae from the study area consisted of diatoms, flagellates, ciliophora and blue-green algae, as further detailed in Table 5.

From Table 5 above, it is obvious to note that the population of cyanophyceae (blue-green) algae dominates other types of species in all the three samples, where its maximum content was at Site 3. The measurement of chloprophyll A and phaeophytin were the highest for Site 3, in agreement to the highest cell count – 64.3 x $10^4$ cells/L – at the location. Identified blue-green algae species are *Trichodesmium thiebautie, Nannochloropsis sp., Tetraselmis sp.,* and *Isochrysis sp.,* the latter being the most dominant species within all the samples locations (40-50% of population count). *Isochrysis sp.* belongs to the microalgae class *Prymnesiophyceae* which is a flagellate cell with dominant golden brown pigment. It has a cell volume of 50-60 $\mu m^3$ with a average diameter of 5-6 µm and spherical rounded shape. Figure 4 depicts the typical morphology of *Isochrysis sp.* Typical contents of carbohydrate, lipid and mineral for *Isochrysis sp* are 5, 20 and 13% respectively.

Plankton bloom occurred at station 3 during which time its mean cell count had already reached a peak value of 64.3 x $10^4$ cells/L. During the bloom, *Isochrysis sp.* and *Trichodesmium thiebautii* was the most predominant alga with a value of > 80% of the total cell density.

High level of phosphate at Site 3 seems consistent with the previous findings in [8] and [9] that relate the presence of *Trichodesmium sp.* bloom with the high amount of inorganic phosphorus, ammonium and nitrate in the Indian Ocean and in the east coast of Lampung, South Sumatera, respectively.

Of the five blue-green algae species identified, four of them - *Nannochloropsis sp., Tetraselmis sp., Chlorella sp.* and *Isochrysis sp.* are commonly cited in various literatures discussing and highlighting on satisfactory and good qualities of biomass yield, lipid content, antioxidant properties and nutritional values they possess [10], [11], [12], [13]. These local species thus can be considered for further studies on the rate and optimization of $CO_2$ fixation from coal-fired power station in Malaysia.

## CONCLUSION

In a gesture to improve sustainability of coal-fired power generation through minimization of emitted $CO_2$ to the atmosphere, this work has completed an early initiative for the purpose. It has scanned some of available marine microalgae species in the vicinity of a coastal coal-fired power station in Malaysia, for the purpose of biological $CO_2$ fixation. It was found out that the dominant microalgae species from the study area consisted of diatoms, flagellates, ciliophora and cyanophyceae algae. The population of cyanophyceae algae appears to be the most dominant species in all the three samples location, and consists of *Trichodesmium thiebautie, Nannochloropsis sp., Tetraselmis sp., Chlorella sp.* and *Isochrysis sp.*, with the latter being the most dominant species within all the samples locations (40-50% of population count). At least four of these identified species are renowned to be commonly cited in literatures, of having satisfactory and good qualities on biomass yield, lipid content, antioxidant properties and nutritional values. With the growing concern and challenge of producing electricity in a carbon-constrained environment, concerted efforts ought to be wielded in minimizing $CO_2$ emission from fossil-fueled power plants. Biological approach can offer a more sustainable means, and at least for the Malaysian context, some local microalgae species identified through this work are eligible for further evaluations.

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