Screening of native microalgae species for carbon fixation at the vicinity of Malaysian coal-fired power plant

Liyana Yahya1,2, Razif Harun1(*) & Luqman Chuah Abdullah1

Global warming has become a serious issue nowadays as the trend of CO2 emission is increasing by years. In Malaysia, the electricity and energy sector contributed a significant amount to the nation’s CO2 emission due to fossil fuel use. Many research works have been carried out to mitigate this issue, including carbon capture and utilization (CCUS) technology and biological carbon fixation by microalgae. This study makes a preliminary effort to screen native microalgae species in the Malaysian coal-fired power plant’s surrounding towards carbon fixation ability. Three dominant species, including Nannochloropsis sp., Tetraselmis sp., and Isochrysis sp. were identified and tested in the laboratory under ambient and pure CO2 condition to assess their growth and CO2 fixation ability. The results indicate Isochrysis sp. as the superior carbon fixer against other species. In continuation, the optimization study using Response Surface Methodology (RSM) was carried out to optimize the operating conditions of Isochrysis sp. using a customized lab-scale photobioreactor under simulated flue gas exposure. This species was further acclimatized and tested under actual flue gas generated by the power plant. Isochrysis sp. had shown its capability as a carbon fixer with CO2 fixation rate of 0.35 gCO2/L day under actual coal-fired flue gas exposure after cycles of acclimatization phase. This work is the first to demonstrate indigenous microalgae species’ ability as a carbon fixer under Malaysian coal-fired flue gas exposure. Thus, the findings shall be useful in exploring the microalgae potential as a biological agent for carbon emission mitigation from power plants more sustainably.

1Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. 2Centre of Bioenergy and Sustainability, Renewable Energy and Green Technology, TNB Research Sdn. Bhd., No. 1, Lorong Ayer Itam, Kawasan Institusi Penyelidikan, 43000 Kajang, Selangor, Malaysia. (*)email: mh_razif@upm.edu.my
photobioreactor design, pH, source of CO₂ supply, temperature and nutrient media. Various studies have been reported on algae capabilities to grow under different flue gas exposure. For example, single phototroph species such as Tetraselmis sp and Chlorella sp were reported to grow well when exposed to flue gas composition containing 10–15% of CO₂ concentration. Also, some studies showed the promising results by using consortia species, for example, mixed freshwater culture with Desmodesmus sp as the dominant species were cultured under actual flue gas that contains up to 11% of CO₂ and Spirulina platensis with mixed algal culture were fed with flue gas at CO₂ concentration up to 15% v/v.

Most algae research in Malaysia focuses on the downstream application at a laboratory scale whereby to achieve economic viability and sustainability of this technology, the challenges in both upstream and downstream processes need to be appropriately addressed. As there are little works on the upstream process, this study explores the potential of native microalgae species as the biological carbon fixers under the Malaysian coal-fired flue gas exposure. The significance of utilizing native microalgae species instead of common species is to expedite the acclimatization period and ease-out the in-situ biological CO₂ fixation process due to the robust and conducive environment for optimum growth of the species. The optimized native microalgae species obtained at laboratory conditions were then tested under actual coal-fired flue gas to screen their potential in mitigating CO₂ emission from industries. A central composite design (CCD) was employed to determine the effect of four operating parameters including gas flow rate, temperature, luminance and pH to obtain the maximum carbon fixation rate ability of the microalgae. The work is significantly important to demonstrate the potential of native microalgae species as the biological carbon fixers towards a more circular economy and environmentally sustainable coal-fired power stations in the long term.

Materials and methods

Sample collection. The sampling of native microalgae species was conducted at the Sultan Azlan Shah TNB Power Station, Perak, Malaysia. This coal-fired power plant generates 3100 MW of electricity and located on a 325 hectare wholly man-made island off the Lekir coast in Janamanjung, Perak, Malaysia. Three sampling locations were identified in the vicinity based on the different site characteristics, as tabulated in Table 1 and mapped in Fig. 1.

The GPS coordinate was measured using Garmin GPSMAP Handheld Navigation Device. Site #1 has less human activities in the area where it is located within a bay of Teluk Rubiah where a resort was once operated. The site #2 is located at the seawater discharge point of the station and site #3 has rich samples of microalgae derived from rich river discharges. All the sites’ depth was also checked to be at least 10 m deep as shown by a bathymetry chart around the power station in Fig. 1.

The samples were collected using a dip net method where a plankton net of 35 μm mesh size, 25 cm mouth diameter and 1.5 m long were used. The net was submerged at least 1.5 m below the surface of water and then pulled it up vertically using a rope and pulley assembly. 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. The physical properties like luminance, temperature, pH and dissolved O₂ were measured at the site using lux meter and Eutech CyberScan PCD 650 portable multi meter. Samples were kept in 500 ml plastic bottles, labeled and deposited in a cool-box during transportation to laboratory and the phosphate content (PO₄³⁻) was later determined using HACH standard procedure in the laboratory. The physical characteristics of the sampling at different sites are shown in Table 1.

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 with penicillin levels ranging from 20–500 mg/l to eliminate contaminants. Air was bubbled through the culture with continuous light supply. After 3–4 days, a narrow range spectrum media was introduced to provide conducive environment for the dominant species to survive. Small volumes (15 ml) samples from the enriched cultures were centrifuged at 3000 rpm for 15 min. The supernatant was removed, and cells were re-suspended in fresh medium. The centrifugation process was repeated for few times to expel the most of microorganisms presented in algal sample. The cells were then streaked onto agar plates using aseptic technique and kept for at least seven days to grow the microalgae. Repeated streak-plating was carried out to peak up a single colony from earlier streaked plates. The single colonies were picked up by a loop and allowed to grow in tubes and vial. The colony was examined for its purity by checking the cells under microscope. Identification of species was done by visual inspection of the morphologies observed under a microscope with reference to the Algae Identification Field Guide and online database.

### Table 1. Details of the sampling sites.

| Site ID | GPS coordinate | Approximate distance from power plant | Luminance (klux) | Temperature (°C) | pH | Dissolved O₂ (%) | Phosphate (mg/L) | Remarks |
|---------|----------------|--------------------------------------|------------------|------------------|----|----------------|-----------------|---------|
| Site 1  | N 4° 9.435' E 100° 37.079' | 2.5 km | 26.6 ± 2.0 | 30.5 ± 0.3 | 7.97 ± 0.05 | 89.85 ± 0.5 | 2.05 ± 0.5 | Near lighthouse at Teluk Rubiah |
| Site 2  | N 4° 8.195' E 100° 38.103' | 2.4 km | 31.25 ± 3.0 | 29.6 ± 0.3 | 8.36 ± 0.3 | 92.3 ± 0.3 | 2.50 ± 0.5 | Near coal jetty |
| Site 3  | N 4° 2.675' E 100° 41.859' | 14 km | 8.05 ± 2.0 | 29.0 ± 0.4 | 7.50 ± 0.5 | 83.3 ± 0.5 | > 4 | At mouth of Perak river |
Laboratory microalgal cultivation. The laboratory microalgal cultivation was performed at the Microbiology Laboratory, TNB Research Sdn. Bhd., Kajang, Selangor. The isolated microalgae species were cultured in 2L flasks using an f/2 medium composed of NaCl (24.32 g/l), MgCl₂ (5.14 g/l), CaCl₂ (1.14 g/l), KCl (0.69 g/l), NaHCO₃ (0.2 g/l), KBr (0.1 g/l), H₃BO₃ (0.027 g/l), SrCl₂ (0.026 g/l), NH₄Cl (0.0064 g/l), NaF (0.003 g/l), NaSiO₃ (0.002 g/l), FePO₄ (0.001 g/l), NaNO₃ (75 g/l), NaH₂PO₄ (5 g/l), Na₂EDTA (4.36 g/l), FeCl₃·6H₂O (3.15 g/l), trace metal stock solution (1.0 ml/l) and vitamin stock solution (0.5 ml/l). The medium preparation was performed in a biohazard laminar flow to minimize contamination. The microalgae were cultivated for a maximum of 14 days at room temperature with an average of 26 °C and illuminated with 18 W fluorescent bulb for 12 h with an average luminosity of 800 lx using a timer switch. Air pump with 0.045 MPa compression and 150 L/min maximum capacity was used and to supply aeration for the algae culture. The samples were taken daily to monitor their growth performance in terms of cell density, chlorophyll A and phaeophytin content.
Carbon fixation experiment. A custom-made two units of bubbling laboratory scale photobioreactor (PBR) was used with a capacity of 10L each. The reactor was made from polycarbonate due to its high resistance and transparency of 92%\(^\circ\). The PBR temperature can vary from −10 to 100 °C with the help of chiller/heater. The system was equipped with other instruments including pH sensor, dissolved O\(_2\) sensor, thermocouple, fluorescent bulbs with timer-controller and data acquisition system for automatic data logging. The layout of the PBR is shown in Fig. 3. The simulated flue gas was supplied throughout the cultivation period and its composition is listed in Table 2. The microalgae were cultivated for up to 14 days and the operating conditions were set based on the optimization statistical model.

The calculation of carbon fixation as shown in Eq. 1 was adopted from balanced photosynthesis formula on the ratio between CO\(_2\) moles and molecular formula of biomass which is about 1.8 g of CO\(_2\) can be fixed by 1 g of microalgae\(^\circ\):

\[
4\text{CO}_2 + \text{nutrient} + \text{H}_2\text{O} + \text{light} \rightarrow 4\text{CO}_0.48\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01} + 3(1/2)\text{O}_2
\]  
(1)

The doubling time of the microalgae cells is the time taken for the population to double its growth and was derived from Monod equation, as in Eq. 2\(^\circ\):

\[
T_d = (t_2 - t_1) \times \frac{\ln(2)}{\ln(N_t) - \ln(N_0)}
\]  
(2)

where \(T_d\)—doubling time (time taken for population to double) (day); \(t_2\)—last day of the population growth curve (day); \(t_1\)—first day of the population growth curve (day); \(N_t\)—number of cells on the last cultivation period; \(N_0\)—number of cells on the first cultivation period.

Analysis of parameters monitoring. Cell count 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 (Optika DM-15, Italy).

![Figure 3. Layout of customize lab-scale photobioreactor.](image-url)
Chlorophyll-A and phaeophytin determination. Chlorophyll-A and phaeophytin were analyzed by a spectrophotometric method. 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 was folded and placed in test tubes containing 15 ml of acetone 90% and was left to be degraded for up to 30 min. 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 Eqs. 3 and 4 as follows:

$$\text{Chlorophyll - A (mg/L)} = (A_b - A_a) \times 2.43 \times 10.48 \times \frac{V}{L}$$

$$\text{Phaeophytin (mg/L)} = [A_b - 2.43(A_b - A_a)] \times 10.48 \times 1.7 \times V$$

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% wt%, concentration) used (15 ml) and $L$ is the width (cm) of cuvette (1 cm).

Optimization statistical analysis. An optimization study using central composite design (CCD) was conducted with four operating parameters including gas flow rate, temperature, luminance and pH to obtain the maximum carbon fixation rate ability of Isochrysis sp. The level of each parameter is shown in Table 3. The response or results gained from experimental work were then analysed by Design Expert 7.0 (Stat Ease Inc. Minneapolis). The ANOVA analysis will be interpreted to understand the effects of each parameters towards the highest carbon fixation rate.

CO$_2$ fixation test under actual flue gas exposure. A portion of flue gas generated at the power plant was tapped at the existing emission monitoring analyzer. The gas flow was maintained at 0.15 ± 0.03 L/min inside the culture. The experimental setup was placed inside the main stack which received the emissions from the three combustion unit as illustrated in Fig. 4. Each experiment was cultivated in 2 × 10L customized photobioreactor for maximum of 14 days. The culture was supplied with light for 12 h with an average luminosity of 15 µmol/m$^2$ s$^{-1}$ at an ambient temperature range of ±28 °C and a pH range of 6–7.
Results and discussion
Dominant microalgae species. The distribution of dominant microalgae species from the vicinity of a coal-fired power plant consisted of several different types of algae include diatom, cyanophyceae, blue-green algae, dinoflagellate and ciliophoran as tabulated in Table 4. The screening of dominant species is crucial to ensure the availability of algae in future use and its robustness to grow at the surrounding ambient.

The population of Cyanophyceae and Blue-green algae were found to be dominated at all the selected sites, where the highest population of the species was identified at Site 3. Chlorophyll is a pigment that responsible for the photosynthesis process and phaeophytin is one of the breakdown products of chlorophyll. A high amount of these two elements indicates a higher population of microalgae. The measurements of chlorophyll A and phaeophytin were at the highest in Site 3 which had the highest cell count of 64.3 × 10⁴ cells/L. This reading might be due to site 3, which is located at the Perak river’s mouth, which enriches nutrients from upstream discharges as indicated by the higher amount of phosphate content than the other two sites. Moreover, the identified

| Type                  | Percentage (%) | Site 1 | Site 2 | Site 3 |
|-----------------------|----------------|-------|-------|-------|
| A. Diatom             |                |       |       |       |
| 1. Rhizosoleniaceae   | 8.4            | 9.3   | 2     |
| 2. Cheatoceaeae       | 6.2            | 5.1   | 2     |
| 3. Bacteriastriaceae  | 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. Eucaniplaeae      | 0.4            | 0.3   | 0.4   |
| 13. Fragilasaceae     | 0.1            | –     | 1.3   |
| 14. Hemialceae        | 0.6            | –     | –     |
| 15. Launderiaceae     | 0.7            | –     | 1.1   |
| 16. Pleurosigmaceae   | 0.1            | –     | –     |
| 17. Skeletonemaceae   | 0.4            | –     | –     |
| 18. Thallasionemaceae | 0.3            | –     | Tr    |
| B. Cyanophyceae       | 30             | 1     | 15    |
| 1. Trichodesmium thiebautii | 30  | 1     | 15    |
| C. Blue-green algae   | 42             | 56    | 60    |
| 1. Nanochloropis sp.  | 1              | 5     | 5     |
| 2. Tetraselmis sp.    | 0.5            | 0.5   | 5     |
| 3. Chlorella sp.      | Tr             | Tr    | –     |
| 4. Isochrysis sp.     | 48.5           | 50.5  | 50    |
| D. Dinoflagellate     | 2.5            | Tr    | 5.7   |
| 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. Proteoperidinium sp.| 0.5           | Tr    | 0.7   |
| 5. Gaunyuallax sp.    | 0.5            | Tr    | 0.7   |
| E. 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 (× 10⁴ cells/L) | 5.4 | 6.8 | 64.3 |
| Chlorophyll A (mg/m³) | 0.2            | 0.3   | 0.61  |
| Phaeophytin (mg/m³)   | 0.1            | 0.1   | 0.60  |

Table 4. Microalgae distribution, expressed as the mean percentage of community, chlorophyll a and phaeophytin values. Values are means of duplicate or triplicate analysed. Standard deviations are omitted for clarity, were normally < 5% (Tr—trace amount, less than 0.05%).
Cyanophyceae and Blue-green algae species in all samples were *Trichodesmium thiebauti*, *Nannochloropsis* sp., *Tetraselmis* sp., *Isochrysis* sp., and traces amount of *Chlorella* sp. According to the microalgae population listed in Table 4, *Isochrysis* sp. was the dominant species within all the sample locations, which amounted up to 40–50% of the total population count. *Isochrysis* sp. belongs to the microalgae class of Prymnesiophyceae which is a flagellate cell-type with dominant golden brown pigment. It has a cell volume of 50–60 µm³ with an average diameter of 5–6 µm and a spherical rounded shape. Out of these blue-green algae species identified, three of them—*Nannochloropsis* sp., *Tetraselmis* sp. and *Isochrysis* sp. are commonly cited in various literature, discussing and highlighting their capability in producing good quality of biomass yield, lipid content, nutritional values and antioxidant properties. Thus, these local species can be considered potential microalgal biomass for scale-up and further studies on the rate and optimization of CO₂ fixation from a coal-fired power station in Malaysia.

**Screening of carbon fixation abilities.** Three dominant isolated species, *Nannochloropsis* sp., *Tetraselmis* sp., and *Isochrysis* sp., were further scale-up and tested with ambient air and pure CO₂ gas to screen for carbon fixation ability. *Isochrysis* sp. showed superior result in carbon fixation rate ability followed by *Tetraselmis* sp. and *Nannochloropsis* sp., as shown in Fig. 5a, b. This explained the dominancy of *Isochrysis* sp. in all the samples. The species is robust with the harsh condition of the power plant's surroundings containing slightly higher CO₂ concentration in its ambient.

It can be observed that *Isochrysis* sp. superseded *Nannochloropsis* sp. and *Tetraselmis* sp. in both culture environments; ambient air and pure CO₂. The growth rate characteristic of these three species was also studied and their doubling time was determined using Eq. 4. Doubling time indicates the growth rate of a species and the rate of CO₂ consumed. The results summarized in Table 5 strengthen the superiority of *Isochrysis* sp. as a better CO₂ fixer where its doubling time is only about two days compared to *Nannochloropsis* sp. and *Tetraselmis* sp. that took up to five to seven days under pure CO₂ exposure. The algae's doubling time is affected by various parameters, such as temperature, pH, sunlight, and CO₂ concentration. The shorter period of doubling time indicates that the species is fast-growing algae and can utilize higher CO₂ as reflects its higher cell density.

**Optimizing the *Isochrysis* sp. carbon fixation.** Based on the screening of potential carbon fixation for the three species under ambient air and pure CO₂ exposure, *Isochrysis* sp. was found to have the highest CO₂ fixation rate. Thus, *Isochrysis* sp. was further optimized and exposed with simulated flue gas containing 4% CO₂, 3% O₂, 105 mg/m³ CO, and 272 mg/m³ NO₂ at 2 x 10L lab-scaled photobioreactor. This approach was crucial for adaptation of the species before being tested with actual flue gas as higher CO₂ concentration will not only improve the photosynthesis rate, however it could also lead to the acidification of the culture. Selection of suitable species that can tolerate with low pH and able to multiply within shorter doubling time is among the crucial parameters to ensure the survival of the culture under actual flue gas exposure as some of the research indicated that the algae culture was inhibited even with 5% of CO₂ concentration. In this study, 21 experimental runs were conducted as tabulated by Design Expert Software as in Table 6 to study the interaction effects of operating parameters on *Isochrysis* sp. CO₂ fixation rate.

### Table 5. Percentage of carbon fixation abilities of selected microalgae.

| Species       | *Isochrysis* sp. | *Tetraselmis* sp. | *Nannochloropsis* sp. |
|---------------|------------------|-------------------|------------------------|
| CO₂ fixation rate (gCO₂/L day) | 0.101 | 0.019 | 0.013 |
| Doubling time (days) | 1.99 | 5.72 | 7.21 |

Figure 5. Carbon fixation rate of microalga species in 2-L culture with (a) ambient air (b) pure CO₂.

---

| Species       | *Isochrysis* sp. | *Tetraselmis* sp. | *Nannochloropsis* sp. |
|---------------|------------------|-------------------|------------------------|
| Species       | *Isochrysis* sp. | *Tetraselmis* sp. | *Nannochloropsis* sp. |
| Doubling time (days) | 1.99 | 5.72 | 7.21 |

---

| Species       | *Isochrysis* sp. | *Tetraselmis* sp. | *Nannochloropsis* sp. |
|---------------|------------------|-------------------|------------------------|
| Doubling time (days) | 1.99 | 5.72 | 7.21 |

---

| Species       | *Isochrysis* sp. | *Tetraselmis* sp. | *Nannochloropsis* sp. |
|---------------|------------------|-------------------|------------------------|
| Doubling time (days) | 1.99 | 5.72 | 7.21 |
The interaction with four parameters were analyzed by Response Surface Methodology (RSM) approach
to determine the optimum parameters for the highest carbon fixation rate of Isochrysis sp. In predicting the
optimal values of CO2 fixation rate within the experimental constrains, the experimental results were analyzed
by regression analysis consisting of the effects of linear, quadratic and interaction which gave the following
regression equation:

\[ Y = 0.82 + 0.037A + 0.12B - 0.047C - \left[ 1.00 \times 10^{-2} \right]D - \left[ 3.75 \times 10^{-3} \right]AB - 0.031AC - \left[ 1.25 \times 10^{-3} \right]AD \\
- 0.0199BC + 0.056BD + 6.25 \times 10^{-3} \cdot CD - 0.088A^2 - 0.098B^2 - 0.16C^2 - 0.017D^2 \]  

where \( Y \) is the CO2 fixation rate and A, B, C and D are the temperature, pH, gas flow rate and lighting respectively.

It was found that the highest carbon fixation rate of 0.350 gCO2/L day was achieved at temperature 35 °C, gas
flow rate of 0.10 L/min, pH 7, and luminosity of 1000lux. Significance and adequacy of the model was analyzed
through the analysis of variance (ANOVA). The summary of ANOVA representing the results of the quadratic
response surface model fitting is shown in Table 7. The quadratic regression model was highly significant, as
evident by the low probability value (\( P_{\text{model}} > F = 0.0051 \)). Overall model’s (quadratic) F-value of 9.80 as per Table 7
implies the model is significant.

In favor to the optimization of CO2 fixation rate, B, A2, B2 and C2 were the significant model terms, which
indicates that these parameters have a significant contribution towards achieving the highest CO2 fixation rate.

At the model level, the correlation measure for estimating the regression equation is the determination of coeffi-
cient, \( R^2 \). The coefficient of \( R^2 \) determines the goodness of the model fitting. In this study, the value of \( R^2 \) is 0.9581
as shown in Table 8, indicates a better correlation between observed and predicted values where only 4.19% of
variations were not explained by the model. The coefficient of variation (CV) indicates the degree of precision
with which the treatments are compared. Usually, the higher the value of the CV, the lower is the reliability of
the experiment. In this study, the value of CV was 16.51%, which indicated a small residue between actual and
predicted values of CO2 fixation rate. The adequate precision value for this study is 9.553, which measured the
signal to noise ratio. A ratio greater than 4 is desirable as it gives better precision and reliability of the carried
out experiments.

The normal probability plot of residuals and the plot of residuals versus predicted values of the response for
the CO2 fixation rate are shown in Fig. 6. A satisfactory correlation between actual and predictive values was
presented, as distribution of plots was balanced throughout the linear line, indicating a good fit of the model.

The 3D response surface and 2D contour plots are graphical representation of the regression equation to
determine the optimum values of the variables. Interaction of each operating parameters in achieving highest
carbon fixation rate of Isochrysis sp. is presented in 2D contour plots and 3D response surface as in Fig. 7. The
maximum activity was obtained near the center points of response surface.

| Run | Factor 1 (A) temperature (°C) | Factor 2 (B) pH | Factor 3 (C) gas flow rate (L/min) | Factor 4 (D) luminance (lux) | Response (Y) CO2 fixation rate (gCO2/L day) |
|-----|-------------------------------|----------------|----------------------------------|-----------------------------|---------------------------------------------|
| 1   | 30.00                         | 6.00           | 0.15                             | 2500.00                     | 0.088                                       |
| 2   | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.031                                       |
| 3   | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.144                                       |
| 4   | 35.00                         | 5.00           | 0.10                             | 2000.00                     | 0.000                                       |
| 5   | 20.00                         | 6.00           | 0.15                             | 1500.00                     | 0.035                                       |
| 6   | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.151                                       |
| 7   | 35.00                         | 7.00           | 0.10                             | 1000.00                     | 0.350                                       |
| 8   | 35.00                         | 7.00           | 0.20                             | 1000.00                     | 0.321                                       |
| 9   | 30.00                         | 8.00           | 0.15                             | 1500.00                     | 0.260                                       |
| 10  | 25.00                         | 7.00           | 0.20                             | 2000.00                     | 0.249                                       |
| 11  | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.132                                       |
| 12  | 30.00                         | 4.00           | 0.15                             | 1500.00                     | 0.000                                       |
| 13  | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.121                                       |
| 14  | 30.00                         | 6.00           | 0.15                             | 500.00                      | 0.049                                       |
| 15  | 35.00                         | 5.00           | 0.20                             | 2000.00                     | 0.037                                       |
| 16  | 25.00                         | 5.00           | 0.10                             | 1000.00                     | 0.000                                       |
| 17  | 30.00                         | 6.00           | 0.15                             | 1500.00                     | 0.125                                       |
| 18  | 40.00                         | 6.00           | 0.15                             | 1500.00                     | 0.000                                       |
| 19  | 25.00                         | 7.00           | 0.10                             | 2000.00                     | 0.347                                       |
| 20  | 25.00                         | 5.00           | 0.20                             | 1000.00                     | 0.000                                       |
| 21  | 30.00                         | 6.00           | 0.05                             | 1500.00                     | 0.125                                       |
According to Fig. 7, the predicted highest CO2 fixation rate was at pH 7.5, temperature 30 °C, luminance of 1500lux and 0.15L/min gas flow rate. A validation experiment was carried out to verify these optimum parameters and the ability of *Isochrysis* sp. carbon fixation rate was further improved by 6% with the carbon fixation rate of 0.370 gCO2/L day.

Table 7. ANOVA for response surface quadratic model with CO2 fixation rate as a response.

| Source | Sum of square | Degree of freedom | Mean square | F-value | P-value > F |
|--------|---------------|-------------------|-------------|---------|-------------|
| Model  | 1.10          | 14                | 0.078       | 9.80    | 0.0051      |
| A      | 0.011         | 1                 | 0.011       | 1.14    | 0.2802      |
| B      | 0.11          | 1                 | 0.11        | 13.83   | 0.0099      |
| C      | 0.035         | 1                 | 0.035       | 4.40    | 0.0807      |
| D      | 8.00 (10^-4)  | 1                 | 8.000 (10^-4) | 0.10    | 0.7624      |
| AB     | 5.625 (10^-3) | 1                 | 5.625 (10^-3) | 7.041 (10^-3) | 0.9359 |
| AC     | 7.813 (10^-3) | 1                 | 7.813 (10^-3) | 0.98    | 0.3609      |
| AD     | 6.250 (10^-6) | 1                 | 6.250 (10^-6) | 7.824 (10^-6) | 0.9786 |
| BC     | 2.812 (10^-1) | 1                 | 2.812 (10^-1) | 0.35    | 0.5746      |
| BD     | 0.013         | 1                 | 0.013       | 1.58    | 0.2549      |
| CD     | 3.125 (10^-4) | 1                 | 3.125 (10^-4) | 0.039   | 0.8497      |
| A²     | 0.20          | 1                 | 0.20        | 24.45   | 0.0026      |
| B²     | 0.24          | 1                 | 0.24        | 30.31   | 0.0015      |
| C²     | 0.62          | 1                 | 0.62        | 77.41   | 0.0001      |
| D²     | 7.227 (10^-3) | 1                 | 7.227 (10^-3) | 0.90    | 0.3783      |
| Residual | 0.048      | 6                 | 0.048       | 8.29    | 0.0331      |
| Lack of fit | 0.047 | 2         | 0.024       | 1.58    | 0.2549      |
| Pure error | 5.200 (10^-4) | 4         | 1.300 (10^-4) | 0.90    | 0.3783      |
| Corrected total | 1.14 | 20          | -           | -       | -           |

Table 8. Analysis of model fitting.

| Elements                 | Values  |
|--------------------------|---------|
| Standard deviation (SD)  | 0.089   |
| Mean                     | 0.54    |
| C.V. %                   | 16.51   |
| PRESS                    | 1.97    |
| R² (R-squared)           | 0.9581  |
| Adjusted R² (adj R-squared) | 0.8603  |
| Predicted R² (pred R-squared) | -0.7214 |
| Adeq precision           | 9.553   |

Figure 6. Normal probability plot for the residuals from CO2 fixation rate model.
Effects of operating parameters. Based on 2D contour plots and 3D response surface as in Fig. 7, the optimal temperature to enhance Isochrysis sp. growth is at 30 °C. The rate of microalgae growth was retarded as the temperature decreases and tend to inhibit as the temperature rises. The range of optimal temperature varies depending on the species, however, most of the microalgae species have an optimum temperature in a range of 20–30 °C\textsuperscript{20}. Determination of optimal temperature is crucial to ensure the survival of selected microalgae during outdoor cultivation as it will be exposed to a large fluctuation in temperature and excessive heat will create shear stress that can disrupt microalgae cell wall\textsuperscript{34,35}. The optimum range for Isochrysis sp. was at pH 7–8\textsuperscript{36,37}. This also agreed well with the observation from Table 7 that pH has a significant influence on the CO\textsubscript{2} fixation rate as indicated by the values of Prob > F, which was less than 0.0500. The CO\textsubscript{2} and SO\textsubscript{2} solubility highly contributes to the variation of pH value as the growth will be affected by the culture’s acidity due to simulated flue gas exposure. This acidic environment may retard and inhibit the growth of microalgae\textsuperscript{38,39}. It is important to control the gas flow rate to moderate the effect of acidic environment in the culture. In this study, the optimum flue gas flow rate was achieved at 0.15 L/min. Higher gas flow rate contributes to the decrement of pH value and produces hydrodynamic stress to the algae, which will inhibit the culture. An optimum gas flow rate is also crucial in maintaining the homogeneity of the culture. Compared to other operating parameters, illuminance gave less impact to microalgae culture in this study as the experiment was conducted indoor and the gap between read-
ings are quite small. Based on studies conducted at the outdoor condition under direct sunlight, the optimum range of luminosity is in a range between 5000 and 10,000 klux.

**Carbon fixation ability under actual coal-fired flue gas exposure.** *Isochrysis* sp. had shown its capability as a carbon fixer under ambient air and simulated flue gas exposure; thus, it was further tested under the power plant's actual flue gas. The cultures were subjected to cycles of growth phase to observe the growth adaptability of *Isochrysis* sp. under harsh flue gas condition containing on average of 4.08% O₂, 200.21 mg/m³ SO₂, 212.29 mg/m³ NO₂, 4.73% CO₂ and 50.72 mg/m³ CO throughout the culture period. Figure 8 shows the four batches of *Isochrysis* sp. culture using a 2×10L customized photobioreactor skid and each cycle lasted up to 8 days. The control culture was first acclimatized using aeration before being exposed to actual flue gas. The control culture and the first two batches under flue gas exposure showed a stagnant growth, which indicates a gradual adaptation of the cultures with the elevated CO₂ concentration in the actual coal-fired flue gas, as shown in Fig. 8. This adaptation might also happen due to the flue gas pollutants such as SO₂, NO₂, and particulate matter. Some studies indicated that these pollutants could inhibit microalgal growth due to decrement in pH value when SO₂ hydrolysis happens. On the other hand, at certain concentrations, NO₂ and particulate matter can be transformed into nutrient and minerals sources for microalgae and promote its growth. However, different species showed different effects on these pollutants, as some studies demonstrated no significant effects on microalgal growth. However, the microalgal growth in this study was not influenced by the pollutant concentrations due to their intrinsic characteristics.

As shown in Fig. 8, after almost 3 weeks of acclimatization phase, the third culture with a slightly higher initial culture density demonstrated the survival of the species under the influence of harsh flue gas conditions. This can be observed by the appearance of the log phase with an increment in optical density, number of cells, and dry weight of the culture as shown in Figs. 8 and 9. This was also supported by a few research that suggested the gradual adaptation of microalgae over high CO₂ concentration. A study carried out by Aslam et al. (2017) took 2 to 4 weeks adaptation period before the mixed freshwater dominated by *Desmodesmus* sp. was tested under
actual flue gas containing 11.24% CO₂. The maximum rate of Isochrysis sp. carbon fixation was achieved at 0.35 gCO₂/L day under this actual flue gas exposure.

However, for the last two days of the culture period, the growth of culture was retarded and started to enter a decay phase. This phenomenon happened due to the decrement of pH value, below pH 6 in the culture. As observed in Fig. 10, dissolved CO₂ was rapidly increased in the last two days of the culture up to 15.2% and resulting in the pH dropped which further inhibit the culture growth. This was also supported by the declination of cell density and cell count by about 40%.

Table 9 summaries several studies reported by other workers using similar dominant species in this study. These data illustrated that higher biomass productivity and CO₂ fixation rate can be achieved with higher CO₂ concentration. The results from this study can be considered quite low as it is a preliminary effort to investigate the potential of indigenous species for CO₂ mitigation. There are few strategies and parameters to address in improving the microalgae productivity such as enhancing culture condition, using consortium microalgae species and improving photobioreactor design⁴³,⁴⁴,⁴⁶. The improvement of microalgae productivity will further enhance the CO₂ fixation ability.

Conclusions
The findings from this study demonstrated proof of concept on the application of microalgae as the biological agent for carbon fixation towards sustainable coal-fired power generation by reducing the CO₂ emission. In this study, dominant indigenous species from the vicinity of Malaysian coal-fired power plant were screened and tested in the laboratory for their fixation capabilities. The interaction of four operating parameters was analyzed by Response Surface Methodology (RSM) approach to determine the highest carbon fixation rate of Isochrysis sp. This superior microalgae species was then adapted to cycles of growth phase under harsh flue gas exposure from coal combustion at Sultan Azlan Shah Power Station, Manjung, Perak. Isochrysis sp. had shown its capability as a carbon fixer under actual flue gas exposure after a certain period of acclimatization. The downstream application of algae biomass in producing valuable downstream products could also be explored to promote industrial symbiosis. With several improvements, including culture techniques, photobioreactor design, and scale-up parameters, microalgae could become a sustainable solution in neutralizing carbon emission from power plants in the years to come.
Received: 12 October 2020; Accepted: 7 December 2020
Published online: 18 December 2020

References

1. Kumar, K., Nag, C., Nayak, R., Lindblad, P. & Das, D. Development of suitable photobioreactors for CO₂ sequestration addressing global warming using green algae and cyanobacteria. *Bioreour. Technol.* **102**, 4945–4953 (2011).

2. Goss, R. M. *BP Statistical Review of World Energy* (2019).

3. Pires, J. C. M., Martins, F. G. & Simões, M. Research developments on carbon capture and storage: An overview. *Chem. Eng. Res. Des.* **89**, 1446–1460 (2011).

4. Aslam, A., Thomas-hall, S. R., Aziz, T. & Schenk, P. M. Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas. *Bioreour. Technol.* **233**, 271–283 (2017).

5. Maeda, K., Owada, M., Kimura, N. K. & Karube, I. CO₂ fixation from the flue gas on coal-fired thermal power plant by microalgae. *Energy Convers. Mgmt* **36**, 717–720 (1995).

6. Chen, H. et al. Application of power plant flue gas in a photobioreactor to grow *Spirulina algae*, and a bioactivity analysis of the algal water-soluble polysaccharides. *Bioreour. Technol.* **120**, 256–263 (2012).

7. Skjønes, K., Lindblad, P. & Müller, J. BioCO₂—A multidisciplinary, biological approach using solar energy to capture CO₂ while producing H₂ and high value products. *Bionol. Eng.* **24**, 405–413 (2007).

8. Kumar, A. et al. Enhanced CO₂ fixation and biofuel production via microalgae: Recent developments and future directions. *Trends Biotechnol.* **28**, 371–380 (2010).

9. Manzolini, G. et al. Techno-economic assessment of SEWGS technology when applied to integrated steel-plant for CO₂ emission mitigation. *Int. J. Greenh. Gas Control* **94**, 102935 (2020).

10. Tang, M., Wang, S., Dai, C. & Liu, Y. Exploring CO₂ mitigation pathway of local industries using a regional-based system dynamics model. *Int. J. Inf. Manag.* **52**, 102079 (2020).

11. Romeo, L. M. & Bailera, M. Design configurations to achieve an effective CO₂ use and mitigation through power to gas. *J. CO₂ Util.* **39**, 101174 (2020).

12. Takayabu, H. CO₂ mitigation potentials in manufacturing sectors of 26 countries. *Energy Econ.* **86**, 104634 (2020).

13. Zhou, W. et al. Bio-mitigation of carbon dioxide using microalgae systems: Advances and perspectives. *Renew. Sustain. Energy Rev.* **76**, 1163–1175 (2017).

14. Kao, C. Y. et al. Utilization of carbon dioxide in industrial flue gases for the cultivation of microalg *Chlorella sp*. *Bioreour. Technol.* **166**, 485–493 (2014).

15. Alomomani, F. et al. Intergraded wastewater treatment and carbon bio-fixation from flue gases using *Spirulina platensis* and mixed algal culture. *Process Saf. Environ. Prot.* **124**, 240–250 (2019).

16. Huynh, M. N. *Algae Identification* (Agriculture and Agri-Food Canada, 2006).

17. Manoylov, K. M. Taxonomic identification of algae (morphological and molecular): Species concepts, methodologies, and their implications for ecological bioassessment. *J. Phycol.* **50**, 409–424 (2014).

18. Guiry, M. D. AlgaBase is a Global Algal Database of Taxonomic, Nomenclatural and Distributional Information. https://www.algabase.org (1996).

19. Nag, C. et al. Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. *Int. J. Hydrog. Energy* **35**, 10218–10238 (2010).

20. Suryata, I., Svarvarsson, H. G., Einarsson, S., Brynjolfsdottir, A. & Maliga, G. Geothermal CO₂ bio-mitigation techniques by utilizing microalgae at the blue lagoon, Iceland. *Proceedings, Thirty-Fourth Workshop on Geothermal Reservoir Engineering* **34**, 1–8 (2002).

21. Chen, X. et al. Lumostatic strategy for microalgae cultivation utilizing image analysis and chlorophyll a content as design parameters. *Bioreour. Technol.* **102**, 6005–6012 (2011).

22. Xiong, J. et al. Intrinsinc kinetic model of photautotrophic microalgae based on chlorophyll fluorescence analysis. *Math. Biosci.* **315**, 108234 (2019).

23. Sun, Z., Wang, X. & Liu, J. Screening of *Isochysis strains* for simultaneous production of docosahexaenoic acid and fucoxanthin. *Algal Res.* **41**, 101545 (2019).

24. Banerjee, A., Guria, C., Matiì, S. K., Banerjee, C. & Shukla, P. Carbon bio-fixation, effect of physicochemical factors and carbon supply strategies by Nannochloropsis sp. using flue gas and fertilizer. *Biomass Bioenergy* **125**, 95–104 (2019).

25. Gao, S. et al. Incorporation of salinity, nitrogen, and shading stress factors into the Fluesseum Algae Biomass Growth model. *Algal Res.* **35**, 462–470 (2018).

26. El Shennawy, E. A. et al. Effect of cultivation parameters and heat management on the algae species growth conditions and biomass production in a continuous feedstock photobioreactor. *Renew. Energy* **148**, 807–815 (2020).

27. Alzahrani, E. O., El-Deesoky, M. M. & Dogra, P. Global dynamics of a cell quota-based model of light-dependent algae growth in a chemostat. *Commun. Nonlinear Sci. Numer. Simul.* **90**, 105295 (2020).

28. de Morais, M. G. & Costa, J. A. V. Isolation and selection of microalgae from coal fired thermodi elektric power plant for biofixation of carbon dioxide. *Energy Convers. Manag.* **48**, 2169–2173 (2007).

29. Cheah, W. Y., Show, P. L., Chang, J. S., Ling, T. C. & Juan, J. C. Biosequestration of atmospheric CO₂ and flue gas-containing CO₂ by microalgae. *Bioreour. Technol.* **184**, 190–201 (2015).

30. Shukla, P., Garai, D., Zafar, M., Gupta, K. & Shrivastava, S. Process parameters optimization for lipase production by *Rhizopus oryzae* KG-10 under submerged fermentation using response surface methodology. *J. Appl. Sci. Environ. Nutr.* **2**, 93–103 (2007).

31. Gao, P., Xiang, W., Li, X. & Liu, S. Optimization of the Maillard reaction of xylose with citruline for modulating aroma compound formation in fermented tilapia fish head hydrolysate using response surface methodology. *Food Chem.* **331**, 127353 (2020).

32. Berkani, M., Kadmi, Y., Bouchareb, M. K., Bouhelassa, M. & Bouzaza, A. Combination of a Box–Behnken design technique with response surface methodology for optimization of the photocatalytic mineralization of C.I. Basic Red 46 dye from aqueous solutions. *Appl. Med. J. Chem.* **3**, 8338–8346 (2020).

33. Khatoon, H. & Rai, J. P. N. Optimization studies on biodegradation of atrazine by *Bacillus badius* ARB6 strain using response surface methodology. *Biotechnol. Reports* **26**, e00459 (2020).

34. Knutson, C. M., McLaughlin, E. M. & Barney, B. M. Effect of temperature control on green algae grown under continuous culture. *Algal Res.* **35**, 301–308 (2018).

35. Wang, C. & Lan, C. Q. Effects of shear stress on microalgae—A review. *Biotechnol. Adv.* **36**, 986–1002 (2018).

36. Kaplan, D., Cohen, Z. & Abeliovich, A. Optimal growth conditions for *Isochysis galbana*. *Biomass* **9**, 37–48 (1986).

37. All, E. B. V. et al. Optimizing conditions for the continuous culture of *Isochysis affinis* galbana relevant to commercial hatcheries. *Archiméer* **326–329**, 106–115 (2012).

38. Cheng, D. et al. Adaptive evolution and carbon dioxide fixation of *Chlorella* sp. in simulated flue gas. *Sci. Total Environ.* **650**, 2931–2938 (2019).

39. Lara-Gil, J. A., Senés-Guerrero, C. & Pacheco, A. Cement flue gas as a potential source of nutrients during CO₂ mitigation by microalgae. *Algal Res.* **17**, 285–292 (2016).

40. Chini Zittelli, G., Rodolﬁ, L., Biondi, N. & Tredici, M. R. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture* **261**, 932–943 (2006).
41. Brennan, L. & Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 14, 557–577 (2010).
42. Ho, S. H., Chen, C. Y., Lee, D. J. & Chang, J. S. Perspectives on microalgal CO2-emission mitigation systems—A review. Biotechnol. Adv. 29, 189–198 (2011).
43. Rahaman, M. S. A., Cheng, L. H., Xu, X. H., Zhang, L. & Chen, H. L. A review of carbon dioxide capture and utilization by membrane integrated microalgal cultivation processes. Renew. Sustain. Energy Rev. 15, 4002–4012 (2011).
44. Van Bergeijk, S. A., Salas-Leiton, E. & Cañavate, J. P. Low and variable productivity and low efficiency of mass cultures of the haptophyte Isochrysis aff. galbana (T-iso) in outdoor tubular photobioreactors. Aquac. Eng. 43, 14–23 (2010).
45. Molina Grima, E. et al. Outdoor culture of Isochrysis galbana ALII-4 in a closed tubular photobioreactor. J. Biotechnol. 37, 159–166 (1994).
46. Chisti, Y. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306 (2007).

Acknowledgements
This study is supported by TNB Seeding Fund (TNBR/SF28/11 & TNBR/SF59/12). The authors wish to thank TNB Research’s Management, UNISEL and UPM for their supports and co-operations in making this work a success.

Author contributions
L.Y. wrote the main manuscript text, R.H. & L.C. improvise the overall content and flow of the manuscript. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to R.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020