

# The Effect of Biochar Application in Microalgal Culture on the Biomass Yield and Cellular Lipids of *Chlorella vulgaris*

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**Abstract**— Microalgal culture may have the potential as a source of raw materials for biodiesel to provide renewable energy services for regional and remote communities. However, current culturing systems face many technical hurdles to be economically feasible, such as high costs for microalgae harvesting and difficulties to make algal cell accumulating lipids. Biochar is an inherent product of biomass pyrolysis; it is a carbon rich and porous substance with high surface area and many surface charges. Biochar could thus be utilised to absorb nutrients and attract and immobilise algae to its surfaces, providing an effective method to alleviate the algae harvesting challenge. In this study, an Oil Mallee biochar of varying particle sizes were added to *Chlorella vulgaris* culture in a Tris-Acetate-Phosphate (TAP) medium to investigate the biochar – algae interaction. Algae growth were severely inhibited when biochar was added at inoculation phase, whereas, adding biochar at later stages of the exponential or stationary growth phase has less or no effect on the algal culture. Based on these observations biochar was tested as filtration aid for algae harvesting. When *C. vulgaris* was gravity-filtrated using a filter paper of 11 µm pore size, the algal mass on filter was increased from 4.7 % of total biomass in the control sample to 8.2 % in a 0.2 g/100mL biochar treated sample. Interestingly, the cellular lipids contents (determined in the form of fatty acid methyl ester) increased by 40 % in the biochar treated culture as compared to the control. The lipid accumulation was thought to have resulted from the stress induced by biochar stripping phosphorus from the medium. These findings provided a scientific basis for an innovative use of biochar to improve the efficiency of the algal culture system and further research to reveal the detailed mechanism of biochar – algae interaction was discussed.

**Keywords**- algae; biochar; cellular lipids; *Chlorella vulgaris*; phosphorus

## I. INTRODUCTION

For many years algae have been thought of as an attractive source for biodiesel production. Due to a simplistic cell structure, algal cells are more efficient at photosynthesis compared to land plants, and as a result produce much greater biomass yields [1]. One of the main attractive qualities algae have in comparison to land plants is that they do not require highly competitive arable lands, currently used for human food production. In addition algae are capable of being grown in wastewaters thus avoiding competition for valuable fresh water [2]. Algal cultures can also be used in industrial plants to mitigate carbon dioxide emissions from exiting flue gases [3, 4]. However, the key limitation in developing industrial use of algae into biodiesel is the lack of an efficient harvesting technique. The small size and relatively low density of algae cells means that harvesting is both difficult and costly [4]. Currently there are three methods available for algae harvesting: centrifuging, filtration, and gravitational sedimentation [2]. However, these methods suffer from either high cost or low efficiency. Centrifuging remains the most reliable and common harvesting technique, however it is also the most cost intensive [2]. Filtration is limited to small volumes of algal biomass with eventual clogging in the filter, with the associated frequent replacement of membranes making it an expensive process. Centrifugal, filtration, and sedimentation must all be preceded with an additional flocculation step [4]. This step is used to aggregate the microalgae cells, thus increasing their effective particle size for ease of separation. Immobilisation of algal cells has been proposed as a harvesting technique [5]. Algae can become immobilised through two means; either through attaching on solid carriers or in being encased in a beaded matrix. Immobilisation for harvesting works on the principal that an additive is

introduced to the algal culture, which causes the immobilisation of the algae cells prior to, during or after cultivation, enabling the biomass to be retrieved more readily. Immobilised algae culture systems are unique in that cells are attached to carriers instead of suspended in the culture media.

Biochar is a porous substance, with high active surface area, similar in its appearance to active charcoal [6]. Depending on the feedstock and pyrolysis conditions, the biochar surface can be either positively or negatively charged. Consequently, biochar can be either scrubber or donor of nutrients when interacting with its environment. Based on the unique properties of biochar, it may be used as an immobilizer for microalgae by simple ionic interactions or by attracting algal cells to micro nutrition domains on biochar surface. The biochar anchored algal cells can be easily separated from the growth medium by filtration, because the biochar particle size can be much larger than that of the algal cells. Biochar aided microalgae harvesting will be able to reduce the cost of this crucial step, and the increase the viability of algal biofuel production. In addition, biochar has been converted to slurry fuel [7] [8] for direct combustion, but the organic matters have been stripped from biochar during pyrolysis. Algae will add valuable organic volatiles such as lipids to the biochar carrier, and give the algae-biochar slurry fuel more favourable combustion properties.

*Chlorella vulgaris* is a common green alga with small globular cells, which grows in freshwaters across Australia. It is highly adaptive to numerous environments, and is capable of producing high biomass [9]. A study also found that *C. vulgaris*, was one of attractive algal species for its lipid production potential [10]. However, microalgae will not accumulate lipid unless they were placed in a stressful environment, such as limitations of nitrogen [11] or phosphorous [12].

In this research, we investigated whether biochar made from Oil Mallee affected the growth of *C. vulgaris* cultures under controlled batch conditions; we also tested the possibility to use biochar as a solid carrier for immobilisation of algal cells; and biochar affecting *C. vulgaris* lipid content.

## II. EXPERIMENTALS

### A. Algal growth condition

*C. vulgaris* was obtained from CSIRO algal collection and grown in a glass flask containing 100ml of Tris-acetate-phosphate (TAP) medium. The culture was kept on a shaker at 22°C and under continuous luminescence at 1800 lux, provided by white fluorescent light. The rotational speed of the shaker was set to 90 rpm. Algal growth rate with or without biochar was monitored by either directed cell counting by using a hemocytometer, or electronic counting through a particle counter (HIAC electronic Model 9703 liquid particle counting system). The HIAC particle counter works on the principle of light extinction to detect particles in the range of 1.3 to 600 µm [13]. Under a standard growth

conditions, the culture will be able to reach stationary growth in 60 to 70 hours.

### B. Oil Mallee biochar

In this study, the biochar was made from oil mallee tree native to Western Australia. The chemical and physical properties have been fully characterized [8]. The biochar was crushed and dry sieved to obtain the desired particle sizes. When size distribution “x” is above 2 mm, the biochar sample is labelled as “+2 mm”; when x is in the range between 2 mm and 1 mm, it is labelled as “+1 mm”; for x is between 1 mm and 212 µm it is labelled as “+212 µm”; and when x is in the range between 125 µm and 75 µm, it is labelled as “+75 µm”.

### C. Determination of interactions biochar has on algal cultures

The physical interaction of biochar and microalgae was observed by an optical microscope.

To investigate chemical changes occurring in the biochar particles before and after incubation, the biochar samples were analysed using a scanning electron microscopy (JEOL 5800LV) fitted with an Oxford INCA energy dispersive spectrometer (SEM-EDS). The samples were analysed at Microanalysis Australia with the samples being prepared on a carbon stubs and then carbon coated to increase the samples conductance under the SEM.

### D. Determination of biochar particles ability as an immobiliser

*C. Vulgaris* cell size distribution was measured by laser diffraction using a Malvern Instrument Mastersizer MS2000, and the average size of algal cells is about 3 µm.

The combined algal biochar samples were inspected under an optical microscope as a qualitative measurement. This was done as an initial study in order to gain an understanding into whether algal growth was evident on the biochar surface. Quantitative measurements were achieved through mass filtration, which assessed if the total algal mass recovery increased with the addition of biochar into algal cultures. With a higher algal mass recovery indicating that algae maybe attached to the surface of the biochar particles.

### E. Fatty acid methyl ester Analysis

The fatty acid methyl ester (FAME) content of algal cells was investigated using a gas chromatograph coupled with mass spectrometry (GC/MS) with the procedure modified as in Matthew *et al.* (2009). A typical procedure for fatty acid analysis is outlined below.

Algal cultures (2 mL) were put into an 2 mL safe-lock tubes (Eppendorf Biopur) The tubes were centrifuged (Eppendorf Centrifuge 5415R) for 5 minutes at 27 °C set at a speed of 16.1 relative centrifugal force. The medium was removed and the cell pellets were methyl-esterified through the addition of 500 µL of a 49:1 100 % methanol (v/v):18 M H<sub>2</sub>SO<sub>4</sub> solution, and incubated at 80 °C for 120 minutes.

Heneicosanoic acid (Sigma) was used as internal standard for quantification. The FAME from the mixture was extracted with n-hexane and subjected to GC/MS analysis. Fatty acid methyl esters were quantified using the area of the total ion current compared with that of the internal standard. Identification of fatty acid methyl esters was based on mass spectral profiles, comparison to standards, and expected retention time from Agilent's RTL method and verified by comparison to those previously described [14].

### III. RESULTS AND DISCUSSIONS

#### A. Effect of biochar on *C. vulgaris* growth.

A series of experiments were conducted to investigate the impact of biochar addition on the growth of algal cultures. Biochars in four different sizes (+2 mm, +1 mm, +212  $\mu\text{m}$ , and +75  $\mu\text{m}$ ), with four different concentrations (8 g/100 mL, 4 g/100 mL, 2 g/100 mL, and 0.4 g /100ml) were added at algal inoculation. It was not surprising to notice that algae growth was severely inhibited by the addition of biochars, and the inhibition was size and dose dependent: the culture with 8 mg/L, +75  $\mu\text{m}$  biochar had the least comparable growth. The inhibitory effect was most likely resulted from biochar blocking light required for photosynthesis.

Subsequently, biochars were added to algal culture at different growth phases to find the condition to minimize the negative impacts. Two biochars (+2 mm and +75  $\mu\text{m}$ ) at concentration of 0.2 g/100 ml were added to cultures at induction phase (12 hours after inoculation, and cell count was  $1.0 \times 10^5$  cells/ml), exponential phase (36 hours after inoculation, cell count is  $1.5 \times 10^6$  cells/ml), and early stationary phase (42 hours after inoculation, cell count is  $3.0 \times 10^6$  cells/ml). The biomass accumulation in term of cell densities was checked at 72 hours after inoculation. The cell count results were shown in Figure 1. The results indicated the cell culture was poorly grown, when biochar was introduced in the early growth stage. However, substantial (greater than 80 %) growth of *C. vulgaris* has been achieved when biochar was added in the exponential growth phase, at which the large algal cell population would buffer the negative impact of biochars. Interestingly, after biochar was introduced at the stationary phase, comparing to the control, algal growth was observed to slightly increase (about 3 %) upon further incubation. Although the exact reason for this phenomenon deserves in-depth investigation, one possible explanation is that biochar might be able to remove so called "quorum sensing molecules" from the medium allowing more algae growth. The presence of quorum sensing molecules is commonly understood in prokaryotic cells such as bacteria [15], but little is known about their presence in eukaryotic cells such as green alga.

The results also allowed us to choose experimental condition to add biochar at stationary phase for the following immobilization studies.

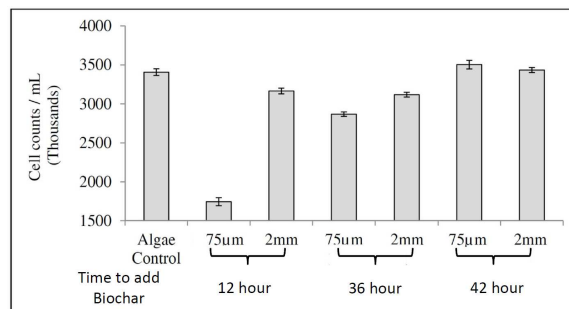


Figure 1: Effect of algal growth stage in which biochar particles are introduced (Error bars: standard deviation of three independent measurements).

#### B. Oil Mallee biochar can be used as algae immobiliser for aiding algal cell harvesting.

The *C. vulgaris* was incubated in TAP medium under above mentioned growth condition for 42 hours. Biochars (0.1 g/ 100 ml, 0.2 g /100 ml +75  $\mu\text{m}$ ) were added and incubated for next 48 hours. Cultures at the same condition, but without biochar were used as controls.

After incubation, biochar plus algal cultures were examined under an optical microscope at 1000 x magnification to check whether algal particles had grown on the surface of the biochar particles. However, as a consequence of the small algal diameter, it was challenging to obtain clearly focussed images of both biochar particles in frame with the algal particles. From **Error! Reference source not found.2**, however, it can be seen that a layer of algal cells are observed to surround the biochar particle, but the immobilization of this layer of cells to biochar surface could not asserted based on this observation alone.

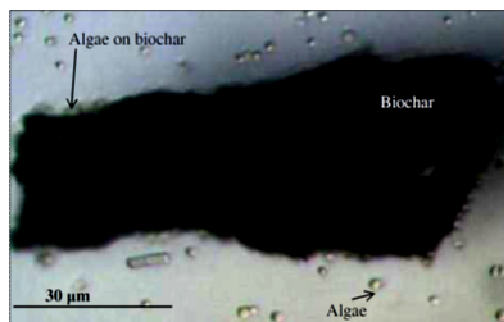


Figure 2. Biochar and algae under an optical microscope.

In order to ascertain whether Oil Mallee biochar particles immobilised algal cultures, and to evaluate the ability of biochar as filtration aid for algae harvesting, A mass filtration method was used for quantitative measurements biochar algae interaction, which assessed if the total algal mass recovery increased with the addition of biochar into algal cultures. With a higher algal mass recovery indicating that algae maybe attached to the surface

of the biochar particles. Mass filtration was determined using Whitman No.1 filters which have a pore size of 11  $\mu\text{m}$ : most algal cells having diameter around 3  $\mu\text{m}$  [16] would pass through the filter; most +75  $\mu\text{m}$  biochar particles, on the other hand, would be retained on the filter. A typical procedure involved filtering 100 mL of the algal culture until dry, and washing with water several times to remove culture medium and free algal cells. The filters were then weighed to four decimal places. Dry algal biomass weight was calculated (grams per litre), after subtracting the filter plus biochar weight from filter plus total weight of sample, leaving an indicative measurement of the algal biomass. As a comparative study, the results obtained above were compared to the total biomass harvested obtained by using Whitman glass microfiber filters (Grade GF/C), which have a pore size of 1.2  $\mu\text{m}$ . The total biomass harvested was  $6.34 \pm 1.1$  g/L. This result obtained through the GF/C filter is comparable to that found in literatures, which state *Chlorella vulgaris* to have biomass yields in the range of 5.5 to 9.5 g/L depending on culture conditions [9, 10, 17].

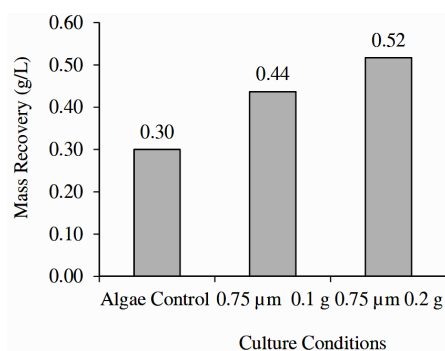


Figure 3. Algal mass harvested with or without biochars.

As illustrated in Figure 3, the harvested algal mass was dramatically increased with aiding of biochar: from 0.30 g/L to 0.52 g/L, and the harvesting rate was increased from 4.7 % to 8.2 % in a 0.2 g/100mL biochar treated sample. The fact that algal cells could not be disassociated from biochar by repeated washing indicated algal cells were at least partially immobilized by the biochar.

### C. Impact of biochar particles on algae lipid accumulation.

Increases in lipid production are a function of algal cell adaption, when the cells are placed in a stressful environment. Adding biochar to the cultural system may stress the algal by light reduction or other parameters. Thus, the impact of biochar particles on algae lipid accumulation was investigated. The investigation involved the biochar at +2 mm and +75  $\mu\text{m}$  size fractions, with the addition of biochar being introduced into the algal cultures at the exponential and stationary phase of the algal growth. The total fatty acid methyl ester (FAME) was used to represent of total lipids, and Figure 4 showed the results of GC/MS analysis of FAME.

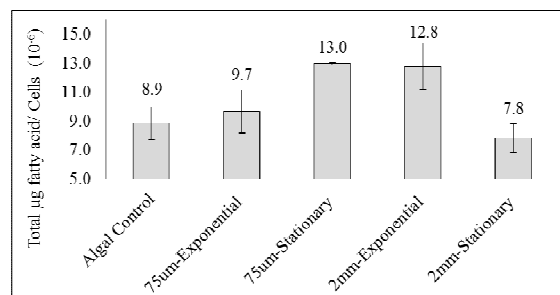


Figure 4. Total FAME of algae from different biochar addition (Error bars: standard deviation of three independent measurements).

The results indicated that biochar addition has resulted a dramatically increased lipid production. There was maximal 40 % lipid increment upon +75  $\mu\text{m}$  biochar addition. In order to understand the factor triggering lipids accumulation, we examined the biochar chemical properties before and after incubation with algal culture. We found the SEM-EDS measured elemental phosphorous in biochar was increased from 0.04 wt% before incubation to 0.1 wt % after incubation, indicating this biochar was able to absorb phosphorous from the culture medium. The reduction of phosphorous concentration might be an additional factor for lipid accumulation.

## CONCLUSIONS

This study was designed to investigate an innovative method of processing algae that would not only lead to an increase in biodiesel production but also alleviate the current algal harvesting problems. We found, as expected, biochar will block light and inhibit algae growth. However, when it was added to algal culture that has reached stationary growth phase, biochar can interact with microalgae culture to promote further growth. The mechanism for the growth promotion has not been understood yet. One possibility is that biochar may be able to remove algae produced putative “quorum sensing” molecules used for population control [15]. We will design new experiments in the future to verify the existence of such molecules and their affinity to bind on biochar. We also observed that adding biochar achieved higher harvesting rate by simple filtration, this may be resulted from immobilization of algal cell to biochar as indicated in our experiment. However, we cannot rule out the high algal cell retention is resultant of biochar blockage of the filter; we will need better technique to measure algal biochar interactions in a quantitative manner. Moreover, adding biochar is likely able to deplete phosphorus from the medium and to stress the cell triggering lipid accumulation. Although, our findings are very preliminary, these results provided the basis for potential industrial application of biochar to streamline microalgae culture. Further research is warranted for detailed mechanisms of microalgae biochar interaction, and for using biochar to increase the energy efficiency for algal biomass production.

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