# DETERMINATION OF FERTILIZING CHARACTERISTICS OF THREE DIFFERENT MICROALGAE CULTIVATED IN RACEWAYS IN GREENHOUSE CONDITIONS

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**Abstract**

Microalgal biomass is rich source of macro elements such as nitrogen, phosphorus, potassium, and calcium. The purpose of this study was to investigate the different algae availability as fertilizer in agriculture. Therefore, three different microalgaes were selected for the experiment. *Chlorella* spp., *Neochloris conjuncta* and *Botryococcus braunii* cultures were grown in basal medium in the raceway which have the capacity of 1.5 tonnes. Experiment was ended when the required value of the number of cell was reached. Microalgaes were harvested by a centrifuge in 6000 rpm. Wet mass of microalgaes were dried in 65˚C at oven. Nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, copper, boron, and organic matter were measured. Nitrogen values for *Chlorella* spp, *Botryococcus braunii* and *Neochloris conjuncta* microalgae were measured 5.45, 6.01, and 5.49 %, respectively. Microalgae can be used as nitrogen sources for plant growth and development due to high nitrogen levels. The concentrations of potassium were measured as 1.34, 0.94 and 1.05%. Organic matters were measured as 67.24, 64.15, and 64.65% for *Chlorella* spp, *Botryococcus braunii* and *Neochloris conjuncta*, respectively. Chlorella strain is currently used for fertilization purpose. However, this study showed that *Neochloris conjuncta* and *Botryococcus braunii* can used as fertilizer, as well.

**Key words**: *Chlorella* spp., *Neochloris conjuncta*, *Botryococcus braunii*, fertilizer, microalgae

Utilization of dry green algae as fertilizer could increase soil fertility and product yield. At the same time, it has been reported to be environmentally friendly approach (Faheed and Fattah, 2008). Liquid bio-fertilizers contains special cell protectants for prevention of spoilage chemicals that promote formation of resting spores or cysts for longer shelf life and tolerance to adverse conditions. Additionally, farmers have to bear considerable initial cost in terms of skill acquisition, trial and failure and risk. The producer firms have serious uncertainty about the demand or sale ability of the product, which deters investment, especially if it is irreversible (Ghosh, 2004). Evaluation of microalgae as a liquid bio-fertilizer has been shown to increase germination rate and plant height of corn and wheat plants. Furthermore, it was highlighted it could be used as a source for good agriculture practice or organic agriculture (Uysal *et al.,* 2015). Recently, the resulting increase in the demand for agricultural products instead of chemical fertilizers in agricultural activities, environmentally-friendly fertilizer use is increasing. Microalgal biomass is a rich source of macro elements in the agriculture, such as nitrogen, phosphorus, potassium, calcium that implemented for improving vegetative growth and yield.

Microalgae are grown in open and closed systems. Although the risk of contamination may exist in open systems, these systems are preferred for microalgae cultivation due to lower initial investment and operating costs. Furthermore, open ponds or raceways are easy to scale up to increase the production mass, therefore, such systems are presently being first considered for practical applications (Chisti, 2007). The purpose of this study was to investigate fertilizing characteristics of three different microalgae (*Chlorella sp.*, *Neochloris conjuncta* and *Botryococcus braunii*) cultivated in raceways in greenhouse conditions.

## MATERIAL AND METHOD

*Chlorella* spp (SAG 242.80), *Botryococcus braunii* (SAG 807-1) and *Neochloris conjuncta* (SAG 78.80) were cultivated in erlenmeyers (500 ml – 2 L) and reactors (10 L –50 L) in laboratory conditions. Basal medium was selected as the nutrient medium (*table 1*). Preparation of micro-elements solution is given in Table 2. The final pH

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of this medium was 7.0 after being autoclaved. The algal cells were grown at a temperature of 25±1°C and PAR (Photosynthetically Active Radiation) of 125 µmol $m^{-2}$ $s^{-1}$ measured by Delta Ohm PAR meter. 50 liters of culture in the laboratory were taken into 250 liter reactor in the greenhouse (6x5m). Afterwards, cultures were transferred to each race-way with a capacity of 1.5 tons once cultures transferred to the raceway reached the desired density. Seven days later, the required microalgal biomasses were obtained and harvested.

Table 1

**Preparation of Basal Medium (SAG, 2016)**

| | Stock solution (g/100 ml) | Nutrient solution (ml) |
|---|---|---|
| $KNO_3$ | 1 | 20 |
| $K_2HPO_4$ | 0.1 | 20 |
| $MgSO_4.7H_2O$ | 0.1 | 20 |
| Microelements solution | | 5 |
| Distilled water | | 935 |
| Total | | 1000 |

Table 2

**Preparation of micro-elements solution (SAG, 2016)**

| | Stock solution (g/100 ml) | Applied solution |
|---|---|---|
| ZnSO4 . 7H2O | 0.1 | 1 ml |
| MnSO4 . 4H2O | 0.1 | 2 ml |
| H3BO3 | 0.2 | 5 ml |
| Co(NO3)2 . 6H2O | 0.02 | 5 ml |
| Na2 MoO4 .2H2O | 0.02 | 5 ml |
| CuSO4 . 5H2O | 0.0005 | 1 ml |
| Distilled water | | 981 ml |
| FeSO4 . 7H2O | | 0.7 g |
| EDTA (Titriplex III, Merck) | | 0.8 g |

The raceway as an open system was used in this study due to the high yield resulted from mixing efficiency compared to other open systems. Raceways have capacity of 1.5 tonnes (*figure 1 and 2*) with a dimension of width=1.5, length =4 meters, and height =60 cm. However, the trial was operated with water height of 30 cm. The sizes of the paddle-wheels used to promote circulation in the raceways are 40 x 40 cm.

Industrial centrifuges (Figure 3) are majorly used for the harvesting of microalgae culture. In this study, harvesting operation was performed by an industrial centrifuge (Extreme Algae Centrifuge 120V/230V, USA) with a rotational speed of 6000 rpm. Schematic of operation of centrifuge is given in *figure 3*.

After harvest, three different microalgal biomasses were dried at 65±5°C and were grounded for nutrient analysis. In order to determine N concentration, 0.5 g grounded sample was weighted into the 250 ml macro-Kjeldahl tubes then 5g of salt mixture and 10 ml concentrated $H_2SO_4$ were added and tubes were placed in the digesting block at 350-400 °C. After digesting, samples were distilled with NaOH (40%). Available phosphorus was measured based on (Kacar, 1995) using ICP spectrometry. Available phosphorus was measured with ICP spectrometry running the samples extracted in 0.5 M sodium bicarbonate solution adjusted to pH of 8.5 (Kacar, 1995). P, Ca, and Mg were measured with ICP spectrometry running the samples extracted in 1 N ammonium acetate solution adjusted to pH of 7 (Kacar, 1995).

Figure 1 **Construction phase of raceway**

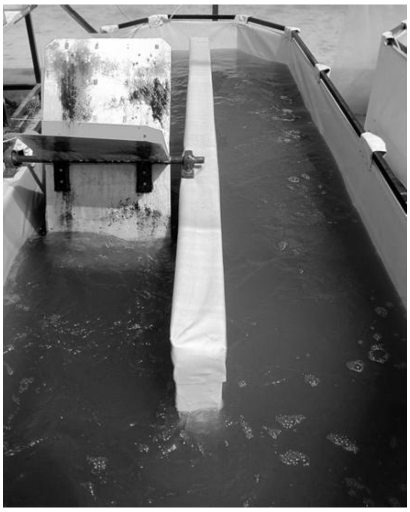

Figure 2 **Cultivation phase in raceway**

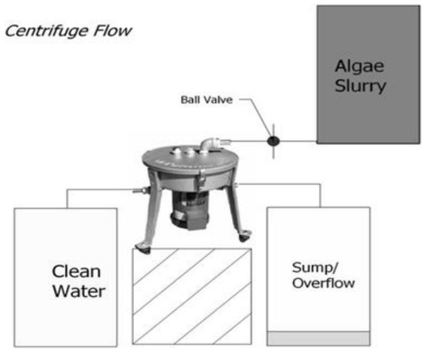


Figure 3 **Industrial centrifuge**

Fe, Mn, Zn, and Cu were measured with ICP spectrometry running the samples extracted in the extraction solution of DTPA (Lindsay and Norwell, 1978). Organic matter content (OM) of dry samples was analyzed after incinerating the samples at 550 °C for four hours.

## RESULTS AND DISCUSSIONS

Chemical analysis of *Chlorella* spp, *Botryococcus braunii* and *Neochloris conjuncta* microalgaes are presented in Table 3. In this study, the results of chemical analysis of microalgal biomasses were compared with those of the finished composts obtained from the study of Tuzel *et al.* (2016) and Oztekin *et al.* (2016). In Figure 4, 5, and 6, Compost 1 was made of rose oil processing waste+separated dairy manure+poultry manure+straw (Oztekin *et al.* (2016), Compost 2 included two phase olive oil mill waste +separated dairy manure+poultry manure+straw) and Compost 3 consisted of three phase olive oil mill waste+separated dairy manure+poultry manure+straw (Tuzel *et al.* (2016). While nitrogen values for *Chlorella sp*, *Botryococcus braunii* and *Neochloris conjuncta* microalgae were measured as 5.45, 6.01, and 5.49 %, respectively, calcium levels were determined as 6.90, 7.51, and 5.97%, respectively (*table 3*). Microalgae can be used as nitrogen sources for plant growth and development due to high nitrogen levels. The levels of nitrogen and calcium for three different microalgal biomasses obtained from this study were higher than those of composts obtained from previous studies. The maximum nitrogen and calcium values were determined for *Botryococcus braunii* (*figure 4*). Phosphorus values for *Chlorella sp*, *Botryococcus braunii* and *Neochloris conjuncta* microalgae were 1.10, 0.64, and 0.66%, respectively, magnesium levels were 1.17, 0.93, and 1.03%, respectively (*table 3*). The highest value was achieved for *Chlorella* spp. as 1.10% for phosphorus and 1.17% for magnesium. Results clearly showed that P and Mg values of microalgal biomasses were higher than those of composts (Figure 5). The results revealed that the levels of potassium for *Chlorella* spp, *Botryococcus braunii* and *Neochloris conjuncta* microalgae as 1.34, 0.94, and 1.05%, respectively were substantially higher than those of composts except for Compost 1 (*table 3* and *figure 6*). At the other side, OM content of both microalgal biomasses and compost were close to each other. As for Fe, Mn, and Zn, composts yielded higher values than those of microalgal biomasses (*table 3*).

Table 3

**Chemical analysis of microalgal biomass**

| Parameter | *Chlorella sp.* | *Botryococcus braunii* | *Neochloris conjuncta* | Compost 1 | Compost 2 | Compost 3 |
|---|---|---|---|---|---|---|
| OM (%) | 67.24 | 64.15 | 64.65 | 66.91 | 71.25 | 56.54 |
| N (%) | 5.45 | 6.01 | 5.49 | 1.88 | 1.09 | 1.15 |
| P (%) | 1.10 | 0.64 | 0.66 | 0.57 | 0.29 | 0.53 |
| K (%) | 1.34 | 0.94 | 1.05 | 1.40 | 0.52 | 0.71 |
| Ca (%) | 6.90 | 7.51 | 5.97 | 3.85 | 4.86 | 5.86 |
| Mg (%) | 1.17 | 0.93 | 1.03 | 0.77 | 0.21 | 0.33 |
| Fe (%) | 0.13 | 0.13 | 0.13 | 0.77 | 0.63 | 0.66 |
| Cu (ppm) | 48.67 | 47.08 | 39.98 | 54.72 | 31.94 | 25.14 |
| Mn (ppm) | 30.46 | 26.8 | 27.72 | 246.2 | 107.5 | 180.9 |
| Zn (ppm) | 43.69 | 47.89 | 39.44 | 268.8 | 115.6 | 186.2 |
| B (ppm) | 20.35 | 21.51 | 20.76 | 25.39 | 23.62 | 24.71 |

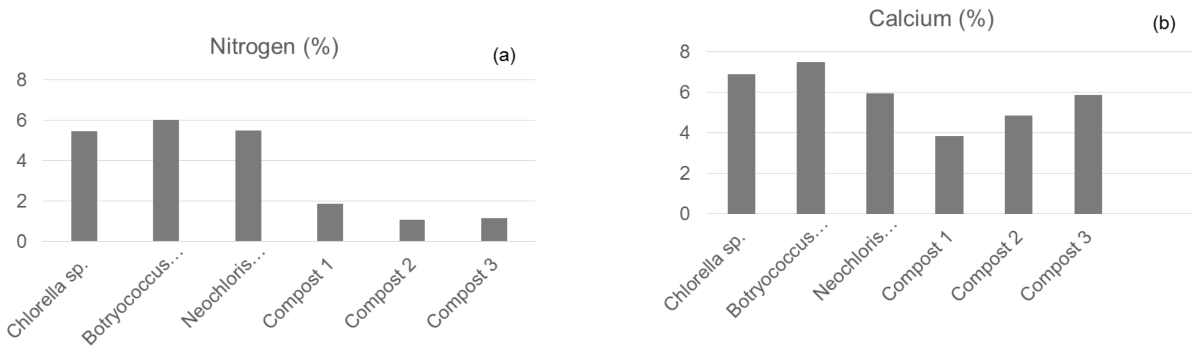


Figure 4 **Comparison of nitrogen (a) and calcium (b) levels with the finished composts**

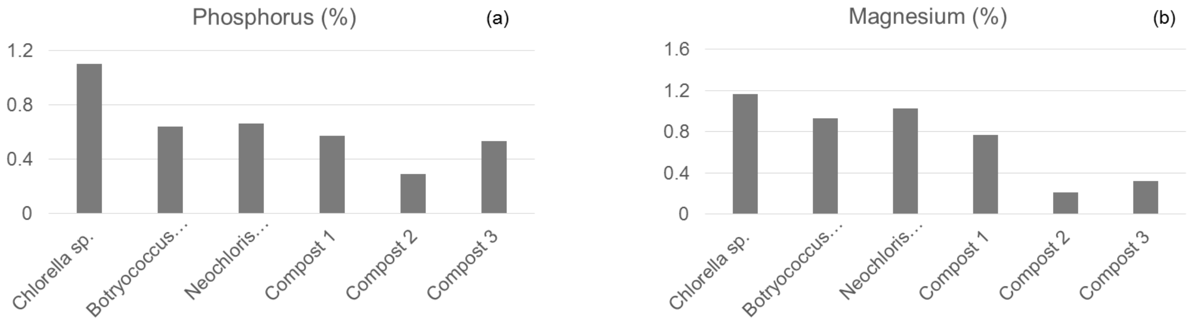


Figure 5 **Comparison of phosphorus (a) and magnesium (b) levels with the finished composts**

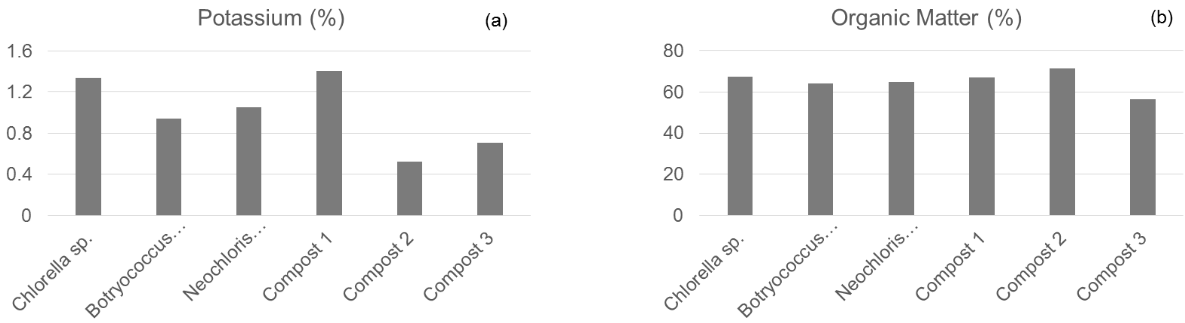


Figure 6 **Comparison of potassium and organic matter levels with the finished compost**

## CONCLUSION

Chemical analysis of *Chlorella* spp, *Botryococcus braunii* and *Neochloris conjuncta* microalgaes were compared with composts. Results on chemical analysis showed that *Chlorella* spp, *Neochloris conjuncta, Botryococcus braunii* are rich in nitrogen, phosphor, magnesium, calsium, potassium. The other parameters are compatible with composts. Therefore, it could be utilized in agriculture as microbial fertilizer. Chlorella strain is currently used for fertilization purpose. However, this study showed that *Neochloris conjuncta* and *Botryococcus braunii* can used as fertilizer, as well. Since microalgal biomasses are in liquid form and the cells are micron size, they do not cause clogging problems in drip irrigation applications. It could reduce the use of chemical fertilizers due to the rich content. Microalgal biomasses improve the soil properties and increase yields in crop production.

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