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# Potential of *Monoraphidium minutum* for carbon sequestration and lipid production in response to varying growth mode

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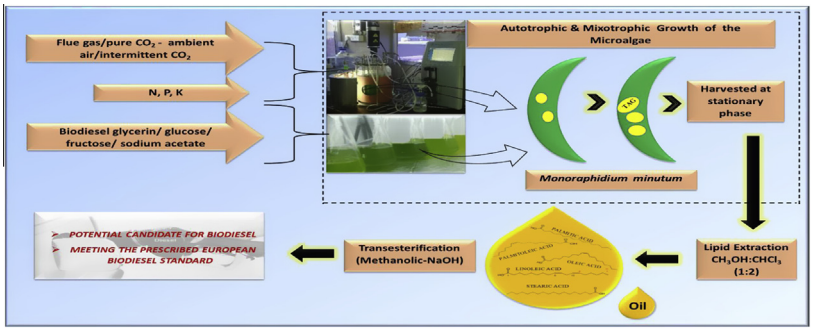
## HIGHLIGHTS

- *Monoraphidium minutum* remediated flue gas.
- Intermittent $CO_2$ supply maximizes lipid accumulation while growing autotrophically.
- Max. biomass productivity was observed in mixotrophic growth.
- Higher C/N of lyophilized biomass showed higher lipid accumulation.
- Resultant biodiesel met with European biodiesel standard.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Mixotrophic growth at flask level and, autotrophic–mixotrophic and autotrophic growth in photobioreactor by utilizing $CO_2$/air/flue gas were checked for the isolated strain of *Monoraphidium minutum* from polluted habitat. Our study confirmed that it is a saturated fatty acid rich (30.92–68.94%) microalga with lower degree of unsaturation oil quality (42.06–103.99) making it potential biodiesel producing candidate. It showed encouraging biomass productivity (80.3–303.8 $mgl^{-1}$ $day^{-1}$) with higher total lipid (22.80–46.54%) under optimum glucose, fructose, microalgal biodiesel waste residue and sodium acetate fed mixotrophic conditions. The pH control by intermittent $CO_2$, continuous illumination with 30% flue gas, and utilization of biodiesel glycerin were effective schemes to ameliorate either biomass productivity or % lipids or both of these parameters at photobioreactor scale (7.5 L working volume). The modulation of environmental variables (pH control, $CO_2$ and organic substrates concentration) could augment % saturated fatty acids, such as C16:0.



## 1. Introduction

Microalgae have maximum aerial/volumetric biomass productivity with reported maximum carbon fixation capability in whole plant kingdom. Many of the microalgae are investigated for their potential of carbon fixation and biofuel production. Green microalgae are attaining significant attraction in sustainable green technology for the future of bioenergy and environment. Out of the all microalgal classes, most of the Chlorophycean microalgae are known as lipid rich and having comparatively higher biomass productivity (Rawat et al., 2013). *Monorphidium* sp. is among the oleaginous Chlorophycean species having favorable growth rates and diverse metabolic lipid pathways (Bogen et al., 2013). However, nutrient uptake rate, light, pH, concentration and bioavailability of nutrients are important factors to affect lipid content, fatty acid

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composition and carbon sequestration (Fields et al., 2014; Yu et al., 2012; Kumar et al., 2010).

Flue gas capturing by microalgae requires an understanding on optimum environmental conditions for the specific strain, which helps in establishing higher biomass and lipid yield (Fields et al., 2014). Many microalgae can grow mixotrophically being able to utilize organic carbon during photosynthesis while utilizing inorganic carbon (Chandra et al., 2014). In this study, nitrate, phosphate and carbon of the growing culture in medium with closed photobioreactor system is monitored at regular intervals under varying $CO_2$/flue gas concentrations. Further, effect of pH control, photoperiod, and $CO_2$ concentration also estimated on the biomass productivity and lipid content. Mixotrophic growth potential of *Monoraphidium minutum* with respect to biomass, total lipid content and lipid profiling at flask level was also carried out.

## 2. Methods

### 2.1. Culture maintenance

*M. minutum* (Accn No.CCNM-1042, CSMCRI culture repository) isolated from contaminated freshwater lagoon from Hazira, Surat, India (21°6.129́N, 72°37.794́E), was propagated as monoalgal culture, maintained in Zarrouk's media (Zarrouk, 1966) under autotrophic condition by providing 100 μmole $m^{-2}$ $s^{-1}$ light for 12:12 dark–light period at 28 °C temp. Detail of isolation and identification is given (Supplementary Text S1).

### 2.2. Experiments under flasks conditions

Monoalgal culture of the late log phase was harvested by centrifugation at 8000 rpm and fresh pellet washed with sterile water twice followed by re-centrifugation. Inoculum in the form of fresh wet pellet (100 mg) was re-suspended in 100 ml autoclaved media for the experiments under sterile conditions. Mixotrophic growth experiments conducted in autoclaved media containing organic substrate (Glucose/fructose/microalgal biodiesel waste residue/sodium acetate) in addition to Zarrouk's media and parallel control was run in each experiment. The inorganic carbon source was sodium bicarbonate and organic carbon source was glucose (G)/fructose (F)/biodiesel glycerin (BG)/sodium acetate (SA) for mixotrophic experiments. All experimental sets were run in triplicates with control. The pH on the initial day adjusted to 8.5 by using autoclaved 0.3 M KOH and HCl. Each flask confronted to 100 μmole $m^{-2}$ $s^{-1}$ light, 25 °C temp. and 12:12 dark–light period. Only manual shaking was provided once in each day of the experiment.

### 2.3. Experiments under photobioreactor conditions

The bioreactor (BioFlo/CelliGen 115; 14 L capacity) externally equipped with lamp (100 μmole $m^{-2}$ $s^{-1}$) and having process control option for pH, dissolved oxygen (DO), agitation, temperature, pump feed, aeration, antifoam and foam/level, was used for the experiments on *M. minutum*. The photobioreactor equipped with mass flow controller up to four ports of gas inlet and mixing control options. The photobioreactor was sterilized before each experiment by autoclaving. Inoculum was added to autoclaved media under sterile condition in the photobioreactor.

The working volume was 7.5 L for each batch of the experiment and, initial optical density for each experiment was 0.5. The rotation per minute (rpm) of impeller throughout all the experiments was 200 rpm and temperature was controlled at 28 °C. The details of different experimental variables of each set are shown in Table 1.

### 2.4. Growth, biovolume, biomass productivity and carbon sequestration rate

In addition to OD measurement of culture at 540 nm by using UV–visible spectrophotometer (Varian Cary 50 Bio, USA), fluorescence based biovolume was measured using Fluoroprobe (bbe, Moldaenke GmbH, Germany) on each day of the experiment. There was a linear relationship between $OD_{540}$ and cell dry weight (Supplementary Fig. 1).

Culture harvesting was done by using centrifugation (8000 rpm for 10 min.) while cells were reached at stationary phase and, then microalgal wet biomass was lyophilized.

Biomass productivity and carbon fixation rate was calculated by following equation:

Biomass productivity (mg/L/day) = Total dry weight (mg)/Total culture volume (L) × Time interval of growth (day)

Carbon fixation rate (mg/L/day) = Biomass productivity × % C of dried biomass on harvesting day (Zhao et al., 2014)

### 2.5. $CO_2$ monitoring on headspace

$CO_2$ analyzer (Thermo Scientific, 410i, USA) employed for $CO_2$ measurement. The sampling tube arranged to connect tightly to the vent over photobioreactor which open up to pass the excess gas of the headspace over culture environment. The flow for sampling the gas for analysis into analyzer system through silicone tube was 0.8 L/min and, readings were taken for three minutes after warm up of 1 min. continuously. This was done regularly after accomplishment of photosynthetic period of 12 h/24 h.

### 2.6. Carbon measurement from culture

Total organic carbon and total dissolved organic carbon was analyzed by TOC analyzer (elementarLiqui TOC/TN) in an interval of 3 days during the growth till microalgae reached to the stationary phase. Dissolved fraction was analyzed after filtration of sample with 0.45 μM whatman filter papers and, suitable dilution was done using Milli-Q water (Millipore, USA) for analysis. The elemental (C, H, N, S) composition (%) of dried biomass (105 °C for 24 h in oven) was analyzed by the CHNS analyzer (elementarvario-Micro) and, sulphanilamide was used as a reference standard. The measured values of the standard had <0.03% variation as compared to theoretical value of its C, H, N, S composition in all analytical results. All samples analysis performed in triplicates.

### 2.7. Dissolved inorganic phosphate, nitrate and nitrite measurement

Samples obtained from sampling port of photobioreactor during the microalgal growth. Samples were filtered by whatman filter paper (0.45 μM) and diluted with Milli-Q water. All analysis was done in triplicates by using methods of Grasshoff et al. (1999).

### 2.8. Lipid estimation, lipid extraction and fatty acid profiling

Microalgal cells were harvested and lyophilized for lipid extraction and analysis. Total lipids were extracted from lyophilized biomass with a solvent mixture of chloroform, methanol (2:1 by vol.) according to the Folch method (Folch et al., 1957). The extract was dried in a hot air oven, and then weighed, re-suspended in chloroform, and finally stored at −20 °C to prevent lipid oxidation. The algal lipid content was measured gravimetrically. Fatty acid methyl esters (FAMEs) were prepared. Briefly, extracted lipid was mixed with a solvent mixture of 1% NaOH in methanol and heated for 15 min at 55 °C, followed by addition of 5% methanolic HCl. Then it was heated for 15 min at 55 °C for producing FAMEs, further extracted with hexane. The FAMEs were analyzed by using