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Fixation of exhaust fume gases by *Spirogyra* species and *Ulothrix* species

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Abstract: The biological fixation of carbon dioxide and other gases using algae has proved to be of major significance in recent times. The present study aims to check algae's ability to lower concentrations of various components of vehicular exhaust fume gases.

Spirogyra species and *Ulothrix* species cultured in Algae Culture Broth were raised and introduced in bioreactors with exhaust fume gases. After the third day of inoculation of the algae in the bioreactor systems laden with exhaust fume gases, significant reductions in free carbon dioxide (42.5%) and sulphate (39.88%) concentrations were observed in *Spirogyra* species as compared to free carbon dioxide (25%) and sulphate (38.76%) concentrations in *Ulothrix* species. Reduction in nitrate concentration (45.71%) and acidic condition of pH (5.75%) were the same in both bioreactor systems.

Keywords: Carbon dioxide, Exhaust fume gases, *Spirogyra*, *Ulothrix*

I. INTRODUCTION

The greenhouse gas emissions have primarily caused the increase in carbon dioxide concentrations which accounts for 68% of the total emissions, mainly from anthropogenic activities [1, 2]. This increase in greenhouse gases has affected all aspects of the natural ecosystems and poses a great threat to worldwide sustainability, such that alleviating greenhouse gases has become a matter of utmost importance in recent times [3, 4, 5]. Carbon dioxide sequestration is a method that deals with the removal of carbon dioxide from the atmosphere and helps reduce GHG emissions [6]. Carbon sequestration can be classified into (a) Abiotic and (b) Biotic sequestration. Abiotic sequestration consists of physical and chemical sequestration like oceanic and geological injection and mineral carbonation. Biotic sequestration is achieved through the photosynthesis of terrestrial plants and many photosynthetic micro-organisms like algae, bacteria, fungi, and yeast [7, 8, 9]. Although plants contribute a 3–6% reduction in global carbon dioxide emissions [10], the carbon dioxide efficiency of algae and certain cyanobacteria is found to be about 10–50 times better when compared to conventional forestry, agricultural and aquatic plants [11, 12, 13, 14, 15].

Algae are primary producers, belonging to a diverse group of unicellular and multicellular organisms, morphologically simpler than other members of the plant kingdom with much faster growth and 10-50 times better carbon dioxide efficiency than terrestrial plants [10, 11, 16]. Algae can be grown in bioreactors, wherein, exhaust/ flue/ waste gases can be used as carbon sources. Studies have proven that algae show tolerance towards high carbon dioxide concentrations, NO_x, and SO_x [17, 18, 19].

This will help to mitigate the carbon dioxide concentration from exhaust/ flue/ waste gases [20]. Biological carbon dioxide sequestration from flue gas is gaining attention because of its eco-friendly and cost-effective nature [19, 21]. It is a strategic alternative that associates environmental and economic interests, especially for those areas with power plants that produce an enormous amount of flue gas. Flue gas is composed of carbon dioxide, oxides of nitrogen, and sulphur, which provide essential nutrients for algae cultivation and biomass production [22, 23]. Algae that have been studied and found suitable for carbon dioxide fixation include *Botryococcus, Chlorella, Chlorococcum, Haematococcus, Euglena, Nannochloropsis, Scenedesmus, Spirulina*, etc [24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35]. Mono and mixed algae cultures have a high potential to reduce nitrate, nitrite, and sulphate [36, 37, 38, 39, 40, 41, 42]. This present study analyses the dissolution of vehicular exhaust fume gases and the ability of filamentous algae to fix the concentrations of carbon dioxide, dissolved oxygen, nitrate, and sulphate in the bioreactors.

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II. MATERIALS AND METHODS

A. Sample collection and identification

Algae samples were collected from different locations: NEHU campus, paddy fields, and Golf link area of Shillong, Meghalaya, India. Algae samples collected from various sites were washed with stream water to remove any loosely attached debris, put in zip-lock plastic bags filled with stream water to avoid desiccation, and transported to the laboratory for further processing. Identification of the samples was carried out by microscopic observations using the Magnus MLM compound microscope according to morphological properties based on descriptions [43].

B. Growth conditions

The algae species were grown and maintained in Algae Culture Broth (HiMedia, India) composed of sodium nitrate (1 g/L), dipotassium hydrogen phosphate (0.250 g/L), magnesium sulphate (0.513 g/L), ammonium chloride (0.050 g/L), calcium chloride (0.058 g/L) and ferric chloride (0.003 g/L). The culture was maintained under fluorescent tube lights of light intensity of 24 μmol photons m^{-2} s^{-1} at pH 7 and 25°C. Aeration was provided using aquarium aerators for adequate mixing.

Figure 1: Growth condition and maintenance of algae

C. Experimental Setup

Polythene tubes (bioreactor systems), each measuring 4m, were filled with 2700 ml of tap water and allowed to aerate using aquarium aerators for 24 hours. Chemical parameters were measured, and this served as a control. Vehicular exhaust fumes were injected into the closed room using a motor vehicle running for 1 hour, after which the chemical parameters were measured. Algae samples along with native bathing sponge *Luffa acutangula* (angled loofah), which acted as algae substratum, were inoculated into the respective polythene tubes, and fumes were run for 1 hour for three consecutive days. Aerators were used to ensure proper dissolution of the fumes in the setup. Chemical parameters were measured after the inoculation of algae samples.

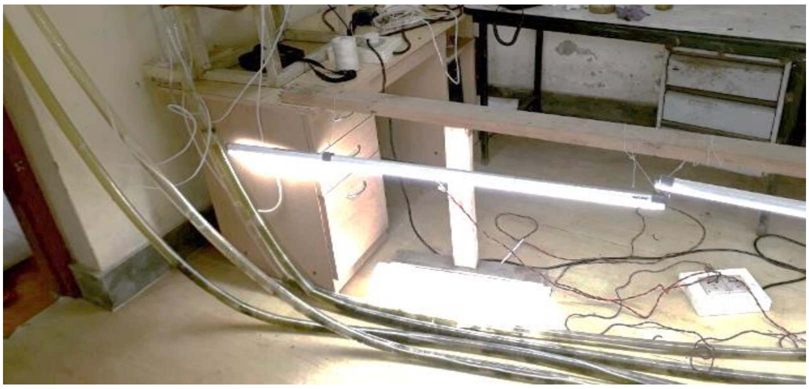

Figure 2: Experimental setup of algae in bioreactors

D. Estimation of chemical parameters

1. pH estimation: pH was measured by the electronic pH meter (EI Deluxe pH Meter -101).

2. Free carbon dioxide estimation: Free carbon dioxide was estimated by the titrimetric method [44].

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3. Dissolved oxygen estimation: The dissolved oxygen concentration was estimated by the azide modification of the Winkler method [44].
4. Nitrate estimation: Nitrate in the samples was estimated by the phenol disulphonic acid (PDA) method [45].
5. Sulphate estimation: Sulphate in the samples was estimated by the turbidimetric method [44].
E. Data analysis: The experiments were carried out in triplicates, and the results are presented as mean and standard error. The significant difference between the control and the experiments was tested using t-test at $p<0.05$ using Microsoft Excel.

III. RESULTS AND DISCUSSION

1. Identification

In this study, the green algae collected from three different sites were studied according to their preliminary morphological identification based on microscopic observations. This showed that the algae belonged to the genus *Spirogyra* and *Ulothrix* of families Zygnemataceae and Ulotrichaceae, respectively.

2. Effect of exhaust fumes on studied parameters

2.1 pH: Figure 3 shows the pH of the control (7.06±0.01, $p<0.05$) and, after the introduction of the fumes (8.7±0.05, $p<0.05$). The pH in control was nearly neutral, whereas, after the introduction of vehicular exhaust fumes, an increase in the pH was reported, which might be due to the variation in temperature, i.e., with an increase in temperature, the alkaline condition of the bioreactor also increased. The assimilation of nitrate and sulphate due to the introduction of vehicular exhaust fumes also affects the pH. This increase in pH also leads to increased dissolution of carbon dioxide in the bioreactors [46]. At the end of the study period, an increase in the pH (9.2±0.03) was found in both the bioreactors inoculated with *Spirogyra* and *Ulothrix*, which led to a more alkaline condition. The decrease in acidity in the bioreactors is due to the removal of carbon dioxide, which is taken up by the algae in the form of carbon for growth. As a result, the pH increases as carbonic acid is removed [47]. Similar findings have also been reported by Scott *et al.* (2010) [48], Craggs *et al.* (2012) [49], and Muriuki *et al.* (2020) [50], suggesting that the breakdown of organic materials in the samples also lead to an increase in the pH.

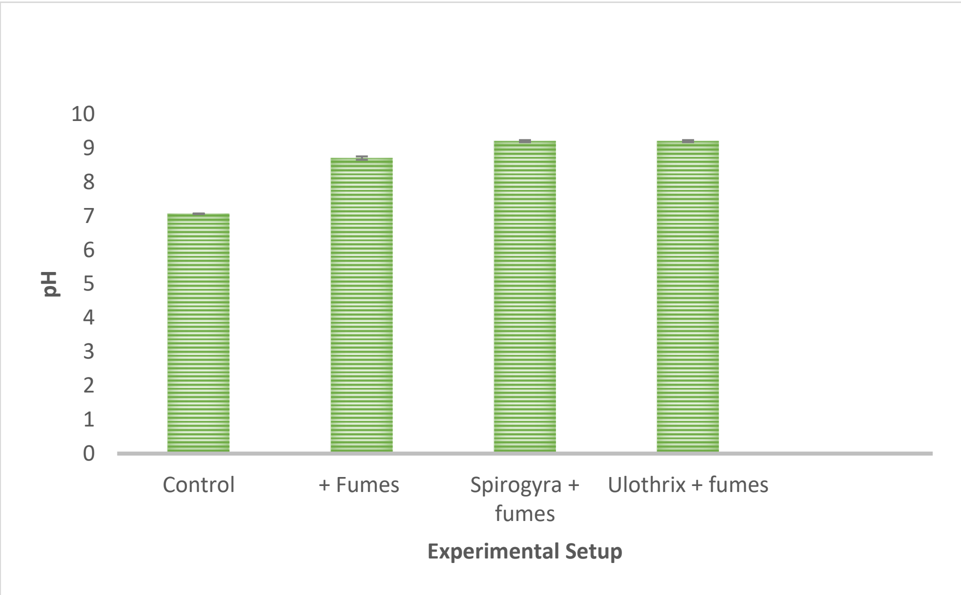

Figure 3: pH values of the different experimental setup

2.2 Free carbon dioxide concentration (mg/L): Figure 4 shows the free carbon dioxide concentration of the control (3.52±0.03 mg/L, $p<0.05$) after the introduction of vehicular exhaust fumes (8.8±0.05 mg/L, $p<0.05$) and after the inoculation of *Spirogyra* (5.06±0.25 mg/L, $p<0.05$) and *Ulothrix* (6.6±0.08 mg/L, $p<0.05$). This decrease in carbon dioxide concentration in the bioreactors containing algae is due to the primary and secondary metabolism of algae that strictly depends on the carbon content of the medium for growth. It can be well equated with a study by Gao *et al.* 1993 [51] wherein, *Garcilaria* species reported higher carbon dioxide absorption in carbon dioxide enriched air than normal air. Certain marine microalgae, like *Ditylum brightwelli*, *Thalassiosira punctigera*, *Rhizosolenia cf. alata*, etc., have also been reported for their ability to absorb increased carbon dioxide concentrations [52, 53].

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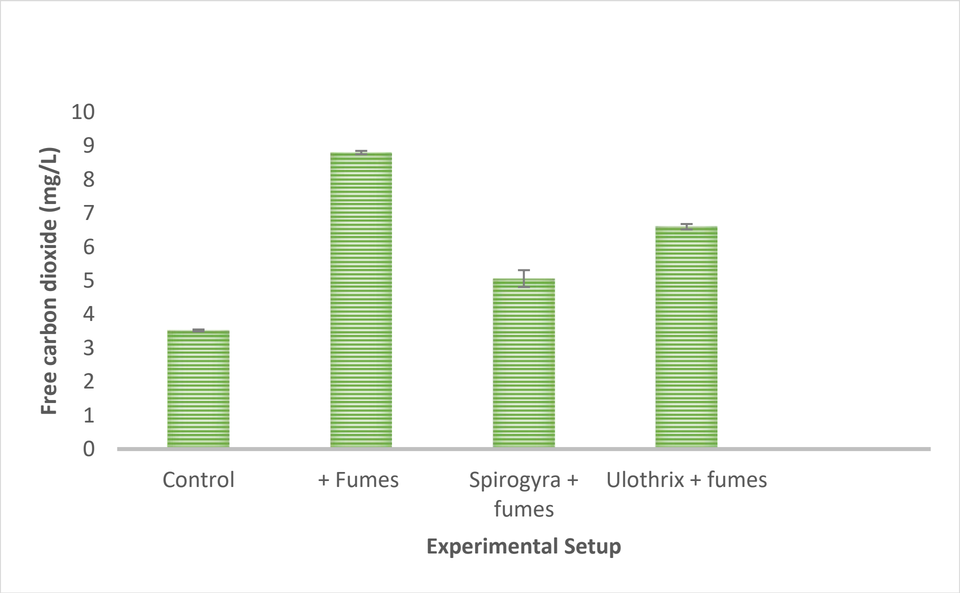

Figure 4: Carbon dioxide concentration (mg/L) of the experimental setup

2.3 Dissolved oxygen concentration (mg/L): Figure 5 shows the dissolved oxygen concentration of the experimental setup i.e; of the control, 6.46±0.26 mg/L ($p<0.05$), and after the introduction of fumes, 7.07±0.05 mg/L ($p<0.05$). The increase in the concentration of dissolved oxygen found after the introduction of vehicular exhaust fumes can be due to continuous aeration of the bioreactor systems with aquarium aerators. The dissolved oxygen concentration of the bioreactor inoculated with *Spirogyra* was 12.52±0.04 mg/L ($p<0.05$), and that of the bioreactor inoculated with *Ulothrix* was 12.92±0.36 mg/L ($p<0.05$). With the inoculation of *Spirogyra* and *Ulothrix* in the bioreactors, a further increase in the dissolved oxygen was reported due to the presence of a nitrogen source in the system that the algae used up for its growth [54]. Kumar *et al.* (2016) [55] and Uddin *et al.* (2019) [56] also reported increased dissolved oxygen levels when *Spirogyra* species were used to treat sugar mill effluent and wastewater, respectively. The higher pH level in the bioreactors also increased the dissolved oxygen concentration, as suggested by Pennington and Agan (2015) [57]. Under illuminated conditions, algae are also known to produce more oxygen than they consume in the dark.

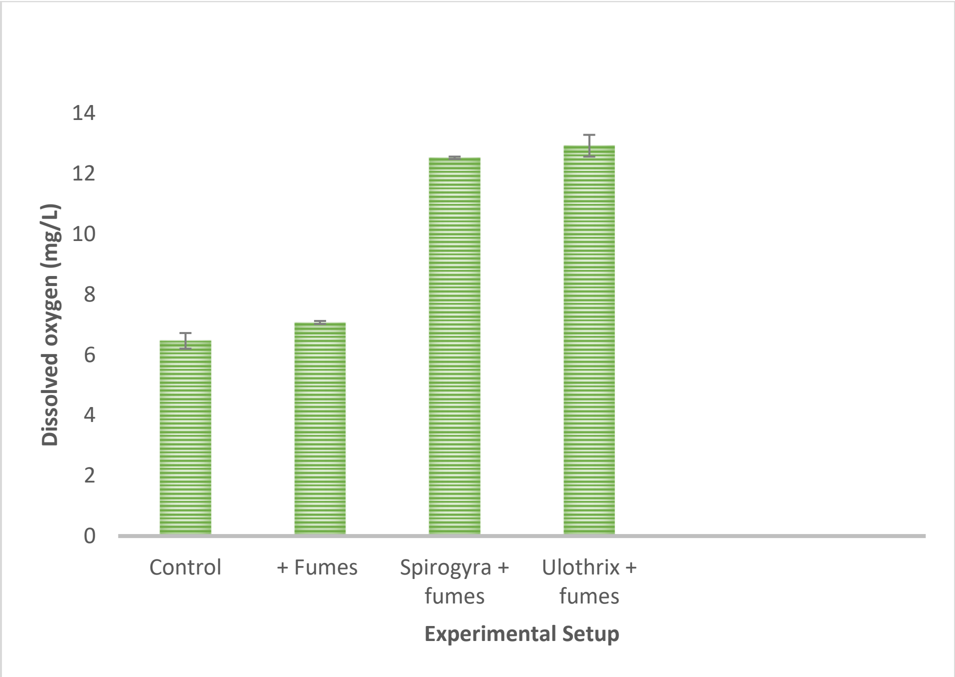

Figure 5: Dissolved oxygen (mg/L) of the experimental setup

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2.4 Nitrate concentration (mg/L): Figure 6 shows the nitrate concentration of the experimental setup. The initial nitrate concentration was 1.2±0.01 mg/L which increased to 3.5±0.008 mg/L ($p<0.05$) after the introduction of vehicular exhaust fumes. Both bioreactors inoculated with *Spirogyra* and *Ulothrix* reported a nitrate concentration of 1.9±0.00 mg/L ($p<0.05$) each. With the inoculation of the algae, a sharp decrease in the nitrate concentration like that of Gao *et al.* (1993) [51] during the study period is seen. This is evidently due to the available nitrogen being used up by the algae as a nitrogen source. Lowering of nitrate ion concentrations was also found in the case of *Spirogyra* species when treated with different levels of carbon dioxide [58]. Sikka & Pramer (1968) [59] reported that *Chlorella pyrenoidosa* and *Euglena gracilis* showed greater absorption during increased nitrate levels in the growth medium resulting in significant growth. *Chlorella vulgaris*, when treated wastewater, also reported lower nitrate levels [60].

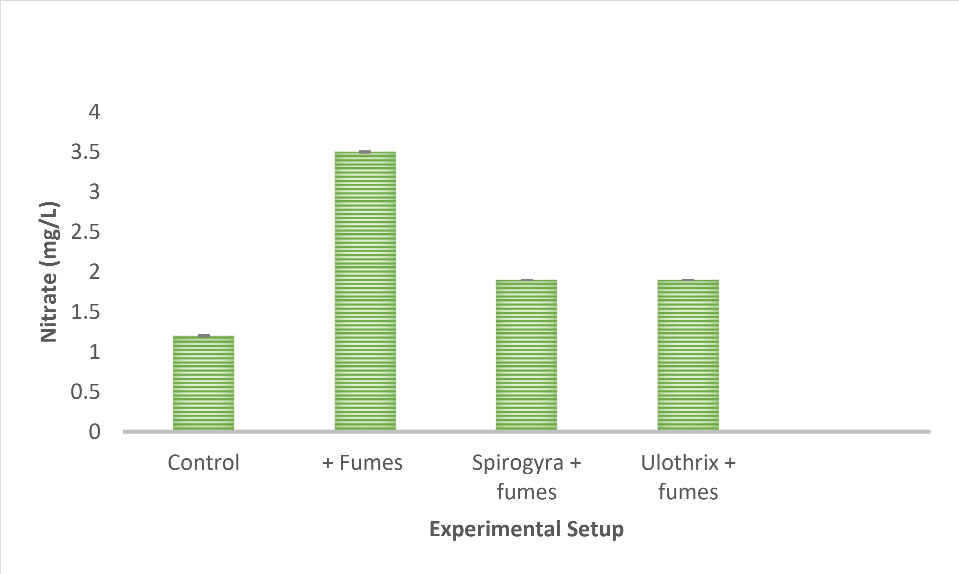

Figure 6: Nitrate concentration of the experimental setup

2.5 Sulphate concentration (mg/L): Figure 7 shows the sulphate concentration of the experimental setup. The control initially had a sulphate concentration of 46±0.01 mg/L, which increased to 178±0.08 mg/L ($p<0.05$) after the introduction of vehicular exhaust fumes. Bioreactor systems inoculated with *Spirogyra* and *Ulothrix* reported sulphate concentrations of 107±0.001 mg/L ($p<0.05$) and 109±0.00 mg/L ($p<0.05$), respectively. The decrease in the concentration after the inoculation of algae can be due to sulphate uptake in the form of sulphur dioxide by algae for photosynthesis. A study on wastewater remediation using *Chlorella vulgaris* [60] reported an initial decrease in the sulphate concentration followed by a subsequent increase till the end of the study period.

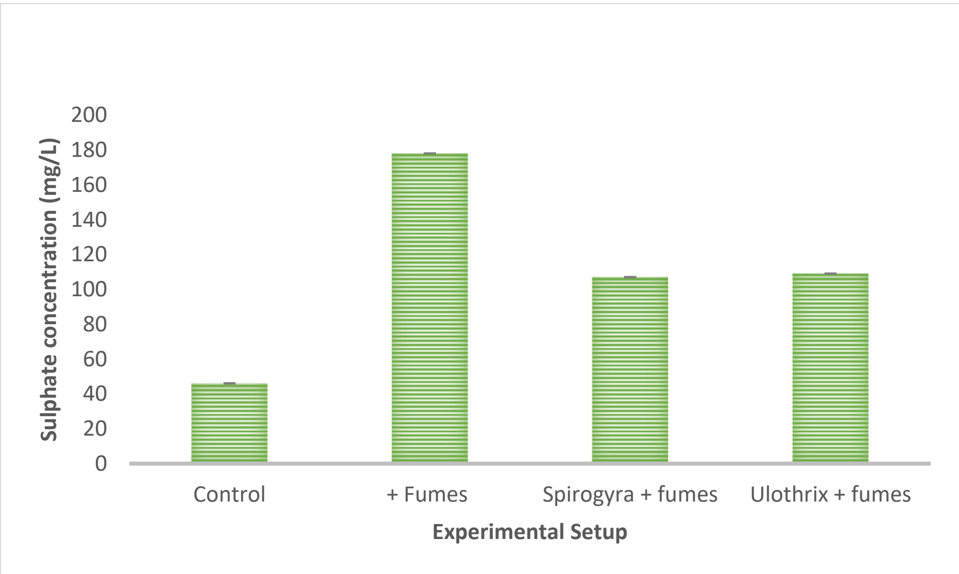

Figure 7: Sulphate concentration of the experimental setup

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IV. CONCLUSION

In the present study, *Spirogyra* exhibited better carbon dioxide, nitrate, and sulphate reduction ability as compared to *Ulothrix*. *Ulothrix* was found to have a higher dissolved oxygen concentration than *Spirogyra*, thus improving the water quality. Although both algae were found to lower the acidic conditions of the bioreactor medium, no apparent significant difference between the two species could be documented. With the widespread concern about reducing exhaust fumes released from vehicles and automobiles into the atmosphere, and the associated deterioration of ambient air quality, algal bioreactor systems can be considered as potential systems for the fixation and removal of carbon dioxide and other polluting gases and help enhance ambient air quality. In the present study, both *Spirogyra* and *Ulothrix* exhibited promising results and can be further explored to analyse their performance under different concentrations of exhaust fume gases and /or under mixed culture conditions using algal consortia and arrive at a promising strategy to establish proper sequestration of gases.

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