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SHORT COMMUNICATION

Influence of Nutrients in Microalgae Cultivation by SEM and EDX Evaluation

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Abstract

Microalgae have recently attracted a lot of attention on a global level because of their numerous application possibilities in the renewable energy, biopharmaceutical, and nutraceutical industries. Microalgae can be exploited to make biofuels, bioactive medicines and food additives at a low cost and with no environmental harmful impact. The media's nutritional content affects the development of microalgae. The role that macro- and micro-nutrients play in the cultivation of microalgae is also significant. For microalgae cultivation, a sample of river water was collected, BG11 and Bold Basal Media (BBM) synthetic media were prepared. Observations of microalgae growth were made after 15 days. On samples of raw water and microalgae grown in a lab, Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray spectroscopy (EDX) were conducted. Raw water and microalgae sample structures were detailed by SEM results, and both samples' chemical compositions were shown by EDX results. The cultivation of microalgae depends heavily on macro and micro nutrients. The growth of microalgae was accelerated in the presence of nutrients.

Keywords: Energy-dispersive X-ray Spectroscopy, Microalgae, Nutrients, Scanning Electron Microscopy

1. Introduction

Global climate change is a serious issue in India, with increasing pollution because of the utilization of fuel in the transportation sector with the emission of environmental pollutants such as nitrogen oxide and chloro-floro carbon. Therefore, scientific attention has been diverted to the use of microalgae as an alternative fuel[1,2]. Microalgae can be used as an alternative source of food, biofuels, bio-fertilizer, and for pharmaceutical purposes[3]. Microalgae contains a high percentage of protein, lipid, and EPA (Eicosapentaenoic acid) by the algal species. In addition, they also contains high amounts of vitamins (A, B1, B2, B6, B12, C and E), inorganic salts (phosphate, zinc, and calcium), antioxidants and pigments[4]. Microalgae are available in various ranges of sizes. Microalgae are eukaryotic microorganisms found in seawater, pond water, river water, and wastewater sources[5]. Microalgae have the potential to survive in the different adverse conditions of hot water bodies, pH and salt concentration and highly polluted waterbodies[6,7]. Microalgae utilizes light and carbon sources to produce biomass with photosynthesis phenomenon with the characteristics of a fast-growing period and exponential growth capabilities in favourable environmental condition[8].

Growth of microalgae is affected by the presence of nutritional content in media. Further, macronutrient and micronutrient are creating vital significance in cultivation of microalgae[9-11]. Different nutrient composition extracted from the cultivated microalgae is influenced by microalgal growth rate and nutritional content in the term of lipid and fatty acid production[12]. Depletive and

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excessive sources of nutritional composition might affect the quality of biomass production. Carbon, nitrogen and phosphorous are considered as a macronutrient which enhances the growth during cultivation, regulating the metabolic activities when provided in certain required form. Potassium, magnesium, calcium and sulphur with a trace amount increases the growth of microalgae as a micronutrient[13,14].

This study deals with the technical feasibility of utilization of macro and micro nutrients for the growth of microalgae. The study highlights the level of oxygen, phosphorus and potassium. This work focuses on the textural analysis of microalgae with SEM and organic and minerals constituents' analysis with EDX.

2. Materials and Methods

2.1 Sample Collection

River sample was collected from the top 20cm of the Sabarmati river (23.0237042, 72.5769516), Ahmedabad Gujarat, India. The collected water sample was taken to the laboratory and maintained at room temperature. It is further treated as a filter with Whatman Filter Paper No 1.

2.2 Media Formulation and Growth Condition

For the cultivation of microalgae BG11 and BBM (Bold Basal Medium), synthetic media was prepared and autoclaved at 121°C for 15 mins and allowed to cool and used for the inoculation. Microalgae were cultivated in the growth media by inoculating (vol/vol) aliquots of growth media to synthetic media in a transparent flask with a ratio of 50:50 (river water:synthetic medium). After the media preparation, the flasks were incubated for 15 days under an artificial light source (16:8, light:dark). The flasks were shaken three times daily to enhance the growth of microalgae, and prevent sedimentation effect, thermal stratification, improve gas exchange between cultural media and air with ensuring that all cells of the population were equally grown under light and nutrient compositions.

2.3 Experimental Setup

The experimental work was carried out at the laboratory of Microbiology Department, Parul University, as shown in Figure 1. All the experiment was performed under room temperature condition.

Figure 1. Experimental set up at Parul Institute of Applied Sciences and Research.

2.4 Scanning Electron Microscope (SEM) and Energy-Dispersive X-ray Spectroscopy (EDX)

Raw water samples and microalgae samples were characterized by SEM and EDX analysis. SEM is used for the detailed structure of raw water and microalgal sample, and the EDX provide analysis of organic and minerals constituents of raw water sample and microalgae water sample.

3. Results

After completion of 15 days cultivation flask appeared green in colour, which indicates the presences of microalgae Figure 2. It was further confirmed by light microscopy. Microalgae image was observed under microscope, as shown in Figure 3.

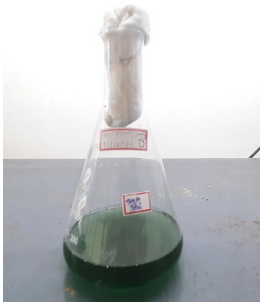

Figure 2. Microalgae flask.

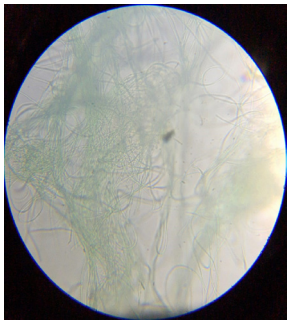

Figure 3. Microalgae.

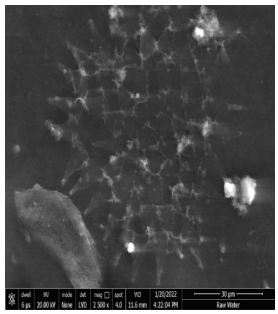

Figure 4. SEM analysis on raw water sample.

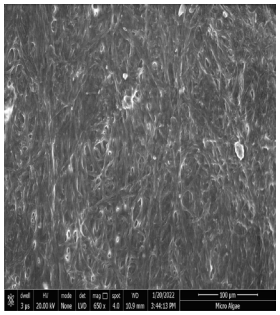

Figure 5. SEM analysis on microalgae sample.

Filamentous structure of microalgae was identified during light microscopy in Figure 3. Comparision of raw water sample and algae sample by SEM analysis shown in Figures 4 and 5. Difference of chemical composition in microalgae sample and raw water sample was analysed by EDX as shown in Figures 6 and 7 (Tables 1 and 2).

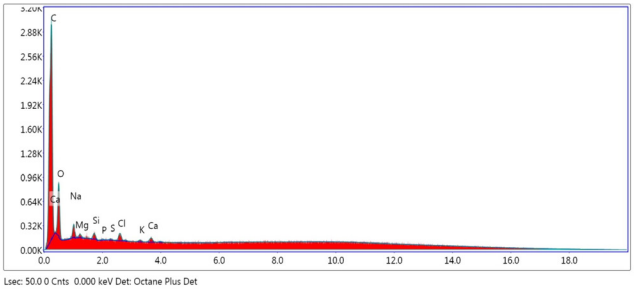

Figure 6. EDX analysis on raw water sample.

Table 1. EDX results of raw water in weight %

Element	Weight %	Atomic %
C	65.94	73.29
O	29.23	24.39
Na	2.16	1.26
Mg	0.28	0.15
Si	0.47	0.22
P	0.10	0.04
S	0.12	0.05
Cl	0.76	0.29
K	0.24	0.08
Ca	0.71	0.24

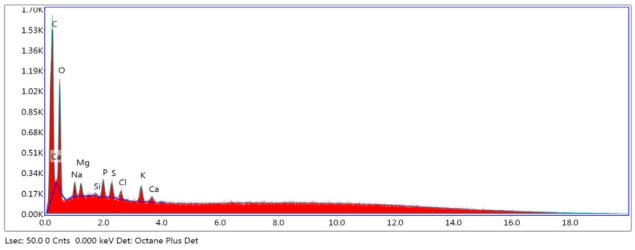

Figure 7. EDX analysis on Algal sample.

Table 2. EDX results of algal water in weight %

Element	Weight %	Atomic %
C	52.78	61.85
O	38.89	34.22
Na	1.91	1.17
Mg	0.94	0.54
Si	0.16	0.08
P	1.22	0.55
S	1.12	0.49
Cl	0.68	0.27
K	1.62	0.58
Ca	0.68	0.24

4. Discussion

SEM is used to examine the detailed structure of raw water before the growth of microalgae. Images show that different organic constituents are aggregated into the water Figure 4. Irregular filamentous structure of microalgae were observed as shown in Figure 5.

Comparison of chemical composition in raw water and algae water sample was done by EDX. For the quantification of chemical elements present in raw water and algal water, both the samples were analysed by EDX analysis. This analysis was conducted at several region of the sample water. Different concentrations of chemical elements were found in different regions. In the raw water sample following chemicals were observed: carbon, oxygen, sodium, magnesium, silicon, phosphorus, sulphur, chlorine, potassium, calcium. In the presence of these chemical compounds, microalgae growth was observed. Carbon, nitrogen, phosphorus and potassium works as macronutrient in the growth of algae. Others are trace elements that are present in the medium in small amount but also enhance the growth of microalgae. Same elements are also present in the algal water sample, but concentration of the elements is differing.

The growth and biochemical synthesis of microalgae depend heavily on carbon. Microalgae media can provide carbon supplies in either organic or inorganic forms. The growth of microalgae, the quantity of metabolites, and the composition of microalgal cells are all significantly influenced by various sources and quantities of carbon. Due to its advantages in reducing greenhouse gas emissions and lower cost, carbon dioxide (CO_2) is preferable to bicarbonate salt in the inorganic form of carbon sources[15-17]. Another necessary substance, phosphorus, is important for the growth of algae, the generation of lipids, the yield of fatty acids, and metabolic processes such as energy transmission, signal transduction, and photosynthesis[18,19]. Phosphorous is a crucial ingredient that contributes just 1% of the total biomass of algae and is needed at concentrations of 0.03–0.06 % in the medium to support algae growth. The generation of cellular components such as phospholipids, DNA, RNA, and ATP for the metabolic pathways involved in energy transfer and nucleic acid synthesis in microalgal cells depends on phosphorus[20-22]. Potassium is the primary macronutrient for the growth of microalgae. Potassium plays significant biological role, because it is an activator for number of enzymes involved in photosynthesis and respiration. Its affects carbohydrate and protein synthesis[21]. For microalgae to grow, potassium is the most important macronutrient. Because it is an activator for numerous enzymes involved in respiration and photosynthesis, potassium performs an important biological role. It impacts the production of proteins and carbohydrates. For the development of microalgae and the creation of biomass, sulphur is a crucial macronutrient. In addition, it is a component of several secondary metabolites that contain sulphur as well as vitamins and regulatory chemicals[23-25]. For the growth of microalgae biomass, magnesium is crucial. Magnesium is an enzyme activator and takes part in the crucial ATP reaction for carbon fixation in cells. Additionally, it consists of the photosynthetic system, particularly the chlorophylls[26-28]. Calcium is a crucial component of cell walls. It plays a key role in the proliferation of microalgae. It is a secondary messenger that influences the body's overall health and influences cell division as well[29,30]. Several other essential micro nutrients like Na, Cl, Fe, Zn, Cu, Mo, Mn, B and Coare also enhanced the growth of microalgae[31].

In this investigation, the chemical elements presented in the sample were identified by using EDX analysis. The following substances were found in the raw water sample: carbon, oxygen, sodium, magnesium, silicon, phosphorus, sulphur, chlorine, potassium, and calcium. When microalgae are exposed to these substances, they act as nutrition for the microalgae that are growing there. Which uses sodium, magnesium, silica, and calcium as micronutrients for microalgae and carbon, phosphorus, and potassium as macronutrients. Further EDX analysis was performed on the microalgae sample in order to compare the chemical components of the raw water sample and the microalgae water sample. In a microalgae sample, the chemical makeup was the same, but the elemental concentrations were different. Therefore, the EDX method was employed to compare the chemical components of a raw water sample and an algal water sample. As a result, the growth of microalgae was affected by the presence of carbon, oxygen, sodium, magnesium, silicon, phosphorus, sulphur, chlorine, potassium, and calcium.

5. Conclusion

This study found that different nutrients play an important role in the proliferation of microalgae. Nutrition composition was confirmed by EDX analysis. In the raw water sample and microalgae sample following chemicals were observed: carbon, oxygen, sodium, magnesium, silicon, phosphorus, sulphur, chlorine, potassium, and calcium. In the presence of these chemical compounds, microalgae growth was observed. Carbon, nitrogen, phosphorus and potassium work as macronutrients in the growth of algae. Others are trace elements that are present in the medium in small amounts but also enhance the growth of microalgae.

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