

**DEVELOPMENT OF MICROALGAE AS FEEDSTOCK FOR BIOFUELS AND BIOPRODUCTS**

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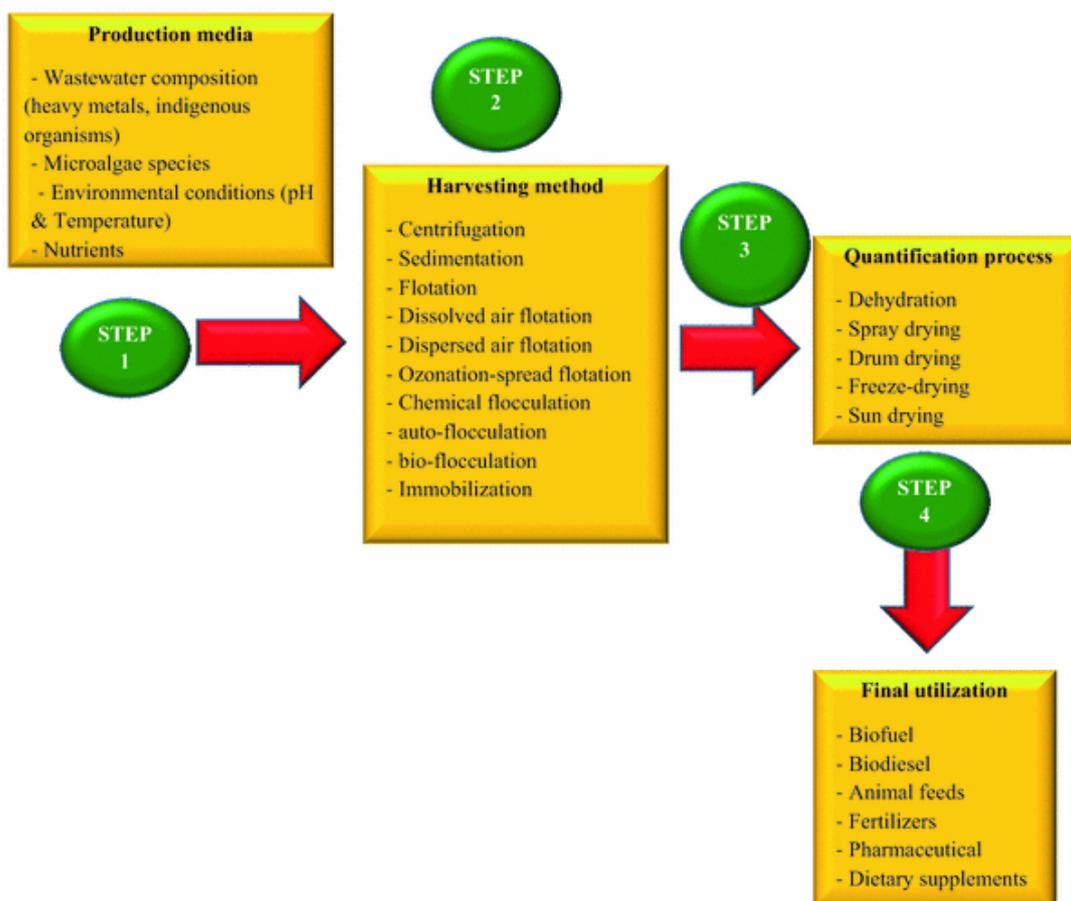
**ABSTRACT**

Microalgae have recently attracted considerable interest worldwide, due to their extensive application potential in the renewable energy, biopharmaceutical, and nutraceutical industries. Microalgae are renewable, sustainable, and economical sources of biofuels, bioactive medicinal products, and food ingredients. Several microalgae species have been investigated for their potential as value-added products with remarkable pharmacological and biological qualities. As biofuels, they are a perfect substitute to liquid fossil fuels with respect to cost, renewability, and environmental concerns. Microalgae have a significant ability to convert atmospheric CO<sub>2</sub> to useful products such as carbohydrates, lipids, and other bioactive metabolites. Although microalgae are feasible sources for bioenergy and biopharmaceuticals in general, some limitations and challenges remain, which must be overcome to upgrade the technology from pilot-phase to industrial level. The most challenging and crucial issues are enhancing microalgae growth rate and product synthesis, dewatering algae culture for biomass production, pretreating biomass, and optimizing the fermentation process in case of algal bioethanol production. The present review describes the advantages of microalgae for the production of biofuels and various bioactive compounds and discusses culturing parameters.

**1. INTRODUCTION**

Microalgae have received much interest as a biofuel feedstock in response to the uprising energy crisis, climate change and depletion of natural sources. Development of microalgal biofuels from microalgae does not satisfy the economic feasibility of overwhelming capital investments and operations. Hence, high-value co-products have been produced through the extraction of a fraction of algae to improve the economics of a microalgae biorefinery. Examples of these high-value products are pigments, proteins, lipids, carbohydrates, vitamins and anti-oxidants, with applications in cosmetics, nutritional and pharmaceuticals industries (Chew et al., 2017). In this studies, Microalgae will be used for the bioremediation of wastewater, protein extraction, antimicrobial activity and 3-hydroxypropionic acid production from glycerol.

The production process of microalgae biomass is illustrated in Figure 1.



**Figure 1: Considerations for production of microalgae biomass in wastewater and the methods of harvesting, quantification and final utilizations (Jais et al., 2017).**

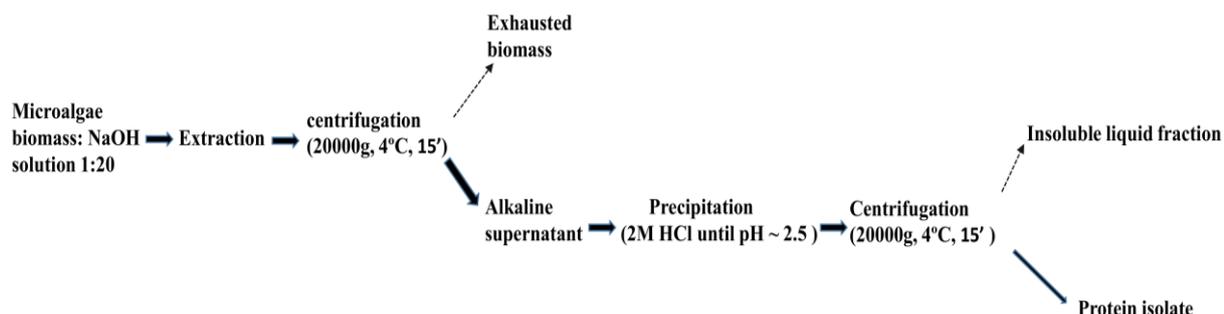
## 2. Biorefinery of Microalgae: Protein extraction

Microalgae have been classified as a rich source of protein for animal and human consumption, with concentrations up to 60% (dry weight). Nevertheless, little has been done to determine the structure and possible application of several protein fraction present into the cells. Further, isolation of microalgae protein has been approached for laboratory studies and there are not reports over protein fractionation for industrial

applications. Hence, the aim of this research is to get a better understanding of the protein fractionation in microalgae and to explore possible applications in food industry.

### Approach

Protein can be isolated by alkaline hydrolysis method. Whole methodology of proteins isolation is being shown in Figure 1.



**Figure 1: Alkaline hydrolysis method.**

Quantification will be carried out by Lowry assay or Bradford assay. The biomass amino acid composition was determined using a well-known standard method (Moore and Stein, 1948). The samples will be

hydrolyzed with 6 N hydrochloric acid at 103°C for 24 h. Then, the hydrolyzed material was adjusted to pH 2.2 with 6 N NaOH and stabilized with a pH 2.2 citrate buffer solution. The final solution will be then filtered to

remove any residual solids remaining in the solution. The analysis was performed by using an amino acid analyzer Biochrome.

### Significance

Extracted protein fraction can be used as raw material for productions of food specialties. For example, proteins for encapsulation, food structural compounds, paper coating, adhesives, edible coating, or the protein hydrolysis that leads to free amino acids production.

### 3. Potential of microalgae in bioremediation of waste water

Microalgae have several industrial applications that can lower the cost of biofuel co-production. Among these co-production applications, environmental and wastewater bioremediation are increasingly important. Heavy metal

pollution and its implications for public health and the environment have led to increased interest in developing environmental biotechnology approaches. Huge load of wastes from industries, domestic sewage and agriculture practices find their way into rivers, pond resulting in large scale deterioration of water quality leading to the availability of potable water (Bilal et al., 2018). There is an urgent need to screen and develop efficient microalgae for the bioremediation of waste water.

### Approach

In this studies, biosorption (Bilal et al., 2018) and bioaccumulation approach has been used to remove heavy metal ions from wastewaters. Figure 2 illustrated the scheme of the biosorption and bioaccumulation processes for heavy metal ions.

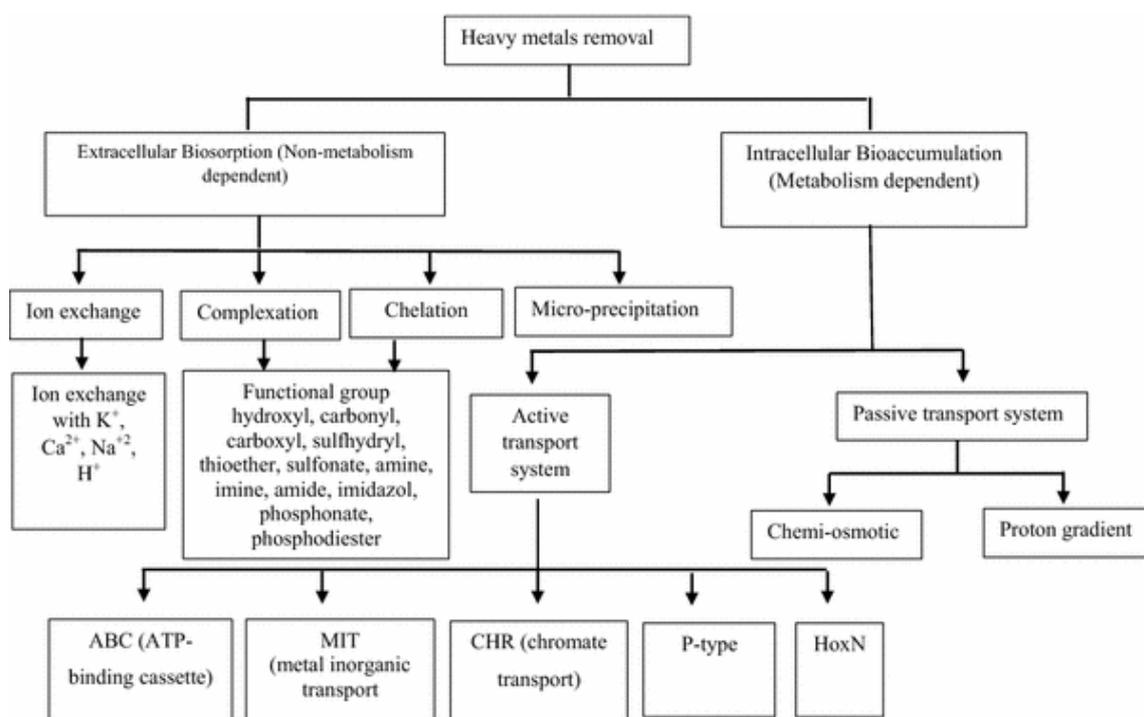


Figure 2: Mechanism of heavy metals removal by microorganisms. It was proposed based on the previous studies (Al-Gheethi et al., 2015).

### Significance

Algal bioremediation is considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted systems. It is widely used for heavy metal removal from waste water.

### 4. Antimicrobial Activities of the Extracts of microalgae

Microalgae contain pharmacologically active compounds such as phlorotannins, fatty acids, polysaccharides, peptides, and terpenes which combat bacterial invasion. The resistance of pathogenic bacteria to existing antibiotics has become a global epidemic. Algae derivatives have shown promise as candidates in novel, antibacterial drug discovery (Shannon and Abu-Ghannam, 2016). In the present study, we will describe

the antimicrobial characteristics of methanol extracts of some microalgae.

### METHODOLOGY

Microalgae samples was counted and concentrated by centrifugation. Subsequently, the pellet was washed with phosphate buffer. The resulting samples was sonicated with a sonicator, and re-centrifuged. A 60% methanol was added to the pellet in a ratio of the initial algae sample of 5 g to 20 mL of methanol. The resulting sample was re-centrifuged. The supernatant was then filtered with 0.22  $\mu\text{m}$  filters and the resulting methanol extract was utilized. The results of antimicrobial activity are expressed as algal cells/mL. Minimum Inhibitory Concentration (MIC) of extracts from microalgae determined using the broth microdilution method,

according to the CLSI guidelines. Briefly, algae extracts was tested on cultures of bacteria in 96-well microtiter plates in Mueller-Hinton broth. After 18–24 h of incubation at 37 °C, the concentration of the extract from algae which prevented a visible bacterial growth was identified as the MIC (Pane et al., 2015).

### Significance

Antimicrobial activity indicates that the presence of active constituents in the extractions of microalgae which can be exploited for the production of innovation drugs for the benefit of the humanity.

### 5. Bioconversion technologies of crude glycerol to 3-Hydroxypropionic acid

Crude glycerol that is produced as the by-product from biodiesel, has to be effectively utilized to contribute to the viability of biodiesel. Crude glycerol in large amounts can pose a threat to the environment (Garlapati et al., 2016). Therefore, there is a need to convert this crude glycerol into valued added products using biotechnological processes, which brings new revenue to biodiesel producers.

### METHODOLOGY

The present investigation was focused on the construction and evaluation of a recombinant strain *Escherichia coli* SH254 that produces 3-HP from glycerol. The strain was developed by cloning two genes, *dhaB* of *Klebsiella pneumoniae* DSM 2026 encoding glycerol dehydratase and *aldH* of *E. coli* K-12 MG1655 encoding aldehyde dehydrogenase, respectively (Lim et al., 2016).

### Significance

3-Hydroxypropionic acid (3HP) is a promising molecule that can be used for the production of an important array of high added-value chemicals, such as 1, 3-propanediol, acrylic acid, acrylamide, and bioplastics.

### CONCLUSIONS

Microalgae are tiny factories and renewable, sustainable and economical sources of biofuels, bioactive medicinal products and food ingredients. Microalgae useful in mitigation of elevated CO<sub>2</sub> level and treatment of waste water. Upgradation of algal fuel and bioproducts technology from pilot scale to commercial level is possible by overcoming the associated challenges and limitations. In this review we describe the extensive applications of the microalgae in bioenergy, nutraceutical and pharmaceutical industry, the associated challenges and limitations and how it can be overcome to make them feasible and viable for commercialization.

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