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Uplifting Technologies to Increase Lipid Content in Scenedesmus Species for Biofuel Production

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Abstract:- Fossil fuels have become a huge part of our life and it has become one of the essential commodities which has become a necessity to most of the day to day activities. As a sustainable alternative microalgae based biofuels have shown a promising approach towards replacing fossil fuels usage. Lipid accumulating microalgae is mainly used for biodiesel production. Several microalgae are capable of producing high quality as well as high quantity of lipids which is suitable for biodiesel production. Hence high lipid content and rapid cell growth is critical for commercial and scale up production. *Scenedesmus sp*. is one of the microalgae species which has low lipid content but high growth rate. Increasing the lipid content in *Scenedesmus* species is a challenging process and extensive research is taking place in this area to increase the lipid content in them. Upstream and downstream stages of the microalgae based biofuel production process have been widely explored to increase lipid as well as biomass productivity in the microalgae. This review is mainly focused on the four of the uplifting technologies which have been used to improve the lipid content especially in Scenedesmus species.

Index terms **-- Biodiesel, biofuel, biomass, fossil fuel, lipid, microalgae and Scenedesmus sp.**

I. INTRODUCTION

In the current scenario fossil fuels have become a huge part of our daily life. Increasing population, industrialization and urbanization have led to the massive usage of fossil fuels. It has made a huge impact on people's life in such a way that we are not able to imagine our life without it [1]. We can say that use of fossil fuels leads to global climate change, environmental pollution which are associated with various health problems, huge fuel demand and energy crisis which leads to irreversible reduction in the source of fossil fuels [2]. All these factors have paved a path for scientists, researchers and governments looking into alternative renewable energy sources in order to replace the usage of these fossil fuels and to minimize the harmful effects of it in the environment[3].

Biofuels have become a promising alternative for fossil fuels and when compared to traditional fossil fuels it has tremendous advantages such as lowering the greenhouse gas emissions, improving energy security, energy export savings, renewability and free of toxicity and sulphur[4]. Biofuels are the fuels that are produced from the biomass either by direct or indirect means. There are three kinds of biofuels according to the generation such as first, second and third [3]. Third generation biofuels are produced from microalgae and microbes which are mainly used for biodiesel production.

Scenedesmus is a genus of microalgae belonging to class Chlorophyceae, which has attracted a huge attention for its usage in the production of biofuel due to its high lipid productivity, desired fatty acid profile and good level of adaptability to various adverse environmental conditions. Scenedesmus sp. lipid content and biomass productivity is 19.6–21.1% and 0.03–0.26 g L^{-1} d^{-1}, respectively [5]. Nannochloris sp. has 56% lipid content and Schizochytrium sp. with 80% lipid content. These species have higher lipid content in them but their growth rate is very slow. While algal species such as Scenedesmus sp. And Chlorella sp. has a higher growth rate but relatively contains lesser lipid content among them.

A considerable balance must be present between lipid content and cell growth to obtain the best economic scenario. Huge amounts of effort are being made in order to obtain this balance in upstream and downstream stages. The upstream process involves screening appropriate microalgal strain and improvement of those microalgal species by genetic engineering with high lipid producing organisms. The downstream process involves implementing various strategies in the harvesting and after harvesting stages which promotes lipid productivity in microalgae [6].

Based on their chemical composition, biofuels such as biodiesel, biohydrogen and bioethanol can be produced from microalgae. Biodiesel can be produced using a high lipid accumulating microalgae and biogas and bioethanol can be producing high carbohydrate accumulating microalgae as a substrate. Hydrogen accumulation based microalgae is used for biohydrogen production [7].

In this paper we have tried to focus on some uplifting strategies which have been researched and implemented with the intention of increasing lipid content in Scenedesmus microalgae species for the production of biodiesel.

II. UPLIFTING TECHNOLOGIES TO INCREASE LIPID PRODUCTIVITY

2.1 Nitrogen ion beam implantation

Ion implantation is one of the processes which is conducted at low temperature in which ions of an element are accelerated into a solid target thereby changing the physical, chemical as well as electrical properties of the chosen target [8]. This method has been used as a novel mutagen and has been increasingly applied in microbial mutagenesis for its higher mutation frequency and wider mutation spectra [9]. Mutation is one of the approaches to obtain desired algal strain with high lipid production [10]. Ion implantation is widely and successfully used in crops and microbial breeding compared to the conventional mutation ways such as UV-rays, X-rays etc. It has advantages such as lesser damage to cells and high mutational probability [11]. Many studies have shown that the usage of heavy ion beam implantation on the strain can significantly improve production of the various metabolites like oil, pigments and polysaccharides [10].

Niwa et al(2009) studied this effect of ion implantation on rhodophyta (red algae species) Porphyra umbilicalis and concluded it was able to produce a stable mutant which showed remarkable increase in total free amino acids, phycoerythrin, alanine and taurine with slight reduction in biomass yield [12]. Studies by Yang et al (2013) on the greasy Nitzschia sp. using C2+ ion irradiation showed that the average lipid content increased by 9.8% when compared with the wild type after four generations [13]. Using N+ ion implantation a highly lipid-producing mutant strain of C.pyrenoidosa was produced. This particular strain showed 25% of higher lipid productivity than that of the wild strain [10]. N+ ion beam implantation was used to enhance Aspergillus niger TA9701 in tannase yield.The results showed that an excellent mutant J-T18 with a yield of 38.5 IU/mL which is five times that of the original strain was obtained in the end of nine implantations under the conditions of 10 keV and 30–40 (×2.6 × 1013) ions/cm2 done successively [9].

Based on many research proofs ion implantation has shown its impact in increasing the lipid content in various algal species and especially N+ ion implantation has shown to increase the lipid content in algal and fungal species. For the main microalgal species i.e Scenedesmus sp. Qu et al (2020) research work has shown a great positive impact. A high lipid content mutant strain of Scenedesmus obliquus was obtained by the N+ ion implantation which was having lipid content 24.1% higher than the original strain along with genetic stability. The mutant strain also showed good adaptability in municipal wastewater and their removal rates of TP, TN, NH4+ and COD were 95.72%, 80.30%, 87.25% and 85.43% respectively after 7 days of cultivation. Thus, N+ ion implantation could both enhance the lipid content of Scenedesmus obliquus and increase its adaptability to wastewater [11].

2.2 Red luminescent solar concentrators

Enhancing microalgal biomass productivity is the main aim of any algal farmer which indirectly helps in improving the lipid content in them [14]. When sufficient nutrients are available light and temperature are the most critical limits to the growth of microalgae [15]. Raceway open ponds are most commonly and majorly used cultivation system for mass algal production. These large scale raceway ponds are operated at a depth greater than 20cm for their operational reasons hence the algal cultures usually receive limited light as light cannot penetrate to a depth below 5cm [16]. Therefore, photo limitation is one of the main obstacles for microalgae grown in raceway ponds resulting in rather low biomass productivity [15]. Temporal and spatial light distribution systems are the two main systems which are used to increase availability of light to microalgal cells [14]. Between these two systems spatial light dilution systems are considered more effective and economical than temporal light dilution systems. Among the spatial dilution systems luminescent solar concentrator (LSC) panels are known to have greater potential for mass microalgae production in commercial fields . LSC panels combine spectrum shifting properties with spatial dilution to channel the light into the culture where it is needed [16]. LSCs do not require a solar tracking system which results in less cost compared to other systems [14].

Red and blue LSC were used for increasing the biomass and phycocyanin productivity of Arthrospira platensis algal species. When red LSCs were used, biomass and phycocyanin productivity of the algal species increased 26% and 44% respectively whereas for the blue LSCs there was no significant increase in their biomass. So red LSCs were considered for increasing biomass productivity [15]. In the study conducted by Raeisossadati et al (2020), usage of red LSCs significantly increased the biomass productivity of Scenedesmus sp. by 18.5%. The protein content (52.9% of biomass) and lipid content(16.5% of biomass) were also 15% and 10% higher respectively with red LSCs than those in the control with no usage of LSCs. Therefore it is observed that protein, lipid and carbohydrate productivity of Scenedesmus sp. are also improved by 35%, 20% and 16% in the presence of red LSCs. Using LSCs has shown that it would require 18.5% cultivation area for the generation of the same biomass productivity which would massively reduce the cost of overall biomass production and as the main focus of our paper it also contributes for enhancing lipid content in Scenedesmus sp. [14].

2.3 UV radiation along with hydrogen peroxide treatment

For increasing the lipid productivity in the cells, strain improvement by mutagenesis is an effective method. Usually mutagenesis in microalgae is performed by a physical method and by a chemical method [17]. Generally UV method of mutagenesis is considered as a good method for microalgae breeding as it can be easily manipulated compared to gene engineering [18].

UV radiation was applied to Chlorella sp. to induce mutagenesis and when they were compared to the control strain the biomass for the UV mutated strain was 7.6% higher. The lipid productivity of the mutated strain also showed a desired increase in its value. These results indicated that the UV method of mutagenesis is one of the effective methods which can be used for lipid production in Chlorella algal species [18]. Oxidative stress increases the lipid peroxidation in microalgae unlike the usual nutrient stress. Oxidative stress increases the reactive oxygen species (ROS) and therefore enhances the lipid content. Lipid content in the Chlorella vulgaris was shown to be increased due to the oxidative stress by H2O2 [19].

Improvement of both biomass and lipid content in various organisms including microalgae by UV mutagenesis and oxidative stress treatment has not been studied much [20]. Most results indicated that UV mutagenesis can improve either the biomass or the lipid content for microalgae and oxidative stress can improve lipid content in the algal species [18]. Scenedesmus sp. obtained by the UV mutagenesis approach had a higher biomass and lipid content when compared to the wild type which showed an increase from 1.9 to 2.4 g/L and from 40 to 55% respectively after 12 days. The hydrogen peroxide treated mutant grown in BG11 medium showed an increase in biomass compared to the H2O2 treated wild type. There was also a 3-fold increase in the yield of lipid of 1.63g/L obtained in the oxidative stress induced mutant compared to the wild type. Overall from all the results obtained we can conclude that prior treatment of UV-mutagenized Scenedesmus with oxidative stress can increase the lipid productivity and can be potentially used for the biodiesel production due to its methyl ester having acceptable biodiesel properties [20].

2.4 Metal ions addition as a form of flocculant.

Microalgae harvesting is one of the key challenges for microalgal production which overall accounts for 20-30% of total biomass production cost. Flocculation- sedimentation process has been employed previously in biomass harvesting of microalgae and is considered to be advantageous than traditional harvesting methods. Metal ions have been regarded as an effective flocculant for microalgae which can neutralize or reduce the surface charges [5].

Studies have been conducted to evaluate the effect of starvation and supplementation of calcium, magnesium and phosphorus on growth and lipid accumulation in microalga Scenedesmus obliquus. Starvation of calcium and phosphate induced lipid accumulation by 52.9% and 47.6% of dry cell weight respectively. Magnesium supplementation of 300mgL-1 induced lipid accumulation by 54.6% of dry cell weight. In this study it can be observed that a combination of nutritional stress adversely affects the growth and lipid productivity [21]. Supplementation of calcium and magnesium ions resulted in a significant increase in the biomass production, fatty acid for biodiesel and docosahexaenoic acid with the both strains Aurantiochytrium sp. DBT IOC-18 and Schizochytrium sp. DBT IOC-1, respectively, on which studies were conducted. These findings explain the importance of calcium and magnesium salts in designing new fermentation strategies [22].

Magnesium played an important role in the flocculation process for Chlorella vulgaris, Chlorococcum sp. , Nannochloropsis oculata and Phaeodactylum tricornutum in the studies conducted by Wu et al (2012) [23]. Al3+ is one of the conventional inorganic flocculants and the flocculation efficiency for Chlorella sorokiniana which was grown in BG-11 medium containing Al3+ can reach about 90% [24]. Another study showed that the settling rate of Scenedesmus obliquus was 80.2% by addition of 69mg L-1 Fe3+ to N11 medium [25].

One of the recent studies investigated the effects of metal ions such as Mg2+, Al3+ and Fe3+ on the growth, lipid accumulation as well as the sedimentation of Scenedesmus sp. in the medium containing fatty acids. The results from this study showed suitable concentrations of Fe3+ and Mg2+ helps in promoting the lipid productivity in microalgae and sedimentation in batch culture. Fe3+ was found to more effective when compared to Mg2+ and the biomass concentration, lipid content and sedimentation efficiency was 1.02 ± 0.6 g L−1, $31.7 \pm 1.7\%$ and $91.2 \pm 2.5\%$ respectively which was obtained at concentration of 45 μM Fe3+. The addition of Al3+ showed an inhibitory effect on biomass and lipid production but there was some enhancement in sedimentation efficiency. The semi-continuous microalgal reactor which was used in this study was steady and efficient, and was able to remove more than 96% of volatile fatty acids in effluent.The average lipid concentration and sedimentation efficiency in semicontinuous mode reached 0.39 g L−1 and 92.7%, respectively. Overall we can say that addition of metal ions such as Fe3+ in a semi-continuous reactor provides a promising process for enhancing lipid production [5].

III. CONCLUSION

We are all aware that the biodiesel demand is growing in India day by day. There is extensive research on lipid production from microalgae, which proves highly dependent on the species and culture conditions. In this review paper focus has been made on four of the technologies which can be used for the enhancement of lipid content especially in Scenedesmus species by giving past research proofs for each one of the technologies which has been conducted on other species. Upstream processes such as UV mutagenesis along with H202 treatment which was able to increase lipid content, red luminescent solar concentrators increased lipid content as well as biomass which can be used for biodiesel and animal feed production respectively, nitrogen ion beam implantation method increased the lipid content and also showed great results in the adaptability to waste water. Downstream processes such as addition of metal ion strategy showed great results in enhancement of lipid content. All the technologies reviewed here have shown positive results in improving the lipid content in the Scenedesmus which can be used for the biodiesel production in a commercial way.

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