



## MICROALGAE *SCENEDESMUS* SP. SAR1 AS A POTENTIAL SOURCE FOR BIOPLASTIC PRODUCTION

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### ABSTRACT

Bioplastic is a biodegradable material produced from renewable sources and used to reduce our dependence on fossil resources which are significantly suffocating the planet and polluting the environment by emitting greenhouse gas. The ability of microalgal cells to absorb carbon dioxide facilitates the reduction of greenhouse gases and also considered as a novel high efficient starch producer. Starch obtained from microalgae can be used as a raw material for bioplastics production. In this study, green microalga *Scenedesmus* sp SAR1 is used as a highly productive source of starch, an alternative source for starch-rich plants in bioplastic production. From *Scenedesmus*, the starch was extracted as granules and analyzed by using FTIR spectroscopy. To create a plastic material with better mechanical properties, the extracted starch granules from the microalga *Scenedesmus* sp SAR1 are mixed with the plasticizer carboxymethyl cellulose. This algal bioplastic is easily degradable and act as a sustainable, biodegradable alternative to single-use packaging.

**Keywords:** *Scenedesmus*, Starch, FTIR, Plasticizer, Bioplastic.

### 1. INTRODUCTION

Plastics are synthetic organic polymers and the topmost threat to our ecological unit due to pollution. Plastic products soon became omnipresent in our daily lives due to their desirable characteristics, like low cost and mechanical resistance. It can be easily shaped into different forms to produce a variety of materials [1]. For many years, we only professed the benefits of plastic and knew slight of the detrimental consequences for human health, natural ecosystem and the climate. It poses a bigger threat to our environment due to their non-degradable nature, the materials used for plastic production and the challenges behind properly discarding them [2].

Generally, one third of all plastic waste ends up in soil or fresh water. Most of the plastic disintegrates into particles smaller than five millimetres, known as microplastics and they breakdown further into nanoparticles (less than 0.1 micrometer in size). These plastic products serve as a conduit for the release and travel of toxins into and through fresh water and food chains. Plastic, mistaken as food is ingested at all levels of

food web and travels through the food web via a process known as bioaccumulation [3].

Since synthetic plastic not only cause harm to environment also they deplete the natural resources like petroleum, coal and natural gas. Thus environmental, economic and safety challenges have provoked many scientists to surrogate petrochemical-based polymers with biodegradable one's which are known as bioplastics [4]. Bioplastics are a type of plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, pea starch or microbiota. Thus bioplastic decrease the dependence on petroleum and reduces the amount of waste material, while still yielding a product that provides similar benefits of traditional plastics [5].

Bioplastics are made from a wide range of renewable bio-based feed stocks. In the first generation, plastics are produced from agriculture biomass, mainly from sugar, starch, plant oil. They are rich in carbohydrate and can be consumed by humans and animals. Although, they have an efficiency to produce bioplastics, because of its potential direct competition with food and animal feed,

politicians and scientists have in the last ten years introduced the idea of using lignocellulosic feedstock as a raw material for bioplastic production [6].

The second generation feedstocks are lignocelluloses which means short-rotation crops such as poplar, willow or miscanthus, or else lignocellulosic agricultural by-products like straw. But the "Food Vs Fuel" polemic continued for non-food crops if grown on land ordained for food production. The use of agricultural waste or residues would not constitute a direct conflict with food unless they are residues from the first generation feed stock. Straw could eventually be considered as animal feed and part of the food chain [7].

Thus the crops used for deriving bioplastics leads to competition with food supplies as well as consumption of large land areas, water and nutrients making this kind of bioplastic production not suitable for long term [8]. In recent times, more and more research is being conceded out into using algae as a feedstock; this is known as a third-generation feedstock. Algae serve as an excellent feed stock for plastic production. Algae are highly effective biomass generators. They are also easy to grow and they are great CO<sub>2</sub> processing machines - they take this gas out of the atmosphere and the sea and turn carbon molecules into starch through photosynthesis, releasing oxygen molecules into the atmosphere as a by-product [9]. They have a higher yield or efficiency than first and second generation feed stock. They don't need fertilizers, pesticides, herbicides or land [10].

Microalgae are microscopic algae, which generally grows in water based environments. Microalgae can be present both separately and in chains or groups. The number of algae species is not known. Different sources vary in the estimated number of species from 30,000 to 1 million. However, closer to 44,000 species of algae are described [11]. One family of species is *Scenedesmus*, which belongs to the class Chlorophyceae [12], which are green algae. The *Scenedesmus* species have shown to have a better ability to capture carbon dioxide than what other examined species have [13]. The *Scenedesmus sp* contain large amount of starch providing the raw materials for bioplastics production [14].

The variation in the nutrient of microalgae can change its metabolism [15]. The possibility of increasing starch content by limitation of a macro element (either nitrogen, phosphorus or sulphur) was investigated as a viable and environmental-friendly option to the control of cell cycle. During essential macro element limitation, biomass synthesis was reduced compared to control

cultures, while starch content increased significantly during the first 12 h of growth. The advantages of algal biopolymers more than conventional plastics are numerous. The realistic side of the use of biopolymer is the profitable advantage for industries and municipal works. The versatility of the algae derived materials means that it has the potential to generate many different types of bio plastics by altering the proportions of polymer, plasticizer and additive in mixture.

Plastic water bottles are a curse to our ecosystem. We utilize them once or twice, and then throw them out, the massive majority of them to wind up in landfills or floating around the oceans. Algae used to create a substitute to plastic water bottles. The algae water bottles maintain its shape whilst full of water, but will start to decompose when empty. Liquids stored in the bottle entirely safe to drink. In relation to the production of packaging, conventional plastics are being replaced by bio plastics at a rapid pace. Bio plastic packaging options include bags for compost, agriculture foils, horticulture products, nursery products, toys and textiles. They are also often used for disposable cups, salad bowls, plates and food containers. The algae bio plastic packaging is sealed with heat rather than glue. The development of sophisticated bio plastic packaging for food products has greatly improved the shelf life of the food.

Starch is a biodegradable & renewable compound which also happens to be the most common and easily obtained natural polymer [16]. Starch based bioplastics with biodegradable plasticizers can be degradable in various environments such as soil, composting and water [17]. Starch-based bioplastics are broadly employed in the medical trade because of their biocompatibility, low toxicity, degradation properties and mechanical properties. In the pharmaceutical industry, starch from the *Scenedesmus sp* algae have the ability to absorb humidity has led to it being widely used for the production of drug capsules. Intended as a replacement for single use or disposable plastics, algae packaging is designed to biodegrade in around two to three months, depending on the thickness of the material and the temperature of the soil. Thus starch-based plastics from algae have emerged very rapidly into the marketplace.

As a result bioplastic from *Scenedesmus sp* SAR1 can help to alleviate the energy crisis as well as reduce the dependence on fossil fuels of our society and also reduces the amount of waste material, while still yielding a product that provides similar benefits of traditional plastics.

## 2. MATERIAL AND METHODS

### 2.1. Sample collection and storage

Water samples from north-eastern estuary of Tamil Nadu, i.e., Muttukadu, Chennai (12°48'36.144"N 80°14'53.9376"E) were collected in sterile containers. The collected samples were aseptically transferred to the laboratory at Nadar Saraswathi College of Arts and Science, Theni and stored at 4°C for further analyses.

### 2.2. Isolation of *Scenedesmus* sp from the water samples

The collected samples were enriched initially in bold basal medium [18] at 24±3°C with 16:8 h photoperiod until algal growth was detected and cultured on bold basal medium [19]. After incubation individual colony of microalgae was picked and transferred to the liquid bold basal medium. The axenic culture was shaken manually for five to six times per day. The purity of the culture was monitored regularly under light microscope.

### 2.3. Morphological and molecular identification

The isolated microalgae was identified and characterized morphologically by studying the morphological features under light microscope at 40X magnification. Axenic culture of *Scenedesmus* sp was maintained in bold basal medium under controlled condition.

The morphologically confined microalgae were subjected to molecular analysis using 16S rRNA gene sequencing quality succession method. PCR products were purified with QIA quick spin columns (Qiagen, Inc., Chatsworth, CA). Nucleotide sequence analysis was carried out in ABI3130 genetic analyzer combined with Big Dye terminator version 3% cycle sequencing kit. The sequence was analyzed at BLAST in NCBI website. The sequences which have maximum identity were selected and aligned on clustalW multiple alignment software. The strains' phylogenetic relationship was assessed by the maximum likelihood method with 1000 replicons. The revolutionary distance was computed using the kimure-2-parameter method. Evolutionary analyses were conducted in MEGA 7.

### 2.4. Determination of optical density(OD)

Optical densities of microalgae cultures were measured at a regular interval of time (24Hrs) by taking absorbance at 680nm ( $A_{680}$ ) with the help of a colorimeter. Every 24 hours, O.D. readings were measured using calorimeter. The concentration of algal biomass was determined by using the formula [20].

Total concentration  $C_b = 13.19 * 10^{-2}$  OD 680nm

Where,  $C_b$  = Concentration of biomass

### 2.5. Determination of biomass dry cell weight

Microalgae dry cell weight was determined gravimetrically [21]. Algal culture was centrifuged at 5,000 rpm for 10 minutes and harvested biomass was dried and weight was recorded using a digital weighing balance.

### 2.6. Determination of starch content

#### 2.6.1. Removal of interfering substances in algal cells [22]

By using perchloric acid method, the algal cells were extracted using 80% ethanol for 15 min at 68°C in order to eliminate the interfering substances (pigments). For whole hydrolysis of starch, 30% of perchloric acid was added to the sediment, stirred for 15 min at 25°C and centrifuged at 2000 rpm for 10 min. The solubilised starch solution was reacted with a mixture of concentrated sulfuric acid and anthrone (2 g anthrone in 1 L of 72% (v/v)  $H_2SO_4$ ) to quantify glucose colorimetrically at 625 nm.

#### 2.6.2. Anthrone method [23]

A 2.5 ml of anthrone solution (2 g anthrone in 1 L of 72% (v/v)  $H_2SO_4$ ) was added to the 0.5 ml of the solubilised starch solution. The mixture was kept in a water bath at 100°C for 8 min. Thereafter, the tubes were cooled rapidly and the absorbance was measured using spectrophotometer at 625 nm. Calibration was carried out simultaneously using glucose as the standard. The values measured for glucose were multiplied by 0.9(monomer unit of starch) to obtain a calibration curve for starch determination.

### 2.7. Treatments for high level production of starch

For high level production of starch in *Scenedesmus* sp. SAR1, the original bold basal medium was removed from the cell suspension by centrifugation, and the cells were resuspended in fresh bold basal medium as control and in one of the following modified bold basal medium [22]. For phosphorus depleted medium,  $KH_2PO_4$  from the original bold basal medium was replaced by 130 mg/L KCl, to keep the concentration of potassium ions the same as in the bold basal medium. For nitrogen depleted medium, urea was omitted from the original bold basal medium. For sulphur depleted medium,  $MgSO_4 \cdot 7H_2O$

from the original bold basal medium was replaced by 168 mg/L  $MgCl_2 \cdot 6H_2O$  to keep the concentration of magnesium ions the same as in the original bold basal medium. For glucose incorporated medium, algal cells were cultivated in the bold basal medium containing 2% of glucose.

### 2.8. Mass cultivation of *Scenedesmus* sp.

Modified bold basal medium (BBM) was prepared and purified form of *Scenedesmus* sp was inoculated and the flask placed for mass cultivation in a growth chamber with light flux of 16:8 hours. Every two days, cell turbidity was measured; Biomass and the amount of starch measured at the end of the period.

### 2.9. Extraction of starch [24]

In the extraction of starch granules, the wet packed cells were suspended in 6 volumes of water, and then subjected to sonic treatment in a 10 kc oscillator for 10 min. The sonicate was centrifuged at 2000 rpm for 5 min. After centrifugation, starch granules and cell debris did not precipitate but remained in the supernatant. The precipitate which contained intact cells was subjected to further sonic treatment, followed by centrifugation. This procedure was repeated three times more. The supernatant solutions were combined and centrifuged at 2000 rpm for 10 min. Starch granules were isolated from the precipitate by toluene treatment. Finally, starch granules were washed with water and 90 % aqueous ethanol, and dried at room temperature for overnight.

### 2.10. Analysis of starch by FTIR

Starch extract of *Scenedesmus* sp SAR1 was analysed in IR spectrum (using electromagnetic radiation) to predict the presence of starch based on its molecular structure. By using an infrared spectroscopy absorption standard chart, the results from the FTIR analysis could be interpreted and important bands could be noted. Band assignments are taken from references [25-27].

### 2.11. Purity of extracted starch

A 10 mg of the obtained starch was assayed by anthrone method to establish the purity of the sample. The purity was calculated as follows [28]:

$$\% \text{ Purity} = \text{Measured starch} / \text{Assayed starch} * 100$$

### 2.12. Film casting

Extracted material and Carboxymethyl Cellulose was mixed in the ratio of 1:2 in presence of 8 ml of distilled water. The mixture was kept at gradient of 90°C to

180°C for about 15mins in the stirrer and molded using manually casting method [29] at 23° C with 50% relative humidity.

### 2.13. Moulded into applicable products

By using the extracted starch, various applicable products were moulded.

### 2.14. Shelf life testing

Shelf life testing determined the durability level of starch bioplastic as plastic packaging. Bioplastics were placed in a plastic spar with limited oxygen. By doing so, the shelf life of starch bioplastic from *Scenedesmus* sp SAR1 could be identified. The testing process was conducted for 30 days. The results were analysed through visual observation [30].

## 3. RESULT AND DISCUSSION

Using 16:8 light flux method, the microalgal strain was isolated and purified after several plating and microscopic observations by using standard microbial techniques. The morphological identification of isolated strain on the basis of its cell shape, size, indicate that it may belongs to Chlorophyta (*Scenedesmus* sp) as described [31]. The isolated microalgae showed the pale green to dark green colour in liquid medium, unicellular, appeared to form colonies (ovoid or fusiform)(Plate 1) and non-motile.

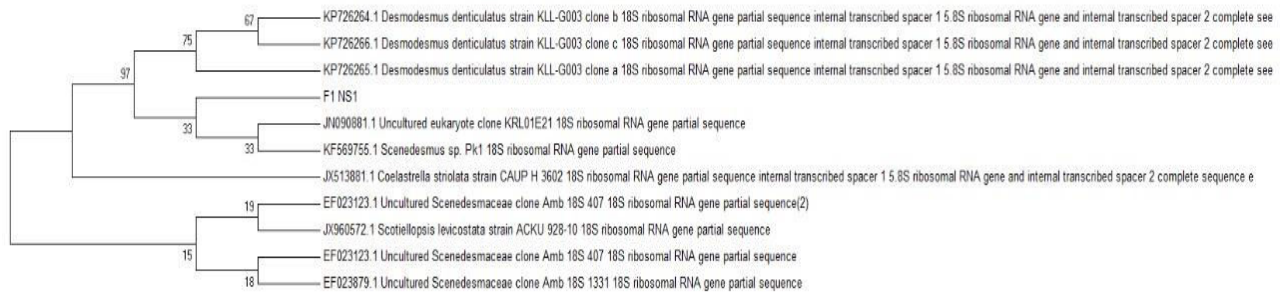


**Plate 1: Microscopic observation of *Scenedesmus* sp SAR1**

It was also observed that this algal strain normally divide by multiple fission forming more than two daughter cells connected in a coenobia. The colony analysis of microalgaerevealed the presence of maximum number of unicells followed by two to three cells arranged in a row in their different developmental stages. In the present study, cells of *Scenedesmus* sp never exceed eight celled

coenobia which is in agreement with Hoek *et al.* [32]. According to the 16SrRNA, partial gene sequence comparisons with GenBank database, the microalgae was identified as *Scenedesmus* SAR1. Nilshammer & Walles,

Trainor and Ho *et al.* [33-35] have also reported the organism with these similar characterization identified as *Scenedesmus* sp. (Fig.1).



**Fig. 1: Molecular characterization of *Scenedesmus* sp.**

Algal cells were grown in light and synthesized the starch in chloroplasts, in the form of starch bodies. Then the starch was used to drive many cellular processes and events, particularly for the highest consumers of energy and carbon such as DNA replication, nuclear division and cytokinesis. Therefore to attain a maximum yield of starch, processes that utilize starch as energy and carbon source must be stopped or minimized, while conditions enabling production of starch should be optimized. The possibility of increasing starch content by limitation of a macro element (nitrogen, phosphorus or sulphur) and incorporation of glucose was investigated as a viable and environmental-friendly option to the control of cell cycle. Similar investigations were also reported by Branyikova *et al.* [22] in *Chlorella vulgaris*.

The study revealed that the accumulated starch of *Scenedesmus* sp SAR1 ranged in 14.8% in nitrogen starved bold basal medium which was higher than the complete bold basal medium. Due to the nitrogen starvation, the macromolecular syntheses of RNA, protein and DNA were arrested in *Scenedesmus* sp SAR1. This diverted the photosynthetic carbon flow into starch without it being consumed as a carbon and energy source. So the starch content was increased in the nitrogen starved medium than complete medium. Ballin *et al.* [36] have also reported that the high level accumulation of starch occurred in the *Scenedesmus* sp when it was grown in the nitrogen starved bold basal medium.

From this study, it was demonstrated that the accumulated starch of *Scenedesmus* sp SAR1 ranged in 14.4% in phosphorus starved bold basal medium which was higher than the complete bold basal medium. Due

to the phosphorus starvation, In *Scenedesmus* sp SAR1 high level accumulation of starch indicated that the rate of photosynthesis was nearly normal during the entire cycle in which RNA synthesis stopped. So the starch content was increased in the phosphorus starved medium than complete medium. Zachleder *et al.* [37] have also reported that the high level accumulation of starch occurred in the *Scenedesmus quadricauda* when it was grown in the phosphorus starved bold basal medium.

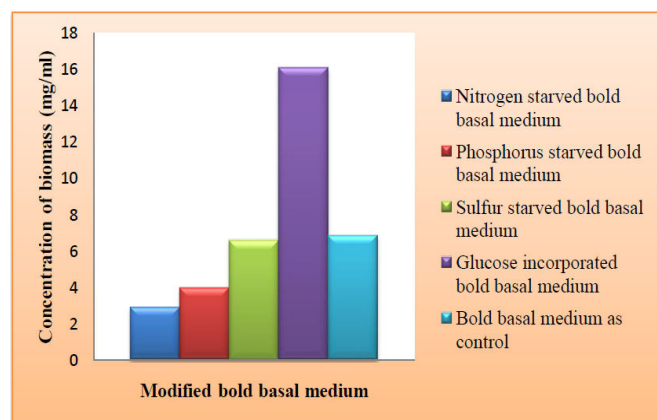
In this study, it was verified that the accumulated starch of *Scenedesmus* sp SAR1 ranged in 14.6% in sulfur starved bold basal medium which was higher than the complete bold basal medium. In *Scenedesmus* sp SAR1 inhibition of RNA synthesis and protein synthesis occurred when it was incubated under photosynthesizing conditions in a sulphur-free medium. Only photosynthesis continued to produce starch at a similar rate to that of normally grown cells. Thus a very large amount of starch accumulated in the sulphur starved bold basal medium than complete bold basal medium. Setlik *et al.*[38] have also reported that the high level accumulation of starch occurred in the *Scenedesmus quadricauda* when it was grown in the sulfur limited bold basal medium.

From this investigation, it was established that the accumulated starch of *Scenedesmus* sp SAR1 ranged in 15.5% in glucose incorporated bold basal medium which was higher than the complete bold basal medium. Due to the incorporation of glucose in bold basal medium, the synthesized starch in *Scenedesmus* sp SAR1 was maintained in chloroplasts in the form of starch bodies. Only the incorporated glucose was used for the

chief cellular processes (DNA replication, nuclear division and cytokinesis). Therefore maximum yield of starch was attained by a *Scenedesmus* sp SAR1 when it was grown in glucose incorporated bold basal medium. Kobayashi *et al.*[24] have also proved that the high level of starch production occurred in the *Scenedesmus* sp when it was grown in the glucose incorporated bold basal medium.

The isolated sample was mass cultivated in bold basal medium (BBM) and incubated in a growth chamber with light flux of 16:8 hours.

The purity of culture was ensured by frequent inoculation in the medium and the cellular morphology was observed under the light microscope. The growth of *Scenedesmus* sp SAR1 was determined colorimetrically at 680nm with regular interval of every two days. After twenty days of incubation *Scenedesmus* sp SAR1 attained a growth range 2.902 mg/ml in nitrogen starved bold basal medium, 3.957 mg/ml in phosphorus starved bold basal medium, 6.595 mg/ml in sulphur starved bold basal medium, 16.092 mg/ml in glucose incorporated bold basal medium and 6.859 mg/ml in bold basal medium as control. Among these investigations, glucose incorporated bold basal medium shows the highest growth concentration of *Scenedesmus* sp SAR1 (Fig.2). Kobayashi *et al.*[24] have also reported that the high growth concentration of *Scenedesmus* sp was attained when it was grown in the glucose incorporated Bold basal medium.



**Fig. 2: Analysis of dry biomass (mg/ml) of the *Scenedesmus* sp SAR1 after twenty days of incubation in various types of modified bold basal medium (BBM)**

For the analysis of starch content, the algal cells were extracted using 80% ethanol for 15 min at 68°C in order to eliminate the interfering substances (pigments).

The removal of interfering substances is exceedingly important because they are able to respond colorimetrically, thus leading to the overestimation of starch values. Then the algal cells were treated with perchloric acid to obtain the solubilised starch solution. Then the starch solution was reacted with a mixture of concentrated sulphuric acid and anthrone to quantify glucose colorimetrically at 625nm. By using the standard graph, the glucose content in the sample was measured. From this glucose value, the starch content was arrived by multiplying with the factor 0.9 (0.9-monomer unit of starch) (Table 1). The starch content of *Scenedesmus* sp SAR1 shows 147.6 µg/ml in the nitrogen starved bold basal medium, 144 µg/ml in the phosphorus starved bold basal medium, 145.8 µg/ml in the sulphur starved bold basal medium, 154.8 µg/ml in the glucose incorporated bold basal medium and 106.2 µg/ml in the bold basal medium as control.

**Table 1: Estimation of starch from glucose concentration in various bold basal medium**

Test Samples	Concentration of glucose (µg/ml)	Concentration of starch (µg/ml)
Starch from nitrogen starved Bold basal medium	164	147.6
Starch from phosphorus starved Bold basal medium	160	144
Starch from sulphur starved Bold basal medium	162	145.8
Starch from glucose starved Bold basal medium	172	154.8
Starch from Bold basal medium as control	118	106.2

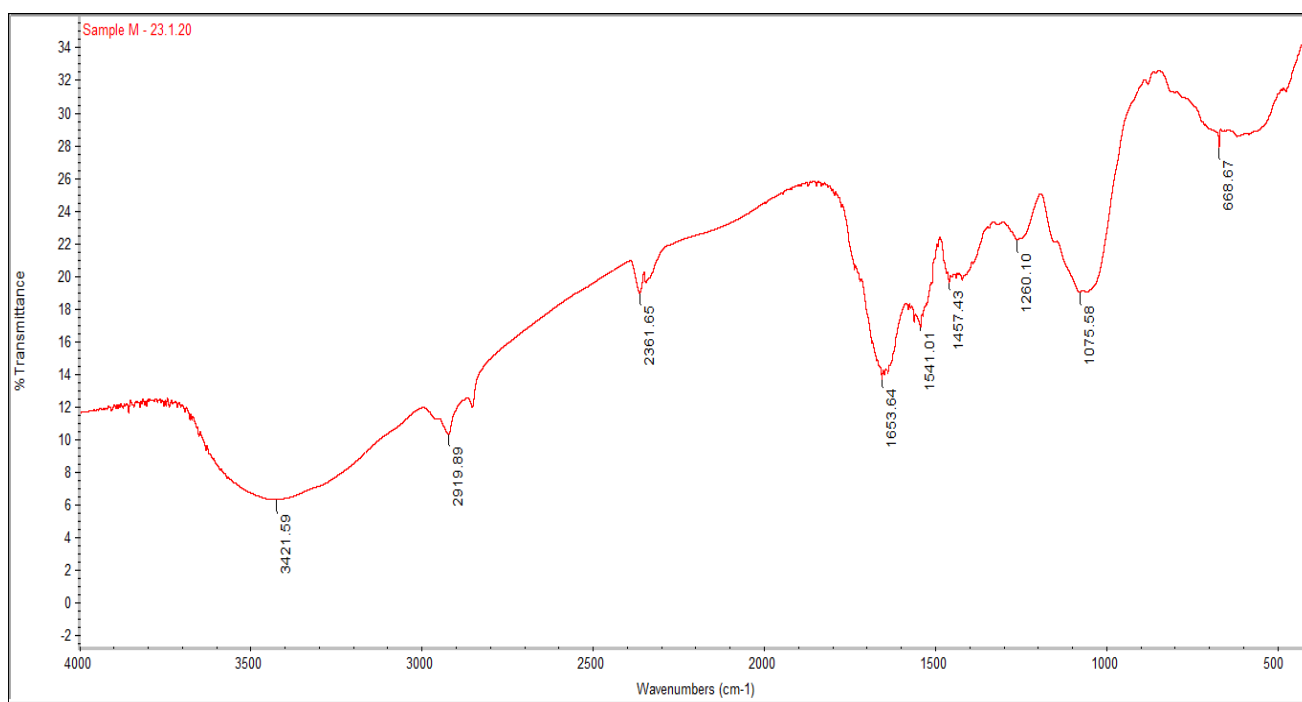
Among these investigations, glucose incorporated bold basal medium shows high level accumulation of starch content. Kobayashi *et al.*[24] have also proved that the high level of starch production occurred in the *Scenedesmus* sp when it was grown in the glucose incorporated bold basal medium.

In the extraction of starch granules, the wet packed cells were suspended in 6 volumes of water and then subjected to sonic treatment in a 10kc oscillator for 10 minutes to break the algal cell wall. The sonicate was centrifuged at 2000rpm for 5 minutes. After

centrifugation, starch granules and cell debris did not precipitate but remained in the supernatant. After repeating this procedure for the precipitate, the supernatant solutions were combined and centrifuged at 2000rpm for 10 minutes. The obtained pellet was subjected to toluene treatment. In the toluene treatment high density starch granules settled as pellet and low density cell debris remains in the upper toluene layer. For further purification, starch granules were washed with 90% ethanol and dried at room temperature for overnight. The extracted starch was

measured.

The FTIR spectrum aids in the identification of molecular components and their structures. The shape of the spectra collected for starch extracted from *Scenedesmus* sp SAR1 showed different peak intensities at particular wave numbers (Fig.3). By using an infrared spectroscopy absorption standard chart, the results from the FTIR analysis could be interpreted and important bands could be noted. Band assignments are taken from references [25-27].



**Fig. 3: FTIR spectrum of the extracted starch from the *Scenedesmus* sp SAR1**

Most important wave numbers were summarized in table 2. The band at  $3421.59\text{ cm}^{-1}$  represents an OH bond.  $2919\text{ cm}^{-1}$ ,  $2361.65\text{ cm}^{-1}$  and  $1457.43\text{ cm}^{-1}$  indicates C-H bond. Wave number  $1653\text{ cm}^{-1}$  shows a C=O bond. Band vibration  $1541.01\text{ cm}^{-1}$  predicted the C=C bond. The band at  $1260.10\text{ cm}^{-1}$  represents C-O bond. Wave number  $1075.58\text{ cm}^{-1}$  shows a C-O-C bond (Table 2).

Similar observations were also reported by Soest and Vliegthast (1997) for the FTIR spectra of starch. According to Soest and Vliegthast [39], the FTIR spectra of starch typically shows bands at  $2900\text{-}3000\text{ cm}^{-1}$  (C-H bond),  $1100\text{-}1150\text{ cm}^{-1}$  (C-O and C-C) and  $1100\text{-}900\text{ cm}^{-1}$  (C-O-C bond). Thus the confirmation of extracted starch can be studied successfully by infrared spectroscopy.

**Table 2: FTIR analysis of extracted starch from *Scenedesmus* sp SAR1**

Wave number ( $\text{cm}^{-1}$ )	Typical band assignment
3421.59	<b>O-H</b>
2919.89	<b>C-H</b>
2361.65	<b>C-H</b>
1653.64	<b>C=O</b>
1541.01	<b>C=C</b>
1457.43	<b>C-H</b>
1260.10	<b>C-O</b>
1075.58	<b>C-O-H</b>

A 10 mg of the obtained starch was assayed by anthrone method to establish the purity of the sample. The purity of the starch was calculated on the basis of the amount

of glucose liberated by means of the hydrolysis of starch and glucose was confirmed as the only product for hydrolysis of the starch. The extracted starch shows 98.6% purity. According to Kobayashi *et al.*[24], the purity of extracted starch from *Scenedesmus* sp G30 shows 99%.

Plastic film was cast using extracted material. Extracted starch was weighed to approximately 100 mg. 50 mg of CMC was used to create a 1:2 ratio between CMC and the extracted material. The co-component of choice was Carboxymethyl Cellulose (CMC) which was used to create a plastic material with better mechanical properties than commonly used plasticizers such as Sorbitol or Glycerol. Both the compounds were mixed with 10 ml of deionized water. The hydrolysis of starch was achieved by heating the mixture at 100°C for 15 minutes with continuous stirring. The created solution was poured into plastic petri dishes which were used as moulds for casting. They were stored to dry in a room

with a relative humidity of 50% and a temperature of 23°C. Finally, the created film was removed from the petri dish. The created plastic film was slightly more flexible and less brittle. To create a material with a wider application area, more or different components should be added. Had a larger amount of algae been available, maybe a less brittle and a thicker material with better mechanical properties could have been cast (Plate 2). Similar observations were also reported by Saga and Isabelle [40] for the casting of plastic film by using the extracted starch from *Scenedesmus* sp.

Starch bioplastic from *Scenedesmus* sp SAR1 maintained its property upto 30 days of incubation in a plastic box with limited oxygen. It shows the shelf life range upto 30 days (Plate 3). So starch bioplastic from *Scenedesmus* sp SAR1 considered as an effective packaging material. Similar observations were also demonstrated by Nanang and Heru [30] for the degradation of bioplastic made from cassava starch.



**Plate 2: Moulded starch into applicable products**



**Before 30 days**

**After 30 days**

**Plate 3: Shelf life testing**

#### 4. CONCLUSION

Algae bioplastics can play a vital role as an environment friendly, biodegradable alternative compared to conventional plastics. Even though bioplastics are expensive they are still considered as a viable option to

improve environmental sustainability. Bioplastics from *Scenedesmus* sp SAR1 have gained utmost importance in the recent times because of their advantages over other biological sources which have already mentioned above. Starch extracted from *Scenedesmus* sp SAR1 majorly used for the bioplastic production. Exploring the production of bioplastics could play a major role in shaping the economics and viability of algal based products. The technology routes for the production of algal based bioplastics are still under research and the use of biotechnological and genetic engineering techniques play a key role in conducting the feasibility and sustainability studies in algal based bioplastics. It is hoped that significant advances made in the bioplastics industry in general will benefit algal based bioplastics industry as well and will make algal based bioplastics a reality in the distant future.



**Conflict of interest**

The authors of this article do not have any conflict of interest in publishing this research article.

**5. REFERENCE**

- Arathi S, Amit B. Algae based bioplastics : Future of green economy, 2021; From the edited volume biorefineries-vision and development-Prof. Krzysztof Biernat.
- Merino V, Ayer D. Plastic pollution primer & Action tool kit, 2018; Produced by earth day network for the end plastic pollution campaign.
- Wilcox C, Erik VS, Britta DH. *Proc Natl Acad Sci USA*, 2015; **112 (38)**:1189-11904.
- Reddy LR, Sanjeevani RV, Anusha G. *Int. J. emerging technology and advanced engineering*, 2013.
- Stevens ES. Green Plastics: An introduction to the new science of biodegradable plastics; 2002. Princeton University Press, Princeton.
- Carus M, Dammer L. *Industrial Biotechnology*, 2013; **9 (4)**.
- Ashworth K, Wild O, Hewitt CN. Impacts of biofuel cultivation on mortality and crop yields. 2013. In: Nature climate change online, **3(5)**.
- Rastogi RP, Madamwar D, Pandey A. *Algal green chemistry*, Elsevier, 2017; 121-138.
- Zhang C, Ho SH, Chen WH. *Trends Biotechnol*, 2020; **38(9)**
- Rajendran N, Sharanya P, Sneha RM, Angeeleena B, Rajam C. *Journal of pharmacy research*, 2012; **5 (3)**: 1476-1479.
- Michael DG. *Journal of Phycology*, 2012; **48**: 1057-1063.
- Fleming FR. *Review of palaeobotany and palynology*, 1989; **59 (1)**:1-6.
- Cervantes TA, Morales M, Novelo E, Revah S. *Bioresource Technology*, 2013; **130**:652-658.
- Matsumoto M, Yokouchi H, Suzuki N, Ohata H, Matsunaga T. *Appl Biochem Biotechnol*, 2003; **105-108**: 247-254.
- Juneja A, Ceballos RM, Murthy GS. *Energies*, 2013; **6 (9)**: 4607-38.
- Abolibda TZ. Physical and chemical investigations of starch based bioplastics; 2015. University of Leicester, Leicester.
- Bastioli C, Magistrali P, Garcia SG. Starch, In biobased plastics : Materials & applications, 2014; Novara, John Wiley & Sons Ltd. 9-28.
- Bischoff HW, Bold HC. Phycological studies IV. Some soil algae from enhanced rock and related algal species. 1963. University of Texas, Austin. **6318**:1-95.
- Andersen RA. Algal culturing techniques, 2005; Elsevier, Amsterdam: 578.
- Chisti Y. *Biotechnol Adv.*, 2007; **25**: 294-306.
- Rai LC, Mallick N, Singh JB, Kumar HD. *J Plant Physiol*, 1991. **138**:68-74.
- Branyikova I, Barbora M, Jiri D, Tomas B, Katerina B, Vilem Z, Milada V. *Biotechnol.Bioeng*, 2011; **108**: 766-776.
- Hodge JE, Hofreiter BT. In: Methods in carbohydrate chemistry (eds Whistler, R.L. and Be Miller, J.N.), 1962; Academic press New York. 17-22.
- Kobayashi T, Ikunosuke T, Akira Obayashi. *Agr.Biol.Chem* , 1974; **38 (5)**:941-946.
- Giordano M, Kansiz M, Heraud P, Beardall J, Wood B, McNaughton D. *J. Phycol* , 2001; **37**:271-279.
- Movasaghi Z, Rehman S, Rehmen IU. *Appl. Spectrosc.Rev.*, 2008; **43**:134-179.
- Murdock JH, Wetzel DL. *Appl. Spectrosc.Rev*, 2009; **44**:335-361.
- Gifunia I, Giuseppe O, Irene RK, Gerardino D, Antonino P, Antonio M. *Chemical Engineering Transactions*, 2017; **57**:1423-1428.
- Vilpoux O, Averous L. Technology, use and potentialities of Latin American starchy tubers, 2004; Sao paulo, NGO Raizes and Cargill Foundation. 521-553.
- Nanang EW, Heru S. *Journal of Mechanical Engineering Science and Technology*.2017. **1(1)**:41-54.
- Hegewald E, Hanagata N. *Algal Stud*, 2000; **100**: 29-49.
- Hoek VC, Mann DG, Jahns HM. Algae: An introduction to phycology, Cambridge University Press, United Kingdom, 1995; 623.
- Nilshammar M, Wallis B. 1974. Electron microscope studies on cell differentiation in synchronized cultures of the green alga *Scenedesmus.Protoplasma*.**79**:317-332.
- Trainor FR. Biological aspects of *Scenedesmus* (Chlorophyceae)-phenotypic plasticity. *Nova Hedwigia Beih*, 1998; **117**:1-367.
- Ho SH, Chen WM, Chang JS. *Bioresour Technol*, 2010; **101**: 8725-8730.
- Ballin G, Doucha J, Zachleder V, Setlik I. *Biol Plant*, 1988; **30**:81-91.
- Zachleder V, Ballin G, Doucha J, Setlik I. *Biol Plant*, 1988; **30**:92-99.
- Setlik I, Ballin G, Doucha J, Zachleder V. *Biol Plant*, 1988; **30**:161-169.
- Soest VJ, Vliegthart JF. *Trends in biotechnology*, 1997. **15(6)**:208-213.
- Saga GJ, Isabelle HS. An analysis of creating plastic material based on the microalgae *Scenedesmus*, 2019; Degree project in technology, First cycle, 15 Credits, Stockholm, Sweden.