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Research Article

EFFECT OF CULTURE MEDIA ON THE POLYHYDROXYBUTYRATE PRODUCTION OF *SPIRULINA* SP.

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ABSTRACT

In reaction to the environmental issues caused by plastics of petrochemical beginning, a decrease in utilization of these materials and their substitution by biodegradable polymers has been sought. Polyhydroxybutyrate (PHB), a biopolymer of biological beginning that has a place to the polyhydroxyalkanoates (PHAs), is comparable to polypropylene in terms of its mechanical properties, thermo degradability and dissolving temperature. Different microorganisms, including cyanobacteria, can synthesize this biopolymer. The objective of this study was to stimulate biopolymer amalgamation by *Spirulina* sp., *Spirulina* was developed under distinctive carbon sources. At first, the growth was conducted with *Spirulina* sp., and at that point neem cake and groundnut cake was utilized as a carbon source. In different nutrient mediums, *Spirulina* appeared the most extreme concentration of biopolymers. The PHB were evaluated at 235nm by utilizing UV spectrophotometer. The pure frame of PHB powder was collected.

Keywords: Biopolymers, Polyhydroxyalkanoates, Microalgae, *Spirulina*, UV spectrophotometer.

1. INTRODUCTION

In the environment, non-biodegradable plastic squander aggregation is evaluated between 4.8 and 12.7 million tons per year, which makes major issues to the marine environments [1]. This can be due to utilization of petrochemicals (non-renewable) as key sources for plastic production right now [2]. Consequently, there's a look for degradable sources of plastics or alternatives to non-biodegradable plastics, to decrease the collection of plastic within the environment. Polyhydroxyalkanoates (PHAs) are biopolymers, their thermal degradation characteristics, melting temperature and mechanical properties are comparable to polypropylene and can act as an alternative to plastics of petrochemical beginning [3]. Polyhydroxybutyrate (PHB) is the foremost studied representative of the PHA group. PHB is delivered by numerous microorganisms from acetyl-CoA through an arrangement of enzymatic reactions utilizing three enzymes. β-Ketothiolase (phaA) is the first key enzyme that changes over acetyl-CoA in to acetoacetyl-CoA molecule; the second enzyme acetoacetyl-CoA reductase (phaB) reduces the acetoacetyl-CoA to 3-hydroxybutyrl CoA and the final enzyme PHB polymerase (phaC) catalyses the polymerization of 3-hydroxybutyrl CoA to PHB molecule. In order to keep the PHB polymerase covalently attached, PHB polymers tend to create amphipathic granules within the cell. Among the microbes utilized for PHA extraction, algae have picked up significant interest as they are sunlight driven cell factories that change over CO_2 into different important bio-products with the O_2 evolution. Moreover, the route of bioplastics production from algal source can be through the polymers from direct biomass or from its auxiliary metabolites [4]. PHB also presents characteristics such as biodegradability, thermo versatility, processibility, hydrophobicity and biocompatibility with cells and tissues, which recommends alluring applications in the food, pharmaceutical and therapeutic ranges [5]. When broken down by microorganisms, PHAs form water and CO_2, which can be reintegrated into nature and close the carbon cycle. These polyesters are delivered by different prokaryotic microorganisms, such as cyanobacteria [6]. The look for options for the production of biodegradable plastics includes the search for modern forms and materials while making utilize of biotechnology, through the utilization of microorganisms and their metabolic items. The knowledge of these microorganisms is exceptionally vital in the change of

certain substances into others and in the conceivable utilize of substrates in getting reasonable products and by-products.

Algae were the first plants to seem on the planet. Billions of years ago, they changed the carbon-dioxide-based environment to an oxygen-rich climate in which other life shapes might advance. Biotechno-logical forms based on cyanobacteria have been getting expanding interest due to their potential to deliver a different run of chemicals and naturally dynamic compounds, such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides [7]. *Spirulina* are multi-cellular and filamentous blue-green algae that has gained significant popularity in the wellbeing food industry and progressively as a protein and vitamin supplement to aquaculture diets. It develops in water, can be collected and handled effortlessly and has exceptionally high large scale- and micro-nutrient substance [8].

The substrate transformation productivity is vital and depends on the physiology and biochemistry included within the synthesis of biopolymers. Among the different nutrients in the medium, the carbon source contributes more essentially to the overall fetching of the production process and to the incitement of the biopolymer synthesis. Carbon source are too vital in PHB union and ought to be restricted in such a way that the metabolic pathway of the microalgae is directed to the generation of biopolymers and not other bio-products. In this way, the objective of this study was to invigorate the generation of biopolymers by *Spirulina* sp. by changing the nutritional conditions.

2. MATERIAL AND METHODS

2.1. Isolation and identification of *Spirulina*

The cyanobacteria utilized in this study were *Spirulina* strain. Zarrouk media was utilized for developing *Spirulina* [9]. Each media of volume 200ml was prepared in two flasks. The pH of each carafe was measured; it was between pH 9-10. Each day each flask was mixed after each 3-4hrs. Conventional pH was checked as often as possible each day. It was recognized utilizing light microscope. *Spirulina* are coiled and helical in shape. After 15 days all tests are sifted by utilizing 300 micron filter paper and the moist weight was measured.

2.2. Culture collection and maintenance

In the present work improvement of species *Spirulina* (filamentous) were utilized to develop on the characterized medium. The culture was kept up in Zarrouk medium in a 1000ml Erlenmeyer carafe inside the standard room temperature, with 12 hours light and 12 hours dim photo period with standard white light and the flask were circulated discuss through artificially. The improvement of the culture was checked as per the laboratory conditions [10] for a period of 15 days and the generation time was calculated [11].

2.3. Influence of different carbon sources

In the Zarrouk medium the Nitrogen, phosphorus and potassium constituents were supplemented with Neem cake, groundnut cake [12] baking soda at 16.8g/litre, sea salt at 1g/litre were included as carbon source and the antacid pH of 10-11 was kept up at 28°C-36°C in open sunlight and PHB extraction and estimation from each medium were carried out.

2.4. Screening for the Generation of PHB Utilizing Sudan Black Staining Technique

The PHB production by the organism can be certified by recoloring with Sudan B dark method [13]. The culture was centrifuged at 6000 rpm for 30 minutes. The pellet was suspended in 5ml of Zarrouk medium. The cell walls are at that point annihilated utilizing ultra-sonic treatment for 5 minutes. A thin spread of it was arranged on glass slide, examined dried and was submerged in Sudan B dark for 5-15 minutes. The slides was discuss dried and the PHB granules were observed under light microscope.

2.5. Extraction of PHB

The 14 days kept up culture was centrifuged at 6000 rpm for 30 minutes. The pellet was dried for 24 hrs, [14] at that point the pellet were suspended in sterile water and homogenized. The cell divider was smashed by utilizing ultrasonic treatment for 5 minutes and 2ml of cell suspension was included in 2 ml of NaCl and warmed to boiling temperature for 30 minutes in water bath. At that point the tubes were centrifuged at 6000 rpm for 20 minutes and 5ml of chloroform was added. The tubes were cleared out overnight. The substance of the test tube were centrifuged at 6000rpm for 20 minutes extracted with 0.1 ml of chloroform and were dried at 40°C.The dried PHB granules were measured.

2.6. Estimation of PHB

Extracted PHB powder was mixed with in 10ml of concentrated sulfuric acid and mixed altogether. The blend was warmed in water shower at 100°C for 10 minutes, the concentrated sulfuric acid changed over into crotonic acid at that point the tubes were allowed to cool [15]. The optical thickness was measured at 235nm

by utilizing spectrophotometer and sum of crotonic acid was calculated.

3. RESULTS AND DISCUSSION

Spirulina is claimed as a non-toxic, nutritious food, in expansion, since it is so high in minerals, protein and fundamental fatty acids it may be a healthy vitality food that's particularly valuable for individuals on low-calorie diets [16]. A culture medium as great as the synthetic medium has been detailed within the writing for the growth of *Spirulina* was obtained, and improved with Neem cake, groundnut cake has been utilized for *Spirulina* cultivation. The cultivation was carried out *in-vitro* for a period 15 days. The growth was observed cultivating in the laboratory conditions [10]. The PHB was focused in this study due to different advantages such as eco-friendly behavior, non-toxic, biodegradable. There are different reports that clearly depict the generation of bio-plastics from the renewable substrates.

3.1. Isolation and distinguishing proof of *Spirulina*:

Spirulina were isolated from freshwater regions and the morphological highlights observed through tiny considers almost, *Spirulina* are coiled and helical in shape and the culture was kept up in Zarrouk medium. The green growth was watched to create a clear mat on the surface.

3.2. Culture collection and maintenance

A culture medium as incredible as the fabricated medium has been detailed within the literature for the improvement of *Spirulina* was obtained; the cultivation was carried out *in-vitro* for a period 15 days. The development was checked amid development inside the laboratory condition.

3.3. Influence of different carbon sources

Growth occurred inside 15 days of incubation, which clearly appears that carbon sources activates the growth as well because it increments the production of PHB. Ordinarily the PHB production of the life form was actuated by α-ketothiolase chemical. The expansion of carbon sources to medium will screen the acetyl CoA action. Consequently the medium was optimized by utilizing groundnut and Neem cake.

3.4. Screening for the Generation of PHB Utilizing Sudan Black Staining Technique

The PHB granules were moreover recognized by their liking for the dye Sudan Black, which may be a hypothetical test for the nearness of PHB. The recolored arrangements were inspected beneath a Light microscope with an oil submersion lens for deciding cellular PHB accumulation. The PHB granules were watched in black color.

3.5. Extraction of PHB

The fig. 1 shows that PHB powder was extricated from Zarrouk medium with groundnut and neem cake. Within the handle the cultures were centrifuged and cell walls were smashed by utilizing Ultrasonic treatment and NaCl was included and recentrifuged. Afterwards prepared PHB was removed with chloroform and allowed to vanish, at last the PHB powder was obtained.

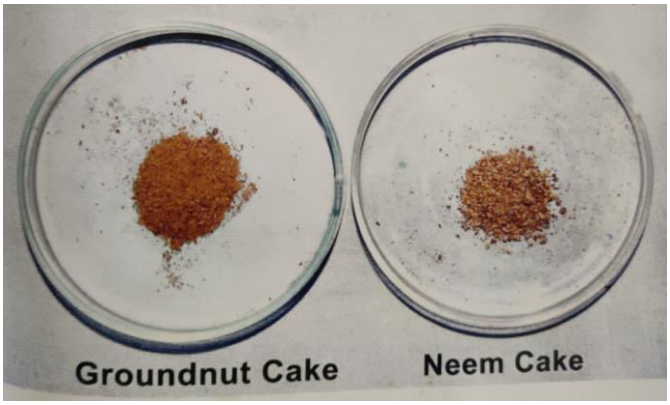

Fig. 1: PHB powder produced from *Spirulina*sp.

3.6. Estimation of PHB

Spectrophotometric investigation method is especially im portant for calculating the concentration of PHB from the test. PHB react with the concentration of sulphuric acid in hot condition to create chrotonic acid. PHB powder was extracted from Zarrouk medium with groundnut cake was measured as 3.232 and neem cake was measured as 3.175 at 235 nm utilizing UV Spectrophotometer. In this study, *Spirulina* have been separated from fresh water sources. PHB is amassed under conditions of supplement sources. PHB is accumulated under conditions of nutrient sources. Two different nutrient sources were included for the production of PHB and each source appeared different. PHB collections was measured at 235 nm utilizing UV Spectrophotometer. The Fig. 2 shows the Comparative test of PHB utilizing different sources. The high PHB production was seen in *Spirulina* developed in Zarrouk medium with groundnut cake when compared with this a somewhat less production was seen in Neem cake.

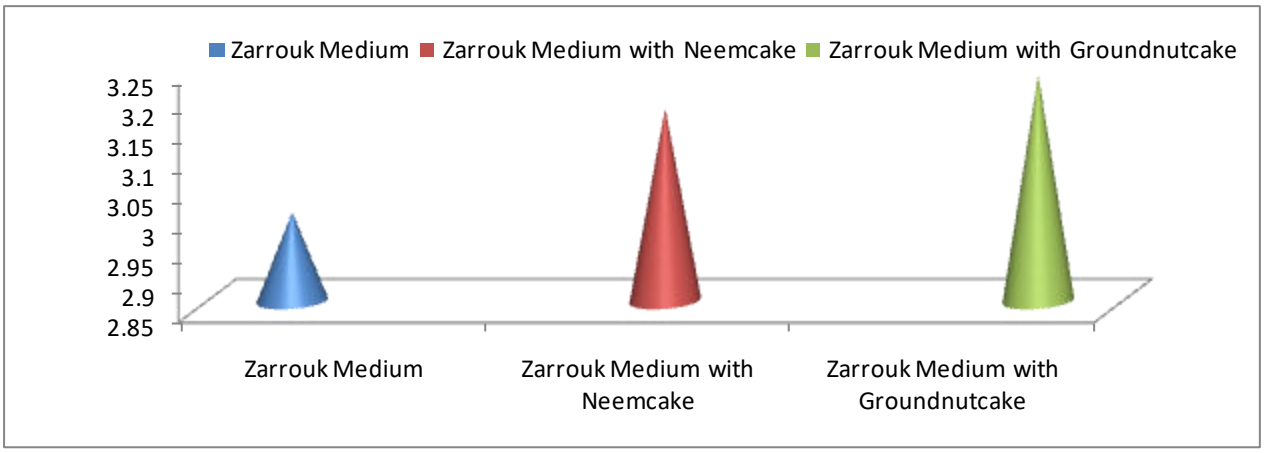

Fig. 2: Comparative Assay of PHB Using Various Carbon Sources

4. CONCLUSION

To conclude, natural conditions do play a critical part within the generation of PHB from *Spirulina*. In spite of the fact that it is isolated from freshwater sources, their PHB production shifts from one source to another. At display, PHB production from photosynthetic micro-organisms to deliver biodegradable plastics as an alternative to ordinary plastics is considered to be cost successful and eco-friendly compared to bacterial production of PHB. Further studies on these isolates for im proved PHB production will move forward the capacity to find unexploited mechanically imperative microalgae present within the environment. *Spirulina* do have the potential to create biopolymers like PHB from groundnut and Neem cake as the sole carbon source, and the yield of PHB may be expanded by different nutrient conditions etc. The technology courses for the production of green growth based bioplastics that are still under the research stage and are distant from commercialization. Algal based bioplastics can play a crucial role as an environment inviting and biodegradable elective com-pared to conventional plastics.

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