ISSN: 2710-0723

Research Progress on Attached Culture Technology of Microalgae

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Abstract

In order to solve the problem of microalgae biomass harvesting in suspension systems, attached culture systems were proposed. In this system, microalgae are fixed on the surface or inner layer of the carrier and grow in an aggregated form. Compared with suspension culture, attached culture has higher microalgae biomass density and easier dehydration and harvesting processes. The biomass can be harvested by simply separating the carrier containing microalgae from the culture medium.

Keywords

Microalgae; Attached Culture; Biomass.

1. Introduction

According to Lee et al. [1], it was found that the biomass yield of attached culture of microalgae was 2.8 times that of the suspension system. From the perspective of sewage treatment, attached culture is easier to harvest microalgae biomass from sewage, is less likely to pollute effluent water quality, and can withstand relatively high pollutant loads [2-4]. During the attachment culture process, the extracellular organic matter of the microalgae or the coated polymer material (sodium alginate) can protect the microalgae cells from external toxic pollutants or bacteria, and resist damage caused by high light intensity or high Effect of nutrient loading on microalgae growth [5].

2. Attached Culture Method

Microalgae attachment culture can usually be divided into immobilized culture and biofilm culture: the former is to fix microalgae cells in gel (such as chitosan or sodium alginate) for culture; the latter is to cultivate biofilm on the surface of solid materials. The form allows microalgae to attach and grow. In the immobilized culture system, the microbeads containing microalgae can be harvested by simply separating them from the culture medium, while contamination can be effectively avoided. However, the gel around the microalgae inhibits the transfer of nutrients and light transmission between the microalgae and the culture medium; resulting in an obvious lag phase in the immobilized culture of microalgae cells and low biomass yield. Although the application of polymer gel materials can improve the above problems, it will also produce a certain degree of toxicity to microalgae cells and affect the downstream applications of microalgae biomass.

In order to solve a series of problems caused by gel-encapsulated biomass in immobilized culture, the biofilm culture method was proposed. Although microalgae biofilm culture has

ISSN: 2710-0723

been proposed relatively recently, biofilms are not a new phenomenon. Biofilms date back to 3.5 billion years ago and are composed of protozoa, bacteria, larvae, and microalgae based on fossils. In aquatic environments, biofilms cover most of the lower solid layers such as ships, rocks, and marine animals. Generally, biofilms exist on almost any solid-liquid contact surface and are usually considered to be surface foulants. Their presence will lead to corrosion of metal surfaces and change the physical and chemical properties of the metal surface. At the same time, biofilms play a very important role in nature, and their existence helps promote the exchange of nutrients and the flow of energy. In recent years, research has found that biofilms can be used in wastewater treatment, bioconversion and fermentation engineering.

Microalgae biofilm is composed of microalgae cells, extracellular organic matter and bacteria. In recent years, many scholars have begun to pay attention to the field of microalgae biofilm culture. Compared with suspension culture, biofilm culture mainly has the following advantages:

1) Low water consumption: In a suspension culture system, 1 ton of microalgae biomass requires 200 tons of water. To produce the same biomass, the microalgae biofilm system only requires 17 tons of water, of which 4 tons of water are used to maintain the biofilm surface moist [3];

2) Low construction cost: Compared with the construction cost of racetrack pools and photobioreactors in suspension systems, the carrier materials that support cells in biofilm culture are relatively cheap, easy to obtain, and can be reused [6];

3) High biomass yield: The biomass yield of microalgae biofilm systems is usually higher than that of suspension systems. According to the study of Gross and Wen [7], the biofilm yield is 302% of that of suspension systems;

4) Easy to harvest: biomass can be harvested by simple scraping [8];

5) The moisture content of biomass is low and no additional dehydration is required. The moisture content of the harvested biomass is similar to that of centrifugal harvesting by suspension culture [9];

6) It has better light utilization efficiency and more uniform illumination [10].

3. Biofilm Formation

The formation of microalgae biofilm is a complex process, and the specific formation process has not been fully demonstrated. But it is certain that the types of microorganisms that make up the biofilm significantly affect the formation process of the biofilm. The current consensus is that the formation process of microalgae biofilm can be divided into two stages: in the first stage, the microalgae cells initially adhere to the surface of the carrier to form an initial film layer. This process is usually reversible; in the second stage, the cells Extracellular organic matter (EPS) secreted from the surface tightly connects cells and carriers, thereby forming an irreversible biofilm. In non-sterile systems, bacteria can also contribute to the initial adhesion of microalgae cells. The surface of bacteria will also secrete EPS, such as polysaccharides, proteins and phospholipids, which will help improve the surface properties of the carrier, allowing more and more microalgae cells to adhere and the biofilm to mature. In addition, EPS can also store necessary nutrients and water for the growth of microalgae, and can also prevent harmful substances in the water from damaging microalgae cells to a certain extent. The height of the mature biofilm gradually increases, forming a multi-layered three-dimensional structure. However, when the biofilm height reaches a certain level, shedding will also occur to varying degrees under the influence of external shear force, impact force and cell death. Biofilm growth is inhibited when the rate of biofilm loss exceeds the rate of growth.

ISSN: 2710-0723

4. Factors Affecting Biofilm Formation

The formation and growth of biofilm are affected by various external environmental factors and the interaction of various internal bacterial groups. In contrast to bacterial biofilms, not many studies have been conducted on the factors affecting microalgal biofilm formation. Currently, most research focuses on designing various biofilm culture devices for biofilm culture, biomass accumulation, and wastewater treatment. However, only by deeply understanding the various influencing factors of microalgae biofilm formation can we better design and develop biofilm culture devices. The specific influencing factors are summarized as follows:

(1) Algae species

The physical and chemical properties of the surface of different microalgae cells vary greatly. Some microalgae cells are easy to attach and grow on solid surfaces to form biofilms, while some microalgae cells are more suitable for suspension culture. For example, when comparing different algal species under sterile conditions, it was found that the initial attachment rate of Chlorella vulgaris was significantly lower than that of Scenedesmus obliquus. In addition, during the formation of biofilm, the stronger the hydrophobicity of the cell surface, the easier it is for biofilm to form on the surface of the carrier. The hydrophilicity and hydrophobicity of the microbial surface can be determined based on its cohesion free energy ($\Delta Gcoh$). When $\Delta Gcoh<0$, the microalgae cell surface is hydrophobic; when $\Delta Gcoh>0$, the microalgae cell surface is hydrophilic.

(2) Carrier material

The physical and chemical properties of the carrier material surface will also affect the formation and growth rate of microalgae biofilms, so understanding the properties of the carrier material is crucial to predicting the formation and growth of biofilms. Generally speaking, the choice of carrier material depends on its roughness, hydrophobicity, surface energy, hardness, biotoxicity, price, porosity, etc. Materials with hydrophobic surfaces are ideal biofilm carrier materials and are easier to adhere to microalgae cells to form biofilms. In addition, according to the research of Barros et al. [11], the adhesion process of microalgae cells on the surface of the carrier material can also be predicted by the thermodynamic theory ($\Delta Gadh$). When $\Delta Gadh<0$, it is conducive to the adhesion of microalgae cells on the surface of the carrier material, and vice versa. The rougher the surface of the carrier material, the more conducive it is to trap biomass. Currently, commonly used methods to increase the surface roughness of materials include: sandpaper grinding, woven mesh bottom layer and laser processing. Although the surface roughness of the material after sandpaper polishing increases significantly, the surface roughness is usually unevenly distributed. Although laser processing can control the surface roughness distribution, it often increases economic costs and is difficult to apply on a large scale.

(3) Culture medium components

Medium composition is a key factor affecting microalgae cell growth and biofilm formation. The growth of microalgae cells is the process of consuming nutrients in the culture medium. The culture medium should contain nutrients necessary for cell growth, such as carbon, nitrogen, and phosphorus. The proportions and concentrations of different nutrients in the culture medium will affect the secretion of EPS by microalgae cells, which in turn affects the adhesion process of microalgae cells to the carrier surface. Schnurr et al. studied the lipid accumulation ability of Scenedesmus obliquus and Nitzschia palea in a biofilm culture system under nitrogen starvation conditions. In contrast to suspension culture, the lipid content did not increase under biofilm culture. However, as the nitrogen concentration in the culture medium increases, the EPS of green algae and diatoms increases, which is beneficial to the adhesion between cells and between cells and carrier materials. At the same time, EPS can also serve as a nutrient reserve to provide nutrients to microalgae cells under nitrogen starvation conditions. Currently, many

ISSN: 2710-0723

researchers use wastewater as a culture medium for the growth of microalgae. Because wastewater contains a large amount of organic and inorganic substances suitable for the growth of microalgae cells, it is an ideal substitute for commercial culture media for microalgae, and the use of wastewater can reduce cultivation costs.

(4) Temperature

Temperature plays a key role in the growth process of microalgae biofilms. By adjusting temperature, the macromolecular structure, metabolic activity, photosynthesis, and energy absorption and consumption of microalgae can be affected. When the temperature rises to a certain level, it will promote the metabolism of microalgae cells, thereby contributing to the increase in microalgae biofilm thickness and biomass productivity. Depending on the species of algae, the optimal cultivation temperature also varies. Generally speaking, for most algae species, 20-25°C is the most suitable temperature for their growth. However, in the natural environment, temperatures vary greatly in different regions, seasons, and time periods. Winter or spring temperatures in low-temperature areas (especially high latitudes) are usually below 0°C; summer temperatures in high-temperature areas are usually above 30°C (http://tianqi.ttcha.net/); there are also obvious temperature differences between day and night. In order for microalgae cells to grow better, an external temperature control device is usually required, which undoubtedly increases the investment and operating costs during the cultivation process. Therefore, it is of important practical and theoretical significance to explore how extreme temperatures or large temperature differences affect microalgae cell biomass accumulation and wastewater treatment under natural conditions.

(5) CO2

Both photoautotrophy and mixed culture require CO2 or inorganic carbon as a carbon source for growth metabolism. Taking CO2 as an example, suspension culture of microalgae requires about 5%-7% volume concentration of CO2. When CO2 consumption is greater than supply, microalgae cell growth will be inhibited. In suspension culture, CO2 must overcome the air-liquid interface before it can enter the liquid medium and be utilized by microalgae cells. However, CO2 dissolves slowly and has poor light permeability in liquid culture media, so the photosynthesis of suspended microalgae cells is severely inhibited. In contrast, in the biofilm culture model, the microalgae biofilm is in direct contact with CO2-rich gas, helping to increase CO2 and light utilization. For example, in a rotating microalgae biofilm system, the biofilm is in direct contact with the air, reducing the loss of CO2 during mass transfer from gas phase dissolution to liquid phase. In this biofilm system, even at lower CO2 concentration levels Under this condition, algae cells can still absorb enough CO2 from the gas phase. The results of this study indicate that cultivating microalgae in a rotating microalgae biofilm system may not require special CO2 enrichment at the air inlet. However, heterotrophic microalgae can obtain carbon sources from wastewater containing organic carbon.

5. Conclusion

Microalgae biofilm culture was proposed in the past 10 years, and current research on culture devices is mostly at the laboratory scale. This method has shown great application potential in the fields of biomass accumulation and sewage treatment. However, there are many factors that affect biomass productivity during the culture process, and each device has its advantages and disadvantages in optimizing biomass productivity and commercial-scale application. At present, there is no recognized optimal microalgae biofilm device system that can effectively remove pollutants in wastewater and efficiently accumulate microalgae biomass in an efficient, economical and easy-to-scale manner. Therefore, in the process of cultivating microalgae biofilm, according to the components of each pollutant in the wastewater and the differences of algae species, the operating parameters should be reasonably optimized, the culture device

ISSN: 2710-0723

should be designed, and the adhesion and film-forming mechanism of microalgae biofilm should be revealed. The long-term and stable operation of the biofilm system is of great significance.

Acknowledgments

Funding: Shaanxi Key Laboratory of Land Consolidation (Chang'an University) Open Fund (Project No.: 300102353506);

The project of Shaanxi Province Land Engineering Construction Group(Project No.:DJTD2023-02).

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