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Studying the Extracellular Polymeric Substances of *Galdieria sulphuraria* as Flocculation Aid for Improving Algal Harvesting Efficiency

Introduction

Some of the most promising sources of biodiesel come from microalgae. Unfortunately, because of their small sizes and low biomass densities, harvesting microalgal biomass in an economical way has proven to be a significant challenge. Filtration and centrifugation consume a considerable amount of energy and can be costly, whereas flocculation uses less energy and is a low-cost process. While this is true, it is also known that alum- and ferric-based inorganic flocculants and synthetic organic flocculants can be toxic to aquatic organisms [1]. However, naturally occurring or microorganism-derived flocculants seem to be a sustainable alternative [1] as they are non-toxic and offer high flocculation efficiency [2]. Extracellular polymeric substances (EPS) is a biopolymer secreted by algae. EPS contains many biomolecules with a wide

range of potential applications. One specific application lies in the flocculation process for algal biomass harvesting. In this study, EPS was extracted from the microalga *Galdieria sulphuraria* and used to conduct flocculation experiments to assess the feasibility of using EPS to improve algal harvesting efficiency.

The strain of the microalga *G. sulphuraria* was cultivated in an enviromentally controlled incubator. The strain was grown under 24 hours of continuous illumination inside of incubators at 42ºC. The carbon levels inside of the incubators were maintained at around 3%. The cultures that were obtained were streaked onto agar plates. The plates then formed single colonies, which were used to create axenic cultures. The cultures were streaked under the Labconco purifier to avoid any contamination. After media preparation, the media was sterilized through an autoclave at 121ºC. All of these conditions were met in order to grow *G. sulphuraria* without any contamination.

The EPS of *G. sulphuraria* was extracted through centrifugation. After the EPS was centrifuged at 4000 rpm for 40 minutes, the supernatant was kept and stored inside a fridge for 90 days. Before conducting any flocculation experiments, the EPS was taken out of the fridge to get to room temperature. It is important to note that each flocculation experiment requires a large amount of culture, which can take over a month to grow for one experiment. In this case, nearly two months were spent growing and maintaining the cultures. Because of the limited amount of large-scale reactors in the lab, only three flocculation experiments were conducted.

Methods

The optical density (OD) value was taken at 750 nm from each sample after a tenfold dilution for every experiment. The OD values were used to calculate the biomass density. Each sample was taken from the center of each reactor as not to disturb the algae that had settled at the bottom. The OD values would decrease as time passed as there would be fewer algae present in the center than in the bottom of the reactor. A lower OD value would indicate that more algae have settled to the bottom of the reactor.

Experiments

For the first flocculation experiment, 50-mL reactors were used, and three different conditions were tested using five replicates for each condition. For Test A, 1 mL/L of EPS was added. For Test B, 2 mL/L of EPS was added. The control samples had no EPS added. The initial OD value of the culture was measured to be 2.25 OD, which roughly translates to 1.125 g/L of algal biomass. After adding the respective amounts of EPS to the reactors of Test A and Test B, the solutions were thoroughly mixed and set to the side. The OD values of all 15 reactors were then taken after 10 minutes, 30 minutes, 1 hour, and 2 hours. A tenfold dilution was made to each sample before measuring its OD value. An interesting observation was made shortly after mixing the EPS into the samples. Floating on the surfaces of the reactors of Tests A and B were unidentified molecules (Fig. 1). This observation was not further examined and maybe an area of interest to future study. Essentially, this experiment was conducted to determine if changing the amount of EPS would affect the harvestability. As can be seen from the results, the EPS had clearly worked best in the first 10 minutes as the OD values for Tests A and B had lowered over 70% compared to the control lowering around 30% from the initial OD value (Fig. 2). Alternatively, after 30 minutes, all 15 reactors seemed to have nearly the same OD values. This shows that the EPS drastically increased the harvesting efficiency for at least the first 10 minutes.

Figure 1. Molecules in Tests A and B

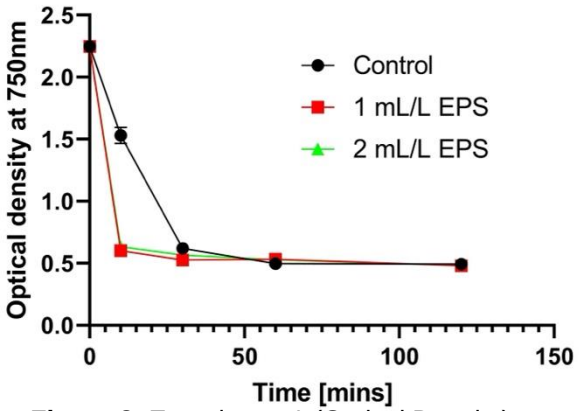

Figure 2. Experiment 1 (Optical Density)

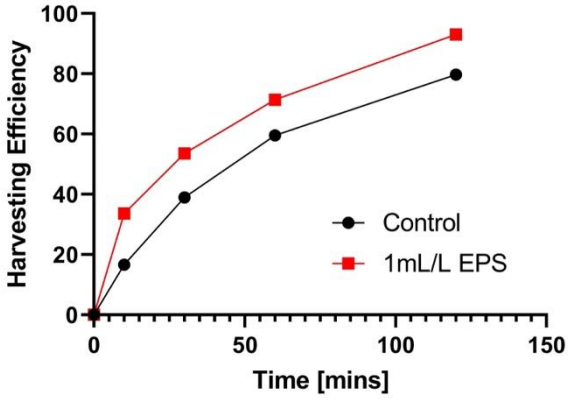

Figure 3. Experiment 2 (Harvesting Efficiency)

For the second flocculation experiment, 1-L column reactors were used, and two different conditions were tested using four replicates. For Test C, 1 mL/L of EPS was added for every 700 mL of working volume, and the control samples had no EPS added. The OD value of the initial culture was 3.30 OD, which means that there was approximately 1.65 g/L of algal biomass. The OD values of all 8 reactors were taken after 10 minutes, 30 minutes, 1 hour, and 2 hours. It was found that the reactors with the added EPS settled faster than the control samples at all times. This indicates that the reactors with EPS had a higher harvesting efficiency (Fig. 3).

For the third flocculation experiment, two conditions were set as the OD values were predetermined to be 0.6 OD (0.3 g/L of algal biomass) and 1.2 OD (0.6 g/L of algal biomass). In order to concentrate the cultures, they were centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded, and the centrifuged algal biomass was kept to make two concentrated cultures. For this experiment, 50-mL reactors were used for two different conditions. Test D used the culture with the value of 0.6 OD, and Test E used the culture with the value of 1.2 OD. Each test had 4 reactors as their control and 4 reactors with 2.5 mL/L of EPS added, so there were a total of 16 reactors used in this experiment. The OD values of all 16 reactors were taken after 10

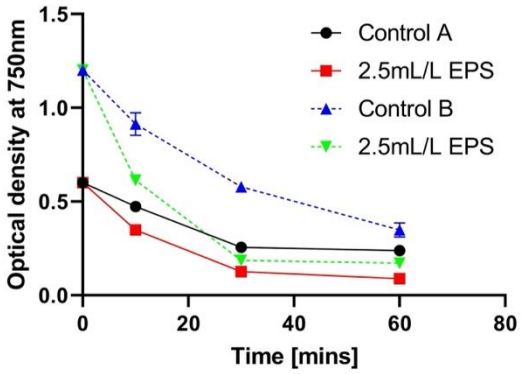

Figure 4. Experiment 3 (Optical Density)

minutes, 30 minutes, and 1 hour. This experiment was conducted to see if a higher amount of EPS (compared to 1 mL/L of EPS in Experiment 1) mixed with varying concentrations of cultures would affect the harvestability. Throughout the experiment, the reactors with the EPS had a higher harvesting efficiency compared to their controls.

Conclusions

Due to the limited time and inavailability of large reactors, only a limited number of experiments were able to be conducted. The addition of EPS seemed to improve short-term harvesting efficiency up to 2 hours. Moreover, the harvesting efficiency was observed to be higher within the first 30 minutes. Even so, more detailed experimentations are needed to make a conclusive argument that the addition of EPS improves algal harvesting efficiency.

References

[1] Yang, L.; Zhang, H.; Cheng, S.; Zhang, W.; Zhang, X. Enhanced microalgal harvesting using microalgae-derived extracellular polymeric substance as flocculation aid. ACS Sustainable Chem. Eng. 2020, 8, 4069−4075.

[2] Rashid, N.; Park, W.; Selvaratnam, T. Binary culture of microalgae as an integrated approach for enhanced biomass and metabolites productivity, wastewater treatment, and bioflocculation. Chemosphere 194 (2018) 67-75.