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Molasses wastewater treatment using microalgae *Bumileriopsis peterseniana* and its hydrogen production

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Abstract

Molasses are used as a substrate in an ethanol production process. During the process, the sewage will be generated leading to oxygen depletion in wastewater. Microalgae offers a promising organism used for wastewater treatments because they provide potentially valuable biomass, which can be widely used in various aspects of application. This research has studied microalgae *Bumileriopsis peterseniana* isolated from molasses wastewater habitats. Even *B. peterseniana* could not grow well in molasses-wastewater modified medium (MWM) as much as in TAP medium; however, the cells of *B. peterseniana* was successfully used in wastewater treatment. After incubation of *B. peterseniana* in molasses-wastewater for 4 days, the chemical oxygen demand (COD) and biological oxygen demand (BOD) values were significantly decreased by 47.83 % and 80.65 %, respectively. Moreover, cells showed the ability in decolorization of molasses-wastewater representing 9.37 %. Besides, *B. peterseniana* was grown in TAP medium under the light intensity of 1500 lux for 1 day. Interestingly, the ability of *B. peterseniana* has been revealed in a way that it could produce hydrogen with a rate of 8.33 µmol/mgDW.

Introduction

Molasses is an important substrate of ethanol production process[1]; however, molasses wastewater contains a high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), as well as a low pH with strong dark brown color[2]. These effects cause a bad smell, in addition, oxygen cannot be dissolved and light to penetrate into the water. In Mae Klong River located in the middle of Thailand, 25 Giant freshwater stingrays were dead because of molasses wastewater contamination[2]. The wastewater treatment can be done in many processes such as chemical, aeration, bioremediation. According to wastewater is an ideal media for a wide range of microorganisms, especially bacteria, viruses and protozoa course, there are a lot of nutrients and organic matters. This research is focused on wastewater treatment using microalgae. Previous studies have reported that numerous algal species, *Anabaena*[4], *Chlamydomonas*[5], *Chlorella*[6], *Nostoc*[3], were used to reduce nitrogen, phosphorus and organic matters from wastewater effect to change BOD and COD values.

Unfortunately, our previous results showed that *Chlorella sp*. and *Tetraspora sp*. CU2551 were able to treat molasses wastewater. However, a new opportunity was initiated for wastewater treatment. Observation from wastewater pond revealed the challenge in new algal strain isolation. This study aims to isolate a new microalgal strain suitable for wastewater treatment in Ethanol Production Plant in Ratchaburi province

Methodology

Microalgae isolation

Wastewater in Ethanol Production Plant in Ratchaburi province was sampled from seven different locations: non-aerated wastewater pond#1, non-aerated wastewater pond#2, aerated pond, effluent pond, CaO-added pond, green-film covered wastewater pond, and green soil. Samplings were made from the top of the water at each location for green algae screening which are able to treat molasses wastewater.

The samples were filtered to remove any solid contaminants and debris. A 100 μL of filtrate were then transferred onto the TAP-agar (Tris-Acetate-Phosphate medium) medium, followed by incubation at 36 ºC with the light intensity of 1,500 lux for 7 days. After green colonies were observed, a number of colonies were picked and restreaked until the pure culture was obtained.

Microscopic analysis and Strains Identification

The general morphological observations of the strains were carried out at seven days of culture in TAP medium. The micrographs were obtained using an Eclipse Ti-U inverted light microscope (Nikon®, Japan). Besides, cell culture was sent to Thailand Institute of Scientific and Technological Research (TISTR) for strain identification.

Growth determination

Bumileriopsis peterseniana were cultured in 50 mL of TAP medium at 36 ºC with the light intensity of 1,500 lux kept on a rotatory shaker at 160 rpm. The one-day culture was used as a starting culture for growth determination. Cells were grown under various concentrations of Molasses wastewater ranging from 0-20% (v/v) with a starting OD_{730} of 0.02. The growth curve was measured by the monitoring culture density at 730 nm using a spectrophotometer.

Wastewater treatment

Molasses wastewaters were collected from Ethanol Production Plant in Ratchaburi province. Pulled wastewaters were filtered using multi-layer gauze to remove large particles. This multi-layer gauze was further used in BOD removal, COD removal and decolorization study.

BOD measurement[7]

BOD method was used to define microorganisms ability in oxidizing organic material to CO_2 and water, using molecular oxygen as an oxidizing agent under the dark at the temperature of 20ºC. Dissolved oxygen of day 1 and day 5 were measured in order to calculating BOD.

COD measurement[7]

COD method was used to define the quantity of oxygen required to oxidize the organic matter in wastewater sample, under specific conditions of an oxidizing agent, temperature, and time**.** In the present study, COD was analyzed by using Closed Reflux Titrimetric technique.

Decolorization[8]

The experiments were conducted in Erlenmeyer flasks containing algal cultures and molasses wastewater at different initial concentrations (1, 5, 10, 15, 20%). The cultures were incubated at 36°C and monitored the color by measuring the absorbance of 475 nm every 24 hours.

Measurement of hydrogen production

Cells were cultured in 50 mL of TAP medium. The initial cell concentration was adjusted to an OD_{730} of 0.2. The cultures then were grown aerobically under continuous illumination of 1,500 lux on a rotatory shaker at 160 rpm at 30 ^{o}C. After incubating for 24, the cells were harvested by centrifugation at 8,000 rpm for 15 minutes at room temperature. The cell pellet was washed twice in the desired medium and resuspended in 2 mL medium in a glass vial. Then the vial was sealed with a rubber septum and bubbled with argon gas for 15 minutes in order to generate an anaerobic condition. After 24 hours incubation on a rotatory shaker at 100 rpm for 24 hours at 30 ^{o}C, the head-space gas was subjected to hydrogen gas measurement. The gas was analyzed using a gas chromatography with argon as a carrier gas as previously described[9]. The linear calibration curve with various H_2 concentrations (0, 0.05%, 0.1%, 0.25%, 0.5%, and 1.0% H_2) was done. The hydrogen production rate was expressed per dry weight.

Results and Discussion

Microalgae isolation

The sampling sites in this study were ponds of water treatment in Ethanol Production Plant in Ratchaburi province. Isolated microalgae pointed out a variety of species living in both wastewater pond and green soil. The algal cells were successfully isolated from 2 sampling sites: 1) aerated pond and 2) green film on soil. These two microalgal strains were later identified by TISTR as *Bumileriopsis peterseniana* (Figure 1a.) which was found in the aerated pond and *Chlorella sp*. (Figure 1b.) which was found in green soil. In this study, *B. peterseniana* were selected for this study since the cells of *Chlorella* sp. could be further cultured in our growth condition.

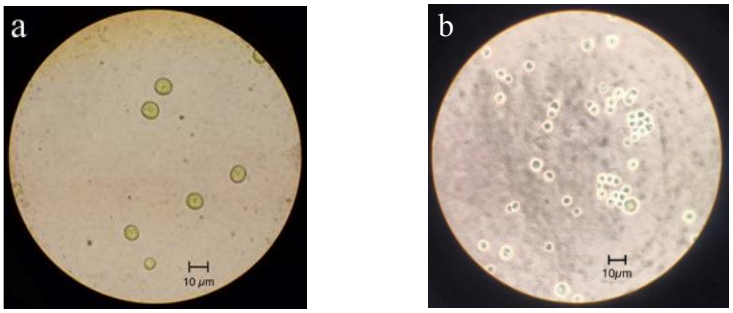

Figure 1. Cell morphology of cells of *Bumileriopsis peterseniana* (a) and *Chlorella sp*. (b) observed under Microscope.

Growth determination

The growth of *B. peterseniana* was examined under various molasses wastewater concentrations ranging from 0 - 20% (v/v). Cells were cultivated at 36 ^{o}C with the light intensity of 1,500 lux, kept on a rotatory shaker at 160 rpm for 4 days. The culture was measured an OD_{730} for the growth every 12 hours. In TAP medium, *B. peterseniana* could grow with the highest rate in 0% wastewater. However, the growth rate was dropped when the wastewater concentration was increased (Figure 3.). This result suggested that cells might use chemical compositions in molasses wastewater and TAP medium as sources for the cell to grow. Unfortunately, the dark color of molasses wastewater interfere light intensity for using in the photosynthesis[10]. In addition, the cells were tested to grow in various molasses wastewater diluted with distillated water (instead of TAP medium). The result of a small growth indicated that *B. peterseniana* could not utilize only carbon sources in molasses wastewater (data not shown).

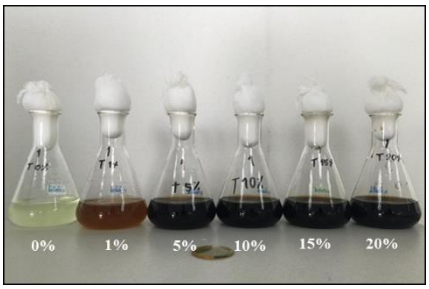

Figure 2. The color of various concentration of molasses wastewater with *B. peterseniana*

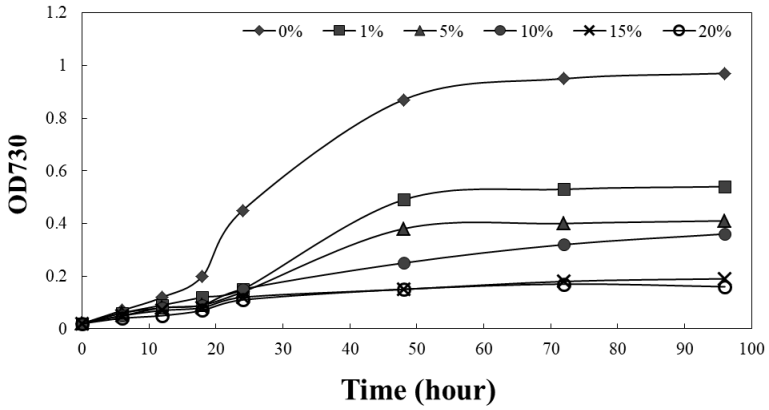

Figure 3. The growth of *B. peterseniana* under various of Molasses wastewater treatment dilution with TAP medium (MWM).

Wastewater treatment

Even *B. peterseniana* showed an inability to grow in wastewater, there are many reports showing that microalgae contained the ability in wastewater treatment such as *Anabaena sp.*[3], *Nostoc sp.*[3], *Chlorella sp.*[6] *and Scenedesmus sp.*[11]. These shreds of information, however, leading to explore the ability of *B. peterseniana* in molasses wastewater treatment. Three parameters were used to monitor the level of treatment: BOD, COD and decolorization.

Harvested *B. peterseniana* cells; grown in TAP medium for 30 hours, were incubated with 20% of molasses wastewater for 5 days. The value dissolved oxygen was measured for BOD and COD calculation. After treatment, the results showed the reduction of both COD and BOD by 52 % and 19 % remaining, respectively (Fig 4). Our finding supported that *B. peterseniana* has an efficient capacity in molasses wastewater treatment.

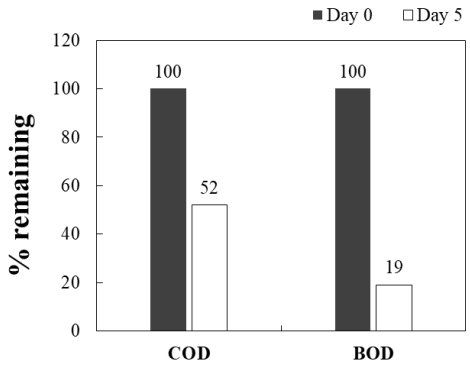

Figure 4. The percentage remaining of BOD and COD after incubation with *B. peterseniana* for 5 days

Moreover, the color of molasses was also determined at various concentrations. Harvested *B. peterseniana* cells; grown in TAP medium for 30 hours, were incubated with 1, 5, 10, 15, 20% of molasses wastewater at 36°C and monitored the color by measuring the absorbance of 475 nm every 24 hours. The results indicated that *B. peterseniana* could also reduce water color as shown in Fig 5. Interestingly, at 20% of molasses wastewater, the color of wastewater was dropped to 31%, leading to 69% color remaining. This should be noted that *B. peterseniana* has a higher color removal capacity than found in other species such as *Chlorella saccharophila*[12] and *Chlorella vulgaris*[12] (Table1.).

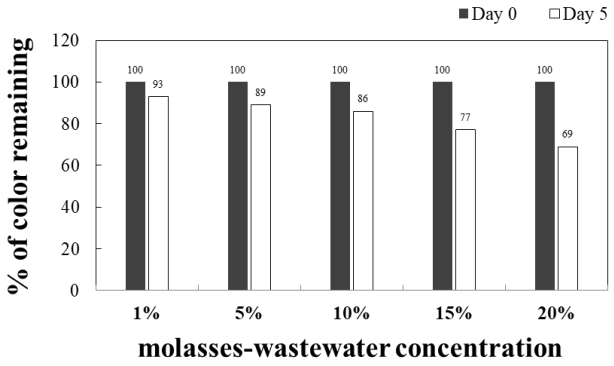

Figure 5. The color of Molasses wastewater remained after the incubation with *B. peterseniana* for 5 days

Table 1. The value of COD and decolorization of *B. peterseniana* comparison with *Chlorella saccharophila* and *Chlorella vulgaris*

	B. peterseniana	***Chlorella saccharophila***	***Chlorella vulgaris***
% COD reduction	48	9	17.3
% Decolorization	31	21.2	17.2

Measurement of hydrogen production

Not only wastewater treatment capacity, but hydrogen production capacity was also observed in *B. peterseniana*. Cells were incubated under the anaerobic condition on a rotatory shaker at 100 rpm for 24 hours at 30 ^{o}C, the head space of vial was collected and subjected to determine the hydrogen amount in every 6 hours interval. The result showed a fast and linear increase of H_2 production over the first 24 hours prior to saturation of H_2 production upward. The highest production at 24 hours was about 8.33 mmolH_2/mgDW (Figure 6). Many studies reported that most of the green algae contained the key enzyme, hydrogenase, which catalyzed

a formation of hydrogen gas from protons and electrons. Hydrogen gas could be used as a renewable energy source in the future.

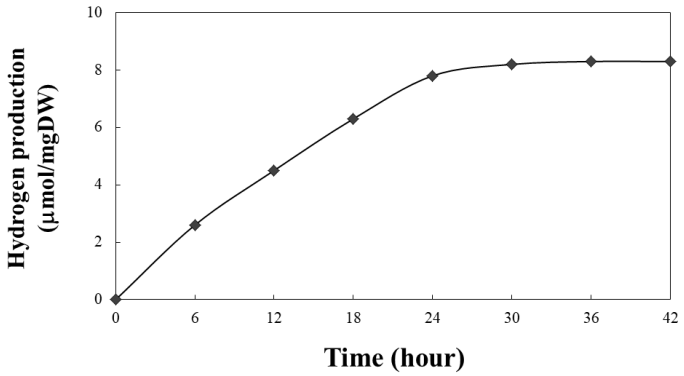

Figure 6. Hydrogen production by *Bumileriopsis peterseniana* under the anaerobic condition on a rotatory shaker at 100 rpm for 24 hours at 30 ºC

Conclusion

Bumileriopsis peterseniana was successfully isolated from wastewater source in Ethanol production Plant. Even *B. peterseniana* could not use wastewater as carbon sources but they showed the high potential in molasses-wastewater treatment indicating by the reduction of COD, BOD, and decoloring representing 48%, 81% and 31 %, respectively. Moreover, *B. peterseniana* also showed hydrogen production capacity with the impressive rate, 8.33 µmol/mgDW. Remarkably, our results suggested that *B. peterseniana* could be further used in a vast aspect from wastewater treatment for an alternative energy production.

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