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Cultivation of Microalgae using Sungai Sura Water Source as a Medium for Biodiesel Production

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Abstract

Objectives: It is to determine the suitability of cultivating green microalgae: Tetraspora sp. and Spirogyra sp. that are cultured in Sungai Sura of Dungun, Terengganu, Malaysia as a water source. **Methods:** The microalgae strains have been cultured in an indoor laboratory of Environmental Research Center, UiTM Dungun for 20 days in the low salinity water medium ranges of 120 to 150 µS/cm. The cultivation process significantly grown in 20 days. **Findings:** It is also shown the positive correlation between growth rate and cultivation days of Tetraspora sp. and Spirogyra sp. However, Tetraspora sp. gives higher densities than Spirogyra sp. when an assessment carried out in the similar days within 20 day cultivation periods. The maximum biomass concentration of Tetraspora sp. is 7.37 mg/ml and Spirogyra sp. is 3.16 mg/ml. **Applications/Improvements:** Tetraspora sp. generate more than double biomass densities than Spirogyra sp. on the similar days within 20 day cultivation periods. Both species were successfully grown indoors in the Sungai Sura water source, where salinity is lower as a cultivation medium.

Keywords: Biodiesel, Cultivation, Low Salinity, Microalgae, Tetraspora sp. and Spirogyra sp

1. Introduction

Transformation of natural resources as alternative bio crude-raw material especially in biofuel industry is much-awaited due to the growing world population, increasing the demand for fossil fuel and commodities. Therefore, sustainable resource in production method for energy and food is required to support for the huge population in the future of years[1]. The research for sustainable and environmental friendly sources of energy has become important in recent years. As a result, a substitute biofuel which can be produced from biomass sources such as microalgae that is also known as third generation biofuel from plants, aquatic plant and organic waste have lead in reducing the world's dependence on fossil fuel. It also could recycle of carbon dioxide gas via photosynthesis process which aid in reduce carbon footprint production[2]. Optional reductions in atmospheric carbon dioxide levels will require significant decreases in consumption of fossil-carbon energy sources. It can help towards effect on carbon emission which is actively that could be recycled between atmospheric and terrestrial pools[3].

They are found in abundance with more than 30000 species and their growth rates are fast as well. The major technical challenges for algae based biofuel include identifying the proper strains with the highest oil content with higher growth rates. Developing cost-effective growing and harvesting methods is the starting point of success for algae based biofuel[4]. There are three primary components for algae to grow such as sunlight, carbon-dioxide and water. Photosynthesis is an important bio-chemical processing which plants, algae and some bacteria

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convert the energy of sunlight to chemical energy[5]. Different types of algae grow in different environments. They also have different nutritional requirements as well[6].

Presently, there is a high demand for commercially viable, renewable alternatives to fossil fuels as energy sources. Recent attention has focused on biofuels such as fuel derived from biomass directly and indirectly, as a solution to the alternative fuel initiative[7]. In addition to providing a renewable energy source, plant-derived biofuels provide a potential solution to the present problem of excess greenhouse gas emissions by sequestering carbon dioxide during photosynthesis[8].

Biofuels can be produced from various types of feedstocks such as pure vegetable oils, waste cooking oils and animal fat. However, the limited supply of this feedstock inhibits the further development of biodiesel production[9]. Moreover, the production of the first generation of biofuel namely oil crops such as soybean, palm oil, corn oil, waste cooking oil and animal fats is not able to replace fossil fuel due to the shortage of this source or food-based raw materials[10]. Furthermore, large-scaled algae cultivation for bioenergy production should be significantly developed to compete with the cost of energy production from other resources, especially petroleum based fuel.

The difference between the cost of bioenergy production from microalgae and presently fossil-based energy is still the major complication that becomes an obstacle for industrialization of the microalgae bioenergy[11]. Advantages of this biofuels sources over traditional fuels that include its can be daily regenerates, greater energy security and reduced environmental impact[12].

An overall carbon dioxide emission is increasing every year that causes climate change and predicted the widespread destruction of ecosystem[13]. Additional to this problem which can consequence to the heavy rain, flood in urban and rural area and slightly increase of sea level and vanish of isolated island[14]. Proposed reductions fossil-carbon energy source will decrease Carbon Dioxide (CO_2) levels of the atmosphere, which add to the pool of carbon that is actively cycled between atmospheric and terrestrial pools.

In water, the algae accumulation becomes a serious problem when end of the growing season, most of algae die. The decomposition in streams or lakes or ponds (aquatic systems) consequence to reduce amount of dissolved oxygen that results in eutrophication, odor, unpleasant taste and kills aquatic life[15]. In addition, domestic effluent water flows to the drainage or stream which consist of the high concentration of phosphate and nitrate that mainly comes from residential sewage tanks, detergents and effluent water from intensive agricultural land. It encouraged the growth of aquatic plant such as macroalgae or microalgae in aquaculture systems[16,17]. This scenario may cause the effect on water flowing systems. The related public authorities have to allocate expenses to remove the aquatic plant growth in the effluents or streams to ensure smooth water flow.

The objective of this research is to investigate the feasibility of cultivating and producing common local microalgae strains grow in aquatic system, especially in Sungai Sura nearby Universiti Teknologi MARA Dungun, Terengganu in Malaysia to be applied as renewable biofuel feedstock.

2. Materials and Methods

2.1 Strains and Culture Medium

In this study, the growth of two of green microalgae (chlorophyta) strains namely Spirogyra sp. and Tetraspora sp. were obtained and investigated from local Sungai Sura water flows outside Universiti Teknologi MARA campus at Sura Hujung in Dungun, Terengganu, Malaysia. The microalgae strains are stored in the freezer at 4ºC to ensure the cells can grow after transfer to the culture medium.

2.2 Culture Medium

Water from Sungai Sura at coordinates (4°42'28.2"N 103°26'12.1"E, Google Map) has been collected as a growing medium for microalgae strains that can be classified as low salinity water due to the river intercept with sea water. The salinity range was determined is 120 to 150 μS/cm. The prepared medium was disinfected in an ozonizer (Hirayama HV-50) to kill bacteria and filter out a wide range of contaminant for 12 hours. Then, the microalgae cells were transferred at regular intervals to the 1-L conical flask, in which the culture medium consists of low salinity water as a growing medium. The pH of the medium has been set up and monitored at a range of 7.0 to 8.0.

2.3 Growth under Controlled Condition

The microalgae strains have been successfully grown in an indoor laboratory of Environmental Research Center, UiTM Dungun for 20 days in the low salinity water medium. Strains were grown in 250 ml Erlenmeyer flasks with an initial cell concentration of approximately 1.0 ml cells in 500 ml culture. The flasks were placed closed indoor room at a temperature (27 ± 2)°C and exposed to a white fluorescent light Philips (36 W) sources that were placed 20 cm above the culture surface. It is to ensure that all cells are equally exposed to the light with 12/12 h light/dark photoperiod which automatically controlled by 24 h timer (Timer Bainian, BND-50/339, China). The light intensity was 325 Lux (32.5 mmol m^2 sec^{-1}) that measured from the culture surface by using a light meter (model MS6612 Digital Lux Meter, Mastech Instruments). The light was supplied in abundance and the cultures were kept dilute enough to neglect self-shading, which allows ignoring the light effect on the photosynthesis and operating at the linear growth phase. The biomass were harvested after three weeks by centrifugation at 3000 RPM for 15 min.

2.4 Environmental Factors

The cultures were aerated by bubbling CO_2 air with a flow rate of 0.35 L min^{-1} which obtained from atmospheric air by using calibrated mass flow controllers (model ST-51, FCI Flow meter, USA). The flasks were tightly sealed and the aeration was introduced through the air filter of Gas Inline Filter specifications (measures 1-3/8" or 35 mm diameter and 2-3/4" or 67 mm long; 60 microns filter element) to prevent any bacterial contaminations. Cultures pH was maintained at range 7.0 to 8.0. Cell examination under microscope (Ray Vision serial no. 33742) 30 magnifications was done to ensure that the available strains were unicellular and entirely free of all other contaminating organisms. The microalgae strains were allowed to grow for three continuous weeks and samples were daily collected to determine the cell concentration in cells mg/ml.

2.5 Determination of Cell Concentration

The cell concentration was monitored daily by measuring the optical density at 680 nm wavelength by using a UV-spectrophotometer (UV Varian's Cary® 50 Spectrophotometers, USA). The daily concentration (cells mg/ml) at any given cultivation time was collected and calculated from a pre-prepared calibration curves of optical density versus cell concentration determined by using Ray Vision serial no. 33742 microscope. The dry weight of algal biomass was also determined from filtering the algal suspension by using a pre-washed and dried Whatman filter paper, which dried overnight for 24 h at 70°C in an oven (Memmert, Germany) until constant weight. A calibration curve between optical density and dry weight for each culture then diluted in 10 ml volumetric flask, which was generated and used in subsequent analysis of biomass concentration (mg/ml). Biomass productivity was determined from the slope of the biomass concentration progress curve, whereas the specific growth rate was determined from the slop of the logarithmic plot of biomass concentration over initial biomass concentration (mg/ml) versus time progress (day). The calibration is calculated by using Equation 1:

Y (Absorbance) = 0.03935 * Concentration (mg/ml) + 0.83747 (1)

2.6 Statistical Analysis

Growth of both microalgae strain date was used to perform a variance analysis from one way ANOVA by using Microsoft Excel 2010 version data analysis. The method was determined that all data have strong significant (P-value < 3.62498E-08) difference in absorbance and the cultivation period that containing different types of microalgae strains.

3. Results and Discussion

3.1 Growth of Microalgae

The growth rate of microalgae species which is Tetraspora sp. and Spirogyra sp. in the low salinity water is illustrated in Figure 1. At the beginning of the first until the seventh day of each species showed that the culture was in lag phase on the eighth day onward (induction phase). It was gradually increased with the concentration of cells in all flasks on the ninth day until the nineteen day, which showed a significant rate of growth for both microalgae strains (exponential phase)[18]. It was determined that the relative ecological growth of both microalgae strains succeeds in adapting to its natural environment or the experimental environment impressed upon it.

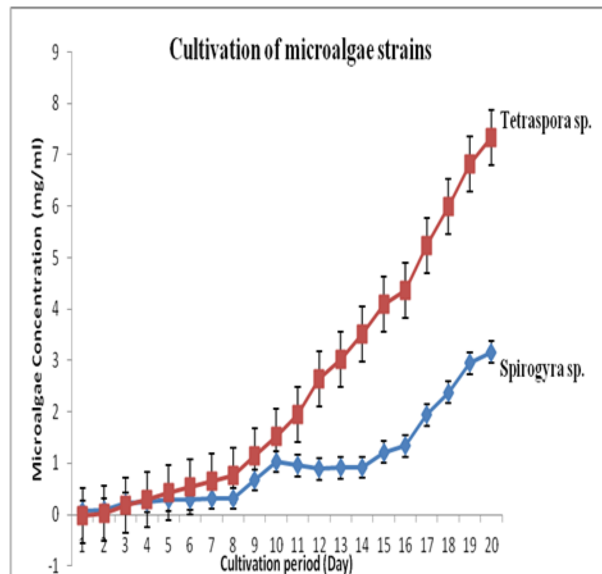

Figure 1. Growth rate of microalgae strains biomass.

However, comparison of densities of both strains is shown that Tetraspora sp. gives higher than Spirogyra sp. on the similar days. The duration of exponential phase in cultures depends upon the size of the inoculums, the growth rate and the capacity of the medium and culturing conditions to support algal growth[19]. The maximum microalgae growth had observed on the twentieth day (densities of Tetraspora sp. is 7.37 mg/ml and Spirogyra sp. is 3.16 mg/ml respectively). The day onwards is the reduction of cell growth that also known as phase of declining relative growth for both microalgae species.

The declining growth process is normally occurs in cultures, when either a specific condition for cell partition is lacking or something else is inhibiting reproduction. In this phase of growth biomass is often very high and an exhaustion photosynthesis component such as nutrient salt, CO_2 or light limitation becomes the main causes of declining growth. When biomass are increasing exponentially, a constant supply of air (or air plus CO_2) will only be in balance with growth at one point during exponential phase. When the densities of cell decreases, excess in CO_2 may lower the pH of the medium and depress growth. The CO_2 limitation at high cell densities causes any further biomass increase to be more linear rather than exponential (with respect to time) and proportional to the input of CO_2[20].

From the previous experiments on the growth of microalgae undergoes lag, log, deceleration, stationary and death phases. In the first phase, initial concentration of microalgae is a significant parameter which can minimize the duration of the lag phase. The cells must be adapted to the growth medium and similar environmental conditions before inoculation[21]. At this stage, the cells should have been young and the inoculums size increases (5-10% by volume). The period of the inoculum has a strong effect on the length of the lag phase. Secondly, an exponential phase where cells can multiply rapidly and cell density increases exponentially with time. Experimental data show the growth of Scenedesmus at various time intervals a operating conditions used in a batch reactor[20].

4. Conclusion

This experiment confirmed that the growth of two of green microalgae (chlorophyta) strains namely Spirogyra sp. and Tetraspora sp. were obtained from local Sungai Sura water. It can be grown in Sungai Sura as a medium for culturing microalgae such as Tetraspora sp. and Spirogyra sp. in 20 days with the maximum biomass concentration of 7.37 mg/ml and 3.16 mg/ml respectively.

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