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BIOETHANOL PRODUCTION FROM ALGAL BLOOM FORMING OSCILLATORIA sp.

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ABSTRACT

This study aims to reduce the usage of fossil fuel and promote the production of bioethanol (as alternative source) obtained from biotic resources. Due to rapid increase of human population and the exponential growth of industrialization, the existing natural resources are depleted very fast. Microalgae (*Oscillatoria sp*.) are pollution causing one in aquatic bodies due to its exponential growth and it must be removed from the environment to reduce carbon dioxide. This alga is easy to cultivate, harvest and can be used as a raw material for biofuel production. Earlier studies showed that bioethanol production was enhanced by using *Saccharomyces cerevisiae* as an intermediate organism to speed up the process. In the present study, the blue green algae Oscillatoria sp., used and the result of this experiment showed that the production of bioethanol is significantly increased upto 700ml/L based on the substrate used. The final product was retrieved from the distillation and analyzed using FTIR. The obtained bioethanol from these microalgae is to be more effective, environmental friendly than petro based fuels. Hence, the bloom forming microalgae and the waste sources can be used as an efficient substrate for ethanol production in future perspective.

Keywords : Bioethanol, Biofuel, Ethanol, Microalgae and Pre-treatment

INTRODUCTION

Bioethanol is usually obtained from carbon based feedstock which is biodegradable, less toxic and reduce the amount of carbon monoxide produced by the vehicle. Bioethanol production is classified as first (raw material based or starch based), second (lignocellulosic materials) and third biofuel generation (microbial biomass), which differ according to the process and raw material used [Silva, 2019]. The principle of ethanol production consists of the cultivation of microorganisms, harvesting of biomass, fermentation and extraction of ethanol. According to the report of Nithi, *et al.*, (2018), a lot of lignocellulosic biomass resources such as agricultural and forestry residues could serve as feedstocks for the eco-friendly bioethanol production. Nowadays, agricultural wastes are used as a feed stock for the cattles and animals. From the research report of Bennan (2018), the algal strains like *Chlamydomonas, Chlorella, Schizochytrium,* and *Chlorococcus* were used to produce ethanol from sugars.

Algae are classified as microalgae and macro algae which can survive in hot conditions and rich in carbohydrates (50% carbohydrates, 30% proteins and 20% lipids). Very limited research has been reported on fermentation of algae for ethanol production. Microalgae get attention due to their high biomass in cell wall. These algae are valuable raw material sources in future generation, because of its proliferating growth in water bodies. There are many advantages of using microalgae as sources of biofuels, oil and ethanol productivity which is higher than the best oilseed crops (Meghwanshi, *et al.*, 2014). According to the report of Chen *et al.*, (2013), the microalgae can grow under sunlight and also produce high amount of biomass in salty water, fresh water lakes, deserts and marginal fields etc. Many species of microalgae can accumulate a significant amount of polysaccharides in their cell structure and provide higher yield than agricultural wastes (Aliya, *et al.*, 2009). Mayur, e*t al.*, (2019), conducted a study that revealed the breakdown of the cell wall of microalgae yield mannose and the fermentation process enhanced by an additional culture of S*accharomyces cerevisiae*. Madhuka, *et al.*, (2015), suggested that the most commercial scale of ethanol fermentation done by bakers' yeast. When the cells are broken down, the yeast is added to the biomass and fermentation begins. In this way, the sugar like glucose, fructose and maltose is converted to ethanol by yeasts. The maximum yield was obtained based on various factors like biomass size, contact time, sample and solvent ratio, extraction method. Yeasts should be harnessed for bioenergy bio-refinery based applications in lines parallel with microalgae. Silva, *et al*., (2019), reported that, the microalgal biomass was pretreated by different methods like autoclaved or enzymatic hydrolysis. The sterilized biomasses can be used for lipid extraction (for biodiesel), simple sugars (for bioethanol) through fermentation or bio-methane production by anaerobic digestion. Now, the solid waste can turn into beneficial raw material. That's why, the work was done for the fulfillment of the society needs, safety and economy based ecofriendly products.

Blue green algae, *Oscillatoria sp.* is bluish green in colour usually free floating, cylindrical or sometimes slightly tapering, unbranched filaments in aquatic environments. As this species forms

blooming in water bodies and biochemically rich content of carbohydrate, the present study is aimed to produce ethanol using this species as substrate and the final product is analysed using FTIR

MATERIALS AND METHODS

Isolation of *Oscillatoria sp.*

The water samples were collected from nearby villages in and around Virudhunagar. These samples were inoculated in BG11 broth and kept in a wooden rack for 15-20 days and the light source provided. The following ingredients like Ferric ammonium citrate (0.006g/L), Disodium magnesium EDTA (0.001g/L), Copper sulphate (0.0056g/L), Di-potassium hydrogen phosphate (0.0314g/L), Calcium chloride (0.0367g/L) and Citric acid (0.0056g/L)were used in BG11 broth for sub culturing of fresh water algae. After 15 days of incubation, the biomass was filtered, dried and also the dry weight was measured (Hermansyah, *et al.,* 2018, Wang *et al.,* 2013). The morphology of the sample (particularly-*Oscillatoria sp.*) was identified using the key given by Barry *et al.,* (2015).

(b) Biological Conversion

According to the report of Hermansyah, *et al., (*2018), 5 g of algal sample was diluted with 100ml distilled water and immediately autoclaved for breaking the mannose in the algal cell wall to glucose molecule. Then, 5 g of yeast pellet was added to sterilized the algal broth and incubated at 37^0C under anaerobic condition for 5 days. Here, the enzymatic conversion of substrate was speed up by the yeast pellets. After 5 days, the fermented sample was filtered by Whatman No.1 filter paper and distillated the supernatant at 60^0C. A cleansing procedure called distillation, used to eliminate the water. The Soxhlet method is one of the most popular method which uses heat plus solvent to extract the preferable products. According to the report of Kim, *et al*., (2013), the sample was subjected to distillation and pure form of ethanol was obtained. Simultaneously, the bioethanol was confirmed by various tests such as Lucas test, Iodoform test and Sodium hydrogen carbonate test.

Quantitative Analysis by Potassium dichromate Method

The quantity of ethanol was determined by adding 2 to 3 drops of acidified 0.1 M potassium dichromate with 2ml of distillated ethanol and kept in a water bath for 5 minutes. After 5 minutes, the green color formation indicated as a positive result and the absorbance of each concentration was measured at 580nm (Nithi, *et al.,* 2018). The quantity of ethanol was measured by plotting standard graph for the obtained value.

(d) FTIR Analysis

Fourier Transform Infra-Red Spectroscopy was used to elucidate the composition of the derived samples. In this experiment, the functional group of the product was analysed by FTIR (BRUKER - ALPHA ECO- ATR, GERMANY). Here, there was no further usage of solvent for predicting the purity and quality of ethanol (Bennan, 2015).

RESULTS AND DISCUSSION

Identification and Cultivation

The morphological study of the algae sample (*Oscillatoria)* showed a lengthy rod like

structure with or without surrounding sheath, fibrous and filaments formation with dense bright coloured expanded mass. In liquid cultures, narrower species formed mucilaginous and membranous growth attached to the glass wall or the surface of medium. All forms in the broth culture were appeared bright and later it became yellow brown due to lack of nutrient depletion. After 15 days of incubation, the biomass of the algae was collected and dried for further use.

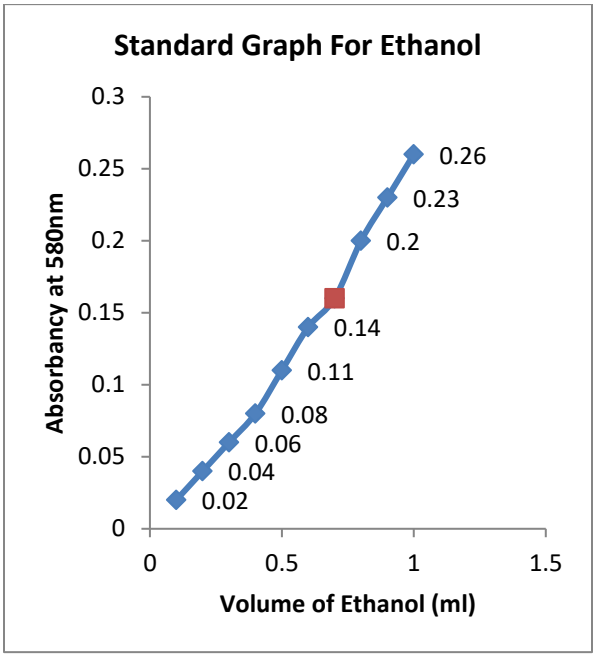
Extraction & Confirmation of Bioethanol

Fermentation is used to convert the sugars in biomass into ethanol by yeast. The distillation process works by boiling the water and ethanol mixture. The ethanol has lower boiling point (78.3^{0}C) compared to that of water (100^{0}C). So, the ethanol turns into the vapour state before the water was condensed and separated. The eluted sample was subjected to detect the confirmation of ethanol by various tests. In Lucas test, the visible formation of turbidity by adding Lucas reagent to the distillate ethanol immediately, indicated as a positive result for primary alcohol. The disappearance of brown colour by adding little bit of iodine solution to the derived ethanol depicted a positive result for Iodoform test. In sodium hydrogen carbonate test, the effervescence formation showed a positive result for the bioethanol.

Quantitative Analysis of Bioethanol

The concentration of ethanol was determined by acidified potassium dichromate method. Figure 1 showed the quantity of ethanol by acidified potassium dichromate method. This sample was subjected to further analysis.

Fig 1: Estimation of bioethanol by potassium dichromate method

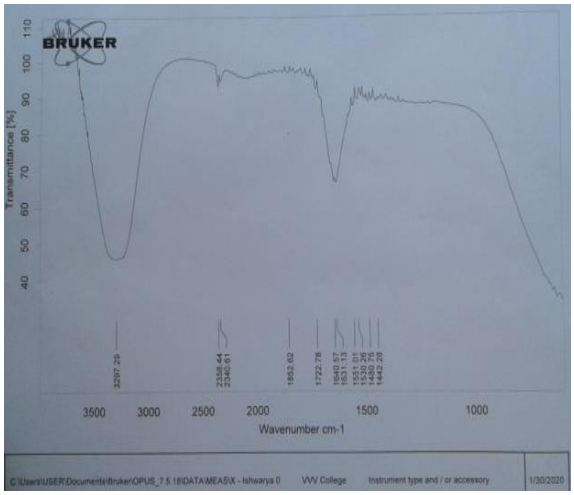


The formation of green colour indicated the confirmation of ethanol by adding few drops of acidified potassium dichromate with the eluted sample and kept in a water bath for 5 minutes. The amount of bioethanol was found that ≈700ul/ml as the OD value of the sample (0.14) corresponded with the standard value (0.7) of ethanol.The quantity of bioethanol was compared with standard graph.

FTIR Analysis

The functional group has been determined by the peak shape and its position in the corresponding region by Fourier transform infrared spectroscopy (FTIR) plot for bioethanol is shown in Figure 2.

Fig. 2, FTIR plot for bioethanol from *Oscillatoria sp.*



From this result, we inferred that the peak formation specifically highlighted the components in bioethanol. The bonding nature of O-H stretching appears at 3000-3500 cm^{-1} with strong and broad peak. The value of 1500 -1700 cm^{-1}indicates the presence of – CH group and this was correlated with the report of Bennan inan, *et al., (*2017).

CONCLUSION

Bioethanol serves mostly in the transport sector as a constituent of mixture with gasoline to push down the usage of conventional fuel. In this study, the bioethanol production was done by using *Saccharomyces cerevisiae* and the functional group was assessed by FTIR. The present findings also the supportive experiment for the production of ethanol using using Oscillatoria sp., Based on the report of Silva *et al., (*2019), bioethanol from corn, beet and sugarcane yield 460L, 100L and 90L per ton biomass respectively (1g of glucose gives 0.511g of ethanol). In the present study, the maximum yield was obtained upto 0.099ml based on the report of Hermansyah, *et al.,* (2018). Little amount of algal biomass can yield high amount of bioethanol compared to other agricultural wastes. In this report, the yield increased (0.700ml) upto the level of the research carried out by Hermansyah, *et al.,* (2018). Instead of agricultural wastes, the algae *Oscillatoria sp.* can be used as an alternative source of raw material for bioethanol production. Recent studies have shown promising results in increasing carbon capture capacity, biomass production and lipid enhancement in genetically modified microalgae. Hopefully, we can move forward our research using recent technologies, one day the toxic petroleum solvents will be replaced by bio-sourced solvents.

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