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Isolation of the Hydrocarbon Producing Algae Botrycoccus braunii Using Serial Dilution Techniques

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Abstract.
The study was carried out to isolate the microalgae species Botrycoccus braunii from a rich algal water sample using the serial dilution technique with modified Chu-13 culture media. Water sample was collected and was examined using the microscope. Botrycoccus braunii was found to be present. Modified CHU-13 culture media was prepared and autoclaved. Under aseptic condition, the media was poured into culture bottles numbered 10^{-1} to 10^{-10}. using micropipette 10ml was collected from the rich water sample and poured into the culture bottle numbered 10^{-1}, again using the micropipette, 10ml was collected from the culture bottle numbered 10^{-1} and was poured into the test tube numbered 10^{-2} , again using micropipette, again 10mls was collected using the micropipette from the test tube numbered 10^{-2} and poured into the test tube numbered 10^{-3}. The process was repeated for 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10}. The culture tubes were maintained at room temperature (25-27^0c) by sunlight with a light dark photoperiod of 15h: 9h. Aeration into the tubes was controlled by covering the culture tubes with cotton wool. The culture was examined after second and forth weeks growth period by preparing slides and viewing under the microscope. Pure Botrycoccus braunii species was found to be present in the test tubes numbered $^{10-8}$ and 10^{-9} at the forth week examination.

1.0 Background.

Algae belong to a large, diverse group of organisms ranging from unicellular to multicellular that produce complex organic compounds from basic inorganic molecules using energy from photosynthesis, inorganic chemical reactions, and heterotrophic fermentation (Evangelista et al., 2008). The algae fossil record dates back approximately three billion years, well into the Precambrian period. Algae are ubiquitous within the biosphere and have generated a large fraction of the oxygen present in the earth's atmosphere and a large quantity of organic carbon in the form of coal and Petroleum (Graham, 2008). Algae's role in the development of the earth's biosphere was of unique importance. The importance of algae has increased with the search for renewable energy sources. Even under highly unfavorable growth conditions, algae can thrive and produce valuable byproducts such as lipids (oils), carbohydrates, proteins, and various feedstock that can be converted into biofuel and other useful materials(Li et al, 2007). Hu et al., (2008) projected a possible yield of 200 barrels of oil per hectare (2.47 acres) of land used for growing photosynthetic algae.

Botrycoccus braunii is a unicellular photosynthetic microalgae. *Botrycoccus braunii* is member of the chlorophyceae (chlorophyta). This colonial microalga is widespread in fresh and brackish waters of all continents (Chisti, 1980). The cosmopolitan nature of the algae is confirmed by the strains originating in the USA (Wolf et al., 1985), Portugal, Bolivia, France, Ivory Coast, Morocco, Philippines, Thailand, and the West Indies (Metzger et al., 1985). These geographical regions belong to various climatic zones, including the continental, temperate, tropical, and alpine zones.

B. braunii is regarded as a potential source of renewable fuel because of its ability to produce large amounts of hydrocarbons. Depending on the strain and growth conditions, up to 75% of algal dry mass can be hydrocarbons. The chemical nature of hydrocarbons varies with the producer strain. Three races of B. braunii have been documented, and these can be differentiated on the basis of the characteristic hydrocarbon they produce. The A race produces odd numbered C25 to C31, n-alkadienes, and trienes. The B race produces triterpenoid hydrocarbons known as botryococcene (CnH2n–10, n = 30–37), apparently of isoprenoid origin (Chisti, 1980). The L race produces Lycopadienes, a C40 tetraterpene. Historically, interest in B. braunii arose because of its geochemical significance.

There are four major techniques for obtaining unialgal isolates: serial dilution, streaking, spraying, and single-cell isolations. Streaking and spraying are useful for single-celled, colonial, or filamentous algae that will grow on an agar surface; cultures of some flagellates, such as *Chlamydomonas* and *Cryptomonas* may also be obtained by these procedures. Many flagellates, however, as well as other types of algae must be isolated by single-organism isolations or serial-dilution techniques.

Serial dilution method of isolating micro algae have been used with great success by; Gross (1937), Parke (1949), and Butcher, (1952) for obtaining unialgal cultures of many interesting marine species but it is doubtful if a pure cultures could ever be obtained by serial dilutions since bacteria occur in far greater number than algae, however there is need for more studies to confirm this assertion.

Chu-13 medium is a culture medium used in microbiology for the growth of certain algal species. it was first published by S.P Chu in 1942. It is used as growth medium for the biofuel candidate alga *Botrycoccus*

braunii (C. Largeau et. al 1980). Chu-13 includes 13 essential minerals and trace element that are required by algae for growth.

Human activities particularly the combustion of fossil fuels, have made the blanket of green-house gases which get trapped in the atmosphere. These greenhouse gases absorb and re-emit radiation from the sun which heats up the world. If we carry on burning fossil fuels the polar ice caps will melt, resulting in a rise in sea levels (This rise could cause areas across the world to flood). The rise in temperature however would cause innocent species to die out because they would not be able to cope with the extra heat; the resulting to increase in global temperature hence altering the complex web of systems that allow life to thrive on earth such as rainfall, wind patterns, ocean currents and distribution of plant and animal species.

The main environmental advantage of biofuel stems from the fact that they are carbon-neutral: the carbon dioxide they release upon combustion is initially extracted from the atmosphere during biomass production, resulting in zero net greenhouse gas emissions. Biofuel also reduce the release of volatile organic compounds. Ethanol also eliminates the need to add lead. In addition, biofuel are biodegradable and non-toxic, meaning spillages represent far less of a risk than fossil diesel spillages.

1.2 Aims and Objectives

1.2.1 Aim

The aim of the study is to identify and isolate Botrycoccus braunii using serial dilution technique.

1.2.2 Objectives

I. To isolate the algal species *Botrycoccus braunii* from the rich algal sample using serial dilution technique
II. To use chu-13 as a media for isolating *Botrycoccus braunii.*

2.0 Material and Method

2.1 Collection of Water Sample.

Water sample was collected from a water pond in the front of Biological sciences Department, Ahmadu Bello University, Zaria, after mixing the water from the bottom to the top, the water sample was collected with a clean container.

2.2 Identification of *Botrycoccus braunii*

Slides were prepared from the collected water sample and were viewed under the microscope to identify the presence of *Botrycoccus braunii* in the collected water sample, *Botrycoccus braunii* was found to be present together with other green algae.

2.3 Preparation of modified CHU-13 media Culture Media.

This culture media was prepared by weighing the individual compound with a digital weighing balance to make the following stock solution for each of the component as shown in the table below.

Table 1: Weights of component compounds for the modified CHU-13 culture media.

S/N	Compound	Mg/l
1	KNO_3	400
2	K_2HPO_4	80
3	$CaCl_2$ dehydrate	107
4	$MgS0_4$ heptahydrare	200
5	Ferric Citrate	20
6	Citric acid	100
7	$CoCl_2$	0.02
8	H_3B0_3	5.72
9	$MnCl_2$ tetrahydrate	3.62
10	$CuSO_4$ pentahydrate	0.16
11	0.072N H_2SO_4	I drop
12	Na_2MoO_4	0.084
13	$ZnSO_4$	0.04

- The remaining volume was pure de-ionized water. Because it was difficult to weigh out some of the trace minerals, all the mixture were made in large concentration and the appropriate amount were mix, and the P.H. was corrected to 7.5 using PH meter.
- The volume was then brought to 1L by adding more distilled water.
- The CHU-13 Medium was autoclaved at 125 °C for 30 minutes at 1atm, together with test tubes.(similar to figure

- The autoclaved media was allowed to Cool to about 50 °C
- The lamina hood was sterilized with methanol
- The media and the test tubes were taken to the Lamina hood
- The CHU-10 Medium was then opened and 9ml (for serial dilution) and 10ml (for capillary method) were poured into 10 and 5 test tubes respectively and all the test tube containing culture media were covered cotton wool to prevent contamination.

2.4 Serial dilution technique for isolating microalgae

9ml of CHU-10 Media was poured into ten test tubes numbered 10^{-1}, 10^{-2},10^{-3}….10^{-10}. From the water sample 1ml was collected and poured into the test tube numbered 10^{-1}, 1ml was then collected from the test tube numbered 10^{-1} and was poured into the test tube numbered 10^{-2}, the processes was continued up to the last test tube numbered 10^{-10}.

Figure 1: Serially diluted algal culture

The cultures tubes were maintained at room temperature (25-27ºC) by a sun light with a light dark photoperiod of 15h: 9h. Aeration into the flask was control by covering the culture tubes with cotton wool.

4.0 Results and Discussions.

Pure *Botrycoccus braunii* species colonies were found in the serially diluted tubes numbered 10^{-7} and 10^{-8} after four week's growth period. The algal species were seen in colony and the individual cells of the colonies were in the range of 3 - 11 µm and the colonies were found to be between 25 - 150 µm. Cells were spherical in shape and the colonies remain attached to one another. Cells are generally green to yellowish green in color. Similar observations were made by Chandra in Miocene lignites of Kerala, India (Chandra 1964). *Botryococcus braunii* is a colonial green micro alga distributed on all the continents in fresh water, brackish water, Saline lakes, reservoirs and small ponds (Aaronson et al., 1983). *Botrycoccus braunii* were mainly distinguished based on colony size and details of cell shape, therefore The Colony characteristics and morphological features of the isolate have demonstrated its similarity with *Botryococcus braunii*.

Figure 2: Image of the *Botrycoccus braunii* snapped using digital camera under the microscope after two weeks growth period

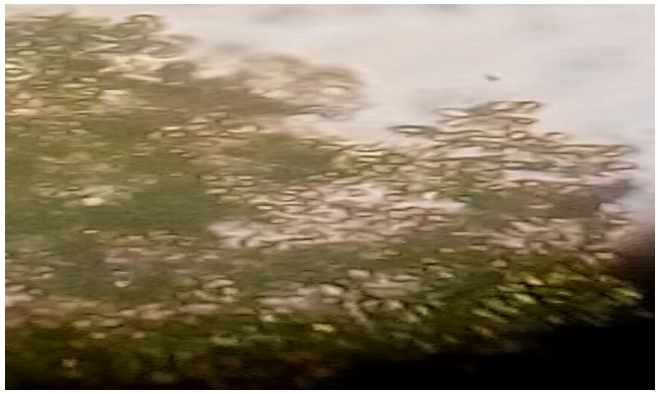

Figure 3: image of the *Botrycoccus braunii* snapped using digital camera under the microscope after forth week's growth period

More so, The result correspond with the result obtained from similar by research work by; Gross (1937), Parke (1949), and Butcher 1952 in which unialgal cultures of many interesting marine species were isolated using serial dilution techniques, however it is doubtful if a pure cultures could ever be obtained by serial dilutions since bacteria occur in far greater number than algae.

5.0 Conclusion

The algal specie *B. braunii* was isolated from the rich algal water sample collected from a water pond at A.B.U Zaria using the serial dilution technique with modified CHU-13 as the culture media and this shows that the technique is a very effective way of isolating the microalgae from a rich sample, therefore this technique is recommended for the isolation of the isolation of the microalgae.

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