Journal of Applied Science and Agriculture, 9(20) Special 2014, Pages: 56-62

AENSI Journals

Journal of Applied Science and Agriculture

ISSN 1816-9112

Journal home page: www.aensiweb.com/JASA

Design and Fabrication of a Biofermenting Machine on a Village Level

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
17 November 2014
Accepted 25 November 2014
Available online 13 December 2014

Keywords
Biofermentor, Spirulina,, Biotreatment

ABSTRACT

This research study was conducted to design and fabricate a biofermentor for treating biologically convertible matter by contacting same with Spirulina, a microalga deposited and immobilized on support carriers. The apparatus is made up of a bioreactor chamber, sludge and collecting chamber and a wastewater conveyor. Fresh cow manure was mixed with a solution consisting of food grade chemicals. The medium was prepared by stirring – in waste matter into the water. Adjustments were made to attain a pH level of 9 – 10.5.Spirulina was cultured in the bioreactor to produce a protein biomass and at the same time absorb, degrade and convert the biologically convertible matter. The physico – chemical quality of wastewater before and after undergoing treatment inside the biofermentor were analyzed and evaluated. Based on the result of the physical analyses the wastewater became odorless, less turbid and the color gradually changed from brownish to yellow – deep green teeming with Spirulina growth. The concentration of potassium was increased while sodium concentration was decreased. There was a marked increase on the biomass yield of Spirulina together with its biochemical composition. Also, based from the microbial assay, the Spirulina grown in a biofermentor was negative with pathogenic bacteria.

To Cite This Article: Guillermo S. Rillon Jr., Design and Fabrication of a Biofermenting Machine on a Village Level. ***J. Appl. Sci. & Agric.,*** *9(20): 56-62, 2014*

INTRODUCTION

Majority of the water used by man are discharged to the rivers and lakes which resulted to the pollution of water sources especially the water table. If this will continue, there will be no safe water for the next generations. Wastewater treatment, therefore, is everybody's responsibility. With the increase in urbanization, the development of industrial technologies and the advancement of the consuming society, there has been a rapid increase in biologically convertible production. To solve this problem, many approaches, based on the nature of organic matter have been adopted. A biofermentor can be designed and fabricated to convert different types of biologically convertible matter utilizing microorganisms into reusable or detoxified materials. This biological method employed *Spirulina*, a microalga which degrade and convert these organic wastes into non-harmful forms. Algal single – cell protein can be cultured in medium preparations from agricultural wastes like animal manure. These microalgae will not only convert organic and inorganic wastes into a high protein biomass but also to prevent pollution of the environment through waste recycling (Ciferri, 1983). Wastewater from agro-industrial system varies on their characteristics, magnitude, volume and degree of contamination. One of the most common ways to handle domestic garbage is to use a sanitary landfill where organic and inorganic biologically convertible matters are deposited and further recovered with a layer of soil. This procedure is the cheapest among biotreatment technologies, however, organic matter which is slowly decomposed by anaerobic process, produce large quantities of methane gas and heavy metals and other groundwater hazardous pollutants. Also, this method leaves huge amount of inorganic matter in the environment. Efforts have been made to recycle and valorize products such as plastics, papers, and metals. Techniques such as bioconversion of biologically convertible matter using flocculation, decantation and microbial treatment of biologically convertible water have been investigated (Leduey, 1979).The biological method or bio remediation of wastewater is the use of microalgae in digesting organic wastes into non-harmful forms (Nobel, 1991). Attempts were made to culture *Spirulina platensis* in human urine directly to achieve biomass production and O2 evolution, for potential application to nutrient regeneration and revitalization in life support system. The culture results showed that *Spirulina platensis* grows successfully in diluted human urine and yields material biomass urine dilution ratios of 140 – 240. Accumulation of lipid and decreasing of protein occurred due to nitrogen deficiency. Oxygen release rate of *Spirulina platensis* in diluted human urine was high (Torzillo *et al*. 1991).A closed biofermentor

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was developed for microalgae mass cultivation. This was developed in order to attain temperature conditions more favorable for the growth of *Spirulina*. The yield of *Spirulin*a grown in biofermentor has been 3.0 – 3.33 kg (dry weight) per square meter – year (average of 5 year experimentation). In the same period the yield of *Spirulina* in open ponds ranged from 1.8 to 2.1 kilogram per square meter – year (Materrasi, 1994).According to the study of Oron (1979), microalgae such as *Spirulina* can be cultivated in a biofermentor using slurry or cow manure. Spirulina species have the ability to convert and recycle organic and inorganic wastes into a high protein biomass.Microalgae such as Spirulina species have the ability to grow in hot and alkaline environments which ensure their hygienic status, as no other organisms can survive to pollute waters in these algae thrive. Unlike the stereotypical association of microorganisms with "germs" and "scum". Spirulina is considered as one of the cleanest most naturally sterile foods found in nature (Materassi, 1994). There is in need therefore, that wastewater must be treated separately using appropriate methods and design. Hence, this study.

Methodology:

Design and Fabrication of a Biofermentor

The machine was constructed with the following features and operations:

a. The waste inside the two chambers were treated utilizing *Spirulina platensis*
b. The residual mass which settled at the bottom of the deposition tank were collected
c. The water circulates continuously on both chambers for biotreatment process
d. Oxidation process took place on the aeration chamber to facilitate the biodegradation of the suspended particles
e. Clean water with unialgal or pure cultures of Spiruliuna

The following materials were used: Poly vinyl chloride (PVC) pipes and tubing, plastic screen,silicon adhesive. Pipe ½ blue, elbow ½, ball valve, silicon sealant, solvent ½ neltex, elbow ¾ and ½, 1 foot pipe, air pump sp.8.50,water pump aerator pr 2,500, tee tube, aquarium 18 in. x18 in.x 18 in. (vertical), sludge screen, regulation tank and air nozzle. These materials are cheaper than the materials used in constructing commercial biofermentor.

The fabricated biofermentor has the following parts essential in carrying out the biotreatment processes

a. <u>Bioreactor chamber.</u> The bioreactor chamber has a volume of water 45 cm X 45 cm X 40 cm and provided in its bottom with eight interconnected holed PVC tubing with a diameter of 0.75 cm attached to an aerator machine to supply the chamber with air. This chamber was connected with the second chamber with three PVC tubes that were 40 cm above its bottom with length of 45 cm and spaced 10 cm. These tubes allowed water and suspended particles to flow the sludge/residual matter and the collection chamber where the sludge settles down as well as the collection of treated water.

b. <u>Sludge and collecting chamber.</u> This chamber has a volume ½ (45 cm X 45 cm X 40 cm) and on of its side was inclined with an angle of 45 degrees to allow solids to settle down quickly. Inside the chamber, was a water pump with a pumping rate maximum of 350 liters per hour connected by a PVC tube going to the first chamber to return untreated water for biodegradation Spirulina on the first chamber.

A collecting tube was attached on its side 42 cm above its bottom where the treated water was collected for intended purposes.

For further treatment, this tube can be connected to another chamber for chlorination or UV disinfection. Both the two chambers were closed or covered with glass sheet to avoid the entrance of contaminants as well as to maintain the pressure and temperature inside them.

c. <u>Waste Water Conveyor</u>**.** The conveyor is made of conical shape with a base diameter of 22 cm and an orifice of 0.75 cm in diameter. The conveyor is connected to the first chamber by PVC tubing to fill in the waste water for fermentation and treatment. The conveyor, for further strength in holding the mass of the water while filling, it is supported by metal rods pivoted on its base.

All the tubings are attached with stopper to control the rate of flow of fluid or gas as well as to avoid the entrance of foreign objects to the chambers. The stopper is made of PVC that suit to the materials used (Becker, 1981).

Biotreatment of Cow Manure:

Cow manure was injected at the top of the biofermentor according to the invention. Spirulina culture taken from the pond was inoculated into the biofermentors. The substrate was supplemented with a source of carbon, phosphorous, iron, and trace element. The culture was propagated under laboratory condition of 30 degree centigrade to 35 degree centigrade and a light source of 6.0 kilowatts. The culture period from juvenile to mature spirulina was from 2 – 3 weeks.

Physico-Chemical Analysis of Water Media:

The Physical -chemical analyses of the water media before and after treatments was conducted. The analysis included pH level; water temperature; color; density and odor. The Chemical analyses included potassium ion and sodium concentrations of the water media.

Biomass Yield of Spirulina after Treatment in a Biofermentor

The yield of Spirulina grown in biofermentor was also measured.

Chemical Composition of the Spirulina Powder:

The Spirulina provide used was subjected to chemical analysis at the FAST Laboratory and supported with secondary data.

Microbiological Assay:

Microbial assay of Spirulina was conducted through standard plate count

Results:

Efficiency of the Biofermentor:

Temperature control:

Temperature control in closed system is characterized by a better temperature profile compared to open ponds. Temperature was maintained at 25 degrees centigrade. Temperature culture differed from open ponds, where the mechanism of evaporative cooling prevents an excessive increase in the temperature of the culture suspension, closed reactors behave as solar collectors. Also, the consumption of water was very much lower compared to the amount of water lost by open ponds through evaporation.

Length of the loop:

Since the photosynthetically produced oxygen cannot escape from the culture, the length of circuit exerts a marked influence on the oxygen concentration in the culture suspension. It has been shown that the short time in which the culture suspension takes to cover the whole circuit length enhances the productivity and biochemical composition of Spirulina.

Diameter of the tubes

Many experiments have been carried out with tubes of 10 and 5 cm diameter. These diameters were selected for achieving a surface to volume ratio similar to that of open ponds. These diameters were selected for achieving a surface to volume ratio similar to that of open ponds.

The Chambers:

A closed biofermentor was designed to increase the surface – to – volume ratio and to control the distribution of photon flux density during the daylight period. A high surface –to-volume ratio will allow the attainment of high biomass concentrations. The chambers were filled with culture suspensions up to 4 -5 cm from the top. The thickness of the culture was 10 mm, cell concentrations of 35 grams per 50 liters.

Mixing and deoxygenation of the culture suspension were achieved by bubbling air at the bottom of the biofermentor. It was reported that at the higher air flow rate, productivity influenced the O2 in the culture.

At the range of values tested, it seems that the turbulence obtained by air bubbling positively influences the growth of the culture suspension and by improving the cells.

Physical Characteristics of the Water Substrate:

Table 1 shows the physical characteristics of water substrate: Sample 1 before treatment, Sample 2 from chamber 1 and Sample 3 from chamber 2 of the biofermentor after two weeks of treatment process using *Spirulina platensis* as a biotreatment agent. Analysiss was conducted by the PHILRICE Analytical Services Laboratory

The results show that the three water samples are all basic with a pH level of 9.81, 9.97, and 9.93, respectively.

The temperatures of all the water samples are constant which read at 25 degrees centigrade. The color of samples 2 and 3 are both yellow while sample 1 (untreated) is brown. It is clear from the result that the color changed and it can be considered evidence that the water had been cleansed.

In a comparative study on the yield of *Spirulina* achieved in open ponds and in biofermentor, the better yield in the latter was attributed to temperature profile maintained inside the biofermentor. This was confirmed by the study of Torzillo *et al*. (1986), in their laboratory experiments which showed that the maximum biomass yield is obtained when *Spirulina* is grown at the optimal temperature of 25 degree Celsius.

As the culture temperature can hardly be modified in outdoors cultures, the use of closed system like the biofermentor that can maintain the temperature as close as possible to the optimum value throughout the day and around the year can be advantageous for obtaining higher biomass outputs.

When it comes to densities; sample 1 has a density of 0.911 gram per milliliter, sample 2 has 0.939 gram per milliliter and sample 3 has 0.938 gram per milliliter. The densities of water sample 2 and 3 were higher than the density sample 1 (untreated).

Two weeks after treatment, samples 2 and 3 became odorless compared to sample 1 which is odorous.

The samples (Sample 1, 2, 3) as shown on the Table 2 have a decreasing absorbance (at 420 nm) of 0.8163, 0.0574 and 0.0622, respectively. Their light absorbance is characterized by their color.

Table 2: Physical Analyses of the water substrates.

Physical components of water media	Sample 1 (Before treatment)	After treatment	
		Sample 2 (from Chamber 1)	Sample 3 (from Chamber 2)
pH level	9.81	9.97	9.93
Water temperature, degree centigrade	25	25	25
Color absorbance (at 420 nm)	0.8163	0.0574	0.0622
Color (visual)	Brown	Yellow	Yellow
Density	0.911 gram/milli liter	0.939 gram/milli liter	0.938 gram/milli liter
Odor	Odorous	Odorless(fishy)	Odorless(fishy)

Chemical Characteristics of the Water Samples:

Table 3 shows the chemical characteristics of three water substrate. The water substrates were analyzed with their available potassium and sodium ion concentrations in ppm (parts per million).Samples 1, 2, 3 have potassium ion concentrations of 134.3, 293.9 and 294.4, respectively. It is obvious that sample 2 and 3 have increased their potassium ion concentration after the treatment.

The sodium ion concentration of the samples: 1, 2, 3 on the other hand, were 2.69, 0.38 and 0.38, respectively. The sodium ion concentration was decreased after the treatment as seen on the result of the chemical analysis. The decrease of sodium ions and increase of potassium ion concentration can be attributed to the capacity of the spirulina to absorb sodium and made insoluble potassium compounds into soluble form. Higa (1994) reported that effective microorganisms through the process of fermentation resulted in the formation of simpler organic compounds such as amino acids, alcohol, sugars, organic acids and ester. It was also assumed that the fermentation process released active oxygen diluted in the wastewaters that consequently activate the biochemical reaction.

Organic compound consisting of carbon, hydrogen and oxygen with additive element of nitrogen, sulphur, phosphate, etc. tend to absorb oxygen. The available oxygen in the wastewater is consumed by the microorganisms to degrade compounds.

Table 3: Chemical analyses of water substrate.

Chemical components of water media	Sample 1 (Before treatment)	After treatment	
		Sample2 (from Chamber 1)	Sample 3 (from Chamber 2)
Potassium ion concentration, ppm	134.3	293.9	294.4
Sodium concentration, %	2.69	0.38	0.38

Biomass Yield of Spirulina Grown in a Biofermentor:

The yield of Spirulina grown in biofermentor has been 34.33g/50 liter (average of 3 months experimentation. It was reported from the results of other studies that the yield of Spirulina in open ponds ranged from 1.8 to 2.1 kilograms per square meter per year (Materassi, 1994).

The better performance of the biofermentor was due to the higher mean daily productivity and to an extended cultivation period. The closed system is characterized by a better temperature profile compared with open ponds since the culture in the biofermentor reaches the optimum temperature for growth (Torzillo *et al.*, 1986).

Table 4: Biomass Yield of *Spirulina platensis* grown in a biofermentor.

Biomass/Duration	Average
2^{nd} week	11.03 g/50 liter
4rth week	23.28 g/50 liter
Total	34.33 g/50 liter

Chemical Composition of Spirulina powder:

One aspect of high quality Spirulina is that the product must have a consistent chemical and physical property. Table 5 shows a typical chemical composition of Spirulina powder grown in a biofermentor together with some physical characteristics.

Table 5: Analysis of Spirulina powder inside the Biofermentor.

General Composition	%
Moisture	3-7
Protein	55-70
Fat (Lipids)	4-7
Carbohydrate	15-25
Minerals (Ash)	7-13
Fiber	4-7
Physical Properties	
Appearance	Fine powder
Color	Dark blue green
Odor and taste	Mild, like seaweed
Particle size	64 mesh through

Microbiological Assay of Spirulina powder:

The cultivation of microalgae in large ponds using water will undoubtedly invite contamination of the harvested product by pathogenic microorganisms. The final microbial load of the product depends on how carefully the culture and product are handled at the various stages. In a closed system, the product conformed to the standards set by different countries where the product is to be marketed (Belay *et al.*, 1994).

Table 6: Microbial assay of Spirulina powder grown in a biofermentor with slurry medium.

Moisture (%)	<7.0
Mold (#/g)	<100
Yeast (#/g)	<40
Coliform	neg
Salmonella	neg
Insect Fragments/10 g	neg
Rodent Hait (#/150g)	neg

Conclusion:

The biofermentor with its essential features was fabricated to treat wastewater by contacting same with *Spirulina platensis* to produce a protein biomass and at the same time degrade and convert biologically convertible matter into food source and for wastewater treatment.

The biofermentor has a better temperature profile compared to open ponds. Temperature was maintained at 25 degrees centigrade which favored the growth of Spirulina. There was a lesser water consumption compared to the amount of water lost by open ponds through evaporation.

The length of the loop of circuit of the biofermentors influenced the oxygen concentration and at the same time enhanced the productivity and biochemical composition of Spirulina.

The diameter of the biofermentor tubes of 10 and 5 cm can achieve a surface to volume ratio similar to that of open ponds. A high surface – to – volume ratio will allow the attainment of high biomass concentrations.

The pH levels of 9.97 and 9.93of the cultures were maintained in the biofermentor which enhanced the growth of Spirulina.

Based from the result of the chemical analysis of the culture medium, there was a decrease of ions and increase of potassium ion concentrations. This can be attributed to the capacity of Spirulina to absorb sodium and made insoluble potassium compounds into soluble form.

There was a marked increase on the biomass yield of Spirulina. Also its chemical composition showed a high protein, minerals and fiber but low carbohydrates and fats.

Based from the microbial assay, spirulina grown in a biofermentor conformed to the standards set by different countries where the product is to be marketed.

Recommendations:

1. Modify the machine in order to provide a modular biofermentor which does not require mechanical aeration and which operates continuously, thereby reducing operational costs.
2. Conduct more studies on the efficiency of the biofermentor using microalgae such as *Chlorella, Diatoms, Spirogyra* and other beneficial microorganisms such as yeasts in the treatment of a wide variety of biologically convertible matter such as piggery, distillery slops and brewery wastewater.

3. *Carry out further modification of the machine with the following features:*

The supports to be used in the bioreactor chamber should be perforated polyster or vinyl membranes having a plurality of orifices varying from 1.6 to 6.5 mm in diameter where each support is maintained apart from each other at a maximum distance of 1.3 mm.

Alternately, the supports to be used for the treatment of a substrate such as pig manure may comprise glass and/or polyester fiber opened lattices covered with PVC with openings of 1.6mm in diameter, such support permitting fixation of microorganisms exchange of oxygen. The lattice is maintained vertically by way of a mechanical support; a framing maintains each support flat and vertical; and the frames are wrapped together with polymer panels.

4. To conduct researches on the process of valorizing biologically convertible matter by producing biomass from microalgae and other beneficial microbes that can be utilized as food, feeds, fertilizer and other pharmaceutical products.

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The Biofermenting Machine which is core of the study is photographed on different perspectives.The said machine is aimed to treat wastewater, production of solid sludge for fertilizer and production of spirulina

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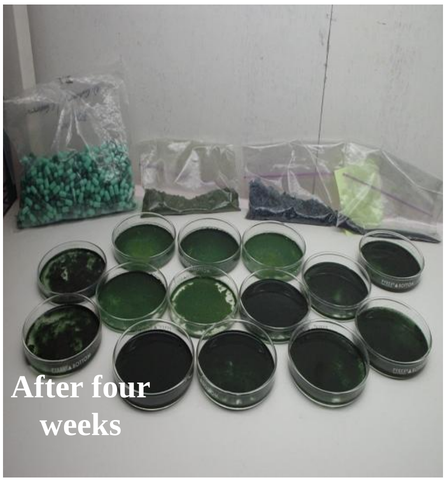

Physico-chemical changes on treated and *Spirulina* biomass yield wastewater.

Pond culture of *Spirulina*.