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Evaluation of Lab Scale Cultivation of *Spirulina* Using Different Substrates and its Nutritional Analysis

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A B S T R A C T

Spirulina is a multicellular, filamentous cyanobacterium with the ability to colonize environments that are unsuitable for many other organisms. It forms populations in freshwater and brackish lakes, as well as some marine environments, primarily alkaline saline lakes. Spirulina contains a high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B12), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides. Spirulina mainly grow in highly alkaline water. Lonar Lake is considered as the largest source of alkaline water in India so the water from Lonar Lake was used to isolate Spirulina. The isolation was carried out in Zarrouk's media. It is specific media used for the growth of Spirulina. Spirulina requires light and cold condition for proper growth so these conditions were maintained during the growth. The media was inoculated with the water sample and kept for incubation at 22˚C for over 15 days. The media was also supplemented with some trace metals for good yield. After incubation period the media was observed under microscope to confirm the Spirulina was isolated. After the isolation was successful Spirulina was cultivated by using various substrates such as cow urine, cheese whey, tap water etc. The substrate were added in the media and the media was inoculated with isolated culture of Spirulina .The media was kept for incubation in similar conditions used during isolation. After 15 days the sample were studied for their protein content, carbohydrate content.

Keywords: *Spirulina* cultivation, Nutritional analysis, Substrate, Zarrouk's media, Protein estimation, Carbohydrate content

1. INTRODUCTION

Spirulina is a multicellular, filamentous cyanobacterium that can colonise environments that are unsuitable for many other organisms. It can be found in freshwater and brackish lakes, as well as some marine environments, primarily alkaline salt lakes [1]. *Spirulina* contains a high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B12), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides [2]. During the 16th century, the Aztecs (Mexicans) referred to *Spirulina* as tecuitlatl, which means stone excrement. Later, due to the outbreak of contagious disease, people adopted new customs such as new food, religious and social changes, and the topic of tecuitlatl was discontinued. *Spirulina* means "small cakes made of mud like algae, which has a cheese-like flavour, and that native took out of the lake to make bread". They are dried into cakes known as "Diha" or "Die" [3]. *Spirulina* are classified as Cyanobacterium (length: 50-500 m, width: 3-4 m), a Gram-negative bacterium that performs photosynthesis similar to that of plants. *Spirulina* is a filamentous, photosynthetic, autotrophic, unbranched, multicellular blue-green algae that uses symbiotic bacteria to fix atmospheric nitrogen. Taxonomically, "*Spirulina*" refers to two species of Cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima*. Cyanobacterium's colour is determined by phycocyanin, a blue photosynthetic pigment, as well as other natural pigments like chlorophyll a, β-carotene, xanthophylls, and allophycocyanin. *Spirulina* is primarily found in natural lakes with high pH values, ranging from 8 to 10, around the world [4]. Because of its nutritional value, *Spirulina* has long been used as a food supplement by humans and animals in a variety of forms such as health drinks, tablets, and powders. Cyanobacteria tablets are sold in Europe, Japan, and North America. *Spirulina* is known to be the richest source of protein and vitamins, and it can be used to treat malnourished children. *Spirulina* is rich in protein, vitamin B12, iron, and essential amino acids, making it a "food of the future" and ideal for astronauts according to NASA [5]. [6] has studied the potential of *Spirulina* in aquaculture as source of nutrient. Conventional fish feed is very high in cost so *Spirulina* is used as source of feed for fish *Spirulina* having high protein content is very effective nutrient source. In this study the use of *Spirulina* in removing heavy metals from water is also studied. In this study it is found out that *Spirulina* is help in reducing usage of water in fish farms. Because of its nutritional value, *Spirulina* has long been used as a food supplement by humans and animals in a variety of forms such as health drinks, tablets, and powders. Cyanobacteria tablets are sold in Europe, Japan, and North America. *Spirulina* is known to be the richest source of protein and vitamins, and it can be used to treat malnourished children. *Spirulina* is rich in protein, vitamin B12, iron, and essential amino acids, making it a "food of the future" and ideal for astronauts according to

NASA [5]. *Spirulina* has a higher quality protein content (55-70 percent of dry weight) than other commonly used plant sources such as dry soybeans (35%), peanuts (25%), and grains (8-10%). The biochemical composition of *Spirulina* is summarised as follows [7]:

Proteins: *Spirulina* contains unusually high levels of protein, ranging from 55 to 70 percent by dry weight, depending on the source. It is a complete protein, containing all essential amino acids but with lower amounts of methionine, cystine, and lysine when compared to standard proteins such as meat, eggs, or milk; however, it outperforms all standard plant proteins, including those from legumes.

Essential fatty acids: *Spirulina* contains a high concentration of polyunsaturated fatty acids (PUFAs), accounting for 1.5-2.0 percent of 5-6 percent total lipid. *Spirulina* is particularly high in linolenic acid (36% of total PUFAs), as well as linolenic acid (ALA), linoleic acid (36% of total PUFAs), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA).

Vitamins: *Spirulina* possess vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E.

Minerals: *Spirulina* contains potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc [8].

This research revolves around the following objectives:

- To Cultivate of *Spirulina* using different substrates.
- To Harvest *Spirulina.*
- Nutritional analysis of *Spirulina*.

2. MATERIALS AND METHODOLOGY

2.1. Materials Required:

Glassware (conical flask, beaker, petri plate, test tube, cuvette, reagent bottle) Instruments: (Autoclave, pan balance, incubator, laminar air flow, spectrophotometer), Chemicals (Follin Lowry reagent, Zarrouk's medium Ascorbic acid, NaOH, O-phthaldialdehyde (OPA) and mercaptopropionic acid (MPA)).

2.2. Isolation of Spirulina:

Spirulina was isolated from alkaline water. Alkaline water was collected from water samples from Lonar Lake. The water was collected in a sterile container. For isolation of *Spirulina* Zarrouk's media was used. The media was prepared and sterilized in autoclave for 15 min at 121˚C. Serial dilution of the water sample were prepared and inoculated in the media. The media was kept for incubation at 22˚C for 15 days under light conditions.

2.3. Microscopic observation:

The isolation of *Spirulina* was confirmed by microscopic observation of the media. A smear was prepared on clean glass free slide. It was allowed to air dry and observed under light microscope.

2.4. Sample preparation:

Different substrates used in this study are collected from local areas viz. rain water, tube well water, cow urine etc. Serial dilution is made and are added in sterile Zarrouk's medium inoculated with 1ml of isolated *Spirulina* culture at pH 9.5 ± 2, temperature 20˚C ± 2 and light intensity 2-3 Klux. pH of the culture varies regularly, so it is adjusted daily to range between 8.5-10 pH. Agitation of the culture is done by manual shaking. The flask was kept for incubation at 22˚C for 15 days in light conditions.

2.5. Total Protein:

Total protein estimation is done by Folin-Lowry method. All the reagents were prepared fresh as per the protocol. The folin reagent was prepared by diluting the standard folin phenol reagent with distilled water 2.5 ml of reagent was diluted with 7.5 ml of distilled water. Bovine serum albumin was used as standard solution. The standard solution is added to the tubes and reagents were added to all the tubes. The sample was taken in a tube. The absorbance was recorded against blank. The concentration of protein was taken from plotting the absorbance against standard concentration at 660 nm.

2.6. Carbohydrate content:

The Carbohydrate content is determined using DNSA method using a standard curve of glucose. DNSA reacts with carbohydrate present in the sample to form amino nitro salicylic acid which has strong absorbance at 540 nm. The concentration of carbohydrate is determined by plotting standard curve of glucose.

3. RESULTS

The *Spirulina* was isolated from the alkaline water sample from Lonar Lake. It was confirmed by microscopic observation. *Spirulina* mainly grow in highly alkaline water. Lonar Lake is considered as the largest source of alkaline water in India so the water from Lonar Lake was used to isolate *Spirulina*. The isolation was carried out in Zarrouk's media. It is specific media used for the growth of *Spirulina*. *Spirulina* requires light and cold condition for proper growth so these conditions were maintained during the growth. The media was inoculated with the water sample and kept for incubation at 22o C for over 15 days. The media was also supplemented with some trace metals for good yield. After incubation period the media was observed under microscope to confirm the *Spirulina* was isolated. After the isolation was successful *Spirulina* was cultivated by using various substrates such as cow urine, cheese whey, tap water etc. The substrates were added in the media and the media was inoculated with isolated culture of *Spirulina*. The media was kept for incubation in similar conditions used during isolation. After 15 days the sample were studied for their protein content, carbohydrate content, chlorophyll content. The detailed results of all the tests are as follows:

Table 1 - Protein Estimation by Folin-Lowry method (Standard Solution)

Sr. No.	Standard Solution	Distilled Water	Lowry's Reagent		Folin's raegent		Conc.	Absorbance at 660 nm
1	0	1.0	5 ml	Incubation at room temp for 10 min	0.5 ml	Incubation at room temp in dark for 30 min	0	00
2	0.2	0.8	5 ml		0.5 ml		50	0.1
3	0.4	0.6	5 ml		0.5 ml		100	0.2
4	0.6	0.4	5 ml		0.5 ml		150	0.3
5	0.8	0.2	5 ml		0.5 ml		200	0.41
6	1.0	0	5 ml		0.5 ml		250	0.5

Table 2 - Protein Estimation by Folin-Lowry method (Sample)

Sr. No.	Sample	Distilled Water	Lowry's Reagent		Folin's raegent		Conc.	Absorbance at 660 nm
1	0.5 ml (Tap water)	0.5 ml	5 ml	Incubation at room temp for 10 min	0.5 ml	Incubation at room temp in dark for 30 min	200	0.40
2	0.5 ml (Cow Urine)	0.5 ml	5 ml		0.5 ml		175	0.35
3	0.5 ml (Cheese whey)	0.5 ml	5 ml		0.5 ml		190	0.38

Table 3 - Carbohydrate estimation by DNSA method (Standard Solution).

Sr. No.	Standard Solution	Distilled Water	DNSA		Distilled Water	Conc.	Absorbance at 660 nm
1	0	1.0	1 ml	Incubation at room temp for 10 min	3 ml	0	00
2	0.2	0.8	1 ml		3 ml	50	0.05
3	0.4	0.6	1 ml		3 ml	100	0.10
4	0.6	0.4	1 ml		3 ml	150	0.15
5	0.8	0.2	1 ml		3 ml	200	0.20
6	1.0	0	1 ml		3 ml	250	0.25

Table 4 - Carbohydrate estimation by DNSA method (Sample).

Sr. No.	Sample	Distilled Water	DNSA		Distilled Water	Conc.	Absorbance at 660 nm
1.	0.5 ml (Tap water)	0.5 ml	1 ml	**Incubation at room temp for 10 min**	3 ml	50	0.05
2.	0.5 ml (Cow Urine)	0.5 ml	1 ml		3 ml	60	0.06
3.	0.5 ml (Cheese whey)	0.5 ml	1 ml		3 ml	40	0.04

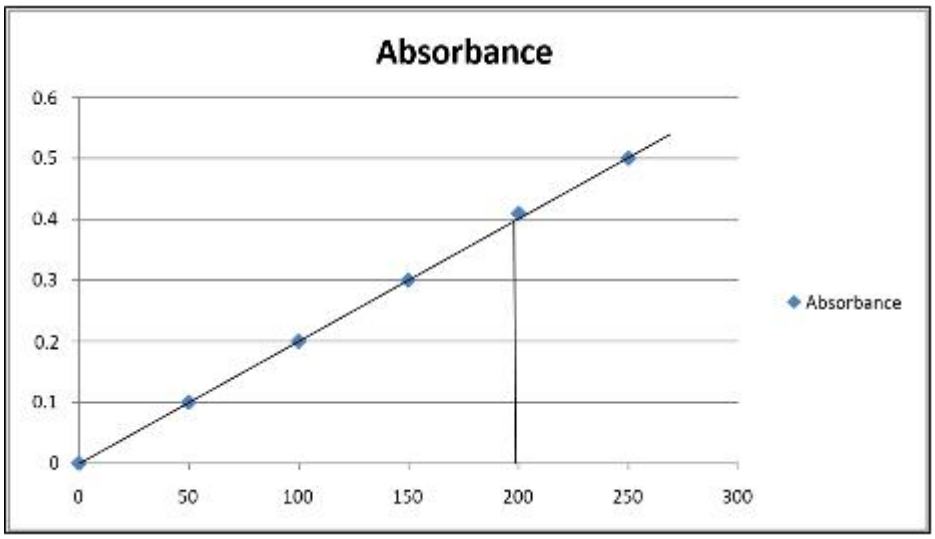

Fig. 1 – Lowry Test (Tap water)

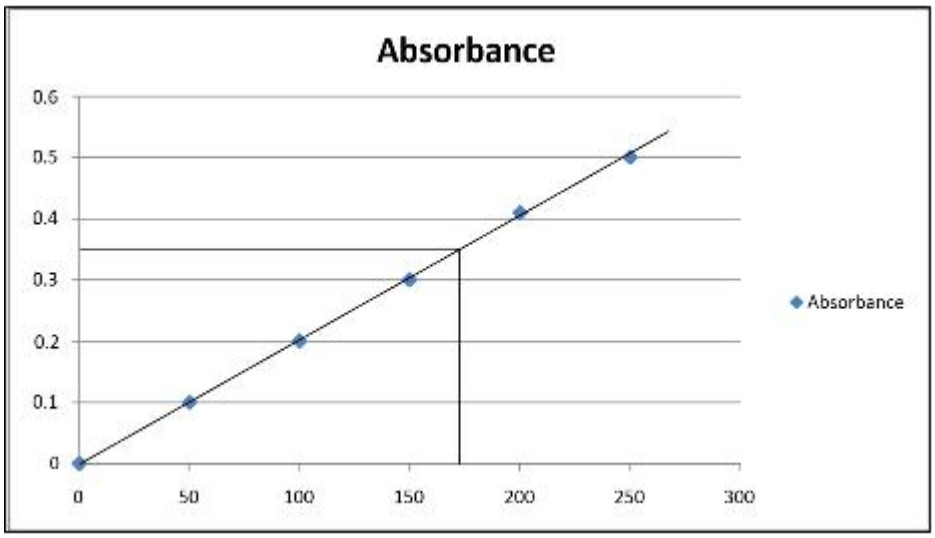

Fig. 2 – Lowry Test (Cow urine)

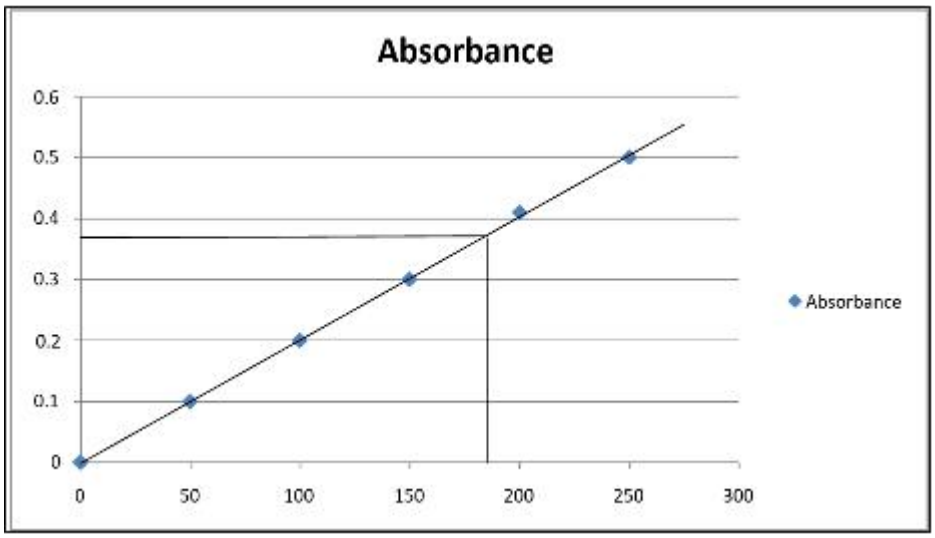

Fig. 3 – Lowry Test (Cheese whey)

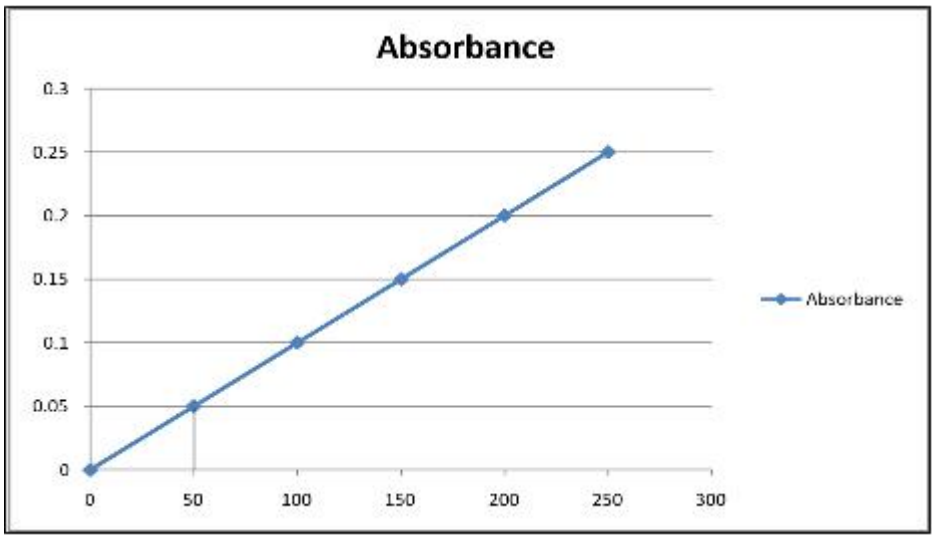

Fig. 4 – DNSA Test (Tap water)

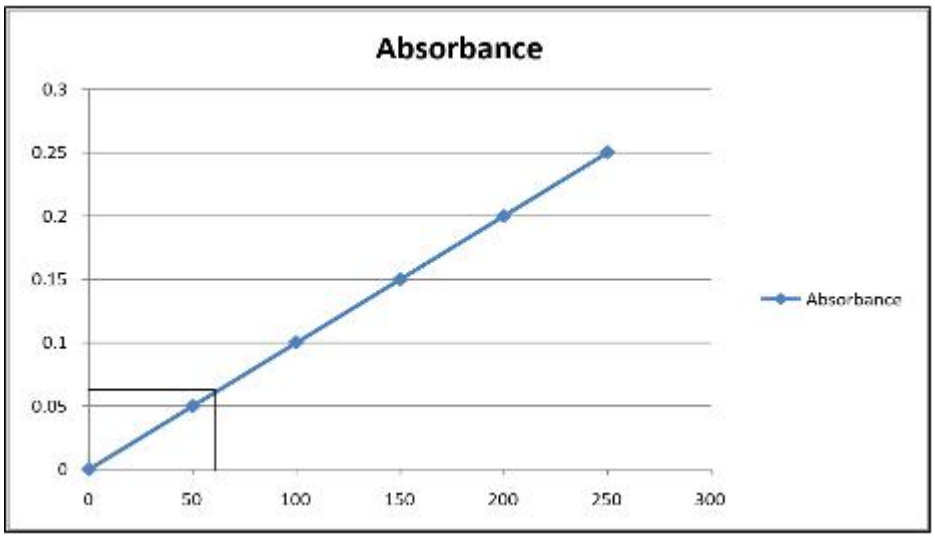

Fig. 5 – DNSA Test (Cow urine)

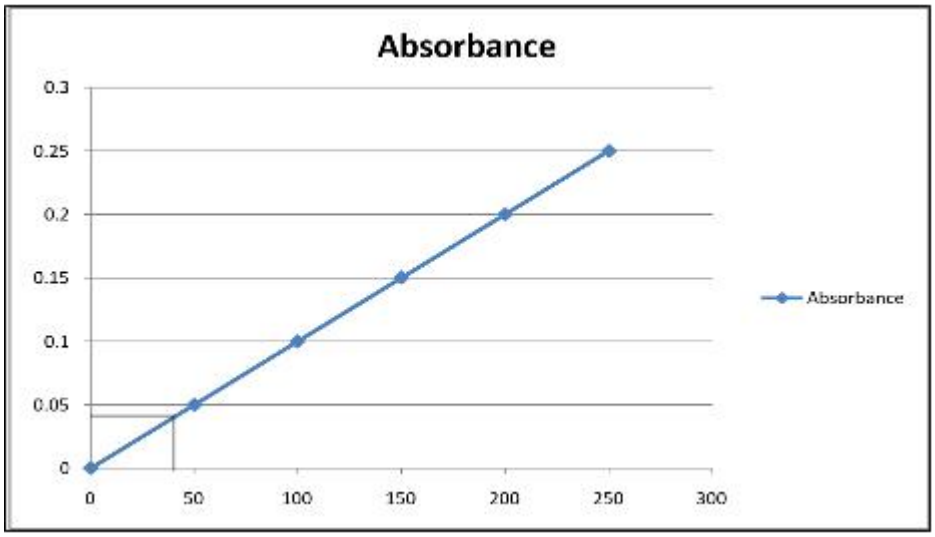

Fig. 6 – DNSA Test (Cheese whey)

Concentration of protein

According to the graph the Concentration of protein in different samples grown with different substrates is

Spirulina grown with tap water as substrate is 200 µg/ml

Spirulina grown with cow urine as substrate is 175 µg/ml

Spirulina grown with cheese whey as substrate is 190 µg/ml

From the graphs and the tables, it can be concluded that the highest amount of Protein was present in *Spirulina* grown with tap water as substrate (200 µg/ml).

Concentration of Carbohydrate:

According to the graph the Concentration of Carbohydrate in different samples grown with different substrates is

Spirulina grown with tap water as substrate is 50 µg/ml

Spirulina grown with cow urine as substrate is 60 µg/ml

Spirulina grown with cheese whey as substrate is 40 µg/ml

From the graphs and the tables, it can be concluded that the highest amount of Carbohydrate was present in *Spirulina* grown with cow urine as substrate (60 µg/ml).

4. DISCUSSION AND CONCLUSION

Since *Spirulina* has very high nutritional value it is used as food source for astronauts. Astronauts use *Spirulina* as protein supplement in space. One major additional advantage of *Spirulina* over most other food stuffs is that it can often be grown in situ and therefore provide a longer-term solution to nutritional strategy development. *Spirulina* can be used as medicine against many problems such as obesity, allergy and fertility problems. It has also been used treat many diseases due to therapeutic characteristics such as strong anti-inflammatory, anti-oxidant properties, immune -boosting qualities and has been recommended as a nutritional supplement to improve the performance of athletes. *Spirulina* Furthermore, *Spirulina* may boost your gut health and has probiotic characteristics, which may have an antioxidant impact by neutralising free radicals (unstable atoms that can harm cells, causing sickness and ageing) [9,13]. *Spirulina* has potential to be used as feed for animals as well as in fisheries. It can be used in water treatment to remove heavy metals from industrial water and make it appropriate for drinking. As *Spirulina* has so many benefits for humans as well as animals it potential should be properly utilized if used properly it can remove problem of malnutrition and hunger from world [7, 10]. *Spirulina* can be cultivated in otherwise rather barren areas without consuming valuable, clean fresh water, because it thrives best even in highly saline water. Other advantages of *Spirulina* cultivation are non-competition with crops for arable land and the possibility of using industrial effluents as nutrient source for their growth therefore it is a sustainable alternative for obtaining renewable energy source for future. The various small scale production systems can be adapted to many post-emergency situations. Indeed, many of the more vulnerable communities – e.g. coastal communities prone to storms, flooding and tsunami damage, as well as arid areas vulnerable to crop-failure and overgrazing show particular potential for small-scale *Spirulina* production development [11].

There is evidence that supplementing with *Spirulina* can lower cholesterol levels and improve overall metabolic health. A 2013 study examined patients with newly high cholesterol levels. A 2019 meta-analysis indicated that *Spirulina* supplementation lowers overall cholesterol levels [12].

Many scientists are researching that *Spirulina* may also protect against cancer, may decrease allergic rhinitis, may help with anemia, may regulate sugar levels, help with Fatty Liver Disease, may help with weight loss and improve oral health [13].

APPENDIX

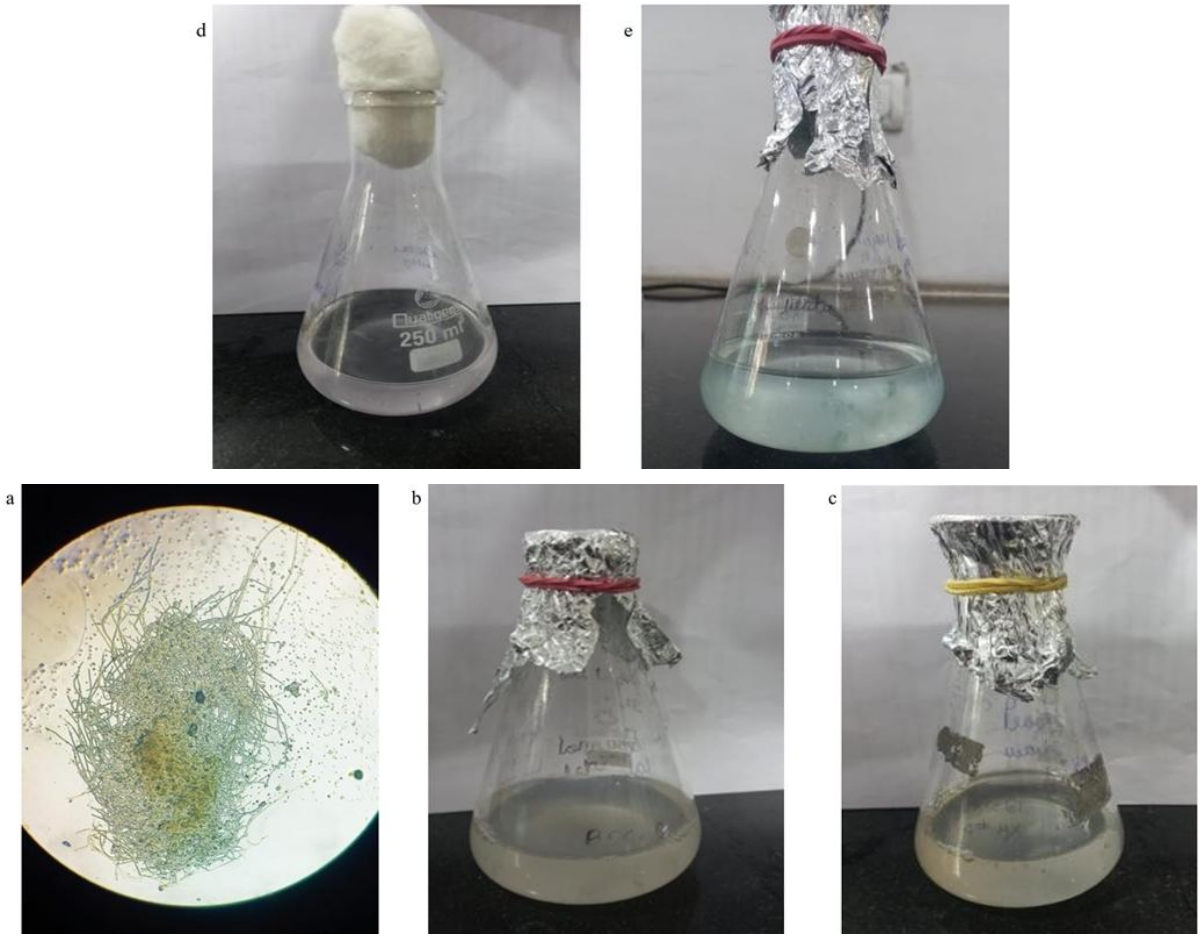

Fig. 7 - (a) Microscopic observation of *Spirulina*; (b) Isolation of *Spirulina*; (c) Cow urine as substrate; (d) Tap water as substrate

(e) Cheese whey as substrate

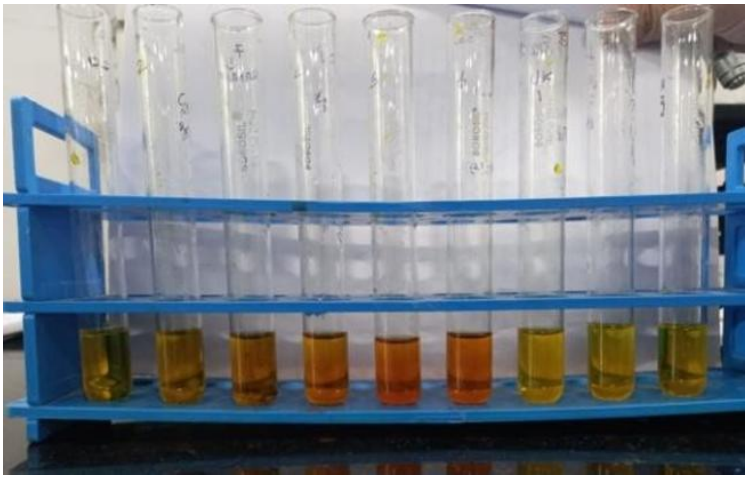

Fig. 8 – DNSA Test

Fig. 9 – Lowry Test

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