Protocol for culturing *Spirulina* under laboratory conditions

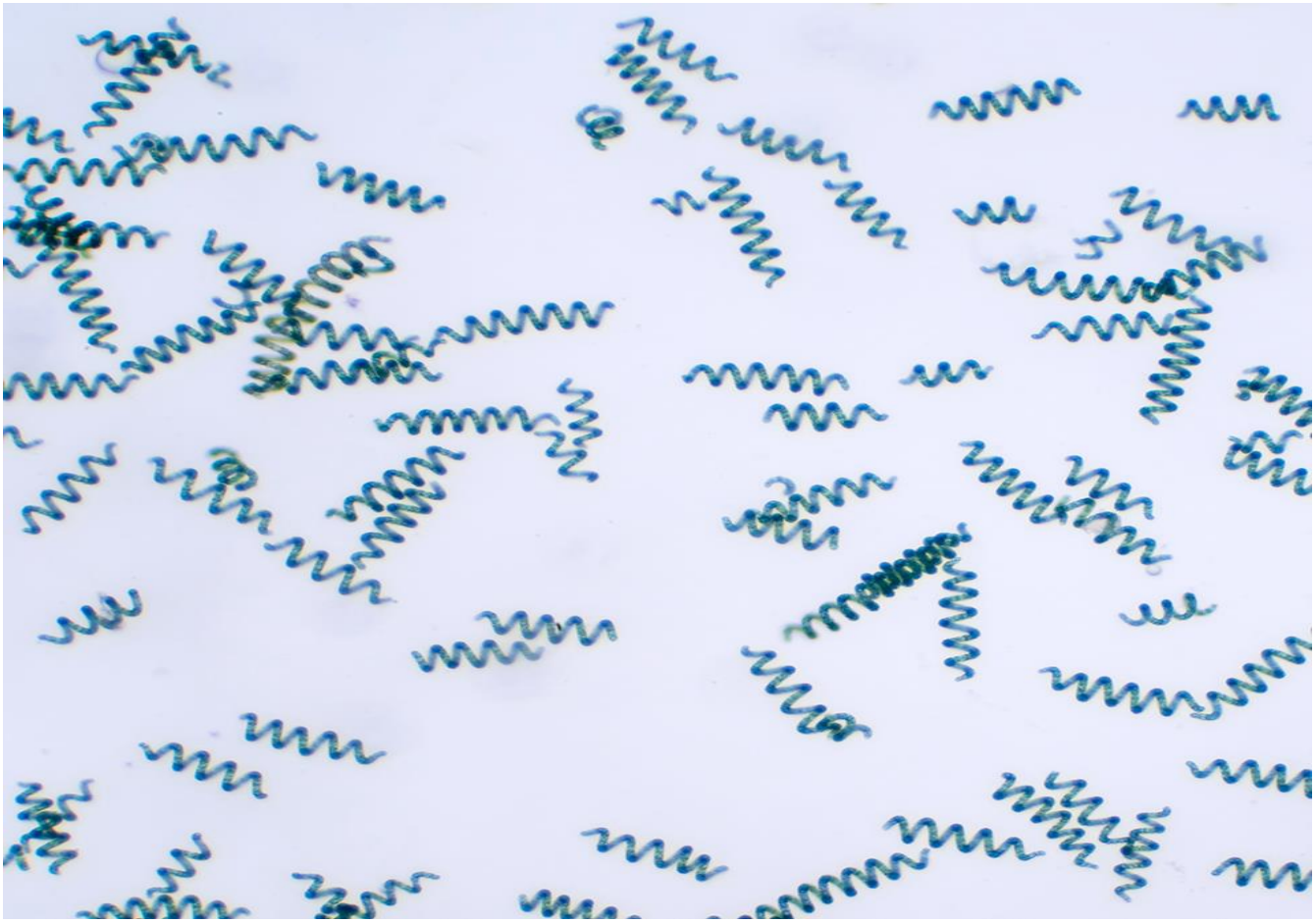

Plate 1: *Spirulina* strands as seen under an inverted microscopic (200X)

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Laboratory Culture Protocol for *Spirulina*

1.0 INTRODUCTION

1.1 Preamble

Spirulina is a blue-green algae genera with several species, but the predominant ones are *Spirulina platensis*, *Spirulina fusiformis* and *Spirulina maxima*. Among several algae genera, *Spirulina* is exploited and has become a commercially important filamentous cyanobacterium due to its inherent ability to accumulate resources such as proteins, minerals, polyunsaturated fatty acids (PUFAs) and a few vitamins (Srivani *et al*., 2017). Hence, several methods have been tried to culture these algae both at laboratory, mass and industrial scales (Hala *et al*., 2015; Borowitzka & Borowitzka, 1988; Avigad & Amos, 1988). Unique attributes of *Spirulina* are autotrophic and alkallophyletic and hence the energy requirements to maintain its culture under laboratory conditions are not necessary. The sunlight that naturally occurs under natural conditions is therefore more than adequate for its growth and replication. Thus, mineral replenishment to ensure ample nutrients in the growth medium only needs to be supplemented. Yet, another important ingredient in the culture medium is pH; *Spirulina* prefers to grow in extreme alkaline medium under sunlight, and this restrains most of the facultative bacteria and fungi which otherwise contaminate the culture medium. Therefore, little sophistication is required for its maintenance under laboratory conditions.

Spirulina has unique attributes that result in its mass biomass production e.g. its photosynthetic pigment (phycocyanin), adaptability, plasticity and its asexual reproduction by binary fission (Srivani *et al*., 2017). Due to its increasing popularity as an abundant nutritional biological resource, it is being used in poultry, aquaculture, animal feed, wastewater treatment and agriculture (Habib *et al*., 2008; Mori *et al*., 1989), and also supplementing human food requirements. Therefore, in view of the importance of *Spirulina*, it is worthy devising a simple and affordable protocol for culturing this alga in our local laboratory environmental setting.

1.2 Purpose of the culture protocol

Spirulina occurs in association with other algae in the wild and hence it has to be identified, isolated and cultured following the optimized protocol. The optimal protocol for this purpose is constituted of several chemical components that aim to eliminate other algae while at the same time maximizing the growth of *Spirulina*. Isolation and culturing of *Spirulina* is aimed at having a desired strain of this algae that will later be mass produced and used for the intended purpose of developing a "Wastewater Cleaning Model" and using the accumulated biomass for processing into a fish feed ingredient.

1.3 Objectives of the activity

The overall objective of the study was to come up with the right culture media that enables *Spirulina* to optimally grow after isolating it from other organisms especially other algae.

Specifically, the studies were aimed at:

i. isolating the right *Spirulina* strain.
ii. optimizing nutrient media for faster growth of *Spirulina*.

2.0 MATERIALS AND METHODS

Water was drawn using 1 L glass jars from National Water and Sewerage Corporation (NWSC) maturation ponds at Kirinya Sewage Works in Jinja, Inner Murchison Bay in Kampala, and Entebbe Bay in Entebbe. From these, 50% of each sample set was taken to NaFIRRI Analytical Laboratory and NARL-Kawanda Molecular Laboratory for *Spirulina* identification, isolation and culturing. The sampled water was concentrated by filtering through a 55µm phytoplankton net and observed for identification and isolation under an inverted or dissecting microscope (Plate 2).



Plate 2: Microscopic observation of *Spirulina* for identification and isolation

Spirulina strands were sorted from the concentrate with the aid of a micro pipette under an inverted microscope (Plate 3(a) magnification of 20X) and/or a dissecting microscope (Plate 3(b) magnification of 10X), and the sorted strands were placed in petri dishes with sterile culture media to clean them of any foreign matter or debris and other algae species.



Plate 3: (a) Inverted microscope; (b) Dissecting microscope

This was done three to four times to ensure clean strands of *Spirulina*, which were then placed in plastic "Culture Well Cells" (CWCs) (of 2 mL capacity; Plate 4) and the constituted culture medium was added to each well.



Plate 4: Culture Well Cells of 2 mL. Each set was composed of 4x6 well cells (i.e. 24 pieces)

The CWCs were covered with a lid and then sealed with a masking tape around the joint of the two covers (bottom & top), and placed in natural light on the windowsill for 12 hours. After three days, the culture solution in the CWCs appeared turbid, and as the days went by, the culture medium in the CWCs turned green due to algal biomass growth. Algae samples were scooped from these CWCs with the aid of a micro pipette (Plate 5) and observed under an inverted microscope (Plate 3(a)) at a magnification of 20X, and/or a dissecting microscope (Plate 3(b)) at a magnification of 10X.

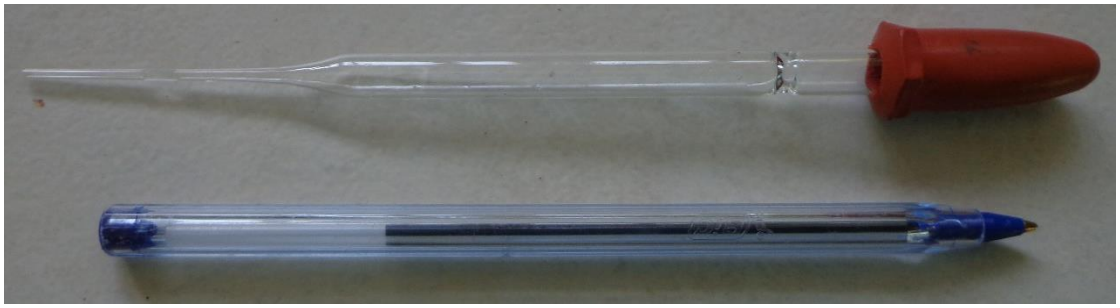

Plate 5: Micro pipette (Its size is compared with a Bic Pen)

The *Spirulina* strands were sorted, placed in clean petri dishes with distilled water to clean them, and were then placed in 50 mL Tissue Culture Flasks (TCFs) (Plate 6).



Plate 6: Tissue Culture Flasks (capacity of 50 mL)

40 mL of the culture medium was added to the TCFs which were then placed on a windowsill where they were exposed to natural light on a daily basis. To note is that the setup is not to be exposed to direct sunlight (i.e. in the open) since exposure to direct sunlight resulted into dying off of all the sorted *Spirulina*. The flasks were occasionally subjected to a gentle swirl so that the spirals are not suppressed by the unwanted yet occurring *Microcystis*, and to avoid the spirals sticking at the bottom of the TCFs. After six days, sub samples were taken from the TCFs to check for presence and growing *Spirulina* strands. After every two days, *Spirulina* strands were sorted and placed on petri dishes that had sterile culture media. This was done several times to get clean (uncontaminated) strands which were then placed in clean TCFs with fresh culture medium. The procedure is repeated until clean/pure strands of *Spirulina* are finally got. The clean strands are finally put in bigger glass containers for mass production of *Spirulina*. It is from this stage that *Spirulina* can be harvested and used for various purposes or subjected to molecular analyses.

Preparation of the liquid culture medium

The liquid culture medium was constituted of the following chemicals with their indicated weights as summarized in Table 1:

Table 1: Constitution of the culture medium for Spirulina

S. No.	Chemical Formula	Common Name	Weight (g)
(A) Stock solution			
1	$NaNO_3$	Sodium nitrate	17.000
2	K_2HPO_4	Dipotassium phosphate/Potassium hydrogen phosphate	3.100
3	$MgSO_4*7H_2O$	Magnesium sulfate heptahydrate	7.400
4	$CaCl_2*2H_2O$	Calcium chloride	3.700
5	Na_2CO_3	Sodium carbonate (or washing soda or soda ash)	2.000
6	$Na_2MgEDTA$	Magnesium Disodium EDTA	0.110
7	$C_6H_8O_7$	Citric acid	0.600
8	$FeNH_3$ citrate plus micro elements	Ferric ammonium citrate plus micro elements	0.600
(B) Micro-elements			
9	H_3BO_3	Boric acid	2.860
10	$MnCl_2*H_2O$	Manganese(II) chloride (Monohydrate)	1.810
11	$ZnSO_4*7H_2O$	Zinc sulphate (Heptahydrate)	0.222
12	$Na_2MoO_4*2H_2O$	Sodium molybdate (Dihydrate)	0.390
13	$CuSO_4*5H_2O$	Copper sulfate (Pentahydrate)	0.079
14	$Co(NO_3)_2*6H_2O$	Cobalt(II)Nitrate (Hexahydrate)	0.049

Each of the stock solution chemicals was separately weighed using a digital weighing scale and separately dissolved in 100 mL of distilled-deionized water. The microelements were weighed separately, mixed and dissolved in 1,000 mL of distilled-deionized water. To 440mls of distilled water in a Glass Digestion Bottle (capacity of 500 mL; Plate 7), 1 ml of each of the solutions was added and the level marked.



Plate 7: Digestion Bottle (withstands high temperatures as in the autoclave)

The mixture was autoclaved at 120°C for 30 minutes. After cooling, 0.84g of $NaHCO_3$ dissolved in 50mls of distilled water was added after filtering it through 0.22µm millipore filter papers. The mixture was swirled gently to make a homogeneous solution, and this constituted the final *Spirulina* Culture Medium.

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