

Fishery and aquaculture

level III

**Based on July 2022, Version- I Occupational
Standard (OS)**



**ModuleTitle: Producing algal and live-feed
cultures**

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Introduction to the module

The Program is designed to develop the necessary knowledge, skills and attitude of the trainees to the standard required by the occupation. The contents of this program are in line with the occupational standard. The Trainees who successfully completed the Program will be qualified to work as Fishery and Aquaculture technician with competencies elaborated in the respective OS. Graduates of the program will have the required qualification to work in the Agriculture Sector in the field of Fishery and Aquaculture.

The prime objective of this training program is to equip the Trainees with the identified competences specified in the OS. Graduates are therefore expected to Maintain water quality, Establish fish farm, Process and utilize fish by- products, Apply aquaculture bio security measure, Perform post-harvest handling, Produce algal and live feed cultures, Apply Agricultural Extension service for rural development, Apply Digital Technology in Agriculture and Prevent and Eliminate MUDA in accordance with the performance criteria and evidence guide described in the OS.

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LG # 17	LO #1: Prepare for algae and live-feed production
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Instruction sheet-1

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Production schedule
- Preparing tools, materials and equipment
- Labour and resource requirements
- Occupational health safety(OHS)
- Risk factors affecting quality of culture
- Planning to minimize risks
- Assembling and commissioning culture systems

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Production schedule
- Prepare tools, materials and equipment
- Labour and resource requirements
- Occupational health safety(OHS)
- Risk factors affecting quality of culture
- Planning to minimize risks
- Assembling and commissioning culture systems

Learning Instructions:

- Read the specific objectives of this Learning Guide.
- Follow the instructions described below.
- Read the information written in the information Sheets
- Accomplish the Self-checks
- Perform Operation Sheets

Information Sheet- 1

1.1 Production schedule

Introduction

Live food organisms include all plants(Phytoplankton) and animal (zooplankton) lives grazed up on by economically important fishes. Phytoplankton are generally eaten by zooplankton. Thus phytoplankton forms the basis of the food chain. Plankton, both phyto- and zooplankton are critical to the health of a marine ecosystem. Live phytoplankton absorbs carbon dioxide and excess nutrients such as nitrates, phosphates and heavy metals. It cleans water as it grows, as it grows it creates food for the tank. Phyto uses the waste nutrients to produce Essential Fatty Acids. These fatty acids are critical to the survival of many marine animals. Zooplankton eat the phytoplankton and in turn get eaten by numerous fish and corals. This is how the essential fatty acid nutrition produced by phytoplankton get into the food chain.

Live Feeds are the highest form of feeding possible. It provides the nutrition found in nature, it elicits natural feeding responses from your tank animals and contributes less to nutrient loading caused typically by feeding or over feeding. In addition, it will create a natural ecosystem in your tank that will make the tank ecosystem healthier and stronger.

PhytoPlankton: At best guess there are over 100,000 to 1 million different types in the oceans. They all have different characteristics that make them unique and important. Some are red, brown, green, blue-green, etc, some swim, some glide others float. They all have different nutritional profiles. As mentioned before, PhytoPlankton will consume waste nutrients such as nitrates, ammonia, phosphates

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and carbon dioxide in the water, which they turn into Essential Fatty Acids (EFAs) that are critical to the marine food chain.

1.1.1 Algal culture techniques

Algae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. The terminology used to describe the type of algal culture include:

- **Indoor/Outdoor.** Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.
- **Open/Closed.** Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.
- **Axenic** (=sterile)/Xenic. Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. The latter makes it impractical for commercial operations.
- Batch, Continuous, and Semi-Continuous.

These are the three basic types of phytoplankton culture which will be described in the following sections.

1.1.2.1.Batch culture

The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 l flasks, 5 and 20 l carboys, 160 l cylinders, 500 l indoor tanks, 5,000 l to 25,000 l outdoor tanks (Figs.1)

Table 1. Inoculation schedule for the continuous production of micro-algae using the batch technique. Every week a serial is initiated with 4 or 7 test tubes, depending on whether a new culture is required for harvesting every 2 days or daily.

Days	New culture available for harvest every 2 days				Harvest required daily							
1	T	t	t	T	T	T	t	t	t	t	T	
2	T	t	t	T	T	T	t	t	t	t	T	
3	T	t	t	T	T	T	t	t	t	t	T	
4	T	t	t	T	T	T	t	t	t	t	T	
5	T	t	t	T	T	T	t	t	t	t	T	
6	T	t	t	T	T	T	t	t	t	t	T	
7	T	t	t	T	T	T	t	t	t	t	T	
8	E	e	e	E	E	E	e	e	e	e	E	
9	E	e	e	E	E	E	e	e	e	e	E	
10	E	e	e	E	E	E	e	e	e	e	E	
11	E	e	e	E	E	E	e	e	e	e	E	
12	E	e	e	E	E	E	e	e	e	e	E	
13	E	e	e	E	E	E	e	e	e	e	E	
14	E	E	e	E	E	E	E	e	e	e	E	
15	E	E	e	E	E	E	E	E	e	e	E	
16	F	E	E	E	F	E	E	E	E	e	E	
17	F	E	E	E	F	F	E	E	E	E	E	
18	F	f	E	E	F	F	f	E	E	E	E	
19	F	f	E	E	F	F	f	f	E	E	E	
20	F	f	f	E	F	F	f	f	f	E	E	
21	F	f	f	E	F	F	f	f	f	f	E	
22	F	F	f	F	F	F	F	f	f	f	F	
23	F	F	f	F	F	F	F	F	f	f	F	
24	L	F	F	F	L	F	F	F	F	f	F	
25	L	F	F	F	L	L	F	F	F	F	F	
26	*	L	F	F	*	L	L	F	F	F	F	
27		L	F	F		*	L	L	F	F	F	
28		*	L	F			*	L	L	F	F	
29			L	F				*	L	L	F	
30			*	L					*	L	L	
31				L						*	L	
32				*							*	

t = 20 ml test tube

e = 250 ml erlenmeyer flask

E = 2 l erlenmeyer flask

f = 30 l fiberglass tank

F = 300 l fiberglass tank

L = use for larval feeding or to inoculate large volume (> 1.5 t) outdoor tanks

* = termination of 300 l fiberglass tank

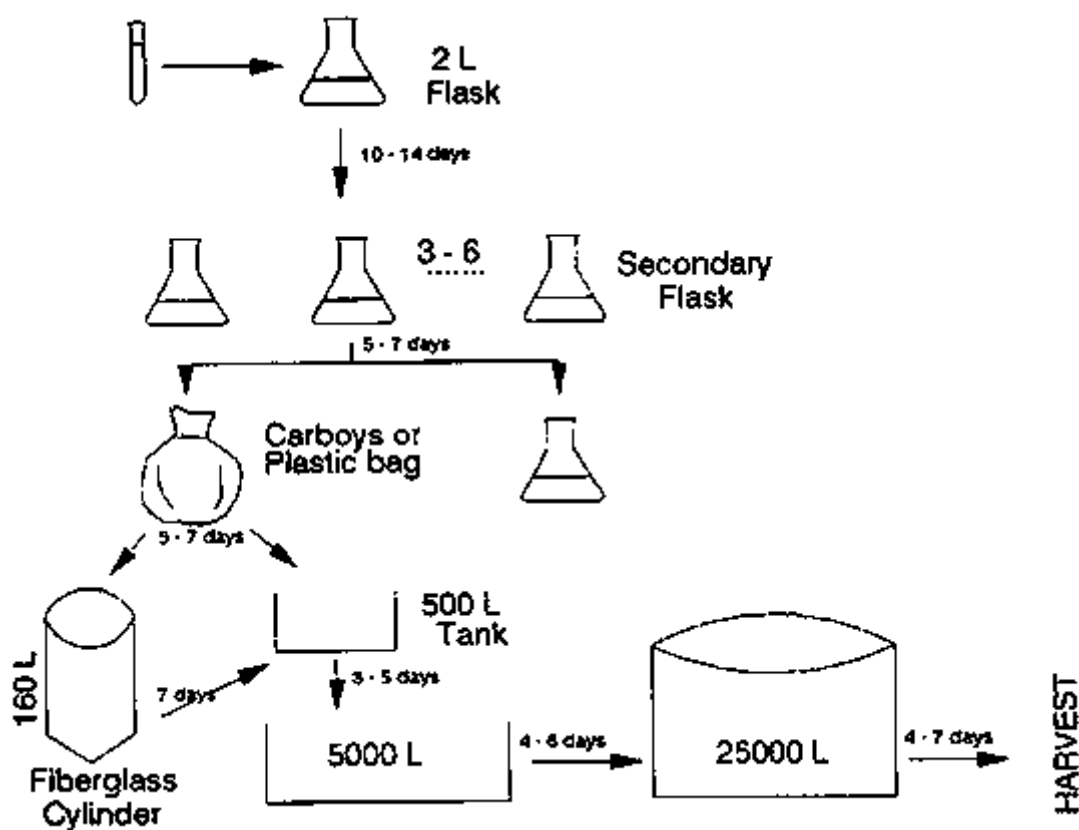


Figure 1.1: Production scheme for batch culture of algae

According to the algal concentration, the volume of the inoculum which generally corresponds with the volume of the preceding stage in the upscaling process, amounts to 2-10% of the final culture volume. An inoculation schedule for the continuous production according to the batch technique

Batch culture systems are widely applied because of their simplicity and flexibility, allowing to change species and to remedy defects in the system rapidly. Although often considered as the most reliable method, batch culture is not necessarily the most efficient method. Batch cultures are harvested just prior to the initiation of the stationary phase and must thus always be maintained for a substantial period of time past the maximum specific growth rate. Also, the quality of the harvested cells may be less predictable than that in continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase).

Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture. Batch cultures also require a lot of labour to harvest, clean, sterilize, refill, and inoculate the containers.

1.1.2.2. Continuous culture

The continuous culture method, (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- **Turbidostat culture**, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- **Chemostat culture**, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

The disadvantages of the continuous system are its relatively high cost and complexity. The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales. However, continuous cultures have the advantage of producing algae of more predictable quality. Furthermore, they are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labor.

1.1.2.3. Semi-continuous culture

The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for

further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.

1.2. Preparing tools, materials and equipment

- **Glass ware**



Figur 1. 2: Glass ware

- **Fibre glass or plastic tanks**

These water storage tanks are made up of steel. Here're a few advantages of using these tanks for water storage

- **Plastic Water Bottles.**

We actually really plastic water bottles for algae cultures. They are inexpensive, readily available, and the water inside is almost sterile! Here is a link to the materials that make up plastic bottles. All over the world people are re-cycling these bottles and using them to culture algae.



Figur 1.3: Plastic water

Algae experiments being done in a water bottle. This experiment was used to demonstrate that a pond was phosphate limited. When phosphate was added to the pond water, an algae bloom resulted.

- **Erlenmeyer Flasks**

Erlenmeyer flasks are used in algae culturing. The shape of the bottom of the Erlenmeyer flask allows for a high surface area to volume ratio. With a small opening at the top of the flask, we can limit the amount of dust and allow for gas exchange to take place. These are often used to maintain a pure culture in a laboratory.



Figur 1.4: Flask

- **Beakers For Growing Algae**

Beakers are less ideal than Erlenmeyer flasks because the top of the lid is much larger and exposes more of the culture to the atmosphere. The risk here is exposure of foreign algae, bacteria, or fungus. We do not recommend using beakers for experiments, but they are great for preparing media and measuring volumes.



Figur 1.5: Beaker

- **Tissue Culture Flasks**

Tissue culture flasks are among any algae growers go-to vessels. These vessels often have a built in filter in the cap that will filter to 0.2uM, preventing nearly all weed algae, fungus and bacteria.



Figur 1.6: Tissue culture flask

- **Glass Fish Tanks**

This is the best method for growing culture that are greater than 10L of total volume. You should consider a lid to prevent water evaporation of the culture media. If your culture loses too much water, the culture will become more and more salty. Always consider measuring the salinity on a regular basis.



Figur 1.7: Glass Fish Tanks

- **Photobioreactors**

These highly automated highly sensed chambers can help researchers dial in exactly how to best grow algae. They often use an impeller or a bubbler to mix the chamber. All of the variables for life can be manipulated inside: temperature, mixing, gas mixture, salinity, pH.



- Figer 1.8: Photobioreactors

- **Aeration**

Aeration can protect your pond and your fish during an algae bloom and die off. Adding an aeration device will provide added oxygen to the water and help buffer the effect of an algae die off

- **Temperature- controlled room.**

is used to conveniently control the temperature of individual rooms. Its cover is provided with self-explanatory icons and coloured status and operation LED for optimum ease of use. The measurement of the room temperature, as compared with the individually adjustable temperature setpoint, along with the different operating modes (comfort, standby, night, and frost protection modes) ensures an optimum and healthy room temperature.

- **Measuring cylinder**

Graduated cylinders are often used to measure the volume of a liquid. Graduated cylinders are generally more accurate and precise than laboratory flasks and beakers



- Figer 1.9: Measuring cylinder

- pipettes and syringes

used to take an accurate amount of concentrate to mix with water or add to your tank. A must for all grow rooms



- Figer 1.10: pipettes and syringes

- **Washing and sterilizing equipments**

machines and automatic cleaning systems for industrial applications. Discover our process cleaning systems for your sector. Request your free demonstration. Cleaning specialists. Custom solutions. Cleaning and blasting. Ready made machine.

- **Filtration**



Figure: 1.11: Filtrator

Is commonly used for harvest and concentration. But with an efficient **filtration** solution

- **Microscope**



- Figer 1.12: Microscope

- **Buckets**

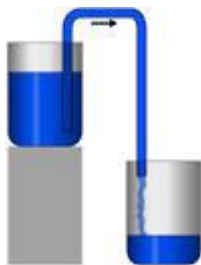
A bucket is a round metal or plastic container with a handle attached to its sides. Buckets are often used for holding and carrying water.

- **Pumps**

A mechanical device using suction or pressure to raise or move liquids, compress gases, or force air into inflatable objects such as tyres.

- **Siphons**

A siphon is a tube that allows liquid to travel upward, above the surface of the origin reservoir, then downwards to a lower level without using a pump.



Figier 1.13: **Siphons**

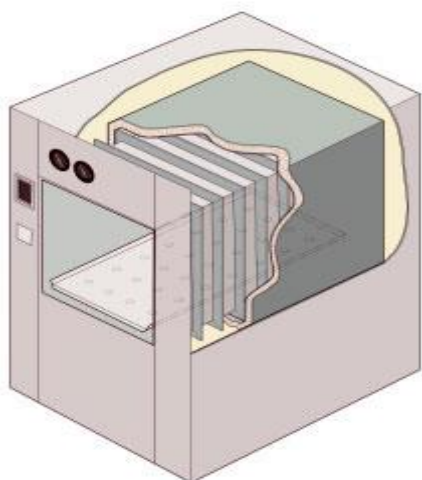
- **Nets, sieves or screens (mesh size generally below 100μm) scoops**



Figier 1.14: **Nets, sieves or screens**

- **Autoclave**

A machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure and/or temperature.



- Figier 1.15: Autoclave

1.2.1. Personal Protective Equipment (PPE)

- Boots



Figier 1.16: Boots

- Sunhats



Figier 1.17: Hats

- sunscreen creams



Figier 1.18: Sunscreen

- Overalls

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Figur 1.19: overall

- Wader



Figur 1.20: Wader

- Life saver jacket



Figure 1.21: life saver jacket

1.2.2. Function of some materials, tools and equipment

All materials, tools and equipment's will be checked and reported their proper functions before going to the activities here are some equipment's will list down how it works;

A) Water Quality testing equipment

Water-quality testing is one of the most important jobs in aquaculture. If the water quality of a culture structure, such as a pond or tank is poor, stock can suffer from health problems such as damage and diseases. A range of tools and test kits are used to test water-quality parameters such as the level of dissolved oxygen, pH, alkalinity, water hardness, and ammonia levels etc.

I. Dissolved oxygen meter

A dissolved oxygen meter is used to measure the level of *dissolved oxygen* in water. It consists of a probe and a meter. The probe is lowered into water and gently moved from side to side, and then a reading is taken from the meter.



Figure 1.22 : Dissolved oxygen meter

- Steps to calibrate and use a dissolved oxygen meter:
 - a) Turn the meter on and inspect the probe for damage.
 - b) Place the probe in a holder that contains a sponge which has been moistened with distilled water.
 - c) Allow time for the probe to "warm up" and for the air in the probe holder to become saturated with water vapor.
 - d) Set the altitude on the meter.

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- e) The probe will now be calibrated to 100% saturation.
- f) Set the salinity of the water sample that you want to measure on the meter.
- g) Put the probe into the water sample and gently move it from side to side.
- h) Wait until the reading on the meter becomes stable, and then record the result.

The methods of calibration can be very similar for different types of dissolved oxygen meters, but you should always check the user manual for the specific dissolved oxygen meter you are using for the correct way to calibrate it.

II. P^H meter

A pH meter is used to measure the pH in water. It consists of a probe and a meter. The probe is lowered into the water sample and the pH of the sample will be displayed on the meter.



Figure 1.23: pH meter

- Steps to calibrate a pH meter:
 - a) Turn the meter on.
 - b) Connect the probe to the meter.
 - c) Place the probe in *buffer 7* solution and wait for the reading to stabilize.
 - d) Press the "Cal" button to enter the calibrate mode.
 - e) Press the "Con" button to set the meter to pH 7.
 - f) This method can be repeated for a buffer 4 and/or a buffer 10 solution.

Press the "Meas" button and Measure will appear

- g) Rinse the probe with distilled water.

h) The pH meter is now calibrated and ready for use.

The methods of calibration are very similar for most pH meters. However, you should always check the user manual for the meter you are using to find out how to calibrate it.

Use-To use the pH meter:

- place the probe in the sample to be measured
- wait for a stable reading to appear on the meter
- Record that reading.

III. Salinity meter

A salinity meter is used to measure the *salinity* of water. A salinity meter has a probe that detects the salinity of a water sample, and a meter that displays the salinity of the water in parts per thousand.



Figure 1.24: Salinity meter

- Most salinity meters don't require calibration. However, some salinity meters require the temperature of the water sample to be set on the meter before it can measure the salinity of the water sample.

To uses a salinity meter:

- insert the probe into the water sample so that the probe is completely submerged
- allow time for the reading on the meter to become stable
- Record the value of the reading on the meter once it stops changing.

IV. Thermometer

A thermometer is used to record the temperature of water. To use it, lower the thermometer into the water and wait a minute or two. Then take the thermometer out and read the temperature recorded on it.

V. Ammonia test kit

Do not smell the reagents.

An ammonia test kit is used to measure the level of *ammonia* in a water sample. It comes with two separate reagents that are added to the water sample.



Figure 1.25: Ammonia test

To use the ammonia test kit:

- fill the container with the water sample
- add the first reagent to the water sample
- add the second reagent, then wait for the water to change color
- compare the color of the water sample to the color chart that comes with the test kit
- Find the color on the chart that matches the color of the water sample, and take a reading of the value on the chart. This is the amount of ammonia in the water sample.

Safety-Ammonia test kits can contain chemicals that can be harmful to you, to stock, or to the environment. Adopt the following guidelines when using an ammonia test kit:

- Always wear clean gloves when using the test kit.
- Always store used waste reagents in a suitable container for disposal later.
- Avoid contact with skin and eyes.
- Do not swallow reagents.

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- Do not smell the reagents.

VI. Nitrite test kit

Description and use-A nitrite test kit is used to measure the amount of nitrite in a water sample. The test kit often comes with two reagents and a sampling container.



Figure 1.26: Nitrite test kit

To use the nitrite test kit:

- Fill the container with the water sample
- Add the first reagent to the water sample
- Add the second reagent and wait for the sample to change color
- Compare the color of the water sample to the color chart that comes with the test kit
- Find the color on the chart that matches the color of the water sample, and take a reading of the value on the chart. This is the level of nitrite in the water sample.

Safety-Nitrite test kits can contain chemicals that can be harmful to you, to stock, or to the environment. Adopt the following guidelines when using a nitrite test kit:

- Always wear clean gloves when using the test kit.
- Always store used waste reagents in a suitable container for disposal later.
- Avoid contact with skin and eyes.
- Do not swallow reagents.

Water treatment

Is any process that improves the quality of water to make it appropriate for a specific end-use. Contamination with bacteria, protozoa or another species of algae is a serious problem for monospecific/axenic

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cultures of micro-algae. The most common sources of contamination include the culture medium (sea water and nutrients), the air (from the air supply as well as the environment), the culture vessel, and the starter culture.

Seawater used for algal culture should be free of organisms that may compete with the unicellular algae, such as other species of phytoplankton, phytophagous zooplankton, or bacteria. Sterilization of the seawater by either physical (filtration, autoclaving, pasteurization, UV irradiation) or chemical methods (chlorination, acidification, ozonization) is therefore required. Autoclaving (15 to 45 min. at 120°C and 20 psi, depending on the volume) or pasteurization (80°C for 1-2 h) is mostly applied for sterilizing the culture medium in test tubes, erlenmeyers, and carboys. Volumes greater than 20 l are generally filtered at 1 µm and treated with acid (*e.g.* hydrochloric acid at pH 3, neutralization after 24 h with sodium carbonate) or chlorine (*e.g.* 1-2 mg.l⁻¹, incubation for 24 h without aeration, followed by aeration for 2-3 h to remove residual chlorine, addition of sodium thiosulfate to neutralize chlorine may be necessary if aeration fails to strip the chlorine). Water treatment is not required when using underground salt water obtained through bore holes. This water is generally free of living organisms and may contain sufficient mineral salts to support algal culture without further enrichment. In some cases well water contains high levels of ammonia and ferrous salts, the latter precipitating after oxidation in air.

1.3. Labour and resource requirements

labor is one of the three factors of production, along with land and capital. Labor is often defined as the physical or mental effort exerted by human beings in the production of goods and services. In neoclassical economics, labor is a broader concept that incorporates all human activity that adds value to a product or service. This includes not only physical and mental effort but also the use of tools, machines, and other equipment. It also surrounds the time spent on planning, organizing, and supervising production. In certain economic models, labor is assumed to be a homogeneous input. This means that all workers are assumed to have the same skills, abilities, and productivity. However, labor is not homogeneous in real life. Workers differ in their skills, abilities, experience, and motivation. These differences can lead to different amounts of output

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per hour of work, even when all other inputs are the same.

Batch cultures also *require* a lot of *labour* to

- ✓ Harvest
- ✓ Clean
- ✓ Sterilize
- ✓ Refill and inoculate the containers

Resources necessary to grow algae sustainably include

- Non-arable land

Land classing looks at whether your land and soil is arable or non-arable, or if it is unsuitable for agriculture. Arable land is suitable for cropping on a regular basis whereas non-arable land is not capable of supporting cropping due to land and soil constraints.

- Non-potable water

Non-potable water is not suitable for drinking but may still be used for other purposes. Potable water is water of a quality suitable for drinking, cooking and personal bathing.

- Waste nutrient streams

the complete flow of waste from its domestic or industrial source through to recovery, recycling or final disposal

- Waste carbon dioxide

Carbon dioxide is a waste product of combusting fossil fuels, and its accumulation in the atmosphere presents a planetary hazard. Carbon dioxide is also managed and used as a resource.

- Sufficient sunlight, and supporting infrastructure to access downstream processing operations.

1.4. Occupational health safety(OHS)

Occupational Health and Safety (OHS), also known as Occupational Safety and Health (OSH), refers to the generic practice of addressing and reducing potential safety and health risks to employees. This can cover anything from risk assessment, injury prevention, work-life balance, safety protocols, workplace hazards, to compensation and benefits, and employee management.

Occupational safety is an important part of any business, as staff safety should always be prioritized before anything else. It's the responsibility of employers to ensure that their staff are well-taken care of and are surrounded by as few risks as possible, so having guidelines in place for OHS can help them greatly.

A guide on OHS and having OHS standards in place will not only ensure a safe workplace and safe and healthy employees, but they could also lead to improvements in business as well.

1.5. Risk factors affecting quality of culture

1.5.1 Illumination

The production of organic matter in the sea by photosynthesis is dependent upon the intensity of incidental light at the sea surface and the depth to which adequate light can penetrate. For algae cultured in controlled rooms, cool-white daylight fluorescent lamps may be used. Two 8-ft, 40-Watt lamps will give a light intensity of about 300 foot candles (3 200 Lux) on a surface 16 inches away. For maintenance purposes, the light from northern exposure (northern hemisphere) may be used to light algal cultures.

These north-facing windows must have no heating vents or radiators below them. If cultures are to be scaled-up for massproduction purposes, larger culture vessels may be subjected to ambient conditions of illumination (where dark and light regimes exist). Finally outdoor culture tanks will have to rely on sunlight for illumination.

1.5.2 Temperature

Temperature normally affects rate of metabolism of an organism. In controlled rooms, temperature is kept within the range of 18°–22°C. In outdoor cultures where temperatures are

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normally higher, the turnover rate is faster. Algal cultures when scaled-up may gradually be subjected to increases in temperature so as to avoid environmental stress. It is generally recommended that indoor to outdoor culture transfer be done early in the morning.

1.5.3 Culture Medium

Phytoplankton usually requires nutrients like nitrogen, phosphorus, potassium and other elements incorporated in the water medium in which it grows.

Stock or maintenance cultures make use of organic compounds in the form of thiamine (B1), Cynocobalamin (B12) and biotin.

Marine phytoplankton is grown in either enriched seawater media, or artificial or synthetic seawater media. Enriched seawater media make use of seawater as base plus small or trace amounts of elements essential for growth. Artificial media, on the other hand, have as a base distilled water plus known amounts of various elements to approximate seawater composition. It has been observed, however, that artificial media show the most constant result for algal culture in contrast to enriched natural seawater which may show varying results depending upon the time and place of collection.

1.5.4 Starter or Inoculum

Starter refers to the “seed” used to start algal cultures. The quality of the starter should be regularly checked for the presence or absence of contaminants. The amount of inoculum to be used is determined by the total volume of culture. In cases where culture conditions may be manipulated, small amounts of starter could be used. For large-scale algal production, however, more starter is required to effect faster harvest of cultures.

The growth period of a particular culture starts from the time the seed is introduced to the point in time where cultures are to be harvested or renewed. Renewal of cultures is necessary to ensure a continuous supply of phytoplankton for the hatcheries.

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1.6. Planning to minimize risks

➤ Frequency of Monitoring

The time period between the moment monitor receives a response from a monitoring agent location and the initiation of a new monitoring session.

➤ Health Advisories

Means that level recognized by the state for which corrective action should be performed.

➤ Analytical Methods

Analytical technique is a method used to determine a chemical or physical property of a chemical substance, chemical element, or mixture. There is a wide variety of techniques used for analysis, from simple weighing to advanced techniques using highly specialized instrumentation.

➤ Factors Likely to Cause Harmful Algal Blooms

Both seaweed and phytoplankton sometimes grow quickly, or bloom. Some blooms can harm people, animals, or the environment. Most harmful blooms that make people and animals sick are caused by phytoplankton.

Blooms can harm people, animals, and the environment when they

- Produce toxins (poisons)
- Become too dense
- Use up the oxygen in the water
- Release harmful gases

These harmful blooms can be caused by many types of phytoplankton. However, three main types of phytoplankton cause most blooms that make people and animals sick

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- Cyanobacteria (sometimes called blue-green algae)
- Dinoflagellates (sometimes called microalgae or red tide)
- Diatoms (sometimes called microalgae or red tide)

Harmful algal blooms need:

- Sunlight
- Slow-moving water
- Nutrients (nitrogen and phosphorus)

1.7. Assembling and commissioning culture systems

- Oversee equipment pre-functional tests and start-ups
Materials that are broken damaged are rejected.



Self-Check-1	Written test
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Name: ID: Date:

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Give \checkmark mark if you are able to answer the question and \times ☐ mark if you are not

able

1. Can you list three types of algal culture -----
2. What are the risk factors affecting quality of culture? (5 point)
3. What are resources necessary to grow algae sustainably? (5points)
4. Which one of culture type is more expensive ? (5points)

LG# 18

LO # 2: Undertake algal and live-feed cultures

Instruction sheet-2

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Production vessels or structures and other equipment
- Physic-chemical requirements of culture organism
- Performing water treatment
- Maintaining sterile conditions and equipment
- Inoculation cultures
- Nutrient formulae or media
- Culture health
- Production activities and equipment operations

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Production vessels or structures and other equipment
- Physic-chemical requirements of culture organism
- Performing water treatment
- Maintaining sterile conditions and equipment
- Inoculation cultures
- Nutrient formulae or media
- Culture health
- Production activities and equipment operations



Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets

Information Sheet-2

2.1. Physic-chemical requirements of culture organism

A characteristic of microorganisms is their ability to grow and form a population of organisms. One of the results of microbial metabolism is an increase in the size of the cell. The many requirements for successful growth include those both chemical and physical.

A, Chemical requirements. In order to grow successfully, microorganisms must have a supply of water as well as numerous other substances including mineral elements, growth factors, and gas, such as oxygen. Virtually all chemical substances in microorganisms contain carbon in some form, whether they be proteins, fats, carbohydrates, or lipids. Perhaps 50 percent of a bacterium's dry weight is carbon. Carbon can be obtained from organic materials in the environment, or it may be derived from carbon dioxide. Both chemoautotrophic and photoautotrophic microorganisms obtain their energy and produce their nutrients from simple inorganic compounds such as carbon dioxide. Chemoautotrophs do so through chemical reactions, while photoautotrophs use photosynthesis.

Other chemical requirements for microbial growth include such trace elements as iron, copper, and zinc. These elements often are used for the synthesis of enzymes. Organic growth factors such as vitamins may also be required by certain bacteria. Amino acids, purines, and pyrimidines should also be available.

B, Physical requirements. Certain physical conditions affect the type and amount of microbial growth. For example, enzyme activity depends on the temperature of the environment, and microorganisms are classified in three groups according to their temperature preferences: psychrophilic organisms (psychrophiles) prefer cold temperatures of about 0°C to 20°C; mesophilic organisms (mesophiles) prefer temperatures at 20°C to 40°C; thermophilic organisms (thermophiles) prefer temperatures higher than 40°C. A minimum

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and a maximum growth temperature range exist for each species. The temperature at which best growth occurs is the **optimum growth temperature**.

2.1.1. Physical and chemical conditions

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. The most optimal parameters as well as the tolerated ranges are species specific and a broad generalization for the most important parameters is given. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another.

2.1.1.1. Culture medium/nutrients

For the maintenance and culture of marine microalgae, the sterilized seawater has to be enriched with substances for growth including nutrients, vitamins, trace metals, chelators and buffer compounds. For this purpose, a variety of media are available for enriching the seawater for algal culture. The most common types of media for enrichment are given below

Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate.

Table 2.1. A generalized set of conditions for culturing micro-algae

Parameters	Range	Optima
Temperature (°C)	16-27	18-24
Salinity (g.l ⁻¹)	12-40	20-24
Light intensity (lux)	1,000-10,000 (depends on volume and density)	2,500-5,000
Photoperiod (light: dark, hours)		16:8 (minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

Silicate is specifically used for the growth of diatoms which utilize this compound for production of an

Table 2.2 Composition and preparation of medium

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride (FeCl_3)	0.8 g ^(a)
Manganous chloride ($\text{MnCl}_2, 4\text{H}_2\text{O}$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA ^(b) , di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate ($\text{NaH}_2\text{PO}_4, 2\text{H}_2\text{O}$)	20.0 g
Sodium nitrate (NaNO_3)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water ^(c)	Heat to dissolve
Solution B	
Zinc chloride (ZnCl_2)	2.1 g
Cobaltous chloride ($\text{CoCl}_2, 6\text{H}_2\text{O}$)	2.0 g
Ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}, 4\text{H}_2\text{O}$)	0.9 g
Cupric sulphate ($\text{CuSO}_4, 5\text{H}_2\text{O}$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water ^(c)	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B ₁	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water ^(c)	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate ($\text{Na}_2\text{SiO}_3, 5\text{H}_2\text{O}$)	40.0 g
Make up to 1 litre with fresh water ^(c)	Shake to dissolve
Solution E	
Vitamin B ₁₂	0.1 g
Make up to 250 ml with fresh water ^(c)	
Solution F (for culture of <i>Chroomonas salina</i> - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate (NaNO_3)	200.0 g
Make up to 1 litre with fresh water ^(c)	

(a) Use 2.0 g for culture of *Chaetoceros calcitrans* in filtered sea water;

(b) Ethylene diamine tetra acetic acid;

(c) Use distilled water if possible.

Table 2.4. Composition and preparation of Guillard's F/2 medium.

Nutrients	Final concentration (mg.l ⁻¹ seawater) ^a	Stock solution preparations
NaNO ₃	75	Nitrate/Phosphate Solution Working Stock: add 75 g NaNO ₃ + 5 g NaH ₂ PO ₄ to 1 liter distilled water (DW)
NaH ₂ PO ₄ .H ₂ O	5	
Na ₂ SiO ₃ .9H ₂ O	30	Silicate Solution Working Stock: add 30 g Na ₂ SiO ₃ to 1 liter DW
Na ₂ C ₁₀ H ₁₄ O ₈ N ₂ .H ₂ O (Na ₂ EDTA)	4.36	Trace Metal/EDTA Solution Primary stocks: make 5 separate
CoCl ₂ .6H ₂ O	0.01	1-liter stocks of (g.l ⁻¹ DW) 10.0 g CoCl ₂ , 9.8 g
CuSO ₄ .5H ₂ O	0.01	CuSO ₄ , 180 g MnCl ₂ , 6.3 g Na ₂ MoO ₄ , 22.0 g ZnSO ₄
FeCl ₃ .6H ₂ O	3.15	
MnCl ₂ .4H ₂ O	0.18	Working stock: add 1 ml of each primary stock solution + 4.35 g Na ₂ C ₁₀ H ₁₄ O ₈ N ₂ + 3.15 g FeCl ₃ to 1 liter DW
Na ₂ MoO ₄ .2H ₂ O	0.006	
ZnSO ₄ .7H ₂ O	0.022	
Thiamin HCl	0.1	Vitamin Solution Primary stock: add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B ₁₂ to 1 liter DW
Biotin	0.0005	
B ₁₂	0.0005	Working stock: add 5 ml primary stock to 1 liter DW

Table 2.5. Various combinations of fertilizers that can be used for mass culture of marine algae (modified from Palanisamy *et al.*, 1991).

Fertilizers	Concentration (mg.l ⁻¹)					
	A	B	C	D	E	F
Ammonium sulfate	150	100	300	100	-	-
Urea	7.5	5	-	10-15	-	12-15
Calcium superphosphate	25	15	50	-	-	-
Clewat 32	-	5	-	-	-	-
N:P 16/20 fertilizer	-	-	-	10-15	-	-
N:P:K 16-20-20	-	-	-	-	12-15	-
N:P:K 14-14-14	-	-	-	-	-	30

2.3.1.2. Light

As with all plants, micro-algae photosynthesize, *i.e.* they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in

this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for erlenmeyer flasks, 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

2.3.1.3. pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture (see below). In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth.

2.3.1.4. Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO₂ originating from the air (containing 0.03% CO₂) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (*e.g.* at a rate of 1% of the volume of air). CO₂ addition furthermore buffers the water against pH changes as a result of the CO₂/HCO₃⁻ balance. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test

tubes, erlenmeyers), aerating (bags, tanks), or using paddle wheels and jetpumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing.

2.3.1.5. Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air - conditioning units.

2.3.1.6. Salinity

Marine phytoplankton are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 g.l⁻¹ have been found to be optimal.

2.2. Performing water treatment

Water treatment refers to the process of improving the quality of water with the purpose of serving an end-use. The most common end-uses include drinking water, industrial water supply, water recreation, and for replenishing environmental sources, such as rivers and lakes.

Water treatment helps in removing contaminants and hazardous substances from the water, making it clean and safe to drink and be used for other purposes. Unfortunately, almost 2 billion people in the world use either untreated drinking water or get water from unsafe or contaminated sources

steps of water treatment

- a. Coagulation. The first step of getting water treated is through coagulation
- b. Flocculation. This step refers to the process of gently mixing the water to create larger heavier particles known as flocs
- c. Sedimentation

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- d. Filtration
- e .Disinfection.

a. **Coagulation**

The first step of getting water treated is through coagulation. This involves adding chemicals with a positive charge to the water which should neutralize the negative charge of dirt and other dissolved substances. Such chemicals include iron and specific types of salt.

b. **Flocculation**

This step refers to the process of gently mixing the water to create larger, heavier particles known as flocs. In most cases, additional chemicals are being added to the water to allow the flocs to form easily.

c. **Sedimentation**

Once flocs form, they settle to the bottom of the water because they are heavier. This is called sedimentation in water treatment, which is one of the processes that water treatment plants use in separating the solids, such as flocs, from the water before going to the next step.

d. **Filtration**

The water again goes through another process of solids separation through filtration. The separated, clear water on top now passes through filters with various pore sizes, made from different materials such as sand and gravel. Ultimately, these filters are in place to help remove dissolved particles and unwanted substances from the water.

e. **Disinfection**

During this step, any remaining parasites, bacteria, and viruses must be eliminated. This can be done by adding one or more chemical disinfectants to water such as chlorine or chlorine dioxide. Why do water treatment plants do this? It's to keep water safe when traveling from the water treatment plant to homes and businesses because chemical disinfectants help eliminate the remaining unwanted microorganisms before the water reaches the intended end-use.

2.3. Maintaining sterile conditions and equipment

Sterile technique is a set of specific practices and procedures performed to make equipment and areas free from all microorganisms and to maintain that sterility

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Principles of sterile technique help control and prevent infection, prevent the transmission of all microorganisms in a given area, and include all techniques that are practised to maintain sterility.

Sterile technique may include the use of sterile equipment, sterile gowns, and gloves

2.4. Inoculation cultures

An inoculum can be defined as the population of microorganisms or cells that is introduced in the fermentation medium or any other suitable medium

All culture media must be checked visually before use for contamination, significant physical imperfections (for example, uneven distribution of media, variable amounts of medium in petri dishes/tubes/bottles, colour, gross deformation of the surface on the media) and expiry date. Culture media should have an identifiable batch or quality control number and have passed QC tests before use. Plates that are beyond their expiry date, contaminated plates, and broth media appearing unusually turbid should be discarded⁴. For the effective detection of the bacterial content of specimens, it is important to achieve growth of individual colonies by using a good technique to inoculate the specimen on culture media. There are many variations and personal preferences for “plating out”, some of which are described in this document.

The initial area inoculated should cover between a quarter and a third of the total area of agar used. Whole plates, half plates, or quarter plates can be used depending on the circumstances. Specimens may be plated out for individual colonies, or seeded directly over an entire segment of a plate and incubated without further spreading. Antimicrobial discs for identification (for example, optochin, bacitracin) may be added as appropriate. Discs should be placed near the edge of the plate, between the areas covered by the first and second spread, to avoid total inhibition of very susceptible organisms. Inoculation loops are designed for quantitative procedures such as sampling, serial dilutions, as well as for bacterial inoculation. Inoculation loops can be ‘wire or disposable loops’. Disposable loops were initially used in safety cabinets to avoid sterilisation with Bunsen burners but now their use is common practice to comply with the health and safety regulations. Disposable loops are also desirable for quantitative purposes. Wire loops are rarely used in clinical microbiology laboratories in the UK to reduce the risk of infection from aerosols of pathogenic organisms and,

crosscontamination from improper sterilisation of the wire loops. Therefore, disposable loops are recommended in this document.

All media should be incubated as soon as possible after inoculation. In particular, plates for anaerobic incubation should be incubated as soon as possible to prevent loss of viability

2.5.1.Types of Inoculum

An inoculum that survives dormant in the winter or summer and causes the original infections in the spring or in the autumn is called a primary inoculum, and the infections it causes are called primary infections. An inoculum produced from primary infections is called a secondary inoculum and it, in turn, causes secondary infections. Generally, the more abundant the primary inoculum and the closer it is to the crop, the more severe the disease and the losses that result.

2.5.2. Sources of Inoculum

In some fungal and bacterial diseases of perennial plants, such as shrubs and trees, the inoculum is produced on the branches, trunks, or roots of the plants. The inoculum sometimes is present right in the plant debris or soil in the field where the crop is grown; other times it comes into the field with the seed, transplants, tubers, or other propagative organs or it may come from sources outside the field. Outside sources of inoculum may be nearby plants or fields or fields many miles away.

2.5. Nutrient formulae or media

like all other living things, microbes require nutrients (food) to grow and live. Microbiological media (singular is "medium") is a mixture of water and nutrients necessary to grow microbes. Different types of media also can be used to provide information about the different characteristics microbes have.

Many types of microbiological media can be ordered in powder form from a science supplier. Instructions for preparing the medium are presented on the medium container. Typically, this involves dissolving a certain number of grams of the media powder in one liter of distilled or deionized water ("grams per liter" which is written as g/L). This does not mean that one must

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always make one liter of medium. The amount can be modified by calculating the amount of microbiological media powder required for the concentration (g/L) listed on the bottle)

Media can be prepared as a broth (liquid), a slant (agar in a test tube that has been slanted when cooling to create a larger agar surface area), a deep (agar in a test tube typically inoculated using an inoculation needle by stabbing into the agar), and a petri plate (a larger surface area for growing microbes on the surface).

How to Make the Medium

1. Measure 500 mL of distilled or deionized water (not tap water) with a graduated cylinder.
2. Weigh 20 g of media powder using a balance.
3. Put a magnetic stir bar into a beaker, flask or bottle (must be larger capacity than 500 mL in for this example - 800 mL or 1000 mL) and add about half of the water.
4. Put the beaker, flask, or bottle onto a stir plate and turn it on so the magnetic stir bar is swirling the water enough to stir it well, but not too much that it is jumping and/or creating lots of bubbles in the water.
5. Gradually add the 20 g of media powder to the stirring water.
6. Add the reminder of the water. By adding the remaining water after the media powder, it will help dissolve any powder floating at the top of the water.
7. Allow to stir until the powder is completely dissolved.

If the medium is agar, the solution will need to be heated just to boiling (but not boiling over) starting after step 3. Keep stirring and heating until the solution is clear. Be careful not to over-heat since it is easy for the solution to boil over.

If the medium will be distributed into test tubes, measure amount into each test tube and add caps.

If the medium is for flasks or bottles, measure the amount into each flask or bottle and add caps or other covers (make sure screw-caps are not screwed on tightly before they are autoclaved).

If the medium is for petri plates, make sure the medium is in a container with a cover (make sure screw-caps are not screwed on tightly before they are autoclaved).

Autoclave.

If test tubes contain agar that will be for slants, prop the rack of test tubes so the agar slants as it cools.

If the medium is for petri plates, disinfect a workspace and pour agar into sterile petri plates and allow to cool completely.

2.6. Culture health and Nutritive value of microalgae

- More than forty different algal species are currently used as live food for aquatic invertebrates and vertebrates.
- Selected microalgae have rich nutritional properties, particularly n-3 highly unsaturated fatty acids (HUFA), which may be helpful for survival of fish larvae juveniles, prawn, and bivalve molluscs.
- Similarly, different microalgae foods have different concentrations of certain polyunsaturated fatty acids (PUFA) which play a very important role in the health status of larvae of many fish.
- The most attractive component of microalgae biomass is crude protein but its utilization by fish larvae depends upon its digestibility.

Why are microalgae preferable to fish larvae?

Important criteria for which microalgae are preferable for larval food may be highlighted as;

- Cell size appropriate to the demands of fish larvae
- Adequate nutritional value
- High digestibility
- Short life cycle
- Tolerance to environmental variations

2.7. Production activities and equipment operations

Operators operate industrial equipment with single or multiple functions and automated to varying degrees. Adjusting equipment, adjusting operating parameters and solving problems during production is an integral part of their work. These activities are generally performed from a workstation, through interfaces (control panels, touch screens, etc.). The operator also performs quality control during the various stages of the industrial process and minor preventive maintenance work on equipment. Eventually, the operator may be called upon to contribute to the improvement of various facets of production.

The operator operates the equipment of a production sequence, a series of equipment or multifunction equipment (performing several production operations). This trade consists mainly of operating production equipment during industrial processing or an industrial manufacturing process. Work is performed individually with a co-worker and occasionally as part of a team of operators. The work requires collaboration with other trades (industrial mechanics, electromechanics, etc.), technical staff (quality control, instrumentation and control, etc.)



Self-Check-2	Written test
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Name: ID: Date:

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Test I: Give short answer. (20 Points)

1. What is Inoculation? (5 point)
2. How to Make the Medium? (5 point)
3. List Sterile technique ?(5 point)
4. What the importance of monitoring the cleanliness of equipments ? (5 point)



Operation Sheet-2

2.1. Performing water treatment

A. materials and equipments

- PPE
- Water
- Filter

B. steps of water treatment

- a. Coagulation.
- b. Flocculation
- c. Sedimentation
- d. Filtration
- e. Disinfection

LAP Test-2	Performance Test
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Name..... ID.....

Date.....

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within 2 hour. The project is expected from each student to do it.

Task 1: Perform water treatment



LG# 19 LO # 3: Harvest culture

Instruction sheet-3

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Harvesting equipment
- Substandard equipment
- Collecting algal and live culture
- Transporting algal and live culture

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Harvest equipment
- Substandard equipment
- Collect algal and live culture
- Transport algal and live culture

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet 3

3.1. Harvest equipment

3.1.1 Algae Harvest Separation

While the potential for algae is enormous, the problem remains that in order to harvest it properly for most commercial uses, you must first dehydrate it – that is, separate it from the aqueous medium in which it grows. And while algae grow two to ten times faster than land crops based on biofuels used as a source of biofuels, the process required to separate and purify them and the oils it produces, from which biofuel is made, has been until very recently too expensive to make industrial-scale algae production commercially viable.

Fortunately, along with increased growth and productivity of algae, techniques for harvesting them that require less energy and overall costs have made significant progress. To understand where these advances are happening, it helps to briefly look at the three most popular algae harvesting processes today.

Flocculation: In flocculation, a chemical flocculant is added to a mixture of algae and water which then causes the algae to aggregate or clump together.

Micro-screening: In micro-screening, also known as membrane separation, the water-algae mixture passes through a filtration system, usually in the shape of a funnel.

Centrifugation: In centrifugation, a mechanized form of separation occurs, often through the use of a continuous flow centrifuge.

One of the most rewarding things about growing algae is harvesting it. I will bet you a dollar that when you harvest your first one, you will think “Green Gold”! There are several ways to get biomass out of water, here is a rundown of the ways we suggest:

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A. Filtration: (Favorite)

The easiest way to harvest algae is to filter it! This assumes a few things, first the algae have to be large enough to be screened out and secondly, you need a screen with a tight enough mesh to catch the algae.

The tightest meshes are in the 30uM range, so that means that you need to have an algae strain that is larger than 30uM. Our only strain that is easily filtered out is spirulina. Spirulina is a giant in the microalgae world with trichomes (colony) lengths from 20uM to 1,000uM. Yes, that means that you can see them with your naked eye!!!

Here is the Algae Research and Supply Spirulina Harvesting screen. It works for spirulina and for zooplankton (brine shrimp and copepods).



Figure 3.1. Filtratin

b. Sedimentation

The lazy-person's way to harvest algae is to sediment! Here, you cause or let the algae to fall to the bottom of the tank, then you decant off the clear water on top, finally collect the concentrate at the bottom. *Chlorella* is very compliant with this process, when it is 'ripe' it simply falls to the bottom. *Nannochloropsis*, on the other hand, has very negatively charged cell surfaces, so they repel each other and sinking is not rapid, even when the nutrients have been used up.

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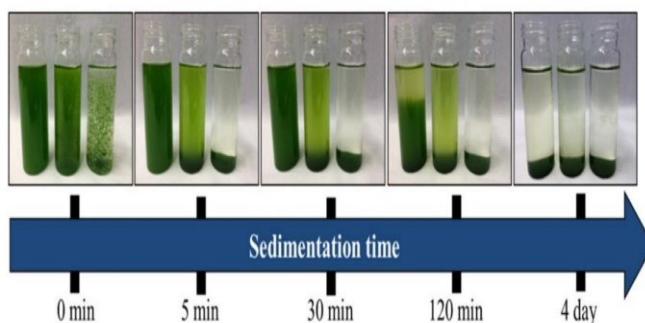


Figure 3.2: Sedimentation

c. Centrifugation

Brute force is what drives the cells from the water using a centrifuge. A centrifuge can add 15,000x the force of gravity to shove the slight more dense algae cells to the bottom of the centrifuge. There are many different types of centrifuges:

Lab bench top: Great for small volumes, generally 50mL tubes.



Figure 3.3: Centrifuge

Flow through : Algae/Water mix/slurry is fed into the top port with gravity or low flow pump. The centrifuge force settles the algae, trapping it in the bowl, under the lip. Out spins clean water. Once the centrifuge is stopped, the liquid contents in the bowl will drain out of the sump. Here is a link to a friend of our who manufactures flow through centrifuges:

3.1.2. Screeners for collecting algae

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With years of experience manufacturing vibratory screening equipment for a host of industries, including food and beverage, chemical, petrochemical, and biofuel production, VibraScreener™ uniquely understands the expectations involved in designing and supporting a screener. of algae harvesting that is robust and profitable enough to ensure profitable commercial use.

Unlike the flocculation process that requires the additional cost of chemical additives, or micro-screening, which generally results in fouling of the membranes, VibraScreener™ has designed its equipment such as the Dynamic Screener™ and Ranger Separator™ to operate efficiently..

Enjoy high-quality equipment and expert advice from VibraScreene

3.2. Substandard equipment

a. Algae magnets.

An algae magnet is a magnet that removes the algae on the aquarium glass in a simple but effective way. The algae magnet is essential and is likely one of the most frequently used tools on your reef tank. This is the exact reason you want to choose an algae magnet that is suitable for your tank, made with strong, high-quality magnets and can safely be left submerged so it is always available for you to quickly clean the tank walls, maintaining that HD quality viewing.



Figure 3.4: Algae Magnets

b. Algae scrapers.

The Pro Scraper 3.0™ Adjustable Aquarium Algae Scraper is great for removing algae from

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aquariums and hard to reach corners. The scraper comes with a stainless-steel blade and a plastic blade to suit all cleaning needs.

c. Brushes.

Heavy duty stainless steel bristles to remove algae from pool walls. Suitable for both concrete and fibreglass pools.

d. Lever pumps

Diaphragm pump for safely moving algae or other particles in suspension. Can be used to move trichomes suspended in water.

Use to feed algae solution into the top of the centrifuge.

- 4 gallons/min
- 40 PSI
- 11x5x4"
- Self Priming
- Flow is restrict able

e. Pliers & grabs.

Pliers with red algae that are usually used for *clamping*, but also cutters vector illustration. Royalty-Free Vector. Pliers with red algae that are usually



Figure 3.5: Pliers & grabs.

f. Crab & fish traps.

Emerald crabs are excellent aquarium scavengers and algae eaters. They will eat leftover food and most types of algae and do a great cleaning up really excessive algae outbreaks. They are most often used to control hair and bubble algae, being one of the few animals that will eat

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bubble algae.

algae, sand, mud, and *invertebrates* is packed into plastic fiber grain sacks (Fig. said that if the algae were placed in the traps alone.

7. Net Covers.

The Oase Algae Nets are very fine mesh construction and are *designed for the removal of silt and algae*. They can also be used for safely netting fish.

3.3. Collect and transport algal and live culture

3.3.1. Collection

Algae grow in almost every habitat in every part of the world. They can be found on very different natural substrates, from animals (snails, crabs, sloths, and turtles are algal hosts) to plants (tree trunks, branches and leaves, water plants, and macroalgae), from springs and rivers to hypersaline lagoons and salt lakes. They also colonize artificial habitats, such as dams and reservoirs, fountains and pools, but cans, bottles, plant pots, or dishes allow algae to extend their natural range. The ubiquities of these organisms together with the plasticity of their metabolic requirements make many algal species easily available for investigation, collection, or simple observation.

Floating microalgae can be collected with a mesh net (e.g., with 25–30 µm pores) or, if in sufficient quantity (i.e., coloring the water), by simply scooping a jar through the water. A small amount of the bottom sediments will also provide many of the algal species that live in or on these sediments. Some algae live attached to other types of substrate, such as dead leaves, twigs, and any underwater plants, which may be growing in the water. Macroalgae and the attached microalgae can be collected by hand or with a knife, including part or all of the substrate (rock, plant, wood, etc.) if possible. Algae growing on soil are difficult to collect and study, many requiring culturing before sufficient and suitable material are available for identification.

3.3.2. Storage

Algae can be stored initially in a glass jar, plastic bottle or bag, or in a vial with some water from the collecting site. The container should be left open or only half filled with liquid and

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wide shallow containers are better than narrow deep jars. If refrigerated or kept on ice soon after collecting most algae can be kept alive for short periods (a day or two). If relatively sparse in the sample, some algae can continue to grow in an open dish stored in a cool place with reduced light. For long-term storage, specimens can be preserved in liquid, dried, or made into a permanent microscope mount. Even with ideal preservation, examination of fresh material is sometimes essential for an accurate determination. Motile algae particularly must be examined while flagella and other delicate structures remain intact, because any kind of preservation procedure causes the detachment of the flagella.

3.1.3. Transportation

Harvest and transport of zooplankton interferes considerably with the survival of these fragile organisms. If it is impossible to convey the material continuously along distribution pipes to the place of consumption, the normal practise is to concentrate and transport the harvested zooplankton in 50 l containers. Under these conditions the survival of the zooplankton depends on the amount of oxygen dissolved in the remaining water. At a concentration of 100 g.l⁻¹, zooplankton can be kept at 10°C without oxygenation for only 15-20 min. At higher temperatures or if the zooplankton is to be kept alive for longer periods, the concentration must be reduced substantially. At a temperature of 18-20°C it can be kept at a concentration of 15-20 g.l⁻¹ without aeration for as long as about 4 - 5 h, although the most sensitive organisms will die. This is certainly the case for *Bosmina*, *Daphnia* and others, that are very sensitive to oxygen depletion. Rotifers, cyclopoid copepods and their developmental stages are less sensitive, and some species of the genus *Moina*, larvae of the genus *Corethra*, and *Daphnia magna* are very resistant to low oxygen levels.

Then the collected zooplankton is transferred from the net to the transport container, part of the material stays in a layer just above the bottom. These organisms are either mechanically damaged or immobilised and could be administered to the fry first. However, when these organisms die, they will soon start to decay. It is useless to administer these dead animals because the fish will refuse it and their decomposing bodies will spoil the water quality of the rearing system. For this reason, dead zooplankton should always be separated from live

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zooplankton by decantation.

Preservation of harvested material for long periods is difficult. At present, freezing is the only method used on a large scale. But even at very low freezing temperatures, (i.e. -198°C) one-third of the free and protein-bound amino acids are lost from the plankton samples through sustained proteases activity and leaching. Dehydration has been used successfully on a small scale, while salting causes mortality in fish. Ensilage, using various acids has also been attempted, but needs further investigations.

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Self-check- 3	Written test
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Name..... ID..... Date.....

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations/answers.

Part 1: Give short answer (6points)

1. What are the three most popular algae harvesting processes
2. How to collect Algae
3. How to transport

LG # 20

LO4- Complete culture production

Instruction sheet-4

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics:

- Refilling production vessel
- Cleaning, repairing and storing of equipment
- Treating and disposing unused cultures and wastes
- Ecologically sustainable development (ESD) principles.
- Recording culture production data
- Reporting work conditions

This guide will also assist you to attain the learning outcomes stated in the cover page. Specifically, upon completion of this learning guide, you will be able to:

- Refill production vessel
- Clean, repair and stor of equipment
- Treat and dispo unused cultures and wastes
- Ecologically sustainable development (ESD) principles.
- Record culture production data
- Report work conditions

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described below.
3. Read the information written in the information Sheets
4. Accomplish the Self-checks
5. Perform Operation Sheets
6. Do the “LAP test”

Information Sheet-4

4.1. Cleaning, repairing and storing of equipment

Waste materials produced during algae and live feed culture work may include;

- Waste water, chemicals, dead fish., aquatic weeds, pond mud and broken components
- Plant debris
- Plastic, metal and paper-based materials
- All these wastes will be either disposed according to industry work procedures or recycled or re-used or returned to manufacturer.

The proper handling of the things we throw away in a manner that does not harm anyone or anything, be it human, animals or the environment. Proper handling includes the collection, transport, processing, recycling or disposal of waste materials produced by human activity in order to reduce their negative effect on the environment. Unwanted materials or substances produced by human activity, which is usually referred to as rubbish, trash, garbage or junk i.e. waste.

Fish Waste - Large amounts of fish guts deposited in an enclosed area can produce foul odors and impair water quality through decreased dissolved oxygen and increased bacteria levels.

Waste handling techniques

- Provide facilities for fish cleaning and carcass disposal.
- Provide a stainless steel sink equipped with a garbage disposal that is connected to a sanitary sewer. (Note: fish heads, large carcasses, and fish skin will clog up the disposal.)
- Provide garbage containers for fish carcasses.
- Empty garbage containers regularly (especially on hot days).
- Prohibit fish cleaning outside of designated areas.
- Implement fish composting where appropriate.
- Use a grinder to make chum out of fish carcasses. Sell the chum at your store.
- Arrange for crabbers to take fish carcasses.

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- Prohibit fish cleaning at your marina.
- Educate people on the water quality problems associated with excess fish waste in lake waters.

All the materials and equipment used in alga and live feed culture should be handled and transported according.

Good handling measurements are:-

- Provide sanitation services to the working devices after and before work
- Maintaining identification and selection of functional equipment from non-functional ones.
- Use safe and well organized storage for tools, equipment and materials.
- Use recommended transportation system as the transported materials nature.
- Make of care during loading and unloading of materials, equipment and tools

4.2. Treating and disposing unused cultures and wastes

- Prohibit fish cleaning outside of designated areas.
- Implement fish composting where appropriate.
- Use a grinder to make chum out of fish carcasses. Sell the chum at your store.
- Prohibit fish cleaning at your marina.
- Educate people on the water quality problems associated with excess fish waste in lake waters.
- Materials, tools and equipment's required to handle and transported properly.
- It requires using guidance for proper handlings and transporting.
- During transporting career should necessary for some fragile and toxic materials and equipment.
- Whenever we are going to our work area we have to handle and transport our equipment materials and tools safely. And also after completing our task we have to take them back to their place (store) safely without any damage on the equipment and ourselves by cleaning and maintaining if necessary.
- materials should handle in a good manner

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- put the same material on the same area don't mix with other
- transport carefully for fragile and toxic material
- Materials should be returned to store or disposed according to the condition. After the accomplishment of task all necessary materials, tools and equipment must be stored properly or if there is need to be disposed should be done accordingly

4.3. Ecologically sustainable development (ESD) principles

The correct management of waste material such as waste water (also called effluent), stock feed and chemicals is an important part of successful ESD in aquaculture. Waste management should focus on: Managing and controlling waste water, Minimizing waste, Disposing of construction waste and Disposing of dead stock.

as a set of principles, which are:

- Decision-making processes should effectively integrate both long-term and short-term economic, environmental, social and equitable considerations;
- If there are threats of serious or irreversible environmental damage, lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation;
- The principle of inter-generational equity—that the present generation should ensure that the health, diversity and productivity of the environment is maintained or enhanced for the benefit of future generations;
- The conservation of biological diversity and ecological integrity should be a fundamental consideration in decision-making; and
- Improved valuation, pricing and incentive mechanisms should be promoted.

4.4. Recording culture production data

Records are sets of information that have been systematically and carefully collected and appropriately stored for a specific purpose. To be able to run any economic enterprise successfully, carefully thought out and properly collected records are a must. Comprehensive record keeping will assist both in tracking farm activities and expenses and in assessing the level

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of investment, the motivation of the investor, and the management skills of the farmer.

4.4.1. Importance of record keeping

Maintaining good records helps you with the following:

- Tracking the activities of your enterprise
- Tracking the expenses of the enterprise
- Monitoring the performance of the enterprise
- Evaluating the performance and operations of the enterprise
- Making decisions about improving operations
- Keeping institutional memory of the enterprise

Good records will, for example:

- Be useful in projection of expected production
- Help in determining the amount of inputs required for specific ponds at various stages of fish production
- Help determine the expected harvesting time
- Determine the economic health of the enterprise

Important aquaculture parameters for record keeping

- Pond identity
- Total area under culture
- Fish species stocked
- Sources of seed
- Stocking densities and time
- Kinds, quantities, and costs of inputs
- Daily events
- Fish production in amounts and values
- Production of other farm crops and their values

i. Classification of fish farming records

Fish farming records can be classified into:

- Fish farming biological management records, e.g.:

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- ✓ Specific pond production (quantity and value), by species
- ✓ Stocking details for each pond (species and numbers)
- ✓ Harvest details for each pond (species, numbers, and weights)
- Financial management records such as:
 - ✓ Purchase of inputs, including quantities and costs
 - ✓ Records of input usage, e.g., feeds and labour
 - ✓ Costs of labour, including the type and duration
 - ✓ Costs of new construction or repairs
 - ✓ Salaries, both in cash and in kind
 - ✓ Sales records, including what was sold, quantities, and prices
 - ✓ Inventory of equipment
 - ✓ Costs of renting or hiring equipment, machinery, services, etc

4.5. Reporting work conditions

Reporting of work outcome started from recording. As a fish farmer, your main objective is to earn money by selling fish at a profit. To understand why you are getting good or poor results, you will need to keep complete and accurate records of everything that goes on at your farm.

As a commercial fish farmer, your main objective is to earn money by selling fish at a profit. To understand why you are getting good or poor results, and more importantly whether or not you are making a profit, you will need to keep complete and accurate records of everything that goes on at your farm.



Self-check 4	Written test
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Name:.....ID:..... Date

Directions: Answer all the questions listed below. Examples may be necessary to aid some explanations.

Part 2: Give short answer (15points)

1. List the Important aquaculture parameters for record keeping
2. List Classification of fish farming records
3. List Cleaning, repairing and storing of equipment

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