PREFACE

In a bid to standardize higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core, generic, discipline specific general elective, ability and skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility of choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry their acquired credits. I am happy to note that the University has been recently accredited by National Assessment and Accreditation Council of India (NAAC) with grade "A".

UGC Open and Distance Learning (ODL) Regulations, 2020 have mandated compliance with CBCS for U. G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Under graduate Degree Programme level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the six semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English/Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

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Netaji Subhas Open University

Under Graduate Degree Programme

Choice Based Credit System (CBCS)

Subject : Honours in Zoology (HZO)

Course : Ecology and Biochemistry Lab

Course Code : CC-ZO-05

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Netaji Subhas Open University

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UG : Zoology
(HZO)

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Unit - 1 ❑ Preparation of nested quadrat and estimation of effective quardrat size

Structure

1.0 Objectives

By studying this unit learners would be able to understand about

- Preparation of nested quadrate
- Estimation of effective quadrate size
- Quadrate number of different plant species

1.1 Introduction

The quadrat is a square sample area of varying size required for the acquisition of dependable data to realise the biotic diversity of the entire community in an ecosystem. It is also effectively used to determine the exact difference or similarity in the structure and composition between two or more communities of related or unrelated regulation.

In a sampling terrestrial ecosystem the objective is not only to study the availability of particular species but also equally to emphasize their distribution frequency and abundance. Although it is not always possible to count and measure the entire biotic community in a large area modern sampling method emphasises for a particular species. With the knowledge on optimum quadrat size, one can have information about the diversity of the entire biotic community.

1.2 Preparation of nested quadrate

Generally a measured area for example 80 × 80 cm^2 are laid down at random in a chosen field at different sites. Different species that are found in each quadrat are recorded; the number of species is then listed in the ascending order (Table-1).

Table-1 : Quadrat number and the plants

Quadrat Number	Plant species
1	*Peparomea* sp., *Eaplia alba*, *Caccinia* sp., *Solanum nigrum*, *Amaranthus* sp., *Oxalus* sp.
2	*Enhydra* sp.
3	*Phylanthus* sp.
4	*Cynodon* sp., *Vandelia* sp.

1.3 Estimation of effective quardrat size

The procedure is to select a point randomly in the study area. A quadrat measurement (20 × 20 cm) is then taken. No of plant species within it are counted. Gradually the size of the quadrat is increased i.e., 40 × 40 cm, 60 × 60 cm and like that. Subsequently plant species are counted in different quadrat of different size. Experiments are then completed when three numbers of same species are consequently recorded (Table-2).

Table-2 : Determination of minimum quadrat size

Sl. No.	Size of quadrat	No. of species	No. of additional species	Total no. of species
1	20 × 20 cm^2	5	0	5
2	40 × 40 cm^2	5	0	7
3	60 × 60 cm^2	7	1	8

Sl. No.	Size of quadrat	No. of species	No. of additional species	Total no. of species
4	80 × 80 cm^2	8	2	10
5	100 × 100 cm^2	10	0	10
6	120 × 120 cm^2	10	0	10

This can be plotted in a graph also. The size of the quadrat used are plotted in the X-axis against the number of species plotted in Y-axis. A curve is obtained and the point at which the curve start flattening indicates the size of the quadrat required for sampling in the chosen study area (Figure-1). Since the curve gets flattened at 80 × 80 cm^2 point, it represents the optimum size of the sample area necessary to determine the plant community present in the ecosystem and it appears that in the chosen study area the optimum size of the quadrat is 80 × 80 cm^2.

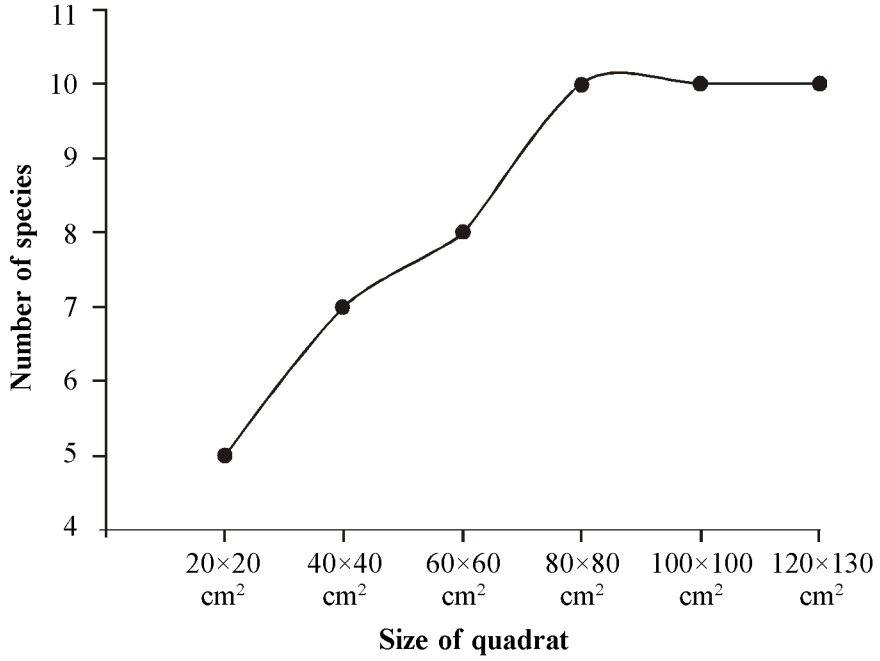

Figure 1 : Curve to show number of quadrats required for sampling

To determine the minimum number of quadrats, in a grassland ecosystem for example, it is necessary not only to study the type of the species which are absent but also it is important to determine the no of quadrats required for biotic diversity analysis (Table-3).

Table-3: To determine the minimum number of quadrats, the following observation can be made

Size of quadrat	No. of quadrats	No. of Species	No. of Additional species	Total no. of species
80 × 80 cm^2	1	5	0	5
	2	5	3	8
	3	8	2	10
	4	10	1	11
	5	11	1	12
	6	12	0	12
	7	12	0	12

Table-3 indicates that total number of species is not increased beyond 5 quadrats. Therefore, the minimum no of quadrat necessary is calculated as 5.

1.4 Selected questions

(i) Mention at least three observation points to determine number of quadrats in any ecosystem.

(ii) Why it is necessary to count the number of plants, for example, in any particular area with quadrats of different sizes?

(iii) What is indicative of flattening of curve in a graph of number of species versus size of quadrats ?

1.5 Suggested readings

1. Magurran, AE (2004). *Measuring Biological Diversity*. Blackwell, UK

Unit - 2 ❑ Biodiversity of ecological communities : calculation of Sorenson's similarity & Shannon-Weiner diversity indices for a community

Structure

2.0 Objectives

By studying this unit learners would be able to understand about

- Definition of Shannon index
- Calculation of Shannon-Weiner index
- Definition of Simpson's index
- Other indices to measure similarities between communities

2.1 Introduction

Measurement of biodiversity is of prime interest to ecologists for explaining structure and function of ecological communities. The measurements are based on

statistical sampling of species and calculation of indices. Consequently methods of measuring several indices of biodiversity have been developed (Magurran, 2004), which are based on two separate components:

(i) Number of species indicating species richness

(ii) Relative abundance of a species indicating dominance or evenness of species.

A community may have huge number of species indicating high diversity of species with a few species showing abundance in number (or dominance) or all species showing equal abundance (evenness).

Three indices of biodiversity have been explored here :

1. The Shannon index (H′), also termed as Shannon-Wiener index indicating both diversity and evenness of a community.
2. Simpson's index of dominance (D).
3. Jaccard's similarity index, which is used to compare diversity among communities.

2.2 Shannon index

The idea behind this index is that the diversity of a community is similar to the amount of information in a code or message. It is calculated in the following way :

$$H' = -\sum p_i \ln p_i$$

Where pi is the proportion of individuals found in species i. For a well-sampled community, we can estimate this proportion as $p_i = n_i/N$, where n_i is the number of individuals in species i and N is the total number of individuals in the community. Since by definition the p will all be between zero and one, the natural log makes all of the terms of the summation negative, which is why we take the inverse of the sum.

This has been explained in Table-4 on a set of hypothetical data.

Table-4 : Calculation of Shannon-Weiner index

Species	No. of individuals (ni)	ni/N	$(ni/N)^2$	ln (ni/N)	ni/N{(ln(ni/N)}
Species – 1	12	0.333	0.111	–1.099	–0.6627
Species – 2	11	0.305	0.093	–1.187	–0.3620
Species – 3	6	0.167	0.028	–1.789	–0.2987
Species – 4	3	0.083	0.007	–2.488	–0.2065
Species – 5	1	0.028	0.001	–3.575	–0.1001
Species – 6	3	0.083	0.007	–2.4889	–0.2065
Total (Σ)	N = 36		0.247		1.8365

Shannon index (H′) = Σpi lnpi or $\Sigma(n_i/N)\ln(n_i/N)$ = 1.8365

Simpson's Dominance index $\{\Sigma(n_i/N)^2\}$ = 0.247

Interpretation : Typical value of H′ generally lies between 1.5 and 3.5 in most ecological communities and the index is rarely greater than 4. The Shannon index increases as both the richness and the evenness of the community increase. The fact that the index incorporates both components of biodiversity (variety and evenness), it has both strength and a weakness. The strength lies in its simple, synthetic summary, but it has a weakness because it makes it difficult to compare communities that differ greatly in richness.

Due to the confounding of richness and evenness in the Shannon index, many biodiversity researchers prefer to stick to two numbers for comparative studies, combining a direct estimate of species richness (the total number of species in the community, S) with some measure of dominance or evenness. The most common dominance measure is Simpson's index.

2.3 Simpson's index

Evenness and dominance are contrast to each other and their measures are thus complimentary to each other. Simpson's index indicates whether individuals of different species in a community are present in equal or unequal proportion. More

the degree of inequality more is the dominance. It is calculated by the following formula (Table-4) :

$$D = \sum p_i^2$$

Where again p_i is the proportion of individuals found in species i. For a finite community, this is

$$D = \sum \frac{n_i(n_i - 1)}{N(N-1)}$$

Interpretation : D is a measure of dominance. As D increases, evenness decreases. Thus, Simpson's index is usually reported as its complement 1-D (or sometimes1/D or -lnD). Since D takes on values between zero and one, D approaches one in the limit of a monoculture, (1-D) provides an intuitive proportional measure of diversity that is much less sensitive to species richness.

2.4 Comparing communities : Jaccard's Index (J)

Of course, we are usually interested not just in the diversity of a single site, but in comparing biodiversity levels across sites. Communities can differ in a number of ways. Considering only the plant component of a system, two communities can differ in species composition (taxonomy), total number of species (richness), and the relative abundance of species (evenness). Species diversity refers to a community-level concept that combines both richness and evenness.

We use a number of different indices to estimate the similarity of two communities. Considerable controversy exists about the effectiveness of these indices as they vary in performance for things like total number of species involved, influence of rare species, etc. A number of papers recently have explored what an appropriate index might be.

An intuitive measure of similarity between two samples can summarize the fraction of species they share.

Jaccard's index is the simplest summary of this, taking the following form :

$$J = \frac{S_c}{S_a + S_b + S_c}$$

Where S_a and S_b are the numbers of species unique to samples a and b, respectively, and S_c is the number of species common to the two samples.

Interpretation : Jaccard's index of similarity is very straight forward since it is simply the fraction of species shared between the samples. Keep in mind, however, that Jaccard's index only utilizes the richness component of diversity, since it does not entail any information on abundance. As a pair wise measure, we can examine how Jaccard's index varies with the distance or environmental differences between the sites.

The Jaccard index, also known as the **Jaccard similarity coefficient** (originally coined coefficient de *communaute* by Paul Jaccard), is a statistical component used for comparing the similarity and diversity of sample sets. The Jaccard coefficient measures similarity between sample sets, and is defined as the size of the intersection divided by the size of the union of the sample sets. This index only uses presence-absence data.

2.5 Other indices to measure similarities between communities

Sorensen-Dice (Sorensen or Sorensen binary) (presence or absence)

$$SSD = \frac{2a}{2a+b+c}$$

Where :

a = number of species in both sites

b = number of species in second site only

c = number of species in first site only

The Sorensen index, also known as Sorensen's similarity coefficient, is a statistics used for comparing the similarity of two samples. It was developed by the Botanist Thorvald Sorensen and published in 1948. It also uses presence-absence data. When we use both the Jaccard and Sorensen index on the same data set, how they differ in performance may be seen below.

Sorensen's index is easily extended to abundance instead of incidence of species. This quantitative version of the Sorensen index is also known as the Bray-

Curtis Similarity index. When using the Bray-Curtis quantitative index, the "minimum" value (# individuals or % cover or Importance Value) for a species when comparing two samples is used for the numerator values.

Bray-Curtis (sometimes called Pielou's percentage similarity or Czekanowski's) **index**

$$S_{BC} = \frac{\sum 2 * \min(n_{1i}, n_{2i})}{\sum n_{1i} + \sum n_{2i}}$$

Where :

n_{1i} = the number of individuals or % cover or importance value of the ith species in sample 1

n_{2i} = the number of individuals or % cover or importance value of the ith species in sample 2

min = refers to the lower abundance value for the species of the two samples being compared

2.6 Laboratory problems

We will use a made-up data set to compare the performance of these similarity indices. Imagine comparing two communities (samples) with 30 species each and 25 species are found common in both samples. Run the calculations. Then modify the proportions in the two samples so we can understand how the indices perform. Change the proportions so both samples keep 30 species, but only 20 are in common, then 15, then 10, then 5. Then contrast a 30 species community with a 20 species community (start with 20 species in common), and make similar proportion shifts.

Now let's see how the total number of species influences things. Start with comparing a two communities of 30 species with 20 species in common (you did this above). Now change it so they both have 30 species, but only 19 in common. Now compare two communities of 15 species each with 10 species in common, and then with 9 species in common. Finally, compare two communities of 3 species each, with 2 species in common, then change it to 1species in common. In this second round, we're only shifting 1 species each time, but because of 'proportion' shifts, the

measures perform quite differently. If we were using Bray-Curtis values, the shifts might not have been changed in the same manner.

2.7 Selected questions

(i) State the significance of Simpson Index and Shannon Index in a biodiversity study.

(ii) What are the indices used to deduce similarities between two species ?

(iii) Why it is important to assess biodiversity index in ecological communities ?

2.8 Suggested reading

1. Magurran, AE (2004). *Measuring Biological Diversity*. Blackwell.

2. Samal, PK, Dollo, M, Singh, J, Lodhi, MS, Arya, SC, Dhyani, PP and Paini, S (2013). Biodiversity conservation through community based natural resource management; G B Pant Institute of Himalayan Environment and Development, Highlanders Communication, Almorah, Uttarakhand.

Unit - 3 ❑ Study of an aquatic ecosystem : major phytoplankton (up to family) and zooplankton (up to genus), temperature, turbidity/ penetration of light, determination of pH, and dissolved oxygen content (Winkler's method) and free CO_2

Structure

3.0 Objectives

By studying this unit learners would be able to understand about

- Different phytoplankton and zooplankton species
- Culture and collection of zooplankton
- Bacterio-plankton and planktonic detritus
- Physico-chemical characteristics of water

3.1 Introduction

Aquatic ecosystem represents a water body which is inhabited by communities of organism that are dependent on each other for their sustenance. The two main types of aquatic ecosystem are freshwater ecosystem and marine ecosystem. Common examples of freshwater ecosystem are ponds, lakes, rivers and various wetlands. Similarly, estuaries, coastal ecosystem, oceans, coral reefs are examples of marine ecosystem. Stagnant water bodies like ponds and lakes represent lentic ecosystem, whereas running water bodies like streams and rivers represent lotic ecosystems. In case of wetlands, soil remains inundated for most of the time of the year.

3.2 Plankton

Plankton are free floating living organism, whose intrinsic power of locomotion is so feeble that their movements depend upon the mercy of water current. They are basic links in the food web of aquatic ecosystem. The optimum biomass of plankton in fertile water is 20-100 mg/l. If the biomass is below 20 mg/l or over 100 mg/l, the water is denoted as oligotrophic or so eutrophic respectively. Plankton can be divided into phytoplankton, zooplankton, bactrio-plankton and planktonic detritus.

3.2.1 Phytoplankton

The freshwater phytoplankton comprises the free floating species of Myxophyceae, (Cyanophyceae), Euglenophyceae, Chlorophyceae, Chloromonadineae, Xanthophyceae (Heteroknotea), Chrysophyceae, Bacillariophyceae (Diatoms), Cryptophyceae, Dinophyceae (Peridineae), Phaeophyceae and Rodophyceae. They are autotrophic primary producers in the aquatic ecosystem. They can absorb CO_2 and other nutritional salts and sunlight for synthesizing organic matters for direct or indirect food of fishes. At the same time O_2 is produced which is essential for life

in water also. The excessive growth of phytoplankton produces water bloom, sometimes called algal blooms, which are potential hazards to fish life. Some species of algal bloom that frequently cause bloom include *Chlorela*, *Nostoc*, *Anabena*, *Oscilatoria*, *Microcystis* etc. This can however be controlled by introducing duck weeds (*Spirodela*, *Lemna*, *Wolfia*) or silver carp of about 500gm weight each and total 500 pieces per hector. Application $CuSO_4$ at a dose of 0.2-0.5 ppm can also be used to control.

3.2.2 Zooplankton

Zooplankton are heterotrophic primary consumers and are mainly composed of free living species of Protozoa, Rotifera, Cladocera and Copepoda (Plate 1). They feed on bacteria, phytoplankton detritus and serve as the most preferred food organism of carp spawn and fry till the time gill rackers develop and feeding habits change. Most widely used starters fish food organisms for fish are densely populated (2000-5000/l) rotifers followed by micro crustaceans such as clodocerans – *Monia*, *Daphnia* and *Bosmina* species. Among rotifers *Brachionus* spp. are considered very good food organisms as they come within 100-150 μ size. Rearing of mixed rotifers in pond water condition provides an excellent starter food for spawn. Copepods often prick the membrane of fertilise eggs of carps and eats the embryos and yolks sac. The larger copepods (cyclopoid copepods) are highly predatory and can destroy large quantity of carps spawn. Thus their control previous to stocking of spawn and fry is mandatory. Somewhere farmers use low dose of insecticide before five days of stocking and this is however not recommended. It is better to use thick mesh-sized net repeatedly. By this way predatory zooplankton can be controlled.

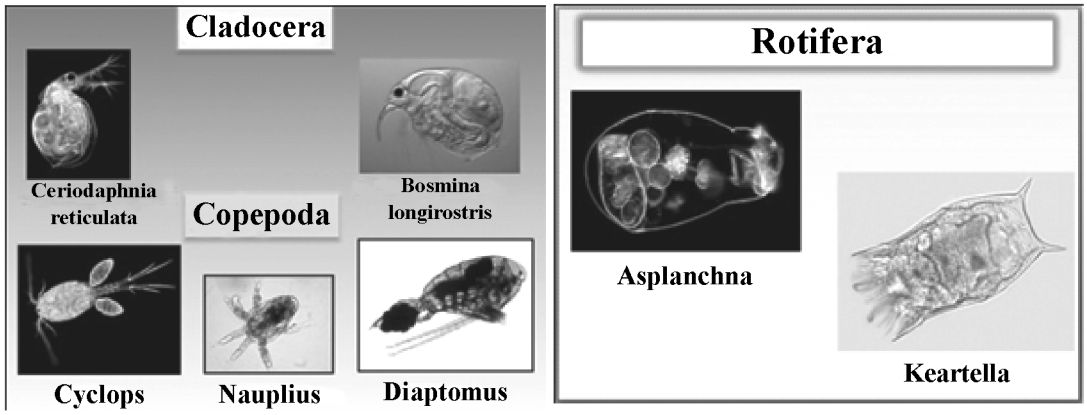

Plate-1: Different zooplankton species found in freshwater

Rotifers are planktonic filter feeders, wherein they ingest many types of food, including algae, yeast, and bacteria or inert food such as microcapsules as long as

the size of the particle is considerably appropriate. So variety of food sources can be used to rear the rotifers, but only the nutritive and palatable food justifies its productivity.

In freshwater aquaculture, more particularly larviculture, feeding of early larval stages is one of the major hurdles faced by the hatchery industry. It is also reported that most of the fish larvae during their early stages feed on passively motile prey organisms and have poor growth and survival when fed with prepared diets. This is attributed to better activity of the digestive enzymes of the larva fed on live foods than dry diets. Even if they accept the diets, poor enzymatic activity and less functional stomach may not allow larvae to digest dry diets. Hence, the success of any hatchery system (shellfish or finfish) virtually rely on the availability of suitable live food. Rotifers and Artemia are the major live food organisms widely used in the rearing of fish larvae.

3.3 Culture and collection of zooplankton

Zooplanktons are cultured in large cement tanks. The tanks are filled with water and fertilized with cow dung. Then the tanks are kept open for 30-60 days. Water of the tanks are sampled periodically by plankton net to check if any zooplankton species has grown (Plate 2). Alternatively, zooplanktons are collected from natural water bodies using the plankton net (Plate 3).

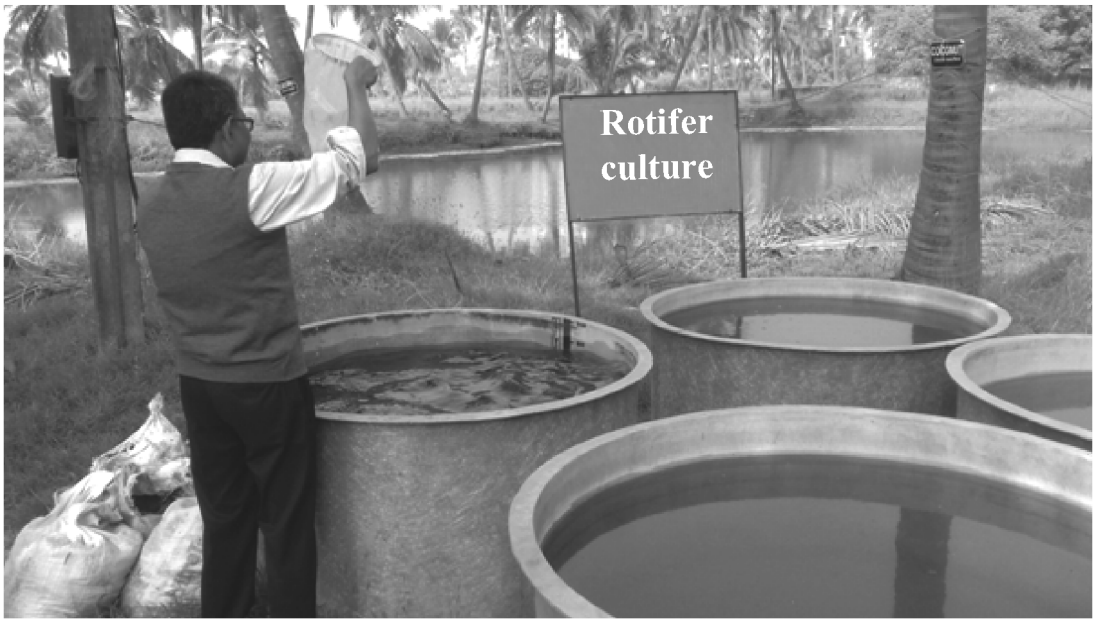

Plate-2 : Zooplankton culture

Plate-3 : Zooplankton collection from natural bodies of water

3.4 Bacterio-plankton and planktonic detritus

These are free floating bacteria called heterotrophic decomposer. They obtain nutrients and energy through the breakdown of organic matter (detritus, dead animals and plants etc). The total bacterioplankton counted on membrane filter constitute the basic food for zooplankton which again form the main food items for filter feeder fish species. Moreover, bacterioplankton and phytoplankton themselves form food for fine filter feeder fishes. Gills of the fish produce mucus to which small particles, even isolated bacteria and small bacterial aggregates adhere and these are aggregated into larger mass and then swallowed by fish. Bacteria constitutes up to 30% of the particulate matter in oligotrophic water and up to 70-80% in eutrophic water. The density of bacteria ranges from 2.4 million/ml in eutrophic water. Together with detritus it contributes to the production through food chain.

Organic detritus is one of the basic nutrient sources for aquatic organisms and has an important role in trophic process of aquatic ecosystem. The detritus are surrounded or even filtered with bacteria. Therefore, the nutritive value of the organic particles for aquatic organisms is dependent on the quality of micro organisms, they contain and that utilize energy of organic matter, associated with the organic matter. Bacterial cells contain amino acids and other nutrients. Detritus forms 20-80% of total particulate matter and calorific value of detritus is estimated to be 1.5 – 4.6 kcal/g. The detritus is community consumed by carp larvae and provide nourishment for their growth.

There are periphyton which are assemblage of microscopic organisms growing upon free surface of submerge objects in water covering them with a slimy coating. They form very good food for carps like rohu.

There are benthic organism in pond bottom which are rich in organism like oligochaete and chironomid larvae and these are food for bottom feeding fishes like mrigal, calbasu, bata etc.

3.5 Physico-chemical characteristics of water

3.5.1 Temperature

Temperature is the single-most important physical factor affecting the life of aquatic organisms including fish. The temperature of water affects activity, behaviour, growth, metabolism and reproduction of fishes. Metabolic rate of fishes becomes double for each 7.8 degree celsius temperature. Fish are categorised into warm water, moderate water and cold water species depending on optimal growth temperature 10-20 degree celsius for coldwater, 21-25 degree for moderate water and 26-35 degree for warm water .

3.5.2 Turbidity

The turbidity in any water body occurs due to suspended solids which is usually associated with clay particles, plankton, fish waste, uneaten fish feed in water. Turbidity caused by clay or soil particles can restrict light penetration and limit photosynthesis. Sedimentation of soil particles may also smother fish eggs and destroy beneficial communities of bottom organisms including bacteria. Adding organic matter slowly to water can reduce clay turbidity. Fish waste particles may irritate the gills of fish and can cause problems to the biological filters including clogging that may cause vitrifying bacteria to die from lack of oxygen.

Transparency and turbidity are inversely proportional. Secchi disc is used for estimation of turbidity. It is a metallic plate of 30 cm diameter with four alternative black and white quadrants on the upper surface and a hook at the centre to tie a thread or ribbon (Plate 4). The procedure is simply to dip the plate and observe the depth at which the disc let down from the water surface just disappear from the view. It is usual to determine the point of disappearance as the disc is lowered (d_1) and allowed to drop a little further to determine the point of reappearance as the disc is raised (d_2). 20-40 cm length is considered optimum for natural productivity of water. The mean of the two readings is taken as Secchi disc transparency. The observation should not be made early in the morning or late in the afternoon.

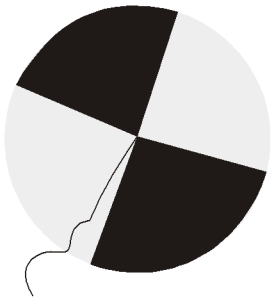

Plate-4 : Secchi disc

3.5.3 pH and its determination

pH is a measure of acidity or alkalinity. The quantity of hydrogen ion (H+) in water will determine if it is acidic or alkaline. A value of 7.0 is neutral, neither acidic nor alkaline, while values below 7.0 are considered acidic and above 7.0 is considered alkaline. The acceptable range for aquaculture is normally between. 7.0 to 8.5; below 6.5 and above 9.0 is not suitable. pH value can be measured directly by pH paper by dipping the paper or by pH metre by dipping electrode into water sample. For a rough estimation, pH paper method is easy and cheaper. Just to dip the pH paper piece in water sample taken in a glass tumbler or beaker and then to compare the changed colour with the standard colour chart provided with the pH paper. Lovibond comparator is also handy for field (pond site) testing of water pH. For this initially it is necessary to do the preparatory test with universal indicator to get the approximate value of pH. Then 10ml of water sample is placed in the glass tube provided with Lovibond comparator and then 0.2 ml universal indicator is added. It is then gently shaken and colour is matched against standard colour disc for the indicator. After ascertaining the approximate value, suitable indicators are used to determine the pH. Bromothymol blue for pH range 6.0 - 7.6; phenol red for 6.8 - 8.4 and thymol blue for 8.0 - 9.6 may be used as indicators.

3.5.4 Dissolved oxygen content

The critical factor governing carrying capacity of a pond is dissolved oxygen content of the water.

Sources of dissolved oxygen : absorption from atmosphere into the water surface and photosynthesis of chlorophyll bearing organisms inhabiting the pond.

Consumption of dissolved oxygen : respiration of aquatic animals and plants for the day and night and decomposition of organic matter.

The oxygen available in a pond at a given time is a balance of these two processes. Values of dissolved oxygen content depend on temperature, atmospheric pressure and water salinity. When temperature increases dissolved oxygen decreases. When partial pressure of oxygen in contact with water at surface increases amount of oxygen dissolved in water also increases. When concentration of dissolved salts (salinity) increases, dissolved oxygen content decreases.

It is generally considered that dissolved oxygen level continuously below 3.0 mg/L is unproductive; between 3-5 mg/L is average productive and 6 - 8.0 mg/L is highly productive.

3.5.5 Determination of dissolved oxygen in water

I. Reagents required :

Winkler's A solution : 480 g manganous sulphate (MnSO4, $4H_2O$) is slowly dissolved in 250 ml distilled water and then volume made up to 1.0 litre.

Winkler's B solution : 700g of potassium hydroxide (KOH) and 150g potassium iodide (KI) are dissolved in 800ml distilled water and then volume made up to 1.0 litre.

N/80 sodium thiosulphate solution ($Na_2S_2O_3$, $5H_2O$) : This solution is prepared by dissolving 3.1g crystalline sodium thiosulphate in 700ml distilled water followed by adding 4.0g borax ($Na_2B_4O_7$,$10H_2O$) and volume made up to 1.0 litre.

Starch solution : 1g of starch powder is added to 5ml distilled water, mixed well and added 95 ml boiling water to which added further 3g boric acid as preservative.

Concentrated sulphuric acid : 36(N) specific gravity 1.84

II. Procedure : Carefully to collect 250 ml water sample to be analysed from 1-1.5 ft. below the water surface from the pond. Immediately after collection, 2.0 ml each winkler's A and B are added using two different pipettes. The bottle is stoppered and mixed the contents by shaking. A brownish precipitate is formed which settles down after about 15 minutes; then added 2.0 ml conc. Sulphuric acid to dissolve the precipitate. 100 ml of this clear solution is then taken and titrated with N/80 soium thiosulphate solution using starch solution as indicator to the colourless end point.

Dissolved Oxygen in the 100 ml sample of water = ml. of N/80 $Na_2S_2O_3$ consumed in the titration or the burette reading.

3.5.6 Dissolved free carbon dioxide

Sources of CO_2 in natural water :

(i) From atmosphere: Rain water contains 0.3 – 0.6 mg/L and air in contact with water surface.

(ii) Respiration of aquatic plants and animals.

(iii) Decomposition of organic matter in water body.

Consumption of CO_2 :

Photosynthesis by aquatic plants and phytoplankton of water

Carbon dioxide is present in three forms, bound $CO_3^=$, half bound HCO_3^- and free state CO_2. In absence of sunlight, when CO_2 comes in contact with water, it produces carbonic acid by the reaction $H_2O + CO_2 = H_2CO_3$, which displays its weak acidic character through dissociation.

$$H_2CO_3 = H^+ + HCO_3^-$$

Just before day-break, concentration of free CO_2 is highest and pond water is therefore, most acidic. Pond which is on calcareous soil contains free $CaCO_3$. This $CaCO_3$ is helpful to prevent the water pH to fall below 5.0 according to the following reaction.

$$CaCO_3 + CO_2 + H_2O = Ca(HCO_3)_2$$

$Ca(HCO_3)_2$ is far less acidic than H_2CO_3. When the pond soil does not contain a free $CaCO_3$, lime should be applied. Lime corrects acidity of water. Ca of lime acts as buffer

Reserve of CO_2 :

The solution of $Ca(HCO_3)_2$ remains stable only in the presence of certain surplus amount of CO_2. Therefore, the CO_2 which is necessary to retain the calcium in solution in the form of Ca $(HCO_3)_2$ is called equilibrium or free CO_2. 2 to 10 ppm of free CO is ideal for good productivity of pond. 20-30 mg/L of CO_2 can be tolerated provided dissolved oxygen is near to saturation. Above 30 mg/L CO_2

concentration cause depletion of O_2 but air-breathing fish may survive even at 100 mg/L free CO_2 concentration.

Determination :

Preparation of N/44 Sodium carbonate (Na_2CO_3) : Dissolve 5.3 g Na_2CO_3 in 1000 ml of distilled water. Dilute 100 ml of this solution (N/ 10) to 440 ml with distilled water to get N/44 Na_2CO_3.

Procedure : Take 50 ml of water sample in a conical flask and add 2 drops of Phenolphthalein reagent. If the water turns pink there is no free carbon dioxide, if not, add N/44 Na_2CO_3 drop by drop from a 10 ml graduated pipette with simultaneous gentle stirring with a glass rod till the colour turns pink.

Free carbon dioxide (mg/L) in the water sample = ml of N/44 Na_2CO_3 required X 20

3.6 Selected questions

(i) Why phytpplankton are primary producers and zooplankton are primary consumers ?

(ii) What do you mean by lentic and lotic ecosystem ?

(iii) Mention at least one point stating the reason of algal bloom formation in stagnant ponds.

(iv) Why rotifers are considered important as fish food organisms ?

(v) What are the sources of dissolved oxygen in water. Mention two factors which may control its level.

3.7 Suggested readings

1. Chattopadhyay, GN (2000). Water quality characteristics, Narendra Publishers, Delhi

2. Nayak AKJR (2006). Sustainable sewage water management, Macmillan India Limited, Daryaganj, New Delhi-2

3. Patil PN (2012). Physico-chemical parameters for testing of water – a review. International Journal of Environmental Science, 3(3) : 1194-1207

Unit - 4 ❑ Estimation of primary productivity by light and dark bottle method

Structure

4.0 Objectives

By studying this unit learners would be able to understand about

- Definition of primary productivity
- Determination of primary productivity
- Definition of gross and net primary productivity

4.1 Introduction

Primary production refers to all or any part of the energy fixed by plants possessing chlorophyll; productivity refers to the rate of production on a unit area basis. The total amount of solar energy converted (fixed) into chemical energy by green plants is called gross primary production (GPP). A certain production of GPP is utilised by plants for maintenance (largely respiratory or energy loss) and the remainder is called net primary productivity (NPP), which appears as new plant biomass. Thus GPP = NPP + R (respiration) or NPP = GPP - autotrophic respiration (R).

4.2 Determination of primary productivity

Primary productivity of a water body (or, aquatic ecosystem) is determined from the difference in dissolved oxygen values of water samples incubated in fixed

quantity Winkler's bottles under light and dark conditions thereby allowing photosynthetic activity in one bottle to take place and the same to be restricted in other bottle. This is known as light and dark bottle method.

The decrease in dissolved oxygen in dark bottle as compared to the initial value represents the amount of dissolved oxygen consumed by respiration by all the biomass in the bottle. The increase in dissolved oxygen in light bottle is due to photosynthesis of autotrophs which exceeded oxygen consumption by respiration/ decomposition. Both GPP and NPP can be calculated from the differences in dissolved oxygen values.

The procedure involves collection of water samples in fixed quantities in three Winkler's bottles. One bottle is designated as initial bottle (IB), the second is light bottle (LB) and the third as dark bottle (DB). LB is left exposed to sunlight till afternoon and DB is wrapped with an opaque dark paper and is left in the dark chamber till afternoon. As per Winkler's method, the dissolved oxygen content is measured immediately after sample collection and the dissolved oxygen values of LB and DB are measured after the incubation time (T).

Let us assume, dissolved oxygen of IB = 1.2 mg/L, that of LB = 1.4 mg/L and DB = 1.0 mg/lit.

$$\text{Now, NPP} = \frac{\text{LB} - \text{IB}}{\text{T}} \times \frac{12}{32} \times 1000 \text{ mg C/m}^3\text{/hr}$$

$$\text{GPP} = \frac{\text{LB} - \text{DB}}{\text{T}} \times \frac{12}{32} \times 1000 \text{ mg C/m}^3\text{/hr}$$

Where T = Time in hours; 12 and 32 stand for molecular weights of C and O_2 respectively. Multiplication by this factor (12/32) is necessary to convert dissolved oxygen values to carbon fixed in carbohydrate produced during photosynthesis. Since 1 m^3 area contains 1000 litre of water, the value is multiplied by 1000 to get value in mg C per m^3 per hour

Say dissolved oxygen value of IB, LB and DB are 1.2, 1.4 and 1.0 mg/L respectively. Using dissolved oxygen values of these 3 bottles we get

$$\text{NPP} = \frac{1.4 - 1.2}{4} \times \frac{12}{32} \times 1000 \text{ mg C/m}^3\text{/hr}$$

= 18.75 mg C/m^3/hr

$$GPP = \frac{1.4-1.0}{4} \times \frac{12}{32} \times 1000 \text{ mg C/m}^3\text{/hr}$$

= 37.5 mg C/m^3/hr

If we collect water from one edge of a pond, the following formula is used to get the value for the whole pond

$$\text{Mean NPP} = \frac{X \times 12}{y} \text{ mg C/m}^2\text{/day}$$

Where X = NPP

Y = Depth of the pond; since NPP value has been derived as per hour, it is multiplied by 12 (day length) to get the value for whole day.

If the depth of the pond is 5 meter

$$\text{The mean NPP} = \frac{18.75 \times 12}{5} \text{ mg C/m}^2\text{/day}$$

= 45 mg C/m^2/day

Comment :

Based on Mean NPP of different ranges, ponds are classified as follows-

Classification of pond/Trophic type	Mean NPP (mg C/m^2/day)
(i) Ultra-oligotrophic	<50
(ii) Oligotrophic	50-300
(iii) Mesotrophic	300-1000
(iii) Eutrophic	>1000

4.3 Selected questions

(i) Briefly discuss on sustainable pond productivity.

(ii) State two factors which influence primary productivity.

(iii) How the primary productivity of an ecosystem is defined ?

(iv) What is gross primary productivity and how it differs from net primary productivity ?

(v) State the significance of secondary productivity.

4.4 Suggested readings

1. Handbook of Aquaculture, Publication Division, Indian Council of Agricultural research, New Delhi-12.

3. Patil PN (2012). Physico-chemical parameters for testing of water – a review. International Journal of Environmental Science, 3(3) : 1194-1207

Unit - 5 ❑ Report on a visit to National park/ Biodiversity park/ Wildlife sanctuary/Sea shore

Structure

5.1 Objectives

By studying this unit learners would be able to prepare reports on a visit to National park/ Biodiversity park / Wildlife sanctuary/Sea shore

5.2 Introduction

Any of these visits should be reported in a manner that has to be informative and will have to be expressed to the point.

5.3 Preparation of report

An outline of the report should be as follows :

(a) Introduction : It will highlight the uniqueness of such a place having national importance with reference to ecological and socio-economic point of view. If it is, for example, in connection with a visit to mangrove forest in Sundarbans, then certain salient features depicting uniqueness of Sundarbans forest, importance of mangrove ecosystem, Sundarbans as Ramsar wetland and natural heritage site, socio-economic aspects of Sundarbans should be described under this heading.

(b) Purpose of the visit : It has to be clearly stated and in what way it would

benefit over and above fulfilling the requirements as laid down in the syllabus should also be mentioned.

(c) **The team members :** Along with the supervisor teacher and the specific responsibility entrusted upon individual / group team member(s) with regard to sample collection, photography, field observations to be done – all these should be recorded under this head.

(d) **Geographical location :** The geographic location including longitude, latitude, height above mean sea level, like that and then land and water remarks, flora and fauna to the extent possible and any other relevant point will also have to be noted.

(e) **Description of the journey :** Since journey is also no less important than the destination itself even if it is not a very long distance from the Institution/ University campus, students should give a description of the journey to make the report interesting.

(f) **Technical details of the field visit :** It will be the major feature in the report. This will have to be supplemented with photograph, sketch, an interview with local peoples, if any. To make the report informative the data should be presented in tabular form as much as possible.

(g) **Comments/feedback :** With regard to the particular tour comments or suggestions, if any, regarding the visiting place, journey, time of visit that may be considered useful for future visits should be made.

(h) **Acknowledgements :** Students should acknowledge the assistance received from institution, teachers, local guide or anybody else, from the planning stage till the end of the tour i.e. back home from the trip - should be incorporated.

5.4 Selected questions

(i) Why it is important to take part in academic excursion periodically?

(ii) Mention certain principles, ethics and guidelines to be followed during a visit, for example, to a location rich in biodiversity.

5.5 Suggested readings

1. India tourism development corporation guidelines to sanctuaries and wildlife parks, ITDC, New Delhi

Unit - 6 ❑ Qualitative tests to identify functional groups of carbohydrates in given solution

Structure

6.1 Objectives

By studying this unit learners would be able to understand about qualitative tests to identify functional groups of carbohydrates.

6.2 Introduction

Qualitative tests are done to detect the presence or absence of monosaccharides or disaccharides.

Monosaccharides : glucose, fructose

Disaccharides : sucrose, lactose.

6.3 Qualitative tests to identify functional groups of carbohydrates

Aldehydes (–CHO) and ketones (+CO) are active groups in carbohydrates. They contain hydroxyl groups as well. The properties of saccharides vary depending on the number of hydroxyl groups and the presence or absence of –CHO/+CO groups. These variations are the basis in the development of colour reactions to identify the saccharides.

Some simple tests can be used to identify the presence or absence of saccharides and these are as follows.

I. Fehling's test

Fehling's solution is prepared just before use by mixing equal volumes of copper sulphate and alkaline sodium-potassium tartarate.

Reagents :

Fehling's solution – A; 35 g. of copper sulphate is dissolved in distilled water and then the volume is made up to 500 ml.

Fehling's solution – B; 120 g of potassium hydroxide and 173 g of sodium-potassium tartarate are dissolved in water and volume is made up to 500 ml.

Method :

1. Equal volumes of solutions - A & B are mixed (1ml of each) to which 5 drops of the test solution is added and then boiled for 2-3 minutes.
2. A brownish yellow precipitate is formed.
3. The blue alkaline cupric hydroxide present in Fehling's solution when heated in the presence of reducing sugar, gets reduced to cuprous oxide which is brownish red in colour.
4. Thus formation of such coloured precipitate indicates the presence of reducing sugar in the test solution.

II. Benedict's method

Benedict's method is a modified Fehling's test that uses single solution and is more stable than Fehling's reagent.

Benedict's reagent preparation :

1. 173 g of sodium citrate and 100 g of sodium carbonate are dissolved in 800 ml of warm distilled water. If required, the solution can be heated to dissolve fully.
2. 17 g of copper sulphate is separately dissolved using a beaker/conical flask and then added to the above solution with stirring using a glass rod.
3. Volume is then made up to 1 litre with distilled water.

Method :

1. To a solution of 2ml of Benedict's reagent 5 drops of the test solution is added.
2. It is boiled for 2-3 minutes. Formation of red to greenish yellow coloured precipitate occurs depending upon the sugar concentration.
3. If no characteristic coloured precipitate is formed it indicates absence of reducing sugar in the given solution.

For non-reducing sugar such as sucrose, the test is as follows :

1. 5 drops of concentrated HCl is added to 5ml of the test solution.
2. It is heated for about 5 minutes on a boiling water bath.
3. Then, a few drops of 10% sodium hydroxide solution is added to make the solution alkaline (can be tested with litmus paper or pH paper).
4. Then Benedict's test can be performed as above to get red to yellow precipitate.

6.4 Selected question

1. State the significance of estimation of sugars in a biological fluid.

6.5 Suggested readings

1. Raghuramalu, N; Nair, KM and Kalyansundaram, S (1999). A manual of laboratory techniques, National Institute of Nutrition, Hyderabad-7.

Unit - 7 ❑ Paper chromatography of amino acids

Structure

7.0 Objectives

By studying this unit learners would be able to understand about the techniques required for paper chromatography of amino acids.

7.1 Introduction

Cellulose in the form of sheets of filter paper can be a useful support phase and has been the basis of paper chromatography. A mixture, for example, of amino acids is spotted onto the paper and then dried using a common hair drier. A solvent mixture is then flown along the sheet either by gravity (descending paper chromatography) or capillary attraction (ascending chromatography). The solvent front is marked and after drying the paper, the positions of the compounds present in the mixture are visualised by staining with a suitable reagent (ninhydrin in case of amino acids). The ratio of the distance travelled by the compound to that moved by the solvent is known as Rf value and is more or less constant for a particular compound, solvent system and paper under carefully controlled conditions of solute concentration, pH and temperature.

7.2 Materials required

- The equipment for paper chromatography

- Whatman No.1 (or any other suitable one) chromatography paper (20 cm × 20 cm.)
- Solvent mixture : Butanol : glacial acetic acid : water : 12 : 3 : 5.
- Ninhydrin colour reagent (0.2 g ninhydrin in 100 ml acetone to be prepared just before use)
- Oven at 100 degree celsius
- Standard amino acids (may be prepared in very small volume using 1% solution in 10% isopropanol. Sometimes a drop of dilute HCl may be needed to bring the compound into the solution).
- The amino acids : Alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine,valine.

7.3 Methodology

(i) At the start, a mixture of three amino acids is prepared.

(ii) 20 µl of this mixture is spotted in one corner of the sheet of chromatography paper.

(iii) The spot is then dried in a current of air. After drying, the sheet is mounted on a metal frame.

(iv) The frame is then placed with one of the edges of the paper to which the sample spot is adjacent to the solvent system and allowed the run for about 4 hours.

(v) The frame is then removed and placed it in the fume cupboard and dried the paper in a current of normal air temperature.

(vi) Rapidly then, paper was dipped in ninhydrin reagent and it was then hung in the fume hood chamber for the solvent to be evaporated.

(vii) The colours were then developed by heating at 105° C for 2 minutes.

(viii) The amino acid map is then constructed and compared with already done chromatogram using standard amino acid mixture.

7.4 Selected questions

1. Why qualitative tests of amino acids and their separation method should be done to begin with ?

7.5 Suggested readings

1. Plummer, DT (1990). An introduction to practical biochemistry, Tata Macgraw Hill company Ltd., New Delhi

Unit - 8 ❑ Estimation of total protein in a given solution by Lowry's method

Structure

8.0 Objectives

By studying this unit learners would be able to understand about estimation of total protein in a given solution by Lowry's method.

8.1 Introduction

Protein (mainly tissue protein) estimation as described by Lowry et al. (1951) is followed internationally as it is considered a very sensitive method.

Protein reacts with the Folin-Ciocalteau reagent to form a coloured complex. The blue colour formed by the reduction of phosphomolbydic-phosphotungstic components in the reagent by the amino acids-tyrosine and tryptophan present in the protein plus the colour developed by the reaction of alkaline copper with the protein form the basis of Lowry's method.

8.2 Materials

- 2% sodium carbonate in 0.1N sodium hydroxide (Reagent-A)
- 0.5% copper sulphate in 1% sodium-potassium tartarate (Reagent-B)
- Alkaline copper solution (Reagent-C)–mixture of 50 ml of A and 1ml of B (to be mixed prior to use only)

- Folin's reagent (Reagent-D)- available from any reputed international chemical company.
- Protein solution (stock standard) – accurately to be weighed 50 mg bovine serum albumin (Cohn Fraction V) and then dissolved it in 50 ml distilled water in a standard volumetric flask.

8.3 Procedure

- 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standards are pipetted into a series of test tubes. In other tubes, taken 0.1 ml, 0.2 ml, of the sample extract.
- The volume in each tube is then made upto 1.0 ml with distilled water.
- Side by side, a tube with 1.0 ml water only is taken as "blank".
- Added then 5 ml of reagent-C to each tube including blank.
- Mixing is then done well using a vortex mixer. It was then allowed to stand for 10 minutes.
- This is to be followed by addition of 0.5 ml of reagent-D and again mixed well and then the entire set of tubes are to be kept for 30 minutes at the room temperature but under dark condition.
- This will allow to form the blue colour. The colour intensity is measured at 660 nm in a spectrophotometer.
- A standard curve is drawn with protein level in X-axis and OD reading in the Y-axis.
- The amount of protein in the sample is calculated from the standard curve and is expressed as mg/g sample.

8.4 Selected questions

(i) State the procedure for estimation of total protein in a given solution.

(ii) State the significance of protein assay.

8.5 Suggested readings

Lowry O.H., Rosebrough, N.J., Farr A.L., Randaal, R.J., 1951. Protein measurement withthe Folin phenol reagent, J. Biol. Chem. 193, 265-275.

Unit - 9 ❑ Studies on activity of salivary amylase under optimum conditions

Structure

9.0 Objectives

By studying this unit learners would be able to understand about the estimation of activity of salivary amylase under optimum conditions.

9.1 Introduction

Amylase presents in saliva catalyses the hydrolysis of polysaccharides and oligosaccharides to produce the disaccharide, maltose.

Its action can be demonstrated by using starch as substrate. At a pH between 6-7 and in the presence of chloride ions, amylase catalyses hydrolysis of starch to maltose with the intermediate formation of dextrin. Starch and the higher dextrins give a blue colour with iodine, the intermediate dextrins give a reddish brown colour and maltose does not react with iodine. Thus the action of amylase can be followed by observing the time taken to reach the point at which the reaction mixture no longer gives a colour with iodine solution.

During analyses, mouth pipetting should not be done to avoid contamination with saliva from mouth.

9.2 Materials required

(i) 0.1 M Phosphate buffer (pH 6.7)

(ii) Buffered starch substrate (0.5% starch in phosphate buffer)

(iii) 0.1 N iodine solution in 3% KI

(iv) 1% NaCl

(v) Glass rod and white tiles

(vi) Water bath at 37° C

9.3 Methodologies

1. A little sample of saliva (preferably own saliva for health and hygiene issues) is collected.
2. 1 ml of saliva is then diluted with 20 ml of distilled water.
3. The diluted saliva is then divided into 5 ml portions.
4. One sample is placed in water bath at 37° C and left to equilibrate, while other is gently boiled for 2 minutes, cooled and placed in water bath at 37° C.
5. The following tubes are then prepared

Reagents	Test	Control - 1	Control - 2
0.5 % starch	5 ml	5 ml	5 ml
0.1 N Phosphate buffer pH 6.7	2 ml	2 ml	2 ml
1% NaCl	1 ml	1 ml	1 ml

6. The tubes are then placed in water bath at 37° C for 5 minutes and 1ml of salivary amylase solution is added to the "Test" tube and thoroughly mixed.
7. The time is noted and exactly 30 seconds later 1ml of salivary amylase solution is added to the "Control-1" and 1ml of boiled amylase is added to the "Control-2" tube and thoroughly mixed.

8. At interval of 1 minute, 1 drop of the reaction mixture is withdrawn from all the tubes and mixed with a drop of iodine on the white tile.
9. The glass rods used must be thoroughly washed between each test.
10. The time is taken when the reaction mixture no longer gives colour with iodine. It is the achromic point.
11. If the achromic point is not reached in 40 minutes, then the time may be taken infinite i.e., zero amylase activity.
12. If the achromic point is less than 4 minutes, the saliva should be diluted to give a more convenient value.

9.4 Selected questions

(i) Write down the functions of amylase.

(ii) What are the experimental materials required for estimation of salivary amylase activity under optimum condition ?

(ii) Write the methodology adapted for estimating salivary amylase activity.

9.5 Suggested readings

1. Jain A, Jain R and Jain S (2020). Study of effect of temperature on Salivary amylase. Basic techniques in Biochemistry, Microbiology and Molecular Bilogy. PP. 227-229.

Unit - 10 ❑ Effect of pH, temperature and inhibitors on the activity of salivary amylase

Structure

10.0 Objectives

By studying this unit learners would be able to understand about the effect of pH, temperature and inhibitors on the activity of salivary amylase.

10.1 Introduction

All living organisms are able to obtain and use energy quite rapidly due to presence of biological catalysts called enzymes. Each enzyme functions best at a specific pH, temperature and again each enzyme has certain inhibitors also which should be worked out.

10.2 Effect of pH

The rate of certain reactions is affected by the pH of the solution. pH is just a measure of the H^+ ion and OH^- ion concentrations. Both the ions are inversely proportional to each other. When H^+ ion is higher the pH is acidic and when OH^-

ion is higher the pH is alkaline. If in a reaction H^+ is a reactant, then a low (acidic) pH will have a higher concentration of that reactant, making for a faster reaction. Similarly, if OH^- is a reactant, than a high (alkaline) pH will make the reaction go faster because at a high pH, the concentration of OH^- is large.

It is well known that all enzymes including salivary amylase are active over a limited pH range and a plot of activity against pH gives a bell shaped curve. The activity of salivary amylase generally ranges between pH 6 and 7. Above pH 7 and below pH 6 the amylase is denaturated. The pH value of maximum activity is known as the optimum. For amylase it is pH 6.8.

The variation of enzymatic activity with pH is due to change in the state of ionization of the enzyme protein and other components of the reaction mixture. Change in pH alters the enzyme activity by affecting the stability of enzyme protein.

10.3 Experiments to test effects of pH

The experiments are same as iodine test describe above. However, the three test tubes used before iodine reaction are marked separately, one with acidic pH (4.0) buffer, one with pH 6.8 buffer (as described above) and another with alkaline pH (9.0) buffer. Add 1 ml of saliva to all test tubes. Mix it well. Then take 1 ml of the mixture and add with iodine as described above. Continue to add the mixture until colour disappears. Not the time as described above.

10.4 Effects of temperature

The rate of an enzyme catalysed reaction like most chemical reactions, increases with temperature. This is due to the effect on velocity constants of the various parts and affinity of the enzyme or cofactors, activators and the like. If, however, the enzyme activity is measured by determining amount of substrate transformed in a given time at different temperatures, then a so called optimum temperature is obtained. The optimum temperature is the result of the balance between the rate of increase of activity and the rate of destruction of the enzyme. The optimum temperature is not a constant value for a given enzyme but depends upon the time during which measurement of activity is made. The shorter the time of measurement, the higher will be the apparent optimum temperature.

10.5 Experiments to test effects of temperature

The experiments are similar to one described for effects of pH. The only difference is that only pH 6.8 buffer is used and the three test tubes are placed in three different beakers / water bath (one in a beaker with ice cubes to maintain low temperature, others two with different water bath, one with temperature 37° C and another with higher temperature). Reaction procedure with iodine remains same.

10.6 Effects of inhibitors

A number of compounds may react with the enzyme and reduce the activity. Such reagents are known as inhibitors and can react with the enzyme protein in a variety of ways; two main types of inhibition are recognized : **competitive and non-competitive type**.

In case of competitive inhibition, the inhibitor reacts with the enzyme by competing with the substrate for the active site. The degree of inhibition depends on the relative concentration of substrate and the inhibitor and almost maximal velocity may be found in the presence of the inhibitor if the substrate concentration is high enough.

In case of non-competitive inhibition, the inhibitor combines with the enzyme but not at the active site so that enzyme can bind both substrate and inhibitor at the same time. The binding site is generally far removed from the active site so that binding of the substrate remains unaffected. The enzyme-substrate -inhibitor complex formed is unable to break down the inhibition and inhibition effectively occurs by the reduction of the enzyme available. Increase of the substrate concentration has no effect on the degree of inhibition.

Most non-competitive inhibitors are not related chemically to the substrate and the same inhibitor may affect a number of enzymes.

Amylase inhibitors are naturally found in many food items and play a crucial role in reducing post prandial blood sugar.

10.7 Selected questions

(i) What is meant by competitive enzyme inhibition and how that differs from non-competitive inhibition ?

(ii) Amylase present in saliva is linked to hydrolysis of polysaccharides and oligosaccharides. What is the end product of such hydrolytic enzyme action ?

10.8 Suggested readings

Plummer, DT (1999). An introduction to practical biochemistry, Tata Macgraw Hill Publishing Company, New Delhi.