INTRODUCTION

There is a very little information on the algal enzymes. The main reasons for this may be the small amount of proteins which can be extracted from the unicellular forms such as Chlorella, Scenedesmus, Anacystis and Chroococcus, which are generally used for physiological and biochemical investigations.

Amongst the workers who have studied enzymes in algae mostly in Chlorella, Scenedesmus and Anacystis are Fredrick (1959, 1960, 1967), Kiyoshi et. al. (1967), Gominski (1972), Murphy and Hoëcha (1973, 1973a).

Catalase:

It was observed early in the 19th century that plant and animal tissues decomposed hydrogen peroxide (H₂O₂). Near the end of the century it became clear that specific enzymes must be responsible for the reaction and Loew (1901) first designated the enzymes concerned as catalases. Thus catalases are enzymes which promote the following catalytic reaction.
They are characterized by the fact that they use $\text{H}_2\text{O}_2$ both as donor of hydrogen and substrate in the catalytic decomposition of $\text{H}_2\text{O}_2$ to form $\text{O}_2$ and $\text{H}_2\text{O}$. In addition to the catalytic reaction the catalases can effect the peroxidatic oxidation of variety of organic donors in the same fashion as peroxidases (Burris, 1960). At the physiological concentrations of enzyme and substrates, catalase also acts peroxidatively on a number of compounds (alcohols, aldehydes, phenols, etc.) (Bjorn, 1967).

According to Boeri and Bonnichsen (1952) catalase has the ability to oxidise $-\text{SH}$ groups without the participation of $\text{H}_2\text{O}_2$. Yokoyama (1956) has pointed out that catalase activity directly affects the oxidation and reduction of cytochrome oxidase system. A high level of catalase and low level of peroxidase activity is related to a higher rate of protein synthesis (Verma and Huystee, 1970). There have been numerous reports that vigour of seed germination is correlated with catalase activity. Galston (1951) reports inverse relation between the catalase activity of plant tissues and their rate of growth. Diesseroth and Dounce (1970) studying the role of $-\text{SH}$ groups suggest that the latter play a role in the catalytic reaction of the enzyme.
in maintaining the structure of the enzyme and in binding the prosthetic groups.

Atkin (1910) found catalase to be of wide spread occurrence among algae, the kinetics of peroxide break-down with an extract of Ulva are essentially the same as with other source, such as blood (Baas, Becking and Hompton, 1920). Direct oxidase tests usually are negative, most algae require the addition of H$_2$O$_2$ to produce colour reaction with guiac alpha napthol or benzidine and hence peroxidase is indicated. The enzyme was shown absent in past by Harvey (1924) in thermal blue green algae Phormidium and in Mesoglea. Gertz (1925, 1926) made a very extensive survey of algal oxidases, finding most of the green and brown algae, to give negative test, as shown by Atkin (1910) where as fifty percent of red algae gave oxidase test positively. Peterson (1940) used ortho toluidine reagent and found that in 15 green, 27 brown and 38 red algae oxidase was distributed widely. But catalase has been reported in thermal blue-green algae by Kubin (1959), in blue-green algae by Shukla (1966), in Oscillatoria by Awasthi (1967). Chu Nam Hai (1971) studied the methyl viologen catalysed Mehler reaction and catalase activity in blue-green algae and green alga Chlamydomonas reinhardii.
Anabaena flos-aquae shows light dependent \( O_2 \) consumption as well as post illumination \( O_2 \) evolution. The rate of \( O_2 \) consumption is stimulated by 1 mM KCN an inhibitor of catalase, but the dark \( O_2 \) evolution was suppressed whereas in Chlamydomonas reinhardtii the cells did not show any net \( O_2 \) exchange during or after illumination. Addition of KCN / 1 mM, however, resulted in immediate \( O_2 \) uptake in light.

Attempts have been made in this investigation to compare the catalase activity in different forms of blue green and green algae.

**Peroxidase and special peroxidase:**

Peroxidase enzyme occurs in nearly all plant cells. The highest concentration recorded in the sap of fig tree. Horse radish root is rich in peroxidase and has been employed frequently for the preparation of peroxidase.

They are porphyrin enzymes catalyzing the oxidation of various substrates by hydrogen peroxide. Among the substrates which are oxidised by the peroxidase-hydrogen peroxide system are nitrites, ascorbic acid, bilirubin and dihydroxy maleic acid as well as inorganic iodides, mono and diphenols monoamines such as aniline, diamines such as
benzidine, aromatic acids such as benzoic acid, salicylic and gallic acid, certain dyes and flavones, adrenaline and amino acids-tyrosine, cystine and tryptophan. Of all the amino acids investigated tyrosine is most readily oxidised by the peroxidase system.

Siegel (1955) has pointed out the broad hydrogen donor specificity of plant peroxidases. There is a regular steep increase in peroxidase activity in all parts of the plant during growth. Peroxidase has been put forward as a constituent of terminal oxidation (Alexander, 1964) as an agent in the oxidation of metabolites by means of H₂O₂ by product (Fruton and Simmonds, 1963). As a key component of the IAA oxidizing system (Bay, 1958; Mecune, 1961) peroxidase is also involved in the formation of amides (Halevy, 1962; Monselise and Halvey, 1962).

Preliminary reports of peroxidase in algae were critically examined from methodological stand point (Reed, 1915; Hampton, 1920; cited by Tamiya, 1935). By more refined methods Bonnerstrand (1943) showed oxidase to be present in a number of marine algae; maximum values found among red algae. Siegel and Siegel (1970) evaluated the substrate specificity among algal peroxidase. They have studied semipurified tissue preparation of 13 red and brown algae. Murphy and h'Bocha (1973) have also gave some details
of peroxidase in *Enteromorpha linza* a green alga and *Cystoclonium* a red alga. Recently Wahal et. al. (1973) demonstrated the localization of peroxidase in the heterocyst of *Anabaena ambiguа*. Much of the work on *Chlorella* was done by the Trubachev (1968), Gurevich and Elkina (1972) for peroxidase and special peroxidase.

Gurevich (1963) has reported special peroxidase enzyme from higher plants such as wheat and corn seedling. It brings about the transfer of hydrogen from ascorbic acid to the substance with very low oxidation reduction potential like ortho-dinitrobenzene to yield yellow soluble product called o-dinitrophenyl hydroxylamine.

Yang (1969) suggested the function of peroxidase as the electron oxidizing agent. There is a positive correlation between respiration rate, catalase and peroxidase in citrus roots. Altman et. al. (1966), Yamazaki and Piette (1961) have shown from their free radical spectrum studies that a radical of ascorbic acid called monodehydro ascorbic acid (MDHA) is formed during its peroxidative oxidation. Siegel and Galston (1967) have ascribed a significant role to peroxidase in the regulation of cell growth and differentiation. Higher activity of hydroperoxidases viz. peroxidase and catalase was noted in reproductive shoots than in vegetative shoots by Srinivasan and Rao (1971).
A lot of work on the free radical producing peroxidase and its active participation in metabolically active cells has been carried out (Chinoy et. al., 1969).

In the present investigation activities of catalase, peroxidase, and special peroxidase in some blue green and green algae are studied.

**Succinic dehydrogenase:**

Enzyme succinic dehydrogenase is widely distributed in plants, animals and microorganisms (Thimann and Bonner, 1950; Fuller et. al., 1961; Thomas et. al., 1957). Many workers have worked on this enzyme in animal tissues, higher plant tissues and yeast in (lower plants) mainly for its importance in the tricarboxylic acid cycle. In TCA cycle succinic acid is transformed to fumaric acid in presence of hydrogen acceptor. The splitting electron hydrogen is accepted by some special chemicals such as methylene blue, toluene blue, thionine, indophenol blue or some other substances like tri-phenyl tetrazolium chloride salts. The colour of the dye can be reduced by the electron or triphenyl tetrazolium chloride may be reduced to form a formazan which is coloured substance. The enzymatic reaction is as follow:
Under aerobic conditions the hydrogen goes to oxygen by means of the action of the cytochrome-cytochrome oxidase system. If cyanide is added, the oxytropic (i.e., oxygen consuming) action is inhibited (see: Boyer, 1972).

The enzyme succinic dehydrogenase (SDH) is probably concerned with respiration of most of the plants and animals cell. It is thought to form a link in the chain reactions which are concerned with the oxidation of lipids, carbohydrates and proteins. Hogeboom (1946) and Morton (1950) have found that the SDH enzyme is not soluble in water, but it has been reported later, to be soluble in weakly alkaline solution. However, this has been contradicted.

Susheela (1964), Patt et al. (1954) have shown that SDH is associated with mitochondria. The enzyme SDH contains -SH groups and these -SH groups are essential for catalytic activity (Hopkins and Morgan, 1938). The energy
liberated by the action of SDH can be utilized for the synthesis of energy rich phosphate bond, and enolphosphates (Colowick et. al., 1941; Schlenk et. al., 1951).

Das and Sen (1951) (see Thimann and Bonner, 1950) have made a survey of fumarase and succinic dehydrogenase in seedlings. Succinoxidase and cytochrome oxidase in mitochondria from the Arum spadix have been studied by Simon (1957), in which he demonstrated the activity of succinic dehydrogenase. Being autotrophic and aerobically respiring lower plants like green alga Chlorella, Millbank (1959) studied the different respiratory enzymes of the cell free extract, in which he studied the oxidation of various organic acids of the tricarboxylic acid cycle.

Peak et. al. (1972) have studied the dehydrogenase of malate in Euglena gracilis cells grown heterotrophically. He found that there was no significant difference in results with malate dehydrogenase between cells grown autotrophically or heterotrophically. Eventhough this is present as mitochondrial enzyme like SDH. He also studied the carbon dioxide, light and other factors. Which are involved in oxidation of acids of TCA cycle. The absence of carbon dioxide does not cause significant reduction of soluble malate dehydrogenase.
Webster and Albert (1953) have studied the respiratory characteristic of *Anabaena* and have also shown that oxidation of organic substrates stimulated, when cytochrome-C was inhibited by carbon monoxide (CO). They also said that cytochrome oxidase is probably present, and polyphenol oxidase might be absent in this organism. Thus oxidative system and electron transfer system of respiration is quite different.

Kratz and Myers (1955) made comparative studies of photosynthesis and respiration in *Anabaena variabilis*, *Anacystis nidulans* and *Nostoc muscorum*. They have found that, there is marked difference in respiration when exogenous organic substrates were applied. They compared this with chlorophyceae like *Chlorella* and other algae.

Stuart and Gaffron (1971) have shown that hydrogen production by photosystem-I of *Scenedesmus* does not require oxygen evolution or cyclic photophosphorylation but this must be due to noncyclic electron flow from organic substances through photosystem-I to dehydrogenase, where molecular hydrogen is released.

An incomplete TCA (tricarboxylic acid cycle) in the blue-green alga *Anabaena variabilis* has been worked out by Pears and Carr (1968). They have indirectly proved
that, there is absence of TCA cycle. They detected some enzymes of TCA cycle, but \(\alpha\)-oxoglutarate dehydrogenase and succinyl -CoA synthetase were absent. They also showed that the succinic dehydrogenase was less active compared to isocitrate lyase and malate dehydrogenase (Pears and Carr, 1967).

Many types of dehydrogenase of respiratory system have been investigated in blue-green and green algae (Kiyohara et. al. 1960, in Tolypothrix tenuis, Fay, 1955 and Hoare and Moore, 1955 in Chloroglea fritschii, Challinor et. al. 1955, 1964 in Chlorella and Frenkel and Riger 1951 in Ulva). Thus reports of succinic dehydrogenase are conflicting at least in blue green algae. These have prokaryotic algae had no mitochondria and thus it was thought interesting to investigate this enzyme in this group. Two blue green algae and four green algae have been investigated for succinic dehydrogenase to screen the metabolic machinery and to find out taxonomic significance of the group.
Method for enzyme analysis:

(a) **Extraction of the crude enzyme:**

Algal suspension, generally 12-15 days old in growth, centrifuged or filtered through Whatmann No. 1 in Buchner funnel, washed 5-6 times with sterile double distilled water, was taken for the extraction of enzymes. Algal samples were weighed and homogenized in prechilled glass pestle and mortar at 4°C in an ice-bath with an equal amount of acid-washed sand. Extraction of the crude enzyme was done with phosphate buffer, 0.015 M with pH 7. Supernatant was collected after centrifuging 2-3 times at about 3000 rpm, to remove sand and cell debris. The cell free extract obtained was used immediately.

**Estimation of catalase activity:**

It was assayed by the manometric technique of Chance and Maehly (1955). The sample is weighed and ground in a pestle and mortar with acid wash sand and calcium carbonate. The extract is made to volume of 20.0 ml and transferred to a reagent bottle 5.0 ml of \( \text{H}_2\text{O}_2 \) (10 vol.) and 2.0 ml phosphate buffer (pH 7.0) was taken in polythene tube 1"x1" and carefully transferred to the reaction bottle. The bottle was connected to the manometer. The mixture was shaken for 2 minutes. On account of evolution of oxygen the
level in the manometer fell down. Difference in reading was noted. The results were calculated per minute, and per gram fresh wt. of algae to express the catalase activity.

Catalase activity = ml. O₂ evolved/min./g.fr.wt.

**Estimation of Peroxidase activity:**

Peroxidase activity was determined by the method of Maehly (1954) and George (1953).

The algal samples were weighed, homogenized made to volume 10 ml and centrifuged. The supernatant served as a source of the enzyme. A reaction mixture was prepared as follows:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer pH 7.0</td>
<td>2.0 ml</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme extract</td>
<td>-</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Guaiacol 20 mM</td>
<td>2.0 ml</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>H₂O₂ 16 mM</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
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</tbody>
</table>

Now these test tubes were read for optical density by colorimeter. The scale of the Klett Summerson colorimeter was zeroed with control mixture and the optical density of the test was read with blue-filter (470 mμ). Then the scale of colorimeter was taken 10-20 divisions ahead from the point of O.D. noted for the test mixture. At this stage
switch was put off and 0.2 ml of H₂O₂ (16 mM) was added by
dipping a thin glass rod in the cuvette containing the
reaction mixture and stopwatch was started. Simultaneously
the time taken in seconds to reach the adjusted O.D. was
noted. The results were expressed as Peroxidase activity =

\[
= \frac{\text{O.D. of colour developed/min/g.fr.wt.}}{}
\]
or

\[
= \frac{\text{O.D. of colour developed/min/1000 \mu g protein}}{}
\]

Estimation of AA-FR-peroxidase (Special peroxidase):

Ascorbic acid free radical peroxidase was assayed
by the method of Gurevich (1963).

The algal samples were homogenized in cold D.W.
and slurry were centrifuged. The supernatant was used for
the enzyme assay. Reagents were added as follows:

<table>
<thead>
<tr>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>o-Dinitrobenzene</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>(Saturated solution)</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>(5 mg/ml)</td>
<td></td>
</tr>
<tr>
<td>GDW</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>H₂O₂ 6%</td>
<td>-</td>
</tr>
</tbody>
</table>
The solutions were allowed to stand at room temperature 26±1°C for 20 minutes. The optical density of the yellow colour was recorded using blue filter (470 mμ). The activity was calculated and expressed as

Special peroxidase activity
= O.D. of the colour developed/20 min/g.fr.wt.

Materials and method for succinic dehydrogenase:

Quantitative estimation of succinic dehydrogenase (SDH):

Method used for SDH determination was that of Kun and Abood (1949).

Principle:

The electron released by the enzyme succinic-dehydrogenase from the substrate are taken up by an electron acceptor i.e. tetrazolium salt, which is reduced to a red formazan. After extracting the formed formazons in acetone the colour intensity is measured in a colorimeter at 470 mμ.

Reagents:

1. 0.15 M phosphate buffer pH 7.6
2. 0.5 M sodium succinate
3. Acetone A.R.
4. Tetrazolium salt (TTC) 1 mg/ml (Freshly prepared)
Procedure:

Algal samples were weighed and homogenized in buffer made to volume 10.0 ml, which was used as the extract. Reagents were added as follows:

- 0.5 ml phosphate buffer pH 7.6, 0.15 M
- 0.5 ml sodium succinate
- 1.0 ml TTC solution
- 1.0 ml aliquots of an enzyme
- 3.0 ml

In control, substrate was omitted and replaced by 0.5 ml of D.W. Now these test tubes were plugged with cotton and placed at 37°C in an incubator for reaction. Incubation period was 20-24 hrs. After incubation 3.0 ml of acetone was added to each test tube and then shaken well and reaction mixture was centrifuged. The supernatant was decanted off in an other test tube and colour of this intensity was read at 470 mμ (blue filter) in Klett Summerson colorimeter.

Standard Curve for SDH:

Weigh exactly 25 mg TTC (Triphenyl tetrazolium chloride) was dissolved in 25 ml of cold double distilled water. From this 5 ml of stock solution was made to 50 ml with water, so each ml contained 100 μg of TTC. Different
aliquots up to 3.0 ml was taken and volume was made 3.0 ml. each of a
In these test tubes pinch of sodium hydrosulphite was added.
The test tubes were allowed to stand for five minutes so
that TTC get reduced to red formazon. The intensity of
colour was read at 470 m\(\mu\) (blue-filter) and regression
equation was made by statistical method.

\[ Y = 0.76078(X) - 0.0083 \]

Where, \(Y\) = concentration of formazon formed in \(\mu\)g
\(X\) = O.D. of the colour intensity

Succinic dehydrogenase activity was expressed as

\[ \frac{\text{Conc. from equation} \times \text{dilution}}{\text{Wt. of the algae in mg}} \]

= \(\mu\)g formazon formed/24 hr/g.fr.wt. OR

= \(\mu\)g formazon/24 hr/mg protein

Protein was estimated by the method of Lowery et. al. (1951).

**Experimental findings:**

1. **Catalase:**

Among algae investigated, here, activities of oxidases differed in different groups of algae.

Catalase has been found in all algae investigated here. Among these *Ulva fasciata* showed maximum activity. In
blue-green alga, Oscillatoria jasorvensis showed maximum evolution of oxygen (Table 5.1 and Plate 5-I). Chroococcus Anabaena and Nostoc showed almost equal activity of catalase.

Unicellular forms like Chlorella and Scenedesmus showed the least activity where as filamentous form like Pithophora showed more than Enteromorpha and Chara. Ulva fasciata showed maximum activities.

2. Peroxidase:

Peroxidase enzyme was found to be present in the algae. But with the substrate guiacol, Nostoc-222 and Chroococcus showed negative test for the peroxidase. However, with Pyragallol they showed positive results for peroxidase activity.

All green algae showed positive test for guiacol and pyragallol both.

Among blue green algae, Anabaena variabilis showed more activity than the Oscillatoria jasorvensis. Where as in green algae Chara corallina showed maximum activity of peroxidase. Chara globularis was studied for its vegetative and reproductive phases and the result showed that vegetative parts of the thallus have more activity then the reproductive parts (Table 5.2).
Ulva fusciata and Pithophora showed feeble activity of peroxidase (Table 5.2, Plate 5-I). The rate of activity of peroxidase was measured in Pithophora and Chara and it was found that Chara showed much greater rate.

3. Special peroxidase (Ascorbic acid free radical peroxidase):

Special peroxidase activity was found in all blue-green and green algae, which were studied here (Table 5.3, Plate 5-II).

Anabaena showed more activity among blue-green algae, and vegetative parts of the thallus of Chara globularis showed more activity amongst the green algae.

Nostoc and Chlorella showed moderate activity of special peroxidase activity.

4. Succinic dehydrogenase:

Out of two blue-green and four green algae investigated here, succinic dehydrogenase activity was found in all algae (Table 5.4, Plate 5-II).

Enteromorpha is found to have maximum activity of the enzyme among all algae (Table 5.4, Plate 5-II) whereas Chlorellia is found to possess less SDH activity.
Blue-green alga Oscillatoria found to have more activity of SDH compared to Nostoc and green alga, Chlorella. Pithophora have more SDH activity than Chlorella and Ulva.

**DISCUSSION AND CONCLUSION:**

1. Catalase:

   The results show that blue green algae have more catalase activity than the green algae. There is not much difference between unicellular and filamentous forms regarding their catalase activity. The evolution of oxygen is higher in Oscillatoria which is a non heterocystous form. Heterocystous Anabaena and Nostoc both stand similar.

   Green algae have different trend, may be due to wide range of thallus organisation. Unicellular alga found to have less enzyme activity of catalase. Pithophora and Chara clearly demarked from the other algae (Table 5-1).

   It is assumed that filamentous branched thallus showed more activity of catalase. The highly evolved thallus of Chara showed maximum among filamentous thallus of green algae. Chara has nodes, internodes branch and branchlet and hence it is distinct from the other algae. So metabolism of each cell may be different due to its work distribution in it.

   Where as in simple thallus like Chlorella or foliose thallii of Ulva and Enteromorpha where no differentiation of
tissues occur, have unique metabolism and each cell has less specific load to produce necessary products.

The results of the study of catalase activity in algae showed that it is more or less uniform in blue green algae. Oscillatoria showed a slightly higher activity. Thus irrespective of simplicity or complexity of thallus structure or of ability to fix nitrogen the catalase activity is moderate in blue green algae. In green algae catalase activity vary amongst the members investigated here. Simpler forms like Chlorella and Scenedesmus showed less activity of catalase while multicellular forms showed more activity of catalase. Pithophora (filamentous thallus) and Ulva (parenchymatous thallus) showed very high catalase activity. These two algae also need a slightly saline environment to grow and possibly the salinity might be increasing catalase activity in these algae. In higher plants the increase in salinity induced higher catalase activity (Chinoy et. al., 1969).

2. Peroxidase:

From the Table 5.2 and Plate 5.II it can be concluded that blue green algae have a limited range of substrate specificity. Whereas green algae have wide range of substrate specificity. This supports the work of Siegel and Siegel (1970). They critically studied the enzyme
peroxidase, in red, brown and green algae. Out of 13 species of green algae showed positive test with seven different substrates. Brown algae have four and Red algae only two. Here only blue green algae Chroococcus and Nostoc-222 gave negative response to guiacol test but positive with pyragallol. This shows that the isozymes of peroxidase of these blue green algae may not be the same as those in green algae which also show positive test with gluiacol.

Unicellular green algae have greater potency for peroxidase as that of the highly evolved thallii of Ulva Pithophora. Chara, however showed tremendously high activity of peroxidase. Altman et. al. (1966) showed the increasing peroxidase activity during the juvenile phase when growth and tissue differentiation is there. It is also associated with increased respiration. Thus the increase respiration rate and degradation of fat may shift the redox balance more towards the oxidative side. Ivanova and Rubin (1963) as well as Gagnon (1968) report that the peroxidase takes part in respiration as it provides an alternate adeninedinucleotide (NAD).

Wahal et. al. (1973) found that peroxidase activity is increasing in Anabaena at the time when heterocyst induction takes place. This investigation has
shown that in *Anabaena* fairly large amount of peroxidase exists and it is much more than that found in non differentiated thallus of *Oscillatoria*. However, it should be recorded that in unicellular forms like *Chroococcus* peroxidase activity is negligible while in *Chlorella* it is quite high, even higher than the differentiated complex thallii of *Pithophora, Ulva* and *Enteromorpha*. Thus it would be difficult to show any correlation between complexity of thallus organization and peroxidase activity.

3. **AA-FR-peroxidase (Special Peroxidase):**

Yamazaki (1962) and Gurevich (1963) have all shown that AA-FR-peroxidase catalyzes the production of free radicals of AA. The increased free radical content as studied by Electron Paramagnetic Resonance spectrometer (EPR) has already been reported by Chinoy et. al. (1969). Gurevich and Elkina (1972) described physiological significance of H$_2$O$_2$ in aerobic respiration. H$_2$O$_2$ activated by a peroxidase catalyst monovalent, oxidises AA and leads to the formation of its free radical, viz. monodehydro-ascorbic acid (MDHA). Due to expenditure of oxidation energy, this free radical is in the activated state and its mobile hydrogen is readily transferable to the corresponding acceptors exposing them to reduction. Thus H$_2$O$_2$ induces hydrogen transfer reaction. The hydrogen acceptor used
consisted of colourless orthodinitrobenzene, which is irreversible reduced to yellow orthonitrophenyl hydroxylamine and can therefore serve as a reliable indicator of hydrogen transfer (Gurevich, 1952, 1953). Considerable evidence has accumulated to highlight the importance of free radicals in the process of energy transfer reaction (Beevers, 1961; Blois et al., 1961; Zhokhovitch et al., 1965; Kols et al., 1966).

Work of Gurevich and Elkina (1972) show that Chlorella cells injured by prolonged starvation, has a deficiency of respiratory \( \text{H}_2\text{O}_2 \). \( \text{H}_2\text{O}_2 \) absorbed by such cells is in a small amount, but in absence of \( \text{H}_2\text{O}_2 \) it will enter into induced reduction reactions and evolve hydrogen transferred from the active free radical of ascorbic acid and pass it to the corresponding biological acceptors, including molecular oxygen; this in turn will lead to accelerated uptake of molecular oxygen as compared with the control (i.e. without addition of \( \text{H}_2\text{O}_2 \)). On the other hand, if Chlorella (cells in the normal state) is well supplied with respiratory hydrogen peroxide, the small amount of \( \text{H}_2\text{O}_2 \) will be decomposed by catalase of the alga with evolution of molecular oxygen. This is manifested in an apparent decrease of molecular oxygen uptake in comparison with the water control. Our observations on
catalase activity in *Chlorella* suggest that since it is moderate there is a scope of $H_2O_2$ respiration in *Chlorella* and apparently it would take less molecular oxygen.

Among algae investigated here AA-FR-peroxidase activity is more in *Scenedesmus* and *Ulva* and it is the highest in differentiated thallus of *Chara* amongst the algae. *Enteromorpha* stands last.

High peroxidase activity is also accompanied by high AA-FR-peroxidase activity in *Anabaena*, *Chlorella*, *Scenedesmus* and *Chara*, it can be said that peroxidase and AA-FR-peroxidase system are parallel in these organisms. However in *Ulva* and *Pithophora* surprisingly peroxidase activity is much less as compared to the activity of special peroxidase. This is indicative of different type of metabolism in these algae. It should be recorded that *Ulva* and *Enteromorpha* though they belong to the same family show this difference in their metabolic behaviour. Orr (1967) reports inhibition of catalase following incubation with ascorbate and $Cu^{+2}$. He suggests that the free radicals generated during ascorbate oxidation are responsible for catalase inhibition. According to Aberg and Johnson (1963) the effect of AA on growth is due to the formation of hydrogen peroxide ($H_2O_2$). If it is so then the catalase activity should increase to remove excess of $H_2O_2$ produced as a result of
ascorbic acid application.

When we compare the results of catalase activity and special peroxidase activity in algae studied here it becomes evident that in order to get rid of the cell from \( \text{H}_2\text{O}_2 \) which is produced as consequence of special peroxidase activity, catalase comes on the scene and scavanges \( \text{H}_2\text{O}_2 \).

4. Succinic dehydrogenase:

From the table and figure it can be concluded that blue-green algae also reduced the triphenyl - tetrazolium chloride (TTC) when provided Na-succinate as substrate. It clearly indicates the presence of succinic dehydrogenase in these prokaryotes where as in green algae it is already reported previously and its presence is further confirmed here in *Chlorella*, *Ulva* and *Enteromorpha*.

Pearce and Carr (1968) have found less activity of SDH in *Anabaena variabilis*, but here *Oscillatoria* *majovansis* is found to show good activity of SDH.

Leach and Carr (1970) showed that electron transport and oxidative phosphorylation in blue-green alga *Anabaena variabilis* there may be electron acceptor system but its details are not yet known.

The structure of the blue-green algae is quite different from the green algae, where mitochondria are prominent Carr and Pears (1966). When, SDH is recognized
as the mitochondrial enzyme (Patt et al. 1954) the presence of SDH in blue green algae is of interest. This enzyme is probably located on lamalae of cytoplasm in blue-green algae (Adachi, 1967).

The reduction of TTC (triphenyltetrazolium chloride) was found in the heterocyst of *Anabaena* (Stewart et al., 1969) while investigating nitrogenase activity. Formation of formazan showed the nitrogenase activity. But here enzyme system was supplied by the Na-succinate as substrate and hence can be concluded that formazan formed in blue-green algae is due to succinic acid dehydrogenase. It can be said that this kind of SDH may play partial role in fulfillment of TCA cycle. This is in support of Pearce and Carr (1967).

The rate of oxidation of organic acid compared to glucose in *Chlorella* had common steps in oxidative path in glycolysis (Millbank, 1957). He also showed that malate inhibition of succinic acid oxidation could not be detected, indicates that succinic acid oxidised by the enzyme.

Hoare and Moore (1965), Carr and Pearce (1966) said that when *Anabaena variabilis* grown autotrophically, the alga metabolised acetate though acetate does not increase the growth or respiration although the key enzyme of the glyoxylate cycle has been detected in extracts of
blue-green algae. Pearce and Carr (1967) could not detect α-oxoglutarate and hence could not detect the formation of succinate, malate or fumarate in this algae. The absence of α-oxoglutarate dehydrogenase has been noted in extracts of photosynthetic, anaerobic facultative autotrophic bacteria by Fuller et. al. (1961) and Calley and Fuller (1967), Adachi (1967).

Thus the role of enzymes of TCA cycles in blue-green algae is still intriguing. Our results have clearly shown the activity of SDH in two blue green algae. One heterocystous and other nonheterocystous. More over experiments in our laboratory (Vaidya and Shah) which are being carried out using ^{14}C labelled glutamate clearly shows that radioactivities enters in aspartate. Short term experiments in this laboratory (Vaidya and Vijayakumari) clearly showed that Oscillatoria and Anabaena (Christian P.J., 1967) consumed considerable oxygen. Thus there is a possibility that TCA cycle is operating in blue-green algae investigated here.

Oscillatoria which is supposed to be a premitive one amongst blue-green algae (Fritsch, 1935), showed more activity of SDH. Thus so far as this physiological aspect is concerned it showed more nearness to green-algae and hence Oscillatoriales on this bases can be considered as an advanced group.
### Table 5.1

**CATALASE ACTIVITY IN BLUE-GREEN AND GREEN ALGAE**

<table>
<thead>
<tr>
<th>Algae</th>
<th>ml O₂ evolved/min/g.fr.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chroococcus minutus</em>, Nag.</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Oscillatoria jaersonensis</em>, Vouk</td>
<td>8.5</td>
</tr>
<tr>
<td><em>Anabaena variabilis</em>, Kutzing ex Born et. Flah</td>
<td>7.14</td>
</tr>
<tr>
<td><em>Nostoc-222</em>, IARI</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em>, Chick</td>
<td>3.7</td>
</tr>
<tr>
<td><em>Scenedesmus</em> sp.</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Pithophora</em> sp.</td>
<td>12.06</td>
</tr>
<tr>
<td><em>Ulva fasciata</em>, Dellie</td>
<td>16.25</td>
</tr>
<tr>
<td><em>Enteromorpha flexuosa</em> (Wulten) J. Ag.</td>
<td>5.25</td>
</tr>
<tr>
<td><em>Chara corallina</em>, Klein ex Willdenow.</td>
<td>6.0</td>
</tr>
</tbody>
</table>
**TABLE 5.2**

PEROXIDASE ACTIVITY IN BLUE-GREEN AND GREEN ALGAE

<table>
<thead>
<tr>
<th>Algae</th>
<th>O.D./min/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcus minutus, Nag.</td>
<td>*</td>
</tr>
<tr>
<td>Oscillatoria jasovensis, Vouk</td>
<td>0.5714</td>
</tr>
<tr>
<td>Anabaena variabilis, kützing ex Born et Flah</td>
<td>6.0000</td>
</tr>
<tr>
<td>Nostoc-222, IARI</td>
<td>*</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa, Chick</td>
<td>1.9677</td>
</tr>
<tr>
<td>Scenedesmus sp.</td>
<td>6.165 O.D./60 Sec./g.fr.wt.</td>
</tr>
<tr>
<td>Pithophora sp.</td>
<td>0.069</td>
</tr>
<tr>
<td>Ulva fasciata, Delile</td>
<td>0.0492</td>
</tr>
<tr>
<td>Enteromorpha flexuosa, (Wulsten) J. Ag.</td>
<td>0.12096</td>
</tr>
<tr>
<td>Chara corallina, Klein ex Willdenow</td>
<td>11.08</td>
</tr>
<tr>
<td>Chara globularis vegetative</td>
<td>10.74 O.D./60 Sec./g.fr.wt. Reproductive 4.95</td>
</tr>
</tbody>
</table>

* Negative test for guiacol
### Table 5.3

**Ascorbic Acid - FR-Peroxidase in Blue- and Green Algae**

<table>
<thead>
<tr>
<th>Algae</th>
<th>O.D./20 min/g.fr.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chroococcus minutus</em>, Nag.</td>
<td>-</td>
</tr>
<tr>
<td><em>Oscillatoria jasorvensis</em>, Vouk</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Anabaena variabilis</em>, Kutzing ex Bornet Flah</td>
<td>3.7</td>
</tr>
<tr>
<td><em>Nostoc</em>-222, IARI</td>
<td>1.47</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em>, Chick</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Scenedesmus</em> sp.</td>
<td>3.3</td>
</tr>
<tr>
<td><em>Pithophora</em> sp.</td>
<td>1.36</td>
</tr>
<tr>
<td><em>Ulva fasciata</em>, Dielie</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Enteromorpha flexuosa</em>, (Wulten) J. Ag.</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Chara globularis</em> vegetative</td>
<td>6.535</td>
</tr>
<tr>
<td><em>Chara globularis</em> Reproductive</td>
<td>1.91</td>
</tr>
</tbody>
</table>
**TABLE 5.4**

**SUCCINIC DEHYDROGENASE ACTIVITY IN BLUE-GREEN AND GREEN ALGAE**

<table>
<thead>
<tr>
<th>Algae</th>
<th>mg Formazan formed/24 hr/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatoria iissorvensis, vouk</td>
<td>0.60909</td>
</tr>
<tr>
<td>Nostoc-222, IARI</td>
<td>0.26421</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa, Chick</td>
<td>0.16000</td>
</tr>
<tr>
<td>Pithophora sp.</td>
<td>0.559902</td>
</tr>
<tr>
<td>Ulva fasciata, Dielie</td>
<td>0.32328</td>
</tr>
<tr>
<td>Enteromorpha flexuosa, (Walten) J. Ag.</td>
<td>0.9445</td>
</tr>
</tbody>
</table>
PLATE - 5.I

Showing catalase, rate of peroxidase activity in Pithophora, Chara and peroxidase activity in blue-green and green algae.

PLATE - 5.II

Showing ascorbic acid free radical peroxidase (Special peroxidase) activity in blue-green and green algae. And Regression formula curve for SDH, TTC (Triphenyl-tetrazolium chloride) salt as standard, and succinic dehydrogenase activity in blue-green and green algae.
ASCORBIC ACID FREE RADICAL PEROXIDASE IN BLUE GREEN ALGAE

REGRESSION FORMULA CURVE FOR SDH, TTC AS STANDARD

SUCCINIC DEHYDROGENASE ACTIVITY IN BLUE-GREEN AND GREEN ALGAE
REFERENCES


*__________ (1953): ibid. 91:543.


Yang, S.F. (1969): Further investigation on ethylene formation from \( \alpha \)-keto-gamma-methylthiobutyric acid or \( \beta \)-methylthiopropionaldehyde by peroxidase in the presence of sulfite and oxygen. J. Biol. Chem. 244(16):4360-4365.
