A study on the influence of storage conditions on the subsequent rate of nitrogen fixation by nostoc commune

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A study on the influence of storage conditions on the subsequent rate of nitrogen fixation by *Nostoc commune*

by

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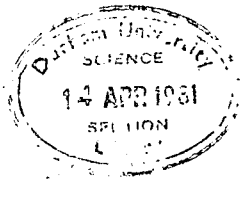

to

CHIANG-YOO-SAE-LOWE

MY FATHER

ABSTRACT

A study was made on nitrogen fixation by Nostoc commune taken from two sites in Northern England, Tarn Moor, Cumbria, and Station Quarry, North Yorks. The rate at which colonies fixed nirtrogen was measured using the acetylene reduction assay technique.

Colonies were dried and rewetted, and the rates of water uptake by dried colonies were measured. Marked variation in rates of water uptake occurred in dried colonies of Station Quarry, with younger colonies taking up water faster. Selected colonies from Tarn Moor were more of the same age, and were found to take up water at a similar rate to young colonies of Station Quarry Nostoc.

Some colonies of N. commune from both sites were dried under different humidity regimes and were found to commence acetylene reduction with different timelags after rewetting: the higher the relative humidity under which the algae were dried, the shorter the lags. Light-dried and dark-dried colonies from Tarn Moor were also shown to have distinctive lags after rewetting. An attempt was made to compare the rates of acetylene reduction by colonies subjected to different treatments. Light-dried Tarn Moor Nostoc colonies had a mean rate 2.5 times that for dark-dried colonies. Tarn Moor Nostoc dried under 75 % relative humidity showed a mean rate of 0.08 $\pm$ 0.06 nMole C_2H_4 μg chl a^{-1} min^{-1} at 72 hours after rewetting, and was two times and five times the mean rate for

algae dried under 50 % relative humidity and 10 % relative humidity sequentially. The mean rate for Station Quarry Nostoc dried under 75 % relative humidity was 0.03 ± 0.02 nMole C_2H_4 µg chl $\underline{a}^{-1}$ $min.^{-1}$ at 72 hours after rewetting, and was also higher than the mean rates for algae dried under 50 % relative humidity and 10 % relative humidity, respectively.

The rates of acetylene reduction of freshly collected colonies from Tarn Moor were compared in three types of incubation: fully submerged, 1/4-submerged and non-submerged. The rates obtained from colonies under submerged condition were significantly different ($p < 0.001$) from the rates obtained from non-submerged colonies. The rates obtained from colonies under 1/4-submerged and non-submerged conditions were however not found to be significantly different from one another ($p > 0.1$).

ABBREVIATIONS AND SYMBOLS

chl *a* = chlorophyll *a*

cm = centrimetre

°C = degrees Celsius

D.F. = degree of freedom

g = gramme

h = hour

l = litre

lx = lux

m = mole

µg = microgramme

mg = milligraade

ml = millilitre

mm = millimetre

mMole = millimole

min = minute

nm = nannometre

nMole = nannomole (text)

N MOLE = nannomole (tables and figures)

% = percentage

p = statistical probability

R.H. = relative humidity

S.D. = standard deviation

T = calculated value of student 't'

= ±

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Charin Rujikietkumjorn

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CHAPTER 1

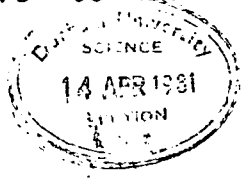
INTRODUCTION

1.1 General introduction

Blue-green algae are widely distributed in soils and waters. They may be free-living or associated forms; many have been found to fix atmospheric nitrogen, contributing to the nitrogen cycle and the fertility of ecosystems.

Work has been carried out with blue-green algae in agricultural areas throughout the world towards increasing the biological conversion of atmospheric nitrogen to a form that plants can use (Postgate, 1978). This helps to minimise the cost which would, otherwise, be high when chemical nitrogen fertilizers are used. The use of free-living Anabaena and Nostoc, and symbiotic Anabaena in paddy fields, for instance, has been found to increase significantly the yield where nitrogenous fertilizers have not been used. The literature contains numerous papers dealing with many aspects of nitrogen fixation by blue-green algae in agricultural systems e.g. Singh (1961), Henriksson, Henriksson and daSilva (1975), Kawaguchi & Kyuwa (1977).

Rychert et al. (1978) presented data showing values of nitrogen fixed by Oscillatoria, Nostoc, Microcoleus and Nodularia in the Sonoran desert, and pointed out that, not only can blue-green algal crusts and/or blue-green algae-lichen crusts fix significant amounts of atmospheric nitrogen into the desert ecosystem, but also the crusts serve to stabilise the



soil surface, to reduce erosion and to increase water retention and infiltration.

Blue-green algae have also been studied in polar and sub-polar regions. Alexander (1975) has reviewed the importance of both free-living and symbiotic blue-green algae in fixing nitrogen in these regions, and has suggested that species such as Nostoc and Collema are a major source of nitrogen in such arid biomes. She also quoted the work of Holm-Hansen (1963, 1964) who had found large populations of blue-green algae in Antarctica and had shown that Nostoc commune was one of the most abundant forms which fixed nitrogen in impure culture.

A considerable number of papers have revealed the significance of nitrogen fixation by blue-green algae in other ecosystems; Saino et al. (1978), on marine Trichodesmium thiebauti; Whitton et al. (1971, 1979), on Nostoc commune of Aldabra Atoll, for instance.

1.2 Nostoc commune

Species of the genus Nostoc are among the most widely distributed of any blue-green algae, and many, including obvious macroscopic forms such as N. commune are probably cosmopolitan (Whitton & Sinclair, 1975).

N. commune normally inhabits damp soils, shallow pools and/or pool margins. N. commune has been found in regions ranging from the poles to the tropics; arctic and antarctic (Holm-Hansen, 1963, 1964), tundra (Tichornirov, 1957), temperate (Granhall, 1975; Henriksson et al., 1975), sub-tropic (Singh,

1961; Renaut et al., 1975), tropic (Venkataraman, 1975). N. commune is more common in temperate than tropical regions(Singh, 1961).

1.3 Effects of light and surrounding humidity on nitrogen fixation

1.31 Light

In his study on Nostoc spp. in Californian streams, Horne (1975) suggested that nitrogen fixation is probably powered by photosynthetic energy from the previous day. It is evident that nitrogen fixation is dependent on the energy supply from photosynthesis. Nitrogenase activity varies from very little to absent in the dark (Stewart et al., 1978).

Padan et al. (1971a) have shown that pre-incubation of Plectonema boryanum in the light produces a marked increase in nitrogen fixation in darkness for several hours. This suggests a comparable situation to when algae are dried and rewetted. For dry algae, the situation in which nitrogen fixation initially increases after rewetting in darkness and declines after sometime is almost certain to be dependent on the stored products when being dried. Such a phenomenon was pointed out by Myers (1974), Donaldson (1978) and Whitton et al. (1979) on rewetting dry colonies of N. commune from the Aldabra atoll and Tarn Moor.

Allison (1937), Fay (1965) and Khoja & Whitton (1971) have shown that dark growth of algae in medium supplied with sucrose is possible. Nitrogenase activity can continue in the dark when

fixed carbon is available, although at lower rates than in the light (Bottomley & Stewart, 1971).

1.32 Environmental humidity

Few details are available regarding the effects of atmospheric humidity on drying algae in relation to nitrogen fixation when being rewetted. Relative humidity of the atmosphere may be one of the important factors influencing nitrogen fixation. In high relative humidity, algae lose their nitrogenase activity slower when being dried, and recover quicker when rewetted (Stewart et al., 1978).

Using the acetylene reduction method, Okafor & MacRae (1973) showed that nitrgoen fixation was greater in 15.0 g samples of air-dried soil moistened with 9.0 ml water than samples moistened with 5.0 ml water. Similar work has been carried out by Steyn & Delwiche (1970) showing the importance of moisture as a limiting factor affecting nitrogen fixation in non-symbiotic microorganisms in some Californian soils. Day et al. (1975) pointed out that the high nitrogenase activity in the top 7.0 cm soil (Broadbalk Wilderness) compared with lower horizons may well be a response to differential wetting rather than a true indication of the relative abundance of nitrogen-fixing organisms. Granhall (1975) and Day et al. (1975) suggested that nitrogenase activity was positively correlated with soil moisture. This implies indirectly that the more humid the soil remained when being dried, the more potential to recover the micro-organisms living in the soil will

have when being rewetted.

In their study on the coastal dune systems of Morocco, Renaut et al .(1975) describes that Nostoc spp. and the lichen Collema develop profusely during the rainy season, but dry up in the dry season and do not fix nitrogen then. They pointed out that in dry season nitrogenase activity occurs in the morning when the algae are wet with dew and declines later in the day. The importance of morning dew in relation to nitrogen fixation was also pointed out by Skujins & West (1974) in their study on the great basin desert (U.S.A.). Experiments carried out by Renaut et al. (1975) also show artificial wetted (with distilled water) samples having higher rates of acetylene reduction than untreated samples. Similar work was carried out by Mayland et al.(1966) showing the differences in rates of acetylene reduction by algal crusts in a semi-arid soil in the United States in that wet algal crusts gave higher rates than wet-dry and dry ones respectively.

It is certain that the higher the atmospheric humidity the less the water potential the plant cells in proportion to the water potential of the atmosphere. Therefore, plant cells are able to maintain more moisture so that physiological activities are more stabilized. Dry algae in low atmospheric humidity could possibly lead to desiccation of the cells bringing about the slow recovery of the activities when being rewetted. Showman & Rudolph (1971) have shown that water loss in lichens (Umbilicaria papulosa) kept in 0% relative humidity was 80% within 10 to 15 minutes in both living and dead lichens. In 53%

relative humidity, the lichens took more than 30 minutes to loose 80% of their water content. The situation shown by Showman & Rudolph (1971) is comparable to that in algae under air-dried conditions.

1.4 Physical and chemical laboratory analyses

1.41 Uptake of moisture in dry algae

There are some distinct advantages of drying algae in algal study. Blue-green algae are resistant to desiccation (Rychert et al., 1978). Dry algae can be kept for a long period of time for installment experiments in laboratory by rewetting them before use. Air-dried algae and lichen crusts were revived after four years of desiccation (Rychert et al., 1978). Dry algae kept in the dark at low temperature, on wetted, dried and rewetted, have been shown by Donaldson (1978) and Whitton et al. (1979) that there is no detectable effect on the levels of chlorophyll a extracted or on the ration of chlorophyll a to phaeophytin a.

1.42 Analysis of chlorophyll a

It is known that there may be a large variation within one population of Nostoc colonies in total nitrogen when related to volume, weight or chlorophyll a content of colonies. It seems probable that this is due to the presence of non-algal nitrogen such as animal excreta (Whitton et al., 1979). The rate of acetylene reduction is frequently expressed in relation to chlorophyll a ie.as nannomoles C_2H_4 μg Chl a $^{-1}$ min^{-1}.

The determination of photosynthetic pigments especially chlorophyll a for the estimation of algal standing crop is widely used method in studying algal communities (Moss, 1967a.). Chlorophyll a is the central reaction in photosynthetic light reaction, and is normally the most abundant and important pigment in plant material.

Apart from the many problems associated with this method, which has been outlined by Strickland (1960), some important problems involved in the extraction and estimation of chlorophyll a have been put forward as outlined below:

1) Algal communities may sometimes contain chlorophyll degradation products, which in some instances can constitute a significant fraction of the total green pigmented materials present (Yentsch & Menzel, 1963; Lorenzen, 1967). These breakdown products can give large errors in estimated chlorophyll a when spectrometry is used, as they absorb in the red part of the spectrum (Lorenzen, 1967).

2) Algae may also associate with local sediments combined with the communities. Hydrogen ion concentration in extracts from hard water may be affected by bicarbonates and precipitated carbonates which directly affect the pH (Marker et al., in press). This would subsequently causes interference in the absorption spectrum at long wavelengths ie. 430 and 410 nm.

3) When the algal population is healthy and degradation products of chlorophyll are at a minimum, the greatest errors will arise from incomplete extraction (Marker et al., in

press).

4) Only a certain percentage of the total pigments may be obtained when using certain solvents. The widely used solvent acetone is unstable for epilithic communities associated with sediments, and a stronger solvent such as methanol is required (Marker, 1972).

5) Methanol has been criticised because of its toxicity (Marker et al., in press). Chlorophyll is also relatively unstable dissolved in methanol (Marker et al., in press) and certain difficulties are known to arise in estimating phaeopigments (Marker, 1972, 1977). Moed & Hallegraeff (quoted by Marker et al., in press) have shown that the formation of dications of phaeophytins is a function of the hydrogen ion concentration and water content of the solvent system as well as the properties of the solvents themselves. They illustrated this effect in acetone, ethanol and methanol.

6) Absorption coefficients are only partly known for some pigments ie. phaeophorbide (Lorenzen, 1967) which is a component of chlorophyll degradation products. These vary from solvent to solvent (Marker, 1972), and some are only calculated for particular solvents. When small instrumental errors occur in conjunction with errors in the absorption coefficients, large inaccuracies in estimation of chlorophyll a result (Marker, 1972).

7) Marker et al. (in press) have shown that the maximum acid ratio is very dependent on wavelength. It can vary by 0-9% between 662 nm and 667 nm which could affect the chlorophyll estimate by 26%. He suggests that it is imperative to set the

wavelength accurately.

On acidification each chlorophyll *a* molecule losses a magnesium atom and is converted into phaeophytin. Lorenzen (1967) advocated this as a method of determining chlorophyll *a* in samples containing phaeopigments, showing that the addition of 1.0 N HCl brings about a change in the absorbance which discriminates between chlorophyllous magnesium containing compounds and those which are magnesium free. The calculation of photopigments assumes that all the phaeopigment is phaeophytin, when in fact a small proportion will be other products (Patterson & Parsons, 1963). Livingstone *et al.* (1953) showed that phaeophytin *a* was stable in acid solution and formed an equilibrium with neutral forms at intermediate acidities. Marker *et al.* (in press) suggest that lower concentrations of water and higher concentrations of acid must be avoided. He pointed out that the conversion of chlorophyll to phaeophytin is generally complete within two minutes in 1.0 M HCl in both acetone and methanol, and from ten minutes to one hour in 0.1 M HCl. The latter process is slow and may hinder routine analysis since because the oxidation of epoxycarotenoids does not contribute significantly to the absorbance at 750 nm and 665 nm only for the first few minutes.

The neutralisation of the acidified extract (e.g. with magnesium carbonate) to bring the solution to neutrality and to compensate for spectral changes on acidification had been advocated by Moss (1967a, 1967b). However, the neutralisation

is an extremely time consuming process which possibly increases degradation of chlorophyll a in the solution (Donaldson, 1978). Potts (1977) noted that the use of magnesium carbonate in neutralisation of extracts seemed to be open to errors. However, he pointed out that there was no direct evidence of the effect of neutralisation and it was difficult to measure the pH in aqueous organic solvents.

In this study, methanol was used as the solvent in all extractions of chlorophyll a, and no extracts were neutralised after extraction. It has been shown by Marker (1972) and Jones (1977) that methanol is much more efficient in extracting pigments than is acetone, especially when algal samples are associated with sediments. 95% was the concentration of the methanol used because it was suggested by Marker (1972) that the use of less than 95% methanol would allow precipitation.

1.43 Acetylene reduction techniques

Methods for the measurement of nitrogen fixation include growth and morphological determinations, N-analysis (including isotopic methods) and reduction of alternate nitrogenase substrates (Hardy et al., 1973).

Although it is one of the simplest method, the correlation between the presence of heterocysts and nitrogen fixation is not absolute (Hardy et al., 1973). N_2 fixation occurs in unicellular algae (Wyatt & Silvey, 1969) and in a filamentous algae devoid of heterocysts (Stewart, 1971).

The Kjeldahl method does not distinguish nitrogen obtained

from other sources (Hardy et al., 1973), though it is helpful in screening new isolates or communities for the ability to fix N_2, and made suitable when substantial quantities of N_2 is concerned (Magué, 1978).

Assays with radioisotope tracer, $^{15}N_2$ is a thousand times as sensitive as the Kjeldahl digestion method (Hardy et al., 1973; Magué, 1978), but involves high expense of $^{15}N_2$, a mass spectrometer and more extensive chemical manipulations than the Kjeldahl method (Hardy et al., 1973).

The acetylene reduction assay technique is by far the most useful and widely applied method for estimation of nitrogen fixation (Hardy et al.,1973) and have been used at cell levels from purified nitrogenase to field samples (Hardy et al., 1973). This method is at least a thousand times more sensitive than $^{15}N_2$ uptake and considerably faster and cheaper than mass spectrometric analysis (Magué, 1978). The advantages of acetylene reduction method are given by Hardy et al. (1973), and in addition the method makes field experiment convenient. The method is based on the indirect measurement of nitrogenase activity by using alternate substrate acetylene which will be reduced to give ethylene by the nitrogen fixing process. There is a ratio of acetylene reduced to nitrogen fixed which is used as a conversion factor to convert measurements from the acetylene-ethylene reduction to absolute values of nitrogen fixation. The value is in fact varied in types of organisms and habitats with a usual value of around 3.0 (3.2 for blue-green algae), although there are some unusually high values of up to

25.Ø for anaerobic soil (Hardy et al., 1973).

1.5 Aims

Although semi-terrestrial species of blue-green algae may be subjected to periods of wet and dry conditions, little work has been carried out regarding these environmental factors in relation to nitrogen fixation. In this study, the main aim is to investigate the effects of environmental humidity during the period for drying Nostoc commune in relation to subsequent nitrogen fixation. It has been shown that Nostoc commune revives when rewetted after long periods of desiccation (section 1.41) and this, coupled with its macroscopic form, wide distribution and frequent occurence in environments subject to a wide range of humidities (section 1.2), makes it a suitable organism for this study.

CHAPTER 2

MATERIALS AND METHODS

2.1 Algae

2.11 Tarn Moor Nostoc commune

Tarn Moor is an upland area of undulating moorland in central Cumberland lying to the west of Sunbiggin tarn in Southern Cumbria, England (map reference NZ 8502, latitude 54° 28'N, longitude 2° 30'W). It is an uneven plateau of about 250 m above Ordnance Datum with ridges to the north and south rising to approximately 300 m. The moor overlies a Carniboforous limestone locally interupted by bands of sandstone, and contains a complex drainage system with numerous springs and swallow holes. The climate is wet with the annual rainfall overranging 1300 mm. The mean annual temperature derived by Myers (1974) (from information given in the Climatological Atlas, 1952) is approximately 9°C, with an average summer temperature of about 14°C and winter of 3°C.

The upper parts of the moor are dominated by Calluna vulgaris with Nardus stricta as the most frequent associate in the drier parts and Sphagnum spp., Molina caerulea and Trichophorum caespitosum on the wetter slopes. There is an interupted belt of "wet flush" vegetation overlying a shallow peat or mineral soil along the sides of the valleys in which many of the springs emerge. Small Carex spp. are dominant in this zone which seperate the valley mires from the Calluna moor

of the ridges.

Further information on the site has been given by Myers (1974) and more details about the area can also be obtained from Holdgate (1955).

The gelatinous pale green-brown colonies of Nostoc commune Vaucher are found on the edges of these flushes near the surrounding vegetation. Some occur in the slight flow riffles but often out of it. Many of them are found shaded in clumps of grasses and mosses. Colonies are irregular in feature and size. They vary from a rounded shape a few mm in diameter to a crust of over 25 mm.

Representative colonies of N. commune from Tarn Moor were collected from the edges of the wet flush areas where they are found growing mixed with the surrounding vegetation which here consists mainly of mosses and grasses.

2.12 Station Quarry Nostoc commune

Information on this site was obtained mainly from the Nature Conservancy Council (Northern Region). Only a general background was available.

The site is situated in the southern part of Middleton in Teesdale, North Yorkshire, England (map reference NY 35/947246, latitude 54° 39'N, longitude 2° 09'W). It is a disused Whin Sill quarry with high vertical crags and spoil heaps, the floor being in parts flooded with shallow water. There are bands of limestone overlying rocks of basaltic composition and drainage from which supplies more alkaline pH water to the

vegetation in the area (Bradshore,per.comm.).

The climate is wet providing a number of wetland habitats important for their flora. A range of species listed by Dalby (1973) includes Eriophorum spp., Salix spp., Orchis spp., small Carex spp. and Sphagnum spp. The site also contains a range of bryophytes.

The gelatinous colonies of Nostoc commune are found on the upper part of the floor where it is drier. Crusts of colonies are yellowish, greenish and brown occurring on the surface of the soil and debris.

Samples of N. commune from Station Quarry were collected from the surface of humid soil intervening between limestone ceramics where grasses and small bryophytes occur. Some colonies were taken from shallow flooded areas.

N. commune from both sites were collected during the experimental period (May - June, 1980). Samples were all collected between 1300 p.m.and 1500 p.m. on cloudy days with sunny and shower intervals ,and were brought to the laboratory in polythene bags or buckets and arrived to the laboratory between 1700 p.m.and 1800 p.m. Colonies were separated from debris and mud by rinsing with distilled water and were air-dried in designed conditions (Fig. 2.1).

2.2 Medium

In order to ensure that dried algal colonies were in a standard environment, a nutrient medium was used for remoistening, in preference to distilled water. The medium used

was a modification from that of Allen and Arnon (1955), which was designed for the growth of blue-green algae. This medium is free of combined nitrogen and contains:

K_2HPO_4	250.00	mg l^{-1}
$CaCl_2.2H_2O$	66.20	mg l^{-1}
$MgSO_4.7H_2O$	200.00	mg l^{-1}
NaCl	230.00	mg l^{-1}
Fe(as EDTA chelate)	4.00	mg l^{-1}
Mn	0.12	mg l^{-1}
Mo	0.08	mg l^{-1}
Zn	0.01	mg l^{-1}
Cu	0.005	mg l^{-1}
B	0.09	mg l^{-1}
Co	0.005	mg l^{-1}
Ni	0.002	mg l^{-1}

A modified Fe-EDTA stock was made from 12.7 g Na.EDTA (ethylenediaminetetra-acetic acid disodium salt) mixed with 9.7 g $FeCl_3.6H_2O$ and made up to 1.0 litre with distilled water. The solution gave 2.0 g Fe per litre.

The medium also contained 5.0 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) and was buffered to pH 7.2-7.5.

2.3 Gasses

Apart from ethylene (BDH Chemicals Ltd), gasses ie.acetylene, gas mixture of argon, oxygen and carbon dioxide,

and the gasses used for gas chromatography, nitrogen, hydrogen and compressed air, were all obtained from British Oxygen Company.

2.4 Humidity control apparatus

This apparatus consisted of a non-hydroscopic leak-proof enclosure made from an ordinary desiccator, the bottom of which contained a saturated salt solution which gave a certain vapour pressure at a certain temperature due to the properties of water-salt systems in relation to humidity as described by Wylie (1965). The use of saturated salt solutions to control humidity is both convenient and cheap (Young, 1967), and chemicals and apparatus involved are available in the laboratory. Most reagents used are available in a reasonably pure form and are also nonvolatile, thus avoiding contamination of the specimen (Young, 1967).

Saturated NaCl (Analar grade, BDH Chemicals Ltd) solution was used to give a relative humidity of 75%. Young (1967) and Hedlin & Trofimenkoff (1965) have shown that the solution will maintain a humidity of 75% at 25°C.* Anhydrous LiCl (commercial grade, BDH Chemicals Ltd) mixed with distilled water in a ratio of 1:1 was used to give a relative humidity of 50%. The use of this mixture was due to the desire to avoid using nitrogen – containing salts ie.$MgNO_3.6H_2O$, $Ca(NO_3)_2.4H_2O$ which are normally used to provide relative humidity of around 50%. Other non-nitrogenous salts normally used for this purpose were not available. Silica gel

* **saturated NaCl can also remain giving a relative humidity of around 75% within a temterature range of 5-60°C with the rate of change of -0.02% per °C (Young, I967)**

was used to provide a very dry atmosphere inside the chamber. The gel was dried daily at 105°C and gave a relative humidity of 10%.

Relative humidity inside the chamber was measured with a simple hygrometer; this was considered to be sufficient for this study. The temperature was kept at 20°C ± 2°C. Air circulation inside the chamber could not be achieved due to the type of desiccator available. However, this did not appear to affect the system since the hygrometer gave consistent readings.

2.5 Physical and chemical laboratory techniques

2.51 Measurement of moisture uptake in dry algae

Individual colonies of algae were used for measuring the uptake of water with time. A dry colony was removed from storage, brushed to remove any debris and was then weighed using an Oertling GC32 balance. The colony was then immersed in distilled water in a plastic petri-dish.

The colony was taken out again at various time intervals for weighing. Excess water was quickly removed by blotting on fine tissue paper before weighing the colony.

Colonies were oven-dried at 105°C for 12 h at the completion of the time course, and dry weights were obtained. Some of these dried colonies were rewetted again.

The percentage moisture content at a particular time was obtained using the formula given by Showman and Rudolph (1971):

$$\%\text{moisture content} = \frac{Wt-Wd}{Ws-Wd} \times 100$$

where:

Wt= wet weight in gram at time t

Wd= dry weight of the colony

Ws= final wet weight of the colony

2.52 Analysis of chlorophyll a

2.521 Extraction of chlorophyll a

The method used for measurement of chlorophyll a was a modified version of that given by Hargreaves and Whitton (1976), which is quick and simple. This enabled daily extraction and facilitated routine analysis of up to 40 samples per day.

The colonies were squashed in a few ml of 95% methanol(Analar) in 29 ml McCartney bottles, then added the rest of methanol (10 ml was the total amount of methanol used in each sample) and screwed loosely with the caps with rubber liners. The bottles were then set standing in a water bath maintained at 70°C. The water bath was covered with a hood allowing the extraction to take place in darkness. A time of 15 minutes given for each extraction was found to allow more than 90% of chlorophyll a to be extracted within the first extraction.

Extracts were made using pressure filtration through 24 mm GF/C (Whatman) glass fibre discs, made the volume to 10 ml or 25 ml with fresh 95% methanol within the 10 ml or 25 ml Pyrex volumetric flask which was used to collect the filtrate. Extracts were transferred into 29 ml McCartney bottles and then the caps with rubber liners were screwed down.

10 ml of the extract was poured into high precision 4.0 cm

optical cells (Thermal Syndicate Ltd). Absorption were read at 665 nm using Perkin Elmer Model 403 ultraviolet spectrophotometer. Absorbance was read again one minute after acidification with 0.1 ml of 1.0 N HCl (BDH Chemicals Ltd).

Chlorophyll degradation during the process was prevented in that all the steps were carried out in shade presumably to minimise light intensity and temperature. In addition, samples which were delayed between the process were kept in ice bucket with the hood providing low temperature and dark condition.

2.522 Estimation of chlorophyll a

Chlorophyll a was calculated using the formula following Marker (1972), but with a constant derived from a different acid factor. Marker (1972,in press) gives an acid factor of 1.5 when using methanol. A mean value of 1.86 for acid factor was used by Whitton et al. (1979) for N. commune from the Aldabra atoll, whereas Donaldson (1978) found acid factor of 1.89 for his algal samples from the same atoll. However, acid factor is varied in species and communities (Whitton & Livingstone, per.comm.), and might also be varied seasonally.

A mean value of 2.21 for the acid factor has been used for all the calculations here. The maximum derived of experimental acid factors were obtained from the extraction of young bright green colonies using the formula:

$$Af = \frac{A_{655}\text{(before acidification)}}{A_{655}\text{(after acidification)}}$$

where:

A_{665} = absorbance at 665, read from the spectrophotometer

Af = acid factor

The actual value obtained was 2.1818 ± Ø.1968 with n=1Ø for Station Quarry N. commune, and 2.2548 ± Ø.1254 with n=58 for Tarn Moor N. commune.

From this mean value for acid factor, a constant was derived using formula:

$$K = \frac{Af}{Af-1}$$

Where K = a constant

Chlorophyll a was calculated by:

$$\mu g \text{ chl } a = K(Ab-Aa) \times 13.1 \times V/l$$

Where:

chl a = chlorophyll a

K = constant,derived from an acid factor

Ab = absorbance read at 665 before acidification

Aa = absorbance read at 665 after acidification

13.1 = Constant, assuming a specific absorption coefficient of chlorophyll a in 95% methanol of 71.Ø7 $lg^{-1}cm^{-1}$

V = volume of solvent (in ml) used in making up extract

l = light path which equals to the width of optical cell

2.53 Acetylene reduction assay techniques

2.531 General method used for experimental assays

In this study the general method of acetylene reduction assay proposed by Stewart et al. (1967, 1971) was followed. All assays were carried out in 29 ml McCartney bottles fitted with rubber stopper liners. Replicates were made in most assays and the number of replicates were varied as indicated.

Approximately 1.5 ml alga was used in the case of fresh colonies, as this was found to give sufficient chlorophyll a. This amount was not absolute, but the volume of algae has little effect upon the composition of the final gas phase (Donaldson, 1978). Whole algal colonies were used in preference in order to avoid error which might be caused by disruption of colonies. Two or three dried colonies and 3-4 ml of medium were used in each case for assays on remoistening.

Acetylene used was conveyed in a football bladder fitted with "T" junction and a bung. Gas samples were taken when required.

3 ml of acetylene gas was injected through the rubber stopper liner into each bottle using Gillette Scimitar disposable syringe and venting was made to adjust the pressure of the gas phase inside the bottle.

After injection of acetylene the bottles were shaken to aid the dissolving of the gasses. The algal colonies were then repositioned by gently tapping the bottles such that they received maximum illumination.

Constant temperatures and light conditions were provided by

setting the incubator (Gallekamp Cooled Incubator, IH-270) to give the conditions required. Illumination inside the incubator was provided using fluorescent tubes.

Sample bottles were laid on their sides in the incubator at an approximate angle of 15° to the horizontal. Assays were incubated for 1-2 hours. At the end of the incubation period samples were shaken to allow maximum amount of gas to be released into the gas phase of the bottles. 1.0 ml of gas sample was removed from each bottle and transferred into a Varian Aerograph Series 1200 gas chromatograph using a 1.0 ml SEBRE syringe.

The gas chromatograph used was fitted with a stainless steel column (1800 mm x 3.2 mm) packed with Poropak R and maintained at 150°C. Nitrogen was used as a carrier gas at a flow rate of 30 ml min^{-1}. The machine was calibrated using dilutions of high purity ethylene (BDH Chemicals Ltd) prepared using a Hamilton gas syringe. Calibration was made every time before determining gas samples. Volumetric flasks were flushed with a gas mixture of 79.97% argon, 20.00% oxygen and 0.03% carbon dioxide for 30 seconds and suba-sealed before filled with standards.

The ethylene contamination of the acetylene was always determined in each experiment by including controls without algae.

Dark controls were also made on samples of algae in most assays by wrapping bottles with aluminium foil.

2.532 Estimation of ethylene produced

From each calibration a standard theoretical linear regression was obtained. The volume of ethylene was then calculated from the standard. Knowing the volume of ethylene and gas phase in the bottle, the total amount of ethylene produced in each bottle could be calculated.

Ethylene produced was expressed as nanomoles obtained from:

$$1.0 \text{ ml ethylene} = \frac{273}{293} \times \frac{10^6}{22.4} \text{ nMoles (at20 } ^{o}\text{C)}$$

2.6 Coding system

A coding system was devised to describe the conditions under which algae were stored and incubated. This is shown in Fig. 2.1. Interpretation of the code is as follow:

1) The first letter indicates the illumination condition, light (L) or dark (D), in which the algae were dried.

2) The number indicates the relative humidity in which the algae were dried.

3) The second letter indicates the illumination condition, light (l) or dark (d), in which dried algae were rewetted under incubation.

All possible combinations are shown in Fig. 2.1.

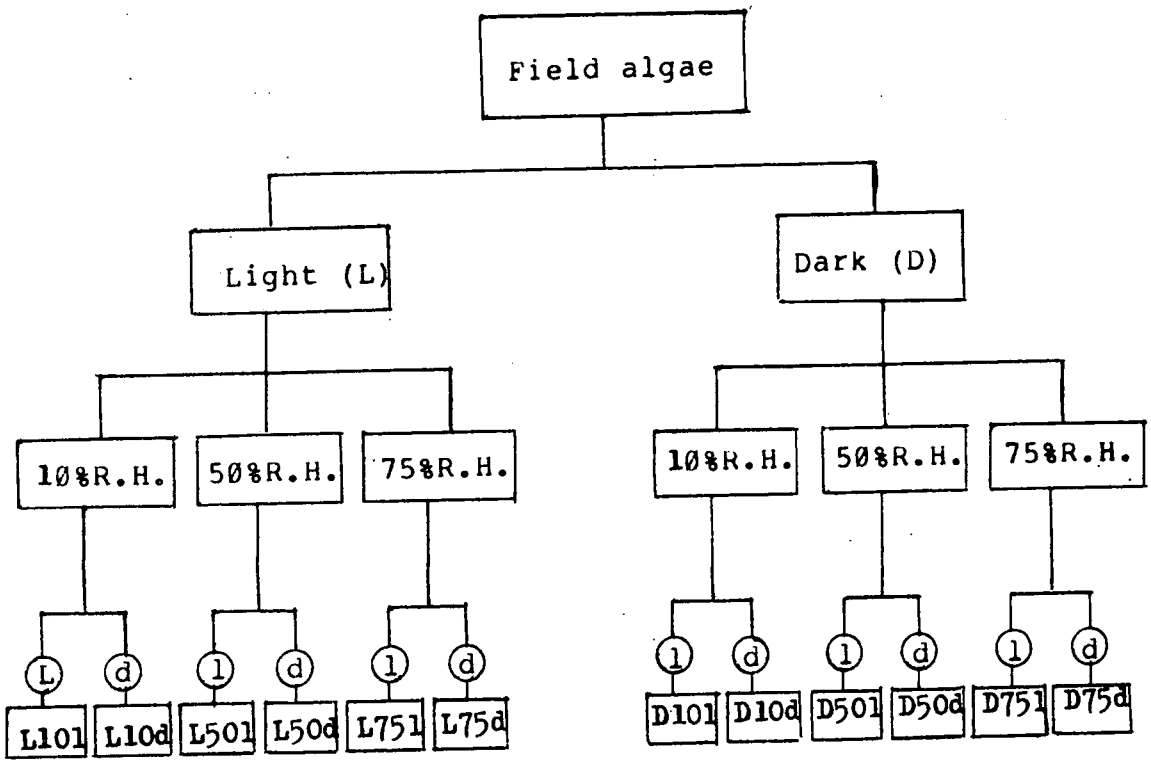


Fig. 2.1 Diagram showing the coding system of conditions in which algae were dried and rewetted under incubation (L or l = light, D or d = dark).

CHAPTER 3

RESULTS

3.1 Moisture uptake experiments

When dry, most colonies of *N. commune* were visually browny-black in colour, a few colonies of Station Quarry *N. commune* were greenish-black. Dry colonies were all hard and brittle, but within a few minutes after immersed in distilled water they became soft and pliable and markedly increased in size and weight.

The rate of water uptake was measured at room temperature with illumination of 1000 lx.

3.11 Moisture uptake by Station Quarry N. commune

Within 15 minutes of distilled water being added, the greenish-black colonies, presumably young, had increased exponentially in size and weight, and had reached a moisture content of about 80% the saturation value. After this period, the rate began to slow down and reached the maximum ceiling level after 20-22 hours (Figs 3.1, 3.2).

The browny-black colonies, presumably old, had reached 80% saturation in about 2 hours, but still the maximum water content was achieved after about 20-22 hours (Figs 3.1, 3.2).

Comparisons between old and young colonies indicate that there is variation in the capacity for moisture uptake in *N. commune*.

(A)

TIME	% WAT.CONTS.					MEAN # S.D.
(H)	S1	S2	S3	S4	S5	
0.016	28	21	28	25	23	25.00 # 3.08
0.033	36	32	34	32	32	33.20 # 1.78
0.083	46	51	40	42	43	44.40 # 4.27
0.167	52	56	50	51	51	52.00 # 2.34
0.250	60	60	52	53	57	56.40 # 3.78
0.500	64	62	56	64	67	62.60 # 4.09
0.750	66	64	58	67	73	65.60 # 5.41
1.000	72	67	64	76	76	71.00 # 5.38
2.000	78	80	80	84	84	81.20 # 2.68
4.000	80	84	82	86	86	83.60 # 2.60
6.000	82	88	84	88	86	85.60 # 2.60
8.000	82	92	86	88	87	87.00 # 3.60
14.000	88	99	90	92	90	91.80 # 4.26
20.000	100	100	98	96	95	97.80 # 2.28
22.000	100	100	100	100	100	00.00 # 0.00
24.000	100	100	100	100	100	00.00 # 0.00

(B)

0.016	31	24	33	24	27	27.80 # 4.08
0.033	39	35	38	37	35	36.80 # 1.78
0.083	47	47	42	46	43	45.00 # 2.34
0.167	53	53	51	52	49	51.60 # 1.67
0.250	59	59	56	56	55	57.00 # 1.87
0.500	63	60	58	63	68	62.40 # 3.78
0.750	69	63	60	69	75	67.20 # 5.84
1.000	71	64	67	74	79	71.00 # 5.87
2.000	80	76	79	83	88	81.20 # 4.54
4.000	82	81	85	86	90	84.80 # 3.56
8.000	86	93	88	89	93	89.80 # 3.11
12.000	90	97	92	90	96	93.00 # 3.31
14.000	94	100	94	93	99	96.00 # 3.24
20.000	98	100	98	96	100	98.40 # 1.67
22.000	100	100	99	99	100	00.00 # 0.00
24.000	100	100	100	100	100	00.00 # 0.00

Table 3.1. Time course of water uptake by dry colonies of Station Quarry N. commune. (A) five old colonies wetted; (B) five old colonies wetted, dried and rewetted.

(A)

TIME	% WAT.CONTS.			MEAN	# S.D.
(H)	S1	S2	S3		
0.016	26	26	34	28.66	# 4.61
0.033	40	47	64	50.33	#12.34
0.083	60	75	82	72.33	#11.23
0.167	75	78	90	81.00	# 7.93
0.250	82	82	93	85.66	# 6.35
0.500	83	83	93	86.33	# 5.77
1.000	84	84	94	87.33	# 5.77
2.000	85	85	95	88.33	# 5.77
4.000	87	89	96	90.66	# 4.72
6.000	91	93	97	93.66	# 3.05
10.000	94	95	97	95.33	# 1.52
14.000	98	96	98	97.33	# 1.15
18.000	99	97	99	98.66	# 0.50
20.000	100	98	100	99.66	# 0.40
22.000	100	100	100	00.00	# 0.00
24.000	100	100	100	00.00	# 0.00

(B)

0.016	28	25	30	27.66	# 2.51
0.033	41	39	55	45.00	# 8.71
0.083	53	64	82	66.33	#14.64
0.167	73	79	91	81.00	# 9.16
0.250	82	84	93	86.33	# 5.85
0.500	84	85	94	87.66	# 5.50
0.750	85	88	95	89.33	# 5.13
1.000	86	91	96	91.00	# 5.00
2.000	93	91	97	93.66	# 3.05
4.000	95	92	97	94.66	# 2.51
6.000	97	94	98	96.33	# 2.08
10.000	98	97	99	97.00	# 1.00
14.000	99	98	99	98.66	# 0.50
18.000	99	100	99	99.66	# 0.40
20.000	100	100	100	00.00	# 0.00
22.000	100	100	100	00.00	# 0.00
24.000	100	100	100	00.00	# 0.00

Table 3.2. Time course of water uptake by dry colonies of Station Quarry N. commune. (A) three young colonies wetted; (B) three young colonies wetted, dried and rewetted.

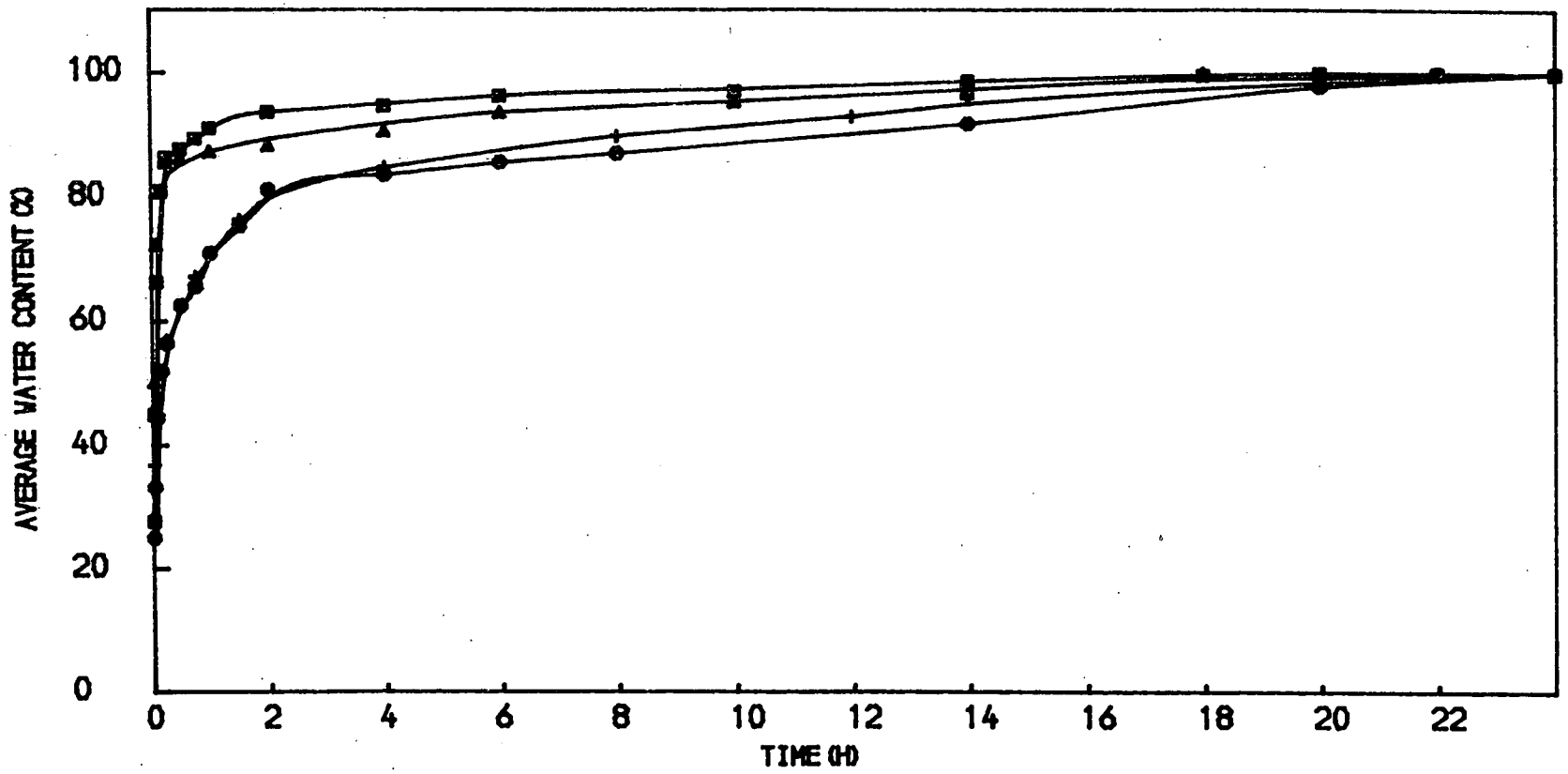


Fig.3.1 Time course of water uptake by dry colonies of Station Quarry N. commune. ▲ mean of five samples of young colonies wetted: ■ mean of five young colonies wetted, dried and rewetted: ● mean of three samples of old colonies wetted: + mean of three old colonies wetted, dried and rewetted.

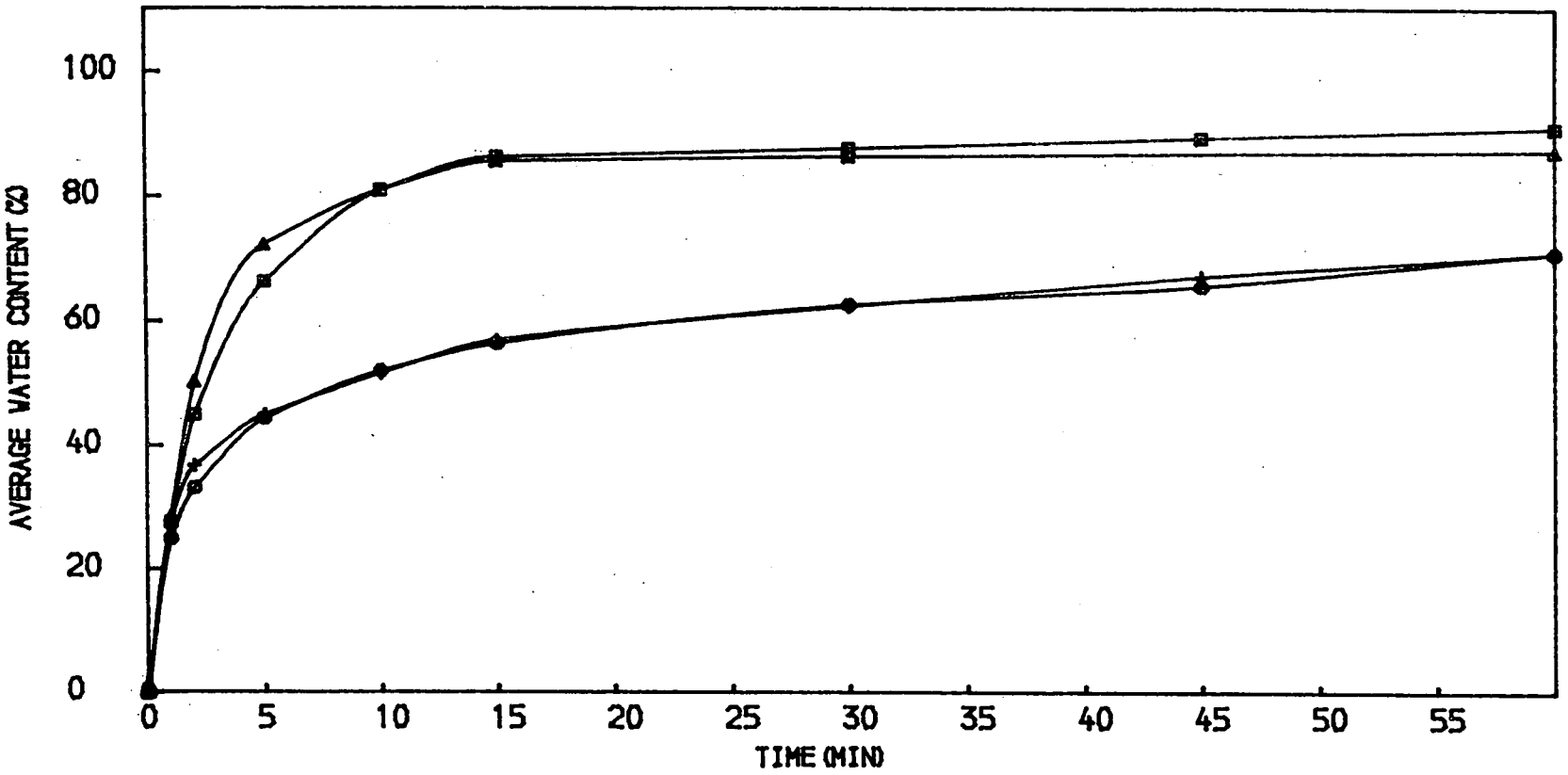


Fig.3.2 Time course of water uptake by dry colonies of Station Quarry N. commune within the first hour after rewetting (picture blown up from Fig.3.1).

TIME	% WAT.CONTS.			MEAN	#	S.D.
(H)	S1	S2	S3			
0.016	29	44	45	39.28	#	9.27
0.033	43	56	52	50.05	#	6.51
0.083	64	63	66	64.26	#	1.28
0.167	71	81	72	75.11	#	5.54
0.250	86	81	83	83.32	#	2.17
0.500	86	81	83	83.32	#	2.17
0.750	86	85	90	86.86	#	2.44
1.000	86	85	90	86.86	#	2.44
2.000	93	89	93	91.62	#	2.37
4.000	93	89	93	91.62	#	2.37
8.000	93	93	97	94.00	#	2.21
12.000	93	93	97	94.00	#	2.21
18.000	99	96	97	97.61	#	2.06
20.000	99	96	99	98.76	#	2.13
22.000	99	99	99	99.99	#	0.001
24.000	99	99	99	99.99	#	0.001

Table 3.3. Time course of water uptake by three dry colonies of Tarn Moor N. commune.

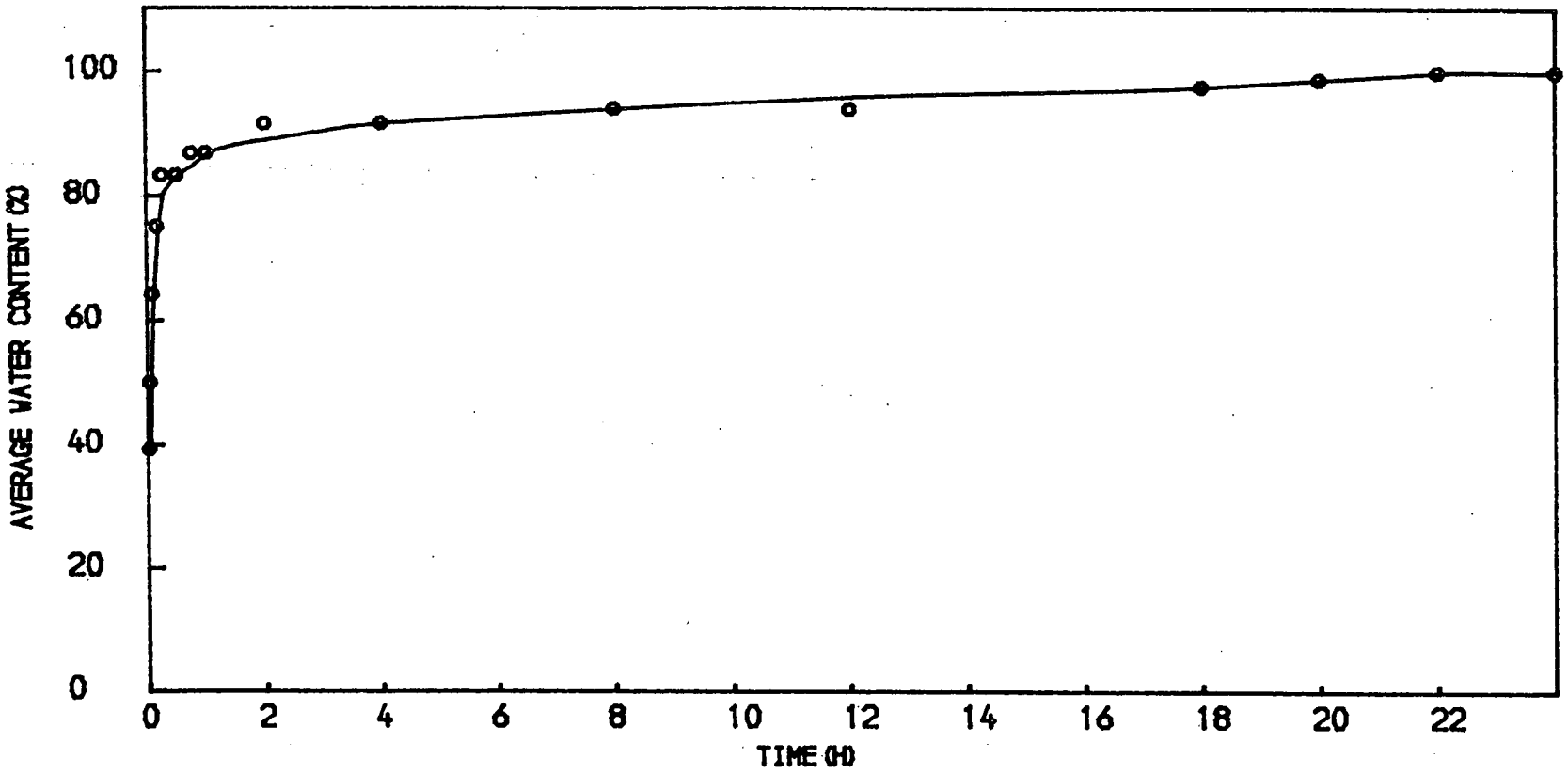


Fig.3.3 Time course of water uptake by dry colonies of Tarn Moor N. commune. Each point represents three replicates.

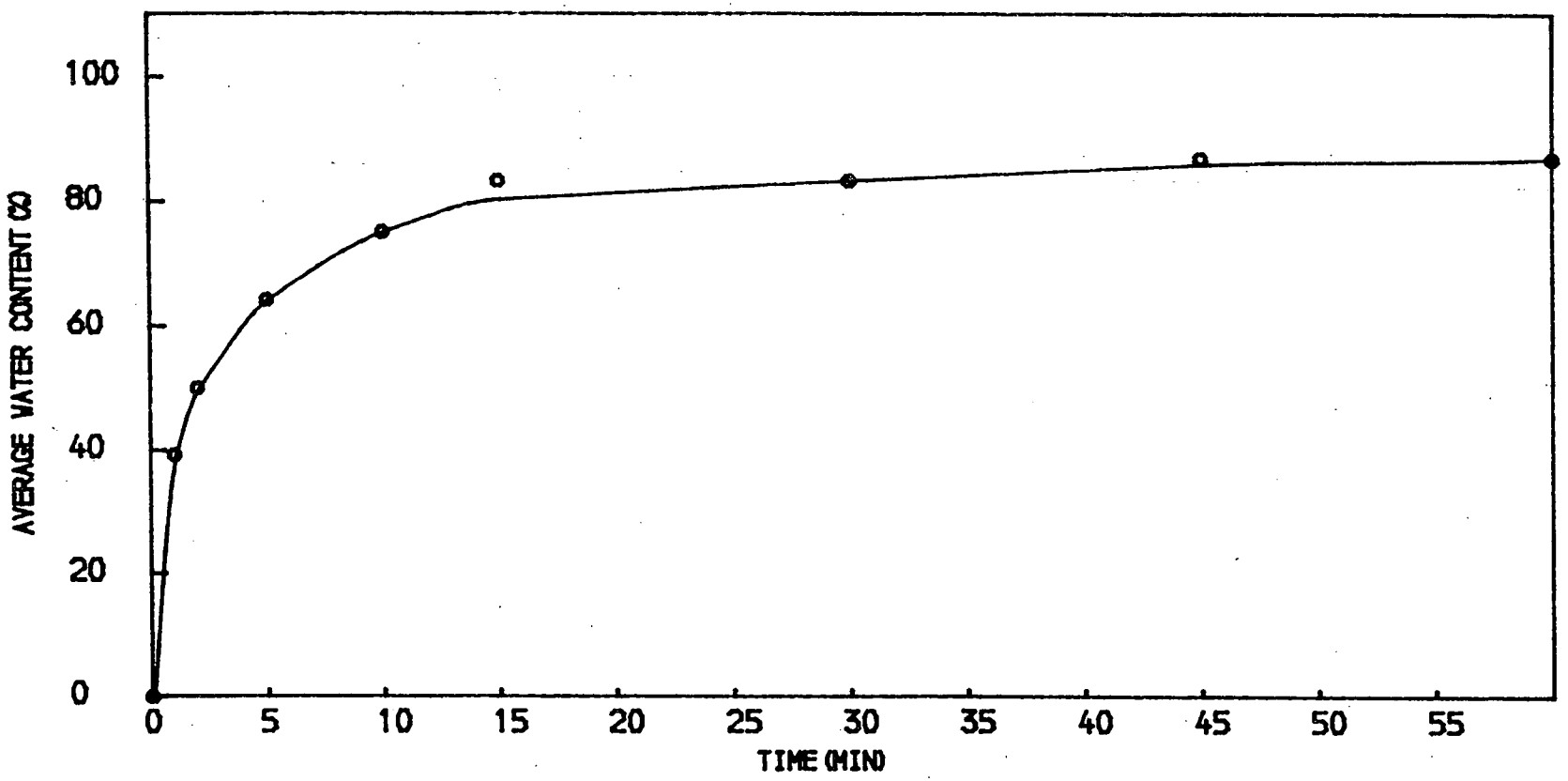


Fig.3.4 Time course of water uptake by dry colonies of Tarn Moor N,commune within the first hour after rewetting (picture blown up from Fig.3.3).

Both old and young colonies which were redried and rewetted gave similar rates of water uptake as the first remoistening (Figs 3.1, 3.2).

3.12 Water uptake by Tarn Moor N. commune

Since the algae collected from Tarn Moor for this experiment were all young colonies (section 2.11, Chapter 2), the samples showed similar rates in water uptake in that 80% saturation was achieved within 15 minutes after remoistening (Fig. 3.4), and maximum level was attained after about 18-22 hours (Fig. 3.3).

3.2 Comparison between rates of acetylene reduction of submerged and non-submerged algal samples

This experiment was carried out initially to investigate the problems of remoistening algae the procedure of which dried algae were normally immersed in the liquid phase (distilled water or medium). Since N. commune from both sites is terrestrial, it was suspected that perhaps submerged conditions may inhibit the acetylene reduction.

Only algae from Tarn Moor were used in this investigation. Fresh algae were brought from the field, and colonies of similar sizes and features were chosen for the investigation. Five samples of approximately 1.5 ml a sample were bottled with 3.5 ml medium which allowed the algae to be submerged. Each of another five algal samples was then placed in bottles, the bottom and one side of which carpetted with filter paper wetted

with medium. This was artificially made to immitate the actual situation in the field.

All sample bottles were gassed and incubated for 1 hour at 20°C, 5000 lx.

The results showed that submerged samples gave lower ethylene on the gas chromatograph. Statistical analysis using Student 't' test of differences have revealed the highly significant difference in the mean rate of acetylene reduction between the two groups. The 't' value obtained from the data was numerically greater than the tabulated value of 't' for $p < 0.002$ (Table 3.4).

This experiment was repeated and similar results were obtained (Table 3.5) with the 't' value of difference greater than the tabulated value for $p < 0.001$.

3.3 Comparisons between rates of acetylene reduction of submerged, 1/4-submerged and non-submerged algal samples

Similar procedures as in experiment 3.2 were carried out with fresh algae from Tarn Moor. They were assayed for acetylene reduction under different conditions: submerged, 1/4-submerged and non-submerged. All samples were incubated at 20°C, 5000 lx for 1 hour. The results have shown that non-submerged and 1/4-submerged algae commenced acetylene reduction at a higher rate than the fully submerged algae. The differences between the means of either between submerged and non-submerged or between submerged and 1/4-submerged were highly significant at $p < 0.001$ (Table 3.5).

$X\ 10^{-4}$ N MOLE ETHYLENE/CHL A/MIN.

	(A)		(B)	
	SUB.	NON-SUB.	SUB.	NON-SUB.
	28.4376	236.2670	85.2539	134.9470
	43.7532	575.5210	17.7520	129.8550
	18.7513	106.6480	47.5608	282.9390
	60.4210	484.0190	28.4376	173.9690
	45.9409	826.9360	45.9409	317.8980
	30.9918	319.3440	58.0408	319.0890
	42.0031	165.2460	39.7344	144.4430
	42.3418	552.6320	30.9918	236.2670
MEAN	39.0803	395.1309	44.2140	217.4259
S.D.	12.7359	264.7955	20.8178	81.7343
T	3.7988		5.8086	
D.F.	14		14	
P	< 0.002		< 0.001	

Table 3.4. (A) Comparison between rates of acetylene reduction in submerged and non-submerged N.commune. The difference between means of the two groups is highly significant with $p < 0.002$. (B) Repeated (A) with $p < 0.001$.

$X\ 10^{-4}$ N MOLE ETHYLENE/CHL A/MIN.

	(A) SUB.	(B) NON-SUB.	(C) 1/4-SUB.
	81.8149	195.9318	271.6078
	190.4045	417.1447	350.0000
	108.8048	542.6017	556.1746
	66.0288	303.8415	476.9269
	274.1869	551.9205	261.0787
MEAN	144.6480	402.2881	383.1576
S.D.	87.2851	153.7637	129.3094

Table 3.5. Comparison of rates of acetylene reduction in submerged (A), non-submerged (B), and 1/4-submerged (C) N.commune. Mean rates of (A) & (B) are significantly different with $p < 0.001$. Mean rates of (A) & (C) are significantly different with $p < 0.001$. Mean rates of (B) & (C) are not signifiantly different ($p > 0.1$).

$X\ 10^{-4}$ N MOLE ETHYLENE/CHL A/MIN.

	NON-SUB.	1/4-SUB.
	253.1570	272.0560
	326.7750	369.1348
	324.9130	480.9030
	357.6100	297.0800
	400.9940	282.2290
MEAN	332.6898	340.2832
S.D.	54.1004	87.3196
T	1.3593	
D.F.	8	
P	> 0.1	

Table 3.6. Comparison between rates of acetylene reduction in 1/4-submerged and non-submerged N.commune. The difference between means of the two groups is not significant ($p > 0.1$).

The mean values of the rates of acetylene reduction between 1/4-submerged and non-submerged algal samples were not different significantly ($p > 0.1$)(Table 3.5).

The experiment was repeated again to compare only between 1/4-submerged and non-submerged samples. Results showed the difference between the rates of acetylene reduction of both sample groups at $p > 0.1$ (Table 3.6) which was similar to the result obtained from the first experiment.

3.4 Time course of acetylene reduction by Station Quarry Nostoc commune

This experiment was carried out as to observe some general background on time course of acetylene reduction by N. commune.

Algae were dried in shade at room temperature of 20°C. Dried N. commune were assayed for acetylene reduction immediately after they had been remoistened. The experiment was continued up to 96 hours.

A sample size of three colonies was taken to ensure a detectable rate of acetylene reduction. Comparison of acetylene reduction between samples rewetted with distilled water and medium were made. Samples were incubated at 20°C, 3000 lx. Dark controls were also determined.

From the knowledge gained in the moisture uptake experiments, the following procedure was carried out. Colonies were rewetted with 3.0 ml liquid phase (medium or distilled water), which covered the algae completely. After an hour when the percentage moisture content of the algae reached >80% the liquid phase would cover only 1/4 of the moisted algae.

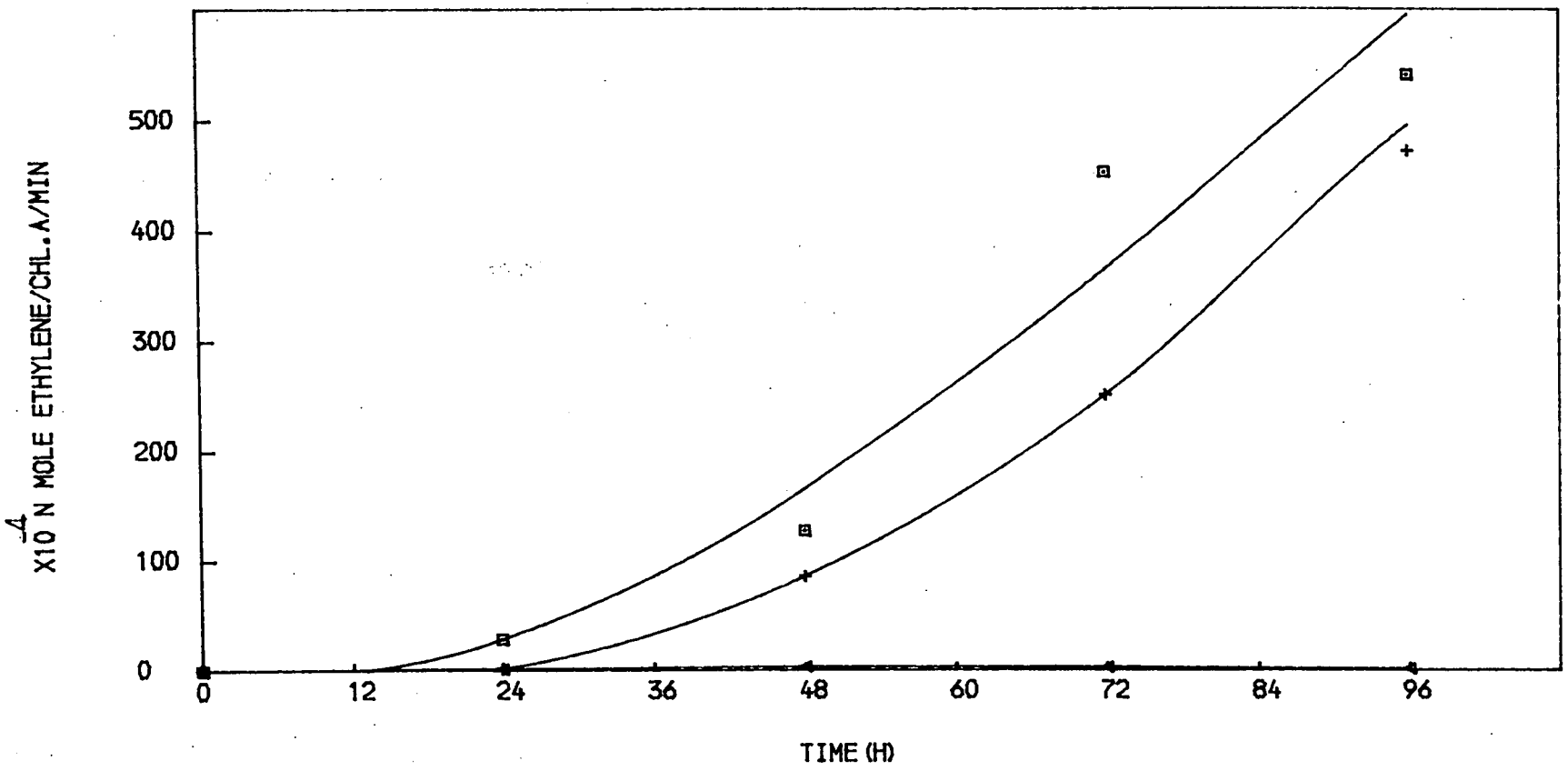


Fig.3.5 Time course of acetylene reduction by rewetted colonies of Station Quarry N. commune. + light-wetted with distilled water: Δ dark-wetted with distilled water: ⊡ light-wetted with medium.

It was found that by this procedure, maximum ethylene produced could be evacuated whereas submerged colonies were prooved to give a highly significant different from non-submerged colonies in terms of ethylene obtained (sections 3.2, 3.3).

It was shown that acetylene reduction commenced between 24-48 hours after rewetting and increased markedly until 96 hours when the experiment was terminated(Fig. 3.5). Colonies rewetted with medium showed an earlier acetylene reduction and increased with a higher rate than colonies rewetted with distilled water.

Reduction in the dark was very low and undetectable.

The use of medium seemed to minimize the timelag before recovery: the results were however not convincing because of the large variation within one population. Small experiments were carried out as attempting to detect the ethylene within the first 24 hours after rewetting. No detectable reduction was found until between 24-48 hours after being rewetted. It was also found that incubation period of up to 6 hours gave the same results.

3.5 Time course of acetylene reduction by Station Quarry N. commune dried in different humidity regimes

Results from experiment 3.4 Suggested that the slow recover of algal activities after rewetting could be caused by the conditions when they were dried. Therefore, in this experiment algae were dried at 20°C with 3000 lx of light intensity in different humidity chambers given relative

humidities of 75%, 50% and 10%.

Three replicates of a sample of two to three colonies were taken as representatives of each humidity. They were assayed for acetylene reduction activity immediately after they had been rewetted and were followed at time intervals up to 4 days. Incubation period was 1-2 hours at 20°C under light intensity of 3000 lx constant.

The results show the difference in rate of acetylene reduction both within the same and between different humidity regimes. Similarity within the same humidity might be recognised by means of recovery timelag. As shown in Fig. 3.6, it looks as if algae dried at a higher humidity have their recover sooner than those dried at lower humidities. Acetylene reduction of algae dried at 75% relative humidity commenced at about 24 hours after rewetting or a little earlier than 24 hours, at 24 hours or later than 24 hours for those dried at 50% relative humidity, and at 36 hours or later for algae dried at 10% relative humidity.

The large variations which occured within the rates of acetylene reduction did not allow meaningful comparisons to be made quantitatively. However, an attempt was made to compare the mean rates of acetylene reduction at 72 hours after rewetting as it was the time at which all the rates had reached maximum equilibrium. A mean rate for L751 samples at this stage was 0.0309 ± 0.0241 nMole C_2H_4 μg chl a^{-1} min^{-1}, and was approximately 1.5 times the mean rate for L501 and two times that for L101 samples.

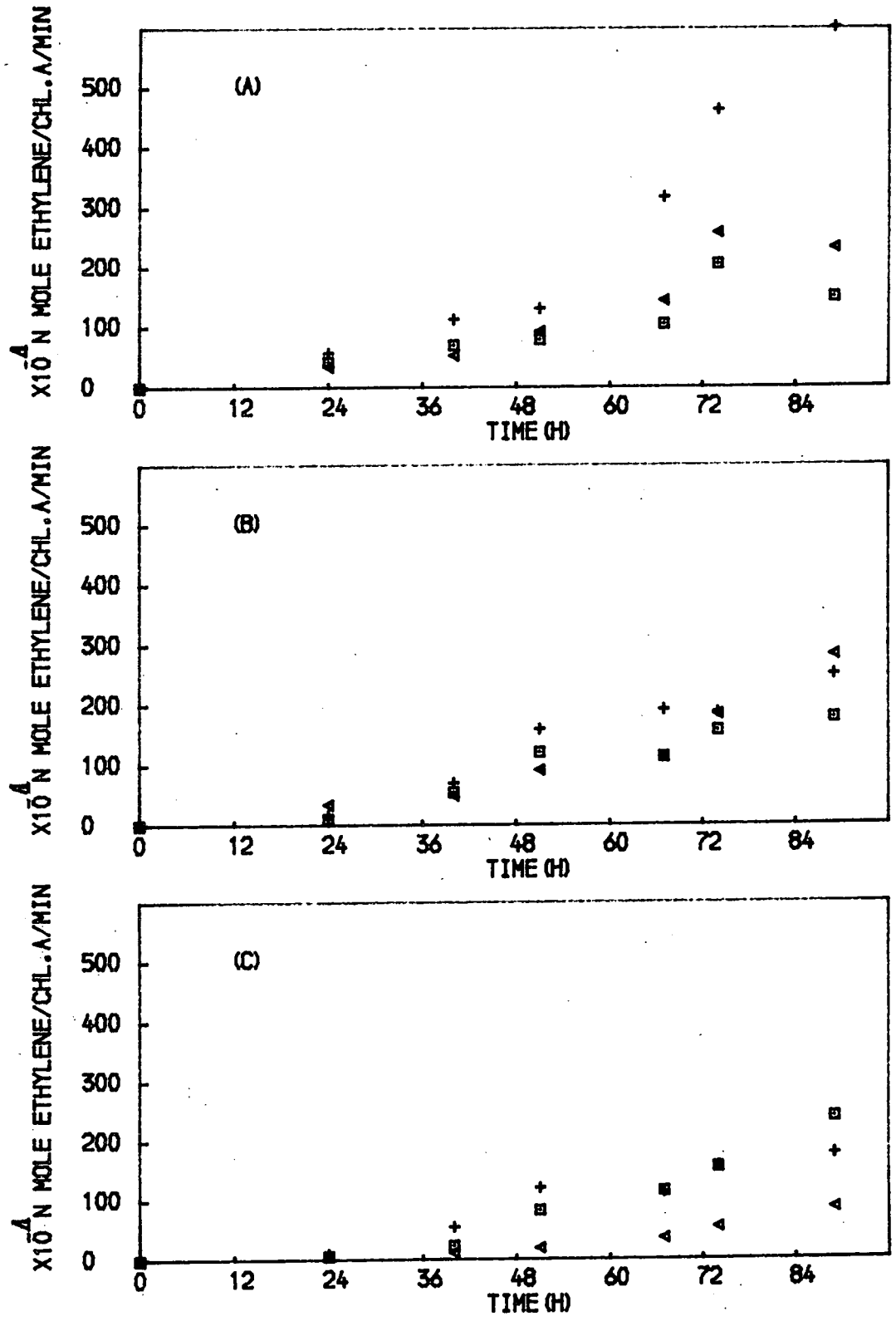


Fig.3.6 Time course of acetylene reduction by light-wetted Station Quarry N. commune. (A) 4 replicates light-dried under 75% R.H.: (B) 4 replicates light-dried under 50% R.H.: (C) 4 replicates light-dried under 10% R.H.

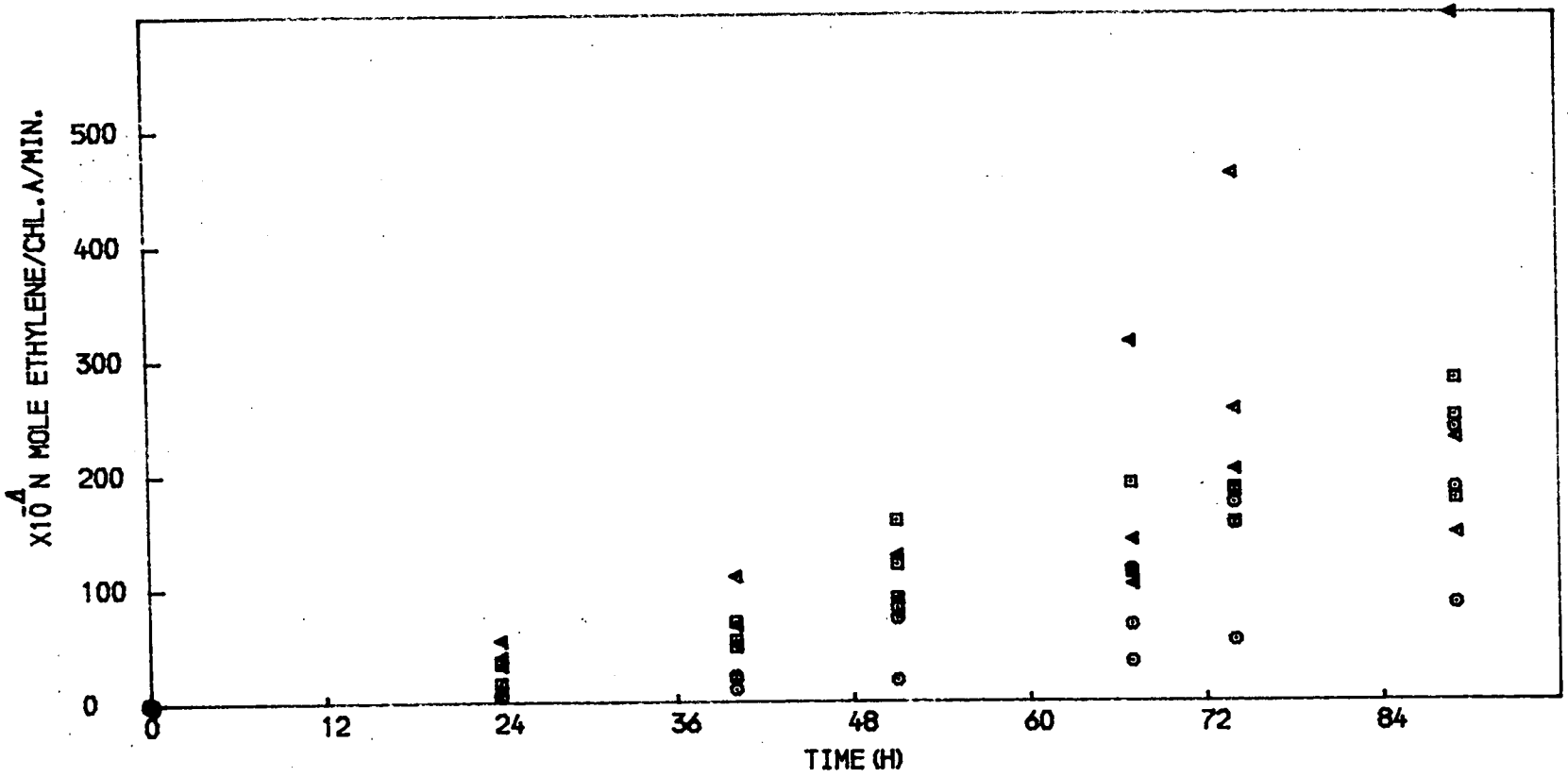


Fig.3.7 Time course of acetylene reduction by light-wetted Station Quarry N. commune. △ 4 replicates light-dried under 75% R.H.: ⊡ 4 replicates light-dried under 50% R.H.: ⊙ 4 replicates light-dried under 10% R.H.

TIME	X 10 N MOLE ETHYLENE/CHL A/MIN								
(H)	75% R.H.			50% R.H.			10% R.H.		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
24.000	56.784	34.060	41.886	16.952	34.112	10.114	5.876	5.564	6.916
40.000	112.528	52.494	69.342	70.668	48.828	54.626	11.336	23.036	20.722
51.000	130.702	91.858	78.754	159.510	91.546	121.030	18.954	82.836	73.710
67.000	317.200	143.468	104.416	191.568	113.022	113.282	35.152	115.986	67.574
74.000	463.060	257.790	205.062	186.966	183.846	157.170	53.846	155.974	174.252
89.000	601.640	231.764	148.824	249.782	282.360	177.450	85.956	239.304	187.044

Table 3.7 Time course of acetylene reduction by light-wetted Station Quarry N. commune light-dried under different humidities.

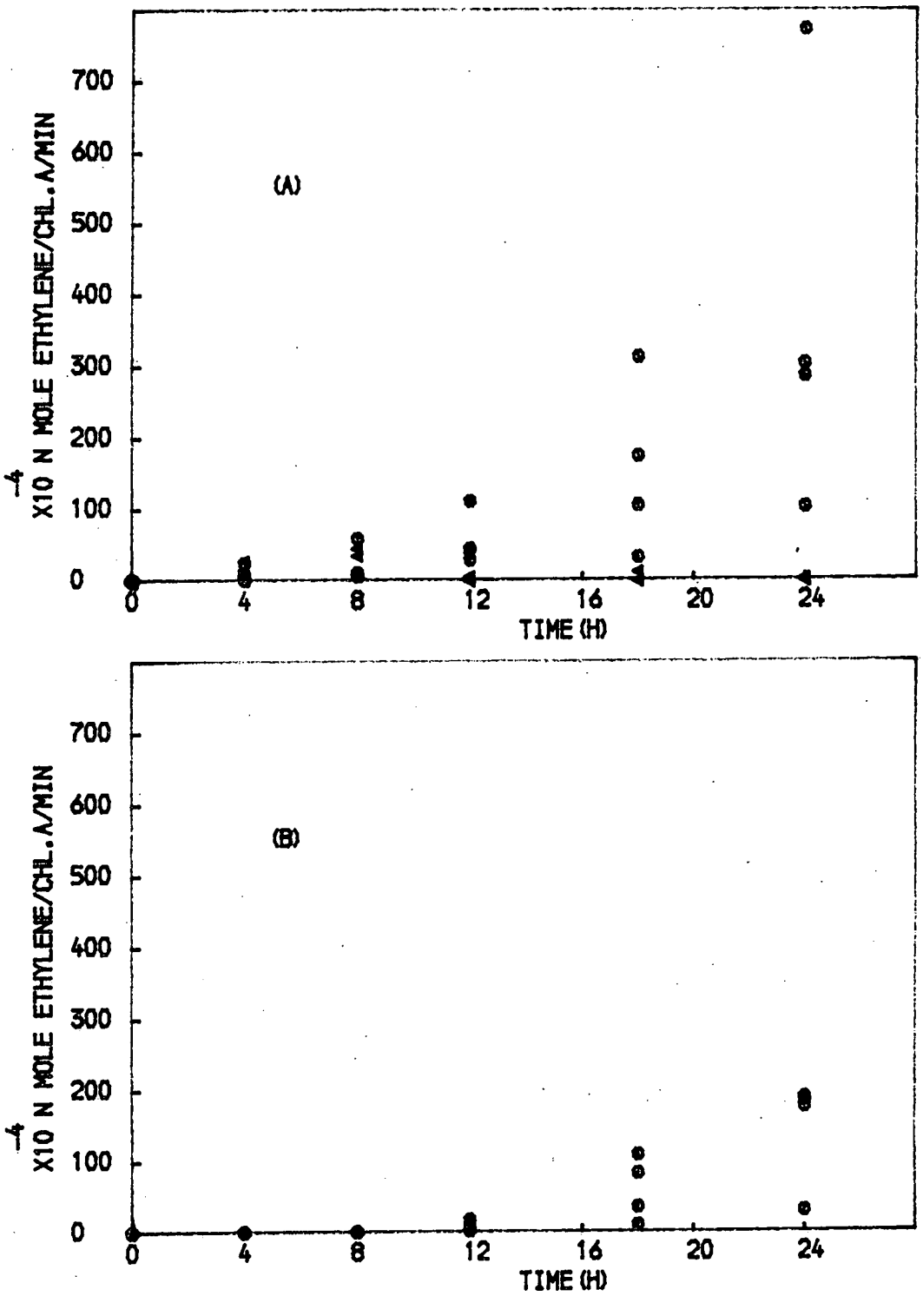


Fig.3.8 Time course of acetylene reduction by Tarn Moor N. commune. (A) Light-dried. ⊙ light-rewetted: △ dark-rewetted. (B) Dark-dried. Only light-rewetted samples are shown here, because no dark-rewetted samples gave any detectable rates.

(A)

TIME	LIGHT-DRIED (X 10^{-4} N MOLE ETHYLENE/CHL A/MIN.)							
(H)	LIGHT-WETTED				DARK-WETTED			
	S1	S2	S3	S4	S1	S2	S3	S4
00.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04.00	2.83	6.22	0.00	24.12	6.95	29.29	16.34	2.09
08.00	4.95	10.18	6.45	58.11	10.59	46.32	35.99	7.46
12.00	27.57	44.11	42.98	110.73	39.50	5.97	5.37	0.00
18.00	31.81	104.61	174.05	312.45	12.64	0.00	0.00	0.00
24.00	102.50	285.56	302.98	772.91	3.16	0.00	0.00	0.00

(B)

TIME	DARK-DRIED (X 10^{-4} N MOLE ETHYLENE/CHL A/MIN.)							
(H)	LIGHT-WETTED				DARK-WETTED			
	S1	S2	S3	S4	S1	S2	S3	S4
00.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
08.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.00	1.41	7.01	17.52	1.68	0.00	0.00	0.00	0.00
18.00	9.90	35.04	82.92	109.12	0.00	0.00	0.00	0.00
24.00	29.69	189.19	185.68	176.27	0.00	0.00	0.00	0.00

Table 3.8 Time course of acetylene reduction by Tarn Moor *N. commune*. (A) Light-dried colonies rewetted in light and dark. (B) Dark-dried colonies rewetetted in light and dark.

3.6 Time course of acetylene reduction when light-dried and dark-dried Tarn Moor N. commune are rewetted

Dark-dried and light-dried colonies were assayed in both light (5000 lx) and dark at 20°C at time intervals (Fig. 3.8) up to 24 hours after rewetting. Four samples of two to three colonies per sample were representatives of each condition.

Results showed clear distinction between samples dried in light and darkness. For light-dried samples, acetylene reduction commenced at the forth hour and progressively increased when rewetted under illumunation, but declined after sometime in those rewetted in darkness (Fig. 3.8).

Dark-dried samples rewetted in light commenced acetylene reduction at the twelth hour after rewetting and continued showing a progressive increase until the experiment was terminated. Dark-dried samples incubated in darkness showed undetectable rates of ethylene production throughout the experiment.

Although a wide range of rates of acetylene reduction was encountered in this experiment, quantitative comparisons were made between the rates for light-dried and dark-dried samples: the former was 0.0366 $\pm$ 0.0286 nMole C_2H_4 μg chl a^{-1} min^{-1} and was 2.5 times the latter (0.0145 $\pm$ 0.0072) at 24 hours after rewetting.

3.7 Time course of acetylene reduction by Tarn Moor N. commune rewetted in different humidity regimes and light condition

Algae were dried in different humidity chambers (section 2.4) giving a relative humidity of 75%, 50% and 10% under illumination of 3000 lx at 20°C for two weeks. Some were dried at 20°C in darkness under relative humidity regimes of 75% and 10%.

Dried algae were rewetted under both illumination of 5000 lx and darkness at 20°C. Four samples of two colonies per sample were representatives of each condition. Combinations of conditions are demonstrated in Fig. 2.1. All samples were assayed for acetylene reduction from time 0 at time intervals up to 84 hours.

Samples dried in darkness commenced acetylene reduction between 12-18 hours after rewetting under illumination for those which were kept in 75% relative humidity (D75l)(Fig. 3.10) and at or later than 48 hours for those which were kept in 10% relative humidity (D10l)(Fig. 3.10). No acetylene reduction commenced for dark-dried samples rewetted in darkness under both 75% and 10% humidity regimes (D75d & D10d) (Fig. 3.10) even at 84 hours after rewetting when the experiment was terminated.

Light-dried algae from all humidity regimes commenced acetylene reduction within 6 hours or shortly after 6 hours of being rewetted under illumination (L75l, L50l and L10l) (Fig.3.9). Light-dried algae rewetted in darkness under 75% relative humidity (L75d) and under 50% relative humidity (L50d) commenced acetylene reduction at or shortly after 6 hours and

the rate of reduction increased progressively until between 18-24 hours after rewetting, then declined (Fig. 3.9). Light-dried algae rewetted in darkness under 10% relative humidity (L10d) commenced acetylene reduction at irregular time from 6 up to 60 hours after rewetting (Fig. 3.9).

The data suggest that samples used in this experiment resemble 'type A.2'* hypothesis which means that humidity level at which samples are drying effects nitrogen fixation when being rewetted. The higher the humidity in which algae are dried, the sooner the algae will restart fixing nitrogen after rewetting. For algae dried in low humidity regime, if not because of the effect of humidity, detectable rate of acetylene reduction should have commenced earlier than it had been, or more or less the same time as those dried in higher humidity regimes.

Despite the fact that large variation within the rates of acetylene reduction did not allow parametric statistics to be made suitably for quantitative approaches, a mean rate at a given time was estimated for each group of samples and was used to compare with means from other groups. The mean rate for L751 samples was 0.0805 $\pm$ 0.0626 nMole C_2H_4 μg chl a^{-1} min^{-1} at 72 hours after rewetting, and was approximately two times the mean rate for L501 samples and five times that for L101 samples. D751 samples showed a mean rate of 0.0324 $\pm$ 0.0091 nMole C_2H_4 μg chl a^{-1} min^{-1} at 72 hours after rewetting while the mean rate for D101 samples was 0.0063 $\pm$ 0.0081 nMole C_2H_4 μg chl a^{-1} min^{-1} at the same stage.

* see section 4.3 page 68

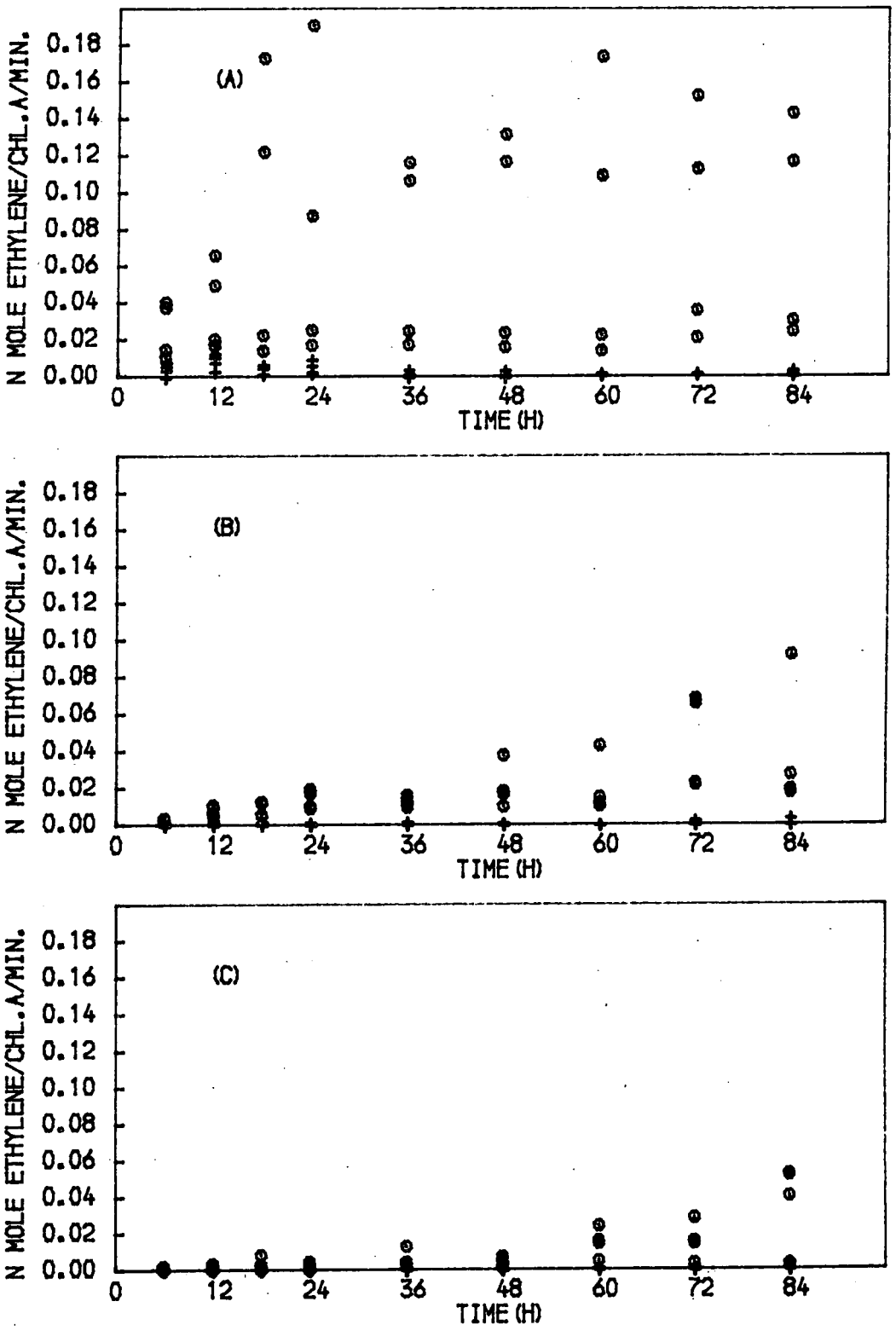


Fig.3.9 Time course of acetylene reduction by Tarn Moor N. commune. (A) Light-dried under 75% R.H. ⊙ 4 replicates of light-rewetted: + 4 replicates of dark-rewetted. (B) Light-dried under 50% R.H. ⊙ 4 replicates of light-rewetted: + 4 replicates of dark-rewetted. (C) Light-dried under 10% R.H. ⊙ 4 replicates of light-rewetted: + 4 replicates of dark-rewetted.

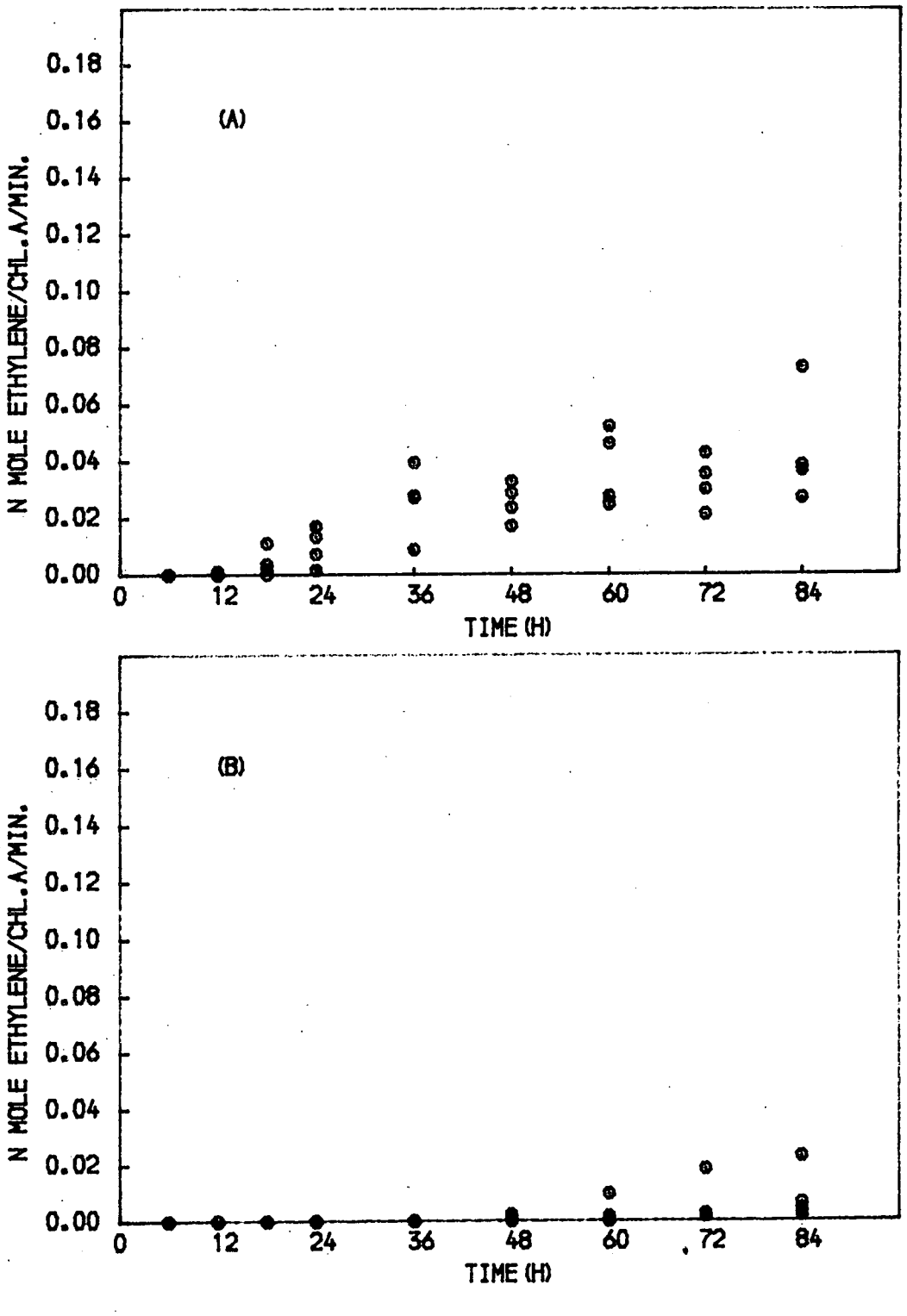


Fig.3.10 Time course of acetylene reduction by Tarn Moor N. commune. (A) Dark-dried under 75% R.H. Only light-rewetted replicates are shown here because no dark-rewetted samples gave any detectable rates. (B) Dark-dried under 10% R.H. Only light-rewetted replicates are shown here. No dark-rewetted samples gave any detectable rates.

3.8 Time course of acetylene reduction by Tarn Moor N. commune rewetted under illumination in different humidity regimes

This experiment was carried out to repeat experiment 3.7 data from which suggested that the humidity conditions under which algae are dried has an effect on nitrogen fixation during subsequent rewetting. It was decided to continue the experiment up to six hours in order to investigate the initial detectable acetylene reduction of representative samples of some conditions ie. L751, L501 and L101 (Fig. 2.1).

Four samples of two colonies per sample were representatives of each condition. The samples were rewetted at 20°C and 5000 lx.

Result has been shown that L751 and L501 algae commenced acetylene reduction at 4 hours and 5-6 hours sequentially after rewetting (Fig. 3.11). L101 samples showed no detectable rates of acetylene reduction throughout the period though the experiment was ultimately extended to 7 hours.

However, a sample from each group of L751 and L501 did not commence acetylene reduction throughout the incubation period. The reason for this is still not evident.

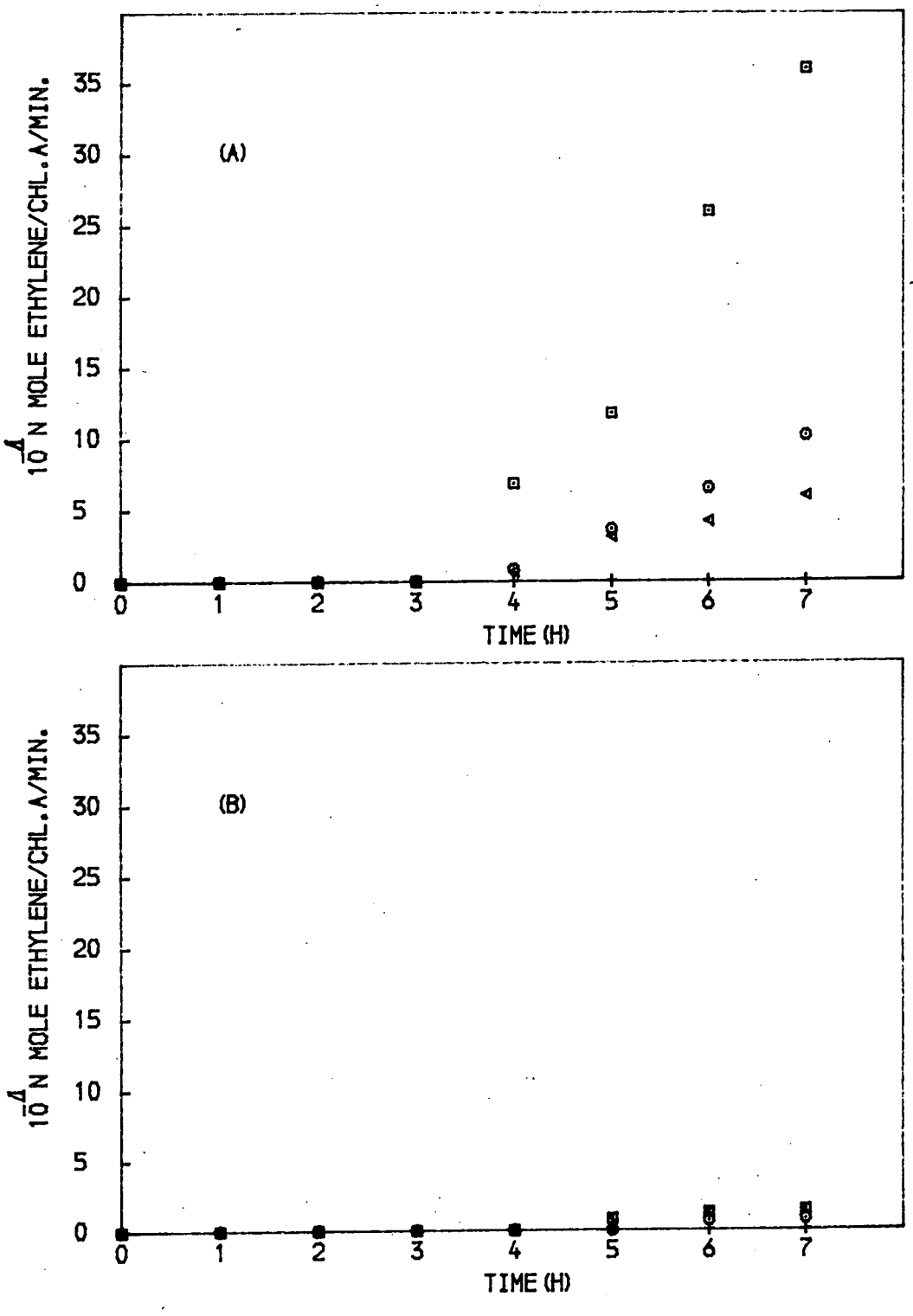
(A)

TIME	$X\ 10^{-4}$ N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
01.0000	0.0000	0.0000	0.0000	0.0000
02.0000	0.0000	0.0000	0.0000	0.0000
03.0000	0.0000	0.0000	0.0000	0.0000
04.0000	0.0000	0.6493	6.8383	0.8396
05.0000	0.0000	3.1166	11.7701	3.6271
06.0000	0.0000	4.1843	25.9717	6.4929
07.0000	0.0000	5.9364	35.9972	10.1711

(B)

TIME	$X\ 10^{-4}$ N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
01.0000	0.0000	0.0000	0.0000	0.0000
02.0000	0.0000	0.0000	0.0000	0.0000
03.0000	0.0000	0.0000	0.0000	0.0000
04.0000	0.0000	0.0000	0.0000	0.0000
05.0000	0.0000	0.8657	0.8288	0.0000
06.0000	0.0000	1.2024	1.2433	0.6625
07.0000	0.0000	1.4429	1.4209	0.7951

Table 3.9 Time course of acetylene reduction within the first seven hours after light-rewetting Tarn Moor *N. commune* light-dried under different humidity regimes. (a) 75% R.H.: (b) 50% R.H.



Symbols = ?

Fig.3.11 Time course of acetylene reduction by Tarn Moor N. commune within the first 7 hours after rewetting. (A) Light-dried under 75% R.H. and light-rewetted: (B) light-dried under 50% R.H. Light-rewetted. No dtectable rates were obtained from samples light-dried under 10% R.H. Rewetted in light.

CHAPTER 4

DISCUSSION

4.1 Difference in rates of water uptake by old and young colonies of Nostoc commune

Young colonies of N. commune initially took up water more rapidly than old colonies (Figs 3.1, 3.2). Such discrimination is likely to be dependent on colony texture in that old colony comprises of more non-living extracellular substance which appears to be dark brown in colour. The situation corresponds to what was found by Showman & Rudolph (1971) between the rate of moisture uptake of living lichen (Umbilicaria papulosa) and dead lichen. Any dry material is hydrophilic to some extent, and physical nature of the material seems to determine the rate of water uptake rather than does the metabolic processes of the living condition (Showman & Rudolph, 1971). The presence of the non-living extracellular substance could simply act as a barrier obstructing water from dispersing to the living algae. This can be observed when immersing colonies into the water, thick dark-brown colonies would remain insoluble for sometime and gradually absorb water afterwards. Pressing the immersed old colony with forceps seems to provide some capillary spaces allowing water to disperse to the sink quicker.

However, there are some common problems involved in the measurement of water uptake by dry algae as outlined below:

1) Dry colonies which are more compact may show a markedly slower rate of water uptake due to their less surface area for

absorbsion.

2) The removal of excess water before weighing the algae was always a snag. Blotting up the excess water with fine tissue paper, though handled carefully, could easily extract water from the colony.

3) At the beginning of the remoistening process when time intervals of removing the colony to weigh were small (seconds or minutes), there was a detention of about 15-20 seconds in blotting up the excess water. This could cause an overestimation in percentage of water uptake.

4.2 Effect of submerged condition on acetylene reduction

The amount of ethylene detected from sample bottles containing submerged colonies were certainly less than from the ones with non-submerged colonies (Table 3.4 & 3.5). It is more likely to be because of less actual acetylene reduced by the algae as a consequence of submerged effects. The presence of surrounding water could interfere with the diffusion of acetylene into the reaction sites. The effects of flooded condition on the gas exchange between the gas phase and algae, which restricting oxygen supply, dark respiration, transpiration and photosynthesis as described by Hardy et al. (1973) and Bergersen (1977), are rather related to long term incubation which was not the nature of this experiment.

In order to overcome the problem of underestimation of the rate of nitrogen fixation on remoistening, 3.0 ml of medium was used to rewet dried algae in a sample. It was found that the

liquid phase would be reduced to cover only a quater of the algae within 1 hour after rewetting. Experiments on the water uptake by dry algae indicated that within 15 minutes after rewetting alga had taken the liquid up to 80% of its saturation.

Such condition was assumed to provide adequate liquid for algae to take up until saturation was achieved, and at the same time having liquid phase small enough to avoid problems of gas diffusion and other possible submerged effects.

Samples containing 1/4-submerged algae was little different from those of non-submerged in rates of acetylene reduction. The mean rate found for samples assayed under 1/4-submerged is similar to the mean rate for non-submerged samples. Statistical tests of differences between two means gave computed values of 't' less than the tabulated value for p=0.1 (Table 3.6). The difference between the sample means is therefore non-significant. With larger numbers of replicates, a clearer non-significant difference of the means could be obtained.

4.3 Influence of storage conditions on the subsequent rate of nitrogen fixation by Nostoc commune

In order to distinguish the effects of light and humidity, it is possible to suggest hypotheses to explain acetylene reduction after algae are rewetted as shown in Fig.4.1.

Type A describes the situation where samples rewetted in the light show progressive increase in acetylene reduction and samples rewetted in darkness show initial increase in acetylene reduction followed by a decline.

A.1 If 'type A' samples commence acetylene reduction soon after rewetting, this may be because when they are dried algae have storage products to be used immediately after being rewetted and the amount of storage is enough to last until the algae is able to maintain the rate of nitrogen fixation by their anabolic process. In this case, humidity may or may not have influence on determining the recovery of algal activities when being rewetted.

A.2 If 'type A' samples commence acetylene reduction late after rewetting with a markedly timelag, it could be that humidity level, when algae are being dried, also have influence on determining nitrogen fixation when they are rewetted.

Type B describes the situation where samples rewetted in the light show progressive increase in acetylene reduction and samples rewetted in darkness show no detectable acetylene reduction. If this situation occurs, no conclusion can be convinced whether atmospheric humidity in which algae are dried has effect on determining the recovery of algal activities when rewetting.

'Type A' normally occurs when samples are dried in the light. 'Type B' often occurs when rewet the dark-dried samples. For algae dried in the dark, catabolic process exceeds assimilation and most storage is exploited. Thus, dark-dried algae are likely to have little storage or none.

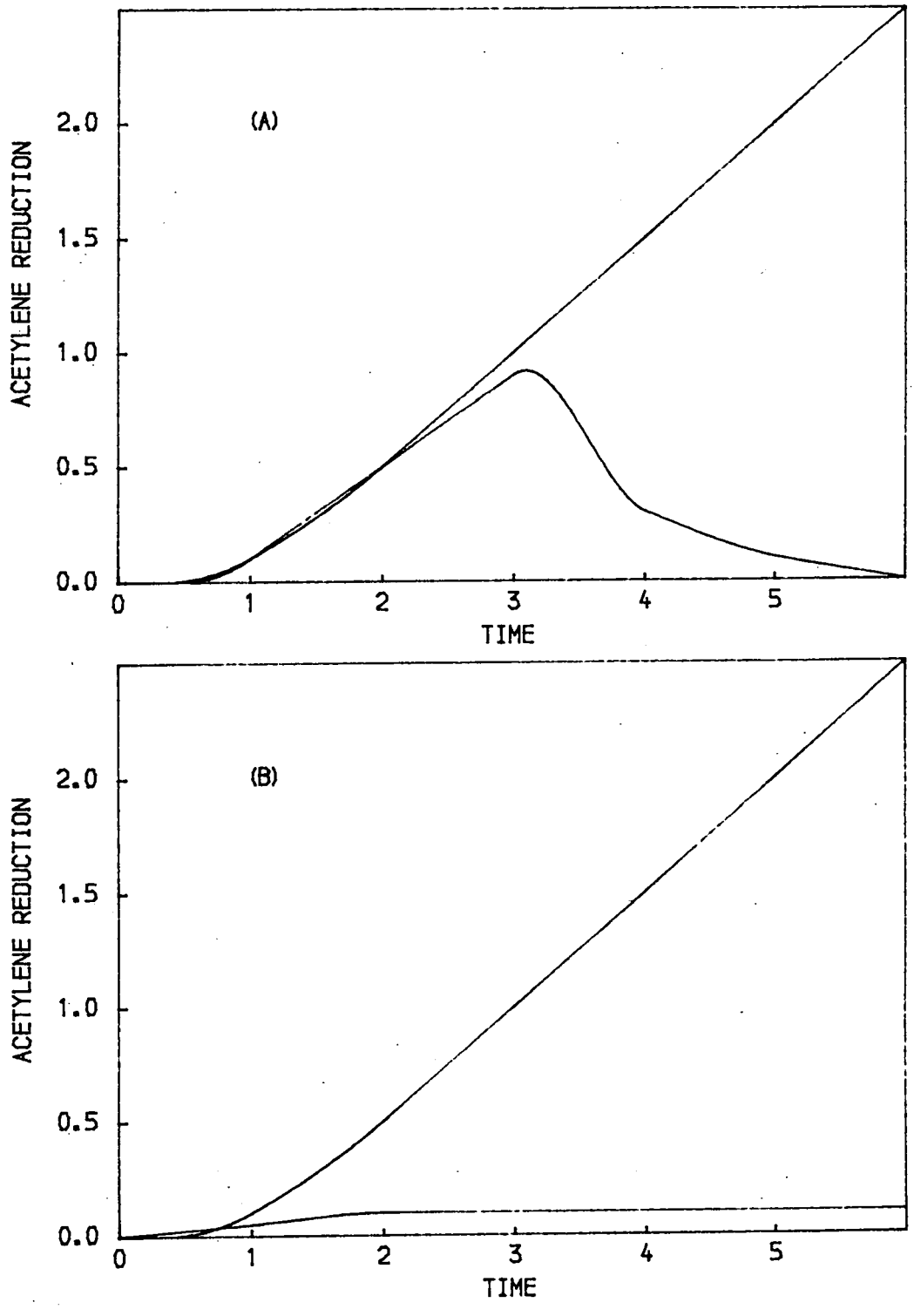


Fig.4.1 Hypothetical time course of acetylene reduction by rewetted N. commune. (A) 'type A' occurs when there are stored products preserved in dry colony. (B) 'type B' occurs when there are no stored products preserved in dry colony.

4.31 Importance of light, when drying Nostoc commune, on determining the recovery of nitrogen fixation after rewetting

As shown in section 3.4, dark control did not give detectable values of acetylene reduction. Therefore, the situation in section 3.5 is likely to fall in 'type B' hypothesis in that the timelags may or may not be due to the humidity effects. However, this was not convinced since it seemed that condition of algae from Station Quarry might be poor. Tests were made on acetylene reduction of fresh algae from the site and it was found that acetylene reduction commenced between 24-48 hours after incubation. Therefore, this could be the reason for the variations occurred.

As the consequence of experiment 3.5, it was thought that experiments using algae from other sites would reveal further information concerning the situation described above. Tarn Moor N. commune were then used as it was shown by Myers (1974) and Whitton et al. (1979) to have a sufficient rate of nitrogen fixation by restart reducing acetylene between 4-6 hours after remoistening.

Light is important both when drying and rewetting colonies of Nostoc commune. All light-dried Nostoc colonies commenced acetylene reduction sooner than the dark-dried ones after rewetting (Figs 3.8, 3.9, 3.10, 3.11). It is certain that the earlier commence of acetylene reduction in light-dried colonies is due to the availibility of stored products from photosynthesis preserved in dry algae. This is evident in light-dried samples rewetted in darkness in that the rate of

acetylene reduction initially increased and declined after sometime (Fig.3.8) presumably when all preserved products had been exploited. It was confirmed in dark-dried samples rewetted in darkness which never gave rise to any detectable rates of acetylene reduction (Fig.3.8).

General observations made from experiments suggest that the amount of preserved material is likely to correlate directly with the level of photosynthetic products before drying. The status of this preserved material in dry algae is also probably dependent on the condition of drying. A considerable amount of photosynthetic products could be all exploited by respiration when drying in darkness.

The mean rate of acetylene reduction for light-dried Tarn Moor Nostoc at 24 hours after rewetting was estimated to be 2.5 times the mean rate for those of dark-dried. Similar situation occurred when mean rates were compared between light-dried and dark-dried colonies under different humidity regimes. The mean rate for L75l samples was 2.5 times higher than that for D75l samles, and equivalently the mean rate for L1Ø1 samples was 2.5 times that for D1Ø1 samples. Regardless of the level of humidity under which algae were dried, these estimations indicate that algae dried under illumination, on rewetting had a higher rate of nitrogen fixation than those dried in darkness.

4.32 Effects of atmospheric humidity during the period for drying Nostoc on subsequent nitrogen fixation after rewetting

The observation that some dry colonies of N. commune, on

rewetting, gave earlier detectable rates of acetylene reduction corresponds to the levels of the atmospheric relative humidity in which they were dried: the higher the humidity, the sooner the detectable rates of acetylene reduction will be obtained after rewetting (Figs 3.6, 3.9, 3.10, 3.11). The condition of light under which colonies are dried seem to effect the lag by means of the availibility of storage preserved in dry algae (see section 4.31). As it was hypothesised in section 4.3, if it had been only light that effects the lags in acetylene reduction, and/or if humidity level had no influence on the lags, all samples should have commenced the reduction with the same or similar timelags. The comparison between samples dried in darkness under different humidity regimes (Fig. 3.10), is also evident for the explanation above in that samples dried in 10% relative humidity should have shown the same lags as the ones dried in 75% relative humidity, if there had been no humidity effects.

Although the variability of the material prevented accurate determinations to be made, it is possible to observe the effect of humidity on acetylene reduction quantitatively. Results from the experiments do not allow parametric statistics to be made suitable in the analyses due to the large variation in rates of acetylene reduction. However, quantitative discrimination between rates of acetylene reduction in samples from different relative humidities could be concluded roughly in terms of ranges. Colonies dried in higher levels of relative humidities, on rewetting, seemed to give higher rates than did samples dried

in lower humidities (Figs 3.7, 3.9, 3.10, 3.11). The estimation made as an attempt to demonstrate the differences in rates of acetylene reduction in Tarn Moor Nostoc at 72 hours after rewetting showed that the mean rate for L751 samples was five times higher than that for L101 samples, so the mean rate for D751 was five times that for D101 samples. This indicates that whether the algae are light-dried or dark-dried, the relation of the rate for samples dried under 75% R.H. to samples dried under 10% R.H. is five times greater.

It is evident that many species of Nostoc can succeed in recovering after long dry periods, the capacity to withstand desiccation and maintain their vitality is likely to vary in species and environments. N. commune from both Tarn Moor and Station Quarry are subject to wet climate, unusual low atmospheric humidity may have a considerable affect on algal activities particularly when remained in such humidity regime for a long period. This suggests a further study on the effects of different periods of drying and storage which would probably reveal a more certain conclusion on such humidity effects.

4.4 Variability of material

Despite the efforts made on selecting similar size and shape of colony to use in experiments in order to minimise variation, large variation in the rate of acetylene reduction occurred in most experiments particularly in quantitative terms. Such variation may be explained as following:

1) Individual colonies, though of similar sizes and shapes

looked at ?

are different in their textures. The same size of colonies may well have different proportions between the non-living extracellular substance and the actual living algae. Colonies which have more non-living substance showed slower rates of water uptake (section 4.1 and Figs 3.1, 3.2). Such detention could consequently give rise to a longer timelag in acetylene reduction by colonies of this type.

2) Because the non-living extracellular material is brown, it could reduce illumination to the algae. Different light intensities would allow different rates of nitrogen fixation, as shown by Miyamoto et al (1979). In addition, such substantial extracellular material may also alter the spectral composition which might interfere the effectiveness of light.

3) It is possible that the amount of chlorophyll a varies between communities. Shaded communities from the field may have more chlorophyll a than the ones exposed to more illumination. Such differences could unpredictably affect the estimation of nitrogen fixation.

SUMMARY

1) A study was made on nitrogen fixation by Nostoc commune taken from two sites in Northern England, Tarn Moor, Cumbria, and Station Quarry, North Yorks. The rate at which colonies fixed nirtrogen was measured using the acetylene reduction assay technique.

2) A standard method was devised for measuring water uptake by colonies. Marked variation in rates of water uptake occurred in dried colonies of Station Quarry, with younger colonies taking up water faster. Selected colonies from Tarn Moor were more of the same age, and were found to take up water at a similar rate to young colonies of Station Quarry Nostoc.

3) Light appeared to be one of the important factors influencing the rate of nitrogen fixation. Light-dried colonies commenced acetylene reduction sooner than dark-dried. The mean rate for light-dried colonies from Tarn Moor measured 24 hours after rewetting was 0.0366 $\pm$ 0.0286 nMole C_2H_4 μg chl a^{-1} min^{-1}; this was approximately 2.5 times those of dark-dried colonies from the same site (section 3.6). The rates of acetylene reduction in both light-wetted and dark-wetted colonies initially increased. The rates continued to increase for light-wetted colonies, but the rates for dark-wetted colonies decreased during 8-12 hours after rewetting (Fig.3.8).

4) Independent of the effect of light, colonies dried to equilibrium with a higher level of humidity commenced acetylene reduction sooner after rewetting. Colonies from Station Quarry

showed detectable rates after a lag of about one day, with longer lags occurring in colonies dried under lower humidities. The mean rate for L751 colonies was 0.0309 ± 0.0241 nMole C_2H_4 μg chl $\underline{a}^{-1}$ min^{-1} and was approximately 1.5 times the mean rate for L501 colonies and 2.5 times that for L101 colonies at 72 hours when all the rates had reached maximum equilibrium (section 3.5). Similar results were obtained from Tarn Moor Nostoc, but with shorter lags in comparison to Station Quarry Nostoc (section 3.7). L751 colonies showed detectable rates within 3-4 hours after rewetting, within 5-6 hours for L501 colonies, and after 7 hours for L101 colonies. The mean rate of ethylene production for L751 colonies was 0.0805 ± 0.0626 nMole C_2H_4 μg chl $\underline{a}^{-1}$ min^{-1} and was two times the mean rate for L501 colonies and five times that for L101 colonies at 72 hours after rewetting.

5) A methodological investigation was made on the effect of submerged condition on acetylene reduction, since the study involved immersing dried algae into a liquid phase. It was shown that acetylene reduction by algal samples incubated under submerged condition was significantly different ($p < 0.001$) from that of samples incubated under non-submerged condition (section 3.2).

6) To avoid such effects of submergence, a method was devised which depended on knowledge gained from the moisture uptake studies. It was found that after 1 hour of rewetting, having 1/4 of the algae submerged seemed to provide an adequate amount of liquid phase for further uptake, and to minimise the

submerged effects at the same time. The difference between the mean rates of acetylene reduction for non-submerged and 1/4-submerged algae were not found to be significant ($p > 0.1$)(section 3.3).

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APPENDIX

Following tables are data from the experiment on time course of acetylene reduction by Tarn Moor N. commune dried and rewetted under different humidities and illuminations.

(A)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0373	0.0103	0.0146	0.0401
12.0000	0.0492	0.0201	0.0167	0.0656
18.0000	0.1219	0.0223	0.0139	0.1727
24.0000	0.0875	0.0250	0.0169	0.1905
36.0000	0.1161	0.0245	0.0172	0.1064
48.0000	0.1314	0.0236	0.0159	0.1166
60.0000	0.1735	0.0223	0.0139	0.1090
72.0000	0.1524	0.0357	0.0210	0.1128
84.0000	0.1429	0.0303	0.0245	0.1166

(B)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0038	0.0082	0.0061
12.0000	0.0109	0.0032	0.0129	0.0080
18.0000	0.0051	0.0016	0.0067	0.0068
24.0000	0.0063	0.0028	0.0021	0.0097
36.0000	0.0014	0.0000	0.0015	0.0039
48.0000	0.0012	0.0000	0.0000	0.0036
60.0000	0.0021	0.0014	0.0014	0.0009
72.0000	0.0020	0.0019	0.0017	0.0011
84.0000	0.0037	0.0016	0.0028	0.0007

Table A1. Time course of acetylene reduction by Tarn Moor N. commune light-dried under 75% R.H. (A) Light-wetted: (B) Dark-wetted.

(A)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0020	0.0015	0.0032	0.0035
12.0000	0.0106	0.0061	0.0055	0.0097
18.0000	0.0119	0.0054	0.0053	0.0124
24.0000	0.0193	0.0089	0.0099	0.0171
36.0000	0.0160	0.0094	0.0134	0.0116
48.0000	0.0376	0.0167	0.0183	0.0099
60.0000	0.0428	0.0114	0.0102	0.0149
72.0000	0.0680	0.0656	0.0218	0.0226
84.0000	0.0918	0.0272	0.0176	0.0193

(B)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0014	0.0000	0.0000
12.0000	0.0000	0.0030	0.0000	0.0015
18.0000	0.0000	0.0016	0.0019	0.0012
24.0000	0.0000	0.0014	0.0000	0.0000
36.0000	0.0000	0.0009	0.0017	0.0000
48.0000	0.0000	0.0011	0.0000	0.0009
60.0000	0.0000	0.0000	0.0000	0.0000
72.0000	0.0000	0.0016	0.0025	0.0000
84.0000	0.0000	0.0000	0.0037	0.0000

Table A2. Time course of acetylene reduction by Tarn Moor *N. commune* light-dried under 50% R.H. (A) Light-wetted: (B) Dark-wetted.

(A)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0012	0.0020	0.0008	0.0000
12.0000	0.0010	0.0033	0.0015	0.0000
18.0000	0.0017	0.0082	0.0028	0.0000
24.0000	0.0015	0.0044	0.0026	0.0000
36.0000	0.0024	0.0128	0.0042	0.0020
48.0000	0.0033	0.0072	0.0047	0.0012
60.0000	0.0159	0.0241	0.0144	0.0048
72.0000	0.0143	0.0284	0.0158	0.0036
84.0000	0.0525	0.0519	0.0404	0.0029

(B)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0000	0.0000	0.0008
12.0000	0.0000	0.0000	0.0000	0.0010
18.0000	0.0000	0.0000	0.0000	0.0000
24.0000	0.0000	0.0000	0.0000	0.0000
36.0000	0.0000	0.0000	0.0000	0.0000
48.0000	0.0000	0.0000	0.0000	0.0000
60.0000	0.0012	0.0000	0.0011	0.0007
72.0000	0.0016	0.0000	0.0000	0.0000
84.0000	0.0040	0.0000	0.0000	0.0007

Table A3. Time course of acetylene reduction by Tarn Moor *N. commune* light-dried under 10% R.H. (A) Light-wetted: (B) Dark-wetted.

(A)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0000	0.0000	0.0000
12.0000	0.0000	0.0000	0.0011	0.0000
18.0000	0.0037	0.0019	0.0111	0.0000
24.0000	0.0134	0.0073	0.0170	0.0016
36.0000	0.0395	0.0271	0.0277	0.0088
48.0000	0.0328	0.0287	0.0236	0.0171
60.0000	0.0522	0.0462	0.0275	0.0246
72.0000	0.0428	0.0354	0.0300	0.0212
84.0000	0.0729	0.0366	0.0383	0.0269

(B)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0000	0.0000	0.0000
12.0000	0.0000	0.0000	0.0000	0.0000
18.0000	0.0000	0.0000	0.0000	0.0000
24.0000	0.0000	0.0000	0.0000	0.0000
36.0000	0.0000	0.0000	0.0000	0.0000
48.0000	0.0000	0.0000	0.0000	0.0000
60.0000	0.0000	0.0000	0.0000	0.0000
72.0000	0.0000	0.0000	0.0000	0.0000
84.0000	0.0000	0.0000	0.0007	0.0000

Table A4. Time course of acetylene reduction by Tarn Moor N. commune dark-dried under 75% R.H. (A) Light-wetted: (B) Dark-wetted.

(A)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
06.0000	0.0000	0.0000	0.0000	0.0000
12.0000	0.0000	0.0000	0.0000	0.0000
18.0000	0.0000	0.0000	0.0000	0.0000
24.0000	0.0000	0.0000	0.0000	0.0000
36.0000	0.0000	0.0000	0.0000	0.0000
48.0000	0.0024	0.0012	0.0000	0.0000
60.0000	0.0096	0.0017	0.0000	0.0000
72.0000	0.0184	0.0025	0.0016	0.0026
84.0000	0.0229	0.0064	0.0022	0.0040

(B)

TIME	N MOLE ETHYLENE/CHL.A/MINUTE			
(H)	S1	S2	S3	S4
00.0000	0.0000	0.0000	0.0000	0.0000
6.0000	0.0000	0.0000	0.0000	0.0000
12.0000	0.0000	0.0000	0.0000	0.0000
18.0000	0.0000	0.0000	0.0000	0.0000
24.0000	0.0000	0.0000	0.0000	0.0000
36.0000	0.0000	0.0000	0.0000	0.0000
48.0000	0.0000	0.0000	0.0000	0.0000
60.0000	0.0000	0.0000	0.0000	0.0000
72.0000	0.0000	0.0000	0.0000	0.0000
84.0000	0.0000	0.0000	0.0000	0.0000

Table A5. Time course of acetylene reduction by Tarn Moor N. commune dark-dried under 10% R.H. (A) Light-wetted: (B) Dark-wetted.