AFRICAN JOURNAL OF HEALTH & ENVIRONMENTAL SCIENCES, ENTREPRENEURSHIP, ENGINEERING & AGRICULTURE

EFFECTS OF pH, LIGHT AND TEMPERATURE ON NITROGEN FIXATION BY INTACT THALLUS AND EXCISED CEPHALODIA OF PELTIGERA APHTHOSA

NGEREBARA, N. N.
Department of Science Laboratory Technology, School of Applied Sciences,
Kenule Beeson Saro-Wiwa Polytechnic;
P.M.B. 20, Bori.

GBOSIDOM, L. V.
Department of Science Laboratory Technology, School of Applied Sciences,
Kenule Beeson Saro-Wiwa Polytechnic;
P.M.B. 20, Bori.

And

AMADI, L.O.
Department of Microbiology, Faculty of Science,
Rivers State University, P.M.B.5080, Nkpolu-Oroworukwo,
Port Harcourt, Rivers State,
Nigeria.

Article history:

Received: FEB 2022;
Received in revised form:
2 FEB 2022;
Accepted: 2 MARCH 2022;

Keywords: Intact thallus, excised cephalodia, nitrogen fixation, pH, temperature, light.

Abstract

The effects of pH, light and temperature on acetylene reduction by the lichen (*Peltigera aphthosa)* was investigated. Intact thallus and excised cephalodia of *Peltigera aphthosa* were incubated at various ranges of pH, temperatures and light intensities, and a comparative nitrogen fixation by the intact thallus and excised cephalodia was used as the method for assessment. Both the intact thallus and cephalodia were incubated at temperature range of 10 $^{\circ}$C to 30 $^{\circ}$C, pH range of 4 to 8, and light intensities as low as 220 lux to a maximum of 11000 lux for 48 hours. Optimum nitrogenize activity was obtained at pH 5 and 7, 4000 lux and 20 $^{\circ}$C for intact lichen material. However, for exercised cephalodia, incubation in the light at various levels over a 48-hour period, the optimum nitrogenase activity was inhibited, showing fairly broad in the range of 5-7, peaking slightly at neutral pH and declining markedly at pH 4 and 8. The study showed that separating the cephalodia from the main thallus lowered nitrogenase activity of the lichen. Obviously, the main thallus affects the cephalodial activity and the cause might be due to: (1) an accumulation of inhibiting nitrogenous compounds in

the nostoc cells on separation from the main sink (medulla), (2) some stimulating effect(s) on the activities of the cyanophyte excreted by any of the main thallus partners is or are lost on separation, and (3) the metabolic activity of the cephalodal fungus may be lowered in the absence of the major sources of photosynthate, indirectly affecting the cyanophyte.

Introduction

Knowledge of nitrogen fixation by blue-green algae has increased substantially within resent years as (1), new groups of nitrogen-fixing algae have been discovered (2), as detailed physiological and biochemical studies on the inter-relations of metabolic processes such as nitrogen-fixation, photosynthesis and respiration have been carried out and (3), as the ecological importance of the group has been recognized and confirmed (White and Silver,2001; Adejumo *et al*.2005; Stephen and Linus, 2002; Ralph and Sylvanus, 2003; Felix and Indubuisi, 2004; Finebone and Kate 2005 and Cornilius and Cornell, 2006). In general, the fixation rates are considered to be rapid.

The ecological significance of nitrogen fixation by lichen has only recently been paid greater attention, especially in the nitrogen deficient arctic tundra where these organisms are very abundant. At the tundra, nitrogen fixation by *Stereocaulon* and *Peltigera* species are considered to be of particular importance in the nitrogen budget (Bone and Bar, 2000 and Alexander *et al.*; 2002).

These associations are widespread on glacial drift on Iceland where they vigorously fix nitrogen (Christopher, 2003). Moreover, in northern Finland, *Nephronma, Solorina* and *Stereocaulon* species are the principal N_2 fixing lichens (Cain *et al*; 2006, Kamalu and Kamalu, 2008). In parts of the subarctic tundra of Sweden species of *Nephroma*, *Peltigera* and *Stereocaulon* cover the ground completely (Soibi, 2007).

This study presents data on the effects of abiotic factors: pH, light and temperature on acetylene reduction rates by *Peltigera aphthosa* wild.

Materials and Methods

The lichen *Peltigera aphthosa* was collected from wet and steep Isiama forest dominated by mosses, ferns and grasses. The lichen materials were kept in glass vials covered with transparent plastic foil to prevent desiccation prior to assay; all adhering materials were carefully removed with forceps and the lichen thalli rinsed in distilled water. In all the tests lichen discs with or without cephalodia or excised cephalodia were used. Lichen discs were punctured from the thallus lobes (9,14 or 16mm) and cephalodia were dissected off with two sharp-edged needles under a low-power binocular microscope.

The method used to determine acetylene reduction in algal cells was that of Steward as was modified by Edward (2001). However, the gas phase was not removed prior to the injection of acetylene nor was the reaction terminated by addiction of trichloroacetic acid. Thallus discs and excised cephalodia were placed in glass serum bottles with a capacity of 7 or 27 ml and fitted with serum liners. The test materials were exposed to an atmosphere containing 10% acetylene in air for 30 to 60 minutes. For the detection of ethylene formation gas, samples were taken and injected into a Varian-Aerograph model 1200-1gas chromatograph.

For pH, light and temperature assessment, both intact lichen thallus and

excise cephalodia of *Peltigera aphthosa* were incubated for 48hrs in ASM medium with Hepe's buffer added ($0.5g^{1-1}$) at 20°C and 8000 lux. The pH values were achieved by adding 0.1m HCL or 0.1m Na0H to the medium.

Results and Discussion

The results of acetylene reduction by intact lichen discs, isolated cephalodia and lichen discs is presented in Figure1. The effect of pH on acetylene reduction by intact lichen thallus and excised cephalodia of *Peltigera aphthosa* is presented in Figure 2. The effect of light intensity on acetylene reduction by intact lichen thallus and excised cephalodia of *Peltigera aphthosa* is presented in Figure 3 and Figure 4. The effect of temperature on acetylene reduction by intact lichen thallus and excised cephalodia of *Peltigera aphthosa* is presented in Figure 5.

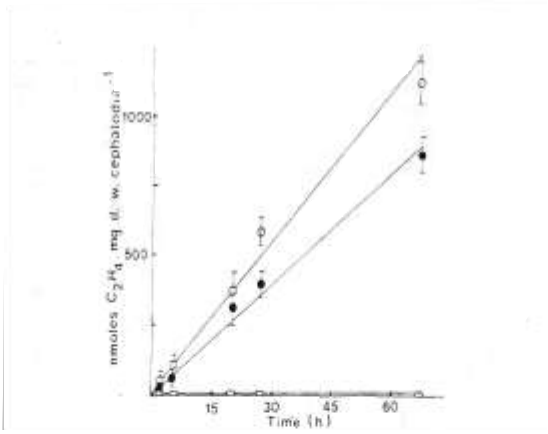

Figure 1. Acetylene reduction by intact lichen discs (O). isolated cephalodia ( ) and lichen discs with cephalodia detached ( ) of *Peltigera aphthosa*. Light intensity was 8000 lux and temperature 20°C. Each point is the mean of triplicate determinations. Bars indicate ± s.e.

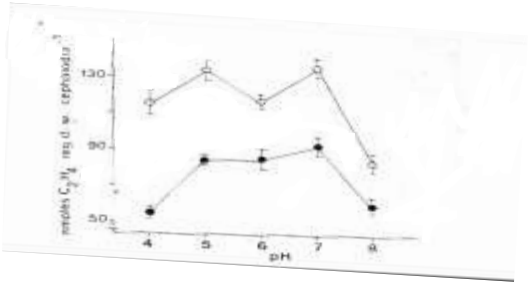

Figure 2. The effect of pH on acetylene reduction by intact lichen thallus (O) and excised cephalodia (■) of *Peltigera aphthosa.* Bars indicate ± s.e.

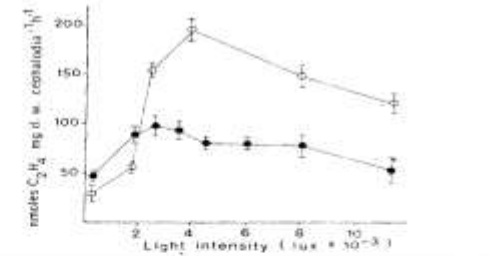

Figure 3. The effect of light intensity on acetylene by intact lichen thallus (O) and excised cephalodia (■) of Peltigera aphthosa. Bars indicate ± s.e.

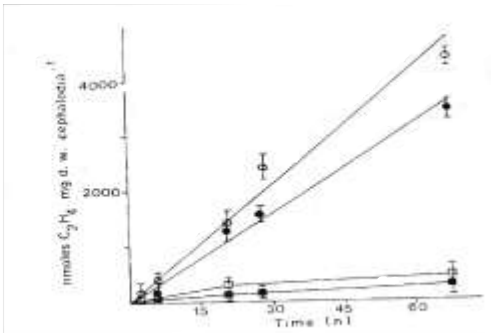

Figure 4. The influence of light (8000 lux: round symbols) and dark (square symbols) on acetylene reduction by intact lichen thallus (O.) and excised cephalodia (■). Each point is the mean of triplicate determinations. Bars indicate ± s.e.

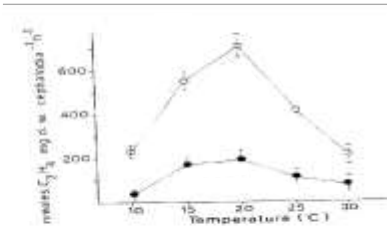

Figure 5. The effect of temperature on acetylene reduction by intact lichen thallus (O) and excised cephalodia (●) of Peltigera aphthosa. The material was incubated for 48 hours at 8000 lux prior to the assay. Each point is the mean of triplicate determinations. Bars indicate ± s.e.

Discussion

The ability of *Peltigera aphthosa* to reduce acetylene was checked in preliminary experiments. From Figure 1, the reduction of acetylene of intact thallus occurs at a linear rate at least over a 68-hours period. Reduction of acetylene by the separated cephalodia and absence of such activity in the lichen thallus without cephalodia indicates that the blue-green phycobiont is the site of nitrogen fixation, which is in agreement with experiment using ^{15}N (Meshack and Mene, 2001). The preliminary experiments also showed that the optimum light intensity for laboratory incubation was 8000 lux, and this intensity was used for all subsequent experiments unless otherwise stated.

The response of excised cephalodia and intact lichen discs to various pH values is presented in Figure 2. When excised cephalodia was incubated in the light at various pH levels over a 48-hour period, the optimum is fairly broad in the range of 5 to 7, peaking slightly at neutral pH and declining markedly at pH 4 and pH 8. This optimum at pH 7 is fairly typical for blue-green algae (Stephen and Peace 2004).

The results using intact discs on the other hand shows less inhibition at pH 4 and pH 8 but, interestingly, a peak of optimum activity at pH 5 as well as pH 7. Such results suggest, a close inter-relationship between all partners in the symbiosis, and that the lower pH values which are optimum for the metabolism of the eukaryotes stimulate nitrogenase activity by the cyanophyte. We assumed this because of the results of Boye and Boyld (2006) that a pH of 4 to 5 supported optimum growth of the isolated mycobiont of *Peltigera aphthosa* and the results of Stephen and Peace, (2004) that pH of 5.6 to 7.4 supported optimum growth of the isolated *Collema tenax* mycobiont. Similarly, the reduced activity of the isolated cephalodia compared with the intact discs may be a reflection of the requirement of the heterotroph of optimum activity of the cyanophyte.

Figure 3 illustrates that the separation of cephalodia from main thallus decreases the rates of acetylene reduction. In addition, Figure 3 shows that low light intensity reduces the reduction rates considerably both for intact lichen discs and excised cephalodia. However, acetylene reduction still occurs at 220 lux, the lowest light intensity tested. Optimum activity

occurs at 3000-4000 lux and a further increase in light intensity up to 11000 lux gives a comparatively slow decline of the reduction rates for both intact materials and cephalodia. The differences noted presumably reflect different light demands of the two phycobiont, with the blue-green phycobiont being light-saturated at a somewhat lower light intensity than the green phycobionts.

The light demand for Peltigera aphthosa is lower than that reported for Stereocaulon species (Kelvin and Miller, 2002, West and Benjamin, 2003) and Lichina (Henshaw et al.,2000), showing increase in the nitrogen fixation activity up to around 20000 lux. The considerably lower maximum activity of 4000 lux for Peltigera aphthosa, in keeping with values reported for Peltigera rufescence (Stephen and Linus 2002) is probably due to the fact that in nature Peltigera aphthosa usually appears on north-facing, shaded walls where light intensities are comparatively low even on sunny days.

A comparison of the acetylene reduction activity by the Peltigera aphthosa material incubated in the dark and at 8000 lux is given in Figure 4. In the dark, acetylene reduction occurs at a reduced level, the activity being about 10% of that in the light after 68 hours of incubation. The finding of a lowered fixation activity in the dark agrees with results obtained for free-living symbiotic blue-green algae (Stephen and Linus 2002 and Henshaw et al., (2000).
The optimum temperature for acetylene reduction by Peltigera aphthosa is obtained at 20°C for both intact lichen discs and excised cephalodia.

The temperature limits of this experiment 10 °C and 30°C reduces the algal activity considerably. After prolonged incubation (3 weeks) of lichen thalli at 25 °C and 30°C the symbiosis seems to break down while samples kept at 10 °C and 20°C still appear healthy

References

Adejumo, T.O.; Nally, N.M.; Kate, Q.V; and Nketeli; O.J. (2005). Nitrogen metabolism in lichen VI. The blue-green phycobiont contents heterocyst frequency and nitrogenase activity in *Peltigera spp-New Phytol*. 74:473-476.

Alexander, V.C.; Billy, G.D.; and Schollar D.M. (2002). The influence of abiotic factors on nitrogen fixation rates in the Barrow, Alaska tundra. *Res*. Stat. 11:3-10.

Boye V.D. and Boyld. B.J. (2006). Nitrogen turnover in marine and brackish habitats. III. The production of extracellular nitrogen by *Calothrix scopulorum*. *J. mar. Biol*. 49:475-488.

Bone, L.K and Barr. G.D (2000). An examination of some symbiotic system for fixation of nitrogen-*Ann. Bot*. 19:67-77.

Cornillus, R.C and Cornell, P.C. (2006). Nitrogen fixation by lichens on glacial drift in Iceland-*New Phytol*. 74:41-49.

Christopher, S.W. (2003). Nitrogen fixation by lichen on glacial drift in Iceland. *New Phytol*. 64:41-50.

Cain, N.T.; Edmund, R.T.; Helen. M.B and Joseph W.O. (2006). Nitrogen fixation in Swedish soils by blue

green algae. *New Phytol*, 72:504-601.

Edward, Y.O. (2001). In situ measurement of nitrogen fixation at low temperatures. Oikos. 25; 283-287

Fine bone, O.G and Kate, N.M (2005) canopy lichens with blue green algae: A Nitrogen source in a Columbian rain forest. *Ecology* 56:1176-1184

Felix, A.G and Indubuisi, W.J. (2004). Nitrogen assimilation and metabolism in blue-green algae J. *Bacteriol*. 120:235-249.

Henshaw, H.J.; Kalio, A.T.; Monday, G.D and John, T.J (2000). Nitrogen turnover in marine and brackish habitats. *J. Mar. Biol*. 49:475-488

Kamalu, K.I and Kamalu, G.D (2008). Nitrogen fixation by *Stereocaullon paschate* under field conditions. *Can. J. Bot* 55: 582-590.

Kelvin, S.O. and Miller, M.U. (2002). Nitrogen fixing in by lichens in Scotland. *New. Phytol*. 72.

Meshack, M.M. and Mene, B.O (2001). Stuches in the physiology of the lichen *Collema Physiol. Plant*. 10:947-950.

Ralph. C.T and Silvanus, P.V. (2003). Nirogenase activity and photosynthesis in Plectonema boryanum. *J. Bacteriol.* 119:255-265

Stephen, O.V and Linus, O.R (2002) Nitrogen fixation in soils of Truelove low land. Northwest territories- *Can. J. Bot* 53:1387-1399

Stephen, S.L. and Peace, K.J (2004). Nitrogen metabolism in lichens 1. Nitrogen fixation in the Cephalodia of *Peltigera aphthosa- New Phytol.* 68:721-729.

Soibi, G.O. (2007). Nitrogen fixation by free-symbiotic *Nostoc* strain Isolated from *Collema*. *Physiol. Plant* 4:540-545.

West, S.L. and Benjamin, J.V. (2003). Saturated solutions for the control of humidity in biological research. *Ecology* 41:232-237.

White, P.W and Silver, P.H. (2001). Nitrogen fixation by free-living microorganisms. Cambridge University press, 471 pp.