A LABORATORY BIOASSAY TOOL TO QUANTIFY THE PERIPHYTIC RESPONSE TO STRESS

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ABSTRACT: A laboratory bioassay tool to quantify the periphytic response to stress. A bioassay methodology is described for the routine monitoring of contaminated waters. The authors propose interpreting the photosynthetic/respiration response of periphytic algae colonizing plastic tube substrata, as a measure of stress. The procedure is based on determining the dissolved oxygen differential between the inflow-outflow ends of a length of tubing through which water is flowing at a known rate. The method yields consistent reproducible data and is unexpensive in terms of time consumed and laboratory resources.

Key-words: periphytic algae, bioassay, photosynthesis, productivity.

RESUMO: Um bioensaio em laboratório para quantificar a resposta do perifiton ao estresse. O presente trabalho descreve uma metodologia para monitoramento de rotina de águas contaminadas, através de bioensaios. Os autores propõem a interpretação de respostas do metabolismo fotossintético e respiratório de algas perifíticas, colonizadas em tubos plásticos transparente, como uma medida de estresse. O procedimento é baseado na determinação do balanço de oxigênio dissolvido entre a entrada e a saída do tubo colonizado, com um fluxo constante de água a uma velocidade conhecida. O método é econômico em termos de tempo e recursos laboratoriais e gera dados consistentes e reproduzíveis.

Palavras-chave: algas perifíticas, bioensaio, fotossíntese, produtividade.

INTRODUCTION

In those instances when algae were included in the toxicological evaluation of a xenobiotic, planktonic forms were generally utilized. Growth was inferred from chlorophyll ***a*** and pheophytin determinations. Less frequently DNA and ATP estimates were utilized to estimate biomass. Some question remains, however, whether phytotoxic effects are faithfully reflected by growth rate or standing crop (Lewis, 1995). Photosynthesis and respiration, on

the other hand, are considered by many researchers as the two most responsive indices of sublethal toxicity (Odum, 1977).

While these indices of stress have been successfully applied in the field as monitoring tools (Tease *et al.*, 1983; Tease & Coler, 1984) they have not made the transition to the laboratory as a standardized methodology. Accordingly, a laboratory protocol is described here for the quantification of the periphytic response to stress as indicated by community periphytic algae. Essentially, the method is predicated on determining the differences in DO concentrations between the two ends of a colonized transparent tube, by respiration and photosynthesis. Water is permitted to flow through at a selected rate and a series of determinations are made during light and dark regimes.

This procedure facilitates generation of effective concentrations (EC50's), maximum allowable toxicant concentration (MATC) and incipient limiting concentrations (ILC's), as well as extrapolation to ecological impacts and identifying limiting nutrients. In this context trends in function characteristics of stressed ecosystems could be identified as well, i. e., increases in community respiration, imbalance of P/R and P/B ratios, increase of nutrient loss. Further, the investigator need only excise a segment of the tubing and scrape off adnate material to determine diversity, chlorophyll and biomass. Reproducible data can thus be consistently generated at only a fraction of the cost in time and laboratory resources of traditional methods. To furnish a laboratory with this capacity is required only: a source of unpolluted (control) water, suitable lighting and supplies common to any wet laboratory.

METHODOLOGY

To implement this procedure, the following steps are outlined. The reader is cautioned not to view the text as a refined procedure. Colonization time, flow rate, illumination, tubing dimensions all require "fine tuning" to maximize oxygen differentials. What is offered here is a prototype that works.

1. Colonization

Deploy tubing lengths (2.5 m x 0.9 cm I.D.) for approximately a month in a shallow stream at a depth of 15-25 cm. Alternatively, colonization with a specific species could be implemented in the laboratory by shunting inoculated culture water back and forth (5-10 $ml.min^{-1}$) between two 25 l carboys for 2-3 weeks. The tubing (4 replicates) is flanked throughout its length by two banks of 40 W (2/bank), cool white fluorescent lamps, situated 20 cm apart. The lamps are left on continuously excepting those instances when the investigator wishes to determine respiration rates.

2. Characterization of Performance

After 2-3 weeks daily monitor dissolved oxygen (DO) concentrations at inflow and outflow were periodically monitored. When the effluent demonstrates a consistently measureable increase of at least 0.3 $mg.l^{-1}$ of oxygen over the influent, colonization has progressed sufficiently to transfer tubes to the control water source. Because the inflow remains constant (unless exposed to sunlight) each outflow determination need not be accompanied by an inflow analysis: four evenly spaced analyses throughout 8 hours of illumination per day, suffice. The authors used tap water allowed to degas in ten 1000 l tanks. Delivery was by gravity feed via PVC tubing. Gross and net primary productivity (outflow-inflow

DO concentrations) generated by a square meter of surface area over an hour's flow were determined. For instance a flow of 30 $ml.min^{-1}$, a 2.5 m length of tubing with a 0,9 cm I.D. has: a surface area of 0.707 m^2, a flow rate of 79 $cm.s^{-1}$ and a time of travel of 5.27 min. As in the present instance titrations should be continued until an acceptable coefficient of variation (C.V.) in DO values is achieved (Table I). Values less than 5% were generally achieved.

3. Administration of Xenobiotic or Nutrient

Upon having accumulated a data base to statistically characterize tube productivity (One Way ANOVA), the investigator may then administer the test factor. We are presently employing the hospital serum/blood infusion apparatus (calibrating flow rate with drops/min) to control flow. The xenobiotic is injected (by gravity feed) directly into the tubing at its source. Assuming a 1l reservoir (situated above the water source) and a minimally reliable controlled flow rate of 1.0 ml/min^{-1} introduced into a 30 $mlmin^{-1}$ stream, the unit could provide a 30 fold dilution for 16.5 hours. Flow rate of a gravity feed set-up must be monitored at hourly intervals because it tends to decrease with time.

RESULTS AND DISCUSSION

The data (Table I) indicate that a flow rate of 30 $ml.min^{-1}$ generated the highest rate of productivity. All flow rates less than 30 $ml.min^{-1}$ did not generate a positive net productivity. At 20 $ml.min^{-1}$ the total gross productivity was diverted to respiration. Because the four tubes were in place for over three months, the colonizing population (almost exclusively cyanophytes: i.e. *Oscillatoria, Anabaena*) had formed congesting mucoid plugs. Upon their removal the productivity of the tubes was greatly diminished making an anticipated 40 $ml.min^{-1}$ tests invalid. This is unfortunate because a review of the literature strongly suggests optimum productivity between 40 and 50 $cm.s^{-1}$. Hynes (1970) reported *Oedogonium kurzii* increased the rate of ^{32}P uptake with increasing current speed *of at least up to 40* $cm.s^{-1}$. Further Welch (1980) noted that the significant enhanced effect of velocity *up to about 45* $cm.s^{-1}$ *is due to the increased rate of diffusion at the plant surface*. Horner stated that the maximum velocity above which loss by erosion becomes greater than accumulation through growth "*is probably around 50* $cm.s^{-1}$" (Welch, 1980). The optimum would therefore be about 50 times our flow rates. This is not, however, to be interpreted as that flow producing the maximum DO differential between inflow and outflow.

Table I – Productivity of periphyton colonized tubes (average + SD) at indicated flow rates, (n=4). C.V.=coefficient of variation.

Flux		**Inflow (mg/l)**	**C.V.**	**Outflow (mg/l)**				**Net Prim. Prod.**	**Respiration**	**Gross Prim. Prod.**
$ml.min^{-1}$	$cm.s^{-1}$			**LON***	**C.V.%**	**LOFF****	**C.V.%**	$mgO_2.h^{-1}.m^{-2}$	$mgO_2.h^{-1}.m^{-2}$	$mg\ O_2h^{-1}/m^2$
5	.13	7.68±0.141	0.02	6.31±0.400	0.06	-	-			
10	.26			7.12±0.220	0.03	-	-			
15	.39			6.98±0.200	0.01	-	-			
20	.52			7.68±0.082	0.01	7.31±0.105	0.01	0	0.625	0.625
30	.79			8.37±0.085	0.01	7.32±0.054	0.01	1.75	0.91	2.7

*LON = fluorescent lamps on
**LOFF = fluorescent lamps off

While it is clearly unacceptable to extrapolate from colonized tubing to laboratory streams, much less to natural streams, some limited value may be derived from putting the data (Table I) in perspective. Hammer reported a gross productivity of 0.1 $gC.m^{-2}.day^{-1}$ in the Rio Negro (Hynes, 1970). This refers to an order of magnitude much greater than ours. Our comparatively low productivity may be attributed to oligotrophic nutrient levels (Watanabe *et al.*, 1989). Phinney & McIntyre (1965) observed a maximum net production of oxygen in a laboratory stream of 439 mg $m^{-2}h^{-1}$; almost 300 times greater than tube productivity.

Thus the comparatively low productivity generated in the tubes is probably largely a consequence of current. Other contributing factors could be light inhibition (not measured), low nutrient levels and the dense build-up of biomass which would inhibit photosynthesis and increase respiration. While it is essential to maximize oxygen generation to identify deviations from the norm, it is not meaningful, to strive for natural levels of productivity.

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