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Influence of Algal Photosynthesis on Biofilm Bacterial Production and Associated Glucosidase and Xylosidase Activities

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ABSTRACT

Natural photosynthetic biofilms were incubated under light (100 μmol m^{-2} s^{-1}) and dark conditions to elucidate the impact of photosynthesis on bacterial production, abundance, biovolume, biomass, and enzyme activities over 24 h. Use of organic carbon-free media limited carbon sources to algal photosynthesis and possibly the polysaccharides of the biofilm matrix. Bacterial production of biofilm communities was significantly higher in light incubations ($p < 0.001$). The greatest differences in production rates between light and dark incubations occurred between 8 and 24 h. Biomass-specific α- and β-glucosidase and β-xylosidase activities were stimulated by photosynthesis, with significantly greater activities occurring at hours 16 and 24 in the light treatment ($p < 0.01$). The results indicate that algal photosynthesis can have a significant impact on bacterial productivity, biomass, biovolume, and enzyme production over longer time periods at low photon flux densities (≤100 μmol m^{-2} s^{-1}).

The decomposition of terrestrial and aquatic plants results in extensive loading of particulate and dissolved organic matter into recipient aquatic ecosystems. Dissolved organic carbon (DOC) is the dominant component of the organic matter pool transported into aquatic ecosystems [40, 41], of which 70–90% is in the form of recalcitrant, polyphenolic humic substances [25]. Labile DOC, comprising 10–30% of the dissolved organic pool [25], is rapidly removed by microbial metabolism or adsorption onto inorganic particles [40]. The selective removal of labile DOC results in an accumulation of recalcitrant humic compounds, that can remain in the dissolved form for extensive periods of time. The availability of dissolved humic compounds for heterotrophic metabolism within attached microbiota (hereafter referred to as biofilm) is dependent on mechanisms that facilitate the transport of carbon substrates to the bacteria. The inability of high-molecular-weight humic compounds to penetrate cell membranes indicates that the acquisition of external carbon substrates is largely hydrolytic, resulting in enzyme-mediated release of small, labile substrates from complex molecules (cf. [5]). Hydrolytic reactions can occur within

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the biofilm by surface-bound enzymes or in the bulk water internal and external to the biofilm via cell-free extracellular enzymes (cf. [8, 18, 24, 30, 38]).

Complementary to dissolved humic substances in the water column, heterotrophs can utilize sources of organic carbon within the biofilm. Release of extracellular organic carbon (EOC) from active, senescent, or lysed algal cells can be an important source of carbon for bacteria [2, 19]. Bacterial metabolism of algal EOC occurs in planktonic [1, 7, 16, 34, 35] and biofilm communities [26, 27]. In biofilms, bacteria are in close juxtaposition to algae and rapidly utilize substances released from algal cells. This interaction between bacteria and algae likely results in an intense internal recycling of nutrients, including carbon, within biofilms [39, 40]. The algal-derived EOC, however, is likely not produced in sufficient quantities to be a major source of carbon for planktonic bacterial growth [6, 7]; the relative importance of algal EOC on bacterial growth within biofilm communities is unknown, particularly over long periods of time (> 12 h) at low photon flux densities (PFD, ≤ 100 μmol m^{-2} s^{-1}).

The influence of algal photosynthesis on bacterial productivity has been previously examined in brief exposures (<2 h) over a range of light intensities [27]. Photosynthesis-induced enhancements in bacterial production within biofilm communities were shown to have marked effects at high photon flux densities (200 and 400 μmol m^{-2} s^{-1}). However, the coupling of bacterial production to algal photosynthesis/metabolism seemed to breakdown around 100 μmol m^{-2} s^{-1} in laboratory experiments [27]. Lentic and lotic biofilm communities frequently are saturated at PFD ranging between 200 and 400 μmol m^{-2} s^{-1} and can experience photoinhibition (see [10]). Generally, biofilm communities, particularly benthic communities, will experience photon flux densities $\leq$100 μmol m^{-2} s^{-1}, as dissolved organic and particulate matter or canopy shading will markedly reduce the incident light available for photosynthesis. The present study examines this algal–bacterial coupling in biofilm communities exposed to PFD of 100 μmol m^{-2} s^{-1} over 24 h ligh–dark incubations. Additionally, the effects of photosynthesis on the abundance, biovolume, and biomass of bacteria, and the activity of the enzymes α- and β-glucosidase and β-xylosidase were examined.

Methods

Biofilm communities were grown *in situ* on glass fiber filters mounted on Plexiglas holders [15]. The Plexiglas holders were submersed in a pool in the Talladega Wetland. The Talladega Wetland Ecosystem (TWE) is a natural 15.1 ha wetland located in the upper Coastal Plain physiographic province in west central Alabama, USA (32° 52′ N, 87° 26′ W) [22, 42]. Biofilm communities were allowed to colonize for 17 days prior to being transported back to the laboratory for experimentation. The biofilm algal communities were dominated by coccoid green algae (e.g., *Chlorococcus* and *Oocystis*) and the euglenophyte *Trachelomonas,* with the occasional green filaments (e.g., *Microspora* and *Bulbochaete*), pennate diatoms (e.g., *Synedra* and *Eunotia*), and chrysophyte *Ophiocytium.*

Experimental Design

The experiment was conducted at 25°C (mean daily water temperature in TWE during summer ranges between 23 and 28°C) in a Percival environmental chamber (Percival Mfg. Co., Boone, IA). Biofilm communities (mean = 0.43 ± 0.39 mg AFDM cm^{-2} (±SE)) were maintained in Moss medium ([23], at saturating nutrient conditions (~10,000 μg N L^{-1} and ~ 890 μg P L^{-1}) under conditions of light or dark to alter the photosynthetic activity of the community. Dark treatments were established by wrapping vials with black tape and then enclosing the vials in cardboard boxes within the environmental chamber. Light treatment biofilm communities were exposed to photosynthetically active radiation (400–700 nm) from fluorescent and incandescent lights that provided PFD of 100 μmol m^{-2} s^{-1}. At each sampling occasion replicated biofilm communities were analyzed for bacterial protein production ($n = 11$), α- and β-glucosidase, β-xylosidase activities ($n = 12$), bacterial abundance, biovolume, and total biomass ($n = 6$) and then removed from the experiment.

Bacterial Protein Production

Bacterial protein production was analyzed by determining rates of incorporation of ^{3}H-leucine into protein [17]. Modifications for greater isotopic dilution of the ^{3}H-leucine were necessary for analysis of intact biofilm communities [15, 37]. Cores (1.13 cm^2) of biofilm communities grown on glass fiber filters were placed into precombusted (500°C) scintillation vials containing 15 mL of Moss medium [23] and incubated for 24 h at 25°C. ^{3}H-Leucine was introduced to the treatment media overlying single biofilm cores. Blanks were inactivated with 37% formaldehyde. After a 45-min incubation period, experimental communities were killed with 37% formaldehyde (final concentration 3%). The cores were placed in 10 ml 5% trichloroacetic acid (TCA) and incubated in a water bath at 90°C for 30 min. The supernatant was cooled for 30 min in an ice bath and then filtered (Millipore GS, 0.22-μm pore size) and washed with cold 5% TCA and 80% ethanol. The filters (MilliporeGS, 0.22-μm pore size) were placed in scintillation vials and solubulized with 1 ml ethyl acetate. After 24 h, 10 ml of Aquasol-2 was added to each sample and radioassayed by scintillation (Beckman 6500 scintillation counter).

Enzyme Analysis

Enzyme activities were determined for α-glucosidase, β-glucosidase, and β-xylosidase with the appropriate fluorescent 4-methylumbelliferyl (MUF) substrate for each enzyme (Sigma Chemical Co.). All fluorescence measurements were corrected for natural

fluorescence and quenching by Moss medium. Standard curves were generated with 4-methylumbelliferone sodium salt (Sigma Chemical Co.). Preliminary experiments using a range of substrate concentrations (10–600 μM) indicated that 400 μM MUF substrate was sufficient to saturate the activity of each enzyme produced by natural communities after a 3-h incubation period.

Replicated cores (1.13 cm^2) of biofilm communities grown on glass fiber filters were placed into precombusted (500°C) glass vials containing 1 mL of the Moss medium and incubated for 24 h at 25°C. An 800 μM solution of the appropriate fluorescent substrate was added to each vial, which resulted in a final substrate concentration of 400 μM. After a 3-h incubation period, 150 μl from each vial was transferred into opaque-walled Costar 96-well plates (Corning Costar Corp.) containing 150 μl $well^{-1}$ of Trizma buffer solution (Sigma Chemical Co., pH 9.5). Fluorescence (360/40 ex., 460/40 em.) was measured with a CytoFluor 2300 plate reader (Millipore Corp.).

Bacterial and Community Biomass/Enumeration

Cores of biofilm communities grown on glass fiber filters were placed into combusted scintillation vials containing 15 mL of treatment medium and incubated in light or dark conditions for 24 h at 25°C. After a 24-h incubation, filter cores were placed in 4% glutaraldehyde and stored at 4°C for later analysis. Bacterial enumeration and biovolume were determined by epifluorescence microscopy with the nucleic acid stain 4′,6′-diamidino-2-phenylindole (DAPI) [28]. Samples preserved in 4% glutaraldehyde were vortexed for 1 min and sonicated in a bath sonicator (Bransonic 220) for 1 min. After sonicating, 0.5 ml of sample was added to a filtration well, diluted with 1.5 ml ultrapure water (Millipore Milli-Q), and stained for 15 min with 30 μL of DAPI. After staining, the solution was filtered onto a black polycarbonate filter (Poretics, 0.2 μm pore size) and transferred to a glass microscope slide. Ten random fields were enumerated at 1,000× with epifluorescence (Zeiss Jenalumar DDR-6900). Cells were measured with an ocular micrometer and categorized into shapes for biovolume determination [11]. Biovolume values were converted to biomass by using the conversion factor of 1 $\mu m^3 = 3.5 \times 10^{-13}$ gC [43].

Statistical Analysis

Sigma Stat software was used for all statistical analyses (Jandel Sigma Stat, 1994). Data normality and variance were tested by using the Kolmogorov–Smirnov test and Levene Median test, respectively. Overall statistical significance between treatments was established by using either a parametric analysis of variance or a nonparametric Kruskal–Wallis test [45].

Results

Bacterial biomass production (gC gC^{-1} h^{-1}) was significantly greater in light than in dark after 16 h (Fig. 1, $p < 0.001$). The overall bacterial biomass production in the dark treatments changed significantly over 24 h ($p < 0.001$), but remained relatively consistent between hours 8 and 16. In contrast, the light treatment changed significantly over time ($p < 0.001$) and displayed an exponential increase between hours 4 and 24.

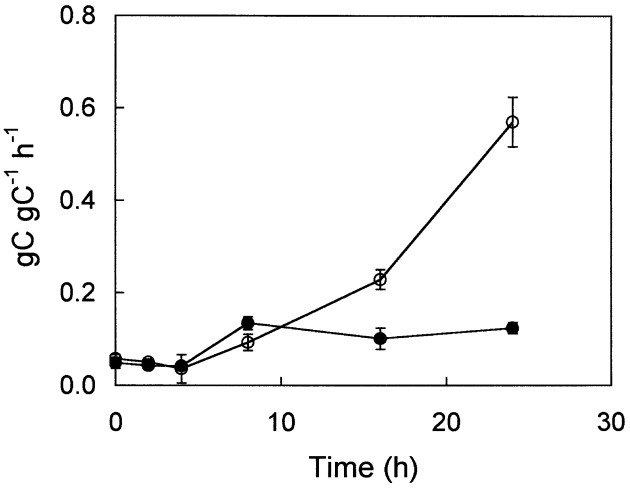

Fig. 1. Mean (±SE) bacterial biomass production (gC gC^{-1} h^{-1}) over 24 h. Biofilm communities cultured in carbon-free (0.2 mg C L^{-1}) medium under light (○) and dark (●) conditions.

The presence of light had a positive influence on overall enzyme activity (Fig. 2). Activities of α- and β-glucosidase and β-xylosidase were significantly greater in light than in dark conditions ($p < 0.01$). All enzymes displayed significant changes over time ($p < 0.001$) with enzyme activities within light treatments increasing and dark treatments decreasing over 24 h. Within time comparison between light and dark treatments indicated statistical differences at hours 16 ($p < 0.02$) and 24 ($p < 0.01$) for all three enzymes.

The number of bacterial cells cm^{-2} was not significantly different between light and dark treatments over 24 h ($p = 0.12$, Fig. 3C). However, there was a significant increase in bacterial biomass ($p < 0.001$, Fig. 3A) and, hence, biovolume ($p < 0.001$, Fig. 3B), which indicated bacteria were accumulating mass without changes in population density.

Discussion

Neely and Wetzel [27] assessed the short-term coupling of autotroph and bacterial productivity in biofilms over a range of photon flux densities (20–400 μmol m^{-2} s^{-1}). Communities were exposed to 20 μm DCMU (3-(3,4-dichlorophenyl)-1, 1-dimethylurea), a photosystem II inhibitor, or not exposed

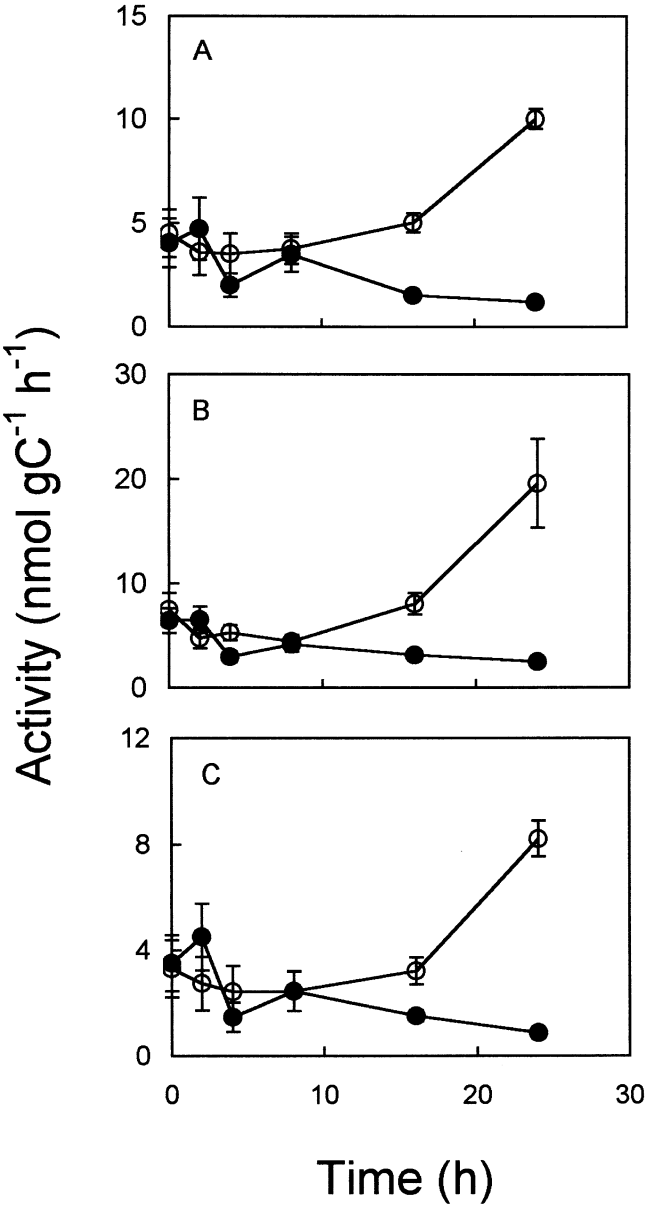

Fig. 2. Mean (±SE) ezyme activity (nmol gC^{-1} h^{-1}) over 24 h for α-glucosidase (A), β-glucosidase (B), and β-xylosidase (C). Biofilm communities cultured in carbon-free (0.2 mg C L^{-1}) media under light (○) and dark (●) conditions.

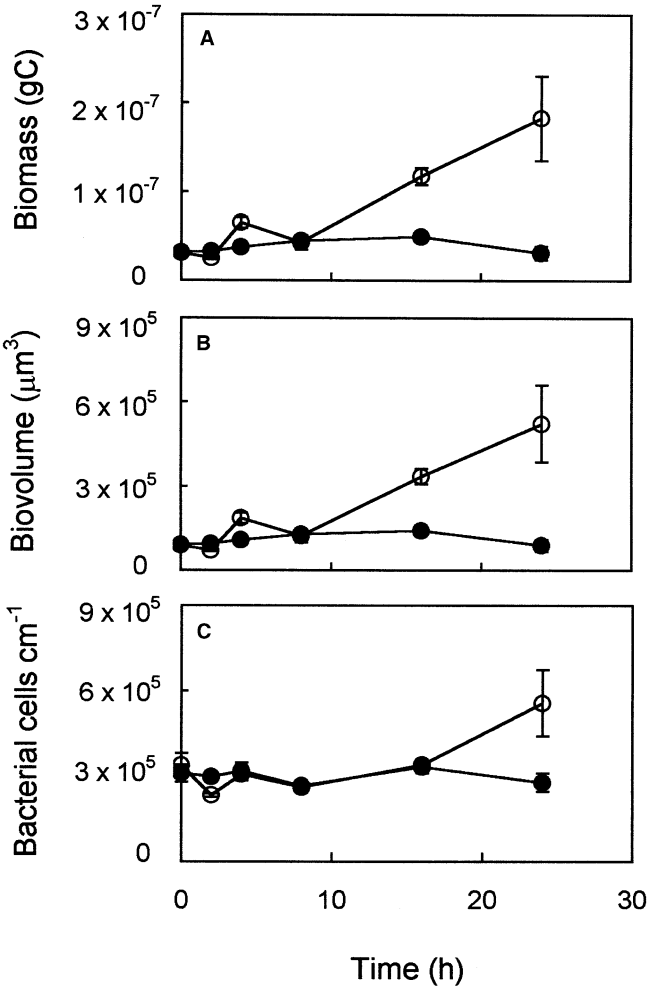

Fig. 3. Mean (±SE) bacterial biomass (gC) (A), biovolume (μm^3) (B), and bacterial numbers (cells cm^{-1}) (C) over 24 h. Biofilm communities cultured in carbon-free (0.2 mg C L^{-1}) medium under light (○) and dark (●) conditions.

to DCMU. Bacterial biomass production (μg C cm^{-2} h^{-1}) of −DCMU treatments were significantly higher at PFD between 200 and 400 μmol m^{-2} s^{-1} compared to DCMU treatments. However, no significant difference occurred at PFD between 20 and 100 μmol m^{-2} s^{-1} over the 2-h exposure. Biofilm communities in the present experiments were cultured under PFD of 100 μmol m^{-2} s^{-1}, and bacterial productivity assessed over a longer time period. Although these results support Neely and Wetzel [27] over the initial 8 h, where production rates were similar between light and dark treatment at PFD 100 μmol m^{-2} s^{-1}, bacterial biomass production in the light became significantly greater than that of dark treatments after 8 h of photosynthesis. These results suggest that although photosynthesis at lower light intensities (≤100 μmol m^{-2} s^{-1}) is less productive, it can have a significant impact on bacterial biomass production over longer time periods. Thus, the coupling of bacterial production to algal photosynthetic output holds true at broader ranges of light intensities than observed by Neely and Wetzel [27], with the overall effect varying with time. It is possible that some carbon supplied to biofilm bacteria in light treatments was from photochemically degraded biofilm extracellular mucopolysaccharides (EPS). High intensity natural PAR irradiance is sufficient to mineralize dissolved organic

matter (reviewed in [40]), which suggests that the mucopolysaccharide matrix of biofilm might be photochemically degraded into more labile carbon forms. However, the extent of any photochemical degradation and release of labile carbon from biofilm EPS is likely small given the low PAR intensity used in this study, which would indicate that the increase in bacterial production in light treatments were generally the result of algal photosynthesis.

The response of bacteria to photosynthesis appears to be an increase in biomass rather than an increase in overall abundance. Haack and McFeters [9] observed higher bacterial activity following seasonal declines in chlorophyll α content of biofilm communities, but did not observe corresponding increases in bacterial abundance. Studies have demonstrated larger increases in cell volumes relative to bacterial abundance grown in UV-exposed DOC [20, 21]. Increased supplies of labile carbon should induce accelerated reproduction rates and, hence, greater bacterial abundance. However, these results, and those of other studies, suggest that enhanced labile carbon supplies, via photosynthetically produced EOC or photochemical mineralization of DOC, may result in the sequestering of carbon by bacterial cells generating greater individual biomass.

If the EOC was sufficiently labile for direct bacterial uptake, then it would be expected that greater enzyme activities would occur in dark treatments, because these communities were under carbon limitation via organic carbon-free media and deprivation of photosynthetic EOC. Furthermore, the production of organic carbon processing enzymes in the light treatment might be under end-product inhibition (cf. [5]). However, if EOC required extracellular enzymatic hydrolysis prior to incorporation, then increased enzymatic activity in the light treatment would be expected because of substrate induction of extracellular enzyme production. In this experiment enzyme activity was stimulated by light, which suggests that the EOC required extracellular enzymatic hydrolysis. This interpretation is supported by the results of Sundh [33], who determined that a majority of EOC released by algae is of high molecular mass (>5,000 Da). It is uncertain whether the observed results were associated with compositional changes in the bacterial community in response to changed conditions or enhanced production of enzymes by existing bacteria responding to higher substrate concentrations. To our knowledge, this experiment is the first published study to demonstrate a direct effect of algal photosynthesis on extracellular enzyme activity within algal/bacterial biofilms. Several previous studies (e.g., [13, 31, 32]) have investigated the influence of algae on biofilm glucosidase and xylosidase activity, but these studies compared enzyme activities of heterotrophic, dark-grown biofilms to auto/heterotrophic light-grown biofilm communities, and thus could not separate the direct effects of photosynthesis from the influence of community composition or other potential causal factors. The present investigation indicated that in auto/heterotrophic biofilms, active photosynthesis could directly stimulate glucosidase and xylosidase activity. Under certain conditions, biofilm protease activity can also be stimulated by algal photosynthesis (Francoeur and Wetzel, submitted manuscript). The mechanism for photosynthetic stimulation of extracellular enzyme activity is not yet known. The simple explanation of active photosynthesis causing increased bacterial biomass, thereby resulting in heightened production and activity of extracellular enzymes (e.g., [4]) cannot fully account for the observed results, because enzyme activities were expressed per unit of bacterial biomass, yet still increased with photosynthesis. It is possible that polysaccharides released from algae during photosynthesis enhanced β-glucosidase production and activity [13, 36] and might have also increased production and activity of α-glucosidase and β-xylosidase. Additionally, a shift in the balance between active and inactive bacterial cells could explain the observed increases in production and enzyme activities without enhanced bacterial numbers. Photosynthetically induced pH shifts would also be expected to influence enzyme activity. Although biofilm pH was not measured in this study, it is well known that algal photosynthesis can cause alkaline pH within biofilm communities [3, 12, 14, 29, 44]. Increased pH did not stimulate glucosidase or xylosidase activity in other TWE biofilms (Espeland and Wetzel, submitted manuscript), suggesting that alteration of pH by photosynthesis may not have caused the increase in extracellular enzyme activity observed in this study. Regardless of the underlying mechanism, the potential exists for photosynthetically mediated diurnal patterns in extracellular enzyme activity. Diurnal patterns in TWE biofilm protease activity have been observed (Francoeur and Wetzel, submitted manuscript). Further study will be required to confirm the occurrence and magnitude of diurnal patterns in glucosidase and xylosidase activity and elucidate their ecological importance.

In summary, algal photosynthesis was stimulatory to biofilm bacterial productivity at lower light intensities than previously reported. However, the stimulatory effect of photosynthesis at lower light intensity took longer to be manifested. Enzyme (glucosidase and xylosidase) activity and bacterial biomass and biovolume, but not bacterial abun-

dance, were also stimulated by active algal photosynthesis. These results indicate that photosynthesis can influence bacterial productivity, biomass, biovolume, and enzyme production over longer time periods at low PFD ($\leq$ 100 μmol m^{-2} s^{-1}).

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