Use of glucose biosensors to measure extracellular glucose exudation by intertidal microphytobenthos in southern Tasmania[1]

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Abstract

Micro glucose biosensors were used to measure net extracellular glucose produced by natural microphytobenthos and three diatom cultures (*Amphora coffeaefirmis*, *Navicula menisculus*, *Nitzschia longissimi)* from southern Tasmania, Australia. They were exposed to a light gradient in either nutrient-replete or nutrient-limiting conditions. Glucose exudation in the natural communities increased with increased light but the response in the cultures was variable. Similarly, nutrient-replete conditions elicited lower rates of glucose exudation in the natural communities but produced variable species-specific responses in the cultures. Increased glucose exudation mostly correlated with a reduction in maximum quantum yield (F_v/F_m). The same trend was observed in the natural communities for relative maximum electron transfer rates ($rETR_{max}$) but responses in the cultures were again variable and species-specific. Responses of the three species to increased light and nutrient deficiency were variable, although glucose exudation, F_v/F_m and $rETR_{max}$ was mostly lower in the nutrient-limited media. In a second set of experiments species/communities were treated with/without antibiotics. In the dark, glucose concentrations in treatments with antibiotics remained unchanged, while in those with bacteria, it fell rapidly. In the sediment communities, glucose consumption in the dark was ~25% the rate of exudation at the highest light level. In culture, exudation rates were up to 100% greater than those with active bacteria. Rates of glucose consumption in the dark in the antibiotic–treated samples were negligible and up to 10^4 times lower than those with active bacteria. These results demonstrate the important role extracellular glucose exudation has on maintaining an active microbial loop.

Keywords: glucose, bacteria, biosensor, light, microphytobenthos, MPB, nutrients

Abbreviations: MPB, microphytobenthos; EPS, extracellular polymeric substances; DOC, dissolved organic carbon; Chl *a*, chlorophyll *a;* RLC, rapid light curve; rETR, relative electron transfer rate; F_v/F_m, photosynthetic efficiency (maximum quantum yield of fluorescence)

INTRODUCTION

Primary production in near shore coastal and estuarine ecosystems is primarily generated by phytoplankton and biofilms of photosynthetic benthic microalgae (microphytobenthos, MPB) with smaller contributions from other sources. MPB is the biofilm of photosynthetic micro-organisms that grows on the bottom or in the intertidal zone of these shallow ecosystems. At depths within the euphotic zone, typically 5 -20 m below the surface, it regularly contributes between 25% and 50% of the total annual primary productivity (Cahoon 1999, Underwood and Kromkamp 1999). Within the euphotic zone, MPB biomass can exceed 500 mg chl $a \cdot m^{-2}$ (Underwood 2010), which is often greater than the depth-integrated phytoplankton biomass. Furthermore, it is the primary food source for many benthic invertebrates (Kang et al. 2007, Lebreton et al. 2011), which then sustains substantial fish and wading bird populations.

Extracellular carbohydrate exudation is widespread in photosynthetic biofilms, being produced by both bacteria and micro algal cells. It has been found to have a number of functions including cell attachment, motility and protection (Hoagland et al. 1993, Cooksey and Wigglesworth-Cooksey 1995, Underwood and Kromkamp 1999, Staats et al. 2000, Cook et al. 2007). Much of the extracellular polymeric substances (EPS) is mucilaginous and is comprised of polysaccharides, uronic acids and sulfated sugars (Underwood and Paterson 2003, Bellinger et al. 2009, Oakes et al. 2010). Dissolved organic carbon (DOC) production and export by microalgae is a core requirement of the microbial food web and is taken up rapidly by both heterotrophic bacteria and the microalgae themselves.

Extracellular release of DOC from microalgae is mostly from either photosynthetic overflow, whereby carbon fixation exceeds the requirements of macromolecular synthesis and excess photosynthate is released directly into the water column, or by cell lysis resulting from grazing or viral attack (Smith and Underwood 2000). The availability of an organic substrate is essential for herotrophic bacterial growth and the effective functioning of the microbial loop (Azam et al. 1983). There is consequently often a close and coupled association between autotrophic producers, such as diatoms, and bacterial consumers (Fenchel 2008).

The composition of DOC exudates from microalgae is dominated by glucose, which often comprises more than 70% of the total (Hama and Yanagi 2001, Haas and Wild 2010). Other important sugars include galactose, mannose, xylose, rhamnose and fucose (Underwood and Paterson 2003). The causes and drivers of photosynthetic overflow are not completely understood. It is likely that any process that decreases cell growth, such as nutrient limitation or high light (Staats et al. 1999, Underwood and Paterson 2003, Cook et al. 2004, 2007) will contribute to overflow photosynthesis. DOC production in culture, for instance, is associated with the low nutrient stationary phase of growth rather the exponential phase (Myklesstad 1977). Unlike phytoplankton, it has usually been thought that MPB is not nutrient limited as it can gain access to re-mineralized nutrients from sediment pore water (Sundbäck et al. 2011), although, several studies have now demonstrated nutrient limitation in MPB (Cook et al. 2004, 2007).

We have previously examined the photophysiological response of MPB in Tasmanian coastal ecosystems to elevated light levels (Jordan et al. 2008, 2009) and extreme temperatures (Lee and McMinn 2012). Cook et al. (2004) examined carbon and nitrogen cycling in a nearby mudflat and concluded that a significant proportion of the production was exported as DOC and EPS rather than cellular production. Other relevant studies on carbon cycling in Australian sediments include Oakes et al. (2010, 2012) and Oakes and Eyre (2004).

Here we use a glucose micro biosensor to examine the exudation and consumption of glucose by MPB and bacteria and assess the potential effects of nutrient limitation and excessive light. These biosensors were developed by Pinnacle Technology Inc., a US biomedical company specializing in biosensors for medical research. The glucose biosensor was specifically developed to study sleep disorders in rodents (Pinnacle Technology Inc. 2017). The application of these biosensors to marine microbial ecology is novel.

MATERIALS AND METHODS

Sample collection and culturing The Penna Beach site, southern Tasmania (42°47'06" S, 147°31'13" E; Fig. 1) is microtidal with a tidal amplitude of < 50 cm. Maximum midday irradiance in summer was 2140 µmol photons · m^{-2} · s^{-1}, salinity varied between 29.6 and 36.6 and temperature between 8.5°C and 22.0°C. The sediment is a sandy mud with >70% of the sediment less than 180 µm in size. The collection site was exposed at low tide.

Short sediment cores were collected from the intertidal zone using a 45 mm diameter hand pushed sediment corer. For the natural biofilm incubations, the cores were transported intact back to laboratory and the top 2 mm of sediment was carefully sectioned and incubated in 50 ml culturing vials in L/1 media (CSIRO, Hobart, Tasmania) prior to the experiments. Natural chlorophyll concentrations at the collection site were 442.051 ± 22.409 µg chl *a* · m^{-2}. Three diatom species, *Amphora coffeaefirmis*, *Navicula menisculus*, and *Nitzschia longissimi,* were isolated from the same sediments for the culture experiments. Each species was isolated using single cell isolation techniques. Once monocultures had been established, they were transferred to 50 mL culturing flasks containing L/1 medium. All cultures were incubated at 25°C at a light intensity of 100 µmol

photons · m^{-2} · s^{-1} provided by a cool white fluorescent tubes (Phillips MASTER TL-D Secura, with a peak emission at 550 nm, spectrum available at http://www.lighting.philips.com/main/prof/conventional-lamps-and-tubes/fluorescent-lamps-and-starters/tl-d/master-tl-d-secura/927921484076_EU/product) and a 12:12 h light:dark regime. Species identification was undertaken with a Hitachi SU-70 Analytical Field Emission Scanning Electron Microscope (FESEM; Hitachi, Naka Division, Ibaraki Prefecture). The cultures were not axenic.

Subculturing was conducted fortnightly, to ensure late-logarithmic growth phase development was reached. The growth medium was replaced weekly to achieve a high biomass, necessary for some of the analyses. Fifteen grams of sediment, collected from the sampling site and sterilized (autoclaved), was added to the bottom of each culture flask to provide a substrate. Immediately prior to the experiments, excess media was carefully removed, until only a thin liquid film remained over the biofilm. This was done to minimize the volume of water and thus maximize the values of the changing glucose concentration.

Experiment design. Two sets of experiments were undertaken; the first to examine the combined effects of high light and high/low nutrients on net DOC production and the second to measure bacterial and algal consumption of glucose in the dark. For the nutrient experiments, the cultures were grown under nutrient replete conditions; this was ensured by replacing the media of the cultures weekly. Nutrient deficiency was obtained by starving the cultures for 20 d prior to the experiments.

Glucose consumption by bacteria was measured as the difference between cultures/communities with and without active bacteria. The bacteria were inactivated by adding 1 mL of the antibiotic, Antibiotic Antimycotic solution (containing 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml, Sigma Aldrich, Australia); 100 µg · L, the cultures/communities were grown

for a week. During the experiments, the cultures/communities were incubated for four hours with four increasing irradiances, as in the nutrient experiment

In each experiment, three replicates of the natural sediment community or diatom culture (*Amphora coffeaefirmis*, *Navicula menisculus*, and *Nitzschia longissimi)* were exposed to a series of increasing irradiances of 67, 140, 300, and 933 µmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. The irradiance, which was provided by a high pressure sodium lamp (Osram Vialox NAV (SON)-E, 400 Watt, sports light, peak emissions 500-650 nm with a high narrow peak at 565 nm, https://infogalactic.com/info/File:High_Pressure_Sodium_Lamp_Spectrum.jpg) was progressively stepped up at hourly intervals. The irradiance levels were adjusted using neutral density filters comprised of a UV blocking film (UV Light Technology Limited, Birmingham, United Kingdom). Glucose exudation rates (mg glucose $\cdot$ h^{-1}) were calculated as the difference between the concentrations at the beginning and end of each irradiance exposure. These were then normalized to chl *a* concentration.

Glucose analysis. A glucose micro biosensor (Pinnacle Technology, Lawrence, KS, USA) was used to measure net glucose exudation (Fig. 1). This biosensor uses the glucose oxidase enzyme to reduce the glucose molecules and produce hydrogen peroxide as a by-product. The hydrogen peroxide is reduced on a platinum electrode to produce electrons that are then detected on a Ag/AgCl reference electrode. This biosensor was designed to measure the glucose concentration in the brains of rodents for the pharmaceutical industry. Hence, modification by the company, specifically to increase sensitivity, was required for the biosensors to be able to measure glucose exudation by MPB in this study. As relatively high glucose concentrations are required for detection (> 2-5 µM), it was important to maintain a high algal biomass within the cultures. The output of each biosensor (pico

amps) was calibrated against a standard curve (D-glucose standard, Sigma Aldrich, St. Louis, MO, USA), which was made by diluting a 10 mg glucose · L^{-1} stock solution, to 1, 0.1, 0.01, 0.001, $1x10^{-4}$, $1x10^{-6}$ mg glucose · L^{-1}. This standard curve was used in the calculation of glucose concentrations in the cultures during the experiment. The biosensors have a 90% response time of ~ 4 s. This is the first time this biosensor has been used to measure glucose concentrations in algal cultures.

The glucose biosensor was mounted onto a manual micromanipulator (Unisense A/S, Aarhus). The glucose concentration was measured by inserting the biosensor to 1 mm above the sediment surface using the micromanipulator. The biosensor was connected to a picoammeter (PA2000, Unisense, Denmark) and the raw data was converted into mg glucose · L^{-1} using the relationship from the standard curve. The exudation rate was then normalized to chl *a* concentrations (mg C · [mg chl *a*]$^{-1}$).

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured using a Pulse Amplitude Modulated (PAM) fluorometer (Diving-PAM; Walz, Effeltrich, Germany) and Rapid Light Curves (RLCs) were obtained following Ralph and Gademann (2005). $rETR_{max}$ was estimated using a multiple non-linear regression to an exponential function (Platt et al. 1980):

$$rETR = rETR_{max} [1-\exp(\alpha E_d/rETR_{max})].$$

Where $rETR_{max}$ represents the maximum potential rETR in the absence of photoinhibition. α is the initial slope of the light curve before the onset of saturation and represents the efficiency of light utilization. E_d is the irradiance (McMinn et al. 2010).

The fibre optic cable of a Diving-PAM was attached to a micromanipulator in order to standardise the distance from the sediment surface, approximately 1 mm. Prior to the incubations, the flasks were wrapped in aluminium foil for 30 min to dark-adapt the cultures. RLCs were obtained in the dark at the start of the incubation period and then hourly at the end of each light level. Between light levels

there was a 5 min period of dark adaption. All measurements were made in the dark. Photosynthetic efficiency (F_v/F_m) and maximum relative electron transport rate ($rETR_{max}$) were acquired from the RLCs. The biomass was manipulated to ensure a Diving PAM gain setting of less than 10.

The biomass of the cultures was determined by extracting the sediment chlorophyll overnight in 10 mL of methanol and then measuring on a Turner Designs 10AU fluorometer (Sunnyvale, CA, USA), which was calibrated against a chl *a* standard (Sigma Aldrich 1 mg $\cdot$ L^{-1} spinach, St. Louis, MO, USA), following the acidification method of Holm-Hansen et al. (1965).

Statistical analysis. Measured data were compiled in Microsoft Excel and statistical analyses were performed with the computing software, R (R Core-Team, 2014). Analysis of variance (ANOVA) was undertaken to study the significance of variation between treatments (three replicates). Reported results are for significant differences at the highest irradiance (933 µmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) between nutrient replete and nutrient deficient treatments (Table 1) and between treatments with and without antibiotics (Table 2).

RESULTS

The MPB community at Penna Beach was dominated by small diatoms, including *Amphora coffeaefirmi, Amphora laevissima, Acnanthes brevipes, Cocconeis peltoides, Gyrosigma balticum, Navicula jeffreyae, Navicula menisculus* and *Nitzschia longissimi;* these species collectively comprised > 80% of the community.

Nutrient experiment. Natural communities: glucose exudation in the natural communities increased with increasing irradiance. (Fig. 2a). Nutrient replete samples showed consistently lower glucose exudation levels than nutrient limited samples. There was an inverse relationship between photosynthetic performance and glucose exudation. (i.e., F_v/F_m and $rETR_{max}$; Fig. 2, b and c). Samples with high F_v/F_m and $rETR_{max}$ values (i.e., those experiencing less stress, consistently had lower rates of glucose exudation).

Single species cultures: There were significant increases ($p < 0.01$) in glucose exudation in *A. coffeaefirmis* cultures with increasing irradiance in nutrient limited conditions but a decrease in nutrient replete conditions (Fig. 3a). Increased glucose exudation was associated with a decline in F_v/F_m in both the nutrient replete and limited cultures (Fig. 3b). However, glucose exudation increased with increasing $rETR_{max}$ in the nutrient-limited cultures but decreased in the nutrient replete cultures (Fig. 3c), Differences in glucose exudation between nutrient treatments, however, were not significant ($F_{1,4}= 0.0872$, $p= 0.777$; Table 1).

Navicula menisculus: glucose exudation by this species was higher under nutrient limited conditions than under nutrient replete conditions (Table 1, Fig. 3d) but correlations (R^2 values) between glucose exudation rates and irradiance, F_v/F_m, and $rETR_{max}$, were poor (Table 2, Fig. 3, d-f). Additionally, glucose exudation remained constant at 1.03×10^{-4} mg C · [mg chl *a*]$^{-1}$ · hr^{-1} at irradiances of 140 µmol photons · m^{-2} · s^{-1} and above in the nutrient replete treatment (Fig. 3d). Significant differences between treatments were observed in F_v/F_m ($F_{1,4}= 379.13$, $p= 1.189*10^{-6}$), $rETR_{max}$ ($F_{1,4}= 8.636$, $p= 0.0260$), as well as rate of glucose exudation ($F_{1,4}= 9.606$, $p= 0.0211$; Table 1).

Nitzschia longissimi: there were no significant relationships between the rate of glucose exudation and light, F_v/F_m, or $rETR_{max}$ in the nutrient replete cultures (Fig. 3, g-i). However, significant correlations were observed between the rate of glucose exudation and irradiance ($R^2= 0.939$), F_v/F_m

(R^2= 0.723) and $rETR_{max}$ (R^2= 0.694) in the low nutrient treatments (Fig. 3, g-i). ANOVA results of both nutrient treatments only showed significant differences with respect to F_v/F_m and no significant differences were observed in the rate of glucose exudation or $rETR_{max}$ (Table 1).

Bacterial glucose consumption. When spiked with an antibiotic, glucose consumption in the dark was inhibited in the natural communities as well as the three diatom cultures (Fig. 4, Table 3). For the natural communities (natural sediment from the field containing bacteria) the control treatment (i.e., no antibiotic), showed a similar rate of glucose exudation to those of the nutrient replete experiments. Samples that were grown with the addition of antibiotics showed higher rates of glucose exudation in the light than the control. However, there was a substantial difference between the rate of glucose consumption in the dark in the control vs antibiotic-treated sample; 1.39 x 10^{-2} vs 8.95 x 10^{-6} mg glucose · [mg chl *a*]$^{-1}$ · hr^{-1}, respectively (Table 3).

There was a large reduction in glucose consumption in the antibiotic treated samples of all three cultures. In *A. coffeaefirmis* cultures the rates were 0 and 7.58 x 10^{-4} mg glucose · [mg chl *a*]$^{-1}$ · hr^{-1} in the antibiotic-treated and control, respectively. In *N. menisculus* cultures the rates were 0 and 2.32 x 10^{-3} mg glucose · [mg chl *a*]$^{-1}$ · hr^{-1}) in the antibiotic-treated and control, respectively and in the *N. longissimi* cultures the rates were 6.57 x 10^{-7} and 3.77 x 10^{-3} mg glucose · [mg chl *a*]$^{-1}$ · hr^{-1}) in the antibiotic-treated and control, respectively (Table 3).

Amphora coffeaefirmis and *N. meniculus* both showed significant differences in the rate of glucose exudation between cultures with antibiotics and those without ($F_{1,4}$= 5.6861, p= 0.0544 and $F_{1,4}$= 112.95, p= 4.088*10^{-5}, respectively; Table 2).

DISCUSSION

This is the first study to use glucose biosensors to measure the rates of glucose exudation and consumption by MPB in real time. While they performed strongly, there were some operational characteristics which may influence future use. The minimum detection limit of the biosensors is ~2 – 10 µM (Pinnacle Tech Inc, https://pinnaclet.com//glucose.html), which is comparable to background levels in sea water (Gocke et al 1981). This level of sensitivity is likely to limit their usefulness in pelagic environments or low biomass cultures. However, glucose concentrations in marine biofilms are often more than an order of magnitude greater than background levels (Underwood et al. 2010, herein) and so the sensors have the strong potential to be used in environments such as sediments and sea ice. The minimum sensor tip size is currently ~80 µm, which will limit their usefulness in flux studies using the diffusive boundary layer approach (Revesbech and Jorgenson 1986, McMinn et al. 2000). The biosensors also have a limited life with a reported operating life of approximately 96 h and they can be stored for up to 21 d after production. This relatively short life could limit their applicability in some remote field locations.

This study examined photosynthetic overflow by MPB in response to light, nutrient limitation and the presence of bacteria. In these experiments, the natural MPB communities and algal cultures were exposed to incremental irradiance increases to examine net glucose exudation in response to the different light levels. The net glucose exudation commenced immediately once the communities and cultures were exposed to light (Figs. 2, 3). Additionally, the rate of net exudation (mostly) increased with higher light intensities (Fig. 3) and this was similar for all cultured species and the natural communities. Glucose can comprise more than 70% of the total extracellular carbohydrate produced by MPB and is associated with overflow metabolism (Smith and Underwood 2000, de Brouwer and Stal 2001, 2002, Hama and Yanagi 2001, Cook et al. 2007, Haas and Wild 2010, Pierre at al. 2012, Passarelli et al. 2015). While extracellular carbohydrate production occurs in both light and dark,

much of the light-weight fraction, principally comprised of monosaccharides such as glucose, is associated with photosynthesis (Underwood and Paterson 1993, Smith and Underwood 1998). In the light, when cells are also nutrient limited, up to 62% of photo-assimilates can be excreted into light-weight water soluble carbohydrates (Smith and Underwood, 2000, Staats et al. 2000). Other studies have also found a relationship between extracellular glucose exudation in MPB and stresses, such as excessive irradiance (Perkins et al. 2001) and nutrient limitation (de Winder et al. 1999, Smith and Underwood 2000, Staats et al. 2000, Underwood and Paterson 2003, Bellinger et al. 2010, Canion et al. 2013).

While elevated rates of glucose exudation were consistently observed in the natural MPB communities when they were exposed to high irradiance and nutrient-depleted growth media, these communities were comprised of many species, each of which was likely to produce extracellular glucose at different, species-specific rates. This variability is thought to relate to species-specific sensitivity to nutrient fluctuations (de Winder et al. 1999, Staats et al. 2000, de Brouwer and Stal 2002, Underwood et al. 2004, Ai et al. 2015). Because of this likely variability, the response of three different MPB species, *A. coffeaeformis, N. menisculus* and *N. longissimi,* was also investigated. Nutrient limiting conditions had a significant influence on glucose exudation in *A. coffeaeformis* and *N. menisculus* but not on *N. longissimi*. *A. coffeaeformis,* and *N. longissimi* but not *N. menisculus* showed increased glucose exudation at higher irradiances. There were also good negative correlations between glucose exudation and photosynthesis (i.e., F_v/F_m and $rETR_{max}$) in *A. coffeaeformis* and *N. longissimi* but not *N. menisculus.* Even when normalized to chl*a*, nutrient limitation and high irradiance in the natural communities consistently elicited a greater response than in the single species cultures.

Photosynthetic overflow often correlates with MPB photosynthetic performance (Perkins et al. 2001). Perkins et al. (2001) found that the percentage allocation of photo-assimilates to low molecular weight extracellular carbohydrate was higher in areas exposed to excessive irradiance,

coinciding with low effective quantum yield (F_q'/F_m') values. In this study also, when glucose exudation in the natural MPB communities increased, there was a coincident decrease in F_v/F_m and $rETR_{max}$.; this relationship was found in both high and low nutrient treatments. However, there was a significant difference in these parameters between the two treatments; MPB biofilms incubated under nutrient-limited conditions had higher rates of glucose exudation and weaker photosynthetic performance than those of nutrient-replete treatments. While increased extracellular carbohydrate production coinciding with decreasing photosynthetic performance has also been reported by Bellinger et al. (2010), Cook et al. (2007) demonstrated that MPB communities were still capable of sustained high rates of photosynthesis despite nutrient limitation.

MPB biofilms constitute a valuable carbon source for heterotrophic bacteria in benthic systems (Decho 1990, Goto et al. 2001, Giroldo et al. 2003, Hofmann et al. 2009, McKew et al. 2013, Van Colen et al. 2014) and bacteria, through consumption, play an important role in determining the extracellular carbohydrate concentration. More than 50% of MPB extracellular carbohydrates can be used by bacteria within 24 hours (Goto et al. 2001) and even up to 60% in as little as one hour (Smith and Underwood 2000).

In the dark, the glucose concentrations in the cultures/communities not treated with antibiotics deceased rapidly while those that were treated showed no significant decline. In the natural sediments without antibiotics the rate of glucose consumption in the dark was ~25% the rate of exudation at the highest light level. In the three cultures, however, the rates were up to 100%. Rates of glucose consumption in the dark in the antibiotic–treated samples were negligible and up to 10^4 times lower than those with bacteria. These results demonstrate again the important role extracellular glucose exudation has on maintaining an active microbial loop. As the natural MPB sediments were collected directly from the sampling site and cultured in the laboratory, the microbial communities would have been comprised of mixed communities of bacteria and diatoms. Different bacteria are likely to have had different rates of consumption potentially leading to greater

rates of glucose consumption. A ^{13}C-tracer study has confirmed the rapid transfer of carbon from extracellular carbohydrates to bacteria (Oakes et al. 2010, 2012). The small decreases seen in the antibiotic treated communities/cultures probably came from both heterotrophic uptake by diatoms and possibly antibiotic-resistant bacteria. Active heterotrophic uptake of glucose in the dark by diatoms has been demonstrated in many studies (Palmisano et al. 1985, Rivkin and Putt 1987, Tuchman 1996, Taylor et al. 2013) and may, in fact, be the norm rather than the exception (Mitra et al. 2016, Selosse et al. 2017).

This is the first study we know of to use glucose micro biosensors to quantify MPB extracellular glucose concentration and exudation. When light levels on the cells were increased, the sensors responded rapidly, with almost instantaneous increases in measured glucose concentrations. While there are some issues regarding their sensitivity, we feel that they have the potential to be a useful tool in future studies of MPB extracellular carbohydrate production and consumption.

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Table 1. Analysis of variance between nutrient-saturated and nutrient-limited treatments of *Amphora coffeaefirmis*, *Navicula meniculus* and *Nitzschia longissimi* at an irradiance of 933 µmol photons · m^{-2} s^{-1}. Significant differences are indicated in bold with ** indicating significant P values (<0.01) and * indicating a significance of P< 0.05. Glucose exudation is measured as mg glucose, rate of glucose exudation is measured as mg glucose · (mg chl *a*)$^{-1}$ · hr^{-1}. Cultures are not axenic

Species	**F_v/F_m**	**$rETR_{max}$**	**Glucose exudation**	**Rate of glucose exudation**
A. *coffeaefirmis*	**0.00285****	**0.0473***	**$8.338*10^{-8}$****	0.777
N. meniculus	**$1.189*10^{-6}$****	**0.0260***	**$8.827*10^{-8}$****	**0.0211***
N. longissima	**0.0276***	0.260	0.2776	0.2344

Table 2. Analysis of variance between cultures of *Amphora coffeaefirmis*, *Navicula meniculus* and *Nitzschia longissima* at an irradiance of 933 µmol photons · m^{-2} . s^{-1} treated with or without antibiotics. Significant values are in bold with ** indicating a P value <0.01 and * indicating a P value < 0.05. Rate of glucose exudation measured as mg glucose · (mg chl *a*)$^{-1}$ · hr^{-1}. Cultures were not axenic

Species	**F_v/F_m**	**$rETR_{max}$**	**Rate of glucose exudation**
A. coffeaefirmis	**0.0128**	0.561	**0.0544**
N. meniculus	0.1043	0.0798	**$4.088*10^{-5}$****
N. longissima	**0.0280***	0.222	0.615

Table 3. Rates of glucose consumption by MPB cultures and natural sediment communities in the presence or absence of an antibiotic.

Species	Rate of glucose consumption (mg glucose · [mg chl. α]$^{-1}$ · hr^{-1})	
	Antibiotic	**Without antibiotic**
A. coffeaefirmis	0	7.58×10^{-4}
N. meniculus	0	2.32×10^{-3}
N. longissima	6.57×10^{-7}	3.77×10^{-3}
Natural sediment	8.95×10^{-6}	1.39×10^{-2}

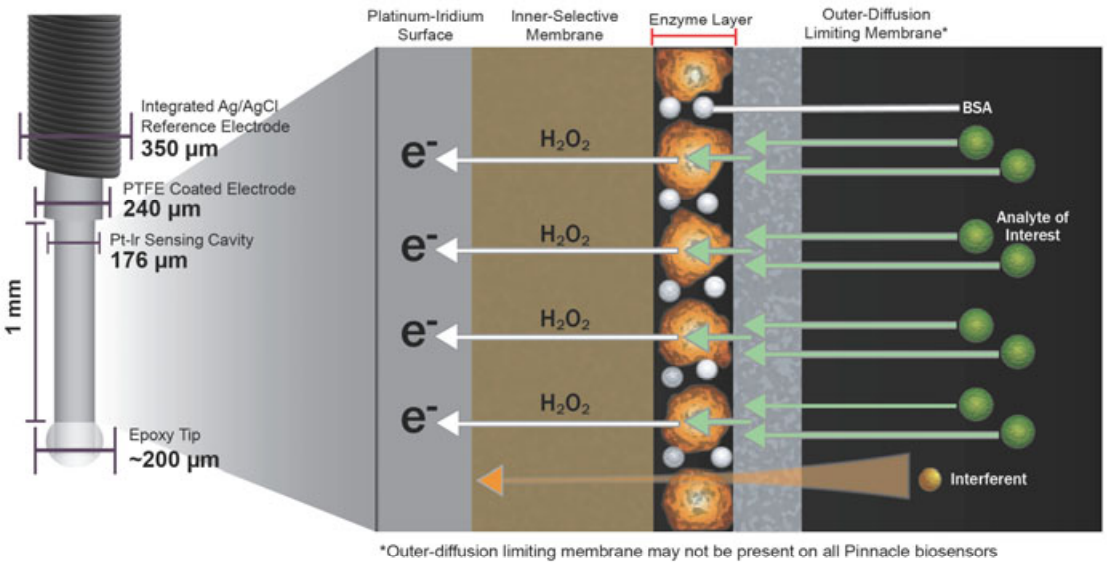
Figure Captions

Figure 1. Pinnacle Technology Inc. glucose biosensor. Reproduced with permission of the company.

Figure 2. Relationship between glucose exudation rate (mg glucose · (mg chl *a*)$^{-1}$ · hr^{-1}) and light (PAR, µmol photons · m^{-2} · s^{-1}), F_v/F_m, and $rETR_{max}$ in the natural MPB communities.

Figure 3. Relationship between glucose exudation rate (mg glucose · (mg chl *a*)$^{-1}$ · hr^{-1}) and light (PAR, µmol photons · m^{-2} · s^{-1}), F_v/F_m, and $rETR_{max}$ in cultures of *Amphora coffeaefirmis* (a, b, c), *Navicula meniculus* (d, e, f) and *Nitzschia longissima* (g, h, i). (○) indicates nutrient saturated media and (●) indicates nutrient limited media.

Figure 4. Bacterial consumption of glucose in *Amphora coffeaefirmis.* (a) is the control with no added antibiotic, while (b) has had antibiotic added. After 160 min the light is turned off.



*Outer-diffusion limiting membrane may not be present on all Pinnacle biosensors