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Customized 3D-printed surface topography governs species attachment preferences in a fresh water periphyton community

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ABSTRACT

Periphyton cultivation systems have few controls over species composition, thereby limiting their applications for production of biomass. A promising approach for controlling species composition in cultivation systems is through design of substratum characteristics. 3-D printing is used to design substratum topographic sections to test for selectivity of colonization of periphyton algae in streams. Experimental tiles were designed with six different topographic sections composed of tightly-packed hemispheres over a range of diameters from 100 to 2000 μm. These tiles were deployed in a local stream for 30 days, retrieved and analyzed for species occurrence and relative abundance on each of the tile sections. Twelve species of periphyton algae in four divisions were identified across all topographic sections. The distribution and relative abundance of these species were found to vary as a function of topographic feature size, with the greatest diversity observed on the surfaces with topographic feature sizes of 500 μm. Of the twelve identified species, two exhibited abundance patterns that were significant. *Microspora wileana* displayed a preference for surfaces with topographic feature sizes <500 μm, and *Stigeoclonium tenue* displayed a preference for surfaces with topographic feature sizes less than or equal to 100 μm and greater than or equal to 1500 μm. These results suggest that substratum design using 3D printing or other technologies may be useful to influence species composition and dominance relationships in mixed communities in engineered periphyton cultivation systems.

1. Introduction

The controlled cultivation of attached algal biofilms and periphyton has been developed and studied for linking remediation of aquatic pollutant nutrients to production of biomass for economic uses such as biofuels precursors [1–5]. Large-scale periphyton cultivation systems have been used for remediation of stormwater, industrial wastewater, and agricultural and municipal wastewaters [6–9]. While the advantages of periphyton cultivation for aquatic pollutant recovery stem from the ease of operation and biomass recovery, the biomass recovered from these systems can be of lower quality than that desired for many biomass use applications, due to the open-reactor approach that allows and encourages competitive colonization of algal species indigenous to the source water [9]. Approaches for controlling species selection in colonization and competition are desirable for ultimate economic use of periphytic biomass.

In the design of processes for periphyton cultivation in open reactors, only a few operational parameters control the algal colonization dynamics that determine biomass quality and product yield. The topography of the solid substratum to which periphyton attach has only recently been investigated as one of these controlling processes [10–12]. In situ studies in streams and laboratory investigations have demonstrated that the physical topographical attributes of the surface, measured through various roughness parameters, can influence the biotic attributes of the colonizing periphyton community by determining attachment of viable colonizing cells or spores [13–16]. For example, in experiments to identify relationships between bacterial cell adhesion and substratum topography, it was shown that the rate of cell attachment and biofilm formation was directly related to average topographic feature size ranging from smooth to 800 μm [17–19]. Algal colonization on surfaces follows a similar pattern, where experiments have shown that algae generally prefer textured over smooth surfaces of the same material [20]. Biofilm formation dynamics in response to substratum topography has been investigated for individual species as well. For example, the rate of cell deposition for *Oscillatoria* sp. on stainless steel tabs was shown to vary directly with increasing surface topographic complexity and inversely with shear velocity [21]. More recently, studies on the effects of engineered surface texture and material on cell attachment of two microalgal species, *Scenedesmus dimorphus* and *Nannochloropsis oculata*, showed that algal cells would colonize on manufactured topographies, but that adhesion of the cells was related

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to the cell's physical geometry, including its shape and size [22]. Species preference for topographic feature size in a flow environment has been demonstrated for *Ulva linza*, where zoospore retention was greatest on substrata with topographic features structures with a vertical average dimension (R_z) of 25 μm, compared to both smooth ($R_z = 1$ μm) and rough ($R_z = 100$ μm) structured surfaces [23]. These and other studies suggest the controlling nature of substratum topography on the development of the colonizing periphytic community in a flow environment.

Despite these past investigations into surface dynamics of algal cell colonization, there has been little research using additive manufacturing to produce surface topographies for attachment and growth of algal communities [13]. Additive manufacturing technologies, also known as 3D printing, build objects layer by layer and are typically associated with the design and fabrication of parts and components in manufacturing. Industries such as aerospace and healthcare have made significant advances in their fields by incorporating the capabilities of 3D printing into their research, development and manufacturing activities. The advantages of the technology include the ability to quickly manufacture shapes and geometries that are difficult, sometimes impossible, to manufacture with traditional methods and without the general need for tooling or fixtures. In addition, 3D printing affords the ability for high-resolution replication of features and, coupled with surface scanning technologies, high fidelity reproduction of existing natural surfaces, making the technology ideal for experimentation in bio-environmental systems involving surface phenomena. While 3D printing has been applied in biomedical fields for tissue and organ engineering, the extension of 3D printing into applications in environmental systems has been limited, despite many biological functions that are dependent upon spatial and topological characteristics [13].

This paper provides an approach for understanding the attachment preferences of certain periphyton species towards substrata topography by using custom designed 3D printed surfaces. The primary objective of this paper is to investigate the effect of surface topography on the differential attachment and colonization dynamics of periphyton species in a mixed community in a natural stream. A secondary objective is to demonstrate the use of 3D printing technologies to design and fabricate replicated platforms that are useful to test selective behavior of species attachment.

2. Materials and methods

The overall methodology consisted of designing and fabricating twelve growth plates, each with six regions presenting different surface textures, using a polyjet-based 3D printer. The plates were then placed in two different locations in a natural stream and left exposed to the ambient conditions over a period of 33 days. After collection, the biomass was carefully harvested in each region and studied microscopically for species identification. Non-parametric statistical analyses were conducted to confirm the effects of surface topography on attachment and growth of identified species. This section provides details in each of the steps in the methods.

Growth plates of acrylic polymer were designed using Solidworks® and fabricated with a Stratasys® Objet30 3D printer with a 28 μm layer thickness. Twelve rectangular plates (55 mm × 74 mm × 4 mm) with four anchor points on corners were designed and printed. Anchor points were designed for ease of attachment to a plastic mesh for placement in the stream. Each printed tile presented six sections with hemispheric topographic features with increasing radii. The sections included an ideally smooth area (area roughness average Sa = 1.19 μm), and five sections with adjacent hemisphere patterns of average area peak-to-valley height (R_z) of 100, 500, 1000, 1500, and 2000 μm (Fig. 1).

The accuracy of the fabrication was confirmed with optical methods using a confocal white-light profilometer (Nanovea ST-400, Irvine, CA) with 20 nm resolution in the vertical direction. Fig. 2 shows the aerial map and the 2D line scan of the surface topography for the 500 μm section. The fabrication dimensional error was <1% for the areas with radii 500, 1000, 1500 and 2000 μm. For the section with radius size of 100 μm, the dimensional error was on the order of 20%, with most radius sizes in this section falling in the range of 77 μm − 100 μm. This is attributed to the feature size approaching the fabrication capability limit of printer in the lateral direction.

The growth plates were attached to two polypropylene screen mats in an alternating order such that different sections on tiles faced upstream/downstream configuration. These mats were deployed in adjacent locations in the stream and left exposed for a period of 33 consecutive days. The sites selected for these deployments were in Chewacla Creek, located in Chewacla State Park, Auburn, Alabama (32°32′51.0″N 85°28′53.7″W). The screen mats were placed proximate to a stream cross-section at which there is USGS station that records information on water discharge and water level every fifteen minutes [24]. The average gage height during experiment period was 0.56 ± 0.04 m, while water discharge during same time was 0.52 ± 0.33 $m^3 s^{-1}$.

In addition, temperature and light intensity were recorded every 15 min using an in-situ data logger (HOBO Pendant, Onset Corp., Bourne, MA) installed at depth on the mats. Over 3000 readings of temperature and light intensity were collected. The mean water temperature during experiment was 27.8 ± 1.2 °C. The locations of deployment were partially shaded with a median daytime light intensity of 223 μmol $m^{-2} s^{-1}$. The pH and conductivity of the water were measured weekly with a handheld combination pH/EC probe (HI 98130, Hanna Instruments, Woonsocket, RI), and averaged 7.36 ± 0.18 and 0.15 ± 0.05 mS cm^{-1}, respectively. Local stream velocity was measured weekly with a Model 2100 current flow velocity meter (Swoffer Instruments, Inc., Tukwila, WA) at the locations of tile installation, and averaged 0.24 ± 0.09 m s^{-1}. Both dissolved P and N concentrations were moderately low (PO_4-P: 0.080 ± 0.020 mg l^{-1}; NO_3-N: 0.52 ± 0.19 mg l^{-1}, $n = 5$), as measured with a YSI 9500 field photometer (YSI Inc., Yellow Springs, Ohio). The growth plates were placed in the stream on July 7th, 2015 and recovered on August 8th, 2015 for a total exposure of 33 days.

After removing the growth plates from screens, each topographic section was imaged and documented at a low magnification (10× to 40×) via digital microscopy. Each section on each plate was harvested with several methods, including vacuuming, mechanical scraping, and washing with low-pressure distilled water, to recover as much biomass as possible. The biomass collected from each section was stored separately in vials containing 30 ml of mixed, harvested samples, DI water, and 0.15 ml of glutaraldehyde, 50% solution, for preservation. There were a total of 72 vials (one for each of the 6 topographic sections across 12 growth plates).

The species identification effort was conducted via digital microscopy (400× to 1000×) using a Motic optical microscope (Motic Corp., Richmond, BC). The microscope sample preparation included a thorough mixing of the vial, which was then transferred onto a glass slide for observation. Three sub-samples, each with a volume of 0.0625 ml, were drawn from each vial (representing the individual growth plate sections) and from which 30 micrographs were obtained from each sub-sample. A total of 216 sub-samples and 6480 micrographs resulted from this approach. The algae observation in the micrographs were keyed to at least the genus level using standard identification keys [25]. A preliminary observation on a subset of the micrographs resulted in the development of a custom species identification key containing those 12 most commonly found species. This key was used to train two laboratory analysts who each scanned the entire set of micrographs independently and recorded observations on presence/absence of each species in each micrograph. The independent observations from the two analysts were compared, showing a high degree of agreement between the two, with discrepancies on the observations found in only 2% of the micrographs. These micrographs were then revisited and jointly analyzed until a consensus on the species identification was reached.

The presence/absence data for the predominant 12 species were recorded and analyzed for patterns and distribution. The numbers of each