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Design and Manufacturing of High Surface Area 3D-Printed Media for Moving Bed Bioreactors for Wastewater Treatment

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Abstract: The specific surface area and topology of a biofilter media carrier is one of the most important parameters that determines the performance and efficiency of the system. In this work, mathematical models and 3D printing technologies were used to design and fabricate complex media designs that provide high specific surface area and refugia to protect biofilm from premature sloughing. Several gyroid based designs were proposed with specific surface area well beyond 2300 m^2/m^3. However, wall thicknesses and pore sizes that are prone to clogging determined a design that yields 1168 m^2/m^3 (133% larger than the baseline commercial K1 Kaldnes). Several moving bed bioreactors were constructed for laboratory testing with inoculation provided by wastewater from a fisheries operation. Preliminary results indicate that the 3D printed media can withstand the prevalent conditions in moving bed bioreactors, and that the NH_3 removal rate of gyroid media is comparable to that of K1 Kaldnes. This work establishes the feasibility of using 3D printing for bioreactor media fabrication and allows for future topology optimization for enhanced operation. Future work is needed to investigate how these complex media designs might stimulate novel microbial assemblages and community metabolism that translate into yield efficiency gains in reactor performance.

Keywords: *specific surface area, biofilter, carrier, topology, gyroid, nitrification, efficiency*

Water pollution is a global problem and is found wherever human activity takes place. Every 20 seconds a child dies from a preventable illness caused by unsafe drinking water, poor hygiene, and inadequate sanitation (UNICEF/WHO 2009). Although the United Nations Millennium Development Goals (MDGs) for water access have been met ahead of schedule, the world did not meet the 2015 goals for sanitation (WHO 2012) as nearly half of the people living in developing regions still lack improved sanitation (WHO/UNICEF 2010). In addition, the Natural Resources Defense Council states that dirty water is the world's biggest health risk and threatens quality of life as well as public health (NRDC 2014). Water is considered polluted when impaired by anthropogenic contaminants and thereby rendered unusable as drinking water. These contaminants typically comprise a combination of organic and inorganic substances, and different remediation methods exist for various pollutants.

One approach for water pollution remediation is through the use of biofilters that employ attached-growth microbial biofilms to metabolize pollutant constituents. In these reactors, microbial biofilms colonize an artificial substratum and capture or transform dissolved pollutants, rendering them less harmful or removing them via losses to the atmosphere. There are different types of biofilm-based bioreactors, including biofilters with suspended (moving bed) media, and biofilters with static (trickling) media, among others. The

potentially low operational costs of biofilters have led to their implementation in many applications for water and wastewater remediation, including for both remediation of organic and nitrogen contaminants (Mendoza-Espinoza and Stephenson 1999).

Moving-bed bioreactors (MBBR) are popular in many wastewater treatment processes because of their simple operation and potential efficiency, combining the advantages of both attached and suspended growth systems. MBBR treatment reactors use carrier media for the growth of biofilms on their surfaces (Biswas et al. 2014). Suitable carrier media present a high surface area per unit volume and are light-weight for buoyancy in the moving fluid flow of the reactor vessel (Ødegaard et al. 2000). Typical carrier media designs employed in biofilter reactors are often simple structures patterned along a single axis of symmetry such that they are amenable to traditional tool-and-die manufacturing processes (e.g., polymer extrusion, injection molding, etc.). Figure 1 shows several types of biofilter media that are commercially available.

Reliably high performance of these media in biofilters has been shown in many pollutant remediation applications. However, limitations in manufacturing processes place restrictions on shape diversity of the media, potentially limiting the ultimate performance gains that might be realized through novel media shapes with recursive surface area complexity for biofilm colonization. The potential exists to develop new media concepts that might stimulate novel microbial assemblages and community metabolism to yield efficiency gains in reactor performance.

The performance of a carrier media in an MBBR is typically evaluated as a function of the media's specific surface area (SSA), which is the ratio of the total surface area to the bulk volume. Media geometries that have high SSA values offer more opportunities for biofilm establishment and are generally considered to be more effective than media with lower SSA values (Guerdat et al. 2010). Other factors, such as material, surface texture (Felföldi et al. 2015), carrier orientation, pore spacing, and geometry (Herrling et al. 2015; Li et al. 2015), have been shown to impact the overall performance of MBBR systems. Young et al. (2016) concluded that high surface area to volume carriers demonstrate a high propensity for clogging due to small pore space configuration, thus reducing the effective surface area of the media and significantly lowering ammonia removal rates in nitrification reactors (Forrest 2014). Herrling et al. (2015) demonstrated that carrier geometry and orientation could dynamically alter local flow velocities in the inner face of the carrier. Odegaard et al. (2000) showed that the shape and size of the carrier do not significantly influence the water treatment process efficiency if the effective surface area is the same. However, the carrier size and surface area often dictate the maximum feed loading rate (Pfeiffer and Wills 2011). Kriklavova and Lederer (2010) proposed the use of a unique design to achieve greater surface area by attaching nano-fibers to commercial bio-carriers.

Carriers made with natural materials have been investigated for their low cost. Various carriers such as sand, gravel, peanut shells, rice husk, coconut shells, cane bagasse, and maize stubble

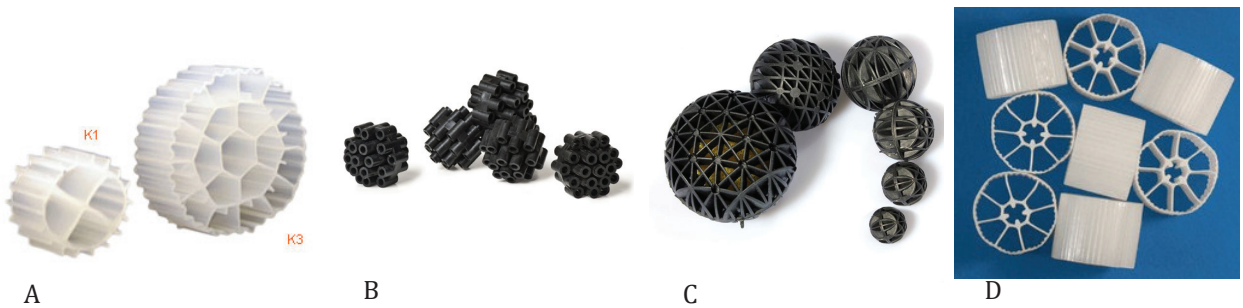


Figure 1. Commercial biofilter media: (A) K1, K3, (B) Atlantic bio-balls, (C) honeycomb bio-balls, and (D) MB3 media .

have been used as carriers in bio-filters (Ramirez-Lopez et al. 2003; Shao et al. 2009). Also, fireclay (Tilaki 2011), bamboo charcoal (Chen et al. 2012), fiber threads (Jin et al. 2012), geotextiles (Mukkulath and Thampi 2012), and peach pits (Soltani et al. 2013) have been investigated as potential carriers in bio-filters. A sample of different kinds of bio-carriers used in a MBBR, along with a brief summary of their characteristics, is given by Shrestha (2013). While these types of media are inexpensive, the variability of carrier characteristics, lack of buoyancy, unpredictability of SSA, and contamination issues stemming from the use of biodegradable materials have diverted attention towards the use of manufactured polymer carrier media to enhance the interaction between the fluid and the biofilm (Bolton et al. 2006; Martinez-Huerta et al. 2009). The patent literature also offers a wide variety of carrier media designs, with most of them aimed at increasing the SSA of the carrier element. From a geometric standpoint, most are variations of a known shape that is amenable to traditional manufacturing. Examples include cylindrical (Brockdorff 2010; Flournoy and Pehrson 2010; McKean et al. 2011), spherical (Cullimore 2013), or cuboidal prisms (Loken 2011; Dempster et al. 2012; Rusten et al. 2014a; Rusten et al. 2014b). Others offer combinations of complex shapes (Barrett 2013). Many incorporate elements such as fins, grooves, and ridges. Several of these designs were too complex for feasible manufacturing at the time of the disclosure and have remained as conceptual designs.

One unexplored yet promising avenue for development and manufacture of biofilter media is additive manufacturing, or 3D printing. Recent advances in 3D printing technologies allow for precise fabrication of complex, biomimetic shapes and topologies in plastic polymer with fidelity at the micron scale. This can be used to design and manufacture ultra-high surface area carriers with complex geometries. Microbial colonization of 3D printed materials has been demonstrated for use in phototrophic biofilm reactors (Kardel et al. 2015; Carrano et al. 2016; Blersch et al. 2017). With 3D printing, shapes might be produced that have no other method of manufacture, and with a complexity that potentially stimulates unique heterogeneous biofilm development through establishment of diverse pathways for diffusion and advective transport of dissolved constituents. For example, media designs might be developed that encourage the growth of mixed bacteria and algal biofilms, creating heterogeneous networks of autotrophic and heterotrophic metabolisms that more completely transform targeted water contaminants. Currently, the potential set of 3-dimensional geometrical patterns for biofilter media remains relatively unexplored, especially with regards to biofilm complexity and biofilter performance. The objective of this work is to challenge the predominant paradigms for microbial biofilm-based reactors by designing and producing novel high-surface area biofiltration media and demonstrate functional feasibility in a mixed-bed bioreactor for nitrogen removal.

Materials and Methods

The overall methodology involved three stages: (*i*) development of mathematical models of various complex shapes of carrier media with ultra-high surface area; (*ii*) fabrication of carrier media with a 3D printer; and (*iii*) preliminary testing and analysis of carrier media performance. In this section, each one of these stages is described.

Mathematical Modeling of Complex Carrier Media

A benchmarking survey of polymer-based biofilter media currently available on the market yields a wide array of shapes, sizes, and performance characteristics. Table 1 presents a list of commercially available products with their corresponding reported SSA values. From this selection, the carrier design with the highest SSA that is commercially available is the MB3 media (604 m^2/m^3). Another similar media shape, the Kaldnes K1, is arguably one of the most popular products due to its pricing, availability, and material (high density polypropylene) and presents a SSA of 500 m^2/m^3. It should be noted that, in many of these designs, much of the area available for biofilm establishment is on the outer perimeter surfaces of the shape, where exposure to high shear stress from local flow velocities may occur and limit biofilm attachment under certain reactor conditions.

Table 1. Polymer-based manufactured biofilter media.

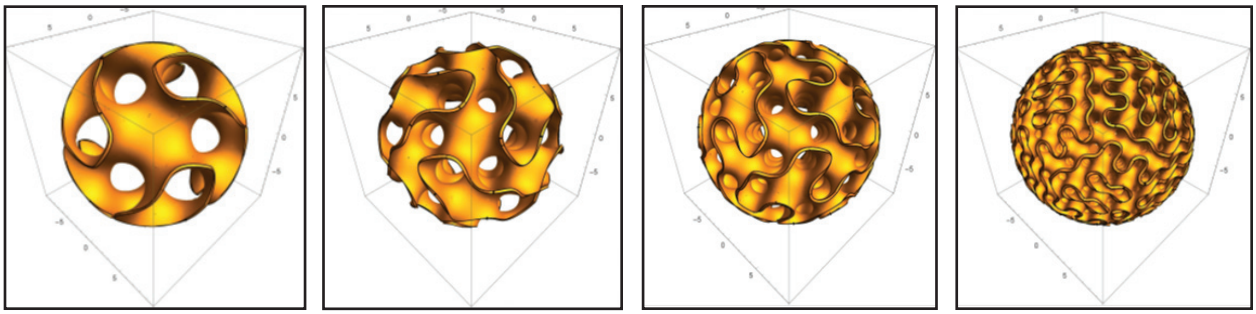
Medium	Specific Surface Area (SSA) (m^2/m^3)	Sources
MB3 Media	604	Pfeiffer & Wills 2011
K1 Kaldnes Media	500	Rusten et al. 2006; Lekang & Kleppe 2000
K3 Kaldnes Media	500	Rusten et al. 2006; Lekang & Kleppe 2000
AMB Media	500	Pfeiffer & Wills 2011
Kaldnes Rings	500	Lekang & Kleppe 2000
BioBall	378	Maslon & Tomaszek 2015
K2 Kaldnes Media	350	Rusten et al. 2006
BactoBalls	300	Godoy-Olmos et al. 2016
Finturf Artificial Grass	284	Lekang & Kleppe 2000
Norton Rings	220	Lekang & Kleppe 2000
Type A Biofill	180	Godoy-Olmos et al. 2016

Modeling was performed for various geometries in an effort to develop shapes with a higher SSA than those found in commercially available products. The approach in this effort incorporated mathematical models of a gyroid, a surface based on *sine* and *cosine* functions of an infinitely connected periodic minimal surface containing no straight lines (Schoen 1970; Osserman 1986). The gyroid is the only known embedded triply periodic minimal surface with triple junctions; does not possess any reflectional symmetries (Große-Brauckmann 1997); and is biomimetic of other surface-area dependent biological processes (Deng and Mieczkowski 1998). The gyroid surface can be described with Equation 1 and further manipulated with scaling parameters.

$$\sin x * \cos y + \sin y * \cos z + \sin z * \cos x = 0 \quad \text{(Equation 1)}$$

Once fabricated, a spherical gyroid exhibits a high degree of internal channel interconnectivity that can potentially facilitate nutrient delivery to attached biofilms. The infinite gyroid function was modeled using Mathematica® software and truncated into a sphere with further mathematical manipulation. A range of options obtained through parametric manipulation is shown in Figure 2. Taking into consideration pore size and other design constraints (e.g., overall size, manufacturability, etc.), the parameters for the chosen carrier media model were determined. It has been shown that efforts to increase SSA in a constant volume might result in topologies that are prone to clogging through accumulation of biofilm thickness (Loupasaki and Diamadopoulos 2013). Clogging in the media can reduce the amount of mass transfer between the water and the biofilm, thereby reducing the efficiency of the system (Colt 2006). Clogging considerations for the spherical gyroid media design yielded estimates for the minimum hole size of a gyroid carrier of 1500 µm.

The chosen gyroid-based media design has an overall diameter of 19 mm and a wall thickness of 500 µm. The model surface area and volume were measured with Netfabb® software (Autodesk, Mill Valley, California) and the specific surface area was estimated at 1168 m^2/m^3, which represents an increase of almost 130% in SSA over the nominal specifications reported for commercial media design Kaldnes K1. Kaldnes K1 was chosen for baseline comparison in this study because of its



SSA= 585 m^2/m^3 SSA= 863 m^2/m^3 SSA= 1168 m^2/m^3 SSA= 2309 m^2/m^3

A B C D

Figure 2. Computer model and mathematical manipulation of a spherical gyroid.

widespread adoption and use in local fisheries. Further confirmation was pursued by performing reverse engineering of the K1 Kaldness media followed by solid modeling (Figure 3) and estimation of the surface and volume. Likely because of manufacturing variability, the bulk SSA for the actual batch of K1 Kaldness media used in this experiment was estimated at a higher SSA of 810 m^2/m^3.

As seen in Figure 2, further mathematical manipulation rendered designs with much higher SSA. However, preliminary testing showed high tendency towards rapid pore clogging in such designs, thus the choice for a gyroid design with larger pore sizes. The spherical gyroid design presented complex internal surfaces, suggesting that this media has increased regions of refugia where biofilm might be protected from fluid shear forces. It is also hypothesized that this could promote the establishment of nitrifying microbial biofilms of *Nitrosomonas* and *Nitrobacter* on the different regions of the carrier (Figure 4), thereby supporting enhanced rates of conversion of ammonia (NH_3^+) into the less toxic nitrate (NO_3^-) (Pfeifeer and Wills 2011).

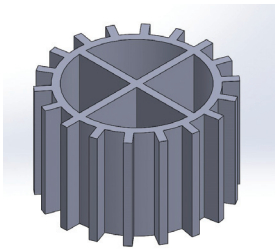


Figure 3. Reverse engineered solid model of K1 Kaldnes media.

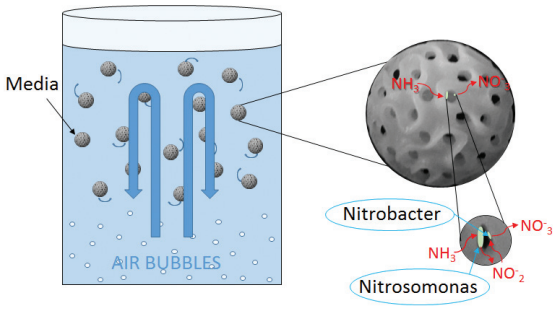


Figure 4. Mixed-bed bioreactor concept for nitrification with spherical gyroids.

Fabrication of Carriers Using 3D Printing

The media design was exported from Mathematica® software (Wolfram, Champaign, IL) into an STL file format and sent to a material jetting 3D printer (Objet30, Stratasys®, Eden Prairie, MN). Like most 3D printers, this one builds an object layer by layer, which allows for the manufacturing of complex shapes. This is especially well suited for media designs that contain complex internal channel geometries, such as the gyroid shape. The 3D printer uses a polyjet principle to deposit droplets of a liquid acrylate-based monomer resin through 200 nozzles. Once the deposition for a layer is complete, ultra-violet lamps ensure proper curing of the resin into a solid. To accommodate the build of hollow structural shapes, the printer also deposits a paraffin support material, allowing for the manufacture of holes, cavities, and channels in the media. This material

is then removed by a combination of chemical dissolution (2% NaOH solution), high-pressure water cleansing, and mechanical scraping. Figure 5 depicts the actual gyroid media fabricated with this process. A batch of 220 gyroid media carriers was fabricated for inoculation and preliminary testing.

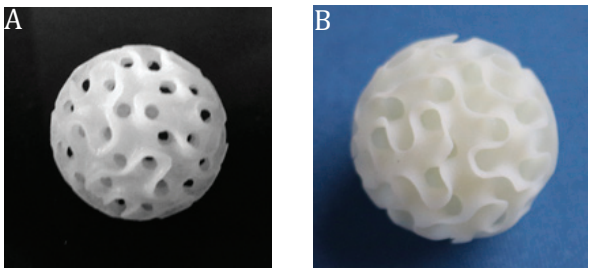


Figure 5. 3D-printed gyroid media with orientation aligned with (A) internal channels, and (B) random orientation.

Preliminary Testing and Analysis of Carrier Media Performance

Several bench-scale bioreactors were designed and constructed for testing, with scaling based on an existing system used in Auburn University's E.W. Shell North Auburn Fisheries Unit (Figure 6A). This system is a MBBR used for nitrification conversion of ammonia to nitrate in a fish aquaculture facility. Two pilot-scale bioreactors (1/10 scale by volume) were designed (Figure 6B) and constructed (Figure 6C) to implement the different test protocols. Each of the bioreactors recirculated to a 5-gallon reservoir with a 1/10 hp sump pump providing the flow. The outlet and inlet channels of the bioreactor were made of nominal ½-in PVC pipe and had valves to allow control over the volumetric flow rate into the bioreactor. Inlet flow velocities from the bottom of the bioreactor provided circulatory velocity vectors that maintained the media in suspension. Using panel-mount air flow meters, the rate of air into the

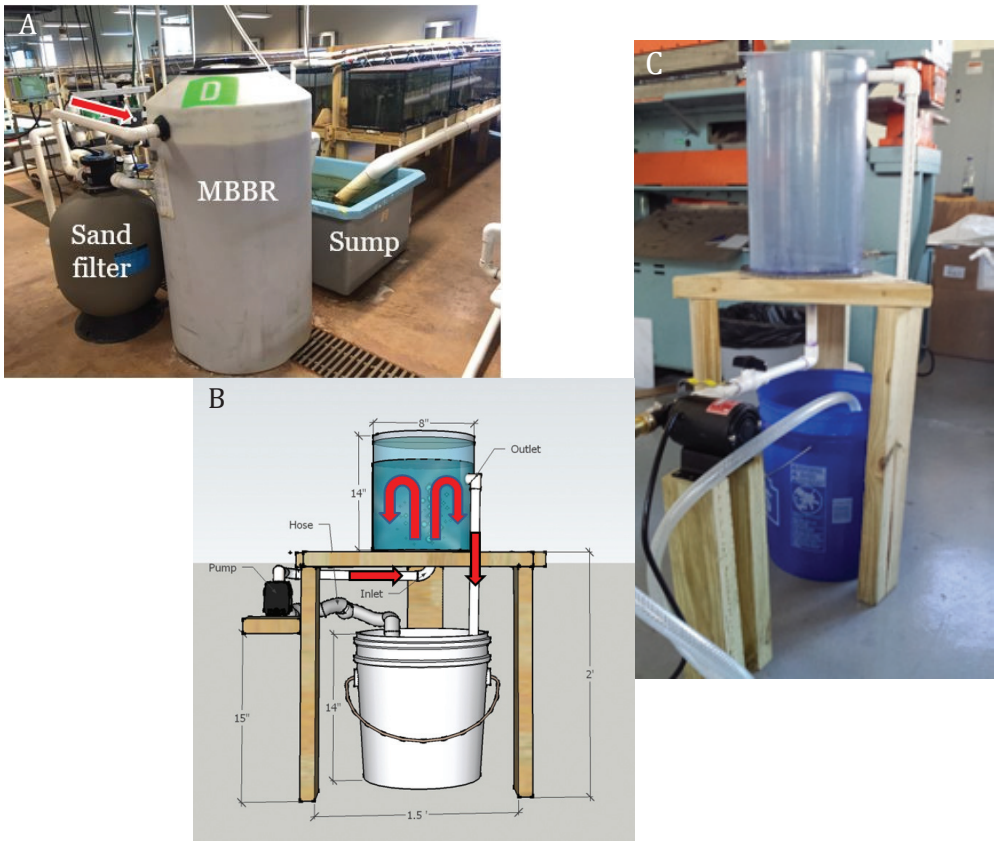


Figure 6. (A) Industrial-scale bioreactor at Auburn University North Auburn Fisheries Unit, (B) sketch of pilot-scale bioreactor for media testing, and (C) photograph of actual pilot-scale bioreactor.

bioreactor was controlled and injected to aerator stones at the bottom of the bioreactor vessel.

A series of functional tests, including tests of buoyancy, durability, and flow, were conducted with small batches of the gyroid carrier media (n<12). The buoyancy test consisted of releasing a gyroid media element at the top of a 500-mL graduated cylinder filled with water (Figure 7A), and recording the time elapsed until it reached the bottom of the water column. This was repeated 25 times and was used to calculate the sinking velocity, an important parameter for moving-bed media, as it determines the energy needed to recirculate the media. The density of each of the media was measured as well by measuring displacement of water of 20 individual elements of known dry mass placed in a graduated cylinder. The wet weight of each element was measured after removal from submergence in water and blot-drying with absorbent towels.

A durability test was conducted to determine the amount of mass, if any, lost during a 48-hour period of vigorous agitation. Ten pre-weighed media elements were placed in 400-mL of water in an Erlenmeyer flask, and agitated with compressed air at an air flow rate of 990 L/hr for 48 hours (Figure 7B). This air flow rate was selected based on the rate used in the bioreactors at the Auburn University Fisheries facility. The media elements were then air-dried for 24 hours and the final mass was recorded.

The flow test involved placing a volume of 0.6 L of gyroid carrier media in a flat plate tank that was built by inserting a sheet of Plexiglas to partition a 10-gallon fish aquarium (Figure 7C). A 1 cm OD silicon tube fitted with air holes was placed at the bottom of the tank to provide air supply. Ten gyroid carriers were marked with permanent ink of different colors so that their motion paths could be tracked through video recording. Using an air flow meter, the pressure was set to 110, 170, 220, and 280 L/hr in order to record the behavior of the media under various flow conditions. These flows were not necessarily meant to mimic the bioreactor in the field, but simply used as a standard to directly compare the media designs to one another based on movement and energy requirements. The edges of the tank were marked with a ruler, and a stopwatch was placed in the recording window so that a frame-by-frame analysis could be conducted. This helped in the determination of patterns of circulation as correlated to air flow, the energy required to adequately circulate the media, as well as the best arrangement of air flow to minimize dead zones.

After the buoyancy, durability, and flow tests were conducted, a batch of gyroid media and K1 Kaldnes was inoculated for a period of 19 days, starting on March 9, 2016 and ending on March 27, 2016. The amount of each media type used in the inoculation and experimentation process was determined by calculating the number needed to occupy 50% of the volume in the bioreactor. The inoculation process involved depositing each media batch into a 5-gallon reservoir containing three gallons of tilapia wastewater from North

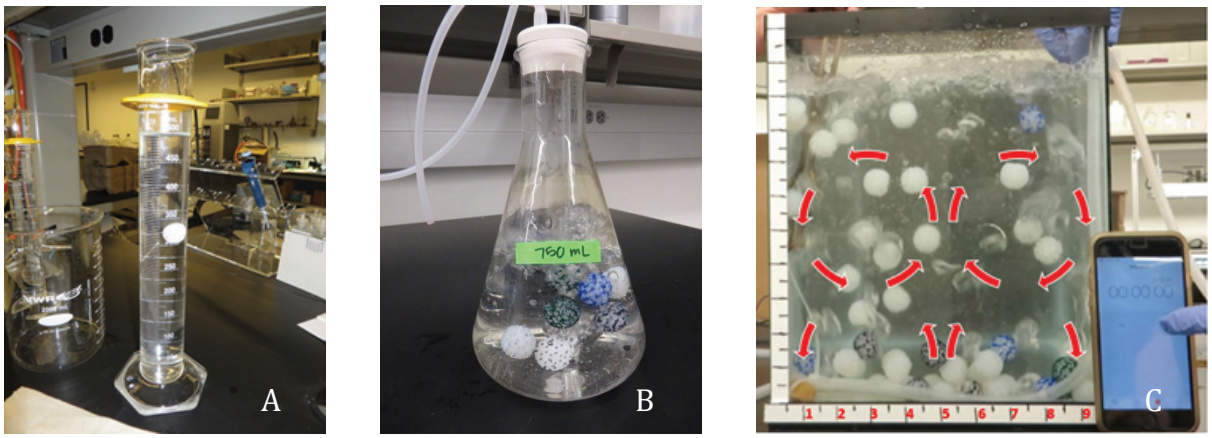


Figure 7. (A) Buoyancy test of Gyroid media, (B) Gyroid media undergoing a durability test, and (C) flow characterization test of Gyroid media.

Auburn Fisheries and keeping it constantly aerated. Each media type was inoculated with a cup (250 mL) of K1 Kaldnes covered in a biofilm of nitrifying bacteria, retrieved from the MBBR at the fisheries facility. Samples of 250 mL of water were taken daily from both reservoirs and measured for pH, temperature, and concentration (in mg/L) of ammonia, nitrite, and nitrate. The nutrient analysis was carried out using an EcoSense 9500 photometer (YSI, Yellow Springs, Ohio) and the temperature and pH were measured using a pH probe (Hanna Instruments, Limena, Italy). Every three days, 25% of the water volume was removed and replaced with fresh wastewater. The data obtained throughout this inoculation process was analyzed graphically to observe the biochemical patterns taking place. The media was deemed inoculated when the ammonia levels had decreased and the nitrate levels had increased.

Once the biofilms reached maturity, the inoculated gyroid and K1 Kaldnes media were incorporated into two separate bioreactors and were monitored during a period of two weeks. The protocol consisted of drawing water samples every six hours and testing for the presence of ammonia and nitrates using standard methods (APHA 2012). Fresh wastewater was added daily to replenish losses due to evaporation and testing, while a 25% wastewater replacement occurred every three days to ensure nutrient levels and pH remained relatively constant.

Results and Discussion

The results from the functional tests indicate that the spherical gyroid carrier, after submergence, was negatively buoyant, with a sinking velocity of 5.5 cm/s in water and a calculated buoyant force of 0.012 N. Density measures for the gyroid result in a dry specific gravity of 0.851±0.036 (n=20), and effective wet specific gravity of 1.033±0.072 (n=20), suggesting that the gyroid becomes negatively buoyant following absorption and adsorption of water. This compares to the positively-buoyant K1 Kaldnes media, found to have a specific gravity of 0.660±0.048 and 0.714±0.065 (n=20) for dry and wet conditions, respectively. Results of the durability testing show that the gyroid carrier experiences an average mass loss of 0.075% by weight over 48 hours. This corresponds to a mass loss of approximately 6.75% over the expected media life of six months in a full-scale bioreactor industry. Also, in the gyroid media design, a shear stress of 7.83 x 10^{-6} lb/ft^2 (47.9 N/m^2) at the spherical surface is estimated.

With respect to the ammonia removal test, the anticipated trends were that the NH_3 levels would decrease, while the concentration of NO_3^- would increase. The graphs below represent the average daily ammonia and nitrate time series curves for the spherical gyroid (Figure 8) and the K1 Kaldnes (Figure 9). For both media, ammonia was generally seen to decrease over time, suggesting nitrification processes were occurring. Additionally, nitrate was seen to decrease over time for both media. Nitrate removal processes are difficult to explain; the loss rate suggests possible denitrification as a removal process for the nitrate, but the high rate of aeration in the bioreactors argues against this as a possibility.

Preliminary data show that the estimated rate of NH_3 removal rates were 1.620 and 0.710 ppm/day for the spherical gyroid and the K1 Kaldnes, respectively. However, due to the difficult, lengthy processing and cost of producing 3D printed media in large batches, only a single, preliminary run was performed, thus these results are not conclusive or statistical significant. However, results do show that the 3D printed media can withstand the conditions that are prevalent in typical MBBR operations for fisheries. It also appears that the performance of the 3D printed media is at least comparable to that from the K1 Kaldnes. Although neither media reached the water quality standards required for these systems during the short nutrient testing period, given more time, it might be expected that the NH_3 and NO_3^- levels would continue to decline.

Conclusions

This preliminary work shows that complex carrier media designs can be achieved with the 3D printing technologies available today and successfully implemented in MBBR environments. Feasibility of such carrier media has been demonstrated with this effort, and features such as sheltered refugia and optimized nutrient delivery flow can be pursued in future work. Also, the

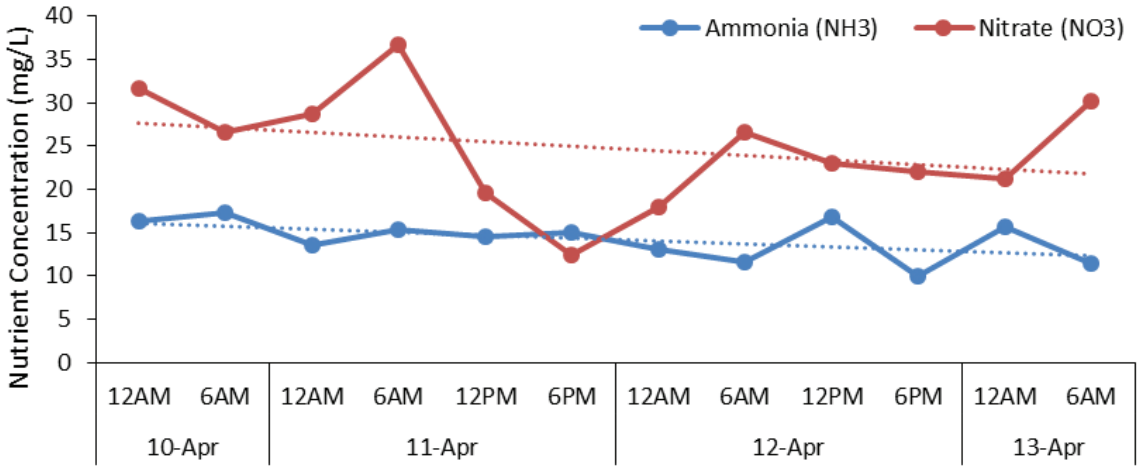


Figure 8. Ammonia and nitrate concentration time series for the bioreactor using gyroid media.

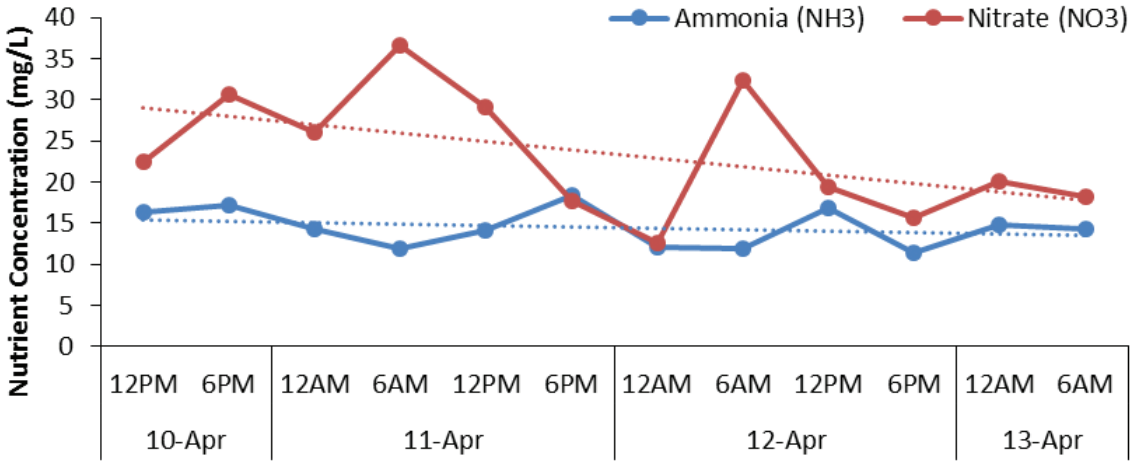


Figure 9. Ammonia and nitrate concentration time series for the bioreactor using K1 Kaldnes media.

approaches with 3D printing can push the frontier on specific surface area limitations. The pursuit of custom topologies that create unique flow pathways through internal features, minimizing sloughing while creating "dead zones" ideal for anaerobic bacteria growth, is now within reach. It is expected that design of heterogeneous flow environments in the media can result in heterogeneous microbial metabolism that can yield increased performance of media-based bioreactors (for example, media design for combined nitrification-denitrification processes). The cost of the 3D production process currently prevents large-scale commercial implementation of the new media designs, but prototyping next generation carrier designs for specialty applications such as high-efficiency wastewater treatment in remote locations or extreme environments can be addressed. There is little doubt that 3D printing will be instrumental in the design of next generation biofilter media.

Undergraduate Education Component

The work presented in this paper was conducted as part of a Phase I award from the Environmental Protection Agency's P3 student design competition (EPA 2016). This program funds student teams to develop solutions to challenges that include a wide range of categories: water, energy, agriculture,

built environment, materials, and chemicals. As a requirement of this program, the student team presented their research at the 2016 National Sustainable Design Expo in Washington D.C. From a curricular standpoint, this work was carried out during the 2015 spring semester and within the Senior Design courses in two departments at Auburn University: Biosystems Engineering and Industrial & Systems Engineering. The team was composed of seven undergraduate students at the senior level and four graduate students. The students relied on each other's expertise to address the multidisciplinary challenge of this project and educated their counterparts in the aspects of the project that were discipline specific. The senior design course constitutes the capstone experience in each of the engineering disciplines at Auburn University and enforces a rigorous engineering design process throughout the semester. This experience emphasizes competencies in areas essential for contemporary new product and process development: multidisciplinary teamwork, technical communication, and the consideration of multidisciplinary design constraints, among others. Other important skills, such as problem solving and awareness of contemporary issues, are imparted through seminars and guest speakers throughout the semester. This project was selected from among 45 entries for Phase II funding for the period 2017-2019 by the Environmental Protection Agency.

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