Journal of Advances in Biology & Biotechnology
3(3): 132-138, 2015; Article no.JABB.2015.040
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Earthworm and Algae Species in a Trickling Filter

K. Dölle[1]* and C. Peluso[1]

[1]Department of Paper and Bioprocess Engineering (PBE), College of Environmental Science and Forestry (ESF), State University of New York (SUNY), Syracuse, New York, 13210, USA.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2015/17861
Editor(s):
(1) James W. Lee, Department of Chemistry and Biochemistry, Old Dominion University, USA.
Reviewers:
(1) Anonymous, Nigeria.
(2) Anonymous, Ghana.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=930&id=39&aid=9122

Original Research Article

Received 28th March 2015
Accepted 14th April 2015
Published 5th May 2015

ABSTRACT

A trickling filter at a Waste Water Treatment Facility in the Village of Minoa in Central New York State represents an entire ecological community with different algae during the spring, summer, fall and winter months. The algae types present are identified as *Cladophora glomerata* and a moss-like species of the genus *Vaucheria*. Earthworm species *Eiseniella tetraedra* and *Dedrodrilus rubidus* are found living in the algae and using the algae as a habitat.

A stable isotope analyses was used to investigate the relationship between the worms and the algae. The analyses showed no relationship between the algae and worms growing on the trickling filter, but rather a direct relationship between the worms and wastewater.

Keywords: Algae; earthworm; stable isotope; trickling filter.

1. INTRODUCTION

In the 1970s, mainly due to anthropogenic sources, the northeastern portion of the United States began experiencing growth of *Cladophora macroalgae* in Lake Michigan and some of the other Great Lakes [1]. With increased use of fertilizers, improper sewage systems and other activities, a threshold amount of phosphorus entering the watersheds has enabled this species to grow. This has led to an increase of eutrophication in lakes that have previously been oligotrophic and is the main problem associated with this species.

**Corresponding author: E-mail: kdoelle@esf.edu;*

A naturally eutrophic lake is a lake high in nutrients and, due to this, typically has low dissolved oxygen (DO) [2]. This is because when plant and algae matter die, their decay uses oxygen and when growing and dying in abundance, low DO will result. Many times, due to anthropogenic reasons as those mentioned above, a lake will become eutrophic over time and this could be an undesirable feature for aesthetics as well as habitat.

Oligotrophic lakes are the opposite. With relatively low nutrient levels, there is typically a high amount of dissolved oxygen and these conditions are suitable for oxygen-requiring types of aquatic life to flourish. For example, lake trout will typically dwell in cold deep waters with high dissolved oxygen. If a lake was becoming eutrophic, the trout would have to travel to areas with higher DO, perhaps leaving the lake entirely to travel in search of these conditions.

Algae species are growing on a trickling filter as part of a secondary treatment at a Waste Water Treatment Facility (WWTF), in Central New York State. Typically algae appear when phosphorus levels in the water are high. It is safe to assume that human lifestyles and activities have a large influence on phosphorus levels, but it is important to keep in mind that this theory may not be so straightforward. For example, because there are many different forms of phosphorus, including but not limited to: orthophosphate. pyrophosphate, longer-chain polyphosphate and organic phosphonates, the reasons for eutrophication can be quite complex [3].

As noticed during the study, the algae species will change to a moss-like form in the winter months. In addition, worms have been discovered living in the constructed wetlands. It was observed upon the first sampling of trickling filter algae that there were worms living in the trickling filter. It is assumed that these worms must have traveled in the water to the trickling filter from the constructed wetlands soils.

At the WWTF, municipal sewage is treated according to the schematic flow chart in Fig. 1.

Approximately 1.8 million l/d of municipal waste water (WW) enter the WWTF through an influent structure where a prescreening process removes large impurities. From the influent structure half of the waste water is pumped to a primary clarifier, the other half is pumped into a sequential batch reactor (SBR) where it is treated and discharged after chlorination. Half of the clarified water from the primary clarifier is directed into an influent box that feeds the trickling filters. The other half of the clarified water is directed to subsurface biofilters (S2BF). The S2BF consist of 3 cells. Cell 1 and cell 2 are operated on a tidal fill drain cycle whereas cell 3 operates as a through flow cell. All three cells of the S2BR are planted half with grass and the other half with Phragmites. The complex root system of Phragmites and grass in the cells as well as bacteria cultures present help take up nutrients and filter the water. The effluent from the S2BR is redirected into the influent box where it mixes with the primary clarified water. The WW from the influent box is then forwarded for final treatment into the trickling filters followed by secondary clarifiers and chlorination before the cleaned WW is discharged into a stream.

The following study explores whether the worms found in the trickling filters' algae mass were using the algae as a habitat, consuming the algae or both. It is important to look into whether the relationship was symbiotic; worms helping the algae grow by helping to cycle important nutrients for growth.

2. METHODOLOGY

The methodology section describes the different methods used to determine and understand the role of the species and their relationship to the entire ecosystem that exists on the trickling filters in greater detail.

2.1 Sample Collection and Preparation

Sample collection for the WW was carried out by collection of 200-800 mL of trickling filter water in 1L Nalgene bottles. Samples for the algae and worms were collected in one gallon zip lock bags. The samples were brought back to the laboratory and subsamples of 50-100 ml were prepared for the WW and 15 gram samples for the algae and worms. The collected sample size of the wastewater, algae and worms was 2-3 times of what was needed.

The smaller WW samples were poured into a 25 mm diameter vacuum filter. The filter contained 0.2 µm pre-combusted glass fiber filters (GFF) to remove any organics and impurities that might influence the signal of the stable isotope analysis. The filter was folded into aluminum foil precisely and lyophilized overnight.

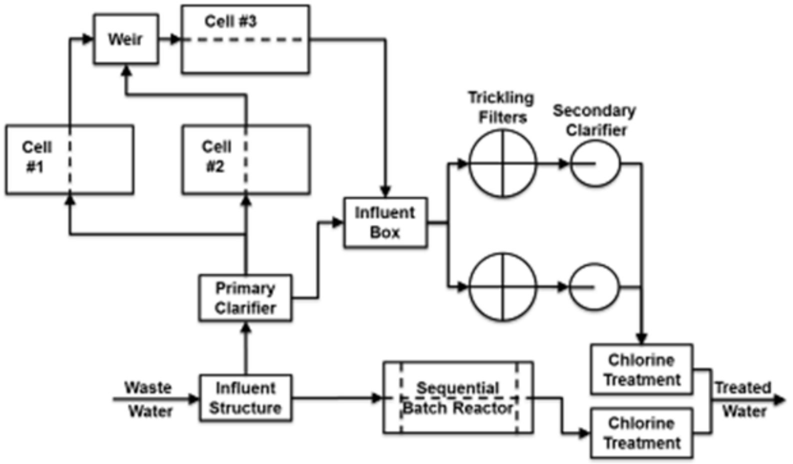


Fig. 1. WWTF schematic flow chart

The worms and algae were washed with de-ionized water frozen overnight and then freeze-dried overnight with a lyophilizer.

2.2 Stable Isotope Analysis

C13 and N15 were among the isotopes tested. Stable isotope analyses-δ^{13}C and δ^{13}C were performed with a Costech elemental analyzer linked via a Thermo Finnigan Conflo III interface to a Finnigan MAT Delta XL Plus stable isotope mass spectrometer (EA-IRMS). Samples were analyzed in triplicate and accuracy and precision of measurements was independently verified using National Institute of Standards and Technology (NIST) standards (RM8573 δ^{13}C = -26.4±0.1‰ and δ^{15}N = -4.5±0.3‰ [*n*=38]; RM8574 δ^{13}C = +37.6±0.2‰ and δ^{15}N = +47.6±0.3‰ [*n*=38]), National Bureau of Standards (NBS) 22 oil (-29.7±0.2‰ [*n*=28]) and NIST 1587 peach leaves (-25.8±0.1‰ [*n*=110]).

Daily precision of the instrument was verified by repeated analyses of internal laboratory standards including acetanilide (-29.9±0.2‰ [*n*=34]), fish muscle tissue (-18.1±0.2‰ [*n*= 32]) and plant tissue (-28.0±0.2‰ [*n*= 22]), which were analyzed repeatedly during the sample runs.

Carbon was chosen because it has the potential to be a food source for the worms. If the carbon in the algae was found in the worms, it could be inferred that the worms were eating the algae. According to Peterson and Frye, diet primarily determines the composition of animal isotopes [4].

2.3 SEM Microbe Analysis

Specimens for the SEM analysis were prepared by first collecting samples from the trickling filter in plastic zip-lock bags. At the laboratory the samples were soaked in 2.5% glutaraldehyde solution for 1 hour, followed by dehydrating them in ethanol. The samples were then sputter coated with gold palladium and analyzed with a JEOL 5800 LV scanning electron microscope.

3. COMMUNITY/ECOSYSTEM

3.1 Algae

During the investigation it was noticed that another green plant species was growing sparsely along with the algae on the trickling filter in the late spring.

The investigated trickling filter at the WWTF represents an entire ecological community with different algae. Algae have the ability to clean WW by phosphorus uptake. In an intensive study on a number of rivers in the US Midwest, an increased phosphorus uptake results in increased growth of *Cladophora glomerata* [5]. However, excess amounts of phosphorus can cause eutrophication in freshwater systems, whereas in the ocean, nitrogen controls primary production [3]. Growth of algae and phosphorus may be an obvious relationship that is influenced by temperature, other environmental factors, as well as the addition of vitamin B12 as a growth enhancement for *Cladophora glomerata* [6-8]. Nutrient levels in ecosystems can directly impact species diversity. For example, if nutrient availability remains at a scale appropriate to the size of the ecosystem, diversity can increase, but if a system becomes overwhelmed with nutrients, diversity can decline [9].

The algae were present at the start of this study and appeared to be a type of algae or moss. However, as the winter months approached, this species began to take over the growth of the algae that dominated during the summer and early fall months (Picture 1).

Picture 1. Moss-like species *Vaucheria* starting to dominate trickling filter [10]

In light of this evidence, it was decided a taxonomical evaluation was necessary to determine exactly what this species was on the filter. Samples of both species were taken from the trickling filter and pictures were taken under a digital light microscope to aid in the identification process. According to the taxonomy guide, Common Freshwater of the U.S., by Gary Dillard [11], the *Cladophora* species shown in Picture 2 was identified as *Cladophora glomerata.* The moss-like species in Picture 3 was identified as the genus *Vaucheria*.

Unfortunately over 70 species exist within this genus and to identify the species, a knowledge and exhibition of the sexual reproduction is necessary and this is slightly beyond the scope of this study [14].

The algae species *Vaucheria* starts to dominate the trickling filter in the colder months October to November, especially as precipitation increases. This observation is congruent with some of the literature, where the North American precipitation map correlates with the appearance of the genus across the United States [15]. Where the higher amounts of rainfall occur, there is a higher abundance of *Vaucheria*. This was described as a “belt” across the map, from Maine to Washington, spanning the middle-northern part of the country, with less found south of this belt (1938).

Vaucheria is typically described in the literature as growing in mats, with a felt-like texture and a moss-like appearance. It is also well documented in areas such as the British Isles as increasing in growth in cooler and wetter months and is found in both freshwater and marine brackish waters [16].

On the trickling filter at the WWTF, we observed over a one year period that this species of *Vaucheria* has its dominant growth in the colder months. Many types of algae, including *Cladophora* and *Vaucheria* have trouble starting growth in colder months. These particular species present at the WWTF trickling filter must be acclimated to the cold in order to keep growing during winter months. Our observation

Picture 2. Summer Algae [12]

Picture 3. Winter Algae [13]

found out that *C. glomerata* had slowed in growth dramatically and that it may have been lying dormant while *Vaucheria* inhabited the filter growing on top of the *Cladophora* during cooler months. It was observed, as spring and summer approached, that *Vaucheria* started to more slowly disappear, as the *Cladophora* once again became the dominant species. Because the area the WWTF is located in has such predominant cloud cover in the winter months, it can be assumed that the lack of sunlight also does not hinder the growth of *Vaucheria*. Perhaps *Vaucheria* could be a species that could be used in colder/cloudy climates or even grown indoors for biomass. The environmental flexibility of this species could prove economically advantageous in certain circumstances.

3.2 Worms

During the study we discovered that worms live in the S2BR wetland cells. It was observed upon the first sampling of C. *glomerata* that there were worms living in the trickling filter alga as well (Picture 4). These worms must have traveled in the water to the trickling filter from the S2BR media and used the algae as a habitat, consuming the algae and or consuming nutrients from the WW or all of the three.

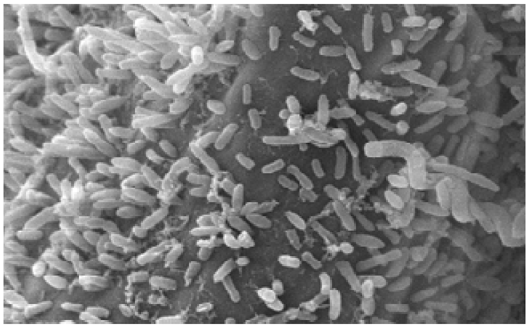
Picture 4. Summer Algae with Worms [17]

Two species were in abundance in the sample collected from a 75 mm by 75 mm plot on the trickling filter. These were identified as common earthworm species *Eiseniella tetraedra* and *Dedrodrilus rubidus*.

In fact, De and Solbe in 1971 identified the exact same worms, *Eiseniella tetraedra* and *Dendrobaena (dendrodrilus) rubida,* growing at two sewage works in Bradford, Yorkshire [18] in other damp habitats and even mountain streams [19].

During this study it was observed that the worms were present from 5% to 20% based on algae mass on the WWTF trickling filter. The worms go through various growth stages, whereupon in the spring months more small worms appear in the algae samples and at the summer months more large and small worms are observed. It can be hypothesized that this may be a part of a reproduction cycle, though different worm species cannot yet be ruled out. In fact, it is not uncommon for both species to exhibit annual changes in numbers, most notably for increased numbers in autumn and winter [18]. During the winter months of this study, small white worms were observed in the algae samples, whereas the large worms are not present. However sampling at the end of the winter and beginning of spring showed an abrupt decline in worms on the trickling filter, little or no worms in the 75 x 75 mm plots could be observed.

A scanning electron microscope (SEM) study found out that the trickling filter media and algae is covered with bacteria (Picture 5) that apparently play an important role in the trickling filter ecosystem and help to remediate the waste water.



Picture 5. Bacteria on Algae [20]

In order to understand what was happening and begin to evaluate the possible relationship between the algae and worms, a stable isotope analysis was needed to follow carbon flows in the system.

4. RESULTS AND DISCUSSION

4.1 Stable Isotope Analyses

To distinguish whether there is a relationship between the worms, algae and WW, a stable isotope analysis was done to determining relationships among and between different groups in the trickling filter ecosystem [4,21].

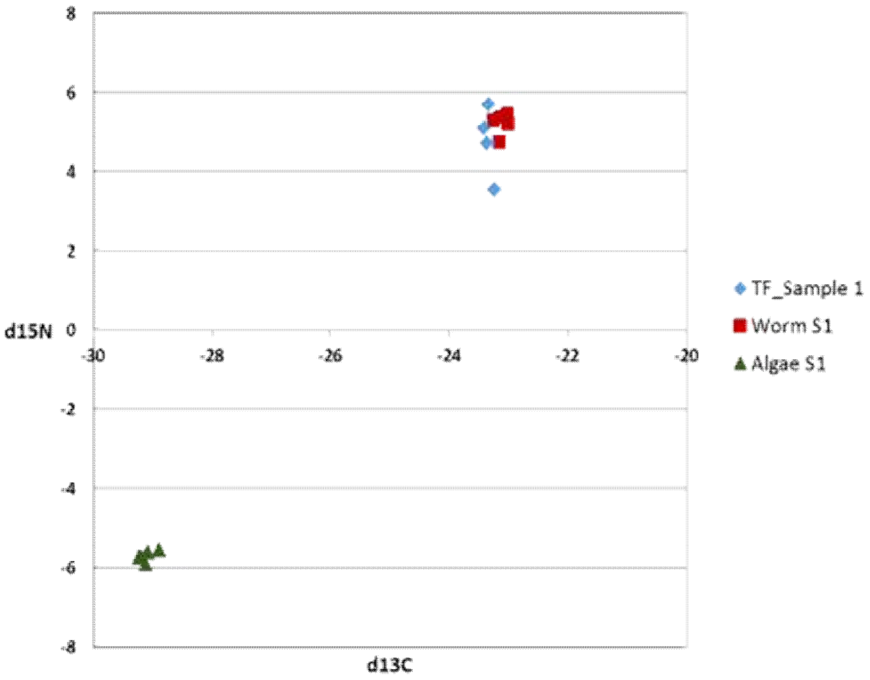


Fig. 2. Trickling filter stable isotope analyses

A stable isotope analysis allows carbon flows in the system to be followed. Stable isotope analysis is a test that measures elements in a sample that have the same atomic number. This means two isotopes of an element have the same number of protons and electrons, but different number of neutrons. They are termed stable because they do not decay. The isotopes commonly measured are those found frequently in nature and they are measured against a universally accepted standard [21]. The analysis provides insight into the food web of the trickling filter, allowing a better understanding of how the worms, the algae species and the WW interact by noting common isotopes that appear in the wastewater at the trickling filter, the worms and the algae and then seeing which isotopes overlap in each sample, makes it possible to analyze the relationship between the three.

The graph in above Fig. 2 shows the results of the stable isotope analysis. It shows that while there is no direct relationship between the worms and the algae, there is a relationship between the worms and the wastewater. This is because the same ratio of elements is found in the worms as are found in the wastewater, suggesting the worms are consuming something from the wastewater. However further understanding the relationship may be more important. Understanding the food web on the trickling filter could help optimize algae growth rates and could provide insight into the complexity of the microbial community. If the worms are consuming and excreting bacteria in the wastewater and the bacteria grows on the algae, then there might be a system or cycle enabling the algae, worms and bacteria to symbiotically thrive. Changing one component in that system may change algal growth rates. A further understanding of the ecosystem on the trickling filter could be of benefit to the waste water treatment facility.

5. CONCLUSION

Stable isotope analyses shows that there is no direct relationship between algae and worms, but there is a relationship between the worms and the wastewater. However the algae are used by the worms as a habitat.

The observation concluded that *C. glomerata* grows during the spring and summer month and starts to fall into a dormant stage in the fall months as the weather becomes colder. Then *Vaucheria* start to take over from its dormant stage during the fall and winter months regardless of sunlight.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Canale RP, Auer MT. Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 5. Model development and calibration. J. Great Lakes Res. 1982;8: 112-125.
2. United States Environmental Protection Agency. National Lakes Assessment [Internet]; 2011. [Cited 2010 Nov 12]. Available:http://water.epa.gov/type/lakes/upload/nla_newchapter5.pdf
3. Correll DL. The role of phosphorus is the eutrophication of receiving waters: A review. J. Environ. Qual. 1998;27:261-266.
4. Peterson BJ, Fry B. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics. 1987;18:293-320.
5. Pitcairn and Hawkes. The role of phosphorus in the growth of *Cladophora*. Water Research. 1973;7:159-171.
6. Anderson RA. Algal culturing techniques. Massachusetts: Elseviar Academic Press; 2005.
7. Lorenz RC, Herdendorf CE. Growth Dynamics of *Cladophora Glomerata* in Western Lake Erie in Relation to some Environmental Factors. Journal of Great Lakes Research. 1982;8.1:42-53.
8. Hoffman JP. Dependence of Photosynthesis and Vitamin B12 Uptake on Cellular Vitamin B12 Concentration in the Multicellular Alga *Cladophora* glomerata (*Chlorophyta)* Limnology and Oceanography. 1990;35(1):100-108.
9. Bracken MES, Nielsen KJ. Diversity of intertidal macroalgae increased with nitrogen loading by invertebrates. Ecology. 2004;85(10):2828-2836.
10. Photo by Klaus Dölle, Moss-like species *vaucheria* starting to dominate trickling filter, jpg-file.
11. Dillard G. Common Freshwater Algae of the United States. Cramer, Berlin. Stuttgart; 1999.
12. Photo by Klaus Dölle, Summer Algae, jpg-file.
13. Photo by Klaus Dölle, Winter Algae, jpg-file.
14. Von Berg KHL, Kowallik KV. Biogeography of *Vaucheria* Species from European freshwater/soil Habitats: Implications from Chloroplast Genomes. *Hydrobiologia.* 1996;336(1):83-91.
15. Prescott GW. A New Species and a New Variety of the Algal Genus *Vaucheria* De Candolle with Notes on the Genus. Transactions of the American Microscopical Society. 1938;57(1):1-10.
16. John DM, BA. Whitton BA, Brook AJ. *The* Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae. Cambridge University Press; 2002.
17. Photo by Klaus Dölle, Summer Algae with worms, jpg-file.
18. De JF, Solbe LG. Aspects of the biology of the lumbricids E. tetraedra (Savigny) and Dendrobaena Rubida (Savigny) F. subrubicunda (Eisen) in a percolating filter. Journal of Applied Ecology. 1971;8(3):854-867.
19. Shen JH, Tsai S, Tsai C. Occurrence of the earthworms Pontodrilus litoralis, (Grube, 1855) Metaphire houlleti, (Perrier, 1872) and Eiseniella tetraedra (Savigny, 1826) from Taiwan. Taiwania. 2005;50(1): 11-21.
20. Photo by Klaus Dölle, Bacteria on Algae, jpg-file.
21. Sulzman EW. Stable isotope chemistry and measurement: a primer. In Stable Isotopes in Ecology and Environmental Science (Lajtha K, Michener RH, eds.), Blackwell Publishing, Oxford; 2007.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=930&id=39&aid=9122