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Application of Algae as a Biomass Feedstock Source at a Waste Water Treatment Facility

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Authors' contributions

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

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ABSTRACT

Algae species *Cladophora glomerata* and Vaucheria are growing in a trickling filter in the secondary treatment process at a Waste Water Treatment Facility. This study investigates the role of the algae and its potential application a as biomass feed source for renewable energy production with and without growth media. Utilizing different growth media, growth rates were found to be the highest on Styrofoam and plastic media. *Phragmites australis* bundles were found to have the most pragmatic application for both growth and harvest. *C. glomerata* exhibited the highest amounts of lipids, or total organic extracts (TOE).

Keywords: Algae; autotrophic; lipids; total organic extracts; eutrophication; and phosphorus.

1. INTRODUCTION

Algae of the species *Cladophora* is growing on a trickling filter in the secondary treatment process at the Village of Minoa Waste Water Treatment Facility (WWTF) in Central New York State. In addition, the common earthworm species *Eiseniella tetraedra* and *Dedrodrilus rubidus* can

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be found living in the algae of the trickling filter [1]. The *Cladophora* algae species has been found in the northeastern portion of the United States, including Lake Michigan and some of the other Great Lakes, since the 1970's, mainly due to anthropogenic sources [2]. With the 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 nutrient levels in streams and lakes, changing them from oligotrophic to eutrophic.

Cladophora will typically appear when phosphorus levels in the water are high. It is safe to assume that human lifestyles and agricultural 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, there are many different forms of phosphorus including but not limited to: orthophosphate (the only form autotrophs can absorb), pyrophosphate, longer-chain polyphosphate, and organic phosphonates [3]. Hence, the reasons for eutrophication can be quite complex.

At the WWTF, a municipal sewage flow of approximately 950,000 l/d is first directed into a primary clarifier. After primary treatment, half of this flow is directed to a constructed wetland. Making up the constructed wetland are 3 cells which the water travels through to filter out organic solids and some nutrients. The complex root system of Phragmites and other species in the cells help absorb nutrients and filter the water. The other half of the primary clarified water flows directly into the headbox of the trickling filter where it mixes together with the treated water from the constructed wetlands. The partially treated waste water from the influent box is then forwarded into a trickling filter. After the trickling filter treatment, the waste water passes through a clarifier and a disinfection unit before it is discharged into a stream [1].

The role of algae in a wastewater treatment facility as a means of water treatment and the possibility of utilizing algal biomass for renewable energy production requires identification of algae and their general characteristics in order to decide how to bio-engineer the species for energy. John, Whitton, and Brook provided an identification guide to freshwater species in 2002 [4], while Vincent Turano provided an overview of *Cladophora glomerata* [5]. In 1938 Prestcott identified a new variety of the genus Vaucheria [6]. Schagerl, and in 2008 Kerchbaumer, studied Vaucheria species with regards to sexual reproduction and morphology [7]. Geographic information was provided about Cladophora with studies by Gordon et al. [8] in southwestern Australia. Simons in 1975 offered information about Vaucheria in the Netherlands, bringing information about the effect of different depths of water on growth [9], while Von Berg and Kowallik in 1996 wrote on similar chloroplast genomes among Vaucheria species found in different geographic locations [10]. Wang et al. [11] studied micro-algal growth on manure. The by-products of an anaerobic digester feedstock of manure were used as substrate to grow the algae. They discovered that algae increased the quality of the byproducts of digestion. Zhang et al. used food waste as feedstock in an anaerobic digester and the yield of methane was particularly high with this approach, especially with vegetables versus meat or rice [12]. Sperling and Grunewald in 1969 found *Mastigocladus laminosus*, a thermophilic algal strain, was able to uptake phosphorus in an artificial stream [13]. Lee *et al.* proved that Algae and worms contain lipids that are used to store energy of the organisms [14]. Vieler *et al.* described that green plant cells typically contain 3 glyceroglycolipids and one phospholipid, typically phosphatidylglycerol [15]. Lodish and Zipursky describe that there are two main portions of a phospholipid, the hydrophilic head and the hydrophobic tail. The tail often made up of fatty acid chains, while the head is made up of phosphate groups [16]. Angelidaki and Ahring and Cirne et al. discussed anaerobic digestion of lipids and how an increase in lipid content would affect methane production [17,18]. This study concluded long-chain fatty acids could actually hinder methane production in an anaerobic digester due to potential mass transfer limitations. This leads to the conclusion that perhaps small amounts of lipids are more desirable as not to inhibit methane production, and algae biomass might be more profitable and usable from species that contain fewer lipids. With increased possibility and potential opportunity for methane production, the lipids should also be evaluated for use as a renewable energy, such as liquid biofuels. Demirbas in 2010 had many great arguments as to why algae should be considered for biofuels production [19]. In 2007, Vieler et al. [15] found different ways to analyze the different types of lipids present in an algal strain by way of matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) and thin-layer

chromatography (TLC). Lee et al. [14] took the approach of using algae for biofuels and addressed its feasibility by studying how to extract lipids, finding the chloroform/methanol method to be more effective and practical than Red Nile staining. Matsunaga et al. [20] investigated a microalgal strain *Scenedesmus* sp. for biofuel applicability in comparison to current-use fuels and found the calorific value to be equivalent to coal one of the reasons for this study was to investigate if algae can help to treat phosphorus and nitrogen-rich wastewater and at the same time can be used as feedstock for biofuel production. At the WWTP it is anticipated in the near future to use a thermophilic digester for biofuel production. Perhaps Cladophora in combination with the worms could be grown at the facility, providing biomass feedstock and an inexpensive media for phosphorus removal from the waste water.

The following study explores the potential of algae growth on a trickling filter, algal and invertebrate (worm) lipids for biofuels that can be used as biomass feed sources for energy production.

2. METHODOLOGY

This section describes the different methods used to determine, fulfill and understand the research project objectives for the algae growth rate characteristics of *C. glomerata* and Vaucheria using various substrates, including lipids content.

2.1 Sample Collection and Preparation

Sample collection for the algae and worms at the WWTP was carried out by collecting the samples in one gallon zip lock bags. The samples were brought back to the laboratory and subsamples of 15 grams of the algae and worms were prepared. The prepared sample size of the wastewater, algae, and worms was 2-3 times what was needed.

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

2.2 Lipid Analysis

The lipids were extracted using a *modified* version of Bligh and Dyer [21]. The collected samples were frozen and lyophilized overnight. Test tube tare weights were recorded, and then the dry sample weight recorded. Approximately 20 mg of the dried algae sample was put in each test tube. A ratio of 1:1 dichloromethane and methanol was used. Dichloromethane was used in place of chloroform for safety reasons. The test tubes were then placed on a vortex for up to 5 minutes, or until thoroughly mixed, followed by centrifugation at 1,000 rpm for 5 minutes. After centrifugation, the solvent was removed with a clean glass pipet. This procedure was repeated 5 times to ensure complete extraction, using 5 ml of clean solvent mixture for each cycle. The extract was then transferred to a pre-weighed round-bottom flask, and the solvent was removed using nitrogen. The total organic extracts (TOE) were re-dissolved in the minimum DCM required to quantitatively transfer to a clean vial fitted with a Teflon cap. The solvent was then removed, with nitrogen gas as the drying agent, under room temperature to produce the dried TOE, which was measured simply as a dry weight.

2.3 Growth Rate of Various Substrates

For this study, sugar maple wood chips, sugar maple solid wood block, recycled cardboard, Styrofoam and plastic bags were used as growth media. The growth media was filled in 28.6 g small plastic nets. The plastic nets' original use was to hold fruits and groceries from a supermarket. The dry weight of the nets with growth media was taken before and after a 24 h water soaking. After soaking, the nets were placed on the trickling filter Brentwood media and the algae growth was observed during a 30 days trial period.

2.4 Growth Rate in Trickling Filter Media

For a growth rate study of the Brentwood media, samples were collected directly from the media itself. Because the perforation in the media is cut and measured identically, 6 squares down and 6 squares across (approximately 1 inch per square) and 3 squares down and 3 squares across (3x3) were picked out and weighed in the laboratory. These weights were then recorded and divided by the number of days in between sampling for the growth rates.

2.5 Growth Rate by Agar Media

For the trickling filter growth study, three different types of agar were used as growth media for the well plates that were inserted into the trickling

filter. The well plates were rectangular in shape, possessing 4 longitudinal wells over an area of approximately 15 square inches. When turned vertically and attached to a horizontal base, they could stand upright on the trickling filter independently, and the water could run over the steep surface of the agar, held back by slight bumps to generate a laminar flow. The well plates were sterilized before agar was fixed onto the well plates.

The agar materials Sigma-Aldrich Phytagel™, Sigma Aldrich Type E, and Fisher Sceintific BD™ Bacto™ Dehydrated Agar were prepared in a 500 ml beaker with a consistency of 1.2% for the Phytagel™ agar and 2% for the Type E and Bacto™ agar. 0.2 g Sima-Aldrich Thiamine Hydrochloride (B1) was added to the Phytagel™ agar and 0.1 g of Thiamine Hydrochloride (B1) to the Type E and Bacto™ agar. After each agar solution was fixed onto each well plate, they were labeled appropriately, and an initial weight was taken to monitor algae growth.

2.6 Growth Rate of Phragmites

For this study, bundles of dried *Phragmites australis* stems were assembled. First they were separated from leafy counterparts and chopped into 100 mm long segments. Then half of the cut stems were husked from their outer dead shell, the other half left with the husk intact. These segments were then bundled together with burlap rope and weighed separately. They were soaked for 48 hours in water and weighed again afterwards. This was to simulate any water weight that would accumulate during their time in the trickling filter. They were placed equidistant around the trickling filter and allowed to grow for 30 days, then were collected and a wet weight was taken.

3. RESULTS

3.1 Lipids

A lipid analysis of the *Cladophora glomerata* and *Vaucheria* algal species and worms was conducted to investigate the TOE content. A total amount of 400 mg were used per sample to determine the TOE. Fig. 1 shows that the alga species Cladophora had the highest amount of TOE with 139.3 mg, followed by the worms with 100.5 mg and Vaucheria with 97.2 mg. The worms exhibited a higher amount than predicted, and perhaps they could be studied further as a lipid source. The TOE is comprised of all the lipids in the sample, i.e., phospholipids, triglycerides, fatty acids, among many other types. All of these lipids are usable in the anaerobic digesting process that is envisioned for the future at the wastewater treatment facility.

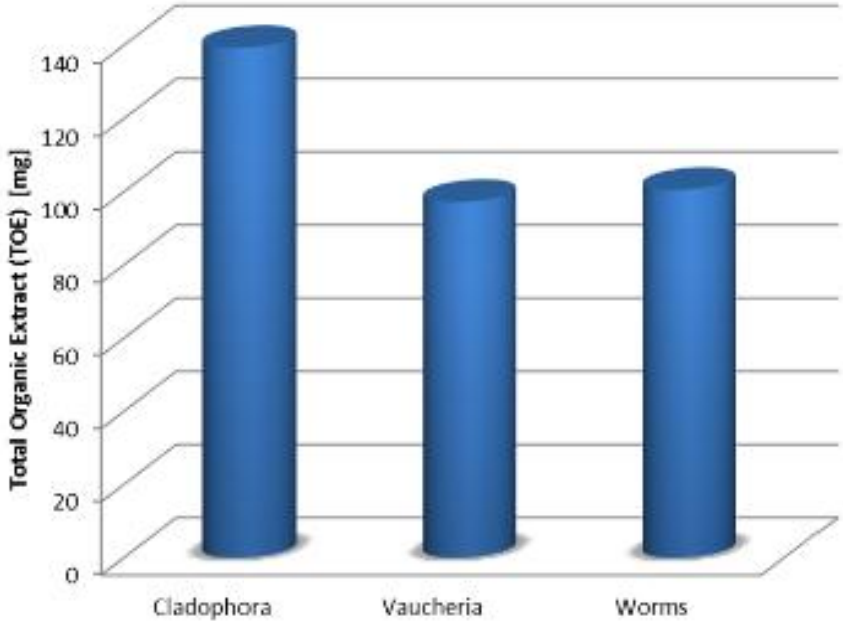

Fig. 1. Lipids content

3.2 Growth of Substrate

Substrate growth rate is shown in grams of algae growth per day based on a 30 day growth rate experiment in Fig. 2. The Styrofoam peanuts had the highest growth rate with 2.27 g/day, followed by plastic bags with 6.19 g/day, softwood wood blocks with 4.26 g/day and sugar maple hardwood wood chips with 2.27 g/day, while the cardboard had the lowest growth rate with 0.84 g/day. The amount of substrate was weighed before and after the study.

3.3 Growth Rate in Trickling Filter Media

Growth rate of the algae specie *Cladophora glomerata* and Vaucheria on the Brentwood trickling filter media was studied over a 30 day period in January. Since the Brentwood media used in the trickling filter is perforated in a square grid-like fashion, it was decided to take consistent measurements of the algae in larger 6 inch x 6 inch plots containing both algae species, and each algae species in a sample 3 inch x 3 inch plot.

Fig. 3 shows the results of the measurements over a 1 month period. The sample containing both algae species showed a 14 g/d increase whereas the sample of Vaucheria and Cladophora showed a decline in algae mass of 1.86 g/d and 0.55 g/d respectively.

3.4 Growth by Rate Agar Media

For this 91 day algae growth study Phytagel, Type E and Bactor Agar was used with and

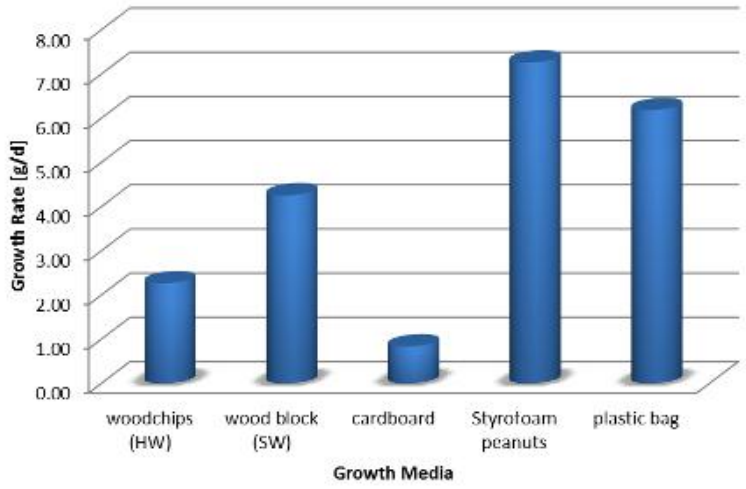

Fig. 2. Support media growth rate

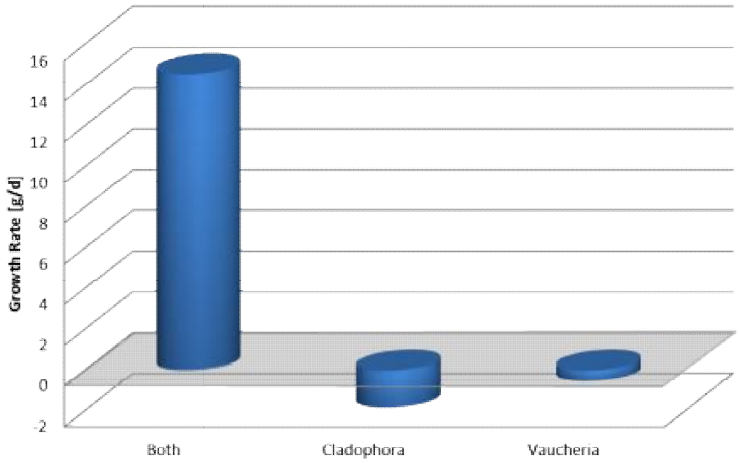

Fig. 3. Trickling filter media growth rate

without thiamine to investigate if agar could be used as a growth media for algae growth. Agar is normally used for plant tissue culturing, but might allow an easy way to grow algae and at the same time use both the algae and growth media as a biomass feed source for anaerobic digestion. Results in Fig. 4 show that the highest growth rate of 0.30 g/day was achieved with Phytagel agar with thiamine added. The second highest growth was the Type E agar with thiamine at a growth rate of 0.19 g/day followed by Phytagel agar without thiamine at 0.18 g/day and Bacto Agar with thiamine and Agar Type E with thiamine at 0.06 g/day and 0.04 g/day respectively. The lowest growth rate was recorded for the Bacto Agar with thiamine at 0.013 g/day.

3.5 Growth Rate of Phragmites

The reed *Phragmites australis* was selected as growth media for this study. It is available in abundance in Central New York State and can serve multiple purposes. If algae grows on the prepared Phragmites bundles, the bundles could provide additional biomass for an energy conversion system. Fig. 5 shows the algae growth on the Phragmites bundles that were placed on the trickling filter for a 30 day trial period. Half of the bundles were husked and half left with the husk on to observe any notable differences.

Phragmites australis with husk had a growth rate of 7.62 g/day and without husk a growth rate of 6.62 g/day.

4. DISCUSSION

Microalgae have a promising future in biofuels production. When studying algae, it is important to measure the lipids [17]. Typically, micro-algal species are known to produce more lipids that macro-algal species, but if there is a macro-algal species that can compete with the lipid content of a micro-algal species, then perhaps that species can be a multi-purpose endeavor and contribute biomass for other biological/biotechnological benefits aside from just lipids. Therefore algae, from a multi-purpose perspective, should not be overlooked. It is appropriate when working with any algae species to test the lipid content, whether directly relevant to the study or not. The lipid contents of this study are at the lower end with 25% to 30%, compared to those of other species such as Botryococcus braunii or Schiochytrium with a reported ranges between 25-75% and 50-77% respectively [22]. Extraction rate can vary between 7 and 93% based on the algae strain and extraction method used. However, it is still be worthwhile to verify the species studied if they can be used as a biofuels strain [20]. For the relevance of this study, the lipid analysis provides information if it is worthwhile to digest algae and worms separately or together. The addition of lipids can increase methane production throughout the digestion phases of hydrolyses, acidogenesis, acetogenesis and methanogenesis. However, lipids can also cause operational problems in anaerobic digesters due to clogging, and may also cause mass transfer problems for soluble substrates because they can become absorbed by the microbial biomass surface [18]. Another study conducted in 1992 showed how long chain fatty acids in the anaerobic digestion of cow manure actually *inhibited* anaerobic digestion by stating that oil can cause inhibition of bacterial growth and biogas production. Slow, continuous feed to biogas reactors is recommended to “allow adaption and maintenance of bacterial population capable of long-chain fatty acid degradation to prevent accumulation of high concentrations of long chain fatty acid [17]. According to Figs. 2 and 3, the highest growth rates of algae occurred on Styrofoam peanuts and Phragmites as substrates. The Styrofoam peanuts had an average growth rate of 7.26 g/day, while the Phragmites without husks had 6.06 g/day and the Phragmites with husk exhibited the top growth rate of 7.62 g/day on average. It should be noted that there is also a possibility that the texture of the Styrofoam of the Phragmites husks are the key attribute. It has been observed during this study that there is a trend in the type of surface the algae prefer to grow on. Hard or rough surfaces appear to be favored over soft and porous surfaces. The rough surface of the husk could allow the filaments to adhere easier versus the smooth surface exposed with the husk removed. Some data observed are consistent with that from other studies. For example, a German study harvested Phragmites *australis* from a lakeshore, cut and placed the reeds into the water, and observed algae growth on the stems in abundance of *C. glomerata* during the summer months [23]. Dow Chemical, in 1962, described Styrofoam to be relatively unaffected by temperature fluctuation and incapable of sustaining mold growth, and therefore can be used as an appropriate and popular substrate for algae growth [13]. Many people have used Styrofoam as a preferred platform for algae growth in ponds due to its

ability to withstand temperature extremes as well as its buoyant nature [24]. The agar media used did not sustain the growth rates that were predicted. Typically agar is used for plant tissue culturing and not looked to for algae growth. The reason behind the proposal of using agar was to find an easy way to harvest the algae and use of a biodegradable substrate in the anaerobic digester. The highest growth rate was 0.3 grams per day on Phytagel with thiamine addition. In retrospect, one hypothesis is that the agar pH may have been too low, given that the trickling filter water pH was 8.00, respectively. Many of the initial experiments were started with burlap, which proved to be a worthy substrate for algae growth. However, by time the algae acclimated and started growing, the microbes in the waste water had already started to decompose the burlap and started to clog the trickling filter media. This made it difficult to harvest the algae and caused operational problems due to the clogging of the trickling filter media. Therefore, burlap was not considered for this growth study. In considering a substrate, it is essential that the substrate can be easily placed in and out of the trickling filter, while not prohibiting or slowing the trickling filter function. While the algae need to have a strong affinity for the substrate, it should also be easily removable. This is the case for the chosen growth media. However, preprocessing steps need to be implemented to remove non biodegradable growth media such as Styrofoam and plastic bags, whereas *Phragmites australis* do not need to be removed during the preprocessing step. One benefit of the *Vaucheria* growth in the winter on the trickling filters Brentwood media is that alga can easily be removed by hand by simply picking it up. One person can traverse the trickling filter by foot and collect the grown algal biomass.

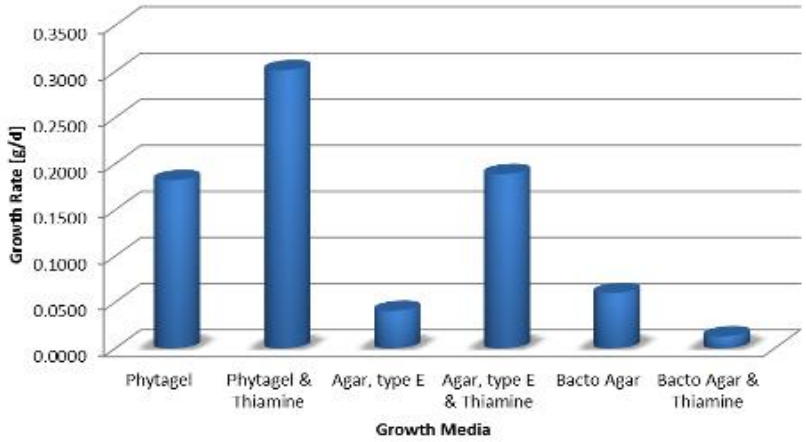

Fig. 4. Growth rate of algae on agar substrate

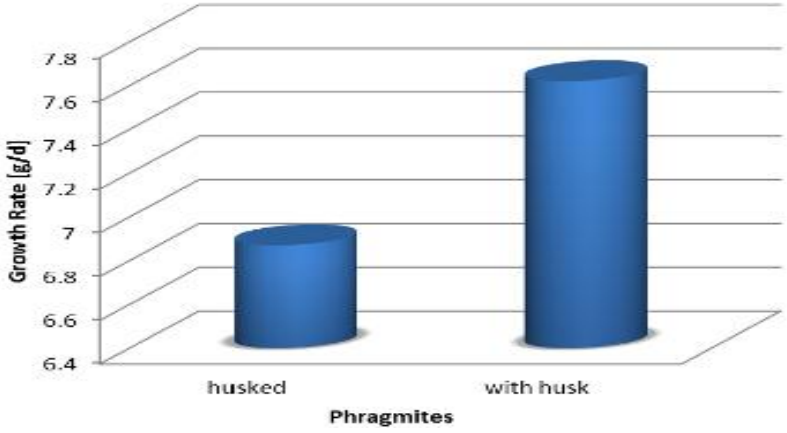

Fig. 5. Growth rate phragmites

5. CONCLUSIONS

Algae can provide a possible biomass source for future fuel and energy production at the Minoa Wastewater Treatment Facility. This study showed that the utilization of algae as feedstock for anaerobic digestion is just one of many biomass energy alternatives for municipalities operating a water treatment facility.

Styrofoam showed to be the best growth media, followed by Pragmites and plastic bags. Commercially available trickling filter media showed good performance in regards to algae growth, but harvesting might be difficult due to seasonable changes.

Agar media did not reveal the anticipated growth rates and is complicated to prepare and monitor. Burlap proved to be a good growth media, but decomposition revealed operational difficulties and provided no advantage to the commercially used trickling filter media.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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