Biosorption Studies of Acid Green 3 Dye

By

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An Undergraduate Thesis Submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Baccalaureate of Science in BioResource Research,
Water Resources

May 23, 2012

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Abstract

Current methods employed in the textile industry are unsustainable because they use an abundance of water in the dying process, and discharge the waste into the environment, causing adverse health effect on aquatic life and local communities. The technology that is in place to remove dye from textile effluents is very expensive and energy intensive. Therefore it is most economically beneficial for textile industries to dump the waste before treating it. This study investigated the potential use of two macro-algae, *Palmaria mollis and Fucus vesiculosus,* and Red Alder biochar to adsorb Acid Green 3 dye from textile effluent. Batch experiments were conducted under different temperature, pH and salinity conditions to determine the adsorption capabilities of *P. mollis, F. vesiculosus* and Red Alder char. *F. vesiculosus* proved to be the most effective sorbant, removing 60% of AG3 dye in a 10g/L dye solution over a course of 8 hours in a saline solution.

Introduction

Many areas of the world, especially developing countries, are experiencing physical and economic water scarcity (Fig. 1). One third of the world's population is suffering from a lack of available water, and water crises in China, India and the United States have become an impending problem (Harvey, 2006). One quarter of the world's population experience physical water scarcity, where groundwater levels have fallen and drought is common due to weather and over-use (Harvey, 2006). One billion people face economic water scarcity, because they lack the infrastructure and/or technology to obtain water. Within the next fifty years, the world population is expected to increase 40-50%

(World Water Council, 2011) and this will lead to greater demands on fresh water. Not only is an increasing population adding to worldwide water stress, but industrialization will lead to greater demands for water, resulting in more permanent water shortages. Many industries already consume an abundance of water and pollute water sources that could have been directed to drinking and sanitary purposes.

The textile industry is one of the most water intensive industries, consuming 80-200 m^3 of water per ton of product and producing 1,650 m^3 of wastewater per day. (Ranganathan, 2007 and Gruwez et al., 2009). Wastewater from the textile industry is one of the most polluting of all industrial effluents (Aksu and Tezer, 2005). Much of the water that is used in textile industries is not reusable and difficult to treat because it is contaminated with dyes or color pigments with complex structures (Aksu and Tezer, 2005; Padmesh et al., 2005).

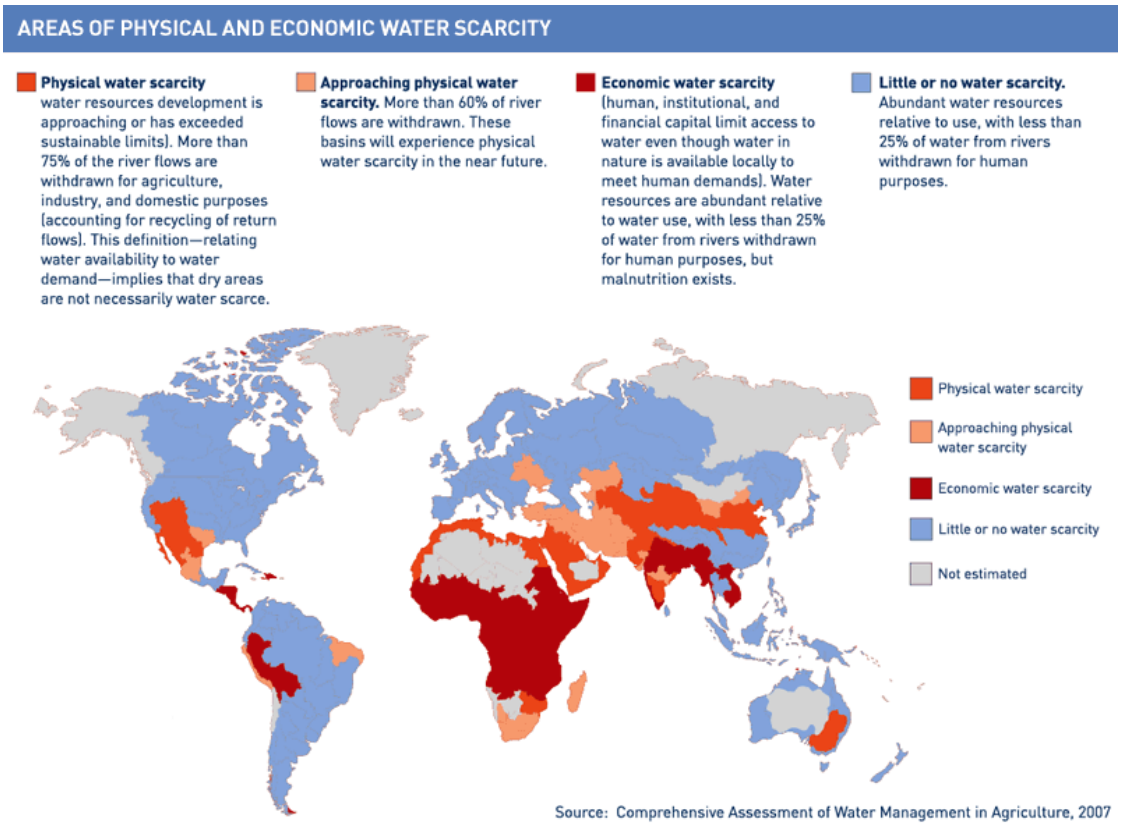

Figure 1. Map of countries facing economic and physical water scarcity

Dye molecules are composed of chromophores and auxochromes. Chromophores are responsible for giving dye their color by absorbing certain wavelengths of light and reflecting others, and auxochromes are responsible for the molecules' solubility and affinity toward fibers, often intensifying the color of the compound (Gupta and Suhas, 2009). Dyes are classified according to their structure, solubility and application to the fiber (Gupta and Suhas, 2009). Some of the most common types of dyes used in the textile industry are acid dyes consisting of azo, anthraquinone and triphenylmethane chromophores (Gupta and Suhas, 2009). Most acid dyes are water-soluble anionic dyes used for nylon, wool, silk, modified acrylics, paper, leather, food and cosmetics (Gupta and Suhas, 2009). Acid dyes are resistant to degradation due to their stable aromatic, structure, can easily pass through most traditional water treatment systems, and are

therefore of most concern in industrial waste waters (Aksu, 2005). This is why our studies focused on removing the Acid Green 3 (AG3) dye from textile effluent. Since most textile industries are located in developing countries, with often lax regulatory enforcement, most of the effluent water is discharged without/with partial treatment into local water bodies, due to the high cost of treatment. Unfortunately, this puts the local communities and our planet at risk. Certain dyes can cause adverse health affects such as allergic dermatitis, skin irritation, cancer and mutations (Aksu, 2005). Even in small quantities, textile dyes discolor water, making it highly visible and aesthetically unappealing. In addition, they also negatively affect photosynthetic activity of aquatic life and ecosystems, by blocking the transmission of light (Aksu, 2005).

Current technologies to treat textile effluent include reverse osmosis, biological oxidation, coagulation and activated carbon. Unfortunately these methods suffer from limitations like high-energy demand, high cost, slow dye removal process, a large amount of chemical requirements, and hazardous byproducts (Aksu, 2005; Padmesh et al., 2005; Vilar et al., 2007). Reverse osmosis forces water under pressure through a membrane that is impermeable to contaminants, but the process is very expensive and energy intensive (Gupta and Suhas, 2009). Biological oxidation process involves effluent water treatment using aerobic and anaerobic bacteria, is expensive, and produces sludge as a byproduct (Gupta and Suhas, 2009). Alternatively, the dyes can be precipitated by the addition of aluminum, calcium and iron ions to the dye effluent. However, this process cannot be used to remove the majority of dyes used in textile industries, and results in formation of sludge with high disposal cost (Gupta and Suhas, 2009). Activated carbon effectively adsorbs synthetic dyes but is expensive which reduces its adaptability. It cannot be

regenerated in a cost effective manner and is not viable at large scales (Aksu, 2005).

Due to the limitation of existing technologies, it is therefore necessary to look into low cost alternatives that could increase water use efficiency by recycling water and reducing effluents. A potential alternative to current technologies is to use the biosorption capabilities of dead algal biomass to adsorb the dyes from textile industry effluents (Mehta and Gaur, 2005; Padmesh et al., 2005). Biosorption is an appealing solution, as it has been demonstrated to have good removal rates of heavy metals, is low cost and uses renewable materials. Biosorption of dyes is a surface process in which the anionic acid dye is bound chemically to active groups in the algal cell wall surface (amino, sulfate and carboxyl) (Ofer et al., 2003). Adsorption is accumulation of dye at the surface of the algae. This is thought to occur in a two step process: an initial rapid process that occurs within minutes to hours, followed by a slow process that can take several hours to a day.

In many adsorption processes involving algae, the algae act as an ion exchanger: an ion from a solution is exchanged for a similarly charged ion attached to an immobile particle (Gupta and Suhas, 2009). Unfortunately, the biosorption process occurring in macro-algae is not well understood, and the roles played by the functional groups of algae have not been thoroughly studied. However, there are hypotheses of how the mechanism works. For example, carboxylate groups of alginate have been identified as the main metal binding sites; therefore these sites may be the binding sites for dye as well (Davis et al. 2000). At pH, 3 carboxyl groups are protonated and as such are positively charged; these positively charged COO groups can attract the negatively charged AG3 dye molecules, allowing for binding (Hazrat and Muhammad, 2008).

In contrast, the Volesky, indicates that many groups (hydroxyl, carboxyl, sulfonate) are neutral when protonated (Volesky, 2003). When the pH of these groups exceeds their pKa, these groups become available for the attraction of cations, which may explain why metals bind to algae. The amine groups are positively charged when protonated and therefore attract anions if the pH is lowered. Since Acid Green 3 dye is an anion, it is possible to hypothesize that the amine group is responsible for AG3 dye uptake. Volesky also state's that in the case of dye with SO_3^- adsorption by chitin, when chitin amide groups were protonated with a positive charge, the anionic sulfate group of the dye could bind to the positively charged chitin amide groups. The same could be said with AG3 dye and macro-algae, since the dye possess SO_3^- groups.

Examples of biomaterials that have been used in different biosorption studies include banana peels, coconut husk, charcoal and algal biomass due to their abundance and low cost (Aksu, 2005). Dead biomass is often preferred in wastewater treatment processes because dead biomass is not affected by toxic waste, does not require continuous nutrient supply and can be regenerated/ recycled. Dead cells can also be stored for a longer period of time and they accumulate contaminants at a greater rate than living cells (Aksu, 2005).

In addition to algal biomass, biochar produced from pyrolysis of biomass has been investigated for its potential to remove heavy metals and organic contaminants through biosorption (Sun et al. 2011). Biochar is formed "through partial or complete carbonization of biomass precursors, with the final physical –chemical properties depending on their heat treatment temperature" (Sun et al. 2011). Biochar has been used to sequester carbon into soil, improve soil quality and reduce greenhouse gas emissions

(Sun et al. 2011). Chars formed at low temperatures (200-300 °C) are only partially carbonized and have low surface area, whereas chars produced at high temperatures (500-700°C) are well carbonized, with higher surface area (Sun et al. 2011). Recent sorption studies indicate that the ability for charred biomass to adsorb dye depends on the pore size, surface area, polarity and functionality of the biochar, and the structure, hydrophobicity and type of contaminant sorbed (Sun et al. 2011).

In general, biosorption capacity depends on biomass type, preparation and specific surface properties such as the functional groups on the biomass, adsorbed molecules/ions, and environmental conditions such as pH and temperature (Aksu, 2005).

Objectives:

Most biosorption studies in literature have focused on the removal of heavy metals from wastewater (Davis et al, 2005, Mehta and Gaur, 2005, Ofer et al, 2003). We hypothesized that it would be possible to use the similar principles to remove anionic acid dyes through biosorption mechanisms. Therefore the overall objective of this study was to investigate the potential for treatment of textile industry effluent water contaminated dyes using algae and biochar. Specific objectives of this work were to:

- Determine optimum pH and temperature conditions for maximum dye adsorption using algae and biochar.
- Conduct batch experiments to determine dye adsorption potential of macro-algae and biochar.

Removing dye from textile industry effluent waters could make this industry more sustainable, as this would lead to reuse of water and to a reduced need for fresh water input, and thus increase overall water use efficiency.

Acid Green 3 (AG3) dye as a model dye:

Acid Green 3 (AG3) dye, also known as Guinea Green B dye, was chosen as a model dye in this research (Fig. 2). The AG3 dye is an anionic triphenylmethane dye, which is considered to be one of the main offenders of environmental pollution by dyes (Kim et al., 2008). Triphenylmethane dyes are animal carcinogens, and certain triphenylmethane dyes may promote tumor growth in some fish species (Hazrat and Muhammad, 2008). AG3 dye was used for the experiments because acid dyes are most widely used within the textile industry and they are one of the most difficult types of dye to treat.

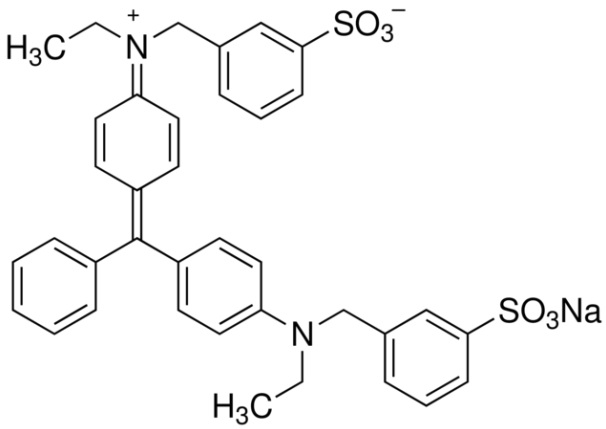

Figure 2. Chemical Structure of Acid Green 3 Dye

Palmaria mollis

The first set of batch experiments conducted used *Palmaria mollis* (*P. mollis*), also referred to as dulse, which is a red marine macro-algae found on the coast of Oregon, to adsorb AG3 dye. This alga is used as feed for abalone, and no other significant uses have been reported (Demertopoulos and Langdon, 2004).

Fucus vesiculosis

F. vesiculosus (*F. vesiculosus*), commonly known as bladderwrack, is a widely available brown marine alga located in the Atlantic and Pacific Ocean, North Sea and Baltic Sea. *F. vesiculosis*, was used extensively in our biosorption experiments because

brown macro-algae have been reported to have the highest adsorption capability of anyt type of algae. The high adsorption capability has been attributed to the presence of alginate (alginic acid) in the cell walls of brown algae. Alginate is an anionic polysaccharide and contains 1, 4-linked B-D mannuronic and α-L-guluronic acid (Li, 1998). The carboxyl group of alginate also plays a vital role in heavy metal uptake by the brown macro-algae *Sargassum fluitans*, which many play a similar role in uptaking dye (Li, 1998). In addition, brown algae contain high concentrations of sulfated polysaccharides and alginic acid, which enable these marine algae to selectively adsorb ions through ion exchange mechanisms (Ofer et al., 2003). Therefore dye binding to the surface of brown algae is based on ion exchange and competition on the algal cell wall binding sites between dyes and hydrogen ions (HCl) (Ofer et al., 2003).

Materials and Methods:

Material collection and preparation

Palmaria mollis collection and preparation:

Palmaria mollis was collected in Newport, Oregon and was brought back to Oregon State University. The samples were immediately washed with distilled water and dried in a convection oven at 50°C for 4 hours. After drying, the algae was pretreated by washing with distilled water and 0.1M HCl and dried in a convection oven at 50°C. The acid protonation using 0.1 M HCl was performed to increase algae adsorption capabilities and to remove any ions from the seawater (Padmesh et al., 2005). Acid protonation induces structural changes in the cell wall, which increases adsorption capacity of the algal biomass (Ofer et al., 2003). The dried algae were ground to a size of less than 2mm using a knife mill.

Fucus vesiculosus collection and preparation:

Fresh samples of *Fucus vesiculosus* were collected at the Hatfield Marine Science Center in Newport, Oregon. The samples were washed, pretreated and ground in a knife mill as described above.

Red Alder char preparation:

John Meidema in Philomath, Oregon donated Red Alder biochar for our experiments. Two different types of char were used in the batch experiments, a low temperature Red Alder char (300°C) and a high temperature Red Alder char (600°). Untreated Red Alder char, kept in its natural state, was used for the first set of batch experiments. The second batch experiments used treated Red Alder char that was washed with distilled water and 0.1M HCl and dried in a conventional oven at 50°C for 4 hours.

Determination of Calibration Curves

One of the practical methods to measure dye concentration is to measure the absorbance of the dye solution at 635 nm in a spectrophotometer. However, the absorbance is dependent on the dissociation of the dye, which in turn depends on the media pH. Therefore, calibration curves were constructed at different dye concentrations (0-50mg/L), under different media pH (2-7) conditions. The calibration curves were determined by linear regression of the absorbance data and dye concentration at a particular pH. The equations were used to determine dye concentrations from absorbance at 635 nm in all experiments.

Batch experiment set 1 with *P. mollis*:

The objective of this set of experiments was to determine the optimum pH for *Palmaria mollis* for maximum dye adsorption. All batch experiments were conducted by adding 0.5g of dried *P. mollis* to 150mL containing 1g/L of dye in 250 mL Erlenmeyer flasks. The flasks were agitated at 150rpm for 27 hours and samples were taken at 0, 30, 60, 90, 120, 240 and 27 hours, diluted 20-400 times and the absorbance was measured at 636nm using a spectrophotometer.

Batch experiment set 2 with *Fucus vesiculosis*:

The objective of these experiments was to determine the optimum temperature for maximum dye adsorption by *Fucus vesiculosus (F. vesiculosis).* All batch experiments were conducted in Erlenmeyer flasks containing 20g of dried *F. vesiculosus* in 150mL of solution at pH 3 containing different dye concentrations. In the first set of experiments, a two factor completely randomized block design with dye concentrations (2.5 and 10 g/L) and temperature (30°C, 35°C, and 40°C) were the factors with a total of six treatments. In the second set of experiments, a two factor completely randomized block design with dye concentrations (2.5, 5 and 10 g/L) and media salinity levels (distilled water and salt water with 30g/L Instant Ocean Sea Salt) were the factors with a total of six treatments. All treatments were conducted in triplicates. The flasks were agitated at 150rpm for 8 hours and samples were taken at 0, 30, 60, 90, 120, 240 and 480 minutes, diluted 20-400 times and measured at 636nm using a spectrophotometer.

Batch experiment set 3 with Red Alder biochar:

The objective of these experiments was to determine the optimum pH for maximum dye adsorption by Red Alder biochar. Batch experiments were conducted

using 10g of dried Red Alder char in 150mL of solution containing 2.5g/L of AG3 dye in Erlenmeyer flasks. Six treatments consisting of three pH levels (pH 3,5,7) and two different types of biochar (high and low temperature Red Alder biochar) were conducted with two sampling times at 4 and 24hours. Another set of experiments were also conducted using pretreated biochar to determine the impact of pretreatment on the biochar dye adsorption capacity.

Analysis:

Langmuir adsorption isotherms were used to determine the equilibrium dye concentrations. Dye uptake (q) was calculated as: $q = V(C_i - C_f)/S$ where v is volume of the dye solution, C_i is the initial concentration and C_f is the final concentration and S is the amount of algae added into the dye solution. The Langmuir isotherm is expressed as: $q = q_{max}\, bC_f / 1 + bC_f$ where (q_{max}) is the maximum amount of dye adsorbed and b is a measure of the biosorption efficiency of the biomass.

For statistical analysis of the batch experiments, a one-way ANOVA was used to compare *F. vesiculosus* adsorption capability under different temperature conditions (30, 35 and 40°C) at dye concentrations of 10g/L and 2.5g/L. A paired t-Test was then used to compare the adsorption potential of *F. vesiculosus* under distilled water and salt-water conditions, at dye concentrations of 10g/L, 5g/L and 2.5g/L.

Results:

The calibration curves for the dye concentrations at various pH for distilled water and salt water are shown in Figs. 3 and 4. For salt-water calibration, pH 6 and pH 7 were excluded from the graph due to inconsistent data. Acid Green 3 dye optimally dissociated at pH2 and pH3 (Figure 3 and 4). The R^2 values for the curves at various pH was (0.9924-

0.9929) and (0.9869-0.9975) for distilled water and salt water respectively. High R^2 values indicate that in the range of absorbencies at 636 nm, dye concentrations could accurately be determined from the absorbance values.

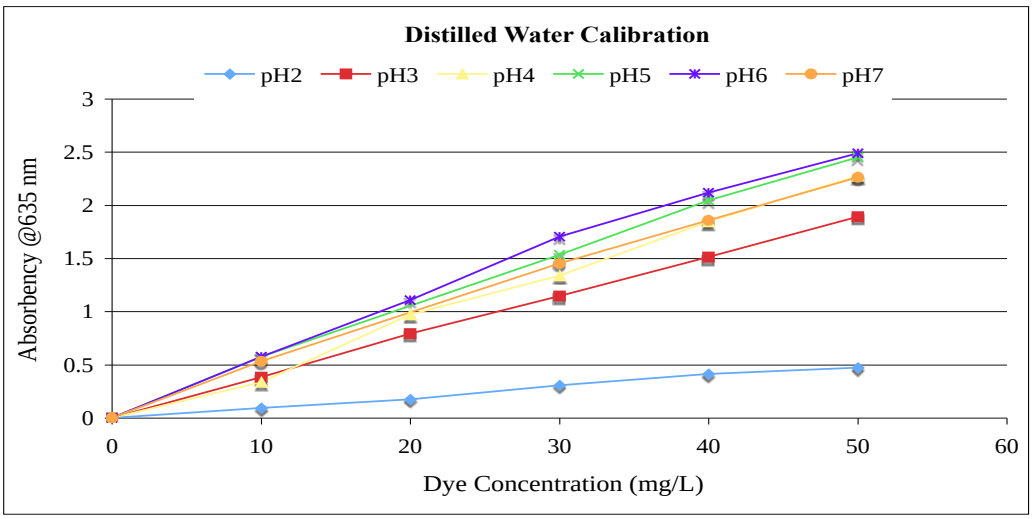

Figure 3. Distilled Water and AG3 Dye Calibration Curve

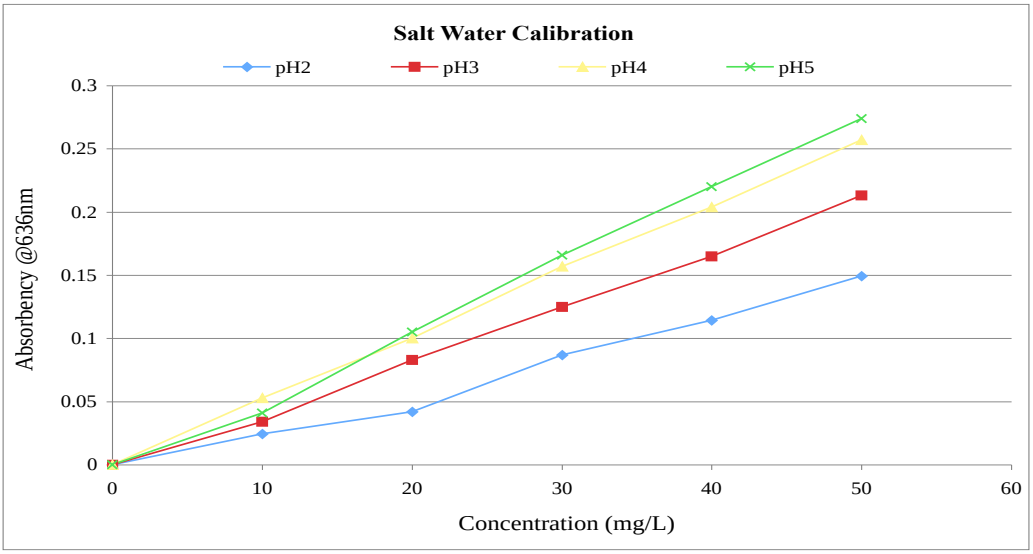

Figure 4. Salt Water and AG3 Dye Calibration Curve

Batch experiment set 1 with *P. mollis*:

P. mollis adsorbed AG3 dye better at a lower pH (2-3) rather than at the higher pH values (4-7). As the pH increased, the rate of dye uptake by the alga decreased (Figure 5).

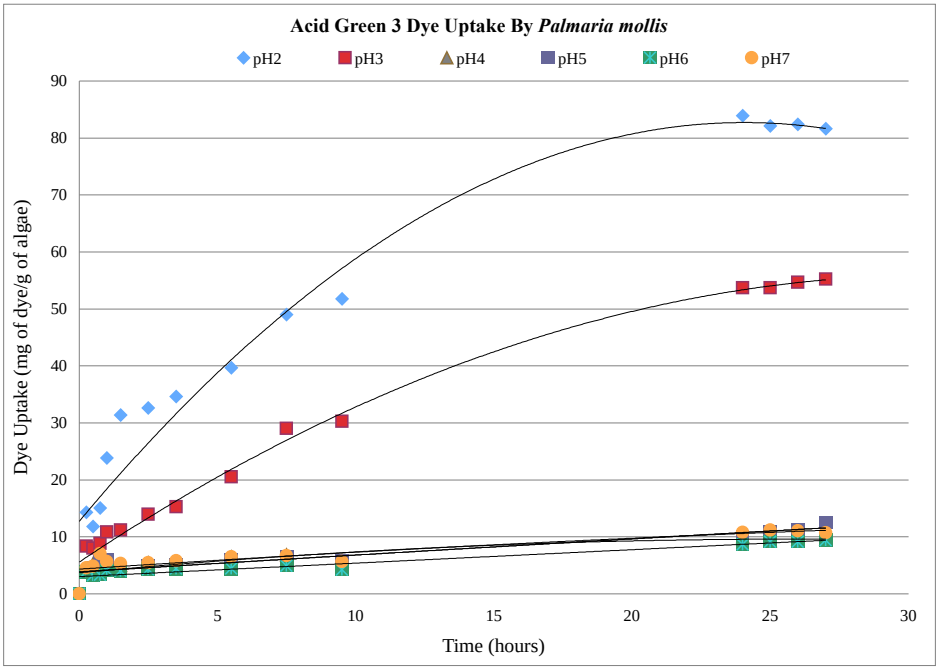

Figure 5. Total amount of AG3 dye adsorbed by *P. mollis*

Batch experiment set 2 with *Fucus vesiculosus*:

There was no significant difference between the adsorption of AG3 by *F. vesiculosus* at 30°C, 35°C and 40°C regardless of the dye concentrations (Figure 6) (p=0.892 and 0.345 for dye concentrations of 10g/L and 2.5 g/L respectively).

F. Vesiculosus adsorbed more AG3 dye in salt water than in distilled water (Fig. 8-10) (p=0.012 and 0.0168 for 10g/L and 2.5g/L respectively). However, the p-value attained for the 2.5g/L dye concentration was not significant (0.586), due to the constant fluctuation of the amount of dye adsorbed.

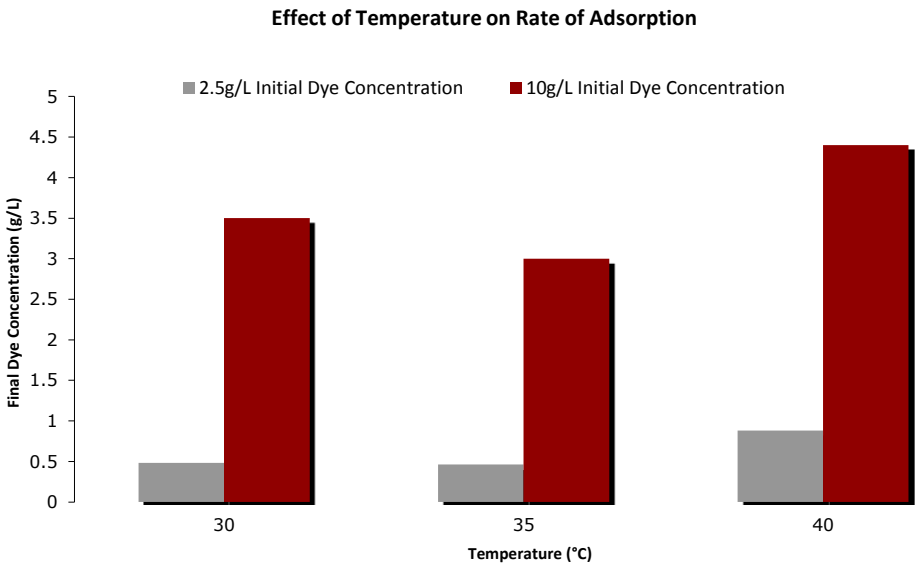

Figure 6. Results from temperature batch experiment using AG3 dye and *F. vesiculosus*

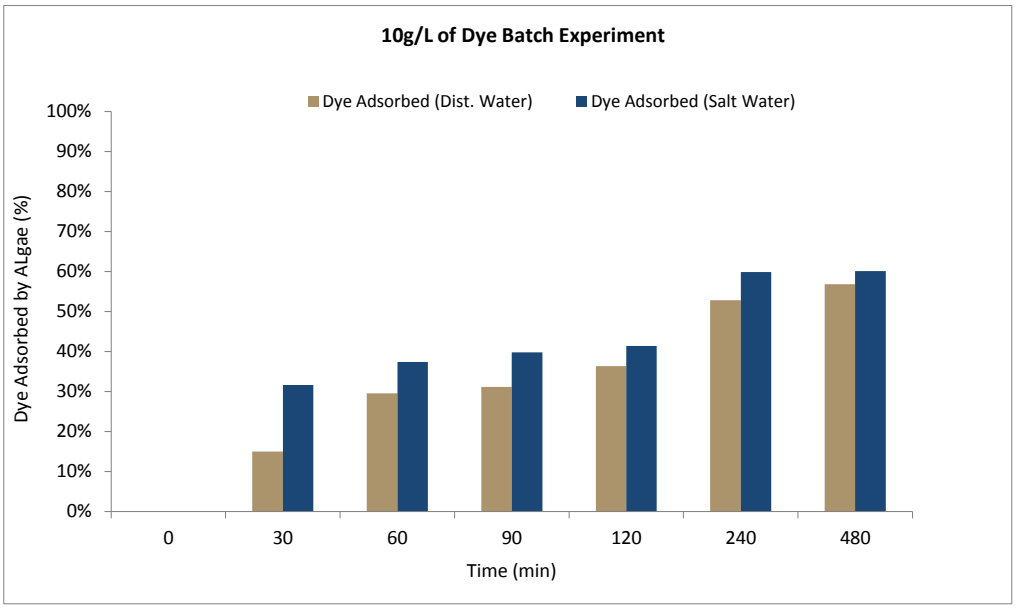

Figure 7. Results from batch experiment testing optimum salinity conditions at 10g/L dye

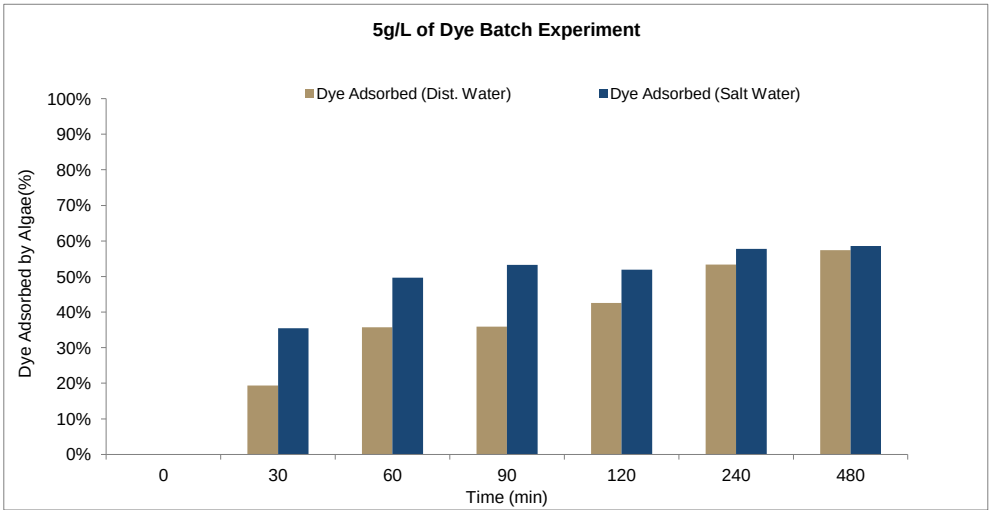

Figure 8. Results from batch experiment testing optimum salinity conditions at 5 g/L dye

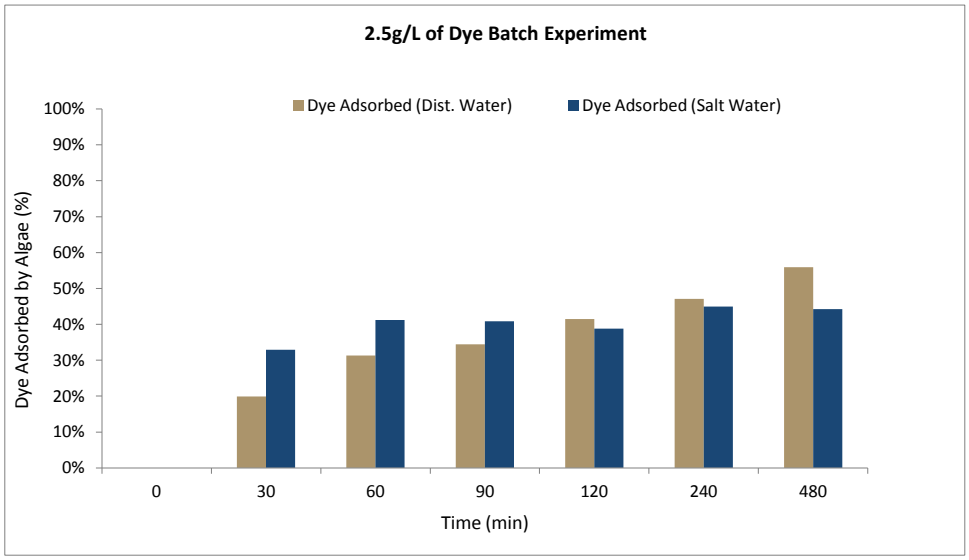

Figure 9. Results from batch experiment testing optimum salinity conditions at 2.5 g/L dye

Batch experiment set 3 with Red Alder biochar:

The low temperature biochar adsorbed less than 7% of the dye (Results not reported).

The untreated Red Alder biochar adsorbed AG3 dye better than the treated biochar and pH 5 was the optimum pH for both experiments (Fig.8 and Fig.9).

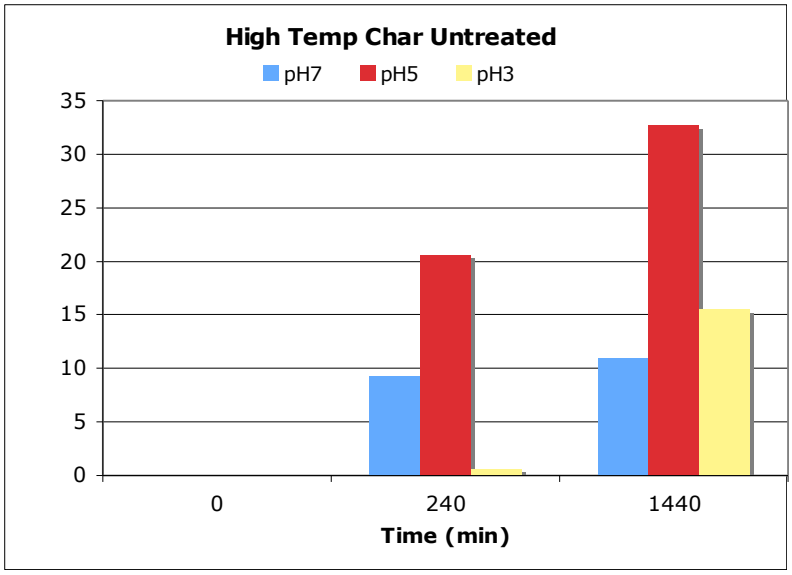

Figure 9. Results from batch experiment using pretreated red alder char

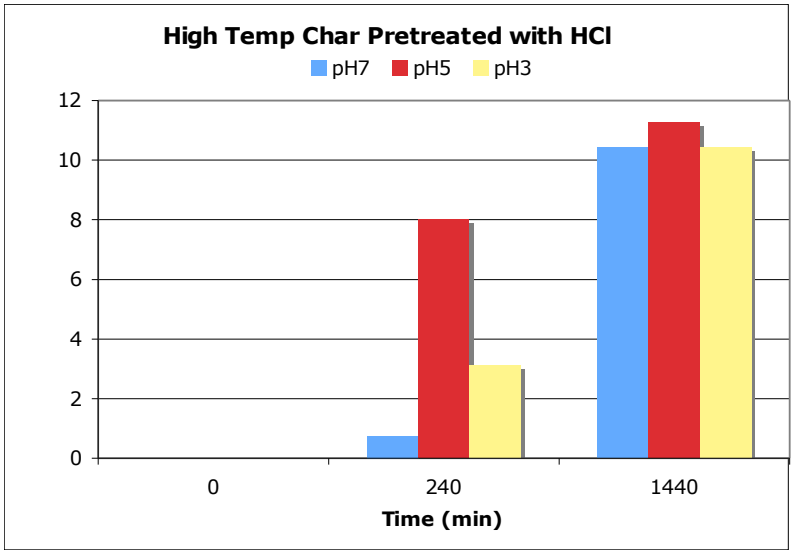

Figure 10. Results from batch experiment using pretreated red alder char

Discussion:

Acid dyes, such as AG3 dye, are anionic dyes because of the negative electrical structure of the chromophore group. As the initial pH increases, the number of negatively charged sites on the biosorbent surfaces (algae) increases and the number of positively charged sites decreases. A negative surface charge does not favor the biosorption of dye

anions due to electrostatic repulsion (Namasivayam & Kavitha, 2002). A low pH affects the active ion-exchange sites of the algae and the ionic state of the dye (Volesky, 2007). In general, AG3 dye uptake is much higher in acidic solutions than those in neutral and alkaline conditions (Chiou & Li, 2002). Therefore, it is expected that lower pH will lead to higher dye adsorption by algae. Although our preliminary results were in agreement with this, and indicated that the algae adsorbed more dye at pH 2 compared to pH 3, pH 3 was chosen for all subsequent experiments since pH 3 is closer to pH 5.5 (the pH of textile industry wastewater). In practice this would reduce any potential pH adjustments to the effluent water to achieve optimum dye adsorption.

Since *F. vesiculosus* adsorbs AG3 dye effectively at a range of temperatures (30-40°C), if implemented in the textile industry, there will be no significant impact on operating temperature in these ranges for optimum dye adsorption. Algae adsorbed a higher amount of dye in salt water compared to distilled water media. One reason for this result could be that the function of the high concentrations of sulfated polysaccharides and alginic acid in brown macro algae is to selectively adsorb metal ions in a saline medium through ion exchange (Ofer et al., 2003). Thus, these functional groups in *F. vesiculosus* would be responsible for adsorption of AG3 dye in a saline solution, the normal environment of algae. Also, since *F. vesiculosus* effectively adsorbed AG3 dye in salt water at concentrations of 5g/L-10g/L, this is beneficial for the textile industry because no secondary step to remove the ions from the wastewater would be necessary. Another reason for the better performance of F. *vesiculosus* in salt water rather than distilled water could be that *F. vesiculosus* naturally grows in seawater. At low dye concentrations (< 2.5 g/L), in salt water, *F. vesiculosus* leaches cellular components into

salt water, which interferes with the measurements. In a control experiment, we tested the absorbency of *F. vesiculosus* in salt water, without any dye. Over time, the algae leached into the solution, discoloring the water. Since 2.5g/L is a relatively low dye concentration compared to 5g/L and 10g/L, the effect of algae leaching into the solution is much greater.

Red Alder biochar produced at high temperature had higher dye adsorption compared to Red Alder biochar produced at a low temperature. However, untreated Red Alder biochar had better dye adsorption compared to pretreated biochars. The dye adsorption after 24 hours was 32% for untreated Red Alder char at pH 5 while for *F. vesiculosus* the adsorption reached 60% in eight hours. Thus Red Alder biochar was not an effective adsorbant compared to *F. vesiculosis* and further studies were conducted using Red Alder char

Unlike *F. vesiculosus,* that increased affinity to AG3 dye when it was pretreated with acid, the Red Alder char did not increase AG3 dye uptake when it was washed with acid. Possible reasons for these results include removal of alkaline ash during HCl washing and protonation of all the dissociated organic functional groups. It is also possible that as the pH of the char decreased, hydrophobic sites of the char increased (Sun et al., 2011). Characterization of the specific char used would provide additional details on the type and nature of the functional groups in the Red Alder biochar that are involved in adsorbing AG3 dye.

Conclusion:

Biosorption technology has proven to be effective in adsorbing AG3 dye from simulated textile industry wastewater using red macro-algae (*P. mollis*) brown macro-

algae (*F. vesiculosis*) and Red Alder char at high temperature. The brown macro-algae, *F. vesiculosus* proved to be the most effective sorbant due to the various functional groups (alginate, carboxyl, sulfate) located in the alga. The optimum conditions for *F. vesiculosus* to adsorb AG3 dye were pH 3 under a saline solution, where temperature had no significant affect. Column experiments would be the next step in this study, and could then be scaled up for textile industry use. If this column technology were to be implemented in the textile industry, it would decrease the water industry's water footprint and reduce their expenses. They would no longer have to import and buy as much water for production, because they could re-use the treated water. Recycling the effluent within the industry will greatly reduce the environmental and health impacts because it will no longer be discharged. This design could also be implemented in other industries that use dyes such as the paper and food industry.

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