Use of Zebra Mussel Shells as an Alternative Mineral Resource for Lime Production as a Phosphorus Precipitant

by

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ABSTRACT

Zebra mussels are an invasive species to North America and are presently found in many rivers and lakes in prolific numbers. Along with many other issues, zebra mussels present a problem when their shells are deposited on shore, carpeting beaches and reducing beach usability. A possible solution presented in this study is to use the zebra mussel shells as an alternative mineral resource to mined calcium carbonate for the production of lime to remove phosphorus in wastewater. Heat-treated coarse (500 µm-1000 µm) and fine (< 75 µm) zebra mussel shell dosed to 10 mg L^{-1} phosphorus containing water at 0.50 g L^{-1} and 0.25 g L^{-1}, removed over 99% phosphorus while maintaining pH levels significantly lower than calcium hydroxide dosed under the same conditions. It was found that ground zebra mussel shells (< 75 µm) heated for 1 hour at temperatures of 600, 700, 800, 900, and 1000 ^{0}C were capable of removing varying levels of phosphorus in water. Shells heated at 800 ^{0}C and dosed at 1.00 g L^{-1} reduced phosphorus in collected real effluent wastewater by 99.48%. It was also shown that shells heat treated at 1000 ^{0}C achieved 98.7% phosphorus removal when dosed at 0.25 g L^{-1}, while maintaining a final effluent pH of 9.13 and demonstrating the lowest energy costs of any of the effective shell treatments. The results indicate that zebra mussel shells show promise as an alternative resource for phosphorus precipitation in wastewater.

Keywords: zebra mussel shells, phosphorus removal, calcium carbonate, calcination, calcium oxide, zebra mussel shell reuse.

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LIST OF ABBREVIATIONS

As – Arsenic

Ba – Barium

Be – Beryllium

Ca – Calcium

CaCl – Calcium Chloride

$CaCO_3$ – Calcium Carbonate

CaO – Calcium Oxide

$Ca(OH)_2$ – Calcium Hydroxide

$Ca_{10}(PO_4)_6(OH)_2$ – Hydroxyapatite

Cl – Chlorine

Co – Cobalt

Cr – Chromium

Cu – Copper

Fe – Iron

GZMS – Ground Zebra Mussel Shell

H^+ - Hydrogen

H_2O – Hydrogen Dioxide

HCl – Hydrochloric Acid

HGZMS – Heated Ground Zebra Mussel Shell

ICP-MS – Inductively Coupled Plasma Mass Spectroscopy

ICP – Inductively Coupled Plasma

K – Potassium

Mg – Magnesium

Min - Minute

Mn – Manganese

Na – Sodium

NaOH – Sodium Hydroxide

Ni – Nickel

OH^- – Hydroxide

Pb – Lead

PO_4^{3-} - Phosphate

RPM – Rotations Per Minute

Sb – Antimony

Si – Silicon

Se – Selenium

V – Vanadium

XRD – X-ray Diffraction

Zn – Zinc

Chapter 1 – Introduction

1.1 Introduction and Objectives

Across the world many lakes, including the Great Lakes and Lake Winnipeg, suffer from two major independent problems: phosphorus overloading resulting in eutrophication which causes the growth of blue green algae, and the presence of the zebra mussels, an invasive species. Despite the independence of each problem, an economic incentive for the collection of zebra mussel shells could be provided through the use of zebra mussel shell material as a phosphorus binding agent for phosphorus removal from wastewater effluents, thereby helping to combat eutrophication. This section aims to review past research on the economic and ecological issues caused by both problems and the potential use of collected zebra mussel shells as a mineral resource and as a phosphorus binding reagent. The need as well as the feasibility of using recycled zebra mussel shells as a phosphorus precipitating material is examined by comparing and analysing current methods and previous research related to zebra mussel invasion, phosphorus overloading and eutrophication, bivalve shell composition, calcium carbonate decarbonation, and oyster and mussel shell re-use for phosphorus treatment. The use of zebra mussels for phosphorus precipitation is studied through original research by examining shell composition, the decarbonation of crushed shell material, and the use and optimization of zebra shell material for phosphorus removal. The objective of this thesis research is to present a novel phosphorus reduction strategy that utilizes recycled calcined zebra mussel shells as a material to chemically remove phosphorus from wastewater effluents.

1.2 Zebra Mussels

Zebra mussels (*Dreissena polymorpha*) are a filter feeding fresh water mussel species belonging to the phylum Mollusca and class bivalve. They are naturally endemic to lakes in eastern Europe, specifically the Black and Caspian Seas. Zebra mussels begin their life cycle as planktonic, microscopic free-swimming larvae known as veligers. The veliger attaches itself to a hard surface to begin adulthood (Benson et al., 2019) and can begin reproduction within the first year of its life (Mackie et al., 1991). Female zebra mussels can produce as many as 1 million eggs per year (Benson et al., 2019). Adult zebra mussels grow on hard structured substrates in lakes including rocks and rock bottoms, man made features such as concrete and metal structures, as well as plants and both naturally occurring and manmade wood structures (Benson et al., 2019). Zebra mussels have a life expectancy of 3-9 years (Benson et al., 2019), however shorter lifespans of 1.5-2 years have been reported in North American waters (Mackie and Schloesser, 1996). In the zebra mussels' native waters their population is controlled through natural predation. In Europe at least 10 fish species have been observed to feed on planktonic zebra mussels (zebra mussel larvae), 27 species of fish have been observed to prey on attached zebra mussels, along with 21 species of birds (Molloy et al., 2008).

Zebra mussels are invasive throughout parts of Europe, Great Britain, and North America. They were first reported in North America in 1988 in Lake St. Claire (Mackie and Schloesser, 1996). It is believed that that they were introduced through the St. Lawrence Seaway in the ballast water of a ship in 1986 (Mackie, 1991). Due to the microscopic size of veliger zebra mussels, the ability of mussels to survive multiple days out of water (Benson et al., 2019), and their prolific reproduction, the control of zebra mussel spread is extremely challenging. Zebra mussels are now invasive to 3 provinces in Canada and 32 states in the United States (Benson et al., 2019). Although a number of fish and birds native to North America have been shown to prey on zebra mussels (Molloy et al., 2008), the predation is not sufficient to counteract the reproduction rate of the mussel, and large populations persist in affected North American waterways.

Many issues, both ecological and societal, arise from invasive zebra mussels. Ecologically, zebra mussels can significantly reduce phytoplankton levels in lakes through filter feeding (Nicholls and Hopkins, 1993). Additionally, zebra mussels can out-compete naturally occurring mussels, snails and invertebrates. 30 freshwater North American mussels are currently endangered due to the presence of zebra mussels (Minnesota Seagrant, 2017). Because zebra mussels are not as commonly preyed on as native mussels, the energy provided by phytoplankton consumed by zebra mussels is not as efficiently passed through the trophic system as with native mussels, in turn adversely affecting fish population. Additionally, zebra mussels increase water clarity through filter feeding, and due to their large population size, this can have profound effects on water turbidity. Due to increased water clarity and therefore increased light penetration, zebra mussels have been shown to increase plant and algae growth, including the growth of blue-green algae, exacerbating eutrophication problems in lakes (Minnesota Sea Grant, 2017).

Societally, invasive zebra mussels present a plethora of problems. As mentioned previously, zebra mussels grow on hard surfaces present in the water including man made structures. This causes issues to infrastructure systems such as marine shipping and transportation, sewage and water intake systems, and hydro-electric damns. It is estimated that zebra mussels cost the US government 1 billion USD annually (Minnesota Sea Grant, 2017). Another study (Connelly et al., 2007) has estimated the annual cost of zebra mussels associated with drinking water treatment and electric power generation facilities alone to be 267 million USD. Another societal issue caused by zebra mussels is the accumulation of washed-up zebra mussel shells on shores and beaches. Washed-up zebra mussel shells are extremely sharp and can cut the bare feet of beach-goers. Additionally, the shells are generally considered aesthetically unpleasing and create an unpleasant odour. Croiser and Molloy (2002) reported zebra mussel growth and decay having impact on recreational activities. Communities that depend on beach tourism are negatively affected economically by both decreased beach tourism and the cost of shell collection and disposal.

1.3 Zebra Mussel Shell Collection

To develop a strategy for zebra mussel shell collection and reuse, it is important to understand the density of zebra mussel shell material available for collection, as well as the methods that are currently used for shell removal and collection in effected areas. Currently throughout North America, zebra mussel shells are collected from piping infrastructure, where shells are removed through chemical and physical means. Additionally, shells are collected from beaches where using manual and tractor pulled rakes and sieves.

1.3.1 Zebra Mussel Shell Density

To assess the feasibility of using zebra mussel shells as an alternative mineral resource and to properly evaluate the best use of collected zebra mussels, an understanding of the available amount of shell material and the density of shell material over a given area must be developed.

Zebra mussel density within piping infrastructure can be extremely high. Mussel colonies are capable of reducing pipe diameter by over two thirds. In one plant in Michigan zebra mussel density was reported to be as high as 700,000 animals/m^2 (Benson et al., 2019). It is unclear weather this density was across the entirety of the plant or within the piping systems. It is likely that the reported density was within the pipes of the plant. Either way, the number of shells that can be collected from infrastructure systems and reused represents an enormous potential.

Zebra mussels can reach densities in the Great Lakes of over 100,000 animals/m^2 of lake floor surface with estimates as high as 779, 000 animals/m^2 (Mackie and Schloesser, 1996). An estimation of dry body mass (without shell) average density in the Great Lakes has been reported as 150 g/m^2/yr (Dermot et al., 1993). Based on shell length to total dry mass weight estimates and dry body mass estimate models developed by Mackie and Schloesser (1996), and using an assumed average shell length of 10 mm, an estimated average shell mass to total mass ratio is predicted to be 3/25. This estimation predicts a shell density of 20.3 g/m^2/yr in the Great Lakes and an annual shell mass production of approximately 5 million tons. In the Hudson River an even higher average shell density, 32 g/m^2, has been reported (Strayer, 2008). If it is assumed that 5% of the shell material produced annually are washed up, then 250,000 tons of shells are deposited annually on Great Lake shores alone.

1.3.2 Collection Methods

Zebra mussel shells are collected through various systems including drain catches, screens, filters, and rake and sifting mechanisms, as well as manual removal. The collection and removal of zebra mussel shells from infrastructure is often necessary for operations in plants, water facilities, publicly held infrastructure (i.e. sewage treatment, water intake. etc.), as well as many other systems. On beaches, the deposition of shells washed up on shore may reduce beach usership and negatively impact tourism industries, making the collection and removal of shells desirable. Given the densities discussed in section 1.3.1, the volume of shell material for re-use as a mineral resource is substantial, thus collection methods of shell material will play an important role in the feasibility of shell re-use.

1.3.2.1 Methods of Collection from Infrastructure

Any piping exposed to zebra mussels may become infested and removal and collection of mussels will become necessary. Zebra mussels can clog or damage these systems leading to negative economical consequences (Banerjee, 2016). Various removal methods are capable of removing mussels from pipes including chemical oxidation, mechanical removal, thermal treatment, foul release coating, low frequency magnetism, pulse acoustics and UV light (Banerjee, 2016). Other commercially available filters for the purpose of mussel removal are also used. For all listed removal methods, mechanical collection methods are still necessary to rid the system of the detached or dead mussels. Physical removal generally happens through screens, filters or drain catches that must be emptied periodically.

1.3.2.2 Methods of Collection from Beaches

Many communities already collect and remove zebra mussel shells from beaches. Shells can be collected manually by shovel or rake, pulling a rake system behind a tractor, or by utilizing sifting machines. Currently, shells collected from most beaches are landfilled.

Collecting zebra mussel shells and removing them from beaches is likely to have a positive effect on beach tourism. Murray et al. (2001) concluded from a study assessing the impact of water quality and beach amenities on beach tourism on Lake Erie, that raking and removing zebra mussel shells would likely help beach managers to increase beach usership.

1.4 Zebra Mussel Shell Composition

An understanding of the chemical composition of zebra mussel shells is necessary to assess the potential for shell re-use as a mineral resource. It is important to know the shells composition to use the shell material as efficiently and safely as possible. Discerning the chemical composition and structure of the shell provides insights into how the material will react and behave under given treatment conditions. Insuring that environmentally hazardous materials are not present in dangerous concentrations is crucial to implement recycled shell material for use in water and wastewater treatment applications.

Limited studies have analyzed the mineral and/or chemical composition of zebra mussel shells. A number of studies, however, have examined the composition of similar bivalve species. Oysters and mussels used in the seafood industry have been examined. Like all bivalves, these studies have found that the shell is primarily composed of calcium carbonate. Studies have reported calcium carbonate percent by mass varying from 60.5% (Hsu, 2009) to 94 %(Lee et al., 2009) and 96% (Yoon et al., 2002). While calcium composition of oyster shells has been reported as 28% by mass (Namasivayam et al. 2004)m suggesting a maximum calcium carbonate composition of 70%. Other chemical compounds found in the oyster shell include SiO_2, Al_2O_3, MgO, SO_3 (Yoon et al. 2002; Hsu, 2009), Fe_2O_3 (Hsu, 2009), P_2O_5, SrO and Na_2O (Yoon et al., 2002).

A recent study conducted by Imel et al. (2016), specifically analysing the chemical composition of zebra mussel shells, has found the shell to primarily consist of calcium carbonate in the crystalline form of aragonite. It was reported that the shells by mass are composed of 35% calcium, thus predicting a maximum calcium carbonate percent mass of 87.5%. A chemical analysis found Na (2297 mg L^{-1}), Si (82 mg L^{-1}), Cl (71 mg L^{-1}), Mg (15 mg L^{-1}), K (10 mg L^{-1})

and P (3.9 mg L^{-1}) also present in the shell material. It is important to note that bivalve shell composition, specifically trace elements, is highly influenced by the water condition that submerses it, and therefore shell composition can vary regionally.

1.5 Potential Uses of Zebra Mussel Shells

Given that zebra mussel shells are composed primarily (>80%) of calcium carbonate, uses of zebra mussels shells as a mineral resource should follow uses of calcium carbonate. Most calcium carbonate, used throughout various industries, is sourced from mined limestone. Collected zebra mussel shells from beaches and pipes could be an alternative source for calcium carbonate.

Calcium carbonate is used in the medical and nutritional fields as a calcium supplement (Chang et al., 2007), a phosphate binder to treat patients with chronic renal failure (Slatopolsky et al., 1986), and as a gastric antacid (Feldman, 1996). It is used in agriculture as a soil neutralizer and buffer (Oates, 2008). Calcium carbonate is used in construction for a variety of purposes including crushed limestone as a road aggregate and for lime (Calcium Oxide) production in cement (Oates, 2008).

A major use of calcium carbonate is in the production of lime. Lime, as mentioned previously, is an ingredient in cement as well as other adhesives (Oates, 2008). Additionally, lime is used in the production of steel slag (Oates, 2008). Calcium Oxide is a reactive material and can also be used as a binding reagent in chemical treatment of sewage and wastewater, specifically for the removal of phosphorus (Tchobanoglous and Burton, 1991).

Given that zebra mussels are invasive to many lakes affected by phosphorus overloading, the use of zebra mussel shells for phosphorus removal could be ideal. Not only does the re-use of zebra mussel shells provide an economic incentive for shell removal from beaches, but shells collected from beaches and infrastructure could be implemented locally in nearby sewage lagoons or wastewater treatment plants. By utilizing shells locally near their collection points, the transportation and energy cost of using shells will be lower.

1.6 Lime Production

Calcium oxide is produced from calcium carbonate through the following chemical reaction:

$$CaCO_3 + HEAT \rightarrow CaO + CO_2 \qquad \text{(Eq. 1)}$$

The threshold heat requirement for the reaction to begin is given by the Gibbs free energy equation:

$$\Delta G°r = 177{,}100 - 158\ T \qquad \text{(Eq. 2)},$$

which yields a temperature of 848 ^oC. However, calcium oxide can be produced at lower temperatures provided that the CO_2 pressure of the calcium carbonate exceeds CO_2 pressure of atmospheric air. The atmospheric pressure of CO_2 is approximately 0.035 kPa at 20 ^oC, thus production of calcium oxide begins under regular atmospheric conditions at around 550 ^oC when the CO_2 pressure of the reaction reaches 0.055 kPa (Haynes, 2011). The greater the CO_2 pressure in the reaction, the more rapid the calcium oxide production. However, in a closed furnace the reaction will result in an increase in CO_2 concentration in the air and therefore increase the furnace CO_2 pressure, slowing or eventually ceasing the reaction.

In most industrial settings, kiln furnaces are used for calcination at temperatures around 1000 ^oC. For practicality and economical feasibility, the reaction partial pressure of CO_2 must be greater than atmospheric pressure of 101 kPa. Thus, the minimum temperature should be no lower than 898 ^oC. Evacuated and purged ovens can reduce the required furnace temperatures while maintaining high and efficient calcium oxide production. However, due to the costs of running these types of furnaces and kilns, they are generally not used in industry and are seen mostly in lab settings.

1.7 Calcium Oxide Precipitation of Phosphorus

Calcium as a free ion will readily react with aqueous phosphate to form one of several forms of calcium phosphate as shown in equation 3, 4, 7, and 8.

$$\begin{aligned} &Ca^{2+} + 2P^{-} + 10H_2O \rightarrow Ca(OH)_2 + 2H_3PO_4 + 12H^{+} \\ &Ca(OH)_2 + 2H_3PO_4 + 12H^{+} \rightarrow Ca(H_2PO_4)_2 + 2H_2O + 12H^{+} \qquad \text{(Eq. 3)} \end{aligned}$$

$$\begin{aligned} &Ca^{2+} + P^{-} + 6H_2O \rightarrow H_3PO_4 + Ca(OH)_2 + H^{+} \\ &H_3PO_4 + Ca(OH)_2 + H^{+} \rightarrow CaHPO_4 + 2H_2O + H^{+} \qquad \text{(Eq. 4)} \end{aligned}$$

As pH of the reaction increases, the form of phosphate decreases its held hydrogen ions, as they are donated to OH^{-} present in solution to form H_2O molecules. The phases of phosphate with respect to increasing pH are shown in equation 5.

$$\begin{aligned} &H_3PO_4 \rightarrow H_2PO_4^{-} \rightarrow HPO_4^{2-} \rightarrow PO_4^{3-} \qquad \text{(Eq. 5)} \\ &\xrightarrow{\qquad\qquad\qquad \text{pH} \qquad\qquad\qquad} \end{aligned}$$

When calcium ions are introduced to solution through the form of calcium oxide (CaO) or calcium hydroxide $Ca(OH)_2$, hydroxide ions are introduced or formed, resulting in increasing pH. As shown by equation 5, the increased pH results in more basic phosphate forms, and causes alternative reactions between calcium ions and phosphate. Equation 6 shows the addition of hydroxide ions when calcium oxide is introduced to water.

$$CaO + 3H_2O \rightarrow Ca(OH)_2 \rightarrow Ca^{2+} + 2OH^- \qquad \text{(Eq. 6)}$$

Provided that calcium oxide is present in excess of 4:1 to phosphate ions, the abundance of resulting hydroxide ions will give the following reaction:

$$3Ca^{2+} + 2PO_4^{3-} \rightarrow Ca_3(PO_4)_2 \qquad \text{(Eq. 7)}$$

with calcium oxide in excess, providing enough hydroxide ions to force phosphate to give up the held hydrogen ions. Provided that calcium oxide is present in excess of 25:6 to phosphate ions, the abundance of resulting hydroxide ions will give the following reaction:

$$10Ca^{2+} + 6PO_4^{3-} + 2OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2 \qquad \text{(Eq. 8)}$$

Equation 8 shows the production of hydroxyapatite, the most thermodynamically stable form of calcium phosphate and thus the most desirable for phosphorus removal. The high ratio amount of hydroxide ions needed to form hydroxyapatite means that the reaction requires a high initial pH. The ratios of calcium oxide to phosphate ions, stated above, are theoretical minimums. In practice pH is used as a measurement of hydroxide ions and are used to predict the form of calcium phosphate produced. A reaction pH of 10 or greater is generally cited for the required pH needed to produce hydroxyapatite (Tchobanoglas and Burton, 1991). Hydroxyapatite requires a greater amount of hydroxide ions than the reaction shown in equation 7, owing to the additional 2 hydroxide ions needed for the completion of the reaction shown in equation 8.

Zebra mussel shells in their raw form consist of calcium carbonate in the form of crystalline aragonite. Calcium carbonate has a k_{sp} of 3.0×10^{-9} (Haynes, 2011), meaning that it has low solubility and thus will not be effective at chemically removing phosphorus from solution. Calcium oxide is much more reactive and has a k_{sp} of 5.5×10^{-6} (Haynes, 2011). Calcium oxide is over 1000 times more soluble than calcium carbonate and therefore calcium ions will more readily dissociate and be available to react with aqueous phosphate. Therefore, for zebra mussel shells to be used for phosphorus precipitation, they need to be first heated to produce calcium oxide. Ultimately phosphorus removal can be achieved through the formation and settling of hydroxyapatite.

1.8 Eutrophication

Eutrophication is the depletion of oxygen in a body of water due to increased algae and vegetation growth caused by nutrient overloading, primarily phosphorus. Eutrophication is a problem seen worldwide, effecting lakes across North America, including many invaded by zebra mussels. Locally, eutrophication is primarily caused by the growth of blue green algae, which is a cyanobacteria (Schindler et al., 2012). The overgrowth of cyanobacteria blooms is detrimental to a lake’s ecosystem. Cyanobacteria has been shown to have adverse affects on fish populations. Rodger et al. (1994), showed that cyanobacteria blooms, in the eutrophic lake Loch Leven, killed a significant number of brown trout. Rodger et al. (2004) concluded that the cause of death in the brown trout could be attributed to toxins produced by the cyanobacteria as well as asphyxiation caused by oxygen depletion resulting from the blooms. Oxygen depletion and dead zones are a major problem caused by cyanobacteria blooms (Schindler et al., 2012).

Cyanobacteria uptakes oxygen both during its life cycle (Rodger et al., 1994) and in larger quantities during its decomposition, robbing the lake of oxygen and suffocating fish populations (Schindler et al., 2012; Rodger et al., 1994).

Controlling nutrient levels released into lakes is an obvious solution to reduce the growth of cyanobacteria. Bunting et al. (2011) and Lewis et al. (2008) argue that controlling nitrogen levels is essential to decreasing cyanobacteria growth, however their results were found only in small scale tests. Paterson et al. (2011) has shown in full lake experiments that phosphorus is the key driver in cyanobacteria growth and that nitrogen levels have a negligible effect. The reason phosphorus is the limiting nutrient for cyanobacteria growth is that cyanobacteria is nitrogen fixing (Schindler et al., 2012), and therefore strives in conditions of low N:P ratios (Smith 1983). Reducing phosphorus is therefore critical to reducing cyanobacteria growth and resulting eutrophication.

1.9 Lake Winnipeg and the Great Lakes

An example of a lake that suffers from both eutrophication and invasive zebra mussels is Lake Winnipeg. Lake Winnipeg, located in the province of Manitoba, is the worlds 10th largest freshwater lake by surface area. Significant economic activity is generated from commercial fishing, recreational fishing, and beach tourism. All three of these industries, on which numerous livelyhoods and communities depend, are adversely affected by both phosphorus over-loading and zebra mussels. Reusing zebra mussel shells as an alternative mineral resource for the chemical precipitation of phosphorus from local municipal sewage lagoons, could provide economic incentive for shell collection from beaches. This could alleviate inauspicious affects to tourism, while simultaneously rerouting shells from the landfill and replacing carbon and land intensive virgin mined calcium carbonate used to combat phosphorus overloading.

Another example of a region that could benefit from the re-use of zebra mussel shells is the Great Lakes, where annual shell production reaches an estimated weight of 5 million tons, and eutrophication presents a major issue. On the Great Lakes, millions of dollars are spent annually for zebra mussel removal from infrastructure. This presents an ideal opportunity for shell re-use, as a ready supply of shells are currently collected.

1.10 Use of Bivalve Shells for Lime Production as a Phosphorus Precipitant

A limited number of studies have been conducted which have examined using oyster and mussel shells from the aquafarm industry as an alternative source of calcium carbonate for lime production and phosphorus precipitation (Abeynaike et al., 2009; Jones et al., 2011; Kwon et al., 2004; Lee et al. 2009; Paradelo et al., 2016). These studies have shown that heat-treated crushed bivalve shells have the potential to be an effective alternative to lime as a phosphorus precipitant. However, there are no reported studies to date investigating the effectiveness of zebra mussel shells for this purpose.

1.10.1 Calcination

Previous studies utilizing bivalve shells for phosphorus removal have heated crushed shell material to promote the conversion of calcium carbonate to calcium oxide via the chemical reaction shown in equation 1. This process is called calcination, and while commonly used for the production of lime from limestone, only a small number of studies have examined this process for calcium carbonate shell material. Past studies have examined the calcination processes under a number of variables including oven atmosphere and air flow, heating temperature and duration, and particle size. The effect to the calcination process in these studies are measured primarily through recorded mass loss and x-ray diffraction (XRD) analysis. Provided that shells are heated to high enough temperatures to combust and volatize all organic material present within the shell as well as evaporate all moisture, recorded mass loss represents the loss of carbon dioxide in equation 1 and therefore relates directly to calcium oxide production. XRD can be used to confirm the calcination process through x-ray diffractograms which represent crystalline structures. Three crystalline stages are observed in the heating of bivalve shells: first the raw crystalline calcium carbonate structure aragonite; secondly the crystalline calcium carbonate structure calcite, which forms after low temperature heating of aragonite; and thirdly calcium oxide. Through XRD analysis each stage can be confirmed and observed.

The calcination of shell material has been studied under regular atmospheric oven conditions and under nitrogen purged conditions. Lee et al. (2009) observed that under atmospheric conditions, shells heated at 750 ^{o}C for 1 hour underwent 41% mass loss, while shells in Nitrogen purged atmosphere, otherwise under the same conditions, experienced 51% mass loss. Similar results were reported by Kwon et al. (2004), who observed 45% mass loss versus 51% mass loss for shells under atmospheric oven conditions versus nitrogen purged ovens. Abeynaike et al (2009) achieved 84 % lime production under nitrogen purged conditions at 800 ^{o}C. Jones et al. (2011) reported optimal heating parameters for shells were nitrogen purged oven with air flow of 0.5 L min $^{-1}$, and a held oven temperature of 800 ^{o}C for 3 hours. Under these conditions 95% calcination or greater was observed regardless of shell particle size.

The effects of particle size on shell calcination has also been studied. Theoretically, smaller shell particles should convert more readily given the greater surface area ratio. Abeynaike et al. (2009) observed that for fine (53-106 μm) and coarse (212-250 μm) shells heated at 750 ^{o}C under the same conditions, mass loss was 30% and 23% respectively. Jones et al. (2011) studied various particle sizes and reported that mass loss increased as particles got smaller, up to the finest studied particle size range (53 – 0 μm). For the finest particle size of crushed shells, it was observed that sintering took place, where clumping and caking of shell material occurred while heating, reducing the effective surface area of the shell powder, and thus reducing calcination.

Temperature is the ultimate driver of the calcination reaction. As discussed in section 1.6, decarbonation happens when the partial pressure of CO_2 of the reaction surpasses atmospheric CO_2 pressure. 600 ^oC is a commonly reported as the temperature for the initial calcination of bivalve shell. Kwon et al. (2004) observed mass loss to begin around 600 ^oC and then to increase with temperature until around 775 ^oC under nitrogen purged conditions. Jones et al. (2011) reported an upper asymptotical mass loss of 44% at temperatures near 775 ^oC under nitrogen purged conditions. Ballaster et al. (2007) reported an upper temperature asymptote for mass loss of 800 ^oC. Temperature affects on shell mass loss appear to have a sigmoidal relationship, where mass loss is not observed until the threshold calcination temperature is reached, mass loss then increases with increasing temperature until a maximum asymptote is reached. Since ovens under nitrogen purged conditions have a much lower CO_2 pressure than ovens under regular atmospheric condition, shells heated under nitrogen purged conditions reach the maximum mass loss at a lower temperature. Due to surface area effects, lower maximum mass loss is reached for finer particles than larger ones, provided sintering does not occur.

1.10.2 Phosphorus Removal

Past studies utilizing bivalve shells for the removal of phosphorus have dosed calcined shell to synthetic phosphate water and examined the effects on phosphorus concentration, pH, time of phosphorus removal and mechanisms of phosphorus removal.

Jar tests are commonly used to examine the effect of calcined shells on phosphorus removal (Abenaike et al., 2009; Jones et al., 2011; Kwon et al., 2004; Lee et al., 2009). Reported results for jar test studies have shown positive results for phosphorus removal. Abeynaike et al. (2009) found that 95 % phosphorus removal is achieved when 5 g L^{-1} of calcined shell is mixed with 10 mg L^{-1} aqueous phosphorus, regardless of particle size or lime conversion. Abeynaike et al. (2009) observed that when optimized shell material was used (fine calcined shell heated under nitrogen purged conditions at 750 ^{o}C for 1 hr) over 90 % phosphorus removal was achieved for doses as low as 196 mg L^{-1}.

For higher concentrated phosphorus water, Kwon et al. (2004) reported 98 % removal of phosphorus for optimized shell removal with doses of 5 g L^{-1}. Results showed that negligible increase in phosphorus removal was seen for shells heated at 800 ^{o}C over those heated at 750 ^{o}C, corresponding to the observed mass loss maximum asymptotes. As expected, lower lime conversion of shells heated in regular atmosphere oven conditions, resulted in lower phosphorus removal. Shells heated at 750 ^{o}C for 1 hour under nitrogen purged and regular atmosphere conditions resulted in 98 % versus 68 % phosphorus removal, respectively.

The time needed for the removal of phosphorus has also been examined. Results show that for lower concentrations of phosphorus (below 12 mg L^{-1}) maximum removal is completed within approximately 5 minutes and always within 10 minutes of the start of the reaction (Abeynaike et al.,2009; Jones et al., 2011; Lee et al., 2009). Lee et al. (2009) observed that for higher phosphorus concentrations (30 mg L^{-1}) maximum removal was achieved within 30 minutes. Past studies have shown that the initial phosphorus removal does not reverse over time (Abeynaike et al., 2009; Jones et al., 2011; Kwon et al., 2004; Lee et al., 2009; Paradelo et al., 2016). Since phosphorus precipitated from solution is not observed to become soluble again over time, the reaction appears to be non-reversable suggesting the formation of hydroxyapatite via equation 8.

1.10.2.1 Mechanism of Phosphorus Removal

The exact mechanisms of phosphorus removal caused by calcined bivalve shells are not known and vary based on a multitude of parameters including particle size, percent lime, dose concentration, water pH, and shell structure and composition. The basic mechanisms of removal are understood and have been examined to varying degrees in past studies. Provided that a sufficient amount of calcium oxide is present in the bivalve shell material, the main mechanism of removal is through the chemical precipitation of phosphorus and the formation of calcium phosphate. Past research has predicted that given the high pH of the reaction and the non-reversibility of the bound phosphorus, it is likely that hydroxyapatite is formed (Abeynaike et al., 2009; Lee et al., 2009). This is the most thermodynamically stable form of calcium phosphate and therefore the most desirable. Abeynaike et al. (2009) extrapolated that providing fine particles are used and that some calcium carbonate is still present in the shell material, heterogenous precipitation will occur along with homogenous precipitation. Abeynaike et al. (2009) observed that for coarse shells a greater amount of phosphorus was contained in suspended particles, which was attributed to homogenous precipitation. For fine particles, due to increased surface area, a greater amount of phosphorus was observed on the shell particle, which was attributed to heterogenous precipitation as well as phosphorus adsorption to calcite. Precipitation of hydroxyapatite, the desired precipitant, happens more readily when a calcite core is available for seeding the precipitant, resulting in heterogenous precipitation. Fine particles have greater surface area and thus higher probability of calcite core seeding. It was therefore recommended by Abeynaike at al. (2009), that partially calcined fine powders are used to increase reaction time. Contrasting these findings, Lee et al. (2009) reported only homogenous precipitation, which was attributed to the near completion of calcination and the corresponding absence of calcite.

Another form of phosphorus removal caused by bivalve shells is through the adsorption onto either calcite or aragonite structures. Research has shown that aragonite has a higher adsorption capacity than calcite (Kitano et al., 1978). Past studies reported phosphorus removal through adsorption onto both raw bivalve shells containing aragonite structures (Abeynaike et al., 2009; Jones et al., 2011: Kwon et al., 2004; Paradelo et al., 2016; Namasivayam et al., 2005) and onto unconverted calcite particles of heat treated shells (Abeynaike et al. 2009; Lee et al., 2009; Paradelo et al., 2016). Phosphorus removal caused by un-calcined shells suggests adsorption as the primary mechanism of removal given the lack of soluble calcium oxide. The use of un-calcined shells, containing aragonite, have resulted in moderate phosphorus removal. Abeynaike et al. (2009) reported 45% phosphorus removal when 5 g L^{-1} of fine (53-106 μm) unheated shells were mixed with 10 mg L^{-1} aqueous phosphorus for 2 hours. Jones et al. (2011) reported 20-30% phosphorus reduction from 10 mg L^{-1} aqueous phosphorus after 1.5 hours of agitation. Kwon et al. (2004) reported approximately 15% reduction when 5 g L^{-1} untreated shells where dosed and agitated with 30 mg L^{-1} aqueous phosphorus for 2 hours.

Namasivayam et al., (2005) conducted a kinetic study into the adsorption of phosphate to bivalve aragonite shells. It was reported that un-calcined shells (24 g L^{-1}) achieved phosphorus removal of 86% from initial 50 mg L^{-1} aqueous phosphorus after 7.7 days of agitation (530 rpm). The effectiveness of un-calcined shells was also studied over a 90-minute mixing time at a lower mixing rate (115 rpm) for 10 mg L^{-1} and 50 mg L^{-1} phosphorus concentrations. Namasivayam et al. (2005) observed that increasing adsorbent (shell) doses resulted in increasing removal efficiency. 1.2 g L^{-1} shell resulted in approximately 45% and 25% phosphorus removal for initial phosphorus concentrations of 10 mg L^{-1} and 50 mg L^{-1} respectively. Calcium ion release was also observed, and it was reported that calcium release increased with time and was closely associated with phosphorus removal. Namasivayam et al. (2005) concluded that phosphorus removal occurs primarily by sorption onto the surface of aragonite shell particles by binding as amorphous calcium phosphate and eventually forms hydroxyapatite. The stated conclusions were based on previous literature and observed moderate fitting retention Freundlich isotherms, predicted by phosphorus sorption (Namasivayam et al., 2005).

Paradelo et al. (2016) conducted a study which examined the removal of phosphorus by both calcined and un-calcined shells in batch (mixed) and column tests. The study concluded that calcined shells removed more phosphorus than un-calcined shells as expected. However, contrary to previous studies and standard literature it was reported that for both calcined shells and un-calcined shells, adsorption and precipitation played near equal roles in the mechanism of phosphorus removal. Paradelo et al. (2016) observed that for increasing phosphorus concentrations phosphorus removal increased for un-calcined shells. Adsorption removal should increase until maximum adsorption is reached. Phosphorus binding rate should decrease as the maximum is approached. This pattern was not observed by Paradelo et al. (2016) suggesting another mechanism of removal. Phosphorus removal mechanisms were examined by Paradelo et al. (2016) by flushing the columns with HCl, H_2SO_4 and $NaOHCO_3$ and testing the phosphorus amount in each dissolvent. HCl and H_2SO_4 are expected to dissolve precipitants while $NaOHCO_3$ dissolves adsorbed phosphorus. From these experiments it was found that precipitation and adsorption occurred in substantial amounts for both calcined and un-calcined shells. Higher amounts of phosphorus removal in both modes were observed for higher concentrations of phosphorus for calcined and un-calcined shells. Higher precipitation was observed for calcined shells, and contrary to expected results, higher adsorption was also observed for calcined shells, despite aragonite having higher adsorption capacity (Paradelo et al., 2016). Similar results were reported by Abeynaike et al. (2009), who observed higher adsorbed phosphorus concentrations in calcined shells than un-calcined shells. The cause for higher adsorption in calcined shells is unclear, however heterogenous precipitation reported by Abeynaike et al. (2009) and supported by Paradelo et al. (2016) may play a role. Although the results reported by Paradelo et al. (2016), contradict the mechanism of removal proposed by Namasivayam et al. (2005), the calcium ion release reported in that study, could explain the role of precipitation observed by Paradelo et al. (2016). Based on past studies, it is believed that most effective phosphorus removal will be achieved from partially calcined shells with remaining calcite particles and calcium oxide particles allowing for adsorption and seeded heterogenous precipitation.

1.10.2.2 Effect of pH

As discussed in section 1.7, the pH of solution controls the form of calcium phosphate precipitated. Higher amounts of calcium oxide results in higher pH values. A pH of over 10 is required for the formation of hydroxyapatite, the most thermodynamically stable and thus most irreversible form of calcium phosphate. Abeynaike et al. (2009), Jones et al. (2011), and Lee et al. (2009) all predicted precipitation in the form of hydroxyapatite due to pH levels of 10 and higher. Abeynaike et al. (2009) reported calcined shells dosed at 5 g L^{-1} raising solution pH to as high as 12. Lee et al. (2009) also reported 12 as a maximum pH, however for lower doses of 0.6 g L^{-1} of calcined shell from an initial pH around 8. Lee et al. (2009) observed that particle size had a strong inverse correlation to pH. Smaller particle sized calcined shell resulted in higher pH. It was also observed that higher pH resulted in higher phosphorus removal. Specifically, pH

10 was shown to be of significance. While approximately 80% removal was achieved at pH values below 9.5, complete phosphorus removal for all particle sizes required a final solution pH of 10 or greater (Lee et al., 2009). The pH 10 requirement for complete phosphorus removal could be due to the reversibility of calcium phosphate forms that occur below pH 10, resulting in an equilibrium of residual aqueous phosphate. While hydroxyapatite precipitated at pH levels above 10 is more thermodynamically stable producing a near non-reversible reaction.

Lee et al. (2009) reported significant differences between calcined shell and chemical grade calcium hydroxide on pH. It was observed that calcined shells posses a time release property, meaning that the initial solution over time reached a maximum pH, when solution was then poured off and replenished with new solution the maximum pH was reached once more over the same period of time. This process was repeated for three water replacements (4 total solutions), each solution resulting in a maximum pH of 11. These results differed from calcium hydroxide which decreases in pH with each water replacement. The cause for the time-release property was suspected to be the remaining calcite core, that holds calcium oxide on its surface. Lee et al. (2009) concluded that the time release property gives calcined shells an advantage in batch reactor settings where solution or batches are replaced overtime. Additionally, Lee et al. (2009) found that for the same pH value, calcined shell required less HCl to lower pH than pure calcium hydroxide. This means that if waste water effluents need to be neutralized post-treatment prior to release, the neutralizing cost of calcined shell will be less than that of chemical grade calcium hydroxide.

1.10.3 Feasibility and Economics

The feasibility of using zebra mussel shells or any calcined shells as a phosphorus binding material hinges on the economics and cost associated with shell collection, treatment and application. For the production cost of using oyster shells in Korea, Kwon et al. (2004) conducted a preliminary economic assessment. They concluded that for a plant to have a production rate of 7500 tons per day, a 40 year investment return period would be required, and after the 40 year return period the plant would be profitable. These calculations were based on the costs of a shell crusher, rotary kiln, and operation. Kwon et al. (2004) determined that per day electricity consumption would be 14 400 kWh and the total cost per ton of calcined shell including the 40 year depreciation capital cost, transportation, energy, and labour would be $42 USD. The cost associated with monthly electricity consumption was estimated to be 65,787 USD. Based on the estimated daily electricity consumption of 14 400 kWh the Manitoba cost of monthly electricity would be approximately 20,000 USD, making shell production significantly cheaper in Manitoba. It is unclear what the sale price was of calcined shell used to calculate the return time. The study, which was conducted in 2004, stated that the cost of heated oyster shell, used in fertilizer and chicken feed, was 126 USD/ton comparable to the 2004 price of quicklime - 120 USD/ton (Kwon et al., 2004). Kwon et al. (2004) concluded that the use of calcined oyster shell as a phosphorus binding material was economically feasible.

The economic feasibility of using zebra mussel shells for the production of lime as a phosphorus reagent depends heavily on the cost of collection. As stated in section 1.2, the US government spends 1 billion USD annually associated with zebra mussels. Washed up zebra mussel shells on beaches have negative economic effects on beach tourism, and on many beaches zebra mussel shells are already collected for removal and disposal. Additionally, removed mussels from pipes and infrastructure are sent to the landfill. If municipal, provincial, and/or federal governments contracted or employed shell collection for the use of calcined zebra mussel shells for the removal of phosphorus in waste water, then the collection costs would be accounted for. This practice could have positive economic effects overall, as an economic incentive would be provided for shell collection, possibly helping to sustain tourism economies.

1.11 Other Potential Uses for Collected Zebra Mussel Shells

Bivalve shells as a mineral resource have many potential uses. Previous studies have examined the use of both calcined and un-calcined bivalve shells for non-phosphorus related environmental applications, as a construction material, and as a nutritional additive both for animal feed and for human consumption.

In addition to phosphorus precipitation and adsorption, shell material can be used to precipitate other contaminates such as arsenic (Seco-Reigosa et al., 2015; Seco-Reigosa et al, 2014), mercury (Pena-Rodriguez et al., 2013; Seco-Reigosa et al., 2014), chromium (Seco-Reigosa et al., 2014), copper, and nickel (Hsu, 2009). Seco-Reigosa et al. (2015) reported that un-calcined shell material increased arsenic adsorption when used with granitic material. Shell material was found to adsorb as much as 83% of arsenic in concentrations of 100 mg L^{-1}. Additionally, the shell material helped to decrease arsenic solubility once amended to the granitic/shell adsorbent. Seco-Reigosa (2014) reported a sewage sludge, wood ash, calcined shell mixture removed significant amounts of mercury (99% removal), arsenic (90-96% removal), and chromium (32% removal). Pena-Rodriguez et al. (2013) reported calcined shells being an effective mercury adsorbent. Mercury adsorption was observed to increase in the presence of aqueous phosphate. Hsu (2009) showed un-calcined shells to be effective at removing copper and nickel from aqueous solutions. The findings of these papers reinforce that calcined bivalve shells could be useful as a binding material for applications in wastewater lagoons and treatment plants. The variety of heavy metals that can be removed from wastewater by calcined shells increases the usefulness of this material, as it could be kept for treatment of phosphorus along with other contaminants.

Shell material has also been studied for applications outside the environmental field. Ballester et al. (2007) and Yoon et al. (2002) have studied un-calcined shell material as an aggregate for mortar production with promising results. Ballester et al. (2007) reported that due to the greater compatibility of shell material over traditional crushed limestone, mortars with shell materials could achieve the same compression strength as mortars with limestone, using less cement and therefore decreasing production costs.

Whole zebra mussels have been studied for the use of a chicken feed additive by Mclaughlan et al. (2014). The use of mussel tissue and shell material was utilized in chicken feed for the experiment. Mixed results were reported, although the shells had no negative effects on the birds and were readily consumed, protein and energy levels proved to be lower than excepted. It was concluded that zebra mussels would best serve as a calcium additive and that shell separation could be beneficial (Mclaughlan et al., 2014). Oyster shells have also been used as a human food additive for calcium supplementation. Chang et al. (2007) found that *Pinctada margatitifera* (black lipped oyster) shells contained below detectible limits of nickel, chromium, cadmium, lead, and vanadium. Concentrations of sodium, magnesium, selenium, iron, aluminum, phosphorus, boron, manganese, copper, and zinc were detected and normalized for dietary use. The normalized concentrations were found to be safe for human consumption, leading Chang et al. (2007) to conclude that *P. margaritifera* was a viable source for calcium supplementation.

1.12 Research Objectives

As discussed previously, research examining the use of zebra mussel shells as a mineral resource is extremely scarce. While past studies have investigated the use of oyster and mussel shells from the seafood industry, no study to date has examined the use of zebra mussel shells for this purpose. Additionally, past studies utilizing bivalve shells for phosphorus removal, have only reported results using synthetic phosphorus water. The use of shell material for phosphorus removal from real wastewater effluents has not been studied.

1.12.1 General Objective

To examine zebra mussel shell composition, the calcination process of collected zebra mussel shells, and the use, effectiveness, and optimization of calcined zebra mussel shells for the removal of phosphorus from collected sewage effluent.

1.12.2 Specific Objectives

- Determine the amount of calcium carbonate in locally collected zebra mussel shells, as well as, identify additional elemental constituents present;
- Investigate the effect of temperature and particle size on shell calcination;
- Determine the effect of shell treatment temperature and application dose has on phosphorus removal from wastewater effluent;
- Determine the effect of shell treatment temperature and application dose has on effluent pH, and subsequent relationships that exist between pH and phosphorus removal;
- Optimize shell treatment, by identifying shell temperature treatments that allow for high phosphorus removal, while using minimal material (small dose) and energy (low temperature) at minimal final pH; and

- Assess the economic and energy cost, and feasibility and potential social implications of using zebra mussel shells for phosphorus removal.

Chapter 2 – Materials and Methods

2.1 Shell Collection and Preparation

A permit was obtained from Manitoba Sustainable Development, Fisheries Science and Fish Culture Section to allow for the legal collection, transportation and experimentation of zebra mussel shells. Zebra mussel shells were collected from various beaches on the east side of Lake Winnipeg. They were transported and prepared as per the permit specifications (specified in section 2.1.1).

2.1.1 Aquatic Invasive Species Permit

An aquatic invasive species permit was obtained for the legal collection, transportation, and the legal holding and experimentation of zebra mussel shells. A total of two permits were obtained, each granting a seasonal collection and transportation time followed by a one-year holding time. Transfer sheets specifying collection date, number of species collected, and location of collection were also filled out and submitted to Manitoba Sustainable Development. Manitoba Parks District Office was also contacted and informed of collection location and dates before collection began. The signed aquatic species permits are included in the appendices (Appendix 4 and 5).

Stipulations of the permit were as follows:

> 1. Purpose(s) of Permit: To authorize the collection by hand, transportation and possession of dead Zebra Mussels (and incidental by-catch of live Zebra Mussels) for research purposes. The Permittee is continuing with his research from 2016 re: determining the extent ground up Zebra Mussel shells can be used to remove phosphorous from waste water.

2. Location: Dead Zebra Mussels will be collected from Beaconia Beach, Patricia Beach, Grand Beach, Winnipeg Beach marina and Gimli. Research will occur at the University of Manitoba (Room E1 251, EIT Complex).

4. Special Conditions:

  a. Permittee must contact the appropriate District Office prior to proceeding with the collection.
  b. Permittee must fill out the attached Introductions and Transfers Form after the collection is complete and the Zebra Mussels are transported back to the University.
  c. See Attachment

C1. Permittee is authorized to collect by hand, possess and transport dead Zebra Mussels. Note Permit also authorizes the possession of live Zebra Mussels which may be collected unintentionally. Quantity of Zebra Mussel shells collected is not to exceed one regular garbage bag size.

C2. Permittee must transport Zebra Mussels in a container with a secure lid.

C3. Permittee must ensure any equipment used to collect and hold live Zebra Mussels is cleaned per Schedule C of Manitoba's AIS regulations before it used anywhere else.

C4. Any water accumulated during the collection and transportation of Zebra Mussels must be drained on the ground away from any surface water or drain.

C5. Permittee must retain this permit (or a copy) while in possession of the Zebra Mussels.

C6. Permit covers individuals working with or for the Permittee. Individuals must have a copy of the permit when they are not with the Permittee.

C7. Permittee must ensure that the Zebra Mussel shells collected are treated in a manner that will kill the incidental presence of live Zebra Mussels, including veligers in source water, and any water or chemical used in the treatment process is disposed of in a manner that it cannot enter any surface water system or municipal waste water system.

C8. Permittee must ensure all organic material and Zebra Mussel shells and ground shell material is disposed of in a landfill away from any water sources.

C9. Permittee is to report as soon as possible *per* section 10 of the AIS Regulations the sighting of an aquatic invasive species when it has been found outside of a control zone that has been established for that species. The exception is the sighting of Zebra Mussels within the Nelson River and /or downstream connecting water bodies. These must also be reported. **Please note confirmation of a species is not required prior to reporting.**

C10. Permittee per C9 must provide the following information: name, phone numbers where they can be reached, location information (GPS coordinates if possible) of sighting; date and time of sighting; photographs and be prepared to take a specimen or sample, to the AIS Director by calling the AIS hotline at: 1-877-867-2470 AND email: candace.parks@gov.mb.ca, laureen.janusz@gov.mb.ca and jeff.long@gov.mb.ca.

C11. Permittee must provide a copy of their final report.

2.1.2 Collection

Zebra mussel shells were collected in the fall seasons of 2017 and 2018. Shells were collected from beaches located on the east side of Lake Winnipeg where shell accumulation was the highest. Once shells were found in sufficient densities, shells were shovelled into sealable bins and pails. Only dead mussels (shell material) were intentionally collected. Shells were separated from rocks, sand, and organic matter within reason, however the collection of these materials was inevitable, therefore the shells were crudely collected to be sorted and cleaned later. Shells were transported directly to the University of Manitoba following all stipulations as per the Aquatic Invasive Species Permit. Tools used for collection (shovels and containers) were sanitized following collection, using alcohol wipes and bleach. The tools were then left to dry for two weeks away from drains.

Fig. 1. Shells on Beaconia Beach, Lake Winnipeg, Manitoba, with sealable container and shovel. Source: The author

2.1.3 Preparation

Once the shells were collected they had to be cleaned and all organic matter removed including any remaining mussel flesh. In addition to cleaning the shells, the process ensured that all live zebra mussels were destroyed so they would not invade any water sources. To achieve both pure and clean zebra mussel shell material, and to ensure that all live zebra mussels were destroyed, the collected shells were treated in the following way. All large stones, twigs or pieces of wood that the mussels had attached to were removed by hand. The removed material was then collected, boiled, strained out and disposed of in the garbage. The boiling solution was then boiled off until the pot was nearly dry and the remaining liquid was poured off on the ground away from drains or ditches. Then the zebra mussels were placed in a 20% bleach solution for 6 hours. Organic material began to float to the top as the shells sat in the solution. Periodically the organic material was removed from the solution using a regular kitchen skimmer. This material was then treated the same way as the previous material and then disposed of in the garbage. The shells were then removed from the solution, washed, and baked in an oven at 80 ˚C for 1 hour. The washing water was boiled until the pot was nearly dry and the remaining water was poured off away from any drains and ditches. Remaining stones were then hand picked from the shell material and thrown in the garbage. All pots and strainers used in the cleaning process were cleaned with 1 mol HCl, rinsed and dried, and wiped with alcohol swabs, and finally boiled, to ensure disinfection was achieved. This process produced clean and pure looking shells, as well as, mitigating any risk involved with handling the zebra mussels.

Fig. 2. In-situ shells (left) vs clean shells (right). Source: The author.

2.2 Shell Grinding and Sieving

The cleaned and dried zebra mussel shells were finely ground using a coffee grinder. Once ground they were passed through a set of sieves. The particles between the 1000 µm and 500 µm mesh sieves were removed and labelled. The fine particles were collected and ground in a planetary ball mill for 50 minutes at 420 rpm, reversing the spin every 5 minutes. This powder was then sieved through a 75 µm sieve. All particles that passed through were collected and labelled.

Fig. 3. Ground zebra mussel shells. Source: The author.

2.3 Shell Characterization

Zebra mussel shells were characterized using three separate elemental examinations: a chemical titration to estimate the amount of calcium carbonate present in the ground shell material, Inductive Coupled Plasma (ICP) analysis to determine the elemental composition of the shells, and X-ray Diffraction analysis (XRD) to determine the crystalline composition of the shells.

2.3.1 Titration for the Determination of Calcium Carbonate

For the determination of calcium carbonate composition present in the zebra mussel shells, a titration was performed. The shells were crushed using a pestle and mortar into a semi fine texture. 0.25 g of crushed shells were dissolved in 58 mL of 0.1 molar hydrochloric acid. Four drops of phenolphthalein was added to the solution. The titration was performed reacting sodium hydroxide, delivered by a glass pipet, with the hydrochloric acid-dissolved shell solution. Once all the remaining acidic hydrochloric acid reacted with the basic sodium hydroxide, the solution was neutralized, and the phenolphthalein indicator turned pink. Since the sodium hydroxide and the hydrochloric acid react in a one to one molar ratio, the volume of sodium hydroxide added to the solution at the point of equilibrium represents the volume of hydrochloric acid that did not react with calcium carbonate present in the zebra mussel shells. The volume that did react can be determined by subtracting the unreacted volume from the initial volume of hydrochloric acid used. Since the reaction of calcium carbonate and hydrochloric acid is known to be:

$$CaCO_3 + 2HCl \rightarrow H_2O + CO_2 + CaCl_2 \text{ (Eq. 9),}$$

from the volume of hydrochloric acid reacted with calcium carbonate and given that the molarity of the acid is 0.1 M, the amount of calcium carbonate can be calculated. From the known molar quantity of calcium carbonate that reacted with the hydrochloric acid, the mass of the shells that reacted can be found, given the molecular mass of calcium carbonate to be 102.17g. The equation for percent calcium carbonate is shown in equation 10.

$CaCO_{3percent} = Mol_{solution}M_{mol}((V_{HCL} – V_{NaOH})) / (2*M_{shell})$ (Eq. 10)

Where: $CaCO_{3percent}$ = percent calcium carbonate of total shell; $Mol_{solution}$ = the molarity of hydrochloric acid and sodium hydroxide used; M_{mol} = the molar mass of calcium carbonate; V_{HCl} = the volume of HCl used to react with calcium carbonate in the ground shell; V_{NaOH} = the amount of sodium hydroxide used to neutralize the remaining HCl; and M_{shell} = the mass of zebra mussel shell used.

2.3.2 ICP Analysis

Inductively coupled plasma mass spectrometry (ICP-MS) measures light frequency and intensity of excited electrons as they drop orbital levels. Samples are dissolved in acid and then super heated into plasma. By measuring the intensity and frequency of emitted light, from the sample after super heating, the elemental composition can be determined by calibrating the light emissions to known concentrations of standard elements.

For the analysis of elemental shell composition ICP-MS (Varian 725-ES ICP-OES with Varian SPS 3 Autosampler, Aligent Technologies Inc.) was utilized. Zebra mussel shells were prepared by dissolving 0.3g of shell in 50 mL of concentrated 1:1 volumetric hydrochloric and nitric acid. Standards were prepared for the analysis of arsenic, barium, manganese, beryllium, cobalt, chromium, iron, nickel, selenium, lead, potassium, zinc, copper, antimony and vanadium.

The measured light intensities were calibrated using the known concentrations of the standard solutions and concentration of dissolved shell.

2.3.3 XRD Analysis

XRD analysis identifies crystalline composition of a powdered material. Material is crushed by hand using a mortar and pestle to achieve extremely fine particles. The powder is packed into a slide and placed into the XRD machine via a magnetic holder. X-rays are then radiated at the powder over a range of angles and the diffracted x-rays are recorded by the machine. The diffracted x-rays over the various angles form a diffractogram which is a visual representation of diffracted x-rays, graphing recorded intensity versus angle. The diffractogram is then analysed by the computer program JADE, which compares the diffractogram with known diffractograms of crystalline material, to determine the sample crystalline composition.

For the analysis of zebra mussel shell composition, the shell was crushed and packed into the slide. X-ray angles from 2θ, 30 to 60, were analysed, over an hour run time. Diffractograms were recorded and analysed using JADE to determine shell crystalline composition.

2.4 Shell Heating

For the preparation of calcined shell material, ground zebra mussel shell (GZMS) was measured out into crucibles. Each crucible contained 1 g +/- 0.07g of GZMS. Two different particle sizes were examined, fine particles (<75 μm) and coarse particles (500 μm-1000 μm). The GZMS was heated in an oven for 1 hour at 105 ˚C, to dry the GZMS of any moisture. The GZMS was then placed in a desiccator to cool. The crucibles with lids, containing the GZMS, were then heated 1 hour +/- 1 minute in a pre-heated Thermolyne Dubuque IV solid state controller muffle furnace oven at temperatures varying from 600 to 1000 ˚C, in increments of 100 ˚C, for 1 hour.

To examine the effect of temperature and particle size on calcium oxide production, shells were weighed after heating was finished. Three samples of the same particle size were heated in the oven at the same time and at the same temperature. 3 samples for each temperature and particle size were heated, resulting in 30 samples. After the samples were heated, they were removed from the oven and cooled in a desiccator. The samples were then weighed using the same scale as before. A total of 30 observations were taken. Observed mass loss was then used to quantify calcination.

2.5 Synthetic Water Jar Tests

The effect of fine (<75 µm) and coarse (500 µm-1000 µm) HGZMS was tested on synthetic phosphate water. Synthetic phosphate water was prepared by mixing 43.86 mg L^{-1} monopotassium phosphate with tap water to achieve 10 mg L^{-1} phosphorus concentration. Both fine and coarse particles, heat treated at temperatures of 600, 700, 800, 900 and 1000 ˚C as described in section 2.4, were mixed (1 g L^{-1}) with the synthetic phosphate water following ASTM D2035 (ASTM, 2012) by mixing at 120 rpm for the first minute, then 50 rpm for the next 20 minutes in round plastic jars. Mixing was stopped and the solution was left to settle for 15 minutes. The pH of the solution was measured and was filtered using 0.45µm paper filters to remove suspended solids. Phosphorus was measured before and after mixing using the ascorbic acid method (APHA, 1995). pH was measured before and after (40 min) treatment using a pH meter. Four jar tests were conducted for each particle size and heat treatment variable, yielding 20 samples for each particle size and a total of 40 samples all together. Three vials were measured for each sample and averaged for one spectrometer reading per jar test.

Fig. 4. Experimental set up of jar tests with mixer. Source: The author.

Fig. 5 Samples of zebra mussel shell treated phosphorus water, with ascorbic acid method reagent b. Source: The author.

Un-heated zebra mussel shells were also mixed with synthetic phosphorus water. Shell powder was dosed in a concentration of 2.00 mg L^{-1}, following the procedure described above. Three jar tests were conducted and the phosphorus and pH was measured before and after mixing.

2.6 Lime Comparison

Chemical grade calcium hydroxide was used to treat synthetic phosphorus water and compared to calcined shell. Dose concentrations of 0.25, 0.50, 1.00 g L^{-1} of calcium hydroxide, fine (<75 μm), and coarse (500 μm-1000 μm) HGZMS were mixed with 10 mg L^{-1} phosphorus water following ASTM D2035 (ASTM, 2012) and the procedures for mixing described in section 2.6. Phosphorus was measured before and after mixing using the ascorbic acid method (APHA, 1995). pH was measured before and after (40 min) treatment using a pH meter. Two jar tests were conducted for each dose, yielding 8 samples. As in section 2.5, three vials were measured for each sample and averaged for one spectrometer reading per jar test.

2.7 Time Release pH Properties of Calcined Shell vs Calcium Hydroxide

The pH effect of time and aqueous batches was examined for calcined shell and compared to calcium hydroxide. 0.5 g L^{-1} calcium hydroxide, fine (<75 μm), and coarse (500 μm-1000 μm) HGZMS were mixed with tap water at 125 rpm continuously. The pH was recorded every 60 seconds for 5 minutes. After 5 minutes the mixing was stopped, and the solution was settled for 1 minute. The supernatant was poured off through 0.45 μm paper filter. The particles on the paper filter were washed back into the jar containing the remaining particles and mixing was resumed at 125 rpm. This process was repeated 3 times for calcium hydroxide, fine and coarse HGZMS.

2.8 Effluent Collection and Characterization

Sewage effluent was collected during the months of July and August 2019, from the Village of Dunnottar Wastewater Treatment Facility (50˚26’39.3’’N, 97˚01’04.0’’W) located near the west shore of Lake Winnipeg’s south basin. Effluent was collected from a secondary cell off of a pier. Samples were collected from approximately one meter of depth. The samples were then transported directly to the University of Manitoba and stored in a fridge at 4 ˚C to maintain stable conditions.

Effluent was analysed for phosphorus content and pH. The effluent was filtered using grade 0.45μm paper filters to remove suspended solids. The effluent was analyzed for phosphorus concentrations using the ascorbic acid method test (APHA, 1995) and an Ultrospec 4300 pro spectrophotometer. The effluent pH was measured using a calibrated pH meter.

2.9 Effluent Jar Tests

Secondary lagoon effluent was added to 1L glass jars and the pH of the effluent was determined once again prior to treatment. The heated fine ground (<75 µm) zebra mussel shell produced for each heating condition (600, 700, 800, 900, 1000 ˚C) was added at dose concentrations of 0.25, 0.50, 1.00, and 2.00 g L^{-1}. Upon addition of the HGZMS the jars were mixed using a paddle mixer. The effluent was mixed following ASTM D2035 (ASTM, 2012), by mixing at 120 rpm for the first minute, then 50 rpm for the next 20 minutes. Mixing was stopped, and the effluent left to settle for 15 minutes. The pH of the effluent was measured, and the effluent was filtered using 0.45µm paper filters to remove suspended solids. The treated and untreated effluent was analyzed for phosphorus concentrations using the ascorbic acid method test (APHA, 1995) and an Ultrospec 4300 pro spectrophotometer. Three sample tests were conducted for each dose and treatment combination, yielding a total of 60 observations, and 20 sample sets. Each observation was poured into three separate plastic vials in the spectrophotometer, yielding 180 spectrometer measurements. The three spectrophotometer measurements were averaged to produce one spectrophotometer reading for each jar test. If outliers were present within the three spectrophotometer readings of the jar tests, the samples were retested to ensure accurate readings.

pH was recorded at the beginning and end (40 minutes from reaction start) with a calibrated pH meter for each jar sample, yielding a total of 60 pH readings (3 for each variable set).

Chapter 3 – Results

3.1 Shell Characterization

The results of three different shell characterization analyses are presented. The followings sections show results of titrations to determine percent mass calcium carbonate, ICP analyses for the determination of percent mass concentration of trace elements, and XRD analysis for the examination of shell crystalline structure.

3.1.1 Titration

Table 1 displays the titration volumes of HCl and NaOH used in the back titration of dissolved ground zebra mussel shells, and the percent mass calcium carbonate calculated from the displayed volumes using equation 10. The average percent calcium carbonate determined from the three titrations was 86.67% (SD = 1.12%).

	Mass of Shells Dissolved (g)	Volume of 0.1 M HCl (mL)	Volume of 0.1 M NaOH (mL)	% Calcium Carbonate by Mass
Titration 1	0.2791	58.00	10.00	87.86
Titration 2	0.2535	58.00	15.50	85.67
Titration 3	0.2635	58.00	13.40	86.47

Table 1. Titration volumes and subsequent percent calcium carbonate of zebra mussel shell.

3.1.2 ICP

Percent elemental composition of zebra mussel shells, for a select group of elements, from ICP analysis are presented in Table 2. For all elements analysed the percent composition represented only trace amounts. The sum of the elements tested for represents 0.094365% of total shell composition.

Element	Percent Composition	Standard Deviation
As	0.00399	0.00165
Ba	0.01319	0.00061
Be	0.00002	0.00001
Co	0.00142	0.00057
Cr	0.00073	0.00010
Fe	0.00843	0.00032
Mn	0.00472	0.00007
Ni	0.00269	0.00045
Pb	0.00324	0.00094
Se	0.00853	0.00079
V	0.00066	0.00017
Cu	0.00057	0.00010
K	0.02801	0.02660
Zn	0.01494	0.00976
Sb	0.00323	0.00068

Table 2**.** ICP analysis of trace elements present in Manitoban zebra mussel shells.

3.1.3 XRD

Figure 6 presents a diffractogram from XRD analysis. The Diffractogram demonstrates peaks representative of crystalline aragonite calcium carbonate (as identified by JADE). Expected calcium carbonate peaks are indicated by the diamond points. No other peaks for other crystalline material were identified, indicating that the only crystalline material present in un-treated zebra mussel shells is aragonite calcium carbonate.

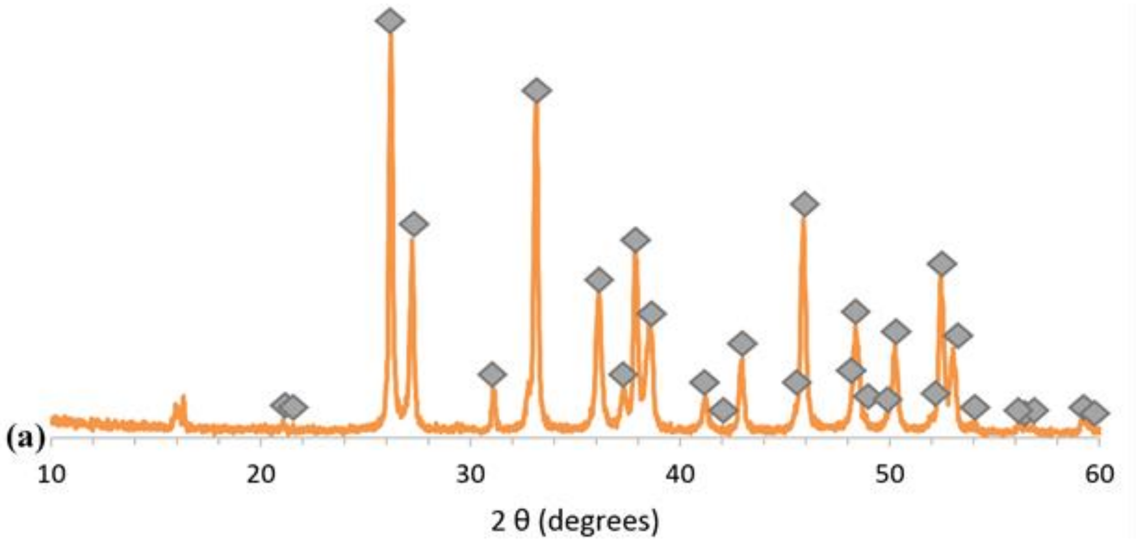

Fig. 6. Diffractogram of un-heated zebra mussel shells.

3.2 Shell Calcination

Shell calcination was examined through two separate processes: firstly, XRD analyses was used too qualify the transitions undergone by shell material through heating; and secondly, mass loss of heated shells was recorded to approximate calcination completion. The results are presented in section 3.2.1 and 3.2.2.

3.2.1 XRD

XRD analysis of unheated shell powder, shell powder heated at 600 ˚C, and shell powder heated at 1000 ˚C (Figure 7), shows the transition of shell material from aragonite calcium carbonate to calcite calcium carbonate, and calcium oxide respectively. The diamond, square, and circle indicate the expected peaks of aragonite, calcite, and calcium oxide respectively. The results confirm the chemical transition expected from heating the shell material at high temperatures.

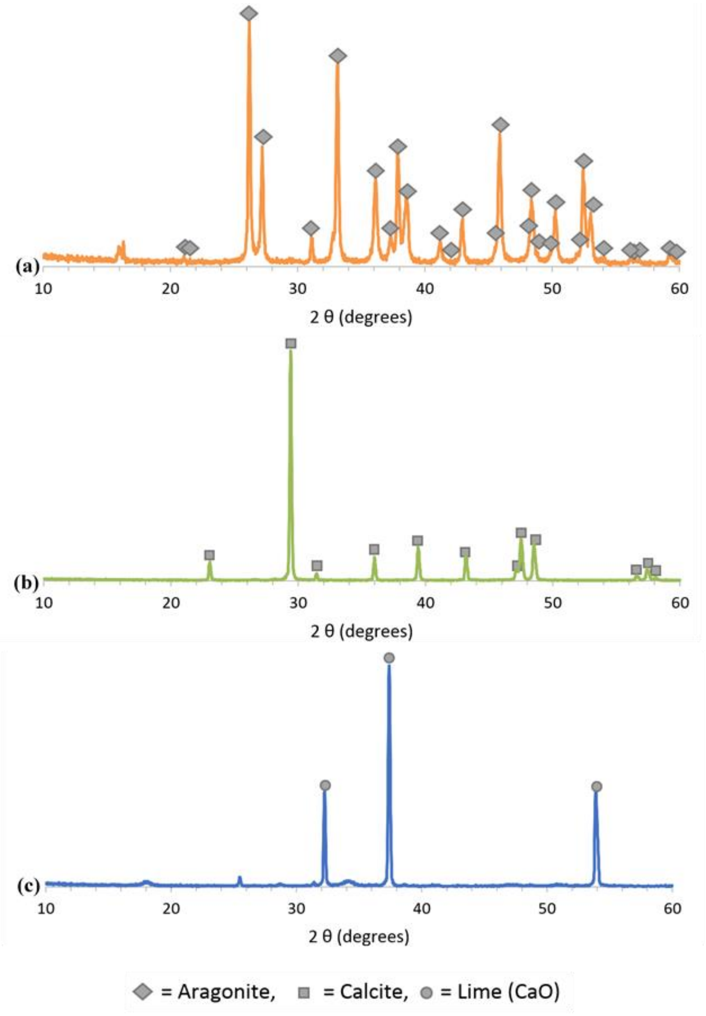

Fig. 7. X-ray difraction results of raw zebra mussel shell (a), zebra mussel shell heated at 600 ˚C (b), and zebra mussel shell heated at 1000 ˚C (c).

3.2.2 Mass Loss Analysis

Calcination completion was approximated through mass loss for coarse (500-1000 μm) and fine (<75 μm) sized particles. Figure 8 and 9 show the relationship between percent mass loss and heat treatment temperature, along with estimated equations modelling the relationship for each particle size. The models predict a maximum mass loss of 45.54 and 43.19%, and mid-point mass loss temperatures of 779.48 and 721.02 ˚C for coarse and fine particles respectively.

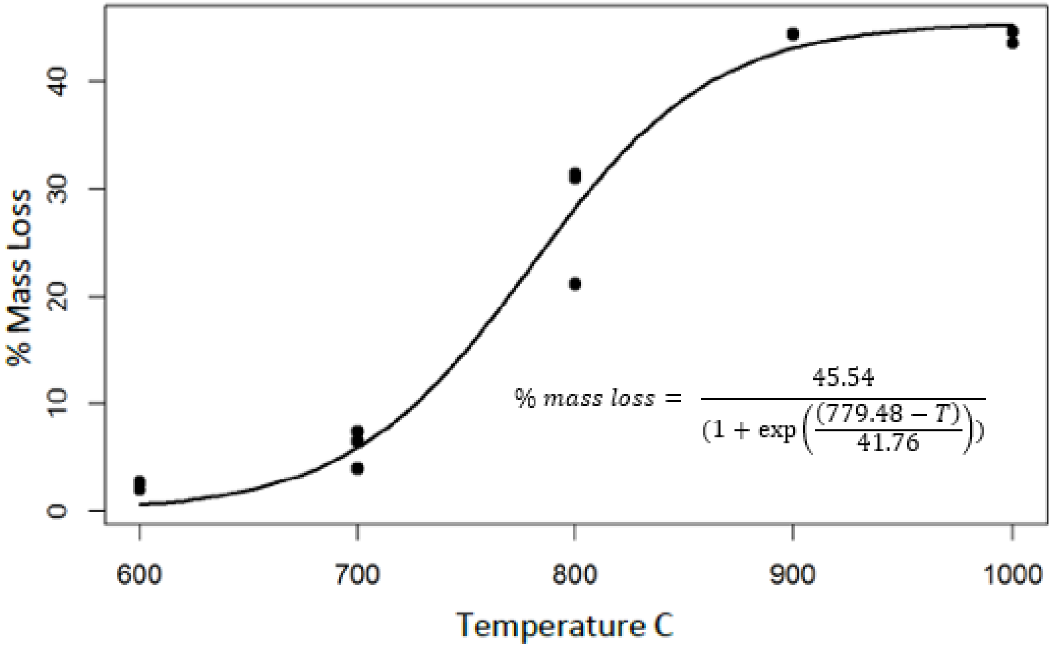

Fig. 8. Temperature treatment vs mass loss of coarse shell with a fitted sigmoid curve (equation 10).

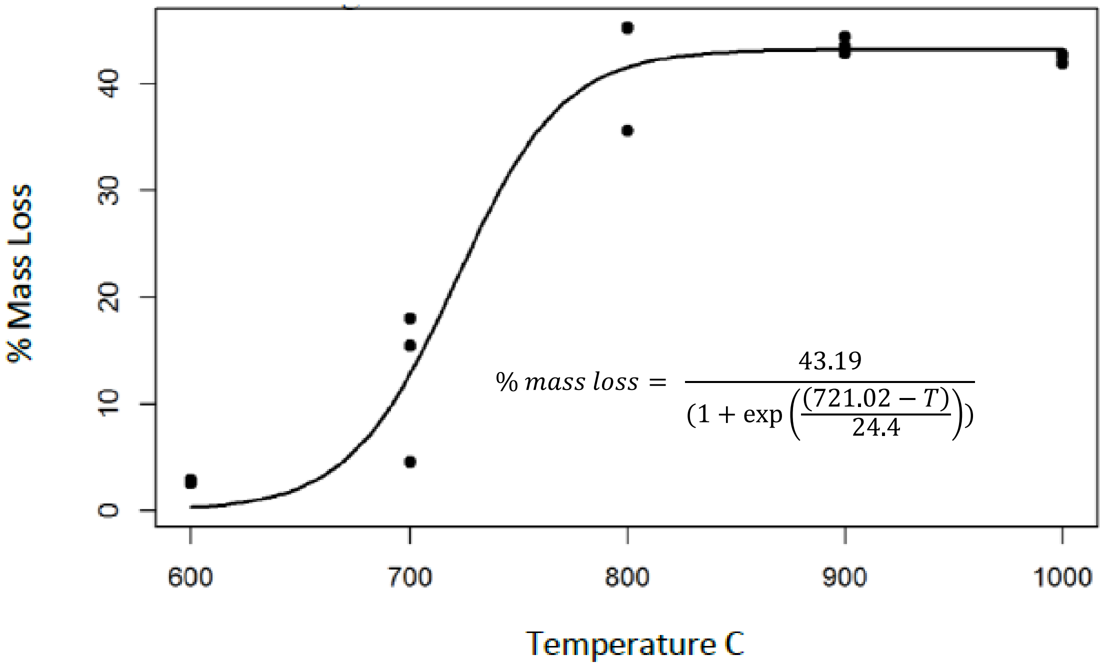

Fig. 9. Temperature treatment vs mass loss of fine shell with a fitted sigmoid curve (equation 11).

3.3 Phosphorus Removal from Synthetic Water

The removal of phosphorus from synthetic water was examined using fine and coarse calcined shells (1 g L^{-1}) heat treated at 600, 700, 800, 900, and 1000 ˚C; fine and coarse shells heat treated at 800 ˚C, and chemical grade calcium hydroxide at doses of 0.25, 0.50, and 1.00 g L^{-1}; and un-heated shell dosed at 2.00 g L^{-1}.

3.3.1 Particle Size Comparison

Figure 10 presents the percent phosphorus removal for both coarse and fine shells heat treated at 600, 700, 800, 900, and 1000 ˚C. Shells heated at 600 ˚C for coarse and fine particles removed an average percent phosphorus of 6.9 and 11.8, respectively. Shells heated at 700 ˚C for coarse and fine particles removed an average percent phosphorus amount of 98.8 and 97.2, respectively. For both coarse and fine particles, heat treatments of 800 ˚C and higher yielded effectively complete phosphorus removal.

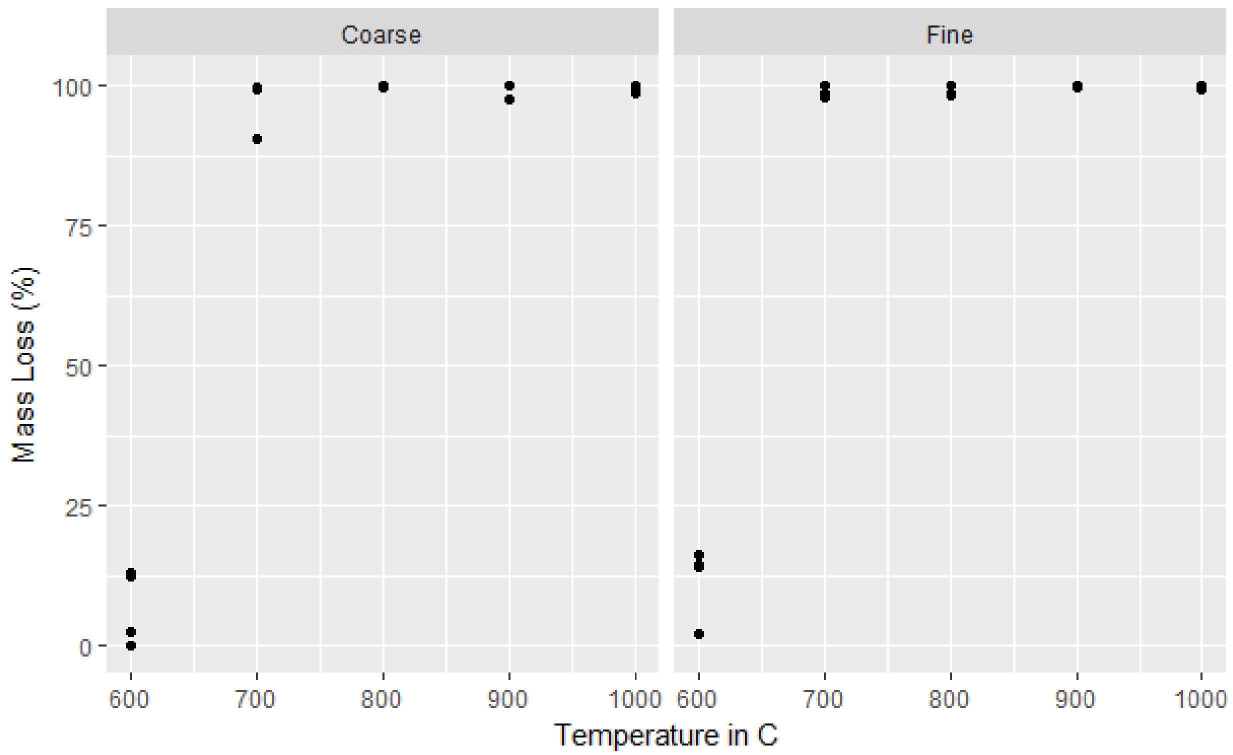

Fig. 10. Percent phosphorus removal, from 10 mg L^{-1} phosphorus water, vs heat treatment temperature for both coarse and fine particles.

3.3.2 Un-treated Shell Phosphorus Removal

Untreated ground zebra mussel shell mixed with 10 mg L^{-1} phosphorus water in concentrations of 2 g L^{-1} removed an average phosphorus of 7.2%.

3.3.3 Comparison to Calcium Hydroxide

Figure 11, 12, and 13 depict percent phosphorus removal, residual phosphorus concentration after treatment, and pH, respectively, for coarse HGZMS, fine HGZMS, and calcium hydroxide, in doses of 0.25, 0.50, and 1.00 g L^{-1}. By comparing figure 11, 12 and 13, it is observed that pH and removed phosphorus show a correlation. The near removal of phosphorus is achieved by all treatment and dose combinations with the exception of coarse shells dosed at a concentration of 0.25 g L^{-1}, which achieved only 80.571% removal. pH is lower with coarse HGZMS, then fine HGZMS, and highest with calcium hydroxide. pH increases with dose concentration. If phosphorus removal is the same for two treatment methods, then the one with lower final pH is desirable.

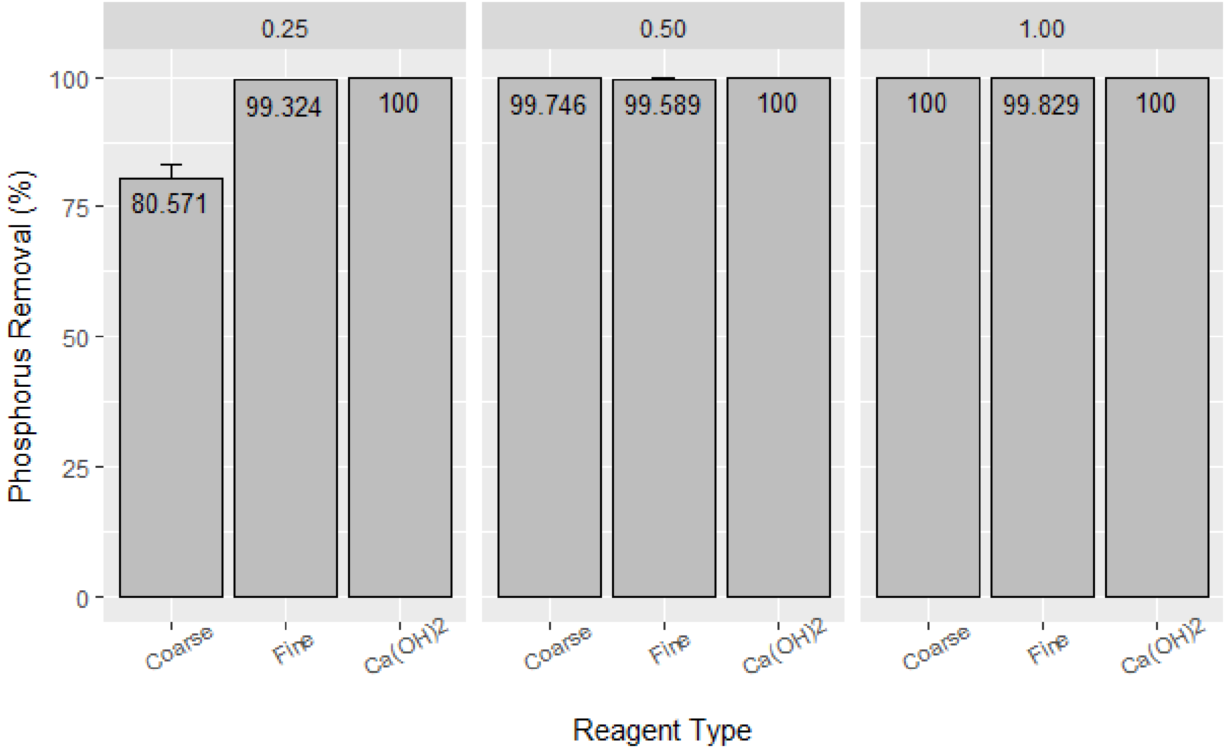

Fig. 11. Percent phosphorus removal from 10 mg L^{-1} phosphorus water for reagent type coarse, fine, and calcium hydroxide, and doses 0.25, 0.50, and 1.00 g L^{-1}.

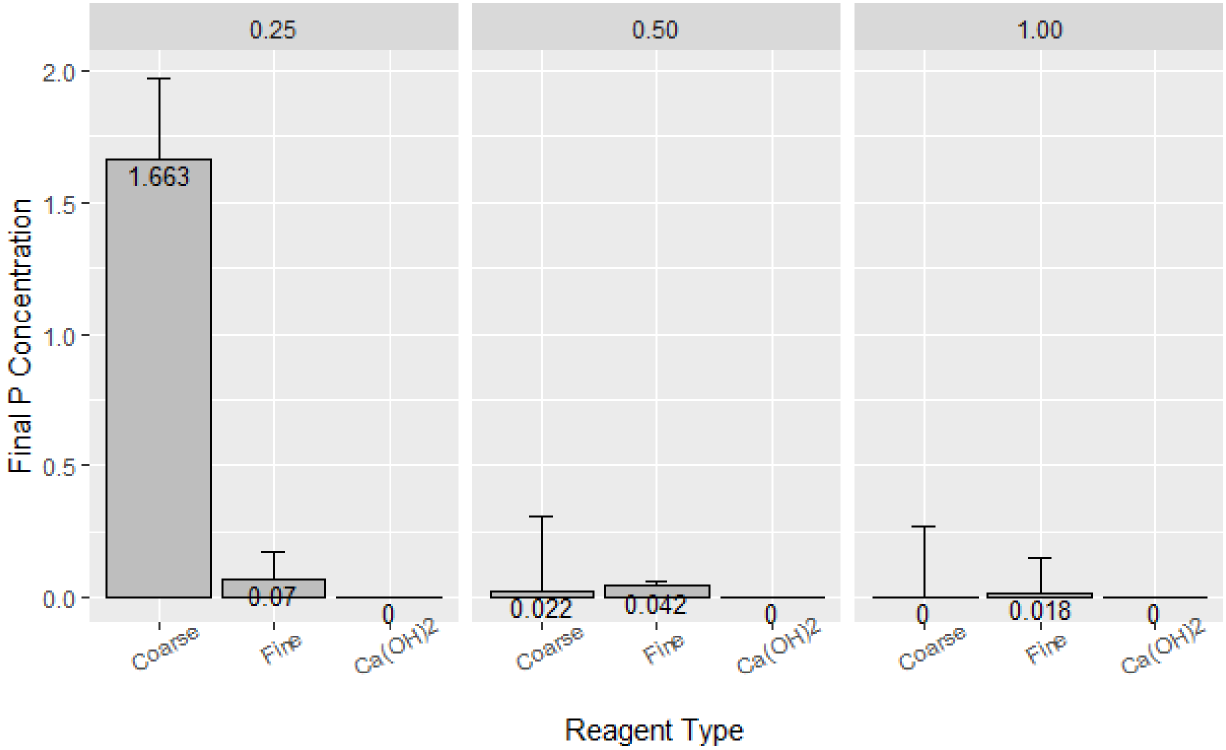

Fig. 12. Residual phosphorus concentration after treatment from 10 mg L^{-1} phosphorus water for reagent type coarse, fine, and calcium hydroxide, and doses 0.25, 0.50, and 1.00 g L^{-1}.

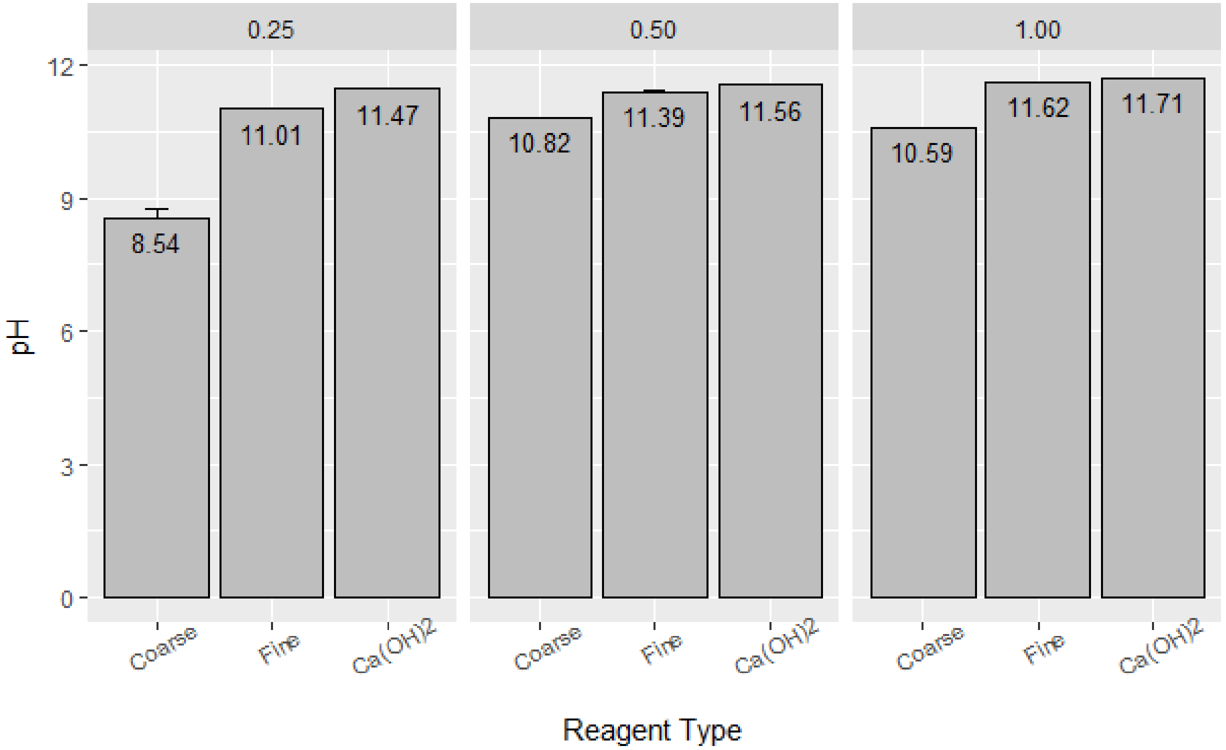

Fig. 13. Final pH (after 40 min) of 10 mg L^{-1} phosphorus water treated with reagent type coarse, fine, and calcium hydroxide, and doses 0.25, 0.50, and 1.00 g L^{-1}.

3.4 Time Release pH Analysis

Figure 14 presents the pH effect of fine and coarse HGZMS, and calcium oxide mixed with tap water in a dose concentration of 0.5 g L^{-1} over time, with supernatant poured of and replaced from the mixture every 5 minutes. After the supernatant was poured off three times coarse HGZMS reached a pH of 10.23 which represents 94.7% of the maximum pH of the first mixture. Fine HGZMS reached a pH of 9.94, after the supernatant was poured off twice, which represents 92.0% of the maximum pH of the first mixture. After the third time the supernatant was poured off the maximum pH reached was 8.1. After the supernatant was poured off just once, calcium oxide reached a pH of only 9.67, after the supernatant was poured off twice the maximum pH reached was 8.06 which represents 71.0% of the maximum pH of the first mixture.

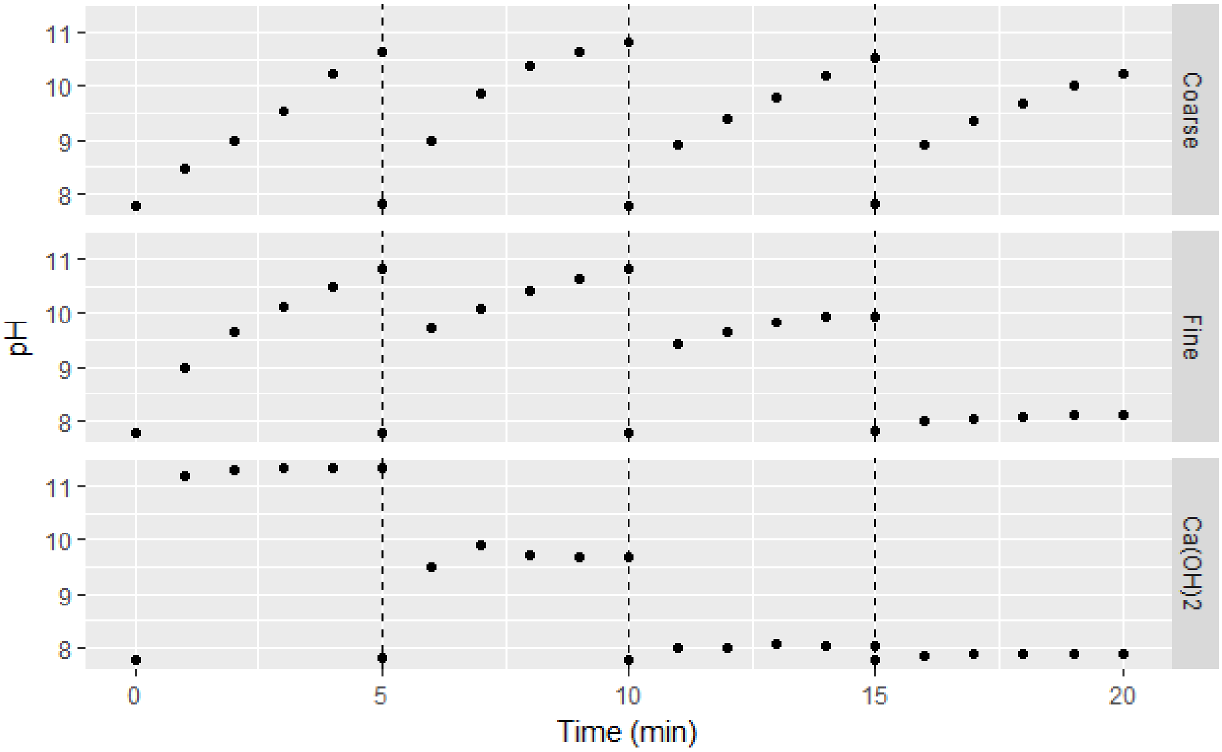

Fig. 14. pH effect over time for 0.5 g L^{-1} Coarse HGZMS, Fine HGZMS, and Calcium hydroxide continuously mixed (125 rpm) with tap water. Dashed line represents poured of and replaced supernatant.

3.5 Collected Effluent Jar Tests and Phosphorus Removal

Results from fine HGZMS, heated at temperatures of 600, 700, 800, 900, and 1000 ˚C, mixed with collected effluent water, in doses of 0.25, 0.50, 1.00 g L^{-1} are shown in Figure 15. Results demonstrate that shells heat-treated at a temperature of 1000 ˚C remove nearly 100% phosphorus at any of the 4 dose concentrations. Removal for shells heat-treated at 700, 800 and 900 ˚C increase with dose concentration up to 1.00 g L^{-1}. Additional increase in phosphorus removal is not observed between 1.00 and 2.00 g L^{-1}.

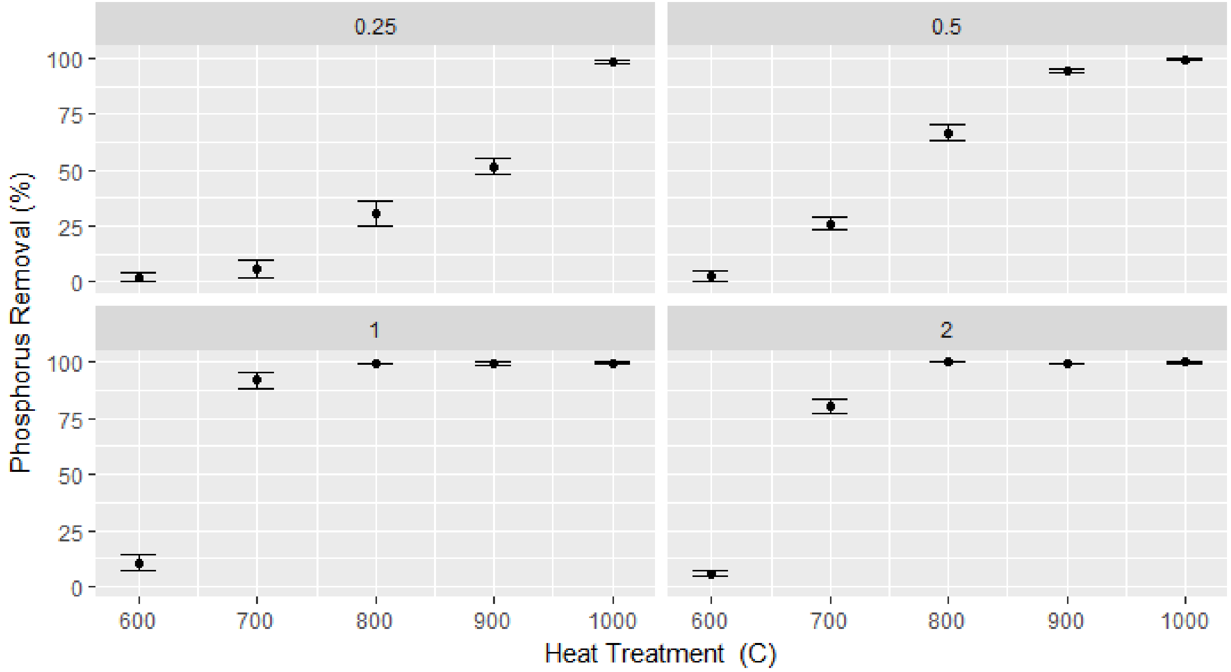

Fig. 15. Average percent phosphorus removal vs shell heat treatment temperature (C) for total HGZMS dose 0.25, 0.50, 1.00, 2.00 g L^{-1}. Error bars indicate 95% confidence interval of boot strapped data (n = 1000).

The results shown in figure 15 were also graphed by plotting percent removal against predicted calcium oxide concentration using the model shown in figure 9. These results are shown in figure 16.

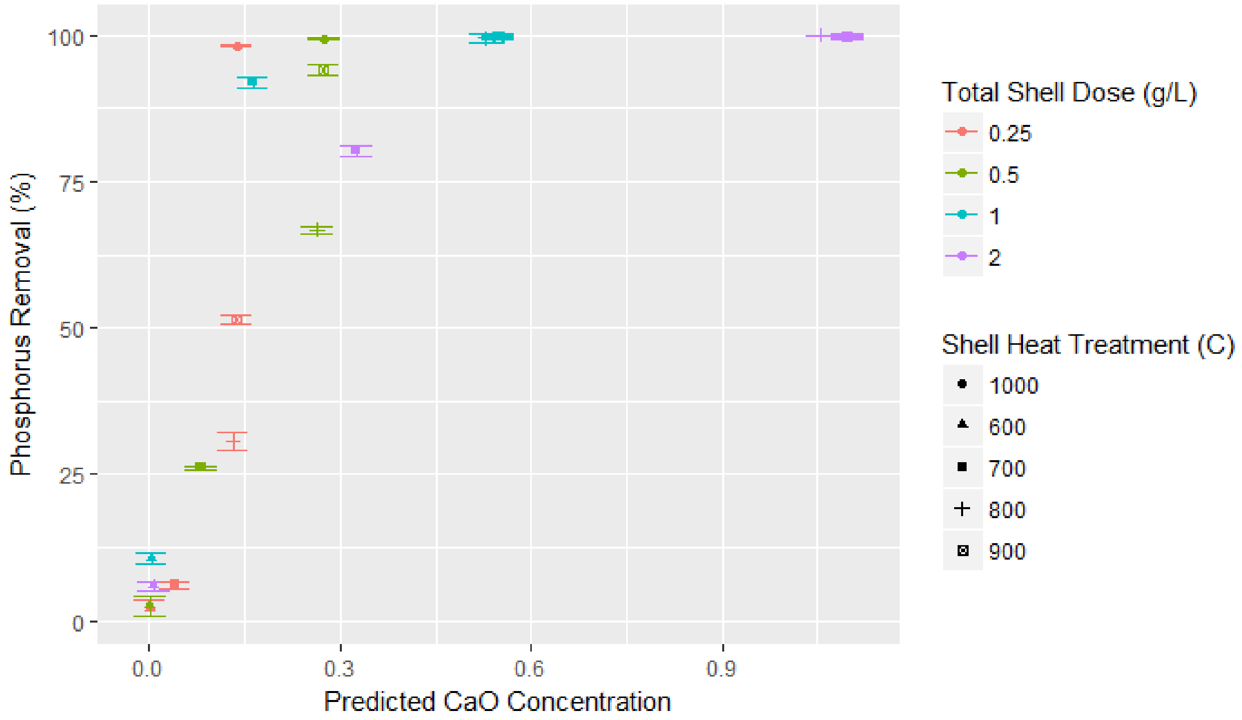

Fig. 16. Average percent phosphorus removal vs model predicted CaO dose concentration (g L^{-1}). Error bars indicate 95% confidence interval of boot strapped data (n = 1000). Legend indicating total HGZMS dose and shell heat treatment.

Figure 17 presents the final pH effluent treated with HGZMS, heat treated at 600, 700, 800, 900 and 1000 ˚C, for dose concentrations of 0.25, 0.50, 1.00, and 2.00 g L^{-1}. The results demonstrate that pH increases with dose and with increased heat treatment temperature. Figure 18 shows the relationship between pH and phosphorus removal. The relationship closely follows the sigmoidal curve shown in figure 18, where removal begins at a threshold pH and reaches maximum removal (~100%) after which point increased pH does not yield higher phosphorus removal.

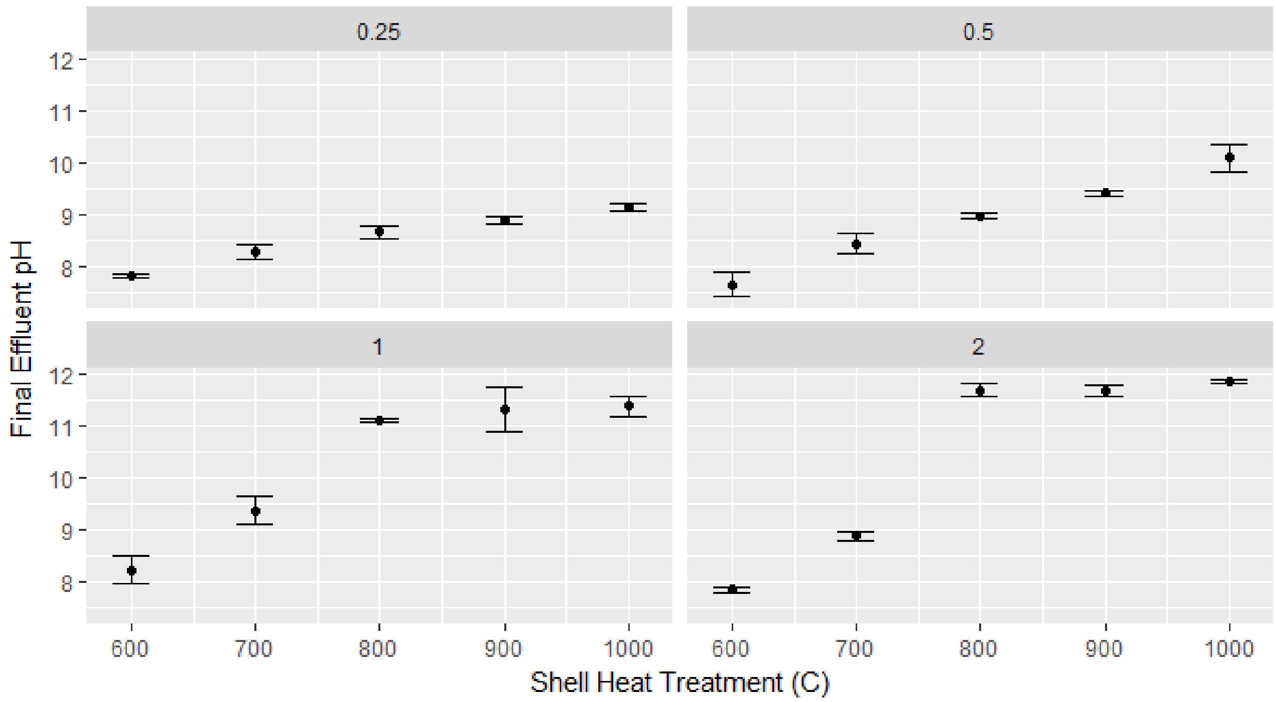

Fig. 17. Final effluent pH vs shell heat treatment temperature (C) for total HGZMS dose 0.25, 0.50, 1.00, 2.00 g L^{-1}. Error bars indicate standard deviation of sample.

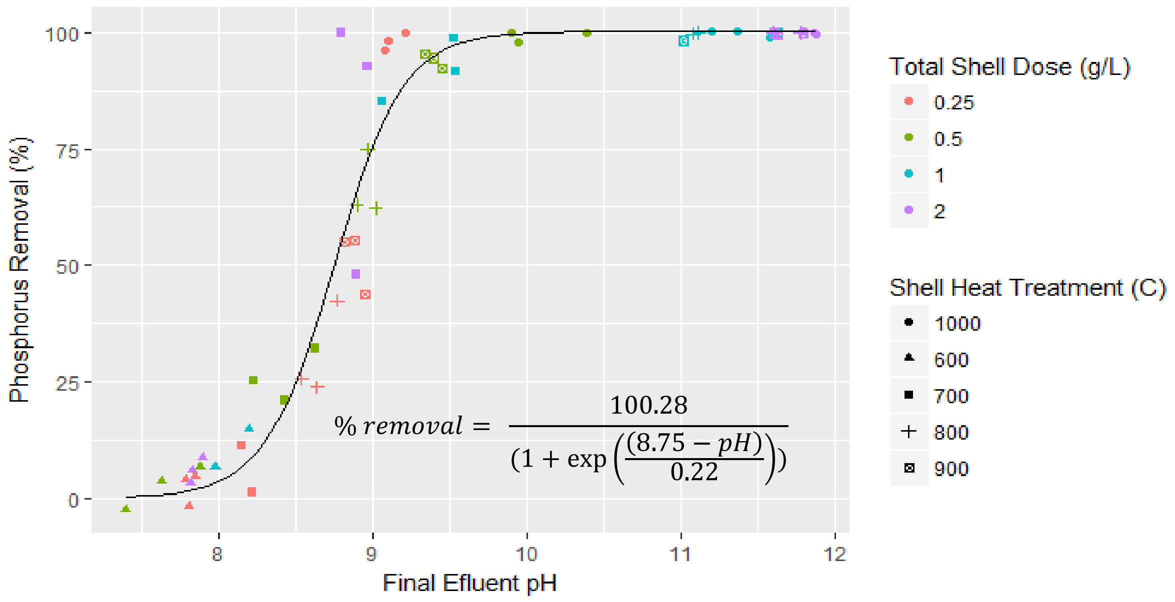

Fig. 18. Phosphorus removal vs final effluent pH fitted with sigmoid curve (equation 4). Legend indicating total HGZMS dose and shell heat treatment.

Chapter 4 – Discussion

4.1 Shell Characterization

The objective of the research presented in this section was to determine the amount of calcium carbonate present in zebra mussel shells and their crystalline structure, as well as to identify other trace elements present in the shells composition.

4.1.1 Calcium Carbonate

As presented in section 3.1.1 zebra mussel shell titration results demonstrate that of the shell material tested, the average calcium carbonate percent mass is 86.67 (SD = 1.12). The determined percent calcium carbonate of zebra mussel shells presented in this study agrees closely to the percent mass of calcium carbonate presented by Imel et al. (2016) of 87.5%.

The percent calcium carbonate determined to be present in zebra mussels is lower than concentrations found in some studies examining the concentration of calcium carbonate in oyster and mussel shells from the seafood industry such as, 94 % (Lee et al., 2009) and 96% (Yoon et al., 2002). However, the calcium carbonate composition is higher than other studies such as, 60.5% (Hsu, 2009) and 70% (Namasivayam et al. 2004). 86.67% calcium carbonate indicates sufficient calcium carbonate composition to be useful as an alternative source to limestone for the production of calcium oxide.

As expected, the XRD analysis of un-treated zebra mussel shell resulted in diffractograms indicating the presence of aragonite calcium carbonate as the sole crystalline structure. Calcium carbonate found in the aragonite crystalline form agrees with the results presented by Abeynaike et al. (2009), Jones et al. (2011), Kwon et al. (2004), Paradelo et al.

(2016), and Namasivayam et al. (2005) for other bivalves, and specifically for zebra mussel shells as presented by Imel et al. (2016).

4.1.2 Trace Elements

Table 2 presented in section 3.1.2 shows the percent mass concentration of tested trace elements. The results indicate that very small amounts of the tested elements are present in zebra mussel shells sourced from Lake Winnipeg. Dangerous and toxic elements such as: arsenic, chromium, lead, cobalt, copper, vanadium, antimony and beryllium were found to be present in extremely low concentrations. Based on the results found from ICP analysis, the concentrations do not present a risk to the environment if the shell product is used for wastewater treatment, as the concentrations in the shells normalized to dosed concentrations for treatment are considerably lower than the maximum allowed concentration levels of these elements in released wastewater effluents.

Of all tested trace elements, the total mass found represented 0.094365% of total shell composition. Combined with the estimated 86.67% calcium carbonate, a total 86.76 % of shell composition is known, leaving 13.24% still unaccounted for. It is likely that sodium (Imel et al., 2016) and potentially magnesium account for a significant portion of the unknown material. Additionally, Hsu et al. (2009) and Yoon et al. (2004) found small but significant amounts of silicon in examined bivalve shells. Given the high amount of silicon present on Lake Winnipeg's eastern shores, it is possible that silicon also could represent a significant portion of the unknown material. Bound elements, undetected by ICP, making up the molecular structures with in the shell, such as oxygen, hydrogen, and carbon, also likely contribute to a significant amount of the remaining 13.24 % shell mass. Further analysis is needed to more completely define the shell materials composition. This would provide added confidence to the safe use of zebra mussel shells for environmental and/or human consumption applications.

4.2 Calcination

Calcination of shell material was qualified through XRD diffractograms to visualize the transition of the shell material at different heating temperatures. The effect of heat treatment was examined for two ground shell particle sizes: coarse (500-1000 µm) and fine (<75 µm). The transition of crystalline structure with heat, as well as the effect of heat and particle size, are discussed in section 4.2.1 and 4.2.2.

4.2.1 Crystalline Structure Transitions

Diffractograms of ground zebra mussel shells, un-heated and heated at 600 and 1000 ˚C are shown in figure 7. For the three treatment methods, the diffractograms demonstrate peaks, as determined through comparison analysis by JADE, indicating crystalline aragonite, calcite, and calcium oxide respectively. The transition of aragonite to calcite at low heat treatment temperatures is expected. Aragonite is coerced to calcite due to calcite having greater thermodynamic stability (Okumura et al., 2018), however no chemical change is observed at this point. At 1000 ˚C a complete transition from calcium carbonate to calcium oxide is observed. Given that 1000 ˚C is a far higher temperature than the threshold temperature discussed in section 1.6 and that the literature value of the partial pressure of calcium oxide at 898 ˚C is 101 kPa (atmosphere), it was expected that complete calcination would be observed.

4.2.2 Mass Loss

Calcination completion was approximated through mass loss observed when heating the shell material. Figure 8 and 9 show mass loss with temperature for coarse and fine ground zebra mussel shells respectively. It was expected that the fine ground zebra mussel shells would decarbonate more effectively and more readily than coarse shells due to the higher surface area to mass ratio. By comparing figure 8 and 9, as well as equations 10 and 11 (modeled mass loss to heat treatment temperature relationships) it can be concluded that particle size does affect mass loss. To better visualize the difference in mass loss between the two particle sizes, the modeled sigmoid curve for each particle size is shown in figure 19.

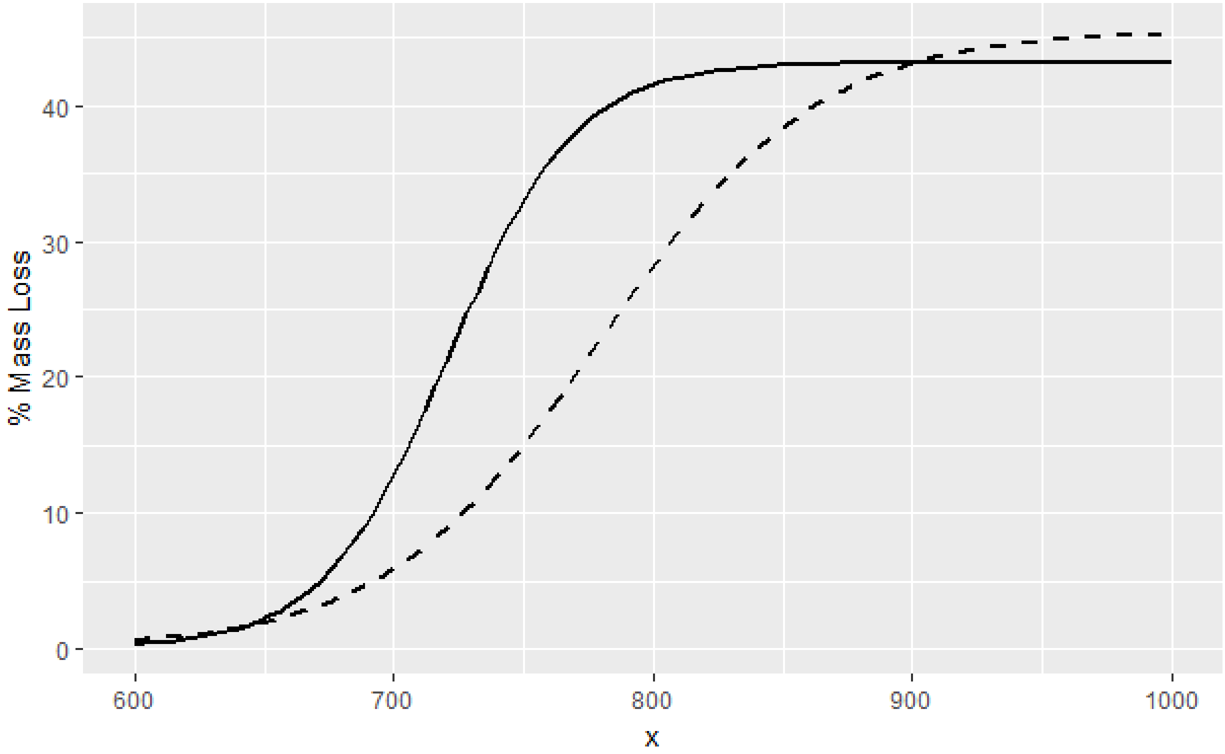

Fig.19. Percent mass loss vs Temperature in Celsius (x) for mass loss to heat treatment temperatures predicted by modeled sigmoid curves (equations 10 and 11) for coarse and fine HGZMS. Dashed line = Coarse HGZMS, solid line = Fine HGZMS.

From figure 19 it can be observed that mass loss is higher at heat treatment temperatures of 700 and 800 ˚C for fine particles than coarse particles. However, at 900 ˚C the mass loss observed between the two particles is effectively the same. At 1000 ˚C the mass loss observed for coarse particles is slightly higher than fine particles, with max mass loss predictions of 45.54 and 43.19 % respectively. It is suspected that while the fine particle size resulted in higher surface area ratio and more effective heat transfer at transitional temperatures (700 and 800 ˚C), at 1000 ˚C small amounts of sintering, as observed by Jones et al. (2011), may have occurred, resulting in slightly less mass loss than coarse ground zebra mussel shells.

Mass losses seen in this study were similar to other past research. Lee et al. (2011) reported 41% mass loss at 750 ˚C under regular atmospheric conditions with oyster shells. Based on the predicted sigmoid curve for fine particles, mass loss at 750 ˚C for the current study would be 33.1%. The reason for the lower mass loss could be attributed to differences in process parameters such as: particle size (particle size was not clear in Lee et al. (2011) study), oven conditions, initial percent calcium carbonate, composition differences between zebra mussel shells and oyster shells, and pre-drying/heating methods. Past studies that utilized nitrogen purged ovens reported maximum mass loss near 800 ˚C (Ballester et al., 2007; Jones et al., 2011; Kwon et al., 2004). In this study maximum mass loss was observed between 900 and 1000 ˚C. Despite higher energy inputs required to produce the same amounts of calcium oxide, atmospheric ovens were chosen because of the impracticality and cost associated with nitrogen purged ovens on an industrial scale.

4.3 Phosphorus Removal (Synthetic Water)

To ensure optimal control for comparison experiments, synthetic water dosed with 10 mg L^{-1} of phosphorus was used. The effect of particle size on phosphorus removal and the comparison of coarse and fine HGZMS to calcium hydroxide, examining phosphorus removal and pH, is discussed in section 4.3.1 and 4.3.2.

4.3.1 Effect of Particle Size

Figure 10 displays percent phosphorus removal achieved by coarse and fine particles for each heat treatment temperature. The results indicate that for temperatures 600 and 700 ˚C fine HGZMS is slightly more effective at removing phosphorus, achieving 11.8 and 98.8 % removal respectively, versus 6.9 and 97.2 % removal for coarse shells. At heat treatment temperatures of 800 ˚C and greater, phosphorus removal is effectively 100% for both particle sized HGZMS, when dosed in a concentration of 1.00 g L^{-1}. The results suggest that the initial particle size of the ground shell prior to heating does not play a significant role in phosphorus removal. It is possible that this observation is due to the calcite core and surrounding calcium oxide ions described by Abeynaike et al. (2009) and Jones et al. (2011). While it is likely that not as many calcium oxide particles are available for phosphorus binding for coarse particles as they are for fine particles, calcium ions are still provided in excess, and enough hydroxide ions are provided to raise the pH above 10, resulting in hydroxyapatite formation and the removal of phosphorus. This would explain why smaller particle size does not increase the reaction equilibrium towards calcium phosphate production as might be expected. Further analysis is necessary to confirm this theory.

4.3.2 Comparison to Calcium Hydroxide

For dose concentrations of 0.25, 0.50, and 1.00 g L^{-1} coarse and fine HGZMS heat treated at 800 ˚C were compared to chemical grade calcium hydroxide. Phosphorus percent removal, phosphorus post-residual concentration, and pH were recorded. The results are shown in figures 11, 12 and 13. For all reagent types and all doses, phosphorus removal was 99.3 % or greater, except for coarse zebra mussel shells dosed at 0.25 g L^{-1}, which removed 80.6% and left a residual phosphorus concentration of 1.663 mg L^{-1}. While it was discussed in the previous section how particle size appears to play little role in phosphorus removal (between the two tested sizes) beyond its effect on calcination in the oven, a significant difference is observed between coarse (80.571%) and fine (99.324%) HGZMS phosphorus removal when dosed at 0.25 g L^{-1}. It is likely that this difference is caused by the resulting pH of the reaction. For coarse HGZMS the final pH was observed to be 8.54 versus fine HGZMS final pH was found to be 11.01. Due to the low pH of coarse HGZMS dosed at 0.25 g L^{-1}, hydroxyapatite would not be formed given it is significantly below the required pH level 10. It is assumed that the low pH is due to the low dose combined with the larger particle size resulting in too few hydroxide ions for pH to increase above 10. These results demonstrate that particle size does effect phosphorus removal when dose concentrations are low enough that hydroxide ions need to be easily dissolved to achieve pH 10 for hydroxyapatite formation. However, when concentrations of hydroxide ions are high by means of HGZMS dose, as in the case for dose concentrations of 0.50 and 1.00 g L^{-1}, particle size does not affect removal.

HGZMS proved to be equally effective as lime for all dose and treatment combinations except for 0.25 g L^{-1} of coarse shells. The HGZMS however, achieved effectively the same phosphorus removal as lime, at lower pH. Lower pH is desirable so that the shell treated effluent can either be released without any further pH adjustment, which would otherwise costly. From figure 13 it can be concluded that coarse HGZMS and fine HGZMS can remove above 99% from 10 mg L^{-1} phosphorus water while maintaining a pH of 10.82 and 11.01 respectively with dosages of 0.25 and 0.50 g L^{-1} respectively.

4.3.3 Un-treated Zebra Mussel Shells

2.00 g L^{-1} un-treated zebra mussel shells removed 7.2 % phosphorus when mixed with 10 mg L^{-1} phosphorus water. It is un-known whether the mechanism of removal was through the conventionally understood adsorption mechanism or through heterogenous precipitation described by Abeynaike et al. (2009) and Jones et al. (2011). The removal rate was not significant enough to be recommended as a removal method.

4.4 Time Release pH Property

Figure 14 shows the results of the time release experiment. The results show that while calcium hydroxide increases pH, it only increases the pH for one “batch” of water. HGZMS however, can increase pH for multiple “batches”. Calcium hydroxide readily dissolves and pH spikes due to the dissolved hydroxide ions. When the supernatant is poured off, all of the dissolved hydroxide ions escape with the supernatant and any remaining particles have little effect on pH. HGZMS however, do not completely dissolve, and likely hold onto calcium oxide particles on the surface of calcite structures, as speculated by Abeynaike et al. (2009) and Jones et al. (2011). Coarse HGZMS were shown to have a stronger time release property than fine HGZMS, supporting the theory that the calcite core releases calcium oxide ions over time, as the ions would be less available to dissolve for larger particles due to smaller surface area ratios. This experiment was a replication of Lee et al. (2011) time lapse experiment, using zebra mussel shells instead of oyster shells and a dosage of 0.5 g L^{-1} instead of 0.6 g L^{-1}. Two particle sizes were examined in this study rather than one, and chemical grade lime was used as a control and comparison. The results of both experiments were similar and demonstrated the time release properties of both heated bivalve shells. The time release pH property of HGZMS could be advantageous for batch treatments where effluent is continuously treated and released. The time release property means that less material could be used for phosphorus treatment than would be used if chemical grade lime was used. Additionally, in a lagoon setting, built up sludge from

HGZMS dosing could provide a residual equilibrium pH, potentially reducing the amount of material needed.

4.5 Phosphorus Removal and pH Effect of Fine HGZMS on Real Wastewater Effluent

The objective of this section was to examine the effectiveness of HGZMS on real secondary wastewater effluent and to identify optimized treatment conditions and dose concentrations for the most efficient phosphorus removal. For the purpose of control, only one particle size (< 75 μm) was used.

4.5.1 Phosphorus Removal

Figure 15 shows the removal of phosphorus from 2.72 mg L^{-1} +/- 0.2 mg L^{-1} (pH was 7.79 +/- 0.3) for heat treatments of 600, 700, 800, 900, and 1000 ˚C and dosages of 0.25, 0.50, 1.00, and 2.00 g L^{-1}. The results show that increasing dose from 0.25 g L^{-1} through to 1.00 g L^{-1} increases removal efficiency, however, increasing dose past 1.00 g L^{-1} does not increase phosphorus removal. Therefore, using a dose above 1.00 g L^{-1} is unnecessry, as there is no observed benefit beyond this point. For dosages 0.25 and 0.50 g L^{-1}, phosphorus removal shows increasing efficiency as heat treatment temperature increases. For dosages 1.00 and 2.00 g L^{-1} maximum phosphorus removal is reached for shells heat treated at 800 ˚C, suggesting that for high concentration doses, heat treating shells at temperatures beyond 800 ˚C has no benefit for phosphorus removal.

The primary mechanism of phosphorus removal is through the chemical precipitation shown in equation 2, therefore, it is expected that the larger the dose of calcium oxide the greater the phosphorus removal. To understand how calcium oxide present in the HGZMS effects phosphorus removal, the relationship can be examined directly as shown in Figure 16. While it is evident from figure 16 that dosed calcium oxide has an effect on phosphorus removal, the variability between points of similar calcium oxide dosing suggests that calcium is not the sole variable effecting phosphorus removal. Residual ions in samples competing for phosphorus and inconsistencies in particle size distribution and processing between samples could explain some of this variability and merits further investigation. Additionally, other mechanisms of phosphorus removal could be exhibited by HGZMS, such as adsorption onto remaining calcium carbonate calcite structures as reported in previous studies (Abeynaike et al., 2009; Namasivayam et al., 2005). Heterogeneous precipitation as observed by Abeynaike et al. (2009) and Jones et al. (2011), where adsorption to unconverted calcite is seeding chemical precipitation, could possibly account for some of the variation in the direct correlation of calcium oxide production to phosphorus removal.

4.5.2 Effect on and of pH

The pH of the HGZMS dosed effluent was recorded for each trial. The results demonstrate that increased dose of HGZMS increases pH. As well, higher temperature heat treatments produce higher pH when dosed. The effect of pH on phosphorus removal closely follows the effect heat treatment has on phosphorus removal as shown in Fig.17. However, for dosages 0.25 and 0.50 g L^{-1} the maximum pH is significantly lower than the maximum pH for dosages of 1.00 and 2.00 g L^{-1}. Since similar phosphorus removal can be achieved at low dosages with high heat treatment temperature as high dosages with high treatment temperature, the low pH exhibited by low dosages is advantageous, since high pH is an undesirable characteristic for released effluent.

Figure 18 indicates that phosphorus removal and pH are strongly correlated. The fitted sigmoid curve demonstrates strong p-values associated with all of the equation’s parameters suggesting that pH level has a strong effect on phosphorus removal. Points from figure 18 that sit above the fitted curve are advantageous for they remove a higher than expected amount of phosphorus for their observed pH.

Real wastewater effluent water was less effected by pH than the synthetic phosphorus water. This is due to buffering capacity that exists in the effluent water.

4.5.3 Optimization and Energy Assessment

If HGZMS were to be implemented as a phosphorus removing reagent in sewage lagoons it would be important to optimize the HGZMS to be as efficient as possible. The efficiency can be characterized by a variety of parameters such as: phosphorus removal, required HGZMS per litre effluent, final pH and energy costs associated with heat-treating the material per litre of effluent. In the province of Manitoba, effluent released from sanitary lagoons and wastewater plants must have a total phosphorus concentration below 1mg L^{-1} (Manitoba Sustainable Development, 2011).

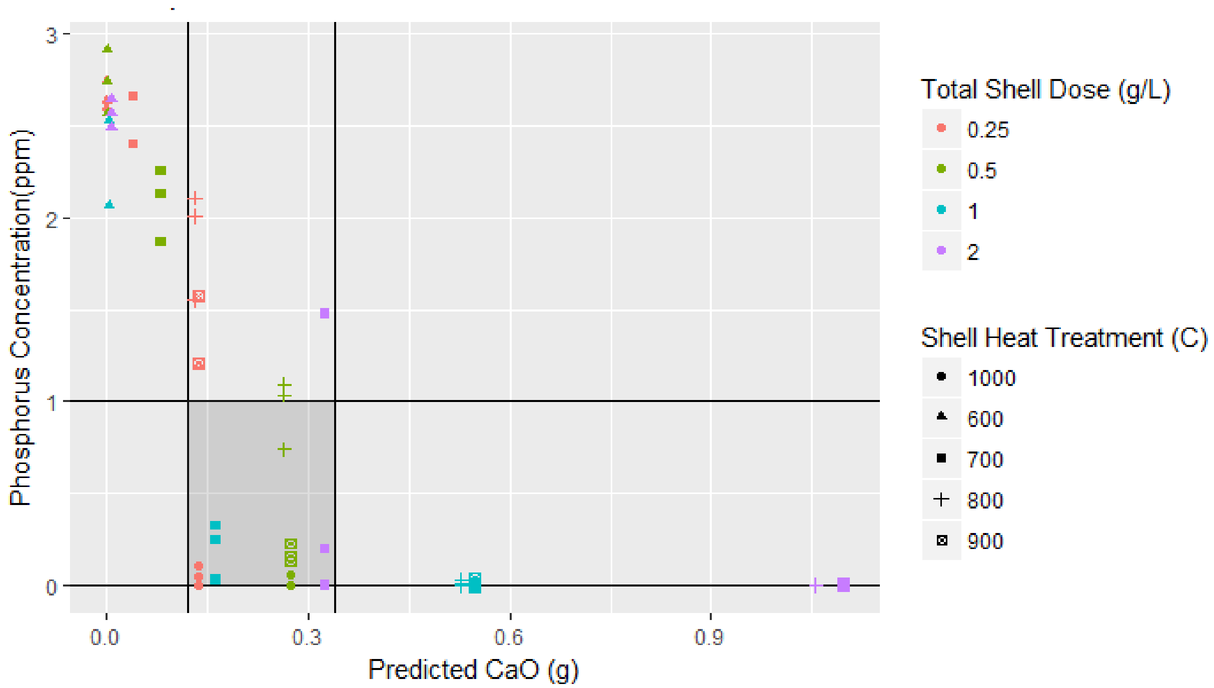

Fig. 20. Achieved phosphorus concentration (mg L^{-1}) vs predicted CaO concentration (g L^{-1}). Optimum removal area shown in shaded region, showing achieved phosphorus levels below local provincial regulation (mg L^{-1}), without redundant CaO concentrations.

All points shown in the shaded region of Figure 20 represent dose and treatments that reduce the collected effluent samples below 1mg L^{-1}. These points can therefore be analysed based on material needs per litre, final effluent pH, and energy costs to determine the optimum HGZMS treatment and dose.

Energy costs were determined based on simple temperature change calculations of air and the shell material. The results (Table 3) demonstrate that dosage far outweighs heating temperature when considering treatment energy. Energy used in shell collection, transportation, cleaning and grinding was not included in these calculations.

Dose (g L^{-1})	**Treatment (C˚)**	**Raw Shell Material Needed per Litre Prior to Heat Treatment (g)**	**Energy Requirement (J L^{-1})**	**pH**
2.00	700	2.29	1301.63	8.89
2.00	800	3.42	2226.99	11.78
2.00	900	3.52	2583.43	11.68
2.00	1000	3.52	2878.40	11.85
1.00	700	1.15	650.82	9.38
1.00	800	1.71	1113.49	11.08
1.00	900	1.76	1291.71	11.33
1.00	1000	1.76	1439.20	11.82
0.50	800	0.86	556.75	8.96
0.50	900	0.88	645.86	9.40
0.50	1000	0.88	719.60	10.08
0.25	1000	0.44	359.80	9.13

Table 3. Chart of Optimization Parameters (All samples shown reduced effluent phosphorus concentrations below 1 mg L^{-1})

From Table 3 it can be observed that HGZMS heat treated at 1000 ^{o}C and dosed at 0.25 g L^{-1} demonstrates the greatest potential for all three optimization parameters. Achieving 98.17 % phosphorus removal, resulting in 0.05 mg L^{-1} final phosphorus concentration, utilizing 0.44 g L^{-1} raw shell material, 359.80 J L^{-1} of energy and a final pH of 9.13.

4.6 Potential Societal Impact

From the presented results and discussion, zebra mussel shells present a viable renewable alternative to mined virgin limestone, for the production of calcium oxide for the use as a phosphorus binding material in waste water treatment. In using zebra mussel shells as a renewable resource, an economic incentive is provided for shell collection, thereby mitigating negative effects that washed-up zebra mussel shells have on beach tourism. The re-use process would require and therefore employ personnel for the collection, transportation, cleaning, crushing, and heat treatment of zebra mussel shells. This employment could potentially be a benefit to a community's economy. If small kilns were located close to affected beaches where shells are collected, and the shell product used in nearby sanitary sewage lagoons that require phosphorus treatment, transportation distance could be minimized, and local micro-industries centred around zebra mussel shell collection and re-use could be established.

Chapter 5 – Conclusions

The presented study examined the use of zebra mussel shells as an alternative source to limestone to produce calcined material for phosphorus removal in wastewater. This study focused on three major areas of research:

1. Zebra mussel shell characterization;
2. Calcination process of the shell material;
3. Effectiveness of heat treated shells as a phosphorus binding reagent.

Shells were characterized through a titration to determine the percent mass calcium carbonate composition, XRD analysis to examine the shell crystalline structure, and ICP analysis to examine tested trace elements. The results led to the following conclusions:

- Zebra mussel shells are composed of 86.67 % calcium carbonate by mass (SD = +/- 1.12).
- Shells crystalline structure is solely calcium carbonate in the crystal form of aragonite.
- Shells do not contain critical concentrations of arsenic, chromium, lead, cobalt, copper, vanadium, antimony or beryllium. Based on the elements tested for in this study, heat treated zebra mussel shells are safe for the treatment of wastewater effluents.
- Given the high calcium carbonate content and the sufficiently low concentrations of harmful trace elements, the shell material is suitable as an alternative to limestone for the production of calcium oxide use for phosphorus removal.

Shells were calcined through decarbonation by means of high temperature heat treatment. Two sieved particle sizes of crushed shells were compared by heating samples at 600, 700, 800, 900, and 1000 ˚C, for 1 hour, and recording mass loss. The recorded observations led to the following conclusions:

- Mass loss of shells follows a sigmoidal curve, where the threshold temperature for mass loss, and presumably decarbonation, occurs at approximately 600 ˚C. Maximum mass loss is dependent on particle size, however for the two particle sizes examined, it occurs between 900 and 1000 ˚C.
- Mass loss is greater for fine particle sized crushed zebra mussel shells at transition temperatures 600, 700, and 800 ˚C. At high temperatures (900 and 1000 ˚C) mass loss is slightly higher for coarse particles. This is likely due to sintering and clumping that occurs in the fine particles at high temperatures.
- If the desired heating temperature is lower than 900 ˚C, it is recommended that fine particle sizes are used. For high temperature heating, particle size between the fine and coarse sizes tested, did not play a critical role in mass loss and calcium oxide production.

The effectiveness of calcined shell for phosphorus removal was tested on both synthetic water and on collected wastewater effluent. The tests on synthetic water compared two sieved particle sizes of calcined shells, coarse (500 – 1,000 μm) and fine (<75 μm), heated at 600, 700, 800, 900, and 1000 ˚C for 1 hour. Additionally, the effectiveness of un-treated zebra mussel shell for phosphorus removal was examined, as well as a comparison of coarse and fine zebra mussel shell heated at 800 ˚C for 1 hour with chemical grade calcium hydroxide, at doses of 0.25, 0.50 and 1.00 g L^{-1}. The tests on phosphorus removal from effluent water examined the effect of dose (0.25, 0.50, 1.00, 2.00 g L^{-1}) and heat treatment (600, 700, 800, 900, 1000 ˚C). The results led to the following conclusions:

- For heat-treated shells dosed to synthetic phosphorus water (10 mg L^{-1}) in a concentration of 1.00 g L^{-1}, particle size had a negligible effect. Coarse particles were nearly as effective at removing phosphorus as fine particles when heat treated at 600 and 700 ˚C, and equally as effective when heat treated at 800, 900, and 1000 ˚C. Effectively 100 % removal was achieved for both particle sizes when shells heat treated at temperatures above 700 ˚C were used.

- When 0.25 g L^{-1} of HGZMS are used to treat phosphorus, coarse shells are less effective than fine shells, removing 80.57% versus 99.23 %, respectively. It is believed the low removal is do to the final pH of 8.54.
- Except for coarse zebra mussel shells dosed at 0.25 g L^{-1}, HGZMS performed comparably well to calcium hydroxide, removing over 99% of phosphorus in solution. HGZMS had an advantage over chemical grade calcium hydroxide because they resulted in a lower final pH. Using 0.50 g L^{-1} of coarse HGZMS, 99.75% removal was achieved producing a final pH of 10.84. Using the same dose of calcium hydroxide, 100% removal was achieved producing a final pH of 11.56. Using 0.25 g L^{-1} of fine HGZMS, 99.32% phosphorus removal was achieved producing a final pH of 11.01. Using the same dose of calcium hydroxide 100% removal was achieved producing a final pH of 11.47.
- It was concluded that HGZMS has a time release pH property significantly different than lime. Coarse HGZMS were shown to produce pH levels over 10 for 4 batches when 0.50 g L^{-1} were dosed to tap water and mixed continuously, while supernatant was poured off and replaced every 5 minutes. For the same process fine HGZMS produced a pH over 10 for two batches and for the third batch maintained a pH of 9.94. Lime was only capable of producing pH levels over or near 10 for the first batch. This is an advantageous

property for HGZMS because it will result in less material needed for wastewater treated in batches.

- Un-heated ground zebra mussel shell dosed at a concentration of 2.00 g L^{-1} removed 7.2 % phosphorus. Un-heated ground zebra mussel shell is concluded to be ineffective at phosphorus removal, due to the low achieved removal and high dose concentrations required.
- 99.48% phosphorus removal from collected wastewater effluent can be achieved by the addition of dosages of 1.00 g L^{-1} of fine (<75 μm) powder heat treated at 800 ^{o}C for one hour. It was also found that optimized HGZMS heat treated at 1000 ^{o}C could achieve 98.7% phosphorus removal when dosed at 0.25 g L^{-1}, while maintaining a final effluent pH of 9.13.
- Fine shell material heat treated at 1000 ˚C for 1 hour, resulted in the lowest energy to phosphorus removal ratio, and an energy requirement of 359.80 J L^{-1}.
- The present study has shown that zebra mussel shells collected from beaches can be used as an effective chemical reagent for the removal of phosphorus from wastewater. By heat treating ground zebra mussel shells, open pit mined calcium carbonate can be replaced by a waste product, providing an economic incentive for the removal of zebra mussel shells from beaches, and thereby sustaining tourism on which beach communities economically depend on.
- Further research is needed to better characterize the zebra mussel shell material and to further optimize heating and conversion energy requirements using techniques such as air and nitrogen purged ovens and rotary kilns, as well as better define the phosphorus removal mechanism displayed by the heat treated shell material.

Chapter 6 – Limitations and Recommendations for Future Work

Several limitations were identified for the presented study, and recommendations are made for future research that should be explored in the field of zebra mussel shell re-use.

- The total composition of zebra mussel shells remains unknown. A more thorough analysis, covering a broader range of trace elements, as well as specifically examining sodium, magnesium, and silicon content, would be beneficial to help further understand shell structure.
- Calcination completion in this study was examined through XRD analysis. However, for expected transition heat treatments, 700 and 800 ˚C, the analysis was not sensitive enough to detect expected residual calcium carbonate. Therefore, mass loss was used to quantify the heating process.
- Only fine particles were used for jar test experiments involving real wastewater effluent. It is recommended that future studies examine the effectiveness of coarse particles for effluent water. It is also recommended that a wider range of particle sizes be examined to further optimize HGZMS use.
- The use of HGZMS as a phosphorus binding reagent was explored only in jar tests where material was mixed with wastewater effluent. It is recommended that for future studies the use of HGZMS for phosphorus removal is also explored through column and/or bed type experiments as was explored by Lee et al. (2011). This would expand the potential means of applying the treated shell material in wastewater treatment settings.
- Soluble phosphorus removal was examined in this study. To further understand the potential of HGZMS as a waste water treatment material, its effectiveness to remove

other metals, as well as its effect on a wider range of water parameters should be explored.

- Given the successful findings for the use of HGZMS in jar tests, it would be beneficial to explore phosphorus removal through a pilot scale study, involving larger scale wastewater treatment, such as periodic application into a secondary wastewater lagoon.
- A limited energy analysis was conducted to help identify optimized shell parameters. It is recommended that a more in-depth energy and economic assessment is conducted, exploring the collection, transportation, treatment, calcination, and use in sanitary sewage lagoons for HGZMS. This would allow a better examination of the economic feasibility of this material as a phosphorus treatment reagent.
- Further studies into the shell collection densities from beaches and infrastructure, both in Manitoba and across North America, are recommended. The seasonality of shell deposition is an important factor and supply consistency to any future industrial process would be of concern. Zebra mussel harvesting through the use of submerged surfaces could be considered to provide a consistent supply of the shell material.
- Further research into other potential uses of collected zebra mussel shell, such as a supplement to livestock or human diet or as a road aggregate material is recommended.

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Appendices

Appendix 1 – Statistics (p-values) associated with predicted model parameters for coarse zebra mussel shell mass loss vs heat treatment temperature.

	Estimate	**Std. Error**	**T value**	**P(>|t|)**
Asymptote	45.54	1.427	31.91	5.665e-13
x-mid	779.5	6.615	117.8	9.359e-20
scale	41.76	5.827	7.166	1.138e-05

Residual standard error: 2.783 on 12 degrees of freedom

Appendix 2 – Statistics (p-values) associated with predicted model parameters for fine zebra mussel shell mass loss vs heat treatment temperature.

	Estimate	**Std. Error**	**T value**	**P(>|t|)**
Asymptote	43.19	1.617	26.71	4.661e-12
x-mid	721	10.71	67.31	7.668e-17
scale	24.4	10.21	2.391	0.03409

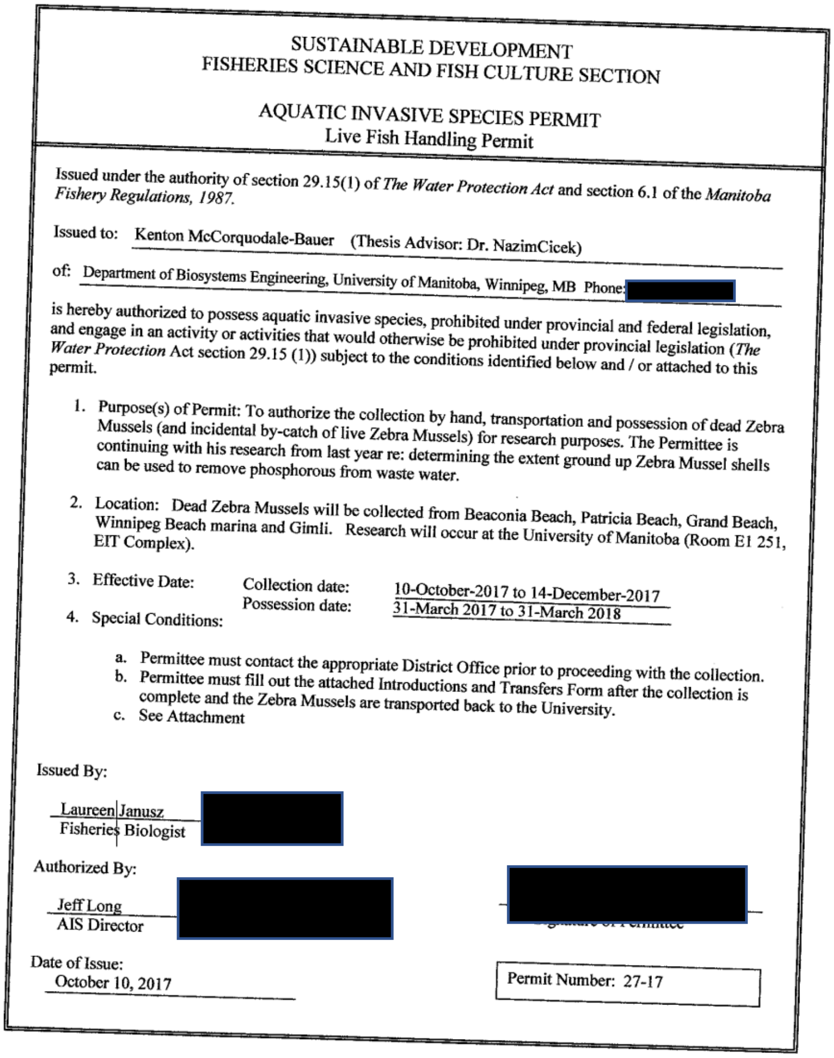
Residual standard error: 3.922 on 12 degrees of freedom

Appendix 3 – Statistics (p-values) associated with predicted model parameters for percent phosphorus removal vs pH.

	Estimate	**Std. Error**	**T value**	**P(>|t|)**
Asymptote	100.3	2.242	44.72	3.53e-43
x-mid	8.75	0.02978	293.8	2.05e-85
scale	0.2247	0.02832	7.935	1.615e-10

Residual standard error: 10.1 on 52 degrees of freedom.

Appendix 4 – Aquatic Invasive Species Permit (March 2017 – March 2018)



SUSTAINABLE DEVELOPMENT
FISHERIES SCIENCE AND FISH CULTURE SECTION

AQUATIC INVASIVE SPECIES PERMIT
Live Fish Handling Permit

Issued under the authority of section 29.15(1) of *The Water Protection Act* and section 6.1 of the *Manitoba Fishery Regulations, 1987.*

Issued to: Kenton McCorquodale-Bauer (Thesis Advisor: Dr. NazimCicek)

of: Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB Phone: [redacted]

is hereby authorized to possess aquatic invasive species, prohibited under provincial and federal legislation, and engage in an activity or activities that would otherwise be prohibited under provincial legislation (*The Water Protection* Act section 29.15 (1)) subject to the conditions identified below and / or attached to this permit.

1. Purpose(s) of Permit: To authorize the collection by hand, transportation and possession of dead Zebra Mussels (and incidental by-catch of live Zebra Mussels) for research purposes. The Permittee is continuing with his research from last year re: determining the extent ground up Zebra Mussel shells can be used to remove phosphorous from waste water.

2. Location: Dead Zebra Mussels will be collected from Beaconia Beach, Patricia Beach, Grand Beach, Winnipeg Beach marina and Gimli. Research will occur at the University of Manitoba (Room E1 251, EIT Complex).

3. Effective Date: Collection date: 10-October-2017 to 14-December-2017
Possession date: 31-March 2017 to 31-March 2018

4. Special Conditions:

   a. Permittee must contact the appropriate District Office prior to proceeding with the collection.
   b. Permittee must fill out the attached Introductions and Transfers Form after the collection is complete and the Zebra Mussels are transported back to the University.
   c. See Attachment

Issued By:

Laureen Janusz
Fisheries Biologist [redacted]

Authorized By:

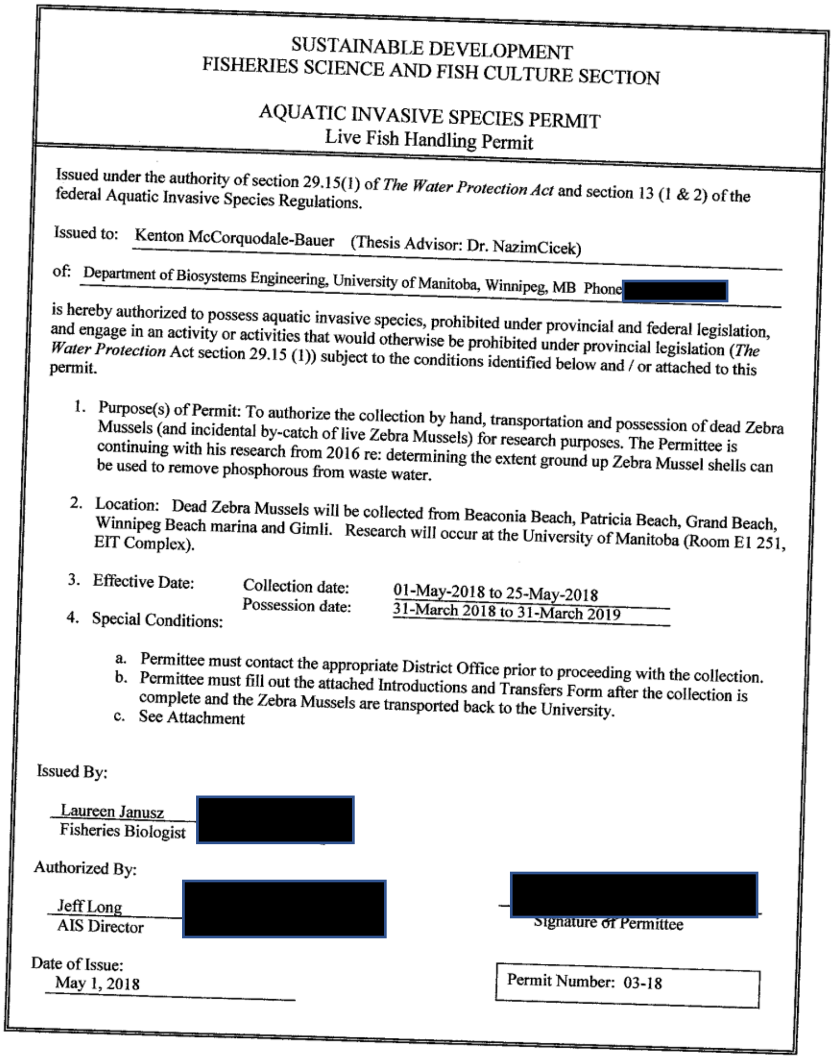
Jeff Long
AIS Director [redacted]

[redacted]

Date of Issue:
October 10, 2017

Permit Number: 27-17

Appendix 5 – Aquatic Invasive Species Permit (March 2018 – March 2019)



SUSTAINABLE DEVELOPMENT
FISHERIES SCIENCE AND FISH CULTURE SECTION

AQUATIC INVASIVE SPECIES PERMIT
Live Fish Handling Permit

Issued under the authority of section 29.15(1) of *The Water Protection Act* and section 13 (1 & 2) of the federal Aquatic Invasive Species Regulations.

Issued to: Kenton McCorquodale-Bauer (Thesis Advisor: Dr. NazimCicek)

of: Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB Phone [redacted]

is hereby authorized to possess aquatic invasive species, prohibited under provincial and federal legislation, and engage in an activity or activities that would otherwise be prohibited under provincial legislation (*The Water Protection* Act section 29.15 (1)) subject to the conditions identified below and / or attached to this permit.

1. Purpose(s) of Permit: To authorize the collection by hand, transportation and possession of dead Zebra Mussels (and incidental by-catch of live Zebra Mussels) for research purposes. The Permittee is continuing with his research from 2016 re: determining the extent ground up Zebra Mussel shells can be used to remove phosphorous from waste water.

2. Location: Dead Zebra Mussels will be collected from Beaconia Beach, Patricia Beach, Grand Beach, Winnipeg Beach marina and Gimli. Research will occur at the University of Manitoba (Room E1 251, EIT Complex).

3. Effective Date: Collection date: 01-May-2018 to 25-May-2018
   Possession date: 31-March 2018 to 31-March 2019

4. Special Conditions:
   a. Permittee must contact the appropriate District Office prior to proceeding with the collection.
   b. Permittee must fill out the attached Introductions and Transfers Form after the collection is complete and the Zebra Mussels are transported back to the University.
   c. See Attachment

Issued By:

Laureen Janusz [redacted]
Fisheries Biologist

Authorized By:

Jeff Long [redacted]
AIS Director

[redacted]
Signature of Permittee

Date of Issue:
May 1, 2018

Permit Number: 03-18