

Freeze/thaw treatment for sludge dewatering,
nutrient recovery and biogas production in
Northern Canadian Communities

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Abstract

Wastewater sludge is considered a valuable source of nutrients and energy. Freeze/thaw treatment is an efficient dewatering method for wastewater sludge management in First Nation communities located in cold climate conditions. Natural freeze/thaw is a simple, practical and low cost solid-liquid separation method, which can effectively dewater sludge. This method is especially effective when used in small treatment plants in remote and cold regions as typical dewatering methods require complex and expensive equipment, skilled operators and special maintenance. The objective of this research is to evaluate dewatering, nutrient recovery and organics separation of wastewater sludge originating from different wastewater treatment processes using freeze/thaw processing. The results of experiments showed the effectiveness of this method in sludge dewaterability and solubilisation of organics and nutrients. The sludge solid content increased by approximately 10-fold after freeze/thaw processing. The treatment was effective in solubilisation of about 15.2%, 33.5% and 21.5% of total nitrogen, total phosphorus and total chemical oxygen demand to soluble one, respectively for the non-BNR sludge. These values were 6.3%, 80% and 16.5%, respectively for the BNR sludge. The released phosphorus and nitrogen in the water can be recovered and used for agricultural purposes, which means sludge can be transformed from a waste product into a marketable product. However, anaerobic digestion of the solid cake post freeze/thaw treatment did not show enhanced methane yield compared with fresh sludge.

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List of Symbols

AANDC	Aboriginal Affairs and Northern Development Canada
ATP	Adenosine Triphosphate
BMP	Biochemical Methane Potential
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DI Water	Deionized Water
DNA	Deoxyribonucleic Acid
ECP	Extracellular Polymers
FIA	Flaw Injection Analysis
F/T	Freeze/Thaw
GC	Gas Chromatography
INAC	Indigenous and Northern Affairs Canada
NEWPCC	North End Water Pollution Control Centre
NH ₄ -N	Ammonium nitrogen
PAOs	Polyphosphate-accumulating organisms
PO ₄ -P	Orthophosphate
RAS	Return Activated sludge

RBC	Rotatory Boil Sludge
RNA	Ribonucleic Acid
SCOD	Soluble Chemical Oxygen Demand
SEWPCC	South End Water Pollution Control Centre
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solid
VS	Volatile Solid
VFA	Volatile Fatty Acids
WAS	Waste Activated Sludge
WEWPC	West End Water Pollution Control Centre

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Chapter 1

Introduction

Rapid global population growth will contribute to environmental degradation and resource depletion in the future. This will require more strict regulations on waste treatment and disposal in order to prevent environmental pollution. Finding alternative renewable resources to substitute with non-renewable ones is critical. Water and wastewater treatment and consequently their waste sludge treatment, reuse and disposal have been some of the most challenging and developing topics in this area. Finding the most practical, cost effective and environmentally friendly strategy for sludge management, based on the real needs of any community, is an important goal for those involved in waste treatment and disposal projects such as researchers, engineering consultants, environmental groups, facility owners, contract operators and community members.

The present research examines the freeze/thaw process as a potentially practical and cost effective sludge management method for Northern Canadian Communities.

1.1 Biological wastewater treatment

The primary objectives of biological wastewater treatment are to use a variety of microorganisms to oxidize the particulate and dissolved biodegradable content to environmentally benign end products; to capture suspended colloidal solids into a biological floc and to remove nutrients, especially nitrogen and phosphorus, from incoming wastewater. Microorganisms (mostly bacteria) oxidize the carbonaceous organics into acceptable end products and biomass. Specific kinds of bacteria are able to oxidize ammonia to nitrite and nitrate (nitrification) and another kind of bacteria transforms the oxidized nitrogen to nitrogen gas (denitrification). Phosphorus removal usually takes place by growing specific bacteria capable of taking up and storing the phosphorus (Metcalf & Eddy et al., 2003).

There are two types of biological treatment methods: suspended growth and attached growth. In suspended growth, the microorganisms are kept in liquid suspension providing enough mixing. Activated sludge process is the most used suspended growth system in wastewater treatment plants. In the attached growth process, the microorganisms are attached to an inert packing material such as rock, gravel, sand, redwood or a variety of plastic and synthetic materials. This study focuses on the solids and biosolids that remain from the activated sludge process both with biological nutrient removal (BNR) and without biological nutrient removal (non-BNR).

1.1.1 Biological oxidation process (BOD removal)

The basic and conventional use of activated sludge treatment includes an aeriated reactor that keeps the microorganisms in suspension with the incoming wastewater and secondary clarifier to separate the solids and liquids. The recycling system returns the activated solids back to the reactor. The BOD can be removed by providing sufficient contact time between the wastewater and heterotrophic microorganisms and enough oxygen and nutrients.

1.1.2 Nitrogen removal

Nitrogen is one of the required nutrients for cell growth. Nitrogen in wastewater is presented in the form of ammonia nitrogen (45 g/m^3), organic nitrogen (10 g/m^3) nitrate and nitrite nitrogen (0.2 g/m^3) (Henze et al., 2008). The sum of organic and ammonia nitrogen is called Total Kjeldahl Nitrogen (TKN). The excess discharge of ammonia can be harmful to aquatic life. Biological nitrogen removal is required to reduce the amount of discharged ammonia and total nitrogen. The removal occurs by ammonification, nitrification and denitrification (van Haandel & van der Lubbe, 2012). First the organic nitrogen converts into ammonium. Ammonium is oxidised to nitrite and then nitrite to nitrate during the nitrification process in aerobic reactors. Denitrification is the biological reduction of nitrate to molecular nitrogen gas in anoxic reactors.

1.1.3 Phosphorus removal

Phosphorus is an essential nutrient for cell growth and is used as a fertilizer for plant growth. However, excess discharge of phosphorus into rivers and lakes can increase plant growth and cause eutrophication. Therefore, phosphorus removal by chemical or biological treatment is needed to accumulate the phosphorus in the sludge and to prevent its discharge to the effluent. The common forms of P in the influent raw wastewater are organic phosphate, polyphosphate and orthophosphate. Biological phosphorus removal is achieved by growing phosphorus accumulating organisms (PAOs) capable of storing phosphorus in their cells. In an anaerobic zone in the presence of carbon, PAOs release phosphorus from stored polyphosphate to obtain the carbon. These bacteria then uptake soluble phosphorus (in excess of what they released in the anaerobic zone) from solution in the subsequent aerobic zone and store it as polyphosphate within their cells. These PAOs are removed from the reactor as a waste sludge residue. In a normal aerobic activated sludge (non-BNR) process, the amount of P that could be stored in the biomass is about 0.02 mg P/mg VS. However, in a BNR activated sludge process this amount will increase to around 0.06-0.15 mg P/mg VS and sometimes up to 0.38 mg P/mg VS (Henze et al., 2008). Higher amounts of P can be removed from the influent and end up in the sludge by growing a higher portion of PAOs in the system.

For chemical phosphorus removal, typically a salt of iron, aluminium or lime is added to the activated sludge. Phosphorus is precipitated from the solution into the sludge. It was reported that this method could increase the sludge volume by 40% and therefore the cost

of sludge disposal. Moreover, the biosolids from this process are not beneficial to use as fertilizer, as the phosphorus is bound to the salts irreversibly (Sargeant, 2009)

1.1.4 Biosolids

Biosolids (sludge) are the by-products of wastewater treatment processes consisting of mostly water, microorganism like bacteria, fungi, and viruses, organic and inorganic particles, heavy metals and micro-pollutants (such as pharmaceuticals and endocrine disrupters). The primary goal of biosolids treatment is to reduce the organic matter and pathogen concentrations in order to have a safe end product for disposal. Biosolids is a term used by the Water Environment Federation (WEF 1998) to redirect the beneficial use of wastewater solids and organic products after treatment (Metcalf & Eddy et al., 2003). The presence of carbon and nutrients in municipal biosolids makes it a valuable resource for renewable energy and fertilizer. Some sludge treatment methods are able to convert the extracellular and intracellular materials of activated sludge flocs such as proteins, sugars and carbohydrates into the soluble phase and allow them to be recovered. Through this recycling, the energy and nutrients in biosolids can be converted from a waste product to a potentially marketable commodity (i.e. biogas or liquid fertilizer).

The primary goals of biosolids treatment are:

- Volume and weight reduction through thickening and dewatering;
- Stabilisation for a controlled degradation of organic ingredients and odour removal;
- Recovering of nutrients and organics;

- Elimination of pathogenic microorganisms.

Additional benefits for the use of municipal biosolids include (CCME, 2012):

- Renewable energy production: Biogas is produced through anaerobic digestion of sludge and can be used for heating or generating electricity, replacing fossil fuels and reducing greenhouse gas emissions.
- Agricultural land and forestry application: The macronutrients (nitrogen, phosphorus and potassium) and micronutrients (copper, cobalt, zinc) of biosolids can enrich soil fertility and help plant growth, thereby reducing the use of chemical fertilizer.
- Land reclamation: Converting marginal, non-productive lands into agricultural land by improving soil carbon content, structure, water retention capacity and fertility.

1.2 Biosolids treatment, reuse and disposal

Wastewater biosolids can be categorised as primary, biological and chemical. The term sludge in general relates to the wastewater treatment process. Primary sludge is produced through mechanical treatments like screening, grit removal and sedimentation and generally contains 5-6% solids (60-70% volatile). The biological solids are mainly waste activated sludge (WAS) or fixed film sludge generated through various processes such as activated sludge, trickling filter and rotating biological contactors. The waste activated sludge generally consists of more than 98% water and 0.8-1.2% total solids (59-88% organics) (Metcalf & Eddy et al., 2003). Table 1.1 shows the characteristics of primary and waste activated sludge. The chemical solids are produced when chemicals are added to the wastewater to remove phosphorus or to coagulate non-settleable solids.

Table 1.1: Characteristic of primary and activated sludge. Adapted from (Metcalf & Eddy et al., 2003)

Parameter	Primary Sludge	Activated Sludge
Total dry Solids (TS)%	5 - 9	0.8 - 1.2
Volatile Solids (% of TS)	60 - 80	59 - 88
Protein (% of TS)	20 - 30	32 - 41
Nitrogen (N, % of TS)	1.5 - 4	2.4 - 5
Phosphorus (P ₂ O ₅ , % of TS)	0.8 - 2.8	2.8 - 11
Potash (K ₂ O, % of TS)	0 - 1	0.5 - 0.7

Reuse and disposal are two different final steps of any sludge treatment process. Reuse refers to the recovery of beneficial components from the sludge such as nutrients and energy. Disposal is finding a low risk and inexpensive method of disposing sludge into

the landfill or applying it to agricultural or forestry land. General sludge treatment steps are thickening, stabilization, conditioning, dewatering and disposal (Fig. 1.1). The current research study will focus on the stabilization and dewatering steps (Fig. 1.2).

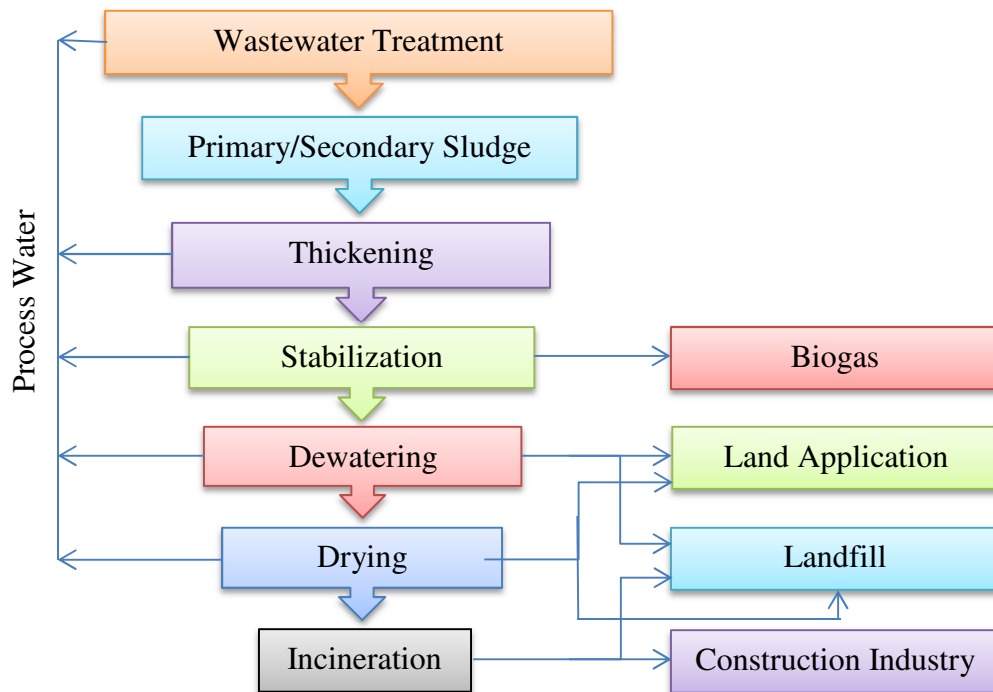


Fig.1.1: Biosolid processing flow diagram, adapted from (Yuan , 2014)

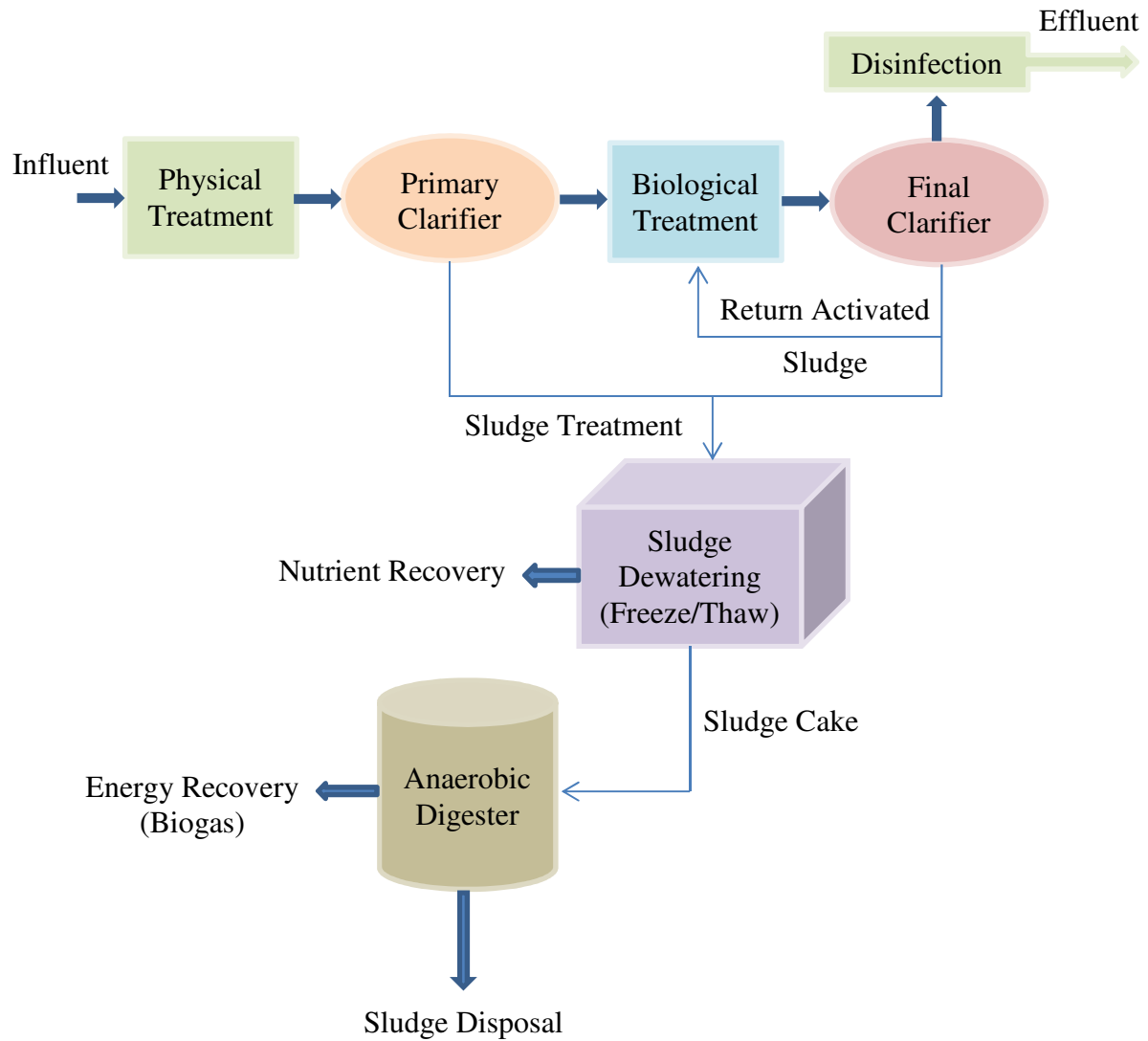


Fig.1.2: Sludge treatment: Dewatering, stabilization and resource recovery

1.2.1 Sludge dewatering

Typically, sludge produced in wastewater treatment processes is liquid or semisolid liquid containing about 0.25 to 12% solids by weight (Metcalf & Eddy et al., 2003). The primary goal of sludge thickening and dewatering is to increase dry solid concentration in the dewatered sludge cake in order to minimize the volume and the cost of hauling and disposal. The sludge cake concentration of 25% or more is recommended prior to disposal (Metcalf & Eddy et al., 2003).

Typical methods of conditioning and dewatering sludge such as gravity thickeners, filter presses, horizontal belt filters, dissolved air flotation and centrifugation are mainly used in larger treatment facilities and require skilled operators and extensive maintenance. The solid contents from a solid bowl centrifuge are in the range of 10 to 30% (Metcalf & Eddy et al., 2003). However, the centrifuge works better on primary sludge when compared with activated sludge since this method cannot remove the bound water in activated sludge (Carrasco, 2013) .

A filter press is used to force the water out of the sludge using high pressure, producing the driest cake. However, this method is not only complex and expensive but also requires the use of inorganic chemicals for flocculation and filter aid. The typical solid concentrations from various mechanical methods are shown in Table 1.2.

Table 1.2: Sludge solid concentration under different dewatering process. Adapted from (Metcalf & Eddy et al., 2003)

Operation	Solids concentration %	
	Range	Typical
Gravity thickeners for mixed primary and activated sludge	2 – 6	4
Flotation thickeners with chemicals	4 – 6	5
Flotation thickeners without chemicals	3 – 5	4
Belt filter press with chemicals	15 – 30	22
Centrifuge dewatering with chemicals	10 – 35	22
Centrifuge dewatering without chemicals	10 – 30	18

There are also some natural dewatering methods such as sludge drying beds, sludge lagoons, and freezing beds that could be used in smaller treatment plants. These natural treatments require low energy consumption, no chemicals and less-skilled operators. However, they may require large areas of land, and there is a potential for odour problems. For the final design, climatic conditions need to be considered (Metcalf & Eddy et al., 2003).

1.2.2 Sludge stabilization and biogas production

Sludge stabilization is defined as a process to convert waste to a stable product, reduce the organics and pathogens and eliminate the odour through different techniques such as aerobic or anaerobic digestion, incineration, gasification, pyrolysis, wet air oxidation and hydrothermal treatment (Tyagi & Lo, 2013). Biogas production through anaerobic digestion technologies using organic waste has many advantages in terms of resource conservation, environmental protection and agriculture waste management (Yadvika,

Santosh, Sreekrishnan, Kohli, & Rana, 2004). Other advantages of biogas plants are stabilization of waste, production of renewable energy, pathogen reduction, odour control, reduction of greenhouse gas emissions and nutrient management (Wilkie, 2005).

Anaerobic digestion of solid organic waste is considered a sustainable and environmentally friendly solution for sludge management and renewable energy production. The process is used for sludge stabilization and biogas production under oxygen-free environments. Methane gas is produced as a source of renewable energy and can be used for heat and electricity production (Tyagi & Lo, 2013).

Anaerobic digestion is a four-stage process including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Fig. 1.3). The first step is hydrolysis which is an important step in the process since it makes substrates available for the next conversion process (Kangle et al., 2012). Complex organic materials are decomposed into simpler soluble compounds, e.g., carbohydrates to sugars, proteins to amino acids and fats to fatty acids. In the second step, acidogenic bacteria convert these soluble compounds to fermentation products such as volatile fatty acids (VFA), ethanol, lactic acid, hydrogen and carbon dioxide. Fermentation products are then converted to acetic acid, hydrogen and carbon dioxide in the acetogenesis stage. The final process is methanogenesis, where methane and carbon dioxide are produced. The biogas composition can be different based on the feedstock type and process conditions. In general, the biogas produced may consist of 60%-70% methane, 30%-40% carbon dioxide, and traces of other gases such as nitrogen, hydrogen, and hydrogen sulfide (Tyagi & Lo, 2013).

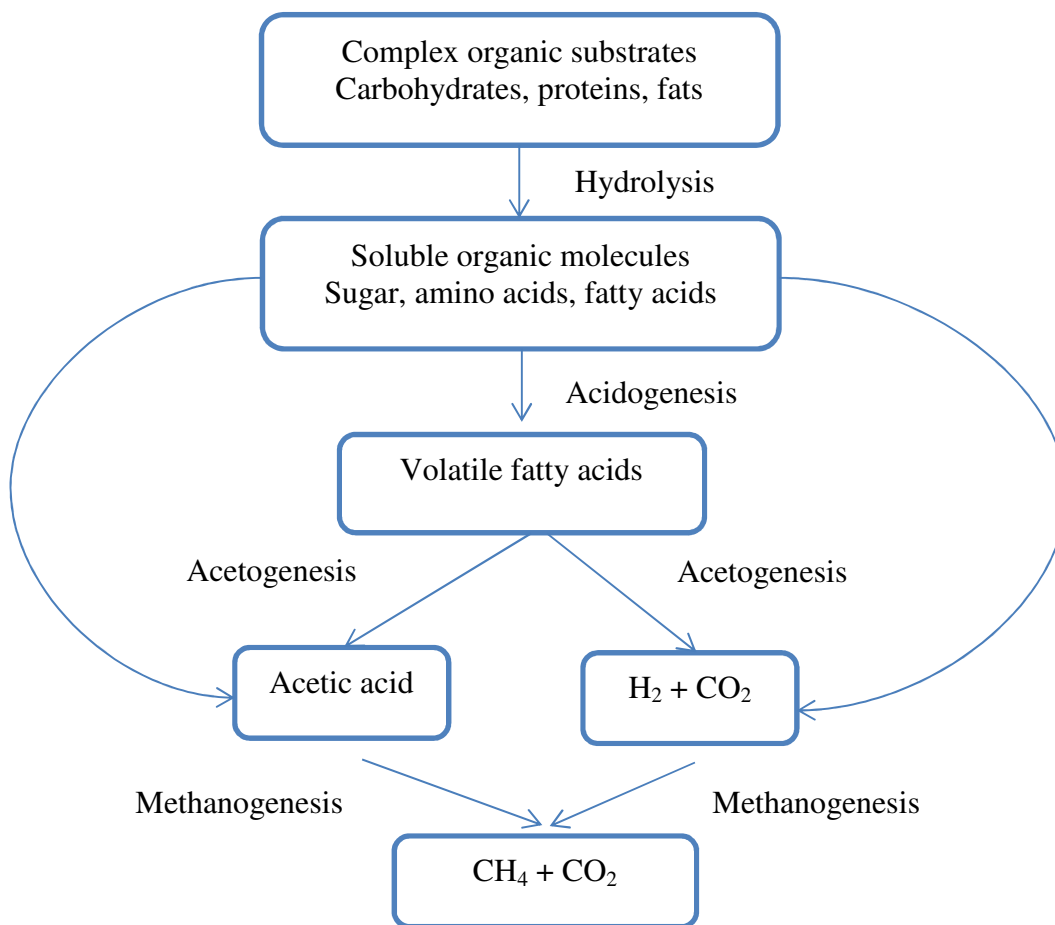


Fig.1.3: Anaerobic digestion flow diagram

The Biochemical Methane Potential (BMP) test is the most well-known method to measure sample biodegradability and methane production in the laboratory. The BMP assay test is usually performed by incubating the waste with anaerobic bacteria for a period of 20 to 30 days. The temperature is generally 35-37°C to provide mesophilic conditions. Biogas volume is monitored during the incubation and analyzed for composition (Hansen, et al., 2004). The process is a simple and inexpensive procedure to

monitor the relative anaerobic biodegradability and biogas potential of a given substrate (Owen et al., 1978).

Typically, the biogas produced from municipal wastewater sludge is between 0.75 to 1 m³/kg VS (Gerardi, 2003). Lim and Fox (2013) conducted an experiment by using a mix Of primary and secondary thickened sludge as substrate and anaerobic granular sludge as inoculum in different ratios (Inoculum/Substrate, I/S) showing that the cumulative methane yields were 51.4, 76.6 and 21.9 mL CH₄/g VS at I/S ratio of 1/1, 1/3 and 1/8, respectively (Lim & Fox, 2013).

However, the presence of large amounts of microorganisms in activated sludge makes biosolids difficult to degrade, requiring longer hydrolysis steps and longer retention times. As a consequence, decreased digester efficiency can be expected when secondary sludge from activated sludge processes is the substrate (Tyagi & Lo, 2011; Carrasco, 2013). Pre-treatment can be used to break the cell wall of microorganisms and make the organic solids available to degrade and thereby enhance biogas production. Different pre-treatment techniques have been studied such as physical, chemical, thermal, mechanical or biological in order to improve the sludge digestibility, digestion efficiency and biogas production. (Tyagi and Lo, 2011)

An experiment conducted using different pre-treatment methods on waste activated sludge showed an enhancement of methane production of up to 64%, 58%, 30% and 27%

with the pretreated sludge using ultrasonic, autoclave, hot water and freezing, respectively, compared with the control (Wang, Chen, Kakimoto, Ogawa, & Kato, 1995). A 50% increase in methane production using thermal pre-treatment on activated sludge at 170°C for 30 minutes was reported in another study (Tyagi and Lo, 2011). Moreover, thermal pre-treatment (170°C to 180°C) for 60 minutes was observed to increase biogas production and COD removal by about 75% and 43%, respectively (Tyagi and Lo, 2011). Microwave irradiation (MW) at 91.2°C for 15 minutes showed 22% COD solubilisation, 23% VS reduction and 79% more methane production under mesophilic anaerobic digestion of sludge (Tyagi and Lo, 2011).

To choose the ideal pre-treatment method, technical feasibility, the extent of extra gas production, energy balance and the cost of the process should be considered.

1.2.3 Sludge resource recovery

Wastewater sludge and biosolids have been considered for many years as a source of macro and micro-nutrients for plant growth such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) (Epstein, 2003). Biosolids contain approximately 3-6% organic nitrogen, 2-4 % phosphorus, 0.2-0.3% potassium, 3% calcium and 1% magnesium by dry weight (Havlin, Tisdale, Nelson, & Beaton, 2014). Nitrogen and phosphorus are the most essential cell growth elements in sludge biomass. Nitrogen is the main component of amino acids which are the building blocks of proteins. Phosphorus is the essential part of DNA (de-oxyribonucleic acid), RNA

(ribonucleic acid), and ATP (adenosine triphosphate) in the cell. These two components can break down and solubilize to the form of ammonia and phosphate under specific treatment processes and can be used as a plant fertilizer (Tyagi & Lo, 2013). Phosphorus is a valuable product in sludge since rock phosphate ores are limited in nature. It was stated that in 150 years no apatite mines will be left (Tyagi & Lo, 2013) and the P shortage would be one of the most important soil fertility problems in the world. Developing an efficient method to recover as much nutrients as possible, especially phosphorus, from other resources is very important.

The general method of phosphorus recovery from sludge is crystallization. This results in the production of calcium phosphate and magnesium ammonium phosphate (struvite). Calcium phosphate is a chemical compound similar to mined phosphate and struvite is considered as a very good slow-release plant fertilizer (Tyagi & Lo, 2013).

Some sludge treatment methods can be used to convert the initial organic N and P to soluble form, ready for uptake by plants. It was reported that by microwave heating of sludge for 5 minutes, up to 76% of the stored polyphosphate and the trapped phosphorus in extracellular material could be released into the solution (Liao, Wong, & Lo, 2005). Another experiment indicated that the combination of H₂O₂ and acid hydrolysis at 100°C and 120°C resulted in about 61% of TP and 36% of TKN solubilisation, respectively (Wong, Chan, Liao, Lo, & Mavrinic, 2006). Hydrothermal treatment on sewage sludge at a temperature of about (180-240°C) for 30 to 90 minutes was also investigated. The

results showed solubilisation of about 40-70% of nitrogen, 50-70% of potassium and 10-15% of phosphorus into the liquid (Sun, Sumida, & Yoshikawa, 2013). Recovering nutrients from a concentrated liquid will allow them to be used as a mineral fertilizer.

1.2.4 Sludge disposal

Common methods of sludge disposal are incineration, land application and landfilling. Incineration converts the organic matter found in sludge into carbon dioxide and water, while the inorganic matter is converted to ash. The greatest sludge volume reduction results from incineration and could be suitable for populated regions (Carrasco, 2013).

Applying the biosolids to land could reduce the need for landfill disposal. Other benefits of applying biosolids to the land include improved soil structure, fertility and carbon content for enhanced plant growth (Vasileski, 2007) .

Local environmental regulations need to be considered prior to disposal, as regulation of sludge disposal has become more stringent in recent years. The United States (U.S) Environmental Protection Agency established regulations for the reuse and disposal of wastewater sludge in 1993 to protect public health and the environment from the hazard of contaminated wastewater biosolids. According to these regulations, biosolids are divided into two categories: Class A and Class B. Class A biosolids require a fecal coliform density less than 1000 MPN/g total dry solids and salmonella density less than 3 MPN per 4 g total dry solids. Class A biosolids must be safe enough to be used by the general public, for application on home lawns or gardens or sold in containers. Class B

biosolids require less treatment for pathogen reduction. The fecal coliform is limited to less than 2×10^6 MPN/g TS (Metcalf & Eddy et al., 2003). There are no national guidelines on best management practice for land application of wastewater biosolids in Canada and provinces have established different categories of biosolids depending on several different qualities such as trace elements, heavy metals, pathogen reduction, vector attraction and odour reduction (CCME, 2010). In terms of pathogens, some provinces have standards for pathogen indicators whereas some have treatment standards. In Manitoba treatment such as anaerobic digestion or equivalent processing is known for pathogen reduction (CCME, 2010). Provinces like Alberta, Quebec and Ontario have their own regulation for pathogen reduction of biosolids. All other provinces the salmonella level less than 3 MPN/ 4 g and fecal coliform less than 1000 MPN / g of the total solids are considered for the highest quality products. For lower quality the fecal coliforms below 2×10^6 MPN/g is accepted (CCME, 2010). Biosolids can undergo processes to further reduce pathogens such as composting, heat drying, heat treatment, thermophilic aerobic digestion, beta-ray or gamma-ray irradiation and pasteurization. Other processes to significantly reduce pathogens include aerobic digestion, air drying, anaerobic digestion, composting and lime stabilization (Metcalf & Eddy et al., 2003).

1.3 Natural sludge freeze/thaw treatment

For facilities located in remote and cold regions, like those in Northern Canadian Communities, finding effective dewatering techniques can be difficult since the typical dewatering methods use complex and expensive equipment, require skilled operators and maintenance. Moreover, simpler methods like drying beds and lagoons are not efficient in cold regions because of the short summer and drying seasons (Martel, 1989). One practical solution for sludge dewatering and disposal in small communities located in cold regions is to use a natural dewatering method such as freeze/thaw. Natural sludge freezing technology could be a reliable solution for sludge dewatering in most Northern U.S. and Canadian regions (Reed, Bouzoun, & Medding, 1986). Sludge freezing and thawing models have been applied successfully (with the exception of coastal regions and southern Ontario) throughout Canada and Northern United States and Alaska (Kinsley, Kennedy, & Crolla, 2012). Freeze/thaw can be performed in a constructed sludge freezing bed similar to a sludge drying bed. Sludge from wastewater treatment can be added to the freezing bed layer by layer during the cold winter months and left to freeze. The sludge freezes from the top to the bottom and pushes all the particles and particulates into larger compacted particles. During the warmer spring weather the sludge is allowed to thaw. The melted water is drained out, collected and the dewatered sludge cake remains as a residue which can be taken off and disposed easily (Wang et al., 2001).

The freezing bed is ideal for sites located in extreme cold climates with more than six months of freezing temperatures. Combining the freezing bed with the drying bed

provides a treatment option for the whole year. The winter sludge production could go to the freezing bed and summer sludge production could be treated in a drying bed (Martel & J. Diener, 1991).

1.3.1 Mechanisms of sludge freeze/thaw process

Several forms of water exist in the sludge such as free water, interstitial water, surface water and bound water. Free water is the water that surrounds the sludge floc but is not associated with the floc (Metcalf & Eddy et al., 2003). It can be removed by typical dewatering processes. The water trapped inside the floc structure that moves with the floc is called interstitial water. The mechanical dewatering system can break the floc and free the water. The water that is held by the surface force is surface water and cannot be easily removed by mechanical devices. Finally, the bound water is chemically bound to the particles and can be released by thermochemical destruction (Vesilind & Martel, 1990). When sludge starts freezing, the free water is the first to be frozen. Freeze/thaw works based on the growth of ice crystals since ice structure is highly organized and cannot join any other molecules. Wherever these ice crystals meet impurities or other atoms, they reject particulate in order to join water molecules. The process of ice crystal growth continues until the accumulated impurities stop the water flow into the crystals. As a result, sludge transforms from a suspension of particles in water to the organized frozen ice crystals and solids (Martel, 1989).

Furthermore, activated sludge containing a large volume of bacteria that are in an agitated and suspended state can prevent effective settling. The gelatinous bacterial cells can also

clog the pores of a filter and inhibit the efficient filtration of sludge. Through freezing, many bacterial cells are lysed or ruptured. Therefore, after thawing, sludge may settle and filter faster (Hadzeriga, 1972). There are studies that have shown freeze/thaw can improve sludge dewaterability and reduce the bound water. Freezing can also aggregate small particles and change the sludge structure from a floc to a more compacted aggregate (Gao, 2011). One study was conducted to evaluate the change in bound water content of activated sludge and mixed digested sludge under various physical and chemical processes. The results showed that there was a significant decrease in bound water (about 70%) after freezing and thawing compared with heat treatment at 130°C that caused the reduction of about 30% of bound water (Katsiris & Kouzeli-Katsiri, 1987). Lee and Hsu (1994) also reported the reduction of bound water content after a sludge freeze/thaw process (Lee & Hsu, 1994).

Freeze/thaw works more efficiently on smaller particles like waste alum sludge and waste activated sludge and is not as effective on raw primary sludge which has a large fraction of bigger particles (Vesilind & Martel, 1990). It was reported that the freezing rate and the curing time for keeping the sludge under frozen conditions as well as the freezing temperature were important factors affecting sludge dewaterability (Hu, et al., 2011, Wang, et al., 2001). In lower freezing temperatures, the ice crystals can extract the surface water and let the particles be in solid-solid contact. Eventually, bigger solid particles are made (Kinsley, Kennedy, & Crolla, 2012). Wang (2001) showed the dewaterability of waste activated thickened sludge that was frozen at a slow rate (-10 to -20°C) was better than fast frozen (-80°C) or unfrozen sludge (Wang et al., 2001).

Ormecci and Vesilind (2001) demonstrated that freeze/thaw worked better on alum sludge than activated sludge. This was attributed to activated sludge containing higher concentration of dissolved organic materials, ions and microorganisms that could impact the effectiveness of freeze/thaw conditioning (Ormecci & Vesilind, 2001). Martel (2000) showed that ice crystal growth would be different in the presence of dissolved solids. In fact, growth for alum sludge occurs in columns whereas growth for activated sludge is dendritic (branching tree-like structure) (Martel, 2000). Gao and Smith (2006) investigated the effects of freeze/thaw on microbial inactivity, particularly *Escherichia coli* (*E. coli*) inactivation capacity under three different temperatures, storage time and freeze-thaw cycle. The results indicated the bacteria frozen at warmer temperature were more sensitive to storage time comparing to ones frozen at colder temperature. Greater microbial inactivation was occurred under longer storage time and warmer freezing temperature (Gao, Smith, & Li, 2006). Sanin et al. (1994) studied the effects of freeze/thaw conditioning on pathogen reduction from sewage sludge and reported a significant decrease in fecal coliforms (Sanin, Vesilind, & Martel, 1994).

1.3.2 Sludge dewatering and freeze/thaw

The effectiveness of freeze/thaw on sludge dewatering has been studied for many years. One of the first studies was conducted in 1931 and reported that freezing alters the draining quality of sludge and results in separation of water from the cake very quickly after thawing. The dried sludge cake had a spongy appearance, little cohesion, no odour and was easily removed from the sand (Martel & J. Diener, 1991). Martel (1989) proved

that this natural conditioning method can improve the drainability of sludge (Martel, 1989).

The concept of a constructed unit operation for sludge dewatering by natural freezing was developed by Martel in 1986, when he conducted a pilot-scale study on a sludge freezing bed. The sludge freezing bed was built at the U.S. Army cold regions research and engineering laboratory in Hanover, New Hampshire, USA. A concrete structure 13.1 m long by 2.6 m wide by 2.4 m deep with a capacity of 17.4 m³ was constructed, where sludge was frozen during winter months in consecutive layers. To avoid snow accumulation the bed was covered with the roof and was surrounded with sidewalls. Sludge was added in layers from 25 mm to 140 mm and was left to freeze during the winter freezing period. The sludge layers were thawed during the spring and summer and the meltwater was drained by opening the bottom drain valves. The bed worked well for 3 years, dewatering aerobically and anaerobically digested sludge. The average total solid content of sludge improved from 6.7% to 39.3% for anaerobically digested sludge (around 83% water removed) and from 1.1% to 24.5% for aerobically digested sludge (about 95% water removed) (Martel & J. Diener, 1991).

Another study conducted by the Lebanon, N.H, Water Treatment Plant on the above constructed freezing bed was done to evaluate the sludge freezing beds' ability to dewater alum sludge. Sludge was added from December 1989 to March 1990 in 18 layers, with a thickness of 20 to 100 mm and then allowed to thaw. The result of this pilot-scale study showed that the volume of alum sludge can be reduced by about 96% in the freezing bed. The solid concentration increased from 0.5% to 13.6% (Martel & J. Diener, 1991). In

northern Sweden, a full-scale sludge freezing ditch, drying ditch and freeze/thawing ditch was studied by Hellstrom (1997) for two consecutive winters. The term ditch was used instead of a bed to indicate that the bed was constructed on the existing ground. From early winter to spring, sludge was pumped in a thin layer of about 100 mm of thickness to the ditch and allowed to completely freeze before adding the next layer. Drainage pipes collected the melted water from the ditch and pumped the water back to the treatment plant. The dry matter content in the freezing ditch was 30-70% and 20-40%, respectively by the end of the first year and second year (Hellstorm & Kvarnstorm, 1997).

A pilot study for residual solid dewatering in the city of Winnipeg, Manitoba was done in 2008. Before starting the pilot study, the desktop evaluation was completed to confirm the feasibility of the project by reviewing other existing ponds. Other facilities such as at the City of Calgary pond in Alberta, the Duluth pond in Minnesota, the city of Regina pond in Saskatchewan and the city of Dauphin sludge dewatering pond in Manitoba were reviewed. A pilot study was completed over a ten week period, resulting in a solid concentration increase from 0.6% by weight before freezing to about 8% by weight after initial thawing and about 20% after a few days of thawing. Based on the desktop studies, the solid concentration was expected to reach a high of 50% over the summer drying period (Mangat et al., 2008).

The laboratory freezing test showed that the sludge with the solids content from 3 to 7% can be frozen at the same rate as tap water. Moreover, the frozen samples drained very fast during thawing and the sludge cake with a solid concentration of 25% remained after

one day (Reed, Bouzoun, & Medding, 1986). The field experiment conducted in Hanover was done in a large-scale outdoor basin with concrete walls and sandy soil. The digested primary sludge with a solid content between 6 to 8% was shipped directly from the plant and applied to the sludge bed to a depth of about 8 cm. Each layer was allowed to freeze completely before adding another layer. In total three layers of liquid sludge to a depth of 26 cm was frozen and then allowed to thaw in 14 days. The solid concentration increased to 35% and the depth of sludge cake reduced to 5 cm, indicating about 80% reduction in volume (Reed et al., 1986). Diak (2011) reported about 86% of melted water collected during the freeze/thaw of wastewater sludge from a rotating biological contactor. Furthermore, the total solid increased from 2.6% to 19% and volatile solid from 2.3% to 17.3%. The volume of sludge cake needed to be disposed after melting and dewatering was reduced by about 88%. A reduction in fecal coliform and salmonella in the sludge was also reported (Diak, Ormeci, & Proux, 2011). The sludge produced from the science-based industrial park wastewater is difficult to dewater. In the experiment the effects of different pre-treatments were analyzed. It was shown that chemical coagulation and thermal heating had no positive effect on sludge dewatering. However, freeze/thaw could release the moisture from the sludge by up to 83%. Moreover, the settleability and filterability of freeze/thaw sludge were greatly improved. A clear supernatant with a height of 70% of initial height was observed in 20 min after settling for frozen/thawed sludge. Whereas, 5% reduction was observed for the thermal heated and flocculated sludge after 24 hour (Chang , et al., 2004). In addition, freeze/thaw treatment was used for oily sludge by petroleum refinery plants, resulting in a separation of about 60% of oil from oily sludge (Jean, Lee, & Wu, 1999).

1.3.3 Sludge nutrient recovery through freeze/thaw

It has been proven that freeze/thawing of activated sludge can increase the concentration of proteins, carbohydrates and cations in the sludge supernatant, which indicated the release of intracellular and extracellular polymers(ECP) from sludge flocs (Ormeçi & Vesilind, 2001). Furthermore, the significant increase in DNA concentration in supernatant after freezing/thawing was observed and indicated the presence of intracellular materials, showing freeze/thaw can cause cell disruption (Ormeçi & Vesilind, 2001). The release of ECP and intracellular materials will result in increments of soluble chemical oxygen demand (SCOD) and ammonium concentration in the freeze/thaw supernatant. The study on the thickened waste activated sludge and the mixture of primary and secondary sludge from a treatment plant at Harbin city, China showed that keeping samples in the curing stage (the time sludge is kept under subfreezing temperature) could increase the solubilisation of organics significantly. The maximum COD solubilisation was reported 7.5% and 10.5% for mixed sludge and waste activated sludge, respectively. Moreover, the maximum release in NH₃-N was reported to be about 45.3% and 74.5% for mixed sludge and waste activated sludge respectively (Hu, et al., 2011). Other experiments examined sludge samples frozen for 24 hours at -10°C and -18°C in the freezer and then thawed at room temperature. The results showed that the organic materials of activated sludge, such as nitrogen and phosphorus in the microorganism cells, can be converted to soluble materials by freezing. In addition, the results indicated that freeze/thaw can increase the soluble chemical oxygen demand (SCOD) 2-8 fold, as well as increase the concentration of ammonia 2-8 times and

orthophosphate concentration 1.2-2.5 times (Gao, 2011). The statistical analysis showed no differences in solubilisation of COD and release of $\text{PO}_4\text{-P}$ and $\text{NH}_3\text{-N}$ when frozen at -10°C versus -18°C and during one or five cycles of freeze/thaw (Gao, 2011). The effects of freeze/thaw on nutrient concentration of mixed primary and waste thickened sludge were evaluated in the lab experiment and resulted in improvements by about 39% and 53% in ammonia nitrogen and phosphorus (as orthophosphate) in supernatant, respectively. As well, there was an increase by about 2 times in soluble COD and VFA concentration (Montusiewicz et al., 2010). Wang (2001) reported an increase of 25.5, 24.6 and 18.8 times the quantity of protein and carbohydrate from the sludge frozen at -10 , -20 and -80°C , respectively compared with unfrozen sludge. Furthermore, the number of viable bacteria decreased by about 96%, 93% and 84%, respectively, indicating cell damage during freeze/thaw (Wang et al., 2001).

1.3.4 Sludge digestability and biogas production through freeze/thaw

Pre-treatment methods usually are used to solubilize the organic matter, create cell disruption and release the cell contents and cell wall polymers such as polysaccharides, proteins and lipids into the liquid by mechanical, chemicals, biological and thermal process. Hydrolysis of organic materials in anaerobic digestion processes usually is a rate-limiting step. Developing a method to enhance the hydrolysis rate could improve the digestion efficiency and biogas production especially for difficult to biodegrade materials (Montusiewicz et al, 2010; Wang et al., 1995).

There were only a limited number of research reports found in the literature regarding the effects of sludge freezing on biogas production. One report stated that there was an increase of about 27% of methane production of waste activated sludge after freeze/thaw processing (Wang et al., 1995). Another study evaluated the effects of freeze/thaw on mixed sewage sludge anaerobic digestion and biogas production in Poland. Primary thickened sludge and waste thickened sludge were shipped to the laboratory and then mixed with a ratio of 60:40 (primary: waste). Some samples went through freezing under -25°C for 24 hours in the freezer and then allowed to thaw at room temperature. The treated and untreated samples were added to two parallel mesophilic anaerobic digesters. One reactor was fed with raw sludge as a control while the other was fed with treated sludge. The result for biogas yield per kg of VS removed were $1.3\text{ m}^3/\text{kg}$ and $0.86\text{ m}^3/\text{kg}$ for the frozen/thawed sludge and the raw sludge, respectively, which indicated about a 50% increase in specific biogas yield (Montusiewicz et al., 2010).

1.4 Objective of this thesis

The present study was designed to find a sustainable, practical and cost-effective treatment for sewage sludge management in First Nation and Northern Communities. According to the Indigenous and Northern Affairs Canada (INAC) protocol (2010) for Centralised Wastewater Systems in First Nations Communities, all Aboriginal Affairs and Northern Development Canada (AANDC) funded First Nations must improve their treatment systems to ensure safe disposal of their solids and biosolids. They also should be restricted by applicable federal or provincial regulations, codes of practice, or guidelines for the management and ultimate disposal of sludge, biosolids, and other process residuals (Aboriginal Affairs and Northern Development Canada, 2010).

According to the National Assessment of First Nation Water and Wastewater Systems final report, January 2011, there are 62 First Nation communities in Manitoba with populations ranging from 43 to 5,869 people. The total number of homes is 15,661 and the average household size is 5.4 people per unit (ppu). There are 61 wastewater systems including 32 facultative or aerated lagoons, 24 mechanical plants, 4 Municipal Type Agreements (MTA) and one communal septic system (Burnside, 2011). Thirty-nine percent of wastewater treatment relies on mechanical treatments which include primary and secondary treatment, where daily sludge is produced during the treatment. Most of the Northern Communities have extremely cold winters and short summers. Sludge that is produced in these communities is generally dumped into sludge lagoons, shipped to the local landfill or transported to the nearest equipped treatment plant for further treatment.

Developing an efficient dewatering and conditioning method has advantages in terms of reducing the cost and lowering the risk of distributing untreated sludge into the environment during shipping or disposal into the landfill. As mentioned earlier, freeze/thaw treatment is a simple, practical and low cost method for sludge dewatering and is applicable for small treatment plants in cold remote regions. In addition, the dewatering process has advantages in terms of resource recovery potential.

The following are the specific objectives of this research study:

- Evaluating the extent of dewatering through freeze/thaw treatment as a sludge dewatering technique. For this, two kinds of sludge were tested:
 - Activated sludge from a biological nutrient removal plant (BNR plant)
 - Activated sludge from a conventional biological treatment plant (non-BNR plant)
- Assessing the potential of nutrient solubilisation from the organic content of two kinds of activated sludge through freeze/thaw process:
 - Activated sludge from a biological nutrient removal plant (BNR)
 - Activated sludge from a conventional biological treatment plant (non-BNR).
- Investigating the digestibility of sludge cake and the possible change in sludge biogas production after freeze/thaw dewatering.

Two wastewater treatment plants in Winnipeg were used for sludge sampling. Activated sludge from the South End plant was used as a source of conventional biological plant

solids (non-BNR sludge) and West End plant as a source of biological nutrient removal solids (BNR sludge).

Chapter 2

Experimental and Analytical Method

2.1 Source and collection

Return activated sludge (RAS) was used in the experiment and accessed from the South End Water Pollution Control Centre (SEWPCC) and West End Water Pollution Control Centre (WEWPCC) in Winnipeg, Canada. Treatment processes in SEWPCC include primary sewage treatment and secondary biological treatment (BOD removal, non-BNR) and ultraviolet disinfection. The centre is currently under construction to upgrade their treatment level to include phosphorus and nitrogen removal i.e. a BNR system; however, the samples were taken prior to this upgrade. The WEWPCC treatment process includes primary treatment and secondary biological treatment including nutrient removal (BNR) and natural-light disinfection in polishing ponds.

Digested sludge samples used as inoculum for BMP tests were obtained from the anaerobic digester in the North End Water Pollution Control Centre (NEWPCC) in

Winnipeg, Manitoba. The sand and pea gravel used as a drainage bed was sourced from a local garden store (Lacoste Garden Centre, Winnipeg, Manitoba).

The experiments were done as batch tests. The first set of batch tests were started on December 2014 using WEWPCC sludge. The second set of batch tests were started on February 2015 using SEWPCC sludge. For the first two sets, only dewatering and nutrient release were evaluated. The third set was started in July 2015 using SEWPCC sludge. For this batch the biogas production of fresh sludge and solid cake after freeze/thaw was measured. The next set of batch tests from SEWPCC sludge were started on October 2015. Biogas production was measured for fresh sludge and the mixture of sludge cake and effluent water after freeze/haw. The last set was set up on January 2016 using the WEWPCC sludge as BNR sludge. However, due to some problems in the treatment process at the WEWPCC, the system did not work fully as a BNR. The anoxic reactor was not working properly and as a consequence the complete biological nitrogen and phosphorus removal did not happen and ferric chloride was added at the plant for phosphorus removal. Consequently, the sludge used in this batch would be considered as BNR/chemical sludge. The amount of water removal, nutrient release and biogas production was measured for fresh sludge and sludge cake after freeze/thaw. The results of the last batch are provided in appendix D.

In addition, to better understand sludge water movement and nutrient release during thawing, two batches of sludge from both west and south end were examined at the same time on April 2015 and the nutrient levels were monitored during thawing.

2.2 Experimental set up

2.2.1 Pre-test phase

A pre-test was done to check the potential effect of sand and gravel on nutrient release during the experiments. A solution of 20 mg/L concentration of nitrogen (using ammonium chloride) and 20 mg/L concentration of phosphorus (using potassium phosphate) was prepared separately. Then sand and gravel were left in the solution for a period of five days. The solution was mixed manually every day and samples were taken to measure $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$. A second pre-test was done preparing the same solution and the sand and gravel were left in a solution for an hourly test, where samples were taken regularly for up to 24 hours and $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were measured.

2.2.2 Freezing phase

The experiment was conducted in a laboratory under controlled conditions. Three big plastic boxes ($0.46 \times 0.65 \times 0.18$ m) were used as beds during the freezing period. The aim was to freeze the fresh sludge in thin layers in the freezer and then thaw and drain the water to separate the solid and liquid fractions for further examination. A period of three weeks was chosen. Every week approximately 20 L of fresh sludge was collected from the wastewater treatment plant and shipped to the university laboratory on the same day of the experiment. The bucket of sludge was mixed and 4 L of fresh sludge was collected. Three litres were added to each box and one litre was used to measure the characteristics of the fresh sludge. The presence of three boxes represents triplicate tests. The boxes

were covered with lids and were placed inside a chest freezer for one week to freeze. The freezer temperature was set at -12°C . After one week a second layer composed of 3 L of fresh sludge was added on top of the initial frozen layer and left in the freezer for another week. At the end of second week the third and last layer of 3 L of fresh sludge was added the same way and left in the freezer for one week (Fig. 2.1).

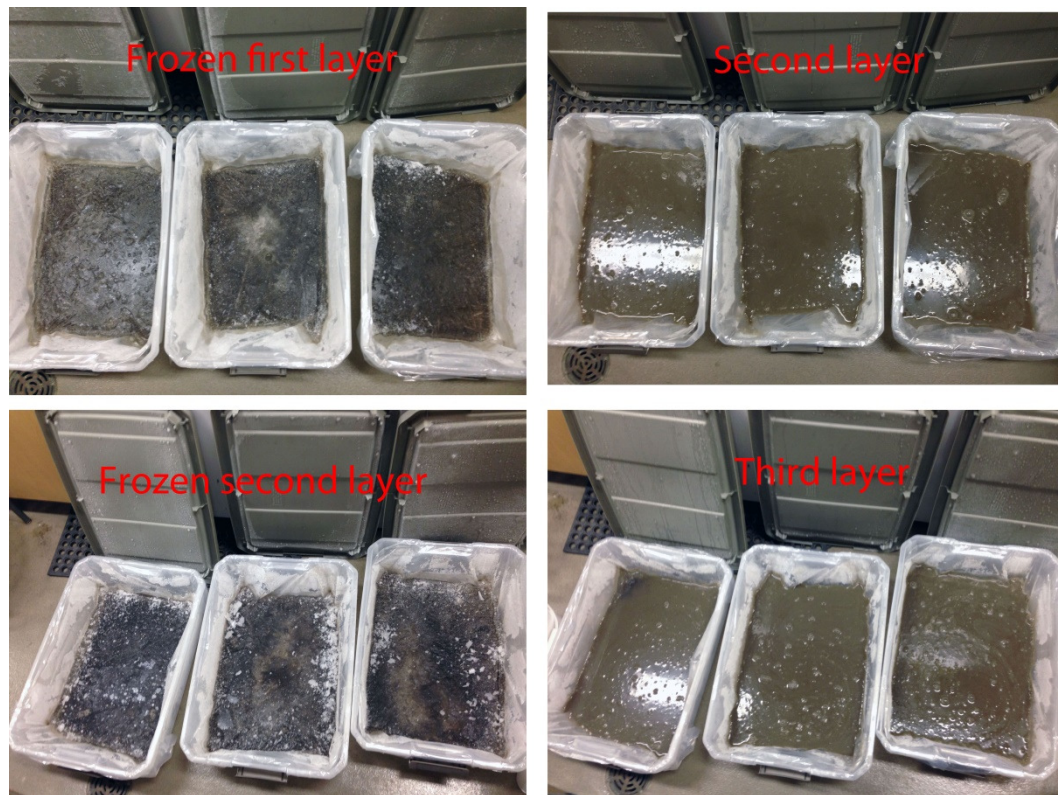


Fig. 2.1: Freezing the fresh sludge

In summary, each box contained three layers of 3 L for a total of 9 L of sludge. TN, TP, TCOD, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, SCOD, TS and VS were measured for each fresh sludge layer. Tables 2.1 and 2.2 demonstrate the fresh activated sludge characteristics collected from both non-BNR and BNR plants, respectively.

Table 2.1: Activated sludge characteristics from non-BNR wastewater treatment plant

non-BNR Sludge	TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	V _s (g/L)
	713.28	143.77	9222.69	42.40	9.45	67.14	7.82	6.60
STD	77.99	44.71	559.02	3.48	2.11	12.90	0.35	0.30

Table 2.2: Activated sludge characteristics from BNR wastewater treatment plant

BNR Sludge	TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	V _s (g/L)
	781.11	270.22	11232.64	4.21	13.84	83.59	9.83	7.96
STD	130.18	31.73	2153.48	0.98	5.42	10.99	1.08	0.75

2.2.3 Thawing and dewatering phase

Three plastic boxes (0.73× 0.45× 0.15 m) were used for thawing and drainage. The boxes were covered with insulation material to replicate natural soil conditions found in sludge freezing beds. Each box was prepared by making two holes and placing tubes to collect the melted water into effluent buckets. Approximately 2 kg of pea gravel and 1 kg of sand were placed in each box as a drainage bed and the melted water drained through the sand and gravel bed. The sludge cake was left inside the box (Fig. 2.2). The frozen samples were placed into the boxes and left at room temperature to melt (21± 1 °C). The fabric screen was used to separate the sand and gravel from the sludge (Fig. 2.3).



Fig. 2.2: Thawing boxes



Fig. 2.3: Placing the frozen sludge for thawing

The weight of the boxes was measured before leaving the samples, after leaving the frozen sludge and after thawing and dewatering. Thawed out water was analyzed for TN, TP, TCOD, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, SCOD, (in triplicates). The sludge cake was analysed for TN, TP, TCOD, TS and VS (in triplicates). Moreover, the sludge cake and sludge cake mixed with effluent water were used in separate tests for biogas measurements using the Biochemical Methane Potential (BMP).

2.2.4 Time-based trend in nutrient release

To find out the process of sludge thawing and to monitor the trend of change in nutrient release during thawing, one batch from each of the waste treatment plants (BNR and non-BNR) was set at the same time. The fresh sludge was obtained from both treatment plants on the same day. About 10 L of fresh sludge was added to two boxes (one box for BNR and one box for the non-BNR sludge). The characteristics of fresh sludge for both samples were measured and analysed. The boxes were left in the freezer for a period of two weeks to freeze. After two weeks, the frozen sludge was left at room temperature to thaw and observed for the next 34 hours until the end of the complete thawing and dewatering process. The first sample was taken after 10 hours and then, every two hours, samples were taken. The container used for collecting water was changed after each sampling. Samples were passed through filter paper and kept in the fridge. At the end of sampling all filtered samples were diluted with DI water and analyzed with Flow Injection Analysis (FIA) for $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$ concentrations.

Moreover, for the one batch from the non-BNR plant, the effluent boxes were kept at room temperature for 2 additional days after complete thawing and dewatering (total 4 days) and were sampled at different times to measure changes in $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$ concentrations.

2.2.5 Respirometer set up

A BMP test was conducted using a respirometer to examine the methane potential of fresh sludge before treatment and sludge cake after freeze/thaw treatment. Inoculum (digested seed) was taken from NEWPCC and shipped to the lab one day before the test set up. The inoculum was collected from a mesophilic sludge digester, operated at 37.5°C , with a sludge age of 14-16 days. It was kept inside the warm chamber at 37°C to maintain the active bacteria. The substrate (RAS) was obtained from SEWPCC and WEWPCC on the day of test set-up. The TS and VS of both inoculum and substrate were measured and the desired volume was calculated based on the inoculum to substrate ratio of one to two (I/S: 1/2) in terms of their VS content. The inoculum and substrate were mixed and then added to 500 mL assay bottles to up to 420 mL. The bottles were closed using a screw cap with butyl rubber septa and the headspace was purged with N_2 gas to remove the oxygen in order to replicate anaerobic conditions (Fig. 2.4). After purging with nitrogen gas for 10 minutes, the bottles were placed on a stirring plate in a water bath at 37°C which was connected to an automated flow-cell system to monitor biogas production (Fig. 2.5). The biogas production was monitored for the period of 20 days and the headspace biogas samples were taken with a pressure-lock syringe twice, and analyzed for CH_4 and CO_2 content using an Agilent 7890A Gas Chromatograph.



Fig. 2.4: Purging N2 gas to provide anaerobic condition

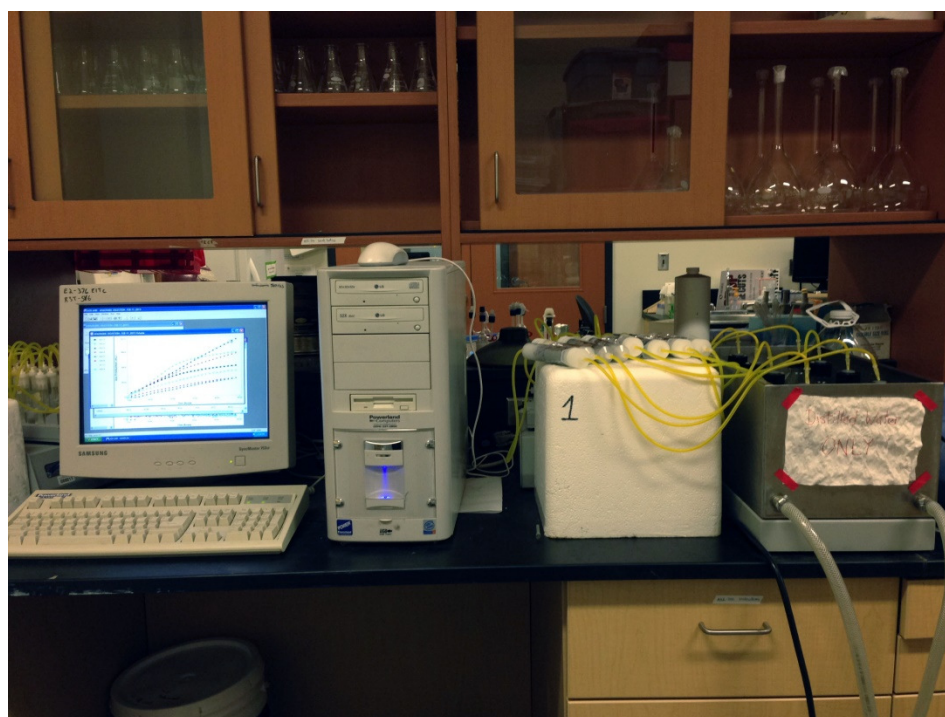


Fig. 2.5: Monitoring biogas production with a respirometer

2.3 Sample analysis

The following methods were used to analyze the fresh sludge, sludge cake and effluent drained water.

The pH, total solids (TS) and volatile solids (VS) of all samples were determined using standard methods (APHA, 1998). The $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were measured with Flow Injection Analysis (FIA) (LaChat QuikChem 8500, University of Manitoba).

The biogas production was monitored by an automated flow-cell system (Challenge AER- 200 Respirometer, University of Manitoba). Biogas composition (CH_4 % and CO_2 %) was analyzed using an Agilent 7890 CG equipped with a TCD using argon carrier gas, University of Manitoba.

2.3.1 Liquid sample analysis

Samples taken from fresh sludge were centrifuged at 10,000 rpm for 10 minutes and then the supernatants were separated and passed through vacuumed filter paper with a pore size of 0.45 μm . The filtrates were diluted with DI water and then $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ were measured using FIA. TCOD of mixed fresh sludge and SCOD of filtrate supernatant were determined using standard methods (APHA, 1998). For the collected effluent water, samples were taken from each box filtered, diluted and analyzed as mentioned above for soluble materials.

The total nitrogen and total phosphorus within the mixed fresh sludge and collected effluent water were determined by a modified method of Kjeldahl Digestion following a Hach procedure using sulphuric acid and hydrogen peroxide (Hach, 1999). Ten millilitres of samples were transferred to a 100 ml digestion flask. Five ml of concentrated sulphuric acid (H_2SO_4) was added to the flasks and then left to digest for about 30 minutes in the Kjeldahl digester apparatus. Once an acid reaction occurred (observation of dark black solution and white acid fumes) hydrogen peroxide was added by drops to obtain a clear solution. Samples were left for another 5 minutes to boil and then left to cool down (Fig. 2.6). Then samples were diluted to 100 mL with DI water, neutralized and analysed with Flow Injection Analysis (FIA).

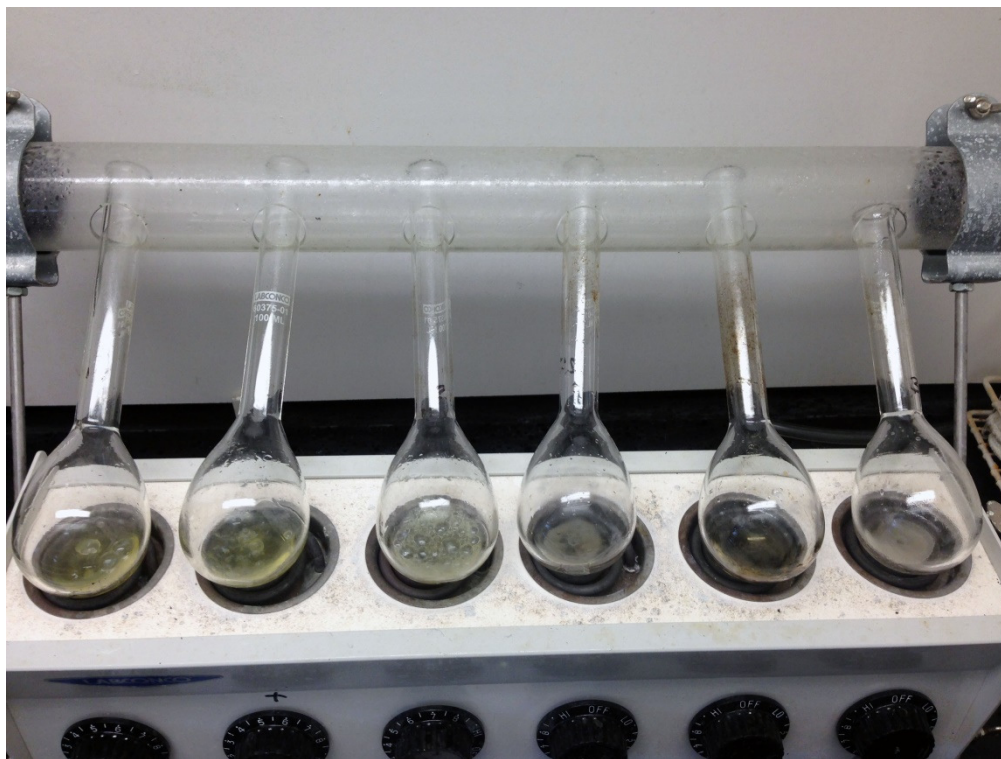


Fig. 2.6: Kjeldahl digester apparatus

2.3.2 Solid sample analysis

To determine the characteristic of the sludge solid cake, first the TS and VS of sludge cake was measured using standard methods cited earlier. Next, a 10 g sample was taken and diluted with DI water to reach a concentration range similar to that of fresh sludge and mixed well. TCOD, TN, TP were analyzed as mentioned for the liquid samples.

2.3.3 Calculations

The concentrations of all the required parameters were measured and according to the influent and effluent volume, the parameters were calculated by mass. Organics solubilisation was determined by subtracting the effluent water from raw soluble supernatant divided by the initial total amount entered to each box calculated by their mass. The same calculation was used to calculate nitrogen and phosphorus release rate. (Hu, et al., 2011)

$$\text{COD solubilisation (\%)} = \frac{(\text{SCOD}_{\text{eff}} - \text{SCOD}_{\text{raw}})}{\text{TCOD}_{\text{raw}}} \times 100$$

$$\text{Nitrogen release (\%)} = \frac{(\text{NH}_4\text{-N}_{\text{eff}} - \text{NH}_4\text{-N}_{\text{raw}})}{\text{TN}_{\text{raw}}} \times 100$$

$$\text{Phosphorus release (\%)} = \frac{(\text{PO}_4\text{-P}_{\text{eff}} - \text{PO}_4\text{-P}_{\text{raw}})}{\text{TP}_{\text{raw}}} \times 100$$

2.3.4 Statistical Analysis

Statistical analysis was performed using SAS version 9.3 (SAS Institute, 2014). Independent t-test was used to compare the effect of freeze/thaw treatment on nitrogen, phosphorus and COD solubilisation of two different kinds of BNR and non-BNR activated sludges. The null hypothesis was that there were no differences between the two types of sludge.

A paired t-test was done to compare the sludge methane potential before and after freeze/thaw treatment. The null hypothesis was that there was no difference in methane production before and after treatment. All tests were evaluated at 0.05 level of probability (95% of confidence).

It was assumed that all the environmental conditions during freezing and thawing were the same for the two different types of sludge for the purpose of analysis.

Chapter 3

Results and Discussion

3.1 Effects of freeze/thaw treatment on sludge dewatering

Frozen sludge left at room temperature thawed and drained completely within 48 hours. The sludge filtered easily through the sand bed. Inside the boxes, the dense and compacted flocs left on top of the flat screen as a sludge cake was easily removed. Fig. 3.1 and Fig. 3.2 illustrate the thawing progression after 24 and 48 hours. Approximately 85% of the sludge water was collected in the effluent boxes. The volume of the water collected after F/T for the non-BNR was 7.57 ± 0.04 L from an initial volume of 9 L raw sludge and for BNR was 7.23 ± 0.2 from an initial volume of 9.6 L raw sludge. In fact, the fraction of moisture removed by this method for the non-BNR and BNR sludge were $84.15 \pm 0.5\%$ and $75.3 \pm 2.1\%$, respectively ($p= 0.0262$). The difference in water removal between the two types of sludge was mostly because of a leakage happened in one box during the thawing process of the BNR sludge.

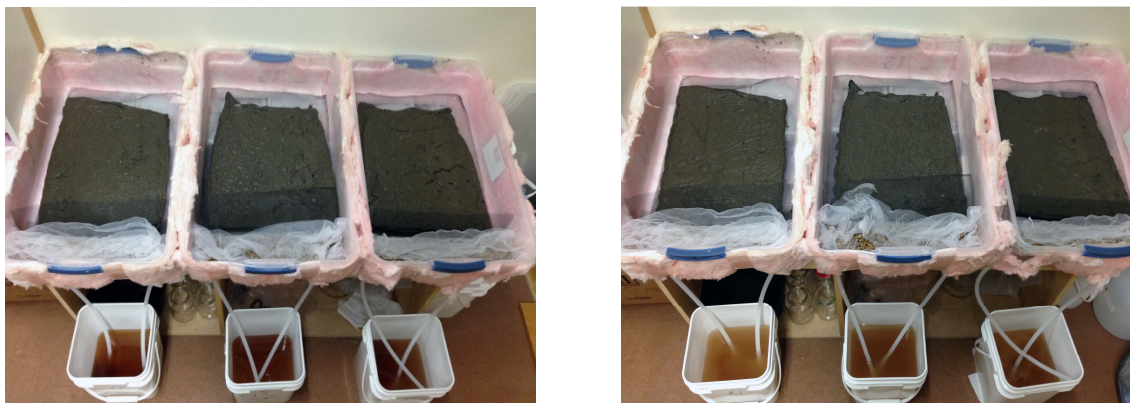


Fig. 3.1: Sludge melting process after 24 h and 48h (non-BNR)

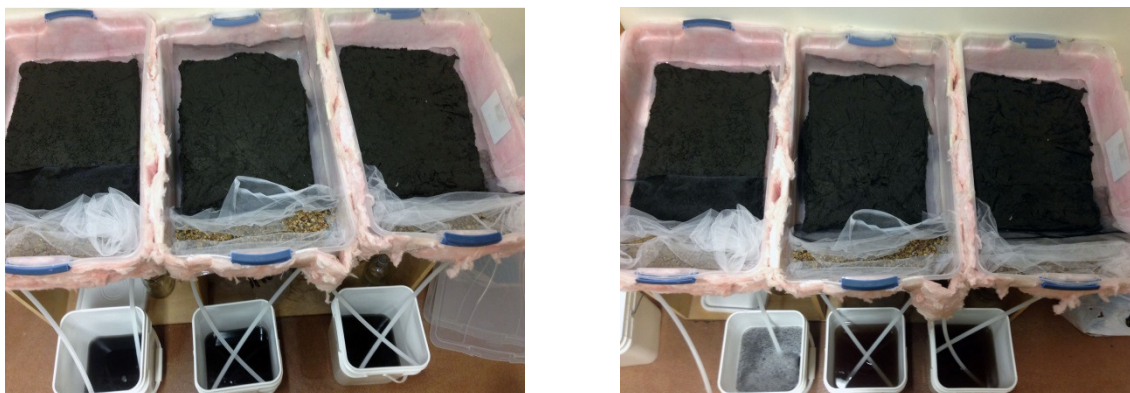


Fig. 3.2: Sludge melting process after 24 h and 48h (BNR)

Overall, considering the evaporation and absorption of the water by the sand and gravel, this method was effective in reducing the sludge water by more than 85%. These results are consistent with other studies reporting about 90% of sludge moisture removal by F/T treatment under natural environment condition (Martel and Diener, 1991; Reed et al., 1986).

According to the previously described mechanisms of freeze/thaw treatment in section 1.3.1, sludge freezing is initiated with freezing of the free water around the sludge flocs. Then, the interstitial water, which is the water molecules inside the flocs, joins the moving ice structure. Providing sufficiently low temperature and long enough freezing time (curing time) can result in freezing of surface water and finally bound water, forcing the particles into the more compacted form (Vesilind, Wallinmaa, & Martel, 1991). The sludge solid content of non-BNR raw sludge was 0.77 ± 0.03 %, which was increased to about 9.3 ± 0.41 % at the end of thawing. The BNR raw sludge total solid was about 0.98 ± 0.11 %, which increased to 9.93 ± 0.38 % in the sludge cake post freeze/thaw treatment (statistic p value = 0.0002 comparing non-BNR and BNR sludge) . Fig. 3.3 demonstrates the sludge solid change before and after freeze/thaw treatment for non-BNR and BNR activated sludge, respectively. However, the thawing phase for this experiment was done under lab temperature and moisture conditions, and it cannot be compared with the results of other studies reporting more than 20% of sludge solid cake content under natural thawing conditions. Higher solid concentration would be expected with a short drying period during the thawing summer season as well as the presence of wind and subsequent evaporation in nature. (Martel & J. Diener, 1991; Hellstorm & Kvarnstorm, 1997; Mangat et al., 2008; Reed et al., 1986).

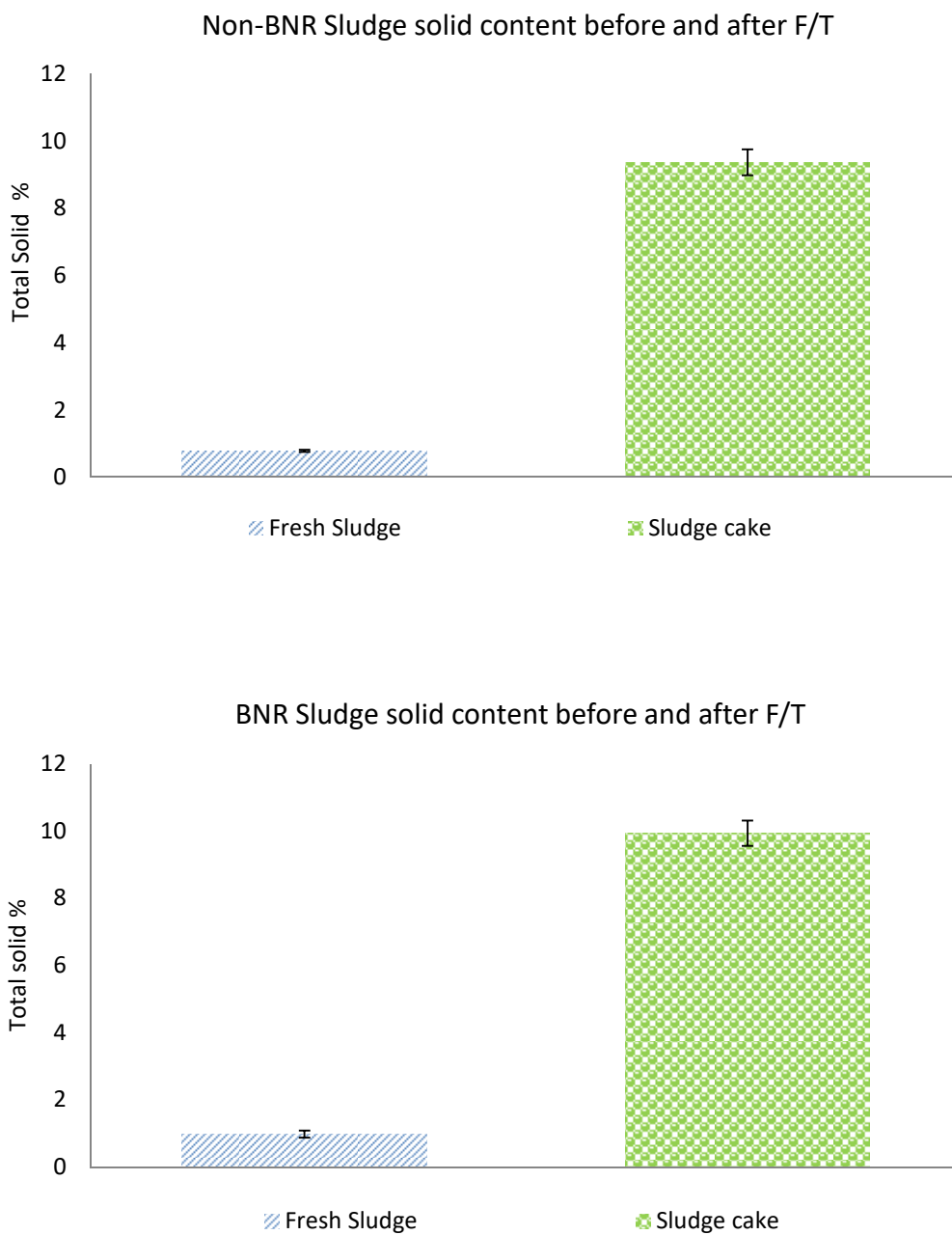


Fig. 3.3: Sludge solid content before and after freeze/thaw for non-BNR and BNR sludge

3.2 Effects of freeze/thaw treatment on sludge solubilisation and nutrient release

The pre-test was done to monitor the possible effects of sand and gravel on nutrient release during thawing periods and the results showed there were no significant effects. Detailed information from the pre-test is presented in Appendix A.

The experiment for BNR sludge was done once and for the non-BNR sludge was repeated three times. The focus of this study was on conventional biological sludge (non-BNR sludge) since current operating wastewater treatment plants in First Nation Communities are performing conventional treatment (Burnside, 2011). The summary of the raw sludge, sludge cake and collected effluent parameters are presented in Tables 3.1, 3.2, 3.3 and 3.4 for the BNR and non-BNR first, second and third set, respectively. More detailed results are presented in Appendix B.

Notably, the results of solubilisation and nutrient release were compared according to the average data of BNR set and the average of non-BNR second set (July, 2015) and third set (Oct, 2015). The first experiment set up for the non-BNR sludge did not consider since the different volume of fresh sludge were used for that batch. Therefore, the results were not included in the statistical analysis and discussion.

Also the total nitrogen, phosphorus and COD were measured before and after F/T treatment for non-BNR sludge and are presented in tables 3.3, 3.4 and Fig. 3.5, 3.7 and

3.9. However, the total amount after F/T treatment for BNR sludge were not measured and the same amounts of raw sludge is considered for presenting the results in bar graph (Fig. 3.4, 3.6 and 3.8). Besides, the different letters on top of bar graphs indicate the statistically differences between the mean of data set. Furthermore, the final freeze/thaw experiment was carried out using a different type of sludge (BNR/chemical). The result of this experiment is presented in appendix D. Moreover, the detailed outputs of the statistical analysis are presented in appendix E.

Table 3.1: Characteristic of raw sludge (a), effluent water (b) and sludge cake(c). BNR Sludge

BNR Dec,2014	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (%)	V _s (%)	Volume (L)
Raw Sludge	7.45	2.59	108.03	0.04	0.13	0.80	0.98	0.8	9.60
STD	0.21	0.00	10.17	0.00	0.01	0.02	0.11	0.07	0.00

(a)

BNR Dec,2014	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	0.51	2.20	18.31	7.23
STD	0.02	0.31	1.55	0.21

(b)

BNR Dec,2014	TS %
Sludge cake	9.93
STD	0.39

(c)

Table 3.2: Non-BNR sludge, Feb, 2015. Characteristic of raw sludge (a), effluent water (b) and sludge cake(c).

Non-BNR Feb,2015	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (%)	Vs (%)	Volume (L)
Raw sludge	8.38	1.39	112.35	0.54	0.09	0.77	93.74	80.44	11.68
STD	0.19	0.00	4.81	0.00	0.00	0.02	0.89	0.39	0.06

(a)

Non-BNR Feb,2015	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	1.99	0.89	29.64	9.50
STD	0.05	0.04	0.92	0.08

(b)

Non-BNR Feb,2015	TS %
Sludge cake	9.55
STD	–

(c)

Table 3.3: Non-BNR set, July, 2015. Characteristic of raw sludge (a), effluent water (b) and sludge cake(c).

Non-BNR July,2015	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ - P (g)	SCOD (g)	TS (%)	Vs (%)	Volume (L)
Raw sludge	5.81	1.13	80.12	0.35	0.10	0.63	0.8	0.67	9.00
STD	0.07	0.02	1.32	0.00	0.00	0.02	0.01	0.01	0.00

(a)

Non-BNR July,2015	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	2.46	0.83	19.96	1.09	0.53	14.90	7.58
STD	0.13	0.10	0.12	0.03	0.01	0.40	0.04

(b)

Non-BNR July,2015	TN (g)	TP (g)	TCOD (g)	TS %	V _s %
Sludge cake	5.50	1.41	93.56	9.62	8.02
STD	0.38	0.07	4.56	0.23	0.21

(c)

Table 3.4: Non-BNR set, Oct, 2015. Characteristic of raw sludge (a), effluent water (b) and sludge cake(c).

Non-BNR Oct,2015	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (%)	V _s (%)	Volume (L)
Raw sludge	7.07	1.74	82.32	0.38	0.08	0.61	0.74	0.62	9.00
STD	0.28	0.10	1.59	0.00	0.00	0.05	0.01	0.01	0.00

(a)

Non-BNR Oct,2015	TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	2.91	0.78	19.68	1.63	0.59	21.35	7.57
STD	0.06	0.09	1.57	0.05	0.08	1.71	0.05

(b)

Non-BNR Oct,2015	TN (g)	TP (g)	TCOD (g)	TS %	V _s %
Sludge cake	5.19	0.85	54.63	8.97	7.05
STD	0.03	0.10	1.05	0.26	0.07

(c)

3.2.1 Effects of freeze/thaw treatment on organic phosphorus release

- BNR sludge

Total phosphorus and soluble phosphorus before and after F/T treatment is displayed in Fig. 3.4. The amount of total phosphorus of raw sludge was 2.59 ± 0.003 (g) and $\text{PO}_4\text{-P}$ of raw sludge supernatant was 0.13 ± 0.008 (g) indicating only 6.5 % of raw sludge was in the form of soluble before F/T. However, the $\text{PO}_4\text{-P}$ of effluent collected water after F/T increased to 2.2 ± 0.31 showing a release of about 80 ± 12 % of P through F/T treatment in to the effluent.

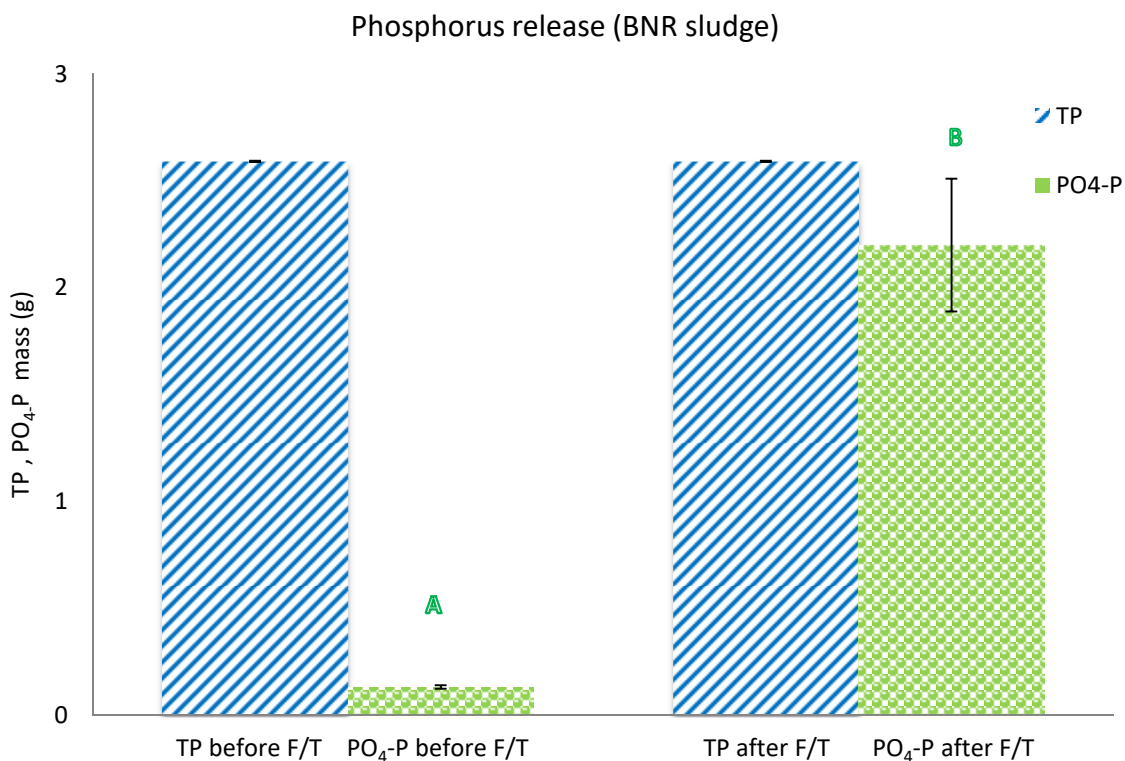


Fig. 3.4: BNR sludge release of phosphorus

- Non-BNR sludge

The amount of total phosphorus entered to the boxes was 1.44 ± 0.31 (g) and $\text{PO}_4\text{-P}$ of raw sludge supernatant was 0.093 ± 0.01 (g) showing 6.45% of raw sludge P was in the soluble form before F/T. However, the $\text{PO}_4\text{-P}$ of effluent collected water after F/T increased to 0.56 ± 0.06 indicating the release of 33.5 ± 5.5 % of P through F/T treatment. Fig. 3.5 presents the results.

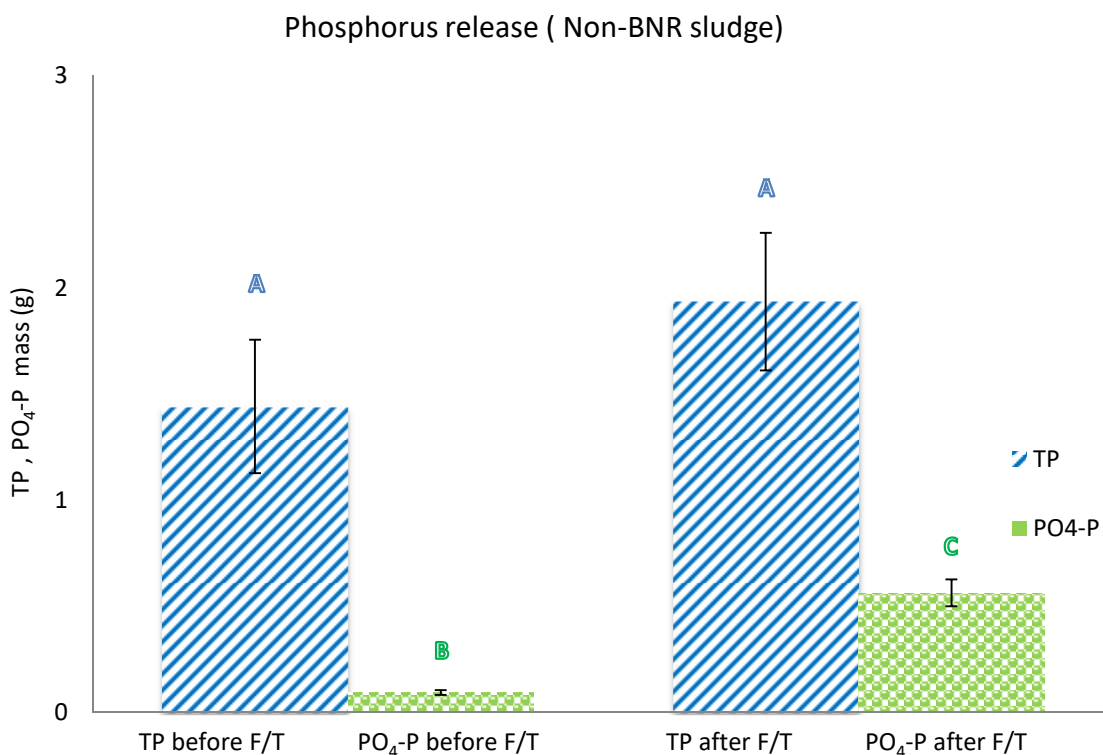


Fig. 3.5: Non-BNR sludge release of phosphorus

Phosphorus is the vital part of DNA, RNA, and ATP in the microorganism's cell. The increase of P in the effluent liquid after freeze/thaw indicated rupture of bacteria cells through freezing and discharge of the intracellular material. The results from this experiment are consistent with those obtained by Ormeci and Vesilind (2001), reporting a

6 times increase in DNA concentration in sludge supernatant after F/T conditioning, which indicates the presence of intracellular material as a result of cell disruption (Ormeçi & Vesilind, 2001). In addition, current findings are in agreement with those by Gao (2001) and Montusiewicz et al (2010) reporting the increase of PO₄-P concentration after freeze/thaw caused by cell disruption.

Comparing the behaviour of the two sludge, there was a significant difference between non-BNR and BNR sludge in terms of organic phosphorus release with F/T treatment ($p=0.0002$). Higher release of orthophosphate from BNR sludge was observed compared with non-BNR (80% and 33.5%, respectively). Considering mechanisms of phosphorus uptake in BNR processes (as described in section 1.1.3), the difference in sludge P content could be attributed to the enhanced presence of PAOs bacteria in the BNR system and their capacity to uptake P and store it as polyphosphate in their cells. The ratio of TP/VS (g/g) of raw sludge in the current experiment was 0.025 and 0.036 for non-BNR and BNR sludge respectively, demonstrating the higher amount of P in the biomass of BNR activated sludge.

3.2.2 Effects of freeze/thaw treatment on organic nitrogen release

- BNR sludge

Organic nitrogen of raw sludge was 7.45 ± 0.21 (g) with small amounts of ammonium at about 0.5%. The freeze/thaw resulted in the release of about $6.31 \pm 0.25\%$ of the cell organic nitrogen in to the collected water (0.51 ± 0.02 g). Fig. 3.6 illustrates the change in organic nitrogen and $\text{NH}_4\text{-N}$ before and after F/T.

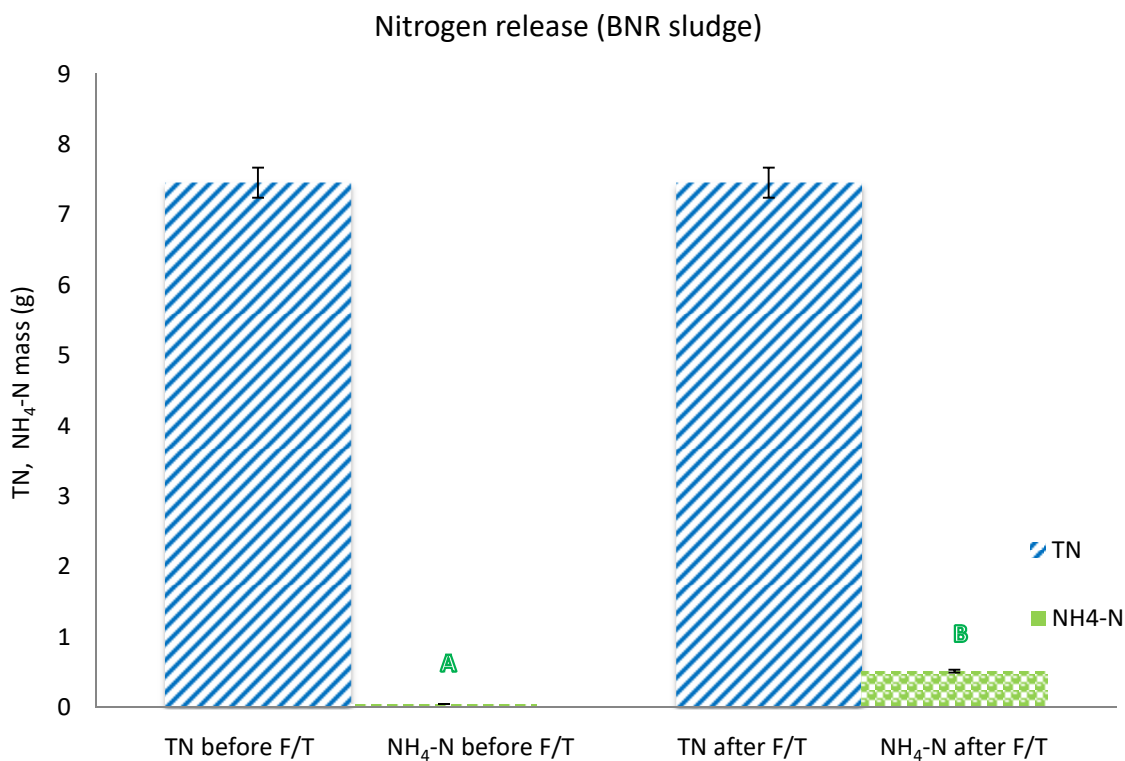


Fig. 3.6: BNR sludge release of nitrogen

- Non-BNR sludge

The organic nitrogen of raw activated sludge from non-BNR plant before F/T was 6.44 ± 0.66 (g), which included 5.6% of $\text{NH}_4\text{-N}$ (0.37 ± 0.02 g). The F/T treatment was effective in hydrolysing about 15.19 ± 2.4 % of organic nitrogen in to the effluent (1.36 ± 0.27 g). The result of nitrogen release is presented in Fig. 3.7.

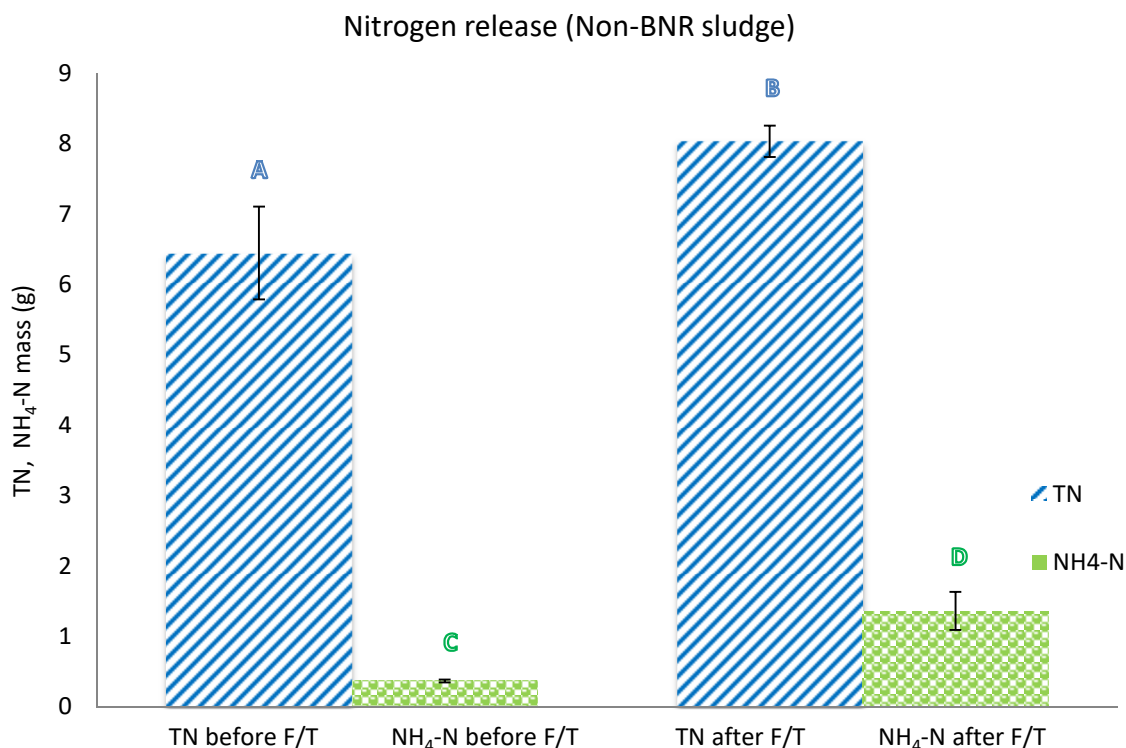


Fig. 3.7: Non-BNR sludge release of nitrogen

Nitrogen is the main component of amino acids which are the building blocks of proteins, vital cell constituents in microorganisms. The increase of ammonium nitrogen in the effluent is the result of degradation of proteins as a consequence of cell rupture through freezing. Ormeci and Vesilind (2001) reported remarkable increase of proteins and carbohydrates by F/T treatment of activated sludge indicating the effectiveness of this

method for releasing the extracellular materials of sludge flocs and intracellular materials of cells to the supernatant. The results of nitrogen release in the present study are in agreement with those from previous studies, which showed an increase of $\text{NH}_4\text{-N}$ concentration in the sludge supernatant after F/T treatment (Hu et al., 2011; Gao, 2011; Montusiewicz et al., 2010; Ormeci & Vesilind, 2001). However, different release rates were reported depending on the freezing temperature and curing time. Hu et al (2011) reported the maximum of 74.5% increase in ammonium concentration of WAS after increasing the curing time from 3 to 72 hour and concluded that curing time was very important in releasing of ammonium nitrogen.

Comparing the non-BNR and BNR sludges, the nitrogen release was significantly different ($p=0.0003$) and higher release occurred for non-BNR sludge. The same observation was reported by Yuan (2010), who studied the fermentation of waste activated sludge generated from BNR and non-BNR treatment plants. The release of $\text{NH}_4\text{-N}$ from non-BNR activated sludge after fermentation was 1.8 times higher than BNR sludge (Yuan, Baranowski, & Oleszkiewicz, 2010). The difference might be related to the differing solids retention time (SRT) in the two treatment plants. Lower SRT of the non-BNR plant resulted in higher amount of active biomass and greater release.

3.2.3 Effects of freeze/thaw treatment on COD solubilisation

- BNR sludge

Results for TCOD and SCOD are presented in Fig. 3.8. Results indicate a release of $16.5 \pm 2.9 \%$ of organics from the sludge to the soluble phase through F/T treatment. The raw sludge supernatant SCOD before treatment was 0.8 ± 0.02 (g), and increased to 18.31 ± 1.55 (g) from the TCOD of 108 ± 10 (g).

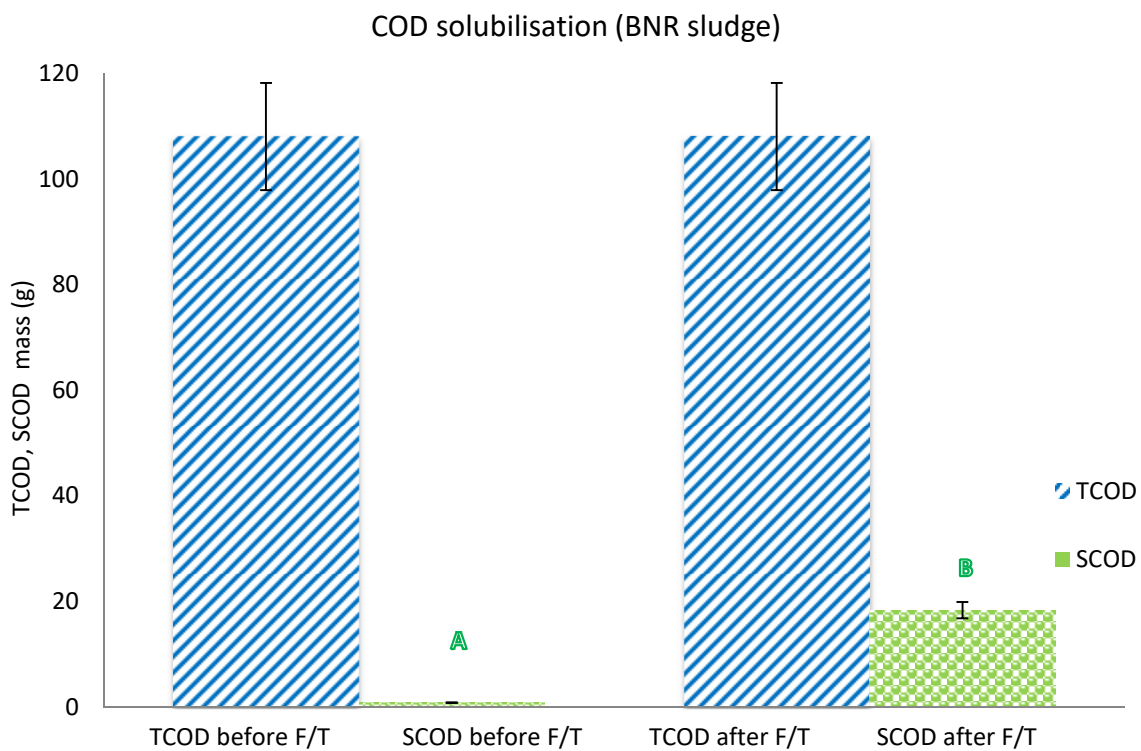


Fig. 3.8: BNR sludge solubilisation of organics

- Non-BNR sludge

The amount of TCOD of the raw sludge was 81.22 ± 1.83 (g) and SCOD before and after F/T treatment was, 0.62 ± 0.04 and 18.12 ± 3.5 (g), respectively. Fig. 3.9 represents the results indicating the sludge organics were solubilized by about $21.5 \pm 4.1\%$.

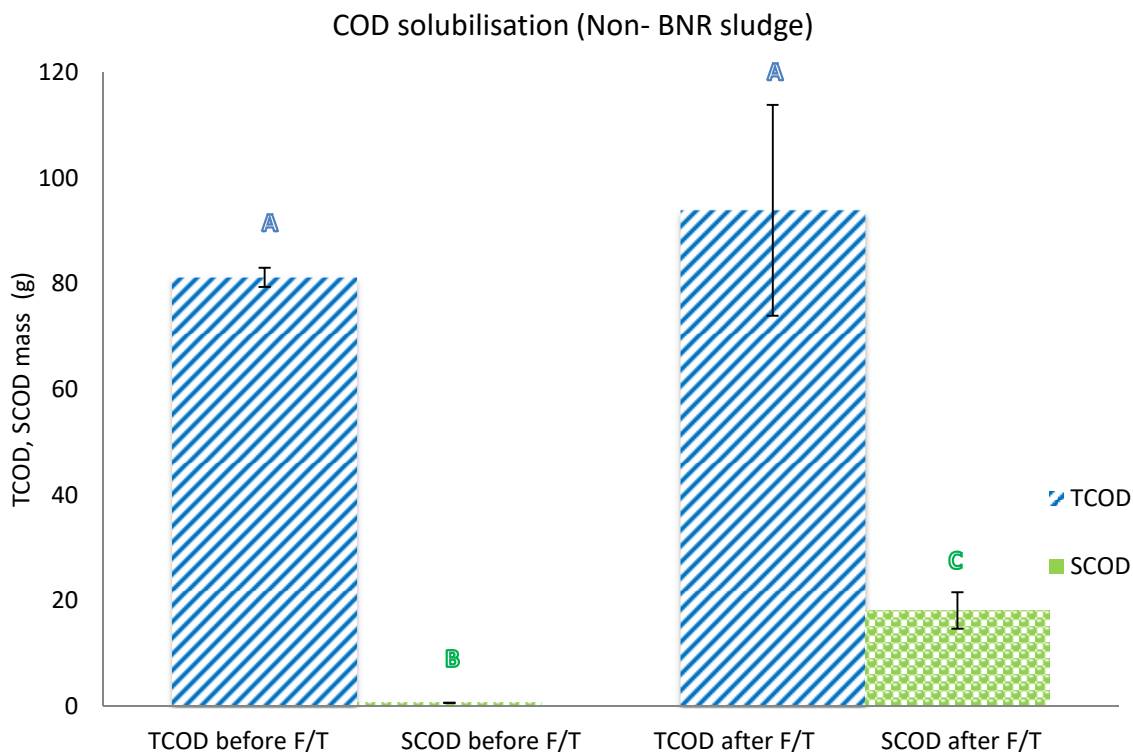


Fig. 3.9: Non-BNR sludge solubilisation of organics

The increase of soluble COD after F/T treatment matches those of previous findings about the release of organic and nutrient contents of activated sludge to the supernatant due to cell lysis during the F/T process. The same observations of COD release were reported by other freeze/thaw studies demonstrating the breakdown of organic particulates in activated sludge flocs into simpler compounds (Diak et al., 2011; Hu et al., 2011; Montusiewicz et al., 2010). However, Hu (2011) observed an increase in COD

solubilisation with increasing curing time (1.6 and 10.5% after 3 h and 72 h), similar to what was observed for nitrogen release, and reported the release of these two parameters mainly depended on the curing time. Further investigation is needed in order to find the ideal conditions for organics release.

The results of COD solubilisation in the current study are comparable with the thermal treatment of activated sludge at 121°C for 30 min that resulted in 17.6% COD solubilisation (Jeongsik et al., 2003). In addition, Bougrier (2008) reported the COD release of about 20% by thermal treatment of activated sludge at a temperature of 100°C (Bougrier, Philippe Delegenes, & Carrere, 2008). In fact, this suggests that freezing/thawing has the same effect as thermal treatment in terms of solubilizing of COD. Gao (2010) has proven that the effect of one cycle of freeze/thaw of activated sludge was the same as the thermal treatment at 103°C for 30 min in solubilizing COD (Gao, 2011).

The SCOD increase after F/T treatment is directly correlated with the reduction of the volatile solids in sludge. According to tables 3.3 and 3.4, the average mass of volatile solid of the sludge prior to F/T was 58.07 ± 2 g, and was reduced to 43.93 ± 4.4 g through the F/T treatment, resulting in 24.5 ± 5.27 % decrease.

Comparing two different types of sludge from BNR and non-BNR plants, it was demonstrated that F/T affected both sludge similarly ($p=0.1258$). The amount of organics

in BNR and non-BNR sludge were 1.5 ± 0.12 and 1.4 ± 0.07 (TCOD/VS g/g) respectively.

3.2.4 Benefits of nutrient release

The released soluble nitrogen and phosphorus in the effluent are in the form of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$, respectively which are ready for uptake by plants, comparable to mineral fertilizers. The ratio of soluble nitrogen to soluble phosphorus for the non-BNR sludge was about 2.3 ± 0.3 . Since all current operating wastewater treatment plants in First Nation Communities are performing conventional treatment (Burnside, 2011), the characteristic of their produced sludge are similar to the non-BNR sludge used in the current study. Therefore, applying sludge freeze/thaw treatment in remote Northern Communities would likely result in similar solubilisation ratios.

Table 3.5: General fertilizer recommendation without a soil test. Adapted from (Manitoba Soil Fertility Guide, 2007)

Crops	N (lb/ac)	P (lb/ac)	Average N/P
Durum wheat	55-90	30-40	2.07
Rye	40-65	30-40	1.50
Oats	55-90	30-40	2.07
Triticale	40-65	30-40	1.50
Canola/rapeseed	70-90	30-40	2.29
Sunflowers	55-90	30-40	2.07
Corn	65-135	30-40	2.86
Potatoes	60-90	45-55	1.50
Field beans	40-90	30-40	1.86

According to Manitoba Soil Fertility guideline the recommended fertilizer ratio for some crops without soil tests are presented on Table 3.5. As a result, there is a potential for using the collected water from F/T treatment directly for agricultural purpose, specifically

for wheat, oats, canola and sunflowers. However, exact fertilizer demands for crops vary depending on the soil type, pH and nutrient availability. Although the N/P ratio of the effluent is suitable, the overall concentrations of N and P in the effluent are low (less than 0.1%), and would need to be amended with additional mineral nutrients to be used as a fertilizer in agriculture or gardening applications. Overall, the effluent from F/T has the potential to be used for irrigation or for nutrient recovery. However, further investigations about pathogenic activity (fecal coliform density) are necessary to allow direct usage of the effluent for food crops (i.e greenhouse applications).

3.2.5 Trend of nutrient release during thawing

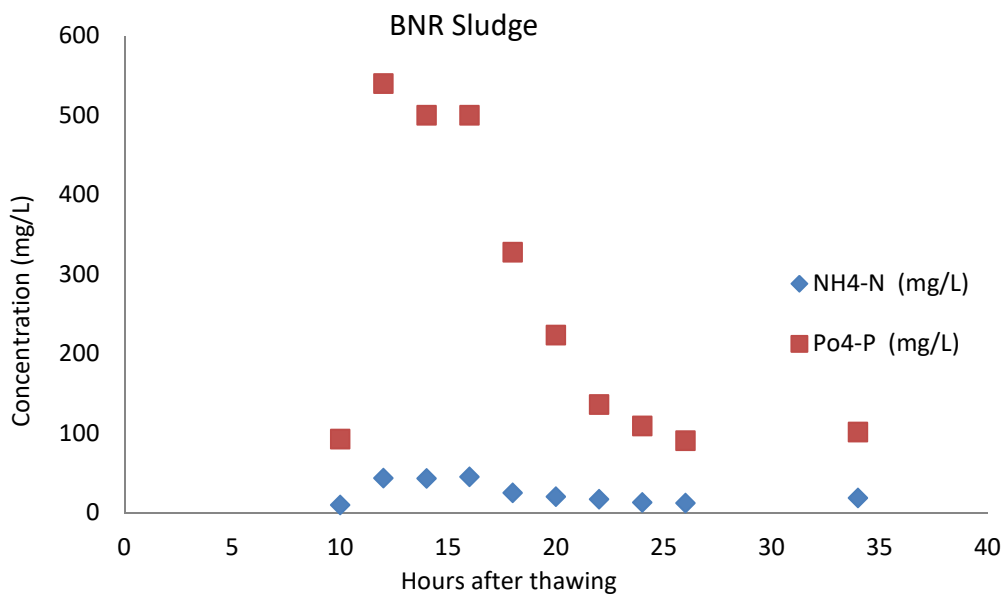
Tests were done to observe time-based trends of nutrient release into the effluent water during thawing. Characteristics of fresh sludge for both BNR and non-BNR are presented in Table 3.6 for these tests. Fig. 3.10 (a and b) demonstrates the concentration of $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ in the effluent for BNR and non-BNR sludge at different times during thawing.

The concentration of nutrients increased during the first hours of thawing and reached its maximum after 16 hours of thawing for both BNR and non-BNR sludge for both ammonium and orthophosphate. This indicated that the majority of nutrients were released during the first 24 hour of thawing. Moreover, the graphs represent the differences of soluble nutrient levels for two kinds of sludge.

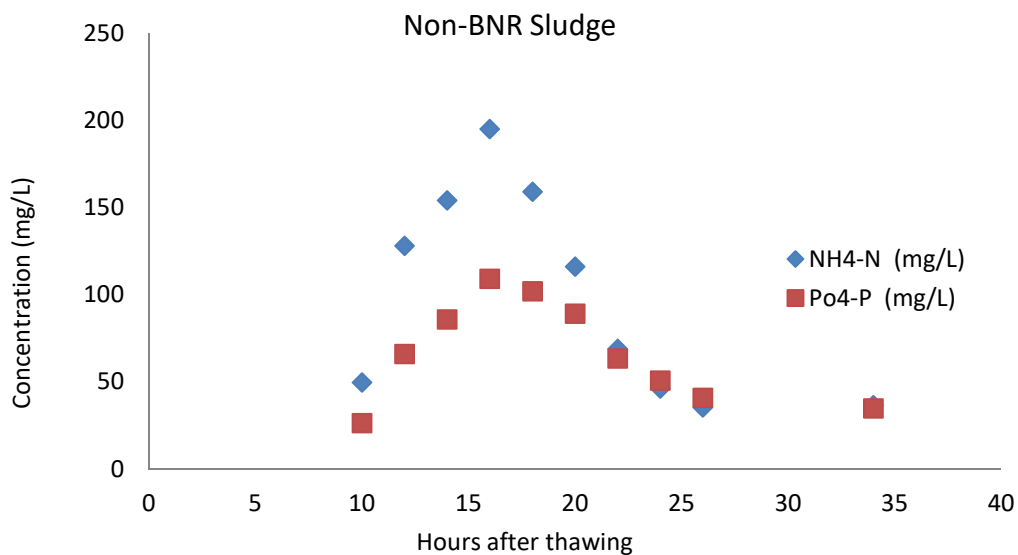
Table 3.6: Characteristic of BNR and non-BNR fresh sludge for hourly test.

	TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	VS (g/L)
BNR	702	288	11610.6	3.27	12	68.66	10.21	7.89
Non-BNR	716	130	9973.6	49.4	9.95	103.13	8.50	7.1

The BNR sludge showed less release of NH₄-N compared with PO₄-P, whereas non-BNR had the higher amount of ammonium released compared with orthophosphate. These results are consistent with the last finding presented in sections 3.2.1 and 3.2.2 regarding the type of treatment and presence of different microorganism in each kind of sludge.



(a)



(b)

Fig. 3.10: Hourly trend of nutrient release for BNR and non-BNR sludge during thawing.

To investigate if any additional nutrient release occurs when the effluent is stored for longer periods of time, a second thawing test was conducted (Fig. 3.11). The collected effluent was left at room temperature for a total duration of 96 hours and sampled every 24 hours.

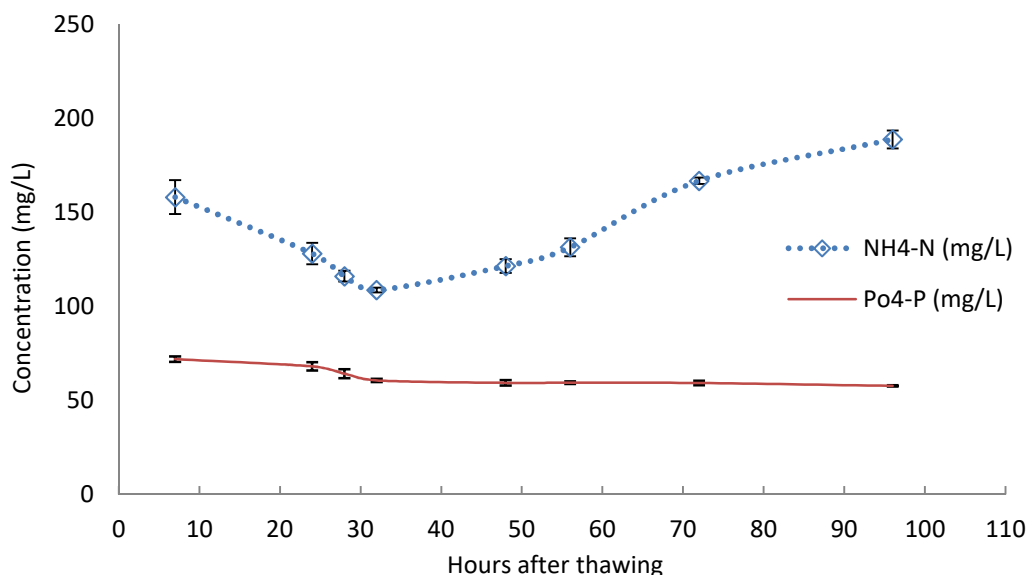


Fig. 3.11: Change in nutrients after thawing

The first sample was taken after seven hours of thawing, with a maximum concentration of 158 ± 9 and 72 ± 1.5 mg/L for $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$, respectively. Then, the concentration decreased to 128 ± 5.7 and 68 ± 2.2 after 24 hours; 121.3 ± 3.7 and 59.3 ± 1.5 after 48 hours when the thawing and dewatering was complete. The next samples were taken after 72 hours and 96 hours, respectively. These samples showed an increase of $\text{NH}_4\text{-N}$, although the $\text{PO}_4\text{-P}$ remained constant. The N/P ratio increased from 1.88 ± 0.02 (after 24 hours) to 3.27 ± 0.1 (after 96 hours). According to previous results presented in Tables 3.3 and 3.4, the majority of the phosphorus in the effluent after thawing is in the form of soluble phosphorus (70%). However, less than 50% of organic nitrogen is solubilized to $\text{NH}_4\text{-N}$ during thawing. The increase in $\text{NH}_4\text{-N}$ during longer-term effluent storage at room temperature indicates that additional hydrolysis of organic nitrogen occurs during this time. This would suggest that extended storage of the effluent

could substantially change the ammonium to orthophosphate ratio, and an updated mineral analysis is necessary prior to application of the water to crops.

3.3 Effects of freeze/thaw treatment on sludge digestibility and biogas production

A biochemical methane potential test was carried out in order to evaluate the digestibility of sludge and methane yield before and after freeze/thaw treatment. Fresh activated sludge before freezing and sludge cake post F/T treatment was considered as a substrate and anaerobically digested sludge from a full scale digester were used as an inoculum. The inoculum and substrate were mixed together at a ratio of 1/2 based on their VS content. The volatile solids and volume of substrate and inoculum before and after F/T treatment are presented in Tables 3.7 and 3.8. Results from the respirometer were corrected for standard conditions (0°C and 1 atm). Also CO₂ and CH₄ percentages from the GC measurements were corrected to 100% and then used for the calculation. The net biogas produced from the substrate was calculated by subtracting the gas production of a control bottle (without addition of substrate) from the treatment bottles. The net biogas production was multiplied by the CH₄% to provide the substrate methane production potential. All the numbers were normalized based on the VS of substrate added to the bottles and the mL CH₄ /g VS were calculated for both fresh sludge and the sludge cake post freeze/thaw treatment. Fig. 3.12 illustrates the normalized CH₄ of both sludge samples. Detailed calculations are presented in Appendix C.

Table 3.7: Volume and volatile solid of fresh sludge and digested sludge. July, 2015.

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	12	6.7	100	320	1.2	2.14	1/1.8
2	12	6.7	100	320	1.2	2.14	1/1.8
3	12	6.7	100	320	1.2	2.14	1/1.8
Blank	12	0	100	0	Filled with DI water		

Table 3.8: Volume and volatile solid of post F/T sludge cake and digested sludge. July, 2015

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	13	83	320	100	4.2	8.3	1/1.9
2	13	83	320	100	4.2	8.3	1/1.9
3	13	83	320	100	4.2	8.3	1/1.9
Blank	13	0	Filled with DI water				

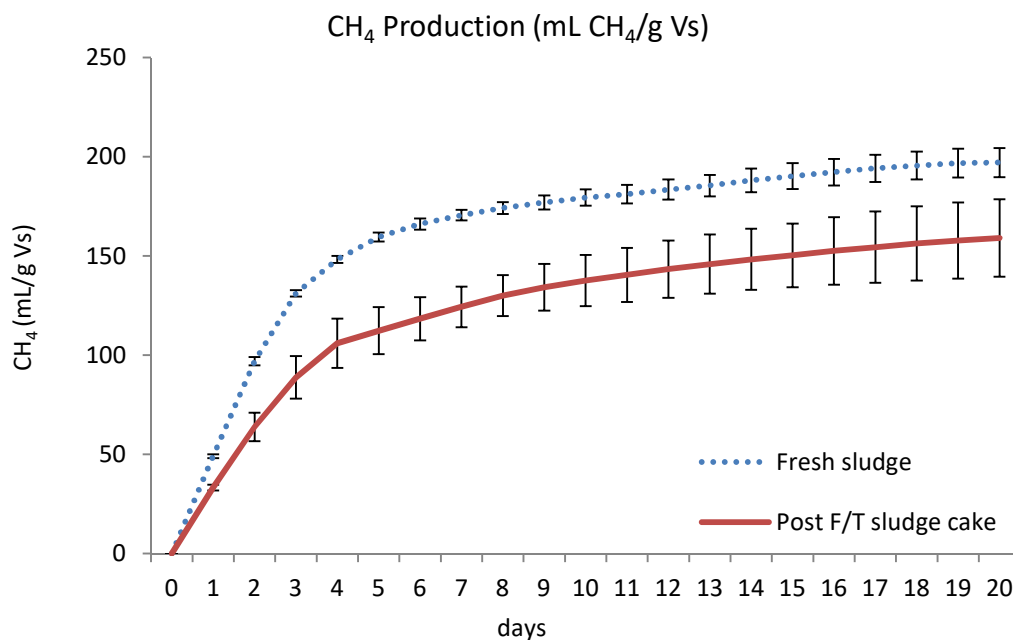


Fig. 3.12: Normalized accumulated methane production of sludge before and after treatment, July, 2015

The accumulated methane produced after 20 days of running the respirometer for fresh sludge and for post F/T sludge cake were 197 ± 7.35 and 159 ± 19.5 (mL/g VS), respectively. The result of paired t test showed no significant change in methane yield before and after treatment ($p = 0.17$). According to previously presented analysis on COD solubilisation (table 3.3) about 25 % of the input TCOD enters the thaw water mostly in the form of soluble COD and 75 % is left in the sludge cake. Therefore, the post treatment F/T sludge cake contained less COD for gas production and considering the stoichiometric relationship of $0.35 \text{ L CH}_4/\text{g COD}$ theoretically 25% less methane would be expected. Accumulated methane yield for post F/T sludge cake was about 20% less than the fresh sludge, indicating no improvement in sludge cake biodegradability after F/T treatment despite the cell disruption by freeze/thaw.

A second batch of BMP tests were conducted to evaluate the methane potential of post freeze/thaw sludge cake mixed with the effluent collected water. The same portion (one-fifth) of sludge cake and effluent water was mixed and TS and VS of mixture were measured. The same ratio of inoculum/substrate as fresh sludge ($I/S = 1/1.9$) was chosen for the BMP test. Tables 3.9 and 3.10 present the detailed information on the batch test based on VS. The normalized methane yield of fresh sludge and post F/T sludge cake mixed with the effluent is presented in Fig. 3.13.

Table 3.9: Volume and volatile solid of fresh sludge and digested sludge. Oct, 2015.

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	10.30	6.20	100	320	1.03	1.98	1/1.9
2	10.30	6.20	100	320	1.03	1.98	1/1.9
3	10.30	6.20	100	320	1.03	1.98	1/1.9
Blank	10.30	0	100	0	Filled with DI water		

Table 3.10: Volume and volatile solid of post F/T sludge cake and digested sludge. Oct, 2015.

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	11	6.5	100	320	1.1	2.08	1/1.9
2	11	6.5	100	320	1.1	2.08	1/1.9
3	11	6.5	100	320	1.1	2.08	1/1.9
Blank	11	0	100		Filled with DI water		

The average accumulated methane potential after 20 days of incubation was 212 ± 8 and 194 ± 8.4 mL/g VS for fresh sludge and post F/T treatment, respectively. The result of paired t-test showed no significant differences ($p = 0.23$). Presence of SCOD in the effluent water after F/T treatment indicated the disintegration of activated sludge microorganism cells and release of intracellular materials. This disruption and release of COD was expected to change methane production during anaerobic digestion. However, comparing the raw sludge and F/T treated sludge plus effluent, no improvements in the accumulated methane yield was observed after treatment. This finding was contrary to what was reported by Montusiewicz et al (2010), who observed a 1.5 times increase of biogas yield from F/T treated sludge. However, this could be attributed to the usage of a mixture of primary and waste activated sludge at the ratio of 60/40 (primary/waste). Primary sludge consists mainly of easily biodegradable organic polymers such as

proteins, lipids and carbohydrates that could break down faster, increase the hydrolysis step and result in higher biogas production after F/T treatment.

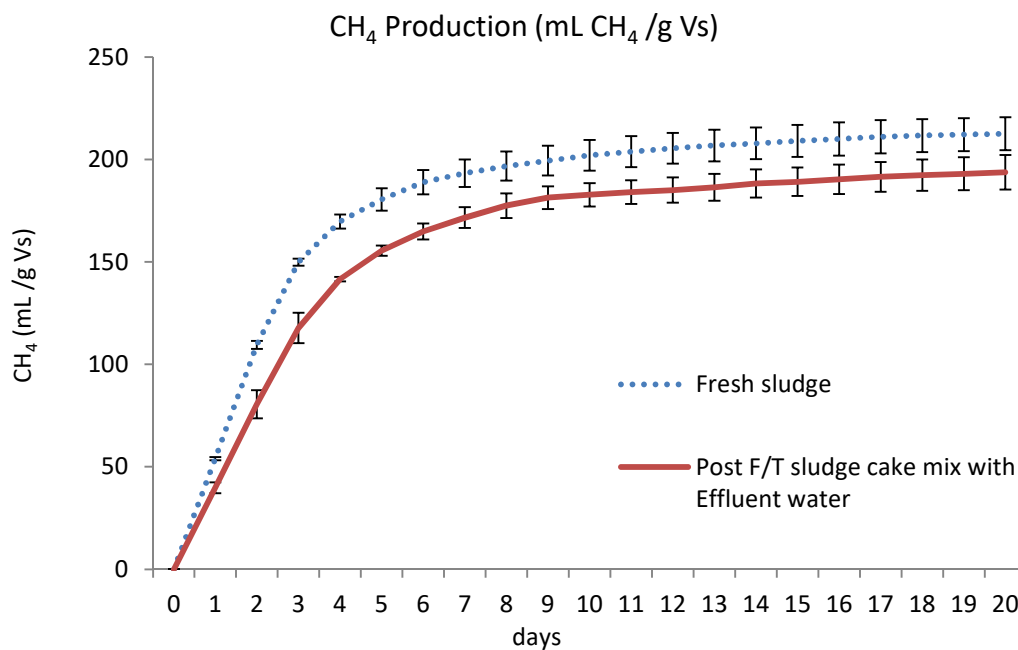


Fig. 3.13: Normalized methane production of sludge before and after treatment. Oct, 2015

Appendix D shows the results for the freeze/thaw treatment as well as the BMP of raw sludge and sludge cake with the BNR/chemical sludge. This substrate is not comparable to the ones presented above, as the high content of ferric chloride for chemical treatment substantially affects sludge characteristics and treatment behavior.

Chapter 4

Conclusion and Recommendations

4.1 Conclusion

Natural sludge dewatering was studied and freeze/thaw treatment was carried out as a sludge dewatering and conditioning method to explore a practical, low cost natural sludge treatment for remote and cold regions. The main objective of the study was to dewater the sludge using natural freezing winter conditions in Northern Communities. This was done successfully through a lab-scale freezing bed. Freeze/thaw treatment experiments showed that freezing can reduce the sludge moisture by more than 85 %. In addition, the method was effective to agglomerate small particles and improve sludge dewaterability. Sludge solid concentration was increased by approximately 10 times and the compacted form of the remaining sludge cake after dewatering was removed easily from the bed. Advantages of sludge volume reduction include lowering transportation and disposal costs and energy consumption. Furthermore, the compacted and concentrated sludge cake is easier to remove, transport and dispose.

The study showed that freeze/thaw is effective in solubilizing complex insoluble organic compounds of activated sludge into soluble ones. Freezing can cause disruption in the living microorganisms in activated sludge and result in the release of intracellular and extracellular materials of cells to the supernatant. Solubilisation of about 15.2% of organic nitrogen, 33.5% of organic phosphorus and 21.5% of total COD were achieved, respectively, for the activated sludge from a non-BNR treatment plant. For the sludge from a BNR treatment plant 6.31%, 80% and 16.5% were observed for nitrogen, phosphorus and COD solubilisation. The effluent water collected after thawing has the potential to be used for agriculture purposes, primarily in irrigation systems or it can be recycled back to the treatment plant.

In addition, the effectiveness of the treatment on sludge biogas production was evaluated by monitoring biogas and methane production from fresh sludge and post freeze/thaw sludge cake by anaerobic digestion processes. The results indicated that no improvement in methane yield can be expected for the type of sludge tested in this research (secondary waste active sludge).

4.2 Recommendation

The majority of the studies about sludge freeze/thaw treatment focused on sludge dewaterability rates and the effect of cycling of freezing and thawing. There were limited studies on the effects of sludge freeze/thaw at different freezing rates or temperature and curing time on organic and nutrient solubilisation. The current study examined sludge F/T treatment at fixed temperature and currying times (-12 °C and one week each layer). However, in view of the northern winter climate and lengthy freezing conditions, further research is required to establish the effects of lower temperatures and longer curing times on microorganism disruption and solubilisation. Designing a pilot scale freezing bed will be beneficial in order to validate results of this research study and also develop a specific design model applicable for Northern Communities, taking into consideration their natural environmental conditions and community infrastructure.

Moreover, measuring and monitoring microbial activity, in particular fecal coliform density for both post freeze/thaw sludge cake and effluent water will be beneficial in order to find the most efficient end use of both the solid cake and filtrate. This will be a function of specific local environmental regulations, and fertilizer use guidelines.

Finally, the cost estimation of design, construction and operation of the freezing bed and comparison with current sludge treatment, shipping and disposal cost could demonstrate the feasibility of this treatment as a cost effective method for use in northern regions. A comparative life cycle analysis could be conducted to demonstrate the benefits and

drawbacks of the F/T treatment over mechanical dewatering processes or raw sludge disposal.

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Appendices

Appendix A: Pre- test result

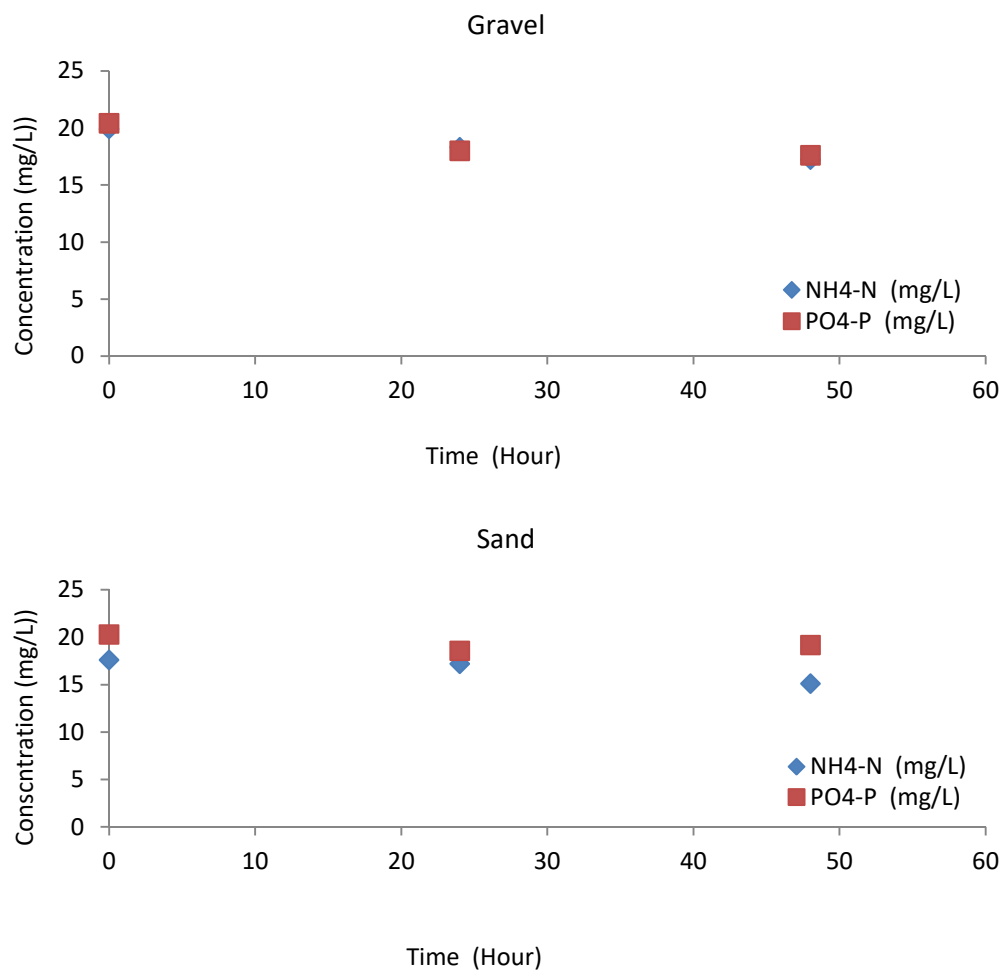


Fig A.1: Sand and gravel test on nutrient absorption during 2 days.

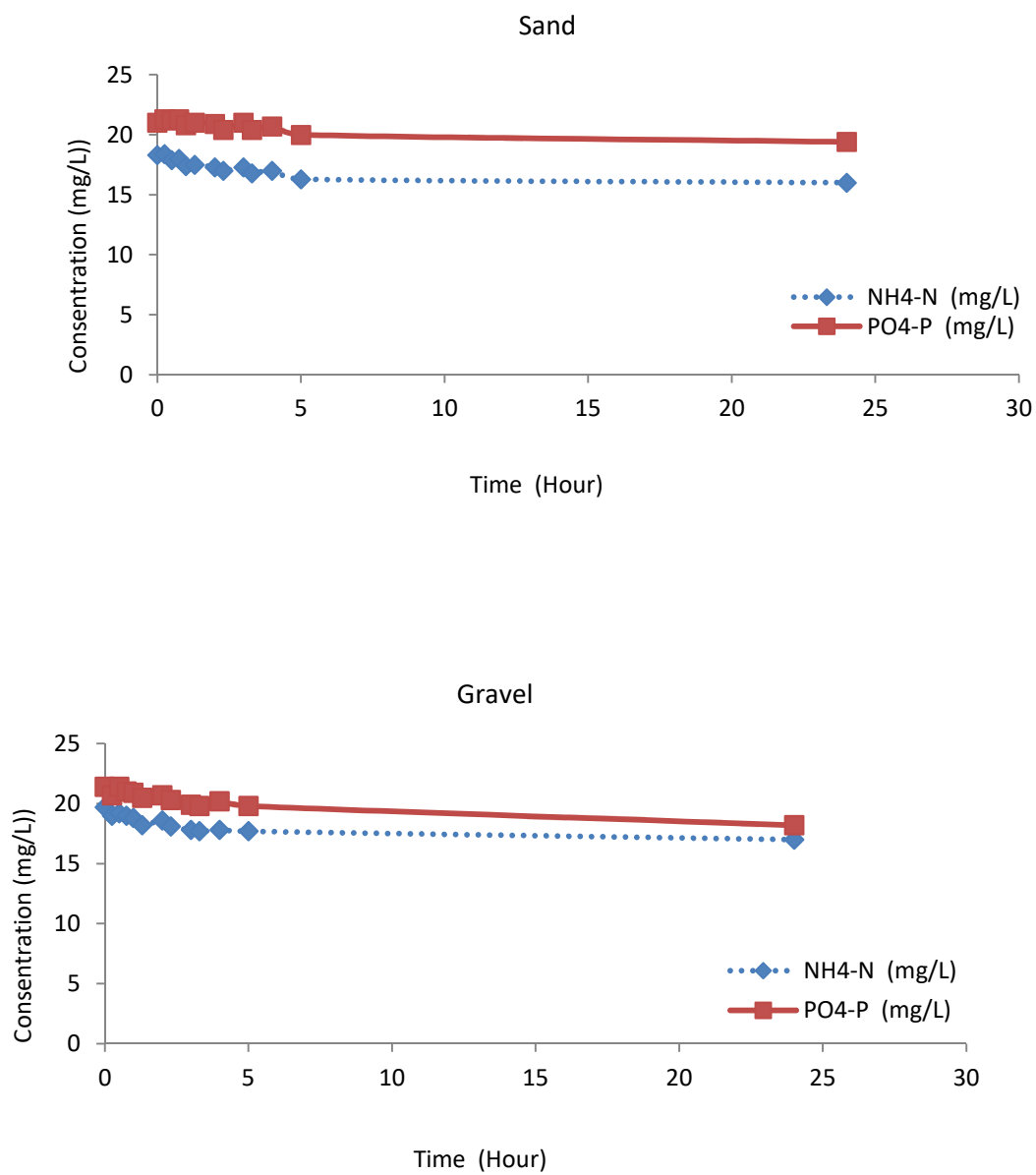


Fig A.2: Sand and gravel test (25 hour)

Appendix B: Detailed results for sludge freeze/thaw and nutrients solubilisation

Table B.1: Characteristic of three layer raw sludge (a),(b), effluent water (c) and sludge cake(d) for BNR plant

BNR Dec,2014		TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	VS (g/L)	Volume (L)
1st Layer Dec 4	Box 1	914	302	11004.4	5.5	23.6	81.86	10.3	8.29	3
	Box2	966	284	10497.6	5.6	20.8	88.37	10.4	8.37	3
	Box 3	952	266	10241.8	5.3	18.9	91.19	10.5	8.46	3
2nd layer Dec 9	Box 1	748	280	16945.4	4.0	11.6	108.24	10.3	8.28	3.3
	Box2	758	308	9512.8	4.2	13.0	80.76	7.1	6.17	3.3
	Box 3	790	304	12312.6	4.1	10.4	84.24	11.1	8.96	3.3
3rd layer Dec 12	Box 1	614	230	9960.2	3.0	8.9	68.87	9.6	7.69	3.3
	Box2	598	220	10509.2	3.1	8.8	74.5	9.6	7.69	3.3
	Box 3	690	238	10109.8	3.1	8.6	74.25	9.6	7.70	3.3

(a)

BNR Dec,2014		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (g)	Vs (g)	Volume (L)
Raw sludge	Box 1	7.24	2.59	121.80	0.04	0.14	0.83	96.57	72.79	9.60
	Box 2	7.37	2.59	97.57	0.04	0.13	0.78	86.31	66.71	9.60
	Box 3	7.74	2.59	104.72	0.04	0.12	0.80	99.81	75.36	9.60

(b)

BNR Dec,2014		NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	Box 1	0.48	1.77	16.39	7.00
	Box 2	0.53	2.34	20.18	7.50
	Box 3	0.51	2.49	18.36	7.20

(c)

BNR Dec,2014		TS %
Sludge cake	Box 1	10.17
	Box 2	9.40
	Box 3	10.22

(d)

Table B.2: Characteristic of three layer raw sludge (a),(b),effluent water (c) and sludge cake(d) for non-BNR plant. Feb, 2015

South Feb 2015		TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	Vs (g/L)	Volume (L)
1st Layer Feb 19	Box 1	744.0	115.0	10117.2	46.40	8.10	73.81	7.64	6.77	3.60
	Box2	772.0	117.0	9384.0	46.65	7.55	88.16	7.83	6.83	3.70
	Box 3	728.0	115.0	9595.4	46.90	7.52	77.22	7.81	6.81	3.75
2nd layer Feb 26	Box 1	700.0	119.0	11012.2	44.50	6.69	65.34	7.79	6.71	4.00
	Box2	698.0	117.0	9323.8	45.45	6.75	69.54	7.79	6.70	4.00
	Box 3	796.0	116.0	9263.6	45.65	6.59	70.70	7.95	6.74	4.00
3rd layer Mar 5	Box 1	680.0	124.0	9668.4	47.00	8.53	52.31	8.52	7.21	4.00
	Box2	666.0	122.0	9177.6	46.10	8.13	46.79	8.36	7.05	4.00
	Box 3	682.0	124.0	9032.6	45.85	8.08	49.59	8.46	7.11	4.00

(a)

Non-BNR Feb,2015		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -p (g)	SCOD (g)	TS (g)	Vs (g)	Volume (L)
Raw Sludge	Box 1	8.20	1.39	119.14	0.53	0.09	0.74	92.74	80.06	11.60
	Box 2	8.31	1.39	108.73	0.54	0.09	0.79	93.57	80.27	11.70
	Box 3	8.64	1.39	109.17	0.54	0.09	0.77	94.91	80.97	11.75

(b)

Non-BNR Feb,2015		NH ₄ - N (g)	Po ₄ - P (g)	SCOD (g)	Volume (L)
Effluent water	Box 1	2.06	0.90	29.79	9.60
	Box 2	1.93	0.93	30.68	9.50
	Box 3	1.97	0.83	28.44	9.4

(c)

Non- BNR Feb,2015		TS %
Sludge cake	Box 1	9.36
	Box 2	NA
	Box 3	9.73

(d)

Table B.3: Characteristic of three layer raw sludge (a), (b), effluent water (c) and sludge cake(d) for non-BNR plant. July,2015

South July 2015		TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	Vs (g/L)	Volume (L)
1st Layer July 9	Box 1	653.90	124.30	8806.8	38.20	9.28	74.56	8.32	6.76	3.00
	Box 2	639.60	112.60	9010.2	37.20	9.78	79.84	8.04	6.62	3.00
	Box 3	645.48	130.50	9537.4	37.60	9.64	66.61	8.06	6.63	3.00
2nd layer July 15	Box 1	655.59	127.38	9331.4	38.35	11.50	58.76	8.10	6.69	3.00
	Box 2	647.86	126.23	9247.8	38.30	11.60	67.88	8.10	6.66	3.00
	Box 3	640.22	124.55	8778.4	38.20	11.30	67.55	8.00	6.73	3.00
3rd layer July 21	Box 1	651.24	130.78	8072.0	39.55	13.40	72.90	7.80	6.63	3.00
	Box 2	620.40	128.83	9021.2	39.05	14.10	72.20	8.00	6.80	3.00
	Box 3	662.32	128.93	8316.6	39.65	14.00	70.11	7.80	6.63	3.00

(a)

Non-BNR July,2015		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (g)	Vs (g)	Volume (L)
Fresh Sludge	Box 1	5.88	1.15	78.63	0.35	0.10	0.62	72.65	60.25	9.00
	Box 2	5.72	1.10	81.84	0.34	0.11	0.66	72.42	60.25	9.00
	Box 3	5.84	1.15	79.90	0.35	0.10	0.61	71.57	59.97	9.00

(b)

Non-BNR July,2015		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	Box 1	2.63	0.76	20.13	1.13	0.52	14.33	7.53
	Box 2	2.32	0.97	19.86	1.05	0.55	15.23	7.58
	Box 3	2.42	0.76	19.88	1.09	0.54	15.13	7.63

(c)

Non-BNR July,2015		TN (g)	TP (g)	TCOD (g)	TS %	Vs %
Sludge cake	Box 1	4.99	1.32	98.49	9.43	7.84
	Box 2	5.61	1.42	94.70	9.49	7.91
	Box 3	5.91	1.49	87.49	9.94	8.32

(d)

Table B.4: Characteristic of three layer raw sludge (a),(b), effluent water (c) and sludge cake(d) for non-BNR plant. Oct, 2015.

South Oct, 2015		TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	Vs (g/L)	Volume (L)
1st Layer Oct 28	Box 1	792.0	199.00	8806.0	43.20	11.20	51.40	7.40	6.20	3.00
	Box2	780.0	206.00	9275.0	44.10	10.40	61.16	7.50	6.30	3.00
	Box 3	804.6	220.50	8885.0	44.45	10.70	64.58	7.50	6.40	3.00
2nd layer Nov 4	Box 1	835.0	131.40	9359.6	38.55	9.08	110.14	7.20	6.09	3.00
	Box2	669.6	132.68	8985.0	40.70	8.75	80.13	7.45	6.20	3.00
	Box 3	776.2	142.91	8527.8	38.95	8.35	67.78	7.40	6.20	3.00
3rd layer Nov 10	Box 1	852.8	206.40	9183.4	46.10	8.32	62.94	7.50	6.20	3.00
	Box2	806.0	280.20	9869.0	43.85	7.97	57.79	7.40	6.20	3.00
	Box 3	756.4	225.60	9425.2	44.20	7.77	54.01	7.38	6.20	3.00

(a)

Non-BNR Oct,2015		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	TS (g)	Vs (g)	Volume (L)
Fresh sludge	Box 1	7.44	1.61	82.05	0.38	0.09	0.67	66.30	55.47	9.00
	Box 2	6.77	1.86	84.39	0.39	0.08	0.60	67.05	56.10	9.00
	Box 3	7.01	1.77	80.51	0.38	0.08	0.56	66.84	56.40	9.00

(b)

Non-BNR Oct,2015		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	Box 1	2.85	0.73	18.57	1.69	0.54	20.70	7.60
	Box 2	2.90	0.70	18.56	1.59	0.53	19.66	7.60
	Box 3	2.99	0.91	21.90	1.59	0.70	23.70	7.50

(c)

Non-BNR Oct,2015		TN (g)	TP (g)	TCOD (g)	TS %	Vs %
Sludge cake	Box 1	5.15	0.98	54.54	9.10	7.00
	Box 2	5.23	0.83	53.40	8.60	7.15
	Box 3	5.18	0.73	55.96	9.20	7.00

(d)

Appendix C: Detailed results for sludge freeze/thaw and biogas production

Table C.1: Biogas and methane gas production. Batch test July, 2015. Fresh sludge

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Substrate CH ₄ 1 (mL)	Accumulated Biogas2 (mL)	Substrate Biogas2 (mL)	Substrate CH ₄ 2 (mL)	Accumulated Biogas3 (mL)	Substrate Biogas 3 (mL)	Substrate CH ₄ 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	202.75	156.38	102.43	210.67	163.35	105.36	211.51	164.09	107.15	25.04
2	383.13	306.85	200.98	404.90	326.00	210.27	401.68	323.17	211.03	34.44
3	520.41	421.96	276.38	535.63	435.35	280.80	536.62	436.22	284.85	40.91
4	591.04	479.78	314.26	600.69	488.28	314.94	607.61	494.37	322.82	45.83
5	636.66	515.72	337.79	646.15	524.07	338.02	657.03	533.64	348.47	50.62
6	663.35	535.07	350.47	675.54	545.79	352.04	688.42	557.13	363.80	55.32
7	686.78	549.47	359.90	700.52	561.56	362.21	711.39	571.13	372.95	62.38
8	702.03	560.43	367.08	716.40	573.07	369.63	729.47	584.58	381.73	65.18
9	714.45	568.10	372.10	729.27	581.14	374.84	746.23	596.07	389.23	68.88
10	726.07	574.90	376.56	740.83	587.89	379.19	762.63	607.08	396.42	72.77
11	731.16	579.38	379.49	745.38	591.90	381.77	771.69	615.05	401.63	72.77
12	740.70	586.66	384.26	753.80	598.19	385.83	783.83	624.62	407.87	74.04
13	751.36	592.90	388.35	763.26	603.37	389.17	796.60	632.71	413.16	77.61
14	762.18	600.67	393.44	773.04	610.23	393.60	811.28	643.88	420.45	79.60
15	769.65	606.61	397.33	780.14	615.84	397.22	823.24	653.77	426.91	80.32
16	779.03	612.75	401.35	789.74	622.18	401.30	834.11	661.22	431.78	82.72
17	787.56	618.52	405.13	798.84	628.44	405.35	843.67	667.89	436.13	84.70
18	794.05	622.32	407.62	805.85	632.70	408.09	852.00	673.31	439.67	86.87
19	802.98	625.64	409.79	815.31	636.49	410.53	863.19	678.62	443.14	92.03
20	807.05	626.28	410.21	819.59	637.31	411.07	868.04	679.95	444.01	95.37

Table C.2: GC analysis, July, 2015, Fresh sludge

July,2015	Biogas % from GC			Corrected to 100%	
Fresh sludge	CO ₂	N ₂	CH ₄	CO ₂	CH ₄
1	33.43	3.09	63.47	34.50	65.50
2	34.70	2.28	63.02	35.51	64.49
3	34.14	1.72	64.14	34.73	65.27
Blank	11.83	65.27	22.90	34.05	65.95

Table C.3: Normalized methane yield. Batch test July, 2015. Fresh sludge

Days	CH ₄ (mL/g VS) 1	CH ₄ (mL/g VS) 2	CH ₄ (mL/g VS) 3	Mean	STD
0	0.00	0.00	0.00	0.00	0.00
1	47.87	49.24	50.07	49.06	0.91
2	93.92	98.26	98.61	96.93	2.13
3	129.15	131.22	133.11	131.16	1.62
4	146.85	147.17	150.85	148.29	1.82
5	157.85	157.95	162.84	159.55	2.33
6	163.77	164.50	170.00	166.09	2.78
7	168.18	169.26	174.27	170.57	2.66
8	171.53	172.73	178.38	174.21	2.99
9	173.88	175.16	181.88	176.97	3.51
10	175.96	177.19	185.24	179.47	4.12
11	177.33	178.40	187.68	181.14	4.65
12	179.56	180.30	190.60	183.48	5.04
13	181.47	181.86	193.07	185.46	5.38
14	183.85	183.92	196.47	188.08	5.93
15	185.67	185.62	199.49	190.26	6.53
16	187.55	187.53	201.77	192.28	6.71
17	189.31	189.41	203.80	194.18	6.81
18	190.47	190.70	205.46	195.54	7.01
19	191.49	191.84	207.07	196.80	7.27
20	191.69	192.09	207.48	197.08	7.35
CH ₄ %	65.5	64.5	65.3		

Table C.4: Biogas and methane gas production. July, 2015. Post freeze/thaw sludge cake

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Substrate CH ₄ 1(mL)	Accumulated Biogas 2 (mL)	Substrate Biogas2 (mL)	Substrate CH ₄ 2 (mL)	Accumulated Biogas 3 (mL)	Substrate Biogas 3 (mL)	Substrate CH ₄ 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	581.36	410.09	266.56	610.66	435.87	262.83	564.80	395.52	253.92	115.35
2	1173.73	870.82	566.03	1090.59	797.65	480.99	990.62	709.68	455.61	184.17
3	1618.25	1220.31	793.20	1495.99	1112.72	670.97	1339.48	974.99	625.94	231.54
4	1922.04	1455.86	946.31	1764.72	1317.42	794.41	1603.85	1175.86	754.90	267.65
5	2020.21	1518.19	986.82	1898.21	1410.83	850.73	1722.67	1256.35	806.58	295.00
6	2097.86	1564.80	1017.12	2043.18	1516.68	914.56	1834.38	1332.94	855.74	319.68
7	2164.83	1604.94	1043.21	2195.56	1631.99	984.09	1933.77	1401.61	899.83	341.03
8	2217.78	1632.36	1061.04	2365.91	1762.72	1062.92	2022.74	1460.73	937.79	362.82
9	2266.97	1656.20	1076.53	2507.10	1867.52	1126.11	2081.81	1493.26	958.67	384.92
10	2311.53	1676.88	1089.97	2614.20	1943.23	1171.77	2136.17	1522.56	977.49	405.99
11	2346.45	1694.12	1101.18	2696.92	2002.54	1207.53	2187.36	1554.12	997.75	421.31
12	2385.78	1715.21	1114.89	2779.09	2061.32	1242.98	2236.69	1584.01	1016.93	436.68
13	2428.39	1739.86	1130.91	2842.34	2104.13	1268.79	2282.26	1611.26	1034.43	451.28
14	2458.93	1758.40	1142.96	2904.27	2150.30	1296.63	2323.75	1639.44	1052.52	460.75
15	2483.77	1772.74	1152.28	2956.77	2188.98	1319.96	2356.10	1660.39	1065.97	469.29
16	2506.36	1784.89	1160.18	3024.70	2241.03	1351.34	2389.35	1681.92	1079.79	478.08
17	2527.09	1795.23	1166.90	3085.22	2286.39	1378.69	2417.43	1698.73	1090.58	487.06
18	2545.96	1807.03	1174.57	3134.68	2325.10	1402.04	2443.75	1717.08	1102.37	492.52
19	2564.35	1817.50	1181.37	3176.13	2355.87	1420.59	2467.49	1732.26	1112.11	499.01
20	2582.83	1828.16	1188.30	3209.62	2379.73	1434.98	2491.91	1748.15	1122.31	505.38

Table C.5: GC analysis, July, 2015, Post freeze/thaw sludge cake

July,2015	Biogas % from GC			Corrected to 100%	
	CO ₂	N ₂	CH ₄	CO ₂	CH ₄
1	34.53	1.53	63.94	35.07	65.00
2	37.60	1.72	60.67	39.71	60.30
3	35.64	0.47	63.88	35.81	64.20
Blank	28.36	4.69	66.89	29.78	70.20

Table C.6: Normalized methane yield. Batch test July, 2015. Post freeze/thaw sludge cake

Days	CH ₄ (mL/gVS) 1	CH ₄ (mL/gVS) 2	CH ₄ (mL/gVS) 3	Mean	STD
0	0	0	0	0	0
1	34.57	33.91	31.23	33.24	1.44
2	73.41	62.06	56.04	63.84	7.20
3	102.88	86.57	76.99	88.81	10.68
4	122.73	102.50	92.85	106.03	12.45
5	127.99	109.77	99.21	112.32	11.88
6	131.92	118.00	105.25	118.39	10.88
7	135.30	126.97	110.68	124.32	10.22
8	137.61	137.15	115.34	130.03	10.38
9	139.62	145.30	117.91	134.28	11.80
10	141.37	151.19	120.23	137.60	12.91
11	142.82	155.81	122.72	140.45	13.61
12	144.60	160.38	125.08	143.35	14.43
13	146.68	163.71	127.23	145.87	14.90
14	148.24	167.30	129.46	148.33	15.45
15	149.45	170.31	131.11	150.29	16.01
16	150.47	174.36	132.81	152.55	17.02
17	151.34	177.89	134.14	154.46	17.99
18	152.34	180.90	135.59	156.28	18.70
19	153.22	183.30	136.79	157.77	19.25
20	154.12	185.15	138.04	159.11	19.55
CH ₄ %	65.0	60.3	64.2		

Table C.7: Biogas and methane gas production. Batch test Oct, 2015. Fresh sludge

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Substrate CH ₄ 1 (mL)	Accumulated Biogas 2 (mL)	Substrate Biogas 2 (mL)	Substrate CH ₄ 2 (mL)	Accumulated Biogas 3 (mL)	Substrate Biogas 3 (mL)	Substrate CH ₄ 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	199.43	153.16	104.76	207.25	160.05	108.35	208.65	161.28	107.57	25.38
2	390.77	309.37	211.61	409.64	325.98	220.69	411.19	327.34	218.34	39.21
3	531.90	428.75	293.27	541.22	436.96	295.82	558.19	451.89	301.41	44.68
4	619.11	501.14	342.78	597.96	482.53	326.67	626.05	507.25	338.34	49.63
5	664.33	538.01	368.00	628.04	506.07	342.61	668.90	542.03	361.54	52.96
6	698.85	562.50	384.75	659.98	528.29	357.66	706.77	569.47	379.84	59.65
7	719.66	575.37	393.55	676.58	537.46	363.86	731.28	585.59	390.59	65.84
8	732.84	586.27	401.01	687.32	546.21	369.79	744.64	596.65	397.97	66.63
9	744.81	595.21	407.13	697.20	553.32	374.60	754.83	604.03	402.89	68.43
10	754.98	600.84	410.98	708.43	559.88	379.04	770.92	614.87	410.12	72.21
11	761.96	606.15	414.61	714.99	564.81	382.38	778.03	620.29	413.73	73.16
12	769.88	609.70	417.03	724.41	569.69	385.68	788.90	626.44	417.83	77.04
13	775.67	613.42	419.58	729.82	573.07	387.97	795.74	631.08	420.93	78.61
14	781.68	616.84	421.92	735.19	575.93	389.90	801.18	634.00	422.88	80.73
15	787.07	620.84	424.66	739.65	579.11	392.06	806.25	637.72	425.36	81.57
16	792.37	622.94	426.09	744.65	580.95	393.30	813.77	641.78	428.06	84.48
17	796.48	626.05	428.21	748.70	584.00	395.37	818.07	645.04	430.24	85.07
18	801.08	627.51	429.21	753.71	585.82	396.60	823.28	647.04	431.58	88.01
19	804.62	628.95	430.20	757.35	587.35	397.64	826.68	648.36	432.46	89.91
20	810.41	629.21	430.38	764.45	588.77	398.60	834.34	650.27	433.73	95.40

Table C.8: GC analysis, Oct 2015, fresh sludge

Oct,2015	Biogas % from GC			Corrected to 100%	
Fresh sludge	CO ₂	N ₂	CH ₄	CO ₂	CH ₄
1	31.04	1.81	67.14	31.62	68.40
2	31.70	1.90	66.39	32.32	67.70
3	32.58	2.10	65.31	33.28	66.70
Blank	9.48	64.99	25.52	27.09	72.91

Table C.9: Normalized methane yield Batch test Oct,2015. Fresh sludge

Days	CH ₄ (mL/g VS) 1	CH ₄ (mL/g VS) 2	CH ₄ (mL/g VS) 3	Mean	STD
0	0.00	0.00	0.00	0.00	0.00
1	52.91	54.72	54.33	53.99	0.78
2	106.87	111.46	110.27	109.53	1.94
3	148.11	149.40	152.23	149.92	1.72
4	173.12	164.99	170.88	169.66	3.43
5	185.86	173.04	182.59	180.50	5.44
6	194.32	180.63	191.84	188.93	5.95
7	198.76	183.77	197.27	193.27	6.74
8	202.53	186.76	200.99	196.76	7.10
9	205.62	189.19	203.48	199.43	7.29
10	207.56	191.43	207.13	202.04	7.50
11	209.40	193.12	208.96	203.82	7.57
12	210.62	194.79	211.03	205.48	7.56
13	211.91	195.94	212.59	206.81	7.69
14	213.09	196.92	213.57	207.86	7.74
15	214.47	198.01	214.83	209.10	7.85
16	215.20	198.64	216.19	210.01	8.05
17	216.27	199.68	217.30	211.08	8.07
18	216.77	200.30	217.97	211.68	8.06
19	217.27	200.83	218.41	212.17	8.04
20	217.36	201.31	219.06	212.58	8.00
CH ₄ %	68.4	67.7	66.7		

Table C.10: Biogas and methane gas production. Oct, 2015. Post freeze/thaw sludge cake mix with effluent

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Substrate CH4 1 (mL)	Accumulated Biogas 2 (mL)	Substrate Biogas 2 (mL)	Substrate CH4 2 (mL)	Accumulated Biogas 3 (mL)	Substrate Biogas 3 (mL)	Substrate CH4 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	155.43	115.81	78.75	158.47	118.48	78.79	178.95	136.51	90.50	23.83
2	299.30	230.82	156.95	306.28	236.96	157.58	358.66	283.05	187.66	37.01
3	432.66	341.05	231.92	448.84	355.29	236.27	501.90	401.98	266.52	45.10
4	538.31	428.60	291.44	559.08	446.87	297.17	556.32	444.44	294.67	51.27
5	597.01	473.99	322.32	622.58	496.50	330.17	602.79	479.08	317.63	58.38
6	627.40	497.68	338.42	667.38	532.86	354.35	637.91	506.92	336.09	61.86
7	652.24	516.78	351.41	700.15	558.94	371.70	661.00	524.49	347.74	64.99
8	679.04	533.01	362.44	734.18	581.53	386.72	687.63	540.57	358.40	73.35
9	700.93	549.08	373.38	748.60	591.03	393.04	703.54	551.38	365.57	76.97
10	711.57	553.34	376.27	759.63	595.63	396.09	713.57	555.10	368.03	82.78
11	721.22	558.68	379.90	767.95	599.80	398.87	719.95	557.56	369.66	86.36
12	726.23	563.09	382.90	772.78	604.05	401.69	721.08	558.55	370.32	86.36
13	733.41	568.46	386.55	779.79	609.28	405.17	724.33	560.47	371.59	87.43
14	741.33	574.17	390.44	788.88	616.02	409.65	731.38	565.42	374.87	88.86
15	748.66	578.11	393.11	794.53	618.47	411.28	735.99	566.96	375.89	91.72
16	755.85	584.43	397.42	798.37	621.85	413.53	737.84	568.59	376.97	91.72
17	768.22	590.48	401.53	806.59	624.25	415.12	745.85	570.79	378.44	97.22
18	772.36	593.89	403.84	810.48	627.43	417.24	747.02	571.59	378.96	97.49
19	775.82	596.93	405.91	814.14	630.65	419.38	747.34	571.87	379.15	97.49
20	779.28	599.98	407.98	818.21	634.23	421.77	748.24	572.66	379.67	97.49

Table C.11: GC analysis, Oct, 2015. Post freeze/thaw sludge cake

Oct,2015	Biogas % from GC			Corrected to 100%	
F/T sludge	CO2	N2	CH4	CO2	CH4
1	31.31	2.15	66.55	31.99	68.00
2	32.77	2.15	65.09	33.49	66.50
3	32.16	4.55	63.29	33.69	66.30
Blank	9.09	74.90	16.00	36.23	63.77

Table C.12: Normalized methane yield. Batch test Oct, 2015. Post F/T sludge cake

Days	CH ₄ (mL/g VS) 1	CH ₄ (mL/g VS) 2	CH ₄ (mL/g VS) 3	Mean	STD
0	0.00	0.00	0.00	0.00	0.00
1	37.86	37.88	43.51	39.75	2.66
2	75.46	75.76	90.22	80.48	6.89
3	111.50	113.59	128.13	117.74	7.40
4	140.12	142.87	141.67	141.55	1.13
5	154.96	158.74	152.71	155.47	2.49
6	162.70	170.36	161.58	164.88	3.90
7	168.95	178.70	167.18	171.61	5.07
8	174.25	185.92	172.31	177.49	6.01
9	179.51	188.96	175.75	181.41	5.56
10	180.90	190.43	176.94	182.75	5.66
11	182.64	191.76	177.72	184.04	5.82
12	184.09	193.12	178.04	185.08	6.20
13	185.84	194.79	178.65	186.43	6.60
14	187.71	196.95	180.23	188.30	6.84
15	189.00	197.73	180.72	189.15	6.95
16	191.07	198.81	181.24	190.37	7.19
17	193.04	199.58	181.94	191.52	7.28
18	194.15	200.60	182.19	192.32	7.63
19	195.15	201.63	182.28	193.02	8.04
20	196.15	202.77	182.54	193.82	8.42
CH ₄ %	68.0	66.5	66.3		

Appendix D: Results for BNR/ Chemical sludge

Table D.1: Characteristic of sludge. Jan, 2016. Fresh sludge

BNR/Chemical sludge	TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	Vs (g/L)
	1411.57	276.25	10953.22	50.65	0.40	68.51	14.85	10.82
STD	329.15	47.48	148.90	4.91	0.43	14.42	2.85	2.15

Table D.2: Three layers of fresh sludge. Jan, 2016.

West Jan 2016		TN (mg/L)	TP (mg/L)	TCOD (mg/L)	NH ₄ -N (mg/L)	Po ₄ -P (mg/L)	SCOD (mg/L)	TS (g/L)	Vs (g/L)	Volume (L)
1st Layer Jan11	Box 1	1774.0	365.3	10671.0	56.00	0.12	74.0	18.3	13.6	3
	Box2	1841.8	306.2	10997.0	53.50	0.06	72.3	18.5	13.8	3
	Box 3	1770.1	279.2	10957.0	55.50	0.10	72.7	18.8	14	3
2nd layer Jan 18	Box 1	1452.0	301.2	10858.0	56.00	0.27	51.0	14.4	9.7	3
	Box2	1444.8	313.0	11016.0	47.40	0.17	48.2	14.45	10.1	3
	Box 3	1432.8	252.2	11275.0	53.50	0.12	51.7	14.4	9.8	3
3rd layer Jan 25	Box 1	941.7	224.7	10936.0	44.15	0.57	79.3	11.7	8.8	3
	Box 2	1072.8	208.0	10949.0	45.15	0.81	72.8	11.4	8.7	3
	Box 3	974.2	236.4	10920.0	44.61	1.41	94.5	11.7	8.9	3

Table D.3: Characteristic of raw sludge (a), effluent water (b) and sludge cake(c).

BNR/chemical Jan,2016		TN (g)	TP (g)	TCOD (g)	NH ₄ - N (g)	Po ₄ -P (g)	SCOD (g)	TS (g)	V _s (g)	Volume (L)
Raw sludge	Box 1	12.50	2.67	97.40	0.47	0.003	0.61	133.20	96.30	9.00
	Box 2	13.08	2.48	98.89	0.44	0.003	0.58	133.05	97.80	9.00
	Box 3	12.53	2.30	99.46	0.46	0.005	0.66	134.70	98.10	9.00

(a)

BNR/chemical Jan,2016		TN (g)	TP (g)	TCOD (g)	NH ₄ -N (g)	Po ₄ -P (g)	SCOD (g)	Volume (L)
Effluent water	Box 1	2.66	0.71	22.94	1.03	0.79	28.40	7.20
	Box 2	2.55	0.87	21.83	1.05	0.89	28.75	7.20
	Box 3	2.83	0.91	19.87	0.95	0.85	32.46	7.20

(b)

BNR/chemical Jan,2016		TN (g)	TP (g)	TCOD (g)	TS %	V _s %
Sludge cake	Box 1	8.66	1.69	76.28	13.80	10.05
	Box 2	8.42	1.61	85.56	13.70	10.10
	Box 3	9.04	1.51	82.28	13.28	9.76

(c)

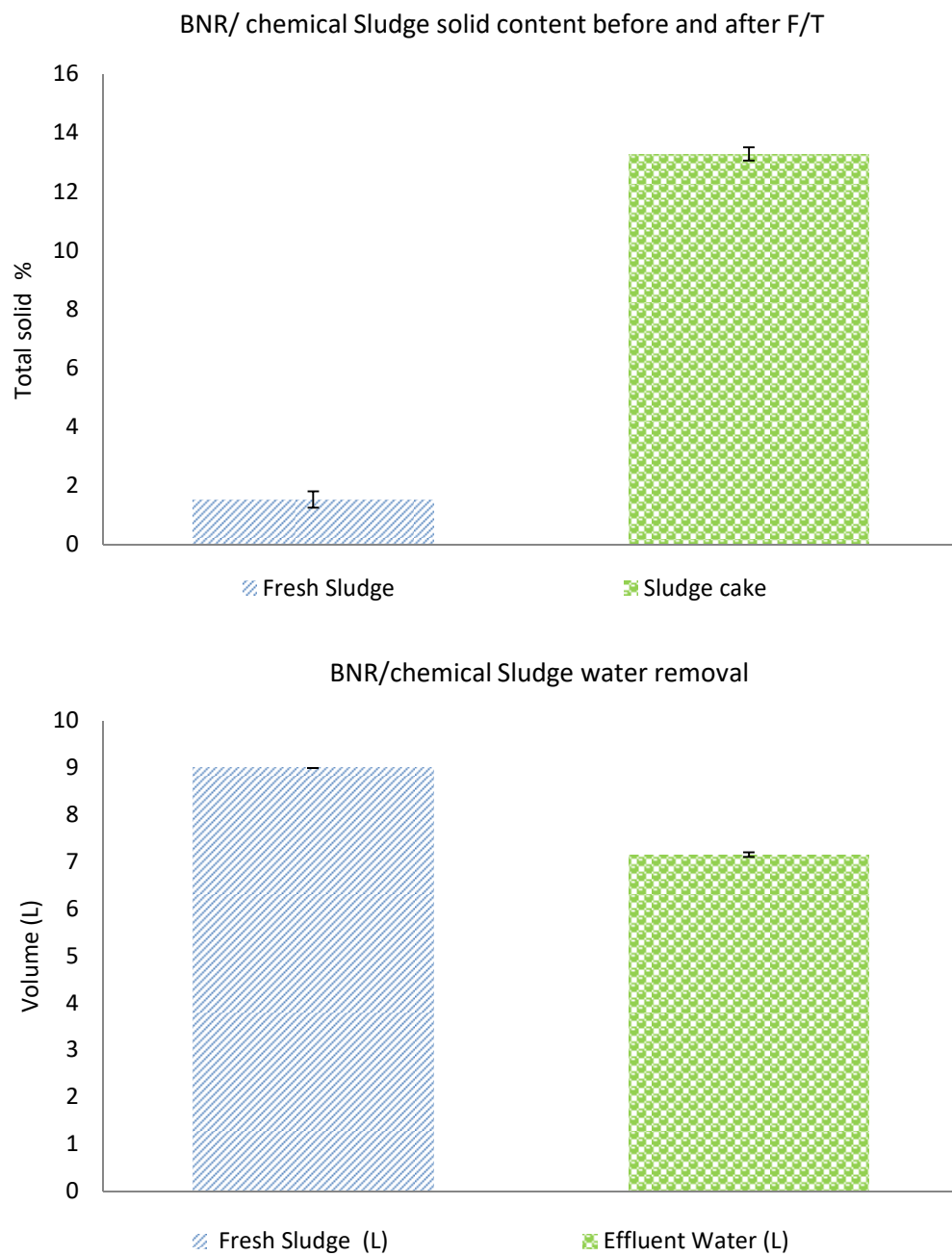


Fig. D.1: Sludge solid content and water removal before and after F/T

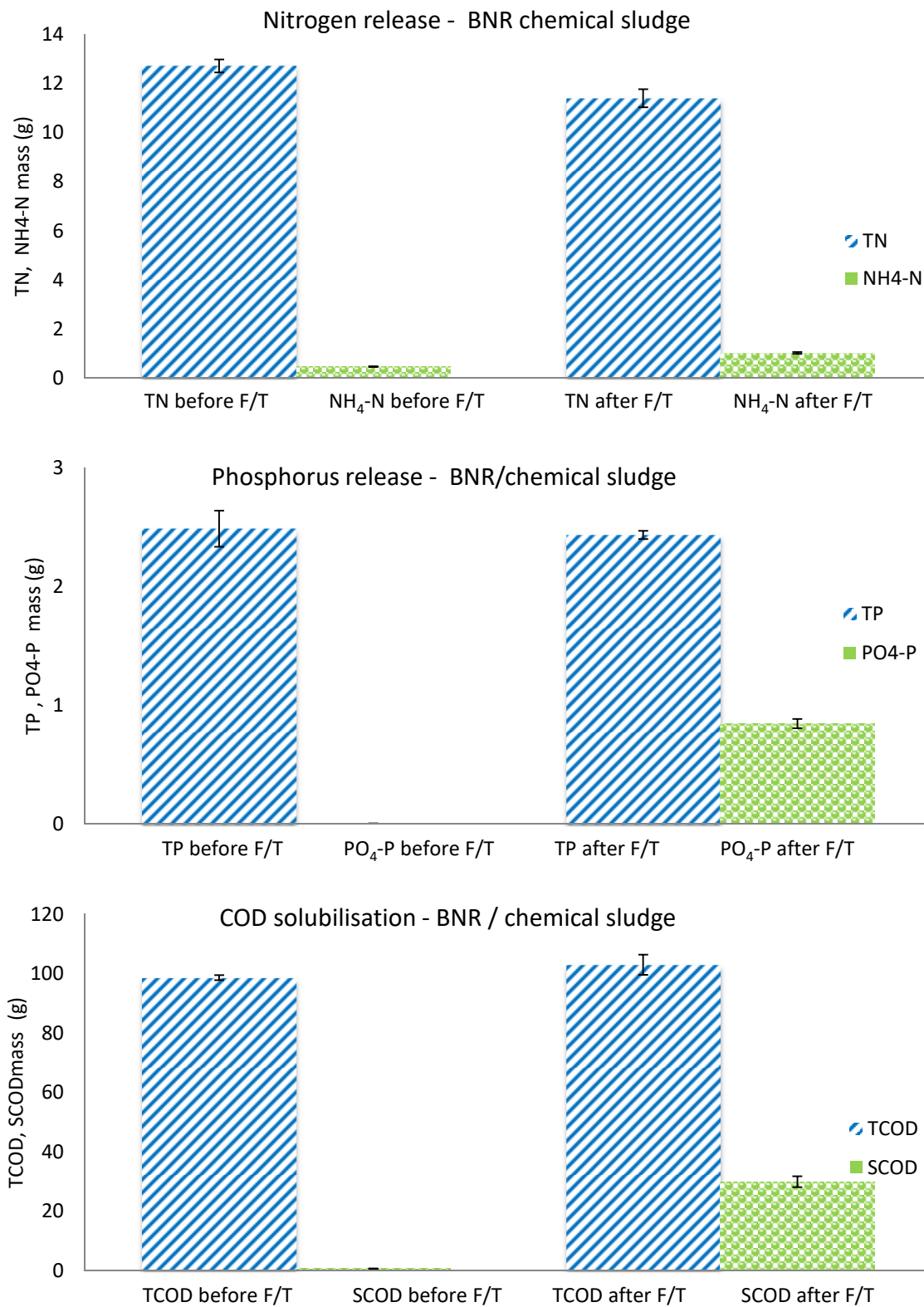


Fig. D.2: sludge solubilisation of nutrients and organics.

Table D.4: Volume and volatile solid of fresh sludge and digested sludge Jan, 2016. Fresh sludge

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	12	13.8	160	260	1.92	3.6	1/1.87
2	12	13.8	160	260	1.92	3.6	1/1.87
3	12	13.8	160	260	1.92	3.6	1/1.87
Blank	12	0	160	0	Filled with DI water		

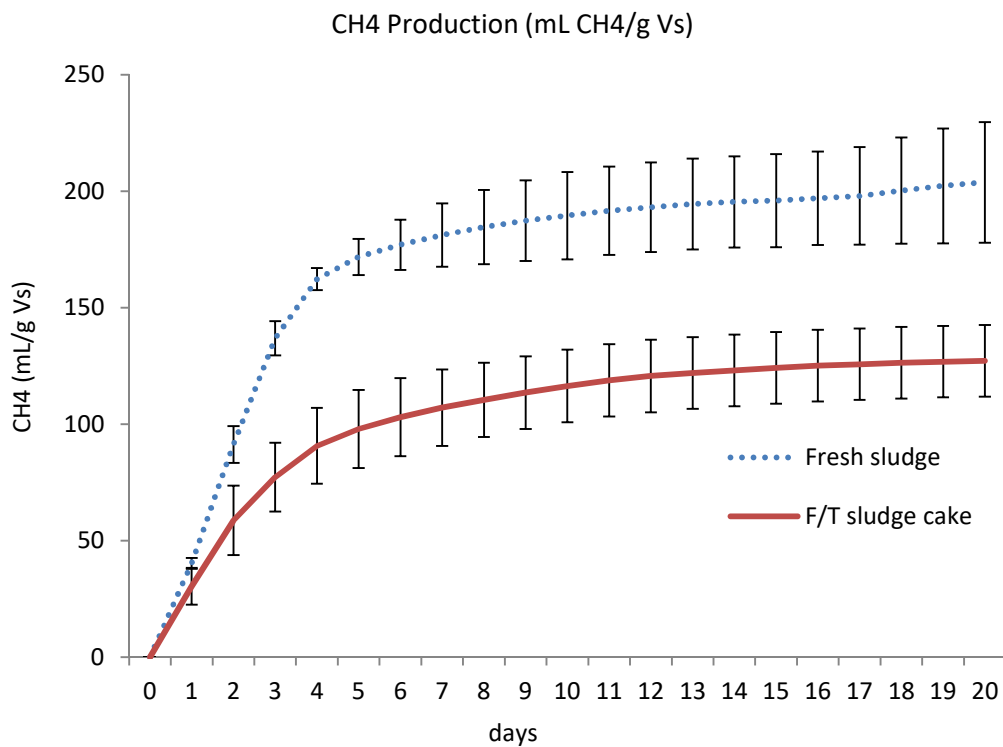


Fig. D.3: Normalized methane production of sludge before and after treatment

Table D.5: Biogas and methane gas production Batch test Jan, 2016. Fresh sludge

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Substrate CH ₄ 1 (mL)	Accumulated Biogas 2 (mL)	Substrate Biogas2 (mL)	Substrate CH ₄ 2 (mL)	Accumulated Biogas 3 (mL)	Substrate Biogas 3 (mL)	Substrate CH ₄ 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	309.63	227.94	156.59	272.65	195.40	138.15	262.82	186.75	139.13	50.60
2	668.80	514.23	353.28	548.46	408.33	288.69	608.18	460.89	343.36	84.44
3	941.35	735.20	505.08	838.23	644.45	455.63	893.69	693.26	516.48	105.89
4	1092.77	849.21	583.41	1033.05	796.66	563.24	1050.51	812.02	604.96	127.75
5	1151.74	882.38	606.19	1099.94	836.80	591.61	1150.90	881.64	656.82	149.03
6	1185.41	893.95	614.14	1142.75	856.41	605.48	1224.77	928.59	691.80	169.55
7	1215.03	903.34	620.59	1175.74	868.77	614.22	1288.90	968.35	721.42	188.50
8	1239.28	911.11	625.93	1203.63	879.74	621.98	1340.56	1000.24	745.18	203.92
9	1257.26	919.46	631.67	1223.56	889.81	629.09	1375.01	1023.08	762.20	212.41
10	1274.86	923.15	634.20	1245.62	897.42	634.47	1411.27	1043.19	777.18	225.82
11	1292.43	925.47	635.80	1280.24	914.75	646.72	1439.57	1054.96	785.94	240.75
12	1303.21	930.82	639.47	1294.62	923.27	652.75	1454.88	1064.29	792.90	245.45
13	1318.27	938.14	644.50	1307.77	928.90	656.73	1470.50	1072.10	798.71	252.20
14	1328.46	942.63	647.58	1317.72	933.17	659.75	1481.32	1077.14	802.47	257.29
15	1342.48	943.99	648.52	1331.77	934.56	660.74	1500.32	1082.89	806.75	269.76
16	1348.89	948.44	651.58	1339.05	939.78	664.43	1507.66	1088.16	810.68	271.11
17	1361.31	950.17	652.76	1351.48	941.52	665.65	1530.76	1099.28	818.96	281.57
18	1368.96	954.38	655.66	1360.93	947.32	669.75	1560.30	1122.76	836.46	284.43
19	1374.28	958.01	658.15	1366.76	951.39	672.63	1587.23	1145.40	853.32	285.63
20	1378.56	961.18	660.33	1371.77	955.21	675.33	1605.92	1161.26	865.14	286.30

Table D.6: GC analysis, Jan, 2016. Fresh sludge

Jan,2016	Biogas % from GC			Corrected to 100%	
Fresh sludge	CO ₂ %	N ₂ %	CH ₄ %	CO ₂ %	CH ₄ %
1	31.35	0.77	67.87	31.25	68.74
2	31.86	1.52	66.61	30.46	69.53
3	32.2	1.03	66.76	25.52	74.47

Table D.7: Normalized methane yield. Batch test Jan, 2016. Fresh sludge

Day	CH ₄ (mL/g VS) 1	CH ₄ (mL/g VS) 2	CH ₄ (mL/g VS) 3	Mean	STD
0	0.00	0.00	0.00	0.00	0.00
1	43.50	38.37	38.64	40.17	2.35
2	98.13	80.19	95.37	91.23	7.88
3	140.30	126.56	143.46	136.77	7.33
4	162.05	156.45	168.04	162.18	4.73
5	168.38	164.33	182.45	171.72	7.76
6	170.59	168.19	192.16	176.98	10.78
7	172.38	170.61	200.39	181.13	13.63
8	173.87	172.77	206.99	184.54	15.88
9	175.46	174.74	211.72	187.31	17.26
10	176.16	176.24	215.88	189.43	18.70
11	176.61	179.64	218.31	191.52	18.98
12	177.63	181.32	220.25	193.06	19.28
13	179.02	182.42	221.86	194.44	19.44
14	179.88	183.26	222.90	195.35	19.53
15	180.14	183.53	224.09	195.92	19.96
16	180.99	184.564	225.189	196.91	20.04
17	181.32	184.904	227.491	197.90	20.97
18	182.12	186.043	232.350	200.17	22.80
19	182.82	186.843	237.036	202.23	24.66
20	183.42	187.593	240.317	203.77	25.89

Table D.8: Volume and volatile solid of post F/T sludge cake and digested sludge Jan, 2016

Sample	VS (I) Inoculum (g/L)	VS (S) substrate (g/L)	Volume Inoculum (mL)	Volume Substrate (mL)	VS(I)*V(I) g	VS(S)*V(S) g	(I/S) ratio
1	11.5	98	340	80	3.91	7.84	1/2
2	11.5	98	340	80	3.91	7.84	1/2
3	11.5	98	340	80	3.91	7.84	1/2
Blank	11.5	0	340		Filled with DI water		

Table D.9: Biogas and methane gas production. Batch test Jan, 2016. Post F/T sludge cake

Days	Accumulated Biogas 1 (mL)	Substrate Biogas 1 (mL)	Sub- strate CH ₄ 1 (mL)	Accumulated Biogas 2 (mL)	Substrate Biogas2 (mL)	Sub- strate CH ₄ 2 (mL)	Accumulated Biogas 3 (mL)	Substrate Biogas 3 (mL)	Sub- strate CH ₄ 3 (mL)	Accumulated Biogas blank (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	386.93	231.92	153.29	551.12	376.40	259.34	629.76	445.61	300.34	123.38
2	698.10	462.78	305.90	970.53	702.48	484.01	1164.92	873.55	588.77	172.25
3	980.18	684.54	452.48	1241.64	914.63	630.18	1437.57	1087.05	732.67	202.28
4	1153.27	815.50	539.04	1460.32	1085.70	748.03	1650.99	1253.49	844.85	226.56
5	1256.47	888.72	587.44	1590.50	1182.66	814.85	1761.27	1332.94	898.40	246.56
6	1347.57	949.97	627.93	1676.47	1239.40	853.95	1851.56	1393.48	939.20	268.05
7	1426.42	1006.97	665.60	1733.65	1277.33	880.08	1921.55	1442.68	972.37	282.13
8	1504.66	1055.76	697.86	1788.93	1305.92	899.78	1987.06	1480.27	997.70	304.92
9	1573.43	1099.41	726.71	1842.76	1336.43	920.80	2046.15	1515.41	1021.38	324.09
10	1630.89	1134.09	749.63	1894.65	1366.20	941.31	2104.09	1550.51	1045.04	342.14
11	1675.67	1162.90	768.68	1940.26	1395.74	961.66	2145.30	1576.18	1062.34	354.18
12	1715.27	1184.67	783.06	1981.97	1419.37	977.94	2184.02	1597.17	1076.49	369.05
13	1752.53	1201.00	793.86	2020.85	1437.12	990.17	2214.59	1607.61	1083.53	387.75
14	1779.09	1213.35	802.02	2052.22	1453.71	1001.60	2238.35	1617.50	1090.19	400.27
15	1795.40	1224.37	809.30	2074.59	1470.05	1012.87	2253.21	1627.24	1096.76	404.07
16	1812.60	1234.53	816.02	2095.29	1483.30	1021.99	2268.92	1636.09	1102.72	409.72
17	1831.03	1242.69	821.42	2114.14	1491.83	1027.87	2284.90	1642.10	1106.77	418.87
18	1843.01	1249.62	826.00	2129.15	1501.42	1034.48	2295.22	1647.56	1110.46	422.98
19	1853.66	1254.38	829.147	2142.66	1508.70	1039.49	2305.19	1651.72	1113.26	428.22
20	1861.79	1257.81	831.418	2153.06	1514.13	1043.24	2312.71	1654.62	1115.22	432.45

Table D.10: GC analysis, Jan, 2016. Post F/T sludge cake

Jan,2016	Biogas % from GC			Corrected to 100%	
F/T sludge	CO ₂ %	N ₂ %	CH ₄ %	CO ₂ %	CH ₄ %
1	31.88	5.8517	62.267	33.862	66.138
2	29.1	6.2871	64.614	31.051	68.949
3	32.45	0.482	67.073	32.602	67.398

Table D.11: Normalized methane yield Batch test Jan, 2016. Post F/T sludge cake

Day	CH ₄ (mL/g VS) 1	CH ₄ (mL/g VS) 2	CH ₄ (mL/g VS) 3	Mean	STD
0	0.00	0.00	0.00	0.00	0.00
1	19.55	33.08	38.30	30.31	7.90
2	39.01	61.73	75.09	58.61	14.89
3	57.71	80.38	93.45	77.18	14.76
4	68.75	95.41	107.76	90.64	16.27
5	74.92	103.93	114.59	97.81	16.76
6	80.09	108.92	119.79	102.93	16.75
7	84.89	112.25	124.02	107.06	16.39
8	89.01	114.76	127.25	110.34	15.92
9	92.69	117.44	130.27	113.47	15.60
10	95.61	120.06	133.29	116.32	15.60
11	98.04	122.66	135.50	118.73	15.54
12	99.88	124.73	137.30	120.64	15.55
13	101.25	126.29	138.20	121.92	15.39
14	102.30	127.75	139.05	123.03	15.37
15	103.22	129.19	139.89	124.10	15.39
16	104.08	130.35	140.65	125.03	15.39
17	104.77	131.10	141.17	125.68	15.34
18	105.35	131.94	141.64	126.31	15.33
19	105.75	132.58	141.99	126.78	15.35
20	106.04	133.06	142.24	127.12	15.36

Appendix E: Result of Statistic Analysis

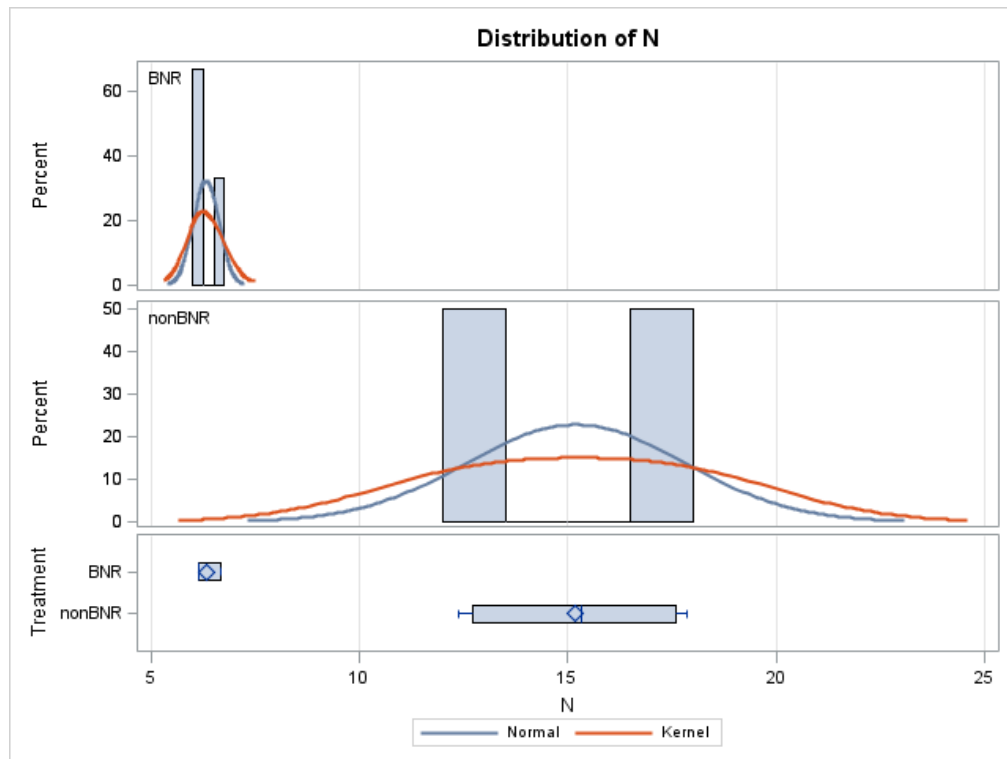
The TTEST Procedure**Variable: N**

Treatment	N	Mean	Std Dev	Std Err	Minimum	Maximum
BNR	3	6.3133	0.3092	0.1785	6.1200	6.6700
nonBNR	6	15.1867	2.6247	1.0715	12.3900	17.8400
Diff (1-2)		-8.8733	2.2244	1.5729		

Treatment	Method	Mean	95% CL Mean	Std Dev	95% CL Std Dev
BNR		6.3133	5.5451 7.0815	0.3092	0.1610 1.9435
nonBNR		15.1867	12.4322 17.9411	2.6247	1.6384 6.4374
Diff (1-2)	Pooled	-8.8733	-12.5927 -5.1540	2.2244	1.4707 4.5273
Diff (1-2)	Satterthwaite	-8.8733	-11.6231 -6.1236		

Method	Variances	DF	t Value	Pr > t
Pooled	Equal	7	-5.64	0.0008
Satterthwaite	Unequal	5.2713	-8.17	0.0003

Equality of Variances				
Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	2	72.04	0.0275



Variable: P

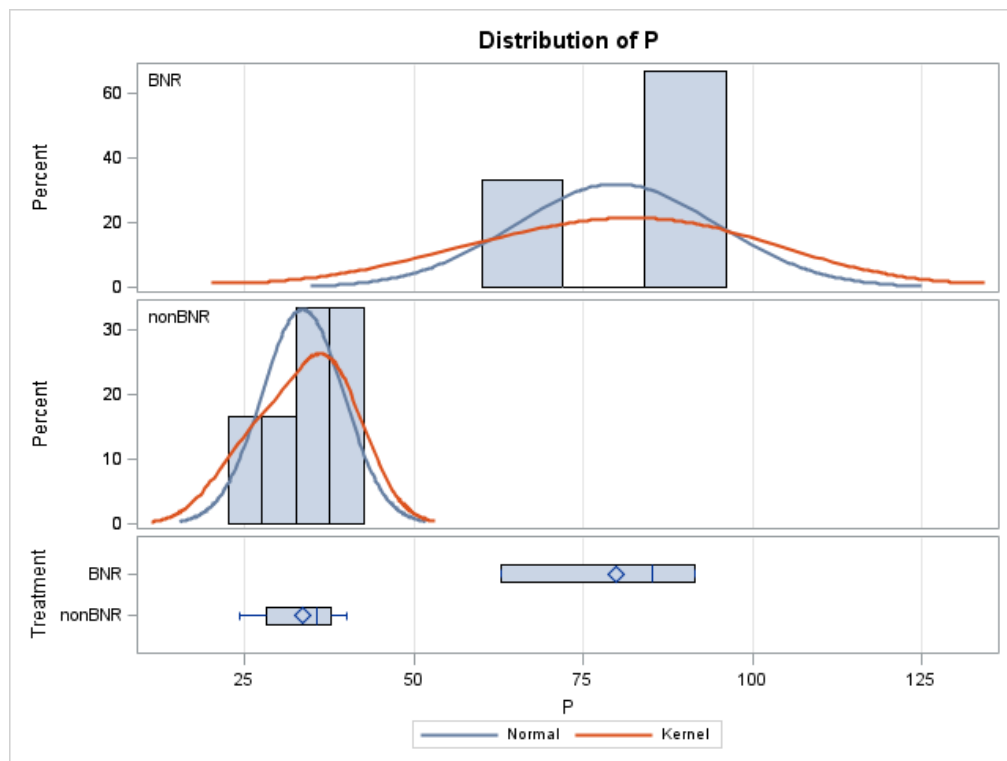
Treatment	N	Mean	Std Dev	Std Err	Minimum	Maximum
BNR	3	79.8300	15.0323	8.6789	62.8700	91.5100
nonBNR	6	33.5300	6.0332	2.4630	24.2200	39.9600
Diff (1-2)		46.3000	9.5165	6.7291		

Treatment	Method	Mean	95% CL Mean	Std Dev	95% CL Std Dev
BNR		79.8300	42.4876	117.2	15.0323
nonBNR		33.5300	27.1985	39.8615	6.0332
Diff (1-2)	Pooled	46.3000	30.3881	62.2119	9.5165
Diff (1-2)	Satterthwaite	46.3000	12.2929	80.3071	6.2920

Method	Variances	DF	t Value	Pr > t
Pooled	Equal	7	6.88	0.0002
Satterthwaite	Unequal	2.3291	5.13	0.0259

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	5	6.21	0.0883



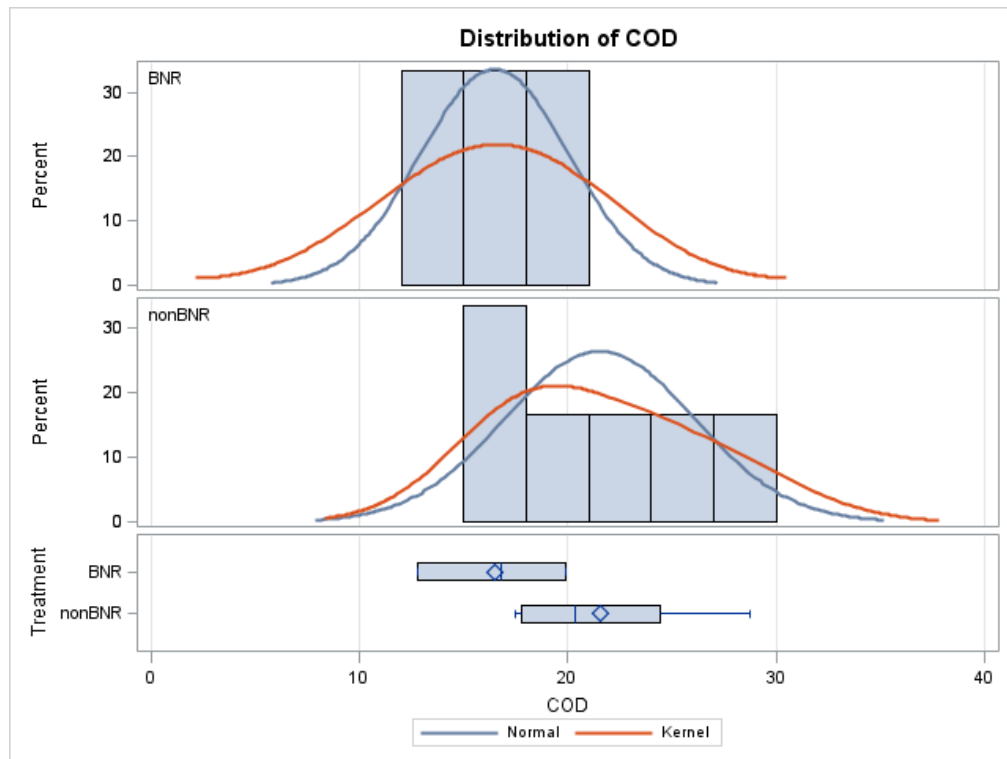
Variable: COD

Treatment	N	Mean	Std Dev	Std Err	Minimum	Maximum
BNR	3	16.4733	3.5643	2.0578	12.7700	19.8800
nonBNR	6	21.5233	4.5456	1.8557	17.4400	28.7400
Diff (1-2)		-5.0500	4.2882	3.0322		

Treatment	Method	Mean	95% CL Mean	Std Dev	95% CL Std Dev
BNR		16.4733	7.6192 25.3275	3.5643	1.8558 22.4005
nonBNR		21.5233	16.7530 26.2936	4.5456	2.8374 11.1485
Diff (1-2)	Pooled	-5.0500	-12.2200 2.1200	4.2882	2.8352 8.7276
Diff (1-2)	Satterthwaite	-5.0500	-12.0915 1.9915		

Method	Variances	DF	t Value	Pr > t
Pooled	Equal	7	-1.67	0.1398
Satterthwaite	Unequal	5.2	-1.82	0.1258

Equality of Variances				
Method	Num DF	Den DF	F Value	Pr > F
Folded F	5	2	1.63	0.8458



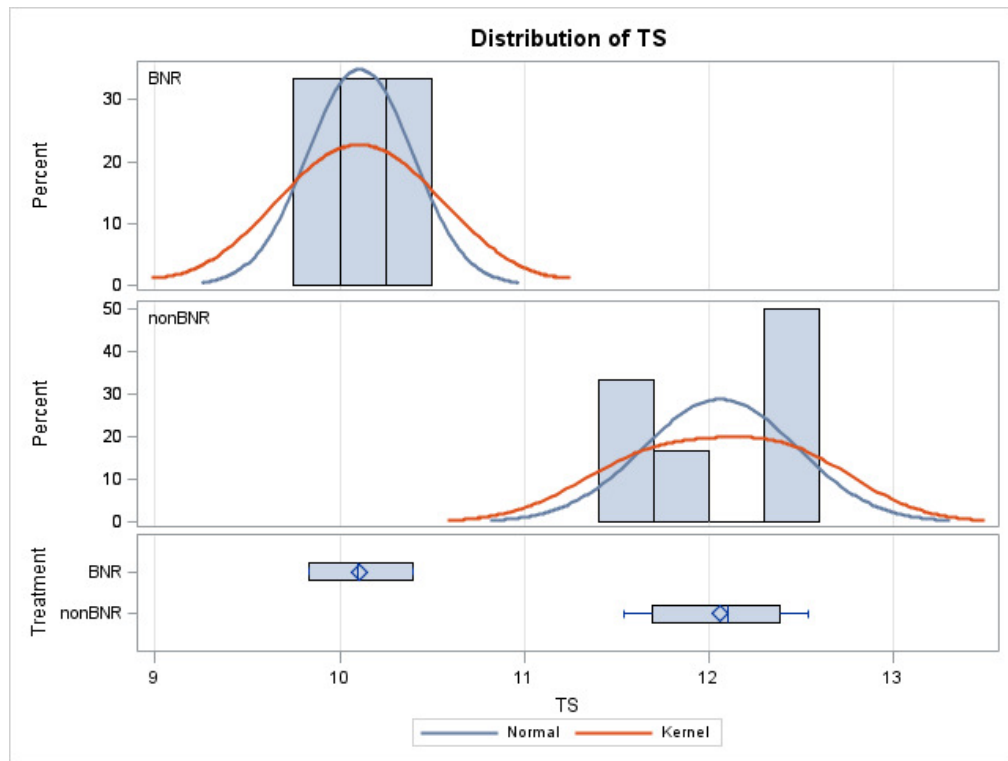
Variable: TS

Treatment	N	Mean	Std Dev	Std Err	Minimum	Maximum
BNR	3	10.1100	0.2851	0.1646	9.8300	10.4000
nonBNR	6	12.0600	0.4157	0.1697	11.5400	12.5400
Diff (1-2)		-1.9500	0.3830	0.2708		

Treatment	Method	Mean	95% CL	Mean	Std Dev	95% CL	Std Dev
BNR		10.1100	9.4017	10.8183	0.2851	0.1485	1.7920
nonBNR		12.0600	11.6237	12.4963	0.4157	0.2595	1.0197
Diff (1-2)	Pooled	-1.9500	-2.5904	-1.3096	0.3830	0.2532	0.7795
Diff (1-2)	Satterthwaite	-1.9500	-2.5319	-1.3681			

Method	Variances	DF	t Value	Pr > t
Pooled	Equal	7	-7.20	0.0002
Satterthwaite	Unequal	5.8622	-8.25	0.0002

Equality of Variances					
Method	Num DF	Den DF	F Value	Pr > F	
Folded F	5	2	2.13	0.7003	



Variable: Volume

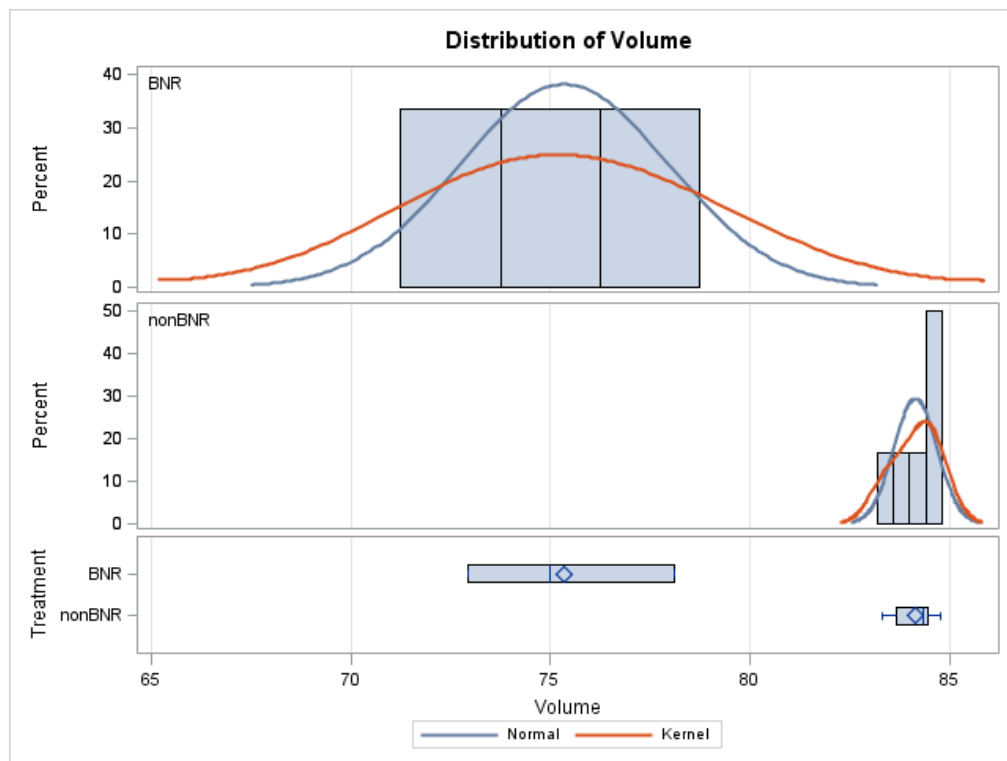
Treatment	N	Mean	Std Dev	Std Err	Minimum	Maximum
BNR	3	75.3467	2.6173	1.5111	72.9200	78.1200
nonBNR	6	84.1433	0.5422	0.2213	83.3300	84.7700
Diff (1-2)		-8.7967	1.4721	1.0409		

Treatment	Method	Mean	95% CL Mean	Std Dev	95% CL Std Dev
BNR		75.3467	68.8450 81.8483	2.6173	1.3627 16.4489
nonBNR		84.1433	83.5744 84.7123	0.5422	0.3384 1.3297
Diff (1-2)	Pooled	-8.7967	-11.2581 -6.3352	1.4721	0.9733 2.9962
Diff (1-2)	Satterthwaite	-8.7967	-15.1138 -2.4795		

Method	Variances	DF	t Value	Pr > t
Pooled	Equal	7	-8.45	<.0001
Satterthwaite	Unequal	2.0864	-5.76	0.0262

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	5	23.30	0.0058



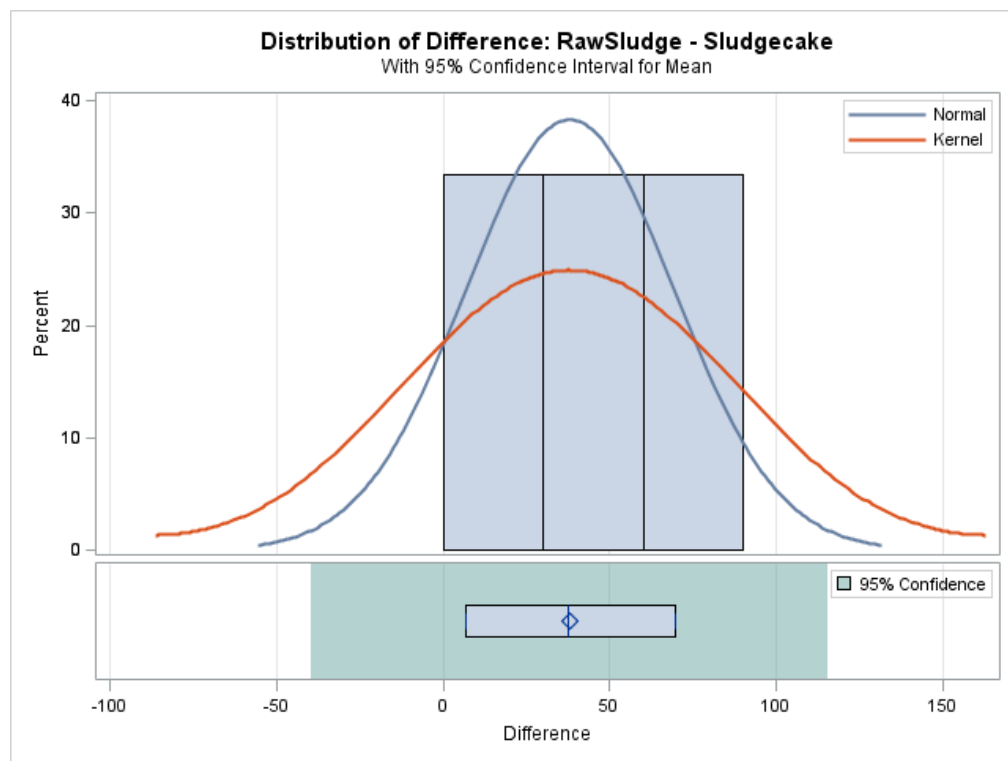
The TTEST Procedure

Difference: RawSludge - Sludgecake

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	37.9770	31.2530	18.0439	6.9320	69.4340

Mean	95% CL Mean	Std Dev	95% CL Std Dev
37.9770	-39.6598 115.6	31.2530	16.2722 196.4

DF	t Value	Pr > t
2	2.10	0.1700



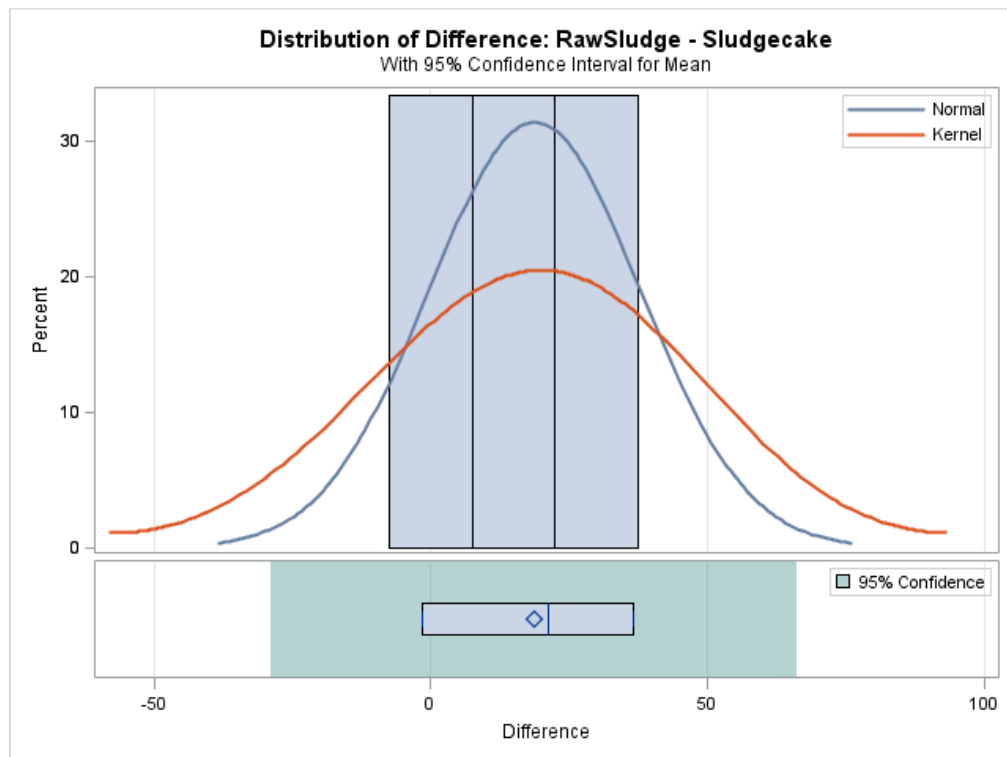
The TTEST Procedure

Difference: RawSludge – Sludgecake + effluent

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	18.7567	19.1085	11.0323	-1.4600	36.5200

Mean	95% CL Mean	Std Dev	95% CL Std Dev
18.7567	-28.7114 66.2248	19.1085	9.9490 120.1

DF	t Value	Pr > t
2	1.70	0.2312



Non BNR		
TN	Before	After
1	5.88	7.62
2	5.72	7.93
3	5.84	8.33
4	7.44	8.00
5	6.77	8.13
6	7.01	8.17

t-Test: Paired Two Sample for Means		
<i>TN</i>	<i>Variable 1</i>	<i>Variable 2</i>
Mean	6.444605	8.03
Variance	0.522370944	0.05972
Observations	6	6
Pearson Correlation	0.23129795	
Hypothesized Mean Difference		0
df		5
t Stat	5.489853646	
P(T<=t) one-tail	0.001368587	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.002737174	
t Critical two-tail	2.570581836	

T valu > t critical so difference

Non BNR		
TP	Before	After
1	1.15	2.08
2	1.10	2.39
3	1.15	2.25
4	1.61	1.71
5	1.86	1.53
6	1.77	1.64

t-Test: Paired Two Sample for Means		
TP	Variable 1	Variable 2
Mean	1.439394	1.933333333
Variance	0.118351762	0.125786667
Observations	6	6
Pearson Correlation	0.967229775	-
Hypothesized Mean Difference	0	-
df	5	-
t Stat	1.746035803	-
P(T<=t) one-tail	0.070621021	-
t Critical one-tail	2.015048373	-
P(T<=t) two-tail	0.141242042	-
t Critical two-tail	2.570581836	-

T valu < t critical so no difference

Non BNR		
TCOD-non BNR	Before	After
1	78.63	118.62
2	81.84	114.56
3	79.90	107.37
4	82.05	73.11
5	84.39	71.96
6	80.51	77.86

t-Test: Paired Two Sample for Means		
<i>TCOD</i>	<i>Variable 1</i>	<i>Variable 2</i>
Mean	81.2189	93.91333333
Variance	4.00969374	478.0446267
Observations	6	6
Pearson Correlation	-0.65973857	
Hypothesized Mean Difference	0	
df	5	
t Stat	1.338330243	
P(T<=t) one-tail	0.119210228	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.238420456	
t Critical two-tail	2.570581836	

T valu< t critical so no difference

Non BNR		
NH4-N	Before	After
1	0.35	1.13
2	0.34	1.05
3	0.35	1.09
4	0.38	1.69
5	0.39	1.59
6	0.38	1.59

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.3651	1.359
Variance	0.000436464	0.0875124
Observations	6	6
Pearson Correlation	0.989297878	
Hypothesized Mean Difference	0	
df	5	
t Stat	8.847313076	
P(T<=t) one-tail	0.000153298	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.000306595	
t Critical two-tail	2.570581836	

T valu > t critical so difference

Non BNR		
PO4-P	Before	After
1	0.10	0.52
2	0.11	0.55
3	0.10	0.54
4	0.09	0.54
5	0.08	0.53
6	0.08	0.70

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.09357	0.56235
Variance	0.000150798	0.004794687
Observations	6	6
	-	
Pearson Correlation	0.507928803	
Hypothesized Mean Difference		
	0	
df	5	
	-	
t Stat	15.06550025	
P(T<=t) one-tail	1.167E-05	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	2.334E-05	
t Critical two-tail	2.570581836	

T valu> t critical so difference

Non BNR		
SCOD	Before	After
1	0.62	14.33
2	0.66	15.23
3	0.61	15.13
4	0.67	20.70
5	0.60	19.66
6	0.56	23.70

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.620185	18.1242
Variance	0.001744057	14.35163403
Observations	6	6
Pearson Correlation	-0.411284822	
Hypothesized Mean Difference	0	
df	5	
t Stat	-11.26617003	
P(T<=t) one-tail	4.81298E-05	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	9.62595E-05	
t Critical two-tail	2.570581836	

T valu > t critical so difference

BNR		
NH4-N	Before	After
1	0.04	0.48
2	0.04	0.53
3	0.04	0.51

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.040075	0.5104
Variance	3.29907E-07	0.00058368
Observations	3	3
Pearson Correlation	0.894858762	
Hypothesized Mean Difference	0	
df	2	
t Stat	34.44965937	
P(T<=t) one-tail	0.000420777	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000841553	
t Critical two-tail	4.30265273	

T valu> t critical so difference

BNR		
SCOD	Before	After
1	0.83	16.39
2	0.78	20.18
3	0.80	18.36

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.801366	18.3072
Variance	0.000708162	3.596544
Observations	3	3
	-	
Pearson Correlation	0.991003581	
Hypothesized Mean Difference		0
df		2
		-
t Stat	15.76893466	
P(T<=t) one-tail	0.001998734	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.003997469	
t Critical two-tail	4.30265273	

T valu> t critical so difference

BNR		
PO4-P	Before	After
1	0.14	1.77
2	0.13	2.34
3	0.12	2.49

t-Test: Paired Two Sample for Means		
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.130697	2.1984
Variance	9.9798E-05	0.145152
Observations	3	3
	-	-
Pearson Correlation	0.802246595	
Hypothesized Mean Difference	0	
df	2	
	-	-
t Stat	9.205455988	
P(T<=t) one-tail	0.005797941	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.011595883	
t Critical two-tail	4.30265273	

T valu > t critical so difference