

Investigating temperature dependence in denitrifying woodchip beds used in onsite septic  
treatment systems

Andrew Jones

A thesis  
submitted in partial fulfillment of the  
requirements for the degree of

Masters of Science

University of Washington  
2015

Committee:  
Michael Brett  
Mark Benjamin

Program Authorized to Offer Degree:  
Civil and Environmental Engineering

©Copyright 2015  
Andrew Jones

University of Washington

Abstract

Investigating temperature dependence in denitrifying woodchip beds used in onsite septic treatment systems

Andrew Jones

Chair of the Supervisory Committee:  
Dr. Michael Brett  
Civil and Environmental Engineering

A 2012 study that tested three pilot onsite denitrifying treatment systems found a Vegetated Denitrifying Woodchip Bed achieved the highest total nitrogen removal. However, denitrification in this system was also much more temperature dependent than the other systems tested. This study examined the nitrogen removal performance of two current Denitrifying Woodchip Bed systems that were recently installed in the Hood Canal watershed. Data from the Hood Canal systems, and the 2012 pilot systems, was analyzed to establish the casual basis for denitrification temperature dependence. The results of this analysis suggest three things. First, the Hood Canal systems had incomplete nitrification, but still had 50% greater input nitrate concentration than the woodchip bed reactor tested in 2012. Second, in all three wood chip reactors tested the available carbon from the woodchips decreased with temperature, reducing the electron donor availability. Third, carbon released from the woodchips was a lower quality electron donor and therefore more sensitive to temperature fluctuations. A benchtop experiment was carried out to test denitrification rates using synthetic wastewater media and woodchip-based media as electron donors in typical summer and winter temperatures for western Washington State. This experiment showed nitrate removal in the high temperature synthetic wastewater treatment was extremely rapid with 98% removal after only 2 days. Conversely,

nitrate removal in the high temperature woodchip-based was 89% after 12 days. In the cold synthetic wastewater treatment nitrate removal averaged 79% after 12 days, whereas in the cold woodchip media nitrate removal averaged 39% after 12 days. This study indicates that woodchip based carbon is a much lower quality electron donor, and the combination of low temperature, low quality organic substrates and low woodchip substrate concentrations may greatly slow nitrate removal. To alleviate these temperature constraints on cold weather nitrate removal in woodchip-based reactors I recommend supplementing the reactors with additional carbon. This can be done effectively by dosing the systems with methanol or another simple form carbon substrate. The amount of methanol required to provide additional carbon sufficient enough for complete denitrification was calculated to cost \$5.25 a month.

## Table of Contents

List of Figures .....	7
List of Tables .....	8
Acknowledgements.....	9
Introduction.....	10
Typical Septic Systems .....	10
N Removal in Onsite Treatment Systems.....	11
Description of Study Treatment Technologies .....	11
Previous UW Study.....	12
Study Objectives .....	13
Hood Canal Site and System Descriptions .....	15
Pacific NW Salmon Center.....	15
Wood & Cock Inn.....	15
Description of Media .....	16
Analytical Methods.....	17
Ammonia.....	18
Nitrate and Nitrite .....	18
Total Nitrogen.....	19
Quality Assurance and Quality Control.....	19
Field Duplicates .....	20
Field Blanks .....	20

Method Blanks .....	21
Sample Spikes .....	21
Snoqualmie Pilot Denitrification Systems .....	22
The Hood Canal Woodchip Bed Denitrification Systems .....	27
Nitrogen Removal .....	28
Secondary Analytes .....	30
System Performance .....	31
Grab Sample Representativeness .....	32
Benchtop Experiment.....	33
Media Preparation .....	33
Bottle Preparation .....	34
Sampling and Analyses .....	35
Results.....	35
Hypothesis Discussion .....	36
Hypothesis 1.....	37
Hypothesis 2.....	37
Conclusion .....	39
Suggested improvements to Hood Canal System design.....	40
Future Work .....	42
Works Cited .....	44
Appendix A1. PNW Salmon Center System Specifications.....	47
Appendix A2. Woodcock Inn System Specifications.....	48

## List of Figures

Figure 1. NO <sub>x</sub> Removal efficiency of the VDWB, VRGF, and ERGF systems.....	24
Figure 2. sCOD in the VDWB, ERGF, and VRGF systems.....	25
Figure 3. sCOD compared to NO <sub>x</sub> Removal efficiency in the VDWB.....	26
Figure 4. Ratios of BOD <sub>5</sub> and sCOD .....	27
Figure 5. Average NO <sub>x</sub> -N in benchtop experiment .....	38

## List of Tables

Table 1. PHENOVA Proficiency Test Results .....	20
Table 2. Average QA/QC Results.....	21
Table 3. Average Total Nitrogen ( $\text{mg L}^{-1}$ ) in the Hood Canal systems .....	28
Table 4. Average $\text{NO}_x\text{-N}$ ( $\text{mg L}^{-1}$ ) in the Hood Canal systems .....	29
Table 5. Average $\text{NH}_4^+$ ( $\text{mg L}^{-1}$ ) in the Hood Canal systems .....	29
Table 6. Average TSS/BOD <sub>5</sub> /FC ( $\text{mg L}^{-1}$ , $\text{mg L}^{-1}$ , CFU's) in the Hood Canal systems .....	30
Table 7. Monthly temperatures ( $^{\circ}\text{C}$ ) during system operation (Weather Underground, 2015) ....	31
Table 8. Grab Sample Variation in the Salmon Center system .....	32
Table 9. Constituents used in synthetic wastewater and woodchip medias.....	34
Table 10. Average sCOD and $\text{NO}_x\text{-N}$ ( $\text{mg L}^{-1}$ , $\text{mg L}^{-1}$ ) measured from benchtop experiment....	35
Table 11. Secondary Carbon Source Calculations.....	42



## **Acknowledgements**

I would like to thank my Advisor Michael Brett for providing his expertise and guidance during the project and my Master's Committee member Mark Benjamin for his assistance with my Master's Thesis. I would also like to thank those who provided assistance and support to me in the UWCEE lab. Songlin Wang, J. Sean Yeung, Nichollete Zhou, Fan Lu, and a number of others made this study possible by providing me with technical support. Crystal Grinell and Stephanie Wei for their work on the original Snoqualmie Denitrification pilot project. The system and project manager, Julian Sammons of the Hood Canal Salmon Enhancement Group, was a great resource for me and a pleasure to work with. He provided overall project support, performed the sampling and transported them to UW. This project would have never been possible if it wasn't for Maureen Woodcock, owner of the Woodcock Inn, and the Pacific Northwest Salmon Center for providing us with the locations to install and study the systems. And I would like to thank the Snoqualmie WWTP for allowing me to take samples for the denitrification experiments.

This research was supported by a grant administered through the Department of Ecology.

## **Introduction**

Nitrogen (N) concentrations in domestic wastewater can range from 40 to 80 mg L<sup>-1</sup> (Robertson et al., 2005). Large centralized wastewater treatment systems, if so designed, can be very efficient at removing N. In rural areas and other places where septic systems are more commonly used, N reducing technologies are far less common. US Environmental Protection Agency (EPA) estimates that over 25% of the United State's population utilizes septic systems for wastewater treatment (U.S. EPA, 2015). Septic systems and other onsite type treatments can have an influence radius on groundwater quality of up to 100 m from their drainfield (Robertson et al., 1991). Without N reduction, the effluent dispersed into the groundwater can have a number of environmental impacts. Nitrate, (NO<sub>3</sub><sup>-</sup>) the most common form of N contamination in wastewater effluents, is very mobile in ground water. If nitrate pollution makes its way into drinking water systems, it can cause methemoglobinemia; a disease which interferes with the oxygen-carrying capacity of the blood in infants (U.S. EPA, 2002). N contaminated effluent may make its way through aquifers into surface waters where it can have significant consequences on the ecosystem. N nutrient pollution has been shown to cause increased eutrophication, toxic algal blooms, alter the aquatic food webs, and reduce aquatic biodiversity (Kellog et al., 2010; Robertson et al., 2005; Shipper et al., 2010; Warneke et al., 2011). Because of these issues, EPA has established a maximum contaminant goal limit (MCGL) of 10 mg L<sup>-1</sup>.

### Typical Septic Systems

Typical onsite treatment systems consist of a septic tank and a drainfield. Septic tanks act as primary treatment for the wastewater and they provide an anaerobic environment to digest the organic waste and settle solids out of the water. This treated effluent contains N primarily in the form of ammonium (Leverenz et al., 2010). The ammoniumified effluent is then dispersed to a

drainfield via a pressurized or nonpressurized distribution system. Once in the ground, the effluent undergoes further treatment where bacteria in the presence of oxygen nitrify the ammonium. The nitrified discharge can then travel to aquifers often with very little subsequent N removed (Kellog et al., 2010)

### N Removal in Onsite Treatment Systems

In order to achieve denitrification in an onsite system, nitrified wastewater must be treated in an anoxic zone. This means there are two alterations to typical septic systems that must be made. Because denitrification requires nitrified effluent, ammonium must first be oxidized. Nitrification is then followed by a tertiary treatment process where nitrified effluent passes through an anoxic zone. In the absence of  $O_2$ , denitrifying bacteria use  $NO_3^-$  as an electron acceptor, reducing the nitrate to nitrogen gas ( $N_2$ ) (Tchobanoglous et al., 2014). The effluent is then discharged to a drainfield for disposal and further treatment by soil bacteria.

### Description of Study Treatment Technologies

In our study nitrification is achieved using a recirculating gravel filter (RGF). RGFs are an attached growth system which can deal with a wide array of flows and waste strengths (Washington Department of Health, 2015). Pressurized pipes distribute septic effluent over the top of the filter and the wastewater trickles through the gravel media and its associated biofilm and is processed by microorganisms. It is then collected in a recirculation basin where 10-20% is discharged to the next step of the treatment train and the rest is recirculated back to the distribution system. Although RGF's in our study were primarily designed for nitrification, RGFs can be designed with both aerobic and anoxic zones and are able to remove up to 50% of N (Crites, 1998). In this combination nitrification/denitrification system, the limiting factor for denitrification is a lack of carbon in the anoxic zone, as well as the recirculation ratio

itself since a fraction of the wastewater equal to one over the recirculation ratio will be discharged after a single pass through the RGF. When nitrification occurs most of the carbon in the wastewater is also oxidized leaving insufficient organic matter for denitrification in the anoxic zone. To optimize N removal, a separate tertiary process with its own carbon source is required.

To achieve denitrification in the systems tested, a denitrifying woodchip bed was used.

Woodchip beds can be very efficient at removing nitrate. Studies have shown that well over 90% nitrate removal can be achieved with nitrate levels reaching concentrations  $< 0.2 \text{ mg L}^{-1}$  (Cameron et al., 2010; Cameron et al., 2011; Greenan et al., 2006; Leverenz et al., 2011; Moorman et al., 2010; Schipper et al., 2010a; Schipper et al., 2010b). Nitrified effluent from the RGF enters the woodchip bed via subsurface flow. Any remaining oxygen in the water is quickly used up along with any leftover carbon from the nitrification process creating an anoxic environment. Woodchips beds are effective because the woodchips themselves provide the carbon source for the denitrifying bacteria to oxidize (Leverenz et al., 2011; Robertson et al., 2005; Sailing et al., 2007).

#### Previous UW Study

In November 2012, Grinnell (2013) and Wei (2013) performed a study to evaluate the performance of three onsite treatment systems. The results of that study clearly showed N reduction below EPA's  $10 \text{ mg L}^{-1}$  could be achieved using a two-step denitrification process with a recirculating gravel filter (RGF) followed by a vegetative denitrifying woodchip bed (VDWB). However, a sharp decline in N removal with colder temperatures was observed. The same temperature dependence was not observed in the second best performing system, a single-step enhanced recirculating gravel filter (ERGF).

## Study Objectives

Three factors can affect denitrification in onsite systems. These are carbon availability, carbon composition, and temperature (Healy et al., 2012; Leverenz et al., 2011; Warneke et al., 2011a; Warneke et al., 2011b). Both of the previously studied systems, the ERGF and VDWB, were fed the same influent from the Snoqualmie WWTP and were exposed to the same temperature fluctuations. Then why is there a more significant temperature dependence in the VDWB than in the ERGF? Although initially the systems were dosed from the same source, due to the difference in system design, the actual carbon composition used to support denitrification was very different for the two systems. Denitrification in the ERGF relied directly on the carbon influent whereas the VDWB used carbon provided by the woodchips. The difference in carbon source may help to explain the difference in temperature dependency.

The carbon-quality temperature hypothesis (CQT) suggests biochemically recalcitrant organic matter will have greater temperature sensitivity to microbial degradation (Craine et al., 2010). Substrates which require a higher activation energy to be oxidized, will be more sensitive to changes in temperature than substrates with lower activation energies (Craine et al., 2010; Bosatta and Agren, 1999). The Arrhenius equation describes how the reaction rate of microorganisms can change relative to temperature and activation energy.

$$k = a \exp\left(-\frac{E_a}{RT}\right)$$

Where  $k$  is the reaction rate;  $a$  is the theoretical reaction rate constant in the absence of activation energy;  $E_a$  is the activation energy;  $R$  is the gas constant ( $8.314 \text{ K}^{-1}\text{mol}^{-1}$ ); and  $T$  is the temperature in degrees Kelvin (Davidson and Janssens, 2006). The Arrhenius equation and the CQT hypothesis give us a potential explanation for why denitrification in the VDWB is more sensitive than in the ERGF.

This study will test two hypotheses which could explain why in VDWB systems denitrification is extremely temperature sensitive. The first hypothesis is that the lower temperatures inhibited the release of carbon from the woodchips, and the lower N removal rates found in the VDWB are a function of less available carbon. The second hypothesis is that the carbon used for denitrification in the VDWB, is more recalcitrant, has a higher activation energy, and biological degradation is therefore more difficult to utilize compared to the carbon used for denitrification in the ERGF.

The goals of this study are threefold. First the temperature, COD, and Nitrogen data from the Grinnel & Wei studies (Grinnel 2013, Wei 2013) will be analyzed to establish the relationship between carbon availability and type, temperature, and nitrogen removal. Second, the BOD<sub>5</sub>, TSS, N, and Temperature data of two denitrifying woodchip bed treatment systems, which were installed in Washington State's Hood Canal area, will be analyzed to further explore the temperature dependence of denitrification performance in a real world setting. Lastly, a benchtop experiment will be carried out to test denitrification rates at different temperatures using different carbon sources. Two sets of reactors will be prepared. One set will use woodchip leachate as a carbon source, while the second set will use synthetic wastewater treatment plant influent as the carbon source. Both groups will contain inorganic media seeded with denitrifying organisms and have a similar spiked NO<sub>3</sub><sup>-</sup> concentrations. The reactors will be maintained at a range of temperatures representative of summer and winter conditions in western Washington and NO<sub>3</sub><sup>-</sup> removal rates will be monitored.

## **Hood Canal Site and System Descriptions**

### **Pacific NW Salmon Center**

The Pacific Northwest Salmon Center is home to the Hood Canal Salmon Enhancement Group, The Farm at Water's Edge, a water quality lab and educational facility. The Salmon Center focuses on research and habitat restoration in the Hood Canal watershed. This was an ideal candidate for a denitrifying system because it is a relatively high profile location. This is also a well utilized site, as the Theler Wetland Trails run adjacent to the property. There are also 8 full-time staff members on the site, 5 days a week. The site is nonresidential, so the system will not be loaded from showers or laundry activity and therefore is expected to have higher strength influent.

Design flow for the system was calculated at 12 employees by 20 GPD per employee equaling 240 GPD. The existing OSS was permitted in 1981 for a four bedroom residence flow of 600 GPD.

The system includes a 1150 gallon septic tank which pumps the wastewater to a 1500 gallon recirculation tank. From there the wastewater is dispersed across an 8' by 10' recirculating gravel filter (RGF) at a loading rate of 3 GPD/ft<sup>2</sup>. The wastewater trickles down through 2' of gravel and drains out into a collection pipe where it is recirculated back through the RGF at a ratio of 6:1. Wastewater passed on from the RGF enters at the end of a 2' by 10.5' woodchip bed (WCB) with 42" of woodchip media. After the wastewater exits the system it is pumped to an existing 528' drainfield.

### **Wood & Cock Inn**

In January 2014 Maureen Woodcock received a permit to run a bed and breakfast from her home. Her home also contains two smaller apartments which house permanent residences and

has an RV site on location which is connected to the onsite treatment system. At least 6 people will be housed at any given time, with up to 12 guests staying on the property. This is an ideal place to test the denitrifying systems because there will always be a significant load to the treatment system, yet the magnitude of the wastewater loading will differ as guests come and go. The variable loading will provide a good opportunity to study the system's adaptability.

The 6 bedroom waterfront residence is located on Hood Canal. The design flow for the system was determined to be 4 bedrooms at 120 GPD each for a total of 480 GPD. This was chosen because the B&B will be approximately 60% occupied during the summer, and 40% or less in the winter. The existing OSS was permitted in 2013 for a six bedroom residential flow of 720 GPD. The system consisted of a 1500 gallon two compartment concrete tank which discharged to a 300' pressurized drainfield in a standard trench design.

The system includes a 1500 gallon septic tank which pumps the wastewater to a 1500 gallon recirculation tank. From there the wastewater is dispersed across an 8' by 20' recirculating gravel filter (RGF) at a loading rate of 3 GPD/ft<sup>2</sup>. The wastewater trickles down through 2' of gravel and drains out into a collection pipe where it is recirculated back through the RGF at a ratio of 6:1. Wastewater passed on from the RGF enters at the end of a 3.5' by 19' woodchip bed (WCB) with 42" of woodchip media. After the wastewater exits the system it is pumped to an existing 300' pressurized drainfield.

#### Description of Media

The gravel media used for both system requires less than 10% of the media to be smaller than 2 to 3 mm (D<sub>10</sub>) with a uniformity coefficient (UC) of less than or equal to 2. The max allowable particle size is 3/8". The gravel must be washed with less than 1% passing through a US #50 sieve.



The woodchip media used in both systems must be Alder woodchips free of bark, leaves, twigs, dirt rocks and other foreign materials. The required length of the woodchips must be ½” to 3” with a width greater than 3/8” and have a minimum thickness of 0.0625”.

## **Analytical Methods**

### Site Sampling

Sampling occurred once a month at each system. Grab sampling methods were performed in accordance with EPA’s Operating Procedure for Wastewater Sampling (US EPA, 1993). Grab samples were taken directly from sample ports located at specific locations along the treatment train. These sampling ports were placed after the septic tank, recirculating gravel filter, and woodchip bed in each of the two systems for a total of 6 sampling areas. For each sampling event a random duplicate sample was also taken, as well as a field blank to insure sample integrity. Most of the grab samples were divided and transported to Hood Canal Salmon Enhancement Group (HCSEG), Centric Analytical Labs (CAL), or University of Washington Civil and Environmental Engineering (UWCEE) for analysis, however Temperature, pH, and DO were determined in situ with a probe.

Upon collection, samples were placed on ice and preserved by reducing their pH below 2 with the addition of H<sub>2</sub>SO<sub>4</sub>. Soon after, the samples were transported to UWCEE or CAL. These samples were then analyzed within 28 days of the sampling date.

### Analytical Methods

The methods used for Nitrogen Analyses at UWCEE are based on Standard Methods (American Public Health Association, 2005). UWCEE was responsible for the NH<sub>3</sub>-N, NO<sub>3</sub> + NO<sub>2</sub> (NO<sub>x</sub>-N), and TN analyses. These parameters were measured for all sampling locations with duplicates and fields blanks for a total of 8 samples per sampling event.

## Ammonia

Ammonia-N was measured using Standard Method 4500-NH<sub>3</sub>-G and Seal Analytical Method G-102-93 Rev 7 with a Bran and Luebbe AutoAnalyzer 3 (AA3). These methods describe the analysis of ammonia in wastewaters within the range of 0 to 4 mg L<sup>-1</sup> as NH<sub>3</sub>-N. Prior to analysis, these samples were filtered using 0.45 µm Millepore Millex filters. Samples were diluted as necessary to bring them within the analytical range. The AA3 measures ammonia via alkaline phenate and dichloroisocyanuric acid reaction which produces a blue color with an intensity proportional to the ammonia concentration. The color is measured spectrometrically at the 660 nm wavelength with a 1 cm flowcell. Further details describing reagent preparation and AA3 operation can be found in the UWCEE Standard Operating Procedure for Ammonia (Appendix B1).

## Nitrate and Nitrite

NO<sub>x</sub>-N was measured using Standard Method 4500-NO<sub>3</sub>-H and Seal Analytical Method G-109-94 Rev 7 with an AA3. These methods describe the analysis of NO<sub>x</sub>-N in wastewaters within the range of 0 to 2 mg L<sup>-1</sup> as NO<sub>x</sub>-N. Prior to analysis, samples were filtered using 0.45 µm Millepore Millex filters, and samples were diluted when necessary. An alkaline hydrazine solution, with a copper catalyst, reduces nitrate to nitrite. Sulfanilimide and N-(1-naphthyl) ethylenediamine dihydrochloride was used to produce a pink coloring with an intensity proportional to the nitrite concentration. The color was measured photometrically at the 550 nm wavelength with a 1 cm flowcell. Further detail can be found in the UWCEE Standard Operating Procedure for NO<sub>3</sub> + NO<sub>2</sub> (Appendix B2).

## Total Nitrogen

Total nitrogen was measured using a two-step process. First, samples were digested using Standard Method 4500-PJ followed by Standard Method 4500-NO<sub>3</sub>-H and Seal Analytical Method G-109-94 Rev 7 with an AA3. Unlike the NO<sub>x</sub> and NH<sub>3</sub> procedures, total nitrogen samples are not immediately filtered, but they were diluted when necessary, to bring them within the 0 to 2 mg L<sup>-1</sup> analytical range. A solution of potassium persulfate and sodium hydroxide was used to oxidize the samples. This converts all nitrogen compounds to NO<sub>3</sub>-N. After the digestion, 200 µL of 3N sodium hydroxide was added to neutralize pH, and the samples were then filtered using a BD 60 mL Luer-Lok Tip Syringe with a 0.45 µm PES membrane Millex-HP syringe driven filter. The samples were then analyzed using the same method as the NO<sub>x</sub> analysis. Additional details are found in the UWCEE Standard Operating Procedure (Appendix B3).

## Quality Assurance and Quality Control

Quality assurance and quality control of sampling and measured water quality parameters were used to ensure the data collected met the project accuracy goals. These QA/QC procedures included attaining EPA accreditation, proficiency testing, field duplicates, field blanks, method duplicates, method blanks, and sample spikes. Proficiency Testing

In order to assure the accuracy of the testing methods, a third party proficiency test was performed. The test samples provided by PHENOVA contained an unknown concentration of Ammonia as N, Nitrate and Nitrite as N, and Total Nitrogen. The analysis was done following standard operating procedures and was blindly reported back to PHENOVA. The results of the proficiency testing are as follows.

Table 1. PHENOVA Proficiency Test Results

NELAC Code	Analyte	Method Description	Units	Assigned Value	Result	Acceptance Limits
1515	Ammonia as N	4500-NH3-G	mg L-1	2.35	2.32	1.7 – 3.07
1820	Nitrate and Nitrite as N	4500-NO3-H	mg L-1	12.5	12.5	10.5 – 14.5
1827	Total Nitrogen	4500-P-J	mg L-1	14.9	14.71	12.4 – 17.3

### Field Duplicates

Field duplicates consist of two samples collected from the same location at the same time. The purpose of field duplicates is to quantify the precision of the sampling and analysis procedures. Inaccuracies in sample collection or analytical procedures may result in discrepancies between the original sample and the duplicate. Field duplicates were delivered to UWCEE without indication of which sample the duplicate was taken from. The acceptance criteria for duplicates were 80%-120% for NH<sub>3</sub>-N and TN, and 90%-110% for NO<sub>3</sub>+NO<sub>2</sub>-N.

### Field Blanks

Field blanks are used to detect bias caused by sample contamination. Field blanks are created by HCSEG personnel and consist of deionized water. These samples are handled, transported, and analyzed in the same manner as normal samples. Contamination may occur due to contaminated sample containers, transportation methods, filtration equipment, or other analytical practices.

### Method Duplicates

Method duplicates are used to measure the precision of the analytical procedures. All of the samples were split into triplicate (including field duplicates, field blanks, and spiked samples),

with each sample split into three aliquots from a single sample container. Variation (SD/mean) between these lab duplicates must be less than  $\pm 15\%$ .

#### Method Blanks

Method blanks consist of mili-Q reagent water and are used to ensure drift does not occur during the course of the analyses. These blanks are prepared in lab, and follow the standard sample preparation procedures. Method blanks are used every 10<sup>th</sup> aliquot during analysis and measure the baseline response of the AA3 to a concentration of zero. If drift is indicated corrective action can be taken.

#### Sample Spikes

Spike recovery samples are used to determine if the sample matrix affects analytical accuracy. An aliquot of a sample is spiked with a known concentration of interest (1 mg L<sup>-1</sup> NO<sub>x</sub>-N & TN, 2 mg L<sup>-1</sup> NH<sub>3</sub>-N). Spike recovery is calculated using the average sample concentration, the known spike concentration and the calculated spike concentration. The calculated spike recovery must be within 85% -115% of the known spike concentration.

Table 2. Average QA/QC Results

		NO <sub>x</sub> -N	NH <sub>3</sub> -N	TN
Duplicate Equivalence (%)	Average	77*	104	94
	Std. Dev.	27*	11	10
Field Blanks (mg L <sup>-1</sup> )	Average	0.06	0.08	0.12
	Std. Dev.	0.03	0.08	0.04
Sample Variation (%)	Average	2.1	0.8	1.9
	Std. Dev.	1.4	0.5	1.5
Spike Recovery (%)	Average	106	97	96
	Std. Dev.	4.8	4.9	7.8

\*NO<sub>x</sub> Duplicate precision is heavily influenced by two instances of poor duplication (34% and 45%). These instances occurred in septic tank measurements where NO<sub>x</sub> measurements were near the detection limit meaning slight variations would result in poor duplicate equivalence %.

## **Snoqualmie Pilot Denitrification Systems**

The studies performed by Grinnell (2013) and Wei (2013) compared the performance of three onsite nitrogen reducing treatment technologies. The systems were a Vegetated Recirculating Gravel Filter (VRGF), an Enhanced Recirculating Gravel Filter (ERGF), and a 2-step Recirculating Gravel Filter followed by a Vegetated Denitrifying Woodchip Bed (VDWB). These systems were located at the Snoqualmie Wastewater Treatment Plant, and each system was dosed with 480 gallons a day over 30 dosing periods. The dosages consisted of influent diverted from the treatment plant's headworks. Treatment effectiveness was measured from August 22<sup>nd</sup>, 2012 to July 27<sup>nd</sup>, 2013 and the measured analytes included pH, Temperature, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD<sub>5</sub>), Carbonaceous Biochemical Oxygen Demand (CBOD<sub>5</sub>), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Alkalinity, Total Nitrogen (TN), Ammonia, Nitrate+Nitrite (NO<sub>x</sub>-N), Total Phosphorous, and Fecal Coliforms.

Over the experimental time period the overall total nitrogen removal rates for the VDWB, VRGF, and ERGF systems averaged 92%, 68%, and 81% respectively. Summer (April-September) nitrogen removal was 97%, 68%, and 79%, while winter (October-March) performance was 84%, 68%, and 83%, respectively. The VDWB system had the highest nitrogen removal overall, with the ERGF system also showing considerable nitrogen removal. The one-step ERGF and VRGF were limited in TN removal by the fact that both nitrification and denitrification occurred simultaneously and by utilizing only the influent as a carbon source. In these systems, the overall nitrogen removal of the systems could be limited in two ways. The influent may not nitrify completely which limits TN removal because only NO<sub>3</sub> is removed via denitrification. A scarcity of available carbon can also limit NO<sub>3</sub> removal. Nitrification and

denitrification in RGF systems has an inherent limitation, due to the recirculation ratio which always allows a portion of influent to leave the system untreated. For instance, in the VRGF where the recirculation ratio is 8.0, the total flowrate from the aerobic zone to the anoxic zone would be 9 times the influent flowrate. Thus 11% of the flow will not have had a chance to go through the nitrification zone, and this fraction of the influent will remain as  $\text{NH}_3$  and will be unavailable for denitrification prior to exiting the system as effluent. The two-step VDWB system used separate stages and carbon sources for nitrification and denitrification. This allowed nitrification and denitrification to be completed in the separate RGF and woodchip reactors. Because of the design differences in each of these systems, different external factors can have varying impacts on the overall system performance. One of the primary external actors on these systems is temperature. By looking specifically at denitrification in each system, we can clearly see how temperature had a greater impact on denitrification in the VDWB than the other two systems.

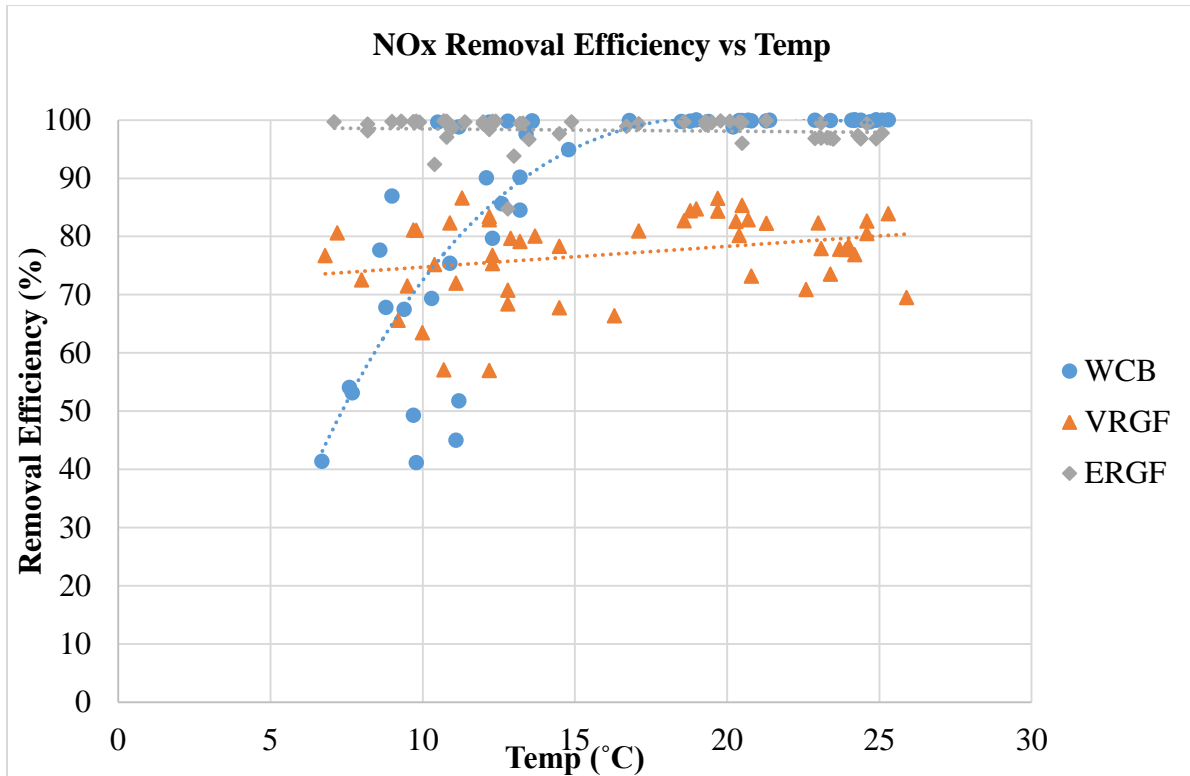


Figure 1. NO<sub>x</sub> Removal efficiency of the VDWB, VRGF, and ERGF systems

This graph shows the NO<sub>x</sub> removal efficiency compared to temperature for each system. The overall NO<sub>x</sub> removal efficiency in the VDWB, VRGF, and ERGF were 88%, 77%, and 98% respectively. In the summer, both the VDWB and ERGF had almost complete NO<sub>x</sub> removal at 99% and 98%, respectively, while the VRGF only had 77% removal. In the winter NO<sub>x</sub> removal in the VDWB dropped, down to only 72% whereas the ERGF and VRGF remained stable at 99% and 76% NO<sub>x</sub> removal, respectively.

In the summer months the VDWB outperformed the ERGF in overall TN removal by 17%, even though denitrification efficiency for both systems was very similar. This was because nitrification in the VDWB system was more complete at 99% whereas the ERGF only nitrified 86% of the influent. In the winter months the overall TN removal of the VDWB dropped 12%,



which was due to the 27% drop in denitrification efficiency. The ERGF saw no drop in either TN or NO<sub>x</sub> removal in the winter.

There are two hypothesis we are investigating to explain these results. One, is that in the winter months the carbon from the woodchips leach at a lower rate causing a reduction in the available electron donors, essentially starving the microbes. The second hypothesis is that the higher quality electron donors in the VRGF and ERGF systems causes the microbial processes in these systems to remain less temperature sensitive than the lower quality electron donors in the woodchip bed system. It is well-known that microbial activity slows down as temperatures lower. In the summer months average temperatures measured from the effluents of the VDWB and ERGF were about 21 °C. In the winter, the average temperatures in the systems dropped to about 11 °C. Because the temperatures in the different systems were nearly identical, it is unlikely that temperature was solely responsible for the decreased winter performance of the VDWB.

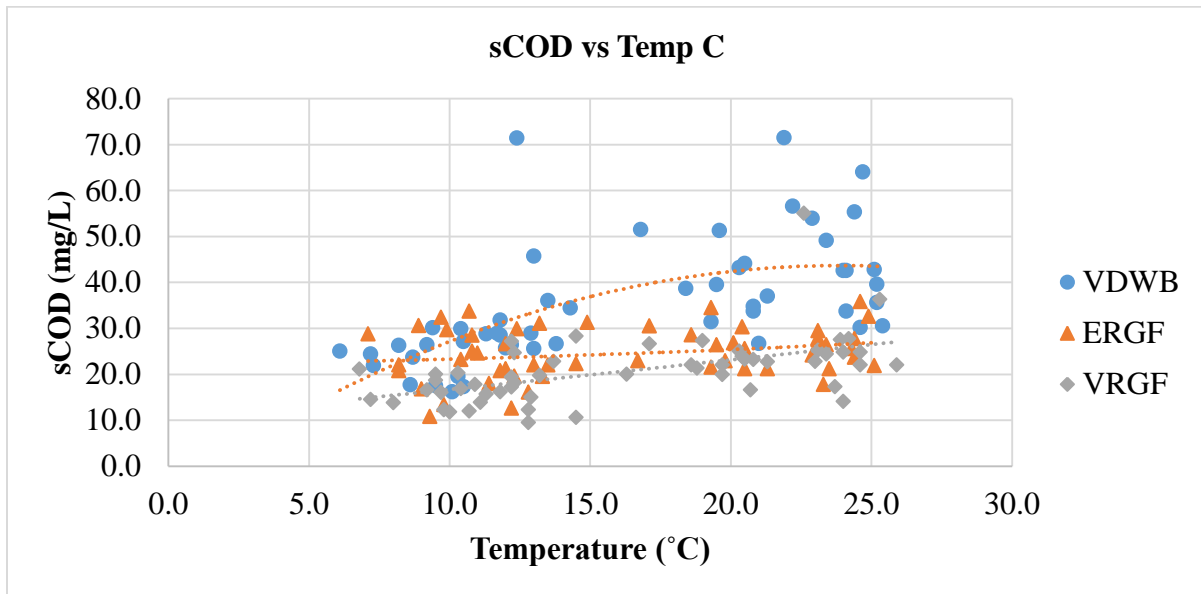


Figure 2. sCOD in the VDWB, ERGF, and VRGF systems

In the summer months the average sCOD in the VDWB and ERGF systems were measured to be 42.2 mg L<sup>-1</sup> and 25.0 mg L<sup>-1</sup>, respectively, in the winter months the average sCOD in the VDWB dropped down to 26.0 mg L<sup>-1</sup> while the average sCOD in the ERGF was nearly the same at 24.2 mg L<sup>-1</sup>. It is clear that temperature has an effect on the sCOD released from the woodchips. However the sCOD concentration in the VDWB were still quite similar to the concentrations in the ERGF.

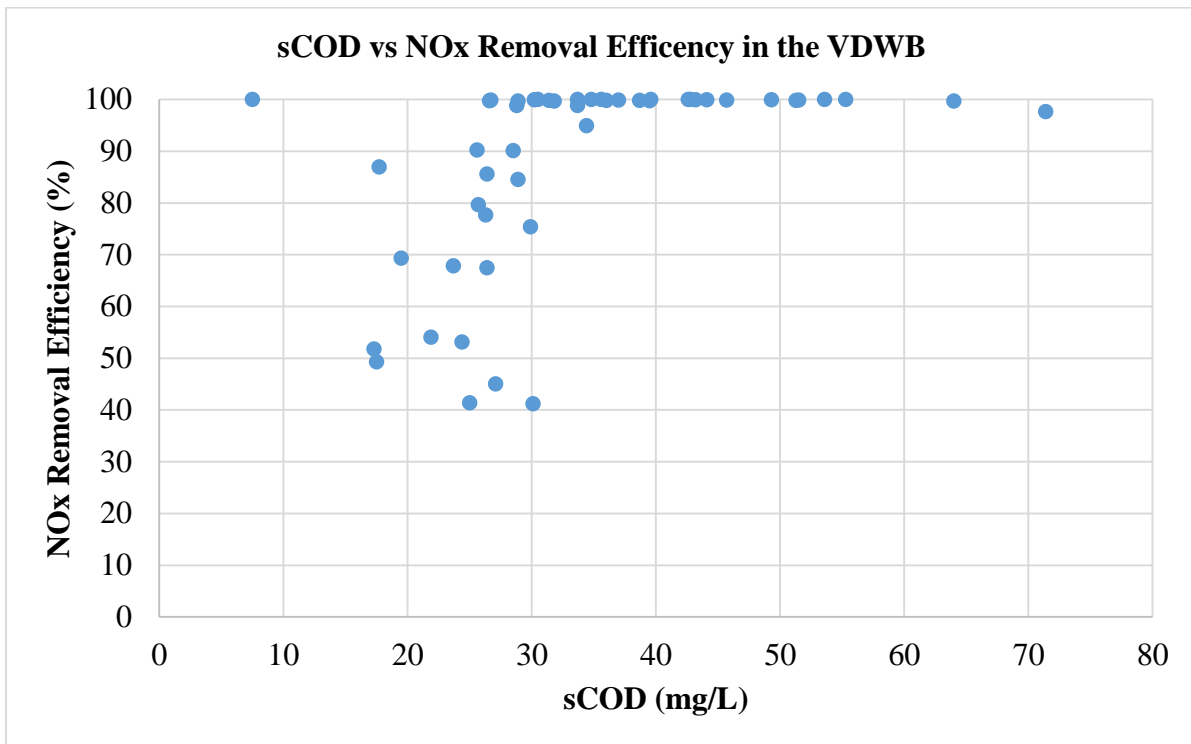


Figure 3. sCOD compared to NO<sub>x</sub> Removal efficiency in the VDWB

Figure 3 shows the sCOD in the VDWB vs NO<sub>x</sub> Removal efficiency. A critical point can be observed at 30 mg L<sup>-1</sup> of sCOD. Above 30 mg L<sup>-1</sup> NO<sub>x</sub> removal efficiency consistently remained at 99%. Below this critical point, there was little correlation between NO<sub>x</sub> removal and sCOD. If sCOD were the limiting factor, a stronger trend between the two would be expected. This suggests that a lack of sCOD alone is not the only cause for the VDWB's temperature sensitivity.

Microorganisms are only able to utilize the bioavailable fraction of the substrate. Though sCOD is a useful indicator, BOD<sub>5</sub> is a more representative of bioavailable substrate.

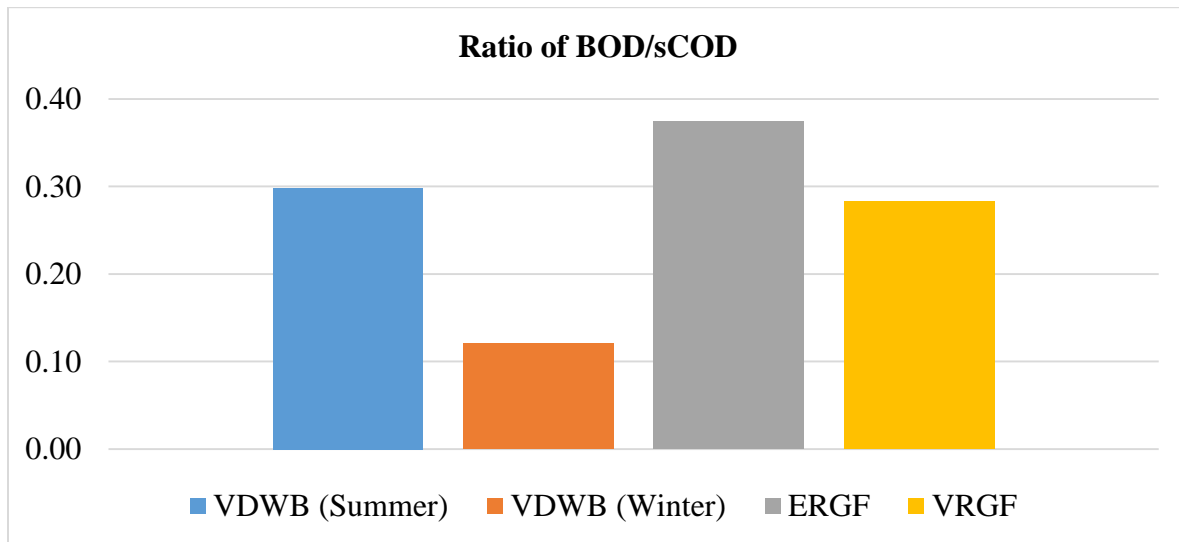


Figure 4. Ratios of BOD<sub>5</sub> and sCOD

In the summer months BOD<sub>5</sub> averaged to be 15.9 mg L<sup>-1</sup> in the VDWB and 8.7 mg L<sup>-1</sup> in the ERGF. In the winter BOD<sub>5</sub> dropped down to 3.5 mg L<sup>-1</sup> in the VDWB, but was unchanged in the ERGF at 8.4 mg L<sup>-1</sup>. This mirrors the sCOD measurements in that the VDWB saw a significant drop from summer to winter, while the ERGF remained stable. Therefore, during the winter, although sCOD concentrations were similar between the two systems, a larger percentage of the available substrate was biologically available in the ERGF. This suggests that the quality of the sCOD is higher in the ERGF.

### **The Hood Canal Woodchip Bed Denitrification Systems**

The Hood Canal systems were installed to test woodchip bed denitrification in an onsite septic system. The systems were initiated August 13<sup>th</sup>, 2014. The start-up period ended 4 weeks later on September 10<sup>th</sup>. The first set of grab samples was collected on September 25<sup>th</sup>.

## Nitrogen Removal

Table 3. Average Total Nitrogen ( $\text{mg L}^{-1}$ ) in the Hood Canal systems

	Septic Tank	RGF	Woodchip Bed	Percent N Removal
<b>Woodcock Inn</b>				
Average	86.3	43.1	28.5	65.2%
Std. Dev.	34.1	6.9	10.8	
<b>Salmon Center</b>				
Average	103.0	45.9	32.2	67.4%
Std. Dev.	64.0	18.5	17.1	

On average, the two Hood Canal systems performed similarly over the course of sampling. The Salmon Center had higher Total Nitrogen (TN) input concentrations than did the Woodcock system at  $103.0$  and  $86.3 \text{ mg L}^{-1}$ , respectively. The overall removal efficiency for both systems was just over 65%, with an average of  $32.2 \text{ mg L}^{-1}$  TN in the Salmon Center system's effluent and  $28.5 \text{ mg L}^{-1}$  TN in the Woodcock system's effluent. Peak removal in both system occurred in September 2014, when each system reduced TN by 86%.<sup>(Table TN)</sup> This was also the warmest of the sampling events with temperatures of  $18 \text{ }^\circ\text{C}$ . During the warmer months, where WCB temperatures at the Woodcock Inn and Salmon Center averaged to be  $14.1 \text{ }^\circ\text{C}$  and  $15.7 \text{ }^\circ\text{C}$ , respectively, TN removal increased to 75% and 78%. When the weather was cooler, with temperatures averaging  $9.1 \text{ }^\circ\text{C}$  and  $10.0 \text{ }^\circ\text{C}$ , TN removal declined to 57% and 59% in the Woodcock and Salmon Center systems, respectively. Overall TN removal in the Hood Canal systems was much less than the Snoqualmie VDWB system which on average had 92% removal.

Table 4. Average NO<sub>x</sub>-N (mg L<sup>-1</sup>) in the Hood Canal systems

	Septic Tank	RGF	Woodchip Bed	Percent NO <sub>x</sub> Denitrified
<b>Woodcock Inn</b>				
Average	0.42	34.1	20.8	39.3%
Std. Dev.	0.41	6.9	12.8	
<b>Salmon Center</b>				
Average	0.37	32.7	19.0	45.2%
Std. Dev.	0.49	13.4	10.7	

The lower TN removal in the Hood Canal systems' becomes obvious in the denitrifying woodchip bed stage of sampling. Denitrification efficiency in the Woodcock and Salmon Center systems was measured to be 39% and 45%, respectively. The overall denitrification efficiency of the Hood Canal system's was far less than the cold weather denitrification efficiency of the Snoqualmie VDWB which was 72%. In the warmer months, denitrification efficiency increased to 63% in the Woodcock system and 55% in the Salmon Center system. In the colder months, denitrification efficiency was greatly impacted by the lower temperatures. In the Woodcock system denitrification efficiency fell down to 20% and in the Salmon Center system a drop down to 38% was observed.

Table 5. Average NH<sub>4</sub><sup>+</sup> (mg L<sup>-1</sup>) in the Hood Canal systems

	Septic Tank	RGF	Woodchip Bed	Percent NH <sub>4</sub> <sup>+</sup> Nitrified
<b>Woodcock Inn</b>				
Average	67.7	6.5	7.2	90.5%
Std. Dev.	10.1	2.7	3.1	
<b>Salmon Center</b>				
Average	62.9	9.8	8.5	88.8%
Std. Dev.	37.3	8.7	8.0	

The RGF's in both systems performed below expectations. Both Hood Canal systems nitrified about 90% of the  $\text{NH}_4^+$  discharged from the septic tanks. Temperature made little difference in nitrification performance. The Woodcock system nitrified 91% of the  $\text{NH}_4^+$  in the warmer months and 90% in the cooler ones, while the Salmon Center system nitrified 90% of  $\text{NH}_4^+$  in the warmer months and 88% in the cooler ones. Nitrification in the Hood Canal systems was lower than the Snoqualmie VDWB system, which nitrified 99% of the  $\text{NH}_4^+$ . The difference in RGF performance is most likely due to the uneven and intermittent dosing that happens in the real world as opposed to a controlled testing environment as was the case for the Snoqualmie study.

## Secondary Analytes

Table 6. Average TSS/BOD<sub>5</sub>/FC (mg L<sup>-1</sup>, mg L<sup>-1</sup>, CFU's) in the Hood Canal systems

	Septic TSS	WCB TSS	Percent Removal	Septic BOD <sub>5</sub>	WCB BOD <sub>5</sub>	Percent Removal	Septic FC	WCB FC	Percent Removal
<b>Woodcock</b>									
Average	71.5	1.1	98%	70.8	< 2	97%	200000**	392.9	99.8%
Std. Dev.	15.9	0.18		10				501.3	
<b>Salmon</b>									
Average	26.3	1.0	96%	25.4	< 2	92%	200000**	143.3	99.9%
Std. Dev.	6.3	0.14		5.49				57.6	

\*September data omitted due to inflated measurements during system start-up

\*\*Fecal Coliforms (FC) samples were not properly diluted, actual values were greater than 200000

Average total suspended solids (TSS) concentration discharged from the septic tanks was 72 mg L<sup>-1</sup> in the Woodcock system and 26 mg L<sup>-1</sup> in the Salmon Center system. These systems' reduced TSS down to 1.1 and 1.0 mg L<sup>-1</sup>, for an average removal of 98% and 96%, respectively. BOD<sub>5</sub> in the septic tanks averaged to be 70.8 mg L<sup>-1</sup> in the Woodcock system and 25.4 mg L<sup>-1</sup> in the Salmon Center system. BOD<sub>5</sub> was reduced down to 36 and 19 mg L<sup>-1</sup>, respectively for an average removal efficiency of 97% and 92%, respectively. Fecal Coliform removal in both

systems was 99.8% beginning with over 200000 CFU's discharged from the septic tanks and being reduced down to 392.9 CFU's in the Woodcock system and 143.3 CFU's in the Salmon Center system. (These values cannot be directly compared to the VDWB because in that system the influent was measured prior to entering the septic tanks whereas the Hood Canal system's only measured the wastewater after it was discharged from the septic tank.)

### System Performance

The primary reason for poor system performance was the low levels of carbon availability in the woodchip bed. Throughout the testing period, with the exception of the first month's sampling, BOD exiting the woodchip beds was below 2 mg/L. This indicates that most of the electron donors in the woodchip bed's had been used up, limiting denitrification.

Table 7. Monthly temperatures (°C) during system operation (Weather Underground, 2015)

	Snoqualmie (2012-13)	Hood Canal (2014-15)
July	66	66
August	69	66
September	63	61
October	54	54
November	48	43
December	43	41
January	38	42
February	44	46
March	47	47
April	50	47
May	58	55
June	64	63
Average	53.7	52.6

A potential reason for the low carbon availability and the system's overall poor performance might be that there was too short of a start-up time in warm weather. The Hood Canal systems were installed in August 2014, and sampling began in September. Temperatures cooled down quickly, and by November effluent temperatures were consistently measured at 10 °C. The

Snoqualmie system on the other hand had a longer period of warm weather during the system's initiation. Start-up in the Snoqualmie VDWB began in July with sampling beginning in August. Effluent temperatures were consistently measured over 20 °C and did not drop below 10 °C until December.

### Grab Sample Representativeness

This study relied on grab samples for analysis. Grab samples consist of a single sampling event that is then used to represent the entire system's performance. It is fair to question the overall representativeness of using grab samples compared to something like composite sampling. In order to examine how representative the grab samples were of the systems' diurnal fluctuations, a small study was done. Five samples were collected at the Salmon Center system over a five hour period. The first sample was taken at 10:30 am with the last sample being taken at 3:30 pm.

Table 8. Grab Sample Variation in the Salmon Center system

	NO <sub>x</sub> -N (mg L <sup>-1</sup> )			NH <sub>3</sub> -N (mg L <sup>-1</sup> )		
	Septic	RGF	WCB	Septic	RGF	WCB
10:30 am	0.10	47.0	22.3	93.3	8.8	6.9
12:00 pm	0.14	51.3	27.0	75.7	11.0	7.0
1:00 pm	0.43	41.0	30.6	71.5	11.7	7.2
2:00 pm	0.12	47.7	32.1	74.6	13.5	7.2
3:30 pm	0.06	47.6	34.6	80.3	12.7	7.4
Average	0.17	46.9	29.5	79.1	11.5	7.1
Std. Dev.	0.15	3.7	4.9	8.5	1.8	0.21
CV %	85.9%	7.9%	16.6%	10.8%	15.8%	3.1%

The septic tank NO<sub>x</sub> variation is not relevant because the measurements were made very close to the method's detection limit, which causes relatively speaking, large method sensitivity even though absolute uncertainty was very low, ± 0.15 mg L<sup>-1</sup>. The next two largest variations were for NO<sub>x</sub> in the WCB and NH<sub>3</sub> in the RGF at 16.6% and 15.8%, respectively. The results show that the grab samples are generally representative of the overall system performance.



## **Benchtop Experiment**

A benchtop experiment was designed to compare denitrification using carbon from wastewater as an electron donor versus the carbon from woodchip leachate. The purpose of this experiment was to test the hypothesis that the carbon used for denitrification in WCBs is more recalcitrant and has a higher activation energy. Therefore biological degradation is slower compared to the carbon found in standard wastewater. Bottles of each media spiked with  $\text{NO}_3^-$  were placed in a warm chamber to represent the summer months and a cool chamber to represent the winter months. Subsequent  $\text{NO}_3^-$  and sCOD removal was monitored over time.

### **Media Preparation**

The synthetic wastewater media was created based on the Organization for Economic Cooperation and Development guidelines (OECD 2009). The media includes the basic inorganic elements required for microbial growth and uses peptone created from animal tissue and meat extract to provide the carbon source. Synthetic wastewater was used instead of actual wastewater so that the two media's contained the same inorganic constituents and only differed when it came to the carbon source. The woodchip media was created by autoclaving about 600 mL of woodchips in 1 L of DI water for 1 hour. The concentrated woodchip leachate was then diluted down to the same sCOD levels as the synthetic wastewater. Six liters of each media were prepared in total.

Table 9. Constituents used in synthetic wastewater and woodchip medias

	Synthetic Wastewater	Woodchip Media
CaCl <sub>2</sub> , 2H <sub>2</sub> O	4 mg L <sup>-1</sup>	4 mg L <sup>-1</sup>
MgSO <sub>4</sub> , 7H <sub>2</sub> O	2 mg L <sup>-1</sup>	2 mg L <sup>-1</sup>
K <sub>2</sub> HPO <sub>4</sub>	28 mg L <sup>-1</sup>	28 mg L <sup>-1</sup>
KNO <sub>3</sub> -N	49.8 mg L <sup>-1</sup>	42.2 mg L <sup>-1</sup>
Alkalinity (HCO <sub>3</sub> <sup>-</sup> )	1 meq L <sup>-1</sup>	1 meq L <sup>-1</sup>
Peptone	90 mg L <sup>-1</sup>	-
Meat Extract (Total Combined sCOD)	60 mg L <sup>-1</sup> (258.1 mg L <sup>-1</sup> )	-
Woodchip Leachate sCOD	-	232 mg L <sup>-1</sup>
pH	7.45	6.75

#### Bottle Preparation

BOD test bottles were used in these experiments. These are clear glass bottles, 300 mL in size, with stopper tops that prevent head space when the experiments are initiated. These bottles were chosen because they were able to retain low DO concentrations over the testing period. In each bottle, two 4 cm x 4 cm fibrous inorganic pads designed for aquarium filters was included to provide media for denitrifiers to grow on. The initial wastewater media was sparged with N<sub>2</sub> gas to reduce DO levels below 0.50 mg L<sup>-1</sup>. The bottles were filled 75% of the way, where they were individually sparged again to remove any DO that may have accumulated in the process of filling up the bottles. The bottles were then seeded with 2 mL of wastewater from the Snoqualmie WWTP's anaerobic denitrifying zone to promote denitrifying bacteria growth. The bottles were then filled to the top and sealed with a stopper. Nine bottles with each media type were placed in a chamber with an average temperature of 24.1 °C and a chamber with an average temperature of 10 °C for a total of 36 bottles. The bottles were kept in a dark environment and were not opened prior to sampling.

## Sampling and Analyses

Initial sampling was done directly from the bulk media solutions. Three samples were taken from each media for both NO<sub>x</sub> and sCOD analyses, this was the day 0. There were three subsequent sampling events, days 2, 6, and 12. On each of these days, three bottles from each media type, and temperature treatment were sampled. Each bottle was only sampled once. During sampling 5 mL was drawn from each bottle. The sample was then pushed through a BD 60 mL Luer-Lok Tip Syringe with a 0.45 µm PES membrane Millex-HP syringe driven filter. NO<sub>x</sub> analysis followed the same procedures as previously described. Hach low range COD digestion vials were used to determine sCOD in the samples.

## Results

Results of the benchtop experiment show a significant difference in the NO<sub>x</sub> removal for both temperature treatments and media types.

Table 10. Average sCOD and NO<sub>x</sub>-N (mg L<sup>-1</sup>, mg L<sup>-1</sup>) measured from benchtop experiment

Media Type		Day 0		Day 2		Day 6		Day 12	
		sCOD	NO <sub>x</sub>	sCOD	NO <sub>x</sub>	sCOD	NO <sub>x</sub>	sCOD	NO <sub>x</sub>
24.1 °C Woodchip	Average	232	42.2	194	31.2	128	14.9	102	4.7
	Std. Dev.	13.9	1.8	4.1	1.3	7.8	1.7	5.0	4.3
10 °C Woodchip	Average			237	42.1	164	30.1	154*	25.8*
	Std. Dev.			5.9	0.73	4.7	0.2	3.25	1.07
24.1 °C Synthetic	Average	258	49.8	84.5	1.0	77.7	2.0	92.4	0.5
	Std. Dev.	7.9	1.2	23.7	0.2	9.6	0.3	30.7	0.5
10 °C Synthetic	Average			254	46.2	170	24.7	109	10.7
	Std. Dev.			4.5	0.7	15.1	8.4	7.9	11.4

\*One of the three bottles showed no NO<sub>x</sub> removal and was omitted from data

The day 2 sampling showed very little NO<sub>x</sub> removal in either of the cold samples, 26% removal in the warm woodchip samples, and almost complete removal (98%) from the warm synthetic samples. Day 6 sampling showed significant removal in all sets of bottles. In the warm woodchip samples 65% of NO<sub>x</sub> was removed. The cold woodchip and synthetic samples had 29% and 50% NO<sub>x</sub> removal respectively. Day 12 sampling showed continued NO<sub>x</sub> removal in the warm

woodchip and cold synthetic samples at 89% and 79% removal respectively. While the cold woodchip samples remained constant at 28%. Overall the warm synthetic samples removed the  $\text{NO}_x$  immediately whereas the other samples required more time for removal and still had not reached maximum removal at the end of the 12 day experiment.

Both warm samples saw the steepest change in  $\text{NO}_x$  between day 0 and day 2. The warm synthetic samples showed quick and complete degradation of  $\text{NO}_x$  outpacing warm woodchip removal by  $37.8 \text{ mg L}^{-1}$ . The cold sample data shows that a longer startup period was required for the denitrifier biomass to build up before  $\text{NO}_x$  removal could match the pace of the warmer samples. Between days 2 and 6,  $\text{NO}_x$  removal in the cooler samples was its greatest. Cold synthetic samples showed the greatest removal in this time period with a removal of  $21.5 \text{ mg L}^{-1}$  followed by the warm woodchip samples at  $16.3 \text{ mg L}^{-1}$  removal and cold woodchip samples at  $12.0 \text{ mg L}^{-1}$ . Between days 6 and 12  $\text{NO}_x$  removal in the cold synthetic samples was the greatest at  $14.1 \text{ mg L}^{-1}$  followed by removal in the warm woodchip samples at  $10.2 \text{ mg L}^{-1}$  and the cold woodchip samples at  $4.2 \text{ mg L}^{-1}$ .

### **Hypothesis Discussion**

- Hypothesis 1- Lower temperatures inhibit the release of carbon from the woodchips, and therefore the lower N removal rates found in the VDWB were due to less available carbon.
- Hypothesis 2 - The carbon used for denitrification in the VDWB is more recalcitrant and has a higher activation energy, and denitrification is therefore less efficient compared to the denitrification in the ERGF.

## Hypothesis 1

The Grinnell and Wei studies along with the Hood Canal systems show that when temperatures drop, carbon released from the woodchips becomes less available (Grinnell, 2013; Wei, 2013).

The Snoqualmie VDWB discharged effluent with sCOD concentrations of  $42.2 \text{ mg L}^{-1}$  in the summer months, and  $26.0 \text{ mg L}^{-1}$  in the winter months, with BOD<sub>5</sub> concentrations mirroring this phenomenon at  $15.9 \text{ mg L}^{-1}$  in the summer and  $3.5 \text{ mg L}^{-1}$  in the winter. The Hood Canal systems had consistent BOD<sub>5</sub> measurements of less than  $2 \text{ mg L}^{-1}$  across both systems throughout the sampling period. This indicates less carbon availability because denitrification efficiency drops in cooler temperatures. If carbon output from the woodchips remained the same, yet denitrification kinetics decreased, then effluent BOD<sub>5</sub> measurements should increase in cooler temperatures. But because BOD<sub>5</sub> decreased instead, it can be reasoned that carbon released decreased as well. The lower temperatures did decrease carbon release from the woodchip beds. This had a direct impact on the availability of electron donors in the VDWB system which negatively impacted denitrification.

## Hypothesis 2

The woodchips produce a cellulose-based carbon product as it leaches out into the bed (Liu et al., 2013). This carbon source is inherently more difficult for the microbes to process compared to carbon found in wastewater as observed in the benchtop experiment.

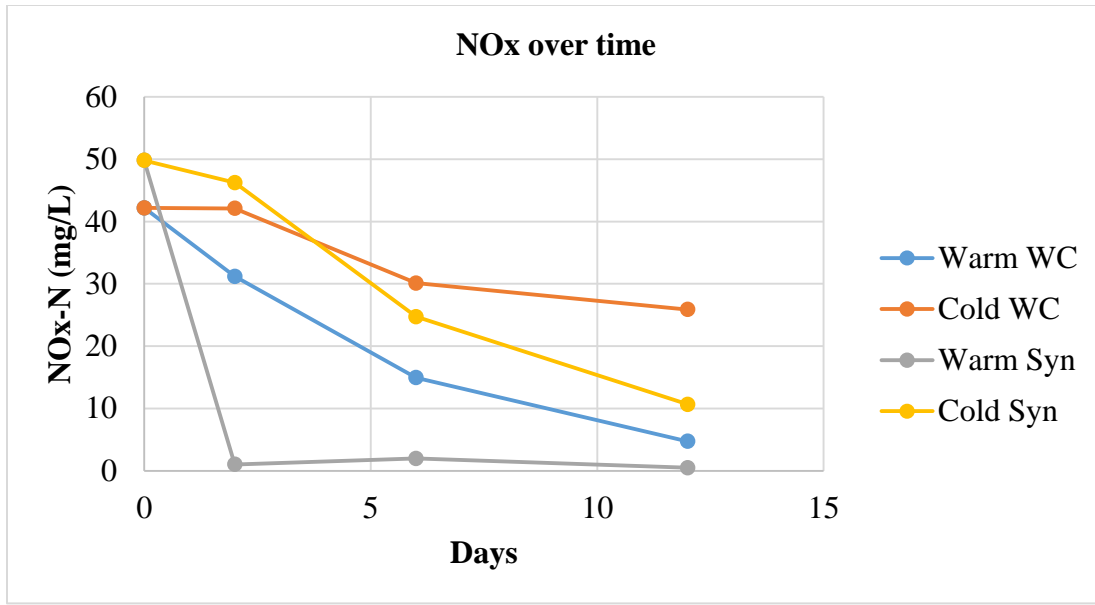


Figure 5. Average NO<sub>x</sub>-N in benchtop experiment

In this experiment, the warm synthetic samples showed very quick and complete removal of NO<sub>x</sub> by day 2. The only other sample with NO<sub>x</sub> removal on day 2 greater than 5 mg L<sup>-1</sup> was the warm woodchip treatment. After day 2 however, the cold synthetic samples removed NO<sub>x</sub> at a faster rate than the warm woodchip samples, with measured total NO<sub>x</sub> removal of 25.1 mg L<sup>-1</sup> by day 6 and 39.1 mg L<sup>-1</sup> by day 12; whereas warm woodchip samples had measured total NO<sub>x</sub> removal of 27.2 mg L<sup>-1</sup> by day 6 and 37.5 mg L<sup>-1</sup> by day 12. This shows that although the colder temperatures induced some lag in denitrifier growth at the start, the synthetic wastewater provided a better carbon source for denitrification. However the results do not indicate woodchip based carbon is more sensitive to cold. Day 6 NO<sub>x</sub> removal rates in the woodchip treatments were 4.5 mg L<sup>-1</sup>d<sup>-1</sup> in the warm and 2.0 mg L<sup>-1</sup>d<sup>-1</sup> in the cold with day 12 NO<sub>x</sub> removal rates measured at 3.1 mg L<sup>-1</sup>d<sup>-1</sup> in the warm and 1.4 mg L<sup>-1</sup>d<sup>-1</sup> in the cold. The average difference between the warm and cold treatments was 1.5 mg L<sup>-1</sup>d<sup>-1</sup>. The difference in NO<sub>x</sub> removal rates for the synthetic wastewater treatments was much more dramatic. After two days the NO<sub>x</sub>

removal rate in the warm synthetic treatments was  $24.4 \text{ mg L}^{-1}\text{d}^{-1}$ . The  $\text{NO}_x$  removal rates in the cold synthetic treatments was  $4.2 \text{ mg L}^{-1}\text{d}^{-1}$  after day 6 and  $3.3 \text{ mg L}^{-1}\text{d}^{-1}$  after day 12. The difference in  $\text{NO}_x$  removal rates was over  $20 \text{ mg L}^{-1}\text{d}^{-1}$ . Based on these results it seems that denitrifiers in this instance preferred the synthetic treatments, but there was also a much larger temperature sensitivity observed.

## **Conclusion**

This study aimed to answer the question of why the two step VDWB system was more temperature dependent than its one step denitrifying ERGF counterpart. To do this, two hypotheses were tested. The first hypothesis was that the lower temperatures inhibited the release of carbon from the woodchips, and the lower N removal rates are a function of that. This hypothesis was found to be true. The second hypothesis was that the carbon used for denitrification in the VDWB, is more recalcitrant, has a higher activation energy, and denitrification is therefore less efficient. This hypothesis was found to be inconclusive. Though benchtop experiment results suggest carbon from wastewater was a more desired substrate than carbon from woodchips, they also indicate that wastewater carbon was more sensitive to temperature differences than a woodchip based carbon.

Both processes (i.e. lower electron donor availability and quality) play a role in the temperature sensitivity of these systems. In fact, the effect on woodchip bed denitrification in low temperatures is threefold. Carbon leaching from the woodchips slows down as temperatures decrease. Woodchip carbon is a lower quality electron donor, however it is not certain if this inherently means this causes denitrification to be more temperature sensitive. Combine the quantity and quality findings with the fact that microbial processes inherently slow down as temperatures drop, and it becomes clear why temperature has such a large impact on this

particular type of system. The woodchip bed system is still very promising for removing N from point source discharges, but it is important to understand its strengths and weaknesses compared to other types N removal systems.

### **Suggested improvements to Hood Canal System design**

There are a few possible strategies for combating WCB temperature sensitivity. Any potential method would have to be as un-invasive as possible because one of the major advantages associated with WCB systems is their low cost and maintenance requirements (Leverenz et al., 2010). Increasing the contact time of woodchip beds would benefit denitrification by allowing more time for the woodchips to release carbon and for denitrifiers to remove nitrates, however this method would increase both cost of installation and the size of the system's footprint. The Snoqualmie VDWB had an empty bed contact time (EBCT) of 2.9 days and actual contact time of 1.2 days. The Woodcock and Salmon Center WCBs had an average EBCT of 3.6 days and 2.3 days, respectively. Assuming a media porosity of 40%, the actual contact time was calculated to be 1.5 days and 0.9 days. This is interesting because the Salmon Center has the smaller woodchip bed and less contact time, yet it denitrifies more efficiently than the Woodcock. Another potential improvement to help promote nitrate removal is adding plant species, such as cattails (*Typha latifolia*), to the denitrifying woodchip bed (Zhang et al., 2011). Plants are thought to increase N removal through root uptake and by promoting the release of carbon from the woodchip media. One negative effect of plants in these systems is that the roots may reduce hydraulic conductivity in the woodchip bed thereby reducing the contact time of the system and lowering time available for denitrification to occur. The cattails added to the Snoqualmie VDWB did not cause too much clogging in the system as their roots did not reach the entire depth of the bed (A. Jones, past observation).



Adding temporary media during the colder months that would be more labile for bacteria than organic matter released by woodchips and would therefore provide the systems with a temporary carbon boost. More labile sources of carbon like maize cobs and wheat have shown the potential for greater N removal rates than woodchip products (Cameron et al., 2010; Warneke et al., 2011), but are more susceptible to degradation and therefore have shorter longevity (Sailing et al., 2007). This source is also difficult to integrate into the current design logistically. The temporary media would need to be added near the beginning of the woodchip bed, and then would need to be done in such a way as to not clog the system.

A simple method for improving denitrification would be to use an easily accessible form of carbon, such as methanol, to supplement the system. Not only would this provide the extra quantity required for more complete denitrification, but through a property known as “priming”, it may improve utilization of the lower quality woodchip carbon (Kuzyakov, 2010). The priming effect describes the phenomenon of microbial utilization of lower quality substrates improving when a higher quality substrate is introduced to the system.

Table 11. Secondary Carbon Source Calculations

	Woodcock Inn	Salmon Center
Winter NO <sub>x</sub> -N concentrations entering the WCB (mg L <sup>-1</sup> )	37.4	43.1
Winter NO <sub>x</sub> -N concentrations exiting the WCB (mg L <sup>-1</sup> )	29.5	26.8
Design Flows (gpd)	480	240
Winter NO <sub>x</sub> -N flux entering the WCB (g d <sup>-1</sup> )	68.0	39.2
Winter NO <sub>x</sub> -N flux exiting the WCB (g d <sup>-1</sup> )	53.6	24.4
Labile C flux required (g d <sup>-1</sup> )	107	48.7
Methanol flux required (g d <sup>-1</sup> )	286	130
Monthly Volume (gal month <sup>-1</sup> )	2.9	1.3

In Table 11 the NO<sub>x</sub> flux entering and exiting the woodchip beds was calculated from the data and design flows. Using a ratio of 2 g of carbon per 1 g of NO<sub>x</sub> (Dawson and Murphy, 1971), the required daily carbon to remove the remaining NO<sub>x</sub> was calculated as 107 g d<sup>-1</sup> for the Woodcock system and 48.7 g d<sup>-1</sup> for the Salmon Center. This converts to a monthly average dose of 2.9 gallons per month for the Woodcock system, and about 1.3 gallon per month for the Salmon Center system. Current methanol prices are about \$1.25 per gallon (Methanex, 2015). The monthly methanol cost for both systems would be less than \$5.25. A low flow, continuous dosing system could be installed so that the methanol is steadily provided to the systems. This would supplement the woodchip carbon and could greatly improve denitrification.

### Future Work

- Continued monitoring of real world WCB systems including the continued monitoring of the Hood Canal systems studied in this report. I predict that N removal in these systems will increase greatly during the warm weather period of the summer of 2015.

- Continued testing and monitoring of single step RGF systems for N removal. Although not as promising in terms of overall N removal, these system show potential (Grinnell 2013; Wei 2013) especially due to temperature having less impact on their performance compared to WCB systems.
- More detailed study of different woodchip types and their sensitivity to temperatures. Different types of woodchips may have differing activation energies, and therefore, differing temperature sensitivities.
- Testing modified performance with the addition of methanol during the winter months.

## Works Cited

- American Public Health Association; American Water Works Association, 2005. *Water Environment Federation Standard Methods for the Examination of Water & Wastewater*; 21st ed.; American Public Health Association: Washington, D.C.
- Bosatta, E., Agren G.I., 1999. Soil organic matter quality interpreted thermodynamically. *Soil Biology and Bio Chemistry*. 31, 1889-1891.
- Cameron, S.G., Schipper, L.A., 2010. Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. *Ecol. Eng.* 36, 1588-1595.
- Cameron, S.G., Schiper, L.A., 2012. Hdraulic properties, hydraulic efficiency and nitrate removal of organic carbon media for use in denitrification beds. *Ecol. Eng.* 41, 1-7.
- Craine, M.J., Fierer, N., McLauchlan, K., 2010. Widespread coupling between the rate and temperature sensitivity of organic matter decay. *Nature Geoscience*. 3, 854-857.
- Crites, R., 1998. *Small and Decentralized Wastewater Management Systems*, McGraw-Hill series in water resources and environmental engineering; WCB/McGraw-Hill: Boston.
- Davidson, E. A., Janssens, I. A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*. 440, 165-173.
- Dawson, R.N., Murphy, K. L., 1971. The temperature dependency of biological denitrification. *Water Research*. 6, 71-83.
- Greenan, C.M., Moorman, T.B., Kaspar, T.C., Jaynes, D.B., 2006. Comparing carbon substrates for denitrification of subsurface drainage water. *J. Eniro. Qual.* 35, 824.
- Grinnell, C., 2013. Innovative onsite wastewater treatment systems for nitrogen removal: a recirculating gravel filter with a preanoxic zone and a recirculating gravel filter with a postanoxic woodchip bed. Master's Thesis, University of Washington, Seattle, Washington.

- Kellog, D.Q., Gold, A.J., Cox, S., Addy, K., August, P.V., 2010. In review. A geospatial approach for assessing denitrification sinks at the local level. *Ecol. Eng.* 36, 1596-1606.
- Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Bio Chemistry.* 42, 1363-1371.
- Leverenz, H., Haunschild, K., Hopes, G., Tchobanoglous, G., Darby J.L., 2010. Anoxic treatment wetlands for denitrification. *Ecol. Eng.* 36, 1544-1551.
- Liu, F., Huang, G., Fallowfield, H., Guan, H., Zhu, L., Hu, H., 2013. Study on heterotrophic autotrophic denitrification permeable reactive barriers for in situ groundwater remediation. Springer Briefs in Water Science and Technology. 103.
- Methanex, 2015. <https://www.methanex.com/our-business/pricing> (accessed August 2015)
- Moorman, T.B., Parkin, T.B., Kaspar, T.C., Jaynes, D.B., 2010. Denitrification activity, wood loss, and N<sub>2</sub>O emissions over 9 years from a woodchip bioreactor. *Ecol. Eng.* 36, 1567-1574.
- OECD, 2009. OECD Guidelines for the testing of chemicals.  
<http://www.oecd.org/chemicalsafety/testing/43735667.pdf> (accessed June 2015)
- Recirculating Gravel Filter Systems – 337-011.pdf  
<http://www.doh.wa.gov/Portals/1/Documents/Pubs/337-011.pdf> (accessed June 2015)
- Robertson, W.D., Cherry, J.A., Sudicky, E.A., 1991. Ground-water contamination from two small septic systems on sand aquifers. *Ground Water* 29, 82–92.
- Robertson, W.D., Ford, G.I., Lombardo, P.S., 2005. Wood-based filter for nitrate removal in septic systems. *American Society of Agricultural Engineers.*48, 121-128.
- Robertson, W.D., 2010. Nitrate removal rates in woodchip media of varying age. *Ecol. Eng.* 36, 1581-1587.
- Septic Systems Fact Sheet - septic\_systems\_factsheet.pdf  
[http://www.epa.gov/owm/septic/pubs/septic\\_systems\\_factsheet.pdf](http://www.epa.gov/owm/septic/pubs/septic_systems_factsheet.pdf) (accessed June, 2015).

Sailing, W.J.B., Westerman, P.W., Losordo, T.M., 2007, Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. *Aquacultural Engineering*. 37, 222-233.

Schipper, L., Cameron, S.C., Warneke, S., 2010. Nitrate removal from three different effluents using large scale denitrification beds. *Ecol.Eng.* 35, 1552-1557.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2014. Wastewater Engineering.: Treatment and Resource Recovery, McGraw-Hill, New York.

U.S EPA, 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100

U.S. EPA, 2002. Onsite Wastewater Treatment Systems, United States Environmental Protection Agency, EPA 600-R-00-008.

Weather Underground. <http://www.wunderground.com/> (accessed June 2015)

## **Appendix A1. PNW Salmon Center System Specifications**

The existing 1150 gallon two compartment concrete septic tank was pumped, cleaned, and leak tested prior to reuse in the new RGF/Woodchip Bed system. Effluent from the septic tank exits a riser and is pumped to a 1500 gallon two compartment recirculating tank where it is then pumped to the recirculating gravel filter. The RGF is 8' wide by 10' long with 2' of gravel. The gravel layer sits on top of 4" slotted collection pips with ¼" wide slots every 4". The RGF is loaded at a rate of 3 GPD/ft<sup>2</sup>. An OSI MVP S1 PT RO Panel operates the recirculation/mixing tank and doses 72 times per day at 20 gallons per dose. Effluent from the RGF is recirculated back through the system at a ratio of 6:1. The next step of the treatment system is the woodchip bed. The woodchip bed is 2' wide by 10.5' long with a depth of 42". RGF effluent enters the bed through a pressurized distribution system utilizing 14" EZFLOW bundles in order to evenly distribute the water to the bed. The outlet the woodchip bed is a stand pipe which holds the water level at 37". Following the woodchip bed the effluent is pumped to the existing 528' drainfield.

## **Appendix A2. Woodcock Inn System Specifications**

The existing 1500 gallon tank was pumped, cleaned, and leaked tested prior to reuse in the new RGF/Woodchip Bed system. Effluent from the septic tank exits a riser and is pumped to a 1500 gallon two compartment recirculating tank where it is then pumped to the recirculating gravel filter. The RGF is 8' wide by 20' long with 2' of gravel. The gravel layer sits on top of 4" slotted collection pips with ¼" wide slots every 4". The RGF is loaded at a rate of 3 GPD/ft<sup>2</sup>. An OSI MVP S1 PT RO Panel operates the recirculation/mixing tank and doses 72 times per day at 40 gallons per dose. Effluent from the RGF is recirculated back through the system at a ratio of 6:1. The woodchip bed is 3.5' wide by 19' long with a depth of 42". RGF effluent enters the bed through a pressurized distribution system utilizing 14" EZFLOW bundles in order to evenly distribute the water to the bed. The outlet the woodchip bed is a stand pipe which holds the water level at 37". Following the woodchip bed the effluent is pumped to the existing 300' pressurized drainfield.