

Anaerobic Digestion Enhancement: Impact on the Volatile Solids Reduction and Biogas
Production Within Municipal Anaerobic Digestion Systems Via Routine Bioaugmentation with a
Biocatalytic Microbial and Enzyme Consortium

By

Jonathan Lee

A Thesis Submitted to the Faculty of Social and Applied Sciences
in Partial Fulfilment of the Requirements for the Degree of

Master of Science in Environment and Management

Royal Roads University
Victoria, British Columbia, Canada

Supervisor: Mickie Noble
October 2020



Jonathan Lee, 2020

COMMITTEE APPROVAL

The members of Jonathan Lee's Thesis Committee certify that they have read the thesis titled Anaerobic Digestion Enhancement: Impact on the volatile solids reduction and biogas production within municipal anaerobic digestion systems via routine bioaugmentation with a biocatalytic microbial and enzyme consortium and recommend that it be accepted as fulfilling the thesis requirements for the Degree of Master of Science in Environment and Management:

Mickie Noble [signature on file]

Audrey Dallimore [signature on file]

Final approval and acceptance of this thesis is contingent upon submission of the final copy of the thesis to Royal Roads University. The thesis supervisor confirms to have read this thesis and recommends that it be accepted as fulfilling the thesis requirements:

Mickie Noble [signature on file]

Creative Commons Statement



This work is licensed under the Creative Commons Attribution-Non Commercial-ShareAlike 2.5 Canada License. To view a copy of this license, visit

<http://creativecommons.org/licenses/by-nc-sa/2.5/ca/>.

Some material in this work is not being made available under the terms of this licence:

- Third-Party material that is being used under fair dealing or with permission.
- Any photographs where individuals are easily identifiable.

Abstract

Wastewater Treatment Plants (WWTPs) frequently use anaerobic digestion (AD) to break down organics to reduce the total volume of biosolids produced. As population increases, cost of biosolids disposal increases while regulatory limits tighten. Bioaugmentation is an innovative process that enhances the biological activity within AD systems to improve performance through the addition of biocatalytic compounds (BC). Currently there is a knowledge gap regarding how the routine use of BCs, containing a consortium of bacteria and enzymes, applied directly within the AD system can affect the system's performance and its by-products (biogas and biosolids). This study reviews the impact of routine bioaugmentation applications using a commercial grade BC on an AD system. An analysis of two full-scale AD systems inoculated with said BC has been completed to determine impacts on biosolids, and biogas production. This study provides significant information substantiating the claim that bioaugmentation enhances AD performance and long-term economic viability.

Keywords: Anaerobic digestion, bioaugmentation, biocatalytic, biogas, wastewater, economics, sustainability, biosolids, bacteria, enzymes

Table of Contents

Thesis Title Page.....	1
Creative Commons Statement.....	3
Abstract.....	4
Table of Contents.....	5
List of Figures.....	8
List of Tables.....	8
List of Abbreviations.....	9
Acknowledgements.....	9
Introduction.....	10
Bacteria and Enzymes.....	13
Research Questions.....	14
Methodology and Procedures.....	15
Full Scale Approach.....	15
Thesis Study Site Participants.....	16
Operational Comparisons.....	17
Average Performance Without Treatment Comparisons.....	19
Lab Tests, Report and Data Collection.....	20
Technical Lab Data Analysis and Calculations.....	23
Van Kleeck Formula.....	25

Mass Balance Formula.....	26
Digital Surveys and Interviews.....	28
Milestones.....	28
Resource Requirements	29
Project Overview	30
Project Details.....	31
Dosage Protocols	33
Results.....	33
SLR.....	34
Volatile Solids Reduction	34
Biogas Production.....	36
SLO.....	44
Volatile Solids Reduction	44
Biogas production.....	46
Interviews.....	54
Interview Results	54
Discussion.....	56
Limitations	57
Results Summary	59
Lessons Learned in Anaerobic Digestion.....	63
Significance of Work	65
Future Work.....	66

Conclusions.....	67
Works Cited	69
Appendices.....	73
Appendix A – Plant Participation Authorization Forms.....	73
Appendix B – SLR – Raw Data.....	77
Appendix C – SLO – Raw Data.....	78
Appendix D – Interview Invitation Email	79
Appendix E – Interview Consent Forms.....	81
Appendix F – SLR and SLO Digital Surveys.....	85
Appendix G – Interview Questions.....	89
Appendix H – SLO – Interview Transcripts.....	91
Appendix I – SLR – Interview Transcripts.....	103
Appendix J – Tables.....	110

List of Figures

Figure 1a. AD system map with application locations at SLO.....	21
Figure 1b. AD system map with application locations at SLR.....	21
Figure 2. Percentage of VS reduced using Van Fleeck method at SLR	35
Figure 3. Organic loading rate kilograms of VS per day at SLR.....	36
Figure 4. Daily total biogas production cubic meters per day from SLR digesters.....	37
Figure 5. Specific biogas yield per kilogram of VSI at SLR.	38
Figure 6. Moving averages of the specific biogas production per VSI at SLR	39
Figure 7. Specific biogas yield per kilogram of VSD at SLR.	40
Figure 8. Moving averages of the specific biogas yield per kilogram of VSD at SLR	41
Figure 10. Daily total biogas production cubic meters per day from SLO digesters.....	47
Figure 11. Specific biogas yield per kilogram of VSI at SLO.....	48
Figure 12. 7 day moving averages of the specific biogas production per VSI at SLO.....	48
Figure 13. 30 day moving averages of the specific biogas production per VSI at SLO.....	49
Figure 14. Specific biogas yield per kilogram of VSD at SLO.	50
Figure 15. Hydrogen sulfide biogas production at SLO	51

List of Tables

Table 1. Wastewater Plant Operational Comparisons	19
Table 2. Reported System AD Performance Comparisons Prior to Bioaugmentation.....	20
Table 3. List of AD system performance tests.....	23
Table 4. Technical analysis calculations.....	27
Table 5. Itemized list of milestones	29
Table 6. AD enhancement dosage schedule at SLR	110
Table 7. AD enhancement dosage schedule at SLO	111
Table 8. AD comparisons between bioaugmented levels in treated to control at SLR.	42
Table 9. AD comparisons during seeding phase compared to augmented phase within the treated digester at SLR.....	42
Table 10. AD comparisons new bioaugmented levels compared to historical averages.....	52
Table 11. AD comparisons during seeding phase compared to after seeding phase within the treated digester.	53

List of Abbreviations

AD – Anaerobic digestion
BA – Bioaugmentation
BC – Biocatalytic compound(s)
BF – Biogas per cubic meter of feedstock
BVSD – Specific biogas yield per kilogram of volatile solids destroyed
BVSI – Specific biogas yield per kilogram of volatile solids introduced
F/M – Food to microorganism
HRT – Hydraulic retention time
JWPCP – Joint Water Pollution Control Plant
CMD – Cubic meters per day
OLR – Organic loading rate
KVSD – Kilograms of volatile solids destroyed
Q – Feed Rate
SLO – San Louis Obispo wastewater treatment plant
SLR – San Luis Ray wastewater treatment plant
TS – Total solids
U – Mass balance formula
USA – United States of America
V/A – ratio of volatile fatty acids to alkalinity
VK – Van Kleeck formula
VS – Volatile solids
VSI – Volatile solids introduced
VSD – Volatile solids destroyed
WAS – Waste activated sludge
WWTP – Wastewater treatment plant(s)

Acknowledgements

I would like to acknowledge SLO and SLR for their willingness to work with me and Acti-Zyme Products Ltd. for supporting the project. I would like to acknowledge Mickie Noble for supporting me with all the project delays, concerns and supporting the development of the project. Lastly, I would like to acknowledge my wife for all her support!

Introduction

Anaerobic digestion (AD) is a process used in wastewater treatment plants (WWTPs) throughout North America where sewage sludge, containing manure and other organics, is separated from wastewater, and is held in large covered tanks called digesters (Huoqing Ge, 2010). Anaerobic digesters create the optimum environment for the biodegradation of organic matter found in raw municipal sewage sludge. There are three main by-products of the AD process: liquid supernatant, biosolids and biogas (Gavala, Yenal, Skiadas, Westermann, & Ahring, 2003). Annually, over 6.5 million dry tons of biosolids are generated from the AD process in the US (Kalogo & Monteith, 2008), and more than 660,000 dry metric tons of biosolids in Canada (Canadian Council of Ministers of the Environment, 2012). There are approximately one thousand two hundred and seventy municipal wastewater systems that utilize anaerobic digestion to reduce raw sludge in the United States as provided in the biogas data report in 2015 by the Water Environment Federation (Water Environment Federation, 2015)

Disposal of biosolids comes at a high cost, both financially and environmentally. The biggest driver of these two costs is the economic cost of treating and disposing of biosolids, as it accounts on average for 30% of the operating cost for WWTPs (Shen, Linville, Urgun-Demirtas, Mintz, & Snyder, 2015). AD systems utilize naturally occurring bacteria and enzymes to further break down the organics found in municipal sewage sludge. The better the AD system functions the more it decreases the total volume of biosolids and increases the production of biogas. The biogas generated from the AD process can be a significant source of renewable energy (Kalogo & Monteith, 2008). Biogas can be captured and used to generate heat or electricity within the WWTPs, thereby reducing their overall energy consumption. However, as reported by Kalogo

and Monteith, only 10% of WWTPs in the USA use 100% of their biogas produced by an AD system (Kalogo & Monteith, 2008), leaving 90% of all WWTPs not fully utilizing their renewable energy potential. There were no data found for WWTPs using biogas in Canada (Statistics Canada, 2015), although similar statistics would be expected. The report also highlighted that the majority of WWTPs flare their biogas, effectively wasting the potential energy that can be derived from the gas and missing the opportunity to improve the long-term sustainability of the operations of the WWTP (Kalogo & Monteith, 2008). WWTPs in the USA face common barriers to using their biogas such as: inadequate levels of biogas production, or low quality of biogas (Kalogo & Monteith, 2008). In addition, systems using biogas are faced with the costs of operating and maintaining biogas equipment and are required to have specialized technical staff onsite (Kalogo & Monteith, 2008). Therefore, in order for WWTPs to improve their economic and environmental sustainability, their systems need to generate an increased supply of high-quality biogas and decrease the production of biosolids.

At the core of the AD system is the “conversion of biodegradable organic matter to methane (CH₄) and carbon dioxide (CO₂) by a consortium of facultative and anaerobic bacteria and methanogenic Archaea” (Duran, et al., 2006). To help improve operating conditions and consequently the sustainability of AD systems, the efficiency of breaking down the raw sewage sludge must be improved, which will increase the production of biogas, and decrease the production of biosolids (Fotidis, et al., 2014). To achieve this increased efficiency of organic breakdown, the biological activity within the AD needs to increase. Each AD system operates with a certain ratio of waste to microorganisms available to consume the waste; this is called the food to microorganism (F/M) ratio (Pennsylvania Department of Environmental Protection, 2017).

Even with a well-designed AD system properly operated, there is a limit to the degradation that can be achieved when relying solely on the naturally occurring bacteria in the waste (National Research Council, 1993). To increase the biological activity beyond these normal operational levels requires a change in the F/M ratio. Bioaugmentation (BA) is the process of enhancing the levels of bacteria, enzymes, and micro-nutrients within an AD system through the addition of a biocatalytic compound (BC) (National Research Council, 1993). BA has the potential to decrease the F/M ratio and enhance the biological activity (Fotidis, et al., 2014), thereby decreasing the biosolids generated, and increasing the biogas produced. If the introduction of BCs into AD systems could significantly decrease the F/M ratio and increase the biological activity, then the use of this technology could save WWTPs millions of dollars in biosolids disposal fees and increase the energy production potential. For instance, the Sanitation District of Los Angeles Joint Water Pollution Control Plant (JWPCP) spends over \$20 million per year disposing of their 121,910 dry tons of biosolids. The JWPCP also uses their biogas and values it at \$0.46 USD per cubic meter (D. Gary, personal communication, June 2, 2017). The implications of improving the breakdown of organics, lowering the amount of residual biosolids produced, and increasing the production of biogas for re-use as a renewable source of energy would save thousands of dollars and improve the sustainability of WWTPs. If successful, this technology could be applied in AD systems around the world.

There are many other factors besides the F/M ratio that contribute to the destruction of organic material in an AD system, including temperature, retention time, and the characteristics of the raw sewage, which vary from facility to facility.

Bacteria and Enzymes

The scientific community has produced several studies reviewing the many different techniques to alter and improve anaerobic digestion (Aydin, 2016; Fotidis et al., 2014; Duran et al., 2006). However, the majority of these studies focus on “pre-treatments and anaerobic co-digestion, while less attention is been paid to the introduction of additives to the digester medium” (Romero-Guiza, Vila, Mata-Alvarez, Chimenos, & Astals, 2016). Many different factors, as mentioned above, affect AD systems. These factors focus on creating the right environment to maintain the right consortium of bacteria and enzymes to complete the digestion process.

The theory behind bioaugmentation is to increase the number of different types of bacteria and/or enzymes within an AD system so that the residual biosolids are digested beyond the typical operation limits. As mentioned by Romero-Guiza et al. (2016), bioaugmentation is the process of dosing a “microbial inoculum with high hydrolytic or methanogenic activity, and enzymes addition to facilitate particulate organic matter solubilization”. In other words, bacteria and enzymes work hand in hand. The enzymes liquefy the organics, after which the bacteria break down the organics into their simpler forms, such as methane gas and water. There have also been many studies reviewing the bioaugmentation process of adding BCs either in the form of purified enzymes (Odnell, Recktenwald, Stensen, Jonsson, & Karlsson, 2016), or specific types of bacteria (Duran, et al., 2006); (Fotidis, et al., 2014). In addition, the application, monitoring and analysis of bioaugmentation compounds have “almost exclusively been investigated in lab-scale digesters”; the use of full-scale plants is “scarce due to its economic uncertainties and risks” (Romero-Guiza, Vila, Mata-Alvarez, Chimenos, & Astals, 2016). While

these studies achieved important results, there seems to be a lack of research on using a BC that contains both bacteria and enzymes in full-scale AD systems. Previous studies that have been conducted by Aydin (2016), Fotidis et al. (2014), Duran et al. (2006), Schauer-Gimenez, Zitomer, Maki, & Struble (2010), and Romero-Guiza et al (2016) explain the need to improve AD systems to reduce costs and bio-waste, while increasing the economic sustainability of WWTPs by the capture and use of biogas as natural renewable resource. The present study has examined the impact on full-scale municipal anaerobic digestion via routine bioaugmentation with a biocatalytic microbial and enzyme consortium developed by Hycura™.

Research Questions

The purpose of the present study was to investigate the impact on the biosolids and biogas production with routine use of a commercial grade BC applied into municipal AD systems, using multiple analyses of full-scale side-by-side and/or historical year-over-year comparisons.

The following are the research questions.

- a) Is there a significant increase in the destruction of volatile solids (VS) in the treated digester vs. the control?
- b) Is there a significant increase in biogas production by the treated digester vs. the control?
- c) Are the performance metrics significantly different between the treated digester system compared to the control digester system throughout the entire project
- e) Are the performance metrics during the augmented phase different than the historical data or controlled digester data?

- f) Are the combined results from the introduction of biocatalytic products into the anaerobic digesters economically significant and sufficient to continue long term use of the biocatalytic products?

Methodology and Procedures

Full Scale Approach

This study of AD bioaugmentation through biocatalytic addition required a stringent quantitative approach based on numerical data collected via lab tests and instruments and subjected to statistical analysis (Creswell, 2009). At the heart of the study was the biological experiment, to determine the influence of biocatalytic treatment on the digestion of biosolids. Creswell (2009) explained that judging the influence of the treatment through the quantitative method is best accomplished when there is a specific treatment that is performed on one group, in this case the addition of the biocatalyst to the anaerobic digester, while it is withheld from another digester acting as the controlled situation. Where possible, in the present study this was accomplished by the side-by-side experiment where one digester was treated with biocatalytic additives while another run in parallel with the same feedstock was the control. When a side-by-side comparison was not feasible, the use of BCs in the AD system was compared to historical averages prior to the use of the BC. Sampling and testing were completed by state certified industry professionals. The application and comparison of each treated digester were conducted independently of each control digester when testing side-by-side. The two different participant sites were also experimentally independent of each other. The factors including temperature, retention time, and the characteristics of the raw sewage are operationally maintained by onsite certified industry professionals. These professionals are officially trained, certified and

recognized by their local state or provincial certification boards. Industry professionals monitored the application of the BC and the operation of the AD systems; did all sampling ; and performed all tests to insure a proper non-biased comparison between the digesters with and without BC could be completed. Furthermore, digital surveys and in-person interviews were conducted to collect both technical and non-technical information. These interviews helped provide more information on the operations and interpretations of results directly from the participants (operators of the digesters).

In each case an analysis was conducted of the AD with and without the BC by using either a comparison against historical records or a side-by-side comparison. Data from each experiment were collected and analyzed to determine biological activity by tracking VS destruction and total biogas production. In the case of the side-by-side experiment one digester was treated with the BC while the other was not and acted as the control. The commercial grade BC used for the purpose of this study contains micronutrients, and a consortium of bacteria and enzymes.

Thesis Study Site Participants

The full-scale tests were conducted at the San Luis Ray Wastewater Treatment Plant (SLR) in Oceanside, CA; and the San Luis Obispo, California, Wastewater Resource Recovery Facility (SLO). These two locations were selected because they (1) were willing to add the BC into their system (2) treat similar municipal feedstocks, (3) are able to complete consistent tests to monitor impacts (4) were willing to provide detailed reports from the operations and testing results, (5) were motivated to reduce high biosolids management costs, and (6) desired to better

utilize the biogas generated from the digestion process. Each participant site completed a participation authorization form (see Appendix A).

SLO is located at 35 Prado Rd, San Luis Obispo, CA 93401, USA. SLO is run by the City of San Luis Obispo, CA. It currently services 47,500 people and has a running capacity of 11,735 cubic meters per day (CMD). The site has three fully operational mesophilic anaerobic digesters, that are run in series one after the other, with a total capacity of 4,050 cubic meters.

SLR plant is located at 3950 N River Rd. Oceanside, CA 92058, USA. SLR is run by the City of Oceanside, CA. It currently services 175,000 people and has a running capacity of 35,204 CMD. They have three fully operational anaerobic digesters that run in parallel, with a total capacity of 10,600 cubic meters.

The project at SLO was not conducted as a side-by-side comparison as they run their digesters in series one after the other, while SLR compared side-by-side digesters. Both AD systems were mesophilic digesters. These facilities provided all relevant data and test results conducted at their facility.

The project sponsor, Acti-Zyme Products Ltd (dba. HycuraTM), was required to complete a participation consent form to release all information collected in the full-scale SLR and SLO projects as well as information on previous and congruent projects.

Operational Comparisons

Optimally it would have been desirable to have two different treatment plant participants with similar if not identical anaerobic digestion systems. This was not the case for this project. The following section compares the important aspects of the anaerobic digestion systems and what comparisons could be made between them. The results in both treated systems were

compared more closely within the individual plants themselves than to each other. The two methods used to compare the two treatment systems were 1) a ratio described below as the Specific biogas yield, which determines the biogas produced per kilogram of organics introduced or destroyed, and 2) the Van Kleeck Formula, which is universally used to calculate the percentage of organics destroyed in anaerobic digestion and is used as a metric for overall system performance.

Both SLO and SLR are run at mesophilic temperatures (approximately 33° Celsius), and they are both continuous stir and feed reactors (see Table 1 Wastewater Plant Operational Comparisons). Both systems use a similar municipal feedstock comprised of a mix of primary sludge and waste activated sludge (WAS). However, they differ significantly in their size and operations. SLR, a 35,204 CMD plant, runs their digesters primarily in parallel, with a secondary tank that acts mainly as a holding tank. All primary digesters feed into this secondary tank. Utilizing any data from the secondary tank would provide inaccurate data as it would contain sludge from both the treated and non-treated digesters. The tests run at SLR were tested in parallel, holding one digester as the Control digester and a second as the Treated digester. In contrast, SLO is about one third of the size of SLR at 11,735 CMD. SLO runs in series with a primary digester, secondary digesters and a third holding tank. These differences between the two participant sites have provided two different pilot project types. SLR compares two digesters as a side-by-side test with one acting as a control, while the SLO treated their entire system and compared the results to historical data.

There are also significant differences in how the two plants mix their sludge in the digesters. SLR digesters use centrifugal mixing pumps while SLO uses recirculated biogas to

mix their sludge. The possible effects of the mixing systems will not be discussed as it is outside of the scope of this project

Table 1. Wastewater Plant Operational Comparisons

	<i>Unit</i>	SLO	SLR
Plant Size	<i>cubic meters per day</i>	11,735	35,204
Total Digester Capacity	<i>cubic meters</i>	4,050	10,600
Treated Digester Capacity	<i>cubic meters</i>	4,050	2,650
Digester Process Phase		Single	Single
Mixing System		Recirculated biogas	Centrifugal mixing pumps
Performance Comparison		Historical	Side by Side
Operation Type		Series	Parallel
Feedstock Type		Primary & WAS	Primary & WAS
Chemical Addition		Ferrous chloride	Ferrous chloride
Annual Production of Biosolids	<i>Wet tons</i>	3,259	18,250

Average Performance Without Treatment Comparisons

The performance of each AD system has other differences and similarities (see Table 2). The total digester capacity of the entire SLO facility is 4,050 cubic meters, while at SLR the test digester's capacity is 2,650 cubic meters. The flow rates between the systems vary drastically. SLO feeds its AD system with on average 58 cubic meters per day with 6.51% total solids (TS) and 85.20% VS. In contrast, SLR feeds each digester with 84 Cubic meters per day with 4.00% TS and 75.33% VS. While both plants utilize a thickening system prior to the digester to increase the solids percentage entering the digester, SLO is able to operate with over 50% higher percentage of solids. This large difference in flow and percentage of solids for both total and organic solids affects both the hydraulic retention time and organic loading. SLO's reported hydraulic retention time of 45 days is almost 50% greater than that of SLR at 32 days. The organic loading rate at SLO of 3,193 kilograms per day is significantly higher than SLR's of

2,522 kilograms per day. Even with these differences the biogas production prior to the project start from the SLO and SLR digesters were relatively close at 1,867 and 2,019 cubic meters per day respectively. With a lower organic loading, the SLR plant is able to achieve a higher level of biogas production. It is important to note that the estimates made above at SLO are based on the entire wastewater plant's digestion system while it is being compared to one single digester at SLR.

Table 2. Reported System AD Performance Comparisons Prior to Bioaugmentation

	<i>Unit</i>	SLO	SLR
Digester Capacity	<i>cubic meters</i>	4,050	2,650
Feeding Rate (Flow)	<i>cubic meters per day</i>	58	84
Average Total Solids Influent	<i>Percentage (%)</i>	6.51	4
Average Volatile Solids Influent	<i>Percentage (%)</i>	85.2	75.33
Average Total Solids Effluent	<i>Percentage (%)</i>	2.69	2.25
Average Volatile Solids Effluent	<i>Percentage (%)</i>	66.7	62.27
Hydraulic Retention Time	<i>Days</i>	45	32
Organic Loading Rate	<i>kgs per day</i>	3,193	2,522
Temperature	<i>Celsius</i>	32.5	36.6
pH	<i>Range 0 to 10</i>	7.15	6.91
Total Alkalinity	<i>ppm</i>	3,996	1,373
Total Volatile Acids	<i>mg/L</i>	0	27.46
Volatile Solids Reduction	<i>Percentage (%)</i>	65.21	45.6
Gas Production	<i>cubic meters per day</i>	1,867	2,019

Lab Tests, Report and Data Collection

All tests and reported data were completed and collected independently from each location by individual operators/lab technicians. Lab reports containing the raw data from all tests conducted are attached in Appendix B and C. With the focus of this project on the impact of the BC on the digestion process, the two main data points reported for analysis were the systems' VS reduction and its biogas production. Each system collected multiple samples from

the influent sludge feed prior to digestion, and from the effluent sludge feed after the digestion process to track any changes of biological activity. Each sludge sample was then tested for percent TS and VS. The biogas quantity and quality (where available) were also measured. These tests for biogas quality were conducted by the lab technicians at the individual plants.



Figure 1a. AD system map with application locations at SLO



Figure 1b. AD system map with application locations at SLR

The following tests and measurements were completed and reported in each plant report: feed rate, TS influent & effluent, VS influent & effluent, Volatile Fatty Acids (SLR only), and Total

Alkalinity, pH, Temperature, biogas production, and biogas quality (SLO only) (see Table 3 for a complete list of tests). Appendix B and C contain the full data reports from both sites. The testing of VS and TS provides numerical data indicating the total biosolids entering and leaving the AD system. The total biogas was tested for its composition (SLO only) and total production. All tests have followed the data collection, sampling and analysis procedures as performed in Aydin (2016), Fotidis et al. (2014), Duran et al. (2006), and Schauer-Gimenez (2010), and as outlined in The Standard Method for the Examination of Water and Wastewater in 2017 (American Public Health Association, 2017).

Table 3. List of AD system performance tests

Test	Unit(s)	Method / Tool	Frequency / Location	Method Source
Feed Rate (Q)	<i>cubic meters</i>	Pressure gage	Daily	
Total Solids (TS)	<i>Grams (g)</i>	Drying sample at 55 C	Once per week day, from input and output of digester	2540 B
Volatile and fixed Solids	<i>Grams (g)</i>	Burning sample at 100 C	Once per week day, from input and output of digester	2540 E
Volatile Acids	<i>50 - 2,500 mg/L</i>	Esterification	Once per week day, from input and output of digester	5560
Alkalinity	<i>50 at 200 ppm</i>	P & T Alkalinity	Once per week day, from input and output of digester	2320 B
pH	<i>Range between 0 and 10</i>	Litmus Test	Once per week day, from input and output of digester	
Temperature	<i>Fahrenheit</i>	Thermometer	Daily	
Gas Production	<i>cubic meters</i>	Pressure gage	Daily	
Biogas Composition (%CH ₄ , %CO ₂ & %H ₂ S)	<i>Parts per million (ppm)</i>	Dräger sampling tube	Weekly	

* *Standard Methods for Examination of Water and Wastewater, 23rd Edition.* (American Public Health Association, 2017)

Technical Lab Data Analysis and Calculations

Based on the tests and data reports from both SLR and SLO, as described above, several calculations and analyses have been completed to provide a deeper understanding of changes that

occur in the AD system. The purpose of this study was to determine any existing relationships within the raw data indicating the impact of using bioaugmentation. Data charts are included to provide visual representation of trends utilizing 7-day and 30-day moving averages, with trend lines and other metrics to highlight any significant changes. Additional secondary data from previous and congruent AD projects will also be referenced to help pull out similarities, differences, trends and changes to AD efficiency.

Organic loading rate (OLR) calculation helped provide an understanding and comparison of the volume of organic waste that was being fed into the digesters at any given day. Because of the relationship between organic waste and production of biogas, a higher OLR increases the potential biogas production. The OLR provided a measure of potential biogas production and was used as a comparison point between the SLR and SLO digesters. While the organic loading rate is an important parameter, understanding the kilograms of total VS destroyed (KVSD) helps move beyond what was fed into the digester to what the biological activity has actually accomplished: KVSD shows the mass of solids reduced or transformed. There are two important ratios that work with OLR and KVSD which show their relationship to biogas production. Using the total biogas production divided by either the OLR or the KVSD provides the specific biogas production per kilogram of organic material, and a means of comparison across different anaerobic digestion systems. As explained above, SLO and SLR have two different retention times, OLRs and a relatively similar biogas production prior to bioaugmentation. These ratios of biogas production to OLR and KVSD will help establish the rate at which either system transforms organic waste into biogas. This rate or specific biogas yield helps provide a fairer comparison between the SLR and SLO systems.

The specific biogas yield is an important way to determine if an AD system is reaching its potential or if it is far from optimal production. Both the theoretical biogas yield and the actual yield were considered to help compare the individual systems to themselves as well as comparing the results to other systems not included in the primary data set. The “total gas production is usually estimated from the percentage of VS reduction. Typical values vary from 0.75 to 1.12 m³/kg of biogas produced per unit mass of VS destroyed” (Metcalf & Eddy Inc., 2019). This theoretical biogas yield range was compared to the actual biogas yield which was calculated by comparing the total biogas production to the total VS destroyed.

The percent of VS reduced was calculated by using standard method 1684, established by the USA Environment Protection Agency (Telliard, 2001) named the Van Kleeck formula. This calculation provided a comparison of the quantity of VS that are destroyed as it calculates the difference between the feed and the residue for each individual AD system. This calculation is widely used to compare the efficiencies between different AD systems. The formula calculates the percentage of volatile organic matter that has been destroyed within the AD system.

Van Kleeck Formula

$$VK = \frac{VS_F - VS_W}{VS_F - (VS_F \times VS_W)} \text{ or } 1 - \frac{VS_W}{VS_F} / 1 - VS_W$$

Where:

- VK = *Van Kleeck*, the percentage of VS reduced
- VS_W = fraction of VS in digestion residue (on solids-only basis)
- VS_F = fraction of VS in feed (on solids- only basis)

There is however another way to measure changes to VS, the mass balance equation. This equation helps provide an accounting for the material that enters and leaves during the AD process.

Mass Balance Formula

$$U = (VS_F - VS_W) / VS_F$$

Where:

- U = *Mass Balance*
- VS_W = concentration of VS in digestion residual (mg/L)
- VS_F = concentration of VS in feed (mg/L)

As stated by Michael Switzenbaum et al. (2003) the two equations are closely connected and have variability between them.

Table 4 outlines all the calculations conducted for comparison and analysis of the above-mentioned data and test results.

Table 4. Technical analysis calculations

Name	Unit	Formula	Source*
Organic Loading Rate	<i>kgs per day</i>	$\text{OLR} = (\text{Feed rate} \times 8.34 \times (\text{Total Solids Influent} / 100) \times (\text{Volatile Solids Influent} / 100)) / 2.2046$	(10-20) p. 1099
Hydraulic Retention Time	<i>days</i>	$\text{HRT} = \text{Feed rate} / \text{Digester capacity}$	(10-17) p. 1093
Kilograms of Volatile Solids Destroyed	<i>kgs</i>	$\text{PVSD} = \text{OLR} - (\text{Feed rate} \times 8.34 \times (\text{Total Solids Effluent} / 100) \times (\text{Volatile Solids Effluent} / 100)) / 2.2046$	
Specific Biogas Yield (per Kilogram of Volatile Solid Introduced)	<i>cubic meter per kg</i>	$\text{BVSI} = \text{Biogas} / \text{OLR}$	
Specific Biogas Yield (per Kilogram of Volatile Solid Destroyed)	<i>cubic meter per kg</i>	$\text{BVSD} = \text{Biogas} / \text{PVSD}$	
Specific Biogas per Cubic meter of Feedstock	<i>cubic meter per cubic meter</i>	$\text{BF} = \text{Biogas} / \text{Feed rate}$	
V/A Ratio (Volatile Fatty Acids to Alkalinity)		$\text{V/A Ratio} = \text{Volatile Fatty Acids} / \text{Alkalinity}$	
Van Kleeck Formula (Volatile Solids Reduction)	<i>Percentage %</i>	$\text{VK} = \text{VS}_F - \text{VS}_W / \text{VS}_F - (\text{VS}_F \times \text{VS}_W) \text{ or } 1 - (\text{VS}_W / \text{VS}_F) / 1 - \text{VS}_W$	(13-16) , p. 1511
Mass Balance Formula	<i>Percentage %</i>	$U = \text{VS}_F - \text{VS}_W / \text{VS}_F$	(13-15), p. 1510

* *Standard Methods for Examination of Water and Wastewater, 23rd Edition.* (American Public Health Association, 2017)

Statistical analysis was also completed utilizing standard *t*-tests to determine the statistical significance of the differences between the means of the different performance metrics.

Microsoft Excel for Mac version 16.26 was used to run the *t*-tests.

Digital Surveys and Interviews

Digital surveys and in person interviews from the two participant plants participating in this project were used to complement the quantitative data described above. Interviews were conducted with questions to reflect upon the start and end of the project in mind. These interviews helped provide greater detail and insight on the perceptions of the participants; non-technical information that is important to understanding the situational differences between participant locations; and any other observations or anecdotal information that could help support the interpretations of the quantitative results of the full-scale tests. An invitation email was sent to each plant with the request to select at least one employee to participate in the interview. Every employee that was to be invited was given the option to opt out of this part of the project. Each interview was recorded, and interviewees were reminded of the option to withdraw at the start of the interview. The interviews were completed by phone. The interviews were transcribed, and a copy was sent to the participant so that they could verify their responses and add any additional information or feedback if desired. The SLR interview was completed by a plant shift supervisor while the SLO interview was completed by the current Interim Utilities Engineer, whom was previously a plant operator. A digital survey was also a part of the initial participant selection and evaluation which included both operational data and open-ended questions for the participants to provide any extra relevant information. The invitation email (see Appendix D), the consent form (see Appendix E), the digital survey (see Appendix F) and the interview questions (see Appendix G) are attached to this paper.

Milestones

Below is an outline of the milestones completed during this thesis study.

Table 5. Itemized list of milestones

Milestone	Complete Date
Prepare/Present poster	August/October 2017
Submit thesis committee	Nov 6, 2017
Revise proposal	Nov 15, 2017
Submit final proposal	October 7, 2018
Collect data	October 2017 to April 2019
Start analysis of tests	April 2018
Literary review	October 2017 – October 2019
Complete data and statistical analysis	April - April 2020
Writing and rewriting thesis	August 2018 – November 2020

Resource Requirements

This project required significant contribution from three major groups. The investigator invested hundreds of hours traveling and visiting the two plants and holding regular meetings with its operators. This travel included significant costs and time in order to identify potential locations, assess willingness to participate and finally agreement to run the project. Once a site agreed to run a bioaugmentation project, there was a series of initial meetings to set up the parameters, project requirements and details. Operational systems, performance measurements, testing protocols, and standards were set prior to the start of the project.

The second contribution came from the plants and their personnel. Plant personnel gave time and effort to developing and running the project. They agreed to share data from their system that were generated during the project as well as historical data for year over year comparisons. In some instances, the plants increased the number of tests completed on a daily or weekly basis to provide more data for this project. This directly affected the operators as they spent additional time working and reporting on the project. The project also represented a certain

level of risk. The common risk of concern was that the addition of a biocatalytic product could cause digester upsets which would lead to costs to dispose of biosolids and return operations to normal parameters. There had been no previous data suggesting that this scenario could occur, however this was a consideration in making the decision to participate in the project.

Lastly, the financial backing of the project was required by the sponsor who provided thousands of dollars' worth of biocatalytic product for use during this project as well as sharing information on previous and congruent projects that added insight and comparisons for this project. Because of difficulties with digesters that arose from the beginning of project at the SLR plant, additional product was required, and that specific site had to restart from the beginning of the seeding process which caused significant delays, and increased usage of product.

Project Overview

The fundamental reason for the development and execution of this thesis project was to determine if the use of bioaugmentation products can improve the performance of anaerobic digesters. Increased performance in anaerobic digesters would make systems more economical to operate due to the increased potential of using its biogas and decreased costs of disposing of its residual biosolids.

It took quite a few months, and dozens of meetings with plants all across California to locate and obtain approvals to complete full size anaerobic digestion testing. This process was difficult because individual systems have different costs, permits, priorities, administration complexities and previously scheduled projects that interfered with participation in the thesis project. Getting the buy in from operators and managers was also challenging. There was initial push back from some staff at certain potential sites due to their previous experience with other

bioaugmentation products. There was also varying appetite to optimize anaerobic digestion systems. Some additional economic factors that contributed to a plant's ability to participate were that many of the systems were not capable of using its biogas while others did not have significant biosolids handling costs.

Both the interviewees at SLO and SLR mentioned that they were not familiar with bioaugmentation technologies. Neither had had any experience using a similar product and did not have any expectations of how it would work. Both plants have co-generation systems which utilize biogas for electrical generation and both also had significant biosolids management costs. This provided two locations that were both willing to optimize the anaerobic digestion systems and were able to take advantage of its potential benefits.

Project Details

Upon approval, both pilot projects set up a timeline to allow the treatment process to last for four months. It was originally anticipated that the project was to start January 2018 but with delays for approvals, product logistics and timing with other projects already slated at the plant the start date for both projects was pushed back.

SLR started its first day of phase 1, the "seeding" phase, on August 20th, 2018 where daily applications of the BC were made following the dosage protocols provided below, in order to allow the biological activity to disperse throughout the whole digester. The seeding phase was completed on September 8, 2018. After the seeding phase the system was dosed weekly based on the maintenance application rate and schedule (see table 6 in appendix J). This weekly dosing continued until the completion of the project on December 20, 2018. It was determined by the plant operators that all applications would be applied through the thief hole on top of Digester 4

(see Figure 1b). This location for application of the product in Digester 4 was chosen to allow for separation between the treated digester and the rest of the system. There were no other locations for applications that would allow for direct application into Digester 4 as the treated digester while maintaining normal operations on the rest, which helped maintain the rest of the digesters as the “control” in the project. Data reports were created upon request with occasional difficulty in communicating with plant staff. As such there were a few delays in obtaining data which led to delays in thesis timelines. Additional approval to share data was also required after completion of the project by the legal department at the City, which caused additional delays prior to data sharing and analysis.

SLO started its first day of seeding on September 16, 2018. It completed its seeding applications on October 5, 2018. Following the seeding applications schedule (see table 7 in appendix J), it continued with a weekly maintenance application which was to be maintained for the rest of the four-month period. All applications were made directly into a spill over box that had easy open-air access to the sludge prior to the injection into the digester (see Figure 1a). After application at the spill over box, the sludge and biocatalyst would continue to move throughout the entire set of digesters as they are one full system connected in series. On November 5, one month after the completion of the seeding application phase, it was decided to stop the weekly maintenance applications as the system had increased its biogas production beyond its capacity to handle it. The co-generation power engines, pipes, and flare stack had reached their maximum abilities to handle biogas, and at this point the biogas was being vented out the release vents on top of the digesters. This was a very promising and positive impact on the biogas, and caused a serious problem meeting the plant’s air permit. As this was a violation

to the plant's permit, they decided to stop applying product and allow the system to return to its regular biogas production rate. This event was an indicator of improved biogas production beyond what the plant operators expected could happen. During the remainder of the 4-month project, monitoring continued but no applications of product were made. Data were shared regularly from the plant and there were a few issues with the completeness of the data. Emails back and forth with the staff resolved the issues and a completed set of data points was received. Even with the completed set of data points, there were sections within the historical records where some data had not been gathered or reported properly. Therefore, some of the comparisons to previous years were difficult due to missing data.

Dosage Protocols

The dosage protocols, utilizing the biocatalytic Hycura AD, provided by the thesis sponsor are as follows: Seeding application rates were 0.45 kg of product per 2200 cubic meters of total digester capacity and then breaking that amount up into 20 equal daily initial applications. Maintenance application rates were 0.45 kg of product per 2200 cubic meters of total digester capacity and then breaking that amount up into 52 equal weekly applications. See Appendix J Table 6 and 7 for the dosage schedule.

Results

As explained previously the two plant locations of SLO and SLR are best compared to their own internal control as the systems differ in the volumetric size of the systems, mixing systems, thickening systems, organic loading rates, hydraulic retention times and perhaps more importantly the setups (in parallel versus in series). Throughout the rest of the results section the comparisons were drawn more within the individual plant system either to the simultaneously

run control or to the historical averages. Utilizing the metrics of the Specific biogas yield and the percentage of VS reduced provided a general comparison of the overall system-to-system performance. There are three main phases the digester goes through during bioaugmentation. First is the seeding phase where the application rates are high to ensure that the BC is spread throughout the AD system. The second is the maintenance phase, where the application rate decreases and is maintained over the rest of the life of the system with a regular weekly dosage. The third phase deals with the actual impacts of the BC on the performance of the AD system. For purposes of this thesis, this was called the Augmented Phase. This was used to indicate the point at which the AD system treated with the BC reached at least a 10% improvement when compared to the control or historical records.

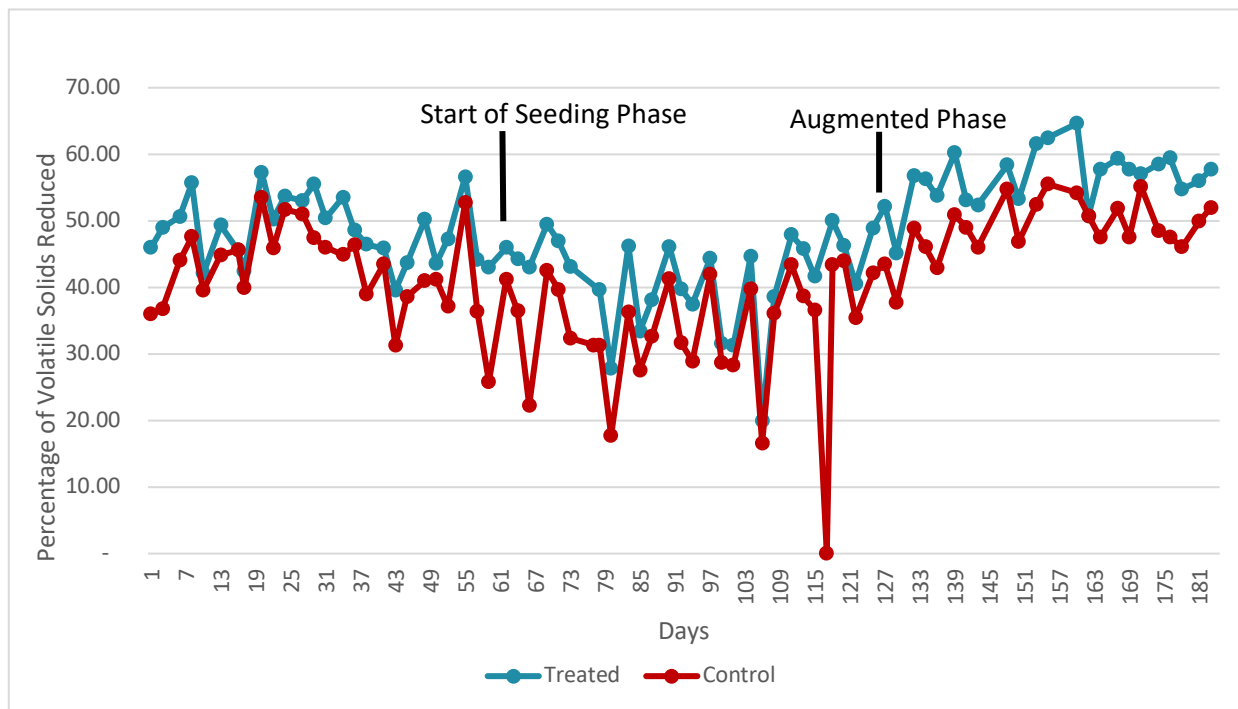
SLR

Volatile Solids Reduction

The SLR plant lab technicians pulled sludge samples to perform regular lab tests on the TS and VS entering the system and leaving the digesters. On average the technicians tested three times a week to determine the digestion system's TS, volatiles solids (VS), Volatile Acids, Alkalinity, and pH. These test results allowed for determination and analysis of the system's organic loading rate, kilograms of VS destroyed, and the percentage of VS reduced. As seen in Figure 2, after the Treated digester had reached the augmented phase, it began to show consistent improved VS reduction compared to the Control. This equates to a 15% overall increase in percentage of VS reduced from 49.72% average reduction in the Control when compared to 57.23% in the Treated digester. The Mass Balance calculation for volatile loss shows that the Treated digester lost 24% more VS with 45.40% of VS remaining, than the Control with 57.23%

of VS remaining. Both of these performance metrics indicate an improvement and were also found to be statistically significant with p values of $p = 1.82 \times 10^{-12}$ and $p = 9.06 \times 10^{-15}$ respectively. The temperature and pH were statistically different but were within normal operational values, and as such were viewed by operators as having negligible impact. The ratio of volatile acids to alkalinity remained unchanged, although it increased in magnitude by 20% from 1373/25 in the Control digester to 1662/31 in the Treated digester.

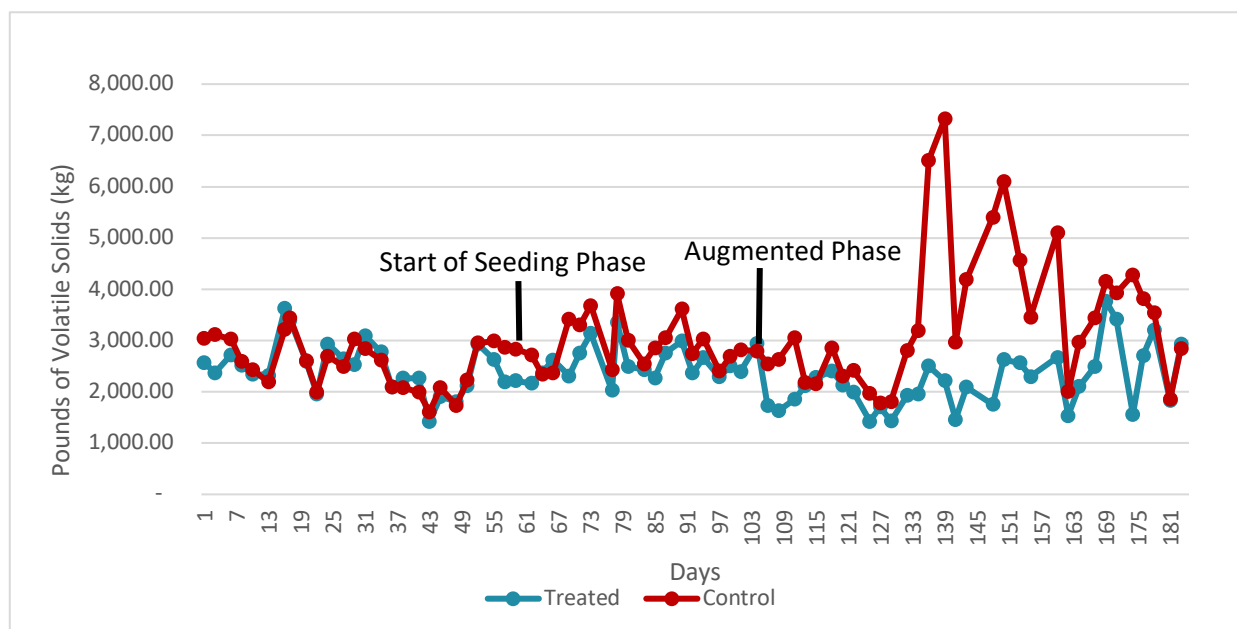
Figure 2. Percentage of VS reduced using Van Fleeck method at SLR



There was an issue that occurred while trying to keep both digesters at the same organic feeding rate which impacted the organic loading rate, the hydraulic retention time, and the kilograms of VS destroyed. As found in Table 8 the Treated digester's feeding rate was lower on average by 42.65 cubic meters per day a -35% difference. This difference translates to an higher loading of 1,658 kgs/day average of volatile organic material into the Control compared to the Treated digester. With the higher feeding rate to the Control, it's hydraulic retention time

decreased to 22.01 days, while the Treated digester remained around 34.08 days. In addition, the total VS destroyed increased in the Control due to the increased feeding on average of 2,403 kgs/day, compared to 1,105 kgs/day in the Treated Digester (see Figure 3). Due to the higher feeding rate and the higher level of organics destroyed, theoretically, there should have been an increased production of biogas in the Control (Evans, Strezov, & Evans, 2015). It was found that the difference in OLR between the two digesters was significant with a $p = 4.23 \times 10^{-7}$ over the whole project timeframe.

Figure 3. Organic loading rate kilograms of VS per day at SLR

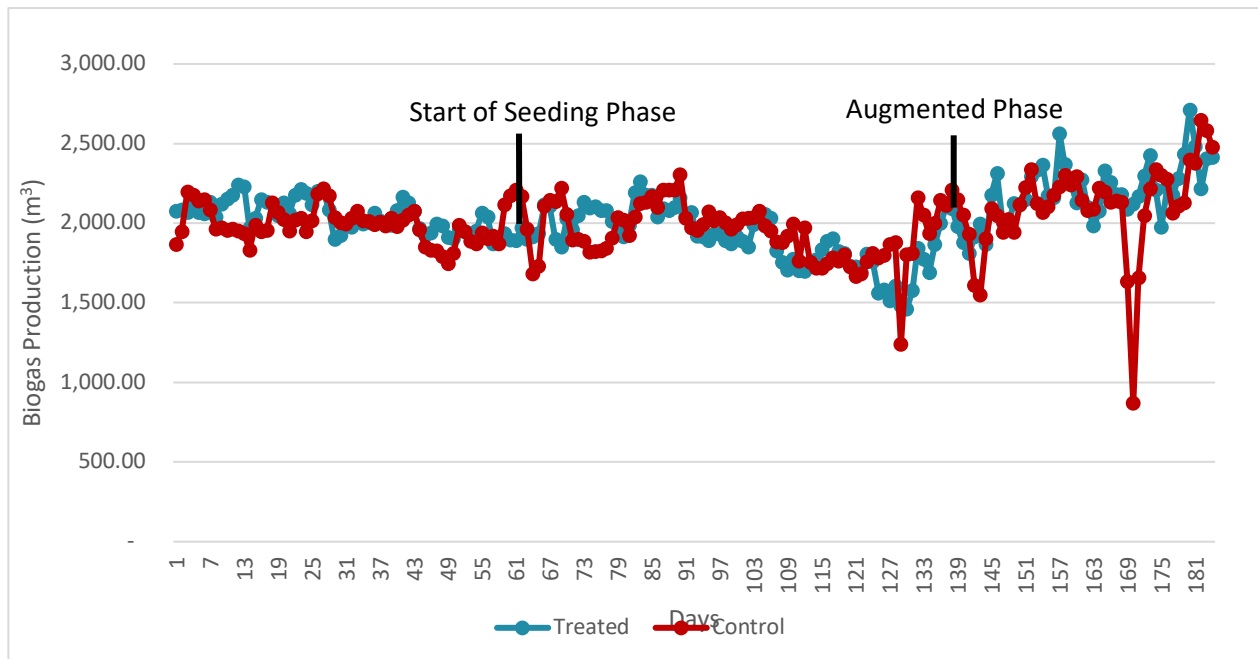


Biogas Production

With all the tests done in the labs at the SLR site, they did not complete any tests on the biogas composition, so no comparison can be made on how the bioaugmentation may have had an effect. This was the decision of the plant manager as they had not historically completed these tests to provide comparison and did not have capacity to perform them under this project. Other

than the biogas composition, the lab completed detailed measurements on the biogas production. Daily readings were taken at the biogas meters of all digesters. The total daily biogas production pre- and post-seeding for both the Control and the Treated systems were found to not be significantly different with a $p = 1.09 \times 10^{-1}$ throughout the entire project. With some exceptions near the end of the project, as shown in Figure 4, the Control and Treated systems move together throughout the entirety of the project. The treated digester did show some marked minor differences near the end of the project. At first glance the digester biogas production for both the Control and the Treated digesters appears to be same.

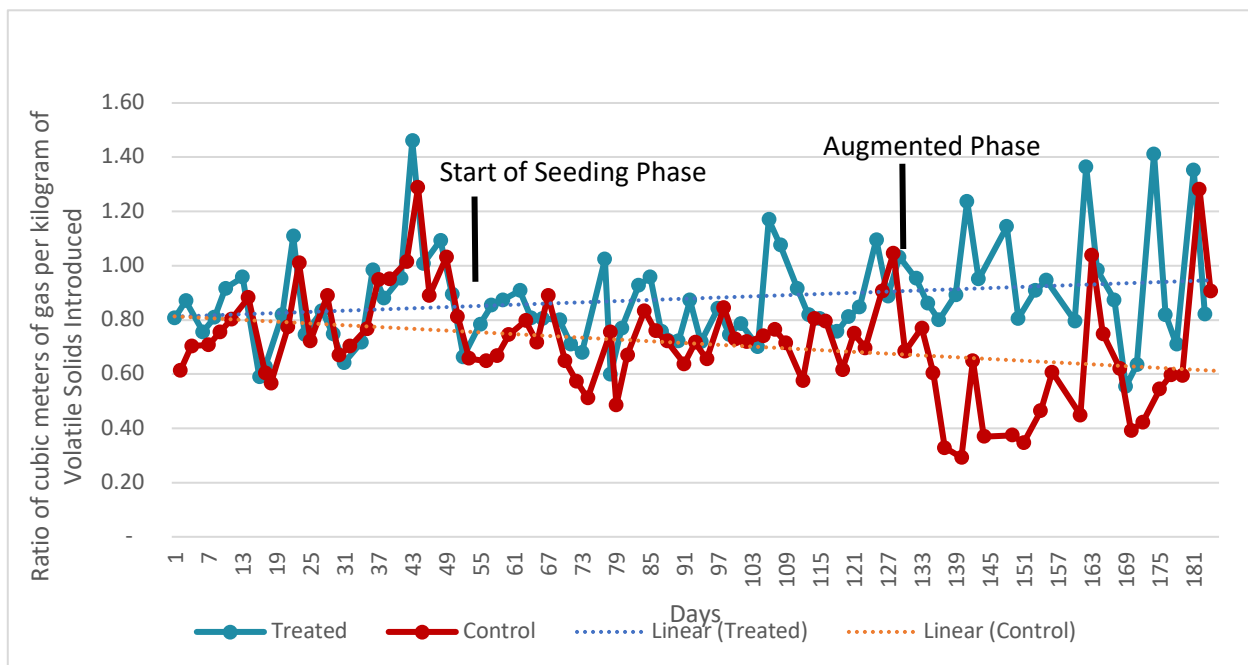
Figure 4. Daily total biogas production cubic meters per day from SLR digesters



Using the data provided on the biosolids destruction and the biogas production, a per unit comparison was done to provide the specific biogas yield for each system. The specific biogas yield per kilogram of VS introduced (VSI) provides a different accounting. If both digesters were performing at the same level the specific biogas produced should be equal. A comparison across

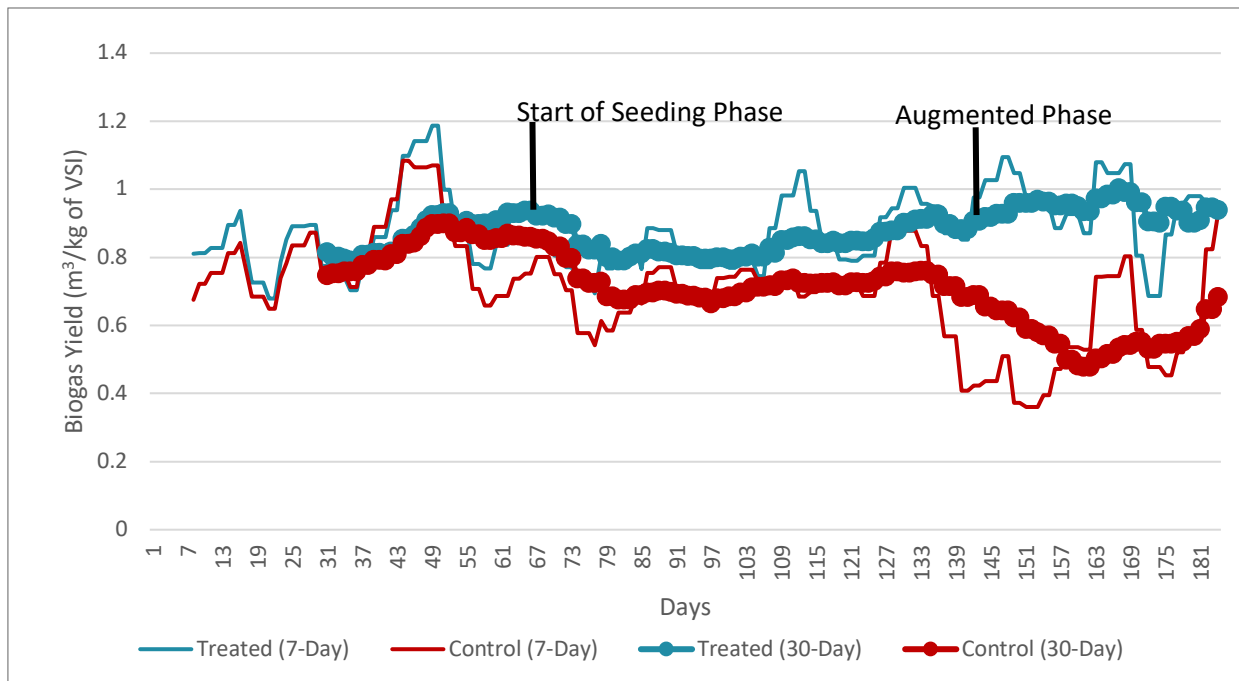
different digesters with different operations and flow can be compared by using the specific biogas metric allowing for the flow into a digester to be higher and still obtain a comparison. Prior to the start of the seeding phase both digesters trended along the same path following each other as seen in Figure 5. During the seeding phase there were some marked differences between the Control and the Treated digesters performance. The major separation between the Control and the Treated digesters occurred after day 106, and again after day 131. After day 131 there began to show an increase in biogas production for the Treated digester with an average of 0.943 cubic meters of gas per kilogram of VSI, and a negative trend downward for the Control with an average of 0.591 cubic meters of gas per kilogram of VSI, 0.352 cubic meter difference or a 60% difference (See Figure 4). The statistical analysis found the difference to be significant with a $p = 4.25 \times 10^{-7}$ during the augmented phase and $p = 2.97 \times 10^{-10}$ throughout the whole project.

Figure 5. Specific biogas yield per kilogram of VSI at SLR.



In addition, 7-day and 30-day moving averages help to depict the overall trends as shown in Figure 6. With the changes to the feeding to the Control this would adversely impact its specific biogas yield and as such the percentage improvement of the Treated digester is most likely not 60%. By using the average specific biogas yield from the Treated digester prior to the Augmented Phase of 0.850 cubic meters of gas per kilogram of VSI to the post-bioaugmented results of 0.944 cubic meters of gas per kilogram of VSI, an 11% increase may be more accurate depiction of the direct impact of the bioaugmentation.

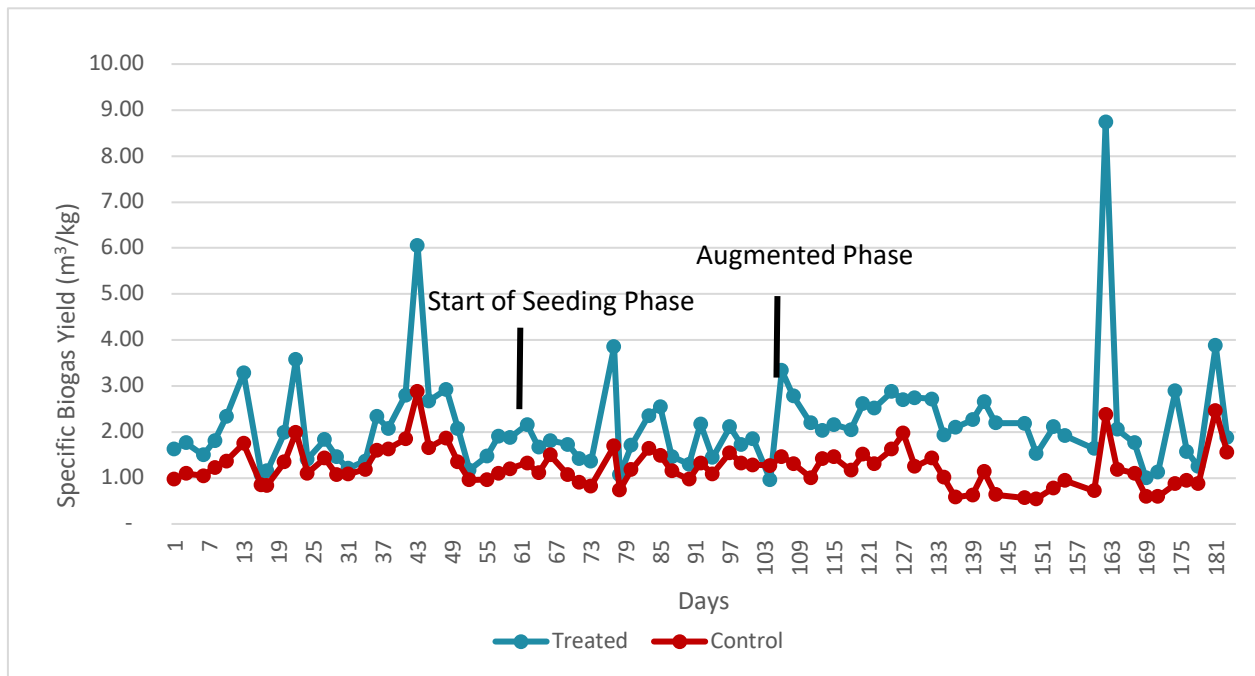
Figure 6. Moving averages of the specific biogas production per VSI at SLR



Another way to consider the specific biogas yield is based on the kilograms of VS destroyed (VSD). This comparison focuses on the resulting biogas from the kilograms of biosolids actually destroyed. In Figure 7, the step change of the Augmented Phase in the Treated digester happens earlier and tracks consistently higher with an average of 2.25 (m³/kg of VSD)

with the Control averaging of 1.17 (m³/kg of VSD). This comparison, taking into consideration of the outlier on day 167, shows a 93% increase in efficiency in biogas production per kilogram of VSD. The difference between biogas production per kilogram of VSD in the Treated and Control digesters was found to be statistically significant with a $p = 3.94 \times 10^{-6}$. As with the specific biogas per kilogram of VSI, a similar concern would be how the feeding rate would have impacted these results. When comparing the result within the Treated digester itself the post augmented phase had an average of 1.923 m³/kg of VSD, and 2.25 m³/kg of VSD in post-bioaugmentation, a 17% increase.

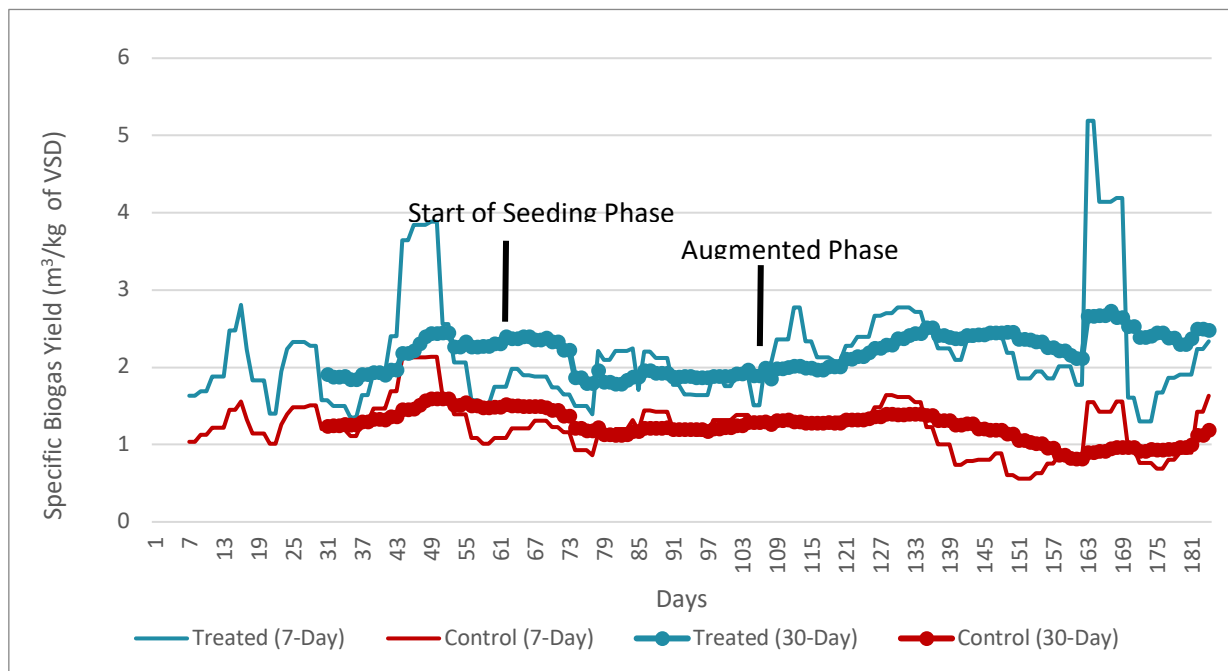
Figure 7. Specific biogas yield per kilogram of VSD at SLR.



The 7-day and 30-day moving averages (see Figure 8) indicate the overall upward trend for the treated digester. It is important to note the specific biogas yield was already higher in Treated compared to the Control before the project started and during the seeding phase. It does appear that the biogas yield of the Treated digester returns to its previous yield and that the

Control decreases. When a statistical analysis was done to compare the data points after the augmented phase to the data prior to the seeding phase, it showed that the augmented phase in the Treated digester is significantly different to the pre-application phase (first 61 days) with a $p = 1.06 \times 10^{-9}$.

Figure 8. Moving averages of the specific biogas yield per kilogram of VSD at SLR



A third way of determining the Specific biogas was assessed by looking at the biogas produced per cubic meter of feed. These details were included in Tables 8 and 9 but were not included in discussion or used for comparison as this calculation does not take into consideration the levels of organics in those cubic meters of water.

Table 8. AD comparisons between bioaugmented levels in Treated to Control at SLR.

	Unit	SLR			
		Control	Treated	Difference	% difference
Digester Capacity	<i>cubic meter</i>	2,650	2,650	-	-
Feeding Rate (Flow)	<i>cubic meter per day</i>	120.40	77.75	(42.66)	-35%
Average TS Influent	<i>Percentage (%)</i>	3.97	3.97	-	0%
Average VS Influent	<i>Percentage (%)</i>	78.11	78.11	-	0%
Average TS Effluent	<i>Percentage (%)</i>	1.92	2.75	0.83	43%
Average VS Effluent	<i>Percentage (%)</i>	64.19	60.38	(3.81)	-6%
Hydraulic Retention Time	<i>Days</i>	22.01	34.08	12.07	55%
pH	<i>Range 0 to 10</i>	6.95	7.05	0.10	1%
Temperature	<i>Celsius</i>	34.44	35.96	1.52	4%
Total Alkalinity	<i>ppm</i>	1,373.81	1,662.38	288.57	21%
Total Volatile Acids	<i>mg/L</i>	25.45	31.45	6.00	24%
Total Biogas Production	<i>cubic meter</i>	114,849	117,335	2,485.54	2%
Gas Production	<i>cubic meter per day</i>	2,088.16	2,133.36	45.19	2%
CH4%		-	-	-	0%
CO2%		-	-	-	0%
H2S		-	-	-	0%
O2 %		-	-	-	0%
Digester Influent (kgs of TS)	<i>kgs per day</i>	5,151.33	3,022.44	(2,128.88)	-41%
Organic Loading Rate (kgs of VS)	<i>kgs per day</i>	4,022.69	2,364.31	(1,658.37)	-41%
Digester Eff. kgs of TS	<i>kgs per day</i>	1,824.68	2,086.05	261.37	14%
Digester Eff. kgs of VS	<i>kgs per day</i>	1,000.08	1,259.48	259.40	26%
Kilograms of VS Destroyed (KVSD)	<i>kgs per day</i>	3,022.60	1,104.83	(1,917.77)	-63%
Specific Biogas Yield (per kgs of VSI)	<i>cubic meter per kilogram per day</i>	0.037	0.059	0.022	60%
Specific Biogas Yield (per kgs of VS destroyed)	<i>cubic meter per kilogram per day</i>	0.073	0.141	0.068	93%
Specific Biogas per cubic meter of Feedstock	<i>cubic meter per cubic meter per day</i>	138.57	209.84	71.28	51%
V/A Ratio (Volatile Fatty Acids to Alkalinity)		0.019	0.019	0.001	4%
Van Fleeck % VSR	<i>Percentage (%)</i>	49.72	57.23	7.51	15%
Mass Balance %	<i>Percentage (%)</i>	59.53	45.40	(14.13)	-24%

Table 9. AD comparisons during seeding phase compared to augmented phase within the treated digester at SLR.

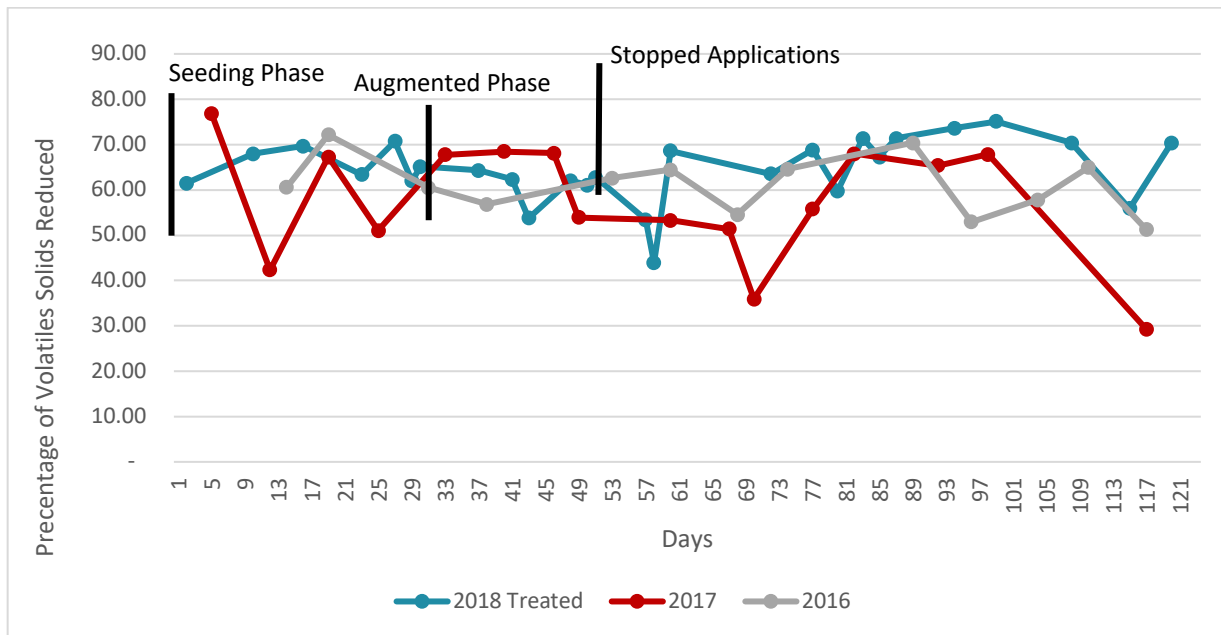
	<i>Unit</i>	SLR		
		Before	After	<i>% difference</i>
Digester Capacity	<i>cubic meters</i>	2,650	2,650	0%
Feeding Rate (Flow)	<i>cubic meters per day</i>	81	78	-4%
Average TS Influent	<i>Percentage (%)</i>	3.94	3.97	1%
Average VS Influent	<i>Percentage (%)</i>	73.66	78.11	6%
Average TS Effluent	<i>Percentage (%)</i>	2.67	2.75	3%
Average VS Effluent	<i>Percentage (%)</i>	60.54	60.38	0%
Hydraulic Retention Time	<i>Days</i>	32.84	34.08	4%
pH	<i>Range 0 to 10</i>	6.9	7.05	2%
Temperature	<i>Celsius</i>	35.77	35.96	0%
Total Alkalinity	<i>ppm</i>	1,408	1,662	18%
Total Volatile Acids	<i>mg/L</i>	31.12	31.45	1%
Gas Production	<i>cubic meters per day</i>	1,976	2,133	8%
CH ₄ %	<i>Percentage (%)</i>	-	-	0%
CO ₂ %	<i>Percentage (%)</i>	-	-	0%
H ₂ S	<i>ppm</i>	-	-	0%
O ₂ %	<i>Percentage (%)</i>	-	-	0%
Digester Inf kgs of TS	<i>kgs per day</i>	3,243	3,022	-7%
OLR Digester Inf. kgs of VS	<i>kgs per day</i>	2,390	2,364	-1%
Digester Eff. kgs. of TS	<i>kgs per day</i>	2,170	2,086	-4%
Digester Eff. kgs. of VS	<i>kgs per day</i>	1,314	1,260	-4%
Pounds of VS Destroyed	<i>kgs per day</i>	1,086	1,105	2%
Specific Biogas Yield (VSI)	<i>cubic meter per kg per day</i>	0.85	0.94	11%
Specific Biogas Yield (VSD)	<i>cubic meter per kg per day</i>	1.92	2.25	17%
Specific Biogas Yield (Flow)	<i>cubic meter per cubic meter per day</i>	24.7588	28.05	13%
Volatile Acids to Alkalinity	<i>ratio</i>	0.02	0.02	-14%
Van Fleeck % VSR	<i>Percentage (%)</i>	44.97	57.23	27%
Mass Balance %	<i>Percentage (%)</i>	44	45.4	3%

SLO

Volatile Solids Reduction

The SLO plant did not complete as many samples and tests on their biosolids as did the SLR plant personnel. As such the data do not support good figures, trendlines or detailed analysis. Figure 8 depicts the sporadic data points in all three years from the treated plant in 2018, and historical from 2016 to 2017. While it appears that the 2018 treated data indicate greater VS reduction than those of the historical periods after day 60, at most points it's hard to notice any real difference through the use of the Figure 8 alone. The average VSR across the project timelines in 2018 was 63.98%, while the two-year average of the two identical calendar periods was 58.58%, a 9% improvement. The Mass Balance calculation also shows a 9% greater loss of organic material from the Treated digester with remaining VS of 56.53% compared to the Historical average of 62.15% of VS remaining. The results of the statistical analysis on these two metrics are split. The %VSR was significant with a $p = 9.15 \times 10^{-2}$ while the Mass Balance was not significant with a $p = 6.03 \times 10^{-1}$ after the augmented phase.

Figure 9. Percentage of VS reduced using Van Fleeck method at SLO



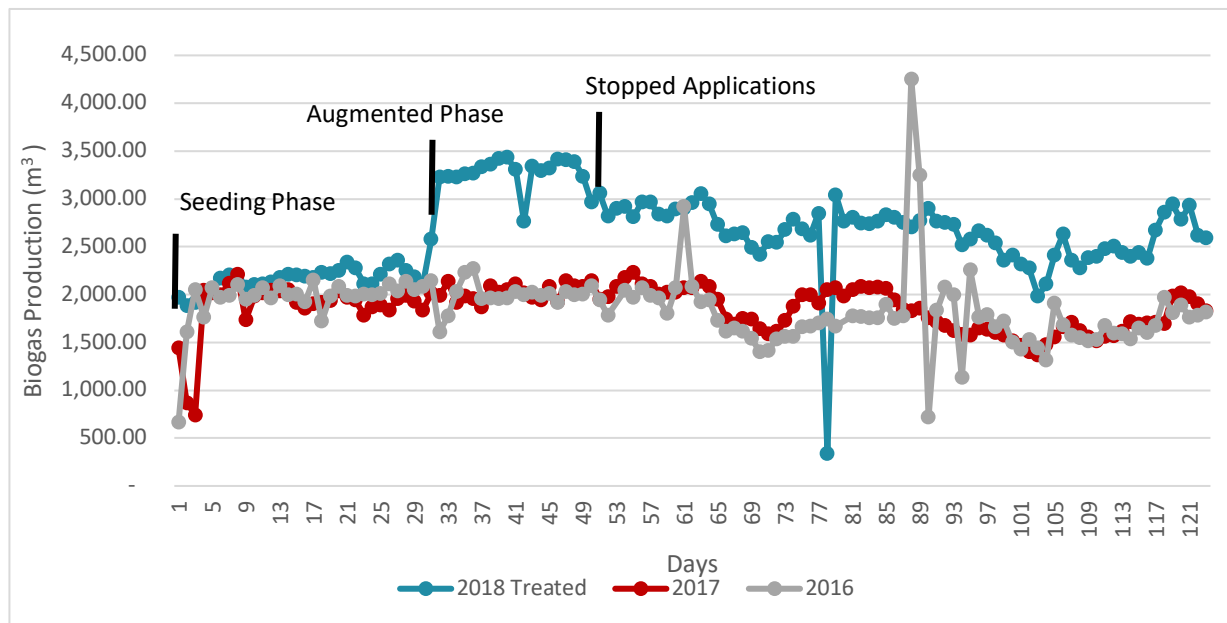
There are performance metrics that show potential impacts on the overall digester performance. There was a decrease in feed into the digester during the project when compared to the two-year historical averages of approximately 1,160 kgs per day on average. This equates to a drop of 15% which increased the retention times from 63 days in the two-year historical data to 75 days during the 2018 Treated period. While there was a drop in flow, the OLR for both digesters were found not to be statistically different with a $p = 2.85 \times 10^{-1}$ throughout the entire project. Temperature during the 2018 Treated period was 3° degrees lower, which was within the normal operational range and the pH remained unchanged. The volatile acids to alkalinity ratio was not calculated as SLO did not test for total volatile acids. Alkalinity exhibited a slight increase of 8% to 4,495 mg/L from 4,180 mg/L historically.

Biogas production

SLO collected accurate and detailed notes on its biogas. It collected both daily biogas production but also it tested for biogas composition throughout the project timeline and historically. With these data points, a detailed comparison on the digester performance was completed. The specific biogas yield comparisons completed at SLO were based on a smaller sample of biosolids data than at SLR, but still had enough data points to conduct statistical significance testing.

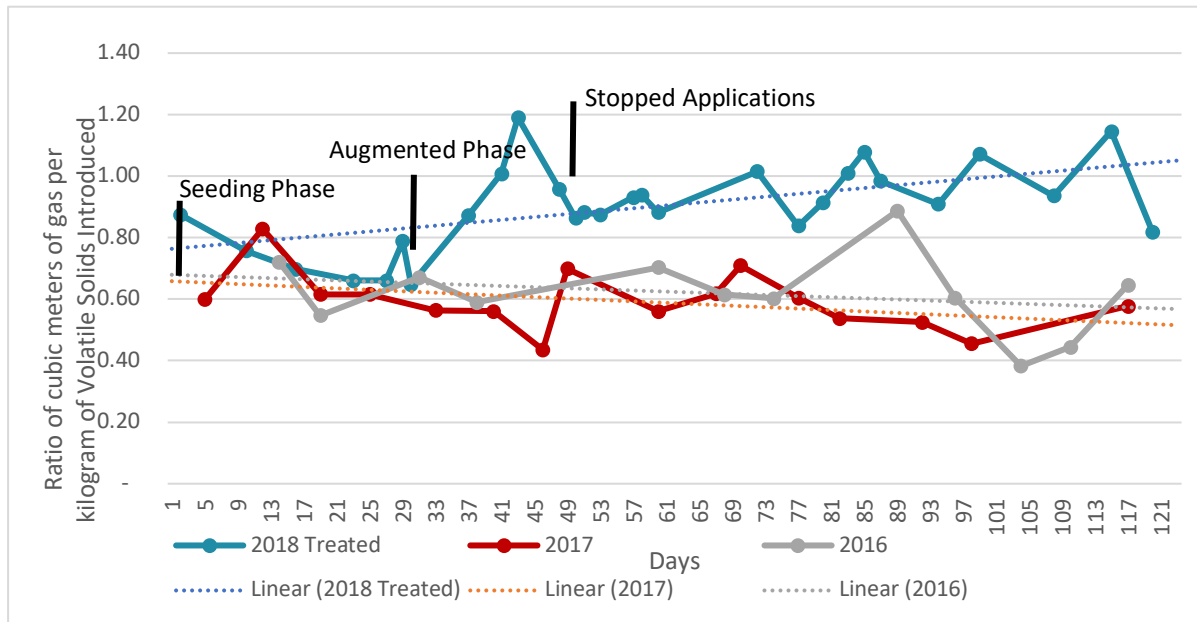
The SLO biogas data exhibited a much clearer picture of the effects of bioaugmentation. During the seeding period the biogas production showed some minor changes and then saw a significant step up into higher levels of biogas production at the start of the maintenance applications. The average biogas as seen in Figure 9 for the 2018 post-bioaugmented period was 2,764 cubic meters per day while the two-year historical average was 1,857 cubic meters per day. This is a 49% increase over the whole remaining bioaugmented phase. This was also found to be statistically significant with a $p = 9.96E-41$. It is important to note the average biogas production for 2018 includes all data post augmentation as it is visually apparent that the biogas production slowly began to decline. This decline began after the weekly applications were stopped on day 51, as explained above. It is reasonable to assume that the increased production of biogas would have remained at a higher level with continued addition of the bio-catalytic product.

Figure 10. Daily total biogas production cubic meters per day from SLO digesters



As mentioned above, the data for VSR at SLO is variable and therefore shows larger variability with the specific biogas production per kilogram of VSI in Figure 11. This chart still shows the significant step up during the augmented phase. The historical two-year average was $0.592 \text{ m}^3/\text{kg}$ of VSI, while the augmented average was $0.958 \text{ m}^3/\text{kg}$ of VSI, a 62% increase, with a $p = 1.04 \times 10^{-2}$. With the flow differences between the historical averages and the flows in 2018, even though the OLR are not significantly different, it would also be beneficial to look at the changes to the specific biogas yield during the seeding phase, which was at $0.727 \text{ m}^3/\text{kg}$ of VSI and the Augmented Phase at $0.958 \text{ m}^3/\text{kg}$ of VSI, a 32% increase within the same digestion system and same year.

Figure 11. Specific biogas yield per kilogram of VSI at SLO.



Using 7-day and 30-day moving averages (see Figures 12, and 13) help to show the smooth upward trend of the specific biogas production.

Figure 12. 7 day moving averages of the specific biogas production per VSI at SLO

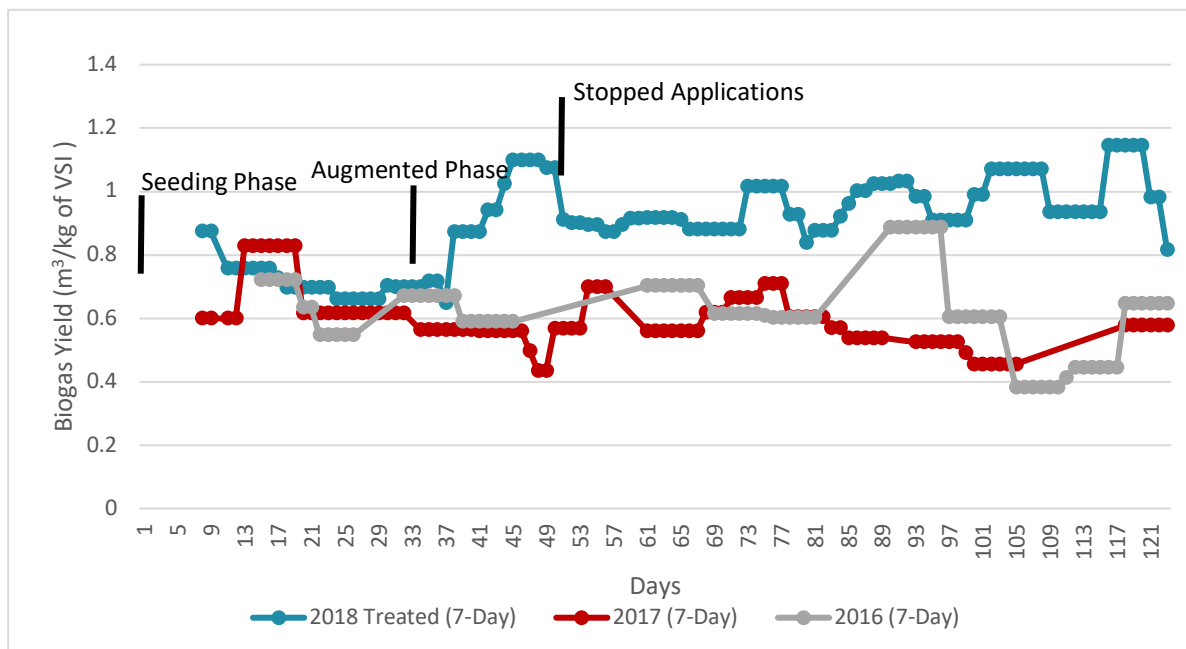
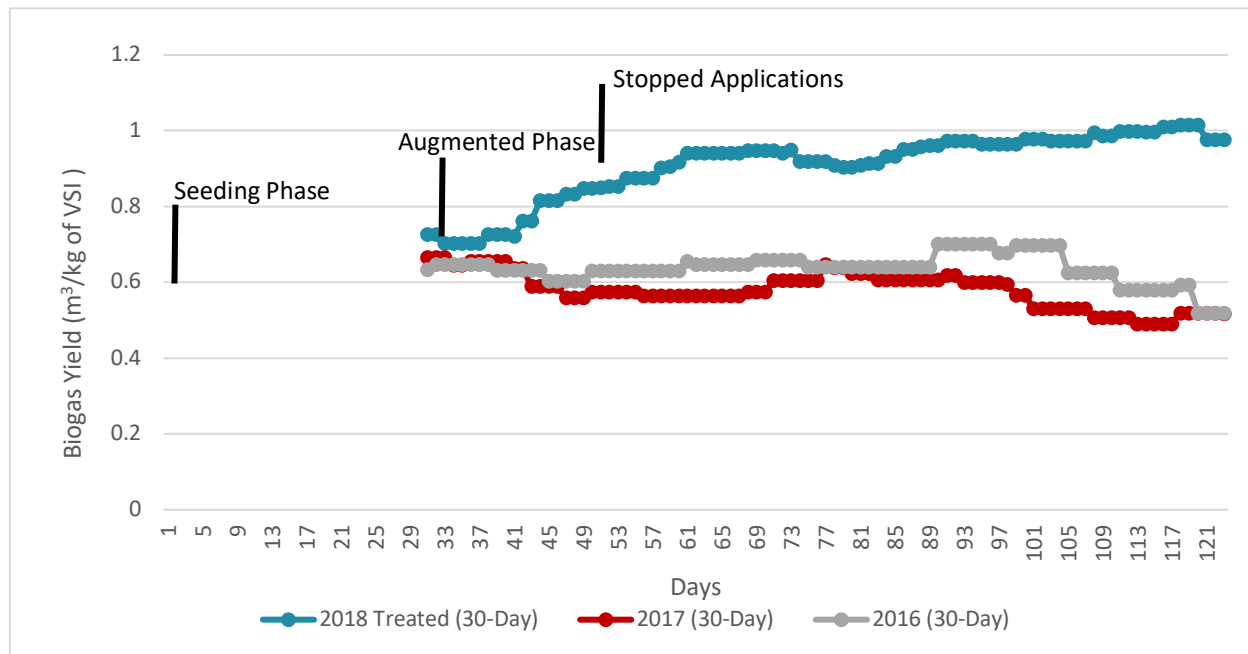
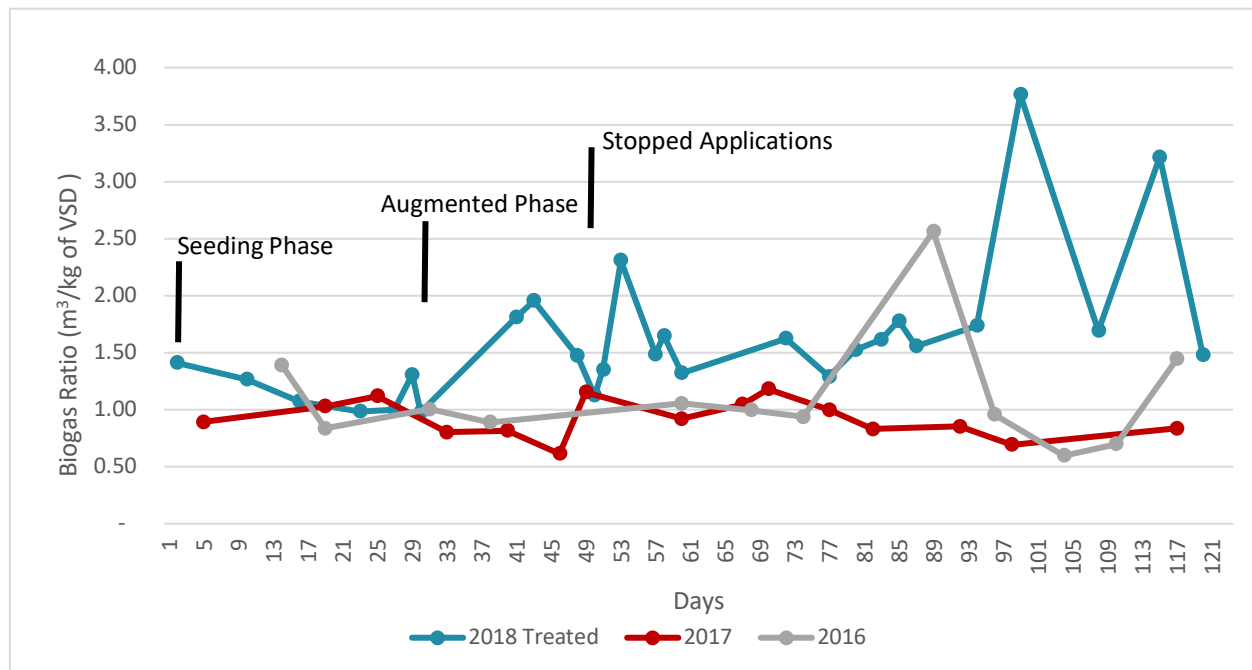


Figure 13. 30 day moving averages of the specific biogas production per VSI at SLO



The last way to compare the biogas production and digester efficiency at the SLO plant is using the specific biogas yield per kilogram of VSD. Again, the data points are not regular, however Figure 14 does portray the effects of bioaugmentation as the 2018 augmented phase reached an average of $1.811 \text{ m}^3/\text{kg}$. of VSD. This is a clear improvement over the two-year historical average of $1.01 \text{ m}^3/\text{kg}$. of VSD, representing an 80% increase, and with a $p = 1.99 \times 10^{-2}$. Figure 14 does highlight that in previous history the digestions system did reach a similar level of performance which appears as either an outlier or on an irregular basis.

Figure 14. Specific biogas yield per kilogram of VSD at SLO.



SLO also provided consistent biogas composition data which provide another viewpoint into the impacts of bioaugmentation. Biogas samples were taken each day by a Dräger sampler and analyzed. Each sample was analyzed for methane, carbon dioxide, and hydrogen sulfide. Figure 15 displays the changes in biogas hydrogen sulfide (H₂S) throughout the project timeframe. During a period of time the H₂S was undetectable. It was confirmed by supervisors and plant operators that the samples were properly taken and analyzed. This confirmed that the hydrogen sulfide had dropped to undetectable levels. It was conveyed by the operators that the impact on the production of the H₂S gas may have been a by-product of the bioaugmentation process, as they had never seen this kind of decrease in H₂S before. This is speculation as there is no other data to confirm this from SLO or from SLR. The resulting decrease in H₂S lagged approximately 33 days after the biogas production had reached the Augmented phase. Subsequently, the H₂S readings returned to previous levels approximately 35 days after the after

the last maintenance application. This may be in part because of the already low levels at the SLO plant. SLO and SLR employ ferrous chloride to reduce H₂S production in the digestion system.

Figure 15. Hydrogen sulfide biogas production at SLO

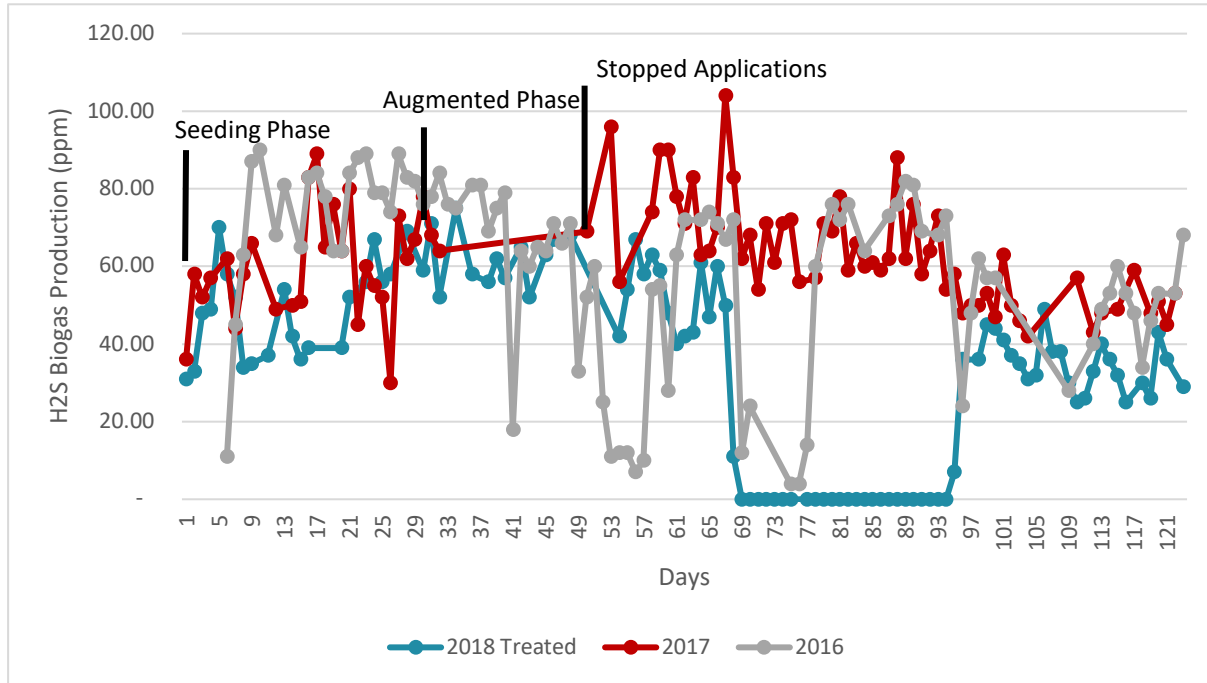


Table 10. AD comparisons of new bioaugmented levels compared to historical averages.

	Unit	SLO			
		Historical Average (2017, 2016)	2018 Treated	Difference	% difference
Digester Capacity	<i>cubic meter</i>	4,050	4,050	-	-
Feeding Rate (Flow)	<i>cubic meter / day</i>	63.38	53.70	(9.68)	-15%
Average TS Influent	<i>Percentage (%)</i>	6.21	6.60	0.39	6%
Average VS Influent	<i>Percentage (%)</i>	84.22	83.13	(1.09)	-1%
Average TS Effluent	<i>Percentage (%)</i>	2.83	3.58	0.75	26%
Average VS Effluent	<i>Percentage (%)</i>	68.68	66.63	(2.05)	-3%
Hydraulic Retention Time	<i>Days</i>	63.90	75.43	11.52	18%
pH	<i>Range 0 to 10</i>	7.25	7.34	0.09	1%
Temperature	<i>Celsius</i>	29.99	31.07	1.08	4%
Total Alkalinity	<i>ppm</i>	4,180.38	4,495.13	314.75	8%
Total Volatile Acids	<i>mg/L</i>	-	-	-	-
Total Biogas Production	<i>cubic meter</i>	169,905.65	257,067.11	87,161.46	51%
Gas Production	<i>cubic meter / day</i>	1,846.66	2,764.16	917.50	50%
CH4%		56.07	57.33	1.27	2%
CO2%		43.65	42.40	(1.25)	-3%
H2S		58.85	30.40	(28.45)	-48%
O2 %		0.19	1.21	1.02	529%
Digester Influent (kgs of TS)	<i>kgs per day</i>	3,771.66	3,648.60	(123.07)	-3%
Organic Loading Rate (kgs of VS)	<i>kgs per day</i>	3,184.11	3,008.15	(175.96)	-6%
Digester Eff. kgs of TS	<i>kgs per day</i>	1,741.40	1,937.90	196.50	11%
Digester Eff. kgs of VS	<i>kgs per day</i>	1,197.45	1,283.04	85.59	7%
Kilograms of VS Destroyed (KVSD)	<i>kgs per day</i>	1,986.65	1,725.11	(261.55)	-13%
Specific Biogas Yield (per kg of VSI)	<i>cubic meter per kilogram per day</i>	0.037	0.060	0.023	62%
Specific Biogas Yield (per kg of VS destroyed)	<i>cubic meter per kilogram per day</i>	0.063	0.112	0.049	78%
Specific Biogas per cubic meter of Feedstock	<i>cubic meter per cubic meter per day</i>	219.82	395.32	175.50	80%
Volatile Acids to Alkalinity		-	-	-	0%
Van Fleck % VSR	<i>Percentage (%)</i>	58.58	63.98	5.40	9%
Mass Balance %	<i>Percentage (%)</i>	62.15	56.53	(5.62)	-9%

Table 11. AD comparisons of the seeding phase and the post-seeding phase within the treated digester.

	Unit	SLO		
		Before	After	% difference
Digester Capacity	<i>cubic meters</i>	4,050	4,050	0%
Feeding Rate (Flow)	<i>cubic meters per day</i>	57.04	53.70	-6%
Average Total Solids Influent	<i>Percentage (%)</i>	6.09	6.60	8%
Average Volatile Solids Influent	<i>Percentage (%)</i>	84.83	83.13	-2%
Average Total Solids Effluent	<i>Percentage (%)</i>	2.83	3.58	27%
Average Volatile Solids Effluent	<i>Percentage (%)</i>	65.69	66.63	1%
Hydraulic Retention Time	<i>Days</i>	71.01	75.43	6%
pH	<i>Range 0 to 10</i>	7.33	7.34	0%
Temperature	<i>Celsius</i>	34.43	31.07	-10%
Total Alkalinity	<i>ppm</i>	4,803	4,495	-6%
Total Volatile Acids	<i>mg/L</i>	-	-	
Gas Production	<i>cubic meters per day</i>	288.35	369.52	28%
Gas Production (Prior vs Augmented)	<i>cubic meters per day</i>	288.35	415.53	44%
CH4%	<i>Percentage (%)</i>	56.80	57.33	1%
CO2%	<i>ppm</i>	41.72	42.40	2%
H2S	<i>Percentage (%)</i>	48.82	30.40	-38%
O2 %	<i>kgs per day</i>	0.24	1.21	399%
Digester Inf lbs of TS	<i>kgs per day</i>	3,506	3,649	4%
Organic Loading Rate (OLR)	<i>kgs per day</i>			
Digester Inf lbs of VS		2,980	3,008	1%
Digester Eff. lbs of TS	<i>kgs per day</i>	1,626	1,938	19%
Digester Eff. lbs of VS	<i>kgs per day</i>	1,069	1,283	20%
Kilograms of VS Destroyed (KVSD)	<i>cubic meter per kg per day</i>	1,911	779	-59%
Specific Biogas Yield (per kilogram of VSI)	<i>cubic meter per kg per day</i>	0.727	0.958	32%
Specific Biogas Yield (per kilogram of VS destroyed)	<i>cubic meter per cubic meter per day</i>	1.145	1.788	56%
Specific Biogas per cubic meter of Feedstock	<i>ratio</i>	37.93	52.85	39%
V/A Ratio (Volatile Fatty Acids to Alkalinity)	<i>Percentage (%)</i>	-	-	0%
Van Fleec % VSR	<i>Percentage (%)</i>	65.76	63.98	-3%
Mass Balance %	<i>Percentage (%)</i>	64	56.53	-11%

Interviews

As an interesting addition to the quantitative data analyzed above, interviews were conducted prior to the start of the project and post-project. These interviews introduced some more qualitative descriptive data to provide some insight from the perspective of the operators who managed the project at the plants directly. It was originally hoped that multiple interviews would be conducted at both the SLR and SLO plants, resulting in identification of common themes, and a deeper understanding of the experiences of operators at each location.

Unfortunately, only two interviews were conducted, one interview at each plant. Both interviewees were seasoned operators with no previous history with bioaugmentation. In spite of the small number of interviews, they did provide insight into how they expected the project to run its course, and positive feedback on its results. The telephone interviews were recorded, and the transcribed copies are found in Appendix H and I.

Interview Results

The two pre-project interviews provided some details on plant operational descriptions, the operator's previous experience with bioaugmentation, thoughts on expected results and impact on the plant performance, insights on methods to be employed during the project, and any potential limitations or issues that could arise and impact the project.

The SLR operator expressed that neither he nor his team had talked about bioaugmentation before and as he says, "We're not familiar with it". It was an idea that had not even been discussed as the operator explained, "We like the plants and the process itself. It's naturally ability to perform the bio-solid solid reduction". On the other hand, the SLO operator was aware of the potential benefits of bioaugmentation and explained that "it would be a great

opportunity, knowing especially that during some months, we don't produce as much biogas". For SLO, this project was in direct response to the direction given from "City Council and our major stakeholders of climate action". Neither of the interviewees felt there were any physical, or operational limitations that could cause an impact on the project, however the SLO operator felt that a potential inhibitor would be "just the fact that kind of convincing management that, this is a good idea, and that it's okay that we add something to the digesters", could be an issue. Both interviewees were excited and expected that by introducing additional biological activity improved performance would happen.

The two post-project interviews provided insight on any changes or issues that came up during the project regarding the systems' previous performance levels and comparisons against the post-augmented phase. The interviewees provided their thoughts on whether or not they felt the project was a success and why, as well as if they felt the project warranted further use of the bioaugmentation products. Lastly, they provided any recommendations, and final comments about the project.

According to the interviewees, the operations of both AD systems at the plants were not changed during the course of the project. The SLR operator expressed that there has been an ongoing issue with managing flows to the digesters because of the inadequate valves. The SLO operator explained that a serious issue arose during their project. The "problems that happened was regarding the Air Pollution Control District Permit and just the capability of burning how much additional biogas we were producing." The Operator continued "When we were in the full force, the bioaugmentation pilot project with [the bioaugmentation product] the capacity of our digester gas piping system was exceeded so that it started venting through our pressure release

valves.” Both operators expressed that the project showed an improved performance in the AD system. The SLO operator explained “there was definitely a significant increase in biogas production... Yes, I think it exceeded our anticipated 30% increase in biogas production.” while the SLR operator felt he was expecting more results, as he put it “I thought it would be doing a little bit better, at least five percent better.”

While both operators reported that during the project both had issues and challenges to overcome, they both expressed increased interest and desire to continue to work with bioaugmentation products. As the SLR operator put it “I see a place for them in the future as restrictions and permit limits get tighter and tighter, as consumption of energy is becoming a greater issue within the wastewater treatment system.” Their last recommendations and comments were to help future operators understand their systems’ capacity and limitations prior to bioaugmentation and provide adequate personal protective equipment for all operators during the process. The SLO operator summed it up with “I wish we could've kept going longer [laughter] I think that we only got the tip of the iceberg with what [the] product can really help us with. And with that I'm excited for future opportunities”.

Discussion

There are significant issues that arise in a decision to optimize a wastewater plant in part, or as a whole. With regulations dictating the level of treatment required and the pressures to protect the environment, AD system optimization has become more prevalent. This project provided an opportunity to contribute to the discussion and look at one particular method of optimization through bioaugmentation. There was a level of difficulty in locating site participants. Each plant’s decision to participate in this project was strongly tied to the plants’

motivations to optimize the performance of their AD system and balance the cost structures that surround them. First, each plant that was approached to participate in the project had different goals. Most goals were to focus solely on reaching and maintaining treatment within the plant's permit. There were only a few plants that had a higher objective to achieve plant efficiency. It appears that there is a hierarchy of needs as a plant moves from the primary needs to meet regulations, to more secondary needs including increasing plant efficiency and reducing costs. Second, when the plant focuses on increasing plant efficiency there is a need to consider all of the costs associated with each proposed modification. As such they have to determine if the modification will improve their economic efficiency, not just plant efficiency, as they still have to remain within their budgetary responsibilities.

Limitations

There were four primary limitations within the scope of this project: the length of time of the project; operational adjustments during the project; potential infrastructure inefficiencies; and the accuracy of the laboratory tests.

The total observation time during this project was 4 months. This short time was determined by the cost of product provided by the sponsor, and the allowable time to carry out the project from each site participant. In retrospect, a full 12 month or multiple year project would provide more in-depth comparison, allow for seasonal variations to be monitored, and allow for the long-term impact of the bioaugmentation to be evaluated.

Operational adjustments to the plant operations happen on a regular basis and are mandatory to meet regulatory requirements. For example, the allowable total suspended solids in effluent are highly regulated and can be a fineable offense if it falls outside of the allowable

limit. If the sludge blankets (level of organics) in primary or secondary tanks get too high, the resulting suspended solids can trickle over into the next treatment process and increase the total suspended solids in the system outfall. Adjusting the solids levels within the primary or secondary settling tanks moves organics from these tanks into the digester. This process may have impacted the study by increasing solids which would have provided above or below normal operating conditions in the anaerobic digesters.

The infrastructure, from valves to gas meters and everything in between, is not 100% reliable or accurate. For example, because of inaccurate valves, SLR reported that they had issues controlling the flow into their digesters. In another plant utilizing the same commercial BC, in the Eastern USA, the biogas production meter readings at the individual digesters did not match the aggregate consumption meters, demonstrating how gas meters are not 100% accurate. This is important to note in the comparison of results but is not expected to completely discredit all observations or results.

The lab test samples taken for laboratory tests can be impacted by sampling error. Most tests were completed using a single sample. Typically, the sample was just a quick grab of waste at one point of time (American Public Health Association, 2017). Also, most tests were run individually and not in parallel with other samples of the same wastewater and time. Completing the lab tests in triplicate would decrease the probability of a poor grab sample, which may contain a singular large piece of biomass, by comparing all three tests and by removing any outliers and increasing the accuracy of testing results (American Public Health Association, 2017). An increase in testing frequency increases the amount of time and money to perform each additional test. This project did not expect site participants to incur increased costs for

testing and each site was asked to carry on testing as per their normal. SLO did increase the number of samples and tests but did not do triplicate samples.

Results Summary

There are two major methods of comparison to determine the impact on the VS reduction and biogas production within municipal anaerobic digestion systems. To measure the impacts of the routine bioaugmentation with a biocatalytic microbial and enzyme consortium, a systems specific biogas production per kilogram of VS and its percentage of VS reduced comparison was completed. The initial comparison of just total biogas production could be misleading as the feedstocks from different plants, digesters and seasons could vary in feedstock characteristics. For this purpose, the use of specific biogas production per kilogram of VS was used. The data analysis in the results section above utilized these two methods and indicates that both the SLO and SLR both saw an increased performance due to the bioaugmentation process.

The increased total biogas production was clearer at the SLO plant as their daily average biogas increased by 50% from 1,847 m³/day to 2,679 m³/day. Looking closer at the plant and using the specific biogas per kilogram of VSI, SLO saw a 62% increase, and an 80% increase in biogas per VSD over historical levels. While SLR saw similar improvements of 60% in biogas per VSI and 93% in biogas per VSD, the AD system had significant issues of providing similar operating conditions compared to the control, as shared during their interview, because of an ongoing issue with managing flows to the digesters because of inadequate valves. A more focused look at the treated digester compared to itself shows a 11% increase in biogas per VSI and a 17% increase of biogas per VSD. This might more accurately portray the results from bioaugmentation and mitigate comparison to the Control digester which experienced the changes

to feed rate and HRT. With all this in mind the specific biogas yield achieved post-augmentation in SLO and SLR of 1.811 m³/kg of VSD, and 2.254 m³/kg of VSD respectively, are higher than the typical “gas production rates from mesophilic digestion” (Kalogo & Monteith, 2008) of 0.749 to 1.124 m³/kg of VSD.

The second method to determine any process improvement is to examine the percentage of VS removed. In using both the Van Fleeck and the Mass Balance calculation for VS reduction provided sufficient data to suggest an improvement occurred. SLO recorded a matching improvement from both calculations, where the VS reduced during the project compared to Historical averages improved by 9% each. While with the SLR plant recorded a 15% improved reduction of VS with the Van Fleeck equation compared to a 24% improvement through the Mass Balance comparison between the Control and the Treated digester. The VS reduction improvement within just the Treated digester was 27%. As stated by Michael Switzenbaum (2003) it was not expected that the Van Fleeck and the Mass Balance equations would provide exactly the same result but would provide closely connected data.

The statistical comparisons provided some evidence that the percentage improvement was not by chance but that a marked improvement was accomplished by utilizing the BC. SLO and SLR had opposite significant results. SLO's changes in OLR were not significant indicating that the overall organic levels were not different. SLO's total biogas production was found to be significantly different. A specific biogas yield comparison confirmed that the increased biogas per kilogram of VSI or VSD were also significant. The biogas production results indicate that the use of BC significantly improved the performance of SLO's AD system. On the other hand, SLR had the flipped results. With the issues of flow regulation, the OLR differences were found

to be significant, indicating that the levels of organics were different. SLR's total biogas production remained the same and was not significantly different. A specific biogas yield comparison for both the VSI and VSD showed that AD system's biogas production was significant. The results shown in SLR also show the impacts of BC on the performance of SLR's AD system. Therefore, this shows that improved digestion and increased AD system performance can occur when either one of the performance metrics, OLR or total biogas production, is not significant while the other one metric is significant. If both OLR and total biogas are significantly different then this would indicate that the differences in OLR were correlated to the changes in the biogas production.

The reduction in hydrogen sulfide gas production recorded at SLO was an indication of additional benefits of bioaugmentation. When H_2S is present in biogas, it acts as a corrosion agent impacting gas scrubbing and combustion devices and increasing the cost of maintenance during the overall biogas to energy process. H_2S also inhibits the removal of other harmful substances from the biogas to energy process found in biogas such as siloxanes and halogenated VOCs (Kalogo & Monteith, 2008). The possibility of reducing H_2S gas to undetectable levels could greatly impact the operation and economic feasibility of wastewater plants. However, with the applications being cut short at SLO, and with no data collected from SLR on biogas quality, there are enough data to suggest that the bioaugmentation process reduces H_2S gas, but not enough to make any definitive conclusions.

Interestingly, the magnitude of the results from measuring the increase in biogas was much larger than the magnitude of results from measuring the destruction of organics. It remains uncertain why the magnitude of results differs; perhaps it revolves more around which phase in

the digestion the BC makes more impact. The process of anaerobic digestion occurs in four phases. It starts with Hydrolysis where complex molecules “are converted into simple sugars, long-chain fatty acids and amino acids” (Evans, Strezov, & Evans, 2015), which is followed by the Acidogenesis phase where “bacteria convert the products of hydrolysis into volatile fatty acids” (Evans, Strezov, & Evans, 2015). The third phase called Acetogenesis is where “further digestion produces acetic acid, carbon dioxide and hydrogen” (Evans, Strezov, & Evans, 2015) and lastly the process is complete with the Methanogenesis phase where “methane, carbon dioxide and water are produced by acetotrophic (primary), hydrogenotrophic and methylotrophic bacteria (Evans, Strezov, & Evans, 2015). The first phase of hydrolysis is where the destruction of organics is achieved, while the conversion of different substrates into biogas occurs in primarily in phase four (Evans, Strezov, & Evans, 2015). If all the multiple phases in the anaerobic digestion process were increased, then it would make sense that the magnitude of results would be increased, as the destruction of organics would be multiplied by the results in the other three phases.

There are two major limiting phases within AD which may provide an explanation of what happened in this study. First, the hydrolysis phase, is a rate limiting step, where the “hydrolysis is dependent on the molecular complexity of the feedstock, with carbohydrates hydrolysed quite quickly and more complex cellulosic feedstocks hydrolysed quite slowly.” (Evans, Strezov, & Evans, 2015). This phase is limited by the ability of the AD system to break down the organics into simple sugars. A review of the VS destruction in both projects, as discussed above, indicates that there was no significant improvement made. The improved biogas production may have come from the methanogenesis phase. As stated by Evans et al.

(2015) “methanogens have a much slower rate of growth than acidogens and so methanogenesis is typically the rate-controlling step, such that the kinetics of methanogenesis describe the kinetics of the entire process”. As there are only two limiting phases, it can be speculated that the use of the biocatalytic compound improves the last step of methanogenesis. The increase in anaerobic bacteria, enzymes and nutrients to the system may have as its main impact on the methanogenesis phase. The exact make-up of the biocatalytic compound is unavailable as it is a proprietary formula created by the Sponsor.

Lessons Learned in Anaerobic Digestion

It is very difficult to find any system that has the exact same infrastructure, operations and feedstock. It is also so hard to control, operate and measure any AD systems to provide exact comparisons even within the same system when compared to historical data or to side-by-side data. These are the lessons learned as each project came up against significant and almost detrimental challenges.

Initially the project was to include up to four locations to provide even more comparison and additional data to showcase the impacts of bioaugmentation. Two of these potential locations had major delays, causing both sites to be dropped out of the project. In addition, SLO and SLR had delays in reporting complete data reports.

With apparent differences in size, operation, flow, percent solids loading, mixing, frequency and type of tests, the SLO and SLR plants were more different than they were similar. Comparisons between them were only made more difficult as SLO stopped applications part way through the test, while SLR had significant flow control issues. These issues led to the

investigator taking an approach of more local comparisons within the system itself or within one digester to historical data.

On reflection, the daily biogas production at SLR did not see an increase over 10% where at SLO there was a large visual and operational improvement in the daily biogas production over 30%. This may be due in part to infrastructure/operational differences that might cause a significant impact on the results achieved. SLR uses a single stand-alone digester compared to another identical digester in series, where SLO uses three smaller digesters run in series one after the other. The Sponsor has provided historical information from two other locations that experienced similar performance results. An AD system in Utah that was run in series using the same bioaugmentation method and product achieved marked results for both the increase in biogas and reduction in biosolids (Acti-Zyme Products Ltd, 2018). While a different AD system in California which ran two similarly sized and operated digesters side by side did not see increases in daily biogas production. Even though their data analysis showed no increases in biogas production they did experienced improved operations of utilizing their biogas during their application period. They were able to run additional gas-powered blowers during the application period until the end of the project, after which they did not have sufficient biogas to run those blowers (Acti-Zyme Products Ltd., 2018). These results provided additional insight. It appears that the inline or in series digestion systems had better results. This comparison led to further investigation into the application dosage rates. It was determined by the sponsor that the application rate into the AD systems with primary and secondary digesters (as shown in Figure 1a) run in series was dosed at 0.120 kg/m^3 into their primary digester. Compared to 0.061 kg/m^3 that went into the individual digesters that were run in parallel (as shown in Figure 1b). The total

dosage rates for both systems used a ratio of total product based on the total capacity of the entire AD system. The systems with digesters in series dosed their primary digester with the full seeding dosage, resulting in a higher concentration per cubic meter of capacity within the primary digester. The higher biogas production from systems run in series and the higher concentration of BC in those ‘primary’ digesters suggests that the difference in biogas production between the plants run in series or single digesters may have occurred because of the higher dosage rate.

Data, sampling, testing procedures and sampling frequency also played an important role in the comparison and analysis of the data. SLR did not sample or test biogas composition, while SLO tested this every day. Conversely, SLO only conducted samples and test on solids once a week while SLR completed these multiple times a week. There were some data outliers that occurred which could be attributed to human error, or sampling error. Most of these data issues could have been resolved with higher frequency of samples and tests repeated in triplicate to ensure accuracy, as explained in the limitations of this project.

Significance of Work

If bioaugmentation with biocatalytic products could improve Anaerobic Digestion operations, reduce total biosolids, and increase biogas production, it would lower costs and provide potential avenues for revenue/energy savings. As mentioned previously, a 10% improvement, as reported by Daniel Gary (2017) from the Joint Water Pollution Control Plant (JWPCP) located in Los Angeles County, one of the largest wastewater facilities in North America, which opted out of participating in this project, would reduce solids by 12,101 dry tons, saving \$2 million per year in transportation costs. A 10% improvement in biogas

production would provide increased energy valued at \$2.3 million per year. This would be a total benefit of \$3.1 million each year for the JWPCP, net of all costs for the biocatalytic compound.

The successful results at SLO and SLR provide evidence that enhanced digestion using BC products could be a very significant economic benefit to the anaerobic digestion operations improving their long-term sustainability and become a template for future applications across the USA and the world. Forecasting the present improvement obtained at each location during this study as the expected improvement in their AD efficiency over a full year of maintenance applications, the biocatalytic products addition can be estimated to provide an annual net expected economic benefit. SLO would have an annual net economic benefit of \$63,123.06 where a \$14,958.49 savings came from the reduction of biosolids at \$51 per wet ton plus \$62,654.32 in savings from the increased biogas at a natural gas equivalent of \$7.00 USD per mcf, minus the cost for product of \$14,489.75 would equal the net benefit of \$63,123.06. SLR would have an annual net economic benefit of \$124,870.74 where a \$123,187.50 savings came from the reduction of biosolids at \$45 per wet ton plus \$26,710.99 in savings from the increased biogas at a natural gas equivalent of \$7.00 USD per mcf, minus the cost for product of \$25,027.75 would equal the net benefit of \$124,870.74.

Future Work

There is lots of future work to be done in anaerobic digestion. This project had a limited scope, project limitations and lessons were learned. As suggested above, the potential of BC in the AD treatment process could positively impact its economic evaluations. Therefore, this suggests the importance of future projects to further understand the implications of BC products

in AD systems. For example, the development of future projects using BC product in AD systems could include additional tests to better understand the impacts of the feedstock composition, biological activity within the digesters, as well as the extended frequency, number and period of testing.

Feedstock composition determines the potential biogas production and ease of organic breakdown. Tests could be conducted to determine the levels of cellulose, protein, lipids in the feedstock. This would provide insights as to how the BCs, containing a consortium of bacteria and enzymes, might be optimized by the manufacturer for the composition of the feedstock.

Tracking the biological activity through DNA sequencing could help show which organisms are being positively or negatively affected by the BC. Being able to track the different types of organisms, with their corresponding roles in digestion, could show which stages of AD are being impacted. This additional information could provide context on the results and allow the manufactures of BC products to make any adjustments to their formulations.

Increasing the length of time for a project like this would show long term trends, account for seasonality, and the effectiveness of bioaugmentation over a longer period.

Lastly, as discussed above, it is important to maintain the same dosage rate. It would be recommended to complete a similar side-by-side project and ensure that the applications rates were 0.120 kg/m^3 per single digester capacity. Completing this type of project could provide a more accurate comparison to the projects that employ an in-series setup.

Conclusions

The impact of routine bioaugmentation with a biocatalytic microbial and enzyme consortium has shown, through this project, to provide Anaerobic Digestion Enhancement on the

reduction of volatile solids and improved biogas production within two municipal anaerobic digestion systems. The use of both of the Van Fleeck formula to calculate percentage of volatile solids reduction and the specific biogas yield providing the ratio of biogas production per kilogram of volatile solid introduced/destroyed, assisted in comparing SLO and SLR, within each individual system and to each other. These ratios and formulae provided the ability to compare plants especially when ones like SLO and SLR had to overcome site specific differences, operational issues and challenges. The data analyzed from SLO and SLR have provided indications that there was in fact an increased reduction of volatile solids on an average across both locations of 12% with an increase of specific biogas production yield of 37.5% per kilogram of volatile solids destroyed.

Works Cited

- Acti-Zyme Products Ltd. (2018a). *Springville AD Enhancement Report*. Calgary: Acti-Zyme Products Ltd.
- Acti-Zyme Products Ltd. (2018b). *San Bernardino digester enzyme addition data report*. Calgary: Acti-Zyme Products Ltd.
- American Public Health Association, A. W. (2017). *Standard Methods for Examination of Water and Wastewater, 23rd Edition*. American Public Health Association, American Water Works Association, and Water Environment Federation.
- Aydin, S. (2016). Enhancement of microbial diversity and methane yield by bacterial bioaugmentation through the anaerobic digestion of *Haematococcus pluvialis*. *Applied Microbiology and Biotechnology*, 100(12), 5631-5637.
- Canadian Council of Ministers of the Environment. (2012). *CANADA-WIDE APPROACH FOR THE MANAGEMENT OF WASTEWATER BIOSOLIDS*. Ottawa: Canadian Council of Ministers of the Environment.
- Creswell, J. (2009). The selection of a research design. In J. Creswell, *Research design: Qualitative, quantitative, and mixed methods approaches* (pp. 3-22). Thousand Oaks, CA, USA: Sage.
- D. L. Pritchard, N. P. (2010). Land application of sewage sludge (biosolids) in Australia: risks to the environment and food crops. *Water Science Technology*, 62(1), 48-57.
- Duran, M., Tepe, N., Yurtsever, D., Punzi, V. L., Bruno, C., & Mehta, R. J. (2006). Bioaugmenting anaerobic digestion of biosolids with selected strains of *Bacillus*,

- Pseudomonas, and Actinomycetes species for increased methanogenesis and odor control. *Applied Microbiology Biotechnology*, 73(4), 960-966.
- Evans, A., Strezov, V., & Evans, T. (2015). Anaerobic Digestion . In *Biomass Processing Technologies* (pp. 177-212). Boca Raton: CRC Press/Taylor & Francis Group.
- Fotidis, I. A., Wang, H., Fiedel, N. R., Luo, G., Karakashev, D. B., & Angelidaki, I. (2014). Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. *Environmental Science and Technology*, 48(13), 7669-7676.
- Gavala, H. N., Yenal, U., Skiadas, I. V., Westermann, P., & Ahring, B. K. (2003). Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature. *Water Research*, 37(19), 4561-4572.
- Huoqing Ge, P. D. (2010). Pre-treatment mechanisms during thermophilic–mesophilic temperature phased anaerobic digestion of primary sludge. *Water Research*, 44(1), 123-130.
- Kalogo, Y., & Monteith, H. (2008). *State of science report: energy and resource recovery from sludge*. Global Water Research Coalition.
- Kelleher Environmental. (2013). *Kelleher Robins Canadian Biogas Study Technical Document*. Ottawa: Canadian Biogas Association.
- Metcalf & Eddy Inc. (2019, September 20). *Wastewater Engineering: Treatment and Reuse*. Retrieved from [VitalSource Bookshelf]:
<https://bookshelf.vitalsource.com/#/books/0077441214/>
- National Research Council. (1993). *In Situ Bioremediation When Does it Work?* Washington, DC: The National Academies Press.

- Nzila, A. (2017). Mini review: Update on bioaugmentation in anaerobic processes for biogas production. *Anaerobe*, 46, 3-12.
- Odnell, A., Recktenwald, M., Stensen, K., Jonsson, B.-H., & Karlsson, M. (2016). Activity, life time and effect of hydrolytic enzymes for enhanced biogas production from sludge anaerobic digestion. *Water Research*, 103, 462-471.
- Pennsylvania Department of Environmental Protection. (2017). *Details on Food to Microorganism Ratio F/M Activated Sludge Systems*. Retrieved from Pennsylvania Department of Environmental Protection:
<https://www.dep.state.pa.us/dep/deputate/waterops/redesign/calculators/FMDetails.htm>
- Romero-Guiza, M., Vila, J., Mata-Alvarez, J., Chimenos, J., & Astals, S. (2016). The role of additives on anaerobic digestion: A review. *Renewable and Sustainable Energy Reviews*, 58, 1486-1499.
- Schauer-Gimenez, A. E., Zitomer, D. H., Maki, J. S., & Struble, C. A. (2010). Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. *Water Research*, 44(12), 3555-3564.
- Shen, Y., Linville, J. L., Urgan-Demirtas, M., Mintz, M. M., & Snyder, S. W. (2015). An overview of biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: Challenges and opportunities towards energy-neutral WWTPs. *Renewable and Sustainable Energy Reviews*, 50(C), 50, 346-362.
- Statistics Canada. (2015, 11 27). *Section 4: Wastewater discharges*. Retrieved from Statistics Canada: <https://www.statcan.gc.ca/pub/16-201-x/2012000/part-partie4-eng.htm>

Switzenbaum, M. S., Farrell, J. B., & Pincince, A. B. (2003). Relationship between the Van

Kleeck and the Mass-Balance Calculation of Volatile Solids Loss. *Water Environment Research*, 75(4), 377-380.

Telliard, W. A. (2001). *METHOD 1684 Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids*. Pennsylvania: U.S. Environmental Protection Agency.

Water Environment Federation. (2015). *Biogas Data*. Retrieved from Resource Recovery Data:
<https://www.resourcerecoverydata.org/biogasdata.php>

Appendices

Appendix A – Plant Participation Authorization Forms

Plant Participation Consent Form

Project Name: Anaerobic Digestion Enhancement: Effects of Adding a Biocatalytic on Volatile Solids Reduction and Biogas Production

Researcher: Jonathan Lee, Student at Royal Roads University
 Dr. Mickie Nobel, Thesis Supervisor, [REDACTED]
 Dr. Audrey Dallimore, Thesis Committee Member, [REDACTED]

Research Purpose: The purpose of the study will be to investigate whether the use of a commercial grade BC affects an AD system, decreases the total biosolids, and improves biogas production by completing multiple analyses of full-scale side-by-side or full system biological experiments.

We invite [REDACTED] to participate in the above-mentioned research study and request that you complete the following consent form for your active participation.

Consent to take part in research

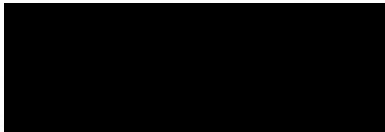
- I, [REDACTED] confirm that I am authorized personnel of City of San Luis Obispo, and voluntarily agree to participate in this research study.
- I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind, up until the thesis is final and approved by the thesis committee.
- I have had the purpose and nature of the demonstration project explained to me in writing and I have had the opportunity to ask questions about the demonstration project.
- I understand that participation involves completing at least a 4-month full-scale application of biocatalytic compounds in the anaerobic digesters located at the above mentioned facility.
- I understand that there is the potential that the addition of any foreign products could upset and decrease the effectiveness of the current anaerobic digestion process.
- I understand that there is historical data from multiple treatment plants that have shown safe and zero negative impact on the use of biocatalytic products. In addition, for large plants percussions will be taken such that these demonstrations are will not completed at a full-scale size but as a side-by-side comparison.
- I understand that I will not benefit directly from participating in this research.
- I agree to my interview being audio-recorded.
- I understand that questions will be asked to understand the anaerobic digestion process and throughout the demonstration period about the conditions, operations and results of using the biocatalytic compound.
- I understand that all information I provide for this study will be treated confidentially.
- I understand that in any report on the results of this research my identity will remain anonymous. This will be done by changing my name and disguising any details of my interview which may reveal my identity or the identity of people I speak about.
- I understand that anonymized extracts from my interview may be quoted in the thesis paper and that the participant's name will not appear on any final documentation whether the report is published or unpublished.
- I understand that signed consent forms and original audio recordings will be retained on external hard drives and be kept in safe keeping by Jonathan Lee until the final thesis defence is completed.

- I understand that I am entitled to access the information I have provided at any time while it is in storage as specified above.
- I understand that if I complete and submit any digital or paper questionnaire, it is assumed that consent has been given.
- I understand that only the principal investigator and the organizational sponsor will have access to raw data until it is destroyed one year after the final thesis report is completed.
- I understand that I am free to contact any of the people involved in the research to seek further clarification and information.
- I understand that the principal investigator, Jonathan Lee, has a direct working relationship with the sponsoring organization Acti-Zyme Products Ltd dba. Hycura.
- I understand that all or part of the research findings, video, or photographs may be used for commercialization purposes by the organizational sponsor.
- I understand that the use of any information from the research findings may lead to sales of the commercialized biocatalytic product. This may provide a direct benefit to the organizational sponsor and its affiliates, including the principal investigator.

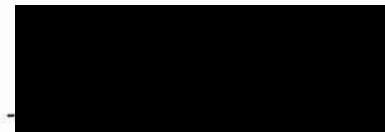
If there are any questions before proceeding with the demonstrations, please forward all inquiries directly to the principal investigator. A copy of this consent form will be sent for your records.

Principal Investigator

Jonathan Lee



Signature of research participant



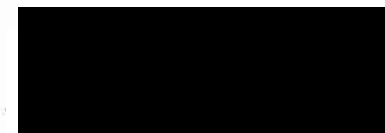
4/2/19

Signature of participant

Date

Signature of researcher

I believe the participant is giving informed consent to participate in this study



4 / 2 / 19

Signature of researcher

Date

Plant Data Utilization Permission Form

Thesis Topic: Anaerobic Digestion Enhancement: Effects of Adding a Biocatalytic on Volatile Solids Reduction and Biogas Production

Principal Investigator / Researcher: Jonathan Lee, Student at Royal Roads University
Dr. Mickie Nobel, Thesis Supervisor, [REDACTED]
Dr. Audrey Dallimore, Thesis Committee Member, [REDACTED]

Purpose of permission form: The purpose of this permission form is to grant approval for the use of raw data from anaerobic digesters at the San Luis Ray Plant, Oceanside, CA for the potential reference and use in the master thesis to be written by Jonathan Lee.

The data comprises the historical and pilot project data from all anaerobic digesters. The pilot project was carried out from August 2018 to May 2019. The pilot project was carried out to investigate whether the use of a commercial grade biocatalytic compound (Hycura AD) affects the Anaerobic Digestion system. The project completed multiple analyses of full-scale side-by-side biological experiments. The project was carried out by treating digester 4 and comparing its operational parameters and metrics to the other digesters.

We ask The City of Oceanside for permission to utilize in the above-mentioned historical and pilot project data for the potential reference and use in the master's thesis. We request that you complete the following consent form for your active participation.

Consent to take part in research

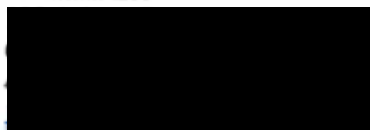
- I, [REDACTED] confirm that I am authorized personnel of **The City of Oceanside, CA**, and voluntarily agree to provide the above mentioned data for the use in this master's research project.
- I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind, up until the thesis is final and approved by the thesis committee.
- I understand that I will not benefit directly from participating in this research.
- I understand that questions will be asked to understand the anaerobic digestion process, the anaerobic digester operational conditions observations during the pilot project and results of using the biocatalytic compound.
- I understand that all information I provide for this study will be treated confidentially.
- I understand that in any report on the results of this research my identity, and the identity of the City and Plant will remain anonymous. This will be done by changing names and disguising any details of my interview which may reveal the identity of myself, the plant, the City or the identity of people I speak about.
- I understand that anonymized extracts from my interview may be quoted in the thesis paper and that the participant's name will not appear on any final documentation whether the report is published or unpublished.
- I understand that signed consent forms and original audio recordings will be retained on external hard drives and be kept in safe keeping by Jonathan Lee until the final thesis defence is completed.
- I understand that only video or photographic media will be shared to the public and will only consist of signage, infrastructure, lab testing.

- I understand that a transcript of my interview in which all identifying information has been removed will be retained for one year in a password secured location.
- I understand that I am entitled to access the information I have provided at any time while it is in storage as specified above.
- I understand that if I complete and submit any digital or paper questionnaire, it is assumed that consent has been given.
- I understand that only the principal investigator and the organizational sponsor will have access to raw data until it is destroyed one year after the final thesis report is completed.
- I understand that all or part of the research findings, raw data and results from the pilot project may be included in the master's thesis and subsequently published.
- I understand that I am free to contact any of the people involved in the research to seek further clarification and information.
- I understand that the principal investigator, Jonathan Lee, has a direct working relationship with the sponsoring organization Acti-Zyme Products Ltd dba. Hycura.
- I understand that all or part of the research findings, raw data, video, or photographs may be used for commercialization purposes by the organizational sponsor.
- I understand that the use of any information from the research findings may lead to sales of the commercialized biocatalytic product. This may provide a direct benefit to the organizational sponsor and its affiliates, including the principal investigator.

If there are any questions before proceeding with the demonstrations, please forward all inquiries directly to the principal investigator. A copy of this consent form will be sent for your records.

Principal Investigator

Jonathan Lee



Signature of research participant



10/10/19

Signature of participant

Date

Signature of researcher

I believe the participant is giving informed consent to participate in this research



10 / 10 / 19

Signature of researcher

Date

Appendix B – SLR – Raw Data

Raw data from SLR plant is found in the excel file named, Appendix B and C – Raw Data.xls. Data available upon request.

Appendix C – SLO – Raw Data

Raw data from SLO plant is found in the excel file named, Appendix B and C – Raw Data.xls. Data available upon request.

Appendix D – Interview Invitation Email

Dear xxx,

As your treatment plant is participating in the Full-Scale application demonstration project using Hycura AD granules in the anaerobic digesters we would like to interview you.

This is not mandatory, and you can opt not to participate. We would be using two different ways to interview you. One would be a digital survey with some technical and non-technical questions. The other would be an in-person interview that would be recorded and transcribed. The purpose of these interviews is to capture more information than raw data. We would like to learn more about your experience, expectations and overall impressions throughout the demonstration project.

Please respond back to this email with a completed participation interview consent form attached to this email. Upon completion we will send out the digital survey and set up a time for the in-person interview. If you have any further questions, please let me know.

Thank you,

Jonathan Lee

Student at Royal Road University

Introductory words

My name is Jonathan Lee, I am conducting a research study as a Student at Royal Road university entitled “Anaerobic Digestion Enhancement: Effects of Adding a Biocatalytic on Volatile Solids Reduction and Biogas Production”. I would like to use your facility to conduct, collect and analyze the application of adding biocatalytic products to your anaerobic digesters. I work with Dr. Mickie Nobel my Thesis Supervisor and Dr. Audrey Dallimore as a Thesis Committee Member. Both of which can be contacted to confirm this research study.

The purpose of the study will be to investigate whether the use of a commercial grade BC affects an AD system, decreases the total biosolids, and improves biogas production by completing multiple analyses of full-scale side-by-side biological experiments. This study will last for approximately 4 months. I will be providing all the product for introduction into your digester.

We would require from you the following:

1. Historical operational, and process data
2. Completion of a pre and post survey
3. Operational, and process data throughout the product demonstration and research study
4. Interviews with operators and managers to capture actual experience, expectations and overall impressions throughout the demonstration

Will you be willing to participate in this research study?

Appendix E – Interview Consent Forms

Interview Participation Consent Form

Project Name: Anaerobic Digestion Enhancement: Effects of Adding a Biocatalytic on Volatile Solids Reduction and Biogas Production

Researcher: Jonathan Lee, Student at Royal Roads University

Dr. Mickie Nobel, Thesis Supervisor, [REDACTED]

Dr. Audrey Dallimore, Thesis Committee Member, [REDACTED]

Research Purpose: The purpose of the study will be to investigate whether the use of a commercial grade BC affects an AD system, decreases the total biosolids, and improves biogas production by completing multiple analyses of full-scale side-by-side biological experiments.

We invite [REDACTED] to participate in the above mentioned research study and request that you complete the following consent form for your active participation.

Consent to take part in research interviews

- I, [REDACTED] voluntarily agree to participate in this research study.
- I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.
- I have had the purpose and nature of the demonstration project explained to me in writing and I have had the opportunity to ask questions about the demonstration project.
- I understand that participation involves completing at least a 4 month full-scale application of biocatalytic compounds in the anaerobic digesters located at the above mentioned facility.
- I understand that there is the potential that the addition of any foreign products could upset and decrease the effectiveness of the current anaerobic digestion process.
- I understand that there is historical data from multiple treatment plants that have shown safe and zero negative impact on the use of biocatalytic products. In addition, for large plants percussions will be taken such that these demonstrations are will not completed at a full scale size but as a side-by-side comparison.
- I understand that I will not benefit directly from participating in this research.
- I agree to my interview being audio-recorded.
- I understand that questions will be asked to understand the anaerobic digestion process and throughout the demonstration period about the conditions, operations and results of using the biocatalytic compound.
- I understand that all information I provide for this study will be treated confidentially.
- I understand that in any report on the results of this research my identity will remain anonymous. This will be done by changing my name and disguising any details of my interview which may reveal my identity or the identity of people I speak about.
- I understand that anonymized extracts from my interview may be quoted in the thesis paper and that the participant's name will not appear on any final documentation whether the report is published or unpublished.
- I understand that signed consent forms and original audio recordings will be retained on external hard drives and be kept in safe keeping by Jonathan Lee until the final thesis defence is completed.
- I understand that only video or photographic media will be shared to the public and will only consist of signage, infrastructure, lab testing.
- I understand that a transcript of my interview in which all identifying information has been removed will be retained for one year in a password secured location.

- I understand that only video or photographic media will be shared to the public and will only consist of signage, infrastructure, lab testing.
- I understand that a transcript of my interview in which all identifying information has been removed will be retained for one year in a password secured location.
- I understand that I am entitled to access the information I have provided at any time while it is in storage as specified above.
- I understand that if I complete and submit any digital or paper questionnaire, it is assumed that consent has been given.
- I understand that only the principal investigator and the organizational sponsor will have access to raw data until it is destroyed one year after the final thesis report is completed.
- I understand that I am free to contact any of the people involved in the research to seek further clarification and information.
- I understand that the principal investigator, Jonathan Lee, has a direct working relationship with the sponsoring organization Acti-Zyme Products Ltd dba. Hycura.
- I understand that all or part of the research findings, video, or photographs may be used for commercialization purposes by the organizational sponsor.
- I understand that the use of any information from the research findings may lead to sales of the commercialized biocatalytic product. This may provide a direct benefit to the organizational sponsor and its affiliates, including the principal investigator.

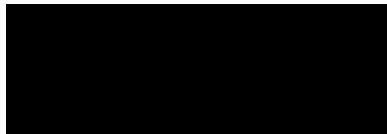
If there are any questions before proceeding with the demonstrations, please forward all inquiries directly to the principal investigator. A copy of this consent form will be sent for your records.

Principal Investigator

Jonathan Lee



Signature of research participant



4/2/19

Signature of participant

Date

Signature of researcher

I believe the participant is giving informed consent to participate in this study



4/2/19

Signature of researcher

Date

Interview Participation Consent Form

Project Name: Anaerobic Digestion Enhancement: Effects of Adding a Biocatalytic on Volatile Solids Reduction and Biogas Production

Researcher: Jonathan Lee, Student at Royal Roads University

Dr. Mickie Nobel, Thesis Supervisor, [REDACTED]

Dr. Audrey Dallimore, Thesis Committee Member, [REDACTED]

Research Purpose: The purpose of the study will be to investigate whether the use of a commercial grade BC affects an AD system, decreases the total biosolids, and improves biogas production by completing multiple analyses of full-scale side-by-side biological experiments.

We invite [REDACTED] to participate in the above mentioned research study and request that you complete the following consent form for your active participation.

Consent to take part in research interviews

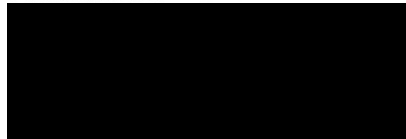
- I [REDACTED] voluntarily agree to participate in this research study.
- I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.
- I have had the purpose and nature of the demonstration project explained to me in writing and I have had the opportunity to ask questions about the demonstration project.
- I understand that participation involves completing at least a 4 month full-scale application of biocatalytic compounds in the anaerobic digesters located at the above mentioned facility.
- I understand that there is the potential that the addition of any foreign products could upset and decrease the effectiveness of the current anaerobic digestion process.
- I understand that there is historical data from multiple treatment plants that have shown safe and zero negative impact on the use of biocatalytic products. In addition, for large plants percussions will be taken such that these demonstrations are will not completed at a full scale size but as a side-by-side comparison.
- I understand that I will not benefit directly from participating in this research.
- I agree to my interview being audio-recorded.
- I understand that questions will be asked to understand the anaerobic digestion process and throughout the demonstration period about the conditions, operations and results of using the biocatalytic compound.
- I understand that all information I provide for this study will be treated confidentially.
- I understand that in any report on the results of this research my identity will remain anonymous. This will be done by changing my name and disguising any details of my interview which may reveal my identity or the identity of people I speak about.
- I understand that anonymized extracts from my interview may be quoted in the thesis paper and that the participant's name will not appear on any final documentation whether the report is published or unpublished.
- I understand that signed consent forms and original audio recordings will be retained on external hard drives and be kept in safe keeping by Jonathan Lee until the final thesis defence is completed.
- I understand that only video or photographic media will be shared to the public and will only consist of signage, infrastructure, lab testing.
- I understand that a transcript of my interview in which all identifying information has been removed will be retained for one year in a password secured location.

- I understand that I am entitled to access the information I have provided at any time while it is in storage as specified above.
- I understand that if I complete and submit any digital or paper questionnaire, it is assumed that consent has been given.
- I understand that only the principal investigator and the organizational sponsor will have access to raw data until it is destroyed one year after the final thesis report is completed.
- I understand that I am free to contact any of the people involved in the research to seek further clarification and information.
- I understand that the principal investigator, Jonathan Lee, has a direct working relationship with the sponsoring organization Acti-Zyme Products Ltd dba. Hycura.
- I understand that all or part of the research findings, video, or photographs may be used for commercialization purposes by the organizational sponsor.
- I understand that the use of any information from the research findings may lead to sales of the commercialized biocatalytic product. This may provide a direct benefit to the organizational sponsor and its affiliates, including the principal investigator.

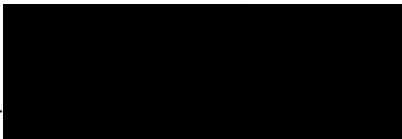
If there are any questions before proceeding with the demonstrations, please forward all inquiries directly to the principal investigator. A copy of this consent form will be sent for your records.

Principal Investigator

Jonathan Lee



Signature of research participant



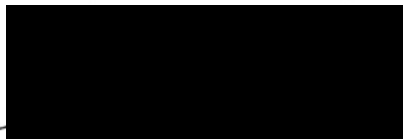
8-9-19

Signature of participant

Date

Signature of researcher

I believe the participant is giving informed consent to participate in this study



8/9/19

Signature of researcher

Date

Appendix F – SLR and SLO Digital Surveys

AD System Initial Survey

Organization Name	City of Oceanside	Date	
System Name	San Luis Rey WWTP	WWTP Flow	9.3 MGD
Main Contact / Title			
Main		Shipping	
Address		Address	0
City		City	0
State		State	0
Zip/Postal		Zip/Postal	0
Phone			
Email			
AD System Capacity		AD Retention	22 Days 33 Calculated Retention Time
	Units		Units
1	700,000 gallons		
2	700,000 gallons		
3	700,000 gallons		
4	700,000 gallons		
5	gallons		
6	gallons		
7	gallons		
8	gallons		
9	gallons		
10	gallons		
11	gallons		
12	gallons		
13	gallons		
14	gallons		
15	gallons		
16	gallons		
17	gallons		
18	gallons		
19	gallons		
20	gallons		
21	gallons		
22	gallons		
AD System Dimensions		System Age	
	28,666.67		
Average Daily Flow (Sludge In)	86,000 gallons/day	Daily Avg. Biogas	158,400 c. f. m3
Average Daily Flow (Sludge Out)	50 Tons/day	How is biogas used?	
Biosolids Disposal Frequency	daily	Flared	Yes
% TS Raw	2.00	Heat	Yes
% VS Raw	80.00	Electricity	Yes
% TS Out	2.70	Sold to grid	No
% VS Out	65.00	% Methane Gas	n/a %
% VSR	53.57142857	% Sulphur Gas	n/a ppm
Volatile Fatty Acids	47	Temp	98 C
		pH	7
AD System Description		Total Alkalinity	1,710
4 total - 3 operating, 1 to 4 - 700,000 - traditional, mesophilic, parallel, all individual metered and tested. Primary to direct, secondary WAS goes into thickener then into the digester. FOG station (new), h2s spike issues. Scum pits goes directly into digester.		Ratio	0.02748538

Biogas Production Evaluation

Biosolids Reduction Valuation

Price of Natural Gas	<input type="text"/>	Power Bill (Press)	<input type="text"/>
Gas Bill (Plant / AD)	<input type="text"/>	Tipping Fee	<input type="text"/>
Price of Electricity	<input type="text"/>	Labour Cost	<input type="text"/>
Electric Bill (Plant/AD)	<input type="text"/>	Transportation Cost	<input type="text"/>
Electric Price to Grid	<input type="text"/>	Chemical Costs	<input type="text"/>
Top Priority		Total Costs	<input type="text"/>
Reduce Biosolids	<input type="text"/>	Cost of Product	
Increase Biogas	<input type="text"/>	Annual Savings	
Decrease Costs	<input type="text"/>		
Decrease Odor	<input type="text"/>		
Improve Efficiencies	<input type="text"/>		
Other	<input type="text"/>		
Additional Comments	<input type="text"/>		
		Additional Comments	<input type="text" value="dewatering, with polymer into centerfige, then to arizona - 9 trucks a week. (25 tons per truck)"/>

Biogas Production Evaluation

Biosolids Reduction Valuation

Price of Natural Gas	<input type="text"/>	Power Bill (Press)	<input type="text"/>
Gas Bill (Plant / AD)	<input type="text"/>	Tipping Fee	<input type="text"/>
Price of Electricity	<input type="text"/>	Labour Cost	<input type="text"/>
Electric Bill (Plant/AD)	<input type="text"/>	Transportation Cost	<input type="text" value="\$166,205"/>
Electric Price to Grid	<input type="text"/>	Chemical Costs	<input type="text"/>
Top Priority		Total Costs	<input type="text"/>
Reduce Biosolids	<input type="text"/>	Cost of Product	
Increase Biogas	<input type="text"/>	Annual Savings	
Decrease Costs	<input type="text"/>		
Decrease Odor	<input type="text"/>		
Improve Efficiencies	<input type="text"/>		
Other	<input type="text"/>		
Additional Comments	<input type="text"/>		Additional Comments
			\$140,810 in 2016 \$48-55/ton, 3258.93 ton/yr, haul to off-site compost facility

Appendix G – Interview Questions

Interview Questions – Start of Project

1. Have you read, understand and completed the consent form previous to this interview?
2. Can you explain the biosolids processes in your plant? From headworks through to biosolids disposal? Please include any chemicals used in the processes.
3. What are (were) your thoughts about using bio-catalytic, bacteria/enzyme, products in anaerobic digesters?
4. What has been your experience so far with these types of products?
5. What are the expected benefits you desire or would be optimal for you and the plant?
6. What do you understand about the bio-augmentation process?
7. Can you see that there would be any limitations or inhibitors for a successful project?
8. Can you explain to me how you are expecting to use the bio-catalytic product?
9. How important would augmentation of the anaerobic digestion process be for the plant?
10. Do you think there will be any issues, operational or other that could be caused during this project? If so, please explain.

Interview Questions – End of Project

1. Have you read, understand and completed the consent form previous to this interview?
2. Were there any changes in the wastewater treatment plant process or operations that are worth noting that might have affected the bio-augmentation project?
3. What were the issues, if any, in running this project? Example, collecting data, operating the digesters, complications in treatment plant operations, measurement tools or methods, etc. If so, please explain.
4. What were the previous biogas production levels? What are the
5. Were you able to archive the desired benefits? Please explain?
6. Would you consider the project a success? If so, Why?
7. Have the results been shared with managers and city officials?
8. Would you consider continuing use of this bio-catalytic product?
9. After completing this project what are your thoughts about using bio-catalytic products?
10. What did you learn during the process of this project?
11. Would you recommend the use of biocatalytic products for bio-augmentation in anaerobic digesters to other operators and wastewater treatment plants?
12. Are there any recommendations that you would give other treatment plant operators looking to use similar products?
13. Any last comments or thoughts about the project and its results?

Appendix H – SLO – Interview Transcripts

Pre-Project Interview

Interviewer: And so, you know, this will be recorded, right? So I can transcribe it for the notes and everything.

Interviewee: That's fine.

Interviewer: Great. So the first question I need to ask is that you've read, understand and completed the consent form? Previously interviewed, correct?

Interviewee: Yeah.

Interviewer: Very good. Okay. So there's kind of two parts. We'll do this in two time. Unfortunately, I didn't have all this kind of figured out before we even started the project, so I'm going to be asking questions as if we had-- was at the beginning of the project, the first few questions.

Interviewee: Okay

Interviewer: And then I'll make a distinct note, okay, now we have to think, okay, now the project is finished and we're looking back on the project. Does that make sense?

Interviewee: Yeah.

Interviewer: Yeah. Okay. Awesome. Okay, so the first question is, can you explain the biosolid process in your plant? From the head works through to disposal of biosolid and you can include different chemicals that you use to process, etc of essentially where are those biosolids are going and how they get treated?

Interviewee: Right. Okay, so we get all of our input wastewater and the first stage is that we have chain rakes in which screenings are removed. They're washed and compressed so that all of the BOD and solid-state in our waste stream, like our process stream. Then we have air rated grit chambers that move all the grit. And then it goes into primary clarification and sludge from the primary clarifiers and scum from the primary clarifiers, get pumped over to our DAFT which is dissolved air flotation thickener].

Interviewer: Yeah

Interviewee: Additionally we have a second clarifier. Downstream of the primary and trickling filter and the scum and sludge from the second clarifier goes to the DAFT as well. And then we have our aeration basins and final clarifiers in which the wasted MLSS gets also sent over to DAFT.

Interviewer: Okay, so there's three different types of solids or sludge that gets moved to the DAFT?

Interviewee: Correct

Interviewer: Okay

Interviewee: For aeration, we add calcium hydroxide as an alkalinity and pH buffer. So there might be residual amount that get moved over there in the sludge, but it's not necessarily for a solid process.

Interviewer: Right. That's for the aeration. Okay, in the basin, yeah? And you guys don't use anything else in this for chemicals for settling out or polymers or Ferric for H₂S?

Interviewee: No. Hmm, yes, but I'm just following the solids right now we use. So then the DAFT, you're familiar with that. We use air to flow and there's a solid, and those solid get put into digester one influent and that's about 6% solid, I would almost say. And then, in our digestion process, we do add, roughly 35 to 40 cubic meters of ferrous chloride a day for each to sulphur gas control.

Interviewer: Right. Okay

Interviewee: And then the solid, then go through digester one, goes through digester two, digestion three is pretty much a holding tank. It's negligible amount of solid destruction or biogas production. And then it goes into our screw press, in which we add a polymer and it gets pressed out and then our cake is roughly 20% solid and that gets shipped off or trucked off.

Interviewer: Good. Great. That's fantastic. Thank you. That's a good detail. Answered the question well. When we first approached and suggested doing a project using biocatalytic products like this, bacteria and enzymes for augmentation and the digestion, what was your initial thoughts when you heard about it?

Interviewee: I approached you guys to follow the direction of our city council and our major stakeholders of climate action. The city is now looking at different avenues of green energy. And I thought it would be a great opportunity, knowing especially that during some months, we don't produce as much biogas. Some of the initial thoughts of our operators that you now have maybe seen this stuff before was skeptical and hesitant because they have experienced an upset digester and so they kind of are protective of putting a different unknown substance into the digestion for fear of process disruption.

Interviewer: Right. Yeah. And then I guess that kind of covers a part of the next question is, that was their experience with the separate products, had you had any experience with other products similar to this type of category?

Interviewee: I had not, no.

Interviewer: Okay.

Interviewee: That's why I was, I guess, I was more willing to try something new because I haven't been negatively influenced.

Interviewer: Right. When you discussed it with your team, and after reviewing everything, what were the expected benefits that you would desire, that would be optimal for you in the plant?

Interviewee: Optimal would be to find a significant amount of biogas increase in production or biogas production increase, enough that it would offset the cost of the product for energy.

Interviewer: Right.

Interviewee: That would be kind of my main goal that energy out put.

Interviewer: Perfect. There are no other goals? Just primarily for biogas production?

Interviewee: Yeah, that was our main driver of this project.

Interviewer: Good. Okay. So before we started the project, what was your understanding about bioaugmentation and the process itself?

Interviewee: I personally had never dealt with bioaugmentation of a digester. I know in the past it's actually with cow manure for start up a digester or to help them if they become upset. So, that would be one source of bioaugmentation that I would be more familiar with. but I kind of just from a different standpoint, I realized that if you add vitamins to a digester or to a human body, things are going to happen and that's kind of how I used and describe it.

Interviewer: Yeah.

Interviewee: [inaudible] it's like you add probiotic to the digester.

Interviewer: Right. I understand that. Yeah. It's a good example. It's very good example. So when starting the project, did you or any of the other operators you worked with, see limitations or perhaps inhibitors that might affect the successfulness of the project?

Interviewee: It was actually very easy to use. We got to the digester on a daily basis to do our rounds and just by adding the bag into the influent channel, that was very simple. I think it wasn't so much of an inhibiting as much as people just didn't believe it would work.

Interviewer: Okay. So perhaps some of their beliefs, well do you think some of the beliefs would be inhibitors or were just, why I say that is, I've experienced before, other situations where if someone doesn't believe it's going to happen; they don't try and make it work or put in the effort to apply the product properly. But you didn't think that was going to be an issue, did you?

Interviewee: No. I think after we got buy-in from the staff, no one was actively trying to disrupt the process. I think one of the main inhibitors is just the fact that kind of convincing management that, this is a good idea, and that it's okay that we add something to the digesters, but I think the whole startup period, just by adding just five bags a day, and it was so quick to respond that quickly people kind of, weren't afraid of it anymore. I think it was more of like a fear-based approach.

Interviewer: Right. And you kind of lightly touched on it. Can you explain how you were expected to use the product day to day?

Interviewee: Yeah, so we figured it out that during morning rounds made the most sense for the operator to apply the products through the digester one influent channel. And we had created a sampling plan that everyone was aware of and we actually added it to our rounds sheet so people do not forget. But, yeah, you just toss it in there.

Interviewer: If you can explain, how important would the potential bioaugmentation of the digestion be for the plant or for the city council?

Interviewee: Important, for what?

Interviewer: Or for like the city council managers, how important would this project be?

Interviewee: The importance would greatly depend on kind of the result. If you were able to offset significant amount of our energy usage, the city council would be very supportive of the use of bioaugmentation. And if how we talked about it was like, even if we didn't necessarily seeing a significant reduction in energy usage, we would still be able to explore avenues for optimization. You can't expect everything to give you results the first try but it would lead us in the direction of more opportunity. I think our city council wants us to go to net-zero energy, we're about to bring two processed online, which will increase energy requirements for the facility. So yeah, something like this could be very important. And it would be more accessible since we're not having to add significant amount of infrastructure compared to adding solar or something. We already have the digesters, and then if we have the capacity to utilize all that additional biogas production, it would be very important.

Interviewer: Right. That's great. So one more last question here before the start of the project is, do you think there-- or did you plan or foresee any issues, either operational or others, that could affect the results and the data of the project?

Interviewee: Issues base on operations, I'm sorry, can you repeat the question?

Interviewer: Did you think there would be any issues, either operational or other, that could cause a difficulty during the project?

Interviewee: Difficulties could be caused, basically, if a digester were to go sour. And that's why we ramped up our sampling plan on alkalinity and pH and kept a closer eye on the digesters during this period to make sure that we were able to proactively change anything so that we didn't end up with an upset digester.

Interviewer: Right, right. That's kind of the big one. There wasn't anything else, either how things were operated or process changes that could cause an issue?

Interviewee: No, I mean, how our biosolid system is set up, it's pretty much just going straight forward. We are concerned maybe the change in sludge would, just as much characteristics to change with the added bioaugmentation, if that would react differently with a polymer, could we do jar testing to figure out a very specific polymer for the best flocculation for our sludge. But now, if there are fluctuation significantly change, how a direct affect our screw press and our dewatering ability,

Interviewer: Right. That's a good consideration. It's a very good one. Okay. Great. Okay, so I'm just going to pause and stop this one, save it and then move on to the next one here.

Post Interview

Interviewer: Okay. So, this will be recorded and I just have a question here at the beginning, like last time, is, you've read, understand and completed the consent form? Previous to the interview? Correct?

Interviewee: Correct.

Interviewer: Perfect. So, the first question was in regards to the project. Were there any changes in the wastewater treatment plant process, or operations that might have been worth noting, that could've affected the bioaugmentation project and its data?

Interviewee: No, we were operating under similar condition from once we had historically operated.

Interviewer: Okay, so it's very historical and no major changes, okay.

Interviewee: Correct.

Interviewer: So, now, were there any issues that came up in running it? You mention in the first interview concerns about sour digesters, about dewatering, maybe there's also issues that

could've come through collecting data or operating the digesters or any kind of complications at all? Or, even measuring different variables, or different tools, was there any issues that came up during the project?

Interviewee: Not so much issues but data collection since there was an increased amount of sampling, sometimes weren't as consistent as we hoped. And, I think our Cogen was offline. It broke down during the project at one time, so although we were able to measure the biogas production rate still we weren't able to necessarily look and see if it cleaner at burning or that had a higher energy content than the historical biogas.

Interviewer: Okay.

Interviewee: Additionally, some of the problems that happened was regarding the Air Pollution Control District Permit and just the capability of burning how much additional biogas we were producing.

Interviewer: Right. Can you explain a little bit more, where there fines? Or were there warnings? What was-- What happened there with the Air District?

Interviewee: Right. So, our digesters are-- pressure release valves are set in a specific pressure into the water column at designated increment. And with that we also have a flare which is online to burn whatever it's not able to go through the Cogen when it maxed out! And, when we were in the full force, the bioaugmentation pilot project with Hycura the capacity of our digester gas piping system was exceeded so that it started venting through our pressure release valves.

Interviewer: Mm-hmm.

Interviewee: You know?

Interviewer: Right.

Interviewee: So that it blow up the concrete topped digester, and we quickly were able to catch it, so it was not a fineable offense because it was mitigated. However, we had to make corrective actions so that we were no longer having periodic release of biogas.

Interviewer: Right. Is that during the time when the digester was down? I mean not the digester the Cogen?

Interviewee: I can look back at the data, let me look. It may have been because otherwise, I think there was more-- the fact that the piping couldn't handle it? And the flares set a specific back pressure as well?

Interviewer: Right.

Interviewee: But, as I go back to-- And it was stopped-- Oh gosh. When was it stopped? The pilot project was...

Interviewer: Was November 11th, I believe, right around then.

Interviewee: November 11th? Yes, it was having issues the Cogen was having faults, some of these sensors in the Cogen were kind of misbehaving? And we had our technician come out and working with him. So yes, that was around the same time.

Interviewer: Okay, got you.

Interviewee: We were having battery failure and it was offline. Yes so, it was just for them now of biogas that was trying to go through the flare, exceeded the flare's capacity.

Interviewer: Right. Had that ever happened before?

Interviewee: No!

Interviewer: Okay.

Interviewee: No.

Interviewer: Great, thank you. So, the next question was, from your opinion and after reviewing all the data and everything, were you able to achieve the desired results? Or benefits that you're looking for?

Interviewee: Yes, we got a-- I guess the desired information it seems that at this time the before we can and continue with the use of the product we will need to make digester and Cogen upgrades? I think it's definitely an opportunity and an avenue to explore the new digester system that will be installed in the next couple of years? And definitely a proven product that-- with additional infrastructure upgrades we would be able to use it.

Interviewer: Right. So, because your objective before was to-- and what was set out kind of by the city officials was to desire to get more energy and reduce the energy consumption that you're paying for? And to have the digester produce more gas, is that correct?

Interviewee: Correct. As long as that meant-- it made economical sense. So, at this time since our Cogen is not correctly sized it would not make sense to produce more biogas just so that is burned by the flare? We're not able to harness the additional energy production?

Interviewer: Correct, right.

Interviewee: But I think while moving forward with energy efficiency projects we could make the case for adding you know bioaugmentation to the digesters and use that to provide a case for

purchasing with additional Cogen so that we could optimize on-site energy production and move towards electricity goals, net-neutral energy.

Interviewer: Right, that's great. Do you recall what the biogas averages were typically before? By augmentation? Like what the daily average production were for like the previous periods?

Interviewee: Right, you probably not just as well as I do, after looking at all of the--

Interviewer: No, I do. I was just more asking a verbal question versus the data but yes.

Interviewee: Yes, yes, yes.

Interviewer: I do know what it is.

Interviewee: [laughter]

Interviewer: [laughter]

Interviewee: Let me look real quick. Yes, it looks like the month between August and December in which, you know the bioaugmentation was still in the system we produced 45% of the annual production of biogas. So, just between August, September, November, December, within four months we produced almost half of the amount of total biogas? So, there was definitely a significant increase in biogas production just by looking at those months alone.

Interviewer: Right.

Interviewee: So, as we continue to have that production I would say that-- what was that turned out to be? Our daily production? Yes, I think it exceeded our 30% our anticipated 30% increase in biogas production.

Interviewer: Exceeded that, you said?

Interviewee: Mm-hmm.

Interviewer: Yes, yes, great, no that's great, thank you. And so, from your opinion then, would you consider that the project was successful?

Interviewee: Was successful?

Interviewer: Successful.

Interviewee: What is it? Totally.

Interviewer: Yes.

Interviewee: I personally believe that we got our answers, that's why going into the project we wanted to see if the product worked? And then being able to use that data and information to go into an engineering and design phase, look at different stakeholders, and who we could partner for additional energy efficiency projects, and now we know that the product is definitely worth continuing exploring those avenues and working with our City Council to try to find grant funding to be able to optimize our on-site energy production.

Interviewer: Well that's great, that's great, thank you. Now, actually kind of goes to the next question, has all these results actually been shared with the plant managers and the city officials? So that they aware of what happened?

Interviewee: They, the results have been shared with the plant manager he's been part of this pilot project with also partnering with Questa College the local community college and using the Federal Work-Study Program to fund interns to help with the additional sampling and testing required.

Interviewer: Right.

Interviewee: Our intern has just completed her final report, and we're going to kind of edit it before sharing it with higher management in the city council but, yes, I anticipate that, especially with these positive results, that we are eager to share this with them? And additionally I'm-- would like to do some presentations of that in conferences and exploring which conferences would be most receptive to this? And additionally where the conferences are that the best could know this kind of data and information.

Interviewer: Very good and that would be very well welcomed from our part to try not be supportive to do that, that's fantastic. So, in connection with that sharing and information, you mentioned a little bit about further investigation. So, the next question was, would you then consider continuing to use about catalytic product like this?

Interviewee: Continue to use it? Well we have to--

Interviewer: In the future, once you do the--

Interviewee: Reconsider... uh?

Interviewer: Sorry, keep going, go ahead.

Interviewee: We've had to discontinue to use it due to our Air Pollution Control District Permit. However we would-- I think that there's opportunities to optimize the dosage? So that we're continuously maxing out our current Cogen and that could-- their capacities without overdosing so that we're wasting some of the products or the additional biofuel? The biogas that's getting produced. Alternatively, during the summer months, our flows drop off significantly because we

live in a college town and our population has significantly decreases? So, I think there's other opportunities to start it while we have lower influence flows and see if we're able to maintain a higher biogas production during those times when we might traditionally have a drop in production rate just because we have less biosolids to digest.

Interviewer: Right, we can continue that discussion afterward for sure. So, now that you've gone through this and thinking of yourself and maybe can slightly talk about other people's thoughts but after completing the project now what are your thoughts about using biocatalytic products.

Interviewee: I think that more people at our facility are open to them. I think that probably they're not all equal in valuableness but I think that a lot of the operators and staff at the facility here might have changed their minds a little bit, at least to be more receptive to learning more about them and considering them products, future opportunities.

Interviewer: Great. Is there something that you feel that you learned during this process of the project?

Interviewee: Yes, I mean I think, definitely a big learning thing is we hadn't even really considered or anticipated the capacity of our digester system, or just our gas system. So, learning about-- anticipating how much our gas pipelines can hold? Would be kind of something to be studied in future projects, to kind of consider-- it's not so much of, how much gas can we produce but what can we do with the additional gas that you're producing? So kind of taking it-- that next step? Also, just kind of importance of communication and maintaining the sampling plan, communicating what it is and then maybe just being a little bit more proactive of things, "Hey! We're producing a lot more digester gas, should we back it off a little bit right now? Or should we just-- so that we are maintaining?"

Interviewer: Yes.

Interviewee: or should we just-- "What is the maximum of gas that we should produce?" so I think that's where the optimization of using a product comes in.

Interviewer: Well, that's great.

Interviewee: And unfortunately we weren't able to meet that because management was fearful of continuing to send biogas to the atmosphere.

Interviewer: Right. And that makes sense. I just have one-- can you hold for just one second?

Interviewee: Mm-hmm.

Interviewer: Thanks.

Interviewee: Okay.

Interviewer: So, from your experience in the project would you recommend the use of biocatalytic products for bioaugmentation to other operators in other treatment plants?

Interviewee: Definitely! Especially those who have room in their digesters? Or are looking for digester upgrades I would encourage them to work with their design engineers to run a pilot project before designing, additional digestion processes so that they could account for-- using resources to maximize the production.

Interviewer: Oh, that's very good. Well said that's a-- it's a good thought. And the last one is, looking at the project and what you've learned from it and the experience you had, would there be any recommendations to plant operators of how to run a project or how to use a product like this?

Interviewee: To the plant operators, I would definitely caution them but it probably will take longer than they think? Just to get it set up, really just kind of regarding what-- how much time do you guys have to spend to the project and looking at the additional sampling and you guys are very receptive in working with-- what sampling points are required which was nice to have so that though additional workload doesn't become burdensome?

Interviewer: Mm-hmm.

Interviewee: And then, also just having a very easy to follow methodology, don't-- even try to keep it as simple as possible so that it doesn't fall to the wayside so that people continue to collect and analyze the data that is needed. So operators, but you know-- specifically operators just, don't be scared I think that people are unwilling to try new things because something has happened before but you never know until you try it and just some proactive analytics can go a long way especially with digester health and help.

Interviewer: Yes, of course.

Interviewer: Well, that's great, great recommendations, thank you. So is this last question, just, do you have any last minute comments or thoughts about the project and its results?

Interviewee: hmm. Last minute comments. I wish we could've kept going longer [laughter] I think that we only got the tip of the iceberg with what your product can really help us with. And with that I'm excited for future opportunities to try Hycura again. Especially as we get our new digesters with different heating and mixing systems and to kind of compare that and maybe how older digesters operated with the new digesters. Additionally, right now we are on an activated sludge plant, and we're going to start doing a-- or we're not only going to do nitrification but in the future we're going to also include the denitrification with methane bioreactors so our waste streams will be different. We're not going to be having a trickling filter or a second clarifiers. So, I'd be curious to see how the characteristics of the waste stream change with the effectiveness of the bioaugmentation product.

Interviewer: Right.

Interviewee: For biogas production.

Interviewer: That's great! Well thank you so much, really, first of all I appreciate the wiliness to have these interviews. But also to do the whole project together. We felt it went very well, and we're grateful for your support as well as the support for all the other operators and managers and city council to do it. And moving forward I will provide all of the details, information's, the transcriptions of these interviews so you can review to make sure that I haven't misquoted you or done things incorrectly? And then I'll eventually at the end with the-- when the thesis is finished I'll send you a copy as well.

Interviewee: Awesome! I look forward to seeing that.

Interviewer: That'd be great.

Interviewee: And we're able to work on that together.

Interviewer: That's fantastic, thanks to much! Have a great day.

Interviewee: Thank you, bye-bye Jonathan.

Interviewer: Bye!

Appendix I – SLR – Interview Transcripts

Pre-Project Interview Transcript

Interviewer: All right. I'm recording. Right on. Okay. The first question is can you explain the bio-solid process in your plant from like headworks through the disposal?

Interviewee: Yes. A biosolid process to our plant is basically the removal of biosolids from the wastewater streams. That's basically three different parts. First is the preliminary, which removes anything larger than a quarter inch. Then, the primary, which removes all the settleable materials and un-settleable materials, floatables. Lastly would be our secondary, which is a biological removal of the coliforms, but I believe, what our concern was with the digesters is the floatables and the settleable from our primary tank. Basically, our wasting process of our biological lifeforms.

Interviewer: Right. Okay. Now, can you explain any chemicals that you use during the process?

Interviewee: There's a number of chemicals. Now, primary, we actually use advanced primary treatment. We use positive displace farther to bionic polymer for our primary. Also, we do Ferris and ferrous injection in our primary and secondary.

Interviewer: Right. Okay. Can you explain why you use those chemicals?

Interviewee: The polymer actually reduces-- I'm not fond between the two reduces. The data protection between the particles. And actually have and it could conglomerate together.

Interviewer: Right.

Interviewee: The ferrous acts as another molecule for the polymer to actually attach to, making it a more successful joining of the masses or the biomass. It all kind of acts like adding benefits by creating iron sulfide inside of our digestives trapping the sulfide itself.

Interviewer: Correct. So, what were your thoughts before, about using biocatalytic compounds or bacterial enzymes in anaerobic digestions like as an additive, what were your thoughts?

Interviewee: Well to tell you the truth, I don't know about that. It's not in our wheel house, we have not thought to talk about that. We generally or thought that chemicals are formed of controlled by not primary process before. We like the plants and the process itself. It's naturally ability to perform the bio-solid solid reduction. Biosolid mass from its wastewater system. But sometimes it doesn't work out too well. And we do have to use chemicals. I never knew about bio-augmentation or whatever the product is referred to I mean the process it does inside us. We're not familiar with it.

Interviewer: Alright. Okay. So then before we started our start a project, what would be the expected benefits you desire or would be optimal for you and the plant?

Interviewee: Before the treatment or after?

Interviewer: Like if you, by using products that provide bio-augmentation what would be the expected benefits that you thought were to be received?

Interviewee: Well greater volatile solid reduction. It's better methane production and actually our added benefits would be less and less bio-solid transportation costs by reducing the number of transfer hauling material.

Interviewer: Right. Okay. That's good. What do you understand about bio-augmentation? How it works or its process?

Interviewee: Very little, to tell you the truth. Very little just only what you've described in the past but I've not read into it. And I just try to care and go through and it seems to work better.

Interviewer: Alright. And then can you see that there would be any limitations or inhibitory things for a successful project? Anything in the plant or its process that you think might inhibit it.

Interviewee: Not necessary a physical inhibits or you know a drawback or one of the things that we're concerned about as operators and wastewater treatment plant supervisors that run process controls is the fact that once you introduce the chemical in, it's very hard to get that process out. You start becoming used to it. The biological life form starts getting used to it. The process gets used to it. Now we start getting used to the outcomes from which the chemicals are intended for. So we become dependent on the chemicals versus adjustment and minor tweaking of the natural processes. It basically it becomes a little bit easier so we become a little bit lazier. And that's one of the fears of using the chemical, right?

Interviewer: Right. Well, that's good point. So how important would bio-augmentation as the anaerobic digestion process be for the plant?

Interviewee: If it does what it says it does. It'd be a huge benefit across the boards. It's be less chemicals, less hauling, less heating, less energy consumption and overall power consumption, right? It'll be a money-saving factor that would actually outweigh the cost of the chemical which is the ultimate goal.

Interviewer: Alright. So do you think there'll be any issues operationally that might come up during the project?

Interviewee: Well the issue of exposure to the operator. That would always the main concern for us. Having them in baglets actually worked out well but when we started walking to measure to

move them out, that was a little bit tricky. And we didn't know the long term used to the effects of the materials.

Interviewer: Okay. So more concern for the operator but there's no other operational procedure that should've affected anything or any issues of concern outside of that?

Interviewee: No, not necessarily. Well, we were wondering what would have happened. That's for sure, but that is why we did it on a trial basis.

Interviewer: Right. Okay. Great. I'm gonna stop recording this and that was the interview for the start of the project.

Interviewee: Cool.

Post-Interview Transcript

Interviewer: Okay. So were there any changes in the wastewater plant process or operations that are worth noting that might have affected about augmentation project? Like not made it work as well?

Interviewee: Well, it seemed that a big production of work we introduced is singular application per week. It seemed not to work as well at the daily consistent application. And that was the general feeling. I know maybe the data didn't show that day to day build up a little bit of drop occasionally in digester gas output. Other than that, not too much. I think a little bit of the increase in dewatering if not the use of the polymer. I believe we switched over commerce that we couldn't really isolate the bioaugmentation that seed of one mitigating factor for the uptake in that dewatering solid.

Interviewer: So, were there any operations that occurred or changes to operations that occurred that caused or could have caused any negative impact on it? So, like changes in procedures or changes in operations that might have negatively affected the--

Interviewee: About the-- during the process?

Interviewer: Yeah.

Interviewee: Make minor changes, but nothing to take note. One big change maybe was a change of polymer in our dewatering process.

Interviewer: Right.

Interviewee: But pumping time, chemical usage times from all the apparatus processes such as primary, preliminary and secondary. None of those actually really changed.

Interviewer: Okay. Were there, or what were the issues, if any, in running a project example: Were there issues and collecting data, operating the digesters complications in the treatment operations, maybe measurements or tools to take tests you know, or managing like flow through the digesters. Is there any of those that have that came up?

Interviewee: Well, we've always had a flow issue because of our inadequate valves and I believe our devices ever measure our flows of all it needed to be calibrated with systems. So getting accurately flows to the digesters was always a challenge. One of major challenges was the physical aspect of physically placing it in there and making sure it got into a place where at the good retention, I believe we found out a good spot to add it to the thief hole.

Interviewer: Right, Yeah. Yeah, by the thief hole, right.

Interviewee: Yeah,

Interviewer: Do you remember -- Now, this is maybe a bit more specific on the data that we reviewed is were, what were the previous biogas productions level at?

Interviewee: I think across the board there were maybe eight to ten percent lower than they are now.

Interviewer: Okay.

Interviewee: I would have to look at it. I'm not standing about right in front of my computer at this time, but I have a general feeling that it was a little bit lower.

Interviewer: Yeah,

Interviewee: Slightly.

Interviewer: Right. So then were you able to achieve the desired benefits that you were looking for in doing the bioaugmentation project?

Interviewee: Well, the overall goal was to increase the process. I think that was achieved, but a shoot for fifteen to thirty percent efficiency. I don't know. I don't know. I would have to look at the data one more time and extrapolate that.

Interviewer: Right. Okay. Right, so that might be, give it a hard to answer the next question was a few-- When you would you consider the project to be successful or not and why? So that might be--

Interviewee: Well determining that successful on the way that it really increased the efficiency, so it is successful on that map. But as to the percentages, I thought it would have done it. I

thought it would be doing a little bit better, at least five percent better. Yeah, Ten percent thought is still an accomplishment.

Interviewer: Right.

Interviewee: Anything that does has ten percent in that industry you're saving-- you're saving a lot of money.

Interviewer: Right. Okay. So has the project results been shared with any like .. managers or city officials?

Interviewee: That would be a question for my immediate supervisor, but I know it's been a topic of conversation throughout the region. But other plans in the area with other operators and CPOs.

Interviewer: Right. So not necessarily like a, as far as you're aware, there wasn't any kind of presentation to other managers or officials on what the results were as of yet?

Interviewee: No, not as of yet. I know for a fact that if a project is not there a certain amount of money, it is not required to give a report--

Interviewer: Alright. Right.

Interviewee: --to City Officials. But once it reaches a certain threshold, then everything has to be their City Officials that be approved.

Interviewer: Okay. So would you consider the continued use of the biocatalytic product?

Interviewee: I would-- I would consider for a two-year run.

Interviewer: For a two-year run to see how it works over two years?

Interviewee: Yeah.

Interviewer: Okay. So now that you've gone through the process, you've completed the project. Now, what are your thoughts about biocatalytic products? If you think back to maybe what it was before and have, have your thoughts changed or where are they now.

Interviewee: They have evolved Jonathan. They definitely have evolved. But I see them both warm in the future. I see a place for them in the future as restrictions and permanent limits get tighter and tighter, as consumption of energy is becoming a greater issue within the wastewater treatment system. I believe at ten percent even at eight percent or twelve percent savings in a profit, will not be denied.

Interviewer: Very good. Was there anything that you'd learned like from this process?

Interviewee: Yeah, When we first met you talk about hummus or the cellular -- what was that? The cellular chasing. What was the technical term?

Interviewer: Yeah. I'm actually not too sure what you're referencing actually.

Interviewee: Sure. What was the term you used, cellulose?

Interviewer: Yeah.

Interviewee: The biomass within the digester that is inert?

Interviewer: Oh, just that it's a bit harder to digest cellulose than carbohydrates.

Interviewee: Yeah. I didn't know there was that much in the digester process.

Interviewer: Oh right. Yeah. Okay.

Interviewee: Yeah.

Interviewer: Great. So, the last couple of questions were more about like future recommendations. So, would you feel that you would be able to recommend the use of biologic products for bioaugmentation in digesters to other plants or operators?

Interviewee: I would and I am. I've actually said they should try it and more people that try it in our industry as a data analysis points work.

Interviewer: That's a great point. It's a very great point. The more data we have, the more we're able to understand how things work and how it affects everything. So a very good point.

Interviewee: Yeah, that's a good point.

Interviewer: Oh, are there any lessons learned or recommendations that you would give to other plant operators, that are looking to do a similar project?

Interviewee: Be aware of PPE, now the PPE wasn't really stated cause it wasn't a standard SOP but there are probably always go above and beyond with PPE. So we are operated well gloved, face mask and eye protection. And the product specifically said you know I mean, they really need to do that.

Interviewer: Yeah.

Interviewee: We erred on the side of precaution.

Interviewer: Right. Okay, just protection of the operators. Now it is, like you said it isn't water-soluble bag. So it's protected and it's not direct contact. But hopefully, that's a good point to give everybody that protection is important. Do you have any last comments or thoughts about the project or its results? This is the last question.

Interviewer: Yeah. Just anything that maybe we haven't talked about. Yeah.

Interviewee: I think we-- I think we went over. I think we need to get more of a regional base, we need to get more data points and we need to do it for a longer period of time. A two year period of time would cover the weather anomalies that we have here and it will give a greater span of how it really, really impacts the wastewater treatment process.

Interviewer: Right, Yeah. And the other thought that came to me is that not only is it seasonal and other, but it's the whole plant versus just part of the plant to be--

Interviewee: Correct.

Interviewer: --there's a lot of things that I've learned anyway over this process is that there are-- there is a project that we did and SLO that did the whole plant, everything was done and there seemed to be an improved, just a certain level, more of improvement as the whole plant, was treated versus just a side by side or just a one off the process, right? So that's something else that's interesting for you. Okay.

Interviewee: Yeah, when it comes to wastewater treatment plants the hipbone is definitely connected to the thigh bone.

Interviewer: Yeah, exactly. It's all interconnected. Perfect. Okay, so I'm going to stop recording there.

Appendix J – Tables

Table 6. AD enhancement dosage schedule at SLR

Application	Date	Suggested Application Rate
Seeding Day 1	August 20, 2018	8 Bags
Seeding Day 2	August 21, 2018	8 Bags
Seeding Day 3	August 22, 2018	8 Bags
Seeding Day 4	August 23, 2018	8 Bags
Seeding Day 5	August 24, 2018	8 Bags
Seeding Day 6	August 25, 2018	8 Bags
Seeding Day 7	August 26, 2018	8 Bags
Seeding Day 8	August 27, 2018	8 Bags
Seeding Day 9	August 28, 2018	8 Bags
Seeding Day 10	August 29, 2018	8 Bags
Seeding Day 11	August 30, 2018	8 Bags
Seeding Day 12	August 31, 2018	8 Bags
Seeding Day 13	September 1, 2018	8 Bags
Seeding Day 14	September 2, 2018	8 Bags
Seeding Day 15	September 3, 2018	8 Bags
Seeding Day 16	September 4, 2018	8 Bags
Seeding Day 17	September 5, 2018	8 Bags
Seeding Day 18	September 6, 2018	8 Bags
Seeding Day 19	September 7, 2018	8 Bags
Seeding Day 20	September 8, 2018	8 Bags
Maintenance Week 1	September 15, 2018	3 Bags
Maintenance Week 2	September 22, 2018	3 Bags
Maintenance Week 3	September 29, 2018	3 Bags
Maintenance Week 4	October 6, 2018	3 Bags
Maintenance Week 5	October 13, 2018	3 Bags
Maintenance Week 6	October 20, 2018	3 Bags
Maintenance Week 7	October 27, 2018	3 Bags
Maintenance Week 8	November 3, 2018	3 Bags
Maintenance Week 9	November 10, 2018	3 Bags
Maintenance Week 10	November 17, 2018	3 Bags
Maintenance Week 11	November 24, 2018	3 Bags
Maintenance Week 12	December 1, 2018	3 Bags
Maintenance Week 13	December 8, 2018	3 Bags
Maintenance Week 14	December 15, 2018	3 Bags

Table 7. AD enhancement dosage schedule at SLO

Application	Date	Suggested Application Rate
Seeding Day 1	September 16, 2018	12 Bags
Seeding Day 2	September 17, 2018	12 Bags
Seeding Day 3	September 18, 2018	12 Bags
Seeding Day 4	September 19, 2018	12 Bags
Seeding Day 5	September 20, 2018	12 Bags
Seeding Day 6	September 21, 2018	12 Bags
Seeding Day 7	September 22, 2018	12 Bags
Seeding Day 8	September 23, 2018	12 Bags
Seeding Day 9	September 24, 2018	12 Bags
Seeding Day 10	September 25, 2018	12 Bags
Seeding Day 11	September 26, 2018	12 Bags
Seeding Day 12	September 27, 2018	12 Bags
Seeding Day 13	September 28, 2018	12 Bags
Seeding Day 14	September 29, 2018	12 Bags
Seeding Day 15	September 30, 2018	12 Bags
Seeding Day 16	October 1, 2018	12 Bags
Seeding Day 17	October 2, 2018	12 Bags
Seeding Day 18	October 3, 2018	12 Bags
Seeding Day 19	October 4, 2018	12 Bags
Seeding Day 20	October 5, 2018	12 Bags
Maintenance Week 1	October 6, 2018	5 Bags
Maintenance Week 2	October 13, 2018	5 Bags
Maintenance Week 3	October 20, 2018	5 Bags
Maintenance Week 4	October 27, 2018	5 Bags
Maintenance Week 5	November 3, 2018	5 Bags
Maintenance Week 6	November 10, 2018	(Applications Stopped) 0 Bags
Maintenance Week 7	November 17, 2018	0 Bags
Maintenance Week 8	November 24, 2018	0 Bags
Maintenance Week 9	December 1, 2018	0 Bags
Maintenance Week 10	December 8, 2018	0 Bags
Maintenance Week 11	December 15, 2018	0 Bags
Maintenance Week 12	December 22, 2018	0 Bags
Maintenance Week 13	December 29, 2018	0 Bags
Maintenance Week 14	January 5, 2019	0 Bags