Date: 7/28/2023

To: [redacted]

Sample(s): Digester, Feed/Manure

Date Received: 6/24/2023

Date(s) Analyzed: 6/27/2023-6/30/2023

Sample Analyzed By: Amy Hall, Digester Doc labs

Results Interpreted By: Dan McKeaton, Aquafix

Objective: Determine if nutrient supplementation would benefit the [redacted] and make recommendations.

Overview:

- Overall, while some levels of all metals were detected in both the Digester and the Feed/Manure samples, cobalt appeared to be the most likely metal to be limiting anaerobic operation.
- Lower levels of nickel and molybdenum may be limiting digester methane production. Selenium levels also appeared somewhat low which may be reducing digester efficiency as well. None of these deficiencies were as likely to be detrimental as the cobalt deficiency.
- While some metals may have been at levels considered slightly inhibitory, it is unlikely any of these will have an impact on typical digester operation. Higher levels of irons may make upset recovery more difficult if the digester pH decreases.
- Low levels of alkalinity, as well as calcium and magnesium were observed suggesting that an alkalinity supplement containing these additives may help improve pH stability.

Results:

DIGESTER DOC

Trace Metals Analysis

Date Received: 6/27/2023

Digester

Macronutrient	Digester Unfiltered	Digester Filtered
Potassium (K)	268.713	271.766
Magnesium (Mg)	59.236	14.790
Calcium (Ca)	45.995	4.492
Sodium (Na)	67.450	70.897

Micronutrient	Concentration Raw	Concentration Filtered
Boron (B)	0.292	0.129
Copper (Cu)	0.595	ND
Iron (Fe)	58.100	1.593
Manganese (Mn)	3.046	0.046
Nickel (Ni)	0.042	0.003
Zinc (Zn)	1.696	0.081
Aluminum	9.867	0.076
Arsenic	ND	ND
Cadmium (Cd)	0.002	0.001
Chromium (Cr)	0.100	0.033
Cobalt (Co)	0.019	0.002
Lead (Pb)	0.011	0.004
Molybdenum (Mo)	0.016	0.002
Selenium (Se)	0.018	0.007
Barium	0.762	0.041

Element	Required Level (mg/L)	Inhibitory Level (mg/L)	Toxic Level (mg/L)
[redacted]			
Magnesium (Mg)	<3,000	3,000	>3,000
Calcium (Ca)	200-2500	8,000	>8000
Sodium (Na)	150	8,000	>8,000
[redacted]			
Boron (B)	30	300	
Copper (Cu)	0.01-5	600	1000
Iron (Fe)	0.4-10	100	1500
Manganese (Mn)	0.5-0.75	5	1,000
Nickel (Ni)	0.01-2	40	100
Zinc (Zn)	0.4-2	7	350-1,000
Aluminum (Al)	0.1-0.2	0.5	
Arsenic (As)			0.1
Cadmium (Cd)	0.02-1	5	>20
Chromium (Cr)	0.025	2	2,000
Cobalt (Co)	0.05-2	6	40
Lead (Pb)			600
Molybdenum (Mo)	0.01-1	11	1,000
Selenium (Se)	0.06-3.0	7.0-80	100
Barium		30	

Lacking Nutrients
Inhibitive Nutrient levels
Toxic, too much of Nutrient level

Good levels of Nutrients

ND = Not Detected

DIGESTER DOC

Trace Metals Analysis

Date Received: 6/27/2023

Feed/Manure Filtered

Macronutrient	Feed/Manure Unfiltered	Feed/Manure Filtered
Potassium (K)	214.620	223.171
Magnesium (Mg)	58.690	40.631
Calcium (Ca)	45.892	15.986
Sodium (Na)	90.278	97.119

Micronutrient	Concentration Raw	Concentration Filtered
Boron (B)	0.249	0.139
Copper (Cu)	2.129	ND
Iron (Fe)	5.525	0.367
Manganese (Mn)	0.829	0.043
Nickel (Ni)	0.026	0.008
Zinc (Zn)	1.307	0.051
Aluminum	3.026	0.135
Arsenic	0.014	ND
Cadmium (Cd)	0.002	0.001
Chromium (Cr)	0.034	0.006
Cobalt (Co)	0.018	0.004
Lead (Pb)	0.001	ND
Molybdenum (Mo)	0.014	0.002
Selenium (Se)	0.020	0.002
Barium	0.290	0.028

Element	Required Level (mg/L)	Inhibitory Level (mg/L)	Toxic Level (mg/L)
Magnesium (Mg)	<3,000	3,000	>3,000
Calcium (Ca)	200-2500	8,000	>8000
Sodium (Na)	150	8,000	>8,000
Boron (B)	30	300	
Copper (Cu)	0.01-5	600	1000
Iron (Fe)	0.4-10	100	1500
Manganese (Mn)	0.5-0.75	5	1,000
Nickel (Ni)	0.01-2	40	100
Zinc (Zn)	0.4-2	7	350-1,000
Aluminum (Al)	0.1-0.2	0.5	
Arsenic (As)			0.1
Cadmium (Cd)	0.02-1	5	>20
Chromium (Cr)	0.025	2	2,000
Cobalt (Co)	0.05-2	6	40
Lead (Pb)			600
Molybdenum (Mo)	0.01-1	11	1,000
Selenium (Se)	0.06-3.0	7.0-80	100
Barium		30	

Lacking Nutrients

Inhibitive Nutrient levels

Toxic, too much of Nutrient level

Good levels of Nutrients

ND = Not Detected

Tested Parameters	Feed/Manure Unfiltered	Digester Unfiltered
TS (%)	8.0	8.4
VS (%)	74.1	65.4
TDS (ppm)	62055	69646
TSS ppm)	18294	13905
COD (mg/L)	24484.19	13515.65
Alkalinity (mg/L)	ND	ND
pH	7.06	8.30
Total Nitrogen	*Pending*	*Pending*

Summary

Anaerobic digesters contain a diverse community of bacteria to complete different phases of the anaerobic digestion process. Generally, the main essential groups are known to be acidogenic bacteria, acetoclastic methanogens, and hydrogenotrophic methanogens. Acidogenic bacteria have a diverse community of rapidly growing organisms allowing them to often acclimate to nutrient deficiencies. This means acidogenic bacteria are unlikely to be of concern in the [redacted] sample as none of the metals tested were completely absent from the system or present at toxic levels. Methanogens are slower to grow and much less diverse than acidogenic bacteria and are more likely to be hindered by sub-optimal nutrient concentrations. The majority of methane production is performed by acetoclastic methanogens which makes these organisms of the highest importance to understanding requirements for micronutrients in a digester. Hydrogenotrophic methanogens perform a less primary function and act as a support group to prevent the accumulation of hydrogen in an anaerobic digester which is undesirable in biogas and potentially inhibitory to acetoclastic methanogens. Therefore, the hydrogenotrophic community is considered of secondary importance to acetoclastic methanogens. Acetoclastic methanogens generally benefit the most from the supplementation of iron, cobalt, nickel, and molybdenum if the levels are deficient. Hydrogenotrophic methanogens also appear to have a selenium requirement which can sometimes be of concern.

Another consideration when analyzing nutrient concentrations is the bioavailability of the nutrients, and assessment to determine if bioavailable nutrients are becoming depleted during the digestion process. As we have received both an influent and digester sample, we will use information from both samples to determine how much bioavailable nutrients were present in the site influent, and if any nutrients were depleted inside the anaerobic system. Nutrients that were present in the influent but became depleted are considered of secondary importance as some of them were still entering the digestion process to facilitate anaerobic function. Nutrients that are fully absent in the plant influent that are necessary to acetoclastic methanogen function are considered of prime importance.

In the case of the [redacted] system, we noted a distinct cobalt deficiency in the feed sludge, with near cobalt depletion in the digester sample. This indicates the supplementation of cobalt is the most likely to improve anaerobic digester operation. Molybdenum and nickel were both nearly depleted in the digester sample which means the addition of some molybdenum and nickel may yield some benefits to digester operation, but likely less so than cobalt. Finally, selenium levels may have been slightly low which could be causing a slight decrease in anaerobic digestion rates and stability, but this impact is likely minor. It is also worth noting that soluble copper was absent in the digester and feed samples. This might have some impact on acid generation rates but as some copper is still present in the digester and feed in the total metals measurement, and this metal is more helpful for heterotrophs than for methanogens, this metal is unlikely to make a major impact.

While total iron levels appeared somewhat high in the digester sample, soluble iron levels were clearly not inhibitory. Total metals can make recovery from upsets where pH decreases more difficult as metal solubility tends to increase at lower pH values which is more likely to hinder anaerobic bacteria. Overall, no metal inhibitions were of major concern in this case.

Cobalt as previously mentioned is considered an essential metal for anaerobic metabolism and is a component in enzymes used in hydrogen metabolism, methane biogenesis, and acetogenesis. Cobalt is also an important cofactor in Vitamin B_{12} dependent enzymes (Degen et al, 2002 cited in Chen et al, 2016) (Chen et al, 2016, cited in Mulroony et al, 2003). Optimal levels of cobalt are around 0.01% of total COD in digester feed (Gerardi, 2003).

Nickel and molybdenum are also considered essential cofactors for enzymes in acetogenic metabolism. Nickel is also involved in hydrogenotrophic methanogen function. Optimal nickel levels are considered to be 0.001% of total COD in digester feed (Gerardi, 2003). Molybdenum has a recommended supplementation rate 0f 0.005% of total COD loading. While these values of metal supplementation are a good estimate, variable levels of all essential metals are required depending on on-site environmental conditions and feed substrate. This means any metal supplementation dose recommendations are guidelines rather than precise requirements.

Recommendations:

- We recommend the addition of BioGas1 at a rate of 2.75-5.5 gallons per 10000 lbs. COD loading. This dose rate is proportional based on cobalt requirements which appear most critical to the [redacted] operation.
- As we observed low alkalinity in the [redacted] sample, as well as relatively low levels of calcium and magnesium, we recommend supplementing alkalinity with BoostNLock which contains a combination of these bases to boost calcium and magnesium levels, as well as alkalinity.

Works Cited

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Matheri, A. N., Belaid, M., Seodigeng, T., & Ngila, J. C. (2016). ***The role of trace elements on anaerobic co-digestion in biogas production***. Proceedings of the World Congress on Engineering, 2.

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Safaric, L., Yekta, S., Svensson, B., Schnurer, A., Bastviken, D., & Bjorn, A. (2020). Effect of Cobalt, Nickel, and Selenium/Tungsten Deficiency on Mesophilic Anaerobic Digestion of Chemically Defined Soluble Organic Compounds. ***Microorganisms***, ***8***(598), 1-18.