DETERMINATION OF OLEANOLIC ACID BASED SAPONIN REMOVAL BY THE WASTEWATER TREATMENT SYSTEM AT SOUTHERN MINNESOTA BEET SUGAR COOPERATIVE

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Abstract:

A quantitative method to determine the amount of one type of beet sugar saponin was used to measure its concentration in wastewater as it passed through treatment at SMBSC. The method included an acid digestion of the sample to cleave the glycosidic linkage and free up oleanolic acid. Its concentration was then determined using liquid chromatography/tandem mass spectroscopy (LC/MS/MS). Glycyrrhetinic acid was used as an internal standard and the samples were spiked with the surrogate hederagenin. The highest concentration of oleanolic based saponins was seen in the water used to wash the sugarbeets. The concentration gradually diminishing as the water passed through the treatment system and was below the method reporting limit in the final effluent.

Introduction:

As a condition of its National Pollution Discharge Elimination Permit (NPDES), Southern Minnesota Beet Sugar Cooperative (SMBSC) is required to conduct Whole Effluent Toxicity (WET) testing on its discharge using three species. For several years SMBSC experienced sporadic and unpredictable toxicity in the effluent from its wastewater treatment plant. The species, Ceriodaphnia dubia, when exposed to the discharge exhibited inconsistent survival. SMBSC investigated the discharge for potential toxic chemicals but was unable to identify any specific one that was affecting this species. At this point, the cooperative conducted a Toxicity Investigation Evaluation (TIE) and Toxicity Reduction Evaluation (TRE).

During these investigations a literature search revealed that saponins were known to be toxic to fish and other lower organisms. Also, they exhibited two other toxicity characteristics seen in SMBSC's discharge: 1) toxicity was reduced by lime softening, and 2) toxicity decreased with time. In addition, foaming was experienced in the post aeration basin, the last treatment before discharge of the water at SMBSC, and saponins are known to cause foaming. Since sugarbeet saponins comprise about 0.1% of the root mass dry solids and are water soluble, they became the focus of the TIE/TRE.

Saponins or sapogenin glycosides are a class of natural products found in plants. Each saponin contains an aglucon or sapogenin and a glycosidic moiety. The primary sapogenin in sugarbeets is the triterpene oleanolic acid. The glycoside moiety is D-Glucuronic acid. In acid solution, the glycosidic linkage is hydrolyzed giving free oleanolic acid.

Traditional chemical methods used to measure saponins were investigated and found not to give quantitative results. SMBSC worked with the Sugar Processing Research Institute, New Orleans Louisiana; Barr Engineering, Minneapolis Minnesota; and Columbia Analytical Services, Inc., Kelso, Washington to develop a quantitative test method and then analyze process, wastewater and discharge waters from SMBSC for beet root saponins.

Objective:

The purpose of this study was to determine if beet sugar saponin was present in the water discharged from SMBSC.

Materials and Methods:

Samples were collected at SMBSC, packed in ice and sent overnight to Columbia Analytical following standard chain of custody protocol. Final effluent samples were 24-hour composites obtained using an auto sampler that takes 250 mL every 15 minutes. All other samples were grab samples, obtained the day they were shipped. Upon receipt, Columbia Analytical Services stored the samples at 4°C.

The method chosen was to hydrolyze the saponins in the samples using sulfuric acid, then analyze for oleanolic acid. A 5 mL aliquot of the sample was added to 5 mL of acetonitrile spiked with the surrogate precursor glycoside hederacoside C., which hydrolyzes to form the surrogate hederagenin. To this was added 1.5 mL of 50 % concentrated sulfuric acid then placed in an 80 °C water bath for two hours. After cooling, 1 mL of the sample was added to an auto-sampler vial with the internal standard glycerrhetinic acid. The compounds were separated chromatographically by injecting a 10 µL aliquot into an HPLC equipped with a reverse phase (C18) column. After elution from the column the compounds were detected by tandem mass spectroscopy (LC/MS/MS). The relative response between the internal standard and the target compounds, including the surrogate hederagenin, was used to determine concentration. Oleanolic acid was used for the laboratory control.

Results and Discussion:

It is necessary to understand the wastewater treatment at SMBSC to interpret the results. Water from the wash loop goes to equalization ponds, in which solids settle and organic loading equilibrates and is biologically reduced. Water from the ponds is fed to an anaerobic digester, which typically removes more than 90% of the organic loading. Effluent from the anaerobic digester is fed to two, identical activated sludge tanks. The effluent from each tank flows to separate settling clarifiers where the aerobic sludge is separated from the water. The discharge from the aerobic system is then filtered through sand beds, combined and called final effluent. The final effluent can be blended with noncontact cooling water at this point. The water, blended or not, can be chlorinated and dechlorinated when applicable. There is a last aeration basin that ensures that the dissolved oxygen level in the discharged water meets the permit requirement. The discharge after blending is called “Final Effluent Blend”.

The analytical results are given below in Tables I, II, and III. Table I gives the results from analysis of final effluent on three different dates. Table II gives the results from the analyses run on samples from the wash loop, before and after the anaerobic digester and the final effluent blend. Table III gives the results from analysis run on samples from the wash loop, before the anaerobic digester, after aerobic treatment and the final effluent.

In most samples the recovery of the surrogate, hederagenin, was acceptable. The most notable exceptions were the two wash loop samples. The sample taken on December 2, 2008 had a recovery of 950% in addition to very high oleanolic acid result. If this result is compared to the analysis of the wash loop water sample taken on March 3, 2008 we see that

not only is the surrogate recovery about 10 times what is expected, but that the oleanolic acid concentration is about ten times higher.

When comparing the wash loop and anaerobic feed samples taken on these two dates, those taken in March are very close, while the samples taken in December are significantly different. In all the testing done, the recoveries from the matrix spikes and controls were good, while the blanks were below the detection limits.

The highest concentration of saponin was found in the wash water loop and anaerobic feed. The anaerobic treatment removed about 80% of the saponin. The aerobic system removed all the remaining except for a few parts per billion.

Table I: Saponin Results from 1/17/2008

Sample	Date Sampled	Result (ng/mL)	Surrogate Recovery (%)	Expected(ng/mL)	Recovery (%)	Relative Difference (%)
Final Effluent	11/05/2007	ND	102			
Final Effluent	12/10/2007	ND	82			
Final Effluent	12/10/2007	ND	100			
Final Effluent	01/07/2008	ND	74			
Final Effluent	01/07/2008	ND	68			
Control		97.8	114	93.3	104	
Control D		83.3	96	93.3	89	16
Matrix Spike		86.7	87	93.8	92	
Blank		ND	102			

Table II: Saponin Results from Analysis on 03/22/2008

Sample	Date Sampled	Result (ng/mL)	Surrogate Recovery (%)	Expected (ng/mL)	Recovery (%)	Relative Difference (%)
Wash Loop	03/03/08	560	149			
Anaerobic Feed	03/03/08	680	68			
Aerobic Feed	03/03/08	127	87			
Final Effluent Blend	03/03/08	ND	63			
Control		339	77	400	85	
Matrix Spike		376	74	400	94	
Matrix Spike D		337	75	400	84	11
Blank		ND	81			

Table III: Saponin Results from Analysis on 01/19/2009

Sample	Date Sampled	Result (ng/mL)	Surrogate Recovery (%)	Expected(ng/mL)	Recovery (%)	Relative Difference (%)
Wash Loop	12/02/08	7,100	950			
Anaerobic Feed	12/02/08	250	59			
N Aerobic Discharge	12/02/08	4.0	98			
S Aerobic Discharge	12/02/08	3.7	86			
Final Effluent	12/02/08	4.1	95			
Control		414	91	400	103	
Matrix Spike		336	75	400	83	
Matrix Spike D		370	81	400	91	9
Blank		ND	83			

*Below the method reporting limit of 25 ng/mL

Conclusion:

The analytical method developed was able to quantitatively detect sugarbeet saponin in the samples taken from SMBSC's wastewater treatment system. The results showed that the wastewater treatment effectively reduced the saponin concentration to a few parts per billion. The results from the wash loop samples suggest that more investigation into this method needs to be done before it can be applied to these samples.