SUGAR BEET PROCESSING APPLICATIONS OF THE DETERMINATION OF FERMENTATION PRODUCTS BY LIQUID CHROMATOGRAPHY

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Introduction:

High performance liquid chromatography (HPLC) and the closely-related technique of ion chromatography (IC) are widely applied in the sweetener industry for the determination of carbohydrates such as glucose, fructose, and sucrose. However, in addition to analyses for these principal components, liquid chromatographic (LC) techniques can be very useful for a variety of other applications including process troubleshooting and emission testing. One area of particular interest is the determination of fermentation products, which may be used to diagnose specific factory problems. For example, the presence of butyrate in diffusion juice has been used as an indicator of the carryover with beets of beet wash water components.[1,2] This report discusses several other specific examples of the use of LC to detect the products of microbiological activity in factory streams and by-products.

Results and Discussion:

A. Organic Acid Determination

One of the more useful LC techniques applied in our laboratory, as well as others in the sugar industry[3,4,5], has been the determination of organic acid profiles in a variety of samples. Organic acids produced by microbiological activity, as well as those naturally present in sugar beet, can readily be separated by eluting samples through an acid-form cation exchange resin LC column (such as Bio-Rad HPX-87H). Elution with very dilute sulfuric acid (0.005 N) keeps organic acids in the protonated form and separation occurs by a combination of ion exclusion and molecular size exclusion mechanisms. Separated acid peaks can be detected using a conductivity detector, variable wavelength ultraviolet (UV) detector, or refractive index detector. UV detection is susceptible to interference from trace components in processed factory juices that are more intense UV absorbers than organic acids; for this reason, our laboratory generally favors conductivity detection for such samples. The ion exclusion technique allows, in a single chromatographic analysis, separation and quantitation of the most common organic acids present in beet juice or fermentation products: lactic, formic, acetic, propionic, butyric, oxalic, citric, and malic acids.

This method of organic acid determination by ion exclusion chromatography has been applied in the analysis of diffusion juice as reported previously[2]. The method was applied again when problems with foaming were experienced in attempts to use raffinate evaporator condensate in boiler feed water. The problem condensate had a strong odor and obviously contained volatile materials carried over with water vapor. Analysis of the condensate for organic acids showed it to contain 140 ppm acetic acid and 150 ppm butyric acid, in addition to lower levels of other acids. Condensate from other evaporator sources contained no detectible organic acids by chromatographic analysis. Since organic acids with soluble calcium salts will carry through the factory process to molasses and are known to then be eliminated in raffinate during chromatographic molasses desugarization, it is not surprising that acids generated anywhere in the factory could end up in raffinate evaporator condensate.

Due to high levels of fermentation-produced organic acids in beet flume streams, this analysis has also proved useful for monitoring parameters in these systems and associated waste water treatment. For example, organic acid analysis can give information on: the course of organic oxidation in aerobic systems; the presence of anaerobic fermentation products; concentration due to evaporation; and flow through components of the system. In one study, organic acid analysis in combination with IC anion analysis and cation determination by atomic absorption spectrophotometry (AA) was used to give a quite good balance of anions to cations in a flume pond water sample. The variation between total cations and total anions in this test was below 3.5% relative to total cations as shown in the following table.

Table 1. Beet Flume Water Analysis

	ppm (mg/liter)	meq/liter
Formate	10	0.22
Acetate	1100	18.6
Propionate	740	10.1
Butyrate	400	4.6
Chloride	948	26.7
Total Anions		60.2
Potassium	396	10.1
Sodium	226	9.83
Calcium	586	29.2
Magnesium	110	9.05
Total Cations		58.2

In a related study, organic acid analysis was used to help demonstrate the lack of seepage of pond water components into ground water. Around a pond containing approximately 600 ppm total acids (lactic acetic, propionic, and butyric), well water samples were found to contain no detectible organic acids (with a 2 ppm detection limit). Studies of this kind based on other waste water components such as cations or common inorganic anions would be less definitive since these ionic species would occur in varying levels in both pond water and uncontaminated ground water as shown in Table 2.

Table 2. Analysis of Waste Water and Well Water.

Component	Waste Water (Level in ppm)	Well Water (Level in ppm)
Chloride	139	41
Sulfate	113	7.0
Lactate	220	<2
Formate	3.7	<2
Acetate	200	<2
Propionate	130	<2
Butyrate	40	<2
Potassium	57	6.7
Sodium	128	62
Calcium	63	156
Magnesium	90	72

Finally, although not directly related to factory processing, extensive use has been made in our laboratory and others[5] of the determination of organic acids in ensiled beet pulp. In general, the organic acid profile of ensiled pulp is an indicator of the type of fermentation occurring in the pile. Good quality materials are higher in lactic and acetic acids while less desirable fermentation processes produce butyric acid. The following table shows analytical results on a typical ensiled pulp sample as well as a sample removed from a hot spot in the pile. Typical ensiled material is expected to have high levels of lactic acid with some acetic acid almost always present but no more than very low levels of butyric acid. The sample removed from a hot spot in the silage pile contains less lactic acid and a significant level of butyric acid, which can present a serious odor problem as well as indicating a less controlled fermentation.

Table 3. Ensiled Pressed Pulp Analysis

	Normal Sample	Sample from Hot Spot
Lactic acid (g/ kg DS)	43.1	15.9
Acetic acid (g/kg DS)	11.0	48.5
Propionic acid (g/kg DS)	0	6.4
Butyric acid (g/kg DS)	0	12.1

B. Investigation of Factory Condensate with High Organic Levels.

A particularly interesting application of HPLC to the investigation of factory processes came about when personnel at one factory noticed significant increases in the chemical oxygen demand (COD) of condensate pond samples late in campaign. Condensate with normal COD levels of 100 - 200 ppm had increased to a COD in the 600 - 800 ppm range. Factory laboratory tests for sugar (α-naphthol test) had already eliminated the obvious possibility of sugar carryover into condensate. Condensate is known to typically contain ammonia due to hydrolysis of amino acid amides in the juice stream but the ammonia present was thought to not be a significant contributor to COD, taking into consideration the normal composition of condensate.[6]

The problem condensate was subjected to a variety of chromatographic analyses in an effort to diagnose the problem. As mentioned above, volatile organic acids could be suspected as contributors to COD but in this case organic acid analysis of combined condensate from several sources showed only low (12 ppm) levels of acetic acid and no other detectible organic acids. Individual condensate samples showed acetic acid levels no higher than 15 ppm. To verify the results of α-naphthol tests showing no presence of sugar, condensate samples were subjected to HPLC on a Bio-Rad HPX-87C column; a normal cation exchange column for the separation of sugars. This test showed no glucose or fructose and only trace levels of sucrose (25 ppm) to be present; however, a significant peak was observed at longer retention time than the monosaccharides. Since the elution order of neutral molecules from cation exchange columns is roughly in the order of high molecular weight to low molecular weight, the observed peak was suspected to be due to a low molecular weight material. Comparison with standards showed the peak to be at the same retention time as ethanol and gas chromatographic (GC) analysis of the condensate verified this assignment. Quantitative standard injections showed the combined condensate sample to contain ethanol at a level of 610 ppm. The stoichiometry of total ethanol oxidation predicts that 610 mg/l of ethanol would require 1270 mg/l of oxygen for complete oxidation. This value is higher than typical COD determination results; however, under the boiling conditions of COD determination it is possible that some ethanol might escape in the vapor phase before oxidation could occur.

Further quantitative HPLC determinations showed ethanol to be present in condensate samples from other factories, although at lower levels. Since ethanol has a lower boiling point (78E C) than water it is reasonable to expect that ethanol produced by sucrose fermentation at any point in the process, from beets in the pile to regions of microbiological growth in the diffuser, could end up in the condensate stream. As a further check on the origins of ethanol in the factory, several juice stream samples were also analyzed for ethanol by HPLC. Results for condensate and juice stream samples are given in the following table. Clearly, the significant level of ethanol measured in condensate does reflect significant levels in the juice stream.

Table 4. Ethanol Levels in Factory Samples

Sample	Ethanol (ppm)
Combined Condensate	610
Diffuser 1, Raw Juice	1040
Diffuser 2, Raw Juice	720

Thin Juice	440

These limited tests did not show whether the production of ethanol by fermentation occurred in beets, within the diffuser, or somewhere else such as in returned press water but did show that somewhere in the system sucrose is being fermented to ethanol. To put this level of ethanol in perspective with respect to sugar loss, if one uses values of 17.2% sucrose/beet and 4 moles of ethanol produced per mole of sucrose then a level of 1000 ppm ethanol in diffusion juice represents a sugar loss of about 0.21%/beet at typical operating conditions of diffusion juice dissolved solids. Of course, this probably represents a minimum value for the actual sugar loss as ethanol because it is likely that high temperature steps around diffusion would result in the evaporative loss of ethanol.

Conclusions:

The determination of fermentation products, particularly organic acids and ethanol, by liquid chromatography can be utilized in a variety of factory troubleshooting, emission investigations, and byproduct quality testing applications. Although liquid chromatography as a sucrose determination method does not always offer the precision and freedom from interferences that one would like in an analytical method, the precision and specificity of LC are very adequate for these kinds of applications. In addition, the ability of LC to separate complex mixtures and quantitate a variety of components is exactly what is needed in this type of project.

References:

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