Advanced Secondary Processing Nutrient Pre-testing To Improve Product Quality and Reduce Nuisance Odor Production

Submitted By

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Table of Contents

Table of Tables

Table of Figures

Executive Summary

Secondary processing nutrients (SPN), also called dissolved air flotation (DAF) float, skimmings or sludge, is a poultry processing wastewater treatment by-product that is often rendered. Facilities collect SPN throughout the processing day and concentrate it with gravity dewatering, a practice that also degrades SPN. Commercially available antioxidants are well tested and effective, but the product is not miscible in water. For SPN that ranges from 5-20% solids, combining an immiscible chemistry can result in globules of unused antioxidant along with large volumes of untreated, thus rancid SPN.

Primary oxidation products (peroxide value or PV) or free fatty acid (acid value or FFA) are typically used for assessing lipid-containing feedstuff (CRN, 2003). Total oxidation (TOTOX) is the combined limit of primary and secondary oxidation products measured as peroxide and anisidine values, reported as a TOTOX value based upon [2 x PV + AN]. Other methods are also employed, e.g., weight gain, conjugated dienes, active oxygen method (Swift test), 2-thiobarbituric acid (TBA) value and carbonyls (hexanal), however, no standard method exists for distinguishing all oxidative changes in lipid-containing materials (Shahidi and Wanasundara, 1998).

This study evaluated methods for monitoring SPN oxidation as part of the broader assessment of SPN quality, nuisance odor generation, and the impact of SPN antioxidants upon wastewater pre-treatment systems. SPN quality evaluation (i.e., accelerated testing at 25, 35, and 40°C for 24-hours) included peroxide value (PV), p-Anisidine (p-A), thiobarbituric acid (TBA) and free fatty acid (FFA) analysis. Hexanal formation was monitored as an indicator of nuisance odors and product quality. Anaerobic bioassays were conducted to evaluate impacts upon biologic pre-treatment systems.

Although the initial goal was to assess both the primary (i.e., peroxide value) and secondary products (i.e, aldehydes) in terms of total oxidation (TOTOX), pigment interferences resulting from SPN sample preparation made iodometric methods for measuring peroxide values impractical.

FFA production increased linearly with time and but production rates varied with temperature. Data indicated free fatty acid value temperature dependence with a bimolecular rate equation. Coupled with the difficulty in discerning trends and relationships for pA and TBA, the ratio of anisidine (pA) and thiobarbituric acid (TBA) values were compared with FFA values. Analysis yielded a transition point that shifted toward initiation with increased temperature.

Antioxidant addition (250-ppm as ethoxyquin) inhibited FFA and TBA formation, especially at 40°C. Hexanal concentrations increased with temperature and were higher without ethoxyquin addition. The greatest rate of hexanal production was at 35°C, with a decrease at 40°C. TBA concentrations were depressed as compared to anisidine for SPN samples with FFA levels below 1.5-mg/L and/or heat and ethoxyquin addition. Data indicates that hexanal formation follows time and temperature relationships similar to that of anisidine. The presence of ethoxyquin derived from the supernate of a 40°C accelerated testing sample did not have adverse effects on anaerobic waste treatment systems. Researchers conclude that comparing anisidine, thiobarbituric acid, or the ratio with either with hexanal or free fatty acid formation will provide a greater indicator of appropriate SPN quality analysis.

Introduction

Secondary protein nutrient (SPN) is a poultry processing wastewater by-product that is typically rendered. Also called dissolved air flotation (DAF) float, skimmings or sludge, principal constituents contain readily biodegradable organic compounds including nitrogen and phosphorus associated primarily with blood, soft tissue, fecal material, cleaning and sanitizing agents. Inorganic polymers enmesh and capture wastewater particulates and some colloidal material in conjunction with air flotation employed to enhance SPN and water separation. Other organic and inorganic material associated with a facility's wastewater is also found. SPN moisture content is generally 80 to 90% or greater before dewatering, with fat (lipid) content at five to ten percent.

SPN production (wet basis, 80 percent moisture) is typically 312- to 520-lbs per 1000-lb live weight (141.8- to 236.4-kg SPN per 454.5-kg live weight) for a poultry facility with further processing. With an estimated 9-billion birds processed annually, approximately 2.8-billion pounds of SPN (wet) must be disposed of or treated. The SPN produced varies primarily based upon the facility water usage, polymer selection, sanitation, and housekeeping practices (USEPA, 2002). If further processing is conducted and/or the facility is part of a larger complex that includes a feedmill, hatcheries, or rendering, additional variability will result. Aguilar *et al.* (2002) reported coagulant remaining in sludge could be reasonably estimated as the difference between fixed solids in sludge and the influent wastewater total suspended solids.

Facilities collect SPN throughout the processing day and concentrate it with gravity dewatering, a practice conducive for SPN fat content spoilage. To prevent fat rancidity and maintain SPN quality for rendering, commercially available antioxidants are dripped into SPN during tanker loading. While antioxidants are well-tested and effective they display low solubility in water. Poor antioxidant mixing due to high SPN moisture content can reduce SPN quality. Residual aqueous-phase antioxidants can gravity flow into wastewater systems. Finally, oxidized SPN creates product and nuisance odor issues for renderers.

Product Quality

Primary oxidation products (peroxide value or PV) or free fatty acid (acid value or FFA) is typically used for assessing lipid-containing feedstuff (CRN, 2003). Total oxidation (TOTOX) is the combined limit of primary and secondary oxidation products measured as peroxide and anisidine values, reported as a TOTOX value based upon [2 x PV + AN]. Other methods are also employed, e.g., weight gain, conjugated dienes, active oxygen method (Swift test), 2-thiobarbituric acid (TBA) value and carbonyls (hexanal), however, no standard method exists for distinguishing all oxidative changes in lipid-containing materials (Shahidi and Wanasundara, 1998).

During lipid oxidation, two types of oxidation products are formed. Early in oxidation, primary products are formed. These transient products, including peroxides and conjugated dienes, are relatively unstable and break down to form secondary lipid oxidation products. Secondary oxidation products consist of aldehydes, ketones and alcohols. Aldehydes are largely considered responsible for the off-flavors and odors in fats and oils due to their low sensory threshold values (Frankel, 1993).

Current lipid oxidation measurement techniques quantify either primary products or secondary products. However, the TOTOX value takes both types of products into consideration using a formula that incorporates both peroxide value (primary products) and anisidine value, a measurement of aldehydes or secondary products. The use of peroxide value as measurement of lipid oxidation is common; however, several studies have shown that it might not serve as a true indicator of the oxidative status of a product (Gray, 1985). The measurement of aldehydes as an indicator of oxidation has been closely correlated with the production of off-odors and overall odor intensity (Tompkins and Perkins, 1999). Taking into account both the primary and secondary products, the use of TOTOX values to describe total oxidation in a product provides a more complete picture of oxidative status of the product.

Hexanal

Hexanal has become a popular indicator of lipid oxidation in foods. Hexanal is one of the dominant volatile secondary products formed during the oxidation of linoleic acid. Hexanal is the only aldehyde formed from both the 9 and 13-hydroperoxide of linoleate (Shahidi and Pegg, 1994). Hexanal, often described as "painty", "grassy" and "fishy", contributes to undesirable off-odors created during lipid oxidation and has a low odor threshold. Gas chromatography (GC) was used to establish strong correlations between hexanal content and sensory scores and traditional lipid oxidation measurements in a number of meat products, including chicken (Ahn et al., 2000) and beef (Spanier et al., 1992). In an oxidation study of chicken myofibrils, hexanal was found to be the major aldehyde produced over 5 days of storage at 50°C (Goodridge et al., unpublished).

Residual Effects on Biological Systems

Clear federal guidelines exist that outline the safe use of ethoxyquin-based stabilizers and acceptable residual concentrations at levels up to 150 ppm in selected animal feeds (21CFR573.380). In 1997, the Food and Drug Administration Center for Veterinary Medicine (CVM) requested that the maximum level for ethoxyquin in complete dog foods be voluntarily lowered to 75 parts per million (ppm) because of concerns about animal health issues.

Environmental toxicity is known to exist under ambient condition for concentrated levels for liquid antioxidants containing ethoxyquin and 4-ethoxyaniline (Solutia Material Safety Data Sheet, 2001), e.g., environmental toxicity at 2.2 mg/L for invertebrate 48-h EC50 and at 7.1 mg/L for fish 96-h LC50. Others have noted that 4-ethoxyanaline is persistent in the environment and toxicological tests showed high toxicity (UNEP, 2004).

The literature, however, does not clearly indicate that ethoxyquin is a health or environmental concern. Sanhueza *et al.* (2000) modeled the thermal stability of synthetic antioxidants frequently applied during the cooking of the by-products (viscera, blood, and feathers) in the poultry feed industry, e.g., butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroxyquinone (TBHQ), and ethoxyquin (EQ). The authors noted that BHA and EQ were inactivated by 70 and 60%, respectively, at 150°C. As proposed by the authors, ethoxyquin is inactivated as an antioxidant by heat.

A variety of references were found that discussed antioxidant levels in animal feeds; however, limited information was found related to the short- or long-term effects upon wastewater systems or nuisance odor generation. A broad discussion is on-going over the

use of antioxidants, but of greater interest is the effect of authorized residual antioxidant concentrations on nuisance odor generation in SPN and subsequent underflow streams following pre-treatment with anaerobic systems.

This study evaluated methods for monitoring SPN oxidation as part of the broader assessment of SPN quality, nuisance odor generation, and the impact of SPN antioxidants upon wastewater pre-treatment systems. SPN quality evaluation included peroxide value (PV), p-Anisidine (p-A), thiobarbituric acid (TBA) and free fatty acid (FFA) analysis. Hexanal formation was monitored as an indicator of nuisance odors and product quality. Anaerobic bioassays were conducted to evaluate impacts upon biologic pre-treatment systems.

Materials and Methods

After subjecting SPN samples to accelerated testing, TBA, p-A and FFA analysis were the primary measures of SPN oxidation evaluated. Hexanal formation was subsequently assessed. All analysis was conducted for SPN samples with and without ethoxyquin.

Secondary Protein Nutrients (SPN)

Samples are gathered from the facility DAF and immediately iced at 4°C for transport. Within 2-hours, either accelerated testing or lipid oxidation analysis commenced.

Accelerated Testing

Accelerated testing was performed to establish the extent of oxidation throughout a simulated 24-hour collection and delivery period, with and without antioxidant addition. For each sample, 125-g aliquots were placed in beakers and immersed in a water bath maintained at temperatures representative of seasonal ambient conditions (25, 30, 35 and 45°C), as well as a 50°C storage temperature typical of military provision storage studies (Sun *et al.*, 2001). Samples are sacrificed at 0-, 30-, 45-min as well as 1-, 2-, 4-, 6- and 24-hr periods. Replicates of each sampling temperature were completed. Ethoxyquin at 250-ppm was added to samples before accelerated testing at 25, 35 and 40°C on two of the sample days; sample blanks were also used and analyzed.

Antioxidant was obtained from the processing location and added to the raw SPN at a rate of 250- mg/kg as ethoxyquin (summer dosage). Because of the antioxidant viscosity, 30 μL will be added to each 125-g aliquot and mixed by hand with a glass rod for 1-min. Aliquots heated for 1-hr or less were stirred every 15-min.

Lipid Oxidation Analysis

P-anisidine, free fatty acid and thiobarbituric acid (TBA) values were measured for lipid oxidation analysis. High pressure liquid chromatography (HPLC) testing with solid phase microextraction (SPME) was used for aldehyde (primarily hexanal) and ketone monitoring. The headspace of selected aliquots was purged to measure the individual volatile carbonyl compounds formed during hydroperoxide degradation. Arrhenius constants were calculated to assess temperature effects.

Peroxide Value

A modified version of AOCS Method 8b-90 was used to measure the degree of lipid oxidation in fats and oils. Specifically, 50-g of sample is placed in 30-mL of glacial acetic acid-chloroform (3:2, v/v). Saturated potassium iodide at 0.5-mL is added and the solution titrated with a 0.01M standardized sodium thiosulfate ($Na_2S_2O_3$) using starch indicator. The peroxide value as meq-peroxide per kg-sample is determined by [(S-B)xNx1000]/[sample weight (g)] where S is sample titration, B is blank titration and N is normality of $Na_2S_2O_3$.

Anisidine Value

A modified version of AOCS Method Cd18-90 is used to determine the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in animal, vegetable fats and oils. Specifically, a 25-g sample is placed in a large test tube. A 15-mL volume of iso-octane (2,2,4- trimethylpentane) [Fisher, reagent grade] is added and extracted for 10-min. A 5-mL volume of the non-polar layer from the bottom of the test tube is withdrawn. The absorbance of each extract is read at 350-nm, with iso-octane used as a blank. Dilution with iso-octane is used to obtain absorbance range.

Thiobarbituric Acid (TBA) Value

Lipid oxidation is assessed on the basis of the concentration of malonaldehyde in the examined samples according to a derivative spectrophotometric method. In brief, 100-g of sample is placed in a 500-mL boiling flask and 75-mL 12N hydrochloric acid is added. Sample is distilled rapidly until the first 50-mL of distillate captured. Distillate is placed in a flask, mixed with 1-mL saturated aqueous thiobarbituric acid solution, and refluxed for 30-min. After cooling at ambient conditions for 30-min absorbance is read at 530-nm. TBA is expressed by converting absorbance readings to mg-malonaldehyde per 1000-g sample

Solid Phase Microextraction (SPME)

A modified version of US EPA Method 524.2 as described by Supelco Application Note 11 is used. Specifically, a 100μm polydimethylsiloxane (PDMS) SPME fiber is placed in the sample headspace for 5-min. Cryogenic focusing to capture early eluting volatiles (specifically hexanal) is performed by placing the initial 0.3-m column length in dry ice for 1-min while solid phase microextraction (SPME) is desorbed in the gas chromatograph (GC) inlet.

For High Pressure Liquid Chromatography (HPLC) Solid Phase Microextraction (SPME), a three-gram sample is placed in a 25-mL vial and sealed. Samples undergo accelerated testing and are sacrificed at the allotted time. A Supelco SPME fiber assembly (60um Polydimethylsiloxane/divinylbenzene fiber) is inserted into the vial through the septum. The fiber is exposed in the vial for thirty minutes, withdrawn and placed in the Supelco desorption assembly. The fiber is allowed to desorb for fifteen minutes and the solvent is injected into a Dionex HPLC using an Alltech 250mm C18 column.

Statistical Analysis

All data will be subjected to analysis of variance (ANOVA). Descriptive statistics will be calculated for the samples gathered over a 24-h period using Analyse-it for Microsoft Excel, Leeds, UK. (http://www.analyse-it.com/)

Residual Affects on Biological Systems

Simulated tanker underflow (with ethoxyquin at 250-ppm and without ethoxyquin) was obtained by submitting 125-g aliquots of SPN to accelerated testing at 40°C for 24-hours. Samples were centrifuged for 15 minutes at 5000-rpm and then filtered through 41 Whatman (>20-25μm) filters. The total volume of the samples was 880mL.

Chemical oxygen demand (COD), pH, alkalinity, total solids (TS), volatile solids (VS), and ammonia were measured by following procedures outlined in *Standard Methods* (APHA, 1998). Total gas production was measured by displacement of an acidified brine solution (10% NaCl w/v, 2% H_2SO_4 v/v) in a graduated burette. Gas composition (i.e., CO_2, CH_4, H_2) and VFAs will be determined by gas chromatography (thermal conductivity detector and flame ionization detector, respectively).

Serum bottles with a total volume of 160mL for each were sealed with rubber stoppers and aluminum crimp seals, then, purged with Helium for 15 minutes at 10psi pressure in order to remove the oxygen trapped inside the bottles. Mineral media added to the serum bottles contains, in g/L, 0.9 K_2HPO_4, 0.5 KH_2PO_4, 0.5 NH_4Cl, 0.1 $CaCl_2.2H_2O$, 0.2 $MgCl_2.6H_2O$, 0.1 $FeCl_2.4H_2O$, 3.5 $NaHCO_3$, 0.5 $Na_2S.9H_2O$ and in ml/L, 2 resazurin, 1 trace metal stock, 1 vitamin stock solutions. For preparation of trace metal stock solution and vitamin stock solutions refer to Shah, 2003.

All the serum bottles were set as triplicates. Seed Blanks were used to determine the excess gas production because of the biodegradable carbon source remained in the inoculum. A set of control series were fed with Dextrin/Peptone solution which resulted in 1200mg COD/L and 2400mg COD/L in the serum bottles.

Antioxidant Measurement

Quantification of dissolved ethoxyquin in bioreactor mixed liquor was performed using a previously reported and modified liquid/liquid extraction technique (Choy *et al.*, 1963). A volume of sample based upon an estimated ethoxyquin concentration is transferred into a 28-mL capacity serum tube (Bellco Glass), followed by 20-mL of iso-octane Tubes will be capped with Teflon-lined rubber stoppers, sealed with aluminum crimps, and vigorously shaken by hand for 2-min and by vortex mixer for 1-min. After mixing, tubes will be centrifuged at 3000-rpm for 20-min for solids separation. Three-mL of supernate will then be transferred to plastic cuvettes for analysis using an HP Model 8453 diode array spectrophotometer equipped with deuterium and tungsten lamps. Ethoxyquin peaks are detected at 255 and 360-nm, however, 360nm will be used to avoid possible interference with plastic cuvette absorbance. Iso-octane served as a blank.

Results and Discussion

Samples were collected over the course of eight months on sixteen separate days. As a result of the accelerated testing, 25, 35, 40°C were chosen as representative operating conditions. Appendix 1 depicts descriptive statistics for the lipid oxidation analysis.

Average SPN sample total solids content was 13.4% ±5% and fat content was 9.3% ± 2.3%.

Total oxidation (TOTOX) testing as a SPN product quality indicator proved unviable due to pigment interferences resulting from sample preparation (i.e., discerning a color change in brown material). Varying SPN dilutions and representative sample sizes did not significantly reduce the iodometric methods error for peroxide determination. Anisidine values were measured, with TBARS and FFA analysis conducted to adjust for difficulties encountered with determining peroxide values.

Lipid Oxidation Analysis

Free fatty acid values increased with time and temperature. Samples dosed with ethoxyquin followed a similar trend, however FFA values were smaller with given equivalent time periods. From 4- to 24-hours, 35°C free fatty acid values (FFA_{35C}) increased at a higher rate than FFA_{40C}. FFA_{25C} increased at a lower rate through 4-hours, but FFA_{25C} production increased at a higher rate than both the FFA_{35C} and FFA_{40C} from 4- to 6-hours. During 6 to 24-hours FFA_{25C} increased at a lower rate but still higher than both FFA_{35C} and FFA_{40C}, with the FFA_{25C} 24-hour value exceeding that of FFA_{40C}. FFA_{30C} and FFA_{50C} (only evaluated one time each) increased at lower rates through 6-hours, however FFA_{30C} exceeded all other temperatures by approximately 30% after 24-hours while FFA_{50C} was approximately 30% less than all other temperatures.

Formation of FFA demonstrated temperature dependence. The overall FFA_{25C} and FFA_{30C} reaction rates were second-order, indicating a bimolecular mechanism. The overall reaction rates for FFA_{35C} and FFA_{40C} were first-order, with a power law best describing the reaction equation for FFA_{50C}.

Without a specific stoichiometry for the FFA reaction, the temperature dependency was examined via determination of the activation energy associated with the reaction rate constants. First-order kinetics was not used. The FFA reaction displayed a change in activation energy with temperature, suggesting a controlling mechanism shift (Levenspiel, 1972). Although the calculated values differ by only one order of magnitude, the transition at FFA_{40C} indicates a shift from low to high activation energy.

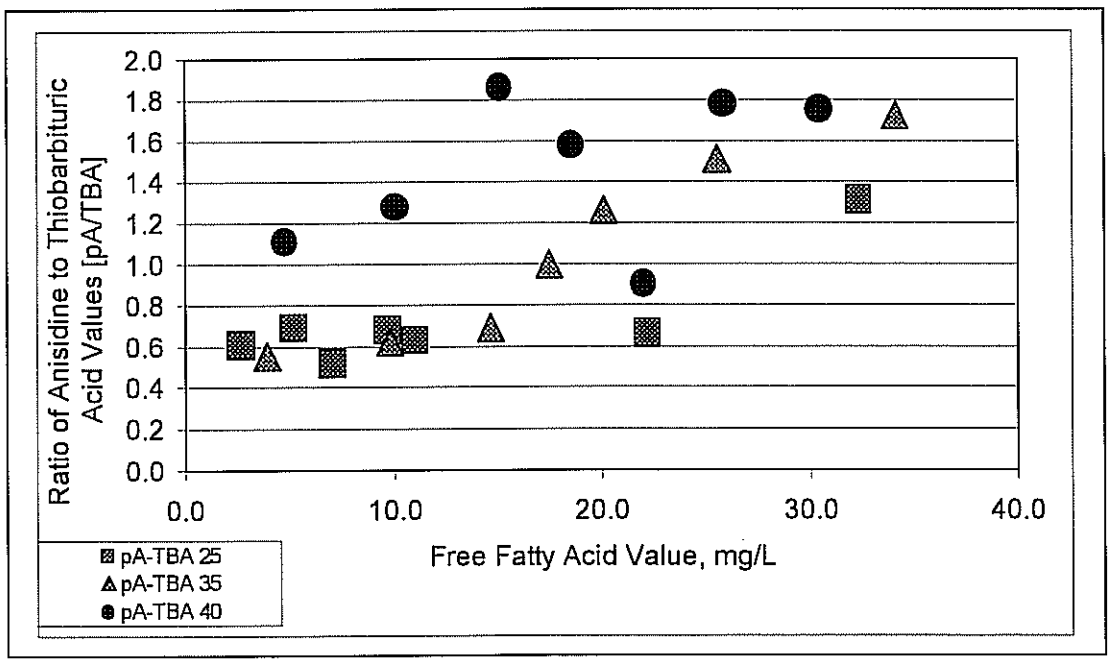

Figure 1. Ratio of Anisidine to Thiobarbituric Acid as compared to Free Fatty Acid Values

Anisidine (pA_{25C} and pA_{35C}) trends lagged thiobarbituric acid (TBA_{25C} and TBA_{35C}, respectively) by 30- to 60-min during the first four-hours, and displayed lower concentrations (mg/L) for equivalent time periods. As temperature was increased, pA exceeded TBA with the transition point shifting toward initiation. Final pA values exceeded TBA for all temperatures. For pA_{40C}, TBA concentrations remained lower during all time periods except at 4-hours. Interestingly, the reaction equation for pA at all temperatures was first-order with a high correlation. Figure 1 depicts the effect of FFA upon the ratio between pA and TBA for each temperature. Unity between pA and TBA demonstrates the shifting transition point as temperature increased. Data distribution by temperature reflects FFA production increased linearly with time but production rates were not equal.

Antioxidant Effects Upon Lipid Oxidation

Single accelerated test sets for each temperature (duplicates for 40°C) were subsequently conducted comparing antioxidant addition (dosed at 250-ppm as ethoxyquin, with results at 319.6-ppm ± 92.7) on pA, TBA and FFA production. Ethoxyquin concentrations were detected at 62.7-ppm±12.7 in SPN control sample filtrate. Testing periods were limited to four hours, with six hours for 40°C. FFA values for non-ethoxyquin samples at 35°C and 40°C (FFA_{35C} and FFA_{40C}, respectively) increased at similar rates during the first hour with comparable but slower increases through four-hours. No increase was measured for FFA_{25C} after two hours but production was recorded after four hours. Overall FFA production for all temperatures and samples were lower by one-half or greater compared with the primary accelerated testing results. Initial FFA values were comparable except for FFA_{40C} which had 1.1 mg/L as FFA.

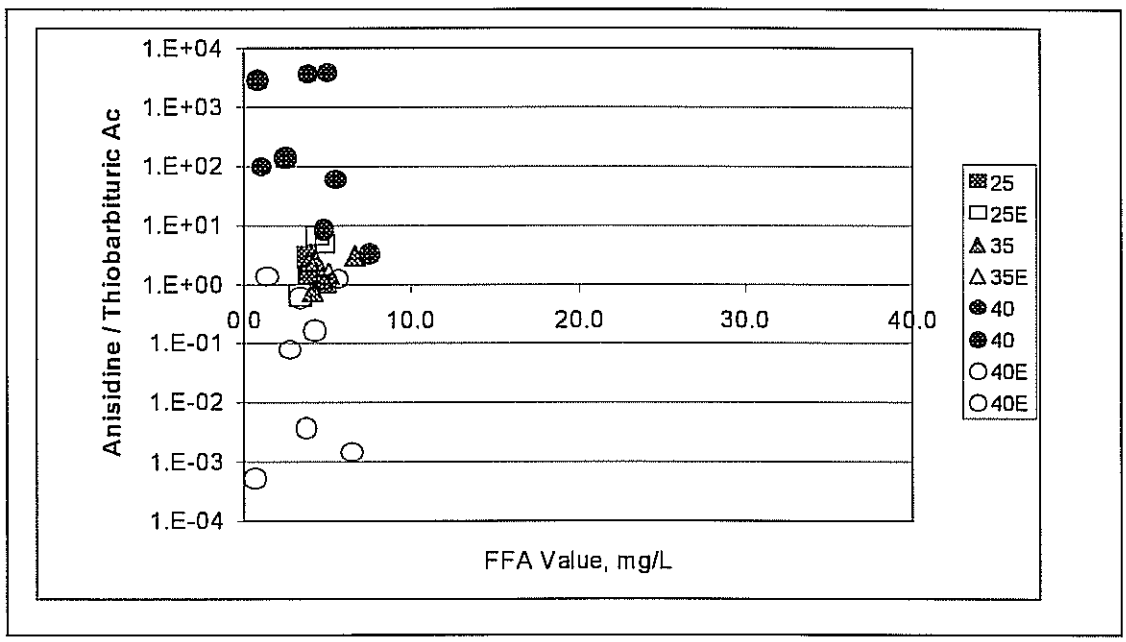

Figure 2. Ratio of Anisidine to Thiobarbituric Acid as compared to Free Fatty Acid values. Samples with and without ethoxyquin are shown based upon temperature.

For all temperatures, the pA to TBA ratio generally exceeded unity. The pA_{25C} / TBA_{25C} value increased after two-hours and then decreased to approximately unity. The pA_{35C} / TBA_{35C} sample was below unity at time zero but exceeded one within two hours. Ethoxyquin-based antioxidants inhibited FFA and TBA production. For SPN initially containing FFA levels less than 1.5 mg/L, TBA production was trivial. Figure 2 depicts the results of antioxidant testing for lipid analysis. Note the abscissa scale (y-values) is logarithmic, indicating that thiobarbituric acid values tended to remain depressed for lower FFA levels.

Hexanal Testing

Initial gas chromatography testing using gas chromatography with cryogenic focusing and solid phase microextraction (SPME) fibers failed to provide repeatable results. Neat hexanal was placed in sealed containers and exposed to SPME fibers with inconclusive results during calibration. GC column replacement failed to detect early eluting VOCs, exhibit distinct peaks, and afford detection limits.

Hexanal production during accelerated testing was measured for samples with and without antioxidants (250-ppm as ethoxyquin) using high pressure liquid chromatography (HPLC). Figure 3 depicts results. For both samples, hexanal concentrations in the aliquot headspace increased with temperature. Hexanal concentrations were higher without ethoxyquin addition, and hexanal formation was increased though 35°C. At 40°C, hexanal production decreased. However, the antioxidant effects of ethoxyquin were greatest at 40°C indicating that ethoxyquin dosage at 250-ppm (summertime loading) reduces hexanal concentrations to levels slightly above that of 25°C. Although not tested (125-ppm as ethoxyquin in wintertime) data suggests that ethoxyquin dosing in conditions where SPN temperature does not exceed 25°C may not be critical.

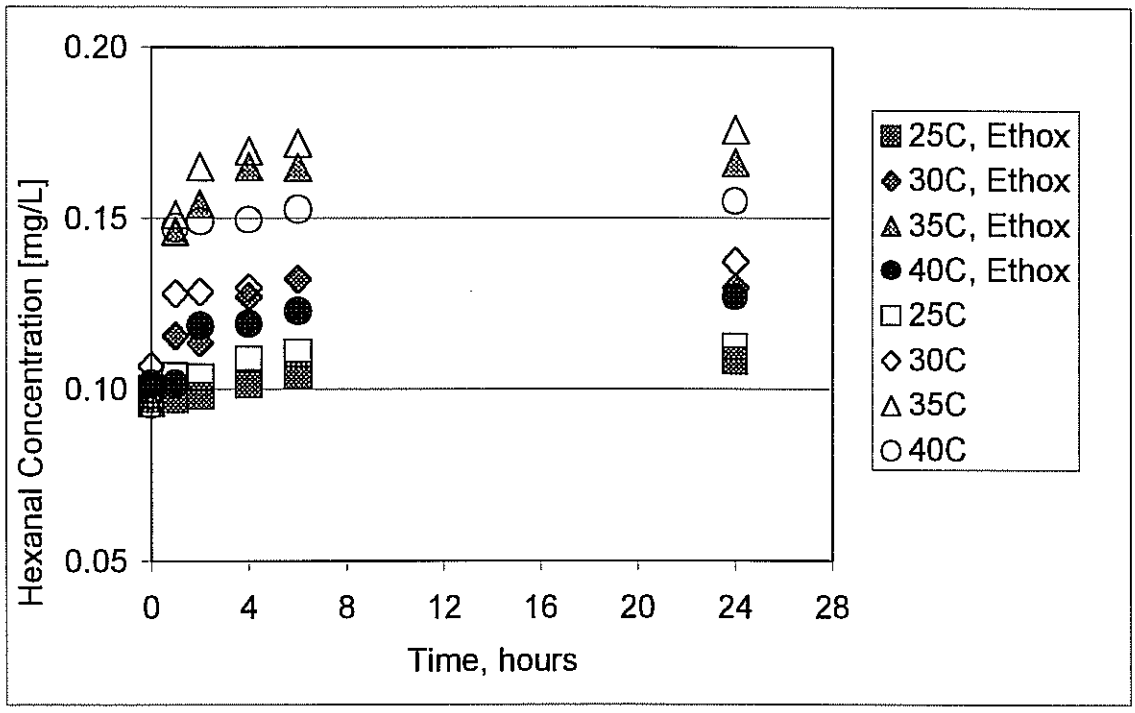

Figure 3. Hexanal Concentration during accelerated testing, with and without antioxidant addition at 250-ppm as ethoxyquin.

As noted, TBA concentrations were depressed as compared to anisidine for SPN samples with FFA levels below 1.5 mg/L and/or heat and ethoxyquin addition. Data indicates that hexanal formation follows time and temperature relationships similar to that of anisidine.

Residual Antioxidant Effects on Biological Systems

The initial pH values of underflow sample (UF) and the underflow with eqthoxyquin (UF+EtQ) was 6.25 and 6.20, respectively. Chemical Oxygen Demand (COD) and Volatile Fatty Acids (VFA) were measured for both samples (Table 1, Table 2). The COD concentrations indicated in Table 3 other than D/P control series are the concentrations contributed only by under flow with and without Ethoxyquin. A 63% diluted stock D/P solution which resulted in COD concentration of 120g/L was used in the experiment

Table 1. COD concentrations of Under Flow samples (UF denotes under flow without antioxidant and UF+EtQ denotes underflow with antioxidant [250-ppm as ethoxyquin]).

Sample	COD, mg/L	Average	Standard Deviation	Coefficient of Variance	Average	Standard Deviation	Coefficient of Variance
UF+EtQ(1/40 Dilution)	20355.84	20732.80	533.10	2.57	20073.12	835.85	4.16
UF+EtQ(1/40 Dilution)	21109.76						
UF+EtQ(1/20 Dilution)	19601.92	19413.44	266.55	1.37			
UF+EtQ(1/20 Dilution)	19224.96						
UF (1/40 Dilution)	17340.16	18471.04	1599.31	8.66	17717.12	1305.83	7.37
UF (1/40 Dilution)	19601.92						
UF (1/20 Dilution)	16586.24	16963.20	533.10	3.14			

UF (1/20 Dilution)	17340.16

Table 2. Initial volatile fatty acid (VFA) concentrations of Under Flow samples (UF denotes under flow without antioxidant and UF+EtQ denotes underflow with antioxidant [250-ppm as ethoxyquin]).

	VFA				
	Acetic acid mg/l	**Propionic acid** mg/l	**iso-butyric acid** mg/l	***n*-butyric acid** mg/l	**iso-valeric acid** mg/l
UF+EtQ(1/3 Dilution)	1043.02	1063.78	44.24	21.82	0
UF+EtQ(1/6 Dilution)	1051.38	1015.19	-	-	-
UF+EtQ(1/12 Dilution)	1045.08	1017.23	-	-	-
Average	**1046.49**	**1032.07**	**44.24**	**21.82**	
Standard Deviation	**4.35**	**27.49**			
UF (1/3 Dilution)	874.82	948.77	39.99	20.41	-
UF (1/6Dilution)	939.15	966.59	-	-	-
UF (1/12 Dilution)	900.35	906.23	-	-	-
Average	**904.77**	**940.53**	**39.99**	**20.41**	
Standard Deviation	**32.39**	**31.01**			

For the spectrophotometric method used for exthoxyquin measurements refer to Shah, 2003. However, differently from the method described in Shah 2003, sample without ethoxyquin was used as a blank in the measurements. The biodegradability of Under Flow was monitored with the total gas production and gas composition. No lag phase in the gas production was noted. The total gas production in the bottles with under flow was significantly lower than the ones that have same COD concentration but fed only with D/P. However, the gas production in the bottles containing under flow and D/P were greater than in the ones having the same COD concentrations but fed only with under flow. These two results on gas production can be concluded as the under flow consisted of more complex organics that are refractory for anaerobic biodegradation thus, the total gas produced in the bottles containing under flow is lower than the ones fed only with D/P with the same COD concentration (Figure 4). Methane production in the bottles containing both under flow and D/P and only D/P were the same (Figure 5).

CO_2 production in the bottles containing underflow was significantly lower than all D/P controls. The cumulative amount of CO_2 produced in the serum bottles which had D/P and underflow was lower than the D/P control with same COD (Figure 6). Results show that, the presence of ethoxyquin in the underflow did not have any effect in terms of total gas production or gas composition. However the spectrophotometric analysis of the samples showed that there was not any ethoxyquin in the samples at the initial time.

Table 3. Serum bottle volumes and contents for underflow sample testing, with Dionized water (DI), Dextrin/Peptone (D/P), and Underflow (UF) with and without (UF+EtQ) ethoxyquin.

Bottle Name	Mixed Liquor (mL)	Media (mL)	DI (mL)	D/P (mL)	UF (mL)	UF+EtQ (mL)
Seed-Blank-1	75	10	15	-	-	-
Seed-Blank-2	75	10	15	-	-	-
Seed-Blank-3	75	10	15	-	-	-

D/P 1200 mgCOD/L-1	75	10	14	1	-	-
D/P 1200 mgCOD/L -2	75	10	14	1	-	-
D/P 1200 mgCOD/L-3	75	10	14	1	-	-
D/P 2400 mgCOD/L-1	75	10	13	2	-	-
D/P 2400 mgCOD/L-2	75	10	13	2	-	-
D/P 2400 mgCOD/L-3	75	10	13	2	-	-
UF 1200 mgCOD/L-1	75	10	8.2	-	6.8	-
UF 1200 mgCOD/L-2	75	10	8.2	-	6.8	-
UF 1200 mgCOD/L-3	75	10	8.2	-	6.8	-
UF 2400 mgCOD/L-1	75	10	1.4	-	13.6	-
UF 2400 mgCOD/L-2	75	10	1.4	-	13.6	-
UF 2400 mgCOD/L-3	75	10	1.4	-	13.6	-
UF-ETQ 1200 mgCOD/L-1	75	10	9	-	-	6
UF-ETQ 1200 mgCOD/L-2	75	10	9	-	-	6
UF-ETQ 1200 mgCOD/L-3	75	10	9	-	-	6
UF-ETQ 2400 mgCOD/L-1	75	10	3	-	-	12
UF-ETQ 2400 mgCOD/L-2	75	10	3	-	-	12
UF-ETQ 2400 mgCOD/L-3	75	10	3	-	-	12
UF+D/P 1200 mgCOD/L-1	75	10	7.2	1	6.8	-
UF+D/P 1200 mgCOD/L-2	75	10	7.2	1	6.8	-
UF+D/P 1200 mgCOD/L-3	75	10	7.2	1	6.8	-
UF-ETQ+D/P 1200 mgCOD/L-1	75	10	8	1	-	6
UF-ETQ+D/P 1200 mgCOD/L-2	75	10	8	1	-	6
UF-ETQ+D/P 1200 mgCOD/L-3	75	10	8	1	-	6

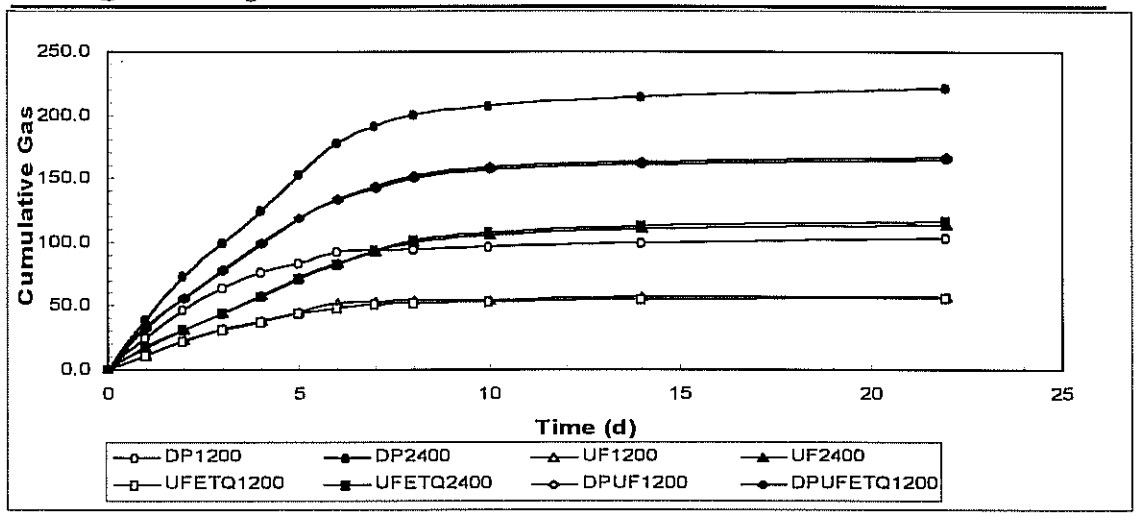

Figure 4. Cumulative gas production in serum bottles.

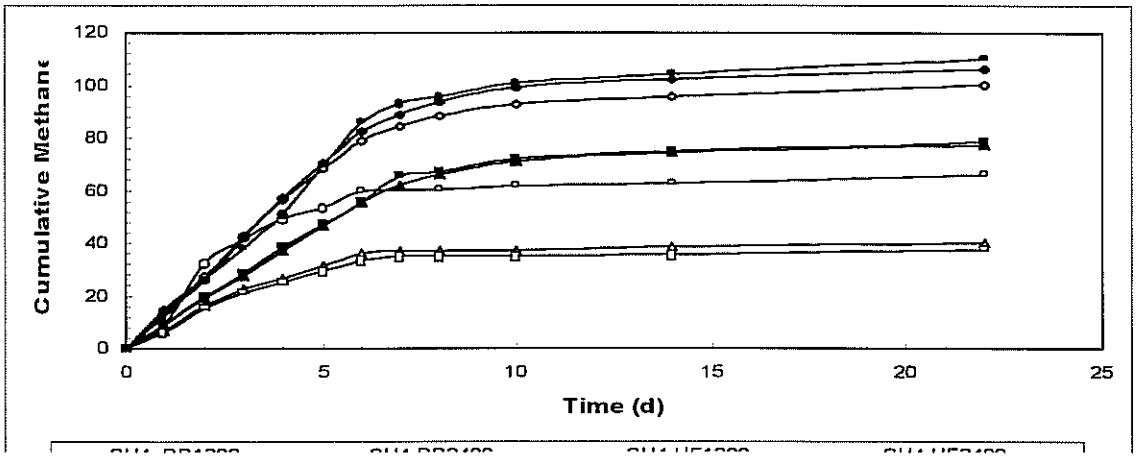

Figure 5. Cumulative methane production in serum bottles.

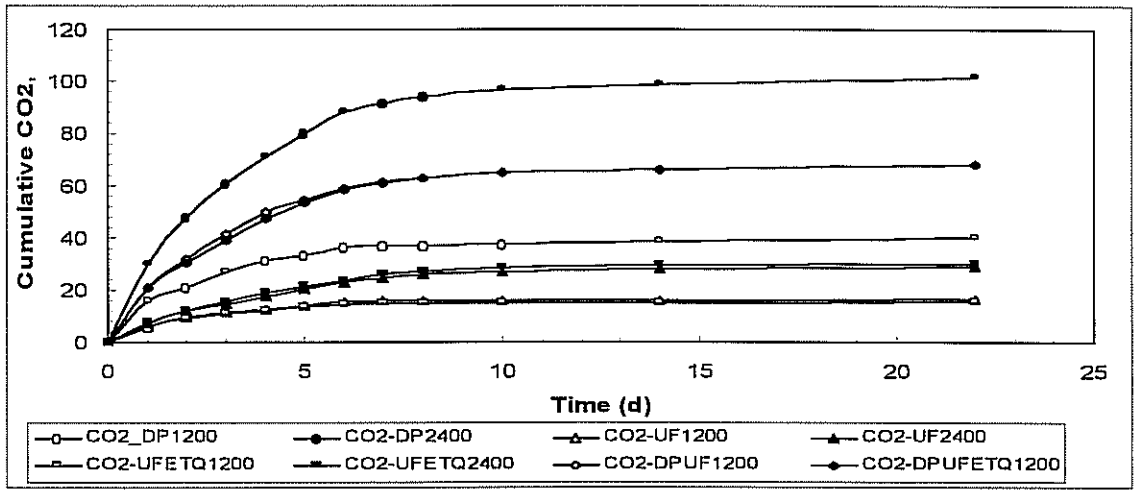

Figure 6. Cumulative CO2 production in serum bottles.

significant differences were noted for pH values between serum bottles. All VFA's were consumed at the end of the experiment.

Table 4. Final pH and volatile fatty acid values for serum bottles.

Bottle Name	pH	VFA, mg/L
Ref-Seed-1	7.62	0
Ref-Seed-2	7.61	0
Ref-Seed-3	7.66	0
D/P 1200 mg/L-1	7.04	0
D/P 1200 mg/L-2	7.06	0
D/P 1200 mg/L-3	7.06	0
D/P 2400 mg/L-1	7.00	0
D/P 2400 mg/L-2	6.99	0
D/P 2400 mg/L-3	6.99	0
UF 1200 mg/L-1	7.40	0
UF 1200 mg/L-2	7.40	0
UF 1200 mg/L-3	7.41	0
UF 2400 mg/L-1	7.31	0
UF 2400 mg/L-2	7.30	0
UF 2400 mg/L-3	7.33	0

UF-ETQ 1200 mg/L-1	7.36	0
UF-ETQ 1200 mg/L-2	7.35	0
UF-ETQ 1200 mg/L-3	7.36	0
UF-ETQ 2400 mg/L-1	7.29	0
UF-ETQ 2400 mg/L-2	7.28	0
UF-ETQ 2400 mg/L-3	7.26	0
UF+D/P 1200 mg/L-1	7.00	0
UF+D/P 1200 mg/L-2	7.01	0
UF+D/P 1200 mg/L-3	6.99	0
UF-ETQ+D/P 1200mg/L-1	7.01	0
UF-ETQ+D/P 1200 mg/L-2	7.04	0
UF-ETQ+D/P 1200 mg/L-3	6.99	0

Conclusions

Pigment interferences resulting from SPN sample preparation make iodometric methods for measuring peroxide values impractical. Free fatty acid values in SPN increase at a linear rate over 24-hour periods at temperatures typical of facility storage and transport. However, the rate of change for FFA values varies with temperature. Data distribution by temperature reflected FFA production increased linearly with time but production rates were not equal. FFA_{25C} increases most predominately after 6-hours while FFA_{35C} production increased after only one-hour. FFA_{40C} production increased immediately with a slowing between hours one and six followed by increased production again through 24-hours.

Anisidine and thiobarbituric acid values varied with time, temperature, and thus FFA value. While individual trends were difficult to discern, the ratio between anisidine and thiobarbituric acid as a function of FFA indicated that unity (between pA and TBA) demonstrated a shifting transition point as temperature increased. With increased temperature, TBA production decreased as compared with anisidine. For SPN samples treated with an antioxidant (250-ppm as ethoxyquin), overall FFA production for all temperatures and samples were lower by one-half or greater compared with the primary accelerated testing results. For all temperatures, the pA to TBA ratio generally exceeded unity. Initial FFA values were comparable except for FFA_{40C} that had 1.1 mg/L as FFA.

Ethoxyquin dosage at 250-ppm (summertime loading) for accelerated testing at 40°C indicated antioxidant effects were improved, with hexanal concentrations reduced to levels similar to those at 25°C. Although not tested (125-ppm as ethoxyquin in wintertime) data suggests that ethoxyquin dosing in conditions where SPN temperature does not exceed 25°C may not be critical. Data indicated ethoxyquin-based antioxidants inhibited FFA and TBA production.

Hexanal concentrations increased with temperature over time, with 35°C producing the highest concentrations for samples with and without antioxidants (250-ppm as ethoxyquin). TBA concentrations were depressed as compared to anisidine for SPN samples with FFA levels below 1.5-mg/L and/or heat and ethoxyquin addition. Data indicates that hexanal formation follows time and temperature relationships similar to that of anisidine. The supernate from SPN samples, inoculated with ethoxyquin at 250-ppm and exposed to accelerated testing at 40°C for 24-hours did not produce an adverse effect on microbial species predominating diverse anaerobic wastewater cultures.

Recommendations for Future Work

Hexanal analysis was not conducted in conjunction with anisidine, thiobarbituric acid and free fatty acid values. Maintaining headspace and sample volumes in existing glassware limited completing the analysis. However, hexanal and free fatty acid values increase with time and temperature therefore comparing anisidine, thiobarbituric acid, or the ratio with either will provide a greater indicator of appropriate SPN quality analysis.

Accurate antioxidant addition to SPN samples is difficult due to viscosity and density. Even when mixing larger volumes of SPN, ensuring the antioxidant is evenly mixed is problematic. Other methods for antioxidant addition are needed to ensure material distribution.

A non-specific, spectrophotometric method was used to quantify ethoxyquin concentrations, however, other compounds may interfere with detection (e.g., ethoxyquin detected in SPN samples not mixed with ethoxyquin). Other techniques such as ion-pair chromatography (IPC) to extract anion-cation ion pair may be more precise. (Schmitt, 1991; Cross and Singer, 1994).

Although residual exthoxyquin contained within the underflow from SPN tankers does not appear to have adverse effects on anaerobic biological systems, supernate derived from 25°C and 35°C samples were not tested. Data indicated that oxidation occurred at similar rates for 25°C samples (with and without ethoxyquin) therefore testing underflows during lower temperature storage may show that non-summer temperatures may actually represent worse case conditions.

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