

Thermophilic Anaerobic Digestion to Increase the Net Energy Balance of Corn Grain Ethanol

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U.S. production of fuel ethanol from corn grain has increased considerably over the last 10 years. Intense debate regarding the true environmental impact of the overall production process has been ongoing. The present study evaluated the utilization of thin stillage (a major byproduct of the dry-mill corn grain-to-ethanol process) in laboratory-scale thermophilic anaerobic sequencing batch reactors for conversion to methane. We found that augmentation of cobalt as a growth factor to the thermophilic anaerobic digestion process is required. After reaching sustainable operating performances, the methane potential in the reactors was 0.254 L CH₄/g total chemical oxygen demand (TCOD) fed. Together with a reduction in the mass of solids that needs drying, methane generation translates to a 51% reduction of natural gas consumption at a conventional dry mill, which improves the net energy balance ratio from 1.26 to 1.70. At the design hydraulic retention time of 10 days, the digesters achieved TCOD, biodegradable COD, volatile solids, and total solids removal efficiencies of 90%, 75%, 89%, and 81%, respectively. We also found that struvite precipitation occurred in the thermophilic digesters during the course of the study, resulting in possibilities for nutrient recovery.

Introduction

Current attitudes toward the environment and a political movement that desires to reduce dependence on foreign oil have bolstered liquid biofuel production in the United States. In addition, restrictions on the fuel additive methyl tertiary butyl ether (MTBE) have promoted ethanol production, resulting in the virtual replacement of MTBE in the United States by a 5–10% addition of ethanol to petroleum-based fuel (1). Total annual U.S. ethanol production has increased considerably between 1997 and 2005 from $\sim 5 \times 10^6$ to $\sim 15 \times 10^6$ m³, mostly from corn (*Zea mays* ssp. *mays*) grain (called kernel in the corn-to-ethanol industry) (1). Corn grain-to-ethanol plants based on dry grinding (i.e., dry mill) constituted 67% of U.S. corn-ethanol production in 2005 with wet

grinding (i.e., wet mill) making up the difference. Further growth in ethanol production is anticipated to be primarily by constructing dry mills because of the relatively lower capital costs (2, 3).

Creating a sustainable biofuel industry requires a holistic assessment that takes into consideration numerous factors, such as deforestation, crop production methods, nonrenewable energy and water consumption, and world food supplies. Some of the environmental-based criticism of corn grain-to-ethanol has mainly focused on the small positive net energy balance that is achieved (1, 4–6). For example, Hill et al. (4) calculated through life-cycle assessment that 26% more energy is gained from ethanol than is contained in nonrenewable fuels required for its production (i.e., a 1.26 net energy balance ratio), but that this gain is mostly due to energy credit of coproduced animal feed. They found a relatively large input of 0.60 units energy per one unit ethanol-energy output for processing the corn grain into ethanol and animal feed (or a 62.5% energy requirement for operating the processing facility out of all the energy inputs). The remaining energy requirements stem from the processing facility construction and laborer household energy use (1%), all energy requirements for farming (31.3%), and crop and biofuel transportation (5.2%) (4).

Because of the small positive net energy balance, corn grain-to-ethanol has been suggested only to be an intermediate step until more favorable technology has been scaled up (7). Because ethanol from corn grain is a reality, the net energy balance can be improved in the immediate future by, for example, finding alternative uses for process streams (e.g., thin stillage) (6, 8). In dry mills, thin stillage is the centrate of distillation bottoms (i.e., the residue after ethanol is distilled from “beer”) and is partially recycled as fermentation broth for ethanol production or dehydrated in evaporators to produce syrup. Usually, less than a 50% recycle ratio for thin stillage as fermentation broth (called backset in the corn-to-ethanol industry) can be utilized due to solids build up and toxicity to yeast by lactic acid, acetic acid, and/or sodium (9–11). Evaporation requires a large energy dedication (often from waste heat), but it enables some of the condensed water to be recycled as makeup water in the fermentation process. Syrup is added to wet distillers’ grains (WDG) and flash dried (often with steam from natural gas) to produce distillers’ dried grains with solubles (DDGS), which is sold as animal feed (wet distillers’ grains dried without syrup is also known as distillers’ dried grains [DDG]).

Anaerobic digestion of thin stillage (called biomethanation in the corn-to-ethanol industry) may be an advantageous process step compared to evaporation and syrup drying because energy is recovered in the form of biogas, which could substantially increase the net energy balance. Anaerobic digestion of stillage from various fermentation feedstocks, such as cane molasses, beet molasses, whey, wheat, and grapes, has been previously studied with a diverse group of reactor types, including continuously stirred tank reactors (CSTR) and upflow anaerobic sludge blanket (UASB) reactors (12–15). For thin stillage treatment from corn grain feedstock, Ganapathi (16) used continuous-flow mesophilic (35 °C) digestion with a CSTR, but this was after complete physical removal of the solids and subsequent dilution with water. Another published study was by Schaefer et al. (17), who studied thermophilic (55 °C) anaerobic treatment of thin stillage in semi batch-fed continuously stirred digesters.

Due to high total chemical oxygen demand (TCOD) concentrations of ~ 100 g/L in thin stillage, cost-efficient digestion requires very high organic loading rates, and thus

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TABLE 1. Characteristics of Five Thin Stillage Samples Used As Substrate for This Study (F1-F5), Two Thin Stillage Samples from Different Full-Scale Dry Mill Corn Grain-to-Ethanol Facilities (Plants 1 and 2), and Average Values for Weekly-Sampled Thin Stillage Batches during Three Different HRT Periods for the Full-Scale MGP Plant (No Statistical Data Reported)^a

thin stillage sample [days fed]	SCOD (g/L)	TCOD (g/L)	VS (g/L)	TS (g/L)
F1 [0–106, 219–238]	39 ± 10, <i>n</i> = 14	97 ± 19, <i>n</i> = 12	37.29 ± 3.08, <i>n</i> = 6	42.09 ± 3.14, <i>n</i> = 6
F2 [107–163]	35 ± 6, <i>n</i> = 6	97 ± 29, <i>n</i> = 6	45.17 ± 4.30, <i>n</i> = 3	49.93 ± 3.91, <i>n</i> = 3
F3 [164–218]	36.5 ± 11, <i>n</i> = 7	77.5 ± 5.5, <i>n</i> = 5	37.11 ± 0.44, <i>n</i> = 5	41.87 ± 0.46, <i>n</i> = 5
F4 [239–295]	39 ± 4, <i>n</i> = 7	73.5 ± 13, <i>n</i> = 8	31.55 ± 0.57, <i>n</i> = 3	36.01 ± 0.56, <i>n</i> = 3
F5 [295–416]	33.5 ± 2, <i>n</i> = 14	75 ± 12, <i>n</i> = 13	31.85 ± 1.51, <i>n</i> = 4	36.63 ± 1.60, <i>n</i> = 4
Plant 1	71 ± 12.5, <i>n</i> = 5	182 ± 4, <i>n</i> = 4	93.76 ± 1.05, <i>n</i> = 3	104.30 ± 0.93, <i>n</i> = 3
Plant 2	66 ± 3, <i>n</i> = 5	123 ± 7, <i>n</i> = 5	84.44 ± 1.04, <i>n</i> = 3	92.82 ± 1.04, <i>n</i> = 3
MGP plant, average for 30-day HRT ^b	59	97.1	61.9	68.9
MGP plant, average for 20-day HRT ^b	76	121	83.5	90.3
MGP plant, average for 15-day HRT ^b	51	96.1	59.1	65.9

^a Standard deviations are given for the variations in average numbers with *n* being the amount of measurements for the F1–F5 samples. ^b From the Midwest Grain Processors (MGP) ethanol plant, Lakota, IA (ref 17).

increased degradation kinetics, to enable reduced reactor volumes. Thermophilic anaerobic digestion is, therefore, advantageous compared to mesophilic anaerobic treatment of thin stillage because of higher metabolic rates (14, 15, 18). In addition, fats, oils, and grease (FOG), which are common at high concentrations in thin stillage from dry mills without fractionation of the corn grain, can accumulate in mesophilic digesters by forming a foam layer, and cause operating problems by washing out active biomass (19). This is not a problem in thermophilic digesters due to sufficient solubilization and degradation of FOG at higher temperatures (20). In many cases, however, due to high effluent volatile fatty acid (VFA) concentrations and increased heating costs, thermophilic digestion has been largely ignored (21, 22). Recent work by Speece et al. (22) has removed the first limitation of high VFA concentrations by supplementing prominent trace elements to prevent inhibition of methanogenesis. It was already known that trace elements are important in the function of many methanogenic enzymes (23), that they are scarce in most digester substrates (24), and that addition of trace elements has been credited with causing dramatic improvements in reactor performance (25, 26), but it seems even more imperative for thermophilic digestion (22). The second limitation of increased heating costs is not valid for thermophilic digestion of thin stillage because the whole stillage is already hot after leaving the distillation column.

This study sought to ascertain the applicability of an integrated method of thermophilic anaerobic digestion of thin stillage from dry mill corn grain-to-ethanol plants by utilizing anaerobic sequencing batch reactors (ASBRs). By allowing biomass to settle in the ASBR before decanting effluent vs no settling in completely stirred digesters, the concentration of active biomass is increased and the sludge retention time (SRT) is elongated compared to the hydraulic retention time (HRT), to pursue higher volumetric degradation rates (high-rate vs low-rate digestion). We will also discuss whether thermophilic digestion with high-rate systems is a better use of thin stillage than evaporation and syrup drying to produce animal feed. From preliminary calculations, we have estimated a substantial increase in the net energy balance ratio when thin stillage is treated with thermophilic anaerobic digestion.

Materials and Methods

Experimental Setup and Operation. The experimental setup of replicates reactor 1 (R1) and reactor 2 (R2) is given in the Supporting Information (S.1 and Figure S1). Each of the reactors was inoculated by adding 1.7 L of thermophilic anaerobic sludge from an anaerobic digester treating a mixture of primary and waste activated sludge at a municipal

wastewater treatment plant (Western Lake Superior Sanitary District, Duluth, MN) to 3.3 L of deionized water. After inoculation, 10 L of natural gas was bubbled through each bioreactor to ensure anaerobic conditions, after which the digesters were allowed 24 h to acclimate before feeding commenced. Thin stillage samples were received periodically from the National Corn-to-Ethanol Research Center in Edwardsville, IL, which is a demonstration-scale dry mill (~1/200 the size of a full-scale plant), and were stored at –20 °C in 1 L bottles until a day before feeding. Five different thin stillage samples were received from the dry mill and fed consecutively (F1–F5); each had somewhat different characteristics and each was fed for different periods of time (Table 1). From day 78 to day 106 of the operating period, 5 mL of a modified trace element solution according to Zehnder et al. (27) (Table 2) and Angenent et al. (28) was added weekly to R1. To R2, we added a 5-mL solution of only FeCl₃·4H₂O (10 g/L) on day 85 of the operating period; FeCl₃·4H₂O (10 g/L) and CoCl₂·6H₂O (2 g/L) on days 92 and 99; and CoCl₂·6H₂O (2 g/L) on day 106. From day 113 until the end of the operating period, a solution of CoCl₂·6H₂O (2 g/L) was added to both reactors at a rate of 1 mL/10 g influent TCOD once a week. All trace element solutions in this study contained EDTA (1 g/L) and HCl (1 mL concentrated HCl/L). A more detailed rationale for this augmentation is given in S.3.

The semibatch ASBRs (R1 and R2) were sequenced through a 24-h cycle with an instant feeding period, a 23-h react period with intermittent mixing, a 58-min settling period (no mixing), and a 2-min decanting period (the volume of feeding and decant solution was the same). Initially, intermittent mixing of the ASBRs was every h for one min, but this was changed to every 15 min for one min on day 69 of the operating period in an attempt to circumvent accumulation of rapidly settling solids. The HRT was decreased in a stepwise manner from 40 days (with an organic loading rate of 2.42 g TCOD/L/d) to 7 days (10.71 g TCOD/L/d) on day 392 after which the HRT was maintained at an 8-day HRT (9.37 g TCOD/L/d) from days 394–417. The HRT was shortened upon achieving pseudo steady-state conditions when stable biogas production rates (within 10% of average values), total VFA concentrations, volatile solids (VS) concentrations, and pH levels were achieved and after a minimum time period of one HRT, except at the 40- and 25-day HRTs during which the reactors were operated for 22 and 21 days, respectively.

We estimated the methane potential and methane yield by plotting the specific volumetric methane production rates against TCOD loading rates or removal rates, respectively, and forcing a linear regression of the points through zero on the y-axis. The specific biogas production rate was found by

TABLE 2. Elemental Analysis of Thin Stillage (F5), R1 and R2 Effluent Samples, Syrup, and Trace Element Solution^a

element	F5 (mg/L)	R1 effluent (mg/L)	R2 effluent (mg/L)	Belyea et. al. ^b (mg/kg dry basis)	Zehnder et. al. ^c (mg/L)
Mg	3.70×10^2	1.66×10^1	1.40×10^1	6870 ± 437	-
P	4.14×10^3	3.37×10^2	3.53×10^2	15200 ± 1280	-
K	5.56×10^3	ND	5.43×10^3	23200 ± 1340	-
Mn	1.17	<DL	<DL	29.2 ± 6.30	1.39×10^2
Fe	6.61×10^{-1}	5.78×10^{-1}	6.58×10^{-1}	138 ± 33.3	2.38×10^3
Co	<DL	2.71	2.77	<DL	4.95×10^2
Ni	1.25×10^{-1}	9.60×10^{-2}	1.00×10^{-1}	4.9 ± 0.90	3.51×10^1
Cu	1.95×10^{-1}	2.16×10^{-1}	2.42×10^{-1}	6.3 ± 3.30	-
Zn	6.24	6.35×10^{-1}	8.8×10^{-1}	126 ± 21.5	2.40×10^1
Na	ND	ND	ND	2360 ± 305	1.63×10^1
Al	ND	ND	ND	10.8 ± 1.10	1.01×10^1
Mo	ND	ND	ND	0.8 ± 0.02	2.64×10^1
Cu	ND	ND	ND	6.3 ± 3.30	1.42×10^1
Sr	ND	ND	ND	2.6 ± 3.40	-

^a <DL = less than detection level, ND = not determined. ^b Values given are for syrup (product of evaporation of thin stillage) (ref 3). ^c Trace element solution (27).

calculating the average biogas production rate (corrected to standard temperature and pressure) during each organic loading rate, except for two periods: the unstable period of R1 (days 284–362) and the time for both reactors beyond the 10-day HRT. The latter data were not included because both reactors were not able to handle the increase in the hydraulic pressure to an HRT of 7–8 days and/or an organic loading rate of 9.37–10.71 g TCOD/L/d. The specific volumetric methane production rate is the product of the specific biogas production rate and the average methane content of the biogas during a TCOD loading rate. Correlation of daily biogas production values were corrected for and linear regressions were performed using the “AUTOREG” and “GLM” procedures, respectively, in SAS software, version 9.1 (SAS Institute Inc., Cary, NC).

Physical and Chemical Analysis. All periodic analyses for evaluating digester performance were performed according to *Standard Methods* (29), unless otherwise indicated. Daily measurements included pH, biogas production, and room temperature and ambient pressure (to correct biogas production to standard conditions). Total VFA (distillation method), total solids (TS), VS, TCOD, soluble chemical oxygen demand (SCOD) (closed-reflux titrimetric method), and total ammonium (i.e., free ammonia and ammonium) concentration (electrode model Orion 9512, Thermo Electron Corporation, Beverly, MA) analyses were performed at least weekly. FOG and sludge volume index (SVI) measurements were also performed periodically. Methane concentration in the biogas was measured biweekly with a gas chromatograph (Series 350, Gow-Mac Instrument Co., Bethlehem, PA) with a thermal conductivity detector. The GC column was a 4 ft. × 1/8 in. o.d. 20% DC-200 on Chromosorb P AW-DMCS, 80/100 mesh (Varian, Inc., Palo Alto, CA). The temperatures for injection port, detection, and column were 50, 115, and 25 °C, respectively. Elemental analysis of influent and effluent was performed with an inductively coupled plasma mass spectrometer (ICP-MS) (model 7500 ICP-MS ChemStation, Agilent, Santa Clara, CA) equipped with an SP-5 autosampler. Precipitate analysis was performed with a scanning electron microscope (SEM) with a 15-kV accelerating voltage (model S-4500, Hitachi High-Technologies America, Schaumburg, IL) equipped with an energy dispersive X-ray spectroscopy (EDX) microanalysis system (Noran, Madison, WI). Further analysis on the precipitate was done by X-ray powder diffraction using Cu K α radiation (Geigerflex D-MAX/A, Rigaku, The Woodlands, TX). Software by Materials Data, Inc. (Livermore, CA) was used to control the diffractometer. Soluble protein concentration was measured in the influent and effluent using the colorimetric BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL). Total protein was

measured by the same method, but prior to analysis the sample was heated in 0.1 M NaOH for 10 min at 90 °C and centrifuged at 9300g for 20 min, after which the supernatant was analyzed. Colorimetric analysis was done using an end point reading in a 96-well plate at 562 nm and ambient temperatures (SPM Synergy HT, Bio-Tek, Winooski, VT). The method for calculating the nitrogen mass balance and measuring the N composition of the precipitate is included in S.2.

Results

Thin Stillage Characteristics. We measured the COD and solids concentrations for seven different thin stillage samples: five from a demonstration-scale dry mill (F1–F5 used as substrate in this study), and two from different full-scale corn grain-to-ethanol dry mills. In addition, we show published data from one full-scale dry mill (Table 1). The average TCOD concentrations for the F1–F5 samples were from 74–97 g/L, while they were 96–182 g/L for thin stillage from the three different full-scale plants (Table 1). The SCOD accounted for 36–53% of the TCOD in the feed substrate from the National Corn to Ethanol Research Center, which was in the same range compared to thin stillage from the full-scale plants (39, 54, and 53–63%). The VS concentration in the substrate batches was lower than those from the full-scale plants (32 to 45 g VS/L compared to 59–84, 84, and 94 g VS/L) (Table 1), and VS contributed 87–92% and 90–92% to the TS in the substrate and the thin stillage from full-scale plants, respectively.

Differences in COD and solids concentrations can be accounted for by varying operating conditions in the dry mill. For example, the solids concentrations from the demonstration-scale facility were lower compared to the full-scale facilities due to a relatively larger capacity of the centrifuge at the demonstration facility compared to a full-scale facility. Elemental analysis of one of the feed substrates (F5) showed that cobalt was not present in detectable concentrations (Table 2). In addition, magnesium, phosphorus, and potassium were all present in relatively high concentrations of 3.70×10^2 , 4.14×10^3 , and 5.56×10^3 mg/L, respectively, while manganese, iron, nickel, copper, and zinc were detected at lower concentrations (Table 2). FOG levels were 3.81 and 2.05 g/L in F4 and F5, respectively. The influent total ammonium concentration remained below 30 mg NH $_4^+$ -N/L over the course of the study, and the total protein concentration in F5 was 7.2×10^3 mg/L (SE = 41.7 mg/L, $n = 3$). pH values of the thin stillage varied from 3.46 to 4.35.

Bioreactor Performance. *Methane Production Rates, Potentials, and Yields.* R1 and R2 were operated similarly

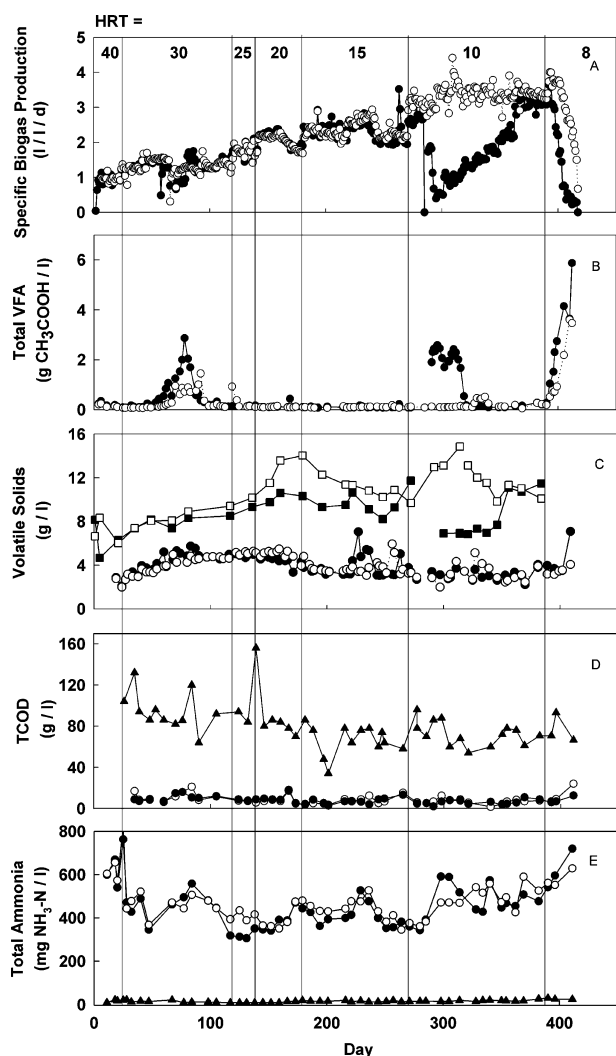


FIGURE 1. Reactor operating conditions and performance for R1 and R2: (A). Volumetric biogas production rate over the operating period (B). Effluent volatile fatty acid concentration over the operating period (C). Volatile solids concentration over the operating period (D). Influent and effluent chemical oxygen demand over the operating period (E). Total ammonium concentrations over the operating period (\blacktriangle - F1–F5 (substrate); \bullet - R1; \circ - R2; \blacksquare - R1 Biomass; \square - R2 Biomass). Vertical lines indicate periods with different HRTs.

over the operating period with stepwise increases in the organic loading rates except during unstable periods due to trace element limitations in both reactors (days 70–106 of the operating period: S.3) and an accidental oxygen influx in R1 (days 284–362: S.4). In addition, the five changes in the feed batch were implemented simultaneously for both reactors on days 107, 164, 219, 239, and 295 (Table 1), which resulted in changing organic loading rates within periods of similar HRTs. Therefore, the performances for R1 and R2 were similar with analogous trends of volumetric biogas production rates following stepwise increases in organic loading rates over the operating time or changing organic loading rates within HRT periods during times of stable performances with low total VFA concentrations (Figure 1A and B). This is reflected in statistically (95% confidence interval) similar methane potentials and methane yields for both reactors (0.253 and 0.254 L CH₄/g TCOD fed; and 0.278 and 0.284 L CH₄/g TCOD removed, for R1 and R2, respectively, with R^2 values of 0.99). A methane potential of 0.254 L CH₄/g TCOD fed, which was estimated from the combined data of both reactors, is used subsequently in this paper (Figure S2).

The methane concentrations in the biogas of both reactors were also similar and were on average 59.6% (SE = 5.1%, n = 30) and 59.7% (SE = 5.3%, n = 36) for R1 and R2, respectively.

Solids and COD Removal Efficiencies. The solids and COD removal efficiencies were determined for each HRT period (Table S1; Figure 1C and D). The 10-day HRT is the design HRT for this study because a stable performance was found for an extended period of operating time at this hydraulic loading. At the HRT of 10 days and an organic loading rate of 7.5 g TCOD/L/d, the removal efficiencies for R1 and R2 were similar, but because more data for R2 are available compared to R1 (10 HRT periods of stable performance vs 3 HRT periods) we report the information from R2 here (data for R1 in S.4). The mean TCOD removal efficiency for R2 measured (effluent vs influent) at the 10-day HRT was 92.2%, which included removal due to methane generation and biomass accumulation in the high-rate bioreactor (biomass was wasted naturally with the effluent, but this occurred periodically and not during the COD measurements at a 10-day HRT). The relative TCOD removal efficiency due to methane formation not including biomass accumulation was 81.1%. This efficiency was calculated from the ratio of the methane yield (0.284 L CH₄/g TCOD removed) to the maximum methane yield (0.350 L CH₄/g TCOD removed). By multiplying the measured and methane formation TCOD removal efficiencies, we estimated the efficiency due to biodegradation to be 74.7% at a 10-day HRT. The biodegradation of VS resulted in a measured 80.3% removal efficiency of VS since VS accounted for 88% of TS in thin stillage. The SCOD removal efficiency based on the TCOD concentration of the influent and the SCOD concentration of the effluent was 95.8% (Table S1). Despite the shorter HRT of 10 days at the end of the operating time compared to the longer HRT of 40 and 30 days at the beginning of the operating period, the TCOD, VS, and TS removal efficiencies were higher (Table S1) because of significantly lower effluent TCOD (α = 0.01), VS (α = 0.01), and TS (α = 0.05) concentrations. This was due to the better settleability of the biomass, which may have resulted in longer SRTs at the end of the operating period compared to the beginning of the operating period. Biomass characteristics of the reactor contents and effluent throughout the operating period are shown in Figure 1C (volatile solids) and described in S.5 and Figures S3 and S4.

Nutrient and Element Removal. The total ammonium concentration in the effluent was stable through the end of the 10-day HRT with average concentrations of 450 mg NH₄⁺-N/L (SE = 96.2, n = 49) and 467 mg NH₄⁺-N/L (SE = 88.0, n = 49) for R1 and R2, respectively (Figure 1E). The resulting high alkalinity levels in the effluent varied with feed and loading rate, but remained between 2,000 and 3,000 mg/L as CaCO₃ through the 10-day HRT (data not shown). As a result, the pH values of the reactor contents were stable without any addition of alkalinity or acid (except after accidental oxygen influx) even during the initial start-up period (Figure S5). The nitrogen balance during the 10-day HRT operation (see details in S.2) shows that the increase in total ammonium concentration in the effluent compared to the influent was due to protein degradation. It also shows an approximately equal concentration of total nitrogen in the influent and the effluent with concentrations of 1180, 1180, and 1190 mg/L N for the influent, R1 effluent, and R2 effluent, respectively. ICP-MS analysis on R1 and R2 effluent during the 10-day HRT period showed a 96% magnesium removal in both reactors when compared to influent levels (Table 2), and 92% and 91% phosphorus removals in R1 and R2, respectively.

Over the operating period, a ~10-cm layer of white precipitate accumulated in the bottom cone of both reactors. SEM/EDX analysis showed that this precipitate contained

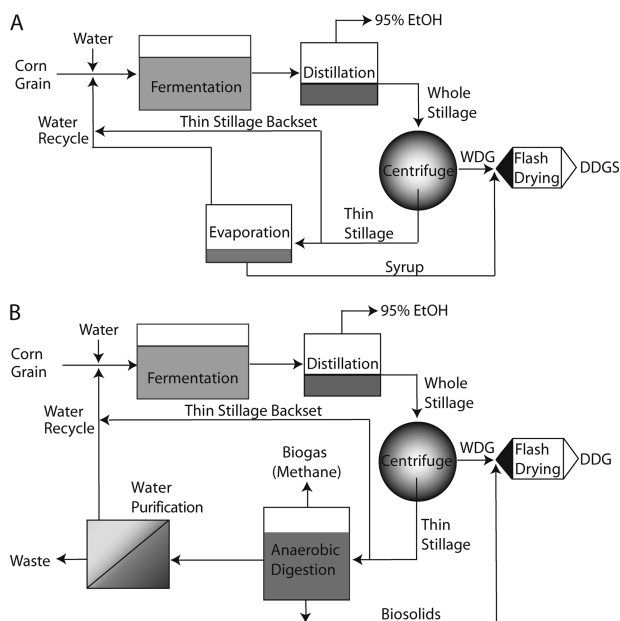


FIGURE 2. Overall process diagram of dry-mill corn grain-to-ethanol processes: (A), conventional facility, and (B), conceptual process diagram with an integrated anaerobic digester (water purification technology and purification requirements are not yet determined).

Mg, P, and O with $16.12\% \pm 0.74$ and $28.93\% \pm 1.58$ (atomic basis) for Mg and P, respectively, which explained the reduction in Mg and P levels in the effluent compared to thin stillage. SEM photographs of the crystals resembled struvite (i.e., $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) (Figure S6A), and the X-ray powder diffraction spectrum of the precipitate was similar to struvite's standard spectra although an amorphous phase peak was present (Figure S6B). We found that the precipitate only contained 0.286 moles of N per mole of precipitate (an equal molar amount is anticipated for crystalline struvite). Because of the relatively low levels of N in the precipitate and a slow accumulation of struvite due to Mg limitations, we were able to close the nitrogen balance without accounting for struvite precipitation in the bioreactors.

Discussion

We operated two similar thermophilic, high-rate anaerobic bioreactors to investigate if anaerobic digestion of thin stillage is a more energetically favorable approach compared to evaporation and syrup drying, which is the conventional process in corn grain-to-ethanol dry mills. A basic schematic of the conventional process of corn grain-to-ethanol fermentation and a conceptual process with an integrated anaerobic digester is shown in Figure 2. We propose a system based on this study that includes thermophilic anaerobic digestion, biomass recovery, and recycling of digester effluent as makeup water. To successfully advance the process technology through this change, the net energy balance ratio must be improved considerably, while recovering nutrients, improving the quality of animal feed, and reducing water consumption.

Digester Methane as an Energy Supplement. The generated methane from thin stillage digestion will partially replace nonrenewable fuels (often natural gas) as energy inputs in the ethanol production process. Using the combined methane potential from R1 and R2 of $0.254 \text{ L CH}_4/\text{g TCOD}$ fed combined with full-scale data, we anticipate a 51% reduction in required energy input from nonrenewable fuels for a $3.8 \times 10^8 \text{ L}$ ethanol per year corn grain-to-ethanol dry mill (see details in S.6). This estimation included a projected reduction

of 45% in the mass of produced animal feed (DDG vs DDGS) and the resulting circumvention of flash drying syrup (with steam from natural gas) (Table S2 and Figure 2). Currently, approximately a third of the total energy input is recovered as waste heat and provides the energy for thin stillage evaporation. Since an integrated anaerobic digestion process would make evaporation of thin stillage redundant, the leftover heat is still available, and therefore a new application for it can be found. However, we have not included this available energy in the calculations for the anticipated 51% energy input reduction because some of this waste heat may be necessary to recover makeup water from digester effluent. Stover et al. (30) found a higher anticipated reduction in energy input of 60% based on methane potential data from Ganapathi (16) due to accounting for the available energy from waste heat by not having to evaporate thin stillage. If these authors had not included waste heat recovery, their anticipated energy input reduction for corn processing would have been 40%. The absence of suspended solids in the thin stillage substrate of the Ganapathi (16) study is one contributing reason for a lower energy input reduction compared to ours. Schaefer et al. (17) published an energy input reduction at the processing plant of 43% based on their methane potential with low-rate thermophilic anaerobic digestion of thin stillage without any correction for the considerable lower mass of produced animal feed. We also estimated a 43% energy input reduction from our data when we did not account for a lower mass of DDG vs DDGS, however, this is an oversimplification, and animal feed quantity reductions should be included in the calculations.

To our knowledge, this is the first report on thin stillage digestion with a high-rate anaerobic digester system. We obtained a shorter design HRT of 10 days and a higher organic loading rate of 7.50 g TCOD/L/d with prolonged stable performance compared to Schaefer et al. (17), who were able to achieve stable operating conditions at a 20-day HRT and an organic loading rate of 6.1 g TCOD/L/d with thermophilic low-rate digesters. The shorter HRT will directly result in smaller reactor volumes, and thus reduced construction costs. Therefore, we anticipate that high-rate anaerobic digestion will be preferential to low-rate digestion and that other high-rate reactor configurations should be evaluated.

Nutrient Recovery. For a practical corn-to-ethanol industry, nutrient recovery for plant growth, yeast fermentation, and animal growth is critical. Direct recycle of up to 50% of thin stillage to the ethanol fermenters (9, 10) will provide nutrients, such as phosphorus (4140 mg/L , Table 2), for yeast metabolism. Subsequently, an integrated anaerobic digestion technology can aid in nutrient recovery because nutrient species are not oxidized and lost to the atmosphere from the process and remain in a closed nutrient cycle, while they are liberated from the VS matrix. Indeed, we were able to obtain a closed nitrogen balance for both bioreactors during formation of total ammonium from protein degradation. Magnesium, phosphorus, and relatively minor amounts of nitrogen were removed from the digester solution by the generation of struvite, which was a mixture of crystalline and amorphous phases, and precipitated out to accumulate on the bottom of the reactor over the operating period. Our results with X-ray powder diffraction were similar to the identification of struvite by Wang et al. (31), including an amorphous region. A close to complete removal of the incoming magnesium was obtained, indicating magnesium to be the limiting species for struvite formation. The precipitated struvite must be removed from a full-scale digester to prevent any operating problems, but it can be used as a slow-release fertilizer for plant growth (32).

Animal Feed Improvement. In the conventional processing scheme of dry mills (Figure 2A), thin stillage is evaporated to syrup, added to wet distiller's grains (WDG),

and flash dried to produce distiller's dried grains with solubles (DDGS), which is used as animal feed. After integrating anaerobic digesters in the dry-mill plant, syrup will no longer be added to WDG (Figure 2B), leaving only distiller's dried grains (DDG) as a higher value animal feed because of increased protein concentrations due to a relatively lower concentration of salts. Long-term effects on animals fed with DDGS may be of concern due to high concentrations of certain elements that stem from syrup (e.g., potassium has a laxative effect and is present at levels of 1.5% in syrup [Table 2]) (8, 15). Our ICP-MS analysis confirmed high levels of both potassium and phosphorus in thin stillage samples (Table 2). By degrading VS from thin stillage in anaerobic digesters, a considerably lower quantity of animal feed (DDG) will be produced albeit at a higher relative protein content. As mentioned above, we anticipate this reduction to be 45% of the total mass of animal feed based on the dry weight of WDG and syrup per quantity of corn grain (Table S2). Currently, animal feed is an important product to ensure economic viability of corn grain ethanol, especially since demand has recently outpaced the increase in supply mostly due to recent growth in exports to China (33). Thus, for anaerobic digestion of thin stillage to be economical, the increased value of a higher quality feed plus the savings due to reduced natural gas consumption must be higher than reduced revenue due to the loss in feed quantity.

Cobalt Augmentation. We discovered that augmentation of the trace element cobalt is necessary for successful long-term anaerobic digestion of thin stillage under thermophilic conditions (S.3). Cobalt is an important factor in enzymatic catalysis of methyl group transfer in methanogenesis (23, 25, 26). We found cobalt levels to be below detection in thin stillage because cobalt is not added as a trace element during yeast fermentation in corn-to-ethanol plants. This is in agreement with Belyea et al. (3), who found that syrup (evaporated thin stillage) contained below-detection levels of cobalt (Table 2). Augmenting cobalt will add cost (~\$0.4 – \$1.20/1,000 kg TCOD), which must be taken into consideration during a full life cycle and economic assessment of the integrated digester system. Codigestion of thin stillage with animal manure would also supplement enough cobalt to sustain long-term anaerobic digestion. For example, cow manure contains levels of cobalt of ~2 mg/kg (dry basis) (34). However, codigestion of thin stillage with animal manure as an integrated technology would result in a digester effluent that is much harder to purify and recycle as makeup water for yeast fermentation. It seems, therefore, an unlikely alternative due to considerably higher water consumption rates at the dry mill.

Outlook. Based on the estimated reduction of 51% for the nonrenewable energy input to process corn grain, the energy input at the facility is anticipated to be 0.30 instead of 0.60 units per unit energy output for ethanol based on published life cycle assessment data (a 45.2% instead of a 62.5% nonrenewable energy requirement for operating the processing facility out of all the energy inputs) (4). Such a decrease in nonrenewable energy input per output would increase the net energy balance ratio from 1.26 to 1.70, which would make corn grain-to-ethanol a more acceptable technology as an existing intermediate step toward lignocellulosic-to-energy technology with a much higher net energy balance. Our calculation included a 45% reduction in animal feed quantity and a resulting loss in energy credit (from 0.203 to 0.112 by using the Hill et al. (4) data) and a 45% lower mass that needs flash drying with nonrenewable energy (17% of nonrenewable fuel in a conventional dry mill goes to flash drying of animal feed (4)). However, the calculation did not include the energetic costs to physically replace the evaporator with the integrated digester system (this will be a relatively small fraction of the energy input

because the percentage of energy input per unit of ethanol energy output for construction of the entire conventional dry mill is 0.2% (4)); the improved quality in animal feed (DDG vs DDGS); nor the available waste heat from circumventing thin stillage evaporation. Since the anaerobic digester will be integrated within the dry mill, all changes will cascade through the plant, affecting water, mass, and energy balances, and to firmly establish the net energy balance ratio, an extensive life cycle and economic assessment should be performed after the changes have become apparent. Further work is necessary to establish the level of purification of recycled anaerobic digester effluent in the dry mill to prevent inhibition of yeast fermentation. Finally, we must assess a new water balance, especially since we project a considerable increase in water reuse (and thus decrease in water consumption) in part due to not releasing water to the atmosphere by circumventing syrup drying.

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Supporting Information Available

This information is available free of charge via the Internet at <http://pubs.acs.org>.

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