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Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks ☆

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Abstract

A technical evaluation of stillage characterization, treatment, and by-product recovery in the ethanol industry was performed through a review of the scientific literature, with particular emphasis on solutions pertinent to a cellulosic-based ethanol production system. This effort has generated substantial information supporting the viability of anaerobic digestion for stillage treatment followed by land application on biomass crops for nutrient recovery. Generally, the characteristics of stillage from cellulosic materials appear comparable to those of conventional sugar- and starch-based feedstocks. However, the data on cellulosic stillage characteristics and treatment parameters are extremely limited and highly variable. This has significant impacts on the capital costs and biogas recovery of anaerobic treatment systems predicted from these data. In addition, technical questions remain unanswered with regard to stillage toxicity from untested feedstocks and the impact of heavy metal leaching when acid hydrolysis reactors are fabricated from corrosion-resistant alloys. Thermophilic anaerobic digestion of ethanol stillage achieves similar treatment efficiencies and methane yields compared to mesophilic treatment, but at almost twice the organic loading rate. Therefore, application of thermophilic anaerobic digestion would improve process economics, since smaller digesters and less stillage cooling are required. Downstream processes for stillage utilization and by-product recovery considered worthy of continued investigation include the production of feed (from single cell protein and/or algae production), color removal, and production of calcium magnesium acetate. This study finds that sustainable and economically viable solutions are available for mitigating the environmental impacts which result from large-scale biomass-to-ethanol conversion facilities. However, further research in some areas is needed to facilitate successful implementation of appropriate technology options.

Keywords: Stillage; Anaerobic digestion; Ethanol production; Cellulosic feedstock; Sugar feedstock; Starch feedstock; By-product recovery; Vinasse; Distillery wastewater; Colour removal

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1. Introduction

Stillage, also termed distillery wastewater, distillery pot ale, distillery slops, distillery spent wash, dunder, mosto, vinasse, and thin stillage, is the aqueous by-product from the distillation of ethanol following fermentation of carbohydrates. The production of ethanol from biomass, whether from sugar crops (sugar beets, sugar cane, molasses, etc.), starch crops (corn, wheat, rice, cassava, etc.), dairy products (whey) or cellulosic materials (crop residues, herbaceous energy crops, bagasse, wood, or municipal solid waste), results in the concurrent production of stillage which exhibits a considerable pollution potential [1,2]. Up to 20 liters of stillage may be generated for each liter of ethanol produced [3] and the pollution potential of stillage can exceed a chemical oxygen demand (COD) of 100 g/L [4]. A medium-sized ethanol facility producing 10^6 L ethanol/yr generates stillage with a pollution level equivalent to the sewage of a city with a population of 500,000 [5].

While large-scale ethanol production from sugar-based and starch-based crops has a considerable history, large-scale ethanol production from lignocellulosic biomass has been limited. However, efforts are underway to improve process economics and to bring cellulosics-to-ethanol conversion into production [6–19]. In contrast to sugar- and starch-based crops, the availability of significant resources of lignocellulosic biomass means that large-scale production of ethanol from lignocellulosic biomass has the potential to replace a major portion of imported liquid fuels [13]. Also, when conversion efficiencies are high, the production and use of fuel ethanol from all biomass sources can reduce greenhouse emissions of CO_2 which would otherwise result from the use of fossil fuels. However, for the production of ethanol to qualify as a sustainable "green energy" process, due consideration for treatment and utilization of the stillage by-product is essential.

An extensive review of the scientific literature was conducted to investigate methods to process and utilize the significant by-product streams associated with ethanol production from conventional and cellulosic feedstocks. A preliminary analysis of ethanol-production wastewater characteristics and treatment revealed a consensus toward anaerobic digestion as an economically viable and sustainable by-product recovery scheme. Therefore, much of this effort focused on examining those aspects of biomass-to-ethanol conversion and effluent characteristics which are expected to impact the technical feasibility of anaerobic treatment. To the extent practicable, an attempt was made to assess the roles of feedstock, hydrolysis method, in-plant recycling, microbial toxicity, by-product recovery (feed and nutrients), reactor type, biogas yield, phytotoxicity and sustainability, in by-product treatment and utilization options.

2. Feedstocks for ethanol production

Conventional feedstocks for the production of ethanol include both sugar-based and starch-based feedstocks, as well as whey from the dairy products industry. The sugar-based feedstocks include crops such as sugar beets and sugar cane, as well as fruit crops such as grapes, and are the most easily fermentable feedstocks. Fresh juices from beets and cane contain reducing sugars which are available to yeast with no pretreatment of the feedstock, other than size reduction and pressing. However, the relatively high market value of sugar has limited implementation of direct conversion to ethanol and, instead, ethanol is often a by-product of sugar production through the fermentation of molasses, also called blackstrap molasses, left over from concentration and precipitation of sugar from the juice [20].

There are several grades of blackstrap molasses depending on the sugar content, ash content, and color. Whereas blackstrap molasses is a by-product of sugar crystallization, high test molasses is a concentration of the virgin juice normally intended for use in food products [21]. High test molasses is often acidified to prevent crystallization of sugars during storage. Unless otherwise stated, we will use the term molasses to mean blackstrap molasses.

Beet molasses and cane molasses are the most common sugar crop-based feedstocks for ethanol production. One other sugar-based feedstock for ethanol production is whey [22], an aqueous by-product of cheese production that contains lactose as the principal sugar. In addition, sweet sorghum contains carbohydrates in fractions of both sugar and starch, and may be considered a sugar-based feedstock due to the sugar fraction.

Starch-based feedstocks include grains such as corn, wheat, rice, barley, and milo (grain sorghum), as well as root crops such as potatoes and cassava. In addition to milling, the conversion of starch-based feedstocks requires an enzymatic hydrolysis step, termed saccharification, to convert the starch to fermentable sugars [23]. Similar to the sugar industry, ethanol can also be produced as a by-product of large wet-milling operations which recover oils, proteins and gluten from grains for food and feed additives, and use the remaining purified starches to produce ethanol [23].

Cellulosic feedstocks for ethanol production include both herbaceous (e.g., grasses) and

woody (softwoods and hardwoods) biomass, as well as industrial wastes (e.g., bagasse, rice hulls, and paper mill sludge) and municipal solid wastes (MSW) of organic origins. The organic fraction of MSW containing waste paper may be a suitable feedstock, as well as refuse derived fuel (RDF), which is a MSW fraction containing mostly paper and plastics. Cellulosic feedstocks typically contain a substantial amount of hemicellulose and lignin, which are bound up in the lignocellulose complex making up the plant fibers. These feedstocks require a more substantial pretreatment to convert the cellulose to fermentable sugars. After shredding, the cellulosic biomass must undergo acid, alkaline, or enzymatic hydrolysis to produce fermentable sugars. Since hemicellulose contains pentose sugars which cannot be utilized by the standard ethanol-producing yeast, *Saccharomyces cerevisiae*, novel organisms must be employed if utilization of these pentoses is desired [24].

3. Ethanol production processes

A successful ethanol production and conversion system that is both economically and environmentally sustainable requires the application of a host of component technologies in a holistic and integrated manner, such that economic risk for the investment is minimized. Fig. 1 displays one schematic representation of an ethanol production system which is classified into four dependent component systems — production, harvesting, storage, and conversion. This study concerns mainly the conversion process. However, conversion may be linked to the production system through the possibility of recovering and utilizing conversion by-products to enhance production efficiency, while providing an economically viable and necessary outlet for by-products which must leave the conversion facility. If the primary output of such a system is the production of liquid fuel ethanol, which leaves the facility in an almost pure state, then every other material input to the conversion facility besides the carbon precursor will eventually require some means of final disposition. Optimal sustainability will occur when each by-product generates maximum value and minimum environmental impact.

Not all of the inputs and losses for the component systems can be completely controlled through process design, although the goal is to minimize the cost of both controllable inputs and losses. For the conversion facility, while minimizing inputs is beneficial for economical ethanol production, there is also a significant incentive to minimize the wasting of "necessary" by-product outputs through treatment and conversion that permits their most valuable utilization.

A more detailed schematic of the unit process steps required to produce ethanol within the conversion facility is depicted in Fig. 2. While some differences exist in the processing of sugar, starch and lignocellulosic feedstocks, many aspects of the ethanol production process remain the same and detailed descriptions can be found elsewhere [25]. Since the total quantity (mass and volume) of the "whole" stillage leaving the distillation column is an order of magnitude larger than any of the other unit process "losses", the focus for minimizing waste at the conversion facility should target by-product recovery from this wastestream. However, since each of the preceding unit processes has a significant impact on the quantity and quality of this stillage wastewater stream, optimal utilization of stillage requires an understanding of how hydrolysis, fermentation and distillation affect the stillage by-product.

4. Pretreatment and hydrolysis

The effect of pretreatment process on stillage characteristics has not been documented. Pretreatment of a feedstock may include mechanical processes (milling and shredding), steam explosion [26,27], steam explosion in the presence of acid [28,29], super-critical explosion by carbon dioxide [30], ammonia freeze explosion (AFEX), solvent delignification (using ethanol, butanol, or acetic acid), and thermal–mechanical processes [18,31]. All of these processes serve to improve access to the substrate for further hydrolytic steps. In the AFEX process [32], the substrate is exposed to ammonia at elevated pressures and

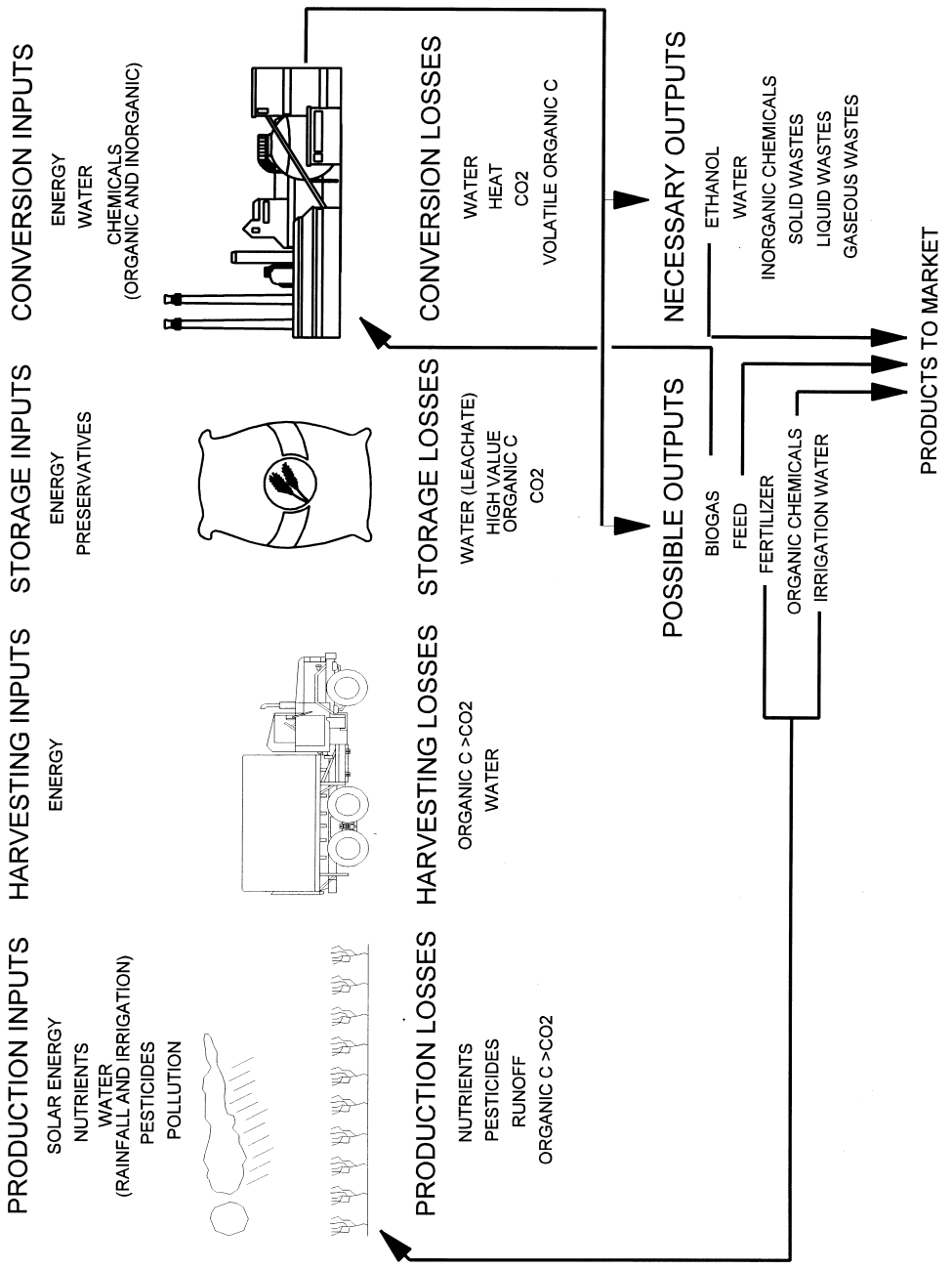

Fig. 1. Schematic representation of an ethanol production system.

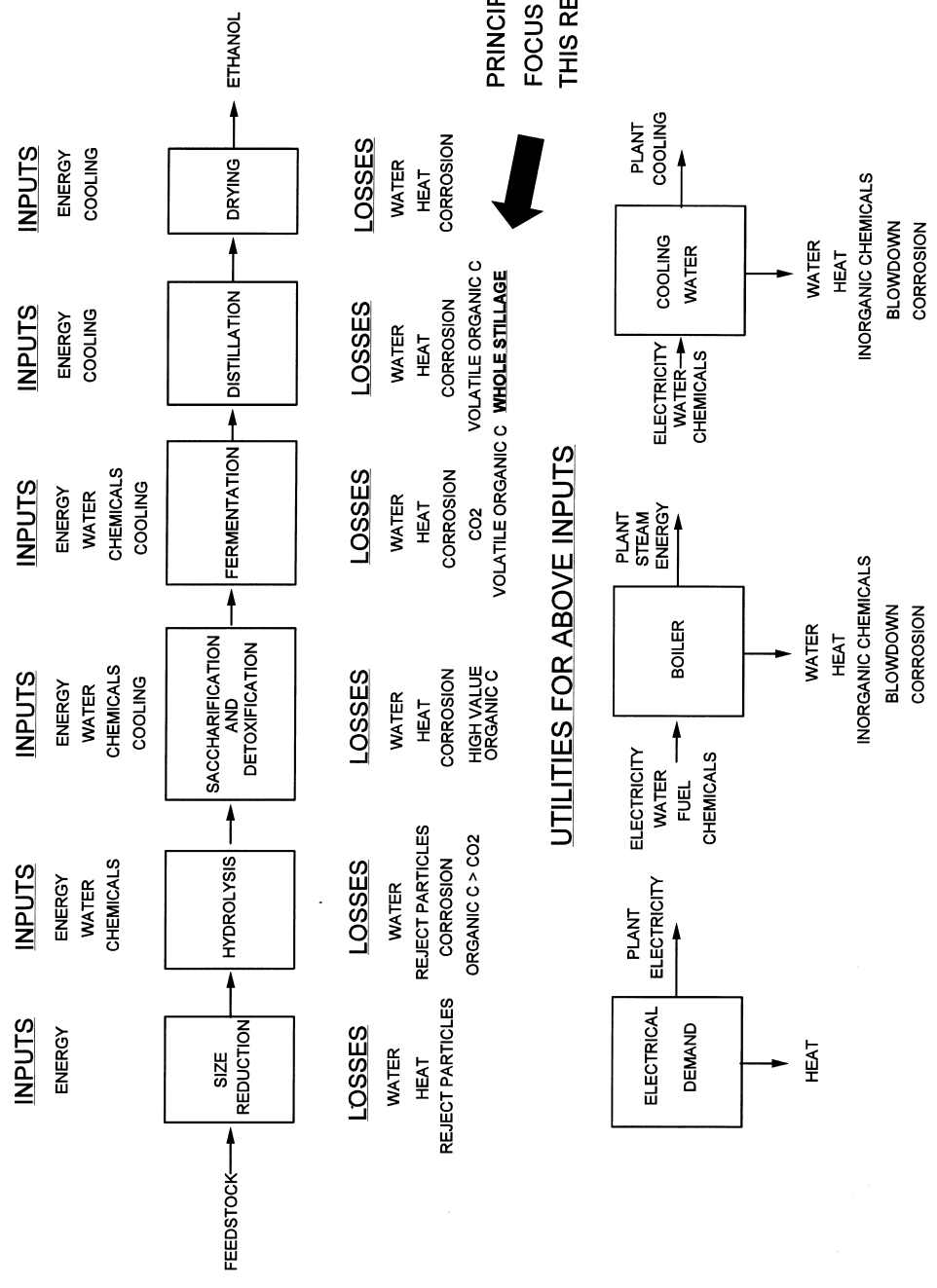

Fig. 2. Ethanol conversion facility: component processes, inputs and outputs.

flashed back to atmospheric pressure to open the cellulose fibers in order to improve enzymatic hydrolysis of the cellulose to fermentable sugars. Much of the ammonia can be recycled except for a fraction remaining on the fibers. Where pretreatments are effected to separate components of the biomass (e.g., bark or pith), it is plausible that such treatments will affect the composition of the fermentation media as well as the final stillage product.

As stated previously, sugar-based feedstocks do not require processing to convert carbohydrates into fermentable sugars. In starch-based feedstocks, a mashing and saccharification process is required to produce fermentable sugars [33]. After milling, the starch-based feedstocks are introduced into a cooker, with make-up water and α-amylase, and heated to 90°C. In this liquefaction process, α-amylase is employed to cleave long starch polymers to dextran. Alpha-amylase requires Ca^{++} for activation and has an optimal pH of 6.6. This is generally achieved by the addition of lime as the Ca^{++} source and additional alkali (usually NaOH) as required to reach the optimal pH, since the pH of most grain-mash is below this optimum. After liquefaction, the mash is cooled to below 60°C and gluco-amylase is added while the pH is lowered to the optimal value of 4.5 for this enzyme [25]. The gluco-amylase enzymes attack the ends of dextran and produce fermentable sugars. At this stage, nitrogen and phosphorous nutrients may be added where the nutrient content of feedstocks is expected to limit fermentation.

Since the pH optima of these two enzymes are different, the salinity of the mash and the final stillage is increased by the salts which are formed as a result of these pH adjustments. The composition of these salts is dependent on the acids and bases employed. The introduction of alkali and acid during mashing and saccharification is an important step for optimization and should receive careful consideration [33]. Minimizing the addition of alkali during mashing will lessen the requirement for acid during saccharification, leading to lower chemical consumption and a lower salinity of final effluent stillage. However, if the time period and pH achieved are insufficient in either step, the presence of unfermentable sugars may increase the COD of the final stillage and thereby impact stillage treatment requirements.

Hydrolysis of cellulosic feedstocks is accomplished through either enzymatic, concentrated acid [34], or dilute acid hydrolysis, or combinations thereof [9]. In enzymatic hydrolysis, extracts of cellulase enzymes obtained from cellulolytic organisms, such as *Trichoderma reesei*, are added to the feedstock, often along with β-glucosidase, to allow conversion of the cellulose to cellobiose and then to individual glucose units [35]. While enzymatic hydrolysis is considered expensive compared to acid hydrolysis, due to the cost of enzymes and the longer time required (days rather than minutes), it possesses the advantage that side reactions which convert some of the carbohydrates in cellulosic feedstocks to non-fermentable sugars are virtually absent.

In acid hydrolysis, the cellulosic feedstock is exposed to concentrated or dilute acids (usually H_2SO_4) at elevated temperatures and pressures for specific time periods to free the hemicellulose and lignin from the cellulose fibers and to hydrolyze the cellulose to glucose [36]. Acid hydrolysis may employ concentrated acids for short periods of exposure or dilute acids for longer periods [37]. A common scheme is to employ a two-stage dilute acid hydrolysis, where the hemicellulose is hydrolyzed to xylose and recovered in the first stage and a more vigorous second-stage hydrolysis is employed for conversion of cellulose to glucose [38]. The two streams may be combined or fermented separately [39]. A consequence of acid hydrolysis is the potential loss of glucose to hydroxymethyl furfural and xylose to furfural in side reactions [18]. In combinational hydrolysis processes, dilute acid hydrolysis or AFEX may be followed by enzymatic treatment to enhance the effect of costly enzyme additions [40]. More complete descriptions of these processes can be found elsewhere [9].

Finally, lignin solids remaining after hydrolysis may cause problems in fermentation when recycling of yeast is desirable. Alkaline (NaOH) or oxidant (H_2O_2) treatments may be employed in

pretreatments to render the lignin to soluble forms [18]. Also, resinous acids and lignin degradation products resulting from acid hydrolysis may be inhibitory to fermentation [41,42], and over-liming prior to fermentation may be employed for detoxification of the mash [36]. The effect of hydrolysis method on stillage characteristics is not documented in the literature.

5. Fermentation

The fermentation process is normally operated as a batch, but the process may also be continuous or partially continuous [43]. In a conventional batch process, an inoculum of yeast culture often close to 10% of the fermenter volume is added to the cooled mash and allowed to ferment to completion, usually in less than 2 days [25]. The volume of stillage which results after distillation is inversely proportional to the concentration of ethanol at the end of the fermentation. Therefore, efforts to assure high ethanol content of the final beer will reduce stillage volume and improve distillation energy consumption and capacity [44]. Also, ensuring that fermentation has reached completion and that residual sugars in the beer are minimized can lower the COD of the resulting stillage. For every 1% of residual sugar (based on glucose), a stillage COD increment of 16 g/L can be expected.

Continuous fermentation with immobilized yeast [45] or recycled yeast [46] is advocated for potentially higher fermenter productivity and ethanol yield, mostly due to a decreased yield of yeast organisms. Any increase in ethanol yield should lower the organic strength of the final stillage, but this may depend on the final disposition of the product yeast. Where yeast is not recovered, continuous fermentation should lower stillage COD, since yeast yield is less than for batch fermentation. Conversely, where yeast is recovered for use in feed products, the impact of continuous fermentation on stillage COD can be expected to be minimal, since the lesser amount of yeast is removed from the stillage. However, continuous fermentation increases the susceptibility to contamination by microorganisms which produce fermentation products other than ethanol, most of which will remain in the stillage and increase stillage COD.

In a partially continuous fermentation, yeast may be partially recovered from the beer prior to distillation and returned to fermentation. The volume of stillage is reduced by the volume of returned yeast [47], but the soluble COD entrained with the yeast increases the COD of the stillage. In a similar manner, when properly used, back-set or stillage recycling (the use of stillage as make-up water for cooking and fermentation) will lower stillage volume [48] but not affect the total amount of COD produced since the stillage strength will be increased by the amount of back-set [49]. An analysis of beet molasses distilleries showed that the use of 30% back-set reduced stillage production from 15.9 to 12.6 L/L ethanol [50]. The use of back-set can reduce consumption of water, steam, and some chemicals, but the accumulation of fermentation products and non-fermentable sugars can inhibit the fermentation process. Therefore, a practical limit of 50% stillage recycling is considered a maximum [25,44,47,48,51–53]. Above this level of stillage recycling, inhibition of the yeast will lower ethanol yield and increase the COD concentration in the stillage beyond that contributed by the recycled stillage alone.

Several different organisms have been proposed for use in fermenting sugars to ethanol, with different strains of the yeast, *Saccharomyces cerevisiae*, being the most widely used due to its robust growth rate and high ethanol tolerance [54]. With proper nutrient and growth conditions, it has been shown that *S. cerevisiae* can tolerate ethanol concentrations up to 23% [54]. There is an interest in the use of thermotolerant yeast in thermophilic ethanol production [55,56], due to the potential for higher fermentation rates and ethanol yields, and the reduced requirements for cooling. Yet, to date, thermophilic fermenting organisms have suffered from low ethanol tolerance, presumably due to leaky cell membranes at the higher temperatures [56].

The bacterium, *Zymomonas mobilis*, has been shown to produce higher ethanol yields due to a lower cell yield, but its lower ethanol tolerance

and lower feed by-product return has limited its widespread application [57]. Difficulty of separation, lower cell yield, and concern for pathogenic contamination in feed have limited the market for bacterial feed additives. In contrast, yeast are more easily separated, generally accepted as safe in feeds, and have an established market [58].

Since *S. cerevisiae* poorly ferments lactose, *Kluyveromyces marxianus* is often employed for fermentation of whey feedstocks [59]. For cellulosic feedstocks containing pentose sugars which are not fermentable by *S. cerevisiae*, the impact of organism selection on stillage COD could be significant since the pentose sugars can amount to 25% of the feedstock carbohydrates [60,61]. Genetically engineered *Escherichia coli, Zymomonas*, and yeast with extended substrate capabilities can utilize both 5-carbon and 6-carbon sugars to produce ethanol [24,62–65]. A significant decrease in stillage COD could be expected from utilizing pentose sugars in the fermentation of cellulosic feedstocks, but this has not been documented.

For cellulosic feedstocks employing enzymatic hydrolysis, saccharification may be aided by the addition of β-glucosidase to the mash to cleave the glucose dimer, cellobiose. Since the activity of β-glucosidase is inhibited by the presence of glucose, the use of saccharification during fermentation, called simultaneous saccharification and fermentation (SSF), is advocated since the fermenting organisms will lower inhibiting glucose concentrations [19,66]. Also, since higher ethanol yields have been achieved using SSF, the resulting stillage should have a lower organic content, although this has not been documented. A simple modification to SSF which was shown to be effective is the periodic application of ultrasound to the fermentation to enhance enzyme contact with the substrate [67].

6. Distillation and dehydration

After fermentation is complete, the beer containing typically 2–12% ethanol is pumped to a continuous distillation process where steam is used to heat the beer to its boiling point in the stripper column [25]. The ethanol-enriched vapors pass through a rectifying column and are condensed and removed from the top of the rectifier at around 95% ethanol. The ethanol-stripped stillage falls to the bottom of the stripper column and is pumped to a stillage tank. With efficient distillation, the stillage should contain less than 0.1–0.2% ethanol but, at times when distillation is not optimal, the stillage may contain a significant ethanol content. For each 1% ethanol left in the stillage, the COD of the stillage is incremented by more than 20 g/L. Due to the potential impact of residual ethanol content, therefore, proper control over distillation can greatly affect the COD of stillage.

The heating of stillage by steam can occur either by direct injection of steam into the bottom of the stripper column or indirectly through a "reboiler" heat exchanger at the bottom of the column [25]. Also true in the cooking process, the direct injection of steam impacts the stillage in two ways. First, the condensed steam adds to the stillage volume and dilutes the contents. In addition, loss of water from the boiler requires the addition of make-up water and increases the blow-down volume from the boiler required to avoid boiler scaling. More boiler feed water use and blow down increases the requirement for boiler chemicals and increases the amount of salts removed in the blow down [68]. Since the blow-down water is normally combined with the stillage, it dilutes the organic content of the stillage and increases the salinity. An analysis at a beet molasses distillery showed that stillage production decreased from 15.9 to 12.7 L/L ethanol when heating was switched from direct steam injection to the use of a reboiler [50].

In order to allow the blending of alcohol with gasoline, the water content must be reduced to less than 1% by volume. Higher water levels can result in the separation of an alcohol/water mixture below the gasoline phase, which may cause engine malfunction when a fuel tank empties. Unfortunately, separation of ethanol from water by distillation is limited to a purity of around 96% due to the azeotropic properties of ethanol/water mixtures. The removal of the water beyond

the last 5% is called dehydration or drying. Traditionally, azeotropic distillation was employed to produce higher purity ethanol by adding a third component, such as benzene, cyclohexane or ether, to "break" the azeotrope and produce dry ethanol [69]. To avoid the illegal transfer of ethanol from the industrial market into the potable alcohol market, where it is highly regulated and taxed, dry alcohol usually requires the addition of denaturing agents which render it toxic for human consumption, and the azeotropic reagents conveniently met this requirement.

Except in the high purity reagent-grade ethanol market, azeotropic drying has been supplanted by molecular sieve drying technology, which is not only more energy efficient but also avoids the occupational hazards associated with the azeotropic chemical admixtures. In molecular sieve drying, the ethanol is passed through a bed of synthetic zeolite with uniform pore sizes which preferentially adsorb water molecules. After the bed becomes saturated, it must be regenerated by heating or evacuating the bed to drive off the adsorbed water. Either liquid or vaporous ethanol can be used, but the dominant and most efficient technology is the vapor-phase "pressure swing" adsorption molecular sieve process [69]. In this case, two molecular sieve beds are placed in parallel with one drying while the other is regenerating. During the regeneration phase a "side stream" of ethanol/water (often around 50%) is produced, which must be redistilled before it can be returned to the drying process. The "bottoms" from side stream distillation is often blended into the stillage, adding to the stillage volume.

7. Stillage production and characterization

The annual production of ethanol from various sugar- and starch-based feedstocks is significant. Production of ethanol in Brazil was 16.2 billion liters in 1997 [70], with 79% produced from fresh sugarcane juice and the balance produced from molasses by-product. In India, 250 distilleries were producing 1.5 billion liters of ethanol in 1995 [71] from sugarcane molasses, with at least 65% of the ethanol used as chemical feedstock. In the US, there were 57 facilities producing an estimated 6.9 billion liters of ethanol in 1999 [72]. Existing feedstocks may support some expansion of production capacity, but significant increases in ethanol production will require the utilization of cellulosic-based feedstocks. Since up to 20 liters of stillage may be generated for every liter of ethanol produced, substantial increases in ethanol production will also require effective solutions for stillage management.

The production and characteristics of stillage are highly variable and dependent on feedstocks and various aspects of the ethanol production process. Wash water used to clean the fermenters, cooling water blow down, and boiler water blow down may all be combined with the stillage and contribute to its variability. However, while the volume and COD concentration of stillage may vary considerably, the total amount of COD produced can be expected to be more consistent with the amounts of feedstock processed and ethanol produced. Table 1 shows yields of ethanol, stillage and COD on the basis of feedstock mass processed and indicates the impact of feedstock on COD produced. Feedstocks yielding higher amounts of ethanol appear to also produce higher amounts of stillage COD, but do not correlate with the COD concentration. Unfortunately, the number of studies which examine stillage production in this manner are limited.

Ignoring stillage production volumes, many studies have examined the characteristics of stillage in terms of its organic strength and nutrient content for various ethanol-producing feedstocks, as shown in Tables 2–6 for sugar beet molasses, for sugarcane juice, for sugarcane molasses, for some additional sugar and starch feedstocks, and for cellulosic feedstocks, respectively. A summary of the data from Tables 2, 3, 4, and 6 is presented in Table 7.

Upon analysis of these values, it is apparent that cane molasses stillage exhibits the highest levels of biochemical oxygen demand (BOD), COD, COD/BOD ratio, potassium, phosphorous

and sulfate, while cane juice stillage exhibits the lowest levels of COD and BOD (Table 7). The concentration of sugars in molasses, through crystallization and evaporation of cane juice, increases the content of non-fermentable organics which remain in the stillage after fermentation, augmenting the COD and increasing the COD/BOD ratio. The high N-content of stillage from barley fermentation (Table 5) stands out and is presumably related to the high protein content in the grain. This level of N is sufficient to produce inhibitory levels of ammonia/ammonium in effluents from barley distilleries [73].

The high sulfate levels of molasses (Table 7) are also noteworthy, and are a result of the type of sulfiting process used in raw sugar production [20]. There is also an instance of high-sulfate cane juice stillage (Table 3) [74], which results from production of direct consumption sugar using a sulfitation process to produce a sugar straight from boiled juice without a second melting and refining step [75]. Such high levels of sulfate can impact further treatment and disposition of stillage.

Organic components of stillage have been studied by several researchers [76–78]. The principal low molecular weight components of cane molasses stillage were found to be lactic acid, glycerol, ethanol and acetic acid, while whey stillage also contained lactose, glucose, arabinitol, and ribitol [77]. Trace amounts of amino acids were found in all stillages tested, with corn stillage containing high levels of alanine and proline [77]. A comparison of barley- versus wheat-based stillage found higher levels of most amino acids in the crude protein of barley stillage but lower levels of crude protein on a stillage dry matter basis compared to wheat stillage [79]. Carbon-13 nuclear magnetic resonance and diffuse reflectance Fourier-transform infrared spectroscopy studies of cane molasses stillage suggested the presence of a fulvic acid (FA) component com-

Table 1
Stillage production from various feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Ethanol production capacity 10^6L/yr	Ethanol yield L/kg feedstock	Stillage yield L/kg feedstock	BOD (COD) g/L	COD yield kg/kg feedstock	COD yield kg/L EtOH	References
Beets fresh and molasses	18.8	0.02	0.22	38 (65)	0.014	0.70	Holmes and Sane [199]
Cane molasses	nd	0.32	3.8	nd (nd)	nd	nd	Chamarro [44]
Cane molasses	50	0.21	2.52	45 (113)	0.28	1.33	Barnes and Halbert [102]
Cane juice	24	0.067	1.33	12 (25)	0.03	0.45	van Haandel and Catunda [3]
Corn	7–70	0.379	6.29	37 (56)	0.349	0.92	Loehr and Sengupta [95]
RDF/CA (dry wt basis)	nd	nd	5.5	37.7 (104)	0.572	nd	Broder [200]
RDF/DA (dry wt basis)	nd	nd	3.8	31.1 (110)	0.418	nd	Broder [200]
Softwood (*Pinus radiata*)	nd	0.25	20.4	13.2 (25.5)	0.52	2.05	Callander et al. [1], Mackie et al. [36]
Whey	2.0	0.012	0.02	5.4 (nd)	nd	nd	Barry [201]
Whey	nd	0.021	0.21	15 (nd)	nd	nd	Singh et al. [202]

[a] nd = no data; CA = Concentrated acid; DA = Dilute acid; RDF = Refuse derived fuel.

Table 2
Stillage characterization for sugar beet molasses feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Stillage yield L/L EtOH	BOD (COD) g/L	N (total) mg/L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH	References
Beet molasses	11.8	27.5 (55.5)	4750	nd	5560	3500	4.3	Vlissidis and Zouboulis [203]
Beet molasses	nd	nd (115.8)	56	175	nd	1042	6.69	Boopathy and Tilche [204]
Beet molasses	nd	69.3 (147)	2700	222	14500	5800	5.5	Basu [205]
Beet molasses	11.8	nd (72)	7340	91	nd	4520	nd	Vlyssides et al. [162]
Beets fresh and molasses	11.3	38 (65)	3000	nd	nd	nd	4.9	Holmes and Sane [199]

[a] nd = no data.

parable to FA extracted from soils and sewage sludge, though exhibiting a higher C/N ratio [76].

Other important characteristics of stillage include color, heavy metals content and the presence of organic priority pollutants. Highly colored effluents can have negative environmental impacts if released into surface waters, where they may disrupt the growth of normal aquatic flora. Phenolics (tannic and humic acids) from the feedstock [80], melanoidins from Maillard reaction of sugars with proteins [75], caramels from overheated sugars [75], and furfurals from acid hydrolysis [81] can contribute to the color of the effluent. In addition, these compounds are known to be inhibitory to fermentation, to rumen microbes [82,83], as well as to biological treatment of the stillage [84–86]. Also, melanoidins are known to be mutagenic [87,88].

Heavy metals have been detected in effluents from ethanol production facilities [89]. Specifically, chromium, copper, nickel and zinc were found at levels significantly above detection limits in effluents from several ethanol facilities. Also, high levels of copper (>150 mg/L) have been found in stillage from cherry/raspberry feedstocks due to the addition of $CuSO_4$ to the mash to bind cyanide in order to meet spirit standards [90]. While some heavy metals may be introduced from the feedstock and chemicals used, corrosion of piping, tanks, and heat exchangers is expected and may contribute to heavy metal levels in the effluent. Processing equipment used in acid hydrolysis is often made of corrosion-resistant alloys [38] to withstand the high temperature and acidic conditions of hydrolysis. Heavy metals contained in these alloys may leach into the feedstock during hydrolysis, resulting in detectable levels in the stillage. However, we found no studies addressing heavy metal levels in hydrolysis stillage.

Organic priority pollutants, including chloroform, methylene chloride, pentachlorophenol, and phenol, were found in wastewaters from at least 2 of 13 ethanol production facilities sampled [89], but no source for these compounds was identified. Since, in the US, large corn-processing plants may only produce ethanol when the demand for high-fructose corn syrup is low (in the winter months), idled ethanol-dehydrating equipment may be employed for drying other industrial chemicals, including organic priority pollutants [91]. Side streams from regenerating the molecular sieves must be redistilled and the chemical-based still bottoms is often combined with ethanol stillage, introducing priority pollutants into the stillage [91]. The presence of organic priority pollutants in stillage is atypical and is not expected when separation processes are not intermingled with other chemical processing.

Generally, the characteristics of stillage from cellulosic materials appear comparable to those of conventional feedstocks (Table 7) and, therefore, methods of stillage treatment and utilization applied to conventional feedstocks should also be applicable to cellulosic feedstocks. Two possible exceptions to the similarity of cellulosic and conventional stillage characteristics which deserve

attention are the potential for higher levels of heavy metals from acid hydrolysis processes and the presence of unusual inhibitors, such as hardwood extractives [92], associated with phenolic compounds present in the feedstock.

8. Stillage treatment and utilization

Solutions for the treatment, utilization and disposal of stillage have been reviewed [2,48,93–95], but the role of anaerobic digestion in stillage treatment received minimal attention in these previous reviews. An early means of treatment and disposal included evaporation of the stillage, neutralization with alkali, followed by incorporation into road building materials [93]. While the fertilizer value of molasses stillage was well recognized, British Guiana banned field application to eliminate odor problems [93]. This led to a process of evaporation followed by incineration in the bagasse furnace, with the ash returned to the fields for fertilizer recovery [93]. From 1926 to 1942, more than 12 patents were issued in the US and UK on processes for treating stillage, including charcoal production, pyrolysis, and various means of fertilizer production [93].

8.1. Physical/mechanical separation

Fig. 3 illustrates the principal stillage treatment technology and utilization options. First, physical/mechanical separation can be applied to the stillage to recover and remove suspended solids containing yeast and other materials. For whole grains (corn), the separated solids can be dried and sold as a high-value animal feed called dried distillers grains (DDG) [96]. The presence of unfermented suspended materials facilitates this recovery process. For sugar crops and cellulosic crops, the separation of suspended solids proves more difficult. Following mechanical treatment, a host of technologies exists for further processing, including evaporation and/or membrane separation, single cell protein production, and anaerobic digestion.

8.2. Evaporation and membrane separation

With evaporation, the stillage is concentrated to a syrup in multi-effect evaporators with the

Table 3
Stillage characterization for sugar cane juice and mixed cane juice/cane molasses feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Stillage yield L/L EtOH	BOD (COD) g/L	N (total) mg/L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH	References
Cane juice	20	12 (25)	400	200	800	nd	3.5	van Haandel and Catunda [3]
Cane juice	nd	15 (22)	400	58	nd	400	3.5	Driessen et al. [206]
Cane juice	nd	16.5 (33)	700	91	1742	760	3.7–4.6	Costa et al. [207]
Cane juice	nd	20 (nd)	nd	nd	nd	nd	3.7–5.9	Barnes and Halbert [102], Willington and Marten [208]
Cane juice	nd	nd (26.0)	1190	320	2100	1470	3.9	Callander and Barford [74]
Cane juice + molasses	nd	19.8 (45)	710	87	3817	3730	4.4–4.6	Costa et al. [207]
Cane juice + molasses	12.5	nd (31.5)	370	24	1300	420	3.9	Souza et al. [209]

[a] nd = no data.

co-production of evaporator condensate which is lower in organics (COD < 10 g/L) and almost devoid of inorganic salts. In whole grain-to-alcohol production, the syrup of concentrated stillage is mixed with DDG and further dried to a saleable product called dried distillers grains and solubles (DDGS) [97]. In the cane molasses ethanol industry, this syrup is sold as a low-value feed additive called "condensed molasses solubles" [20] which is typically high in potassium, limiting its use in feed formulations due to the laxative effect [98].

While evaporation serves to concentrate stillage components into a smaller volume, the significant energy required to evaporate the stillage (equivalent to 10% of the energy content of the ethanol) can negatively impact the energy balance of ethanol production [96]. Also, while the evaporator condensate is significantly lower in organic content than stillage, it still contains volatile organics including ethanol, acetic acid, and formaldehyde. The use of evaporator condensate for make-up water in the cooking process is possible. This can allow for higher levels of water recycling than achieved using 50% stillage back-set, but a build up of inhibitors prevents 100% water recycling [99]. Evaporator condensate has also been used for both boiler and cooling make-up water,

Table 4
Stillage characterization for cane molasses feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Stillage yield L/L EtOH	BOD (COD) g/L	N (total) mg/L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH	References
Cane molasses	nd	25 (65)	1610	127	6497	6400	4.2–5.0	Costa et al. [207]
Cane molasses	16	25.8 (48)	820	157	nd	nd	4.4	de Menezes [48]
Cane molasses	nd	27 (88)	2000	nd	nd	4000	4.3–4.6	Shrihari and Tare [210]
Cane molasses	nd	30 (120)	1600	61	1920	4600	4.1	Harada et al. [211]
Cane molasses	nd	32 (nd)	205	6.8	nd	nd	4.6	Sahai et al. [212]
Cane molasses	nd	35.7 (77.7)	1780	168	8904	4360	4.2	Sheehan and Greenfield [2]
Cane molasses	13–15	39 (100)	1030	33	7000	9500	3.4–4.5	Driessen et al. [206]
Cane molasses	nd	40 (nd)	345	38.8	nd	69.5	4.4	Srivastava and Sahai [213]
Cane molasses	nd	40 (80)	nd	45	4013	nd	4.5–5.0	Silverio et al. [214]
Cane molasses	12	45 (113)	nd	nd	nd	nd	4.8	Barnes and Halbert [102], Willington and Marten [208]
Cane molasses	12	45 (130)	1000	130	nd	nd	4.5	Yeoh [4]
Cane molasses	nd	48 (nd)	382	10.4	nd	67	4.1	Sahai et al. [215]
Cane molasses	15	50 (108)	nd	nd	8298	4700	4.5	Lele et al. [5]
Cane molasses	20	60 (130)	2500	200	nd	3000	4.8	Halbert and Barnes [165]
Cane molasses	nd	60 (98)	1200	1500	1200	5000	3.8–4.4	Goyal et al. [216]
Cane molasses	nd	nd (68.9)	nd	nd	4484	1640	4.72	Espinosa et al. [217]
Cane molasses	nd	nd (66)	nd	nd	nd	nd	4.5	Calzada et al. [138]
Cane molasses	10	nd (75)	975	20	nd	nd	4.4	Garcia Garcia et al. [218]
Cane molasses	nd	nd (100)	2500	300	1750	700	4.6–5.1	Sanchez Riera et al. [219]
Cane molasses	13	nd (22.5)	1192	247	nd	nd	5.2	Cho [220]
Cane molasses	nd	27.5 (65)	750	nd	10370	nd	4.2–4.5	Sen and Bhaskaran [221]
Cane molasses	nd	41 (118)	1135	nd	5070	4200	3.5–3.7	Damodara Rao and Viraraghavan [190]
Cane molasses	nd	nd (24.6)	812	29	1980	607	4.17	Casarini et al. [222]
Cane molasses (rum)	nd	42 (105)	1450	100	nd	4000	4.0–5.0	Szendrey [223–225], Szendrey and Dorion [226]
Cane molasses (stored)	nd	27.5 (64.0)	1300	nd	nd	2800	4.5–5.5	de Bazua et al. [120]

[a] nd = no data.

but the acidity may cause problems in the boiler and the organics often result in excessive slime growth in the cooling system, which lowers heat exchanger efficiencies. Finally, the evaporator condensate can undergo aerobic or anaerobic biological treatment, if required nutrients and buffers are added [100].

Membrane separation has also been employed for concentration of stillage and recovery of permeate for recycling in cooking and mashing

Table 5
Stillage characterization for other sugar and starch feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Stillage yield L/L EtOH	BOD (COD) g/L	N (total) mg/L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH	References
Agave tequilana (tequila)	10	nd (66.3)	nd	nd	290	880	3.4	Ilangovan et al. [227]
Apple/pear	nd	22 (48.9)	380	62	nd	nd	3.4	Robertiello [228]
Banana	nd	nd (53.7)	1530	150	3830	nd	nd	Hammond et al. [229]
Barley spirits (shochu)	1.5	83 (97)	6000	nd	nd	nd	3.7–4.1	Kitamura et al. [73]
Barley and sweet potato	nd	nd (29.5)	nd	9.1	nd	1370	4.2	Shin et al. [230]
Cassava	16	31.4 (81.1)	650	124	nd	nd	3.5	de Menezes [48]
Cherry (morello)	nd	nd (80.0)	nd	nd	nd	34	3.5–4.0	Stadlbauer et al. [90]
Cherry/raspberry	nd	nd (60.0)	nd	nd	nd	1975	2.7–2.9	Stadlbauer et al. [90]
Corn (thin stillage)	nd	26.9 (64.5)	755	1170	nd	nd	3.3–4.0	Ganapathi [231]
Corn (thin stillage)	nd	43.1 (59.4)	546	228	nd	299	nd	Dahab and Young [232]
Figs	nd	20.4 (35.4)	880	170	nd	900	3.6	Vlissidis and Zouboulis [203]
Grapes (cognac)	nd	nd (26)	nd	nd	800	nd	3.0–3.2	Henry et al. [233]
Grapes (wine)	nd	nd (30)	450	65	nd	250	3.5–4	Driessen et al. [206]
Grapes (wine)	nd	nd (40.0)	nd	130	nd	nd	3.8	Borja et al. [234]
Grapes (wine)	nd	16.3 (27.5)	650	nd	nd	120	4.2	Vlissidis and Zouboulis [203]
Pear	nd	nd (47.5)	nd	nd	nd	157	3.4–3.8	Stadlbauer et al. [90]
Potato	nd	nd (52.0)	2100	nd	nd	nd	4.8	Temper et al. [235]
Potato	nd	nd (39.0)	1000	430	4000	nd	nd	Wulfert and Weiland [236]
Milo (thin stillage)	nd	34.9 (75.7)	nd	1280	nd	nd	2.5–4.0	Stover et al. [237], Ganapathi [231]
Milo (thin stillage)	nd	40.4 (45.5)	nd	nd	nd	nd	4.1	Hunter [238]
Raisins	nd	30 (57.5)	750	220	nd	480	3.2	Vlissidis and Zouboulis [203]
Raisins (raki)	nd	nd (14.0)	250	50	nd	nd	3.9	Eremektar et al. [239]
Raspberry	nd	nd (70.0)	nd	nd	nd	37	2.9–3.8	Stadlbauer et al. [90]
Rice spirits (shochu)	nd	25 (50.9)	nd	129	nd	nd	3.5	Yang and Tung [240], Yang [241]
Rice spirits (shochu)	1.5	84 (nd)	nd	389	nd	nd	4.26	Kida et al. [118]
Sweet potato (shochu)	nd	14.2 (30.7)	1200	140	nd	nd	4.5	Nagano et al. [242]
Sweet sorghum	16	46.0 (79.9)	800	1990	nd	nd	4.5	de Menezes [48]
Wheat (shochu)	nd	25.9 (50.1)	1500	170	nd	nd	4.6	Nagano et al. [242]
Whey	1.7	5.4 (nd)	nd	nd	nd	nd	nd	Barry [201]
Whey	nd	15 (nd)	nd	nd	nd	nd	nd	Singh et al. [202]

[a] nd = no data.

[101]. While energy consumption is less than for evaporation, membrane fouling is problematic [102,103] and low molecular weight organics still pass through the membranes, eliminating the potential for 100% water recycling in the ethanol production process [104]. Membrane separation could also be applied to the evaporator condensate but, since this stream only contains low molecular weight organics, separation efficiencies would not be sufficient to remove fermentation inhibitors.

8.3. Single cell protein production

A potentially viable use of stillage is for single cell protein (SCP) production [99], where a second aerobic culture is employed to remove residual sugars and soluble proteins in the stillage and lower the COD and nutrient content [105]. Also, a portion of the stillage can be used to produce inoculum for ethanol production. Finally, the sludge from biological treatment of stillage could be processed into feed materials [106,107].

Five different filamentous fungi were grown on rum stillage, resulting in a COD reduction of up to 60% with *Gliocladium deliquescens* performing best [108]. Several species of *Candida* were grown on molasses stillage along with various additives and the best protein and biomass production occurred using *Candida krusei* with a phosphoric

Table 6
Stillage characterization for cellulosic feedstocks (values are calculated from data in literature sources)[a]

Feedstock/Process	Stillage yield L/L EtOH	BOD (COD) g/L	N (total) mg/L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH	References
Eucalyptus/DA	nd	nd (22.5)	200	40	nd	260–360	5.8–6.3	Good et al. [243]
Hardwood/TS-DA	nd	nd (19.1)	2800	74	nd	900	nd	Strickland et al. [244]
Hardwood (willow)/ SE-Enz	nd	19.8 (33.3)	nd	nd	nd	nd	nd	Larsson et al. [12]
Mixed (herbaceous)/ nd	nd	56.2 (140)	nd	nd	nd	602	nd	CH2M Hill [245][b]
Mixed (biomass)/nd	nd	46.8 (119)	nd	nd	nd	617	nd	CH2M Hill [245][b]
Mixed (softwood)/nd	nd	26.7 (72.0)	nd	nd	nd	589	nd	CH2M Hill [245][b]
MSW/TS-DA-SF	nd	32.1 (72.0)	140	nd	nd	nd	5.5	Broder [200]
MSW/nd	nd	20.9 (61)	nd	nd	nd	599	nd	Larsson et al. [12]
Pinus radiata/DA-SF	16.7	13.2 (25.5)	95.3	10.3	38.5	600	4.5–5.0	LFTB [246], Callander et al. [1]
RDF/CA	nd	37.7 (104)	13760	14.0	nd	nd	5.0	Broder [200]
RDF/DA	nd	31.1 (110)	2100	0.68	nd	nd	5.9	Broder [200]
RDF/TS-DA-SF	nd	nd (38.1)	nd	nd	nd	nd	5.5	Broder and Henson [247]
RDF/nd	6.7	6.5 (nd)	nd	nd	nd	nd	nd	DiNovo et al. [168]
Softwood (spruce and pine)/SE-Enz	nd	12.8 (26.5)	nd	nd	nd	nd	nd	Larsson et al. [12]
Timothy grass/SE	6–15	nd (50)	2100	nd	nd	nd	4.5–5.0	Belkacemi et al. [159]
Timothy grass/AFEX	6–15	nd (26)	1100	nd	nd	nd	nd	Belkacemi et al. [248]

[a] nd = no data; AFEX = Ammonia freeze explosion; CA = Concentrated acid; DA = Dilute acid; MSW = Municipal solid waste; RDF = Refuse derived fuel; SE = Steam explosion; SE-Enz = Steam explosion and enzymatic hydrolysis; SF = Saccharomyces fermentation; TS = Two stage.

[b] CH2M HILL (1991) values are predicted estimates.

acid addition [109]. A mixed culture of *Geotrichum candidum, C. krusei*, and *Hansenula anomala* was used to reduce the COD of whiskey stillage by 54.9%, which was higher than achieved by any of the organisms in pure culture [110]. Cultivation of pure and mixed cultures of *Aspergillus niger, Penicillium fellutanum*, and *Mucor hiemalis* on cane molasses resulted in an optimal process using a spore inoculum of 70% *A. niger* and 30% *P. fellutanum* [111]. Beet molasses stillage was used to propagate a mixed culture of both *Trichosporon* and *Candida* species in continuous culture, resulting in a 70% COD reduction at a loading of 66 g COD/L/day [112].

A two-staged culture of beet molasses, with *H. anomala* J 45-N-5 followed by an unknown soil yeast isolate I-44, resulted in an overall organic carbon reduction of 75% [113]. A two-staged culture of sugarcane molasses stillage, by *Candida utilis* followed by *Paecilomyces variotii*, resulted in a COD reduction of 92% [114]. Cane molasses stillage was also used to produce *C. utilis* var. major NRRL 1087, where large-scale production (7000 L) was prone to bacterial contamination which could be controlled by lowering the media pH [115]. A thermotolerant strain of *Candida rugosa* was found to achieve a higher rate of COD reduction at 40°C than at lower temperatures and this higher temperature also improved flocculation of the yeast, which would improve the economics of recovery [116,117]. Beet molasses stillage was also used in the cultivation of a *Hansenula* sp., isolated from stillage effluent, resulting in a 35.7% COD reduction and the amino acid profile of the biomass compared favorably with other food protein sources [118,119]. Shochu stillage was used to cultivate *Aspergillus awamori* var. *kawachi* which resulted in almost 50% reduction in organic carbon and improved the rate of anaerobic treatment of the resulting filtrate [120,121].

The use of SCP grown on malt whiskey stillage as an aquaculture feed has been studied [122]. A mixed culture of *G. candidum, C. krusei*, and *H. anomala* was substituted for casein protein in diets of rainbow trout and up to 50% of the protein could be replaced using the mixed culture without affecting growth. However, the N-utilization was less for the SCP-amended feed and amino acid supplementation did not improve N-uptake. In another study, *C. utilis* was found to be a suitable protein source for rainbow trout but the yeast was not grown on stillage wastes [123]. *C. utilis* grown on cane molasses stillage (rum) has also been used in laying hen diets and, though it proved to be inferior to soy protein, was found to give adequate performance at a 10% level in the feed [124]. Ultimately, the econ-

Table 7
Summary of stillage characterization for beet molasses, cane juice, cane molasses, and cellulosic feedstocks[a]

Feedstock		Stillage yield L/L EtOH	BOD g/L	COD g/L	COD/ BOD	N (total) mg/ L	P (total) mg/L	K mg/L	Total S as SO_4 mg/L	pH
Beet molasses	— Average	11.6	44.9	91.1	1.95	3569	163	10030	3716	5.35
	— std dev	0.3	21.7	38.9	0.21	2694	66	6322	2015	1.02
	— *n*	3	3	5	3	5	3	2	4	4
Cane juice	— Average	16.3	16.7	30.4	1.96	628	130	1952	1356	4.04
	— std dev	5.3	3.4	8.2	0.35	316	110	1151	1396	0.49
	— *n*	2	5	6	4	6	6	5	5	7
Cane molasses	— Average	14.0	39.0	84.9	2.49	1229	187	5124	3478	4.46
	— std dev	3.3	10.8	30.6	0.57	639	350	3102	2517	0.35
	— *n*	7	19	22	16	20	17	12	16	25
Cellulosics	— Average	11.1	27.6	61.3	2.49	2787	28	39	651	5.35
	— std dev	4.14	15.2	40.0	0.54	4554	30	nd	122	0.53
	— *n*	4	11	15	10	8	5	1	6	7

[a] nd = no data; std dev = standard deviation; *n* = number of literature values used.

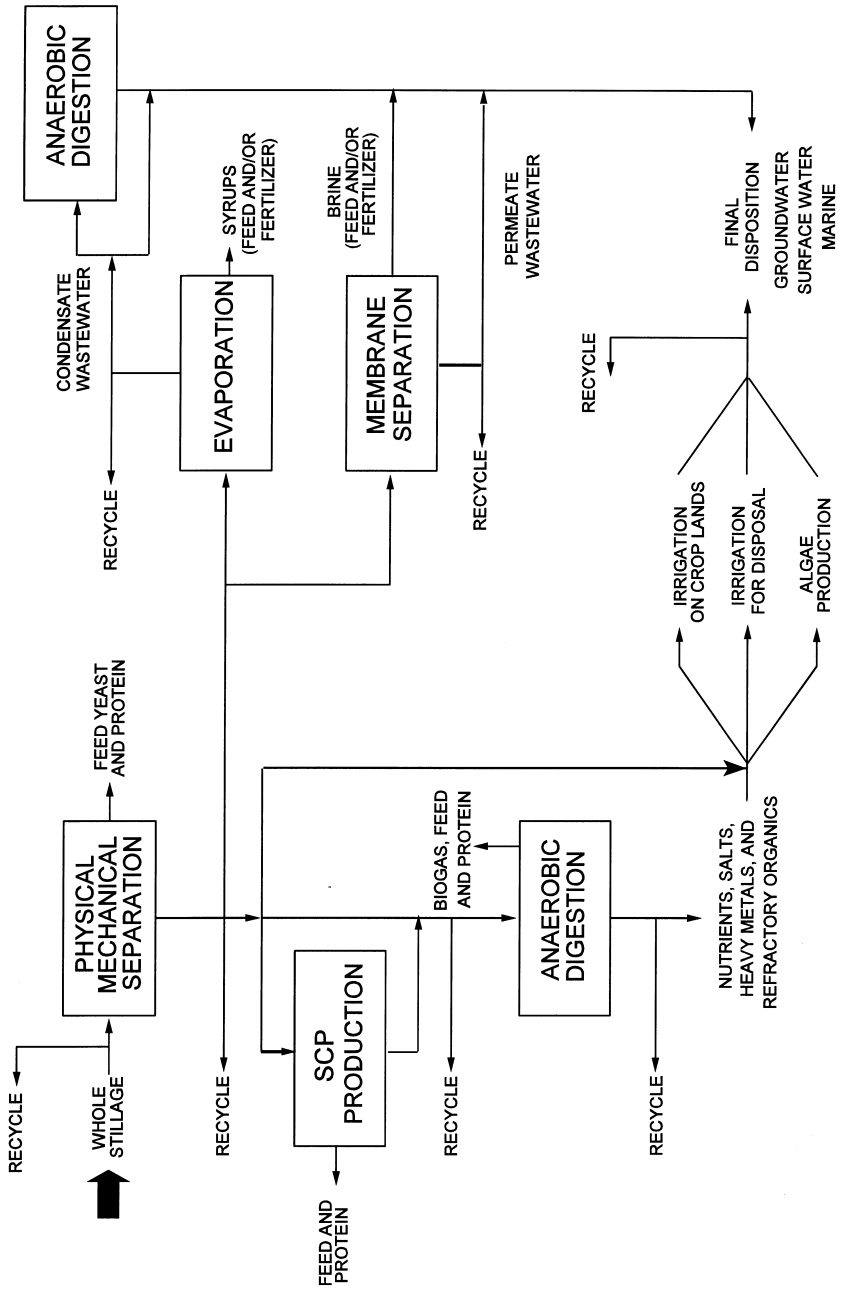

Fig. 3. Stillage treatment technology and utilization options.

omics of SCP production will be highly dependent on the market for SCP.

8.4. Calcium magnesium acetate

The production of organic acids from stillage for sale in the industrial chemical market has received recent attention [125]. In North America, the use of NaCl for winter time de-icing of roads and bridges is known to cause environmental degradation and to enhance corrosion rates of structures and vehicles, resulting in significant economic loss. Calcium magnesium acetate (CMA), as well as potassium acetate, are considered the most suitable substitutes for de-icing salt as they produce less environmental damage and are less corrosive. The market for CMA could grow significantly as restrictions on the use of road salt are mandated. One means of CMA production is through the fermentation of carbohydrates by *Clostridium thermoaceticum* followed by the precipitation and recovery of the organic acids, of which acetic acid is the major end-product. To lower the cost of nutrients required in the fermentation, the use of stillage has been investigated and found to be as effective as more expensive media additives [125–127].

8.5. Other bioproducts

The use of stillage for the production of potentially viable biological products including enzymes, chitosan, astaxanthin, plant hormones and the biopolymers, alternan and pullulan, has been studied. Shochu stillage was used to produce *A. awamori* var. *kawachi* for fodder, which also produced an effective saccharifying enzyme [128]. Shochu stillage was also employed in the production of both a protease, using *Aspergillus usami* mut. *shirousami* [129], and chitosan using *Gongronella butleri* which resulted in a 49% COD reduction [130]. Astaxanthin, a staining agent and quencher, has uses in both food processing and medical diagnostics, and its production by *Phaffia rhodozyma* was enhanced by supplementation of the media with molasses stillage [131]. A 1:4 dilution of stillage was found to enhance the production of the plant hormones, gibberellic acid, abscisic acid, indole acetic acid, and cytokinin by both *Funalia trogii* and *Trametes versicolor* [132]. Finally, condensed solubles from wet-milled corn stillage was used to supplement the media for *Leuconostoc mesenteroides* for the production of the biopolymer, alternan, which has uses in foods and cosmetics [133], and a similar substrate was also used for the production of pullulan by *Aureobasidium* [134].

8.6. Anaerobic digestion

Anaerobic digestion can serve as an effective means for removing COD from stillage and converting it to biogas, which is a readily usable fuel for the ethanol facility. This treatment option is examined in more detail in the next section. While sugar mills have bagasse in excess of fuel requirements [135], generation and sale of electricity can allow complete utilization of the bagasse by-product [136], as well as the biogas from anaerobic digestion of stillage. In addition, while sludge production from anaerobic digestion is low compared to that produced using aerobic treatment, the high COD of stillage will result in anaerobic sludge production which could be processed into feed materials [121,137]. The nutrients contained in the stillage are generally conserved through anaerobic digestion. After the majority of the organic content of the stillage has been removed by anaerobic digestion, only refractory organic compounds and inorganic compounds remain, including plant macro-nutrients (N, P, and K), plant micro-nutrients (Fe, Zn, Mn, Cu, and Mg), and nonessential metals. The application of anaerobic digester effluents to croplands returns these nutrients to a productive nutrient cycle. However, these nutrients may cause environmental degradation if over-applied to crops, or if the treated stillage is discharged into surface or marine waters [48].

8.7. Algae production

One potential means for removing the most environmentally detrimental of these nutrients (N and P) is via the growth of algae on the treated effluent [138,139]. *Spirulina platensis* is most often

considered for nutrient polishing of effluents due to its high productivity, ease of harvesting, and potential market as an animal feed supplement. *Spirulina maxima* has been grown satisfactorily on dilutions of stillage while reducing the COD by 74% [140]. However, unless the final disposition is to marine waters, the high sodium requirements for Spirulina production could damage some soil and surface water ecosystems. *Chlorella vulgaris* has also been used for nutrient removal and to measure the stimulative effect of stillage on algae growth [141]. *Chlamydomonas reinhardii* growth was found to be stimulated by additions of 5% molasses stillage, with levels over 10% causing decreases in growth [142]. Also, algae may have potential in the removal of heavy metals from distillery effluents [143].

8.8. Color removal

Reducing the color of stillage, in addition to COD reduction and nutrient removal, may be required to allow the discharge of treated effluents into surface waters without degrading water quality. Highly colored wastewater can reduce the penetration of solar energy into shallow waters, which is required by aquatic plants for maintaining oxygen levels through photosynthesis. These colored effluents can cause death and decay of aquatic plants, which then contribute to oxygen demand and cause eutrophication. Color may be measured after removal of suspended solids and appropriate dilution and compared with a platinum–cobalt standard, but most of the work on color in stillage has relied merely on spectrophotometric absorption at a wavelength of 475 nm. While the removal of color may not be required for land application of stillage effluents, some facilities may not have adequate land area and must discharge effluents into surface waters.

Methods studied for color removal of stillage effluents include flocculation and coagulation, photocatalytic color removal, and microbial color removal by bacteria and fungi. A polymer of ferric-hydroxy-sulfate was used in the flocculation and coagulation of both fresh and anaerobically digested cane molasses stillage resulting in 32 and 87% reduction in absorbance at 475 nm, respectively [144]. In another study, alum, commercial inorganic flocculants, and commercial cationic polymers, were all capable of 86% color removal (absorbance at 475 nm) of anaerobic–aerobic treated molasses stillage, while less than 3% color removal was obtained for raw effluent [145]. Photocatalytic color removal after anaerobic treatment of stillage was shown to be effective [146], where digested cane molasses stillage (rum) exhibited a range of 75,000–100,000 Pt–Co color units (456 nm). A 10% dilution of this treated stillage was required to allow UV light penetration and, using a titanium dioxide catalyst, 99% color removal occurred within 1 day.

Microbial color removal has received considerable attention. An unknown bacterial soil isolate capable of agar liquefaction was found to remove, under anaerobic conditions, 71% of the color (absorbance at 475 nm) from anaerobically digested cane molasses stillage, while raw stillage underwent only 50% color removal [147]. An aerobic soil bacterial isolate from an Indian distillery resulted in a 36.5% color removal of digested cane molasses stillage in 8 days under aerobic conditions when nutrients and glucose were provided [148]. A culture of *Lactobacillus hilgardii* was capable of melanoidin conversion to lactic acid and produced 28% and 40% decolorization of cane and beet molasses, respectively [149]. *L. hilgardii* was also capable of continuous decolorization under anaerobic conditions [150]. An immobilized isolate of the bacteria *Lactobacillus casei* was found to achieve a decolorization of 52% and a COD reduction of 57%, and to simultaneously produce 11.3 mg/mL of lactic acid, when fermenting digested cane molasses stillage supplemented with nutrients and glucose [151].

Filamentous fungi have also shown promise. After 8 days, an isolate resembling *Mycelia sterilia*, with proper nutrient and glucose additions, resulted in 93% decolorization of a molasses pigment solution prepared from molasses stillage [152,153]. White-rot fungi have also been employed for decolorization, commonly using *Coriolus versicolor*. *C. versicolor* was found to achieve 71.5% color removal along with a 90%

Table 8
Mesophilic anaerobic treatment of stillage from beet and cane molasses feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Reactor type (size — L)	Influent BOD (COD) g/L	HRT (days)	OLR (g COD/L/day)	Temp (°C)	Treatment efficiency % removed BOD (COD)	Methane yield (Prod) L/g COD (L/L/day)	References
Beet molasses	HABR (165)	nd (116)	5.78	20.0	37	nd (70)	0.08 (1.69)	Boopathy and Tilche [204]
Beet molasses	UASB (5×10^3)	nd (10)	0.58	13.8	39	nd (55.4)	0.36 (4.95)	Pipyn and Verstraete [249]
Beet molasses	UFF (500)	nd (48)	1.3	36.0	42	nd (50)	0.36 (12.4)	Braun and Huss [250]
Beet molasses	DFF (5×10^3)	30 (73)	9.1	8.0	37	nd (70)	nd (nd)	Athanasopoulos [251]
Cane molasses	UASB (11×10^3)	nd (15.2)	0.83	18.3	nd	nd (76)	0.28 (5.2)	Costa et al. [207]
Cane molasses	UASB (42.5)	39 (100)	10	10	nd	87 (67)	nd (nd)	Driessen et al. [206]
Cane molasses	UASB (42.5)	43 (109)	6.8	16	nd	85 (67)	nd (nd)	Driessen et al. [206]
Cane molasses	2-GACF (5.25)	nd (70)	10	7	27	nd (81)	0.25 (1.77)	Goyal et al. [216]
Cane molasses	UASB (2.3)	nd (68.9)	3.2	21.5	35	nd (58)	0.17 (3.6)	Espinosa et al. [217]
Cane molasses	DFF (nd)	nd (50)	10	25.0	35	nd (78.1)	0.17 (nd)	Shrihari and Tare [210]
Cane molasses	ACR (20)	60 (130)	10	4.6	36	90 (85)	0.37 (nd)	Halbert and Barnes [165]
Cane molasses	2-CSTR (6.0)	13.7 (22.5)	4.1	5.4	37	89 (63)	0.20 (0.35)	Cho [220]
Cane molasses	UASB (100)	nd (46)	2	23.3	40	nd (71.3)	0.22 (5.1)	Sanchez Riera et al. [219]
Cane molasses	FB (300)	nd (67.7)	5	13.5	30–37	nd (66.3)	0.15 (2.04)	de Bazua et al. [120]
Cane molasses	2-CSTR (2.5×10^6)	49 (132)	5.6	5.1	35–40	84.3 (63.2)	nd (nd)	Yeoh [4]
Cane molasses	HUASB (5)	40 (103)	0.25	36	30	nd (80)	0.4 (14.4)	Shivayogimath and Ramanujam [252]
Cane molasses	UASB (nd)	nd (88)	4.4	20	35	nd (61)	0.28 (nd)	Morris and Burgess [253]
Cane molasses	ACR (160×10^3)	nd (80)	16	5	33	nd (80)	0.22 (0.74)	Karhadkar et al. [254]
Cane molasses (rum)	ACR (1890)	32.9 (74.8)	19	3.6	35	nd (67.8)	0.19 (0.70)	Shea et al. [255]
Cane molasses (rum)	ACR (30)	nd (54.6)	6.8	8.0	35	nd (78)	0.37 (2.96)	Roth and Lentz [256]
Cane molasses	UFF (5.25)	nd (66.1)	5.6	11.76	35	nd (71.8)	0.23 (2.7)	Seth et al. [257]
Cane molasses (rum)	DFF (1.7×10^6)	20.5 (57.6)	3.8	15	35	60 (85)	0.22 (3.5)	Bories et al. [258]
Cane molasses (rum)	UFF (10×10^3)	nd (55.0)	2.8	20	36	88 (70)	0.24 (4.8)	Arnoux et al. [259]
Cane molasses (rum)	DFF (13.2×10^6)	42 (105)	8.2	12.8	38	85 (70)	0.21 (nd)	Szendrey [223–225], Szendrey and Dorion [226]

[a] nd = no data; ACR = Anaerobic contact reactor; 2-CSTR = 2-staged continuously stirred reactor; DFF = Downflow fixed film; FB = Fluidized-bed; 2-GACF = 2-phased granular activated carbon fixed film; HABR = Hybrid anaerobic baffled reactor; HUASB = Hybrid UASB; UASB = Upflow anaerobic sludge blanket; UFF = Upflow fixed film.

COD reduction in anaerobically digested cane molasses stillage when the effluent was amended with glucose [154]. The same organism achieved only 53% color removal when using fresh cane molasses stillage [155].

In all cases where decolorization was applied to anaerobically digested stillage compared to raw stillage, the level of decolorization was enhanced. In one case, *G. candidum* was grown on winery stillage to remove phenolic compounds prior to anaerobic digestion in order to improve anaerobic treatment performance [156]. Similarly, *Penicillium decumbens* was grown on beet molasses stillage to reduce phenolics which substantially improved digestion [157]. Most of these microbial decolorization studies required effluent dilution for optimal activity and, in cases where aerobic fermentation is required, the energy demand could be significant. Decolorization technology has not been applied at full-scale and cannot yet be considered a developed technology.

8.9. Other treatment processes

Several additional processes have been studied which hold potential for stillage processing and these include both thermal and electrochemical processes. First, thermal pretreatment using direct wet air oxidation of stillage followed by char recovery and incineration for steam production showed the potential for higher energy recovery than stillage evaporation followed by syrup incineration [5]. Also, supercritical water oxidation of stillage, using H_2O_2 at elevated temperatures of 673–773 K, has been shown to result in rapid reduction in organic strength [158]. Attempts at solid-catalyzed wet oxidation of stillage, using pure oxygen and MnO_2/CeO_2 mixed oxide catalyst at elevated temperatures (620 K) and pressures (20 MPa), were successful at reducing stillage strength, but resulted in inactivation of solid catalyst by carbonaceous fouling and reaction inhibition by stable intermediates [159]. Thermochemical liquefaction of stillage, using a sodium carbonate catalyst at elevated temperatures (300°C) and pressures (12 MPa), produced a maximum oil yield of 60% [160]. Finally, electrochemical treatment of stillage using NaCl, resulted in the production of chlorine and other oxidants which destructively oxidized stillage COD [161,162]. None of these processes have been attempted at large scale and they cannot be considered as proven or economical stillage treatment methods at this time.

8.10. Final disposition

Nutrients contained in ethanol feedstocks are drawn from the soils on which these crops are grown and, therefore, should be returned to these soils for the ethanol production system to be truly sustainable. Thus, land application is the most appropriate method for final disposition of ethanol stillage. In Brazil, much effort has been focused on the proper utilization of stillage nutrients [48] and methods for land application of untreated stillage onto sugar cane fields prior to planting have been developed. Experience has shown that application of untreated stillage to standing pasture can result in phytotoxicity [91], presumably due to N-immobilization. This was overcome by amending the untreated stillage with ammonia, but this increased the land area required and the cost of disposal. The use of surface and marine waters for final disposition should be discouraged but there may be site-specific circumstances in which these options have to be considered. Where surface water discharge is required, methods for tertiary treatment [163] (nutrient removal) should be considered, along with decolorization.

Odor control alone is sufficient incentive to consider appropriate treatment for stillage prior to discharge. Finally, the long-term impact of Na salts in stillage effluents on agronomic properties of soils has not been adequately studied and the replacement of sodium-based chemicals in plant operations should be investigated. The final disposition of stillage and treated stillage effluents will be considered in depth in a subsequent review [164].

9. Anaerobic treatment of stillage

Anaerobic treatment of ethanol stillage has

Table 9
Mesophilic anaerobic treatment of stillage from other conventional feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Reactor type (size — L)	Influent BOD (COD) g/L	HRT (days)	OLR (g COD/L/day)	Temp (°C)	Treatment efficiency % removed BOD (COD)	Methane yield (Prod) L/g COD (L/L/day)	References
Agave tequilana (tequila)	UASB (2.3)	nd (66.3)	3	25	32	nd (80)	nd (nd)	Ilangovan et al. [227]
Barley and sweet potato	2-UASB (4.7)	nd (29.5)	1.2	25	37	nd (90)	0.28 (7.0)	Shin et al. [230]
Cherry (morello)	2-PAF (1200)	nd (80)	8	10	32	nd (80)	nd (nd)	Stadlbauer et al. [90]
Corn (thin stillage)	ACR (11.2)	8.8 (16)	5	3.2	35–38	nd (97.3)	nd (3.6)	Stover et al. [237], Ganapathi [231]
Evaporator condensate (corn)	UASB (2×10^6)	nd (5.7)	0.46	12.3	35	nd (89)	0.25 (3.1)	Lanting and Gross [100], Gross and Lanting [260]
Evaporator condensate (sugar beet)	UASB (145×10^3)	nd (2.6)	0.17	15	nd	nd (85)	nd (nd)	Driessen et al. [206]
Grape (cognac)	DFF (140)	nd (26)	2.2	16	35	nd (91)	nd (5.48)	Henry et al. [233]
Grape (brandy)	UASB (127×10^3)	25 (30)	2.2	15	35	nd (82)	nd (nd)	Cheng et al. [261]
Grapes (wine)	UASB (42.5)	nd (30)	1.4	22	nd	nd (92)	nd (nd)	Driessen et al. [206]
Grapes (wine)	DFF (15×10^3)	nd (25)	1.7	15	36	nd (89)	0.34 (5.2)	Arnoux et al. [259]
Grapes (wine)	ITR (2.9)	nd (25)	2.4	10.7	36	nd (nd)	0.22 (2.35)	Buhlert et al. [262]
Grapes (wine)	UASB (10.5)	nd (31)	3.4	9	30	nd (90)	0.08 (0.72)	Moosbrugger et al. [263]
Grapes (red wine)	AFB (8.0)	10.2 (17)	1.13	15	37	nd (80)	nd (nd)	Ehlinger et al. [84]
Grapes (red wine)	DFB (5.0)	nd (15)	1.3	15	35	nd (85)	0.30 (4.48)	Garcia-Calderon et al. [264]
Malt whiskey (pot ale)	UASB (1.05)	nd (43)	2.8	15.3	35	nd (90)	nd (nd)	Goodwin and Stuart [265]
Mixed (wheat and sweet potato shochu)	MCR (5500)	20 (40)	5.7	7	37	nd (98)	0.28 (2.3)	Nagano et al. [242]
Mixed (potato, beets, wheat, and corn)	UFF (1.8×10^6)	nd (20–55)	5	10	37	nd (75–95)	0.3 (3.25)	Weiland and Thomsen [266]
Potato and beet	UFF (1400)	nd (40)	4	10	36	nd (90)	nd (nd)	Weiland and Wulfert [267]
Whey	ACR (26×10^6)	17 (27)	3.7	7.3	35	nd (nd)	0.08 (0.63)	Mawson [59]
Whey	ACR (nd)	nd (7)	nd	nd	36	nd (85)	0.37 (nd)	Reesen and Strube [268]
Whey	CSTR (3×10^6)	nd (nd)	nd	20	35	nd (85)	nd (nd)	Stafford [170]

[a] nd = no data; ACR = Anaerobic contact reactor; AFB = Anaerobic fluidized bed reactor; CSTR = Continuously stirred reactor; DFB = Downflow fluidized bed; DFF = Downflow fixed film; ITR = Inclined tube reactor; MCR = Membrane contact reactor; UASB = Upflow anaerobic sludge blanket; UFF = Upflow fixed film; 2-PAF = 2-staged pulsed anaerobic filter; 2-UASB = 2-staged UASB.

Table 10
Thermophilic anaerobic treatment of stillage from conventional feedstocks (values are calculated from data in literature sources)[a]

Feedstock	Reactor type (size — L)	Influent BOD (COD) g/L	HRT (days)	OLR (g COD/L/day)	Temp (°C)	Treatment efficiency % removed BOD (COD)	Methane yield (Prod) L/g COD (L/L/day)	References
Barley (shochu)	UFB (0.45)	12.6 (21.4)	0.18	115	53	nd (78.0)	0.27 (31.6)	Kida and Sonoda [269]
Beet molasses	UASB (2×10^6)	35 (43.2)	10.5	6.57	52.7	88.0 (86.0)	0.43 (0.81)	Vlissidis and Zouboulis [203]
Beet molasses	UASB (5.75)	nd (15.4)	0.18	83.6	55	nd (59.6)	0.26 (22.1)	Wiegant et al. [270]
Cane molasses	UASB (140)	2.5 (10)	0.43	23.5	55	88.2 (40)	0.12 (3)	Harada et al. [211]
Cane molasses	UASB (5.8)	nd (3.8)	0.16	24.0	55	nd (66)	nd (nd)	Harada et al. [211]
Cane molasses	2-CSTR (8.6)	45 (130)	5.6	20.0	55	90.2 (65.2)	0.17 (1.23)	Rintala [271]
Cane juice + molasses	UASB (70×10^3)	nd (31.5)	0.45	26.5	56	nd (71.7)	0.22 (5.88)	Souza et al. [209]
Grapes (wine)	AFB (0.25)	nd (15)	0.46	32.3	55	nd (82.5)	0.33 (5.8)	Perez et al. [272]
Grapes (wine)	UFF (2.0)	nd (15)	0.82	19.6	55	nd (47.9)	0.18 (3.55)	Perez et al. [273]
Grapes (wine)	CSTR (1.8)	11.7 (16.6)	4	4.15	55	nd (88)	0.25 (1.04)	Romero et al. [274]

[a] nd = no data; AFB = Anaerobic fluidized bed reactor; CSTR = Continuously stirred reactor; 2-CSTR = 2-staged continuously stirred reactor; UASB = Upflow anaerobic sludge blanket; UFB = Upflow-fluidized bed; UFF = Upflow fixed film.

often been cited as an effective and economic treatment option [2,3,48,102,165,166]. However, some studies [8,9,89,167,168] overlooked the potential of anaerobic digestion and considered the application of aerobic treatment for ethanol wastewaters. The high COD of stillage means that significant aeration power would be required for aerobic treatment and that about 50% of the COD would be converted to sludge requiring further disposal [99,169]. Anaerobic digestion can convert a significant portion (>50%) of the COD to biogas, which may be used as an in-plant fuel, and also saves the energy that would be required for aeration using aerobic treatment. In addition, anaerobic digestion has about 10% of the sludge yield and lower nutrient requirements compared to aerobic treatment [170].

A considerable amount of research has been conducted on anaerobic digestion of ethanol stillage from conventional feedstocks, especially cane molasses. Cane molasses stillage with a COD of over 100 g/L has been found to inhibit stable digestion and this may be overcome by dilution to a COD of around 50 g/L [171] using other waste streams at the plant. High potassium levels [172], high levels of metals [173], high stillage sulfate levels [174], and the presence of phenolic compounds [156,157,175] have been implicated in molasses stillage digestion deficiencies.

The effects of wastewater sulfate levels on anaerobic treatment have received considerable attention [176,177]. In anaerobic treatment, wastewater sulfate is converted to more toxic sulfide at the expense of methane production and leaves the reactor as either sulfide in the effluent or hydrogen sulfide in the biogas. Effluent sulfide levels contribute to odors, corrosiveness and effluent oxygen demand, while hydrogen sulfide in the biogas causes corrosion problems in engines and boilers. In general, sulfide inhibition is not encountered in anaerobic treatment when the wastewater COD/SO_4 ratio is above 10 g/g, while inhibition is severe when the ratio is below 0.5 g/g [177]. This is caused by the stripping effect of higher biogas production rates which rapidly remove sulfide as it is formed. Digestion of wastewater with an intermediate COD/SO_4

Table 11
Anaerobic treatment of stillage from cellulosic feedstocks (values are calculated from data in literature sources)[a]

Feedstock/ process	Reactor type (size — L)	Influent BOD (COD) g/L	HRT (days)	OLR (g COD/L/day)	Temp (°C)	Treatment efficiency % removed BOD (COD)	Methane yield (Prod) L/g COD (L/L/ day)	References
Eucalyptus/DA	UFF (2.0)	nd (22.5)	2.1	10.7	35	nd (86.6)	0.4 (2.7)	Good et al. [243]
Eucalyptus/DA	UFF (2.0)	nd (22.5)	2.25	10.0	55	nd (84.4)	0.38 (2.4)	Good et al. [243]
Eucalyptus/DA	CSTR (2.0)	nd (22.5)	9.5	2.4	35	nd (85.5)	0.4 (0.6)	Good et al. [243]
Hardwoods/TS-DA-SF	CSTR (1.0)	nd (19.1)	nd	nd	35	nd (nd)	nd (nd)	Strickland et al. [244]
Pinus radiata/ DA-SF	CSTR (8.0)	nd (25.5)	6.4	4.0	37	nd (92)	0.28 (1.2)	LFTB [246], Callander et al. [275]
Pinus radiata/ DA-SF	UASB (10)	13.2 (25.5)	1.6	16.0	37	93 (86)	0.21 (3.3)	LFTB [246], Callander et al. [276]
Pinus radiata/ DA-SF	UASB (8.0)	13.9 (27.5)	2.0	13.8	37	92 (82)	nd (4.0)	LFTB [246]
RDF/CA	BMP (0.125)	37.7 (104)	2–5	nd	35	96.3 (67)	0.16 (nd)	Broder [200]
RDF/DA	BMP (0.125)	31.1 (110)	2–5	nd	35	93.6 (85)	0.27 (nd)	Broder [200]

[a] nd = no data; BMP = Batch assay; CA = Concentrated acid; CSTR = Continuously stirred reactor; DA = Dilute acid; RDF = Refuse derived fuel; SF = Saccharomyces fermentation; TS = Two stage; UASB = Upflow anaerobic sludge blanket; UFF = Upflow fixed film.

ratio may be handled by diluting the wastestream to a COD of 15 g/L so that the sulfide is removed in the effluent at the higher flow rate that dilution allows [177]. Finally, high reactor sulfide levels can also be mitigated by adding soluble Fe^{3+}, which promotes precipitation of ferrous sulfide.

Table 8 lists treatment parameters for mesophilic anaerobic digestion of stillage from beet and cane molasses. Table 9 lists treatment parameters for mesophilic anaerobic digestion of stillage from some other conventional feedstocks. Table 10 lists treatment parameters for thermophilic anaerobic digestion of stillage from beet and cane molasses. Table 11 lists treatment parameters for anaerobic digestion of stillage from cellulosic feedstocks. Finally, Table 12 summarizes the anaerobic treatment parameters from Tables 8–11.

For the mesophilic studies, the average organic loading rate (OLR) applied is 9–12 g COD/L/day, with an average COD treatment efficiency greater than 70% and average methane yield greater than 0.25 L/g COD added (Table 12). Thus, the treatment efficiencies and loading rates for mesophilic anaerobic treatment are quite high and indicate that anaerobic digestion is a suitable method for biological treatment of the waste. Since stillage leaves the distillation process at about 90°C, cooling is required to bring the waste down to mesophilic temperatures (<42°C).

Application of thermophilic digestion would only require cooling the stillage to under 60°C, which occurs naturally during temporary stillage storage. Table 12 shows that thermophilic treatment of molasses stillage achieves similar BOD treatment efficiencies at almost twice the OLR of mesophilic systems. While the average COD treatment efficiency for thermophilic molasses stillage digestion appears lower than that for mesophilic, this difference is most likely due to variations in the refractory COD of the molasses stillage and the lower thermophilic methane yields tend to confirm this. The higher thermophilic OLRs indicate that smaller digesters are required which should improve process economics. It is interesting to note that, in 1932, Boruff and Buswell advocated thermophilic an-

Table 12
Summary of anaerobic treatment of stillage from conventional and cellulosic feedstocks. Statistics are compiled from studies using reactors larger than 1000 L, except for thermophilic and cellulosic studies where data was limited[a]

Temperature/Feedstock		OLR (g COD/L/day)	Treatment efficiency % removed BOD	Treatment efficiency % removed COD	Methane yield (L/g COD)	Methane productivity (L/L/day)
Mesophilic/molasses	— Average	12.25	79.33	71.20	0.26	3.84
	— Std dev	5.72	12.98	9.33	0.06	1.85
	— *n*	8	4	8	6	5
Mesophilic/other	— Average	12.16	nd	87.25	0.25	2.90
	— Std dev	4.08	nd	5.60	0.10	1.66
	— *n*	10	nd	8	5	5
Thermophilic[b]/molasses	— Average	23.50	89.20	60.73	0.17	3.37
	— Std dev	2.68	1.41	14.12	0.05	2.35
	— *n*	4	2	4	3	3
Mixed/cellulosic	— Average	9.48	93.73	83.56	0.30	2.37
	— Std dev	5.35	1.84	7.27	0.10	1.28
	— *n*	6	4	8	7	6

[a] nd = no data; std dev = standard deviation; n = number of literature values used.
[b] Data from Vlissidis and Zouboulis [203] excluded due to impacts of P and SO_4 precipitation on process; Data from Wiegant et al. [270] excluded as outlier.

aerobic digestion of stillage [178], and yet the literature indicates that only two full-scale thermophilic digesters have been built (Table 10).

The failure to implement thermophilic stillage digestion is caused by a number of factors, including: (1) a lack of availability of thermophilic inocula; (2) a perceived higher sensitivity of thermophilic digestion compared to mesophilic digestion; (3) concern about restart of intermittently operated thermophilic digesters; and (4) perceived higher COD levels in thermophilic effluent compared to mesophilic effluent. A number of studies have shown that the use of mesophilic inocula does not limit the development of thermophilic biomass [179–183]. Likewise, some studies have shown that thermophilic digestion is more tolerant to organic overloads than mesophilic digestion when immobilized reactor designs are used [184,185]. In tropical climates between sugarcane harvests, the temperature in an idled thermophilic digester would drop to an ambient temperature of 25–35°C. At the start of the next cane harvesting season, the reactor must be brought to design operating temperature and loading rate within a reasonable period. There is no indication that this restart period is longer for thermophilic digesters [186] than for mesophilic digesters [187,188]. Finally, a comparison of effluent COD from thermophilic versus mesophilic digestion of cane molasses stillage in Brazil concluded that higher effluent COD was a disadvantage of the thermophilic process [189]. However, the thermophilic reactor was only installed to provide biogas for yeast drying and the bulk of the stillage was land applied without treatment, so there was no incentive to limit effluent COD from the reactor [189]. If appropriate loading rates and nutrient supplementation are maintained, there is no reason for effluent COD levels from thermophilic reactors to exceed those of mesophilic reactors treating the same wastewater [184]. Thus, lower cooling demand and increased loading rates should make thermophilic anaerobic stillage treatment preferable in future installations.

Table 13 lists some of the full-scale anaerobic digesters currently treating stillage by supplier, reactor type, country and range of OLR. This list indicates that at least 149 facilities have been built, and that 87 of these are in India. While most of these digesters are of the upflow anaerobic sludge-blanket (UASB) or expanded granular sludge-bed (EGSB) design (78 UASB; 3 EGSB), a significant number (27) of bulk volume fermenters (BVF) have been commissioned as well. There are also 22 downflow fixed film (DFF) digesters, 10 anaerobic contact (AC) digesters, six hybrid (Hybr) digesters, and three upflow fixed film (UFF) digesters. The immobilized sludge reactors (UASB, EGSB, DFF, UFF and Hybr systems) have significantly higher OLRs, with a trend of higher OLRs in developed countries. In contrast, the BVF have much lower OLRs, with a trend of higher OLRs in developing countries. This may suggest that a higher level of control in developed countries allows high OLRs in the immobilized sludge type digesters, while less stringent discharge requirements may allow higher OLRs for BVF in developing countries. In India, the BVF is regarded as being the most inexpensive and stable design which is applicable where land area is not restrictive [190]. The number of full-scale anaerobic digesters operating on stillage wastes is a valid testament to the feasibility of this treatment technology.

Finally, the limited data regarding anaerobic treatment of stillage from cellulosic feedstocks (Table 11) are comparable with treatment parameters from other feedstocks (Table 12). The OLR applied and treatment efficiencies achieved indicate that cellulosic stillage is amenable to anaerobic treatment. However, the limited number of studies on stillage from different cellulosic feedstocks and hydrolysis methods means that predictions of treatment performance are prone to error. Improved predictions could be made if a larger data set of cellulosic stillage characteristics and treatment parameters were developed.

10. Summary and conclusions

This technical review was developed from research conducted at the University of Florida

Table 13
Full-scale anaerobic digester facilities treating distillery wastes worldwide by supplier [203, 209, 277–284], reactor type, country and effluent type[a]

Supplier	Reactor type	Country	Effluent type	No. of plants	OLR (g COD/L/day)
ADI	BVF	India	Cane molasses	20	0.9–3.6
ADI	BVF	Kenya	Cane molasses	1	3.00
ADI	BVF	USA	Evaporator condensate	3	0.26–0.58
ADI	BVF	Colombia	Cane molasses	1	2.30
ADI	BVF	Pakistan	Cane molasses	1	0.9–3.6
ADI	BVF	Nepal	Cane molasses	1	3.50
Bacardi	DFF	Puerto Rico	Cane molasses	1	12.8–14.4
Bacardi	DFF	Dominican Republic	Cane molasses	1	nd
Bacardi/Lars Enviro	DFF	India	Cane molasses	2	8.65–9.5
Biometano Consultoria	UASB thermophilic	Brazil	Cane juice and molasses	1	26.5
Biothane	EGSB	Germany	Evaporator condensate	1	11.20
Biothane	EGSB	USA	Evaporator condensate	2	15.6–15.9
Biothane	UASB	Canada	Evaporator condensate	1	15.40
Biothane	UASB	India	Evaporator condensate	1	12.60
Biothane	UASB	Slovakia	Evaporator condensate	1	10.50
Biothane	UASB	USA	Evaporator condensate	2	8.3–10
Biothane	UASB	Thailand	nd	1	15.00
Biothane	UASB	Germany	Evaporator condensate	1	9.00
Biothane	UASB	Netherlands	Evaporator condensate	1	19.40
Biotim	AC	Germany	Wheat starch	1	3–4.7
Biotim	Hybr	Thailand	Cane molasses	1	nd
Biotim	Hybr	Belgium	Beet and cane molasses	1	9
Biotim	Hybr	Korea	Barley, sweet potatoes and tapioca	1	8
Biotim	Hybr	Indonesia	Cane molasses	1	16.5
Biotim	UASB	India	Cane molasses	2	15
Biotim	AC	Korea	Barley, sweet potatoes and tapioca	2	3.3
Biotim	Hybr	India	Cane molasses	1	9.2
Biotim	Hybr	Portugal	Wine	1	13
Degremont	AC	France	Grape wine	1	4.42
Degremont	AC	Germany	Cereals	2	13–15
Degremont	UFF	Germany	Cereals	1	12.11
Degremont	UASB	India	Cane molasses	23	12–19.5
Degremont	AC	Paraguay	Cane juice	1	nd
Degremont	UFF	Spain	Grape wine	2	10.36
Degremont	AC	Switzerland	Mixed	1	1.05
Degremont	AC	USA	Corn	1	nd
Degremont	AC	Venezuela	Cane molasses	1	8.61
Lars Enviro	DFF	India	Cane molasses	13	8.65–12.3

Table 13 (*continued*)

Supplier	Reactor type	Country	Effluent type	No. of plants	OLR (g COD/L/day)
Paques	UASB	Japan	Distillery	1	28.6
Paques	UASB	Japan	Sweet potato	1	30
Paques	UASB	Turkey	Grape wine	4	11.2–12.6
Paques	UASB	India	Molasses	25	9.9–15.2
Paques	UASB	S. Africa	Grape wine	1	13.95
Paques	UASB	Germany	Grape wine	1	12.00
Paques	UASB	Taiwan	Chinese wine	1	10.67
Paques	UASB	Switzerland	Grape wine	1	13.16
Paques	UASB	Guatemala	Cane juice	1	17.50
Paques	UASB	Netherlands	Beet molasses	1	14.48
Paques	UASB	Venezuela	Cane juice	2	16.2–18.5
Paques	UASB	Brazil	Cane juice	2	15.00
SGN	DFF	France	Grape wine	2	11.0–11.8
SGN	DFF	Spain	Grape wine	2	15
SGN	DFF	Guadeloupe	Cane molasses (rum)	1	14.1
Vlissidis	UASB thermophilic	Greece	Beet molasses	3	7
			Total installations	149	

[a] AC = anaerobic contact; BVF = bulk volume fermenter; DFF = downflow fixed-film; EGSB = expanded granular sludge bed; UFF = fixed-film; Hybr = hybrid reactor; UASB = Upflow anaerobic sludge blanket.

Table 14
Commercial full-scale cellulosics-to-ethanol projects under development in North America [198][a]

Feedstock	Location	Process technology	Annual production L/yr	Company
Cellulosics	Ottawa, Canada	Enzymatic	3.8×10^6	Iogen
Bagasse	Jennings, LA	2-stage dilute acid	38×10^6	BCI
MSW	Middletown, NY	Concentrated acid	38×10^6	Masada [285]
Rice straw	Sacramento, CA	Concentrated acid	45×10^6	Arkenol
Rice straw	Gridley, CA	Enzymatic	76×10^6	BCI/Gridley LLC
Softwood wastes	SE Alaska	nd	$23–30 \times 10^6$	Sealaska
Softwood wastes	Chester, CA	Enzymatic	76×10^6	BCI/Colling Pine

[a] nd = no data.

to assess the feasibility of an integrated biomass-to-energy system in Central Florida which resulted in several related publications [16,191–197]. Table 14 lists some cellulosic-to-ethanol conversion projects currently under development in North America [198]. Large-scale production of ethanol from lignocellulosic biomass has considerable potential due to the availability of significant resources of lignocellulosic biomass. However, substantial increases in ethanol production also require effective solutions for stillage management. This effort has contributed to an appreciation of the potential impacts of the biomass-to-ethanol production process on cellulosic stillage characteristics and utilization. It can be concluded from this study that existing research supports the application of anaerobic digestion for cellulosics-to-ethanol stillage treatment and biogas recovery. However, there is a need for further information on the characteristics and treatment of cellulosic-based stillage.

The results of some of the research currently underway, both in the US and in other countries at the forefront of commercially viable biomass-to-ethanol technology development (e.g., Canada, Brazil, New Zealand, etc.), are not widely available and not immediately accessible to the authors. Specific research efforts resulting in greater information dissemination would facilitate government and industry progress toward economically and environmentally sustainable biomass-to-ethanol energy production systems. Areas of research apparent to the authors which merit further investigation include:

1. hydrolysis stillage characterization data should be obtained for pertinent feedstocks, hydrolysis methods, and fermentation schemes, and these results should be considered during feedstock and process selection/optimization;
2. as final selection of feedstock/process is approached, corresponding hydrolysis stillage treatability studies should be performed prior to preliminary process design and cost estimation.

Thermophilic anaerobic digestion of ethanol stillage achieves similar BOD treatment efficiencies and methane yields, at almost twice the organic loading rate, compared to mesophilic treatment. Therefore, application of thermophilic anaerobic digestion would improve process economics, since smaller digesters and less stillage cooling are required. Downstream processes for stillage utilization and by-product recovery considered worthy of continued investigation include the production of feed (from single cell protein and/or algae production), color removal, and production of calcium magnesium acetate. The results of this study suggest that sustainable and economically viable solutions for mitigating environmental impacts which result from large-scale biomass-to-ethanol conversion facilities are available. However, further research in some

areas is needed to facilitate successful implementation of appropriate technology options.

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