Effect of Recycled Laboratory Backset on Fermentation of Wheat Mashes

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Distillers' solubles, soluble components and the liquid obtained after distillation of fermented mash and removal of distillers' grains, are often recycled as "backset" to reduce effluent treatment costs and water usage. In this study, recycling of laboratory distillers' solubles was carried out over five successive fermentations. When 50% backset was used in a 30-gal mash (30 U.S. gal/56-lb bushel, a term used by corn distillers) using wheat as the substrate, yeast growth and ethanol production remained unaffected over five successive fermentations. The final cell yields approximated 9×10^7 yeasts/mL. The alcohol yields ranged from 5.7 to 6.0% w/v. No undesirable substances accumulated in high enough concentrations to inhibit normal yeast metabolism. In fact, calcium, lactic acid, and acetic acid were utilized by the yeasts and removed from the mash. Wheat mashes contained low levels of utilizable free amino nitrogen. Yeast extract added to the mash stimulated the rate (but not the amount) of alcohol produced. Optimal supplementation allowed a 40% reduction in fermentation time compared to that of unsupplemented controls. Although yeast extract contains unassimilable substances, no compounds in the backset were found to impede yeast fermentation through four cycles of backsetting at 50%.

Stillage is fermented mash from which alcohol has been removed by distillation. It consists of both soluble and insoluble matter. Stillage is high in biological oxygen demand (Maiorella *et al.*, 1983b), and in a typical fuel ethanol distillery, it is produced at 8–13 times the volume of the distilled ethanol (Kujala, 1979). Hence, the disposal of this effluent requires capital and energy-intensive waste treatment methods. To reduce effluent volumes and costs, 25–75% of the thin stillage (the soluble portion of whole stillage) is reused in a subsequent mash, replacing a percentage of the water (Easley, 1987; St. Julian *et al.*, 1990). This is known in the industry as backsetting.

A controversy exists, however, with regard to the benefits and problems occurring in the use of backset. Products from lysis of yeast cells during dewatering, recycling, or fermentation, products of metabolic activity of yeasts (Panchal and Stewart, 1980), products made by undesirable contaminants (Ingledew, 1993), and inhibitory constituents from the feedstock utilized all can accumulate (Keim, 1983; Maiorella *et al.*, 1984). These lead to physical and chemical imbalances in the fermentor and reductions in growth and fermentation rate in both batch and continuous systems. For example, Chen (1990) found that backsetting at levels exceeding 20% resulted in inhibition of yeast growth. Tajima et al. (1966) reported that salt tolerance of yeast during alcoholic fermentation is one of the most important factors to consider, particularly when the substrate in use contains high quantities of inorganic salts. The accumulation of protein metabolites and the increased osmotic pressure in the media also have been shown to impair fermentations (Panchal and Stewart, 1980).

Lactic acid bacteria and acetic acid bacteria, common contaminants found in breweries, distilleries, and fuel alcohol plants (Ingledew, 1993), can inhibit yeast metabolism and compete for nutrients, resulting in the production of lactic acid and acetic acid. Ethanol yields are stoichiometrically reduced as lactate and acetate are made, and the presence of these contaminants is indicated by an increase in titratable acidity in the fermentation medium.

Inhibitory constituents in feedstock and nonmetabolized medium components all build up as stillage is recycled and will limit yeast productivity. Maiorella *et al.* (1984) showed the inhibition order (80% reduction of cell mass production) in continuous culture for a number of salts and sugars (in molar concentration): $CaCl_2$ (0.23), $(NH_4)_2SO_4$ (0.24) > NaCl (0.45), NH_4Cl (0.46) > KH_2PO_4 (0.73) > xylose (0.92), $MgCl_2$ (0.92) > $MgSO_4$ (0.97) > KCl (1.74). From their study, calcium ion was found to be the most potent inhibitor. They suggested that, in a typical ethanolic fermentation of cane molasses, the recycling of stillage must be limited to less than one-third of the feed rate to avoid inhibitory effects. The elevated salt concentration was said to cause interference with enzymes and with transport of various ions (Maiorella *et al.*, 1984). Mineral salts also interfere with fermentation by entering and bursting the cells through increased osmotic pressure (Keim, 1983).

Some positive effects on the use of backset have been reported. Dissolved or suspended materials, including grain components in the fermentation medium, added nutrient supplements, and materials leaked from yeast cells, remain in the residual stillage following a fermentation. These materials, when recycled, may accelerate yeast growth and fermentation rate and improve ethanol tolerance in the subsequent fermentation (Ingledew, 1993; Murphy *et al.*, 1982; Wall *et al.*, 1983). As an example, Fujikawa et al. (1983) have purified and identified growth-promoting factors such as inositol and inositol 2-phosphate.

Wall et al. (1983) observed no adverse effects on yeast growth and fermentation when 100% recycling of corn distillers' solubles (seven runs) was carried out. Ronkainen *et al.* (1978) used up to 80% stillage water and found no detrimental effects on alcohol yield, and St. Julian *et al.* (1990) carried out 100% recycling of thin corn stillage for six successive fermentations and noticed no major changes in ethanol yields. Rossell (1988) has also reported that yields and productivity were not affected by stillage recycling when sugarcane was used as substrate. Dellweg and Luca (1988) concluded that in a beet molasses distillery one can permanently recycle 40% waste effluent without significant decreases in productivity.

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Most studies on backset have been carried out using corn, sugarcane, sugar beet, or molasses as substrates. Little is known about the use of backset in fuel alcohol plants based on wheat. In this study, fermentation of wheat mashes with and without nitrogenous supplementation was carried out with successive recycling of laboratory distillers' solubles at the 50% level.

MATERIALS AND METHODS

Grinding and Mashing of Wheat. Commercial red spring wheat was ground with a plate grinder (Type KT-30; Falling Number AB, Stockholm, Sweden) at setting 2. For mashing, 365 g of wheat was dispersed with constant stirring into 1653 mL of distilled water at 60 °C containing 1 mM calcium chloride. This was followed by the addition of 2.5 mL of a high-temperature α-amylase (Alltech Biotechnology Center, Nicholasville, KY). The temperature was elevated and maintained at 80 °C for 30 min. The mash was then heated to boiling for 1 h with continuous agitation. Water lost through evaporation was replaced by adding sterile distilled water. After cooling to 80 °C, the gelatinized starch was liquefied by adding 2.5 mL of high-temperature α-amylase and allowing it to react for 30 min. As prepared, the mashes all contained about 14 g of dissolved solids/100 mL. After the mash cooled, diethyl pyrocarbonate (DEPC, Sigma Chemical Co., St. Louis, MO) was added to 0.01% v/v and the mash was refrigerated at 4 °C for 48 h.

Following cold-sterilization, 2-L aliquots were transferred aseptically to sterile, jacketed 2-L Wheaton bioreactors (Wheaton Instruments, Millville, NJ), connected to a D3-G water bath circulator (Haake Inc., Saddle Brook, NJ) at 30 °C. Glucoamylase (4 mL of Allcoholase II, Alltech Biotechnology Center) was added to each fermentor to saccharify the dextrins to fermentable sugars. After 30 min, the temperature of the fermentors was reduced to 20 °C for fermentation. When nitrogenous supplementation was desired, concentrated solutions of yeast extract (Difco Laboratories, Detroit, MI) which had been presterilized at 121 °C (15 min) were mixed into the wheat mash to concentrations of 0.20 or 0.45% (w/v).

Preparation of Inocula. Ten grams of active dry yeast (*Saccharomyces cerevisiae*, Alltech Biotechnology Center) was dispersed into 99 mL of prewarmed (39 °C) 0.1% sterile peptone water and incubated at 39 °C for 20 min. This is an adaptation of the prescribed conditioning recommended by yeast manufacturers. The required volume of inoculum was added to each fermentor to obtain 1.4×10^7 yeast cells/mL of mash [based on a viable count of ADY of approximately 2.2×10^{10} cells/g and a recommended inoculation rate (Casey and Ingledew, 1985) of 10^8 cells °Plato^{-1} mL^{-1}].

Fermentation Conditions. Throughout fermentation, mashes were maintained at 20 °C and stirred at 100 rpm using a Wheaton Biostir 6 (Wheaton Instrument). The fermentation was halted when the specific gravity (DMA 45 densitometer, see below) leveled at or near 0 °Plato.

Backset. At the end of fermentation, the alcoholic mash was centrifuged (10300*g* for 30 min at 4 °C) and the supernatant was collected for simple distillation. A significant change in temperature at the condenser inlet (from 78 to 97 °C) was an indication of the completion of ethanol distillation. The residual liquid was then cooled, made up to the original volume with distilled water, and recentrifuged at 10300*g*. The resulting supernatant, termed laboratory distillers' solubles (or backset), was used in subsequent fermentations. It differs from industrially produced backset in that all insoluble materials including yeast were removed prior to recycling. It was made up to original volume to ensure exact quantitation of substrate or yeast-derived solutes. Backset was frozen until the next sequential mashing. For 50% recycling of backset, half of the volume of the water into which ground wheat was added in the first step of mashing was replaced by backset from the previous fermentation. A total of five fermentations with four recyclings of laboratory distillers' solubles were carried out in each experiment.

Assay Methods. The membrane filtration technique was used to monitor viable cell counts (Ingledew *et al.*, 1980), with membranes incubated aerobically at 27 °C on yeast extract–peptone–dextrose (YPD) agar.

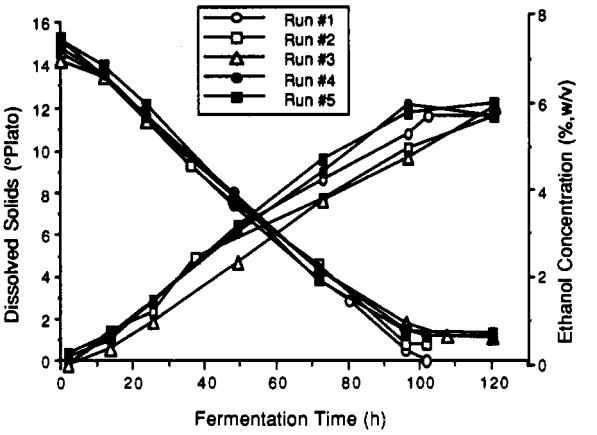

Figure 1. Utilization of dissolved solids and production of ethanol over five sequential fermentations of wheat mashes made with 50% backset.

Total dissolved solids were determined by measuring the specific gravity of the water-soluble portion of the mash and "beer" after centrifugation at 10300*g* for 30 min and measured at 20 °C by a DMA 45 density meter (Anton Paar KG, Graz, Austria). Readings were converted to grams of dissolved solids (as sucrose) per 100 g (°Plato).

Ethanol content in the mash and beer samples was measured enzymatically using alcohol dehydrogenase (Thomas and Ingledew, 1990). Free amino nitrogen (FAN) was determined using the European Brewery Convention (EBC) ninhydrin method (European Brewery Convention, 1975).

Acid titrations of mash to detect excessive bacterial contaminants were carried out by following the Alltech Biotechnology Center's laboratory procedure, "Acid Titration". Laboratory distillers' solubles were also assayed for lactic acid content by the D/L-lactic acid UV enzymatic method and for acetic acid by the acetic acid UV method (Boehringer Mannheim, Mannheim, Germany).

The mineral contents of laboratory distillers' solubles were analyzed by the Saskatchewan Soil Testing Laboratory, University of Saskatchewan, Saskatoon, SK, using the "plant tissue extract analysis package" where inductively couple plasma (ICP) was used to determine concentrations of nine elements (calcium, magnesium, copper, iron, manganese, zinc, phosphorus, potassium, and sulfur).

RESULTS

Backset without Supplementation. The initial study utilized distilled water for the first mashing, and a mixture of distilled water (50%) and laboratory distillers' solubles (50%) for the next four sequential mashings. All five wheat mashes of 14 °Plato were fermented at similar rates to completion at 100 h (Figure 1). The use of the DMA 45 density meter to follow the fermentation relies on the fact that most of the solutes in mash are sugars and are converted to gaseous CO_2 and ethanol (density of 0.789) in an approximately 50:50 ratio. Therefore, specific gravity decreases significantly as fermentation proceeds. Alcohol yields ranged from 5.7 to 6.0% (w/v) (Figure 1). In industry, a typical grain mash has been reported to consist of about 14% dissolved carbohydrate with 6.9% (w/v) alcohol produced (Maiorella, 1985; Shelton and Rider, 1980). That only 83–87% of the industrial expectation was realized in these experiments is probably the result of the lowered starch levels in wheat compared to corn and because less than a bushel of wheat was used in the mash calculation (56 lb). No attempts were made to incorporate these factors into the 30-gal mash definition used or to adjust to the more normal 8% (w/v) alcohol levels seen in industry.

These fermentations proceeded without the complications or consequential yield reductions caused by microbial

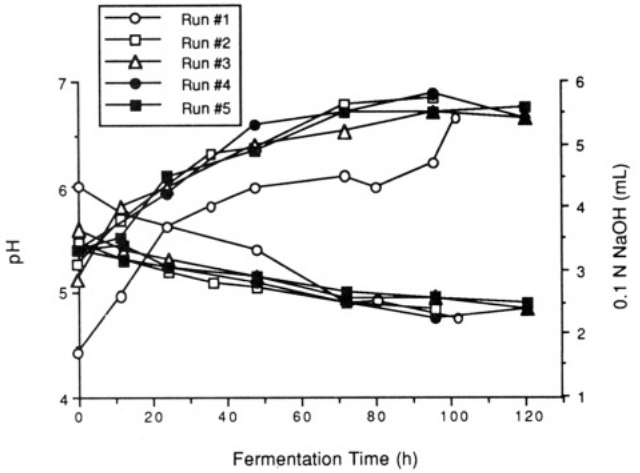

Figure 2. Changes in acidity in fermentation media as measured by pH and by acid titration over five sequential fermentations of wheat mashes made with 50% backset.

contamination. Bacterial infection is expected in fuel alcohol plants, but without serious contamination problems; the pH of fermenting mash in industry drops from 5.5 to between 3.8 and 4.2, and an acidity equivalent to 7–14 mL of 0.1 N sodium hydroxide is found (Ingledew, 1993). In our experiments, an increase in acidity was seen as each fermentation progressed (Figure 2). The pH decreased from initial values of 5.5–6.0 (at zero time) to approximately pH 5 by the end of the fermentation. The acid titration values increased from the initial 1.6–3.2 to 5.4–5.8 mL. These results suggest that no bacterial infection took place through five sequential fermentations; the drop in pH and the increase in acidity were due to the formation of minor metabolic end-products. The acidity of mashes varies widely depending on the grain and the water used. Each plant must establish its own normal values.

The patterns of yeast growth graphed from the five fermentation cycles closely resembled each other (data not shown). This was expected because ethanol production and carbohydrate utilization proceeded in each of the five sequential runs at similar rates, and previous work has shown these to be directly related to the growth and vigor of yeast cells. The mashes were inoculated at an average of 1×10^7 cells/mL. After 40 h, a maximal cell number of 9×10^7/mL was reached. The cell population, therefore, increased by almost 9-fold (over three doublings). Subsequently, the yeasts remained viable throughout fermentation.

All wheat mashes contained between 37 and 52 mg/L of free amino nitrogen (FAN) (Figure 3), of which 30–34 mg/L was available for yeast growth. Although the nitrogenous contents in the mashes were considered to be extremely low, utilization of carbohydrate was complete since wheat reportedly provides the "right" kind of amino acids for yeast cell growth (Thomas and Ingledew, 1990).

Since the accumulation and recycling of organic acids and inorganic salts can adversely affect fermentation and ethanol yield, their levels in laboratory distillers' solubles were also monitored. As shown in Figure 4, the lactic and acetic acid concentrations increased between fermentation runs 1 and 2 to maximum levels of 0.45 and 0.26 g/L, respectively. This was followed by a reduction in the concentrations to 0.24 and 0.10 g/L, respectively. The decrease in the amount of the acids may be due to the fact that *S. cerevisiae* is capable of consuming lactic acid and acetic acid as carbon and energy sources for growth (Leao and van Uden, 1986).

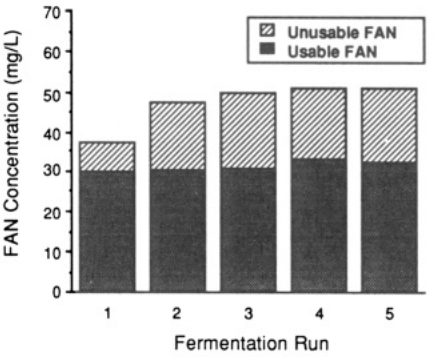

Figure 3. Total, usable and unusable (final), free amino nitrogen (FAN) concentrations measured over five sequential fermentations of wheat mashes made with 50% backset.

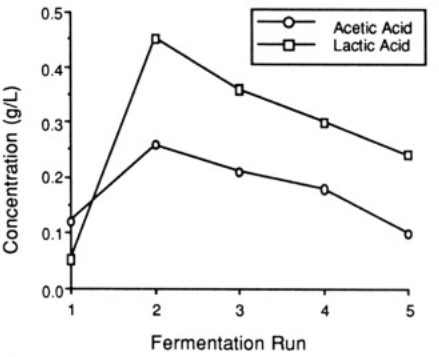

Figure 4. Changes in acetic and lactic acid in laboratory distillers' solubles measured over five sequential fermentations of wheat mashes made with 50% backset.

Mineral analysis of backsets showed no detectable levels of copper or zinc. Iron and manganese were found in very low levels (0.001 and 0.008 g/L, respectively). The concentrations of the latter two minerals remained relatively constant during backsetting as did the concentrations of potassium, sulfur, and magnesium. Phosphorus was the only element that showed a definite increase in concentration as recycling continued. It accumulated from 0.4 to 0.6 g/L after four rounds of recycling (data not shown).

Calcium is of interest since it is generally considered as a potent yeast growth inhibitor. In this study, calcium was added to the mash as an enzyme stabilizer in the form of calcium chloride. On the basis of the rate of calcium supplementation and backset recycling, it was estimated that calcium concentrations should accumulate from 0.04 g/L in backset 1 to 0.06 g/L in backset 2 and eventually up to 0.0775 g/L in backset 5. However, calcium increased from 0.026 to 0.050 g/L after one recycling and then leveled off at this concentration during subsequent recyclings. These results, therefore, suggest that calcium (and other elements) was taken up by the yeast cells, leaving less than expected to accumulate in distillers' solubles. In general, no significant ion buildup was evident in five cycles of backset as practiced in these fermentations. It should be noted that no pH adjustments of mash were made in this work—such practices could affect these results profoundly.

Backset with Nitrogen Supplementation. The above series of fermentations reconfirmed that wheat mashes contain low levels of assimilable nitrogen. Since nitrogenous substances are fermentation stimulants (O'Connor-Cox and Ingledew, 1989; Thomas and Ingledew, 1990), yeast extract was added to backsets in this study. It is known that amino acids and small peptides from yeast extract will be used, but *S. cerevisiae* is unable to

metabolize proteins, most peptides, and proline under fermentative conditions (Ingledew *et al.*, 1986); and therefore, these soluble but unusable nitrogenous constituents (although in low concentrations) will remain in the beer at the end of fermentation. Hence, the practice of backsetting yeast food-adjusted mashes may result in the accumulation of these compounds along with minerals and other substances from yeast extract that are not taken up by yeast cells.

Preliminary work showed that maximal rates of substrate utilization and alcohol production were obtained in the presence of as low as 0.45% (w/v) yeast extract (data not shown). In the presence of 0.2% (w/v) yeast extract, approximately 150 mg/L FAN was made available in the mash, which represents the recommended level. These two levels of yeast extract were selected for further examination.

To determine whether the recycling of backset from nitrogen-supplemented mashes might eventually lead to the buildup of undesirable substances that may inhibit alcohol production, three sets of fermentations were performed. The initial wheat mashes were prepared with distilled water, and each was supplemented with yeast extract at 0, 0.2, or 0.45% (w/v). Following the first fermentation run, 50% recycling of laboratory distillers' solubles was carried out for the remaining four sequential fermentations. Each subsequent mash was supplemented with the preselected level of yeast extract for that particular series of fermentations.

Figure 5 shows the fermentation profiles of the three sets of five fermentations. An unsupplemented 14 °Plato mash required 120 h to ferment to completion. The attenuation time was shortened to 60 and 45 h by the addition of 0.2 and 0.45% yeast extract, respectively. The marked reduction in fermentation time was directly related to increases in yeast growth. All wheat mashes were inoculated with approximately 10^7 yeast cells/mL, and maximal cell counts were reached after 40 h of fermentation in all cases. Values of 1.5×10^8 cells/mL were obtained as the mean maximal cell yield in the presence of 0.2% yeast extract compared to 1.0×10^8 cells/mL in the unsupplemented mashes. With further addition of yeast food (0.45%), 2.0×10^8 cells/mL was found, twice the cell yield found in the control.

The increased cell number and corresponding catalytic activity improved the rates of ethanol production. The nitrogenous supplement, therefore, helped to speed the rates at which alcohol was made. In the presence of 0.2 and 0.45% yeast extract, 60 and 45 h, respectively, were required for the maximal level of ethanol to be reached (Figure 6b,c). Production rates were much slower in control fermentations (Figure 6a). Nonetheless, the actual yield of alcohol did not vary with addition of nitrogen; 5.5–5.8% (w/v) ethanol was produced regardless of the supplement.

The concentrations of FAN in the wheat mashes were also determined. Within each individual set of fermentations, approximately the same amount of assimilable FAN was present and utilized. It ranged from 27 to 30 mg/L for the control series, from 120 to 130 mg/L when 0.2% yeast extract was added, and from 230 to 240 mg/L when 0.45% yeast extract was available. The increasing level of assimilable nitrogen present in the three series of fermentations corresponded to the amount of yeast extract added. It is apparent that the unusable nitrogen in the yeast food was recycled and accumulated together with the unusable portion normally present in each single wheat mash.

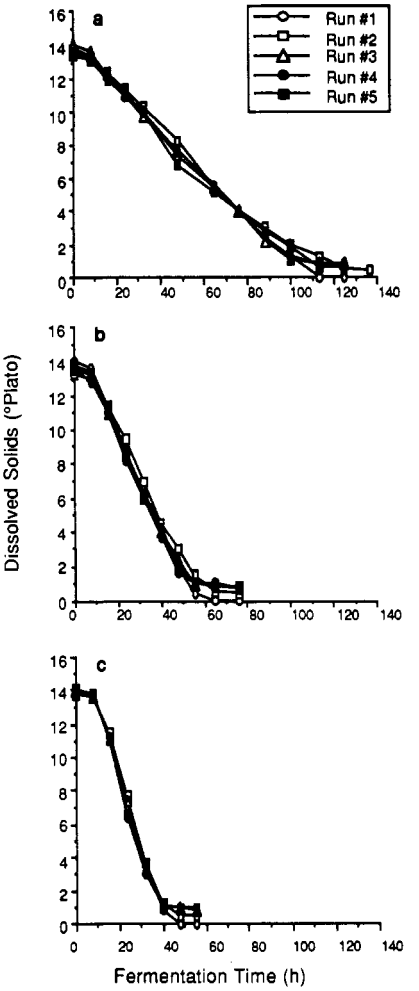

Figure 5. Utilization of dissolved solids over five sequential fermentations of wheat mashes supplemented with yeast extract and containing 50% backset: (a) unsupplemented, fermentation series 1; (b) 0.2% yeast extract, fermentation series 2; (c) 0.45% yeast extract, fermentation series 3.

DISCUSSION

The practice of backsetting resulted in the recycling and accumulation of certain substances, but in general, ions and acids failed to accumulate significantly in the spent fermentation media. We did find that the final specific gravity of the beer increased slightly with each recycling of backset. Since cell growth and ethanol production were not affected in any of the five runs, it appears that all assimilable substrate was being fully attenuated and that the increase in final specific gravity was due to the accumulation of other unusable substances found in wheat mash (none of which were inhibitory).

Yeast cells produce ethanol and CO_2 as the major end-products, but a number of minor products in low concentrations are also formed by yeast, including malic acid, fumaric acid, succinic acid, oxaloacetic acid, citric acid, α-ketoglutaric acid, and glutamic acid (Ingledew, 1993). Acidity results from such compounds. The pH of these wheat mashes decreased from 6 to about 5.5 after the first recycling. As recycling continued, the initial pH values of each mash maintained a similar level. This trend was also reflected in the acid titration curves, indicating that, upon addition of backset, acidic substances from the distillers' solubles of fermentation run 1 were recycled to the next fermentation and contributed to the increase in acidity. The recycling of distillers' solubles from runs 2–4 did not result in similar increases in acidity.

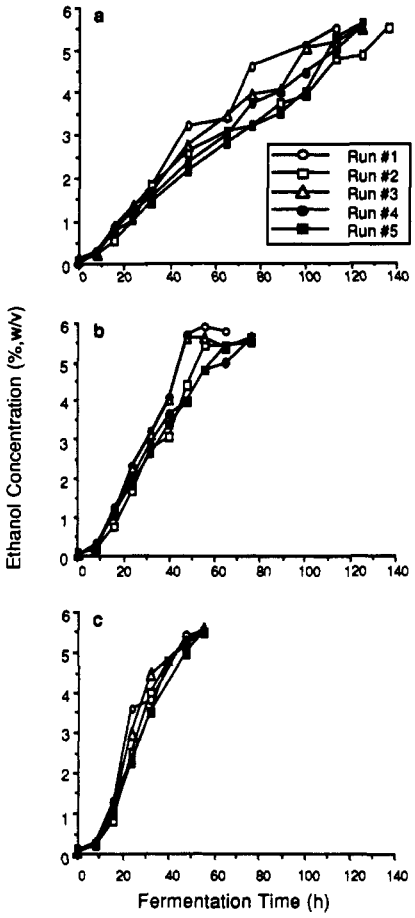

Figure 6. Ethanol production over five sequential fermentations of wheat mashes supplemented with yeast extract and containing 50% backset: (a) unsupplemented, fermentation series 1; (b) 0.2% yeast extract, fermentation series 2; (c) 0.45% yeast extract, fermentation series 3.

Low molecular weight assimilable nitrogen is needed by yeasts for growth. It is generally believed that 150 mg/L of FAN is required for normal brewery fermentations (Ingledew, 1993). The assimilable nitrogen provided by this wheat was approximately one-fifth of the level needed in brewing fermentations When nitrogen is deficient in worts and grape juices, stuck or sluggish fermentations often occur (O'Connor-Cox and Ingledew, 1989). This did not occur in this work. Even unsupplemented fermentors completely fermented out. This phenomenon was previously noted in wheat mashes by Thomas and Ingledew (1990). Moreover, as the fermentation runs continued, a small amount (17 mg/L in run 1) of soluble, unusable nitrogenous materials recycled and accumulated in the wheat mashes. With backsetting, a portion of this FAN was introduced to the next mash. The unassimilable FAN concentration increased by 10 mg/L after one recycling. Following the second fermentation run, the FAN value increased slightly to 52 mg/L in the mash of run 3. The total FAN readings thereafter remained at approximately the same level.

In all three cases above where recycling of substances was observed, the accumulation rates appeared to be similar. Following the first fermentation run, as laboratory distillers' solubles were recycled, the accumulation of the substances showed a marked increase. When the additional backsetting was carried out, further accumulation occurred at a much lower level or no accumulation at all was detected. This was expected. When the same proportion (50%) of distillers' solubles was reused each time, the percentage of increase in each fermentation run would be reduced. This concept is explained as follows. As an example, assume that wheat provides each mash 7 mg/L of unusable FAN. When the first 50% backsetting is carried out, theoretically 50% of the unusable nitrogen in the first mash is introduced to the second mash. At the end of this second fermentation, the amount of unassimilable FAN left in the mash is equivalent to 10.5 mg/L, the amount recycled (and diluted) from the last fermentation (3.5 mg/L) plus the inherent amount of unusable FAN normally provided by wheat (7 mg/L). As backsetting continues, the mathematics dictate that the unusable FAN in each fermentation continues to increase. However, the amount at each increase is only half of the last fermentation. Hence, the FAN accumulation in mash increased rapidly in the first few fermentation runs. As the series continued, the accumulated FAN eventually leveled out at a value twice that of the unused FAN in the first fermentation. The formula for the change in concentrations of FAN can be written

$$Y_n = x(a^0 + a^1 + a^2 + a^3 + \ldots + a^n) \qquad (1)$$

where Y_n is the amount accumulated after n cycles, x is the concentration of unused material in the first mash, a is the backset as a fraction of total mash volume (e.g., the 50% backset value is 1/2), and n is the number of cycles carried out. All substances, if they are introduced and/or produced in each fermentation run at a reproducible concentration, would follow this same pattern of accumulation. The data indicate that after the fourth or fifth fermentation run, the accumulated substance will no longer increase by a significant amount. This finding allowed us to conclude each experiment after four or five recyclings.

Importantly, the use of 50% backset over five consecutive fermentations reduces water usage in mashing (and effluent in stillage) by 40%. This value also increases asymptotically to 50% if recycling continues. This demonstrates the importance of this practice to the fuel alcohol industry. This experiment has also demonstrated that at 50% recycling of backset over five fermentation runs yeast growth and ethanol production were not affected. Values of around 6% (w/v) ethanol were consistently produced from a 30-gal wheat mash of 14 °Plato. The (recycling and) accumulation of a number of known inhibitors was monitored. Although some did appear to accumulate, none apparently reached a concentration sufficient to impede yeast growth and fermentative ability. The results suggest that in wheat-based ethanol plants safe reuse of distillers' solubles at 50% over at least five runs can be contemplated without adverse effects on yeast fermentation. The above relationship suggests that with no further inputs or system disturbances 50% backset can be continued indefinitely without problem. This consideration would be severely compromised, however, by bacterial action, by sporadic additions of acids or bases which would unbalance the system, and by the use of very hard water in mash preparation.

It is also clear from this work and other studies where urea, ammonium salts, and amino acids were used (Thomas and Ingledew, 1990, 1992) that there is a deficiency of nitrogenous nutrients in wheat mashes and that recycling of backset as described in this study does not overcome the lack of usable nitrogen. Nitrogenous supplementation helps to dramatically improve alcohol production rates. The final attenuation time was reduced to 40% of the original time (approximately 45 h). Although the few unusable constituents found in yeast extract were recycled and accumulated in the distillers' solubles, they did not

affect yeast growth and fermentation. In each individual series of fermentations, no inhibition in yeast metabolic rate was observed.

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