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Properties and Performance of Glucoamylases for Fuel Ethanol Production

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Abstract

Studies were conducted on maltodextrin saccharification and on simultaneous saccharification and fermentation (SSF) with various commercial glucoamylases. In kinetics studies, none of the glucoamylases were able to completely convert maltodextrin into glucose. Typically, about 85% conversion was obtained, and glucose yields were about 75%. Typically, the kinetics were biphasic, with 1 h of rapid conversion, then a significant reduction in rate. Data were consistent with strong product inhibition and/or enzyme inactivation. Some glucoamylases followed first-order kinetics, initially slower at dextrin conversion, but eventually achieving comparable conversion and glucose concentrations. Most of the glucoamylases were more active at 55°C than at 35°C, but pH had little effect on activity. Screening studies in an SSF system demonstrated little difference between the glucoamylases, with a few exceptions. Subsequent targeted studies showed clear differences in performance, depending on the fermentation temperature and yeast used, suggesting that these are key parameters that would guide the selection of a glucoamylase.

Index Entries: Glucoamylase; kinetics; fermentation; saccharification.

Introduction

Glucoamylase is an exoglycosidase responsible for hydrolyzing the terminal α-1,4 glucosidic bonds of dextrins and related oligo- and polysaccharides. The reaction involves a proton transfer by acid catalysis, followed by formation of a transition state analogous to an oxocarbonium ion, and finally, a base-catalyzed nucleophilic attack of water *(1)*. Glutamic acid present in different regions of the enzyme-active site is thought to act as the acid and base catalysts required for the reaction. A typical three step reaction scheme is as follows:

$$E + S \xleftrightarrow{K_1} ES_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2 \xrightarrow{k_{cat}} E + P \qquad (1)$$

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where E represents the enzyme, S represents the substrate, P represents the product, ES_1 and ES_2 are enzyme–substrate complexes, k_{cat} is the reaction rate constant for the species in question, and K_1 is its equilibrium binding constant.

Two of the three reaction steps are pH-dependent, involving the protonation of the enzyme before catalysis. The kinetics schemes used to describe maltodextrin saccharification have ranged from simple Michaelis-Menten expressions *(2)* to complex schemes that account for enzyme inhibition *(3)* and glucose condensation reactions into maltose and isomaltose. Additional complexity arises owing to the fact that each reaction product from maltodextrin hydrolysis acts as a substrate for subsequent conversion. For example, assuming a maltodextrin with a degree of polymerization (DP) equal to six, the following hydrolysis reactions may occur:

$$DP6 + H_2O \rightarrow DP5 + DP1$$

$$DP5 + H_2O \rightarrow DP4 + DP1$$

$$DP4 + H_2O \rightarrow DP3 + DP1$$

$$DP3 + H_2O \rightarrow DP2 + DP1$$

$$DP2 + H_2O \rightarrow 2\ DP1$$

where, DP1 = glucose; DP2 = maltose; DP3 = maltotriose; DP4 = maltotetraose; DP5 = maltopentaose; and DP6 = maltohexaose. Each species may possess different binding (K_1) and reaction (k_{cat}) rate constants, in accordance with scheme *(1)*.

Additional products may form owing to condensation reactions between various components in the system. In a typical mash following liquefaction, maltodextrins of size DP15–DP20 may be present.

Peeva and Yankov *(4)* proposed a mathematical model to describe the key steps in the conversion of maltodextrins into ethanol. Reactions to form dextrins DP5 and larger were simulated by a lumped first order model; subsequent reactions to form DP4, DP3, DP2, and DP1 were described using a standard Michaelis-Menten model, with different reaction velocities (V_{max}) and Michaelis constants (K_m) for each species. Finally, the conversion of glucose into cells and into ethanol was described using Monod kinetics, wherein the expression for the specific growth rate of yeast cells was modified to account for inhibition by glucose.

Maltodextrin hydrolysis can be conducted in batch saccharification tanks at about 55°C for about 3–4 h, followed by fermentation, or a simultaneous saccharification and fermentation (SSF) step may be adopted, in which the mash, glucoamylase, yeast and nutrients are all added to the fermenters and incubated at about 33°C for up to 70 h. SSF processing is most common, since it reduces the risk of infection, and also reduces the impact of product inhibition on glucoamylase activity, because glucose/maltose are

consumed by yeast, and their concentrations do not build up appreciably. In this latter case, the glucose and maltose (DP1 and DP2) produced via scheme one, above, are converted into ethanol, carbon dioxide and other products by the yeast.

The objective of this study was to compare the activities of various glucoamylases under standard saccharifying conditions, and identify characteristics of importance for ethanol production. Subsequent "mini-fermenter" trials were conducted to assess the performance of these enzymes for ethanol production under SSF conditions.

Materials and Methods

Glucoamylase Kinetics

Dextrin (5 or 10 g), citrate buffer 25 mL and pH 4.8 (0.025 *M*) and glucoamylase were combined in an 80-mL jacketed glass reactor. The temperature was controlled at 55°C by water flowing through the jacket, and a magnetic stirrer was used to mix the contents of the vessel. The duration of each experiment ranged from 4 to 48 h, depending on whether initial rate data or a full set of kinetics data were desired.

One mililiter samples were collected during the trial at designated time intervals. The sample was immediately inactivated with 0.10 mL HCl solution (pH=1.05, 25°C). From the inactivated sample, 0.2 mL was added to 1.8 mL of filtered deionized water and 1 mL of 0.075 *M* mannose (internal standard). This preparation was subsequently centrifuged to eliminate solid impurities. The supernatant was collected and transferred to vials for subsequent high-performance liquid chromatography (HPLC) assay. The HPLC system was equipped with a Bio-Rad HPX 87C carbohydrate analysis column and a Shodex RI-71 refractive index detector. Deionized, filtered (0.2 nm) water was used as the mobile phase, with a flow rate of 0.6 mL/min and temperature of 85°C. The run time for each sample was 14.1 min. For fermentation trials, an HPX 87H column was used, with a run time of 22.5 min. Samples were injected using an autoinjector. Glucoamylases studied in these experiments were obtained from a variety of commercial suppliers. For the purpose of comparison, glucoamylases are labeled Glu-1 through Glu-11.

Effects of pH

The baseline kinetics assay was conducted, except that the buffer pH was set to 3.6, 4.0, 4.4, or 5.2, to complement the data originally collected at pH 4.8.

Effects of Temperature

The baseline kinetics assay was conducted, except that the reaction temperature was set at 35°C, to more closely replicate the conditions in an SSF system.

Mini-Fermentation Trials

These trials were based on the procedure proposed by Allain et al. *(5)*, wherein small-scale fermentations are tracked based on loss of mass from the system. The premise is that, as glucose is fermented to ethanol, carbon dioxide is also produced and emitted from the system, according to the reaction:

$$C_6H_{12}O_6 \rightarrow 2\,CO_2 + 2C_2H_5OH$$

On a mass basis, each 100 g of glucose should produce 48.9 g of carbon dioxide and 51.1 g of ethanol, assuming no side reactions. Thus, a mini-fermenter should experience a decrease in mass equal to about one-half of the original substrate mass added to the vessel. Note that this does not account for the mass gain owing to dextrin hydrolysis. For example, 100 g of 100% maltohexaose (DP6) would produce about 109 g of glucose.

This is a relatively crude method, in that it does not account for mass losses owing to evaporation of volatile components in the vessels, such as ethanol or acetaldehyde. Nonetheless, data collected from these "mass-loss" experiments correlated well with HPLC measurements from parallel experiments wherein samples were collected. Thus, these mass-loss experiments provide a reasonable representation of the SSF process.

In these trials, different glucoamylases were compared under equivalent conditions to assess their efficacy for fermentation. Each trial used a control which received all necessary reaction components except yeast and glucoamylase. In some trials, maltodextrin was used as the substrate, using citrate buffer as the reaction medium. In other trials, corn mash was prepared by 3 h of slurry/liquefaction using α-amylase, and then transferred to the mini-fermenters for a subsequent SSF trial. Before adding yeast or glucoamylase, the mash was cooled to about 35°C, and the pH was adjusted to about 4.5 by addition of HCl.

Initial screening tests were performed to establish the dose of yeast and glucoamylase. Initial experiments were conducted with baker's yeast; subsequent trials used brewer's yeast (Red Star, LeSaffre, Headland, AL).

Reactions were conducted in a 125 mL (unjacketed) vessel. Thus, the temperature followed ambient conditions, which ranged between 23 and 27.5°C. For each 10 mL of liquefied corn mash added to the vessel, five drops of 5% (w/v) HCl and 0.09 g of yeast were added. The glucoamylase dose was varied from 4 to 50 μL. The mass of the empty vessel was recorded, and the mass was also recorded after each item was added. The final mass after adding parafilm and an elastic seal was also recorded. A small (approx 2 mm) hole was punched in the parafilm to allow CO_2 to escape. Typically, several vessels were run simultaneously in a single trial, along with a control vessel (to account for evaporative losses). The mass of each vessel was recorded at several points in time, over 48–120 h. The mass loss was corrected to account for the mass loss in the control vessel (which did not contain enzyme or yeast), and plotted to compare the glucoamylases used in each trial.

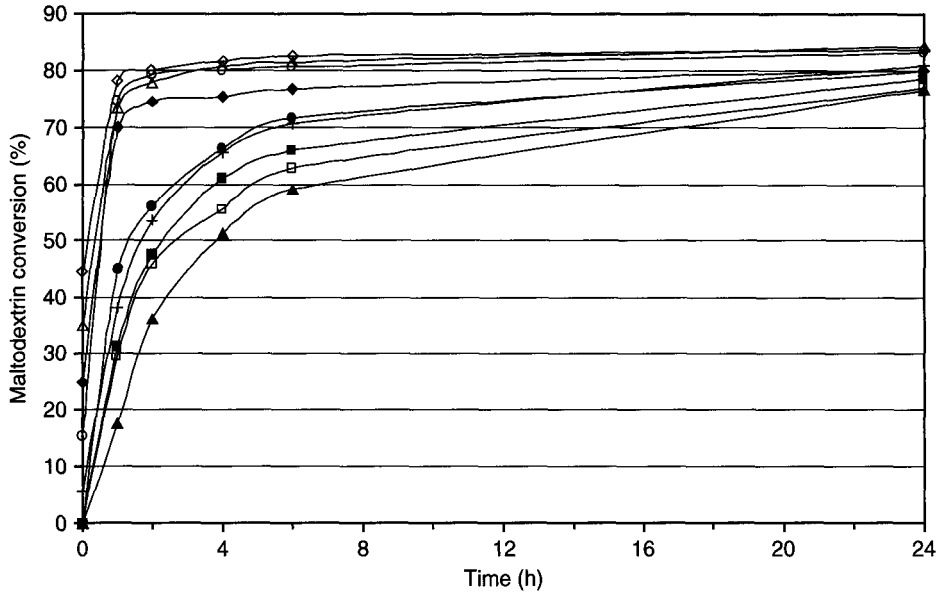

Fig. 1. Dextrin conversion with various glucoamylases at 55°C, 10 g maltodextrin, and 125 µL of glucoamylase at pH 4.8. ♦, Glu-1; ■, Glu-2; ○, Glu-3; •, Glu-4; □, Glu-5; ▲, Glu-6; +, Glu-7; Δ, Glu-8; ◊, Glu-9.

Owing to slight differences in substrate, mixing conditions and temperature histories encountered in the different trials, comparisons among trials are not justified. However, within each trial, comparisons can be made because all parameters within a trial were kept constant, and each vessel would experience the same temperature history.

Results and Discussion

Glucoamylase Kinetics

Experiments were conducted to compare the different glucoamylases, the effect of enzyme dilution on conversion, and the effect of enzyme dose. Subsequently, the kinetics of each reaction was compared using simple models.

Figure 1 shows the maltodextrin conversion profiles arising from studies at pH 4.8 and 55°C for nine of the glucoamylases. There was a dramatic difference in conversion rate. For example, Glu-1 reached a plateau within 4–5 h, corresponding to about 80% conversion of maltodextrin. Conversely, Glu-4, -5, and -6 converted the dextrin more slowly, but sustained this conversion over 24 h. Ultimately, there was very little difference in the dextrin fractional conversion after a 24-h reaction; all enzymes had converted about 80% of the available dextrin. The observed plateau in substrate conversion indicates either strong end product inhibition or enzyme inactivation. A similar lack of complete conversion was observed by Peeva and Yankov (4), who studied the SSF of maltodextrins from starch using Diazyme L-200.

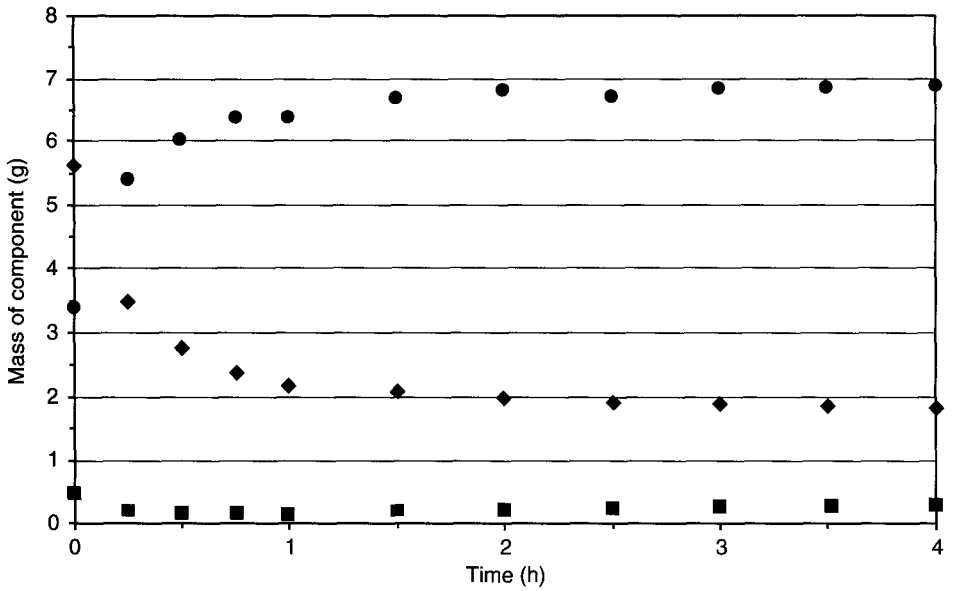

Fig. 2. Maltodextrin conversion by Glu-9, 10 g maltodextrin, and 125 µL of glucoamylase at pH 4.8. ♦, maltodextrin; ■, maltose; •, glucose.

The glucose production rates arising from the kinetics trials were generally consistent with the dextrin conversion profiles. Some enzymes (e.g., Glu-1, -3, -8, and -9), exhibited very rapid glucose production, with yields approaching 70–75% of the originally available dextrin within as little as 15 min. However, the glucose levels then reached a plateau, with little additional glucose produced over the remaining 24 h of reaction. Such a rapid increase in glucose concentration may not be suitable for fermentation; high glucose levels can inhibit yeast *(6)*, and gradual feeding of glucose to yeast is generally preferred. Conversely, glucose profiles arising from Glu-4, -5, and -6 did not plateau, but continued to increase over the duration of a 24-h reaction. Although the initial glucose production rate was slower with Glu-4, -5, and -6, there was sustained glucose production throughout the reaction, which is thought to be beneficial for fermentation.

Generally, maltodextrin profiles of Glu-1 and Glu-3 were comparable, regardless of enzyme dose or reaction conditions. However, glucose levels were about 5–10% lower when Glu-3 was used and maltose levels were correspondingly higher. Thus, whereas the rate constants (k_{cat} values) for larger dextrins were comparable, the rate constant for conversion of maltose to glucose was clearly greater for Glu-1. Glu-9 showed much more rapid maltodextrin conversion—almost 65% in the first 15 min of reaction, with a corresponding rapid increase in glucose (Fig. 2). However, there was less overall conversion to glucose, with only about 70% of the maltodextrin converted to glucose.

Glu-4, -5, and -6 are different strength formulations of the same enzyme. The dextrin conversion (Fig. 1) and glucose production profiles

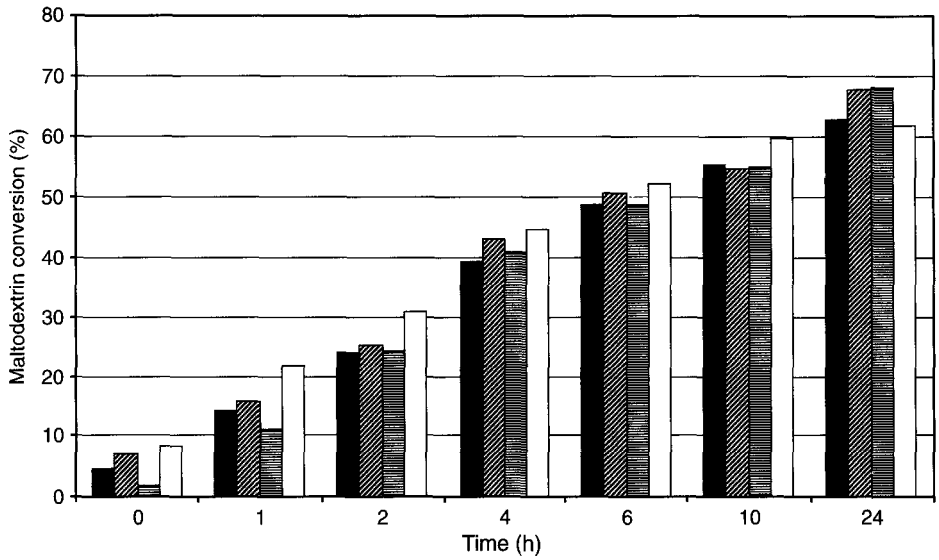

Fig. 3. Effect of pH on dextrin conversion by Glu-5, 10 g maltodextrin, and 125 µL of glucoamylase at 35°C. ■, pH 3.6; ▤, pH 4.4; ▧, pH 4.8; □, pH 5.2.

with Glu-4, -5, and -6 show different initial dextrin conversion rates, but the profiles converge after about 24 h. Similarly, Glu-4 produces more glucose during the early stages of the reaction, but by 24 h, the total glucose produced is essentially equivalent. Glu-6, the weakest formulation of the three, shows the lowest rates of maltodextrin conversion and glucose production, reaching about 80% conversion after about 32 h of reaction. Nonetheless, the different formulations demonstrate the opportunity to control the rate of glucose delivery to the fermenters. There is also a likely opportunity to tailor the formulation to the particular needs of a plant or process, to match the glucose production rate with the glucose requirements demanded by the yeast.

Effects of pH

The effect of pH on the kinetics of maltodextrin conversion at 35°C is shown in Fig. 3, using Glu-5. There is little effect of pH during the first 12 h of reaction, but, at 24 h, the conversion is slightly better at pH 4.4 and 4.8 (approx 68%) than at pH 5.2 and 3.8 (approx 60%). Interestingly, the greater dextrin conversion at pH 4.4 and 4.8 does not correspond to higher glucose concentrations; instead, at about 24 h, glucose concentrations are about 10% higher if the pH is 3.8 or 5.2 (Fig. 4). Collectively, these results imply greater conversion of the maltodextrin substrate at pH 4.4 and 4.8, but reduced conversion of reaction intermediates (maltotriose, maltose, and so on) into glucose. Consequently, k_{cat} values for large polysaccharides exhibit a maximum at about 4.6 as the pH is increased from 3.6 to 5.2, whereas k_{cat} values for smaller oligosaccharides exhibit a minimum over the same pH range. Nonetheless, these differences in dextrin conversion

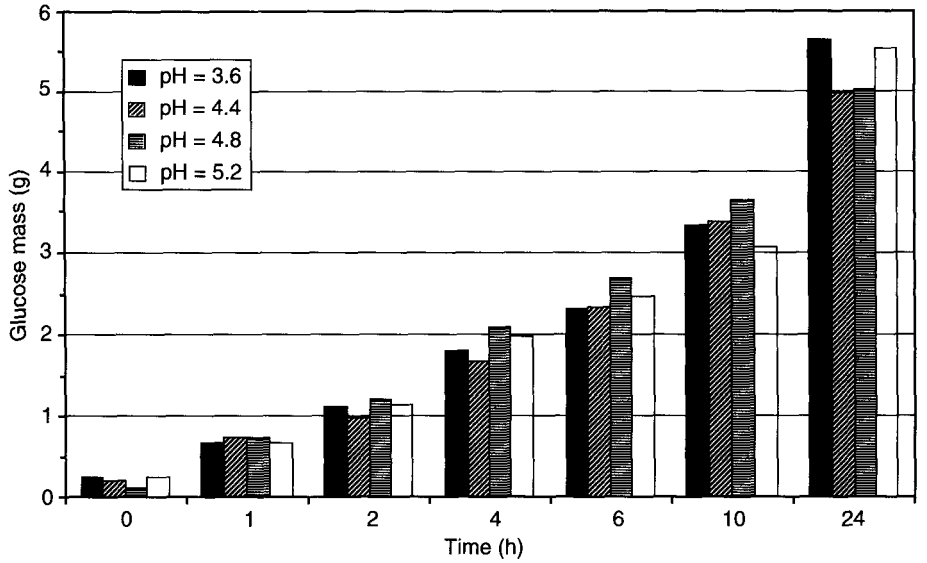

Fig. 4. Effect of pH on glucose production by Glu-5, 10 g maltodextrin, and 125 µL of glucoamylase at 35°C. ■, pH 3.6; ▤, pH 4.4; ▧, pH 4.8; □, pH 5.2.

and glucose production are quite small. Considering that the normal pH during fermentation is between 4.2 and 4.8, it is apparent that the pH-activity profile for Glu-5 is suitable for SSF systems. Although other glucoamylases showed a slightly greater dependence on pH, the overall effect of pH was still fairly mild, affecting dextrin conversion and/or glucose production by up to 20%.

Effects of Temperature

The effects of temperature on maltodextrin conversion and on glucose production by Glu-5, -7, and -8 are shown in Figs. 5 and 6, respectively.

Increasing the temperature from 35°C to 55°C caused the dextrin conversion by Glu-5 to increase from about 68–80% over 24 h of reaction. Glucose concentrations increased by about 40% owing to the increase in temperature, suggesting that temperature had a large effect on k_{cat} values for small oligosaccharides, but a comparatively smaller effect on k_{cat} values for the larger polysaccharides. Glu-8, regardless of temperature, produced glucose much more quickly than Glu-7, and Glu-7 produced nearly twice the amount of glucose at 55°C than at 35°C during the first 5 h of reaction. However, at longer times, the effect of temperature on glucose profiles from Glu-7 was less. Furthermore, Glu-7 eventually caught up with Glu-8; at 55°C, glucose levels are nearly equivalent after about 24 h of reaction. Surprisingly, temperature had little effect on maltodextrin conversion by Glu-8; profiles were nearly equivalent at both temperatures, with a very rapid initial rise, then a plateau (Fig. 5). For Glu-7, the effect of temperature was much more pronounced. The initial maltodextrin conversion rate with Glu-7 was actually faster at 35°C, but beyond the 1 h of

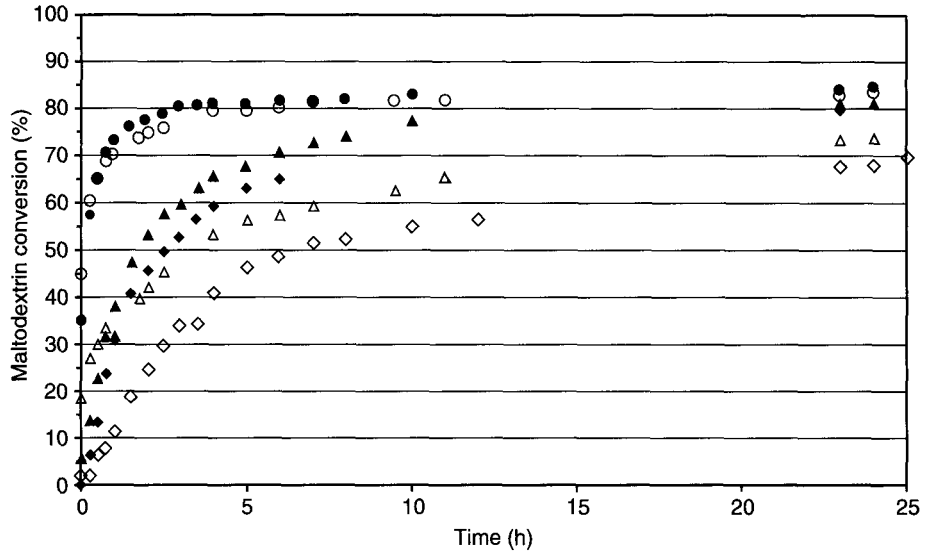

Fig. 5. Effect of temperature on maltodextrin conversion, 10 g maltodextrin, and 125 µL of glucoamylase at pH 4.8. ♦, Glu-5, 55°C; ▲, Glu-7, 55°C; ●, Glu-8, 55°C; ◇, Glu-5, 35°C; Δ, Glu-7, 35°C; ○, Glu-8, 35°C.

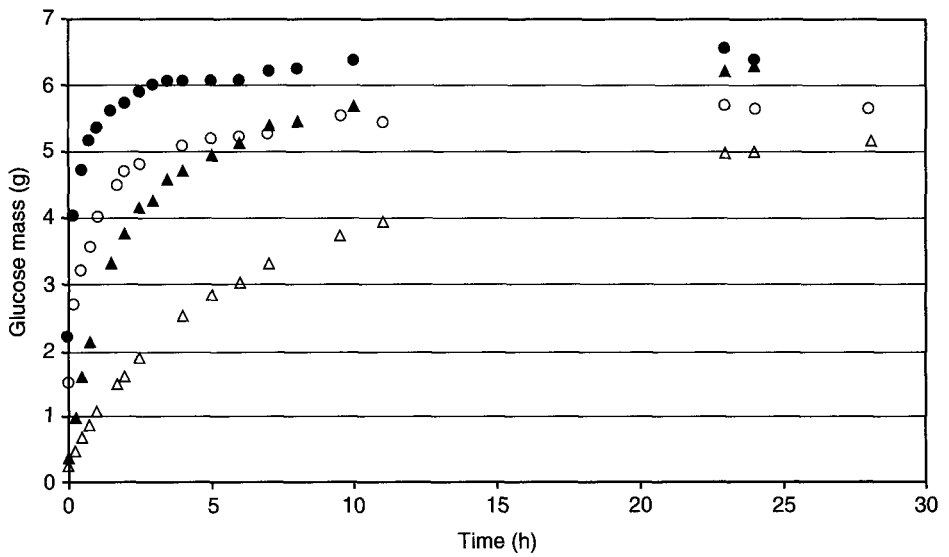

Fig. 6. Effect of temperature on glucose production, 10 g maltodextrin, and 125 µL of glucoamylase at pH 4.8. ▲, Glu-7, 55°C; ●, Glu-8, 55°C; Δ, Glu-7, 35°C; ○, Glu-8, 35°C.

reaction, the conversion (and rate) was greater at 55°C. The glucose profiles, conversely, consistently showed a greater rate of formation at 55°C vs 35°C. Thus, the higher degree of maltodextrin conversion during the first hour of reaction at 35°C did not translate into additional glucose, but rather, an accumulation of intermediate products.

The kinetics profile for Glu-8 suggests that temperature has little effect on k_{cat} for the larger polysaccharides, but has a significant effect on k_{cat} for

Table 1
Fermentation Efficiency of Various Glucoamylases

Enzyme	Average mass loss rate over 99 h, mg/h
Glu-3	48
Glu-4	45
Glu-7	28
Glu-8	46
Glu-9	46
Glu-11	9
Glu-10	46

the smaller species, such has maltotetraose and maltotriose. Experimentally, there was little difference in maltose concentrations at the two temperatures, but the material balance was much worse at 35°C. Thus, more of the original maltodextrin was unaccounted for at 35°C, which implies greater quantities of maltopentaose and maltotetraose, compounds that are not easily detected by the current HPLC assay. The kinetics profiles for Glu-7 indicate an enzyme designed for greater activity at lower temperatures (as evidenced by the higher initial dextrin conversion rate at 35°C), but also subject to end product inhibition, since the reaction rate slows dramatically as the concentration of glucose increases. Such end product inhibition is more severe at lower temperatures, based on the data obtained.

Mini-Fermentation Trials

Preliminary mini-fermentation (SSF) trials were conducted to compare the performance of various glucoamylases. In these experiments, 50 mL of liquefied corn mash was treated with 20 μL of glucoamylase and 0.45 g of baker's yeast. The mass loss was determined at regular intervals over 99 h, and corrected by subtracting the mass loss in a control vessel (i.e., without enzyme or yeast). The average mass loss produced using each enzyme is shown in Table 1. For the most part, overall mass loss rates were comparable, except for Glu-7 and Glu-11, which were substantially poorer than Glu-3, -4, -8, -9, and -10. Glu-11 consistently lagged all other glucoamylases throughout the experiment, whereas Glu-7 performed comparably with the other glucoamylases over the first 24 h, but lagged thereafter.

Figure 7 demonstrates the effect of enzyme dose on fermentations conducted with Glu-4 and Glu-7. The baseline dose, 20 μL, is close to that used in dry-mill plants, on a volume per substrate mass basis. The reproducibility of the procedure is evident, with a 2–5% variation in mass loss from replicate trials. As expected, the mass loss rate increased with dose; at low doses of Glu-7, the mass loss rate dropped off significantly after the initial 24 h of fermentation. In subsequent studies to examine the effect of dose on the performance of Glu-4 (data not shown), it was observed that each 5 μL increase in enzyme dose increased the average mass loss rate by

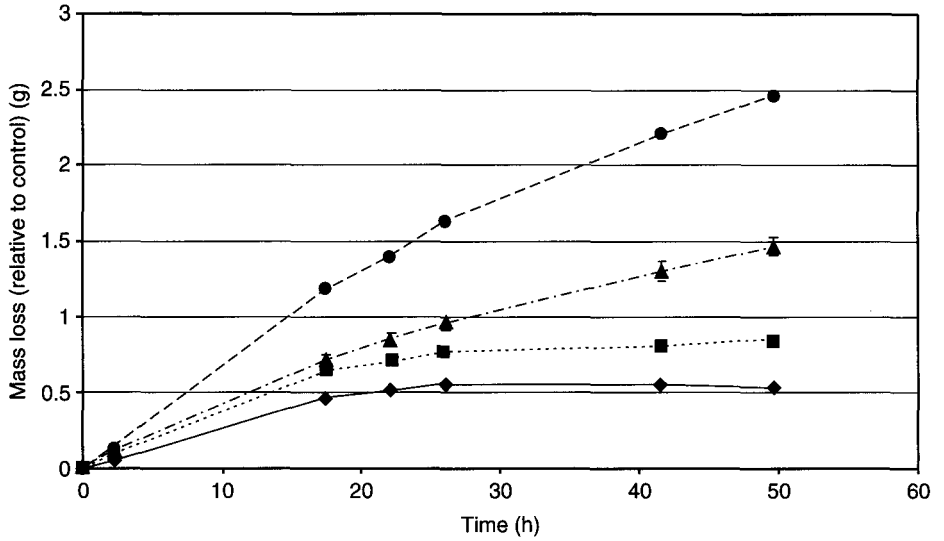

Fig. 7. Fermentation of corn mash with glucoamylase. ■, Glu-7 (8 µL); ♦, Glu-7 (4 µL); ▲, Glu-7 (20 µL); ●, Glu-4 (20 µL). Error bars represent ± SD. Error bars for Glu-4 are contained within the size of the symbol.

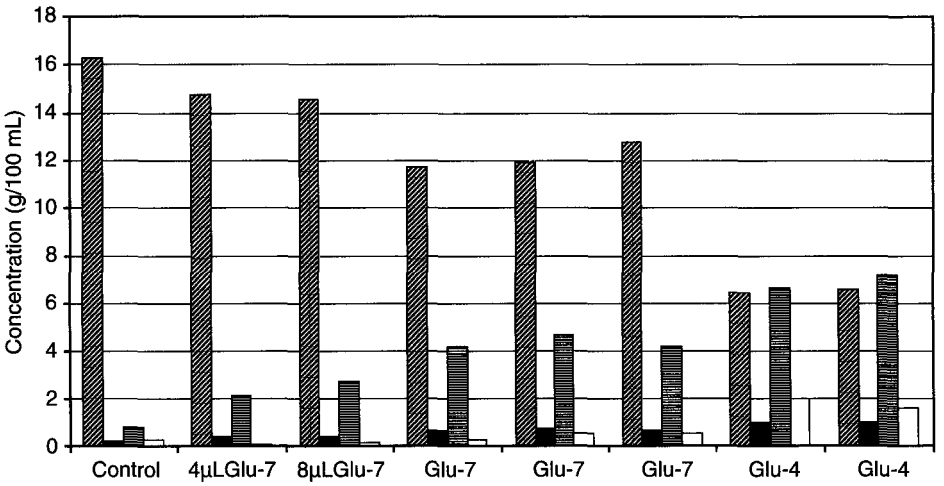

Fig. 8. HPLC analyses of carbohydrates and fermentation products from SSF studies. Liquefied corn mash treated with 0.45 g baker's yeast and 20 µL glucoamylase (unless otherwise specified); Control has no yeast or enzyme. ▨, Maltodextrin; □, Glycerol; ▤, Ethanol; ■, Glucose.

about 7 mg/h. Furthermore, at each enzyme dose, Glu-4 outperformed Glu-7, in spite of the observation that, during kinetics studies, Glu-7 produces glucose more rapidly.

Figure 8 shows that the carbohydrate and ethanol profiles (as measured by HPLC) are consistent with the mass loss data shown in Fig. 7. The HPLC results further support the observation that Glu-4 is superior to

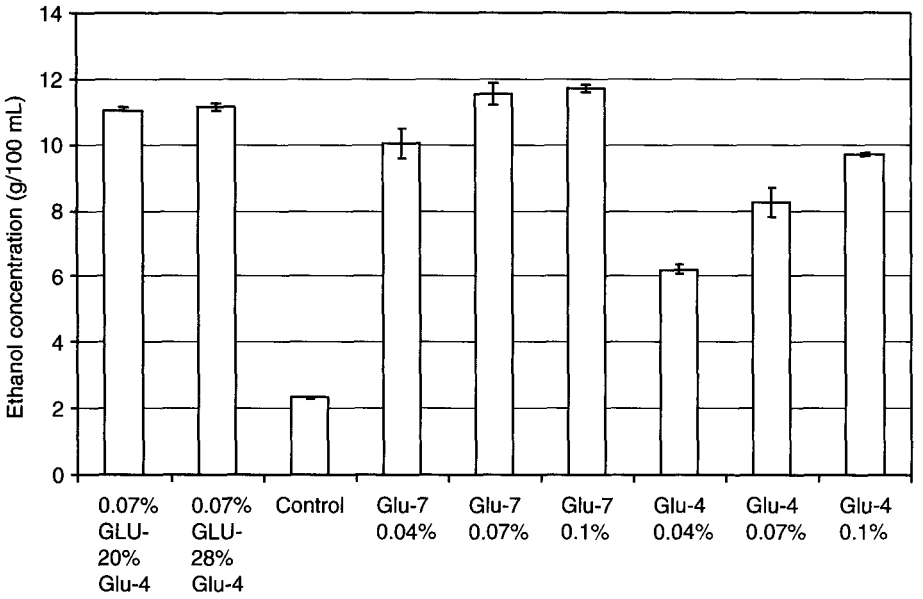

Fig. 9. Effect of glucoamylase, dose, and blends on ethanol production in SSF. Liquefied corn mash (pH 4.5) treated with 0.35 g brewer's yeast; control has no enzyme. Data shown as mean ± SD; For single enzymes, percentages refer to dose in wt%; For blends, the first percentage refers to the total glucoamylase dose (GLU) in wt%, and the second percentage refers to the percentage of Glu-4 in the Glu-4/Glu-7 mixture.

Glu-7, based on these trials with baker's yeast. Interestingly, the HPLC results also show that Glu-4 provided superior dextrin conversion than Glu-7, contrary to prior observations from kinetics studies. However, Fig. 5 also shows that Glu-7 is sensitive to temperature, and thus, the reduced performance of Glu-7 might be owing to the lower fermentation temperatures used in these studies.

Subsequent mini-fermentation studies were performed using brewer's yeast, with the temperature controlled at 33°C. In these studies, the performance of Glu-4 and Glu-7 was compared, and the performance of several blends of these two glucoamylases was also studied, to replicate conditions in some dry-mill plants that use a blend of commercial glucoamylases. The effect of enzyme dose was also examined, with doses ranging from 0.04 to 0.10 wt%. The results from these studies are shown in Figs. 9 and 10. When blends were used, the total dose was maintained at 0.07 wt%, and the percentage of Glu-4 in the blend was varied. Trials using blends are designated with "GLU" in Figs. 9 and 10, with a total glucoamylase (GLU) dose of 0.07 wt%.

It is apparent that, under these conditions, Glu-7 outperforms Glu-4, regardless of dose. Glu-7 produced higher ethanol levels, lower dextrin levels, and lower glucose levels than Glu-4. However, blends of Glu-4 and Glu-7 produced ethanol levels comparable to those obtained when Glu-7 was used alone, in spite of higher dextrin and glucose levels at the end of

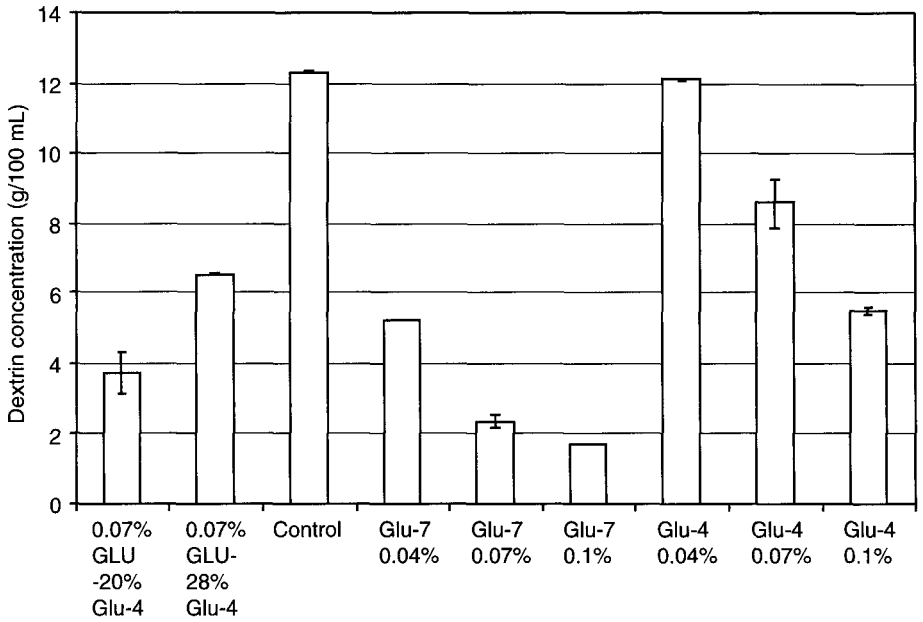

Fig. 10. Effect of glucoamylase, dose, and blends on dextrin conversion in SSF. Liquefied corn mash (pH 4.5) treated with 0.35 g brewer's yeast; control has no enzyme. Data shown as mean ± SD. For single enzymes, percentages refer to dose in wt%; For blends, the first percentage refers to the total glucoamylase dose (GLU) in wt%, and the second percentage refers to the percentage of Glu-4 in the Glu-4/Glu-7 mixture.

fermentation. This result suggests that the yeast is making more effective use of sugars produced using the glucoamylase blend, but the comparably higher dextrin and glucose levels also indicate the need to optimize the fermentation to use all of the available sugars.

The difference in relative performance with Glu-4 and Glu-7 in these mini-fermentation studies underscores the important relationship between glucoamylase function, temperature, and yeast selection. Although Glu-4 proved superior at low temperatures with baker's yeast, Glu-7 was superior in trials at 33°C using brewer's yeast. Temperature has a substantial effect on glucose production rate, and the rate of glucose production will also affect yeast performance. It is expected that some yeasts will be better able to tolerate high levels of glucose than others, and thus, selection of a glucoamylase (or blend) for use in an ethanol plant must also consider the nature of the yeast used during fermentation. These studies show dramatic variations in glucoamylase performance, with some producing glucose very rapidly but unable to sustain the rapid initial production rate, whereas others generate glucose gradually, and maintain that production over an extended period. Ultimately, a combination of glucoamylases may be desirable, aiming to produce sufficient initial glucose to launch the yeast, and then sustaining glucose production to ensure yeast viability. Glucoamylases also differed substantially in their dependence on

temperature, confirming that this is also an important parameter to consider when selecting a glucoamylase. Changing the pH may lead to subtle changes in glucoamylase performance; evidence suggests a greater impact on glucose production than on overall dextrin conversion.

Conclusions

During kinetics trials, dextrin conversion did not exceed 85%, with a plateau in the quantity of glucose produced, suggesting product inhibition and/or deactivation. The kinetics of Glu-1 and Glu-3 were virtually equivalent under all reaction conditions and enzyme doses. Conversely, Glu-9 produced glucose much more rapidly than other glucoamylases, but also exhibited lower overall dextrin conversion (approx 70%) and a more rapid onset of the plateaus in glucose and dextrin concentrations. This indicates that Glu-9 is more sensitive to product inhibition, or is less stable than other glucoamylases. Glu-7 and Glu-8 also produced glucose fairly quickly.

Dextrin conversion rates with Glu-4, -5, and -6 were slower than those with other glucoamylases over about the first 4 h, but equivalent conversion was obtained within 12–24 h, depending on the formulation used. The persistent production of glucose suggests either a reduced sensitivity to inhibition or a more stable enzyme formulation, and enables "spoon-feeding" of the yeast if desired. pH variations between 3.6 and 5.2 led to small (approx 10%) changes in dextrin conversion and glucose production. Dextrin conversion was maximized at pH 4.0 to 4.8, whereas more glucose was produced at pH 3.6 and 5.2.

Generally, the glucoamylases were significantly more active at 55°C than at 35°C, with the exception of Glu-8, which had nearly equivalent activity at both temperatures. Temperature had a greater effect on glucose production than on maltodextrin conversion.

The mini-SSF trials demonstrated the key relationships between important fermentation parameters: temperature, glucoamylase, and yeast. Mass-loss measurements were consistent with independent measurements using HPLC. Furthermore, the mass loss consistently increased in response to an increase in glucoamylase dose, indicating that the systems were enzyme-limited.

Results from ambient temperature trials with baker's yeast suggested that Glu-4 was superior to Glu-7, but the opposite conclusion was reached from trials at 33°C with brewer's yeast. The strong effect of temperature on glucose production rate and the susceptibility of some yeast to inhibition by glucose may account for these differences, underscoring the importance of these parameters when contemplating selection of a new yeast and/or glucoamylase. There is also some evidence that blends of certain glucoamylases may be beneficial, particularly in case the blend can produce glucose at a controlled rate over an extended time, minimizing inhibition while maintaining yeast viability.

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References

1. Christenson, U. (2000), *Biochem. J.* **349,** 623–628.
2. Nagy, E., Belafi-Bako, K., and Szabo, L. (1992), *Starch/Staerke* **44,** 145–149.
3. Cepeda, E., Hermosa, M., and Ballesteros, A. (2001), *Biotechnol. Bioeng.* **76,** 70–76.
4. Peeva, L. and Yankov, D. (2000), *Bioprocess Eng.* **22,** 397–401.
5. Allain, E. (2004), Lab-Scale Modelling of Fuel Alcohol Fermentations, presented at the 20th Fuel Ethanol Workshop, Madison, WI.
6. Ingledew, M. (1999), In: *The Alcohol Textbook, 3rd edition,* Jacques, K. A., Lyons, T. P., and Kelsall, D.R. (eds.), Alltech, Inc.