FUEL ALCOHOL PRODUCTION FROM RYE AND TRITICALE BY NORMAL AND VERY HIGH GRAVITY FERMENTATION, **S. *WANG,*[2] KC. *THOMAS,*[2]** *W.M.* ***INGLEDEW,*[2]** *K.* ***SOSULSKI,*[1]** *and F. W.* ***SOSULSKI*[3],** 'Saskatchewan Research Council, 15 Innovation Boulevard, Saskatoon, SK Canada S7N 2X8 *Department of Applied Microbiology and Food Science, 'Department of Crop Science and Plant Ecology, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK Canada S7N 5A8.

OBJECTIVES

- To determine the fermentability of rye and triticale by normal and very high gravity fermentations.
- To determine the fermentation rates, ethanol yields, and fermentation efficiencies for normal and very high gravity fermentation.

INTRODUCTION

The current commercial practice in Saskatchewan is to use wheat as the main basic raw material for fuel alcohol production. Considerable laboratory studies and pilot plant trials have been carried out on the fermentation of wheat for fuel alcohol production (Ingledew, 1993, Jones and Ingledew, 1994, Sosulski and Sosulski, 1994, Thomas et al., 1996). As wheat prices have increased significantly in recent years, the need to use less expensive crops, such as rye and triticale, became more and more pressing for industry in order to remain competitive. However, knowledge of the fermentability of rye and triticale for fuel alcohol production is not widely available. A laboratory study designed to address these questions could provide the alcohol industry with information necessary to choose the most economic raw material available in the region at particular time. Increased levels of fermentable carbohydrates in mash, in the Very High Gravity (VHG) fermentations, would lead to increased concentrations of ethanol in the beer, and improved plant productivity.

The current study was undertaken to investigate the fermentability of rye and triticale by normal and VHG fermentations, containing 20.5 and 28.5 g of dissolved solids per 100 ml of mash liquid, respectively.

MATERIALS AND METHODS

One fall rye (PRIMA) and one triticale (AC COPIA) obtained from Agriculture and Agri-Food Canada (Saskatoon, SK), were used in the current study. The starch contents (dry basis) were 63.1 1±0.79% and 64.95±0. 10%, respectively.

Normal gravity fermentations, with an initial 20.5 g dissolved solids per 100 ml, were achieved through mashing at water to grain ratio of 3:1. VHG fermentations, with an initial 28.5 g dissolved solids per 100 ml, were prepared at water to grain ratio of 2: 1.

Ground rye or triticale cereals were dispersed in 300 ml (for normal gravity fermentations), or 200 ml (for VHG fermentations) prewarmed (40°C) distilled water. Calcium chloride solution was added to the slurries to a final concentration of 1 mM. The mash temperature was raised to 60" C. The high temperature α-amylase preparation (Alltech Biotechnology Center, Nicholasville, KY) was added to mash in two portions, each at a concentration of 0.5% (v/w enzyme to ground cereal). Starch hydrolysis with the first portion of enzyme was carried out at about 96° C for 45 min. After lowering the temperature to 80° C, the second portion of enzyme was added and liquefaction was continued for an additional 30 min. For the preparation of VHG rye mashes, 0.0002 g of viscosity-reducing enzyme, Roxazyme G (Hoffmann-La Roche), was added per gram of ground cereal, prior to and after mashing, and incubated at 40° C for 30 min to break down viscosity-causing non-starch polysaccharides. Rye or triticale mash was then transferred into a jacketed fermentor (Wheaton Instruments, Millville, NJ). Saccharification with glucoamylase (Allcoholase II, Alltech Biotechnology Center, Nicholasville, KY) was carried out at 30° C for 30 min at an enzyme concentration of 0.8% (v/w enzyme to ground cereal). Either 5 ml of sterilized distilled water or 5 ml of urea solution was added to the fermentor to a final concentration of 8 or 16 mM for normal and VHG fermentations, respectively. Inoculum (10^7 cells per gram of mash) of Saccharomyces cerevisiae yeast (Alltech Biotechnology Center, Nicholasville, KY) was added to mash cooled to 27°C or 20° C for normal and VHG fermentations, respectively.

Progress of fermentation was monitored at 0, 6, 12, 24, and every 24 hours thereafter until all dissolved solids were consumed. A number of fermentation parameters were measured at each time. Results on mash viscosity, fermentation rate and efficiency, ethanol yield and concentration, and chemical composition of stillages, are presented in the current poster.

Fermentation rate, or rate of utilization of dissolved solids, expressed as grams of sugar per 100 ml of fermenting liquid, were determined at 20°C with a digital density meter (DMA-45, Anton Paar, Graz, Austria). Ethanol was measured by the alcohol dehydrogenase assay (Sigma Bulletin no. 331 U.V., Sigma Chemical Co., St. Louis, MO). Mash viscosity was measured with a starch tester (Model AV 30, Haake Inc., Saddle Brook, NJ), under standard conditions, at 40" C. Parallel fermentations without sampling were set up simultaneously and the total alcoholic mashes were distilled at the end of fermentations for the determination of ethanol yields and fermentation efficiencies.

RESULTS AND DISCUSSION

Mash Viscosity Reduction

Triticale mashes were low in viscosity even when the water to grain ratio used during mashing was 2:1 (Table 1). Mash viscosity lower than 500 Brabender Units (BU) would cause no problems during fermentation.

Table 1 - Mash Viscosity After the Completion of Mashing

	Mash Viscosity (Brabender Units)			
Mashes	Rye		Triticale	
Water to Grain Ratio	3:1	2:1	3;1	2:1
Without Enzyme Treatment	600	1980	102	338
With Enzyme Treatment	N/A	200	N/A	N/A

Rye mashes were high in viscosity (1980 BU) when the water to grain ratio was 2: 1. However, rye mashes could be treated with the viscosity-reducing enzyme, Roxazyme G, to effectively lower the viscosity from 1980 to 200 BU (Table 1). Such treatment was necessary if low water to grain ratio of 2:1 was used for preparation of mash for VHG fermentation.

Fermentability of Rve and Triticale

Successful fermentation of rye and triticale at normal and very high gravities were confirmed by the rates of utilization of dissolved solids in mashes (Fig.1 and Fig.2). Normal gravity fermentations were completed within 72 hours at 27 °C (Fig.1), while VHG fermentations were completed within 144 hours at 20 °C (Fig.2). Rye had higher level of nutrients; its free amino acid nitrogen content was 50% higher than that in triticale. This helps to explain faster fermentation rates for rye. Supplementation with urea significantly increased fermentation rates and shortened time required for the completion of fermentation by 1/3 for triticale, and by 1/6 to 1/4 for rye.

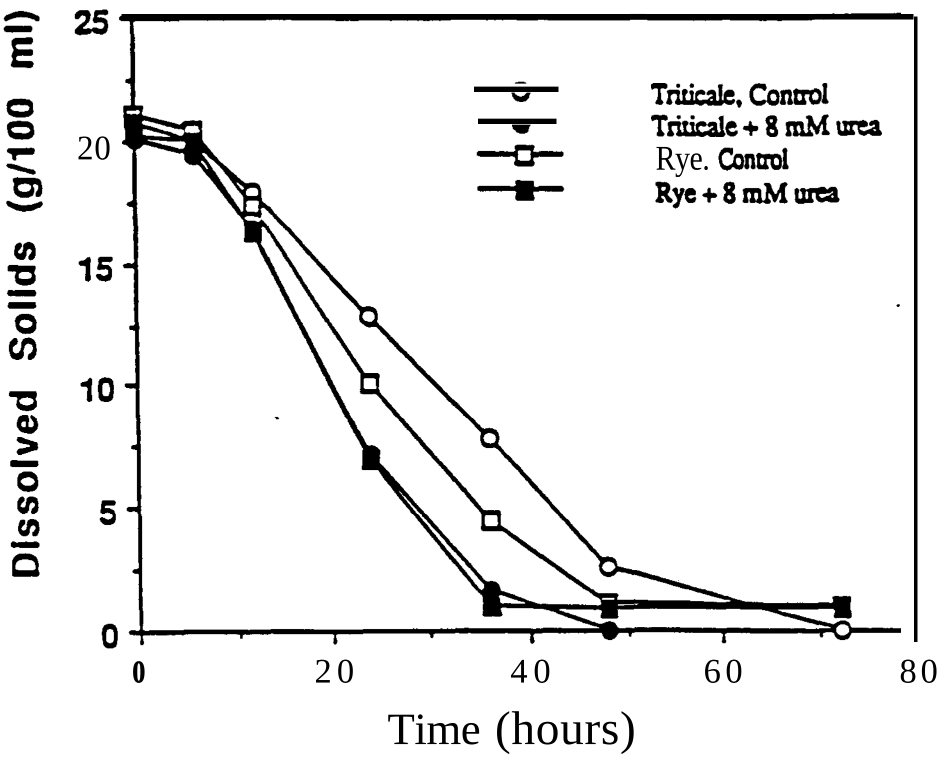

Figure 1 --- Rates of Utilization of Dissolved Solids in Rye and Triticale Mashes During Normal Gravity Fermentations, Fermentation Temperature: 27 "C.

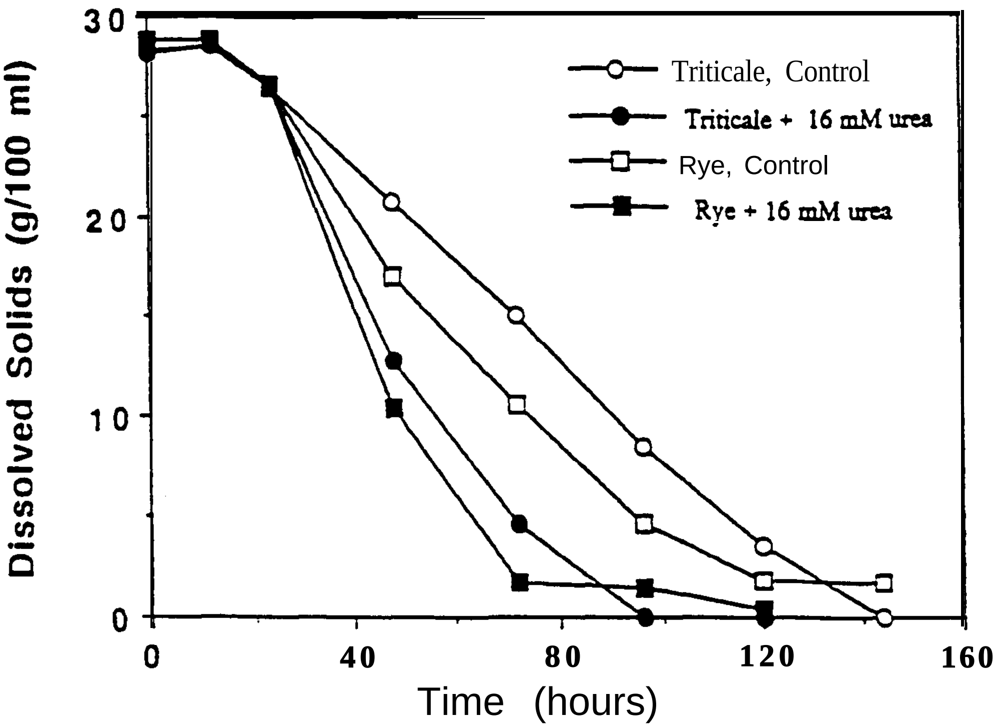

Figure 2 --- Rates of Gtilization of Dissolved Solids in Rye and Triticale Mashes During Very High Gravity Fermentations, Fermentation Temperature: 20 °C.

Ethanol Yields and Fermentation Efficiencies

The reduction in water to grain ratio from 3: 1 to 2:1 did not affect significantly fermentation efficiencies and final ethanol yields per unit weight of grain fermented (Table 2). Urea supplementation accelerated fermentation rates without significantly reducing final ethanol yields and fermentation efficiencies.

Table 2 --- Ethanol Yields and Fermentation Efficiencies"

	Ethanol Yields (Liter/ Tonne of 14% Moisture Grain)				"Fermentation Efficiencies (%Theoretical)			
Cereal	Rye		Triticale		Rye		Triticale	
'Urea Supplementation	No Urea	With Urea	No Urea	With Urea	No Urea	With Urea	No Urea	With Urea
Normal Gravity	363±6	356±11	367±13	362±2	92.9±1.7	91.3±2.9	91.4±3.1	90.2±0.5
Very High Gravity	352±8	352±3	374±1	359±12	90.1±2.1	90.1±0.9	93.0±0.1	89.4±3.1

Average nf two fermentations
"Fermentation efficiency was calculated as the ratio between actual ethanol yield and theoretical ethanol yield, assuming all starch was converted to glucose and then to ethanol.
'Normal gravity fermentations were supplemented with urea to a final concentration of 8 mM, very high gravity fermentations were supplemented with urea to a final concentration of 16 mM.

Fermentation efficiencies were 90-93% for rye and triticale. These fermentation efficiencies were comparable to those of wheat. The final ethanol yields were 350-360 L/tonne for rye, and 360-370 L/tonne for triticale, at 14% grain moisture. For a constant volume of the fermentation vessel, 1/3 more grain was processed in the VHG fermentation than in the normal gravity fermentation. This represented a 35-56% increase in ethanol concentrations in the beer (Table 3). The 1/3 reduction in water use would result in significant saving in energy consumption for mash heating, fermentor cooling, and ethanol distillation. Further increase in alcohol are possible but adjunct addition, or "double mashing", will be needed (Thomas et al., 1996), or the amount of mash insolubles must be reduced by gram preprocessing (Sosulski and Sosulski, 1994, Sosulski et al., 1996) and/or post-mashing separation techniques (Ingledew, 1993).

Table 3 --- The Effects of Very High Gravity Fermentation on Ethanol Concentration.

	Ethanol Concentration in the Beer (%v/v)			
Cereal	Rye		Triticale	
[a]Urea Supplementation	No Urea	With Urea	No Urea	With Urea
Normal Gravity	9.59 ±0.09	9.46 ±0.12	10.03 ±0.06	9.67 ± 0.00
Very High Gravity	12.91±0.08	14.33±0.41	14.75 ±0.16	15.08 ±0.28
Ethanol Concen. Increase	34.6%	51.5%	47.0%	55.9%

[a]Normal gravity fermentations were supplemented with urea to a final concentration of 8 mM, very high gravity fermentations were supplemented with urea to a final concentration of 16 mM.

Chemical Comnosition of Stillages

Analysis of the chemical composition of stillage samples showed that protein and total dietary fibre each accounted for about 1/3 of stillage dry weight, while the remaining 1/3 were ash, fat, residual starch, and fermentation by-products (Table 4).

Table 4 -- Chemical Composition of Fermentation Stillages in Dry Basis

Fermen-tations	Cereals	Urea Supplemen.	Starch (%)	Ash (%)	Fat (%)	Protein (%)	TDF (%)
Normal Gravity	Rye	No Urea	1.08±0.24	5.35±0.01	2.64±0.06	25.00±0.29	32.84±0.32
		8 mM Urea	1.09±0.56	5.32±0.03	2.29±0.22	25.04±1.08	33.83±0.35
	Triticale	No Urea	1.01±0.01	4.94±0.03	4.10±0.09	33.48±0.57	34.00±1.25
		8 mM Urea	1.98±0.39	4.71±0.01	3.55±0.08	34.18±0.66	35.84±0.36
Very High Gravity	We	No Urea	1.52±0.08	5.66±0.01	3.32±0.01	27.72±0.20	28.24±0.72
		16 mM Urea	1.47±0.09	5.42±0.01	3.24±0.13	29.27±0.09	27.89±0.18
	Triticale	No Urea	1.67±0.51	5.04±0.03	3.93±0.06	34.54±0.71	32.61±1.03
		16 mM Urea	0.86±0.24	4.88±0.07	4.22±0.13	35.90±0.16	33.66±0.26

CONCLUSIONS

(1) Normal and very high gravity rye and triticale mashes could be fermented to completion with or without urea as an external nitrogenous source.
(2) Triticale is more effectively mashed than rye due to its low mash viscosity. High viscosity of rye mash can be lowered by treatment with Roxazyme enzyme.
(3) Fermentation efficiencies and ethanol yields obtained for rye and triticale were comparable to those reported for wheat.
(4) Fermentation efficiencies and ethanol yields per unit weight of grain fermented were not affected significantly by the type of fermentation.
(5) Ethanol concentrations in the beer were increased by 3556% for VHG fermentations.
(6) Reduction in water use by 1/3 in VHG fermentations preparation will result in significant saving in energy consumption for mash heating, cooling, and ethanol distillation.

REFERENCES

INGLEDEW, W.M. 1993. Yeasts for production of fuel ethanol. Pages 245-291, in: The Yeasts, Vo1.5, Yeast Technology, 2nd ed., ROSE, A.H. and HARRISON, J.S., eds., Academic Press, New York.

JONES, A.M. and INGLEDEW, W.M. 1994. Fuel alcohol production: Optimization of temperature for efficient very-high-gravity fermentation. Appl. and Environ. Microbiol. 60: 1048.

SOSULSKI, K. and SOSULSIU, F. 1994. Wheat as a feedstock for fuel ethanol. Appl. Biochem. and Biotechnol. 45/46: 169.

SOSULSKI, K., WANG, S., INGLEDEW, W.M., SOSULSKI, F.W. and TANG, J. 1996. Preprocessed Barley, Rye and Triticale as a Feedstock for an Integrated Fuel Ethanol-Feedlot Plant. Appl. Biochem. and Bioeng. - in press.

THOMAS, K.C., HYNES, S.H. and INGLEDEW, W.M. 1996. Practical and theoretical considerations in the production of high concentrations of alcohol by fermentation, Proc. Biochem.31:321.