J Ind Microbiol Biotechnol (2004) 31: 362–368
DOI 10.1007/s10295-004-0156-3

ORIGINAL PAPER

Dennis P. Bayrock · W. Michael Ingledew

Inhibition of yeast by lactic acid bacteria in continuous culture: nutrient depletion and/or acid toxicity?

Received: 22 August 2003 / Accepted: 24 June 2004 / Published online: 15 July 2004

Abstract Lactic acid was added to batch very high gravity (VHG) fermentations and to continuous VHG fermentations equilibrated to steady state with *Saccharomyces cerevisiae*. A 53% reduction in colony-forming units (CFU) ml^{-1} of *S. cerevisiae* was observed in continuous fermentation at an undissociated lactic acid concentration of 3.44% w/v; and greater than 99.9% reduction was evident at 5.35% w/v lactic acid. The differences in yeast cell number in these fermentations were not due to pH, since batch fermentations over a pH range of 2.5–5.0 did not lead to changes in growth rate. Similar fermentations performed in batch showed that growth inhibition with added lactic acid was nearly identical. This indicates that the apparent high resistance of *S. cerevisiae* to lactic acid in continuous VHG fermentations is not a function of culture mode. Although the total amount of ethanol decreased from 48.7 g l^{-1} to 14.5 g l^{-1} when 4.74% w/v undissociated lactic acid was added, the specific ethanol productivity increased ca. 3.2-fold (from 7.42×10^{-7} g to 24.0×10^{-7} g ethanol CFU^{-1} h^{-1}), which indicated that lactic acid stress improved the ethanol production of each surviving cell. In multistage continuous fermentations, lactic acid was not responsible for the 83% (CFU ml^{-1}) reduction in viable *S. cerevisiae* yeasts when *Lactobacillus paracasei* was introduced to the system at a controlled pH of 6.0. The competition for trace nutrients in those fermentations and not lactic acid produced by *L. paracasei* likely caused the yeast inhibition.

Keywords Very high gravity · Continuous culture · Fuel alcohol · Lactic acid · Growth inhibition · Nutrient limitation

D. P. Bayrock · W. M. Ingledew (✉)
Department of Applied Microbiology and Food Science,
University of Saskatchewan, 51 Campus Drive,
Saskatoon, Saskatchewan, S7N 5A8, Canada
E-mail: ingledew@sask.usask.ca
Tel.: +1-306-9665028
Fax: +1-306-9668898

Introduction

In the fuel alcohol industry, microbial contamination is the largest ongoing problem challenging ethanol productivity and eroding profits. The majority of contaminants are *Lactobacillus* species [14].

In theory, there are two ways by which a contaminant can compete and overtake another organism in a culture. First, a contaminant can produce end-products such as organic acids that inhibit the growth of the other microbe. With lipophilic, weak carboxylic acids (e.g. lactic acid, acetic acid), it has been shown that only the undissociated form is able to inhibit yeast growth and fermentation [15, 19, 22, 23]. The undissociated forms of short-chain carboxylic acids diffuse into yeast and (if the intracellular pH is higher than the extracellular pH) dissociate, resulting in an acidification of the cytoplasm [3, 4, 9, 12, 17, 24]. Glucose-repressed yeast cells do not transport the dissociated forms of short-chain carboxylic acids, so anions outside the cell do not alter the pH of the yeast cytoplasm [3].

The inhibitory effects of added fermentation end-products on yeast in continuous culture have been assessed. First, the concentrations of ethanol, lactic acid, and acetic acid that caused 80% reduction in yeast biomass under steady-state conditions were 70, 38, and 7.5 g l^{-1}, respectively [13]. Others reported that a lactic acid concentration of 8 g l^{-1} in a beet molasses batch fermentation reduced yeast viability by 95% and the alcohol production rate by 80% [18].

Second, a contaminant can compete with the production culture by scavenging the trace nutrients required for its optimal growth. Examples exist in the literature to indirectly support this concept. The threshold level of *L. casei* that influenced the specific growth rate of yeast was found to be 10^4 cells ml^{-1} during beet molasses fermentation [18]. In that study, the pH of the 14% w/v molasses medium was adjusted to 5.0 and the medium was inoculated with 3×10^6 yeast cells ml^{-1} [18]. In other work, ethanol productivity in a

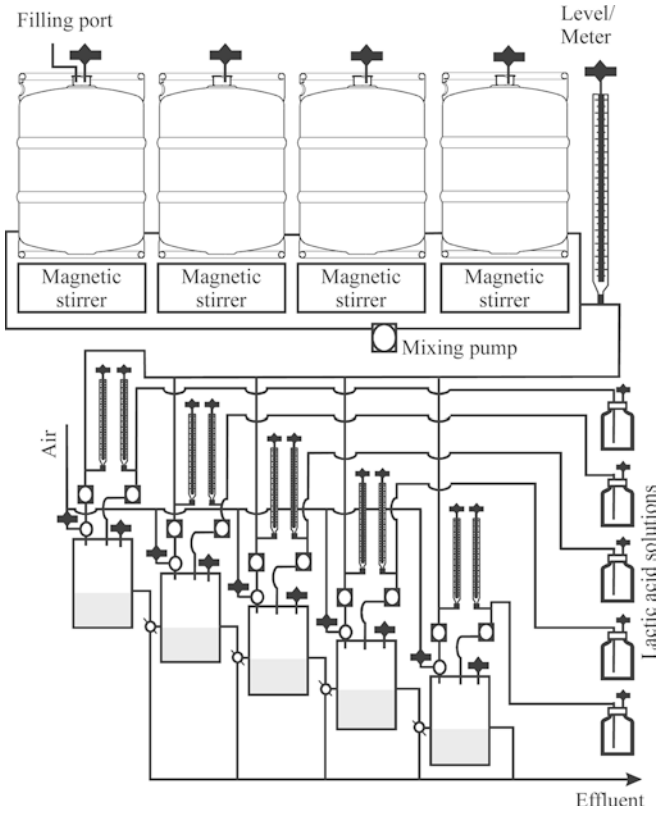

Fig. 1 Schematic diagram of modifications to the constructed MCCF system

cell-recycle continuous culture using a 20°P glucose medium (20°Plato = 20 g glucose in 100 g medium) fell by 30% when the system was inoculated with *L. fermentum* at 3×10^8 colony-forming units (CFU) ml^{-1} [6]. Production losses of 7% of total ethanol were shown when 10^9 CFU ml^{-1} of *L. fermentum* were added to 10^6 CFU ml^{-1} of yeast at the beginning of a 22–24°P wheat mash batch fermentation. More than 2% of the total ethanol was "lost" with the introduction of only 10^5 CFU ml^{-1} of either *L. paracasei* or *Lactobacillus* no. 3 at the beginning of each batch fermentation [15]. Other work showed effects on yeast by lactic acid at 0.9% w/v and by acetic acid at 0.04% w/v [16]. Yeast cell viability decreased 60% in fermentations with contaminated backset (*L. fermentum* or *L. delbrueckii*) that had been treated to inactivate viable bacteria [5]. A lactic concentration of 14 g l^{-1} was observed during fermentation [5]. Although all of the experiments in the literature mentioned above contained a contaminant in the fermentation that would compete for nutrients, it was not possible to clearly separate the magnitude of inhibition provided by the effects of competition for nutrients vs the effects of end-products produced by the contaminant. In addition, no evidence is available in the literature to show that competition for nutrients, rather than inhibition by metabolic end-products of contaminants, is the major reason for the loss of ethanol productivity and yeast vitality in fuel alcohol production.

In recent work, very high gravity (VHG) and multistage continuous culture technologies (MCCF) were merged successfully for the first time to produce fuel ethanol concentrations as high as 17% v/v [1]. This was accomplished without the use of conditioned or genetically modified *Saccharomyces cerevisiae* and without major changes to existing production equipment. Deliberate contamination of this VHG MCCF system in subsequent work yielded data on how *L. paracasei* and *S. cerevisiae* co-existed in the system, and what effects *L. paracasei* has on yeast growth and ethanol production [2].

The present work was designed to examine the effects (in the absence of a microbial contaminant) that lactic acid has on the growth and ethanol productivity of *S. cerevisiae* in VHG fermentations operated in continuous and batch modes. Additionally, the issue was examined as to whether inhibition by lactic acid and/or competition for nutrients by *Lactobacillus* spp pose a threat to the growth and ethanol production of *S. cerevisiae* in a mixed continuous fermentation.

Materials and methods

Continuous fermentation system

Five Bioflo III fermentors (New Brunswick Scientific, New Brunswick, N.J.) were connected in series to produce a MCCF system, as described previously [1]. On each fermentor effluent port line, an additional connection was made so that a choice could be made either to allow the fermentor contents to proceed to the next fermentor in the MCCF, or to drain the overflow into a common effluent line (Fig. 1). This arrangement allowed the system to be used either in MCCF or in individual continuous stirred-tank reactor (CSTR) mode and also allowed switching from one mode to another without the fear of contamination while the system was operating. Each fermentor had an independent supply of medium, sterile air, and lactic acid. The medium feed was supplied to individual fermentors by an external peristaltic pump and the lactic acid feed was supplied by the on-board Bioflo pump. Both feeds were calibrated and monitored during operation, using in-line burettes. All fermentors in the MCCF were operated at 2.8 l.

Fermentation and equilibration in individual identical CSTRs

Individual CSTR mode fermentations were initially started in MCCF mode. The system was switched 24 h later to create five individual and identical CSTR fermentations (each matching the F1 conditions of a MCCF system). This was accomplished in each fermentor by redirecting the effluent flows, by providing sterile air at 2 standard liters per minute (SLPM), and by providing sterile medium. Fermentations were

conducted at 28°C with 100 rpm agitation. The fermentors were run for at least 7 days (11 working vol. displacements) at a chosen dilution rate to allow the system to reach steady state, as evidenced by glucose concentrations that varied less than 5% in each fermentor over three consecutive days of sampling. Then, diluted lactic acid (not *Lactobacillus*) was introduced into each fermentor in the system.

Each CSTR in the present work was matched to conditions found previously for F1 in an MCCF system, since F1 provided *L. paracasei* with the best opportunity to thrive [2]. This would thus provide a comparison of the magnitude of inhibition of yeast growth by the growth of *L. paracasei* [2] and the inhibition of yeast growth due to the levels of lactic acid added.

Lactic acid addition to CSTR fermentors

Sterile medium and diluted lactic acid (Purac, Lincolnshire, Ill.) were pumped into the fermentor at the rate of 3.1 ml min^{-1} and 0.5 ml min^{-1}, respectively. With these rates of pumping, the effective dilution rate was 0.077 h^{-1}. The lactic acid concentration in the fermentor was varied by adjusting the concentration of the lactic acid feed while maintaining a constant dilution rate ($D = 0.077\ h^{-1}$). A control fermentor with only the endogenous level of medium lactic acid (0.41% w/v, contributed by the corn steep powder medium component) was run with a feed of sterile distilled water at 0.5 ml min^{-1}. Following the introduction of acid, the individual CSTR fermentors were run for 7 days (13 working vol. displacements) to allow equilibration to new steady states. This was confirmed when the glucose concentrations (via HPLC) in each fermentor varied by less than 5% over three subsequent days of sampling.

Batch growth of *S. cerevisiae* with lactic acid

Batch growth of yeast was followed using a medium which contained the same concentration of glucose (26% w/v), corn steep powder (CSP; 2% w/v), and $(NH_4)_2HPO_4$ (20 mM) as used in CSTR experiments. A clarified and concentrated solution of CSP with $(NH_4)_2HPO_4$ was prepared and autoclaved at 121°C for 15 min, simulating normal medium preparation for CSTR experiments. It was then clarified by centrifugation at 10,200 g for 15 min and filter-sterilized through a 0.45-μm membrane filter (Gelman Sciences, Ann Arbor, Mich.). A concentrated sterile solution of lactic acid was also prepared by first autoclaving the solution at 121°C for 30 min to hydrolyze all lactic anhydride to lactic acid, followed by filter-sterilization. A concentrated solution of glucose was sterilized similarly. Media for batch studies were combined aseptically from concentrated stocks to provide (in duplicate) a 0–7% w/v range of added lactic acid. The pH of each medium was measured and a 10-ml aliquot from each flask was frozen and stored for subsequent HPLC analysis.

Aliquots (100 ml) of each medium were dispensed into sterile 250-ml screw-capped, side-armed Erlenmeyer flasks. One milliliter of an inoculum of *S. cerevisiae* was added and each flask was incubated at 28°C at 100 rpm in a rotary shaker incubator. The growth of *S. cerevisiae* was monitored by measuring absorbance using a Klett–Summerson colorimeter (Klett Mfg Co., New York, N.Y.) equipped with a no. 66 red filter (640–700 nm). When the yeast entered the stationary phase, a 10-ml sample from each flask was removed, filtered through a 0.45-μm membrane filter and frozen for subsequent HPLC analysis.

Analysis of batch growth curves

All growth data for *S. cerevisiae* were analyzed mathematically according to the procedure described by Zwietering et al. [25]. The curve-fitting mathematical routines calculated the maximum slope (and therefore maximum growth rate) for exponential growth of yeast in each flask. Statistical analysis (*t*-test in Microsoft Excel) of each original and curve-fitted sigmoidal curve pair indicated no significant difference in any curve pair (95% confidence interval).

HPLC analysis

Lactic acid, glycerol, ethanol, and glucose concentrations were determined by HPLC analysis [2]. The concentration of undissociated lactic acid at the pH of the fermentation medium was determined by calculation using the Henderson–Hasselbach equation [16]. Unless otherwise stated, all lactic acid concentrations recorded herein refer to undissociated lactic acid concentrations.

Determination of number of viable cells

Viable cells in a sequential series of dilution blanks (0.1% peptone) were determined by triplicate enumeration of CFU by the membrane filtration procedure, using yeast extract/peptone/dextrose plates at an incubation temperature of 30°C [11]. A maximum 10% variability between triplicate samples was observed for viable counts of yeast after 48 h of incubation. No additional colonies were detected after 72 h or 96 h of incubation.

Results and discussion

Figure 2 portrays steady-state levels of viable *S. cerevisiae* when 1–7% w/v undissociated lactic acid was fed to the individual CSTR fermentors. As evident in Fig. 2, the steady-state number of viable yeast cells in the control decreased by 53% when the concentration of undissociated lactic acid was increased to

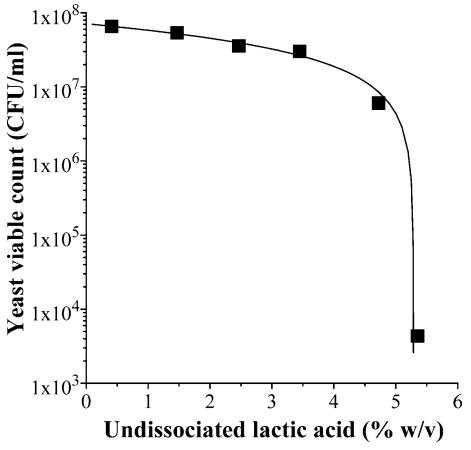

Fig. 2 Levels of *S. cerevisiae* in steady-state continuous cultures with various amounts of undissociated lactic acid. The control value of 0.41% lactic acid is contributed by the CSP used in the medium. No viable cells (< 10 CFU ml^{-1}) were present at an undissociated lactic acid concentration of 70.2 g l^{-1}. The total (and undissociated lactic acid) concentrations (% w/v) at the corresponding pH values were: 0.52 (0.41) at pH 3.16, 1.67 (1.46) at pH 2.88, 2.77 (2.46) at pH 2.82, 3.81 (3.45) at pH 2.76, 5.15 (4.73) at pH 2.68, and 5.81 (5.36) at pH 2.66

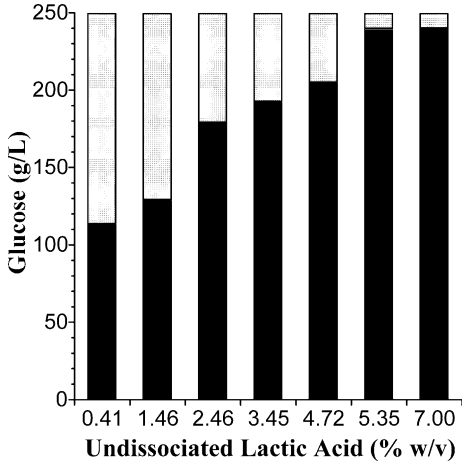

Fig. 3 Residual (*black*) and consumed glucose (*white*; corrected for metered lactic acid additions) in steady-state continuous cultures of *S. cerevisiae* in the presence of increasing undissociated lactic acid concentrations. *L* Liters

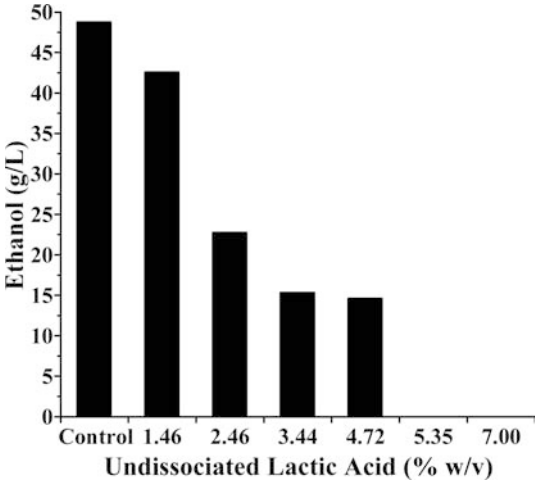

Fig. 4 Ethanol production at steady-state continuous cultures of *S. cerevisiae* in the presence of increasing undissociated lactic acid concentrations

3.44% w/v. An endogenous 0.41% w/v amount of lactic acid was present in the CSP component used in all medium formulations. At an undissociated lactic acid concentration of 5.35% w/v, *S. cerevisiae* was inhibited by more than 3.5 orders of magnitude. No viable yeasts were recovered at 7% w/v undissociated lactic acid. Based on the shape of the curve in Fig. 2, total elimination (washout) of viable *S. cerevisiae* from the CSTR would appear to occur between 5.5% and 6.0% w/v undissociated lactic acid. A maximum pH change of 0.2 units was observed across all undissociated lactic acid concentrations tested. Separate batch fermentations in identical media over a pH range of 2.5–5.0 did not show any differences in the growth rate of *S. cerevisiae* (data not shown). Thus, as shown in Fig. 2, the undissociated lactic acid (and not the minor change in pH) influenced the steady-state levels of *S. cerevisiae* in the CSTR fermentations.

Changes in steady-state glucose concentrations with lactic acid concentrations are shown in Fig. 3. As expected, glucose concentrations at steady state increased with increasing undissociated lactic acid concentrations, since the numbers of viable cells of *S. cerevisiae* declined. In the control, nearly 50% of the glucose supplied was not used. Glucose consumption decreased by a further 50% when 2.46% w/v lactic acid was present. As the undissociated lactic acid level increased to 5.4% w/v, more glucose remained in each fermentor, until at 5.4% and 7.0% w/v lactic acid virtually all glucose remained unused. Steady-state ethanol concentrations under all lactic acid conditions are shown in Fig. 4. Ethanol concentrations were reduced by 50% at 2.5% w/v undissociated lactic acid and by 70% with 3.45% and 4.72% w/v undissociated lactic acid conditions. No ethanol was detected when the medium contained 5.4% or 7.0% w/v lactic acid.

Specific rates of ethanol production (g produced CFU^{-1} h^{-1}) and glucose consumption (g consumed CFU^{-1} h^{-1}) were calculated to determine how *S. cerevisiae* responded to increasing concentrations of lactic acid in the medium. These results are presented in Fig. 5. Cell-specific glucose consumption remained constant at ca. 2×10^{-6} g glucose CFU^{-1} h^{-1} until 3.5% w/v lactic acid was present. It then increased 4-fold at 4.7% w/v lactic acid and 4,400-fold at 5.4% w/v lactic acid. In other words, as the lactic acid concentration increased beyond 3.5% w/v, the surviving yeasts dramatically increased the consumption of glucose on a per viable cell basis. One hypothesis to account for this increase is that *S. cerevisiae* needs the energy (ATP) produced from the increased catabolism of

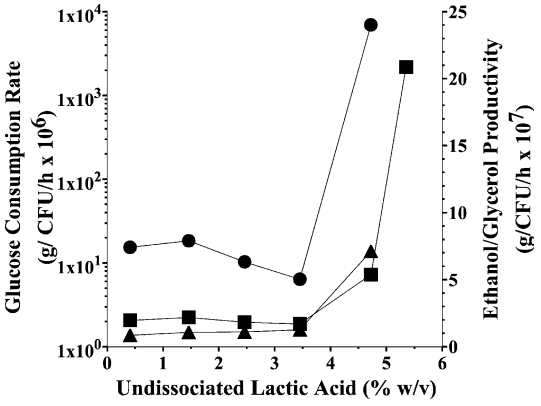

Fig. 5 Specific glucose consumption and specific ethanol and glycerol productivities in steady-state continuous cultures of *S. cerevisiae* in the presence of increasing undissociated lactic acid concentrations. *Squares* Specific glucose consumption, *circles* specific ethanol productivity, *triangles* specific glycerol productivity)

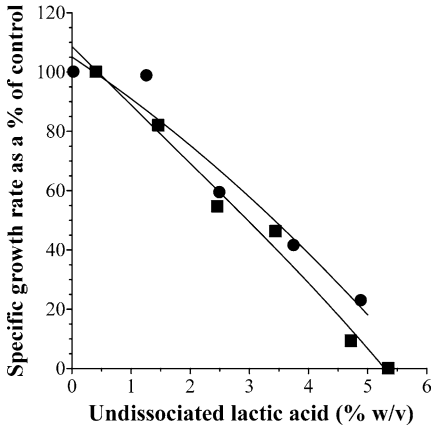

Fig. 6 Comparison of maximum specific growth rates in CSTR and corresponding batch fermentations with increasing undissociated lactic acid. *Squares* Percentage of maximum specific growth rate in CSTR fermentations, as compared with CSTR control. *Circles* Percentage of maximum specific growth rate in batch fermentations, as compared with batch control

glucose to expel the increased hydrogen ion concentration resulting from the influx of undissociated lactic acid (which dissociates inside the yeast cell at the increased pH). This hypothesis is borne out by the work of Verduyn et al. [24] using benzoic acid (non-metabolizable by yeast), where they document that the specific glucose flux (uptake) increased when benzoic acid levels were increased in continuous culture. Other authors discuss this possibility in relation to fuel alcohol production [17].

Since specific glucose consumption in *S. cerevisiae* increased with increasing lactic acid concentrations and *S. cerevisiae* could not metabolize aerobically at the sugar concentrations present in the fermentations [8, 20], it is reasonable to assume that specific ethanol and glycerol production rates would also increase. These are shown in Fig. 5. Both the specific ethanol and glycerol production rates increased in parallel with the specific glucose consumption rate. High concentrations of undissociated lactic acid not only caused a decrease in viable cell numbers, but also forced the remaining cells of *S. cerevisiae* to produce more ethanol. Unfortunately, this boost in specific productivity came at the price of reduced cell numbers. If cell recycle was implemented in the CSTR fermentors to raise viable numbers of *S. cerevisiae* at the point of highest specific ethanol production rate (4.7% w/v lactic acid), ethanol might be produced much faster and translate into a higher ethanol productivity for the alcohol producer.

The apparent resistance of *S. cerevisiae* to high levels of lactic acid shown in Fig. 2 may be due to the natural resistance of this strain of *S. cerevisiae* to lactic acid, or due to medium composition, or due to the fact that the experiment was operating in continuous mode. To address whether the culturing mode affects the apparent resistance of yeast, similar experiments were performed in batch cultures using identical concentrations of glucose and CSP as used in the CSTR experiments, but with varying lactic acid concentrations. The results are summarized in Fig. 6. Here, a comparison can be made between the specific growth rates from CSTR experiments and those from corresponding batch experiments, where both sets of data were plotted as a percent of their respective (maximum) control values. Both resulting plots showed similar inhibition profiles and slopes when undissociated lactic acid concentrations in the media were increased to 5% w/v. A 50% reduction (inhibition) in both plots occurred when the lactic acid concentration reached ca. 3.0% (CSTR fermentations) and 3.5% w/v (batch fermentations).

One can conclude from these data that there appears to be no discernable difference in the inhibition of *S. cerevisiae* by undissociated lactic acid when yeast ferments in batch rather than in continuous culture. Thus, the apparent resistance of *S. cerevisiae* to high levels of lactic acid must be due to either the natural resistance to lactic acid of this strain of *S. cerevisiae*, or yeast metabolism, and/or possibly protective components in the medium. Evidence in other work has shown that medium components can sequester and/or buffer *S. cerevisiae* from increasing concentrations of undissociated lactic acid [21]. In experiments where minimal media with or without CSP were fermented with high concentrations of lactic acid at pH 4.4 and pH 2.2, *S. cerevisiae* grew in the supplemented media at pH 2.2, while no growth was observed in minimal media when lactic acid concentrations were matched [21]. Subsequent titrations of both types of uninoculated media (previously poised at pH 2.8–5.5) to pH 2.8 with NaOH or HCl showed that, at all pH values tested, the medium supplemented with CSP required more titrant to bring the medium to a pH of 2.8. The authors concluded that medium components in fermentation media could "protect" the yeast from the damaging inhibition caused by lactic acid and acetic acid—especially at low pH [21].

In previous work [2] which involved direct contamination of a yeast-equilibrated MCCF, it was concluded

that either the production of lactic acid by *L. paracasei*, or competition for nutrients by *L. paracasei*, or a combination of both was responsible for the 83% reduction in yeast viability when viable *L. paracasei* increased by more than 2,500-fold (4.4 log) upon the initiation of pH control. At the highest level of viability in that experiment, *L. paracasei* produced a maximum of 2% w/v total lactic acid. At a controlled pH of 6.0, the calculated concentration of the undissociated fraction of lactic acid in that fermentor would be 0.01% w/v. Since the previous work was performed under nearly identical operating conditions to the present work (the only difference being a slight increase in the dilution rate from $D = 0.06$ to $D = 0.077$, due to the addition of the lactic acid flow), it is possible to determine the inhibition of *S. cerevisiae* by lactic acid in that study. By extrapolating 0.01% w/v with data from Fig. 2, it becomes very clear that lactic acid could not have been responsible for the 83% reduction seen for *S. cerevisiae* during the second steady state. Since the concentration of undissociated lactic acid was so small, competition for nutrients by *S. cerevisiae* and *L. paracasei* would appear to be the only other possible factor that could be responsible for inhibition of *S. cerevisiae* in the mixed continuous culture. If this is true, these results contradict the commonly held belief that it is the production of organic acids produced during a contaminated fermentation that inhibit yeast growth and ethanol production [7]. These data raise the question as to what concentration of lactic acid needs to be produced by a contaminant (e.g., *Lactobacillus* spp) in order to impact yeast growth. From Fig. 2, a 50% reduction in yeast numbers in continuous operation corresponds to ca. 3.4% w/v undissociated lactic acid concentration in a fermentor. This translates to a total lactic acid concentration at corresponding pH values of: 9.84% w/v at pH 4.0, 23.7% w/v at pH 4.5, 67.5% w/v at pH 5.0, 206% w/v at pH 5.5, and 644% w/v at pH 6.0. It is obvious that the latter values are not possible and that inhibition by lactic acid at those pH values does not exist. This also raises the question at higher lactic acid concentrations as to how (or if) dissociated lactic acid could inhibit the yeast, as seen in other work [21]. The results in this work suggest that the effects on yeast of competition for trace nutrients by the contaminant may be lessened by the addition of trace nutrients from the CSP fraction. This approach may be a cost-effective method for improving yeast levels (and thus ethanol yield) without the need for adding expensive antibacterial substances. Thus, the contaminant would be ''tolerated'' in such fermentations. The present work also suggests that, in addition to the well documented chemical stresses that a yeast may encounter during fermentation due to a contaminant [7, 10], nutritional stresses for trace nutrients due to competition by contaminating bacteria may inhibit yeast performance.

Acknowledgements The authors are grateful for financial support from the Canadian Wheat Board, the Natural Sciences and Engineering Research Council, Alltech Inc., the Chippewa Valley Ethanol Co., Corn Plus Corp., and Delta T Corp. The very capable assistance of Mrs. Sandra Hynes and Dr. K.C. Thomas during the course of the project is gratefully acknowledged.

References

1. Bayrock DP, Ingledew WM (2001) Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. J Ind Microbiol Biotechnol 27:87–93
2. Bayrock DP, Ingledew WM (2001) Changes in steady state in multistage continuous fuel ethanol fermentation on introduction of a *Lactobacillus* contaminant. J Ind Microbiol Biotechnol 27:39–45
3. Cásio F, Leâo C, Uden N van (1987) Transport of lactate and other short-chain monocarboxylates in the yeast *Saccharomyces cerevisiae*. Appl Environ Microbiol 53:509–513
4. Capucho I, San Romao MV (1994) Effect of ethanol and fatty acids on malolactic activity of *Leuconostoc oenos*. Appl Microbiol Biotechnol 42:391–395
5. Chin PM, Ingledew WM (1994) Effect of lactic acid bacteria on wheat mash fermentations prepared with laboratory backset. Enzyme Microb Technol 16:311–317
6. Chang IS, Kim BH, Shin PK, Lee WK (1995) Bacterial contamination and its effects on ethanol fermentation. J Microbiol Biotechnol 5:309–314
7. Connolly C (1999) Bacterial contaminants and their effects on alcohol production. In: Jacques KA, Lyons TP, Kelsall DR (eds) The alcohol textbook: a reference for the beverage, fuel and industrial alcohol industries, 3rd edn. Nottingham University Press, Nottingham, pp 317–334
8. DeDenken RH (1966) The Crabtree effect: a regulatory system in yeast. J Gen Microbiol 44:149–156
9. Eklund T (1983) The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J Appl Bacteriol 54:383–389
10. Ingledew WM (1999) Alcohol production by *Saccharomyces cerevisiae*: a yeast primer. In: Jacques KA, Lyons TP, Kelsall DR (eds) The alcohol textbook: a reference for the beverage, fuel and industrial alcohol industries, 3rd edn. Nottingham University Press, Nottingham, pp 49–87
11. Ingledew WM, Burton JD (1980) Membrane filtration: survival of brewing microbes on the membrane during storage at reduced humidities. J Am Soc Brew Chem 38:125–129
12. Kashket ER (1985) The proton motive force in bacteria: a critical assessment of methods. Annu Rev Microbiol 39:219–242
13. Maiorella B, Blanch HW, Wilkie CR (1983) By-product inhibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*. Biotechnol Bioeng 25:103–121
14. Makanjuola DB, Springham DG (1984) Identification of lactic acid bacteria isolated from different stages of malt and whisky distillery fermentations. J Inst Brew 90:13–19
15. Narendranath NV, Hynes SH, Thomas KC, Ingledew WM (1997) Effects of lactobacilli on yeast-catalyzed ethanol fermentations. Appl Environ Microbiol 63:4158–4163
16. Narendranath NV, Thomas KC, Ingledew WM (2000) Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium. J Ind Microbiol Biotechnol 26:171–177
17. Narendranath NV, Thomas KC, Ingledew WM (2001) Acetic acid and lactic acid inhibit growth of *Saccharomyces cerevisiae* by different mechanisms. J Am Soc Brew Chem 59:187–194
18. Ngang JJE, Letourneau F, Wolniewicz E, Villa P (1990) Inhibition of beet molasses alcoholic fermentation by lactobacilli. Appl Microbiol Biotechnol 33:490–493
19. Pampulha ME, Loureiro-Dias MC (1989) Combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast. Appl Microbiol Biotechnol 31:547–550

20. Pertik M, Kappeli O, Fiechter A (1983) An expanded concept for glucose effect in the yeast *Saccharomyces uvarum*: involvement of short and long-term regulation. J Gen Microbiol 129:43–49
21. Thomas KC, Hynes SH, Ingledew WM (2002) Influence of medium buffer capacity on inhibition of *Saccharomyces cerevisiae* growth by acetic and lactic acids. Appl Environ Microbiol 68:1616–1623
22. Verduyn C, Postma E, Scheffers WA, Dijken JP van (1990) Energetics of *Saccharomyces cerevisiae* in anaerobic glucose limited chemostat cultures. J Gen Microbiol 136:405–412
23. Verduyn C, Postma E, Scheffers WA, Dijken JP van (1990) Physiology of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures. J Gen Microbiol 136:395–403
24. Verduyn C, Postma E, Scheffers WA, Dijken JP van (1992) Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcohol fermentation. Yeast 8:501–517
25. Zwietering MH, Jongenburger I, Rombouts FM, Riet K van'T (1990) Modeling of the bacterial growth curve. Appl Environ Microbiol 56:1875–1881