Journal of Industrial Microbiology & Biotechnology (1997) 19, 12–17

Ethanol production from spent cherry brine

H Park and AT Bakalinsky

Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602, USA

Spent cherry brine is an acidic byproduct of maraschino cherry processing and typically consists of variable amounts of glucose and fructose of up to 11% fermentable solids, 0.5–1.5% $CaCl_2$, up to 0.4% sulfur dioxide, sorbitol, and lesser amounts of other cherry constituents. Disposal of brine represents a significant cost to processors because of its high biological oxygen demand. As an alternative, brine was tested as a substrate for ethanol production. Initially, the toxic level of sulfur dioxide was reduced by raising brine pH to 8.0 to precipitate calcium sulfite. Because alkalinization was subsequently found to result in a 10-fold reduction in phosphorous, brines were titrated with phosphoric acid to pH 6.0 prior to inoculation with *Saccharomyces cerevisiae*. All strains of *Saccharomyces cerevisiae* tested were able to ferment all lots of $Ca(OH)_2$-treated and phosphorous-enriched brines efficiently. One lot of brine containing 10% (w/v) fermentable sugar yielded 4.7% (w/v) ethanol in 4 days.

Keywords: yeast; *Saccharomyces cerevisiae*; sulfite; sulfur dioxide; fermentation; maraschino

Introduction

Spent cherry brine is an acidic by-product of maraschino cherry processing whose composition is dependent on that of the fruit, and on the formulation used by processors. In general, on a weight per volume basis, spent brine contains approximately 4–11% glucose and fructose, 0.5–1.5% $CaCl_2$, sulfur dioxide, sorbitol, and lesser amounts of other soluble constituents leached from the fruit [5–6,17,19]. Although variable, sorbitol usually accounts for about 20% of the total sugar content [14,22]. Brine disposal is costly due to its high biological and chemical oxygen demand and high acidity [20]. Within municipalities, brine is neutralized chemically prior to discharge in water treatment facilities. Outside of municipalities, brine is typically held in vigorously aerated ponds supplemented with fertilizer, prior to discharge into waterways or onto neighboring fields. Aeration is required to minimize emissions of noxious hydrogen sulfide which would readily form from biological reduction of brine sulfur under anaerobic conditions. Future discharges onto fields may be restricted by regulatory agencies who view such practices as contrary to the long-term health of agricultural lands.

Various procedures have been proposed as alternatives to disposal: treatment with activated charcoal to permit limited reuse of brine [6,20], chemical removal of the sulfite by precipitation and oxidation, anaerobic oxidation of the neutralized brine [19], and removal of the sugars by reverse osmosis (C Payne, personal communication, Oregon Cherry Growers, Salem, OR, 1995). Here we describe a simple process to ferment brine with the yeast *Saccharomyces cerevisiae* to produce fuel alcohol. The process produces a value-added product, reduces the biological and chemical oxygen demand of the effluent, and generates a new stream, a calcium sulfite precipitate that has potential value as a liming agent for acidic soils.

Correspondence: AT Bakalinsky, Dept of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602, USA
Technical paper number 11 068 of the Oregon Agricultural Experiment Station
Received 24 December 1996; accepted 14 April 1997

Materials and methods

Yeast strains

Saccharomyces cerevisiae 2896 is a heterothallic derivative of strain AB2 derived from an isolate of the wine strain *S. cerevisiae* Pasteur Champagne [4], which was originally obtained from Universal Foods Corporation (Milwaukee, WI, USA). An independent isolate of the Pasteur Champagne strain was also used (Universal Foods Corporation). *S. cerevisiae* Allyeast was obtained from Alltech (Nicholasville, KY, USA). Single colony isolates of all strains were used and maintained on YEPD agar slants at 4°C.

Media and chemicals

YEPD is 2% Difco Bacto peptone (Difco Laboratories, Detroit, MI, USA), 1% Difco Bacto yeast extract, and 2% dextrose. YEPD + cyh is YEPD supplemented with 6 mg L^{-1} cycloheximide (Sigma Chemical Co, St Louis, MI, USA). Nutrient agar (NA) is 0.3% Difco Bacto beef extract and 0.5% Difco Bacto peptone. Media were solidified by addition of 2% Bacteriological agar (American Biorganics, Niagra Falls, NY, USA) and were sterilized by autoclaving. All chemicals were reagent grade.

Spent cherry brine

Spent brines were obtained from Oregon Cherry Growers (Salem, OR, USA). The major chemical constituents of brines No. 1 and No. 2 are shown in Table 1.

Pre-fermentation brine treatments

Excess sulfite was removed from spent brine prior to fermentation by raising the pH to 8.0 with solid calcium hydroxide, and holding the brine for approximately 2 h at room temperature to allow precipitation of calcium sulfite. The supernatant was transferred to another vessel and its pH adjusted to 6.0 with concentrated phosphoric acid to a final addition of 7.4 mM. Alternatively, the pH was

Table 1 Composition of spent cherry brine

Major components	Brine No. 1 (pH 3.4) (g L^{-1} ± SEM)[a]	Brine No. 2. (pH 3.3) (g L^{-1} ± SEM)[a]
Glucose	24.5 ± 1.3	71.4 ± 0.1
Fructose	17.8 ± 1.6	63.0 ± 1.1
Chloride	12.6 ± 1.0	11.8 ± 0.5
Sorbitol	4.6 ± 0.2	32.4 ± 1.0
Calcium	4.4 ± 0.1	4.4 ± 0.2
Sulfur dioxide	4.1 ± 0.1	4.1 ± 0.1
Sodium	3.4 ± 0	3.3 ± 0.1
Organic matter	78.6 ± 10.5	172.5 ± 6.4
Inorganic matter	23.2 ± 0.3	26.8 ± 2.1
Trace elements	**(mg L^{-1} ± SEM)**	**(mg L^{-1} ± SEM)**
Potassium	124 ± 5.7	140 ± 4.2
Phosphorous	122 ± 2.8	160 ± 4.2
Magnesium	8.5 ± 0.4	10.6 ± 0.6
Iron	0.4 ± 0.1	0.3 ± 0
Manganese	0.2 ± 0	0.3 ± 0.1
Barium	0.2 ± 0	0.1 ± 0
Zinc	0.2 ± 0.1	0.2 ± 0.1

[a]Data are means of duplicates ± SEM (standard error of the mean). Copper was not detected (<0.1 mg L^{-1}).

adjusted to 6.0 by addition of 5 N hydrochloric acid, or 5 N hydrochloric acid following addition of diammonium phosphate (DAP) or urea to final concentrations of 7.4 mM.

Fermentations

Four sorts of brine were fermented following the aforementioned pre-treatments: brine No. 1 containing 4.2% glucose and fructose, brine No. 1 enriched with 5.8% d-(+)-glucose to a final fermentable sugar concentration of 10%, brine No. 2 containing 13.4% fermentable sugar, and brine No. 3 containing 8% fermentable sugar. Yeast strains were grown overnight in YEPD at 30°C and 300 rpm, pelleted by centrifugation ($850 \times g$), washed twice, and resuspended in distilled water. Duplicate pre-treated brine samples (2 L) were dispensed into 4-L sterile glass carboys fitted with fermentation locks, and were inoculated to a final concentration of 2×10^6 yeast cells ml^{-1}. The brines were not sterilized prior to inoculation. Fermentations were conducted at room temperature without shaking, and were sampled daily as described below for the assay of sugars, glycerol, and ethanol. Dilutions of daily samples were plated in duplicate on YEPD plates and were incubated at 30°C for 3–5 days to monitor yeast growth during the course of fermentation.

Microbial contamination

To determine the initial level of microbial contamination in the brine, 60 ml of the spent brine, or the $Ca(OH)_2$-treated and reacidified brine (pH 6.0) were centrifuged at $3700 \times g$ for 10 min at 4°C. The pellets were washed twice, resuspended in sterile distilled water, and aliquots were plated in duplicate on YEPD (for detecting yeast), YEPD + cyh (for detecting bacteria), and NA (for detecting bacteria), representing a total of 48 ml of undiluted brine. At the end of fermentation, 3-ml samples were pelleted by centrifugation at $12\,000 \times g$ for 60 s at room temperature. The pellets were washed twice, resuspended in sterile distilled water, and aliquots were plated in duplicate on YEPD, YEPD + cyh, and NA, representing a total of 2.4 ml of undiluted fermented brine.

Sugars, glycerol, and ethanol

Samples of brine or fermenting brine were centrifuged at $12\,000 \times g$ for 10 s at room temperature to remove yeast cells, and the supernatants were held at −80°C until assay. Glucose, fructose, sorbitol, glycerol, and ethanol were determined enzymatically using assay kits from Boehringer-Mannheim (Indianapolis, IN, USA).

Free sulfur dioxide

Free sulfur dioxide was measured by a modified Ripper method [2] by redox titration using a platinum redox electrode according to the manufacturer's instructions (Orion Research, Boston, MA, USA). To an appropriately diluted sample, 0.4 ml of 1 : 3 H_2SO_4 : H_2O and 20 ml of distilled water were added. The solution was then titrated with 0.002 N iodine solution and the redox potential recorded after each incremental addition of 0.05 ml. The end point was considered the initial rise in slope (ml iodine solution added *vs* mV).

Wet and dry weights and composition of the $Ca(OH)_2$-induced precipitate

The wet volume (v/v) and wet weight (w/v) of the calcium sulfite sludge, precipitated by raising brine pH to 8.0 with $Ca(OH)_2$, were measured following sedimentation by gravity and removal of the supernatant. To determine dry weight, the sludge was dried overnight at 70°C in a vacuum oven and maintained in a dessicator until weighing. The dried precipitate was dissolved in 5% nitric acid and diluted appropriately for elemental analysis by inductively coupled plasma emission spectrometry using a Jarrell-Ash ICPA-9000 instrument (Thermo Jarrell Ash-Baird Corp, Franklin, MA, USA) in the Central Analytical Laboratory of the Department of Crop and Soil Science at Oregon State University. Because the volatility of sulfur dioxide in the nitric acid interfered with the sulfur determination, sulfur was measured in a separate nitric acid sample following treatment with 5 N NaOH to raise the pH to 5.0, and vigorous overnight aeration to oxidize sulfur dioxide to non-volatile sulfate. Any reduction in volume by evaporation was corrected by addition of distilled water. Chloride was determined by dissolving the dried precipitate in sulfurous acid and diluting appropriately using a Dionex 2000i ion exchange chromatography system (Dionex Corporation, Sunnyvale, CA, USA) with an AS4A-SC anion exchange column and electrical conductivity detector in the Central Analytical Laboratory of the Department of Crop and Soil Science at Oregon State University.

Other analytical assays

Organic and inorganic matter were determined using standard methods for wastewater analysis [1]. Assimilable nitrogen was determined using a bioassay with *S. cerevisiae* 2896 [13]. $CaCO_3$ equivalents in the $Ca(OH)_2$-induced brine precipitate were determined in the Central Analytical Laboratory of the Department of Crop and Soil Science at Oregon State University [9].

Results

Brine composition

An analysis of spent cherry brines No. 1 and No. 2 is presented in Table 1. Glucose, fructose, and sorbitol together accounted for 4.7% soluble solids in brine No. 1 and 16.7% soluble solids in brine No. 2. Total sugar levels in cherry brines are variable, but are dependent on the variety and date of harvest. Non-sugar organic matter amounting to 3% and 0.5% soluble solids in brines No. 1 and No. 2, respectively, likely included unmeasured constituents leached from the cherries such as acids, polysaccharides, pigments, amino acids, and proteins. Potassium and phosphorous levels were 13% and 30% higher, respectively, in brine No. 2 than in brine No. 1. The major inorganic components of brine are not derived from the fruit, but are added by processors: calcium chloride, sulfur dioxide, and sometimes sodium chloride.

Pre-fermentation treatments

The brines were found to be toxic to yeast, presumably due to the combination of a high sulfur dioxide content and low pH. Indeed, no viable yeast cells were recovered following inoculation of a sample of untreated brine No. 1 with 10^6 cells ml^{-1} of any of the strains. The brine itself was subsequently analyzed for the presence of viable microorganisms. No yeasts and fewer than 10 bacterial cells were found in 48 ml of brine.

In order to make the brine a suitable medium for yeast fermentation, the sulfur dioxide concentration was reduced by making the brine alkaline and causing precipitation of $CaSO_3$ [19]. The sulfur dioxide level of brine No. 1 was reduced 90% by raising the pH to 8.0 with $Ca(OH)_2$ (Figure 1). Increasing the pH to 12 did not result in a further decrease in sulfur dioxide. NaOH was found to be a less effective precipitating agent than $Ca(OH)_2$, per unit increase in pH. Titration of brine No. 2 yielded a nearly identical pH *vs* free sulfur dioxide profile. On a volume basis, about 100 ml of wet sludge formed per liter. An analysis of the mineral composition of the precipitates revealed great similarity: 27–28% calcium, 15–16% sulfur, 2% potassium, 1% phosphorous, and trace amounts of magnesium and iron (Table 2). An analysis of oxygen, hydrogen, carbon, and nitrogen was not undertaken. The precipitate from brine No. 1 was found to contain 4.6% organic matter and both precipitates were pink in color, presumably due to co-precipitation of cherry anthocyanin pigments. On a dry weight basis, the precipitate from brine No. 1 was found to consist of 49.4% $CaCO_3$ equivalents.

Fermentations

At pH 8.0, sulfur dioxide-depleted brine No. 1 was still not a suitable substrate for fermentation because *S. cerevisiae* cannot grow under alkaline conditions. In a preliminary experiment, brine No. 2 containing 13.4% fermentable sugar (glucose and fructose) was treated with $Ca(OH)_2$, supplemented with 0.01, 0.03, 0.05% or no diammonium phosphate (DAP), adjusted to pH 6.0 with HCl, and inoculated with *S. cerevisiae* 2896 (data not shown). All DAP-supplemented brines fermented at the same rate, whereas the unsupplemented control fermented more slowly and produced only about 70% as much alcohol. In the brine supplemented with 0.01% DAP, the initial pH adjustment to 6.0 was found to result in faster ethanol production and faster utilization of the fermentable sugar than adjustment to 5.5, 6.5, or 7.0. Stimulation of fermentation by DAP suggested that either nitrogen, phosphorous or both were deficient in the $Ca(OH)_2$-treated brine and that a 0.01% addition of DAP was sufficient remedy. Repetition of the experiment using brine No. 3 containing 8% fermentable sugar supplemented with 0.01, 0.03 or 0.05% sodium phosphate produced results similar to those observed follow-

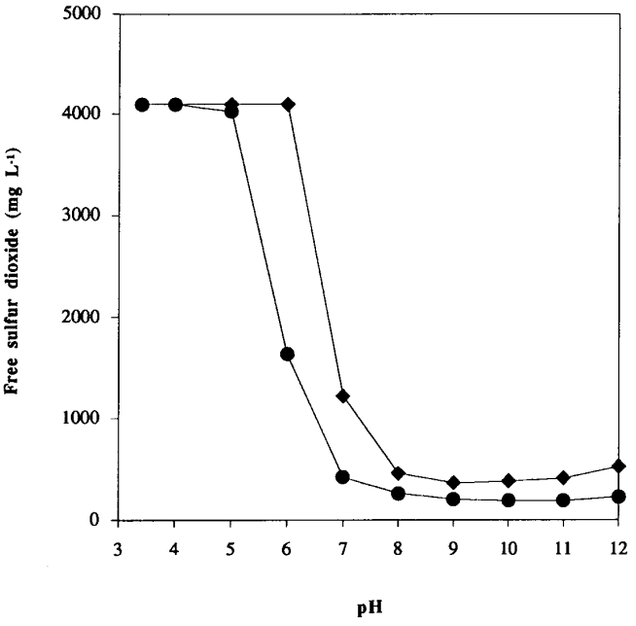

Figure 1 Reduction of brine sulfite by pH adjustment. Aliquots of solid calcium hydroxide (—●—) or sodium hydroxide (—◆—) were added to 100 ml of stirred brine No. 1 until the pH stabilized. One 2-ml sample per addition was withdrawn and held for 2 h at room temperature prior to centrifugation at 12 000 × *g* for 30 s to remove precipitate. Free sulfite was measured in the supernatant liquid. Data points are means of duplicate assays. Standard errors were less than 10% of the means.

Table 2 Mineral composition of precipitate formed after adjustment of brine pH to 8.0 with $Ca(OH)_2$[a]

Element	Precipitate from brine No. 1 mg g^{-1} precipitate (dry weight) ± SEM[b]	Precipitate from brine No. 2 mg g^{-1} precipitate (dry weight) ± SEM
Calcium	279 ± 7.1	270.5 ± 10.6
Sulfur	161 ± 4.2	149.5 ± 3.5
Potassium	17 ± 1.4	21.6 ± 1.2
Phosphorous	10.5 ± 0.3	14.5 ± 1.5
Magnesium	0.3 ± 0.1	0.2 ± 0
Iron	0.2 ± 0	0.3 ± 0

[a]Per liter of treated brines No. 1 and No. 2, 12 g of precipitate formed on a dry weight basis. On a wet weight basis, 115 g of precipitate formed per liter of treated brine No. 1. The wet weight of precipitate formed in brine No. 2 was not measured. The compositions of brines No. 1 and No. 2 are given in Table 1.

[b]Data are means of duplicate assays ± SEM (standard error of the mean). Sodium, chloride, manganese, copper, barium, and zinc were not detected (<0.1 mg g^{-1} precipitate).

ing DAP-supplementation suggesting a phosphorous deficiency. Brine phosphorous and nitrogen were subsequently determined in brines No. 1 and No. 2. The $Ca(OH)_2$ treatment was found to have reduced the phosphorous content at least 10-fold. Untreated brines No. 1 and No. 2 contained 122 and 160 mg L^{-1}, respectively, whereas following treatment, the content was reduced to below the limit of detection, 15 mg L^{-1}. It is likely that relatively insoluble forms of calcium phosphate [21] co-precipitated with $CaSO_3$. The assimilable nitrogen contents of $Ca(OH)_2$-treated brines No. 1 and No. 2 were found to be 945 and 988 mg L^{-1}, respectively, which is about two-fold more than that needed by a wine strain to utilize 20% glucose in a synthetic grape juice containing excess amino acids [11]. Assimilable nitrogen was not measured in the untreated brine, because growth of the yeast needed to perform the bioassay was not possible.

To confirm the phosphorous deficiency, brine No. 1 was pre-treated with $Ca(OH)_2$ to reduce the sulfur dioxide content, supplemented with 7.4 mM DAP, urea, or phosphoric acid, and adjusted to pH 6.0 with 5 N hydrochloric acid, if necessary. The unsupplemented brines (controls) were brought to pH 6.0 by addition of 5 N hydrochloric acid. (Addition of 7.4 mM phosphoric acid was sufficient in itself to reduce the pH to 6.0.) Because these operations were not performed aseptically, the treated brines were examined for contaminating microorganisms prior to yeast inoculation. A small number of organisms were detected: 42 yeast and bacteria ml^{-1} on YEPD, 48 bacteria ml^{-1} on YEPD + cyh, and 68 bacteria ml^{-1} on NA. The brines were then inoculated with *S. cerevisiae* 2896 (Figure 2). Cell viability and rates of sugar utilization, and ethanol and glycerol production were essentially the same in brines supplemented with phosphoric acid or DAP. These fermentations yielded ethanol and glycerol levels consistent with the initial fermentable sugar concentration [10] and were complete in 3 days. Ethanol yields were 80% of the theoretical yield (assuming two moles of ethanol per mole of fermentable sugar with no correction for cell growth or maintenance). In contrast, in the control brine and that supplemented with urea, about 30% of the fermentable sugar remained unconsumed after 7 days. Levels of ethanol, glycerol, and viable yeast cells were the same and correspondingly lower as well. Ethanol yields were 52–55% of the theoretical yield. As expected, the sorbitol content in all brines did not change following fermentation because *Saccharomyces cerevisiae* is unable to ferment this sugar. To determine if contaminating bacteria grew during fermentation, samples of 2.4 ml of the undiluted and fermented brines taken on day 7 were concentrated and plated on YEPD, YEPD + cyh, and NA. As expected, a lawn of yeast was observed on the YEPD plates from all samples. However, no microbial colonies were observed on the YEPD + cyh or NA plates, indicating a maximum potential level of contamination of less than one cell per ml. The pH of the fermented brines ranged from 4.3 to 4.9, likely due to production of a small amount of acetic acid by the yeast [10].

To test commercial strains of *S. cerevisiae* in brine No. 1 containing a higher level of fermentable sugar, glucose was added to a total sugar level of 10%. The glucose-enriched brine was treated with $Ca(OH)_2$, separated from the $CaSO_3$ precipitate, and either supplemented or not with 7.4 mM phosphoric acid. The unsupplemented brine was brought to pH 6.0 by addition of 5 N hydrochloric acid and inoculated with *S. cerevisiae* 2896, Pasteur Champagne, or Allyeast (Figure 3; 2896, data not shown). The initial rates of sugar utilization were approximately the same among all strains in both the phosphorous-supplemented and unsupplemented brines. However, after the second day, sugar consumption by strains in the phosphorous-supplemented brines was faster than in the unsupplemented brines. After

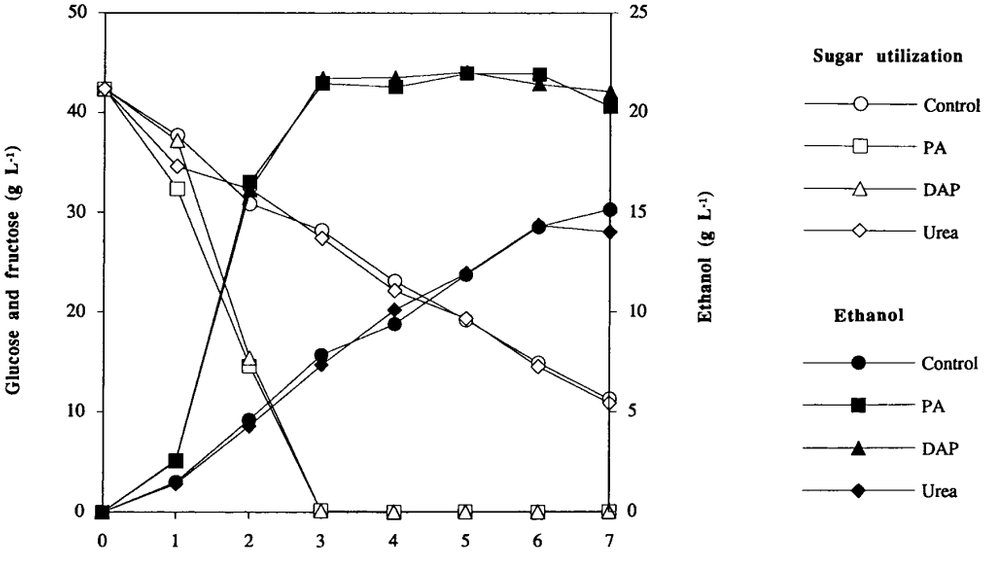

Figure 2 Fermentation of calcium-hydroxide treated brine No. 1 containing 4.2% (w/v) fermentable sugar (glucose and fructose) by *Saccharomyces cerevisiae* 2896 following addition of phosphoric acid (PA), diammonium phosphate (DAP), or urea to final concentrations of 7.2 mM, and subsequent adjustment to pH 6.0 with hydrochloric acid, where necessary. The addition of 7.4 mM phosphoric acid was sufficient in itself to reduce brine pH to 6.0. The control is a calcium hydroxide-treated brine adjusted to pH 6.0 with hydrochloric acid without addition of phosphate or nitrogen. Data points are means of four determinations from duplicate fermentations. Final glycerol yields in the control, PA-, DAP-, and urea-supplemented fermentations were 0.12, 0.21, 0.21, and 0.12% (w/v), respectively. Standard errors were less than 20% of the means of all determinations.

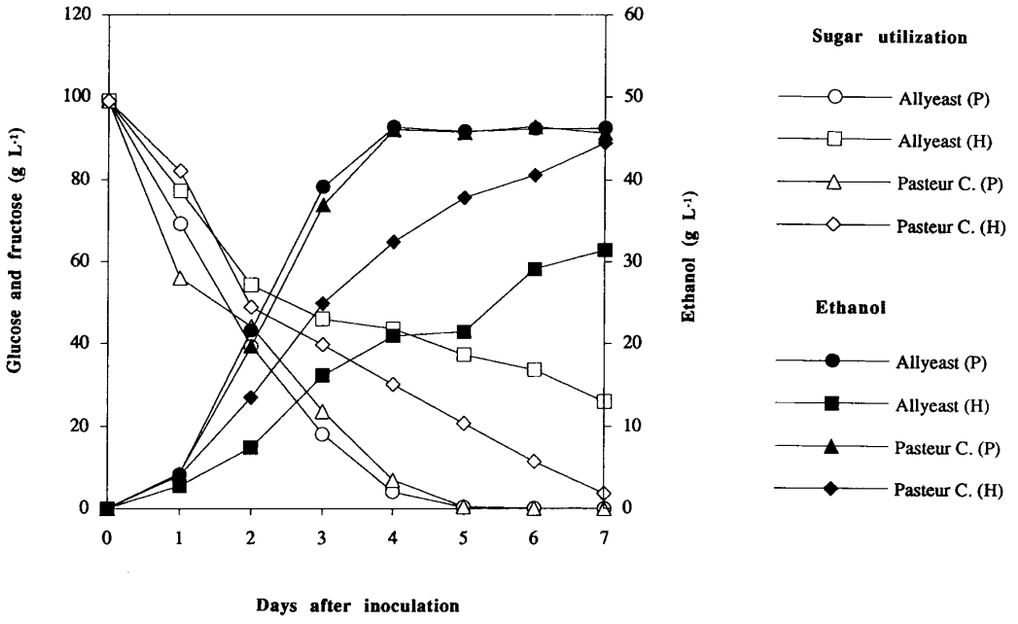

Figure 3 Fermentation of calcium-hydroxide treated brine No. 1 supplemented with glucose to a total fermentable sugar level of 10% (w/v) by *Saccharomyces cerevisiae* Allyeast or Pasteur Champagne following addition of phosphoric acid (P) to a final concentration of 7.4 mM to reduce brine pH to 6.0. (H) indicates control brines whose pH was adjusted to 6.0 with hydrochloric acid without addition of phosphorous. Final glycerol yields of the P- and H-supplemented Allyeast and P- and H-supplemented Pasteur Champagne fermentations were 0.34, 0.21, 0.30, and 0.23% (w/v), respectively. Data points are means of four determinations from duplicate fermentations. Standard errors were less than 20% of the means except for the H-supplemented Allyeast fermentation, where the error was less than 40%.

7 days in the latter brines, about 35% of the fermentable sugar remained in the fermentations involving 2896 and Allyeast, and about 4% in the brine fermented by Pasteur Champagne. In contrast, no fermentable sugar remained in the phosphorous-supplemented brines after 5 days. In the phosphorous-supplemented brines, ethanol production was the same among the three strains, reaching a maximum of about 4.7% (v/v) after 4 days, representing 72–73% of the theoretical yield. In the unsupplemented brines, 2896 and Allyeast produced less ethanol after 7 days, while Pasteur Champagne produced about the same as it did in the phosphorous-supplemented brine, suggesting that this strain used the limiting phosphorous more efficiently than the others, or was better able to mobilize internal phosphorous reserves, such as vacuolar polyphosphate [7]. Glycerol, a natural fermentation byproduct, accumulated to a greater extent in the phosphoric acid-supplemented brines (Figure 3). The levels produced in all fermentations (Figures 2 and 3), 0.12–0.34% (w/v) were comparable to that observed in wine fermentations, 0.1–0.9% (w/v) [15]. Brine was sampled at the end of the fermentations (day 7) for bacteria and yeast as described above for the fermentations that compared DAP, urea, and phosphoric acid supplementation. The same results were obtained: no colonies were observed on the NA or YEPD + cyh plates, and a yeast lawn was found on the YEPD plates, indicating a maximum potential level of non-*Saccharomyces* contamination of less than one cell per ml. As before, the pH of the fermented brines decreased, ranging from 4.1 to 4.7.

Free sulfur dioxide was measured at the end of the fermentations shown in Figures 2 and 3. Initially, brine No. 1 contained 296 mg L^{-1}, having been reduced from 4096 mg L^{-1} by treatment with $Ca(OH)_2$. In the fermentations where all the fermentable sugar was consumed, free sulfur dioxide was reduced about 50%, to 120–170 mg L^{-1}. When all the fermentable sugar was not used, the free sulfur dioxide was reduced about 86%, to 36–52 mg L^{-1}. Initially colorless, the brines turned pink during the course of fermentation likely due to hydrolysis of anthocyanin sulfonates, as this is the expected color of the free pigments at acid pH.

Discussion

Disposal of spent cherry brine, an acidic byproduct of maraschino cherry processing, is a significant problem for processors because of its high biological and chemical oxygen demand. As an alternative to disposal, brine was tested as a substrate for ethanol production by the yeast *Saccharomyces cerevisiae*. Initially, it was found necessary to reduce the growth-inhibitory level of sulfur dioxide by raising brine pH to 8.0 with $Ca(OH)_2$ to precipitate calcium sulfite. Because brine phosphorous was found to co-precipitate under these conditions, the $Ca(OH)_2$-treated brines were subsequently supplemented with phosphoric acid which had the beneficial side-effect of reducing brine pH to 6.0, a more suitable starting pH for yeast fermentation. All strains of *Saccharomyces cerevisiae* tested were able to ferment all lots of $Ca(OH)_2$-treated and phosphorous-enriched brines efficiently. Interestingly, the Pasteur Champagne strain was found to ferment the $Ca(OH)_2$-treated brine without phosphorous addition, albeit more slowly. The process produced ethanol, a value-added product, reduced the biological and chemical oxygen demand of the effluent, and generated a new stream, a calcium sulfite precipitate, which because of

its alkalinity, has potential use as a liming agent for acidic soils.

Sulfur dioxide is added to brine as a preservative and for the purpose of bleaching natural color through reaction with cherry anthocyanins to form colorless sulfonates. The toxicity of sulfur dioxide to microorganisms is a function of pH. The dissociation equilibria for sulfurous acid are given below [12]:

$SO_2 + H_2O = H_2SO_3$	Hydration of sulfur dioxide
$H_2SO_3 = HSO_3^- + H^+$	$pKa_1 = 1.77$
$HSO_3^- = SO_3^{-2} + H^+$	$pKa_2 = 7.2$

At low pH, as in cherry brine, a significant proportion is in the form of undissociated sulfurous acid, the toxic species. Its toxicity is due to it being the form of sulfur dioxide that readily crosses microbial cell membranes [3]. In contrast, at neutral pH, yeast is much more tolerant of sulfur dioxide because the proportion present as sulfurous acid is so small. In fact, a yeast fermentation designed for the overproduction of glycerol was developed by Neuberg early in this century that requires growth in a neutral brine containing 3–3.5% free sulfur dioxide [18]. Under these conditions, acetaldehyde reacts preferentially with sulfur dioxide to form 1-hydroxyethane sulfonate [16] which cannot be reduced to ethanol. Instead, regeneration of NAD^+ is coupled to the reduction of dihydroxyacetone phosphate which produces glycerophosphate, and ultimately glycerol, through the action of a phosphatase [8]. An independent cause of glycerol formation is the diversion of pyruvate for biosynthetic reactions and away from ethanol production. When this occurs, a stoichiometric amount of acetaldehyde is unavailable for regeneration of NAD^+, causing dihydroxyacetone phosphate to be reduced instead, as described for the Neuberg fermentation [10].

The high sulfur dioxide level in untreated acidic brine acts as an antimicrobial agent and has the beneficial property of preventing premature fermentation without need for an additional sterilization treatment. Following $Ca(OH)_2$ addition, and adjustment of brine pH to 6.0 with phosphoric acid, it is likely that a variety of microorganisms would grow if a large inoculum of *S. cerevisiae* were not added. Because *S. cerevisiae* usually produces a small amount of acetic acid during fermentation, the pH dropped to about 4.5, which made the residual sulfur dioxide a more effective preservative. While *S. cerevisiae* is relatively tolerant of this amount of sulfur dioxide, contaminating bacteria and other yeasts may not be, which would explain their absence at the end of the alcoholic fermentation in spite of the fact that the $Ca(OH)_2$-treated brine was not sterilized prior to yeast inoculation.

While use of $Ca(OH)_2$ to precipitate excess sulfur dioxide was found to be efficient, it led to formation of a new waste stream, solid $CaSO_3$. On a mass basis, the material has about half the liming value of $CaCO_3$, which is normally used to lime acidic agricultural soils. In practice, this potential will likely only be realized if the material can be used within close proximity to the site where it is produced and if the water content can be reduced to a practical level economically.

Acknowledgements

The authors thank the Oregon Sweet Cherry Commission, the Northwest Cherry Briners Association, Parallel Products, and the Agricultural Research Foundation for financial support; Oregon Cherry Growers for providing brine samples; Carl Payne, Tom Koehler, and Professors Daniel F Farkas and Robert F Cain for helpful discussions.

References

1 American Public Health Association. 1975. Standard Methods for the Examination of Water and Waste Water, 14th edn. American Public Health Association, New York.
2 Amerine MA and CS Ough. 1974. Wine and Must Analysis. John Wiley and Son, NY, NY.
3 Bakalinsky AT. 1992. Yeast biopreservatives. In: Food Biopreservatives of Microbial Origin (Ray B and MA Daeschel, eds), pp 347–371, CRC Press, Boca Raton, FL.
4 Bakalinksy AT and R Snow. 1990. Conversion of homothallic wine strains of *Saccharomyces cerevisiae* to heterothallism. Appl Environ Microbiol 56: 849–857.
5 Beavers DV, CH Payne and RF Cain. 1971. Quality and yield of brined cherries. Oregon Agr Exp Station Tech Bull 118.
6 Beavers DV, CH Payne, MR Soderquist, KI Hildrum and RF Cain. 1970. Reclaiming used cherry brines. Oregon Agr Exp Station Tech Bull 111.
7 Bostian KA and JM Lemire. 1983. Physiological control of repressible acid phosphatase gene transcripts in *Saccharomyces cerevisiae*. Mol Cell Biol 3: 839–853.
8 Gancedo C, JM Gancedo and A Sols. 1968. Glycerol metabolism in yeasts: pathways of utilization and production. Eur J Biochem 5: 165–172.
9 Horneck DA, JM Hart, K Topper and B Koepsell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University, Corvallis, OR.
10 Ingledew WM. 1993. Yeasts for production of fuel alcohol. In: The Yeasts, Vol 5, Yeast Technology (Rose AH and JS Harrison, eds), pp 245–291, Academic Press, London.
11 Jiranek V, P Langridge and PA Henschke. 1995. Amino acid and ammonium utilization by *Saccharomyces cerevisiae* wine yeasts from a chemically defined medium. Am J Enol Vitic 46: 75–83.
12 King AD Jr, JD Ponting, DW Sanshuck, R Jackson and K Mihara. 1981. Factors affecting death of yeast by sulfur dioxide. J Food Prot 44: 92–97.
13 Monterio FF and LF Bisson. 1991. Biological assay of nitrogen of grape juice and prediction of sluggish fermentations. Am J Enol Vitic 42: 47–57.
14 Neubeller J and R Stösser. 1977. Das zuckermuster bei süβkirschen in den einzelnen teilen der frucht und in verschiedenen untersuchungsjahren. Gartenbauwissenschaft 42: 222–225.
15 Noble AC and GF Bursick. 1984. The contribution of glycerol to perceived viscosity and sweetness in white wine. Am J Enol Vitic 35: 110–112.
16 Ough CS. 1993. Sulfur dioxide and sulfites. In: Antimicrobials in Foods (Davidson PM and AL Branen, eds), pp 137–190, Marcel Dekker, NY, NY.
17 Payne CH, DV Beavers and RF Cain. 1969. The chemical and preservative properties of sulfur solution for brining fruit. Oregon Agr Exp Sta. Circular of Information 629, Oregon State Univ, Corvallis.
18 Phaff HJ, MW Miller and EM Mrak. 1978. The Life of Yeasts. Harvard University Press, Cambridge, MA.
19 Sapers GM, O Panasiuk and LR Ross. 1977. Disposal of sweet cherry processing brines. J Food Sci 42: 1454–1456.
20 Soderquist MR. 1971. Activated carbon renovation of spent cherry brine. J Water Poll Contr Fed 43: 1600–1608.
21 Windholz M (ed). 1976. The Merck Index, 9th edn. Merck & Co, Rahway, NJ.
22 Wrolstad RE and RS Shallenberger. 1981. Free sugars and sorbitol in fruits—a compilation from the literature. J Assoc Off Anal Chem 64: 91–103.