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Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium

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Specific growth rates (μ) of two strains of *Saccharomyces cerevisiae* decreased exponentially (R^2>0.9) as the concentrations of acetic acid or lactic acid were increased in minimal media at 30°C. Moreover, the length of the lag phase of each growth curve (h) increased exponentially as increasing concentrations of acetic or lactic acid were added to the media. The minimum inhibitory concentration (MIC) of acetic acid for yeast growth was 0.6% w/v (100 mM) and that of lactic acid was 2.5% w/v (278 mM) for both strains of yeast. However, acetic acid at concentrations as low as 0.05–0.1% w/v and lactic acid at concentrations of 0.2–0.8% w/v begin to stress the yeasts as seen by reduced growth rates and decreased rates of glucose consumption and ethanol production as the concentration of acetic or lactic acid in the media was raised. In the presence of increasing acetic acid, all the glucose in the medium was eventually consumed even though the rates of consumption differed. However, this was not observed in the presence of increasing lactic acid where glucose consumption was extremely protracted even at a concentration of 0.6% w/v (66 mM). A response surface central composite design was used to evaluate the interaction between acetic and lactic acids on the specific growth rate of both yeast strains at 30°C. The data were analysed using the General Linear Models (GLM) procedure. From the analysis, the interaction between acetic acid and lactic acid was statistically significant ($P \leq 0.001$), i.e., the inhibitory effect of the two acids present together in a medium is highly synergistic.
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Keywords: *Saccharomyces cerevisiae*; acetic acid; lactic acid; synergy; specific growth rate

Introduction

Extensive research has been carried out to understand and characterize the inhibitory actions of various organic acids on growth of microorganisms. Organic acids have both fungitastic and fungicidal effects which are maximal at low pH [15]. In this context, both acetic and lactic acids are of special interest to alcohol manufacturers, since both these acids are potential inhibitors of yeast growth. Maiorella *et al.* [12] reported an 80% reduction in biomass yield of *Saccharomyces cerevisiae* when 7.5 g/l of acetic acid or 38 g/l of lactic acid was present in the medium. Acetic acid is a very minor endproduct of fermentation by *S. cerevisiae*, but inhibitory amounts may be produced by contaminating lactic acid bacteria and/or acetic acid bacteria. Lactic acid is the major metabolite of lactic acid bacteria and may cause a pH change in the growth medium sufficient to antagonise microorganisms [17], including yeast in an alcohol fermentation [14]. A pH change in the medium resulting from accumulation of this weak acid is, however, not extensive because a large amount of lactic acid does not dissociate at the pH value used (pK_a for lactic acid=3.86). The extent of any pH change is also influenced by the medium composition, medium pH and the degree of buffering provided.

Early experiments by Levine and Fellers [10] demonstrated that acetic acid was more lethal to microorganisms than lactic or hydrochloric acid. They concluded that this toxicity was not due to hydrogen ion concentration alone, but seemed to be a function of the concentration of undissociated acid. Acetic acid (pK_a=4.74) has between two and four times more molecules in the undissociated form over a pH range between 4.0 and 4.6 compared to lactic acid [11]. With acetic acid in the medium, a lowering of the pH increased the inhibitory activity, confirming that the undissociated molecule was the effective inhibitor [4]. Thus, the inhibition by organic acids used as antimicrobial agents would increase with decreasing pH depending on their dissociation constants. This implies that efficacy relies upon the undissociated form of the molecule which diffuses across the cell membrane passively due to its high solubility in the phospholipid portion of the plasma membrane. The molecule then dissociates inside the cell with the extent of dissociation depending on the intracellular pH. The membrane is impermeable to the dissociated acid [5,7], unless yeast is metabolizing aerobically. Then, a mediated transport system for acetic acid behaving as an electroneutral proton symport for the anionic form of the acid can be seen in *S. cerevisiae* IGC 4072 grown aerobically in medium with acetic acid [1]. However, it cannot be generalized that only the undissociated form is active, as Eklund [3] demonstrated cellular effects attributable to both dissociated and undissociated forms of sorbic acid above pH 6 in experiments to determine the minimum inhibitory concentration (MIC). The inhibitory action of undissociated acid was 10–600 times greater than that of dissociated acid. But the latter caused more than 50% growth inhibition of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at pH levels above 6, where more than 95% would be present as sorbate anion.

Studies have been carried out on the effects of fatty acids on microorganisms [4,8,25] and on the combined effects of alcohols and fatty acids on yeasts [16,21]. However, not much is known about the synergistic action of these compounds. Moon [13]

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Table 1

Independent variable	Code level				
	−1.414	−1	0	+1	+1.414
(a) Levels of acetic acid and lactic acid corresponding to coded values as designated by the central composite design (Alltech strain)					
Acetic acid (% w/v)	0	0.037	0.125	0.213	0.25
Lactic acid (% w/v)	0	0.07	0.25	0.43	0.5
(b) Levels of acetic and lactic acids corresponding to coded values as designated by the central composite design (ATCC 26602)					
Acetic acid (% w/v)	0	0.051	0.175	0.299	0.35
Lactic acid (% w/v)	0	0.102	0.35	0.598	0.7

studied the inhibition of yeast growth by mixtures of acetic, lactic and propionic acids at one pH value and derived simple polynomial expressions linking growth rate with concentrations of the preservatives. Formulae included interactive terms that implied synergisms, although it was not stated whether differences were statistically significant.

In this paper, we report the effects of acetic and lactic acids on the specific growth rate of yeast, fermentation of glucose by yeast and the MICs of these two acids for yeast. A response surface central composite design was used to evaluate the interactive effects of acetic and lactic acids on yeast growth.

Materials and methods

Organism

The two strains of *S. cerevisiae* used were an isolate purified from an industrial strain of active dry yeast obtained from Alltech (Nicholasville, KY) and ATCC 26602 (American Type Culture Collection, Rockville, MD).

Medium

A chemically defined (minimal) mineral salts medium with glucose (2% w/v) and vitamins was used. The final concentrations of ingredients in the medium were: (mmol/l) $(NH_4)_2SO_4$, 37.85; K_2HPO_4, 0.86; KH_2PO_4, 6.83; $MgSO_4$, 2.03; NaCl, 2.05; and (μmol/l) H_3BO_3, 24; $MnSO_4$, 20; Na_2MoO_4, 1.5; $CuSO_4$, 10; $CoCl_2$, 1.5; $ZnSO_4$, 100; KI, 1.8; $FeCl_3$, 100; $CaCl_2$, 82; and (μg/l) biotin, 200; calcium pantothenate, 2000; folic acid, 20; myo-inositol, 10,000; niacin, 400; pyridoxine HCl, 400; riboflavin, 200; thiamine HCl, 200. The vitamin solution was prepared as a 1000-fold concentrated stock and kept frozen at −20°C. When needed, an aliqout was thawed and filter-sterilized (0.2-μm pore size membrane filter) and the required amount was added to the medium.

Growth conditions

Growth was measured turbidometrically using a Klett Summerson colorimeter (Klett Manufacturing, New York, NY) equipped with a no. 66 red filter (420–660 nm). Calibration curves of Klett units plotted against cell number and cell mass were constructed. Starter cultures were grown with shaking (100 rpm) (Model G25 Controlled Environmental Shaker, New Brunswick Scientific, Edison, NJ) at 30°C for 24 h in 50 ml of pH 4.5 minimal medium in 250-ml Erlenmeyer flasks without added acetic or lactic acid. Then, $\sim 2\times10^7$ or $\sim 4.5\times10^7$ cells of the Alltech strain and ATCC 26602, respectively, were inoculated into experimental flasks and grown at 30°C in the shaker (100 rpm). The flasks used were 250-ml screw-capped, side-arm Erlenmeyers with 50 ml medium and a range of concentrations of the acid (0, 0.1, 0.2, 0.3, 0.4 and 0.5% w/v for acetic acid and 0, 0.2, 0.4, 0.6, 0.8, and 1.0% w/v for lactic acid). Experiments were done in duplicate. The specific growth rates (μ in h^{-1}) and lag times (h) were calculated for both yeast strains at various concentrations of both acetic and lactic acids.

Determination of MIC

The MIC of each acid for both yeast strains was determined. For this work, MIC was defined as the smallest concentration of the acid that inhibited growth of the chosen yeast for a period of at least 72 h. The concentrations of acetic and lactic acids tested were 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.9% w/v and 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% w/v, respectively. Experiments were done in duplicate.

Fermentation rates at various concentrations of acetic and lactic acids

The yeast strains were grown in minimal media with glucose (2% w/v), minerals and vitamins along with different concentrations of the acids (0, 0.1, 0.2 and 0.3% w/v acetic acid and 0, 0.2, 0.4, and 0.6% w/v lactic acid). Samples were withdrawn at 3, 6, 9, 12, 15.5, 20 and 24 h, filtered through a 0.45-μm pore size filter, diluted in an equal volume of 2% w/v boric acid (internal standard) and analysed for glucose consumed and ethanol produced using high-performance liquid chromatography (HPLC).

HPLC analysis

A 5-μl aliquot from a suitably diluted sample was analyzed using a HPX-87H column (Bio-Rad Laboratories, Mississauga, Ontario, Canada) maintained at 40°C which analyzes sugars, alcohols and organic acids. Sulphuric acid (5 mM) was used in the mobile phase at a flow rate of 0.7 ml/min. The components were detected with a differential refractometer (model 410; Waters Chromatographic Division, Milford, MA). The data were processed using the Maxima 820 computer program (Waters Chromatographic Division).

Experimental design for the evaluation of the interactions between acetic and lactic acids

The experiment was planned and conducted using response surface central composite design [2] for two variables at five levels (Table 1a and b). The maximum concentrations of acetic and lactic acids selected were based on the criteria that they should not completely

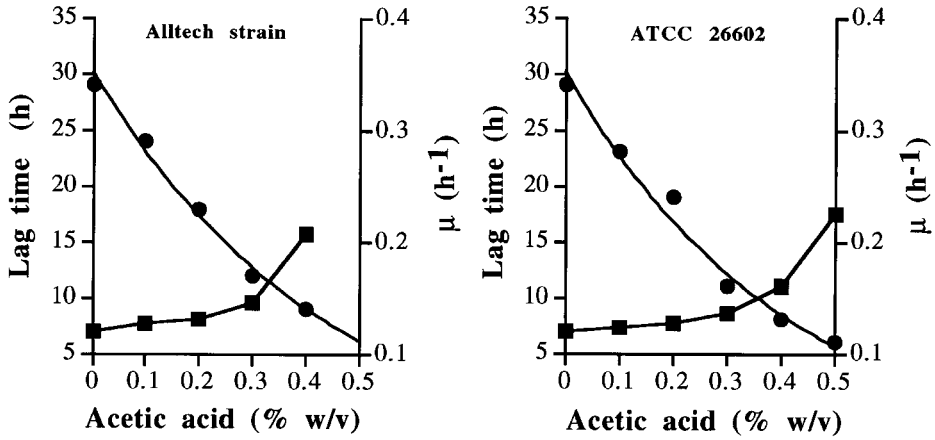

Figure 1 Effect of acetic acid on specific growth rates (●) and lag times (■) of two strains of *S. cerevisiae* in minimal medium at 30°C.

inhibit the metabolic activity of the two yeasts studied. Two replicate experiments were conducted. There were 13 treatment combinations of the two acids, including five centre points. The growth of the yeasts was monitored as a measure of turbidity in each of the 13 experimental flasks for 24 h at 3-h intervals. The specific growth rates in the log phase of growth were calculated.

Statistical analysis of data

Data were analysed using the General Linear Model (GLM) of SAS Institute [19]. Estimates for the linear, quadratic and interaction effects of each acid, which fit the following equation, were developed:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \epsilon$$

where y is the specific growth rate (μ) at a certain level of acetic and lactic acid; x_1 the concentration of acetic acid; x_2 the concentration of lactic acid; β_n the parameter estimates; β_0 the estimate for the y-intercept; β_1 the estimate for the linear effect of acetic acid concentration; β_2 the estimate for the linear effect of lactic acid concentration; β_{11} the estimate for the quadratic effect of acetic acid concentration; β_{22} the estimate for the quadratic effect of lactic acid concentration; β_{12} the estimate for the interactive effect between acetic acid and lactic acid; and ε the error term.

Results and discussion

Growth of yeast is always faster in complex than in minimal media. Moreover, the presence of components such as yeast extract in yeast extract–peptone–dextrose (YEPD) broth offers some protection against stress conditions. It is difficult (and in some cases impossible) to quantitate the uptake of substrates in complex media and to study the effects of stress conditions. Use of chemically defined media overcomes many of the limitations of complex media, although growth rates are reduced and are not representative of industrial fermentations. The studies reported here were carried out at 30°C in a chemically defined medium with glucose (2% w/v) and added vitamins.

Inhibitions of yeast growth by acetic and lactic acids

The specific growth rates of both yeast strains decreased exponentially while lag times increased exponentially as the concentration of the acids in the medium was increased (Figures 1 and 2). Similar increases in lag times of yeast growth were observed by Lambert and Stratford [9] for increasing concentra-

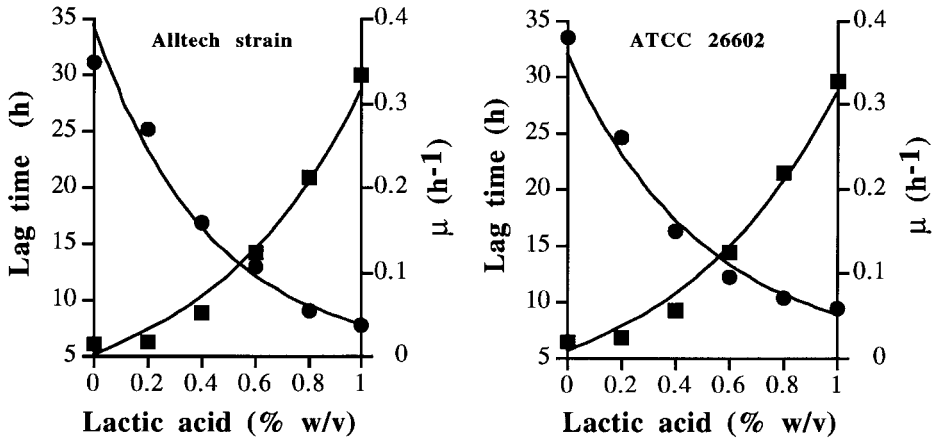

Figure 2 Effect of lactic acid on specific growth rates (●) and lag times (■) of two strains of *S. cerevisiae* in minimal medium at 30°C.

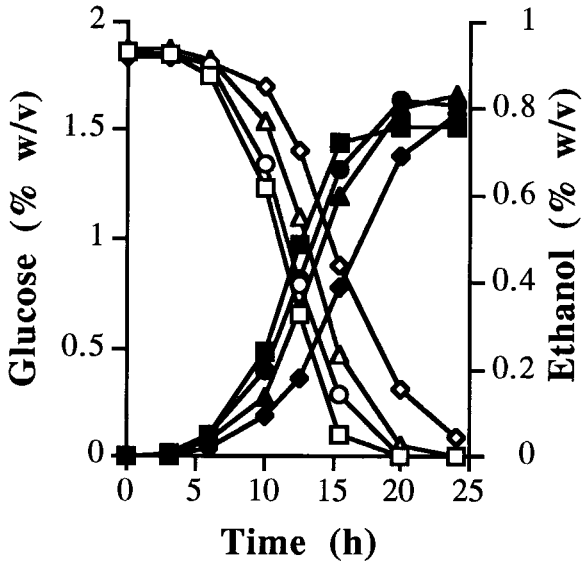

Figure 3 Glucose depletion (open symbols) and ethanol production (filled symbols) by *S. cerevisiae* (Alltech strain) in minimal medium at 30°C in the presence of increasing concentrations of acetic acid. Symbols: (□,■) 0% w/v (control); (○,●) 0.1% w/v (17 mM); (△,▲) 0.2% w/v (33 mM); and (◇,◆) 0.3% w/v (50 mM).

tions of the weak acid preservative, sorbic acid, in the medium. These authors proposed a model to show that the increase in the duration of the lag phase observed at increasing weak acid concentrations reflected the time taken by yeast to pump out excess protons to achieve the required intracellular pH for growth. The MIC of acetic acid was 0.6% w/v (100 mM) and that of lactic acid was 2.5% w/v (278 mM) for both yeast strains tested (i.e., these were concentrations at which no growth of the yeast strains was detected for at least 72 h after inoculation). Stratford and Anslow [23] reported a similar concentration of 90 mM acetic acid to be the MIC for *S. cerevisiae* X2180-1B. Concurrently, we noted that lactic acid concentrations of 0.8–1.0% w/v reduce the growth rate of yeast sharply, and that acetic acid reduces the growth rate of yeast at concentrations as low as 0.05–0.1% w/v in minimal medium with glucose (2% w/v) as the carbon source. Similar values for acetic acid were reported by Maiorella *et al.* [12]. Acetic acid is inhibitory to yeast at a much lower concentration than is lactic acid. At a given acidic pH (because of the higher pK_a value of acetic acid), there is more undissociated acetic acid present than would be found with an equal concentration of lactic acid [11]. The undissociated forms of these acids (due to their lipophilic nature) diffuse into yeast cells through the cell membrane, and at higher intracellular pH, they dissociate, producing hydrogen ions and thereby causing changes in yeast metabolic activity [5,7].

Table 2 Maximum yeast cell mass (mg/ml dry weight) obtained in minimal medium with various concentrations of acetic or lactic acid in 24 h at 30°C

Acid	Concentration (% w/v)	Dry weight (mg/ml)	
		Alltech strain	ATCC 26602
Control (no acid)	0	2.1952	1.8224
Acetic	0.1 (17 mM)	2.0776	1.5664
	0.2 (33 mM)	1.7248	1.3338
	0.3 (50 mM)	1.4014	1.0998
Lactic	0.2 (22 mM)	1.8228	1.4430
	0.4 (44 mM)	1.1270	0.6630
	0.6 (66 mM)	0.0918	0.1794

Effects of acetic and lactic acids on the fermentation rate of S. cerevisiae

There was a reduction in the rates of glucose consumption and ethanol productions as the concentration of acetic acid was increased to exceed 0.1% w/v in the medium (Figure 3). The total biomass produced also decreased with increasing concentrations of acetic and lactic acids (Table 2). We needed to ensure that the decreases in biomass observed in the presence of the acids were not due just to the lowering of pH of the medium (which resulted from the addition of these acids). To verify this, both yeasts were grown in minimal media at pH levels of 2.6 and 3.0 at 30°C without acetic or lactic acid. The total biomass values produced after 24 h of growth were 1.38 and 1.4 mg/ml, for the Alltech strain and for ATCC 26602, respectively, when the initial media pH was 2.6. Biomass values were 2.117 and 1.833 mg/ml for the Alltech strain and for ATCC 26602, respectively, when the initial media pH was 3.0. Values for dry weight when acetic or lactic acid was added (and pH was therefore poised near 2.6 or 3.0) were more than eightfold less than when the medium was adjusted to the same pH values without the organic acids. Therefore, it can be concluded that the reduction of total biomass of both yeast strains observed (Table 2) is due to the presence of acetic or lactic acid in the media which, at low pH values (2.64 or 3.19; Table 3), exists predominantly in the undissociated/uncharged form. Even though biomass production decreased with increasing concentrations of acetic acid in the medium, all of the glucose was consumed and the same levels of maximum ethanol were produced in 24 h by the Alltech yeast (Figure 3). Similar results were obtained with the yeast, ATCC 26602 (data not shown). This can be explained by the classic weak acid theory, i.e., that undissociated molecules freely diffuse through the cell membrane and dissociate in the cytoplasm due to the higher intracellular pH, thereby acidifying the cytoplasm. The cell, however, tries to maintain its internal pH homeostasis by pumping out the excess protons *via* the H^+ translocating plasma membrane ATPase which utilizes ATP for its activity. The interference of acetic acid, therefore, results in an increased ATP requirement for cell maintenance [12]. In other words, the ATP required for

Table 3 Percentages of undissociated acid and anions of acetic and lactic acids in minimal medium at pH values attained corresponding to the various acid concentrations

Acid	Concentration (mM)	pH[a]	Undissociated acid (%)[b]	Anion (%)[b]	Mole concentration of undissociated acid (mM)
Acetic	17	3.48	94.63	5.37	16.08
	33	3.31	96.37	3.63	31.80
	50	3.19	97.25	2.75	48.63
Lactic	22	2.95	87.68	12.32	19.29
	44	2.76	92.06	7.94	40.48
	66	2.64	93.97	6.03	62.98

[a]Values are means of duplicate samples.
[b]Values were calculated using the Henderson–Hasselbach equation [$pH = pK_a + \log([A^-]/[HA])$] and p$K_a$ values of acetic (4.74) and lactic acids (3.86).

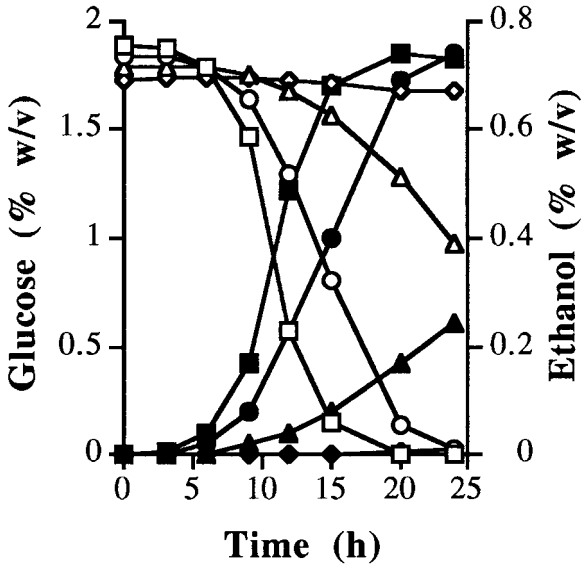

Figure 4 Glucose depletion (open symbols) and ethanol production (filled symbols) by *S. cerevisiae* (Alltech strain) in minimal medium at 30°C in the presence of increasing concentrations of lactic acid. Symbols: (□,■) 0% w/v (control); (○,●) 0.2% w/v (22 mM); (△,▲) 0.4% w/v (44 mM); and (◇,◆) 0.6% w/v (66 mM).

production of cell mass is channelled for maintenance of pH homeostasis inside the cell rather than growth [22]. This causes a reduction in the total biomass produced. According to van der Rest *et al.* [24], ATPase activity is estimated to consume 10–15% of the ATP produced during yeast growth and has a reaction stoichiometry of one proton extruded per molecule of ATP hydrolyzed.

Lactic acid appears to have a different effect than acetic acid on the Alltech yeast (Figure 4). Similar results were obtained with ATCC 26602 (data not shown). While an increased acetic acid concentration delayed both the utilization of glucose and production of ethanol, lactic acid at a relatively low concentration (0.6% w/v) totally shut down glucose utilization and ethanol synthesis in glucose–mineral salts medium. Lesser concentrations of 0.2–0.6% w/v greatly affected glucose utilization and the rate of ethanol production (Figure 4). A level of 0.6% w/v lactic acid is an industrially relevant concentration and is easily produced through the action of lactic acid bacterial contaminants in fermentation. Lactic acid bacteria can be serious contaminants in the fuel alcohol fermentation since they compete with yeast for nutrients. Yeast viability is reduced, carbohydrate may be unused at the end of fermentation and yields of ethanol are reduced as a portion of glucose is converted by these bacteria to lactic (and acetic) acid. These acids are inhibitory to yeast growth and are recycled when backset and process condensate are used as make-up water in mashing [6].

The inhibitory activity of acetic and lactic acids in the medium is determined by the pH of the medium, the dissociation constants of the acids and by their molar concentrations. These values are given in Table 3. Taking this into consideration, different effects are observed for glucose uptake and ethanol production in both yeast strains as caused by acetic and lactic acids, although both acids have similar molar concentrations of undissociated acid in the medium. Studies on the mode of action of these acids have indicated that they may not act in the same manner on the cell, as Maiorella *et al.* [12] reported that acetic interference with yeast metabolism resulted in an increase in ATP requirement for cell maintenance whereas the mechanism of lactic acid inhibition was probably different. Data for the action of acetic, lactic and propionic acids on yeasts showed growth inhibition different from that predictable on the basis of dissociation constants, indicating that these acids may not act in the same manner [13].

Interaction of acetic and lactic acids on the inhibition of yeast growth

It is difficult to demonstrate that two or more agents act synergistically or antagonistically on the specific growth rate of a culture. Only by very careful experimental design can such interactions be assessed. Response surface central composite design is one way of detecting interactions between two or more agents. However, when concentrations of weak acids are set at particular values, the proportions of dissociated and undissociated weak acid at any given pH will vary depending upon the dissociation constant of the acid. In this study, the interactive effect of acetic and lactic acids on the specific growth rate of *S. cerevisiae* was evaluated based on the concentrations of these acids (at particular values) in the medium (i.e., fluctuations in the

Table 4

Source	*df*	Type III SS	Mean square	*F* value	Probability
(a) Computer-generated[1] *ANOVA for specific growth rate of S. cerevisiae (Alltech strain)*					
Trial	1	0.0000087	0.0000087	0.29	0.5961
Acetic	1	0.0011091	0.0011091	37.25	0.0001
Lactic	1	0.0002367	0.0002367	7.95	0.0110
Acetic×Acetic	1	0.0000211	0.0000211	0.71	0.4098
Lactic×Lactic	1	0.0035682	0.0035682	119.85	0.0001
Acetic×Lactic	1	0.0005281	0.0005281	17.74	0.0005
(b) Computer-generated[1] *ANOVA for specific growth rate of S. cerevisiae (ATCC 26602)*					
Trial	1	0.0000203	0.0000203	0.10	0.7513
Acetic	1	0.0029339	0.0029339	14.91	0.0011
Lactic	1	0.0031810	0.0031810	16.17	0.0007
Acetic×Acetic	1	0.0001114	0.0001114	4.57	0.0001
Lactic×Lactic	1	0.0006541	0.0006541	3.33	0.0001
Acetic×Lactic	1	0.0007031	0.0007031	3.57	0.0011

[1](SAS/STAT®) — see Ref. [19].

molecular species were not taken into consideration). Table 4a and b show the analyses of variance (ANOVA) for the two independent variables (acetic and lactic acids) for both yeast strains. Several criteria such as R^2 values, coefficient of variation (CV) and model significance were used to judge the adequacy of the models. For a good fit of any model, R^2 should be at least 80%, CV should not exceed 10% and model significance (P value) should be <0.05 [26]. The models developed in this study were adequate since the levels of R^2, CV and model significance agreed to the criteria for a good fit of any model (Table 5).

Higher maximum concentrations of acetic and lactic acids were chosen for strain ATCC 26602 (Table 1b) because this strain was capable of growth at higher concentrations of both these acids compared to the Alltech strain (although the MICs of both the acids for both strains were similar). If disproportionate inhibitory concentrations of the two acids are used, the ratios will shift to one end of the spectrum, thus appearing to be additive [18]. This is probably why it has been reported that acetic and lactic acids, when present together, exert an additive inhibitory effect on *Salmonella gallinarum* [20].

The statistical significance of linear, quadratic and interactive effects of acetic and lactic acids on the specific growth rates was determined by ANOVA procedure for the Alltech strain (Table 4a) and for ATCC 26602 (Table 4b). Experiments conducted at different times yielded similar results. There were no significant differences observed between the trials ($P=0.5961$ for the Alltech strain and 0.7513 for ATCC 26602). All the other effects (linear, quadratic and interaction) of acetic and lactic acids were highly significant ($P\leq0.001$). The linear effect of lactic acid is still significant for the Alltech strain since $P=0.011$, but the quadratic effect of acetic acid is not significant ($P=0.4098$). Therefore, in Figure 5, a smooth quadratic surface is not seen for the Alltech strain, but is seen with ATCC 26602 (which has a significant quadratic term for acetic acid with an acceptable fit). The interaction term, x_1x_2, between acetic and lactic acids (in the reduction of the specific growth rate of both strains of *S. cerevisiae*) is highly significant ($P\leq0.001$), indicating synergy. Figure 5 indicates the influence of acetic and lactic acids on the specific growth rate (μ) of *S. cerevisiae*. These acids, when present together in the medium, exerted a higher inhibitory effect (due to synergy) on the specific growth rate of yeast than when each acid was present alone. When 0.5% w/v lactic acid was present in the media, the presence of even 0.04% w/v acetic acid (which did not cause a significant change in yeast growth rate when present by itself) caused a significant reduction in the growth rate of *S. cerevisiae* ($P\leq0.001$) (Figure 5). This provides the explanation to a phenomenon noted in the fuel alcohol industry that small concentrations of acetic acid have an enormous inhibiting effect in a fermentation (which already has significant levels of lactic acid made by contaminating lactic acid bacteria).

Although the effects of acetic and lactic acids on the specific growth rates, lag times and the fermentation rates have been elucidated, it is difficult at this stage to explain the findings in terms of specific cellular events, i.e., what the mechanism might be for the

Table 5 Models for the response variable (specific growth rate) obtained from the GLM procedure for the two strains of *S. cerevisiae*

Variable and source	*df*	Sum of squares	*F* value	*P*>*F*
(1) Alltech strain				
Model	6	0.1097708	614.47	0.0001
Error	19	0.0005657		
Corrected total	25	0.1103365		
$R^2=0.9948$				
Coefficient of variation (CV) = 2.895%				
Coefficients for response surface model				
Specific growth rate				
$y=0.306+(0.001/2)-0.354x_1-0.081x_2+0.158x_1^2-0.508x_2^2-0.513x_1x_2$				
(2) ATCC 26602				
Model	6	0.0866123	73.38	0.0001
Error	19	0.0037370		
Corrected total	25	0.0903500		
$R^2=0.9586$				
Coefficient of variation (CV) = 9.625%				
Coefficients for response surface model				
Specific growth rate				
$y=0.26+(0.002/2)-0.409x_1-0.214x_2+0.184x_1^2+0.112x_2^2-0.302x_1x_2$				

y = Specific growth rate (μ).
x_1 and x_2: concentrations of acetic and lactic acids, respectively.

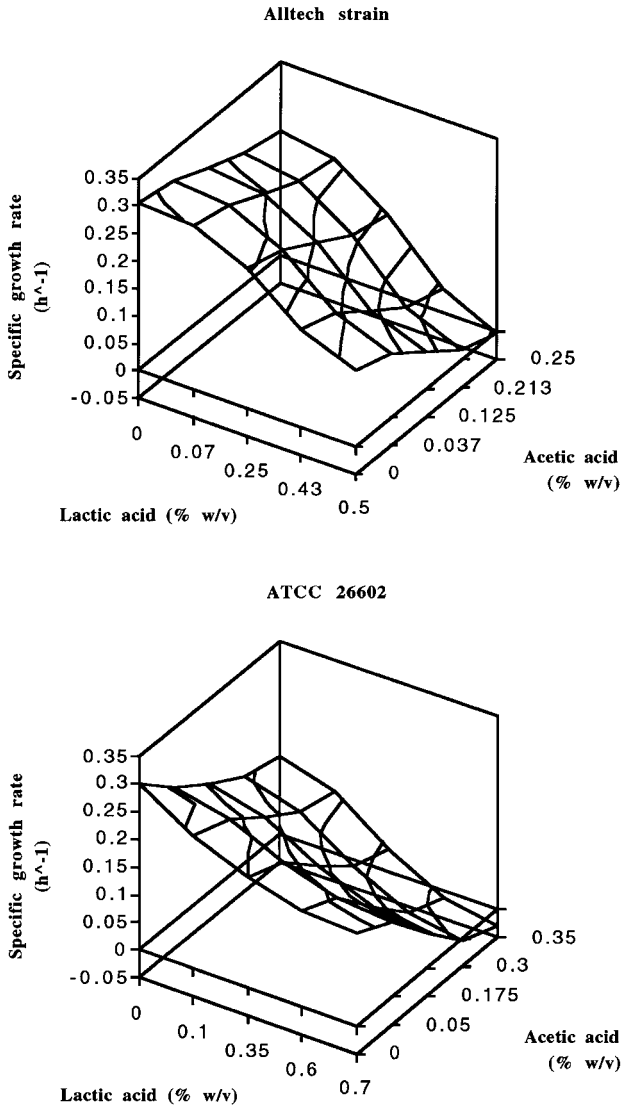

Figure 5 Influence of acetic and lactic acids on the specific growth rate (μ) of *S. cerevisiae* in minimal medium at 30°C. A negative (synergistic) interaction between the compounds is shown.

action of lactic acid on yeast. The present work, however, has shown that growth of both strains of yeasts is inhibited in a glucose–mineral salts medium synergistically by acetic and lactic acids and that both these acids may not inhibit yeast in the same manner. Work on the details of the mechanism of action is currently in progress.

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