International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:11, 2014

Effect of Mineral Ion Addition on Yeast Performance during Very High Gravity Wort Fermentation

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***Abstract*—**The effect of Zn^{2+}, Mg^{2+}, and Ba^{2+} on *Saccharomyces pastorianus* performance was evaluated in this study at independent and three variable combinations. After 96 h of fermentation, high wort fermentability (%F) = 29.53 was obtained in medium containing 900:4 ppm Mg^{2+} + Ba^{2+}. Increased ethanol yield 7.35 %(v/v) and 7.13 %(v/v) were obtained in media containing 900:4 ppm Mg^{2+} + Ba^{2+} and 12:900 ppm Zn^{2+} + Mg^{2+}. Decrease %F = 22.54 and ethanol yield 6.18 % (v/v) was obtained in medium containing 12:4 ppm Zn^{2+} + Ba^{2+}. In media containing the individual ions, increased %F = 27.94 and 26.03 were recorded for media containing 700 ppm Mg^{2+} and 2 ppm Ba^{2+} , with ethanol yield of 7.88% (v/v) and 7.62% (v/v) respectively. Reduced %F and ethanol yield was observed for 10 ppm Zn^{2+} and 4 ppm Ba^{2+} media. The impact of Ba^{2+} at 1 and 2 ppm was significant.

***Keywords*—**Ethanol yield, fermentability, mineral ions, yeast stress, very high gravity fermentation.

I. Introduction

In recent years, high and very high gravity (VHG) fermentation technology has been seen as a viable and economic approach in fermentation processes and has been progressively adopted in the brewing and biofuel industry worldwide. Various benefits have been associated with the technology and have been drivers for further investigation into optimization techniques [1], [2]. Significant challenges associated with the technology include decreased material efficiency, reduced foam stability of beer, problem of flavor matching, inhibitory feedback effect on hydrolyzing enzyme and most importantly, the negative effect of high ethanol concentration on the yeast physiology [3], [4]. Several studies have linked this last setback to the effect of three inter-related factors namely increased ethanol concentration, increased osmotic pressure and nutrient limitation [5]-[7].

Research findings have provided evidence on mechanisms through which these factors affects yeast cells during fermentation. It is reported that the extent of these stress factors on yeast performance is dependent on the genetic make-up of the yeast strain, physiological state of the yeast cell, physicochemical nature of the fermentation medium and the presence of other competitive microbes like wild yeast and bacteria [8], [9]. Research evidence has indicated that yeast tolerance to stressful conditions is strongly dependent on the nutritional composition of the fermentation medium [10], [11]. Nutritional imbalance resulting from compromised nutrients during the preparation and fermentation of high and VHG wort, has been shown to prevent the optimal performance of yeast cells during fermentation [12]. These nutrients which include carbon and nitrogen sources, vitamins and minerals have been described under various conditions (including HG and VHG fermentation) with much attention to the impact of carbon and nitrogen sources, vitamins and certain metal ions on yeast performance. Till date, the effect of metal ions on yeast performance in high and VHG fermentations have been poorly demonstrated.

Yeast mineral composition reveals range of metal ions required by yeast cells [12]. Metal ions have been found to maintain structural integrity and functionality of intracellular organelles, induce cell-cell interaction such as flocculation, govern gene expression and nutrient uptake mechanisms, activate arrays of enzymes intimately involved in metabolism, and also, acting as stress-protectants [12]-[17]. Under variant fermentation condition, increased fermentability and ethanol yield, improved yeast viability and vitality, desirable flocculation behavior and enhanced stress tolerance, has been reported upon yeast-preconditioning or media supplementation with metal ions [18], [19].

Zinc ions have been solely implicated with the function of activating alcohol dehydrogenase enzyme which is essential for ethanol formation and more importantly as a key structural element of zing-binding motifs of many transcriptional activators [20], [21]. Its availability is paramount to fermentation efficiency, as it plays diverse roles in yeast metabolism which drives important biochemical reactions that ensures efficient fermentation process. Zn-deprivation has also been reported to prevent budding and arrest cell growth in *S. cerevisiae* which often result in slow or stock fermentation [22]. In other hand, Mg^{2+} availability in fermentation medium have been shown to directly influence the rate of sugar consumption, ethanol production and yeast growth in both conventional and high gravity wort [23]. Magnesium is necessary for the activation of several glycolytic enzymes (such as glucokinase, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase and enolase), acting as size transducer and growth enhancer and as well as stress protectant during fermentation [17], [24]. Thus in practical terms, this implies that Mg-deficient medium can suppress yeast metabolism, which may consequently affect fermentation progress.

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Literature on the effect of Ba^{2+} on yeast physiology and its role in fermentation processes is grossly lacking. Generally the effect of Ba^{2+} on cells has been recorded as toxic [25]. Study on the effects of various cations on the flocculating behavior of yeast, showed Ba^{2+} to inhibit yeast flocculation after La^{3+} and Sr^{2+} [26]. Functional comparison of plant inward-rectifier channels expression in yeast in [27], revealed potassium channel genes (KAT1 and AKT1) to be impeded by Ba^{2+} with moderate effectiveness of 30-50% at 10 mM. Reference [28] reported a good enzymatic activity when Ba^{2+} was combined with strontium ions, although a lower activity was recorded for Ba^{2+} alone. Reference [29] also reported similar effect, where Ba alginate was found to exhibit good cell activity and gel stability. It is apparent from these reports that Ba^{2+} exhibits more of an inhibitory than promotory effect on the functionality of cells. The effect of Ba^{2+} during fermentation of all-malt wort has not been reported, thus this study evaluates the effect of Ba^{2+} in combination with Zn^{2+} and Mg^{2+} on yeast performance during VHG wort fermentation.

II. Materials and Methods

A. Yeast Strain and Propagation

A lager brewing yeast (*S. pastorianus*) was used in the experiment and was purchased from Brewmaster Incorporation, Johannesburg, South Africa. The cells were first grown in a 250 ml conical flask with composition (g/ 100 ml); glucose (20), yeast extract powder (5), peptone water buffered (3) and was allowed to grow for 2 days at 18°C. Upon observation of an active growth, the cells were harvested at 4000 rpm for 5 min. The harvested cells were washed with distilled water and propagated in a nutrient broth with composition (g/ 100 ml): Lab-lemco powder (1), yeast extract (2), peptone (5), and sodium chloride (5), and was allowed to grow for 24 h at 18°C.

B. Wort and Fermentation Condition

Wort gravity of 21°P was prepared according to the standard production procedure [30]. All wort was prepared using well modified barley malt, hops and water. A ratio of 4:1 of water-malt was maintained to achieve the desired wort strength. The pH of the wort was 5.25 ± 0.02. A model (Table I) was adapted for the study. Upon aeration of the wort metal-replete media, which was achieved by gently swirling for 5 min, a static fermentation was initiated and maintained at a temperature of 18°C for 96 h [6]. Samples were taken every 24 h until the end of fermentation. The control (C) medium which contained no added $ZnSO_4.7H_2O$, $MgSO_4.7H_2O$, $BaCl_2.2H_2O$ salts was used to measure the effects of the mineral ions and their combination on wort fermentability, pH and ethanol yield. Fermentation was performed in 50 ml volumetric flasks in triplicate, with 30 ml working volume.

C. Fermentation Analysis

Sample preparation was done according to [31] for wort analysis, which entails de-carbonation of the fermented wort sample by shaking gently at first and then vigorously. After 24-96 h of fermentation, 10 ml each of the fermented wort samples was withdrawn and centrifuged at 4000 rpm for 5 min. Upon centrifugation, the supernatant was decanted and analyzed. Wort and beer gravity (°P) was measured using a refractometer. The brix value obtained which is Plato equivalent was used to calculate the fermentability using the equation:

$$\text{Fermentability (\%)} = \frac{\text{Original gravity} - \text{Final gravity} \times 100}{\text{Original gravity}}$$

TABLE I
Model for the Metal Ions

S/N	Metal ions	Concentration range (ppm)	Fermentation properties/ comments	References
1	Mg	500 – 900	Increases cell growth, ethanol yield, increases attenuation rate. Increased tolerance to ethanol, high temperature and osmotic pressure.	[32]
2	Zn	8-12	Increases yeast growth, fermentation rates, improves ethanol yield, avoid stuck fermentation. Improves tolerance to temperature, ethanol and osmotic stress.	[33], [34]
3	Ba	1-4	Increases attenuation rate (or fermentability) and ethanol yield.	[35]

The pH and ethanol content of the beer were determined using a pH meter and gas chromatography equipped with flame ionization detector (FID) with chromosorb mesh. Absolute ethanol was used as standard internal solution with N_2 as carrier gas.

D. Statistical Analysis

Statistical analysis was carried out on the triplicate data obtained from the fermentations. A Duncan multiple comparison test was used to analyze the data using SPSS version 9, with statistical significance accepted at 5% probability levels ($p \leq 0.05$).

III. Results

A. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Wort Fermentability

Table II illustrates the effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and their combinations (Zn + Mg, Zn + Ba, Mg + Ba) on wort fermentability with time. During the 24–96 h of fermentation, a general increase in wort fermentability with time was observed for the entire wort media.

In the first 24 h of fermentation for the metal ions, the effect of Mg^{2+} at the evaluated concentrations (500, 700 and 900 ppm) was found to have a significant effect on wort fermentability compared to Zn^{2+} and Ba^{2+}. After 96 h of fermentation, wort media supplemented with 500 ppm Mg^{2+}, 1 ppm Ba^{2+} and 8 ppm Zn^{2+}, was found with increased wort fermentability of %F = 25.08, %F = 23.81 and %F = 23.17, which were not significantly different ($p \geq 0.05$). Unlike in the control medium, reduced fermentability of %F = 10.90 was recorded. In media containing the metal combinations, the cationic combinations (Mg^{2+} + Ba^{2+}) and (Zn^{2+} + Mg^{2+}) displayed a rapid increase in fermentability in the first 24 h of fermentation unlike in the media supplemented with (Zn^{2+} + Ba^{2+}), where low fermentability was observed. After 96 h of fermentation, low wort fermentability of %F = 14.29 and %F = 13.65 were obtained for wort medium supplemented with the combinations 500:1 ppm (Mg^{2+} + Ba^{2+}) and 8:500 ppm (Zn^{2+} + Mg^{2+}), respectively. The least wort fermentability of %F = 10.48 was obtained in wort medium containing the combination 8:1 ppm (Zn^{2+} + Ba^{2+}), which is not significantly different ($p \geq 0.05$) from the control medium (%F = 10.90).

For the second concentration level tested for the metal ions and their combinations, high wort fermentability of %F = 27.94 and %F = 26.03 ($p \geq 0.05$) were obtained after 96 h of fermentation in wort media supplemented with 700 ppm Mg^{2+} and 2 ppm Ba^{2+}. A low wort fermentability of %F = 11.75 was recorded in wort medium supplemented with 10 ppm Zn^{2+}. In wort media containing the metal combinations, high wort fermentability of %F = 27.00 and %F = 26.35 were obtained for wort media supplemented with the combinations 700:2 ppm (Mg^{2+} + Ba^{2+}) and 10:2 ppm (Zn^{2+} + Ba^{2+}). The least wort fermentability of %F = 15.87 was obtained for wort medium supplemented with 10:700 ppm (Zn^{2+} + Mg^{2+}).

For the third concentration level tested for the metal ions and their combinations, increased wort fermentability of %F = 27.38 and %F = 25.40 ($p \geq 0.05$) was recorded after 96 h of fermentation in wort media supplemented with 900 ppm Mg^{2+} and 12 ppm Zn^{2+}, respectively. Compared to the control medium (%F = 10.90), low wort fermentability of %F = 10.80 was obtained in wort medium supplemented with 4 ppm Ba^{2+}. In media containing the metal combinations, high wort fermentability of %F = 29.53 was obtained in wort medium supplemented with 900:4 ppm (Mg^{2+} + Ba^{2+}). A relatively high wort fermentability of %F = 27.30 was obtained for wort medium containing 12:900 ppm (Zn^{2+} + Mg^{2+}). The least wort fermentability of %F = 22.54 ($p < 0.05$) was obtained for wort medium containing 12:4 ppm (Zn^{2+} + Ba^{2+}).

B. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Wort pH

Table III demonstrates the effect of the three cationic concentration level tested for Zn^{2+}, Mg^{2+}, Ba^{2+} and their combinations (Zn^{2+} + Mg^{2+}, Zn^{2+} + Ba^{2+}, and Mg^{2+} + Ba^{2+}) on wort pH. During 24 – 96 h of fermentation, a general decrease in wort pH was observed in the entire wort media. It was observed that the addition of Zn^{2+} and Ba^{2+} resulted in an initial increase in wort pH for all concentration level tested, thereafter a decrease (increasing acidity).

After 96 h of fermentation for the individual metal ions and their combinations, a significant decrease in wort pH (4.78) was recorded for wort medium supplemented with 500 ppm Mg^{2+}, compared to wort pH of 4.88 and 4.83 for wort medium containing 8 ppm Zn^{2+} and 1 ppm Ba^{2+} ions, respectively. In wort media containing the metal combinations, reduced pH of 4.82, 4.85 and 4.90 was recorded for media supplemented with the combination ratios of 500:1 ppm (Mg^{2+} + Ba^{2+}), 8:500 ppm (Zn^{2+} + Mg^{2+}) and 8:1 ppm (Zn^{2+} + Ba^{2+}), respectively.

After 96 h of fermentation for the second concentration level tested both for the individual metal ions and their combinations, a significant decrease in wort pH of 4.75 was recorded for wort medium supplemented with 700 ppm Mg^{2+} compared to wort pH of 4.96 and 4.87 for wort medium containing 10 ppm Zn^{2+} and 2 ppm Ba^{2+}, respectively. In wort media supplemented with the metal combinations, pH of 4.77 and 4.78 was obtained for wort media supplemented with the metal combination ratios of 700:2 ppm (Mg^{2+} + Ba^{2+}) and 10:700 ppm (Zn^{2+} + Mg^{2+}), respectively. The least effect on wort pH was recorded for wort (medium) supplemented with 10:2 ppm (Zn^{2+} + Ba^{2+}) with pH of 4.87.

Compared to pH 4.88 and 4.98 in medium containing 12 ppm Zn^{2+} and 4 ppm Ba^{2+} ions; a significant decrease in wort pH (4.78) was recorded for wort medium supplemented with 900 ppm Mg^{2+}, for the third concentration level tested for the metal ions. In wort media containing the metal combinations, no significant difference in wort pH; 4.78, 4.79 and 4.79 was observed for medium supplemented with 900:4 ppm (Mg^{2+} + Ba^{2+}), 12:900 ppm (Zn^{2+} + Mg^{2+}) and 12:4 ppm (Zn^{2+} + Ba^{2+}).

TABLE II
EFFECTS OF ZN, MG, BA IONS AND THEIR COMBINATIONS ON WORT FERMENTABILITY

Fermentability (%F)							
Period (h)	Control	Zn	Mg	Ba	Zn + Mg	Zn +Ba	Mg + Ba
24	4.76 ± 0.0^{a}	4.13 ± 0.55^{a}	6.67 ± 0.00^{b}	3.17± 1.10^{c}	10.48 ± 0.00^{d}	5.71 ± 0.00^{e}	10.80 ± 0.55^{d}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		5.24 ± 0.48^{a}	10.16 ± 0.55^{b}	4.76 ± 0.00^{a}	12.38 ± 0.00^{c}	3.49 ± 0.55^{d}	9.20 ± 0.55^{e}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700: 2 ppm)
		9.52 ± 0.00^{b}	12.06 ± 0.55^{c}	5.71± 0.00^{d}	11.43 ± 0.00^{e}	3.49 ± 0.55^{f}	11.43 ± 0.00^{g}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
48	5.39 ± 0.55^{a}	7.62 ± 0.00^{b}	11.75 ± 0.55^{c}	7.62 ± 0.00^{b}	10.48 ± 0.00^{d}	6.99 ± 0.55^{b}	10.81 ± 0.58^{d}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		6.03 ± 0.55^{a}	14.92 ± 0.55^{b}	10.48 ± 0.00^{c}	13.33 ± 0.00^{d}	8.89± 0.55^{e}	12.70 ± 0.55^{d}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		11.43 ± 0.00^{b}	16.19 ± 0.00^{c}	6.03 ± 0.55^{a}	16.19 ± 0.00^{c}	7.94 ± 1.10^{d}	15.62 ± 0.49^{c}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
72	8.57 ± 0.95^{a}	11.75 ± 1.10^{b}	15.87 ± 0.55^{c}	12.38 ± 0.00^{b}	12.00 ± 0.00^{b}	8.57 ± 0.00^{d}	12.38 ± 0.00^{b}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		8.89± 0.55^{a}	20.32± 0.55^{b}	16.82± 0.55^{c}	14.29 ± 0.00^{d}	14.61 ± 0.55^{d}	17.46 ± 1.98^{c}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		16.82± 0.55^{b}	20.95 ± 0.00^{c}	8.10± 0.74^{a}	19.37 ± 0.55^{d}	13.97 ± 0.00^{e}	20.63 ± 0.55^{c}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
96	10.90± 0.48^{a}	23.17 ± 3.07^{b}	25.08 ± 3.85^{b}	23.81 ± 1.65^{b}	13.65 ± 0.55^{c}	10.48 ± 0.00^{a}	14.29 ± 0.00^{c}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		11.75 ± 1.45^{a}	27.94 ± 1.10^{b}	26.03 ± 0.55^{b}	15.87 ± 0.55^{c}	26.35 ± 2.20^{b}	27.00 ± 0.58^{b}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		25.40 ± 1.46^{b}	27.38 ± 1.15^{b}	10.80 ± 0.55^{a}	27.30 ± 0.55^{b}	22.54 ± 2.74^{c}	29.53 ± 2.52^{d}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)

Values in the same row having different superscript for different concentration of each metal are significantly different ($p < 0.05$)

TABLE III
EFFECT OF ZN, MG, BA IONS AND THEIR COMBINATIONS ON WORT PH

Wort pH							
Period (h)	Control	Zn	Mg	Ba	Zn + Mg	Zn +Ba	Mg + Ba
24	5.32 ± 0.02^{a}	5.30 ± 0.01^{b}	5.23 ± 0.02^{c}	5.00 ± 0.03^{c}	5.19 ± 0.01^{d}	5.31 ± 0.01^{a}	5.18 ± 0.02^{d}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		5.30 ± 0.06^{a}	5.17 ± 0.03^{b}	5.26 ± 0.00^{c}	5.17 ± 0.01^{b}	5.25 ± 0.01^{c}	5.15 ± 0.02^{b}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		5.26± 0.05^{b}	5.21 ± 0.02^{c}	5.32 ± 0.03^{a}	5.17 ± 0.03^{c}	5.27 ± 0.01^{b}	5.13 ± 0.01^{d}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
48	5.14 ± 0.04^{a}	5.08 ± 0.02^{b}	5.01 ± 0.03^{c}	5.00 ± 0.03^{c}	5.02 ± 0.02^{c}	5.10 ± 0.03^{b}	4.99 ± 0.02^{c}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		5.08 ± 0.02^{b}	5.00 ± 0.04^{b}	5.04± 0.08^{b}	4.98 ± 0.03^{b}	5.03 ± 0.03^{b}	4.93 ± 0.02^{c}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		5.05 ± 0.03^{b}	4.94 ± 0.03^{c}	5.11 ± 0.02^{a}	4.99 ± 0.02^{d}	5.07 ± 0.02^{b}	4.91 ± 0.02^{c}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
72	5.01 ± 0.01^{a}	4.93 ± 0.01^{b}	4.89 ± 0.02^{b}	4.91 ± 0.02^{b}	4.94 ± 0.02^{c}	4.97 ± 0.02^{c}	4.92 ± 0.04^{b}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		5.03± 0.06^{a}	4.85 ± 0.07^{b}	4.91 ± 0.05^{c}	4.90 ± 0.04^{c}	4.92 ± 0.01^{c}	4.86 ± 0.02^{b}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		4.91 ± 0.0 5^{b}	4.87± 0.0 4^{c}	5.01 ± 0.02^{a}	4.86 ± 0.02^{c}	4.89 ± 0.02^{b}	4.84 ± 0.01^{c}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)
96	4.99 ± 0.05^{a}	4.88 ± 0.03^{b}	4.78 ± 0.03^{c}	4.83 ± 0.02^{d}	4.85 ± 0.05^{d}	4.90 ± 0.01^{b}	4.82 ± 0.02^{d}
	(0 ppm)	(8 ppm)	(500 ppm)	(1 ppm)	(8:500 ppm)	(8:1 ppm)	(500:1 ppm)
		4.96± 0.12^{a}	4.75 ± 0.02^{b}	4.87 ± 0.05^{c}	4.78 ± 0.01^{b}	4.87 ± 0.01^{c}	4.77 ± 0.02^{b}
		(10 ppm)	(700 ppm)	(2 ppm)	(10:700 ppm)	(10:2 ppm)	(700:2 ppm)
		4.88 ± 0.03^{b}	4.78 ± 0.03^{c}	4.98 ± 0.03^{a}	4.79 ± 0.01^{c}	4.79 ± 0.01^{c}	4.78 ± 0.02^{c}
		(12 ppm)	(900 ppm)	(4 ppm)	(12:900 ppm)	(12:4 ppm)	(900:4 ppm)

Values in the same row having different superscript for different concentration of each metal are significantly different ($p < 0.05$).

C. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Ethanol Yield

Figs. 1 (a)-(f) illustrate the effect of increasing amounts of Zn^{2+}, Mg^{2+}, Ba^{2+} and their combinations (Zn + Mg, Zn + Ba, Mg + Ba) on ethanol yield with time. A general increase in ethanol yield was observed for the entire medium during the set period of fermentation.

After 96 h of fermentation for the first concentration levels tested for the individual metal ions and their combinations, high ethanol yield of 6.6815% (v/v), 6.3526% and 6.2492% ($p \geq 0.05$) were obtained for wort media supplemented with 1 ppm Ba^{2+}, 500 ppm Mg^{2+}, and 8 ppm Zn^{2+} respectively (Fig. 1 (a)), unlike in the control where ethanol yield of 4.7980% (v/v) was recorded. In wort media containing the metal combinations (Fig. 1 (d)), high ethanol yield of 5.9720% (v/v) was recorded in wort medium containing 500:1 ppm (Mg^{2+} + Ba^{2+}). Lower ethanol yield of 4.7838% (v/v) and 4.4635% (v/v) was obtained for wort media supplemented with 8:500 ppm (Zn^{2+} + Mg^{2+}) and 8:1 ppm (Zn^{2+} + Ba^{2+}).

International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:11, 2014

In the first 24 h of fermentation for the second concentration level tested for the metal ions and their combinations, a rapid increase in ethanol yield was observed for wort medium supplemented with 700 ppm Mg^{2+} compared to wort medium containing 10 ppm Zn^{2+} and 2 ppm Ba^{2+}. After 96 h of fermentation, high ethanol yield of 7.8844% (v/v) and 7.6245% (v/v) ($p \geq 0.05$) were obtained in wort media supplemented with 700 ppm Mg^{2+} and 2 ppm Ba^{2+} respectively (Fig. 1 (b)). Low ethanol yield of 4.9867% (v/v) was obtained for wort medium supplemented with 10 ppm Zn^{2+}. In wort media containing the metal combinations (Fig. 1 (e)), high ethanol yield of 7.4668% (v/v) and 7.2880% (v/v) were obtained for the metal combination ratios of 10:2 ppm (Zn^{2+} + Ba^{2+}) and 700:2 ppm (Mg^{2+} + Ba^{2+}) respectively. A lower ethanol yield of 5.0808% (v/v) was obtained for wort medium containing 10:700 ppm (Zn^{2+} + Mg^{2+}).

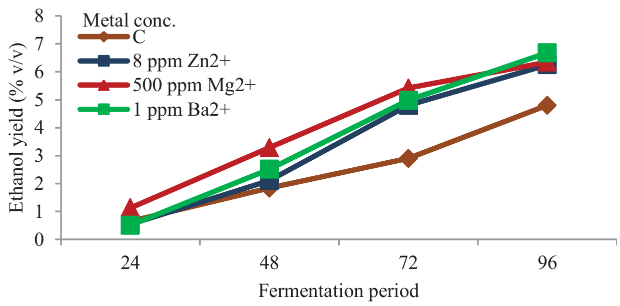

Fig. 1 (a) Effect of increasing amount zinc, magnesium and barium on ethanol yield

Control (C): 0 ppm. First concentrations level of Zn^{2+} (8 ppm), Mg^{2+} (500 ppm) and Ba^{2+} (1 ppm)

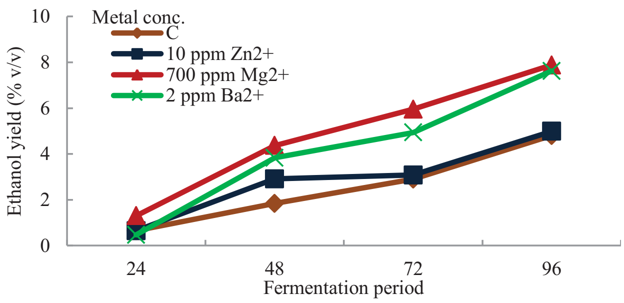

Fig. 1 (b) Effect of increasing amount zinc, magnesium and barium on ethanol yield

Control (C): 0 ppm.). Second concentration level of Zn^{2+} (10 ppm), Mg^{2+} (700 ppm) and Ba^{2+} (2 ppm)

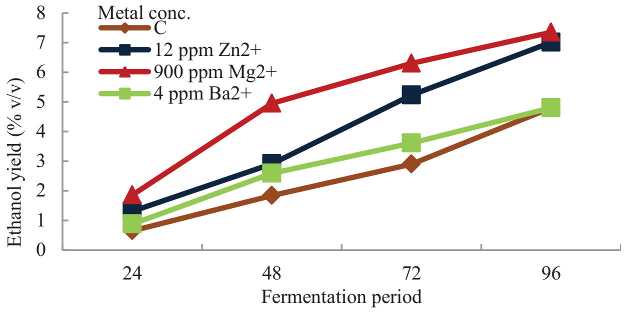

Fig. 1 (c) Effect of increasing amount zinc, magnesium and barium on ethanol yield

Control (C): 0 ppm.). Third concentration level of Zn^{2+} (12 ppm), Mg^{2+} (900 ppm) and Ba^{2+} (4 ppm)

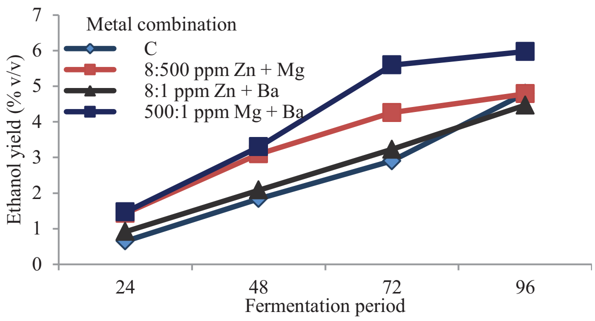

Fig. 1 (d) Effect of zinc, magnesium and barium combinations on ethanol yield

Control (C): 0 ppm. Combinations of the metal ions for the first concentration level of Zn + Mg (8 ppm + 500 ppm); Zn + Ba (8 ppm + 1 ppm); Mg + Ba (500 ppm + 1 ppm)

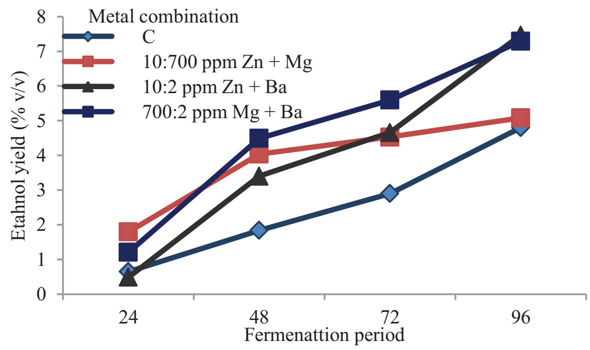

Fig. 1 (e) Effect of zinc, magnesium and barium combinations on ethanol yield

Control (C): 0 ppm.). Combinations of the metal ions for the second concentration level of Zn + Mg (10 ppm + 700 ppm); Zn + Ba (10 ppm + 2 ppm); Mg + Ba (700 ppm + 2 ppm)

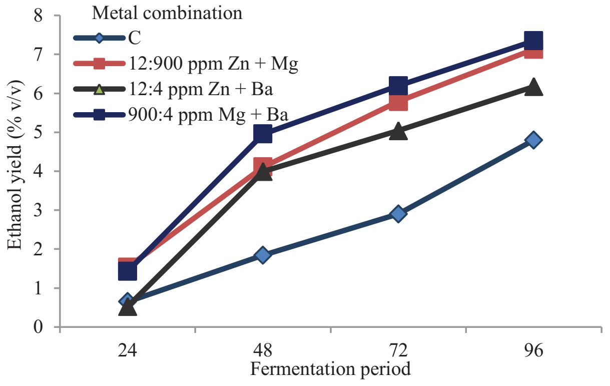

Fig. 1 (f) Effect of zinc, magnesium and barium combinations on ethanol yield

Control (C): 0 ppm.). Combinations of the metal ions for the third concentration level of Zn + Mg (12 ppm + 900 ppm); Zn + Ba (12 ppm + 4 ppm); Mg + Ba (900 ppm + 4 ppm)

At the end of 96 h of fermentation for the third concentration level tested for the metals and their combinations, high ethanol yield of 7.3529% (v/v) and 7.0164% (v/v) were obtained in wort media supplemented with 900 ppm Mg^{2+} and 12 ppm Zn^{2+}at $p \geq 0.05$, respectively (Fig. 1 (c)). Compared to the control (4.80% v/v), low ethanol yield of 4.80% (v/v) was recorded for wort medium containing 4 ppm Ba^{2+}. In wort media supplemented with the metal combinations (Fig. 1 (f)), highest ethanol yield of 7.3491% (v/v) and 7.1313% (v/v) were obtained for wort medium containing 900:4 ppm (Mg^{2+} + Ba^{2+}) and 12:900 ppm (Zn^{2+} + Mg^{2+}) respectively. A low ethanol yield of 6.1757% (v/v) was obtained for wort medium containing 12:4 ppm (Zn^{2+} + Ba^{2+}).

IV. Discussion

A. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Wort Fermentability

In the experiment, Zn^{2+} addition in the concentrations 8, 10 and 12 ppm was found to have variable effect on wort fermentability during the course of fermentation. Supplementation of 12 ppm Zn^{2+} resulted in high fermentability of %F = 25.40, followed by 8 ppm Zn^{2+} (%F = 23.17); with 10 ppm Zn^{2+} having a lesser effect on wort fermentability (%F = 11.75). Hypothetically, the effect in the wort fermentability at concentration of 10 ppm Zn^{2+} could be attributed to its repressive effect on Zn-uptake systems (the Zrt1-high affinity and Zrt2-low affinity system) at the set concentration. This observation agrees with the report of [36], [37] where high intracellular Zn^{2+} levels was said to inhibit the function of ZAP1 gene (zinc uptake regulatory gene in yeast) which consequently down-regulate the expression of Zap1 target genes (ZRT1, ZRT2), responsible for Zn^{2+} uptake. This finding agree with the report of [12] where Zn^{2+} levels between 0.05 - 1.07 ppm Zn^{2+} was found to enhance wort fermentability of lager yeast strain of *S. cerevisiae*, than at 10 ppm Zn^{2+}. On the other hand, Zn^{2+} levels as high as 65.5, 327.5 and 1300 ppm have been reported to improve fermentation rates [23], [38]. Thus, further research as to what underlying mechanism that improves yeast performance at higher concentrations should be considered.

In this work, Mg^{2+} addition in the concentrations of 500, 700 and 900 ppm was found to impact positively on wort fermentability, with high fermentability of %F = 27.94 obtained at concentration of 700 ppm Mg^{2+}. No significant increase in wort fermentability was observed above 700 ppm, as 900 ppm showed no significant increase in fermentability (%F = 27.38). This could indicate that the yeast cell have reached its optimum requirement for the mineral ion. The finding of 700 ppm (700 mg/L $MgSO_4$. $7H_2O$) as optimal concentration for yeast fermentative performance; agrees with the report of [39], [40] where optimal yeast attenuating strength was observed at concentration ≥ 700 mM. Therefore, Mg^{2+} supplementation or preconditioning in fermentation media should be considered in concentration factor of 7 ppm, for improved fermentation in media with high sugar concentration.

Literature on the role of Ba^{2+} on yeast physiology as well as in fermentation processes are grossly lacking. The effect of Ba^{2+} on yeast fermentative performance has not being previously reported and in this work, Ba^{2+} was found to enhance wort fermentability. Ba^{2+} was found to improve wort fermentability at concentration of 1 and 2 ppm. Fermentability of %F = 26.03 and %F = 23.81 was recorded at 2 and 1 ppm Ba^{2+} respectively. This result suggests that the metal ion has a positive effect on sugar uptake through an undefined mechanism at the set concentration. Four parts per millions (4 ppm) Ba^{2+} was found to reduce wort fermentability with time. This process impact at 4 ppm Ba^{2+} could be as a result of Ba^{2+} having reached its toxic level, whereby the cells are unable to maintain active cellular metabolism to efficiently ferment the sugars. This finding supports the report of [27] where negative effect of Ba^{2+} on the gating kinetics of K^+ was demonstrated. The result also supports the view of [41] who postulated that cell division and expansion can be impaired by Ba^{2+} toxicity. Thus, the need to elucidate the mechanism through which Ba^{2+} improves yeast performance should be considered, as it could be useful in yeast precondition processes as well as supplementation for high performing yeast cells.

The combined effects of Zn^{2+}, Mg^{2+} and Ba^{2+} on yeast performance have not been reported. The combinatory effect of Mg^{2+} + Ba^{2+} at concentration of 900:4 ppm was found to impact optimally (%F = 29.53) on wort fermentability. This effect could be a result of an unknown relationship between the metal ions, which could have cooperatively enhanced the fermentative ability of the yeast cell. This finding suggests a higher amount of Mg^{2+} to trace metal for optimal yeast performance. This report supports the report of [42], [43]; where high ratio of Mg^{2+} to trace elements was found to enhance fermentation rate. Though the wort medium containing Zn^{2+} + Ba^{2+} displayed a comparable fermentability at concentration level of 10:2 ppm (%F = 26.35), reduced wort fermentability (%F = 10.48 and %F = 22.54) was observed for other concentration levels tested (i.e. 8:1 ppm and 12:4 ppm) respectively. The result suggests a strong inverse relationship between Zn^{2+} and Ba^{2+} at somewhat higher or lower concentration on yeast performance.

B. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Wort pH

Although not considered much as a critical factor affecting yeast performance, wort pH plays a dictating role in metal uptake in yeast cell during fermentation, as such, controls the impact of the metal ions on the fermentation progress. As shown in Table III, pH range of 4.75–4.88 was found to favour the fermentative performance of *S. pastorianus*; with pH of 4.75 found to be optimal for the yeast activity. These results indicate that the stimulatory effect of the mineral ions on yeast performance is greatly affected by pH. This effect could be explained by the impact of the mineral ions on the ATPase-dependent H^+ activity, which is known to be associated with cation uptake in cells [44], [45]. Also, medium pH has been reported in [46] to have a predictory role on the binding competence of metal cations for absorption sites on the yeast cell wall. The results presented agree with the reports of [47], [48] where yeasts optimal ability to ferment sugars, although with different medium composition, was found at pH range of 4.5-5.

C. Effect of Zn^{2+}, Mg^{2+}, Ba^{2+} and Their Combinations on Ethanol Yield

In this study, supplementation of Zn^{2+} in the concentration of 8, 10 and 12 ppm resulted in enhanced ethanol formation, with concentration of 8 ppm (6.24% v/v) and 12 ppm (7.01% v/v) having beneficial effect on ethanol yield. The results support the report of [49], [50] where lager brewing strains were found with increased ethanol yield upon Zn^{2+} addition. Also, the result agrees with the reports of [43], [51] where increased ethanol yield was reported at increasing (0.01-1 g/L and 0.30–0.48 mg/L) amounts of Zn^{2+}. The decrease in ethanol yield (4.99% v/v) recorded at 10 ppm Zn^{2+}could be attributed to the amount of Mg^{2+} and Mn^{2+} that are present in the wort, and or the repressive effect of high amount of Zn^{2+} on Zn-uptake systems during ethanol formation. This observation agrees with the report of [12] where 10 ppm Zn^{2+} was found to have a lesser effect on ethanol productivity. Also, the result supports the report of [38] where yeast-Zn requirement was shown to be affected by the availability of Mn^{2+} in wort. Taking into account the like causality, supplementing Zn^{2+} in somewhat lower or higher concentration would allow for improved ethanol production.

In each concentration level evaluated for Mg^{2+}, a rapid initial increase in ethanol yield was observed compared to Zn^{2+} and Ba^{2+}. This observation can be explained by the growth stimulating effect of Mg^{2+} on yeast cells, which has a direct impact on ethanol formation. As illustrated in the results, Mg^{2+} concentration of 500, 700 and 900 ppm was found to promote ethanol yield. This result agrees with the reports of [5], [23] where increased ethanol productivity was recorded in both standard and high gravity wort upon 500 ppm Mg^{2+} addition. High ethanol yield of 7.8844% and 7.3529% (v/v) ($p \geq 0.05$) was recorded for Mg^{2+} at concentrations of 700 and 900 ppm, respectively. The result supports the reports of [39], [52] where enhanced ethanol tolerance and ethanol productivity upon increasing amount of Mg^{2+} was demonstrated.

Addition of Ba^{2+} in the concentration of 1 and 2 ppm was found to impact positively on ethanol productivity – with concentration of 2 ppm found optimal (7.6245% v/v). This result suggests that Ba^{2+} has a promotory effect on ethanol formation. This observation could be explained in part by the promotory effect of Ba^{2+} on protein synthesis, which may have impacted positively on yeast sugar metabolism. This observation supports the report in [29] where increased protein synthesis for *Cephalosporium acremonium* cells that were entrapped in Ba-alginate was observed; however, the report of [25] states otherwise of the effect of Ba^{2+} on protein synthesis in seedling growth. Addition of 4 ppm was found to reduce ethanol productivity (4.80% v/v) with time, which infers that the Ba^{2+} can be toxic to the yeast cells at concentrations ≥ 4 ppm. Retardation in cell division and expansion has been shown to be key factors to Ba^{2+} mediated growth inhibition [25], [41]. This process impact can be attributed to the negative effect of high amounts of Ba^{2+} on yeast cells in terms of decreasing its protein synthesis and increasing peroxidase activity which is an indication of cell toxicity.

Novel relationship between Zn^{2+}, Mg^{2+} and Ba^{2+} was established in the evaluated combinations. Synergistic relationship was observed for Mg^{2+} and Ba^{2+} in which high ethanol yield of 7.3491% (v/v) was recorded for combination of concentration 900:4 ppm (Mg^{2+} + Ba^{2+}). This could imply that Ba^{2+} have a promotory effect on yeast performance in the presence of Mg^{2+} concentration. The interactive effect of Zn^{2+} and Mg^{2+}, and Zn^{2+} and Ba^{2+} was found to have variable impact on ethanol yield. Low ethanol yield was observed more in combinations of Zn^{2+} to Mg^{2+} and Ba^{2+}, although at high concentration of Mg^{2+} to Zn^{2+} and Ba^{2+}; enhanced ethanol production was recorded. It was observed that high concentration of Mg^{2+} to the trace metals had a beneficial impact on ethanol yield. This observation supports the view of [42], [43]. It is understandable from the results that a counteractive relationship exists at somewhat higher concentration of Zn^{2+} to Mg^{2+} and Ba^{2+}. Hypothetically, this could be explained by a competitive reactivity of Zn^{2+} with Mg^{2+} for binding sites either on chelating molecule in the wort or on the yeast membrane, which may have reduced the uptake of the ions by the yeast cells. This observation supports the view of [53] on ion-ion interaction of Zn^{2+}, where metal ions such as Ca^{2+}, K^+, Mn^{2+} and Mg^{2+} were found to influence the effect of Zn^{2+} on yeast performance.

V. Conclusion

It is evident from the results that Mg^{2+} has a profound effect on yeast performance, with 700 ppm Mg^{2+} found optimal for the yeast fermentative performance. It was found that increasing amount of Zn^{2+} does not necessarily translate into high yeast fermentative performance, as variable concentration range had different effects; thus maintaining Zn^{2+} concentration at somewhat higher or lower levels will enhance yeast performance. It is noteworthy from the results that Ba^{2+} has beneficial effect on yeast fermentation process. The effect of Ba^{2+} on yeast fermentative performance was established at concentration range of 1-2 ppm, with concentration of 4 ppm Ba^{2+} found to reduce yeast performance with time. Therefore, the need to investigate the effect of Ba^{2+} on yeast physiology regard to its enhancing and inhibitory effects on yeast performance is required - as it could suggest a biotechnological significance, not only to the brewing and biofuel industry but as well as health. Also, it is apparent from the results, that maintaining high concentration of Mg^{2+} to trace elements enhances the fermentative performance of lager brewing yeast. The metal combination ratio of 900:4 ppm Mg^{2+} + Ba^{2+} had 'best' impact on the evaluated parameters. Nevertheless, their impact on beer character needs further investigation.

ACKNOWLEDGMENT

The authors appreciate the financial assistance to HOU through the Research Incentive: Project number: SARDF/13/FST/02 of the University of Venda, South Africa.

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