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Response of *Saccharomyces cerevisiae* to Cadmium and Nickel Stress: The Use of the Sugar Cane Vinasse as a Potential Mitigator

Ricardo Pinheiro de Souza Oliveira · Luiz Carlos Basso · Adalberto Pessoa Junior · Thereza Christina Vessoni Penna · Marco Del Borghi · Attilio Converti

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Abstract Most of the metals released from industrial activity, among them are cadmium (Cd) and nickel (Ni), inhibit the productivity of cultures and affect microbial metabolism. In this context, the aim of this work was to investigate the capacity of sugar cane vinasse to mitigate the adverse effects of Cd and Ni on cell growth, viability, budding rate and trehalose content of *Saccharomyces cerevisiae*, likely because of adsorption and chelating action. For this purpose, the yeast was grown batch-wise in YED medium supplemented with selected amounts of vinasse and Cd or Ni. The negative effects of Cd and Ni on *S. cerevisiae* growth and the mitigating one of sugar cane vinasse were quantified by an exponential model. Without vinasse, the addition of increasing levels of Cd and Ni reduced the specific growth rate, whereas in its presence no reduction was observed. Consistently with the well-proved toxicity of both metals, cell viability and budding rate progressively decreased with increasing their concentration, but in the presence of vinasse the situation was remarkably improved. The trehalose content of *S. cerevisiae* cells followed the same qualitative behavior as cell viability, even though the negative effect of both metals on this parameter was stronger. These results demonstrate the ability of sugar cane vinasse to mitigate the toxic effects of Cd and Ni.

Keywords Cadmium · Nickel · Toxicity · *Saccharomyces cerevisiae* · Sugar cane vinasse

R. P. d. Oliveira · A. P. Junior · T. C. V. Penna
Department of Biochemical and Pharmaceutical Technology,
Faculty of Pharmaceutical Sciences, São Paulo University,
Av Prof Lineu Prestes, 580, Bl. 16,
05508-900 Piracicaba, São Paulo, Brazil

L. C. Basso
Biological Sciences Department, Escola Superior de Agricultura
Luiz de Queiroz, São Paulo University,
Avenida Pádua Dias, 11,
13418-900 Piracicaba, São Paulo, Brazil

M. Del Borghi · A. Converti (✉)
Department of Chemical and Process Engineering,
Genoa University,
Via Opera Pia 15,
16145 Genoa, Italy
e-mail: converti@unige.it

Introduction

The characteristics of metals, their toxicity and action mechanism are being discussed in recent years all over the world [1]. A major cause of widespread dispersion of metals at trace levels into the environment is their high concentration in some sewage sludge [2–4]. The soil can be considered contaminated when toxic chemicals (pollutants or contaminants) are present in high enough concentrations causing risk to human health and/or ecosystem. Moreover, even when the levels of contaminants in soil are not sufficient to cause some kind of risk, soil pollution can occur due to the simple fact that the contaminant levels exceed those present naturally in soil [5].

Environmental pollution by toxic metals has been increasing throughout the world as a consequence of the industrial progress. Owing to the rapid development of industry, especially in developing countries, wastes containing metals are directly or indirectly discharged into the environment, having brought serious environmental pollution, and threatened the biolife [6, 7]. Therefore, it is important to establish an efficient and low-cost method for the removal of metal pollutants [8].

Traditional methods for removing metal ions from aqueous solution have been tested, among them, chemical

precipitation, ion exchange, electrochemical treatment, adsorption on activated carbon. However, the main disadvantage of their use is the high cost. One alternative process is biosorption, which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. [9, 10].

The relationship between the various microorganisms, especially bacteria and metals is fairly well documented [11, 12]. However, little information about the resistance of yeasts to metals is available in the literature, despite a wide variety of microorganisms (fungi, algae, bacteria, etc.) is capable of sequestering metal ions [12, 13]. The study of interactions between yeast and metals has a high scientific interest. Among the fungi, yeasts are by far the most scientifically explored, because they are eukaryotes easily to be handled. Thus, they can effectively be used as models for investigating many important problems in biology of eukaryotes [10].

Yeasts are also known to accumulate large quantities of metals in aqueous media [14]. Thus, when these organisms enter the food chain, they can become dangerous. An example of ecological risk may be the industrial production of *Saccharomyces cerevisiae*, as this microorganism is used in human and animal feed as a protein source and is widely used in fermentation processes [10].

In general, some metals can be highly toxic and may disturb the microbial metabolism. The mechanism of their bioaccumulation has been explored extensively. For instance, cadmium or nickel ions are transported across the cell membrane, probably by transport proteins, to the cytoplasmic matrix, where they adhere to various organelles or bind to metalloproteins [15]. Many authors [16, 17] have reported that sugar transport into the yeast cell is inhibited by metals, and this inhibition may be involved in the interaction of these metals (e.g., nickel, cobalt, aluminum, and cadmium) with the polyphosphate membrane, causing a conformational change in some active sites, which would hinder binding with the sugar.

Vinasse is a byproduct from the fermentation industry producing yeast, alcohol, citric acid, ephedrine and other substances from beet and cane molasses [18]. In the bioethanol industry, after alcohol removal, it results in the proportion of 10 to 18 l of vinasse per liter of alcohol produced. Sugar cane vinasse is a waste rich in organic matter and mineral elements, which is also used to raise the pH of the soil as well as to improve its physical, chemical, and biological properties [19, 20]. According to Cortez and Pérez [21], the mean composition of Brazilian sugar cane vinasse is (% *w*/*w*): 0.48 K, 0.01 P, 0.04 N, 0.07 Ca, 0.02 Mg, 1.95 ash, 46.5 total solids, 4.63 organic solids (pH 4.8). Such a byproduct, which is hugely abundant in ethanol-producing countries like Brazil, was already shown to reduce the toxic effect of aluminum to the same yeast [22].

Based on these considerations, sugar cane vinasse has been tested in this study as a potential mitigator of the toxic effects of Cd and Ni on yeast metabolism at different concentrations of these metals. The toxic effects of Cd and Ni were quantified by an exponential model described later.

Material and Methods

Microorganism

S. cerevisiae (Fleischmann's baker yeast) was used as a microbial model to test cadmium (Cd) and nickel (Ni) toxicity to biosystems. The freeze-dried microorganism stored at 4°C was reactivated for 24 h at 30°C in 30 mL of yeast-extract dextrose (YED) media (5.0 g/L yeast extract plus 20 g/L glucose) contained in 125-mL Erlenmeyer flasks agitated at 150 rpm [22].

Vinasse

After sugar cane had been processed in a Brazilian sugar industry to produce crystalline sugar, its pulp and molasses were fermented to alcohol. The sugar cane vinasse, obtained as the final byproduct after ethanol distillation, was used in this study to mitigate the toxic effects of Cd and Ni to the yeast.

Growth Tests

The growth tests were conducted in triplicate on a rotary shaker at 30°C and 150 rpm in 125-mL Erlenmeyer flasks sealed with anhydrous cotton caps and containing 30 mL of YED medium supplemented with the selected amounts of vinasse (0, 20 and 40 g/L) and Cd or Ni. The pH was finally adjusted to 4.0 using 0.1 N H_2SO_4 and the resulting medium sterilized at 121°C for 15 min in autoclave. Experiments were done separately in the presence of these metals (0, 3.75, 7.50, 11.25, and 15.00 mg/L), in the form of cadmium acetate ($CdC_4H_6OH{\cdot}2H_2O$) or nickel chloride ($NiCl_2{\cdot}6H_2O$), respectively. Preliminary tests with cadmium chloride ($CdCl_2{\cdot}2H_2O$) instead of cadmium acetate did not reveal any significant influence on growth. Moreover, no appreciable pH change occurred during yeast growth; therefore, no pH adjustment was required.

The first three columns of Table 1 show the conditions under which the growth tests were performed. The media were inoculated with 2.0 mL of 20 g/L (dry matter) yeast suspension, corresponding to 10^5 cells/mL. Cell growth was followed by determinations of cell mass concentration every 2 h within a whole time period of 24 h.

Table 1 Experimental design and results of maximum specific growth rate (μ_{max}) of *S. cerevisiae* in cultivations performed at different Cd and Ni concentrations with or without vinasse contents of the medium

Run	Cd or Ni concentration (mg/L)	Vinasse content (g/L)	μ_{max}Cd (h^{-1})	μ_{max}Ni (h^{-1})
1	0	0	0.46±0.04	0.45±0.03
2	3.75	0	0.44±0.04	0.43±0.04
3	7.50	0	0.41±0.03	0.41±0.02
4	11.25	0	0.37±0.03	0.38±0.02
5	15.00	0	0.35±0.02	0.35±0.02
6	0	20	0.60±0.05	0.58±0.05
7	3.75	20	0.57±0.04	0.57±0.03
8	7.50	20	0.55±0.04	0.56±0.04
9	11.25	20	0.52±0.04	0.54±0.04
10	15.00	20	0.51±0.04	0.51±0.04
11	0	40	0.90±0.03	0.91±0.03
12	3.75	40	0.88±0.03	0.90±0.04
13	7.50	40	0.87±0.02	0.88±0.02
14	11.25	40	0.83±0.02	0.85±0.02
15	15.00	40	0.80±0.02	0.82±0.02

Analytical Measurements

Cell mass concentration was determined by optical density (OD) measurements at 570 nm. OD was related to dry weight biomass concentration (X) by a calibration straight line ($OD=8.1031X$) obtained using yeast suspensions with known dry biomass contents.

Cell viability and budding rate were determined at the end of growth runs (24 h) by optical microscopy in a Neubauer chamber. In particular, for cell viability, the cells were stained with 0.25% erythrosine according to Bonneu et al. [23]. Samples were diluted so as to obtain counts in the range of 200–400 cells.

Trehalose was extracted from 60 mg of washed cells (fresh weight) with 2 mL of 0.5 mol/L trichloroacetic acid in ice bath for 20 min, during which the suspension was shaken frequently [24]. After centrifugation, 0.2 mL of each supernatant was submitted to the anthrone reaction.

The experimental data of cell viability, budding rate, and trehalose were presented as mean values, while variations with respect to the mean values were presented as standard deviations.

Results and Discussion

Growth Kinetics

Figures 1 and 2 show the simultaneous effects of both metals (Cd or Ni) and vinasse on cell mass concentration of *S. cerevisiae* during aerobic growth. It may be noted that Cd and Ni showed very similar inhibition of yeast growth, i.e., when the level of these metals was raised, the growth decelerated, whereas an opposite effect was exerted by vinasse, thus confirming the mitigating effect already observed in the presence of aluminum [22].

For instance, compared with the control (0 g/L of both vinasse and Cd, run 1 in panel A of Fig. 1), the final cell mass concentration decreased by approximately 35% and 70% using 7.5 mg/L (run 2) and 15 mg/L (run 3) of Cd, respectively. The ability to remove Cd by different living microorganisms, among which *Sphaerotilus natans* [25, 26], *Zoogloea ramigera* [27], and *Rhizopus arrhizus* [28], was already demonstrated. In almost all cases, the toxic effect of such a metal was related to a biosorption mechanism mediated by specific carriers [25, 29]; therefore, taking into account its abiotic nature along with its low incidence in the environment, one can think to a mechanism of cell penetration in competition with some other biotic metal.

On the other hand, very little is known about the biosorption and the possible inhibiting effect of Ni on microbial growth [29]. Using the same reasoning, in the presence of Ni under the same conditions (Fig. 2) these percentages were almost coincident (37% and 69%, respectively), hence suggesting that the negative effect of this metal on *S. cerevisiae* growth was similar to that of Cd. On the one hand, this result may appear quite strange, because Cd is recognized as one of the most toxic metals. For example, Ahmad et al. [30] found minimum inhibitory concentrations of Cd and Ni for different fungi, including *Aspergillus* and *Penicillium* species, of 125–550 and 300–850 μg/L, respectively. But, from the other hand, it is quite interesting, because it proves an unexpected tolerance of *S. cerevisiae* to Cd, which suggests its possible use as biosorption material for effective removal of this metal from polluted wastewater.

As stressed by Gadd [12], tolerance of most microorganisms to toxic metals could depend on some change in metal speciation leading to mobility variations. In fungi,

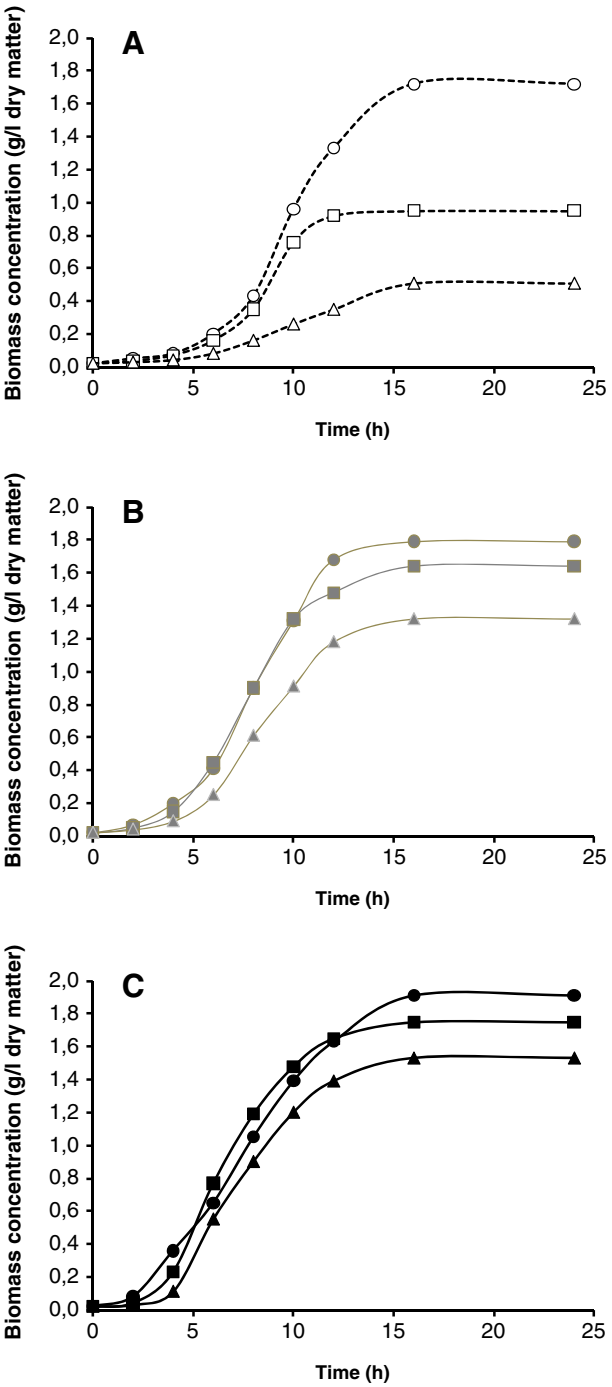

Fig. 1 Cell mass concentration curves of *S. cerevisiae* in YED medium with and without 20–40 g/L sugar cane vinasse in the presence of different Cd concentrations. **a** Runs without vinasse: 0 mg/L Cd (run 1, *white circle*); 7.5 mg/L Cd (run 2, *white square*); 15.0 mg/L Cd (run 3, *white upright triangle*). **b** Runs with 20 g/L vinasse: 0 mg/L Cd (run 4, *gray circle*); 7.5 mg/L Cd (run 5, *gray square*); 15.0 mg/L Cd (run 6, *gray upright triangle*). **c** Runs with 40 g/L vinasse: 0 mg/L Cd (run 7, *black circle*); 7.5 mg/L Cd (run 8, *black square*); 15.0 mg/L Cd (run 9, *black upright triangle*)

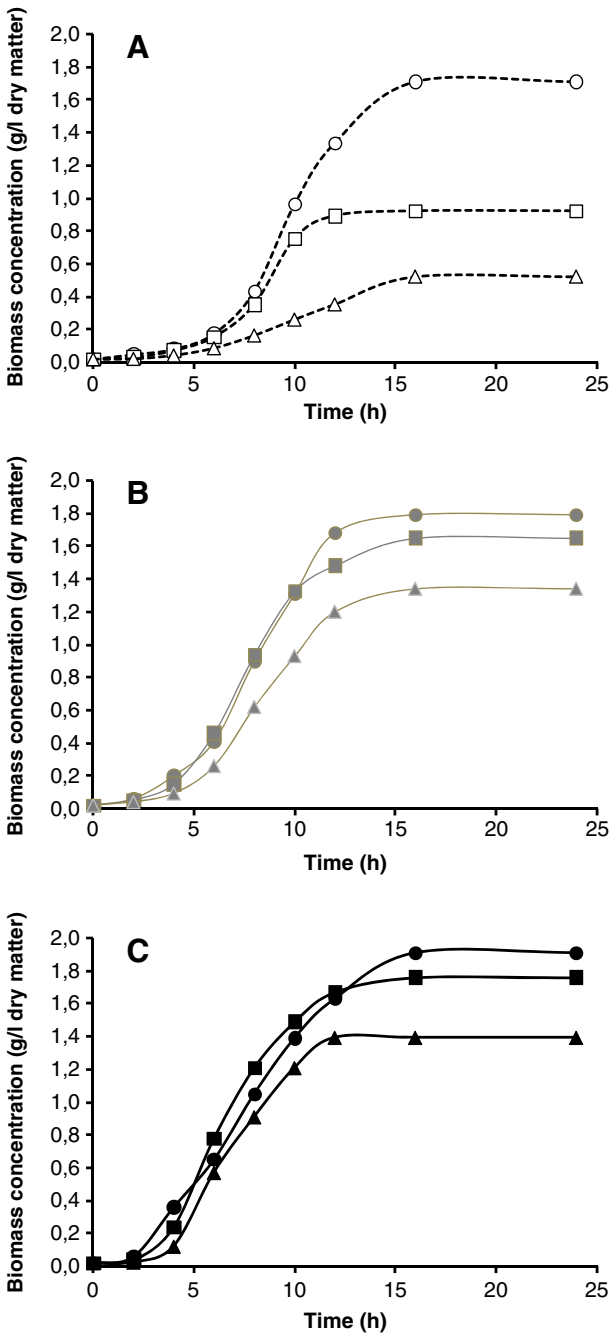

Fig. 2 Cell mass concentration curves of *S. cerevisiae* in YED medium with and without 20–40 g/L sugar cane vinasse in the presence of different Ni concentrations. **a** Runs without vinasse: 0 mg/L Ni (run 1, *white circle*); 7.5 mg/L Ni (run 2, *white square*); 15.0 mg/L Ni (run 3, *white upright triangle*). **b** Runs with 20 g/L vinasse: 0 mg/L Ni (run 4, *gray circle*); 7.5 mg/L Ni (run 5, *gray square*); 15.0 mg/L Ni (run 6, *gray upright triangle*). **c** Runs with 40 g/L vinasse: 0 mg/L Ni (run 7, *black circle*); 7.5 mg/L Ni (run 8, *black square*); 15.0 mg/L Ni (run 9, *black upright triangle*)

many metals, like Ni, mainly accumulate and are detoxified in vacuoles, but, due to the absence of these organelles, *S. cerevisiae* is known to have an increased sensitivity to them. On the other hand, Cu and Cd are preferentially sequestered in the cytosol by an induced phytochelatin, a glutathione-derived isopeptide that in this yeast has a

Table 2 Kinetic parameters of yeast growth inhibition by Cd and Ni according to the model of Ciftci et al. [33]

Metal vinasse level (g/L)	Cd			Ni		
	0	20	40	0	20	40
$\mu_{max}°$ (h^{-1})	0.64	0.76	0.92	0.71	0.63	0.95
K_1 (h^{-1})	0.175	0.163	0.022	0.258	0.040	0.041
K_2 (L/mg)	0.035	0.031	0.110	0.022	0.069	0.077
r^2	0.996	0.974	0.973	0.999	0.998	0.997

(γGlu-Cys)$_2$-Gly structure. Even though metallothionein was detected in Cd-resistant *S. cerevisiae* strains, transport phenomena and extracellular precipitation were also suggested as determinants of toxicity [12].

Interestingly, when vinasse was used to mitigate the toxic effect of Cd, there was a generalized rise in the final cell mass concentration (Fig. 1 in panel B and C) compared with controls (runs 1–3 in panel A), which became more marked when the initial concentration either of Cd (Cd_o) or vinasse (V_o) was increased. In particular, increasing V_o up to 20 and 40 g/L, these increases were about 73% (run 5 in panel B) and 84% (run 8 in panel C) at Cd_o=7.5 mg/L, and 159% (run 4 in panel B) and 200% (run 7 in panel C) at Cd_o=15 mg/L. One could believe that the enhancement due to vinasse addition could have been the result of a stimulating action on growth exerted by some vinasse component; however, comparing the final growth curves of runs 1–3, it is evident that such an effect was too small (4–11%) to justify the above marked effects. In addition, these results confirms previous observation that vinasse affected only slightly the cell mass concentration and viability of two different strains of *S. cerevisiae* [31].

Using the same reasoning for Ni (Fig. 2), an increase in V_o up to 20 and 40 g/L led to an increase in the final cell

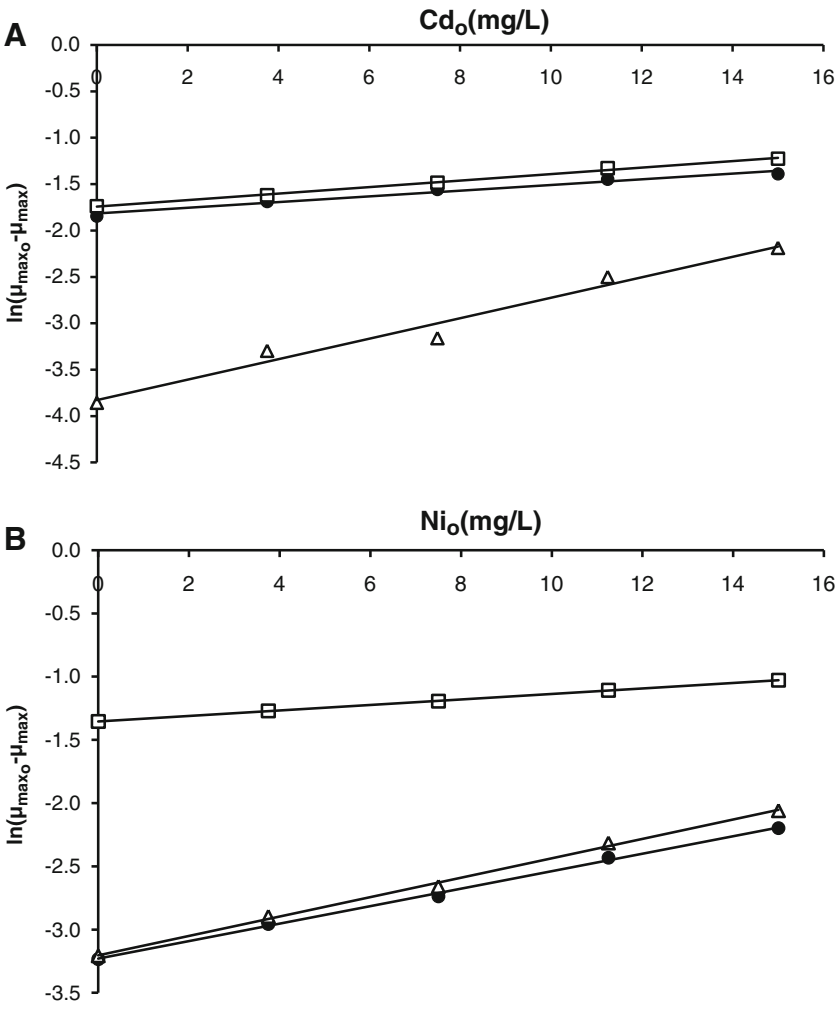

Fig. 3 Semilog plot of the maximum specific growth rate of *S. cerevisiae* in YED medium versus Cd (panel **a**) and Ni (panel **b**) concentrations without sugar cane vinasse (*white square*); with 20 g/L sugar cane vinasse (*black circle*) and 40 g/L sugar cane vinasse (*white up-right triangle*)

mass concentration by about 79% (run 5 in panel B) and 91% (run 8 in panel C) at Ni_o=7.5 mg/L, and 153% (run 4 in panel B) and 164% (run 7 in panel C) at Ni_o=15 mg/L, with respect to the controls (runs 1–3 in panel A). These results taken together demonstrate that, contrary to Ni, the lower the level of Cd, the higher the vinasse ability to mitigate its toxicity, and suggest that higher vinasse/metal ratios should be investigated to detect the maximum detoxifying capacity of this byproduct, with particular concern to Cd.

The progressive decrease in the specific growth rate (μ_{max}) observed increasing either Cd or Ni concentration (Table 1) can be explained by the decrease in sugar absorption by yeast cells, owing to the complexation of the phosphate groups of the plasmatic membrane by these metals [32]. Such an inhibiting effect has been mathematically described by the equation proposed by Ciftci et al. [33] for ethanol inhibition of anaerobic yeast growth:

$$\mu_{max} = \mu_{max}^{\circ} - K_1 e^{K_2 M} \quad (1)$$

where μ_{max}° (h^{-1}) is the theoretical value of μ_{max} in the absence of any metal (M_o=0), and K_1 (h^{-1}) and K_2 (L/mg) are two empirical parameters.

This model was already applied with success to investigate not only the toxic effect of aluminum on the same yeast and the mitigating effect of vinasse [22], but also the ethanol inhibition of starch hydrolysate fermentation by *S. cerevisiae* [34] as well as that of vanillin of ferulic acid-to-vanillin bioconversion by a recombinant strain of *Escherichia coli* [35].

Table 2 lists the values of the parameters appearing in Eq. (1) for *S. cerevisiae* growth either with 20 or 40 g/L vinasse or without vinasse. To this purpose, the experimental data of $\ln\mu_{max}$ were plotted in Fig. 3 versus the initial concentrations of Cd (panel A) and Ni (panel B), and the parameters estimated by using a standard technique for error minimization. The high values of the determination coefficient of the straight lines obtained either with Cd ($0.973 \le r^2 \le 0.996$) or Ni ($0.997 \le r^2 \le 0.999$) demonstrate the applicability of this model also to the metals under consideration as well as its general validity.

As one can see, the values of all kinetic parameters depended mainly on the level of vinasse, while the type of metal exerted an appreciable effect only at the intermediate vinasse level. In particular, as far as the mitigating effect of vinasse on Cd toxicity is concerned, the addition of 20 g/L vinasse to the YED medium led to values of the kinetic

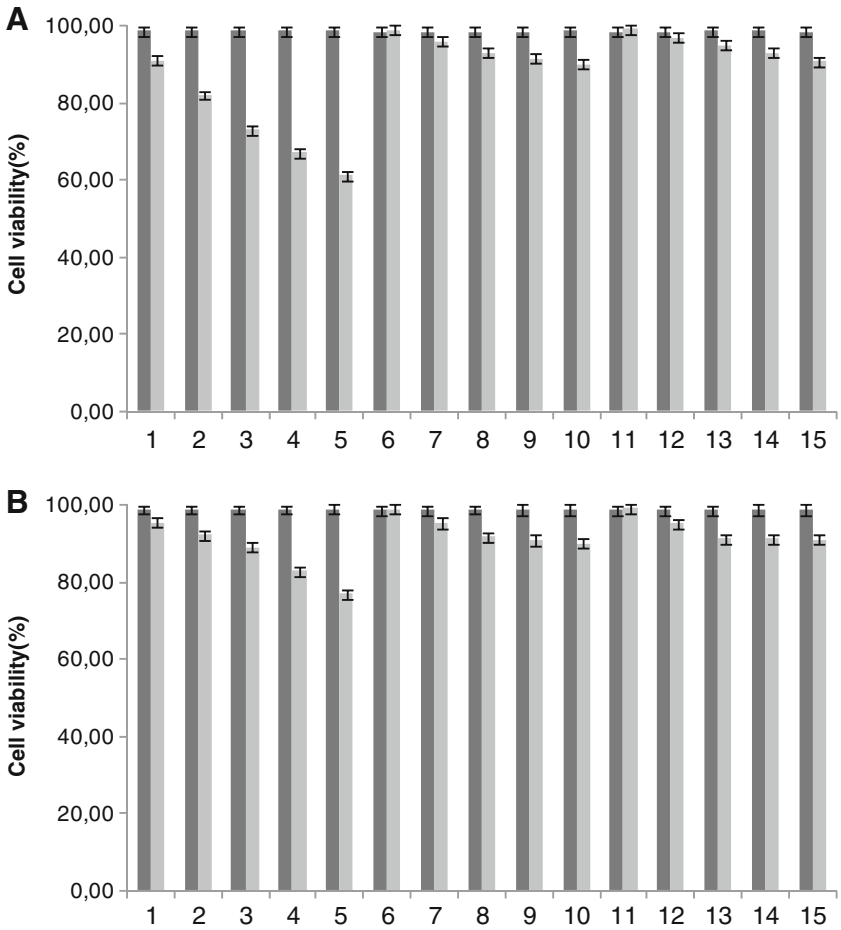

Fig. 4 Cell viability at the beginning (*gray square*) and the end (*light gray square*) of *S. cerevisiae* cultures in YED medium without sugar cane vinasse (*runs 1–5*), with 20 g/L vinasse (*runs 6–10*), with 40 g/L vinasse (*runs 11–15*), in the presence of different Cd (panel **a**) and Ni (panel **b**) concentrations. Run numbering and related conditions are the same as those listed in Table 1

constants comparable to those obtained in the absence of vinasse, and to an increase in $\mu_{max}°$ of only 20% (from 0.64 to 0.76 h^{-1}), while a further increase in the vinasse level up to 40 g/L brought about significant rise in $\mu_{max}°$ (up to 0.92 h^{-1}) and K_2 (up to 0.110 L/mg) and decrease in K_1 (to 0.022 h^{-1}). These results, taken together, suggest that whereas at the intermediate level vinasse simply behaved as a source of some limiting nutrient or growth factor, at the highest one, such an effect was accompanied by a mitigating one. This behavior was almost opposite to that observed in the presence of Ni. The dramatic decrease in K_1 and the nearly constant value of $\mu_{max}°$ do in fact reflect a strong mitigating effect of vinasse already at 20 g/L, while the quasi-invariability of both kinetic parameters and the strong increase in $\mu_{max}°$ at V_o=40 g/L revealed a stimulation of growth by additional nutrients.

This is the second attempt to use the above model to describe metal toxicity mitigation; therefore, it is almost impossible to make comparison with literature. Nevertheless, we can compare these results with those obtained with Al at higher levels either of metal (0–54 mg/L) or of vinasse (150 g/L), for which K_1 and K_2 values resulted to be about one order of magnitude higher and lower, respectively, and $\mu_{max}°$ substantially lower either with or without vinasse (0.48–0.49 h^{-1}). Although these results on the whole cannot be used to make any toxicity comparison, they certainly allow to claim about the stronger mitigation effect of vinasse on Al toxicity, compared with Cd or Ni.

As far as the inhibition of Cd and Ni on the growth is concerned, it is possible to get an idea of its significance by a comparison with inhibitions observed for other biosystems. The impressive capability of vinasse to mitigate Cd and Ni toxicity is also demonstrated by the fact that the K_1 value in the absence of this byproduct was about one order of magnitude higher than those obtained for two less inhibitory phenomena, i.e., excess substrate inhibition on ferulic acid-to-vanillin bioconversion (0.0147 h^{-1}) [33] and alcoholic fermentation of starch hydrolysate (0.0164 h^{-1}) [34], whereas in its presence it became even of the same order of magnitude.

Unexpectedly, contrarily to these biosystems and to the removal of Al, the exponential parameter K_2 significantly increased when vinasse was added in the presence of both metals, even though this effect was more marked with Cd than with Ni. Such an increase should not be ascribed to any increased toxicity induced by vinasse, because it would be in contrast with the slight stimulation of growth observed in Figs. 1 and 2. Thus, it suggests a possible change in the mechanism of metal removal by vinasse. We can suppose that the addition of low vinasse levels to the metal solution could

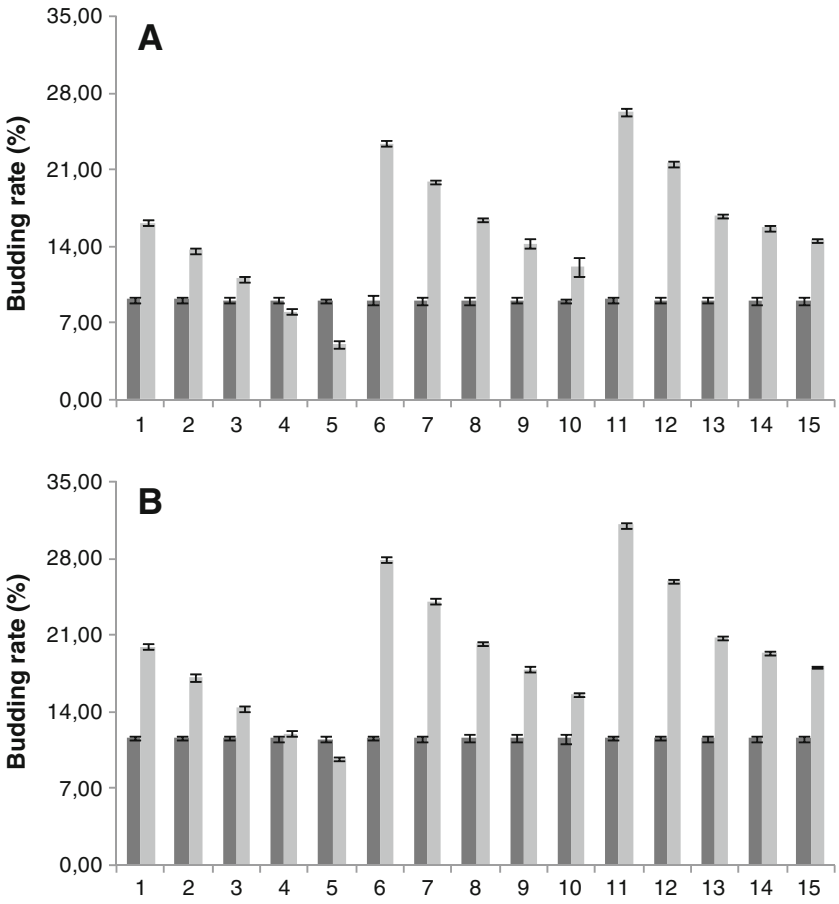

Fig. 5 Cell budding at the beginning (*gray square*) and end (*light gray square*) of *S. cerevisiae* cultures in YED medium without sugar cane vinasse (*runs 1–5*), with 20 g/L vinasse (*runs 6–10*), with 40 g/L vinasse (*runs 11–15*), in the presence of different Cd (panel **a**) and Ni (panel **b**) concentrations. Run numbering and related conditions are the same as those listed in Table 1

have induced a simple metal adsorption (K_2) at the beginning of the process and then a stronger chelation of metal ions by vinasse (K_1). In the presence of excess adsorbent, i.e., 20 and 40 g/L vinasse for Ni and Cd, respectively, such a stable structure could have been broken by the presence of vinasse, thus favoring the simple adsorption (K_2 increase) to the detriment of chelation (K_1 decrease).

Cell Viability and Budding Rate

Figure 4 shows the results of cell viability both at the start and the end of cultivations, in the presence of different initial concentrations of Cd (panel A) and Ni (panel B). It should be noted that, as expected, the initial viability was always very close to 100% in all the runs, and that the final viability progressively decreased with increasing the concentration of both metals, consistently with their well-proved toxicity. However, the higher the vinasse level, the lower the final viability loss induced by both metals, which provides an additional proof of the mitigating effect of this byproduct. As an example, taking as a reference the run 5 (panel A), carried out at the highest Cd concentration (15 mg/L) without vinasse, the cell viability decreased by 38%, whereas in the presence of 20 and 40 g/L vinasse it decreased by only 8.7% (run 10) and 7.8% (run 15), respectively. However, using Ni (panel B), the viability decreased by only 22% without vinasse (run 5) and by approximately 8% either with 20 g/L (run 10) or 40 g/L vinasse (run 15). These results, taken as a whole, demonstrate that, contrary to the cell mass concentration that was almost equally affected by Cd and Ni, the former metal had a stronger negative effect on cell viability than the latter, and that 20 g/L vinasse was likely sufficient to achieve its maximum mitigating effect.

A different behavior is evident in Fig. 5 for the budding rate, which remarkably increased during all cultivations as the result of the normal growing ability of the yeast. However, in the absence of vinasse, the budding rate progressively decreased from 16.2 to 5.1% when the Cd concentration was increased from 0 to 15 mg/L (panel A), consistently with the Cd toxicity. A similar trend was observed for Ni (panel B) even though the budding rate was always higher, because of the less toxicity of this metal. But the most interesting finding was the dramatic increase in the budding rate under all tested conditions when progressively more vinasse was added to the system. To give only a few examples, when 40 g/L vinasse was added, the budding rate increased in the presence of Cd_o=15 mg/L from 5.1% (run 5) to 14.5% (run 15) (panel A), and in the presence of Ni_o= 15 g/L from 9.8% (run 5) to 18.1% (run 15) (panel B).

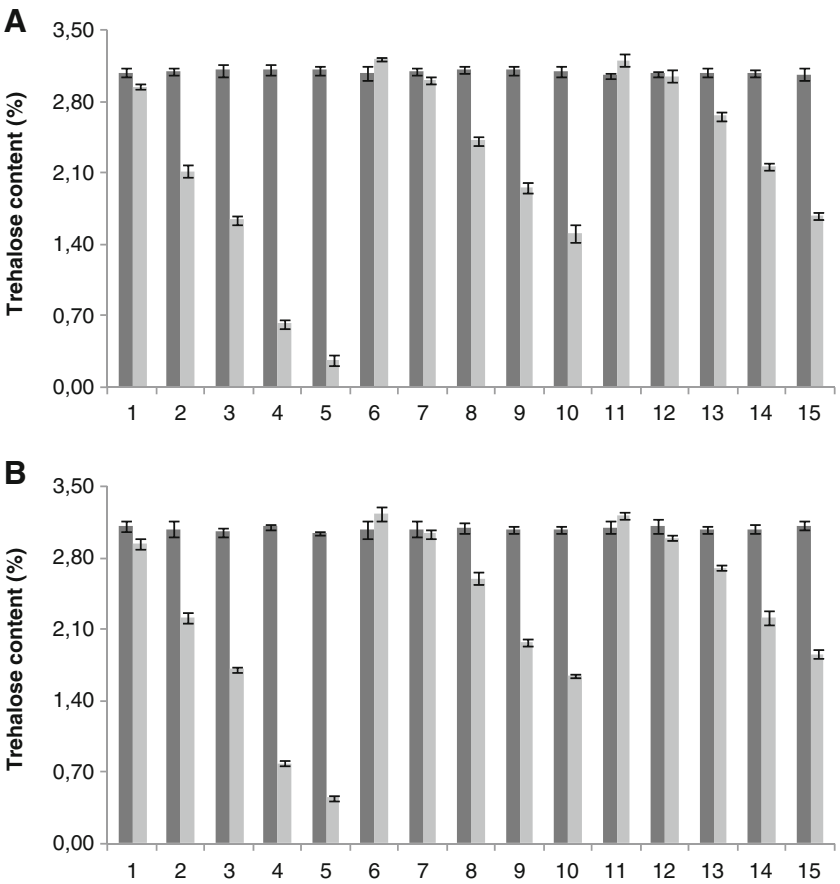

Fig. 6 Trehalose content of cells at the beginning (*gray square*) and end (*light gray square*) of *S. cerevisiae* cultures in YED medium without sugar cane vinasse (*runs 1–5*), with 20 g/L vinasse (*runs 6–10*), with 40 g/L vinasse (*runs 11–15*), in the presence of different Cd (panel **a**) and Ni (panel **b**) concentrations. Run numbering and related conditions are the same as those listed in Table 1

Trehalose Content

Trehalose is a nonreducing disaccharide composed of two glucose units that seems to exert a protective effect on the yeast cells during the stress [36]. As trehalose is associated mainly with carbohydrate storage in fungi during the nonproliferation period [37], the yeast survival depends on the stored trehalose level [38]. As a consequence, the trehalose content of *S. cerevisiae* cells (Fig. 6) followed the same qualitative behavior as the cell viability, even though the negative effect of both metals on it was much stronger. For instance, in the absence of vinasse, the trehalose content decreased from 2.95% without Cd to 0.26% with Cd_o=15 mg/L (panel A) and from 2.94% without Ni to 0.45% with Ni_o=15 mg/L (panel B), which means that under these conditions the accumulation of trehalose in the cell was almost completely hindered. However, the addition of 40 g/L vinasse progressively improved this situation, leading to 1.68% and 1.86% trehalose at the highest levels of Cd and Ni, respectively, confirming the mitigating effect of this byproduct also in this aspect. These results seem to be consistent with the hypothesis of inhibition of sugar transport into the yeast cell by metals [16, 17], which would hamper binding with sugar.

Conclusions

Cadmium and nickel, even at low concentrations, affected the growth of *S. cerevisiae*. In the presence of cadmium or nickel, the use of vinasse showed a clearly protective effect of this byproduct, in that it minimized the negative effects of both metals on cell mass concentration, cell viability and budding rate. The trehalose content of cells showed a good correlation with both cell viability and yeast growth, and this parameter proved to reflect well the state of physiological stress to which the yeast was subjected. The use of vinasse as a toxicity mitigator, not only of cadmium and nickel but also of other metals, promises to have potential environmental applications and deserves to be targeted for more detailed examination. In addition, the ability of sugar cane vinasse to mitigate the toxic effects of Cd and Ni could be exploited by using this waste as an amending material of soil for crop cultivation.

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