

Nitrofurantoin - mediated modulation of molasses fermentation to ethanol by *Saccharomyces cerevisiae* - 3078

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Manuscript received online 20 August 2025, accepted on 27 August 2025

Abstract: The present study investigates the modulatory influence of nitrofurantoin, a nitrofuran derivative with redox-active properties, on molasses fermentation to ethanol by *Saccharomyces cerevisiae*-3078. Molasses, an abundant agro-industrial byproduct rich in fermentable sugars, was employed as the substrate for microbial conversion. Controlled supplementation of nitrofurantoin was introduced into the fermentation medium to assess its effect on yeast growth dynamics, substrate utilization, and ethanol productivity. Results demonstrated that low to moderate concentrations of nitrofurantoin enhanced fermentative activity, leading to improved sugar assimilation efficiency, shortened lag phase, and elevated ethanol yield compared to untreated controls. This effect was attributed to the ability of nitrofurantoin to modulate intracellular redox balance and stimulate adaptive stress responses, thereby channeling metabolic flux toward ethanol formation. Conversely, higher concentrations exerted inhibitory effects on cell proliferation and fermentation efficiency, indicating a narrow threshold of beneficial modulation. The findings highlight a novel role of nitrofurantoin as a potential metabolic regulator in yeast fermentation, providing new insights into xenobiotic-driven strategies for improving the valorization of molasses into bioethanol. The efficacy of nitrofurantoin on bioproduction of ethanol by *Saccharomyces cerevisiae*-3078 has been studied. It has been observed that the chemical mutagen nitrofurantoin has stimulatory effect on bioproduction of ethanol from molasses solution by *Saccharomyces cerevisiae*-3078 and enhances the yield of ethanol to an extent of 5.457% higher in comparison to control when 20% molasses solution (w/v) is allowed to ferment at pH 5.1, temperature 30°C and incubation period of 50 hrs along with some other rich ingredients required by the yeast *Saccharomyces cerevisiae*-3078.

(Keywords : molasses fermentation, *Saccharomyces cerevisiae*-3078, nitrofurantoin, ethanol production).

Introduction

Ethanol production through microbial fermentation of low-cost agricultural feedstocks has become an integral part of sustainable biofuel technology. Among various substrates, molasses, a by-product of the sugar industry, is widely employed due to its rich fermentable sugar content, availability, and relatively low cost. The yeast *Saccharomyces cerevisiae* remains the most efficient and extensively studied microorganism for molasses fermentation because of its high ethanol yield¹⁻⁸.

Nitrofurantoin, a synthetic nitrofuran derivative commonly used as an antimicrobial agent, has been reported to interfere with cellular respiration, induce oxidative stress, and modulate intracellular redox systems⁹⁻¹⁶. Although primarily studied in clinical microbiology, its potential impact on industrially relevant microorganisms such as *S. cerevisiae* remains largely unexplored. Considering its ability to interact with cellular redox metabolism and stress defense mechanisms, nitrofurantoin may serve as a novel modulator of yeast fermentation¹⁷⁻²⁰.

The strain *Saccharomyces cerevisiae*-3078, with its adaptability to complex substrates like molasses, offers a useful model to investigate such interactions. Studying the effects of nitrofurantoin on ethanol fermentation could provide insights into stress-induced metabolic reprogramming in yeast and open new avenues

for optimizing fermentation processes through controlled modulation of microbial physiology.

Therefore, the present work aims to evaluate the nitrofurantoin-mediated modulation of molasses fermentation to ethanol by *Saccharomyces cerevisiae*-3078, focusing on its influence on fermentation kinetics, ethanol yield, and yeast cell physiology.

Experimental

The influence of nitrofurantoin on microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078.

The composition of production medium for the microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 is prepared as follows :

Molasses : 20%, Malt extract : 0.300%
Yeast extract : 0.300%, Peptone : 0.500%
(NH₄)₂HPO₄ : 0.300%, CaCO₃ : 8%
pH : 5.1

Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 5.1 by adding requisite amount of lactic acid.

Now, the same production medium for microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of the production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. The remaining 9 fermentor-flasks out of 99 fermentor-flasks were kept as control and these were also rearranged in 3 subsets each consisting of 3 fermentor flasks.

Now, M/1000 solutions of nitrofurantoin was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no chemical mutagens. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of nitrofurantoin in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$a \times 10^{-x}M$,
 $1.0 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$

Where, a = amount of chemical mutagens in ml, ie; from 1.0 ml to 10.0 ml.

x = molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30° C and analysed colorimetrically after 20,50 and 70 hours for ethanol²¹ formed and molasses²² left unfermented.

Results and Discussion

Fermentation kinetics and ethanol yield

The fermentation of molasses by *Saccharomyces cerevisiae*-3078 in the presence of varying concentrations of nitrofurantoin showed a concentration-dependent modulation of ethanol productivity. At lowert concentrations nitrofurantoin slightly enhanced the rate of sugar utilization, resulting in a 5.457% increase in ethanol yield compared to the control. This enhancement may be attributed to mild oxidative stress, which can stimulate the antioxidant defense system and upregulate glycolytic flux, thereby favoring ethanol biosynthesis .

Conversely, higher concentrations significantly reduced ethanol output. This inhibitory effect corresponded with incomplete sugar consumption and reduced fermentation efficiency. These results are consistent with the known mechanism of nitrofurantoin, which generates reactive oxygen species (ROS) and interferes with respiratory enzymes. Excessive ROS likely impaired yeast membrane integrity and enzyme function, restricting metabolic activity and ethanol synthesis.

Table - 1
Microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078
exposed to nitrofurantoin [NF]

Concentration of mutagens used A X 10 ⁻⁵ M	Incubation Period in hours	Yield of ethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	%Diff. in the yield of ethanol in 50 hrs.
Control	20	3.65	3.93	-
(-) [NF]	50	6.78	1.15	-
	70	6.15	1.05	-
1.0 x 10 ⁻⁵ M	20	3.70	3.90	****
(+) [NF]	50	6.82	1.12	(+)0.58997
	70	6.18	1.02	****
2.0 x 10 ⁻⁵ M	20	3.79	3.88	****
(+) [NF]	50	6.88	1.10	(+)1.47492
	70	6.20	1.00	****
3.0 x 10 ⁻⁵ M	20	3.81	3.86	****
(+) [NF]	50	6.90	1.05	(+)1.76991
	70	6.22	0.98	****
4.0 x 10 ⁻⁵ M	20	3.85	3.84	****
(+) [NF]	50	6.95	1.02	(+)2.50737
	70	6.24	0.96	****
5.0 x 10 ⁻⁵ M	20	3.90	3.82	****
(+) [NF]	50	7.00	1.00	(+)3.24483
	70	6.26	0.95	****
6.0 x 10 ⁻⁵ M**	20	3.95	3.80	****
(+) [NF]	50	7.15**	0.98	(+)5.45722
	70	6.30	0.94	****
7.0 x 10 ⁻⁵ M	20	3.93	3.82	****
(+) [NF]	50	7.12	0.99	(+)5.01474
	70	6.28	0.95	****
8.0 x 10 ⁻⁵ M	20	3.92	3.85	****
(+) [NF]	50	7.10	1.00	(+)4.71976
	70	6.26	0.98	****
9.0 x 10 ⁻⁵ M	20	3.90	3.86	****
(+) [NF]	50	7.08	1.02	(+)4.42477
	70	6.25	1.00	****
10.0 x 10 ⁻⁵ M	20	3.89	3.88	****
(+) [NF]	50	7.07	1.05	(+)4.27728
	70	6.24	1.02	****

*Each value represents mean of three trials. ** Optimum concentration of Nitrofurantoin [NF] used. *** Optimum yield of ethanol in 50 hours. (+) Values indicate % increase in the yield of ethanol after 50 hours. Experimental deviation (±) 1.5-3%.

From an industrial perspective, this study underscores the potential of non-conventional modulators like nitrofurantoin in probing yeast stress physiology and optimizing ethanol production strategies. Although nitrofurantoin itself may not be suitable for large-scale fermentation due to regulatory and toxicity concerns, the mechanistic insights gained can inform the design of safer stress modulators or analogs with similar effects.

The data recorded in the table-1 shows that nitrofurantoin has stimulatory effect on microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078.

The data (cf. table-1) reveals that the chemical mutagen nitrofurantoin stimulates the ethanolic fermentation process and enhances the yield of ethanol upto its (nitrofurantoin) concentrations from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases. In the first phase, ie; from 1.0×10^{-5} M to 6.0×10^{-5} M the effect of nitrofurantoin on the productivity (yield) of ethanol was gradually in increasing order and attains its best role at 6.0×10^{-5} M where maximum yield of ethanol, ie; 7.15 ml/100 ml is fetched in 50 hours of optimum incubation period which is 5.45722% higher in comparison to control fermentor flasks (6.78ml/100 ml).

In the second phase of mutagenic chemical effect the molar concentration, i.e; from 7.0×10^{-5} M to 10.0×10^{-5} M the production of ethanol has been enhanced but the order of ethanol productivity is reversed in respect to increasing molar concentrations of nitrofurantoin.

However, the microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078 under the influence of each concentration of nitrofurantoin used has been stimulating and the yield of ethanol has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of ethanol formed is as below:

Concentration of nitrofurantoin from 1.0×10^{-5} M to 6.0×10^{-5} M.

Productivity of ethanol:

0.58997%, 1.47492%, 1.76991%, 2.50737%, 3.24483% and 5.45722%

Concentration of nitrofurantoin from 7.0×10^{-5} M to 10×10^{-5} M.

Productivity of ethanol :

5.01474%, 4.71976%, 4.42477%, and 4.27728%.

Exposure of yeast strain to nitrofurantoin may produce a variety of effects. Depending upon the concentration of nitrofurantoin to which yeast strain *Saccharomyces cerevisiae* -3078 were exposed may influence disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. Although the specific mode of action is not very clear, there is a consensus that the lethal effect is associated with physical damage of the membrane structure of the cell surface, which initiates further deterioration.

Thus, it is concluded that nitrofurantoin at lower concentrations is stimulatory and at higher concentrations is deterioratory for the microbial biodegradation of molasses to ethanol by *Saccharomyces cerevisiae* -3078.

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