

Modulatory effect of maleic hydrazide (MH) in fermentative bioethanol production by microorganisms

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Abstract: Maleic hydrazine (MH) is a plant growth regulator and mild mutagens. In microbial biotechnology, it may be used to modulate metabolic pathways, possibly affecting bioethanol yield. It has also been investigated in fermentation technology due to its chemical and biochemical activities. Maleic hydrazide can be a useful biotechnological tool in bioethanol fermentation. In the present communication modulatory effect of maleic hydrazide in fermentation bioethanol production by microorganisms viz. *Thermoanaerobacter mathereanii* ARC-1603, *T. pentosaceus* ARC-1605, *Zymomonas mobilis* ARC-1606 and *Saccharomyces cerevisiae* ARC-1607 has been assessed. It has been found that the microbial strain of *S. cerevisiae* ARC-1607 is most successful and significant bioethanol producer. It has been observed that maleic hydrazide has stimulatory effect on bioethanol production and enhances the yield of bioethanol to an extent of 28.243% higher in comparison to control when 23.5% (w/v) molasses solution is allowed to ferment for 60 hour of optimum incubation period, 4.8 pH, and 34.5°C temperature along with some other nutritional ingredients required by the fungal strain under trial.

(Keywords : Bioethanol, maleic hydrazide and *S. cerevisiae* ARC-1607).

Introduction

Mutagens can significantly impact bioethanol production by altering, the genetic

makeup of microorganisms used in fermentation¹⁻¹⁰. While some mutations can enhance ethanol production, others can be detrimental, decreasing production or even rendering the microorganism non-viable¹¹⁻¹⁶. Not all mutagens are beneficial. Some can negatively impact cell growth, ethanol production, or stress tolerance, highlighting the need for careful strain selection. Mutagens can be a powerful tool for improving bioethanol production by creating strains with enhanced ethanol tolerance and sugar utilization. However, the effects of mutagenesis are not always predictable, and careful selection of mutants is crucial for maximizing ethanol yields¹⁷⁻¹⁸. Mutagenesis helps in developing high yielding and stress tolerant microbial strains especially *S. cerevisiae* using mutagens in biotechnology significantly enhances bioethanol production efficiency. *S. cerevisiae* is one of the most promising unicellular fungi on account of its vital applications in biotechnology as well as bioethanol production. Each year all kinds of industries increase globally, therefore, every research has also dramatically increased. Several environmental problems and global climate change rapidly appeared as a result of fossil fuels and their derivatives consumption. In recent years, bioenergy as well as bioethanol demand has become greater than before as a source of eco-friendly and safe alternative energy. Production of bioethanol can contribute to the solution of these problems due to its nature as a clean, renewable and carbon neutral fuel. Mutagens can help and promote process of

Table – 1
Studies on the production of bioethanol readily when maleic hydrazide (MH) is applied

Mutagen concentration used $A \times 10^{-x} M$	Hourly Incubation Period	Production of bioethanol* per 100 milliliters	g/100 ml of unfermented molasses sugars*	% change in bioethanol yield from control after 60 hours
Control	35	3.04	9.662	-
	50	4.54	8.361	-
	60	6.89	7.665	-
	70	5.56	6.565	-
	80	3.02	5.869	-
$1.0 \times 10^{-5} M$	35	****	-	-
	50	4.59	8.321	-
	60	6.97	7.586	+1.204
	70	5.62	6.505	-
	80	****	-	-
$1.5 \times 10^{-5} M$	35	****	-	-
	50	4.68	8.231	-
	60	7.11	7.446	+3.193
	70	5.72	6.405	-
	80	****	-	-
$2.0 \times 10^{-5} M$	35	****	-	-
	50	4.77	8.141	-
	60	7.24	7.316	+5.137
	70	5.83	6.295	-
	80	****	-	-
$2.5 \times 10^{-5} M$	35	****	-	-
	50	4.82	8.091	-
	60	7.32	7.236	+6.313
	70	5.89	6.235	-
	80	****	-	-
$3.0 \times 10^{-5} M$	35	****	-	-
	50	5.00	7.551	-
	60	7.60	6.416	+10.319
	70	6.11	5.565	-
	80	****	-	-
$3.5 \times 10^{-5} M$	35	****	-	-
	50	3.36	7.551	-
	60	8.14	76.416	+18.243
	70	6.56	5.565	-
	80	****	-	-
$4.0 \times 10^{-5} M$	35	****	-	-
	50	5.53	7.381	-
	60	8.40	6.156	+22.002
	70	6.78	5.345	-
	80	****	-	-

Contd

4.5×10 ⁻⁵ M	35	****	-	-
	50	5.62	7.291	-
	60	8.55	6.006	+24.208
	70	6.89	5.235	-
	80	****	-	-
5.0×10 ⁻⁵ M**	35	****	-	-
	50	5.81	7.101	-
	60	8.83***	5.726	+28.243
	70	7.11	5.015	-
	80	****	-	-
5.5×10 ⁻⁵ M	35	****	-	-
	50	5.58	7.331	-
	60	8.48	6.076	+23.076
	70	6.83	5.295	-
	80	****	-	-
6.0×10 ⁻⁵ M	35	****	-	-
	50	5.32	7.591	-
	60	8.08	6.476	+17.271
	70	6.50	5.625	-
	80	****	-	-
6.5×10 ⁻⁵ M	35	****	-	-
	50	5.18	7.731	-
	60	7.88	6.676	+14.368
	70	6.33	5.795	-
	80	****	-	-
7.0×10 ⁻⁵ M	35	****	-	-
	50	4.95	4.961	-
	60	7.53	7.026	+9.288
	70	6.06	6.065	-
	80	****	-	-
7.5×10 ⁻⁵ M	35	****	-	-
	50	4.86	0.051	-
	60	7.38	7.176	+16.090
	70	5.94	6.185	-
	80	****	-	-
8.0×10 ⁻⁵ M	35	****	-	-
	50	4.73	8.181	-
	60	7.19	7.366	+4.354
	70	5.79	6.405	-
	80	****	-	-
8.5×10 ⁻⁵ M	35	****	-	-
	50	4.67	8.241	-
	60	7.11	7.446	+3.193
	70	5.72	6.405	-
	80	****	-	-

Contd...

9.0×10 ⁻⁵ M	35	****	-	-
	50	4.64	8.271	-
	60	7.05	7.506	+2.322
	70	5.63	6.445	-
	80	****	-	-
9.5×10 ⁻⁵ M	35	****	-	-
	50	4.59	8.451	-
	60	6.98	7.651	+1.306
	70	5.63	6.495	-
	80	****	-	-
10×10 ⁻⁵ M	35	****	-	-
	50	4.55	8.361	-
	60	6.91	7.646	+0.290
	70	5.57	6.555	-
	80	****	-	-

** Optimal concentration of the employed chemical mutagen.

*** Optimal bioethanol yield following 60 hours.

*The mean of three trials is represented by each number.

(+)The figures indicate the bioethanol yield increase as a percentage over the control.

The experimental deviation (+) is 1.5-2.3%.

bioethanol enhancing its yields significantly. Literature survey reveals that a little work has been done on modulatory effect of mutagens¹⁹⁻²⁷ in fermentative bioethanol production, therefore, the authors have employed maleic hydrazide on bioethanol production by *S. cerevisiae* ARC-1607.

Experimental

The impact of maleic hydrazide (MH) on *Saccharomyces cerevisiae* ARC-1607 yeast's bioethanolic fermentation. The following is the preparation of the production medium for the *Saccharomyces cerevisiae* ARC-1607 yeast's bioethanolic fermentation :

Molasses : 23.5%, Malt extract : 1.45%
Yeast extract : 1.45%, Peptone : 1.45%
(NH₄)₂HPO₄ : 0.35%, pH : 4.8, Temperature : 34.5
I.P. : 35, 50, 60, 70, 80

The capacity was raised to "100 ml" by adding distilled water. The medium's pH was

brought to 4.8 by adding the necessary quantity of lactic acid. The same fermentation medium, *Saccharomyces cerevisiae* ARC-1607, was now created for 315 fermentor-flasks, or 100 ml of production medium per flask, for the purpose of bioethanolic fermentation. Then, 20 sets of 15 fermentor-flasks each were assembled from these fermentor-flasks. Out of the 315 fermentor flasks, the remaining 15 were preserved as controls and were rearranged into 5 subsets, each with 3 fermentor flasks. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 ml of the m/1000 maleic hydrazide (MH) solution were added to the fermentor-flasks of the first 21 sets, accordingly. There were no chemical mutagens present in the control fermentor-flask. Each fermentor-flask was filled to a capacity of "100 ml" by adding the necessary amount of distilled water.

As a result, the Maleic hydrazide (MH) concentrations in the first through twentieth subsets were roughly as follows:
a × 10⁻⁵M,

1.0×10^{-5} M, to 10×10^{-5} M respectively. Where, a = amount of mutagens in ml, i.e.; from 1.0 ml to 10.0 ml. X = Molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 34.5° C, and colorimetrically analyzed after 35, 50, 60, 70, and 80 hours for bioethanol²⁸ produced and blackstrap molasses sugars²⁹ left unfermented.

Results and Discussion

Table - 1 displays results indicating that maleic hydrazide (MH) stimulates the production of bioethanol by the yeast *Saccharomyces cerevisiae* ARC-1607.

The data (table-1) reveals that the chemical mutagen maleic hydrazide (MH), stimulates the bioethanolic fermentation by the yeast *Saccharomyces cerevisiae* ARC-1607 and enhances the yield of bioethanol upto its (maleic hydrazide (MH), concentrations from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases :

Maleic hydrazide (MH) had a gradual but increasing effect on bioethanol productivity (yield) in the first phase, i.e., from 1.0×10^{-5} M to 5.0×10^{-5} M. It reached its peak at 5.0×10^{-5} M, where the maximum yield of bioethanol, i.e., 8.83 ml/100 ml, was obtained in 60 hours of optimal incubation period, which is 28.243% more than in control fermentor flasks (6.89 ml/100 ml).

The synthesis of bioethanol has increased in the second phase of the mutagenic chemical impact, from 5.5×10^{-5} M to 10.0×10^{-5} M, however the order of bioethanol productivity is reversed in relation to rising molar concentrations of maleic hydrazide (MH). Nonetheless, the *Saccharomyces cerevisiae*

ARC-1607 yeast has demonstrated promoting bioethanolic fermentation in response to varying concentrations of maleic hydrazide (MH), with a yield of bioethanol determined to be higher than that of the control fermentor flasks. The productivity and percentage of bioethanol generated after 60 hours in both phases are as follows :

Maleic hydrazide (MH) concentration increased from 1.0×10^{-5} M to 5.0×10^{-5} M.

Productivity of bioethanol: 1.204%, 3.193%, 6.313%, 10.319%, 18.243%, 22.002%, 24.208% and 28.243%

Concentration of maleic hydrazide (MH) from 5.0×10^{-5} M to 10×10^{-5} M .

Productivity of bioethanol: 23.076%, 17.271%, 14.368%, 9.288%, 7.111%, 4.345%, 3.193%, 2.322%, 1.306% and 0.290%

Maleic hydrazide (MH) exposure of yeast strain *Saccharomyces cerevisiae* ARC-1607 can result in a number of different outcomes. The amount of maleic hydrazide (MH) that the yeast strain *Saccharomyces cerevisiae* ARC-1607 was exposed to affect various aspects of the yeast, including enzyme inactivation, cell disruption, precipitation of cell protein, and leakage of amino acids from the cells. There is general agreement that the deadly impact is linked to physical destruction of the cell surface's membrane structure, which starts additional degeneration, even though the precise mechanism of action is unclear.

Therefore, it may be said that maleic hydrazide (MH) stimulates the fermentation of bioethanol by yeast at lower concentrations and degrades it at greater amounts.

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