

Studies on facile biotic production of bioethanol exposed to L-methionine-DL-sulfoximine (MSO)

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Abstract : In the present communication some of the important steps has been taken for the higher facile biotic production of bioethanol exposed to some chemical mutagens specially L-methionine-DL-sulfoximine (MSO) by some microbial strains such as *Thermoanaerobacter matheranii* ARC-1603, *T. pseudoethanolicus* ARC-1604, *T. pentosaceus* ARC-1605, *Zymomonas mobilis* ARC-1606 and *Saccharomyces cerevisiae* ARC-1607. It has been noted that *Saccharomyces cerevisiae* ARC-1607 has been found most successful bioethanol producer. It has been observed that the chemical mutagen, i.e, L-methionine-DL-sulfoximine (MSO) has stimulatory and significant impact on bioethanol production and enhances the yield of bioethanol to an extent of 17.142% higher in comparison to control, i.e., 6.65ml/100 ml when 23.5% (w/v) molasses solution is allowed to ferment for 60 hrs 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, fermentation, mutagen, L-methionine-DL-sulfoximine (MSO) and *Saccharomyces cerevisiae* ARC-1607)

Introduction

Each year all kinds of industries increase globally; therefore, energy 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 (Farrell *et al.*¹ 2006; Hill *et al.*² 2006). The industrial development and continuous rise of crude oil cost has improved the competitiveness of bioethanol against fossil fuels. Over the past years, discussion and focus on the green impacts of bioethanol and other biofuels have appeared (Anex and Lifset³ 2009).

A genetic alteration that is present for the first time in one family member as a result of a variant (or mutation) in a germ cell (egg or sperm) of one of the parents, or a variant that arises in the fertilized egg itself during early embryogenesis. Also called de novo mutation, de novo variant, and new variant.

Mutagens can be classified into 3 types based on their origin. They are as follows: Physical mutagens: These include ionizing radiation, such as X-rays, gamma rays and alpha particles. Ultraviolet radiations can also behave as potential mutagens. Chemical mutagens: Elements such as arsenic, nickel and chromium are considered to be mutagens.⁴⁻¹⁰

Early studies by Ames showed around 90% of known carcinogens which can be identified in Ames test as mutagenic and 80% of the mutagens identified through Ames test may also be carcinogens. Mutagens are not necessarily carcinogens, and vice versa. Sodium Azide for example may be mutagenic (and highly toxic),

but it has not been shown to be carcinogenic.¹¹⁻¹⁸

Literature survey reveals that a very little work has been done on facile biotic production of bioethanol by the yeast *Saccharomyces cerevisiae* ARC-1607 exposed to chemical mutagens¹⁹⁻²⁷, therefore, the authoress have employed L-methionine-DL-sulfoximine (MSO) on facile biotic production of bioethanol by yeast *Saccharomyces cerevisiae* ARC-1607.

Experimental

The influence of L-methionine-DL-sulfoximine (MSO) on facile biotic production of bioethanol by yeast *Saccharomyces cerevisiae* ARC-1607. The constitution of production medium for the fermentative bioproduction of bioethanol from molasses by *Saccharomyces cerevisiae* ARC-1607 is prepared as follows :

MS Molasses Solution : 23.5 % (w/v) Malt-Extract : 1.45%, Yeast-Extract : 1.45%
Peptone : 1.45%, Distilled water : To make up 100 ml pH : 4.8

Distilled water was added to make up the volume up to '100 ml'. The pH of the medium was adjusted to 4.8 by adding requisite amount of lactic acid.

Now, the same production medium for facile biotic production of bioethanol from molasses by *Saccharomyces cerevisiae* ARC-1607 was prepared for 315 fermentor-flasks, i.e., each containing hundred ml of production medium.

These fermentor-flasks, i.e., each containing hundred ml of production medium. These fermentor-flasks were then arranged in twenty one sets each comprising fifteen fermentor-flasks. The remaining fifteen fermentor-flasks out of 315 fermentor-flasks were kept as control and these were also rearranged in five subsets each consisting of three fermentor flasks.

Now, M/1000 solutions of L-methionine-DL-sulfoximine (MSO) was made and 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 this solution was added to the fermentor -flasks of first 20 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 L-methionine-DL-sulfoximine (MSO) in 1st to 20th 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 mutagen in ml, ie; from 1.0 ml to 10.0 ml.

and x = molarity of the solution respectively.

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

Results and Discussion

The influence of L-methionine-DL-sulfoximine (MSO) on facile biotic production of bioethanol by *Saccharomyces cerevisiae* ARC-1607.

The data recorded in the table-1 shows that L-methionine-DL-sulfoximine(MSO) has stimulatory effect on facile biotic production of bioethanol by the yeast *Saccharomyces cerevisiae* ARC-1607.

The maximum yield of bioethanol, i.e., 7.79 ml/100 ml in the presence of L-methionine-DL-sulfoximine (MSO) was observed at $7.0 \times 10^{-5} M$ molar concentration in 60 hours of optimum incubation period which is 17.142% higher in comparison to control fermentor flasks, ie; 6.65 ml/100ml in the same times course and other same experimental parameters.

Table – 1
Studies on facile biotic production of bioethanol exposed to
L-methionine-DL-sulfoximine (MSO)

Concentration of mutagen used $A \times 10^{-x} M$	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in the yield of bioethanol in comparison to control after 60 hrs
Control	35	2.80	9.802	-
	50	4.30	8.606	-
	60	6.65	7.905	-
	70	5.32	6.804	-
	80	2.78	6.103	-
$1.0 \times 10^{-5} M$	35	****	-	-
	50	4.32	8.581	-
	60	6.67	7.902	+ 0.300
	70	5.35	6.770	-
	80	****	-	-
$1.5 \times 10^{-5} M$	35	****	-	-
	50	4.34	8.562	-
	60	6.69	7.900	+ 0.601
	70	5.37	6.098	-
	80	****	-	-
$2.0 \times 10^{-5} M$	35	****	-	-
	50	4.36	8.541	-
	60	6.74	7.811	+ 1.353
	70	5.40	6.723	-
	80	****	-	-
$2.5 \times 10^{-5} M$	35	****	-	-
	50	4.39	8.511	-
	60	6.80	7.751	+ 2.255
	70	5.44	6.683	-
	80	****	-	-
$3.0 \times 10^{-5} M$	35	****	-	-
	50	4.45	8.451	-
	60	6.86	7.691	+ 3.157
	70	5.50	6.623	-
	80	****	-	-
$3.5 \times 10^{-5} M$	35	****	-	-
	50	4.50	8.401	-
	60	6.95	7.601	+ 4.511
	70	5.54	6.583	-
	80	****	-	-

Table-1 Contd.

Concentration of mutagen used A X 10 ^{-X} M	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in the yield of bioethanol in comparison to control after 60 hrs
4.0×10 ⁻⁵ M	35	****	-	-
	50	4.52	8.381	-
	60	6.96	7.581	+ 4.661
	70	5.55	6.573	-
	80	****	-	-
4.5×10 ⁻⁵ M	35	****	-	-
	50	4.54	8.361	-
	60	6.98	7.571	+ 4.962
	70	5.56	6.563	-
	80	****	-	-
5.0×10 ⁻⁵ M	35	****	-	-
	50	4.55	8.351	-
	60	6.99	7.562	+ 5.112
	70	5.58	6.543	-
	80	****	-	-
5.5×10 ⁻⁵ M	35	****	-	-
	50	4.57	8.331	-
	60	7.07	7.481	+ 6.315
	70	5.60	6.523	-
	80	****	-	-
6.0×10 ⁻⁵ M	35	****	-	-
	50	4.62	8.281	-
	60	7.19	7.361	+ 8.120
	70	5.63	6.493	-
	80	****	-	-
6.5×10 ⁻⁵ M	35	****	-	-
	50	4.69	8.211	-
	60	7.47	7.081	+ 12.330
	70	5.68	6.443	-
	80	****	-	-
7.0×10 ⁻⁵ M**	35	****	-	-
	50	4.75	8.151	-
	60	7.79	6.761***	+ 17.142
	70	5.75	6.373	-
	80	****	-	-
7.5×10 ⁻⁵ M	35	****	-	-
	50	4.73	8.171	-
	60	7.72	6.831	+ 16.090
	70	5.72	6.403	-
	80	****	-	-

Table-1 Contd.

Concentration of mutagen used $A \times 10^{-X} M$	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in the yield of bioethanol in comparison to control after 60 hrs
$8.0 \times 10^{-5} M$	35	****	-	-
	50	4.70	8.201	-
	60	7.32	7.231	+ 10.075
	70	5.70	6.423	-
	80	****	-	-
$8.5 \times 10^{-5} M$	35	****	-	-
	50	4.69	8.211	-
	60	7.26	7.299	+ 9.172
	70	5.69	6.433	-
	80	****	-	-
$9.0 \times 10^{-5} M$	35	****	-	-
	50	4.56	8.341	-
	60	7.00	7.550	+ 5.263
	70	5.57	6.553	-
	80	****	-	-
$9.5 \times 10^{-5} M$	35	****	-	-
	50	4.45	8.451	-
	60	6.90	7.651	+ 3.759
	70	5.49	6.638	-
	80	****	-	-
$10 \times 10^{-5} M$	35	****	-	-
	50	4.35	8.551	-
	60	6.77	7.781	+ 1.805
	70	5.38	6.743	-
	80	****	-	-

* Each value represents mean of three trials.

** Optimum concentration of the chemical mutagen used.

*** Optimum yield of bioethanol in 60 hours.

(+) Values indicate % increase in the yield of bioethanol in comparison to control.

Experimental deviation ($\pm$) 1.5–3%.

The higher molar concentrations of L-methionine-DL-sulfoximine (MSO) were not much favourable for the facile biotic production of bioethanol by the yeast *Saccharomyces cerevisiae* ARC -1607. So the gradual addition of the mutagen L-methionine-DL-sulfoximine (MSO) after certain concentrations were not beneficial for the bioethanolic fermentation by the yeast *Saccharomyces cerevisiae* ARC-1607.

It has been observed that molar concentration of the mutagen, ie., L-methionine-DL-sulfoximine (MSO) from $1.0 \times 10^{-5} M$ to $7.0 \times 10^{-5} M$ enhances the yield of bioethanol to a certain order being 0.300%, 0.601%, 1.353%, 2.255%, 3.157%, 4.511%, 4.66%, 4.962%, 5.112%, 6.315%, 8.120%, 12.330% and 17.142% higher in comparison to control flasks.

It has been observed further that after optimum concentration, i.e; 7.0×10^{-5} M, the addition of the same mutagen to the production medium causes fall in the yield of bioethanol gradually and reached to 1.805%. However, at

all the experimental concentrations of L-methionine-DL-sulfoximine(MSO), used, the yield of bioethanol by submerged fermentation has been found higher in comparison to control fermentor flasks.

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