

Natural production of bioethanol exposed to acrylonitrile

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Abstract : The global bioethanol market size was accounted at USD 40.8 billion in 2021 and it is expected to reach around USD 124.5 billion by 2030. What will be the CAGR of global bioethanol market? The global bioethanol market is poised to grow at a CAGR of 13.2% from 2022 to 2030. The efficacy of chemical mutagen, i.e., acrylonitrile (AN) on natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922 has been assessed. It has been found that the chemical mutagen, i.e., acrylonitrile at its molar concentration of 6.0×10^{-5} M has moderate stimulatory impact on natural production of bioethanol to an extent of 5.769% higher in comparison to control fermenter flask, i.e. 5.20 mL/100mL while molar concentration of acrylonitrile under trial at 7.0×10^{-5} M and onwards slow down and retards the bioethanol production. Experimental variables has been optimized viz. : 32°C temperature, 4.6 pH, 55hrs incubation period with 15.0% (w/v) molasses solution alongwith other nutritional ingredients required by the yeast *Saccharomyces cerevisiae* PK-1922 .

(Key words: Lactic Acid Molasses, acrylonitrile and yeast *S. cerevisiae* PK-1922)

Introduction

Bioethanol has evolved as a potential source for biofuel production at the current state of energy insecurity and in environmental safety challenges over fossil. Bioethanol is ethanol (C_2H_5OH), or ethyl alcohol, produced by biological methods. It is among the best established of biofuels. Bioethanol is used mostly as an additive to gasoline (petrol). Engines capable of burning pure or anhydrous ethanol are now available and coming into increasing use. This article discusses bioethanol and the key

technologies used in producing it. These include microbial fermentation of sugars to ethanol; pretreatment of carbohydrate polymers (e.g., starch, cellulose) to release the fermentable sugars; separation of ethanol by distillation from an aqueous fermentation broth; and further dehydration to fuel-grade bioethanol.¹⁻⁵

Bioethanol produced from lignocellulosic biomass serves as an alternative promising carbon neutral biofuel. Advantages of bioethanol as a biofuel include high octane number (108), low boiling point, higher heat of vaporization, and comparable energy content. Blended gasoline with up to 85% (v/v) bioethanol can be used in vehicles without modification of the existing engine. Blending can significantly reduce greenhouse gas emissions as well as petroleum use. Although several bioethanol pilot plants have been commissioned throughout the world, a lot of effort is still needed to bring down the production cost. Major cost constraints are the cost of feedstock, cost of enzymes, detoxification cost, and ethanol recovery cost.⁶⁻¹²

Bioethanol production from lignocellulosic biomass involves three major unit operations: pretreatment, enzymatic saccharification, and fermentation. One of the major limitations is that the processing cost is high, which makes the process economically unviable. Since 2008, a lot of progress has been made in cost reduction, such as using enzyme cocktails for better saccharification, using engineered microorganisms for improved product yield, and producing some low-volume high-value-added products to improve the overall

process economics. Ethanol production at high temperature using thermophiles helps in the removal of ethanol by gas stripping or solvent extraction. The production of bioethanol from rice straw was reported by Takano and Hoshino . Simultaneous saccharification and fermentation of steam exploded rice straw, yielding 13.2 g/L of bioethanol. One of the major advantages of using brown algae is that saccharification is relatively easy because of the absence of lignin. One major limitation is the presence of alginate, which cannot be metabolized by microorganisms; this can be overcome by employing metabolically engineered microorganisms that can utilize alginate. Bioethanol production from brown algae is still in its infancy and commercialization is possible once cost-effective strategies for brown algae cultivation and integrated bioprocessing are developed¹³⁻¹⁸.

Chemical mutagens react with DNA and lead to faulty base pairing that can be classified into different categories. Base analogues mimic a particular nucleobase in nucleic acid and are misread by the replicating machinery as a normal base. Following incorporation into DNA, they form non-Watson pairing with the DNA. The most studied base analogues are halouracils and uridine derivatives that include 5-bromodeoxyuridine (BrdU). This potent mutagen is capable of inducing point mutations by substituting thymine residues and pairing with guanine instead of adenine. Such base pair changes are deleterious and are more prone to alterations by physical mutagens such as X-rays, UV rays, or gamma rays relative to normal DNA. Certain chemicals such as nitrous acid, hydroxyl amine, and sodium azide can modify the bases by deamination, thus modifying the regular base pairing. Nitrous acid deaminates adenine, guanine, and cytosine substituting adenine to hypoxanthine, guanine to xanthine, and cytosine to uracil. These substitutions induce AT to GC transitions leading to faulty base pairing. Chemical mutagens are also comprised of alkylating agents and DNA intercalating agents. Ethyl methyl sulfate, nitrogen

mustards, mitomycin, methyl methane sulfonate (MMS), diethyl sulfate, and nitrosoguanidine (NTG, NG, MNNG) are few examples of alkylating agents as well as DNA-intercalating agents, which include acridine orange, ethidium bromide (EtBr), proflavin, daunorubicin, among others.

Acridine orange or EtBr introduces frameshift mutations either by addition or deletion of base pairs. Certain pesticides such as rotenone, paraquat, or maneb have the capacity to induce mutations such as base pair changes in genes and could lead to neurodegenerative diseases such as Parkinson disease (PD).

Acrylonitrile (AN) is an important intermediary for the synthesis of a variety of organic products, such as artificial fibres, household articles and resins. Although acute effects are the primary concern for an exposure to AN, potential genotoxic, carcinogenic and teratogenic risks of AN have to be taken seriously in view of the large number of workers employed in such industries and the world-wide population using products containing and possibly liberating AN. An understanding of the effect of acrylonitrile must be based on a characterization of its metabolism as well as of the resulting products and their genotoxic properties. Tests for mutagenicity in bacteria have in general been positive, those in plants and on unscheduled DNA synthesis doubtful, and those on chromosome aberrations *in vivo* negative. Wherever positive results had been obtained, metabolic activation of AN appeared to be a prerequisite. The extent to which such mutagenic effects are significant in man depends, however, also on the conditions of exposure. It appears from the limited data that the ultimate mutagenic factor(s), such as 2-cyanoethylene oxide, may have little opportunity to act under conditions where people are exposed because it is formed only in small amounts and is rapidly degraded. The carcinogenic action of AN has been evaluated by various agencies and ranged from 'reasonably be anticipated to be a

Table - 1
Natural production of bioethanol exposed to acrylonitrile

Concentration of mutagen used A x 10 ^{-x} M	Incubation Period in hours	Yield of bioethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	% Diff. in the yield of bioethanol in 55 hrs
Control	55	5.20	2.890	—
1.0 x 10 ⁻⁵ M	55	5.22	2.860	(+) 0.384
2.0 x 10 ⁻⁵ M	55	5.25	3.770	(+) 0.961%
3.0 x 10 ⁻⁵ M	55	5.30	3.720	(+) 1.923%
4.0 x 10 ⁻⁵ M	55	5.35	3.630	(+) 2.884%
5.0 x 10 ⁻⁵ M	55	5.40	3.410	(+) 3.846%
6.0 x 10 ⁻⁵ M	55	5.50	3.205	(+) 5.769%
7.0 x 10 ⁻⁵ M	55	5.45	3.430	(+) 4.807%
8.0 x 10 ⁻⁵ M	55	5.38	3.660	(+) 3.461%
9.0 x 10 ⁻⁵ M	55	5.32	3.260	(+) 2.307%
10.0 x 10 ⁻⁵ M	55	5.28	3.182	(+) 1.538%

* Each value represents mean of three trials. ** Optimum concentration of mutagen used.

*** Optimum yield of bioethanol in 55hours. (+) Values indicate % increase in the yield of bioethanol after 55 hours. Experimental deviation ($\pm$) 1.5-3%.

human carcinogen' to 'cannot be excluded', the most recent evaluation being 'possibly carcinogenic to humans'. Animal data that confirm the carcinogenic potential of AN have certain limitations with respect to the choice of species, type of tumors and length of follow up. Epidemiological studies which sometimes, but not always, yielded positive results, encounter the usual difficulties of confounding factors in chemical industries. Exposure of workers to AN should continue to be carefully monitored, but AN would not have to be considered a cancer risk to the population provided limitations on releases from consumer products and guidelines on AN in water and air are enforced. AN is teratogenic in laboratory animals (rat, hamster) at high doses when foetal/embryonic (and maternal) toxicity already is manifest. Pregnant workers should not be exposed to AN. In view of the small concentrations generally encountered outside plants, women not professionally exposed would appear not to be at risk of teratogenic effects due to AN. Future research should concentrate on the elucidation of the different degradation pathways in man and on epidemiological studies in workers

including pregnant women, assessing also, if possible, individual exposure by bio-monitoring.

Treatment with mutagenic agents can increase mutation frequencies by order of magnitude¹⁹⁻²⁰. The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers²¹⁻³¹. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes³²⁻³⁸.

Thus, from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for alcoholic fermentation and in view of this the author has studied the influence of acrylonitrile (AN) by *S. cerevisiae* PK-1922.

Experimental

The influence of acrylonitrile (AN) on natural production of bioethanol by *S. cerevisiae* PK-1922.

The composition of production medium for the natural production bioethanol by

Saccharomyces cerevisiae PK-1922 is prepared as follows :

Molasses :15 % (w/v), Malt-Extract: 0.250%
Yeast-Extract : 0.250%, Peptone : 0.250%,
Distilled water : To make up 100 ml , pH : 4.6

Distilled water was added to make up the volume up to '100 ml'. The pH of the medium was adjusted to 4.6 by adding requisite amount of lactic acid.

Now, the same production medium for natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922 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 acrylonitrile (AN) 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 acrylonitrile (AN) 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, 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 32^o C and analysed colorimetrically after 55 hours for natural production bioethanol formed³⁹ and

molasses⁴⁰ pollutant left unfermented.

Results and Discussion

The influence of acrylonitrile (AN) on natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922

The data recorded in the table-1 shows that the chemical mutagen, i.e., acrylonitrile (AN) has been found stimulatory for the natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922 at all its concentrations, i.e; from $1.0 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$ in two phases.

In the first phase the molar concentration of acrylonitrile (AN), i.e; from $1.0 \times 10^{-5} M$ to $6.0 \times 10^{-5} M$ is significant and the productivity of bioethanol gradually increases till a maximum is reached at $6.0 \times 10^{-5} M$, i.e.; 5.50ml/100ml in 55 hours of optimum incubation period which is 5.769% higher in comparison to control, i.e; 5.20ml/100ml.

In the second phase of mutagenic effect, i.e., at $7.0 \times 10^{-5} M$ and onwards molar concentration of acrylonitrile (AN) on the natural production of bioethanol has been increased but in lesser quantum in the presence of acrylonitrile (AN). So, the gradual addition of acrylonitrile (AN) after the optimum concentration of $6.0 \times 10^{-5} M$ gradually slows down the natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922 on the part of % increase at $7.0 \times 10^{-5} M$, $8.0 \times 10^{-5} M$, $9.0 \times 10^{-5} M$ and $10.0 \times 10^{-5} M$ molar concentration of acrylonitrile (AN) is as follows: 4.807%, 3.461%, 2.307% and 1.538% respectively.

From the present investigation it is obvious that the chemical mutagens used, i.e., acrylonitrile (AN) is useful for the natural production of bioethanol by *Saccharomyces cerevisiae* PK-1922 and therefore, it can be employed for the improved/enhanced yield of bioethanol.

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