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Alcohol production on co culture Method

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ABSTRACT

The Zymomonas mobilis used in this experiment may be a mutant type. The S. cereviciac may be wild type which may be the reason behind the low ethanol yield. The parameters responsible for very low ethanol production by co-culture should be studied further. The number of live Z mobils was studied after 12 hours and 24 hours. When compared to years the number of Z mobils cell were rapidly reduced. It was identified by plating the serially diluted culture sample on Macconkey agar. The reduction may be due to the ethanol intolerance of Z-mobilis. Zuobilis is a non-lactose fermenting organisms (NLF). Hence when lactose is used as a sugar source ethanol is not produced by Zmobilis. In search of a bacteria, which can covert lactose in to glucose E coli came as a topper of the list. Ecoli is a lactose fermenting organisms (LF). By producing B-galactosidase enzyme Ecoli is able to convert lactose into glucose and galactose. An overnight E coli culture was inoculated in a RML medium in which lactose was the only sugar source. It was incubated at 37 C for 12 hours. After 12 hourse Z.mobilis was introduced into E.coli culture with the expectation of glucose production (Before distillation the ethanol production was qualitatively confirmed by smell). After 24 hours the culture was subjected to distillation. Distillations gave only 1.7 ml of thanol. The number of E.coli was reduced. It was identified by plating the serially diluted culture sample on Macconkey medium. It was done before and after inoculation of Zmobilis. The ethanol produced by Zmobilis may be a reason for the reduction of E.coli cells. Though the reasons are yet to be identified. This work was done to test the practicability of theoretical possibility. The little success obtained in this study is yet to be confirmed. Ethanol may be produced using this method. But how for it will be helpful industrially should clearly be studied.

Keywords: Zymomonas mobilis, Alcohol, E.coli, Saccharomyces crevisiae, Sugar Fermentation

I. INTRODUCTION

Alcohol is an essential chemical used in lots of prescription drugs products which includes allopathic and homeopathic preparations. As a solvent. It is utilized in various chemical reactions. Nowadays alcoholic beverages production is at height which is incomes crores and crores: commonly alcohol is produced via two methods 1) Chemical methods 2) organic methods. From the duration of Pasteur microbial manufacturing of alcohol is gaining greater importance and appeal. The chemical manufactures the brewer, the distiller, the baker, the vinegar producer, the scientist, the housewife and many others rely in one way or every other at the ability of micro organism to transform sugars to alcohol, Carbondioxide and other and products. But unknowingly alcohol turned into produced and fed on by using human beings from time immemorial (Doelle, 1985).

The traditional microorganism used for alcohol manufacturing is yeast, an unicellular fungus called Saccharomyces. The usage of pressure improvement method and many others., it's been modified as a good alcohol-generating organism. Even as searching fro alcohol generating micro organism scientists have come upon Zymomonas mobilis, a terrible bacilli, a quick growing and green alcohol manufacturer. Wastes containing greater sugar the sort of molasses is generally used as a source of alcohol manufacturing can be because of the increasing production of alcohol the initial organism E.Coli had been identified by using plating serial dilutions of sample on MacConkey agar. The bacterial of the genus Zymomonas are polarly flagellated, gram terrible rods that occur in fermentative plant materials (Miller, 1959). Like enteric micro organism, they're facultative anaerobes which have each respiration

Enteric micro organism, they're facultative anaerobes that have both respiration and fermentative ability Zymomonas mobilis ferments glucose to provide ethyl alcohol through the Entner-Doudoroff pathway, with formation of one

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mole of ATP permole of glucose; yeasts in contrast ferment glucose to ethyl alcohol via Embden-Meyerhoff pathway yielding molds of ATP according to mole of glucose. Yeasts produce extensively greater cells than Zymomonas mobilis according to mole of glucose fermented by using approximately the equal amount in line with mole ATP produced by way of the fermentation (Doran, 1994).

In this study Zymomonas mobilis a known bacteria fermenting glucose, fructose and sucrose was used. The same sugars can also be used by Saccharomyces cerevisiae. They were cultivated separately and together In grape juice and their efficiency in alcohol production was recorded. The Zomononas mobilis is actually a non-lactose fermenting organism. But Escherichia coli are a Lactose fermenting organism (Acabal, 1992).

E.Coli turned into inoculated in lactose medium wherein the sugar source was lactose best. The organisms had been allowed to ferment lactose (the usage of B-galactosidase enzymes). In this response lactose is transformed to glucose. Glucose dis fermented via Zymomonas mobilis and converts the equal into alcohol. In order that lactose is transformed into alcohol by means of the mechanism of those two organisms. The quantity of ethanol was determined through distillation of culture broth (Guinaraes, 1992)

For quantitative analysis various parameters such as time of production of glucose by Ecoli and the time when Zmobilis should be introduced, the quantity inoculum of both and the effect of byproducts as alcohol production were not studied because of time limit.

II. MATERIALS AND METHODS

SAMPLE COLLECTION

Zymomonas mobilix strain obtained from M.K. University was used for ethanol production. The first source used for the ethanol production was grape juice. 250 ml of grape juice was inoculated with seed culture of Zymomonas mobilis. This seed culture was grown in fermentation medium called RM medium, for 18 hrs at 30°C and 10% V/V of this culture used as inoculum. Fermentation was carried out with 250 ml Erlenmeyer flask at 30°C (Acabal, 1992).

Similiary Saccharomyces cerevisiae strain obtained from M.K.University was also used for ethanol production using 250 ml grape juice. Seed cultures were grown in fermentation medium called YPD medium for over night. And 10% v/v of this culture used as the inoculated fermentation experiments was carred out 250 ml Erlenmeyer flask at room temperature. Then similar quantity of grape juice was inoculated with both Zymomonas mobilis and Saccharomyces cerevisiae and all similar condition were maintained. E.coli obtained from NCCT, Chandigarh,

DISTILLATION OF ETHANOL

After 48 hrs the fermented grape juice was taken and allowed for distillation ethanol. The boiling temperature of ethanol is 80 C at which vaporization of the same occurs. The ethanol vapour was condensed into liquid ethanol. Then the quantity of ethanol obtained from Zmobiles fermeration and S. cerevisiae fermentation and their combined fermentation were measured and compared with each other.

Z mobilis, an ethanologenic bacterium has been of considerable interest in recent years for ethanol production as an alterative to the conventional yeast strains. It gives near theoretical yields of ethanol from glucose and fructose. However ethanol production from sucrose by Zymomonas mobils is considerably reduced due to the formation of by production such as levan and sorbitorl (Dawes, 1966, Viikri, 1984).

Zymomonas mobilis is basically a non lactose fermenter, so ethanol cannot be produced when lactose is used as a sugar source. E.coli obtained from NCCT, Chandigarh was inoculated in 100 ml of newly formulated RMI. medium.

Ecoli is a lactose fermenting organism so that they were allowed to ferment lactose present in RML. medium. Lactose is converted into glucose and galactose by an enzyme called B-galactosidase, with that theoritical idea Zymomonas mobilis was introduced into overnight culture of E.coli in RML medium. (Guinaraes, 1992).

THE VARIOUS STEPS INVOLVED IN THE ABOVE CO-
CULTURE METHOD:

- 80 ml of RML. medium was prepared in 250 ml of flask. For the preparation of RML medium, all ingredients except sugar were added in 20 ml of distilled water and sterilized at 121 C for 15 minutes.
- 2 pms lactose added into 20 ml of distilled water was filter sterilized and aseptically transferred to the above medium so that, the medium was made into 100 ml with 2 gms lactose,
- Overnight culture of E.coli was inoculated in the RML. medium and incubated at 37°C for

12 hrs. Then overnight culture of Zymomonas mobilis was introduced into the medium which would have got glucose due to the B-galactosidase enzyme produced by the E.coli

- As the organisms are facultative anaerobic organisms the mouth of the flask was tightly closed with polythene paper and incubated at 30°C for 24 hrs.
- The number of E.coli present in the medium before and after the introduction of Zymomonas mobilis was studied by taking 1 ml of the medium as a sample. 1.0 ml of the sample medium was serially diluted and plated for the identification number of E.coli on Macconkey medium.
- The medium was taken after 24 hrs and subjected to distillation process. The quantity of ethanol obtained in distillation process was noted (Mali, 1984: Torres, 1987; Doran, 1984).

BIO CHEMICAL CHARACTERS OF E.coli:

Various bio-chemical characters such as sugar fermentation IMVIC test, catalase test, oxidase test and motility test were done to confirm the purity of E.coli culture.

SUGAR FERMENTATION:

The organisms were inoculated into sugar media with an indicator bromothymol blue and incubated at 37°C for 24 hrs. After 24 hrs the change of colour from green to yellow was observed which indicates the acid production during the fermentation of sugar, gas production was also tested using durhams tube. Various sugars such as glucose, fructose, maltose, lactose, sucrose, mannitol were added separately in sugar media for studying the fermentation ability of the organisms (Gunasekaran, 1986).

BROMOTHYMOL BLUE:

Bromothymol blue 2 gm per litre (0.2% W/V) - 12.5 ml

Preparation of Bromothymol blue:

0.1g of Bromothymol blue was mixed 2.5 ml of 0.1 mol/lit of (N/10) NaoH. 47.5 ml of Sterile distilled water was added and mixed well. It was stored in dark bottle for further use.

MOTILITY TEST:

The hanging drop technique was followed to observe the motility of the organisms. It was observed under microscope for the darting or cork screw movement of the organism. The results were noted.

INDOLE TEST:

The colony from the agar slant was inoculated into the indole medium in the tube and then incubated at 37 °C for 24 hrs,

The formation of red ring due to the addition of 1 ml of kovacs indole regent indicates the positive reaction.

METHYL RED TEST:

The colony inoculated into the MR-VP broth tubes and incubated at 37°C for 24 hrs.

Formation of red colour due to the addition of methyl | red indicator, indicates the positive reaction.

VOGES-PROSKUER TEST:

The same colony was inoculated into the MR-VP broth tubes and incubated at 37°C for 24 hours.

Development of pink to bright red colour by the addition of Baritt's reagent to the medium indicates the positive reaction.

CITRATE UTILIZATION TEST:

Stant of Simon's citrate agar medium was inoculated with the organisms and incubated for 24 hours at 37°C.

Change of colour from green to blue indicates the positive reaction.

CATALASE PRODUCTION:

One loopful of 3% Hydrogen peroxidase was added to the culture on the glass slide. Prompt effervescence indicates the positive result production.

OXIDASE TEST:

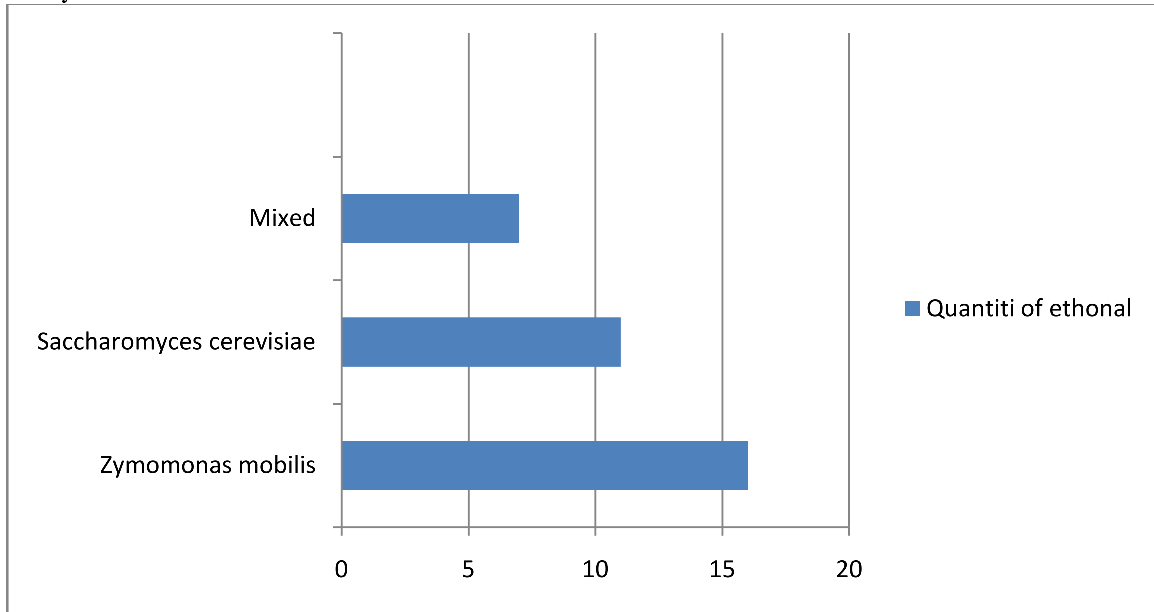
The colony to be tested was smeared on the filter paper, soaked in the oxidase reagent, (1% tetramethy I paraphenylene diamino dihydrochloric acid). In a positive reaction the smeared area turns dark purple in 10 to 60 seconds.

BIO-CHEMICAL CHARACTERISTICS OF Zymomonas mobilis:

All the test is done for the indentification Ecoli were also performed for the confirmation of purity of Zmobilis. The results were observed and tabulated.

RESULT

Quantity of Ethonal



250 ML OF GRAPE JUICE

FIG 1

GRAPH REPRESENTING VIABILITY OF ORGANISMS

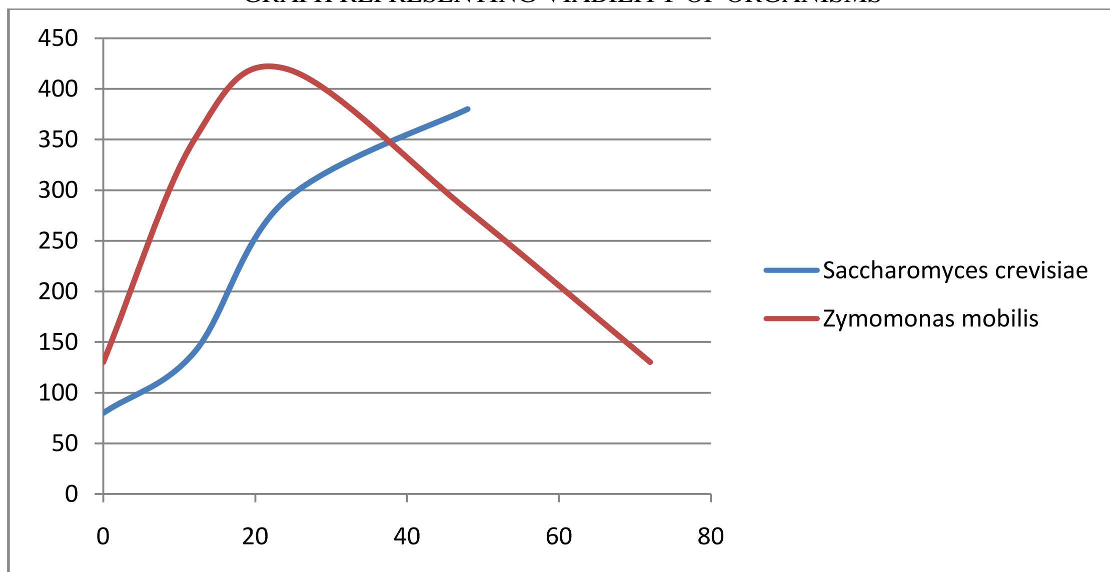


FIG 2

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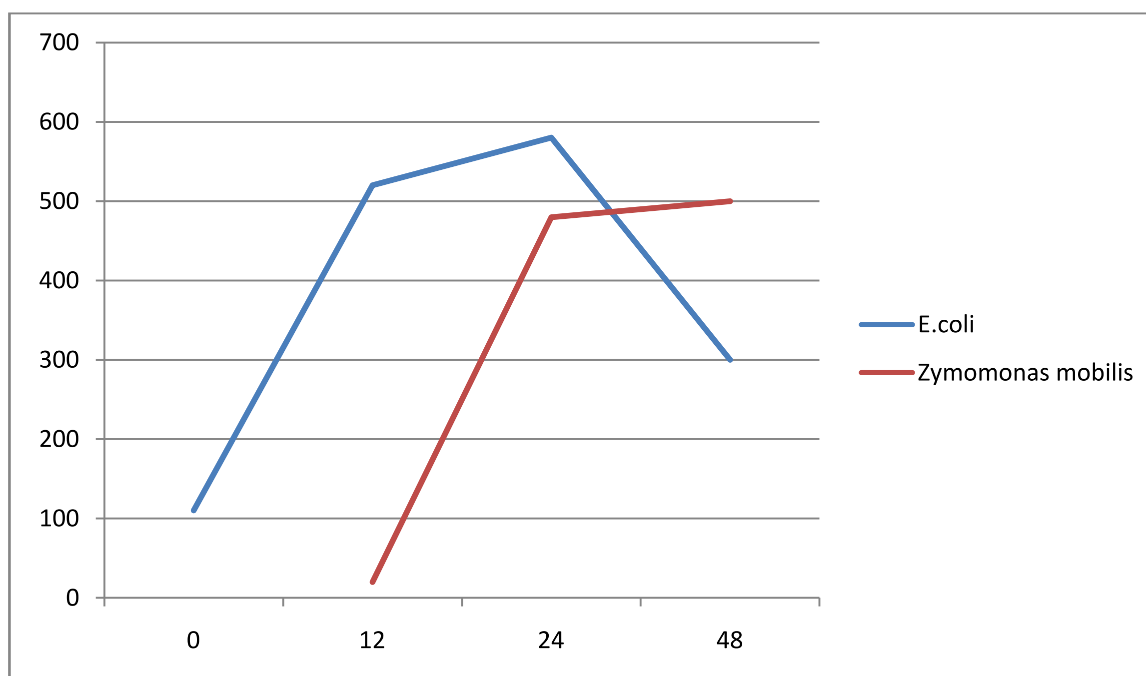

III. RESULTS

ETHONAL CONCENTRATION IN Zmobilis, S.Cerevisiae AND MIXED FERMENTATION:

The quantity of ethanol obtained in yeast and Z mobilis fermentation in grape juice in indicated Figure 1. Saccharomyces cerevisiae in 250 ml of grape juice yield 11.2 ml of ethanol. Whereas Zmobilis produced 16.5 ml of ethanol 250 ml grape juice. In mixer culture of both the yeild was only 6,7 ml.

The number of live Zymemonas mobilis was increased upto 24 hrs and it was reduced at 40-70 hrs. But the number of Saccharomyces cerevisiae was increased at 24 hrs and it was reduced at 72 hrs. The results were graphically represented in Figure1.

ETHANOL CONCENTRATION IN E.coli and Z. mobilis FERMENTATION:

In flask containing RML medium with E.coli and Z.mobilis the ethanol production was qualitatively confirmed by its smell. Only 1.7 of ethanol was obtained after distillation. The number of E.coli were reduceed which was identified in plates containing serial dilutions of medium. It was graphically represented in Figure.2

ORGANISMS

TABLE-1
BIO-CHEMICAL CHARACTERS OF E.coli AND Z.mobiles
I-Indole, MR-Methys red, VP-Voges-proskauer, C-Citrate utilisation

Organism	Motility	I	M	VP	C	Catalase	Oxidase	H_2S Prodn
E. coli	+	+	+	–	+	+	–	–
Zymomonas mobilis	+	–	–	+	–	+	–	D

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+ = Positive
- = Negative
d = doubtful

TABLE-2
SUGAR FERMENTATION CHARACTERS OF E.coli AND Z.mobiles
+ = Positive

Organism	Glucose	Fructose	Maitose	Sucrose	Lactose	Mannitol
E.coli	+	+	+	D	—	+
Zymomonas mobilis	+	+	—	D	+	—

- = Negative
d = doubtful

TABLE -3
QUANTITY OF ETHANOR PRODUCTION

S.No	MICRO ORGANISMS	ETHANOL(%)
1	Zymomonas mobilis	16.5 %
2	Saccharomyces crevisiae	11.2 %
3	Mixed	6.7 %

MORPHOLOGICAL & BIO-CHEMICAL CHARACTERS OF E.coli and Z.mobilis.

The results of the motility and IMVIC Test of E.coli were positive (Motility, IMVIC, Catalase and Oxidase). (Table-I).

The results of the motility and IMVIC Test of Zmobilis were positive (Motility, Vi and Catalase). (Table-I)

The fermentative ability of E.coli was observed in the carbohydrate test (Glucose, Fructose, Lactose, Maltose, Mannitoi). (Table 2)

The fermentative ability of Z.mobilis was observed in the carbohydrate fermentation test (Glucose, Fructose). (Table-2)

The results of morphological & Bio-chemical characters were tabulated in table 1 and 2.

Graph juice fermentation

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Graph juice after fermentation

IV. DISCUSSION

A flasks with 250 ml of grape juice was inoculated with Saccharomyces cerevisiae. Z mobilis and mixer of both respectively. The yield of ethanol in grape juice by Zymomonas mobilis was greater than Saccharomyces cerevisiae The wider variation may be due to the nature of both these strains. (Hobley, 1994). The results indicated that the Zymomonas mobilis strain resembled more with mutant strain. Such kind of improved strains are often results in higher ethanol production during fermentation. The ethanol concentration is very low in mixer culture where, Saccharomyces cerevisiae and Zymomonas mobilis were inoculated. It may be due to an over growth of ethanol tolerant S. cereviceae. As Zymomonas mobilis cannot tolerate higher ethanol concentration, they may be killed by ethanol produced by Saccharomyces cerevisiae. (Agrawal, 1994). Reduced number of non-lactose fermenting Zymomonas mobilis colonies were identified in Macconkey agar indicated the death of more Zymomonas mobilis here the environmental changes created by Saccharomyces cerevisiae such as, the effect of by products should be studied (Mendoza, 1986). The availability of sugar for both the organism may vary when organisms are inoculated simultaneously. Z.mobilis may be killed due to lack of nutrients mainly in sugar. Through many morphological and Bio-chemical of E.coli and Z. mobilis were known, they were separately studied during the course of this work to know their purity (Rogers, 1982).

In successive culture technique the E.coli was introduced and allowed to ferment lactose in RMI. medium. After 12 hours Zymomonas mobilis were introduced into the E.coli culture with the aim of production of glucose by previous organism. The production of ethanol in the successive culture technique was quantitavely confirm by its smell. When the same culture was allowed for distillation only 1.7 ml of ethanol /1.0 ml of RML medium was obtained. Here the time of introduction of Zymomonas mobilis in E.coli culture was only approximately calculated. But, it is understood that thetime of glucose production should be correctly identified because, one glucose is produced, it can be utilized by both E.coil as well as Zymomonas mobilis. The death of more lactose. fermenting Ecoil was confirmed by plating them on Macconkey agar plate from the serial dilutions of the culture. But the exact factors involved in killing of Ecoil are not clearly known. The size of inoculum, the generation time and environmental alteration ability of both the organism should be studied. The effect of Ecoil on Zmobilis and vice versa should also be studied. It is also important to study we there the ethanol was originally produced by Zymomonas mobilis or due to other factors (Skontnicki, 1981).

The results of these studied can be interpreted is such a way that there are chances for production of alcohol in these successive culture technique. But how far it will be useful industrially should be determined by the time factor only.