SUMMARY

SUMMARY

Capability of yeast to produce acetic acid from different natural substrates with the help of alcohol dehydrogenase & aldehyde dehydrogenase suggests usefulness of this enzyme & the organism itself for different applications including cost effective industrial production. Such microbial production of acetic acid, can reduce the production cost & also almost eliminate the probability of contamination. Such type of bio fermentation method could find application for generating valuable protein chemical products.

Glucose is one of the chief natural products of acetic acid producing industry. It is cheap, easily available & readily fermentable by any microorganism.

In Chapter 1, for the purpose of production of acetic acid from glucose by alcohol dehydrogenase & aldehyde dehydrogenase, a strain of *Saccharomyces cerevisiae* was taken. In some previous study, it was found that alcohol & temperature resistant *Saccharomyces cerevisiae* can produce alcohol & acetic acid very efficiently. Mother culture of *Saccharomyces cerevisiae* have 1.45% alcohol producing capacity. To achieve a high alcohol yielding strain, resistance was introduced by treating the parent strain with alcohol from 5.0% to 10.0% in temperature range 28-30°C - 37-39°C. Finally the *Sacharomyces cerevisiae* Y_{B23} was selected as the most potent organism for high alcohol production.

The appropriate maintenance medium for the *Sacharomyces cerevisiae* Y_{B23} to retain the alcohol producing capacity was selected in Chapter 2. The composition of the most suitable maintenance medium for *Sacharomyces cerevisiae* Y_{B23} is:

Glucose – 1.0%

$(NH_4)_2SO_4$ – 0.5%

KH_2PO_4 – 0.1%

$MgSO_4,7H_2O$ – 0.025%

$FeSO_4,7H_2O$ – 0.002%

Biotin – 0.2μg/ml

Agar-4.0%

pH – 5.0

Environmental parameters such as, period of fermentation, temperature of fermentation, initial pH of the medium, volume of fermentation medium, density of inoculum and age of inoculum; are very crucial factors which control the overall fermentation process and affect the production of the desired product. The optimum conditions for alcohol production by *Sacharomyces cerevisiae* Y_{B23} were determined by a series of surface culture fermentation experiments in Chapter 3 –

i)	Initial pH of the medium	:	4.5
ii)	Volume of the fermentation medium	:	100.0 ml
iii)	Period of fermentation	:	48hours
iv)	Cell density	:	2.05×10^5cells/ml
v)	Temperature of fermentation	:	30^0C
vi)	Age of the inoculums	:	48hours

The acetic acid producing capability was examined using various ethanol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B ; we found in Chapter 4 that *Saccharomyces cerevisiae* Y_{B23} is the desired strain which produces the maximum amount of acetic acid.

As the production of acetic acid is self inhibitory the alcohol & temperature resistant *Saccharomyces cerevisiae* Y_{B23} was treated consecutively with 1.0-3.0% concentration of acetic acid in Chapter 5. The isolated acetic acid resistant strain *Saccharomyces cerevisiae* Y_{B100} produces maximum amount of acetic acid (0.2874gm/100ml).

A synthetic medium has been prepared for acetic acid production by the acid resistant *Saccharomyces cerevisiae* Y_{B100} in Chapter 6. The media consists of-

Sucrose: 12.0%

$(NH_4)_2SO_4$: 0.5%.

KH_2PO_4: 0.1%

$MgSO_4,7H_2O$: 0.05%

pH : 4.5.

Saccharomyces cerevisiae Y_{B100} was named *Saccharomyces cerevisiae* Y_{Bmax} for convenience.

The optimum conditions for acetic acid production from sucrose by *Saccharomyces cerevisiae* Y_{Bmax} using a synthetic medium were determined in Chapter 7. The conditions are as follows-

i)	Initial pH of the medium	:	4.5
ii)	Volume of the fermentation medium	:	85.0 ml
iii)	Period of fermentation	:	96hours
iv)	Cell density	:	10.25×10^5cells/5ml
v)	Temperature of fermentation	:	30^0C
vi)	Age of the inoculums	:	48hours

In Chapter 8 suitable carbon and nitrogen source for the proper growth and multiplication of *Saccharomyces cerevisiae* Y_{Bmax} was optimized. Glucose and Ammonium sulphate were found to be the most suitable carbon and nitrogen sources for this purpose respectively. The optimum concentration of glucose and ammonium sulphate were also determined in this chapter-

Optimum concentration of Glucose : 10.0%

Optimum concentration of Ammonium sulphate : 0.5%

Chapter 9 deals with both macro and micro nutrient requirement of *Saccharomyces cerevisiae* Y_{Bmax}. Among macro elements, both KH_2PO_4 & K_2HPO_4 , $MgSO_4,7H_2O$, NaCl, $CaCl_2$, Na_2HPO_4 & boric acid showed stimulatory effects, whereas among the trace elements iron, manganese and zinc aided growth and enzyme production, but Mo^{6+} , Cu^{++}, Ni^{++} & V^{5+} show negative effect on cell growth and acetic acid production. The composition of the synthetic medium for acetic acid production by *Saccharomyces cerevisiae* Y_{Bmax} is as follows:

Glucose- 10%
$(NH_4)_2SO_4$- 0.5%
KH_2PO_4- 0.125%
K_2HPO_4- 0.125%
$MgSO_4.7H_2O$- 0.05%
NaCl- 0.1%
$CaCl_2$- 0.01%
Na_2HPO_4- 1.0%
Boric acid- 0.5 mg/L
Fe^{++} ion (as $FeSO_4.7H_2O$)- 10 µg/ml
Mn^{++} ion (as $MnSO_4.H_2O$)- 10 µg/ml.
Zn^{++} ion as ($ZnSO_4.7H_2O$)- 10 µg/ml.

Effect of Complex nutrient on acetic acid production was described in Chapter 10. In commercial acetic acid production, separate provision of all mineral nutrients is not economic. Complex nutrients are cheap sources of many such nutrients along with growth promoting factors like vitamins, amino acids etc. 10 different natural and commercially available complex nutrients from plant and animal origin were added to the fermentation medium to observe their effect on acetic acid production. Among all complex nutrients meat extract, soybean meal, malt extract, beef extract and tryptone showed positive influence. Peptone, rice bran extract, paddy soak liquor, wheat bran extract & corn steep liquor declined the fermentation rate.

Chapter 11 deals with the effect of amino acids, vitamins, metabolic inhibitors and antibiotics on cell growth of *Saccharomyces cerevisiae* Y_{Bmax} and acetic acid production.

Biotin has a significant positive effect on cell growth & acetic acid production; Nicotinic acid, Vit B_{12}, Ca-pantothenate and Riboflavin have a minute positive effect on acetic acid production and on the activities of alcohol dehydrogenase and aldehyde dehydrogenase, while Thiamine-HCl, Folic acid, Inositol and p-Amino benzoic acid show negative effect at higher concentrations.

L-Glutamic acid and L-Tryptophan exert positive effect on acetic acid production and on the activities of alcohol dehydrogenase and aldehyde dehydrogenase, while other amino acids show negative effect at higher concentrations.

2,4 DNP, Sodium arsenate and Mercuric chloride exert a very high negative effect on cell growth, acetic acid production, and activities of alcohol dehydrogenase and aldehyde dehydrogenase, while other metabolic inhibitors show comparatively lower negative effect.

Among all tested antibiotics Chlotrimazole, Terbinafine and Amphotericin B exert a very high negative effect on cell growth, acetic acid production, and activities of alcohol dehydrogenase and aldehyde dehydrogenase, while other antibiotics show comparatively lower negative effect. Though Chlotrimazole is a fungal antibiotic, it does not exhibit any drastic declination of cell growth or acid production. At higher concentration it shows little negative effect on the same.

Chapter 12 describes the effect of surface active agents on acetic acid production by *Saccharomyces cerevisiae* Y_{Bmax}. Sodium lauryl sulphate exerts high inhibitory effect on cell growth, acetic acid production, and activities of alcohol dehydrogenase and aldehyde dehydrogenase, while Tween 20 shows comparatively lower negative effect. On the other hand, Tween 80 shows positive effect on cell growth but negative effect on acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase. The effect is also dependent on the time when the agent is added to the acetic acid production medium.

Chapter 13 explains the biochemical changes which take place in the fermentation medium and the cell growth.

At the end of fermentation, 94.0% glucose was utilized. The rate of uptake of glucose has increased from 24hours and reaches its maximum between 24hours and 96hours. After 96hours of fermentation, the rate of utilzation of glucose gradually decreases and after 108hours, the organism shows almost no uptake of glucose.

As the fermentation advanced the rate of uptake of nitrogen in cell increased till 96hours of incubation and then decreased. Whereas, ammonia nitrogen came in the broth gradually after 24 hours and reach the maximum at 96hours. Amino nitrogen started to appear in fermentation medium after 24hours and gradually increased with time. Decrease in rate of cellular nitrogen uptake was due to the autolysis of the organism after 96hours. Unutilized N content in the broth decreased. 90.36% of the total available N was utilized after 96 hours of fermentation.

In Chapter 14 immobilization of the resistant strain was done in Ca-alginate & Agar agar for better yield of acetic acid, reusability of the organism & for its protection from environmental stress. Immobilzation in Ca-alginate increased the acetic acid production almost by 28.98%. But in case of Agar agar, the acetic acid production decreases than the free cells.

From the study it can be concluded that *Saccharomyces cerevisiae* Y_{Bmax} which resists alcohol, temperature & acid can be a potent and efficient organism for acetic acid production from glucose.