MICROBIAL BIOTRANSFORMATION OF VOLATILE OILS

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Microbial Biotransformation

Chemical reaction performed by micro-organisms and catalyzed by the enzymes within the microbial cell.

It depends on nature of micro-organism and nature of substrate.

Industrial applications

- Wine and Beer industry
- Ayurvedic medicines eg. Asava and aristha
- Antibiotics e.g *Penicillium notatum*[4]
- Perfumery:

  Biotransformation by bacteria, fungi and yeast of compounds such as linalool, cineole and alpha pinene to their respective epoxides, alcohols and diols, aldehydes, ketones and acids etc.

Experimental:

Aim: to study the effect of fermentation by *S. cerevisiae* on different different volatile oil component.

Microbe used

Saccharomyces cerevisiae is commonly known as yeast

Widely used in Brewing and Bakery industry

ATCC no. confirmed by NCCS Pune- 204508

Substrates

Substrates used are:

Whole essential oils

- Clove oil
- orange peel oil (Extracted by Hydrodistillation)

Oil components

- Vanillin
- Menthol

Crude powder

- Ajowan powder (Crude powder was used)

Materials and methods:

Preparation of media: The 3 % Sabouraud dextrose broth was prepared and sterilized.

Inoculation of YEAST: Inoculation of *Saccharomyces cerevisiae* into the culture media

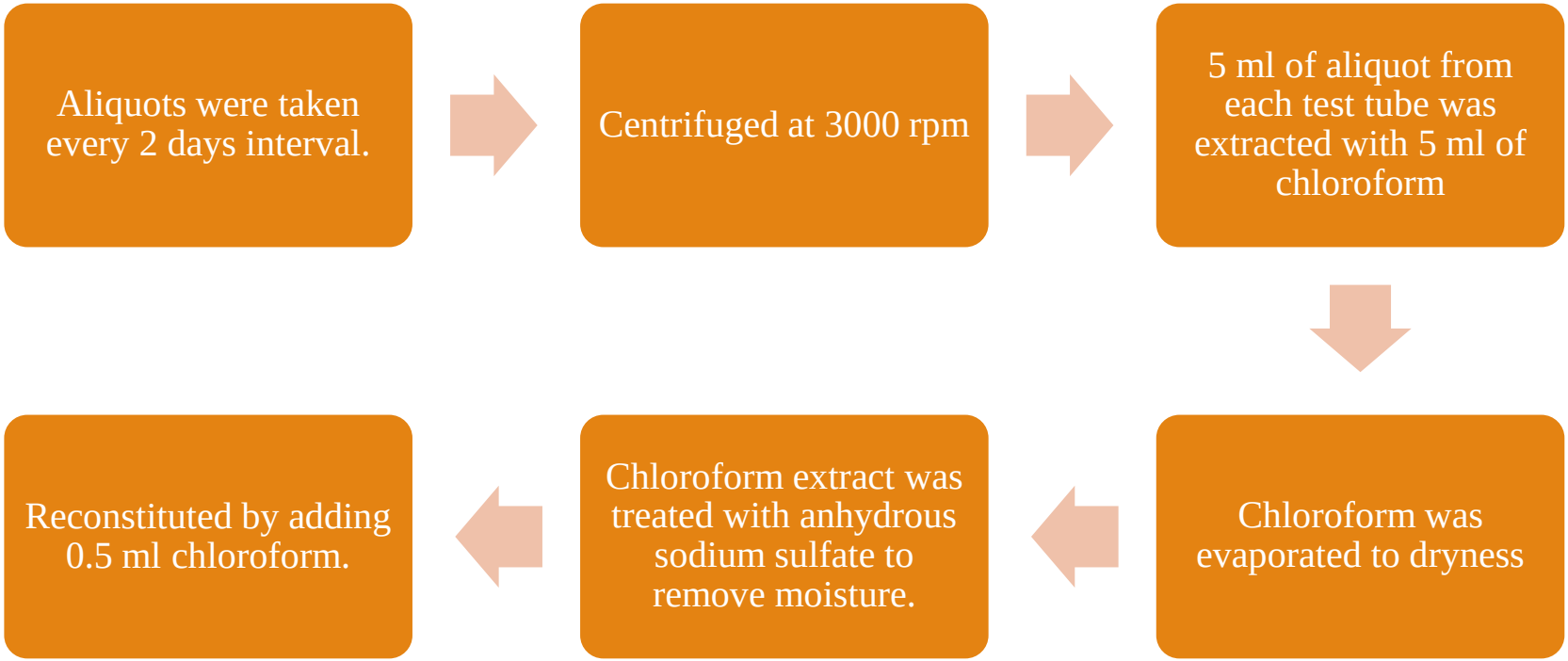
Addition of substrates:

- Solids: added 0.1 to 0.5 % W/V(Alcohol)
- Oily Liquids: added in 0.1 to 0.5% V/V(using tween 80 surfactant)

Incubation : at 37º C for the period of 10 days

Analysis : Using HPTLC method of analysis

Analysis :


Aliquots were taken every 2 days interval.
Centrifuged at 3000 rpm
5 ml of aliquot from each test tube was extracted with 5 ml of chloroform
Chloroform was evaporated to dryness
Chloroform extract was treated with anhydrous sodium sulfate to remove moisture.
Reconstituted by adding 0.5 ml chloroform.

Why Chloroform?

Immiscible in aqueous phase

Adequate solubility of all substrates

Changes in orange oil and Cove oil

Orange peel oil

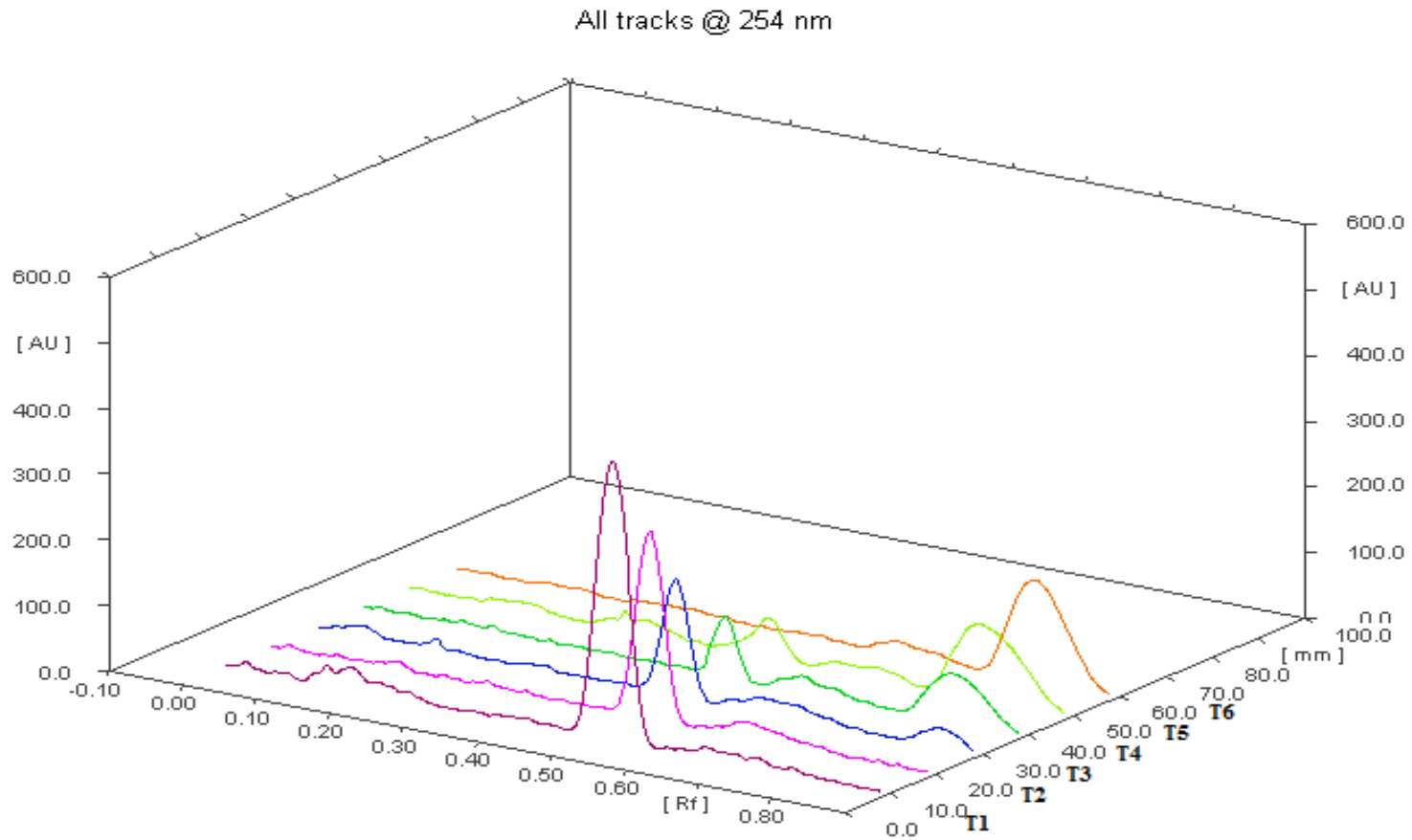
Clove oil

HPTLC

- TLC Plate - silica gel 60 F254 coated aluminim plates
- Sample applicator- CAMAG LINOMAT 5
- Scanner- CAMAG SCANNER 3
- Chromatograph Plate Development

Mobile phase- Hexane: ethyl acetate: glacial acetic acid
Proportion - (65:35:5)

3D Chromatogram of vanillin biotransformation



track	Incubation period
T1	0 days
T2	2 days
T3	4 days
T4	6 days
T5	8 days
T6	10 days

Peak Table

Track	peak	Start Rf	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	1	0.45	0.52	420.1	84.41	0.58	0.3	14928.7	90.08
2	1	0.44	0.51	283	88.4	0.56	0.4	8961.4	84.78
3	1	0.53	0.56	111.8	4.09	0.57	11.5	172.1	2.02
3	2	0.79	0.84	27.6	9.55	0.88	6.7	1111.8	13.07
4	1	0.54	0.59	24.6	12.24	0.62	13.9	846.3	10.78
4	2	0.72	0.8	71.4	35.49	0.88	0.9	4312.7	54.92
5	1	0.51	0.53	16	6.16	0.68	0	554.1	4.34
5	2	0.79	0.81	111.4	42.94	0.88	6.5	7934.4	62.13
6	1	0.54	0.6	19.3	11.32	0.62	13.7	661.3	6.7
6	2	0.76	0.78	151.1	88.68	0.88	1.2	9212.9	93.3

Result and Discussion

Track of incubation period upto2 days (Track 1 and 2) shows no change in TLC pattern

A second peak is observed after incubation period of 4 days

As concentration of substrate decreases the concentration of product increases

Conclusion

- The yeast Saccharomyces cerevisiae is capable of converting vanillin to more simple compound which can prove to be an important starting material for new flavours.
- This is an important finding for the point of view of flavouring properties of product of biotransformation.

Future aspects

Biotransformation can be used as tool to explore new and cost effective methods of bio-catalysis using selected metabolic pathways from cells.

References

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Thank you