Fermentation: An age old technology with brand new challenges

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our future through science

Double, double toil and trouble;
Fire burn, and cauldron bubble

Macbeth Act IV, Scene I

INTRODUCTION

Fermentation (from Wikipedia)

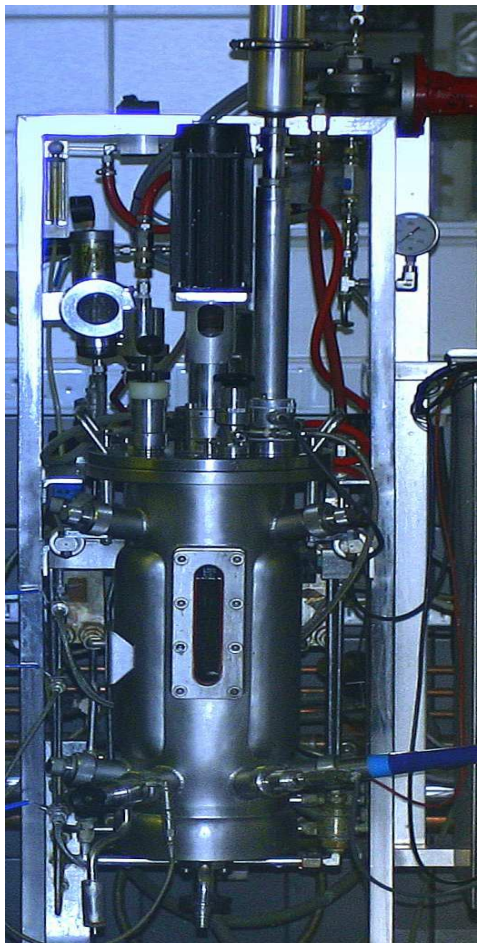
- Formerly called zymosis - the anaerobic metabolic breakdown of a nutrient molecule, such as glucose, without net oxidation
- An age old technology
  Babylon circa 5000 BC, ancient Egypt circa 3000 BC
- Also used more broadly to refer to the bulk growth of microorganisms on a growth medium
- Usually implies that the action of the microorganisms is desirable

From natural occurring phenomenon to powerful tool

- Earliest fermentation was a natural occurring process
- Still prevalent in Africa in production of foods such as Gari
- 1836 - Cagniard-Latour studied yeast in beer
- 1900 to 1930 - ethyl alcohol and butyl alcohol were the most important industrial fermentations
- 1928 - Alexander Fleming, penicillin
- 1960's - chemical synthesis of alcohols and other solvents became less expensive
- 1982 - first genetically engineered product - human insulin produced by Eli Lilly using *E. coli*
- Move towards beneficiation of waste materials

The challenges of fermentation

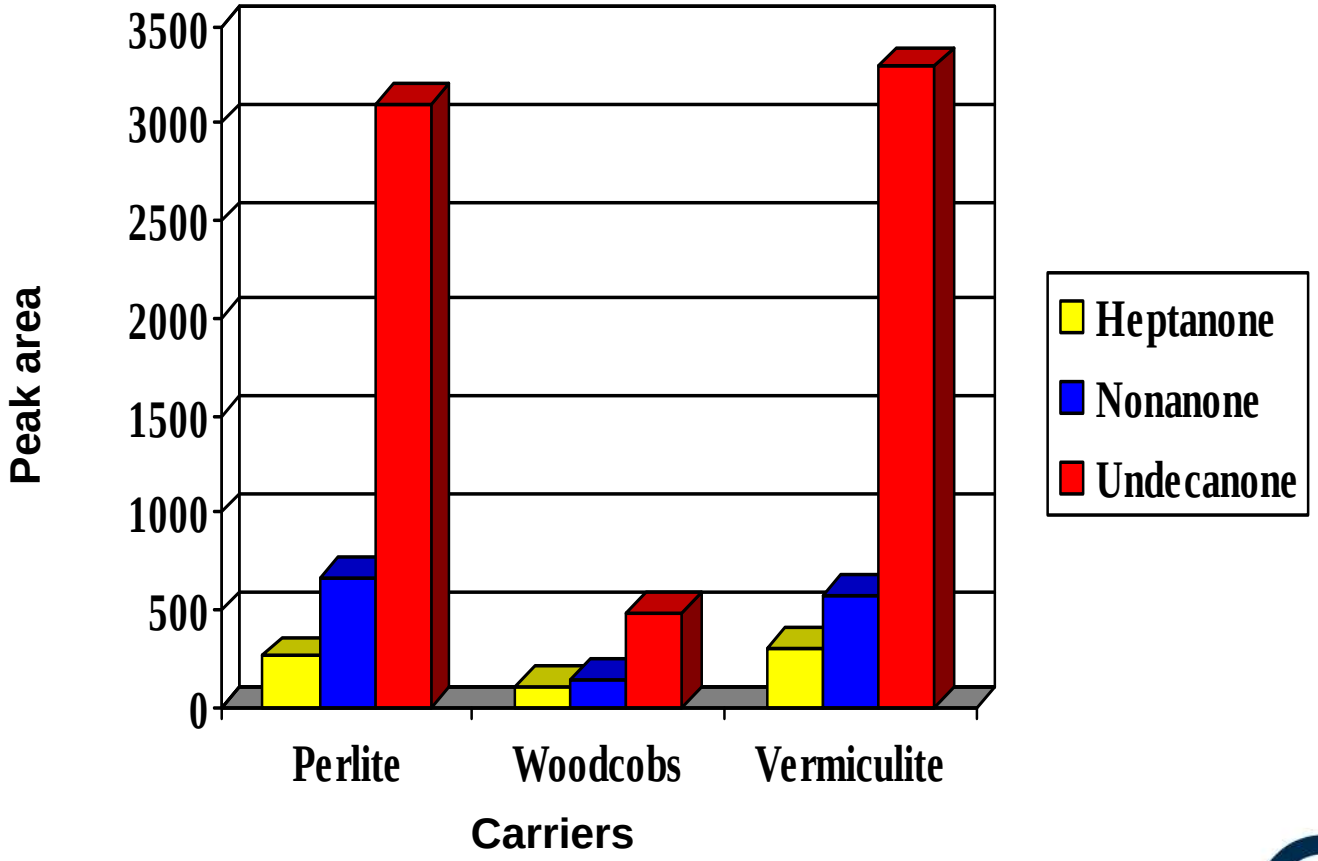
- New challenges
- Reactors designed for specific processes
- Metabolic regulation through physical parameters
- Product purification and formulation
- Modified organisms to produce desired products
- Can we meet the challenges?



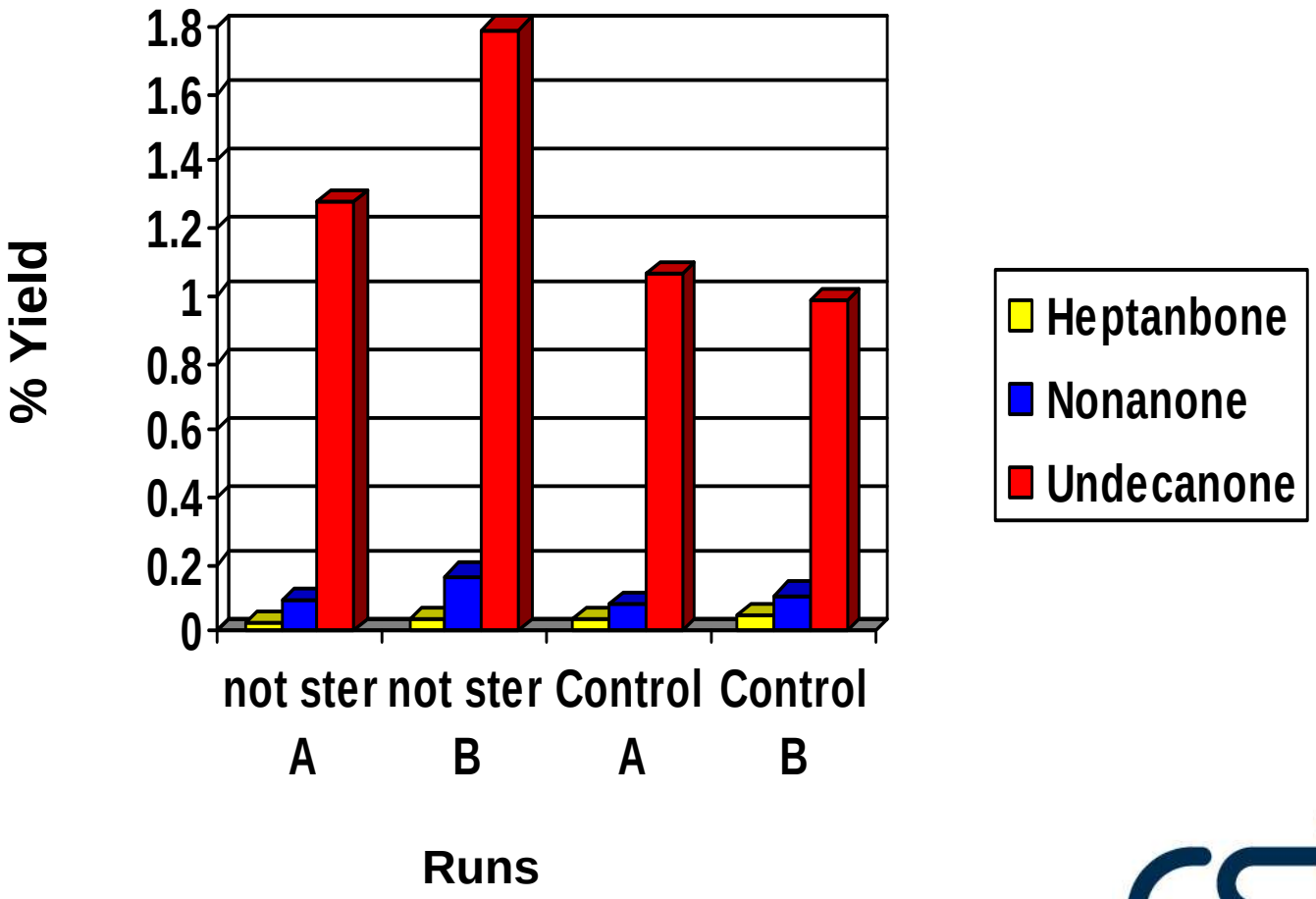
Reactor design
The right tool for the right job

- Challenge of developing a technology that requires low capital input
- Danger of falling into the trap of developing technology that is bucket science
- Methyl ketones give the blue cheese flavour
- Flavour is caused by a mixture of: heptanone, nonanone and undecanone
- Solid state production of methyl ketones

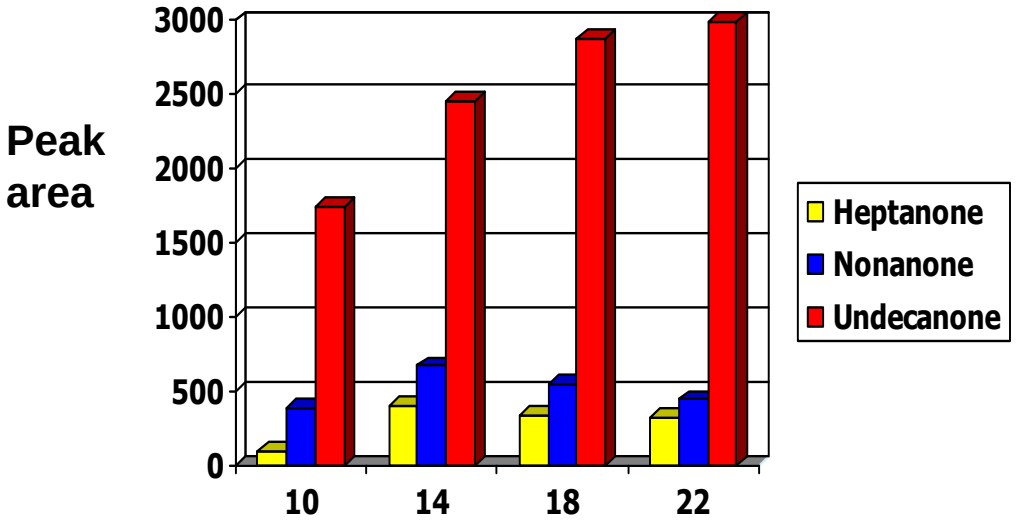
Selection of support



Sterility requirement

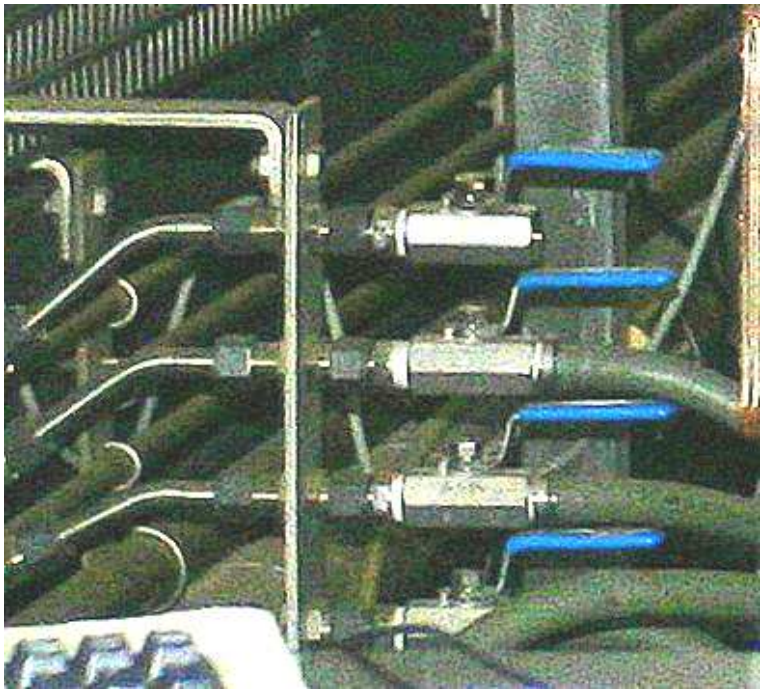


Solid state production of methyl ketones



Regulating metabolism to produce the product of choice

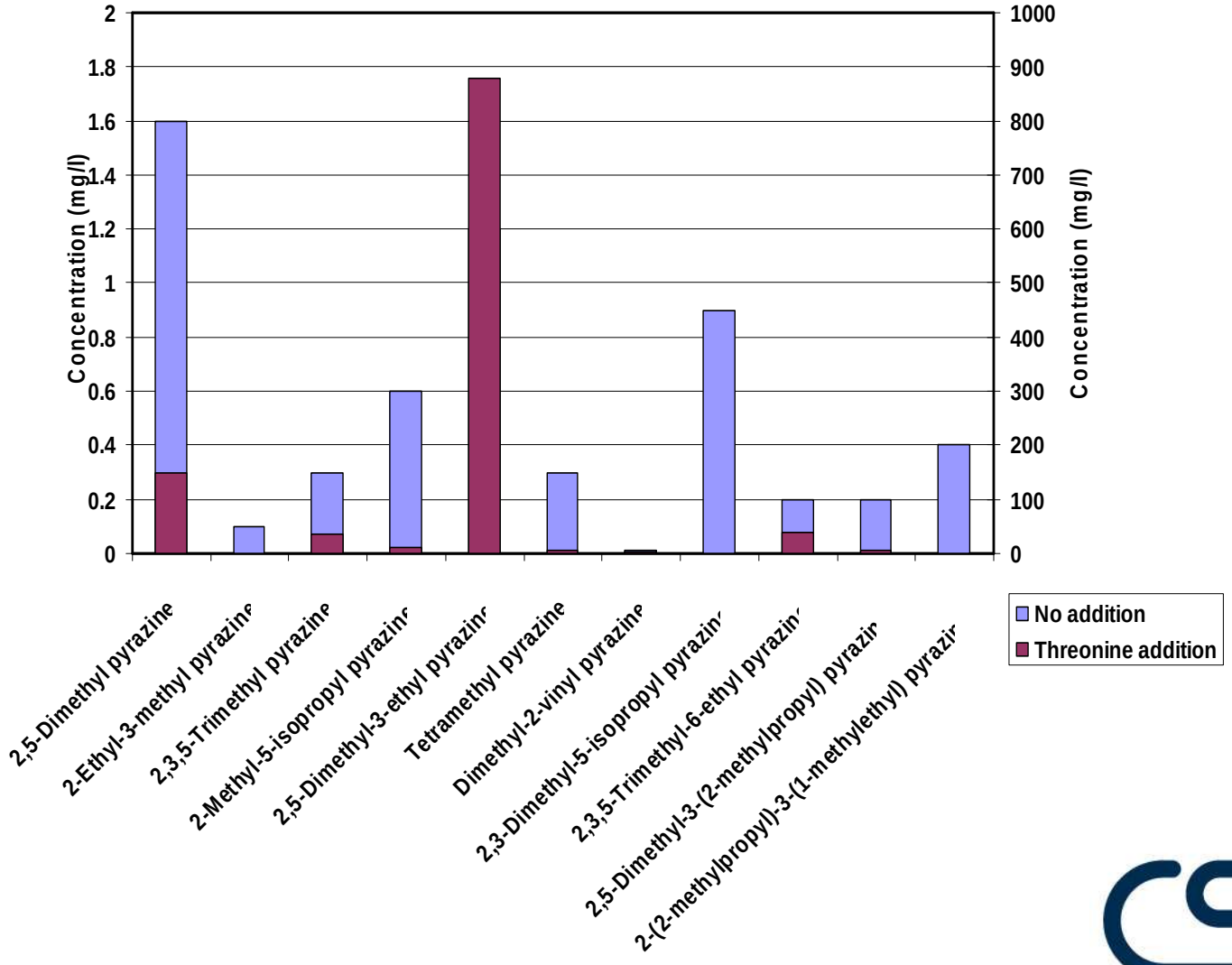
- How do you open the right tap?
- Metabolic engineering
- Wild type produces 19 different products that are closely related



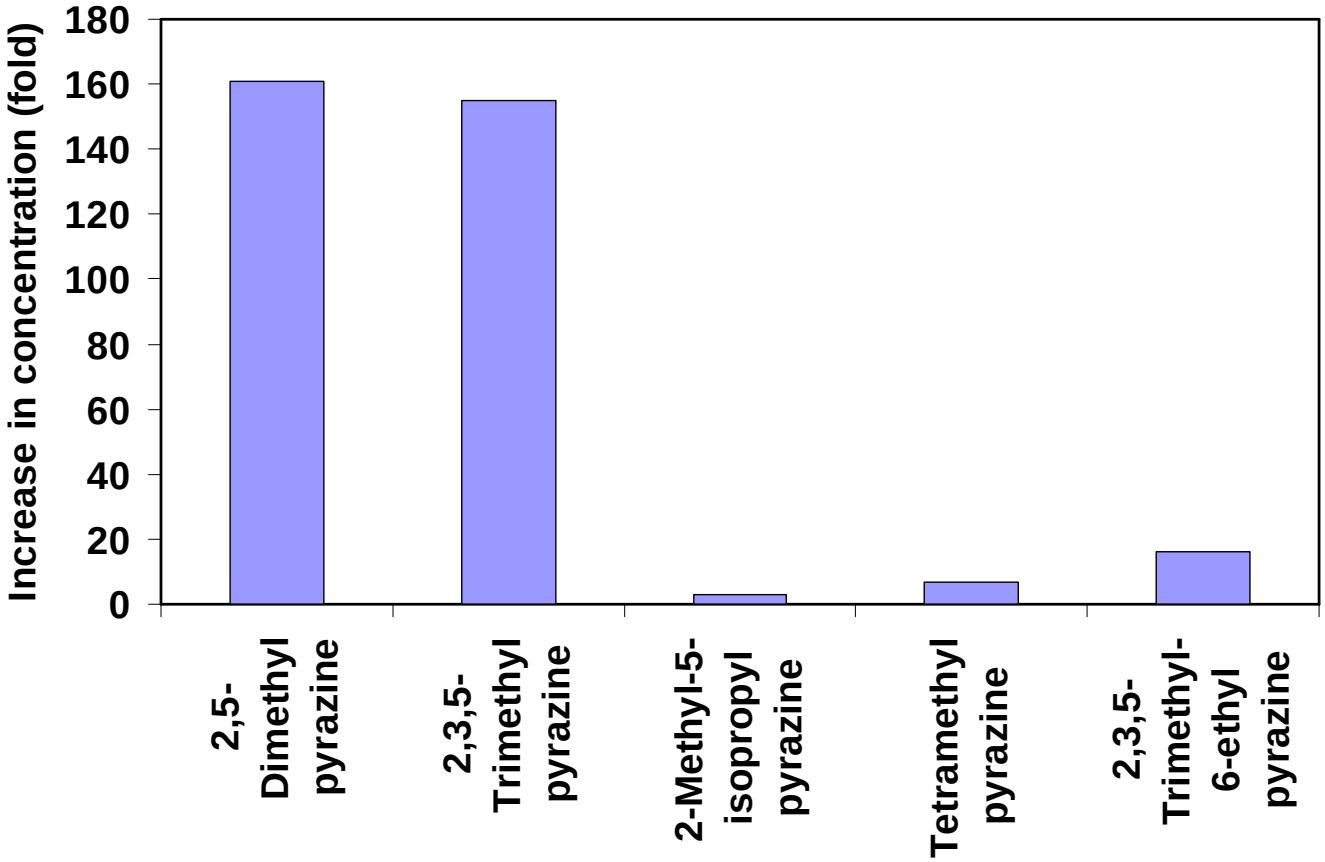
Pyrazines

Pyrazine name	Detection threshold (ppb)	Aroma and/or taste
2-methylpyrazine	60,000	Green, nutty, cocoa, musty, potato, fishy-ammoniacal notes
2-ethylpyrazine	6,000	Musty, nutty, buttery, peanut odour, chocolate-peanut taste
2,3-DMP	2,500	Green, nutty, potato, cocoa, coffee, caramel, meaty notes
2,5-DMP	800	Chocolate, roasted nuts, earthy, chocolate taste
2,6-DMP	200	Chocolate, roasted nuts, fried potato odour
2,3,5-trimethylpyrazine	400	Nutty, baked potato, roasted peanut, cocoa, burnt notes
2,3,5,6-tetramethylpyrazine (TTMP)	1,000	Weak, nutty, musty, chocolate odour, chocolate taste
2-ethyl-3-methylpyrazine	0.4	Potato, burnt nutty, roasted, cereal, earthy
2-ethyl-5-methylpyrazine	100	Nutty, roasted, somewhat grassy
2-ethyl-3,5-DMP	1	Cocoa, chocolate, nutty (burnt almond) notes

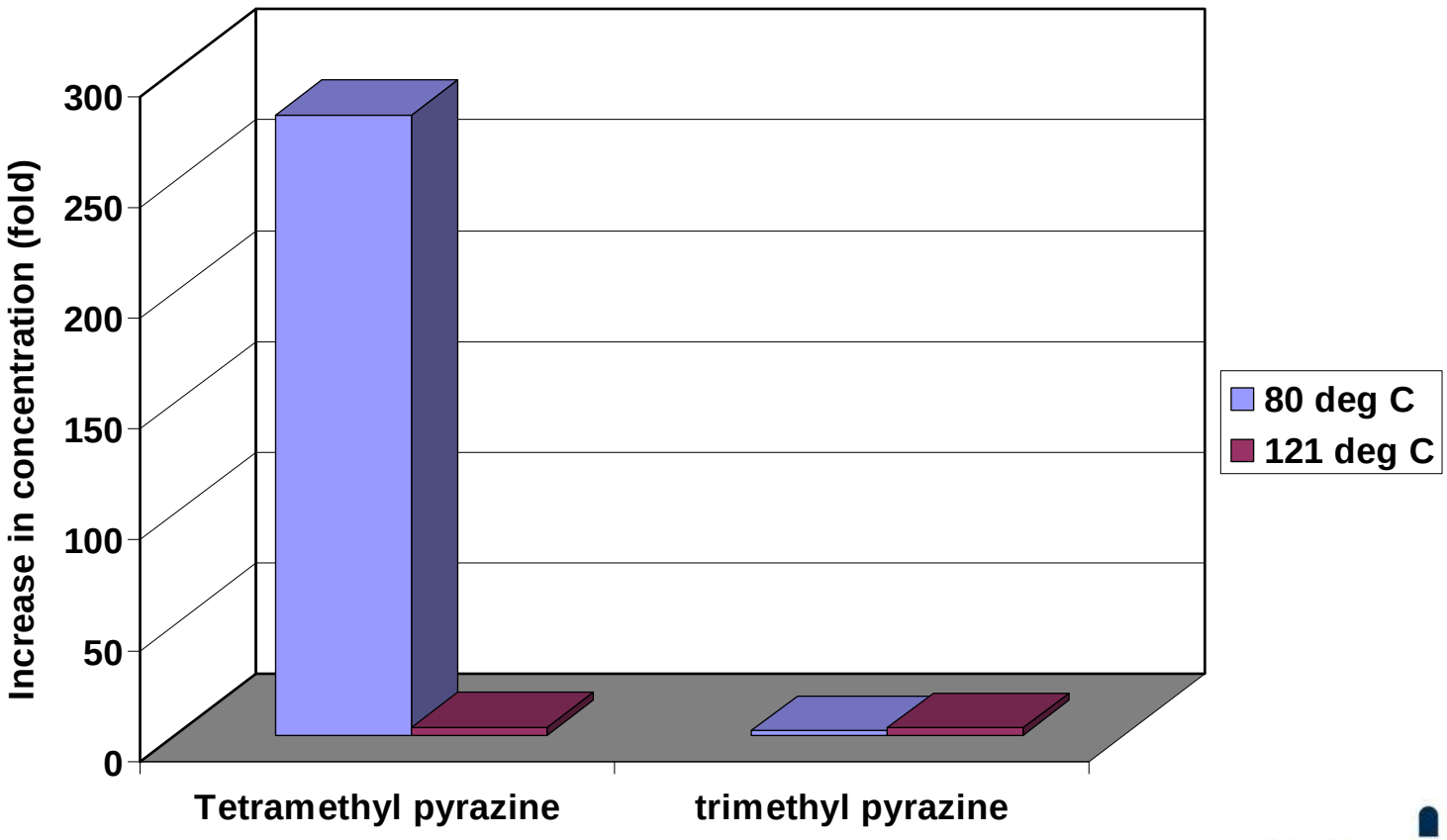
19 different pyrazines produced by B. polymyxa



Effect of threonine as precursor on the concentration of key pyrazines

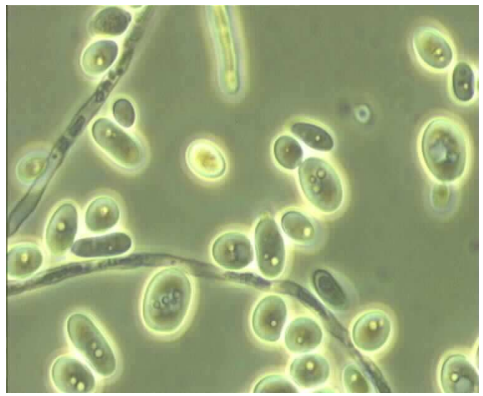


Effect of temperature on concentration of key pyrazines

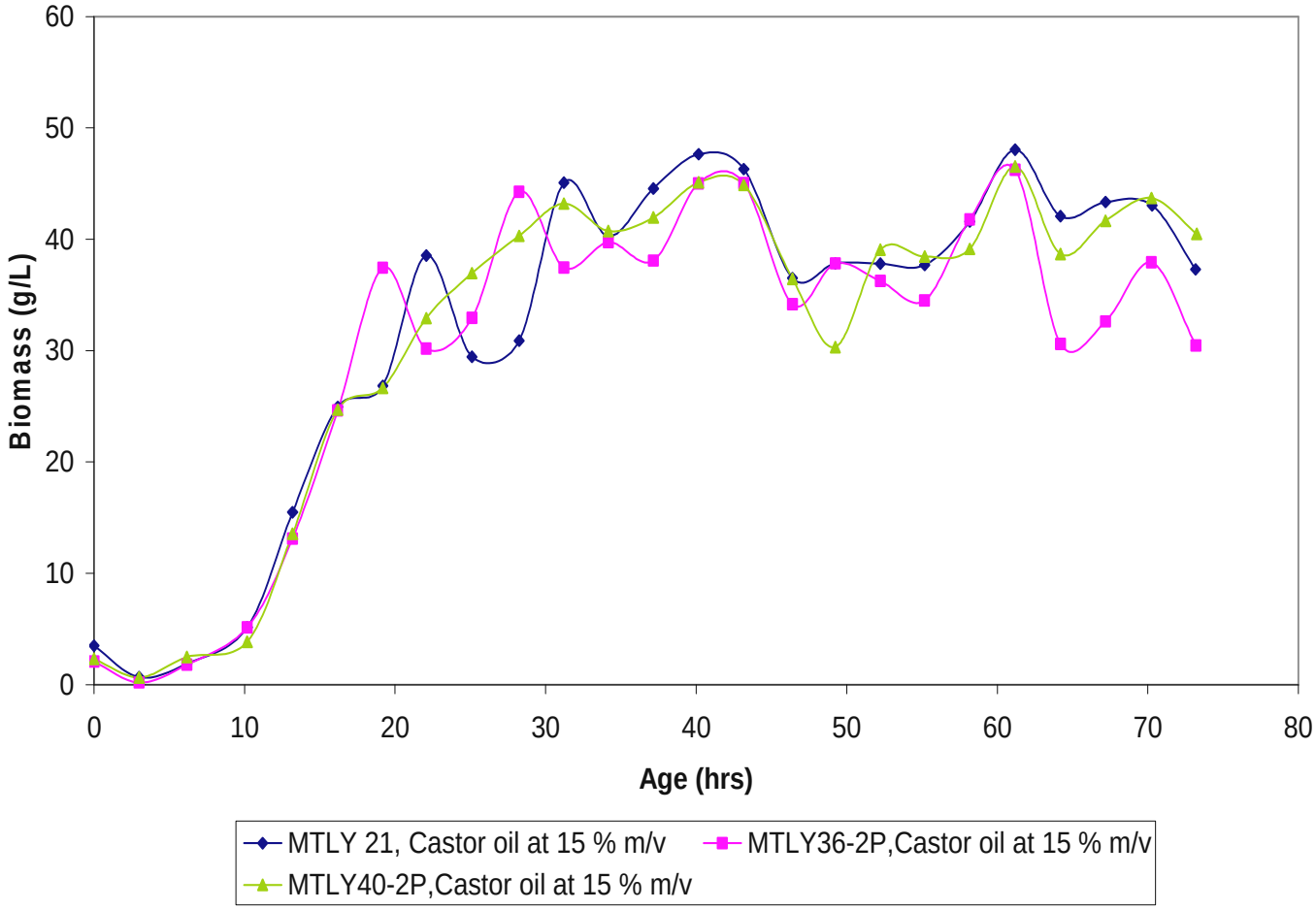


Modification of metabolic pathways

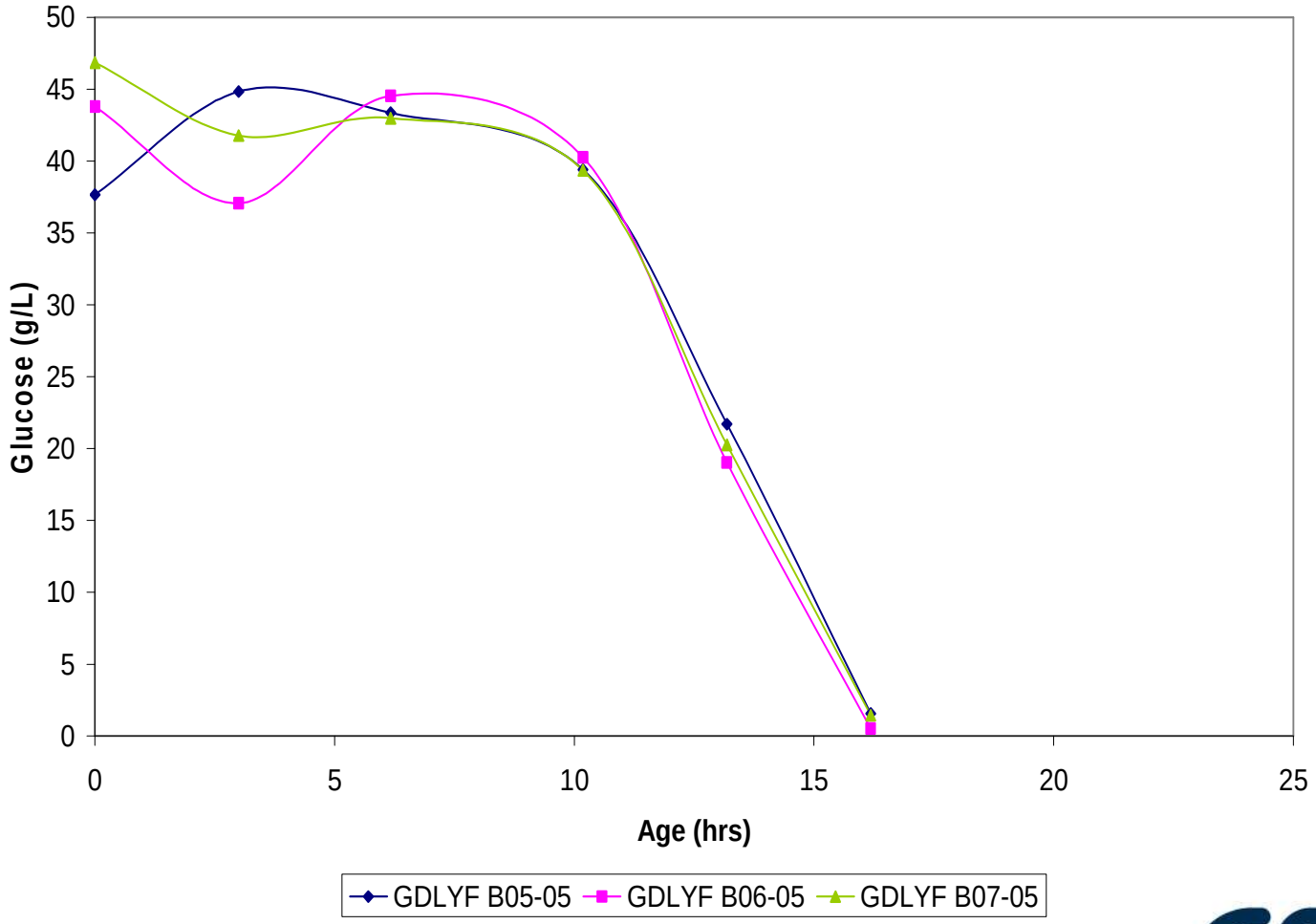
- Gamma decalactone production (INRA)
- *Yarrowia lipolytica* has five acyl-CoA-oxidase genes namely ACO1 to ACO5
- Each ACO has specificity towards a fatty acid chain length
- Selective insertion of combinations of ACO
- POX promoter
- Castor oil as substrate



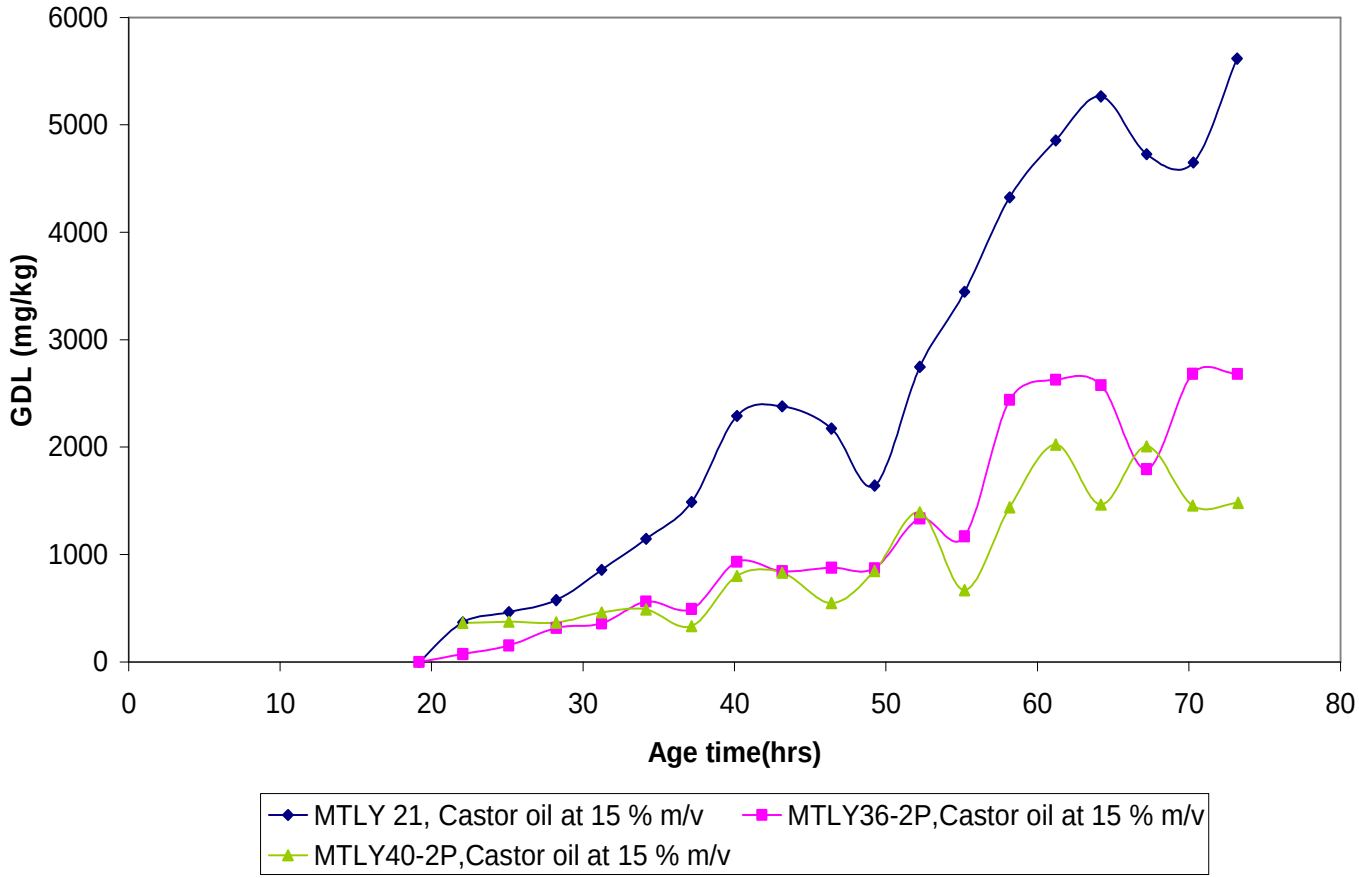
No observed difference in growth



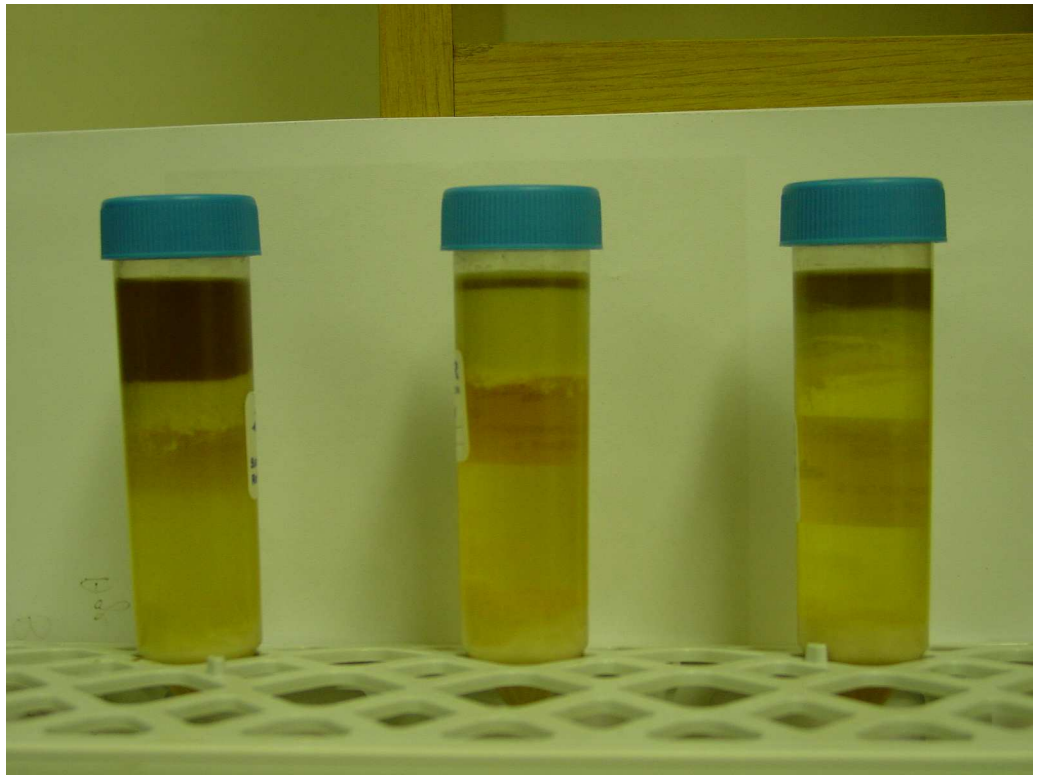
Glucose consumption



GDL production by different mutants

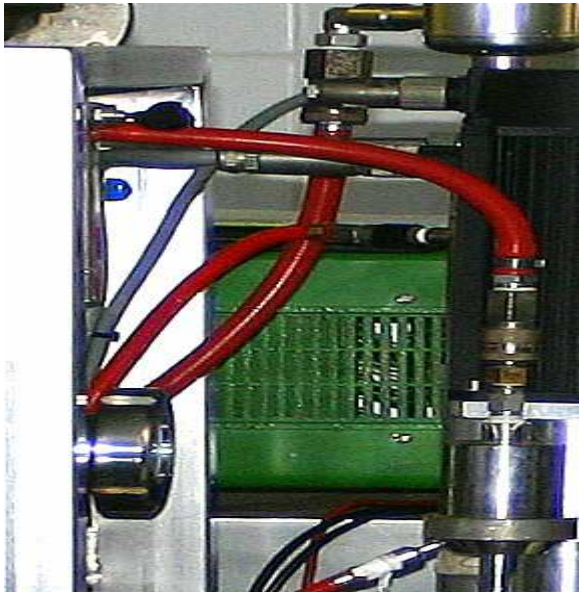


Difference in layering of centrifuged samples



Modified organisms to produce desired products

- Gene inserted in host organism = Product ✗
- Gene expressed in host organism = Product ✗
- Above + fermentation technology = Product ✔



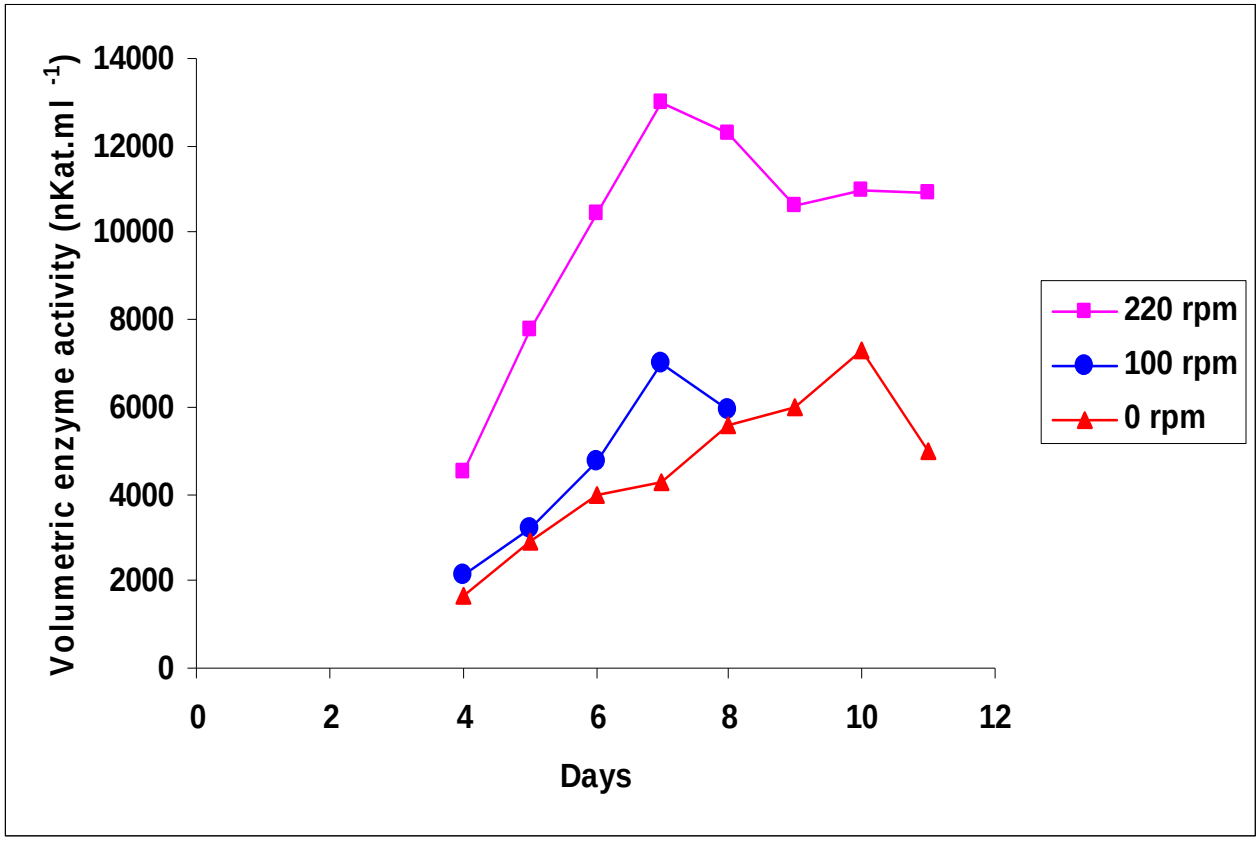
Aspergillus niger as an expression host

- Project in partnership with Prof van Zyl from University of Stellenbosch
- *A. niger* is known to produce high amounts of homologous proteins
- GRAS status
- Grows on a range of cheap nutrients
- Problematic when it comes to production of heterologous proteins
- Low secretion

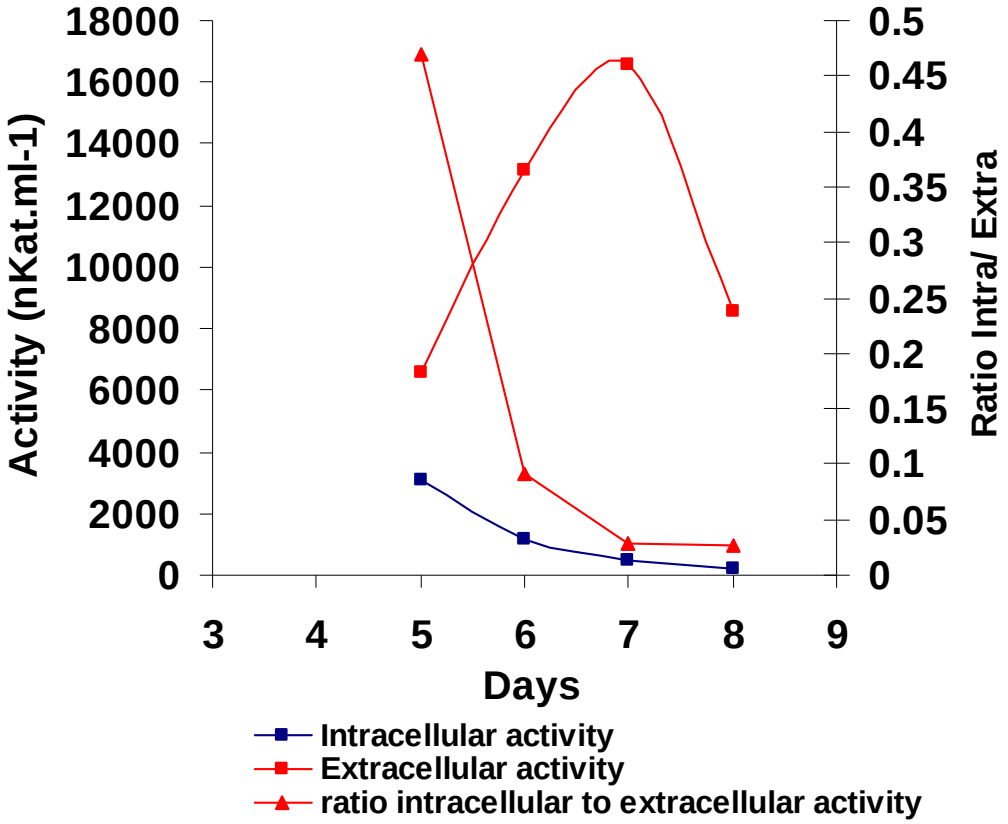
Factors influencing secretion

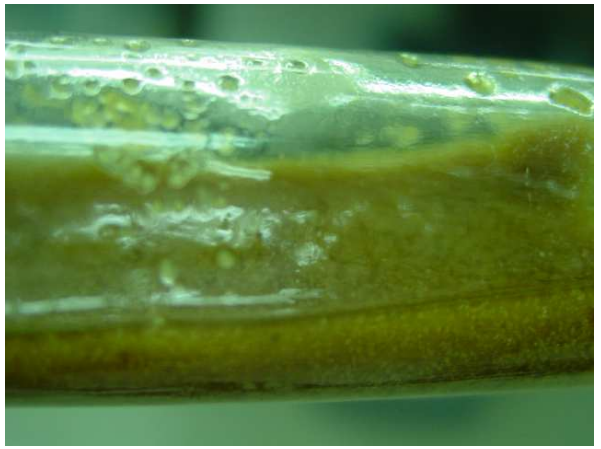
- Secretion takes place through the hyphal tips
- The more tips the better secretion
- Factors that influence the branching of the mycelia, influence secretion
- Fungi grow as pellets in submerged cultures
- Research on the optimum pellet size for maximum production
- Oxygen and nutrient transfer

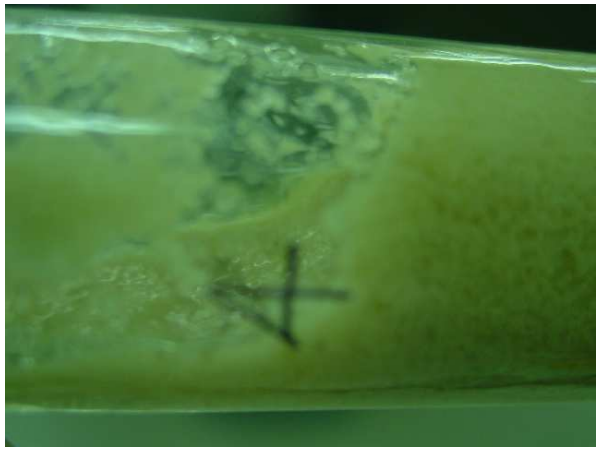
Effect of agitation on mannanase production



Effect of nutrients on growth and mannanase production







Summary

- A brief look into the challenges that one is confronted with
- Reactor design – solid state production of methyl ketones
- Regulation of metabolic pathways through substrates – pyrazine production
- Regulation of metabolic pathways through genetic engineering – GDL production
- Product purification - pyrazine production
- Expression hosts – mannanase production

The future of fermentation is only limited by our imagination

- As Biotechnology develops new applications, new challenges will arise
- Bio-nanotechnology
- Can we meet the challenges?