Fermentation: An age old technology with brand new challenges

Dr P J van Zyl
CSIR Biosciences
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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

![Graph showing peak area for Perlite, Woodcobs, and Vermiculite carriers with Heptanone, Nonanone, and Undecanone peaks.](image)
Sterility requirement

![Bar chart showing yield comparison between different runs and sterility conditions. The chart includes bars for 'not ster A', 'not ster B', 'Control A', and 'Control B' with different compounds indicated by colors. The compounds include Heptanone, Nonanone, and Undecanone.](image)
Solid state production of methyl ketones

![Image of fermentation vials]

![Image of fermentation trays]

**Days of fermentation**

- Peak area
- Heptanone
- Nonanone
- Undecanone

![Bar graph showing peak area over days of fermentation]

- Days of fermentation:
  - 10
  - 14
  - 18
  - 22

- Peak area:
  - Heptanone
  - Nonanone
  - Undecanone

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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

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

- Tetramethyl pyrazine (80 deg C)
- Trimethyl pyrazine (121 deg C)
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

![Graph showing glucose consumption over age (hours) for different groups.](image-url)
GDL production by different mutants

![Graph showing GDL production by different mutants.](image-url)
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

![Graph showing the effect of agitation on mannanase production. The x-axis represents days, ranging from 0 to 12. The y-axis represents volumetric enzyme activity (nKat.mL⁻¹), ranging from 0 to 15000. Three curves are shown: 220 rpm (pink square), 100 rpm (blue dot), and 0 rpm (red triangle). The graph shows an increase in enzyme activity over time for all agitation rates, with 220 rpm reaching the highest activity.]
Effect of nutrients on growth and mannanase production

![Graph showing the effect of nutrients on growth and mannanase production. The x-axis represents days, ranging from 3 to 9. The y-axis represents activity (nKat.ml-1), ranging from 0 to 18000. There are three lines: Intracellular activity, Extracellular activity, and the ratio intracellular to extracellular activity. The ratio line peaks around day 6, indicating the highest ratio during this period.](image-url)
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?