# Industrialization of a biotech process - a multidisciplinary approach

## $CO_2$ capture and storage(CCS), using microbial, thermostable enzymes as a model system

**Understanding Microbial Communities**
**Developing the Potential**

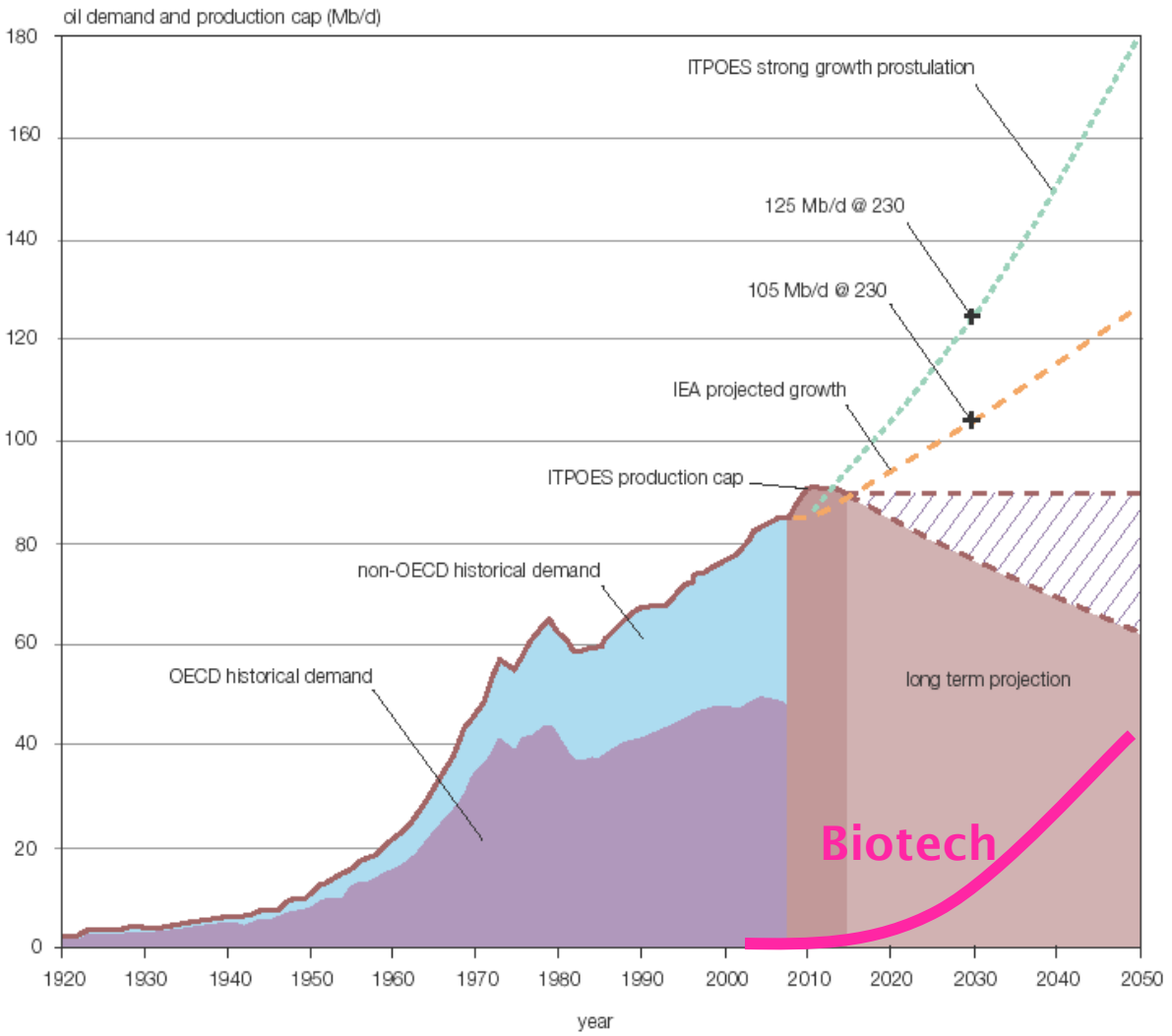
Speaker: Hans Kristian Kotlar
Ex: Senior specialist biotechnology

# Outline of the talk

***Industrialization of a biotech process – a multidisciplinary approach!***

- Aspects -/influence of peak oil and onset of biotech
- OECD's 3rd wave of biotech and conversion to bio-economy
- The sciences are available now - Not "future"
- Assembled or integrated in a "new" way ?➔ future use
- CCS as an adequate industry model
- Economy driven or solution driven ?
- Outside the box thinking: At the interface of nature and engineering.
- "You must have it all" - you have to be multidisciplinary

# “Peak oil”, energy security – and onset of biotech




Source: UK Industry Taskforce on Peak Oil & Energy Security (ITPOES)

**No time to loose.**
**START NOW !!**

# 3rd wave of biotechnology

Table 3. **Current R&D expenditures versus future markets for biotechnology**

| Application | Share of total OECD business expenditures on biotech R&D in 2003 | Estimated potential share of total biotechnology gross value added (GVA)[1] in the OECD area[2] for 2030 |
|---|---|---|
| Health | 87% | 25% |
| Agriculture | 4% | 36% |
| Industry | 2% | 39% |
| Other | 7% | - |
| | 100% | 100% |

Source: OECD Bioeconomy 2030

**Commitments**
**Long term**
**Predictable**

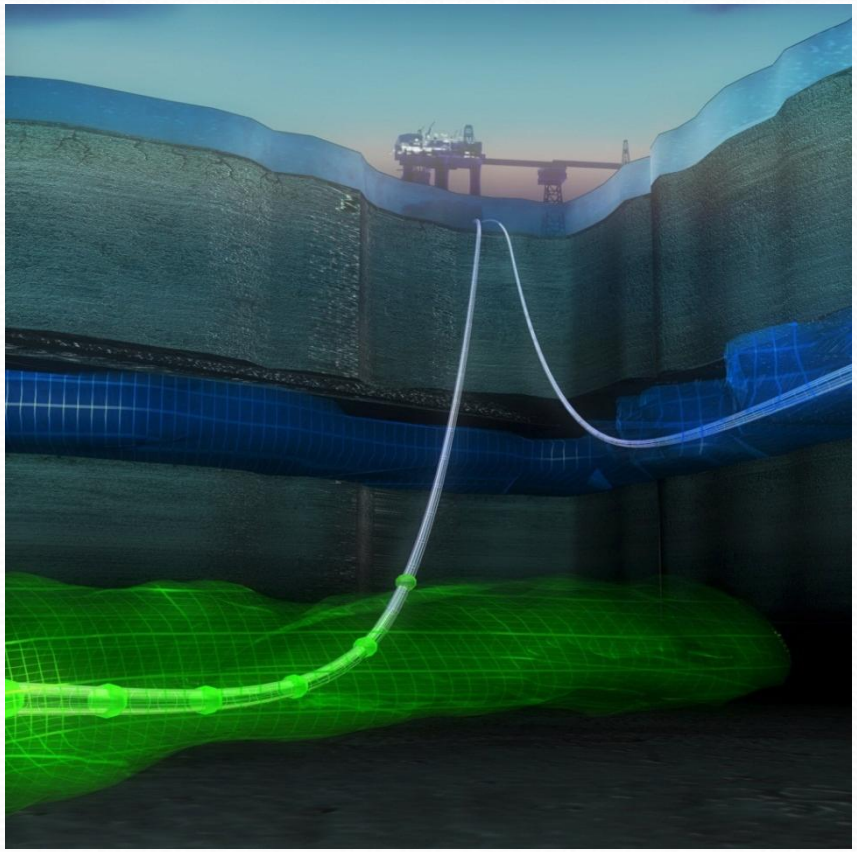
**Incitement for new:**
**Industrialization**
**Commersialization**
**- IPR**

**Projects ➔ more focused: Why?**
**What shall be achieved ?**

# Communities of subsurface microorganisms («Superman»-bugs)

## Where do they come from ?

## Indigenous microorganisms originating from the extra deep subsurface



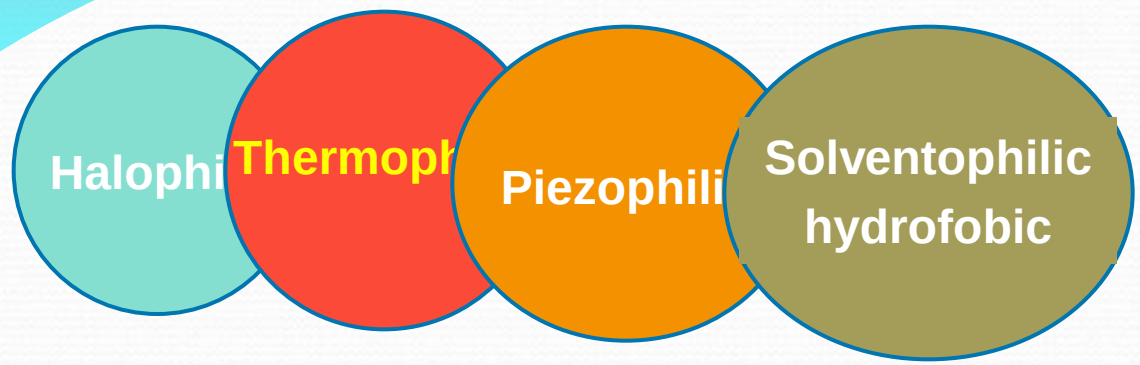
2000 – 3500 m subsurface there is a new, unknown microflora mankind has just recently, started to explore.

Reservoir temperatures: 50 - 120˚C - (170)˚C

Who are they ?
What are they doing ?
Metagenome-project
Mapping the entire biodiversity of the oil reservoir

**Internal strain collection(BTC) of more than 5000 isolates**

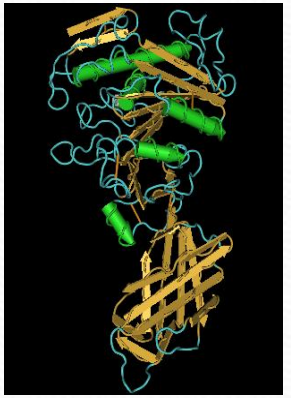
# "Extreme to the 4th power!"




## Properties of cellular components from extremophilic microbes.

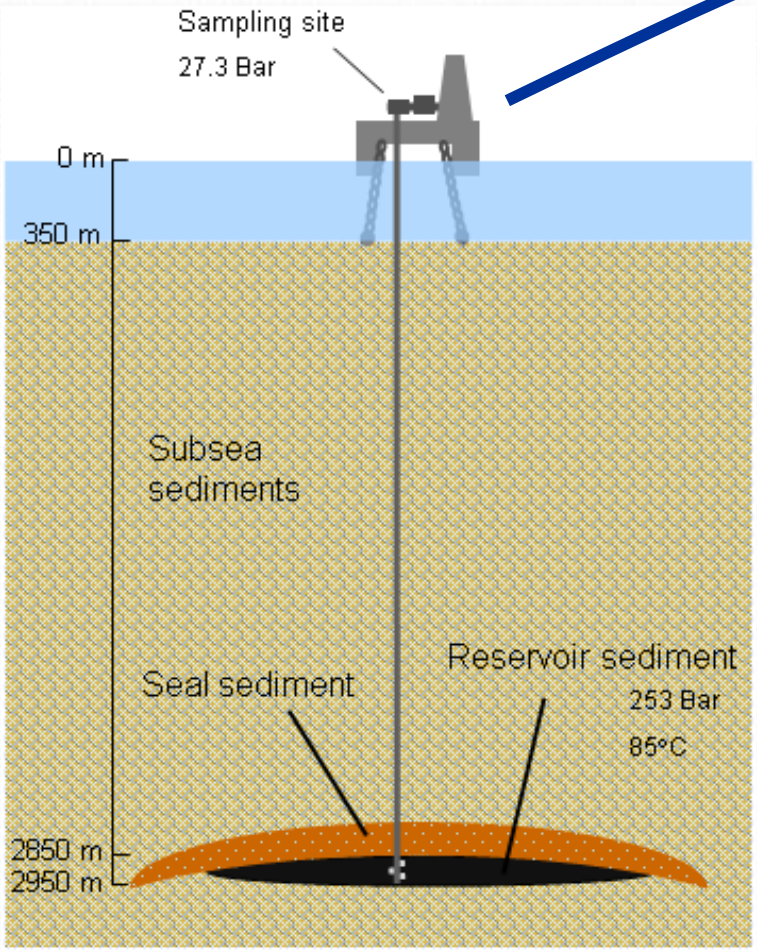
**Enzymes, proteins and nucleic acids**

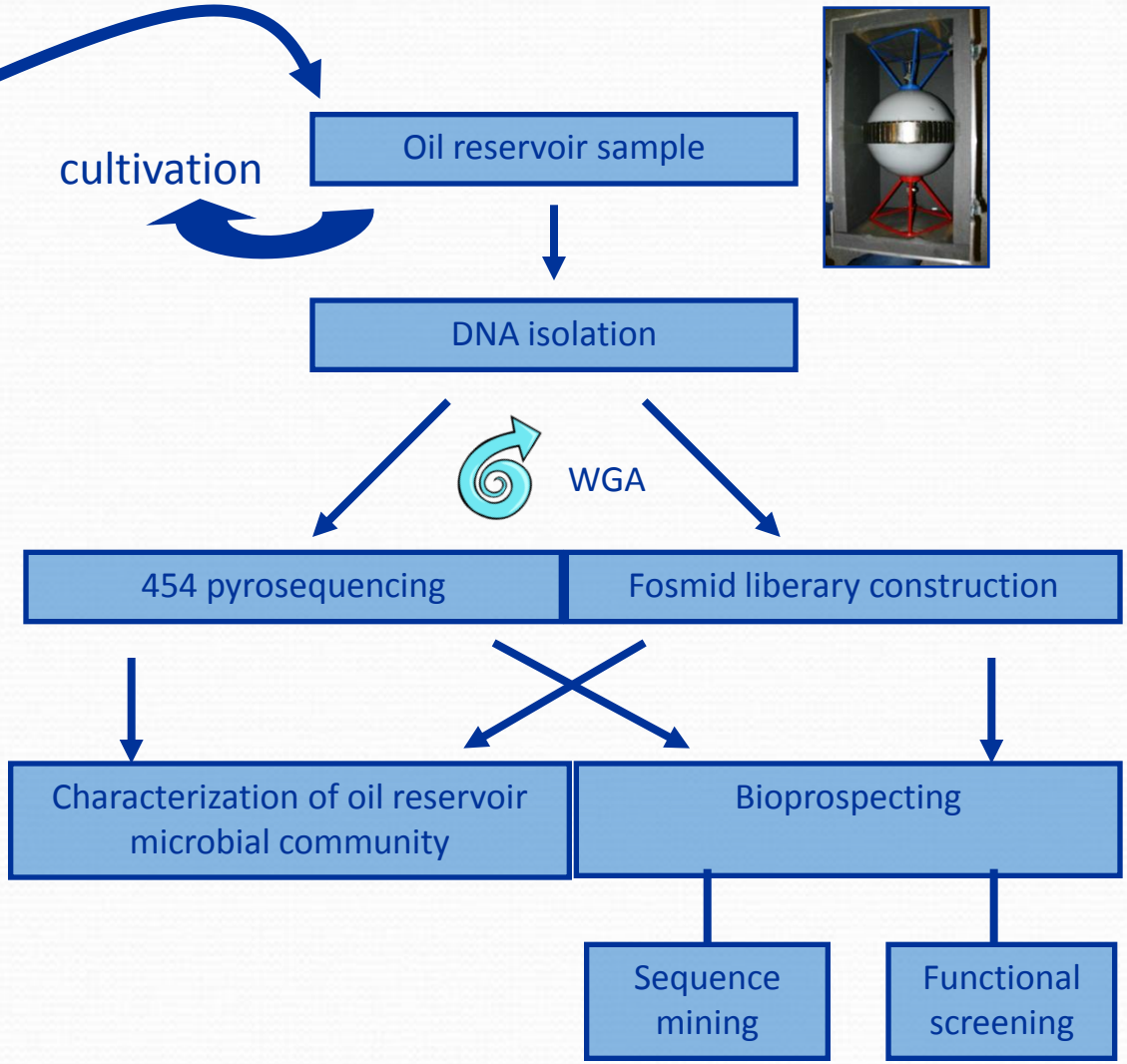
- Thermostability
- Stability against denaturing substances like detergents, organic solvents
- Stability against extreme pH conditions
- Suitable for fermentative processes, etc.
  - solubility of many substrates significantly improved
  - contamination/undesired complications reduced at higher temp



– **Search for the specific enzyme in the appropriate extremophile organism**

# Oil reservoir sampling/ Metagenome strategy



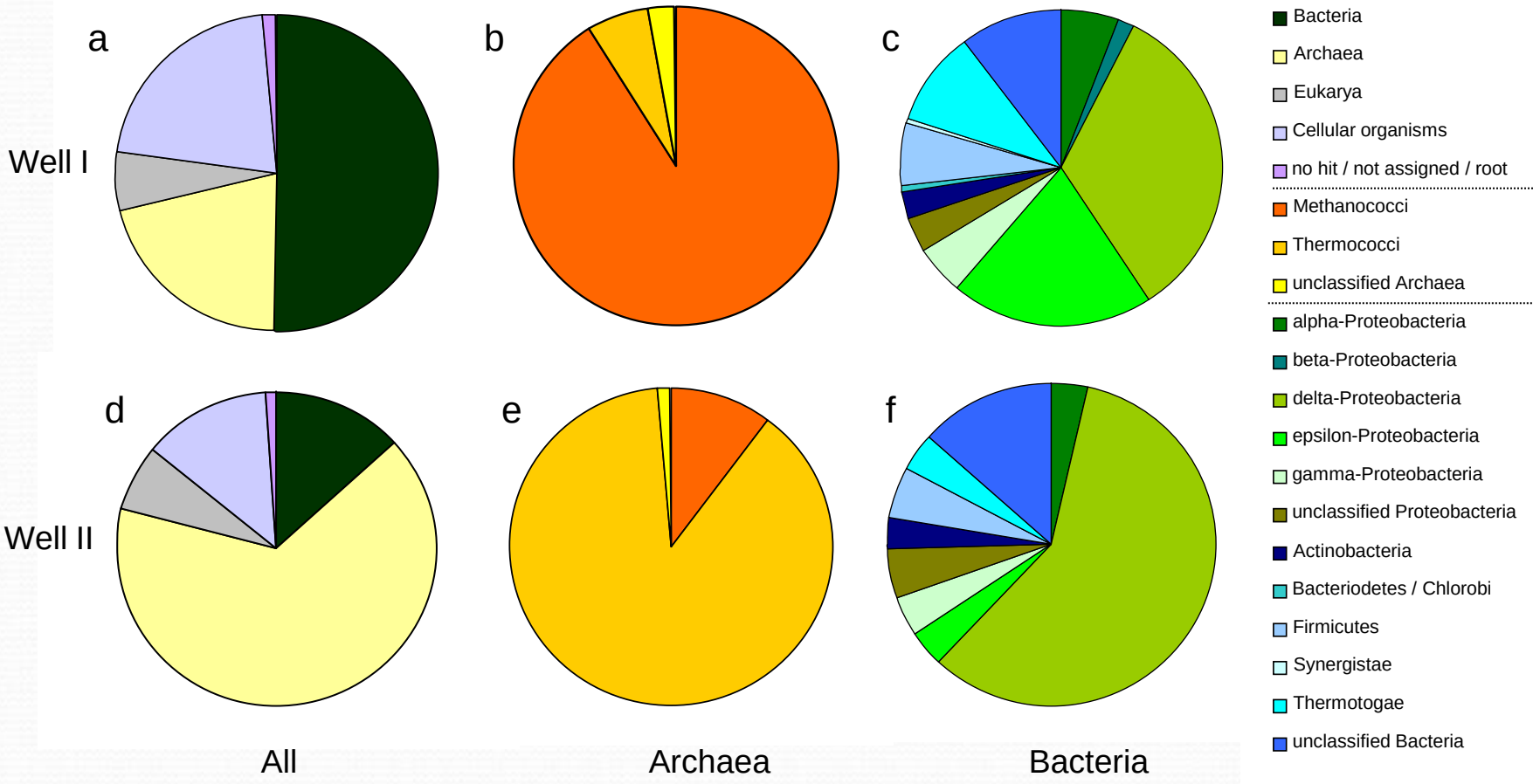





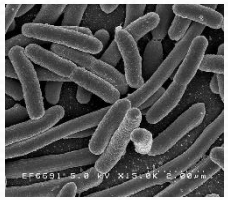

Kotlar, H.K. *et al.*: Environ. Microbiol. Reports 3(6) 674-681, 2011

# Domain-, Family- and OTU distribution in oil reservoir water phase samples of well I and II.

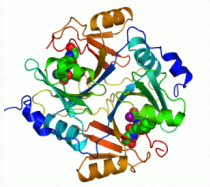
**Bioinformatics/classification:**



# POSSIBLE SPIN-OFFS FROM BIOPROSPECTING FOR ENZYMES AND MICROORGANISMS IN OIL-WELLS

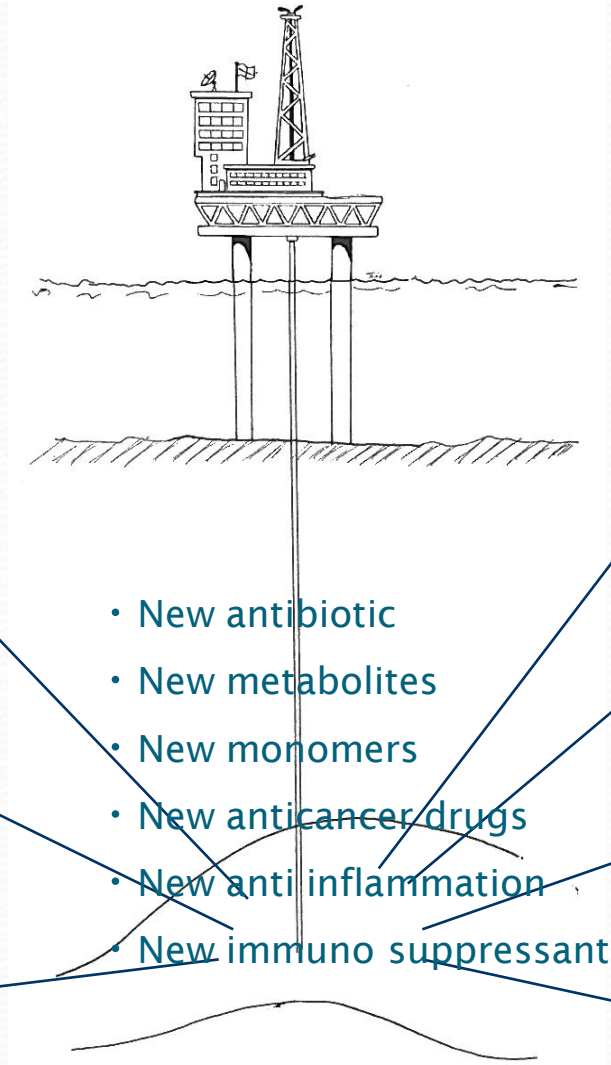


Extremophiles



Extremozymes

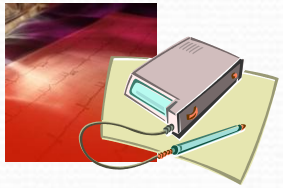
New drugs



- New antibiotic
- New metabolites
- New monomers
- New anticancer drugs
- New anti inflammation
- New immuno suppressants

Anticancer drug ?

Anti malarial drug ?



Diagnostic applications

Drug delivery systems

# Bioprospecting of oil reservoirs for industrial enzymes

- **Homology based sequence data mining**
  - Fast and cheap
  - Independent of gene expression, production host and screening system
  - Limited to at least some sequence homology (restricting novelty)

- **Functional library screening**
  - Independent of known sequences (new discoveries possible)
  - Dependent on functional expression systems and screening procedures
  - More time consuming and more costly, specialized equipment

- **Complementarity of approaches craves for a combined approach for maximum output**

# Mining for carbonic anhydrases – potential industrial use in CCS

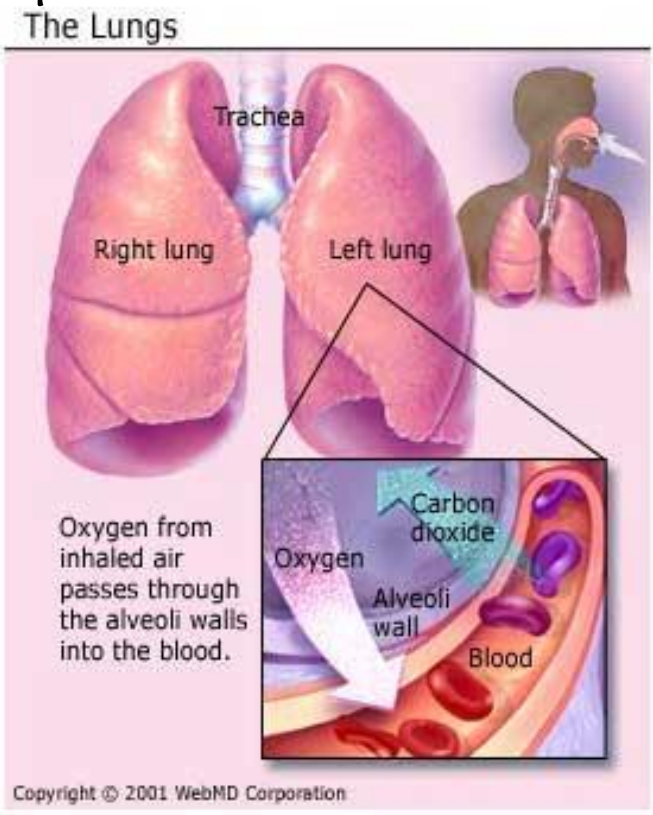
- **alpha-CA family:** mammalian enzymes (14 isoforms)
- **beta-CA family:** prokaryotic and plant chloroplast enzymes (clades A – D)
- **gamma-CA family:** enzymes from methane producing bacteria
- **delta-CA family:** enzymes from diatoms
- **epsilon-CA family:** enzymes from some lithotrophic bacteria and marine cyanobacteria

**CA, is one of the most active enzymes found in nature. It catalysis the reaction:**

$$CO_2(g) \rightarrow CO_2\ (aqua)$$

$$CO_2\ (aqua) + H_2O \rightarrow HCO_3^- + H^+$$

**and is thus vital to the process of life.**



# Bioprospecting for enzymes: Novelty of sequences: IP issue

**Identification and cloning of a thermophilic CA (tCA)**

1: The a.a sequence for the Bovine CA (BCA: NP_848667.1) was used as a search sequence to identify α-CA enzymes in the genomic DNA sequences of the thermophilic archaea and bacteria.

2: A sequence was found that possessed >30% identity to BCA .

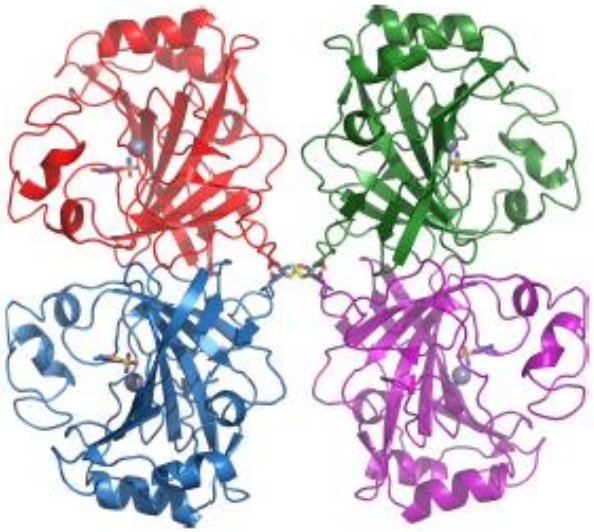
3: The enzyme has been synthesised with the codon usage optimised for *Escherichia coli* expression and sub-cloned into the pLATE51 expression vector (Thermofisher).

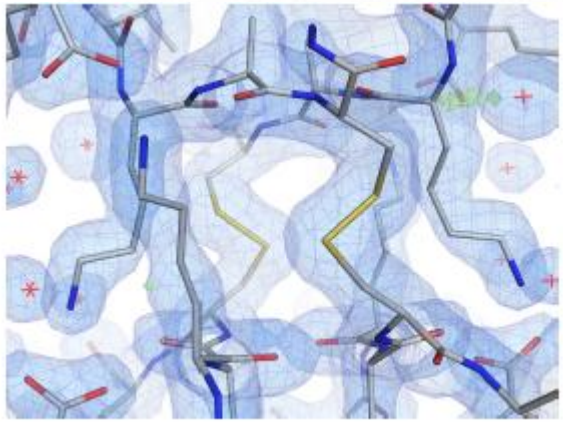
4: The enzyme has been expressed, purified, and biochemically and structurally characterised.

Patents:

US7521217 ($CO_2$ solution): seqID 1 (variants of human enzyme, alpha-CA family)
US2010/0209997 (Codexis): seqID 2 P40881 (*Methanosarcina thermophila*)
WO2010/151787 (Novozymes): seqID 2/13 A6DCH2 (*Caminibacter mediatlanticus* TB-2)
US2010/0297723,WO2008/095057 (Novozymes):

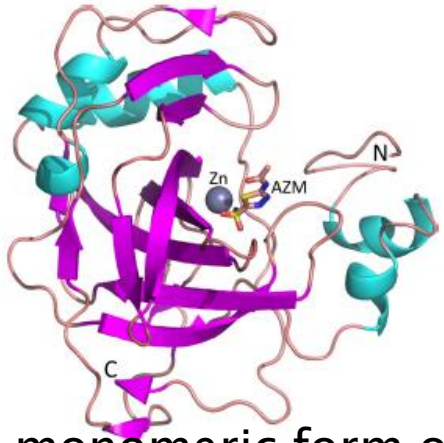
# Crystallographic structure:
## *α-carbonic anyhdrase from Thermovibrio ammonificans*





***Thermostability***
Retains 90 % activity after incubation at 70 ˚C for one hour

The oligomeric state of the enzyme is a tetramer held together by two pairs of disulphides at central core. This is unique for this thermostable α-carbonic anydrase.



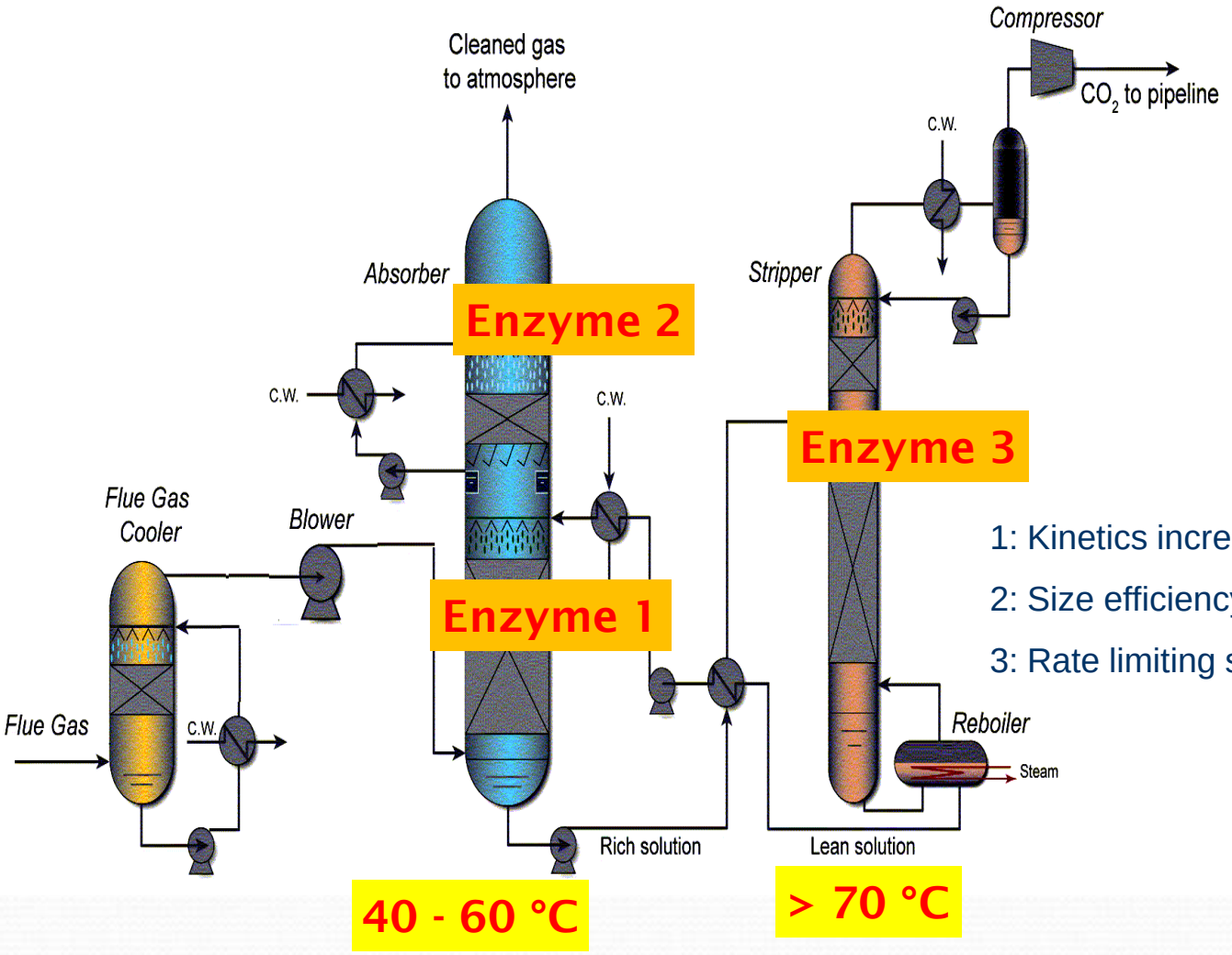

***Thermostability***
Retains 30 % activity after incubation at 70 ˚C for one hour

The monomeric form of the enzyme.

James, P. *et al*: Acta Cryst. (2014). D**70**, 2607–2618

# Chemical engineering: Schematics of a typical CCS process unit

$$H_2O + CO_2 \xrightarrow{CA} HCO_3^- + H^+ \quad pH > 7 \qquad HCO_3^- + H^+ \xrightarrow{CA} H_2O + CO_2 \quad pH < 7$$



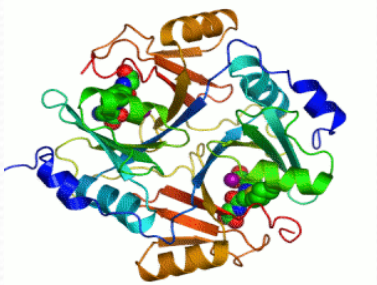

1: Kinetics increased by 20 x

2: Size efficiency of $CO_2$ adsorber ≈ $1/r^2$

3: Rate limiting steps: $C0_2 + H_2O$ ➔ $HCO_3^- + H^+$

# Potential bio-mining for other interesting industrial enzymes

Extremophile enzymes: Extremozymes

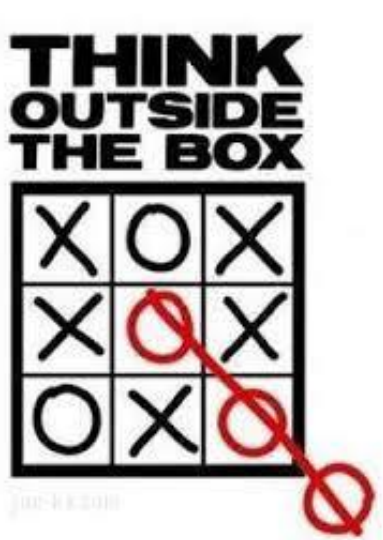


Lipases
Oxygenases
Carbonic anhydrases * CCS to boost kinetics
alkMa/alkMb
CoM
Sulfate reductases
Cellulases
Glucanases
Esterases
Transaminases
Proteases
etc

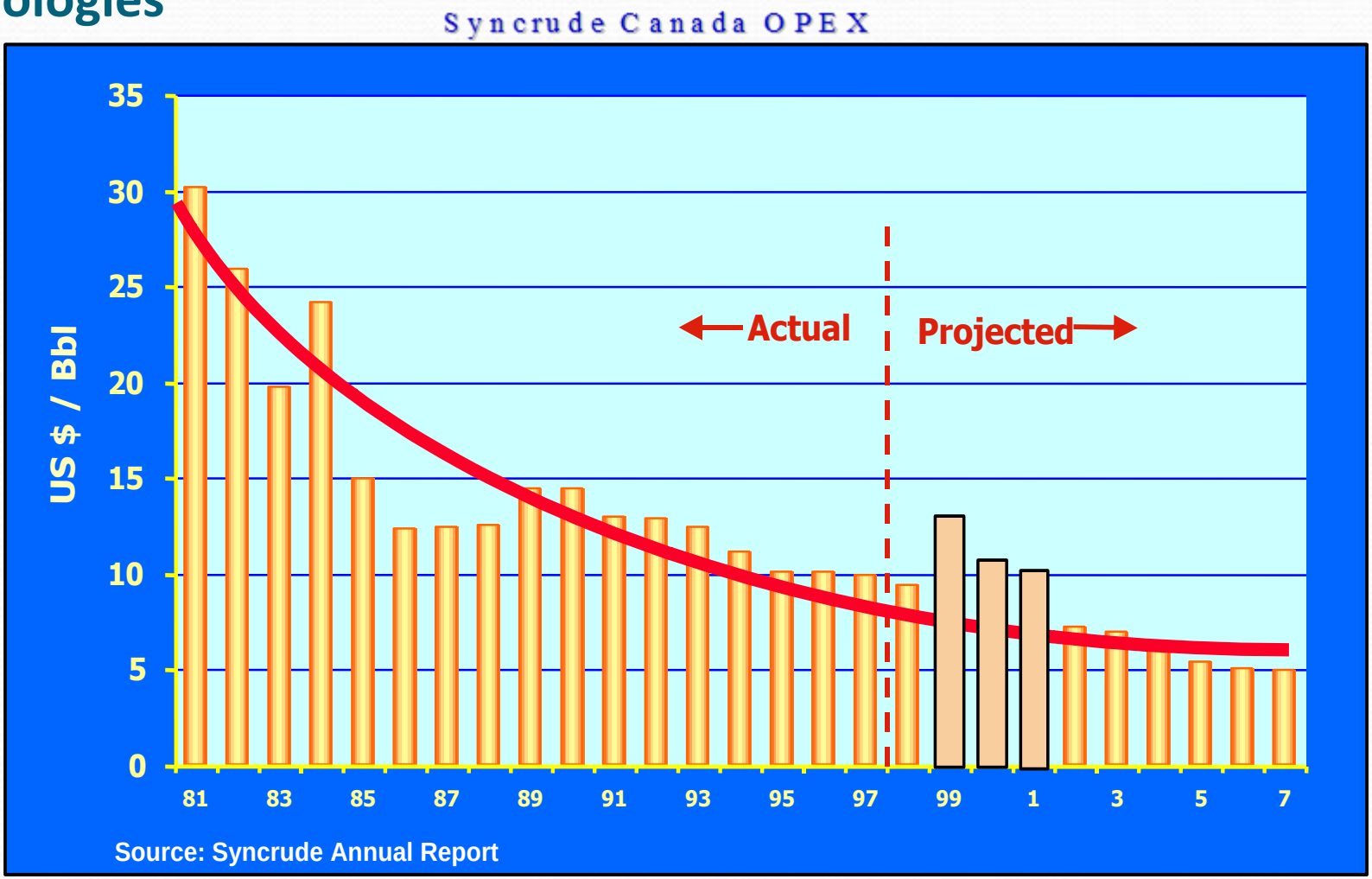
* 16 – 17 possible candidates through mining of the metagenome library

# Future aspects

- ✓Very conservative industry: cost efficient
- ✓Type of organism:
  - Bacteria vs Archaea
- ✓Metagenome approach, thermophiles ?
- ✓Industrial adaptation
  - Optimize different steps in the process
- ✓Employment
  - During production, a whole new process, or ?
    - Pre-refining
    - Post -refining




# The Canadian lesson: OPEX profile in developments of new technologies




For mining bitumen, $10 OPEX is probably the lower limit

OPEX profile in developments of new technology for mining bitumen. The curve shows the measured cost until 1998, then the further projection. The bars in 99, 00 and 01 are the actual cost. (Maurice B. Dusseault, personal communication)

**Courtesy of George Stosur**

# You must have it all

## Total integration

1. Physical knowlegde of the habitat: The oil reservoir
2. Classical sequencing 454 pyrosequencing, SOLiD etc
3. Functional fosmids library
4. Bioinformatics
5. Bioprospecting
6. Gene synthesis from putative sequence
7. Cloning and production
8. Testing of function and thermostability
9. Testing/pilot in industrial process
10. Generate protective IP
11. Outside the box thinking
12. For the sucess of a product: **You must have it all !**