# Process Technology Strategy & Methods

## Fermentation, Biofilm Process and Biosensor process

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# Process Engineering

**Biochemical Engineering** | **Biomedical Engineering**

**Fermentation process, Separations process**
**Biotransformation and Biocatalysis**

**Bacterial adhesion, biofilm formation, biosensor**

**Process: optimization**（**design methods: Plackett–Burman design, central composite design, orthogonal matrix methods, and uniform design, etc.**）
**modeling, controlling, kinetic parameters.**

**Fermentation: bacterial culture, fungus culture, mammalian culture, etc.**
**scale-up and scale-down in stirred-tank, airlift and biofilm reactors**

**Purification: Affinity chromatography, Ion exchange chromatography, and Size exclusion chromatography, etc**

**Adhesion: specific binding, non-specific binding**

**Biosensor: enzyme technology**

# PART I.
# Optimization & Modeling of Fungal Cultures

**Design methods:** Plackett–Burman design, central composite design, orthogonal matrix methods, and uniform design, etc.
**Unstructured model:** Logistic equation, the Luedeking–Piret equation and Luedeking–Piret-like equations.

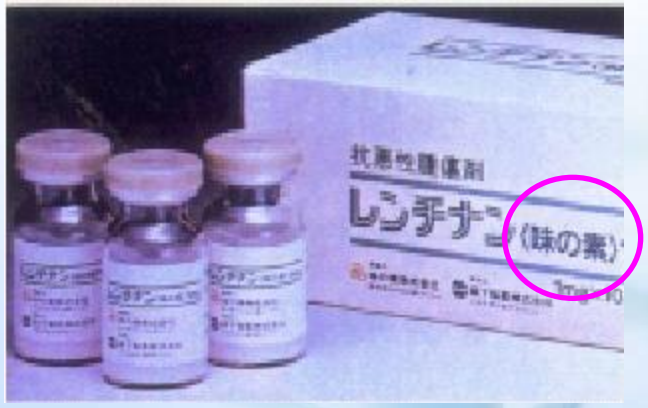
**Statistical optimization of fermentation process for exo-biopolymer production from fungal cultures (*Paecilomyces tenuipes*, *Auricularia polytricha*, and *Cordyceps militaris*)**

## ❖ Medicinal activities of exopolysaccharides from mushrooms or enthomopathogenic fungi

Immunostimulating activity , Antitumor activity

Hypoglycemic activity, Enhancement of stamina , Tonic promoting longevity

## ❖ Industrial application


## ❖ Comparison of different statistical experiment design methods

| | Full Factorial design | Orthogonal design | Response Surface design | Uniform design |
|---|---|---|---|---|
| Factor number | 3 | 3 | 3 | 3 |
| Level number | 5 | 3 | 5 | 5 |
| Experiment number | 125 | 9 | 20 | 5 |

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Process Biochemistry 38 (2003) 1025–1030

PROCESS BIOCHEMISTRY

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# Optimization of submerged culture conditions for mycelial growth and exo-biopolymer production by *Paecilomyces tenuipes* C240

Chun-Ping Xu[a], Sang-Woo Kim[a], Hye-Jin Hwang[a], Jang-Won Choi[b], Jong-Won Yun[a,*]

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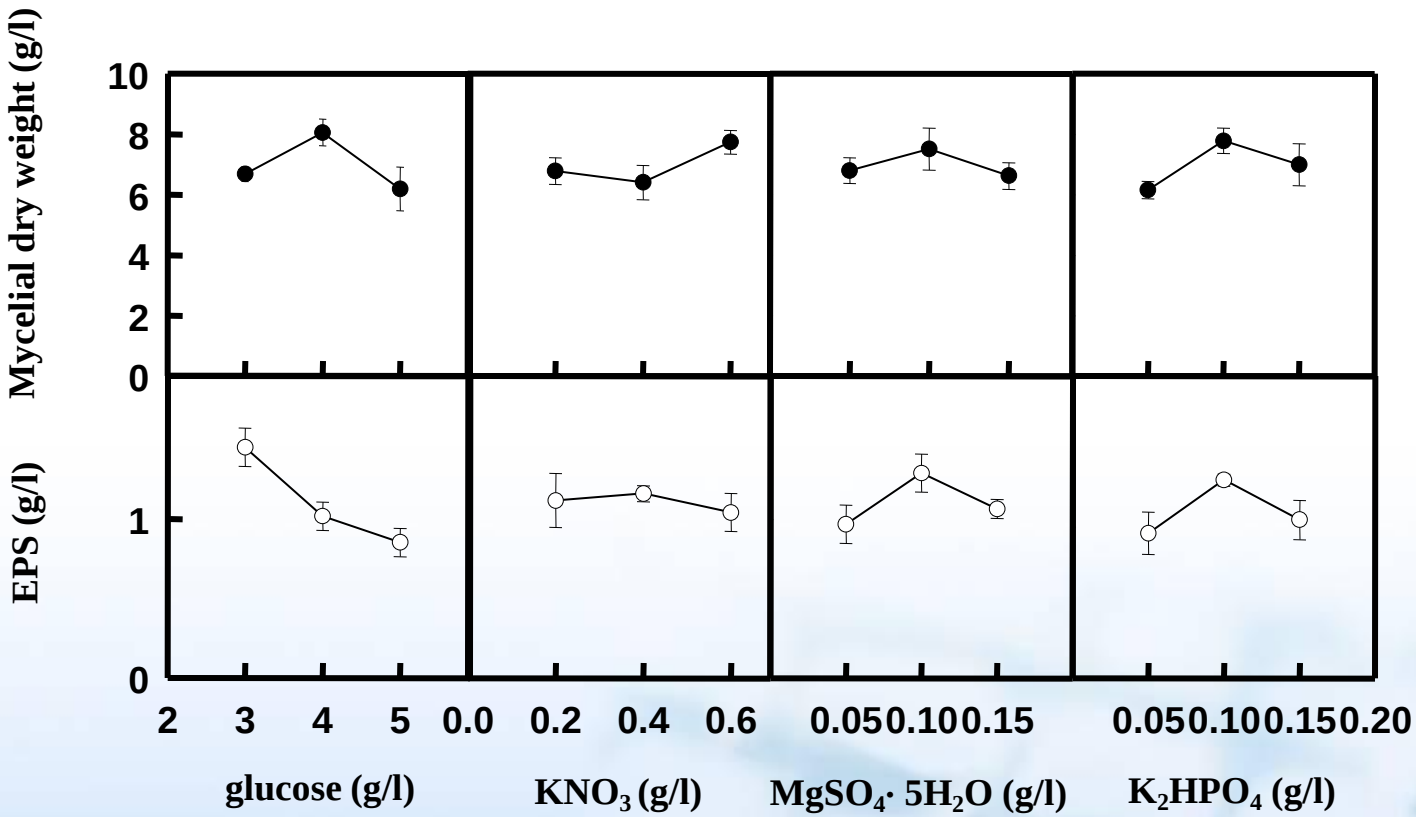
# ❖ Analysis of media with Orthogonal Projects

| | Dry cell weight (g/l) | | | | EPS (g/l) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| $K_1$ | 20.05[a] ± 0.71 | 20.35 ± 1.31 | 20.40 ± 1.26 | 18.46 ± 0.88 | 4.35 ± 0.37 | 3.35 ± 0.51 | 2.90 ± 0.36 | 2.88 ± 0.41 |
| $K_2$ | 24.17 ± 1.32 | 19.22 ± 1.72 | 22.52 ± 2.06 | 23.35 ± 1.27 | 3.05 ± 0.28 | 3.48 ± 0.16 | 3.94 ± 0.37 | 3.94 ± 0.11 |
| $K_3$ | 18.57 ± 2.17 | 23.22 ± 1.78 | 19.87 ± 1.31 | 20.98 ± 2.06 | 2.56 ± 0.27 | 3.13 ± 0.35 | 3.19 ± 0.19 | 3.14 ± 0.40 |
| $k_1$ | 6.68[b] ± 0.24 | 6.78 ± 0.44 | 6.80 ± 0.42 | 6.15 ± 0.29 | 1.45 ± 0.12 | 1.12 ± 0.17 | 0.97 ± 0.12 | 0.96 ± 0.14 |
| $k_2$ | 8.06 ± 0.44 | 6.41 ± 0.57 | 7.51 ± 0.69 | 7.78 ± 0.42 | 1.02 ± 0.09 | 1.16 ± 0.05 | 1.31 ± 0.12 | 1.31 ± 0.04 |
| $k_3$ | 6.19 ± 0.72 | 7.74 ± 0.39 | 6.62 ± 0.44 | 6.99 ± 0.69 | 0.85 ± 0.09 | 1.04 ± 0.12 | 1.06 ± 0.06 | 1.05 ± 0.13 |
| R | 1.87[c] ± 1.16 | 1.33 ± 0.96 | 0.88 ± 1.13 | 1.63 ± 0.71 | 0.57 ± 0.21 | 0.12 ± 0.17 | 3.22 ± 0.24 | 0.20 ± 0.18 |
| Optimal level | 2 | 3 | 2 | 2 | 1 | 2 | 2 | 2 |

**[a] $K_i^A$ = Σ mycelial yield at $A_i$**
**[b] $k_i^A = k_i^A/3$**
**[c] $R_i^A$ = max $\{k_i^A\}$ − min$\{k_i^A\}$.**

## ❖ Intuitive analysis of the relationship



Biotechnol. Appl. Biochem. (2002) 36, 127–131 (Printed in Great Britain)

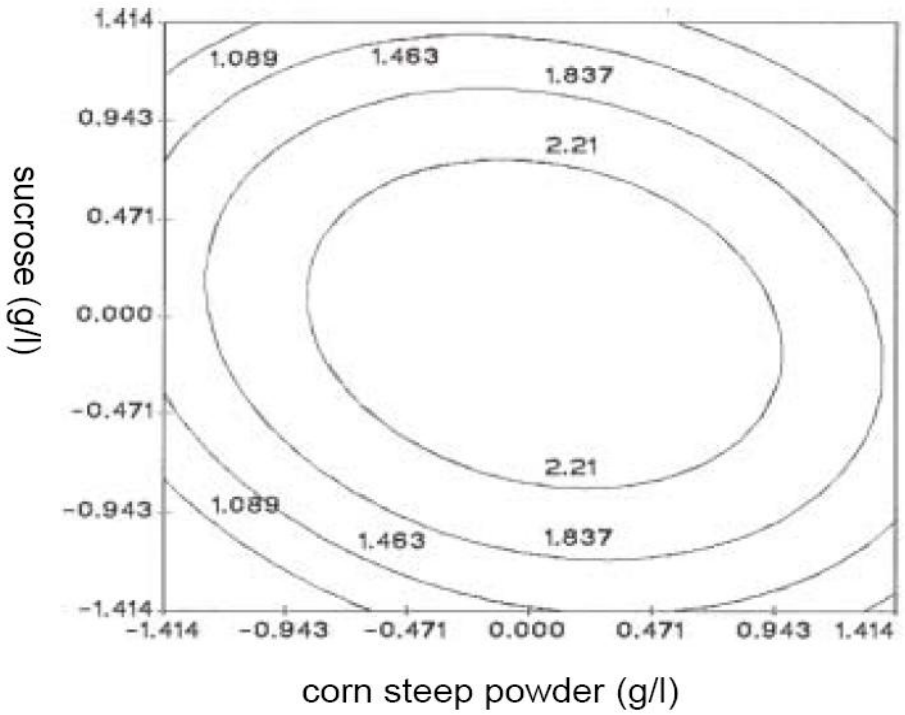


# Application of statistically based experimental designs for the optimization of exo-polysaccharide production by *Cordyceps militaris* NG3

**Chun-Ping Xu, Sang-Woo Kim, Hye-Jin Hwang and Jong-Won Yun[1]**

*Department of Biotechnology, Taegu University, Kyungsan, Kyungbuk 712-714, South Korea*

# Contour plot of the model equation fitted to the data of the central composite design experiment



Biotechnol. Appl. Biochem. (2003) 38, 193–199 (Printed in Great Britain)



# Optimization of submerged-culture conditions for mycelial growth and exo-biopolymer production by *Auricularia polytricha* (wood ears fungus) using the methods of uniform design and regression analysis

**Chun-Ping Xu and Jong-Won Yun**[1]

*Department of Biotechnology, Daegu University, Kyungsan, Kyungbuk 712-714, Korea*

## ❖ Programme

**DPS (Data Processing System, supplied by Tang, QY, Zhejiang University, Zhejiang, China)**

Table 6 Application of uniform design U6($6^3$) to the mycelial growth and exobiopolymer production by *A. polytricha*

The arrangements of column $X_1$, $X_2$, and $X_3$ were decided by uniform design for 3 (factor) × 6 (run number); every row of run number represents one experimental replicate, every run was replicated twice.

| | Variables levels | | | Responses | |
|---|---|---|---|---|---|
| Run no. | $X_1$ | $X_2$ | $X_3$ | Dry cell weight (g/l) | Exo-polymer (g/l) |
| 1 | 1(1) | 2(0.5) | 4(0.20) | 1.40 ± 0.12 | 0.283 ± 0.03 |
| 2 | 2(2) | 4(1.5) | 1(0.05) | 3.00 ± 0 | 1.081 ± 0.01 |
| 3 | 3(3) | 6(2.5) | 5(0.25) | 4.79 ± 0.22 | 0.957 ± 0.04 |
| 4 | 4(4) | 1(0) | 2(0.10) | 0.59 ± 0.06 | 0 ± 0 |
| 5 | 5(5) | 3(1) | 6(0.30) | 2.76 ± 0.10 | 1.813 ± 0.09 |
| 6 | 6(6) | 5(2) | 3(0.15) | 4.88 ± 0.12 | 0.929 ± 0.02 |

## ❖ Equations

**$Y_{mycelia}$=1.017621994-0.4442459335X1+1.6732637907X2+0840661245X1X1+0.2605374823X2X3 (R=0.999997, F=40349.55, P<0.004).**

**$Y_{exo\text{-}biopolymer}$=0.851147884-0.5173888854X1+0.03335286087X2X2-19.060431923X3X3+0.3520500186X1X3 (R=0.999999, F=124999.81, P<0.003).**

## Production of exopolysaccharides by submerged culture *P. tenuipes* C240 in stirred-tank and airlift reactors

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Bioresource Technology 97 (2006) 770–777

BIORESOURCE TECHNOLOGY

# Production of exopolysaccharides by submerged culture of an enthomopathogenic fungus, *Paecilomyces tenuipes* C240 in stirred-tank and airlift reactors

Chun Ping Xu, Sang Woo Kim, Hye Jin Hwang, Jong Won Yun *

*Department of Biotechnology, Daegu University, Kyungsan, Kyungbuk 712-714, Republic of Korea*

Received 11 February 2004; received in revised form 4 January 2005; accepted 4 January 2005
Available online 13 June 2005

# Production and characteristics of exopolysaccharides in a stirred tank reactor under different aeration conditions

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Enzyme and Microbial Technology 35 (2004) 33–39

ENZYME and MICROBIAL TECHNOLOGY

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## Influence of aeration on the production and the quality of the exopolysaccharides from *Paecilomyces tenuipes* C240 in a stirred-tank fermenter

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*Department of Biotechnology, Daegu University, Kyungsan, Kyungbuk 712-714, South Korea*

Received 19 May 2003; accepted 11 March 2004

# Application of uniform design for optimization of the physical parameters for exopolysaccharides production in a batch bioreactor

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

## Optimization of physical parameters for exo–biopolymer production in submerged mycelial cultures of two entomopathogenic fungi *Paecilomyces japonica* and *Paecilomyces tenuipes*

Chun Ping Xu, Jayanta Sinha, Jun Tae Bae, Sang Woo Kim and Jong Won Yun

Department of Biotechnology, Daegu University, Kyungbuk, Korea

## ❖ Experimental factors and their levels for uniform design

| Factor | Levels | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| $X_1$ (pH) | 4 | 5 | 6 | 7 | 8 |
| $X_2$ (rpm) | 150 | 200 | 250 | 300 | 350 |
| $X_3$ (vvm) | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |

***Symbols $X_1$, $X_2$, and $X_3$ represent factors of pH (controlled), agitation speed, and aeration rate.**

**Symbols 1, 2, 3, 4, and 5 represent concentration levels of each factor.**

## ❖ Application of uniform design U5($5^3$)

| Run No. | Variable levels | | | Responses | |
|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | Mycelial biomass (g/l) | EPS production (g/l) |
| 1 | 1(4) | 2(200) | 4(3.5) | 13.68±0.170 | 0.986±0.03 |
| 2 | 2(5) | 4(300) | 3(3.0) | 9.601±0.153 | 2.962±0.10 |
| 3 | 3(6) | 1(150) | 2(2.5) | 10.94±0.129 | 1.208±0.04 |
| 4 | 4(7) | 3(250) | 1(2.0) | 2.899±0.044 | 0.813±0.01 |
| 5 | 5(8) | 5(350) | 5(4.0) | 0.910±0.014 | 0.048±0.01 |

***The arrangements of column $X_1$, $X_2$, and $X_3$ were decided by uniform design for 3 (factor) ×5 (run number); every row of run number represents one experimental replicate, every run was carried out in duplicate.**

# ❖ The quadratic model interpreted using DPS

## ❖ Programme

**DPS (Data Processing System, supplied by Tang, QY, Zhejiang University, Zhejiang, China)**

## ❖ Equations

**The two equations relate the coefficients obtained for mycelial growth and exo-polymer production to the experimental variables as follows:**

$$Y_{\text{mycelia biomassl}}=-92.86889+37.5187303X_1-3.548434103X_1*X_1+0.00016327119565X_2*X_2$$

**($R$=0.999405 $F$=279.8472 $P$<0.05)**

$$Y_{\text{exopolysaccharides}}=8.01491343-0.03529643881X_2-0.0533113428X_1*X_1+0.000065662687X_2*X_2$$

**($R$=0.999660 $F$=489.890 $P$<0.05)**

## ❖ Conclusions

**The optimal values of the test variables obtained by DPS are as follows:**

**for high mycelial growth, the optimum combination : pH (controlled)-4.88, aeration-2 vvm and agitation-350 rpm.**

**for high EPS production, the combination : pH (controlled)-4, aeration-2 vvm and agitation-150 rpm.**

# An unstructured model for exopolysaccharide production by *Paecilomyces tenuipes* C240

*Journal of Life Science* 2005, Vol. 15. No. 1. 15~20



## A Kinetic Study for Exopolysaccharide Production in Submerged Mycelial Culture of an Entomopathogenic Fungus *Paecilomyces tenuipes* C240

Chung Ping Xu and Jong Won Yun*

Department of Biotechnology, Daegu University, Kyungsan, Kyungbuk 712-714, Korea

Received November 29, 2004 / Accepted December 29, 2004

**1. The Logistic equation for mycelial growth, the Luedeking–Piret equation for exopolysaccharide production and Luedeking–Piret-like equations for glucose consumptions were successfully incorporated into the model.**

**2. The value of the key kinetic constants were:**
**maximum specific growth rate ($\mu_m$), 0.7281 $h^{-1}$;**
**growth-associated constant for exopolysaccharide production ($\alpha$), 0.1743 g(g cells)$^{-1}$;**
**non-growth associated constant for exopolysaccharide production ($\beta$), 0.0019 g(g cells)$^{-1}$;**
**maintenance coefficient ($m_s$), 0.0572 g (g cells)$^{-1}$.**

**3. When compared with batch experimental data, the model appeared to provide a reasonable description for each parameter during the growth phase.**

**4. The model showed that the production of exopolysaccharide in *P. tenuipes* C240 was growth-associated.**

# PART II.
# Mechanism & Modeling of Biofilm Formation Process

**Adhesion:** specific binding, non-specific binding

# Mechanism of Adsorption Enthalpy in Becateral Adhesion

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Colloids and Surfaces B: Biointerfaces 54 (2007) 193–199

COLLOIDS AND SURFACES B

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## Calorimetric comparison of the interactions between salivary proteins and *Streptococcus mutans* with and without antigen I/II

Chun-Ping Xu[a], Betsy van de Belt-Gritter[a], Henk J. Busscher[a], Henny C. van der Mei[a,*], Willem Norde[a,b]

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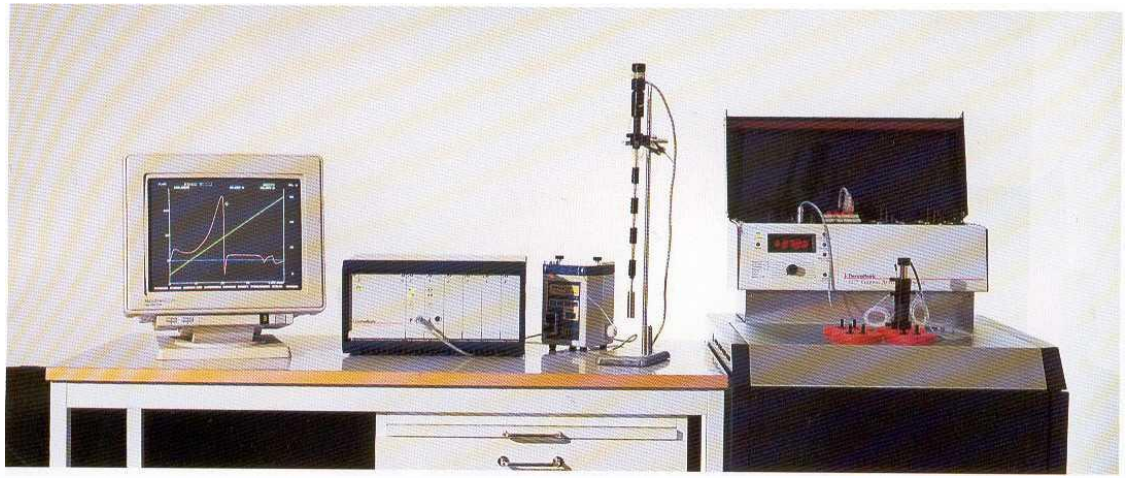
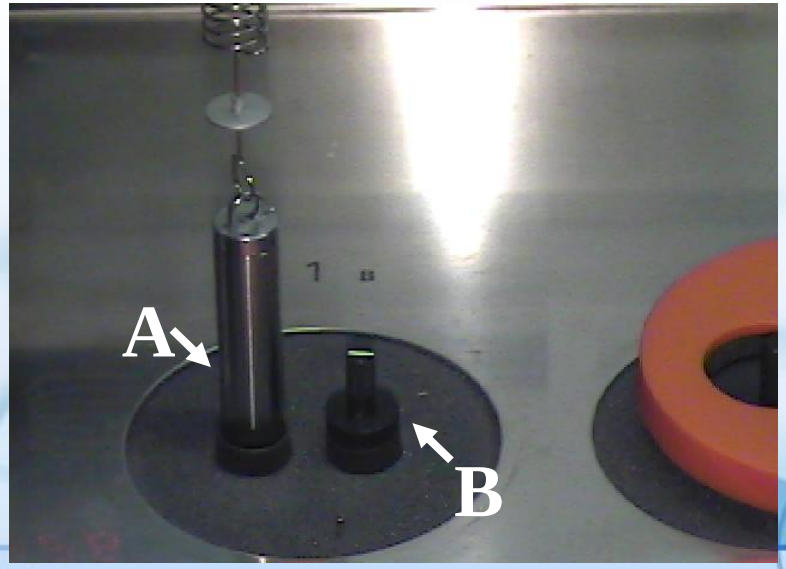
[b] *Laboratory of Physical Chemistry and Colloid Science, Wageningen University, P.O. Box 8038, 6700 EK Wageningen, The Netherlands*

Received 4 September 2006; received in revised form 13 October 2006; accepted 13 October 2006
Available online 24 October 2006

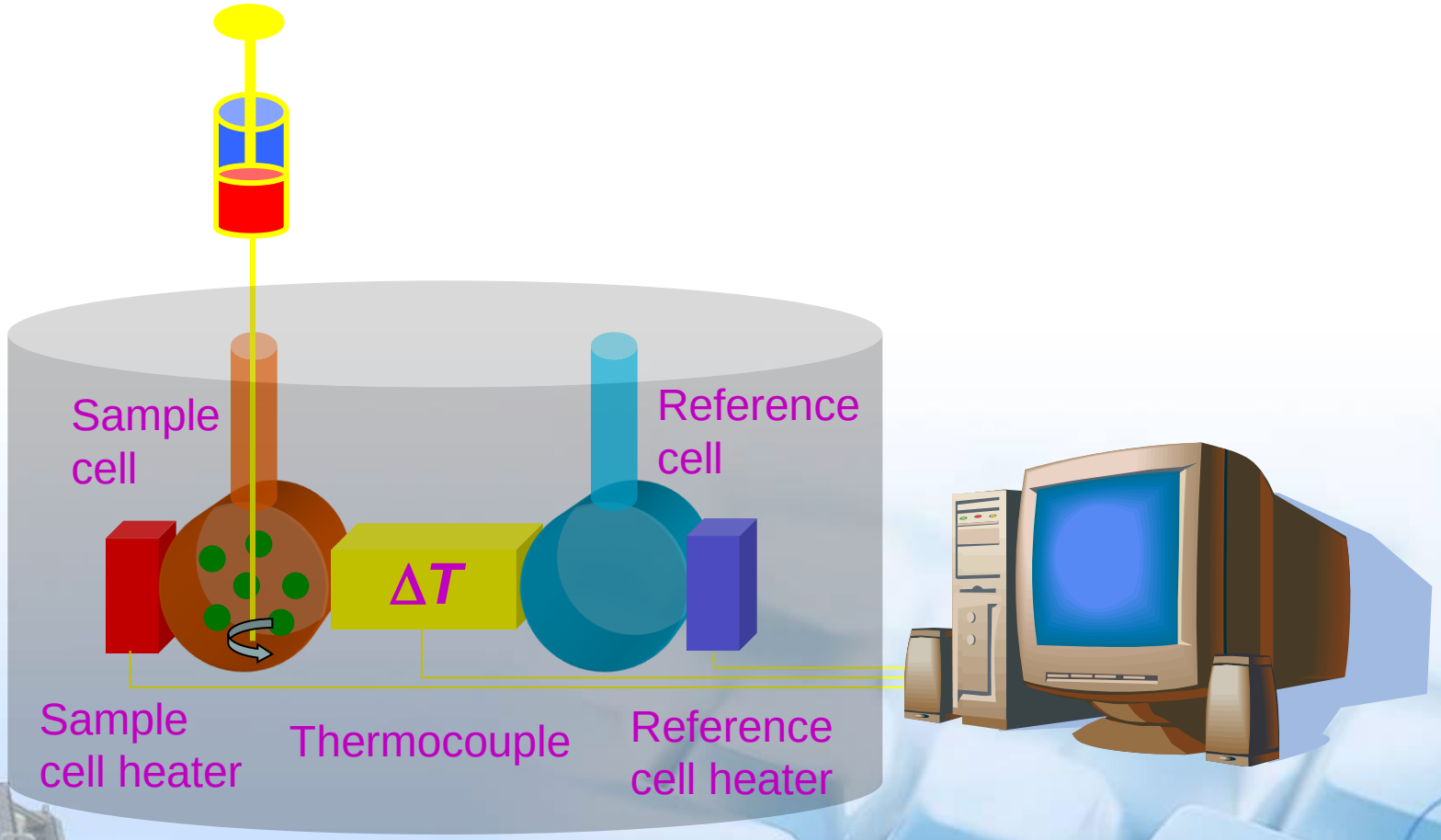
# Mechanism of Adsorption

# Isothermal titration calorimetry

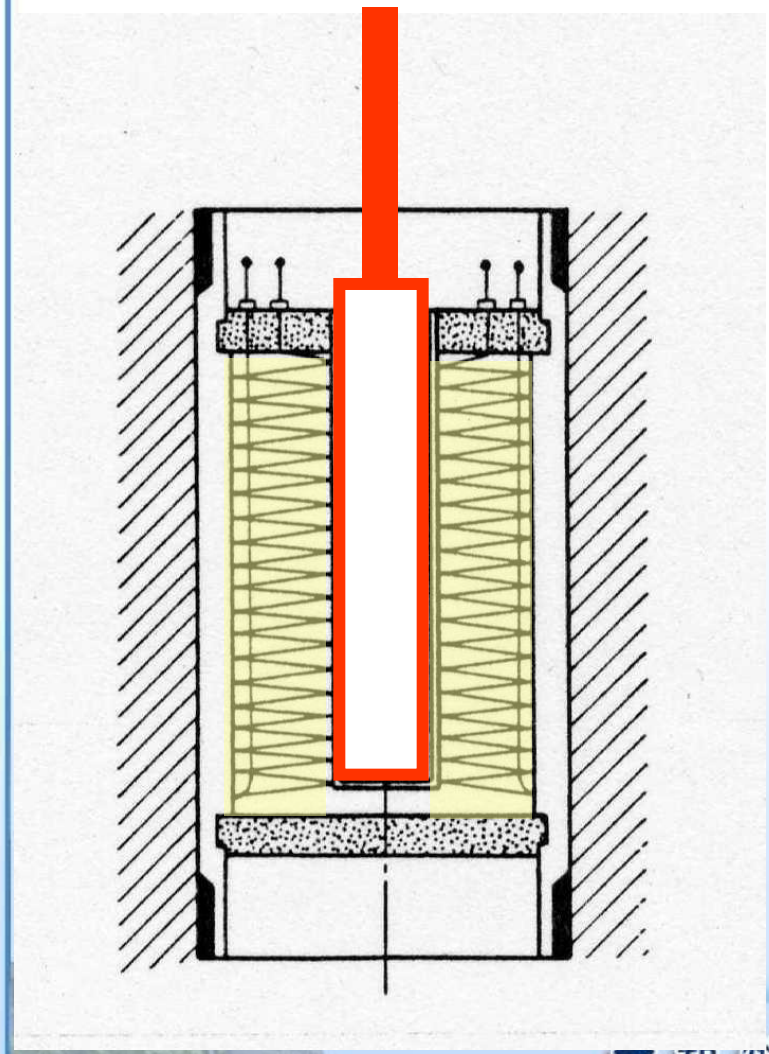




# Principle of ITC

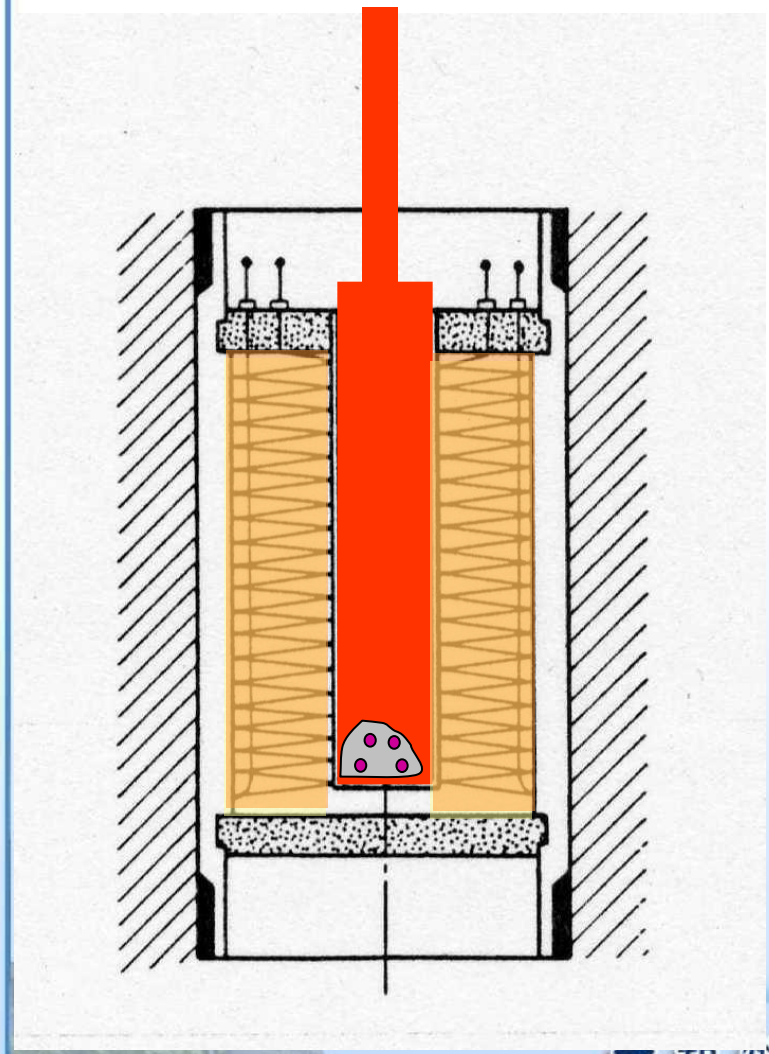


# The Calorimetric Element



- The sample cell is placed into a calorimeter element
- The cell is surrounded by a thermopile made of more than 400 thermocouples in series
- Thermopile has 2 functions:
  - transfers heat
  - generates signal

# Heat and Heat Flow

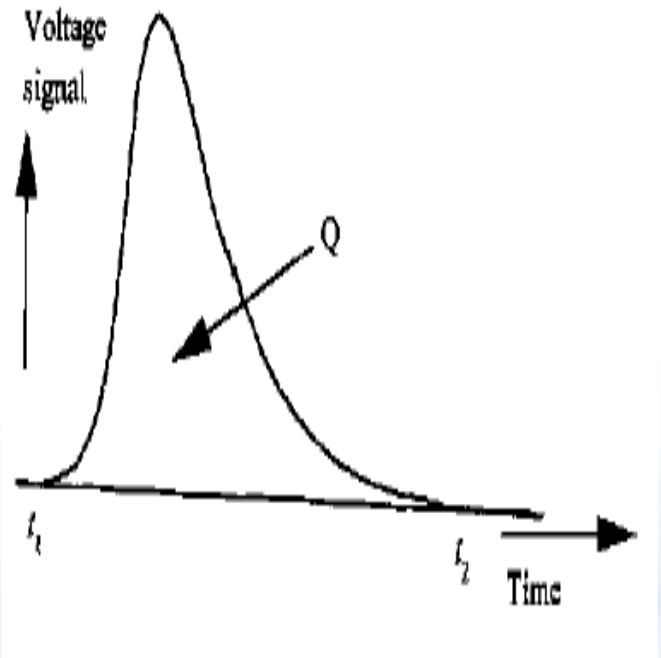


- **The heat produced by the reaction is consumed by two processes**

- **1. Increase of the temperature of the sample cell**

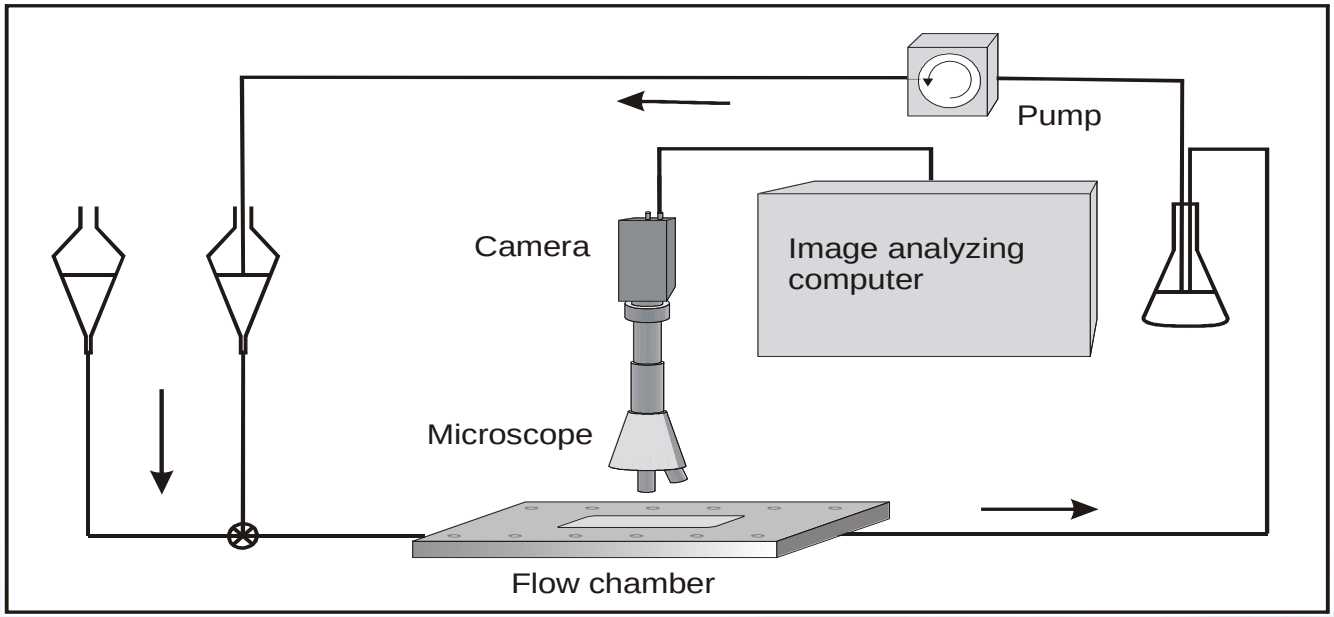
- **2. Once there is a temperature gradient between cell and surrounding block, heat flow through the thermopile**

# Analysis Principle of Programme

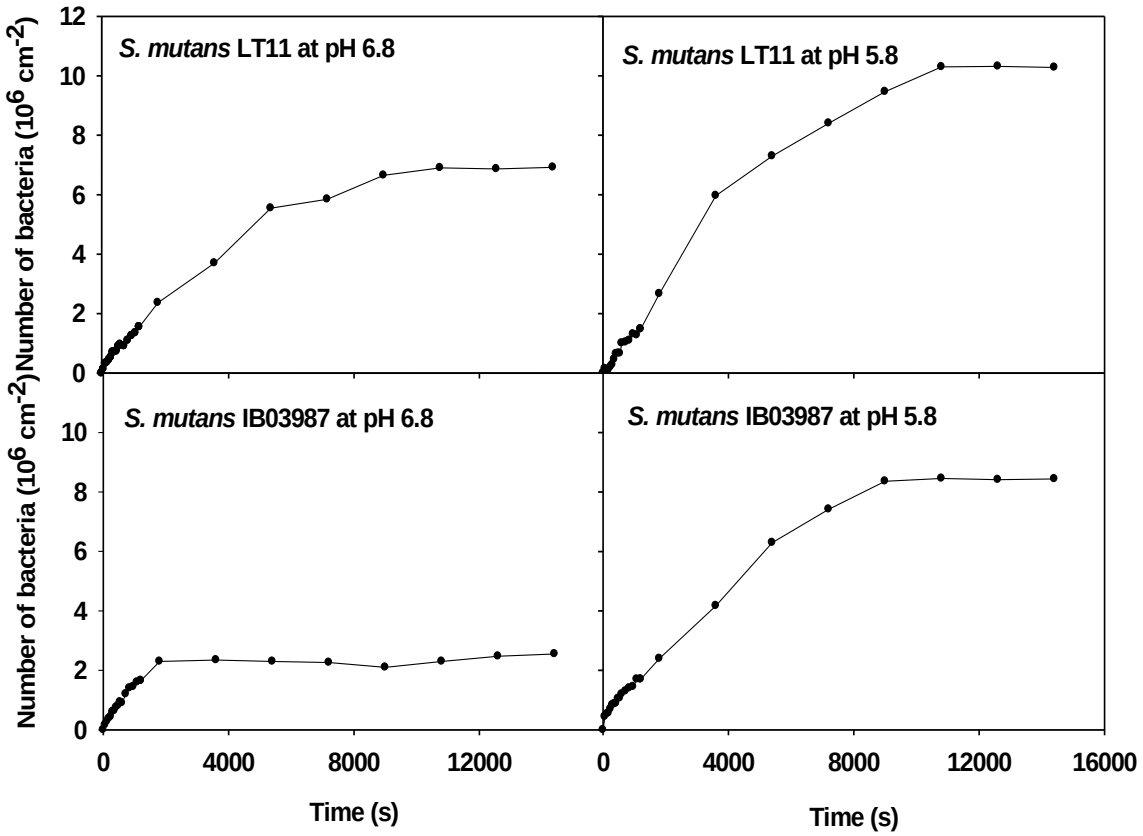



**Typical heat flow signal (curve) generated by the micro-calorimeter during adsorption**

# Parallel plate flow chamber



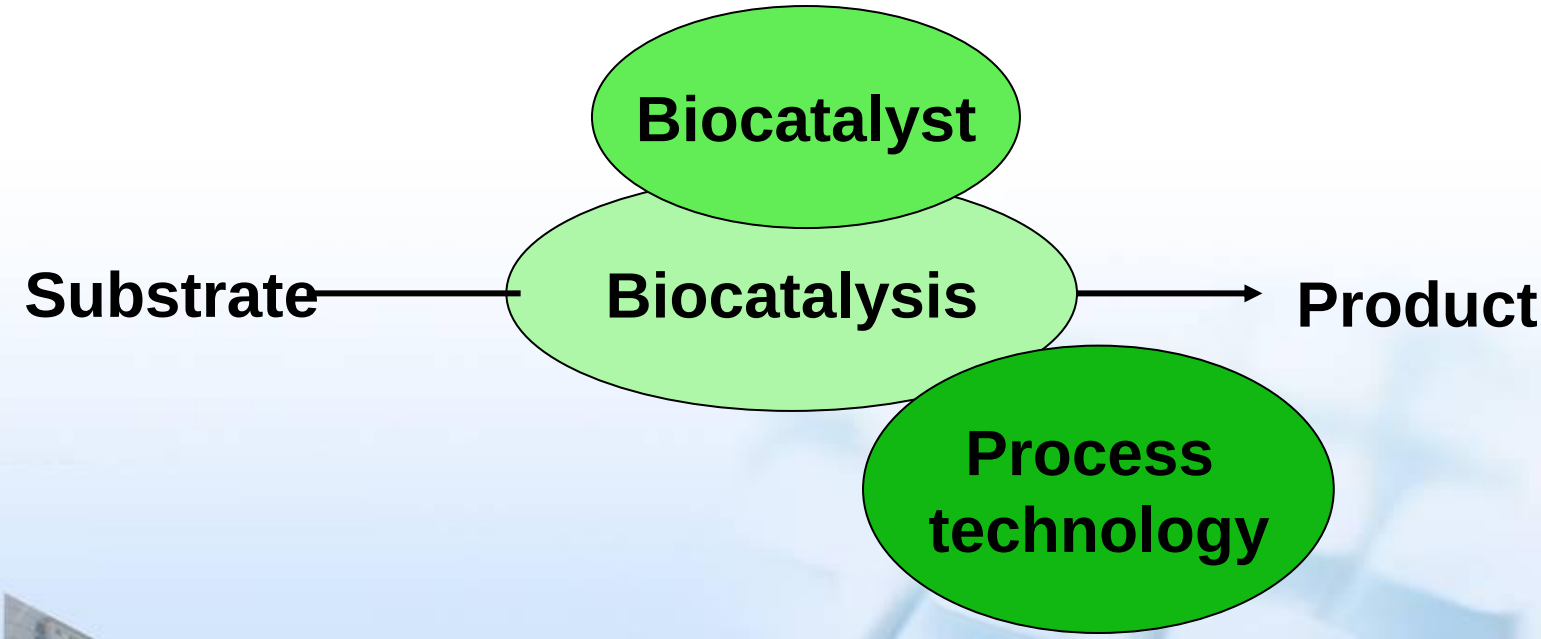
# Representative examples of the adhesion kinetics



# PART III.
# Biocatalysis & Biotransformation of Tobacco Chemicals

## Biotransformation 简介：

生物催化（biocatalysis）是指利用酶或者生物有机体（全细胞、细胞器、组织等）作为催化剂进行化学转化的过程，这种反应过程又称为生物转化（biotransformation）。生物催化中常用的有机体主要是微生物，其本质是利用微生物细胞内的酶进行催化。



# 国内外有关生物催化的意义和研究现状

- 生物催化或生物转化是生物学、化学、过程科学的交叉学科，是目前学科发展的前沿技术。世界经合组织指出，以生物催化或生物转化为核心的工业生物技术是可持续工业发展最有希望的技术。

- 我国在生物催化领域起步与发达国家相差不多，真正能够参与国际竞争之处正在于此。为此，国家将生物催化技术列入“973”计划项目，目标是要建立完善的理论和方法体系，使我国在生物催化与生物制造领域的基础研究与工业技术取得突破性进展，全面提升我国的生物制造能力，促进基础物质加工产业的可持续发展。

# || 烟草的化学成分和生物催化研究的意义

- **Tobacco synthesizes more than 3,000 Natural Products (NPs)**
- **Tobacco is the most studied plant on earth in terms of its chemistry**
- **Its compound's bioactivity has not been fully investigated**
- **'Genetic chemistry' can be used to generate unlimited chemical diversity**

## LEAF INTERNAL CHEMICALS (~10% DW)

*Alkaloids*
nicotine
anabasine
nornicotine, etc.

*Carotenoids*
β-carotene
lutein
zeaxanthin, etc.

*Phenolics*
chlorogenic acid
rutin
kaempferol
quercetin, etc.

## LEAF SURFACE CHEMICALS (~16% DW)

a. Trichome exudates

*Diterpenoids*
Cembranoids
Labdanoids

*Sugar esters*
Sucrose esters

b. Cuticular compounds

Fatty acids
Fatty alcohols
Hydrocarbons
Solanesol (prenol)
Wax esters

# Biocatalysis: 烟草公司的 Strategy

人们在吸食卷烟的同时，或多或少地摄取了某些烟草化学成分，而作为具有生物活性的天然化合物，它们会对人类的健康产生一定负面影响。目前，世界许多国际型的烟草公司的研发中心，其研发重点已经从单一的烟草加工，新型卷烟的研制向以下几个方面转移：

1) 烟草产品使人患上疾病的原因以及其病理过程；

2) 分子改造从而合成有生物活性的新型有机化合物，应用于精细化工和制药业。

3) 以烟草细胞为载体，进行基因改造，合成新型的抗体蛋白等产品

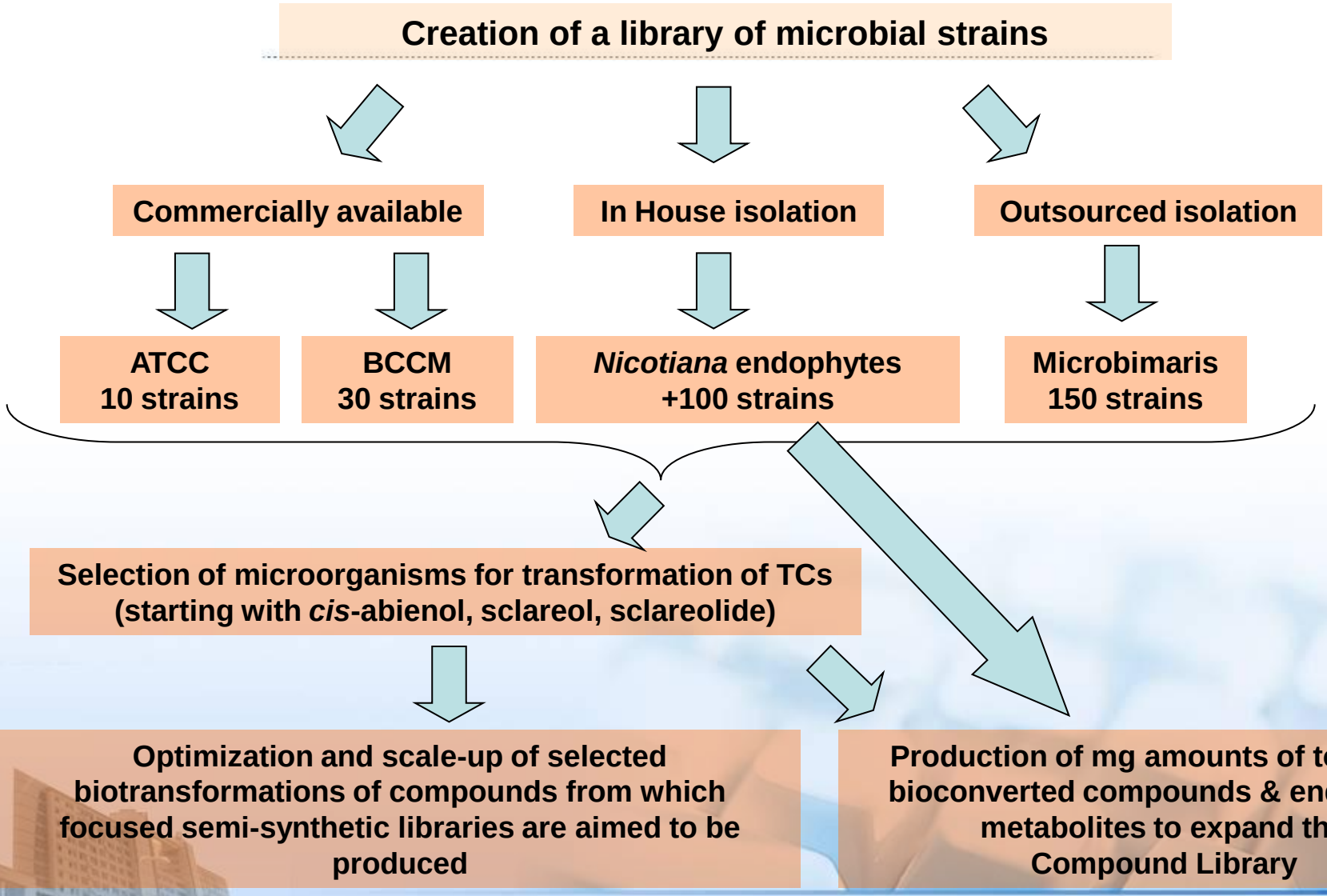
PHILIP MORRIS INTERNATIONAL

BRITISH AMERICAN TOBACCO

JTI

中国烟草 CHINA TOBACCO

# Biotransformation Program



# 建立菌株库进行烟草分子的生物催化

## The selection criteria of microorganisms:

**Previous successful biotransformation of compounds structurally similar to *Nicotiana* metabolites**
**Taxonomical diversity**
**Commercial availability**
**Biosafety level is 1 (as defined by the Swiss Agency for Environment, Forest, and Landscape)**
**Ecological relation to *Nicotiana* spp.**
**Ability to grow on selected *Nicotiana* compounds as sole carbon source**

## 菌株随机筛选方法来进行生物催化

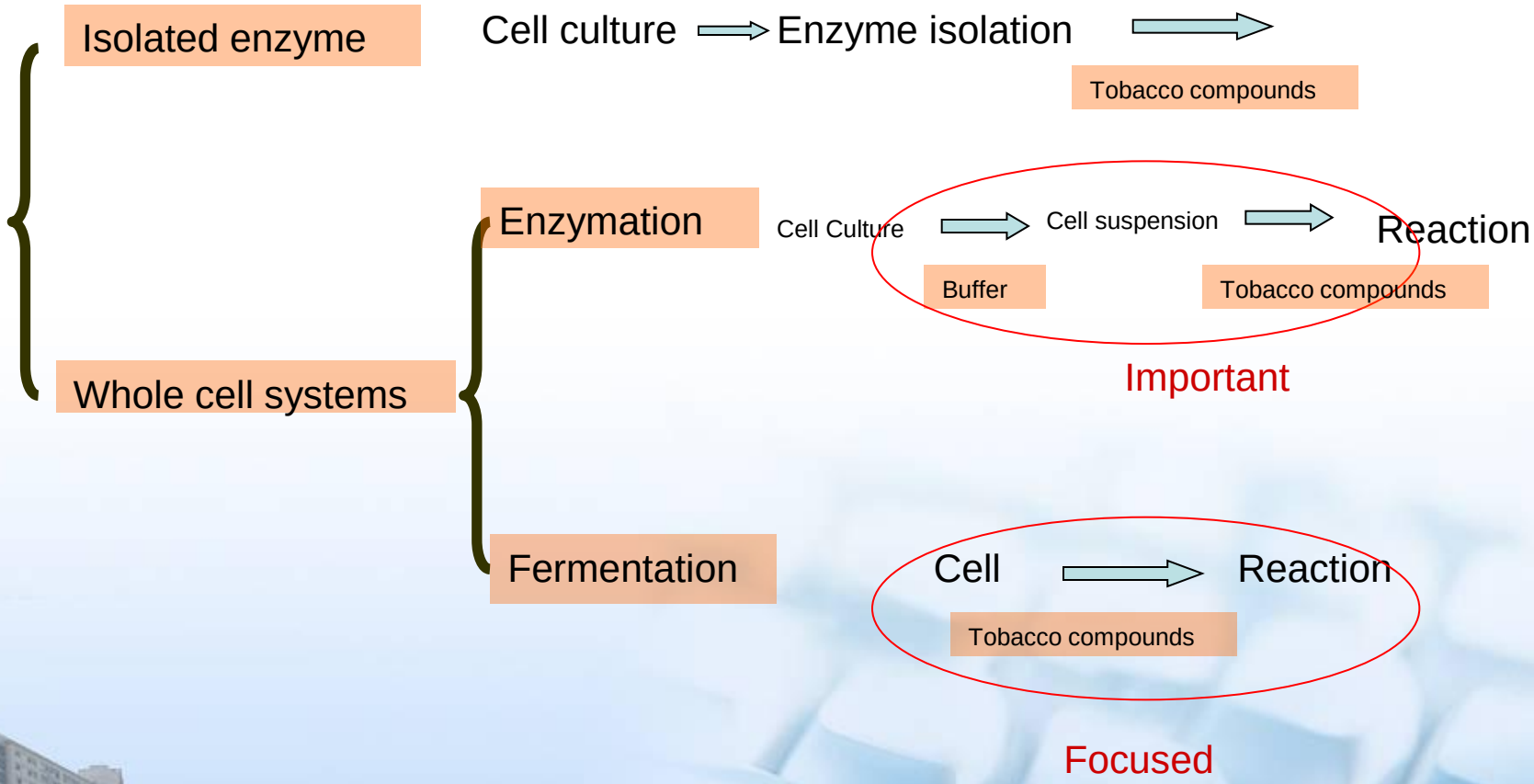
## This comprises:

**Define culture parameters (media composition, time of culture, and others)**
**Determine best method for substrate application**
**Establish chemical analysis methods**

## Desired properties:

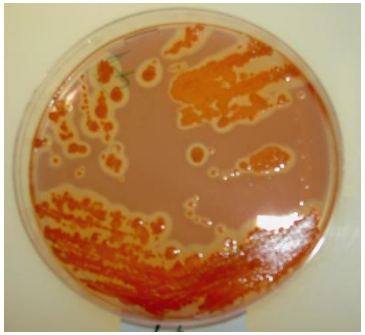
**Small scale**
**Reproducible**
**Flexible: can be easily adapted to different substrates**
**Time efficient**
**Optimized for detecting highest number of biotransformation events**
**Low number of false positives**

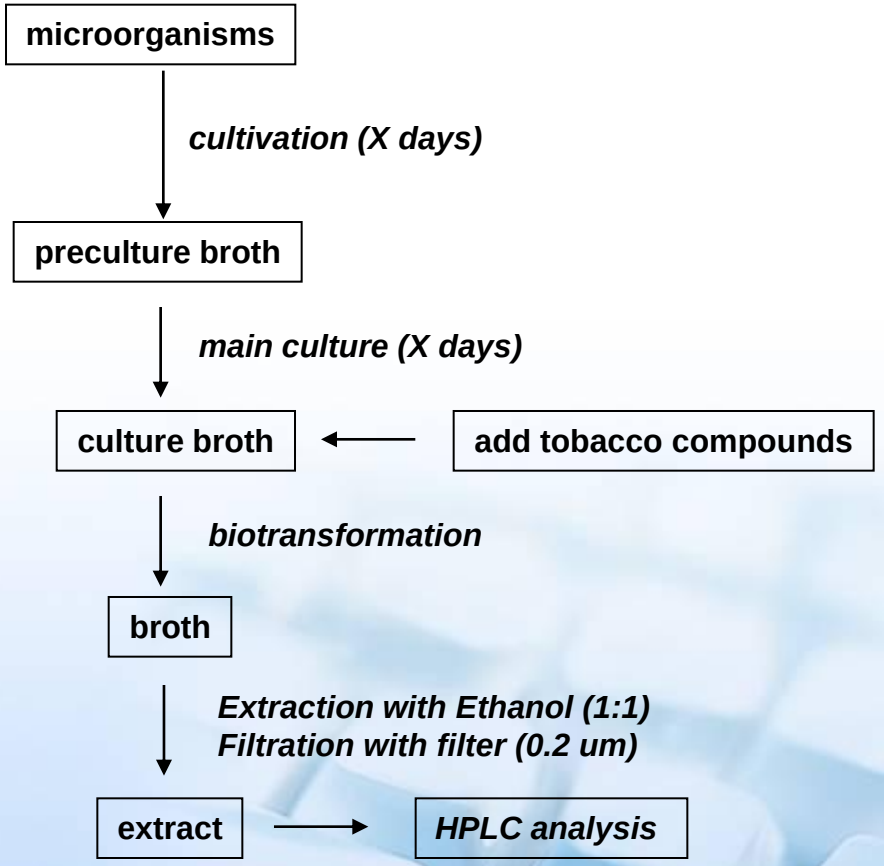
# Methods of tobacco biotransformation



# 技术路线

该研究整体上分四大部分，即紫苏醇（sclareol）和香紫苏内酯（sclareolide）的生物转化过程，转化产物的分离分析，产物分离纯化成分分析的药理学性质研究，目的菌株、发酵过程及分离纯化工艺的优化。具体实验方案的技术路线如下图：





# 过程优化和大规模发酵生物催化

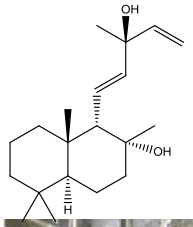
## 优化培养基和发酵过程

**Goal:** To define culture parameters that allow obtaining high conversion yields

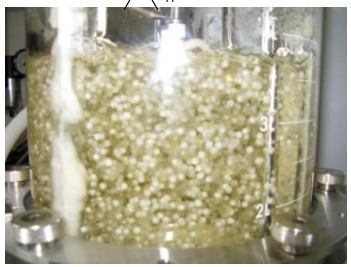
## 大规模培养

**Goal:** To obtain sufficient amounts of bioconversion products, either for conducting bioassays (~5 mg) or further structural diversification (>1 g)


# 解决分离纯化工艺，用NMR对其结构进行表征




**sclareol**



biotransformation (4L)

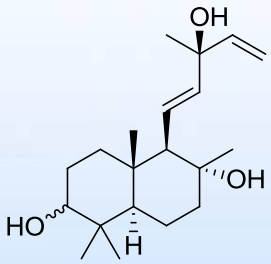
*Filtration*

**Column absorption on XAD-16**

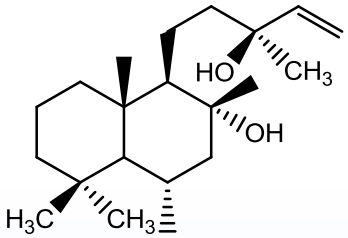
Product Purification

Product


Structure elucidation by NMR + MS



## Biotransformation from Sclareol

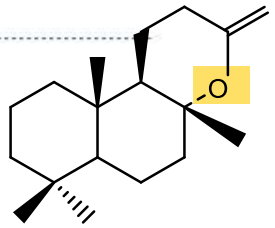



Sclareol

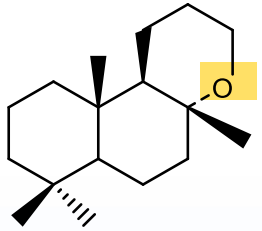
***Cryptococcus albidus* ATCC** 20918

***Cryptococcus albidus* ATCC 20920**

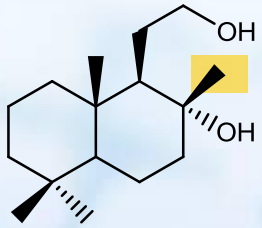
***Mucor plumbeus* ATCC 4740**




***Sclareolid***



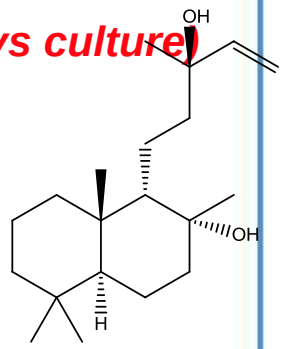

***lactone***




***diol***

脱水反应 **Dehydration**

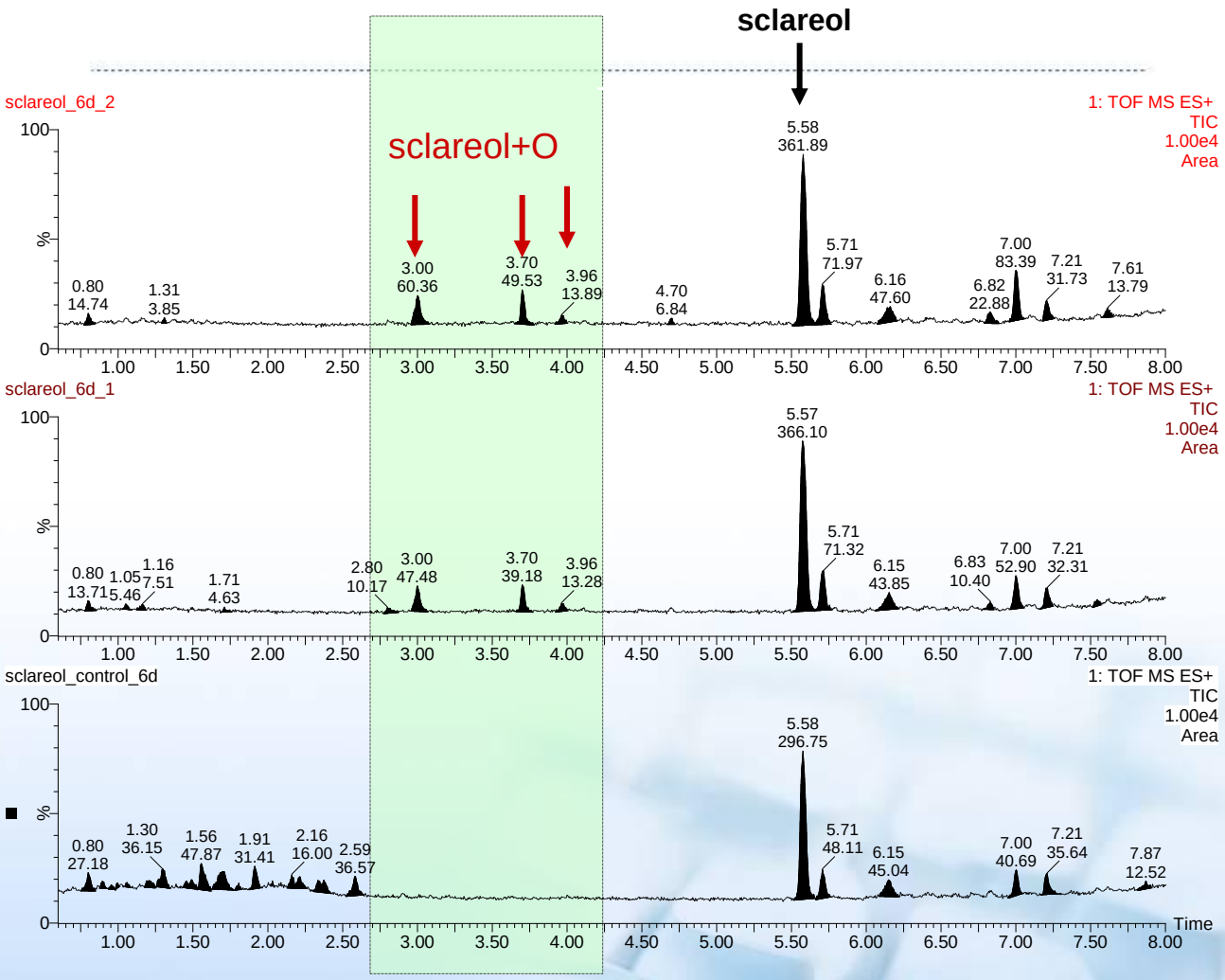
# *HPLC results of biotransformation of sclareol by* Mucor plumbeus *(6 Days culture)*



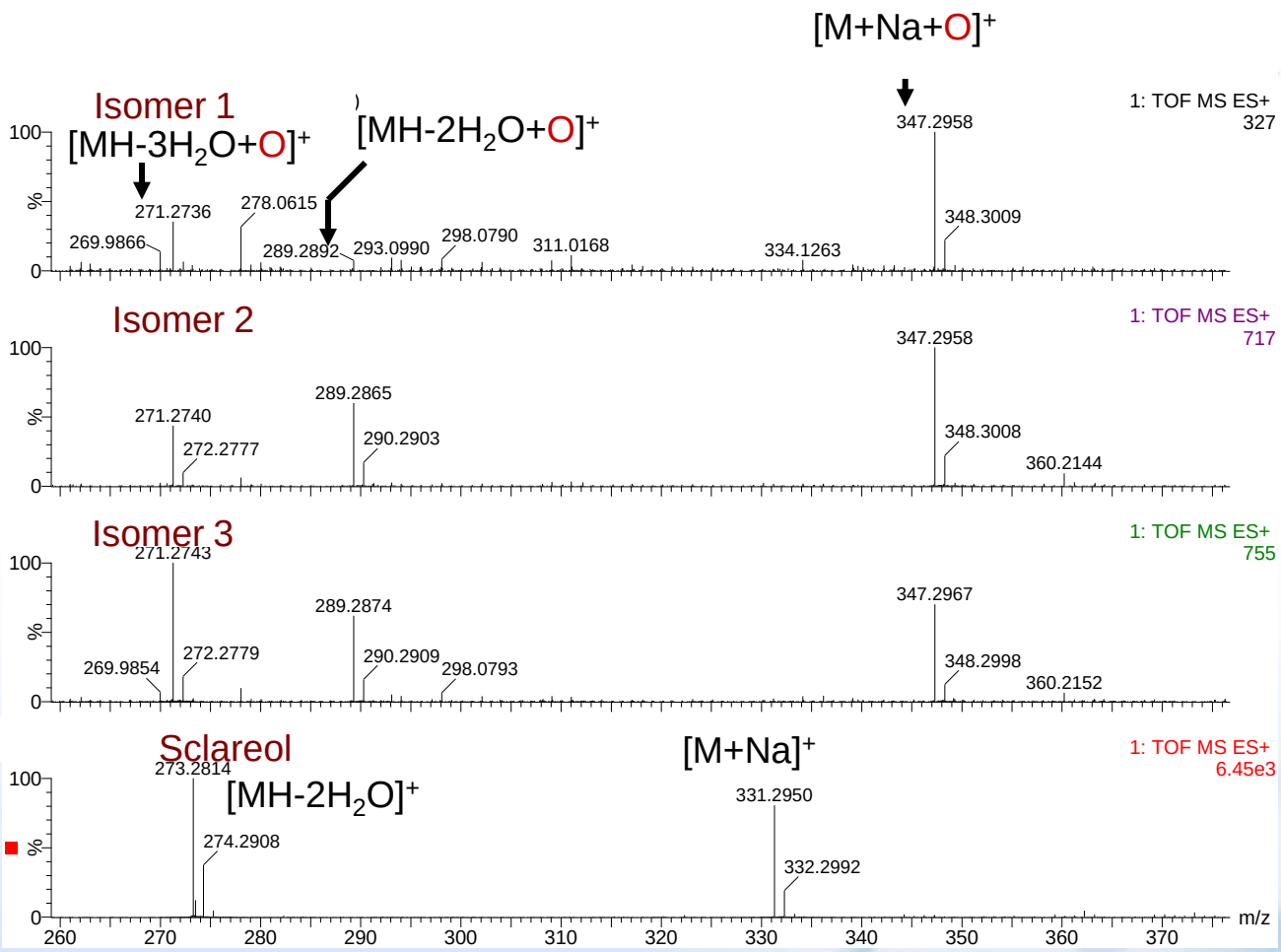

Replicate 1

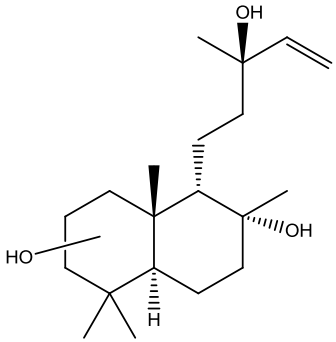
Replicate 2

Control



# Comparison ESI MS data of sclareol and scareol derivatives



# 烟草生物技术实验室主要研究任务

## 1. 烟草分子的生物转化：

- 由烟草生物活性有机分子的生物转化合成新型香料和药物：包括的烟草有机化合物有烟酸，香紫苏醇和其类似物香紫苏内酯，豆甾醇，β-石竹烯，顺-冷杉醇，烟草三烯-4，6-二醇。（许春平，王建民，阎克玉）
- 由烟草分子衍生物（如：白桦脂酸）的生物催化合成新型药物。（许春平，王吉中，刘苏萌）

## 2. 新型香料合成方向：

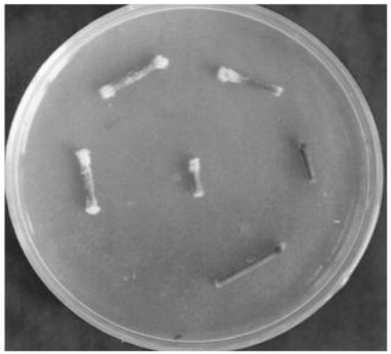
- 利用真菌资源发酵产物开发烟用天然菌类香料：包括香菇，灵芝等真菌（许春平，何培新，王吉中）
- 利用产香植物表面优势菌株开发烟草香料：香紫苏植物 （许春平，王吉中，程传玲）

## 3．烟草微生物方向：

- 烟草内生菌的分离鉴定与活性分子的生产，用于农药等（许春平，王吉中，刘苏萌）
- 烟草酶制剂，微生物制剂的开发（许春平，王吉中，刘苏萌）

# Endophyte Isolation & Testing Program

**Isolation of endophytes from fresh tobacco materials** → **Cultivation of endophytes and analysis of metabolic profile** → **Selection of strains producing unique metabolites** → **Optimization and upscaling of metabolite production**



**3X**

**Isolation**

**Several cycles of isolation until obtaining single clone strains**

- 被认为是烟草的国度，烟草是传统经济作物，著名的“哈瓦那雪茄”，驰名世界。
- Cuba and cigars



Havana Cigars

*Taken by Chun-Ping XU in Key-west*

***CUBAN CIGARS?***

***The states had better not wait for Washington to deliver on trade***

# West Havana Scientific Pole

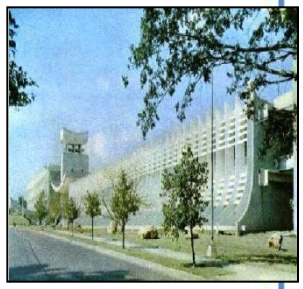
**CIGB**

**IPK**

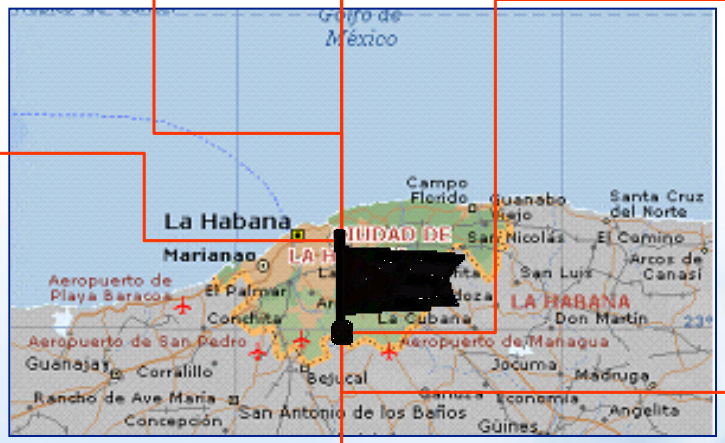
**CNB**

**CIE**

**NEUROSCIENCES**

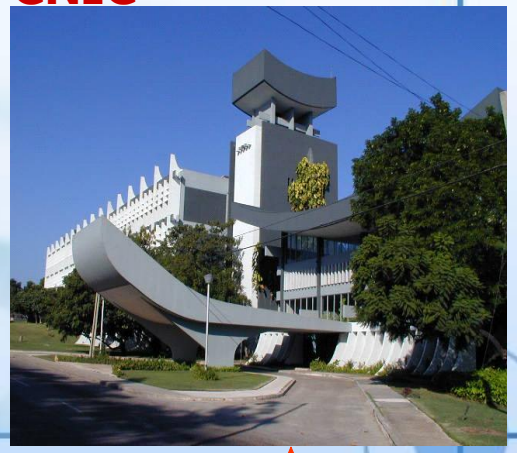


**CIM** (Centre of molecular immunology)




**I. FINLAY**

**CNIC**



**CENPALAB**

**40 ORGANIZATIONS** 超过40个研发中心

**12 000 WORKERS**超过1.2万名工作人员

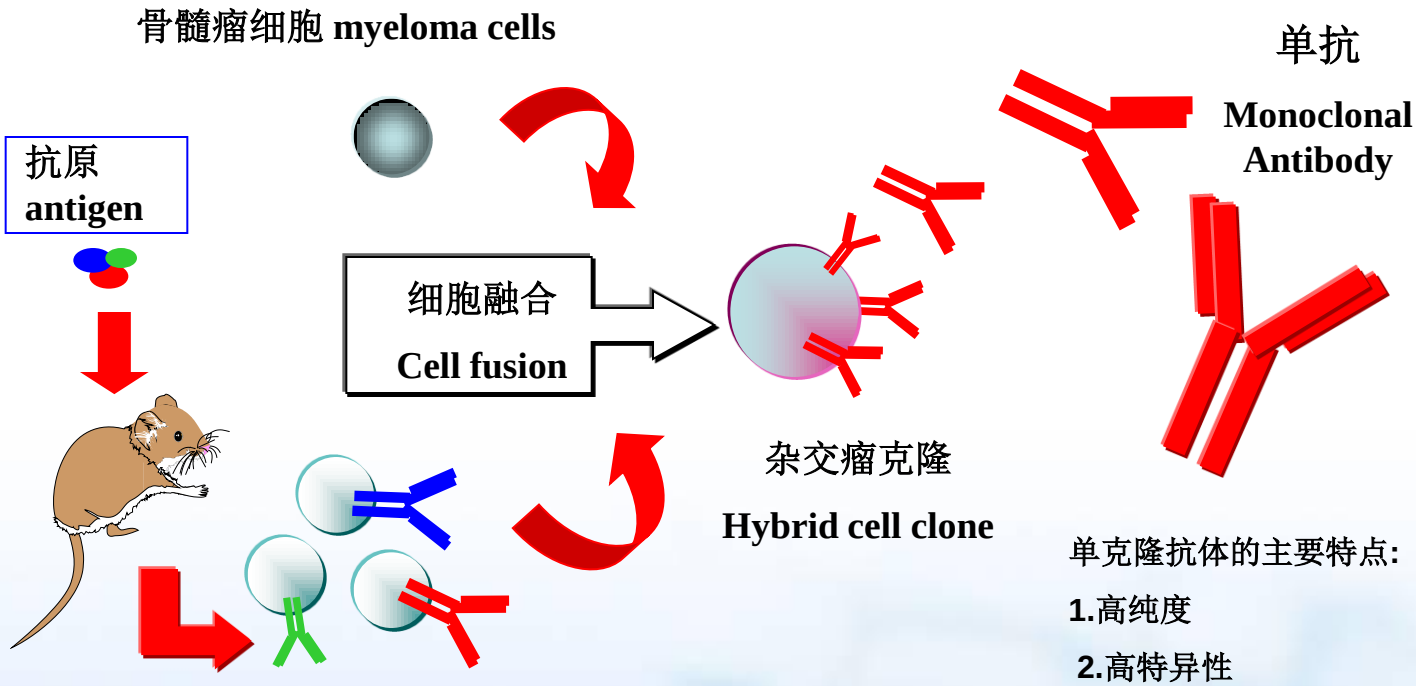
**7 000 SCIENTISTS and ENGINEERS**超过7千名科学家和工程师

**150 RESEARCH PROJECTS** 超过150个研发项目

# PART IV.
# Fermentation & Separation of Antibody from Mammalian Culture

# 来自古巴的动物细胞发酵生产抗体技术

## 单克隆抗体的来源h-R3　　Preparation of Monoclonal Antibody h-R3




单克隆抗体的主要特点:

1.高纯度

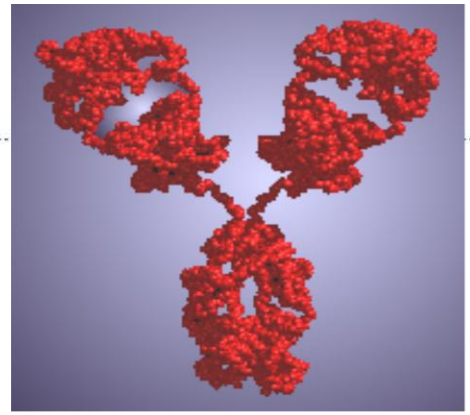
2.高特异性

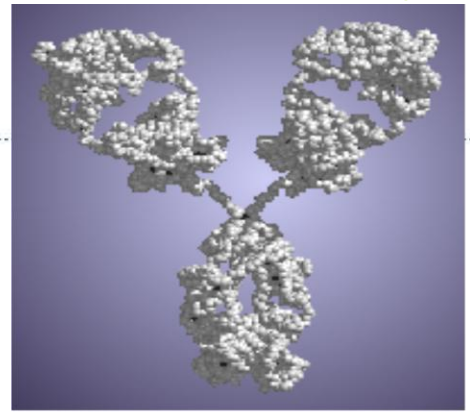
3.高重复性

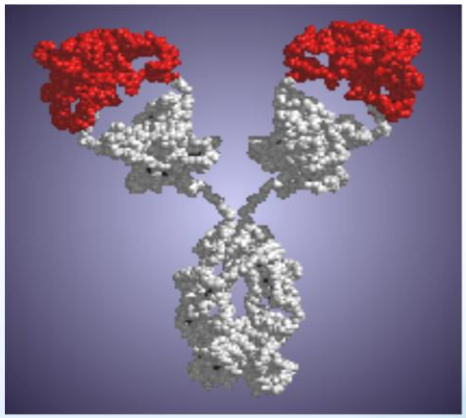
high purity, specificity and activity

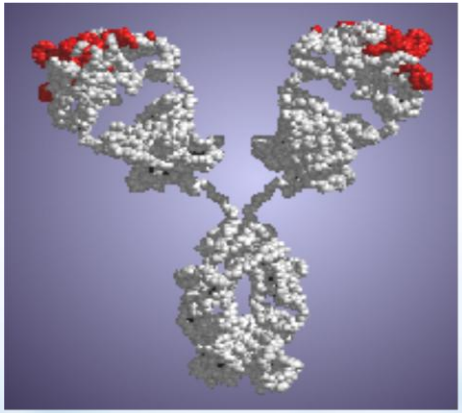
鼠源单抗 murine antibody

人Humanized Antibody









嵌合单抗
Combined antibody
30 % 鼠
70 % 人

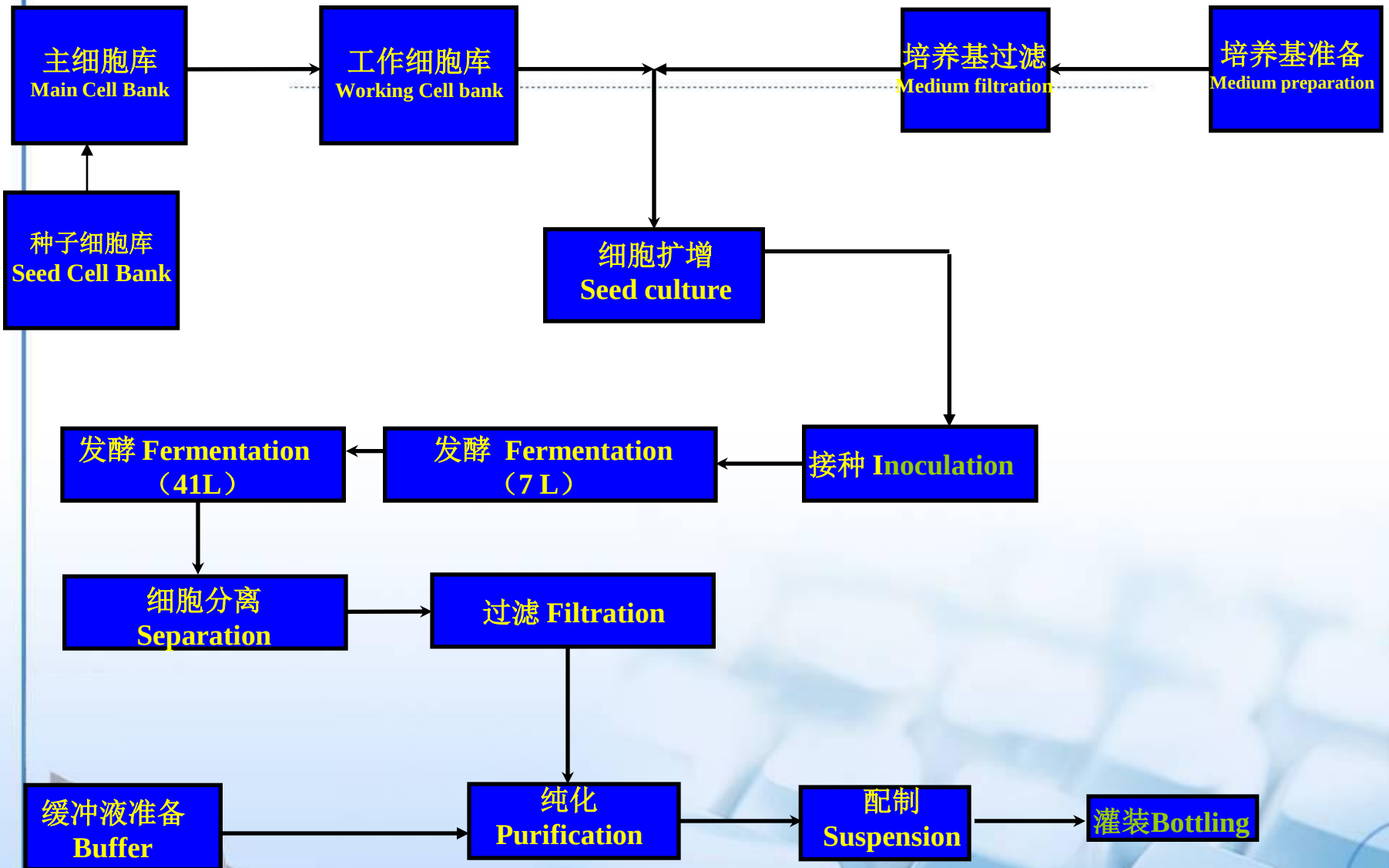
人源化单抗
Humanized antobody
5 % 鼠
95 % 人

高

HAMA氏免疫排斥反应
immune reaction

低

# h-R3生产工艺流程 Summary of producation process



## *细胞扩增 Seed culture*

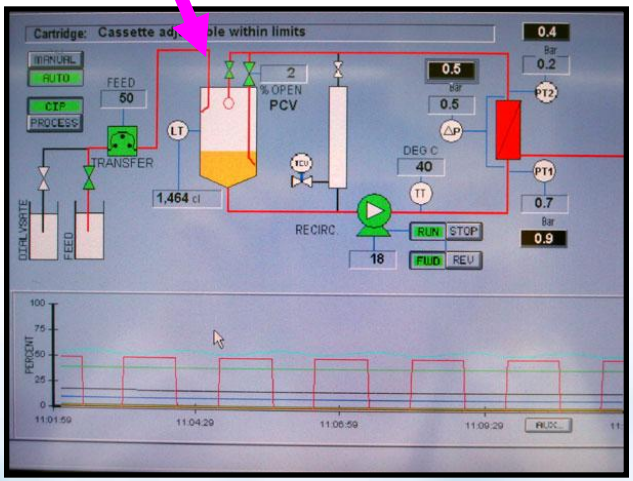
动物细胞对周围环境十分敏感；对理化因素敏感，如：渗透压、pH、离子浓度、剪切力、微量元素等。细胞生长需贴附于基质,并有接 触抑制现象。

Animal cells are more difficult to culture than microorganisms because they require many more nutrients and typically grow only when attached to specially coated surfaces.

Culture condition: Serum free media at 37$^{o}$C and 7.3 pH.

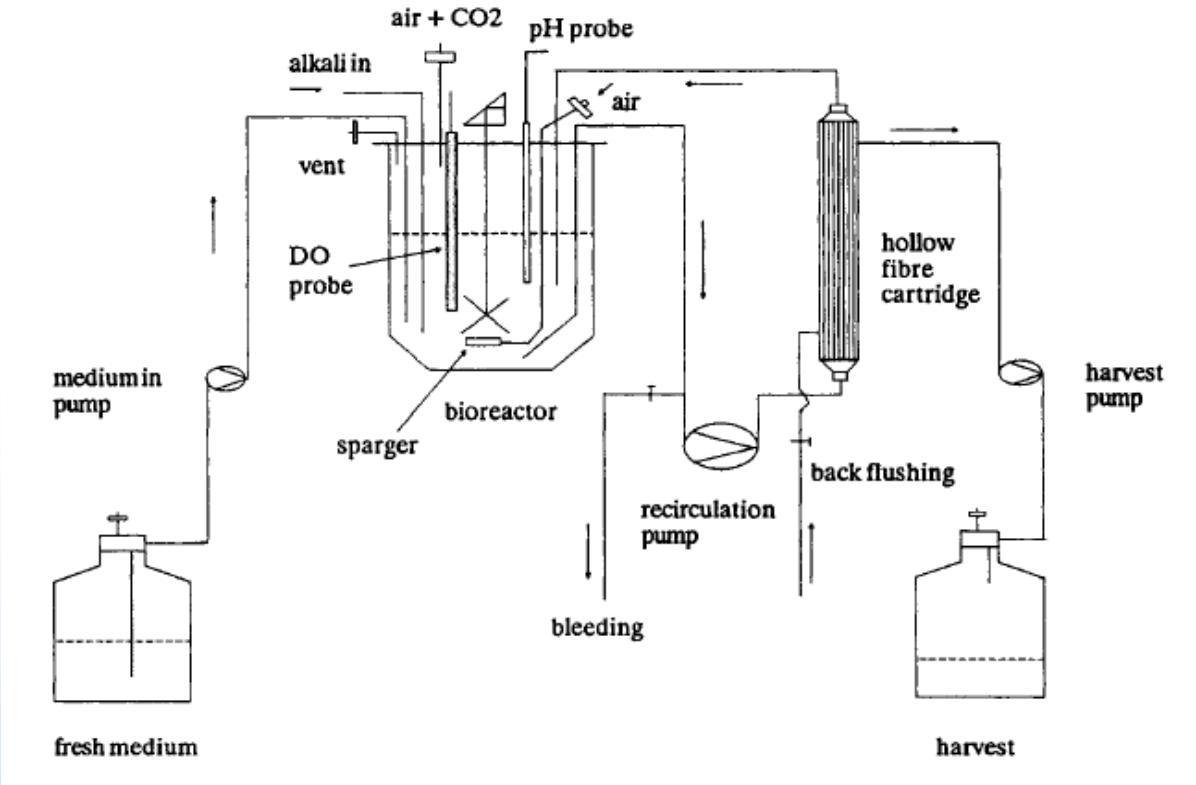
# 发酵 *Fermentation and harvest processing*

biosensor



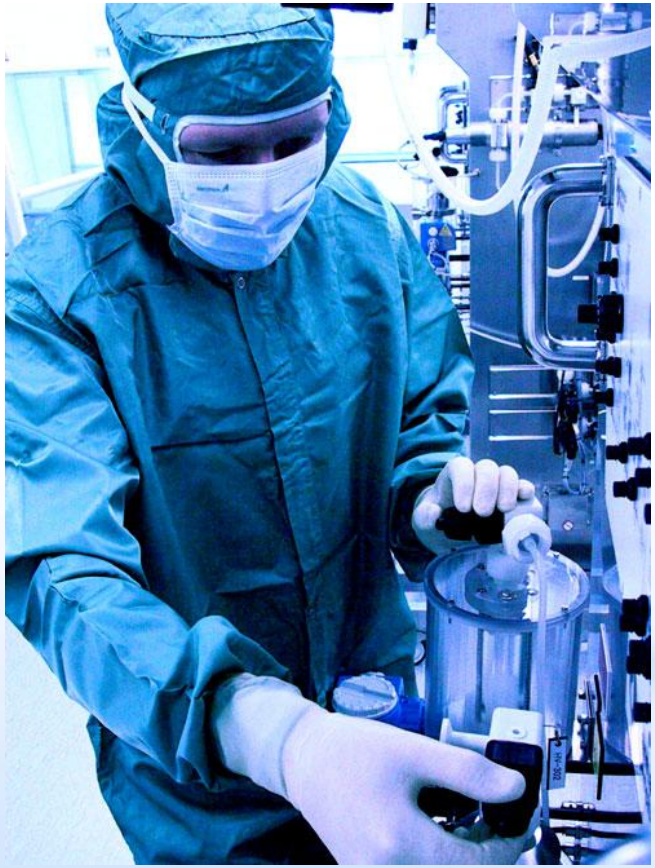
# *灌流式细胞培养 Perfusion culture*

细胞和培养基一起加入反应器，反应过程中不断地将部分条件培养基取出，同时不断地补充新鲜培养基。它与连续式操作不同之处在于取出部分条件培养基时，绝大部分细胞仍保留在反应器内，而连续式培养则同时也取出了部分细胞。

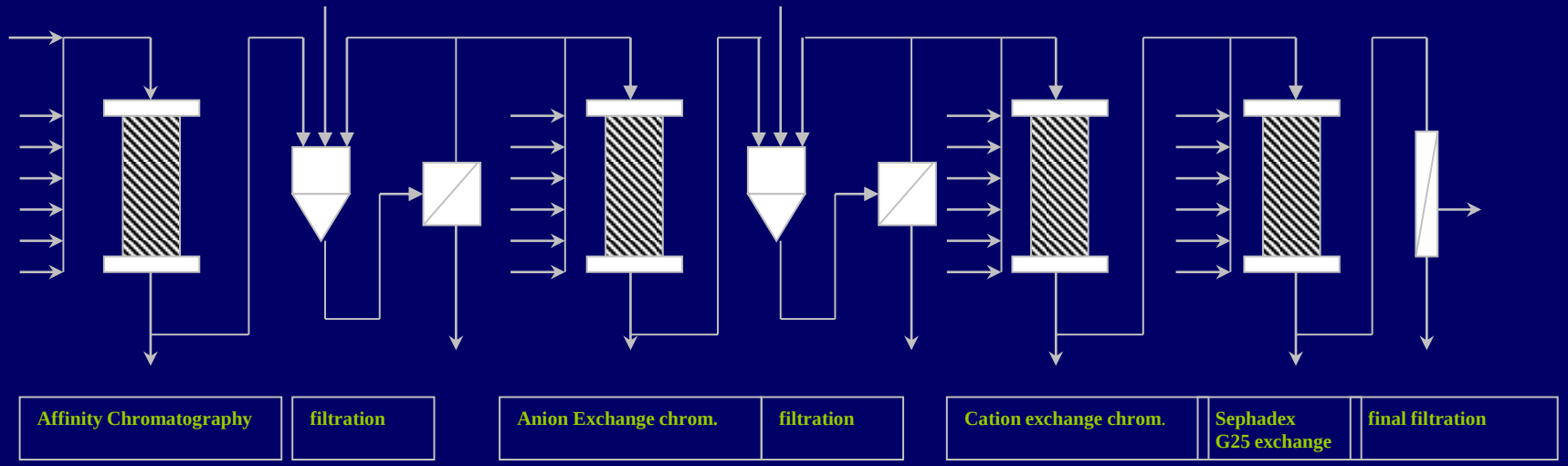



S. Zhang, A. Handa-Corrigan,and R.E. Spier, BIOTECHNOLOGY AND BIOENGINEERING, VOL. 41, NO. 7, MARCH 25, 1993

# 大规模纯化　　Large-scale purification



# 純化 Purification procedure



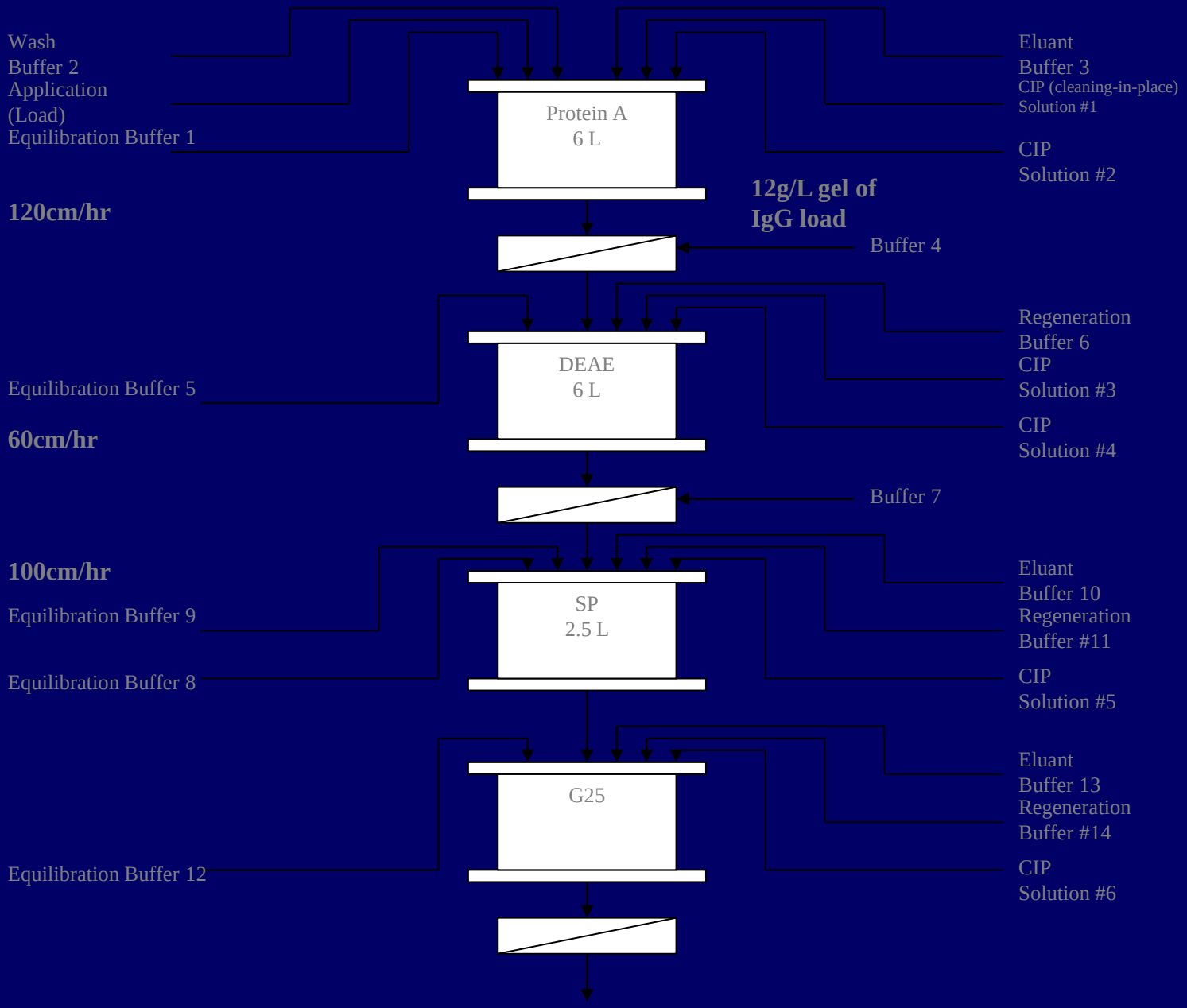

## Affinity Chromatography

- Used in many applications
- Purification of substances from complex biological mixtures
- Removal of small amounts of biomaterial from large amounts of medium broth

## Ion Exchange Chromatography

- Principle is to separate on basis of charge "adsorption"
- Positively charged proteins are reversibly adsorbed to immobilized negatively charged beads/polymers
- Negatively charged proteins are reversibly adsorbed to immobilized positively charged beads/polymers

# 纯化工艺示意图 Purification process



# PART V.

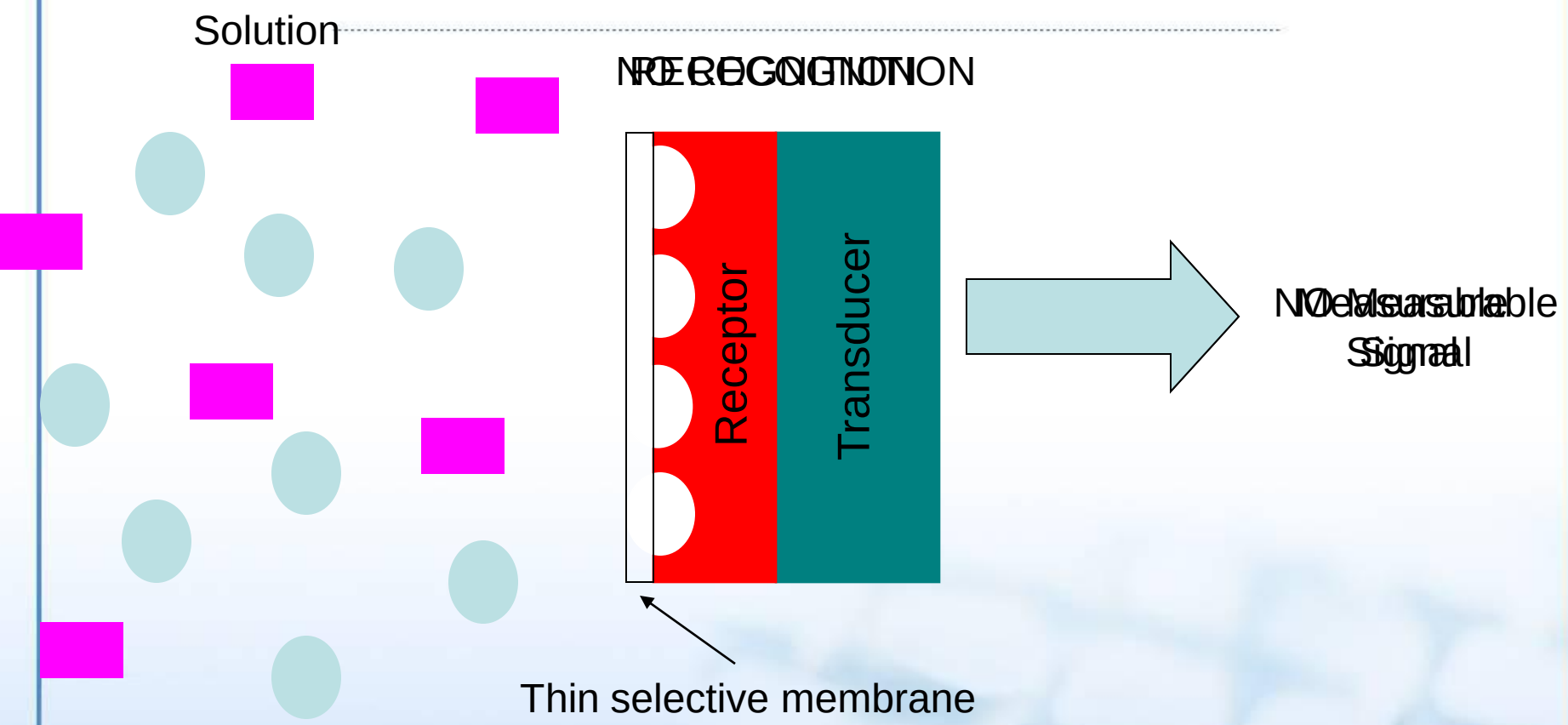
# Mechanism & Fabrication of Biosensor Based on Enzyme Technology

# 生物传感器—大规模发酵技术的过程控制

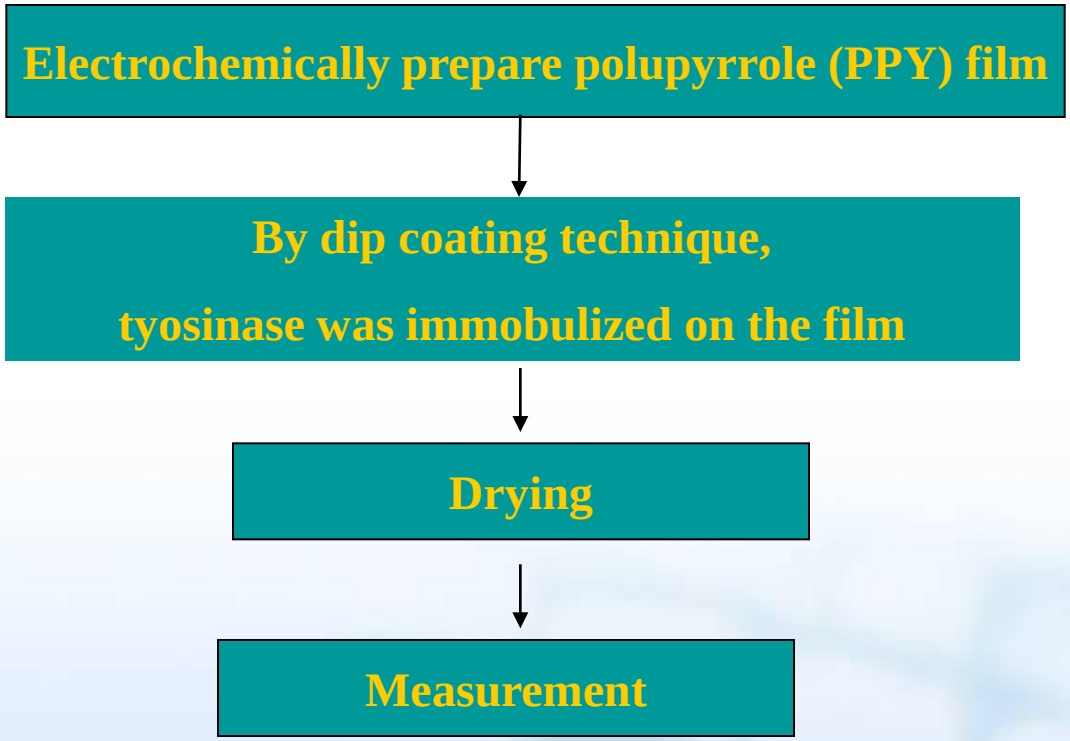
## Conducting Polymer BASED BIOSENSOR

Biosensors are analytical devices widely used due to the possibility to combine the specificity of biomolecules with the electroanalytical methods. The biomaterial, giving the selectivity to the system, is immobilised in some inert matrix and fixed to the surface of the basic electrode. In oxygen sensor based systems, where the oxygen content is studied, oxidoreductases are used as the selective component of the biosensor most frequently.

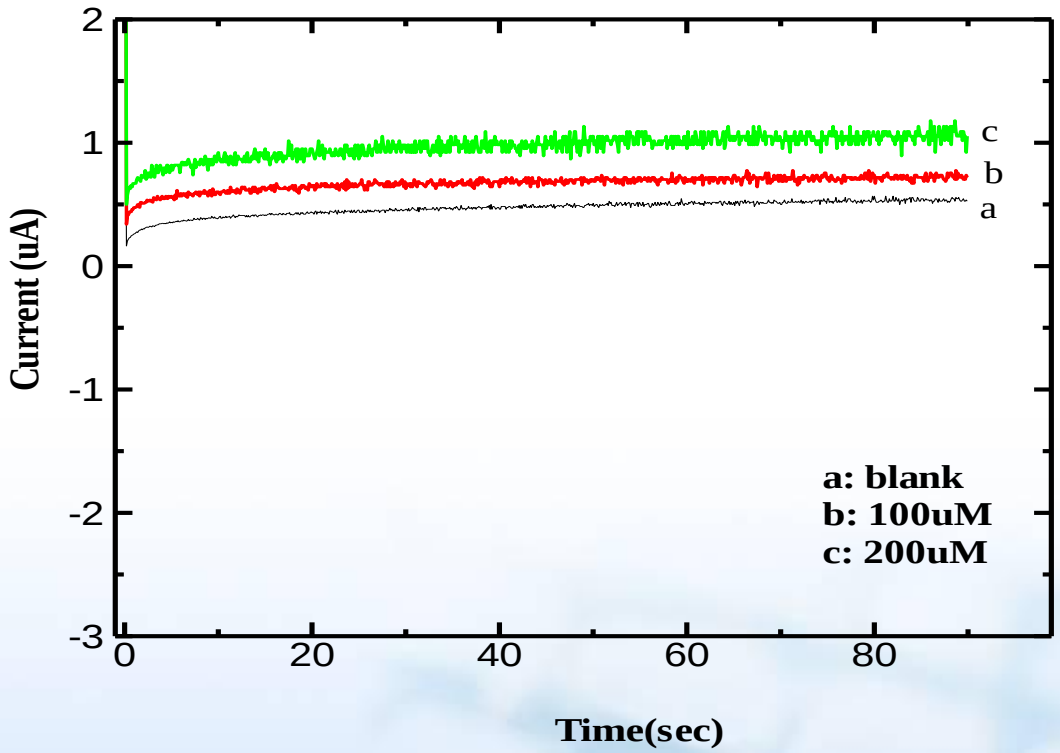
# Mechanism of a Biosensor



# Fabrication Methodology



# Typical steady state current response of the biosensor




Typical steady state current response of the biosensor to an increasing concentration of phenol

# Cymo B.V. , The Netherlands

**Cymo B.V. , The Netherlands is a company who is expert on medical and industrial endoscopy imaging and voice instruments.**

**With 40 years research experience on voice instruments and 15 years endoscopy equipments developing history, Cymo B.V., a spin-off company of University of Groningen, the Netherlands, is officially registered on June 8, 2007. The company is supported by Technology Foundation STW, a branch of NWO, the Dutch research council, and Groningen Voice Research Lab (HKS).**


## About us

# 谢谢大家！