Vantaggi e problematiche dell’approccio enzimatico per la produzione di bioetanolo da biomassa lignocellulosica

University of Milano Bicocca

Department of BioTechnology and BioSciences

- The Future is ... under way!!
- 5+1 Requirements for scale up
- How is EtOH produced from starch (1st gen)?
- How is EtOH produced from biomass (2nd gen)?
- What are the key biological barriers
- What and How at Bicocca
- Not only EtOH!
- Not only Biofuels!
- Conclusions
- Acknow.
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## Requirements for industrial scale-up

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- Continuous R & D: adaptation of (micro)organisms to technical constraints (development of superior hosts)

**Starting cheap – ending cheap?**

The proper choice of the substrate is crucial

Gain and/or Loss of functions lead(s) the way to production (gr/L), productivity (gr/l/hr) and yield (gr/gr).

Bio-process optimization: technical support of natural or engineered abilities

Starting cheap – ending cheap?

Purification of product adds significantly to the production costs

**Not only EtOH !**

**Not only Biofuels !**

**Conclusions**

**Acknow.**
Patents and Patent Applications

- 1997: Yeast Strains for the Production of Lactic Acid, (Patents)
- 2000: Ascorbic Acid Production from Yeasts, (Patents)
- 2000: Production of heterologous proteins from Z. bailii, (US Patent)
- 2002: Process for expression and secretion of proteins by the non-conventional yeast Zygosaccharomyces bailii (Patents)
- 2005: Ascorbic acid production from D-glucose (Patents)
- 2005: Improved strains for the production of organic acids (Patents)
- 2006: Improved strains for the production of organic acids (Patents)
- 2007: Increase in stress tolerance with ascorbic acid during fermentation (PCT patent application)
- 2008: Methods for improving acid and low pH tolerance in yeast (PCT patent application)
- 2008: Improved yeast strains for organic acid production (EU Patent application) University of Milano-Bicocca
- 2009: A new one will be filed within 3 months (EU Patent application) University of Milano-Bicocca

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How is EtOH produced?

1st Generation

1. Pretreatment (Heat, pressure, or acid treatments).
2. Solid-Liquid Separation.
3. Enzymes Production.
4. Cellulose Hydrolysis.
5. Fermentation of Cellulosic and Hemicellulosic Sugars (C5 and C6)

A. Milling.
B. Liquefying and Heating the Corn meal.
C. Starch Hydrolysis.
D. Fermentation of Starch Sugar (C6)

2nd Generation

E/6. Distillation (96%)
F/7. Dehydration.

US Department of Energy - Office of Science
How is EtOH (2\textsuperscript{nd} Gen) produced?

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1. Biomass production and delivery: main challenges

- Sequence DNA from energy crops
- Identify genes and pathways that improve biomass productivity
- Develop crops optimized for enzyme degradation

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2. Pretreatment: main challenges

- Identify enzymes that rescue the severity of pre-treatments
- Minimize production of inhibitory by-products

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3. Cellulose hydrolysis: main challenges

- Screen natural environments for the most efficient enzymes produced by fungi, bacteria and animals
- Understand how enzyme system(s) interact with cellulose
- Increase the catalytic rate and thermal tolerance of enzymes

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4. Sugar fermentation: main challenges

- Integrate Biomass hydrolysis and fermentation
- Engineer metabolic pathways to produce diverse biofuels
- Increase product tolerance and yield
- Develop strains capable to efficiently fermenting mixed substrata
- Engineer strains to increase tolerance to by-products

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Novel high performance Enzymes and Micro-Organism for conversion of lignocellulosic biomass to bioethanol

WP2: Efficient enzymes
- Screening from nature and culture collections
- Metagenomic and cDNA libraries
- Protein engineering

WP3: Optimal enzyme mixtures
- Hydrolysis efficiency
- Enzyme induction
- Feasibility of production

WP4: New strains through mutagenesis and screening
- Screening from nature and culture collections
- Mutagenesis
- Evolutionary engineering

WP5: Targeted strain engineering
- Pathway engineering
- Sugar uptake engineering
- Redox & energetics
- Metabolic modelling

WP6: Process assessment
- Biomass hydrolysis and fermentation
- Process options (SHF, SSF, modified)
- Temperature regimes
- Pilot scale assessment

WP7: Process calculations
- Economics & ecoefficiency
- Optimal process regimes

Novel micro-organisms

Novel enzymes
- Thermo and mesophilic enzymes with improved efficiency, and reduced nonproductive binding
- Optimal enzyme mixtures for biomasses of European relevance

Ecoefficient and economic process options for efficient 2nd generation bioethanol production

2009-2013

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EU Framework VII - NEMO

- Acetic Acid 60mM pH 3
- Lactic Acid 45 g/l pH 3
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**BBC** is a structure based on the most advanced scientific and technological platforms required for upstream, bioreactor production, downstream and analysis of the product (GMP-like env.)

**BBC** is part of **CEBIB** (Centre of Excellence Bicocca Industrial Biotechnology), Regione Lombardia - Università degli Studi di Milano Bicocca.
5th Floor - Upstream and Production

4th Floor - Downstream and Analysis
What is a good Biofuel?

(a) … it is highly combustible, but not explosive;
(b) … it is something with a high energy to mass ratio;
(c) … it is stable for long-term storage;
(d) … it is transportable;
(e) … it is inexpensive;
(f) and it is renewable!!
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For millennia, human societies …

… depended on biological sources for energy and materials. Plants were combusted for heat, used for building materials and clothing. Animals were used for building materials and animal power was harnessed for transportation.

A little bit more than a century ago, society underwent a **HUGE** transition: from horse to automobile, from whale oil to crude oil.

That time was similar to today in that many material and fuel sources were being tested, production was somehow settled, but this new industry was not yet integrated and consolidated.

... a similar situation is under way ...
Conclusions

The Future … is under way …

-Presidenza del Consiglio dei Ministri

Comitato Nazionale per la Biosicurezza, le Biotecnologie e le Scienze della Vita

- ItSusChem

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… sviluppare vere Bioraffinerie per favorire la produzione di fine-chemicals, bioprodotti, biomateriali e biocombustibili dalle proprietà superiori da impiegare nelle applicazioni esistenti, o con caratteristiche innovative per nuove applicazioni industriali, con particolare riferimento ai processi biotecnologici in uso nell'industria chimica, nutraceutica, alimentare, farmaceutica, cosmetica, tessile, etc…

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ACKNOWLEDGEMENTS

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...thanks for your attention...

It is a joint challenge …

Paola Branduardi

Simone Passolunghi

…and ALL the past and current (2009) young Researchers:

Dato Laura Fossati Tiziana

Codazzi Vera Rossi Giorgia

Longo Valeria Posteri Riccardo Solinas Nicola