

# **Recent Applications in Bioprocess Engineering**

## **Hämeenlinna, Finland 2005**

**Proceedings**



**Helena Kautola & Tuija Pirttijärvi (ed.)**

**Recent Applications in Bioprocess Engineering  
Hämeenlinna, Finland 2005**

**Helena Kautola & Tuija Pirttijärvi (ed.)**

**HAMK University of Applied Sciences**

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Helena Kautola & Tuija Pirttijärvi (ed.)

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## Forewords

The “Symposium on Recent Applications in Bioprocess Engineering” 9-10 November 2005 held in Hämeenlinna City Hall was the second seminar of the series of International Bioprocess –seminars in Hämeenlinna, Finland.

The first seminar “Challenges of Biotechnology in the Future” took place in Vanajanlinna 22nd May 2002 with lecturers Prof. Murray Moo-Young, University of Waterloo, ON, Canada and Prof. Carmen Prieto, Universidad de Chile, Santiago de Chile.

The “Symposium on Recent Applications in Bioprocess Engineering” in 2005 was a jubileum seminar for the 5-year old Bioprocess Technology Laboratories at Häme Polytechnic (HAMK University of Applied Sciences). Because of this, the theme of the seminar followed the subjects close to the performance of the laboratories. The first day was related to food technology and the second day was related to environmental biotechnology and bioenergy. The seminar was also extended with a poster session.

In both seminars Deputy Director General Paula Nybergh from the Finnish Ministry of Trade and Industry has honoured us with giving the opening lecture about the overview on Finnish biotechnology on different aspects.

The next International Bioprocess –seminar in Hämeenlinna will be held on 12th of June 2007 in Visamäki, which is the year of a 10-year old Degree Programme of Biotechnology and Food Engineering at HAMK University of Applied Sciences.

The organizer Degree Programme of Biotechnology and Food Engineering at HAMK University of Applied Sciences thanks all the participants of the seminars and wishes a fruitful co-operation also in the future.

Hämeenlinna 18th May 2007

Helena Kautola and Tuija Pirttijärvi



- Seminar at Häme City Hall



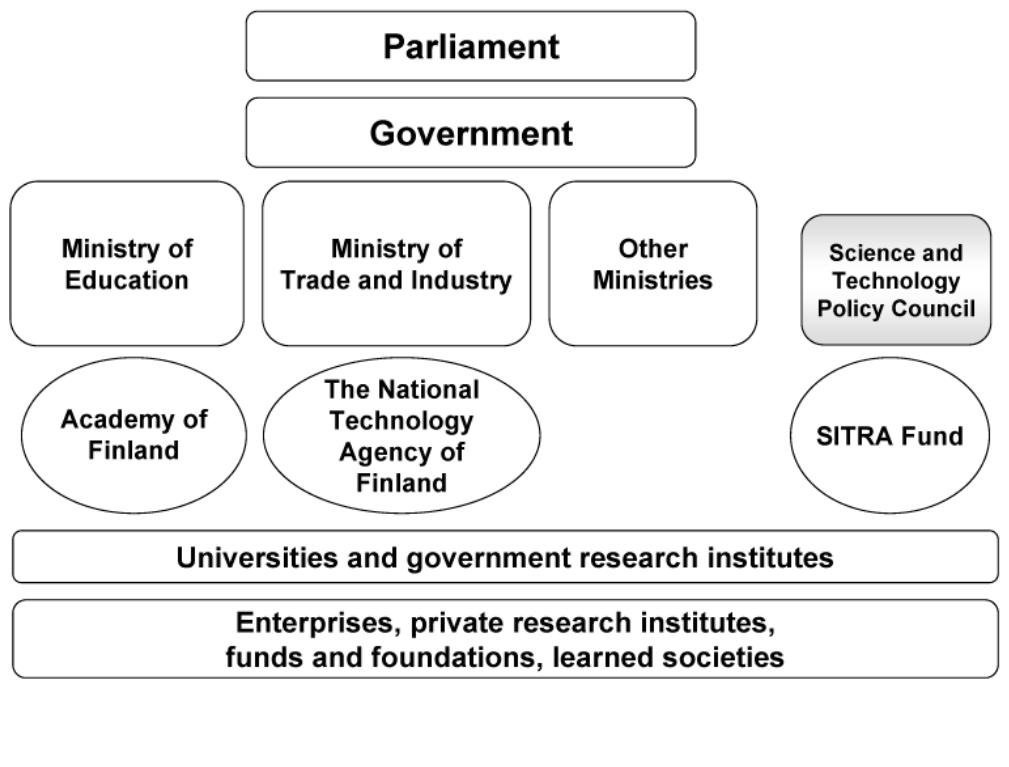
- Prof. Verstraete (left), prof. Tavares, prof. Bisping, dr. Kautola, prof. Rymovicz, prof. Vasavada and dr. von Weymarn

# Finnish innovation policy and biotechnology

Paula Nybergh  
Deputy Director General  
Ministry of Trade and Industry  
Technology Department

## Innovation policy

- Increase in r&d –funding
- From science and technology to innovations
- Utilisation of r&d results
- Development of public research structures
- Productivity programme
- Regional policy



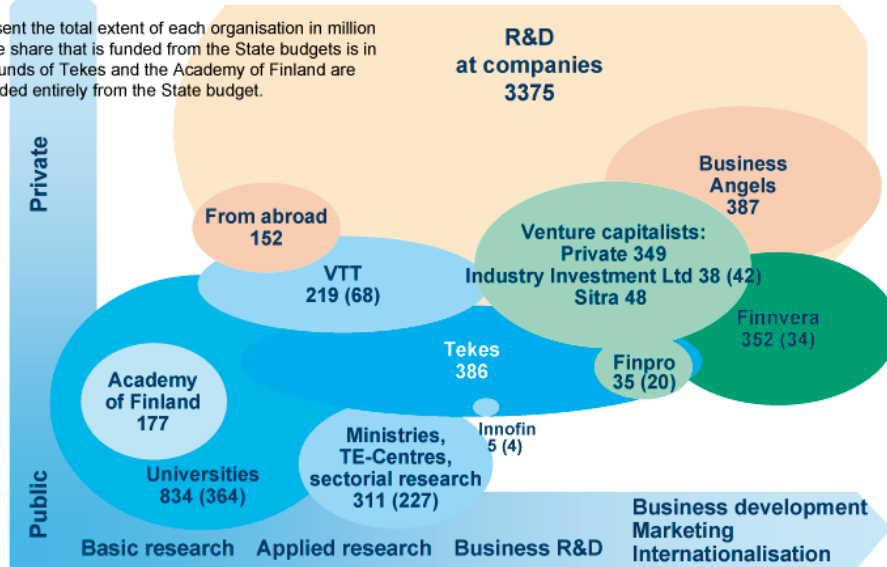
## Key actors of the Finnish innovation environment





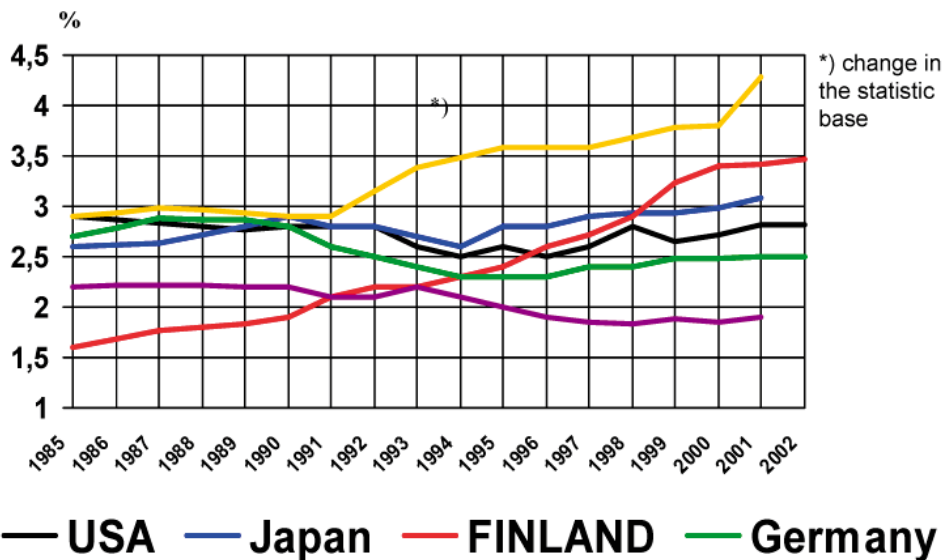
## Innovation system Resources and funding in 2002

The figures represent the total extent of each organisation in million euros in 2002. The share that is funded from the State budgets is in brackets. The funds of Tekes and the Academy of Finland are funded entirely from the State budget.



Source: Tekes 04-2004

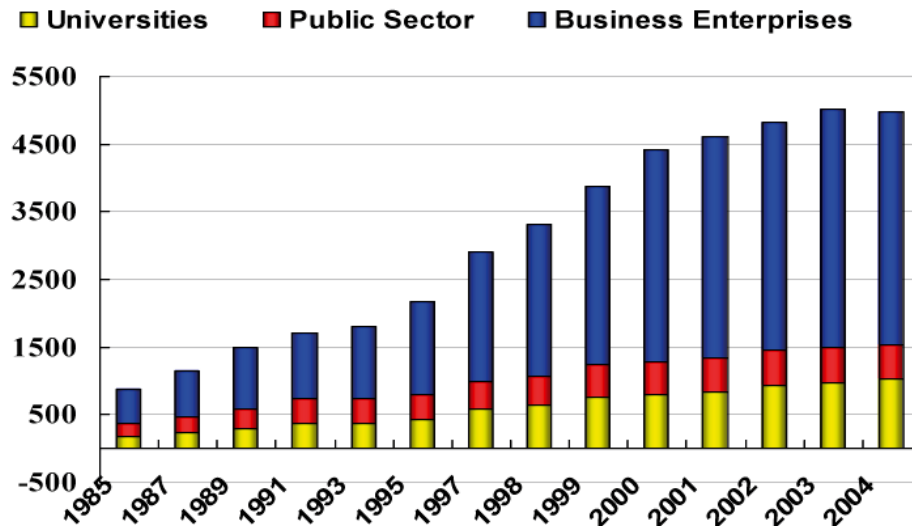
## SHARE OF R&D IN GDP IN SOME OECD COUNTRIES



Source: OECD/MSTI 2003/2, European Commission/Key Figures 2003-2004 and national statistics agencies

6/2004

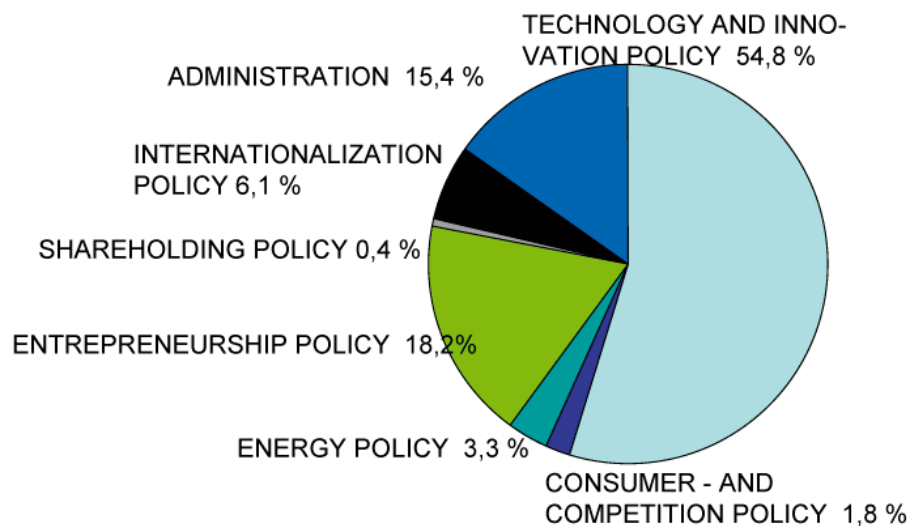
## R&D EXPENDITURES BY SECTOR 1985-2004 (million €)



3/2005

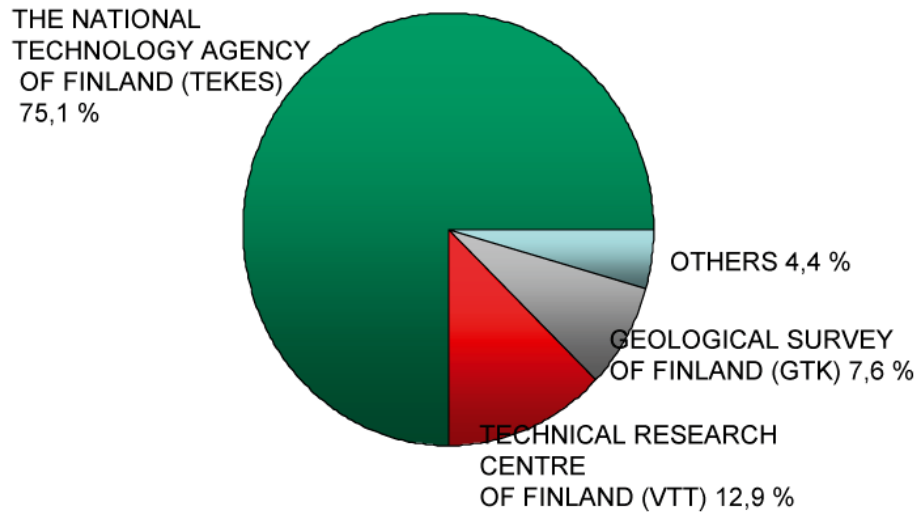
Source: Statistics Finland

## MTI Budget 2005 Total 976 milj. €



3/2005

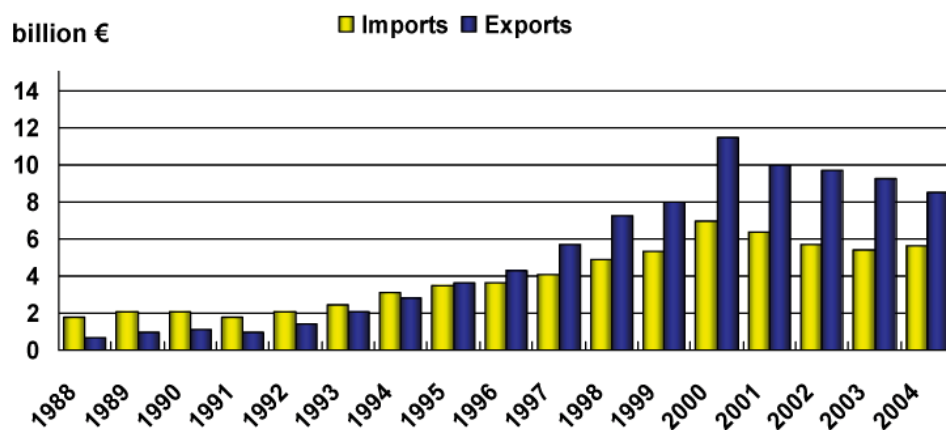
## TECHNOLOGY AND INNOVATION POLICY BUDGET 2005 Total 535 milj. €



3/2005

## FINNISH TRADE ON HIGH TECH PRODUCTS

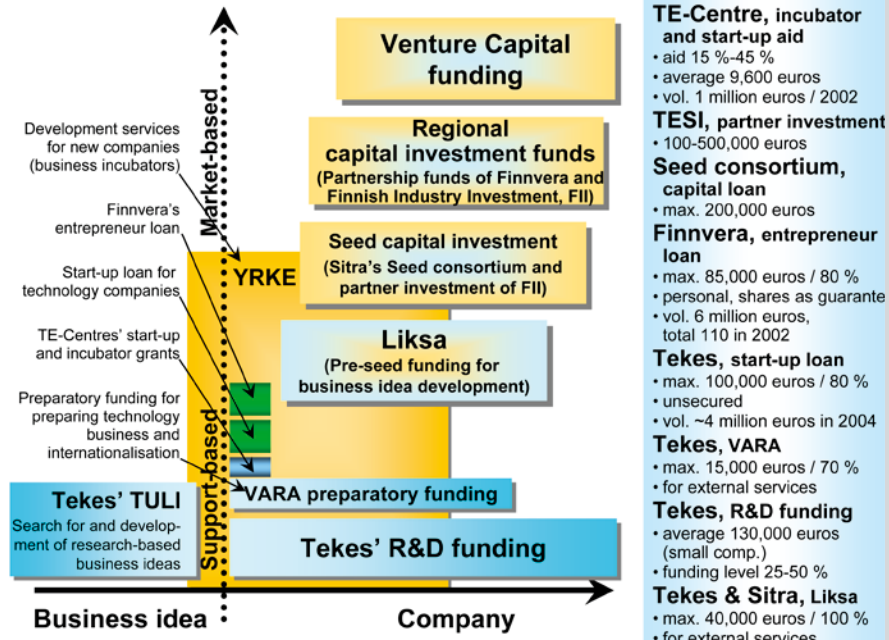
Finnish high technology exports totalled € 8,5 billion  
and imports 5,6 billion in 2004



3/2005

Source: National Board of Customs

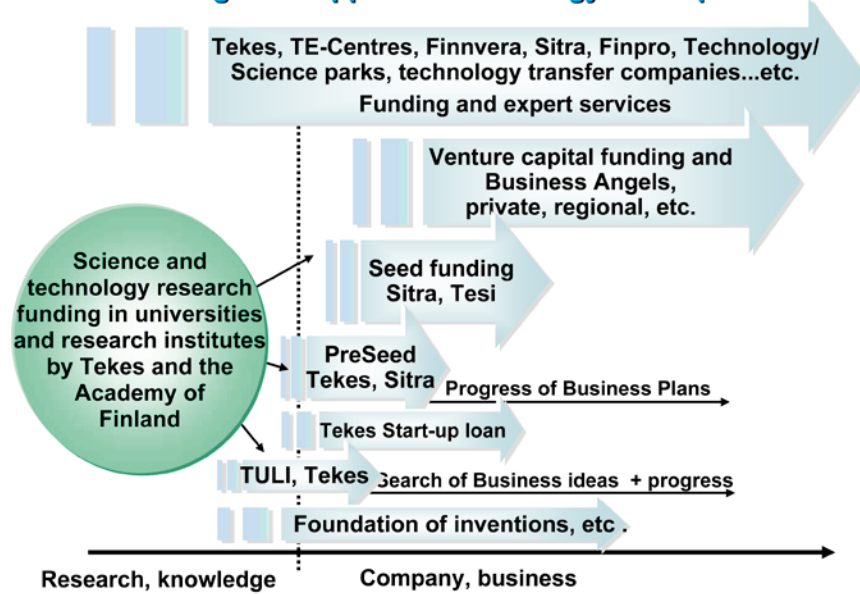
## Funding for new technology companies



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## New business start up's

### Funding and support of technology start up's



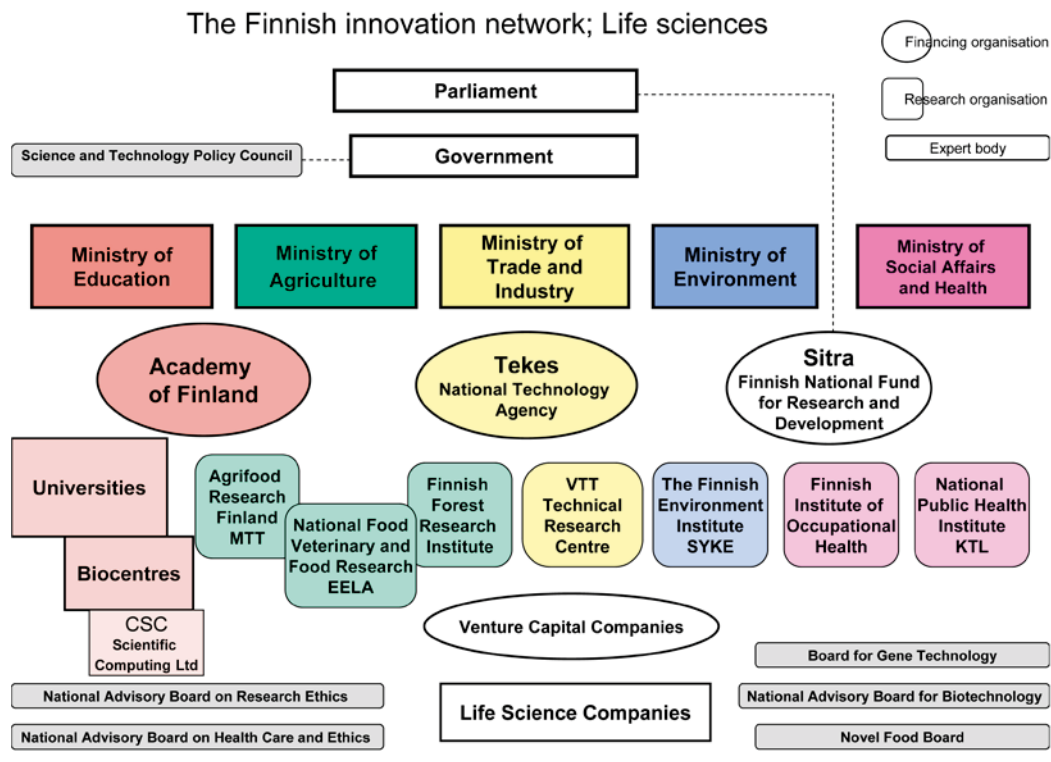
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## Biotechnology and Europe

- A key area in science and technology policy
- Financing available for r&d
- Lack of seed and venture capital
- Understanding of biobusiness – in different bioareas
- Unclear and unpredictable regulation

## Biotechnology in Finland

- Special public programmes:
  - The National Technology Agency Tekes, since 1984
  - The Academy of Finland, 1987
  - The Ministry of Education, 1987
- Several evaluations
- number of biocompanies



## Biocenters

- 5 Biocenters:
  - Helsinki: Bioinstitute in Viikki and Biomedicum in Meilahti
  - Turku
  - Oulu
  - Tampere
  - Kuopio

## Other biotech units

- Espoo/Otaniemi: TKK and VTT
- Other universities
- Other research institutes
- Polytechnics

- Basic sciences
- Medical biotechnology
- Plant biotechnology
  - Bioinformatics
- Bioprocess technology

## What do we need in the future

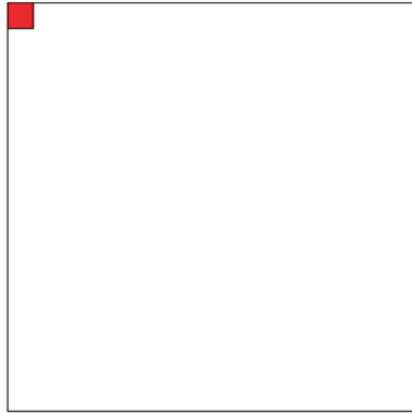
- High level research and training
- Multi/cross disciplinary research
- Business skills
- Entrepreneurship
- Understanding of customers' needs
- Clear and predictable legislation and regulation

Is there a future for bioinventions?

Yes, but  
Lot of work and  
co-operation  
is still needed



## Finland in Global R&D



## Novel applications of lactic acid bacteria

**Group Manager, D.Sc. Niklas von Weymarn**  
**VTT Technical Research Centre of Finland, Espoo, Finland**

### Introduction

Lactic acid bacteria (LAB) are a large group of Gram-positive, non-spore forming rods or cocci that are functionally related to each other by their ability to produce lactic acid as a sole or major end product of fermentation. They prefer anaerobic conditions, but some species also tolerate oxygen in low concentrations. LAB are part of the normal microbial flora of healthy humans, where they proliferate in body cavities such as the oral cavities, the gastrointestinal (GI) tract and the female urogenital tract. On the other hand, some streptococci are human pathogens and certain LAB are responsible for the formation of dental plaque and the initiation of dental caries.

The first classification of the LAB group, based on appearance, fermentative pathway, growth at 10 °C and 45 °C, and range of sugar utilization, was introduced by Orla-Jensen in 1919. Since then the LAB have been divided into two sub-groups based on their fermentative metabolism: 1) the homofermentatives that produce two moles of lactate from one mole of glucose, and 2) the heterofermentatives that produce one mole of lactate, carbon dioxide and ethanol each from one mole of glucose. Since the introduction of the molecular-level identification methods some 15 years ago many genera and species have been re-named. Genetically, the LAB are highly diverse with GC contents varying from 34 to 53 % (Renault, 2002). Most LAB (e.g. *Lactobacillus*, *Leuconostoc* and *Lactococcus* sp.), however, belong to a sub-group having a GC content below 50 % (Coenye and Vandamme, 2003).

The first pure culture of a lactic acid bacterium was obtained in 1873 by Joseph Lister, when he succeeded in isolating *Bacterium lactis* from soured milk (Axelsson *et al.*, 1998). The species was later re-named first to *Streptococcus lactis* and then to *Lactococcus lactis* subsp. *lactis*. In the beginning of the 20<sup>th</sup> century the use of LAB in manufacture of edible fermented food products began its development into the controlled food and feed fermentation industry we know today. In industrialized countries, about 1/3 of the food is modified by fermentation, usually utilizing blends of defined LAB strains, so-called starter cultures, but also the raw materials' natural strains (Schmid, 2003). Because of the long use and safe track record in foods many LAB have a GRAS-status (generally recognized

as safe). In industrial fermentations LAB contribute to e.g. conservation, flavour and texture.

A more recent, but highly popular application of LAB is the field of functional foods and especially use of LAB as so-called probiotics. It has been shown that the consumption of certain LAB strains, especially members of the *Lactobacillus* genus, offer a number of benefits to the human and animal health and well-being. The phenomena behind this are still not fully understood, but they include competitive exclusion of pathogens, stimulation of mucosal immunity, and/or a positive effect on the normal intestinal microflora. The probiotic effect is often a result of a metabolite(s) produced by bacteria. For instance organic acids, carbon dioxide, hydrogen peroxide, diacetyl, bacteriocins (e.g. nisin), low molecular weight substances (e.g. reuterin), and adhesion inhibitors are believed to be important in creating the health-beneficial effects.

The use of LAB as cell factories is also increasing in importance. In a cell factory the cells of a specific microbe are used to produce a high-value product. The product is typically separated from the cells and purified to high purity and then sold to another industry as raw material. Industrial LAB cell factory products include L-lactic acid, nisin and dextrans. L-lactic acid is used as the raw material for production of e.g. biodegradable polylactide polymers (PLA), nisin is used as a natural food preservative, and dextrans in manufacture of plasma expanders and Sephadex® gels and beads for protein separation.

Much of the early genetic engineering development was focused on LAB. For instance, the transformation techniques suitable for many LAB species were available already in late 1980s (Luchansky *et al.*, 1988). Since then, several food-grade cloning systems and expression vectors have been developed (see list in Renault, 2002). To date, the most widely used expression system is utilizing nisin as the inducer (de Vos and Hugenholtz, 2004). Notably, about twenty LAB genomes are being sequenced or have already been sequenced (de Vos *et al.*, 2004). According to the latest information (de Vos *et al.*, 2005) the number of completed LAB genome sequences is over a dozen. These include species like *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*. Not all sequences are, however, publicly available. The figures also contain some parallel work, i.e. genomes from different strains of the same bacterium have or are currently sequenced. In general, the LAB genomes are fairly small ranging from about 2 to 4 Mb (Klaenhammer *et al.*, 2002).

## New developments

Many of the starter culture strains used for food processing have been improved by either natural or random mutagenesis techniques. The strain improvement efforts have aimed at enhancing the suitability of the strains for industrial processing, increasing the reliability of the food making processes, as well as improving the safety (e.g. increase phage resistance) and quality (e.g. flavour development and batch-to-batch uniformity) of the end-product.

The many studies on physiology and biochemistry as well as the developments in genetic engineering, the simple metabolism and the availability of complete genome sequences make LAB interesting targets for metabolic engineering. Hence, LAB metabolic engineering has received extensive attention (see review by de Vos and Hugenholtz, 2004). It still, however, seems unlikely that metabolically

engineered strains would be accepted for industrial food use in the near future, because of the suspicion consumers may have concerning the use of such genetically engineered organisms. Metabolic engineering in combination to red (medical and health) and white (industrial) biotechnology areas hold currently more promise. Examples of novel LAB white biotechnology application developments include e.g. the production different sugar alcohols (von Weymarn *et al.*, 2003; Aarnikunnas *et al.*, 2003; Nyysölä *et al.*, 2005), ethanol (Gold *et al.*, 1996), L-alanine (Hols *et al.*, 1999), and diacetyl (Hugenholtz *et al.*, 2000).

The mechanisms by which probiotic bacteria exert their effect are not fully understood. Hence, it still requires significant basic research to enable rational selection and controlled modification of probiotic strains. One key element behind the benefit of probiotics is believed to be the ability of LAB to adhere to specific regions of the GI tract. In fact, both live and inactivated LAB cells have been shown to affect the mucosal immune response of humans and animals. As an example, the addition of inactivated *Lactococcus lactis* and *Lactobacillus plantarum* cells was shown to induce the production of IL-12 and IFN- $\gamma$  in murine spleen cell cultures (Repa *et al.*, 2003). Studies of the interaction are also believed to open up new ways of preventing allergic diseases.

Other attractive red biotechnology applications include the use of LAB as live delivery vehicles for biologically active compounds, such as therapeutic proteins, antibodies, enzymes and vaccines. An example of this strategy was introduced by Robinson and co-workers (1997), where oral intake of recombinant *Lactococcus lactis* cells expressing the fragment C of tetanus toxin successfully elicited a protective immune response against tetanus in mice. In another example, Steidler (2000) developed a recombinant Interleukin-10 (IL-10)-producing *Lactococcus lactis* and used it as the delivery vehicle of IL-10 to the colon of mice for treatment of inflammatory bowel diseases.

Finally, a biosensor applying genetically engineered *Streptococcus thermophilus* cells has been developed for the detection of biocides in milk.

The presentation will focus on new cell factory-type of applications of LAB.

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## **Lactose-free milk drinks – new products with new technology**

**Olli Tossavainen / 26.10.05**

Completely new types of product are only rarely generated in dairy product markets. Valio lactose-free milk drinks, however, fits the description because they possess some genuinely new properties. Lactose-free milk drink differs from low-lactose milk in that it contains no lactose (residual lactose <0.01%) and tastes like normal, pasteurized milk. In fact, the product has already attracted new consumers as milk drinkers – both those who had given up milk and those who do not favour the sweetness of low-lactose milk. New chromatographic and membrane technologies have made these products possible.

### **Lactose intolerance in Finland and low-lactose products**

Finns have traditionally drunk a lot of milk. Consumption at this time is approximately 130 l/ person/ year, while in the 1970s it was amongst the highest in the world at 230 l/person/year. The occurrence of lactose intolerance in Finland is relatively low (ca. 17%) by international comparison, and of that percentage only some people develop symptoms from dairy products. The problem is nevertheless significant due to the high consumption of milk. A general awareness of lactose intolerance arose in the early 1970s and Valio started to search for a solution to the problem. After the first commercial lactase enzymes became available, the solution found was to hydrolyze the lactose in milk enzymatically into glucose and galactose. Valio launched the first lactose-hydrolyzed product – HYLA milk powder – for test marketing in 1978. The product became fully available in 1980, after which the range began to grow slowly. Then from 1985 onwards the HYLA product range was vigorously expanded. A low-lactose alternative was introduced in each product group and the HYLA range has continued to grow. Valio currently offers over 100 different HYLA products.

### **The development of chromatography technology**

At the same time, Valio was researching and developing whey processing. The amount of whey generated as a byproduct of cheese manufacturing is almost the same as the amount of milk used in the production. In addition to water, the main component of whey is lactose at around 4.5%. Valio commenced production

of lactose in the late 1970s. The product was exported and mainly used in infant formulae. In the early 1980s, production was expanded from the Toholampi dairy to Lapinlahti and Joensuu.

Valio began its research into chromatographic separation in the mid-1980s, in order to improve the lactose yield. Chromatography was already commonly used in the sugar industry to improve the yield in sugar production. The test results showed that chromatography allowed a specific separation of lactose from the mother liquor in lactose production, which improved the manufacturing yield. In addition, it was possible to separate lactose from skim milk, and, consequently, another product besides lactose was a fraction containing all the salts and proteins of milk. This way of separating lactose from skim milk was new to milk separation technology. Used for a long time, ultrafiltration also enabled the separation of lactose from milk, but the salts were removed from the protein fraction at the same time. The process of removing lactose chromatographically from whey and milk was protected with patents. This technology later enabled the production of lactose-free milk drink. Between 1996 and 1998 a TEKES supported project was carried out. Also the analytical methods to detect residual lactose were developed to support development and production of lactose free milk drink.

Chromatography was introduced into the manufacture of lactose in Joensuu in 1988, where it was employed until lactose production was ended in 1996. Thereafter chromatography was applied to the processing of skim milk. The resulting protein fraction was first used in making low-fat ice cream, and from September 2001, as a raw material of lactose-free milk drink.

### **Lactose-free milk drink**

Lactose-free milk drink was launched in Finland in autumn 2001. It tastes like ordinary milk. The product was named “milk drink”, because according to the present EU Directive, lactose may only be removed from milk products enzymatically. The physical separation of lactose using chromatography resulted in the product being labelled milk drink.

The composition of the product is similar to that of semi-skim milk, except for its carbohydrate content. It contains less than 0.01% lactose, which is the maximum permitted for lactose-free products by the Finnish authorities. Due to the lower carbohydrate content, the product also contains less energy than a corresponding traditional product: lactose-free milk drink holds approximately 83% of the energy content of semi-skim milk.

The product was eagerly received though little advertised. Its innovative value is so great that it generated press coverage in national newspapers. The product reached the sales target set for the whole year in just three months. Sales grew vigorously and continue to do so. Exports to Sweden commenced in November 2002 and again exceeded all expectations. In spring 2002, Valio lactose-free milk drink was selected star product of the year in the drinks category of the Finnish Food Product of the Year competition. At the same time a membrane based process technology for lactose free milk drinks was developed and patented.

What was the reason for such a positive reception? Based on consumer feedback, the product has obviously attracted a new group of milk users: people who had given up milk due to lactose intolerance and dislike the sweetness of low-lactose

(hydrolyzed) milk. These consumers are once again able to enjoy the good taste of milk without the fear of developing stomach disorders. Valio's range of lactose-free products has gradually expanded. This means that a totally new product category was generated. Valio has also licensed the technology behind lactose-free milk abroad, and at the moment products based on this technology are in market in six different countries.

Author:

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**Symposium on Recent Applications in Bioprocess Engineering  
Hämeenlinna, 9-11.11.2005**

## **Traceability in Bioindustry**

**D. Tech. Jarno Tuimala**

**Logistics and traffic research and development centre  
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### **Abstract**

Tracking systems are largely utilized to enhance the performance of any supply chain or manufacturing process. By tracking technology, companies may improve their business transactions with suppliers, subcontractors, distributors, retailers and logistics service providers. Tracking supports the companies to shorten lead times in their delivery process. By real-time tracking, companies are updated real-time of their supply chain performance and any mistakes occurring there. Tracking also helps the management of business networks. By tracking, the companies may gain better process performance with fewer quality defects. Problems, such as delivery of wrong products to wrong customers with bad quality and a couple of dates too early or late, can be avoided.

Traceability is nowadays a “hot topic” in logistics research when dealing with tracking issues especially in food industry processes. Food industry has altered to a subcontracting business when producers have outsourced many of their earlier “home made” production parts into suppliers and subcontractors all over the world. Due to global sourcing, customers and authorities are aware to be informed of food quality and safety aspects. At the same time, various regulations force food industry companies to collect much data about used raw-materials, production, hygiene and other critical data.

The use of electronic data interchange methods has been increasing dramatically last years. Due to quality expectations, companies increasingly need to manage information in their business processes. Also tracking technology available has been both cheapened and generalized. Especially, the costs of RFID (radio frequency based identification method) technology have been decreasing last years very much. RFID technology has many advantages compared to traditional data collection methods. Some to mention are its ability to carry more information and its ability to read data from a short distance.

In this study, the motivation for traceability in food industry is presented with regard to customer service and influences in supply chain management related issues. This study presents the basic motivation for information collection and share with different participants in global supply chains. Mostly, the study considers Finnish food and bio industry.

## **Developments in microbiological methods for rapid detection of emerging pathogens and prions”.**

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Food safety has emerged as one of the most important topic in the food industry. The threat of emerging pathogens in causing outbreaks of foodborne illnesses and prospect of wide spread recall of foods contaminated with pathogens have underscored the need for rapid methods and automation in food microbiology. Food safety testing also involves detection of allergens, GMO, toxins and residues that may be unsafe for human consumption. Rapid detection of viruses and prions has posed new challenges for rapid detection and diagnostics in food industry. Historically, the bulk of food safety testing was done using conventional cultural methods involving detection and enumeration of various microorganism of interest and isolation and characterization of cultures grown in broths and agar media. Although, the majority of microbiological testing in food industry is done using conventional cultural methods, advances in miniaturization, mechanization and automation, and computerization as well as developments in immunology and molecular biology have lead to many notable improvements in the conventional methods. Some of the methods and approaches for food pathogen testing in vogue include new generations of immunoassays, and molecular methods based on hybridization and amplification of nucleic acid, e.g. polymerase chain reaction (PCR) , and methods involving the use of a specific nucleic acid fragment (genetic sequence) with or without amplification of sequences. The development of the so called biosensors and micro array assays on chips are some of the recent promising developments that may be applicable to detection and characterization of microorganisms and toxins. The new techniques for rapid microbiology now available present unprecedented choices to microbiologists. What was considered futuristic just a decade ago, is now within the realm of practical use in food industry for rapid detection and for microbiological surveillance of ingredients, products, process and environment.

## **Anaerobic wastewater treatment in Finland – status and possibilities**

**Dr. Juhani Suvilampi, Watrec Ltd. Symposium on recent applications in bioprocess engineering, 9.11-10.11.2005 Hämeenlinna, HAMK**

### **Anaerobic wastewater treatment**

Anaerobic wastewater treatment is especially used in industrial wastewater treatment. Commonly practised in closed reactors, where gas and water are discharged in separate streams and sludge is entrapped in reactor by gravity. During the process organic substances are biologically converted into methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), cells, and water. In comparison to the aerobic treatment the process typically results in low sludge yield. It produces energy, 1 kg of removed COD yields approximately 350 l CH<sub>4</sub>, in pure energy that corresponds with 3,5 kWh. The operating temperature in anaerobic processes can be in the range from 10°C to 70°C, especially the upper temperature limit is higher than with aerobic treatment. Anaerobic process tolerates high loading rates, which is based on high amount of sludge in reactor.

### **Applications**

The upflow anaerobic sludge bed (UASB) is based on the reactor systems, where granular sludge is bottom of the reactor and treated water flows through the sludge bed. The granules are well-settleable and subsequently easily retained in reactor. The reactor system is compact with low footprint. The internal circulation (IC) reactor, is a new generation of UASB. In principal it has two UASB reactors connected together, high-loaded systems is in the bottom and polishing reactor on the top. Granular sludge bed is expanded by and lifted up the reactor with produced biogas and it drops back to the bottom of the tank after the gas is separated. This yields an aggressive circulation of sludge and water from the bottom to the top and back inside the reactor. IC reactor can tolerate markedly higher loading rates than UASB systems. Expanded granular sludge bed (EGSB) reactor has the same principal but the circulation is conducted with pump.

### **Status of anaerobic wastewater treatment in Finland**

All anaerobic wastewater treatment plants in Finland are built for industrial wastewater treatment. First plants were established in 1980's for ethanol production

plant, fluting plant, sugar mill and dairy. Among the first anaerobic wastewater treatment plants in Finland, a Finnish Taman process was designed. The treatment is a two stage systems, with acid reactor and methanogenic reactor. There were 4 installations; 1 for dairy wastewater, 2 for pulp and paper mill wastewater, and 1 for ethanol (liquor) production. Plant performances were not success stories and today only 1 plant is in use. First UASB reactor in Finnish industry was built in 1982 by YIT. The treatment plant was designed for sugar mill wastewater and has been successfully operated from more than 20 years. UASB reactors showed markedly more stable performance than domestic plants but still only few plants were built after the first failures. Few plants have also been built to Finnish food industry.

### **Case: Upgrading of contaminated wetlands water treatment with UASB.**

Contaminated wetlands in Central Finland were spoiled by a black liquor spill during the years 1935-1967 from an acid sulphite mill. The spill was conducted to the wetlands because the lake near the pulp mill was already so much contaminated by the black liquor so that the manufacturing process was interfered.

Restoration project initiated 1980's, there were several attempts to solve the problem. The water was used e.g., for dust removal in roads and military used the wetlands for dumping of caustic. In the 1990's the aim was to drain the wetlands. In 1992-1993 the water from the wetlands was pumped via 25 km sewer to a municipal wastewater treatment plant. The project was completed at 1993, after which the sewer pipe was dismantled. After a year the wetlands had the same amount of polluted water 100.000 m<sup>3</sup> than there was before. The second phase started with new idea to construct new wastewater treatment for the polluted water and to pump the water directly into a lake. Treatment system was the combination of trickling filter and flotation and the plant was started in 1997. Within 4 years it was realised that the drainage of the wetlands will not solve the problem, new water came always when old was removed via rainfall and as groundwater. The organic load in the area did not reduce since the polluting substance was mainly attached into the peat fraction of the wetlands. Only 1% of the organic load was in the water fraction. Two projects were established to complete the restoration programme: i) isolation of the contaminated wetlands from the rainfall and groundwater and ii) to develop more efficient treatment and drainage of the existing waters.

### **Water treatment upgrading project.**

The project was established to develop more efficient wastewater treatment prior the existing trickling filter/flotation. The project was started by conducting field studies, where different treatments were compared. These systems included aerated lagoon, high-rate aerobic biofilm process, and anaerobic treatment (UASB). Anaerobic treatment resulted in highest COD and BOD removals, as the existing treatment removed 50% of BOD and 20% of COD, the UASB removed BOD up to 70-80% and COD from 30 to 40%. Project continued with the design and commissioning of the anaerobic process. Within one year the COD removal was shown to stable to 40-45% and BOD removal to 90-95%. The lignin removal (as sodiumlignosulphonate, NaLS) was up to 40-50%. After 1.5 year of operation the wetlands was finally covered with soil and the wastewater flow was diminished.

## Possibilities for anaerobic wastewater treatment in Finland

Industrial wastewaters owe the potential for anaerobic wastewater treatment. The largest volume of water is used in pulp and paper industry. Food industry has high concentrations of organics and nutrients, and other industries can have challenges which anaerobic treatment can solve. Municipal wastewaters have also an interesting future in the sense of biological nutrient removal. With pulp and paper industry there are 37 paper and board mills and 19 pulp mills, in total 41 separate mills. The industry produces wastewater approximately 800 million cubic meters annually, of which 80% is treated in activated sludge process. There exists 1 anaerobic treatment (UASB) plant. 1 anaerobic treatment plant is replaced with aerobic treatment. 150 000 m<sup>3</sup>/d (from 6 mills) is still treated in aerated lagoons, with mechanical treatment or with chemical treatment. This wastewater volume has a potential to be treated in anaerobic systems since these treatments will probably be upgraded within next 5-8 years. The biggest competitor is the aerobic biofilm treatment, which can also be used as a high-rate pretreatment to remove organic load. One big attraction is also in closed water cycles since anaerobic treatment can be operated at high temperatures up to 70°C.

Most of the existing anaerobic wastewater applications exists in food industry sector, the industry produces 3.8 million cubic meters of wastewater annually. There are already one application in process water treatment for water reuse, and in total there are 2-3 anaerobic wastewater treatment plants in use. Probably the most attractive industry for anaerobic wastewater treatment – however, in meat industry high nitrogen concentration is a risk to be considered.

Special applications, such as anaerobic metal removal in mining industry, can also be a good possibility for anaerobic treatment. So far there are no full-scale installations but some research and development projects have been conducted with promising results.

## **Biovakka Oy – The first centralised co-digestion plant in Finland**

**Dr. Juhani Suvilampi, Watrec Ltd. Symposium on recent applications in bioprocess engineering, 9.11-10.11.2005 Hämeenlinna, HAMK**

### **Waste management challenges in the agriculture - pig manure**

Pig manure is a challenge for the farms in Finland. According to regulations the sludge must be stored for approximately 6 months since the land use is allowed only during specific periods, spring and fall. Subsequently spreading the half year production to land within few weeks causes several unwanted effects, such as greenhouse gas emissions, odour, nutrient leaching, and a heavy work load to farmers along with traffic problems. Since the farms are getting larger in size, the problem with manure and nutrient management is concentrating to certain areas, such as Vehmaa commune.

### **Biovakka Oy – Vehmaa biogas plant**

The company was established in 2002 to solve the farmers challenge in manure management. Company is 100% owned by the local farmers. The challenge was to solve the nutrient and manure management problems. The project was initiated in 2002. The goal of the project was to implement a local manure treatment facility, which would result in the sustainable use of pig manure. Project was divided into 9 steps:

1. Pre-design of the first centralized co-digestion plant in Finland
2. Evaluation of the competitiveness of anaerobic treatment
3. Applying the required permissions; Environmental Impact Assessment Procedure, Environmental permit, Application from the veterinary authorities according the EU side-product regulations, Chemical law
4. Tendering of the potential local and international suppliers for the biogas plant
5. Application of the governmental grant for the renewable energy production facility

6. Selection of the applicants for constructing the biogas plant
7. Design of the plant facilities and final concept
8. Building the biogas plant
9. Starting up the plant

## Process

In anaerobic digestion sludge is processed biologically so that its organic fraction is mineralized. During the process biogas is formed, odor is reduced, nutrients are converted for more available form to use of plantation. Also the reduced amount of organic carbon leads to reduced consumption of oxygen in soil during the land spreading. Additionally the potential of methane emission is diminished. In other words; pig manure is soundly processed into an organic fertilizer. The Biovakka biogas plant process consists of homogenization and crushing (particle size less than 12 mm), hygienisation (1 h 70°C), biological treatment (6700 m<sup>3</sup> steel digester, SRT 21 d), and decanter centrifuge. Biogas is treated to remove H<sub>2</sub>S and moisture and it is used in CHP, which currently produce 820 kW electricity. .

## Investment

Investment and pay-back calculations were conducted before and during the tendering process. It was assumed that there is a base line for investment, after which the price increases accordingly to the plant capacity. From the start it was obvious that in Finland the anaerobic digestion will not pay back the investment without the incomes from industrial and municipal wastes. In Finland the market price for electricity is currently 2,5-3,5 eurocent/kWh. These calculations led the company to decide that co-digestion plant is a right option for their manure processing plant.

## Legislation

Biological waste treatment facilities are regulated by environmental authorities and biogas plants treating animal by-products are controlled by EU regulation (EC 1774/2002). Legislation demands the EIA procedure if biological treatment capacity exceeds 20.000 tn/a. Biovakka biogas plant has the treatment capacity of 120.000 tn/a.. The EIA was initiated as soon as the basic design (process and the treatment capacity) was completed. Duration of the project was 1.5 years to finish the program and to get the permit from environmental authorities. The EIA procedure is conducted in two steps, firstly the program is evaluated and after that the actual EIA is conducted. The target of the procedure is to give tools for the legislative bodies for the assessment of the environmental impacts of the plant and to keep the stakeholders informed.

In Vehmaa biogas plant the environmental permit describes the limitations for the plant operation, such as the amount of treated wastes (120 000 tn/a), required efficiency for the treatment of off-gases (90% reduction / <3000 smell units), and characteristics for the wastewater if it is conducted to sewer (COD <1000 mg/l, nitrogen < 150 mg/l).

According to the 'side-product' legislation, there are several demands for biogas plants. These demands can be divided into three categories: i) traceability, ii) hygienic operations, and iii) HACCP. Traceability concerns the documentation of the biogas plant. The plant must be able to verify where the wastes are brought for treatment, what has been the treatment chain and treatment conditions, and where the treated waste has been transported from the plant. Also all the exceptions in the plant operations must be documented. The hygienic requirements concern the transportation equipments, vector control, prevention of recontamination, and the maintenance of machinery and equipments. Every part of the treatment process must be designed so that the hygienic risk is managed. The specific hygienisation requirements in biogas plants are the thermal treatment (70°C, 60 min / 133°C 20 min for II class) and the criteria today include the particle size of the material conducted into the hygienisation (12 mm for 70°C and 50 mm for 133°C). The process must produce treated waste, which has the Salmonella, enterobacteria, *Escherichia coli.*, and *Clostridium perfringens* (if only pig manure is treated) under certain levels. These criteria are under re-evaluation and they will probably be changed so that the enterobacteria is excluded from the requirements. HACCP is used to evaluate the working procedures in the plant.

## **Tendering**

The tendering process was initiated during the summer 2002. Requests were sent to Finnish companies and to European biogas suppliers. There were two options: to collect individual companies and build the plant by ourselves or to purchase the plant from one main contractor as a turn-key delivery. The whole tendering process took over 2 years. The most challenging part was found to be the treatment for the wastewater after the decanter – more than 98% removal of nitrogen was required if the wastewater should be conducted into sewer.

After long term negotiations it was concluded that the cost-wise solution was to combine the two initial options with one main contractor, who supplies the core of the biogas plant and the rest was done by the purchaser himself. These items were the civil works, construction of the buildings, the site electricity and pipe works, the insulation works. Also the CHP unit and decanter centrifuge were purchased by Biovakka itself. Wastewater treatment was excluded and it was decided that the reject water is used as a fertilizer.

## **Treated wastes**

The biogas plant has the capacity and permission to treat pig manure, solid manure, biowaste and industrial sludges, and wastewater sludge so that the total amount is 120.000 tn annually. As the plant has the maximum TS limit of 12% the treatment capacity is 14.400 tn TS/a / 40 tn TS/d.

## **Building the biogas plant**

Construction started late fall 2003 with excavations of the site. On May 2004 the actual construction started with concrete tanks and underground pipe works. Erection of the equipments were conducted in June 2004 – January 2005. Starting up the plant initiated in February 2005 and is presently on the phase of the guarantee runs.



## Results

During the start up the biogas plant has treated 40 000 tn of waste including pig manure, molasses, fish waste, potato waste, enzyme waste, and wastewater sludge. During the period the digestion process has produced 700 000 m<sup>3</sup> of biogas (60-65% CH<sub>4</sub>). After the decanter the TS of the humus is 32% and TS in reject water 1.4%. 90% of the digested sludge is separated as water fraction and 10% as dry fraction. 85% of the ammonia load is in the wastewater and 90% of phosphorus is in the humus. Odor removal is efficient, 98% as an average is removed from compounds causing odor emissions.

Financially the conclusion is open, the plant can offer the treatment for e.g. 40 000 tn/a of wastewater or industrial sludge, which would result in an economical success.

## Conclusions

It is possible that anaerobic co-digestion will be a success story in Finland. However, the calculations and design must be based on the gate fees – so far the price for renewable energy is too low. If manure is treated in co-digestion plant, the capacity should be 20.000 + manure (60.000 tn/a) to ensure financial viability.

## Biogas plants in Finland

### Case 2: Laihia Co-Digestion Plant

Mr. Martti Jormanainen, Managing Director  
Envipro Partnership Company

#### Background

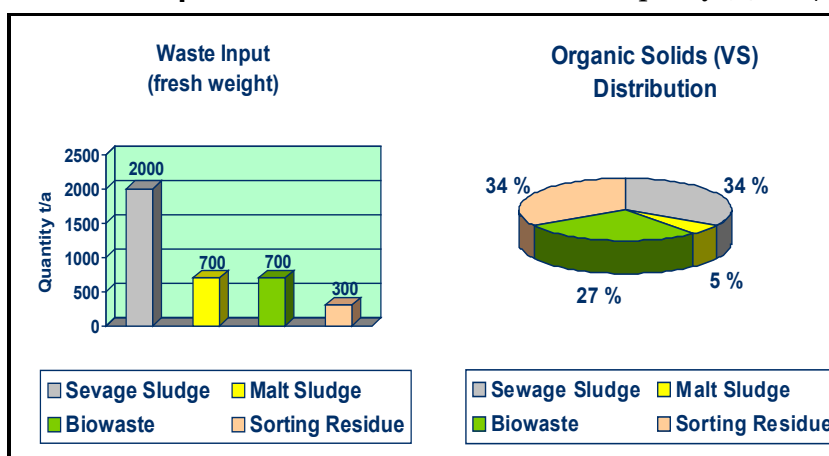
Laihia is a medium size community in the western Finland. It covers the area of 508 sq. kms and has population of 7500. The main sources of livelihood are service trade (51 %), industry and construction (35 %), and agriculture and forestry (11 %). The main industrial plant is the malt factory founded in 1910.

Originally there was the plan to build a composting plant. However, due to the people's resistance this plan had to be abandoned. The new bid inquiry allowing either composting or digestion was issued in 2001. The digestion proved to be the most attractive choice and the contract was awarded to RMG Pointo Ltd on turnkey basis. The contract was signed in April 30, 2003, the construction works were commenced in June 2003 and the plant was ready for start up in December 2003.

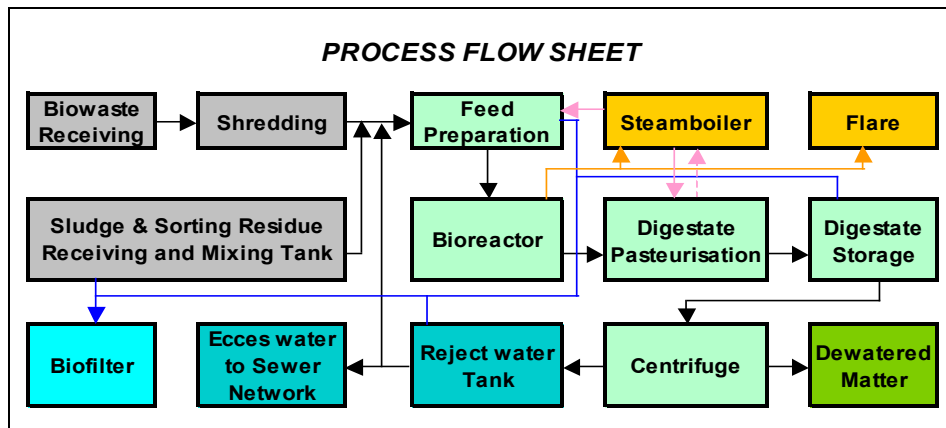
Because of the large number of small communities in Finland, digestion plants with small capacity are of special interest. Further, co-operation between municipality, local industry and local farms would improve the economic viability of small-scale digestion plants.

#### Plant description

Capacity 3 700 t/a



## Process



## Plant

Plant area	2 330 m <sup>2</sup>
Building area	230 m <sup>2</sup>
Floor area	277 m <sup>2</sup>
Building volume	1 572 m <sup>3</sup>

## Tank volumes:

Sludge & Sorting Residue Receiving and Mixing Tank	30 m <sup>3</sup>
Feed Preparation Tank	50 m <sup>3</sup>
Bioreactor	325 m <sup>3</sup>
Hygienisation Tank	25 m <sup>3</sup>
Digestate Storage Tank	41 m <sup>3</sup>

## Design Data

Total Waste Input	3 700 t/a
– total solids TS	818 t/a
– organic solids VS	660 t/a

## Feed Batch

– no of batches	125 batch/a
– waste input per batch	30 t
– dilution water per batch	10 t
– heating steam per batch	0,83 t
– solid content of feed substrate TS-%	16 %

## Bioreactor

– organic loading rate (OLR)	6,0 kgVS/r-m <sup>3</sup> /d
– hydraulic retention time (HRT)	23 d
– specific yield of biogas	450 m <sup>3</sup> /tVS

## Start up and trial run

Initial heating on 8.12.2003

- pumping 49 m<sup>3</sup> hot water (t = 43°C) into the bioreactor

Removal of oxygen on 9.12.2003

- pumping 5 m<sup>3</sup> raw sewage sludge (t = as received) into the bioreactor

Adding of inoculum on 10.12.2003

- pumping 40 m<sup>3</sup> digestate brought from the Forssa Sludge Digester (t = ~20°C, heated to 40°C)

Organic loading of the bioreactor (started on 11.12.2003)

- OLR was gradually increased from 0,5 kgVS/r-m<sup>3</sup>/d to 2,0 kgVS/r-m<sup>3</sup> during first 57 days– – – – –
- feed substrate varied from TS 3 % to TS 12 %, t = 65°C
- in spite of the cold outside temperature the temperature inside the bioreactor was well controlled

Methane content of biogas

- 58 vol-% after 34 days from start up–
- 62 vol-% after 50 days from start up–

Removal of digestate

- the bioreactor became full and the first removal of digestate was made after 50 days from start up

## Operation experiences

The following experiences are gained during first two years operation.

- AD process has performed quite well. Average yield of biogas 467 m<sup>3</sup>/tVS<sub>fed</sub>. Methane content of biogas 62 vol-%. Degradation rate 55,65 %.
- Wastewater treatment unit (ammonia stripping) failed. New system, which consists of a pumping station and transfer sewer to the public sewerage network is built.
- The following equipment has been replaced because of inadequate performance:
  - ◻ Waste shedder (particle size requirement was not fulfilled)
  - ◻ Biogas booster fan (inadequate gas tightness)
  - ◻ Filter press (high solid content in reject water, cake not dry enough, high use of rinsing water)
  - ◻ Steam boiler (frequent operation disturbances because of the over-sized burner)

## The biogas promise large scale practice

**W. Verstraete, Ghent University, Labmet.ugent.be**

The anaerobic digestion of organic matter by means of the methane producing bacteria is one of the most effective ways to recover energy from biomass. Indeed, the bacteria convert all biodegradable matter in the absence of oxygen to methane and carbon dioxide. The energy present in the organic matter distils all by itself off in the form of a very valuable gas. The latter can be used as such or converted to electrical energy. Overall, the AD conversion can reach conversion efficiencies up to 100% and this with minimal input of chemicals or other externalities.

Yet the bottlenecks of the technology are situated in the fact that the conversion process is based on a food chain of decomposing bacteria which are all interdependent. As a consequence, conversion rates are generally limited to some 25 kg organic matter per m<sup>3</sup> reactor per day corresponding to energy outputs in the form of electricity of the order of some 1 kW per m<sup>3</sup> reactor per day.

The large-scale practices in AD are fully present nowadays. Numerous reactors have been built which are surpassing the 1000 m<sup>3</sup> volume, often reaching up to 10 000 m<sup>3</sup> volume. These systems are functional for the digestion of sewage sludge, industrial wastewater, domestic sewage and solid organic wastes. The concept of landfills as super-large scale bioreactors has emerged in the last decade and certainly holds more potential than considered thus far, particularly for dealing with farm surpluses and crop-to-energy conversion in the tropics.

The promises of the AD technology are largely related to the evolution of the world energy market. In case of a strong market driven economy in a time span of economic growth, it can not be doubted that biomass-to-biogas will be a major axis. The AD technology will be an essential component of all biorefinery plants and will fit perfectly in an overall policy of renewable energy and CO<sub>2</sub>-emission rights trading.

Clearly, the future of biogas is bright.

## Recent developments in microbial fuel cells

**K. Rabaey & W. Verstraete, Ghent University, Labmet.ugent.be**

Microbial fuel cells (MFCs) provide new opportunities for the sustainable production of energy from biodegradable, reduced compounds. MFCs function on different carbohydrates but also on complex substrates present in the wastewaters. As yet there is limited information available about the energy metabolism and nature of the bacteria using the anode as electron acceptor; few electron transfer mechanisms have been established unequivocally. To optimize and develop energy production by MFCs fully this knowledge is essential. Depending on the operational parameters of the MFC, different metabolic pathways are used by the bacteria. This determines the selection and performance of specific organisms. Here we discuss how bacteria use an anode as an electron acceptor and to what extent they generate electrical output. The MFC technology is evaluated relative to current alternatives for energy generation.

### **Willy Henry VERSTRAETE, biography**

W. VERSTRAETE is born on April 25, 1946 in Beernem (Belgium). He graduated in 1968 from the Gent University as bio-engineer. He followed a summer course on Soil Microbiology at the Pasteur Institute of Paris. In 1971, he obtained a Ph D degree in the field of microbiology at the Cornell University, Ithaca (USA).

Since 1971, he works at the Gent University, first as assistant and since 1979 as professor and head of the Laboratory of Microbial Ecology and Technology (LabMET - Faculty of Bioscience Engineering). His R & D has a central theme: processes mediated by microbial mixed cultures. His team deals with microbial transformations in waters and soils and the gastro-intestinal tract. A variety of biotechnological processes, based on microbial consortia, are subject to R&D at LabMET.

He received in 1975 the Intermediair price for a review article entitled "Environmental hygiene from a microbial-ecological perspective". In 1976, he received from the Belgian Comité of the International Association for Water Quality (IAWQ) the price for the design of a treatment plant dealing with concentrated wastewaters. In 1982, he received the price of the Technological Institute of the Royal Society of Flemish Engineers for his work in the field of anaerobic digestion. In 1997, he was awarded the Francqui chair at the University of Louvain-la-Neuve. In 1999, he re-

ceived the Altran price (Fr) for his research about nitrogen removal technologies in wastewaters. In 2002, he received a 4 years' appointment as Honorary Professor in the Advanced Wastewater Management Centre of the University of Queensland, Brisbane, Australia. In 2005, he was awarded by an international jury the Five Yearly Prize for Applied Sciences of the Flemish Science Foundation.

W. Verstraete was co-chairman of the Working Party on Environmental Biotechnology of the European Federation for Biotechnology (EFB) (1989 -1993) and was General-Secretary of the European Environmental Research Organization (EERO) (1991-1997). He was a member of the OECD Workgroup on Environmental Biotechnology (1990-1998). In 1993, he was elected to the Fellowship of the International Institute of Biotechnology. In 1991, 1997 and 2004 he co-chaired the International Symposium on Environmental Biotechnology of the EFB at Ostend (B). In 1994 he chaired the International Symposium on Anaerobic Digestion of the International Water Association (IWA) at Cape Town (SA). In 2001, he was for the second time chairman of this international event, this time organised in Antwerp. He was president of the Royal Society of Flemish Engineers (Koninklijke Vlaamse Ingenieursvereniging) for the period 1997-1999. He is a member of the Royal Academy of Sciences and Arts of Belgium. He was chairman of the Belgian branch of the IWA (2000-2005) and of the Centre of Environmental Studies of the Ghent University (2000-2005). W. Verstraete is appointed member of the Technical Commission for Soil Protection of the Netherlands. He is a member of the Flemish Council for Scientific Affairs (VRWB). He is also member of the scientific council of the Belgian food industry (FEVIA).

W. Verstraete has field experience with respect to design and operation of drinking water production plants (slow sand filtration), aerobic wastewater treatment (in particular with respect to nitrification-denitrification), anaerobic digestion of wastewaters and sludges, solid state fermentation of organic residues and bioremediation processes of soils and sediments. He has also gained experience in various aspects of pre- and probiotics used in human and animal nutrition.

For further information, see  
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## Bioethanol in Europe

**Director Harri Enwald**  
**Valcon Ltd., Valkeakoski, Finland**

### What is bioethanol?

Bioethanol is ethanol produced by fermentation from renewable resources. Main uses for ethanol are alcohol beverages, industrial use (mainly as solvent) and fuel. Here bioethanol is referred to ethanol produced to be used as fuel or as fuel additive for transport purposes. This is the largest and most rapidly growing application of ethanol in the world.

### Reasons for use of bioethanol

The main reasons for fuel use of ethanol are

- reduction of harmful emissions
- less dependence on oil
- new markets for agricultural products

As fuel additive ethanol or its derivatives act as oxygenate and octane enhancer. Due to the oxygen content of ethanol gasoline will burn better and harmful emissions will be reduced. Because bioethanol is produced from renewable resources its use will also lead to net reduction of carbon dioxide emissions.

At the moment the driving force for increasing ethanol production and use in Europe is the Directive 2003/30/EC. The directive states that each member state of EC shall set a national target for biofuels use for transport purposes. The ambitious reference values for the national targets are 2 % of petrol and diesel use in 2005 and 5.7 % of petrol and diesel use in 2010.

### Use of bioethanol

Ethanol utilization for fuel purposes can be divided to use as fuel additive and use as fuel. As fuel additive ethanol can be used as direct mixture with gasoline or it can be further processed to derivatives like ETBE or TAAE. Majority of the ethanol use in Europe is as fuel additive. In Germany, France and Spain ethanol is mainly



processed to ETBE but in Sweden ethanol is directly mixed with gasoline as 5 % mixture.

The main advantages for ethanol use as fuel additive compared to ethanol use as fuel are

- no modifications to existing vehicles are needed
- existing fuel delivery systems can be used
- efficiency of ethanol use is better, because of improved burning of gasoline
- competitiveness of ethanol is better because it competes with high price alternative fuel additives and not with gasoline

When used as fuel ethanol suffers from the lower energy content compared to gasoline and the market price of ethanol per litre can be expected to be only 60 – 70 % of the gasoline price. Especially in Sweden the number of flexifuel cars that can use either gasoline or 85 % ethanol / gasoline blend (E85) is steadily increasing.

### **Raw materials for bioethanol**

Practically any starch or sugar based raw material can be used for bioethanol production. Raw material price is the dominant cost of ethanol production. In Europe mainly grain (barley, wheat, triticale and rye) and in lesser extend sugar beet are used for ethanol production. In tropical countries sugar cane, molasses and tubers like cassava are common raw materials for ethanol plants.

A lot of research and development work has been done to commercialise the production process of ethanol from low cost lignocellulose based materials. So far commercial scale production units have not been built but theoretical calculations show that lignocellulosic ethanol may be competitive with ethanol from traditional raw materials in the near future.

### **Fuel ethanol production in Europe**

Large scale fuel ethanol production in Europe began at the middle of 1990's in France. The main purpose for ethanol production was to create new markets for agricultural products that suffered from overproduction. The French ethanol plants are mainly integrated with sugar production. Raw materials are mainly beet juice and wheat.

Spain became the leading ethanol producer in Europe in 2000 when Abengoa started the barley based plant in Cartagena. At the moment Abengoa is the biggest ethanol producer in Europe and will start its third fuel ethanol plant in Spain by the end of 2005.

The fastest growth in ethanol production in Europe during the last years has been in Germany where the tax exemption for biofuels for traffic purposes came into force in 2004. In 2005 Germany will surpass Spain as the biggest ethanol producer in Europe.

In 2005 the fuel ethanol production in Europe is expected to be about 700 000 t. In addition considerable amounts of ethanol is imported to Europe, mainly from Brazil. Despite the recent growth of production ethanol is still a marginal fuel in

the European markets. Biodiesel is so far the dominant biofuel for traffic purposes in Europe.

### **Barriers for ethanol production in Europe**

Despite the EC biofuel directive and tax exemptions in some countries several barriers for larger ethanol production in Europe still remain. The main obstacle is the high price of raw material that keeps the production costs in Europe higher than in USA and Brazil which are the biggest fuel ethanol producers in the world. The amount of starch or sugar containing raw materials available for ethanol production is also limited. Increased ethanol production will also lead to saturation of the by-product markets. The most important by-products of grain based ethanol production is DDGS that is used in fodder components.

Ethanol production is very capital intensive and many projects have been postponed or cancelled because of lack of financing. The final product price fluctuates with oil prices and the ethanol producers shall be prepared to face years when the product price is too low for profitable plant operation.

Many oil companies regard ethanol as a competitor to their products and are not willing to by ethanol to be mixed with gasoline. In Europe oil companies have partly solved this problem by using ethanol for ETBE production. This enables the oil companies to have control over the ethanol use. For ethanol producers this means that the number of potential buyers is limited. In addition the oil companies can easily turn from ETBE to MTBE production, if that becomes more profitable than ETBE production. Use of ethanol requires also receiving capacity and mixing stations and in many countries there simply does not exist enough capacity to receive ethanol for fuel purposes.

Bioethanol production in Europe is not competitive without subsidies. The EC biofuel directive enables the member states to use tax exemptions to promote the use of biofuels. The tax exemptions are temporary and vary from state to state so investors considering starting of ethanol production or ethanol mixing with gasoline are unaware of future taxation policy and the location of most favourable markets.

Because of the high investment cost of ethanol production units, uncertainty of future price development and uncertainty of future political decisions to promote biofuels the investors for ethanol production shall have considerable risk taking potential.

### **Competitiveness of bioethanol produced in Europe**

Ethanol production in Europe costs more than in USA and especially in Brazil. To be competitive in the future European producers shall be able to use multiple raw materials and to produce various high value by-products. Integrating ethanol production to other industries like co-generation plants, oil refineries, sugar plants, paper mills or biogas production is among the key factors for competitive ethanol production in the future. Lignocellulotic raw materials shall be used in the future to lower the ethanol production costs.

Some ethanol buyers certainly will prefer long term purchase contracts with a reliable nearby suppliers to being dependent on fluctuating world market prices. This may give some advantage to local producers compared to imports from overseas.

It is hardly probable that ethanol produced in Europe could even in the future compete with the production costs of tropical cane ethanol. However, it can be expected that future development of ethanol markets will adjust the market price of ethanol to follow the price of gasoline and the price of alternative fuel additives like MTBE. Therefore the crucial factor for ethanol production profitability will be the ability to produce ethanol with costs that can compete with alternative fuel additives and finally with costs of gasoline. Looking at the rapid increase of oil price during the last months and the continuing development of better ethanol processes it is obvious that in the near future ethanol production costs compared to oil price can be low enough to enable large scale ethanol production in Europe even without subsidies. However, for creating conditions for the growth of ethanol production and use, subsidies or mandates for biofuels use will still be necessary.

EC = European Community

MTBE = Methyl tertiary butyl ether Gasoline additive produced from methanol and isobutylene. Used as oxygenate and octane enhancer

ETBE = Ethyl tertiary butyl ether Gasoline additive produced from ethanol and isobutylene. Used as oxygenate and octane enhancer

TAAE = Tertiary amyl ethyl ether. Ethanol derivate used as gasoline additive. TAME is the corresponding methanol based product.

DDGS = Distiller's dried grains and solubles. Valuable by-product of grain based ethanol plants. Contains lot of fibre and proteins. Used as fodder component.

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## **Bioethanol – a Possibility in Finland**

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### **Background**

In the EU the use of biofuels, such as bioethanol, biodiesel and biogas, for transport purposes is increasingly recommended. The EU Directive 2003/30/EU on the promotion of the use of biofuels or other renewable fuels for transport directs that these fuels shall be 2% based on energy content, of all petrol and diesel by the end of 2005, and 5,75% by the end of 2010.

As a background for the directive, the Commission White Paper, called European Transport Policy for 2010: Time to Decide, has been published. According to this Paper, the dependence on oil (currently 98%) in the transport sector should be reduced by using alternative fuels such as biofuels because of the ecological reasons, mainly CO<sub>2</sub> emissions. This is really an important point in Europe today, when we live the time of the Kyoto Agreement and Emissions Trading. In general, the EU aims to reduce its dependence on imported energy and thus to increase its own energy supply in the long term. This is stressed in the Commission Green Paper, called "Towards a European strategy for the security of energy supply". It aims to substitute 20% of conventional fuels by alternative fuels in the road transport sector by the year 2020. At the same time, the fuel quality, vehicle emissions and air quality requirements have to keep at good level.

There're also two Directives which make possible for the member countries to apply reduced tax or no tax at all for biofuels compared to fossil fuels: EU Directive 1992/21/EU (Mineral Oil Directive) and EU Directive 2003/96/EU (Restructuring the taxation of energy products and electricity). The more favorable rate of duty concerns only the part of the transport fuel which is biofuel, and, this tax relief can be allowed maximum for 6 years.

All the above mentioned acts together with the current high price of oil favor the use of biofuels. Despite these, so far, Finland has not been very active in the field of biofuels for transport purposes. In July 2005 the EU gave to Finland an official comment on its exceptionally low level of biofuels and obligated Finland to make a proposal how to increase the use of biofuels in the near future.

Starting from summer 2005 biofuels have got significant publicity in Finland. Nowadays the public pressure demands that something has to be done to find the ways to promote the use of biofuels in Finland.

## Fuel Bioethanol Use in Vehicles

Bioethanol can be used blended with petrol, for instance, as 5% (v/v), E5 or 10% (v/v), E10. In so low concentrations, ethanol actually works as oxygenate. So far, in Europe there is a standard (EN 228) which is valid only up to 5% (v/v). The use of E5 is a common practice in Sweden, E10 respectively in the USA. Both of these can be used in normal petrol cars – there is no need for engine modifications. Also, when these petrol blends are introduced, fuel delivery (refilling) infrastructure is available, so only ethanol receiving and blending systems are needed. If the EU is going to settle for the above mentioned current standard, the reference requirement of 5.75% as energy based can not be achieved: 5% (v/v) corresponds to energy content of 3.3%. So, E10 would be a desirable type of petrol in the future also in Europe.

A special biofuel is the blend of 85% (v/v) E85 ethanol with petrol. This a common practice in the USA, and increasingly also in Sweden. The vehicles are called Flexi-Fuel Vehicles (FFV), and compared to typical petrol cars, certain engine modifications are necessary. In addition to vehicle renewal, the delivery (refilling) infrastructure needs to be rebuilt.

One very typical way to utilize bioethanol in petrol is in the form of other oxygenates, such as ethers, ETBE (ethyl tert-butyl ether) or TAEE (tert-amyl ethyl ether). Based on the opinions of oil refinery and car industry people, the ethers have better blending and fuel properties compared to ethanol. A Finnish oil refining and marketing company, Neste Oil Oyj, has ETBE production at its Porvoo refinery, started in 2004. The whole production (around 100 000 tons/a) is exported and the bioethanol for the production is imported. Also, another ether plant, TAEE plant, is under planning to be built by Neste Oil in Finland. In the future, ethanol based ethers will increasingly replace the methanol based ones. Thus the demand for ethanol in this sector will certainly increase.

## Raw Material Supply of Bioethanol in Finland

Traditional raw materials for bioethanol production are all starch and sugar containing agro products – in Finland this means, first of all, grains (barley, wheat, rye) and sugar beet. Novel raw materials, largely studied by VTT (Technical Research Centre) in Finland, are lignocellulosic materials, such as forest waste, energy plants and agricultural residues, like straws.

The availability of the above mentioned agro-based raw materials, to some extent, depend on the coming agro-political decisions and trends. What will be the EU's "sugar reform" and its effects on the profitability of sugar beet cultivation for sugar production in Finland – is still an open question. Will the reducing trend of the grain price continue in the EU? How the current overproduction of some grains in Finland should be utilized? Could the set-asides be used for more productive purposes? And, how the cultivation support system will be renewed – will the cultivation be supported differently whether the grains are for energy or food purposes? In the very end, the availability of agro-based raw materials solely depends on their price for ethanol production – i.e. how much the plant can afford to pay for the materials.

The reference value of the EU, 5,75% of the petrol use by the end of 2010, corresponds to the need of around 145 000 tons of ethanol per year, at the moment in Finland. But in the future, when the consumption of fuels will be reduced the need will be less, for instance in 2020 of around 110 000 tons of ethanol per year. If the current EN standard for the blend of 5% (v/v) will limit the use of bioethanol in Finland, the need would be only 82 000 tons of ethanol per year at the moment. As a comparison, if all sugar beet cultivated in Finland today (around 1 million tons per year), would be used for bioethanol production, around 90 000 tons of ethanol could be produced. This corresponds to the cultivation area of around 30 000 hectares. In the case of barley, around 540 000 tons are needed for the production of 145 000 tons of ethanol per year. The amount would be around one third of the current production amount of barley in Finland.

In Figure 1 different raw materials are compared based on their ethanol yield both per mass, here in liters per ton of raw material, and per cultivation area, here in liters per hectare.

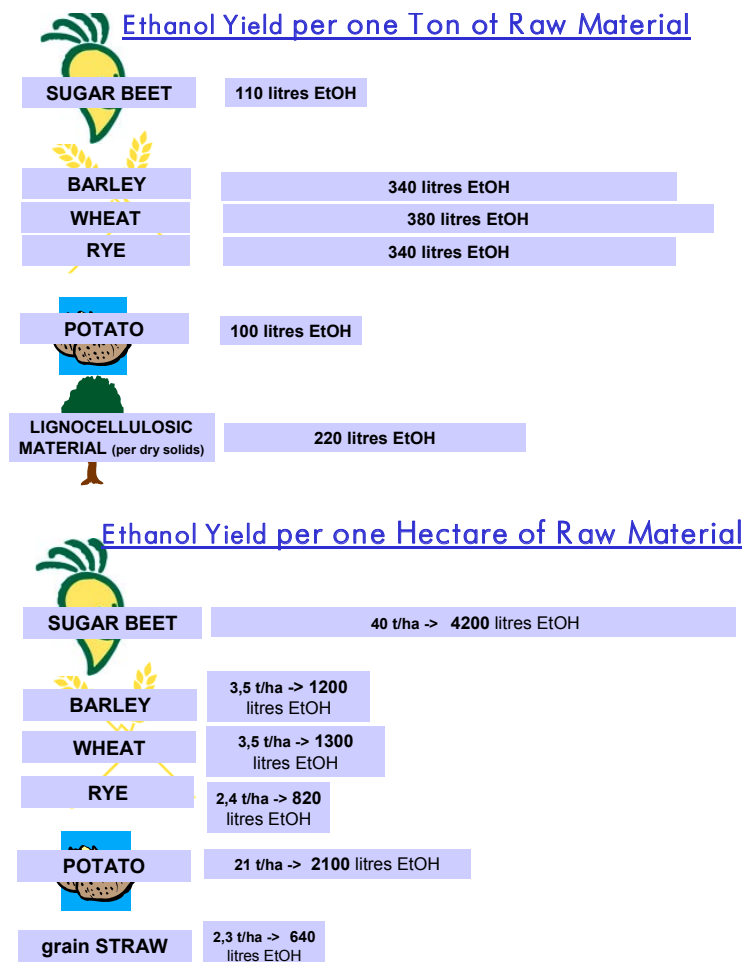


Figure 1. Typical ethanol yields of different potential raw materials for the bioethanol production.

As shown in Figure 1, the ethanol yield per wet weight of sugar beet is clearly lower than that of grains. This is due to the high water content, around 75%, of beet. On the other hand, sugar beet is very high-yielding material: around three times more ethanol based on hectare can be produced from sugar beet compared to grains. The starch content of wheat is slightly higher than that of barley or rye, resulting in the higher ethanol yield of wheat, as shown in Figure 1. Potatoes are a very heterogenic group of material – the starch content may vary between 10 and 25%. This corresponds to the ethanol yield of around 70 to 140 liters per ton of potatoes. Also, lignocellulosic materials are a very heterogenic group of different potential materials, and typically the yields between 200 and 250 liters per ton of material have been reported.

### **Process Technology and Integration**

Commercial process technology and technology know-how based on traditional raw materials (grains and sugar beet) are available in the world, also in Finland. Finnish technology suppliers have been active already in the middle of 1990s in Europe. As an example, the Swedish bioethanol plant, Agroetanol Ab in Norrköping, is a turn key delivery of a Finnish consulting and engineering firm, Jaakko Pöyry Oy in 1999-2001.

Production technology based on lignocellulosic raw materials is under development. The enzymatic hydrolysis stage – the degradation of cellulose and hemicelluloses to sugars – has become more and more reasonable due to remarkable reduction in the price of enzymes. A pilot (200 tons of ethanol per year) plant based on soft wood and straw has started in Örnsköldvik in Sweden in 2004, and a demo (2500 tons of ethanol per year) plant based on straw (wheat, barley, oats) has been operating in Ottawa in Canada already since 1997. Competitive technology based on lignocellulosic materials has been estimated by VTT to be available within five or ten years.

By integrating the ethanol production to other industries the competitiveness can be increased. In the world, the integration to sugar plants is a common practice. In Finland, a sugar company Danisco is studying this possibility in Finland and even, the possibilities to build the ethanol plant based on the former sugar production process facilities. Integration possibilities in Finland would also be, e.g., biogas plant, CHP (combined heat and power) –plant or pulp and paper mill.

### **Economy**

Based on earlier experiences the minimum capacity of a competitive ethanol plant would be around 50 000 tons of ethanol per year. Clearly larger plants, around 100 000 - 150 000 tons per year are in use in the world, like in the USA and also in middle-Europe in the near future. The capacity bigger than 50 000 tons per year seem, however, unrealistic in Finland due to the shortage of raw materials and lack of markets for fodder in the close surroundings of the production plant.

The total investment costs of a traditional grain based ethanol plant of a capacity of 50 000 tons per year would be around 50 millions euros. The estimation of the operation costs for that plant is presented in Figure 2. The share of the raw material, here barley (110 euros/ton), is remarkable, being over 50% of the total costs. Based on these operation costs and taking into account the income of the sale

of fodder (by-product of the process), the production costs of bioethanol would be around 40–50 cents per liter. Nowadays, the costs is not competitive with the costs of petrol. Subsidies are needed to support the production and use of bioethanol in Finland.

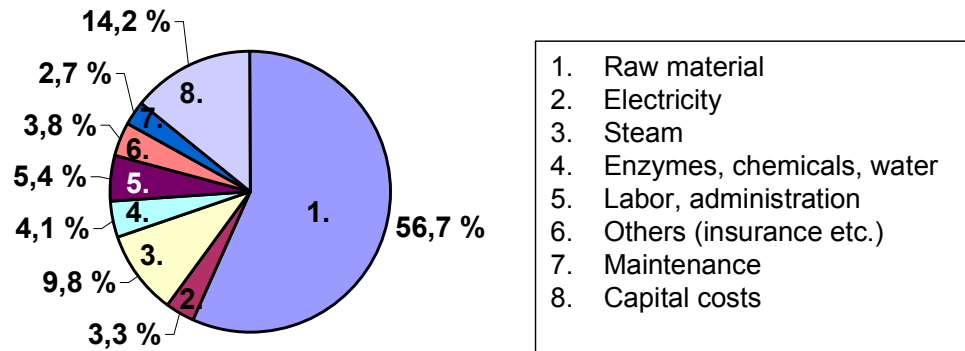


Figure 2. The distribution of the operation costs of bioethanol from barley. The plant capacity: 50 000 tons ethanol per year.

## Future in Finland

The target values set by the EU for the use of biofuels in traffic sector are far beyond the practice today in Finland.

The potential biofuels, besides bioethanol, are biogas and biodiesel. Bioethanol is not used nor produced in Finland today. Biogas is produced, but not used as a traffic fuel else but in a trial run. A large biodiesel plant (170 000 tons biodiesel per year) is under construction by the Finnish oil refining and marketing company, Neste Oil Oyj, in Porvoo, Finland. The production should start in 2007. But, so far, the company has stated to sell the whole production aboard due to the fuel taxes in Finland. If the biodiesel would be used in the future in Finland, that would account for around 5 to 8% of the domestic diesel demand.

A bioethanol plant (50 000 bioethanol per year) integrated to a biogas plant is under planning in Häme, in southern part of Finland. The study is carried out by Häme Polytechnic and a group of local farmers. The plant would utilize local sugar beet supply in addition to barley. Thus, the plant would not only help Finland to achieve the EU targets concerning biofuels but would have many positive impacts in the agriculture sector, especially after the coming “Sugar Reform” in the EU. One local aim is to ensure the living of around 500 farms which are dependent on sugar beet cultivation and the related sectors, such as harvesting and transport.

Because of the above mentioned pressures set by the EU and national publicity, a working group, appointed by Minister of Trade and Industry in Finland, has started to prepare a proposal of the promotion of biofuel production and use in Finland. The proposal should be ready by the end of February 2006.



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## Brazilian bioethanol strategy

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### Summary

The Brazilian bioethanol strategy covers historical and recent measures toward the largest biomass energy experience that required surpass politic, economic, technology, logistic and social difficulties. Present and further development in sustained basis foresees internal and international changes that can influence production in many countries and, ultimately, improvement in technology and biomass products to invest in novel markets.

### A. brazilian bioethanol strategy: historical and recent actions toward energy of biomass

At the oil crisis in the 70's Brazil was investing heavily in hydroelectricity, nuclear energy and domestic oil production when promoted the National Ethanol Program (PROALCOOL). That required the expansion of sugarcane plantation areas, to improve existing industries and build up new ones, to control ethanol prices lower than gasoline and lower taxes for cars. Challenged by low sugar export, producers from Northeast and Southeast adopted the program in new and existing sugarcane areas, some settled in colonial times alike the *plantation system* developed in the *Canary and Madeira Islands* by Portuguese. *Then with sugar lower prices the Colony gained markets in Europe, a feat sustained by production technology, equipment and mills construction, payments by a share-cropping leasing system and African slavery trade financed by the Dutch allies that controlled refining and distribution, as raw sugar was exported to Portugal and then to Holland.* In 1612, production reached 14,000 tons counting 99 sugar mills in Pernambuco and 50 sugar mills in Bahia and in the decade of 1640, Pernambuco exported more than 24,000 tons of sugar annually to Amsterdam. Expulsed from Brazil in 1624, the Dutch moved to The Caribbean Islands and later the French and English with advanced technologies dominated the sugar market. Ultimately, sugar motivated European expansion by a violent colonial structure mercantile oriented centered in raw materials and slave trades, and some rules are still preserved<sup>(4-7)</sup>. Abolishing slavery immigration from Europe was stimulated to labor in coffee plantations in São Paulo and soon the Italians bought lands to plant sugarcane to be crushed for the distillate product "aguardente" (the same used

today for caipirinha drinks). By 1910, these little units became mills and São Paulo the largest sugar producing center. Pedro Morganti, Carbone brothers and others formed the Company of Refiners Union (Cia. União dos Refinadores) and by 1920, the mechanics group of the Italian Mario Dedini become the first equipment industrial unit for sugar production in Brazil <sup>(8)</sup>.

Following France and Germany, Brazil started to run cars on ethanol since the 20's after Usina Serra Grande in Pernambuco developed a fuel ethanol. Gasoline was imported from USA and the new fuel would mean energy independence and currency economy. Car performance tests showed a consumption of 5,466 Km/l for gasoline; 4,800 Km/L for USGA and 4, 266 Km/L for Ethanol 42°GL. Tells the story USGA was removed off market by dumping of the multinational oil companies allied to automaker industries and the fuel distributor The Great Western Brazil Railway. Before these experiences France, in 1902, had engines on anhydrous ethanol (Alkomine) industrially produced and sold in large scale. In 1903, Germany consumed 648,000 gallons of ethanol in many fuels like Eletrina (50% Ethanol, 50% Benzol) and Leuchtspiritus (about 35% benzene hydrocarbons, a quantity of naftalene and the rest of ethanol). The anhydrous ethanol French process directed Brazil to impose by decree (1931) to gasoline importers to buy ethanol 96° GL at the equivalent amount of 5% of the gasoline imported till equip the industries to produce anhydrous ethanol. A Decree of June 1, 1933 created The Institute of Alcohol and Sugar (IAA) to coordinate production and commerce of alcohol and sugar <sup>(9)</sup>.

The car and fuel market in Brazil had been shaped over the years by foreign companies, a situation that started to change after founding of Petrobras in 1953, by [Law 2004](#), with the objective of executing, on behalf of the Federal Government, the activities of the [oil sector](#) in Brazil <sup>(10)</sup>. And the implantation of the national automotive industry in Brazil started in 1956 with the first truck industry with a national engine <sup>(11)</sup> and grew up favored by special protections, subsidies and tax cuts.

The foundation of the Cooperative of Producers of Sugarcane, Sugar and Alcohol (COPERSUCAR) in 1959, formed by 10 industries and the cooperatives Coopira and Coopereste aimed to improve sugar commerce and the development of technologies at the Center of Technology Copersucar<sup>(12)</sup> have a remarkable presence over the years and embraced PROALCOOL in 1975, when ethanol was added gasoline in the percentage of 20% to 22%. Only in 1980 hydrated ethanol moved adapted gasoline engine vehicles stimulating the car industry to sell 94.4% of cars on pure ethanol by 1984. The percentage of hydrous ethanol cars felt persistently since 1986 with the anti-inflation politics and the industry shifted to sell gasoline cars (Fig. 1). The Brazilian Government reduced the financial support to PROALCOOL since 1987 inducing uncertainty to continue the program. Meanwhile sensitive to sugar price of US\$0.15/Pound more sugar was made reducing ethanol production causing long lines at the pumps. In 1989 methanol was imported for fuel and 5% of gasoline added to ethanol, a situation aggravated by the fall of oil prices from US\$30.00 to US\$20.00 challenging PROALCOOL to a serious credibility crisis <sup>(13)</sup>. Ultimately, in the 90's the Government abolished selling taxes for ethanol fuelled cars, opened the market for imported cars and stimulated the production of 1000cc cars not adapted for ethanol. These measures combined discontinued production of hydrous ethanol powered cars. In the critical analysis of Carvalho (2005), the Government the traditional mentor of sugarcane politics - since the era of the Hereditary Captaincies - left the control of the sugar-alcohol industry to the private enterprise "a rarity in the international market of sugar and alcohol

since the state interference in the sector is really global”<sup>(14)</sup>. And the unimportance given to hydrous ethanol ceased any further technological advances ignoring a fuel that need less investment and have more useful energy power that gets on 42% and proved to make 52% with a patented equipment to pre-evaporate the fuel, a feat of Brazilians (Zanetti and Camerini) as theoretical yield would be 60% maximum as 40% is heat<sup>(15)</sup>.

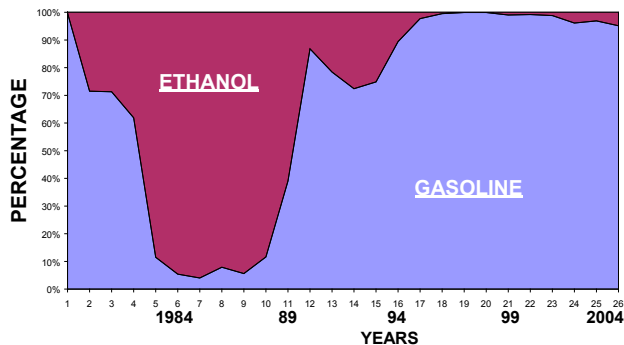


FIGURE 1 - Domestic wholesale of cars by fuel type - Share in percentage

Source: ANFAVEA 2004 Vehicles - Production, domestic sales and exports<sup>(16)</sup>.

Brazil is still selling hydrous ethanol and enthusiastic with increasing sales of flexible fueled vehicles (FFV) that run on gasoline, alcohol or any of the two in the tank with the mix to combustion adjusted by electronic sensors. The great acceptance by consumers both for the option of fuel kind and price, closing sales of October 2005 Brazil is expected to sell over one million FFV after 31 month sells<sup>(17)</sup>.

## B. Present and further development

The Brazilian bioethanol gets attention for the very large scale production and distribution in great distances to fuel millions of vehicles for years sustaining more than 25000 gas stations with hydrous ethanol pumps. The experience started with PROALCOOL after the impacts of raising oil prices and become an example renewable energy resource of environmental significance. As the large use of petrol started in the XIX century and traded worldwide for energy and chemicals, gasoline an idle surplus had to be burned by cars. To reduce pollutant gas emissions ethanol added to gasoline nowadays is the best alternative for liquid fuels under the rules of the Kyoto Protocol. Thus the international market for ethanol in 2003 had been of 34.4 billion litres of alcohol where 4.6 billion/L of beverages, 7.3 billion/L industrial and 22.5 billion/L for fuel<sup>(2)</sup> and in 2005 Brazil and USA alone are producing 34 billion liters for fuel a proved confidence on bioethanol. And production is growing in many countries showing that USA corn ethanol percentages about the same as Brazil sugarcane ethanol (Fig. 2) followed by and others with a growth trend in Europe for starch (corn, wheat, rye, etc.) bioethanol compared to sugarbeet.

## World Ethanol Production 2004

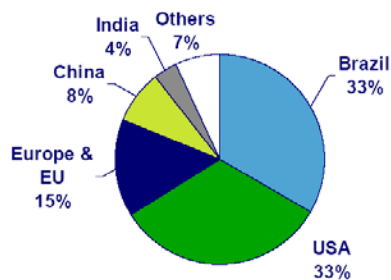
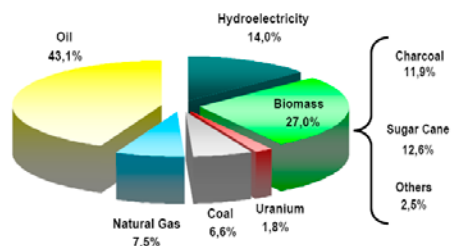


FIGURE 2 – Bioethanol producer leading Countries (18).

The figures about Brazil energy consumption and fuel production are described in Fig. 3. It is important to mention that Brazil is the 10th largest energy consumer in the world and the third largest after USA and Canada in Americas with total energy production growing and almost self sufficient in domestic oil production. The categories of energy consumption compared reveal 12% for bioethanol fuel and 43% for oil and the total of fossil energy accounts for 57.2% of the total from a variety of energy sources <sup>(19)</sup>.



Source: MME/2004

FIGURE 3 – Brazil: categories of energy consumption percentage.

Being the first sugarcane producer (61% for domestic and 39% for export products), the largest sugar exporter, the third largest sugar consumer, and the largest bioethanol producer, Brazil in 2005 will produce about 17 billion liters of ethanol (85% for domestic market and 15% to export) and 219,183,000 ton/sugar (37% for internal market and export 63%) in a crop area of 5.8 million hectares<sup>(20)</sup>. The sugar cane production costs estimates of about US\$180/ton and US\$165/ton for São Paulo, these best prices in the World followed by Australia (US\$335/ton) and sugar prices in EU varies from US\$565 to US\$713/ton (18). As subsidies and quotas are about to be reduced the EU sugar companies are associating to sugarcane industries as seen in Brazil either for strategic sugar market and foreseeing ethanol production overseas or to attend the Kyoto Protocol. Thus the French group Louis Dreyfus bought the Usina Cresciunial and Usina Luciânia, the group French/Italian Béghin-Say bought two units of Usina Guarani, the largest Brazilian group COSAN (47,5%) allied to the French groups Union SDA (47,5%) and Sucden (5%) to buy the Usina Univalem and the Swiss Glencore bought the Usina Portobelo (21).

For the present crop season of 2005 estimates from the Union of Sugarcane Ag-  
rindustry of São Paulo (UNICA) the sector output is summarized below:

Table 1 - BRAZIL PRODUCTION MARKET (2004/2005 CROP SEASON)

	DOMESTIC (%)	EXPORT (%)
SUGAR	37	63
ETHANOL	85	15
SUGARCANE	61	39

Source: UNICA ([www.portalunica.com.br](http://www.portalunica.com.br))

The State of São Paulo alone crushes about 60% of the total sugarcane for sugar and ethanol production. Some units have the experience of co-generation of electricity and sell to energy distributors, a market that Nastari et al (2005) <sup>(21)</sup> consider as the second wave of the economic use of sugarcane bagasse. Considering the sugarcane products sugar and ethanol and converting to a sucrose common index they found a growth from 7.3 million tones in 1975/76 to 55.42 million tones in 2004/05, an will reach 58.06 million tones sucrose in 2005/06. They foresee a third wave to come using sugarcane residues (trash), tops and leaves that will lower the ethanol production cost from total biomass. Regarding land use Nastari et al (2005) point out that conservation and preservation areas of Brazil reached 95 million hectares in 2004 (11% of the territory, of an area of 858 million hectares), the entire crop area is 60.4 million hectares: about 7% for agriculture (half by soybean and corn), cattle pastures 35%, forests 55% and among them sugarcane occupy 0.6% of the territory and available areas for expansion at least 12%. The distribution of areas occupied with sugarcane plantations and grain crops are shown in Fig. 3.

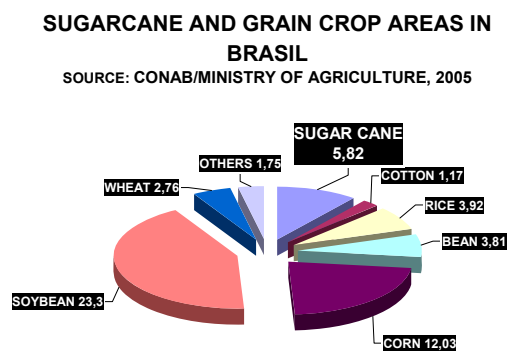


FIGURE 3 – Sugarcane and Grain Crop Areas in Brazil (numbers in Million Hectares)

Source: CONAB/MAPA, 2005 ([www.conab.gov.br/](http://www.conab.gov.br/)) and IBGE 2005 ([www.ibge.gov.br/home/presidencia/noticias/](http://www.ibge.gov.br/home/presidencia/noticias/)).

The industrial units in Brazil total 329 operating in 2003, from which 21 were producing sugar only, 98 alcohol only and 210 sugar and alcohol producing units classified by regions as seen in Table 2. The plantation areas, total ethanol and sugar production estimates for 2005 are presented in Table 3 and the values show 85.3% of sugarcane production for the Center-South region of Brazil.

Table 2 - NUMBER OF MILLS IN THE 2003/04 HARVEST SEASON

MILLS	São Paulo	Center South	North Northeast	TOTAL	% of total
Sugar	2	6	15	21	6,4
Alcohol	22	62	36	98	29,8
Sugar+Alcohol	109	158	52	210	63,8
Total	133	226	103	329	100,00

Source: UNICA([www.portalunica.com.br](http://www.portalunica.com.br))

Table 3 – SUGARCANE, TOTAL ETHANOL (NEUTRAL, HYDROUS, ANHYDROUS) AND SUGAR PRODUCTION IN BRAZIL (SEASON 2005/2006 ESTIMATES)

REGION	AREA (x 10 <sup>6</sup> Ha)	SUGARCANE (x10 <sup>3</sup> ton)	TOTAL ETHANOL(x10 <sup>3</sup> l)	SUGAR
NORTH/ NORTHEAST	1169.20	64,701.60	1,183,446.9	4,052,401.80
CENTERSOUTH	4763.70	375,321.60	15,154,565.5	23,157,490,80
BRAZIL	5932.90	440,023.20	17,028,012.40	27,209,892,6

Source: CONAB/MAPA, 24/10/2005 ([www.conab.gov.br/](http://www.conab.gov.br/))

### C. Conclusions

The development requires to foresee internal and external influences on bioethanol production, ultimately learning from history and the recent PROALCOOL, and to invest in research and technology. The market growth are trends in Brazil, USA, EU and others, and expansions shall consider the Global Bioenergy Partnership launched by G8 leaders to support biomass and biofuels use mainly in developing countries. Private groups are doing acquisitions and partnerships in Brazil that must spread over expecting better ethanol processes and productivity increases. It should be reconsidered to use better the superior hydrous ethanol top fuel efficiency and its lower production costs. The co-generation of energy and total sugarcane processing require financial investments along with research to evaluate gasification technologies and to run simple stationary engines. Technology advances are necessary in agriculture and for industrial residues conversion, to recover evaporated ethanol and to incentive Co<sub>2</sub> lost in fermentation for biomass and more ethanol production such as the conversion by the bacteria *Clostridium ljungdahli*. So far economic progress shall reflect improvement of social conditions for workers and the society.

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## Preparation of chitin out of shrimp wastes by two proteolytic *Bacillus licheniformis* strains isolated from Indonesian shrimp shells

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### Abstract

Deproteinization of shrimp shell wastes should be accomplished by proteolytic microorganisms. Bacteria from Indonesian shrimp waste were isolated and characterized using API test systems and 16S rRNA analysis. A culture of two thermophilic *Bacillus licheniformis* strains showed the best properties in hydrolyzing shrimp protein of fresh and processed *Penaeus monodon* waste. In a 1 L fermentation approach at 55 °C and pH 8.0, 98.8 % of the shrimp waste protein was hydrolyzed.

### Introduction

Chitin (poly  $\beta$ -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine) and its deacetylated derivative chitosan have a large range of applications in chemistry, medicine, pharmacy, water treatment etc. concerning their unique properties including the ability to form films, chelate metal ions and antimicrobial activities (Kumar, 2000). The limitations on the utilisation of this resource are directly connected to the relatively high costs of its purification by the traditional chemical process (Muzzarelli, 1990). In this process proteins and calcium carbonate are removed by an alternate treatment with acid and alkali which results in high disposal costs for the large volumes of aqueous wastes generated. An alternative to the chemical process represents a biotechnological working up of the shrimp shell waste. The biotechnological process includes the deproteinization of shrimp waste protein by proteolytic microorganisms or enzymes and a removal of calcium carbonate by lactic acid fermentation (Healy et al., 1994). In this work a process for deproteinization of shrimp shell waste is optimized with two newly isolated and characterized *Bacillus licheniformis* strains.

### Results and Discussion

240 Strains were isolated from Indonesian shrimp wastes at different conditions. Thermophilic strains of the genus *Bacillus* showed the best properties in hydrolyzing shrimp waste protein. The total nitrogen content of the deproteinized waste was reduced from 7.2% without addition of a starter culture to 2.7% with addition of a mixed culture of the isolates F5 and F11. These two strains showed 100% identity to strains of *Bacillus licheniformis* determined by 16S rRNA analysis and the API test system. The advantage of a process performed at thermophilic conditions lies in the repression of potentially pathogen microorganisms of the shrimp waste spoilage flora. Furthermore high energy costs for sterilization could be avoided.

Shrimp shell wastes contain ~30 % protein in dry matter. Most of these proteins have only a slight adherence to the shrimp shells and so easily can be washed out. After grinding of the shell wastes and several washing steps about 70 % of the protein is removed and a notable better efficiency of deproteinization can be achieved. The amount of protein to be hydrolyzed was lowered, so nearly the whole amount of residual protein in the shrimp waste could be hydrolyzed within 48 h by proteolytic enzymes produced during fermentation. The protein content could be reduced from 10% in the chitin product of untreated waste material to 2.1% in the chitin product of pretreated wastes (Tab 1.)

## Fermentation of tempe with *Bacillus megaterium* for supplementation with vitamin B<sub>12</sub>

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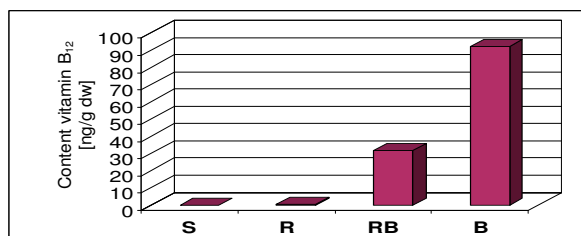
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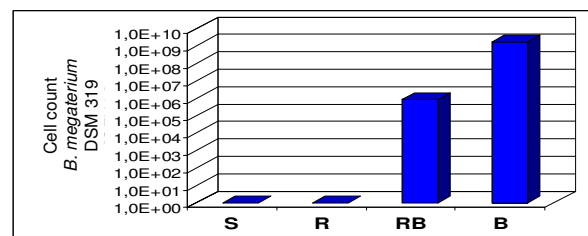
The Indonesian food tempe is produced by fermentation of soybeans with strains of the mold *Rhizopus* spp. Tempe is of high nutritional value because of the high content of free amino acids, various vitamins and the pattern of free fatty acids as a result of the fermentation process. In traditional fermentations also contaminating bacteria contribute to this.

In industrial processing defined starter cultures are necessary to obtain a safer product with more defined quality. Therefore, in former studies, the starter culture of *Rhizopus* spp. has been optimized by using complementary strains of *R. oligosporus* and *R. oryzae* concerning vitamin production (Wiesel et al. 1997). For supplementation of tempe with vitamin B<sub>12</sub>, bacterial co-fermentation is necessary. We use *Bacillus megaterium*, which is also used for industrial production of vitamin B<sub>12</sub>. By co-fermentation with *B. megaterium* DSM 319, tempe could be enriched with vitamins, mainly vitamin B<sub>12</sub> and biotin (Hampf and Bisping 2003).

In this study we investigated the influence of co-fermentation of *R. oligosporus* MS5, *R. oryzae* EN and *B. megaterium* DSM 319 during tempe fermentation. Tempe was prepared by fermentation of soybeans with a mixed starter culture of *R. oligosporus* MS5 and *R. oryzae* EN with *B. megaterium* DSM 319 (sample RB) and without *B. megaterium* DSM 319 (sample R) for 34h at 32°C. For reasons of comparison fermentation of soybeans was performed with *B. megaterium* DSM 319 only (sample B). Furtheron we compared also unfermented soybeans (sample S). As shown in figure 1, tempe could be enriched with vitamin B<sub>12</sub> by co-fermentation with *B. megaterium* DSM 319. Determination of vitamin B<sub>12</sub> was performed by microbiological assay using *Lactobacillus delbrueckii* ssp. *lactis* DSM 20355 (Okada et al. 1985; Strohecker and Henning 1963; Augustin et al. 1985). Vitamin B<sub>12</sub> analogs were calculatively subtracted. A vitamin B<sub>12</sub> content of 31,6 ng/g dry weight was obtained in sample RB. An about three fold content of vitamin B<sub>12</sub> was obtained by fermentation of soybeans with *B. megaterium* DSM 319 only (sample B).



**Figure 1:** Content of vitamin B<sub>12</sub> in soybeans and various tempe preparations.



**Figure 2:** Cell count of *B. megaterium* DSM 319 after fermentation.

Cell counts of *B. megaterium* DSM 319 in tempe co-fermented with *Rhizopus* spp. are shown to be significantly lower than after fermentation with *B. megaterium* DSM 319 only (figure 2). This effect could result from competition for nutrients or from antibacterial substances produced by *Rhizopus* spp. Antibacterial activity of extracts of tempe and of *R. oligosporus* against various grampositive bacteria was described earlier (Wang et al. 1969; Wiesel et al. 1997).

## Fodder yeast production from raw glycerol

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The aim of these studies was to identify a suitable yeast strain for fodder yeast production on media containing a glycerol fraction from bio-diesel plants (rapeseed oil ethyl esters). Single or mixed cultures of *Yarrowia lipolytica*, *Candida lipolytica*, *Candida utilis* and *Candida tropicalis* were employed for biomass yeast production.

All experiments were conducted on the media containing 30 g/l of a glycerol fraction, as substrate, in a stirred tank reactor at the pH ranging from 3.5 to 4.0. The glycerol fraction contained 45% (w/w) of raw glycerol, 3-4% ethyl esters, 46% of fatty acids and large amounts of potassium soaps. Based on the results of these experiments, a mutant UV of *Y. lipolytica* was found to be the most suitable for biomass production from the glycerol fraction. This strain simultaneously utilized raw glycerol, fatty acids and ethyl esters during batch cultivation. *C. utilis* and *C. tropicalis* strains utilized first fatty acids and next (very slowly) raw glycerol with lower biomass productivity and the yield. Concentration of protein in the biomass yeasts ranged from 36 to 42.5%, depending both on the amount of nitrogen in broth and the yeast strain. The highest protein concentration (42.5%) and its higher biological value were found in the *Y. lipolytica* yeast strain. The glycerol fraction from bio-diesel production proved to be a very good substrate for biomass production by *Y. lipolytica* giving excellent yields and productivity at low pH. The results are very promising as they may lead to cheap processes for fodder yeast production from renewable lipid resources.