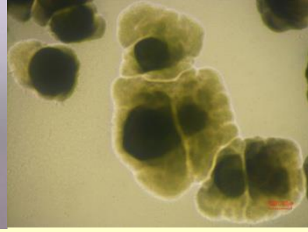
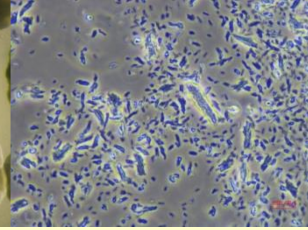
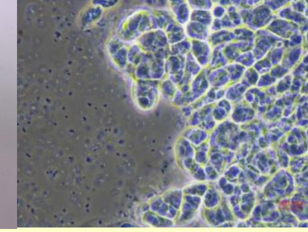
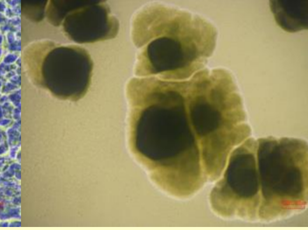
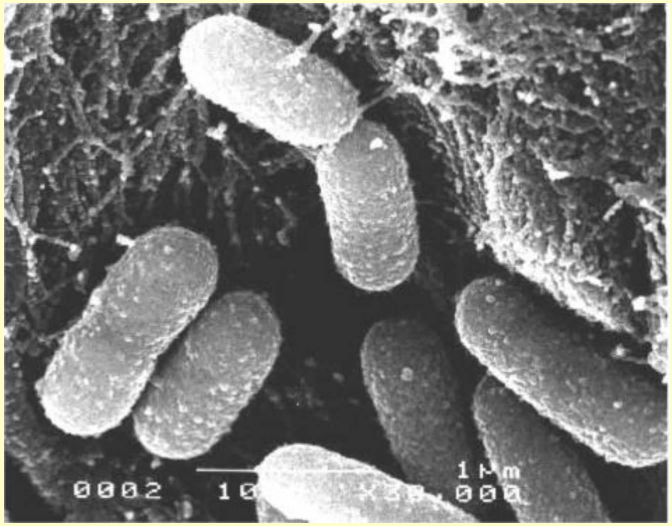




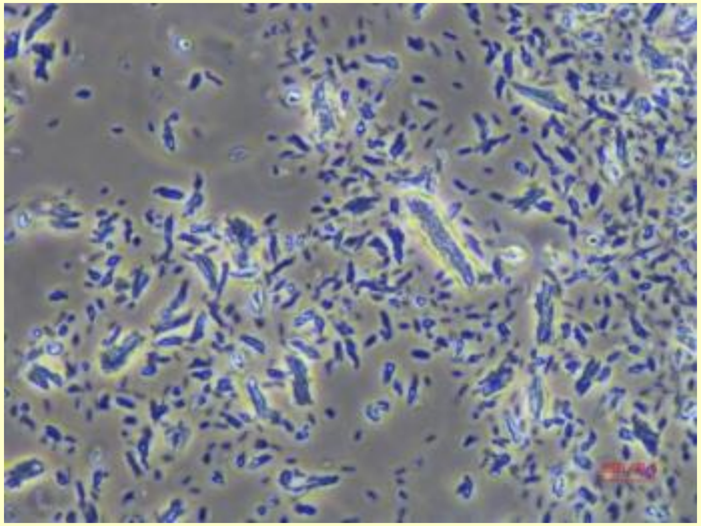




COMBINATION OF NATURAL MICRO-FLORA WITH INDUSTRIAL STRAINS IN BIO REFINERIES

Adj. Prof. Elias Hakalehto, University of Eastern Finland

MICROBES ARE INVISIBLE TO THE NAKED EYE

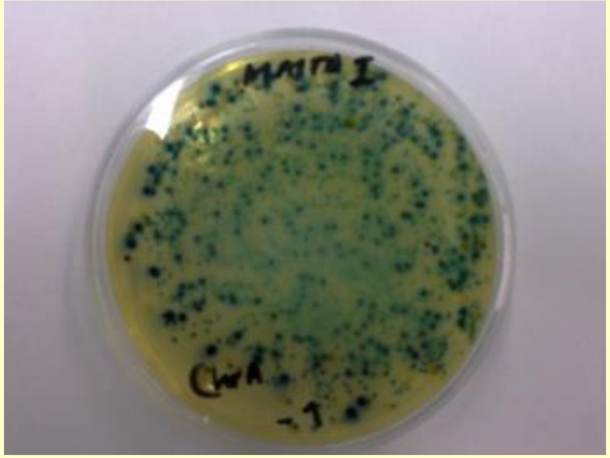




BUT WE NOTICE THEIR INFLUENCE ON US

MICROBES LIVE IN THE INTERFACES OF THE THREE PHASES:

1. SOLID
2. LIQUID, AND
3. GAS PHASE



MICROBES ARE UNIVERSAL

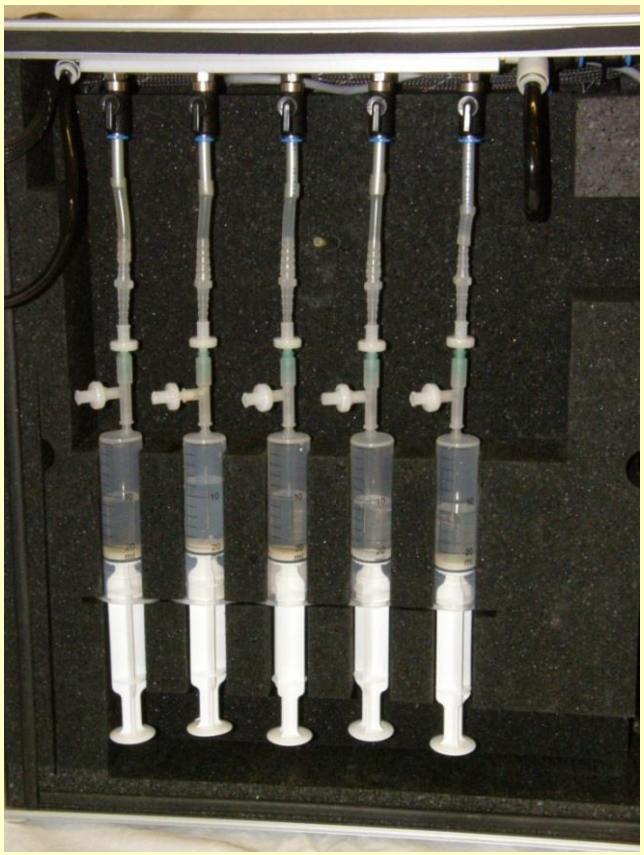
IN NATURE THEY EXIST AS MIXED COMMUNITIES

CONTRARY TO THE ANIMAL SPECIES, MICROBIAL STRAINS ARE GLOBALLY DISTRIBUTED. THEY LIVE IN COEXISTENCE WITH ANIMAL AND PLANT KINGDOMS, AND WITH MAN.

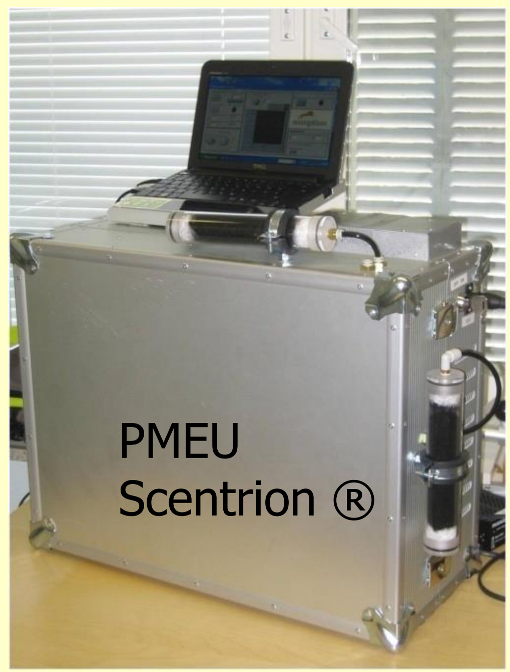
IN ORDER TO STUDY THE INTERACTIONS BETWEEN VARIOUS MICROBES,
WE NEED TOOLS.

PMEU versions

PMEU Spectrion ®



Syringes in the PMEU (Portable Microbe Enrichment Unit)



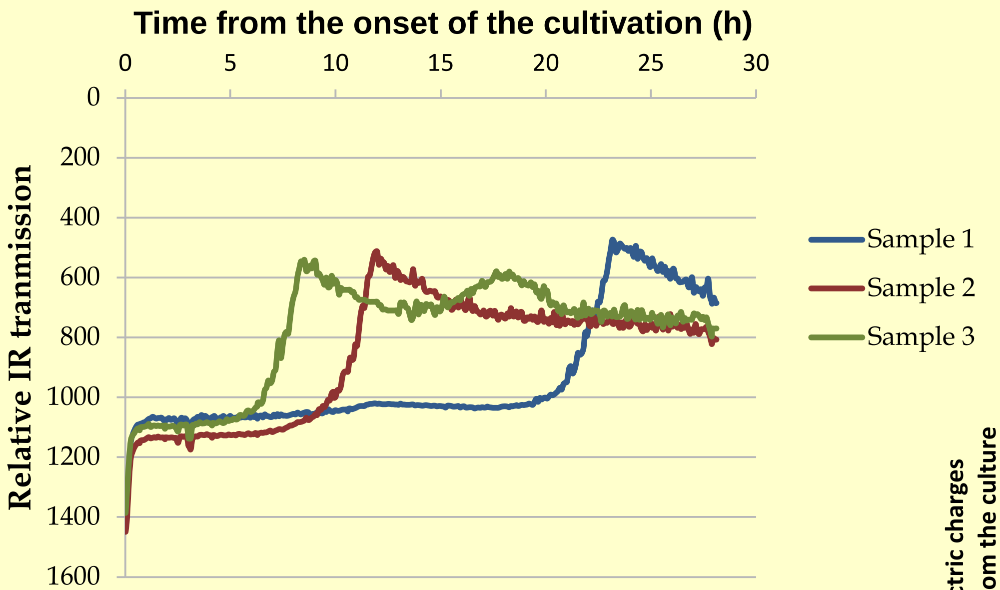
PMEU Scentrion ®

PMEU with Automated Sample Collection System

EXHAUST GASES OF ONE MICROBIAL CULTURE EXPLOITED BY THE SUBSEQUENT ONES

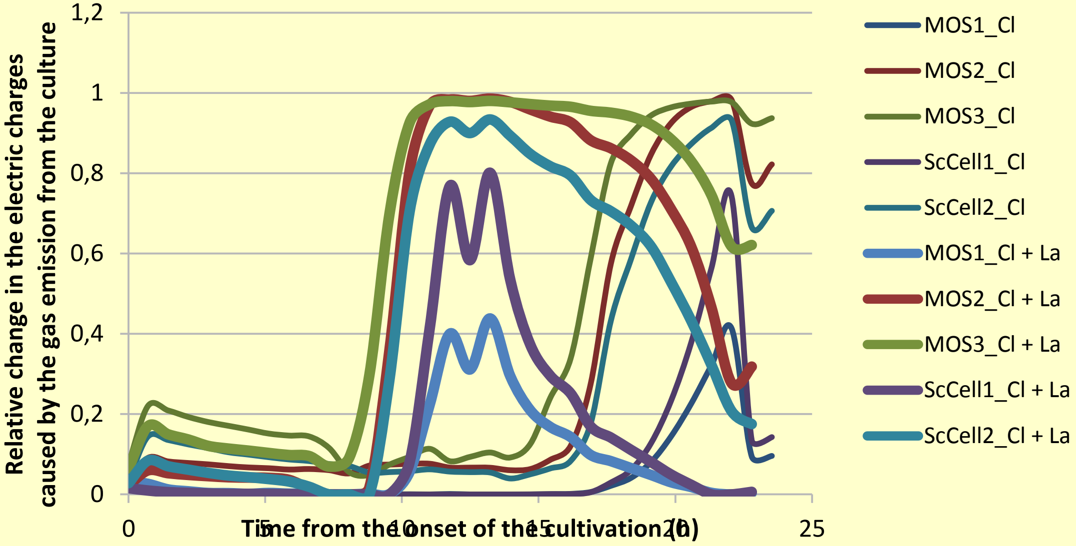
Hakalehto, E. 2013. Interactions of *Klebsiella* sp. With other intestinal flora. In Pereira, L.A. & Santos, A. (eds) *Klebsiella* infections: Epidemiology, pathogenesis and clinical outcomes. Nova Science Publishers, Inc. New York, USA.

CARBON DIOXIDE SPEEDS UP THE BACTERIAL GROWTH



Effect of CO_2 from preceding PMEU syringe culture on the onset of clostridial growth

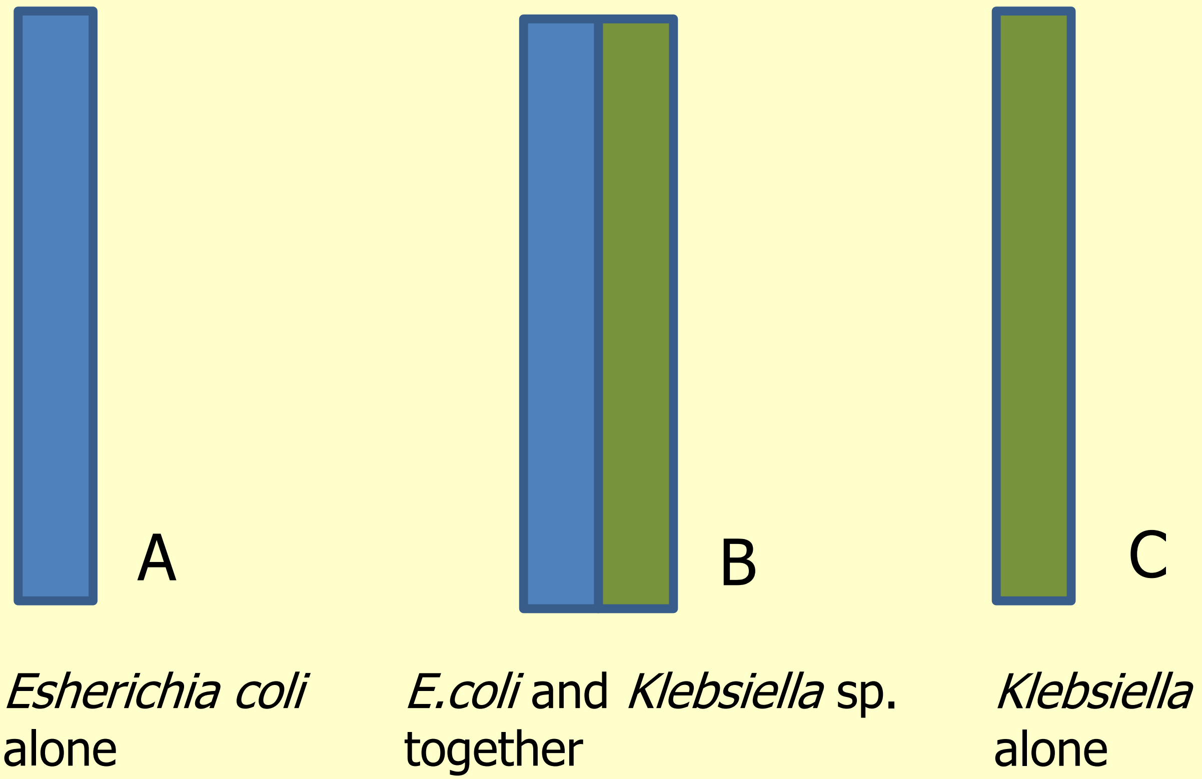
Thin lines = *C. butyricum* alone
Thick lines = *C. butyricum* connected from *L. brevis* culture



Hakalehto, E. & Hänninen, O. (2012): *Can. J. Microbiol*. 58: 928-931.

Gaseous CO2 signal initiates growth of butyric-acid-producing *Clostridium butyricum* in both pure culture and mixed cultures with *Lactobacillus brevis*

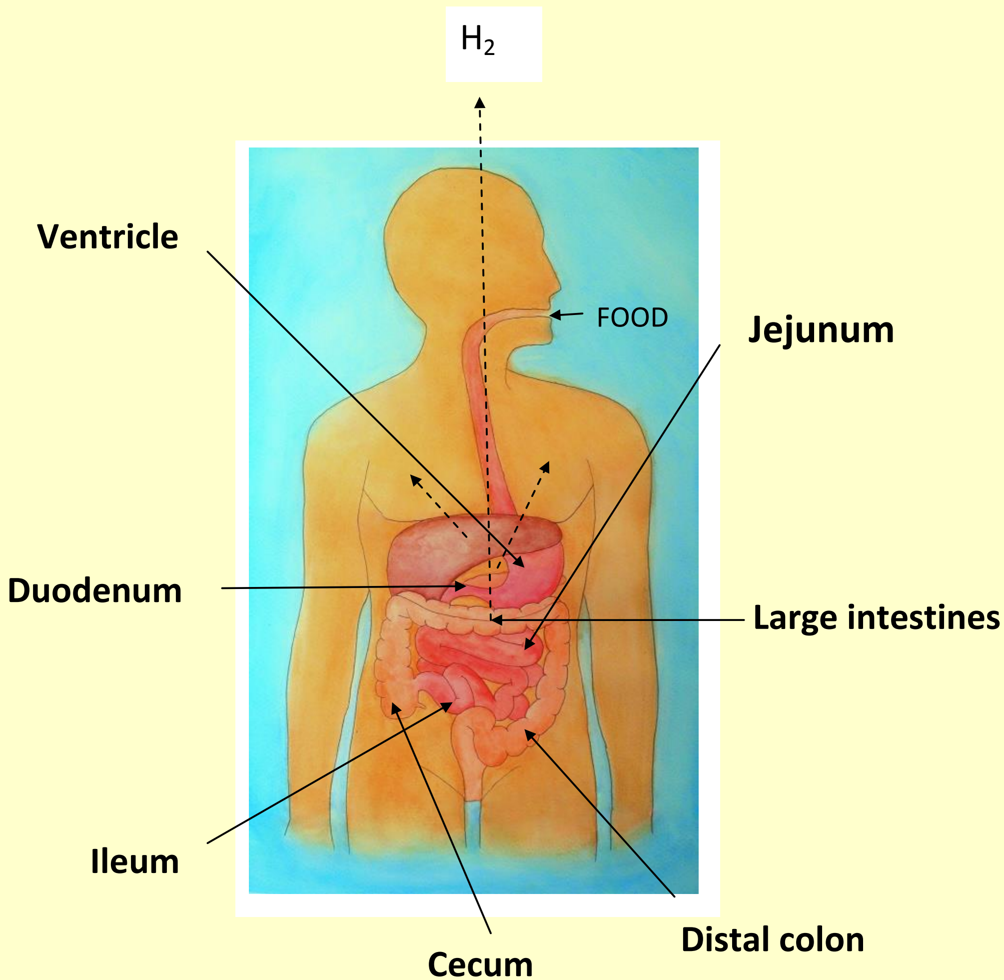
DUALISTIC BALANCE IN THE SMALL INTESTINES

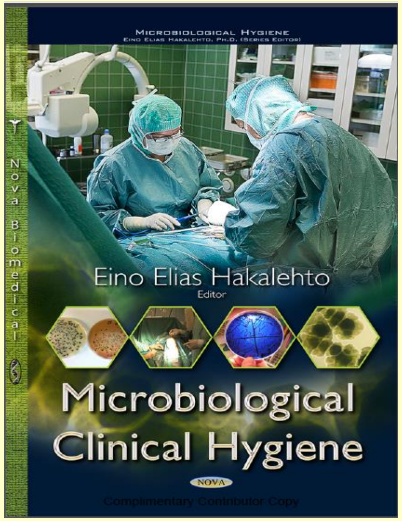


GROWTH LEVELS IN PURE CULTURES (A,C) AND IN A MIXED CULTURE (B) *Klebsiella* and *E. coli* GROW UP TO SAME CONCENTRATION AS EACH OF THEM SEPARATELY. TOGETHER THEY KEEP THE PH OF DUODENUM AT 6 WITH SIMULTANEOUS EMISSION OF VOLATILES, CO_2 AND H_2.

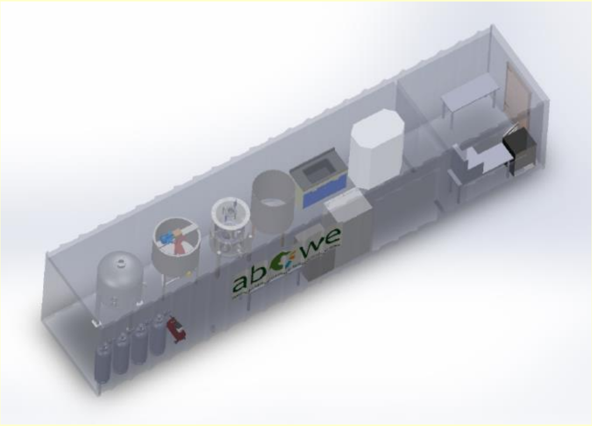
Hakalehto, E, Humppi, T., Paakkanen, H. 2008. Dualistic acidic and neutral glucose fermentation balance in small intestine: Simulation *in vitro.* Pathophysiology 15: 211-220.

HUMAN GASTROINTESTINAL TRACT…

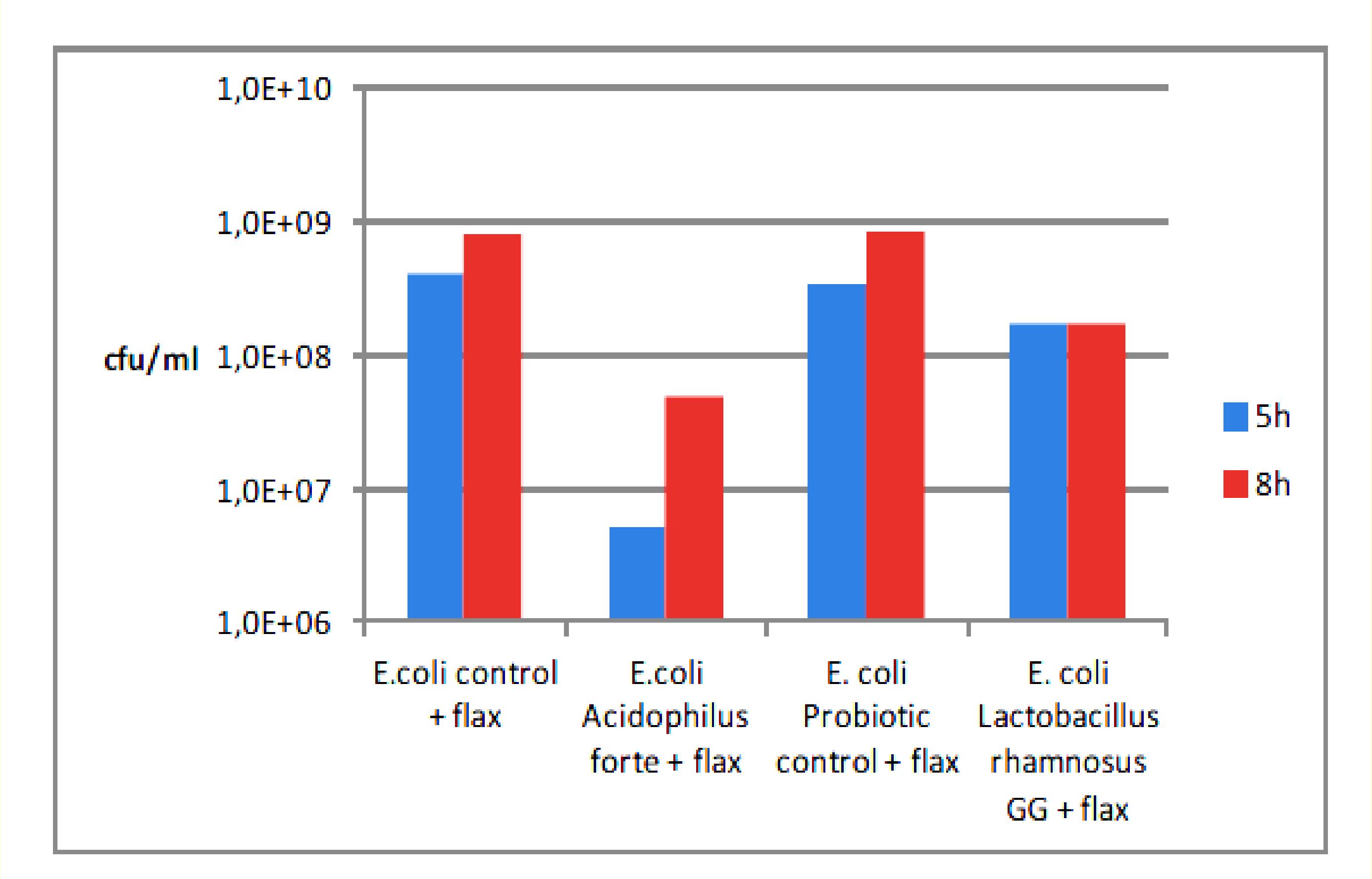




…IS A BIOREFINERY



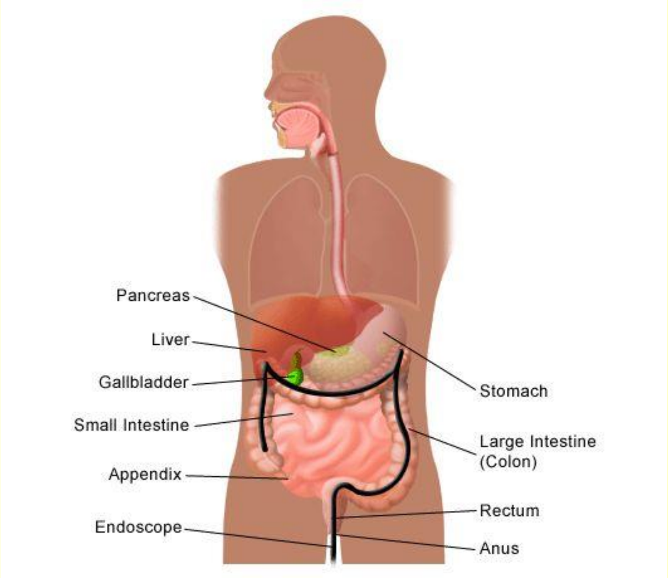
The joint effect of a probiotic mixture of *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *B. lactis* with prebiotic flax on *Escherichia coli* growth in the PMEU. Note that the logarithmic scale indicates approximately 100-fold restriction of the colibacteria.



Hakalehto E. & Jaakkola K. 2013. Synergistic effect of probiotics and prebiotic flax product on intestinal bacterial balance. Poster in 35th ESPEN Congress on Clinical Nutrition and Metabolism, September 2013, Leipzig, Germany. *Clinical Nutrition* 2013 Vol. 32, Supplement 1, S200.

Patient no	Cardia	Antrum	Pylorus
1	*Enterococcus faecalis* (2)[a] *Str. thermophilus* *L. salivarius ssp. salivarius*	*L. salivarius ssp. salivarius (2)*	*L.s salivarius ssp. salivarius*
2	*S.. salivarius* (2)		
4	*S..sanguinis* (2) *S..salivarius* *E. faecalis*	*S. salivarius*	
5	*S. salivarius*	*Str. salivarius*	*L. salivarius*
6		*L. reuteri* *L.casei* 97%	*L.reuteri*
7		*L. reuteri* (3) *S.salivarius*	*L. reuteri*
8		*Str.sanguinis*	*Lactobacillus reuteri* (2)
9	*Lactococcus lactis ssp. lactis (2)*		*S. thermophilus*
10	*S. sanguinis*	*S. sanguinis*	*S.. sanguinis*
11	*S.salivarius* (2)	*Str. salivarius* (2)	*L. reuteri* (3)
12	*S. salivarius*	*Str.salivarius* (3) *L. reuteri* (2)	*S.salivarius*
13	*S. salivarius* (2)		*S. salivarius*

Identified LAB (lactic acid bacteria) species enriched from the biopsies obtained from different gastric sites and cultivated with PMEU



Hakalehto, E; Vilpponen-Salmela, T; Kinnunen, K; von Wright, A. Lactic Acid bacteria enriched from human gastric biopsies. *ISRN Gastroenterol,* 2011.

Journal of proteome research

Article

pubs.acs.org/jpr

Composition and Antimicrobial Activity of the Skin Peptidome of Russian Brown Frog *Rana temporaria*

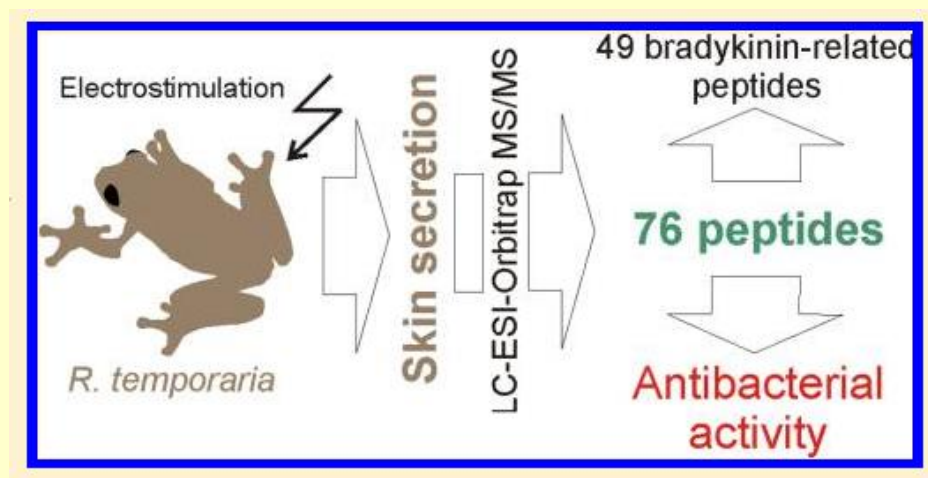
T. Yu. Samgina,[1] E. A Vorontsov,[1] V. A. Gorshkov,[1] E. Hakalehto,[2] O. Hanninen,[3] R. A. Zubarev,[4] and A. T. Lebedev[1,*]

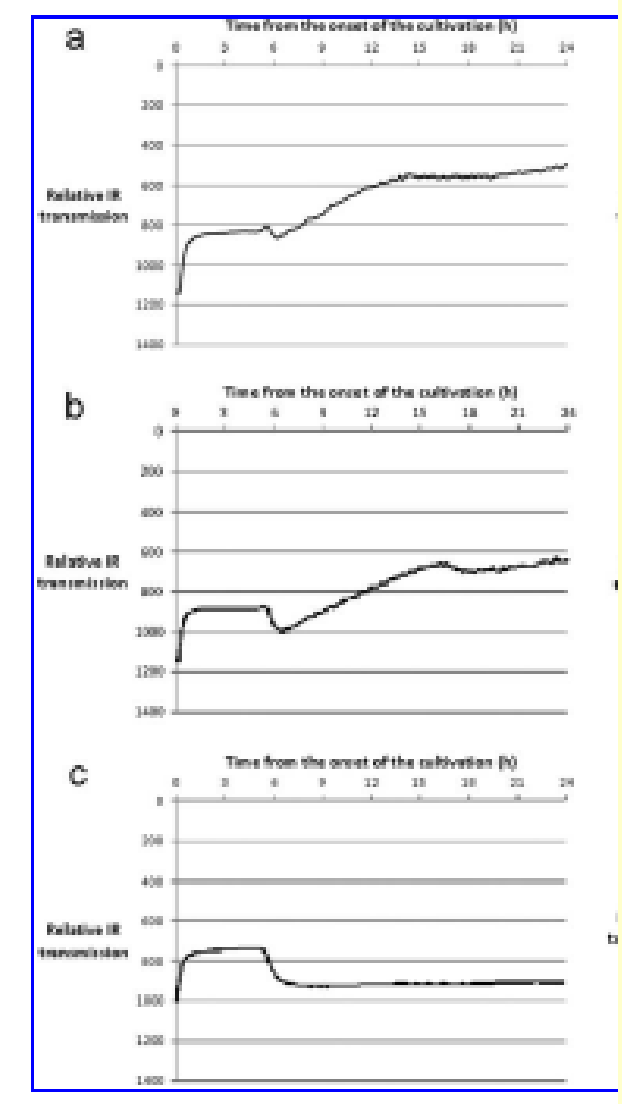
[1]Organic chemistry Department, Moscow State University, Moscow, Russia

[2]Department of Biosciences, University of Eastern Finland, P.O.B. 1627, FI-70211 Kuopio, Finland

[3]Department of Physiology, University of Eastern Finland, P.O.B. 1627, FI-70211 Kuopio, Finland

[4]Department of Medicinal Biochemistry and Biophysics, Division of Molecular Biometry, Karolinska Institutet, Stockholm, Sweden



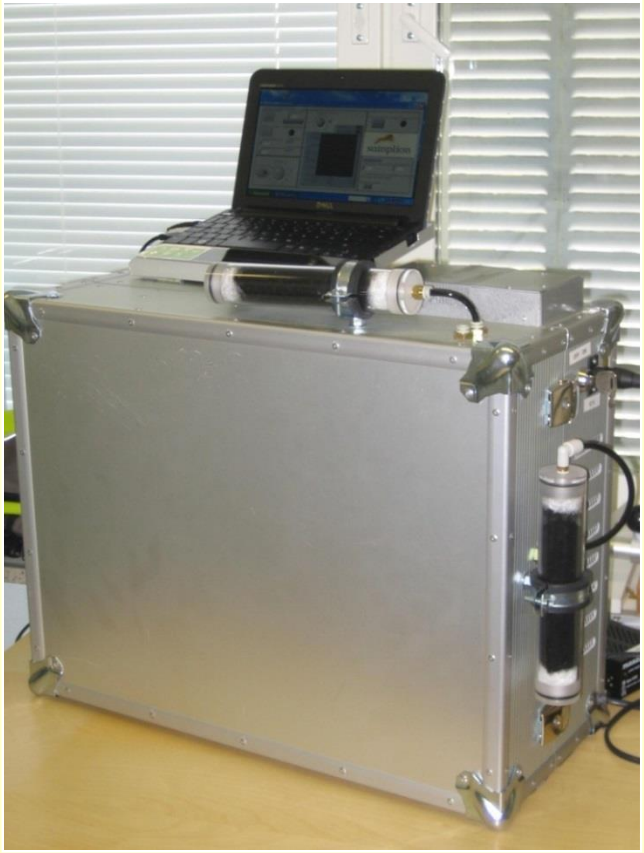


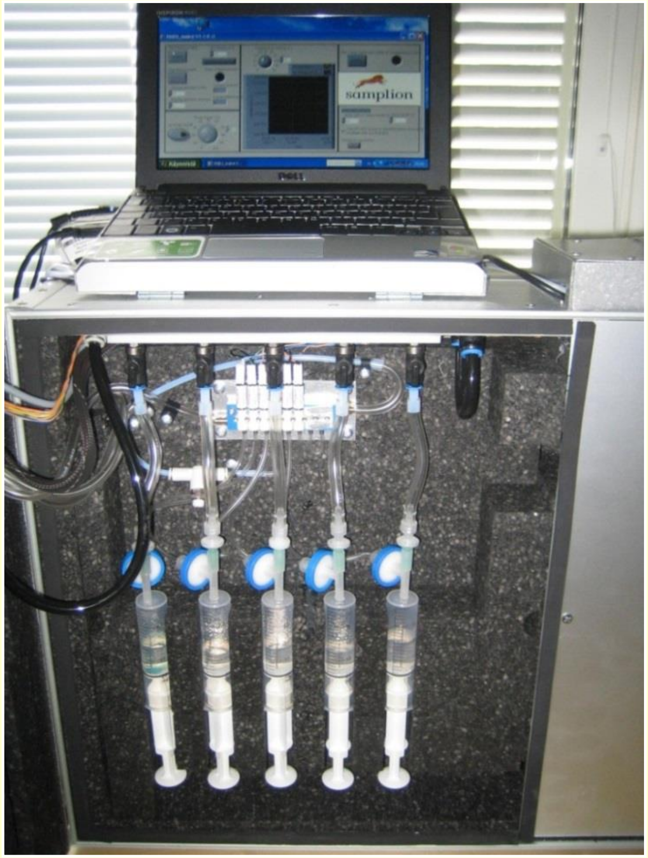
Samgina, T. Yu et *al*. 2012.
Journal of Proteome Research
American Chemical Society

Figure 4. (a) Growth of *Staphylococcus aureus* strain in the TYG medium without peptide additions; (b) growth of *Staphylococcus aureus* strain in the presence of 0.01 mg/mL of M3 sample; (c) growth of *Staphylococcus aureus* strain in the presence of 0.03 mg/mL of M3 sample.

Study method: PMEU Scentrion®

Hybrid analyzer designed by Adj. Prof. Elias Hakalehto (Finnoflag Oy, Finland) and Dr. Heikki Paakkanen, (Environics Oy, Finland)





Finnoflag Oy and Samplion Oy, Siilinjärvi, Finland

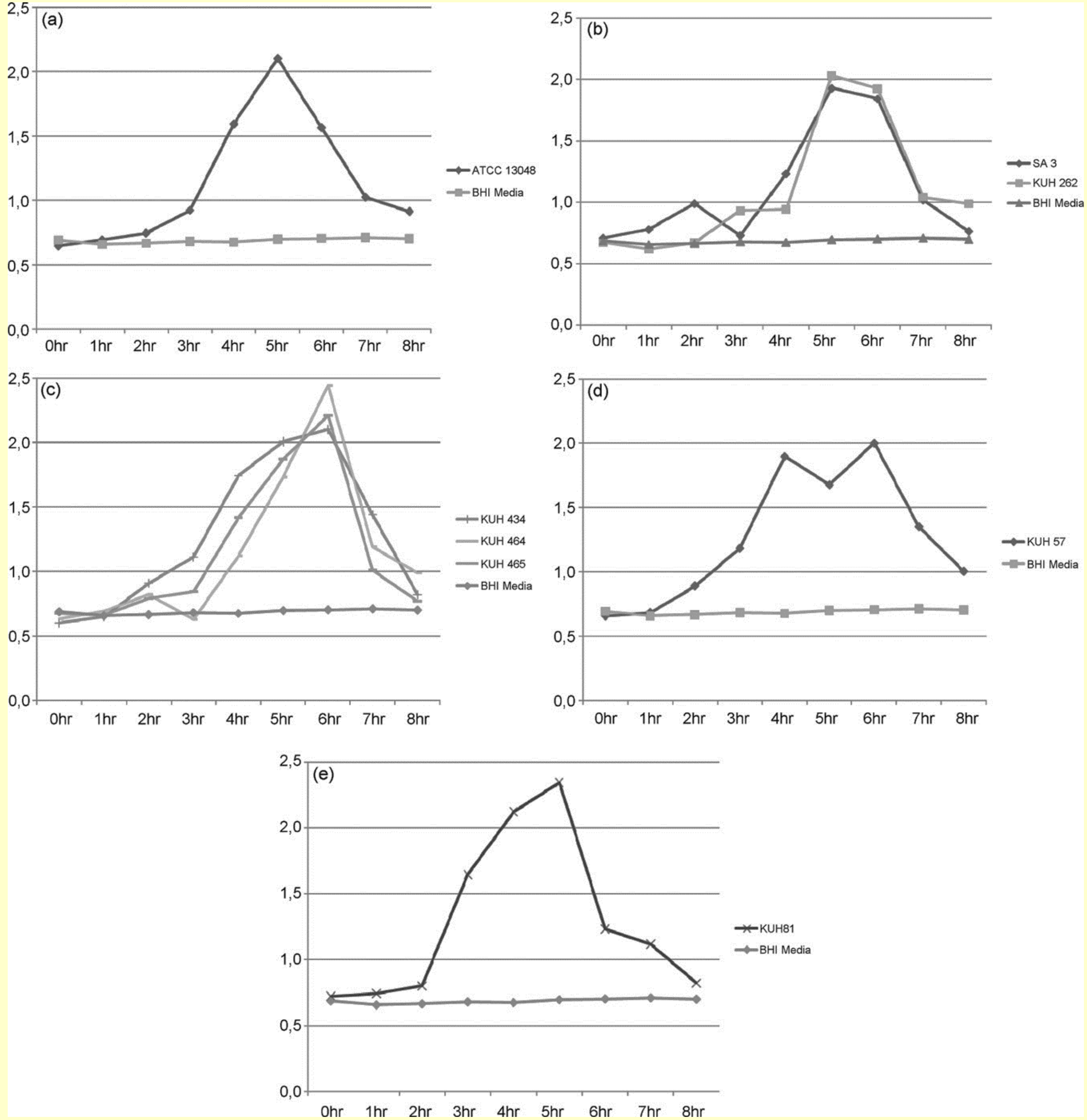
PMEU Scentrion® in rapid detection of bacterial strains isolated from neonatal blood cultures.

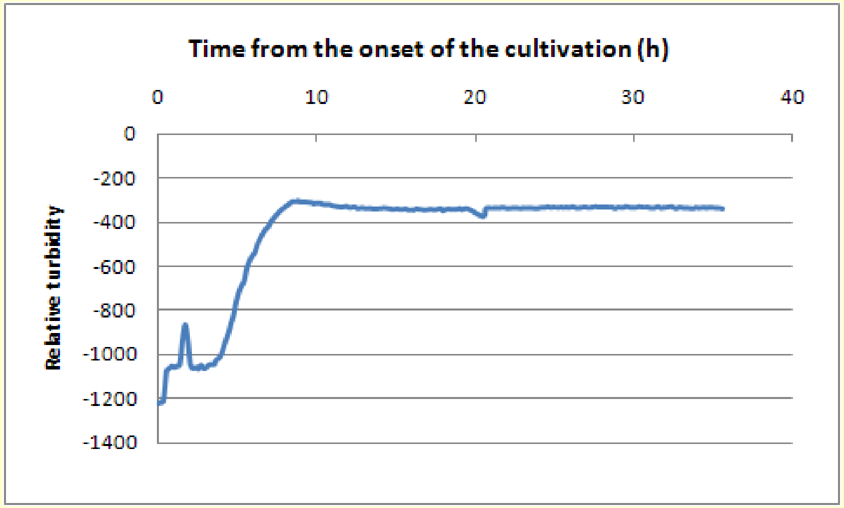
When initial bacterial concentration was approx. 1 x 10^6 cells / ml, the growth was detected after 2 - 4 hours of PMEU cultivation.:

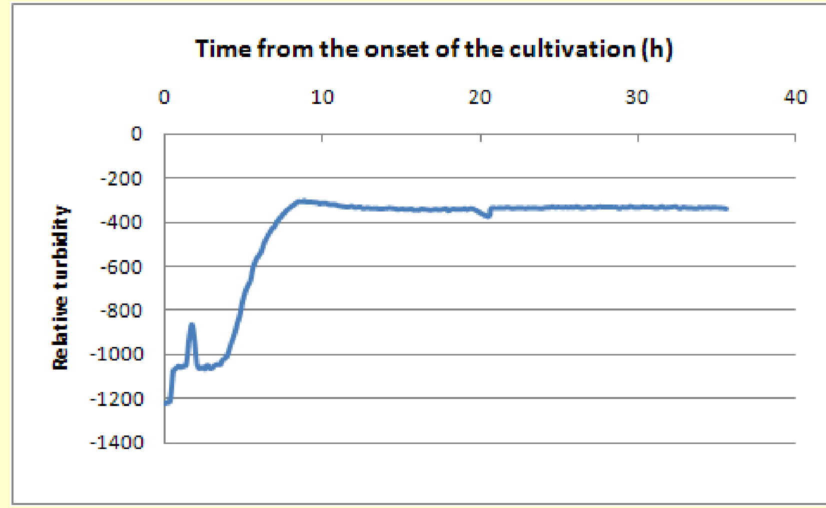
a) *Klebsiella mobilis*
b) *Staphylococcus aureus*
c) *Staphylococcus epidermidis*
d) Coagulase negative *staphylococcus*
e) *Streptococcus agalactiae*

Also with bacterial concentration of 0.5 - 600 cells / ml, the growth was detected within 4 - 5 hours.

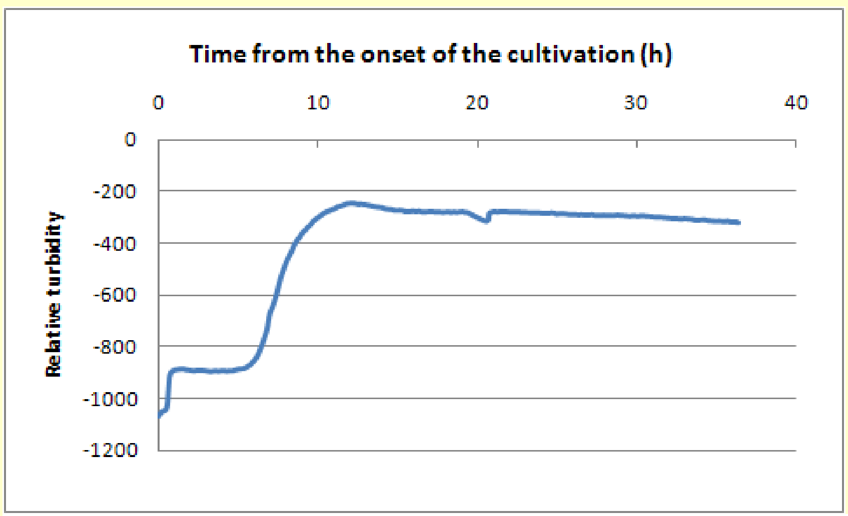
(Hakalehto *et al.*, *Pathophysiology* 2009)

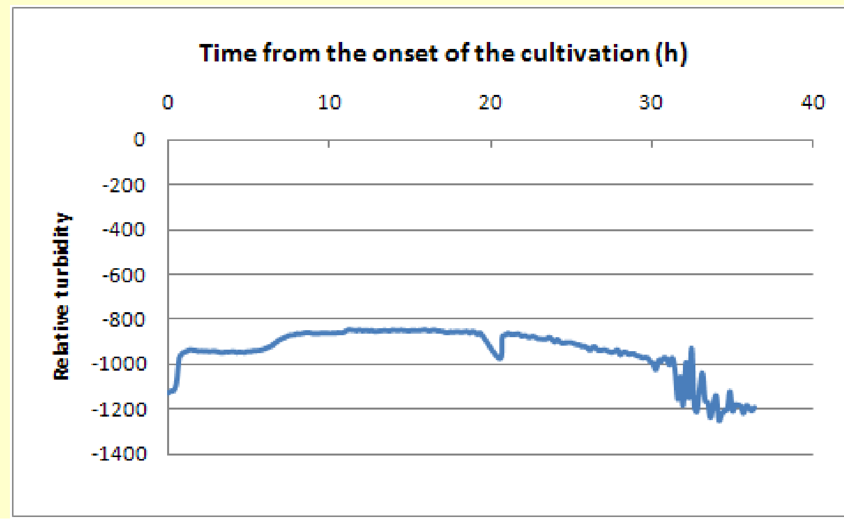






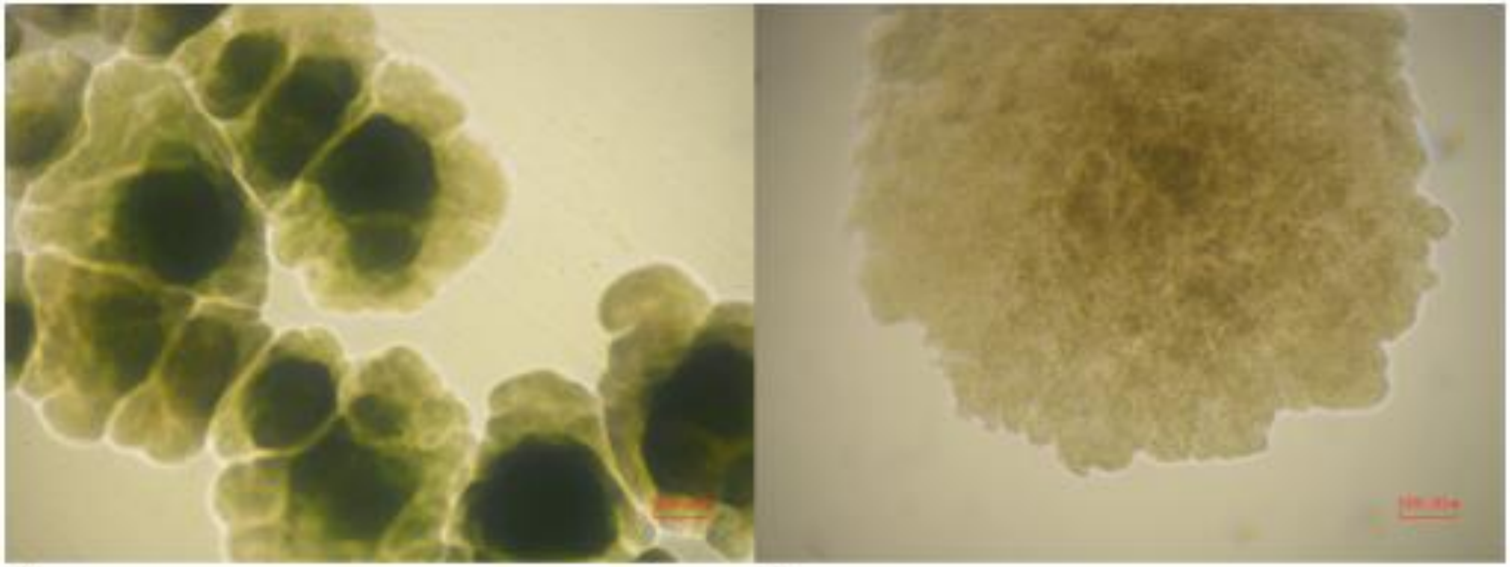
The influence on growth of *Enterobacter cloacae* in the PMEU Spectrion® in TYG medium in presence of different antibiotics. a- *E. cloacae* in TYG medium (control), b – with penicillin G, c – with cefuroxime, d – with netilmicin.





Hakalehto, E. 2011. Antibiotic resistance traits of facultative *Enterobacter cloacae* strain studied with the PMEU (Portable Microbe Enrichment Unit). In: Antonio Méndez-Vilas (ed.) *Science against microbial pathogens: communicating current research and technological advances*, Formatex Research Center, Badajoz. Spain: Microbiology Series N:o3. Vol. 2. pp.786-796.

Fast detection of *Mycobacterium* sp.



A B

Fig. 3. (A and B) Close images of the mycobacterial colonies. On the left growth of *Mycobacterium* sp. E40 isolated from river water on CromAgar™ medium. On the right a colony of *M. marinum* strain ATCC 927 grown on M7H9 agar (1.25%).

Hakalehto, E. *Pathophysiology* 2013

Enhanced mycobacterial diagnostics in liquid medium by microaerobic bubble flow in Portable Microbe Enrichment Unit

Elias Hakalehto *

Pathophysiology 20 (2013) 177–180

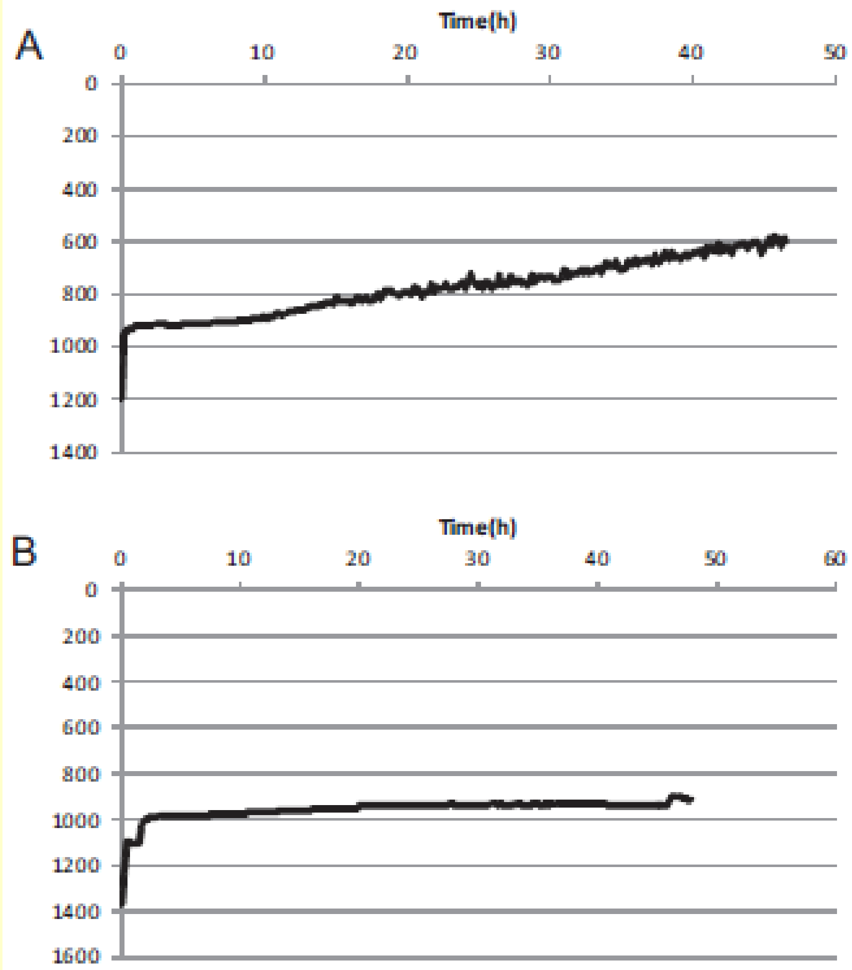
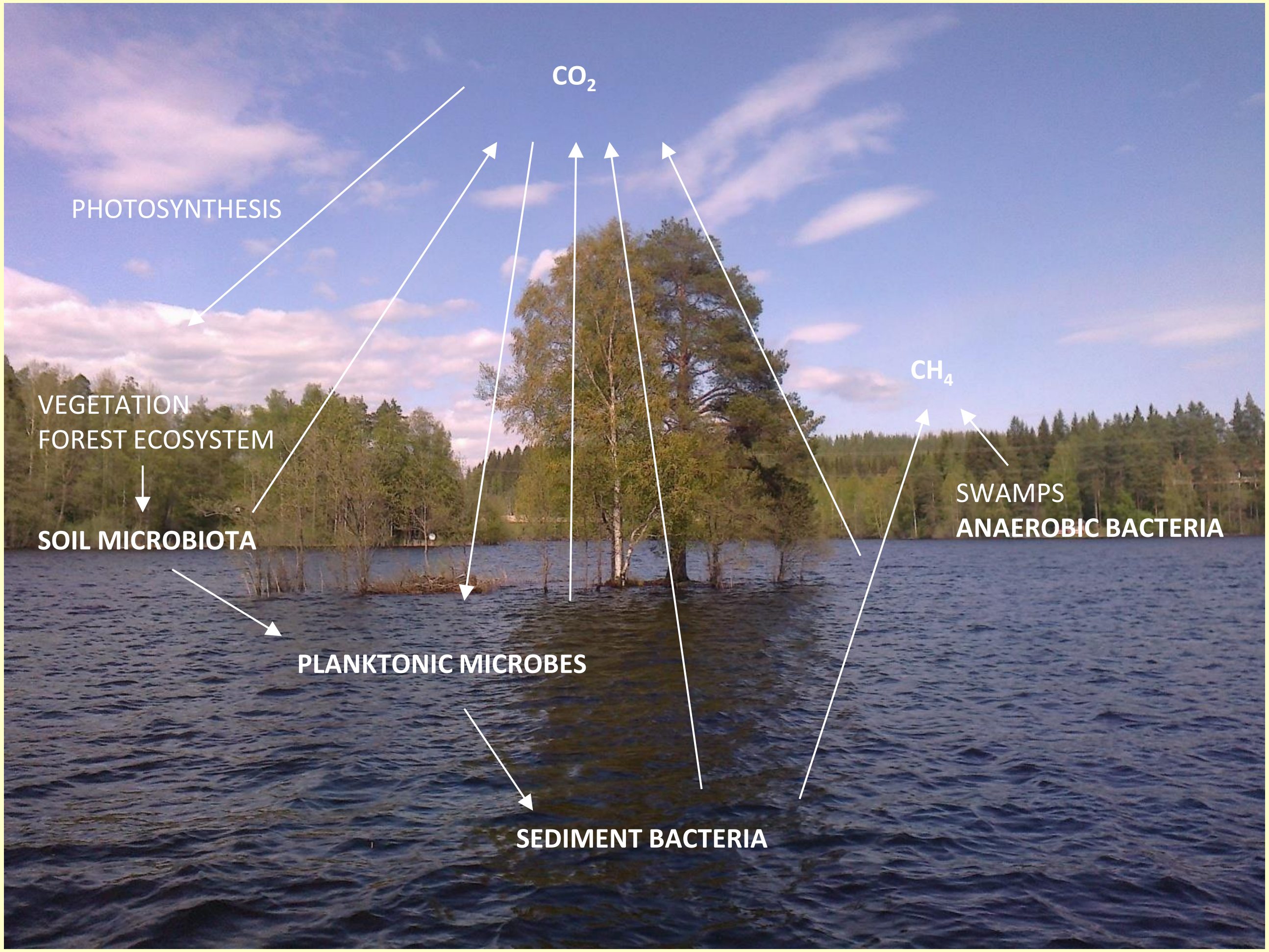


Fig. 1. Growth curves on *M. fortuitum* from PMEU Spectrion® analysis. The culture with gas bubble flow above (A), with static reference culture below (B). In the former one A case, the onset of growth was recorded in less than 12 h.

MICROBES CIRCULATE DIFFERENT ELEMENTS

1. CARBON
2. NITROGEN
3. PHOSPHORUS
4. SULPHUR   ETC.

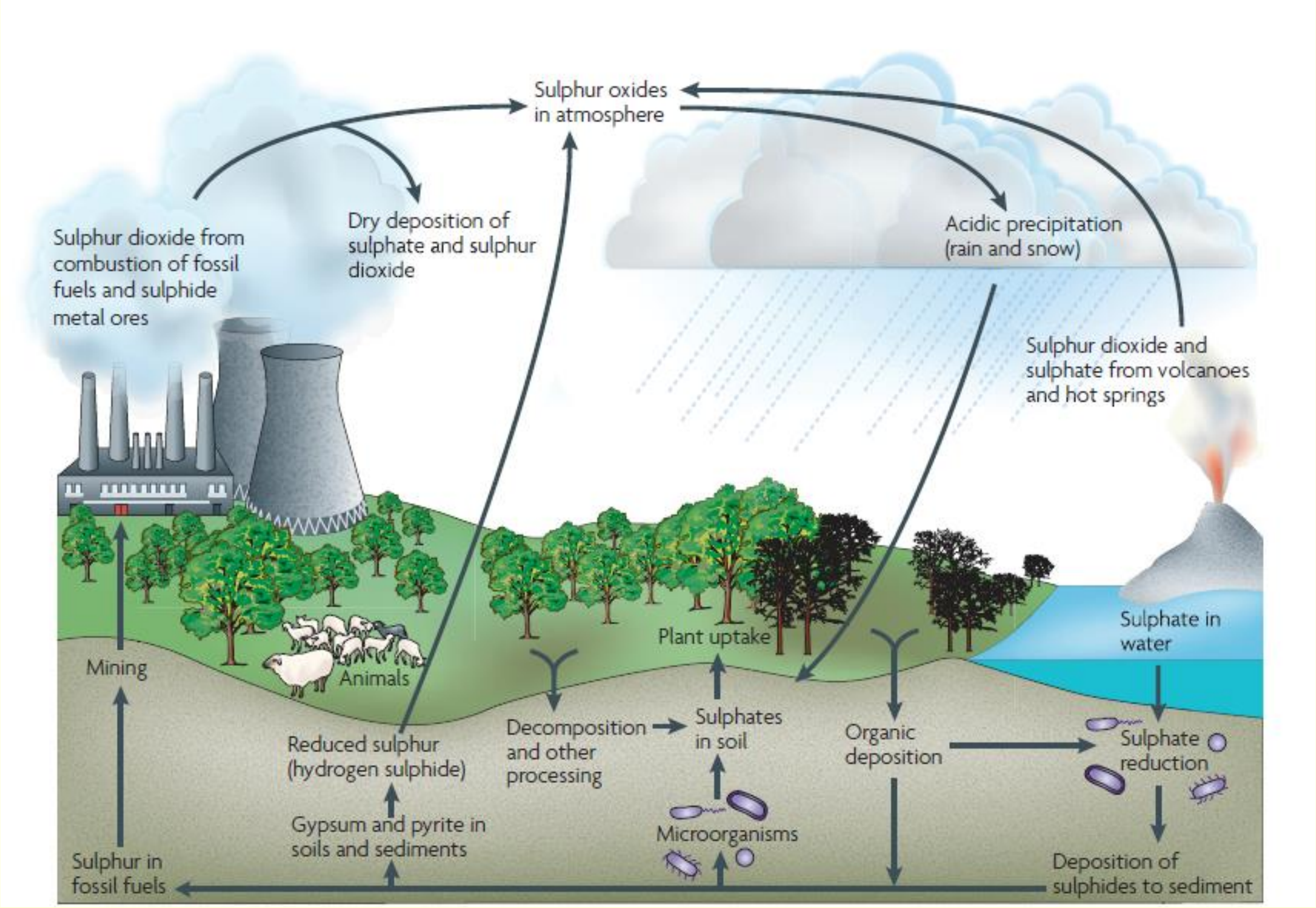
FOR A SUSTAINABLE FUTURE. WE NEED TO UTILIZE THEIR POTENTIAL TO MAINTAIN THE BALANCES.



Microbial carbon circulation in the Finnish Nature. Micro-organisms liberate carbon into the air mostly as CO_2 in respiration and fermentation, and CH_4 in symbiotic fermentation (by acid producing eubacteria and methane producing archae). Any anthropogenic impact on any of the parts of these sequences will eventually effect all the parts, and consequently shake the balance.

Hakalehto, E. 2015. Bacteriological indications of human activities in the ecosystems. In: Armon, R. & Hänninen, O. (eds.) Environmental indicators.. Springer Dortdrecht, Germany.

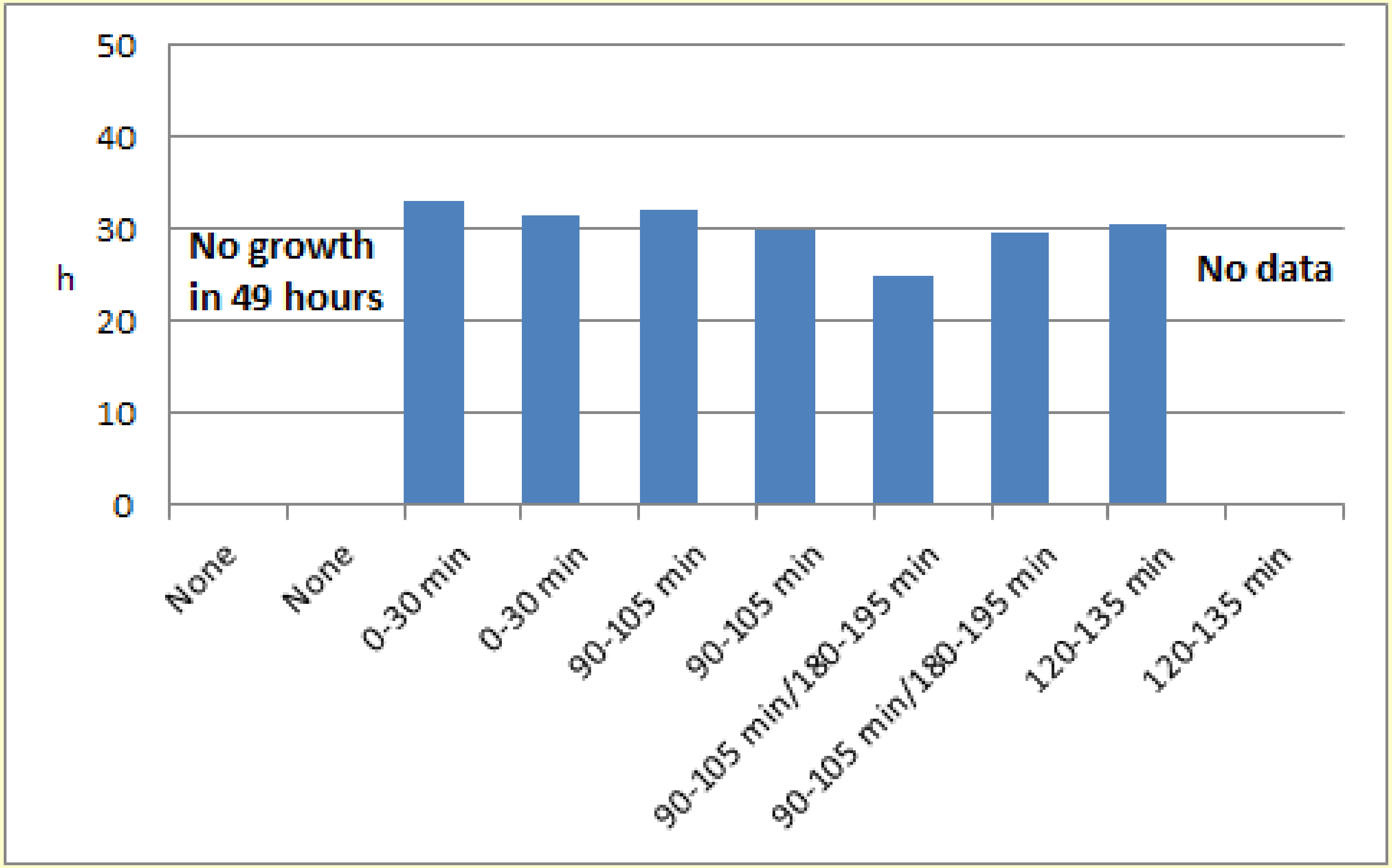
The Sulphur Cycle



Muyzer & Stams, Nature Reviews, Microbiology, 6, 2008

MICROBIAL GAS EMISSIONS REGULATE THEIR COMMUNITIES

Shortening of the onset of *Clostridium acetobutylicum* strain ATCC 185 growth by carbon dioxide in the PMEU Spectrion® cultures [4]. Then a constant nitrogen (100%) flow was in some cases interrupted with pulses of 45% CO_2 with 15-30 min duration near the beginning of the cultivation. The triggering CO_2 impulses produced bacterial growth.

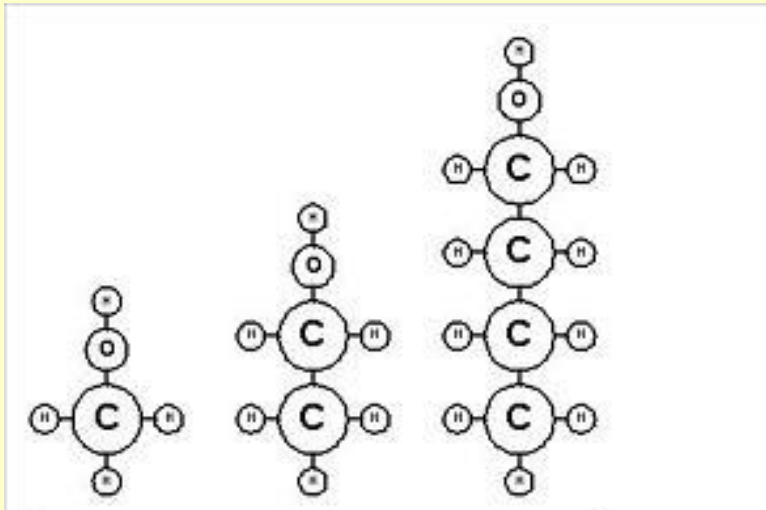


Hakalehto, E., 2015. Enhanced microbial process in the sustainable fuel production. In: Boehm, R.F., Yang, H., Yan, J. (Eds.), 2015. Handbook of clean energy systems. John Wiley & Sons, Inc, Chichester, West Sussex, UK.

SUSTAINABLE BIOECONOMICS

BIOTECHNOLOGY IN THE PRODUCTION OF ENERGY AND CHEMICALS

- Acetone-butanol-fermentation; *Clostridium acetobutylicum*
- Products: acetone, butanol, ethanol, hydrogen
- Butanol is important raw material for chemical industry and a potential liquid fuel
- Tested during ABOWE



Methanol CH_3OH	Ethanol C_2H_5OH	Butanol C_4H_9OH	Gasoline Many - Many
Energy Content (Btu's per Gallon)			
63 K	84 K	110 K	115 K
Motor Octane			
91	92	94	96
Air to Fuel Ratio			
6.6	9	11-12	12-15
Vapor Pressure (psi@100F) Reid V.P. - Safety			
4.6	2	0.33	4.50

Source: www.butanol.com


Pilot A:
Novel Biorefinery
abowe
Implementing Advanced Concepts for Biological Utilization of Waste
SAVONIA
UNIVERSITY OF APPLIED SCIENCES
Finnoflag
Baltic Sea Region
Part-financed by the European Union (European Regional Development Fund)
www.abowe.eu

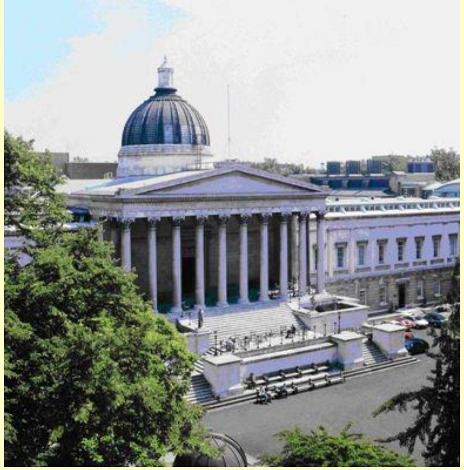

samplion

UNIVERSITY COLLEGE LONDON
BIOENGINEERING PILOT PLANT

- Prof Jack Drummond: vitamins 1930

-studies on antibiotic and enzyme fermentation started about 50 years ago, e.g. by Prof Malcolm D. Lilly and Prof. Peter Dunnill

- development of bioreactors



EU Baltic Sea Region Projects

REMOWE

Regional Mobilizing of Sustainable Waste-to-Energy Production 2009-2012

ABOWE

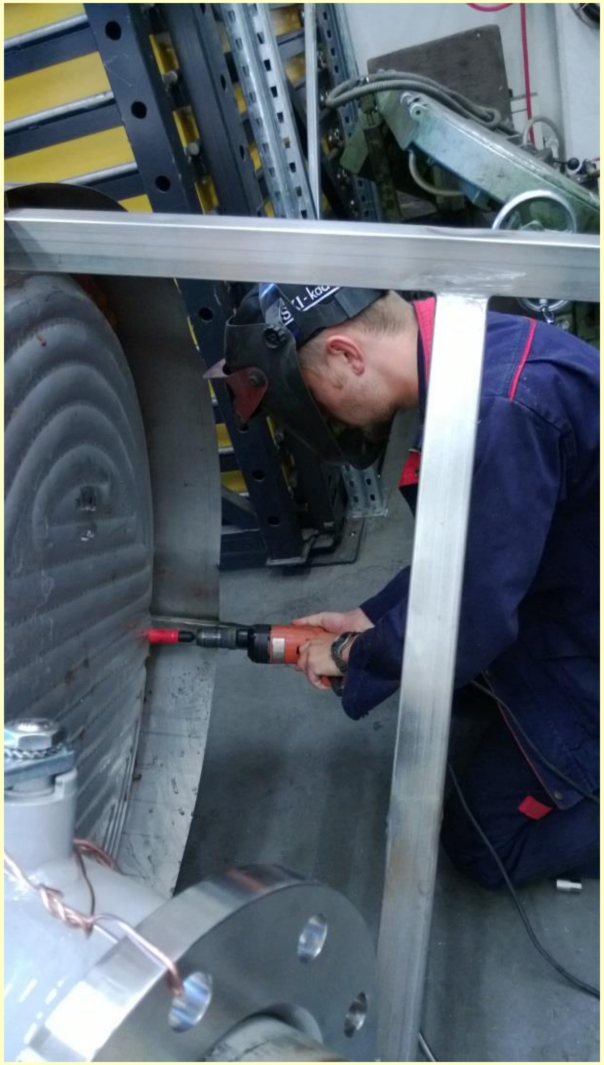
Implementing Advanced Concepts for Biological Utilization of Waste 2012-2014

Participating countries:

Estonia, Finland, Germany, Lithuania, Poland, Sweden

www.abowe.eu

Construction of Pilot A in Kuopio, Finland
Autumn 2013



Reactor manufacturing in Kuopio, Autumn 2013

BIOREFINERIES

ABOWE up and running

Interesting possibilities ahead

GEOGRAPHICAL SCOPE OF ABOWE

As REMOWE project (Regional Mobilisation of Sustainable Waste to Energy Production) was finalised, at the same time started the extension stage project ABOWE (Implementing Advanced Concepts for Biological Utilisation of Waste) to work with two promising technologies, unlocking investments with support from the Baltic Sea Region programme.

Two mobile pilot plants will be built and tested in several Baltic Sea Region countries.

Pilot plant A is planned in Finland for testing enzymatic hydrolysis and microbiological processing (biocatalysis approach) of various wastes by Savonia UAS and Finnoflag Oy.
Pilot plant B is a German dry fermentation process, invented by Ostfalia University of Applied Sciences.

The pilots form the basis for know-how transfer, compilation of investment memos and upcoming investor events as well as evaluating the new processes' economic and climatic impacts in each region.

The desired outcome from ABOWE is implementer/investor driven continuation projects targeting full scale plant investments of the two technologies.

In these Newsletters, we will inform about proceeding of these ABOWE activities. Updated information will be available at www.abowe.eu

Operational time for ABOWE is 10/2013 - 9/2014.

Ari Jääskeläinen
Project Coordinator
Savonia University of Applied Sciences

ABOWE MICROBES

Klebsiella sp., *Escherichia coli*, *Clostridium* sp. and lactobacilli were studied with different natural microbes as the production organisms.

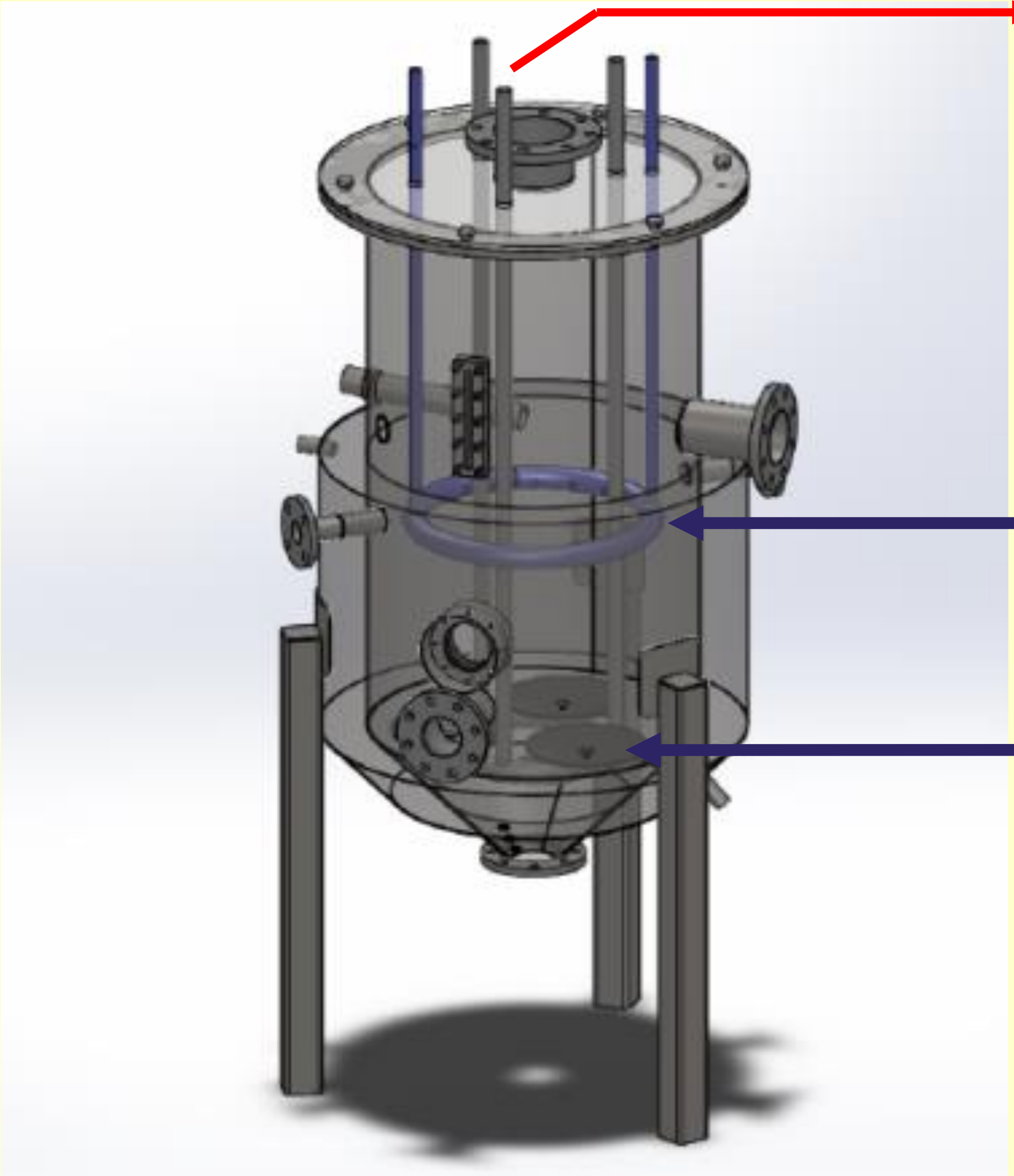
The same cycles that prevail in the environment circulate the substances in the bioreactors

We can monitor the microbes and their production of

- biofuels
- chemicals (bulk, platform and speciality chemicals)
- organic fertilizers

by measuring their gas emissions

ABOWE bioreactor (patented)



volatile measurement

upper gassing level

lower gassing level

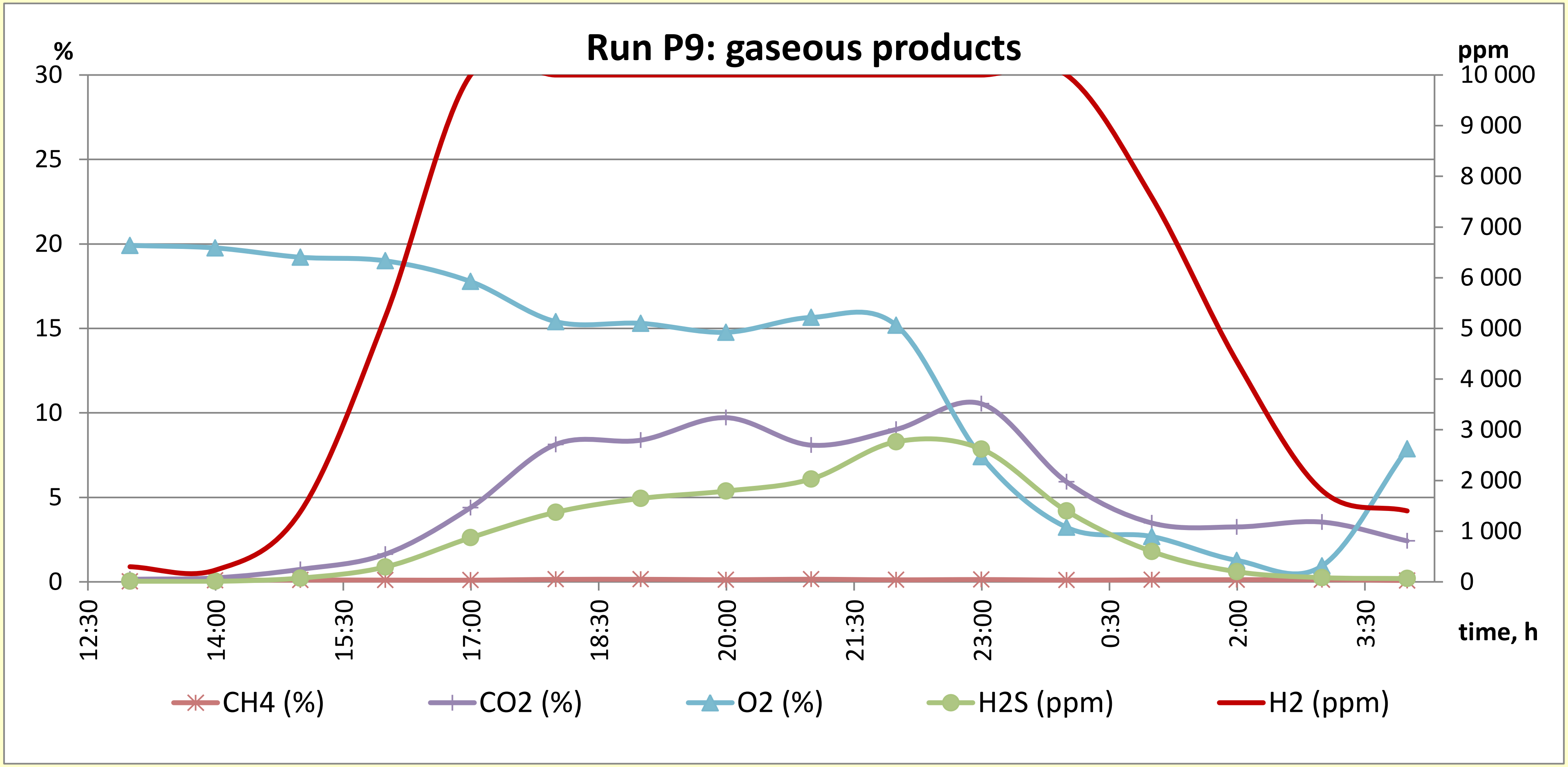
The ABOWE Pilot A interior

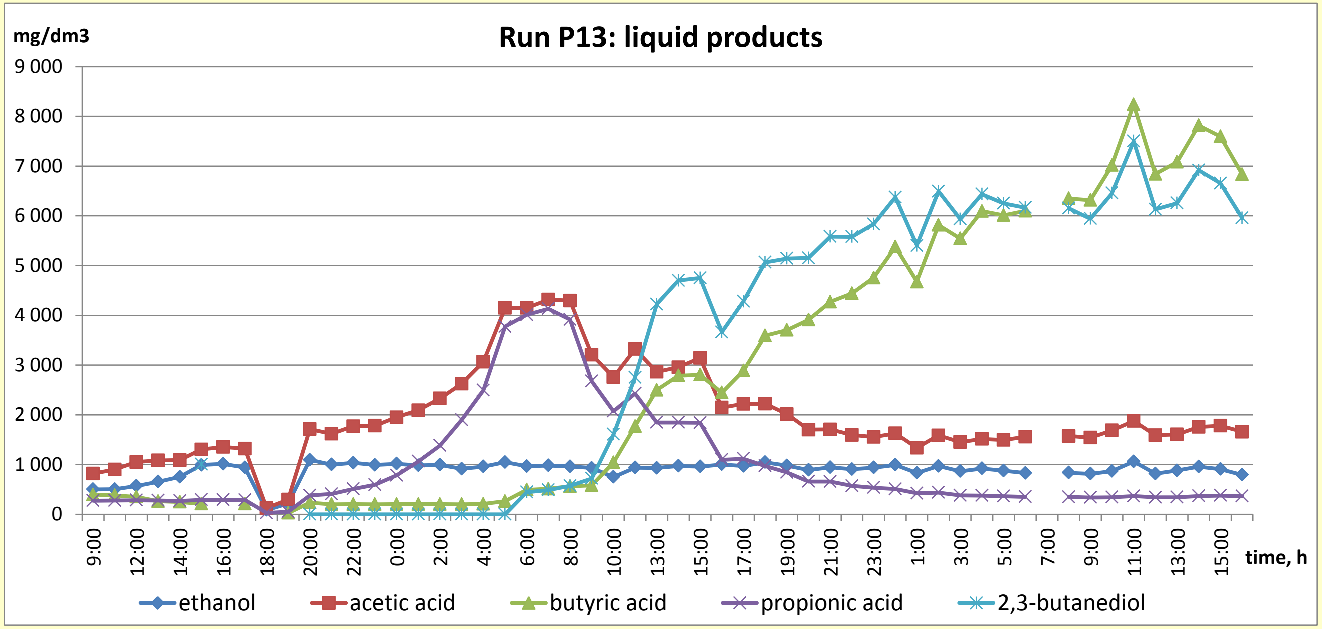
Pilot A in Poland

- Testing site: ZGO Gac Ltd
- (Zaklad Gospodarowania Odpadami Sp. z o.o.)
- Primary waste to feed in: potato waste from a chip factory near Gac
- Target product is 2,3 Butanediol -> plastics, synthetic rubber, anti-icing agents, cosmetics, textiles…
- Also separately collected biowaste has been added to the process

www.abowe.eu

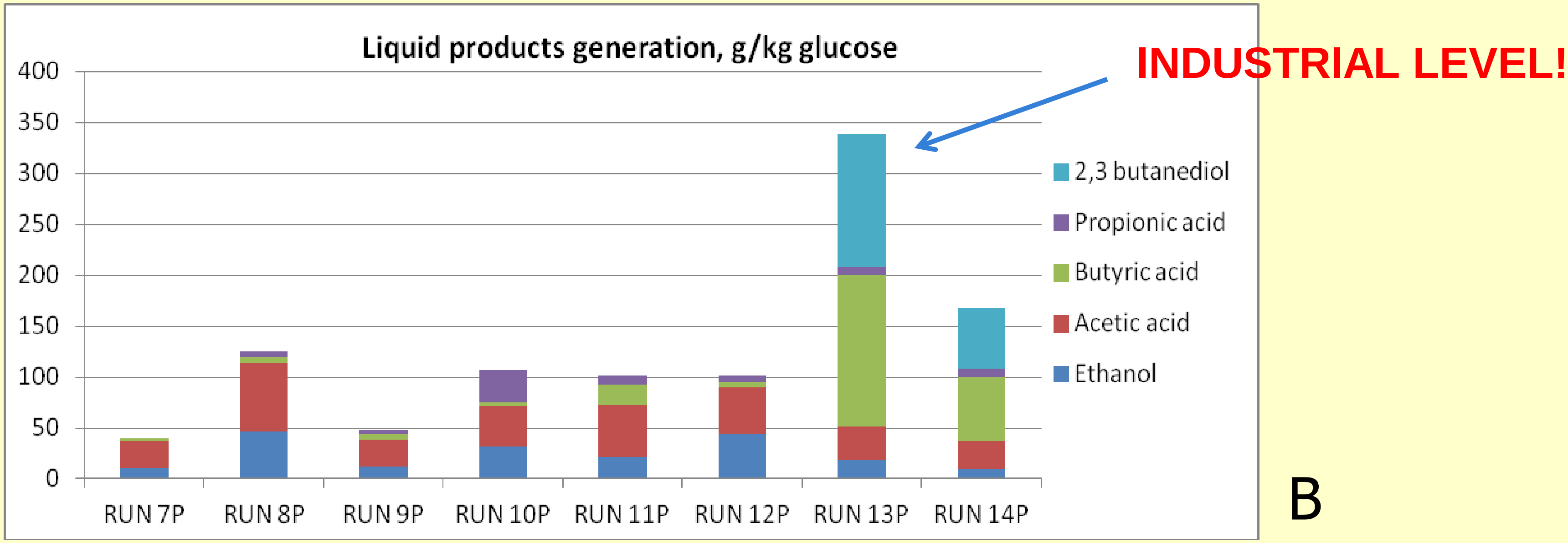
Biohydrogen production (ppm) and other gases liberated (%) in ABOWE Pilot A from potato industry waste mixed with sorted restaurant biowastes. This pattern of gas formation was measured from a mixed culture of natural microbes and *Klebsiella mobilis* (HAMBI 1271). Graph by Prof. Emilia den Boer, Wroclaw University of Technology, Poland. Hydrogen in red and oxygen in turquoise color.





A

ABOWE, Poland, liquid products during run 13P (A) and their generation of glucose in different runs (g/kg) (B).



B

Adj. Prof. Elias Hakalehto, Beneficial Microbes, 25.8.2015, Valencia, Spain

Polish testing 5-6/2014

ZGO Gac Ltd waste management centre, Lower Silesia	**SITE**
Potato industry waste, municipal source-sorted biowaste	BIOMASS
Initial conversion to lactate, acetate, H_2S	PROBLEM
Optimization of the fermentation conditions	SOLUTION
2,3 butanediol, butyrate, propionate, valerate, hydrogen	PRODUCTS

ABOWE SWEDISH TESTING 8-9/2014

Hagby organic chicken farm from Enköping, Sweden. Slaughter waste and chicken manure as raw material (dowm)

Joakim Jansson and Elias Hakalehto peeling apples for sugar source for the test run.

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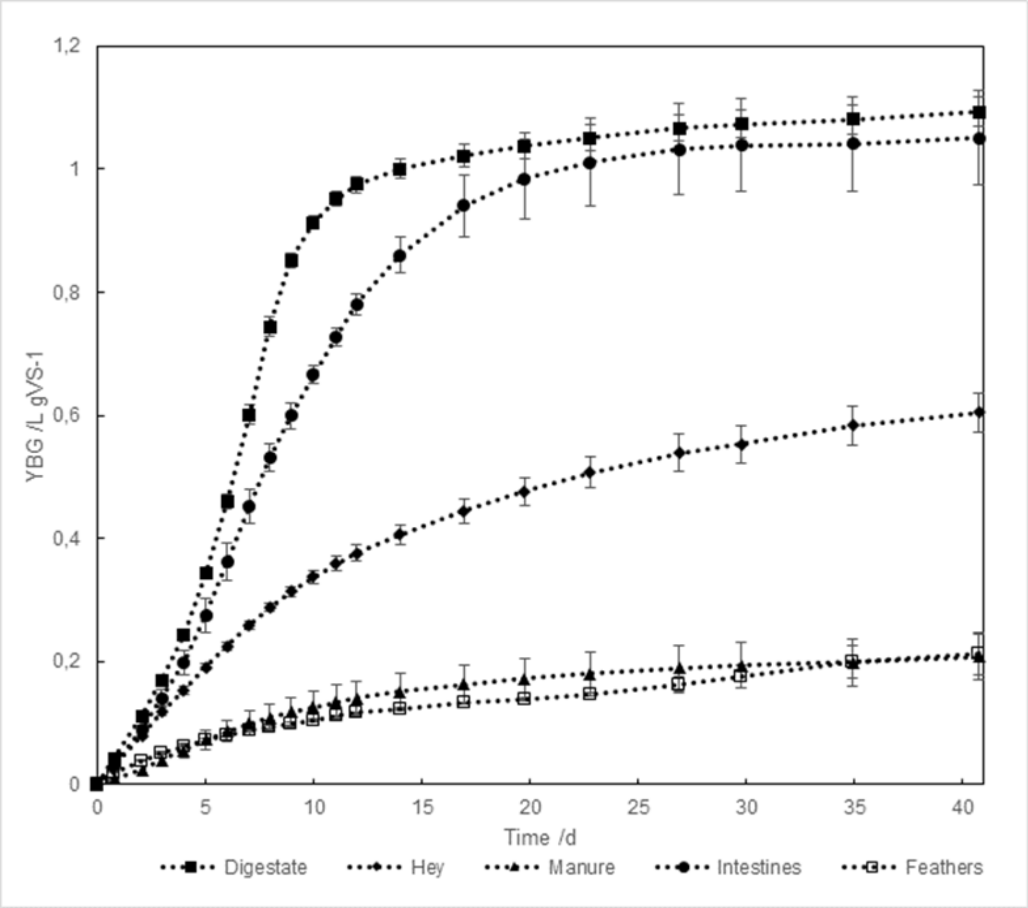
BIOGAS PRODUCTION FROM DIFFERENT SUBSTRATES

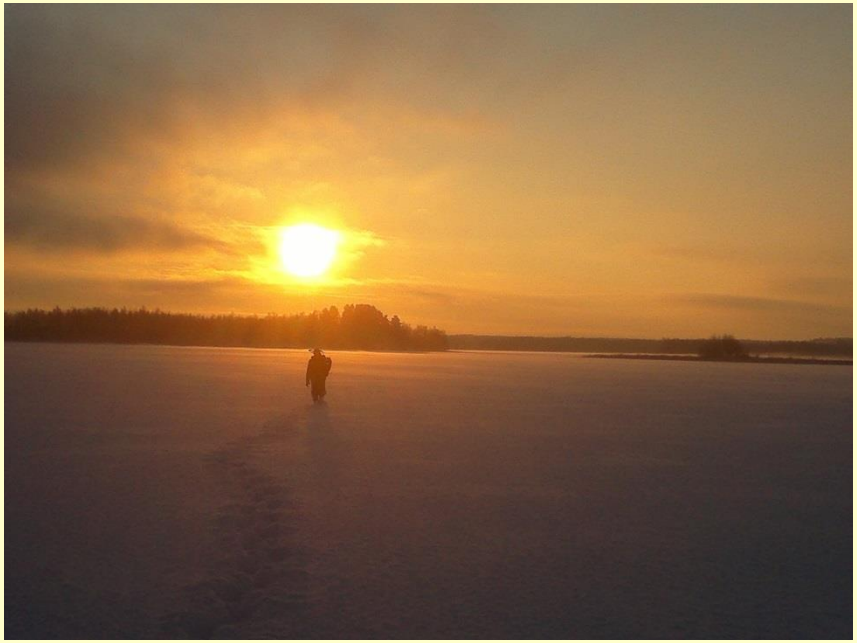


Biogas yield of the different substrates used in the biorefinery pilot runs in Sweden and the residues/digestate after the first pilot run. The y-axis shows the yield in liter biogas per grams of volatile solids (ABOWE Swedish Test Report 2014-2015).

Swedish testing 8-9/2014

Hagby farm (slaughterhouse), Enköping	SITE
Slaughterhouse waste, chicken manure, saw dust	BIOMASS
Inefficient hydrolysis, low soluble carbohydrate	PROBLEM
Conversion to organic acids	SOLUTION
Propionate, valerate, butyrate, butanol, hydrogen, ammonium salts	PRODUCTS

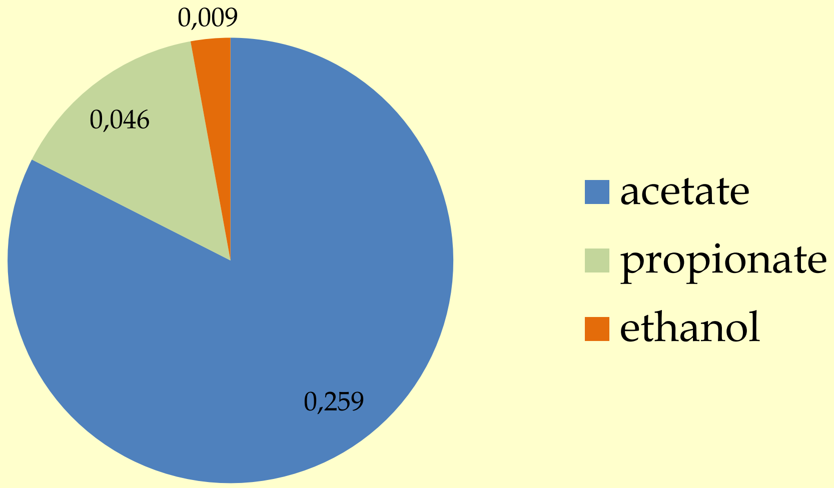
The pioneer of biotechnological NMR studies, Prof. Reino Laatikainen, School of Pharmacy, University of Eastern Finland, Kuopio, Finland. He has developed remarkable skills and software for interpreting the NMR graphs.

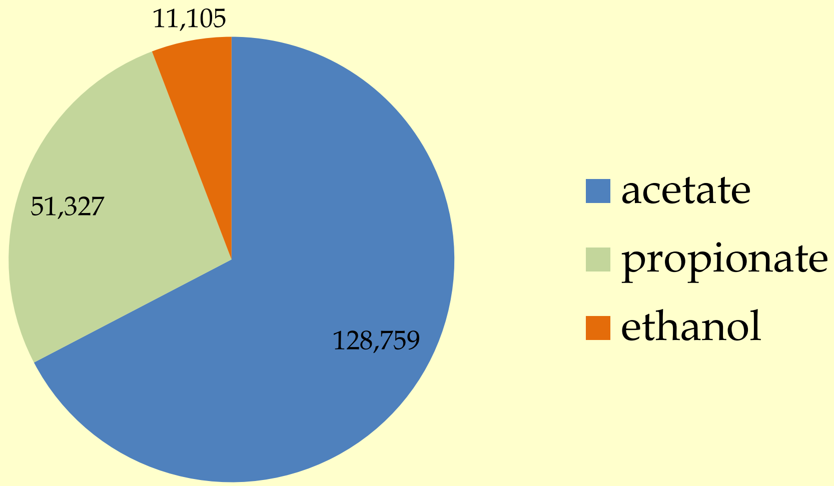


FINNISH LAKE SHORE IN THE MIDDLE OF WINTER (-10 C). BENEATH THE SURFACE MICROBES keep the mud unfrozen by their metabolism (left).

Hakalehto, E. 2015. Bacteriological indications of human activities in the ecosystems. In: Armon, R. & Hänninen, O. (eds.) Environmental indicators.. Springer Dortdrecht, Germany.

EQUAL PROPORTION OF ORGANICS IN WINTER LAKE MUD AND SLAUGHTERHOUSE WASTE

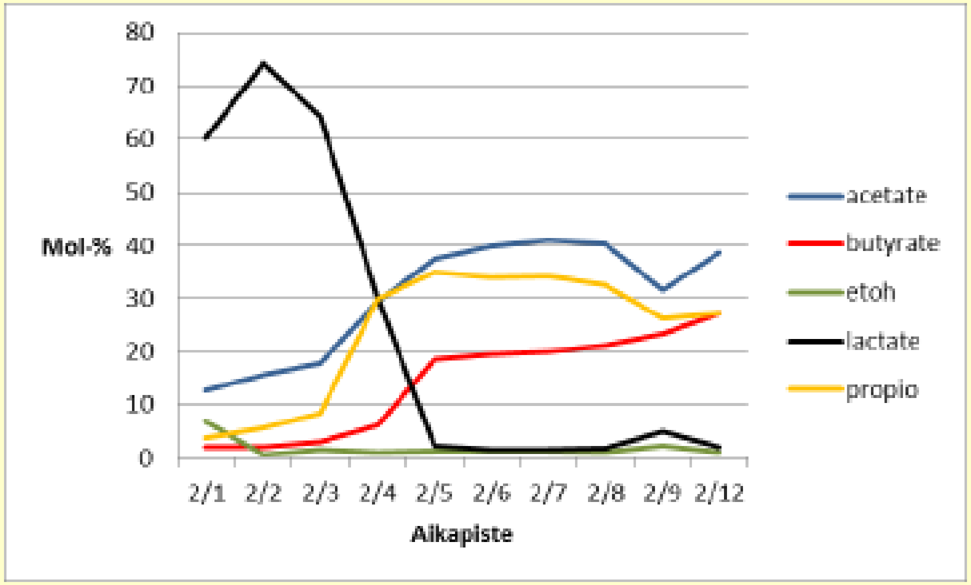




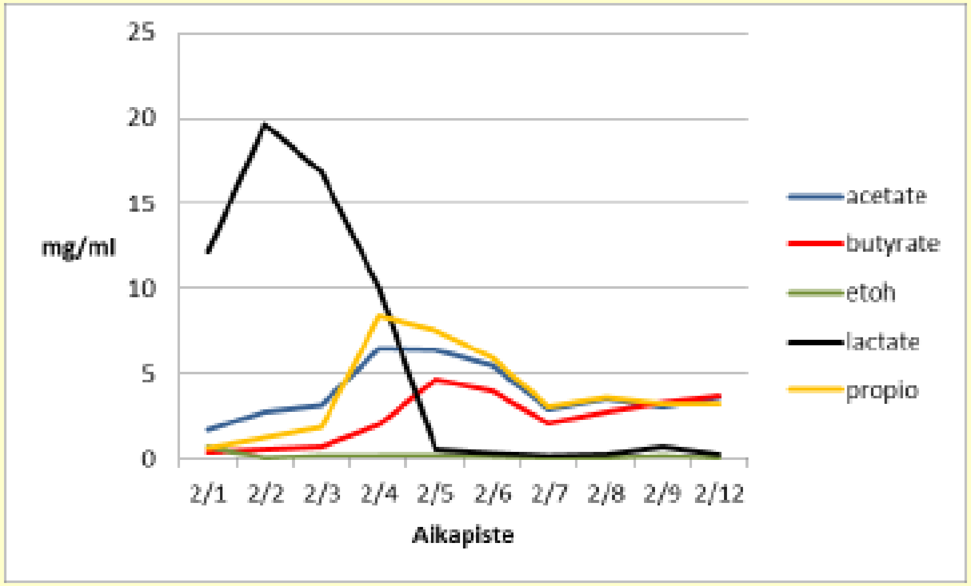
Proportions of acetate, propionate and ethanol in the natural mud sludge (above) and in the industrial waste treatment process of a slaughterhouse (below). Microbial metabolic activities in the latter one produce higher relative amounts of the latter two molecules. However, proportions to each other remained unchanged. Even in the Finnish winter conditions anaerobic metabolism in the mud was high enough to keep it unfrozen under the thick snow belt (approximately 70cm). The mud sludge was slightly basic, which also indicated the role of microbiological phenomena in keeping it unfrozen.

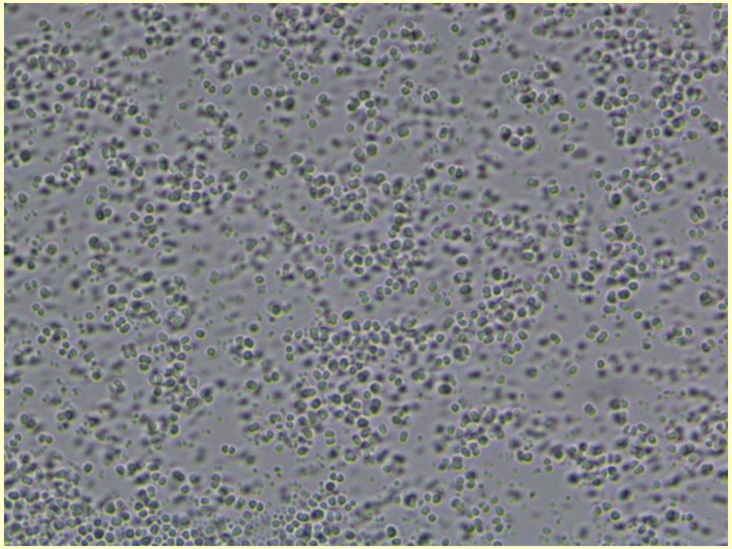
Hakalehto, E., 2015. Enhanced microbial process in the sustainable fuel production. In: Boehm, R.F., Yang, H., Yan, J. (Eds.), 2015. Handbook of clean energy systems. John Wiley & Sons, Inc, Chichester, West Sussex, UK.

Conversion of Lactate into Other Organic Acid in Slaughterhouse Waste Treatment



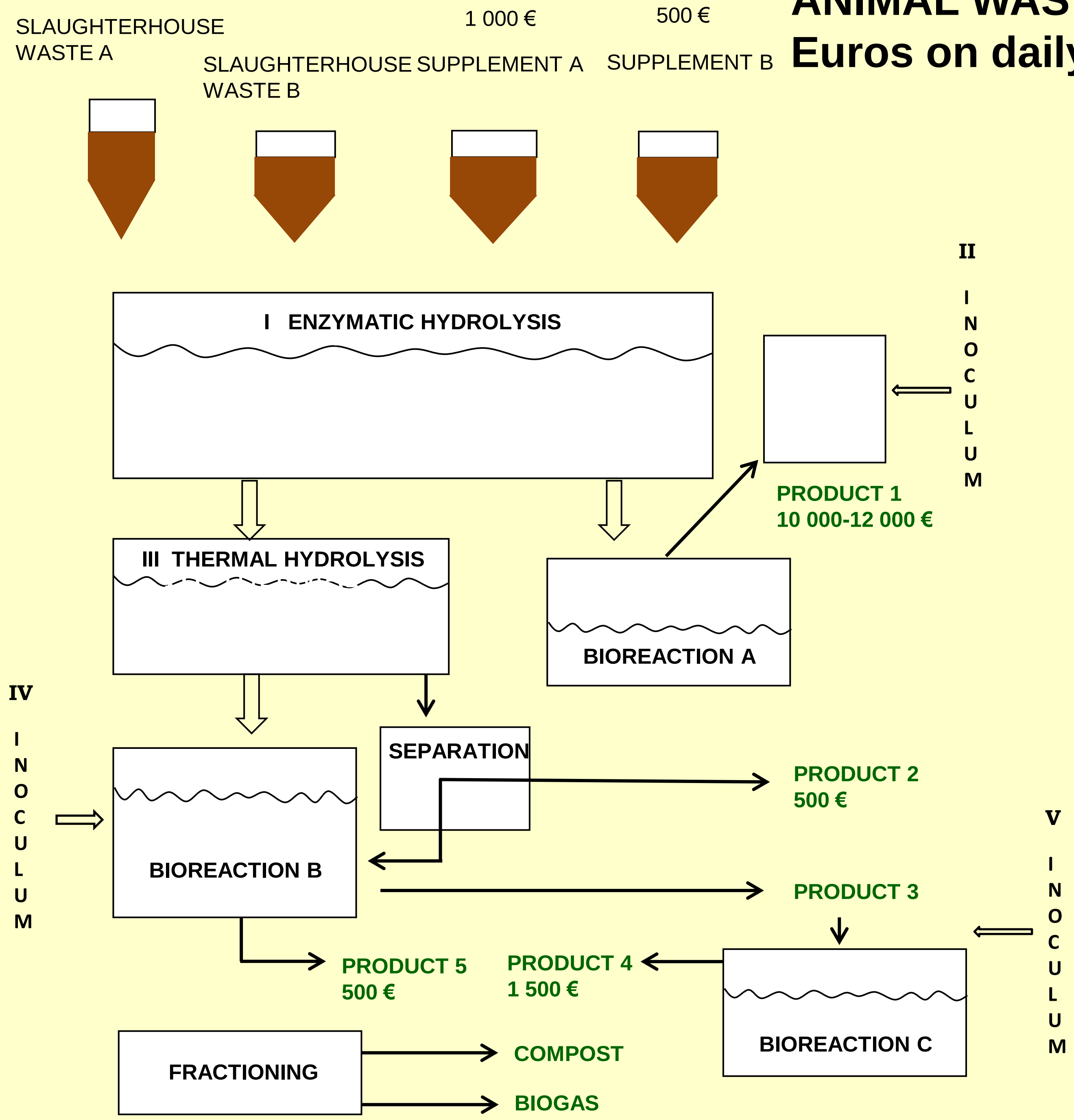
Hakalehto, E. 2015. *Microbial Food Hygiene*. Nova Science Publishers, Inc. New York, USA. In print





***Propionibacterium acidipropionici* is accepted as safe production organism by EFSAn, (European Food Safety Association). It is used for conserving wheat or chicken meat, for example.**
http://www.journalofdairyscience.org/article/S0022-0302(98)75663-2/abstract

ANIMAL WASTE PROJECT
Euros on daily basis



PATENTED

Pharm Dev Technol. 2013 Mar 26. [Epub ahead of print]

Why is mannitol becoming more and more popular as a pharmaceutical excipient in solid dosage forms?

Ohrem HL, Schornick E, Kalivoda A, Ognibene R.

Source

Merck KGaA , Darmstadt , Germany.

Abstract

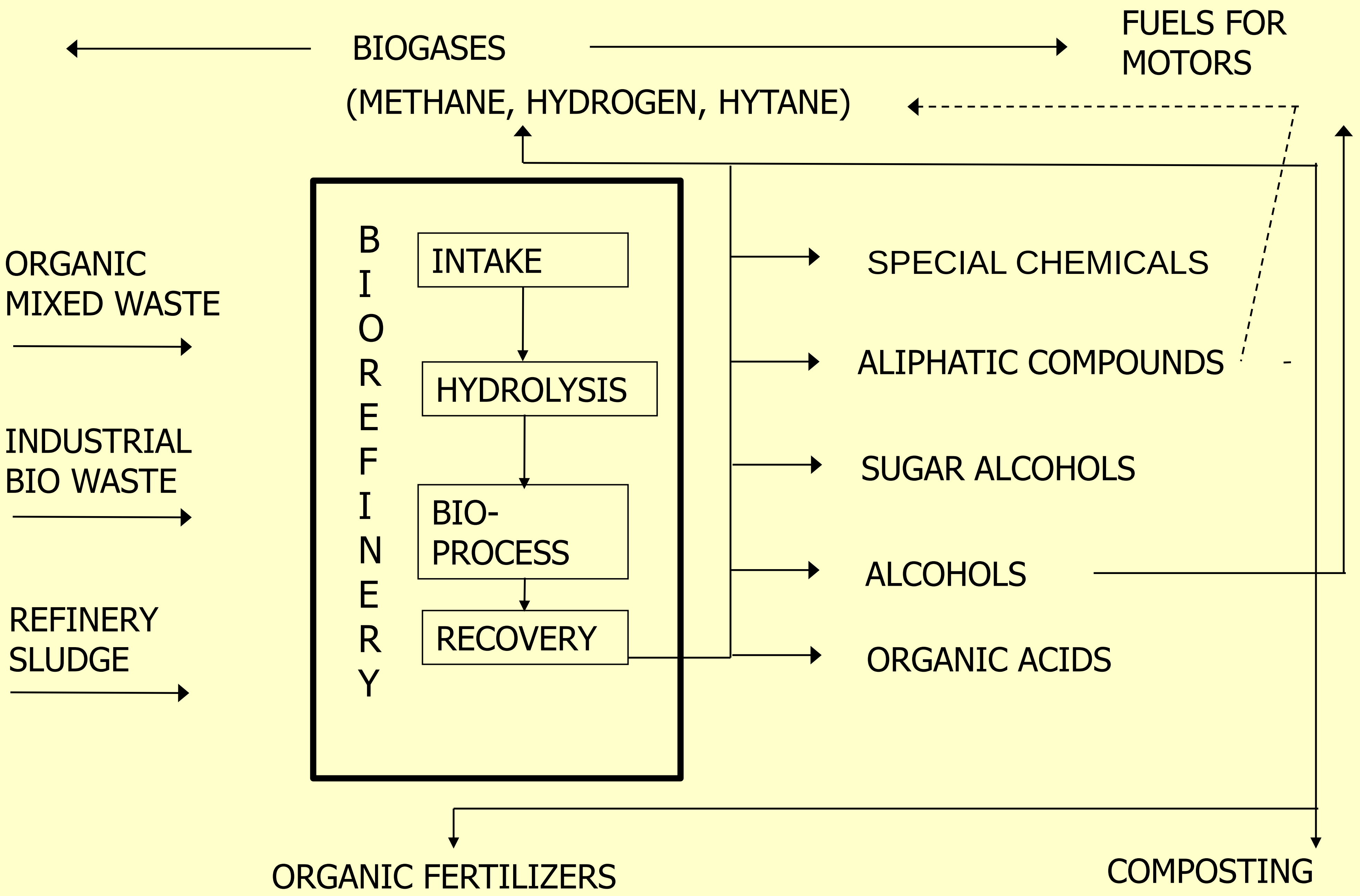
Abstract Various fillers/binders which are applied for the formulation of solid oral dosage forms are assessed for their benefits and drawbacks, including lactose, sorbitol, mannitol, microcrystalline cellulose and calcium hydrogen phosphate dihydrate. A focus of this work was to evaluate the application of mannitol in comparison to other common fillers/binders as it was observed that this excipient is gaining more and more attention in pharmaceutical formulation development and production. While one of the main advantages of conventional fillers/binders such as lactose, microcrystalline cellulose and calcium hydrogen phosphate dihydrate is their low price level, mannitol excels regarding its physicochemical characteristics such as a low hygroscopicity, a strong inertness towards both the API and the patient's body, its good compactibility and the ability to produce extremely robust tablets. Additionally, the suitability of mannitol for the emerging formulation technology of orally disintegrating tablets is pointed out. In summary, it is emphasized that the selection of the filler/binder is highly individual, depending, for example, on the preferred characteristics of the final solid dosage form, the applied API and the available budget. However, mannitol exhibits many strong advantages which can be expected to result in a more widespread application in the near future.

PMID: 23528124
[PubMed - as supplied by publisher]

SAVON SELLU WASTE WATER TREATMENT PLANT

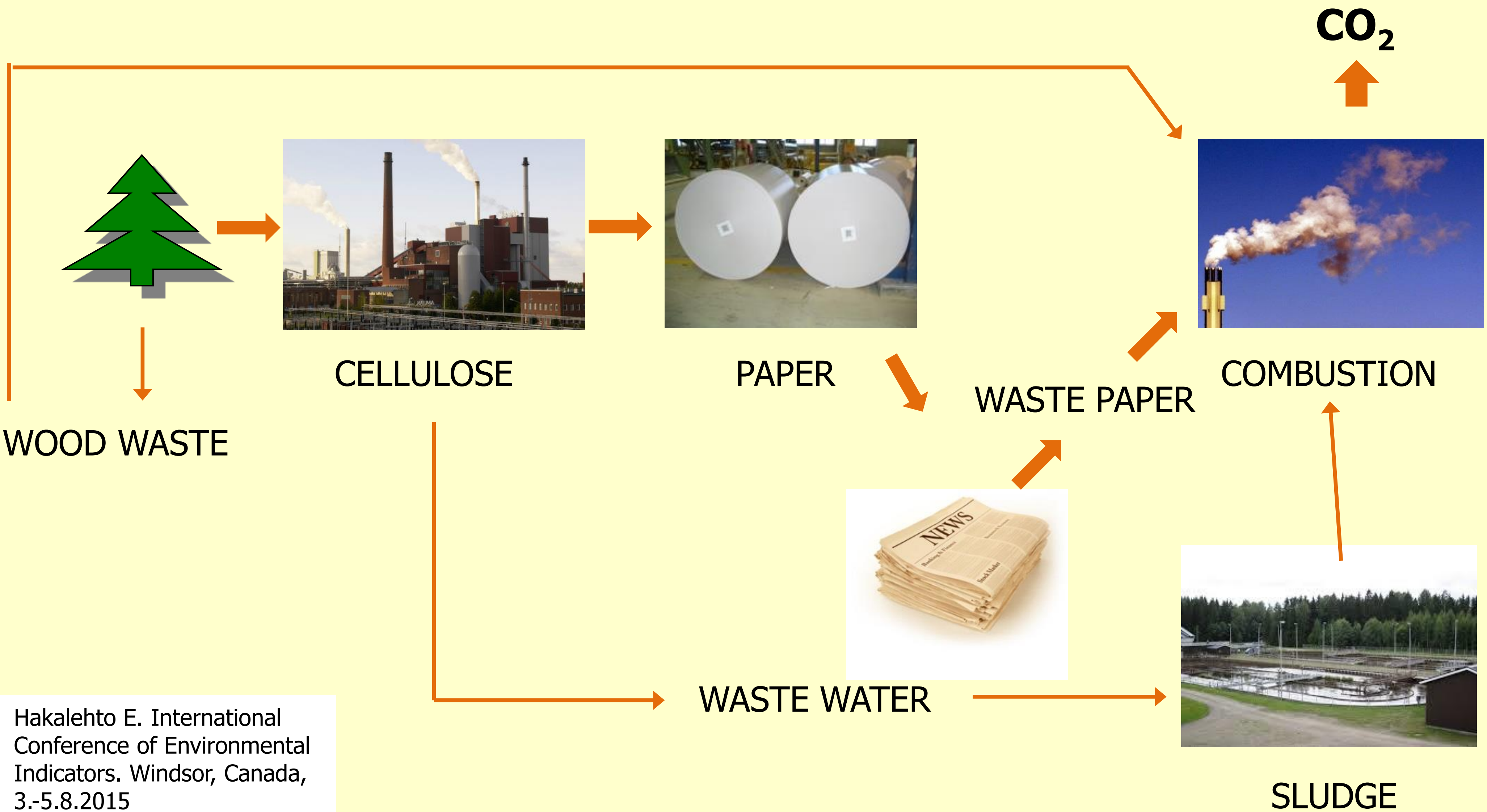
Hakalehto, E., 2015. Enhanced microbial process in the sustainable fuel production. In: Boehm, R.F., Yang, H., Yan, J. (Eds.), 2015. Handbook of clean energy systems. John Wiley & Sons, Inc, Chichester, West Sussex, UK.

BIOREFINERIES AS A BASIS FOR CIRCULATION ECONOMY



FATE OF CARBON IN INDUSTRIAL SYSTEMS

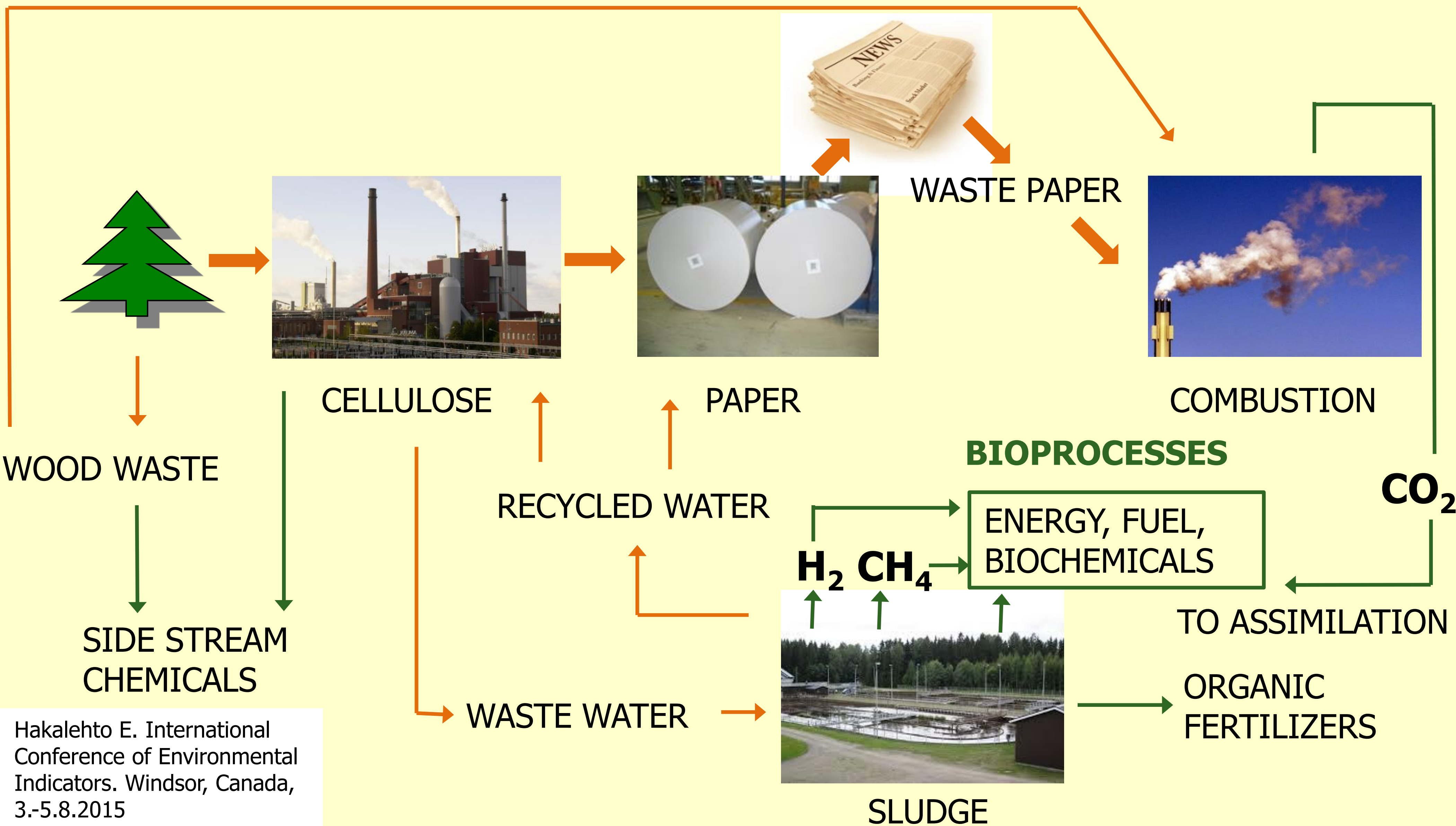
PAST IN PAPER AND PULP PRODUCTION



Hakalehto E. International Conference of Environmental Indicators. Windsor, Canada, 3.-5.8.2015

FATE OF CARBON IN INDUSTRIAL SYSTEMS

FUTURE IN FOREST BIOREFINERIES



Hakalehto E. International Conference of Environmental Indicators. Windsor, Canada, 3.-5.8.2015

SAVON SELLU WASTE WATER TREATMENT PLANT'S NATURAL H_2 (HYDROGEN) PRODUCTION/DAY BY MICROBES CORRESPONDING TO
10 000 -20 000 KM WITH A FCV (Fuel Cell Vehicle)

H_2

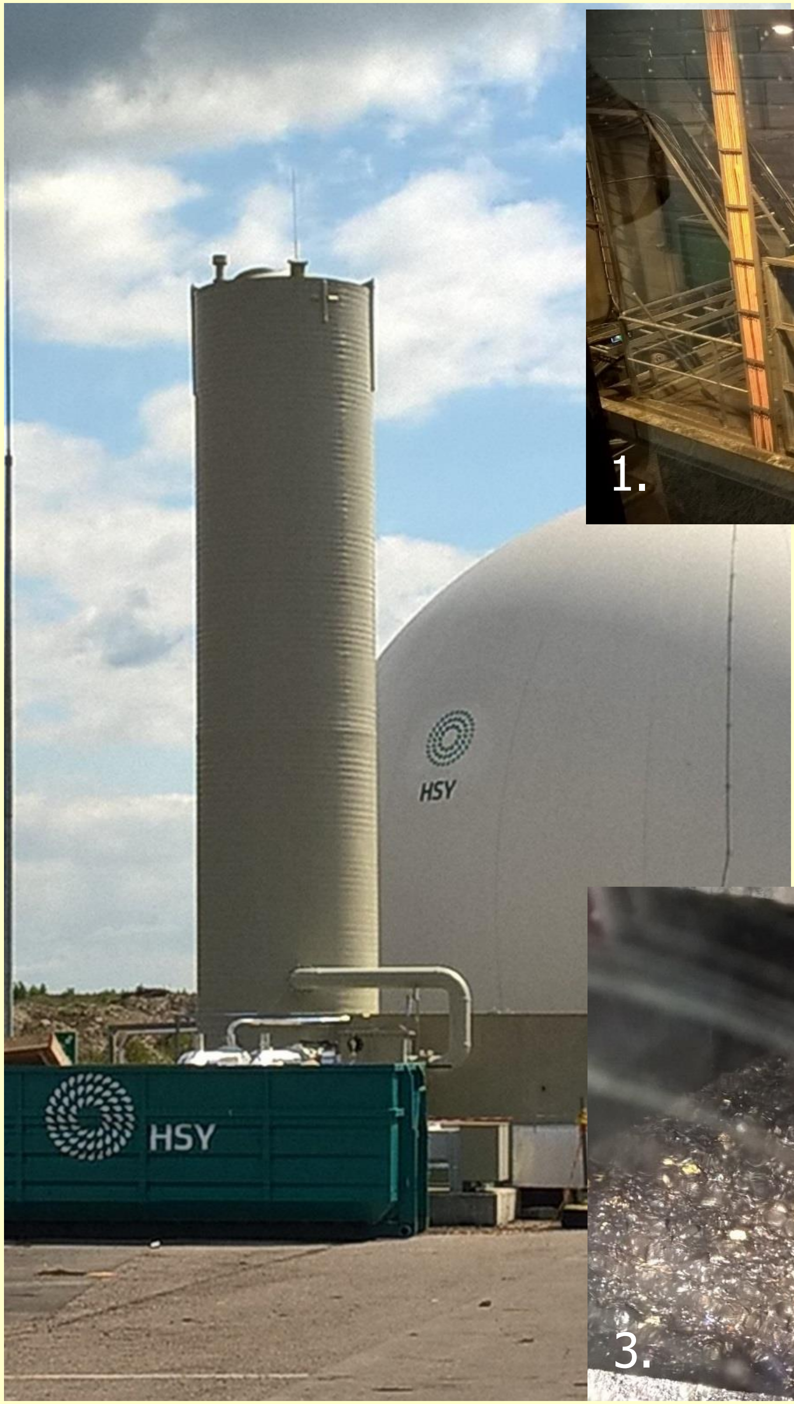
Full cell vehicle F-Cell -car, which is based on model B. Production 200 cars (source: Mercedes-Benz).

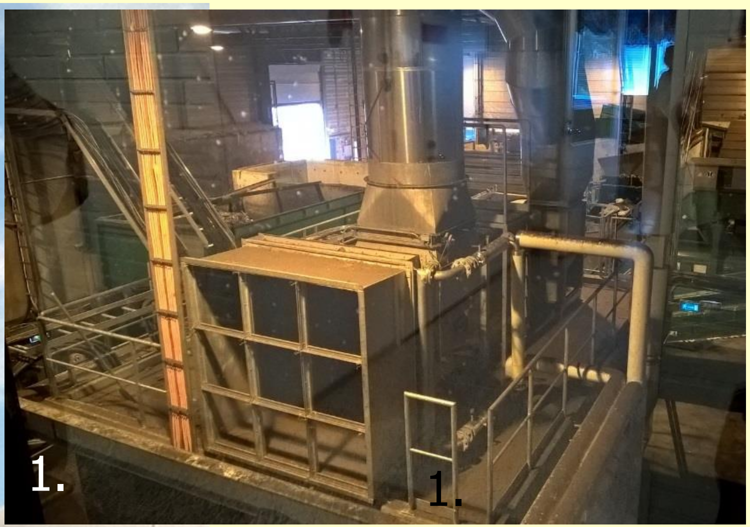
www.abowe.eu

Finnish testing 1-3/2014

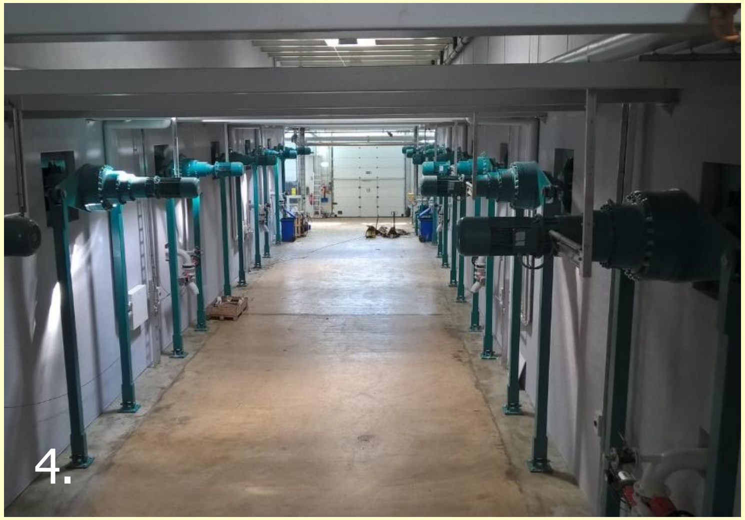
Savon Sellu fluting factory, Kuopio	SITE
Cellulosic waste	BIOMASS
Too high H_2S production	PROBLEM
Nutrient bed with seed bacteria	SOLUTION
2,3 butanediol, ethanol, butanol, butyrate,propionate, hydrogen	PRODUCTS

DESIGN OF A NOVEL HYDROGEN PLANT

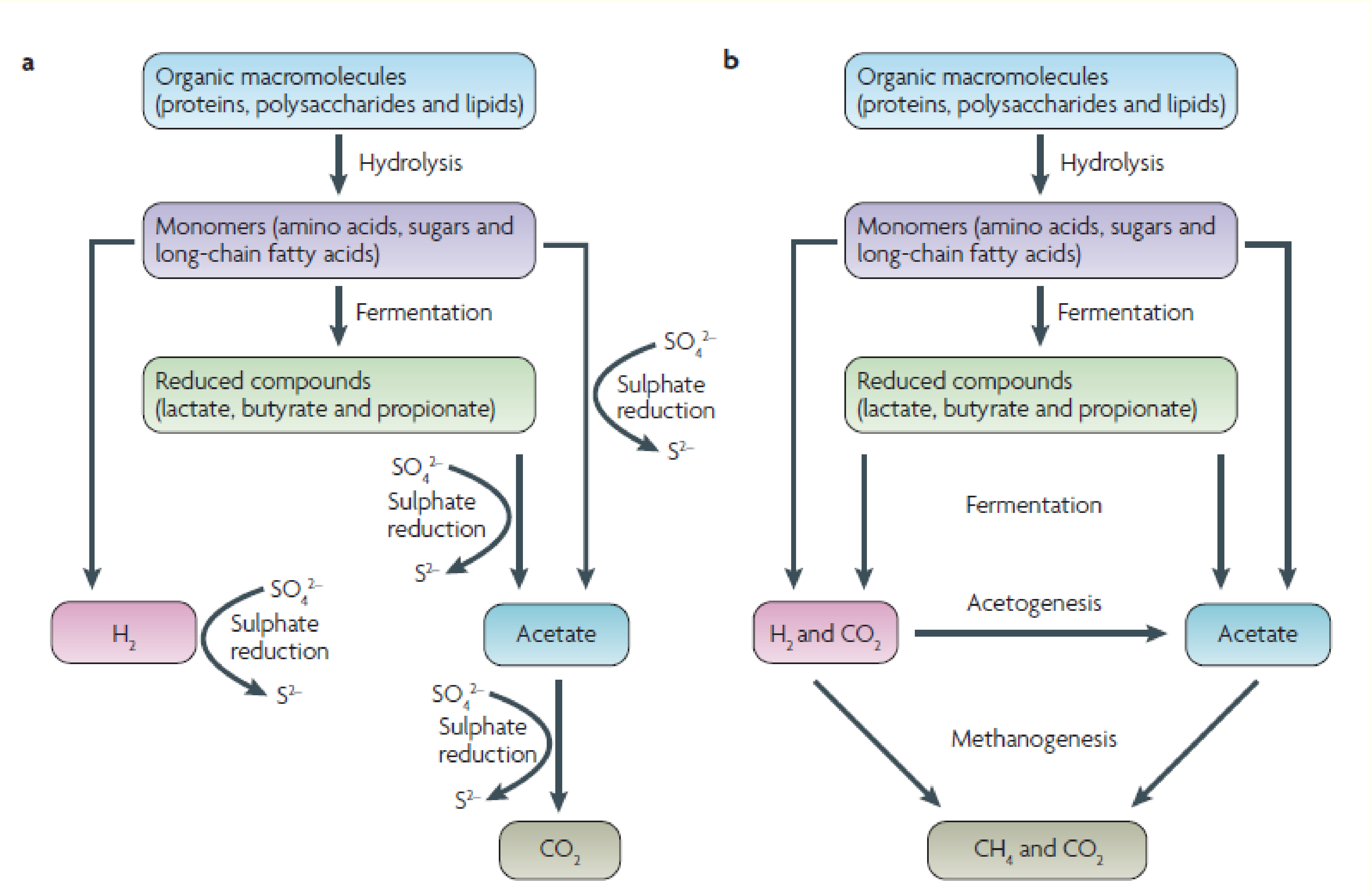




Biowaste treatment in the city of Helsinki



Microbial degradation of complex organic matter in anoxic environments in a) the presence and b) absence of sulphate



Muyzer & Stams, Nature Reviews, Microbiology, 6, 2008

Microbiological bioprocess can lower the environmental burden of the combustion of brown coal and other fossilic fuels

PATENTED

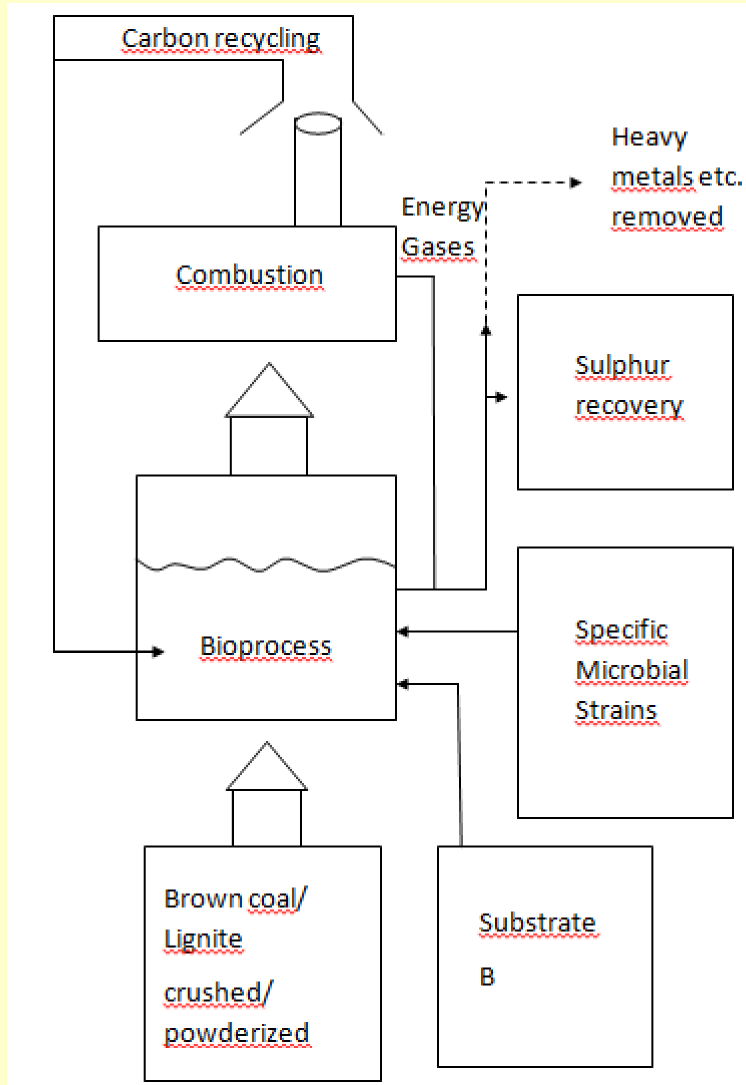
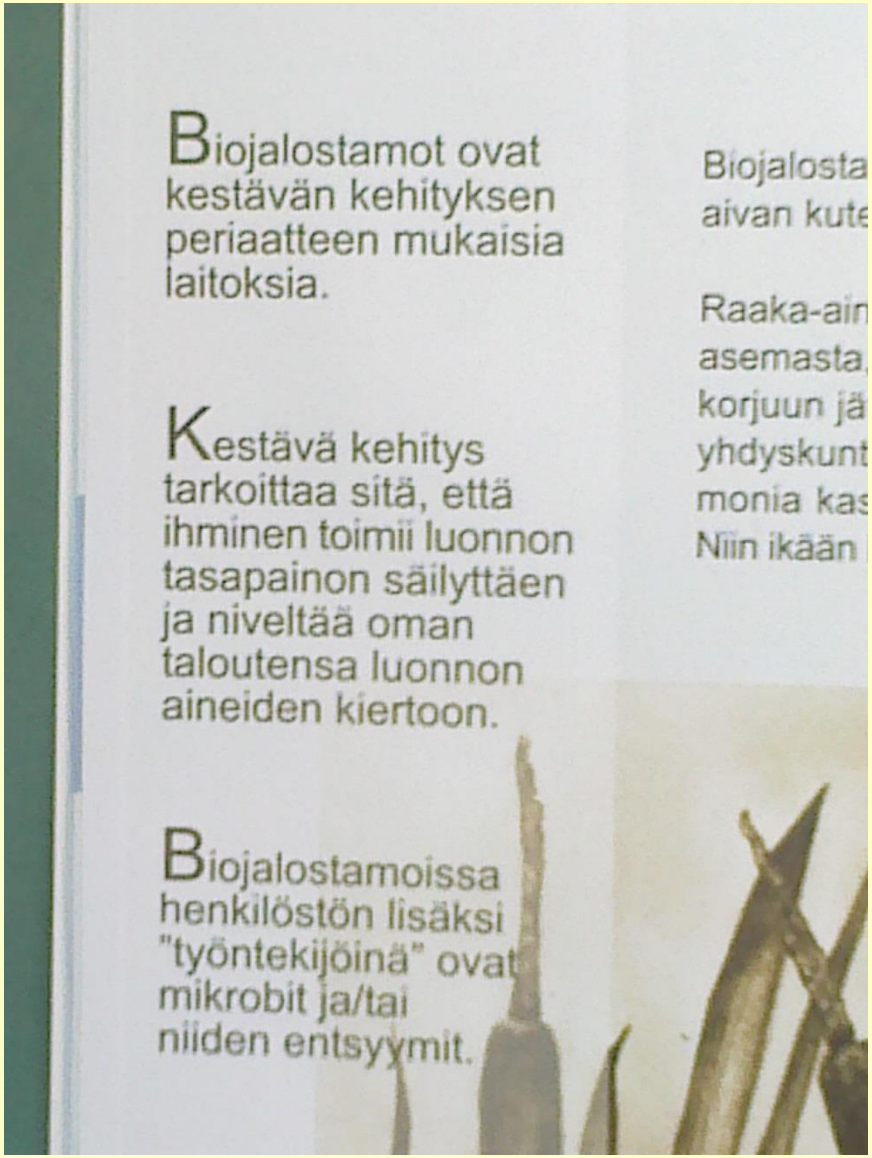


Fig. Ecological combustion of brown coal with Finnoflag biorefining principles (patented 2014)

Fig.: National Geographic

Adj. Prof. Elias Hakalehto, Beneficial Microbes, 25.8.2015, Valencia, Spain



BIOREFINERIES are production units based on sustainable principles.

Sustainability refers to human activities integrated with the balances in the Nature, as well as with the circulation of substances.

In the biorefineries the ”workers” are the microbes and their enzymes.

Industry Like Nature®

Thank You!