

BEACON



From plants to products
O blanhigion i gynhyrchion

Novel yeasts for a microbiological biorefining platform

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Bio Pacific Rim, Summit on Industrial Biotechnology and Bioenergy,
October, 10-12, 2012, Vancouver, Canada.

IBERS

Sefydliad y Gwyddorau Biolegol, Amgylcheddol a Gwledig
Institute of Biological, Environmental and Rural Sciences





IBERS – staff

Plant breeders

Agronomists

Molecular geneticists

Biochemists

Physiologists

Rumen/silage expertise

Enzymes/microbiologists

Other sources

Phenomix Centre



Platform for non-destructive dynamic imaging of plant growth & development



**Controlled environment
conveyor based system
radio-tagged plants**



**state-of-the art
imaging stations:**

- visible
- near IR
- Thermal IR
- fluorescence
- laser scan 3-d
- both canopy and root imaging
- DNA sequencing for rapid phenotypic associations

Novel yeasts for a microbiological biorefining platform (BEACON)

Isolated 100s of yeasts from diverse environments
Yeast diversity studies from forest backs, gut of insects, fruits, biogas reactors and frozen lakes of Himalayas.

Identified using Molecular characterization and chemotaxonomic studies

Reported novel yeast sp like *Candida hyderabadensis* sp. nov., *Pichia cecembensis* sp. nov., *Rodotorula himalayansis* sp. nov., *Cryptococcus shivaji* sp. Nov., *Candida northwykensis* sp. nov., *Blastobotrys serpentis* sp. nov.

Cultures submitted to NRRL-USDA

NRRL Y-48184 =	<i>Aureobasidium</i> sp.	YS67	(AM236013)
NRRL Y-48185 =	<i>Cryptococcus</i> sp.	YS DN8	(AM397841)
NRRL Y-48186 =	<i>Cryptococcus</i> sp.	YS NB5	(AM397842)
NRRL Y-48187 =	<i>Cryptococcus</i> sp.	YS 92	(AM397843)
NRRL Y-48188 =	<i>Cryptococcus</i> sp.	YS 88	(AM397844)
NRRL Y-48189 =	<i>Cryptococcus</i> sp.	YS 87	(AM397845)
NRRL Y-48190 =	<i>Rhodospiridium</i> sp.	YS 69	(AM397846)
NRRL Y-48191 =	<i>Debaryomyces</i> sp.	YS DN2	(AM397847)
NRRL Y-48192 =	<i>Hanseniaspora</i> sp.	YS DN19	(AM397848)
NRRL Y-48193 =	<i>Hanseniaspora</i> sp.	YS 82	(AM397849)
NRRL Y-48194 =	<i>Kodamaea</i> sp.	YS 85	(AM397850)
NRRL Y-48195 =	<i>Kluyveromyces</i> sp.	YS NB1	(AM397851)
NRRL Y-48196 =	<i>Kluyveromyces</i> sp.	YS 9F	(AM397852)
NRRL Y-48197 =	<i>Lodderomyces</i> sp.	YS 83	(AM397853)
NRRL Y-48198 =	<i>Metschnikowia</i> sp.	YS W1	(AM397854)
NRRL Y-48199 =	<i>Torulaspota</i> sp.	YS DN16	(AM397855)
NRRL Y-48200 =	<i>Sporidiobolus</i> sp.	YS NB7	(AM397856)
NRRL Y-48201 =	<i>Bullera</i> sp	YS 105	(AM397857)
NRRL Y-48202 =	<i>Issatchenkia</i> sp.	YS 110	(AM397858)
NRRL Y-48203 =	<i>Clavispora</i> sp.	YS 109	(AM397859)
NRRL Y-48204 =	<i>Pichia</i> sp.	YS DN22	(AM397860)
NRRL Y-48205 =	<i>Pichia</i> sp.	YS 73	(AM397861)
NRRL Y-48206 =	<i>Pichia</i> sp.	YS 78	(AM397863)
NRRL Y-48208 =	<i>Rhodotorula</i> sp.	YS 75	(AM397865)
NRRL Y-48209 =	<i>Rhodotorula</i> sp.	YS 74	(AM397866)
NRRL Y-48210 =	<i>Trichosporon</i> sp.	YS 93	(AM397867)
NRRL Y-48211 =	<i>Rhodospiridium</i> sp.	YS DN7	(AM397868)

Exploitation of yeasts

The new isolates used for the production value added products like ethanol and xylitol from D-xylose, Extracellular enzymes and polysaccharides.

Carbohydrate Polymers (2010) 82:728-732

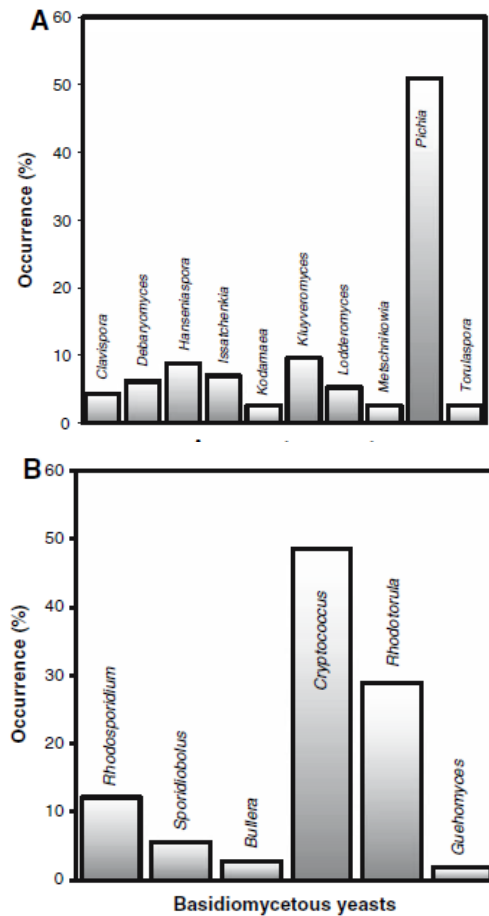
Letters in Applied Microbiology (2008) 47: 19-24

Current Microbiology (2008) 56: 489-494

Current Microbiology, (2007) 55: 441-446

Yeasts and Yeast-Like Fungi Associated with Tree Bark: Diversity and Identification of Yeasts Producing Extracellular Endoxylanases

Bhaskar Bhadra · R. Sreenivas Rao · Pavan K. Singh · Partha K. Sarkar · Sisinthy Shivaji



Abstract A total of 239 yeast strains was isolated from 52 tree bark samples of the Medaram and Srisailam forest areas of Andhra Pradesh, India. Based on analysis of D1/D2 domain sequence of 26S rRNA gene, 114 strains were identified as ascomycetous; 107 strains were identified as basidiomycetous yeasts; and 18 strains were identified as yeast-like fungi. Among the ascomycetous yeasts, 51% were identified as members of the genus *Pichia*, and the remaining 49% included species belonging to the genera *Clavispora*, *Debaryomyces*, *Kluyveromyces*, *Hanseniaspora*, *Issatchenkia*, *Lodderomyces*, *Kodamaea*, *Metschnikowia*, and *Torulaspota*. The predominant genera in the basidiomycetous yeasts were *Cryptococcus* (48.6%), *Rhodotorula* (29%), and *Rhodospordium* (12.1%). The yeast-like fungi were represented by *Aureobasidium pullulans* (6.7%) and *Lecythophora hoffmanii* (0.8%). Of the 239 yeast strains tested for Xylanase, only five strains of *Aureobasidium* sp. produced xylanase on xylan-agar medium. Matrix-assisted laser desorption ionization-time of flight analysis and N-terminal amino-acid sequence of the xylanase of isolate YS67 showed high similarity with endo-1-4- β -xylanase (EC 3.2.1.8) of *Aureobasidium pullulans* var. *melanigenum*.

Yeast and fungi biofilms

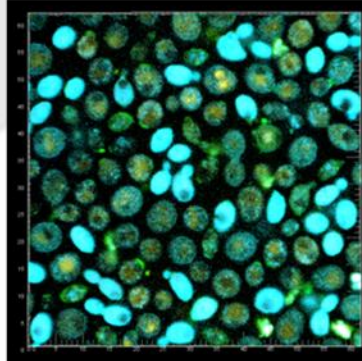
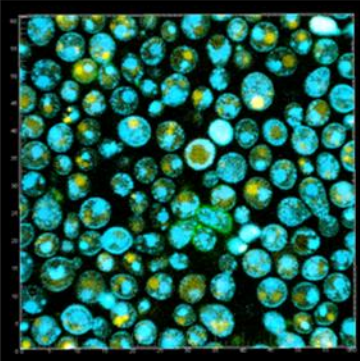
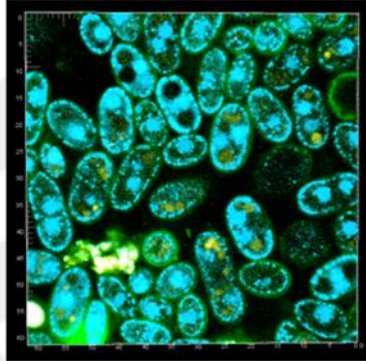
3 Days

A. pulluans

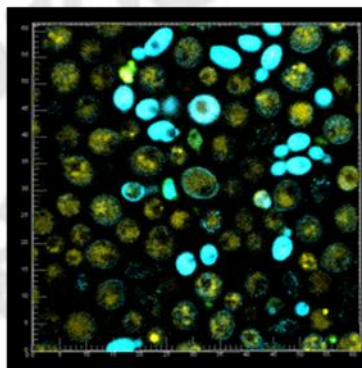
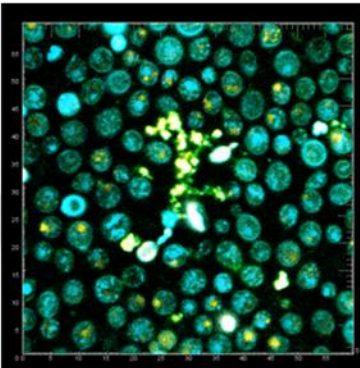
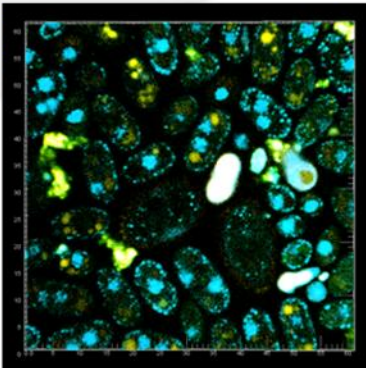
C. shivajii NCYC 3541

C. laurentii

GS-II
ConA
DAPI



IB₄
ConA
DAPI



From P. O. blanchigio

BEACON - Biorefining of High-Sugar Perennial Rye-grass

- Products
- Juice, fibre
- Chemicals, fuels
- Animal feed, biocomposite products
- Crude protein
- Fractionated sugars
- Waxes, lipids and pigments
- Lignin, yeast



Juicing separating juice and fibre

Analysis such as:

Yield

WSC

Digestibility

Protein

ADF NDF

Calorific value (animal feed)

Chemical isolation

Fermentation

Demonstration/pilot scale



BEACON and what it will do

- Welsh European Funding Office (WEFO) funded initiative with a value of £20m
- Partnership between Aberystwyth, Swansea and Bangor Universities to develop biorefining R&D expertise in Wales
- Enable academic and a wide range of industrial partners to develop and demonstrate scale-up processes for economically viable industrial applications

Strategic objectives of our funder

To develop an innovation based platform through:

- Establishing links between the business community and academia within Wales
- Development of new products and processes or IP that will support economic growth
- Creation of highly skilled jobs in the area of green/clean biotech
- Supporting inward investment
- Promoting science excellence in and from Wales

WEFO Metricated Objectives

Outputs

- **202 enterprise assists**
- **25 R & D collaborations**

Enterprise has to be in Wales
(or have a registered office)
If leading to inward investment then
outside companies can also be counted

Results

- **67 Jobs Created**
- **3 Enterprises Created**
- **Profit Benefit £1,680,000**
- **Investment Induced £3,360,000**
- **7 Products, process or services registered**
- **16 New/improved products, processes or services launched**

BEACON

Facilities and equipment for
development of biorefining expertise
and process scale-up

From plants to products
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Aberystwyth

New pilot scale facility

Primary processing area

- ~1000kg/hr wet feedstock processing line
- ~400kg/hr dry pelleting line

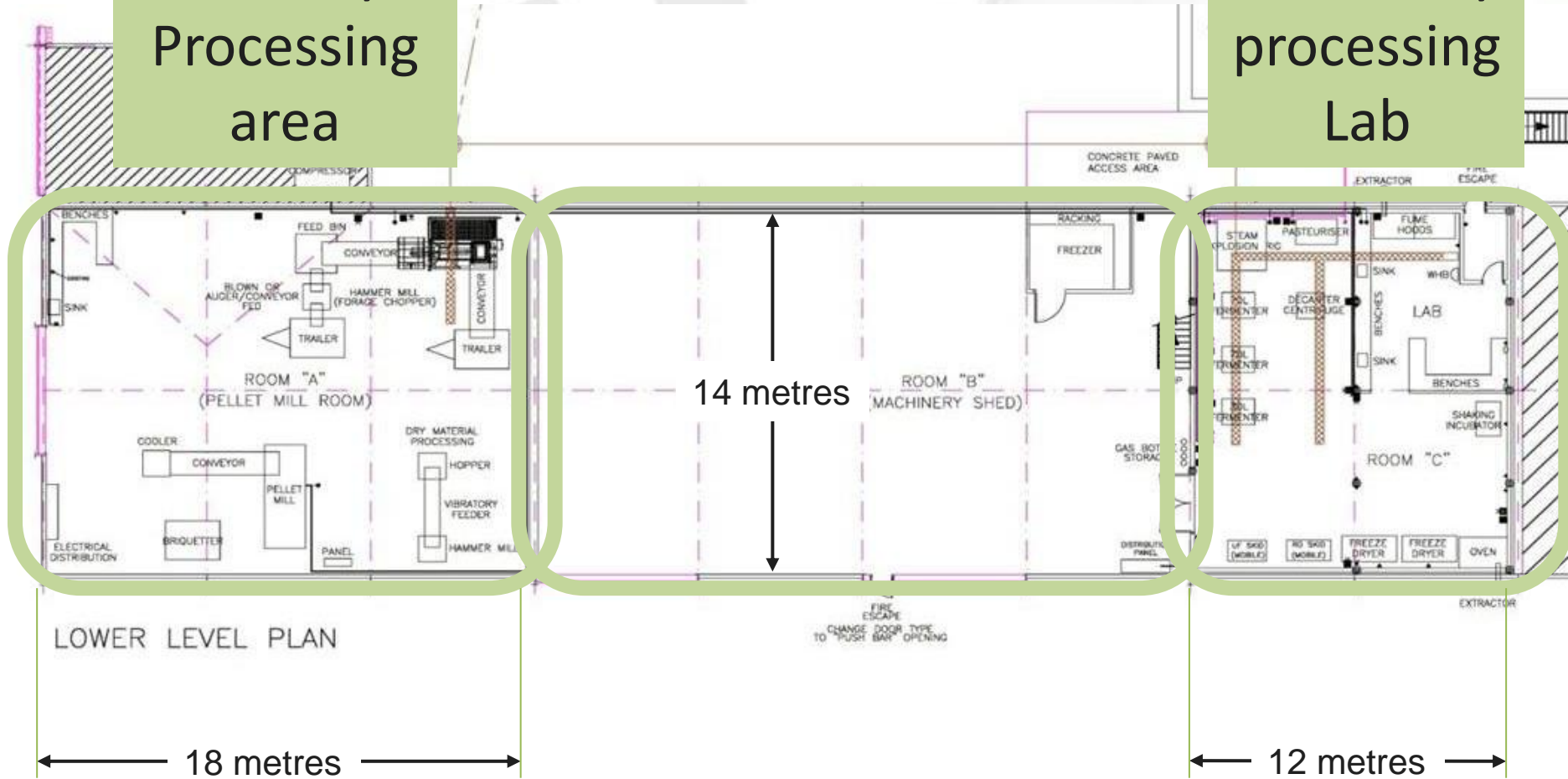
Secondary processing laboratory

- Liquid handling, solids pretreatment, fermentation and extraction:
 - Pasteuriser, centrifuges (decanter & CEPA),
 - Cross-flow filtration rigs: UF/MF & NF/RO
 - SIP fermenters
 - Batch steam explosion unit
 - 100 litre pressurised reactor
 - Flash chromatography units & 25 litre rotary evaporator

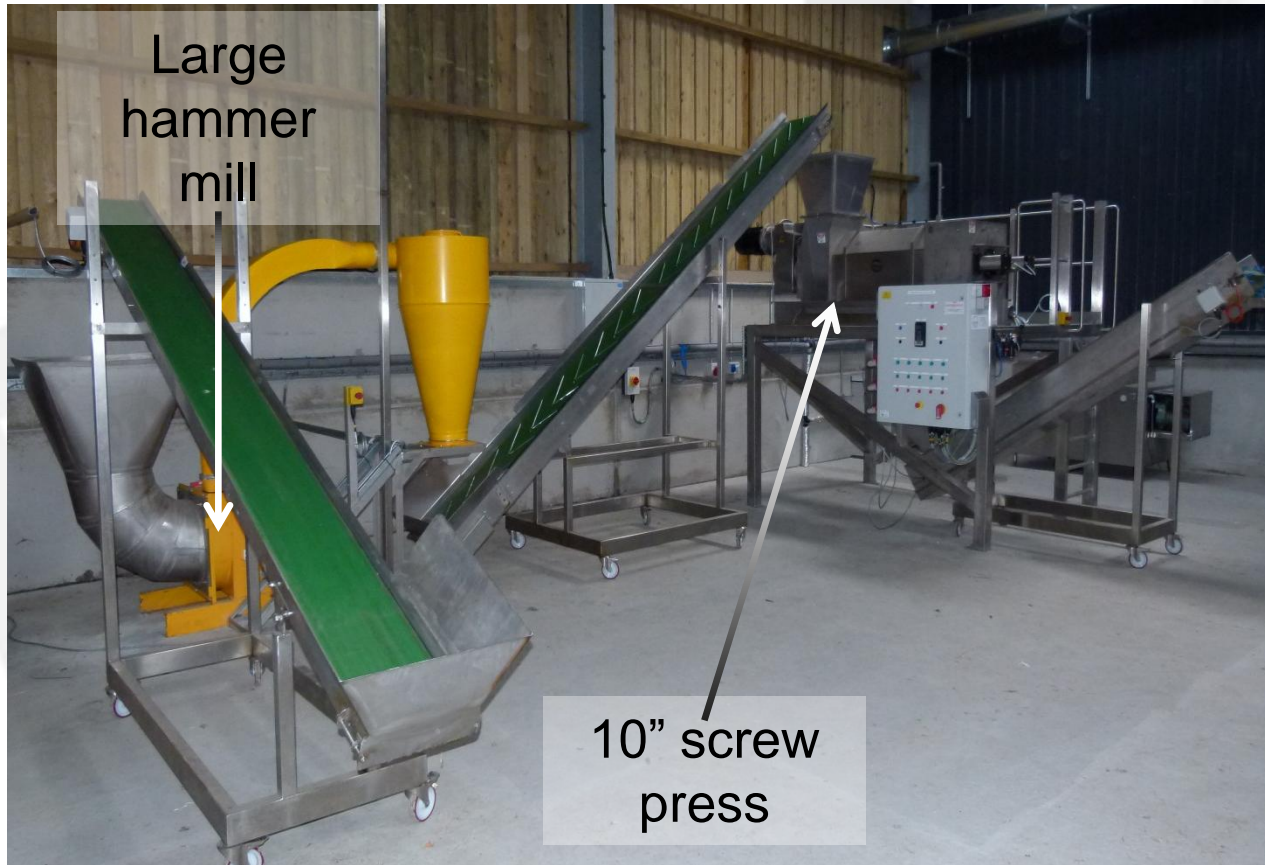
Biomass treatment and fermentation pilot facility lay-out

Primary Processing area

Secondary processing Lab



Primary Processing Area



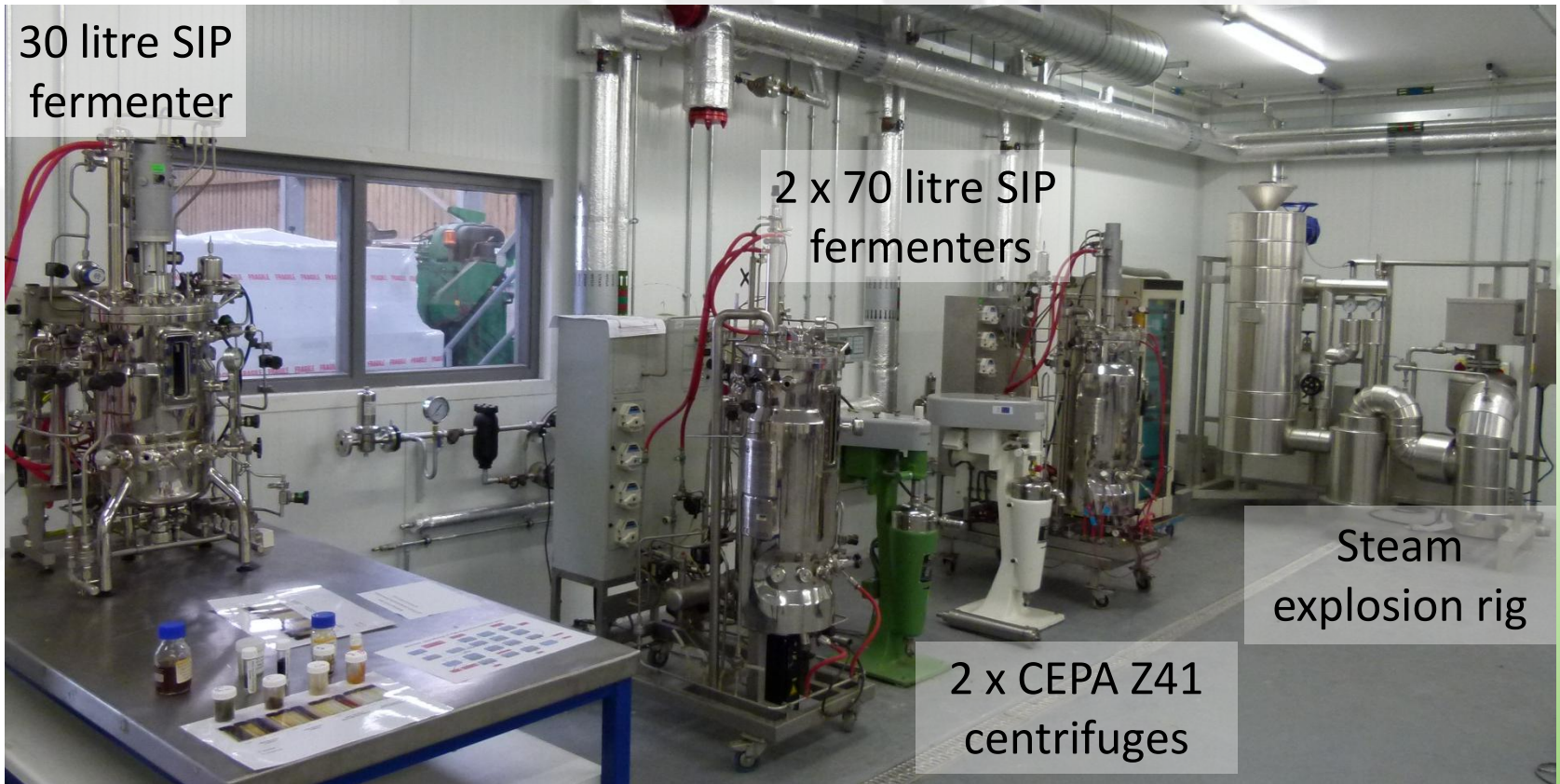
Integrated wet processing line



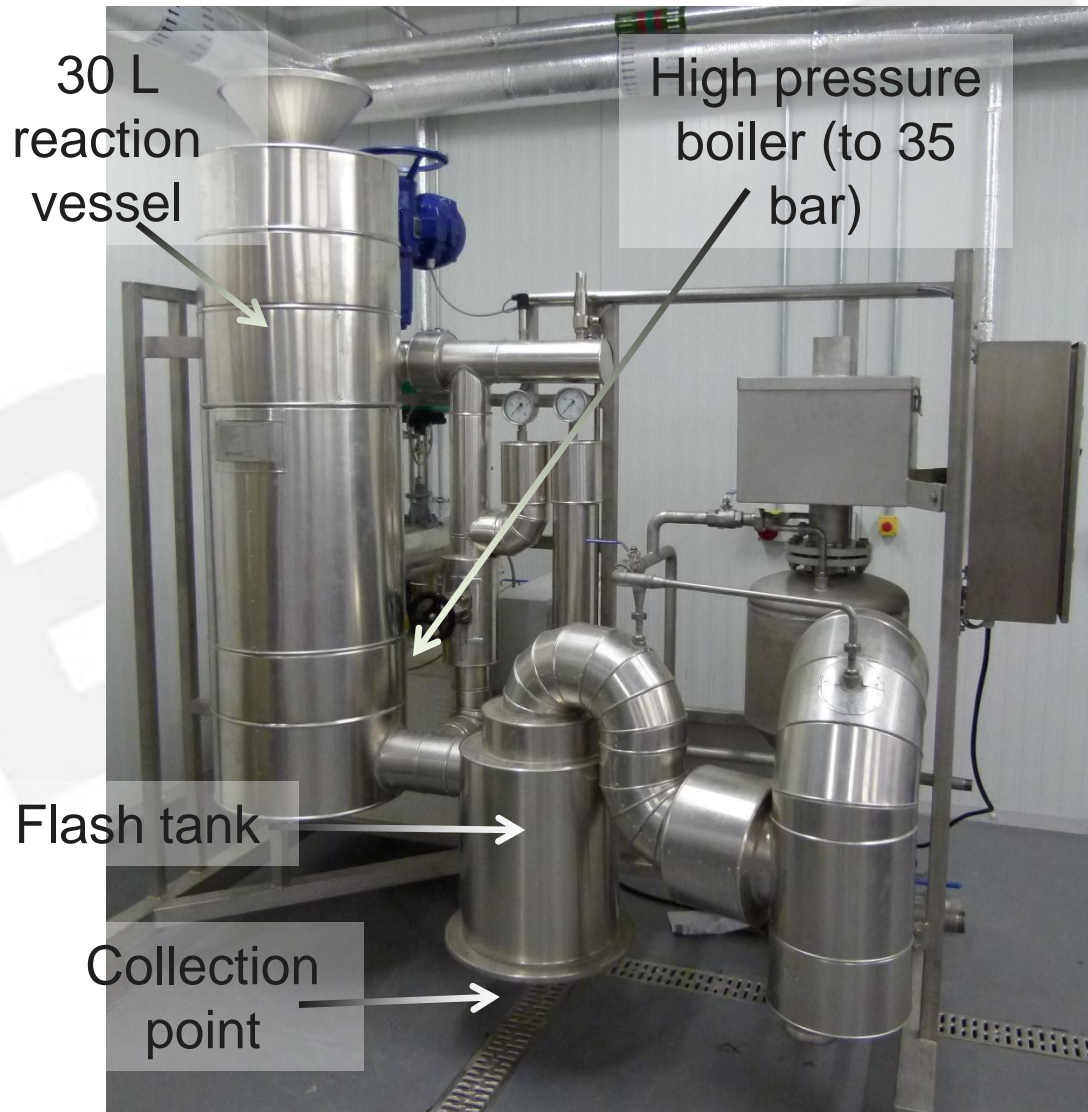
Dry processing:
Pellet mill

Secondary processing Lab

Fermentation and pre-treatment



Steam explosion rig by Cambi



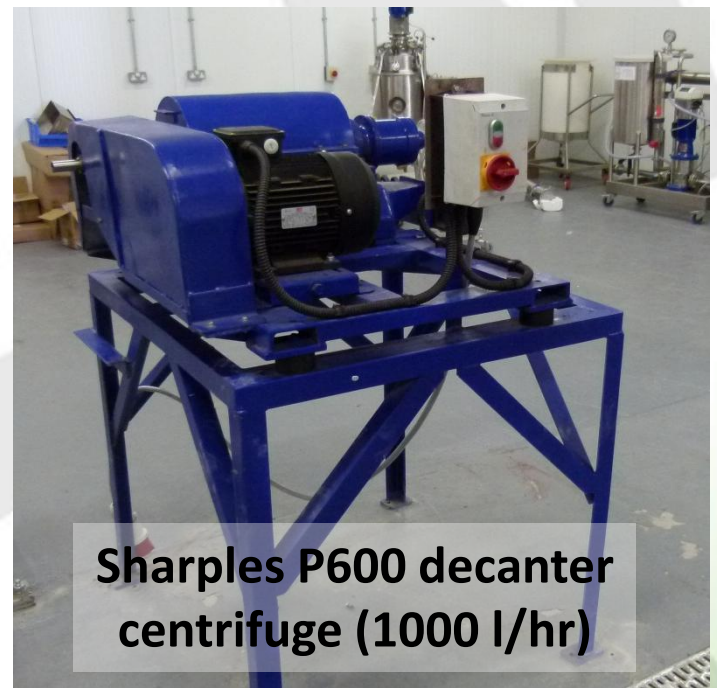
Pre-treated biomass from the flash tank



SE is common scalable pretreatment for lignocellulosic biomass. Above material will liquefy in under 1 hour with standard cellulosic enzymes.



**Dual duty (50/500 litre/hr)
pasteuriser**



**Sharples P600 decanter
centrifuge (1000 l/hr)**



**100 litre
reaction tank**



NF/RO filtration skid



UF/MF filtration skid

Biochar Facility



**Oven
(500kg
capacity)**

Pre-heater

**Hot gas
recirculation**

**Condenser for
pyrolysis oils**

From *pyrolysis*
© *blanck*

Biocomposites Centre pilot facility: BioProducts and BioRefining TTC (BPR-TTC)



Supercritical CO₂ extraction



Lab scale:
0.01, 0.1 and 1 litre

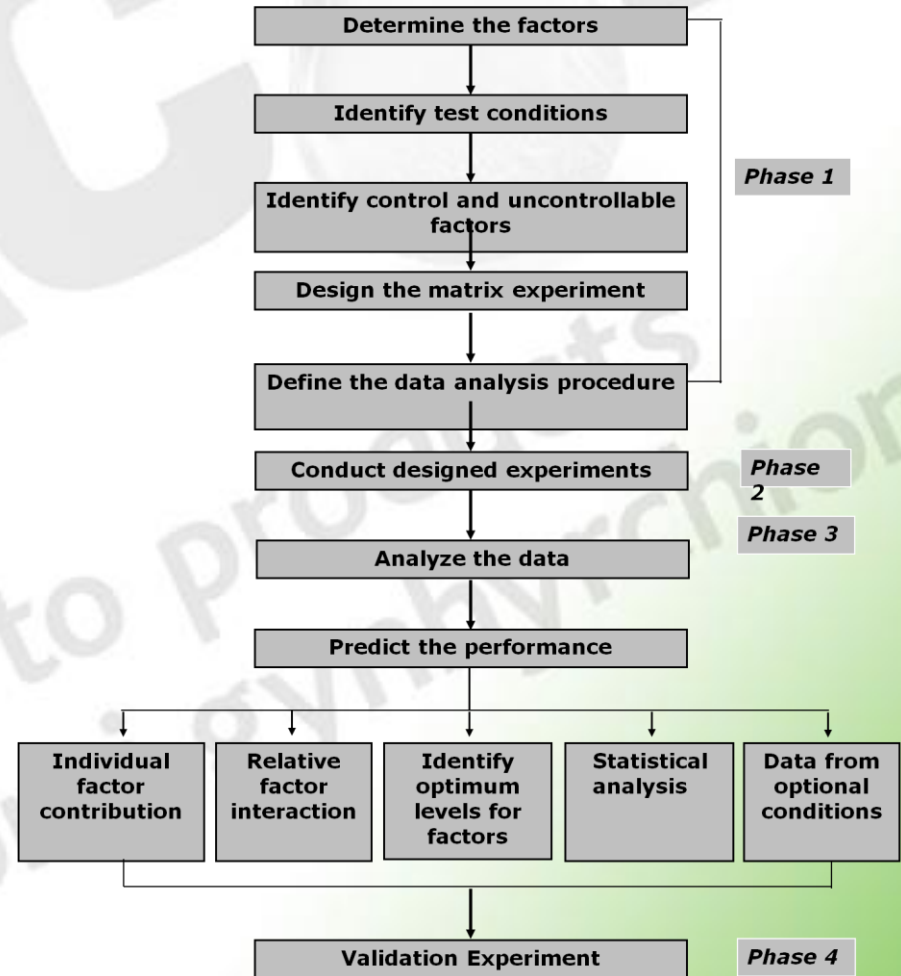
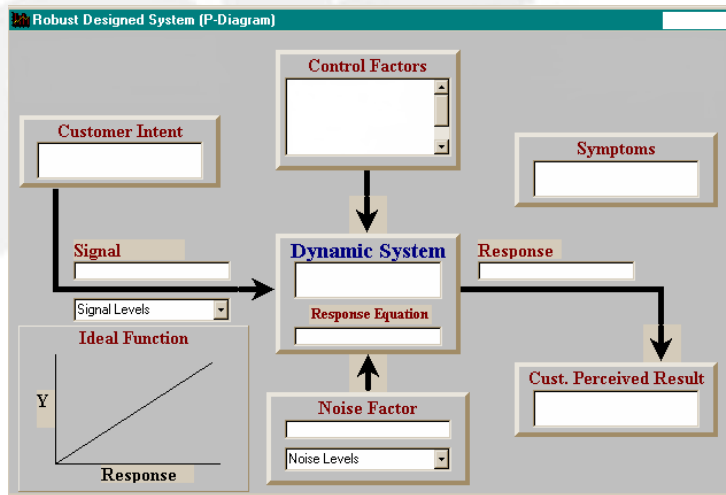
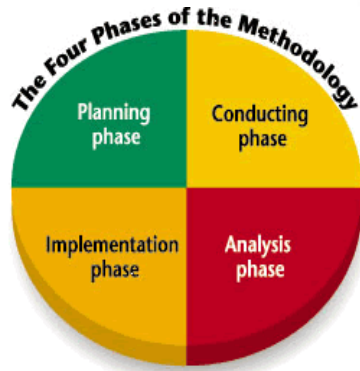


Pilot scale:
30 litres

Extraction of non-polar high value actives, eg:

- Omega 3 fatty acids
- Carotenoids/ wax extraction
- Essential oils and aromas
- Polymer cleaning/ LCD recycling

Taguchi methodology for process optimization



BEACON



Thank you

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