wetlands

Engineering

Detoxifying Waste Water through Immobilized Laccase Based Bioremediation

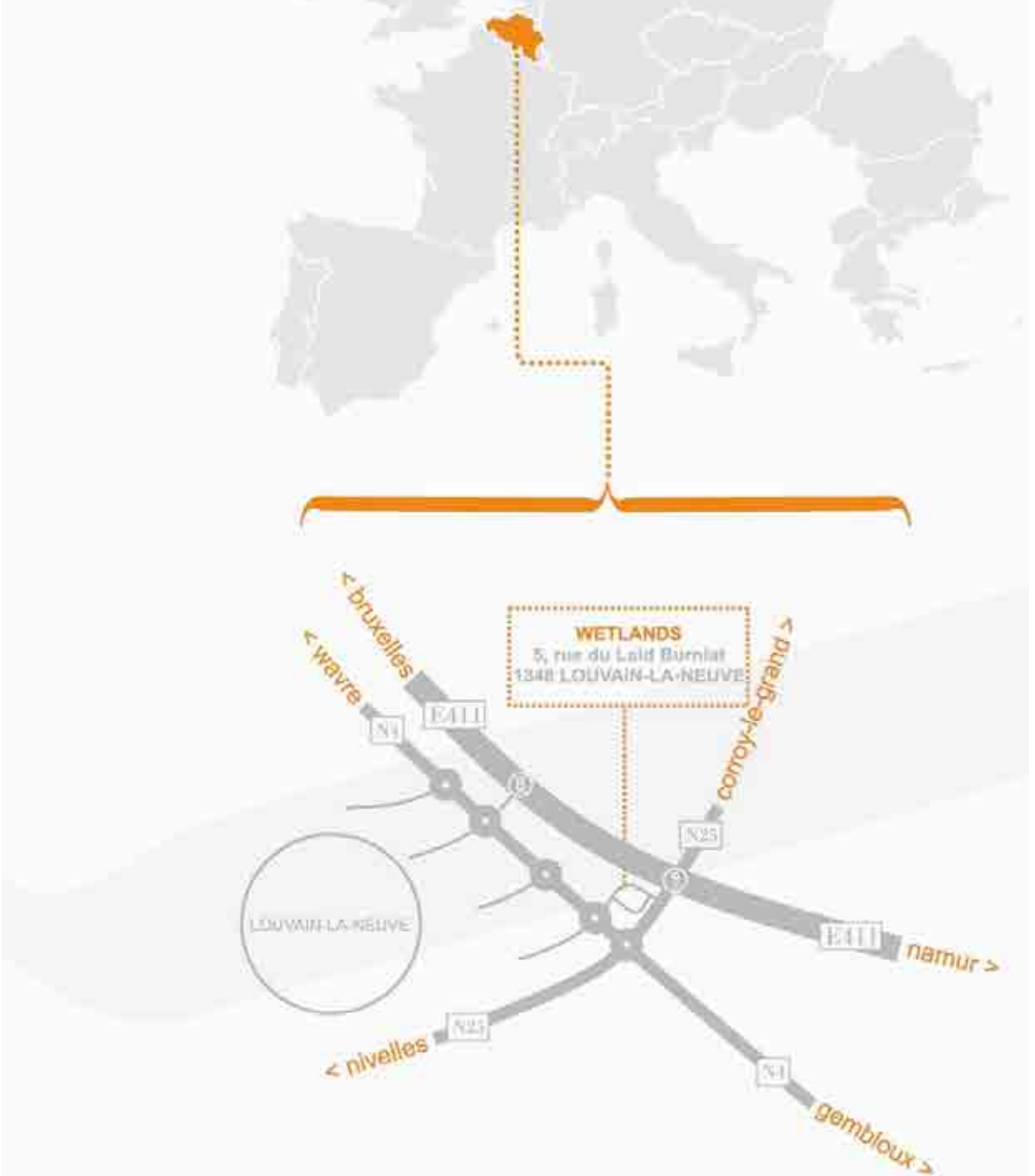
Rob Onderwater, Sophie Vanhulle

www.wetlands.be

Wetlands key figures:

- created in 2001
- 23 employees
- networking with 50 research centres and universities through Europe
  in microbiology, nanotechnology, engineering, bioremediation, fermentation fields.
- 2 major implications in FP6 European projects

www.sophied.net	development of novel sustainable bioprocess for colour indutries
www.quorumsensing.eu	development of models for new nanotechnology-based processes


WETLANDS
5, rue du Laid Burniat
1348 LOUVAIN-LA-NEUVE
< bruxelles
< wavre
E411
N4
N25
corroy-le-grand >
LOUVAIN-LA-NEUVE
E411
namur >
< nivelles
N25
N4
gembloux >

Wetlands competences:

LIFE SCIENCE
>>> microbiology, toxicology, agronomy

PROCESS BIOENGINEERING
>>> design and production, automation, process software development

INDUSTRIAL BIOTECHNOLOGY
>>> fermentation, bioremediation, biosynthesis, composting technology, production of lab equipment

Wetlands product range:

umic to master organic waste biodegradation

>>> composting plate-form, laboratory, certification organisation

zymm to manufacture enzyme

>>> research centers, textile, pulp/paper, cosmetic, beverage and pharmaceutical sectors

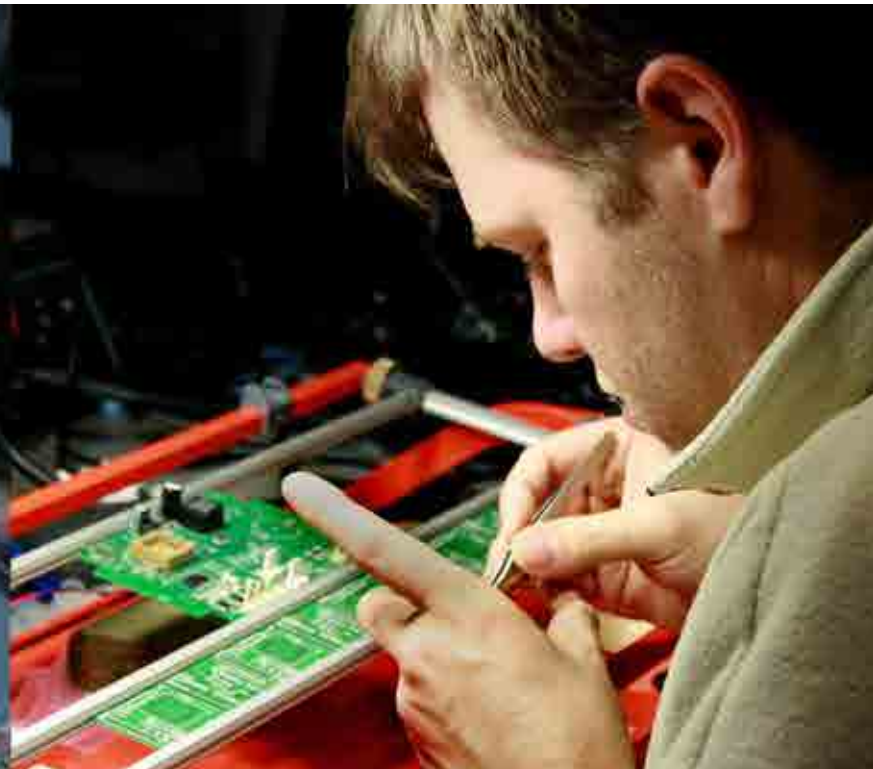
TOXX to screen toxicity *in vitro*

>>> municipal, pulp/paper, chemical manufacturing, petrochemical, food and beverage sectors

ENZYCLEAR to treat industrial waste water

>>> municipal, dye, textile, plastic and cosmetic sectors

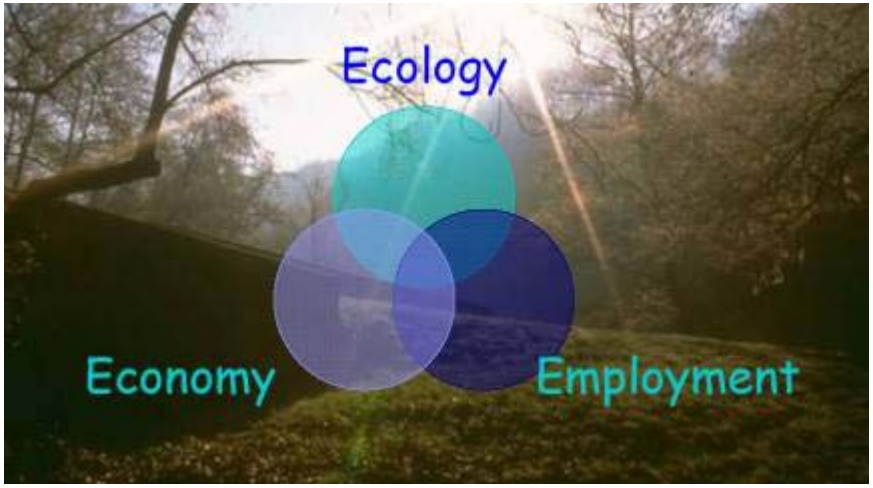
From a dedicated team able to provide customized solutions throughout Europe



Sustainable bioprocesses for the European colour industries

Development of new bioprocesses at 3 levels

- New bioremediation technology to detoxify coloured waste waters
- New safe enzyme-assisted processes for the production of existing dyes
- New dyes which are less toxic and synthesized biotechnologically for high added value markets



Industrial wastewaters of colour industry

Class	Loss to effluent (%)	Rejection (*1000 tons)
Acid	**5-20**	**5-20**
Basic	**0-5**	0-2,5
Directs	**5-30**	**4-21**
Disperse	**0-10**	0-16
Reactive	**10-50**	**11-57**
Others (Sulfur, vat, metallic…)	**2-40**	4-80

Waste water treatment
Decolourization

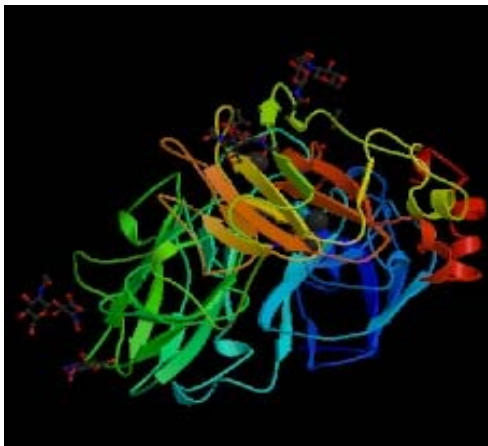
- Adsorption to activated carbon
- Filtration (with sedimentation)
- Ozonation
- Electrolytic treatment
- Treatment with HOCL, H_2O_2 or H_2O_2 and Fe^{2+}
- UV treatment (with H_2O_2)
- Microbial (sludge)

- White Rot Fungi or Laccase enzymes from WRF

White Rot Fungi

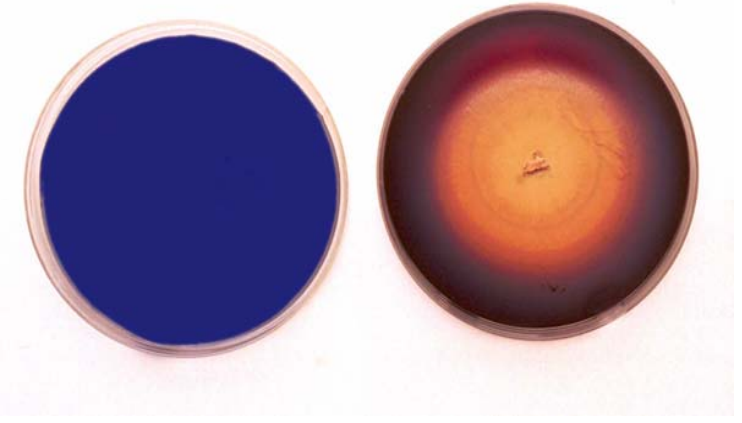
Laccase enzyme

- Laccases (EC 1.10.3.2) are multi-copper-containing oxidase enzymes
- Found in many plants, fungi, and microorganisms
- Involved in lignin breakdown
- The copper is bound in several sites
  - Type 1, Type 2, and/or Type 3.
- Laccases act on phenols and similar molecules
- Perform a one-electron oxidation, reducing O_2 to water

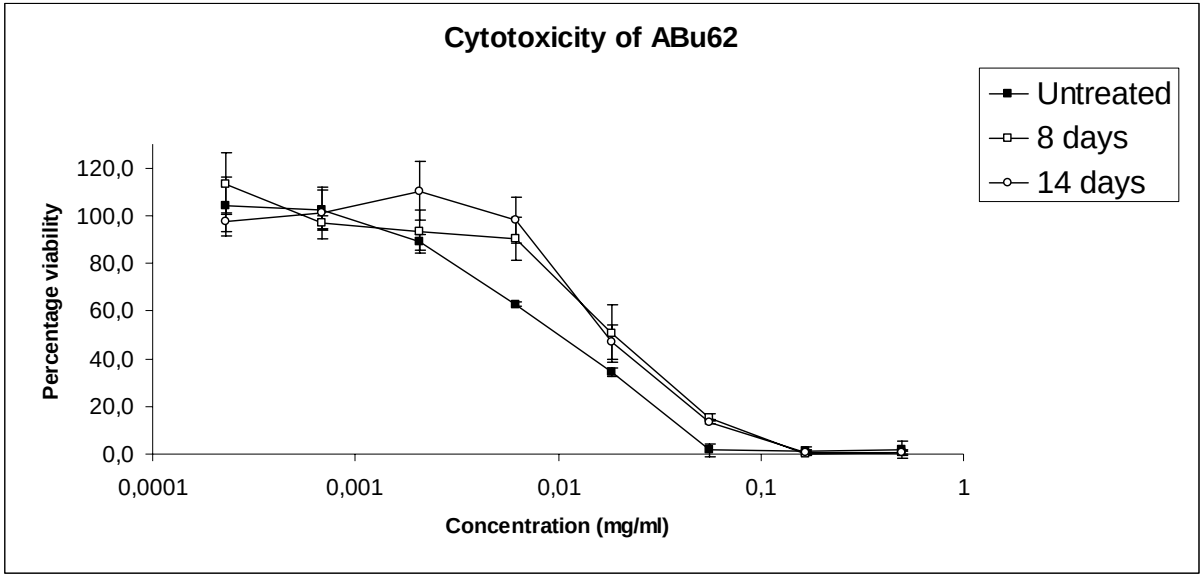


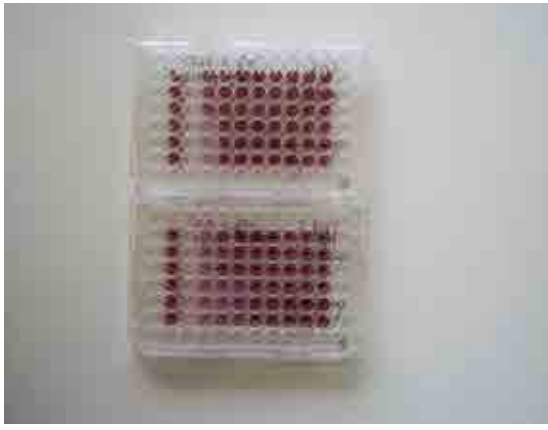
Laccase enzyme

- Have varying optima for pH and temperature
- Capable of decolourizing and detoxifying industrial dyes and industrial waste waters
- A mediator molecule is often required



Toxicity of model dye ABu62 towards the Caco-2 human intestinal cell line





- Model dye treated in liquid culture for 8 and 14 days

European mycotheques

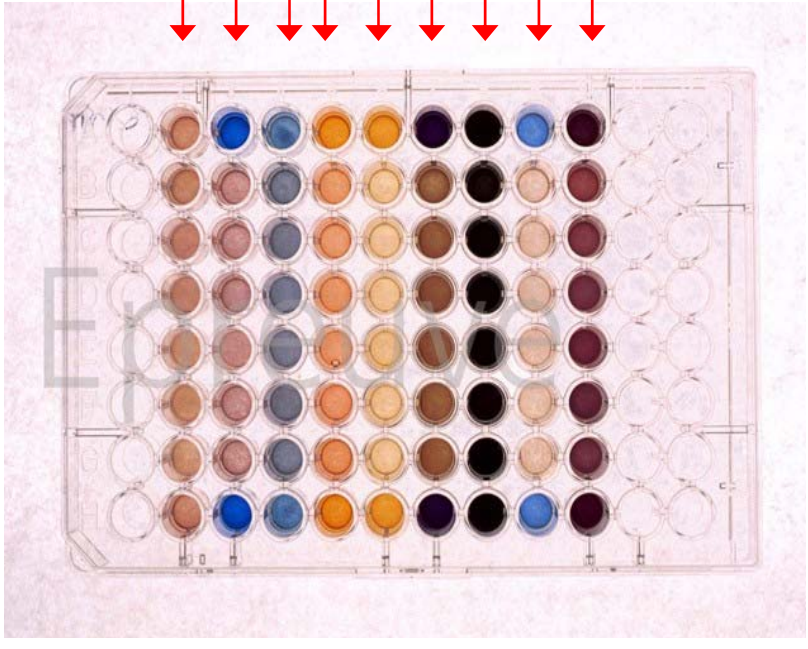
FUNGAL BIODIVERSITY

Internal culture collection

➢600 microorganisms already identified as potential dye decolourisers in project proposal

Normalized procedures for screening of strains from 7 european laboratories

Each column contains a model dye



Uninoculated line = blank

6 replicates of dyes decolourized by White Rot fungi

Uninoculated line = blank

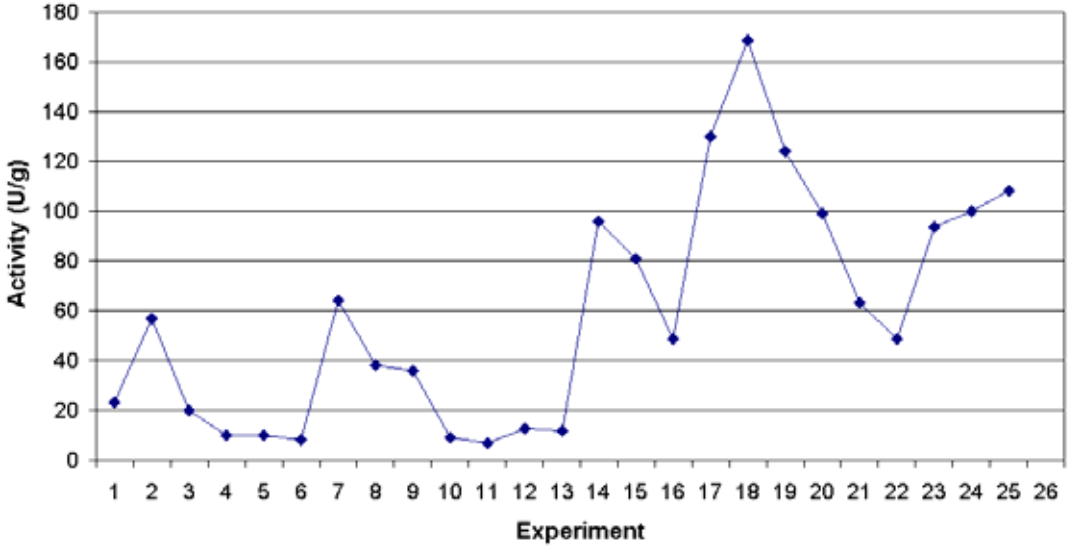
➢Of these 300 strains of WRF were ultimately tested

Laccase immobilisation

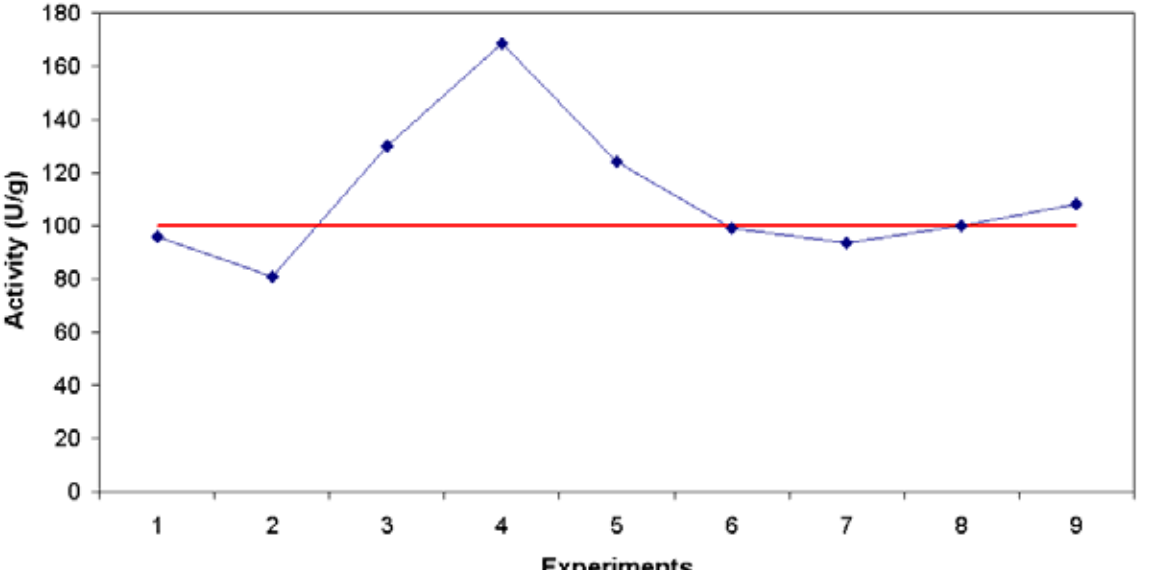
- Laccase : *Coriolopsis Polyzona* 36
  - *(*Zimbabwe, Hwange national park, savanna)
  - Produced by Wetlands in 150L bioreactor
- High activity broth (~ 50.000 U/l)
- Purification & immobilization at once
- Immobilization :
  - Initially used porous support (Perlite)
  - Now use non porous inert support (glass beads; 0.4 – 0.6 mm)

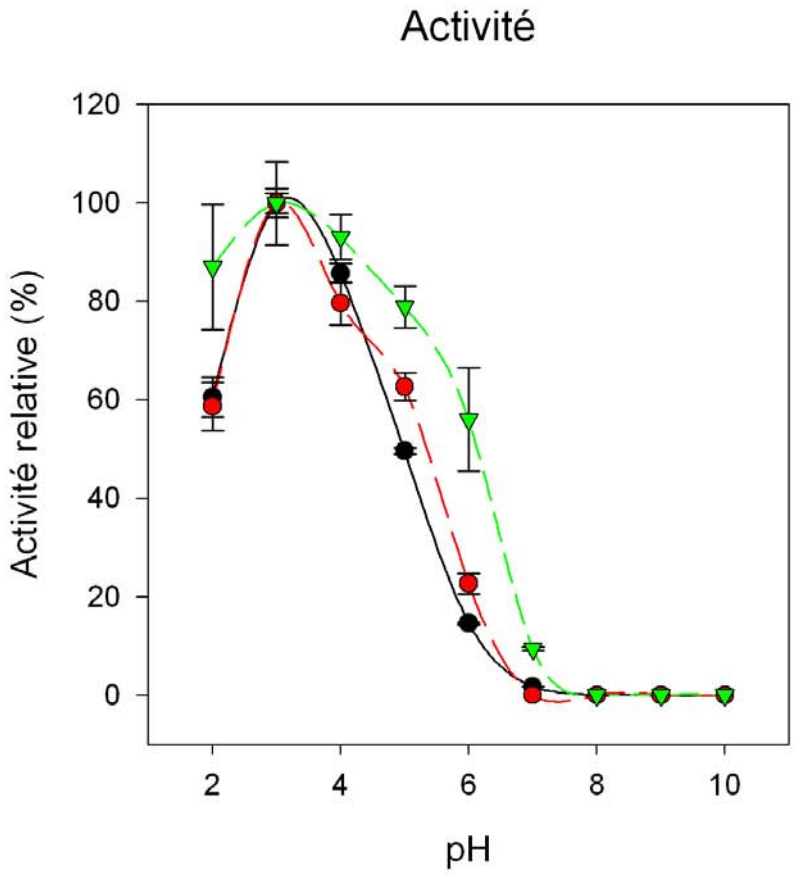
- Porous supports adsorb dyes

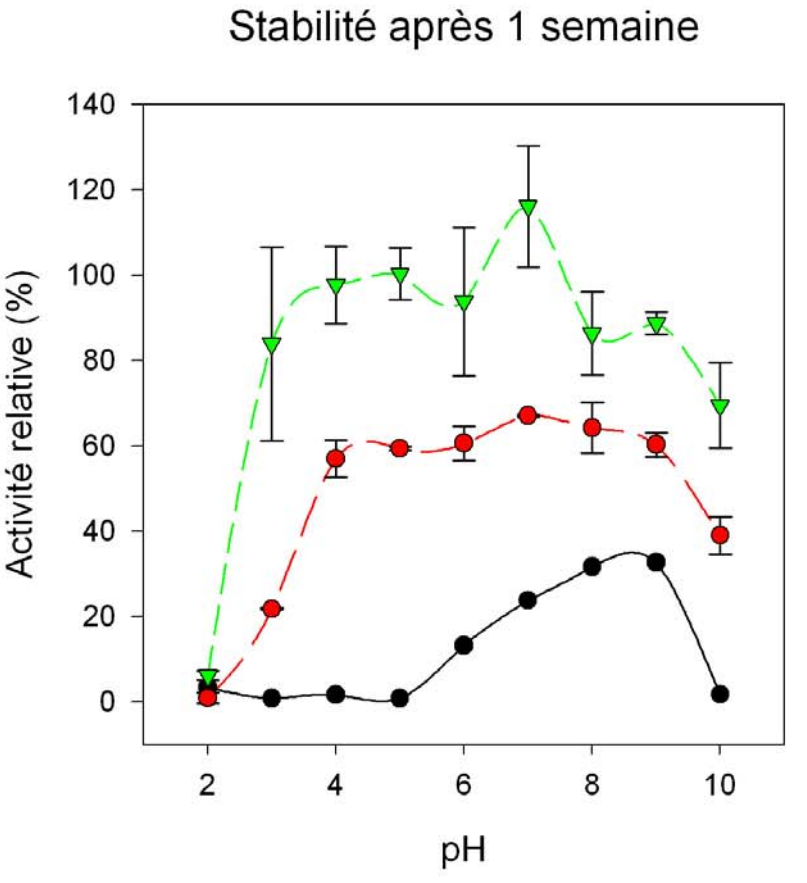
Improvements in activity of immobilized laccase

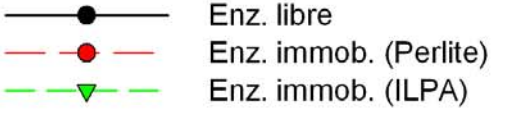


Routine activity of immobilized laccase




Activité
Activité relative (%)
120
100
80
60
40
20
0
2
4
6
8
10
pH


Stabilité après 1 semaine
Activité relative (%)
140
120
100
80
60
40
20
0
2
4
6
8
10
pH


Enz. libre
Enz. immob. (Perlite)
Enz. immob. (ILPA)

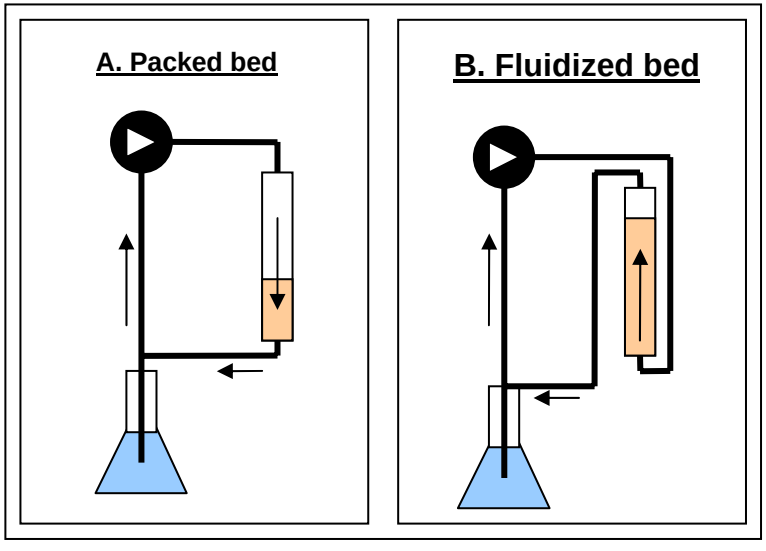
First trials…

Problems reported :

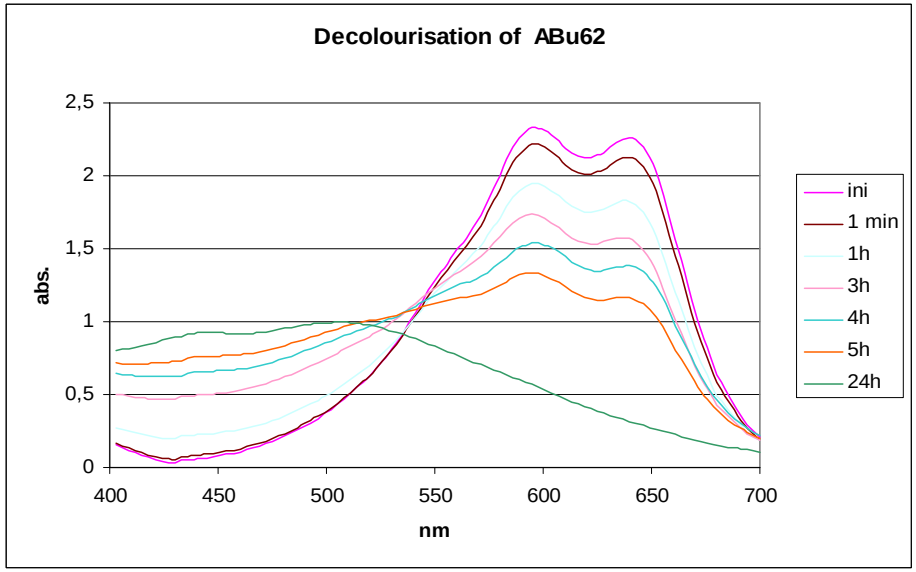
- overpressure events
- flow regulation not accurate
- no data about the oxygen demand
- frequent leakage of the equipment

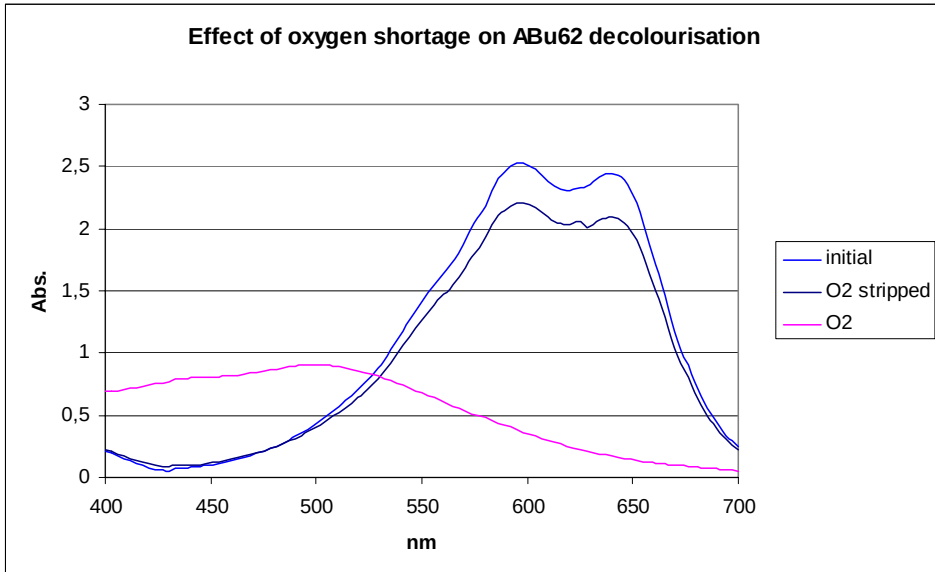
Issues discovered :

- differences in the use of the immobilized Laccase packed bed filters / fluidized bed
- oxygen mass transfer rate
- pH logging / monitoring
- salt content / conductivity
- continuous logging of the spectrum
- temperature control

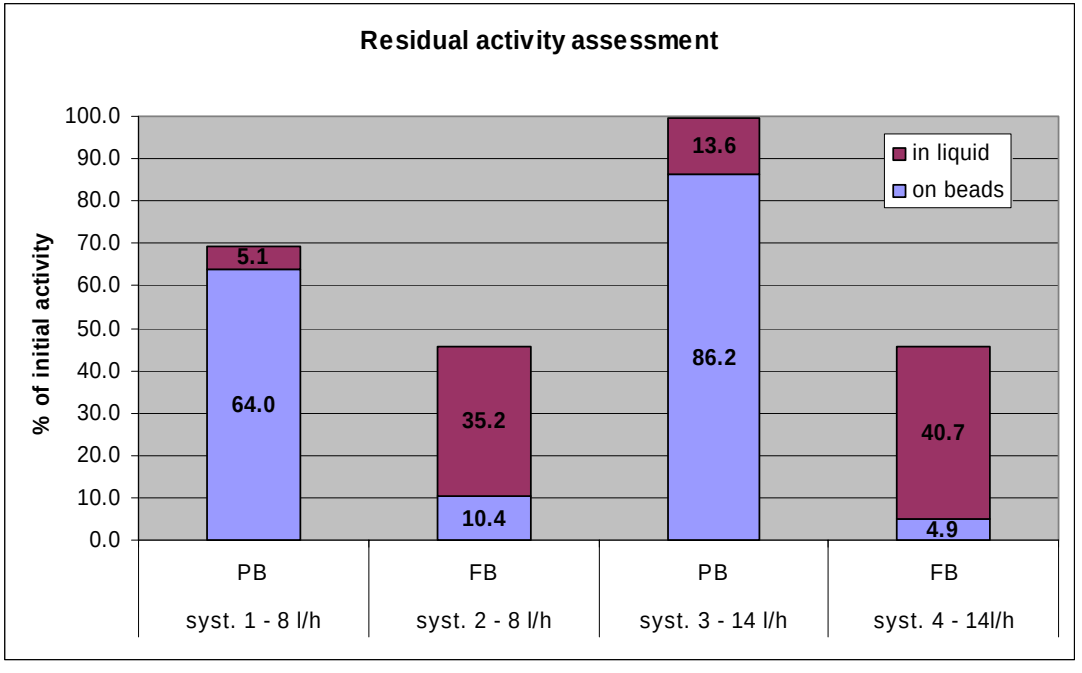


Decolourization of ABu62 a model Dye





Packed *vs* Fluidized bed



PB – Packed bed

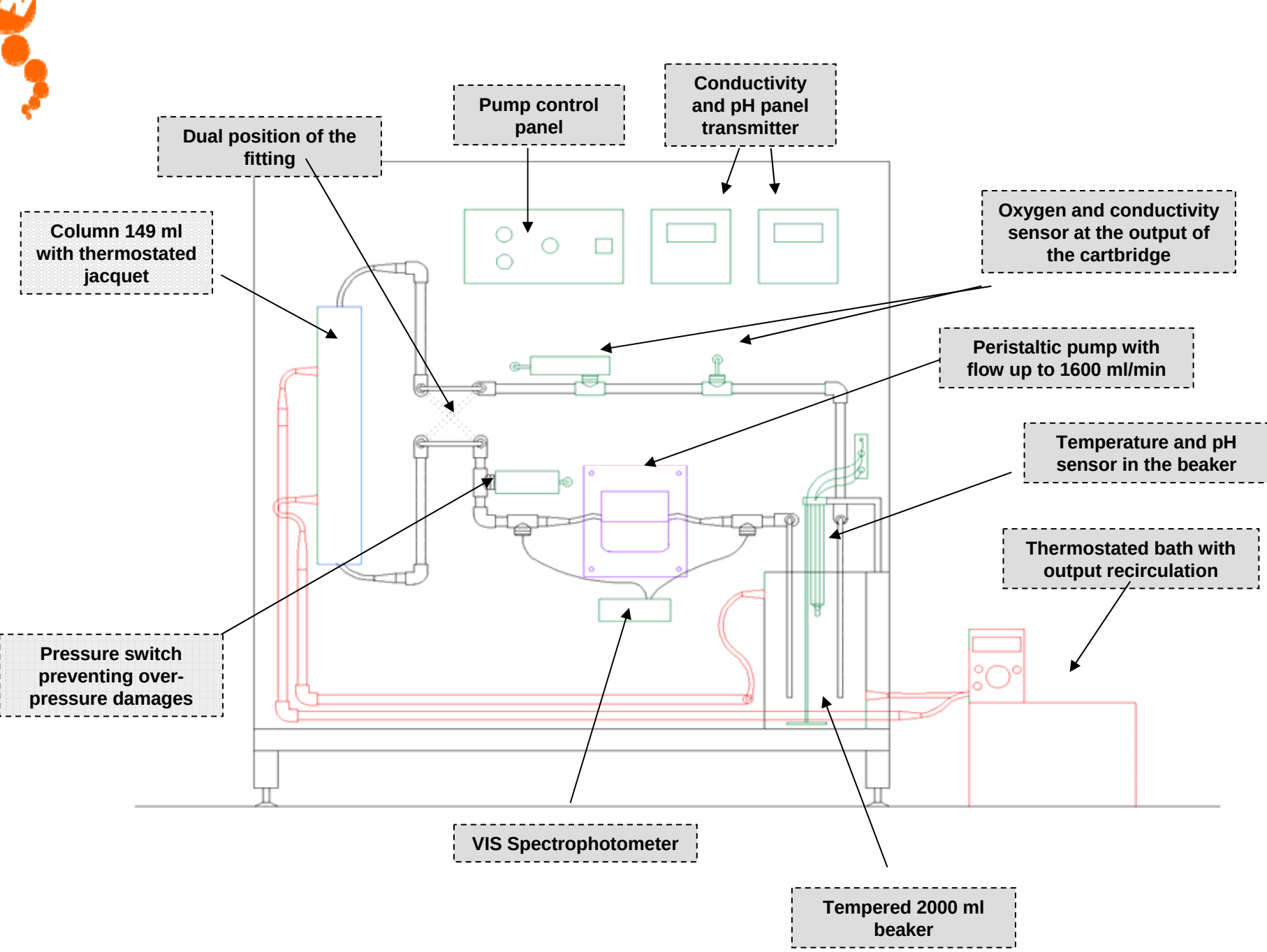
FB – Fluidized bed

➢Faster decolourization in fluidized bed but larger decrease of the activity fixed on the beads

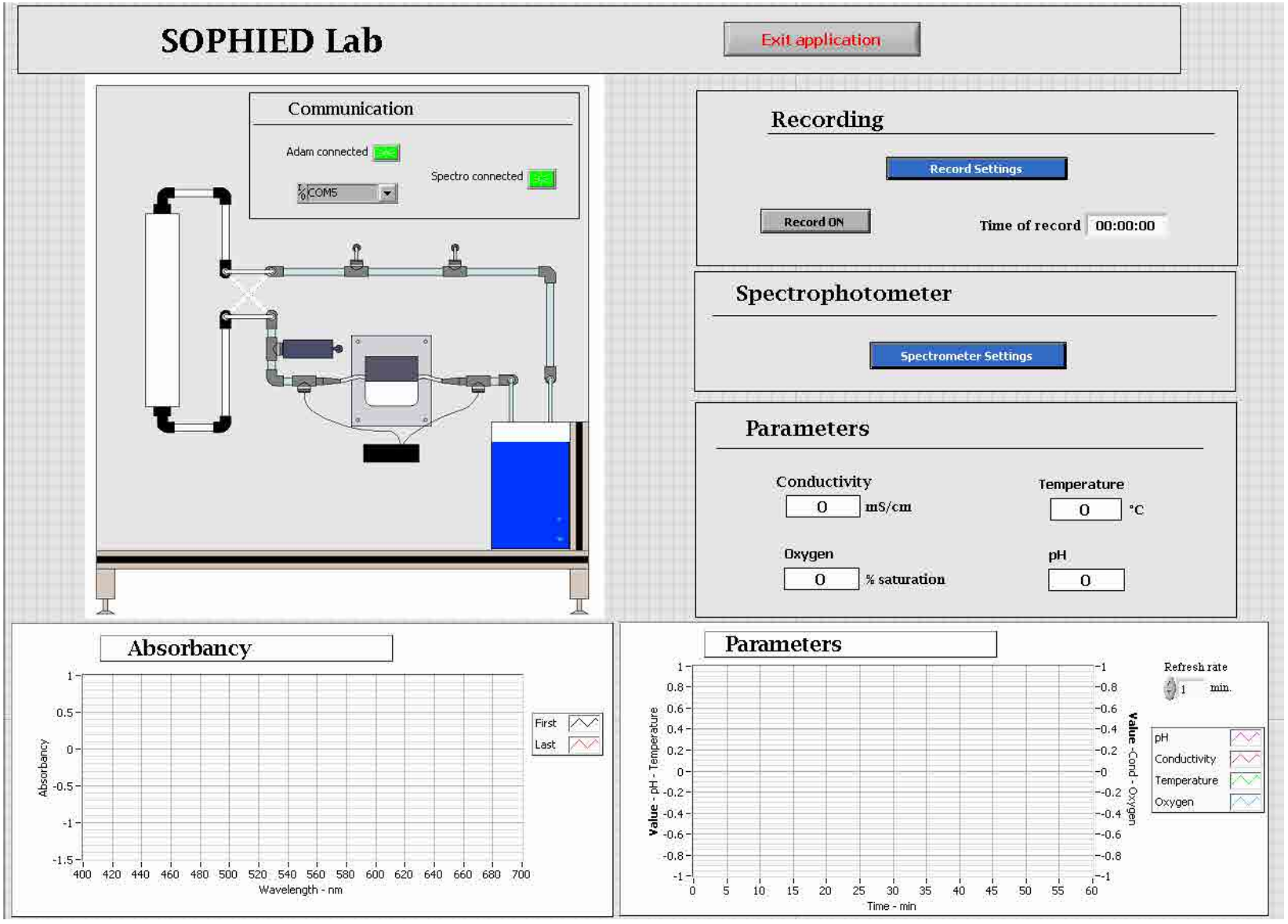
Labscale system

Equipment summary :

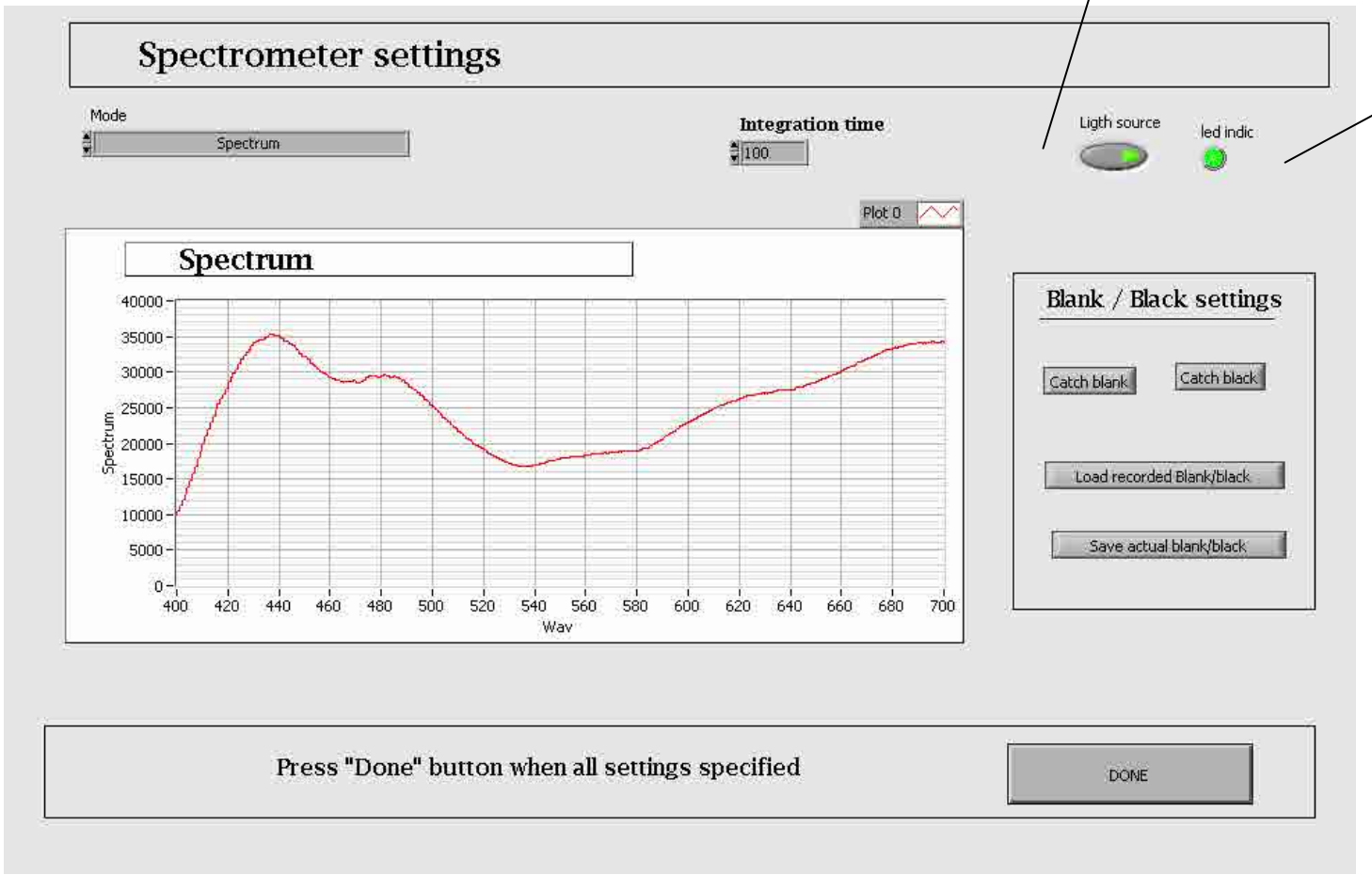
- Working volume : 500 ml – 2000 ml;
- Continuous spectral absorbency record (until 1.5);
- 149 ml of reactor for immobilized laccase or living biomass;
- Pump : 0-1600 ml/min;
- Conductivity, Temperature, pH, Oxygen logging;
- Temperature control by double jacket circuit (beaker and reactor);
- Software for an easy calibration and log of the equipment.
- **Also useful for enzyme catalyzed synthesis**

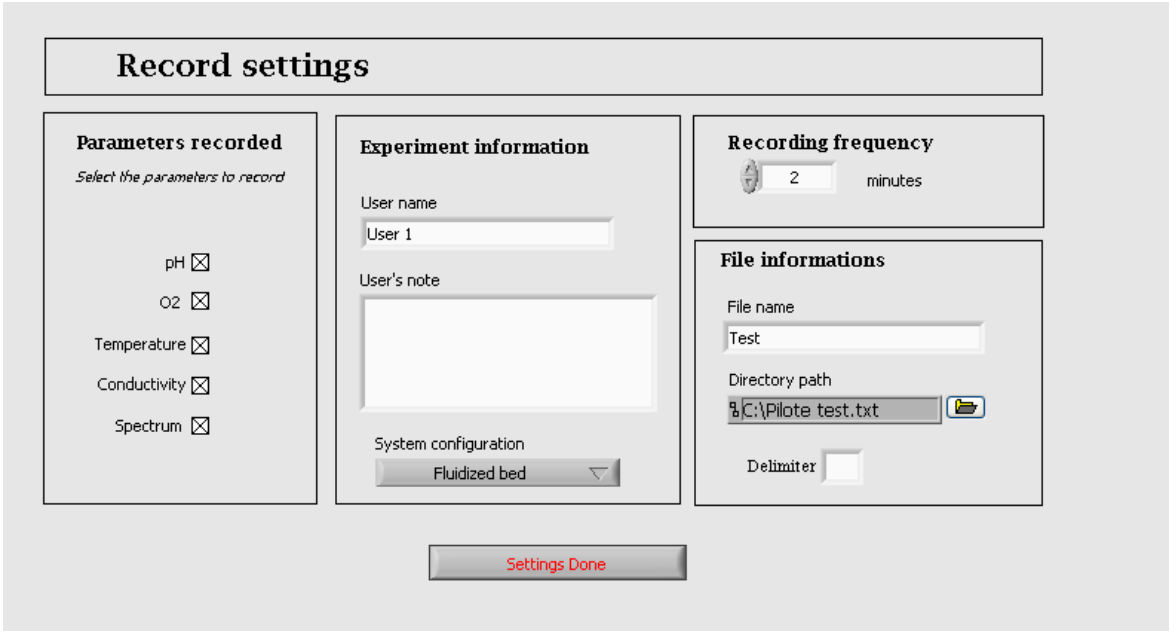

Dual position of the fitting
Pump control panel
Conductivity and pH panel transmitter
Column 149 ml with thermostated jacquet
Oxygen and conductivity sensor at the output of the cartbridge
Peristaltic pump with flow up to 1600 ml/min
Temperature and pH sensor in the beaker
Thermostated bath with output recirculation
Pressure switch preventing over-pressure damages
VIS Spectrophotometer
Tempered 2000 ml beaker

Software interface



Spectrophotometer calibration and settings



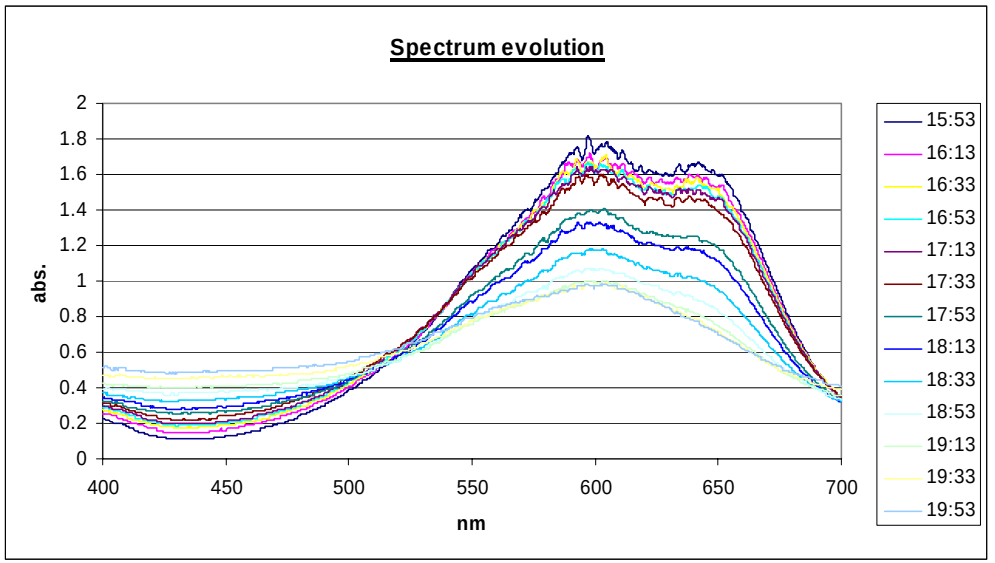


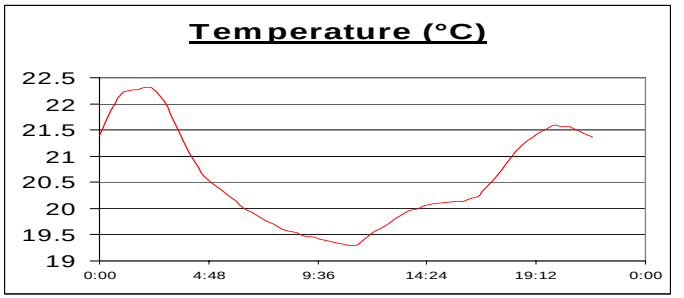
Easy data export …

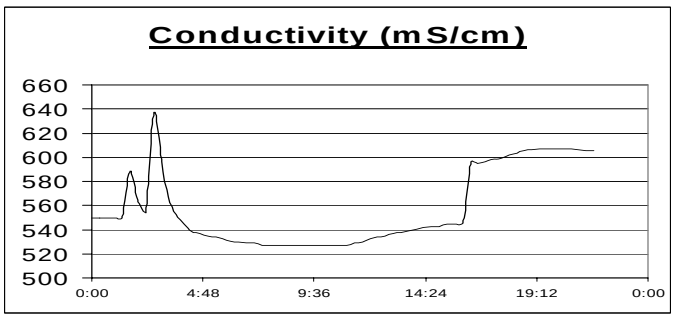
Trailer lab; ;
Xavier; ;
Lare one 1 gr/l speed 2; ;
11/03/2008;11/03/2008;11/03/2008
15:33;15:35;15:37
Fluidized bed;Fluidized bed;Fluidized bed
pH;3.99;4.38
O2 (mg/l);2.49;2.50
Temperature (°C);20.93;21.06
Conductivity (mS/cm);0.00;552.47
Spectrum; ;
nm; ;
345.74;NaN;NaN
345.95;NaN;NaN

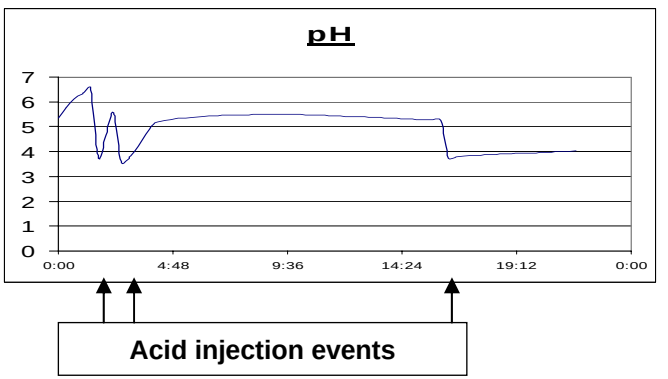
	A	B	C	D
1	Trailer			
2	Xavier			
3	Lar1 1 gr/l speed 2			
4	11/03/2008	11/03/2008	11/03/2008	
5	15:33	15:35	15:37	
6	Fluidized bed	Fluidized be	Fluidized bed	
7	pH	3.99	4.38	
8	O2 (mg/l)	2.49	2.5	
9	Temperature(	20.93	21.06	
10	Conductivity (	0	552.47	
11	Spectrum			
12	nm			
13	345.74	NaN	NaN	
14	345.95	NaN	NaN	

Typical results



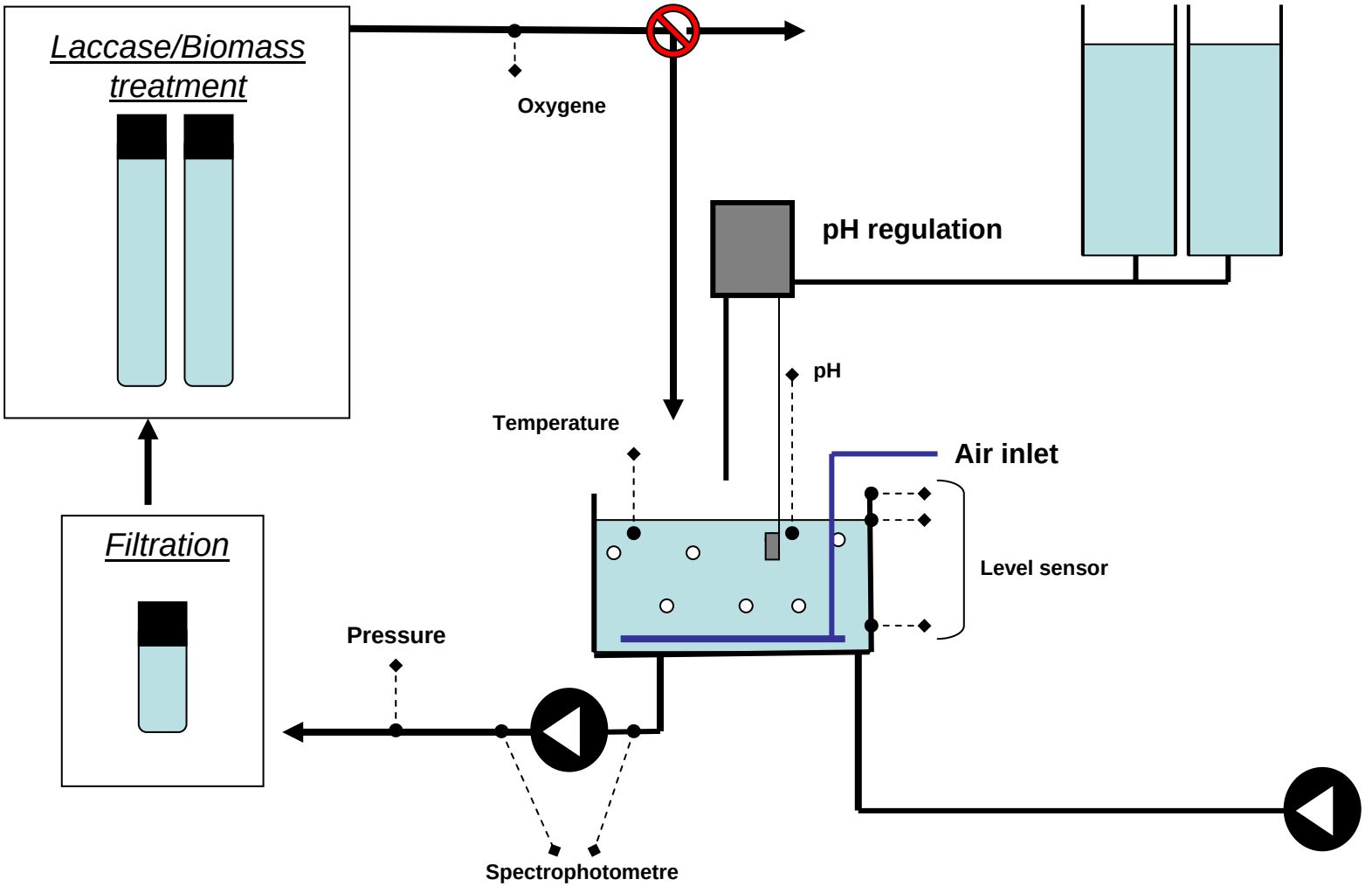


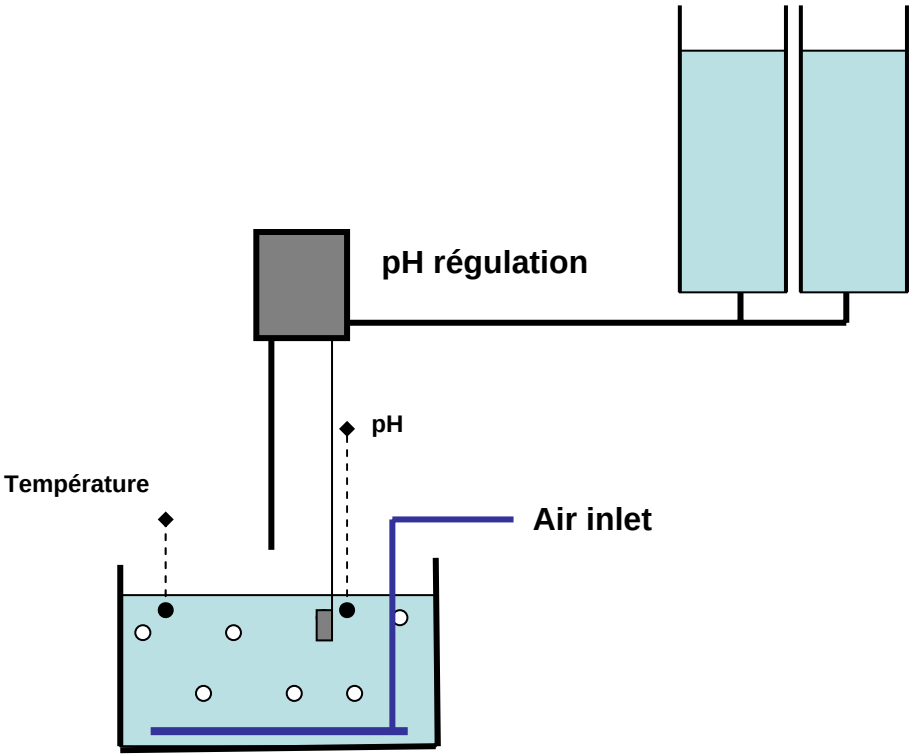


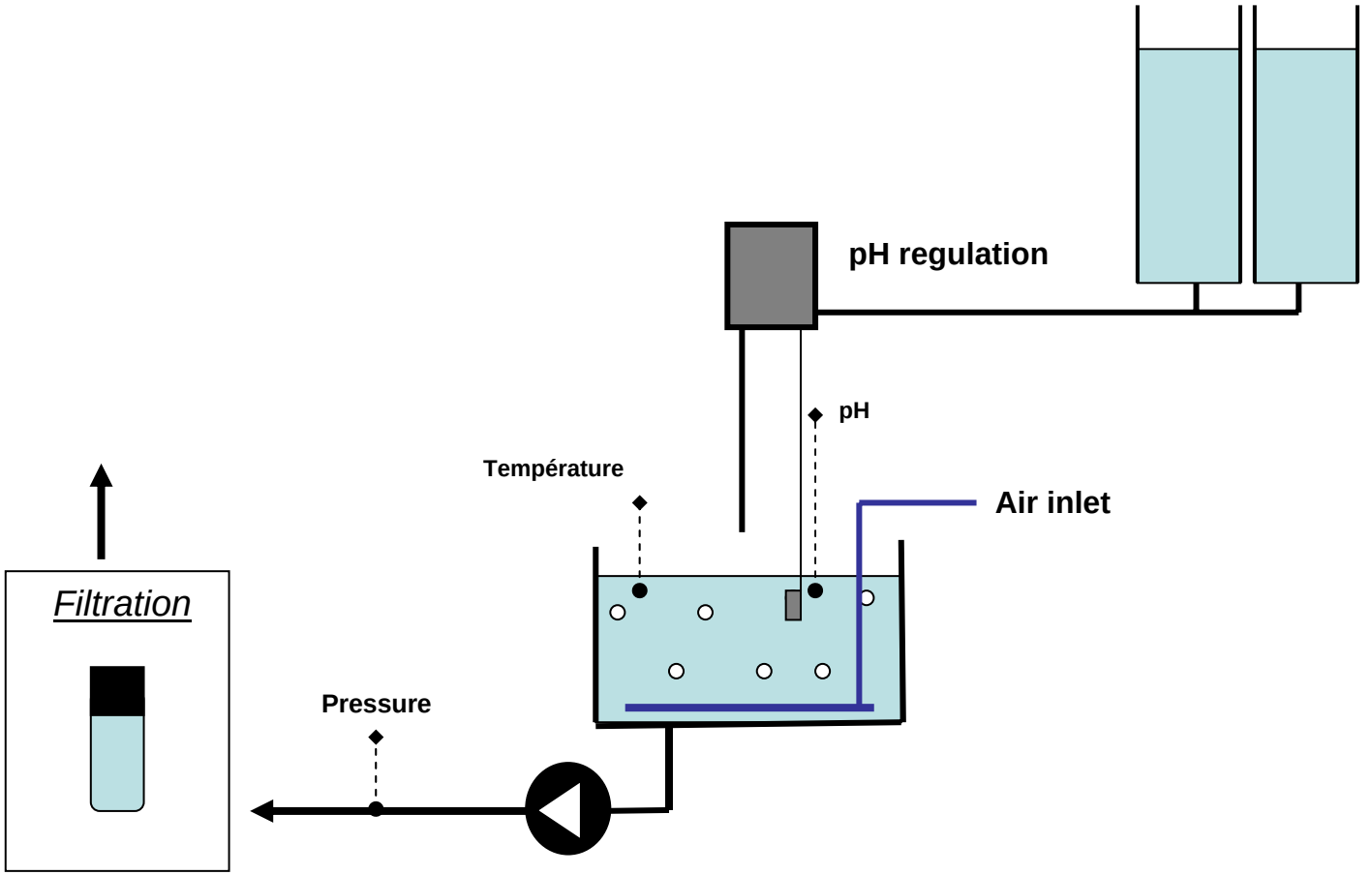


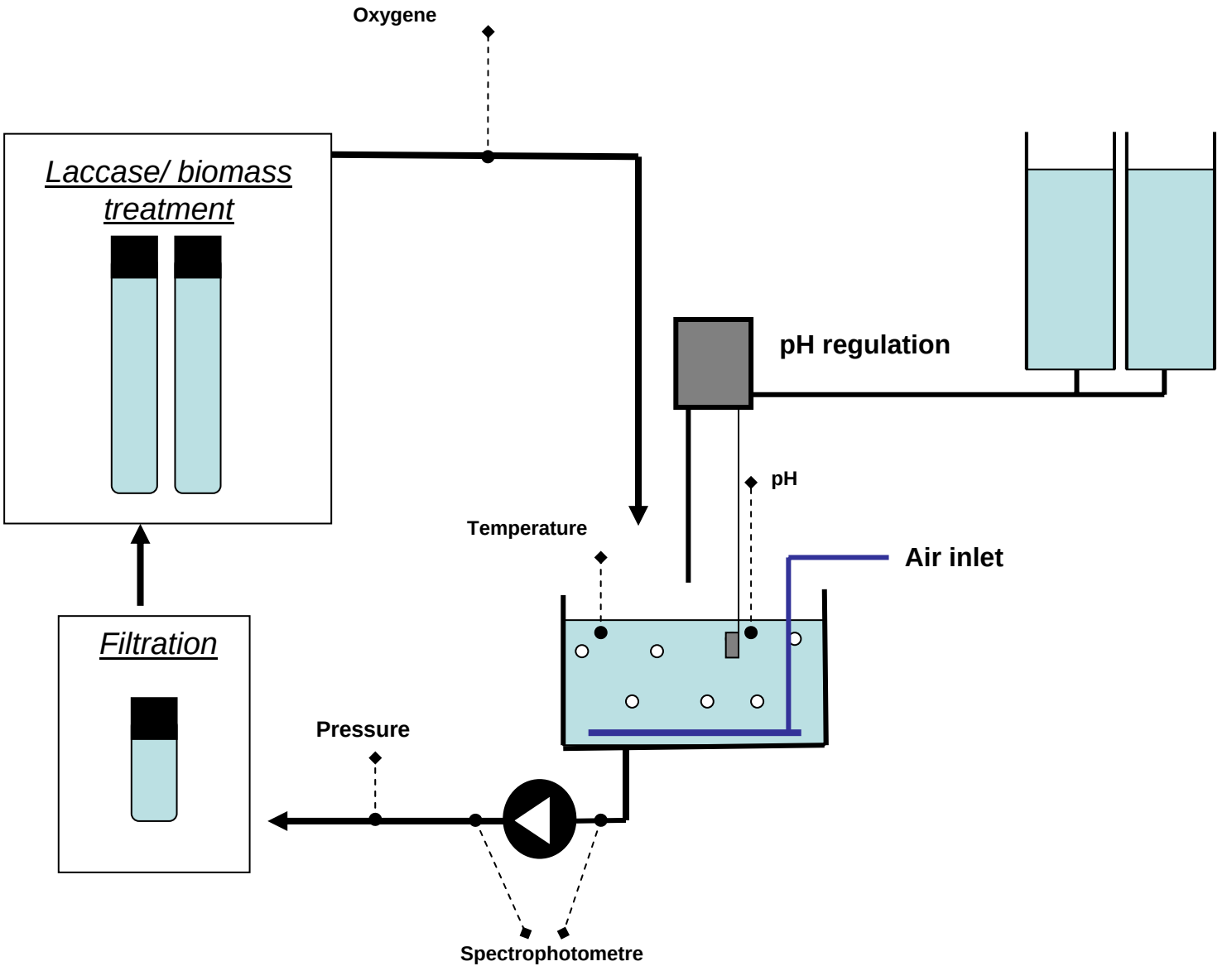
Prototype *pre*-industrial treatment system

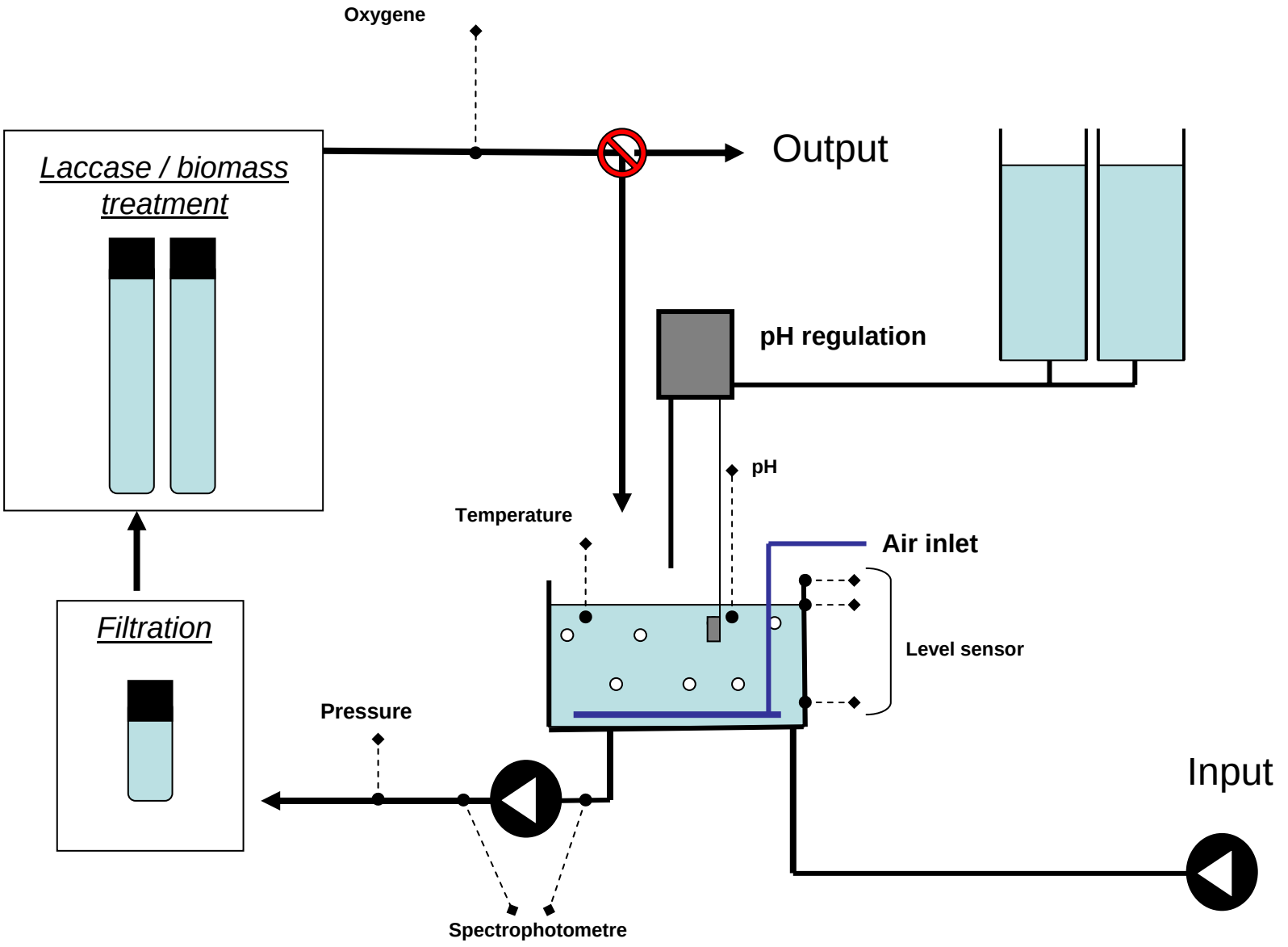
Equipment : global overview




pH régulation
pH
Température
Air inlet

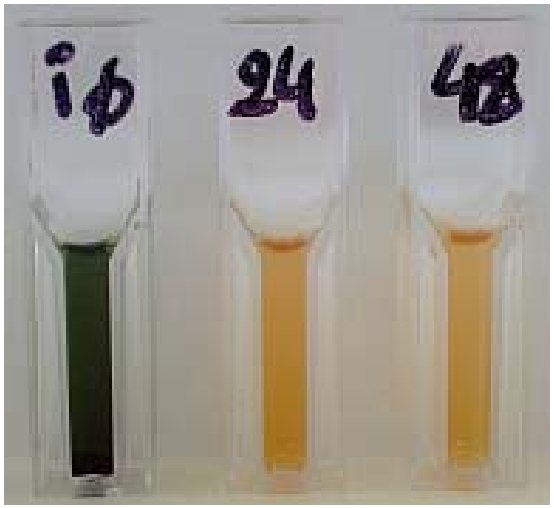

pH regulation
pH
Température
Air inlet
Filtration
Pressure


Oxygene
Laccase/ biomass treatment
pH regulation
pH
Temperature
Air inlet
Filtration
Pressure
Spectrophotometre


Oxygene
Laccase / biomass treatment
Output
pH regulation
pH
Temperature
Air inlet
Level sensor
Filtration
Pressure
Input
Spectrophotometre

Decolourization of model dye waste water

- Acid Dye bath for Wool, 50 U/g in cartridge
- Decolourisation after 24h and 48h continuous run
- TLC : disappearing of blue ABu62 after 24h, decrease of red AR266 and yellow AY49
- No decrease of immobilized Laccase activity after 48h ru
- DCO : decrease from 2050 mg O_2/l to 1670 mg O_2/l after 24h (81%)



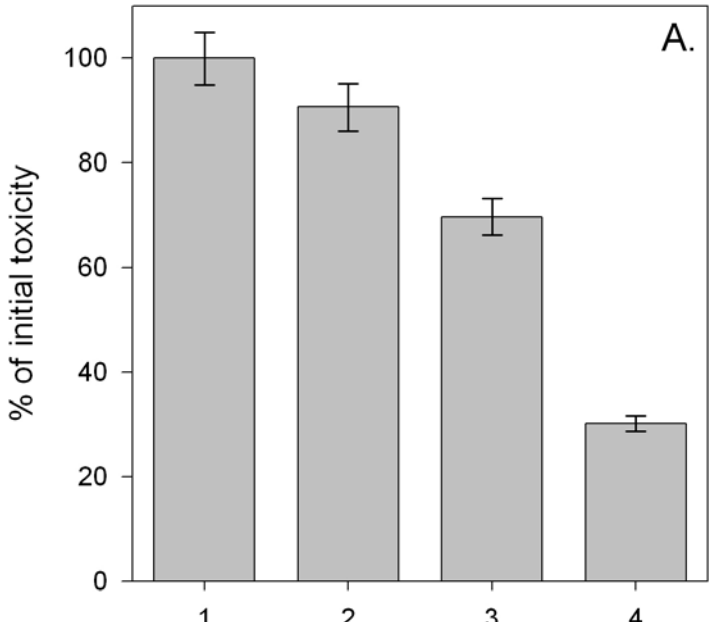
Percentage of initial cytotoxicity towards the Caco-2 human intestinal cell line

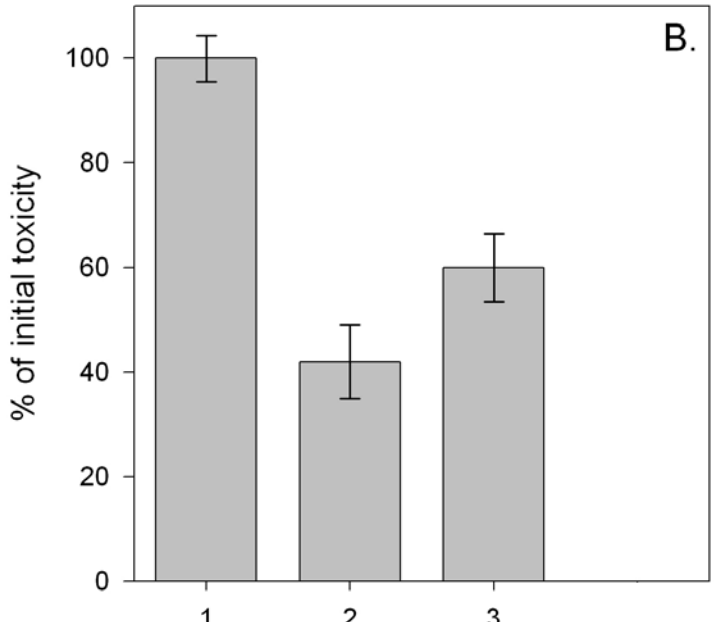
A. Whole cell

(1) crude effluent
(2) ozonised effluent
(3) crude effluent treated by WRF
(4) ozonised effluents treated by WRF

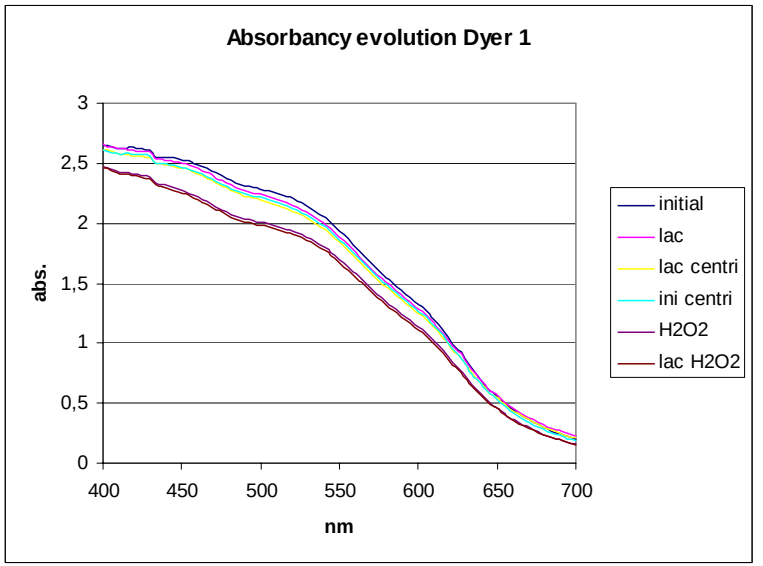
B. Enzymatic treatment

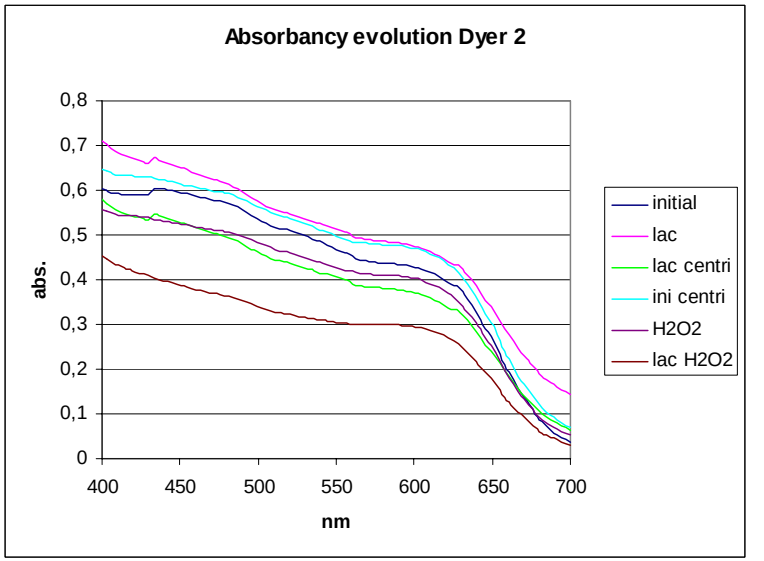
(1) crude effluent
(2) treatment with immobilized Laccase from Wetlands
(3) treatment with Laccase immobilized perlite

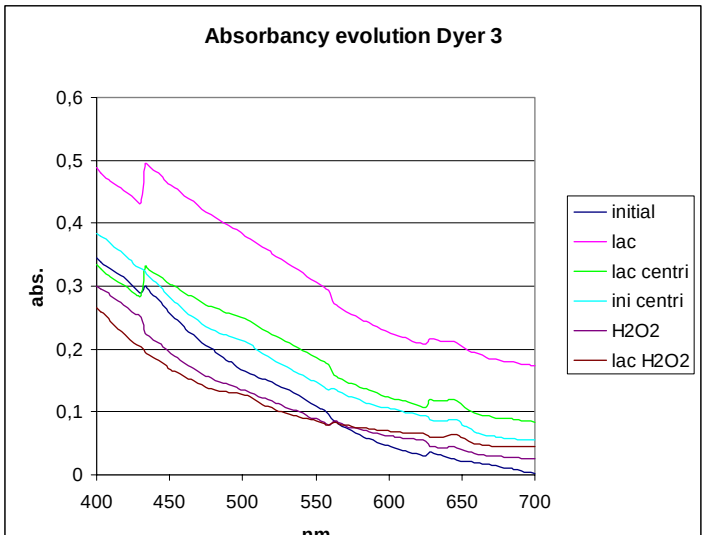




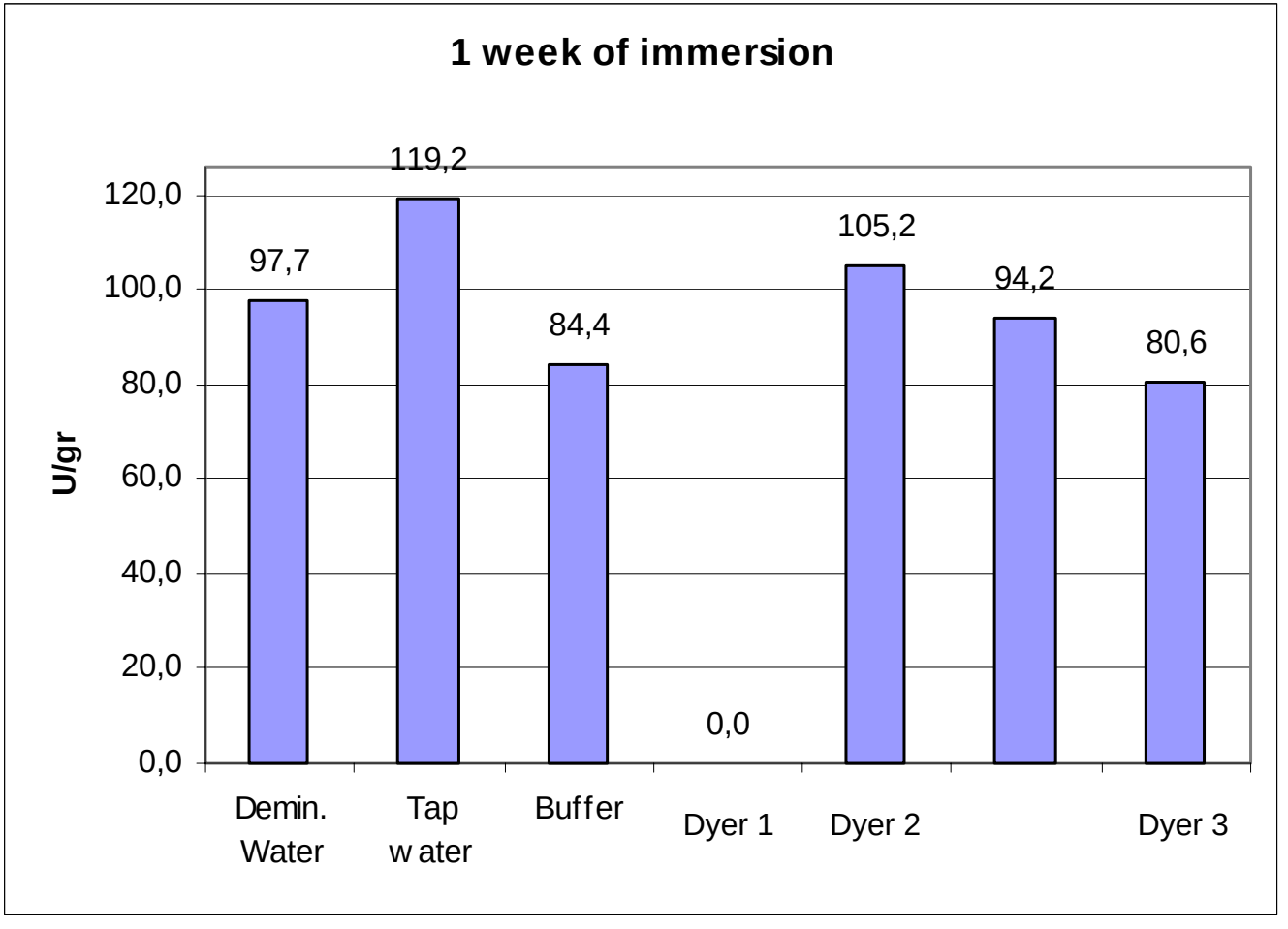
Decolourization of real Dyer waste water





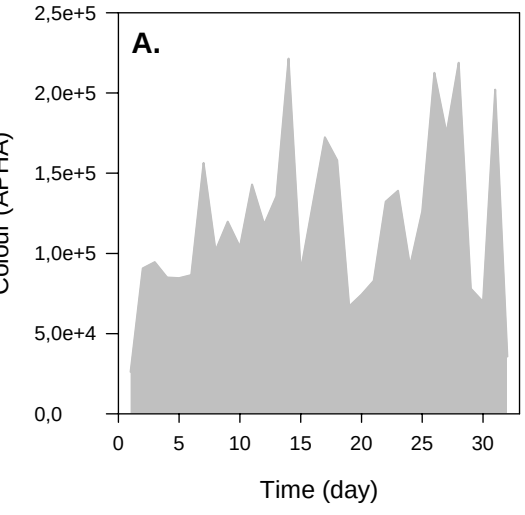


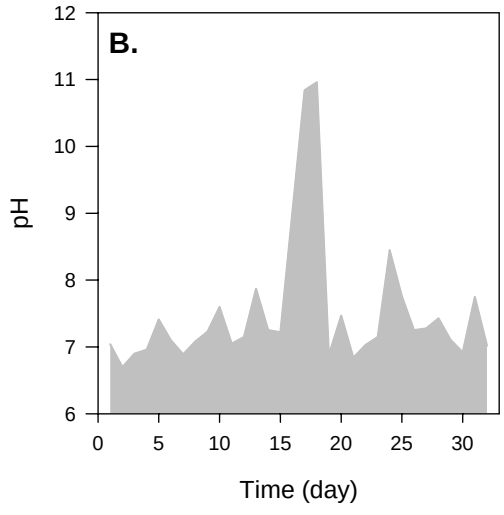
Remaining activity of immobilized Laccase after immersion in real Dyer waste water

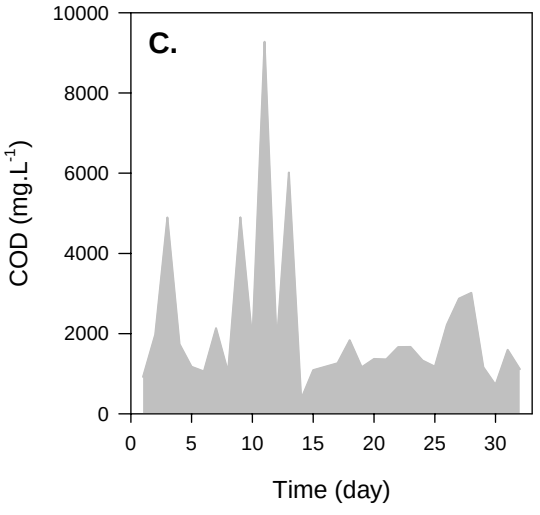


Characteristics of waste waters

Colour (APHA) pH Chemical Oxygen Demand







- Great variability of industrial waste water in time
- Buffer tank may be required to even out characteristics

Conclusions

- Laccase from WRF can be used for waste water treatment
  - Decolourization, detoxification, COD reduction
  - Some additives to dying process inactivate enzyme
    - Buffer tank?

- Fluidized bed reactors are most effective
  - High oxygen mass transfer rate required

- Non-porous support material is needed
  - Wetlands proprietary method for immobilization results in high activity bound per gram with high yield

- Reactors for waste water treatment can also be used for enzyme-catalyzed synthesis


Thank You!