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Exploring enzymatic behaviour in xylo- and phytophagous insects consortia. What could be learned from Nature ?

Claire Dumas, Lucile Chatellard, Amandine Gales, Adèle Lazuka, Lucas Auer, Guillermina Hernandez Raquet, Hélène Carrère, Jean-Philippe Steyer, Jean-Jacques Godon, Michael O'Donohue

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HAL Authorization

Exploring enzymatic behaviour of xylo- and
phytophage insects microbiota.
What could be learned from Nature ?

Claire Dumas,

*Lucile Chatellard, Amandine Gales ,
Adèle Lazuka, Lucas Auer, Guillermina
Hernandez-Raquet,*

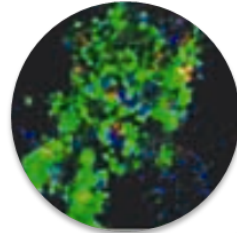
*Hélène Carrère, Jean-Philippe Steyer
Jean-Jacques Godon, Michael O'Donohue,*



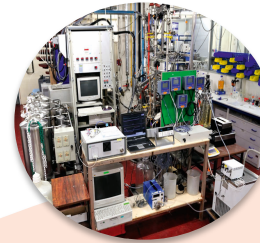
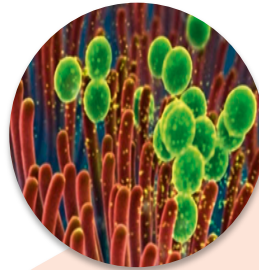
PARTNERS



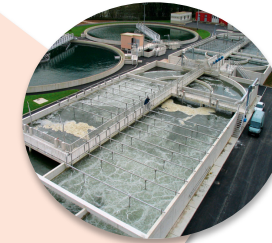
MICRO-ORGANISMS



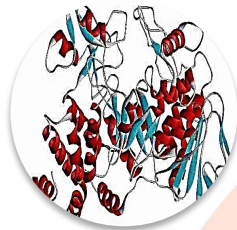
BIOFILMS, FLOCS,
GRANULES



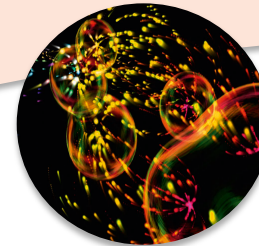
PILOTS



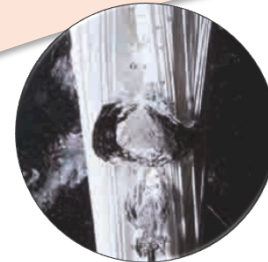
APPLICATIONS



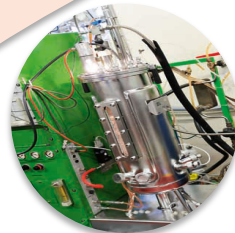
BIOCATALYSIS



BUBBLES
DROPS
PARTICLES



MEMBRANES

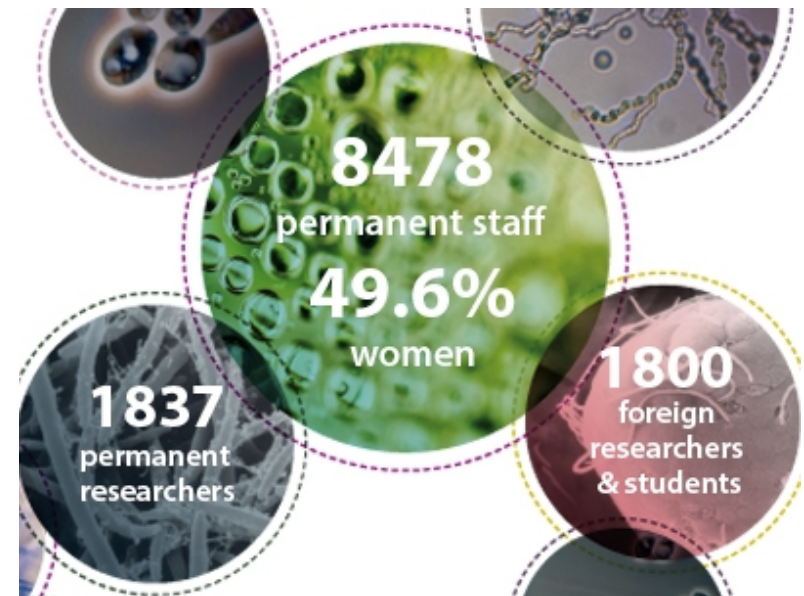
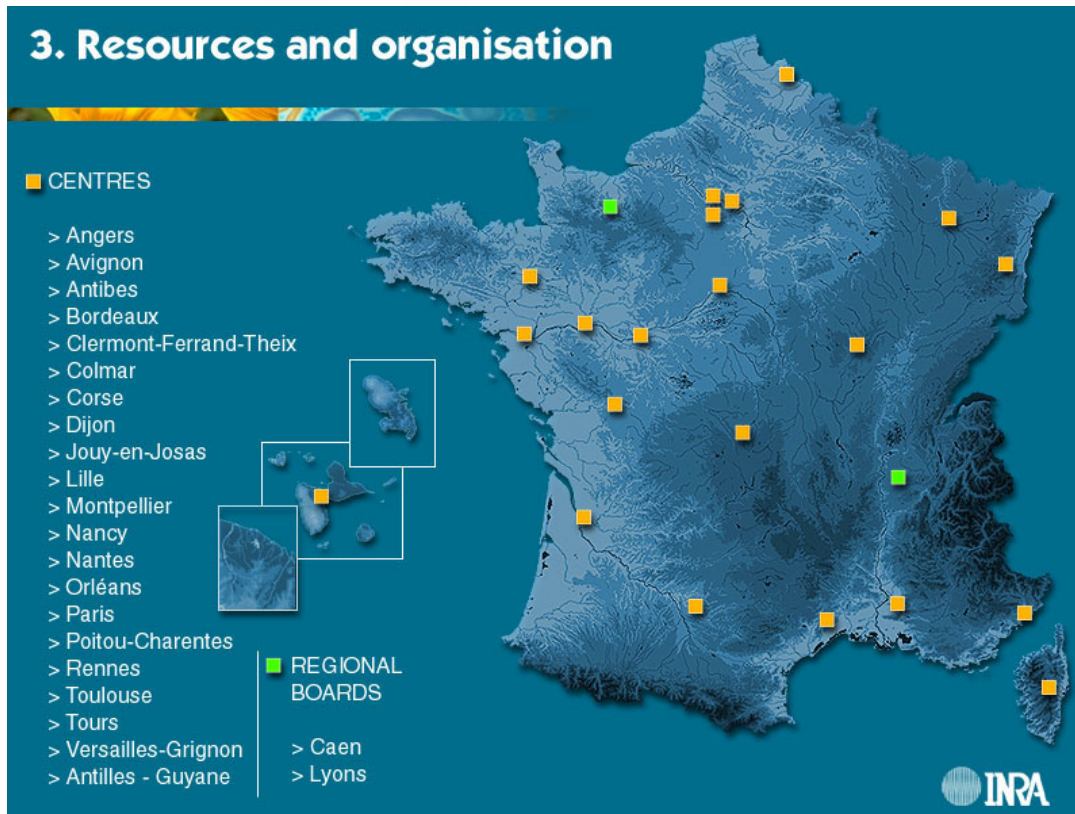


CHEMICAL
CATALYSIS,
NANOPARTICLES

Fields of Application

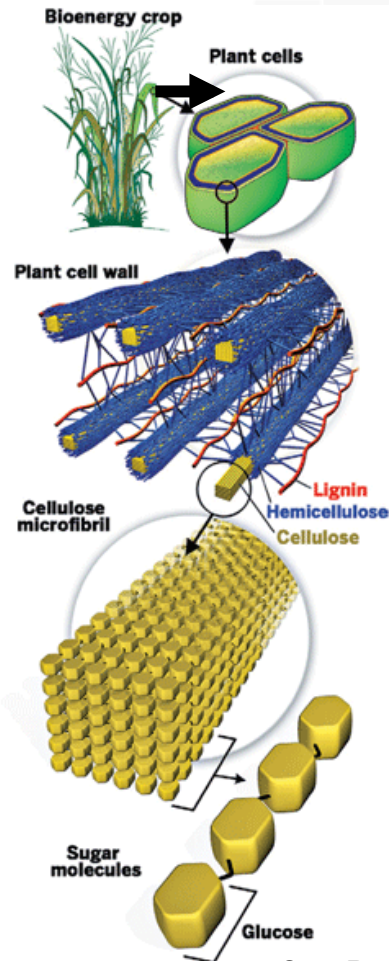
- Agriculture and food industries
- Water and environment
- White biotechnology
- Pharmaceuticals and cosmetics
- (Bio)chemical engineering

The French National Institute for Agricultural Research

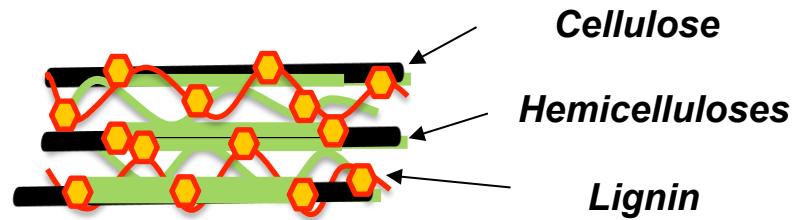


CONTEXT

Lignocellulose (LC)



- Complex chemical structure → difficult biological degradation

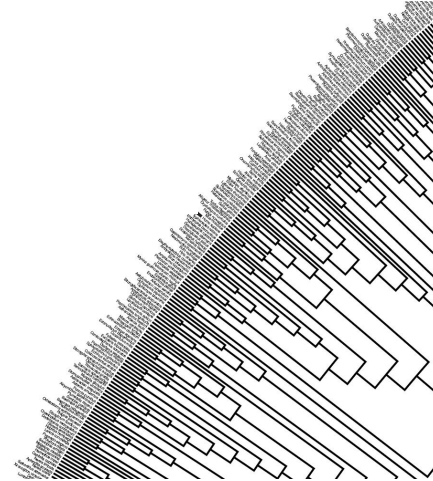


Deconstruction
=
Major technological challenge

Sun, R.-C.: Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels
Guo XM, Trably E, Latrille E, Carrere H, Steyer JP. 2010. IJHE. 35(19): 10660-10673.

CONTEXT

Microbial consortia



Many advantages

- High genetic diversity and metabolic versatility
- Large range of enzymatic activities
- High stability and robustness in a broad range of conditions
- Non-sterile operating environments
- Metabolism can be directed towards the production of carboxylates thank to operating conditions
- Attractive industrial tools that could be cost competitive for bioconversion of LC



CONTEXT

Microbial consortia habitats

Environmental sample degrading raw material



Rumen



Soil or compost

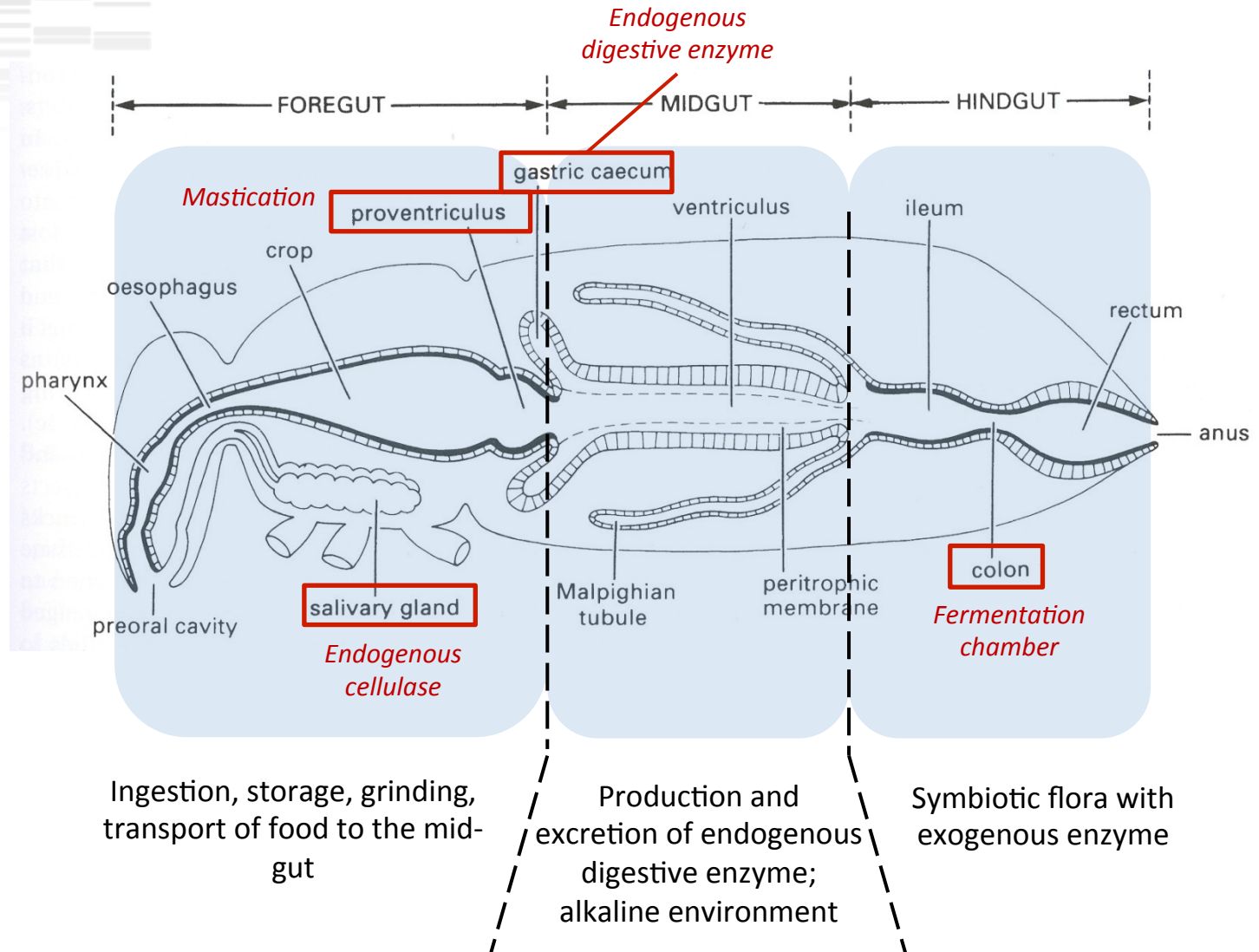


**Phyto-
Xylophage
Hindgut**

INSECT GUTS STRUCTURE



Mandibles:
Mastication organ
Enhance access
of cellulolytic enzyme
to cellulose

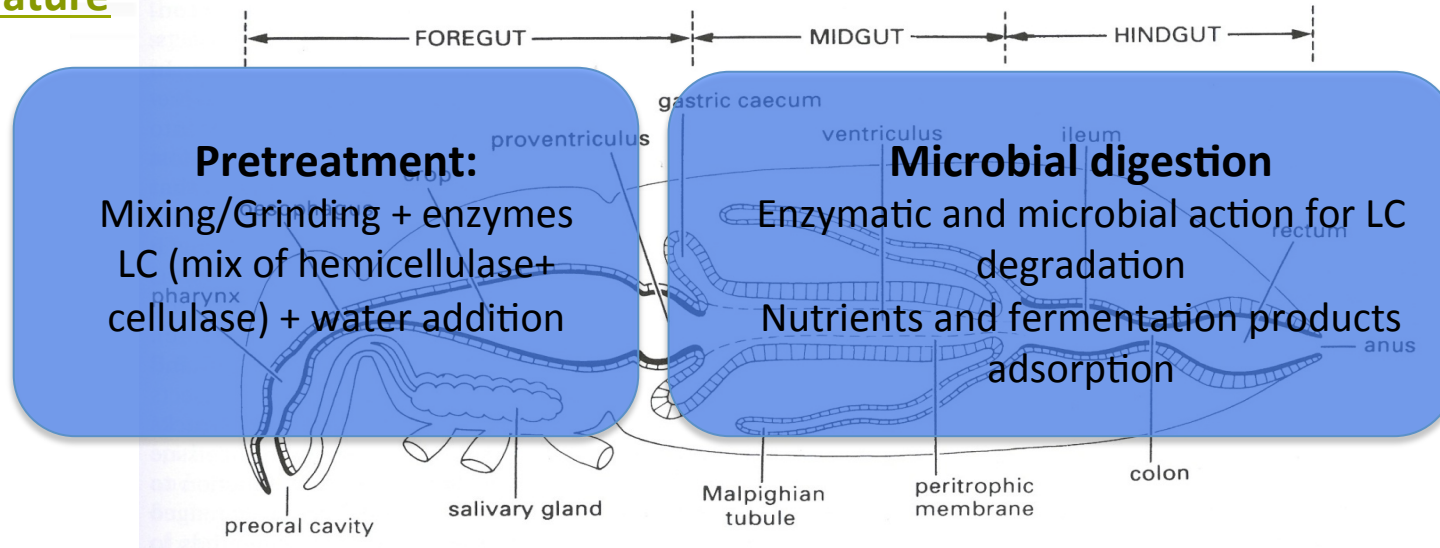


Gullan P.J., et al, 2010; Sun, J.-Z., et al, 2010; Huang, S.-W., et al, 2010

C.DUMAS Exploring enzymatic behaviour of xylo- and phytophagous insects microbiota.

BIOMIMICRY

The Nature



Bioprocesses

*Wheat straw
milled*

*Fermentation reactors
Insect guts + Wheat straw*

Godon, J.-J., Arcemisp  h  re, L., Escudi  , R., Harmand, J., Miambi, E., Steyer, J.-P.: BioEnergy Res. 6, 1063–1081 (2013).

OUR PROJECT

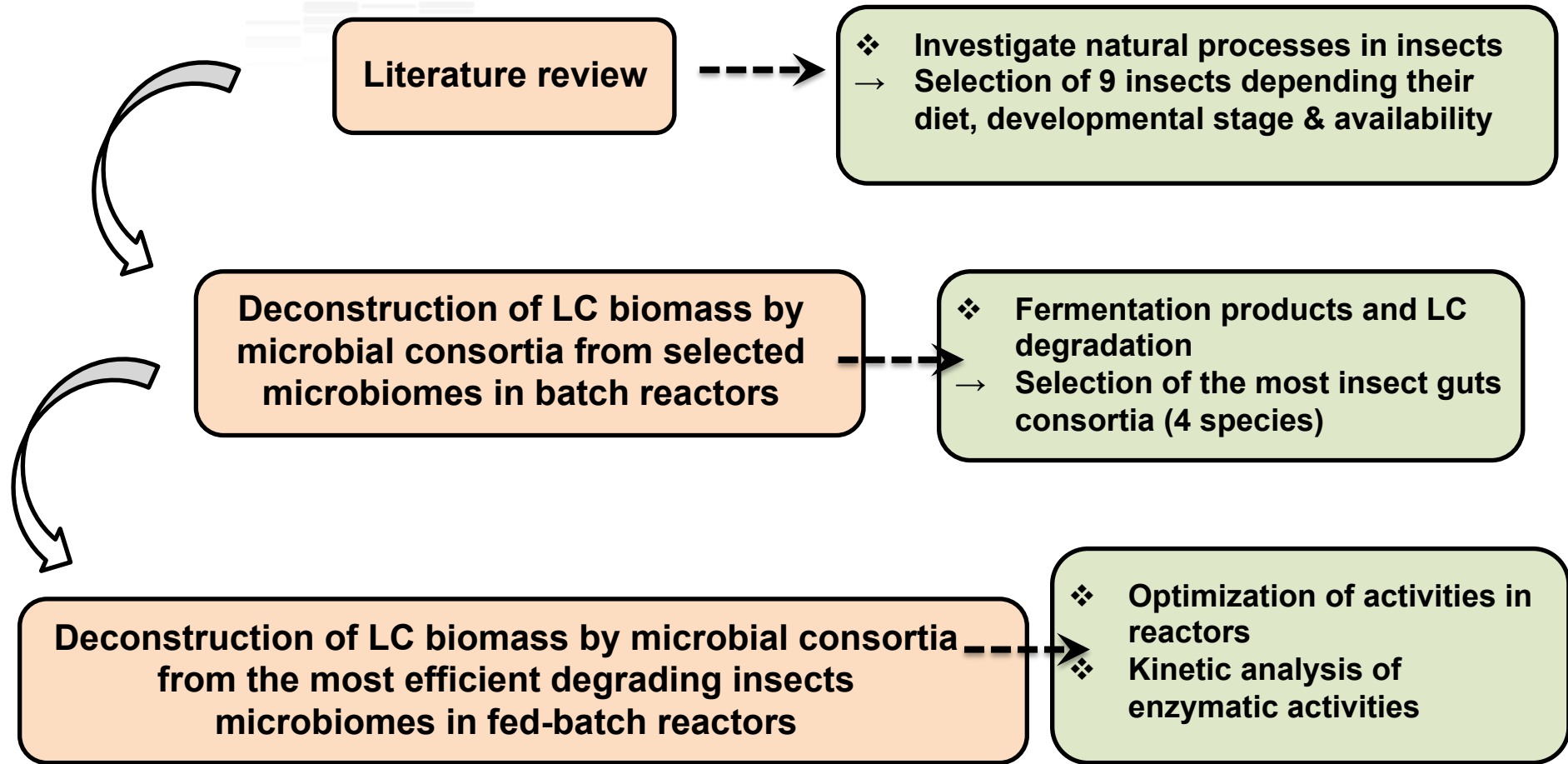
Challenges of the project

- ✓ **Manage to implement microbial consortia from insect guts in reactors**
- ✓ **Manage to degrade LC biomass and produce metabolites**
- ✓ **Ensure the maintenance of microbial consortia and the propagation of activities in reactors**

Goals

- ✓ **Select the most efficient microbiomes**
- ✓ **Quantify and characterize the degradation performances of insects guts**
- ✓ **Identify enzymatic activities expressed during biomass deconstruction**
- ✓ **Characterize the functional diversity that express this enzymatic activities**

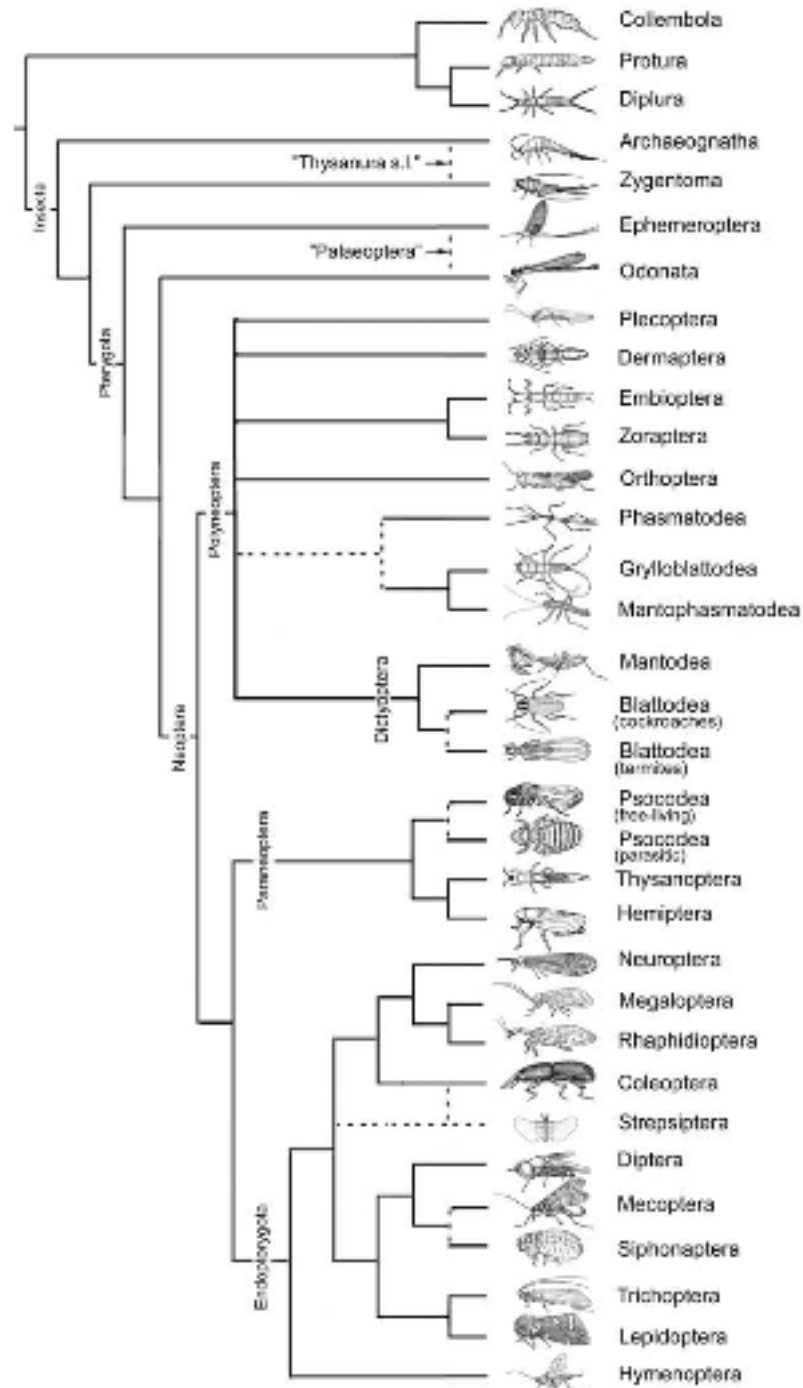
PLAN OF THE STUDY



Choice of insects

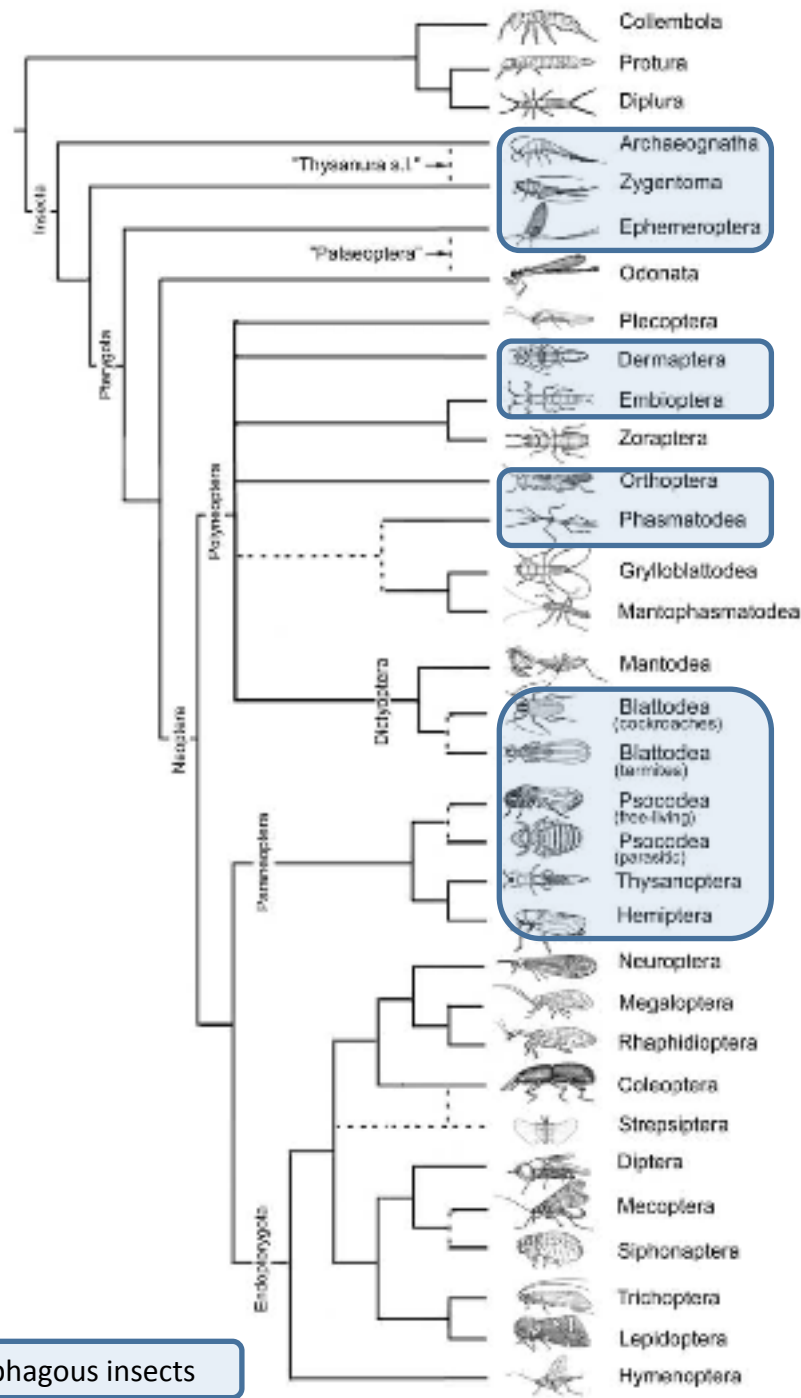
Insects of interest

- 33 orders



Insects of interest

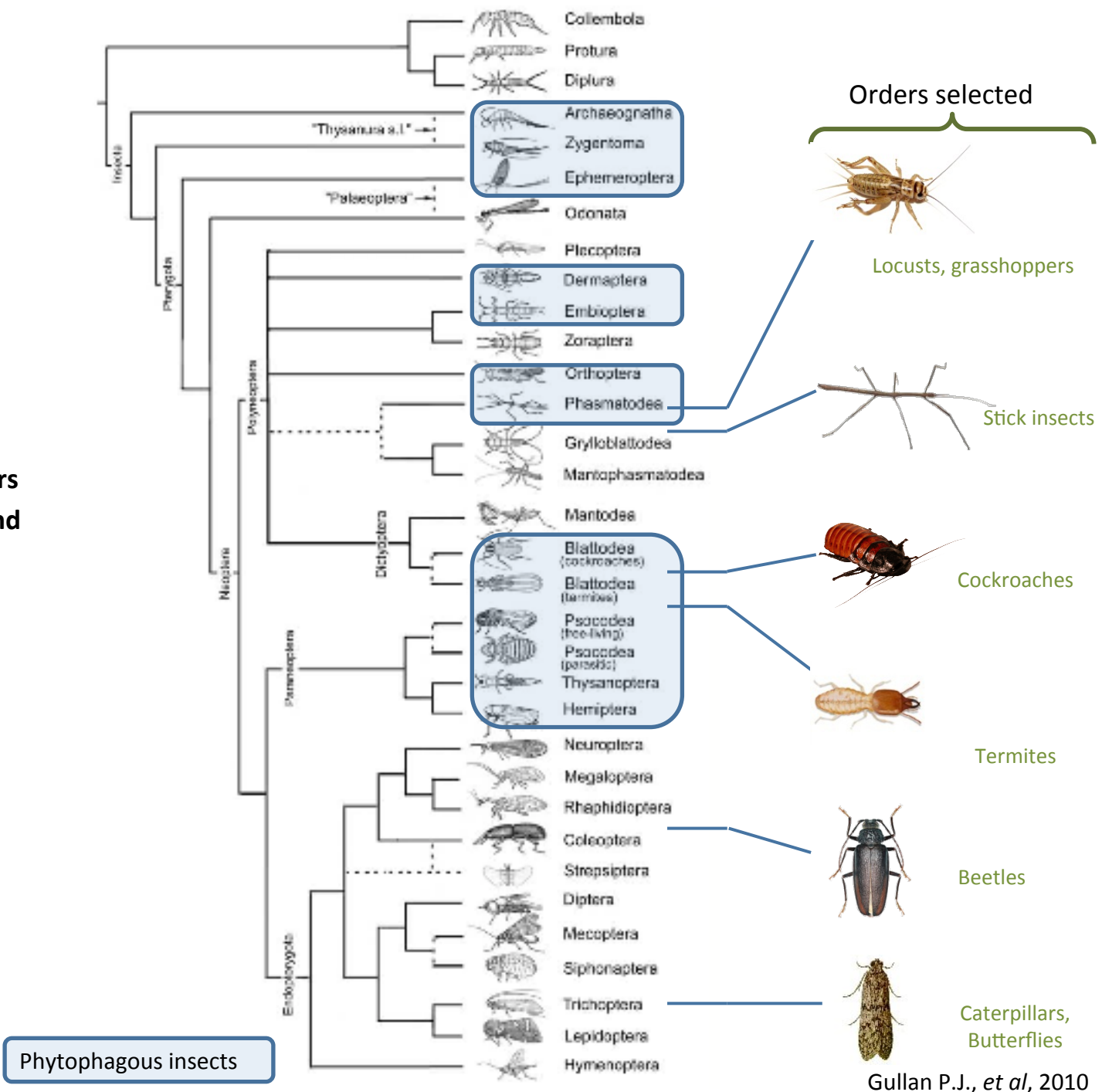
- 33 orders
- **18 orders phytophagous**



Phytophagous insects

Insects of interest

- 33 orders
- 18 orders phytophagous
- **6 interesting orders**
- **9 species at the end**

















Gullan P.J., et al, 2010

METHODS

Selected insects from literature review



Cockroaches		Beetles					
<i>Gromphadorrina portentosa</i> (Blaberidae) Madagascar giant hissing cockroach		<i>Ergates faber</i> (Cerambycidae): Long-horned beetle		<i>Potosia cuprea</i> (cetoniidae) : Copper Chafer		<i>Propomacrus bimucronatus</i> (Euchiridae)	
							
Locusts, grasshoppers			Caterpillars, Butterflies		Stick insects		
<i>Gryllus bimaculatus</i> (Gryllidae) : Domestic cricket		<i>Locusta migratoria</i> (Acrididae) : Migratory locust		<i>Cossus cossus</i> (Cossidae) :		<i>Carausius morosus</i> (Diapheromeridae) :	
							



Termites

Higher termites,
Not fungi eater
Not protozoa

Termes hospes,
Microcerotermes parvus,
Nasutitermes ephratae,
Nasutitermes lujae

Fermentation in batch reactors

METHODS

Preparation of insects guts



INSECTS

Insects anaesthetic
Under CO₂ flow



Surface sterilization
Ethanol 70%



Dissection & extraction
of insects guts



Extracted guts weighing



Mixing/cutting in minimum media pH 7 + preparation
of 40g/L insect guts



TERMITES

Termites anaesthetic
On ice



Aseptic experimentations



Dissection & extraction
of termite guts



Freezing at -80°C



Vortex homogenizing in growth media, ~ 890 mg of
guts, pH 6.15

METHODS

Preparation of batch reactors



INSECTS



TERMITES

Substrate-fed reactors

Blank reactors
(without substrate)

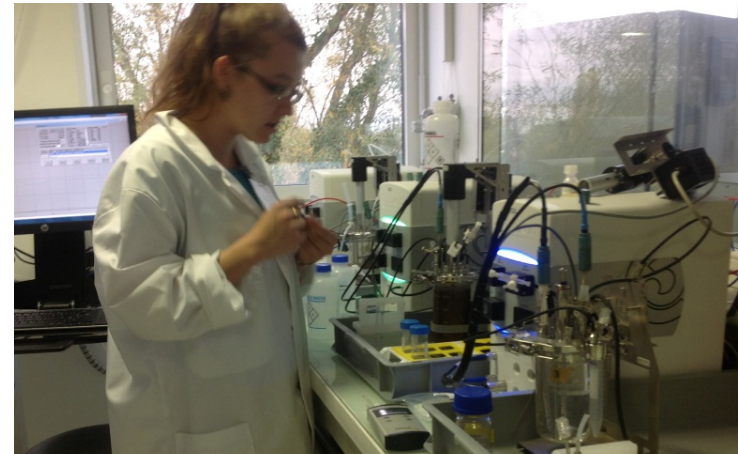
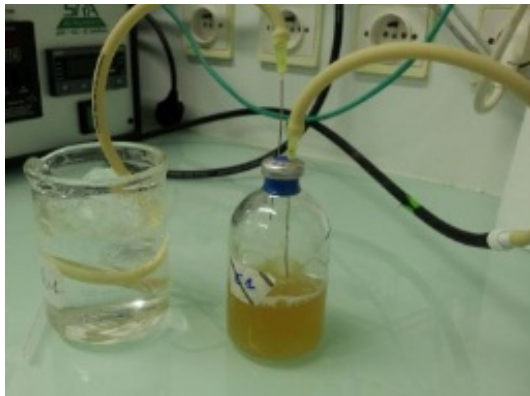
Guts residues

Mesophilic
Anaerobic

Wheat straw as sole carbon source

Substrate - fed reactors

Blank reactors
(without inoculum)



METHODS: INSECTS

Biochemical and microbiological analysis

Quantification and analysis of structural carbohydrates (FTIR, HPLC)		Quantification and analysis of structural carbohydrates (FTIR, HPLC)
Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)
Microbial communities		Microbial communities
Production of biogas	Production of biogas	Production of biogas
Sequencing + enzymatic activities		Sequencing + enzymatic activities

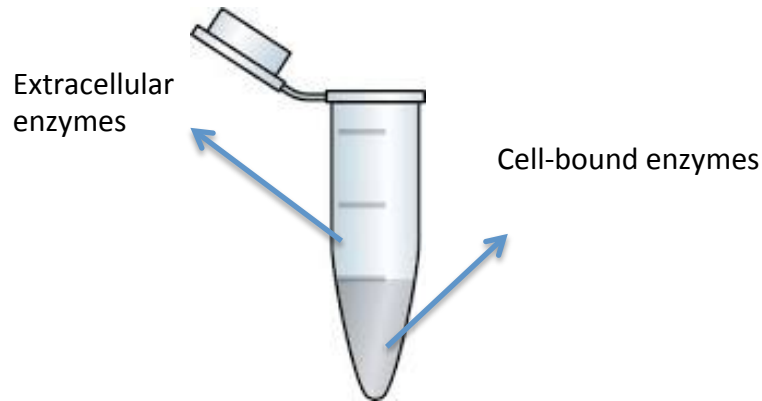


METHODS: INSECTS

Enzymatic activities and microbiological analysis

Specific enzymatic activities

Centrifugation/ separation
Analyses on solid and liquid part of the samples



Cellulase

Endoglucanase: CMC (Na)
Exoglucanase: Avicel
Beta-glucosidase: Cellobiose

Hemicellulase

Xylanase: xylan birchwood

Secondary activities

Xylosidase
Arabinase
Galactanase
Estrase
...

Chromogenic substrates

Microbiological analysis

Analysis of diversity of populations

- Single-Strand Conformation Polymorphism (SSCP)
- Illumina sequencing

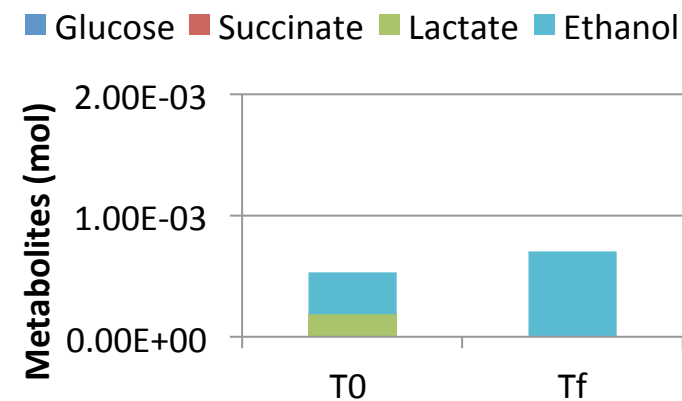
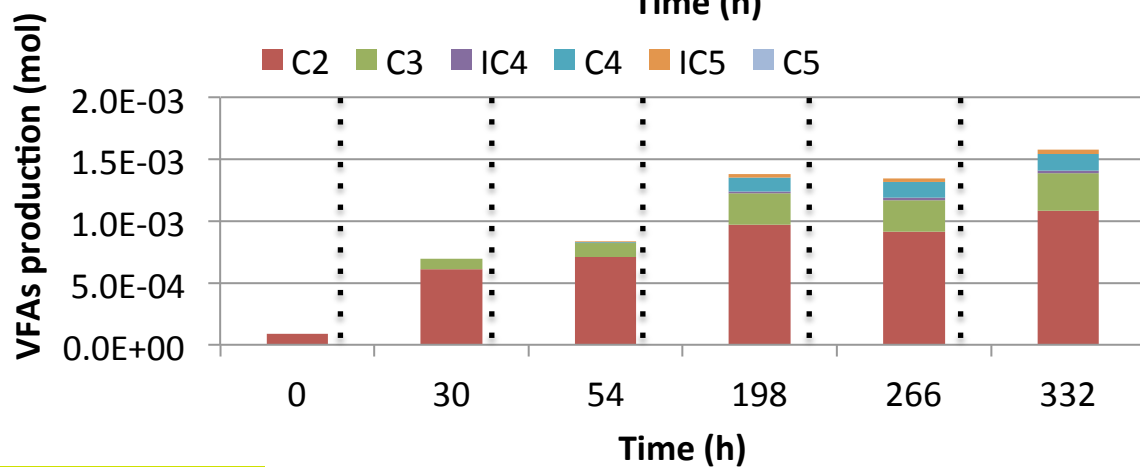
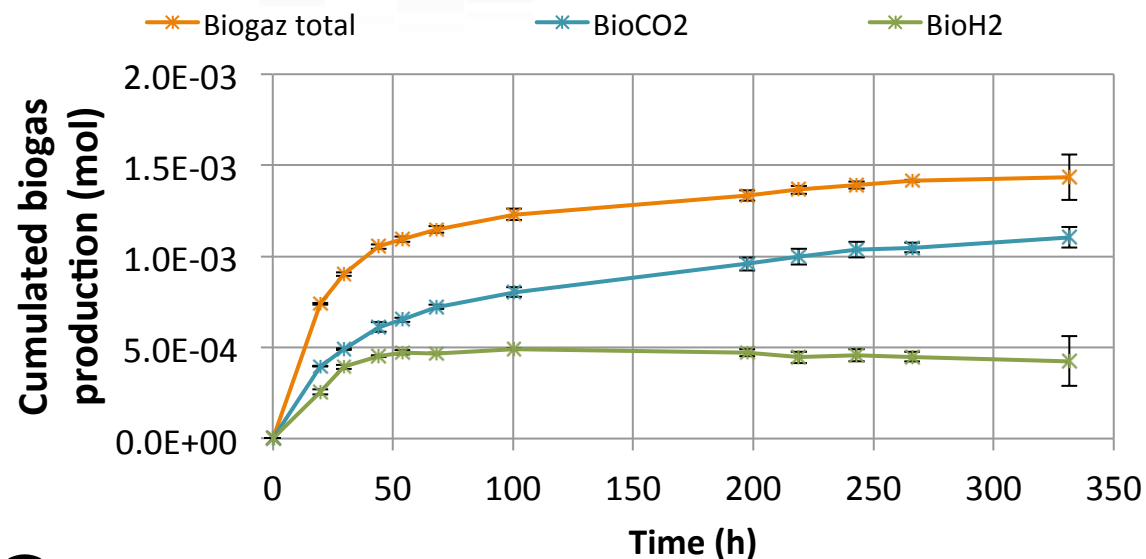


02

Results

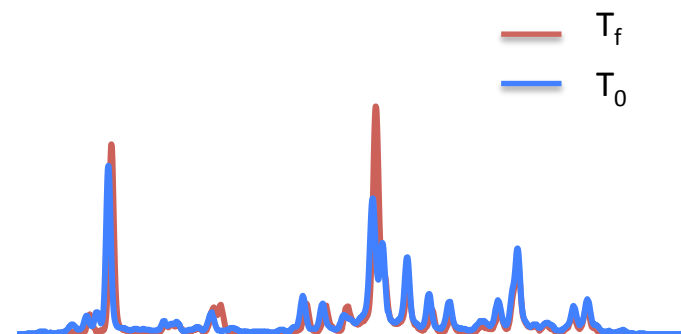
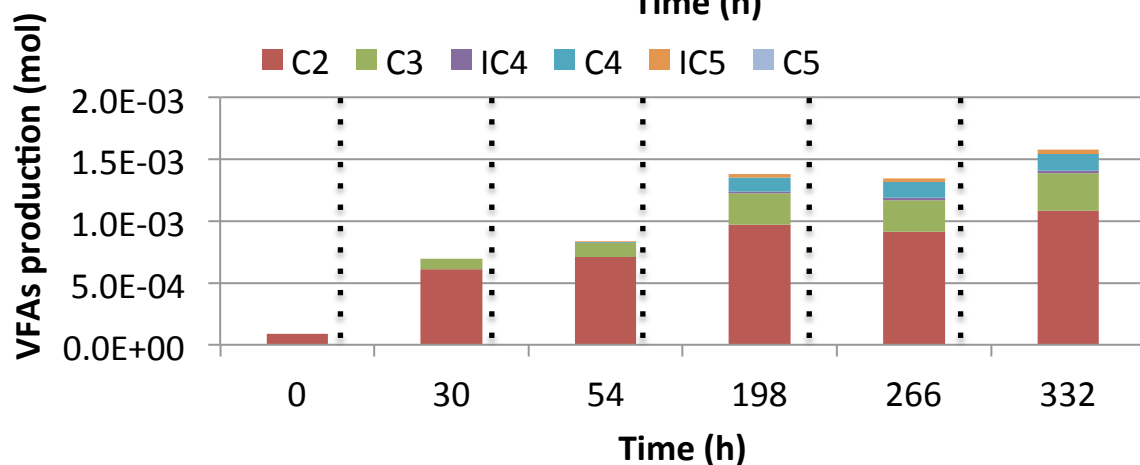
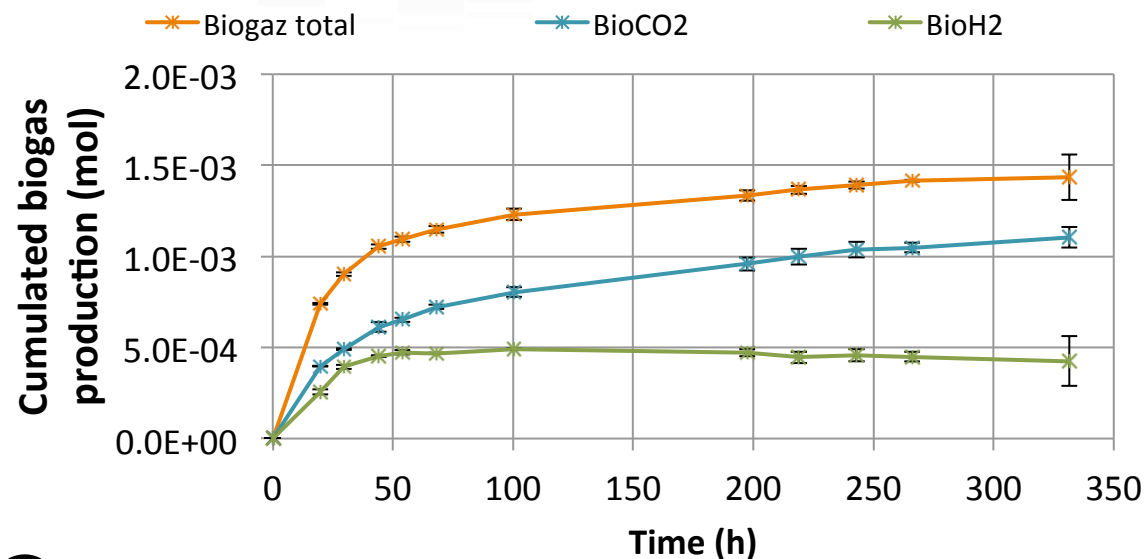
RESULTS

1ST Batch : *Gromphadorrina portentosa* case



RESULTS

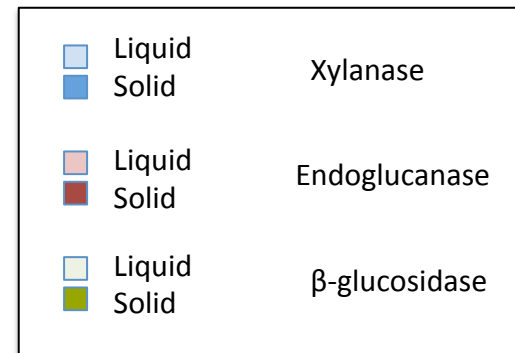
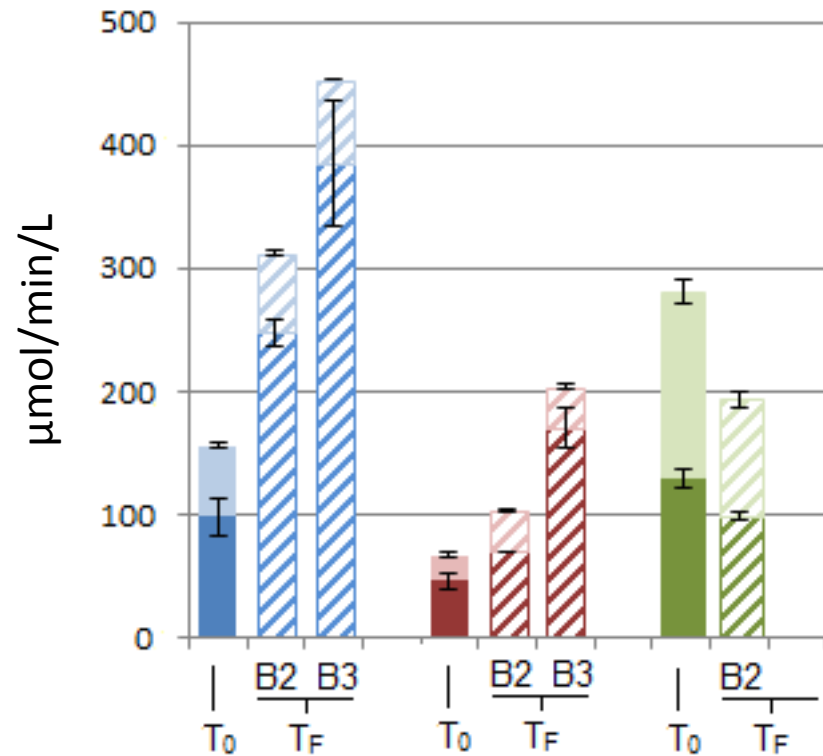
1ST Batch : *Gromphadorrina portentosa* case



- Small variation of microbial communities
 - Growth
- Degradation of LC due to initial communities

RESULTS– Enzymatic activities

1ST Batch : *Gromphadorrina portentosa* case



Higher activity of cell- bound enzymes

RESULTS– all insects



Products (mmol)	<i>Gryllus</i>	<i>Locusta</i>	<i>Ergates</i>	<i>Potosia</i>	<i>Propomacuscus</i>	<i>Gromphadorrina</i>	<i>Cossus</i>
Total biogas total	1.4	2.2	1.7	0.9	0.5	1.4	1.2
BioH ₂	0.4	0.8	0.7	/	0.2	0.5	0.4
Total VFAs	1.3	1.5	0.7	1.7	0.7	1.6	0.6
Lactate	/	0.1	/	/	n.d	/	n.d
Ethanol	0.7	0.2	/	/	n.d	0.7	n.d

High production of VFAs

Optimization

OPTIMIZATION – In situ-pH for biomimicry



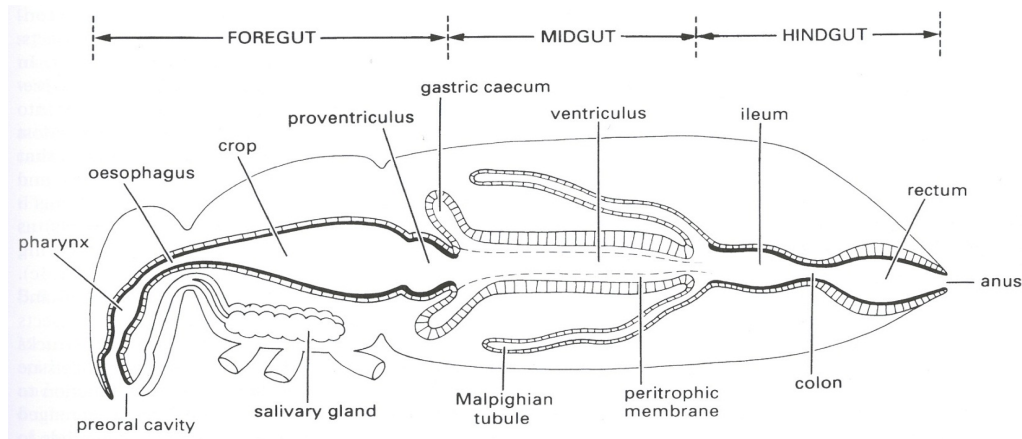
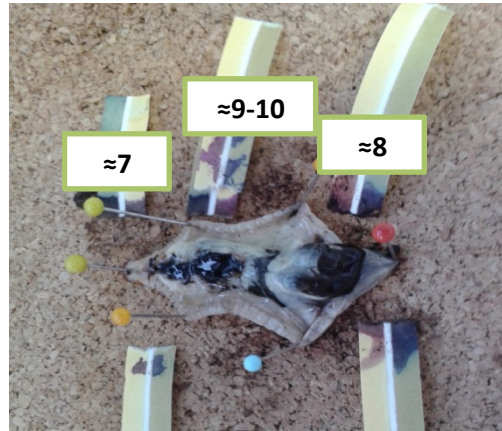
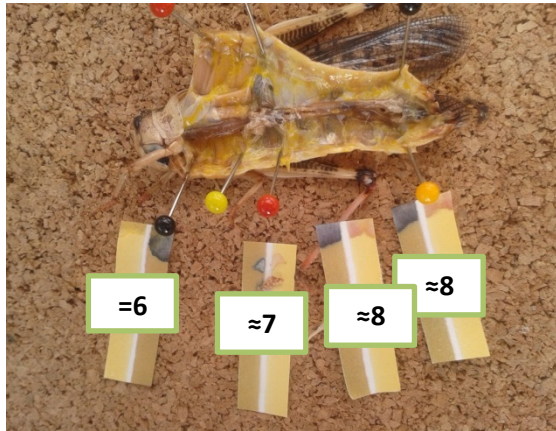
Locusta migratoria



Potosia cuprea



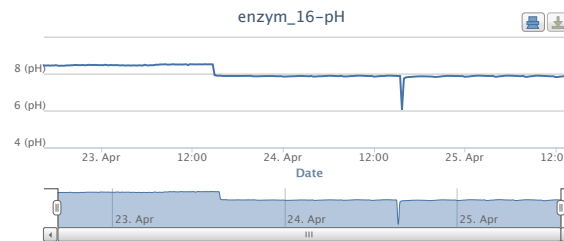
Gromphadorrina portentosa



OPTIMIZATION – controlled pH, normalized microbial biomass quantity



- Monitored and controlled pH



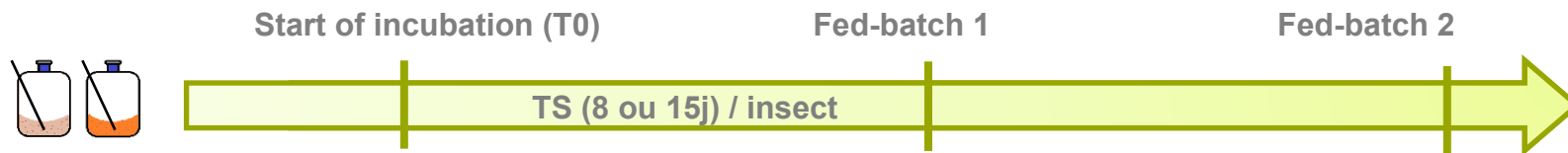
pH=8
 Wheat straw as sole carbon source
 Normalized quantity of biomass (16S copy)



Species	g of guts	insects	DNA 16S copies
<i>Locusta migratoria</i>	1	7	1.10 ⁺⁸
<i>Potosia cuprea</i>	1	0.6	5.5.10 ⁺⁹
<i>Gromphadorrhina portentosa</i>	1	1	6.10 ⁺⁸

METHODS– biochemical & microbiological analysis

Quantification and analysis of structural carbohydrates (FTIR, HPLC)		Quantification and analysis of structural carbohydrates (FTIR, HPLC)
Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)
Microbial communities	Work in progress	Microbial communities
Production of biogas	Production of biogas	Production of biogas
Sequencing + enzymatic activities	Sequencing + enzymatic activities	Sequencing + enzymatic activities





03

Conclusions & prospects

Conclusions and prospects

Conclusions

- ❖ Succeed in realising batch reactors implemented with insect microbiomes
Production of biogas, VFAs and metabolites in few hours
- ❖ Differences between insects
- ❖ Maintenance of microbial consortia

Prospects

- ❖ Check and optimized performances in fed-batch reactors
 - ❖ Characterize enzymatic activities and functional diversity during the reaction
- ✓ **Final goal: Improve degradation yield of LC biomass thanks to discovery of new enzymatic activities and microbial consortia**

Acknowledgement



- *Chatellard Lucile*
- *Gales Amandine*
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Hervé Brustel – Entomologist INP-EI Purpan

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Thank you for your attention