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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,  
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# 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,*

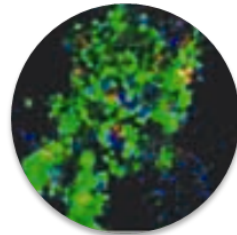
*Hélène Carrère, Jean-Philippe Steyer  
Jean-Jacques Godon, Michael O'Donohue,*



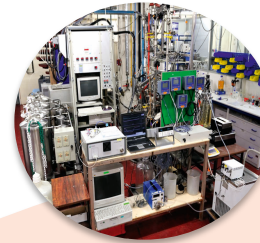
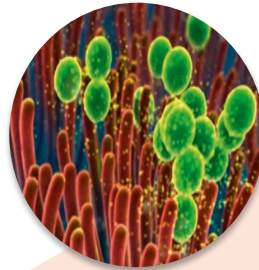
# PARTNERS



MICRO-ORGANISMS



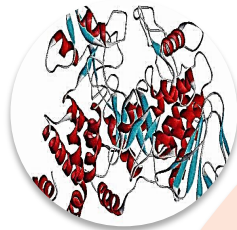
BIOFILMS, FLOCS,  
GRANULES



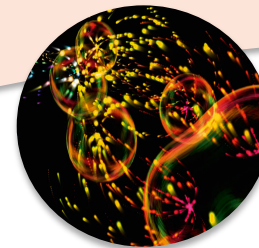
PILOTS



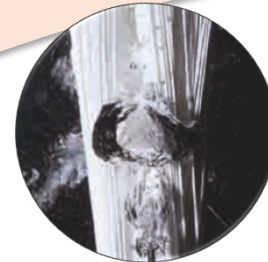
APPLICATIONS



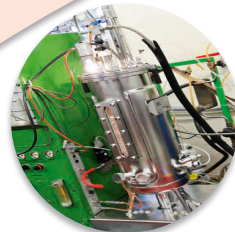
BIOCATALYSIS



BUBBLES  
DROPS  
PARTICLES



MEMBRANES

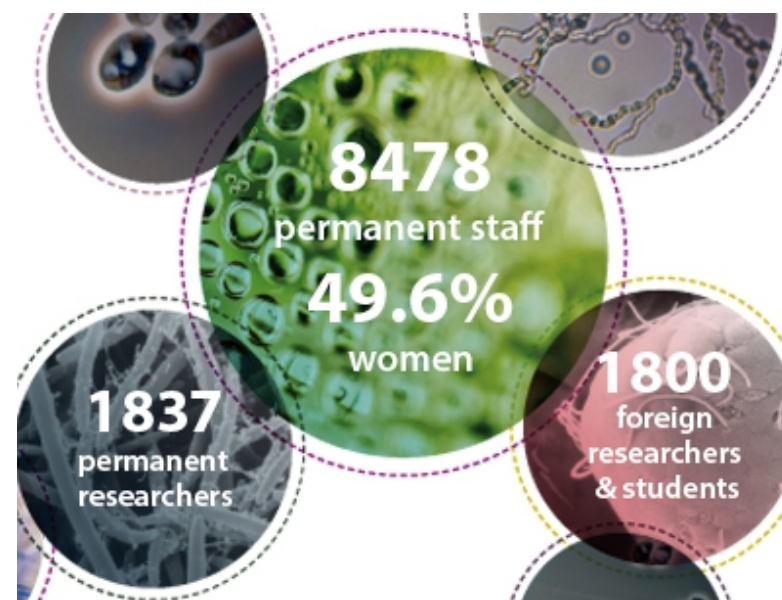
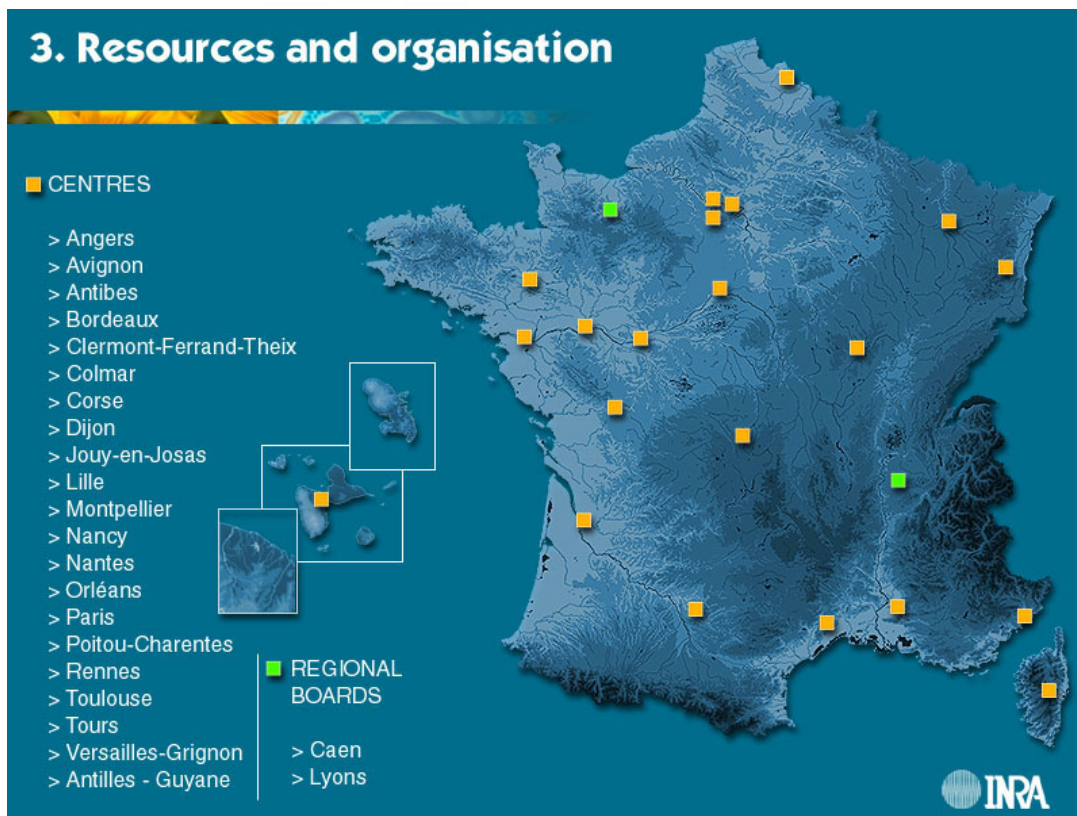


CHEMICAL  
CATALYSIS,  
NANOPARTICLES

## Fields of Application

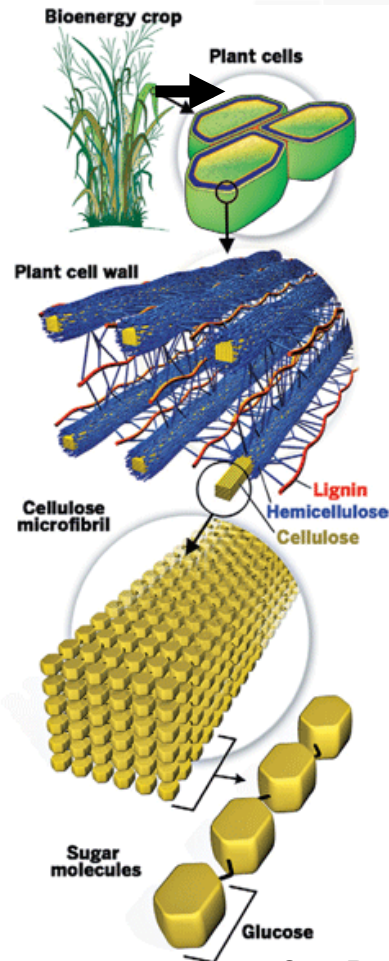
- Agriculture and food industries
- Water and environment
- White biotechnology
- Pharmaceuticals and cosmetics
- (Bio)chemical engineering

# The French National Institute for Agricultural Research

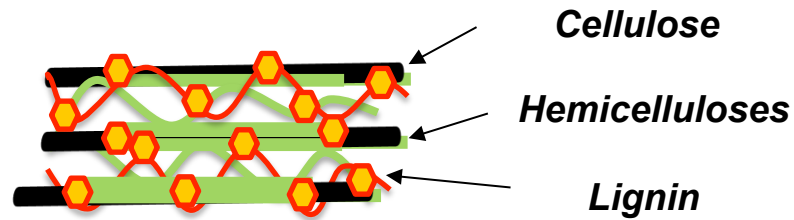


# CONTEXT

## Lignocellulose (LC)



- Complex chemical structure → difficult biological degradation

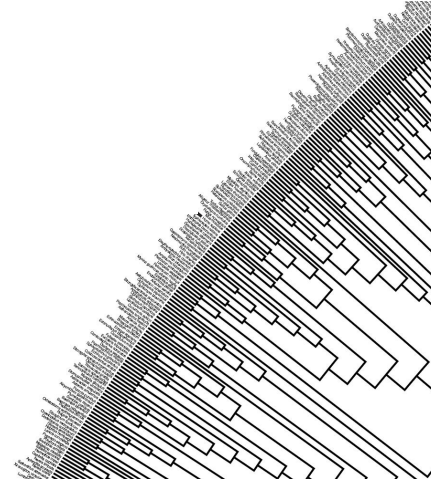


**Deconstruction**  
=  
**Major technological challenge**

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**

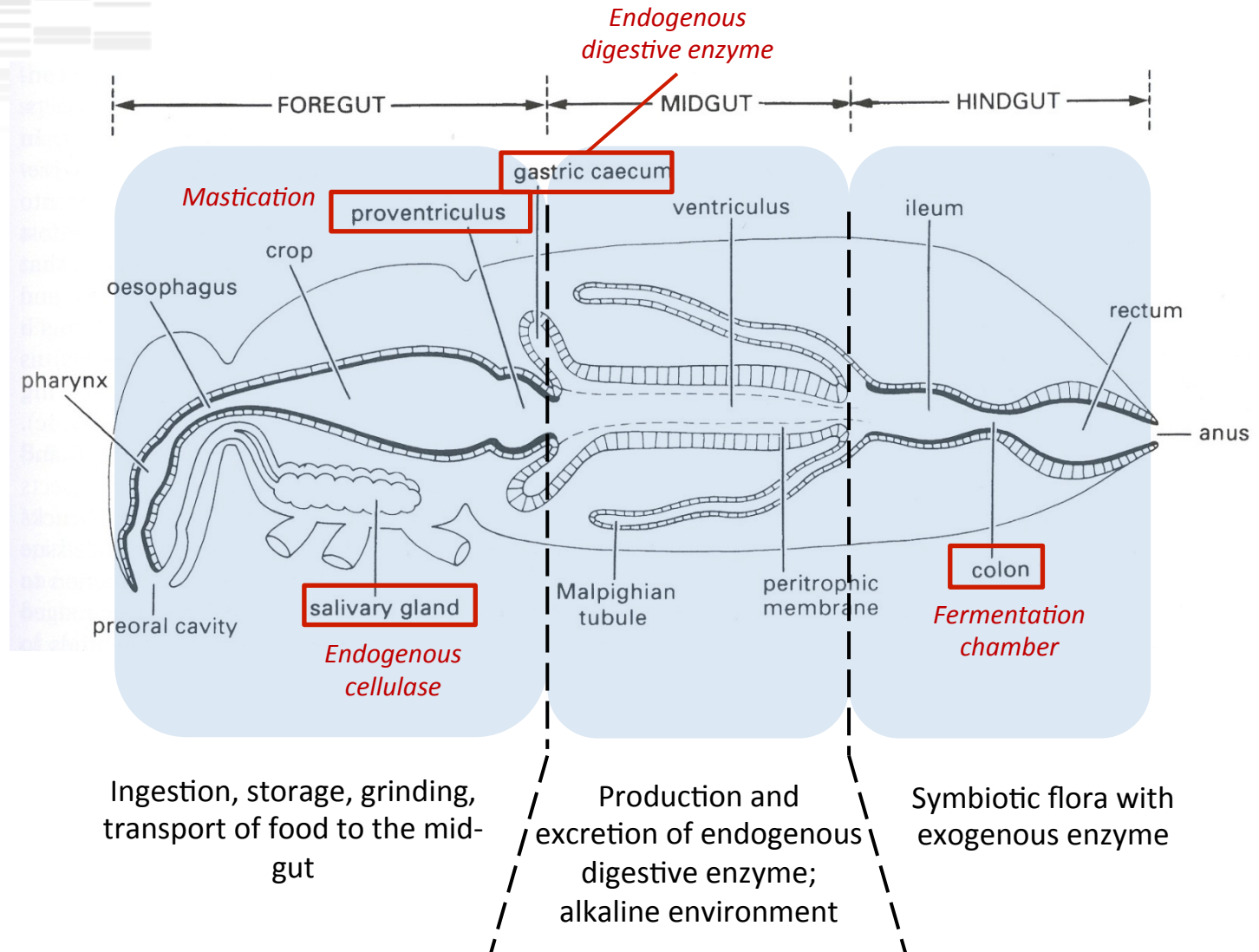


**Phyto-  
Xylophage  
Hindgut**

# INSECT GUTS STRUCTURE



**Mandibles:**  
Mastication organ  
Enhance access  
of cellulolytic enzyme  
to cellulose



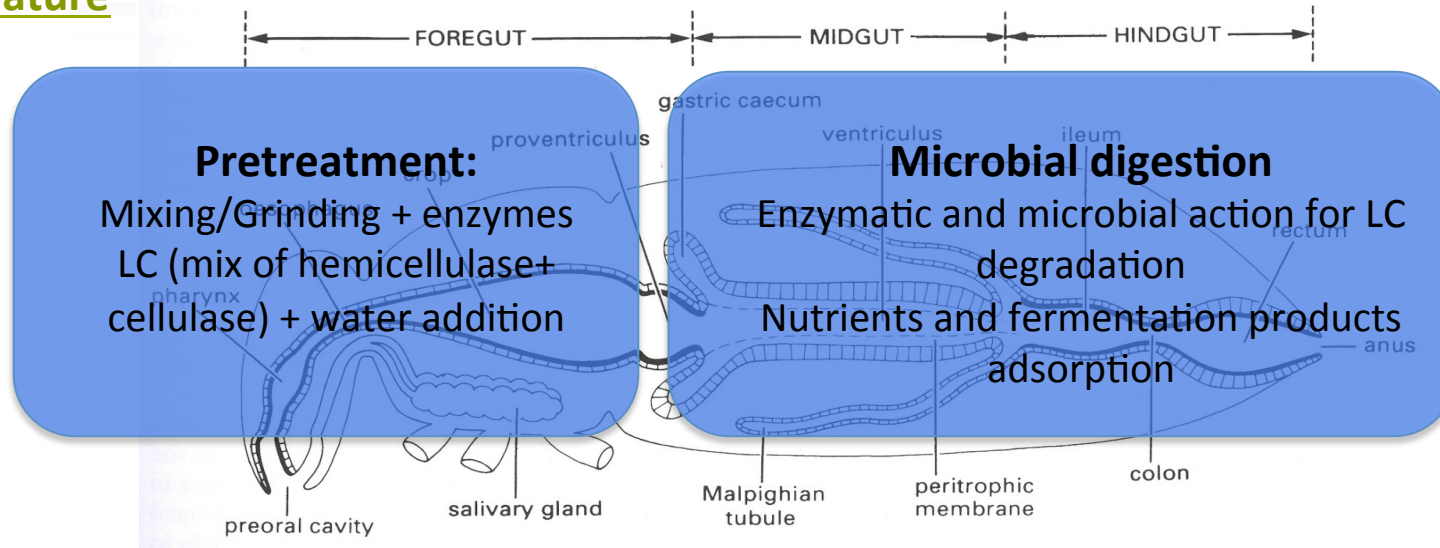
Gullan P.J., et al, 2010; Sun, J.-Z., et al, 2010; Huang, S.-W., et al, 2010

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

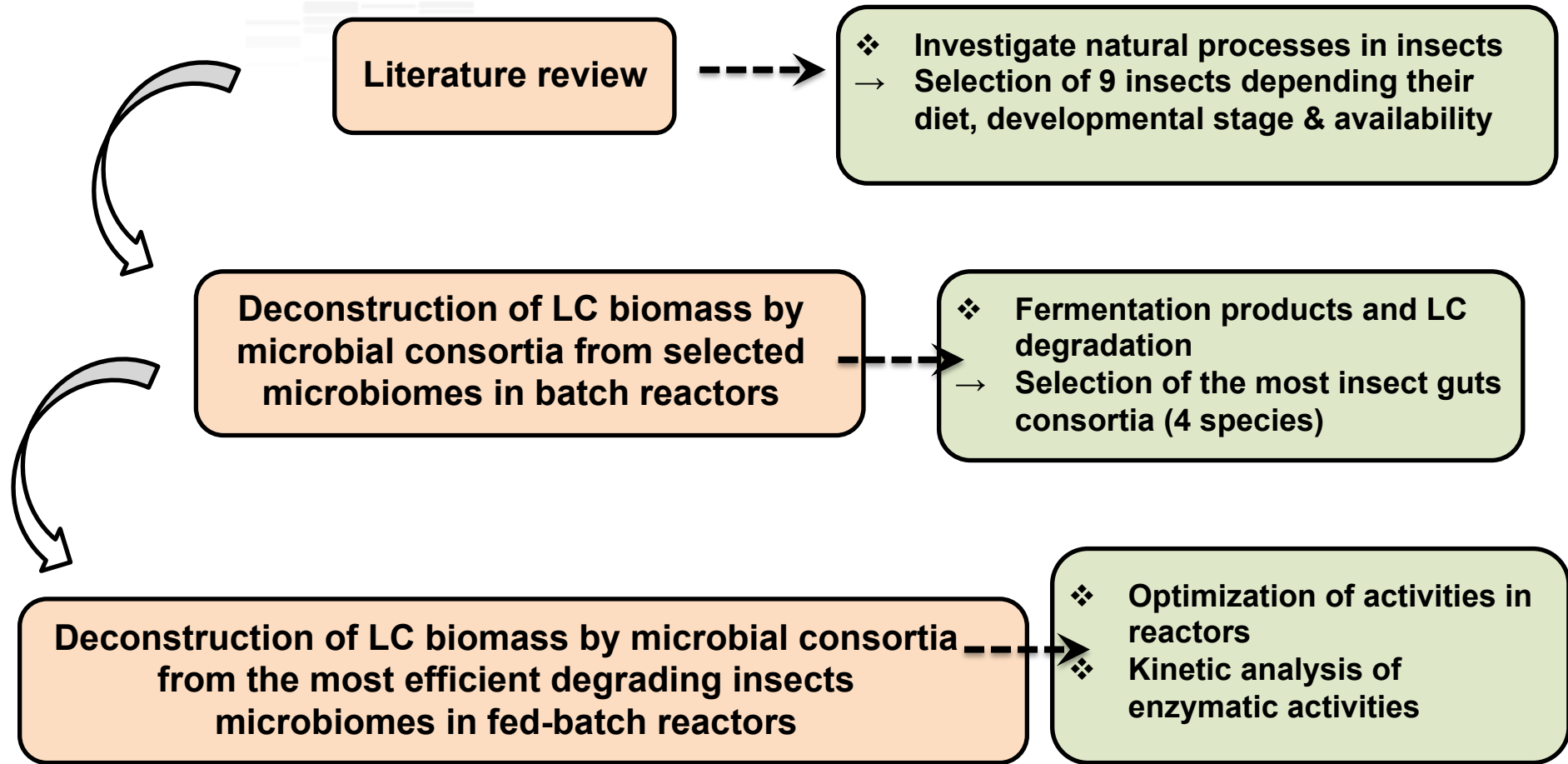
## Challenges of the project

- ✓ **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**

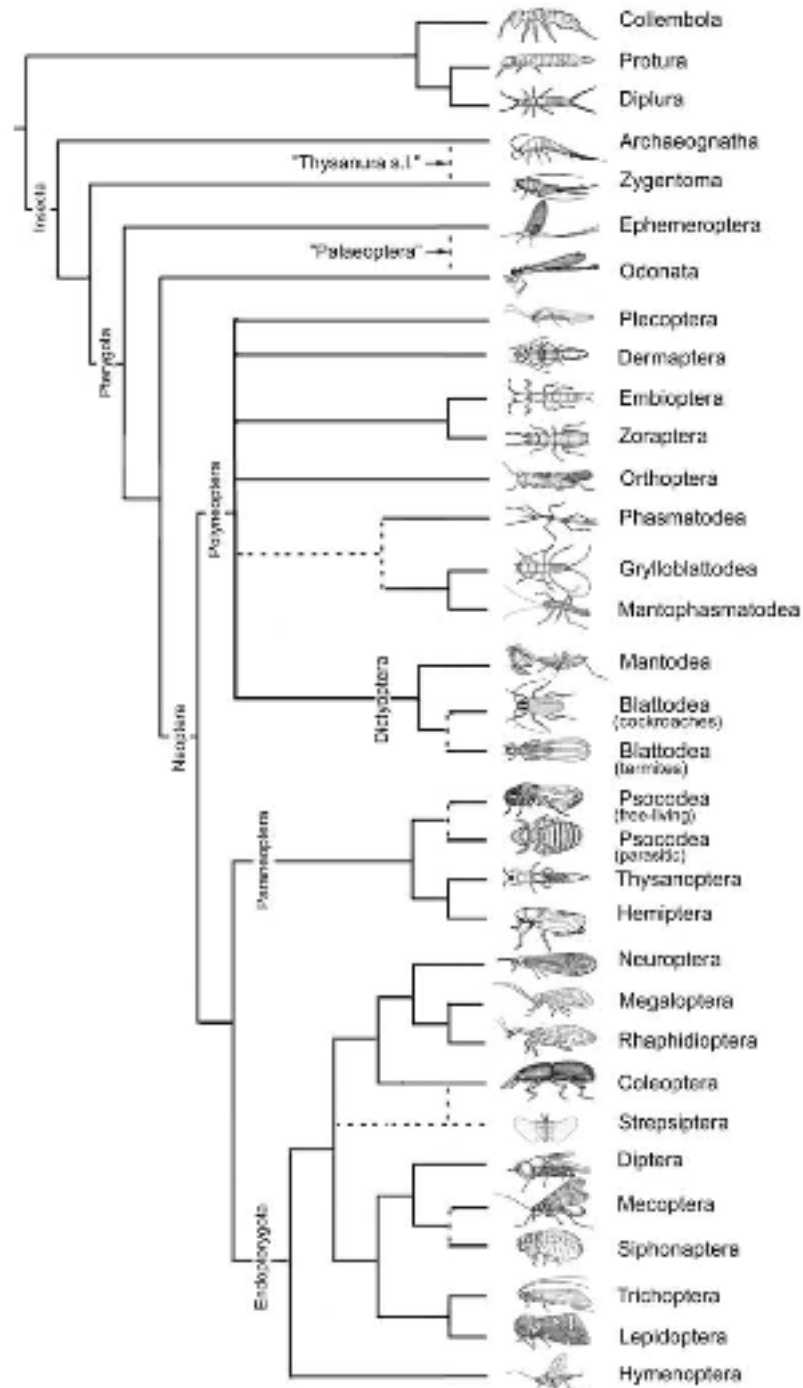
# PLAN OF THE STUDY



# Choice of insects

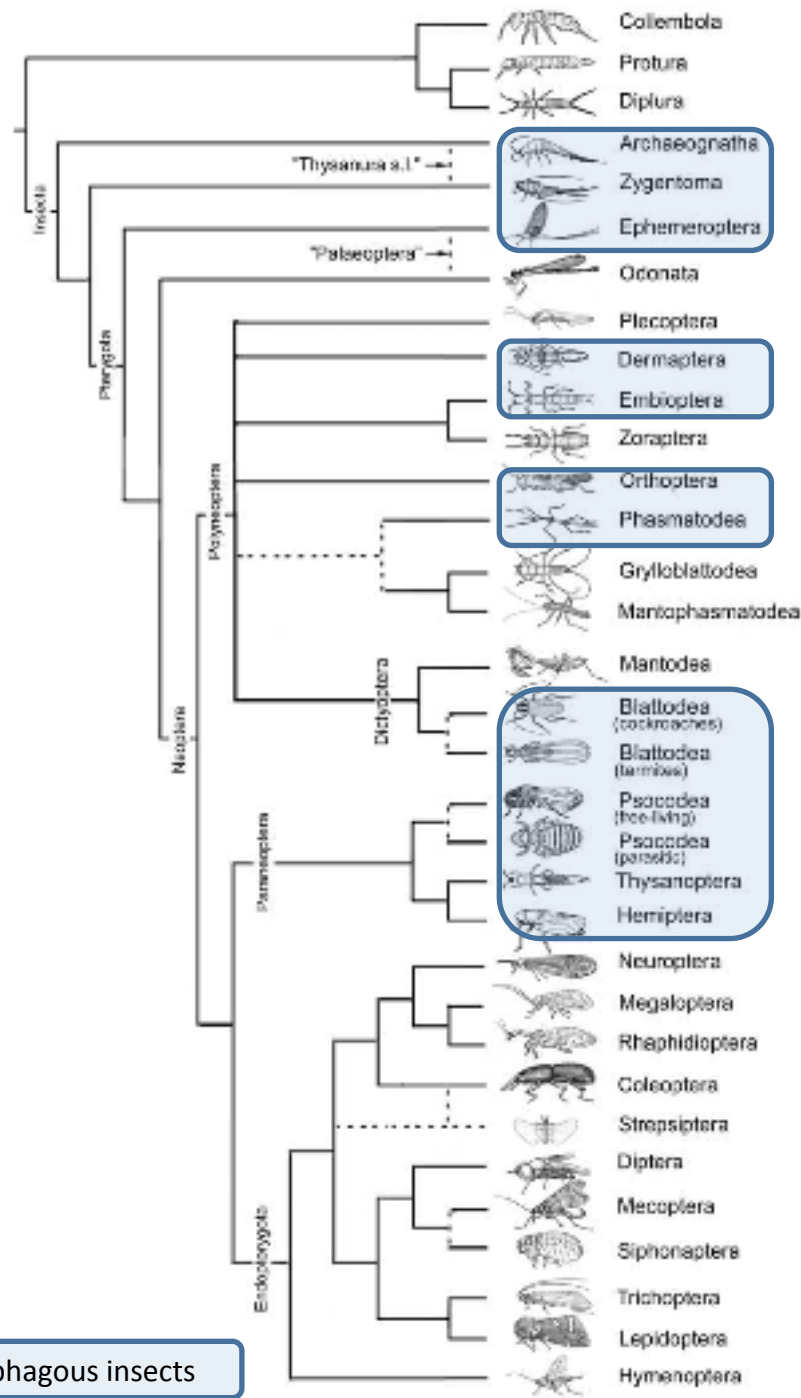
# Insects of interest

- 33 orders



# Insects of interest

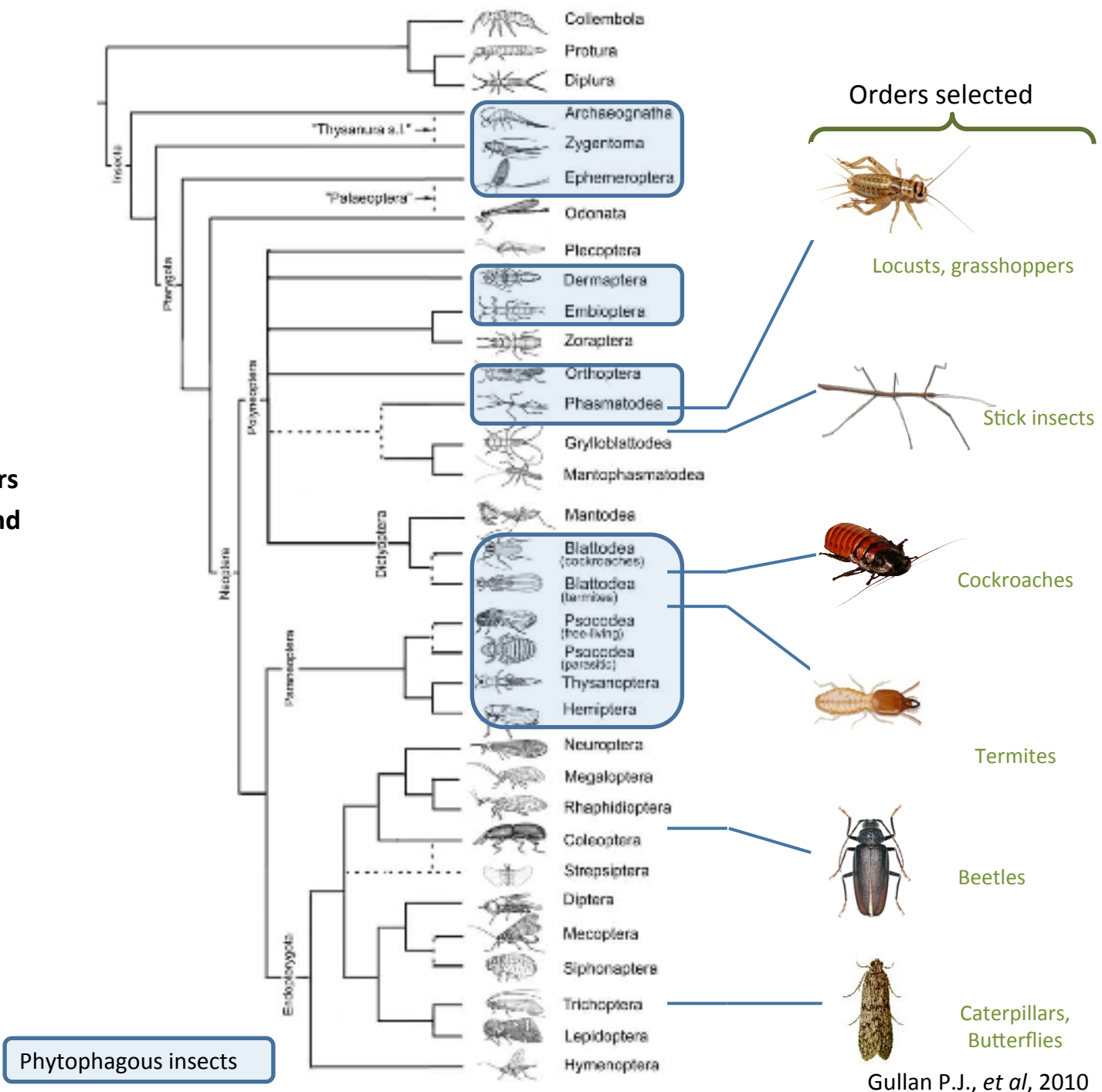
- 33 orders
- **18 orders phytophagous**



Phytophagous insects

# Insects of interest
















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



# 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) :	
					
					



### 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 CO<sub>2</sub> 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)

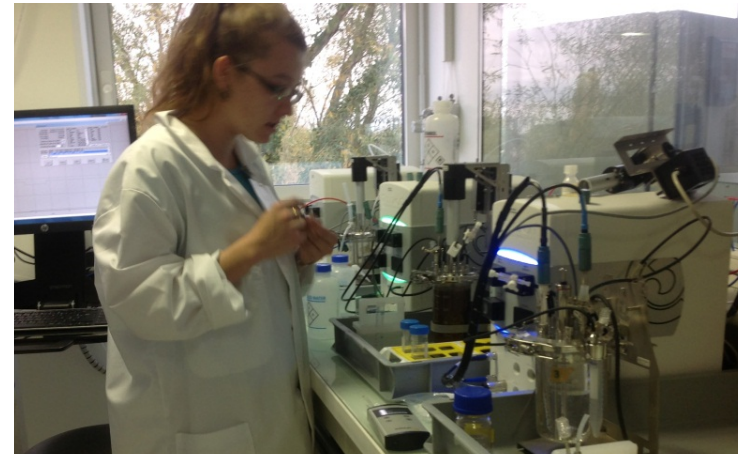
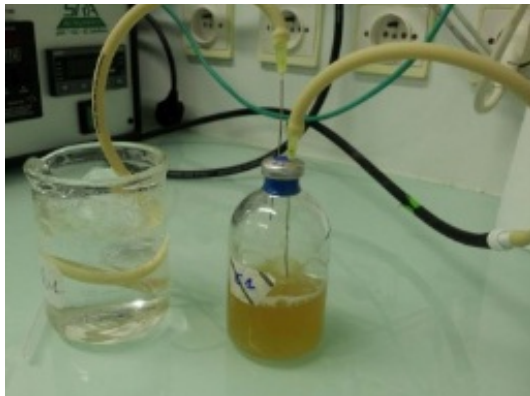
*Guts residues*

**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

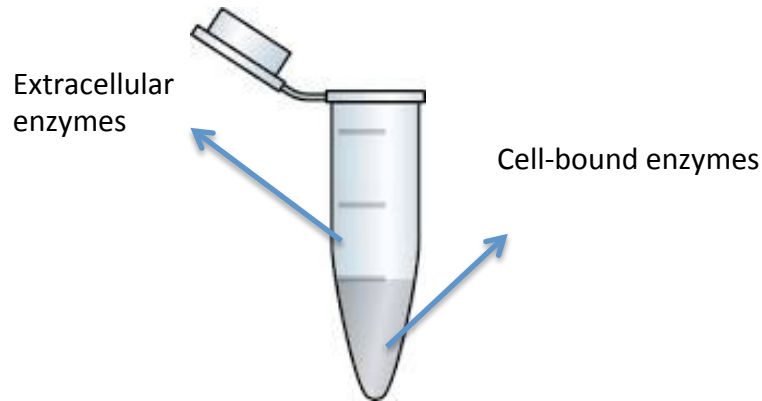


# METHODS: INSECTS

## 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

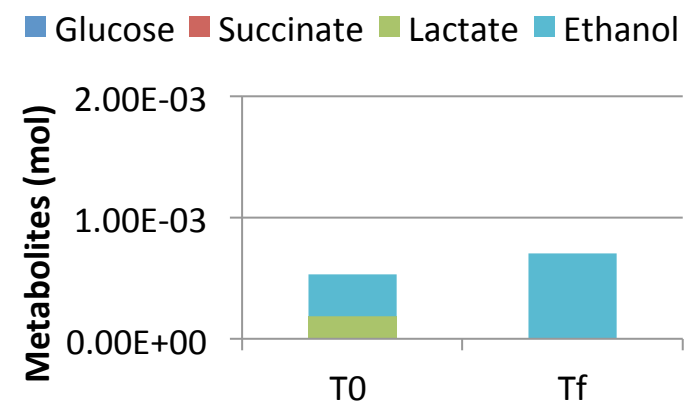
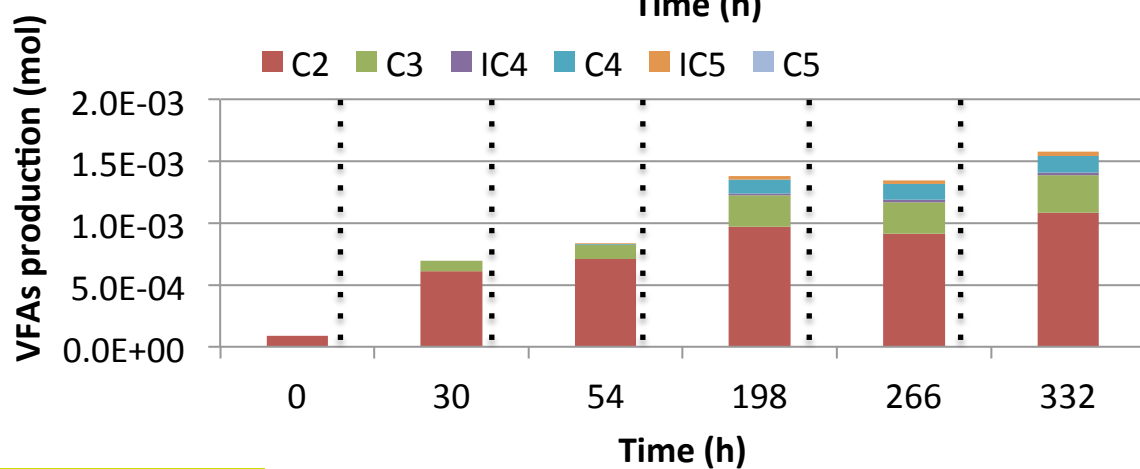
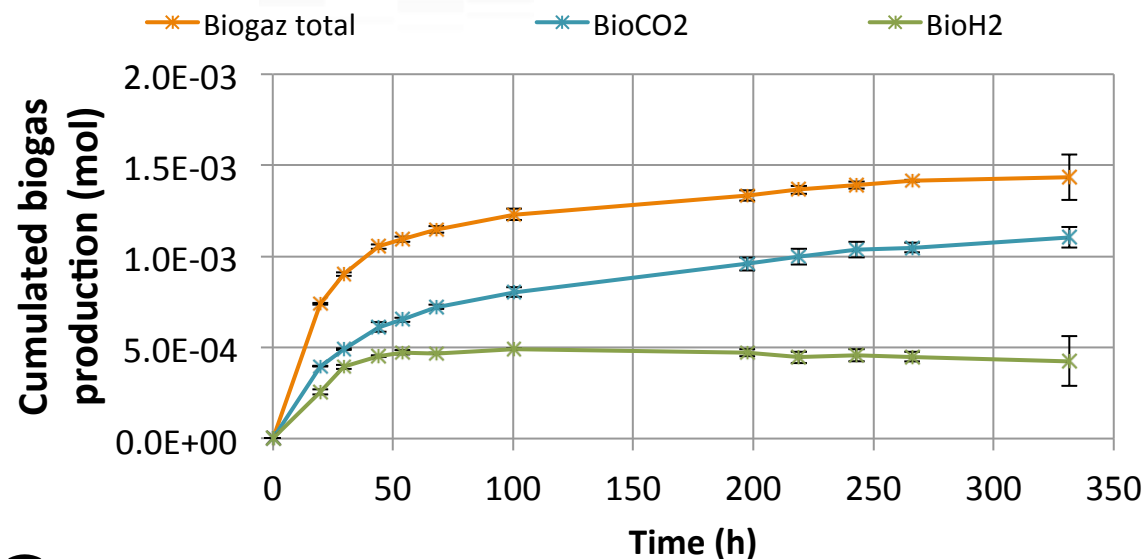


02

Results

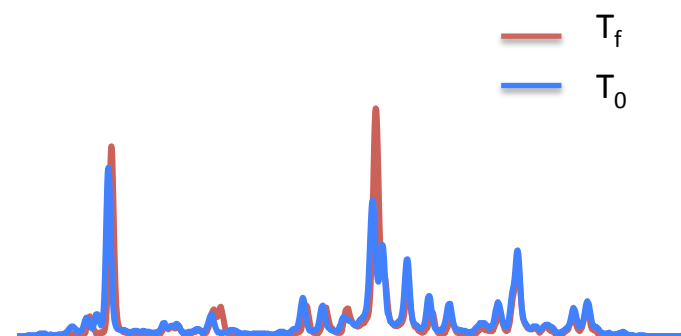
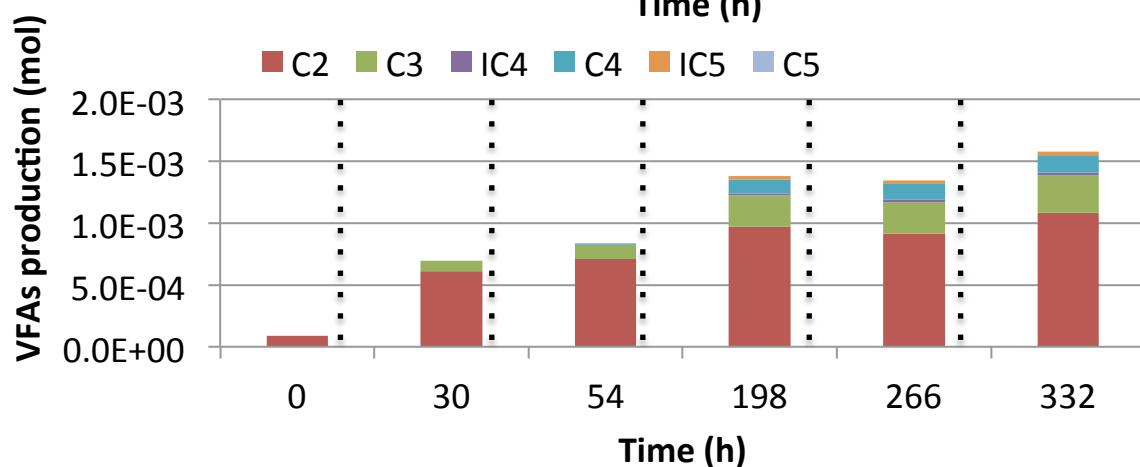
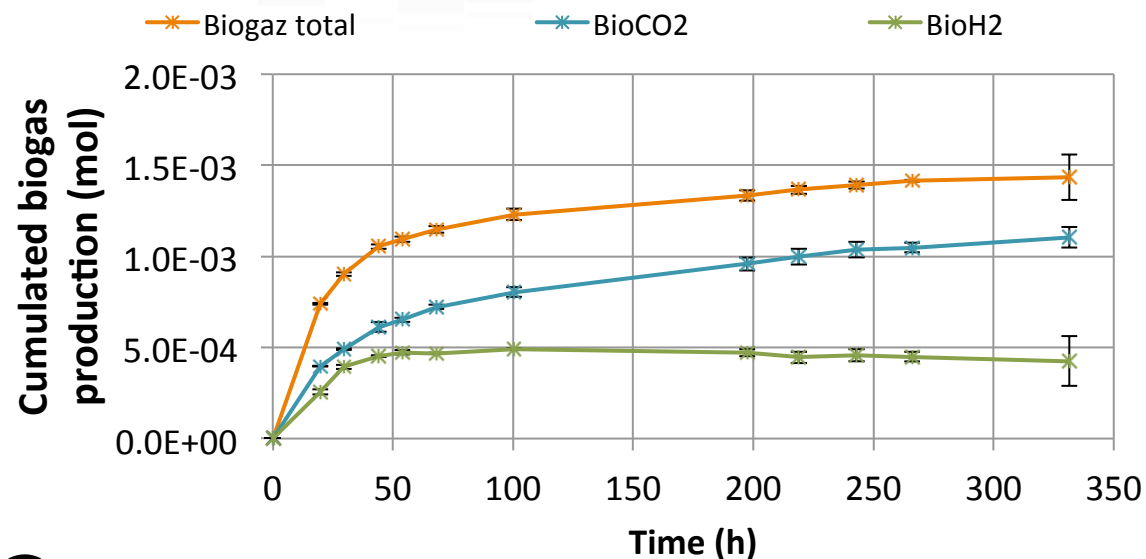
# RESULTS

1<sup>ST</sup> Batch : *Gromphadorrina portentosa* case



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1<sup>ST</sup> Batch : *Gromphadorrina portentosa* case

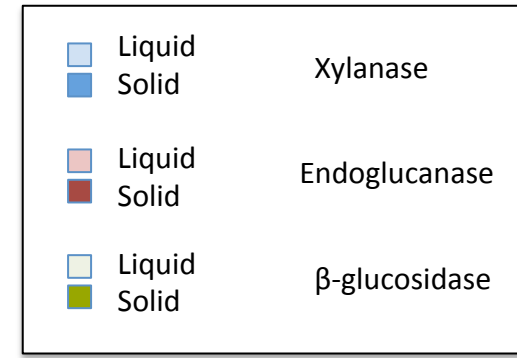
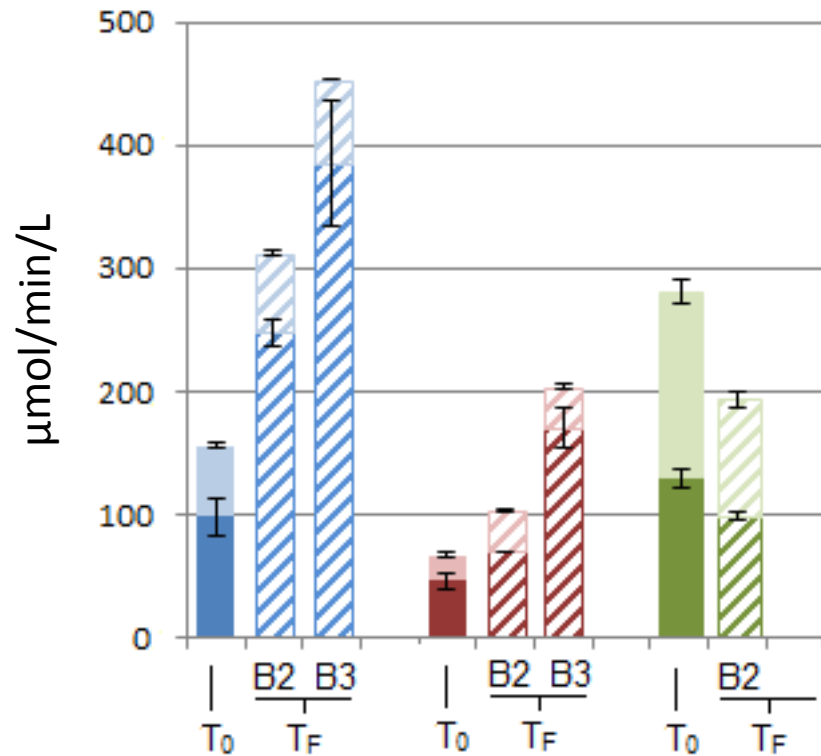


- Small variation of microbial communities
  - Growth
- Degradation of LC due to initial communities



# RESULTS– Enzymatic activities

1<sup>ST</sup> 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>Propomacrus</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 <sub>2</sub>	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



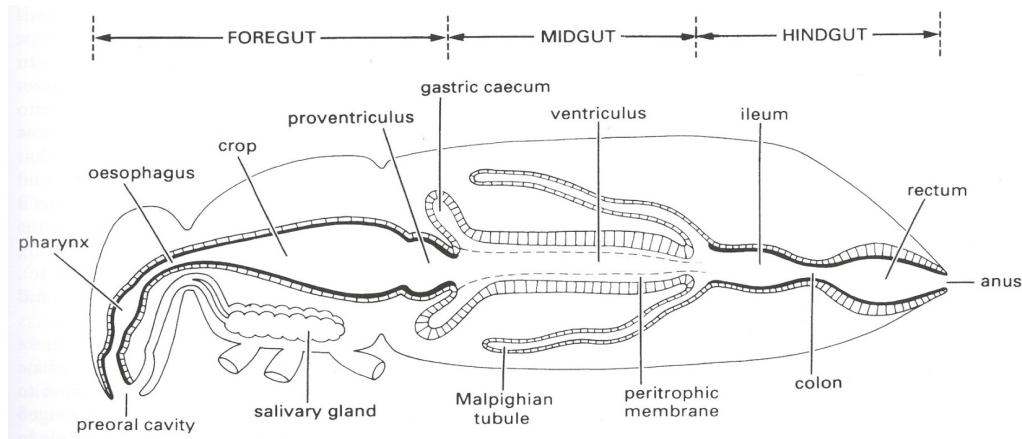
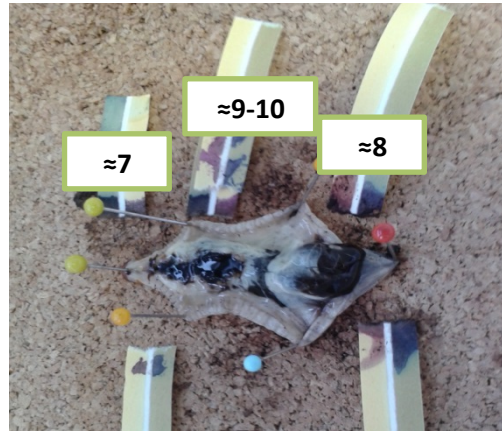
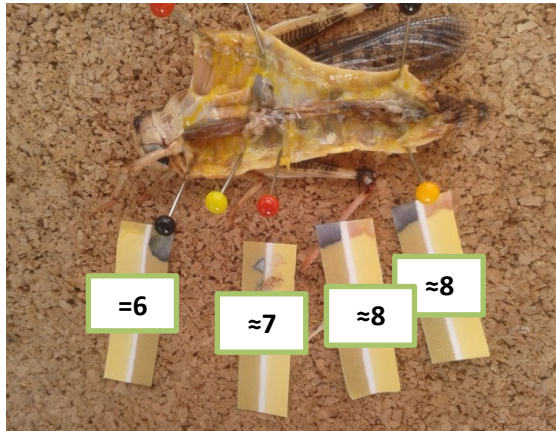
*Locusta migratoria*



*Potosia cuprea*



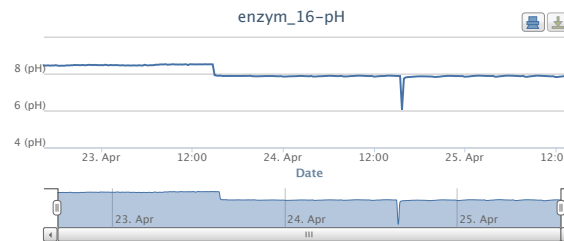
*Gromphadorrina portentosa*



# OPTIMIZATION – controlled pH, normalized microbial biomass quantity



- Monitored and controlled pH



**pH=8**

Wheat straw as sole carbon source

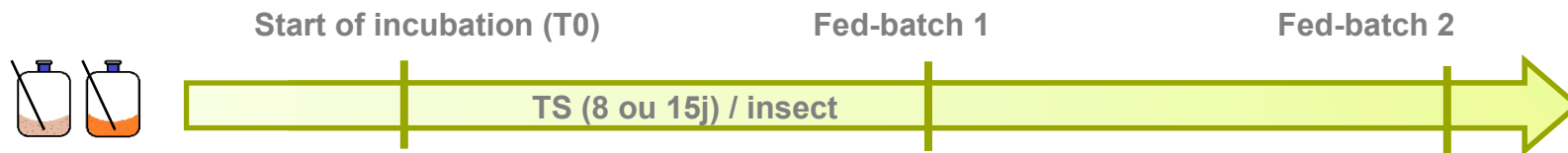
Normalized quantity of biomass (16S copy)



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

# METHODS– biochemical & microbiological analysis

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Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)	Quantitative analysis of metabolites (HPLC)
Microbial communities	<b>Work in progress</b>	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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- *Dumon Claire*
- *O'Donohue Michael*
- *Hernandez-Raquet Guillermina*



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*Hervé Brustel – Entomologist INP-EI Purpan  
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Thank you for your attention