Microbiological options to enhance the anaerobic digestion of lignocellulosic biomass

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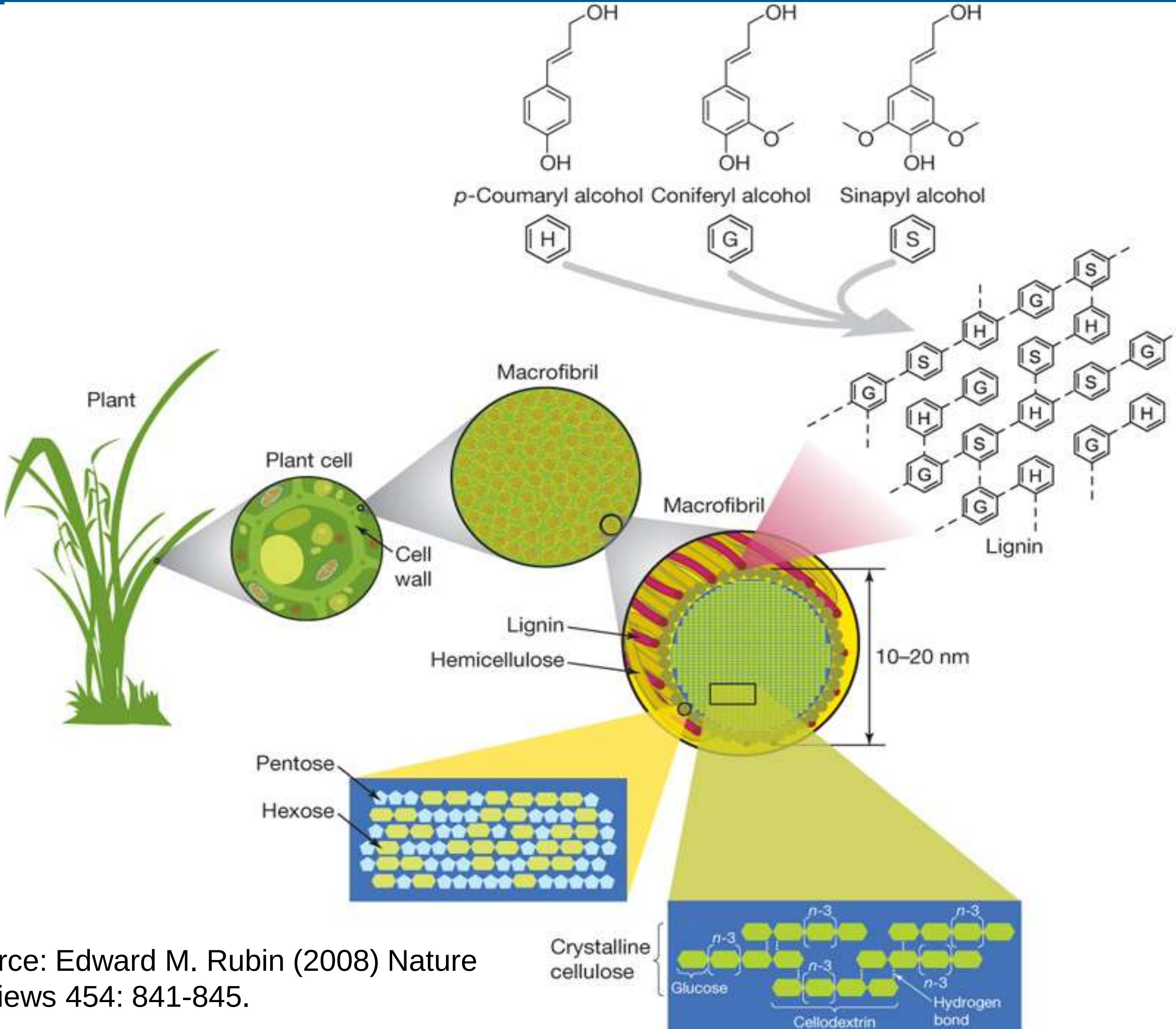
Introduction

Vasa warship exhibited in Stockholm
(1628 – 1961 stayed underwater)

Reasons for lack of wood degradation

- Cold temperature
- Anoxic conditions
- Low salinity
- Lack of shipworms (*Teredo navalis*)

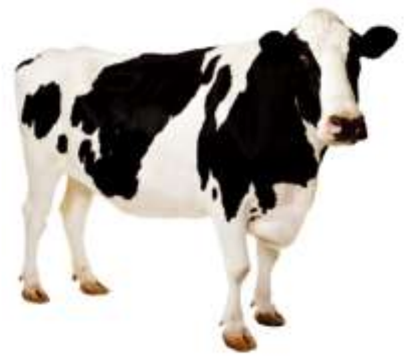
Lignocellulose structure



Source: Edward M. Rubin (2008) Nature Reviews 454: 841-845.

Animal digestion versus AD process

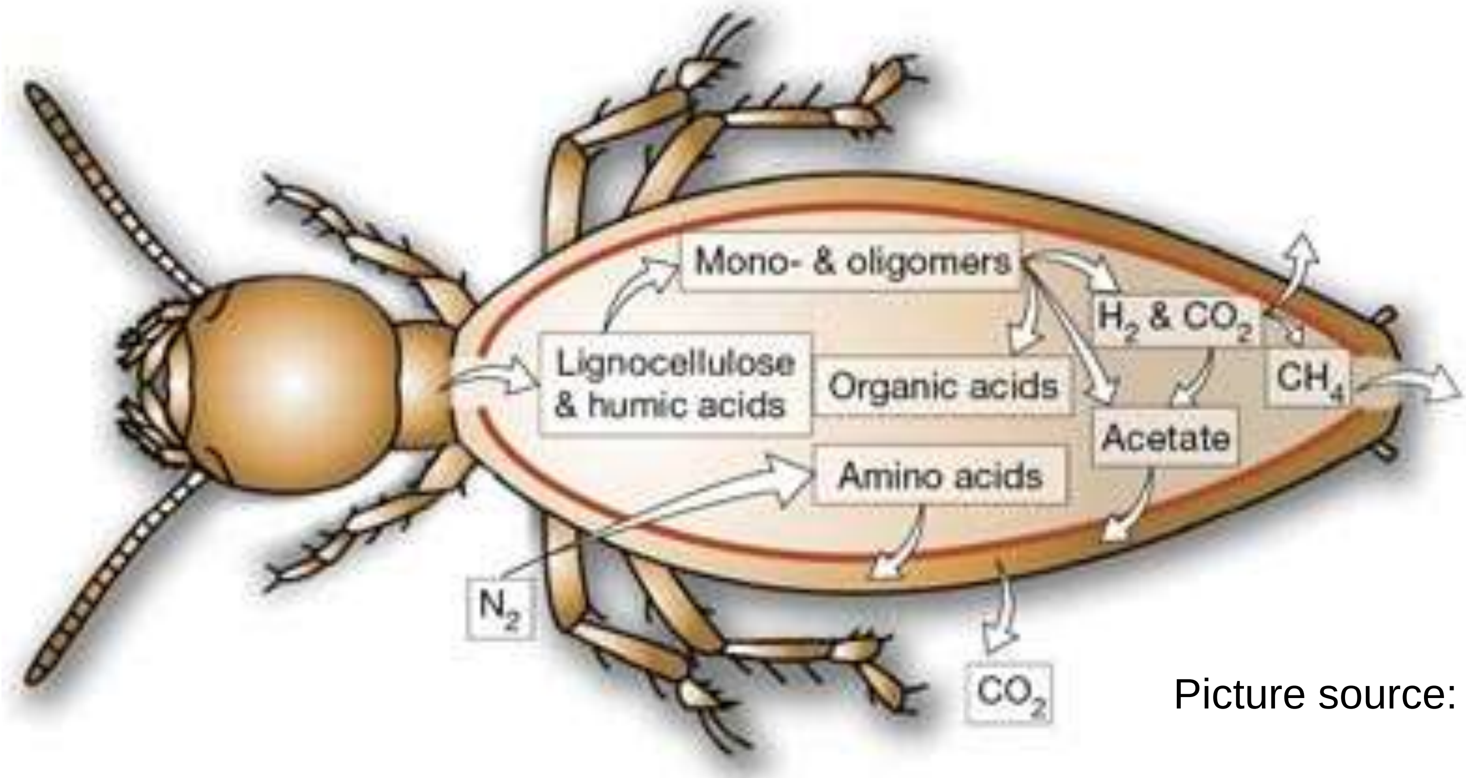
VFA production rate

Conventional anaerobic digester	Cow rumen	Termite gut system
		
6 g COD-based VFA/L d	18 g COD-based VFA/L d	225 g COD-based VFA/L d

Bayane A., Guiot S.R. (2010). Animal digestive strategies versus anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass. Rev Environ Sci Biotechnol. 10:43-62

Animal digestion versus AD process

- **Higher termites employ an array of specialized microbes in their hindguts to break down the cell walls of plant material and catalyze the digestion process**



Picture source: Microbe Wiki

- **The precise identity and role of the microbes from their digestive tract is still a mystery**

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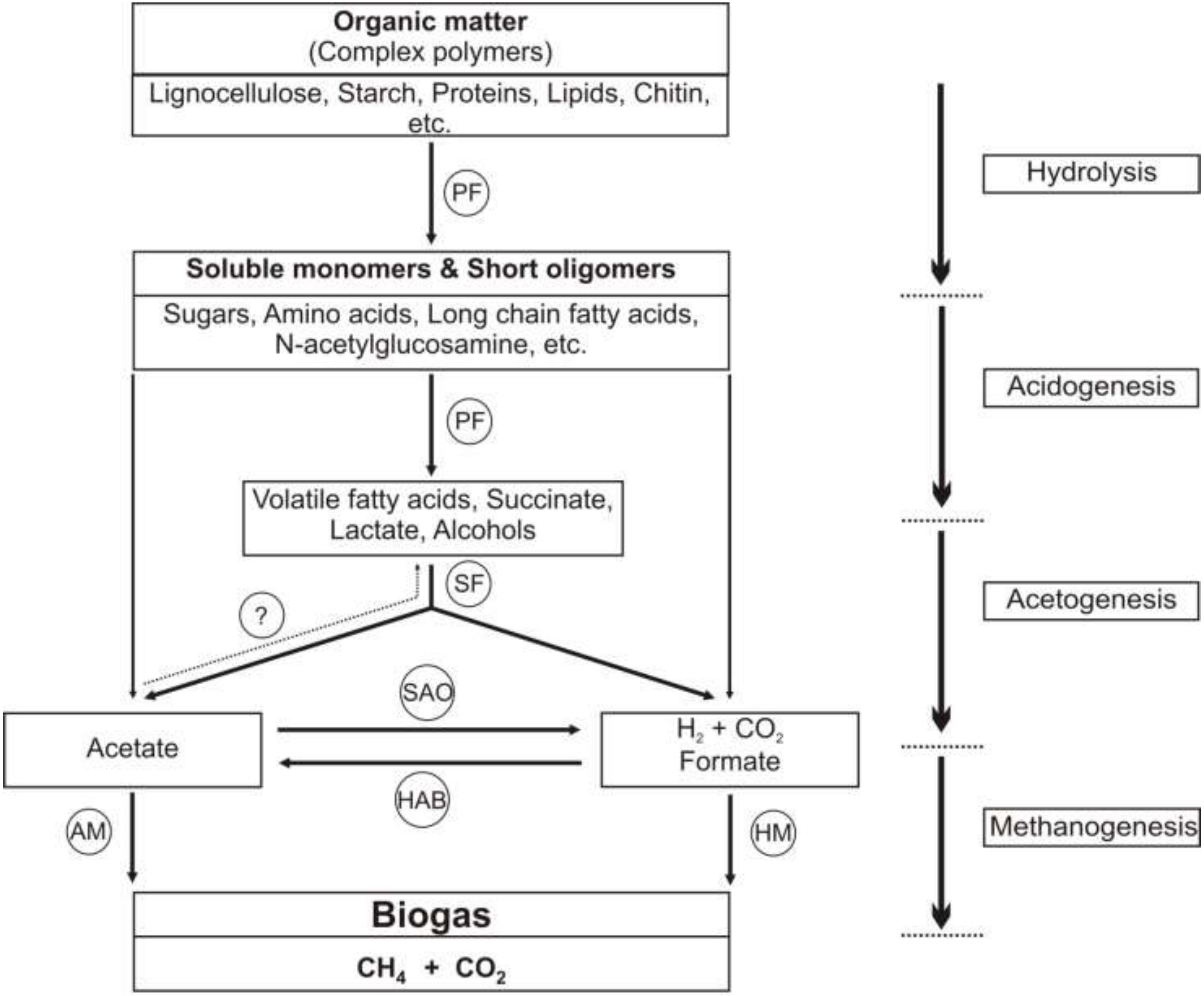
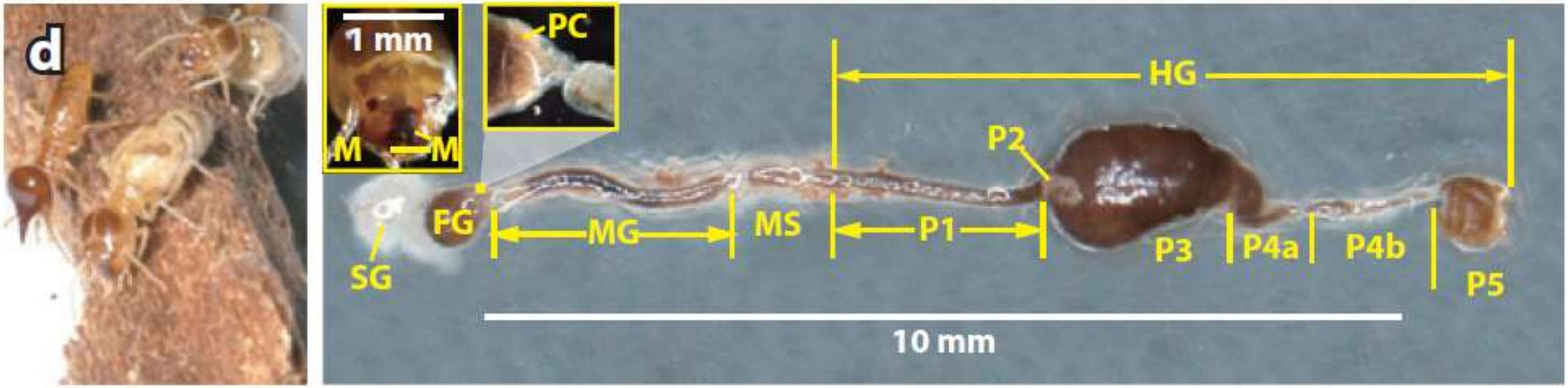
Anaerobic digestion

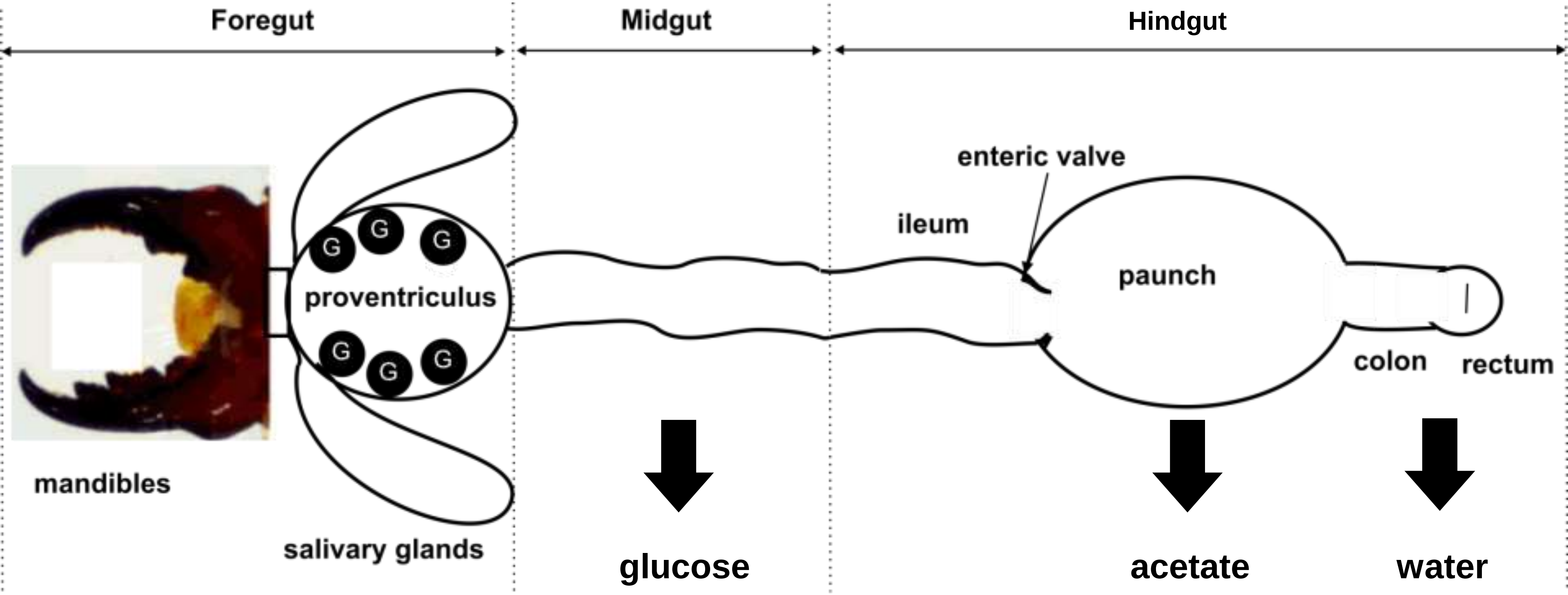


Gut system of *Nausitermes takasagoensis*

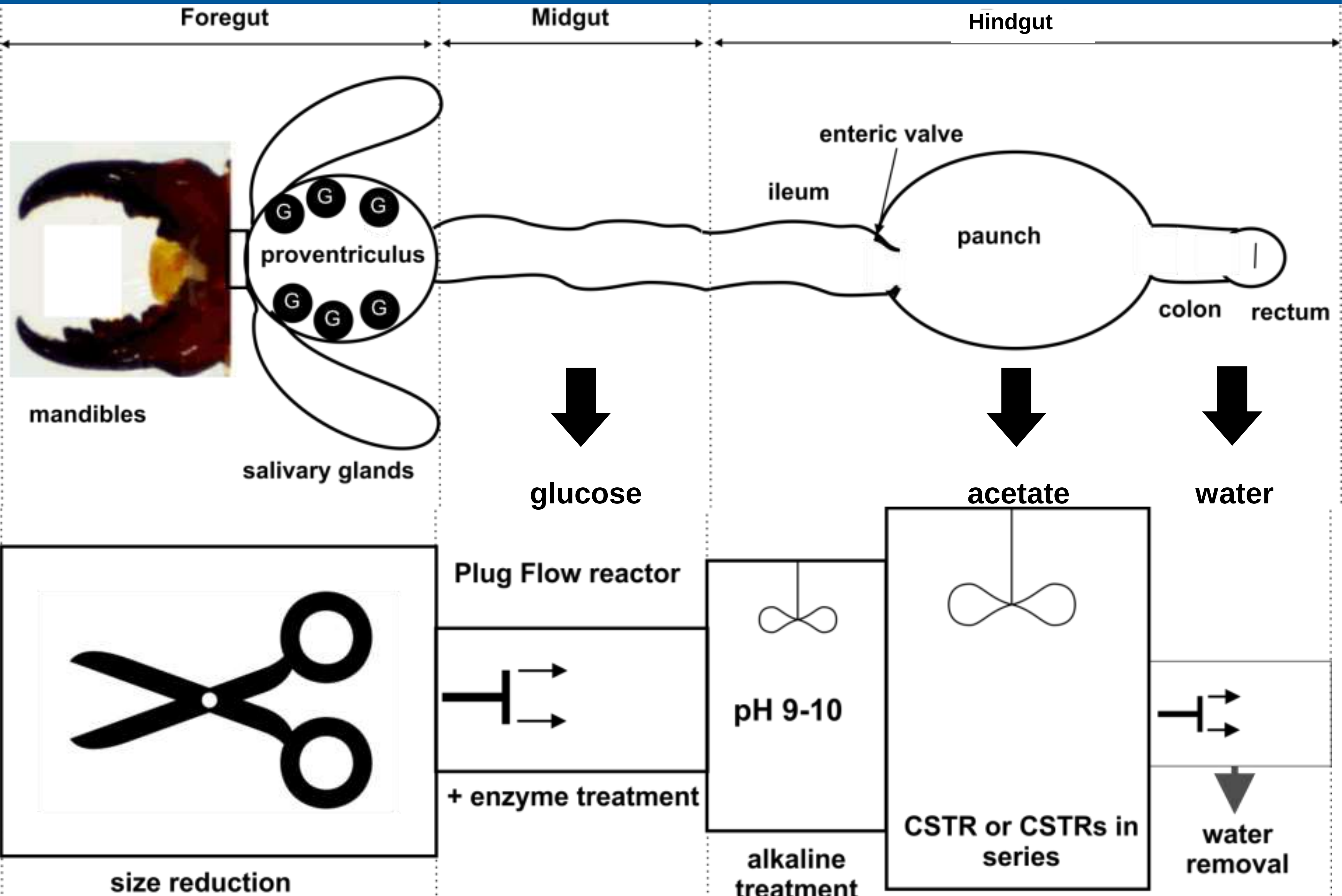


Watanabe & Tokuda (2011) Cellulotic Systems in Insects. Annu. Rev. Entomol. 55:609-632.

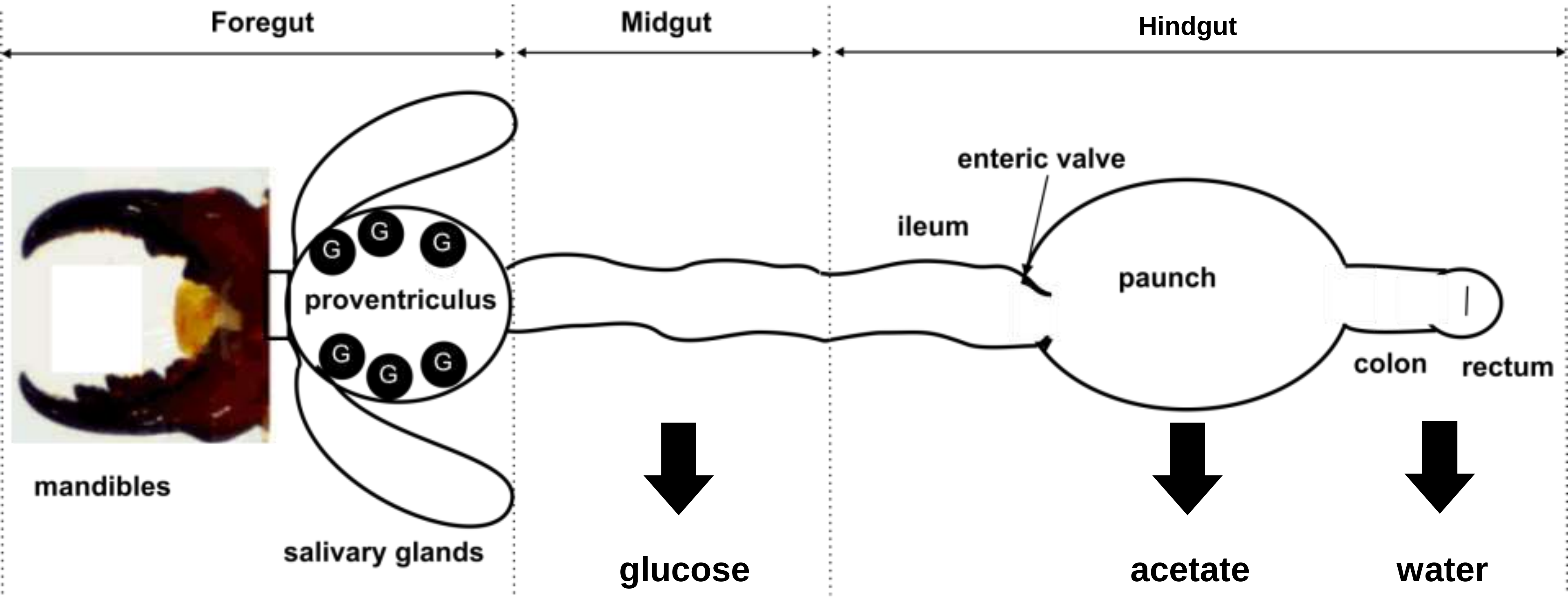
Termite gut system



Termite gut system

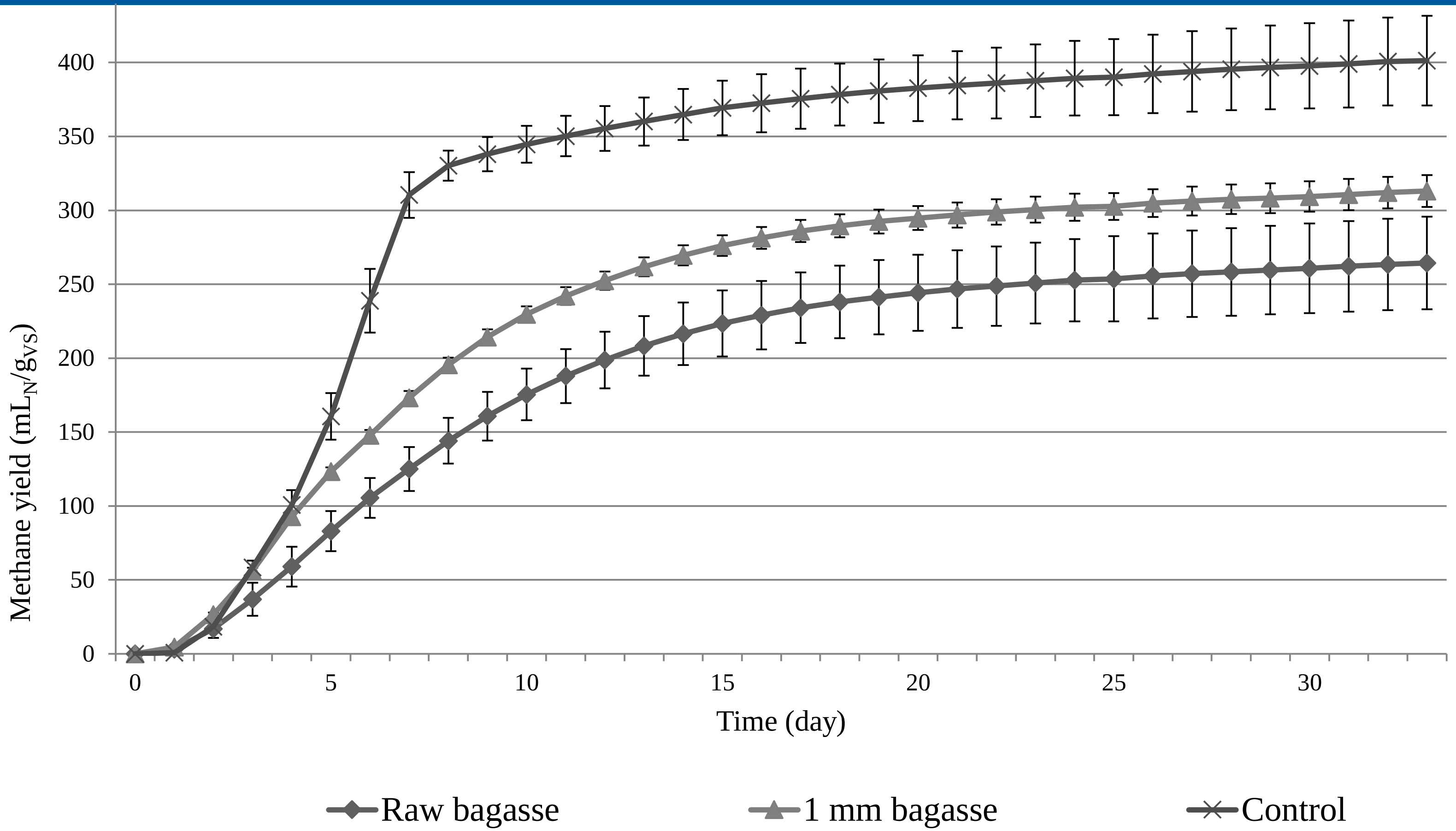


Termite gut system



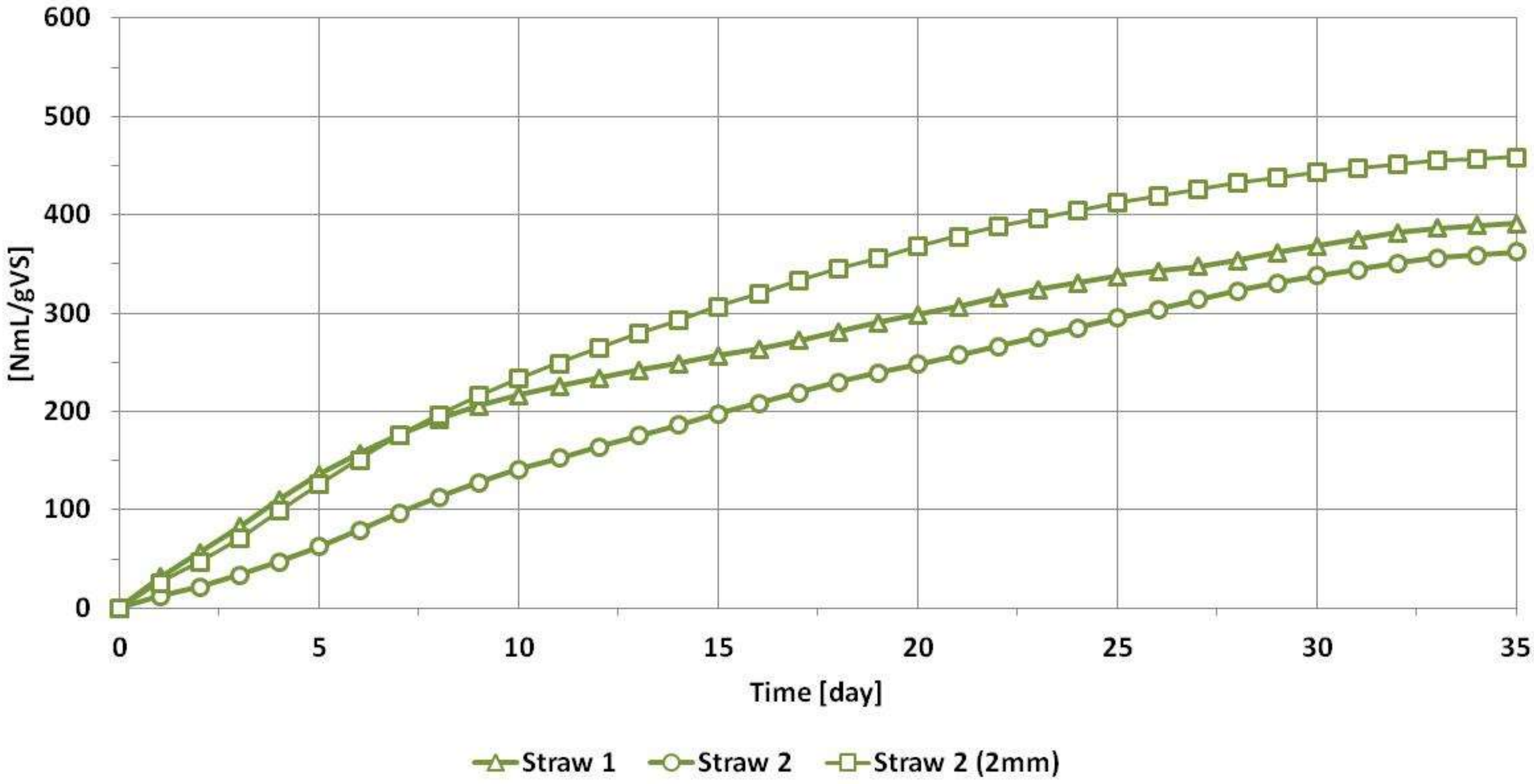
- Mechanical grinding (10-50 µm)
- Mandibles and proventriculus plays a key role
- Proventriculus has cuticular teeth-like structure
- Salivary glands secrete cellulolytic enzymes (endoglucanases, β-glucosidases) and laccases, phenoloxydases, esterases

Effect of size reduction



Leite *et al.* (2015) Assessment of the variations in characteristics and methane potential of major waste products from the Brazilian bioethanol Industry along an operating season. Energy&Fuels. 29 (7):4022–4029

Effect of size reduction



Janke et al. (2015) Biogas Production from Sugarcane Waste: Assessment on Kinetic Challenges for Process Designing. Int. J. Mol. Sci. 2015, 16:20685-20703

Effect of size reduction

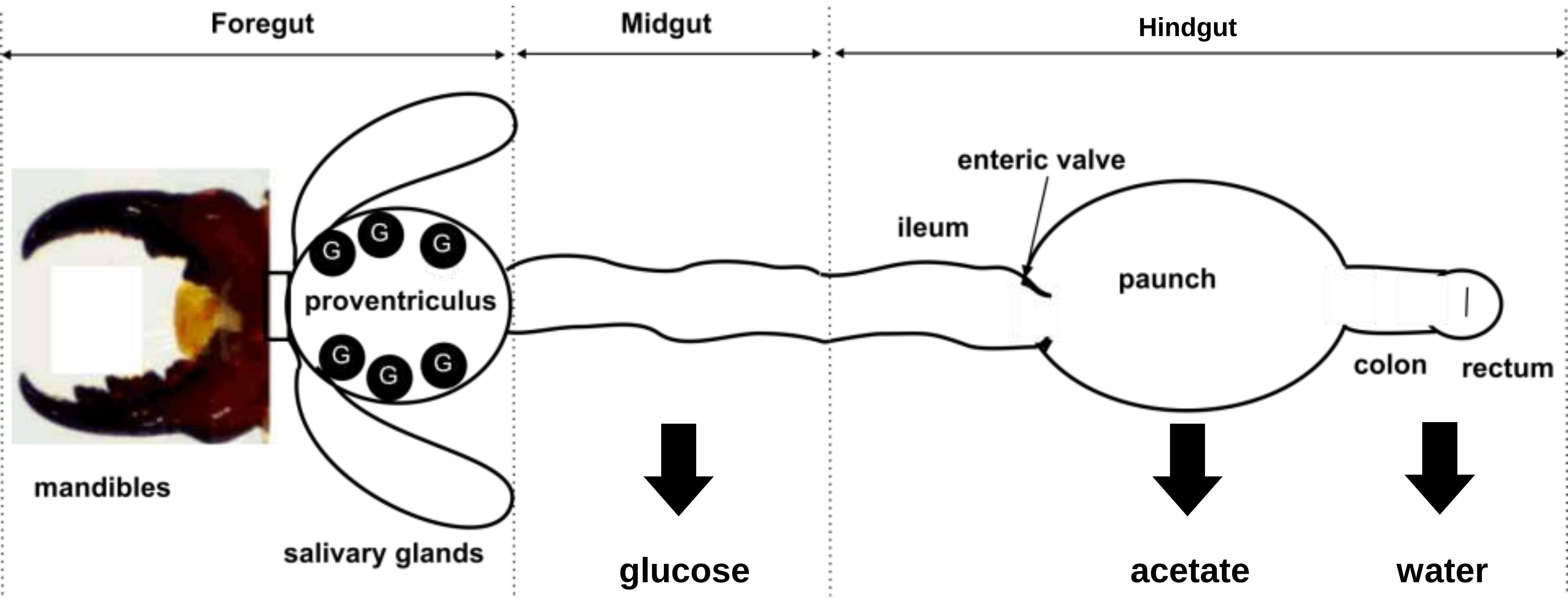
- Grinding lignocellulosic substrates promotoes the rate and extent of hydrolysis (increased surface, reduced crystallinity)

- Ball milling (100 µm) is as effective as steam explosion (Ghizzi et al (2012))

- Energy consuming and cost effective

Ghizzi *et al.* (2012) Effects of grinding processes on enzymatic degradation of wheat straw. Bioresour Technol 103(1):192–200

Termite gut system



- Very high concentration of endogenous enzymes (e.g. cellulase 3 mg/mL; 10^3U/mL)
- Lignin and hemicellulose degradation is probably due to the combined action of laccases, phenoloxydases, esterases/carboxylesterases

Enzyme pretreatment

- Enzymatic treatment is routine procedure in 2nd generation bioethanol production
- Only few positive examples for the enhancement of biogas production

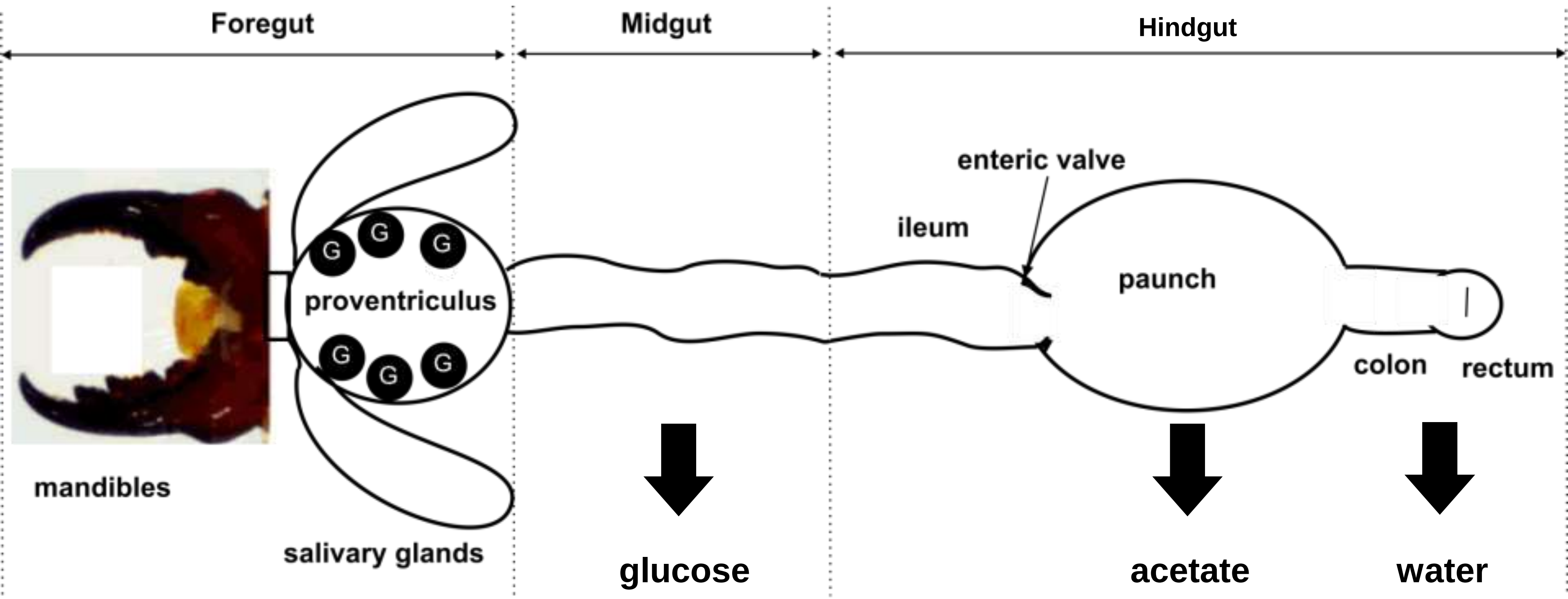
Substrate	Treatment	Effect	Reference
Maize	Sil-all 4x4	+10% methane	Vervaeren et al. (2010)
Manure fibers	Steam+NaOH+ laccases	+34% methane	Bruni et al. (2010)
Wheat grass	Commercial enzyme mixture	No effect	Romano et al. (2009)

Vervaeren et al. (2010). Biological ensilage additives as pretreatment for maize to increase the biogas production. Renew Energ, 35, 2089–2093.

Bruni et al. (2010) Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. Bioresour Technol 101(22):8713–8717

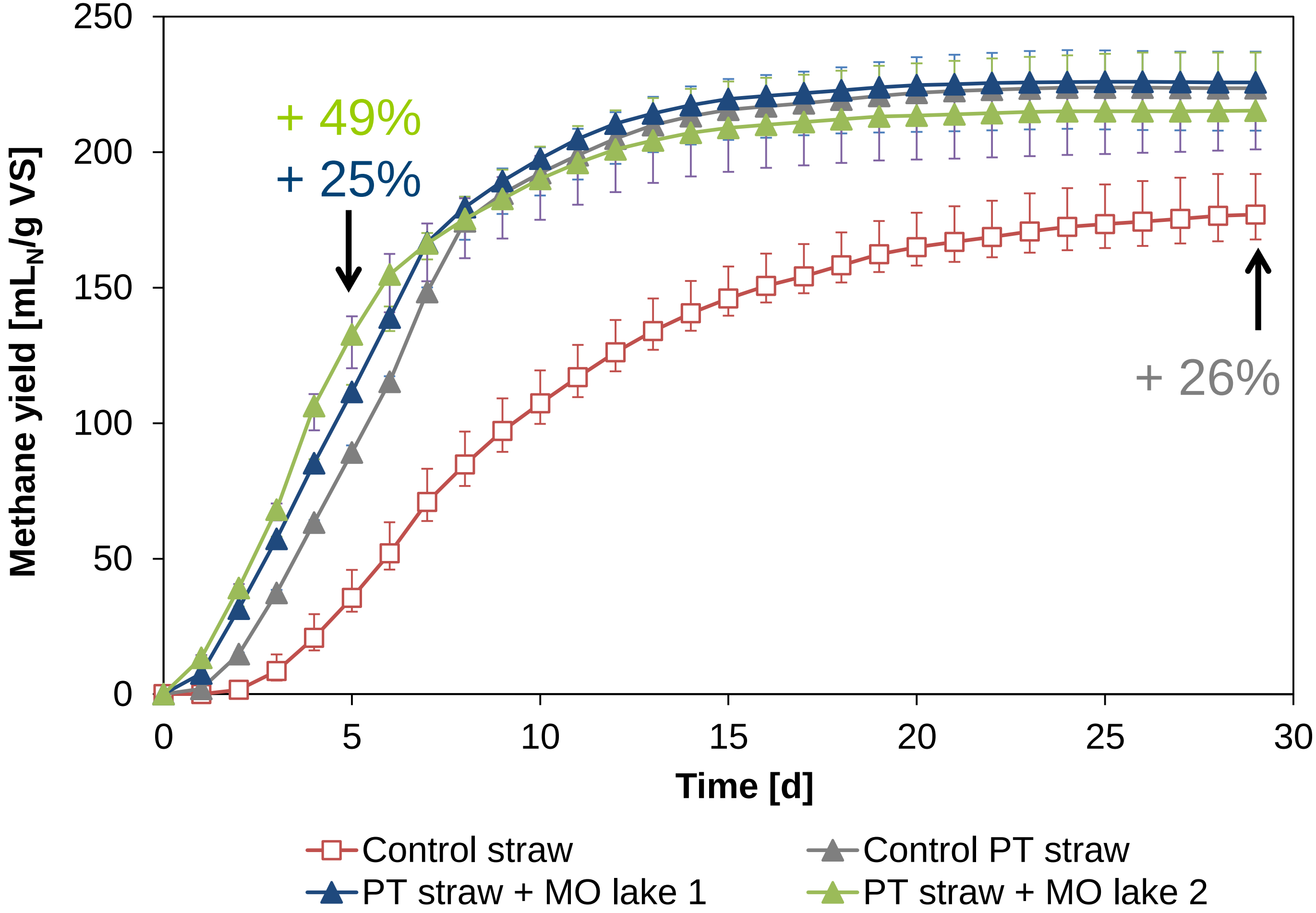
Romano et al. (2009). The effects of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresour Technol, 100, 4564–4571.

Termite gut system



- Ileum has a very high pH (9-12)
- Alkaline pre-treatment
- Paunch is a fermentation chamber (pH 6-7.5)
- Oxygen diffuses to the peripherial part
- Only 40% (lumen) is completely anoxic
- Microorganisms (protozoa and bacteria) are involved in the final degradation of lignocellulose

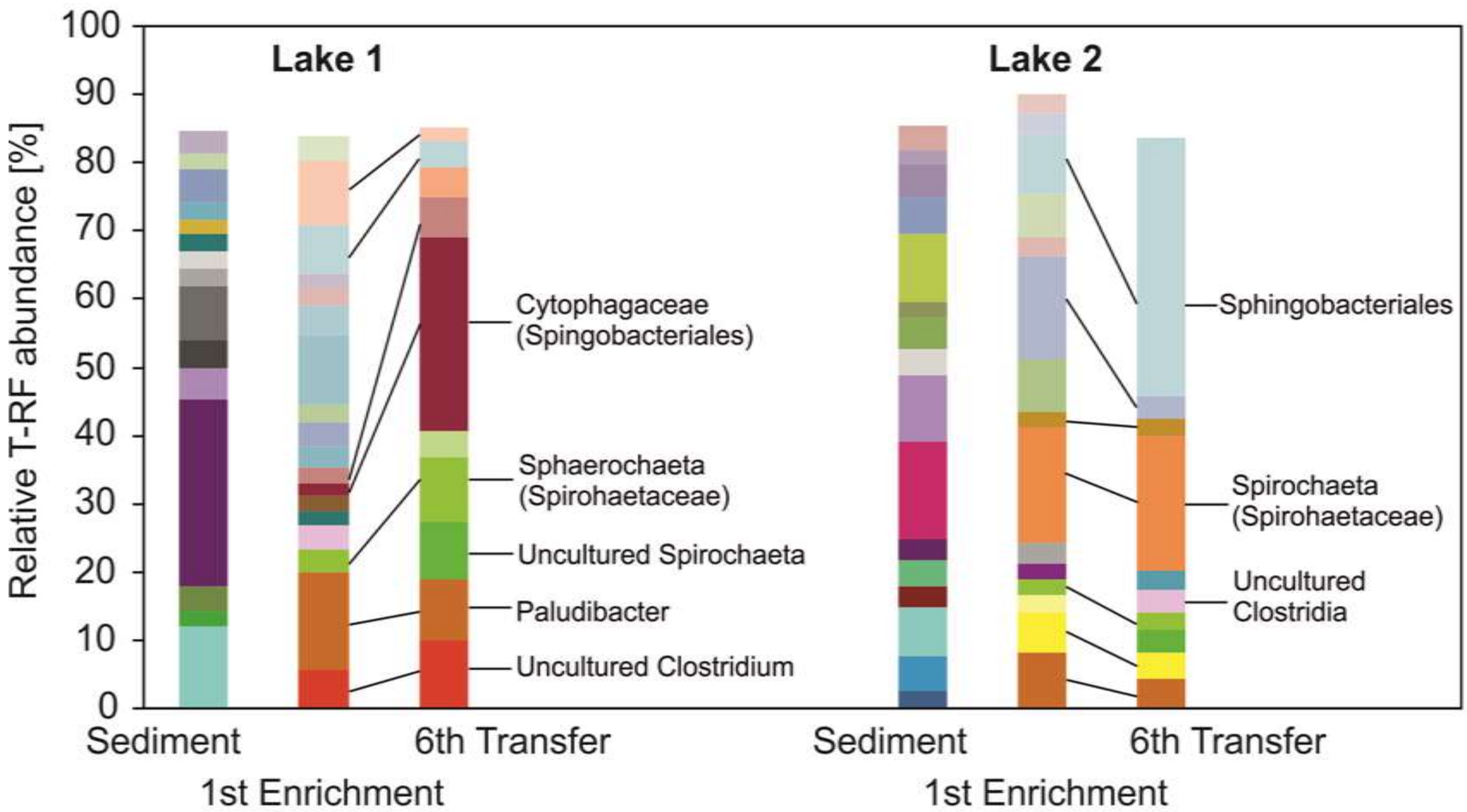
Alkaline pre-treatment



→ Chemical pre-treatment enhanced methane yield significantly

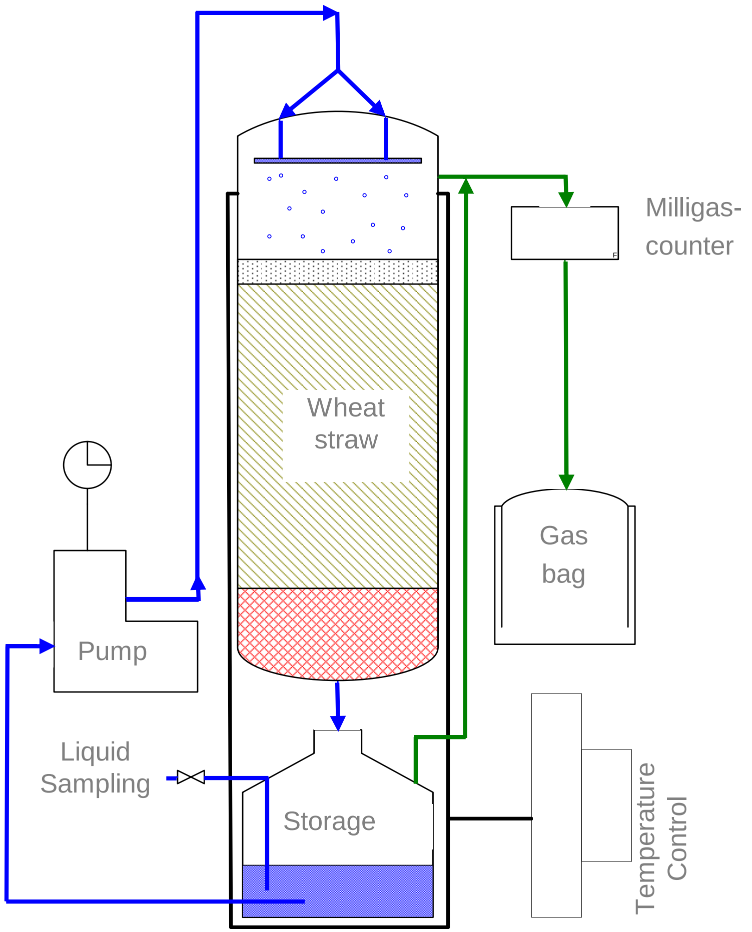
→ Faster degradation of the pre-treated straw with enrichment cultures

Molecular characterization - Bacteria



Porsch *et al.* (2015) Characterization of wheat straw-degrading anaerobic alkali-tolerant mixed cultures from soda lake sediments by molecular and cultivation techniques. Microbial Biotechnol. 8(5):801-814

Bioaugmentation potential – solid state fermentation



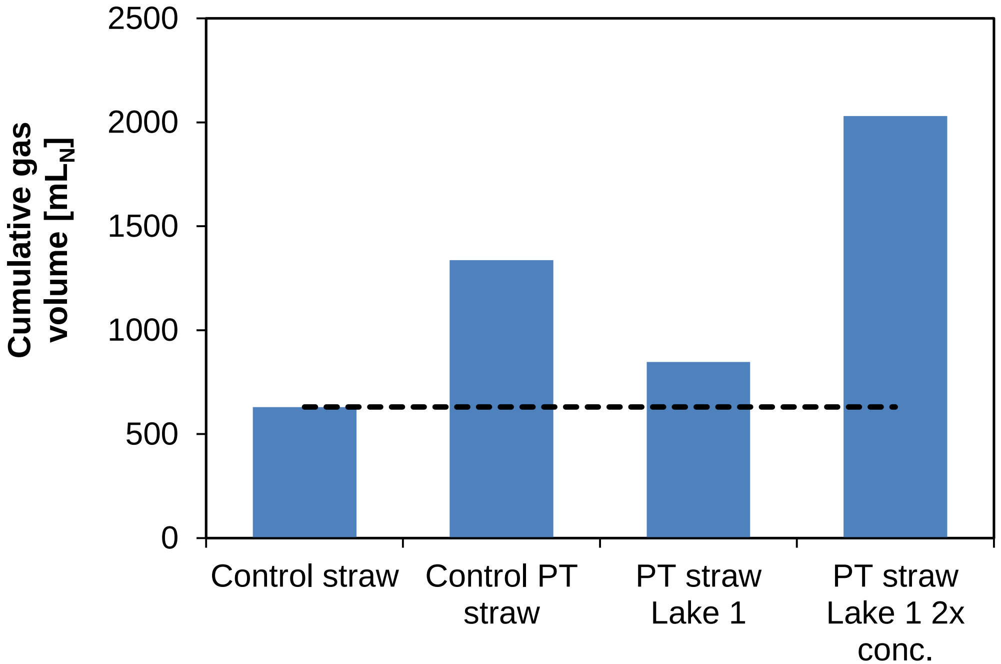
Sträuber *et al.* (2015) Improved anaerobic fermentation of wheat straw by alkaline pre-treatment and addition of alkali-tolerant microorganisms. *Bioengineering.* 2:66-93

Set-up in duplicates:

- Wheat straw was pre-treated with 57 mM $Ca(OH)_2$ (23 mL/g straw) for 24 h
- Straw was filled in 1.7-L-column reactors with percolation
- Process liquid (tap water) was inoculated with old percolation liquid or enrichment culture
- Running time: 2 weeks

Wheat straw	Microbes
No pre-treatment	Old percolation liquid
Pre-treatment	Old percolation liquid
Pre-treatment	Culture Lake 1
Pre-treatment	Culture Lake 1 double conc.

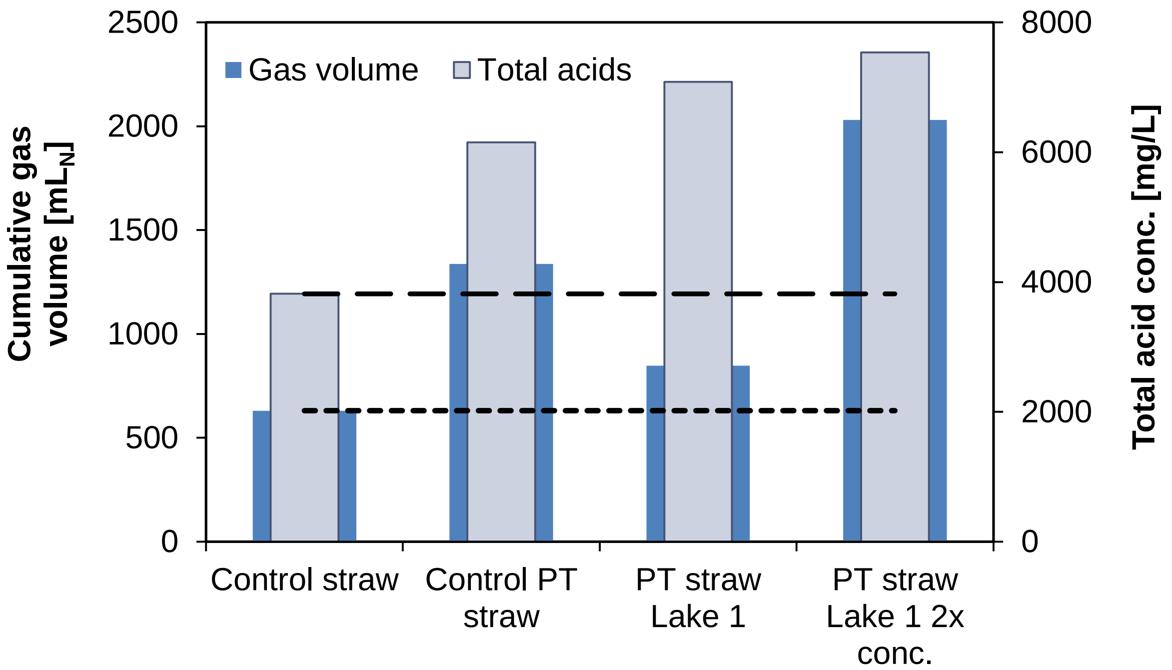
Pre-treatment and bioaugmentation – solid state fermentation



	Gas composition [%]			
CO_2	96	98	76	98
H_2	4	2	-	2
CH_4	-	-	24	-

Sträuber *et al.* (2015) Improved anaerobic fermentation of wheat straw by alkaline pre-treatment and addition of alkali-tolerant microorganisms. *Bioengineering.* 2:66-93

Pre-treatment and bioaugmentation – solid state fermentation



	Acid conc. [mg/L]			
Acetate	2300	3900	4100	4500
Butyrate	1000	1500	900	1900
Propionate	100	300	500	500
Others	400	500	300	700

→ Better hydrolysis and acidogenesis after chemical pre-treatment and bioaugmentation

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Examples for bioaugmentation

Substrate	Treatment	Sytem	Effect	Reference
Rice straw	Complex community (Firmicutes, Bacteroidites, Proteobacteria)	batch	+9% methane	Yan *et al.* (2010)
Corn straw	Complex community (Yeasts, cellulolytic bacteria, lactic acid bacteria)	batch	+33% biogas	Zhong *et al.* (2011)
Wheat straw	*Clostridium cellulolyticum*	batch	+13% methane	Peng *et al.* (2014)
Celluloses, maize, and grass silage	Rumen anaerobic fungi (best strain: *Anaeromyces* sp. KF8)	Batch, semi-continuous	Batch: 22% Semi-cont.: 4%	Prochazka et al. (2012)
Cattail, corn silage	Rumen fungus (*Piromyces rhizinflata*)	two-stage system	No significan effect (faster process)	Nkemka *et al.* (2015)
Cellulose	Ruminal content + waste treatment sludge	batch	No effect	Chapleur *et al.* (2014)
Corn stower	Repeated inoculation (Proprietary bioculture, Clostridia)	two-phase AD system	+56% methane	Martin-Ryals *et al.* (2015)

Yan et al. (2012) Bioresour Technol 111:49–54

Zhong et al. (2011) Bioresour Technol 102(24):11177–11182

Peng et al. (2014) Bioresour Technol 152:567–571

Nkemka et al. (2015) Bioresour Technol 185 79–88

Chapleur et al. (2014) FEMS Microbiol Ecol 87:616–629

Martin-Ryals et al. (2015) Bioresour Technol 189:62–70

Prochazka et al. (2012) Eng Life Sci.12(3):343–351

Fungal pre-treatment used by wood-feeding animals

Fungus-growing termites (*Termitidae, Macrotermitinae*)

▪Abundant in Asian and African tropics

▪Consume more than 90% of dry wood in some arid tropical areas

▪Specific symbioses with basidiomycete white-rot fungi (genus *Termitomyces*)

▪Termite nest has an optimal, controlled humidity and temperature for the growth of *Termitomyces*

▪Fungi have the ability to degrade lignin

▪Cellulose degraded partially by the cellulase produced by the termite

▪Fungi supplies also cellulase and xylanase to act synergistically with the enzymes produced by the termite

Ohkuma (2003) Appl Microbiol Biotechnol 61:1-9

Fungal pre-treatment used by wood-feeding animals

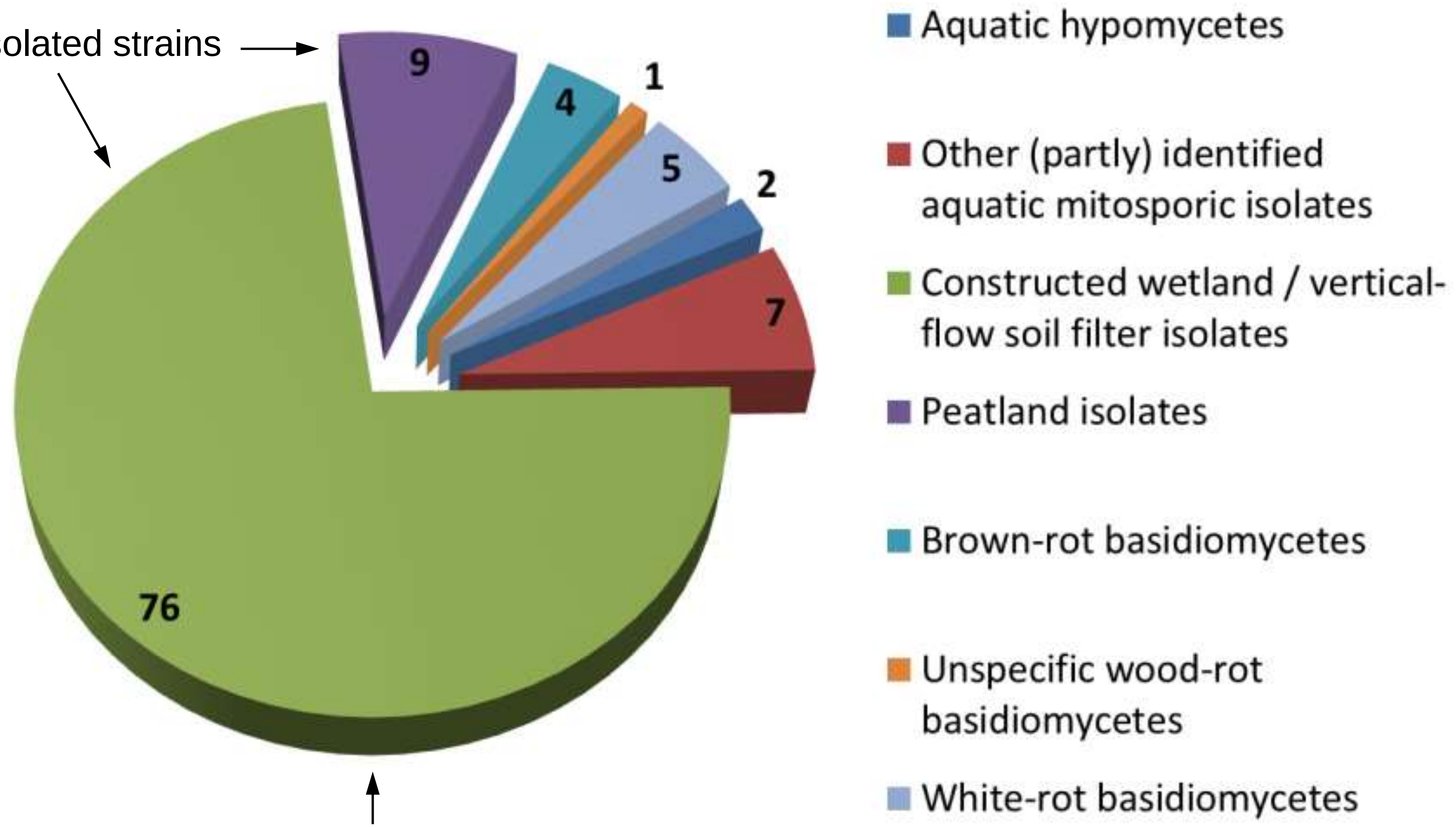
Woodwasps (Siricidae)

- Woodwasp carry arthrospores of basidiomycete fungi (*Amylostereum*)
- Females make holes into new host trees and deposit fungal arthrospores together with their eggs
- Fungi decompose the cellulose and/or lignin in the wood
- Larvae acquire several fungal enzymes while ingesting mycelium tissue and wood

Kukor J.J., Martin M.M. (1983) Acquisition of digestive enzymes by siricid woodwasps from their fungal symbiont. Science. 220: 1161-1163.

Fungal pretreatment

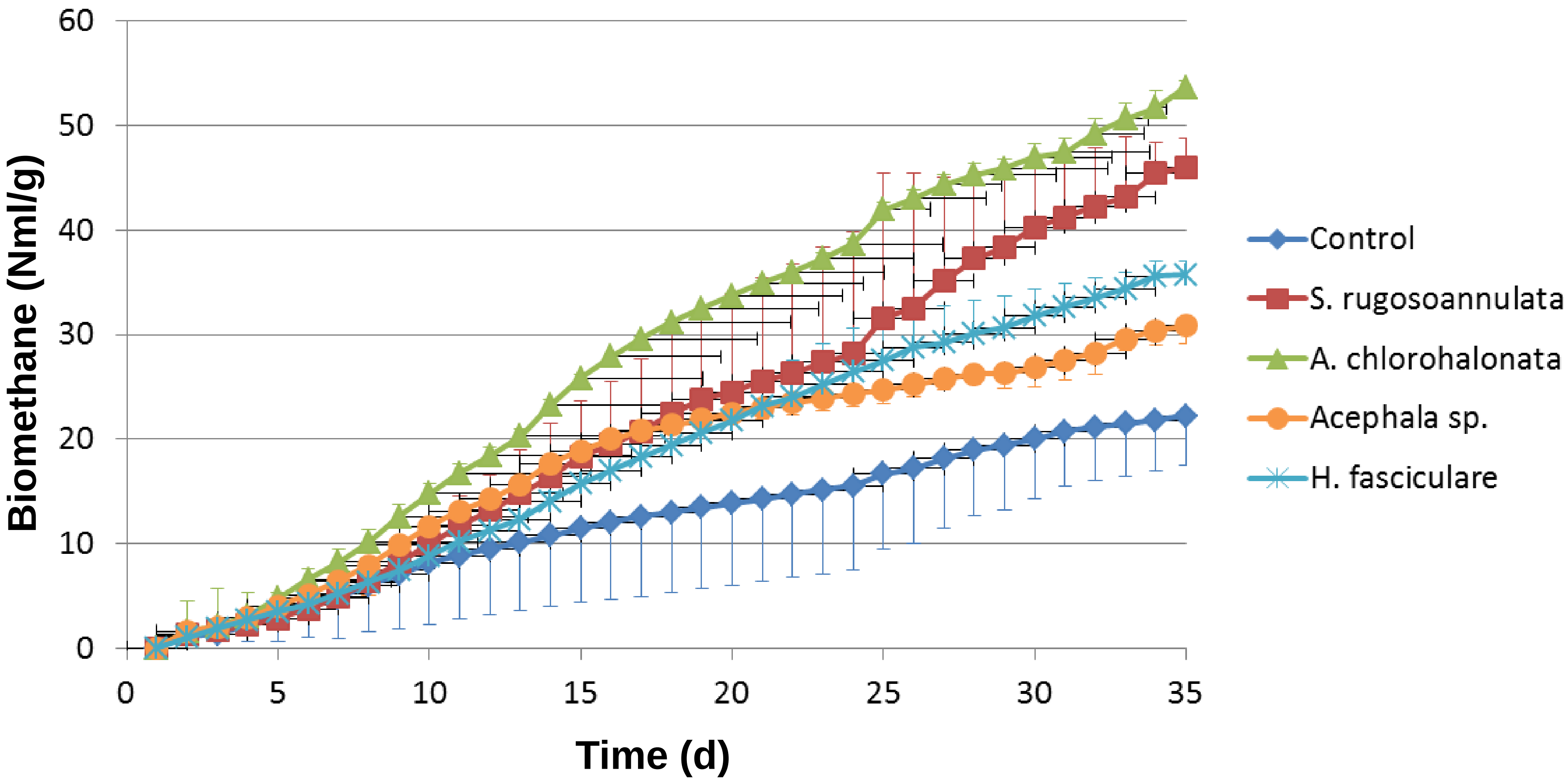
Newly isolated strains



Constructed wetlands & vertical-flow soil filters; CoTra (compartment transfer) pilot-scale groundwater treatment plant (Leuna, Saxony-Anhalt, Germany)

Fungal pretreatment

Wheat straw: lab scale pre-treatment with the most promising strains + subsequent discontinuous biogas tests:



Conclusions

Animal gut systems

- Combination of various treatments integrated to the microbial AD process is responsible for the effectiveness of the animal gut systems
- Enzymatic treatments with a variety of enzymes improve the yield of the microbial digestion
- Microoxic conditions (radial and axial gradient of oxygen) might be responsible for the improved delignification
- Continuous removal of the VFAs (absorption) and H_2 (methanogenesis) improves the fermentation
- Retention of microorganism is important (adhesion to the epithelium, trapping in the mucus)
- Compartmentalization is an important feature of the gut systems (CSTR vs multiple-stage systems)

Engineered systems

- The highest methane yield can be achieved by combining pre-treatment types and using mixed inocula
- Economic considerations should be taken into account

Thank you for your attention !!

MicAS Group

We know how to perform Anaerobic Digestion!

with