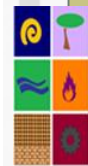


**FEASIBILITY OF BIOFUELS PRODUCTION: COMBINING  
H<sub>2</sub>-CH<sub>4</sub> AND LIPID PRODUCTION FROM FOOD WASTES  
USING MIXED ANAEROBIC MICROFLORA**

X. Gómez, C. Fernández, E.J. Martínez, J. Fierro, F.  
González-Ándres



universidad  
de León



Instituto de Medio Ambiente,

Recursos Naturales y

Biodiversidad

# TABLE OF CONTENT

- **IRENA**
- **Food waste valorisation**
- **Digestion and fermentation**
- **Coupling H<sub>2</sub>-CH<sub>4</sub> production**
- **Feasibility of lipid production from the process**



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Biodiversidad



The research Group of Chemical Engineering, Environmental and Bioprocess (located at IRENA) has dedicated its activity to the valorisation of waste

In the last ten years our research work has focus on:

**Energy recovery from organic waste: anaerobic digestion and pyrolysis.**

**Wastewater treatment by MEC and MFC.**

**Production of bio-fertilizers.**

**Hydrogen production from biological fermentation of organic waste**

**Development of prototypes for energy recovery**



# FOOD WASTES VALORISATION

- **Food wastes: food losses – food processing wastes**



Edible food

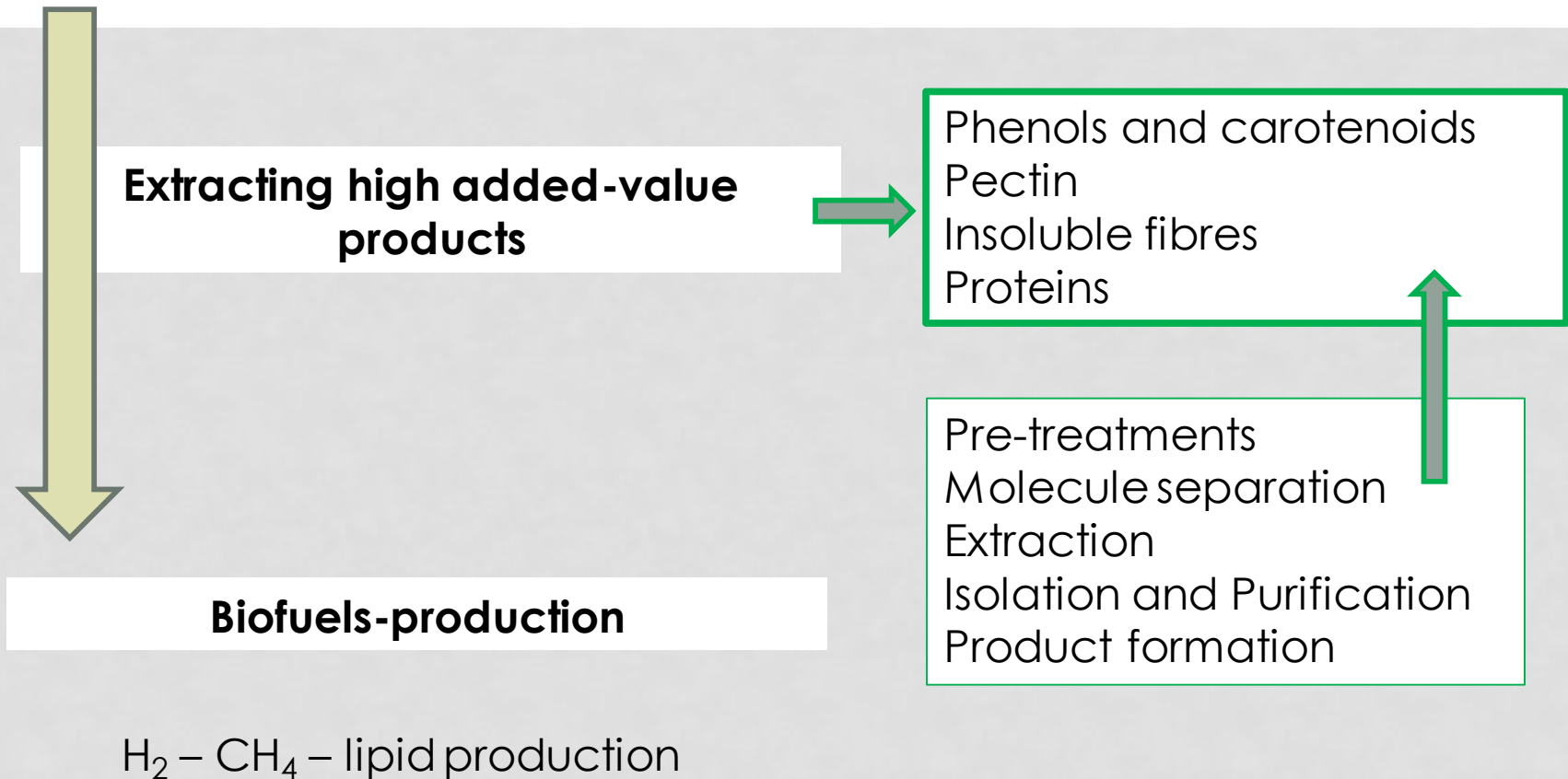
Discarded in different stages  
of the food supply chain

- Production
- Postharvest
- Handling
- Processing
- Distribution
- Consumption



utilization and disposal of food waste should  
yield benefits to the industry, society, and  
environment

# FOOD WASTES VALORISATION



# CHEESE WHEY VALORISATION

Cheese whey (CW) is a by-product from cheese factories that represents between 85 and 90% of the total volume of processed milk.

**Lactose** is the main component (4.5–5%), but other substances are also present:

proteins (0.6–0.8%),  
lipids (0.4–0.5%)  
and salts (0.7%)



**Proteins concentrates**  
**Saccharide mixtures**

## Whey treatment

- Skimming for casein fines and whey cream removal
- Protein concentration by membrane filtration
- separation of glycoproteins in liquid form (heat coagulation, cooling, precipitation and centrifugation)
- Lactose recovery: time-temperature depended crystallisation by adding seed crystals



**Animal feeding**



**Biofuels-production**

H<sub>2</sub> – CH<sub>4</sub> – lipid production



# FOOD WASTES FERMENTATION

REFERENCIA PROYECTO: IPT-2012-0144-120000



MINISTERIO DE  
ECONOMÍA Y  
COMPETITIVIDAD



## ANAEROBIC DIGESTION FERMENTATIVE H<sub>2</sub> PRODUCTION

Single stage process

Two-stage: Evaluating hydrolysis and H<sub>2</sub> production

Stream Recycling



BIOMASA PENINSULAR

# CHEESE WHEY FERMENTATION

## ANAEROBIC DIGESTION FERMENTATIVE H<sub>2</sub> PRODUCTION



**Single stage process**

**Two-stage: Evaluating hydrolysis and H<sub>2</sub> production**

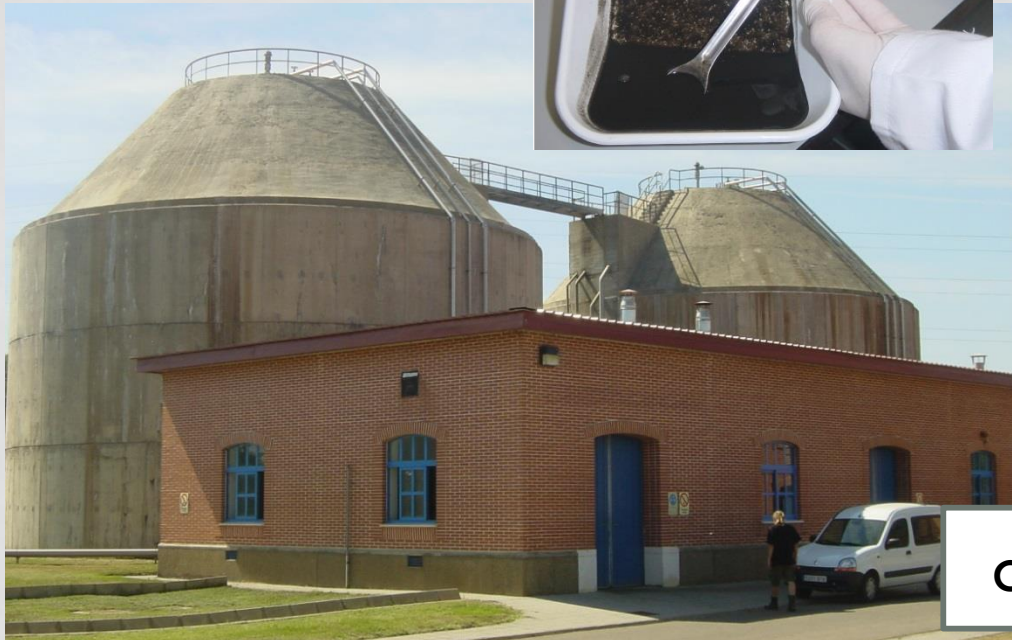
**VFA accumulation**

**Membrane separation for effluent clarification**

# MATERIAL & METHODS

Inoculum: WWTP of city of León

Digesters: WAS and primary sludge



Parameters	Units	Inoculum
TS	(g L <sup>-1</sup> )	18.3 ± 0.1
VS	(g L <sup>-1</sup> )	9.1 ± 0.1
COD	(g L <sup>-1</sup> )	25.6 ± 4.6
Alkalinity	(g L <sup>-1</sup> )	1.8 ± 0.2
KN	(g L <sup>-1</sup> )	1.3 ± 0.1
TP	(g L <sup>-1</sup> )	0.5 ± 0.1
NH <sub>4</sub> <sup>+</sup>	(mg L <sup>-1</sup> )	578 ± 21
K <sup>+</sup>	(mg L <sup>-1</sup> )	19.1 ± 5.2
Na <sup>+</sup>	(mg L <sup>-1</sup> )	49.8 ± 9.1

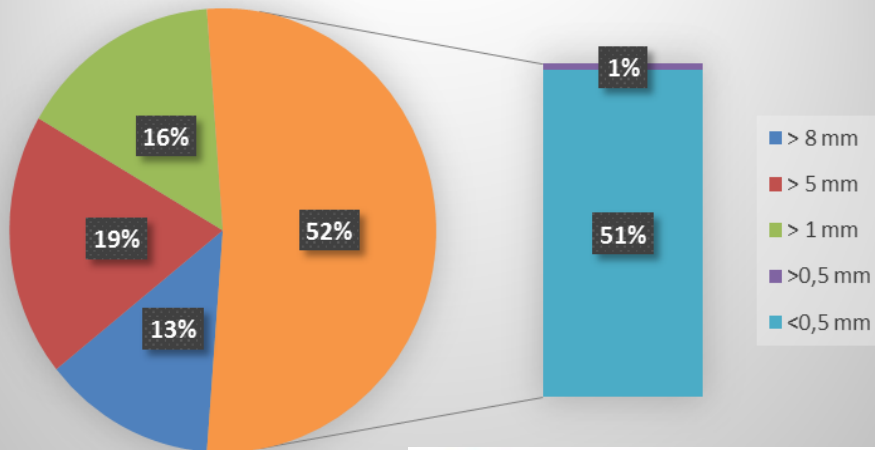
Chemical characteristics of inoculum<sub>1</sub>

# MATERIAL & METHODS

## Substrate

Food wastes: Obtained from food processing industry elaborating salads and fresh packed fruits.

### Particle size distribution



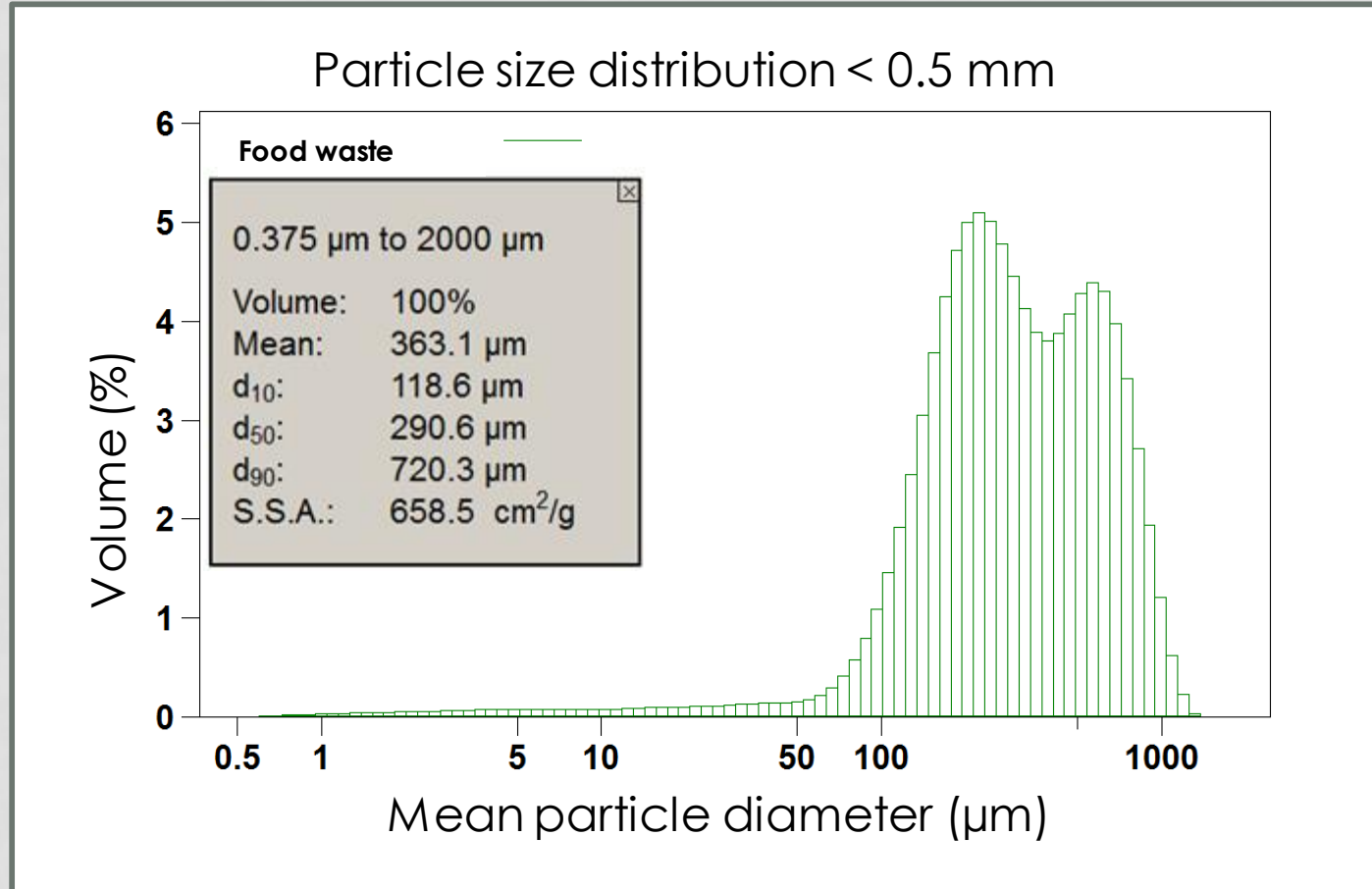
Parameters	Units	Food wastes
TS	(g kg <sup>-1</sup> )	123.7 ± 2.2
VS	(g kg <sup>-1</sup> )	115.1 ± 2.7
COD	(g Kg <sup>-1</sup> )	163.8 ± 7.6
Organic matter	(%)	86.4 ± 4.0
N	(%)	1.5 ± 0.1
Carbohydrates	(%)	44.5 ± 2.2
Cellulose	(%)	16.7 ± 0.8
Hemicellulose	(%)	19.2 ± 1.2
Ash	(%)	7.1 ± 0.4

JWCE Muffin Monster



**Chemical characteristics of Food Wastes**

# MATERIAL & METHODS



Particle size analysis was carried out using a Laser Diffraction particle Size Analyser LS 13 320 Beckmann Coulter. Samples were previously diluted in tap water for analysis. 10 measurements were performed for each sample

# MATERIAL & METHODS

## Substrate

Cheese whey: Obtained from Cheese processing factory (Zamora (Spain))

The CW liquid effluent was obtained after treatment with a membrane separation unit designed to recover proteins, thereby resulting in a liquid waste stream with poor nitrogen content

Parameters	Units	CW
TS	(g L <sup>-1</sup> )	33.1 ± 0.1
VS	(g kg <sup>-1</sup> )	30.1 ± 0.1
COD	(g Kg <sup>-1</sup> )	38.4 ± 1.4
Alkalinity	(mg L <sup>-1</sup> )	370 ± 10
KN	(g L <sup>-1</sup> )	0.4 ± 0.1
NH <sub>4</sub> <sup>+</sup>	(mg L <sup>-1</sup> )	26 ± 2.2
K <sup>+</sup>	(mg L <sup>-1</sup> )	639 ± 30
Na <sup>+</sup>	(mg L <sup>-1</sup> )	167 ± 10
Cl <sup>-</sup>	(mg L <sup>-1</sup> )	487 ± 20

**Chemical characteristics of CW**



# MATERIAL & METHODS

## Digestion and fermentation: Batch

Experiments were carried out in Erlenmeyer flasks of 250 mL

Substrate – Inoculum ratio: Controlled based on desired process performance.

Tap water was added when needed to complete a 250 mL volume

Replicates: measuring gas production and composition.

Control: Batch reactor containing only inoculum was used as blank.

Temperature: 34°C, this being controlled by a water bath. Agitation was provided by means of magnetic stirrers.



# MATERIAL & METHODS

Digestion under mesophilic conditions  
3 L volume reactor



## Analytical techniques

Kjeldahl nitrogen, TS, VS, COD,  
alkalinity, ammonium, and pH

Biogas composition and VFA  
(Varian CP 3800 GC)

Thermal analysis  
FTIR spectroscopy  
NMR

Soxhlet method



Digestion under  
thermophilic conditions  
5 L volume reactor

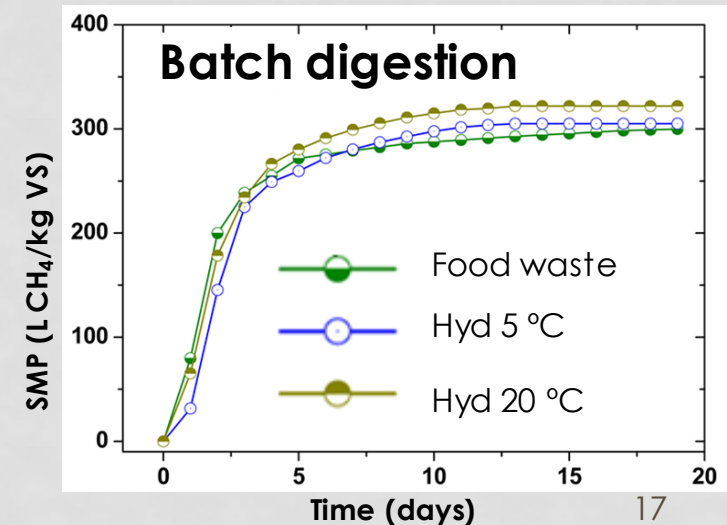
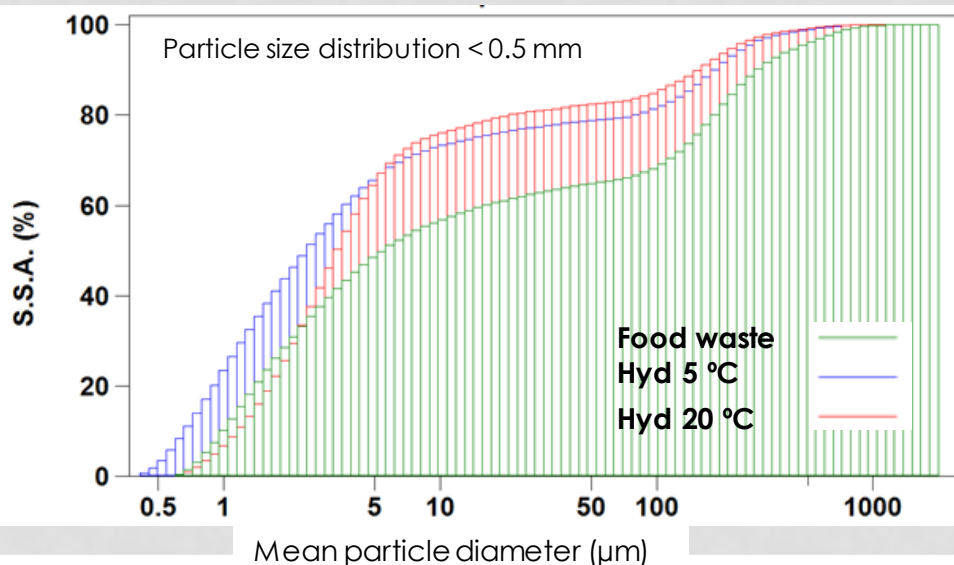


# RESULTS

Hydrolysis of vegetable wastes (3 d period) at 20 and 5 °C.

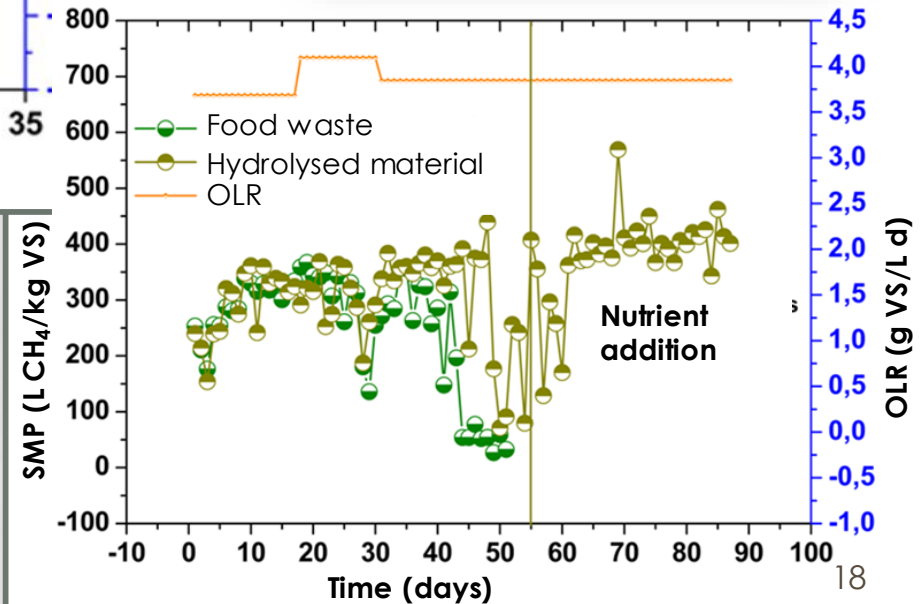
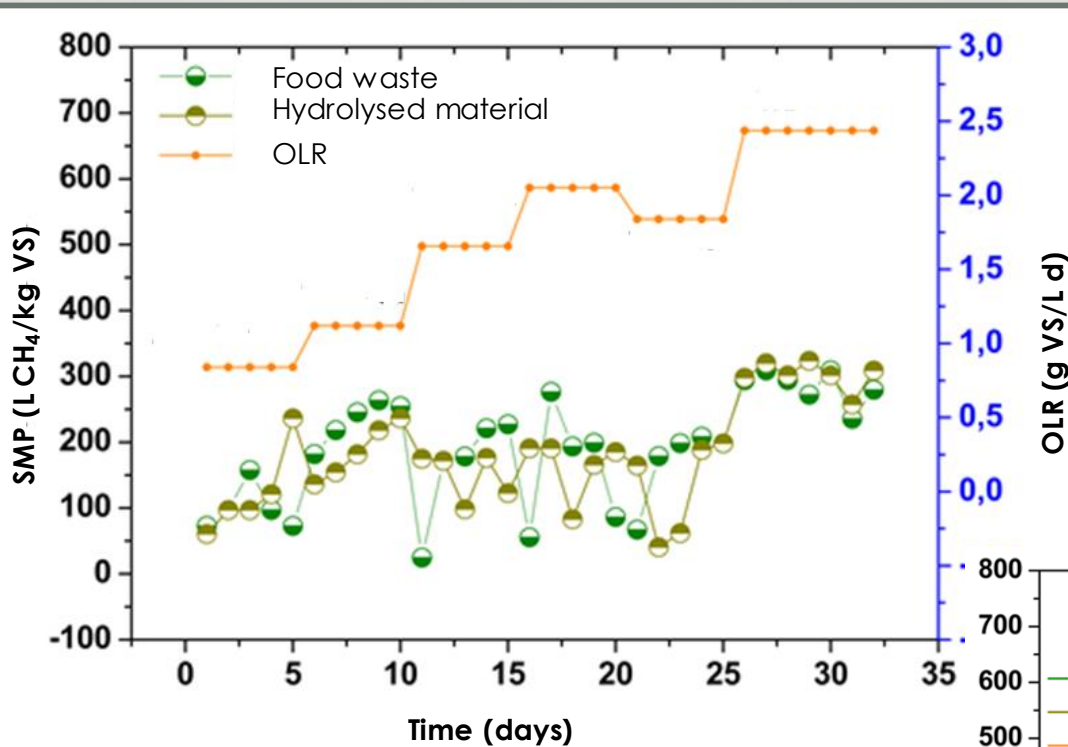
	Mean µm	S.S.A. cm <sup>2</sup> /g
Food waste	91.1	659
Hydrolysis 5°C	45.7	1312
Hydrolysis 20°C	37.6	1596

Hydrolysis		20°C	5°C
Gas production (mL)		272.5	140
Gas Composition	% CO <sub>2</sub>	99.2	99.6
	% CH <sub>4</sub>	0.8	0.4
COD soluble (g COD/L)		49.7	38.5
VFA			
Acetic (mg/L)		526	374
Butyric (mg/L)		65	69
Valeric (mg/L)		232	114



# RESULTS

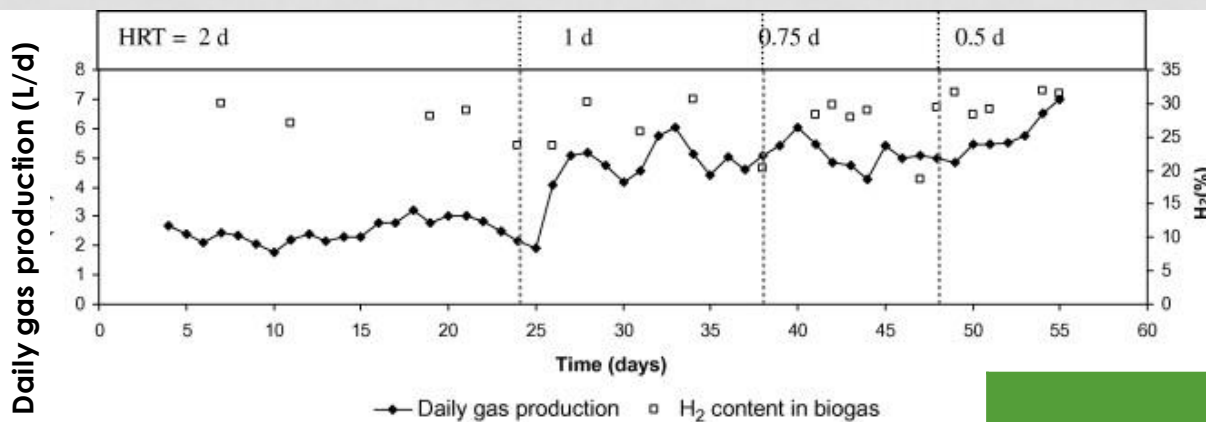
## Digestion of vegetable wastes



Parameters	Food Waste			Hydrolysed material		
	HRT (d)	30	30	30	30	30
OLR (g VS/L d)	3.68	4.09	3.84	3.68	4.09	3.84
Biogas (L)	5.4	5.9	3.8	5.3	6.1	6.5
CH <sub>4</sub> (%)	58	61	58	59	60	60
SMP (LCH <sub>4</sub> /kg VS)	290	299	195	291	300	350
pH	7.4	7.2	7.1	7.4	7.3	7.1

# RESULTS

## Coupling H<sub>2</sub> – CH<sub>4</sub> Production

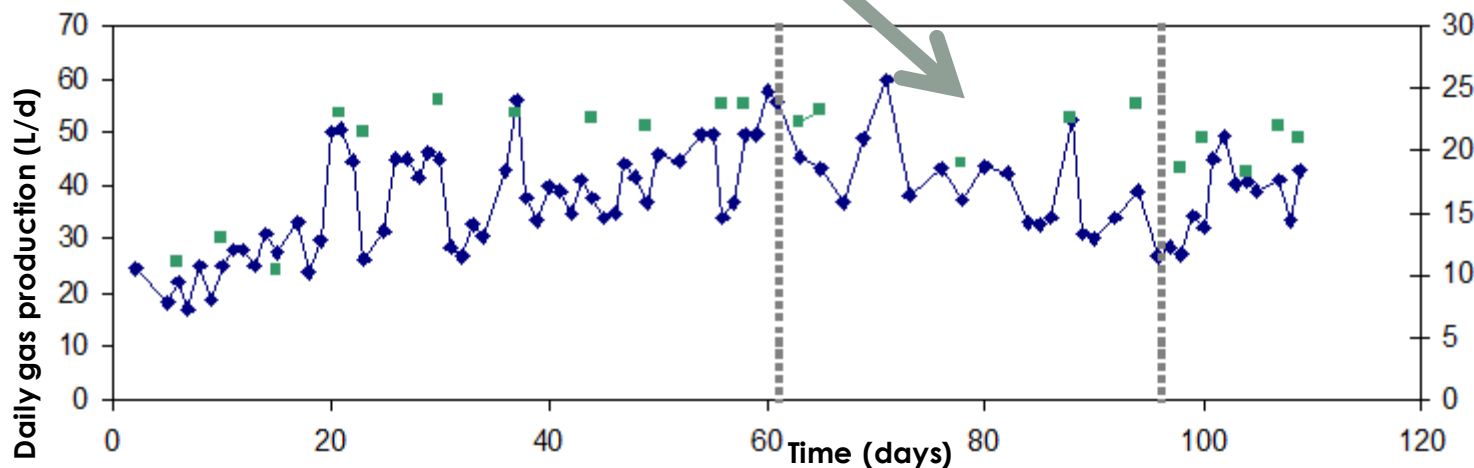


3 L reactor

V. Redondas, X. Gómez, S. García, C. Pevida, F. Rubiera, A. Morán, J.J. Pis. **Hydrogen production from food wastes and gas post-treatment by CO<sub>2</sub> adsorption.** Waste Management, Volume 32, Issue 1, 2012, 60 - 66

Supernatant  
recirculation (20% v/v)  
7500 mg CaCO<sub>3</sub>/L

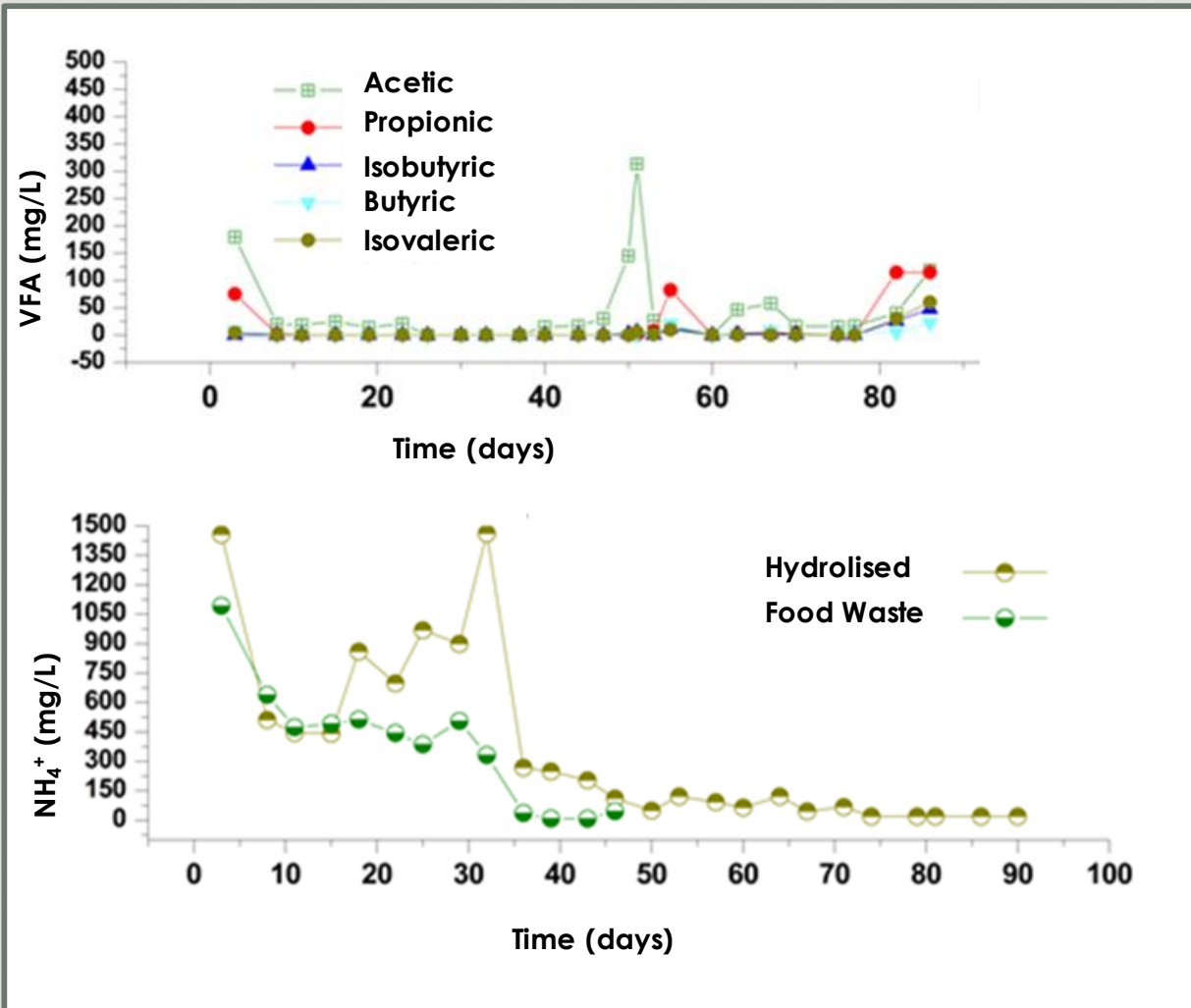
H <sub>2</sub> producing reactor				
HRT (d)	2	1	0.75	0.5
Daily gas production (L/d)	2.5	4.7	5.1	5.8
H <sub>2</sub> production (L/ Lr d)	0.23	0.42	0.44	0.6
SH <sub>2</sub> P (L/g VS)	20.6	18.5	14.5	13.1



25 L reactor  
HRT: 3 d

# RESULTS

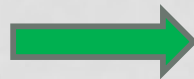
## CH<sub>4</sub> Production



# FEASIBILITY OF LIPID PRODUCTION

Single cell oil production from low cost substrate

Food wastes  
Municipal wastewater



*Cryptococcus curvatus*,  
*Yarrowia lipolytica*,  
and *Rhodotorula glutinis*



Biomass concentration: 0.58 – 1.5 g/L

Lipid content: 18,7 – 28.6%

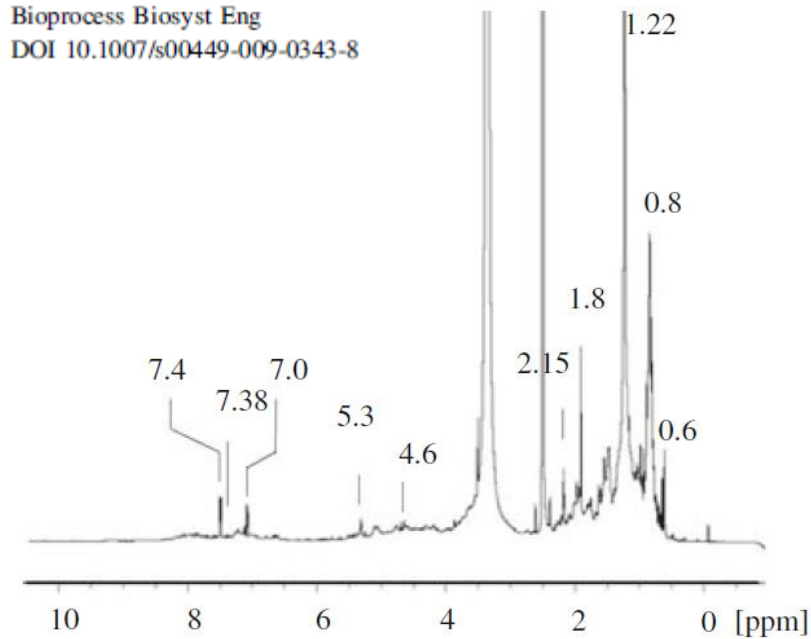
**Lipid Production by Culturing Oleaginous Yeast  
and Algae with Food Waste and Municipal Wastewater  
in an Integrated Process**

Zhanyou Chi • Yubin Zheng • Anping Jiang •  
Shulin Chen

Appl Biochem Biotechnol (2011) 165:442–453  
DOI 10.1007/s12010-011-9263-6

# RESULTS

Bioprocess Biosyst Eng  
DOI 10.1007/s00449-009-0343-8



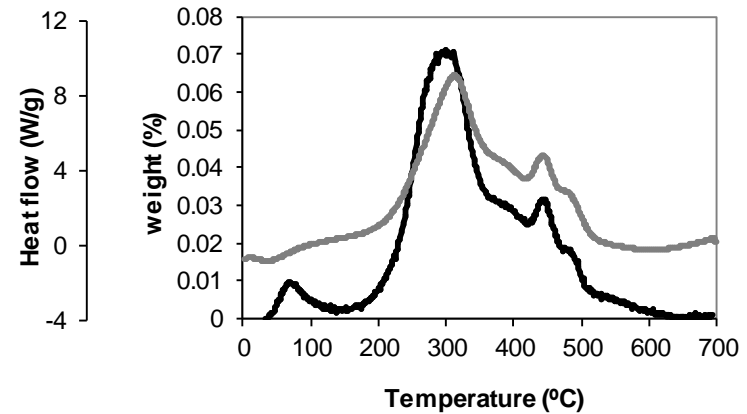
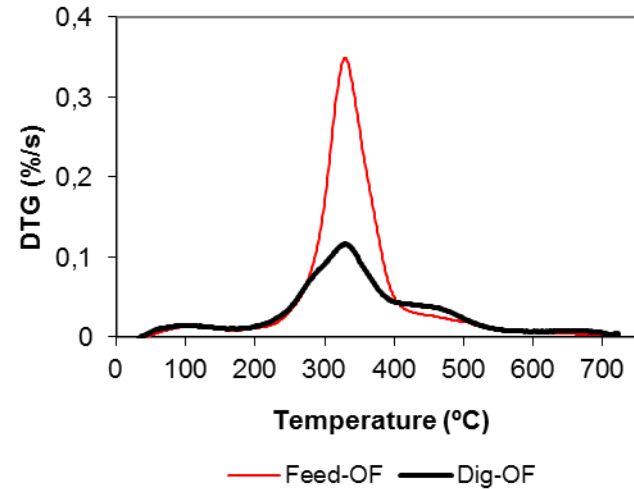
A comparison of analytical techniques for evaluating food waste degradation by anaerobic digestion

X. Gómez · M. J. Cuetos · B. Tartakovsky ·  
M. F. Martínez-Núñez · A. Morán

**Lipid extraction**

**2.15%** (Solid content: 23 g/L)

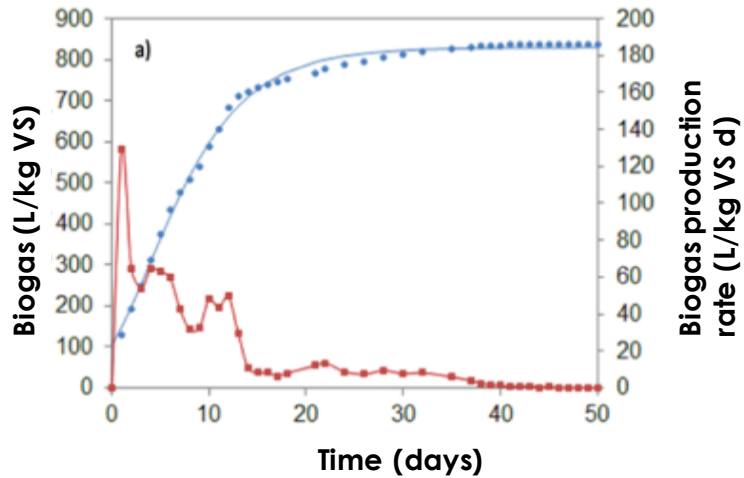
**Inoculum: 0.8%**



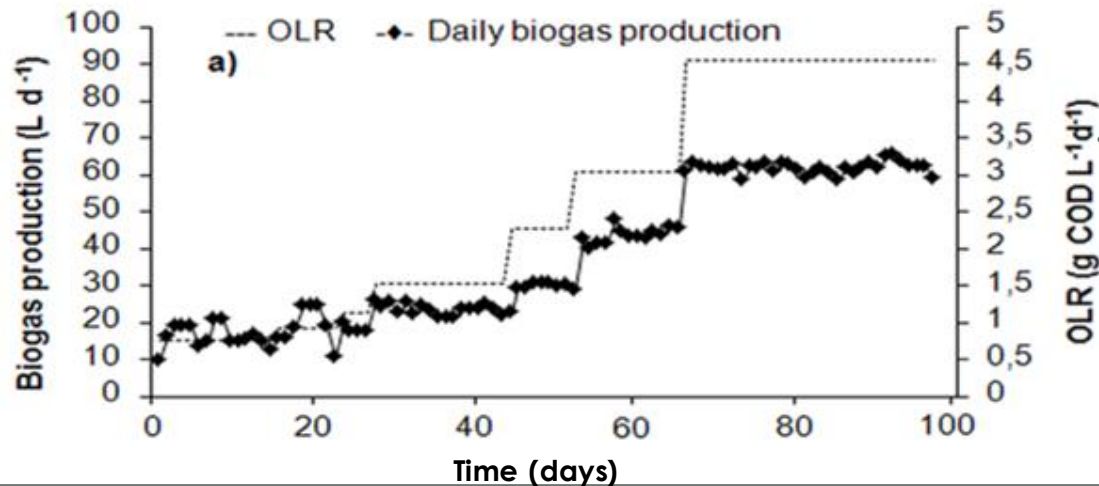
# RESULTS: Thermophilic

CH<sub>4</sub> Production: Cheese whey

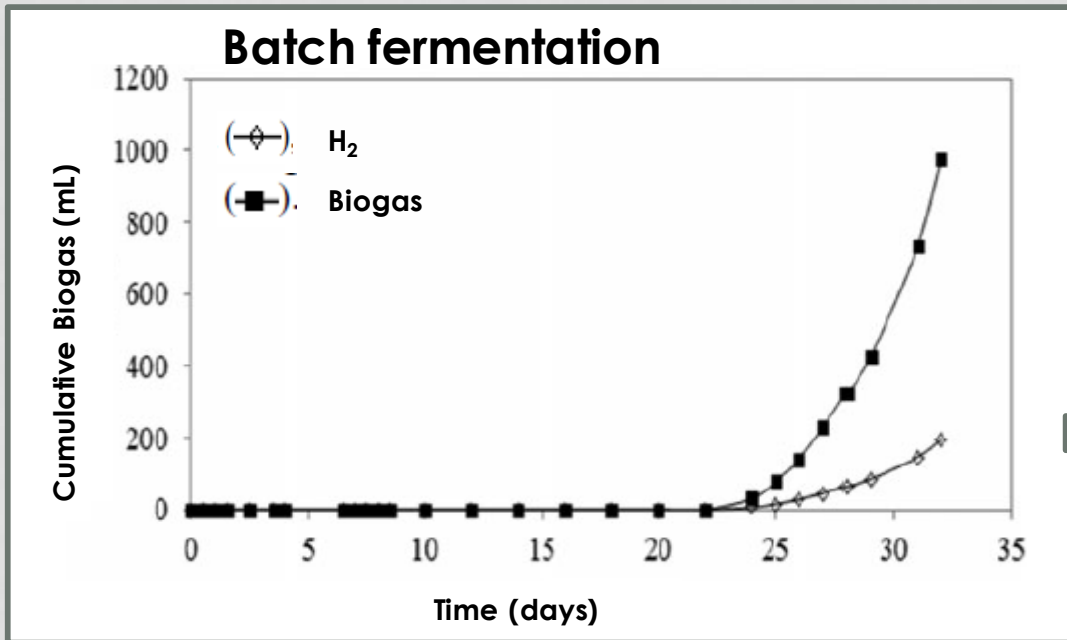
## Batch digestion



## 5 L semi-continuous digestion

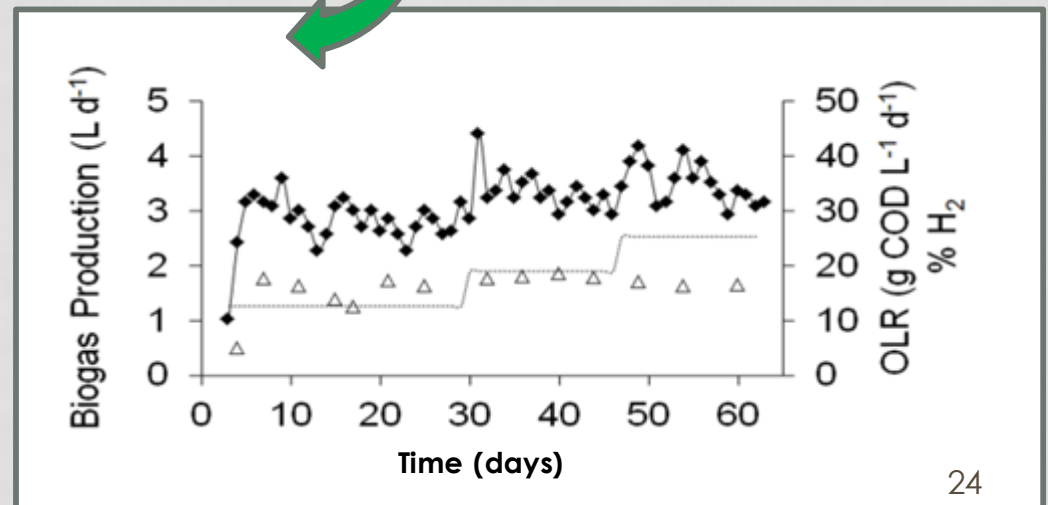


# RESULTS: H<sub>2</sub> - mesophilic



DGGE analysis: revealed that the genus *Clostridium* was dominant, *Clostridium acetobutyricum* (at 24 h) *Clostridium butyricum* (at 32 h)

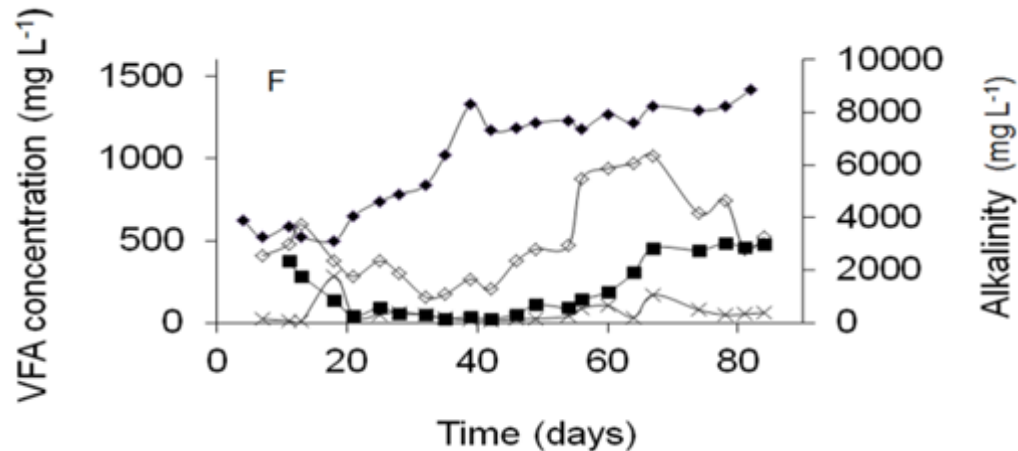
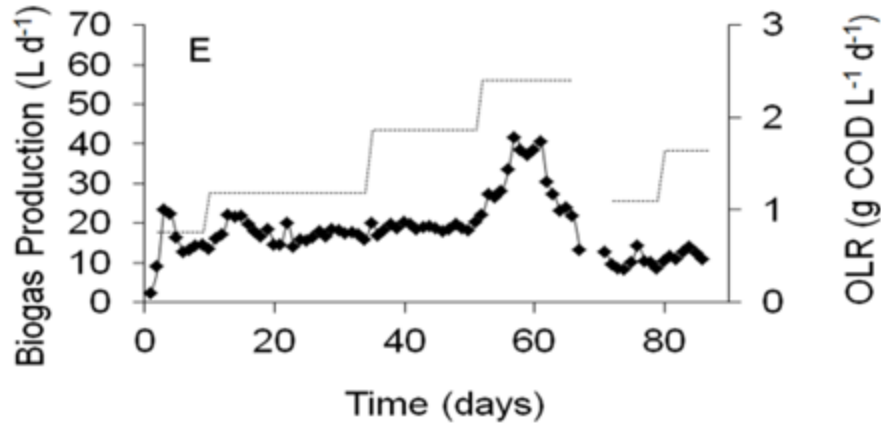
3 L reactor  
Mesophilic conditions





# RESULTS

## Coupling H<sub>2</sub> – CH<sub>4</sub> Production



Second Stage (25 L reactor)  
Thermophilic conditions  
Biomass accumulation

**Lipid extraction**

**Single digestion  
HRT 8.3 d: 1.61%**

**Second phase  
HRT 16.7: 0.81%**

# CONCLUSIONS

Food waste (Fruit and vegetables) and cheese whey valorisation by means of fermentative processes was evaluated. Results indicated high methane potential from these wastes. The need of nutrient addition to keep steady operation becomes an economical burden to the process.

Exploring the alternative of increasing the amount of lipid extracted from digestates may be a novel option for producing polar lipids.

Further studies are needed to explore the characteristics of polar lipids obtained from anaerobic microflora and its industrial application

## THANKS FOR YOUR ATTENTION



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Elia Judith Martínez Torres



Julio Fierro Fernández



Fernando González Andrés

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