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## **Valorisation of the organic fraction of Municipal Solid Waste: Improving succinic acid production efficiency by immobilized continuous fermentation**

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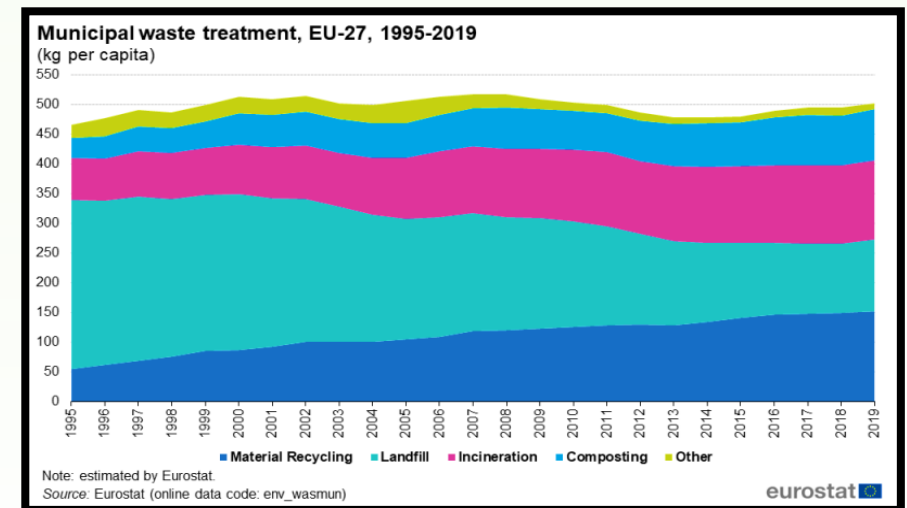
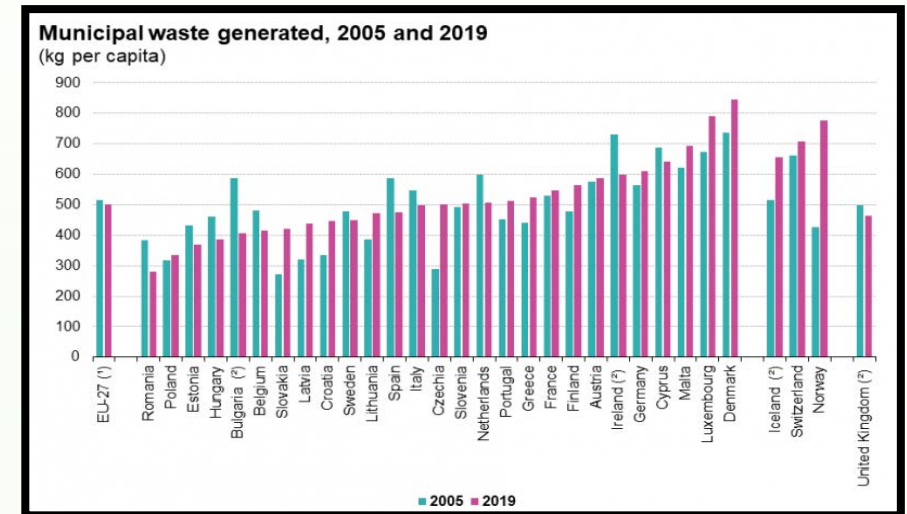
# Objectives

- Characterisation of biowaste fraction of municipal solid waste (MSW)
- Valorisation of biowaste fraction of MSW as potential fermentation feedstock for succinic acid production with *Actinobacillus succinogenes*
  - Batch fermentation
  - Fed-batch fermentation
  - Continuous fermentations in a cell free system and immobilised bacterial cells using glucose and OFMSW hydrolysate as carbon source



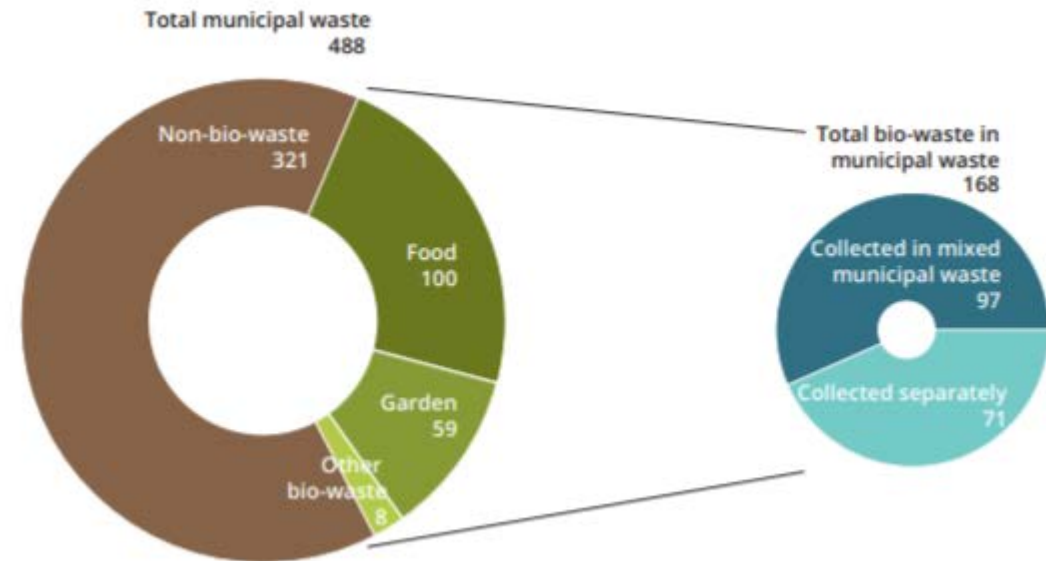
# Municipal solid waste (MSW) in Europe

- Municipal solid waste management is an important issue in Europe
- 502 kg of municipal waste per capita were generated in the EU in 2019
- 48% of municipal waste in the EU was recycled in 2019
- The total amount of municipal waste landfilled has diminished
- The landfilling rate in the EU dropped from 61 % (1995) to 23 % (2019)

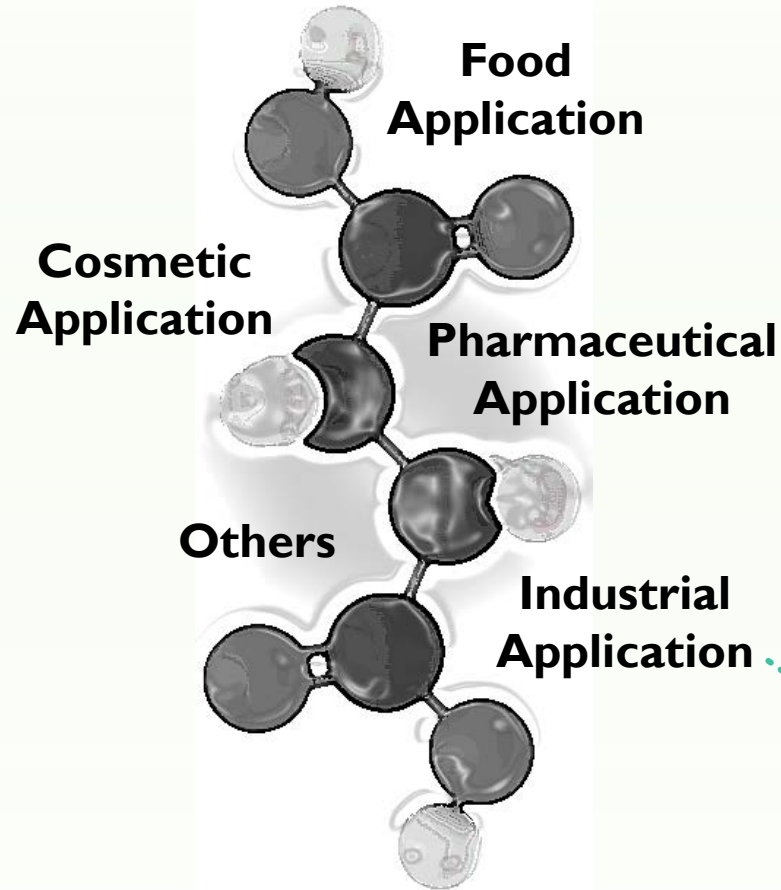


# Organic fraction of municipal solid waste (OFMSW) in Europe

- Bio-waste represents an important share of European municipal waste generation
- In 2017, the EU-28 generated 249 million tonnes of municipal solid waste of which about 34 % (86 million tonnes) was the bio-waste fraction
- Total municipal bio-waste in the EU-28 consist of:
  - 60 % Food waste
  - 35 % Garden waste
  - 5 % Other



# Potential of succinic acid as platform chemical



- One of the top 12 value-added bio-based chemicals
- Total succinic acid market demand is estimated around 50,000 metric tonnes in 2016 and is expected to reach 94,000 metric tonnes at the end of 2025 with an annual growth rate of 6.5%
- Intermediate in several chemical processes

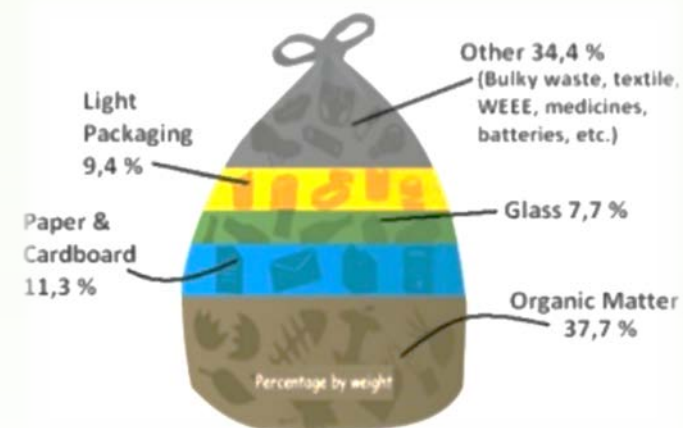
- 1,4-Butanediol (BDO) and Derivatives
- Polyesters polyols
- Resins, Coatings, Pigments, & Inks
- Solvents and Lubricants
- Polybutylene succinate (PBS)
- Plasticizers



# OFMSW hydrolysate composition

Liquid fraction composition		
Component	Aver	StDev
pH	5.12	0.06
Free amino nitrogen (mg/L)	203.6	0.32
Inorganic phosphorus (mg/L)	100.6	0.55
<b>Total sugars conc. (g/L)</b>	<b>89.9</b>	<b>2.10</b>
Sucrose	1.8	0.10
Glucose	75.9	0.60
Xylose	7.6	0.40
Galactose	1.4	0.50
Arabinose	0.5	0.40
Mannose	0.0-0.5	
Fructose	0.0-0.5	
Glycerol	2.0	0.60
Organic acids (g/L)	13.2	2.00
Lactic acid	10.7	2.00
Acetic acid	2.9	0.02

Solid fraction composition		
Component	Aver	StDev
Total dry weight of suspension (g/L)	114.2	
Moisture content of centrifuged solids (%)	54.3	0.41
Ash content (% dry basis)	25.0	0.60
Lipid content (% dry basis)	7.3	0.60
Protein content (% dry basis)	8.4	0.02



# Experimental design

## Batch and Fed-batch fermentations

### Bacterial strain:

*Actinobacillus succinogenes* 130Z

### Fermentation conditions:

Fermentation volume: 500 mL

Temperature: 37 °C

Agitation: 100 rpm

pH: 6.7

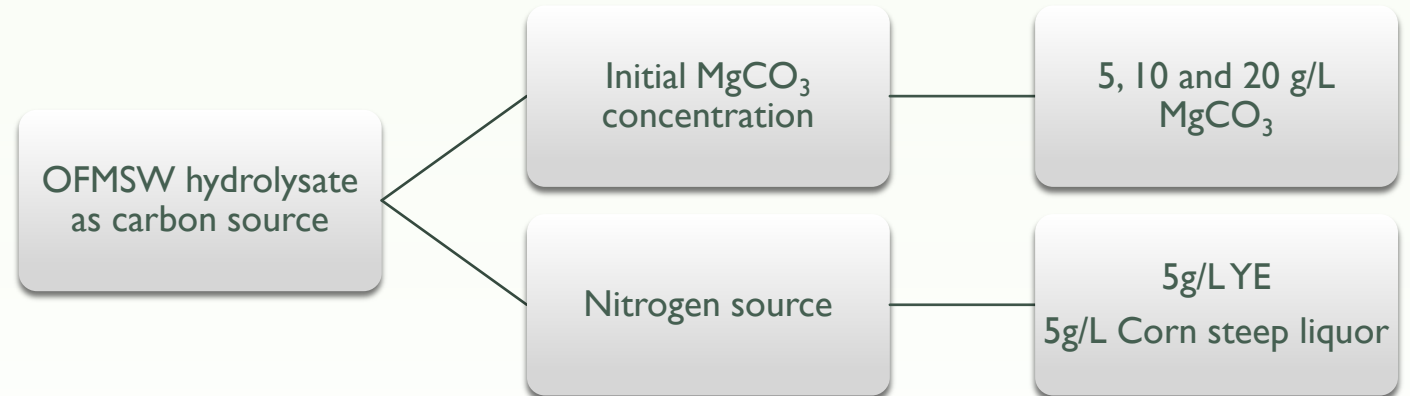
CO<sub>2</sub> flow : 0.5 L/min



### Batch fermentations

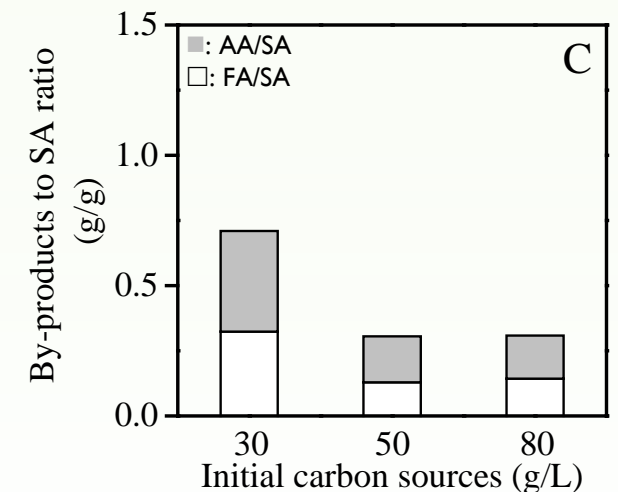
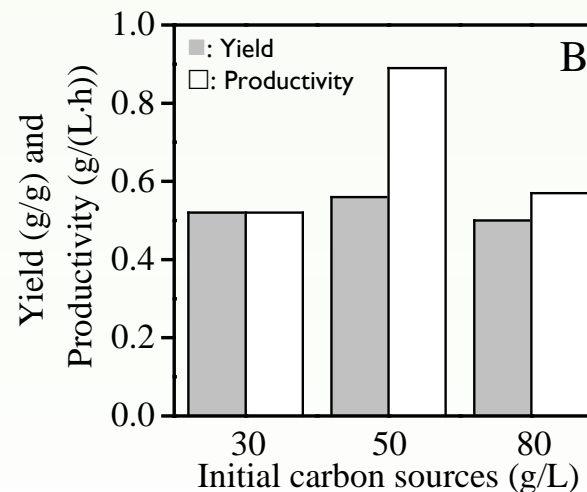
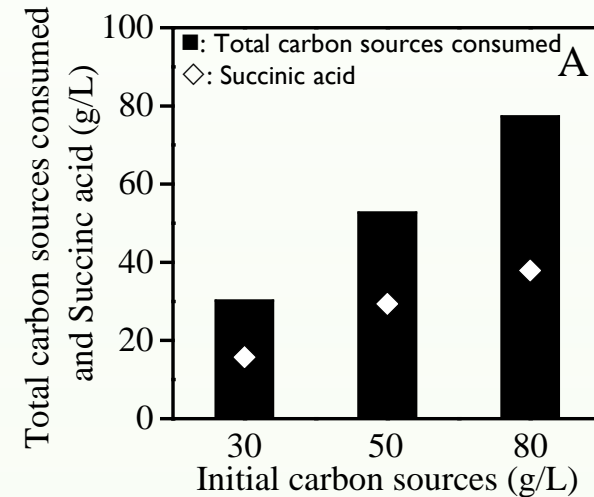


### Fed-batch fermentations



# Batch fermentations using OFMSW hydrolysate

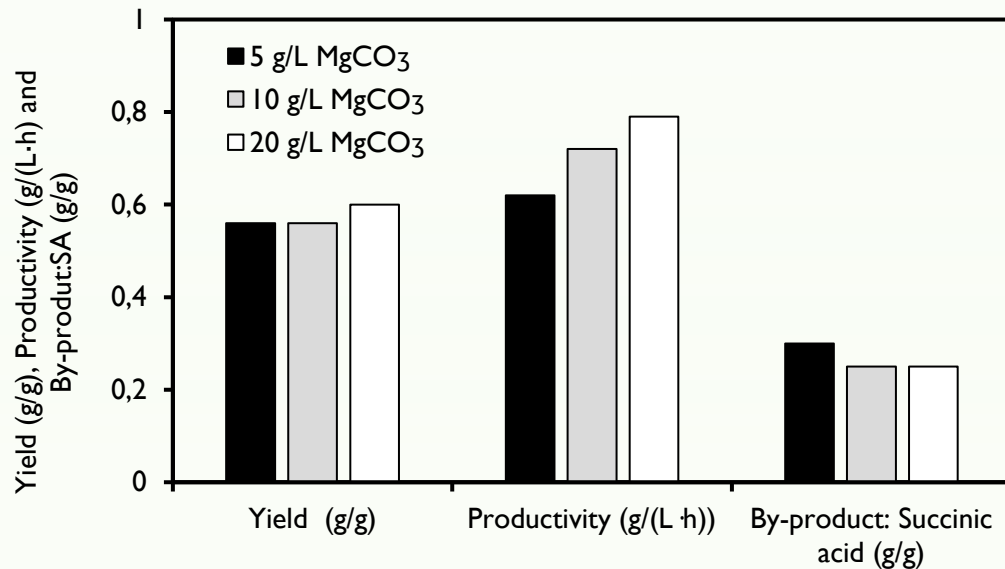
- OFMSW hydrolysate can efficiently used as fermentation feedstock for succinic acid production
- The total by-products to succinic acid ratio was decreased with increasing the initial total carbon source concentration
- Highest yield and productivity was observed at 50 g/L initial carbon source concentration



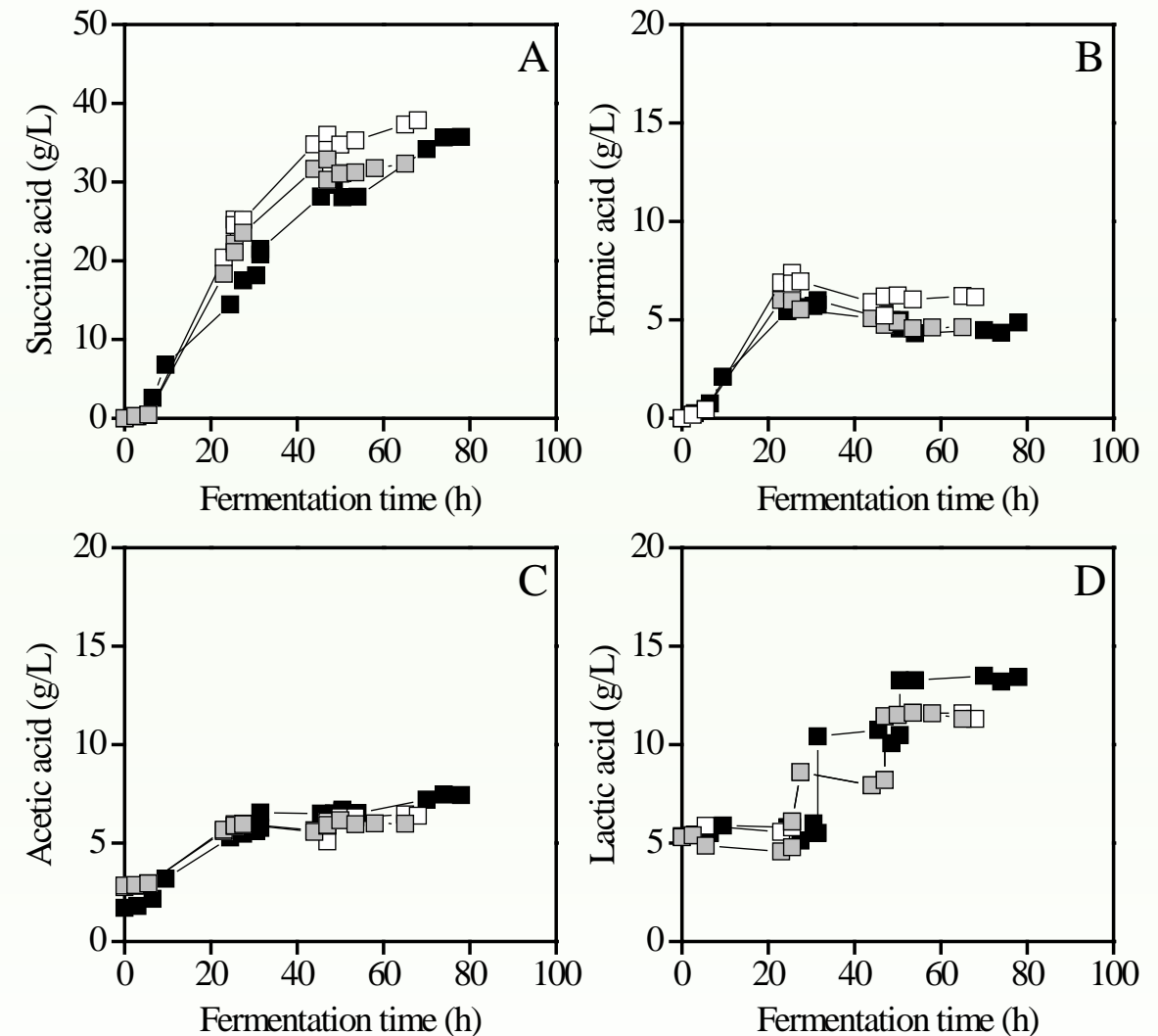


# Fed-batch fermentations

## Initial $MgCO_3$ concentrations



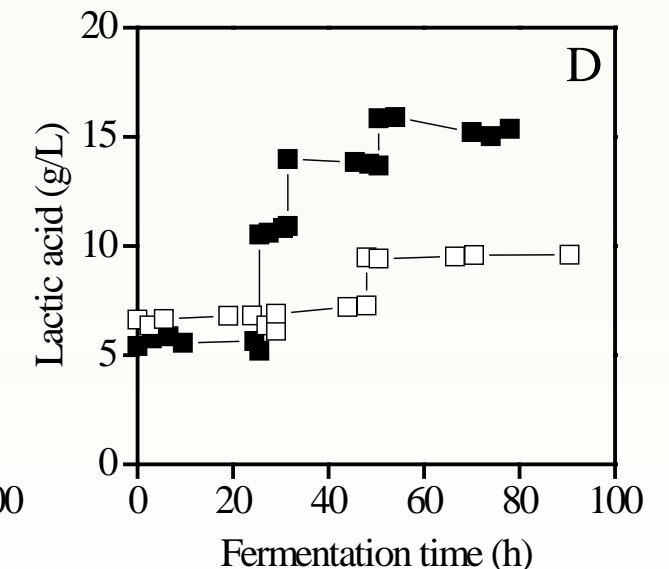
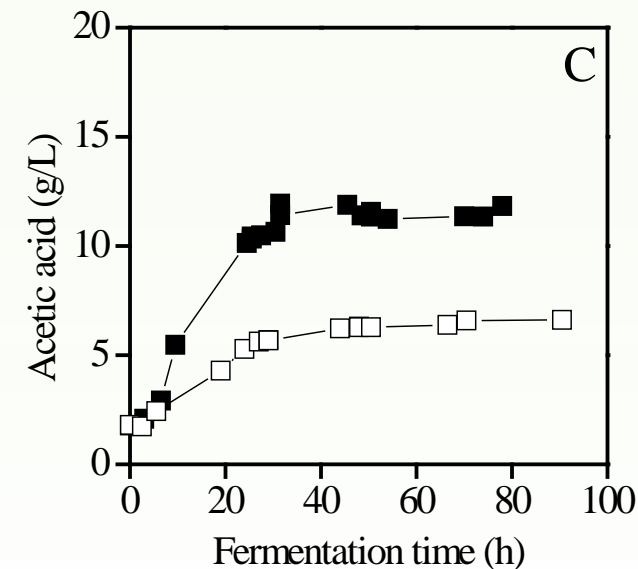
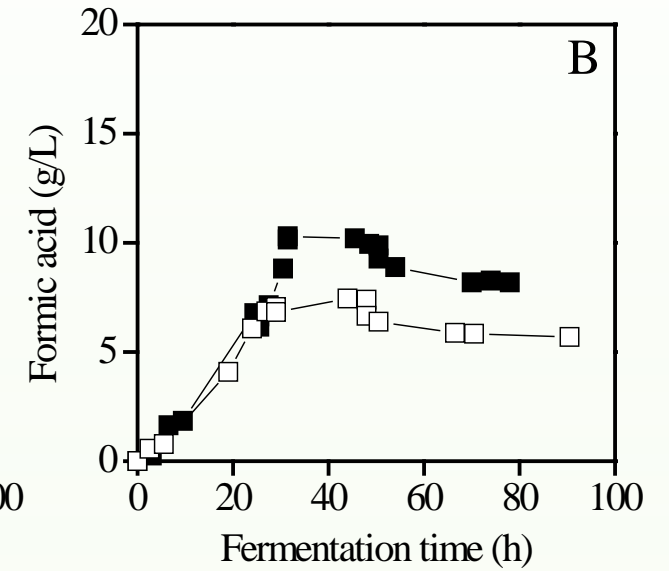
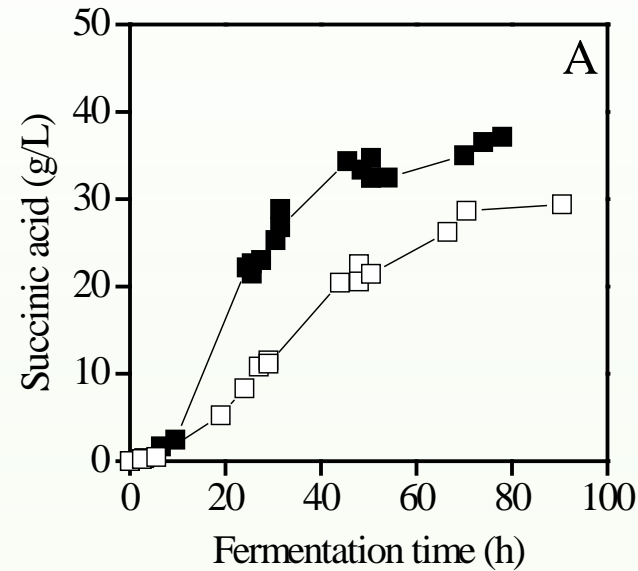
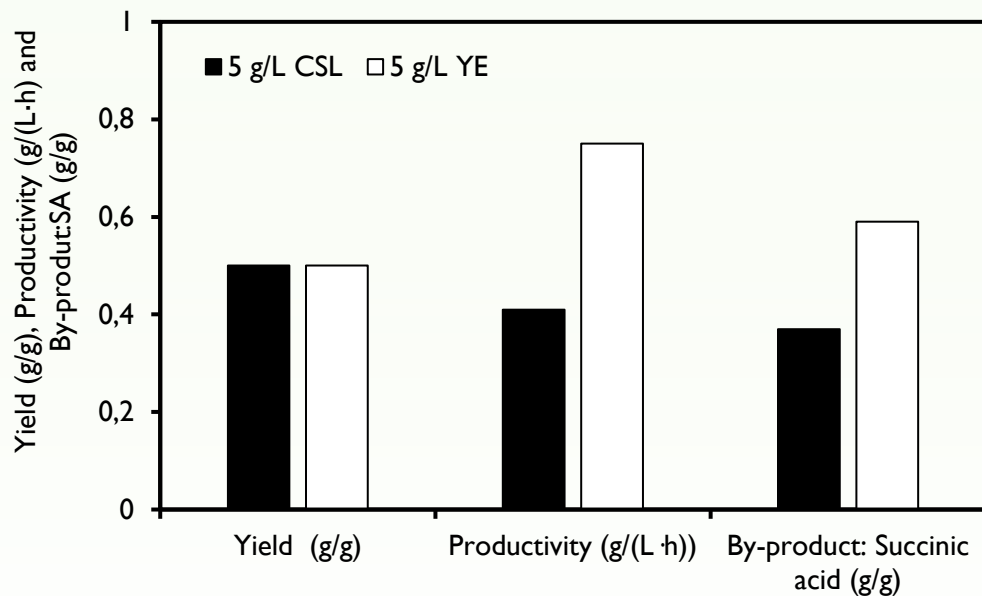
- Succinic acid production improved with increasing  $MgCO_3$  concentration
- No significant differences were observed in By-product:SA ratio



# Fed-batch fermentations

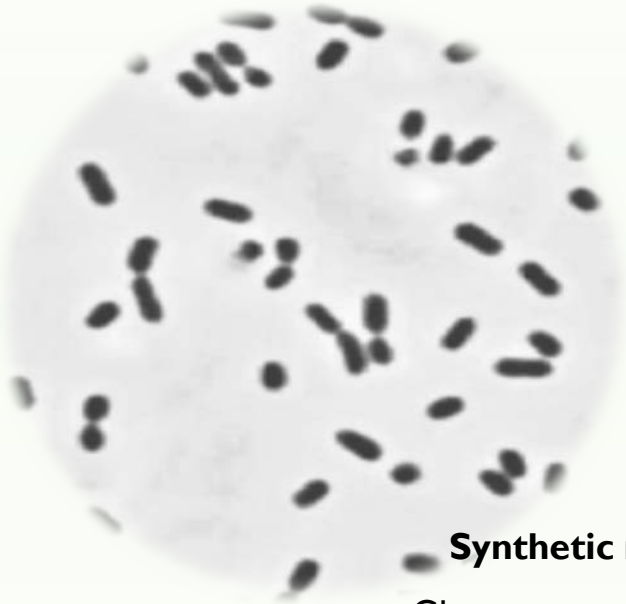
## Nitrogen source

- Supplemented the hydrolysate with 5 g/L YE resulted in significant higher succinic acid production of 37 g/L



# Experimental design

## Continuous fermentation



### Bacterial strain:

*Actinobacillus succinogenes* 130Z

### Fermentation conditions:

Fermentation volume: 500 mL

Temperature: 37 °C

Agitation: 100 rpm

pH: 6.7

CO<sub>2</sub> flow : 0.5 L/min

### Synthetic medium:

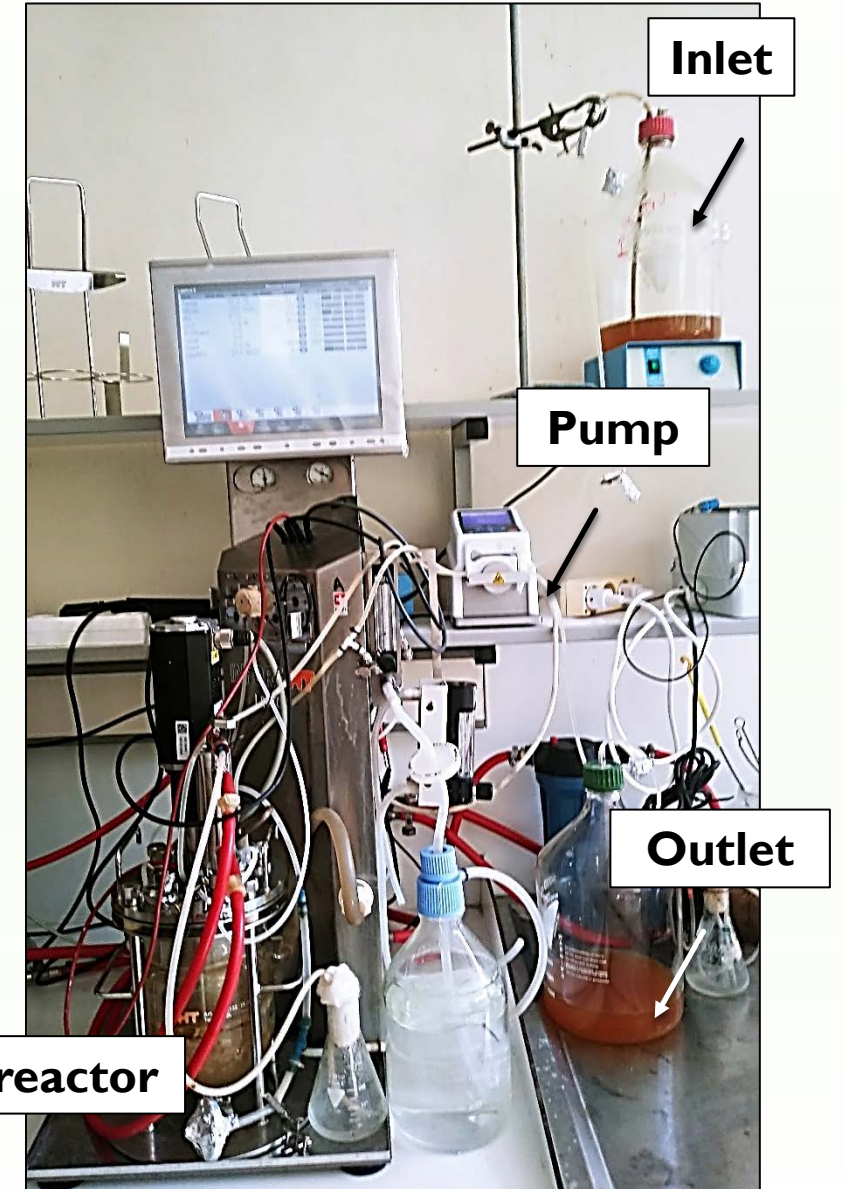
Glucose as carbon source

Yeast extract: 5g/L

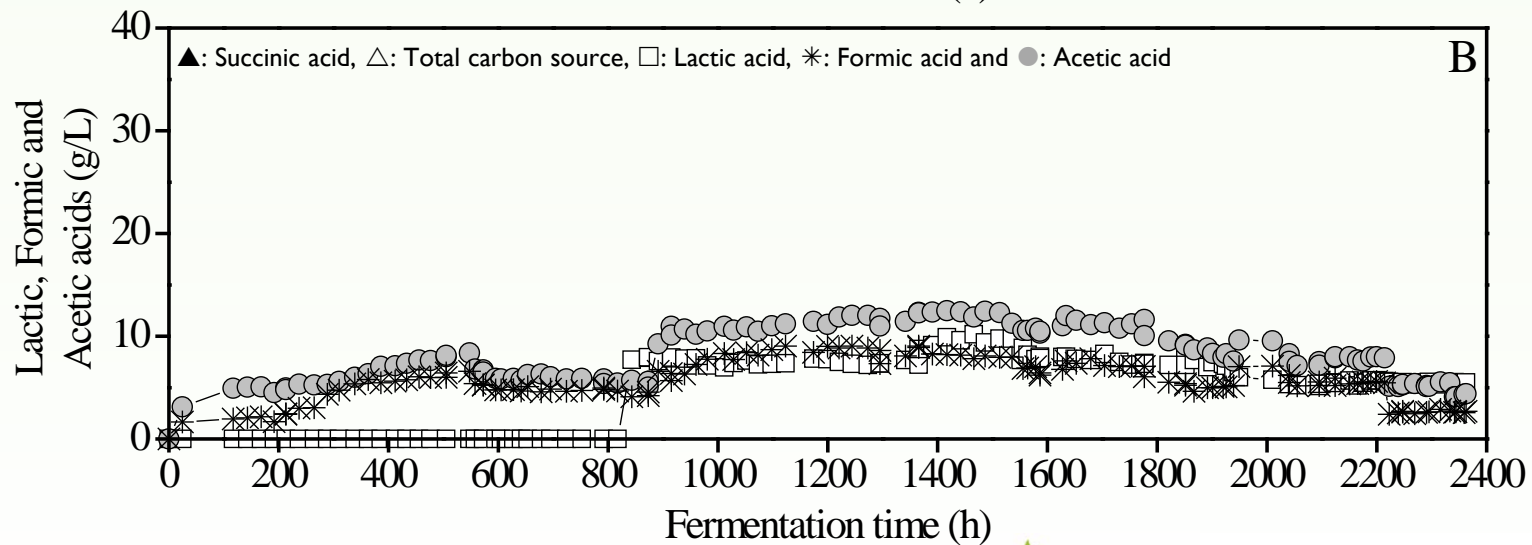
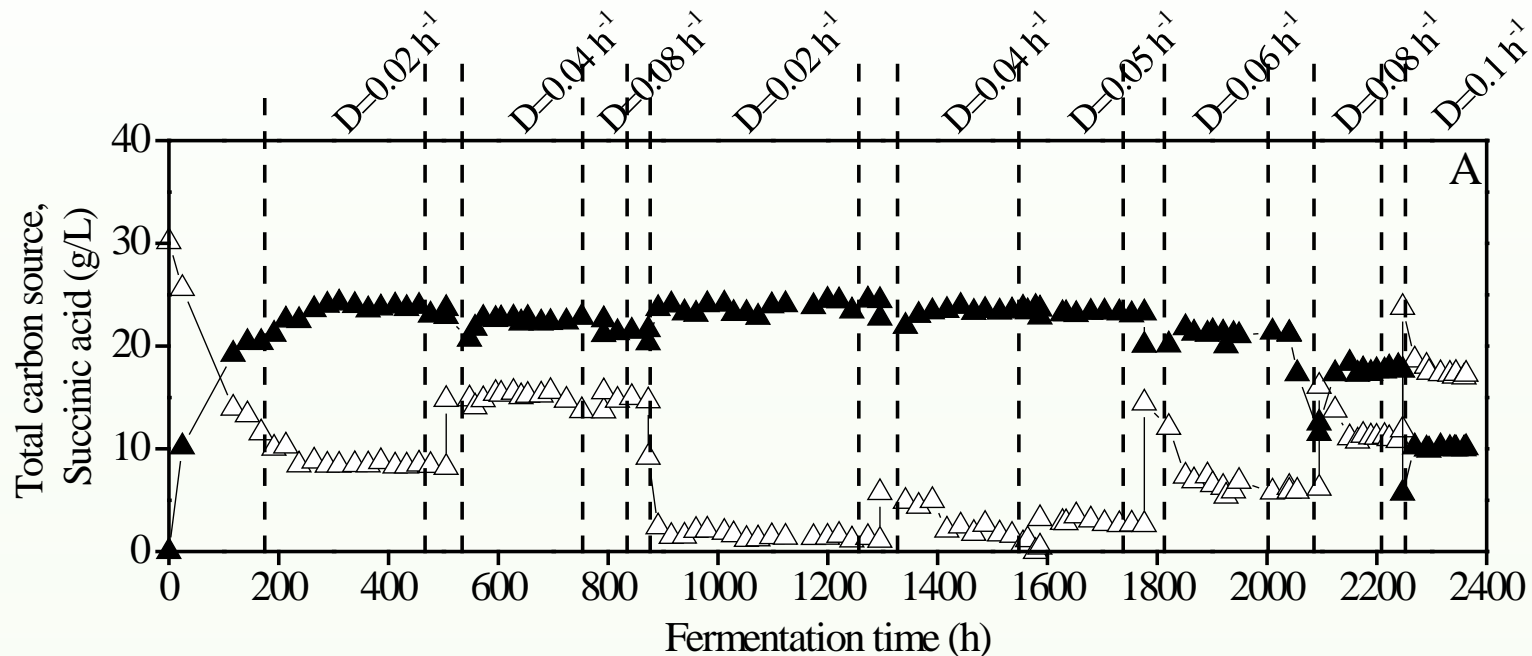
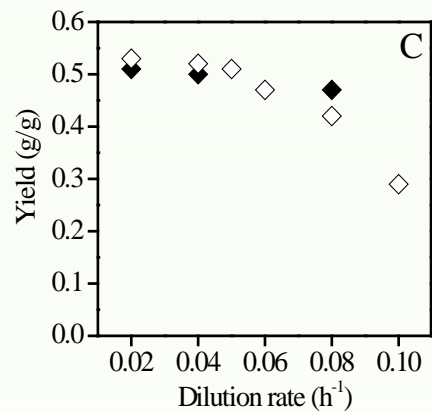
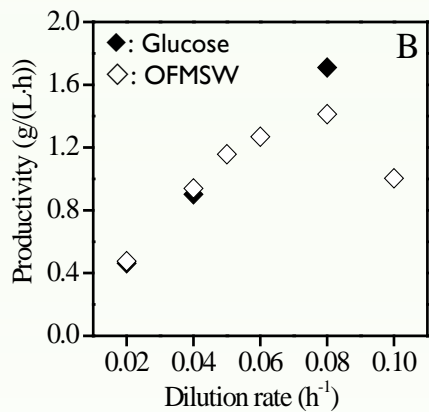
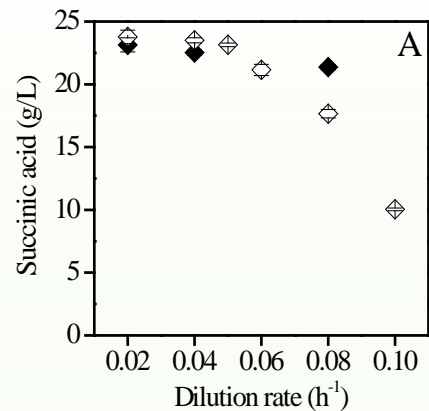
Minerals solution

### OFMSW hydrolysate:

Yeast extract: adjust the Free Amino Nitrogen at 500 mg/L

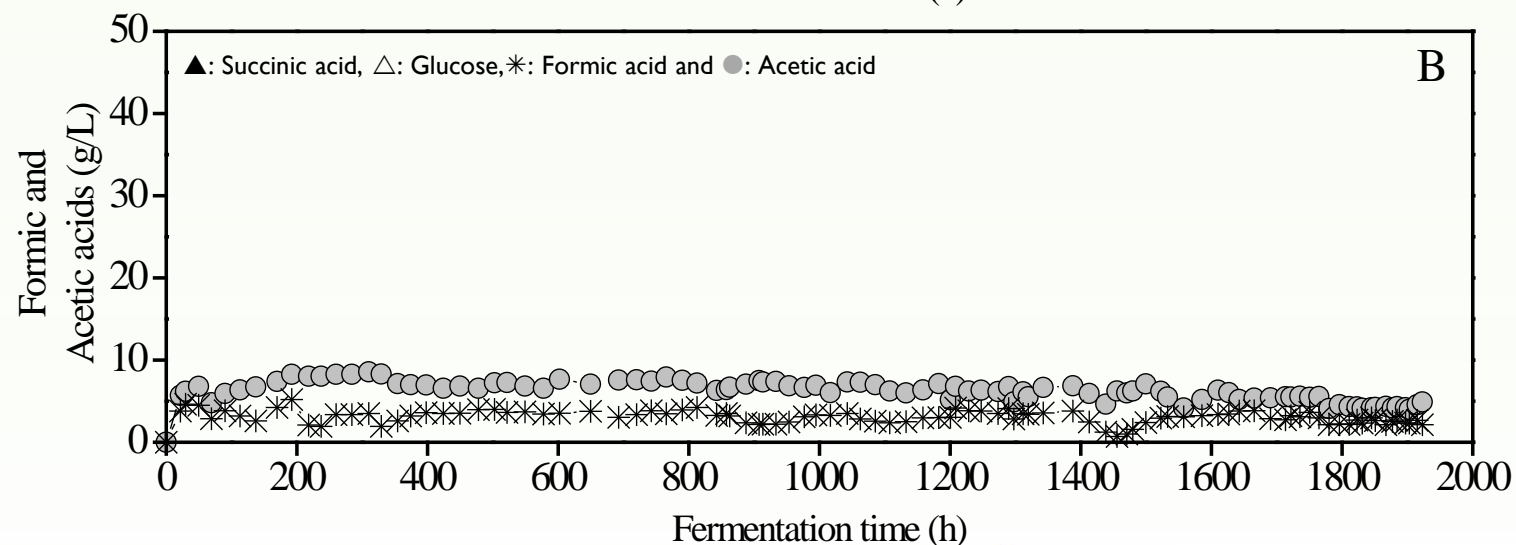
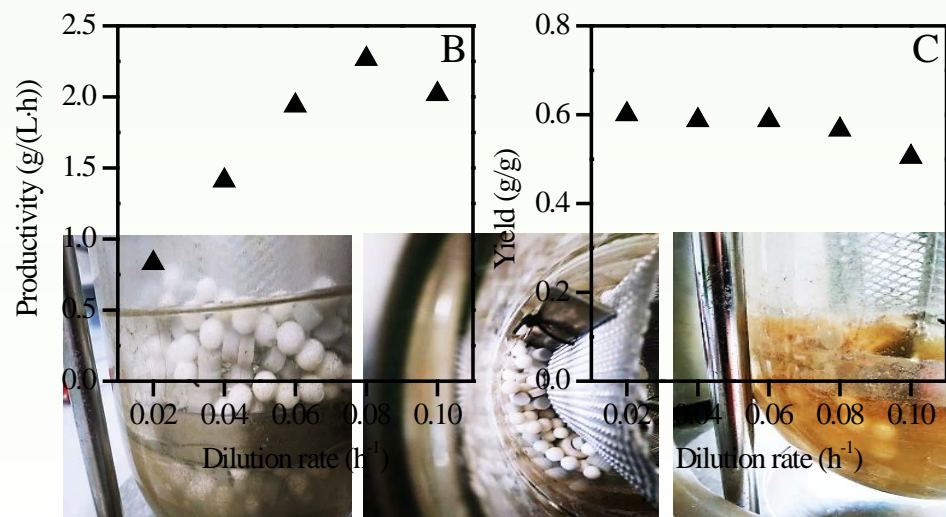
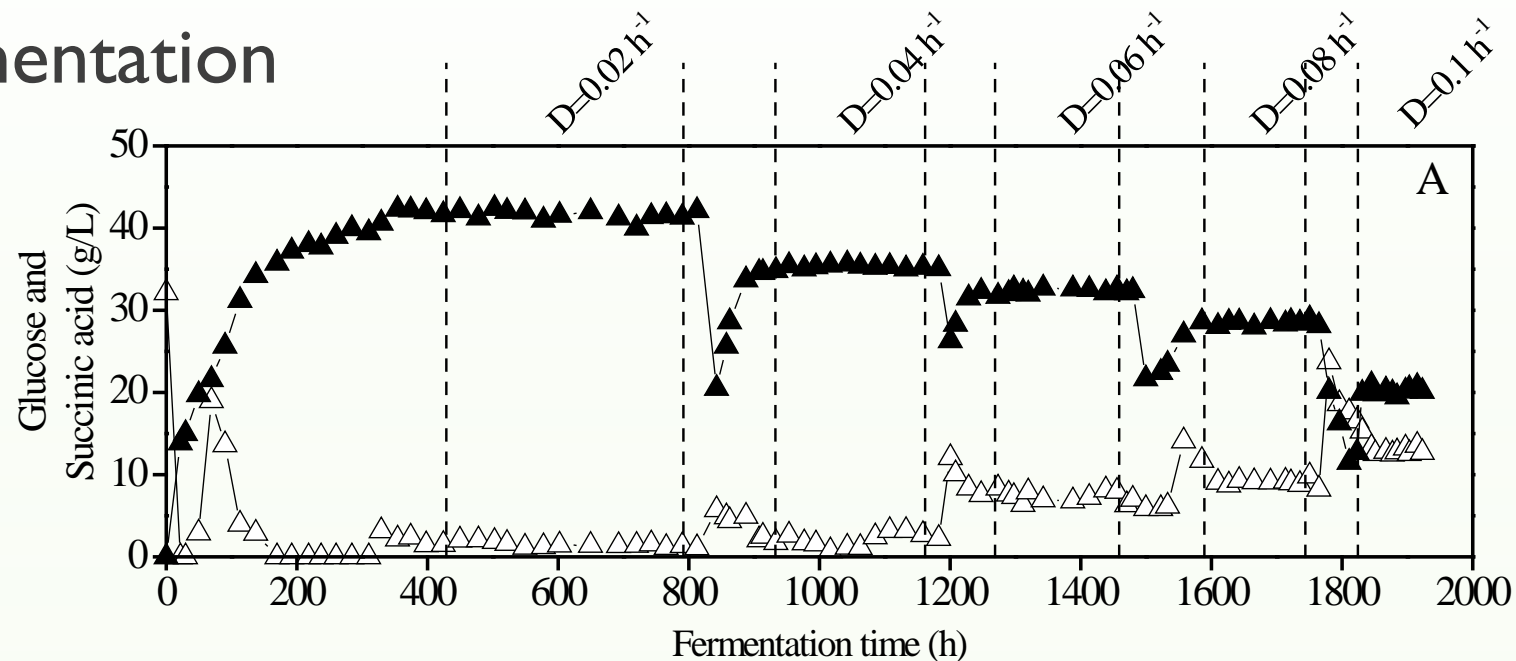
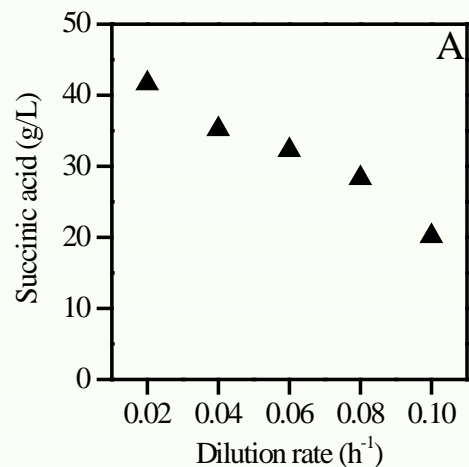


# Continuous fermentation



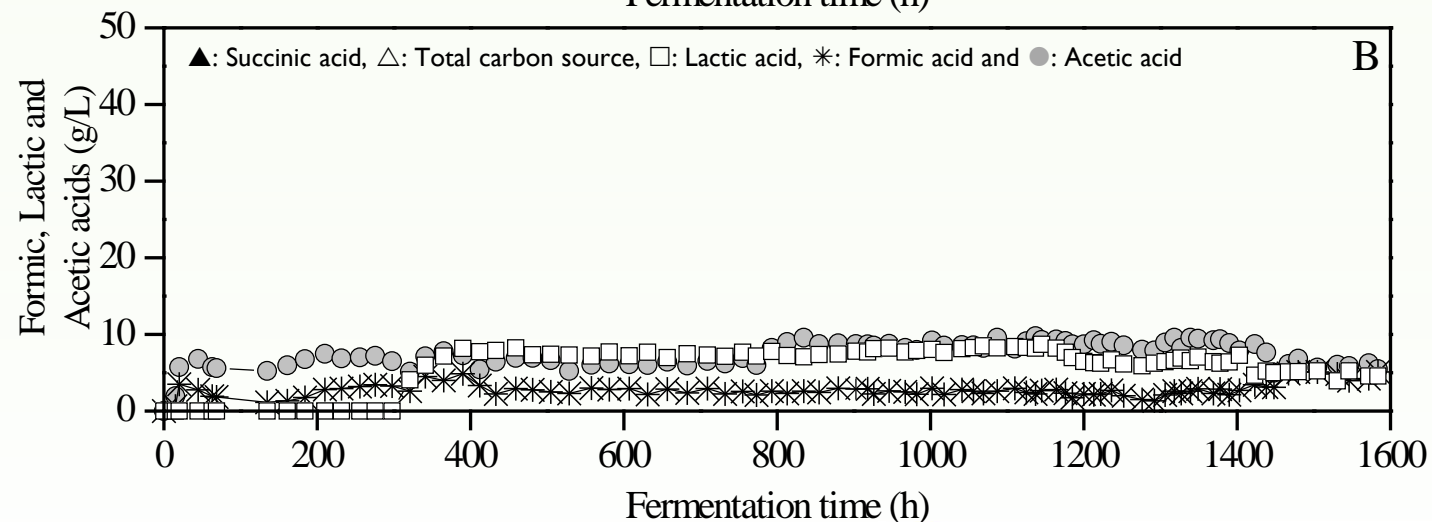
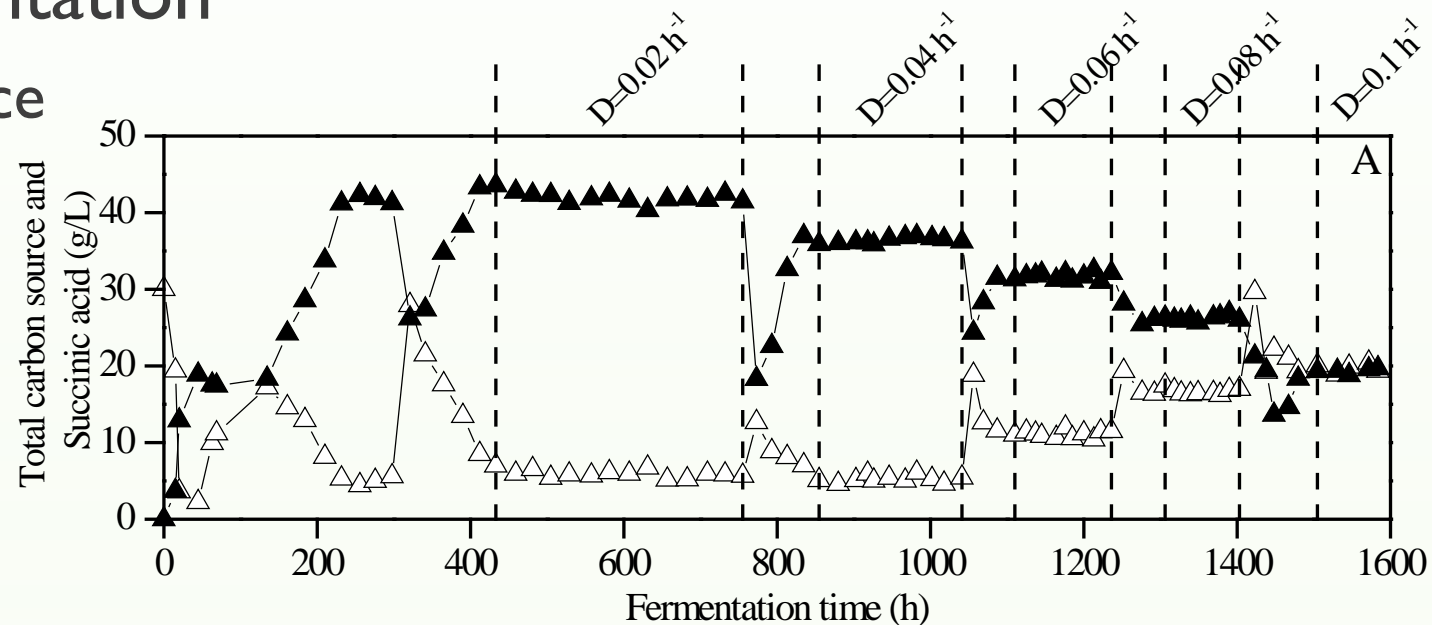
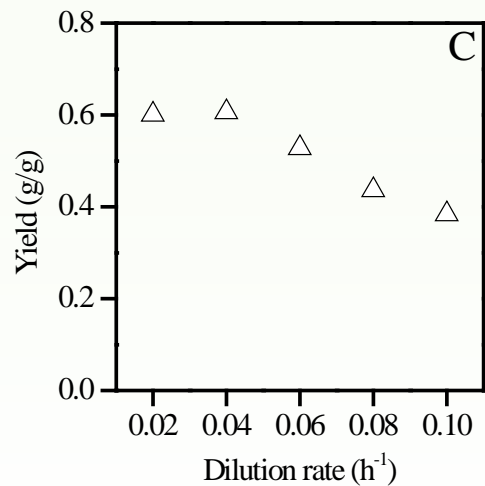
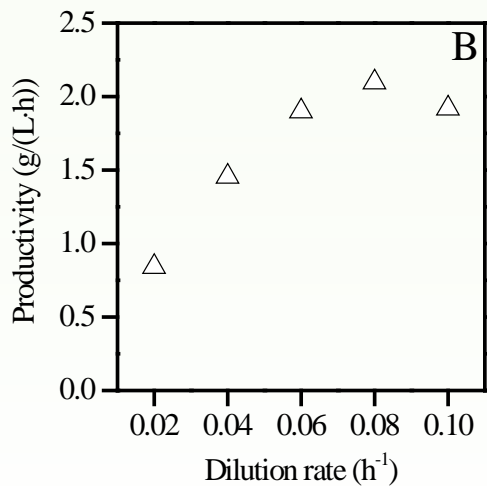
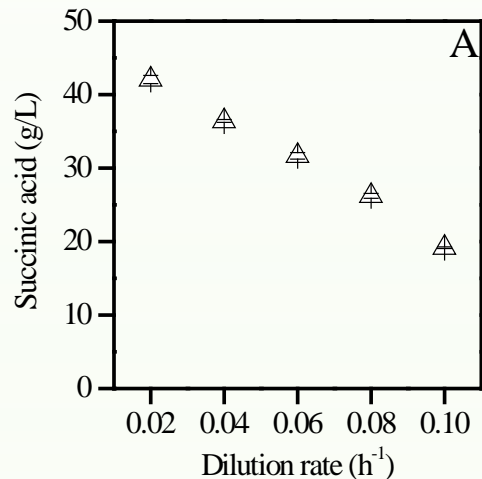
# Immobilised continuous fermentation

## Glucose as carbon source

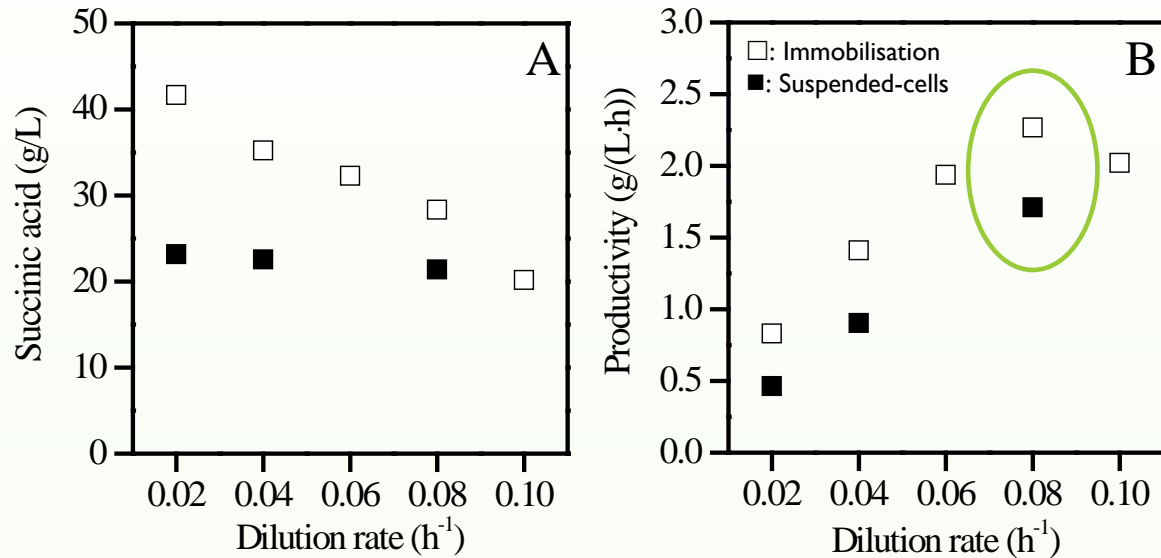


# Immobilised continuous fermentation

## OFMSW hydrolysate as carbon source



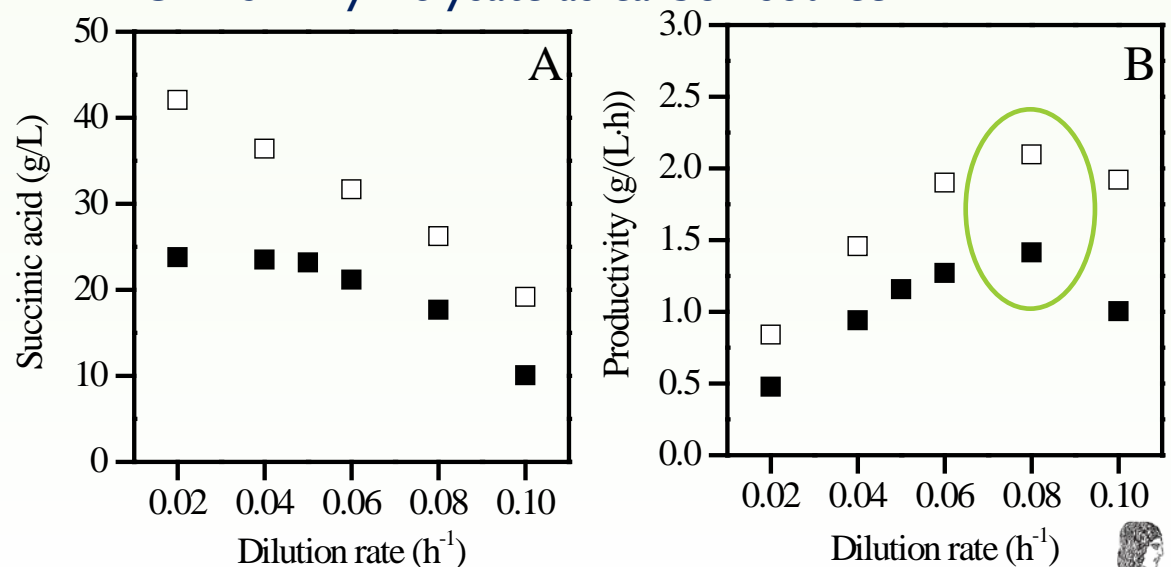
### Glucose as carbon source



**Immobilisation of the culture under continuous operation resulted in higher productivities and final succinic acid concentration at all dilution rates**

- The productivity increased by 32.8 % in glucose-based medium at a dilution rate of 0.08 h<sup>-1</sup>, where succinic acid concentration was 28.3 g/L
- When OFMSW hydrolysate was used as feeding solution the productivity increased by 22.8 % with succinic acid concentration of 26.2 g/L at a dilution rate of 0.08 h<sup>-1</sup>

### OFMSW hydrolysate as carbon source



# Conclusions

- The OFMSW hydrolysate can be considered as a promising feedstock for the production of bio-based products via fermentation
- OFMSW could replace commercial nutrients currently used for the production of fermentation products
- *A. succinogenes* I30Z proved to be a natural biofilm former
- The use of immobilized cells in continuous cultures could further improve the biotechnological production of succinic acid





# THANK YOU FOR YOUR ATTENTION!

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