Production of microbial oil from vegetable wastes

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UNIÓN EUROPEA
Fondos Estructurales y de Inversión Europeos

Comisión Europea

Comunidad de Madrid

Proyecto BIOTRES-CM (P2018/EMT-4344), financiado por la Comunidad de Madrid y el Fondo Europeo de Desarrollo Regional.

1. Introduction

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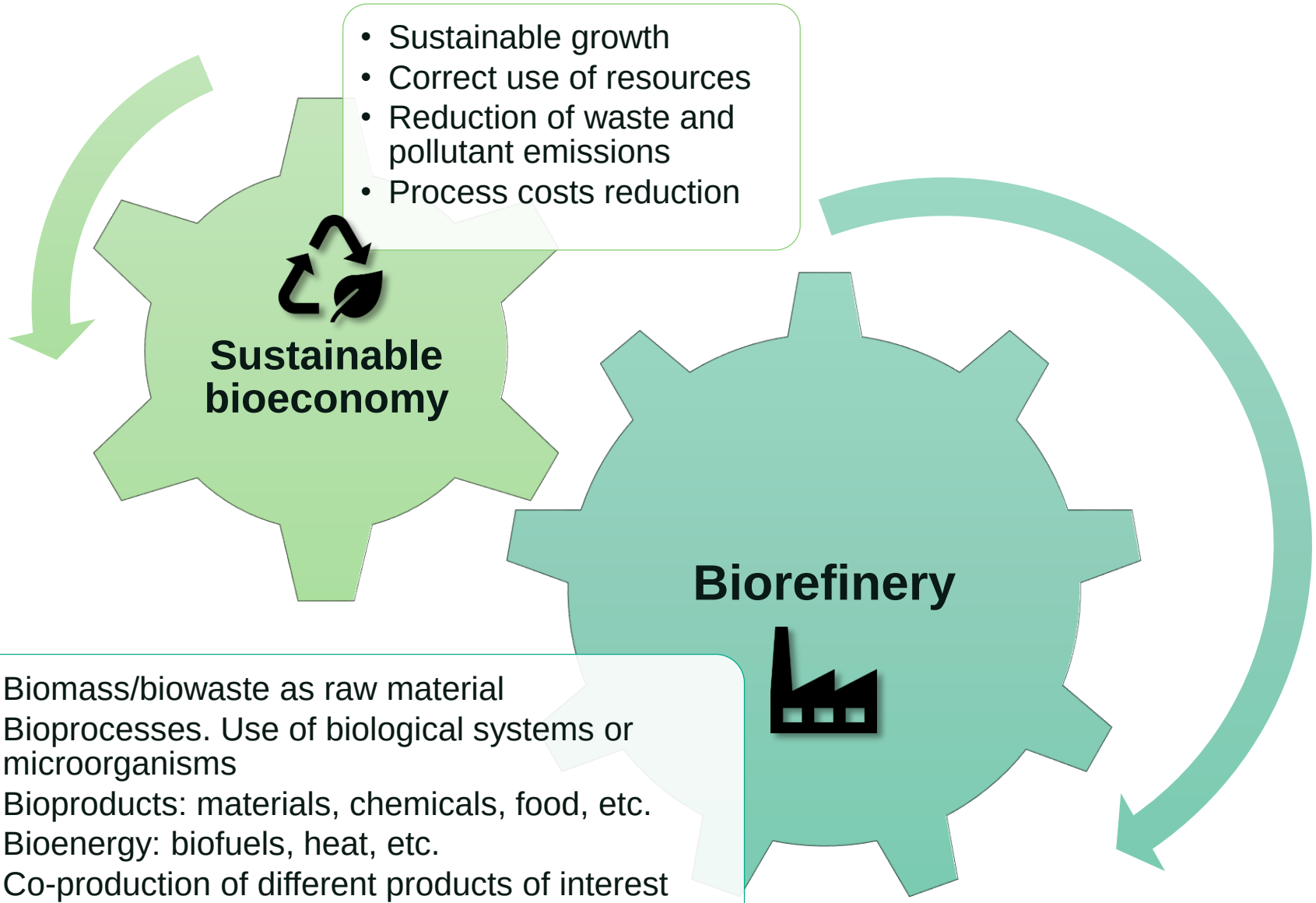
• Sustainable growth
• Correct use of resources
• Reduction of waste and pollutant emissions
• Process costs reduction
Sustainable bioeconomy
Biorefinery
• Biomass/biowaste as raw material
• Bioprocesses. Use of biological systems or microorganisms
• Bioproducts: materials, chemicals, food, etc.
• Bioenergy: biofuels, heat, etc.
• Co-production of different products of interest

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Conventional process

Oil extraction

Vegetable oil

Biodiesel production

Biodiesel

Alternative scenario

Vegetable/Fruit waste

Carbohydrate extraction

Microbial culture

Microbial oil

Biodiesel production

Biodiesel

Obtaining other products with high-added value

Exploitation in other sectors

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Waste availability

Main industries that generate organic waste

Fruit and vegetable sector

WASTE

~5%

E.g.:
3 types of crops

Rejected tomato, pepper and cucumber → greenhouse production

115,000 tons/year

Only in the province of Almería (2019)

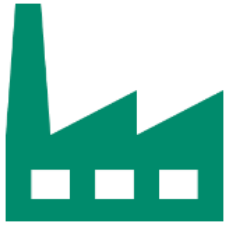
Alternative scenario

Vegetable/Fruit waste

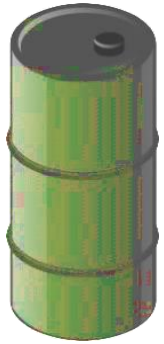
Carbohydrate extraction

Microbial culture

Microbial oil



Biodiesel production



Biodiesel

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Satellite view – “Google maps” - Greenhouses Almería

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2. Objetives

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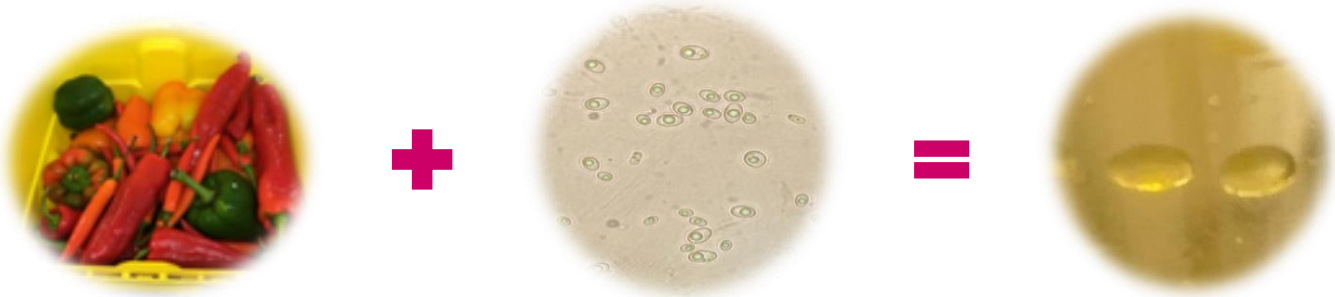
Produce microbial oil using defective pepper (culls) as raw material

- Generate a culture medium from discarded pepper
- Study whether the obtained medium is appropriate for lipid accumulation using oleaginous yeasts
- Generate appropriate C/N ratios to enhance lipid accumulation
- Establish optimal conditions in well-controlled bioreactors to cultivate oleaginous yeasts and promote lipid accumulation



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3. Methodology

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3.1. Fractionation of culled pepper

Raw material homogenization (crushing) and centrifugation

Insoluble fraction (IF): Pulp

Soluble fraction (SF): Sugary juice

Sterilization by filtration

Sterile culture medium

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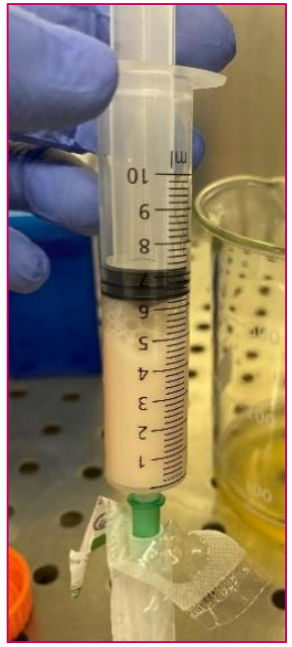
3.2. Microorganisms and cell propagation

Plating – Petri dish
YPD medium
Yeast:
Cryptococcus curvatus
(CL6032 from "Biobanco Nacional del Instituto de Salud Carlos III (BBN-ISCIII)")

Conditions:
- Temperature: 25 ˚C
- 2-3 days

Preculture
YPD medium
~ 50 ml

Conditions:
- Temperature: 25 ˚C
- Shaking: 180 rpm
- pH: 6
- 24 hours



Inoculum

Bioreactors
Synthetic medium[1]:

- Glucose
- Peptone
- Yeast extract

Bioreactors
Studied medium:

- **Soluble fraction from pepper residue**

[1](Chang y col., *biomass and bioenergy*, 2015, vol. 72, p. 95-103)

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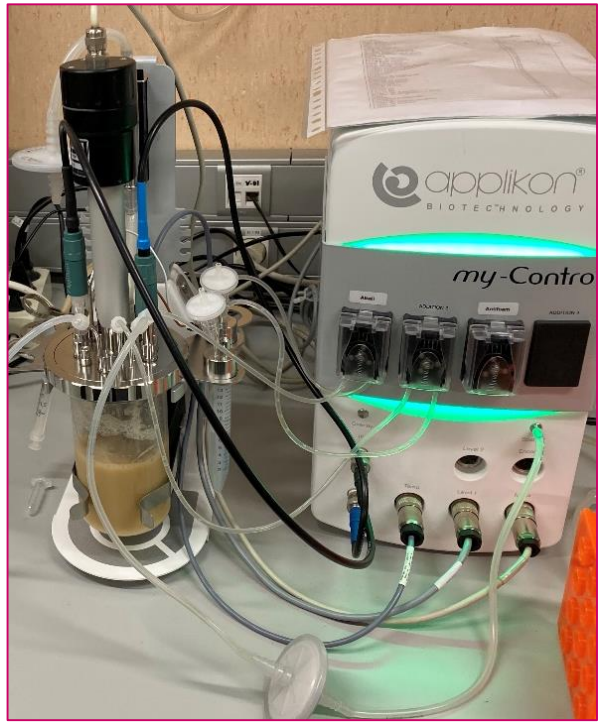
3.3. Culture conditions in bioreactors

Medium dilution	pH	T ˚C	OD (600nm) Inoculum	Aeration vvm (L/min)	% minimum O_2 dissolved
undiluted	6	28	1 – 1.5	1	20
1/2	6	28	1 – 1.5	1	20
1/3	6	28	1 – 1.5	1	20

3.4. Parameters analyzed

Parameter	Method
Sugar consumption (g/L)	HPLC
Dry weight (g/L)	Gravimetric
Total lipids (%)	Gravimetric
Fatty acid profile	Gas Chromatography (GC)
Fatty acid (%)	Gas Chromatography (GC)



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4. Results

4.1. Available sugars in the different fractions obtained from pepper residue

1 kg discarded pepper
(120 g dry pepper)

Insoluble fraction
Available sugars: 11.5 g /100 g dry pepper
Glucose: 7.8 g/100 g dry pepper

Soluble fraction:
Available sugars: 40.5 g/100 g dry pepper
Glucose: 15.9 g/100 g dry pepper
Fructose: 23.6 g/100 g dry pepper

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4.2. Composition of the soluble fraction

Medium	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)	Total (g/L)
SF Pepper	23.4	34.0	0.9	**58.3**

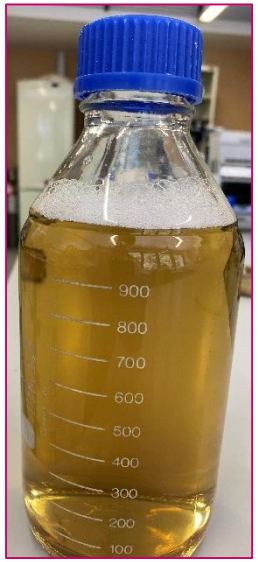
Raw material

Medium	Nitrogen (g/L)
SF Pepper	**1.7**

Calculation with the **C** derived from the main sugars

C/N (g/L) Undiluted	C/N (g/L) Dilution 1/2	C/N (g/L) Dilution 1/3
15	30	50

SF Culture medium



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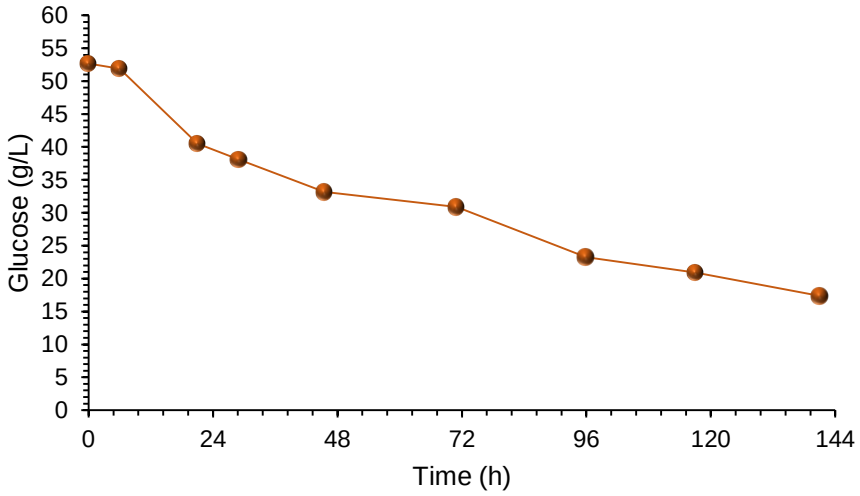
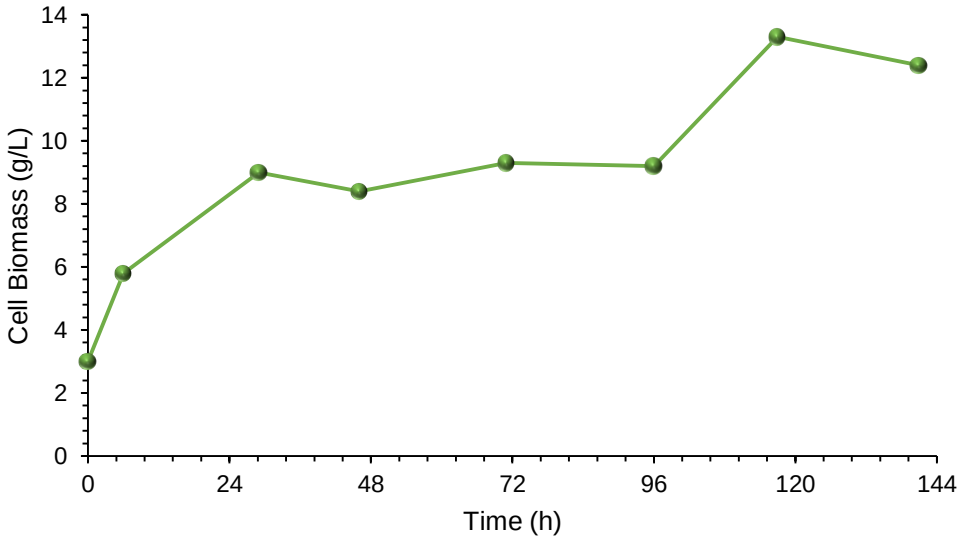
4.3. Lipid accumulation in synthetic media (C/N 100)

Glucose consumption



Cell biomass



Sample (C/N 100)	Lipids (%) Gravimetric method	Lipids (%) GC method	Lipids (g/L)
140 h	45.0	40.0	5.4

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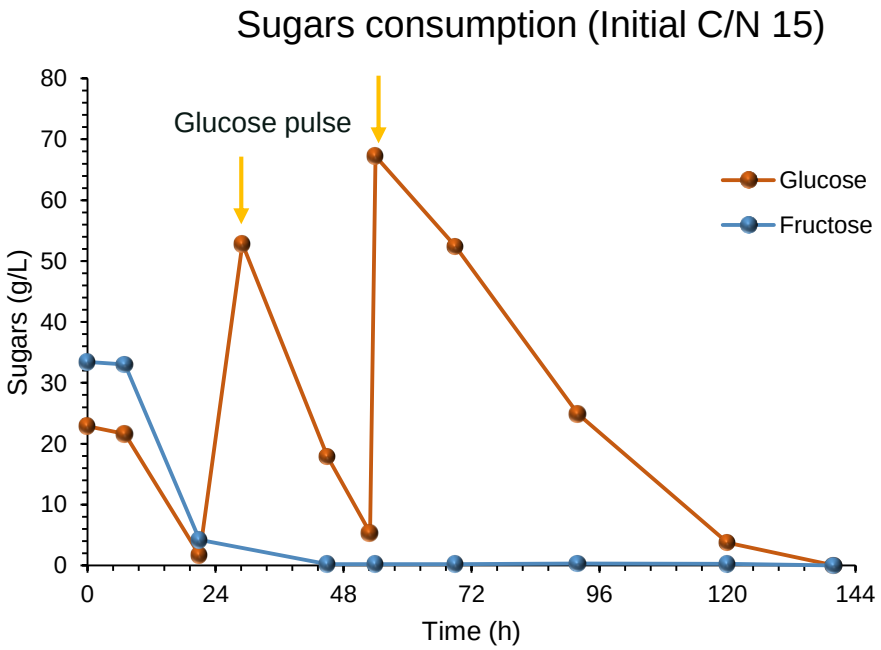
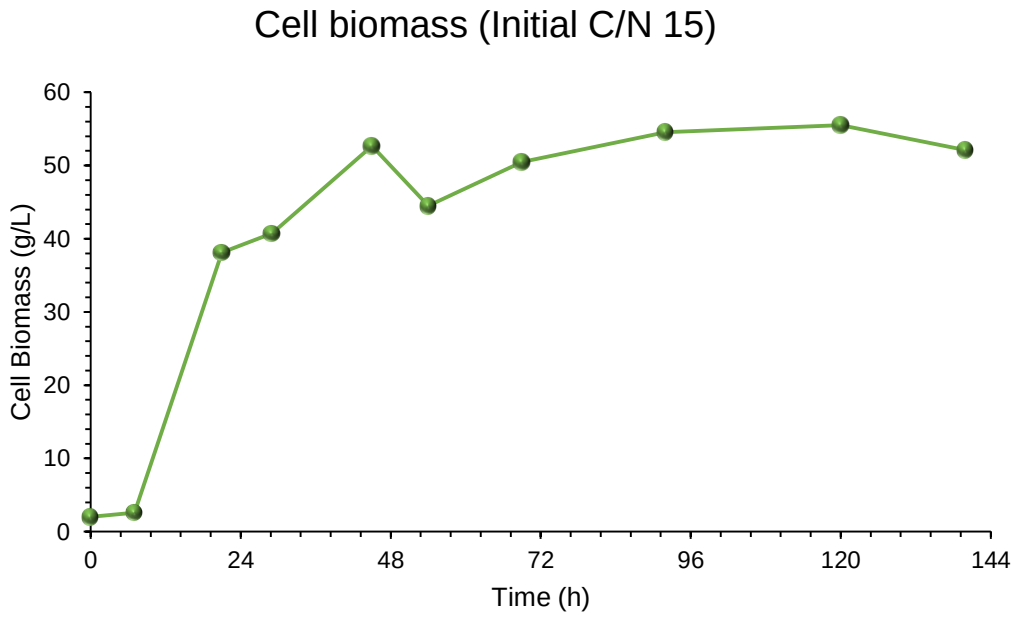
4.4. Lipid accumulation in pepper-derived medium (Initial C/N 15)





Sample (Initial C/N 15)	Lipids (%) Gravimetric method	Lipids (%) GC method	Lipids (g/L)
29 h	8.6	7.3	3.3
53 h	14.3	12.5	6.2
140 h	**32.7**	**29.2**	**16.8**

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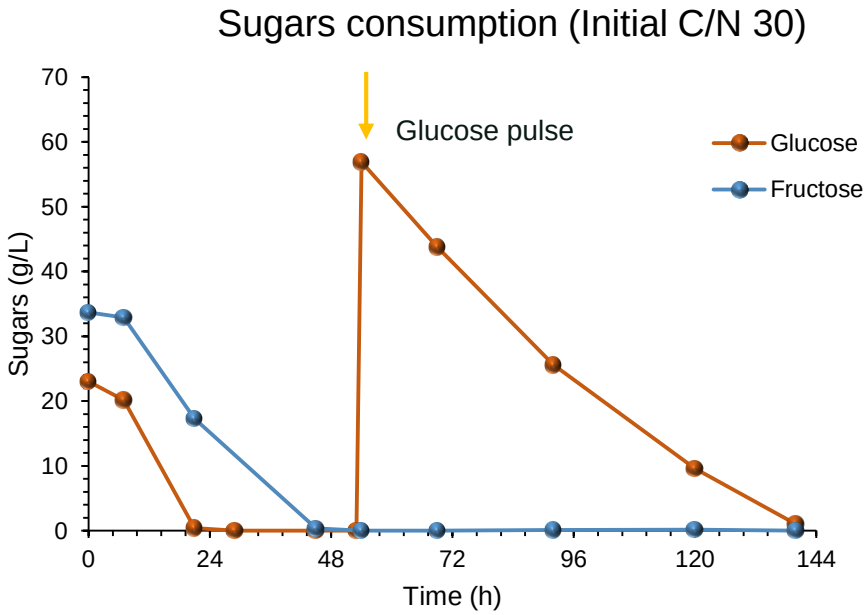
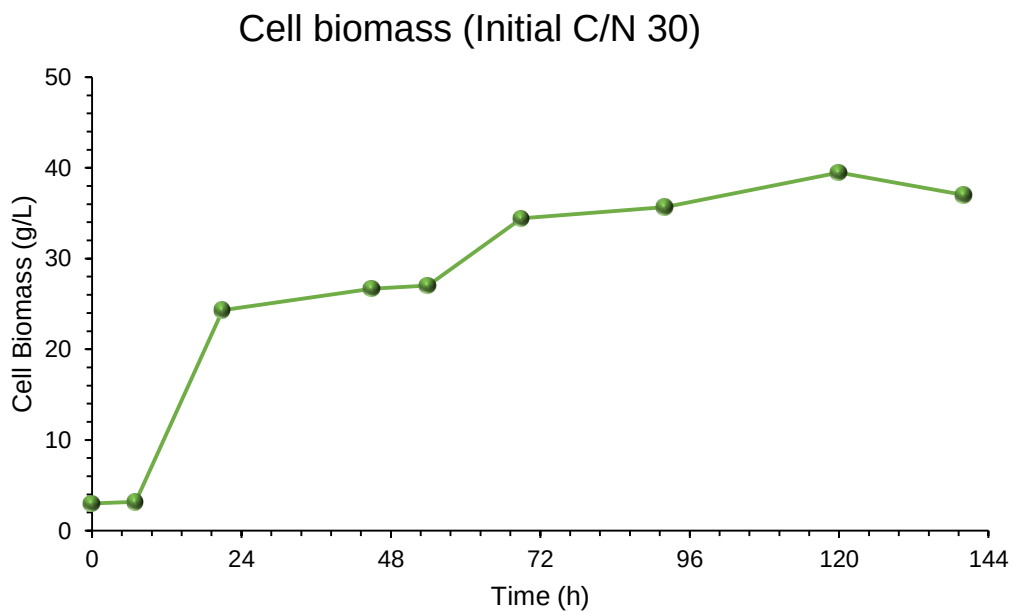
4.5. Lipid accumulation in pepper-derived medium (Initial C/N 30)





Sample (Initial C/N 30)	Lipids (%) Gravimetric method	Lipids (%) GC method	Lipids (g/L)
53 h	18.4	14.8	4.8
140 h	**36.8**	**31.0**	**13.4**

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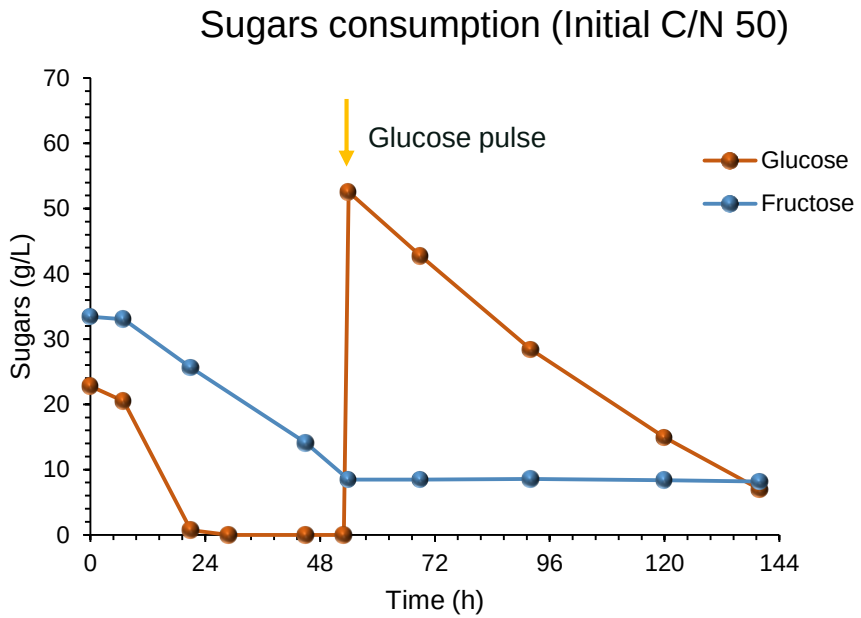
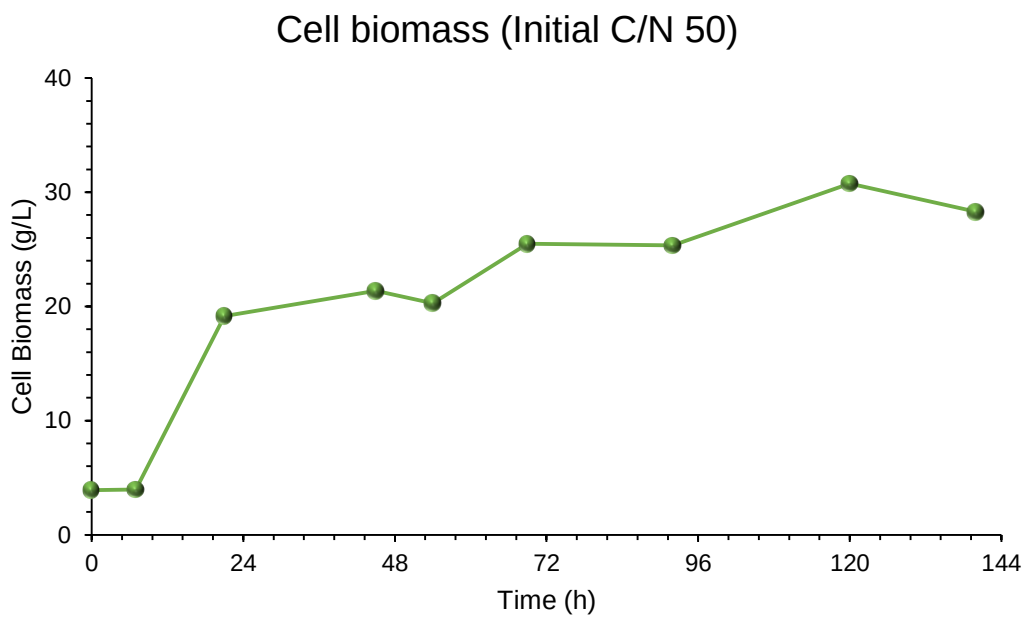
4.6. Lipid accumulation in pepper-derived medium (Initial C/N 50)





Sample (Initial C/N 50)	Lipids (%) Gravimetric method	Lipids (%) GC method	Lipids (g/L)
53 h	20.8	15.9	4.0
140 h	**40.9**	**37.2**	**11.4**

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4.7. Fatty acid profile obtained in different media

	Fatty acid profile (%)							
C. curvatus	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C24:0
0 h	-	11.1	-	9.9	30.2	35.4	13.5	-
140 h C/N 100	0.2	22.2	0.5	9.0	53.2	11.9	1.6	1.3
140 h initial C/N 15	0.7	26.3	1.2	5.2	52.6	12.1	2.1	-
140 h initial C/N 30	0.3	25.7	0.9	9.7	54.4	10.5	1.9	0.6
140 h initial C/N 50	0.2	23.1	0.6	9.0	55.4	9.3	1.6	0.7
Palm oil[2]	1.1	39.7	0.3	4.5	43.5	10.9	-	-

[2](Demirbas. *Biodiesel: A Realistic Fuel Alternative for Diesel Engines*, 2008, p. 121-140)

C14: 0 → Myristic
C16: 0 → Palmitic
C16: 1 → Palmitoleic
C18: 0 → Stearic
C18: 1 → Oleic
C18: 2 → Linoleic
C18: 3 → Linolenic
C24: 0 → Lignoceric

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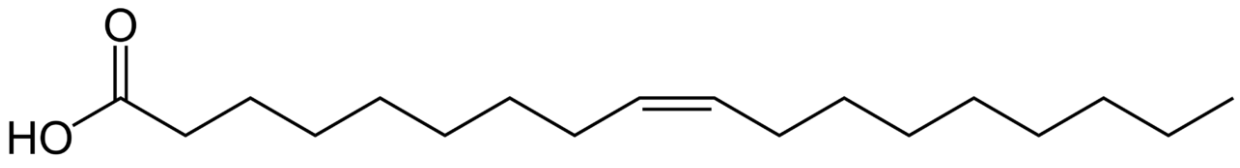
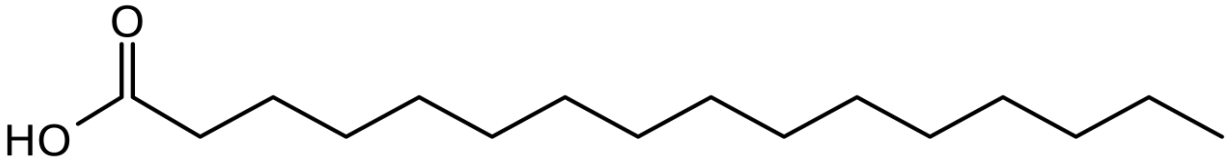
Summary	
Initial C/N ratio	Lipids (%) GC
15	29.2
30	31.0
50	37.2

Synthetic C/N	Lipids (%) GC
100	40.0

Oleic acid (18:1)



Palmitic acid (16:0)



~80% of the total produced

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5. Conclusions and Future actions

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Conclusions

- **Sugars can be easily extracted** by mechanical methods (crushing and centrifugation)
- **High content of sugars** in the **soluble fraction** of the residual pepper
- **Increase C/N ratio** favors the **accumulation** of intracellular **fatty acids**

- **Fatty acid profile** obtained is **similar** to the **profile of vegetable oils** that are used for conventional **biodiesel** production

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Future actions

- Increase C/N ratio:
  - Combination with other waste-derived media → Avoid making dilutions or pulses with synthetic media
  - E.g.: Sugars obtained from lignocellulosic waste materials with lower N content
- Carry out assays with the insoluble fraction and combine with the soluble fraction
- Co-production of high-added value compounds together with bio-oils
  - **Carotenoids** *

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Acknowledgements

- “Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación, Fondo Social Europeo (FSE), Fondo Europeo de Desarrollo Regional (FEDER)”: Grants for predoctoral contracts Ref. **PRE2018-086317** and ACMIBIO Project Ref. **ENE2017-86864-C2-1-R”**

- “**Albaida Residuos S.L**.” for providing the residues used in this study

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Thanks for your attention

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Proyecto BIOTRES-CM (P2018/EMT-4344), financiado por la Comunidad de Madrid y el Fondo Europeo de Desarrollo Regional.

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