A biorefinery approach for the simultaneous production of biofuels and bioplastics

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Background

3 step process for simultaneous biohydrogen and polyhydroxyalkanoates (PHA) production:

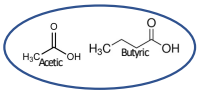
- Dark fermentation using food waste for biohydrogen and SCOA production (I)
- Bacterial selection for enrichment of PHA accumulating bacteria using pinewood pyrolysis bio-oil (II)
- PHA batch production using SCOA (III)

Food waste

Dark Fermentation (I)

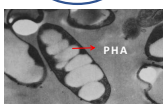
BioH2

SCOA



SCOA for batch production (III)

PHA



- 100% Biodegradable
- Biocompatible
- Widespread industrial application

Pinus pinaster and *Pinus pinea*

Forest waste

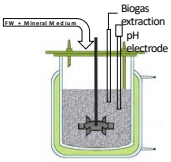
Fast-pyrolysis

Aerobic fermentation (Direct – for selection) (II)

Biomass

Methods

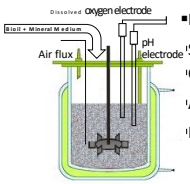
Dark Fermentation (I)



- Bioaugmentor: *Clostridium butyricum*
- Substrate: Catering industry (CIW) food waste
- Batch system
- Initial sugar concentration: 20 g/L total sugars
- C/N/P ratio of 100:3:1, pH= 5.5

Experimental setup for BioH₂ and SCOA production

Bacterial Selection (II)



- Inoculum: Active sludge from an aerobic tank of WWTP
- Substrate: Bio-oil (Fast pyrolysis of pinewood residues)
- OLR: 30C-mM/day of biodegradable carbon
- ADF conditions
- HRT= 2 days; SRT= 10 days; C/N/P ratio of 100:3:1; pH= 7.95 – 8.25

Experimental setup for biomass selection

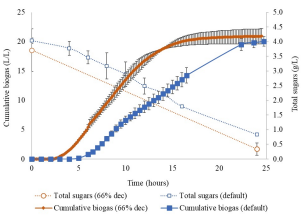
Results

Dark Fermentation (I)

- Biomass was submitted to microwave (MW) pretreatment for contamination control, 550 W for 4 mins.
- Batch fermentations were performed under non-sterile conditions during a period of 25 hours.
- Two nitrogen concentrations were tested: default (12 g/L NH_4Cl) and 66% reduction (4 g/L NH_4Cl).

Composition of the processed CIW used in the fermentation experiments, by dry weight (d.w.).

Component	Concentration (% d.w.)
Total sugars	62.1 ± 0.1
Crude protein	10.4 ± 0.2
Total fat	26.3 ± 2.2
Ash	1.2 ± 0.1



Time-course of biogas production and sugar consumption during MW-pretreated non-sterile fermentation of CIW: default and 66% nitrogen reduction

Experimental parameters attained in the fermentation assays by *C. butyricum*.

Supplementation	Total sugars consumption (%)	Total biogas production (L/L)	Max. biogas productivity (mL/(L h))	H_2 production yield (mL/g^{TS})	Acetate (g/L)	Butyrate (g/L)
12 g/L NH_4Cl	79.3 ± 0.0	4.1 ± 0.1	264.4 ± 13.8	77.9 ± 4.1	2.0 ± 0.1	4.8 ± 0.2
4g/LNH_4Cl	90.1 ± 3.3	4.2 ± 0.1	420.1 ± 11.9	141.9 ± 18.1	1.9 ± 0.1	4.2 ± 0.1

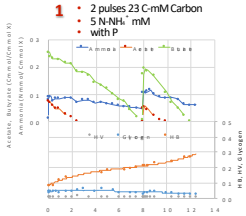
- Total biogas production did not vary significantly for both tested conditions
- The biogas productivity increased significantly, approximately 59% compared to the normal NH_4Cl supplementation.
- After decreasing the NH_4Cl concentration, the acid production reached a total of 6.1 g/L, mainly composed by butyrate and acetate.

PHA Accumulation (III)

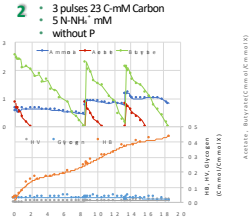
Batch production

- The fermented stream with the lowest nitrogen supplementation was used to perform three batch PHA production essays.

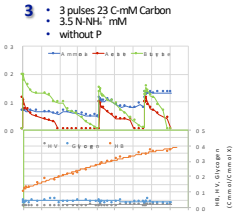
1
- 2 pulses 23 C-mM Carbon
- 5 N-NH_4^+ mM
- with P



2
- 3 pulses 23 C-mM Carbon
- 5 N-NH_4^+ mM
- without P



3
- 3 pulses 23 C-mM Carbon
- 3.5 N-NH_4^+ mM
- without P



Experimental parameters for the three batch essays

Sample	Content*	$Y^{HA/S}$ máx	$Y^{X/S}$ máx	q^{Acet} máx**	q^{But} máx**	q^{HB} máx**
1	24.1	0.50	0.70	0.031	0.053	0.017
2	27.2	0.73	0.46	0.039	0.044	0.065
3	23.7	0.92	0.63	0.027	0.044	0.038

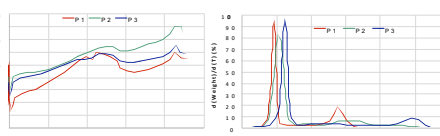
*(%dw); **(C-mmol/ CmmolX.h)

- The major impact of the different conditions used was the absence of phosphorus addition reflected both on the content and polymer yield on substrate.

Polymer Characterization

Polymer's properties

Sample	HB:HV	%Purity	T_m^0 (°C)	ΔH_m^0 (J/g)	Crystallinity (%)	T^d (°C)	Mw (Kda)	PDI
P1	98:2	57%	165.75	32.17	24%	242.89	624	2.9
P2	94:6	68%	167.19	41.77	32%	255.84	637	2.8
P3	95:5	54%	164.94	29.67	20%	274.05	724	3.4



- The three polymer samples had very similar properties, reflecting that the changes in the accumulation conditions did not have an impact in the polymer produced

Take Home Message

- Non-sterile dark fermentation was able to convert CIW into a butyrate/acetate solution with concurrent bioH₂ production.
- Decreasing NH_4Cl in the medium impacted positively the dark fermentation performance, reaching a maximum biogas productivity of 420 mL/(L h).
- The enriched biomass although selected with a different substrate was able to achieve a maximum polymer content of 27% PHA using the fermented stream from the DF.
- The values of melting temperature (Tm), enthalpy (ΔHm), crystallinity and molecular weight are in range of what is reported for polymers with similar HV content, and meet the standards required for use in commercial plastic applications.

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