SCALIBUR

LEADING A REVOLUTION
IN BIOWASTE RECYCLING

Enzyme cocktails for the valorization of organic waste

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Contents

1. Challenge

Development of enzyme cocktails for the release of sugars and other fermentable compounds from OFMSW

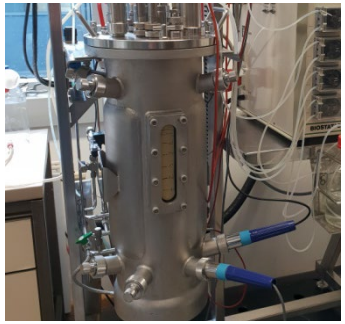
- fermentation processes for

biobased and biodegradable polyesters and biopesticides

- Demonstrate the **production of fermentable sugars** from the OFMSW in an operational environment.

- Validate the robustness and performance of **specific enzymatic cocktails** prepared for OFMSW.

2. Solution
2.1 Description of the solution



Enzyme production

Cellulase
Hemicellulase
Pectinase
Amylase
Protease
Lipase

OFMSW:
Cellulose
Hemicellulose
Pectin
Starch
Protein
Fat

Test with organic fractions of OFMSW

Optimized enzyme cocktails

Optimized enzyme cocktails

Degradation of OFMSW

Most effective Enzyme cocktails

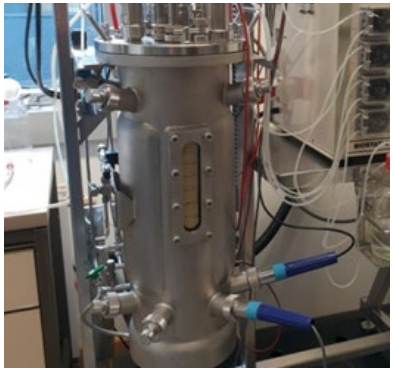
Production of enzyme cocktails

CENER

Enzyme characterization

2. Solution
2.1 Description of the solution

Production of enzyme cocktails



Task 4.2: Enzymatic hydrolysis of the OFMSW for the production of sugars. (CENER)

Task 4.3: Fermentation into building blocks for biodegradable polyesters (NOVAMONT)

Task 4.4: Bacterial biopesticides production through fermentation (CENER, AERIS)

2. Solution - 2.2 Results

Laboratory test protocol for the selection and characterization of enzyme cocktails: Release of sugars

- grinding of the OFMSW-sample (CENER), particle size approx. 0.5 – 1.0 cm
- suspend 1.0 g OFMSW-sample in 10.0 ml 0,05 M Na-McIvaine-buffer (pH 3 – 7)
- addition of enzyme mixture: 50 µl per gram grinded OFMSW-sample
- incubation for 20 h at 50°C, 120 rpm (or at 20 – 60°C at pH 5,0 for temperature dependency)
- centrifugation (supernatant1): detection of reducing sugars
- washing
- drying of the residue (dry matter)
- determination of ignition loss in the residue

2. Solution - 2.2 Results
2.2.1 Selection and characterization of enzyme cocktails

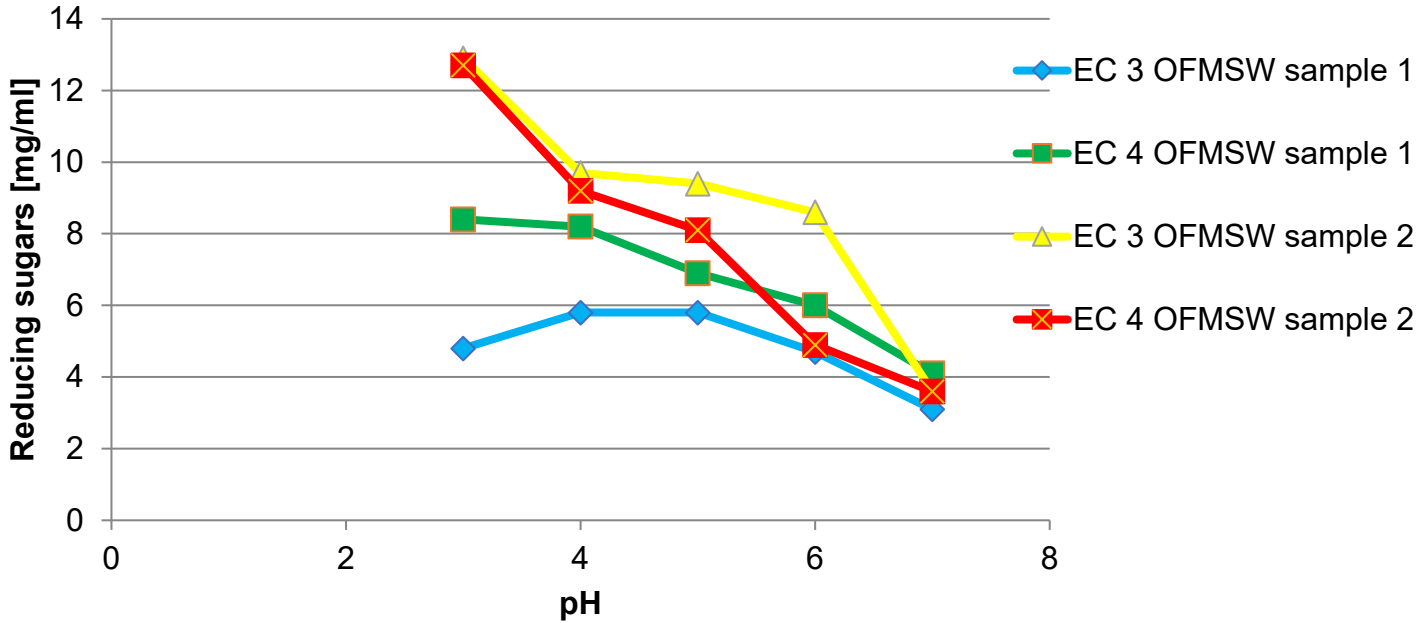


Figure 1: pH-dependency of the release of sugars from two different OFMSW samples

EC = Enzyme cocktail

2. Solution - 2.2 Results

2.2.1 Selection and characterization of enzyme cocktails

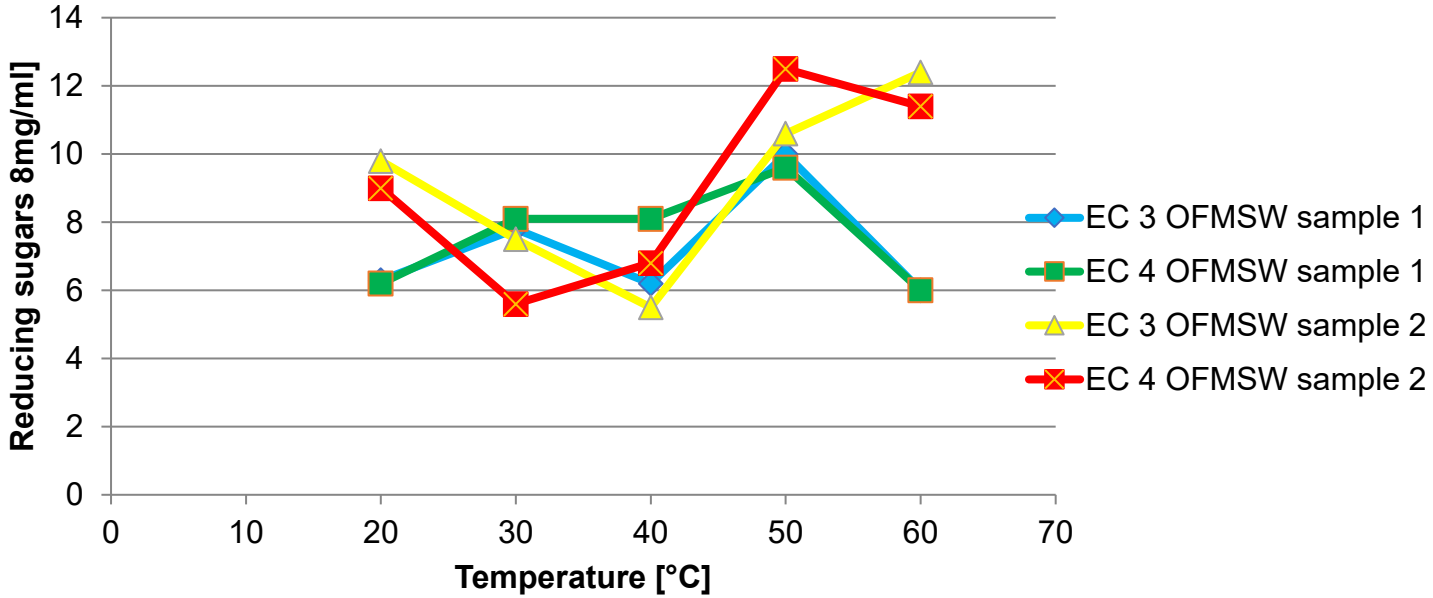


Figure 2: Effect of temperature on the release of sugars from two different OFMSW samples

EC = Enzyme cocktail

2. Solution - 2.2 Results
2.2.1 Selection and characterization of enzyme cocktails

Table 1 Characterization of enzyme cocktails no. 3 and 4

Enzyme cocktail	OFMSW sample	pH-optimum		Temperature optimum [°C]	
		Release sugars	Red. Org. matter	Release sugars	Red. Org. matter
3	1	4 - 5	5,0	50	50
4	1	≤ 3,0	5,0	50	50
3	2	≤ 3,0	4,0	≥ 60 (?)	30
4	2	≤ 3,0	4,0	50	40

2. Solution - 2.2 Results

2.2.2 Adaption of high-performance fungi strains by using OFMSW as fermentation substrate

Procedure

1.1 Fungi strains

Penicillium funiculosum (ASA Nr. 150)
Aspergillus nidulans OSE 8 (ASA Nr. 266)
Aspergillus nidulans OSE 12 (ASANr. 264)

1.2 OFMSW-pretreatment

OFMSW-sample no. 2 from CENER (ca. 200 g dry matter), 08.10.19
8 g OFMSW + 232 ml tap water
5 min stirring at RT
centrifugation
30 g pellet (wet mass) per liter main medium

2. Solution - 2.2 Results

2.2.2 Adaption of high-performance fungi strains to OFMSW

Table 2: Enzyme activities of lyophilizates produced by different fungi strains on OFMSW

Strain	Enzyme activities [U/g]			
	C1-Cellulase	CMCase	ß-Glucosidase	Xylanase
P. funiculosum 27.02.20	28,3	3.750	8,8	1.350
P. funiculosum new	83	17.650	2,84	2.490
A. nidulans OSE 8	4,8	3.970	0,83	1.020
A. nidulans OSE 12	2,3	156	14	720
	endo-Pektinase	ß-1,3(4)-Glucanase	ß-1,3-Glucanase	FA-Esterase
P. funiculosum 27.02.20	473	2.390	703	≤ 0,5
P. funiculosum new	971	4.220	785	< 1,6
A. nidulans OSE 8	0	3.750	58,0	< 1,9
A. nidulans OSE 12	0	959	47,9	< 2,1

2. Solution - 2.2 Results

2.2.2 Adaption of high-performance fungi strains to OFMSW

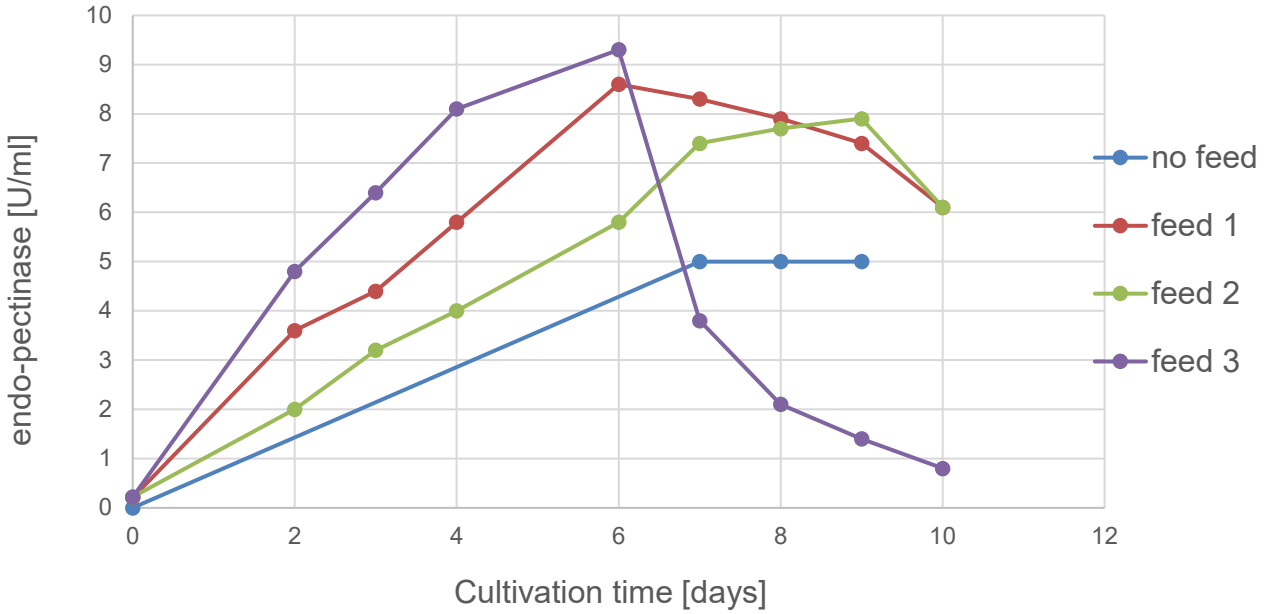


Figure 3: Formation of endo-pectinase by P. funiculosum by applying different feed strategies

Feed 1: 1 g OFMSW polymer per 100 ml and day
Feed 2: 2 x 1 g OFMSW polymer per 100 ml and day
Feed 3: 1 g OFMSW polymer + 0,5 g potato starch per 100 ml and day

2. Solution - 2.2 Results

2.2.2 Adaption of high-performance fungi strains to OFMSW

Table 3: Enzyme activities measured in lyophilizates of Penicillium funiculosum generated by three different feed strategies

Description	Enzyme activities [U/g]			
	C1-Cellulase	CMCase	ß-Glucosidase	Xylanase
Batch	83,0	17.650	2,84	2.490
Feed 1	54,3	21.600	9,0	3.980
Feed 2	42,4	24.530	3,0	3.180
Feed 3	24,4	19.530	0,9	3.740

	endo-Pektinase	ß-1,3(4)-Glucanase	ß-1,3-Glucanase	FA-Esterase
Batch	970	4.220	785	< 1,6
Feed 1	615	5.310	1.150	< 0,1
Feed 2	536	5.030	1.170	< 0,1
Feed 3	120	4.350	2.280	< 0,1

2. Solution - 2.2 Results

2.2.2 Adaption of high-performance fungi strains to OFMSW

Table 4: Degradation of OFMSW by newly developed enzyme cocktails.
Enzyme dosage: 50 µl; temperature: 50° C; pH 4.0

Enzyme cocktails no.	dm0 [mg]	Ignition loss [%]	Red. sugars [g/L]
4.4	555	62,0	16,8
4.5	663	74,3	17,5
P. funiculosum 1	591	66,8	13,4
P. funiculosum 2	657	67,8	11,3
P. funiculosum 3	638	60,1	11,5

2. Solution - 2.2 Results

2.2.3 Further optimization of best enzyme cocktails

Table 5: Compilation of the results for the best enzyme cocktails
(1 g OFSMW M3 per 10 mL buffer; T=50°C, Enzymzugabe 50 µl)

E.C. No.	Increase red. sugars [g/L] after h		Contained enzymes [%]				Price
	4	24	exo-cellulase/ hemicellulase	endo-cellulase/ ß-glucosidase	pectinase	α-amylase	[€/kg]
4.30	7,8	20,8	38	62	0	0	16,4
4.33	11,3	20,9	20	32	48	0	20,1
4.25	9,3	20,1	20	32	20	28	17,6
4.4	10,9	16,6	15	20	18	15	12,5
4.5	8,6	20,6	8	32	18	15	12,5

2. Solution - 2.2 Results

2.2.4 Comparison of different OFMSW samples

Table 6: Enzymatic release of sugars from different OFMSW samples by EC 4.5. Reducing sugars in the supernatant after 24 h incubation. ph 4.0, 50°C

OFMSW sample	Reducing sugars [mg/mL]
CENER 1	10.2
CENER 2	17.5
CENER 3	20.6
DYADEMA, Kouzani	16.0

2. Solution - 2.2 Results
2.2.5 Summary

- A laboratory test procedure was developed for the identification and characterization of enzymes suitable for the degradation of OFMSW
- New enzyme cocktails for the disintegration and saccharification of OFMSW were designed and delivered to CENER.
- High content of endo-cellulose/ß-glucosidase with low exo-cellulose/hemicellulose seems to give the highest release of sugars
- Due to the results of CENER further optimizations were undertaken and led to EC 4.5 as the most effective enzyme preparation for the release of sugars from OFMSW.
- Several batches of this enzyme preparation were produced in a scale up to 50 liters for demonstration purposes at CENER's plant.
- Cultivation of Penicillium funiculosum on OFMSW as substrate delivered taylor-made enzyme preparations containing very high activities of endo-cellulase, pectinase and ß-glucanases. However, characterization experiments showed, that the efficacy concerning the release of sugars is lower compared to the best enzyme cocktail 4.5.

2. Solution - 2.3. Opportunities for marketing of the developed enzyme cocktails

- Enzyme cocktails and enzymatic process (technology) for the conversion and especially for the saccharification of OFMSW.

- The enzymatically converted OFMSW can be used as substrate for the fermentation of value-added products as
  - PHA/PHB
  - building blocks for formulation of biodegradable polyesters
  - bacterial pesticides.

- Potential customers: municipalities collecting the OFMSW.
- Organic waste can be enzymatically converted to valuable raw material.
- Thus the enzymatic process reduces the disposal costs

Thank you so much for your attention !

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