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Optimisation of the production process of polyhydroxyalkanoates from waste biomass

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INTRODUCTION

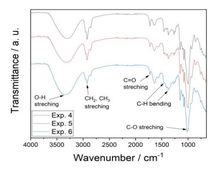
- The plastic of today – synthetic polymers made from petroleum and petroleum derivatives
- Problems with synthetic polymers – excessive production and accumulation; non-biodegradable
- Scientific research → Biodegradable polymers – polyhydroxyalkanoates (PHA)
- PHA – produced by fermentation (submerged fermentation (SmF) and solid state fermentation (SSF))
- PHA – Reserve energy source in the cytoplasm of microorganisms
- Agro-industrial waste – PHA production and appropriate disposal
- **This research** → waste starch, pretreatment of starch by ultrasound and NaOH solutions (Full factorial design), and SSF

pretreatment of starch by ultrasound and NaOH

SSF = starch + suspension of microorganisms

optimal conditions for pretreatment

t (US) / min	*P* (US) / W mL^{-1}	*c*(NaOH) / mol L^{-1}
30,00	1,71	0,01



PHA

MATERIALS & METHODS

Substrate → **1° Isolation of microorganisms** → **2° Identification of microorganisms** → **3° Pretreatment of starch** → **4° Solid state fermentation (SSF)** → **5° PHA characterization**

Starch

1° Isolation of microorganisms

- **Bacteria**
- **Fungi**

Yeast

2° Identification of microorganisms

- Gram staining
- Sudan black staining
- KOH test
- Biochemical tests: catalase, oxidase and nitrate-reductase tests
- API Strips

- Sudan black staining
- API 20 C aux

3° Pretreatment of starch

- The pretreatment of starch was performed according to the Full factorial design.
- Three factors were tested at three levels (Table 1).
- The starch was suspended in deionized water at a ratio of 1:20.

Table 1. Design experiment

Experiment	t (US) / min	P (US) / W mL^{-1}	c(NaOH) / M
1	30	1	0,01
2	30	1	0,05
3	30	1	0,1
4	30	2	0,01
5	30	2	0,05
6	30	2	0,1
7	30	3	0,01
8	30	3	0,05
9	30	3	0,1
10	60	1	0,01
11	60	1	0,05
12	60	1	0,1
13	60	2	0,01
14	60	2	0,05
15	60	2	0,1
16	60	3	0,01
17	60	3	0,05
18	60	3	0,1
19	90	1	0,01
20	90	1	0,05
21	90	1	0,1
22	90	2	0,01
23	90	2	0,05
24	90	2	0,1
25	90	3	0,01
26	90	3	0,05
27	90	3	0,1

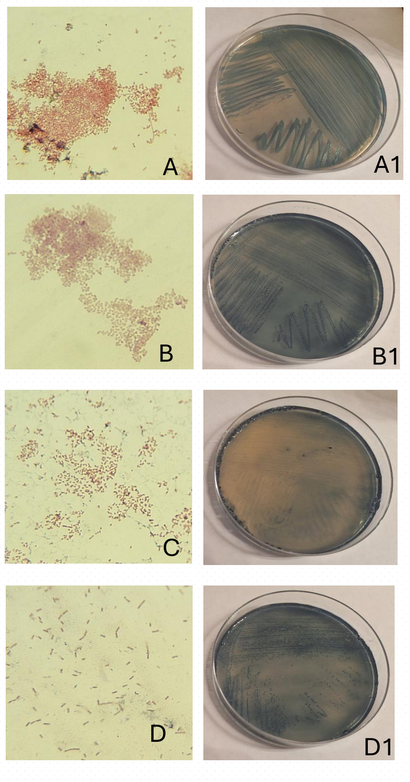
4° Solid state fermentation (SSF)

- SSF was performed for 7 days at room temperature
- in 100 g of pretreated starch, 10 ml of the suspension of the mixed culture was added
- extraction and purification of PHA was performed with chloroform and 4% sodium hypochlorite

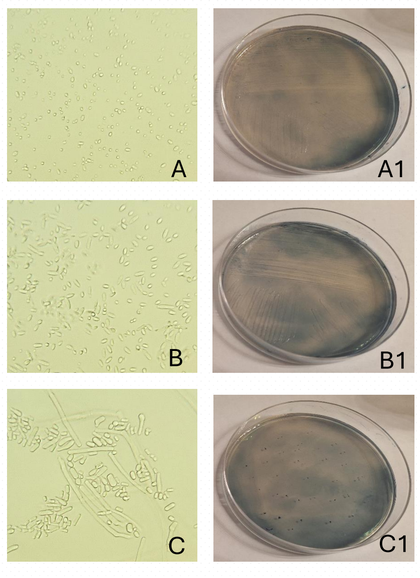
5° PHA characterization

- FTIR – ATR spectroscopy in wavelenght range: 4000 – 650 cm^{-1}

RESULTS



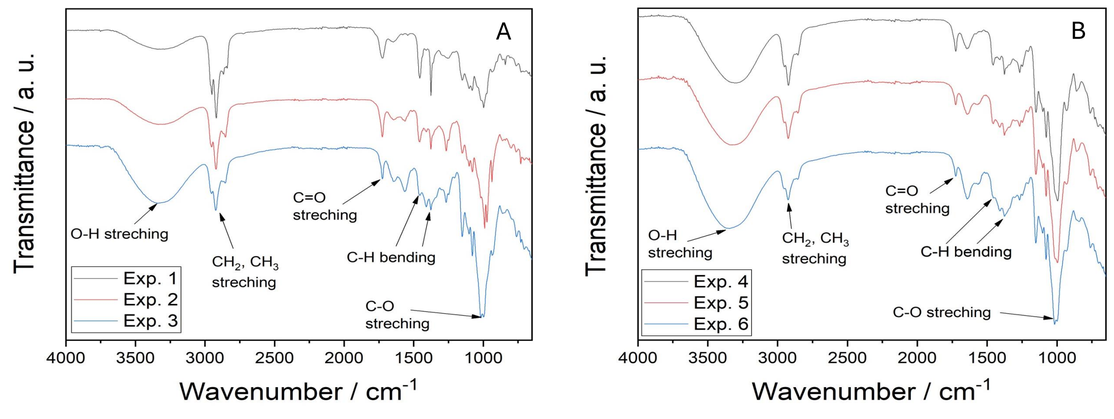
Microphotographs of Gram staining and photographs of Sudan Black staining of bacteria *Leukonostoc sp*. (A, A1), *Bacillus licheniformis* (B, B1), *Citrobacter freundii* (C, C1), *Staphilococcus lentus* (D, D1), respectively, isolated from starch.

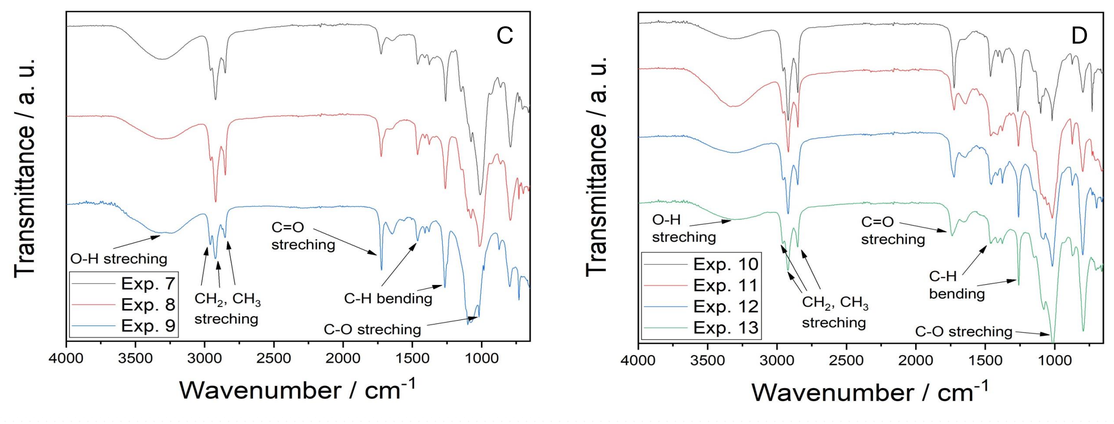


Microphotographs of Gram staining and photographs of Sudan Black staining of yeasts *Candida krusei* (A, A1), *Cryptococcus humicola* (B, B1), and *Geotrichum klebahnii* (C, C1), respectively, isolated from starch.

Accumulation of PHA obtained by SSF of waste starch after 7 days.

Experiment	PHA accumulation / %
1	00670
2	0.3184
3	0.1498
4	0.5572
5	0.1872
6	0.2145
7	0.0063
8	0.0582
9	0.0278
10	0.0311
11	0.0033
12	0.0023
13	0.0069
14	0.0256
15	0.0684
16	0.0274
17	0.0098
18	0.0060
19	0.0212
20	0.1347
21	0.0404
22	0.0388
23	0.0308
24	0.1223
25	0.0333
26	0.0302
27	0.5105





FTIR spectra of PHA obtained by SSF of starch in experiments 1, 2, and 3 (A), 4, 5, and 6 (B), 7, 8, and 9 (C), 10, 11, 12, and 13 (D) after 7 days.

Optimal conditions for starch pretreatment to achieve the highest PHA accumulation.

t (US) / min	*P* (US) / W mL^{-1}	*c*(NaOH) / mol L^{-1}
30.00	1.71	0.01

Characteristic functional groups of PHA obtained by FTIR-ATR spectroscopy.

Bond	Wavenumber, cm^{-1}
C = O streching	1728, 1730
C - H bending	1380, 1383, 1454, 1456, 1462
C – O streching	990, 1005, 1015, 1263
O - H streching	3300
CH_3, CH_2 streching	2850, 2852, 2920, 2922, 2925, 2960

CONCLUSION

- 4 bacteria and 3 yeasts suitable for PHA production were isolated and identified from waste starch
- Starch pretreatment was performed according to a Full factorial design by varying the duration of the ultrasonic bath, the ultrasonic power, and the NaOH concentration, *c*(NaOH)
- PHA production was carried out by solid state fermentation
- The bonds observed in the FTIR spectrum at approximately 1730, 1380-1465, 1000-1300, 3300 and 2850-2970 cm^{-1} represent C=O ester, -CH, C–O, -OH, CH_3 and CH_2 bonds, respectively, which are characteristic of PHA

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