Cyprus University of Technology

Environmental Bioprocessing Laboratory

Use of vineyard prunings biochar as a carrier for cell immobilization and ethanol overproduction by *Saccharomyces cerevisiae*

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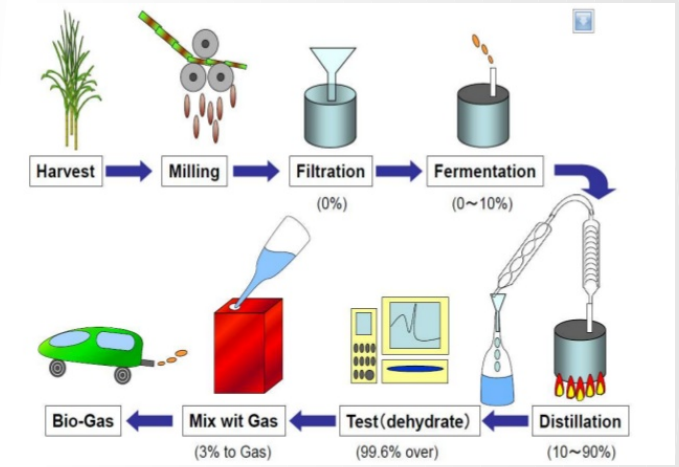
Technology of bioethanol

- Biodiesel and bioalcohols constitute the most important biofuels



- Highlights the need to identify alternative feedstocks for sustainable manufacturing
- Current limitations of bioethanol technology:
  - **Cooling and distillation cost**
  - **Lack of rigid carriers for recirculation of yeasts**

Biochar as a yeast immobilization carrier

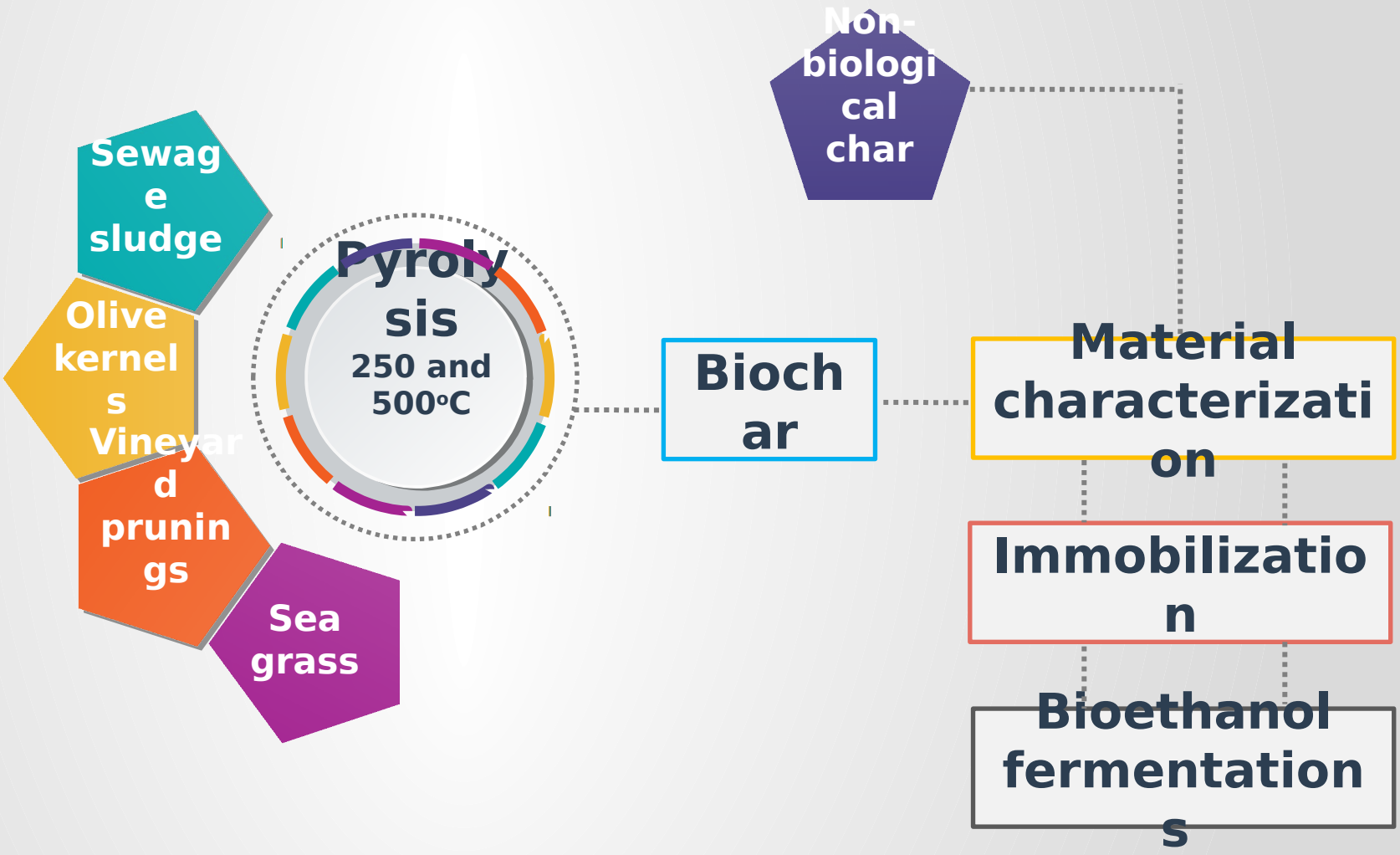
- **Substrate** and **product** inhibition
- **Immobilized biocatalysts**
- Alginate gel beads have **poor mechanical properties**
- **Biochar**
- Environmental management and soil amendment
- Immobilization of heavy metals
- Enhance methane production in anaerobic digestion
- **Promotes interspecies electron transfer**
- Improves cell activity and growth
- Assists buffering capacity and nutrient adsorption into their surface

Aim of the study

- Evaluate the use of biochar as a renewable and low-cost support material for **whole-cell immobilization**

- **Enhance bioethanol production** emphasizing the importance of employing **elevated** temperatures in order to **reduce the energy** demand of manufacturing.

Pyrolysis for biochar production



Material characterization

EDX

Energy Dispersive X-Ray analysis
Elemental analysis

XRD

X-Ray Diffraction analysis
Phase identification of crystalline materials

NaCl Si KCl $CaCO_3$

BET

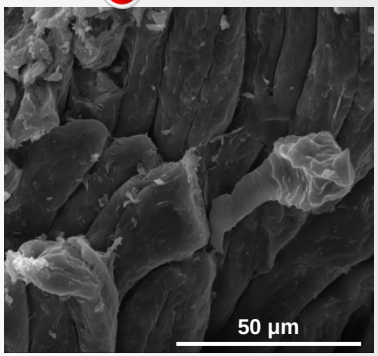
Brunauer–Emmett–Teller method
Determination of specific surface area

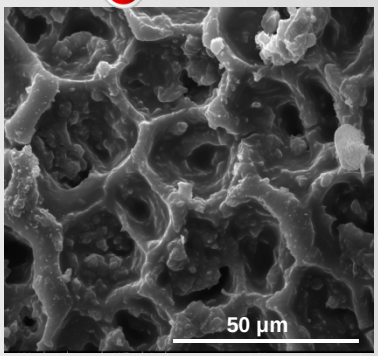
SEM

Scanning Electron Microscopy
Porosity and Structure characteristics

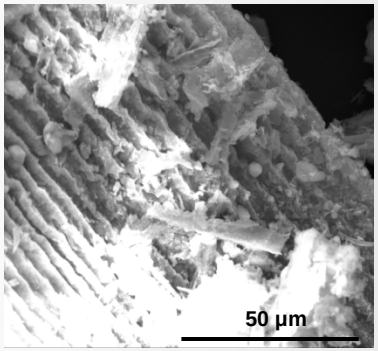
Scanning electron microscopy

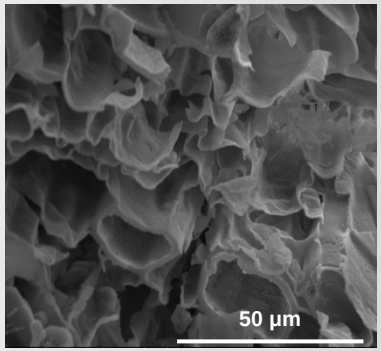
Olive kernels



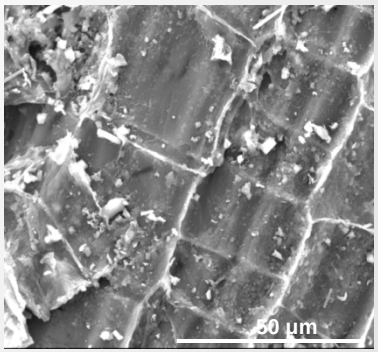


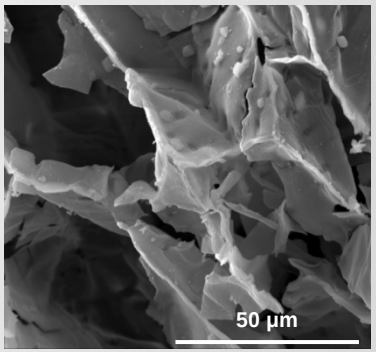
Sea grass





Vineyard prunings





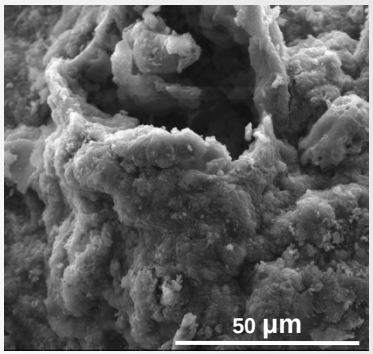
SEM

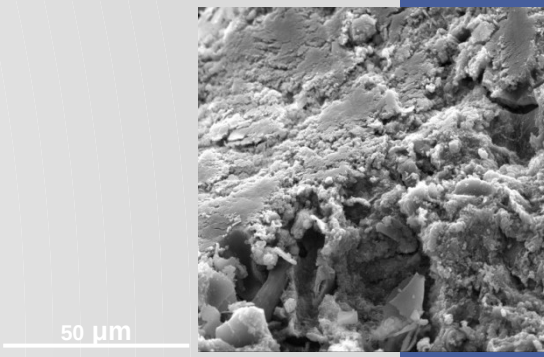
Scanning electron microscopy

250° C

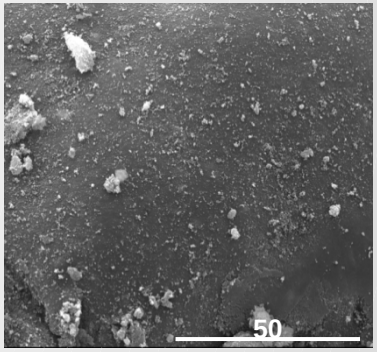
500° C

Sewage sludge





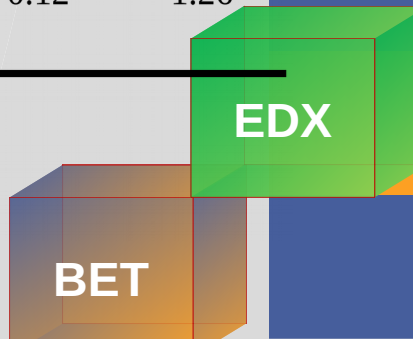
Non-biological char



SEM

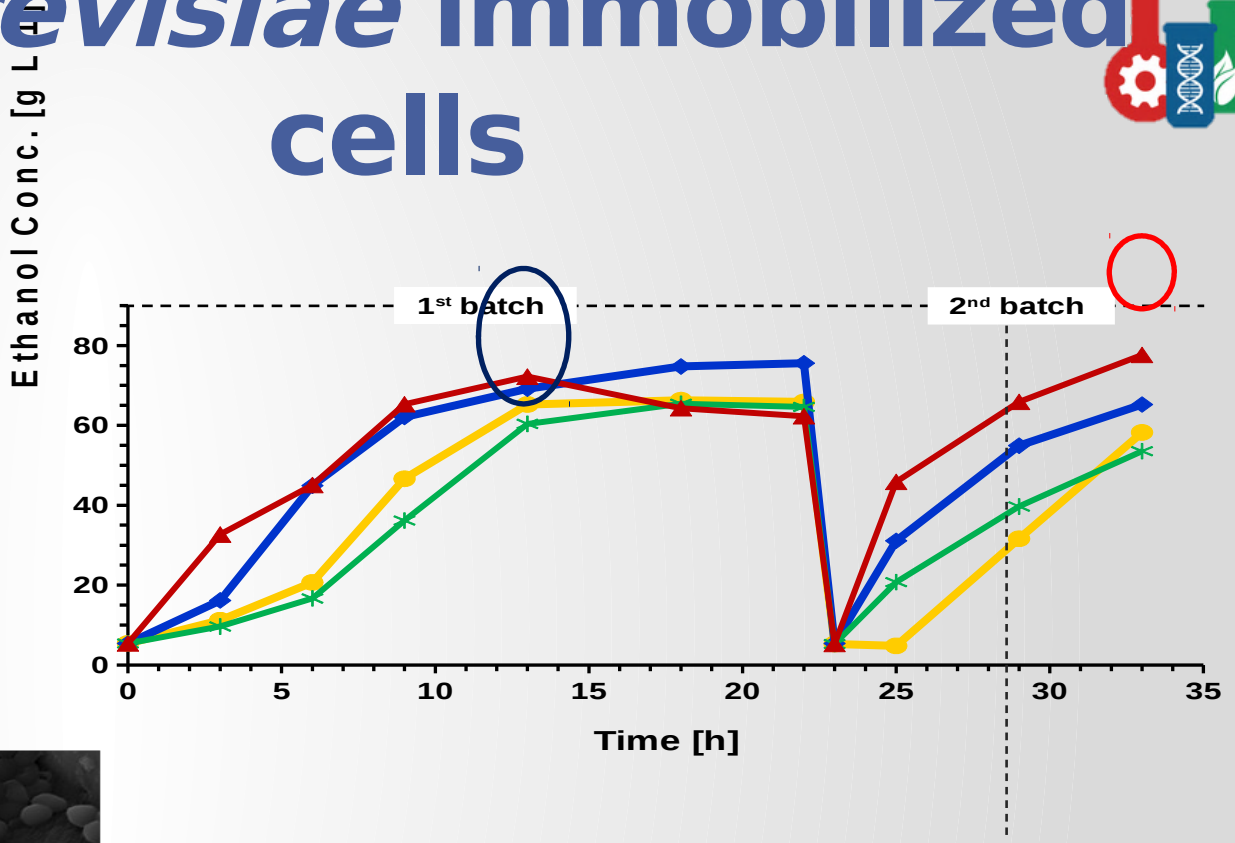
Surface area and elemental composition

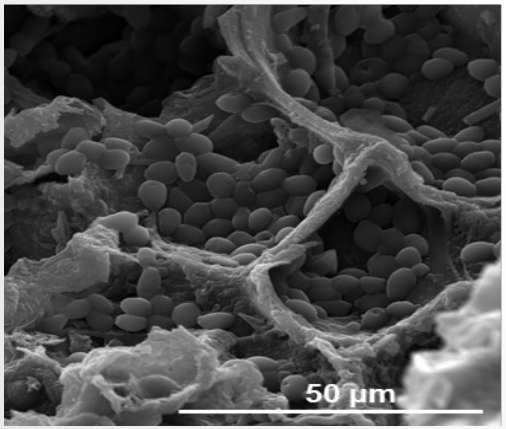
Sample	Temperature (℃)	Specific surface area ($m^2 g^{-1}$)	C (%)	O (%)	Ca (%)	Cl (%)	Si (%)
Olive kernels	250	0.15	61.72	21.42	1.14	0.30	-
	500	1.5	65.73	20.02	2.23	0.64	0.23
Vineyard prunings	250	0.5	69.62	24.65	3.04	-	-
	500	41.7	71.65	21.59	1.24	-	-
Sewage sludge	250	0.7	33.11	34.38	6.30	-	3.48
	500	1.4	55.78	23.47	3.76	-	1.73
Sea grass	250	1.9	60.52	24.07	0.61	3.90	0.18
	500	5.3	60.97	22.86	0.6	5.14	-
Non-biological char	-	73.0	88.0	3.16	0.32	0.12	1.26



S. cerevisiae immobilized cells

Temperature
37 ºC



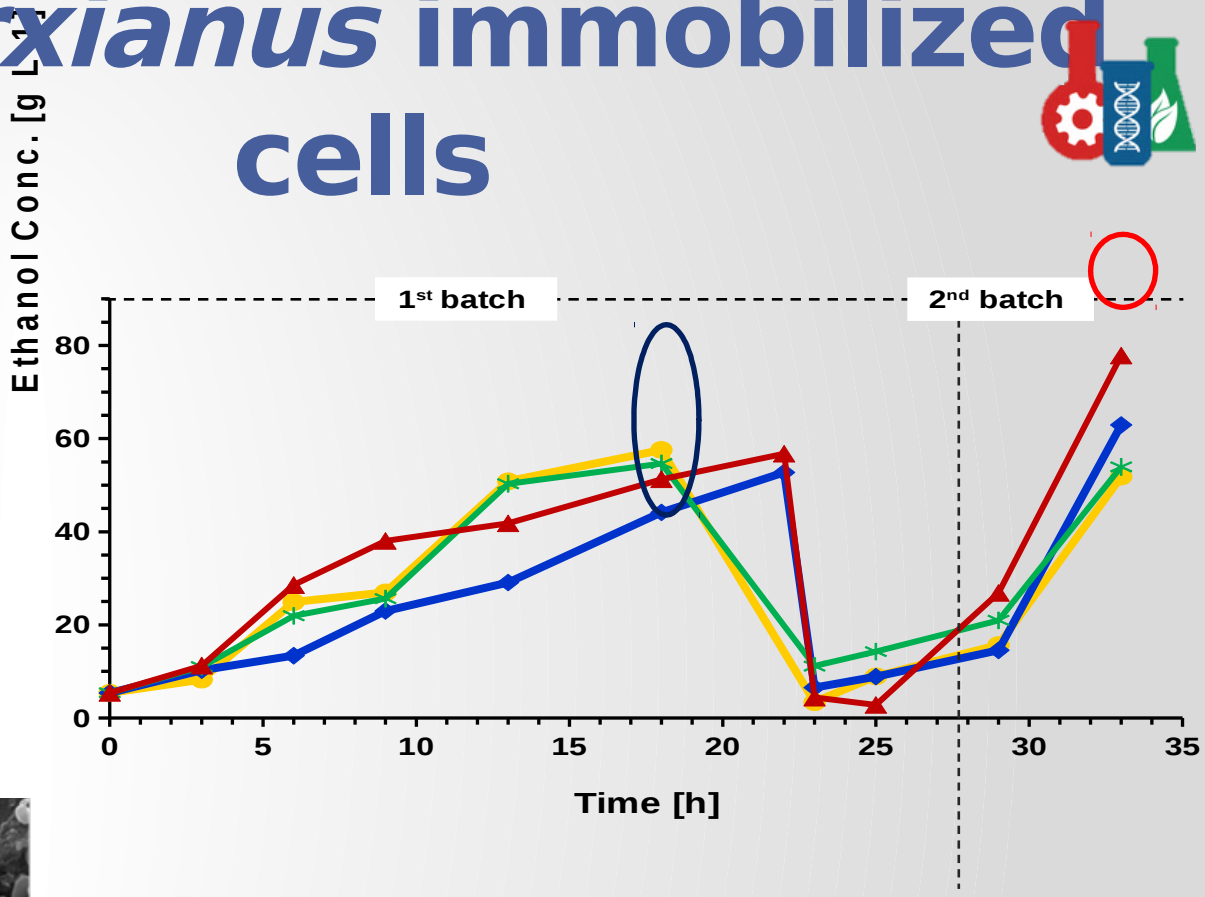


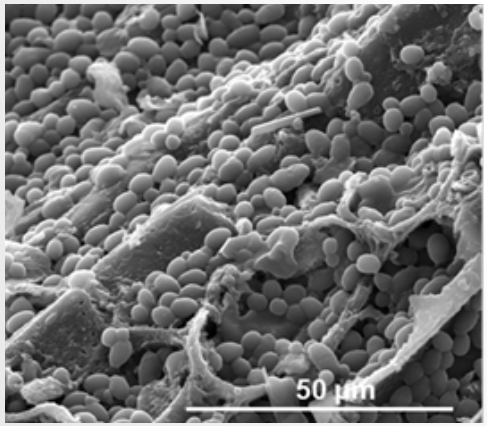
S. *cerevisiae* immobilized on vineyard prunings

- **Vineyard prunings biochar 7.2 g L^{-1} h^{-1}**
- **Non biological char 6.0 g L^{-1} h^{-1}**
- **Sea grass biochar 4.8 g L^{-1} h^{-1}**
- **Free cells 5.3 g L^{-1} h^{-1}**

K. marxianus immobilized cells

Temperature
42 ºC



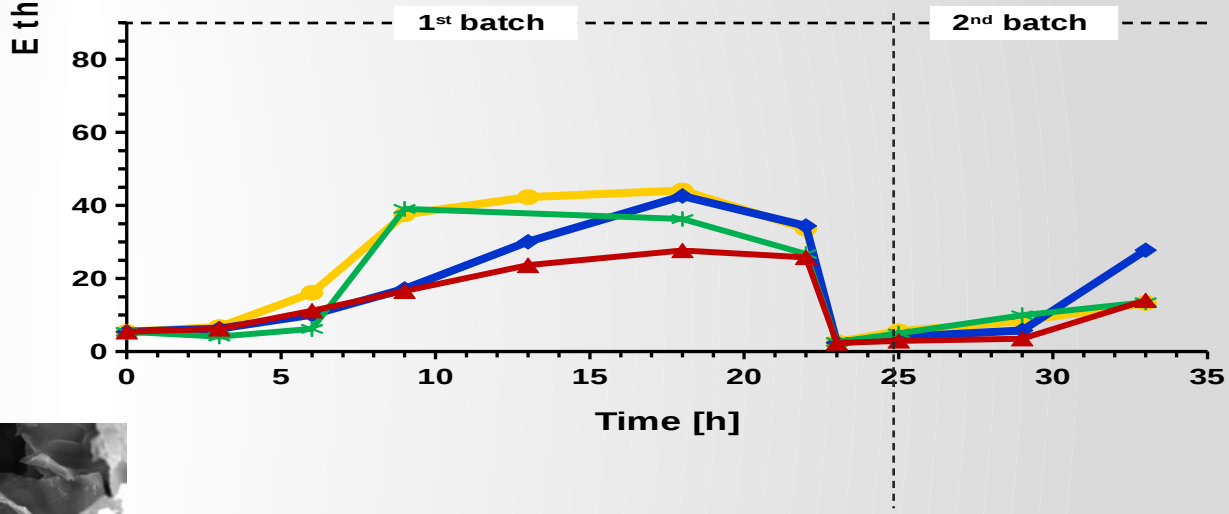


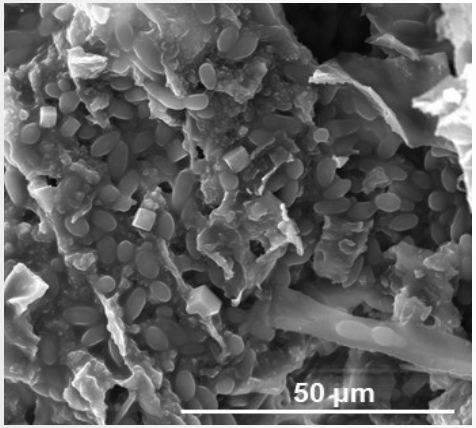
S. *cerevisiae* immobilized on vineyard prunings

Vineyard prunings biochar 7.3 g L^{-1} h^{-1}
Non-biological char 5.6 g L^{-1} h^{-1}
Sea grass biochar 4.3 g L^{-1} h^{-1}
Free cells 4.9g L^{-1} h^{-1}

P. kudriavzevii immobilized cells

Temperature
42 ºC





Vineyard prunings biochar
Non-biological char
Sea grass biochar
Free cells

S. *cerevisiae* immobilized on vineyard prunings

Bioethanol production from immobilized *S. cerevisiae*

Feedstock	Carrier	Ethanol conc. ($g\ L^{-1}$)	Ethanol prod. ($g\ L^{-1}\ h^{-1}$)	Specific surface area ($m^2\ g^{-1}$)
Sugar molasses	Alginate-based MCM-41 mesoporous zeolite composite	78.6	6.55	-
Cane molasses	Bacterial cellulose-alginate sponge	92	1.92	-
Glucose and sucrose	Sorghum bagasse	92.7	5.72	3.0-5.0 [54]
Sugarcane bagasse	Sugarcane bagasse	15.4	0.43	3.0-12.7 [55]
Blackstrap molasses	Thin-shell silk cocoon	80.6	1.85	-
Sugar beet thick juice	Sugar beet pulp	52.3	1.09	3.0-16.5 [55]
Sorghum juice	Sweet sorghum stalks	98.5	1.37	3.0-5.0 [54]
Mahula flower	Calcium alginate	25.8	0.27	-
Corn meal	Calcium alginate	88.9	2.34	-
Wheat straw	Calcium alginate	37.1	0.38	-
Glucose	Mineral Kissiris	48	3.06	2.2 [62]
Molasses	Orange peel	58.9	4.17	0.4 [63]
Orange peel waste hydrolysate	Non- biological char	60	6.0	73.0
Orange peel waste hydrolysate	Vineyard prunings biochar	72	7.2	41.7
Orange peel waste hydrolysate	Non- biological char	56	5.6	73.0
Orange peel waste hydrolysate	Vineyard prunings biochar	73	7.3	41.7

Application of other materials

- High specific surface area
- High concentration of micropores

Pistachios

Peanuts

Corks

Pyrolysis 500°C

Biochar

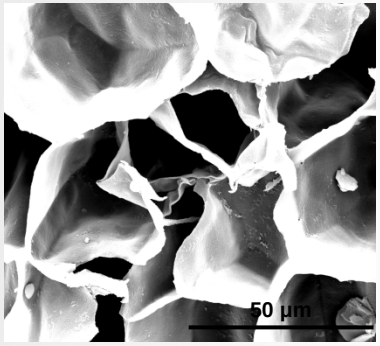
Carbon foam

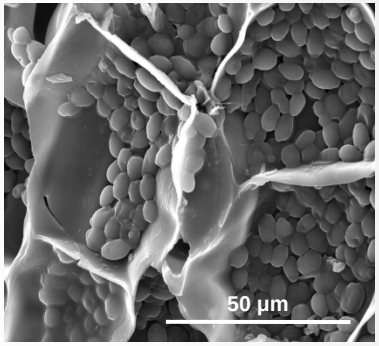
Immobilization

Bioethanol fermentations

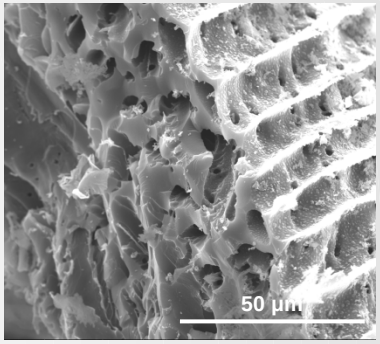
Scanning electron microscopy

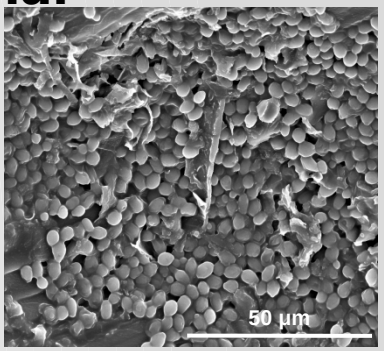
Corks biochar



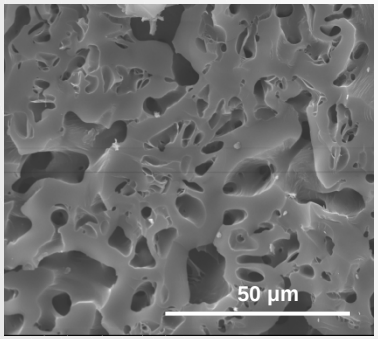


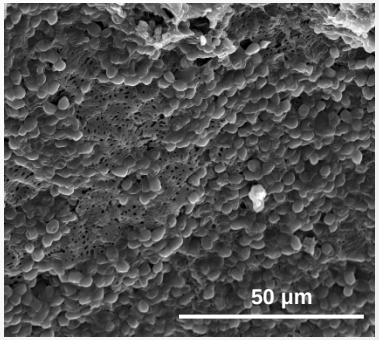
Peanuts biochar



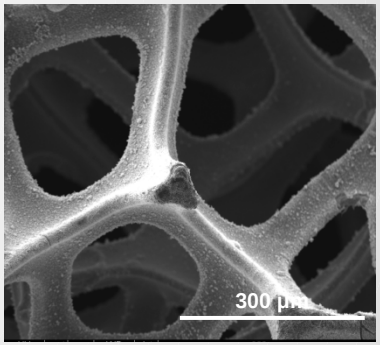


Pistachios biochar

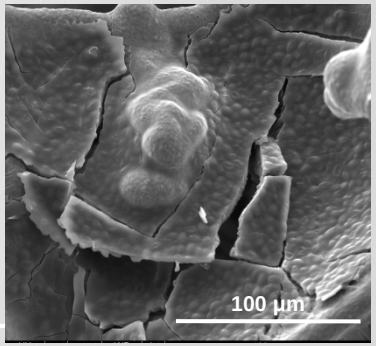




Carbon foam



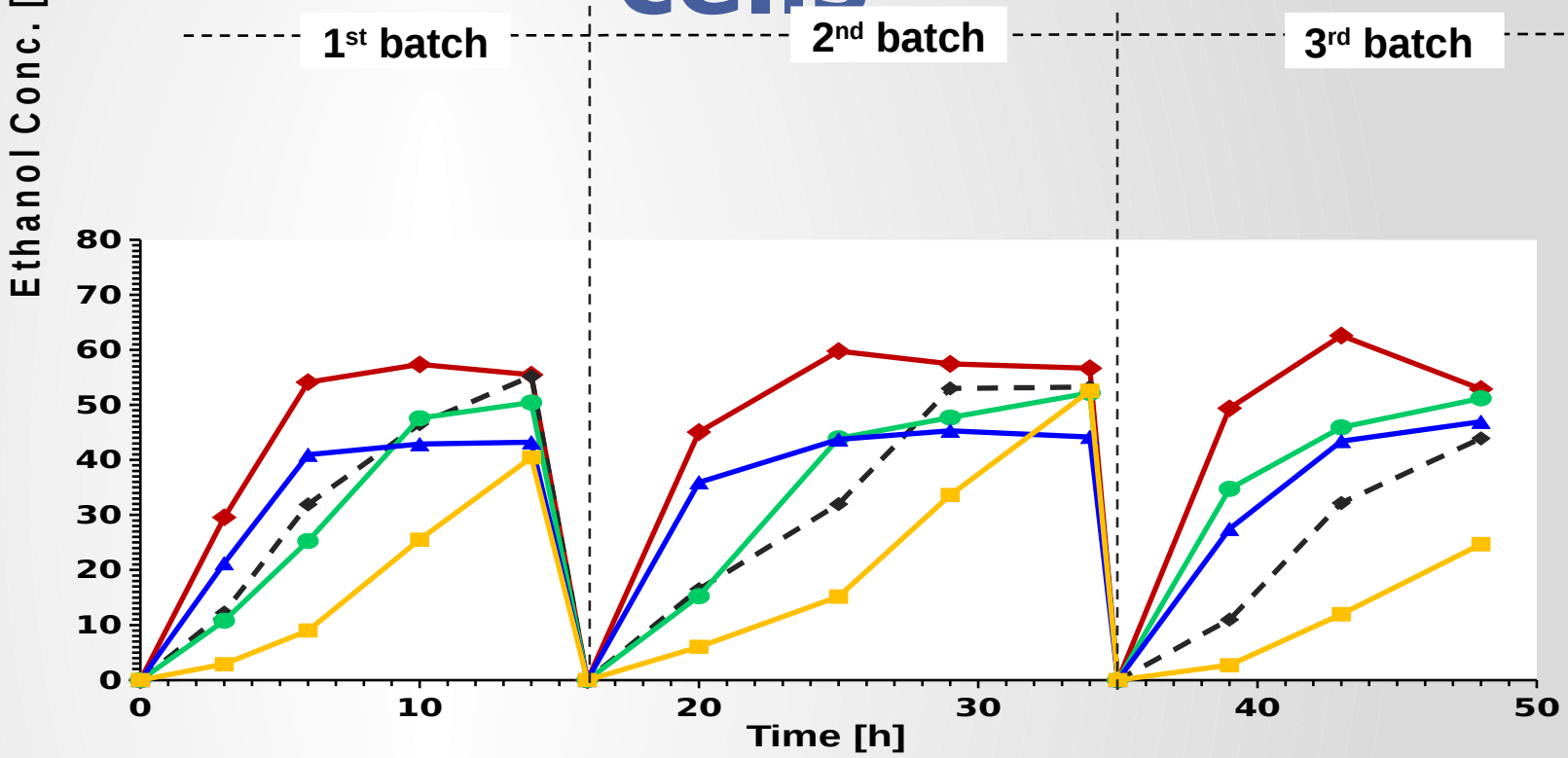
x 500



x 1500

SEM

S. cerevisiae immobilized cells



Temperatur e 37 ºC

- **Pistachios biochar 7.8 g L^{-1} h^{-1}**
- **Peanuts biochar 5.4 g L^{-1} h^{-1}**
- **Corks biochar 5.7 g L^{-1} h^{-1}**
- **Carbon foam 1.5 g L^{-1} h^{-1}**
- **Free cells 4.0 g L^{-1} h^{-1}**

Conclusions

- Pyrolysis conditions strongly affect:
  - Morphology
  - Physicochemical properties

↑ Pyrolysis temperature ↑ specific surface area and porosity

37 ºC — *S. cerevisiae*

42 ºC — *K. marxianus*

→ **Vineyard prunings biochar** → ↑ High ethanol concentration

37 ºC — *S. cerevisiae* → **Pistachios biochar** → ↑ Improvement ethanol productivity

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