Synthesis of biosurfactants using winery residues for potential applications to treat hydrocarbon-contaminated soils

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SiCon 2020

Roma 12-02-2020

Circular economy in olive oil and wine sectors
The CREIAMO project in the Lombardia region (Italy)

Circular economy

Olive and winery wastes

Non-pathogenic bacteria → **Rhamnolipids** → **Remediation of contaminated soils**

Symbiosis network with the active participation of both SMEs and local stakeholders

CREIAMO

Systemic eco-innovation through industrial symbiosis

UNIVERSITÀ DEGLI STUDI DI BRESCIA

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ENEA

Fondazione CARIPLO

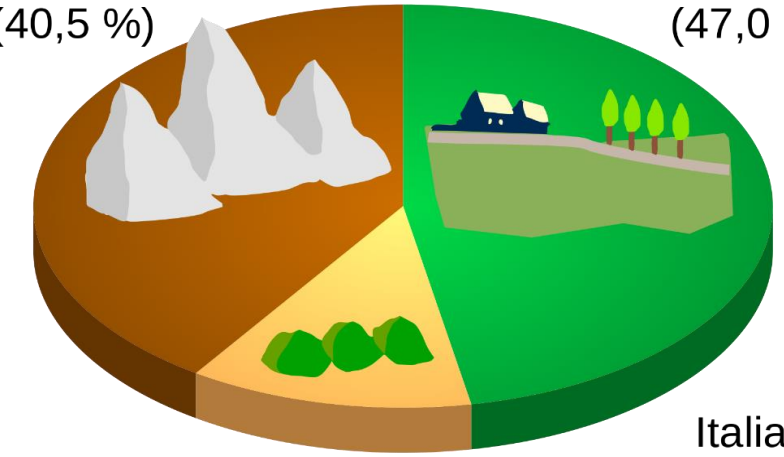
Italy: Lombardia as a model for the circular economy
Olive and winery cultivation

Lombardia

(2.386.285 ha)

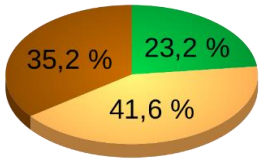
Montagna
967.281
(40,5 %)

Pianura
1.122.642
(47,0 %)



Collina
296.362
(12,4 %)

Italia



CREIAMO

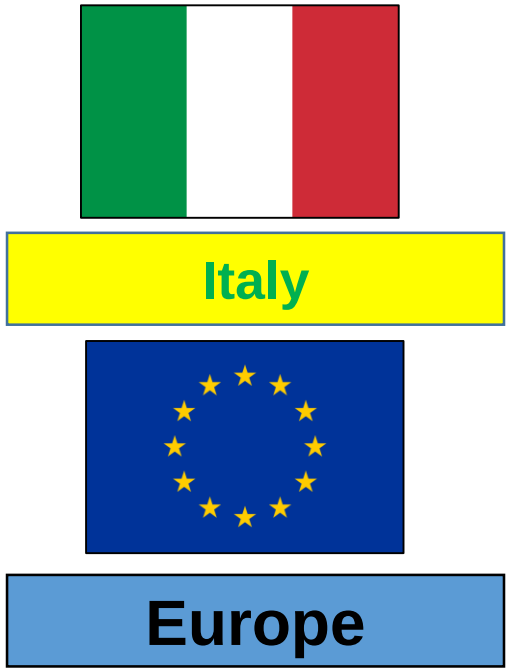
7.2 Million tonnes

1.47 million hectoliters 2016

150 million olive trees

1,100,000 hectares

Winery wastes in numbers



The vineyard area covers a total of 21220 hectares

535 thousand hectoliters

wine grape production :12 t/ha

1 ha → **2 t marc grape/ season**

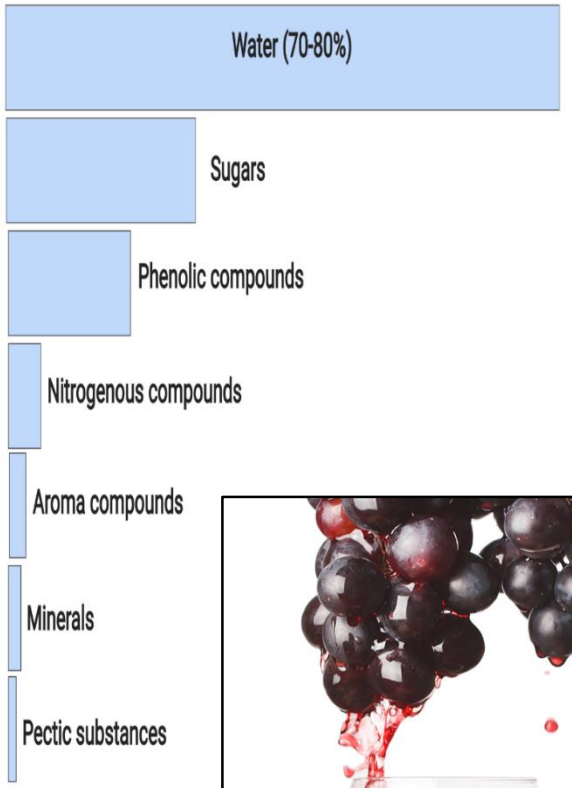
2 * 21220 = 42440 t marc/season

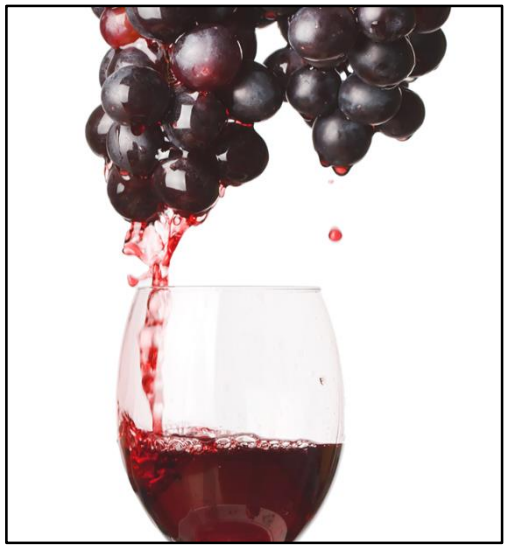
3.2 million hectares in the EU → **6,400,000 t marc grape/ season**

Muhlack et al., Waste Management (2018)

Grape vs grape marc composition

(*Vitis vinifera* L.)





(*Vitis vinifera* L.)

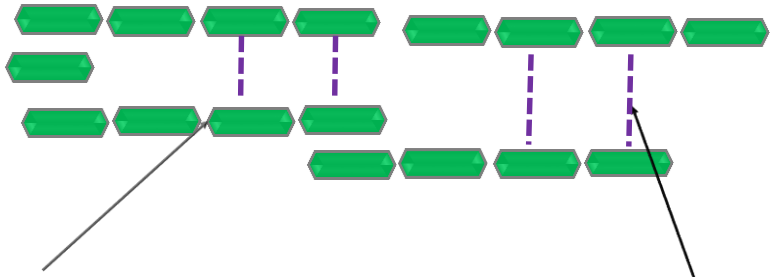
Table 1
Physical and chemical properties of grape marc.

Parameter	Value	Reference
pH	3.6 ± 0.2	Lafka et al. (2007) (red grape marc)
Moisture	73.6 ± 2.6% w/w	
Reducing sugars	1.5 ± 0.3% w/w	
Ash	4.6 ± 0.5% w/w	
Cellulose	20.8% w/w	Mendes et al. (2013) (grape skins only)
Hemicelluloses	12.5% w/w	
Tannins	13.8% w/w	
Proteins	18.8% w/w	
Ash	7.8% w/w	

Muhlack et al., Waste Management (2018)

Hemicellulose vs Cellulose

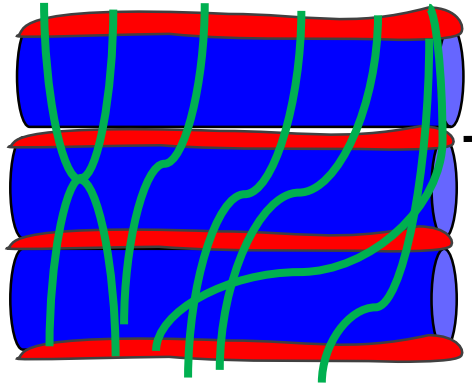
Cellulose

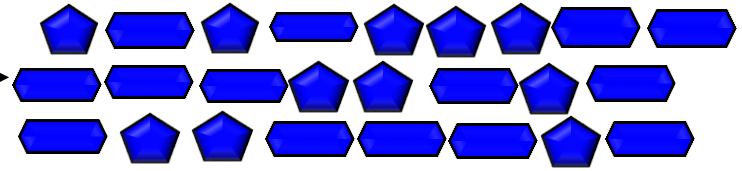


β-(1,4)-glycosidic bond

Hydrogen bond

Hemicellulose

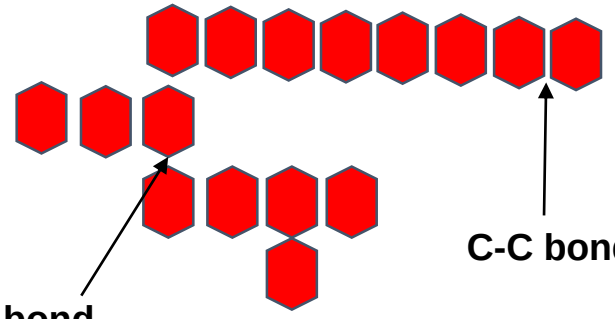




β-(1,3)-glycosidic bond

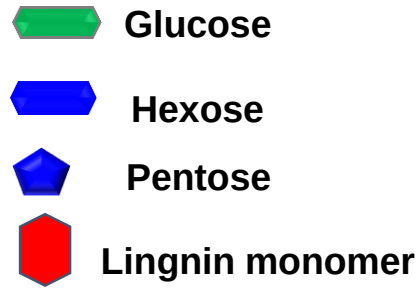
Lignin

Lignocellulosic biomass



C-C bond

Aryl ether bond



Sustainable wineries through waste valorization

White and red grape marc from wineries

Chemical treatment

Thermal treatment

Biological treatement

e.g., extraction of phenolics, antioxidants

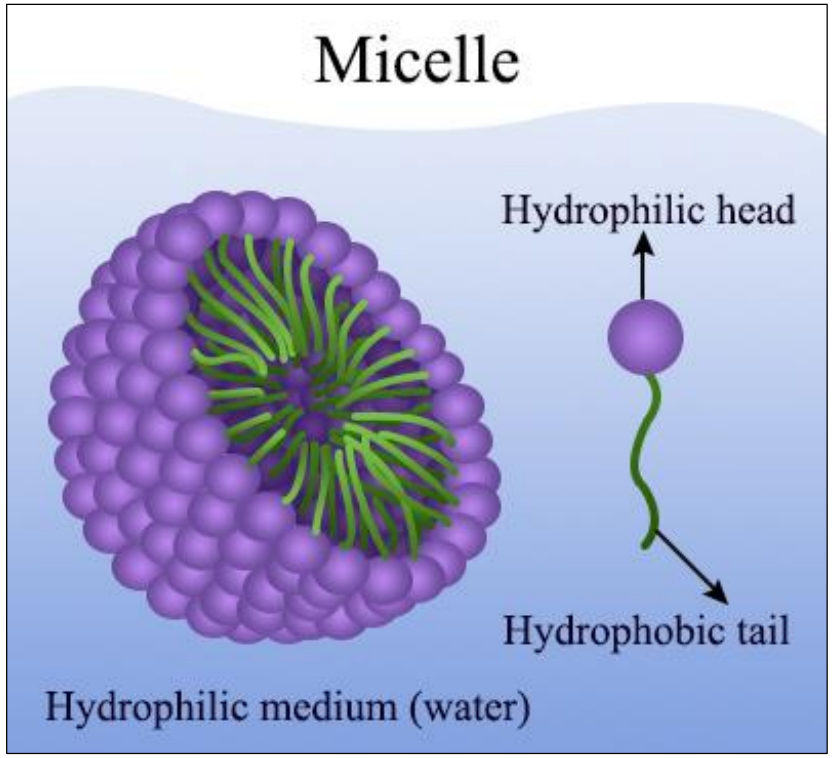
e.g., bioenergy biochar

e.g., beverage alcohol, biofuels, compost, animal feed, Biosurfactants

Muhlack et al., Waste Management (2018)

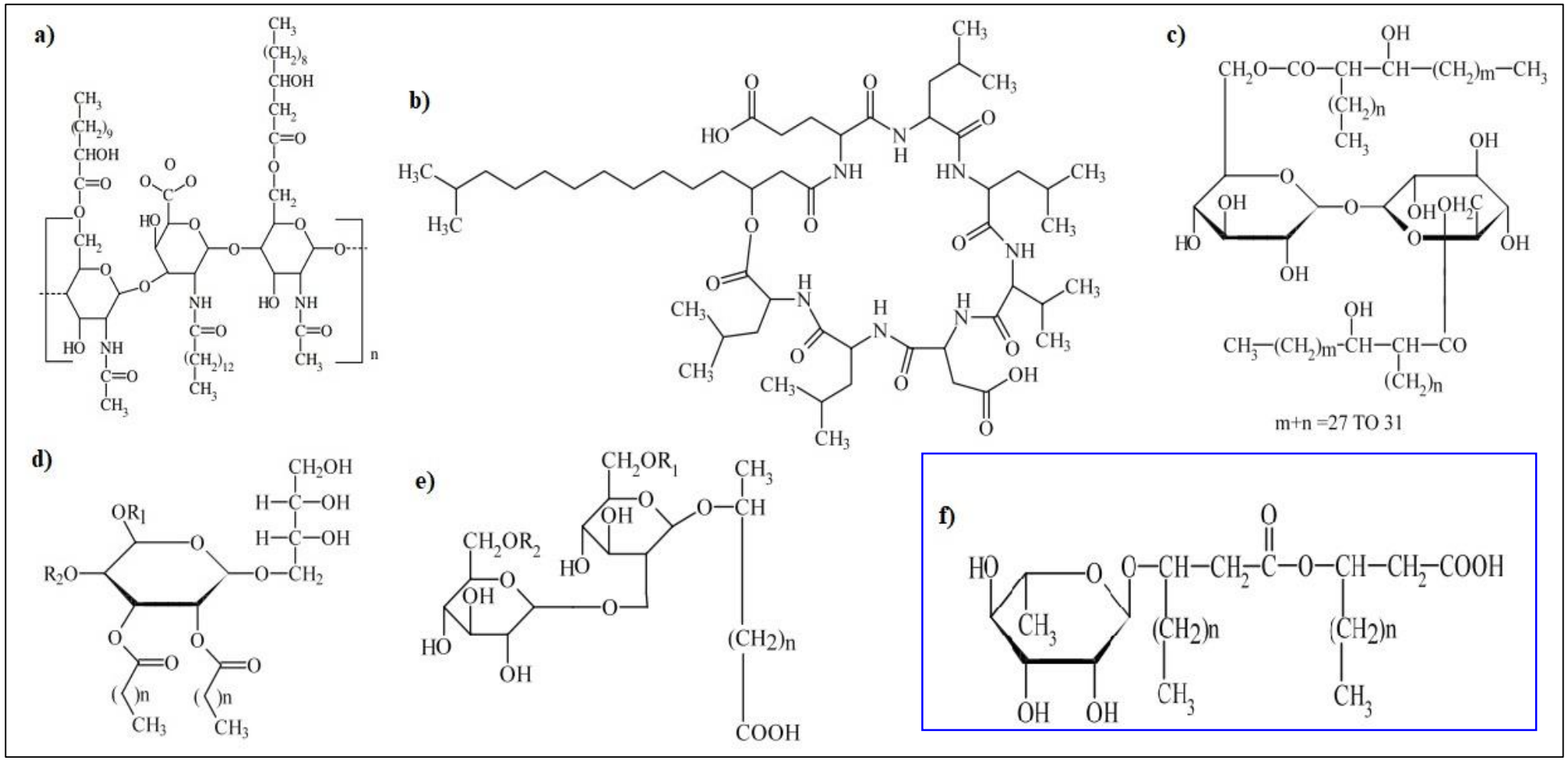
Biosurfactants

- **Glycolipids (rhamnolipids)**
- Lipopeptides
- Phospholipids and fatty acids
- Polymeric biosurfactants
- Particulate biosurfactants



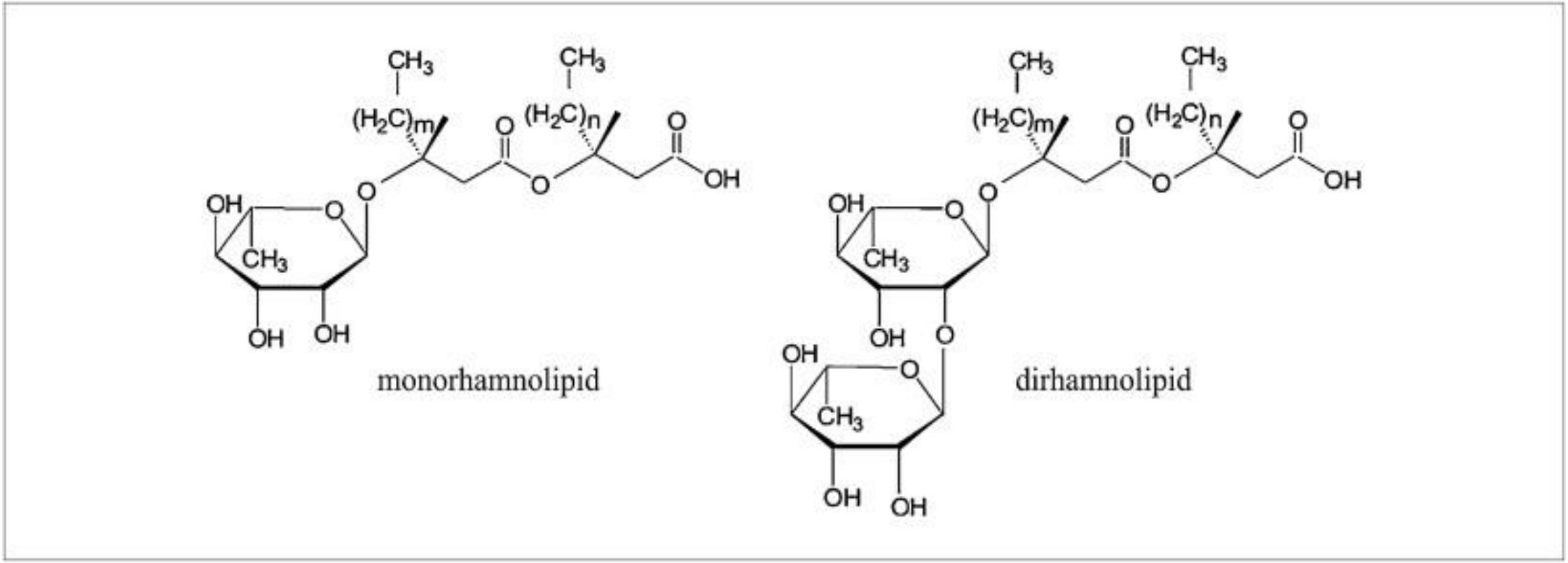
Franzetti et al., Adv Exp Med Biol (2010)

Biosurfactants:
Various Structures and Wide Applications



Biosurfactants: applications of rhamnolipids -

major applications of rhamnolipids



rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-C10-C10)

rhamnosyl-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-Rha-C10-C10)

Bioremediation and enhanced oil recovery (EOR):

Pharmaceuticals and therapeutics

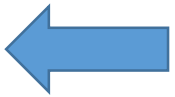
Detergents and cleaners

Agriculture

Banat IM et al., Appl Microbiol Biotechnol. (2010)

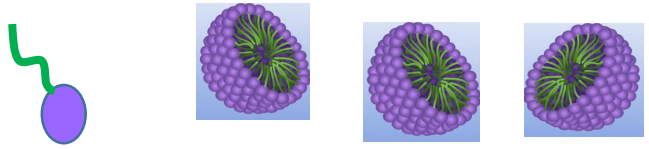
Competitive Matrix (an example)

Important product or technology characteristics	Dodecyl-sulphate	Triton	**Jeneil (BS-Rhls)**
CMC, mg/l	260	650	20-100
Interfacial tension, mN/m	0,1	0,4	0,07
Foaming capacity	8	6	9
Price, Euro/kg (approximate)	140	90	**280**

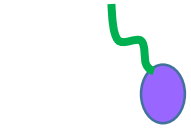


Remediation of contaminated soils

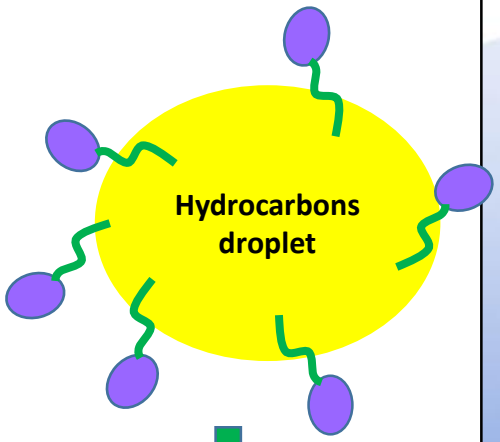
Bioremediation of contaminated soils

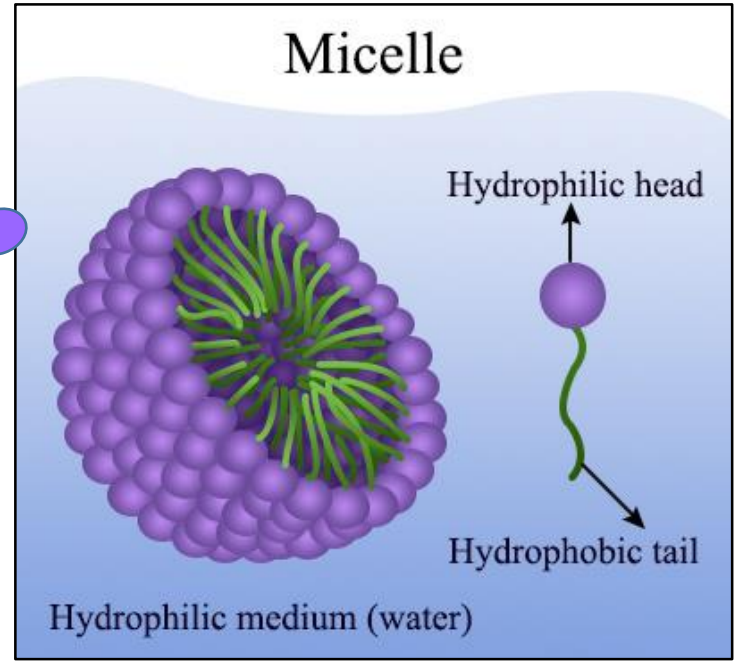


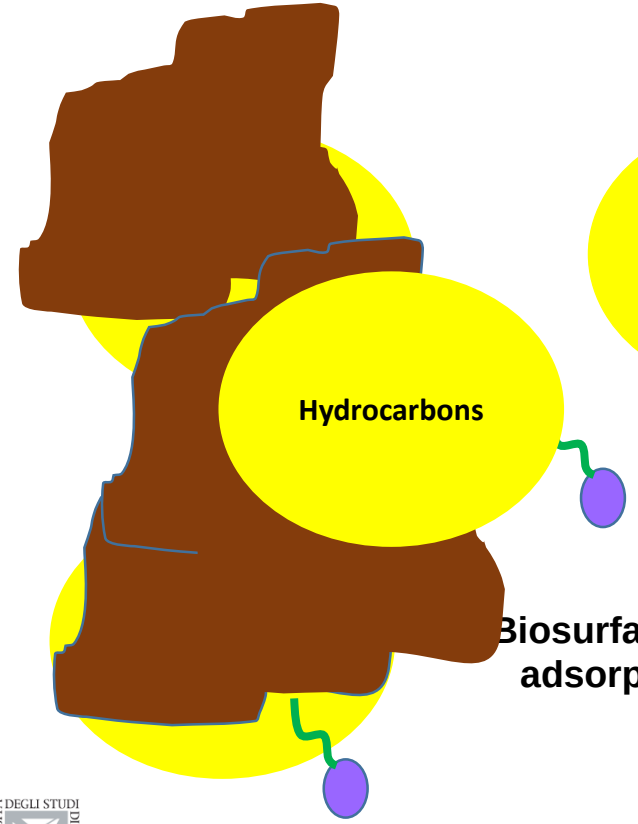
micelles

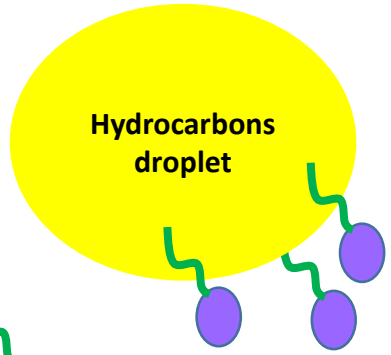


Biosurfactant adsorption

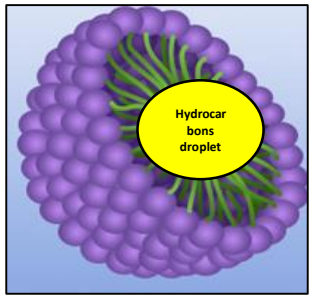


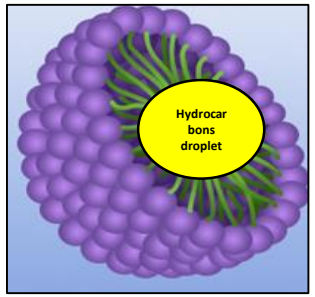




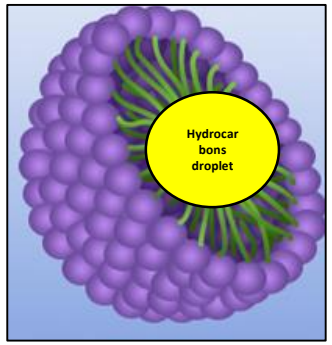


Biosurfactant adsorption





Emulsified hydrocarbons



Transport to
- Bacterial cells
- or Remobilization

Biosurfactant producing companies around the globe

1	TeeGene Biotech	UK	Rhamnolipids and Lipopeptides	Pharmaceuticals, cosmetics, antimicrobials and anti-cancer ingredients
2	AGAE Technologies LLC	USA	Rhamnolipids (R95, an HPLC/MS grade rhamnolipid)	Pharmaceutical, cosmeceutical, cosmetics, personal care, bioremediation (*in situ* & *ex situ*), Enhanced oil recovery (EOR)
3	Jeneil Biosurfactant Co. LLC	USA	Rhamnolipids (ZONIX, a bio-fungicide and RECO, a rhamnolipid used in cleaning and recovering oil from storage tanks)	Cleaning products, EOR
4	Paradigm Biomedical Inc.	USA	Rhamnolipids	Pharmaceutical applications
5	Rhamnolipid Companies, Inc.	USA	Rhamnolipids	Agriculture, cosmetics, EOR, bioremediation, food products, pharmaceutical
6	Fraunhofer IGB	Germany	Glycolipids, Cellobiose lipids, MELs	Cleansing products, shower gels, shampoos, washing-up liquids, pharmaceutical (bioactive properties)
7	Cognis Care Chemicals	China, Germany, USA	Alkyl polyglucoside APG®, Plantacare 1200 GLY (green surfactant for use in oral-dental formulations), Rheocare TTA (for cleansing formulations)	Used in formulations for household cleaners, bath/shower gels, dish washing, laundry detergents and in agrochemicals
8	Saraya Co. Ltd.	Japan	Sophorolipids (Sophoron, a low-foam dishwasher detergent)	Cleaning products, hygiene products
9	Ecover Belgium	Belgium	Sophorolipids	Cleaning products, cosmetics, bioremediation, pest control, pharmaceuticals
10	Groupe Soliance	France	Sophorolipids	Cosmetics
11	MG Intobio Co. Ltd.	South Korea	Sophorolipids (Sopholine—functional soap with Sophorolipids secreted by yeasts)	Beauty and personal care, bath supplies e.g., soaps with new functions
12	Synthezyme LLC	USA	Sophorolipids	Cleaning products, cosmetics, food products, fungicides, crude oil emulsification
13	Allied Carbon Solutions (ACS) Ltd	Japan	Sophorolipids (ACS-Sophor—first bio-based surfactant from Indian mahua oil)	Agricultural products, ecological research
14	Henkel	Germany	Sophorolipids, Rhamnolipids, Mammoslyerthritol lipids	Glass cleaning products, laundry, beauty products
15	Lion Corporation	Japan	Methyl ester sulfonates (MES)	Detergents formulations, cleaning products
16	Lipo Chemicals	USA	Lipomulse Luxe (high-temperature resistance emulsifier)	Skin care, sun-lotions hair care formulations, thickening polymers, rheological modifiers, natural gums
17	Kaneka Co.	Japan	Sophorose lipids	Cosmetics and toiletry products

Horizon 2020

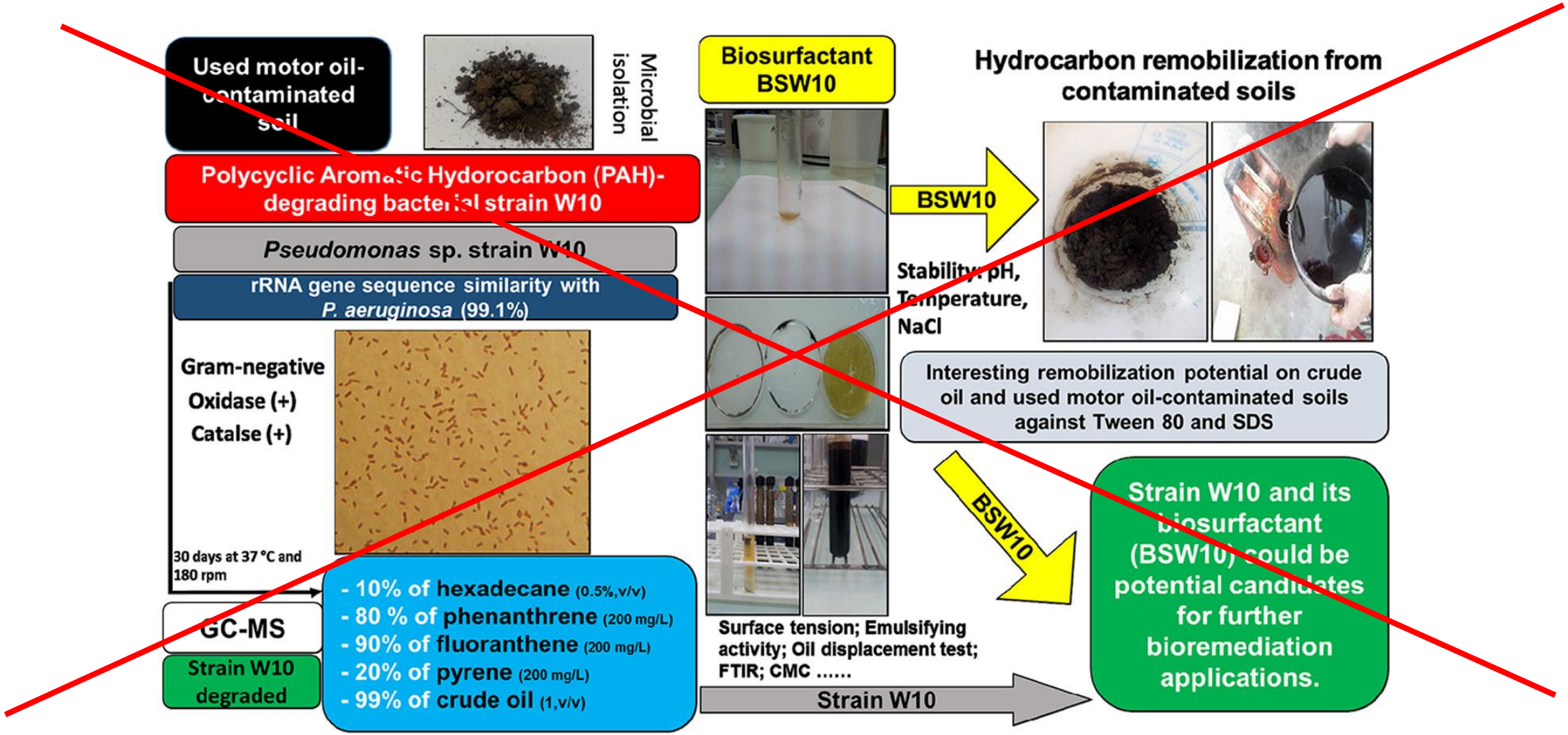
CARBOSURF

Bio-based Industries Consortium

Bio-based Industries Joint Undertaking: a €3.7 billion partnership between the EU and the Bio-based Industries Consortium

(Universiteit Gent (Belgium) Bio Base Europe Pilot Plant (Belgium) Fraunhofer IGB (Germany) VITO (Belgium) IMD Natural Solutions (Germany) CIMV (France) Evonik Industries (Germany) Croda Europe (United Kingdom) Inbiose (Belgium) Carbosynth (United Kingdom) EOC Belgium (Belgium)

Rhamnolipids' production by
Pseudomonas aeruginosa W10



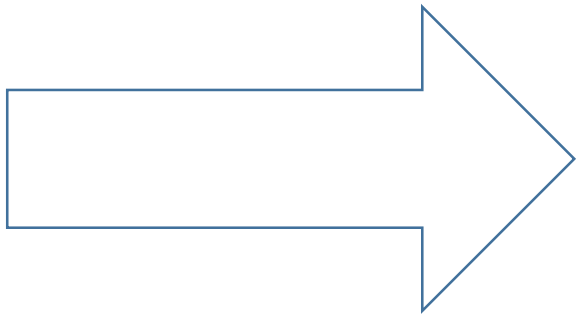
Chebbi et al 2017, Inter. Biodeterioration and Biodegradation
Chebbi et al, 2017, Journal of Basic Microbiology

Nonpathogenic organisms for rhamnolipids: a lab move

Safe but?

***Pseudomonas aeruginosa* PAO1**

di-RL with C10-C10



Pseudomonas putida CB-100
Pseudomonas chlororaphis
Pseudomonas fluorescens Migula 1895
Pseudomonas fluorescens P:V:10
Burkholderia thailandensis E264
Burkholderia pseudomallei NCTC 10274
Burkholderia pseudomallei 1026b
Burkholderia plantarii DSM 9509
Burkholderia. kururiensis KP23T
Burkholderia glumae AU6208

pathogenicity

di-RL with C12-C14, C14-C14

Irorere al 2018 Applied Microbiology and Biotechnology

Strategy for biosurfactant production (Unimib)

BIOSURFACTANT PRODUCTION CAPACITY : VALIDATION

Microbial growth
Surface tension reduction
Emulsification index (E24)

GRAPE WINERY WASTES

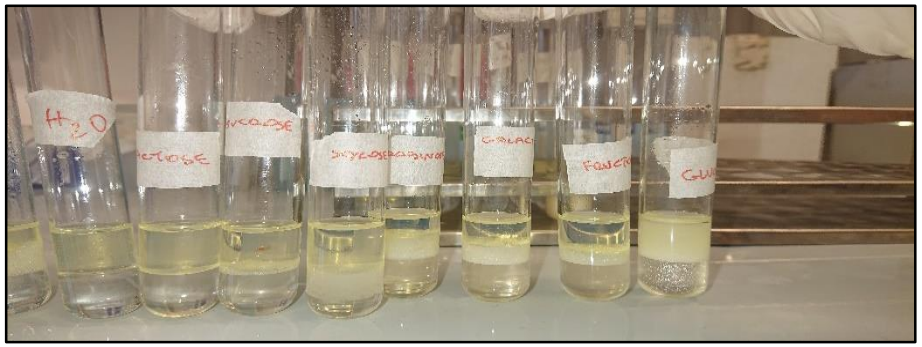
Nonpathogenic organisms for rhamnolipids

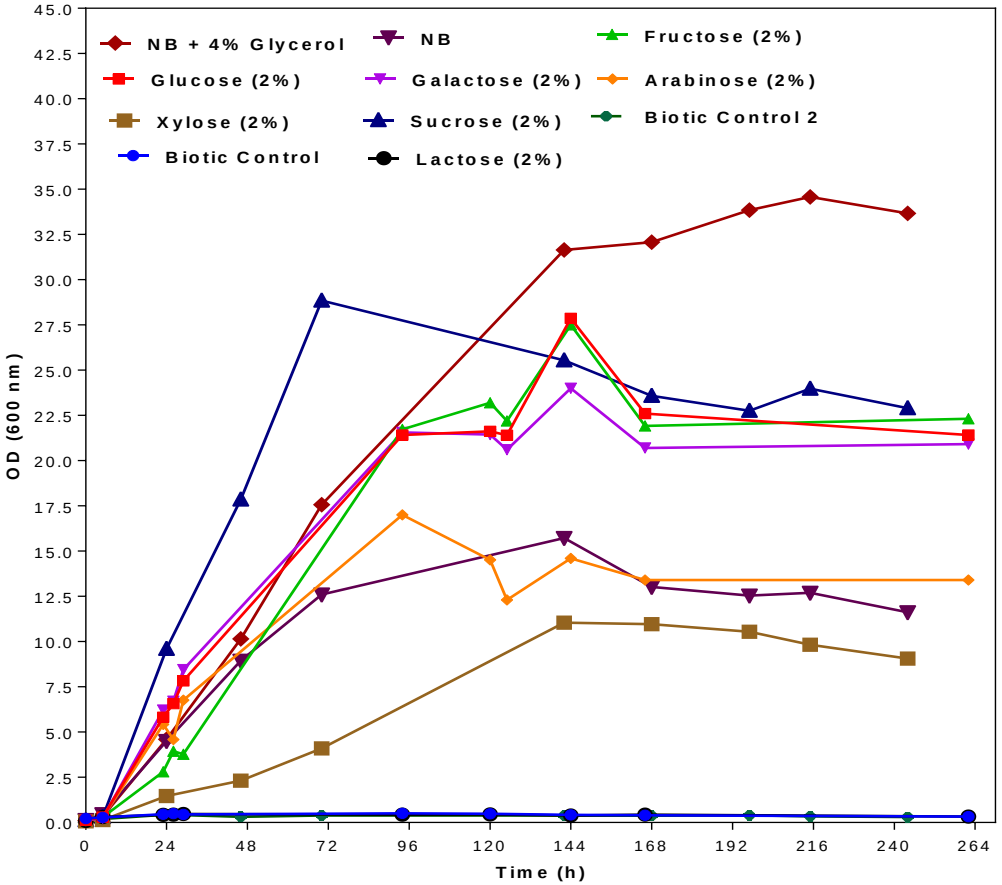
Pseudomonas chlororaphis **DSM 50083^T,**
Burkholderia thailandensis **DSM 13276^T**
Paraburkholderia kururiensis **DSM 13646^T**

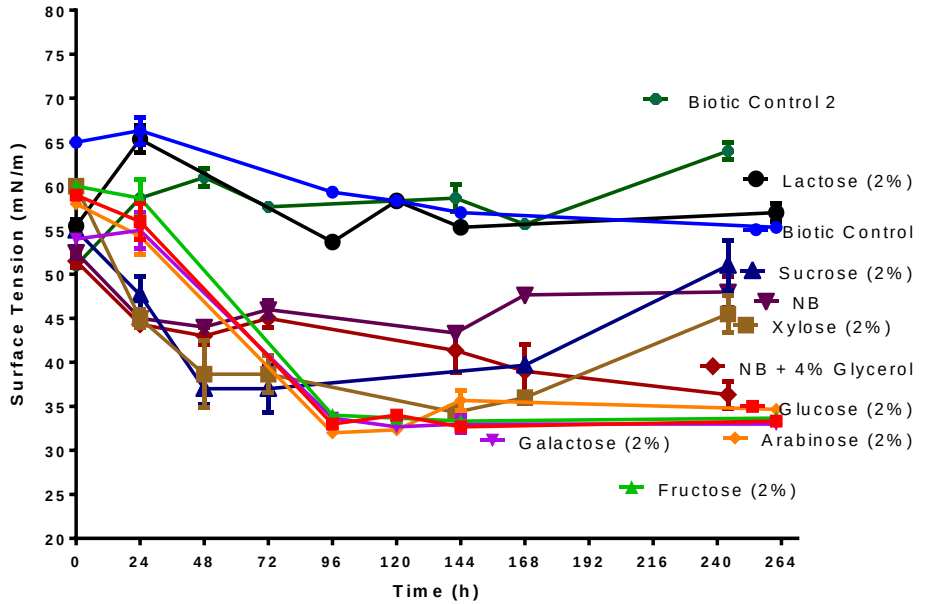
Glucose
Xylose
Fructose
Mannose
Arabinose
Sucrose
Lactose
Nbroth
NB + glycerol

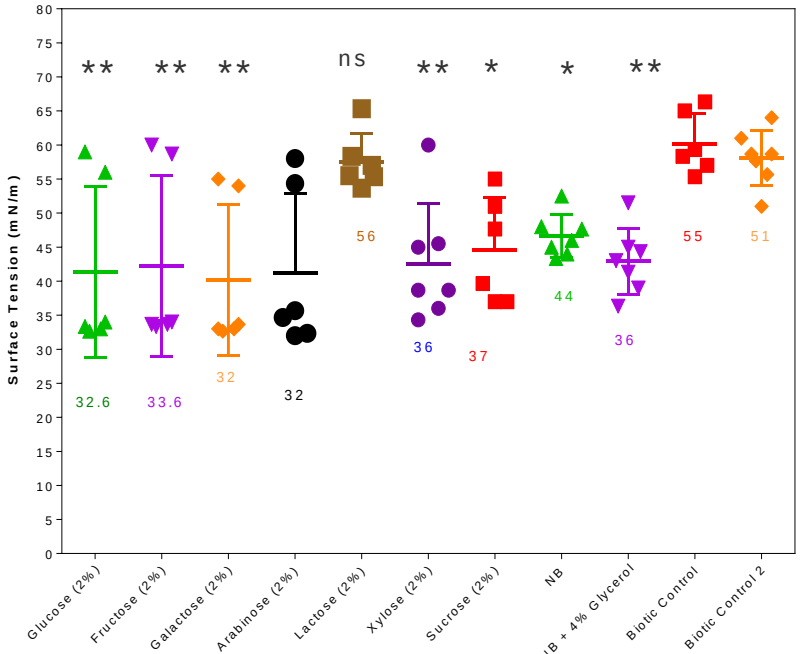
Biosurfactant production capacity

B. thailandensis DSM 13276^T (E264) growth and surface tension reduction after incubation during 262 h at 30 °C and 150 rpm



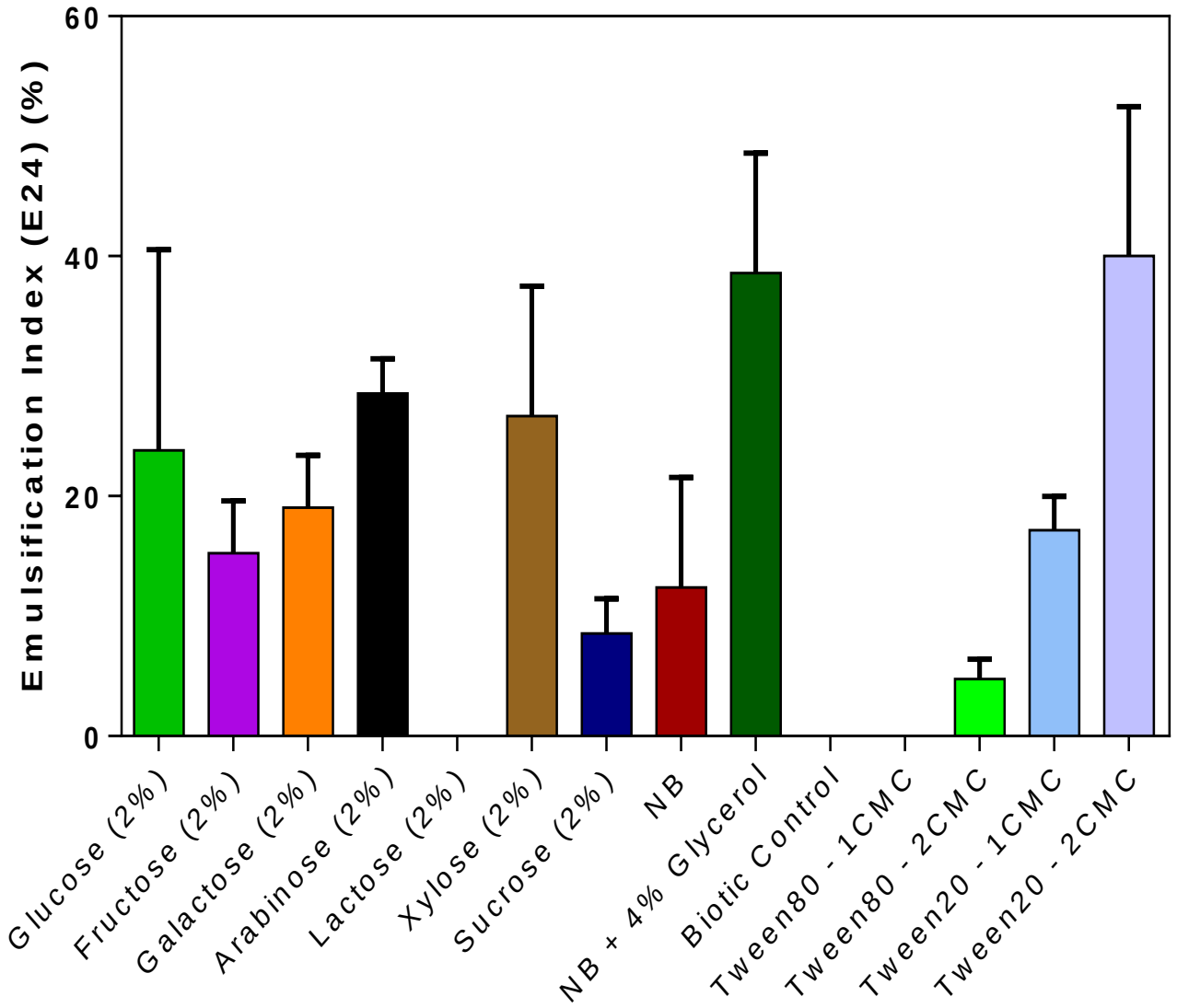






Emulsifying potential (E24)

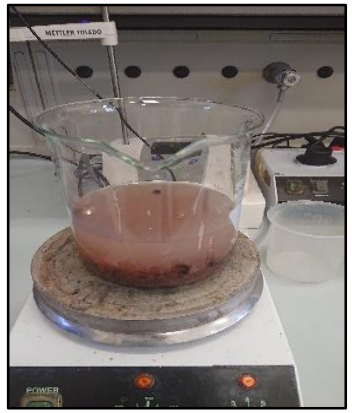
after incubation during 144 h at 30 °C and 150 rpm (MSM medium) *Burkholderia thailandensis DSM 13276^T*



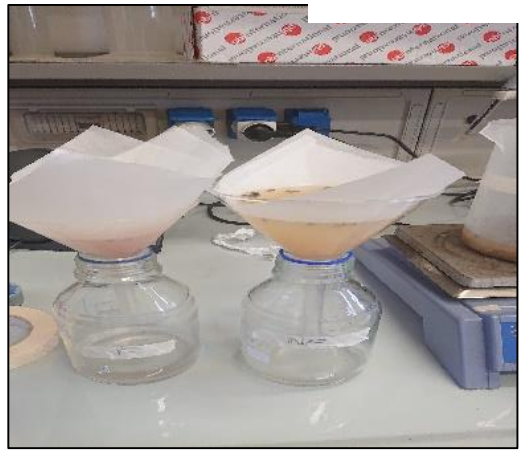
Grape marc pretreatments

1) Raw broth

Oscar Manuel Porteilla et al (2007)



(10g/g) + 30 min stirred
Solid/liquid

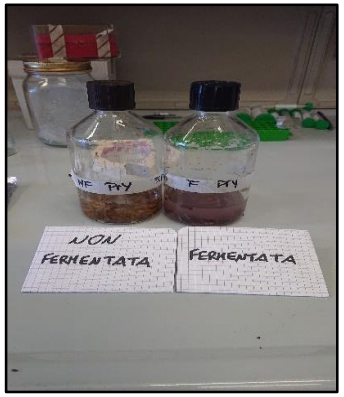


Autoclave during 20 °C
Temp 120 °C

Glucose
Xylose
- Other monomeric sugars

2) Acid Hydrolysis

Oscar Manuel Porteilla et al (2007)



(10g/g) + 30 min stirred
Solid/liquid

Autoclave during 30 min
Temp 120-130 °C

$CaCO_3$
pH neutralized
$CaCO_4$ (filtration)
calcium carbonate

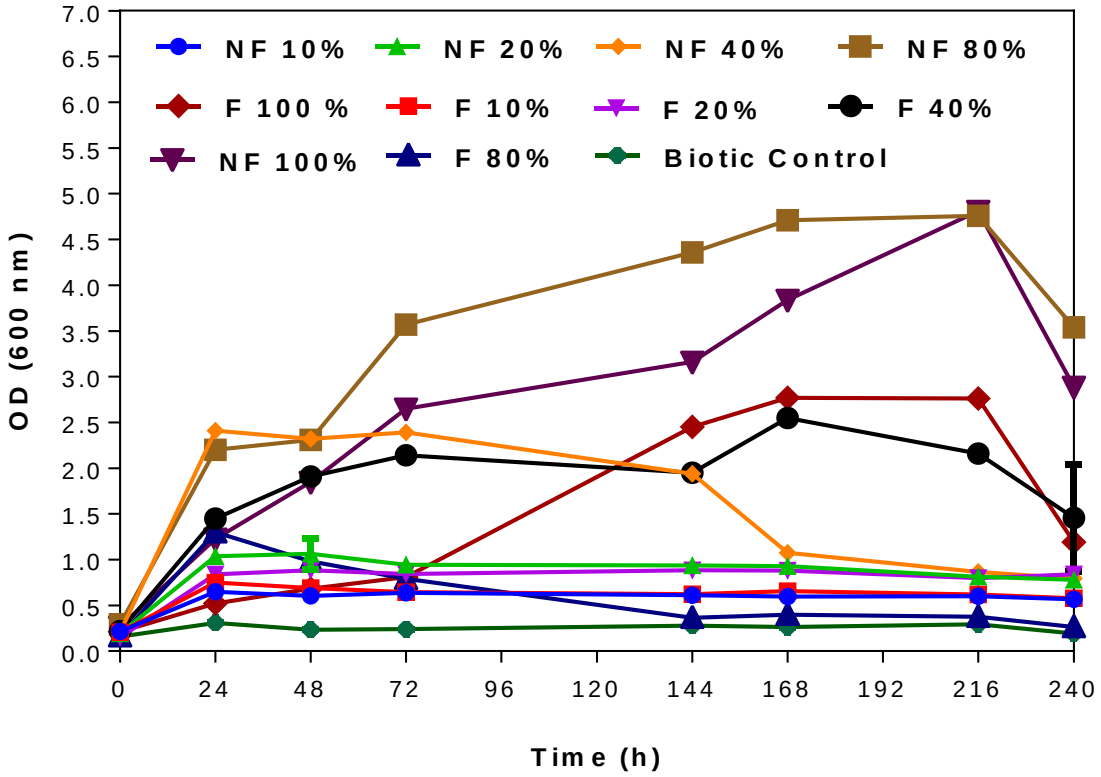
3) Enzymatic hydrolysis

Enzymatic hydrolysis (For the future)

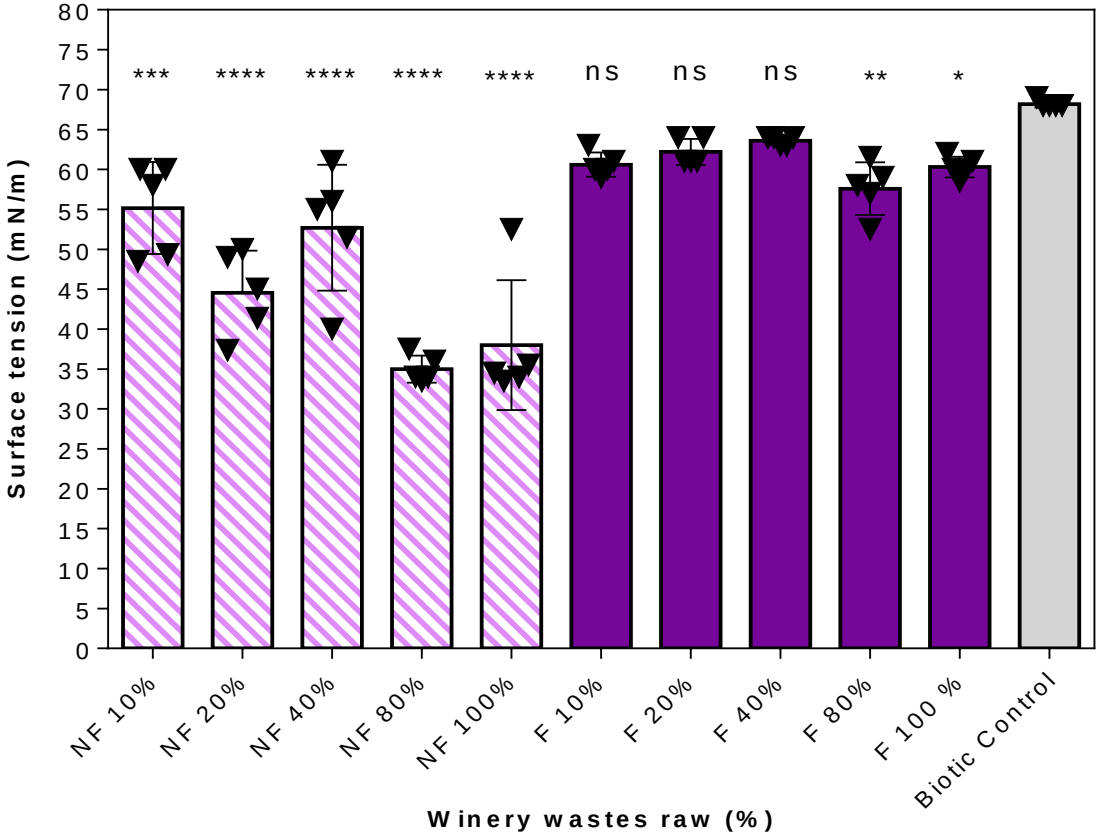
Cellulase
Hemicellulase
Pectinase

Strain E264 Growth and surface tension reduction on grape marcs
Fermented vs. nonfermented (Raw broth)

A



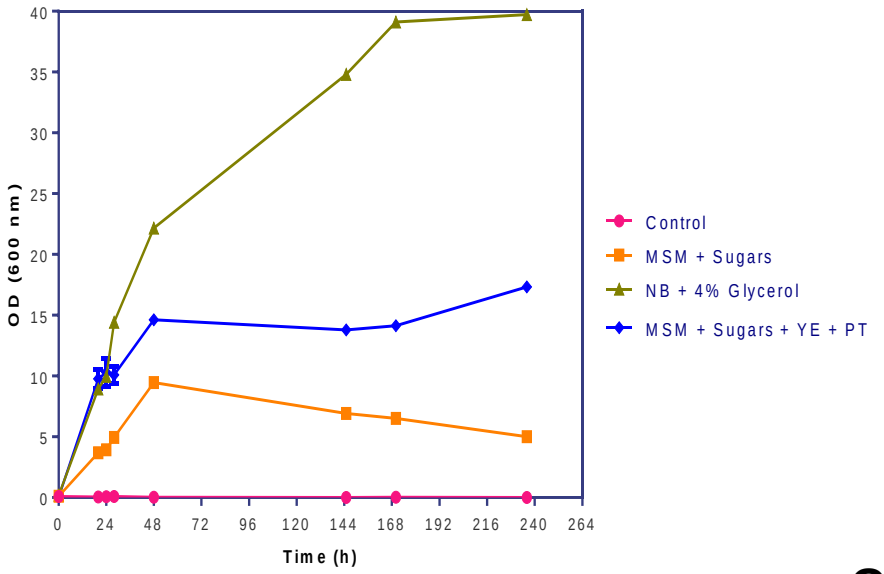
B



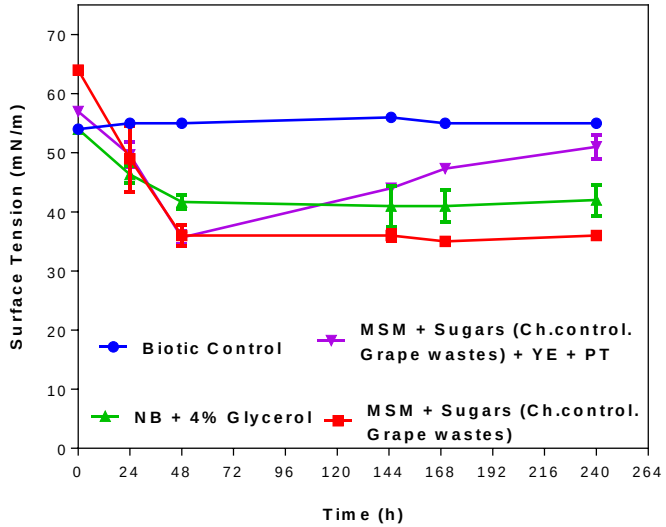
Rhamnolipids production

B. thailandensis E264 after 236 hours of incubation at 30 °C and 150 rpm

A



B



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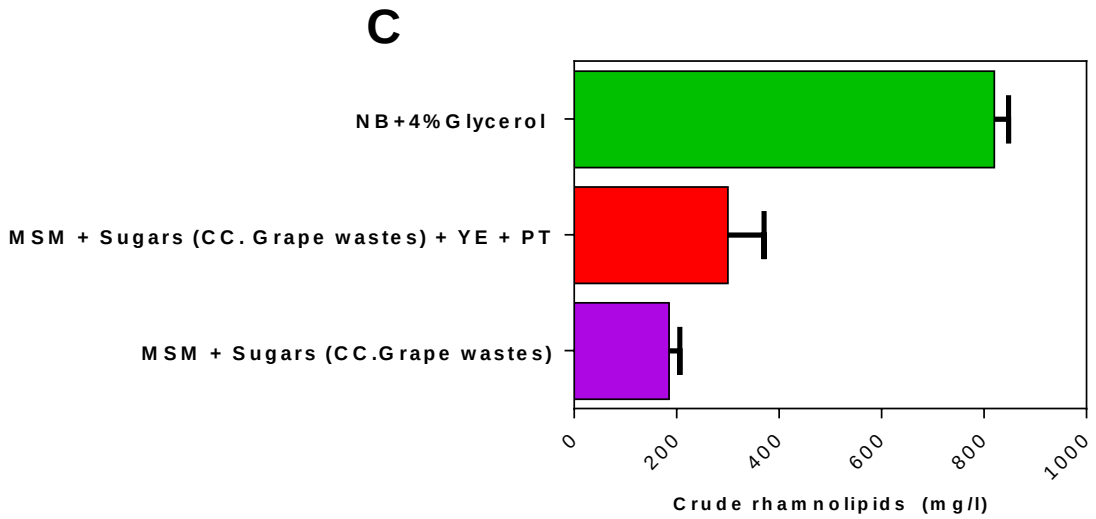
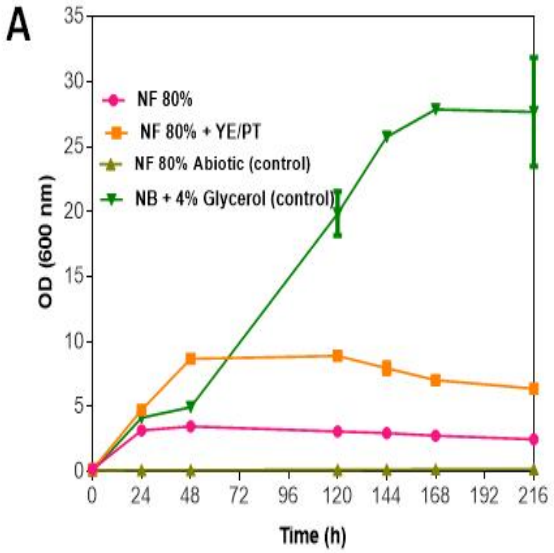
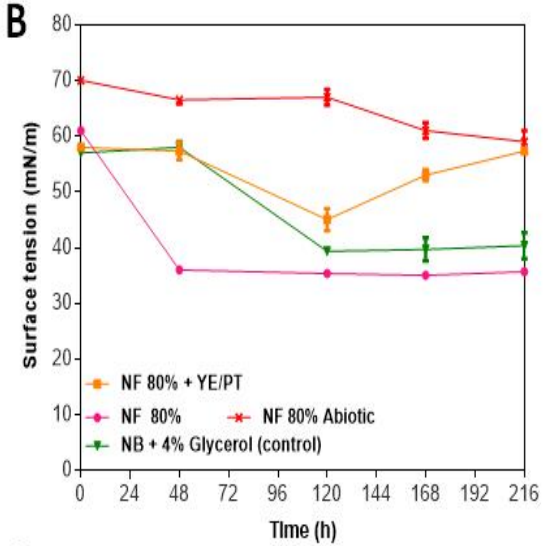
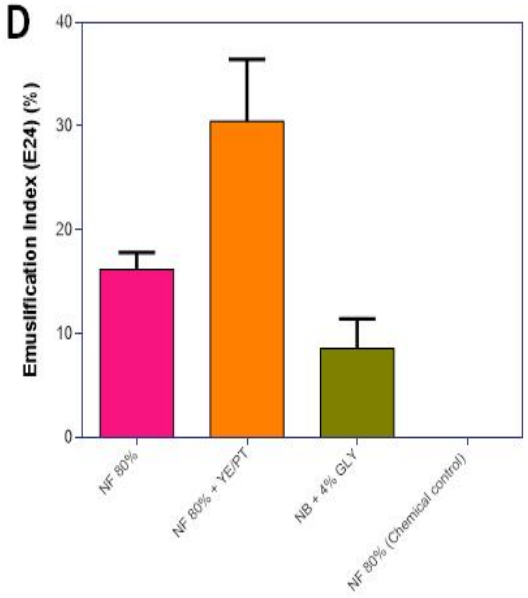


Rhamnolipids' production by B. thailandensis E264 using nonfermented grap marc





Foaming
(Schiumogeno)



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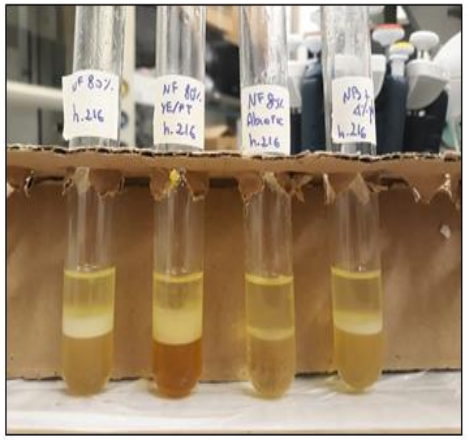


Ocrinol

Chemical CT
Grape wastes

Strain E264 +
grape wastes

Work in progress

- physico-chemical parameters: Winery wastes and olive cake mill:
- Biodiversity/ enrichment /isolation of BS producers
- Ecotoxicity testing on soils: (Validation ?)
- Large scale production and testing the effects on contaminated soils

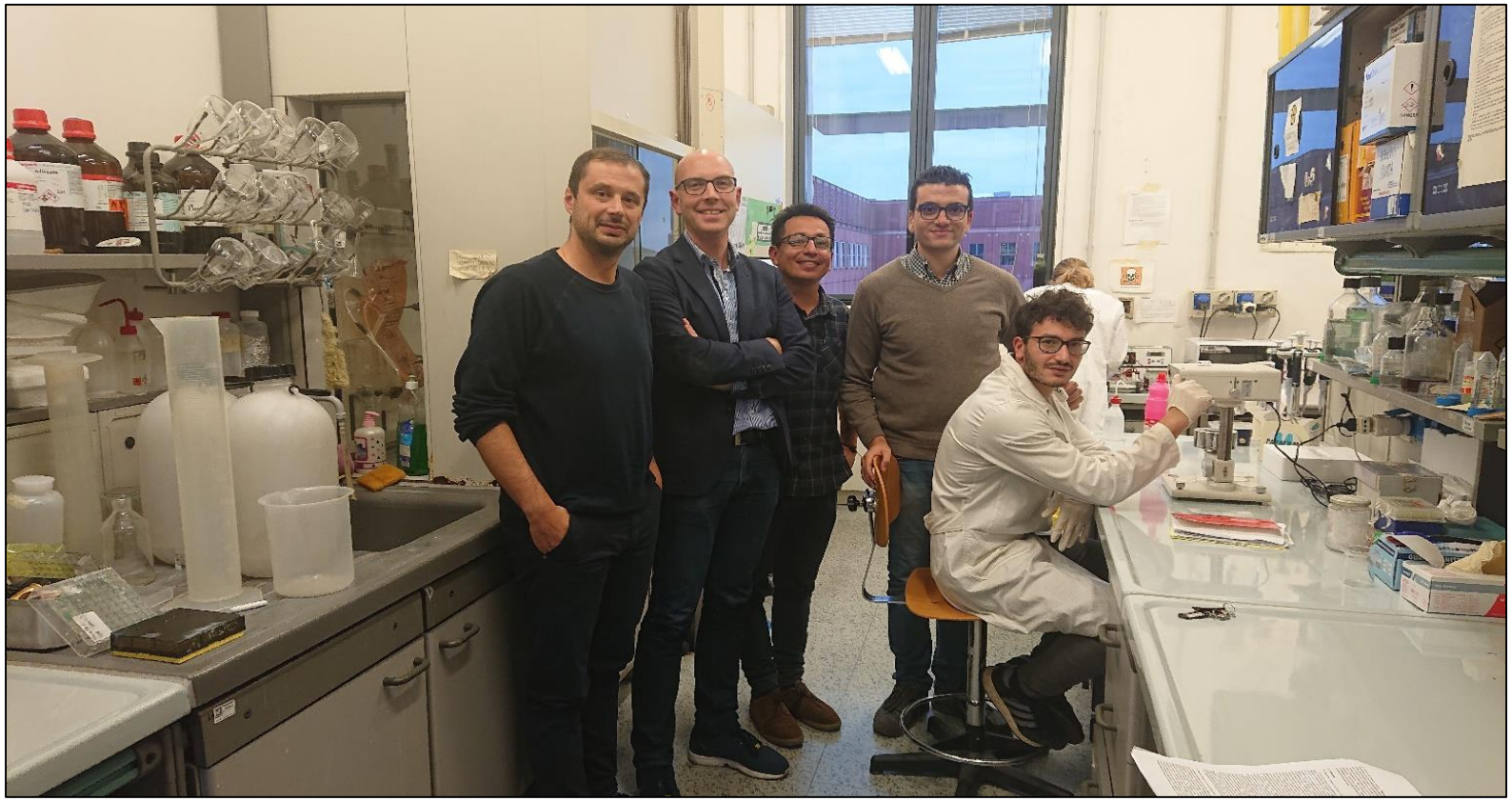
pH
Moisture (%)
Reducing sugars and type of sugars
Ash : dry residue: yes)
Cellulose
Hemicellulose
Taninis
Proteins
C/N ratio
COD
Ethanol
Glycerol
Polyphenols
Tartaric Acid
Malic Acid
Lactic Acid
Acetic Acid

CREIAMO: a close collaboration with farmers in Lombardy region

Sirmione

Valtenesi

CREIAMO



Dr. SILVIA SBAFFONI

Twitter: @**CreiamoE**

Grazie a tutti…… *SiCon 2020*

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E-mail Creaimo: contact@creiamo-circulareconomy.com

UNIVERSITA' DEGLI STUDI DI MILANO BICOCCA

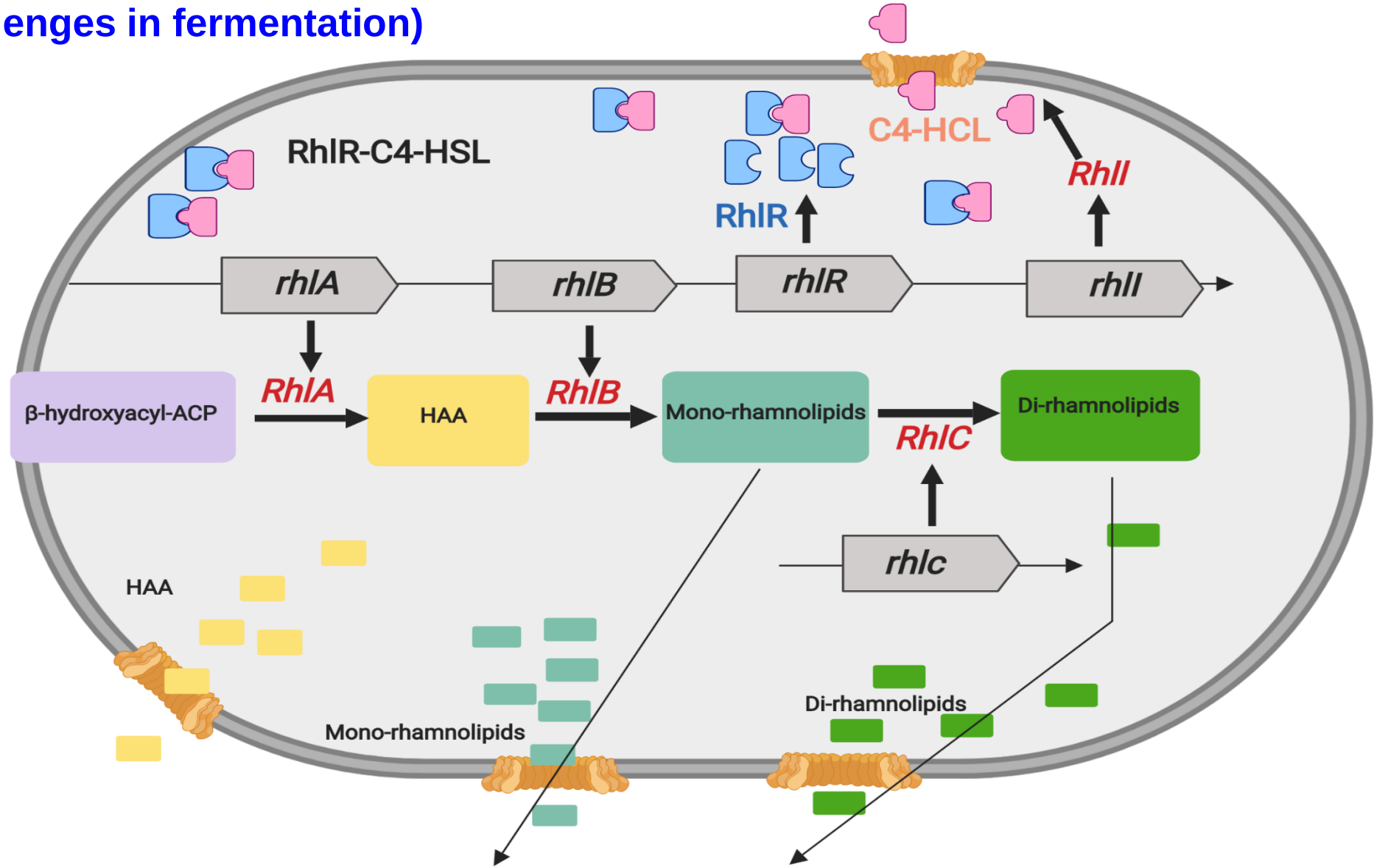
UNIVERSITÀ DEGLI STUDI DI BRESCIA

ENEA

Fondazione CARIPLO

Rhamnolipids' production by *Pseudomonas aeruginosa* PAO1

(challenges in fermentation)



Reg: Quorum sensing

Winery wastes

Solid winery wastes

- **Grape stalks**

- **Grape pomace**

- **Grape seeds**

Liquid winery wastes

- **Wash water**

Muhlack et al., Waste Management (2018)