Convegno conclusivo del Progetto CREIAMO:

Economia Circolare delle filiere olivicola e vitivinicola. Valorizzazione dei sottoprodotti e degli scarti tramite processi innovativi e nuovi modelli di business

Martedì 25 gennaio 2022, Ora 9:00 – 13:00

Sala Consiliare di Ingegneria, DICATAM - Università degli Studi di Brescia
Via Branze n. 38, Brescia

Biotensioattivi ottenuti a partire dagli scarti di produzione di vino e olio di oliva

Alif CHEBBI

25 01 2022

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

Systemic eco-innovation through industrial symbiosis

UNIVERSITÀ DEGLI STUDI DI BRESCIA

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ENEA

Fondazione CARIPLO

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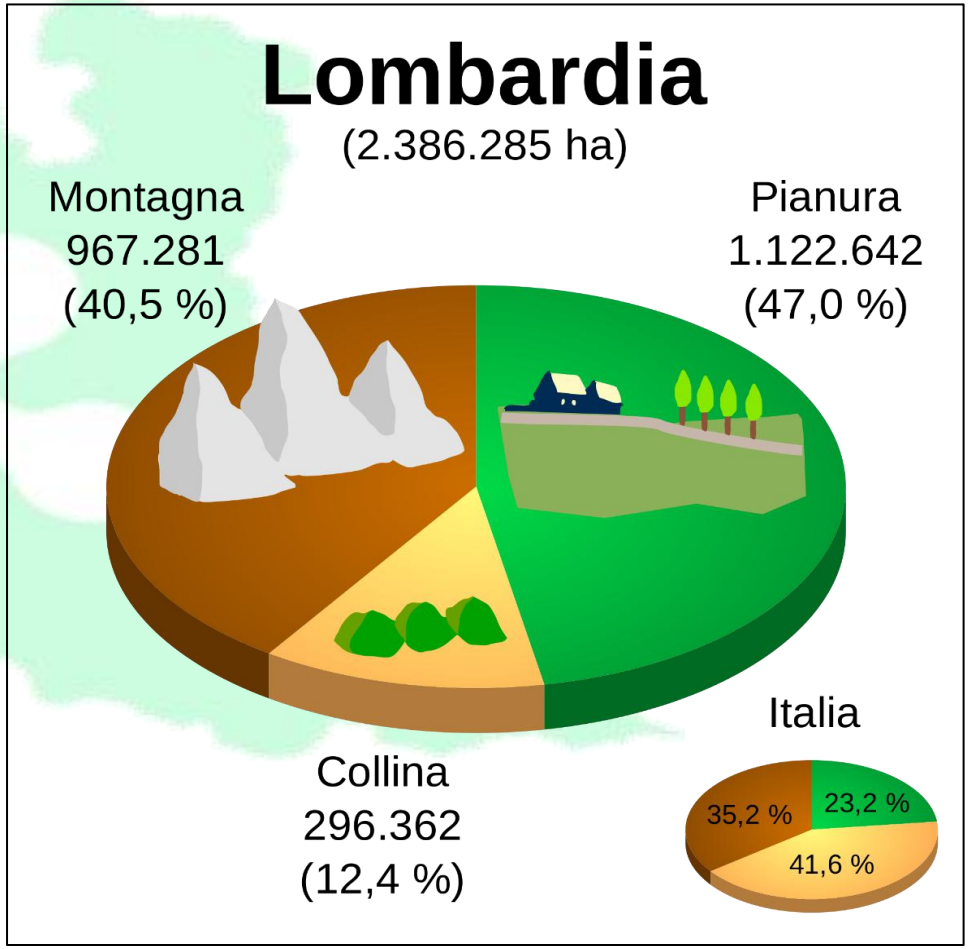
Italia: La Lombardia come modello per l'economia circolare
Olivicoltura e viticoltura



Gli scarti delle industrie vinicole in numeri

Italy

Europe

- In Lombardy , the vineyard area covers a total of 21220 hectares
- >1.5 million hectoliters (2019)_ >45 M hectoliters in Italy
- 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)

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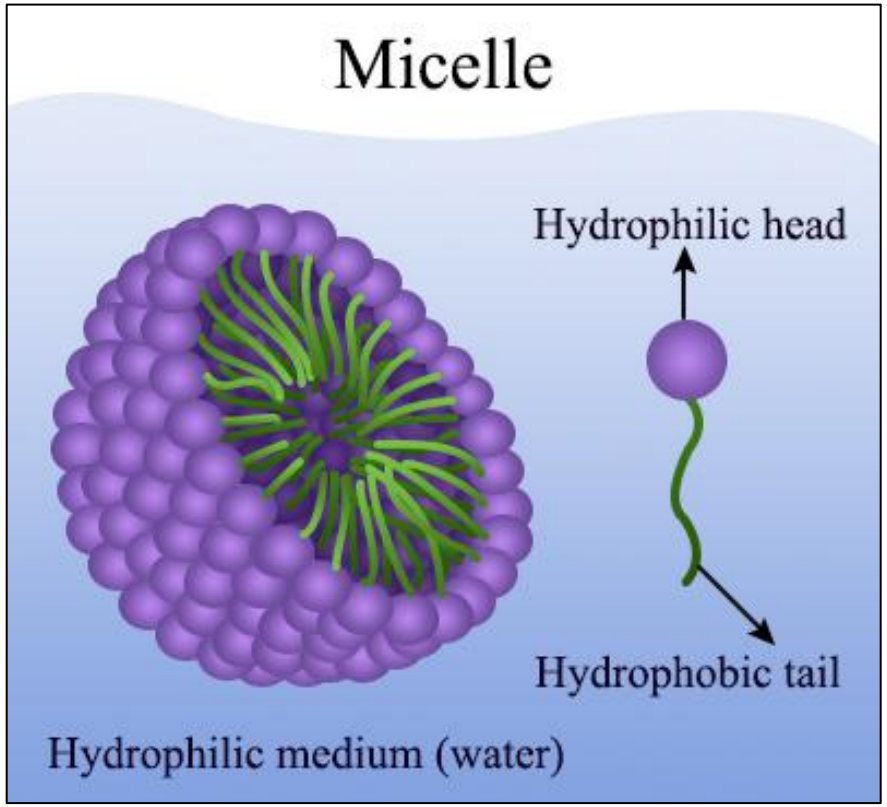
Biosurfattanti e tensioattivi microbici

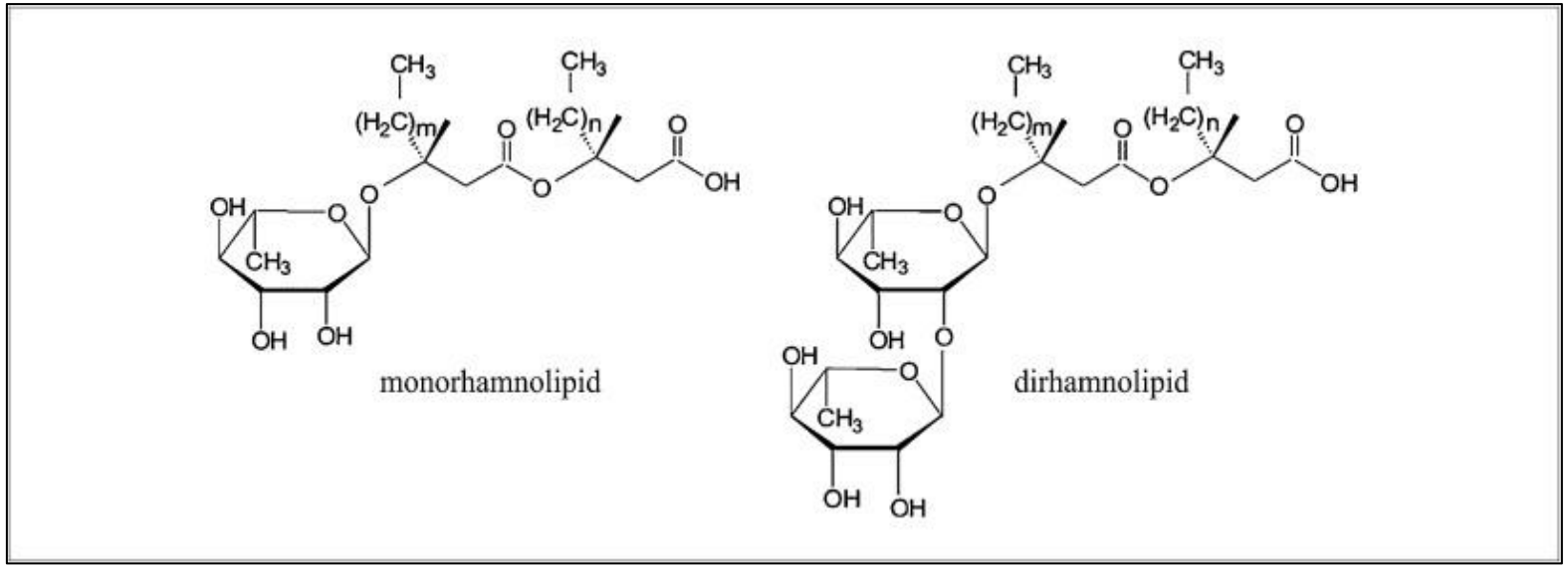
- Glycolipids
- Lipopeptides
- Phospholipids and fatty acids
- Polymeric biosurfactants
- Particulate biosurfactants



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

Composti tensioattivi microbici (SACs)

Principali applicazioni dei ramnolipidi



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.,. (2021)

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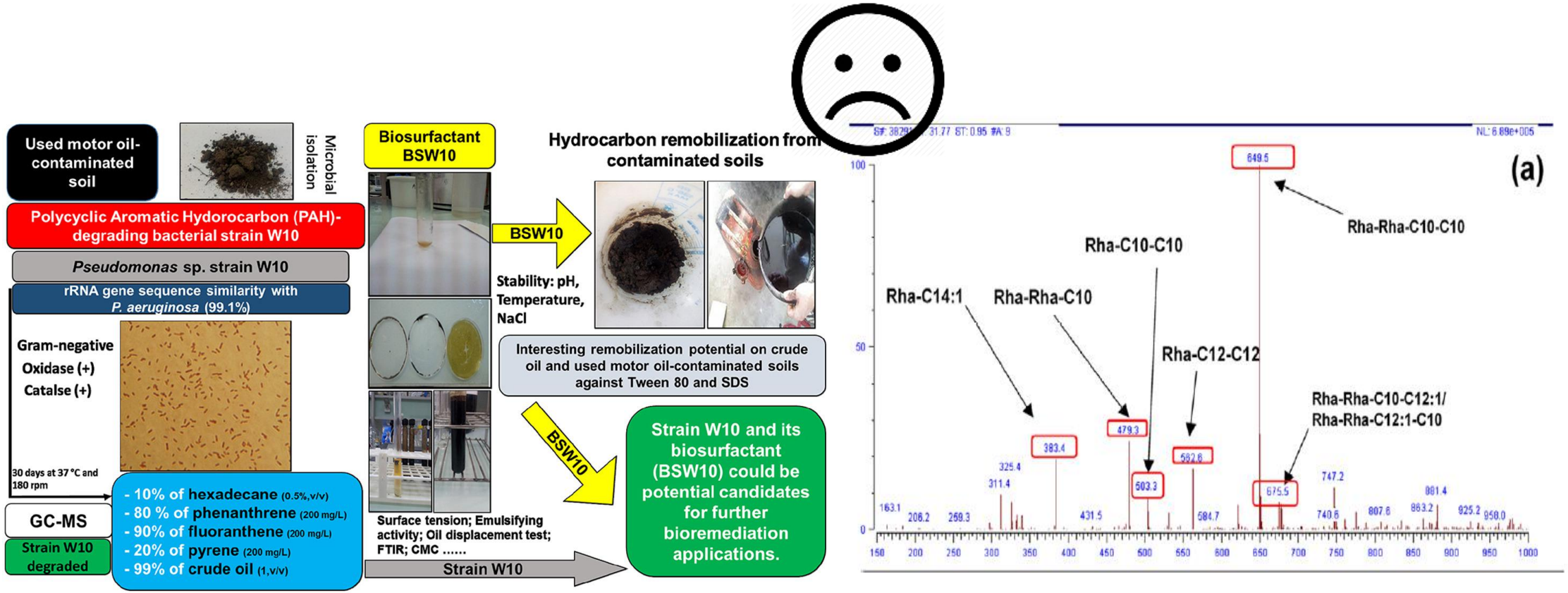
Produzione di ramnolipidi da *Pseudomonas aeruginosa*



Chebbi et al 2017, Inter. Biodeterioration and Biodegradation

Chebbi et al 2017b, JOBM

Chi sono i produttori principali del mercato dei biosurfattanti?

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

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)

Bio-based Industries Consortium

CARBOSURF

Horizon 2020

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**

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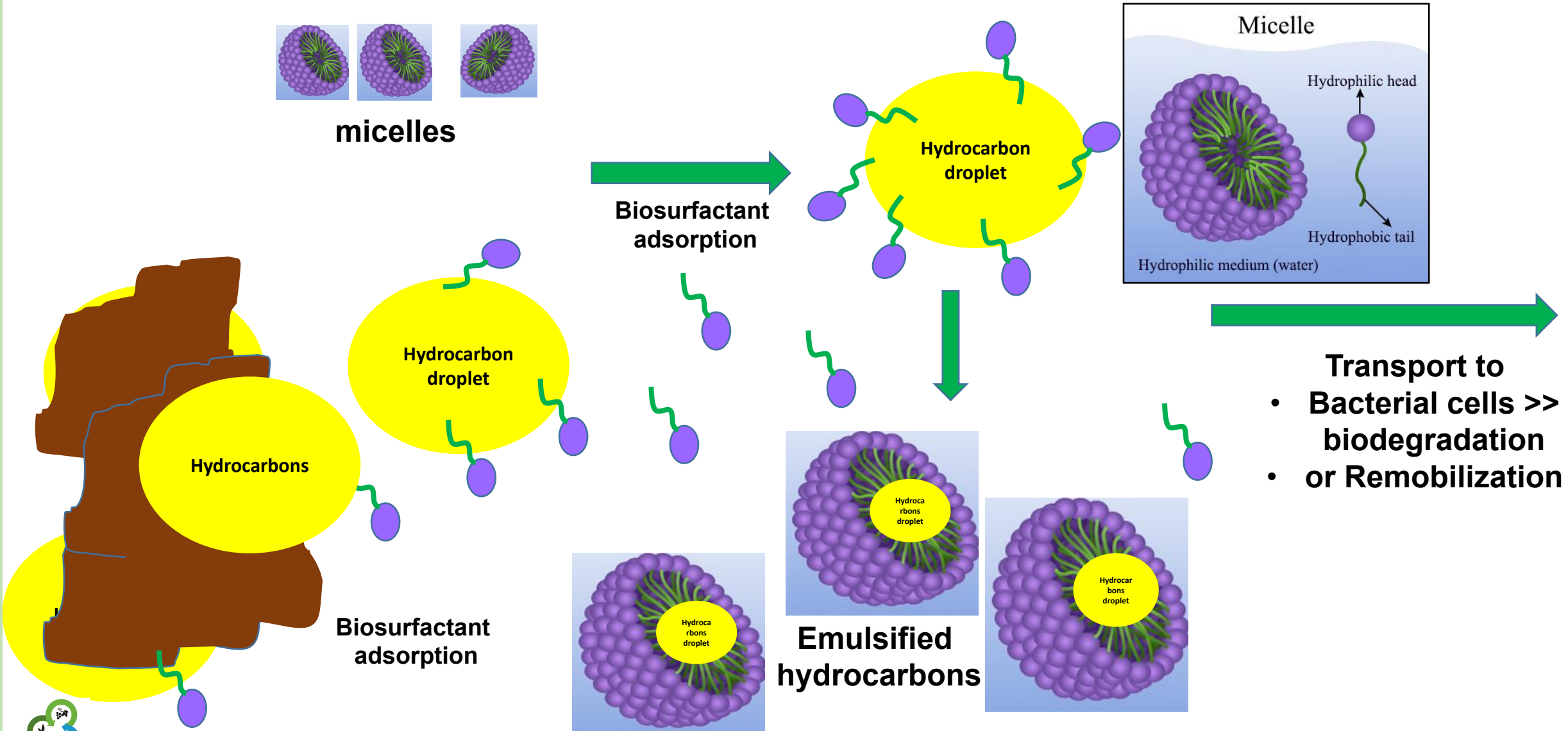
Rhamnolipidi come promettenti tool naturali

Effetti fisici, chimici e biologici verso l'economia circolare
nelle tecnologie di bonifica del suolo e dell'acqua



Una visione globale:

Organismi non patogeni per la produzione di ramnolipidi

Safe but?

***Pseudomonas aeruginosa* PAO1**

di-RL with C10-C10

pathogenicity

A lab move

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* KP23^{T}
Burkholderia glumae AU6208

di-RL with C12-C14, C14-C14

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Irorere al 2018 Applied Microbiology and Biotechnology

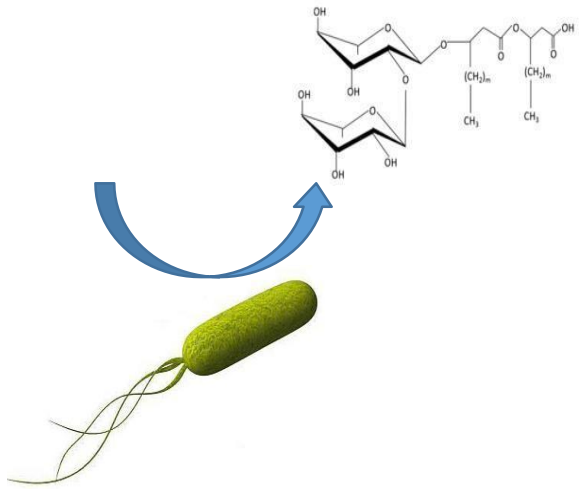
CREIAMO fa luce sui produttori di ramnolipidi non patogeni:

Sviluppare percorsi per l'economia circolare

Pathogenic

***Pseudomonas aeruginosa* PAO1**

Prd. RhaRha-C10C10
(Abdel-Mawgoud et al. 2010)



Non pathogenic

***Pseudomonas chlororaphis* DSM 50083**
(Gunther et al. 2005) Prd. Rha-C10C10

***Burkholderia thailandensis* E264**
(Dubeau et al. 2009; Funston et al. 2016)
Prd. RhaRha-C14C14

***Paraburkholderia kururiensis* KP23^T**
(Tavares et al. 2013)

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Caratterizzazione fisico-chimica: rifiuti di produzione vinicola e di olio d'oliva

	Nonfermented grape marc (NF)	Fermented grape marc (F)	Wine lees (WL)
pH	4.01	3.6	5.9
Protein (g/kg)	39	34	47
at 100-105 °C (%)	62.53%	74.52	66.54
C/N ratio	30.6	25.1	24.5
ASH (%)	4.94	8.18	5.31
Alcoholic grade (ml/100 g)	0.7	7.3	0.2
Total polyphenol content (TPC) (g/kg)	6.7	6.8	6.3
Malic acid (g/kg)	4.4	1.9	n.q (<0.1 g/kg)
Lactic acid (g/kg)	n.q (<0.1)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Tartaric acid (g/kg)	14.0	22.3	0.8
Acetic acid (g/kg)	1.23	0.71	0.79
Glycerin (g/kg)	4.39	5.35	0.35
Arabinose (g/kg)	n.q (<0.1)	0.3	n.q (<0.1 g/kg)
Fructose (g/kg)	61.3	0.2	0.3
Galactose (g/kg)	0.1	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Glucose (g/kg)	65.4	0.4 g/kg	0.3
Mannose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Rhamnose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Ribose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Saccharose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Trehalose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)
Xylose (g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)	n.q (<0.1 g/kg)

	Olive oil pomace (OMP)
pH	4.95
Total nitrogen (%)	0.93 %
At 100-105 °C	56.94%
Total acidity	5.0 g/kg
Ash (%)	2.79%
Calcium (mg/kg)	1025
Phosphorous (mg/kg)	340
Magnesium (	158
Potassium (mg/kg)	4615
Total polyphenolic (g/kg)	1.1
Glycerin (g/kg)	0.82
Fructose (g/kg)	n.q (<0.1 g/kg)
Glucose (g/kg)	0.2
Arabinose (g/kg)	n.q (<0.1 g/kg)
Galactose (g/kg)	0.2
Mannose (g/kg)	n.q (<0.1 g/kg)
Ramnose (g/kg)	n.q (<0.1 g/kg)
Ribose (g/kg)	n.q (<0.1 g/kg)
Saccharose (g/kg)	n.q (<0.1 g/kg)
Trealose (g/kg)	n.q (<0.1 g/kg)
Xylose (g/kg)	n.q (<0.1 g/kg)

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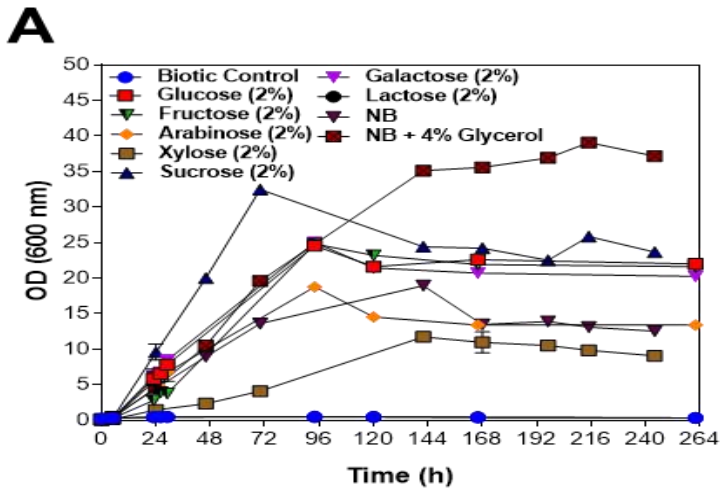
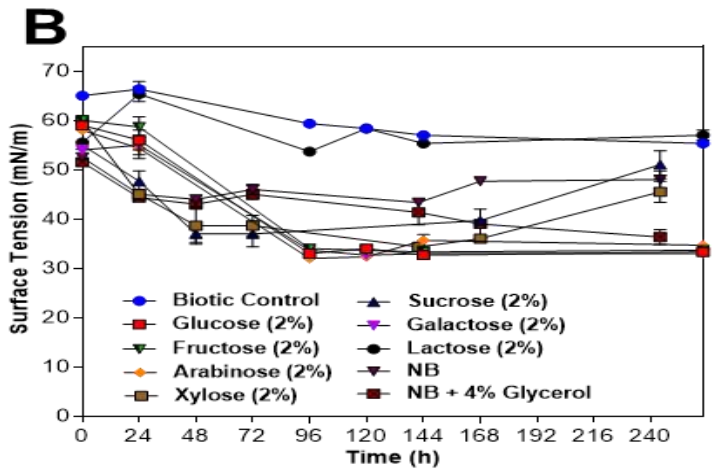
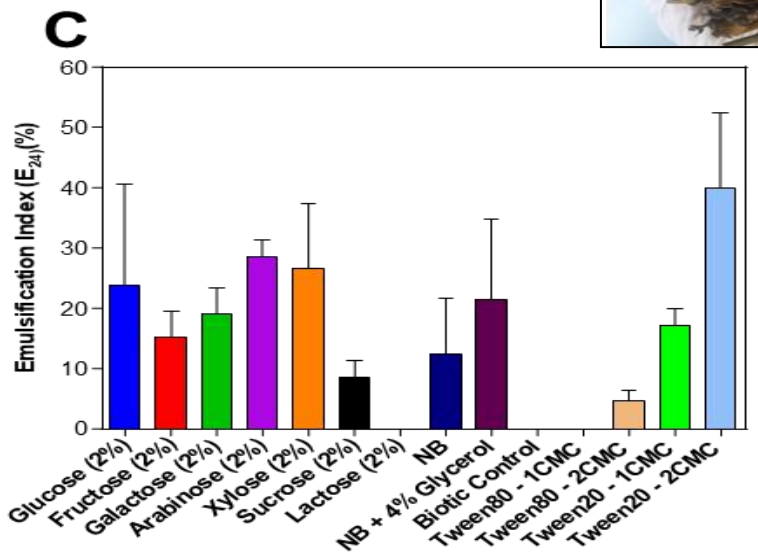
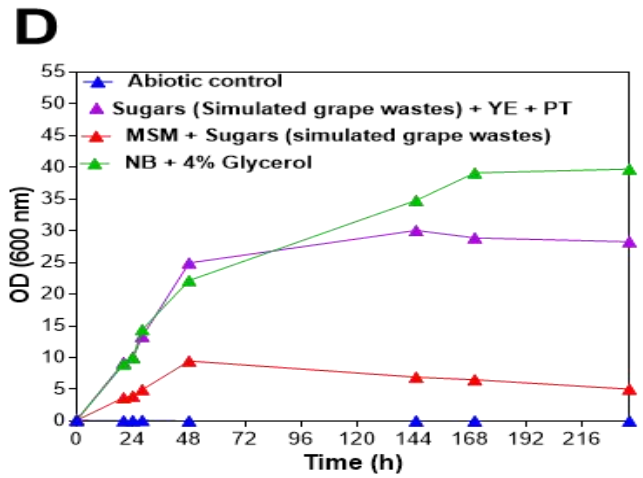
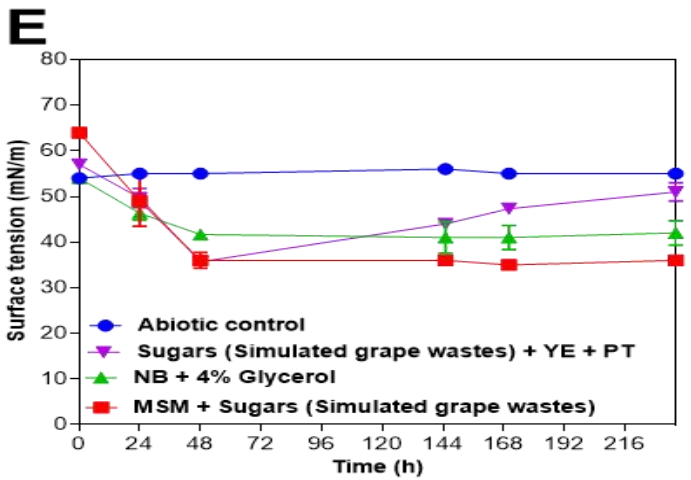
Residui di vinificazione come substrati

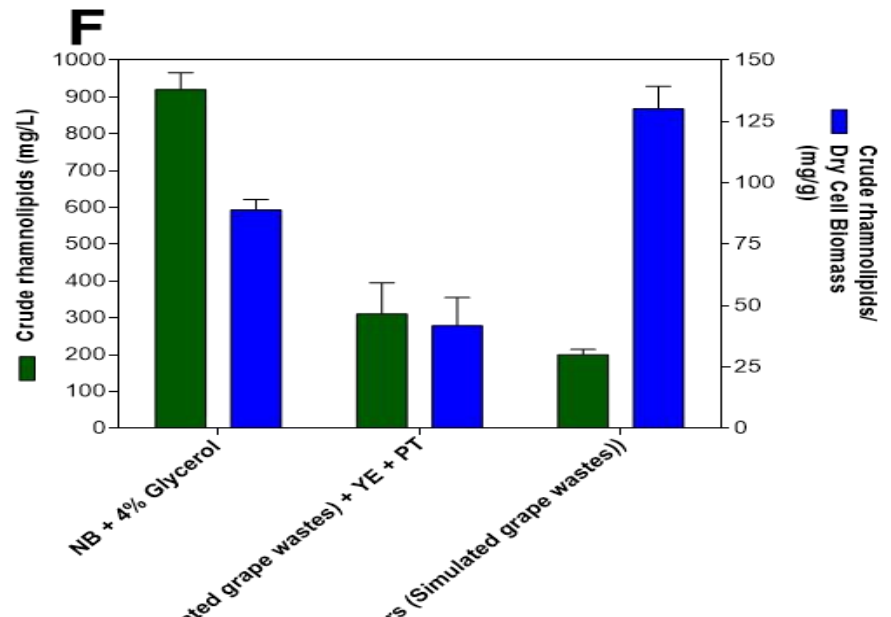












Residui di vinificazione come substrati

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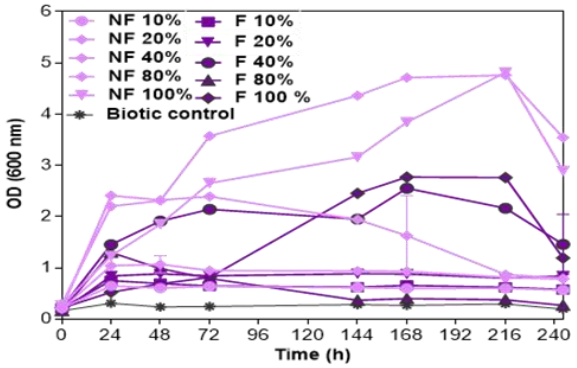

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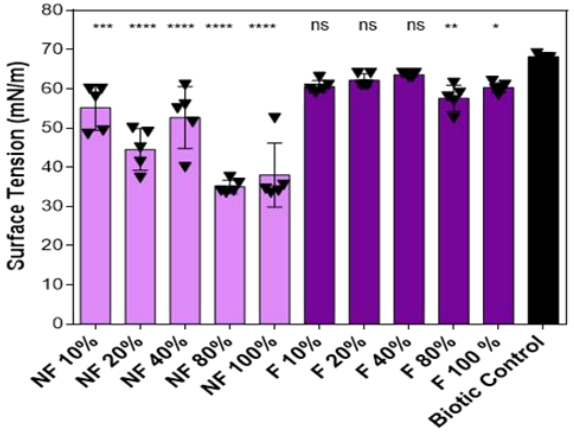

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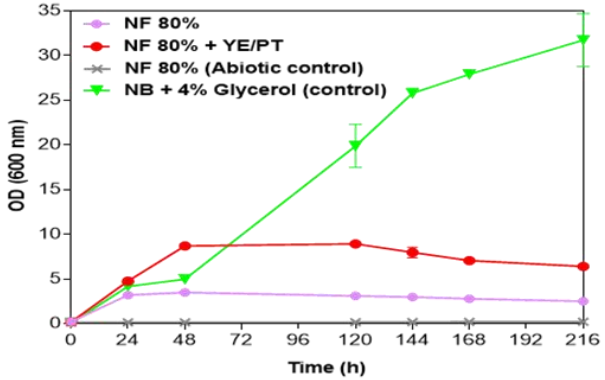

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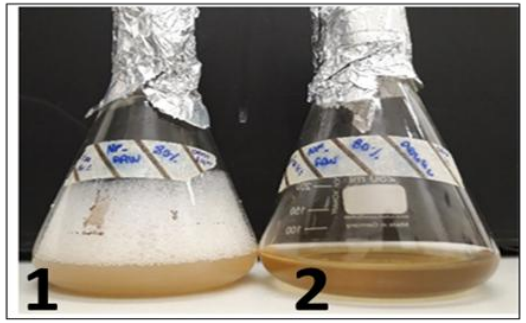

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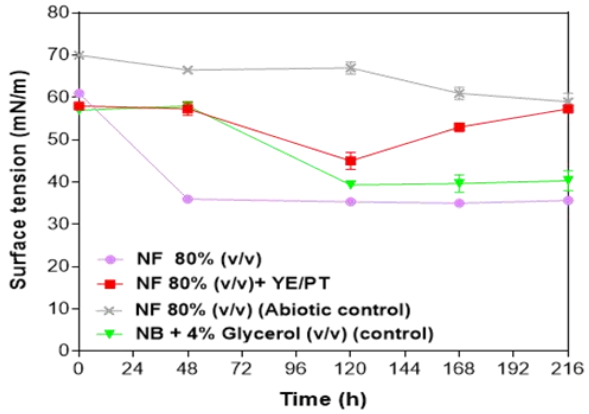

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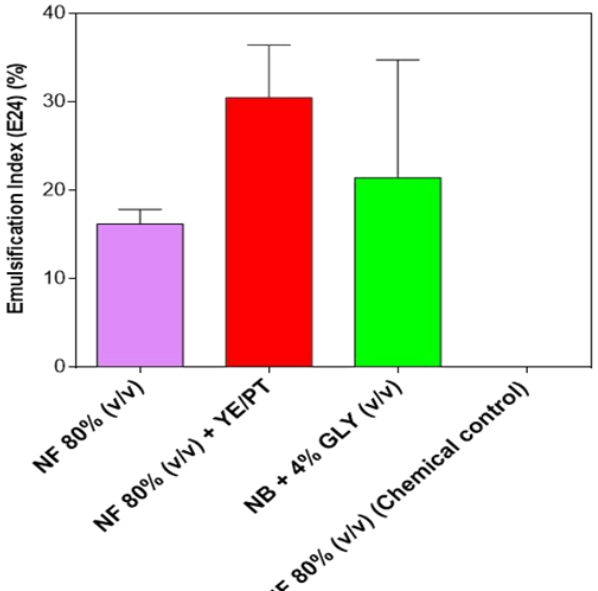

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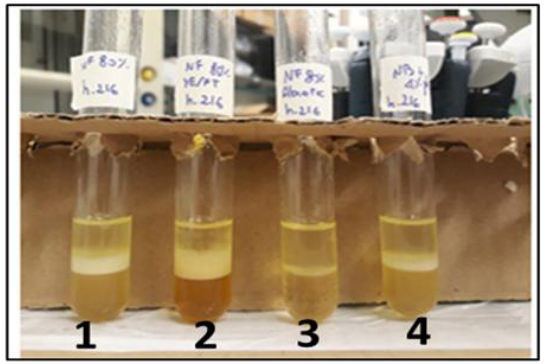

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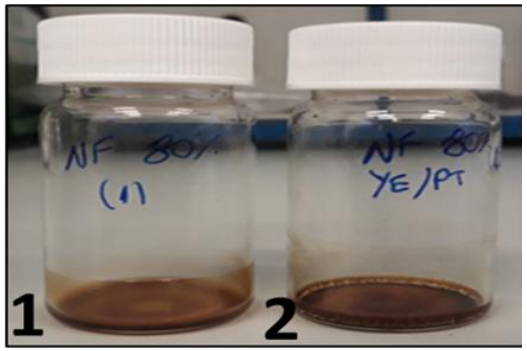

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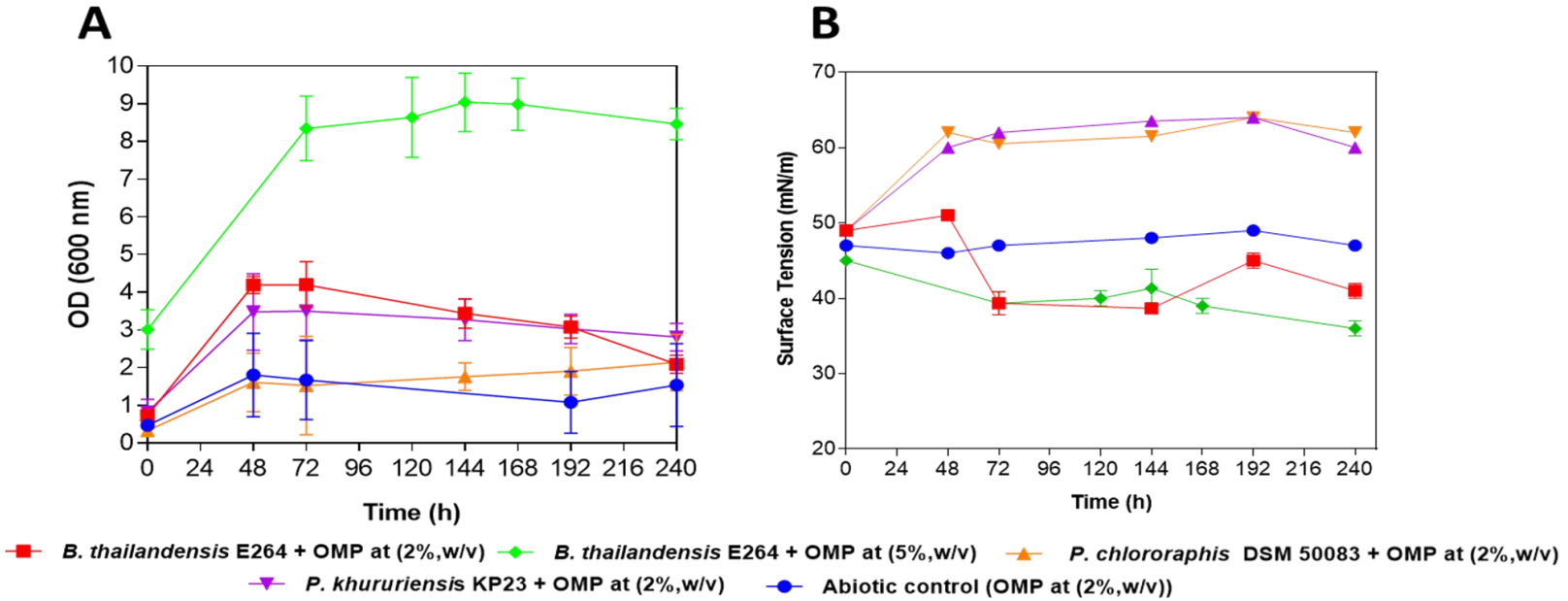
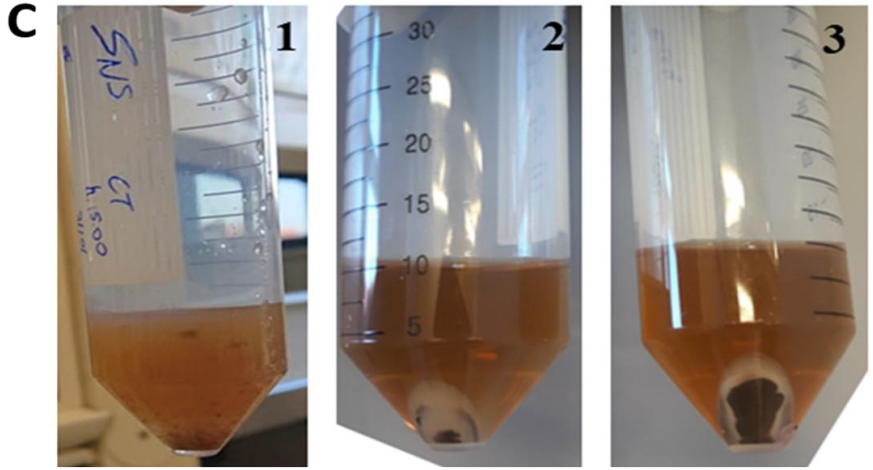
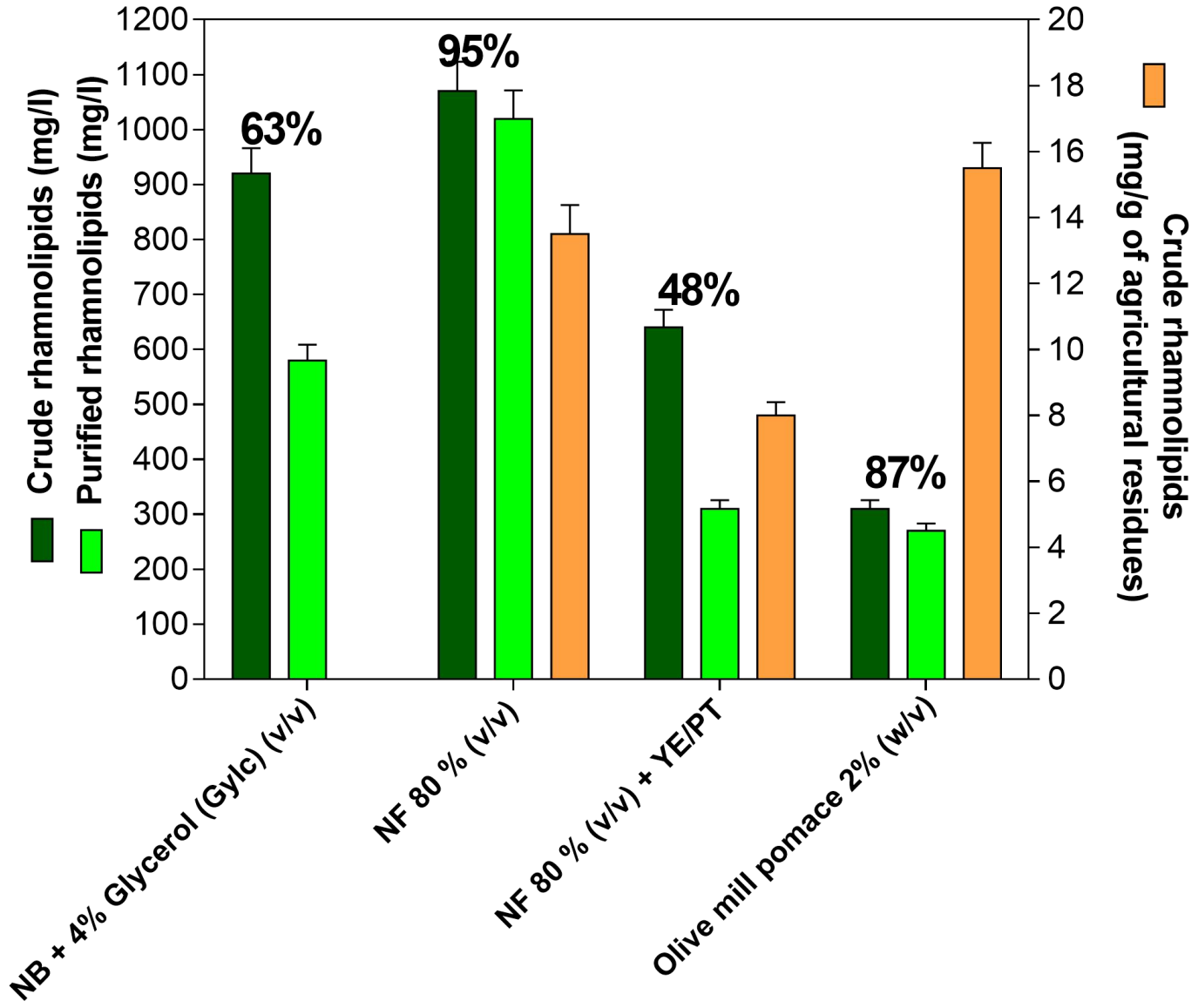
Sansa di olio d'oliva come substrato



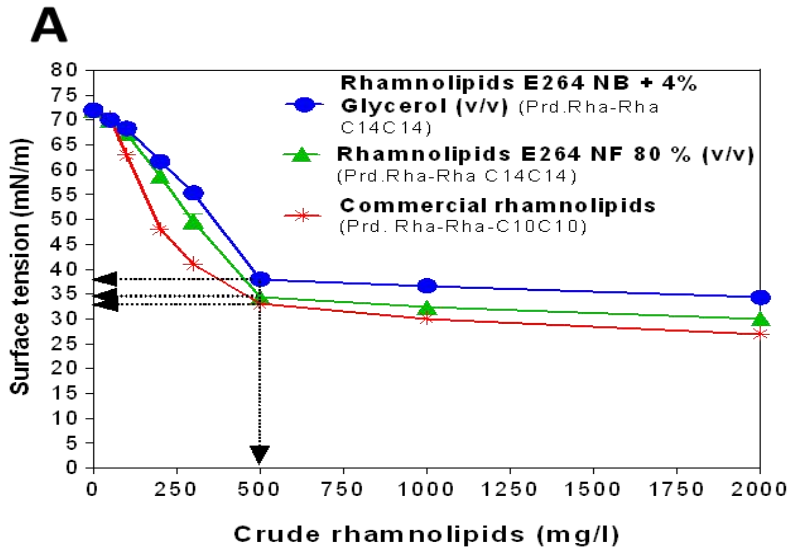


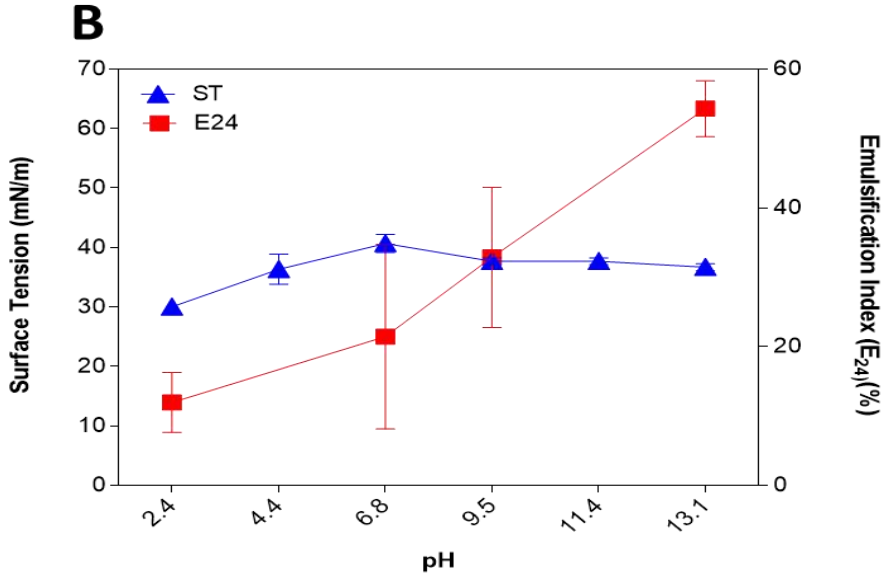
Produzioni di rhamnolipidi a catena lunga da *B. thailandensis* E264: Residui di vinificazione e di olio d'oliva

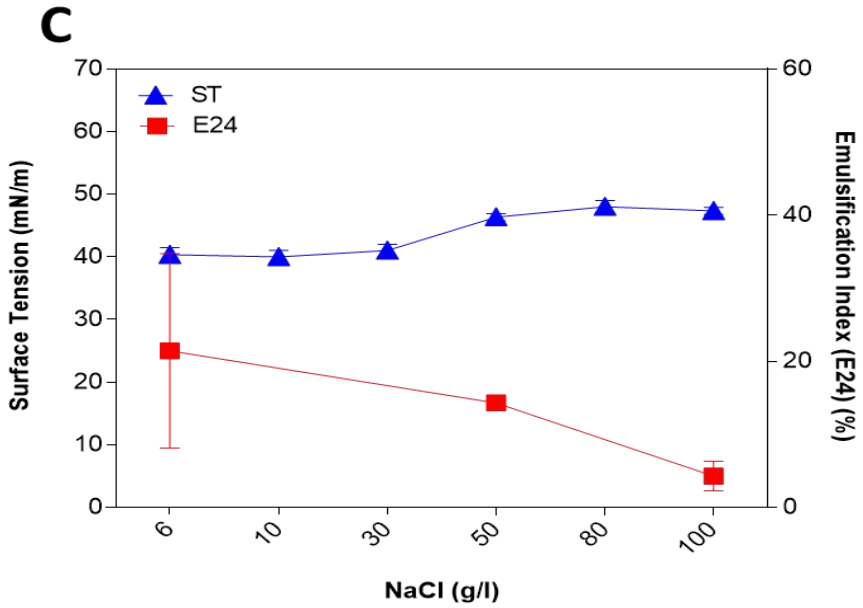


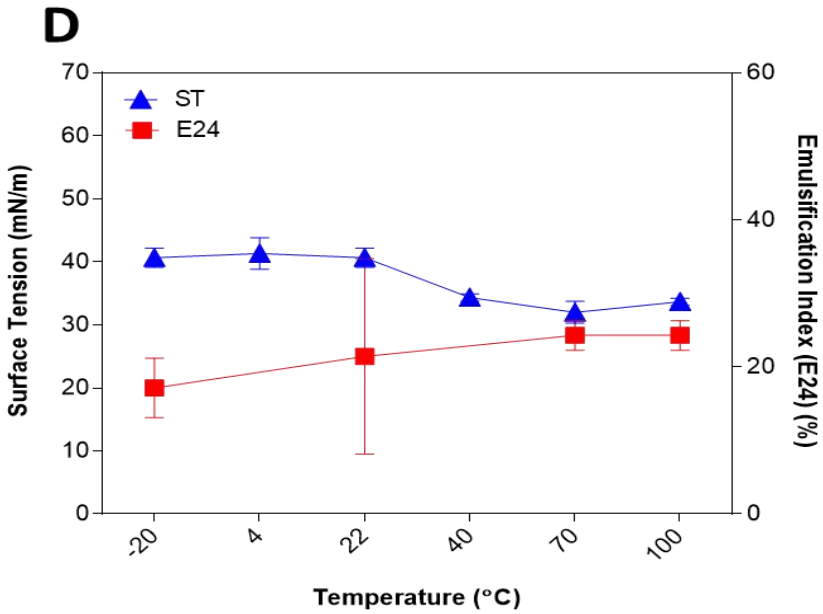
Caratterizzazione del rhamnolipid prodotto da *B. thailandensis* E264

La concentrazione micellare critica (CMC) e gli studi di stabilità (pH, temperatura e salinità)









Analisi UPLC-MS

Agricultural wastes	Rhamnolipid congener	Pseudomolecular (m/z)	Retention time (min)	Relative abundance (%)
Nonfermented grape marcs (NF)	Rha-C12-C14/Rha-C12-C14	587	10.98	**12.56**
	ND	643	10.9	**2.39**
	Rha-Rha-C12-C14/Rha-Rha-C12	733	8.88	**27.02**
	Rha-Rha-C14-C14	761	12.37	**53.16**
	Total			**100%**
Olive mill pomace (OMP)	Rha-C14-C14	615	8.78	**93.44**
	Rha-C16-C14/Rha-C14-C16	789	13.28	**6.55**
	Total			**100%**

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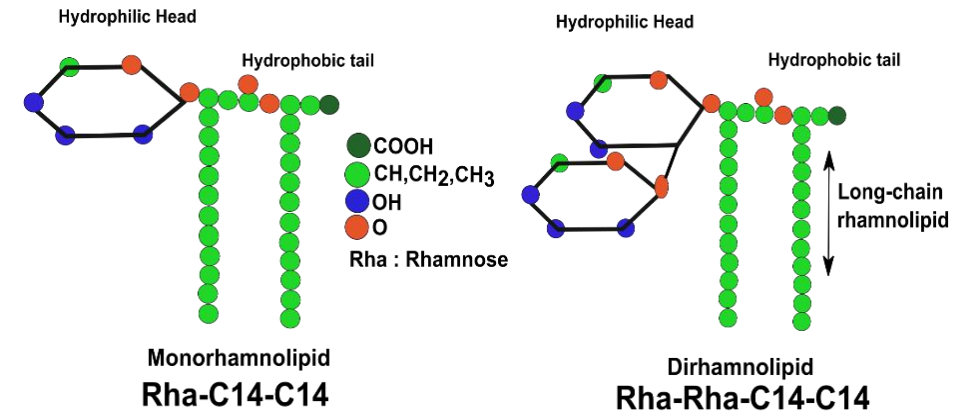

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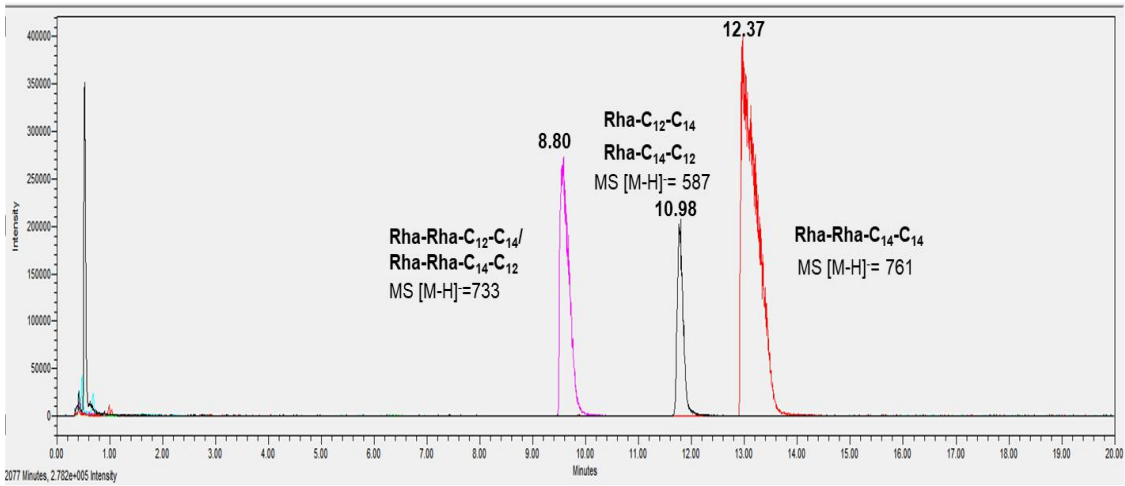

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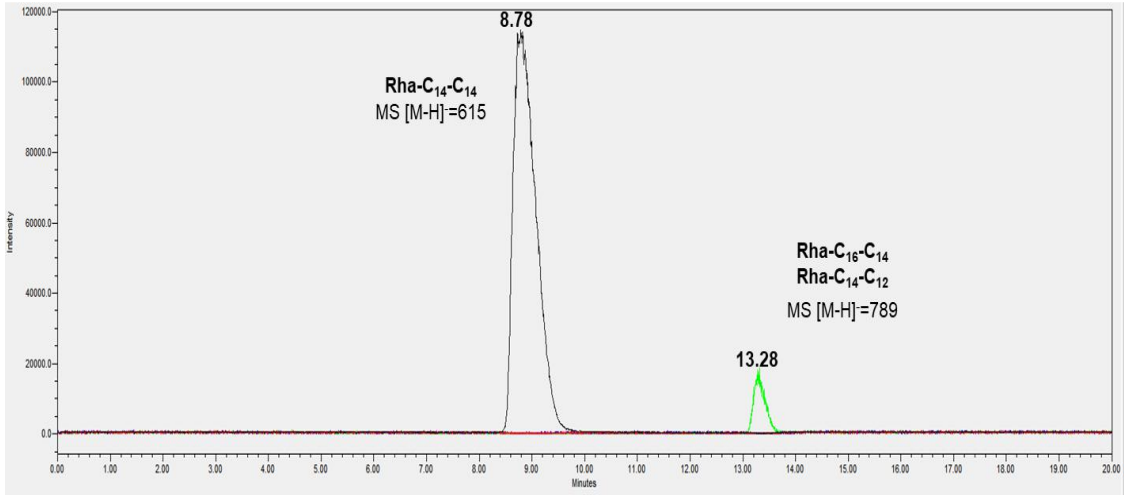

Rispetto alla letteratura precedente ?

Substrate(s)	Pretreatments	Strain	Biosurfactant type	Biosurfactant production (mg/L)	References
Winery wastes					
Trimming wine shoots	Acid hydrolysis + detoxification	*Lactobacillus pentosus* CECT-4023^T	Bioemulsifier	6500	(Moldes et al. 2007)
Trimming wine shoots	Acid hydrolysis + detoxification	*Lactobacillus acidophilus* CECT-4179 and *Debaryomyces hansenii* Y-7426	Not characterized	NA	(Portilla et al. 2008)
Grape marcs	Acid hydrolysis	*Lactobacillus pentosus* CECT-4023^T	Bioemulsifier	4.8	(Portilla-Rivera et al. 2008)
Trimming wine shoots + wine lees	Acid hydrolysis + enzymatic hydrolysis (shoots) Recovery of tartaric acid (lees)	*Lactococcus lactis* ssp. lactis CECT-4434	Not characterized	1.5	(Rodríguez et al. 2010)
Wine lees	NO	*Lactococcus lactis* ssp. lactis CECT-4434	Glycolipopeptide	6.64	(Vera et al. 2018)
Grape marcs (nonfermented) (white)	**Raw broth 80% (v/v)**	***Burkholderia thailandensis* E264**	**Safe long-chain rhamnolipids**	**1070**	**Our study**
Olive oil wastes					
Olive oil Mill effluent (OOME)	Dilution + $NaNO_3$	*Pseudomonas* sp.JAMM	Rhamnolipid	1400 >	(Mercadé et al. 1993)
Olive mill waste (OMW)	NO	*B. subtili* N1 and *P. aeruginosa* PAO1	Surfactin and Rhamnolipid	3.12 (Surfactin) 191.46 (Rhamnolipid)	(Moya-Ramírez et al. 2015)
Olive mill waste (OMW)	i) acid hydrolysis ii) enzymatic hydrolysis iii) acid hydrolysis + enzymatic hydrolysis	*B. subtilis* N1 and *P. aeruginosa* PAO1	Surfactin and Rhamnolipid	26.5 (Surfactin) 299 (Rhamnolipid)	(Moya Ramírez et al. 2016)
Olive mill waste (OMW)	NO	*P. aeruginosa* #112	Rhamnolipid	5100	(Gudiña et al. 2016)
Olive mill pomace(OMP)	Autoclaved in the culture medium 2% (w/v)	*Burkholderia thailandensis* E264	Safe long-chain rhamnolipids	300	**Our study**

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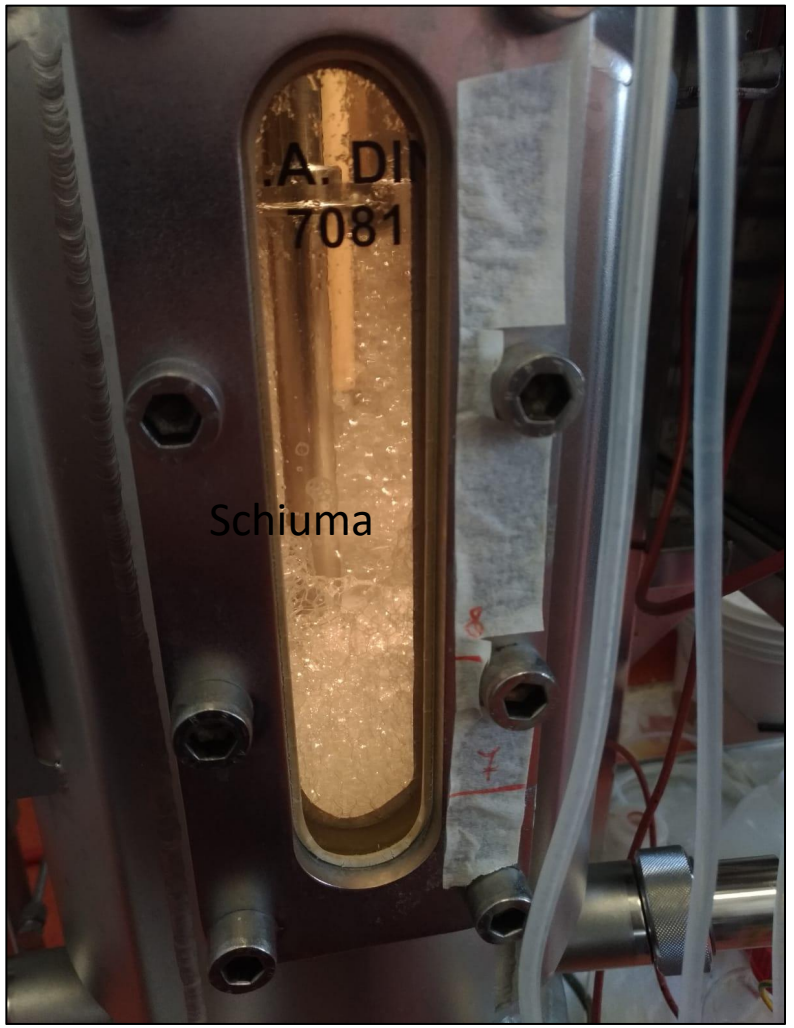
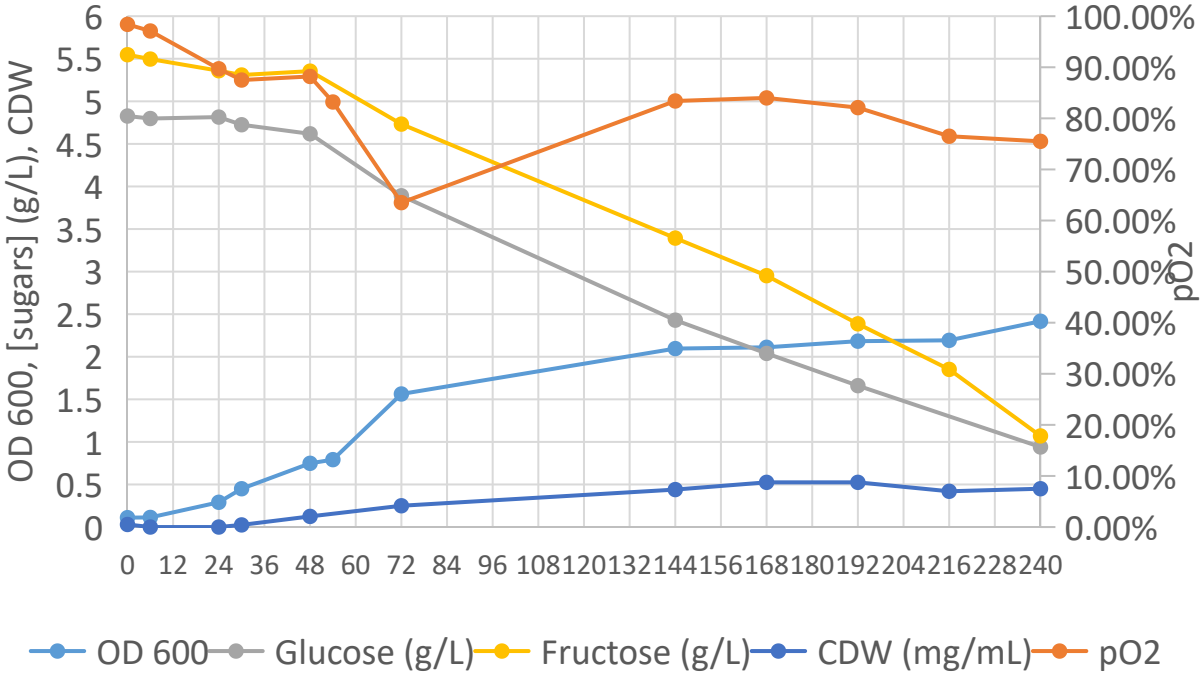
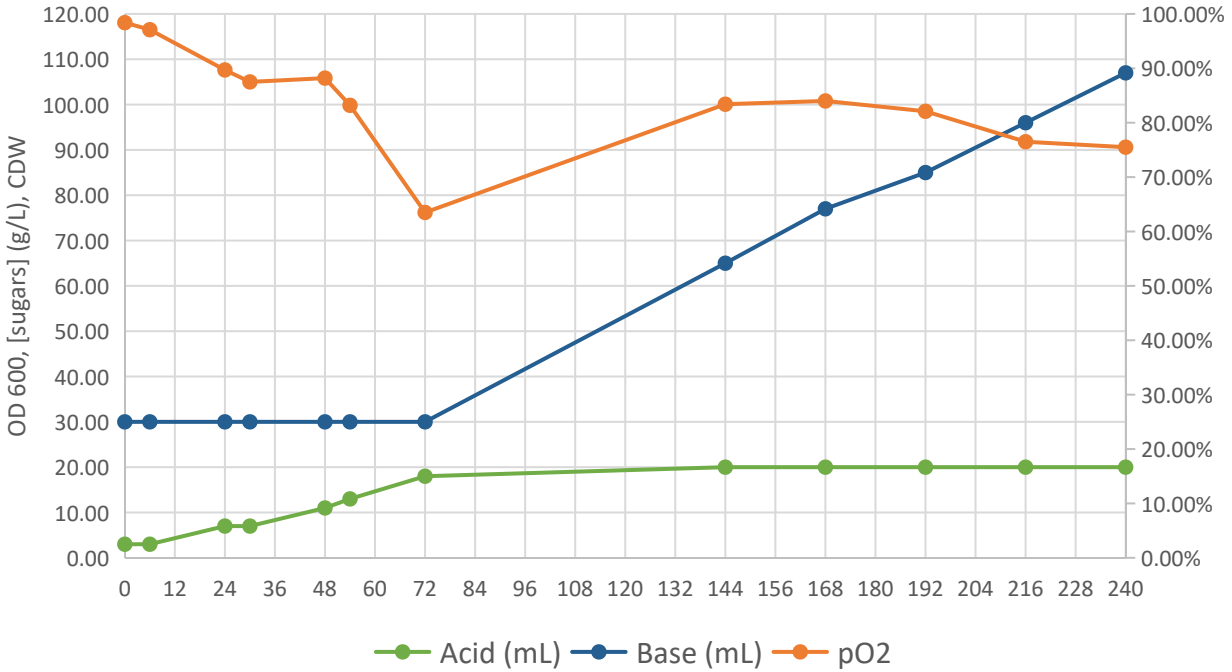
Fermentazione su larga scala utilizzando i residui della vinificazione



Fermentazione su larga scala utilizzando i residui della vinificazione

Come unica fonte di carbonio e di energia (fermentatore da 10L: volume di lavoro 4l)





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Grazie

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