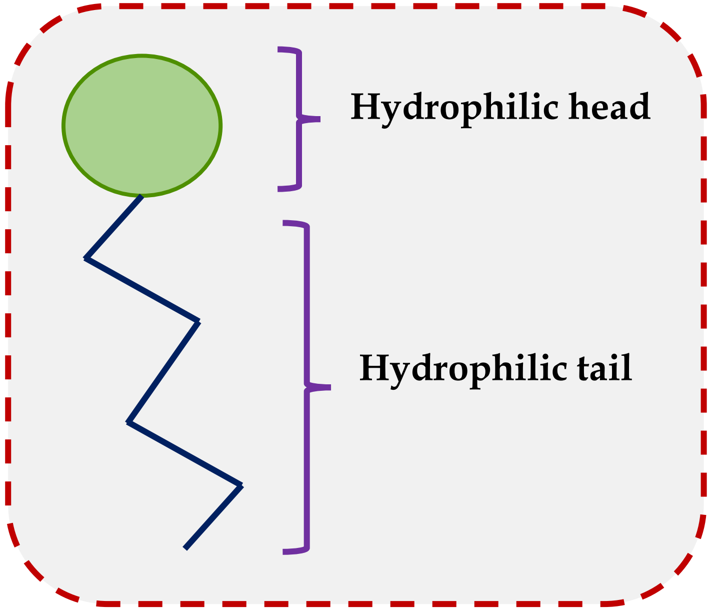
Evaluation of diversified bioprocessing schemes for biosurfactants production from *Lactobacillus* strains using cheese whey

V. Kachrimanidou
Department of Food Science and Technology, Ionian University

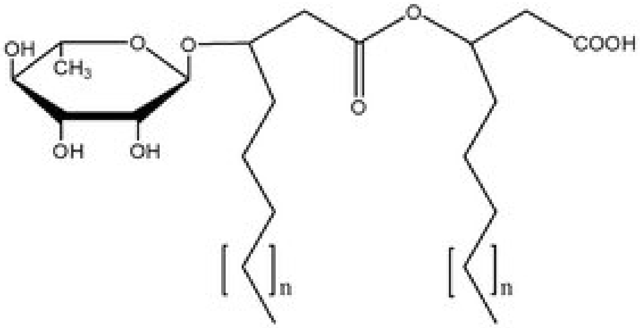
Presenting author email: **vkachrimanidou@gmail.com**,
*Corresponding author email: **kopsahelis@ionio.gr**

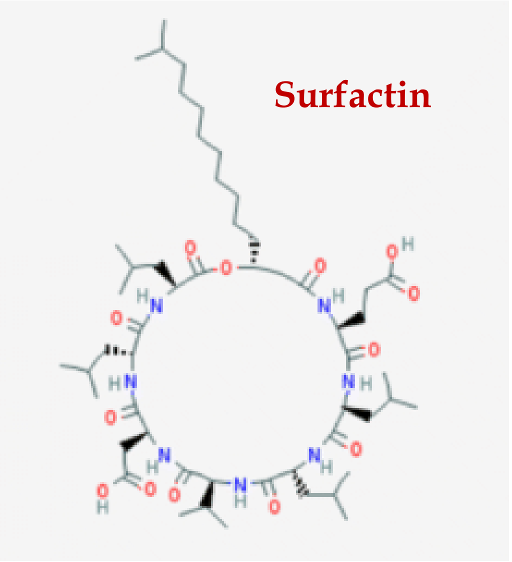
Biosurfactants

Biosurfactants or microbial surfactants constitute a group of amphiphilic molecules, comprising of both a <u>hydrophobic</u> (e.g. long-chain fatty acid, hydroxyl fatty acid) and a <u>hydrophilic</u> moiety (e.g. carbohydrate, amino acid, peptides, phosphate, alcohol).



Mono-rhamno-di-lipid

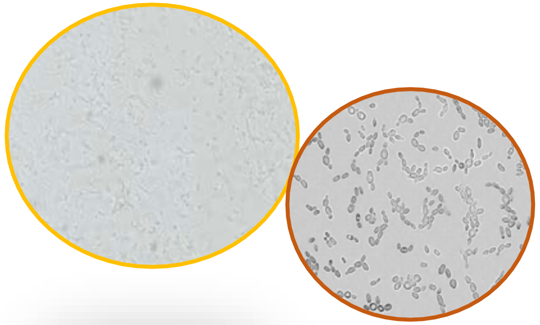




Biosurfactants

Microbial surfactants

- ✓ Biodegradable-Environmentally benign
- ✓ Surface active properties
- ✓ Moderate to low toxicity
- ✓ Numerous applications



Bacteria, yeast and fungi

Biosurfactant production

Bioremediation-Environment

Soil washing

Pharmaceuticals

Food industry

Cosmetic formulations

Agriculture

However…

Industrial production is hindered

- ✗ High cost of production-cost of raw materials
- ✗ Pathogenic strains-restrict food applications
- ✗ Characterisation of the produced structures
- ✗ Low productivities
- ✗ Downstream separation

Biosurfactants

Biosurfactant advantages

- ✓ Surface activity properties
- ✓ Effective Critical micelle concentration
- ✓ Stability to several factors: pH, temperature, salt concentrations

Significant characteristics specifically for the food industry

Potential applications the food industry

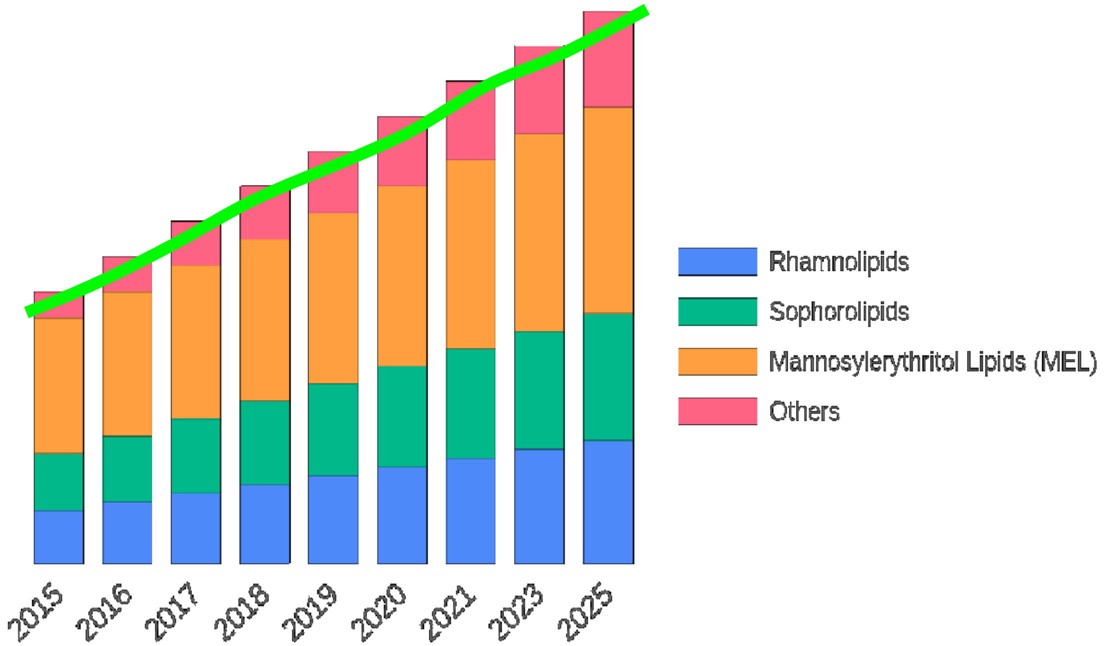
- ✓ Antimicrobial agents
- ✓ Biofilm formation inhibition
- ✓ Emulsifying agents
- ✓ Antioxidant properties
- ✓ Novel food formulations

✓ Could replace the chemically derived counterparts

Biosurfactants market

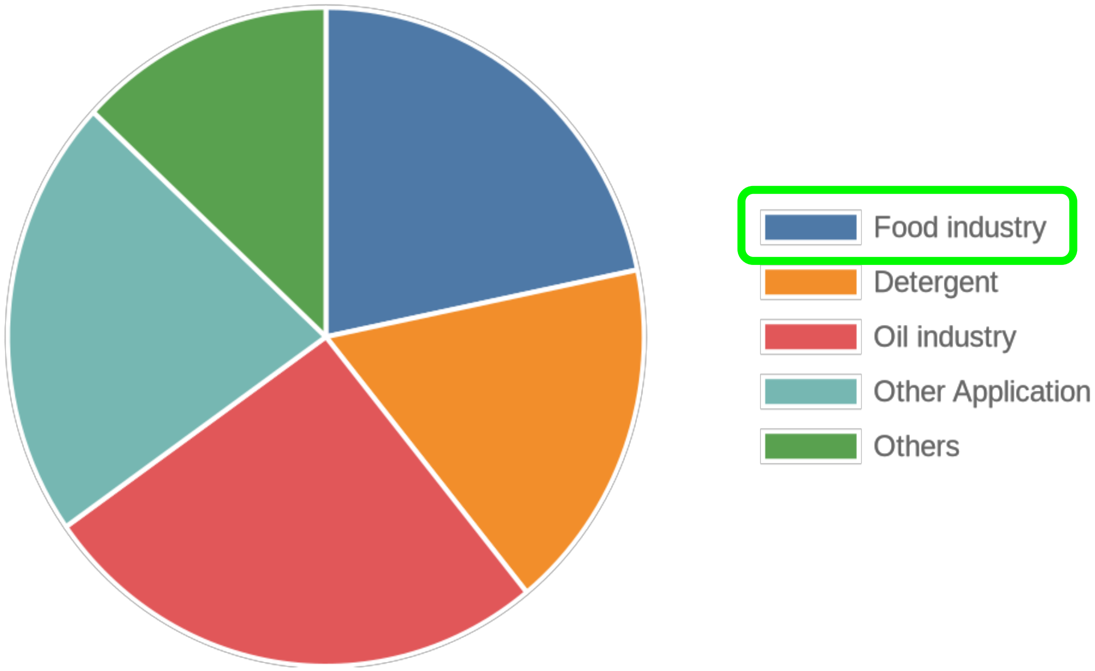
In 2018, the global biosurfactants market was > $1.5 billion

Global market size and growth forecast by product type



Misailidis, N., Petrides, D. Intelligen, Inc.

Market share and forecast by applications



https://bulletinline.com/2020/08/21/microbial-biosurfactants-market-outlook-recent-trends-and-growth-forecast-2028akzonobel-basf-innospec-clariant-stepan/

Biosurfactants

Cost competitive BS production

✕ High cost of production-cost of raw materials

Utilisation of renewable resources

(e.g. agro-industrial waste and by-product streams)

Molasses

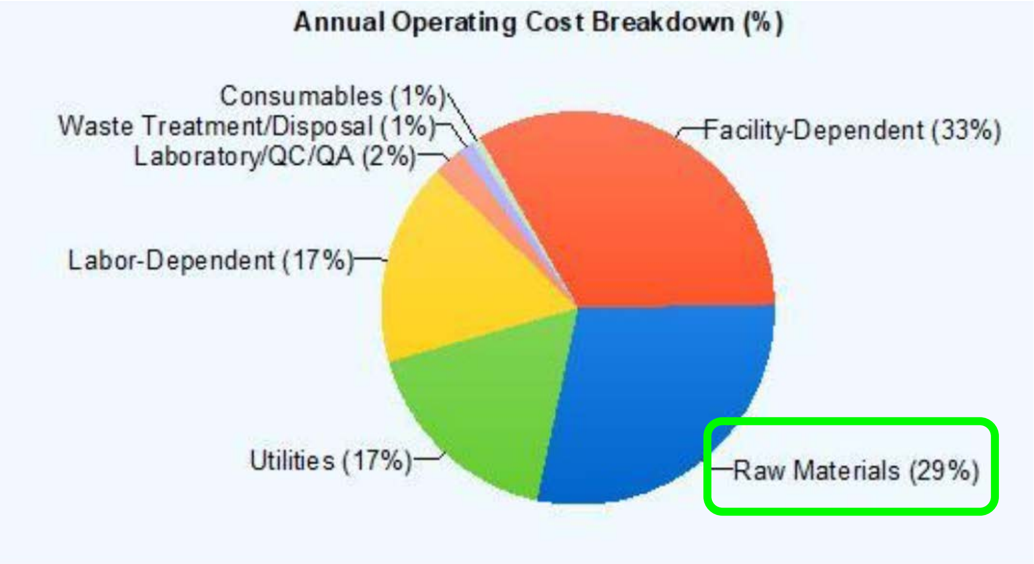
Waste frying oil

Glycerol

Winery by-products

Lignocellulosic biomass

Cheese whey



Annual Operating Cost Breakdown of a plant producing rhamnolipids via fermentation with *Pseudomonas* strain

Misailidis, N., Petrides, D. Intelligen, Inc.

Biosurfactants

Cost competitive BS production

✕ Pathogenic strains-restrict food applications

- Identification of novel GRAS strains (e.g. *lactobacilli* strains)
  - Isolation of strains found in the microbiota of fermented foods (e.g. dairy industry)

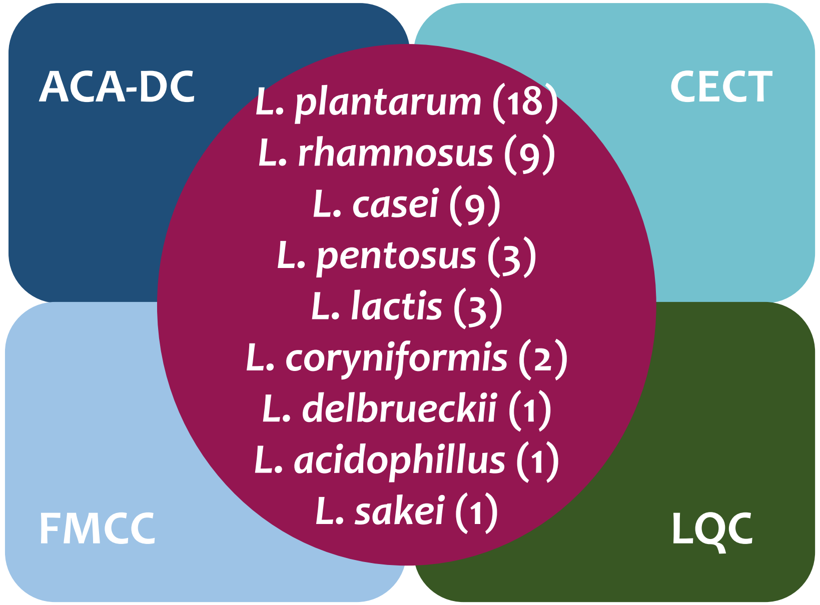
- Establishing novel end product formulations with increased added value will mediate the sustainability of BS production
- Integration in biorefinery concepts within the concept of circular bio-economy

Biosurfactants production

Twofold approach of this study:

A. Identification of potential biosurfactant producers selected from lactobacilli isolated from several sources

✓ *Four different culture collections were employed*



Screening for BS producers →

✓Ten (10) strains were selected after the screening →

✓ *Evaluation of bioprocessing strategies*

Microtiter fermentations

Shake flasks

Bioreactor studies

Renewable resources for BS production

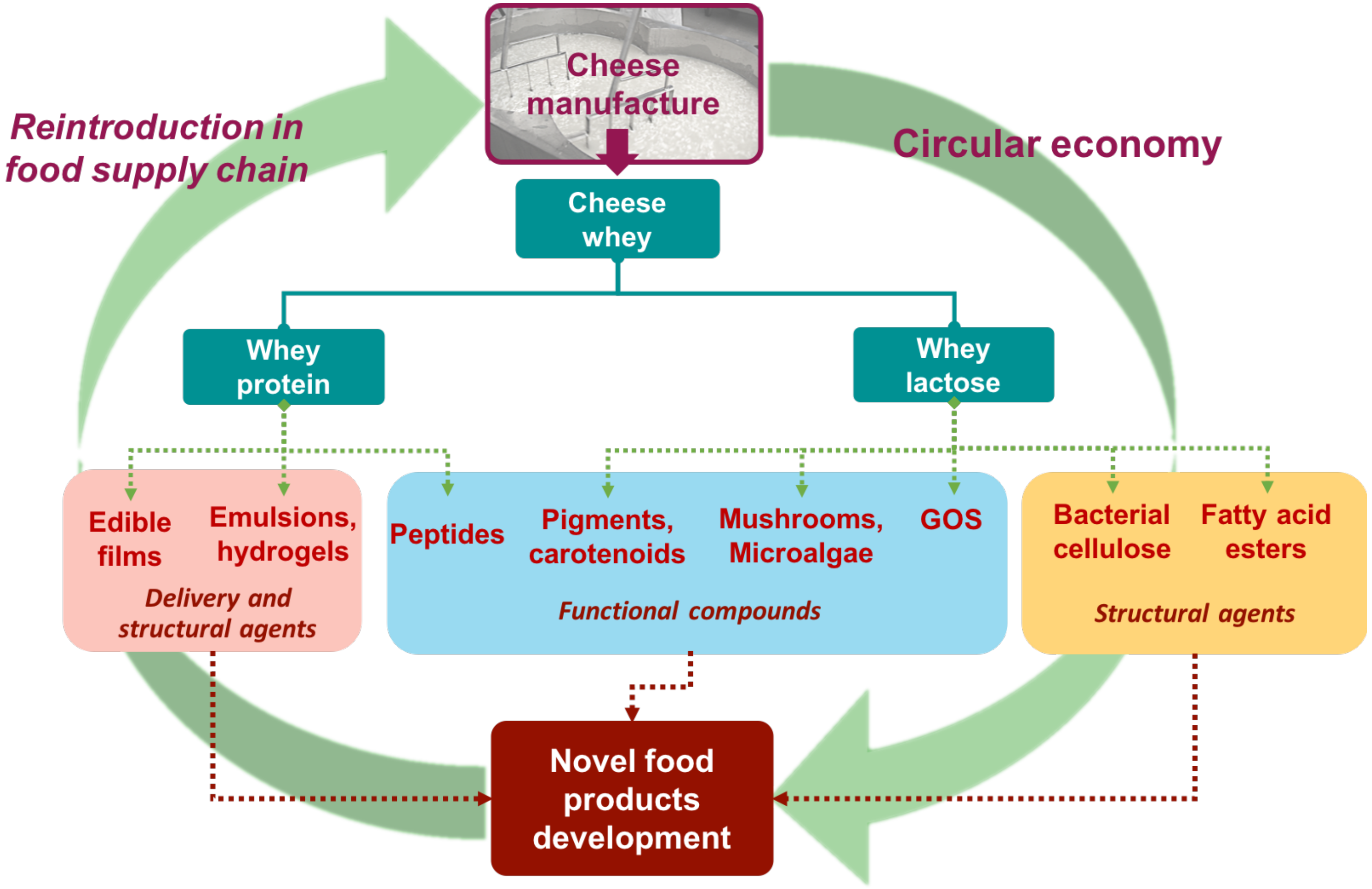
B. Implementation of cheese whey as a low-cost fermentation feedstock

Cheese whey: by-product stream of the dairy industry

Mainly water, but also:
lactose (66–77%, *w/w*)
protein (8–15%, *w/w*)
minerals salts (7–15%, *w/w*)

~ 9 L of whey are obtained for every 1 kg of cheese produced

Cheese whey-integrated biorefining approaches within circular economy



Lappa et al., Foods 2019, 8, 347.

Experimental design for BS production

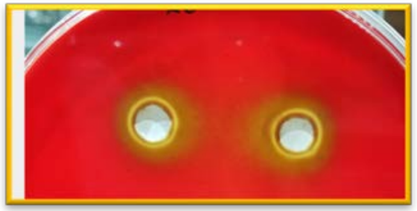
LAB culture on commercial medium and cheese whey

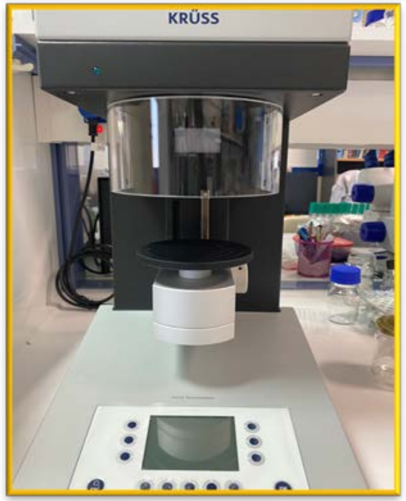
Biosurfactant evaluation

- ✓ Surface tension measurements
- ✓ Blood agar test- Haemolytic activity
- ✓ Oil displacement test
- ✓ Emulsification index (E_{24}, E_{48})
- ✓ Lactose consumption

LAB BS production → Extracellular or cell-bound → Supernatant and PBS-extracts were tested → Selection of potential BS producers

Several methods for screening of LAB strains





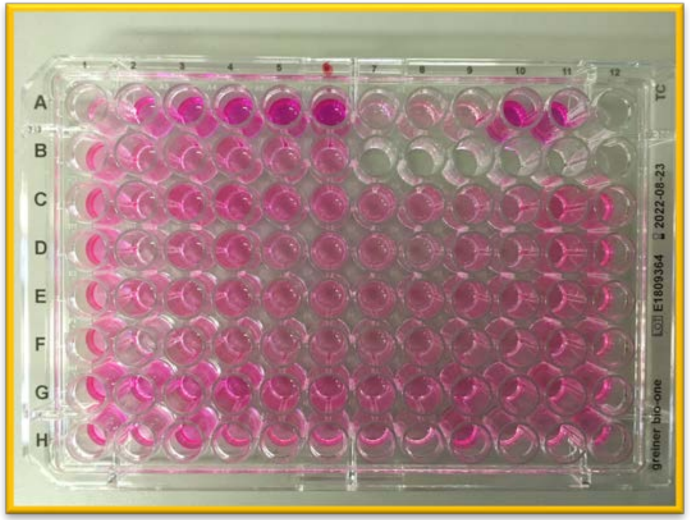
Experimental design for BS production

✓ Selection of potential BS producers

A. Shake flask fermentations

Effect of pH and incubation temperature (°C)

B. Microplate experiments



Effect of selected nitrogen sources and micronutrients (μ, h^{-1})

C. Bioreactor fermentations

Controlled pH and temperature

Shake flask fermentations

- Cheese whey was the sole fermentation substrate
- Initial lactose: ~20 g/L, pH: 4, 5, 6.8 and T: 25, 30,37 and 40 °C

Lactose consumption was significantly low and fermentation was prolonged

E_{24}(%) was higher at pH 5 and pH 6.8 and T:37 and 40 °C

pH 4 and T:25 °C did not sustain microbial growth

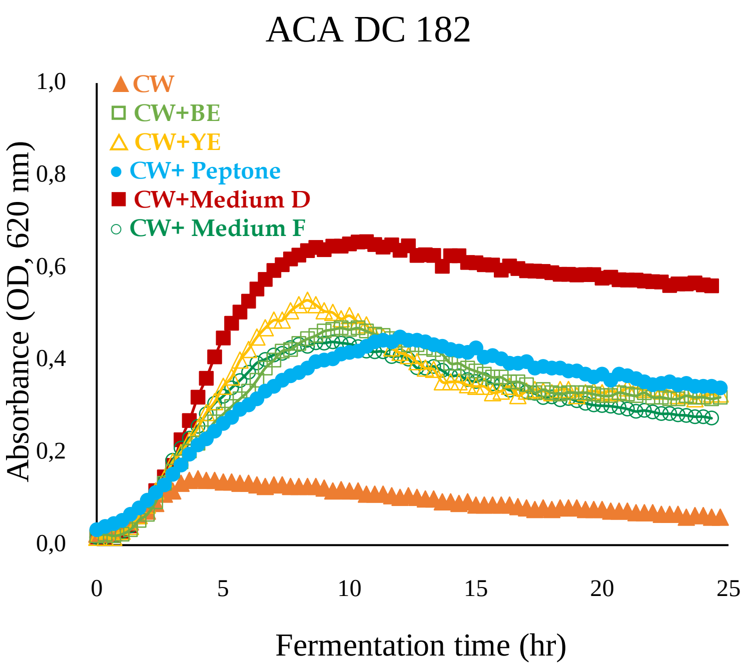
Low Lactic Acid (LA) and Total Dry Weight (TDW, gL^{-1}) production

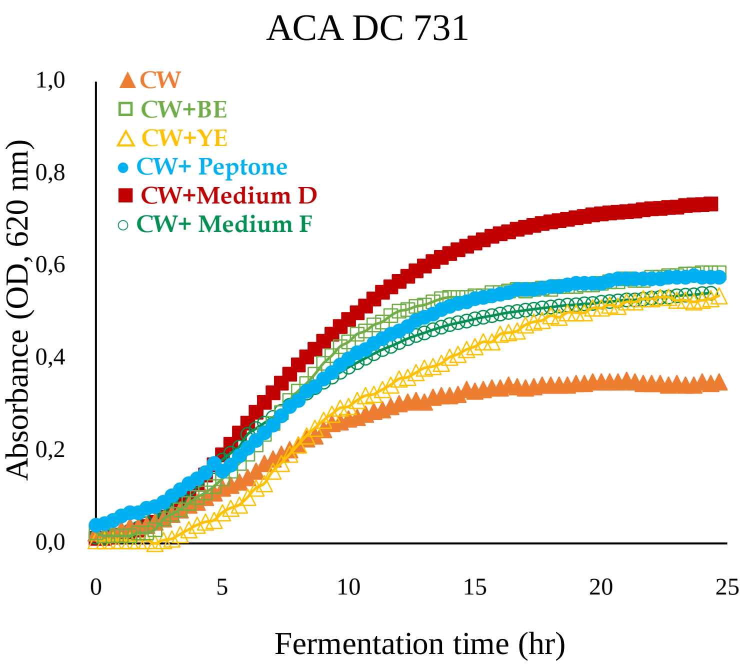
Shake flask fermentations

	Strain	T_f (h)	Lactose (g/L)	Lactic acid (g/L)	TDW (g/L)	E_{24} PBS extracts (%)	E_{24} supernatants (%)
pH 4	ACA 182	95	5.44	2.13	0.55	8.70	17.39
	ACA 276	95	5.63	4.53	0.60	13.04	21.74
	ACA 731	95	4.39	5.29	0.85	8.70	21.74
	ACA 4052	95	3.86	0.35	0.35	13.64	17.39
	CECT 278	95	8.51	8.03	2.95	13.64	18.18
	CECT 4023	70	2.31	1.87	0.25	13.50	15.00
	LQC 752	119	3.95	3.58	0.80	13.64	17.39
	LQC 753	119	1.50	1.26	0.85	13.64	21.74
	LQC 854	95	1.70	0.00	0.15	11.00	27.27
	FMCC E108	95	4.52	4.61	0.90	13.64	25.00
pH 5	ACA 182	119	15.84	2.54	2.65	9.38	19.23
	ACA 276	95	14.65	4.53	0.30	9.38	21.74
	ACA 731	70	2.21	4.69	0.55	9.38	15.38
	ACA 4052	95	11.73	3.07	0.30	12.50	19.23
	CECT 278	95	17.22	7.56	4.60	9.38	23.08
	CECT 4023	119	2.15	1.60	0.60	17.39	30.43
	LQC 752	95	10.12	2.16	0.25	9.09	13.04
	LQC 753	95	10.40	1.80	6.95	9.00	66.67
	LQC 854	119	15.78	0.00	4.05	13.04	39.13
	FMCC E108	46	10.15	2.05	0.10	9.09	48.00
pH 6.8	ACA 182	95	6.04	1.79	1.10	13.04	21.74
	ACA 276	119	15.51	8.22	0.70	10.87	39.13
	ACA 731	119	15.33	12.81	1.85	13.04	30.43
	ACA 4052	119	13.72	11.76	3.15	13.04	39.13
	CECT 278	70	14.28	9.00	2.00	19.05	26.09
	CECT 4023	46	9.16	0.00	0.90	13.04	34.78
	LQC 752	95	2.33	2.05	1.15	13.64	21.74
	LQC 753	95	2.35	0.73	0.80	16.67	21.74
	LQC 854	95	7.71	2.55	0.25	13.04	26.09
	FMCC E108	70	5.85	2.19	0.50	18.18	21.74

Microplate experiments

- ❑ Cheese whey was supplemented with several nitrogen sources
- ❑ Yeast extract, peptone, beef extract and their combinations



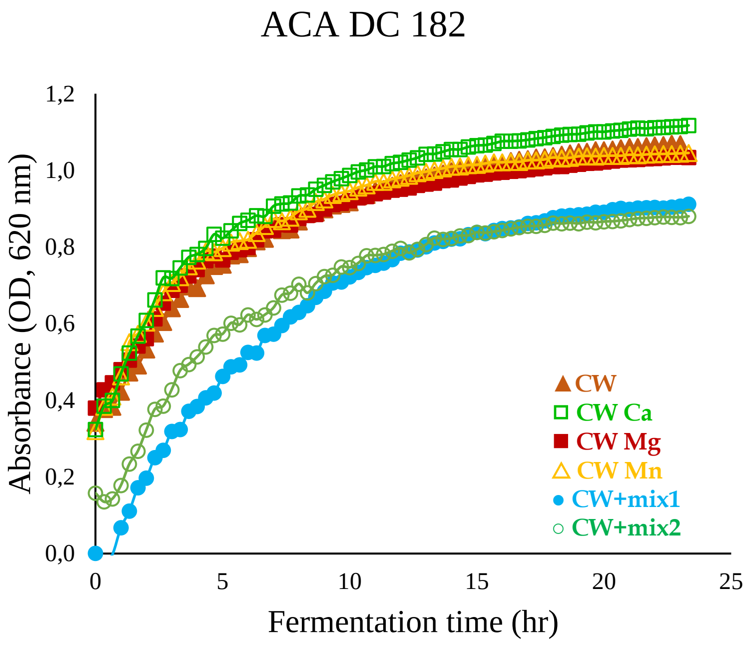


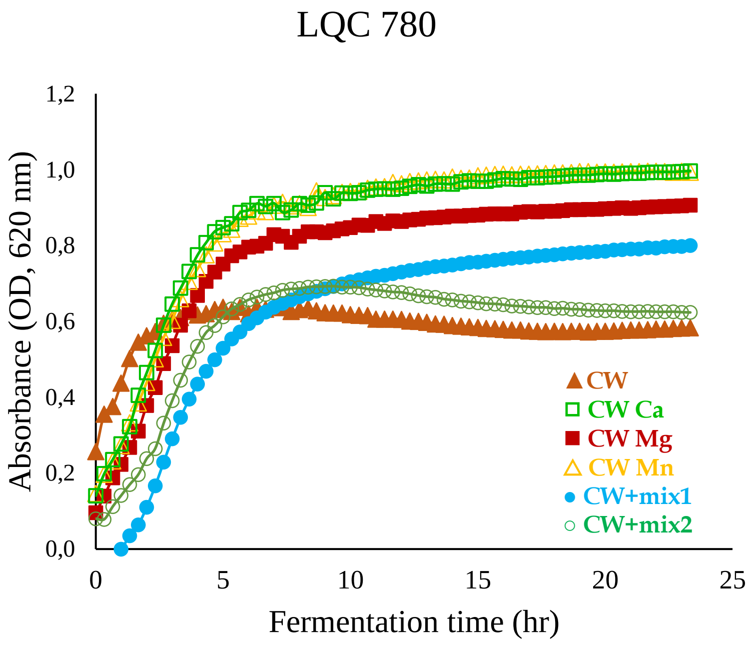
- ✓ The combination of yeast, peptone and beef extract indicated higher specific growth rates
- ✓ Cheese whey was supplemented for further experiments

Medium D: yeast extract 4 gL^{-1}, peptone 10 gL^{-1} , beef extract 8 gL^{-1}
Medium F: yeast extract 4 gL^{-1}, 5.3 gL^{-1} ammonium citrate, peptone 10 gL^{-1}

Microplate experiments

- Cheese whey was also supplemented with specific micronutrients: Ca, Mg, Mn, and two combinations of micronutrients.
- Initial lactose: ~20 g/L, pH: 6.8 and T:37 °C



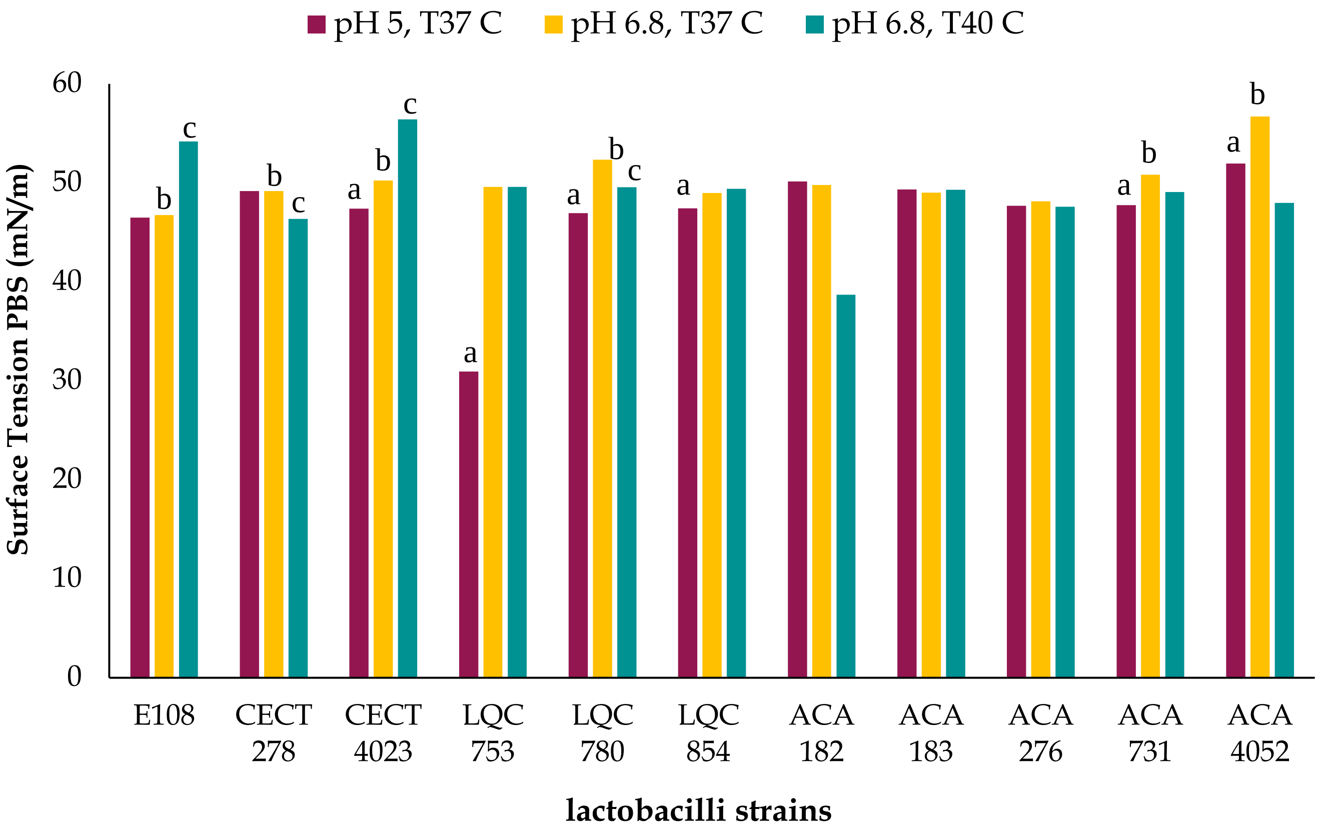


✓ The addition of Ca improved microbial proliferation, followed by Mg

mix1: 7 gL^{-1} KH_2PO_4; 2.5 gL^{-1} Na_2HPO_4; 1.5 gL^{-1} $MgSO_47H_2O$; 0.15 gL^{-1} $FeCl_36H_2O$; 0.02 gL^{-1} $ZnSO_47H_2O$; 0.06 gL^{-1} $MnSO_4H_2O$; 0.15 gL^{-1} $CaCl_22H_2O$
mix2: 1.6 gL^{-1} K_2HPO_4; 0.4 gL^{-1} KH_2PO_4; 0.1 gL^{-1} NaCl; 0.1 gL^{-1} $MgSO_47H_2O$; 0.02 gL^{-1} $CaCl_22H_2O$; 0.5 mgL^{-1} $CuSO_45H_2O$; 10 mgL^{-1} H_3BO_3; 10 mgL^{-1} $MnSO_45H_2O$; 7 mgL^{-1} $ZnSO_4$

Shake flask fermentations

- Cheese whey was supplemented with yeast extract, peptone and beef extract
- Initial lactose: ~20 g/L, pH: 5, 6.8 and T: 30, 37 and 40 °C



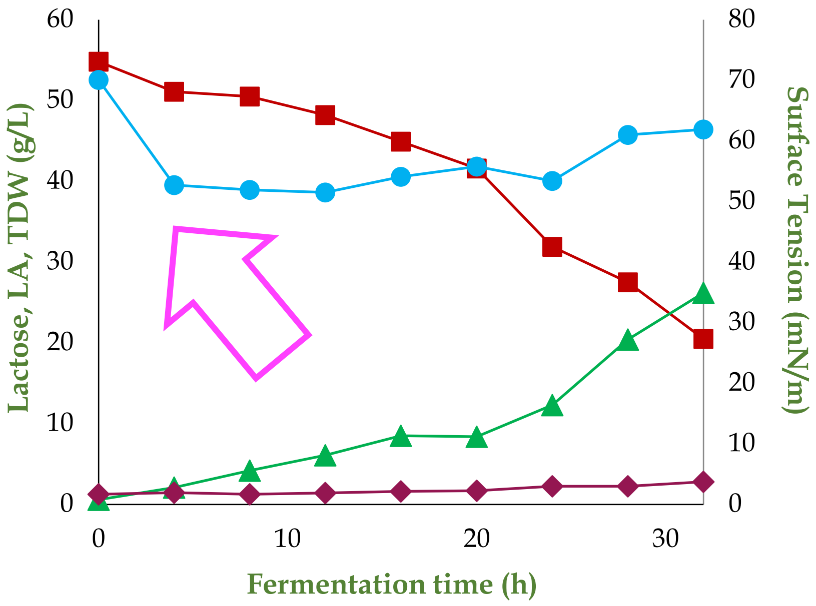
In most cases pH 5 led to lower BS production

T:40 °C inhibited BS secretion in some strains

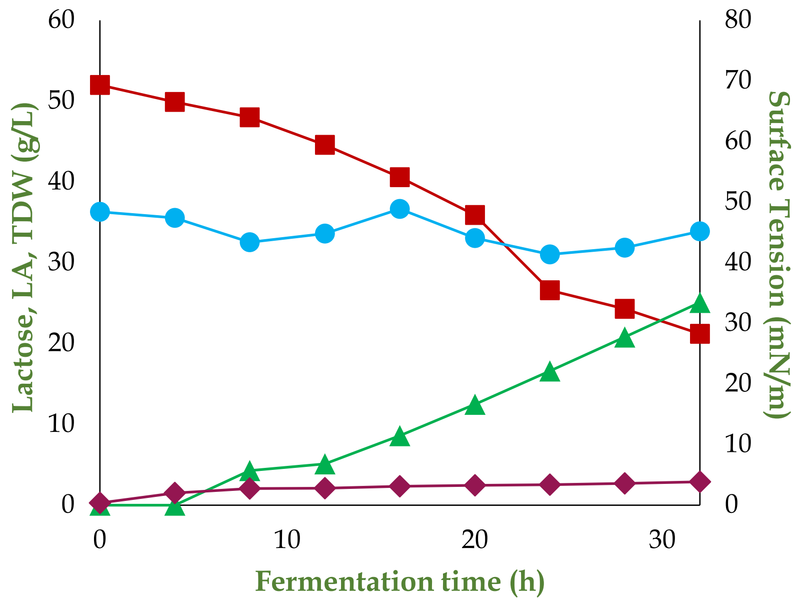
Bioreactor studies were performed with less strains to further study the fermentation conditions

Bioreactor fermentations

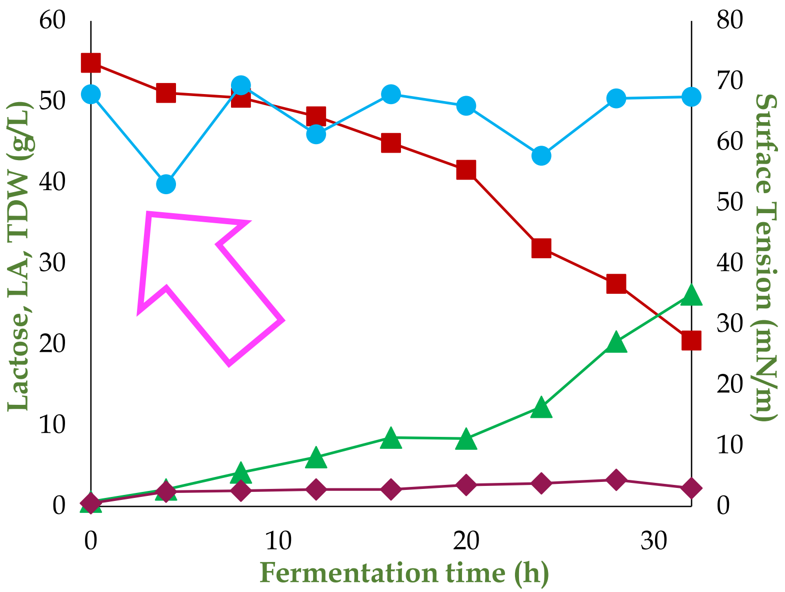
Lacticaseibacillus rhamnosus CECT 278



Cheese Whey



Cheese Whey with yeast extract and peptone



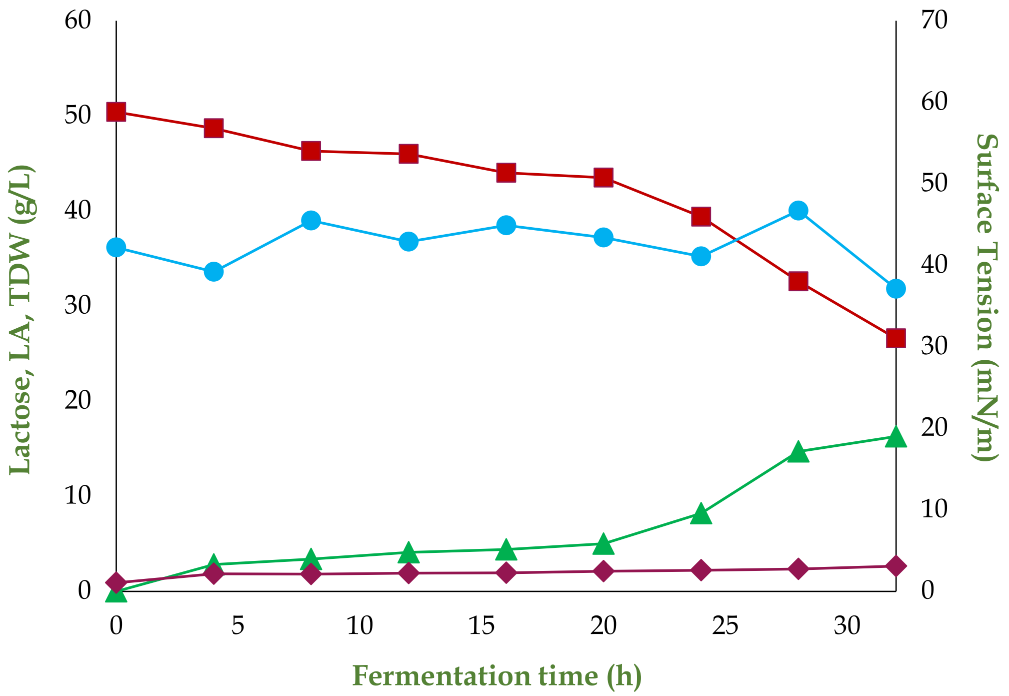
Cheese Whey with yeast extract, beef extract and peptone

- ■ Lactose
- ● Surface Tension
- ▲ Lactic acid
- ◆ TDW

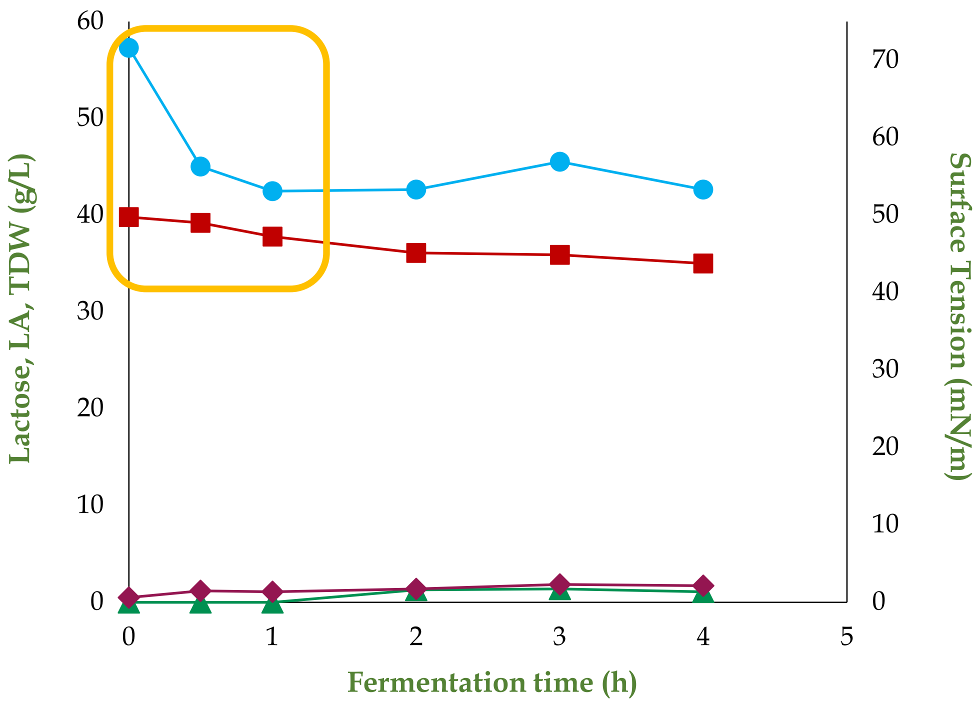
Significant reduction occurs the first hours

Bioreactor fermentations

Limosilactobacillus fermentum ACA DC 183



Cheese Whey with yeast extract and peptone

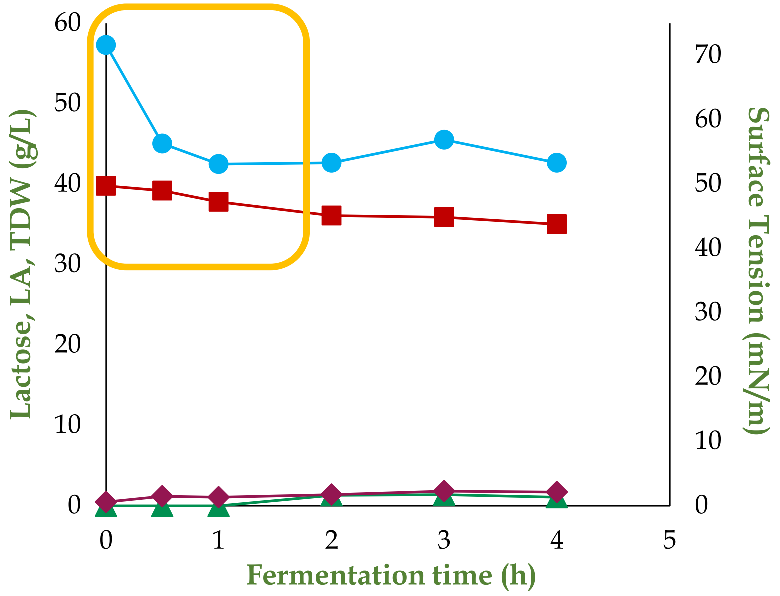


Cheese Whey

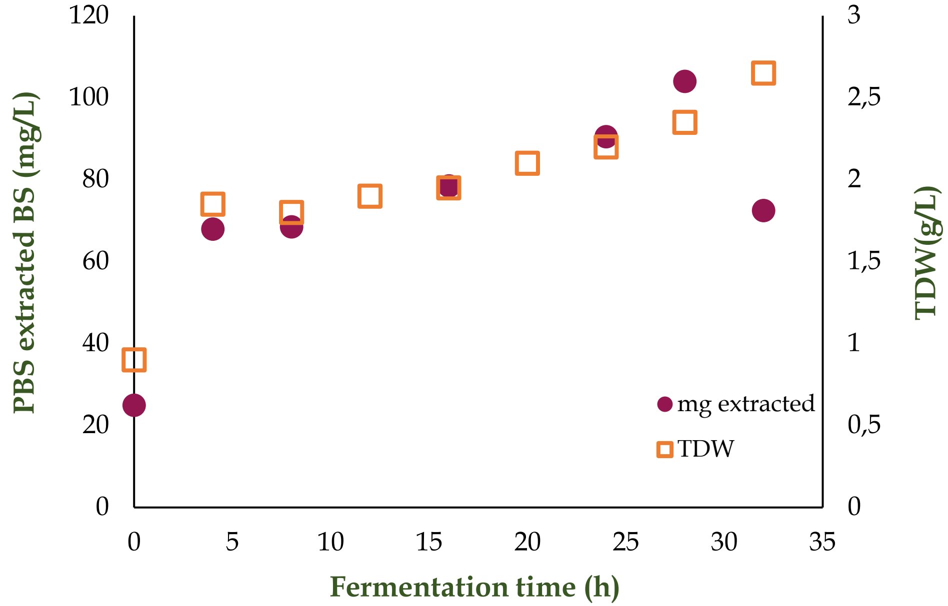
- ■ Lactose
- ● Surface Tension
- ▲ Lactic acid
- ◆ TDW

Bioreactor fermentations

Limosilactobacillus fermentum ACA DC 183



Cheese Whey



Maximum reduction in ST occurs the first hours of fermentation when TDW increases two-fold

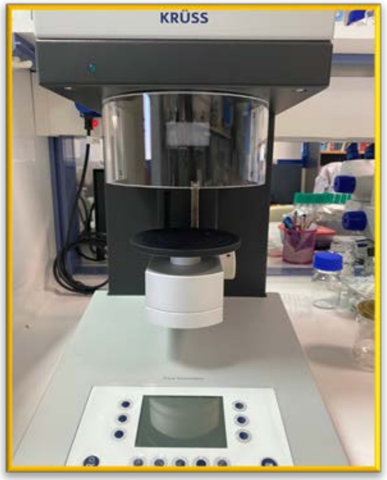
Crude BS increase along with biomass increase

Preliminary BS characterisation

Downstream of BS

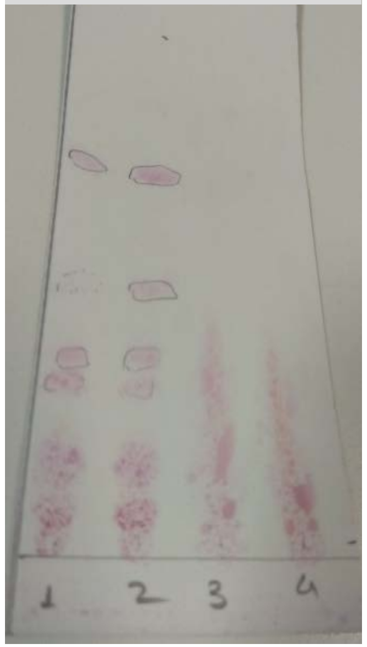
Extraction of cell bound BS with PBS → *Centrifugation* → Dialysed with membranes → Freeze Drying → Crude BS extract → Characterisation

Dialysed with membranes → Surface tension measurements



Characterisation → Protein and Carbohydrates content

Ninhydrin



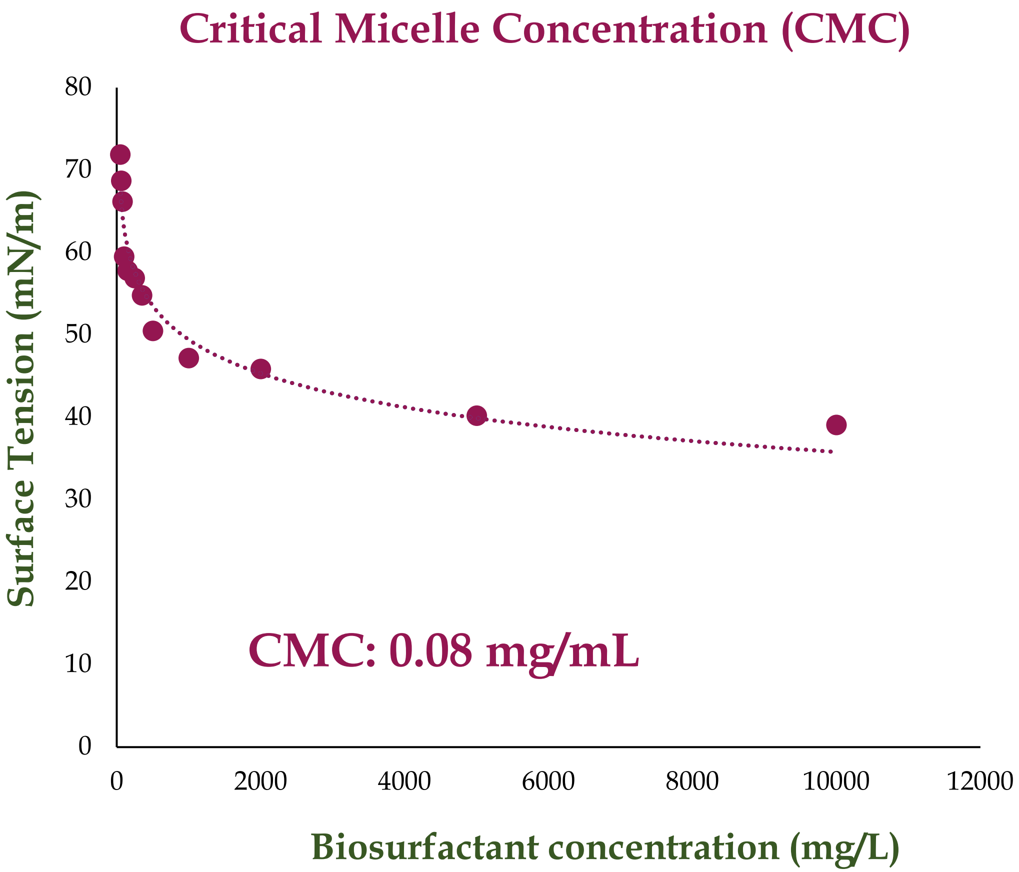
protein-based BS molecule

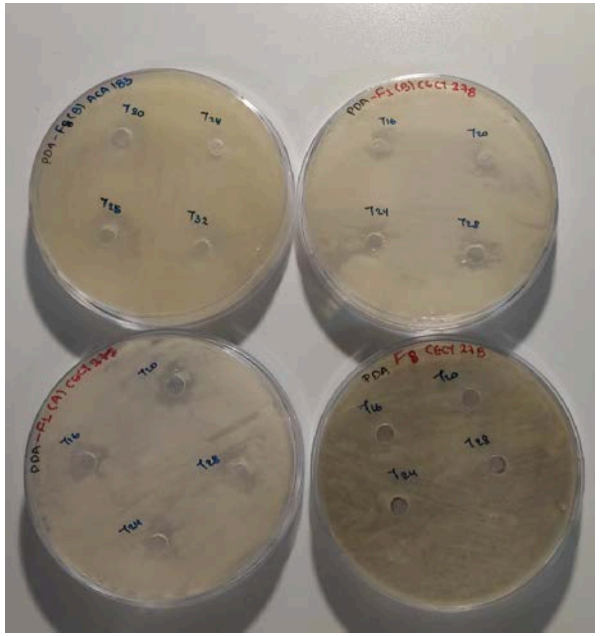
60-70% protein content

20% carbohydrates

Bioreactor fermentations

Limosilactobacillus fermentum ACA DC 183





Antimicrobial activity against fungal strains

Future studies

Evaluation of bioprocessing strategies

Target: To further increase biomass production within the first hours

- Evaluate aeration conditions (facultative anaerobic strains)
  Trigger biomass production instead of lactic acid

- Evaluate repeated fed-batch

- Stability studies and antimicrobial activity to identify potential applications

- Detailed characterisation of produced BS

- Investigate emulsion stability and surface tension for cosmetic/food formulation

8TH INTERNATIONAL CONFERENCE
ON SUSTAINABLE SOLID WASTE MANAGEMENT
23-26 JUNE 2021, THESSALONIKI, GREECE

THESSALONIKI2021
23-26 JUNE

HELLENIC REPUBLIC · IONIAN UNIVERSITY · 1984

Thank you for your attention

Dr Vasiliki Kachrimanidou

Department of Food Science and Technology, Ionian University

vkachrimanidou@gmail.com

kopsahelis@ionio.gr

Acknowledgments

Dimitra Alimpoumpa

Dr Aikaterini Papadaki

Dr Iliada Lappa