

«THE MICROALGAE HUB»

Microalgae for the treatment and valorization of agro-zootechnical waste and dairy by-products

How to use by-products from the food industry to grow microalgae and their valorization

SaltGae, final meeting

Ljubljana , 25 September 2019

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Fondazione
CARIPLO 

 **Regione
Lombardia**

Dairy by-products characterization

Campioni	N-NH4 (mg/L)	P-PO4 (mg/L)	TKN (mg/L)	COD tot (g/L)	COD sol (g/L)	ST (g/kg)	SV (g/kg)	N/P	COD/SV	SV/ST
Whey	65	455	1336	87,3	65,6	64,2	59,22	2,9	1,47	0,92
Exhausted whey	10	320	720	61,6	50,9	55,87	46,94	2,3	1,31	0,84
Buttermilk	7,7	130	223	15-20	5,9	14,53	12,84	1,7	1,60	0,88

Whey: very high COD, competition with bacteria

Exhausted whey : very high COD, competition with bacteria

Buttermilk: COD acceptable, N / P unbalanced vs P, N need to be hydrolyzed, competition with bacteria

2,5 milion of tons/year in Lombardy region

Experimental Design

Laboratory evaluation of microalgal species able of growing on dairy by-products

- *Chlorella sorokiniana*
- *Scenedesmus acuminatus*
- *Acutodesmus obliquus*
- *Desmodesmus armatus*
- *Chlamydomonas debaryana*
- *Porphyridium cruentum*



- *Euglena gracilis*
- *Arthrospira platensis* ("Spirulina")



Buttermilk



Exhausted whey

Arthrospira platensis (Spirulina)

In 1967 Spirulina was recognized as a 'future food source' by the International Association of Applied Microbiology



Generally recognized as safe (GRAS) since 1981 (FDA)

- **Antioxidant**
- **Anti-inflammatory**
- **hypoglycemic**
- **antihypertensive**
- **immunostimulant**



Content in 100 g

Proteins 35.4–70.0 g

Amino acids
Glutamate 7.0–7.3 g

Leucine 5.9–8.4 g

Aspartate 5.2–6.0 g

Lysine 2.6–4.6 g

Tyrosine 2.6–3.4 g

Phenylalanine

2.6–4.1 g

Methionine 1.3–2.7 g

Fat 4.0–16.0 g

% of total fatty acids

Palmitic

25.8–44.9%

Gamma-linoleic

17.1–40.1%

Linoleic 11.1–12.0%

Oleic 10.1–16.6%

Palmitoleic 2.3–3.8%

Stearic 1.7–2.2%

Carbohydrates

14.0–19.0 g

Crude fiber 3.0–7.0 g

Minerals

Potassium 2.0–2.6 g

Sodium 1.5–2.2 g

Total phosphorus

1.3–2.2 g

Iron 273.2–787.0 mg

Magnesium 330

Calcium 120–900 mg

Vitamins

B12 5.7–38.5 µg

B2 3.0–4.6 mg

B6 0.5–0.8 mg

Niacin (B3) 13–15 mg

Folic acid

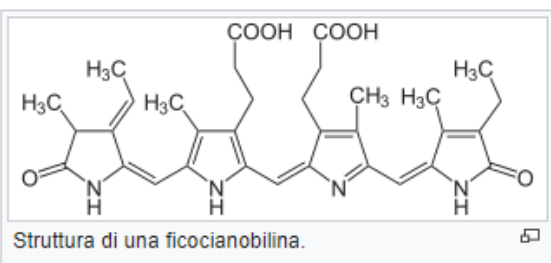
0.05–9.92 mg

Carotenoids 0.3–2.6 g

Tocopherol 0.4–9.8 g

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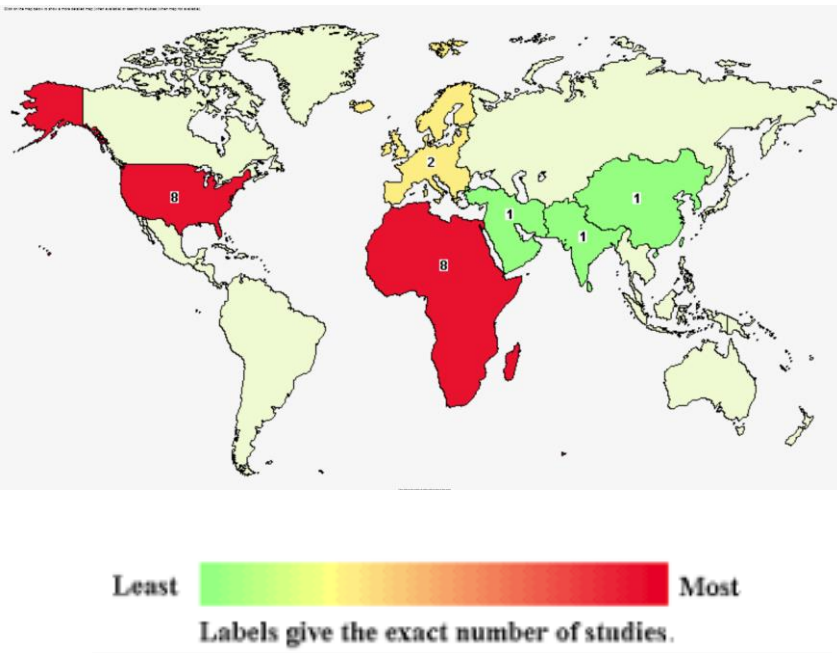
Valorisation of *Arthrospira platensis* in the cosmetic and nutraceutical sector



PHYCOCYANIN: association of proteins of the phycobiliprotein family, and of water-soluble photosynthesis pigments, the phycocynobilins, of the phycobiline family.

- powerful antioxidant (40 times higher than that of vitamin C and vitamin E). Contrast oxidative stress.
- it counteracts inflammatory states by acting similarly to non-steroidal anti-inflammatory drugs (Fans) and by inhibiting various enzymes involved in the inflammatory response (colitis, inflammation to the ear, arthritis and tumors).
- it contributes to the reinvigoration of the immune system by strengthening the cell membrane and increasing the protection of cells from virus attacks.
- alleviate the symptoms of seasonal allergies.
-

CLINICAL TRIAL



ClinicalTrials.gov (last access 10/09/2019)

"Spirulina"

-23 "completed" studies in the world

-17 studies in the world "being completed"

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1	<input type="checkbox"/>	Completed	Vitamin A Value of <i>Spirulina</i> Carotenoids in Humans
2	<input type="checkbox"/>	Completed	Spirulina Supplementation and Infant Growth, Morbidity and Motor Development
3	<input type="checkbox"/>	Completed	Effect of <i>Spirulina</i> on Zinc, Vitamin E and Linoleic Acid Levels in Palm Skin Following Chronic Exposure to Arsenic
4	<input type="checkbox"/>	Completed	Carbon-13 (13C)-<i>Spirulina</i> <i>Platensis</i> Gastric Emptying Breath Test (GEBT) Has Results
5	<input type="checkbox"/>	Completed	Effects of <i>Spirulina</i> Supplementation on Overweight or Obese Adults
6	<input type="checkbox"/>	Completed	Effects of <i>Spirulina</i> on Cardiac Functions in Children With Beta Thalassemia Major
7	<input type="checkbox"/>	Completed	Effect of <i>Spirulina</i> on Liver Iron Concentration in Beta Thalassemic Children With Hepatitis C
8	<input type="checkbox"/>	Completed	Effect of <i>Spirulina</i> on Serum Hyaluronic Acid in Beta Thalassemic Children With Hepatitis C
9	<input type="checkbox"/>	Completed	Effect of <i>Spirulina</i> on Liver Fibrosis by Transient Elastography in Beta Thalassemic Children With Hepatitis C
10	<input type="checkbox"/>	Completed	The Effects of <i>Spirulina</i> <i>Platensis</i> on Insulin Resistance in HIV-infected Patients

Valorisation of Arthrospira in the cosmetic sector



LIGNE ST BARTH
 SHAMPOO ALLA
 SPIRULINA 125 ML
 COD: 27160 7015001

DISPONIBILE
 € 32,00

**ERNO
LASZLO**


DISPONIBILE
 € 135,00
 Spedizione gratuita



INSTITUT ESTHERDERM |




ESTHERDERM
 Intensif Spiruline Cream 50ml

 69.00€



ESTHERDERM
 Intensif Spiruline Serum 30ml

 80.00€



69,00 €



Wang et al. Bioresource Technology 2015 184:355–362

Spirulina in animal feed



- In common carp it improves growth and increases proteins and fat content (Abdulrahman and Ameen, 2014)



- It stimulates pigmentation in ornamental fish, such as Koi carp (Gouveia, 2003)



- It stimulates the immune system of trout juveniles increasing resistance to pathogens (Yeganeh, 2015).



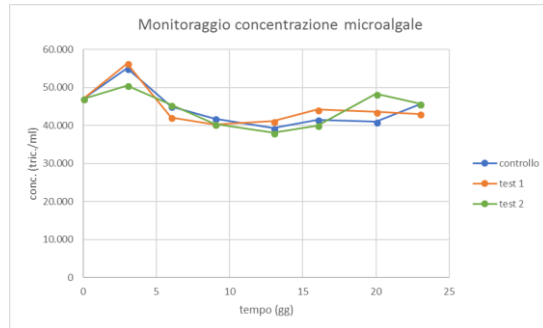
- It induces increasing in fat, protein and lactose content in cow's milk (Simkus et al., 2007-2008), whose production can increase by 21% (Kulpys et al., 2009).
- Improves growth and fertility (Holman, 2013)

What's the aim of the work?

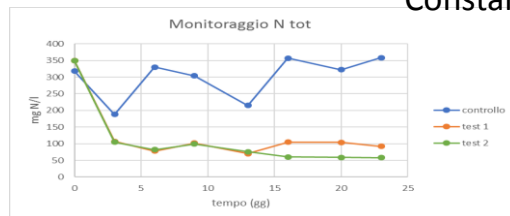
- decrease the cost of the biomass production by reducing the cost of synthetic medium;
- valorize the dairy by-product, reducing at the same time the nutrient content (N, P, COD)

Cultivation of *A. platensis* for nutrients removal from dairy by-products

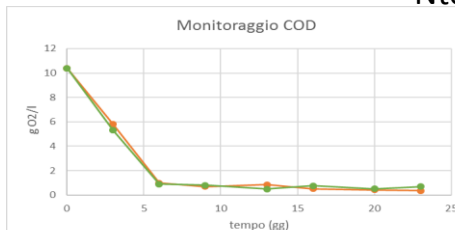
Arthrospira platensis (spirulina) cultivation test on exhausted whey: pilot scale test (semi-batch mode)



Constant growth



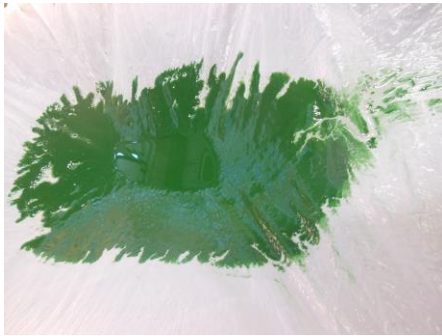
N_{tot} removal : 76%



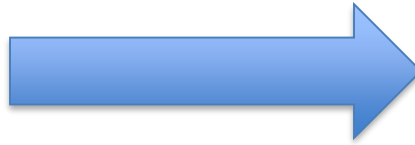
COD Removal: 94%

Parameters	Characteristics exhausted whey	Characteristics effluent
pH	5,5	9
COD (g/L)	50	0,5
N-NO ₃ (mg/L)	<1	0
N-NH ₄ (mg/L)	10	0
N-tot (mg/L)	720	50-100

Harvesting and conservation



Filtration



lyophilization





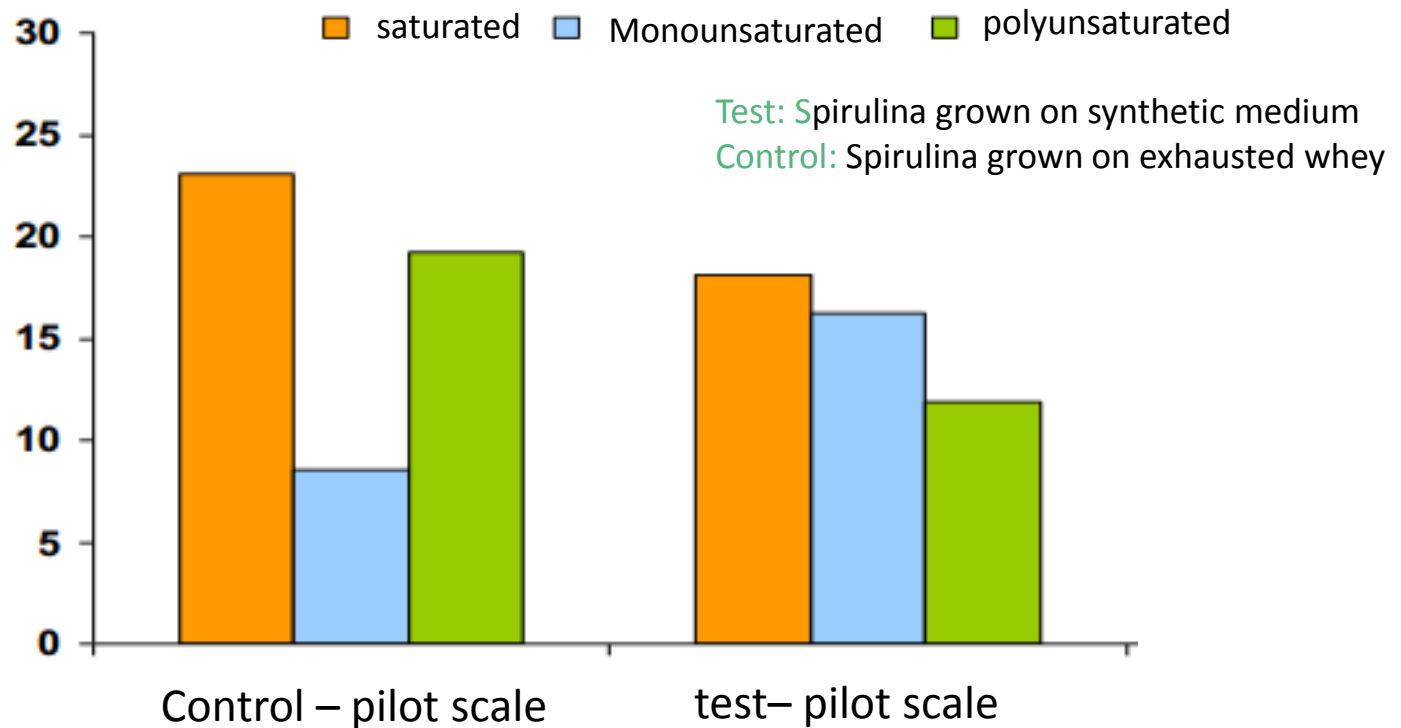
Biochemical and microbiological analysis

Samples	Total microbial charge	Enterobacteriaceae	E. coli	sporogenous sulfite reducers	FA	Salmonella
Arthrospira platensis (control)	9,5E+04	<100	<100	<100	-	assente
Arthrospira platensis (hexausted whey)	1,2E+05	<100	<100	<100	-	assente

Data expressed as ufc/g

g/100g	Humidity (%)	Protein (%)	Carbohydrates (%)	Lipids (%)
Arthrospira platensis (control)	8,5	51.8	10.3	12,5
Arthrospira platensis (hexausted whey)	11,3	51.1	11,2	10,5

Fatty acids composition of Spirulina (test vs control)



VALORISATION OF ARTHROSPIRA IN THE FEED INDUSTRY

Feeding trial on Sturgeon juveniles (*Acipenser baeri*)

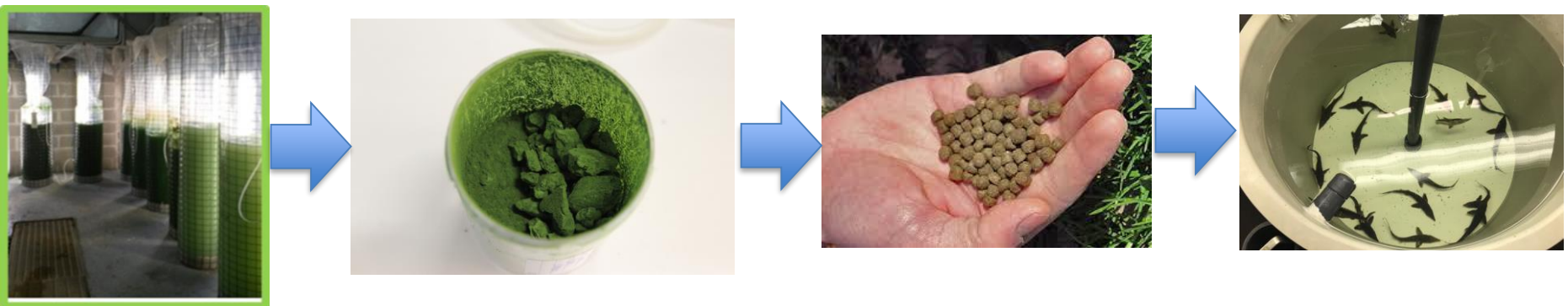


The sturgen: *Acipenser baeri*

- Freshwater fish of zootechnical interest, especially for caviar production. Caviar market, world production: 250 ton / year (over € 400 million per year), with great growth potential. Italy is the European leader for caviar production (50 tonnes in 2018) and second worldwide (between China and Russia)
- Sturgeons are mainly carnivorous and opportunistic omnivorous. At all stages, they spend most part of their life at the bottom of the river, to feed on algae attached to rocks, zooplankton, small invertebrates and fish.
- The main problems of these fish, during the juvenile phase, are above all associated with the adaptation of new diets and, depending on the species, at this stage the mortality is often very high



Feeding trial on Sturgeon juveniles (*Acipenser baeri*)



Microalgae
biomass
production (from
standard synthetic
medium (MS) and
HEXAUSTED
WHEY

Harvesting and
drying

Aquafeed
preparation

Feeding trial on
sturgeon
juveniles

External Collaborations: AIA-Veronesi (Verona), University of Almeria (Spain) and University of Milan

Ljubljana, 25 settembre 2019

Feeding trials and growth performance

Monofactorial random design ➡ diets tested in triplicate (control vs test)

Control: groups of fish fed by control diet, based on fish meal;

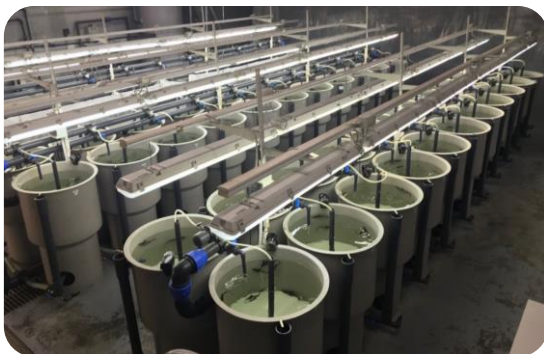
Test: groups of fish fed by experimental diet, based on 10% inclusion of spirulina.

- Fish



~ 100 *A. baerii* (IBW $12,8 \pm 0.4$ g)
randomly divided among 6 groups

- Experimental facilities



circular tanks

(Volume 130 L)
RAS T $18.9 \pm 0.6^{\circ}\text{C}$,
salinity $1 \pm 0,02\%$,
DO $9.6 \pm 1.2\text{mg/l}$,
pH 8.1 ± 0.1 ,
 $\text{NH}_4\text{-N} < 0.06\text{mg/l}$,
 $\text{NO}_2\text{-N} < 0.2\text{mg/l}$,
light-dark cycle 12:12

- Experimental protocol

- Fish/tank: 16
- Feed ratio: 3% g/w
- Feeding period: 6 weeks (6 days per week)
- Diets offered in two daily meals
- Fish were tank/group weighted every week



Feeding trials and growth performance

The following parameters were calculated per group over 40 days of feeding trial (until fish have quadruplicated in weight):

Feed intake during the trial

Specific growth rate (SGR): $100 \times [(\ln \text{ final body weight} - \ln \text{ initial body weight}) / \text{days}]$

Feed conversion ratio (FCR): Feed intake/weight gain

Fish Survival (%)

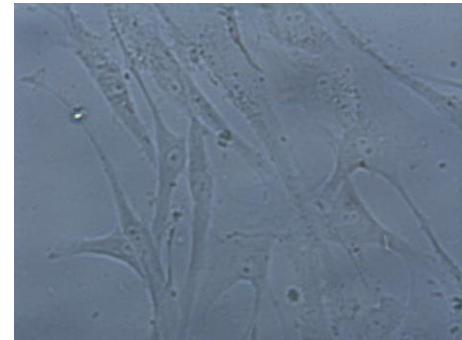


No differences in Growth Performance were found between control and test groups

Valorization of *Arthrospira platensis* in cosmesis

PRELIMINARY TRIAL

Cytotoxicity and antioxidant activity assay on human fibroblasts, in order to test phyto-complex based on **PHYCOCYANIN protein**, extracted from *Spirulina* grown on a synthetic medium (Zarrouk)



Cytotoxicity Test

No negative effects of PHYCOCYANIN complex were found on human fibroblasts in the range of concentration tested

Membrane Potential Test and ROS Evaluation Test

Protection of the human fibroblasts against oxidant agent (H_2O_2 : 1,5-3 mM)

Valorization of *Arthrospira platensis* in cosmesis

Naviglio instrument: Solid / liquid extraction

The generation of a negative pressure gradient between the outside and the inside of a solid matrix containing extractable material, followed by a sudden restoration of the initial equilibrium conditions, induces the forced extraction of the compounds in the solvent.



Spirulina



Extraction

Phycocyanin complex



Valorization of *Arthrospira platensis* in cosmesis and aquafeed industry

UNDER STUDIES :

- Extraction of phycocyanin from Spirulina grown on exhausted whey;
- Cytotoxicity and antioxidant power test on fibroblasts
- Comparison of the results with those obtained using phycocyanin complex grown on synthetic medium (Zarrouk)



POLITECNICO
MILANO 1863

Elena Ficara



Katia Parati



Valeria Mezzanotte



Aldo Tava



Federico
Castillo



Luciano
Foglio



Lorenzo
Proietti



Tiziana
Bongiorno



Katia
Parati



Camera di Commercio
Cremona

Ufficio Statistica e studi

Ilaria Casadei

Thanks for your attention!



**Provincia
di Cremona**

Barbara Pisaroni



ENTE REGIONALE PER I SERVIZI
ALL'AGRICOLTURA E ALLE FORESTE



Regione Lombardia

Donatella Melani