Valorization of microalgal extracts obtained by pulsed electric field in lactic acid fermentation

Aleksandra DjUKIĆ-VUKOVIĆ 1, Dragana MLADENOVIC 1, Christian GUSBETH2, Ljiljana MOJOVIĆ 1

1Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Serbia
2Karlsruhe Institute of Technology, Institute for Pulsed Power and Microwave Technology, Germany
Microalgae

Lipids
- Polyunsaturated fatty acids

**Chlorella vulgaris**

**Pigments**

**Proteins**

\[
\text{CO}_2 \quad \rightarrow \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{Biodiesel}
\]

1000 L photobioreactor, KIT

Not enough!

More products to make it economically viable!
Pulsed Electric Field - PEF treatment

✓ of adequate strength and duration
✓ on eukaryotic and prokaryotic cells
✓ causing increase in cell membrane permeability, if increase in transmembrane voltage surpasses certain value
Microalgae biomass

PEF4AlgBiotics

**Lactobacillus rhamnosus**

**Lactobacillus paracasei**

1. **Valorization of released components**
   - Nutrients recycling
   - Release of small water soluble components

2. **Valorization of proteins**
   - Water soluble proteins

3. **Biofuel**
   - Lipids

**Process scheme from IHM/KIT**

- Microalgae biomass
- PEF treatment
- Ethenolic extraction
- Anaerobic digestion
- Thermo-chemical conversion

- **Valorization of proteins**
  - Lactic acid + probiotics

**PEF4AlgBiotics** is a process scheme from IHM/KIT.
PEF obtained aqueous fraction of microalgae

*Lactobacillus rhamnosus*
*Lactobacillus paracasei*

Lactic acid

As chemical
As preservative
As monomer for poly(lactic) acid
Content of proteins in PEF obtained extracts

*C. vulgaris* suspension was concentrated (of approx. 0.8 g/l)

Centrifugation: 10000 g, 6 minutes and resuspended – adjusting conductivity

**PEF treatment**

- Continuous treatment,
- 10 kV/cm, 100 ns, 4.5 s⁻¹
- 20 h incubation, 23 °C

- 20-23% of proteins extracted
- Protein concentration: 0. 950 g/L (by Lowry)
- Role of C/N ratio for lactic acid fermentation

Supplementation with fermentable sugars

FERMENTATION
• Growth of *L. rhamnosus* and *L. paracasei* on extracts

Recommended number of viable cells in probiotics $10^6$-$10^9$ CFU/g
Antimicrobial activity of lactic acid bacteria against:

- *Salmonella enteritidis*
- *Staphylococcus aureus*
- *Shigella sonnei*

Resistance to gastrointestinal stress (low pH, pepsin, pancreatin, bile salts)

Antibiotic resistance to:

- *Gentamicin*
- *Kanamycin*
- *Steptomycin*
- *Vancomycin*

Susceptibility to:

- *Erytomycin*
- *Tetracycline*
- *Chloramphenicol*
- *Penicillin G*
- *Ampicillin*

Important probiotic characteristics of selected bacteria

Antioxidant activity of intact cells and intracellular cell free extracts

Survival rate of *L. rhamnosus* ATCC 49196 (%)

Gastric juice

Intestinal juice

Time (h)
Lactic acid fermentation

- Batch open fermentation – avoiding thermal treatment
- 37 °C, 24h, 100 rpm
- 5% (v/v) inoculum concentration
- ~30 g/L initial sugar concentration

<table>
<thead>
<tr>
<th>Strain</th>
<th>LA concentration</th>
<th>LA yield</th>
<th>LA productivity</th>
<th>Stereoselectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. rhamnosus</em></td>
<td>24.24 g/L</td>
<td>85%</td>
<td>1.01 g/Lh</td>
<td>&gt; 97% L-lactic acid</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>27.3 g/L</td>
<td>91%</td>
<td>1.14 g/Lh</td>
<td>&gt; 97% L-lactic acid</td>
</tr>
</tbody>
</table>

Not significant changes were observed in free amino nitrogen concentration!
Antioxidant activity

ABTS

Before fermentation

L. paracasei

L. rhamnosus

control

DPPH

Before fermentation

L. paracasei

L. rhamnosus

control
Revalorisation of PEF obtained extracts

*Chlorella vulgaris* – FDA approved for human nutrition

*L. rhamnosus*
*L. paracasei* – GRAS status

✓ Can be performed in open fermentation mode – avoiding thermal sterilisation or filtration

✓ But… changes in colour, oxidation, temperature, pH …

✓ After the LA fermentation high antioxidant activity in fermented extracts

---

**Fermented extracts**

- **Lactic acid extraction**
- **centrifugation**
- **Lyophilisation**
- **Food additive - probiotics**

- **liquid**
- **solids**
Thank you for your attention!

adjukic@tmf.bg.ac.rs

Department of Biochemical Engineering and Biotechnology
Faculty of Technology and Metallurgy
University of Belgrade
Serbia