



**2022 MELISSA CONFERENCE**  
8-9-10 NOVEMBER 2022

CREATING  
A CIRCULAR  
**FUTURE**

# Green algae for sustainable edible proteins production

**Prof. Matteo Ballottari**

**Federico Perozeni, Margherita Angelini, Eliana Gasparotto, Stefano Cazzaniga, Nicola Frison, Elisa Maricchiolo, Andrea Pompa**





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Solar Energy Bio-exploitation Lab



## *Green algae for sustainable edible proteins production*

**Prof. Matteo Ballottari**

[matteo.ballottari@univr.it](mailto:matteo.ballottari@univr.it)

# Bio-products from algae



Animal feed



Bio-fertilizer



Nutraceutical



Human nutrition



Medicine and drugs



Cosmetics



Biofuels

Several species approved by FDA and EFSA as novel food

*Spirulina*  
*Chlorella*  
*Euglena*  
*Dunaliella*  
*Tetraselmis*



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FROM THAT

...



TO THIS

- ✓ *Smaller volume*
- ✓ *Higher productivity*
- ✓ *Adaptation to harsh conditions*
- ✓ *Tailored production of bioproducts*
- ✓ *Circular approach to provide nutrients*



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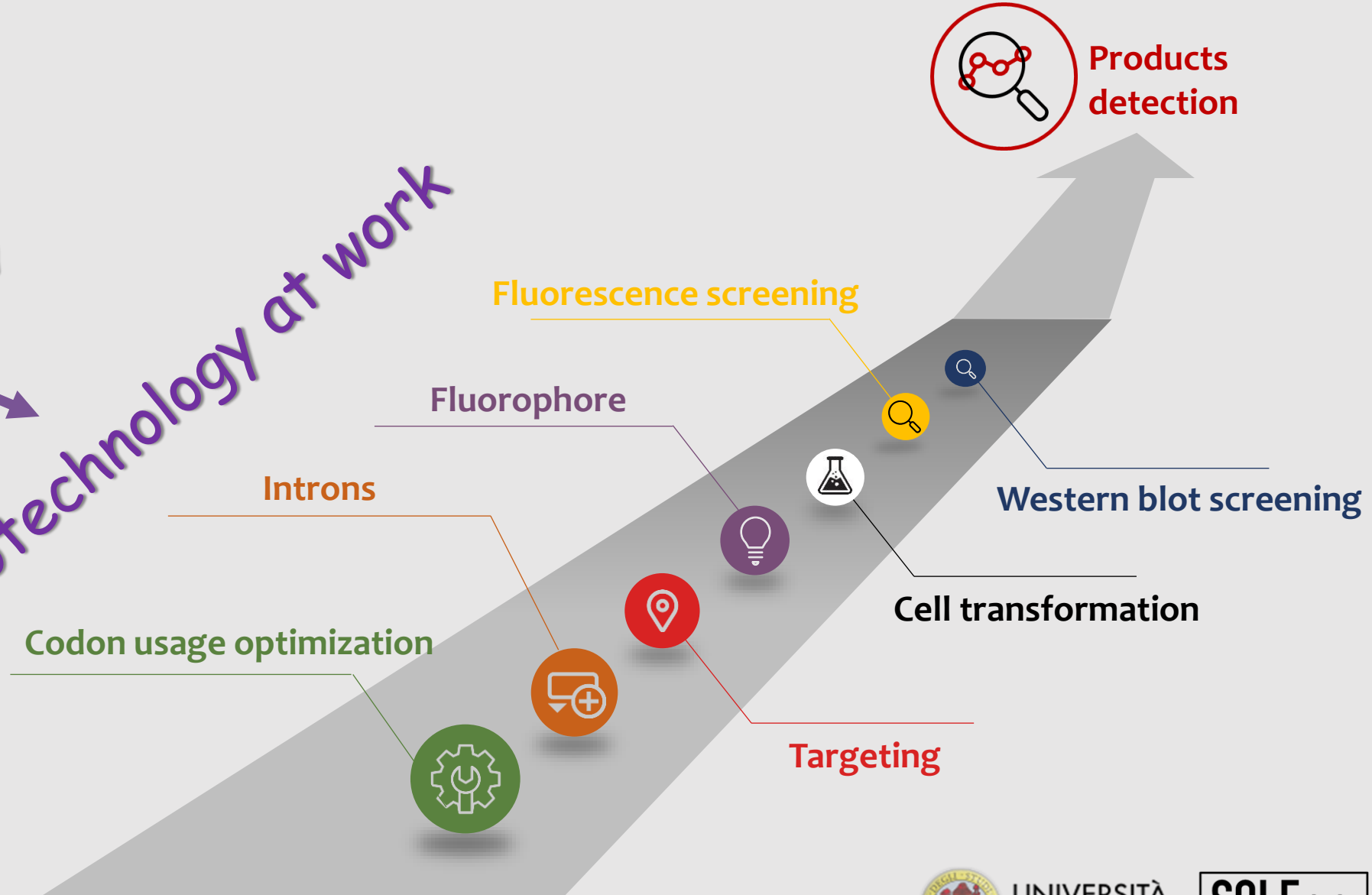
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# How we do it...

Species and strains selection

Biotechnology at work



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# THE CASE OF *CHLAMYDOMONAS* *REINHARDTII*:

- Model organisms for green algae
- Biotechnological tool available
- Strains with improved productivity already available
- Approved from FDA

## THE CASE OF *CHLAMYDOMONAS REINHARDTII*:

- Protein content: 40%
- Essential amino acid score (EAAS): 0.9
- Fatty acid predominantly unsaturated (42% ALA)
- Iron content ~1mg/g of dry weight
- Selenium content ~10 µg/g DW

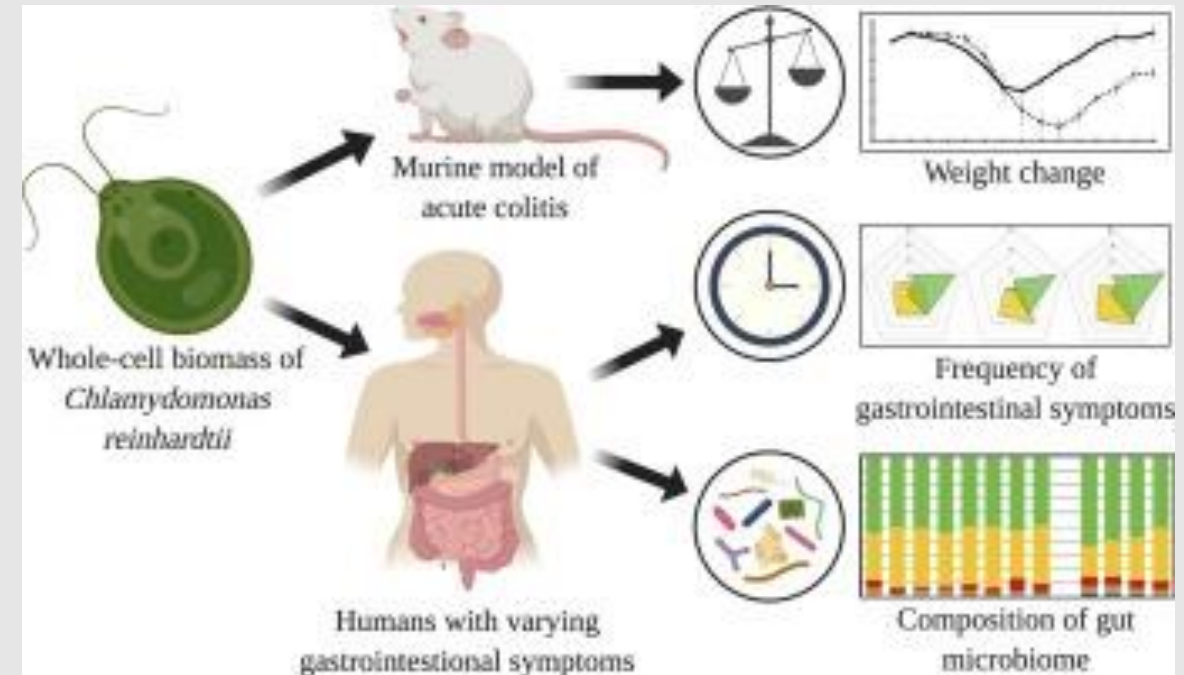




# THE CASE OF *CHLAMYDOMONAS REINHARDTII*:

## THE CASE OF *CHLAMYDOMONAS REINHARDTII*:

- *C. reinhardtii* significantly mitigated weight loss in a murine model of acute colitis.
- *C. reinhardtii* positively impacted gastrointestinal symptoms in humans.
- *C. reinhardtii* had no adverse effect on the microbial composition of participants





FROM THAT

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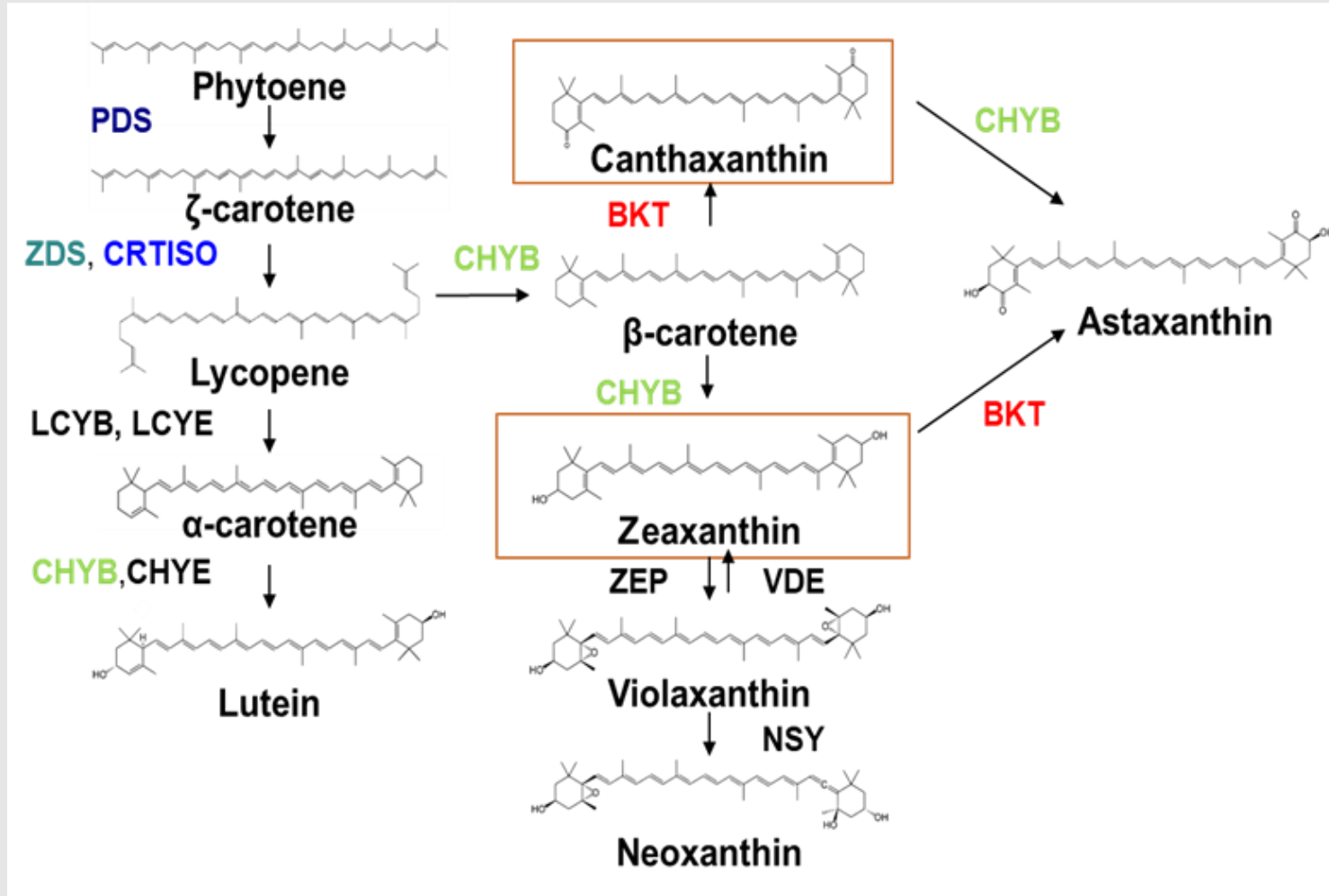
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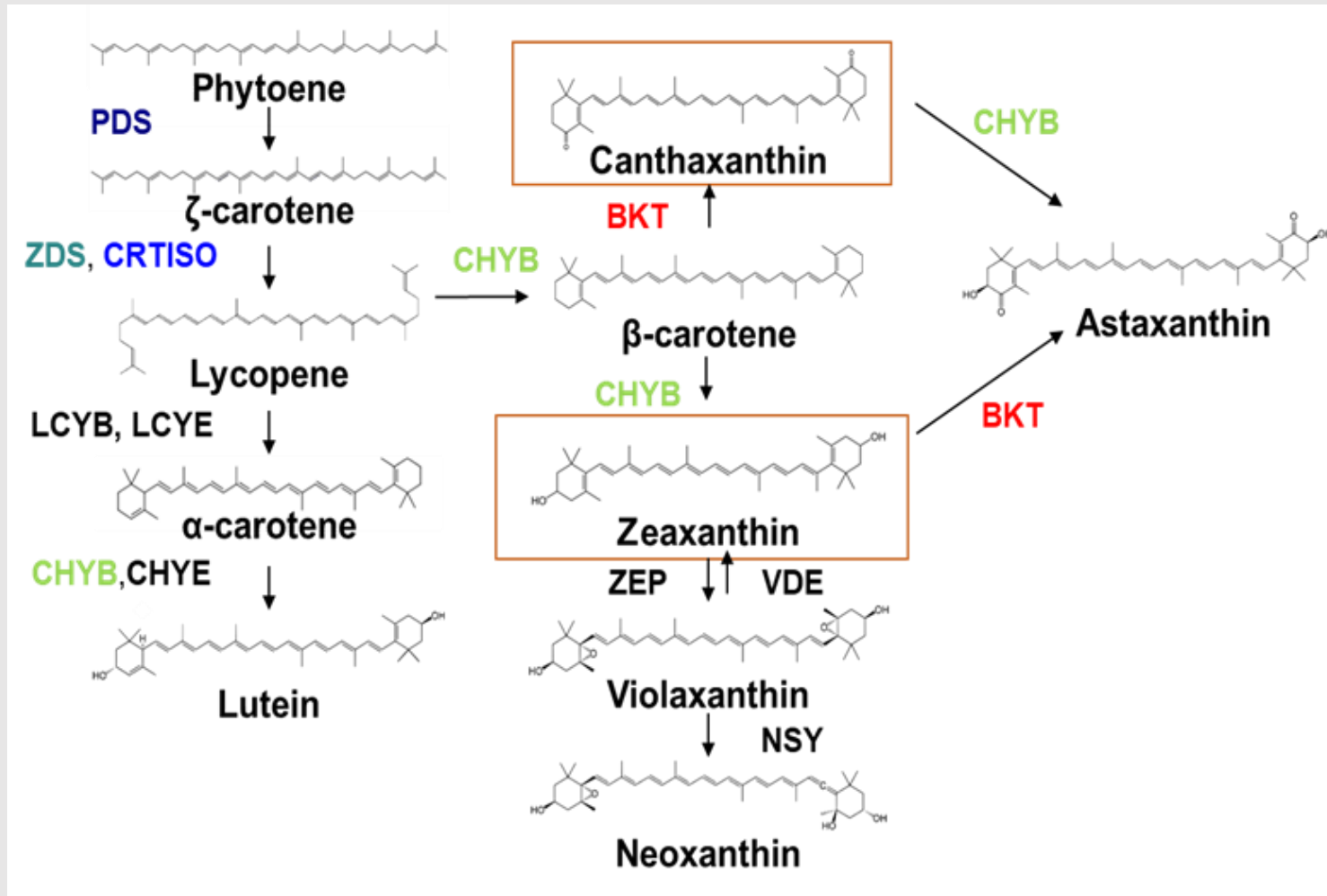
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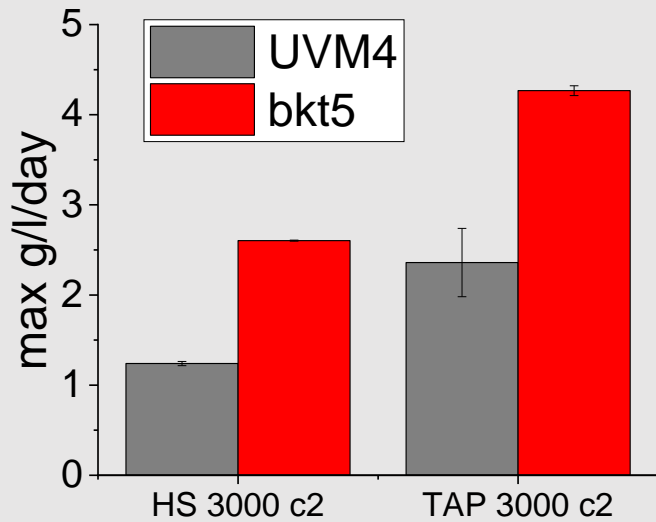
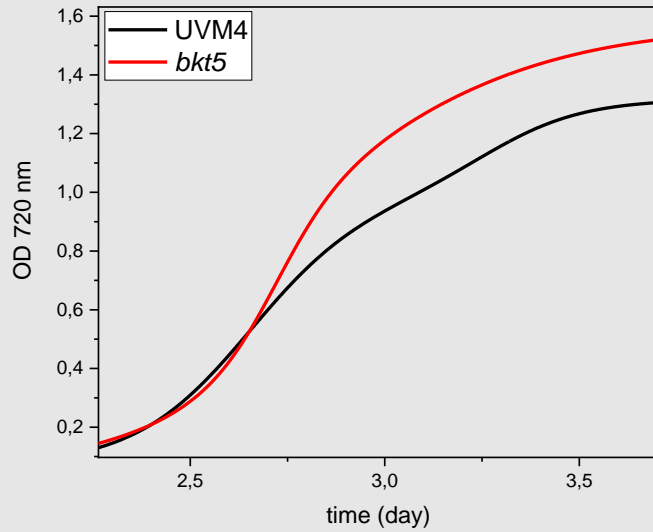
# Astaxanthin production in *Chlamydomonas reinhardtii* strongly increase biomass production and resistance to photooxidation



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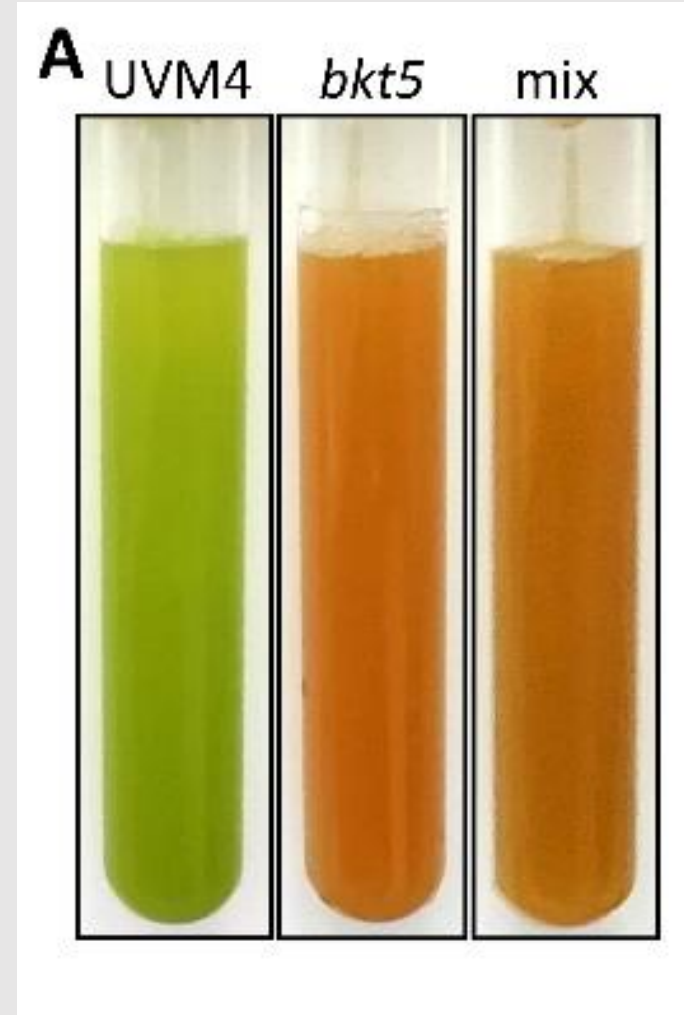


# Astaxanthin production in *Chlamydomonas reinhardtii* strongly increase biomass production and resistance to photooxidation



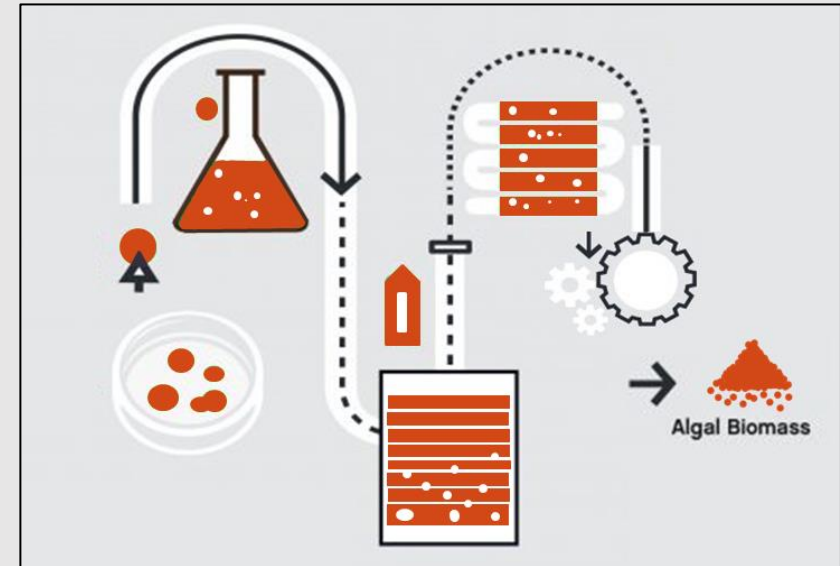
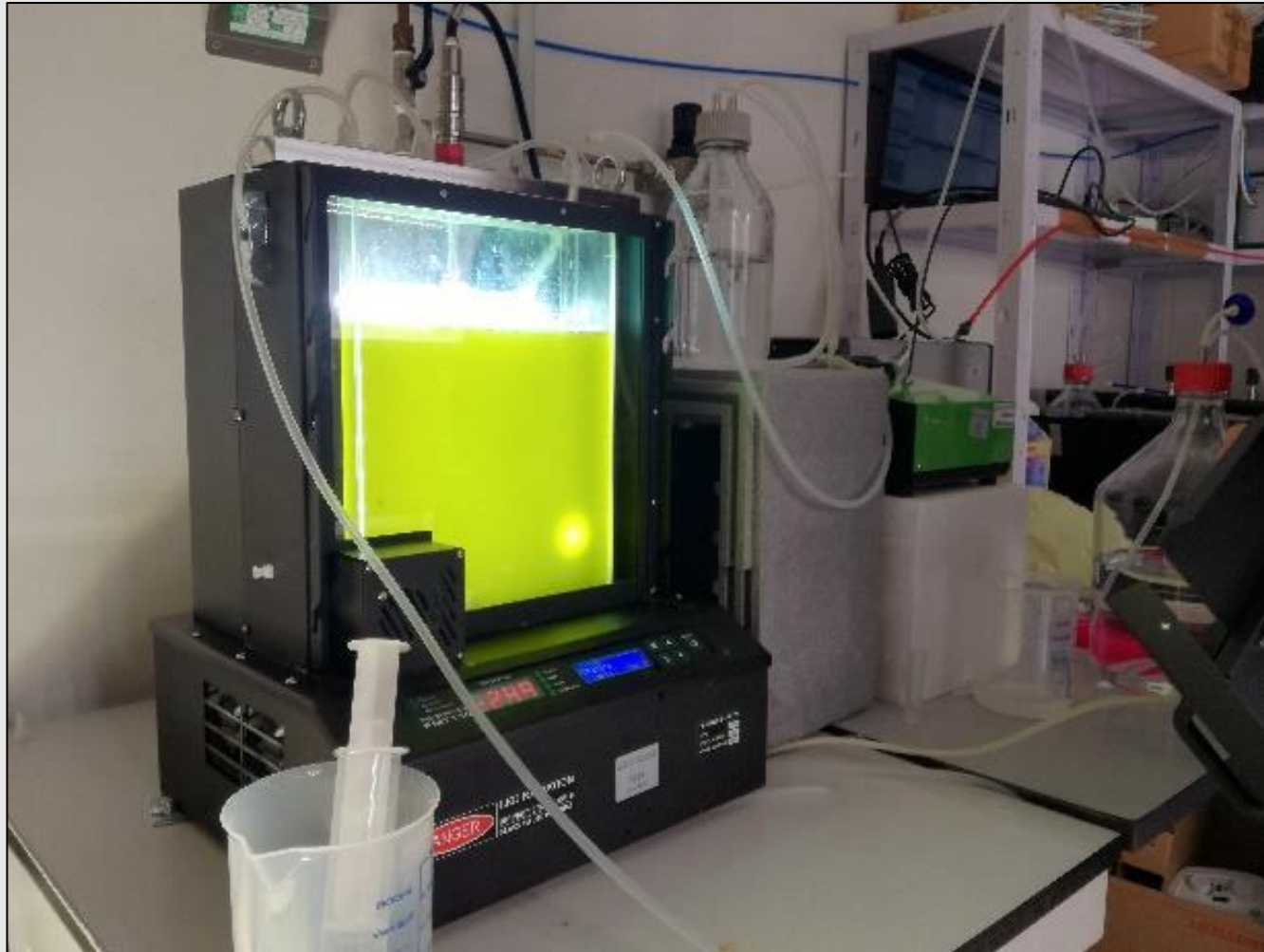
80%-110% increase in biomass productivity in high light conditions

40% of protein content



**Patent pending**

# Astaxanthin production in *Chlamydomonas reinhardtii* strongly increase biomass production and resistance to photooxidation



14-16 harvesting/month

# Astaxanthin production in *Chlamydomonas reinhardtii* strongly increase biomass production and resistance to photooxidation



Astaxanthin is employed as healthy food and many clinical studies reveal its possible role in human disease treatment



Eye Health



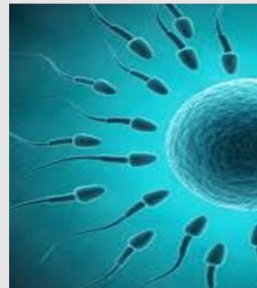
Cardiovascular support



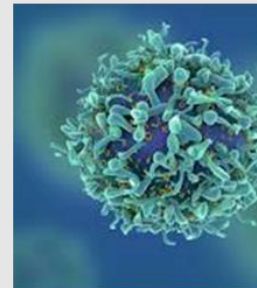
Immune system modulation



Brain Health



Male Fertility



Anti-Aging & Cellular health



Joint, Tendon and muscle support



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*How can we improve protein content and quality in *Chlamydomonas reinhardtii*?*



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# Why zeolin?

Phaseolin and zein are storage proteins produced in seeds



**Phaseolin:** water-soluble glycoprotein, high in Lys and Trp, accumulated in vesicles



**Zein:** insoluble, high in sulphured AA, forms protein bodies accumulated in the ER.

**Can we accumulate seed storage proteins in Chlamydomonas?**



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# Why zeolin?

Phaseolin and zein are storage proteins produced in seeds



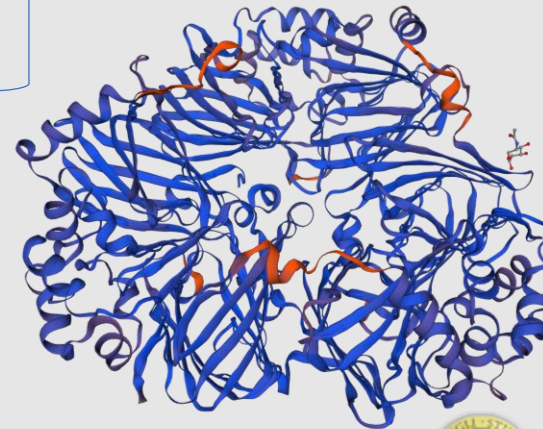
**Phaseolin:** water-soluble glycoprotein, high in Lys and Trp, accumulated in vesicles



**Zein:** insoluble, high in sulphured AA, forms protein bodies accumulated in the ER.

**Zeolin:** chimeric protein composed by phaseolin + 89aa  $\gamma$ -zein

Formed inclusion bodies and accumulating into ER



## Can we accumulate seed storage proteins in Chlamydomonas?

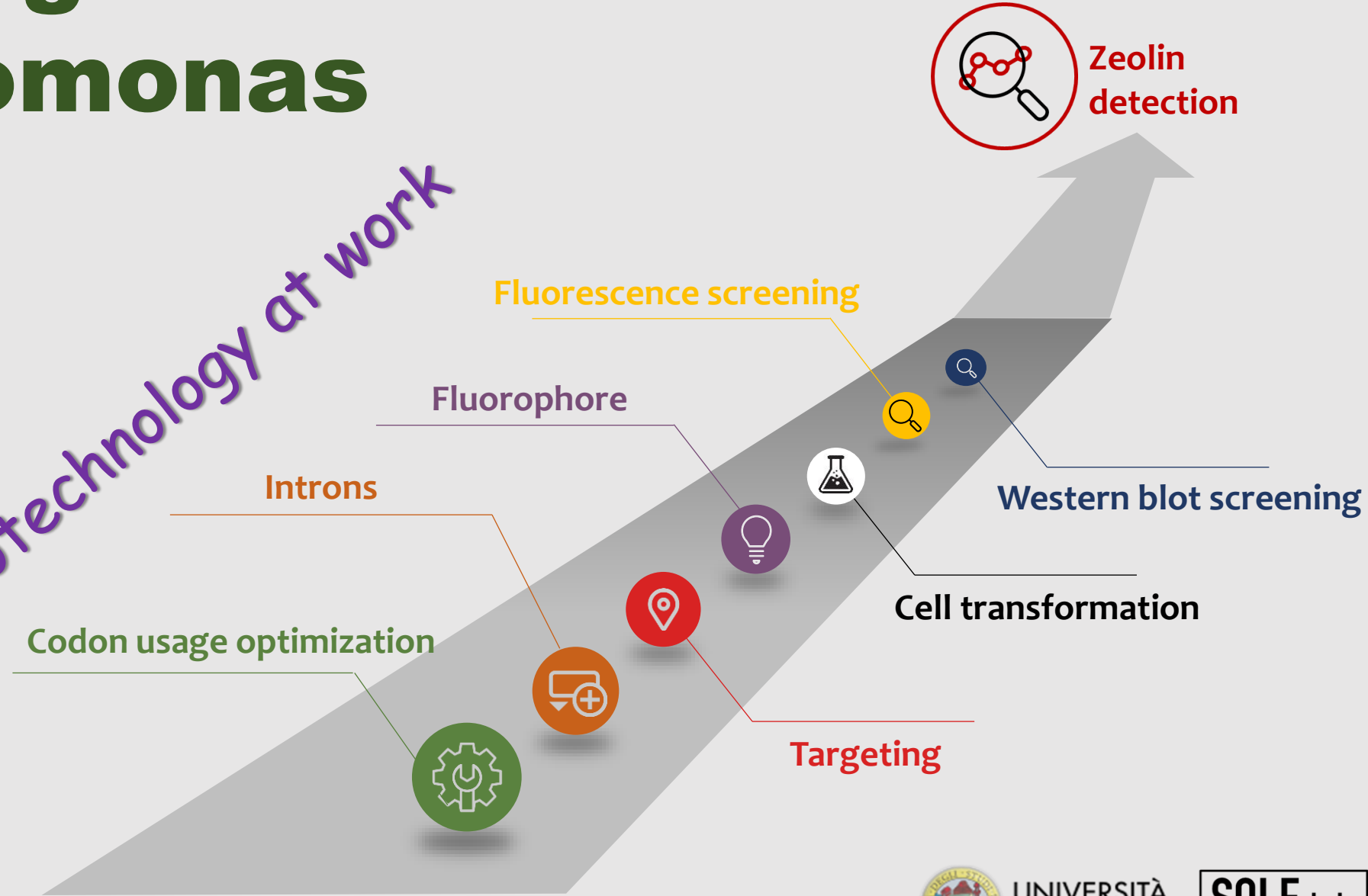


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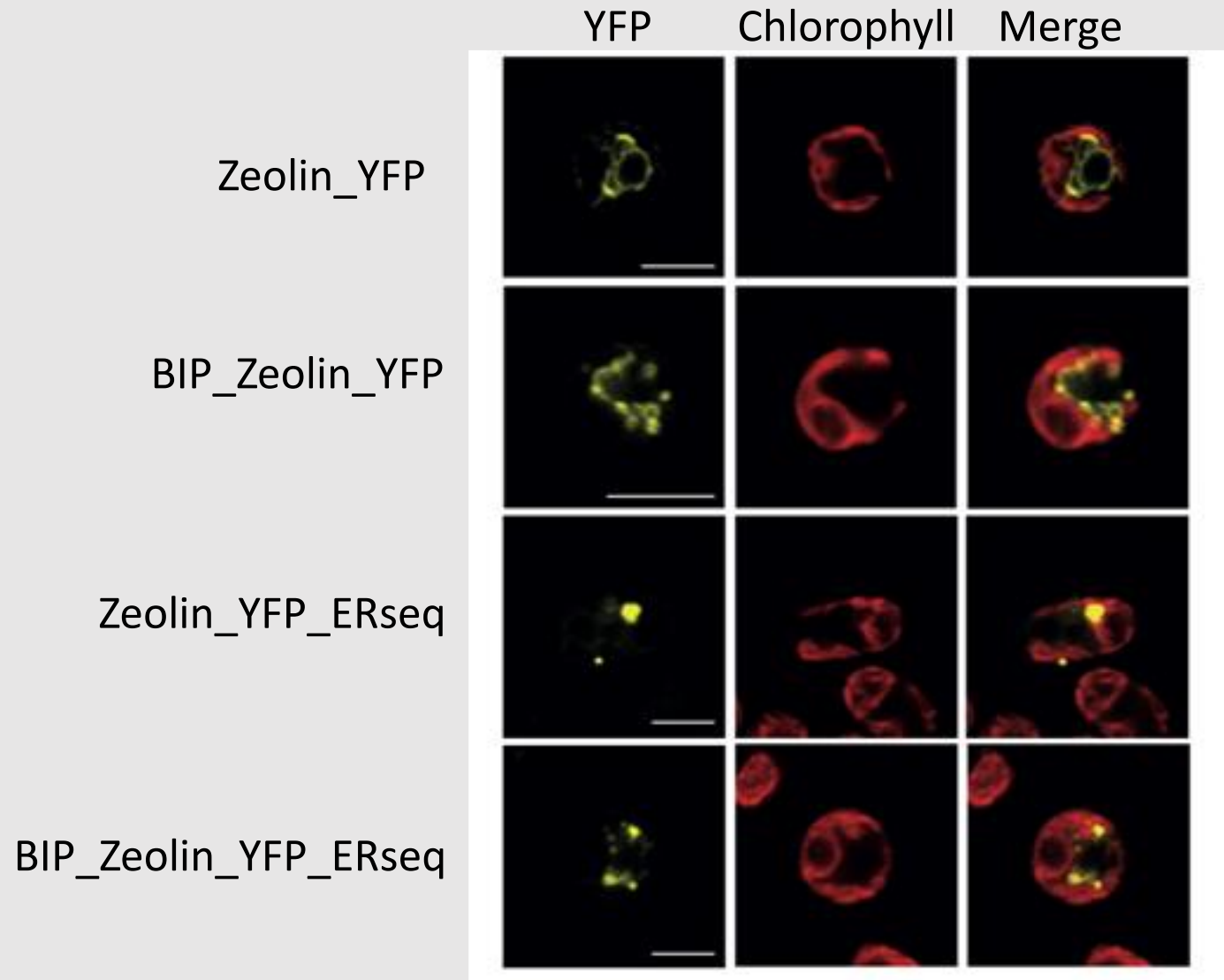
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# Expressing zeolin in Chlamydomonas

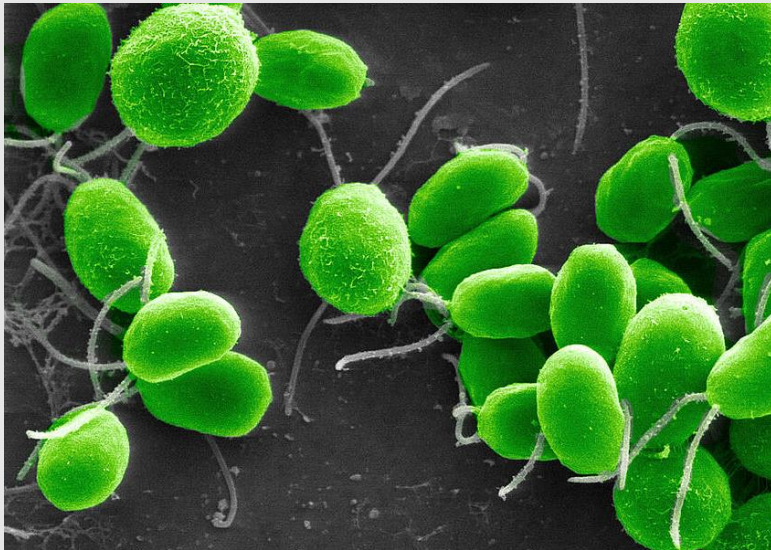
Biotechnology at work



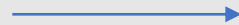
# Both inside and outside cells



# Towards the production of a superfood:



+BKT



+ZEOLIN



- ✓ **HIGH PRODUCTIVITY**
- ✓ **HIGH PROTEIN CONTENT**
- ✓ **COMPLETE AMINO ACID PROFILE**
- ✓ **HIGH ANTIOXIDANT PROPERTY**



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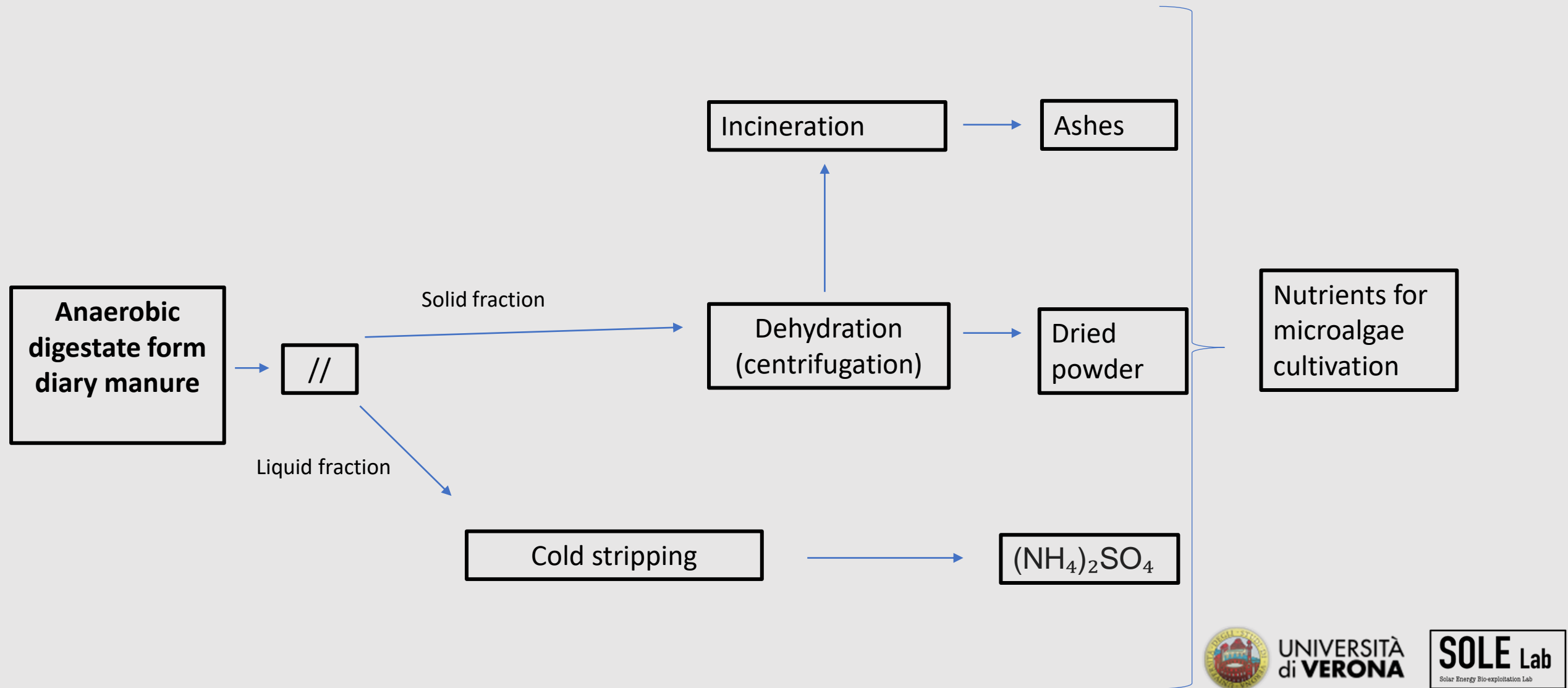
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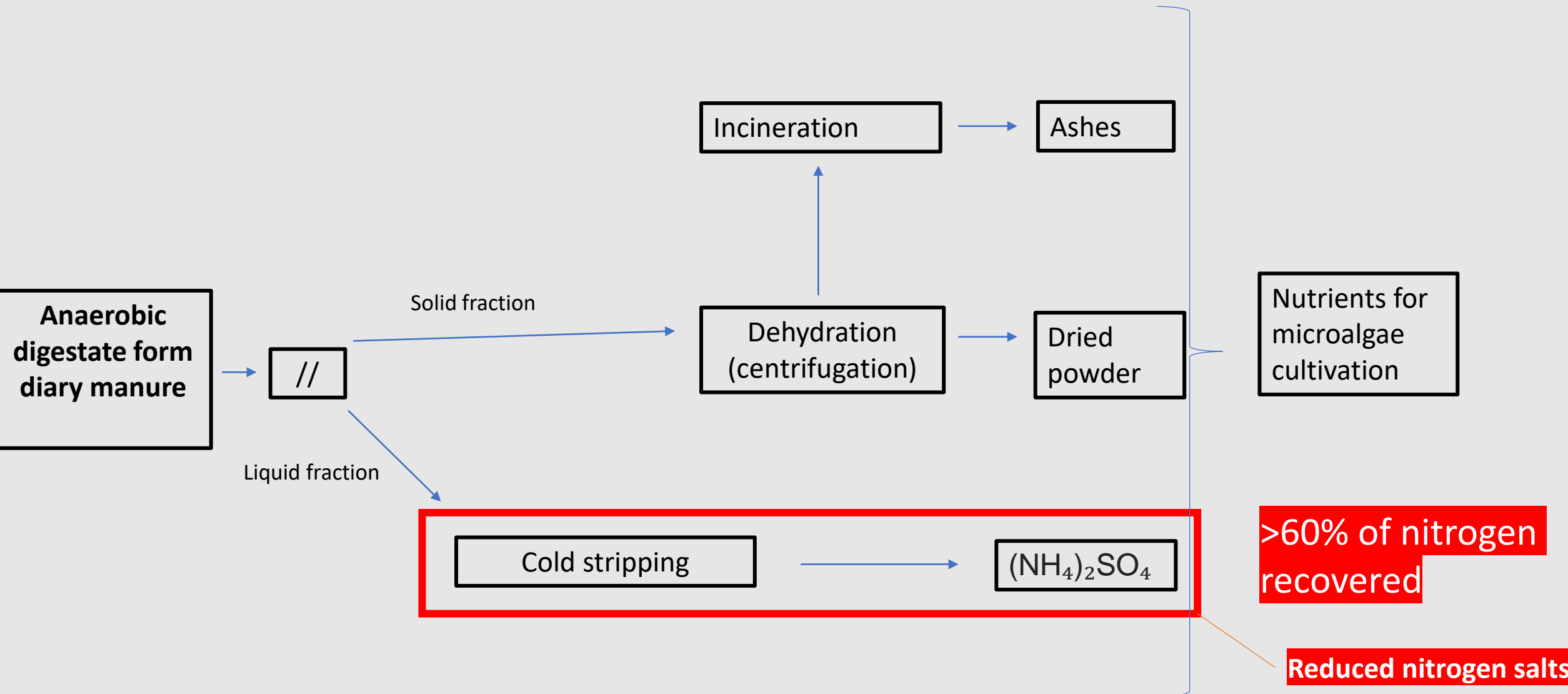
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# Nutrients from wastes:





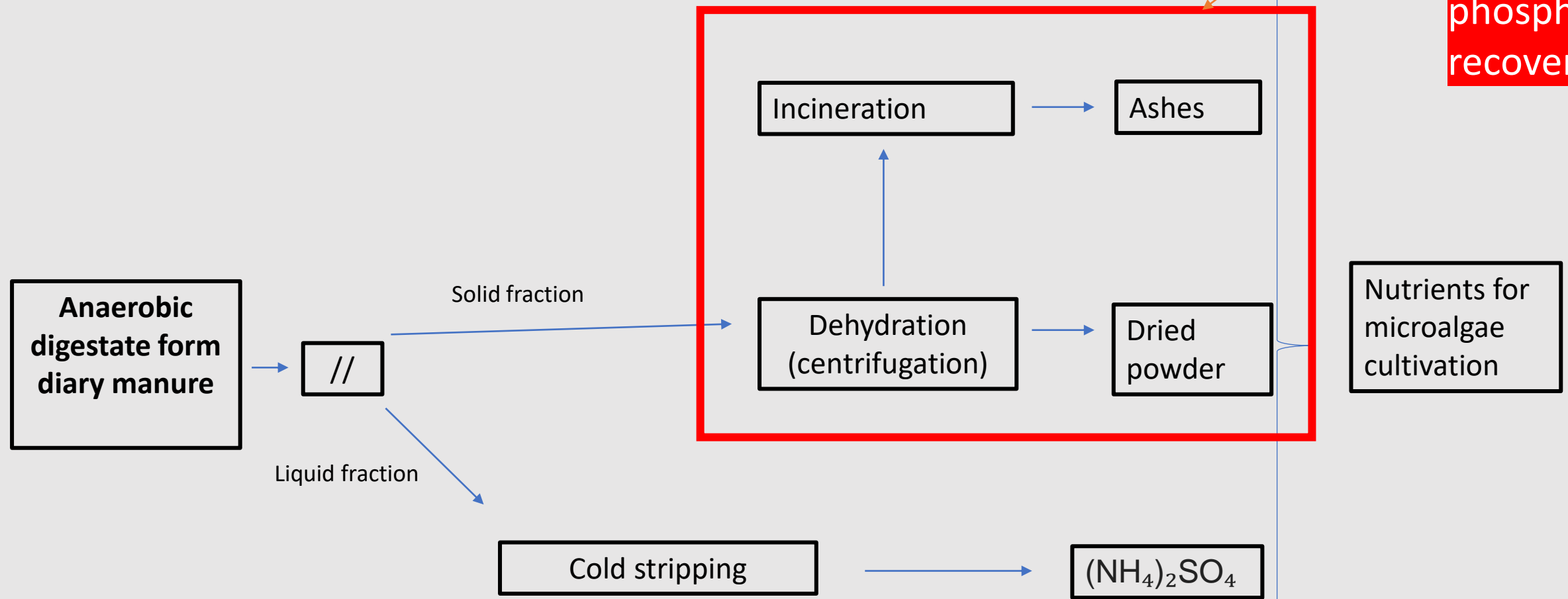
# Nutrients from wastes:



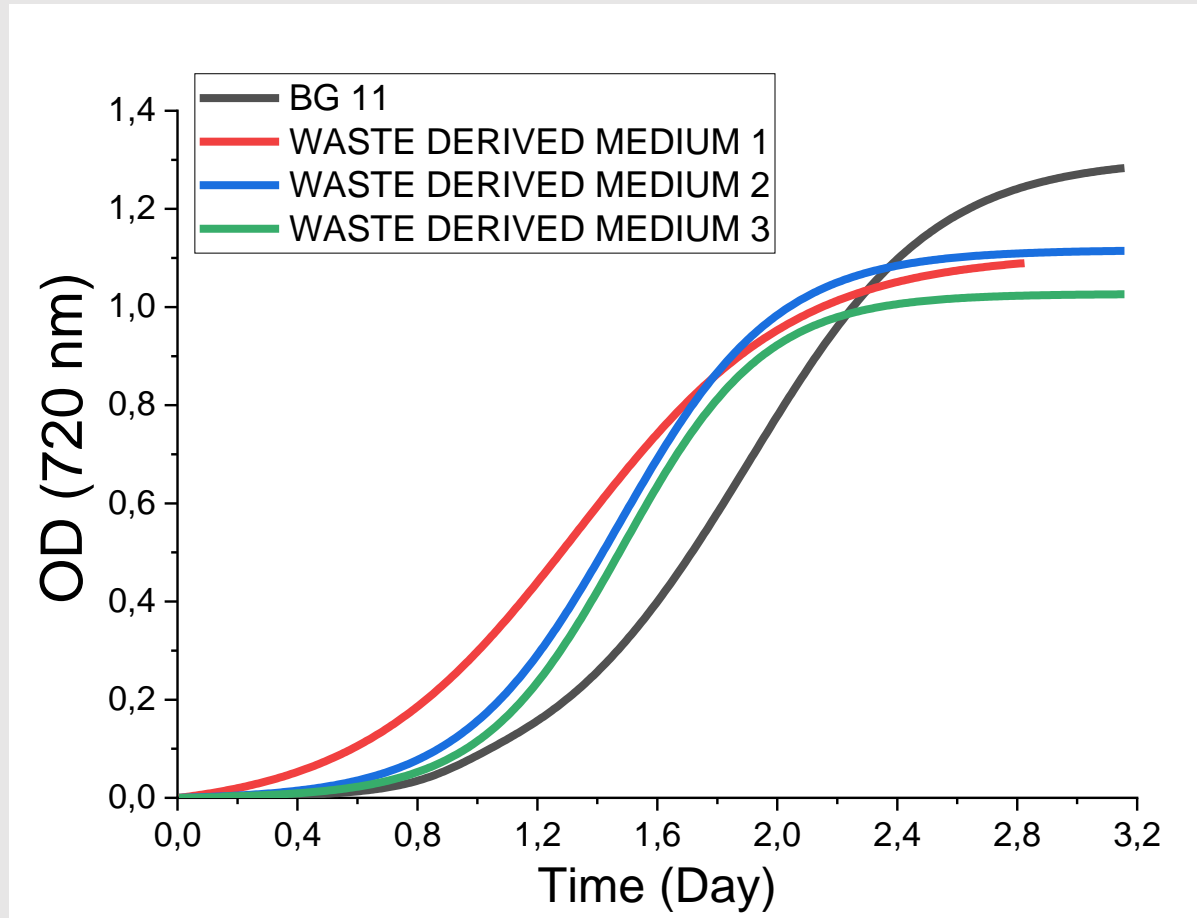
# Nutrients from wastes:

Phosphate salts

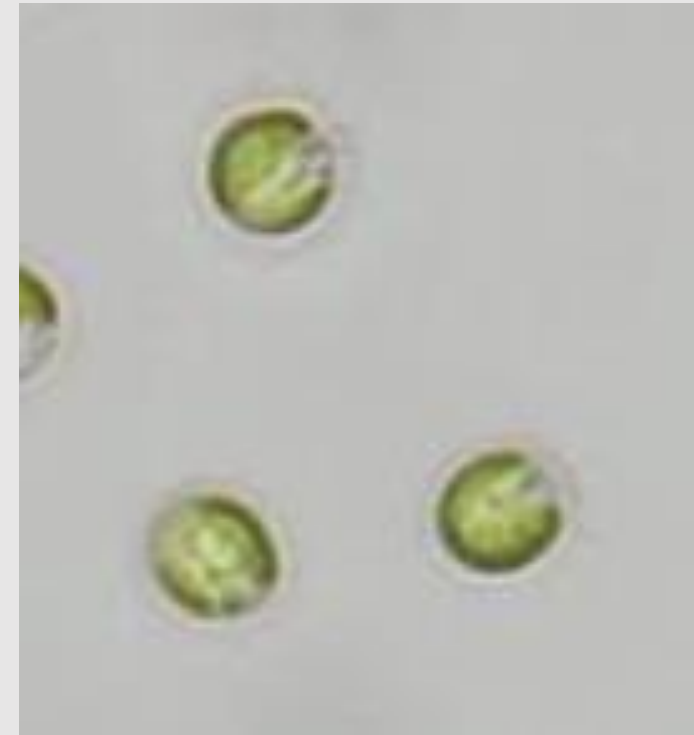
>80% of phosphates recovered



# Nutrients from wastes:



Growth medium for the green algae *Chlorella vulgaris* was prepared using only the substrates produced from digestate



***Chlorella vulgaris***  
**FDA AND EFSA approved**  
**>60% of proteins!**



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# Nutrients from wastes:



Growth medium for the green algae *Chlorella vulgaris* was prepared using only the substrates produced from digestate

Continuous cultivation, 1L volume, 0.2L/h flow:

0.17 g/L/day produced of edible biomass

*Stable nitrogen and phosphorous content in the medium: nutrient consumption and biomass production are balanced*



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# CONCLUSIONS



Improved biomass productivity, antioxidant properties, protein content and quality by genetic modification of the green algae *Chlamydomonas reinhardtii* (>4 gr/L/day in extremely high light)

Possibility to design circular approaches to valorize waste as nutrients for microalgae cultivation



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**THANK YOU.**

Prof. Matteo Ballottari  
University of Verona

[matteo.ballottari@univr.it](mailto:matteo.ballottari@univr.it)



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