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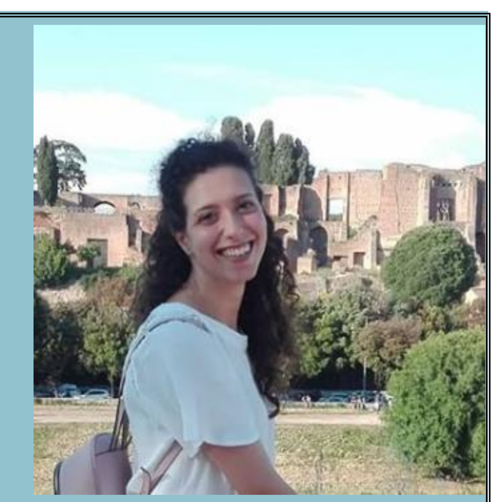
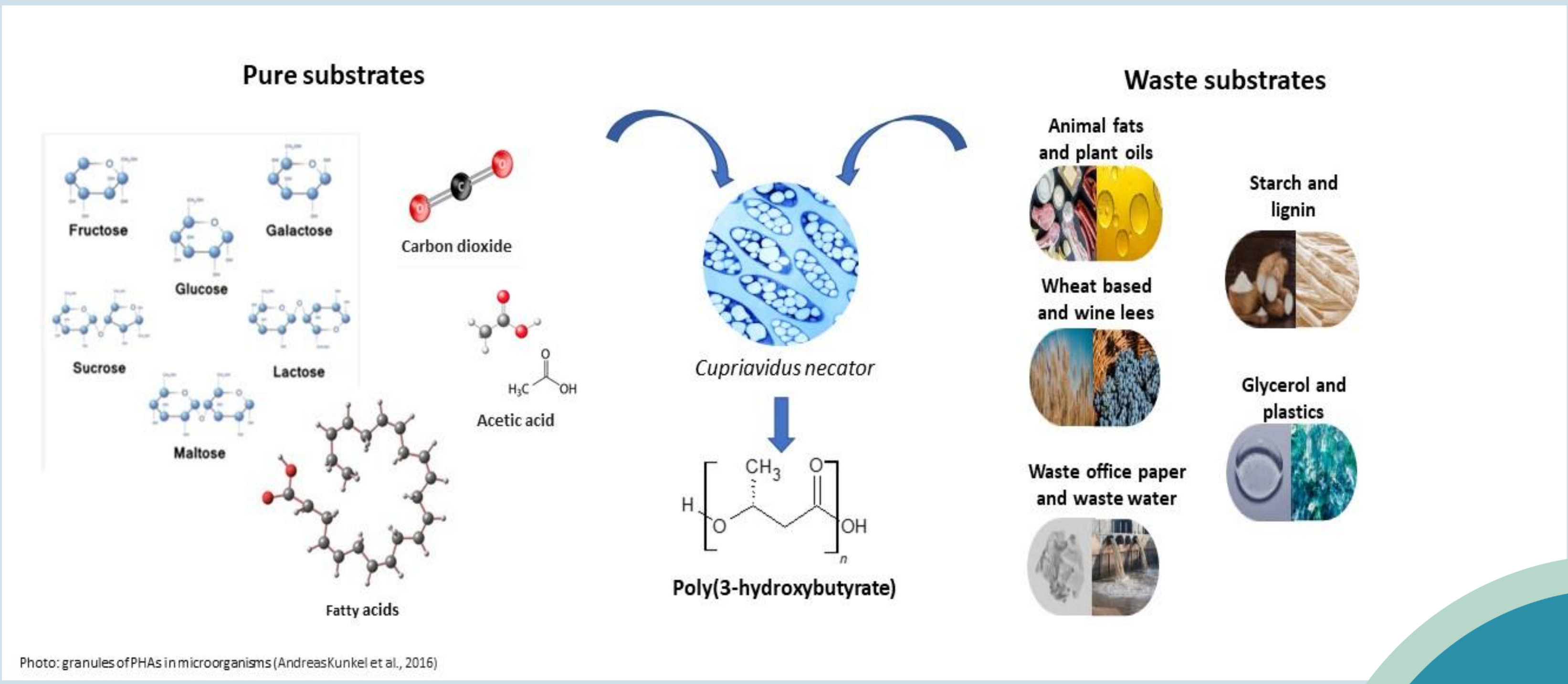
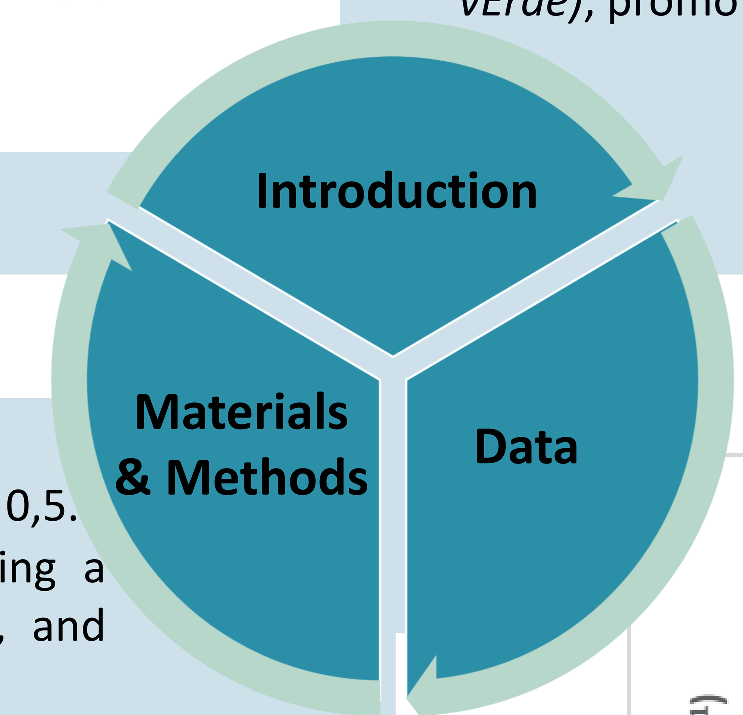


S. Bellini [a,b], T. Tommasi [a] and D. Fino [a,b]*

[a] Politecnico di Torino, Department of Applied Science and Technology, Torino, Piemonte, Italy
[b] Istituto Italiano di Tecnologia, Center for Sustainable Future Technologies, Torino, Piemonte, Italy
*corresponding author, *(presenting author e-mail: silvia.bellini@polito.it)*



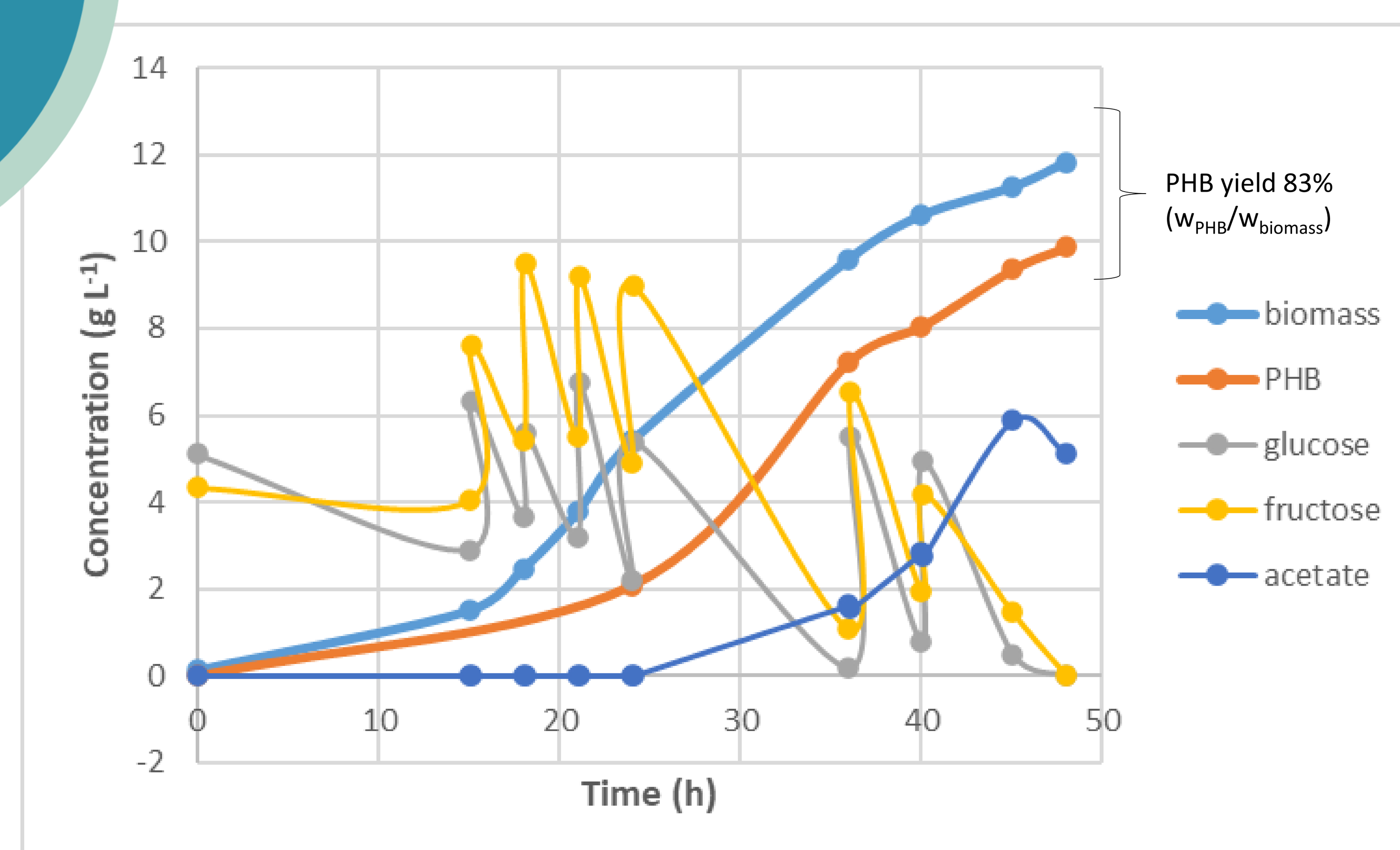
- ***Cupriavidus necator*** produces large amounts of the polyester poly (3-hydroxybutyric acid), or **PHB.**
- It is a **versatile bacterium:** it can grow both autotrophically and heterotrophically, and on a wide range of organic substrates, including **waste feedstocks** (Bellini et al., 2022).
- In this work, *C. necator* DSM 545 strain has been fermented to reach high yield of PHB by using **two waste substrates**.
- **Circular economy** approach for bacterial PHB biosynthesis
- This research is part of the ***PRIME* project** *(Processi e pRodotti Innovativi di chiMica vErde)*, promoted by Novamont® and Piedmont Region.

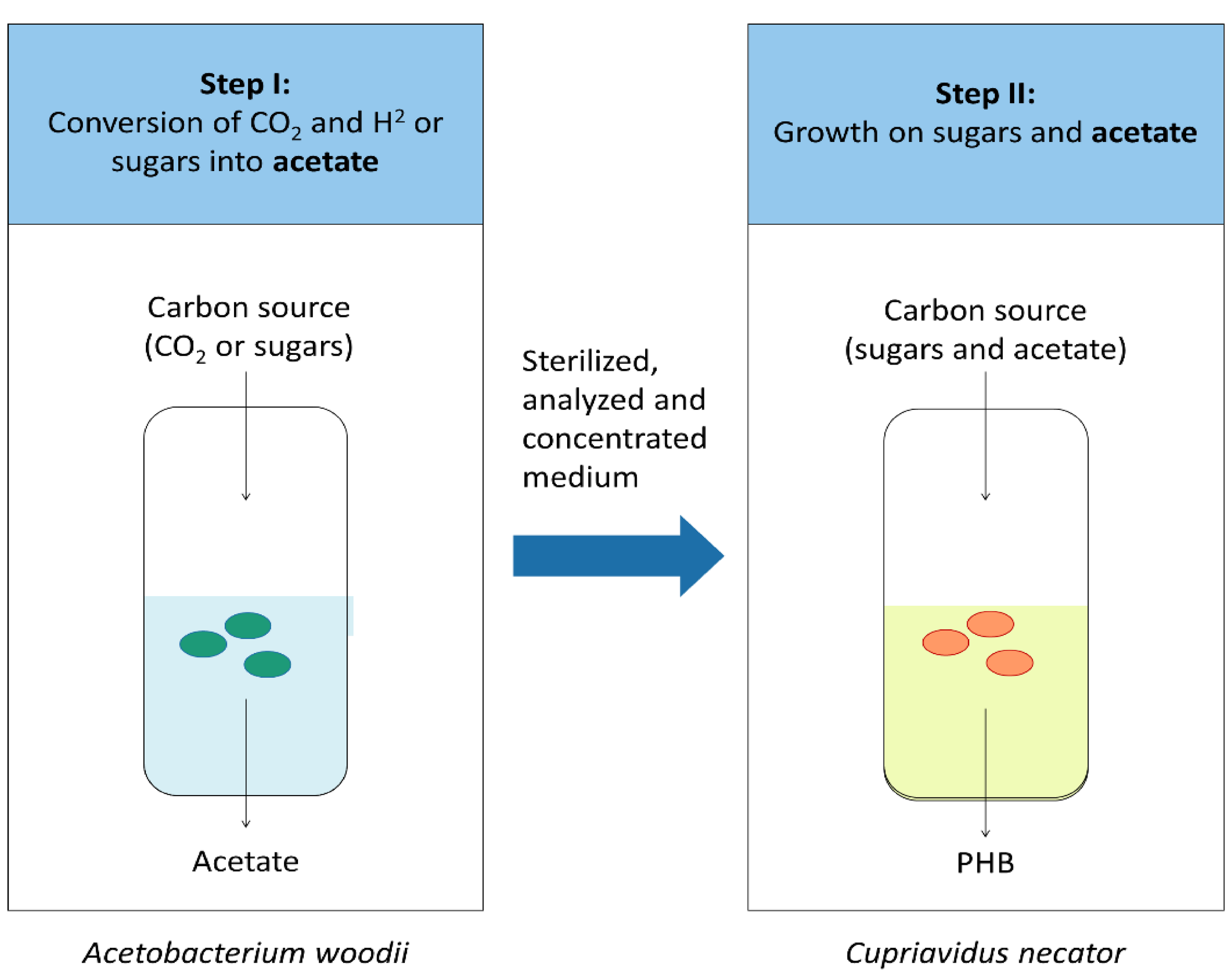


- 1L bioreactor Sartorius®, working volume 0,5 L, pH 6.8, 30°C, vvm 0,5.
- *C. necator* DSM 545 grown using a growth medium containing a **carbon source**, **phosphate**, **sulphate** and **magnesium** sources, and **metals** (Mozumder et al. 2014.
- **Biomass** sampled at 15, 24, 36, 40, 45 and 48 hours.
- **Two different carbon waste sources:**

1. a **syrup** (**glucose** and **fructose** in equal amount) from PRIME project supply chain (partner Sedamyl©)
2. the sterilized and concentrated waste medium of an acetogenic bacterium (*Acetobacterium woodii*) fermentation containing **acetate.**

- **Sugars and acetate** in the medium and **PHB concentration** (extracted by acid digestion using sulphuric acid 96%): analyzed at HPLC using a Resex18 column and a mobile phase of H_2SO_4 5 mM (flow rate of 0.7 mL/min).





Results & Discussion

- The graph shows the fermentation of *C. necator* DSM 545 performed through a **"two-step"** fermentation strategy, as shown in the scheme on the left.
- **Sedamyl© syrup** (containing glucose and fructose equal concentrations) has been furnished at different concentrations during the whole **fed-batch fermentation** ("spike feeding").
- After 24 hours, **spike feeding** of the acetogenic bacterium medium containing **acetate** (2 g L^{-1} each feed).
- The highest PHB concentration, almost **10 g L^{-1}**, has been reached after 48 hours of fermentation and the biomass reached about **12 g L^{-1}** at the same hour.
- **83% of PHB content,** $w_{PHB}/w_{biomass}$.
- PHB is mainly accumulated in *C. necator* under **unbalanced growth conditions**, e.g. when shortage of N and P occur.

Conclusions

- High concentration of PHB and biomass: **yield of 83% of biopolymer** (w/w), using valuable **waste substrates**, using a **circular economy** approach.
- *C. necator* DSM 545 easily convert glucose, fructose and acetate into PHB.
- **Optimization** of fermentation operative conditions:

i. A **three phases C/N ratio** fermentation approach (three different concentration of C and N and relative ratio) could be used to test PHB biosynthesis improvement (Garcia-gonzalez and Wever, 2018).
ii. Exponential feeding and an alkali-addition monitoring strategies (Mozumder et al., 2014)
iii. Utilization of **carbon dioxide waste** (e.g. industrial gas-off) as carbon source for acidogenic bacterium fermentation, to improve the whole **Life Cycle Assessment analysis** of the process.

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