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Production of polyhydroxyalkanoates from dark fermentative digestate of organic waste

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Abstract:
Polyhydroxyalkanoates (PHAs) are a potentially sustainable alternative to fossil-based polymers. We concentrated on producing PHA using mixed microbial cultures (MMC) fed with dark fermentative digestate derived from organic waste to find a less environmentally damaging and more cost-effective way than the current one. A 5L lab-scale reactor was built to conduct the microbial culture selection process to obtain a PHA-accumulating MMC, which will subsequently be employed as a source of biomass for PHA accumulation in one or more reactors. accumulation. The initial experiment was designed to examine the process, determine the ideal operating parameters, and establish a reactor monitoring strategy. The appropriate airflow was then established to define a feast-to-famine (F/F) ratio adequate for carrying out a proper selection process. Following that, the first accumulation experiments were performed, which revealed that the selected biomass could accumulate more than three times the quantity of PHA accumulated during the selection process. Based on these findings and the literature, a second experiment was carried out to investigate a complex digestate and higher organic loads, to apply more selection pressure and select a biomass with better accumulation performance. The analyses completed allowed us to discover probable preferential metabolic pathways for the various VFAs, and certain essential parameters for reactor control under these conditions (for example, HRT), and build the groundwork for a third trial, which has recently begun.

Keywords: Polyhydroxyalkanoates; PHA; Mixed microbial cultures; Circular bioeconomy; Organic Waste; Bioplastics

Introduction

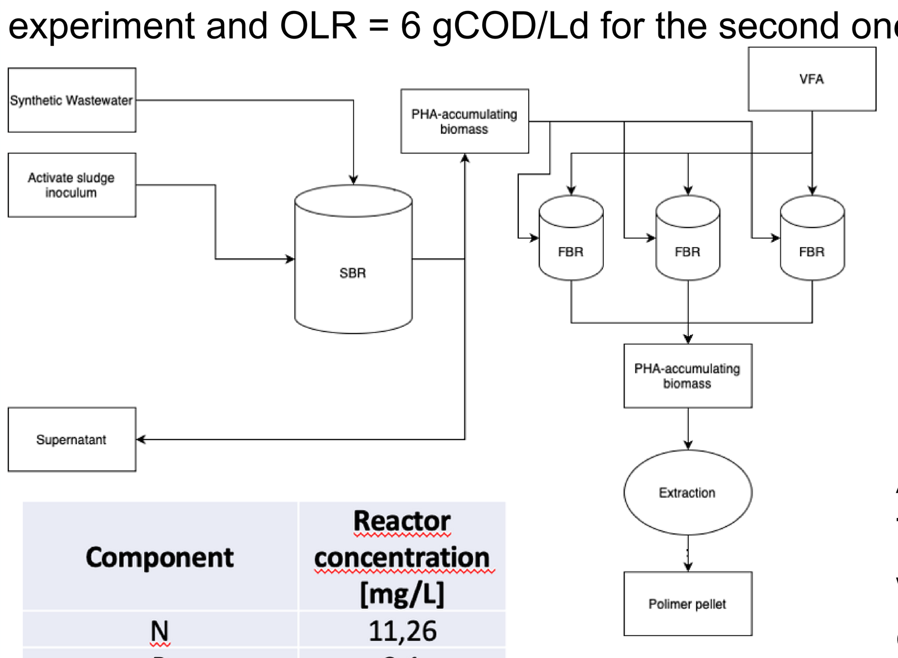
- **Plastics are not sustainable: have a fossil origin, are not easy to recycle and often characterized by a linear life cycle and are one of the most serious emerging pollutants in the environment (microplastics and nanoplastics) [1].**
- **Polyhydroxyalkanoates are a good solution: they are easily biodegradable and can be produced from renewable feedstock (e.g., organic waste) [2]. Though anaerobic digestion could be recycled leading to a circular life cycle [3].**
- **Through acidogenic fermentation, the first phase of anaerobic digestion, it is possible to produce volatile fatty acids (VFAs). These can be used by specific bacteria as a substrate for the production of PHA [3].**
- **Inside a Sequenced Batch Reactor (SBR), within which a feast-to-famine selective pressure is applied, it is possible to select from activated sludge biomass specialized in the production of PHA starting from a substrate rich in VFA [2].**

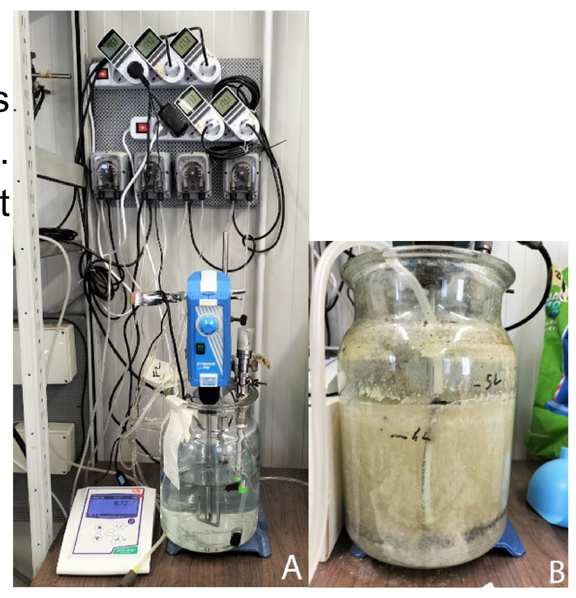
Aim of the study

- **Define the process that allows the selection of a pha-accumulating biomass and the production of PHA using VFA as a substrate.**
- **Identify the main operating parameters that influence the operation and performance of the reactor. Study elements and phases of the process that make PHAs environmentally and economically disadvantageous (e.g. aeration).**
- **Study the nutrient cycle associated with the selection process and the influence of aeration on nutrient assimilation and reactor performance.**
- **Study the degradation of complex feedstocks and the different metabolic patterns associated with the different VFA species, calculating kinetics, yields and other parameters as a function of the different composition of the feedstock.**

Materials and methods

Selection process
An SBR with a working volume of approximately 5 litres was developed, an HRT equal to 1 day and an SRT equal to 4 days. A 12 h cycle was set, with OLR = 0.6 gCOD/Ld in the first experiment and OLR = 6 gCOD/Ld for the second one.



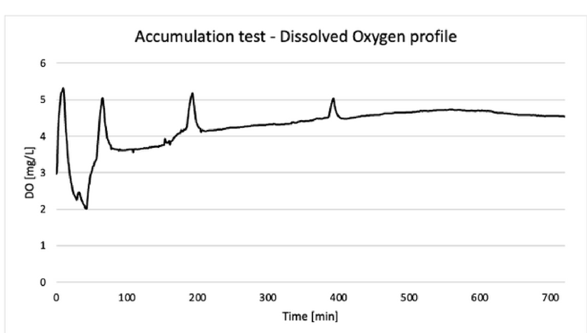


SBR reactor setup: stirrer, Do probe, ph meter, peristaltic pumps, aeration and timed sockets.

Component	Reactor concentration [mg/L]
N	11,26
P	2,1
SO_4^{2-}	5,7
K	1,9
Mg	0,9
Zn	1,0
Ca	0,5
Mn	0,3
Fe	0,053
Co	0,072
Cu	0,074
Mo	0,023
Cl	2,4
Acetate	240 (mgCOD/L)
Propionate	60 (mgCOD/L)

Feeding composition in the first experiment.

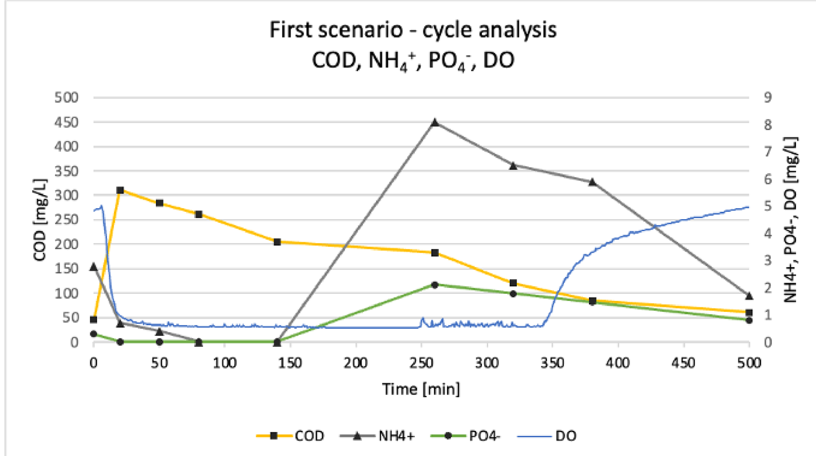
Accumulation process
Three Fed batch reactor (FBR) were set up, within which the accumulation tests were carried out according to the spiking strategy outlined by Papa et al [4].
Below is an example of the application of this strategy, aimed at maximizing the VFA conversion into PHA.



Results

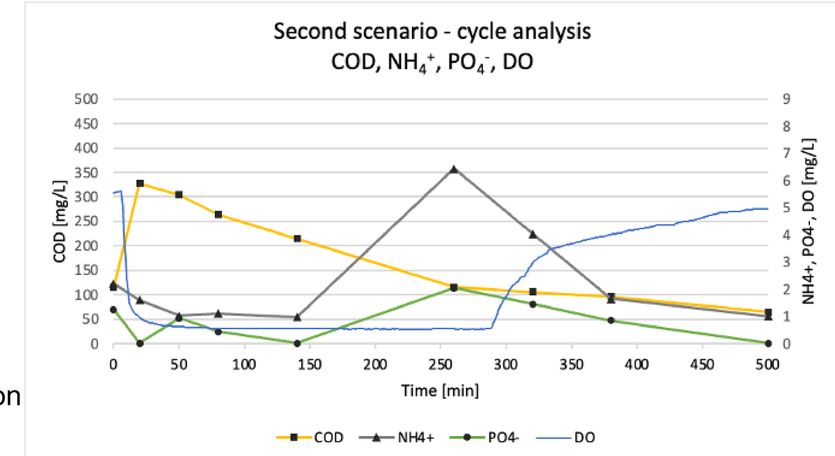
Parameter	Mean End of Famine	Standard deviation	Consumption	Standard deviation
COD	57.00	26.15	81%	9%
$N\text{-}NH_4^+$	2.53	0.38	79%	3%
$P\text{-}PO_4^-$	0.50	0.37	76%	17%

First scenario (VVM = 0.08)
- Biomass ⟹ TSS = 0.69 g/L and VSS = 0.39 g/L;
- Supernatants ⟹ TSS = 0.21 g/L and VSS = 0.19 g/L;
- Slow COD trend;
- DO rising where COD drops below 100 mg/L and F/F ≈ 0.82;
- Reduced COD consumption in presence of nutrients, probably due to the preference of internal stock for bacteria replication.



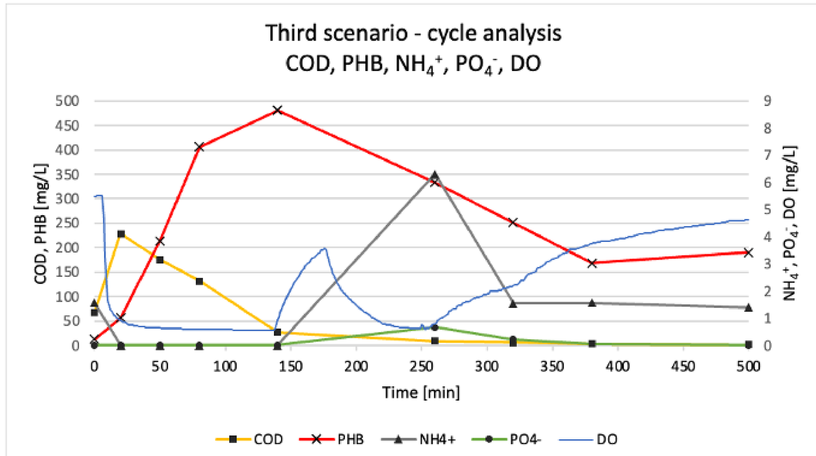
Parameter	Mean End of Famine	Standard deviation	Consumption	Standard deviation
COD	69.40	11.67	77%	4%
$N\text{-}NH_4^+$	1.70	0.34	85%	3%
$P\text{-}PO_4^-$	0.41	0.25	77%	14%

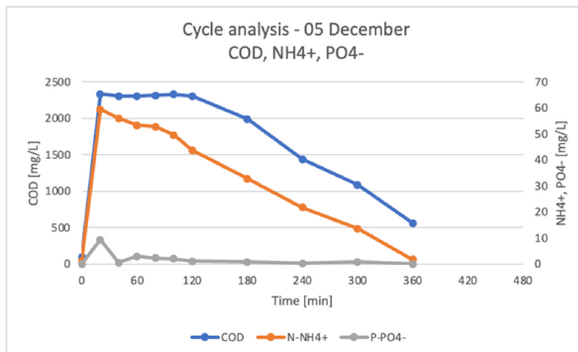
Second scenario (VVM = 0.16)
- Biomass ⟹ TSS = 0.75 g/L and VSS = 0.56 g/L;
- Supernatants ⟹ TSS = 0.16 g/L and VSS = 0.14 g/L;
- Similar trend for COD consumption;
- DO rising 100 min before ⟹ F/F ≈ 0.67;
- A more rapid decrease of ammonia indicates higher replication thanks to the higher accumulation.



Parameter	Mean End of Famine	Standard deviation	Consumption	Standard deviation
COD	60.00	6.78	80%	2%
$N\text{-}NH_4^+$	1.55	0.03	86%	0%
$P\text{-}PO_4^-$	0.02	0.04	99%	2%

Third scenario (VVM = 0.32)
- Biomass ⟹ TSS = 0.99 g/L and VSS = 0.48 g/L;
- Supernatants ⟹ TSS = 0.06 g/L and VSS = 0.04 g/L;
- DO rising after about 120 min ⟹ F/F ≈ 0.16;
- Rapid consumption of ammonia and phosphate ⟹ replication phase visible even though DO profile;
- PHB profile depicts the rapid conversion of VFA in PHB and the partial consumption for the replication.





Second sperimentation
VFA is not rapidly consumed by the biomass ⟹ F/F > 0.33
In the first phase, VFA is not degraded, but NH4+ and PO4- are consumed for replication.
The reactor progressively transformed into an accumulation, due to some technical problems, of a proliferation of filamentous substances. Furthermore, it was verified that butyric and valeric acids are preferentially converted into PHA compared to acetic and propionic acids.

Conclusions

- **Aeration is a crucial parameter for an effective selection, but large part of the famine phase is characterized by the absence of aerobic reactions. Microaeration or anoxic phase could be applied in this last part for less energy consumption.**
- **High OLR implies technical difficulties at our scale, due to the lack of efficient aerators but consistent volumes of biomass in the reactor. The reduction of the OLR could be the best solution for the study of complex digestate.**
- **In addition to DO, a key parameter for verifying metabolic activity, pH and redox potential could also represent useful parameters for monitoring and identifying the different processes that occur within the reactor.**

Future perspectives and activities

- **Study the evolution of the microbial composition inside the reactor, identifying the main associated bacterial species, those most involved in the accumulation process and strategies aimed at better selection.**
- **A third trial was started with OLR = 3 gCOD/L and the same composition as the previous one. The first objective, once the process has been stabilized, will be to study the different metabolic pattern to which the VFA are subjected during the selection and accumulation phase, and then to study how the variability of the input profile influences the various process parameters, the composition of the microbiological community and the characteristics of the final polymer.**

References:
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