

# DETERMINATION OF INTRACELLULAR POLYHYDROXYALKANOATES (PHA) IN AEROBIC PULSE FEEDING BATCH TESTS USING CHROMATOGRAPHY AND MICROSCOPY TECHNIQUES

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## 1. Introduction

Polyhydroxyalkanoates (PHA) are biodegradable polymers that could be synthesized using mixed microbial cultures and treating organic wastes in a three-stage process<sup>[1, 2]</sup>. The first stage consists of the production of volatile fatty acids (VFAs) through acidogenic fermentation, which is used as feedstock for the selection of PHA-storing microorganisms alternating the presence (feast phase) and absence (famine regime) of VFAs (second stage). The increasing of PHA of the sludge purge is usually performed maintaining feast conditions (third stage) under aerobic conditions.

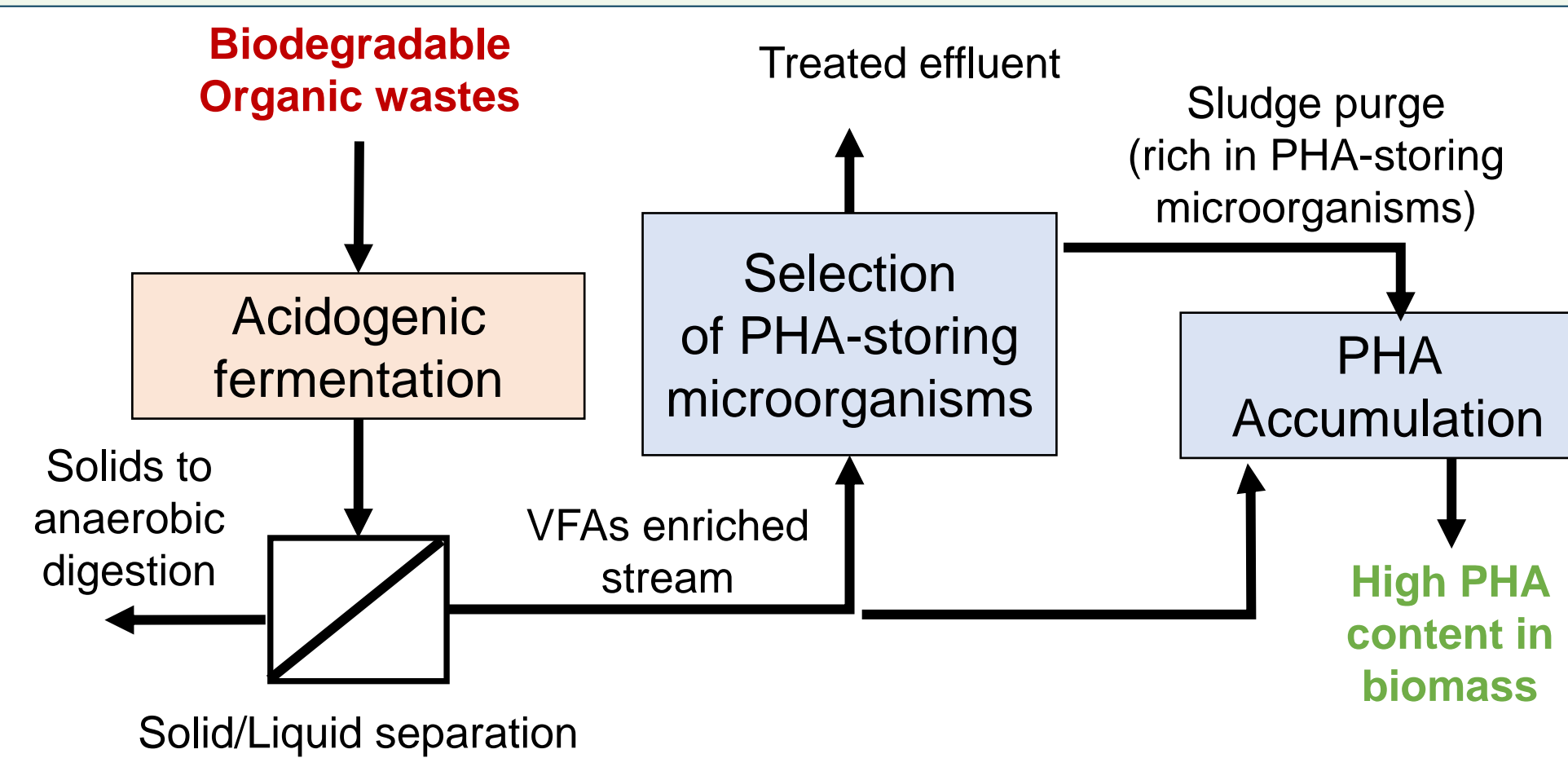


Figure 1. General scheme of the PHA production process using mixed microbial cultures.

## 2. Objective

To monitor and quantify, using analytical and microscopy techniques, the accumulation of PHA in a biomass purged from a selection reactor using synthetic VFAs with 3.5 g COD<sub>VFA</sub> L<sup>-1</sup> where feast/famine conditions were alternated.

## 3. Materials and methods

### 3.1 - Selection SBR

A sequencing batch reactor (SBR) of 3.75 L at 35 °C was used to select PHA accumulating microorganisms. The SBR was inoculated with 750 mL of previously acclimated biomass with 2.1 g VSS L<sup>-1</sup> and fed with synthetic wastewater.

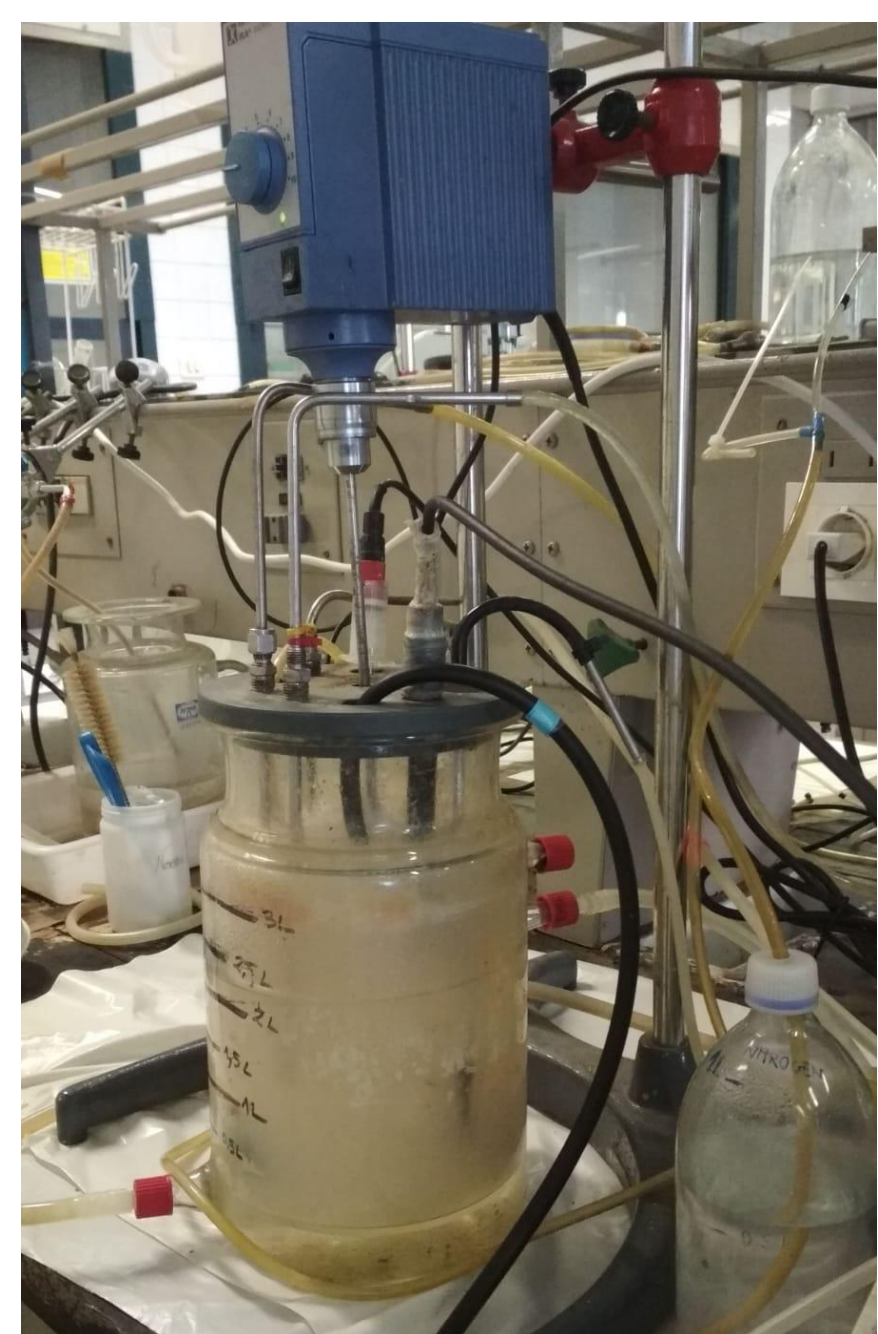


Table 1. Operating parameters of SBR.

Parameter	Units	Period I	Period II
Hydraulic retention time	days	1.1	1.1
Solid retention time	days	4.2	4.2
Feast/cycle ratio	%	29 ± 19	24 ± 6
Organic loading rate	g COD <sub>VFA</sub> L <sup>-1</sup> d <sup>-1</sup>	3.1	3.1
Nitrogen loading rate	mg NH <sub>4</sub> <sup>+</sup> -N L <sup>-1</sup> d <sup>-1</sup>	94	160
Ratio OLR/NLR	-	33.3	19.6
%PHA	% SS basis	18.6 ± 3.2	11.2 ± 3.6
%PHB	% PHA basis	88.7 ± 2.9	89.1 ± 3.0
VFA content (sludge purge)	mg COD L <sup>-1</sup>	1.5 ± 4.5	14.7 ± 2.0
Effluent's total NH <sub>4</sub> <sup>+</sup> -N	mg NH <sub>4</sub> <sup>+</sup> -N L <sup>-1</sup>	0.5 ± 0.7	13.5 ± 2.0

### 3.2 - Accumulation batch reactor

A reactor of 1.5 L at 35 °C was used for accumulation tests increasing the PHA content in the biomass with pulse feeding of VFA using the same synthetic VFA-rich wastewater without N presence.

Table 2. Operating conditions of accumulation tests.

Parameter	Units	Value
SBR purge (inoculum)	mL	450
Duration	h	7-8
Number of VFA <sub>feed</sub> pulses	pulses	5-6
Volume of VFA <sub>feed</sub> /pulse	mL	80



### 3.3 - PHA analysis

PHA content was characterized by using gas chromatography techniques (Shimadzu GC 2010 plus) and microscopically using Sudan Black and Nile Blue. Other analyses were performed according to the Standard Methods.

## 4. Results and discussion

### 4.1 - Selection SBR performance

The performance of a sSBR was monitored for 30 days and different periods could be established depending on the ratio of VFA and NH<sub>4</sub><sup>+</sup>-N uncoupled feeding during feast and famine periods, respectively (Table 1). The highest COD<sub>VFA</sub>/N ratio (Period I) led to a complete depletion of the nitrogen source during the famine step and yielded a higher PHA content in the purged biomass (Figure 1)

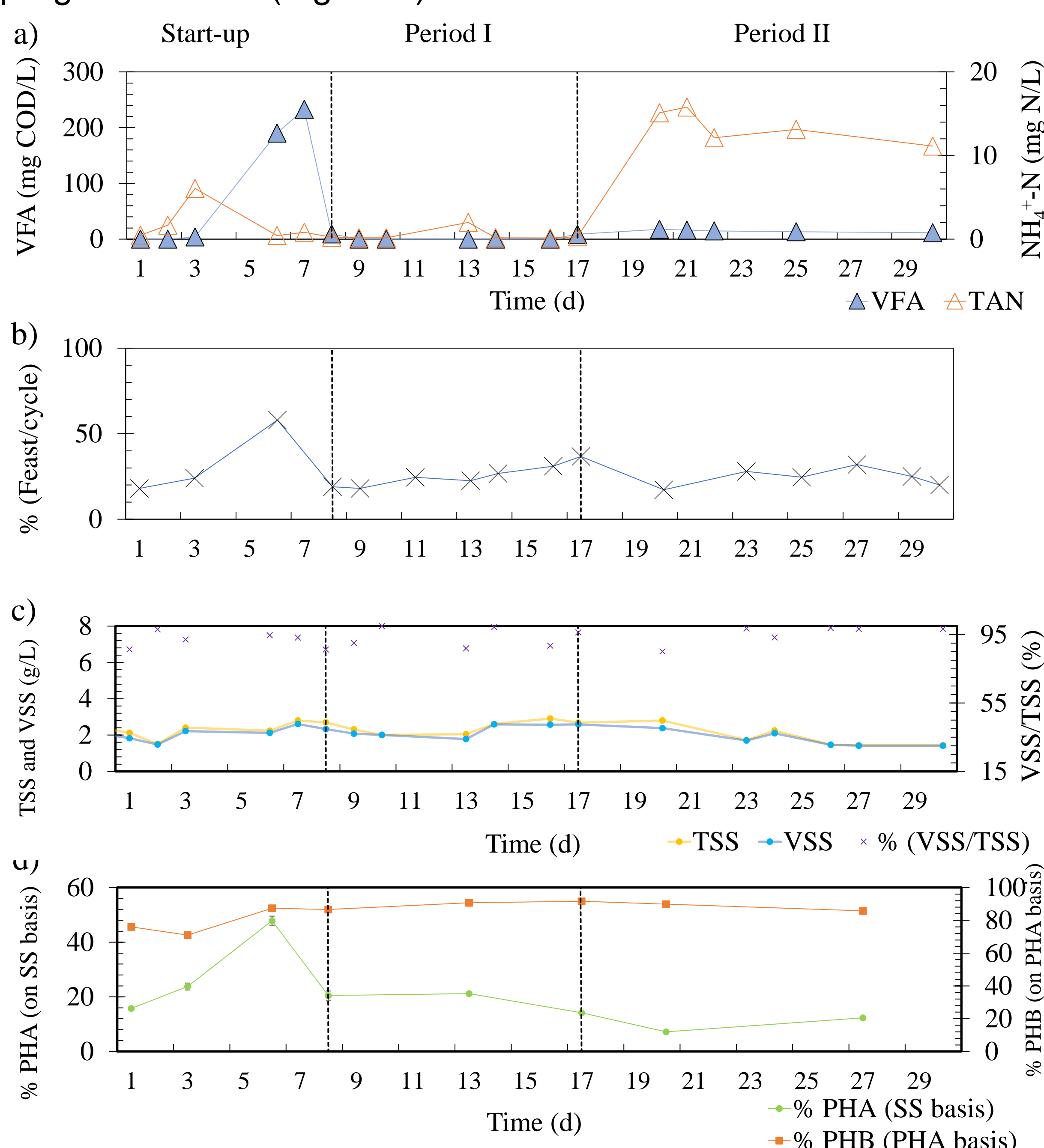
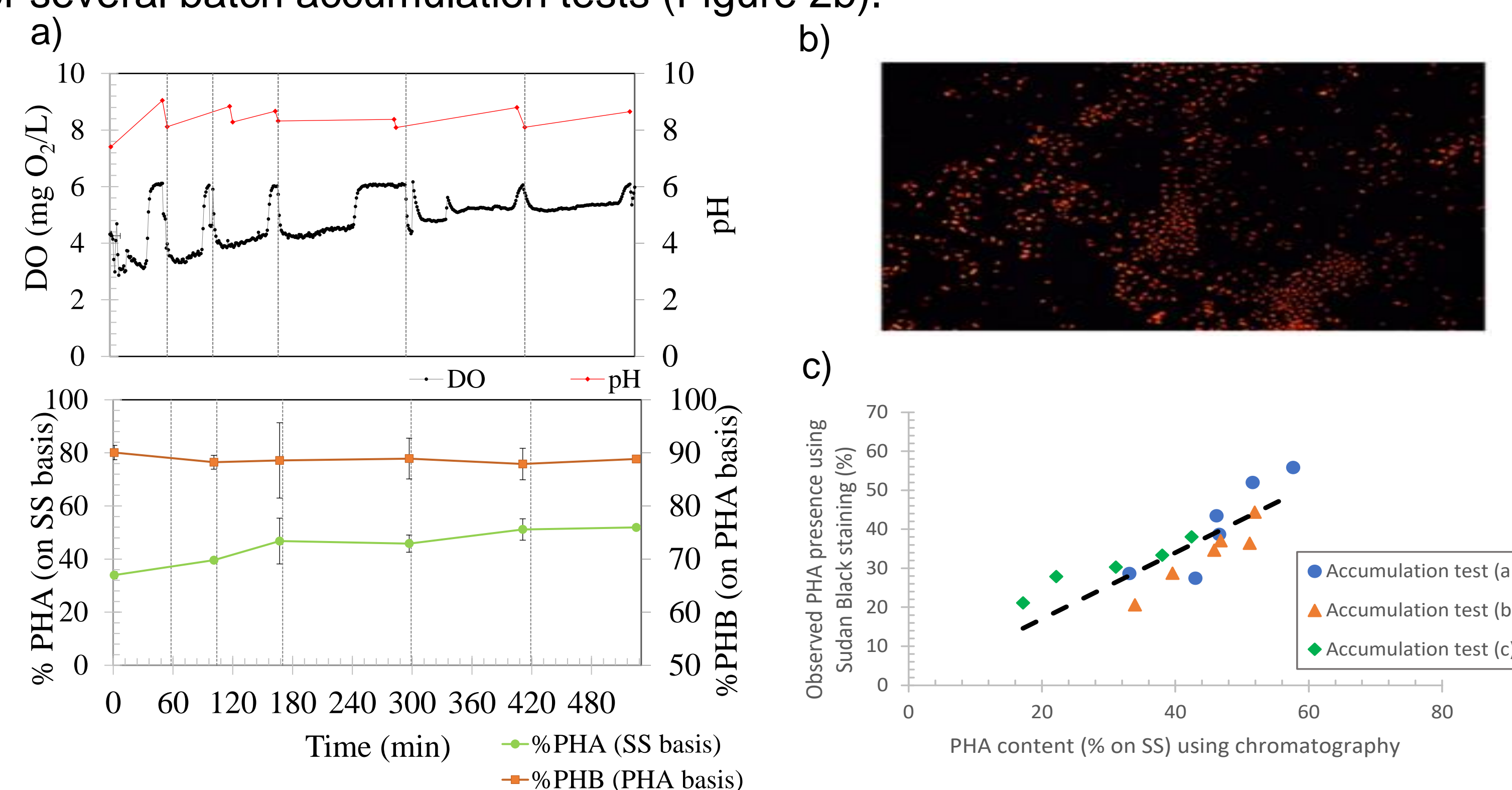


Figure 1. Evolution of VFA, NH<sub>4</sub><sup>+</sup>-N, feast/cycle time ratio, TSS, VSS, %PHA and %PHB during the operation of the selection SBR.

### 4.2 - PHA Accumulation tests using different PHA assessment techniques

After the accumulation tests, the PHA content reached values in the range of 42-52% PHA on SS basis after the 5<sup>th</sup> or 6<sup>th</sup> pulse, which is a higher PHA content than the rough threshold of 40% that is believed to make the recovery commercially viable<sup>[3]</sup>. Figure 2a shows the monitoring of a representative accumulation test using sludge purge from Period I when PHA was analysed using chromatography. Both the chromatography and microscopy (using Sudan Black and Nile Blue) techniques showed similar correlation in the PHA content for several batch accumulation tests (Figure 2b).



Figures 2. a) Accumulation test of a Period I of the selective reactor; b) Analyzed sample using Nile Blue with fluorescence; c) Correlation between the techniques used.

## 5. Conclusions

- ✓ A PHA content of 42-52% on SS basis was obtained in a sludge purge previously enriched in PHA-storing biomass using synthetic wastewater
- ✓ The monitoring of the PHA content could be performed using chromatography, but also using microscopic detection of PHA with Sudan Black and/or Nile Blue staining.

## Acknowledgement

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## 6. References

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