tutor/s

Dr. Joan Dosta Parras
Carme Vidal Antich
Departament d'Enginyeria Química i Química Analítica

Grau en Enginyeria Química

Treball Final de Grau

Study of polyhydroxyalkanoates production using acetic, propionic and butyric acids

Miriam Sánchez Fitschen

September 2020

UNIVERSITAT DE BARCELONA

"Siempre he creído que no importa cuántos disparos falle... Acertaré en el siguiente."

Jonathan Swift

D'entrada, vull agrair al meu tutor, Dr. Joan Dosta Parras, per la paciència i la dedicació que m'ha mostrat.

Agrair també a la meva co-tutora, Carme Vidal Antich, per la seva constant implicació, paciència i consells.

A més agrair als meus companys del laboratori pel suport moral i ajuda que m'han aportat en les hores de treball.

Tot seguit donar les gracies als meus companys d'universitat i amics fora d'ella per donar-me energia en els mals moments, incloent als que ja no formen part de la meva vida.

Per acabar, a tota la meva família que sempre em brida suport incondicional.

CONTENTS

SUMMARY I

RESUM III

1. INTRODUCTION 5

1.1. Petroleum-based and bio-based polymers 5

1.2. Polyhydroxyalkanoates (PHA) 6
1.2.1. Definition and applications 6
1.2.2. Production process using pure or mixed cultures 7

1.3. Use of wastewaters for PHA production 9
1.3.1. Temperature effect 10
1.3.2. pH effect 11
1.3.3. Feast and famine ratio 11
1.3.4. ADD (aerobic dynamic discharge) 12
1.3.5. Uncoupled nitrogen supply 12

2. OBJECTIVES 15

3. MATERIALS AND METHODS 17

3.1. Sequencing batch reactor for mixed microbial cultures selection 17
3.1.1. Inoculum 20
3.1.2. Carbon source feeding 20
3.1.3. Nitrogen source feeding 22

3.2. Batch reactor for mixed microbial cultures enrichment 24

3.3. Analytical methods 26
3.3.1. T*otal suspended solids and volatile suspended solids* 26
3.3.2. A*mmonium nitrogen* 26
3.3.3. Volatile fatty acids 27

4. RESULTS AND DISCUSSION 28

4.1. Selection sequencing batch reactor 28
4.1.1. Start-up 28
4.1.2. Selection SBR performance 29
4.1.3. Monitoring of selection SBR cycles 33

4.2. Batch accumulation (Phase 2) 39
4.2.1. Accumulation test operation 39
4.2.2. Monitoring of accumulation test 39

5. STUDY OF PHA PRODUCTION IN A MUNICIPAL WWTP 44

6. CONCLUSIONS 49

REFERENCES AND NOTES 51

ACRONYMS 59

SUMMARY

Nowadays, to replace the use of plastic is needed due to its low degradation capacity and its low efficiency in recycling, which usually leads to pollutants and toxic substances. To promote a circular system, the use of a bio-polymer such as polyhydroxyalkanoates (PHA) is proposed.

This bio-polymer is produced from a four-stage system: fermentation, selection of microorganisms, accumulation of intracellular PHA and extraction, using wastewater or other wastes as the municipal waste organic fraction as substrates.

In this work, a selection of PHA-producing microorganisms with an organic loading ratio of 3.5 g COD · L^{-1}·d^{-1} with synthetic feeding is carried out in a 3L reactor where the ratio carbon-nitrogen is decreased (56.39 g COD /g N, 48.59 g COD / g N and 43.00 g COD / g N) to find the appropriate ratio. The process is performed with an HRT of 1.16 days and an SRT of 4.87 days. Accumulation test have also been carried out in a 1 L reactor where a ratio of 43.00 g COD / gN is worked. The pH, oxygen concentration, ammonium concentration and volatile fatty acids consume are monitored along the process.

Moreover, the effect of added the PHA production process in a wastewater treatment plant where secondary sludge is mixed with municipal organic fraction has studied.

The results obtained are to see the evolution of a system that it is trying to stabilize. Nevertheless, a reactor that works with a coupled feeding have a volatile suspended solid and totals ratio lower than another that works with an uncoupled feeding. In addition to that, the microorganism PHA content is twice as many in a reactor with coupled feeding as in an uncoupled feeding, according to the bibliography. However, it is observed that the microorganisms have the same maxim PHA content independent of the organic rate load, according to the bibliography.

Regarding the nitrogen limitation, a correlation is observed between the increase in biomass and the increase in the nitrogen dose. Besides the C-N ratios of this study were insufficient. It is also observed that the acid consumption speed on the number of carbon in the molecular chain.

Lastly, it has been concluded that the PHA production process could be implemented in a municipal wastewater treatment plant using VFA produced by the acidogenic fermentation of waste secondary sludge and squeezed OFMSW

Keywords: Polyhydroxyalkanoate (PHA) - Nitrogen - Mixed culture - Bioplastic - Wastewater treatment - aerobic dynamic feeding

RESUM

Avui en dia hi ha una necessitat de substituir el plàstic degut a la seva baixa capacitat de degradació i la seva poca eficiència en el reciclatge, que normalment deriva a substancies contaminants i tòxiques. Per tal de promoure un sistema circular, es proposa la utilització d'un bio-polímer com el polyhidroxialcanoats (PHA).

Aquest bio-polímer es produeix a partir d'un sistema de tres quatre: fermentació, selecció de microorganismes, acumulació de PHA intracel·lular i l'extracció, utilitzant les aigües residuals o altres residus com la fracció orgànica residual municipal com a substrats.

En aquest treball es duu a terme una selecció de microorganismes productors de PHA amb una carrega orgànica de 3,5 g $DQO \cdot L^{-1} \cdot d^{-1}$ a partir d'un aliment sintètic en un reactor de 3L on es va disminuint la relació carboni-nitrogen (56,39 gDQO/gN, 48,59 gDQO/gN i 43,00 gDQO/gN) per trobar la relació adequada. . El procés es porta a terme amb un temps de retenció hidràulic (TRH) de 1,16 dies i un temps de retenció cel·lular (TRC) de 4, 87 dies. També s'han realitzat assajos d'acumulació en un reactor de 1L. Durant els diversos assajos s'ha monitoritzat el pH, la concentració d'oxigen, l'amoni i els àcids grassos volàtils consumits.

Per una altra part, s'ha estudiat l'efecte d'accionar el procés de producció de PHA en una planta de tractament d'aigües residuals on es barreja fangs secundaris amb fracció orgànica municipal.

Els resultats obtinguts es veu la evolució d'un sistema que s'intenta estabilitzar. Encara i això, també es pot que un reactor que treballa amb un aliment acoplat té una relació sòlids suspesos volàtils i totals més baixa que un altre que treballa amb aliment desacoplat, A més de que en relació en el contingut de PHA en les cèl·lules després del consum dels àcids es gairebé el doble en un reactor amb aliment acoplat que un desacoplant, segons bibliografia. Encara i així, s'observa que els microorganismes tenen la mateixa màxima concentració de PHA independentment de la carrega orgànica, segons bibliografia.

En relació a la limitació de nitrogen s'observa una correlació entre el creixement de la biomassa i l'augment de la dosis de nitrogen. A més de les relacions C-N treballades son insuficients. També s'observa que la velocitat de consum dels àcids depenen del numero de carbonis en la seva cadena molecular.

Finalment, s'ha conclòs que el procés de producció de PHA es podria implementar en una planta de tractament d'aigües residuals municipals mitjançant VFA produït per la fermentació acidogènica de fangs secundaris de residus i OFMSW comprimit.

Paraules claus: Polihidroxialcanoat (PHA) - Nitrogen – Cultiu mixta - Bioplàstic – Tractament d'aigües residuals – Alimentació dinàmica aeròbica

.

1. INTRODUCTION

1.1. PETROLEUM-BASED AND BIO-BASED POLYMERS

Currently, polymers are one of the most used materials by society. The immense production of these arises from non-renewable sources of petrochemical industry being a problem. These polymers are essential for industries of almost any nature, among them the industries dedicated to textiles, transportation, construction, automobiles, aviation, medicine, etc., can be count.

This type of material has a huge environmental and economic impact not only on its production but also on its entire life cycle. Its long period of degradation together with its overcrowding, cause an accumulation of plastic waste. A clear example of this problem is the so-called "garbage island" that has been generated in the middle of the Pacific Ocean due to the convergence of surface currents that have carried these remains.

To avoid these drawbacks, there are currently recycling or reuse processes to treat this waste, however, incineration is still the most widely used process. This process is expensive, dangerous and generates gases harmful to the health and the environment, such as hydrogen chloride or hydrogen. (Ojumu al et. 2004)

A recent discovery deals with a bio-based polymer called "polihidroxialcanoate" or PHA. This is a biodegradable material that arises from renewable sources and, besides, it has properties very similar to polymers of petrochemical origin (Poirier al et. 1995). This makes it very attractive as a substitute for petroleum-derived polymers. The PHA prodution would represent a more than significant advance in the field of sustainability and a considerable improvement for the environment.

1.2. **POLYHYDROXYALKANOATES (PHA)**

1.2.1. Definition and applications

Polyhydroxyalkanoates (PHA) are a biodegradable nature polyester obtained from bacteria. These bacteria produce PHA in order to use it as an energy and carbon reserve under conditions of environmental stress in form of granules (Anderson and Dawes 1990).

PHAs are polymers formed by hydroxyalkanoic acids. The formation of these happen through an ester bond of the carboxy group of one monomer with the hydroxyl group of the next (Khanna and Srivastava 2005). An example of PHA is given in Figure 1:

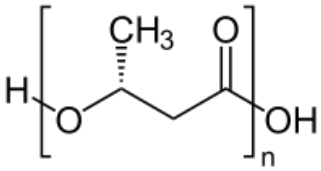


***Figure 1**. Poly-(R)-3-hydroxybutyrate (P3HB) (Mexpolimeros s. f.)*

This material is appreciated for having similar properties to oil-derived plastic. Moreover, this material can have changes in its structure and composition changing the substrate properties used on its production.

One way to PHA organize is classified into short chain length PHA, which has 3 to 5 carbon atoms, and medium chain length PHA, which has 6 to 14 carbon atoms (Lee and Choi 1999), but it is not the most used. The most common system of PHAs classify is sorting based on their composition.

The most common PHAs are:

- *Polyhydroxybutyrate (PHB)* that is highly crystalline, stiff, and brittle, with a glass transition temperature and a melting point about 5 and 175 ºC, respectively (Mitomo et al. 1999).

- *Poly (3-hydroxybutyrate / 3-hydroxyvalerate) copolymer P (HB / HV)* being a polymer that improves the thermal and physical propierties, depending on the amount of hydroxyvalerate (HV) units. Increasing the HV fraction decreases the melting temperature, in addition to increasing elasticity and flexibility (Lee, 1996).

The PHA bioplastic can be used for packaging (Bugnicourt et al. 2014), medical industry (Brigham y Sinskey 2012; Zhang et al. 2018), tissue enginyeering (Misra et al. 2006; Williams et al. 1999), agriculture (Amelia et al. 2019) and cosmetics (Sudesh 2013). Moreover, the PHA can be used to produce chemical compounds by hydroxylated fatty acid monomer that can be introduced as chiral chemical components (Ren et al. 2005; Anderson and Dawes 1990).

1.2.2. Production process using pure or mixed cultures

Nowadays, the commercial production of PHA is carried out using pure crops that can be genetically modified, using sugars and agricultural raw materials as a substrate. In these cases is necessary to work with sterilized conditions because pure cultures are vulnerable to contamination (Chen, 2009). Since it works under aseptic conditions, the production costs are very expensive, being higher than the production of plastic from petroleum. An alternative proposed to produce PHA is the use of open system mixed microbial cultures (MMC) avoiding, the needs of an aseptic system which leads to lower consumption of energy and a lower equipment cost (Davide Dionisi et al. 2004). Hence, the substrate used is also an important economic part of the process and the alternative is to replace the materials used in a pure crop with low-value raw materials such as wastewater. The use of waste as a resource provides a lower cost eliminating economic competition with food, but against it is a substrate where the composition is not constant, affecting the composition of PHA. Although the cost for further processing could increase, the advantages greatly outweigh the drawbacks. Besides, another advantages are the possibility of producing continuously and it can be better socially accepted (Johnson et al. 2009; Serafim et al. 2008). Moreover, it must be considered that the quality of polymer obtained by MMC is like that of pure cultures. This fact is due to work with a hard

selection pressure were the crop could be dominated by an organism due to its competitive PHA production capacity carrying out a behavioural system similar to a pure cultivation process, but without the need for aseptic conditions (Johnson et al. 2009).

Another important factor to consider is the stability of the process. The MMC system is more stable than a pure culture system because has better adaptability. When conditions change, the MMC has a bacterial variety that facilitates the event that one type of bacteria dominates over others with a better yield than in a pure crop that undergoes the same change. Another fact could be contamination of the pure culture that will be avoided using MMC that could not be affected or could improve the culture. This could happen if the microorganism added were more competitive producer PHA (Johnson et al. 2009). As an example of the stability of the MMC process, Serafim et al. (2008) worked with this process for more than 2 years.

The typical PHA production process consist in 4 phases: fermentation, selection of PHA accumulating organisms, accumulation of PHA and polymer extraction.

1. **Acidogenic Fermentation**

   This process consists of transform organic waste into volatile fatty acids (VFA) or other low molecular weight organic compounds in anaerobic conditions. The product obtained at this stage of the process represents a suitable substrate for the selection stage and the PHA accumulation stage.

2. **Selection of PHA accumulating organisms**

   This stage is based on enriching the culture, which works with PHA-producing microorganisms, using active sludge through aerobic dynamic feeding system (ADF), which is explained in the following section (1.1.3). As mentioned before, the substrate that is used as carbon source for the organisms to produce of PHA is the VFA. This stage is key to the optimization of the entire production process since a good selection of the crop will obtain a greater amount of PHA at the end of the stages.

3. **Accumulation of PHA**

The microorganisms selected in the previous stage are taken to a new reactor where a pulse feed will be performed in order to achieve a maximum accumulation of PHA inside the microorganisms.

4. PHA extraction

Once the bacteria with the maximum intracellular PHA content have been obtained, the polymer must be extracted. The common method is an extraction with chloroform, where the lysis of the microorganisms is caused. Subsequently, the polymer is purified.

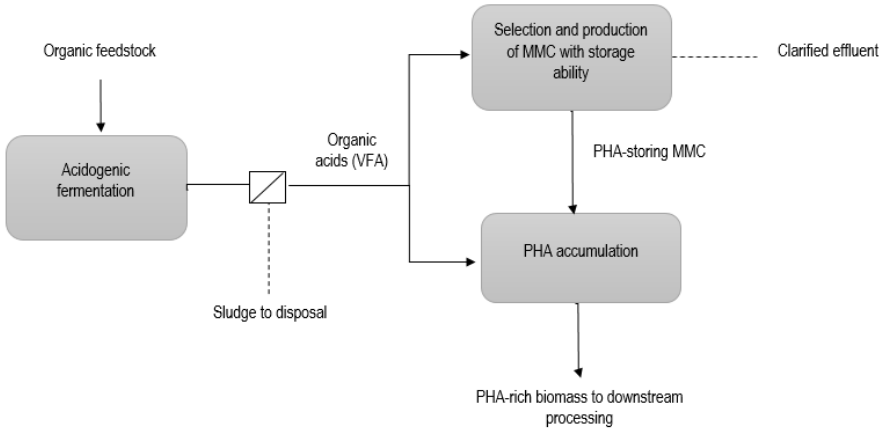


***Figure 2**. Scheme of the steps MCCs process (Valentino et al. 2017)*

1.3. USE OF WASTEWATERS FOR PHA PRODUCTION

In Europe, approximately 90 million tons of waste that satisfy the above conditions are generated. The strategy of using this waste as a substrate helps to promote the circular economy, in addition to lowering the PHA process cost (Valentino et al. 2018). Through the

integration of the process, better efficiency could still be obtained (Morgan-Sagastume et al. 2015; Valentino et al. 2018).

The substrate used for obtaining PHA represent 30-40% of the total process cost (Chanprateep 2010). This fact indicates the importance of obtaining an economic substrate. In addition to the price of the substrate, it must be considered that the raw material must be rich in easily degradable organic matter. The substrate must also mostly contain VFA and / or can be maximized through Acidogenic fermentation (Valentino et al. 2017).

Even so, it must be taken into account that the manipulation of the VFA profiles causes a change in the production of PHA with different monomer compositions (Lemos, Serafim, y Reis 2006; H. Chen y Li 2008)

In recent years, several studies have been carried out with different low-cost substrates, such as fermented sugar cane molasses (Albuquerque et al. 2010; Albuquerque, Torres, y Reis 2010; Albuquerque et al. 2007; Bengtsson et al. 2010); paper mill effluent (Bengtsson et al. 2008); cheese whey (Duque et al. 2014; Valentino, Riccardi, et al. 2015); palm oil (Możejko y Ciesielski 2013; W. S. Lee et al. 2015); municipal wastewater (Coats et al. 2007; Morgan-Sagastume et al. 2014; 2014); olive oil mill wastewater (Serafim et al. 2004; Beccari et al. 2009; Oliveira et al. 2017; D. Dionisi et al. 2005; Dias et al. 2006; Sierra et al. 2001; Campanari et al. 2014); and hardwood spent sulfite liquor (Queirós et al. 2014).

The production of PHA is affected by several parameters and variables that will be discussed above.

1.3.1. Temperature effect

Temperature plays a role in microbial competition in the selection reactor. Since this parameter influences that at certain temperatures one community of microbes or another dominates, producing changes in the production of PHA (Jiang et al. 2011; Pérez et al. 2019; Johnson et al. 2010). It has been found that at working temperatures of 30 and 37 ºC the microorganisms have

a higher PHA accumulation than compared to selected biomass at 25 ºC (Pérez et al. 2019). Other studies also conclude that working at 30 ºC is more favourable than at lower temperatures (Jiang et al. 2011; Johnson et al. 2010).

1.3.2. pH effect

The pH is a parameter that can influence the process. For this reason, the influence of modifying this parameter is determined. Keep in mind that two different reactors are working, the selection reactor and the accumulation reactor.

In the PHA-producing microorganism selection reactor, according to Serafim et al. (Serafim et al. 2004) states that the PHA on tent and polymer storage yield is higher in the case that the pH is not controlled. Other researchers also provide the no need for pH control (Montiel-Jarillo, Carrera, y Suárez-Ojeda 2017). However, Villano et al. (2010) obtained a high content of HV at pH 8.5, giving a strategy to control the type of polymer that is synthesized within the reactor.

In contrast, in the accumulation reactor, a difference in PHA production is observed by controlling the pH. At pH ≥ 7.5,there is a higher production of PHA (Montiel-Jarillo, Carrera, y Suárez-Ojeda 2017; Chua et al. 2003; Villano et al. 2010). At pH of 9.5 a higher production would be obtained in the proportion of the HV polymer (Villano et al. 2010).

1.3.3. Feast and famine ratio

The aerobic dynamic feeding (ADF) or Feast and Famine strategy is used as a selection method for mixed cultures selection. This strategy consists in excess substrate period (Feast phase) and then another period of substrate limitation (Famine phase). During the feast phase, the microorganisms consume the substrate. Subsequently, in the famine phase since there is a lack of nutrients, the bacteria that are capable of accumulating PHA can survive during this period consuming the PHA produced since it is its source of carbon and energy that allows the catabolic part to be carried out (Reis et al. 2011; Dias et al. 2006). In this way the population of

cells that do not fulfil the function of producing or accumulating PHA is reduced (Beccari et al. 1998).

Therefore, it is important to measure the proportion that each phase lasts when looking at each phase, since if the famine phase is too short (F / F is too high), non-PHA-producing bacteria might be able to survive (Wang et al. 2017). This would cause both bacteria, producing PHA or not, to grow and store less amount of PHA (D. Dionisi et al. 2005).

Optimal F / F working with a raw material with dominant acetate is below 0.3 ~ 0.4. (Davide Dionisi et al. 2006; Oliveira et al. 2017; Hao, Wang, y Wang 2018). Moreover, Dionisi et al. (2006) contributes that the Feast phase length between the cycle length cannot exceed 0.25.

1.3.4. ADD (aerobic dynamic discharge)

The problem that arises in the selection system is the accumulation of sludge (Martins, Karahan, y van Loosdrecht 2011; Wen et al. 2012). The intracellular storage of PHA causes an increase in the density of bacteria cells («Environmental Biotechnology: Principles and Applications - PDF Free Download» s. f.),being a selective physical pressure. Aerobic Dynamic Discharge (ADD) takes advantage of this principle as an improvement in the selection method. It consists of a decanting phase followed by an extraction phase at the end of each cycle. In this way, the bacteria that store the least amount of PHA will be the most difficult to decant. Consequently, it will be easier to extract them, and therefore, enrich the bacteria system with a higher PHA storage performance. This method was tested where it was claimed that the ADD method improved performance in less time (Z. Chen et al. 2015).

1.3.5. Uncoupled nitrogen supply

Nitrogen is an essential component that acts as a macronutrient for the bacteria, because is part of proteins, enzymes and nucleic acids (Sharma et al., 2004). This fact results in an interest in seeing how this substance can affect PHA production.

Firstly, it is studied how the proportion of nitrogen affects the system. Serafim et al. (2004) and Lemos et al.(2006) studied the limitation of nitrogen in the substrate for PHA selection process. They observed a cellular decrease in the proportion of cell growth with the limitation of nitrogen with a decline in the production and storage performance of PHA.

On the other hand, (Davide Dionisi et al. 2006) studied that the excess or absence of nitrogen does not provide a significant impact on the storage performance of PHA.

In other matters, the studies by Ince et al.(2012), Venkateswar et al. (2012), Hong et al. (2009), Albuquerque et al. (2007) and Basak et al. (2011) concluded that the deficiency of nitrogen in the selection sequencing batch reactor (SBR) produced biomass with greater PHA storage capacity, suppressing growth in the substrate. In the case of Johnson et al. (2010) and Valentinno et al.(2015) they also affirm that there is an increase in the production of PHA with nitrogen limitation but they also show that the limitation strategy of carbon in the selection step and reserve the nitrogen limitation in the PHA production process is a more effective strategy.

As mentioned above, PHA production occurs under limited growth conditions (Daigger y Grady 1982; Anderson y Dawes 1990). To increase PHA productivity and storage performance, crop selection should be improved. Since nitrogen is an important factor for biomass growth, a different strategy was studied, the uncoupled carbon and nitrogen feeding strategy. This strategy consists of working with two substrates, one that represents the carbon source and the other that represents the nitrogen source. These two sources are separated feed with carbon feeding at the feast phase to select the PHA producer's microorganism and feed the nitrogen at the beginning of the famine phase. In this way, an attempt is made to improve the selection by reinforcing it, since the addition of nitrogen at the beginning of the famine stage aims to cause the growth of the biomass selected in that cycle, causing a faster selection. This strategy is reinforced by the increase in PHA accumulated in the microorganisms in the results obtained in different experiments (Silva et al. 2017; Oliveira et al. 2017; Lorini et al. 2020; Norhafini, Huong, y Amirul 2019). Using this strategy, it was found that the production of PHA doubled, compared to that obtained with the coupled carbon and nitrogen feed.

2. OBJECTIVES

The general objective of this work is to study the selection and accumulation processes to obtain microorganisms capable to store PHA inside itself through the mixed microorganism culture.

To achieve this main objective, several specific objectives were proposed:

- To find the correct ammonium concentration to operate correctly the SBR reactor to select PHA producer microorganisms. Moreover, it is important to observe the effect of nitrogen in biomass growth.
- To study the difference and similarities between a reactor that works with C-N coupled and uncoupled feeding in the selection process.
- To establish the necessary time to arrive an efficient PHA concentration in the accumulation reactor.
- To measure the PHA content at the end of the feast phase and the end of accumulation tests.

Due to extraordinary success of COVID, another objective was proposed which consist in study the PHA production in a wastewater treatment plant. Moreover, the PHA content will be estimated with the bibliography.

3. MATERIALS AND METHODS

3.1. SEQUENCING BATCH REACTOR FOR MIXED MICROBIAL CULTURES SELECTION

The mixed microbial cultures selection was performed in a crystal reactor with 3.0 L working volume, where active sludge was inoculated. The temperature was fixed to 30ºC, with a thermostatic jacket that allows maintaining the reactor at this temperature. To ensure the homogeneity inside the reactor, a mechanical agitator was added. Moreover, oxygen supply was achieved using air pumps air connected to ceramic diffusers located inside the reactor.

The reactor worked with an HRT of 1.16 day and an SRT of 4.87 days, which were implemented by adjusting the volume dosage of several peristatic pumps. Moreover, these peristatic pumps, the air pumps and the mechanical agitator were connected to timers to operate at selected times. This configuration allows the reactor to work properly with SBR cycles that were repeated every 6 hours. Figure 3 schematizes the selection reactor.

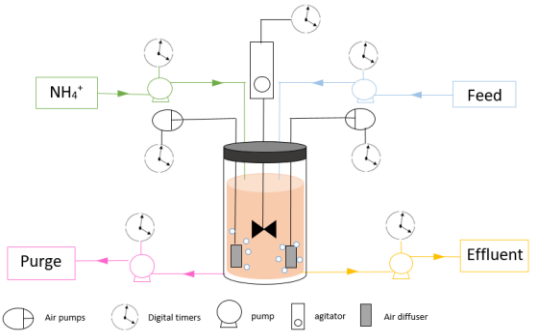


Figure 3. *Scheme of sequencing batch reactor for mixed microbial cultures selection (Adapted from (Pérez, 2019)*

The reactor was equipped with two probes to monitor the pH (Mettler Toledo HA405-DPA-SC-S8/225) and dissolved oxygen (DO) concentration (CellOx 325.WTW connected to oxi 3310WTW portable meter) along the time.

The cycles of the selection reactor had a length of 6 h, with mean 4 cycles per day. During the cycle period, the reactor goes through a series of phases, bearing in mind that the FF (Feast-Famine) strategy is to be carried out.
The phases are the following:

- **Synthetic wastewater feeding [(15 min: 750 mL) organic carbon rich feeding]**: this phase consists of adding C-feeding to the reactor without stirring and aeration. In Phase 1, this synthetic wastewater did not include NH_4^+-N, while during Phase 2, nitrogen was supplied at the same time the C-feeding was added.
- **Feast phase (136 min):** In this stage, the agitation and aeration of the reactor starts, allowing the biomass of the system to consume the substrate. During the Feast phase, a complete consumption of the organic carbon source added is expected.
- **Purge phase (1 min: 150 mL):** consists of the extraction of a sample volume where the microorganisms have the maximum PHA content. It is performed after the feast phase, when the microorganisms have the maximum PHA content during the cycle.
- **Nitrogen source feeding (1min: 50 mL)** In this step, carbon and nitrogen uncoupled feeding strategy was carried out in the first phase introducing the nitrogen when famine starts. This stage did not take place in Phase 2 when it was decided to add nitrogen with the feeding at the beginning of the cycle to avoid the carbon and nitrogen uncoupled strategy (as will be discussed later).
- **Famine phase (164 min):** In this step of the SBR cycle, the mixed liquor lacks easily biodegradable organic matter, since it has been previously consumed, and, therefore, the biomass has to use the stored carbon sources (intracellular PHA) to produce energy and continue growing for its survival. Moreover, those microorganisms that were not able to store organic matter in the Feast stage would decay (death-

regeneration process) during this step. In the Phase 1, at the beginning of the famine phase, nitrogen source feeding was added to apply the uncouple feeding strategy.

- **Sedimentation (30 min):** After the famine phase, the digital timers switched off the air compressors and the mechanical stirrer, leading to the settling of the suspended biomass inside the reactor.
- **Extraction of the treated effluent (15 min: 650mL):** It is the phase where the excess volume is extracted, allowing the appropriate volume to be obtained in the following cycle so that after the next substrate addition the working volume is obtained. The volume being extracted is the supernatant volume.

Tables 1 and 2, as well as Figures 4 and 5, summarizes the layout of the timers to see the configuration of the Phase 1 and 2, respectively:

***Table 1**. Cycle time distribution of the selection Sequencing Batch Reactor (SBR) in Phase 1*

Stage	Start time		Finish time	Duration	Units
Carbon source feeding	0:00	-	0:15	15	min
Feast period	0:15	-	2:31	136	min
Purge	2:30	-	2:32	2	min
Nitrogen source feeding	2:45	-	2:46	1	min
Famine period	2:31	-	5:15	164	min
Sedimentation	5:15	-	5:45	30	min
Effluent withdrawal	5:45	-	6:00	15	min

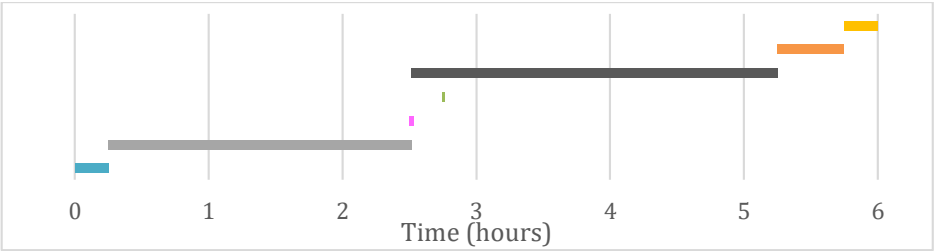


***Figure 4.** Cycle time distribution of selection SBR in Phase 1*

***Table 2**. Cycle time distribution of SBR for selection (Phase 2)*

Stage	Start time		Finish time	Duration	Units
Carbon source feeding	0:00	-	0:15	15	min
Nitrogen source feeding	0:14	-	0:15	1	min
Feast period	0:15	-	2:31	136	min
Purge	2:30	-	2:31	1	min
Famine period	2:31	-	5:15	164	min
Sedimentation	5:15	-	5:45	30	min
Effluent withdrawal	5:45	-	6:00	15	min

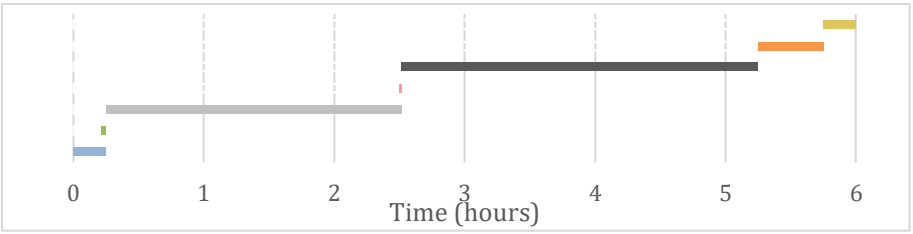


***Figure 5.** Cycle time distribution of selection SBR in Phase 2*

3.1.1. Inoculum

At the beginning of the process, an inoculum of a previous selection reactor that worked at 7 g COD · L^{-1} was used to inoculate the selection reactor.

3.1.2. Carbon source feeding

For the growth, maintenance and production of PHA from bacteria, the culture needs a carbon source. A synthetic feeding based on organic loading ratio (OLR) of 3.5 g COD · L^{-1} · d^{-1} was

chosen to do this study. The synthetic feeding used was composed by acetic acid, propionic acid and butyric acid in the concentration proportion of 62,5% acetic acid (HAc), 18,8% propionic acid (HPr) and 18,8% butyric acid (HBu) (see Table 2) to imitate the real organic fraction studied by Dosta (2018). It is intended to obtain results with a synthetic source to be able to later compare them with a real one.

Table 3. *Components concentration of organic loading ratio of 3.5 g COD·L^{-1}·d^{-1}.*

Component	Value	Units	COD	Units	Purity	Units
acetic acid	1.69	mL·L^{-1}	1.44	g·L^{-1}	99	%
propionic acid	0.51	mL·L^{-1}	0.62	g·L^{-1}	99	%
butyric acid	0.51	mL·L^{-1}	0.74	g·L^{-1}	99	%
total	2.71	mL·L^{-1}	2.8	g·L^{-1}		

Furthermore, micro and macronutrients were added to the synthetic to ensure the needs of microorganisms which are used an adaptation of the nutrient described for (Dapena-Mora et-al-, 2004), where they are described in the Table 4:

Table 4. *Concentration of micronutrients and macronutrients (Adapted from (Dapena-Mora et al. 2004)*

Component	Value	Units	Component	Value	Units
K_2HPO_4	0.58	$g \cdot L^{-1}$	**$FeCl_3 \cdot 6H_2O$**	1.5	$mg \cdot L^{-1}$
KH_2PO_4	0.23	$g \cdot L^{-1}$	**H_3BO_3**	0.15	$mg \cdot L^{-1}$
$MgSO_4 \cdot 7H_2O$	0.09	$g \cdot L^{-1}$	**$CuSO_4 \cdot 5H_2O$**	0.03	$mg \cdot L^{-1}$
$CaCl_2 \cdot 2H_2O$	0.07	$g \cdot L^{-1}$	**KI**	0.03	$mg \cdot L^{-1}$
EDTA	0.02	$g \cdot L^{-1}$	**$MnCl_2 \cdot 4H_2O$**	0.12	$mg \cdot L^{-1}$
NH_4Cl	2.81	$g \cdot L^{-1}$	**$Na_2MoO \cdot 2H_2O$**	0.06	$mg \cdot L^{-1}$
			$ZnSO_4 \cdot 7H_2O$	0.12	$mg \cdot L^{-1}$
			$CaCl_2 \cdot 2H_2O$	0.12	$mg \cdot L^{-1}$

Moreover, additional alkalinity (2.0 $g \cdot L^{-1}$) was added in form of sodium bicarbonate to ensure the pH between 8-9.

3.1.3. Nitrogen source feeding

Since biomass requires nitrogen for growth, it is necessary to add it to the reactor. Due to this fact, the nitrogen was supplied in form of NH_4Cl. The theoretical amount needed to add to the reactor depends on two factors: the specific carbon source fed and the observed heterotrophic yield. Through these factors, the need for ammonia required by the reactor had been calculated (see Annex 1), but as the experiment was carried out, these concentrations varied to obtain better efficiency.

In an initial stage (Phase 1), the concentration of nitrogen used in the synthetic wastewater was 1.05 g $N \cdot L^{-1}$ (4.03 g $NH_4Cl \cdot L^{-1}$) and was added separated to the synthetic feeding after feast phase. This strategy was carried out to follow the strategy of uncoupled carbon and nitrogen

feeding. Along the Phase 1, diverse nitrogen concentrations were carried out to obtain a better selection of biomass. There are two sub-phases distributed by different concentrations:

- Phase 1.1: the initial concentration of nitrogen used in the synthetic feeding was 1.05 g $N\text{-}NH_4^+ \cdot L^{-1}$ (4.03 g $NH_4Cl \cdot L^{-1}$).
- Phase 1.2: how the nitrogen concentration was insufficient, the concentration was increased 15% from 1.05 to 1.22 g $N\text{-}NH_4^+ \cdot L^{-1}$ (4.63 g $NH_4Cl \cdot L^{-1}$).

Then, another increment of 15% in nitrogen was done and a second stage was carried out (Phase 2) feeding the nitrogen with the carbon source Hence, a synthetic wastewater with 1.39 g $N \cdot L^{-1}$ were added.

It must be taken into account that in each modification in the reactor that is submitted, such as the increase in nitrogen that was provided in the reactor, the reactor needs a minimum adaptation time of 3 times the SRT, before seeing a clear trend and that each increase cannot be extremely large so as not to harm the system.

Figure 6 shows the different nitrogen concentrations in the different Phases:

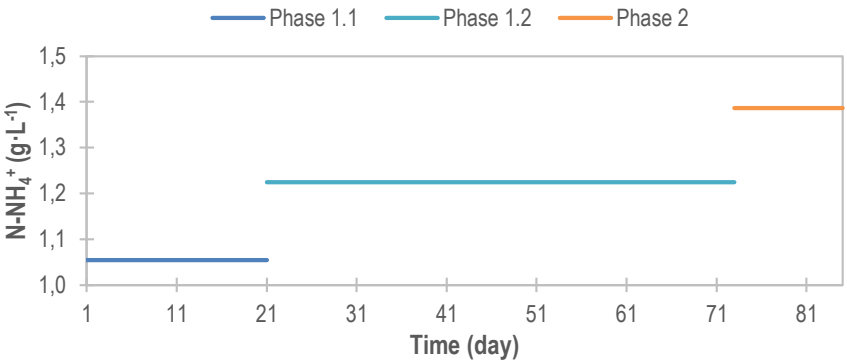


Figure 6. *Different nitrogen concentrations of synthetic nitrogen feeding*

3.2. **BATCH REACTOR FOR MIXED MICROBIAL CULTURES ENRICHMENT**

The reactor used for the enrichment of PHA-accumulating biomass was simpler than SBR used in selection phase. This reactor was a glass jacketed reactor with 1L of working volume connected to a thermostatic bath to maintain the operating temperature. In this case, all the feedings and extractions were performed manually with a graduated syringe. Moreover, the reactor was equipped with two probes to measure the pH and DO in the reactor as in the selection reactor (see Figure 7).

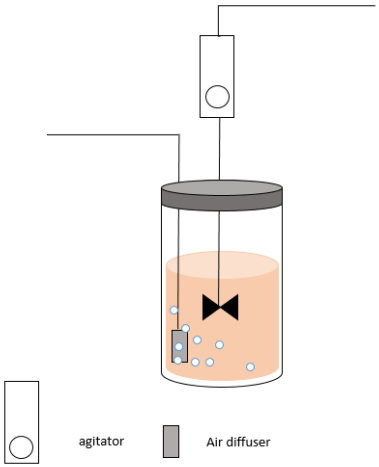


***Figure 7**. Scheme of enrichment batch reactor (Adapted from (Pérez, 2019))*

The PHA accumulation reactor objective is to maximize the PHA production. To promote PHA accumulation in the microorganism, a limitation in the growth rate was needed. The nitrogen is a substance that allows the biomass growth; due to this fact, the nitrogen source feeding was not performed in the PHA accumulation reactor. Moreover, the same carbon source feeding of SBR is added for PHA production.

In order to carry out this test, the one-day purge (4 cycles) of the SBR was taken, which corresponds to 600 ml of mixed liquor, having a concentration equal than the reactor. The purge was introduced into the reactor as inoculum and throughout the test, 4 additions of synthetic carbon source feeding were performed. Every feed addiction was 200 mL. The time needed to

consume each addition was governed by the oxygen profile, since it was the indicator of the depletion of the carbon source. This could be seen through a sharp increase of the dissolved oxygen concentration inside the reactor followed by a stabilization. The working volume of the accumulation reactor is 1000mL and, therefore, it is necessary to settle the biomass and extract the supernatant after finishing two additions. At the beginning and at the end of every feast, a sample was taken to quantify PHA and volatile fatty acids concentration.

The duration of batch accumulation tests were approximately 9-10 hours.

3.3. **ANALYTICAL METHODS**

The analytical methods used in that experiment were performed following the *Standard methods for the examination of water and waste water* (APHA,2005):

3.3.1. T*otal suspended solids and volatile suspended solids*

This analysis consists of determining the organic matter of the sample. To have the tare, the first weight (P1) is performed, weighing a 0.45 µm filter and an inert aluminium weighing dish. Once the weight has been obtained, a known volume (V) is filtered, in this case 10 mL with the help of a kitasate where the sample will be sucked until the water is removed. Subsequently, the filter was removed from the filtration system and transferred to an inert aluminium weighing dish. It was dried in an oven at 105ºC for approximately 24 hours. After this process, the sample was weighed, thus obtaining the second weight (P_2) that provides the information of total suspended solids (TSS). Next, to measure the volatile suspended solids (VSS), the sample was introduced into a muffle furnace at 550ºC, where the last weight (P_3) was obtained. To obtain the TSS and VSS equation 1 and 2 respectively are used.

$$TSS = \frac{P_2 - P_1}{V} \quad (1)$$

$$VSS = \frac{P_2 - P_3}{V} \quad (2)$$

P_1 = weight of dish and filter, mg.

P_2 = weight of residue + dish and filter before muffle, mg

P_3 = weight of residue + dish and filter after muffle, mg.

V = volume of sample used, mL

3.3.2. A*mmonium nitrogen*

For this analytical technique, a selective ammonia probe (Orion 9512HPBNWP) has been used, which measures the conductivity of the sample analysed. In order to measure the concentrations of the samples, a preliminary calibration was performed. The calibration was

carried out using standards of 9.99 ppm, 24.99 ppm, 49.99 ppm and 99.99 ppm of N_NH4 obtaining is process consists of achieving a relationship between the concentration and the conductivity of $N\text{-}NH_4^+$ standards. The standards used in the calibration are 9.99 ppm, 24.99 ppm, 49.99 ppm and 99.99 ppm of $N\text{-}NH_4$ obtaining the corresponding conductivity. The samples measured must be filtrated by a 0.45 µm filter and then can be measured. The reader will indicate the potential difference that is obtained with the probe electrolyte and with the relation obtained in the calibration of the sample concentration. In the sample we have a base acid balance of NH_3, in order to displace it, a few drops of 10M NaOH were added. Furthermore, it must be taken into account that the values obtained were expressed in mg $N\text{-}NH_4^+ \cdot L^{-1}$.

3.3.3. Volatile fatty acids

In order to quantify the concentration of volatile fatty acids (VFA), gas chromatograph was used. The procedure for this analysis was to collect a sample that will be filtered with a 0.45µm filter. Subsequently, a known volume that was filtered in a vial was taken considering that the concentration of this sample cannot exceed 1,000 mg $AGV \cdot L^{-1}$ of each acid, since it was the limit that the instrument can measure. If the value is exceeded, the sample was diluted considering the degree of dilution. Next, 25 µL of phosphoric acid was added for each millilitre of sample that had been collected and homogenized. The equipment used analyse the sample was the gas chromatography (GC-2010 Plus, Shimadzu) equipped with a Nukol ™ column 15m x 0.53mm x 0.5µm that will provide the acid concentrations.

4. RESULTS AND DISCUSSION

4.1. SELECTION SEQUENCING BATCH REACTOR

4.1.1. Start-up

Firstly, the reactor was inoculated with the previously selected purge by Perez (2019) who worked with 7 g COD · L^{-1}. Hence, on the first day, the reactor had the following characterization: 1.97 SST, 1.89 SSV and 0.96 SSV/SST ratio.

The start-up of the selection reactor was carried out using the C and N uncouple feeding strategy (Phase 1) trying to obtain a better selection. Working with FF and uncoupled feeding strategy, only carbon absorption occurs in the feast period, favouring the absorption of carbon by PHA-producing microorganisms. Later, at the beginning of the famine, nitrogen is introduced allowing the growth of microorganisms. On the other hand, a reactor that works with only the feast and famine strategy without decoupled nutrients allows the growth of microorganisms from the beginning of the cycle, being a slower selection system. Furthermore, it has been found that the reactors that work with FF have a slight decrease in biomass compared to the reactors that work with FF and uncoupled feeding, in addition to its production (Oliveira et al. 2017).

In the start-up phase, the concentration of ammonia used was 4.03 g $NH_4Cl \cdot L^{-1}$ (56.39 g COD/ g N) (Phase 1.1) which was obtained from a calculation explained in Annex 1. In this first phase, the samples, taken in the effluent, gave constantly zero ammonium concentration due to the total consumption of the nitrogen by the microorganisms. This fact indicated insufficiency of this nutrient turning it into a limiting substrate. Therefore, it was decided to increase the initial ammonium concentration by 15%.

4.1.2. Selection SBR performance

In order to observe how the reactor evolves, daily monitoring results of the SBR selection are presented with the following parameters: TSS, VSS and ammonium concentration:

4.1.2.1. Selection SBR performance in Phase 1.2

Phase 1.2 was started in 21st day of the experimentation with an increment of 15% of ammonium concentration as has been explained before resulting in a concentration of 4.65 g $NH_4Cl \cdot L^{-1}$ (48.59 g COD/ g N). The daily data obtained in this phase was represented above (see Figure 8 and Figure 9):

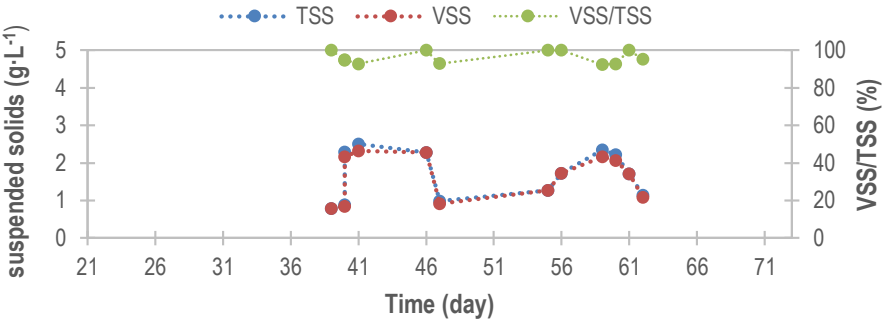


Figure 8. *Profile of volatile suspended solids (VSS) and total suspended solids (TSS) of SBR in Phase 1.2*

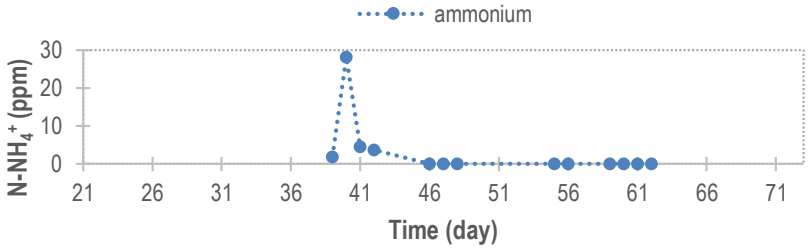


Figure 9. *Profile of ammonium concentration in the effluent of SBR in Phase 1.2*

First of all, it is important to note that the figures have a lack of data until the 39th day. This fact is due to several failures in the operation of the reactor that did not allow the reactor track. The failure was because the nitrogen pump was broken giving several problems in the reactor. Hence, was replaced but the reactor did not work efficiently.

Due to the malfunction of the entire system, it was decided to incorporate the biomass purge of two days before at 39th day to help reactor with the same conditions of this phase. At 39th day the reactor had the following characterization: 0.79 $g \cdot L^{-1}$ SST, 0.79 $g \cdot L^{-1}$ SSV and 1.00 SSV/SST ratio. Hence, a huge increase in VSS and TSS was observed in the following days (Figure 8) reflecting the increase in biomass concentration due to the inoculation. Even so, the biomass decreases rapidly, due to the lack of nitrogen (Figure 9), which is the consequence of the biomass growth (Johnson et al. 2010). Moreover, on day 40 it was detected that the nitrogen pump stopped again. It was decided to dose double the amount of nitrogen feeding volume to solve the deficiency inside the reactor. In this manner, a peak of ammonium is observed in the profile on 40th day.

In Figure 8 was observed that slower growth of biomass at 47th day probably due to a lack nitrogen dose of 46th day. Even so, seemed that the reactor was recovery its stability. Later, a deficiency of nitrogen occurs again on 55th day as can be seen in Figure 9, causing a consequence decrease in VSS and TSS. This fact is due to a lack of nitrogen that implies a decrease in the biomass concentration as was studied by Johnson et al.. The effluent ammonium concentration was zero again implying a decreasing of VSS and TSS. Hence, the ammonium concentration on the nitrogen feeding was needed to be increased again and the strategy changed coupling C and N nutrients (Phase 2) to simplify the operation of the reactor after several failures of Phase 1.1 and Phase 1.2.

4.1.2.2. Selection SBR performance in Phase 2

Phase 2 was started on the 73rd day of the experimental work and was characterized by the simplification of the double selection strategy to a nitrogen and carbon coupled feeding system.

In this phase, the ammonium concentration was increased again 15% with a concentration of 5.06 g $NH_4Cl \cdot L^{-1}$ (43.00 g COD/ g N). To start this phase, the reactor purge of days before was added to help reactor in the stability process of new conditions at 73th day. The reactor had the following concentration: 3.65 $g \cdot L^{-1}$ SST, 3.29 $g \cdot L^{-1}$ SSV and 0.9014 SSV / SST ratio. The daily results are shown in the Figures 10 and 11:

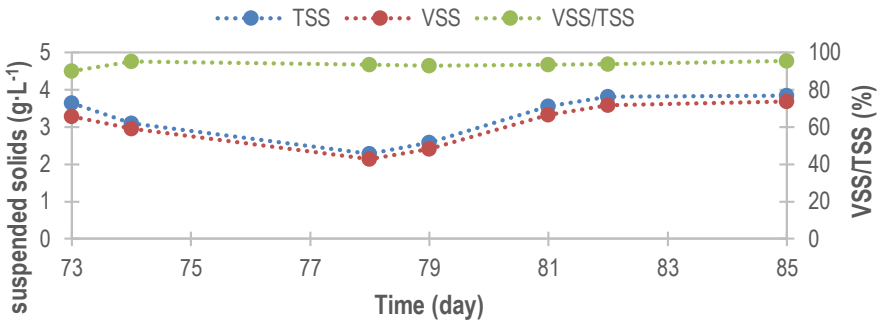


Figure 10. *Profile of volatile suspended solids (VSS) and total suspended solids (TSS) of SBR in Phase 2*

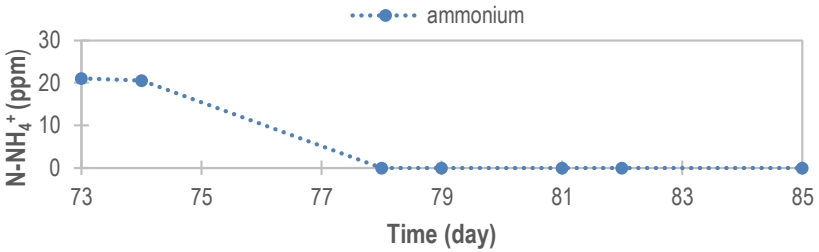


Figure 11. *Profile of ammonium concentration in the effluent of SBR in Phase 2*

In Figure 10, a decrease in TSS and VSS can be observed with a decrease in ammonium concentration in Figure 11. The fact can be associated with the biomass stability process that allows the biomass growth in new conditions. From a 78th day, the suspended solid

concentration increased with a stabilization indicating a better biomass growth inside the reactor.

In Figure 11, since 78th day it shows how throughout Phase 2 the microorganisms consumed all the added ammonium. No action was taken to increase the ammonium concentration in nitrogen feeding, since it is intended to see if there was an evolution in the system with the biomass stabilization. This last phase only be operated for 12 days due to the alert situation (COVID-19) that forced the closure of the installations.

If the data obtained from Phase 1.2 (Figure 8) was compared with and Phase 2 (Figure 10), it was observed that working with couple feeding, lower VSS/TSS ratio was obtained than when working with uncoupled feeding. This success is consistent with the study carried out by Oliveira et al. (2017) who observed the same phenomenon, working with one reactor with only feast and famine strategy and other reactor with uncoupled feeding and feast and famine strategy. But instead, the concentration of totals suspended solids was higher in Phase 2 probably due to the increase in nitrogen concentration (Johnson et al. 2010).

4.1.3. Monitoring of selection SBR cycles

Once the biomass was more stable although the ammonium was still totally consumed at the end of each cycle, a more detailed analysis was carried out to determine how the reactor operates Hence. the reactor was monitored, analysing the dissolvent oxygen, pH and ammonium during a cycle:

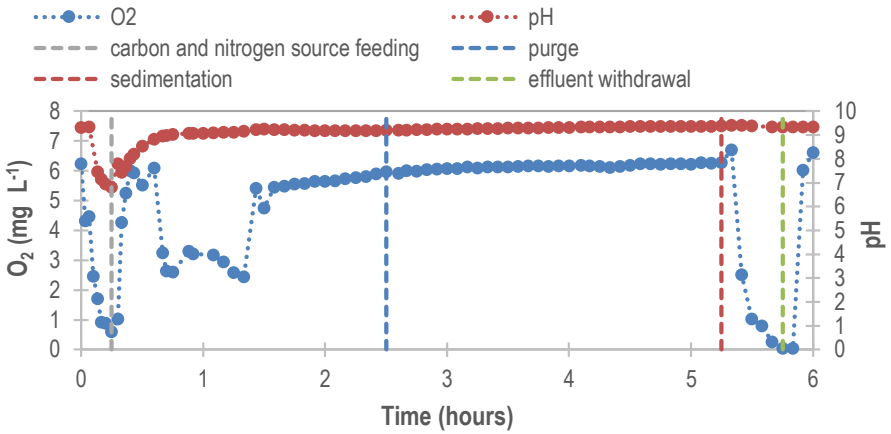


***Figure 12**. Profiles of dissolvent oxygen and pH of SBR cycle in Phase 2 at 82nd day*

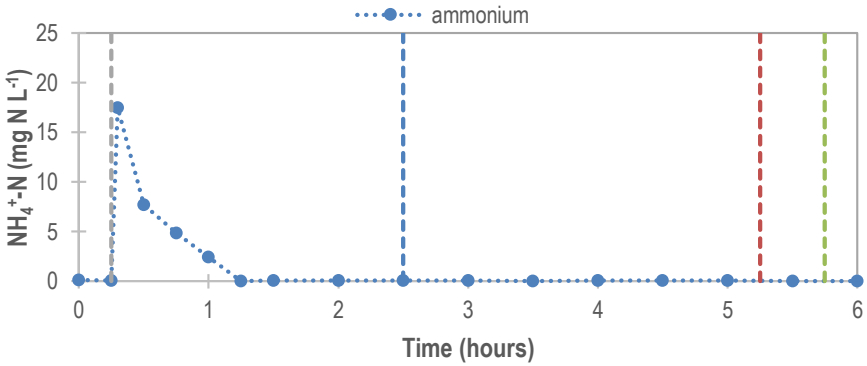


***Figure 13.** Ammonium profile of SBR cycle in Phase 2 at 82nd day*

The track of the reactor carried out at 82nd day had a TSS concentration of 3.82 g TSS·L^{-1} and VSS of 3.58 g VSS·L^{-1} with a VSS/TSS ratio of 96%. More details can be obtained in the Table 7 (Appendix 3).

In Figure 12 the length of the feast phase can be observed. This phase is characterized by the substrate exhaustion which is easily observed in the oxygen profile. The first drop in the oxygen profile indicates the supply of feed to the reactor in anaerobic phase. Subsequently, an increase in the oxygen concentration was observed due to the start-up of the air pumps that supply air to the system. After this huge increase, a slight drop in the oxygen concentration occurred indicating the feast time where the bacteria need consume oxygen to consume the VFAs inside the reactor. In this specific case, the feast phase length was 65 min. Hence, there was again an increase in the oxygen concentration that becomes stable begin, the famine phase which is characterized by the lack of VFA. Hence, there was a decrease due to the interruption of the oxygen supply in the reactor, since sedimentation proceed. Finally, an increase was seen again caused by the probe remaining in contact with the air caused by the removal of the supernatant. This same trend was observed by Villano et al. (2014) who worked with coupled feeding a synthetic mixture of organic acids with the same reactor cycle length as this study.

To ensure a good selection, the feast phase needs to be shorter than the famine phase (Albuquerque et al. 2011; Korkakaki et al. 2016). Since the feast phase was 65 min, the ratio between feast duration and the cycle length in this cycle was 18%. This ratio is lower than 25% that seems to be important to allow a good selection of PHA-storing biomass (Dionisi et al. 2006).

The Figure 13 shown that all ammonia introduced into the reactor in the first period was totally consumed along the cycle. This fact indicated that more nitrogen concentration could be required to not limiting the bacteria growth. But, since it is not yet known if the procedure is completely stable and the nitrogen is only completely consumed when the feast period ends, before taking action it was necessary to carry out another cycle monitoring.

Few days later, another cycle reactor monitoring was made to contrast previous results:

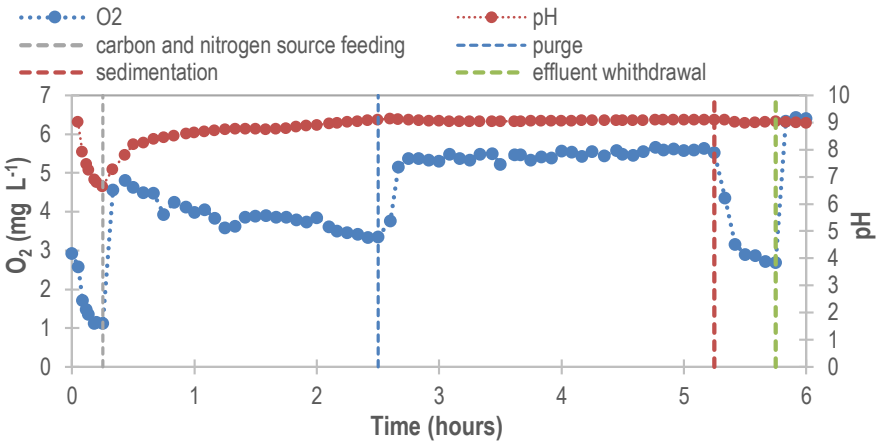


***Figure 14**. Profiles of dissolvent oxygen and pH of SBR cycle in Phase 2 at 85th day*

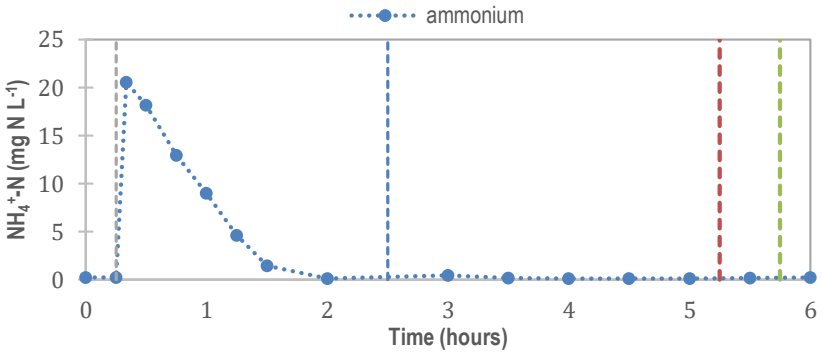


***Figure 15.** Ammonium profile of SBR cycle in Phase 2 at 85th day*

At 85rd day, the TSS and VSS concentration inside the reactor was 3.85 g TSS·L^{-1} and 3.68 g VSS·L^{-1} with a relation of 96% VSS/TSS. More information could be found in Table 8 (Appendix 3).

Figure 14 shows a different feast phase length. How the synthetic feed was not changed, it may be made by an air flow fluctuation. In this case, the phase length was 131 min. The ratio

between feast duration and the cycle length in this cycle 39% was bigger than 25%. Since working with a lower ration than 25% seems to better to obtain a good selection of PHA-storing biomass as has been commented before, this cycle probably had a worse selection than the previous assay. As, the ratio was bigger than 25%, the air flow was increased to provide a better feast/cycle ratio in the previous days.

As it can be seen in Figure 15, all the ammonia that was introduced into the reactor was totally consumed again before the first period ends confirming that the nitrogen concentration was insufficient in the process and an increment of nitrogen concentration was needed. At 82nd day (Figure 13), the ammonia is completely consumed just at the end of the feast period. However, at 85th day (Figure 15), all the ammonia that was introduced into the reactor was totally consumed before the first period ends. The possible reason for this event is the slight growth of the biomass where 3.58 g VSS·L^{-1} is passed to 3.68 g VSS·L^{-1}, which causes the insufficiency of ammonia to satisfy the need for growth for all biomass. It should be noted that it is intended to work with carbon as a limiting nutrient, not nitrogen as a limiting component.

Another increment of nitrogen concentration is not unreasonable, as others studies that SBRs works without nitrogen limitation as Lorini al et. (2020) who worked with C/N ratio of 33.4 g COD/g N compared to 43.00 g COD/g N used in Phase 2.

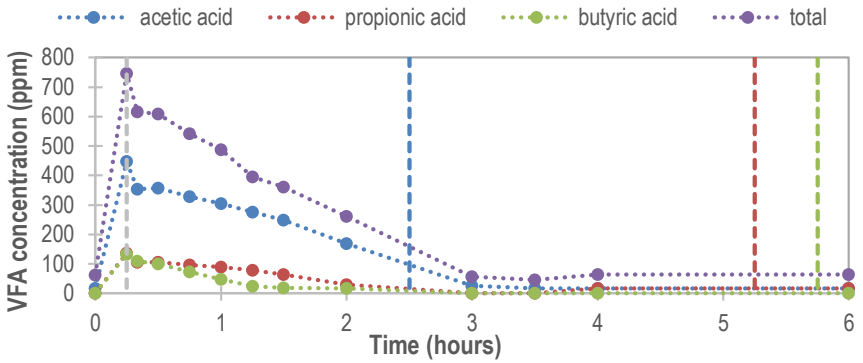


***Figure 16**. Profile of all volatile fatty acids of SBR cycle in Phase 2 at 85th day*

Table 5. *Table of consummation rate of volatile fatty acids of the SBR monitoring cycle in Phase 2 at 85th day*

Acid	straight	consumption rate	
Acetic acid	y = -3243.3x + 437,97	-3243.3	ppm VFA · h^{-1}
Propionic acid	y = -1103x + 132,51	-1103	ppm VFA · h^{-1}
Butyric acid	y = -1133.4x + 113,53	-1133.4	ppm VFA · h^{-1}
Total	y = -5575.2x + 725,99	-5575.2	ppm VFA · h^{-1}

Figure 16 and Table 5 gives information about the consumption rate of VFAs. It was observed how acetic acid had a higher consumption rate than propionic and butyric acid that had the same speed. The reason this happed was the carbon amount of acid molecular structure. Breaking a molecule of 2 carbons is easier than a molecular of more carbons. A similar consumption trend of volatile fatty acids have studies like Albuquerque et al (2010) who used fermented molasses and Campanari et al. (2017) who used olive oil mill wastewater.

Although, the PHA obtaining in the purge of this SBR monitoring was not able to be analysed, the result obtained was roughly estimated using the bibliography:

Table 6. *Parameters monitored in the selection sequencing batch reactors*

Parameters	References			
	(Perez, 2019)	(Silva et al. 2017)	(Valentino et al. 2014)	(Villano et al. 2014)
OLR (g COD ·L^{-1}·d^{-1})	3.5	8.5	8.5	8.5
C-source	Synthetic VFA mixture	Synthetic VFA mixture	Synthetic VFA mixture	Synthetic VFA mixture
C-N feeding in SBR	uncoupled	uncoupled	coupled	coupled
Cycle length (h)	6	6	6	6
Feast phase and cycle length ratio (%)	17 ± 1	35 ± 2	20 ± 1	18 ± 1

PHA content at the end of the feast(g PHA/g VSS)	0.14 ± 0.01	0.28 ± 0.02	0.18 ± 0.05	0.19 ± 0.01

In the Table 6, it was observed different studies carried out to obtain PHA that are similar as this study. How the same ORL for coupled C-N feeding in SBR was not found, but it was found for uncouple feeding, it was wanted compere first the PHA content at the end of the feast between the different strategies with similar parameters. As the table shows, the uncoupled reactor used by Valentino et al. (2014) had approximately two times bigger than couple feeding, as Silva et al. (2017) with the same OLR and cycle length using synthetic VFA mixture as feeding.

Pérez (2019) obtains a purge with around 14% PHA by weight from a similar reactor but working with the nitrogen carbon uncoupled feeding strategy. Assuming that The production in the case of uncoupling N- and C-source, is two times bigger than coupled feeding as had been commented before the PHA content of the biomass purge was estimated to have a content of approximately 7% PHA by weight.

4.2. **BATCH ACCUMULATION (PHASE 2)**

4.2.1. Accumulation test operation

In order to carry out this test, the one-day purge of biomass (4 cycles) of the SBR was taken to inoculate the accumulation reactor, which corresponds to 600 ml. This inoculum was introduced into the reactor and throughout the test, 4 additions of carbon source feeding with a concentration of 3.5 mg COD · L^{-1} were introduced. The time of addiction was governed by the oxygen profile, since it was the indicator of the end of consumption of the VFAs. This could be seen through an augment ended with stabilization of dissolved oxygen. As the accumulation reactor had a volume of 1000mL was necessary to settle and extract the supernatant after finishing analysing two additions.

4.2.2. Monitoring of accumulation test

Assuming that the SBR selection is close to reaching a state of stability in the Phase 2 and in order to observe the efficiency of the reactor at that moment, accumulation tests were carried out:

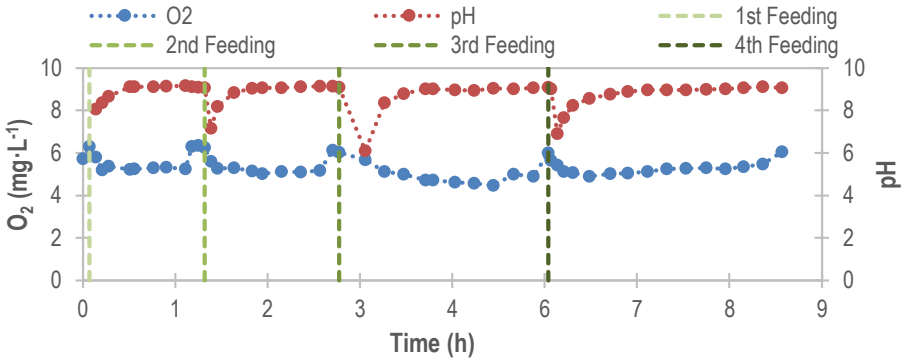


***Figure 17**. Profile of dissolved oxygen and of pH in PHA accumulation test in Phase 2- fist accumulation*

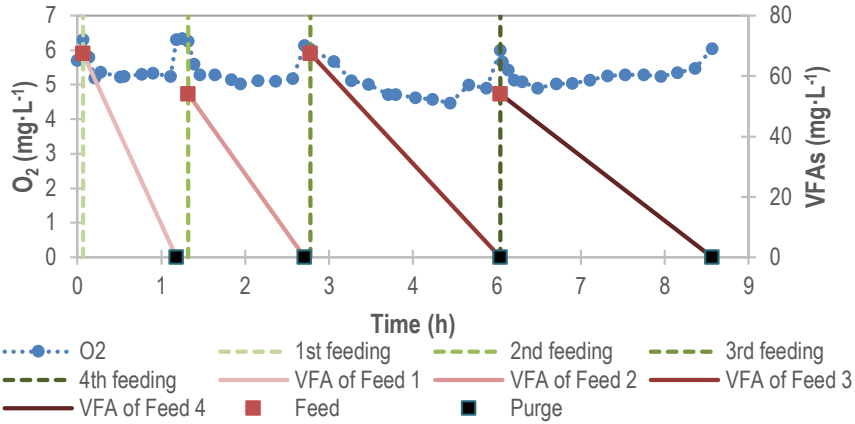


Figure 18. *Profile of dissolved oxygen and theoretical VFA concentrations – first accumulation*

Table 7. *VFAs consume rate in accumulation batch reactor test – first accumulation*

stage	VFAs consume ratio	
1	-50,6	mg VFA ·L^{-1}·h^{-1}
2	-32,4	mg VFA ·L^{-1}·h^{-1}
3	-24,8	mg VFA ·L^{-1}·h^{-1}
4	-17,8	mg VFA ·L^{-1}·h^{-1}

Figure 17 shows the profile of dissolvent oxygen and the pH in the accumulation test carried out. Through the dissolvent oxygen profile, it was shown that it became a slower process of VFAs consumption as another dose of synthetic feeding was added, where PHA production was increasing. Moreover, through the dissolvent oxygen profile of Figure 17 and theoretical VFA concentrations in the beginning and the end of every dose of synthetic feeding, the VFAs consume ratios were calculated (Table 7 and Figure 18). In Table 7, a slower VFA consume ratio was observed as another dose of synthetic feeding was added. This happened because microorganisms increase more intracellular PHA contain that makes carbon absorption difficult. This is in agreement with Campanari et al. (2017) who worked with fermented olive oil mill wastewater. Also, It was consistent with Valentino et al. (2019) who worked with an

accumulation reactor that was fed with fermented organic fraction of municipal solid waste and secondary sludge mixture rich in VFA that was similar of synthetic feed of this study.

In addition, this study provided approximately 9 hours were necessary to carry out 4 feeds additions. The time it takes for each addition of synthetic feeding, together with the PHA content obtained, are essential parameters to take into account to consider the efficiency it would integrated the process in a pilot plant that will work with reactors of these characteristics.

Besides, in Figure 17 shown that pH was around 7 when the feeding was added and increase around 9 when the VFAs was being consumed. If more accumulation reactor parameters are required, the table 9 in Appendix 4 shows it.

Three days later, the same test was made to ensure the reproducibility of the results obtained in the previous assay. Hence, the same parameters were monitored, and the operation of accumulation test was made in the same mode:

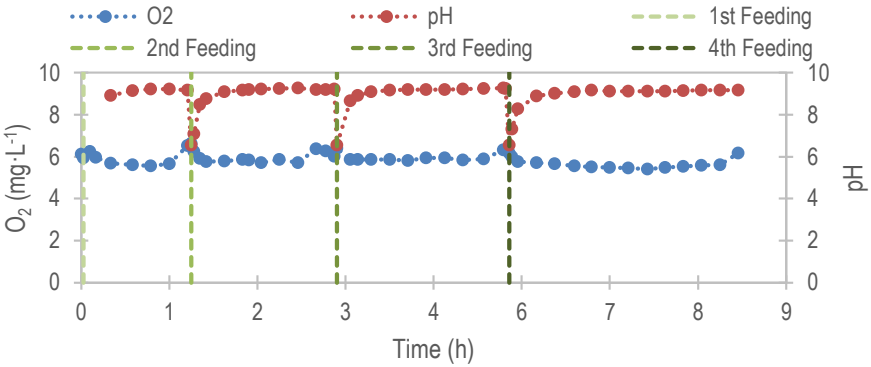


Figure 19. *Profile of dissolved oxygen and pH of Accumulation test (Phase 2) – second accumulation*

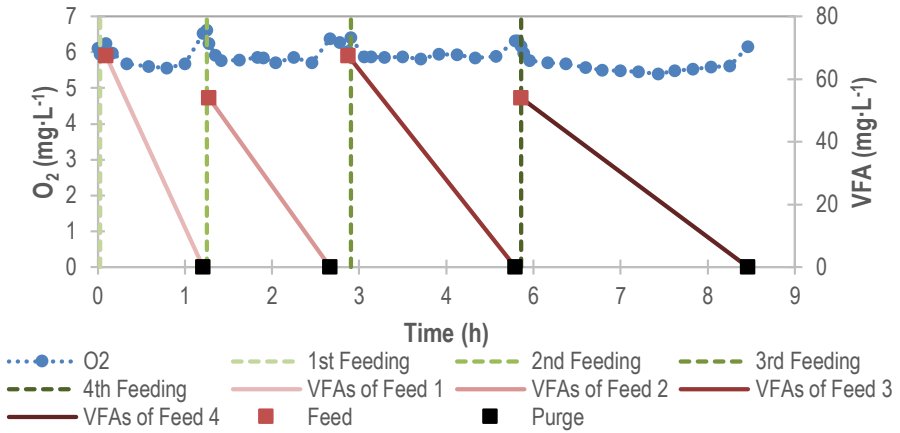


Figure 20. *Profile of dissolved oxygen and theoretical VFA concentrations – second accumulation*

Table 8. *VFAs consume rate in accumulation batch reactor test – second accumulation*

stage	VFAs consume ratio	
1	-46,6	mg VFA ·L^{-1}·h^{-1}
2	-32,4	mg VFA ·L^{-1}·h^{-1}
3	-29,3	mg VFA ·L^{-1}·h^{-1}
4	-17,3	mg VFA ·L^{-1}·h^{-1}

Figure 29 illustrates that in each addition the microorganisms took longer to consume the VFA, being in accordance with the previous accumulation test (Figure 17). Furthermore, it was observed again that the process takes approximately the same time than the previous test. If more information is required, Table 10 of Appendix 4 can be consulted.

Besides, Figure 20 and Table 8 show that the VFA consume speed was lower as another dose of synthetic feeding was added as was observed in the previous assay.

Although, the PHA obtaining in accumulation batch reactor test monitoring was not able to be analysed, it had proceeded to roughly estimate the result that should be obtained. Since the both accumulation tests were similar, probably both should have the same PHA production. To

estimate the maximum PHA content in both accumulations tests, similar studies of PHA production was researched (Table 9):

***Table 9**. Parameters monitored in the accumulations tests.*

Parameters	References					
	(Perez, 2019)	(Perez, 2019)	(Valentino et al. 2014)	(Albuquerque, Torres, y Reis 2010)	(Villano et al. 2014)	(Campanari et al. 2014)
OLR (g COD $\cdot L^{-1} \cdot d^{-1}$)	5	7	8.5	8.5	8.5	4.7
C-source	Synthetic VFA mixture	Synthetic VFA mixture	Synthetic VFA mixture	Synthetic VFA mixture	Synthetic VFA mixture	fOMW *
Maxim PHA (g PHA/g VSS)	0.44 ± 0.01	0.46 ± 0.01	0.45-0.53	0.38-0.52	0.46 ± 0.02	0.32 ± 0.05

* Fermented olive oil mill wastewater

As expected, literature with the same conditions of this study was not found. Table 9 presents different maxim PHA results that were achieved with accumulation tests for different accumulation batch reactors fed with feedings like this study. Although the reactors have different ORL, it seems that the percentages of maxim PHA were similar among them. This fact seems to indicate that the maximum content of PHA in microorganisms does not depend on the ORL. A possible reason to this succeed is that the microorganisms have the same PHA storage capacity, though if it was used a biomass with more PHA content.

Through these data, it was concluded that the maxim PHA result that can be obtained in both accumulation tests was 0.38-0.53 g PHA / g VSS.

5. STUDY OF PHA PRODUCTION IN A MUNICIPAL WWTP

In this chapter, the possible configuration of a municipal wastewater treatment plant is discussed Figure 21 shows a typical municipal wastewater treatment plant (WWTP) (Galí, 2006)

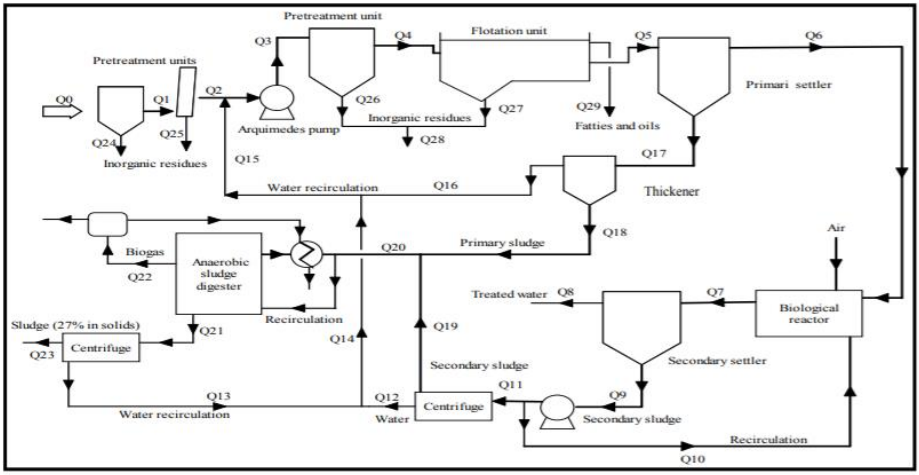


***Figure 21**. Typical municipal wastewater treatment Process (Galí, 2006)*

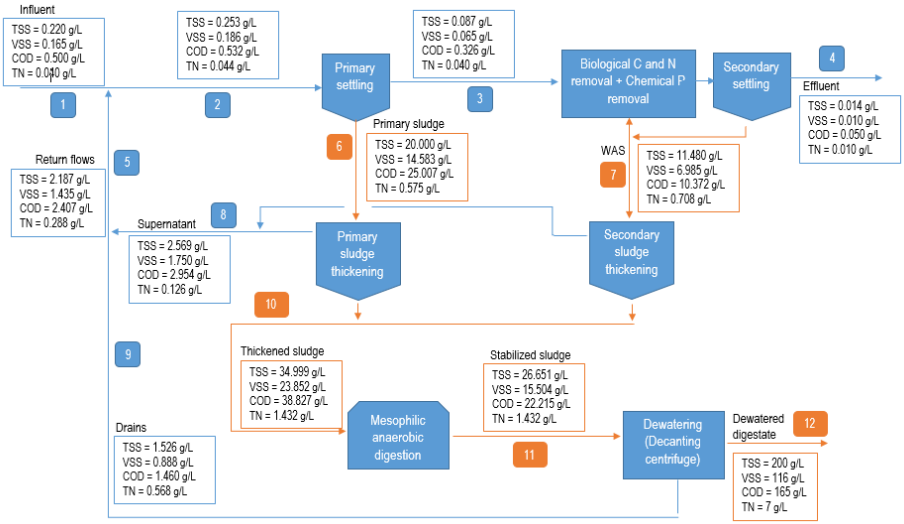
Figure 22 shows the main characteristics of the streams of a WWTP analysed by Mininni et al. (2015). The influent flowrate of the WWTP of this study consists of 136,500 m^3/d with a nominal capacity for 500,000 population equivalents (Mininni et al. 2015). As can be seen in Table 10, it falls within the range of wastewater influent flowrates that are treated in some municipal WWTPs of the Barcelona Metropolitan Area.

Table 10. *Flows and treatment capacity of Barcelona wastewater treatment AMB (2020)*

WWTP	Influent Flowrate		Treatment capacity	
Prat de Llobregat	419,904	m^3/d	2,000,000	population equivalents
Sant Feliu de Llobregat	72,000	m^3/d	320,000	population equivalents
Gavà i Viladecans	64,000	m^3/d	300,000	population equivalents
Vallvidrera	1,200	m^3/d	5,000	population equivalents
Besòs	525,000	m^3/d	3,000,000	population equivalents
Montcada i Reixac	72,000	m^3/d	360,000	population equivalents
EDAR de Begues	1,200	m^3/d	4,000	population equivalents

Considering the TSS, VSS, COD and Total Nitrogen (TN) mass balances of Minini et al. (2015) (Figure 22), it can be seen how the organic material and nitrogen are distributed in the System. In relation of chemical organic demand, in Figure 22, it was shown that in the anaerobic digestion it is treated the 58% of the COD entering with pretreated wastewater and this COD is composed by 68% of primary sludge and 32% of secondary sludge.

The PHA production process in a municipal WWTP could be carried out following the configuration proposed by Valentino et al. (2019). This alternative consists in mixing the thickened secondary sludge with organic fraction municipal solid waste (OFMSW) for PHA production. The diagram flow is presented in Figure 22:



Stream	Flowrate (m^3/d)
1	136500
2	138840.5
3	137690.8
4	136375.5
5	2348.5
6	1157.7
7	1315.3
8	1489.3
9	859.1
10	983.7
11	983.7
12	124.5

Figure 22. *Flow diagram of a municipal wastewater treatment plant (Adapted from Mininni et al. 2015)*

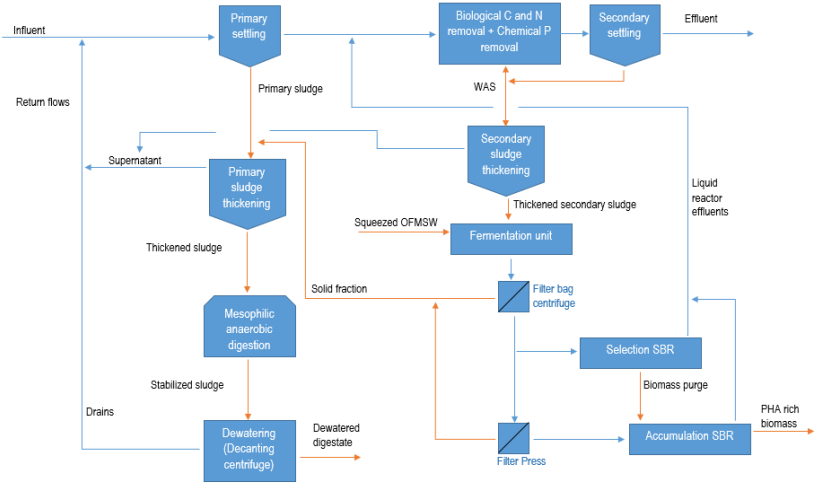


Figure 23. *Alternative municipal wastewater treatment with PHA production considering the results of Valentino et al. (2019)*

According to Valentino et al. (2019) results, secondary sludge could be mixed with an OFMSW squeezed stream (OFMSW representing the 30-35% by volume) that helps to generate more VFAs (Valentino et al. 2019). The fermentation unit could work as F-III fermentation reactor of Valentino et al (2019) and the overall process yield of PHA production from selected organic waste has been estimated to 65 g PHA/kg TVS of untreated waste stream, composed by the source sorted OFMSW and sewage sludge (Valentino et al., 2019)

The addition of the OFMSW squeezed stream involves introducing nutrients such as nitrogen and phosphorus that will not be eliminated in the PHA production process and therefore these extra nutrients addition should be treated in the same plant. However, in this study it was supposed that the higher nitrogen and phosphorous load could be treated in the same main wastewater line.

6. CONCLUSIONS

Through the work carried out with the selection operation and accumulation tests to produce PHA, various conclusions are obtained and explained above.

On the one hand, the selection reactor operation was able to observe the following conclusions:

- Nitrogen is an essential nutrient for biomass growth. If the nitrogen concentration is not enough, the bacteria growth is limited. Due to this fact, it is an important parameter to regulate in the selection process for the correct reactor operation to avoid failures in the biomass growth.
- The ratios between COD and N used in the diverse phases were too high (Phase 1.1: 56.39 g COD / g N, Phase 1.2: 48.59 g COD / g N and Phase 2: 43.00 g COD / g N). The first value calculated theoretically was lower than the value needed in the reactor operation. Even so, the two increments carried out seemed to be insufficient although an increment of biomass was presented in Phase 2.0.
- The VSS/TSS ratio was lower when the C-N coupled strategy is used in comparison to the C-N uncoupled strategy.
- The lower carbon chain acids, such as acetate acid, have a higher rate of consumption than longer chain acids, such as butyric acid.
- The selection process with an SRT of 5d responds slowly to the changes. This fact requires long periods of time to observe the results of conditions changes.
- Through the bibliography, it can be concluded that the PHA content obtained with C-N coupled strategy is half of the PHA obtained in uncoupled feeding strategy. Hence, the PHA content in the biomass purge of this study reactor in Phase 2 could be approximately 7% PHA/biomass.

- The use of an automatic monitoring system to collect the oxygen and pH data could be very beneficial. This improvement would provide reactor information constantly helping to detect failures in the system more quickly and would help to provide information more comfortably.

On the other hand, the accumulation tests carried out were useful to obtain the following conclusions:

- The VFA consumption process slows down after each addition of synthetic food, taking up to 9 hours to make 4 additions. Moreover, the VFA consumption process is slower with each synthetic feeding addition, since the increasing intracellular PHA storage.
- The maxim intercellular PHA storage seems to be intrinsic in the microorganisms, since in the bibliographic there are no chances in the PHA concentration in different ORLs. That lead to conclude that the maxim PHA content of this study reactor in the Phase 2 was 0.38-0.53 g PHA / g VSS

Finally, the PHA production process could be implemented in a municipal wastewater treatment plant using VFA produced by the acidogenic fermentation of waste secondary sludge and squeezed OFMSW.

REFERENCES AND NOTES

Albuquerque, M. G. E., S. Concas, S. Bengtsson, y M. A. M. Reis. 2010. «Mixed Culture Polyhydroxyalkanoates Production from Sugar Molasses: The Use of a 2-Stage CSTR System for Culture Selection». *Bioresource Technology* 101 (18): 7112-22. https://doi.org/10.1016/j.biortech.2010.04.019.

Albuquerque, M. G. E., M. Eiroa, C. Torres, B. R. Nunes, y M. A. M. Reis. 2007. «Strategies for the Development of a Side Stream Process for Polyhydroxyalkanoate (PHA) Production from Sugar Cane Molasses». *Journal of Biotechnology* 130 (4): 411-21. https://doi.org/10.1016/j.jbiotec.2007.05.011.

Albuquerque, M. G. E., V. Martino, E. Pollet, L. Avérous, y M. A. M. Reis. 2011. «Mixed Culture Polyhydroxyalkanoate (PHA) Production from Volatile Fatty Acid (VFA)-Rich Streams: Effect of Substrate Composition and Feeding Regime on PHA Productivity, Composition and Properties». *Journal of Biotechnology* 151 (1): 66-76. https://doi.org/10.1016/j.jbiotec.2010.10.070.

Albuquerque, M. G. E., C. A. V. Torres, y M. A. M. Reis. 2010. «Polyhydroxyalkanoate (PHA) Production by a Mixed Microbial Culture Using Sugar Molasses: Effect of the Influent Substrate Concentration on Culture Selection». *Water Research* 44 (11): 3419-33. https://doi.org/10.1016/j.watres.2010.03.021.

AMB (2020) accessed at 31th of August of 2020. https://www.amb.cat/web/ecologia/aigua/instalacions-i-equipaments/llistat?p_p_id=EquipamentSearchListPortlet_WAR_AMBSearchPortletportlet&p_p_lifecycle=1&p_p_state=normal&_EquipamentSearchListPortlet_WAR_AMBSearchPortletportlet_pageNum=1&_EquipamentSearchListPortlet_WAR_AMBSearchPortletportlet_subambit=medi_ambient.aigua_xarxapotable&_EquipamentSearchListPortlet_WAR_AMBSearchPortletportlet_format=map&_EquipamentSearchListPortlet_WAR_AMBSearchPortletportlet_type=medi_ambient.aigua_depuradora.

Amelia, Tan Suet May, Sharumathiy Govindasamy, Arularasu Muthaliar Tamothran, Sevakumaran Vigneswari, y Kesaven Bhubalan. 2019. «Applications of PHA in Agriculture». En *Biotechnological Applications of Polyhydroxyalkanoates*, editado por Vipin Chandra Kalia, 347-61. Singapore: Springer. https://doi.org/10.1007/978-981-13-3759-8_13.

Anderson, A. J., y E. A. Dawes. 1990. «Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates.» *Microbiology and Molecular Biology Reviews* 54 (4): 450-72.

APHA (2005). Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington, DC.

Basak, Bertan, Orhan Ince, Nazik Artan, Nevin Yagci, y Bahar Kasapgil Ince. 2011. «Effect of Nitrogen Limitation on Enrichment of Activated Sludge for PHA Production».

Bioprocess and Biosystems Engineering 34 (8): 1007-16. https://doi.org/10.1007/s00449-011-0551-x.

Beccari, M., L. Bertin, D. Dionisi, F. Fava, S. Lampis, M. Majone, F. Valentino, G. Vallini, y M. Villano. 2009. «Exploiting Olive Oil Mill Effluents as a Renewable Resource for Production of Biodegradable Polymers through a Combined Anaerobic–Aerobic Process». *Journal of Chemical Technology & Biotechnology* 84 (6): 901-8. https://doi.org/10.1002/jctb.2173.

Beccari, M., M. Majone, P. Massanisso, y R. Ramadori. 1998. «A Bulking Sludge with High Storage Response Selected under Intermittent Feeding». *Water Research* 32 (11): 3403-13. https://doi.org/10.1016/S0043-1354(98)00100-6.

Bengtsson, Simon, Ana R. Pisco, Peter Johansson, Paulo C. Lemos, y Maria A. M. Reis. 2010. «Molecular Weight and Thermal Properties of Polyhydroxyalkanoates Produced from Fermented Sugar Molasses by Open Mixed Cultures». *Journal of Biotechnology* 147 (3): 172-79. https://doi.org/10.1016/j.jbiotec.2010.03.022.

Bengtsson, Simon, Alan Werker, Magnus Christensson, y Thomas Welander. 2008. «Production of Polyhydroxyalkanoates by Activated Sludge Treating a Paper Mill Wastewater». *Bioresource Technology* 99 (3): 509-16. https://doi.org/10.1016/j.biortech.2007.01.020.

Brigham, Christopher John, y Anthony J. Sinskey. 2012. «Applications of Polyhydroxyalkanoates in the Medical Industry». *International Journal of Biotechnology for Wellness Industries* 1 (1): 52-60.

Bugnicourt, Elodie, Patrizia Cinelli, Andrea Lazzeri, y Vera Alejandra Alvarez. 2014. «Polyhydroxyalkanoate (PHA): Review of Synthesis, Characteristics, Processing and Potential Applications in Packaging», June. https://doi.org/10.3144/expresspolymlett.2014.82.

Campanari, Sabrina, Floriana Augelletti, Simona Rossetti, Fabio Sciubba, Marianna Villano, y Mauro Majone. 2017. «Enhancing a Multi-Stage Process for Olive Oil Mill Wastewater Valorization towards Polyhydroxyalkanoates and Biogas Production». *Chemical Engineering Journal* 317 (June): 280-89. https://doi.org/10.1016/j.cej.2017.02.094.

Campanari, Sabrina, Francisca A. e Silva, Lorenzo Bertin, Marianna Villano, y Mauro Majone. 2014. «Effect of the Organic Loading Rate on the Production of Polyhydroxyalkanoates in a Multi-Stage Process Aimed at the Valorization of Olive Oil Mill Wastewater». *International Journal of Biological Macromolecules*, Special Issue: Biodegradable biopolymers, 71 (November): 34-41. https://doi.org/10.1016/j.ijbiomac.2014.06.006.

Chanprateep, Suchada. 2010. «Current Trends in Biodegradable Polyhydroxyalkanoates». *Journal of Bioscience and Bioengineering* 110 (6): 621-32. https://doi.org/10.1016/j.jbiosc.2010.07.014.

Chen, Guo-Qiang. 2009. «A Microbial Polyhydroxyalkanoates (PHA) Based Bio- and Materials Industry». *Chemical Society Reviews* 38 (8): 2434-46. https://doi.org/10.1039/B812677C.

Chen, Hong, y Xiaolin Li. 2008. «Effect of Static Magnetic Field on Synthesis of Polyhydroxyalkanoates from Different Short-Chain Fatty Acids by Activated Sludge». *Bioresource Technology* 99 (13): 5538-44. https://doi.org/10.1016/j.biortech.2007.10.047.

Chen, Zhiqiang, Zirui Guo, Qinxue Wen, Long Huang, Rune Bakke, y Maoan Du. 2015. «A New Method for Polyhydroxyalkanoate (PHA) Accumulating Bacteria Selection under Physical Selective Pressure». *International Journal of Biological Macromolecules* 72 (enero): 1329-34. https://doi.org/10.1016/j.ijbiomac.2014.10.027.

Chua, Adeline S. M, Hiroo Takabatake, Hiroyasu Satoh, y Takashi Mino. 2003. «Production of Polyhydroxyalkanoates (PHA) by Activated Sludge Treating Municipal Wastewater: Effect of PH, Sludge Retention Time (SRT), and Acetate Concentration in Influent». *Water Research* 37 (15): 3602-11. https://doi.org/10.1016/S0043-1354(03)00252-5.

Coats, Erik R., Frank J. Loge, Michael P. Wolcott, Karl Englund, y Armando G. McDonald. 2007. «Synthesis of Polyhydroxyalkanoates in Municipal Wastewater Treatment». *Water Environment Research* 79 (12): 2396-2403. https://doi.org/10.2175/106143007X183907.

Daigger, Glen T., y C. P. Leslie Grady. 1982. «The Dynamics of Microbial Growth on Soluble Substrates: A Unifying Theory». *Water Research* 16 (4): 365-82. https://doi.org/10.1016/0043-1354(82)90159-2.

Dapena-Mora, Ana, Stijn WH Van Hulle, J. Luis Campos, Ramón Méndez, Peter A. Vanrolleghem, y Mike Jetten. 2004. «Enrichment of Anammox Biomass from Municipal Activated Sludge: Experimental and Modelling Results». *Journal of Chemical Technology & Biotechnology* 79 (12): 1421-28. https://doi.org/10.1002/jctb.1148.

Dias, João M. L., Paulo C. Lemos, Luísa S. Serafim, Cristina Oliveira, Marta Eiroa, Maria G. E. Albuquerque, Ana M. Ramos, Rui Oliveira, y Maria A. M. Reis. 2006. «Recent Advances in Polyhydroxyalkanoate Production by Mixed Aerobic Cultures: From the Substrate to the Final Product». *Macromolecular Bioscience* 6 (11): 885-906. https://doi.org/10.1002/mabi.200600112.

Dionisi, D., G. Carucci, M. Petrangeli Papini, C. Riccardi, M. Majone, y F. Carrasco. 2005. «Olive Oil Mill Effluents as a Feedstock for Production of Biodegradable Polymers». *Water Research* 39 (10): 2076-84. https://doi.org/10.1016/j.watres.2005.03.011.

Dionisi, Davide, Mauro Majone, Viviana Papa, y Mario Beccari. 2004. «Biodegradable Polymers from Organic Acids by Using Activated Sludge Enriched by Aerobic Periodic Feeding». *Biotechnology and Bioengineering* 85 (6): 569-79. https://doi.org/10.1002/bit.10910.

Dionisi, Davide, Mauro Majone, Giovanni Vallini, Simona Di Gregorio, y Mario Beccari. 2006. «Effect of the Applied Organic Load Rate on Biodegradable Polymer Production by Mixed Microbial Cultures in a Sequencing Batch Reactor». *Biotechnology and Bioengineering* 93 (1): 76-88. https://doi.org/10.1002/bit.20683.

Dosta , J., Mata, J., 2017. «La obtención de bioplásticos a partir de materia orgánica residual y el proyecto RES URBIS». *Retema: Revista técnica de medio ambiente* 29 (197): 88-93.

Dosta, J., A. Martin-Ryals, M. Garrigó, V. Ortiz-Roca, I. Fernández, R. Torres-Castillo, y J. Mata-Álvarez. 2018. «Acidogenic Fermentation and Anaerobic Co-Digestion of Mechanically Sorted OFMSW and Polyethylene Glycol». *Waste and Biomass Valorization* 9 (12): 2319-26. https://doi.org/10.1007/s12649-018-0294-x.

Duque, Anouk F., Catarina S. S. Oliveira, Inês T. D. Carmo, Ana R. Gouveia, Filipa Pardelha, Ana M. Ramos, y Maria A. M. Reis. 2014. «Response of a Three-Stage Process for

PHA Production by Mixed Microbial Cultures to Feedstock Shift: Impact on Polymer Composition». *New Biotechnology*, Polyhydroxyalkanoate (PHA) by Mixed Microbial Cultures: Fermentation, Control and Downstream Processing, 31 (4): 276-88. https://doi.org/10.1016/j.nbt.2013.10.010.

«Environmental Biotechnology: Principles and Applications - PDF Free Download». s. f. Epdf.Pub. Accedido 20 de abril de 2020. https://epdf.pub/environmental-biotechnology-principles-and-applications8a39108418890fdace465348b092d10165536.html.

Galí (2006) Optimisation of biological nitrogen removal processes to treat reject water from anaerobic digestion of sewage sludge. Doctoral Thesis. Universitat de Barcelona. Available on line: http://hdl.handle.net/10803/1527 [Accessed 09/2020]

Hao, Jiuxiao, Hui Wang, y Xiujin Wang. 2018. «Selecting Optimal Feast-to-Famine Ratio for a New Polyhydroxyalkanoate (PHA) Production System Fed by Valerate-Dominant Sludge Hydrolysate». *Applied Microbiology and Biotechnology* 102 (7): 3133-43. https://doi.org/10.1007/s00253-018-8799-6.

Hong, Chen, Hao Huang, y Haiyun wu. 2009. «Process optimization for PHA production by activated sludge using response surface methodology». *Biomass and Bioenergy* 33 (abril): 721-27. https://doi.org/10.1016/j.biombioe.2008.11.004.

Ince, Orhan, Bertan Basak, Bahar Kasapgil Ince, Zeynep Cetecioglu, Sükriye Celikkol, y Mustafa Kolukirik. 2012. «Effect of nitrogen deficiency during SBR operation on PHA storage and microbial diversity». *Environmental Technology* 33 (16): 1827-37. https://doi.org/10.1080/09593330.2011.634441.

Jiang, Yang, Leonie Marang, Robbert Kleerebezem, Gerard Muyzer, y Mark C. M. van Loosdrecht. 2011. «Effect of Temperature and Cycle Length on Microbial Competition in PHB-Producing Sequencing Batch Reactor». *The ISME Journal* 5 (5): 896-907. https://doi.org/10.1038/ismej.2010.174.

Johnson, Katja, Jorien van Geest, Robbert Kleerebezem, y Mark C. M. van Loosdrecht. 2010. «Short- and Long-Term Temperature Effects on Aerobic Polyhydroxybutyrate Producing Mixed Cultures». *Water Research* 44 (6): 1689-1700. https://doi.org/10.1016/j.watres.2009.11.022.

Johnson, Katja, Yang Jiang, Robbert Kleerebezem, Gerard Muyzer, y Mark C. M. van Loosdrecht. 2009. «Enrichment of a Mixed Bacterial Culture with a High Polyhydroxyalkanoate Storage Capacity». *Biomacromolecules* 10 (4): 670-76. https://doi.org/10.1021/bm8013796.

Johnson, Katja, Robbert Kleerebezem, y Mark C. M. van Loosdrecht. 2010. «Influence of the C/N Ratio on the Performance of Polyhydroxybutyrate (PHB) Producing Sequencing Batch Reactors at Short SRTs». *Water Research* 44 (7): 2141-52. https://doi.org/10.1016/j.watres.2009.12.031.

Khanna, Shilpi, y Ashok K. Srivastava. 2005. «A Simple Structured Mathematical Model for Biopolymer (PHB) Production». *Biotechnology Progress* 21 (3): 830-38. https://doi.org/10.1021/bp0495769.

Korkakaki, Emmanouela, Mark C. M. van Loosdrecht, y Robbert Kleerebezem. 2016. «Survival of the Fastest: Selective Removal of the Side Population for Enhanced PHA Production in a Mixed Substrate Enrichment». *Bioresource Technology* 216 (septiembre): 1022-29. https://doi.org/10.1016/j.biortech.2016.05.125.

Lee, Sang Yup. 1996. «Bacterial Polyhydroxyalkanoates». *Biotechnology and Bioengineering* 49 (1): 1-14. https://doi.org/10.1002/(SICI)1097-0290(19960105)49:1<1::AID-BIT1>3.0.CO;2-P.

Lee, Sang Yup, y Jong-il Choi. 1999. «Production and Degradation of Polyhydroxyalkanoates in Waste Environment». *Waste Management* 19 (2): 133-39. https://doi.org/10.1016/S0956-053X(99)00005-7.

Lee, Wee Shen, Adeline Seak May Chua, Hak Koon Yeoh, Tadashi Nittami, y Gek Cheng Ngoh. 2015. «Strategy for the Biotransformation of Fermented Palm Oil Mill Effluent into Biodegradable Polyhydroxyalkanoates by Activated Sludge». *Chemical Engineering Journal* 269 (junio): 288-97. https://doi.org/10.1016/j.cej.2015.01.103.

Lemos, Paulo C., Luísa S. Serafim, y Maria A. M. Reis. 2006. «Synthesis of Polyhydroxyalkanoates from Different Short-Chain Fatty Acids by Mixed Cultures Submitted to Aerobic Dynamic Feeding». *Journal of Biotechnology* 122 (2): 226-38. https://doi.org/10.1016/j.jbiotec.2005.09.006.

Lorini, Laura, Francesco di Re, Mauro Majone, y Francesco Valentino. 2020. «High Rate Selection of PHA Accumulating Mixed Cultures in Sequencing Batch Reactors with Uncoupled Carbon and Nitrogen Feeding». *New Biotechnology* 56 (mayo): 140-48. https://doi.org/10.1016/j.nbt.2020.01.006.

Martins, António M. P., Özlem Karahan, y Mark C. M. van Loosdrecht. 2011. «Effect of Polymeric Substrate on Sludge Settleability». *Water Research* 45 (1): 263-73. https://doi.org/10.1016/j.watres.2010.07.055.

Mexpolimeros. s. f. «PHA | Polihidroxialcanoatos». Polimeros termoplasticos,elastomeros y aditivos. Accedido 29 de junio de 2020. https://www.mexpolimeros.com/pha.html.

Mininni, G., G. Laera, G. Bertanza, M. Canato, y A. Sbrilli. 2015. «Mass and Energy Balances of Sludge Processing in Reference and Upgraded Wastewater Treatment Plants». *Environmental Science and Pollution Research* 22 (10): 7203-15. https://doi.org/10.1007/s11356-014-4013-2.

Misra, Superb K., Sabeel P. Valappil, Ipsita Roy, y Aldo R. Boccaccini. 2006. «Polyhydroxyalkanoate (PHA)/Inorganic Phase Composites for Tissue Engineering Applications». *Biomacromolecules* 7 (8): 2249-58. https://doi.org/10.1021/bm060317c.

Mitomo, H, T Takahashi, H Ito, y T Saito. 1999. «Biosynthesis and Characterization of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) Produced by Burkholderia Cepacia D1». *International Journal of Biological Macromolecules* 24 (4): 311-18. https://doi.org/10.1016/S0141-8130(98)00064-6.

Montiel-Jarillo, Gabriela, Julián Carrera, y María Eugenia Suárez-Ojeda. 2017. «Enrichment of a Mixed Microbial Culture for Polyhydroxyalkanoates Production: Effect of PH and N and P Concentrations». *Science of The Total Environment* 583 (abril): 300-307. https://doi.org/10.1016/j.scitotenv.2017.01.069.

Morgan-Sagastume, F., F. Valentino, M. Hjort, D. Cirne, L. Karabegovic, F. Gerardin, P. Johansson, et al. 2014. «Polyhydroxyalkanoate (PHA) Production from Sludge and Municipal Wastewater Treatment». *Water Science and Technology* 69 (1): 177-84. https://doi.org/10.2166/wst.2013.643.

Możejko, Justyna, y Slawomir Ciesielski. 2013. «Saponified Waste Palm Oil as an Attractive Renewable Resource for Mcl-Polyhydroxyalkanoate Synthesis». *Journal of*

Bioscience and Bioengineering 116 (4): 485-92. https://doi.org/10.1016/j.jbiosc.2013.04.014.

Norhafini, Hambali, Kai-Hee Huong, y A. A. Amirul. 2019. «High PHA Density Fed-Batch Cultivation Strategies for 4HB-Rich P(3HB-Co-4HB) Copolymer Production by Transformant Cupriavidus Malaysiensis USMAA1020». *International Journal of Biological Macromolecules* 125 (marzo): 1024-32. https://doi.org/10.1016/j.ijbiomac.2018.12.121.

Ojumu, T. V., J. Yu, y B. O. Solomon. 2004. «Production of Polyhydroxyalkanoates, a Bacterial Biodegradable Polymers». *African Journal of Biotechnology* 3 (1): 18-24. https://doi.org/10.4314/ajb.v3i1.14910.

Oliveira, Catarina S. S., Carlos E. Silva, Gilda Carvalho, y Maria A. Reis. 2017. «Strategies for Efficiently Selecting PHA Producing Mixed Microbial Cultures Using Complex Feedstocks: Feast and Famine Regime and Uncoupled Carbon and Nitrogen Availabilities». *New Biotechnology*, Biopolymers Eu Symposium, 37 (julio): 69-79. https://doi.org/10.1016/j.nbt.2016.10.008.

Pérez, N. (2019) Posada en marxa de bioreactors per a la producció de bioplàstics a partir de residus orgànics. Treball de Fi de Grau. Universitat de Barcelona (UB), Barcelona, Catalonia.

Pérez, Rebeca, Sara Cantera, Sergio Bordel, Pedro A. García-Encina, y Raúl Muñoz. 2019. «The Effect of Temperature during Culture Enrichment on Methanotrophic Polyhydroxyalkanoate Production». *International Biodeterioration & Biodegradation* 140 (mayo): 144-51. https://doi.org/10.1016/j.ibiod.2019.04.004.

Poirier, Yves, Christianae Nawrath, y Chris Somerville. 1995. «Production of Polyhydroxyalkanoates, a Family of Biodegradable Plastics and Elastomers, in Bacteria and Plants». *Bio/Technology* 13 (2): 142-50. https://doi.org/10.1038/nbt0295-142.

Queirós, Diogo, Simona Rossetti, y Luísa S. Serafim. 2014. «PHA Production by Mixed Cultures: A Way to Valorize Wastes from Pulp Industry». *Bioresource Technology* 157 (abril): 197-205. https://doi.org/10.1016/j.biortech.2014.01.099.

Reis, M., M. Albuquerque, M. Villano, y M. Majone. 2011. «6.51 - Mixed Culture Processes for Polyhydroxyalkanoate Production from Agro-Industrial Surplus/Wastes as Feedstocks». En *Comprehensive Biotechnology (Second Edition)*, editado por Murray Moo-Young, 669-83. Burlington: Academic Press. https://doi.org/10.1016/B978-0-08-088504-9.00464-5.

Ren, Qun, Andreas Grubelnik, Mirjam Hoerler, Katinka Ruth, René Hartmann, Helene Felber, y Manfred Zinn. 2005. «Bacterial Poly(hydroxyalkanoates) as a Source of Chiral Hydroxyalkanoic Acids». *Biomacromolecules* 6 (4): 2290-98. https://doi.org/10.1021/bm050187s.

Serafim, Luísa S., Paulo C. Lemos, Rui Oliveira, y Maria A. M. Reis. 2004. «Optimization of Polyhydroxybutyrate Production by Mixed Cultures Submitted to Aerobic Dynamic Feeding Conditions». *Biotechnology and Bioengineering* 87 (2): 145-60. https://doi.org/10.1002/bit.20085.

Serafim, Luísa S., Paulo C. Lemos, Cristiana Torres, Maria A. M. Reis, y Ana M. Ramos. 2008. «The Influence of Process Parameters on the Characteristics of Polyhydroxyalkanoates Produced by Mixed Cultures». *Macromolecular Bioscience* 8 (4): 355-66. https://doi.org/10.1002/mabi.200700200.

Sierra, J, E Martı, G Montserrat, R Cruañas, y M. A Garau. 2001. «Characterisation and Evolution of a Soil Affected by Olive Oil Mill Wastewater Disposal». *Science of The Total Environment* 279 (1): 207-14. https://doi.org/10.1016/S0048-9697(01)00783-5.

Silva, Fernando, Sabrina Campanari, Stefania Matteo, Francesco Valentino, Mauro Majone, y Marianna Villano. 2017. «Impact of Nitrogen Feeding Regulation on Polyhydroxyalkanoates Production by Mixed Microbial Cultures». *New Biotechnology*, Biopolymers Eu Symposium, 37 (July): 90-98. https://doi.org/10.1016/j.nbt.2016.07.013.

Sudesh, Kumar. 2013. «Potential Applications of PHA». En *Polyhydroxyalkanoates from Palm Oil: Biodegradable Plastics*, editado por Kumar Sudesh, 79-100. SpringerBriefs in Microbiology. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-33539-6_6.

Valentino, Francesco, Mario Beccari, Serena Fraraccio, Giulio Zanaroli, y Mauro Majone. 2014. «Feed Frequency in a Sequencing Batch Reactor Strongly Affects the Production of Polyhydroxyalkanoates (PHAs) from Volatile Fatty Acids». *New Biotechnology*, Polyhydroxyalkanoate (PHA) by Mixed Microbial Cultures: Fermentation, Control and Downstream Processing, 31 (4): 264-75. https://doi.org/10.1016/j.nbt.2013.10.006.

Valentino, Francesco, Lamija Karabegovic, Mauro Majone, Fernando Morgan-Sagastume, y Alan Werker. 2015. «Polyhydroxyalkanoate (PHA) Storage within a Mixed-Culture Biomass with Simultaneous Growth as a Function of Accumulation Substrate Nitrogen and Phosphorus Levels». *Water Research* 77 (June): 49-63. https://doi.org/10.1016/j.watres.2015.03.016.

Valentino, Francesco, Giulia Moretto, Laura Lorini, David Bolzonella, Paolo Pavan, y Mauro Majone. 2019. «Pilot-Scale Polyhydroxyalkanoate Production from Combined Treatment of Organic Fraction of Municipal Solid Waste and Sewage Sludge». *Industrial & Engineering Chemistry Research* 58 (27): 12149-58. https://doi.org/10.1021/acs.iecr.9b01831.

Valentino, Francesco, Fernando Morgan-Sagastume, Sabrina Campanari, Marianna Villano, Alan Werker, y Mauro Majone. 2017. «Carbon Recovery from Wastewater through Bioconversion into Biodegradable Polymers». *New Biotechnology*, Biopolymers Eu Symposium, 37 (June): 9-23. https://doi.org/10.1016/j.nbt.2016.05.007.

Valentino, Francesco, Carmela Riccardi, Sabrina Campanari, Donatella Pomata, y Mauro Majone. 2015. «Fate of β-Hexachlorocyclohexane in the Mixed Microbial Cultures (MMCs) Three-Stage Polyhydroxyalkanoates (PHA) Production Process from Cheese Whey». *Bioresource Technology* 192 (September): 304-11. https://doi.org/10.1016/j.biortech.2015.05.083.

Venkateswar Reddy, M., y S. Venkata Mohan. 2012. «Effect of Substrate Load and Nutrients Concentration on the Polyhydroxyalkanoates (PHA) Production Using Mixed Consortia through Wastewater Treatment». *Bioresource Technology* 114 (June): 573-82. https://doi.org/10.1016/j.biortech.2012.02.127.

Villano, Marianna, Mario Beccari, Davide Dionisi, Silvia Lampis, Alfredo Miccheli, Giovanni Vallini, y Mauro Majone. 2010. «Effect of PH on the Production of Bacterial Polyhydroxyalkanoates by Mixed Cultures Enriched under Periodic Feeding». *Process Biochemistry* 45 (5): 714-23. https://doi.org/10.1016/j.procbio.2010.01.008.

Villano, Marianna, Francesco Valentino, Andrea Barbetta, Lucrezia Martino, Mariastella Scandola, y Mauro Majone. 2014. «Polyhydroxyalkanoates Production with Mixed Microbial Cultures: From Culture Selection to Polymer Recovery in a High-Rate Continuous Process». *New Biotechnology*, Polyhydroxyalkanoate (PHA) by Mixed Microbial Cultures: Fermentation, Control and Downstream Processing, 31 (4): 289-96. https://doi.org/10.1016/j.nbt.2013.08.001.

Wang, Xiaofei, Adrian Oehmen, Elisabete B. Freitas, Gilda Carvalho, y Maria A. M. Reis. 2017. «The Link of Feast-Phase Dissolved Oxygen (DO) with Substrate Competition and Microbial Selection in PHA Production». *Water Research* 112 (Abril): 269-78. https://doi.org/10.1016/j.watres.2017.01.064.

Wen, Qinxue, Zhiqiang Chen, Changyong Wang, y Nanqi Ren. 2012. «Bulking Sludge for PHA Production: Energy Saving and Comparative Storage Capacity with Well-Settled Sludge». *Journal of Environmental Sciences* 24 (10): 1744-52. https://doi.org/10.1016/S1001-0742(11)61005-X.

Williams, Simon F, David P Martin, Daniel M Horowitz, y Oliver P Peoples. 1999. «PHA Applications: Addressing the Price Performance Issue: I. Tissue Engineering». *International Journal of Biological Macromolecules* 25 (1): 111-21. https://doi.org/10.1016/S0141-8130(99)00022-7.

Zhang, Junyu, Ekaterina I. Shishatskaya, Tatiana G. Volova, Luiziana Ferreira da Silva, y Guo-Qiang Chen. 2018. «Polyhydroxyalkanoates (PHA) for Therapeutic Applications». *Materials Science and Engineering: C* 86 (May): 144-50. https://doi.org/10.1016/j.msec.2017.12.035.

ACRONYMS

ADD aerobic dynamic discharge

COD chemical oxygen demand

fOMW: fermented olive oil mill wastewater

FF feast and famine strategy

HB hydroxybutyrate

HRT hydraulic retention time

HV hydroxyvalerate

MMC mixed microbial culture

OFMSW organic fraction municipal solid waste

OLR organic loading ratio

OOMW olive oil mill wastewater

OUR specific oxygen up-take rate

PHA polyhydroxyalkanoates

PHB polyhydroxybutyrate

P(HB/HV) poly(3-hydroxybutyrate/3-hudroxyvalerate)

PHV polyhydroxyvalerate

SBR sequencing batch reactor

SRT sludge retention time

SS secondary sludge

VFA volatile fatty acids

APPENDICES

APPENDIX 1: CALCULATIONS

Volatile fatty acid calculation:

The organic loading that is worked is 3.5 COD·L^{-1}·day^{-1}:

The volatile fatty acid proportions in the synthetic feed are 62.5% acetic acid, 18.8% propionic acid and 18.8% butyric acid in the total concentration.

$$\frac{0.625\cdot x\,g\,acetic\,acid}{L}\cdot\frac{1\,mol\,acetic\,acid}{60.05\,g\,acetic\,acid}\cdot\frac{2\,mol\,O_2}{1\,mol\,acetic\,acid}\cdot\frac{32\,g\,O_2}{1\,mol\,O_2}=\frac{0.666\cdot x\,g\,O_2}{L}$$

$$\frac{0.188\cdot x\,g\,propionic\,acid}{L}\cdot\frac{1\,mol\,propionic\,acid}{74.04\,g\,propionic\,acid}\cdot\frac{3.5\,mol\,O_2}{1\,mol\,propionic\,acid}\cdot\frac{32\,g\,O_2}{1\,mol\,O_2}=\frac{0.284\cdot x\,g\,O_2}{L}$$

$$\frac{0.188\cdot x\,g\,butyric\,acid}{L}\cdot\frac{1\,mol\,butyric\,acid}{88.05\,g\,butyric\,acid}\cdot\frac{5\,mol\,O_2}{1\,mol\,butyric\,acid}\cdot\frac{32\,g\,O_2}{1\,mol\,O_2}=\frac{0.342\cdot x\,g\,O_2}{L}$$

The sum of the chemical oxygen demand of this three VFA must be 3.5 COD·L^{-1}·day^{-1}:

$$0.666\cdot x+0.284\cdot x+0.342\cdot x=3.5$$

$$1.292\,x=3.5$$

$$x=2.709$$

Substituting the unknown, the concentration of each acid was obtained:

$$acetic\,acid\,concentration: 0.625\cdot x\,g\cdot L^{-1}=0.625\cdot 2.71\,g\cdot L^{-1}=1.69\,g\cdot L^{-1}$$

$$propionic\,acid\,concentration: 0.188\cdot x\,g\cdot L^{-1}=0.188\cdot 2.71\,g\cdot L^{-1}=0.51\,g\cdot L^{-1}$$

$$butyric\,acid\,concentration: 0.188\cdot x\,g\cdot L^{-1}=0.188\cdot 2.71\,g\cdot L^{-1}=0.51\,g\cdot L^{-1}$$

NH_4Cl calculation:

The daily COD that the reactor works is calculated:

$$acetic\ acid\ COD = \frac{ci}{HRT} = \frac{0.666 \cdot x}{HRT} = \frac{0.666 \cdot 2.71\ g\ O_2 \cdot L^{-1}}{1\ day} = 1.556\ g\ COD \cdot L^{-1} \cdot day^{-1}$$

$$propionic\ acid\ COD = \frac{ci}{HRT} = \frac{0.284 \cdot x}{HRT} = \frac{0.284 \cdot 2.71\ g\ O_2 \cdot L^{-1}}{1.16\ day} = 0.663\ g\ COD \cdot L^{-1} \cdot day^{-1}$$

$$butyric\ acid\ COD = \frac{ci}{HRT} = \frac{0.342 \cdot x}{HRT} = \frac{0.342 \cdot 2.71\ g\ O_2 \cdot L^{-1}}{1.16\ day} = 0.799\ g\ COD \cdot L^{-1} \cdot day^{-1}$$

$$1.556\ g\ COD \cdot L^{-1} \cdot day^{-1} + 0.663\ g\ COD \cdot L^{-1} \cdot day^{-1} + 0.799\ g\ COD \cdot L^{-1} \cdot day^{-1} = 3.018\ g\ COD \cdot L^{-1} \cdot day^{-1}$$

Then, observed heterotrophic yield was calculated using a theoretical Y_H value of 0.35:

$$Y_{OBS} = \frac{Y_H}{\left(1 + k_d \cdot \theta_x\right)}$$

$$Y_{OBS} = \frac{0.35}{(1 + 0.1 \cdot 5)} = \frac{0.233\ g\ cellular\ COD}{g\ removed\ COD}$$

Obtained the yield, the daily grams of NH_4Cl were calculated taking into account the reactor volume:

$$3.00\ L \cdot \frac{3.018\ g\ COD}{L \cdot day} \cdot \frac{0.233\ g\ cellular\ COD}{1\ g\ COD} \cdot \frac{1\ g\ C_{25}H_7O_2N}{1.42\ g\ cellular\ COD} \cdot \frac{1\ mol\ C_{25}H_7O_2N}{113\ g\ C_{25}H_7O_2N} \cdot \frac{1\ mol\ N}{1\ mol\ C_{25}H_7O_2N} \cdot \frac{1\ mol\ NH_4Cl}{1\ mol\ N} \cdot \frac{53.5\ g\ NH_4Cl}{1\ mol\ NH_4Cl} = 0.710\ g\ NH_4Cl \cdot day^{-1}$$

The pump has a flow rate of 44 mL · min-1 working for 1 minute in each cycle. Therefore, 44 mL of NH_4Cl solution is added , for each cycle. With this data the concentration of the ammonium chloride solution must be:

$$\frac{1}{\frac{44\,mL}{\min}\cdot\frac{1\,\min}{1\,cicles}\cdot\frac{4\,cicles}{1\,day}\cdot\frac{1\,day}{0.710\,g\,NH_4Cl}\cdot\frac{1\,L}{10^3\,mL}} = 4.034\ g\,NH_4Cl\cdot L^{-1}$$

APPENDIX 2: PUMP CALIBRATION

First pump calibration:

Tabla 1. *Flow rates by carbon source feeding pump speed number*

NUMBER	V (mL)	time (s)	q (mL/s)	q (mL/min)
20	25	22,5	1,11	66,67
25	25	17,71	1,41	84,70
30	25	14,61	1,71	102,67
35	25	12,95	1,93	115,83
40	25	11,23	2,23	133,57

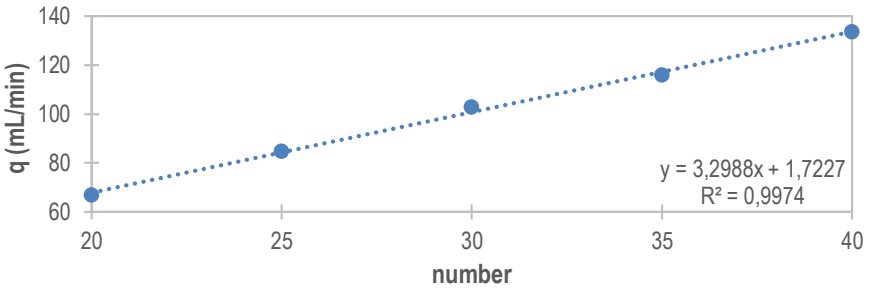


Figura 1*. Regression line of flows on the speed number of the carbon feeding pump*

Tabla 2. *Flow rates by speed of the nitrogen feeding pump*

NUMBER	V (mL)	time (s)	q (mL/s)	q (mL/min)
20	25	32,88	0,76	45,62
25	25	26,16	0,96	57,34
30	25	22,04	1,13	68,06
35	25	19	1,32	78,95
40	25	16	1,56	93,75

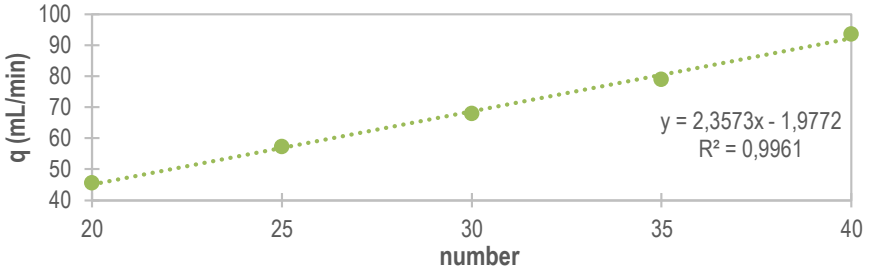


Figura 2. *Regression line of flows on the speed number of the nitrogen feeding pump*

Tabla 3. *Flow rates by purge pump speed number*

NUMBER	V (mL)	time (s)	q (mL/s)	q (mL/min)
-	25	70,72	0,35	21,21

Second calibration:

Tabla 4. *Flow rates by carbon feeding pump speed number*

NUMBER	V (mL)	time (s)	q (mL/s)	q (mL/min)
20	100	93,13	1,074	64,43
25	100	74,62	1,340	80,41
30	100	63,59	1,573	94,35
35	100	55,49	1,802	108,13
40	100	48,94	2,043	122,60
45	100	44,74	2,235	134,11
50	100	41,01	2,438	146,31
55	100	38,03	2,630	157,77
60	100	32,35	3,091	185,47
65	100	30,55	3,273	196,40
70	100	27,85	3,591	215,44

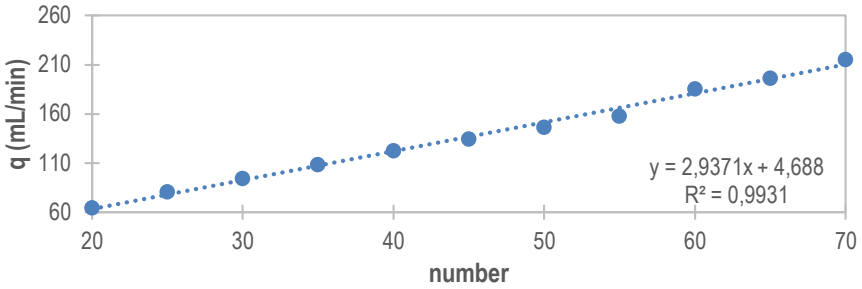


Figura 3. *Flow rates by carbon feeding pump speed number*

Tabla 5. *Flow rates by speed of the nitrogen feeding pump*

NUMBER	V (mL)	Time (s)	q (mL/s)	q (mL/min)
20	50	155,66	0,3212	19,27
25	50	125,47	0,3985	23,91
30	50	107,31	0,4659	27,96
35	50	91,92	0,5440	32,64
40	50	81,87	0,6107	36,64
45	50	74,31	0,6729	40,37
50	50	66,46	0,7523	45,14
55	50	61,45	0,8137	48,82
60	50	54,38	0,9195	55,17
65	50	50,07	0,9986	59,92
70	50	46,73	1,0700	64,20

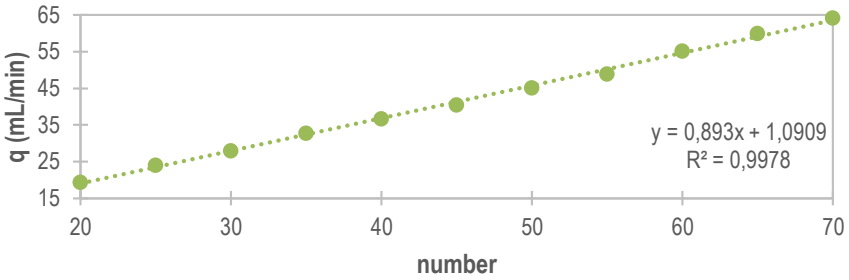


Figura 4. *Regression line of flows on the speed number of the nitrogen feeding pump*

Tabla 6. *Flow rates by purge pump speed number*

NUMBER	V (mL)	Time (s)	q (mL/s)	q (mL/min)	q media (mL/min)
-	100	106,77	0,937	56,20	
-	100	107,37	0,931	55,88	56,20
-	100	106,52	0,939	56,33	

APPENDIX 3: PARAMETERS OF SELECTION SEQUENCING BATCH REACTORS

Tabla 7. *Tracking parameters of sequencing batch reactor monitoring of 82nd day in Phase 2*

Parameter		Value	Units
Working Volume		3	L
Cycle duration		6	h
Time distribution:			
	Carbon source feeding	15	min
	Nitrogen source feeding	1	min
	Feast duration	65	min
	Purge	1	min
	Famine duration	231	min
	Sedimentation	30	min
	Effluent discharge	15	min
Feast /Cycle ratio		18	%
HRT		1.16	day
SRT		4.85	days
OLR_{VFA} (Organic Loading rate due to VFA)		3.02	g COD_{VFA}/(L day)
VFAs Feeding percentage:			
	Acetic acid	62.5	%
	Propionic acid	18.8	%
	Butyric acid	18.8	%
SS in the reactor		3100	mg TSS/L
Biomass concentration		2950	mg VSS/L
VSS/TSS		95	%
Temperature		30	ºC
DO during Feast period		(3.35-2.61)	mg O_2/L
NLR (Nitrogen Loading rate due to NH4+-N)		0.087	g N/(L day)
pH range		6.8-9.4	-
PHA content at the end of Feast			mg PHA/(g VSS)

Tabla 8. *Tracking parameters of sequencing batch reactor monitoring of 85th day in Phase 2*

Parameter		Value	Units
Working Volume		3	L
Cycle duration		6	h
Time distribution:			
	Carbon source feeding	15	min
	Nitrogen source feeding	1	min
	Feast duration	141	min
	Purge	1	min
	Famine duration	155	min
	Sedimentation	30	min
	Effluent discharge	15	min
Feast/cycle ratio		39	%
HRT		1.16	day
SRT		4.85	days
OLR_{VFA} (Organic Loading rate due to VFA)		3.5	g COD_{VFA}/(L day)
VFAs Feeding percentage:			
	Acetic acid	62.5	%
	Propionic acid	18.8	%
	Butyric acid	18.8	%
SS in the reactor		3820	mg TSS/L
Biomass concentration		3680	mg VSS/L
VSS/TSS		96	%
Temperature		30	ºC
DO during Feast period		(4.49-3.77)	mg O2/L
NLR (Nitrogen Loading rate due to NH_4^+-N)		0.087	g N/(L day)
pH range		6.7-9.1	-
PHA content at the end of Feast		-	mg PHA/(g VSS)

APPENDIX 3: PARAMETERS OF PHA ACCUMULATION BATCH REACTORS

Tabla 9. *Tracking parameters of accumulation reactor monitoring in Phase 2 – first accumulation*

Value	Units	Feeding on demand strategy	
		Initial biomass	
600	mL	V_0 (Volume of purged biomass)	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
		1st feeding	
200	mL	V_1 (WW volume added)	
800	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
1.33	h	time spent	
		2nd feeding	
200	mL	V_2 (WW volume added)	
1000	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
1.67	h	time spent	
3rd feeding (after settling and separating a clarified effluent with volume V1+V2)			

200	mL	V_3 (WW volume added)	
800	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
2.72	h	time spent	
		4th feeding	
200	mL	V_4 (WW volume added)	
1000	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
3.00	h	time spent	

Tabla 10. *Tracking parameters of accumulation reactor monitoring in Phase 2 – second accumulation*

Value	Units	Feeding on demand strategy
		Initial biomass
600	mL	V_0 (Volume of purged biomass)
-		SS
-		VSS
-		% PHA
-		% HB
-		%HV
		1st feeding
200	mL	V_1 (WW volume added)
800	mL	working volume
-		SS

		VSS	
-		% PHA	
-			% HB
-			%HV
1.45	h	time spent	
		2nd feeding	
200	mL	V_2 (WW volume added)	
1000	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
1.67	h	time spent	
3rd feeding (after settling and separating a clarified effluent with volume V_1+V_2)			
200	mL	V_3 (WW volume added)	
800	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
2.30	h	time spent	
		4th feeding	
200	mL	V_4 (WW volume added)	
1000	mL	working volume	
-		SS	
-		VSS	
-		% PHA	
-			% HB
-			%HV
3.12	h	time spent	