Integrating the selection of PHA storing biomass and nitrogen removal via nitrite for the treatment of the sludge reject water

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Integrating the selection of PHA storing biomass and nitrogen removal via nitrite for the treatment of the sludge reject water

N. Frison, E. Katsou, S. Malamis, F. Fatone

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Department of Biotechnology, University of Verona,

June 8-14, 2014
Otranto (Lecce), ITALY
Conventional plastics

- Conventional plastics require petroleum for their production
  - 4% of the global petroleum and natural gas goes for plastics production
  - Another 3-4% is consumed as energy for the production process
- 30 million tonnes of plastic waste were produced in EU in 2005
- Little amount is actually recycled
- Main environmental problems
  - Contribute to the Pacific Ocean Garbage Patch
  - Accumulate in landfills (require hundreds of years > 300 to degrade)
  - Plastic bags that end up in seas/oceans are consumed by fish and end up in the food chain

US EPA, 2007, in Morgan et al., 2010
The need....

✓ Resource consumption – PROBLEM 1
Daily crude oil consumption in 2012: 83,477 million barrels
.....Global reserve: 1,652,600 million barrels
✓ But sufficient for how long?
1,652,600,000,000 barrel / 83,477,295 barrel/day = 19.797 days
That is equal to 54.2 years
According to OPEC ~ 64 years
✓ Consumption and global reserve may fluctuate significantly

Waste production? - PROBLEM 2
The United Nations Environment Program estimated by 2006 every square mile of ocean already hosted 46,000 pieces of floating plastic

A biodegradable plastic can be the solution

© Institute for Sanitary Engineering, Water Quality and Solid Waste Management
What are bioplastics?

- Bioplastics are plastics that are either biobased (meaning derived from a renewable energy) or biodegradable or both.
- Polyhydroxyalkanoates (PHA) are both biodegradable and biobased.

Diagram:

- Non biodegradable
- Biodegradable
- Biobased
- Fossil based
- European bioplastics, 2014
What are polyhydroxyalkanoates (PHA)?

- PHA are biopolymers which are produced by specific types of microorganisms
- PHA is an intracellular energy and carbon reserve (like fat is produced in humans)
- PHA have similar properties to petrochemically derived plastics (thermoplastic)
- But PHA are also biodegradable and biocompatible
  - Time of biodegradation depends on temperature, light, moisture, exposed surface area, pH and microbial activity
  - Can degrade both under aerobic and anaerobic conditions
- Current price 3-5 € / kg
- More than 300 different microorganisms that synthesize PHA have been isolated
- Approximately 150 different types of PHA (i.e. biopolymers)

Kleerebezem et al., 2013
How are PHA produced?

- Different PHAs have different properties
  - Flexibility
  - Gas permeability
  - Temperature tolerance
- PHAs are commercially produced using expensive, pre-sterilized, high-tech equipment and substrates such as glucose by pure cultures
- As a result the production cost is significantly higher than that of conventional plastics (ten times more)

- Solutions to reduce cost:
  - Use waste material as substrate; fermented molasses, agro-industrial waste, paper mill wastewater, chocolate waste, waste glycerol, waste frying oil, food waste, olive mill wastewater, fermented sewage sludge
  - Use mixed cultures that are readily available to grow the bacteria that produce PHA (activated sludge)

- In WWTPs we have both the substrate (wastewater) and the culture (activated sludge) to produce PHA!
Methane versus PHA

- Yield of methane: 0.350 m³/kgCOD
- Methane sale price: 0.2 €/m³

- Yield of PHA: 0.35 kgPHA/kgCOD
- PHA sale price: 5 €/kg

Internal rate of return (IRR) & emissions of non-renewable CO2 eq.

Gurieff & Lant, 2007
Moving towards the biorefining concept
Producing bioplastics in WWTPs

Kleerebezem et al., 2013
Typical steps involved in PHA production in WWTPs

Step 1: Sludge Fermentation
Produce VFA

Step 2: Selection of PHA storing biomass
Select microorganisms that have a high PHA storing capacity

Step 3: Maximize PHA
Maximize the production of PHA within the biomass

Step 4: Recovery of PHA from biomass
Separate and recover the PHA from the biomass

Biomass and PHA

VFAs
VFAs
**Step 1: Fermentation to produce VFAs**

- This step is necessary when the substrate has poor content in volatile fatty acids (VFA). The hydrolysis that takes place releases VFA in the liquid phase.

- In WWTPs sewage sludge is fermented (either alkaline or acidic conditions) producing an effluent that is rich in VFAs.

**Step 2: Selection of PHA storing biomass**

- Bacteria are subjected to an alternation of high and low substrate availability under aerobic conditions.

- The feast and famine regime creates favourable conditions for microorganisms capable of storing VFAs as PHA.
Step 3: Maximize PHA within biomass

- PHA accumulation occurs when growth is limited by external factors such as a lack of nutrients or internal factors such as an insufficient amount of RNA or enzymes required for growth.

Kleerebezem et al., 2013

Johnson et al., 2009

**PHA = 89% wt**

The accumulation of PHA within the biomass is usually accomplished by feeding with carbon source (preferably VFAs) and at the same time depriving one of the key growth nutrients (nitrogen, phosphorus).

This way growth is avoided/limited and the biomass uptakes the VFAs and stores them as PHA in order to be able to use them when growth conditions will be feasible.
Step 4: Recovery of PHA from biomass

Need to separate the PHA (biopolymers) from the rest of the biomass

Table 1. PHA Recovery Methods: Advantages and Disadvantages

<table>
<thead>
<tr>
<th>method</th>
<th>advantage</th>
<th>disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent extraction</td>
<td>high product purity, no degradation of the polymer, low endotoxin content of PHA extracted from Gram-negative bacteria</td>
<td>with most solvents only applicable to small scale, often dried cells required, economically not feasible, hazardous to human beings and the environment, most suitable for lab scale</td>
</tr>
<tr>
<td>disruption by sodium hypochlorite</td>
<td>high product purity, no drying of cells necessary, applicable to large scale, applicable to environmental samples</td>
<td>digestion of the cell matter strongly exothermic, hypochlorite may digest also the polymer, thereby reducing the molecular weight</td>
</tr>
<tr>
<td>disruption by surfactants</td>
<td>PHA can be recovered directly from the culture broth, limited degradation of the polymer</td>
<td>high costs, SDS is difficult to recover and to remove from the isolated polymer</td>
</tr>
<tr>
<td>disruption by chelate-hydrogen peroxide treatment</td>
<td>high product purity</td>
<td>depending on the conditions, degradation of the polymer may occur with concomitant reduction of molecular weight</td>
</tr>
<tr>
<td>disruption by dissolution of non-PHA cell mass by acids</td>
<td>inexpensive and ecologically friendly, high yield and purity of PHA</td>
<td>acids may degrade PHA, leading to a reduced molecular weight</td>
</tr>
<tr>
<td>enzymatic cell disruption</td>
<td>high recovery rate and purity of the polymer, mild operating conditions</td>
<td>high costs for enzymes</td>
</tr>
<tr>
<td>disruption by bead mill</td>
<td>no chemicals required</td>
<td>requires efficient cooling, several passes necessary for a reasonable recovery, difficult to scale up</td>
</tr>
<tr>
<td>disruption by high pressure homogenization</td>
<td>good performance at high biomass concentrations, applicable for large scale treatment</td>
<td>poor disruption at low biomass concentrations</td>
</tr>
<tr>
<td>disruption by ultrasonication</td>
<td>combination with other extraction methods leads to high purity of the product</td>
<td>difficult to apply to large scale</td>
</tr>
<tr>
<td>supercritical fluids</td>
<td>low cost chemical treatment with moderate operating conditions, nonflammable, low toxicity and reactivity</td>
<td>requires strict process parameters, further chemicals needed for a high degree of disruption</td>
</tr>
<tr>
<td>cell fragility</td>
<td>efficient and gentle release of the polymer, high recovery and purity of the product, applicable to several bacteria</td>
<td>genetically engineered production strains required</td>
</tr>
<tr>
<td>air classification</td>
<td>high purity of the product</td>
<td>numerous steps to recover the polymer</td>
</tr>
<tr>
<td>dissolved-air flotation</td>
<td>no chemicals necessary</td>
<td>consecutive batch flotation steps required</td>
</tr>
<tr>
<td>spontaneous release of PHA granules</td>
<td>no chemicals necessary</td>
<td>genetically engineered producing strains required, not all cells may secrete PHA granules</td>
</tr>
</tbody>
</table>
Different PHA yields achieved

<table>
<thead>
<tr>
<th>Substrate used</th>
<th>PHA yield (gPHA/gTSS)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionate</td>
<td>60</td>
<td>Jiang et al., 2011a</td>
</tr>
<tr>
<td>Butyrate</td>
<td>88</td>
<td>Marang et al., 2013</td>
</tr>
<tr>
<td>Lactate</td>
<td>92</td>
<td>Jiang et al., 2011b</td>
</tr>
<tr>
<td>Glycerol</td>
<td>67</td>
<td>Moralejo-Gárate et al., 2011</td>
</tr>
<tr>
<td>Paper mill wastewater</td>
<td>77</td>
<td>Jiang et al., 2012</td>
</tr>
<tr>
<td>Wastewater (acetate)</td>
<td>34 (gPHA/gVSS)</td>
<td>Morgan-Sagastume et al. (2014)</td>
</tr>
<tr>
<td>Mars chocolate</td>
<td>68</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>
The complex bacterial flora in a WWTO can be employed instead of using a pure culture of PHA-producing bacteria.

The concept of ANOXKALDINES – ‘Using biological wastewater to produce PHA’
Objectives

‘The build-up of internal electron donors as storage compounds is of great importance for N removal’

- To develop and test an alternative scheme for the treatment of the anaerobic supernatant from sewage sludge digestion
- To integrate the nitrogen removal via nitrite with the selection of PHA storing biomass in the side stream treatment line using one single reactor
- To produce PHA using the mixed culture of activated sludge, where sludge fermentation is used as substrate
- To apply an alternative feast/famine regime: feast under aerobic conditions and famine in anoxic conditions
- To increase the availability of nitrite (efficiency of nitritation) for the subsequent denitrification process for the famine conditions
- To control the properties of the carbon source by using wollastonite in the fermentation process
Real added value can emerge if PHA production can be integrated within the normal operation of WWTPs.

In this process short-cut nitrogen removal is combined with the selection of PHA biomass for the treatment of sludge reject water.

It is carried out in 5 steps:
- Sewage sludge fermentation
- Solid/liquid separation of fermentation effluent
- Simultaneous nitritation/denitrification and PHA selection
- Maximization of PHA in biomass
- Recovery of PHA from biomass

A novel process for integrating BNR and PHA production
The «short-cut» process (i.e. nitritation/denitritation)

Main advantages compared to the conventional nitrification / denitrification process:
- Reduction of aeration requirements up to 25%
- Less carbon source requirement up to 40%
- 30% lower sludge production
- 20% Lower CO₂ emissions

How is it accomplished?
- Need to inhibit completely (or washout) the nitrite oxidizing bacteria (NOB) and promote the growth of ammonium oxidizing bacteria (AOB)
- In highly nitrogen effluents (such as the sludge reject water) this can be achieved by maintaining a high free ammonia in the reactor (free ammonia > 1 mgL⁻¹ causes complete inhibition of NOB)
Consider the case study of 100,000 PE:

- COD loading factor: 0.12 kgCOD/PEd
- COD inlet in WWTP: 100,000 PE x 0.12 kgCOD/PEd = 12,000 kgCOD/d;
- Amount of PHA produced: 12,000 kgCOD/d x 0.03 gCOD(PHA)/gCOD (VFA) = 360 kg of PHB, equivalent to ~100,000 biodegradable bags (Weight of polyethylene bag - 8 l: 3.5 g)
Parameters affecting the PHA production process

Variables influencing

Raw material
- Hydrolysis
- Basification
- Thermal treatment

Pretreatment process
- Acidification
- Acidogenic fermentation

Selection of PHA producers in SBR
- Substrate composition
- Feeding strategy
- Sludge retention time

SBR
- Transient carbon or oxygen conditions

PHA accumulation
- Carbon source
- Absence of nutrients!

Optimized operating conditions
- Temperature and pH
- Inoculum type

The ‘selection’ plays the most important role for the maximization of the efficiency of the process
Influential parameters of the PHA production process

<table>
<thead>
<tr>
<th>SBR enrichment step</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT</td>
<td>15 d</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>We controlled through the addition of carbon source and the applied NLR</td>
</tr>
<tr>
<td>pH</td>
<td>Not controlled</td>
</tr>
<tr>
<td>Temperature</td>
<td>Not controlled 25°C</td>
</tr>
<tr>
<td>Nutrients availability - Limiting substance</td>
<td>We applied carbon limitation</td>
</tr>
<tr>
<td>Feeding strategy</td>
<td>We applied the feast (aerobic) / famine (anoxic) regime</td>
</tr>
<tr>
<td>Length of the feast / famine phase</td>
<td>Our aim: to ensure effective nitritation &amp; sufficient PHA selection</td>
</tr>
</tbody>
</table>

Fed batch reactor for PHA accumulation

<table>
<thead>
<tr>
<th>Feeding strategy</th>
<th>Pulse feeding – on demand through OUR control</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/P/N ratio –</td>
<td>We used wollastonite in the fermentation process that resulted in limited release of ammonia and phosphate in the FL - we maximized the C/P/N ratio</td>
</tr>
<tr>
<td>The sludge fermentation liquid results in the presence of nutrients – limiting the efficiency of the process !</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Not controlled</td>
</tr>
<tr>
<td>pH</td>
<td>Not controlled</td>
</tr>
</tbody>
</table>
Step 1: Production of volatile fatty acids from sewage sludge alkaline fermentation

- Semi-continuous reactor (500 L)
- Use of wollastonite (10 g/L) for pH adjustment, improvement of sludge dewatering characteristics and fermentation liquid properties (N, P)
- Temperature = 37°C, HRT = 5 days, SRT = 15 days
- TS feed = 3-7 g/L
- Produced VFAs = 8-10 gCOD/L

Step 2: Solid/liquid separation of fermentation effluent

- Ultrafiltration membrane modules are used for solid/liquid separation
- PVDF membranes, internal diameter of 8 mm and molecular weight cut (MWCO) of 15 kDa.
- Filtration area = 0.32 m².
- Maximum pressure = 600 kPa
Step 3: Simultaneous nitritation/denitritation and selection of PHA storing biomass

- The short-cut SBR (26 L) treated sludge reject water from the WWTP of Carbonera
- Inoculated with biomass acclimatized to “short-cut” nitrogen removal
- A modified feast/famine regime was applied with aerobic feast and anoxic famine conditions (feast time / famine time = 0.2)
- Initial COD/N = 3, SRT = 15 days
- Spiking with sludge fermentation liquid during the aerobic phase but NO carbon source addition in the anoxic period
Our feast and famine process

**Feast:**
- Bacteria store VFAs as PHA;

**Famine:**
- Recovery of energy for growth by PHA consumption

**Aerobic - Feast:**
- Bacteria store VFAs as PHA;
- Nitritation

**Anoxic - Famine:**
- Bacteria use PHA to accomplish the via-nitrite denitrification and growth
The «feast and famine» process...selecting the biomass...

1) Feeding of sludge reject water and sludge fermentation liquid to create feast conditions in an aerobic environment
2) Nitritation and simultaneous VFA uptake and PHA production
3) Long anoxic phase (famine) to allow the denitrification by using the previously stored PHAs.
The main stages of our process

**VFAs production from primary sludge**
- Fermented liquid: 248 gCOD\(_{VFA}\)/kgTVS\(_{fed}\)
- Ultrafiltration with tubular membrane
- VFA:NH\(_4\)-N:PO\(_4\)-P = 100:7.8:0.06 in FL

**Sequencing batch reactor**
- 26 L reaction volume
- Aerobic/Anoxic-Feast/Famine biomass enrichment
- COD(VFA):NH\(_4\)-N ≈ 2.5

**Batch PHA accumulation**
- \(Y_{PHA/VFA}\) 0.33±0.04 gCOD/gCOD
- \(Y_{X/VFA}\) 0.22±0.09 gCOD/gCOD
- PHA 0.18±0.04 gCOD/gCOD (in 8 h)
Step 3: Selection of PHA storing biomass

PERIOD 1: Stable reduction of the VFAs uptake rate and the PHA storage rate

PERIOD 2: ‘Stable’ recovery of the PHA storage rate by the extension of the aerobic phase (more nitrite are available)
**Selection - Low Nitrogen Loading Rate Applied**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Run 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>vNLR influent (gN/m3d)</td>
<td>110±0.03</td>
</tr>
<tr>
<td>vOLR (kgCOD/m3d)</td>
<td>0.62±0.12</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>5.6±1.1</td>
</tr>
<tr>
<td>F/M (kgCOD/kgMLVSS)</td>
<td>0.33±0.08</td>
</tr>
<tr>
<td>n°cycle</td>
<td>4.0</td>
</tr>
<tr>
<td>MLSS (g/L)</td>
<td>2.1±0.9</td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td>SRT (d)</td>
<td>12-15</td>
</tr>
<tr>
<td>Time aerobic (h/d)</td>
<td>5.1</td>
</tr>
<tr>
<td>Time anoxic (h/d)</td>
<td>17.4±2.4</td>
</tr>
</tbody>
</table>

**Diagram**

- **Nitrogen (mgN/L)**
  - PO4-P
  - NH4-N
  - NO2-N
  - NO3-N
  - NO2-N spiked

- **Nitrite, Nitrate (mgN/L)**
  - AEROBIC
  - ANOXIC

- **Volatile Fatty Acids (mgCOD/L)**
  - AEROBIC
  - ANOXIC

- **PHA (gCOD/gCOD)**
  - HAc
  - HPr
  - HBt
  - %PHA

- **Time (min)**
  - 0 50 100 150 200 250 300

- **PHA (gCOD/gCOD)**
  - 0 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50

- **PHA (gCOD/gCOD)**
  - 0 100 200 300
Selection - High nitrogen loading rate rate applied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>vNLR influent (gN/m3d)</td>
<td>380 ± 0.09</td>
</tr>
<tr>
<td>vOLR (kgCOD/m3d)</td>
<td>0.74</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>F/M (kgCOD/kgMLVSS)</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>n°cycle</td>
<td>4.2</td>
</tr>
<tr>
<td>MLSS (g/L)</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>SRT (d)</td>
<td>12-15</td>
</tr>
<tr>
<td>Time aerobic (h/d)</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>Time anoxic (h/d)</td>
<td>13.4 ± 1.1</td>
</tr>
</tbody>
</table>

- **NH4-N**
- **NO2-N**
- **NO3-N**

- **HAc**
- **HBt**
- **HPr**
- **PHA**
Step 5: Recovery of PHA from biomass

- Solvent extraction method used
- Biomass from step 4 was placed for lyophilization (freeze drying under low temperature and vacuum). The extract was obtained and the following chemicals were added:

Extraction of dry biomass with chloroform at 70°C for one hour in a ratio of 50ml/g dry biomass

Purification: Mixing the extract with methanol (five times the volume) for precipitation of bioplastic and then filtration

Collection of the solid and re-dissolution in chloroform to obtain bioplastic films

Lyberatos, 2013
## Step 4: Maximizing the PHA production (accumulation reactor)

### Graph
- **%PHA (gCOD/gCODx100)** vs. **Time (h)**
- Lines represent different carbon sources:
  - **PS+W**
  - **HAc**
  - **PS**
  - **Mix VFA**
- PS: primary sludge
- FL: fermentation liquid

### Table
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 2</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon source</td>
<td>Acetate</td>
<td>Sludge FL + Wollastonite</td>
<td>Synthetic Mixture of VFA</td>
<td>Sludge FL</td>
</tr>
<tr>
<td>COD(VFA):NH₄-N:PO₄-P</td>
<td>100:0:0</td>
<td>100:7.8:0.06</td>
<td>100:0:0</td>
<td>100:9.7:2.1</td>
</tr>
<tr>
<td>-qTVFA (mgCOD/gCODh)</td>
<td>90.3 ± 1.18</td>
<td>133.3 ± 38.7</td>
<td>177.4 ± 15.7</td>
<td>94.9 ± 22.3</td>
</tr>
<tr>
<td>qPHA</td>
<td>41.5 ± 12.3</td>
<td>40.0 ± 4.6</td>
<td>81.7 ± 12.6</td>
<td>25.3 ± 6.7%</td>
</tr>
<tr>
<td>PHA (gCOD/gCOD) in 8-9 h</td>
<td>0.29 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.35 ± 0.04</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Y_{PHA/VFA} (gCOD/gCOD)</td>
<td>0.46 ± 0.10</td>
<td>0.33 ± 0.04</td>
<td>0.51 ± 0.06</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>Y_{X/VFA} (gCOD/gCOD)</td>
<td>0.20 ± 0.05</td>
<td>0.22 ± 0.09</td>
<td>0.20 ± 0.02</td>
<td>0.28 ± 0.06</td>
</tr>
</tbody>
</table>
Nitrogen (mgN/L)

Time (min)

NH₄⁻N  NO₂⁻N  NO₃⁻N

Volatile Fatty Acids (mgCOD/L)

Time (min)

HAc  HBt  HPr  PHA

COD removed (mgCOD/L)

COD stored and growth

VFA uptake

growth

Before

After
The high range of applications of the PHAs

**Industrial**
- Cover paper or cardboard to make water-resistant surfaces
- Foils, films and diaphragms
- Combs, pens and bullets
- Pressure sensors for keyboards, stretch and acceleration measuring instruments

**Medical**
- Bone plates, osteosynthetic materials and surgical sutures
- PHA fibres are sought after to make swabs and dressing materials for surgery
- Pericardial patches, artery augments, cardiological stents, vascular grafts, heart valves
- Tissue engineering
- Controlled drug release

**Household**
- Disposable razors, utensils, diapers, feminine hygiene products

**Agricultural**
- Biodegradable agricultural film
- Controlled release of insecticides

**Blending PHAs with high or low molecular weight molecules help improve their material properties.**
### What is the balance?

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Points that must be improved – potential drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>It uses both activated sludge and sludge reject water to produce PHA. All materials are available in the WWTPs. It reduces the amount of sludge that must be handled.</td>
<td>Increased competition between the heterotrophic &amp; autotrophic bacteria in the aerobic phase for the DO in the presence of VFAs.</td>
</tr>
<tr>
<td>Integrates reject water treatment with PHA selection in the one reactor.</td>
<td>Nitritation is not always effective → lack of NO₂⁻N for denitrification. → nitrogen removal is limited.</td>
</tr>
<tr>
<td>The previously stored PHA are the electron donor for denitritation without the requirement for carbon source.</td>
<td>It is crucial to ensure effective and stable nitritation and nitrite as electron acceptors for denitrification.</td>
</tr>
<tr>
<td>The short-cut via nitrite process results in lower aeration and external carbon source requirements than conventional nitrification/denitrification.</td>
<td>The duration of the feast &amp; famine phase should be adjusted.</td>
</tr>
<tr>
<td>The PHA selection with the famine–anoxic phase requires less O₂ compared to the typical feast/famine process under aerobic conditions.</td>
<td>Nutrient release in the fermented liquid → Use of wollastonite.</td>
</tr>
<tr>
<td>The use of wollastonite in the fermentation can adjust the ration of C/N/P during PHA accumulation.</td>
<td></td>
</tr>
</tbody>
</table>
Coupling the short-cut nitrification/denitrification in SBR with an adequate feast and famine regime it is possible to allow the production of PHA within the WWTP by using the selected biomass from the reactor in a parallel aerobic batch reactor that can be fed with sludge fermentation liquid, to accomplish the PHA accumulation.

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Thank you!