Spring 6-10-2014

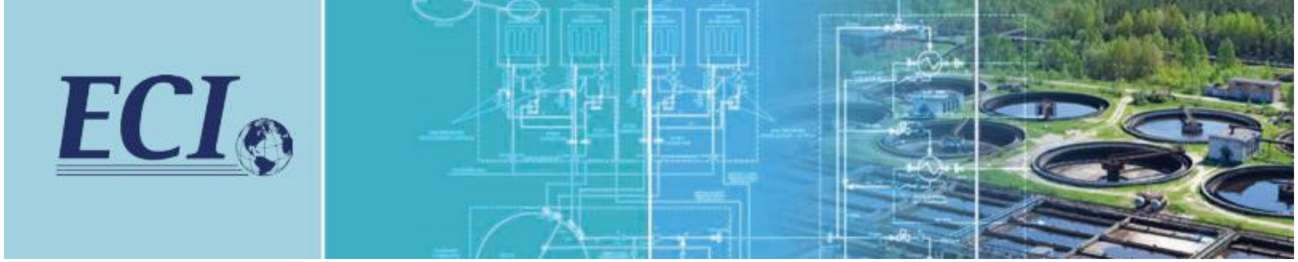
Integrating the selection of PHA storing biomass and nitrogen removal via nitrite for the treatment of the sludge reject water

F. Fatone
University of Verona

N. Frison
University of Verona

E. Katsou
University of Verona

S. Malamis
University of Verona



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

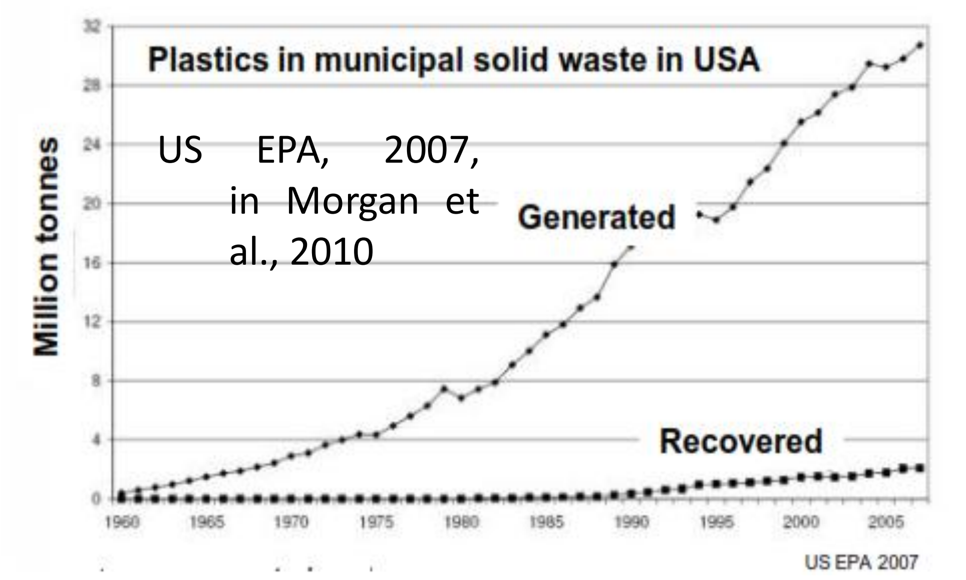
N. Frison, E. Katsou, S. Malamis, **F. Fatone**

Department of Environmental Sciences, Informatics and Statistics,
University Ca'Foscari of Venice,
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



Rentagreen.com

The need….

✓ **Resource consumption <u>– PROBLEM 1</u>**

Daily crude oil consumption in 2012: 83,477 million barrels

…..Global reserve: 1,652,600 million barrels

✓ **But sufficient for how long?**

1652.600.000.000 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? **<u>- PROBLEM 2</u>**

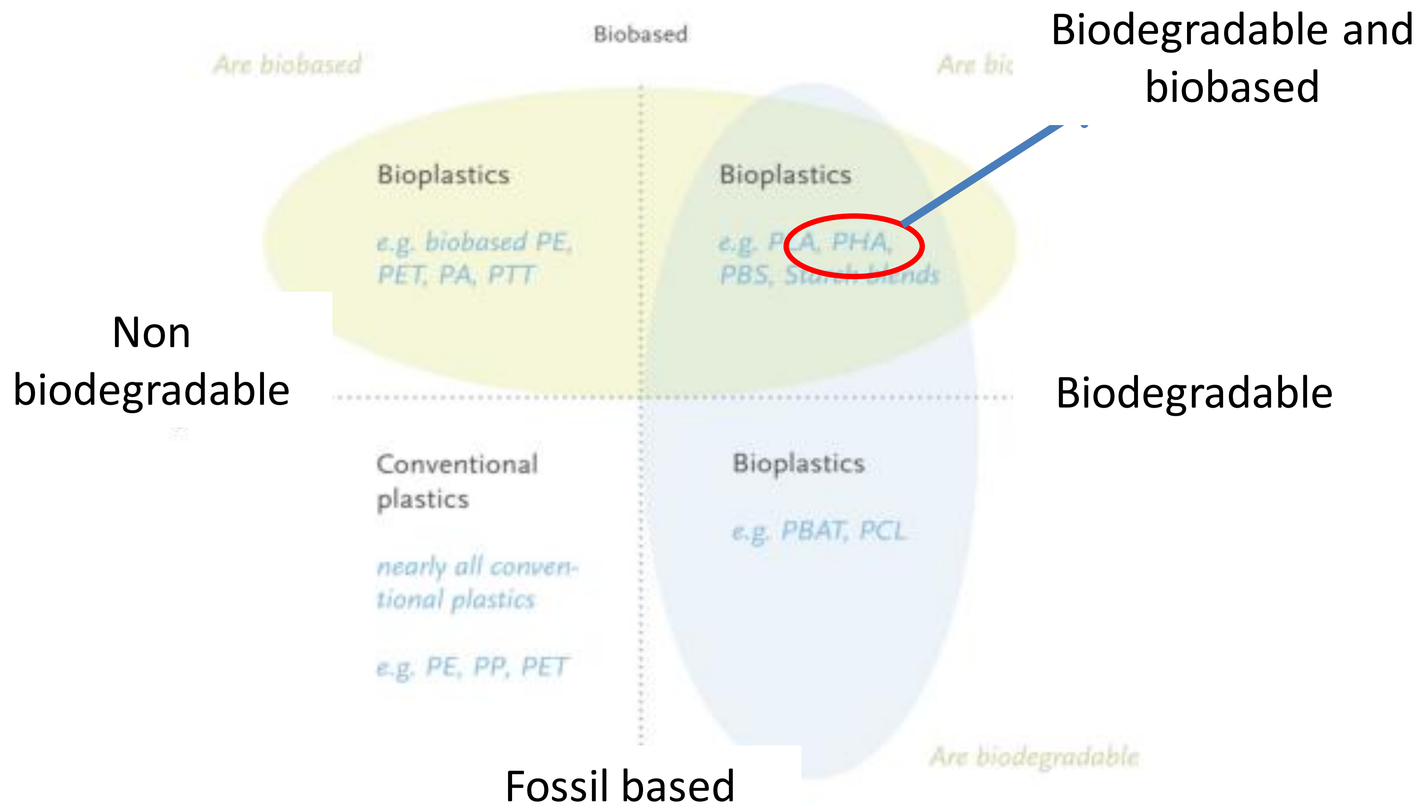
The United Nations Environment Program estimated by 2006 every square mile of ocean already hosted **46,000** pieces of floating plastic

A biodegradable plastic can be the solution

- BP Statistical Review of World Energy, 2012
- Institute for Sanitary Engineering, Water Quality and Solid Waste Management
- U.N. Environment Program, www.unep.org

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



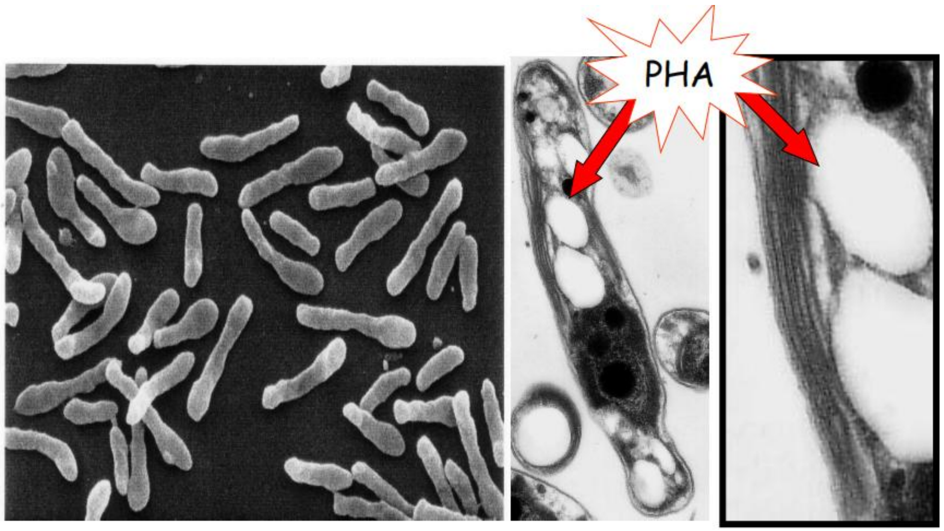
European bioplastics, 2014

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

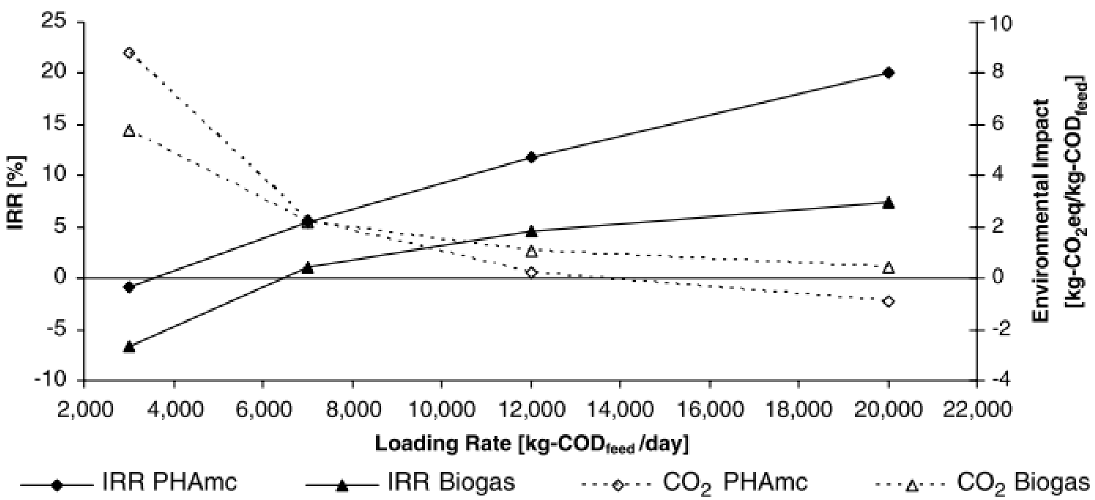
- ✓Yield of methane: 0.350 m3/kgCOD
- ✓Methane sale price 0.2 €/ m^3

→ 0.07 €/ kgCOD

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

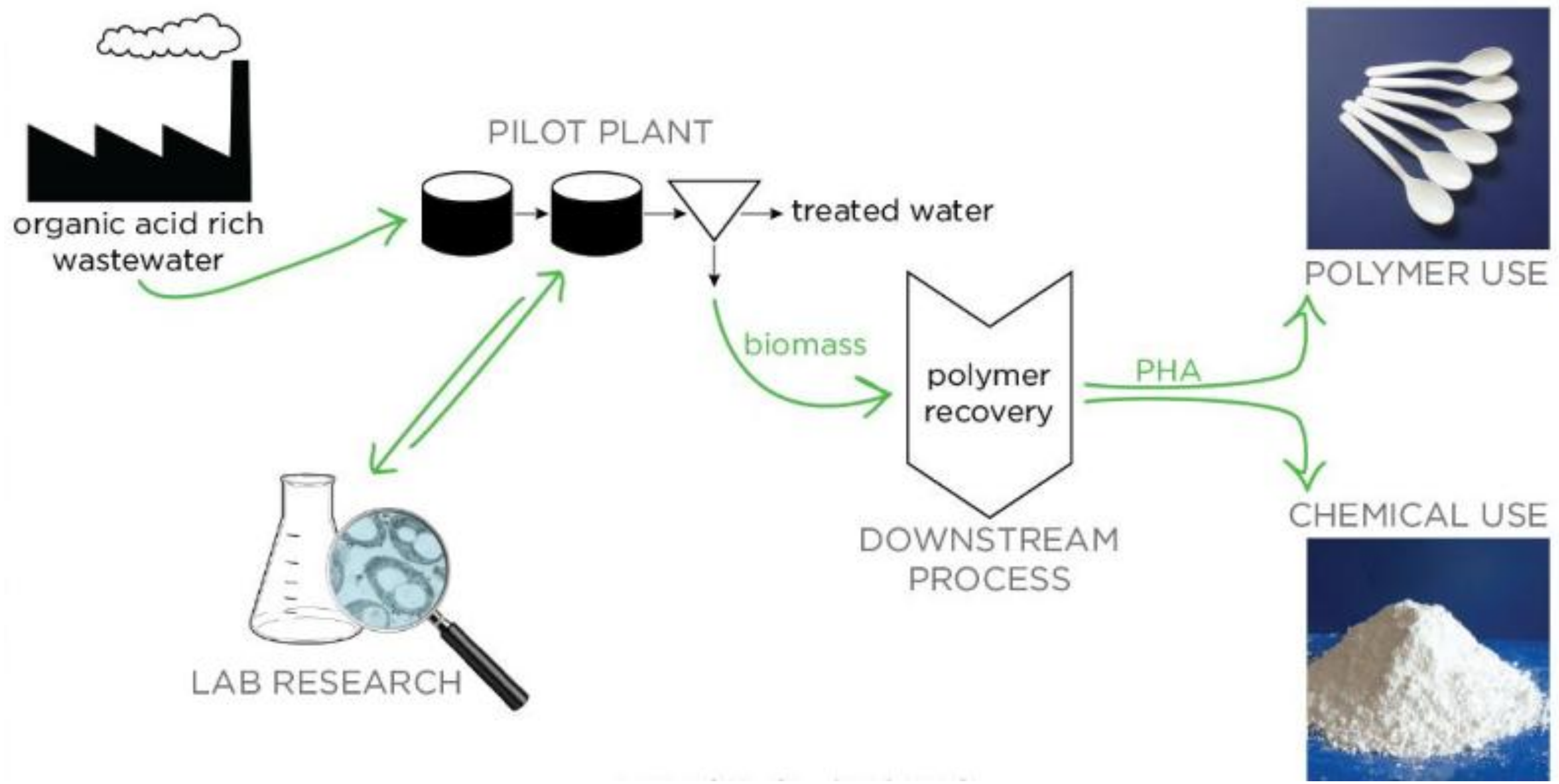
→ 1.75 €/ kgCOD

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



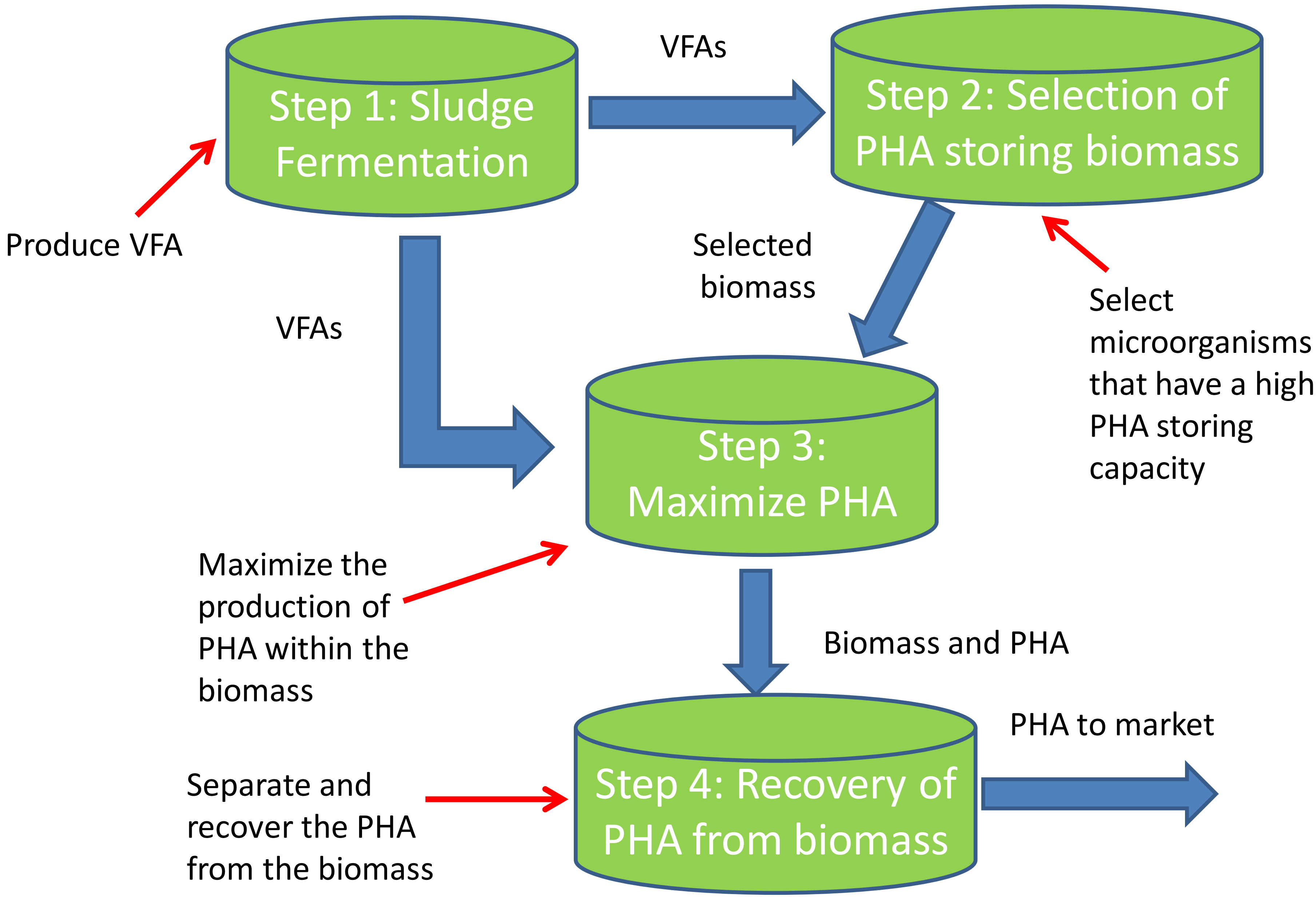
Gurieff & Lant, 2007

Moving towards the biorefining concept
Producing bioplastics in WWTPs



Kleerebezem et al., 2013

Typical steps involved in PHA production in WWTPs

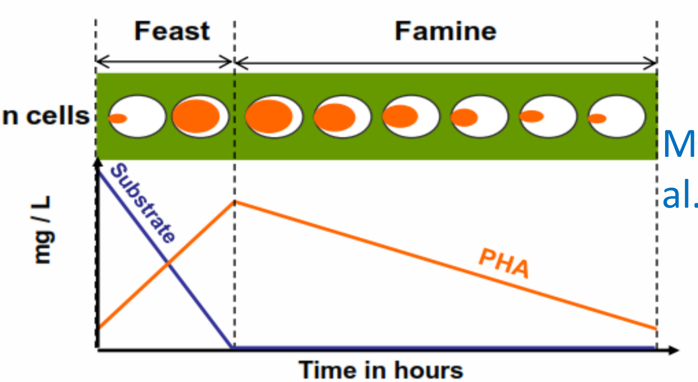


Step 1: Fermentation to produce VFAs

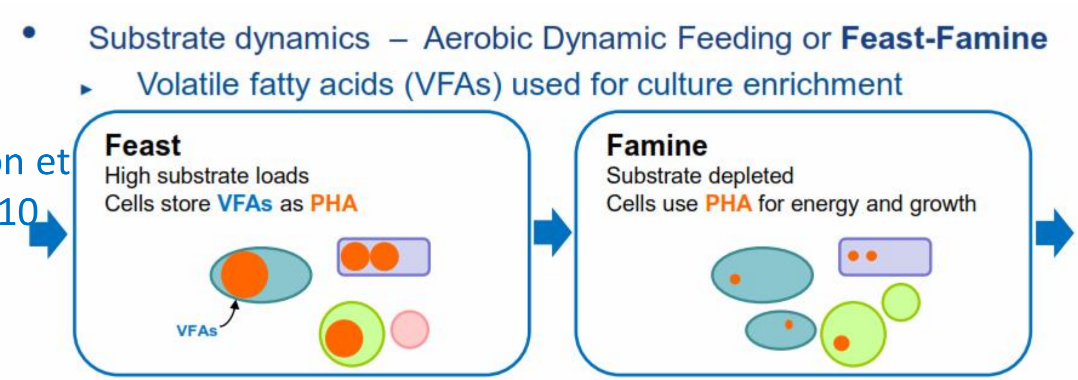
✓This step is necessary when the substrate has poor content in volatile fatty acids (VFA). The hydrolysis that takes place releases VFA in the liquid phase.

✓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

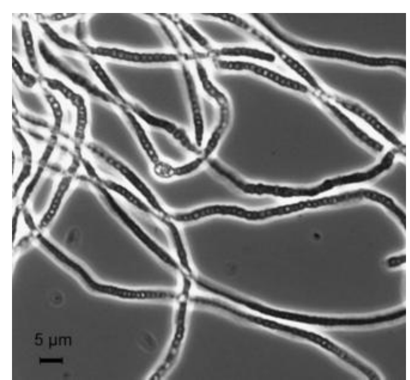


Morton et al., 2010



✓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



Micrograph of filamentous PHA accumulating bacteria, Bengtsson et al., 2008

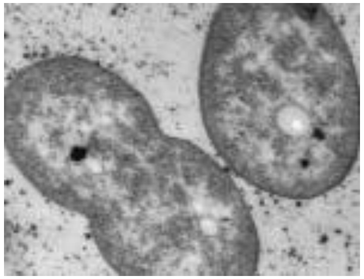
Step 3:Maximize PHA within biomass

➢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

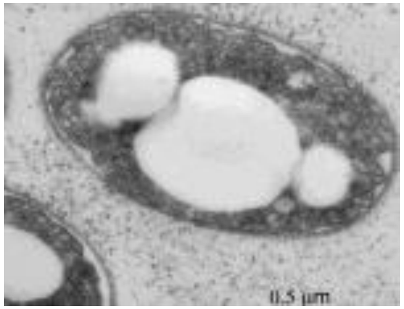
Johnson et al., 2009

PHA = 89% wt

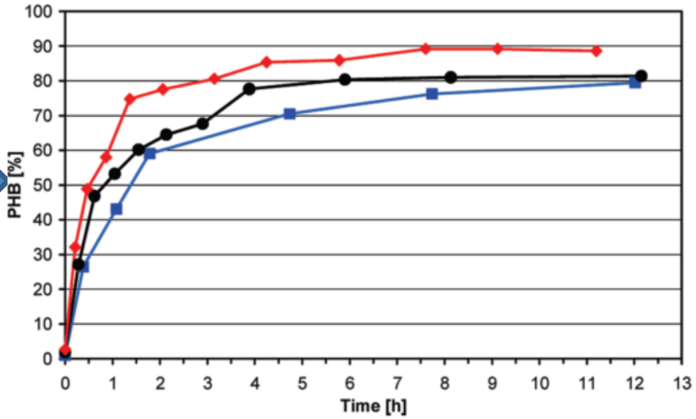
Kleerebezem et al., 2013



Before step 3



After step 3



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

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

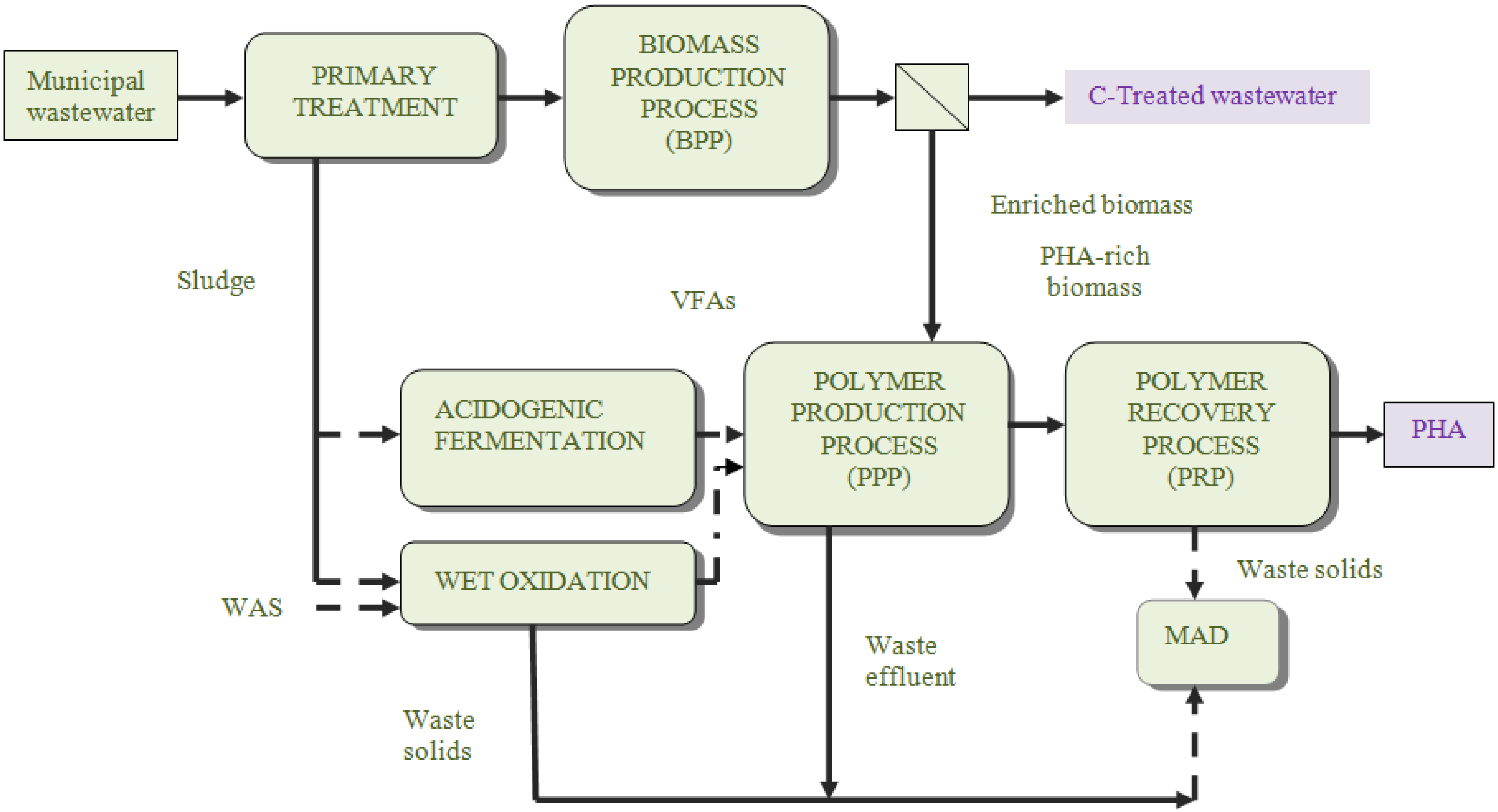
Different PHA yields achieved

Substrate used	PHA yield (gPHA/gTSS)	Reference
Propionate	60	Jiang et al., 2011a
Butyrate	88	Marang et al., 2013
Lactate	92	Jiang et al., 2011b
Glycerol	67	Moralejo-Gárate et al., 2011
Paper mill wastewater	77	Jiang et al., 2012
Wastewater (acetate)	34 (gPHA/gVSS)	Morgan-Sagastume et al. (2014)
Mars chocolate	68	Unpublished

WWTP as biorefinery for PHA production

The complex bacterial flora in a WWTO can be employed instead of using a pure culture of PHA-producing bacteria

The concept of ANOXKALDNES – 'Using biological wastewater to produce PHA'



Objectives

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

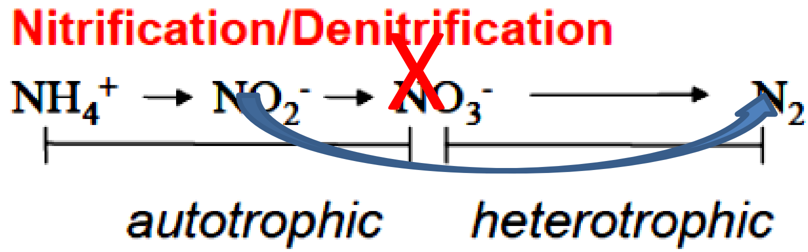
- To develop and test an alternative scheme for the treatment of the anaerobic supernatant from sewage sludge digestion
- To integrate the nitrogen removal via nitrite with the selection of PHA storing biomass in the side stream treatment line using one single reactor
- To produce PHA using the mixed 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 denitritation process for the famine conditions**
- **To control the properties of the carbon source by using wollastonite in the fermentation process**

A novel process for integrating BNR and PHA production

- 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/denitritation and PHA selection
  - Maximization of PHA in biomass
  - Recovery of PHA from biomass

The «short-cut» process (i.e. nitritation/denitritation)

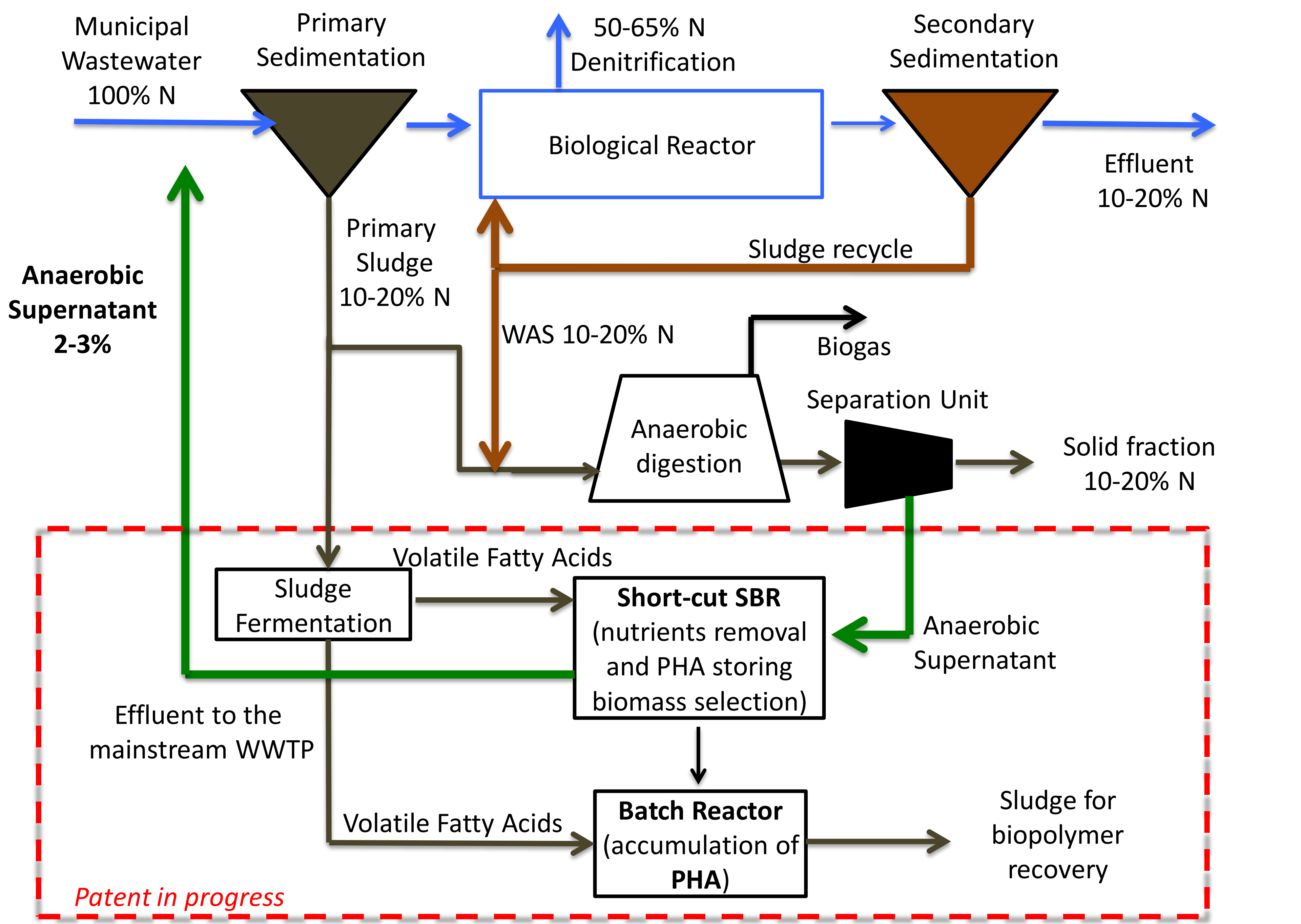


Main advantages compared to the conventional nitrification / denitrification process :

- Reduction of aeration requirements up to 25%
- Less carbon source requirement up to 40%
- 30% lower sludge production
- 20% Lower CO_2 emissions

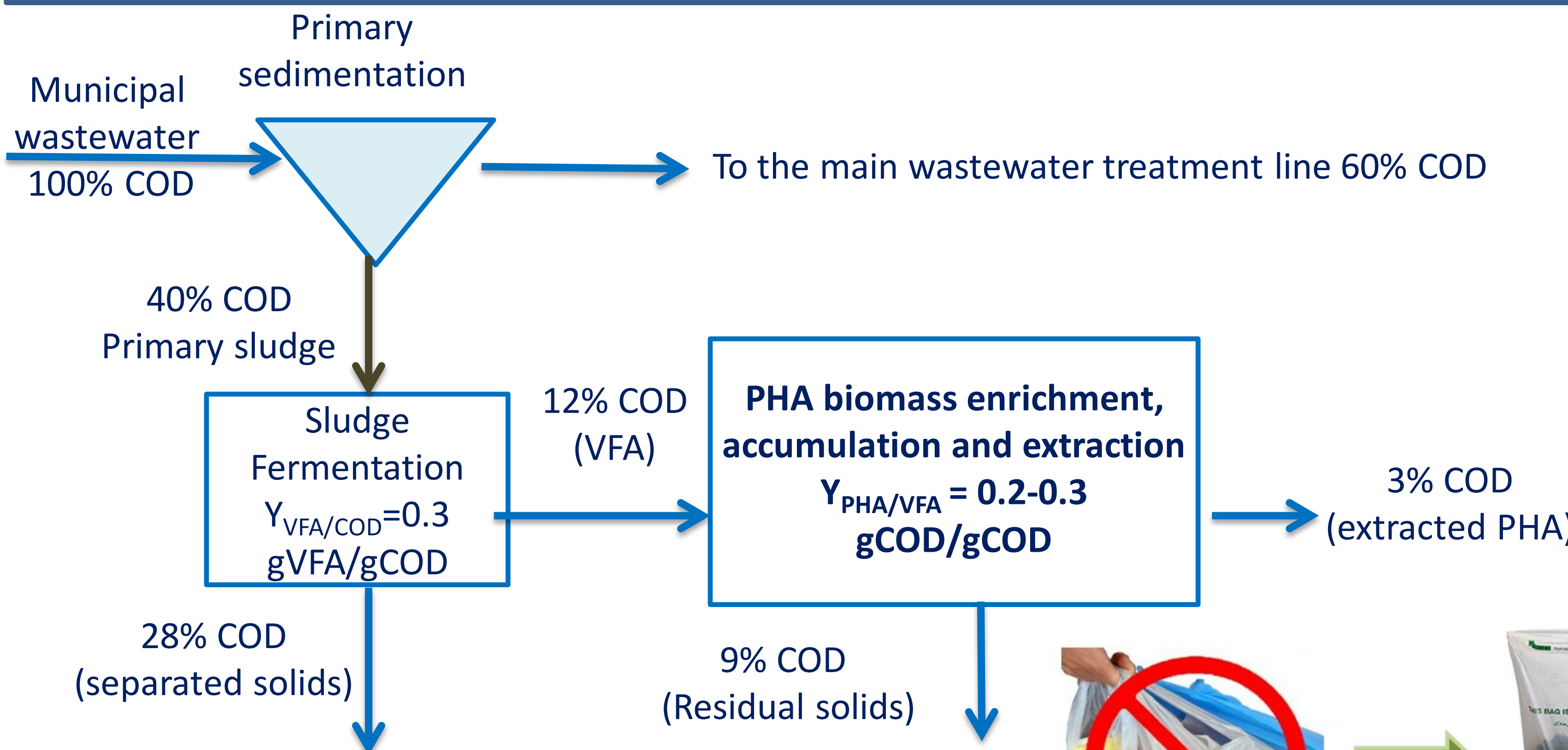
How is it accomplished ?

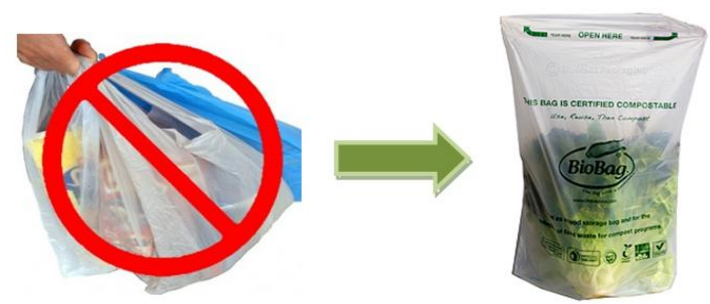
- Need to inhibit completely (or washout) the nitrite oxidizing bacteria (NOB) and promote the growth of ammonium oxidizing bacteria (AOB)
- In highly nitrogen effluents (such as the sludge reject water) this can be achieved by maintaining a high free ammonia in the reactor (free ammonia > 1 mgL^{-1} causes complete inhibition of NOB)



Municipal Wastewater 100% N
Primary Sedimentation
50-65% N Denitrification
Secondary Sedimentation
Biological Reactor
Effluent 10-20% N
Primary Sludge 10-20% N
Sludge recycle
Anaerobic Supernatant 2-3%
WAS 10-20% N
Biogas
Separation Unit
Anaerobic digestion
Solid fraction 10-20% N
Volatile Fatty Acids
Sludge Fermentation
Short-cut SBR (nutrients removal and PHA storing biomass selection)
Anaerobic Supernatant
Effluent to the mainstream WWTP
Volatile Fatty Acids
Batch Reactor (accumulation of PHA)
Sludge for biopolymer recovery
Patent in progress

PHA production in the sludge line





Considerering the case study of 100000 PE:

- COD loading factor: 0.12 kgCOD/PEd
- COD inlet in WWTP: 100000 PE x 0.12 kgCOD/PEd = 12000 kgCOD/d;
- Amount of PHA produced: 12000 kgCOD/d x 0.03 gCOD(PHA)/gCOD (VFA) = 360 kg of PHB, equivalent to ~100000 biodegradable bags {Weight of polyethylene bag - 8 l: 3.5 g}

Parameters affecting the PHA production process

Variables influencing

Raw material

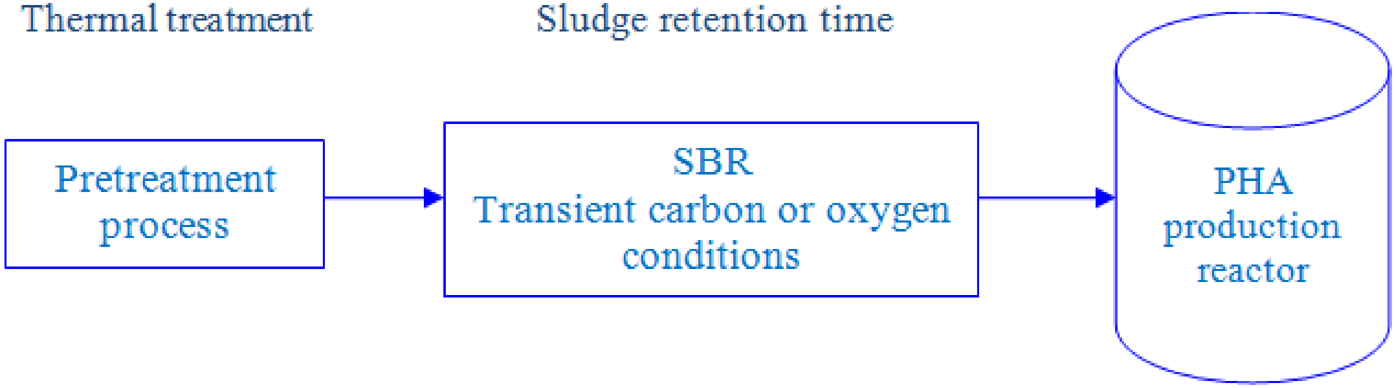
the Selection of PHA producers in SBR

the PHA accumulation

Hydrolysis
Basification
Thermal treatment

Substrate composition
Feeding strategy
Sludge retention time

Carbon source
Absence of nutrients!



Acidification
Acidogenic fermentation

Nutrients
Temperature and pH

Optimized operating conditions
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

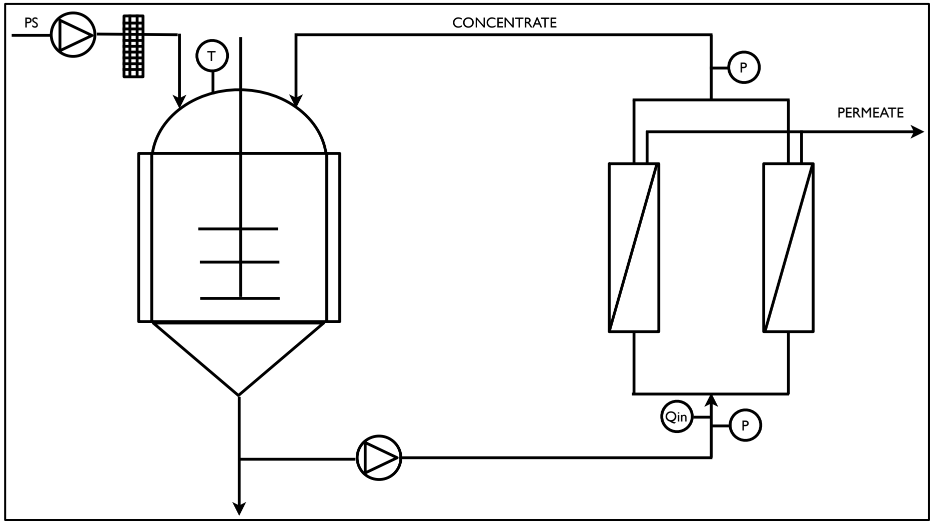
SBR enrichment step	
SRT	15 d
C/N ratio	We controlled through the addition of carbon source and the applied NLR
pH	Not controlled
Temperature	Not controlled 25^{o}C
Nutrients availability - Limiting substance	We applied carbon limitation
Feeding strategy	We applied the feast (aerobic) / famine (anoxic) regime
Length of the feast / famine phase	Our aim: to ensure effective nitritation & sufficient PHA selection
Fed batch reactor for PHA accumulation	
Feeding strategy	Pulse feeding –on demand through OUR control
C/P/N ratio – The sludge fermentation liquid results in the presence of nutrients – limiting the efficiency of the process !	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
Temperature	Not controlled
pH	Not controlled

Step 1: Production of volatile fatty acids from sewage sludge alkaline fermentation

- ✓Semi-continuous reactor (500 L)
- ✓Use of wollastonite (10g/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 m^2.
- ✓Maximum pressure = 600 kPa

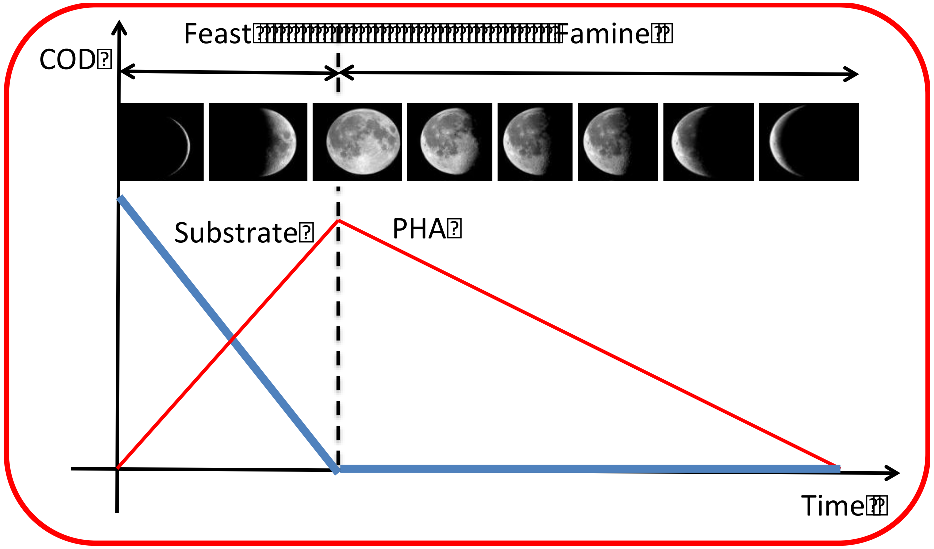


Step 3: Simultaneous nitritation/denitritation and selection of PHA storing biomass

- 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

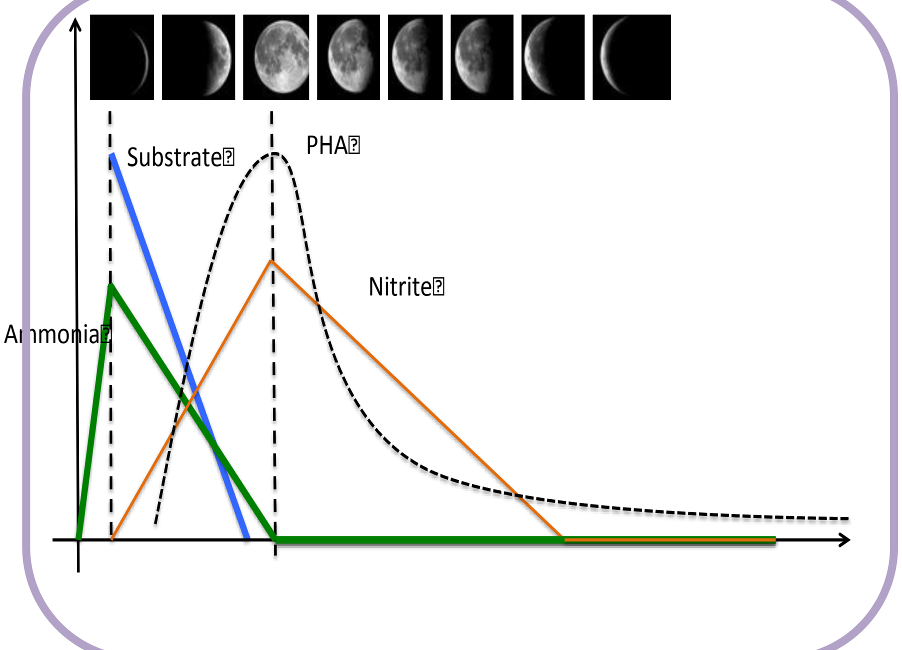
Pilot short cut SBR in Verona

Our feast and famine process



Feast:
-Bacteria store VFAs as PHA;

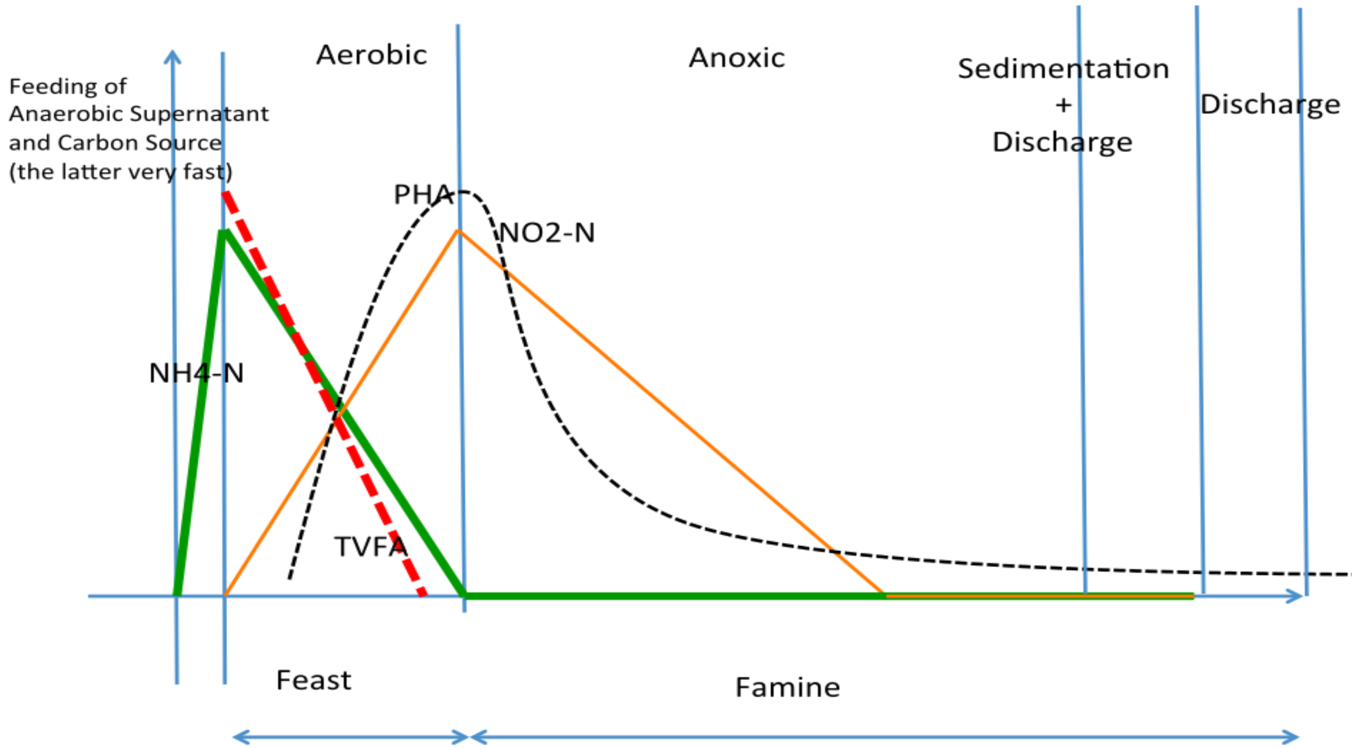
Famine:
-Recovery of energy for growth by PHA consumption



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:PO4-P = 100:7.8:0.06 in FL

Sequencing batch reactor

26 L reaction volume

Aerobic/Anoxic-Feast/Famine biomass enrichment

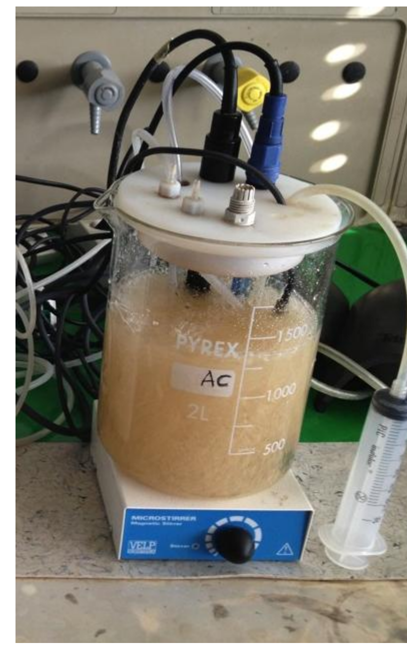
COD(VFA):NH4-N ≈ 2.5

Batch PHA accumulation

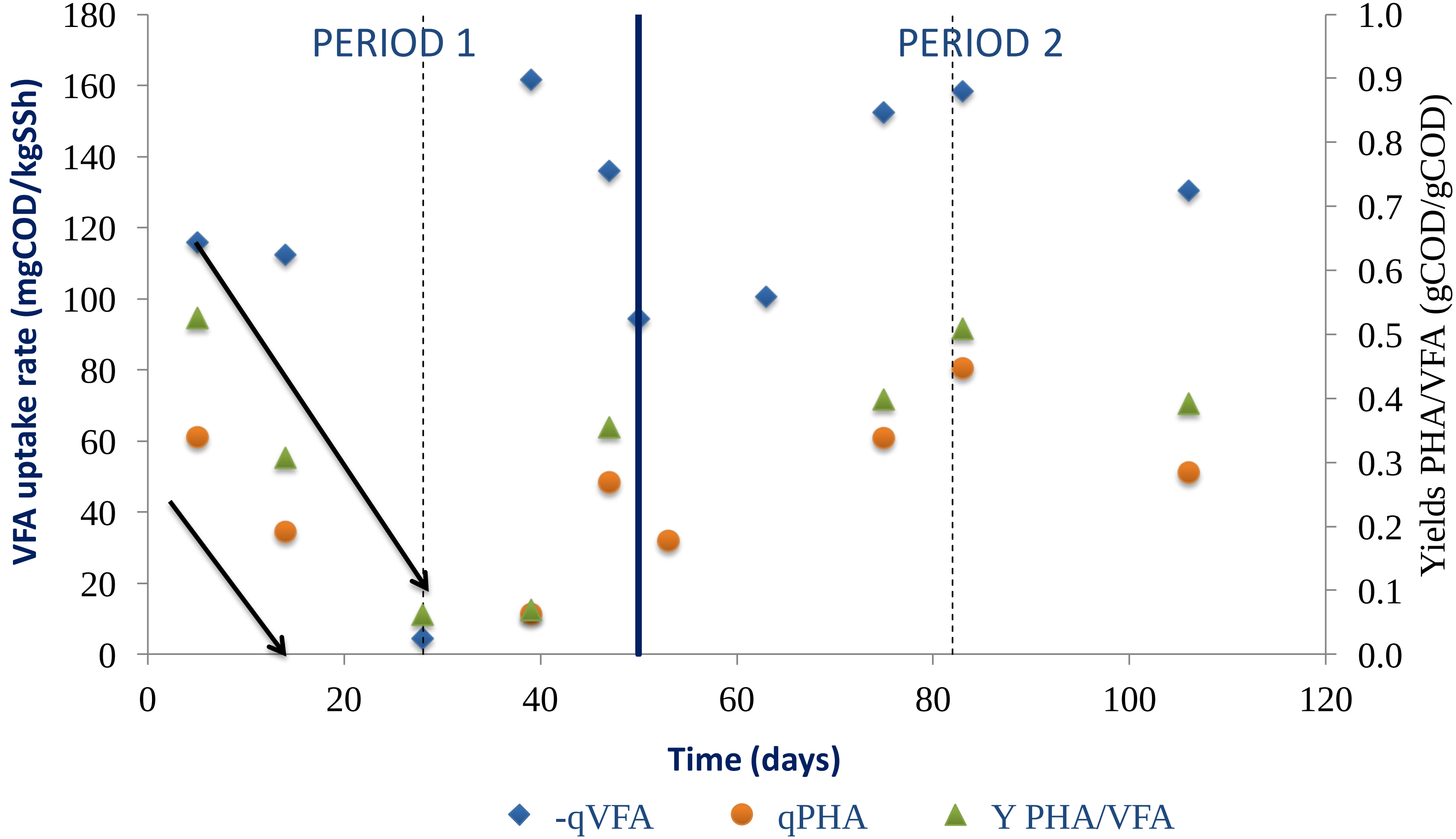
$Y_{PHA/VFA}$ 0.33±0.04 gCOD/gCOD

$Y_{X/VFA}$ 0.22±0.09 gCOD/gCOD

PHA 0.18±0.04 gCOD/gCOD (in 8 h)



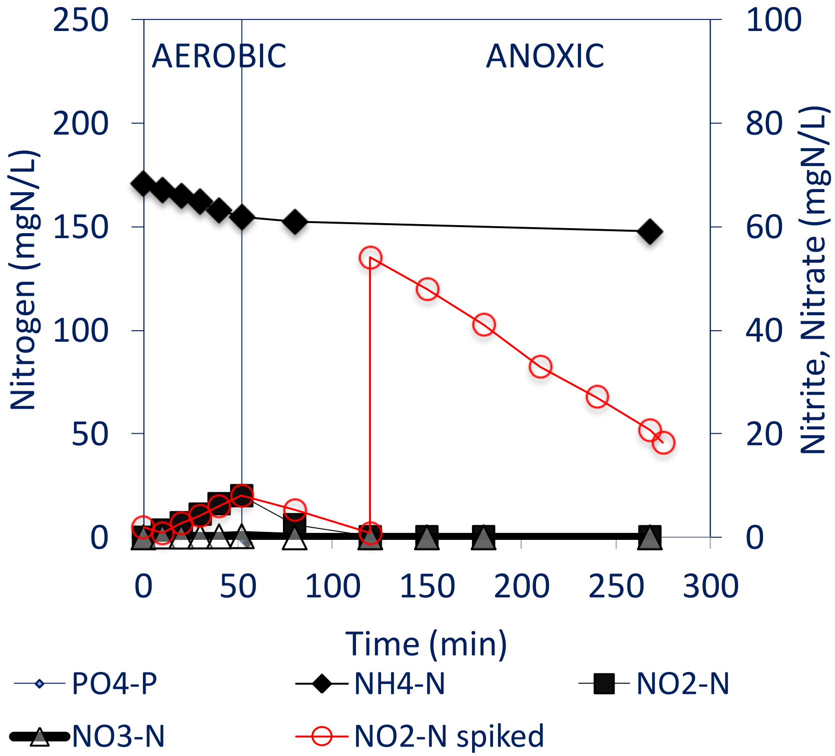
Step 3: Selection of PHA storing biomass

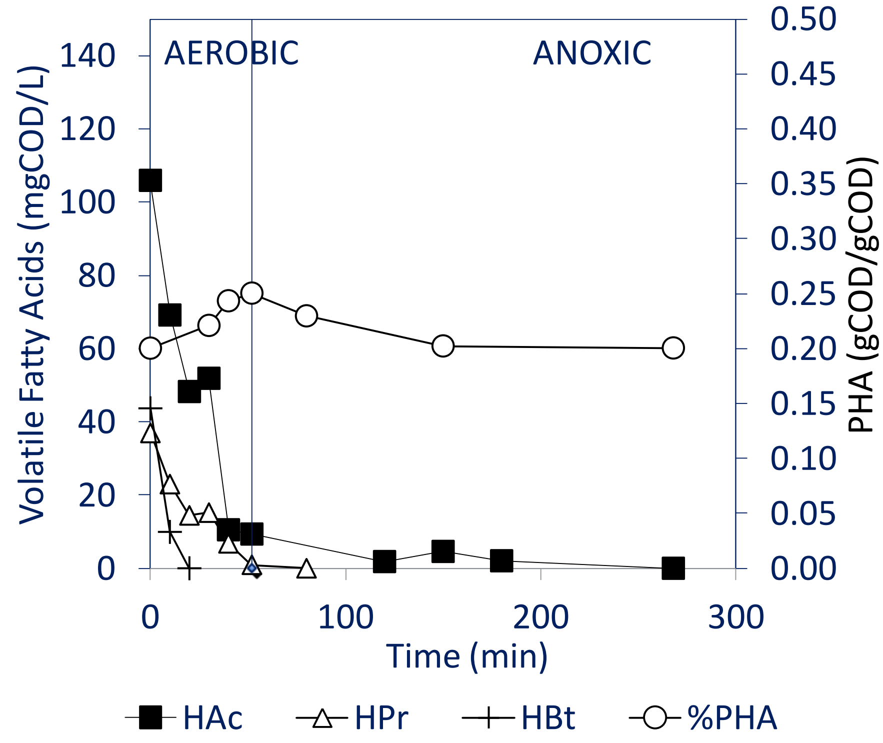


- PERIOD 1: Stable reduction of the VFAs uptake rate and the PHA storage rate
- PERIOD 2: ‘Stable’ recovery of the PHA storage rate by the extension of the aerobic phase (more nitrite are available)

Selection - Low Nitrogen Loading Rate Applied

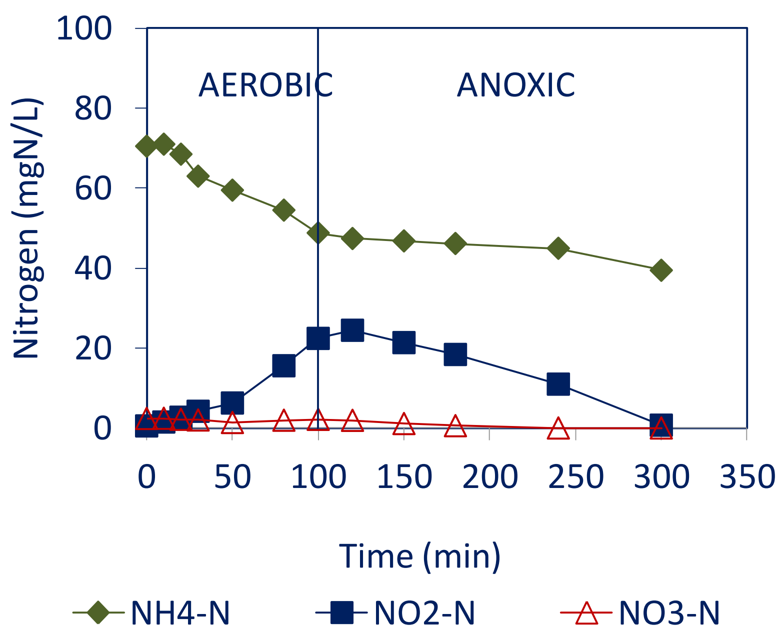
Parameter	Run 1
vNLR influent (gN/m3d)	110±0.03
vOLR (kgCOD/m3d)	0.62±0.12
C/N ratio	5.6±1.1
F/M (kgCOD/kgMLVSS)	0.33±0.08
n°cycle	4.0
MLSS (g/L)	2.1±0.9
MLVSS (g/L)	1.9±0.8
SRT (d)	12-15
Time aerobic (h/d)	5.1
Time anoxic (h/d)	17.4±2.4

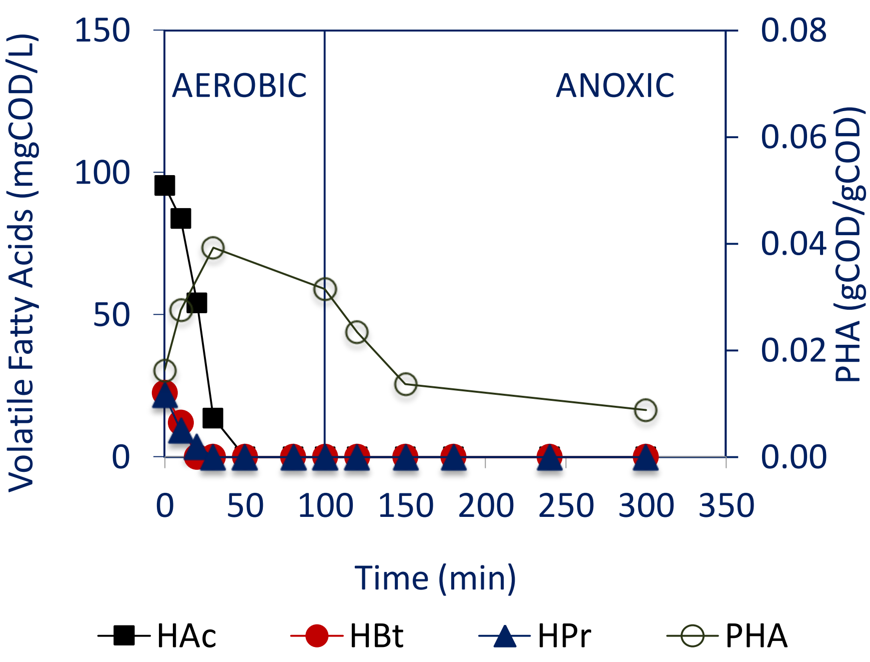




Selection - High nitrogen loading rate applied

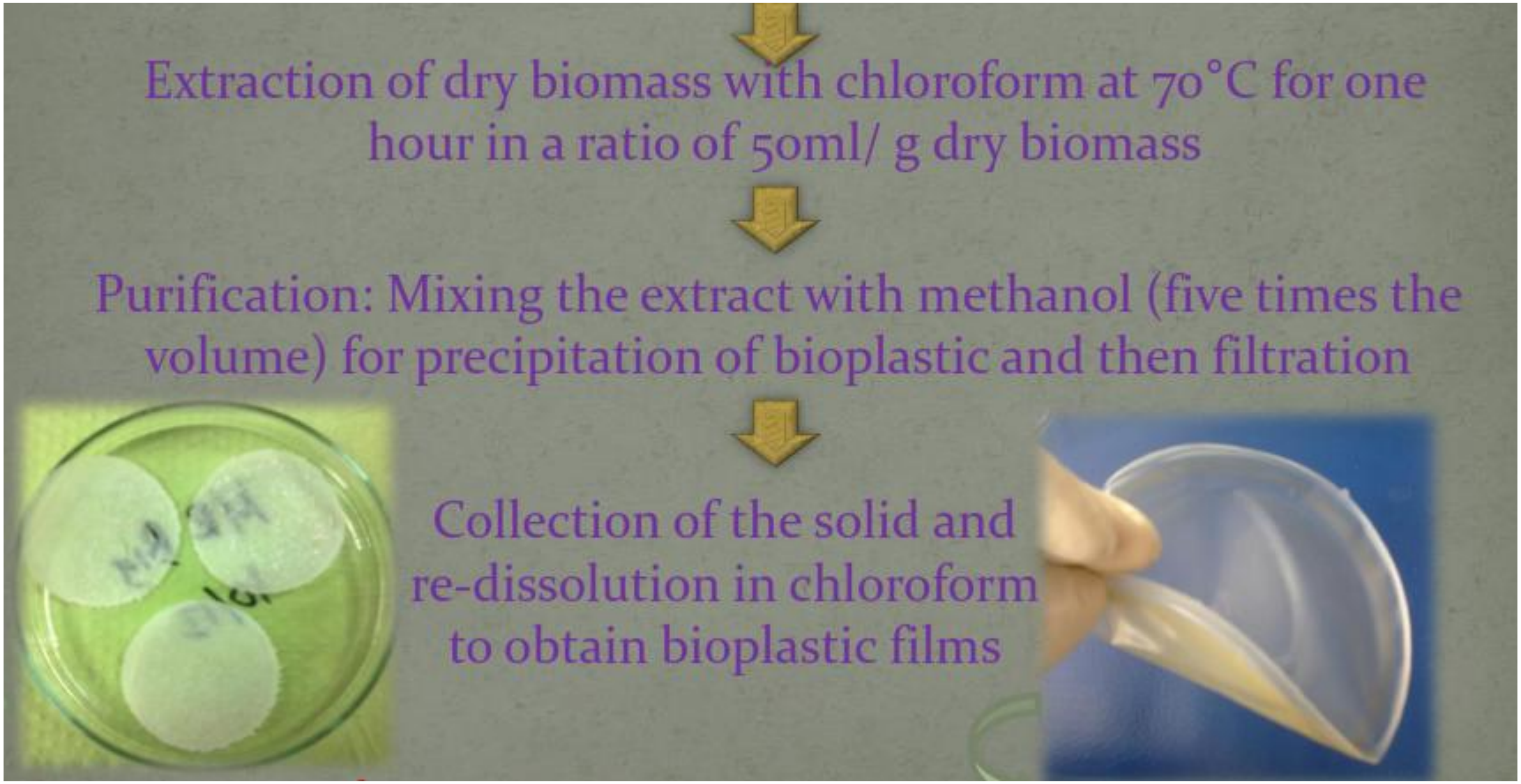
Parameter	Run 2
vNLR influent (gN/m3d)	380±0.09
vOLR (kgCOD/m3d)	0.74
C/N ratio	1.9±0.1
F/M (kgCOD/kgMLVSS)	0.22±0.05
n°cycle	4.2
MLSS (g/L)	4.2±0.5
MLVSS (g/L)	3.3±0.3
SRT (d)	12-15
Time aerobic (h/d)	9.1±0.6
Time anoxic (h/d)	13.4±1.1





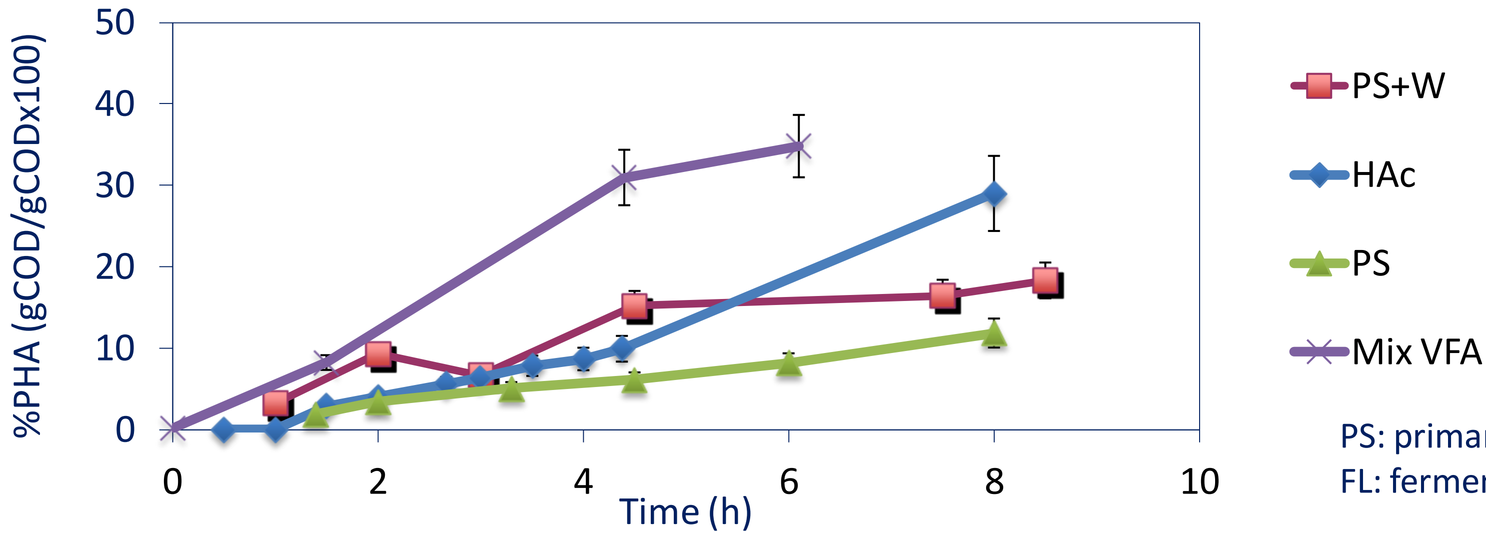
Step 5: Recovery of PHA from biomass

- 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:



Lyberatos, 2013

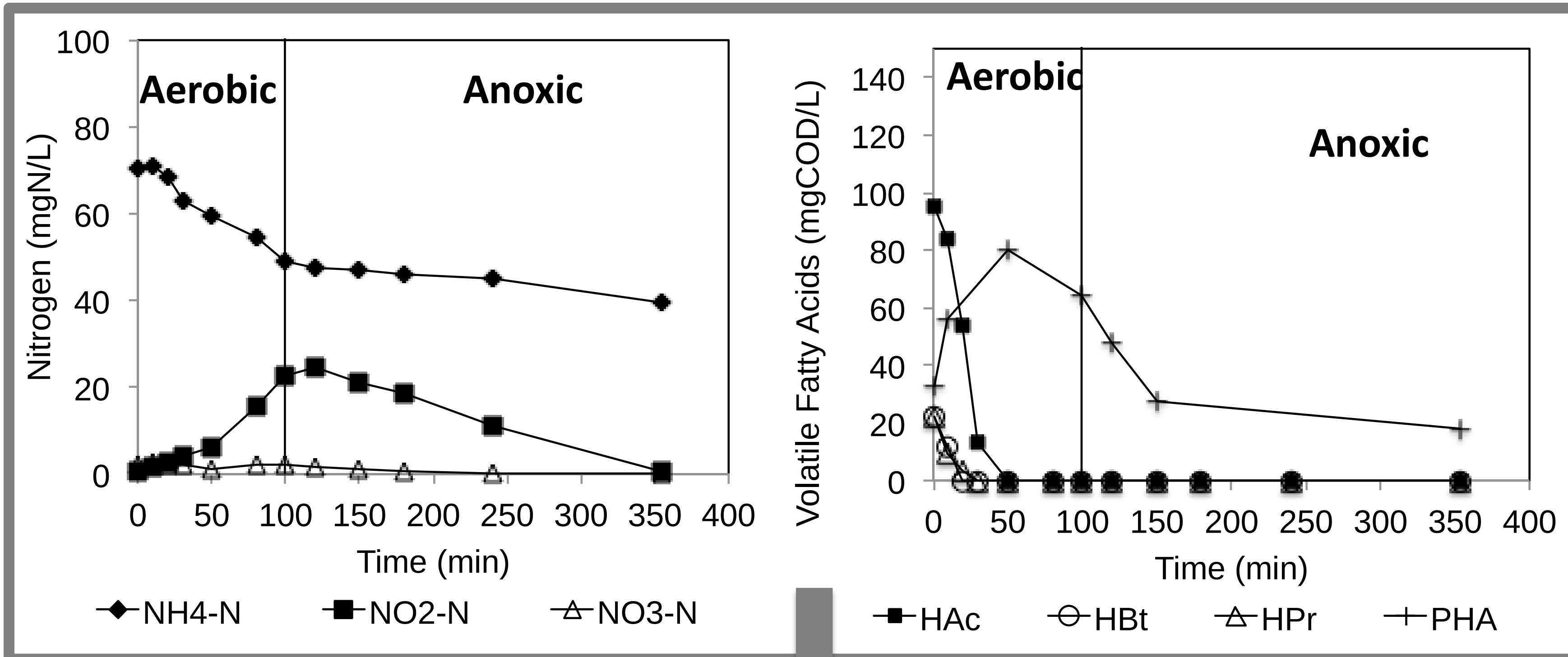
Step 4: Maximizing the PHA production (accumulation reactor)



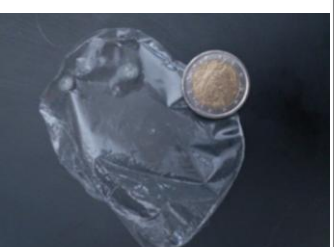
PS: primary sludge
FL: fermentation liquid

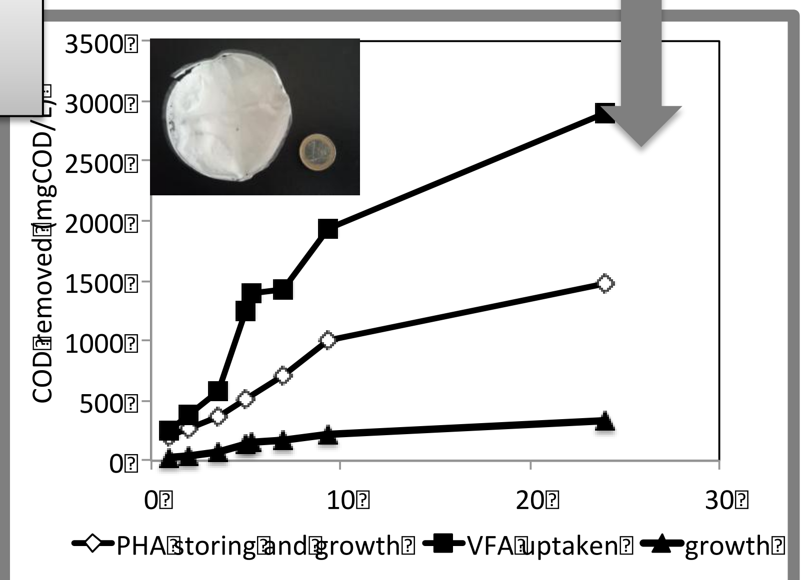
Parameter	Period 1	Period 2	Period 2	Period 2
Carbon source	Acetate	Sludge FL + Wollastonite	Synthetic Mixture of VFA	Sludge FL
COD(VFA):NH_4-N:PO_4-P	100:0:0	100:7.8:0.06	100:0:0	100:9.7:2.1
-qTVFA (mgCOD/gCODh)	90.3 ±1 1.8	133.3 ± 38.7	177.4 ± 15.7	94.9 ± 22.3
qPHA	41.5 ± 12.3	40.0 ± 4.6	81.7 ± 12.6	25.3 ± 6.7%
PHA (gCOD/gCOD) in 8-9 h	0.29 ± 0.03	0.18 ± 0.04	0.35 ± 0.04	0.16 ± 0.02
$Y_{PHA/VFA}$ (gCOD/gCOD)	0.46 ± 0.10	0.33 ± 0.04	0.51 ± 0.06	0.27 ± 0.04
$Y_{X/VFA}$ (gCOD/gCOD)	0.20 ± 0.05	0.22 ± 0.09	0.20 ± 0.02	0.28 ± 0.06

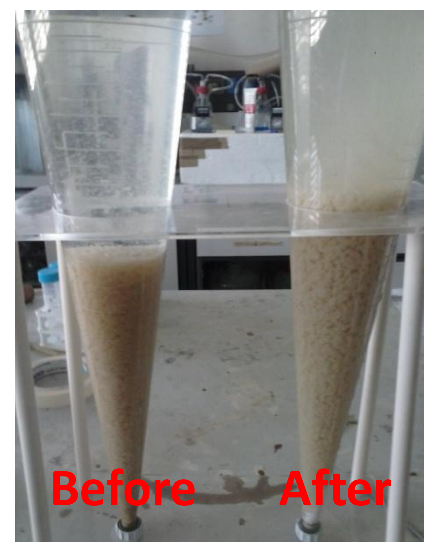
PHA selection



PHA accumulation







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, ostheosynthetic 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

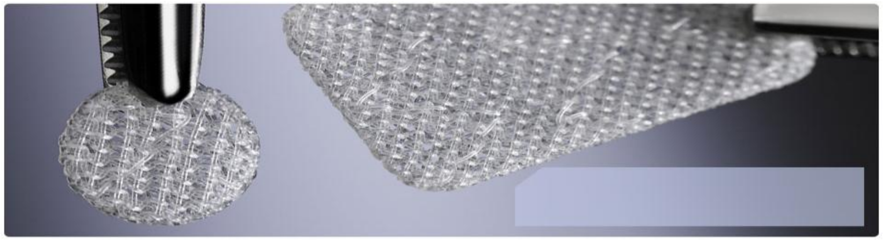
Household

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?

Advantages	Points that must be improved – potential drawbacks
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	Increased competition between the heterotrophic & autotrophic bacteria in the aerobic phase for the DO in the presence of VFAs
Integrates reject water treatment with PHA selection in the one reactor	
The previously stored PHA are the electron donor for denitritation without the requirement for carbon source	Nitritation is not always effective → lack of NO_2-N for denitritation → nitrogen removal is limited **It is crucial to ensure effective and stable nitritation and nitrite as electron acceptors for denitritation**
The short-cut via nitrite process results in lower aeration and external carbon source requirements than conventional nitrification/denitrification.	
The PHA selection with the famine–anoxic phase requires less O2 compared to the typical feast/famine process under aerobic conditions	
The use of wollastonite in the fermentation can adjust the ration of C/N/P during PHA accumulation	The duration of the feast & famine phase should be adjusted
The proposed scheme could be integrated with processes applying nitritation as a stage of the treatment plant.	Nutrient release in the fermented liquid → Use of wollastonite

Moving towards a new perspective.....

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.

the international water association

ecoSTP EcoTechnologies for Wastewater Treatment

Technical, Environmental & Economic Challenges

Verona, Italy | 23-27 June 2014

'ECOSTP2014' – VERONA 23-27 JUNE 2014

www.ecostp.org

Thank you !

Organized by

In collaboration with

Acque Veronesi · AGS Azienda Gardesana Servizi · ATO Veronese · Banca Popolare di Verona

With Sponsors and Supporters

Passavant Impianti · xylem · Veolia Water · invent · Kemira · Koch Membrane Systems · SIAD

Endorsed by

Utilitatis · AIDIC · Comune di Verona · provincia di verona

Technical tour by

Veritas