The 2nd International
Resource Recovery
Conference

VIA-NITRITE SELECTION OF PHA STORING BIOMASS THROUGH FERMENTATION LIQUID OF CELLULOSIC PRIMARY SLUDGE

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SMART-Plant

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Nutrients **Water** **Energy**

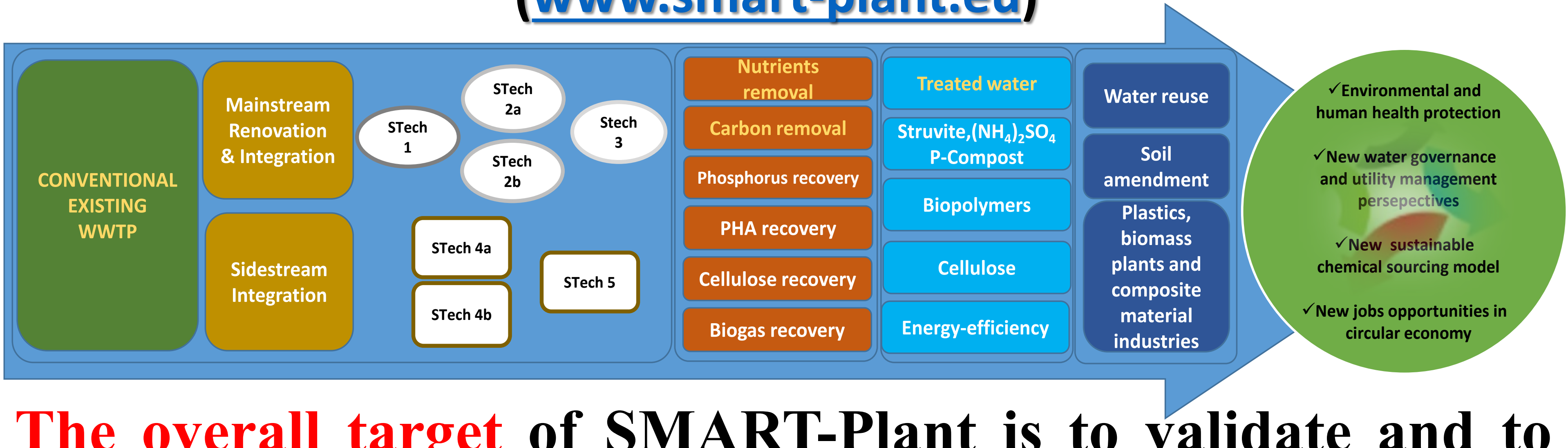
Outline

- The Horizon2020 «SMART-Plant» Innovation Action
- Cellulose in wastewater and recovery/valorization routes
- Controlled fermentation to recover propionate-rich volatile fatty acids
- Bio(co)polymers (PHB-coPHV) production in the sidestream SCEPPHAR system
- What's next: scale-up to demo in real environment at Carbonera WRRF

The Horizon2020 “SMART-Plant” Innovation Action

Scale-up of low-carbon footprint MAterial Recovery Techniques for upgrading existing wastewater treatment Plants

(www.smart-plant.eu)


CONVENTIONAL EXISTING WWTP

Mainstream Renovation & Integration

Sidestream Integration

STech 1

STech 2a

STech 2b

Stech 3

STech 4a

STech 4b

STech 5

Nutrients removal

Carbon removal

Phosphorus recovery

PHA recovery

Cellulose recovery

Biogas recovery

Treated water

Struvite,$(NH_4)_2SO_4$ P-Compost

Biopolymers

Cellulose

Energy-efficiency

Water reuse

Soil amendment

Plastics, biomass plants and composite material industries

✓Environmental and human health protection

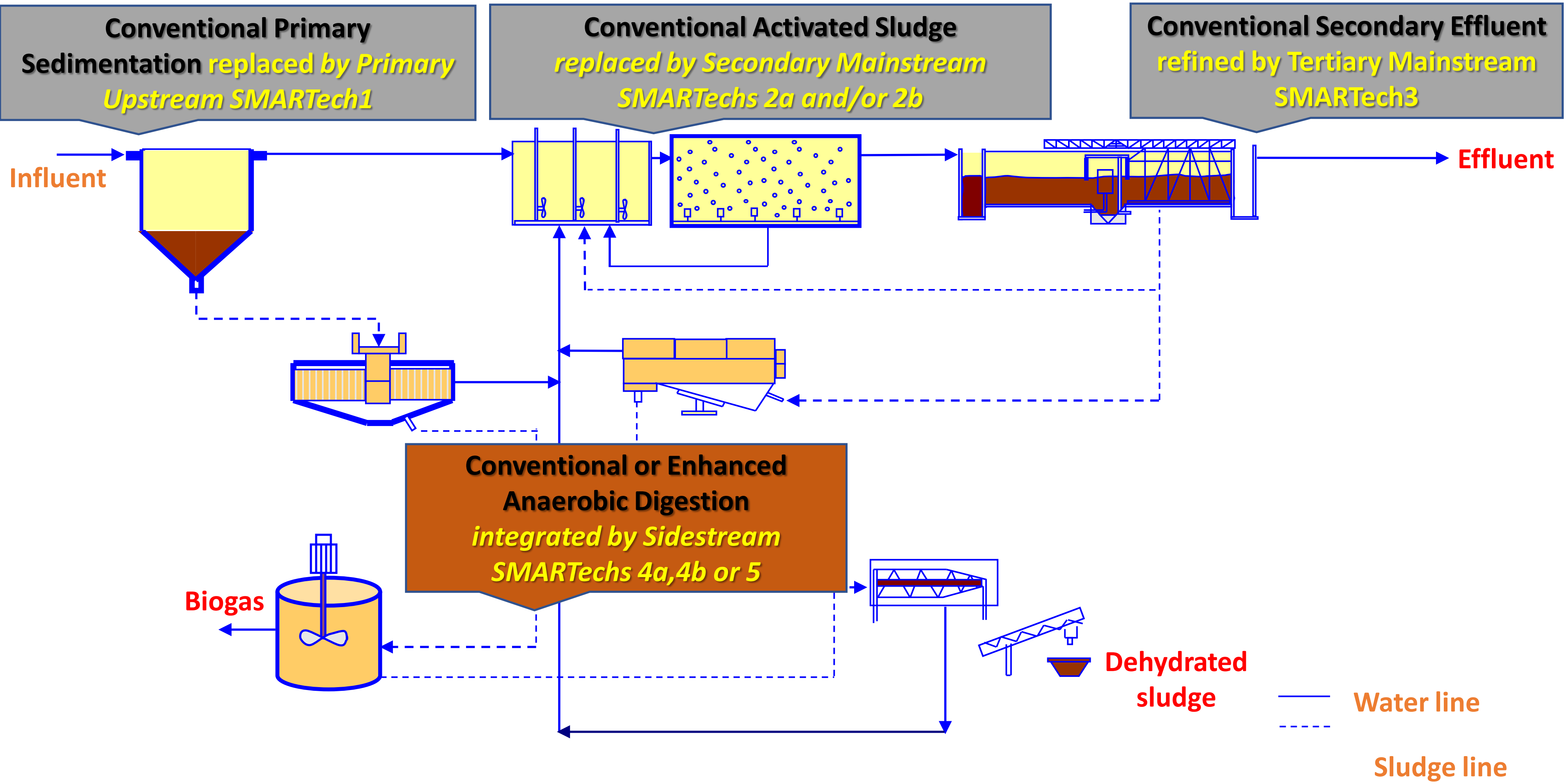
✓New water governance and utility management persepectives

✓New sustainable chemical sourcing model

✓New jobs opportunities in circular economy

The overall target of SMART-Plant is to validate and to address to the market a portfolio of SMARTechnologies that, singularly or combined, can **renovate and upgrade existing wastewater treatment plants** and give the added value of instigating the **paradigm change towards efficient wastewater-based bio-refineries**.

The SMARTechnologies to integrate and renovate existing WWTPs



Conventional Primary Sedimentation *replaced by Primary Upstream SMARTech1*

Conventional Activated Sludge *replaced by Secondary Mainstream SMARTechs 2a and/or 2b*

Conventional Secondary Effluent *refined by Tertiary Mainstream SMARTech3*

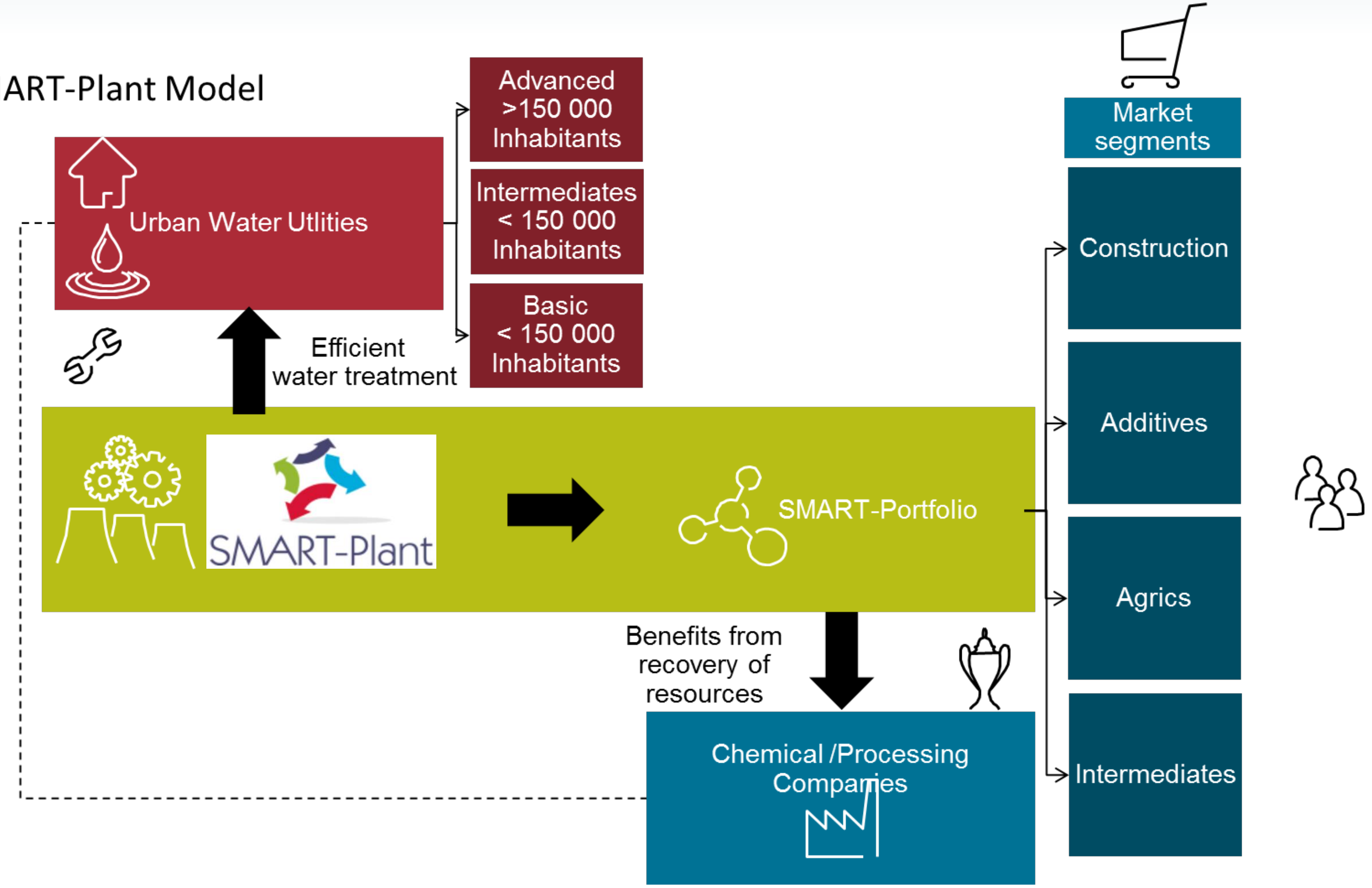
Conventional or Enhanced Anaerobic Digestion *integrated by Sidestream SMARTechs 4a,4b or 5*

Key Enabling Strategy: upstream solid concentration, integration and innovation of the sewage sludge treatment, energy efficient mainstream treatment for water reuse

The WWTPs integrated by SMART-Plant to WRRFs

SMARTech n.	Integrated municipal WRRF	Key enabling process(es)	**SMART-product(s)**
1	Geestmerabacht (Netherlands)	Upstream dynamic fine-screen and post-processing of cellulosic sludge	**Cellulosic sludge, refined clean cellulose**
2a	Karmiel (Israel)	Mainstream polyurethane-based anaerobic biofilter	**Biogas, Energy-efficient water reuse**
2b	Manresa (Spain)	Mainstream SCEPPHAR	**P-rich sludge, PHA**
3	Cranfield (UK)	Mainstream tertiary hybrid ion exchange	**Nutrients**
4a	Carbonera (Italy)	Sidestream SCENA+conventional AD	**P-rich sludge, VFA**
4b	Psyttalia (Greece)	Sidestream SCENA+enhanced AD	**P-rich sludge**
5	Carbonera (Italy)	Sidestream SCEPPHAR	**PHA, struvite, VFA**

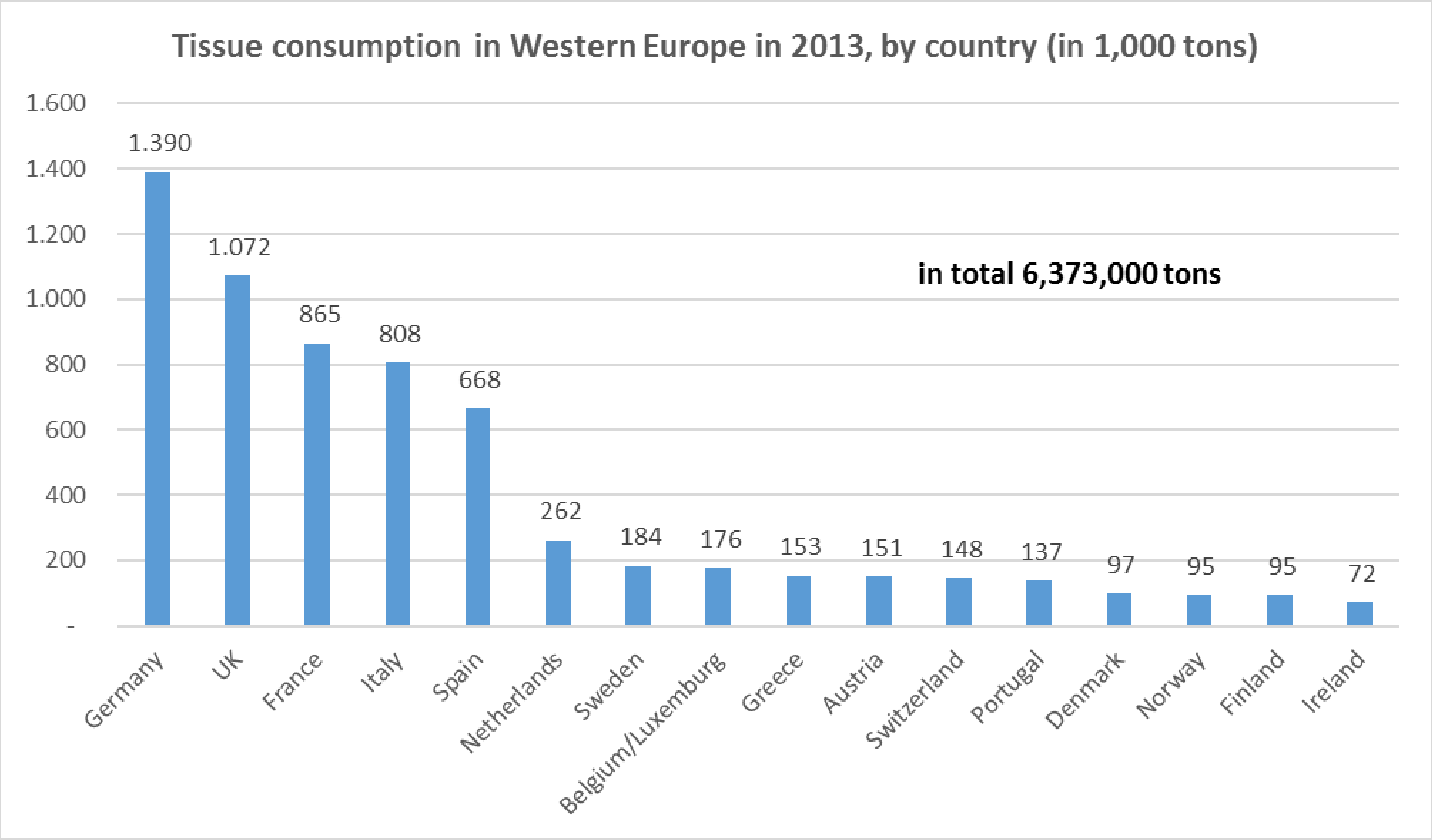
SMART-Plant value chains, business plan and market deployment strategy



Resource recovery optimization? What about toilet paper?

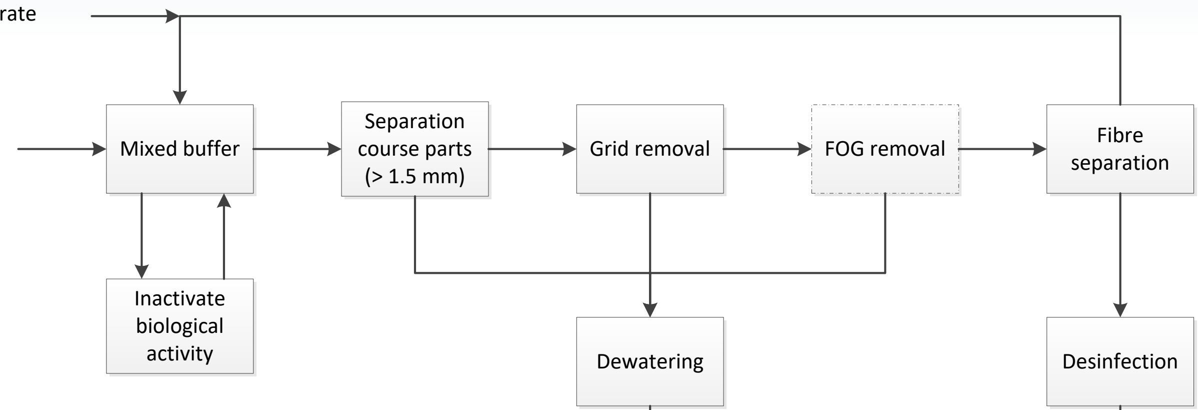
- ✓ 12 to 18 kg per person per year
- ✓ 8.5 pieces of paper per visit to the restroom
- ✓ On average, a person spends 43 hours a year on the toilet
- ✓ 70% folds the sheets before using them, 29% make a proper use

Facts and figures



Source: www.statista.com

SMARTech1: Primary (upstream) dynamic sieving and clean cellulose recovery


Flitrate

Screenings

Mixed buffer

Inactivate biological activity

Separation course parts (> 1.5 mm)

Grid removal

FOG removal

Fibre separation

Dewatering

further upgrading

Desinfection

Product sales


Inactivation biological activity


Separation course parts


Sand-/grid removal


Fibre separation

SMARTech1: Primary (upstream) dynamic sieving and clean cellulose recovery

- 79% cellulose fiber,
- 5 % other organics,
- 6% inorganic (ash),
- 10% other contaminants (average in The Netherlands).

Potentially marketable product, but the economic feasibility depends mainly on savings at the WWTP

salsnes Filter

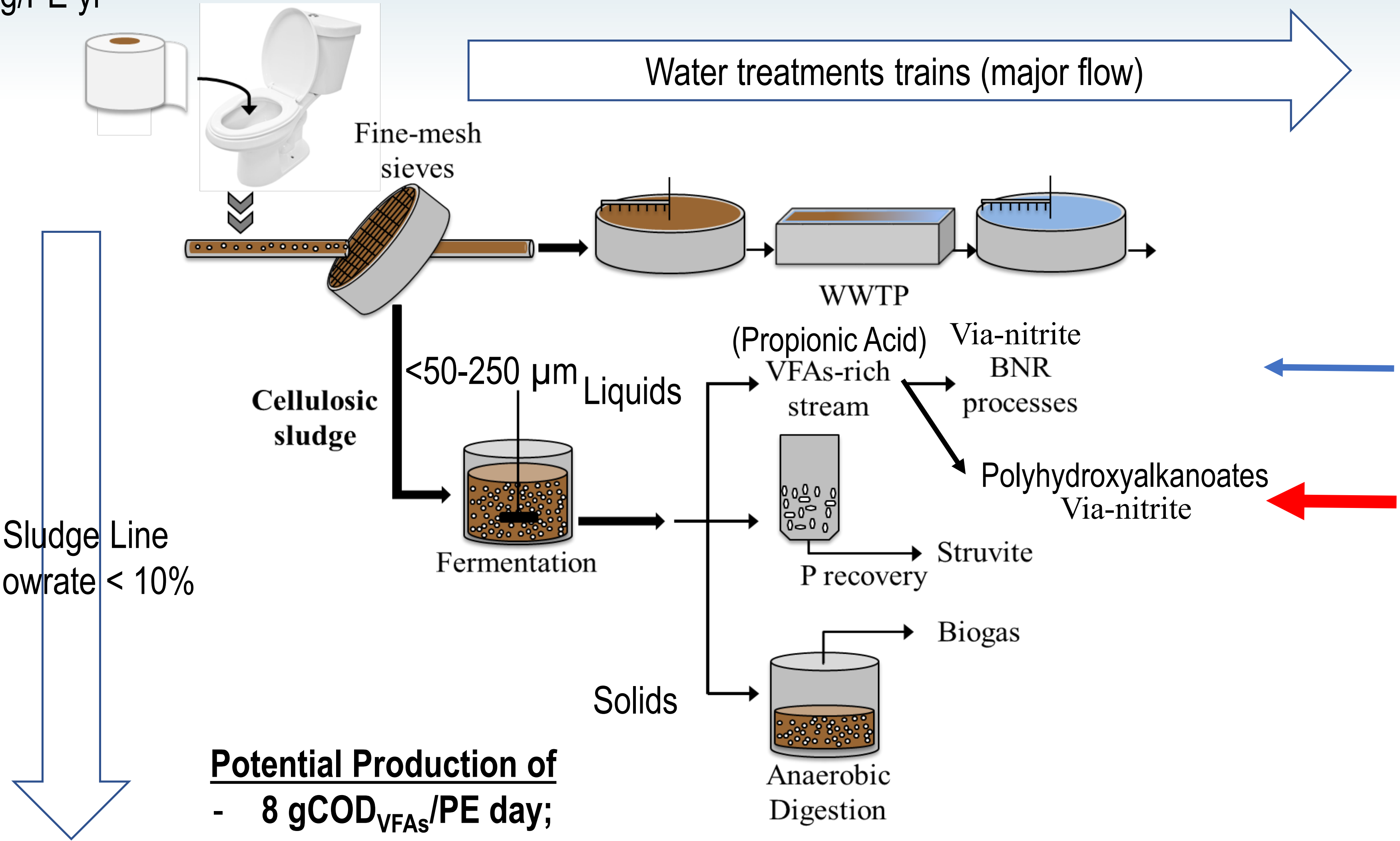
BWA

Market development

Marketing and valorization of recovered cellulose

- ✓ Reuse in asphalt
- ✓ Raw material for composite
- ✓ Insulation materials (In development, not sure yet)

Novel SMART-Plant demos for valorization of cellulosic sludge



5-15 kg/PE yr

Water treatments trains (major flow)

Fine-mesh sieves

WWTP

Cellulosic sludge

<50-250 µm

Liquids

(Propionic Acid) VFAs-rich stream

Via-nitrite BNR processes

Polyhydroxyalkanoates Via-nitrite

Fermentation

Struvite

P recovery

Biogas

Solids

Anaerobic Digestion

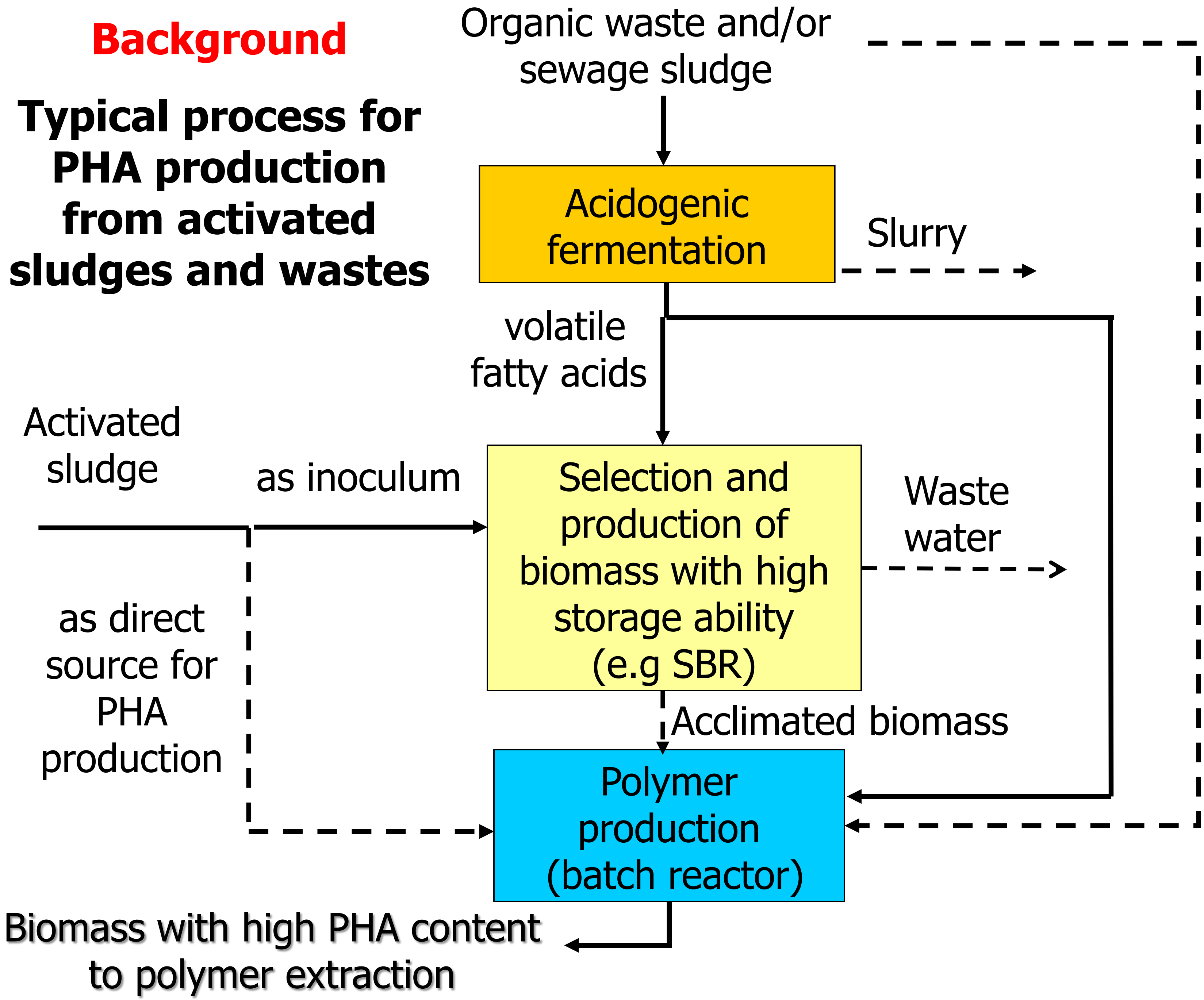
Sludge Line Flowrate < 10%

Potential Production of

- **8 $gCOD_{VFAs}$/PE day;**
- **10 gStruvite/PE day**

Background

Typical process for PHA production from activated sludges and wastes



Organic waste and/or sewage sludge

Acidogenic fermentation

Slurry

volatile fatty acids

Activated sludge

as inoculum

Selection and production of biomass with high storage ability (e.g SBR)

Waste water

as direct source for PHA production

Acclimated biomass

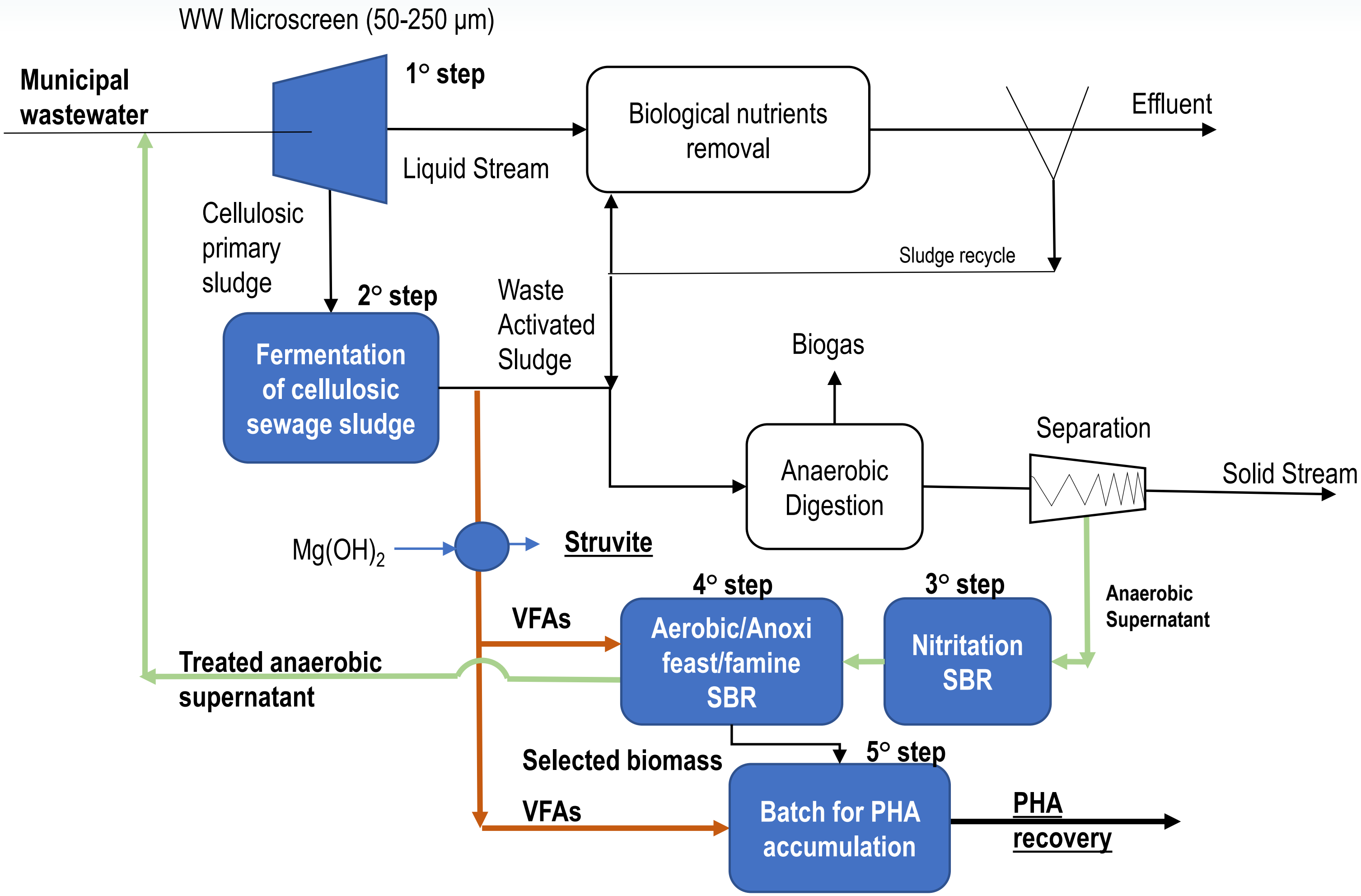
Polymer production (batch reactor)

Biomass with high PHA content to polymer extraction

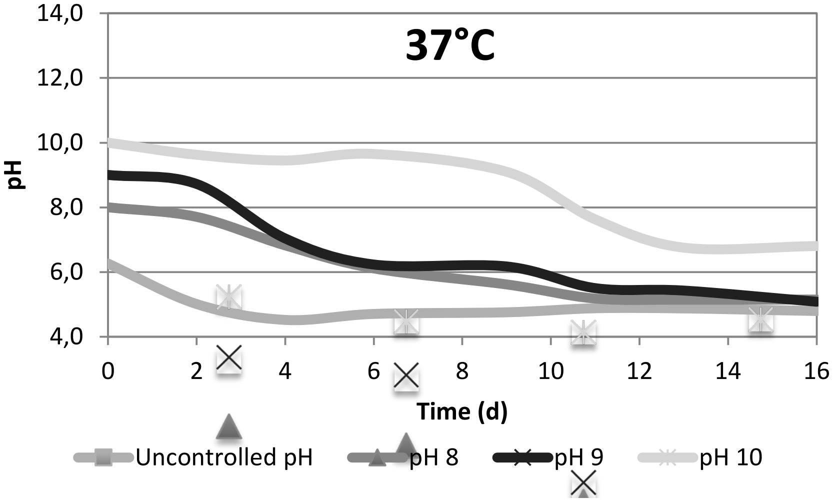
The SMART-Plant innovation:

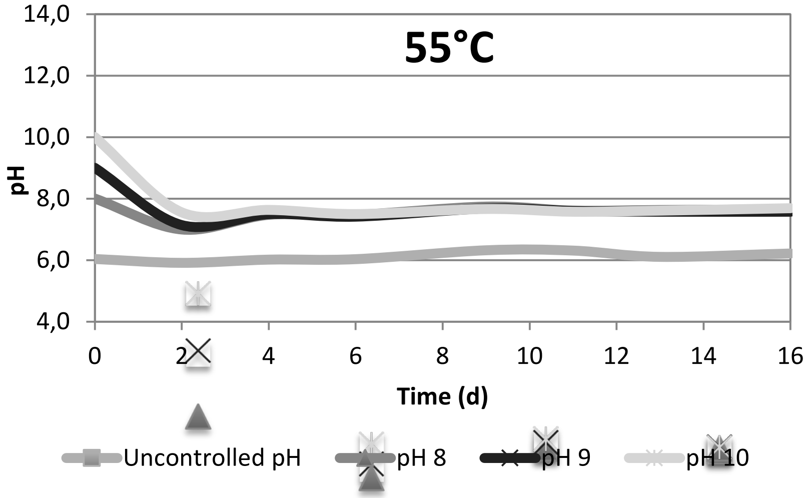
- To integrate the energy-efficient via-nitrite nitrogen removal with the PHA recovery → major interest of the water utility
- To adopt anoxic (via-nitrite) famine conditions to optimize energy consumptions
- Phosphorus (struvite) recovery even to support the balance of nitrogen and phosphorus to the PHA recovery system
- **To optimize the system using primary cellulosic sludge → IRRC2017**

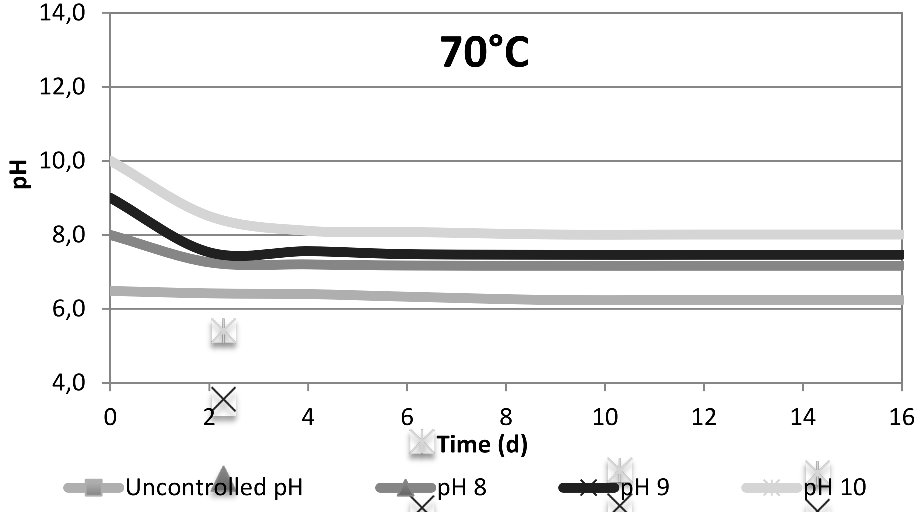
Sidestream S.C.E.P.P.H.A.R.: Short-Cut Enhanced Phosphorus and PHA recovery



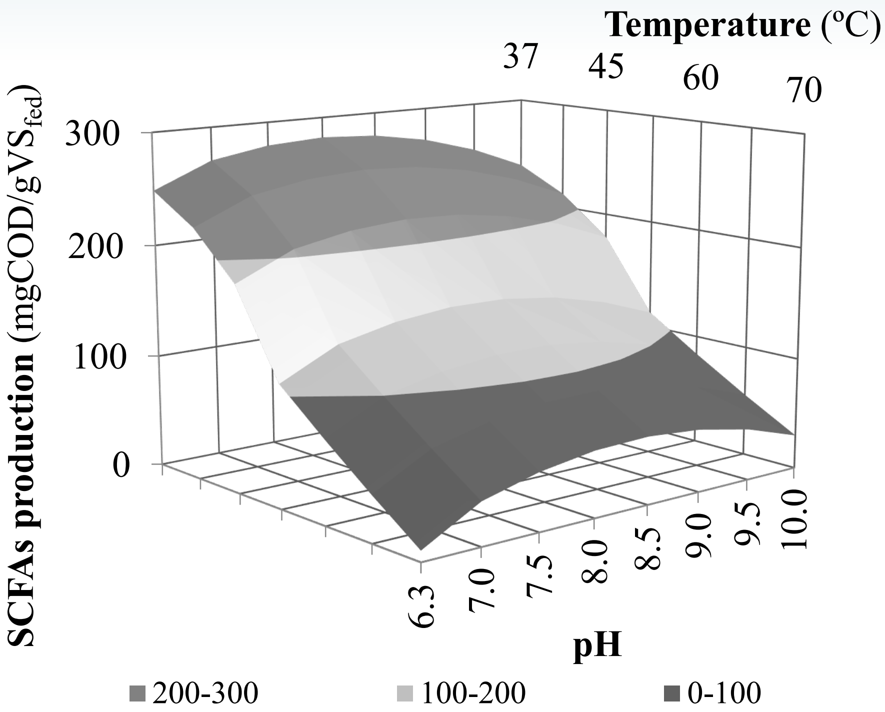
Outline of the batch Cellulosic Fermentation experiments

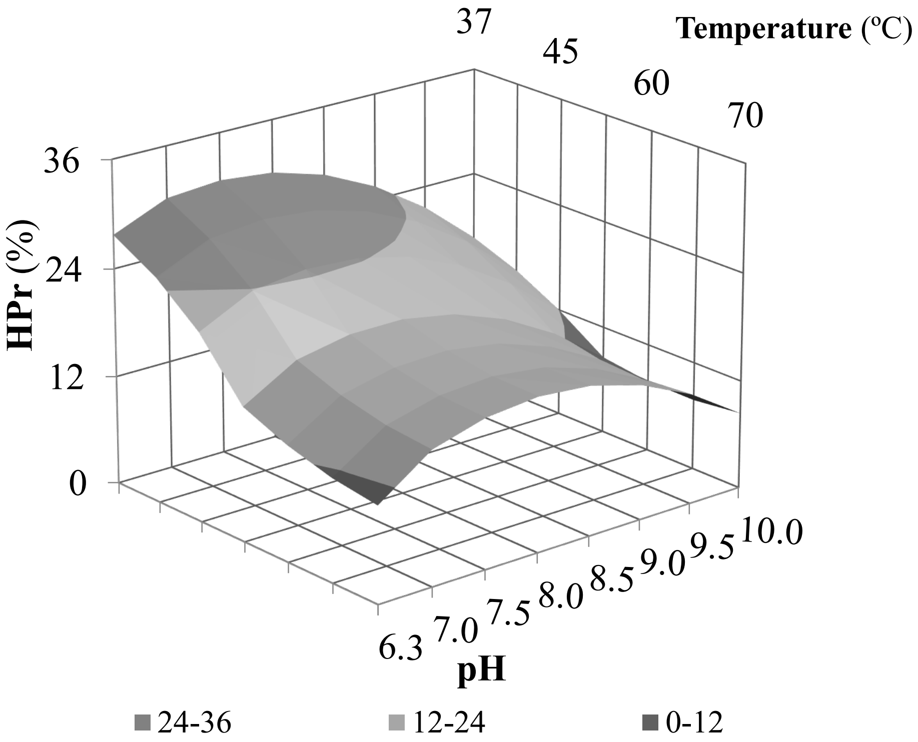






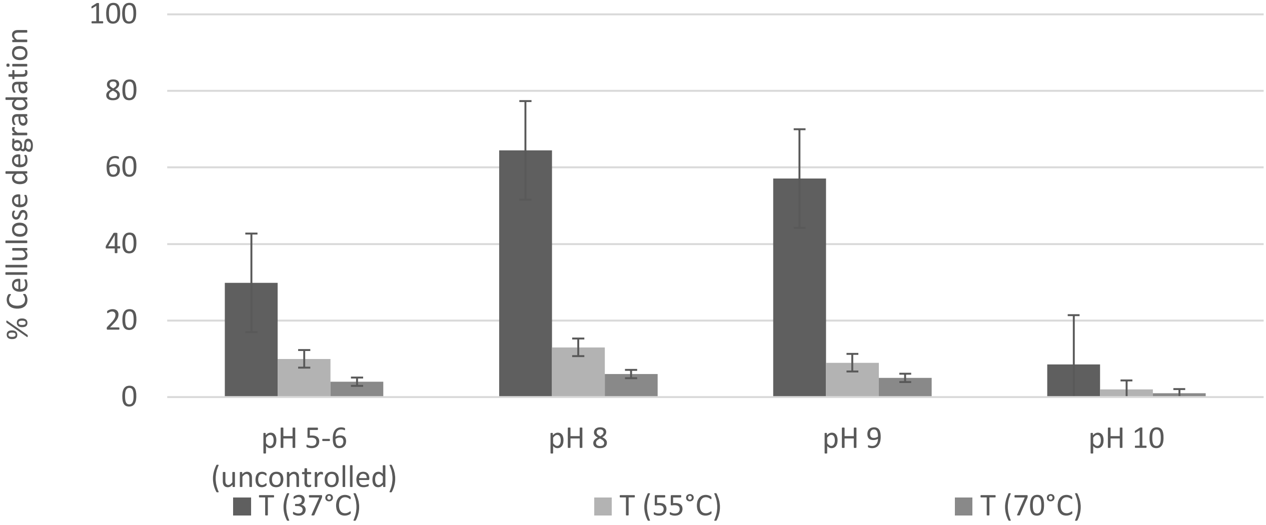
Optimal parameters for the recovery of Propionic Acid





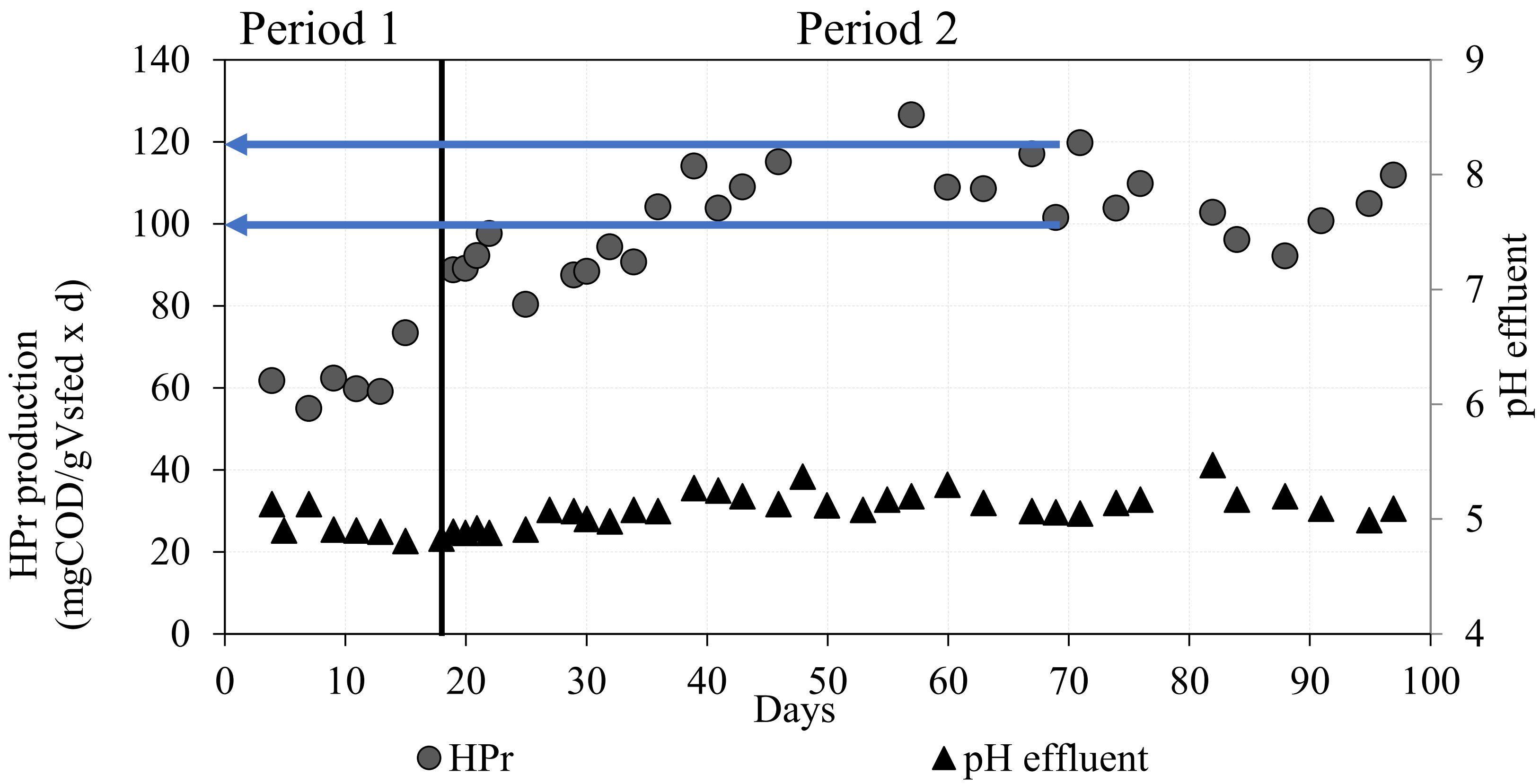
- Initial pH 7.5-8
- Temperature 37°C

Cellulose biodegradation



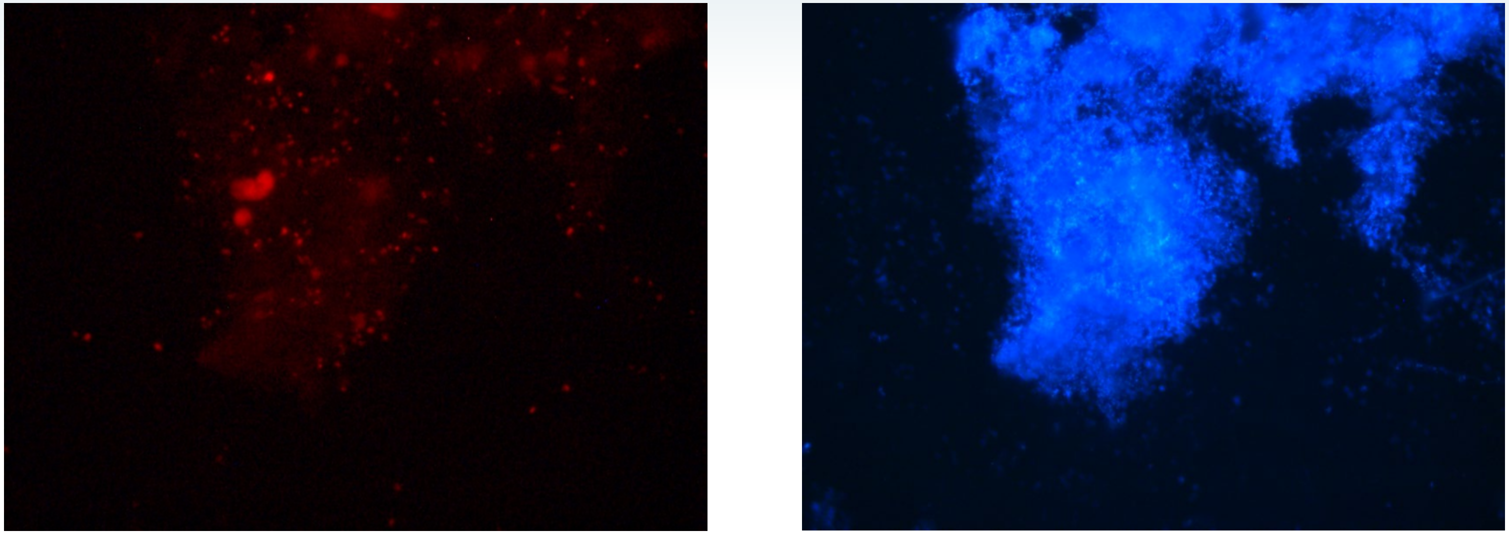
Residual sieved material (250 µm) after fermentation

Sequencing Batch Fermentation Reactor



- Period 1: uncontrolled pH; **Period 2: influent pH 8**
- Stable production of propionic acid during period 2
- Propionic acid: 30-35 % SCFA

FISH Analyses on *Propionibacterium acidopropionici*

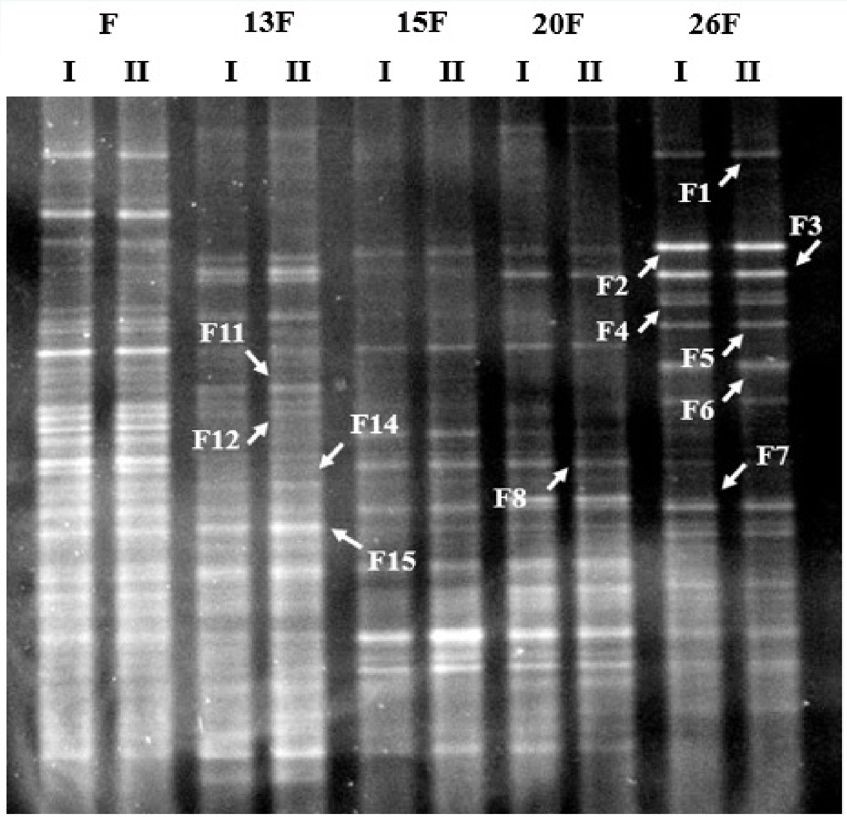


Temperature (°C)	37 Aver. ± St.Dev	55 Aver. ± St.Dev.
Bacteria detected with probe Apr820 (%)	34±15.7	24±28
Propionate Production (mgCOD/gVSS)	100-120	20-40

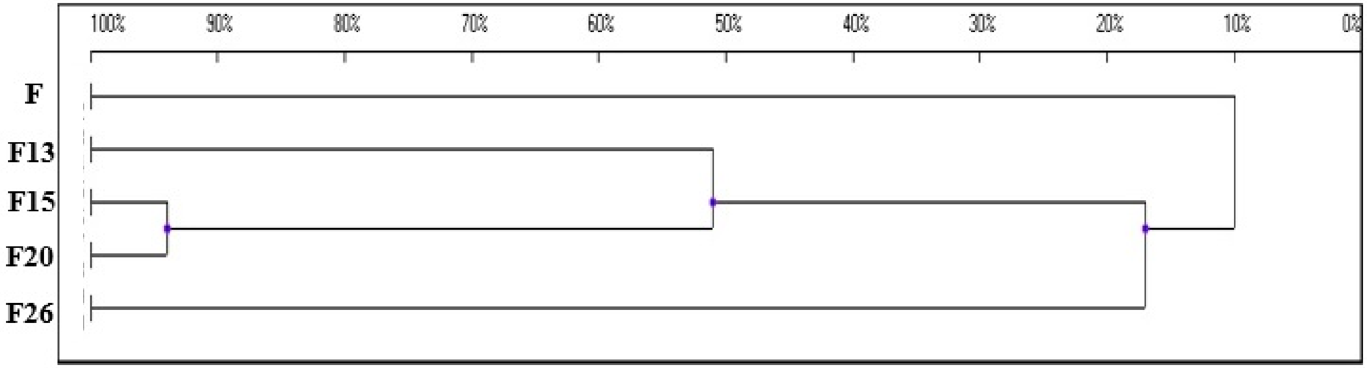
- *Propionibacterium acidopropionici* are more present at a temperature of 37 rather than 55° C.
- % of bacteria is correlated with the amount of propionate and they both decrease as the temperature increases

Microbial Community Analyses of the Fermentation Unit

DGGE Analyses



The PCR-DGGE results showed heterogeneous bacterial population in the fermentation unit



Phylogenetic analyses

Band	Closest bacterial strain	Accession number	Percentage of identity (%)	Class	Phylum
F1	Uncultured *Rhodopila* sp. CJ	FJ495222	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Parvibaculum* sp. CH	FJ495220	99	Alphaproteobacteria	Proteobacteria
F2	Uncultured bacterium	AF390917	100	Betaproteobacteria	Proteobacteria
	Uncultured beta proteobacterium	HE856465	99	Betaproteobacteria	Proteobacteria
	Comamonas sp. RPWA5.3	KC584758	98	Betaproteobacteria	Proteobacteria
	Curvibacter delicatus	NR_028713	98	Betaproteobacteria	Proteobacteria
	Acidovorax sp. DQS-01	KP126996	98	Betaproteobacteria	Proteobacteria
F3	Uncultured alpha proteobacterium	JQ919505	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Rhodopila* sp. CJ	FJ495222	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Parvibaculum* sp. CH	FJ495220	99	Alphaproteobacteria	Proteobacteria
F4	Uncultured *Proteiniphilum* sp. partial 16S rRNA gene, isolate OTU 224	LT625110	98	Cytophagia	Bacteroidetes
	Uncultured *Bacteroidetes* bacterium	FJ754858	98		Bacteroidetes
	Bacteroidales bacterium	GU129048	98		Bacteroidetes
F5	Uncultured alpha proteobacterium	JQ919505	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Rhodopila* sp. CJ	FJ495222	98	Alphaproteobacteria	Proteobacteria
	Uncultured *Parvibaculum* sp. CH	FJ495220	98	Alphaproteobacteria	Proteobacteria
F6	Uncultured *Bacteroidetes* bacterium	CU917950	100		Bacteroidetes
	Bacteroidales bacterium	GU129081	99		Bacteroidetes
	Proteiniphilum sp. I28	KP262056	99	Cytophagia	Bacteroidetes
	Porphyromonadaceae bacterium enrichment culture	HQ133064	99	Bacteroidales	Bacteroidetes
F7	Uncultured alpha proteobacterium	JQ919505	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Rhodopila* sp. CJ	FJ495222	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Parvibaculum* sp. CH	FJ495220	99	Alphaproteobacteria	Proteobacteria
F8	Uncultured bacterium clone	KP279931	100		
	Flavobacterium sp. enrichment culture clone SA NR2 1	GU726988	99	Flavobacteria	Bacteroidetes
	Uncultured *Leadbetterella* sp. SSP-AOLR-2-1	KR705991	99	Cytophagia	Bacteroidetes
	Cytophaga sp. INT-18	AB046729	98	Cytophagia	Bacteroidetes
F11	Uncultured bacterium gene	AB746716	96		
F12	Uncultured alpha proteobacterium	JQ919505	99	Alphaproteobacteria	Proteobacteria
	Uncultured *Rhodopila* sp. CJ	FJ495222	98	Alphaproteobacteria	Proteobacteria
	Uncultured *Parvibaculum* sp. CH	FJ495220	98	Alphaproteobacteria	Proteobacteria
F14	Uncultured *Saprospiraceae* bacterium	KP717501	100	Sphingobacteria	Bacteroidetes
	Uncultured *Haliscomenobacter* sp. C-115	JX415430	99	Sphingobacteria	Bacteroidetes
F15	Uncultured *Clostridium* sp. D7	KM494503	96	Clostridia	Firmicutes

Focus on cellulolytic activity

Firmicutes

Firmicutes, (e.g. like Clostridium and Bacillus) are able to produce cellulases such as carboxymethyl cellulase (CMCase). It plays an important role to hydrolyse cellobiose to fermentable glucose

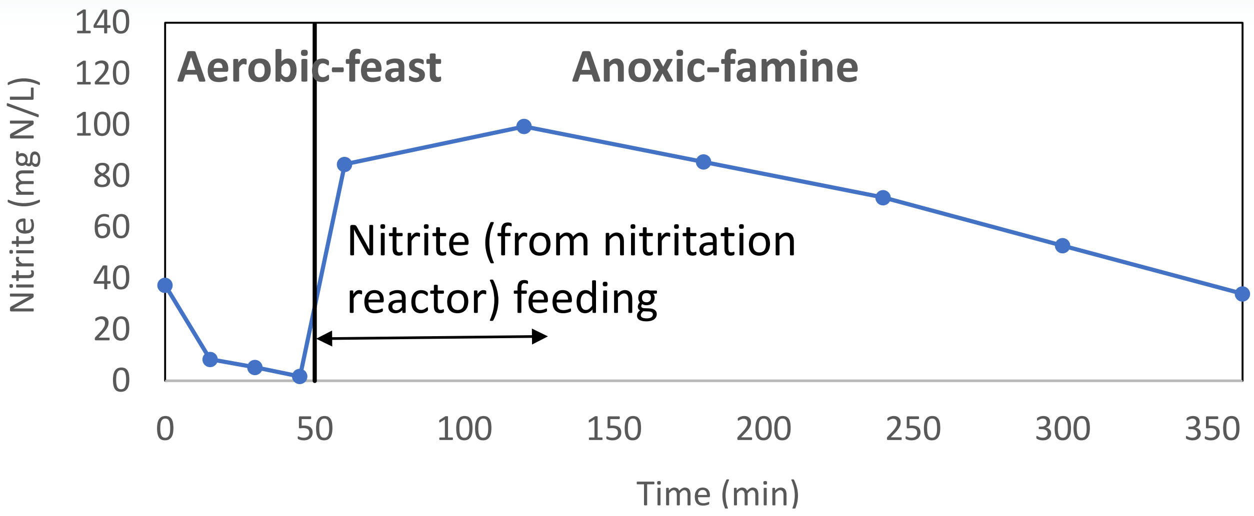
Bacteroidetes

Bacteroidetes are able to decompose long chained polymers such as cellulose and so contribute to the hydrolysis step in fermentation.

Operating conditions for the PHA-storing community selection

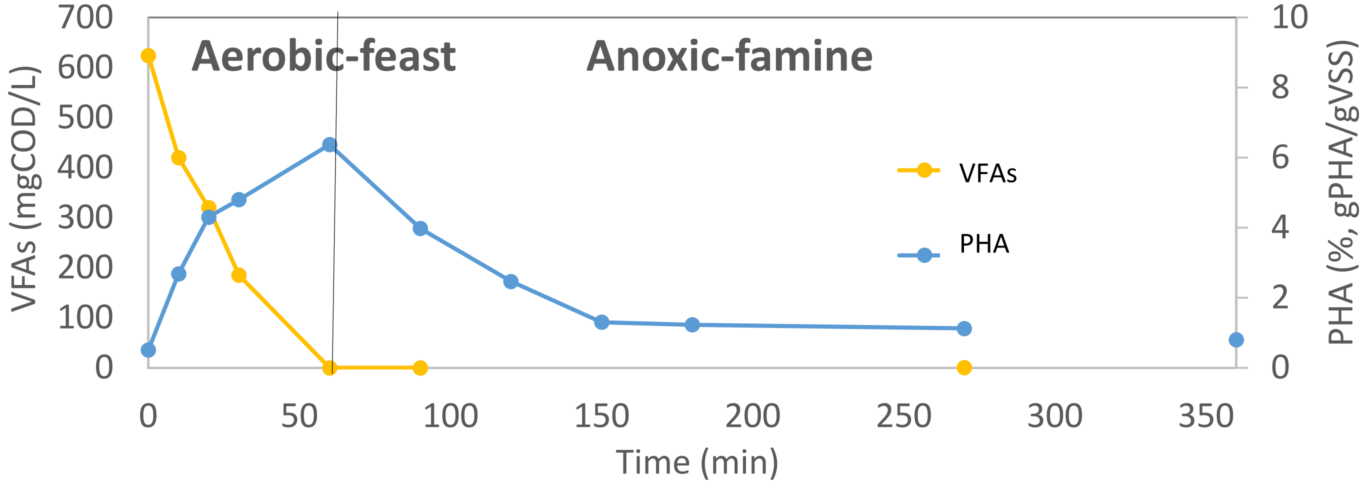
Parameter	Period I (0-39 days)	Period II.1 (40-106 days)	Period II.2 (107-145 days)
vNLR (kgN/m^3d)	0,610	0,480	**0,56**
vOLR(kgCOD/m^3d)	1,3	1,32	**1,2**
HRT (d)	1,5	1,1	**1,2**
Aerobic/Anoxic	1	0,44	**0,10-0,20**
SRT (d)	5	7-10	**7-10**
Carbon source	VFAs from synthetic acetic and propionic acid	VFAs from synthetic acetic and propionic acid	**VFAs from real cellulosic primary sludge (C.P.S)**
Nitrified anaerobic supernatant	NO2 and NH4+ from synthetic anaerobic supernatant	NO2 and NH4+ from synthetic anaerobic supernatant	**NO2 and NH4+ from real anaerobic supernatant**

Selection of PHA-storing microorganisms: S.C.E.P.P.H.A.R. cycle



Nitrite denitritation driven by PHA

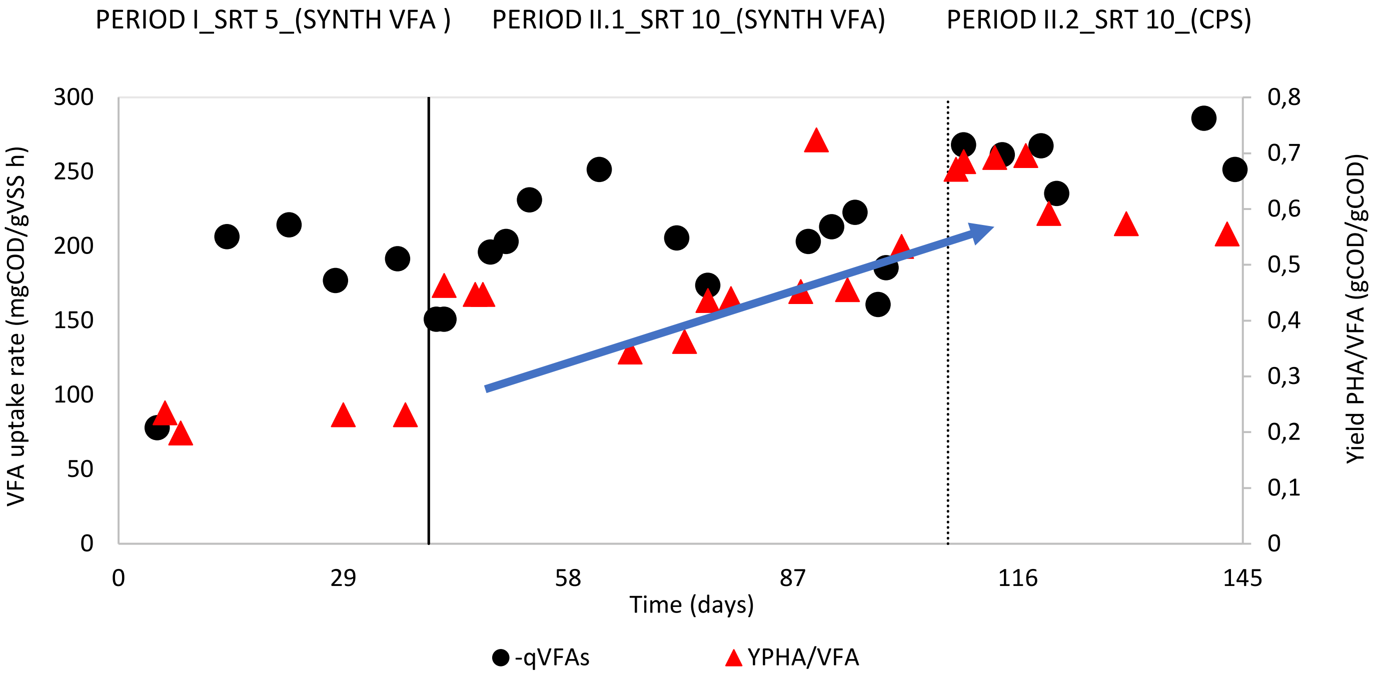
Kden(20°C)=
6-8 mgNO$_2$-N/gVSS h



Feast phase 16% of the total cycle

Performance of the selection of PHA-storing microrganisms

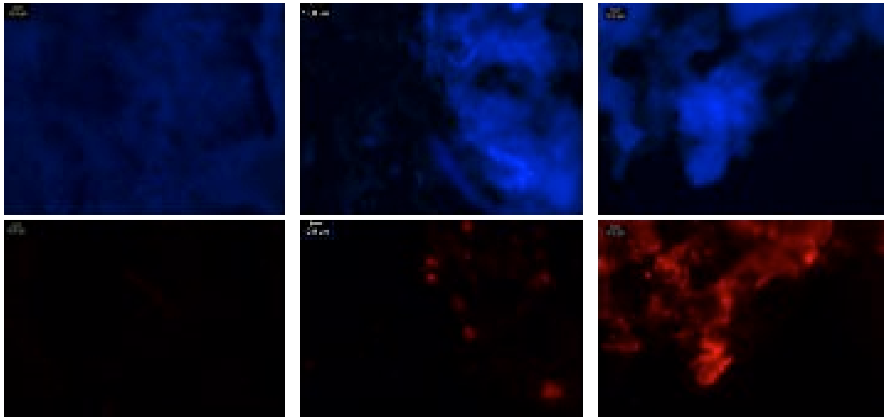
From the day 40, the $Y_{PHA/VFA}$ gradually increased (from 0.22 to 0.51 $gCOD_{PHA}/gCOD_{VFA}$), reaching the better results when the CPS was used as C-source in the period II.2 (up to 0.65 $gCOD_{PHA}/gCOD_{VFA}$).

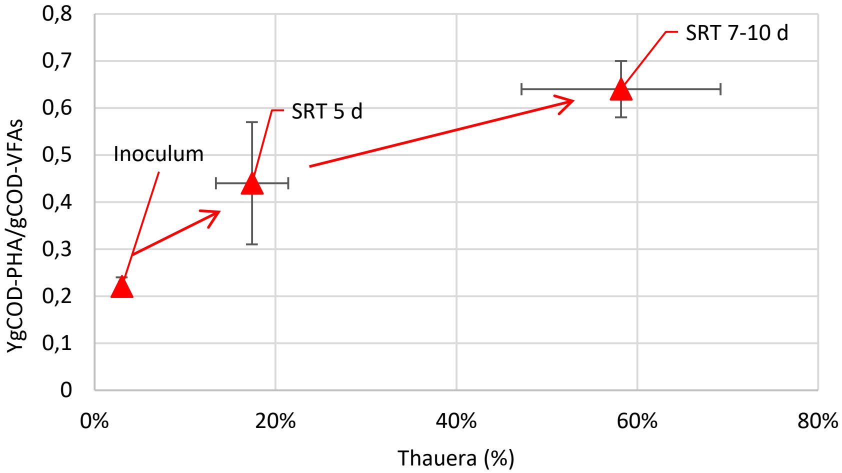


Analyses of the microbial community

Link between process performance characteristics and microbial population

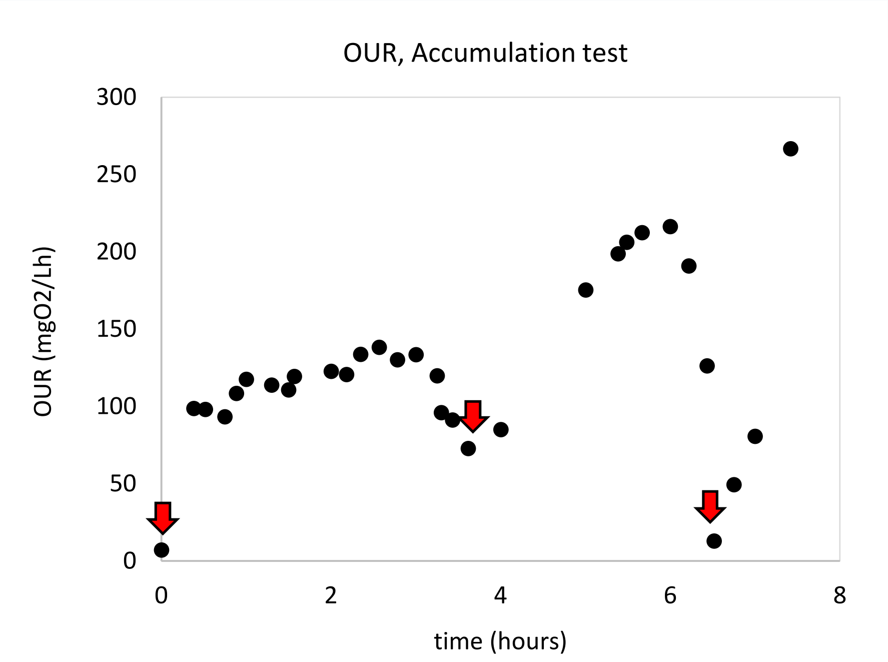
Significant increase of **Thauera** concentration from 3±0 (Period I) to 58±11% (Period II.2), according with the increase of the PHA storage yields at SRT of 7-10 days.

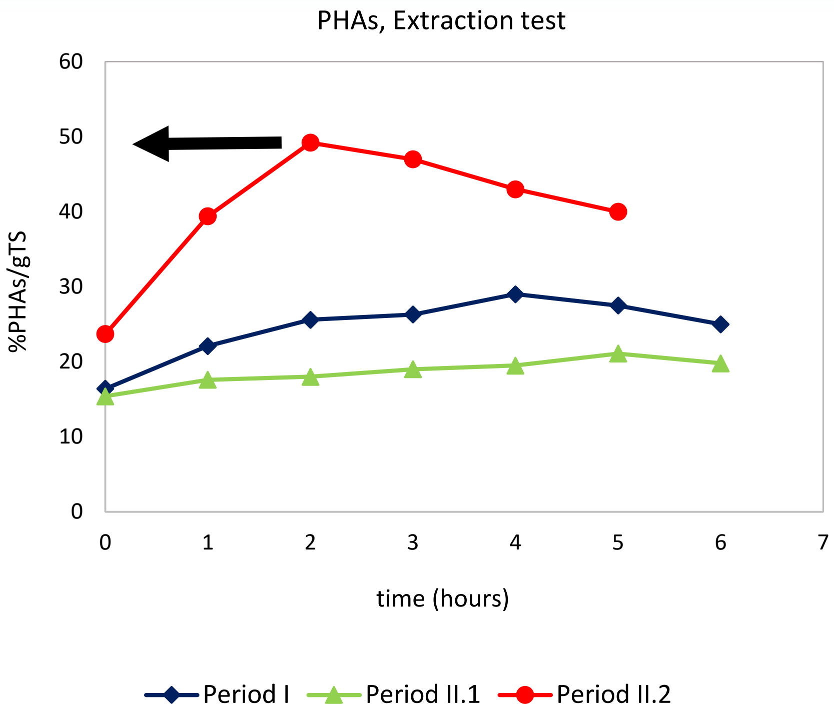




The increase of the $Y_{PHA/VFA}$ from 0.42 (with SRT 5 days) to 0.64 (with SRT 7-10) $gCOD_{PHA}/gCOD_{VFAs}$ could be attributed to the presence of other type of organisms such as Paracoccus and Azoarcus.

Accumulation and PHAs Extraction





- The presence of carbon substrate was monitored via the Oxygen Uptake Rate (O.U.R) profiles;
- No significant differences between the period I and II.1 in terms of %PHAs/gTS (25% PHA/gTS);
- The carbon source from cellulosic sludge maximized the PHAs content up to 50 %PHA /gTS.

From SCEPPHAR 1.0 to 2.0 (using cellulosic sludge)

Parameter	Original configuration SRT 15 days (Frison et al., EST, 2015)	Period II.2 (107-145) SRT 10 days (current study)
Carbon Source	VFAs from mix sludge fermentation	VFAs from real cellulosic primary sludge
Denitrification efficiency (%)	89,4 ± 4	86,21 ± 4,85
F/M (kgCOD/KgMVLSS)	0,37 ± 0,07	0,63 ± 0,09
feast/famine	0,13	0,10 ± 0,01
-qVFA (mgCOD/gVSS h)	239 ± 7	260,24 ± 18,77
$Y_{PHA/VFAs}$ (gCOD-PHA/gCOD-VFA)	0,42 ± 0,03	0,64 ± 0,06

Conclusions: via-nitrite PHA production

- The fermentation of cellulosic primary sludge from sieved wastewater provides a suitable source of propionate-rich VFAs for the PHA production;

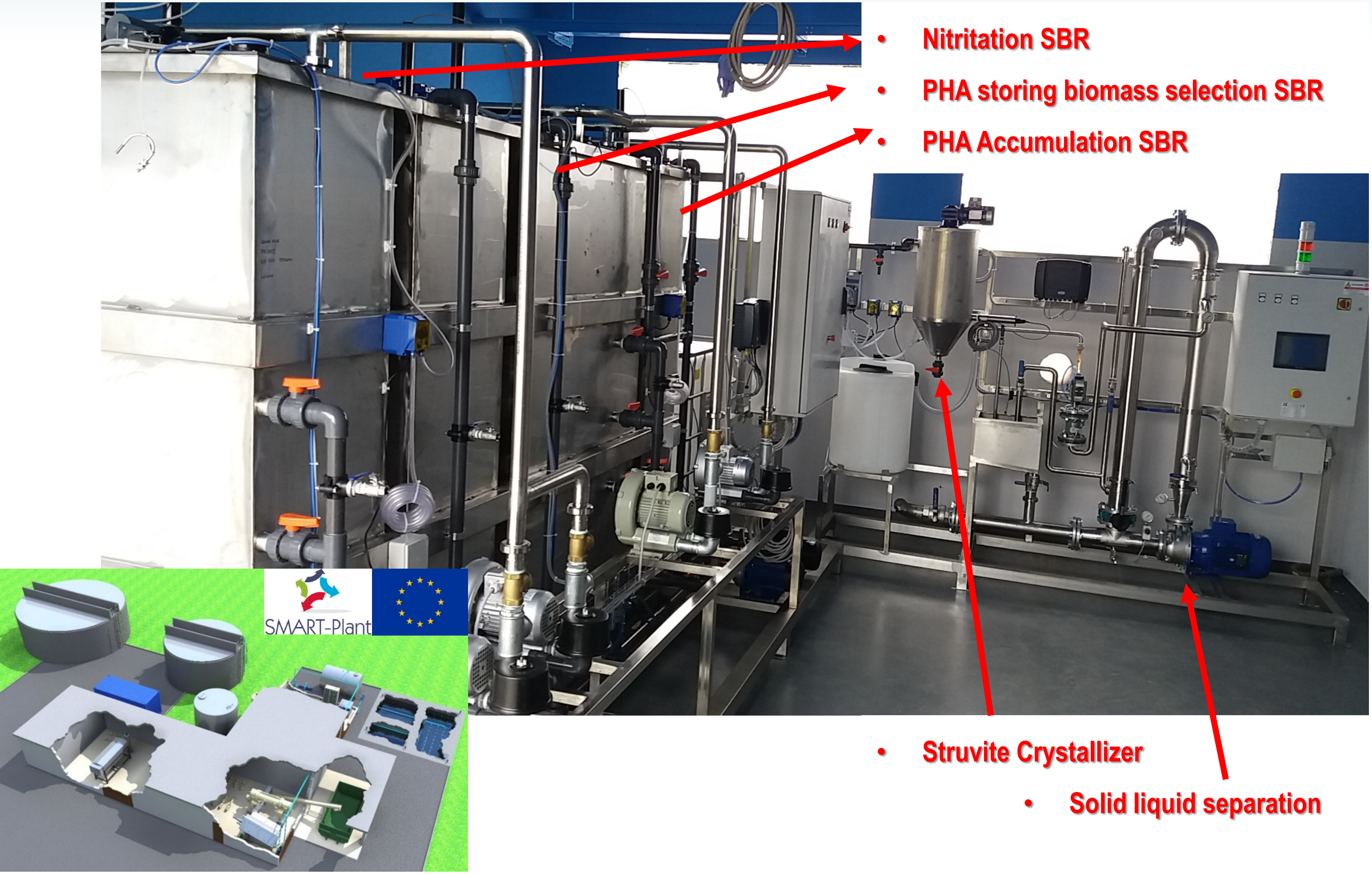
- Aerobic/Feast with Anoxic/Famine regime was coupled with the via-nitrite route to treat high nitrogenous anaerobic effluent;

- After 2 hours of accumulation, the PHA content was as much as 50% (gPHA/gTVS).

- Struvite recovery from cellulosic primary sludge can be a strategy to promote the PHA storage during the accumulation stage.

- The Sidestream S.C.E.P.P.H.A.R. is the **SMARTech5** of the **Horizon2020 Smart-Plant** which will be scaled up at pilot scale (potential 0.5-0.8 kgPHA/d) within the WWTP of Carbonera (TV);

SCEPPHAR pilot scale at the Carbonera WWTP (Smartech 5)



- **Nitritation SBR**
- **PHA storing biomass selection SBR**
- **PHA Accumulation SBR**
- **Struvite Crystallizer**
- **Solid liquid separation**

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