

**ANOXKALDNES**

## Production of bioplastics as by-products of waste treatment

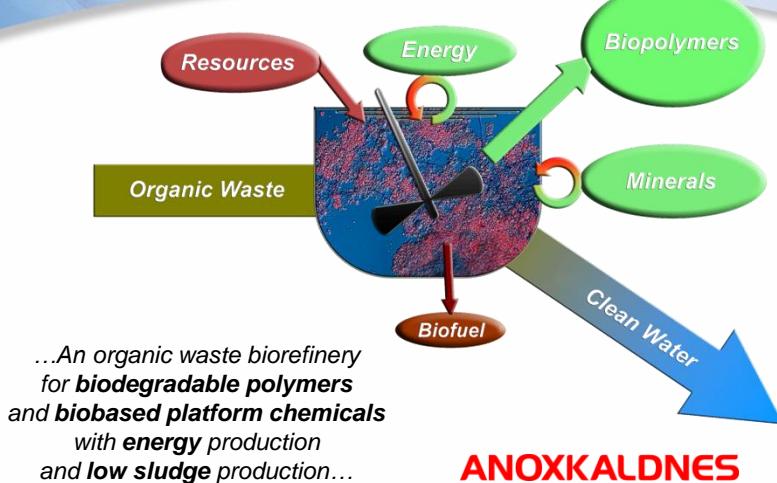
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\*Neptune Partners  
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### Wastewater treatment - biorefinery for environmental protection



## Content

- Objective
  - ▶ To present a promising vision in organic waste cycling
- Linear life of conventional plastics
- Polyhydroxyalkanoate (PHA) bioplastics
- Industrial wastewater treatment plants as biorefineries producing PHAs
- PHAs from wastewater and biosolids

## Research & Development → Application

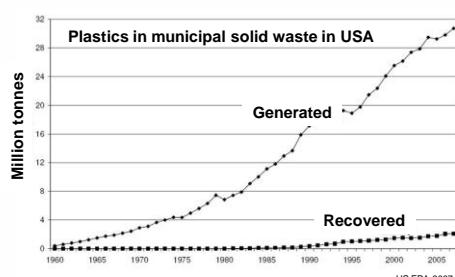
F Morgan-Sagastume et al. 2010. AnoxKaldnes AB

## Petroleum-based plastics

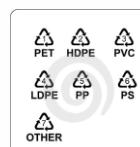
- Extensively used, but produced from a non-renewable resource

Municipal solid waste	
Region / Country	Plastic waste (million tonnes / yr)*
EU 2005	30
USA 2007	31
OECD Countries 2005	70

\*OECD Environmental Data 2008; US EPA 2007

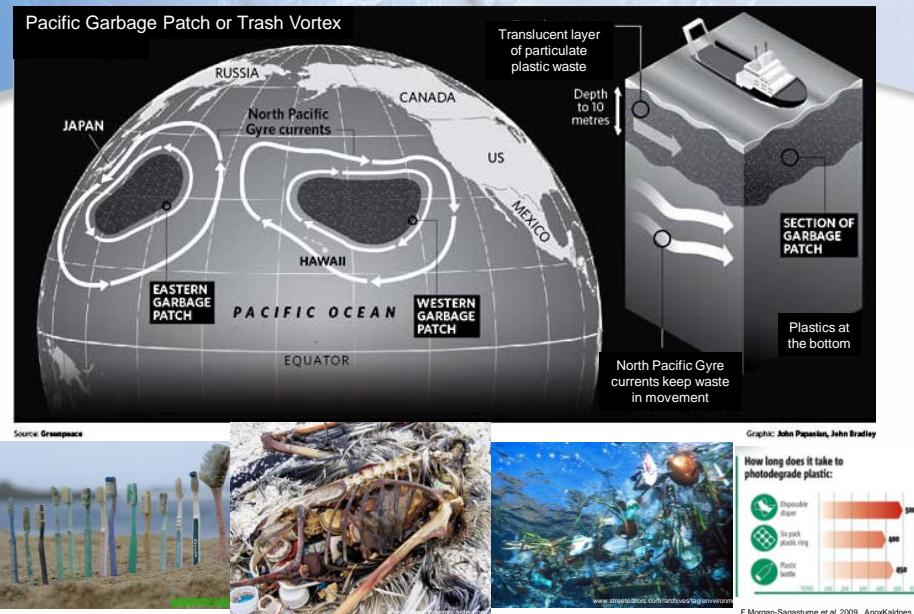


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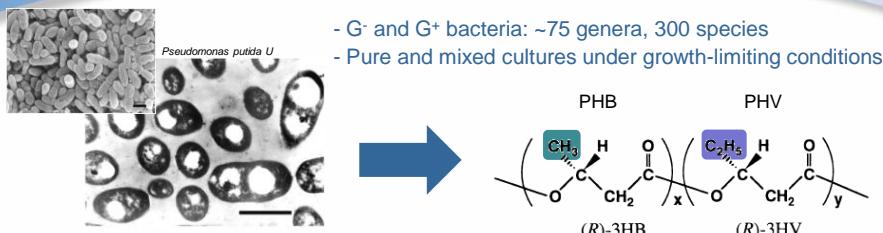
Recovery

## Plastics accumulate and impact ecosystems



## Polyhydroxyalkanoate (PHA) bioplastics

- Microbial short-chain length (scl) polyesters: 3-5 C
- Intracellular storage compounds – energy and C source



Luengo et al. 2003. Current Opinion in Microbiology, 6:251-260

**Biologically produced from renewable resources**  
**Biodegradable**  
**Thermoplastic**  
**Biocompatible**

F Morgan-Sagastume et al. 2010. AnoxKaldnes AB

*Journal of Chemical Technology and Biotechnology*      *J Chem Technol Biotechnol* 82:233–247 (2007)

**Review**  
**Polyhydroxyalkanoates: biodegradable polymers with a range of applications**  
S. Philip, T. Keshavarz and I. Roy<sup>a</sup>  
<sup>a</sup>School of Biosciences, University of Westminster, London, UK

- Injection moulded products  
- Films and coatings  
- Foams  
- Biomass products

**Gilland D - Metabolix**  
  
**Procter & Gamble - Nodax**

**SCI**  
where science meets business

## Production of commercial PHAs

Pure cultures and transgenic plants

Product name	PHA type	Company	Price	Production (tonne/yr)
<b>Homopolymers</b>				
Biomer®	P(3HB)	Biotechnology Co., Germany	20€/kg (2003) <sup>a</sup> 3-5€/kg (2010) <sup>a</sup>	50 (2003) <sup>b</sup>
Biocycle	P(3HB)	PHB Industrial S/A, Brazil	-	60
Biogreen®	P(3HB)	Mitsubishi GAS Chemical, Japan	10-12€/kg (2003) <sup>a</sup> 2.5-3€/kg (2010) <sup>a</sup>	1400 (2003), 30-60,000 (2010) <sup>a</sup>
	P(3HB)	Metabolix, USA (BASF, ADM) <sup>b</sup>	ca. 2.2 €/kg (2010) <sup>c</sup>	-
	P(3HB)	Jiangsu Nantian Group, China	-	-
	P(3HO)	Metabolix, USA (BASF, ADM) <sup>b</sup>	ca. 2.2 €/kg (2010) <sup>c</sup>	-
<b>Copolymers</b>				
Biopol®	P(3HB-co-3HV)	Metabolix, USA (BASF, ADM) <sup>b</sup>	10-12€/kg (2003) <sup>a</sup> 3-5€/kg (2010) <sup>a</sup>	1100 (2003) <sup>b</sup>
	P(3HB-co-3HV)	PHB Industrial S/A, Brazil	-	50 (2003) <sup>b</sup> , 10,000 (2006) <sup>a</sup>
ENMAT®	P(3HB-co-3HV)	Tianan Biologic Material, China	-	1000
Nodax	P(3HB-co-3HHx)	Procter & Gamble, USA (Kaneka)	2.5 €/kg (2010) <sup>a</sup>	250 (2003) <sup>a</sup> , 20-50,000 (2010) <sup>a</sup>
	P(3HB-co-3HHx)	Jiangsu Nantian Group, China	-	-
	P(3HB-co-3HHx)	Lianyi Biotech, China	>5 US\$/kg <sup>c</sup>	2000 <sup>c</sup>

<sup>a</sup> Techno-economic feasibility of large-scale production of bio-based polymers in Europe, Utrecht University and Fraunhofer Institute for Research (2003) System Innovation Research (2003)

<sup>b</sup> The strategic alliance between ADM and Metabolix will lead to a production of PHAs of 50,000/ tonnes/year in 2008

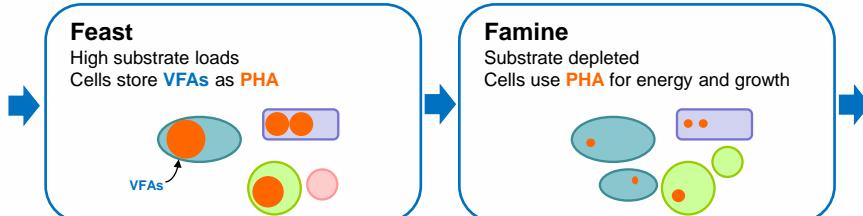
<sup>c</sup> The fourth Knowledge Millennium Summit on Biotechnology & Nanotechnology, India, O'Bier Technology Co., Ltd (March 2006)

Jacquel et al. (2008). Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochemical Engineering Journal 39:15-27.

F Morgan-Sagastume et al. 2010. AnoxKaldnes AB

## PHA production with open, mixed microbial cultures

- Towards cost effectiveness
  - ▶ Recombinant organisms: bacteria, plants, yeasts
  - ▶ Other C sources: molasses, sucrose, lactose, glycerol, oils
  - ▶ **Open, mixed cultures:** no sterilization and cheaper C sources
    - Harnessing a selective pressure by dynamic conditions
- Substrate dynamics – Aerobic Dynamic Feeding or **Feast-Famine**
  - ▶ Volatile fatty acids (VFAs) used for culture enrichment

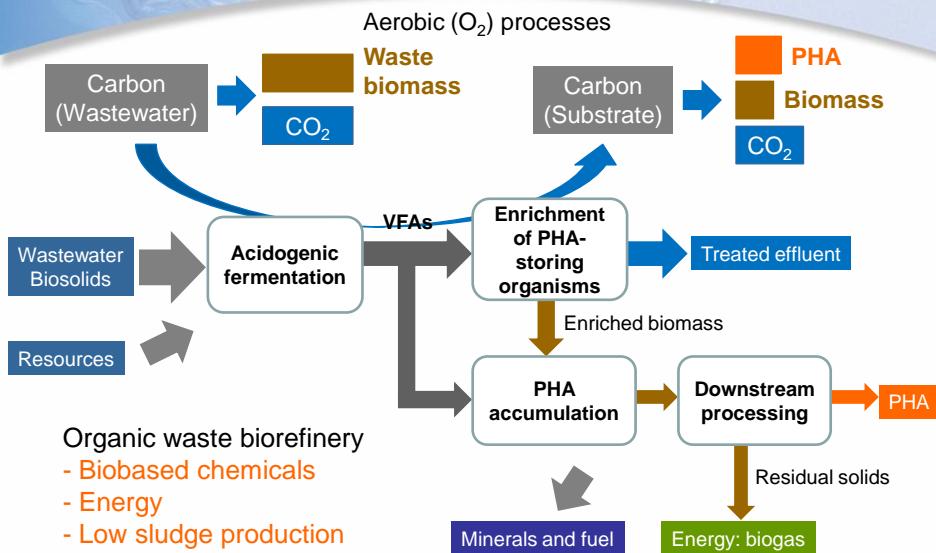


- Electron- acceptor (oxygen) dynamics – anaerobic/aerobic cycling

Bengtsson et al., 2008. Production of polyhydroxyalcanoates by glycogen accumulating organisms treating a paper mill wastewater. Water Sci. Technol. 58 (2): 323-30  
F Morgan-Sagastume et al. 2010. AnoxKaldnes AB

## Mixed culture synergies and opportunities

### Wastewater biotreatment ↔ PHA production



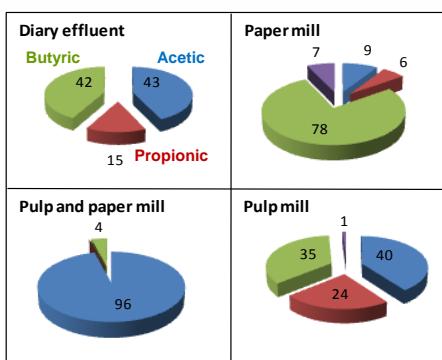
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## Industrial wastewaters for PHA production

- VFAs mixtures(acetate, propionate)
- Food waste
- Olive and palm oil mills effluents
- Sugar-cane molasses
- Diary effluent
- Paper mill effluents
- Fruit and tomato cannery effluents
- Brewery effluent
- Municipal wastewaters

- VFA spectrum depends on wastewater, pH and retention time
- Control of VFA and final PHA monomers in fermenters
- However, full VFA fermentation may not be required with readily degradable carbohydrates (Fruit cannery effluents; Gurieff, 2008)

Acidogenic fermentation  
VFA production from  
Carbohydrate-rich effluents



Bengtsson, Hallquist, Werker, Welander (2008). Acidogenic fermentation of industrial wastewaters: effects of chemostat retention time and pH on volatile fatty acids production. Biochemical Eng. J. 40: 492-499.

F Morgan-Sagastume et al. 2010. AnoxKaldnes AB

## PHAs production from wastewater at lab scale

Fermentation → Enrichment → Accumulation → DSP

Effluent	Enrichment reactor Feast-Famine	Reference
Sugar cane molasses	SBR	Albuquerque <i>et al.</i> , 2007
Paper mill	Activated sludge	Bengtsson <i>et al.</i> , 2008
1° sludge or cannery	SBR	Gurieff, 2008



Available online at www.sciencedirect.com  
ScienceDirect  
Bioreactor Technology 99 (2008) 509–516



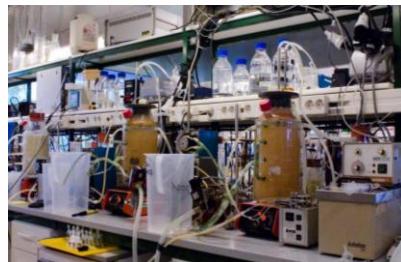
Production of polyhydroxalkanoates by activated sludge treating a paper mill wastewater

Simon Bengtsson <sup>a,b,\*</sup>, Alan Werker <sup>a</sup>, Magnus Christensson <sup>a</sup>, Thomas Welander <sup>a</sup>

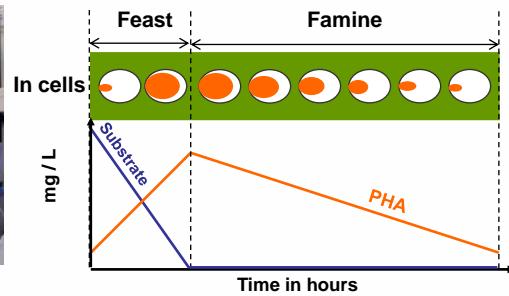
<sup>a</sup>AnoxKaldnes AB, Klostergårdsgatan 11A, SE-226 47 Lund, Sweden

<sup>b</sup>Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Sequencing batch reactors (SBRs)  
enriching for PHA- accumulating  
organisms

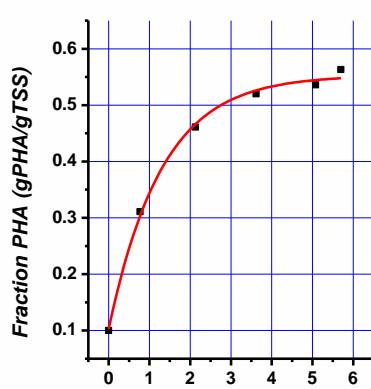


Diary effluent  
F Morgan-Sagastume *et al.* 2010. AnoxKaldnes AB



## PHA accumulation in fed batch

Effluent	Batch PHA production (g PHA / g dry biomass)	Reference
Sugar cane molasses	0.30	Albuquerque <i>et al.</i> , 2007
Paper mill	0.48	Bengtsson <i>et al.</i> , 2008
1° sludge or cannery	0.32-0.39	Gurieff, 2008



- Yields of 50% polymer in biomass accumulation attainable
- Overall yields 0.10 to 0.20 g PHA/g COD influent



F Morgan-Sagastume *et al.* 2010. AnoxKaldnes AB

## PHA from biosolids

SIXTH FRAMEWORK PROGRAMME

-Neptune  
FP6 Project

- Biosolids – major operating costs in WWTPs
- Waste activated sludge and 1<sup>o</sup> solids pretreated with high-pressure thermal hydrolysis (solubilised sludge)



### Fermentation into VFAs

- VFA levels = 20 g VFA<sub>COD</sub>/L
- Yield

$$50\% \text{ (g VFA}_\text{COD}/\text{g COD}_\text{sol. in)}$$



Semi-continuous fermenters

### Enrichment in SBRs

- At high organic loadings 6 g COD/Ld
- High N and P levels
- Non-VFA COD



Two parallel SBRs

### Batch PHA accumulation

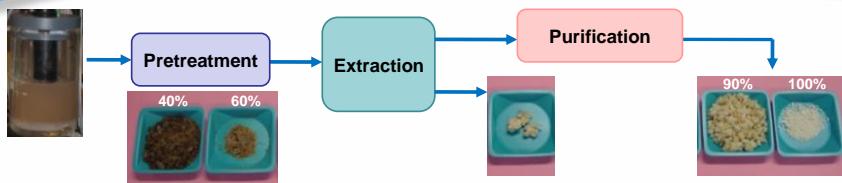
- 0.25 g PHA/g dry biomass
- 0.33-0.46 PHA Cmol/VFA Cmol
- Under high N and P levels



Parallel batch accumulation tests

## Downstream processing to final product

- Most costly stage in PHA production and critical
- Optimise for lower PHA content and other matter



### Polymer properties by a suite of techniques



Available at [www.sciencedirect.com](http://www.sciencedirect.com)  
ScienceDirect  
journal homepage: [www.sciencedirect.com/science/journal/01674811](http://www.sciencedirect.com/science/journal/01674811)

Alan Werker<sup>a,1</sup>, Petter Lind, Simon Bengtsson, Frida Nordström

<sup>a</sup>AnoxKaldnes Biopolymer AB, Elstervägen 11A, 322 47 Lund, Sweden

### Final product

- PHB-PHV with flexible processing potential
- High molecular weights: 500,000 – 1,000,000 g/mol
- High thermal stability

<sup>1</sup>Morgan-Sagastume et al. 2010. AnoxKaldnes AB

- Content / composition → GCMS and FTIR
- Molecular weight → SEC
- Thermal properties → DSC, TGA
- Viscoelasticity → Melt rheology

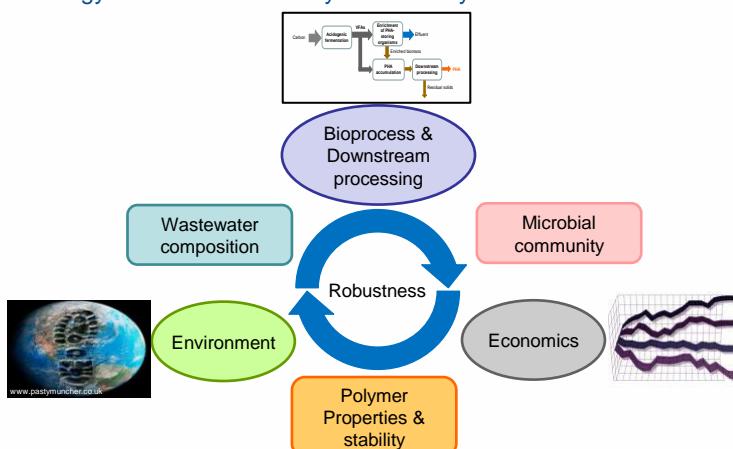
## Pilot testing and PHA production



F Morgan-Sagastume *et al.* 2010. AnoxKaldnes AB

## Concluding remarks

- Wastewater treatment can be coupled to the production of PHA bioplastics taking advantage of the existing open, mixed cultures and renewable resources
  - ▶ Reduction of waste sludge, aeration and nutrient costs complementing energy and mineral recovery - biorefinery



F Morgan-Sagastume *et al.* 2010. AnoxKaldnes AB

## Acknowledgements

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F Morgan-Sagastume *et al.* 2010. AnoxKaldnes AB