

Biological wastewater treatment II

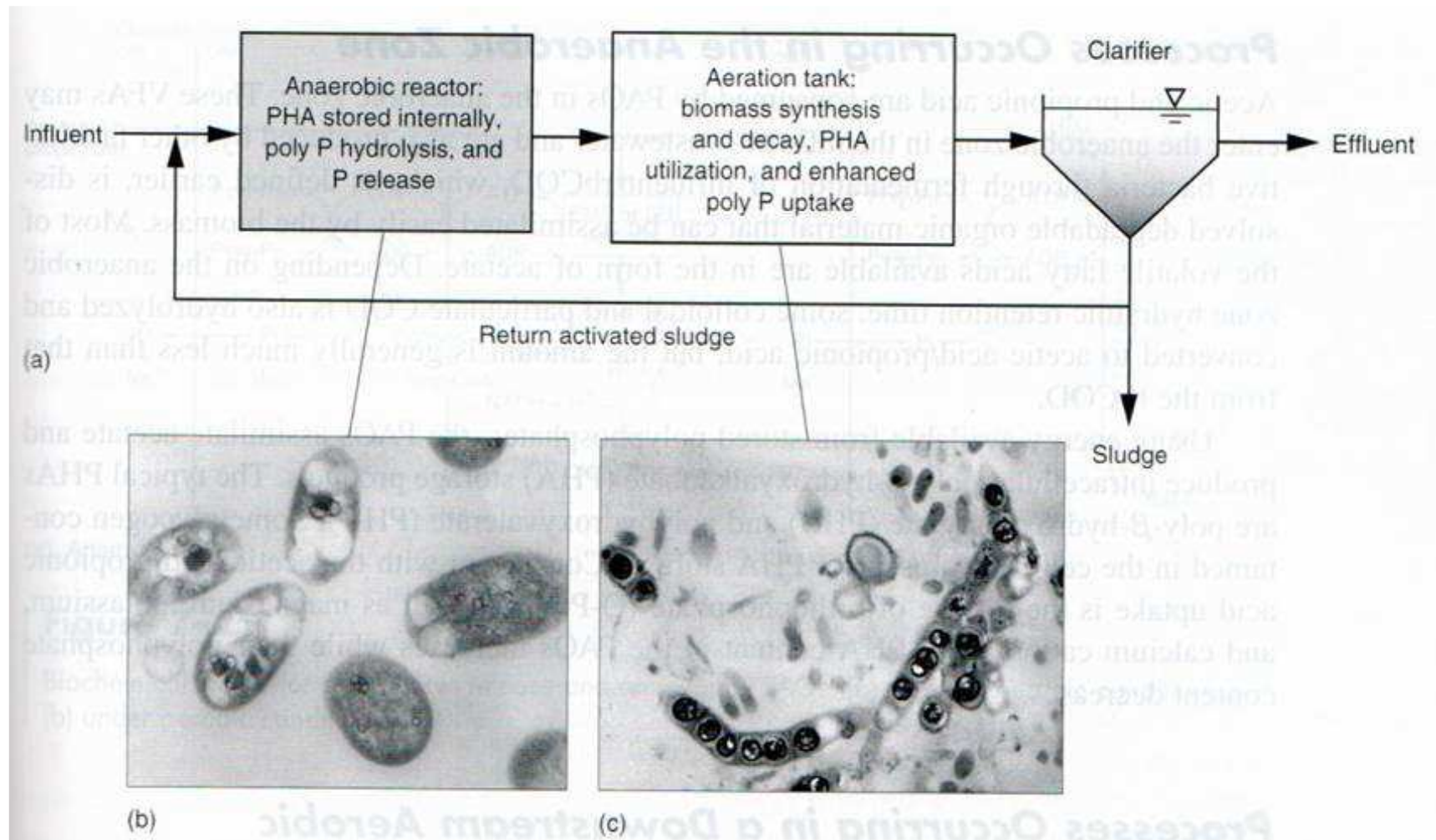
Today's class

- Biological nutrient removal (cont'd)
 - : *Conventional strategies to improve P removal efficiency in the secondary treatment*
- Biopolymer production

Enhanced biological P removal

- Involves incorporation of P in the biomass produced in the treatment system and subsequent removal of the biomass as waste sludge
- Biomass of heterotrophic bacteria contains ~ 0.015 g P/g VSS
 - Insufficient to remove P from influent wastewater (only 10~20% of total)
- Use **phosphorus accumulating organisms (PAOs)** for ***enhanced* biological phosphorus removal (EBPR)**
- Reduced chemical costs and less sludge production compared to chemical precipitation

Enhanced biological P removal



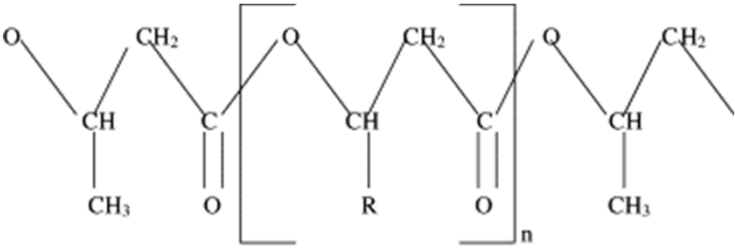
EBPR: Process description

- Place an anaerobic tank ahead of the aeration tank
 - Provide selectivity for growth of PAOs
- In the anaerobic tank, PAOs consume energy stored in the form of polyphosphates
 - The energy generated is used to convert volatile fatty acids into carbohydrate storage products (PHA)
- In the aerobic tank, PAOs consume COD & stored PAH for biomass growth
 - Use some of the energy for enhanced P uptake to store polyphosphates
- So:
 - Anaerobic tank: PHA accumulation & P release
 - Aerobic tank: excessive P uptake & PHA utilization
- PAOs form very dense floc with good settleability – additional benefit

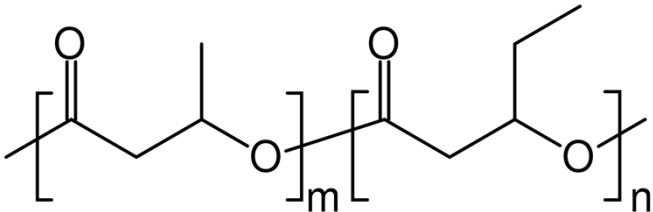
EBPR: Process description

- **Process occurring in the anaerobic zone**
 - Volatile fatty acids (VFAs) are produced by fermentation
 - VFAs are assimilated by PAOs into PHAs by energy available from stored polyphosphates
 - Typical PHAs: poly(3-hydroxybutyrate) (P3HB) & poly(3-hydroxyvalerate) (P3HV)
 - Some glycogen contained in the cell is also used
- **Processes occurring in the aerobic/anoxic zone**
 - Stored PHA is metabolized to provide energy for cell growth
 - Some glycogen is produced from PHA metabolism
 - Soluble orthophosphate in solution is taken up by PAOs to form polyphosphates in the existing cells and the new cells
 - Portion of the biomass is wasted → P removal
 - The process can occur in the anoxic zone as well (NO_3^- or NO_2^- as e^- acceptors)

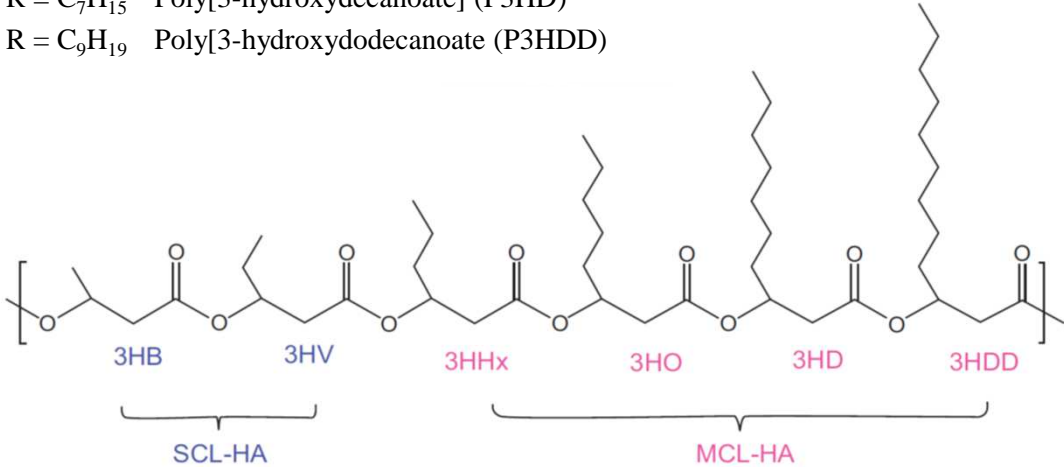
PHAs: A class of biopolymer



- R = CH₃ Poly[3-hydroxybutyrate] (P3HB)
- R = C₂H₅ Poly[3-hydroxyvalerate] (P3HV)
- R = C₃H₇ Poly[3-hydroxyhexanoate] (P3HHx)
- R = C₅H₁₁ Poly[3-hydroxyoctanoate] (P3HO)
- R = C₇H₁₅ Poly[3-hydroxydecanoate] (P3HD)
- R = C₉H₁₉ Poly[3-hydroxydodecanoate] (P3HDD)



Poly[3-hydroxybutyrate-co-3-hydroxyvalerate]
(P[3HB-co-3HV] or PHBV)



SCL = short chain length

MCL = medium chain length

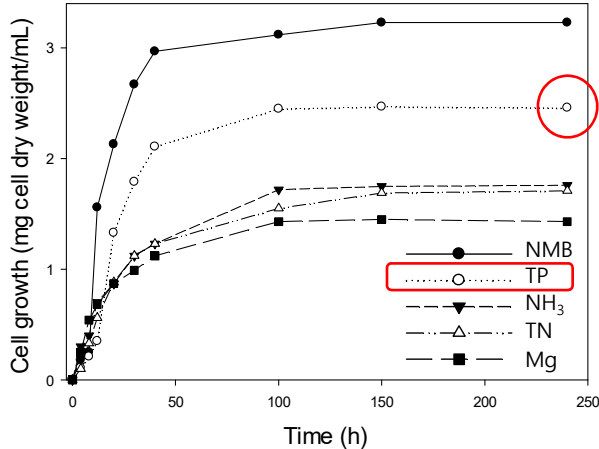
Table 3

Comparison of several PHAs biosynthesis processes in recombinant bacteria.

Strain	Cultivation mode	PHAs biosynthesis genes source	Type of PHAs	Carbon sources	Biomass (g/L)	PHA content (%)	PHA yield (g/L)	References
Short-chain-length PHAs								
<i>Escherichia coli</i> K24KL	Fed-batch	<i>Cupriavidus necator</i>	P(3HB)	Glycerol	41.9	63	26.4	Nikel et al. (2010)
<i>Escherichia coli</i> K24KP	Aerobic batch	<i>Azotobacter</i> sp. (FA8)	P(3HB)	Glucose	9.43	37.2	3.5	Almeida et al. (2010)
<i>Escherichia coli</i> K24KP	Batch	<i>Azotobacter</i> sp. (FA8)	P(3HB) P(3HB)	Glycerol Glycerol	4.75 12.23	30.1 27	1.4 3.3	Almeida et al. (2011)
<i>Escherichia coli</i> JM109	Batch	<i>Bacillus megaterium</i> NBRC15308 T	P(3HB)	Glucose	9.3	80	7.4	Tomizawa et al. (2011)
<i>Escherichia coli</i>	Batch	<i>Bacillus cereus</i> YB-4	P(3HB)	Glucose	5.35	18.41	1.0	Meng et al. (2015)
Medium-chain-length PHAs								
<i>Escherichia coli</i>	Batch	<i>Pseudomonas</i> sp.LDC-5	mcl-PHA	Molasses	4.05	75.5	3.06	Saranya and Shenbagarathai (2011)
<i>Escherichia coli</i>	Batch	<i>Pseudomonas aeruginosa</i> PAO1	mcl-PHA	Glucose	–	15	–	Agnew et al. (2012)
Copolymers								
<i>Escherichia coli</i> JM109	Batch	<i>Comamonas</i> sp. EB172	P(3HB-co-3HV)	Glucose	1.6	46.1	0.7	Yee et al. (2012)
<i>Burkholderia</i> sp. USM (JCM 15050)	Fed-batch	<i>Aeromonas caviae</i>	P(3HB-co-3HHx)	Crude palm kernel oil	1.7	66	1.1	Chee et al. (2012)
<i>Cupriavidus necator</i>	Fed-batch	<i>Burkholderia</i> sp. USM (JCM 15050)	P(3HB-co-4HB)	Crude palm kernel oil	2.4	66	1.6	Lau and Sudesh (2012)
<i>Shimwellia blatae</i>	Two step fed-batch	<i>Ralstonia eutropha</i>	P(3HB-co-3HP)	Glycerol	23.2	30.7	7.1	Sato et al. (2015)
Terpolymers								
<i>Cupriavidus necator</i>	Batch	<i>Aeromonas caviae</i>	P(3HB-co-3HV-co-3HHx)	Palm kernel oil	7.9	79	6.2	Bhubalan et al. (2008)
<i>Cupriavidus necator</i>	Fed-batch	<i>Chromobacterium</i> sp. USM2.	P(3HB-co-3HV-co-3HHx)	Sodium valerate	9.4	86	8.1	Bhubalan et al. (2010)
<i>Delftia acidovorans</i> DSM39	Batch	<i>Pseudomonas stutzeri</i> BT3	P(3HB-co-3HV-co-4HB)	Lard	–	39.33	–	Romanelli et al. (2014)

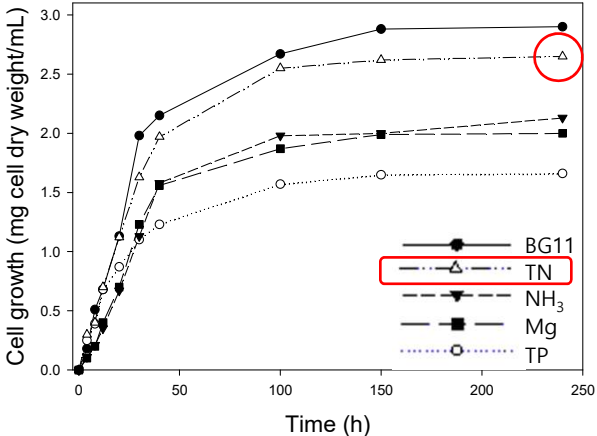
Biopolymer production case study

M. parvus MK
Utilizes CH₄



Deficient condition	Biopolymer accumulation (mg polymer/mg cell dry wt)
NMB (nutrient-sufficient)	0.96±0.11
TP	3.05±0.21
TN	2.40±0.42
NH ₃	2.39±0.17
Mg	2.59±0.09

M. putida MK1
Utilizes CO₂



Deficient condition	Biopolymer accumulation (mg polymer/mg cell wt)
BG11 (nutrient-sufficient)	0.38 ± 0.11
TN	2.8 ± 0.80
NH ₃	2.37 ± 0.67
Mg	1.65 ± 0.62

Courtesy: Dr. Moonkyung Kim