Biological wastewater treatment II

Today's lecture

- Biological nutrient removal (cont'd)

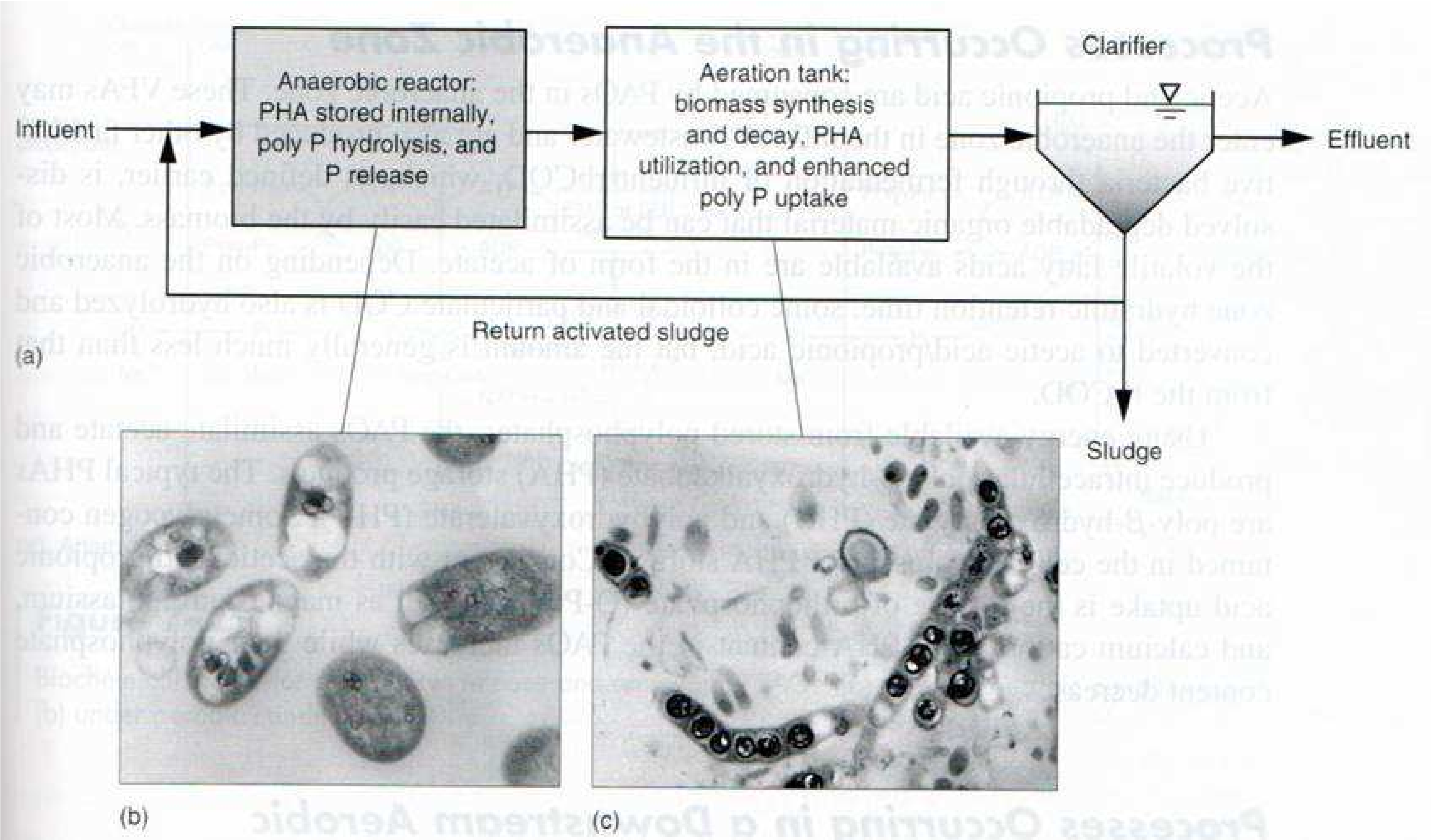
  : *Conventional strategies to improve P removal efficiency in the secondary treatment*

- 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



#1

PHA: polyhydroxyalkanoates

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

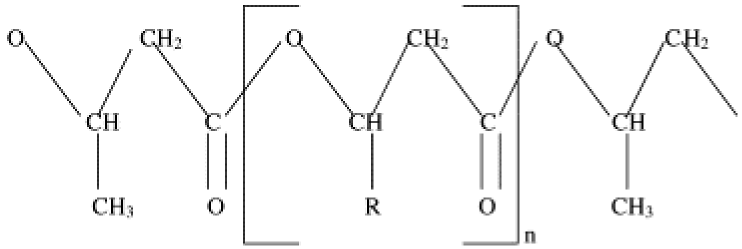
EBPR: Process description

- **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 in 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)

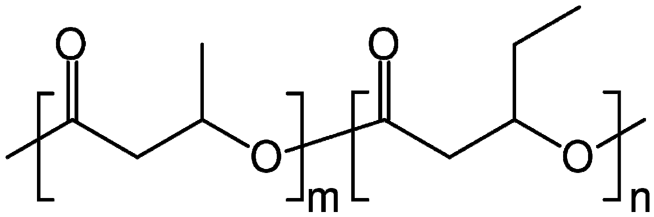
PHAs: A class of biopolymer

#2



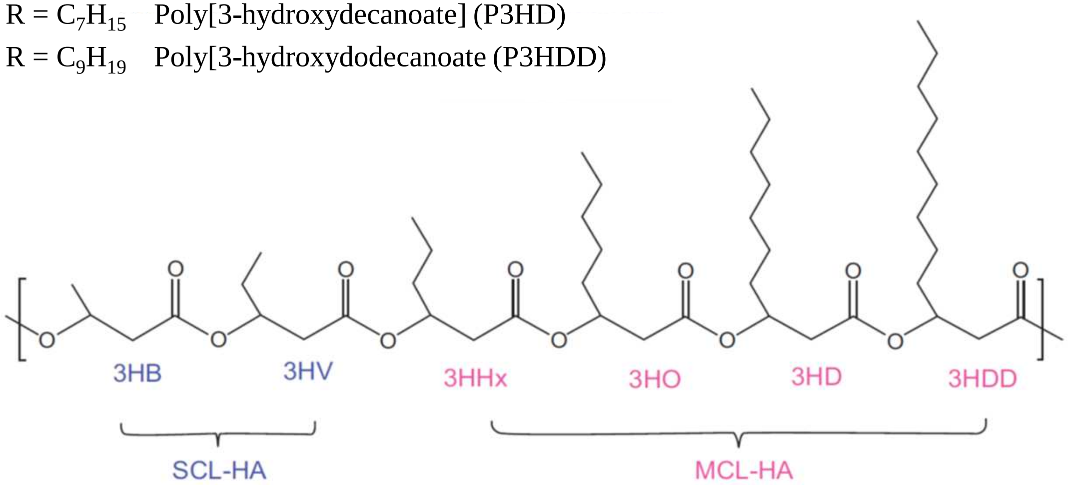
R = CH_3	Poly[3-hydroxybutyrate] (P3HB)
R = C_2H_5	Poly[3-hydroxyvalerate] (P3HV)
R = C_3H_7	Poly[3-hydroxyhexanoate] (P3HHx)
R = C_5H_{11}	Poly[3-hydroxyoctanoate] (P3HO)
R = C_7H_{15}	Poly[3-hydroxydecanoate] (P3HD)
R = C_9H_{19}	Poly[3-hydroxydodecanoate (P3HDD)

#4



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

#3



SCL = short chain length

MCL = medium chain length

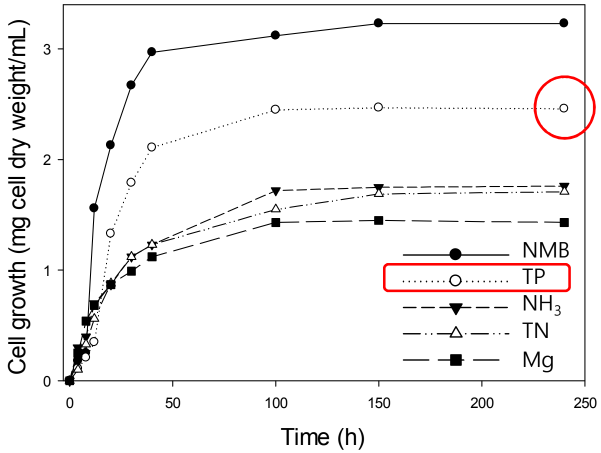
Table 3
Comparison of several PHAs biosynthesis processes in recombinant bacteria.

#5

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

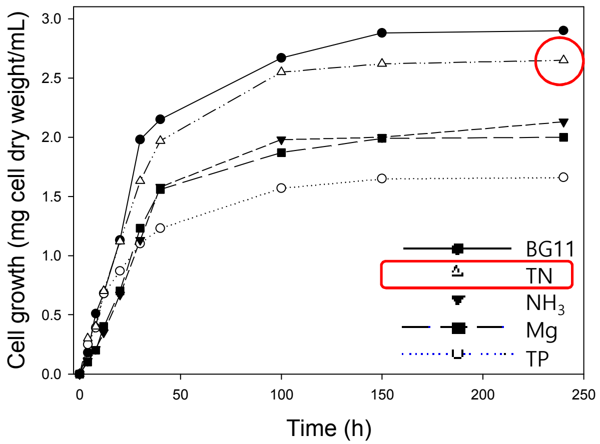
Biopolymer production case study

M. parvus **MK**
Utilizes CH_4



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 CO_2



Deficient condition	Biopolymer accumulation (mg polymer/mg cell wt)
BG11 (nutrient-sufficient)	0.38 ± 0.11
TP	1.88 ± 0.53
TN	2.8 ± 0.80
NH_3	2.37 ± 0.67
Mg	1.65 ± 0.62

Courtesy: Dr. Moonkyung Kim

References

#1) Metcalf & Eddy, Aecom (2014) Wastewater Engineering: Treatment and Resource Recovery, 5th ed. McGraw-Hill, p. 649.

#2) Khanna, S. K., Srivastava, A. K. (2005) Recent advances in microbial polyhydroxyalkanoates. Process Biochemistry, 40(2): 607-619.

#3) Li, Z., Yang, J., Loh, X. J. (2016) Polyhydroxyalkanoates: Opening doors for a sustainable future. NPG Asia Materials, 8: e265.

#4) https://en.wikipedia.org/wiki/PHBV

#5) Mozejko-Ciesielska, J., Kiewisz, R. (2016) Bacterial polyhydroxyalkanoates: Still fabulous? Microbial Research, 192: 271-282.