A BIOTECHNOLOGICAL APPROACH TO CONVERT METHANE INTO BIO-POLYMER

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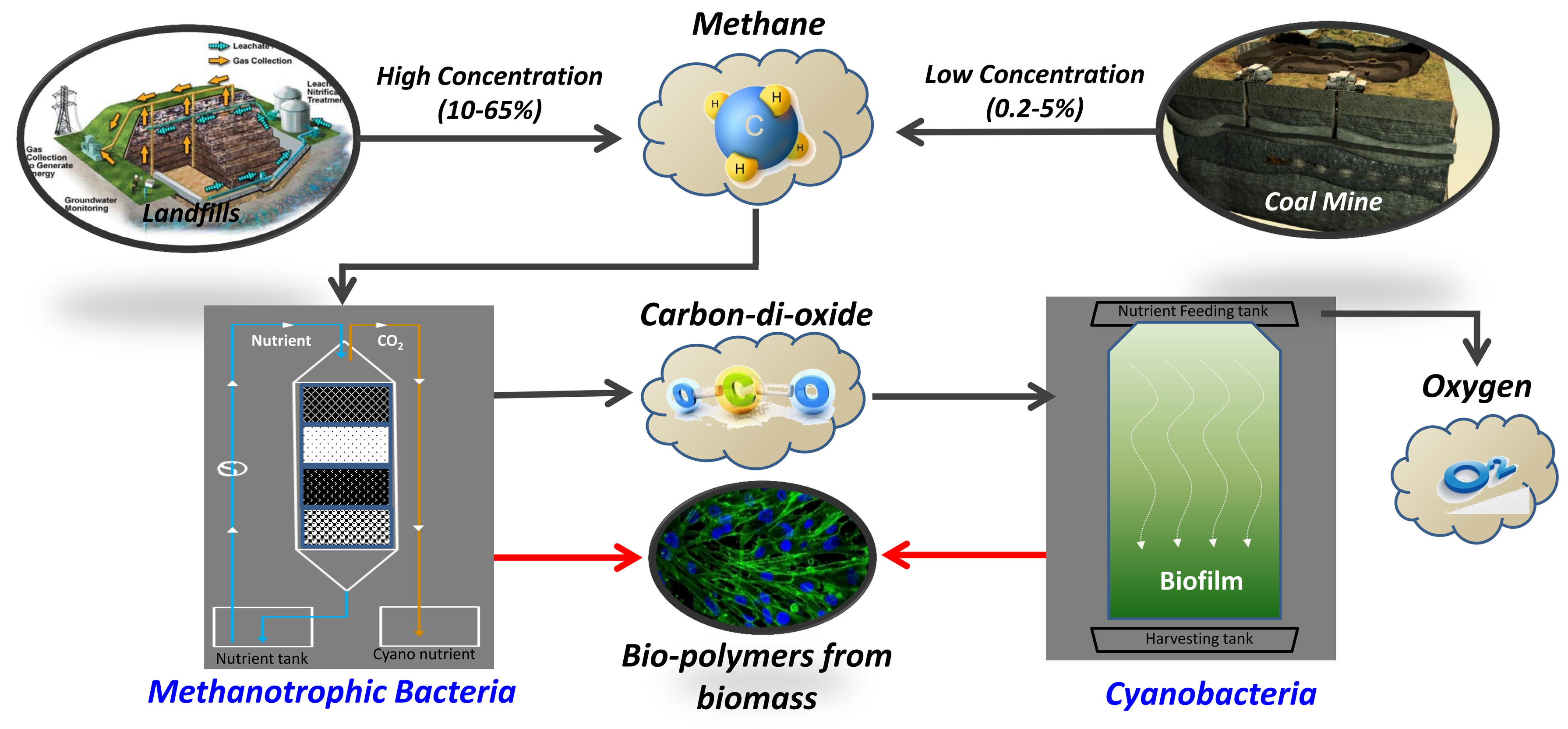
Research Overview



Bio- (vs) Conventional Plastics

- ✓PHAs are bio-polyesters characterized by high molecular mass similar to that of conventional fossil fuel-derived plastics (Table 1).
- ✓PHAs are stored (Figure b) as Carbon and Energy sources in many bacteria under nutrient starved conditions, including ***methanotrophic bacteria***.
- ✓Different types of PHA monomers were identified
  - ✓**Polyhydroxybutyrate (PHB);** Polyhydroxyvalerate (PHV); poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) (PHBO); etc.
- ✓Wide applications in different fields including drug and fine chemicals, biofuels, bio-implants, food and feeds, industrial fermentation, bio-plastics, etc.

Table 1. Characteristic difference between bio- and conventional- polyesters

Particulars	PHA/PHB biopolymer	Petroleum based polymer
Glass transition temperature (Tg°C)	10	0-275
Crystalline melting temperature (Tm°C)	177	98-310
Toughness (Mpa)	NA	15-65
Youngs'Modulus (Mpa)	3500	156-674
Tensil strength (Mpa)	40	13-27
Elongation (%)	0.4	126-576
Biodegradability	6 – 60 months	Over 100 years
Source: Chanprateep, 2010.		

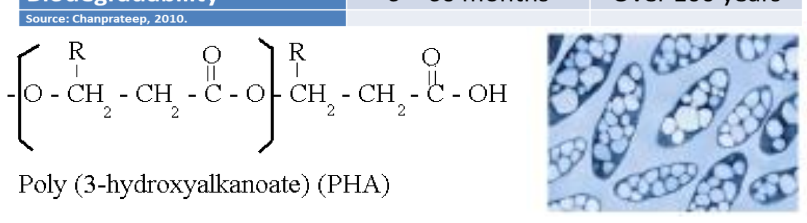


Figure 1. Molecular structure and accumulation of PHA in methanotrophs

Techno-Economic Scenario

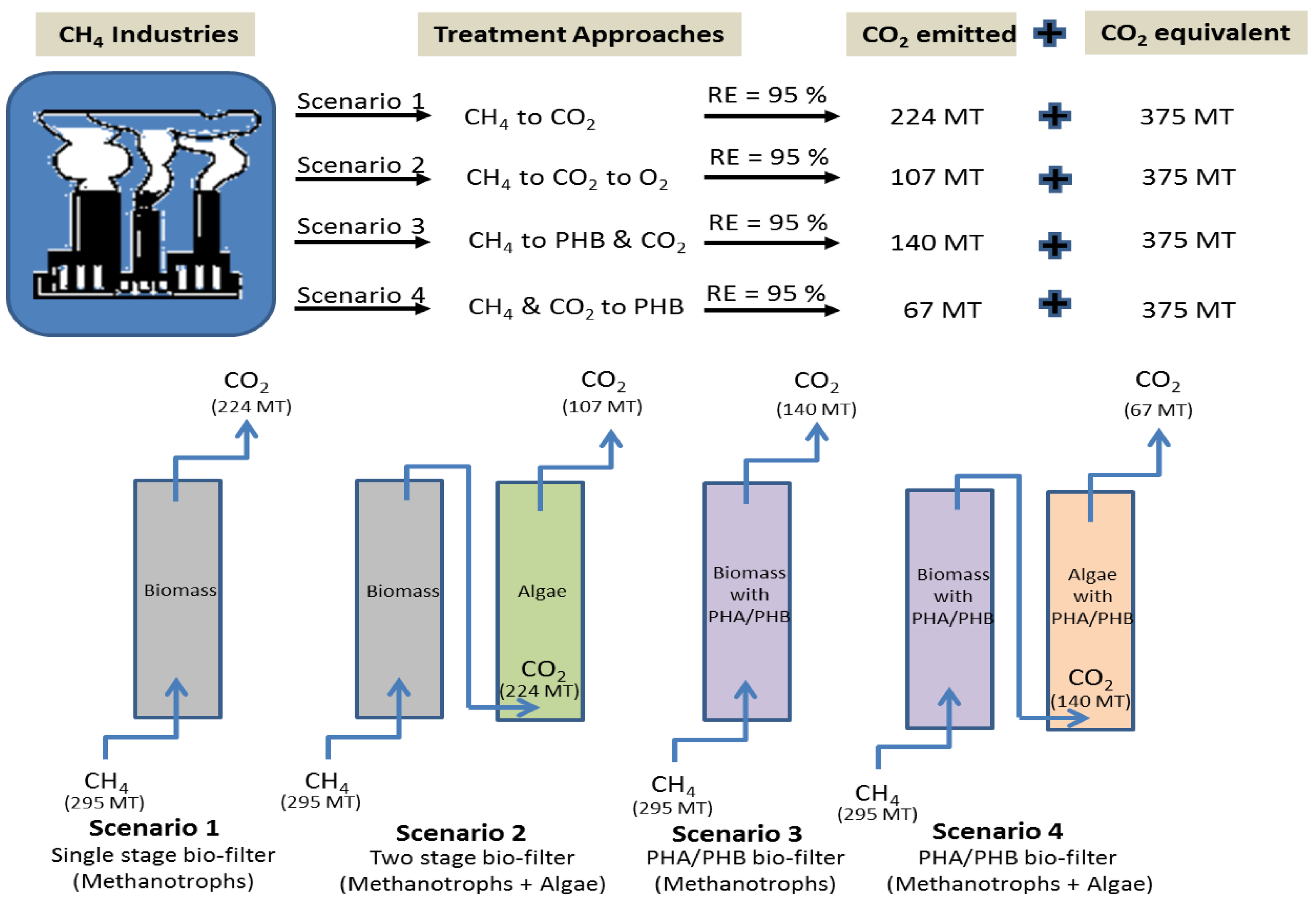


Figure 2. Techno-economic scenario development for CH_4 remediation using dual culture system

Table 2. Total GHG elimination capacities and revenue generated from re-routing of CH_4 into PHA cycle

Particulars	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Total carbon sequestration (MT)	6776	6893	6860	6933
Total bacterial biomass (MT)	98.3	98.3	147.5	147.5
PHA/PHB content from bacterial biomass (MT)	-	-	95.5	95.5
Total cyanobacterial biomass (MT)	-	65	-	40
PHA/PHB content from cyanobacterial biomass (MT)	-	-	-	8.8
Revenue from bacterial biomass as bio-char (billion USD)	6.47	6.47	-*	-*
Revenue from cyanobacterial biomass as animal feed (billion USD)	-	32.50	-	-*
Revenue from pelleted PHA/PHB (billion USD)	-	-	15,280	16,688
Carbon credits according to GS (billion USD)	8.81	8.96	8.92	9.01
Total revenue generated (billion USD)	15.28	47.93	15,288.90	16,696.07

Methanotrophs and PHB potential

- ✓ Type II methanotrophs store CH_4-carbon as phospholipid fatty acids (PLFA; ex. 18:1ώ8C) and biopolymers (mainly PHB) into glycolipid and polar biomass fractions, respectively.
- ✓ Theoretically (based on eq. 1 and 2), PHA/PHB accumulation of 67 % (i.e., 0.68g/g of dry biomass) is possible via the serine cycle.

$$2CH_4 + 3O_2 + 6NADH_2 + 2CO_2 \rightarrow C_4H_6O_2 \text{ (PHB monomer)} + FPH_2 \quad (1)$$

$$8CH_4 + 12O_2 + FP \rightarrow C_4H_6O_2 \text{ (PHB monomer)} + 4CO_2 + 12ATP + FPH_2 \quad (2)$$

- ✓ High PHB accumulation has been reported for ***Methylocystis parvus* – *up to 70 % and Methylosinus trichosporium up to 40% .***

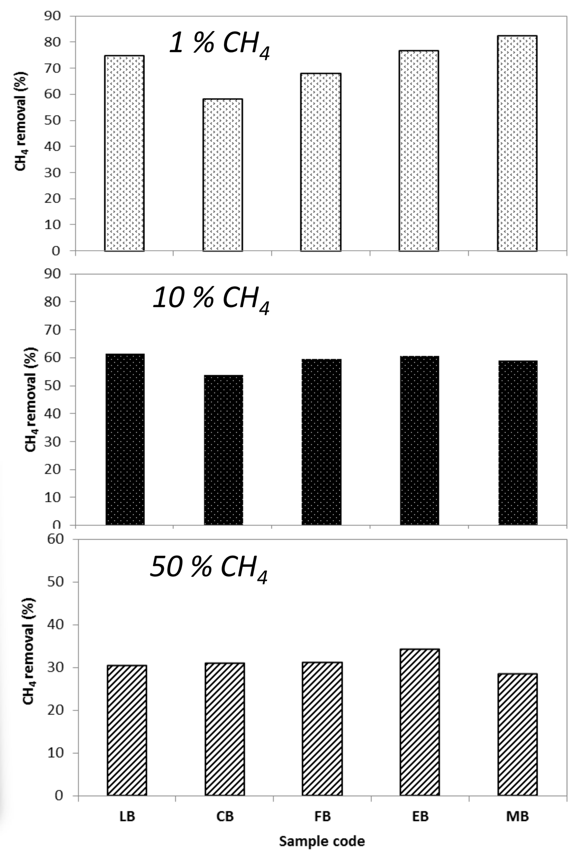


Figure 3. CH_4 oxidation potential of indigenous methanotropic consortia

Table 3. Total lipid production and FAME analysis of enriched mixotrophs in bioreactors

Particulars	Bioreactor 1	Bioreactor 2
Biomass enrichment	Estuarine biomass	Marine biomass
Dry weight of biomass (g/l)	0.118-0.122	0.124-0.579
PHB potential (mg/l)[a]	70.8-73.2	74.4-347.4
FAME (C18:1, trans-Δ[11]) mg/g Dwt of biomass)	1.02 -1.67	0.0-1.58

[a] assumed that 60% of biomass enrichment contained PHB

Figure 4 . Bioreactors for biomass enrichment

Table 4. Testing of biofilters with different packaging material for CH_4 oxidation

Particulars	unit	Biofilter 1	Biofilter 2	Biofilter 3
CH_4 removal	%	28-45	30-66	28-54
Size of the materials	mm	≤ 1-3	≤ 20	≤ 5-7
Bulk density	$Kg.m^{-3}$	568 ± 11	233 ± 20	1642 ± 65
Porosity	%	55	73	32
Surface area	cm^2	0.50	50.6	4.52
True residence time	s	35.8	21.7	54.1

Figure 5 . Biofilter (miniature)

Cyanobacteria and PHB potential

- ✓PHB production is widespread in cyanobacteria (circa 80% of the strains) but yield is species-specific.
- ✓PHB-producing strains may be screened by microscopy, e.g., Nile Red staining and fluorescence excitation at 460 nm (Balaji et al. 2013) followed by GC-MS quantification.
- ✓With CO_2 as the sole carbon source, cyanobacterial PHB accounts for 1-20% of dry weight. PHB production is enhanced by nutrient (N,P) starvation and reaches up to 77% by addition of organic C sources (glucose, glycerine, acetate).

Figure 6. Calothrix sp. (field sample)

Table 5. Potential PHB production in cyanobacterial strains from the methane bioremediation project

Strain	Biomass ($g\ DW\ L^{-1}$) [a]	Lipid content (% DW)	Potential PHB production ($g\ L^{-1}$) [b]	
			Low yield	High yield
An siamensis NQAIF306	1.5	11.1	0.01	0.3
Calothrix sp. NQAIF310	0.9	8.7	0.009	0.2
Fischerella sp. NQAIF311	0.7	9.5	0.007	0.14
Nostoc sp. NQAIF313	1.3	10.1	0.01	0.3
Tolypothrix sp. NQAIF319	1.3	7.8	0.01	0.3

[a] nitrogen- depleted medium
[b] PHB productions estimated from published PHB contents (% DW). Low yield, 1% DW, high yield, 20% DW.

Preliminary outcomes

- ✓Mixotrophic cultures are more suitable than pure cultures for effective CH_4 remediation and to produce high levels of PHB. Isolation of pure strains and PHB quantification/production optimization are on-going.
- ✓N_2-fixing cyanobacteria represent an inexpensive source of PHB (1-20% dry weight) with CO_2 (product of CH_4 oxidation) as the sole carbon source. Therefore dual culturing would be ideal.
- ✓ Globally, conventional plastic production consumes ≈ 270 million tons of oil and gas annually resulting in high GHG emissions. Therefore, synthesis of PHA/PHB from waste gas such as CH_4 would be an economically feasible and sustainable approach.

References

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