Use of Various Amendments to Control Methane Production During Environmental Applications

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Methanogenesis

- Methanogens are microorganisms that produce methane
- They are ubiquitous, and they are often dominant in numbers, averaging 2% to 15% of all soil microbes
- They are important members of synergistic, fickle anaerobic communities
- They are genetically unique and belong to their own domain, Archaea
- They can double cell numbers in one hour and are problematic when overactive

Methane Control

- Natural statin compounds, including Monacolin K (also known as Lovastatin), can effectively inhibit methanogens while permitting other biodegradation processes to occur.
- The statins inhibit the growth and development of Archaea hence minimizing methanogenic activity. Bacteria cell walls contain peptidoglycan (murein), whereas the cell walls of methanogens cell walls contain pseudomurein.
- Pseudomuerin is biosynthesized via activity similar to that of 3-hydroxyl-3-methyl-glutaryl-coenzyme. A (HMG-CoA) reductase, which is a key enzyme in the cholesterol biosynthesis pathway in humans.
- Statin containing substances have the ability to inhibit HMG-CoA reductase, interrupt the pseudomurein biosynthesis pathway, and as a result the methanogens are restricted from growth, development and proliferation (Figure 1).

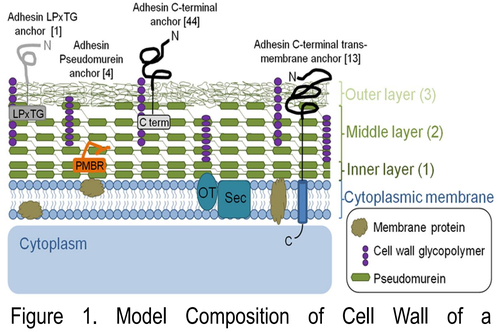

Figure 1. Model Composition of Cell Wall of a Methanogen

Methane Control

•Essential Oils are mixtures of aromatic chemicals present in plant material such as leaves, buds, flowers, fruit, bark, root, or wood, and are comprised of various terpenes, acids, aldehydes, alcohols, esters, and ketones.

•Saponins are glucosides with foaming characteristics. They consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30).

•The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part.

•The utilization of essential oils and/or saponins can disrupt the enzyme and coenzyme systems, that are integral parts of the methanogenesis process.

•The disruption limits the growth and reproduction of the methanogens and thus limits the amount of methane produced during in situ remediation processes.

•At a wide range of concentrations the essential oils/saponins are typically found to be harmless to most other bacteria that may be present in the environment (e.g., an aquifer system).

•The essential oils/saponins utilized to disrupt the enzyme and coenzyme systems in order to limit the methanogenesis process may include, but not limited to, garlic oil (Allium sativum), lemon oil (Cymbopogon citratus) and/or cinnamon oil (Cinnamomum zeylanicum).

Competing Reactions

✓In-situ reductive dehalogenation processes have been found very effective for the remediation of numerous recalcitrant and toxic compounds, including chlorinated ethenes.

✓One seemingly universal phenomenon has been the biological production of methane, especially during the early phases of the process.

✓Production of methane is a direct indication that hydrogen generated from the electron donor amendments was used by methanogens instead of the target microbes (e.g., Dehalococcoides spp.), substantially reducing application efficiency.

✓Active measures to control the production of methane can offer multiple advantages in terms of cost, treatment efficiency, and safety.

Bench Scale Studies – Red Yeast Rice

- Red yeast rice, an Asian dietary staple made by fermenting yeast (Monascus purpureus) on rice, contains active ingredients of the statin drugs such as Lovastatin.
- RYR can successfully inhibit the key enzyme hydroxymethylglutaryi-SCoA (HMG-CoA) reductase, resulting in the inhibition of methanogenic activity.

Figure 2. Reactor Setup

Table 2. Methane Concentration Changes by RYR.

Activity	Time (days)	Control (%)	Test (%)
dosed Test (40 mg/L)	0	57	62
	2	61	47
	4	68	32
	6	59	20
dosed Control (20 mg/L)	7	65	13
	9	51	6
	11	31	0
	13	22	0
	15	8	0
	17	0	0

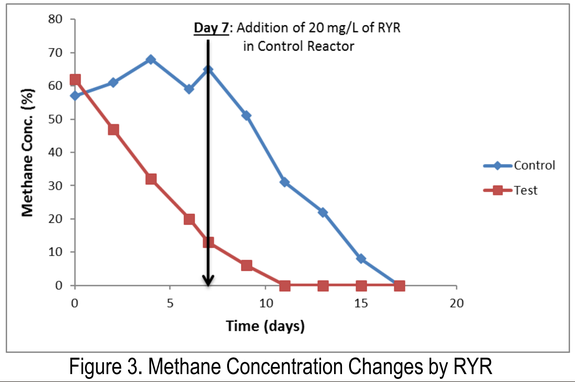

Figure 3. Methane Concentration Changes by RYR

Conclusions – Red Yeast Rice

- Prior to the addition of the RYR extract, methane concentrations in the biogas varied from approximately 55% to 70%, which indicated an active methanogenic culture.
- Following the addition of RYR to 40 mg/L in the Test Reactor, the methane content of produced biogas was rapidly reduced from 62% to below detection (0.05%) within 11 days and remained below detection levels until Day 17.
- Addition of RYR at 20 mg/L to the Control Reactor on Day 7 reduced the methane content of biogas from 65% to below detection (0.05%) by day 17 (i.e., after 10 days).

Bench Scale Studies – Essential Oils

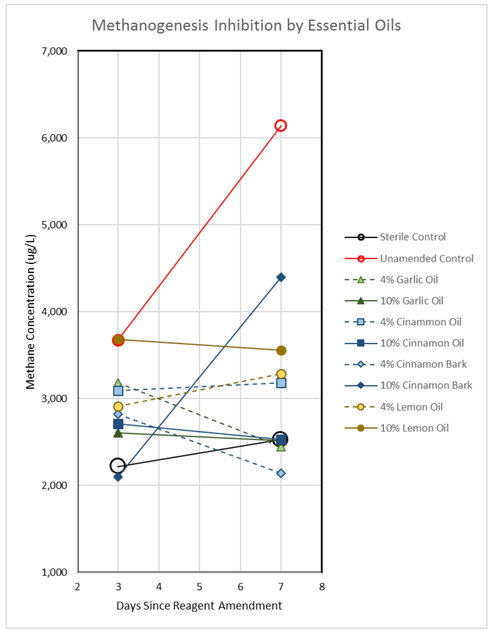

Figure 4. Methane Concentration Changes by Essential Oils

Conclusions – Essential Oils

- ✓ Based on the laboratory findings it is apparent that the majority of the essential oils were successful in decreasing the amount of methane produced.
- ✓ Garlic Oil was the most effective of all by gradually decreasing the methane production by 29%, 7 days after the initiation of the experiments, by 60% after 11 days and 79% after 16 days.

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