Rapid Start-up of Batch Thermophilic Anaerobic Digestion and Overcoming Sodium Toxicity by Utilizing Turf Grass as Inoculum

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DECLARATION

I declare that this thesis is my own account of my research work undertaken which has not been previously submitted for a degree at any tertiary educational institution.

Suwat Suwannoppadol

The following papers have been presented and published from this research:

Suwannoppadol, S., Ho, G., Cord-Ruwisch, R., 2011. Rapid start-up of thermophilic anaerobic digestion with the turf fraction of MSW as inoculum. Bioresource Technology. 102, 7762-7767.

Suwannoppadol, S., Cord-Ruwisch, R., Ho, G., 2012. Distribution of methanogenic potential in fractions of turf grass used as inoculum for the start-up of thermophilic anaerobic digestion. Bioresource Technology. 117,124-130.

Suwannoppadol, S., Cord-Ruwisch, R., Ho, G., 2012. Overcoming sodium toxicity by utilizing grass leaves as co-substrate during the start-up of batch thermophilic anaerobic digestion. Bioresource Technology. 125, 188-192.

Abstract

Thermophilic anaerobic digestion offers certain advantages over mesophilic anaerobic digestion such as a higher rate of biogas production and significant pathogen reduction. Despite such advantages, there remain some key hurdles preventing the wider adoption of thermophilic digestion, one of them being that of a difficult start up procedure. To overcome this limitation, this thesis aims to determine suitable start-up conditions and inoculum sources suitable for thermophilic anaerobic digestion. Cow manure, a well-known source of methanogens, was compared to municipal solid waste (MSW) as seeds for of thermophilic anaerobic digestion start-up. Results indicated that in contrast to cow manure, MSW (collected from the metropolitan area of Perth, Western Australia) has a higher potential to be a thermophilic anaerobic seed as compared to cow manure. After incubating of MSW at 55 $^{\circ}$C, methane was produced within 3 days with an initial methane production rate of 0.7 $L*L^{-1}*day^{-1}$.

In an attempt to narrow down the source of thermophilic methanogens in a typical MSW, components of a typical MSW (vacuum cleaner dust, banana peel, kitchen waste, and garden waste), which might contain the thermophilic methanogens, were tested as inoculum for thermophilic methanogenesis with acetate as the substrate. Results singled out grass turf as the key source of thermophilic acetoclastic methanogens. Within 4 days of anaerobic incubation at 55^{o}C, anaerobically incubated grass turf samples produced methane accompanied by acetate degradation enabling the successful start-up of thermophilic anaerobic digestion. This indicates that turf has the ability to act as an alternative seed for the start-up of thermophilic anaerobic digestion.

Theoretically, methanogens could be divided into two main groups: hydrogenotrophic and acetoclastic methanogens. Hydrogenotrophic methanogens generate methane from the reduction of carbon dioxide by utilizing hydrogen as an electron donor while acetoclastic methanogens cleave acetate to form methane and carbon dioxide. Therefore, investigation of both groups of methanogens in each fraction of turf was addressed in the current study. Characterization of the incubated turf sample identified the main hydrogenotrophic and acetoclastic methanogens present in turf to be *Methanoculleus* sp. and *Methanosarcina* sp. respectively.

The current study also investigated the effects of co-digestion of grass leaves in anaerobic digestion on sodium toxicity. It was motivated by results of observations that with appropriate addition of sodium bicarbonate (330 mM), to tackle the early build-up of volatile fatty acids, successful start-up of MSW and grass leaves were established. A study was conducted to evaluate to what extent grass leave) could help in overcome the sensitivity of methanogenic populations to high sodium content. Results demonstrated the complete failure of the start-up of thermophilic anaerobic digestion at sodium concentration of 7.8 g Na^+/L. Grass leaves addition could, however, reduce the effects of sodium toxicity in both mesophilic and thermophilic digestion.

Moreover, in an attempt to narrow down the component of grass leaves which is responsible for elevating sodium toxicity, different likely compounds present in grass leaves such as potassium and betaine were tested. Results revealed that addition of betaine (GB) (1, 5, 10 mM) was observed to be effective in increasing methane production in the presence of high sodium.

To correlate the effect of GB to the effect caused by grass leaves, the GB content in the turf grass was determined. Results of betaine analysis showed that there was 3.9 mg of GB present per g of fresh grass leaves, resulting in final expected GB concentration of 3.3 mM. This implies that GB is one of the organic compounds contained in grass leaves, which plays an important role in the antagonistic effect of grass leaves toward sodium toxicity during thermophilic anaerobic digestion.

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List of Abbreviations

ΔG^o	standard Gibbs free energy
$\Delta G^{o'}$	Change in Standard Gibbs Free Energy
APHA	American publish Health Association
ATP	Adenosine Triphosphate
atm	atmosphere
CH_4	Methane
CO_2	Carbon dioxide
CSTR	Continuously Stirred Tank Reactor
d	day
DNA	Deoxyribonucleic acid
GC	Gas Chromatography
g	gram
HRT	Hydraulic Retention Time
hr	hour
H_2	Hydrogen
kg	kilo Gram
kJ	kilo Joule
L	Litre
mg/L	milli Gram per Litre
mL	milli Litre
mM	milli Moles per Litre
MSW	Municipal Solid Waste
NH_3-N	Ammonia Nitrogen
OFMSW	Organic Fraction of Municipal Solid Waste
ORL	Organic Loading Rate
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
rRNA	ribosomal RiboNucleic Acid
rDNA	ribosomal DeoxyRibonucleic Acid
TAN	Total Ammonia Nitrogen
TS	Total Solid
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids