Enhancing Anaerobic Degradation of Lipids in Wastewater by Addition of Co-substrate

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DECLARATION

I declare that the work in this thesis is my own account of my research and the contents has not been submitted for a degree at any university.

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PUBLICATION

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Y. Kuang, M. Lepesteur, G. E. Ho (2001). “Identification and quantification of the anaerobic bacterial subpopulation with oligonucleotide probe hybridization under the inhibition of long chain fatty acid” Proceeding of 9th Word Congress on Anaerobic Digestion 2001, Antwerpen, Belgium

Y. Kuang, M. Lepesteur, P. Pullammanappallil and G.E. Ho (2002). "Enhancing the lipids anaerobic digestion by stimulating bacteria growth with co-digestion proteins and carbohydrates in two-phase UASB reactor" Accepted as oral presentation by VII Latin American Workshop and Symposium on Anaerobic Digestion.

Y. Kuang, M. Lepesteur, P. Pullammanappallil and G.E. Ho (2002). “Lipid hydrolysis in mesophilic and thermophilic two-phase anaerobic treatment systems with co-digestion with protein and carbohydrates” Submitted to *Water Research*

Y. Kuang, M. Lepesteur, P. Pullammanappallil and G.E. Ho (2002). “Enhanced recovery of methanogenesis from LCFA inhibition by feeding substrates in a single stage UASB reactor” Submitted to *Bioresouce Tech.*

ABSTRACT

Anaerobic treatment systems are becoming increasingly popular to treat complex organic wastes that contain carbohydrates, proteins and lipids. Lipids are widely found in sewage and industrial wastewaters. Dairy, edible oil, fat refining, slaughterhouse, wool scouring, meat processing plants and grease-trap wastes from restaurants generate wastewater high in lipids. Although it is well known that lipids can be degraded by biological process, they have been reported to inhibit anaerobic processes by causing sludge flotation and wash-out. The inhibitory effect of lipids in anaerobic process has also been attributed to the long-chain fatty acids (LCFAs) which are the hydrolysed products of lipids. It has been shown that LCFA and lipids inhibit the formation of granular sludge in Upflow Anaerobic Sludge Blanket (UASB) reactors and that the adsorption of LCFAs on to the granules can result in its flotation and washout. It was also found that the degradation of LCFA was very poor.

Various techniques have been employed to enhance degradation of lipids and these include physico-chemical pre-treatment, application of two stage treatment employing new reactor designs like Expanded Granular Sludge Bed (EGSB).

This thesis investigated the influence of co-substrates, both in the form of hydrolysed products and polymeric form, on reducing the toxicity and enhancing the degradation of LCFA and lipids in a single stage and two stage upflow anaerobic sludge blanket (UASB) reactors. The investigations were carried out on both microbiological and physico-chemical aspects. A combination of techniques including the use of light

microscopy (LM), confocal laser scanning microscopy (CLSM), transmission electron microscopy (SEM) and Fluorescent In Situ Hybridisation (FISH) was used to study the characteristics of microbial aggregates and to locate microbial populations within these aggregates. The microbial populations visualised using FISH techniques were *Bacteria*, *Archaea*, *Methanobacteriaceae*, *Methanomicrobiales* and *Methanosarcinaceae*. The performance of digesters was also monitored by measuring bulk parameters such as concentration of residual substrates, intermediate products (LCFAs, volatile fatty acids), methane (or gas) production rate and chemical oxygen demand of treated effluent.

Initially batch assays were carried out to determine the effects of glucose (hydrolysis product of carbohydrate) and cysteine (hydrolysis product of protein) on the toxicity of sodium oleate (hydrolysis product of lipid) to methanogenesis. The results showed that glucose and cysteine addition could reduce the toxicity of sodium oleate on the methanogenesis and enhance the degradation of sodium oleate. While the addition of glucose had a better effect than cysteine on decreasing the toxicity of sodium oleate, the combination of glucose and cysteine had the optimal result to stimulate the degradation of sodium oleate.

Secondly the effect of addition of glucose, cysteine and sodium oleate as co-substrates on the characteristics of granules in an LCFA fed single stage UASB were investigated. It was shown that the addition of glucose produced the best results on the formation of granules while both cysteine and sodium oleate adversely affected the granule formation. In a LCFA inhibited digester glucose and cysteine addition enhanced the recoveries of

different anaerobic microbial communities. Although the effects of glucose and cysteine on the various microbial groups were different, the combination of glucose and cysteine had the optimal results on recoveries of all bacterial groups.

The next half of the thesis investigated the influence of starch and yeast extract on the hydrolysis and degradation of canola oil by application of one and two stage UASB reactors. The results showed that the combined addition of protein and carbohydrate had an optimal effect on enhancing the hydrolysis of lipid compared to the addition of only protein or carbohydrate by promoting a balanced growth of the microbial groups. It was also demonstrated that a two- stage UASB reactor performed better in terms of extent of lipid hydrolysis and methanogenesis than a one-stage UASB reactor.

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LIST OF ABBREVIATIONS

ABR	anaerobic baffled reactor
CLSM	confocal laser scanning microscopy
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
EGSB	expanded granular sludge bed reactor
ELISA	immunosorbent assay
FISH	fluorescent in-situ hybridization
GC	gas chromatography
HPLC	high performance liquid chromatography
LCFA(s)	long chain fatty acid(s)
HRT	hydraulic retention time
SO	sodium oleate
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
UASB	upflow anaerobic sludge blanket
VFAs	volatile fatty acids

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