final report

Project Code: A.ENV.0150
Prepared by: Damien Batstone, Jurg Keller & Huoqing Ge

Date published: August 2013

PUBLISHED BY
Meat and Livestock Australia
Limited Locked Bag 991
NORTH SYDNEY NSW 2059

High rate aerobic treatment combined with anaerobic digestion and anammox

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

1. Milestone

Since the project has been approved to be continued for the third year, the originally proposed milestone due in August, 2013, the final report, will be delivered later. This report covers the project progress from March to August, 2013.

2. Abstract

The characterisation of the biological phosphorus (Bio-P) removal process at 2 days sludge retention time (SRT) is ongoing, with the main research focus being the identification of the functional microorganisms involved in the process. Molecular analysis by using flow cytometry and pyrosequencing has been performed to obtain the cells rich in polyphosphate (polyP) and identify the community structure. Preliminary pyrosequencing results showed that *Haemophilus*, *Streptococcus* and *Pseudomonas* are dominant populations in the polyP enriched sample and likely to be the functional phosphate accumulating organisms (PAOs) in the Bio-P removal process at 2 days SRT. More pyrosequencing analysis is being conducted to confirm this finding.

The thermophilic and mesophilic anaerobic digesters are being operated to treat the waste sludge produced from the high-rate sequencing batch reactor (SBR). Both digesters achieved approx. 50% volatile solids (VS) destruction, with the effluent from the thermophilic digester showing relatively high residual methane potential. Thus the hydraulic retention time (HRT) of the thermophilic digester has been extended from 5 days to 8 days to further evaluate the digestion process. Effects of temperature on the digestion process are also being evaluated.

3. Project objectives

The outcome of this project will be the evaluation of a novel, innovative technology to maximise the COD and nutrient removal performance while minimising the energy demand for the treatment of meat processing wastewater. While this project will focus on the development and demonstration of the process at the laboratory scale, it will identify major design and performance parameters that will be essential for the evaluation of the possible suitability and economics of the process once implemented at full-scale.

Therefore, this project will have as a key output the design, operating and performance parameters for this innovative technology that could provide an economic alternative to current treatment options in situations where nutrient removal is important and/or space availability is limited and hence anaerobic lagoons plus SBRs is not an ideal option.

Further work would then be required to demonstrate the technology on site at a pilot scale, but this is not part of the current project scope at this stage.

4. Success in achieving milestone

The high-rate SBR is being operated with 2 days SRT and 0.5 day HRT to further characterise the Bio-P removal at short SRT conditions. The current investigating focus is to identify the functional microorganisms mediating the Bio-P removal at 2 days SRT. Three fresh biomass samples were collected from the SBR periodically and washed by following the fixation procedures for flow cytometric analysis of environmental bacteria. Subsequently, the samples were subjected to flow cytometry analysis by staining with 4´, 6´- diamidino-2-phenylindole (DAPI) to separate the potential PAOs from non-PAOs based on the detection of cellular polyP at a higher DAPI concentration (0.24 μM for DNA staining and 1μM for polyP staining). This analysis was performed by using a BD fluorescence-activated cell sorting (FACS) high speed cell sorter, where the DAPI-DNA blue fluorescence was passed through a 450/50 band pass (BP) filter, and DAPI-polyP yellow fluorescence was passed through a 575/25 BP filter. After the polyP containing sub-populations were separated from the entire bacterial population, the sort gates were set to collect the polyP containing cells and non-polyP containing cells, respectively. The cells rich in polyP were then examined by

pyrosequencing analysis to identify the microbial community structures. So far, the pyrosequencing result of one sorted sample is available, indicating *Haemophilus*, *Streptococcus* and *Pseudomonas* are dominant populations in this sample and likely to be the functional PAOs in the Bio-P removal process at 2 days SRT. The results for another two sorted samples are still being analysed by Australian Centre for Ecogenomics to confirm this identification. Meantime, the analysis by using a transmission electron microscope (TEM) combined with an energy dispersive spectroscopy (EDS) is being organised to further investigate bacteria morphology and elements (e.g. phosphors) existed in bacteria.

At the same time, two anaerobic digesters are being operated to stabilize the waste sludge generated from the high-rate SBR. One digester is operated at 55°C with 5 days HRT, and the other digester is operated at 35°C with 10 days HRT. VS destruction is a key performance indicator used in the project to evaluate the digestion process for effectiveness of sludge stabilization. The average VS destruction achieved in both digesters was approximately 50%, as shown in Table 1. The residual biochemical methane potential (BMP) tests were then carried out by using the effluents from both digesters to further assess the digestion processes. Figure 1 shows the cumulative methane production from the residual BMP tests. As indicated in the figure, the residual methane potential of the effluent from the mesophilic digester is below 100 mL per VS added, which meets the disposal requirement for anaerobic digestate. However, the residual methane potential of the effluent from the thermophilic digester is relatively high, suggesting some biodegradable materials are residual in the thermophilic effluent and can be further converted to methane after the current 5 days thermophilic digestion. Figure 2 shows a summary of digestion performance in the mesophilic and themophilic digesters, which consists of three parts, VS destruction in the digester, degradable methane from the effluent and the non-degradable recalcitrant materials. Thus, the HRT of the thermophilic digester has been extended to 8 days to further assess the digestion performance, which showed the VS destruction was improved nearly to 60%. In addition, another thermophilic digester is being operated at 60°C with 5 days HRT to evaluate the temperature effects on the digestion process. Preliminary results showed that the achieved VS destruction was approximately 52%.

Table 1. A summary of the performance of anaerobic digesters operated in this project.

Digesters	Periods	Operating conditions	VS destruction
Thermophilic digester 1	Day 0 - Day 247	55°C / 5 days HRT	52%
	Day 248 – Day 260	55°C / 8 days HRT	60%
Thermophilic digester 2	Day 0 – Day 15	60°C / 5 days HRT	52%
Mesophilic digester	Day 0 - Day 247	35°C / 10 days HRT	50%

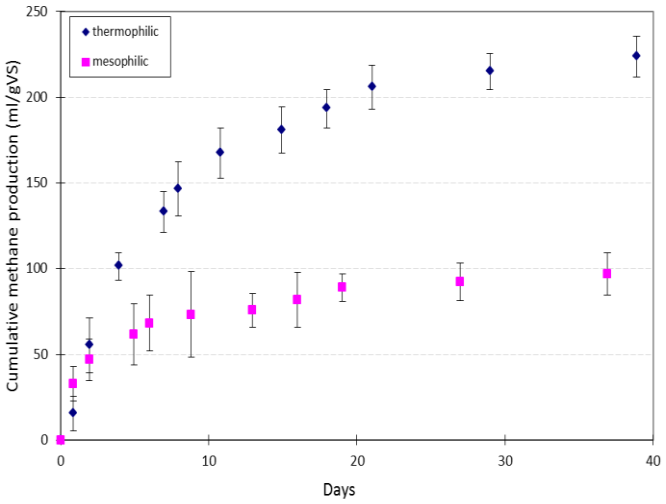

Figure 1. Cumulative methane production from the residual BMP tests by using the effluents from the thermophilic (55°C/5 days HRT) and mesophilic (35°C/10 days HRT) digesters.

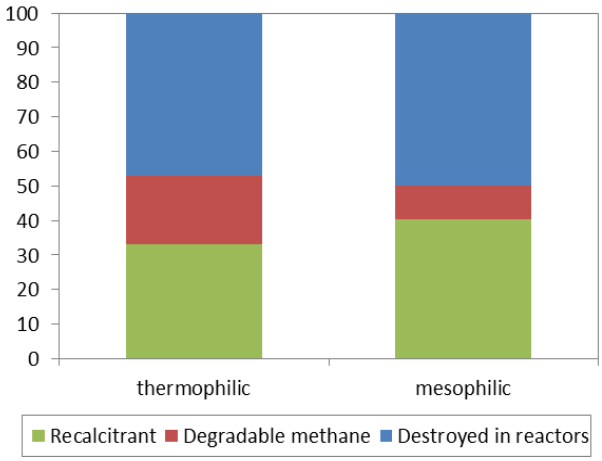

Figure 2. A summary of digestion processes in the thermophilic (55°C/5 days HRT) and mesophilic (35°C/10 days HRT) digesters.

5. Overall progress of the project

The characterisation of the Bio-P removal process at 2 days SRT is still ongoing. The next research focus will be on the investigation of the biochemical transformations involved in the Bio-P removal to fully understand the process. Cycle studies are being performed by feeding the SBR with a sole carbon source (e.g. acetate, propionate). The profiles of polyhydroxyalkanoates (PHA), glycogen, volatile fatty acids (VFAs) and phosphate will be monitored during the cycle studies, to characterize the biochemical activity of the Bio-P removal process. Stoichiometric ratios of carbon uptake, PHA production and glycogen consumption will also be determined in the cycle studies. This will be compared with previously proposed metabolic models for Bio-P removal process to reveal the metabolism of Bio-P removal at the short SRT.

Future work on the anaerobic digesters will be focusing on optimisation of the digester operations, including the effects of temperature and HRT. After the anaerobic digesters optimised, the digesters will be linked with the established Anammox-type process, which is used to treat the sludge dewatering liquor after the anaerobic treatment.

The project operating expenses to August 2013 are outlined in Table 2. The chemical analysis cost has been raised due to the cycle studies being performed to determine the biochemical activities of the Bio-P removal process. Overall, the budget for the current financial year has been achieved as planned.

Table 2. Operating expenditure for period Mar-Aug 2013.

Description	Budget	Cost
Expenses	$18,000	$ 18,000
Total chemical analysis		$ 9,300
Total microbial analysis		$ 5,300
Other consumables & maintenance		$ 2,200
Travel costs for wastewater collection		$ 1,200
Fees	$36,000	$36,000
TOTAL	**$54,000**	**$54,000**

6.Recommendations

- That this report be accepted as completion of Milestone 5 in Project ENV.0150.