ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

Quantitative Analysis of Methyl-Coenzyme M Reductase (MCRA) Gene in a Biogas Producing Reactor Treating Brewery Wastewater

[1] Abimbola M. Enitan, [2] John O. Odiyo, [3] Feroz M. Swalaha
[1, 2] Department of Hydrology and Water Resources, School of Environmental Sciences, Thohoyandou, X5050, Limpopo, South Africa
[3] Department of Biotechnology and Food Technology, Durban University of Technology, P.O. Box 1334, Durban, 4000, South Africa

Abstract:-- **Among the organisms that are involved in digestion of organic matter degradation methanogens are the major microbial group responsible for methane production. This study quantify the concentration of total bacteria and methyl-coenzyme M reductase α-subunit (mcrA) gene, specific functional gene for methane-producing Archaeal in an anaerobic reactor treating brewery wastewater using quantitative real-time polymerase chain reaction (Q-PCR). Primer sets targeting mcrA gene and total bacteria were used to detect and quantify the concentration present in the sludge samples. Q-PCR results showed that a high amount of mcrA gene that codes for the functional enzyme in methane producing Archaea are present in the reactor. However, the ratio of Archaea to bacteria concentration is lower and this revealed that the quantity of methane producing communities need to be enhanced in this reactor, in order to increase biofuel production. The results further increased our understanding on the ability of methanogens to grow in high concentration at an optimum reactor performance in anaerobic condition to transform organic substrate present in industrial wastes into biogas as source of renewable energy.**

Keywords **— Archaea, brewery wastewater, methanogens, quantitative PCR.**

I. INTRODUCTION

Anaerobic treatment of wastewater have been widely adopted for the treatment of high strength wastewater using different anaerobic technology and for a proper functioning of any bioreactor, the biodiversity of the microbial community is very important [1]. Brewery wastewater consist of complex organic compounds and the breakdown of these organic matters during anaerobic digestion process using UASB reactor involves the ultimate action of several groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic bacteria) through a variety of intermediates to biogas production [2,3,16,17]. Due to the huge structural complexity of the granular sludge, it is hard to assess the diversity, colonization and topological distribution of these groups of microorganisms using normal conventional methods (isolation, plate-counting, etc). Advances in understanding the microbial ecology of anaerobic systems are needed for an efficient and better effluent quality as well as to enhance bioenergy production. Knowledge on the quantification of microbial communities using culture-independent molecular tools to determine the impact of shallow reactor on microbial concentration Granules from a full-scale UASB reactor treating brewery wastewater in Durban, South Africa was investigated in this study. Sample collection and treatment was earlier described in [6]. The direct isolation of total genomic DNA from granular sludge samples was carried out according to phenol-chloroform extraction method described by [7]. The concentration of the DNA was checked by Nanodrop ND-1000 Spectrophotometer. The purified DNA

Target group	Target microrganisms	Primer names	Sequences(5'→3')	Reference
McrA	Functional gene for methanaogenic Archaea	MLf	GGTGGTGTMGGATTCACACARTAYGCWAC	[15]
		MLr	AGC	
			TTCATTGCRTAGTTWGGRTAGTT	
16S rDNA	Bacterial	27f	AGA GTT TGA TCM TGG CTC AG	[9]
		1492r	TAC GGY TAC CTT GTT ACG ACT T	

molecular techniques, such as the rRNA-approach: FISH, cloning and sequencing of 16S-rRNA genes [2]; DGGE: denaturing gradient gel electrophoresis and pyrosequencing [18,20] are been used to know the interaction of bacterial populations of anaerobic sludge granules [5,11,19,20]. Little work has been done on the distribution and quantity of microbial community across shallow reactors. The aim of this study was to quantify the spatial distribution of microbial communities especially methyl coenzyme M reductase (mcrA) genes. The functional genes that are responsible for methanogenic activity during fermentation and anaerobic digestion of wastes using Q-PCR as compared to the total bacteria.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

II. MATERIALS AND METHODS

A. Sample Collection and Genomic DNA Extraction from Granular Sludge Sample

B. Amplification of Genomic DNA Using Quantitative PCR Purified PCR amplicons for methyl coenzyme M reductase (mrcA) genes and total bacteria were used as a template for the standard curve. DNA from eac amplicon was diluted a 10-fold and bacteria series concentration using PCR grade water. Range of 101 to 108 target DNA copies/µl was generated and analyzed in duplicate by qPCR with its corresponding primer set. Quantitative real-time PCR (qPCR) to quantify 16S rRNA copies in DNA extracted from the samples were performed using a thermal Cycler instrument (C-1000 Touch, CFX 96, Biorad Laboratories Pty Ltd, USA) using two primer sets targeting domain bacteria and mrcA gene (Table 1). QPCR reaction mixture for the amplification was carried out in a final volume of 20µl containing PCR-grade water, 1 µl of each primer (final concentration, 10 µM), 10 µl of the Sso fast Eva green Master Mix (Biorad Laboratories Pty Ltd, USA) and 4 µl of template DNA. Two-step amplification of the target DNA was carried out using the protocol described by [6] as follows: initial denaturation for 3.5 min at 94°C followed by 40 cycles at 95°C for 30 s and annealing at 55 °C for 30 s and final extension with image capturing at 72°C for 30s. The temperature was increased at 0.5˚C every 10 s from 40 to 95˚C for melting curve analysis. Each QPCR assay was conducted in duplicates. For all experiments, appropriate negative controls containing no genomic DNA were subjected to the same procedure to exclude any possible contamination or carry-over. For each qPCR assay, the value of the logarithmic starting quality for the different 16S rDNA gene were plotted against the threshold cycle (Cq) numbers and the linear ranges of the standard curves were selected based on the R2 of the slope greater than 0.990. For quantification of 16S rDNA gene concentration that were present in the DNA obtained from the different compartment, the Cq values for each sample were compared with the corresponding standard curves. The 16S rDNA gene copy was calculated with the average molecular weight of 660 Da and avogadro's numbers (6.02 ×1023) per base pair was of double-stranded DNA [8].

III. RESULTS AND DISCUSSION

A. Quantification of 16S rDNA gene concentration of microbial community using real-time PCR

The validation and accuracy of quantified the 16S rDNA gene copy numbers were determined using coefficients (R2) values of 0.991 and 1.000 respectively for mrcA gene and total bacteria in the reactor. The 16S rDNA gene copies of mrcA in the samples were calculated against the total bacterial 16S rDNA gene copies. The compartment showed a noticeable disparity in terms of the composition of bacteria and methanogenic population using real-time PCR (Fig. 1). It is observed that the concentration of mrcA gene decreases with an increase in the total bacteria concentration along the compartments of the full-scale reactor. Real-time PCR revealed high percentage of mrcA gene in Compartment 1 as compared to bacteria concentration. The percentage of 16S rDNA gene copies of mrcA gene per nanogram of sample in Compartment 1 was much higher (34.90%) than the total bacterial (2.62%). Similar variation in spatial distribution of this gene colonizing the lower and middle parts in the anaerobic reactor was reported by Kubota et al. [12]. However, the bacterial concentration increased to 95.80% in the last compartment with a decrease in the amount of mrcA 16S rDNA gene copies. Fluctuation in the quantity of bacterial in the different compartments was noticed and this might be as a result of production of metabolites by some group of bacteria to inhibit or suppress the growth of other bacterial in the reactor [14]. The abundance of mrcA genes at the lower compartment of the UASB reactor as determined by real-time PCR suggest the presence of lower toxic substances that supports the growth of methanogens in the reactor. The reduction in mrcA gene concentration or cell number indicates the production of intermediates metabolites that do not favour or inhibit the growth of methanogens [10,13]. The study further confirms that there is variation in the microbial population in each compartment. It can further be deduced that different compartment in the reactor might be involved in different phases of anaerobic degradation of organic matter in brewery wastewater with different concentration of metabolic products been produced as confirmed by the qPCR assays.

IV. CONCLUSIONS

Quantitative PCR-based technique produced a better concentration of the microbial consortium present in the UASB reactor treating brewery wastewater. This technique helps us to quantify the microbial population in each compartment and possibly the phases at which anaerobic fermentation takes place in the investigated UASB reactor.

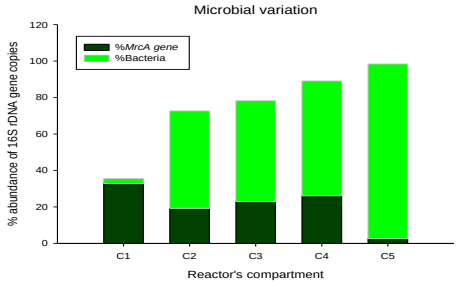

Fig. 1. Comparison of microbial population in samples taken from different compartment of UASB reactor treating

brewery wastewater as determined by domain-specific and methyl-coenzyme M reductase 16S rDNA-targeted oligonucleotides primer sets

REFERENCES

[1] Amani T, Nosrati M, Mousavi SM, et al. (2011) Study of syntrophic anaerobic digestion of volatile fatty acids using enriched cultures at mesophilic conditions. Int. J. Environ. Sci. Tech. 8: 83-96.

[2] Amann RI, Ludwig W and Schleifer KH. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59 143–169.

[3] Appels L, Baeyens J, Degréve J, et. al. (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energ. Combust 34: 755–781.

[4] Bialek K, Kim J, Lee C, et. al. (2011) Quantitative and qualitative analyses of methanogenic community development in high-rate anaerobic bioreactors. Water RES 45(3): 1298-1308.

[5] Chan O-C, Liu W-T and Fang HHP. (2001) Study of microbial community of brewery-treating granular sludge by denaturing gradient gel electroforesis of 16S rRNA gene. Wat. Sci. Technol 43: 77-82.

[6] Enitan, A.M., 2014. Microbial community analysis of a UASB reactor and application of an evolutionary algorithm to enhance wastewater treatment and biogas production. Durban University of Technology.

[7] Enitan, A.M., Kumari, S., Swalaha, F.M., Adeyemo, J., Ramdhani, N., Bux, F., 2014a. Kinetic modelling and characterization of microbial community present in a full-scale UASB reactor treating brewery effluent. Microbial Ecol. 67, 358-368.

[8] Enitan, A.M., Kumari, S., Swalaha, F.M., Bux, F., 2014b. Real-time quantitative PCR for quantification of methanogenic Archaea in an UASB reactor treating brewery wastewater. Conference of the Inter J Arts Sci. 07, 103–106.

[9] Giovannoni SJ. (1991) The polymerase chain reaction. In:Stackebrandt E, Goodfellow M (eds) Nucleic Acid Techniques in Bacterial Systematics. John Wiley and Sons, Chischester. 177-203.

[10] Irianni-Renno, M., Akhbari, D., Olson, M.R., Byrne, A.P., Lefèvre, E., Zimbron, J., Lyverse, M., Sale, T.C., Susan, K., 2016. Comparison of bacterial and archaeal communities in depth-resolved zones in an LNAPL body. Appl. Microbiol. Biotechn. 100, 3347-3360.

[11] Krakat N, Westphal A, Schmidt S, et. al. (2010) Anaerobic Digestion of Renewable Biomass: Thermophilic Temperature Governs Methanogen Population Dynamics. Applied and Environmental Microbiology 76: 1842–1850.

[12] Kubota K, Hayashi M, Matsunaga K, et. al. (2014) Microbial community composition of a down-flow hanging sponge (DHS) reactor combined with an up-flow anaerobic sludge blanket (UASB) reactor for the treatment of municipal sewage. Bioresource Technology 151: 144-150.

[13] Langenhoff AAM and Stuckey DC. (2000) Treatment of a dilute wastewater using an anaerobic baffled reactor: Effect of low temperature. Water Research 34: 3867-3875.

[14] Lee S-H, Kang H-J, Lee YH, et. al. (2012) Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. Journal of Environmental Monitoring 14: 1893-1905.

[15] Luton PE, Wayne JM, Sharp RJ, et. al. (2002) The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148: 3521–3530.

[16] Mirzoyan N, Parnes S, Singer A, et. al. (2008) Quality of brackish aquaculture sludge and its suitability for anaerobic digestion and methane production in an upflow anaerobic sludge blanket (UASB) reactor. Aquaculture 279: 35-41.

[17] Mumme J, Linke B and Tolle R. (2010) Novel upflow anaerobic solid-state (UASS) reactor. Bioresour. Technol. 101: 592–599.

[18] Muyzer G, Waal ECd and Uitterlinden AG. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. App. Environ. Microbiol. 59: 695-700.

[19] Zhang L, Sun Y, Guo D, et. al. (2012) Molecular diversity of bacterial community of dye wastewater in an

anaerobic sequencing batch reactor. African Journal of Microbiology Research: 6444-6453.

[1] Ziganshin A, Schmidt T, Scholwin F, et. al. (2011) Bacteria and archaea involved in anaerobic digestion of distillers grains with solubles. Applied Microbiology and Biotechnology 89: 2039-2052