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Methane yields and methanogenic community changes during co-fermentation of cattle slurry with empty fruit bunches of oil palm

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HIGHLIGHTS

- Empty fruit bunches of oil palm are a suitable source for biomethanisation.
- Thermophilic digestion was more efficient than mesophilic AD.
- At 55 °C a consortium of *Methanoculleus* + *Methanosarcina* replaced *Methanobrevibacter*.

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ABSTRACT

The biomethane potential and structural changes of the methanogenic community in a solid-state anaerobic digestion process co-digesting cattle slurry and empty fruit bunches were investigated under mesophilic (37 °C) and thermophilic (55 °C) conditions. Phylogenetic microarrays revealed the presence of two hydrogenotrophic genera (*Methanoculleus* and *Methanobrevibacter*) and one acetoclastic genus (*Methanosarcina*). *Methanosarcina* numbers were found to increase in both mesophilic and thermophilic treatments of empty fruit bunches. *Methanobrevibacter*, which dominated in the cattle slurry, remained constant during anaerobic digestion (AD) at 37 °C and decreased in numbers during digestion at 55 °C. Numbers of *Methanoculleus* remained constant at 37 °C and increased during the thermophilic digestion. Physicochemical data revealed non-critical concentrations for important monitoring parameters such as total ammonia nitrogen, free ammonia nitrogen and volatile fatty acids in all treatments after AD. The biomethane potential of empty fruit bunches was higher under thermophilic conditions than under mesophilic conditions.

1. Introduction

The production of palm oil to meet the growing demand for vegetable oils is becoming increasingly important. According to the USDA (2007), 37 Mt of palm oil were consumed worldwide in 2006/2007, representing approximately one-third of all vegetable oil consumption. Moreover, it is expected that worldwide vegetable oil consumption will continue to increase up to 280 Mt by 2050 (Corley, 2009).

In addition to palm oil and palm kernel oil, both extracted from the pulp (endocarp) of the oil palm (*Elaeis guineensis*) fruit, huge amounts of lignocellulose-rich by-products including empty fruit bunches (EFB), fronds, mesocarp fibres, trunks and shells are produced throughout the oil manufacturing process. Although some of these by-products are already deployed in the industrial production of bio-plastics, organic acids, animal feedstuffs, furniture and cigarette and bond papers, the quantities produced are too high for immediate processing. Increasing energy costs as well as strong competition in the international palm oil market have resulted in the need to reduce the existing operating costs for the production of palm oil. One possibility to overcome the issues of increasing costs and excess wastes could be the production of biomethane from the energy-rich by-products via an anaerobic digestion (AD) process.

Numerous possibilities regarding the operational mode and reactor design for the AD are known, and depend primarily on existing reactor infrastructure and type as well as the amounts and types of digestible organic residues available. Sewage sludge or food waste comprising 0.5–15% total solids (TS) are generally

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treated in a liquid-state AD process (LS-AD), while organic fractions with a lower water content (e.g. plant residues) can be processed by solid-state AD (SS-AD; TS > 15%; Li et al., 2011). Biogas production from SS-AD and LS-AD is comparable, although there are several benefits for SS-AD, including smaller reactor volume, lower energy requirements for heating and minimal material handling (Li et al., 2011). There are also some disadvantages, including up to three times longer retention times, the need for larger amounts of inocula and a higher potential for process instability (Li et al., 2011). The aim of this work was to determine the biomethane potential (BMP) of EFB, as well as to investigate the adaption process of a methanogenic community originating from cattle slurry (CS) in a SS-AD process co-digesting the EFB.

2. Methods

2.1. Sampling and pre-treatment

Fresh fruit bundles of *E. guineensis* (oil palm) grown in Bogota, Columbia (4° 35′ 56″ N, 74° 4′ 51″ W) were harvested as whole units, and subjected to an oil extraction process. Lignocellulose-rich EFB were frozen and shipped to Innsbruck, Austria, for physical, chemical and microbial analyses.

For lab-scale experiments, EFB were cut with a commercial garden shredder. Smaller fractions were additionally cut with scissors to obtain fibres smaller than 5 cm in length. Homogenized fibres were stored at −20 °C until use. Fresh CS (methanogen inoculum source) was collected from a dairy farm in Innsbruck, Austria (47° 16′ 2″ N, 11° 23′ 34″ E), and particulate matter (>2 mm) was removed from the slurry by sieving. Degassing of the CS was conducted by storing the slurry at 37 °C for one week. Physicochemical characteristics of the inoculum CS and the co-substrate EFB are listed in Table 1.

2.2. Reactor operation and BMP measurement

AD was conducted in an Automatic Methane Potential Test System instrument (AMPTS II; Bioprocess control, Lund, Sweden). Treatments were conducted in triplicate, and an inoculum/substrate ratio of 2 was used (66% FW CS and 33% FW EFB), resulting in a composition with 16.8% TS. Control reactors containing only CS were included. Reactors were operated in batch-mode in 400 ml volumes without agitation at 37 °C and 55 °C for 22 days to simulate SS-AD. The BMP of EFB under mesophilic conditions (EFB37) and under thermophilic conditions (EFB55) was calculated by subtracting the BMP measured in the control reactors containing only CS (CS37 and CS55).

2.3. Physicochemical parameters

Physical and chemical parameters of the CS inoculum, EFB and all sludge samples after AD (CS37, EFB37, CS55, EFB55) were determined. The parameters pH, electrical conductivity (EC), TS, volatile solids (VS), C/N ratio, total ammonia nitrogen (TAN), free ammonia nitrogen (FAN) and volatile fatty acids (VFAs) were investigated as described by Franke-Whittle et al. (2014).

2.4. DNA extraction, 16S rRNA gene amplification and ANAEROCHIP microarray hybridisation

DNA extraction of CS, CS37, EFB37, CS55 and EFB55 was conducted using the NucleoSpin® Soil extraction kit (Macherey-Nagel, Düren, Germany), according to the instructions provided by the manufacturer.

DNA extracts from CS, EFB37 and EFB55 were subjected to PCR using 16S rRNA gene specific primers, prior to being hybridised with the ANAEROCHIP microarray, an oligonucleotide microarray targeting methanogens. PCR amplifications, DNA purification, DNA quantification, DNA digestion, array hybridisation, washing and scanning were performed as described by Franke-Whittle et al. (2009). All hybridisations were conducted in triplicate. All signals with a signal-to-noise ratio (SNR) above 2 were considered positive.

2.5. Real-time quantitative PCR

To quantitate methanogens in CS, CS37, EFB37, CS55 and EFB55, qPCR was conducted with specific 16S rRNA gene primers (Table 2) for the three genera *Methanosarcina*, *Methanoculleus* and *Methanobrevibacter*, according to microarray results. qPCR was performed with 1 µl of template DNA as described in Franke-Whittle et al. (2014). After an initial denaturation at 95 °C for 5 min, thermal cycling comprised 45 cycles of 20 s at 95 °C, 20 s at 58–65 °C (annealing temperature) and 20 s at 72 °C. The annealing temperatures were as follows: 64 °C for *Methanosarcina*, 65 °C for *Methanoculleus* and 59 °C for *Methanobrevibacter*.

2.6. Statistical evaluation

The PASW-SPSS 21.0 software (SPSS, Chicago, IL, USA) was used to determine correlations between physicochemical parameters

Table 1
Physicochemical properties of EFB, CS and all sludge samples after AD.

Parameters	EFB	CS	CS37	CS55	EFB37	EFB55
pH	nm	7.90 (±0.08)	7.64 (±0.02)	8.20 (±0.01)	7.78 (±0.05)	8.36 (±0.02)
EC (mS cm^{-1})	nm	18.1 (±0.17)	21.0 (±0.54)	21.8 (±0.24)	22.0 (±0.90)	22.0 (±0.08)
TS (% FW)	33.6 (±1.90)	8.07 (±0.19)	8.22 (±0.11)	6.84 (±0.09)	9.98 (±0.17)	11.90 (±0.23)
VS (% TS)	92.2 (±0.71)	70.7 (±0.74)	27.5 (±0.64)	34.1 (±0.46)	20.5 (±0.64)	19.5 (±0.32)
C/N total (%)	57.5 (±13.7)	16.7 (±2.95)	14.6 (±0.24)	14.8 (±0.33)	21.2 (±2.46)	23.5 (±1.84)
TAN (mg L^{-1})	nm	1895 (±223)	2275 (±182)	2033 (±103)	1907 (±5.48)	1033 (±47.1)
FAN (mg L^{-1})	nm	77.1 (±2.04)	115 (±13.1)	735 (±45.5)	131 (±16.1)	461 (±27.4)
Acetate (mmol L^{-1})	nm	11.7 (±0.95)	0.80 (±0.24)	12.6 (±0.49)	0.60 (±0.19)	1.90 (±0.26)
Propionate (mmol L^{-1})	nm	4.89 (±0.50)	4.05 (±0.17)	8.39 (±0.95)	4.11 (±0.18)	2.84 (±0.34)
Butyrate (mmol L^{-1})	nm	nd	nd	nd	nd	nd
Isobutyrate (mmol L^{-1})	nm	0.38 (±0.13)	nd	0.41 (±0.07)	nd	nd
Valerate (mmol L^{-1})	nm	nd	nd	nd	nd	nd
Isovalerate (mmol L^{-1})	nm	0.21 (±0.04)	nd	1.83 (±0.37)	nd	nd
CH_4 (g FW^{-1})	nm	nm	5.13 (±0.29)	4.11 (±0.16)	29.6 (±0.78)	65.9 (±1.13)
CH_4 (g VS^{-1})	nm	nm	89.9 (±5.01)	72.1 (±2.80)	94.7 (±2.50)	211 (±3.61)

Note: nm – not measured; nd – not detected; EFB – empty fruit branches of oil palm fibres; CS – cattle slurry; CS37 – sludge from AD of cattle slurry at 37 °C; CS55 – sludge from AD of cattle slurry at 55 °C; EFB37 – sludge from AD of EFB at 37 °C; EFB55 – sludge from AD of EFB at 55 °C.