Co-cultivation of *Thermoanaerobacter* strains with a methanogenic partner enhances glycerol conversion

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**Summary**

Glycerol-rich waste streams produced by the biodiesel, bioethanol and oleochemical industries can be treated and valorized by anaerobic microbial communities to produce methane. As current knowledge of the microorganisms involved in thermophilic glycerol conversion to methane is scarce, thermophilic glycerol-degrading methanogenic communities were enriched. A co-culture of *Thermoanaerobacter* and *Methanotrophobacter* species was obtained, pointing to a non-obligately syntrophic glycerol degradation. This hypothesis was further studied by incubating *Thermoanaerobacter brockii* subsp. *finnii* and *T. wiegelii* with glycerol (10 mM) in pure culture and with different hydrogenotrophic methanogens. The presence of the methanogen accelerated glycerol fermentation by the two *Thermoanaerobacter* strains up to 3.3 mM day −1, corresponding to 12 times higher volumetric glycerol depletion rates in the methanogenic co-cultures than in the pure bacterial cultures. The catabolic pathways of glycerol conversion were identified by genome analysis of the two *Thermoanaerobacter* strains. NADH and reduced ferredoxin formed in the pathway are linked to proton reduction, which becomes thermodynamically favourable when the hydrogen partial pressure is kept low by the hydrogenotrophic methanogenic partner.

**Introduction**

Worldwide demand for biodiesel increased in the last decade, leading to a global biodiesel production of $36 \times 10^9$ l in 2016 (OECD/FAO, 2017). Glycerol is co-produced in quantities that match approximately 10% of the total biodiesel production, leading to a surplus of this compound. Consequently, glycerol prices have decreased, changing glycerol from a commodity chemical to a surplus by-product, and even a waste product (Viana et al., 2012; Clomburg and Gonzalez, 2013; Ciriminna et al., 2014). Glycerol is also generated in ethanol production by yeast (Navarrete et al., 2014) and is frequently present in different wastes/wastewater as e.g. from the oleochemical industry, where waste streams can contain up to 90% glycerol (Clomburg and Gonzalez, 2013).

Anaerobic microbial processes can provide a solution for these glycerol-rich wastes producing a wide range of valuable compounds (Viana et al., 2012; Clomburg and Gonzalez, 2013). Since glycerol is a highly reduced compound, fermentative microorganisms must be able to dispose of the excess reducing equivalents, which is generally accomplished by the production of 1,3-propanediol (1,3-PDO), a product that is more reduced than glycerol (Clomburg and Gonzalez, 2013; Schindler et al., 2014). Microorganisms that lack the 1,3-PDO formation pathway generally transfer the electrons to hydrogen or formate, as well as to pyruvate, generating organic compounds such as ethanol, butanol or succinate (Murarka et al., 2008; Scholten et al., 2009; Clomburg and Gonzalez, 2013). The problem of the release of excess electrons in glycerol fermentation has been studied with diverse mesophilic bacteria, including studies on electron transfer to electrodes (Emde et al., 1989; Emde and Schink, 1990) or medium sparging with inert gases for $\text{H}_2$ removal (Dhandadi et al., 2006; Murarka et al., 2008).

The high energy content of glycerol makes it also an interesting substrate for biogas production, individually or
in co-digestion with different feedstocks – e.g. sewage sludge or the organic fraction of municipal solid wastes (Kolesárová et al., 2011; Yang et al., 2012). The generated biomethane may be stored or injected into the natural gas grid and used as biofuel (Beauchamp et al., 2014; Hengeveld et al., 2014).

The production of biodiesel and bioethanol typically generates waste streams at temperatures between 40 to 65°C. Therefore, thermophilic conditions are beneficial for valorization of glycerol. Moreover, anaerobic digestion is generally faster when performed by thermophilic than by mesophilic microorganisms (Ho et al., 2013). Some thermophilic bacteria were reported to grow with glycerol in pure culture, e.g. Thermoanaerobacter wiegeli (Cook et al., 1996), Moorella glycerini (Slobodkin et al., 1997) and Pseudothermotoga lettingae (Balk et al., 2002), but the thermophilic conversion of glycerol by mixed communities is only scarcely studied (Yang et al., 2008; Zhang et al., 2015).

This work aims to gain insight into the different microbial key players involved in glycerol degradation in mixed thermophilic anaerobic cultures. Thermophilic glycerol-degrading cultures were enriched, and a co-culture of Thermoanaerobacter brockii and a methanogenic partner was obtained, pointing to the possibility of facultatively syntrophic glycerol degradation. The influence of different methanogenic partners on glycerol degradation by two Thermoanaerobacter species was then investigated.

Results

Enrichment of glycerol-degrading microbial cultures

A stable thermophilic (55°C) glycerol-degrading enrichment (culture Gly(9)) was obtained through repeated transfers to fresh medium containing glycerol as sole substrate over a period of approximately one year (Fig. S1 and Table S1). This culture converted $6.5 \pm 0.3$ mM of glycerol mainly to methane ($6.2 \pm 0.1$ mM) and acetate ($6.7 \pm 0.1$ mM) during the first 6 days of incubation (Fig. 1). Propionate was also detected, but at concentrations lower than 1 mM (Fig. 1). No other fermentation products, such as lactate, ethanol, butanol, 1,3-PDO, 1,2-PDO or hydrogen, were detected.

Culture Gly(9) was mainly composed by microorganisms from the genera Methanothermobacter, Thermoanaerobacter, Pseudothermotoga and Acetomicrobium, as shown in Table 1. Taxonomic identification was not possible for approximately 25% of the retrieved sequences.

This culture was further incubated in agar-shake cultures at 70 and 40°C, considering that several members
of the *Thermoanaerobacter* genus and all the known *Acetomicrobium* species can grow at this last temperature. A methanogenic glycerol-degrading culture designated Col-Gly was obtained at 40°C, which presented very low diversity when examined by phase contrast microscopy (Fig. S2). Microbial community analysis showed the presence of only two microorganisms belonging to *Methanothermobacter* and *Thermoanaerobacter* genera, with relative abundances of 24–30% and 70–76% respectively (Table 1).

When culture Col-Gly was incubated at 65°C (the optimal growth temperature for both identified microorganisms), 10 mM glycerol was completely degraded within 6 days of incubation (data not shown), associated with the formation of methane (8.0 ± 0.2 mM), acetate (8.7 ± 1.8 mM) and lactate (2.8 ± 0.3 mM) (Fig. 2). Hydrogen was detected at residual concentrations (< 0.01 mM) during the experiment (data not shown). Similar glycerol consumption (i.e. glycerol was not detectable after 7 days of incubation) and products profile (Fig. S3) were obtained in the incubations at 55°C (the original incubation temperature of the enrichment Gly(9)), and therefore further experiments were performed at 65°C.

When the enriched co-culture Col-Gly was incubated with BrES, a selective inhibitor of methane-producing archaea (DiMarco et al., 1990), no methane was detected in the headspace of the bottles and only vestigial glycerol consumption was observed during 7 days of incubation (Fig. 3). Hydrogen accumulated at very low amounts (< 0.1 mM), while ethanol and lactate were not detected (Fig. 3). No noteworthy effect of BrES could be detected in glycerol consumption and products formation by the *T. brockii* subsp. *finnii* type strain (Fig. S4). These results raised the hypothesis that the presence of the methanogen could influence the observed glycerol conversion rates in the enriched co-culture Col-Gly.

### Glycerol degradation by *Thermoanaerobacter* species, in pure culture or in co-culture with methanogens

Within the *Thermoanaerobacter* genus, only *T. wiegellii*, *T. siderophilus*, *T. brockii* subsp. *finnii* and *T. subterraneus* were reported to grow with glycerol (Cook et al.,

![Fig. 2. Methane (A) and organic acids (B) production by culture Col-Gly at 65°C.](#)
1996; Slobodkin et al., 1999; Fardeau et al., 2000; Alves et al., 2016), although growth of T. brockii subsp. finnii was described as poor (Alves et al., 2016). Therefore, to further assess the possible positive effect of methanogens on glycerol fermentation, T. brockii subsp. finnii DSM 3389T and T. wiegelii DSM 10319T were grown individually in pure culture or with a methanogenic partner. Methanothermobacter sp. strain GH (the culture obtained after incubation of the enrichment Col-Gly with \( \text{H}_2/\text{CO}_2 \) for 15 transfers) and M. marburgensis were the selected methanogens. The results obtained are shown in Figs 4 and 5.

After approximately 16 days of incubation, T. brockii subsp. finnii and T. wiegelii consumed 52 ± 4% and 39 ± 5% of the glycerol added, respectively, with corresponding volumetric substrate depletion rates \( (R_g) \) of 0.3 and 0.2 mM day \(^{-1} \) (Figs 4A and 5A). Acetate was the main product (around 2 mM in both cases, Figs 4B and 5B) and hydrogen, lactate and ethanol were obtained in small amounts (0.5–1.2 mM, Figs 4C and 5C). Product yields were calculated relatively to the amount of glycerol consumed, and were similar for both species, i.e. around 0.5 mmol mmol \(^{-1} \) for acetate, 0.25 mmol mmol \(^{-1} \) for hydrogen and between 0.15 and 0.20 mmol mmol \(^{-1} \) for lactate and ethanol (Table 2). Considering the stoichiometry of the possible reactions involved (Table 3), the products measured accounted for 87% and 75% of the glycerol consumed by T. brockii and T. wiegelii, respectively. When the Thermoanaerobacter type strains were incubated in co-culture with the methanogens, glycerol consumption rate was substantially accelerated, i.e. all the constructed co-cultures (Thermoanaerobacter strain + methanogen) completely degraded the added glycerol in 4–7 days with volumetric substrate depletion rates \( (R_g) \) 8–12 times higher than the bacterial pure cultures (i.e. between 2.1 and 3.3 mM day \(^{-1} \), Figs 4A and 5A). Acetate and methane were the main products obtained (Figs 4B, D and 5B, D), with respective yield of 1.0 and 0.63–0.82 mmol mmol \(^{-1} \) relatively to the amount of glycerol consumed, which are close to the theoretically expected values (Tables 2 and 3).

Discussion

Glycerol (1,2,3-propanetriol) can sustain growth of a diverse microbial community, as shown by the composition and activity of culture Gly(9) enriched at 55°C (Table 1, Fig. 1). The main bacterial genera identified were probably involved in glycerol conversion, since some of the characterized strains within the genera Pseudothermotoga, Acetomicrobium and Thermoanaerobacter have been reported as glycerol degraders (Rees et al., 1997; Menes and Muxi, 2002; Maru et al., 2013; Alves et al., 2016). Acetate and methane were the main products of glycerol conversion, indicating that methane was produced from formate or \( \text{H}_2/\text{CO}_2 \) (Fig. 1). This was reinforced by the identification of Methanothermobacter sp., a hydrogenotrophic methanogen, as the only archaeon in this community (Table 1).

When applying a lower temperature (40°C) as selective factor, a co-culture composed by Thermoanaerobacter and Methanothermobacter was enriched (culture Col-Gly). This co-culture was capable of fast glycerol degradation (< 6 days) coupled to good growth evaluated by visual inspection. Also in the work of Zhang et al. (2015), Thermoanaerobacter spp. and hydrogenotrophic methanogens (mainly Methanothermobacter thermautotrophicus) were the dominant microorganisms in the community developed in a continuous bioreactor operated with glycerol at 70°C. However, as previously mentioned, Alves et al. (2016) reported that glycerol was only poorly utilized by T. brockii subsp. finnii. When we incubated pure cultures of T. brockii subsp. finnii or T. wiegelii with glycerol at 10 mM, glycerol was hardly fermented, i.e. more than 16 days were required to convert 40–50% of the added glycerol (Figs 4 and 5). In spite of that, these two strains easily ferment glucose in pure culture, e.g. Alves et al. (2016) reported degradation of 20 mM of glucose by T. brockii subsp. finnii and T. wiegelii in 7 and 3 days respectively. This difference is probably related to the more reduced nature of glycerol, which leads to the generation of twice the number of reducing equivalents per pyruvate molecule formed, compared with glucose (Clomburg and Gonzalez, 2013).

The two Thermoanaerobacter strains studied in this work did not produce 1,3-PDO, and the analysis of their genomes confirmed that these bacteria lack the genes encoding for the enzymes involved in the pathway of 1,3-PDO formation (enzymes 1 and 2, Fig. 6). Therefore,
Fig. 4. Glycerol consumption (A) and production of acetate (B) by *Thermoanaerobacter brockii* subsp. *finnii* (DSM 3389T) when incubated in pure culture or in co-culture with methanogens. Lactate, ethanol and H₂ production by *T. brockii* subsp. *finnii* in pure culture (C) and methane production in co-culture with methanogens (D). *M. sp*. GH, culture obtained after 15 transfers of the enriched culture Col-Gly with H₂/CO₂; *M. mar*, *Methanothermobacter marburgensis* DSM 2133T. $R_g$ Volumetric glycerol depletion rate.
Fig. 5. Glycerol consumption (A) and production of acetate (B) by Thermoanaerobacter wiegelii (DSM 10319\textsuperscript{T}) when incubated in pure culture or in co-culture with methanogens. Lactate, ethanol and H\textsubscript{2} production by T. wiegelii in pure culture (C) and methane production in co-culture with methanogens (D). M. sp. GH, culture obtained after 15 transfers of the enriched culture Col-Gly with H\textsubscript{2}/CO\textsubscript{2}; M. mar, Methanothermobacter marburgensis DSM 2133\textsuperscript{T}. $R_\text{g}$, Volumetric glycerol depletion rate.
Table 2. Product yields of glycerol fermentation, calculated relatively to the amount of glycerol consumed (mmol mmol⁻¹), by T. brockii subsp. finnii (DSM 3389T) and T. wiegelli (DSM 10319T), when incubated in pure culture or in co-culture with methanogens.

| Culture        | Acetate     | Lactate     | Ethanol     | H2          | Methane |
|----------------|-------------|-------------|-------------|-------------|---------|
| T. brockii     | 0.50 ± 0.05 | 0.18 ± 0.01 | 0.20 ± 0.04 | 0.25 ± 0.02 | n.a.    |
| T. brockii + M. sp. GH | 1.00 ± 0.04 | n.d.        | n.d.        | n.d.        | 0.63 ± 0.02 |
| T. brockii + M. mar | 1.00 ± 0.00 | n.d.        | n.d.        | n.d.        | 0.82 ± 0.01 |
| T. wiegelli     | 0.46 ± 0.06 | 0.15 ± 0.05 | 0.15 ± 0.04 | 0.24 ± 0.04 | n.a.    |
| T. wiegelli + M. sp. GH | 1.00 ± 0.04 | n.d.        | n.d.        | n.d.        | 0.63 ± 0.03 |
| T. wiegelli + M. mar | 1.00 ± 0.04 | n.d.        | n.d.        | n.d.        | 0.75 ± 0.03 |

M. sp. GH, culture obtained after 15 transfers of the enriched culture Col-Gly with H2/CO2; T. wiegelli + T. wiegelli 0.46
reoxidation of NADH
zyme 16) oxidizes ferredoxin and, using protons as
genes (Fig. 6
vate formate lyase and formate hydrogen lyase encoding
functions as biological electron acceptor (Fig. 6). In fact,
glycerol conversion to acetate becomes more exergonic and thermodynamically more favourable if the hydrogen
produced is used by hydrogenotrophic methanogens to
produce methane, as shown by the Gibbs free energy
changes of ΔG° = −73.1 and −174.7 kJ reaction⁻¹
respectively (reactions 1 and 3, Table 3). In these co-cul-
tures, ethanol production could not be detected, and only
small amounts of lactate were produced by the enriched
culture Col-Gly (Figs 4 and 5).

The positive effect of the methanogen was also exper-
imentally confirmed when the methanogenic activity in
culture Col-Gly was inhibited by BrES, which caused
glycerol degradation to proceed at a much lower rate
(Fig. 3). The relationship between the Thermoanaerobacter strains and the methanogen points to a facultative
syntrophy, since glycerol fermentation can be performed
by these bacteria in pure culture but their growth and
metabolic products are directly influenced by the hydro-
gen scavenger (Stams and Plugg, 2009). This
syntrophic relationship is energetically advantageous for the
Thermoanaerobacter bacteria, compared with glycerol
fermentation in pure culture, since it allows higher ATP
gain, i.e. 2 ATP are formed from glycerol to acetate
instead of 1 from glycerol to lactate and/or ethanol
(Fig. 6). Since syntrophic glycerol fermentation by the
two Thermoanaerobacter strains does not involve a
pyruvate formate lyase, the ability of the methanogenic
partner to consume formate is not needed for this inter-
species relationship. Only a slight delay of approximately

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2 days was observed in the incubations of *T. wiegellii* and the *Methanothermobacter* sp. strain GH (Fig. 5).

The importance of an external electron acceptor for improving glycerol conversion has been previously reported, for example in cultures of *Actinobacillus succinogenes* grown in the presence of dimethylsulfoxide (DMSO) as external electron acceptor (Carvalho et al., 2014; Schindler et al., 2014). Moreover, glycerol fermentation by *Escherichia coli* was shown to be impaired by hydrogen accumulation (Dharmadi et al., 2006; Gonzalez et al., 2008), which could be overcome by co-cultivation with the methanogen *Methanobacterium formicicum* (Richter and Gescher, 2014; Kim et al., 2017). Likewise, glycerol fermentation by *E. coli* and *Propionibacterium freudenreichii* can be supported through electron transfer to electrodes mediated by potassium ferricyanide (Emde et al., 1989; Emde and Schink, 1990). For *Thermoanaerobacter brockii* subsp. *brockii*, the addition of thiosulfate or *Methanobacterium* sp. as electron acceptors improved the oxidative deamination of aminoacids (Fardeau et al., 1997). The consumption of glucose and pyruvate by *Thermoanaerobacter brockii* was enhanced as well by using acetone as electron acceptor (Ben-Bassat et al., 1981). Also, Vipotnik et al. (2016) showed inhibition of glucose consumption by *Thermoanaerobacter* strain AK68 when exposed to high hydrogen partial pressure and that the addition of thiosulfate or co-cultivation with *Methanothermobacter* strain M39 (as electron scavenger) increased the utilization of glucose and acetate production (Vipotnik et al., 2016). In summary, we show that the presence of a hydrogenotrophic methanogenic partner, acting as biological electron acceptor, enhances glycerol conversion by *Thermoanaerobacter* species, since it facilitates the redox balance and contributes to a higher energy gain by these bacteria. Therefore, syntrophic glycerol fermentation promotes faster anaerobic treatment of glycerol-rich waste streams coupled to methane production.

**Experimental procedures**

**Biomass source**

Thermophilic anaerobic sludge was collected from a lab-scale up-flow anaerobic column reactor operated at 55°C, fed with a mixture of skim milk and sodium oleate (50:50% of the chemical oxygen demand, COD) at a COD concentration of 10 g l⁻¹ and hydraulic retention time of 1 day. Additional details of the reactor operation are provided in Supporting Information. Degradation of the substrate accumulated during the reactor operation was promoted by incubation in batch at 55°C for 18 days, before starting the enrichments.

**Medium composition and cultivation**

All the experiments were performed using a bicarbonate-buffered mineral salt medium (basal medium, BM) prepared as described by Stams et al. (1993). BM was...
dispensed in serum bottles which were sealed with butyl rubber septa and aluminum crimp caps. The headspace of the bottles was flushed with a gas mixture of N₂ and CO₂ (80:20% v/v), at a final pressure of 1.7 × 10⁵ Pa. Before incubation, the medium was reduced with 0.8 mM sodium sulfide and supplemented with salts and vitamins. All inoculations and transfers were done aseptically using sterile syringes and needles.

**Enrichment of glycerol-degrading microbial cultures**

Enrichments (coded Gly(x), where x represents the number of transfers) were started by inoculating 120 ml serum bottles, containing 50 ml BM, with 10% (v/v) of the sludge. Glycerol was added from a sterile stock solution to a final concentration of 10 mM, based on the works of Fardeau et al. (2000) and Alves et al. (2016). Successive transfers of the cultures to new medium (10% v/v) and serial dilutions were made after confirming microbial growth and activity, based on microscopic observations and methane measurements (more than 30% of the theoretical value expected). All cultures were incubated at 55°C, statically and in the dark. Schematic representation of the experimental procedure applied is shown in Fig. S1.

Physiological characterization was performed after nine successive transfers (enrichment Gly(9)), in triplicate 500 ml bottles containing 250 ml BM (Fig. S1) and glycerol (10 mM). Methane content in the headspace, volatile fatty acids (VFA), lactate, glycerol, ethanol, butanol, 1,3-PDO and 1,2-PDO were measured daily. The final hydrogen content of the headspace was also measured. In addition, duplicate samples were collected at the end of the incubation period for DNA extraction and measurements (more than 30% of the theoretical value expected). All cultures were incubated at 55°C, statically and in the dark. Schematic representation of the experimental procedure applied is shown in Fig. S1.

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Analytical methods

Phase contrast microscopy was performed using an Olympus CX41 RF microscope, and micrographs were obtained with an Olympus Altra 20 image acquisition system. The software used with this setup was the AnalySIS getiT (Olympus soft imaging solutions GmbH).
Methane and hydrogen were quantified by gas chromatography. For methane quantification, a GC-2014 Shimadzu gas chromatograph was used with a Porapak Q column and a flame ionization detector. N$_2$ was used as carrier gas. Injection port, column and detector temperatures were 110, 35 and 220°C respectively. Hydrogen was analysed using a Molsieve column (MS-13x 80/100 mesh) and a thermal conductivity detector Bruker Scion 456 Chromatograph (Bruker, Billerica, MA, USA) with argon (60 ml min$^{-1}$) as the carrier gas. The injector, column and detector temperatures were 100, 35 and 130°C respectively. Volatile fatty acids (VFA), lactate, glycerol and other alcohols were analysed by high-performance liquid chromatography (HPLC; Jasco, Tokyo, Japan). For organic acids quantification, an Agilent Hi-Plex H (300 × 7.7 mm) column was used, with a mobile phase of 2.5 mM H$_2$SO$_4$ at a flow rate of 0.6 ml min$^{-1}$. The column temperature was set at 60°C and spectrophotometric ultraviolet (UV) detection was performed at 210 nm. Glycerol and other alcohols were analysed using a Varian Aminex 87H (300 × 7.8 mm) with a mobile phase of 5 mM H$_2$SO$_4$ at a flow rate of 0.7 ml min$^{-1}$, with the column temperature set at 60°C and refractive index (RI) detection.

**Microbial composition of the glycerol-degrading enrichment cultures**

Aliquots of well-homogenized sludge were collected from cultures Gly(9) and Col-Gly, and immediately frozen at −20°C. Total genomic DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH) and purified by ethanol precipitation. DNA amplification, Illumina library preparation, amplicon sequencing (Illumina MiSeq, Inc. San Diego, CA, USA) and bioinformatics analysis of the data were performed at Research and Testing Laboratory (Lubbock, TX, USA). Samples were amplified for sequencing using the universal primer pair 515f and 806r (Caporaso et al., 2011), targeting the prokaryotic 16S rRNA gene. Details on the sequencing and bioinformatics data analysis can be found elsewhere (Paulo et al., 2017). All sequencing reads were submitted to the European Nucleotide Archive (ENA) under the study accession number PRJEB30535 (http://www.ebi.ac.uk/ena/data/view/PRJEB30535). A comparison of 16S rRNA gene sequences of OTU to the NCBI database was also performed using the BLASTN alignment tool (http://ncbi.nlm.nih.gov/blast).

**Genome analysis of Thermoanaerobacter strains**

Analysis of *Thermoanaerobacter brockii* subsp. *finnii* (DSM 3389$^T$) and *Thermoanaerobacter wiegeli* (DSM 10319$^T$) genomes, was performed using the Integrated Microbial Genomes (IMG) (https://img.jgi.doe.gov/) and The National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) genomic platforms.

**Acknowledgements**

The authors thank Ruben Gonçalves for preparing the thermophilic biomass and Andreia Salvador for the support with the microbial communities’ analysis. This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2019 unit, Project SAICTPAC/0040/2015 (POCI-01-0145-FEDER-016403) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 – Programa Operacional Regional do Norte. The authors also acknowledge the financial support of FCT and European Social Fund through the grants attributed to C.P. Magalhães (SFRH/BD/132845/2017) and A.L. Arantes (PD/BD/128030/2016).

**Conflict of interest**

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Experimental procedure applied for the enrichment of thermophilic glycerol-degrading microbial cultures at 55°C.

Figure S2. Phase contrast micrograph of culture Col-Gly.

Figure S3. Methane (A) and organic acids (B) production by enriched culture Col-Gly at 55°C.

Figure S4. Glycerol consumption (A) and production of acetate (B) and H₂ (C) by T. brockii subsp. finnii type strain, when incubated with BrES (w/BrES) and without BrES (w/o BrES).

Table S1. Methane production from glycerol by the different generations (coded Gly(x), where x represents the number of transfers), during the enrichment process.

Table S2. Additional information about the enzymes involved in the metabolic pathway for glycerol conversion of Thermoanaerobacter brockii subsp. finnii (DSM 3389T) and Thermoanaerobacter wiegelii (DSM 10319T). The data were retrieved from NCBI genomic platform.