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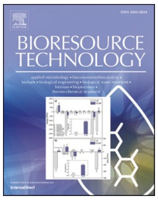

Short Communication

# Non-conductive silicon-containing materials improve methane production by pure cultures of methanogens

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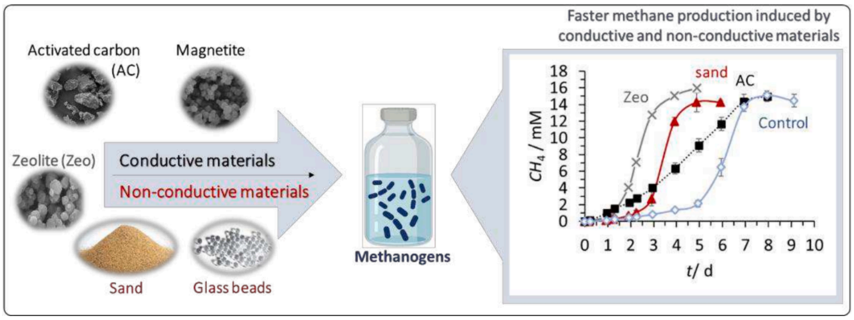
## HIGHLIGHTS

- Non-conductive materials like sand and glass improve methanogenesis.
- Zeolites and activated carbon accelerate methane production similarly.
- Silicon, present in the materials, positively affect methane production.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Conductive materials (CM) enhance methanogenesis, but there is no clear correlation between conductivity and faster methane production (MP) rates. We investigated if MP by pure cultures of methanogens (*Methanobacterium formicicum*, *Methanospirillum hungatei*, *Methanothrix harundinacea* and *Methanosarcina barkeri*) is affected by CM (activated carbon (AC), magnetite), and other sustainable alternatives (sand and glass beads, without

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Conductive materials
Non-conductive materials

conductivity, and zeolites (Zeo)). The significant impact of the materials was on *M. formicicum* as MP was significantly accelerated by non-CM (e.g., sand reduced the lag phase (LP) duration by 48 %), Zeo and AC (LP reduction in 71 % and 75 %, respectively). Conductivity was not correlated with LP reduction. Instead, silicon content in the materials was inversely correlated with the time required for complete MP, and silicon *per se* stimulated *M. formicicum*'s activity. These findings highlight the potential of using non-CM silicon-containing materials in anaerobic digesters to accelerate methanogenesis.

## 1. Introduction

The production of biomethane, a renewable energy source derived from waste and wastewater treatment through engineered anaerobic digestion (AD) process, is crucial for the emergent energy transition and independence from fossil fuels. Therefore, the research on methane production (MP), especially in emerging technologies, holds a key position in accelerating the transformation of biowaste to biomethane, thereby advancing the clean energy transition and waste valorization.

The use of conductive materials (CM) as a strategy to enhance the efficiency of methane production (MP) from various types of wastes in anaerobic engineered systems has been reported. The positive effects, such as the increase in methane production rates (MPR) and biogas yields, along with the changes in the composition of methanogenic communities and the interspecies interactions (including the shift to interspecies electron transfer (IET) mediated by CM), have been observed (Castilho et al., 2022; Martins et al., 2018; Rotaru et al., 2021).

Due to many factors, the application of CM and its role in AD to boost MP efficiency is a hot topic. From a microbiology point of view, a new mechanism of IET was discovered, in which CM serves as an electrically conductive bridge between bacteria and methanogens (e.g., *Geobacter metallireducens* with *Methanosarcina barkeri*), avoiding the need for the production of hydrogen or formate as electron shuttles (Rotaru et al., 2021). This discovery was very relevant, although whether it occurs in all methanogenic systems containing CM is unclear. Some CM were also found to improve methanogenesis by directly enhancing the MPR by pure cultures of different methanogens (see supplementary materials), which will be reflected in the efficiency of the entire AD process. From an applied point of view, the fact that, generally, CM accelerate MPR is highly relevant mainly because one disadvantage of AD is the lower rate of anaerobic conversions. Despite some authors report the improvements in MP kinetic parameters (e.g., reduction of lag phase duration and increase of MPR), the reasons behind the observations when CM are present remain unknown. To date, there are only a few studies on the effect of CM (e.g., carbon nanotubes (CNT), activated carbon (AC), magnetite (Mag)) directly on methanogens (see supplementary materials). Besides, there is no evidence that the material's conductivity is a requisite for accelerating methanogenesis. It is worth investigating if more eco-friendly materials, conductive or non-conductive, can improve the MP kinetic parameters of methanogens as well. We selected a set of materials to evaluate their potential to improve the methanogenic activity of pure cultures of hydrogenotrophic and acetoclastic methanogens commonly found in anaerobic bioreactors (i.e., *Methanobacterium formicicum*, *Methanospirillum hungatei, M. barkeri*, and *Methanothrix harundinacea*). The materials were AC and Mag, commonly used in AD studies to improve MP kinetic parameters, and materials never studied in pure cultures of methanogens, such as zeolites (Zeo), sand and glass beads.

Several studies are reporting the beneficial application of Zeo in AD (Tang et al., 2023), for example, as adsorbents of inhibitory compounds (Cardona et al., 2021; Szerement et al., 2021), and as support for microbial growth (Cardona et al., 2021; Montalvo et al., 2012), which ultimately contributed for improving MP efficiency. The electrical conductivity of Zeo (Yimlamai et al., 2011) is much lower than the electrical conductivity of CNT, AC or Mag, and the Zeo effect directly on the activity of methanogens was never assessed. Similarly, non-conductive sand was never investigated in pure cultures of methanogens. However, sand was reported to improve AD efficiency by contributing to biomass immobilization (Montalvo et al., 2005). Glass beads were only used in assays with defined cocultures as a non-conductive control material (Rotaru et al., 2018). Still, glass beads effect directly on MP by methanogens was not explored.

Zeo, sand and glass beads are low-cost materials with minimal environmental impact that could serve as alternatives to CM to improve MP efficiency. Methanogenesis is crucial for converting organic waste to methane. Without methanogens, this conversion process cannot occur, regardless of the activity levels of other microorganisms. If these sustainable materials can improve the activity of methanogens, they will enhance the overall efficiency of AD processes, which is of significant importance from an applied viewpoint. Despite the differences in the physicochemical properties of all materials, the presence of silicon (Si) in the composition of materials was a common characteristic observed. For this reason, the effect of silicic acid was tested and compared with the effect of these materials, considering their Si content.

## 2. Materials and methods

### 2.1. Preparation and characterization of materials

Mag (nanopowder, particle size 50 – 100 nm, Iron (II, III) oxide, $Fe_3O_4$), Zeo (Molecular sieve, type 13X − beads, 4–8 mesh) and glass beads (acid washed, 212–300 μm, 50–70 U.S. sieve), were purchased from Sigma-Aldrich (USA). Granular AC (Norit ROX 0.8, pellets with 0.8 mm of diameter and 5 mm of length) was supplied by Norit. Sand sample was collected from Apúlia beach (Esposende, Portugal), washed multiple times with distilled water, and heated at 550 °C, for 2 h, to remove organic contaminants. In the experiments, AC and Zeo were crushed, sieved (particle size $\leq$ 280 μm) and used as a powder (increasing the external surface area). Glass beads and sand were also sieved to limit the range of particle size in the assays to a particle size $\leq$ 280 μm.

Materials were characterized regarding the following surface and textural properties: Brunauer-Emmett-Teller (BET) specific surface area ($S_{BET}$), mesopore surface area ($S_{meso}$), micropore volume ($V_{micro}$), total pore volume ($Vp_{\ P/P0=0.95}$), and $pH_{pzc}$, as described elsewhere (Pereira et al., 2010; Silva et al., 2021). The chemical elemental composition of AC and Mag was attained by scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS). Electrochemical impedance spectroscopy (EIS) was used to measure the electrical resistance of Mag and Zeo samples to characterize their electrical conductivity, as described elsewhere (Figueira et al., 2014), using a GAMRY analyzer (GAMRY Instruments, Reference 600+, Potentiostat/Galvanostat/ZRA, 39008). Mag and Zeo powder samples were pressed into circular pellets using a lab manual press machine. Pellet thickness was measured using a Digimatic Micrometer IP54 (Mitutoyo) (0.994 mm for Mag and 0.388 mm for Zeo). EIS measurements were performed in triplicate. The Gamry Echem Analyst software was used to access the data regarding the frequency response displayed in a Nyquist plot. The same software was used for data fitting purposes, which allowed the Mag sample's resistance value to be obtained. The resistance value for Zeo was obtained using the tools of Origin software due to inadequate model fitting in the Gamry Echem Analyst software. Conductivity values were calculated as described elsewhere (Figueira et al., 2014). Conductivity analysis for sand and glass beads was not feasible, as preparing the pellets with those materials was impossible. Due to the physical characteristics of powder AC, it was also impossible to obtain a firm pellet and perform the impedance analysis. The physicochemical characterization of the materials is shown in Table 1.

### 2.2. Incubation conditions

Two hydrogenotrophic methanogens (*M. formicicum* (DSM $1535^T$), *M. hungatei* (DSMZ $864^T$)), and two acetoclastic methanogens (*M. harundinacea* (DSM $17206^T$) and *M. barkeri* (DSM $800^T$)) were purchased from DSMZ (Braunschweig, Germany) and maintained in our laboratory. In the first set of experiments, *M. formicicum* was incubated with 0.5 $g\cdot L^{-1}$ of Zeo, glass beads and sand. An additional experiment was performed with this methanogen to evaluate the direct effect of silicic acid (99.9 %, 20 μm, purified by refining, Sigma-Aldrich) on methanogenic activity. Silicic acid (in aqueous solution sterilized by autoclaving) was tested at a final concentration of 1 $mg\cdot L^{-1}$. The effect of silicon *per se* was investigated as it is a common element in the composition of all tested materials. The amount of silica in the tested materials ranged from 1.1 $mg\cdot L^{-1}$ in Mag to 325 $mg\cdot L^{-1}$ in sand. However, 1 $mg\cdot L^{-1}$ of silicic acid was the lowest soluble concentration in the aqueous media used in the assays; higher amounts cause precipitation of silicic acid/*ortho*-silicic acid (water-soluble forms) as hydrated silica, which are stable only in highly diluted aqueous solutions. Another set of experiments was performed to investigate variations in MP among the different species of methanogens when incubated with Zeo and with CM, which were previously recognized to accelerate methanogenesis, i.e., AC and Mag. A material concentration of 0.5 $g\bullet L^{-1}$ was chosen based on preliminary assays in which the best results were obtained with 0.5 $g\bullet L^{-1}$ of AC when testing concentrations of 0.1, 0.5, 1, 2 and 5 $g\bullet L^{-1}$ (data not shown). Similar results with CNT were reported by (Salvador et al., 2017).

The incubation of methanogens and abiotic assays were carried out as described by (Salvador et al., 2017). Acetate (at 20 $mmol\cdot L^{-1}$ and 10 $mmol\cdot L^{-1}$) was the substrate for MP in *M. barkeri* and *M. harundinacea* incubations, respectively, and a mixture of hydrogen and carbon dioxide ($H_2/CO_2$, 80:20 %; 170 kPa) was the substrate for *M. formicicum* and *M. hungatei*. Control assays without materials were conducted. All assays were performed in triplicate. Abiotic assays were carried out in duplicate to detect eventual adsorption/retention of the gaseous substrate ($H_2/CO_2$, 80:20 %; 170 kPa) or liquid substrate (acetate, 10 $mmol\cdot L^{-1}$) on the materials.

**Table 1**
Physicochemical characterization of materials regarding specific surface area ($S_{BET}$), mesopore surface area ($S_{meso}$), micropore volume ($V_{micro}$), total pore volume ($Vp_{P/Po=0.95}$), pH at zero-point charge ($pH_{pzc}$), electrical conductivity and elemental analysis (values measured in weight, %).

| Characterization | AC | Mag | Zeo | Sand | Glass beads |
|---|---|---|---|---|---|
| $S_{BET}$ (±10)/($m^2\bullet g^{-1}$) | 1002 | 8 | 488 | – | – |
| $S_{meso}$ (±5)/($m^2\bullet g^{-1}$) | 165 | – | 43 | – | – |
| $V_{micro}$ (±0.005)/ ($cm^3\bullet g^{-1}$) | 0.347 | – | 0.184 | – | – |
| $Vp_{P/P0=0.95}$ (±0.005)/ ($cm^3\bullet g^{-1}$) | 0.525 | 0.015 | 0.247 | – | – |
| $pH_{pzc}$ (±0.2) | 7.3 | 4.6 | 11.6 | 9.5 | 8.8 |
| Conductivity/($S\bullet cm^{-1}$) | * | $(2.13 \pm 0.69) \times 10^{-5}$ | $(0.33 \pm 0.07) \times 10^{-5}$ | – | – |
| **Characterization by SEM-EDS** | **AC** | **Mag** | – | – | – |
| C/(% wt) | 88.80 | 4.50 | – | – | – |
| O/(% wt) | 8.29 | 20.70 | – | – | – |
| Na/(% wt) | 0.00 | 0.00 | – | – | – |
| Al/(% wt) | 0.45 | 0.00 | – | – | – |
| Si/(% wt) | 1.05 | 0.22 | – | – | – |
| P/(% wt) | 0.00 | 0.84 | – | – | – |
| S/(% wt) | 1.44 | 0.00 | – | – | – |
| Cl/(% wt) | 0.00 | 0.00 | – | – | – |
| Fe/(% wt) | 0.00 | 73.70 | – | – | – |

[a] (–) values not determined.

[b] (*) Values found in literature for conductivity of AC: the range varied between $(1.68 \times 10^{-4})$ $S\bullet cm^{-1}$ and $(8.6 \times 10^{-3})$ $S\bullet cm^{-1}$ (Lee et al., 2020; Martins et al., 2018; Song et al., 2019).

Acetate (in acetoclastic incubations), hydrogen consumption (in hydrogenotrophic incubations) and MP were monitored over time. Redox potential (ORP), pH and conductivity of the anaerobic growth media were measured at the beginning, middle of the exponential phase of MP, and end of the incubations, as previously described by (Salvador et al., 2017).

### 2.3. Analytical methods

Hydrogen and methane were quantified by gas chromatography using the equipment BRUKER SCION 456 (Billerica, MA) under the conditions described elsewhere (Salvador et al., 2017). Acetate was monitored by high-performance liquid chromatography (Jasco, Tokyo, Japan) (Salvador et al., 2017). ORP and pH were measured using multiparameter analysers (Salvador et al., 2017), and the conductivity of the growth medium was measured using a Consort multi-parameter analyser C3010.

### 2.4. Mathematical and statistical analysis

The modified Gompertz model was used to obtain the values of kinetic parameters (lag phase duration and exponential MPR (EMPR)) as described elsewhere (Sousa et al., 2013). Standard errors were calculated for each kinetic parameter, and $R^2$ fitted experimental data to the modified Gompertz model. The initial methane production rate (IMPR), i.e., MPR at incubation start-up, was also calculated considering the period of lag phase duration of the corresponding assay without materials. For example, if the lag phase of the assay without materials lasted 3 days, the IMPR in the assays with materials was calculated for the first 3 days of incubation. The aim was to compare each material's effect in accelerating the process's onset.

Using Excel tools, statistical significance for assessing differences in kinetic parameters between conditions was determined through single factor analysis of variances (ANOVA). The statistical significance was set at the $p < 0.05$ level. In addition, SPSS software (IBM SPSS statistics 26) was used for correlation analysis, combining the kinetic parameters and the physicochemical properties of the materials.

## 3. Results and discussion

The methanogenic activity of *M. formicicum* was significantly accelerated when it was incubated with non-CM (glass beads and sand) and also with Zeo (Fig. 1a), which presents a relatively low conductivity $(((0.33 \pm 0.07) \times 10^{-5})$ $S\cdot cm^{-1}$, Table 1). Lag phase duration was reduced from 5 d (without material) to 3.5 d with glass beads, 2.6 d with

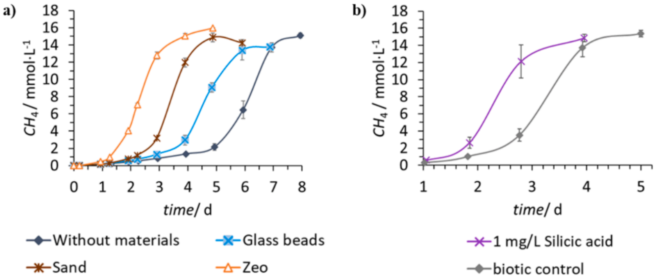


**Fig. 1.** Cumulative MP by pure cultures of *M. formicicum* incubated a) with and without sand, glass beads and Zeo, and b) with and without silicic acid. The results are the average and standard deviation of triplicate assays.

sand, and 1.5 d with Zeo, resulting in a faster IMPR (Table 2). EMPR increased up to 1.5 times, and the time for the complete conversion of $H_2/CO_2$ to methane was reduced up to 3 days (Table 2).

A common feature of Zeo, sand and glass beads is the presence of high amounts of silicon dioxide ($SiO_2$), also known as silica (Busca, 2014; Montalvo et al., 2005). We found a statistically strong correlation ($-0.883$, $p = 0.020$) between the reduction of the total time required to achieve a complete MP by *M. formicicum* and silicon content in materials' composition, suggesting a role of silicon on MP enhancement (see supplementary materials). From the experiments performed with other methanogens incubated with Zeo, AC and Mag (Fig. 2), a statistically significant moderate correlation ($-0.568$, $p = 0.043$) was also found between the material's silicon content (Table 1) and the reduction of the total time for complete MP (see supplementary materials), reinforcing the importance of Si. These results corroborate the investigation of the effect of Si *per se*. Indeed, there was a decrease of 32 % in the lag phase duration when *M. formicicum* was incubated in growth medium supplemented with silicic acid (Fig. 1b) (results statistically different, $p < 0.05$, corresponding to a decrease from (2.50 ± 0.02) d without to (1.68 ± 0.05) d in the presence of Si). Silicon is not reported as essential for the activity of methanogens, but it was found to accelerate organic matter decomposition in peatlands and consequently increase methane concentrations (Reithmaier et al., 2017). Besides, some types of Zeo were reported as having an *ortho*-silicic acid ($H_4SiO_4$)-releasing property, which results in the release of a bioavailable form of silicon for microorganisms (Jurkić et al., 2013). Zeo and other silicon-containing materials can probably release Si to the growth medium, which might stimulate methanogenesis. AC and Mag, previously shown to accelerate MP (Castilho et al., 2022; Liu et al., 2023), also contain small amounts of silicon in their composition (Table 1). The results obtained in this study indicate that silicon *per se* may partially explain MP improvements by stimulating *M. formicicum* metabolism. However, the exact biological roles of silicon towards methanogenic activity are still unknown. There are reports of biosilicification in a thermophilic methanogen, *Methanocaldococcus jannaschii*, which is the only methanogen with the ability to convert Si to $SiO_2$, although the benefits of biosilicification to the methanogen are not known yet (Ikeda, 2021; Orange et al., 2009). We have no experimental data to evaluate if biosilicification was occurring

**Table 2**

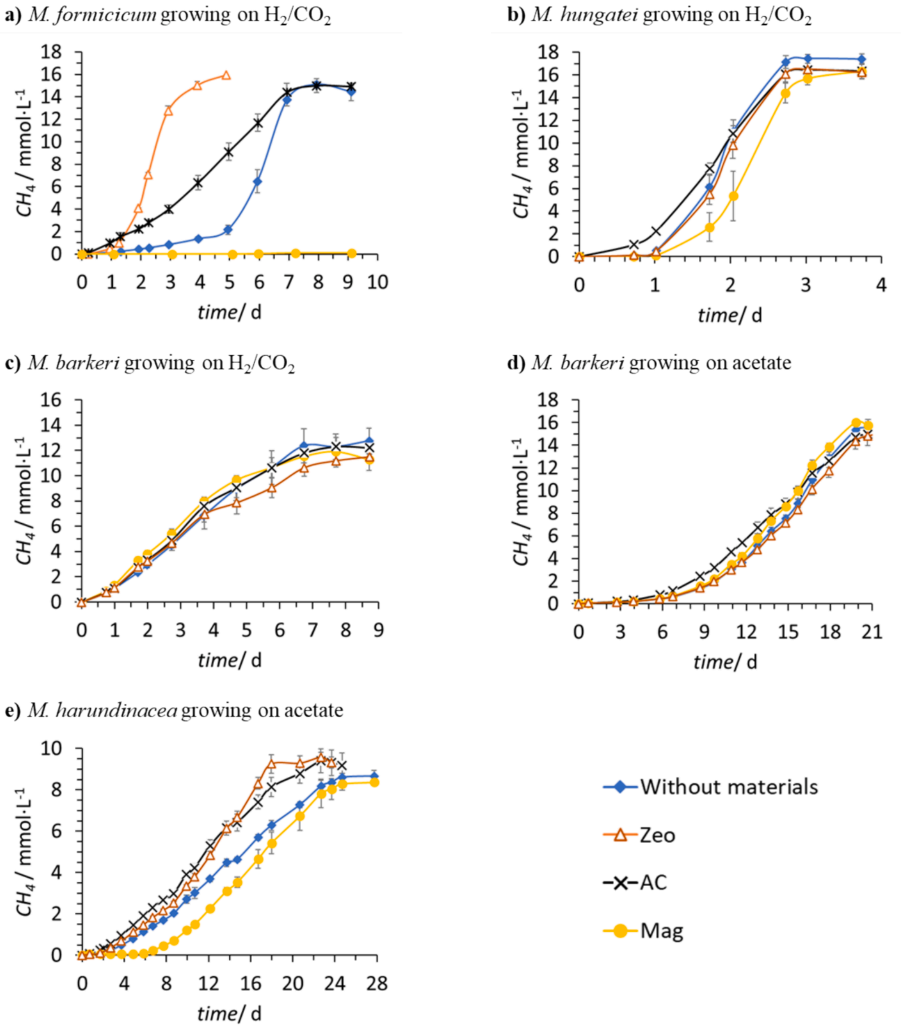
Values of the initial concentration of substrates ($C_i$), duration of lag phase, IMPR, EMPR, and time for complete MP (t), for the incubations with different species and materials. n × represents the number of times that IMPR and EMPR increases, or decreases (>1 or < 1, respectively), relatively to the assay without materials.

| Cultures | Materials | $C_i$/ mmol·L$^{-1}$ | Lag phase[a]/ d | IMPR[b]/ mmol·L$^{-1}$·d$^{-1}$ | $R^2$ | n× | EMPR[a]/ mmol·L$^{-1}$·d$^{-1}$ | $R^2$ | n× | t/ d |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. formicicum*/($H_2/CO_2$) | None | 64.1 ± 0.3 | 5.10 ± 0.44 | 0.30 ± 0.04 | 0.973 ± 0.006 | 1.0 | 7.64 ± 0.93 | 0.981 ± 0.004 | 1.0 | 8 |
| | Sand | 66.0 ± 0.7 | 2.63 ± 0.00 | 1.08 ± 0.06 | 0.78 ± 0.02 | 3.6 | 11.10 ± 0.63 | 0.99 ± 0.00 | 1.5 | 5 |
| | Glass beads | 66.3 ± 0.3 | 3.51 ± 0.13 | 0.47 ± 0.02 | 0.93 ± 0.01 | 1.5 | 6.93 ± 0.22 | 0.98 ± 0.01 | 0.9 | 6 |
| | Zeo | 64.6 ± 1.1 | 1.47 ± 0.05 | 4.74 ± 0.14 | 0.864 ± 0.005 | 15.6 | 9.62 ± 0.82 | 0.997 ± 0.001 | 1.3 | 5 |
| | AC | 64.9 ± 1.6 | 1.29 ± 0.16 | 1.43 ± 0.13 | 0.988 ± 0.001 | 4.7 | 2.98 ± 0.60 | 0.995 ± 0.003 | 0.4 | 8 |
| | Mag | 62.1 ± 0.6 | 13.14 ± 0.43 | – | – | – | 1.50 ± 0.24 | 0.993 ± 0.005 | 0.2 | 24 |
| *M. hungatei*/($H_2/CO_2$) | None | 60.5 ± 1.4 | 1.40 ± 0.04 | 0.45 ± 0.13 | 0.701 ± 0.112 | 1.0 | 18.26 ± 1.60 | 0.998 ± 0.001 | 1.0 | 4 |
| | Zeo | 59.8 ± 3.3 | 1.42 ± 0.03 | 0.40 ± 0.12 | 0.791 ± 0.043 | 0.9 | 17.25 ± 1.38 | 0.997 ± 0.000 | 0.9 | 4 |
| | AC | 61.4 ± 1.2 | 0.89 ± 0.03 | 2.06 ± 0.16 | 0.929 ± 0.006 | 4.5 | 10.06 ± 0.49 | 0.992 ± 0.002 | 0.6 | 4 |
| | Mag | 61.3 ± 1.7 | 1.66 ± 0.16 | 0.09 ± 0.08 | 0.521 ± 0.000 | 0.2 | 15.71 ± 0.46 | 0.997 ± 0.001 | 0.9 | 4 |
| *M. barkeri*/($H_2/CO_2$) | None | 57.8 ± 0.4 | 0.88 ± 0.07 | – | – | 1.0 | 2.49 ± 0.31 | 0.998 ± 0.001 | 1.0 | 9 |
| | Zeo | 52.7 ± 0.4 | 0.57 ± 0.07 | – | – | – | 2.14 ± 0.40 | 0.994 ± 0.000 | 0.9 | 9 |
| | AC | 57.3 ± 0.5 | 0.76 ± 0.07 | – | – | – | 2.56 ± 0.28 | 0.998 ± 0.001 | 1.0 | 8 |
| | Mag | 55.4 ± 1.9 | 0.63 ± 0.11 | – | – | – | 2.76 ± 0.23 | 0.995 ± 0.005 | 1.1 | 8 |
| *M. barkeri*/(acetate) | None | 17.2 ± 0.3 | 9.25 ± 0.25 | 0.24 ± 0.01 | 0.912 ± 0.005 | 1.0 | 1.44 ± 0.07 | 0.996 ± 0.000 | 1.0 | 22 |
| | Zeo | 17.4 ± 0.5 | 9.09 ± 0.20 | 0.25 ± 0.01 | 0.920 ± 0.001 | 1.0 | 1.31 ± 0.08 | 0.997 ± 0.000 | 0.9 | 22 |
| | AC | 16.5 ± 0.8 | 7.22 ± 0.22 | 0.43 ± 0.04 | 0.933 ± 0.003 | 1.8 | 1.20 ± 0.05 | 0.998 ± 0.000 | 0.8 | 21 |
| | Mag | 15.8 ± 0.8 | 8.87 ± 0.20 | 0.28 ± 0.03 | 0.909 ± 0.007 | 1.2 | 1.54 ± 0.05 | 0.994 ± 0.001 | 1.1 | 21 |
| *M. harundinacea*/ (acetate) | None | 8.6 ± 0.2 | 5.09 ± 0.79 | 0.26 ± 0.01 | 0.987 ± 0.002 | 1.0 | 0.48 ± 0.01 | 0.994 ± 0.007 | 1.0 | 25 |
| | Zeo | 8.2 ± 0.6 | 4.83 ± 0.35 | 0.33 ± 0.01 | 0.995 ± 0.000 | 1.3 | 0.75 ± 0.09 | 0.987 ± 0.006 | 1.5 | 18 |
| | AC | 8.5 ± 0.1 | 3.16 ± 0.06 | 0.41 ± 0.01 | 0.996 ± 0.001 | 1.6 | 0.59 ± 0.03 | 0.998 ± 0.000 | 1.2 | 23 |
| | Mag | 8.3 ± 0.2 | 8.58 ± 0.46 | 0.00 ± 0.01 | 0.259 ± 0.266 | 0.0 | 0.60 ± 0.10 | 0.998 ± 0.002 | 1.2 | 25 |

[a] kinetic parameters obtained by modified Gompertz model.

[b] IMPR was calculated for the early MP during the following periods of time: between days 1 and 3 in *M. formicicum* experiments; between days 0 and 1 in *M. hungatei* assays; between days 4 and 9 in *M. barkeri* growing in acetate; and between days 2 and 6 for *M. harundinacea* experiments.

[c] (–) not determined because there was no MP (in *M. formicicum* pure cultures assays with Mag) or there was no long lag phase duration with or without materials (in *M. barkeri* growing in $H_2/CO_2$ assays) to consider the existence of an IMPR.


**a)** *M. formicicum* growing on $H_2/CO_2$

**b)** *M. hungatei* growing on $H_2/CO_2$

**c)** *M. barkeri* growing on $H_2/CO_2$

**d)** *M. barkeri* growing on acetate

**e)** *M. harundinacea* growing on acetate

**Fig. 2.** Cumulative MP profiles over incubation time for the following assays with and without materials: a) *M. formicicum*, b) *M. hungatei*, and c) *M. barkeri*, growing on $H_2/CO_2$; d) *M. barkeri*, and e) *M. harundinacea*, growing on acetate. In each assay, the effect of Zeo, AC, and Mag was investigated. The control condition without material is represented as "without materials". The results are the average and standard deviation of triplicate assays.

in our methanogenic cultures, but it is something to investigate in the future.

This is the first report of the improvement of MP by Si. To date, the improvements in MP were mainly associated with the materials' conductivity (Castilho et al., 2022; Martins et al., 2018). However, if the conductivity was a main factor explaining the improvement of methanogenic activity, one would expect methanogens to produce methane much faster when incubated with AC or Mag than with Zeo. The conductivity of Mag is at least 6 times higher than the conductivity of Zeo, and AC shows the highest conductivity (Table 1). On the contrary, for *M. formicicum* and *M. harundinacea*, MP was faster with Zeo than with Mag (Fig. 2a and 2e, respectively). And despite the high difference in conductivity between Zeo and AC, their effect on MP by the majority of the cultures was quite similar, except *M. formicicum* (Fig. 2a), which performed better in the presence of Zeo than with materials presenting higher conductivity (Fig. 2; Table 2). A similar effect was verified with *M. harundinacea* cultures (Fig. 2e), although to a lesser extent (Fig. 2e). On the other hand, Mag was even inhibitory for cultures of *M. formicicum* (Fig. 2a) and *M. harundinacea* (Fig. 2e), as the lag phase preceding MP was much longer in the presence of Mag (Fig. 2, Table 2). Inhibition might be related to Mag's chemical composition, as Fe(III) was previously found to inhibit hydrogenotrophic methanogenesis (Van Bodegom et al., 2004).

The observation that materials with a lower conductivity than CM, i.e., Zeo, exert a comparable, or even superior effect, to that of more CM, such as AC, suggesting that conductivity is not the main parameter dictating the improved performance of methanogens, is a novel finding of this study. Indeed, there is no significant correlation between lag phase reduction and conductivity (see supplementary materials). However, a moderate correlation exists between conductivity and IMPR (0.465, $p = 0.039$), indicating that while conductivity does not contribute to lag phase reduction, it tends to increase the IMPR slightly. The specific and mesopore surface area also showed a moderate correlation with the IMPR ($S_{BET}$ (0.465, $p = 0.039$) and $S_{meso}$ (0.552, $p = 0.012$)). The increase in the surface area of materials might contribute to microbial cell attachment and retaining compounds, making the compounds more available for microorganisms. Nevertheless, abiotic experiments did not show hydrogen or acetate adsorption (see supplementary materials). The presence of AC and Zeo caused a decrease in ORP values, particularly AC (e.g., the presence of AC decreased the ORP from (−284 ± 31) mV to (−363 ± 5) mV in the assays conducted with *M. formicicum* cultures, while ORP reduction with Zeo was slightly lower, from (−284 ± 31) mV to (−324 ± 4) mV) (see supplementary materials). Methanogenesis benefits from low ORP (optimal ORP range, from −200 mV to −400 mV), but AC decreased ORP more than Zeo, and yet both materials exerted similar effects on MP for the majority of the methanogens (Fig. 2), showing that ORP reduction was not a determinant factor, as it was also previously reported

(Salvador et al., 2017).

Our results show that a unique physicochemical characteristic does not explain the observations. Instead, a synergistic effect can be partially explained by the presence of Si and, to a lesser extent, by the available surface area and conductivity of materials.

## 4. Conclusion

This study shows that various materials highly affect both hydrogenotrophic and acetoclastic methanogenesis regardless of their conductivity. Non-CM, like sand or glass beads, were effective in accelerating methanogenesis, and Zeo exerts superior or similar effects to those of AC. A set of physicochemical properties was found to contribute to MP efficiency improvement, with Si content being the most significant. Non-CM appears as a low-cost and environmentally friendly alternative for application in engineered anaerobic systems.

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## CRediT authorship contribution statement

**Cátia S.N. Braga:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gilberto Martins:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition. **O. Salomé G.P. Soares:** Writing – review & editing, Methodology, Investigation. **M. Fernando R. Pereira:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Inês A.C. Pereira:** Writing – review & editing, Supervision. **Luciana Pereira:** Writing – review & editing, Supervision, Investigation, Formal analysis. **M. Madalena Alves:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Andreia F. Salvador:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131144.

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