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## Introduction

Estonian graptolite-argillite contains significant quantities of U, Mo, V, Pb and Co, as well as Zn, Re, Ni and other elements. Metals are in argillite as sulfide minerals or in the composition of organometallic compounds. Extensive methane generation from organic matter is so far postulated to be feasible only with the archaeal *mcrA* gene needed in the final stage of methanogenesis (Fig.1). Only recently other metabolic pathways including those belonging to bacteria have been discovered<sup>1</sup>.

## Objectives

- To describe the argillite indigenous community ARGCON5 and culturable strains isolated thereof aiming at compiling new communities degrading argillite organic matter;
- Testing these communities with **native and sterilized argillite** to specify the distinct methane producing archaea / bacteria.

## Materials and methods

- Methanogenesis of argillite indigenous community ARGCON5 was monitored by pressure increase with OxiTop system (Fig. 2);
- Taxa from this community were identified by 16S rRNA gene 454 sequencing;
- The isolates were tested by 16S rRNA gene Sanger sequencing, the ability to degrade argillite organic component (by methanogenesis), outplating and by biochemical properties.



Figure 2. Pressure increase measurement with OxiTop system (WTW, Germany).

## Results

Heterotrophic facultative anaerobes and methanogenic archaea of argillite community ARGCON5 (CELMS No EEUT ARGCON5)<sup>3</sup> under anaerobic conditions are able to decompose the organic matter resulting in methane and CO<sub>2</sub> generation with releasing the metals (Fig. 3)

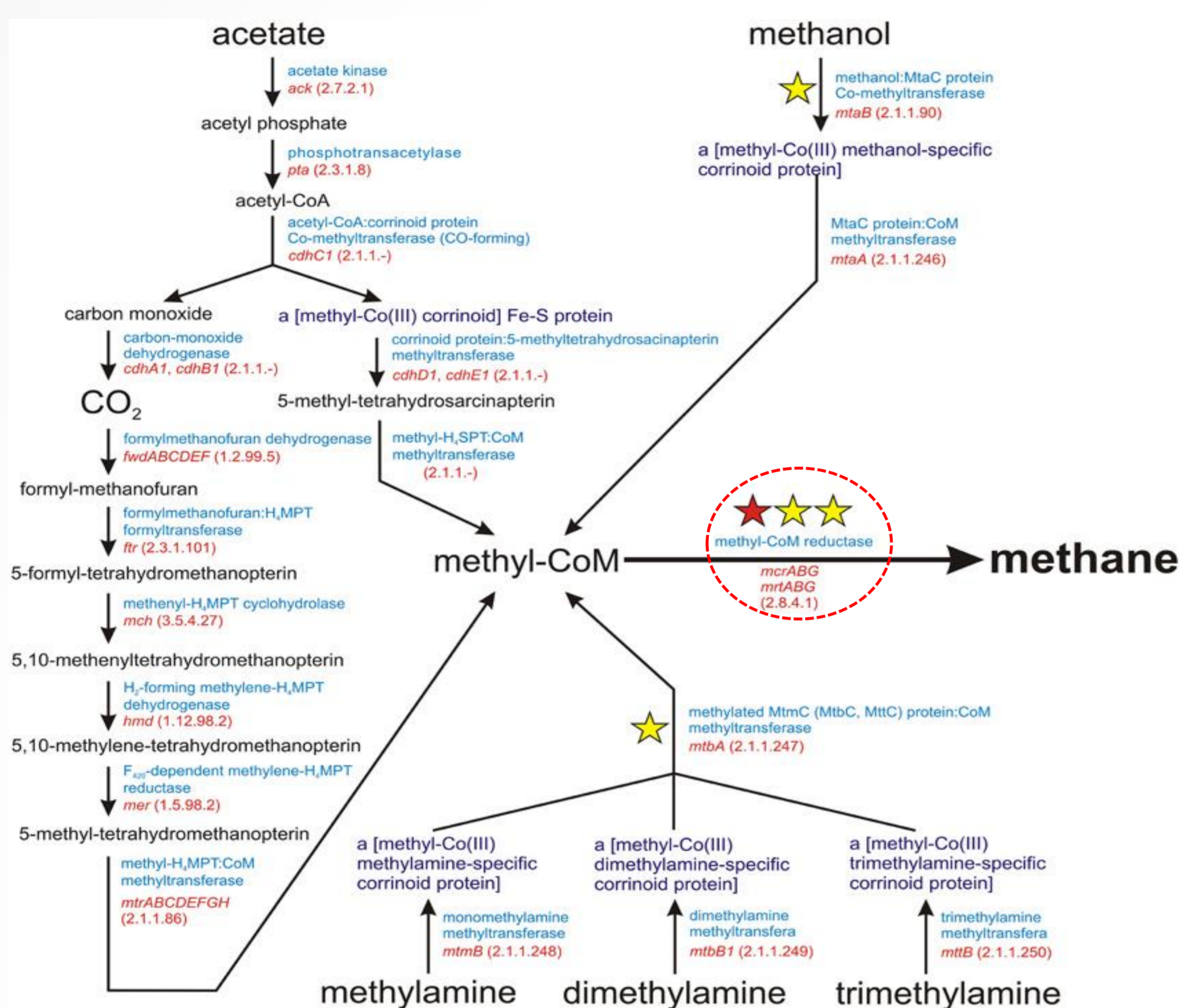


Figure 1. Schematic diagram of the pathway of methanogenesis. E.C. numbers for enzymes are shown in parentheses. The red star indicates the *mcrA* gene encoding subunit  $\alpha$  of a methyl-coenzyme M reductase I, which is commonly used as a molecular marker for the detection of methanogens. The yellow stars denote molecular markers developed in study<sup>2</sup>.

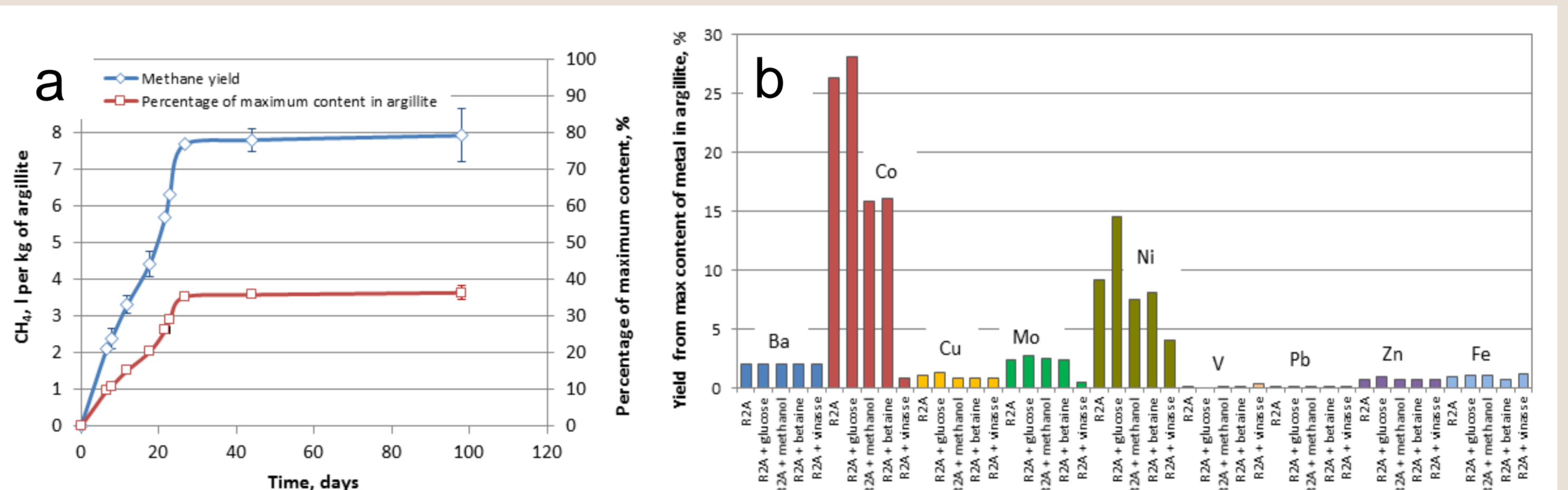


Figure 3. a. The dynamics of methane evolution from argillite with inherent adapted culture ARGCON5. b. Leaching of metals from argillite with various cultivation media, yield of metals from maximum content of metal in argillite (enrichment value) on experiment day 76.

The microbial community stimulating methanogenesis is dominated by bacterial class *Bacilli* with the presence of archaeal genus *Methanosarcina*. Microbial communities lacking methanogenesis are dominated by the class *Clostridia*, mainly the genus *Desulfotomaculum* known to be involved in sulfur metabolism (Fig. 4).

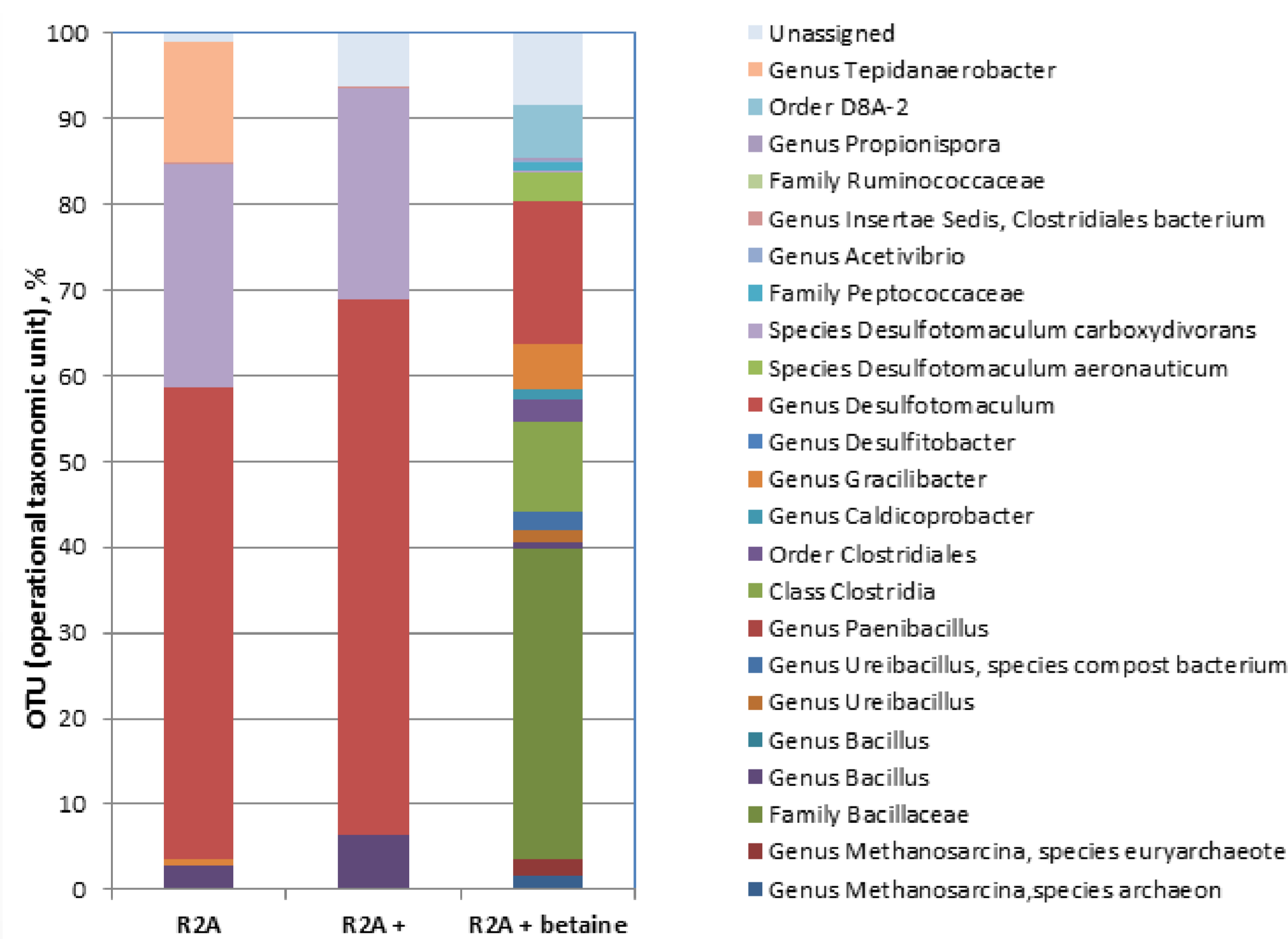


Figure 4. Taxa identified from the methane producing community grown in various culture media. The highest methane yields were obtained in R2A medium in the presence of betaine. Microbial community was characterized by 454-tag pyrosequencing of archaeal 16S ribosomal RNA gene V2 region using primers Arch349F-A934b. In line with methanogens also bacterial taxa were identified.

## Conclusions

- The balance between sulfate reducers and methanogens is inevitable to secure the processes towards methanogenesis.
- Facultative anaerobes isolated from indigenous consortium of argillite are able to biodegrade the argillite organic matter.
- Is methanogenesis in these experiments initiated by methanogenic archaea in a co-culture with bacteria or by a separate bacterial pathway remains to be solved in the BiotaTec's H2020 SME Instrument phase 2 project.

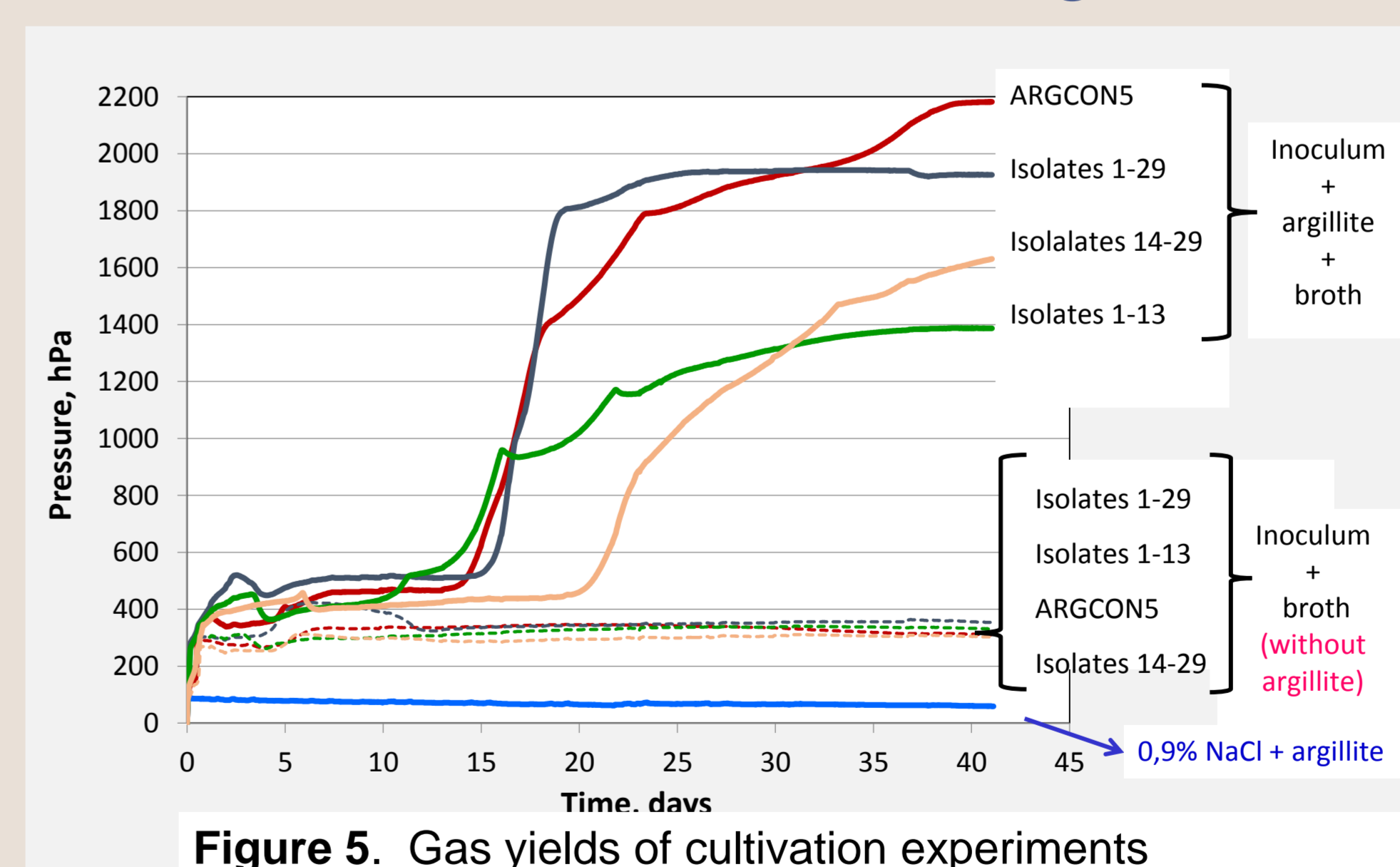


Figure 5. Gas yields of cultivation experiments

>50% of indigenous argillite strains from cultivation experiments had catalase and urease activities, necessary for degrading the complex organic matter. Among the culturable taxa identified were *Bacillus* sp, *Staphylococcus warneri*, *Pseudomonas stutzeri*, *Enterobacter cloacae*, *Microbacterium oxydans* and *Rothia* sp. The gas and methane yields were higher in cultivation experiments with the whole list of isolates than in cultivations with a smaller number of isolates (Fig. 5).

## Acknowledgements

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