Evaluating microbial robustness in continuous vs. feast-famine bioreactors via methane-oxidizing activity by q-PCR.

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ABSTRACT

Methanotrophs are responsible for methane (CH_4) abatement in nature and off-gas treatment bioreactors. Their activity is crucial in bioreactors treating this potent greenhouse gas at the low concentrations typically found in emissions from waste treatment facilities. The ability of methanotrophs to rapidly respond to intentional or accidental stress conditions derived from operational failures or process fluctuations is of utmost importance to guarantee a robust CH_4 abatement. In this work, the microbial robustness of three stirred tank reactors (STRs) treating CH_4 at low concentrations (4-5 % v/v) operated under feast-famine and continuous feeding mode was systematically evaluated through the analysis of the *pmoA* gene encoding the enzyme methane monooxygenase. In addition, the elimination capacities (EC) and CO_2 production rates of the microbial communities in the STRs were also assessed. Feast-famine operation is a well-known low-cost alternative to control biomass in bioreactors.

Three STRs were operated under continuous regime (R1) or under feast-famine conditions (two alternate units-R2 and R3- under 5d:5d cycles) for 177 days. CH_4 was continuously fed to R1 at an inlet load of 127.5 ± 6.4 g m^{-3} h^{-1} and to R2 and R3 (during feast periods) at 132.4 ± 6.7 g m^{-3} h^{-1} and 130.2 ± 6.1 g m^{-3} h^{-1}, respectively. A low CH_4-free air flow rate was supplied to R2 and R3 during famine to prevent anaerobic conditions. Diluted real centrate supplemented with SO_4^{2-} was used as a nutrient solution (150 mg L^{-1}). At day 178, a complete feast-famine cycle in R2 and R3 was exhaustively analysed by sampling biomass (for *pmoA* expression analysis by quantitative PCR) and the inlet and outlet gas samples (for CH_4 and CO_2 concentration by GC-TCD) periodically at 0,03, 0.25, 0.5, 1.0, 1.5, 2.0, 6.0, 12.0, 24.0, 72.0, 120.0 hours (h) after the application of feast or starvation conditions. A feast-famine cycle was also applied to R1 to compare the recovering capacity of biomass enriched under feast-famine conditions with that of biomass continuously exposed to CH_4.

R1 showed higher levels of *pmoA* transcripts (≈ 2 orders of magnitude) than R2 and R3 during feast and starvation periods (Fig. 1a). Results of the analyses of *pmoA* DNA (higher in R1) (data not shown) suggests that gene:transcript ratios are similar for the three units, indicating similar levels of physiological activity between methanotrophs. The 3 STRs were able to rapidly recover (within 2 h) their steady ECs after the 5-day famine period (EC_{R1} = 11.65 ± 4.7 g m^{-3} h^{-1}; EC_{R2} = 11.05 ± 4.2 g m^{-3} h^{-1} and EC_{R3} = 13.01 ± 4.4 g m^{-3} h^{-1} (Fig 1b). A maximum in *pmoA* gene expression was observed between 2 h and 6 h, which highlighted the rapid physiological adaptation – mediated by a rapid synthesis of *pmoA* mRNAs - to feast conditions. Then, the *pmoA* gene expression gradually decreased to basal levels (Fig. 1a). Surprisingly, an increase in the levels of *pmoA* transcripts was observed during the first 12 h of the famine period

following an initial descrease (graphs not shown), which agrees with previous observations (Tavormina et al. 2017). Indeed, the *pmoA* gene can be activated to assimilate nitrogen using carbon storage polymers as a source of reducing power (Pieja et al. 2011). Lower *pmoA* mRNA levels, which remained as basal levels in the 3 units, were observed during CH_4 starvation after t = 12 h compared to feast conditions level (Fig. 1a). This finding has been previously observed in ammonia-oxidizers, which suggests that these bacterial groups are able to maintain basal mRNA levels that allow a quick start-up of NH_4 or CH_4 oxidation (Bollman et al. 2005).

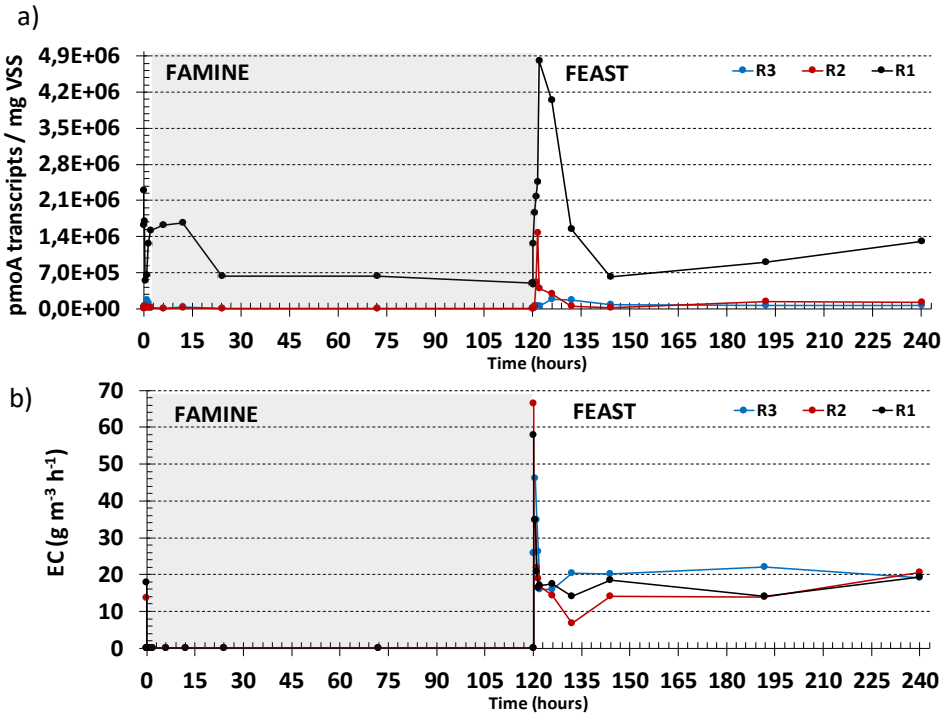

Figure 1. *pmoA* gene transcripts (per mg VSS) (a) and EC (g m^{-3} h^{-1}) (b) in R1, R2, R3.

CONCLUSIONS

This work revealed the extremely fast recovery of CH_4 biodegradation activity of methanotrophs after a 5-days starvation period regardless of the previous history of the microbial community. The fact that methane-oxidizing activity was not damaged under long starvation periods suggested the high robustness of biofiltration for the treatment of diluted CH_4 emissions.

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