

# Suppression of methanogenic activity in anaerobic granular biomass for hydrogen production

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## Abstract

**BACKGROUND:** Several pretreatment methods at lab-scale have been developed to inactivate methane-producing and hydrogen-consuming microorganisms. The need to obtain a hydrogen-producing inoculum by a more practical method is still necessary. The objective of this study was to evaluate the adaptation of anaerobic granules to suppress the methanogenic activity for hydrogen production.

**RESULTS:** Preliminary tests in discontinuous operation indicated that methanogenic bacteria were hardly suppressed. The continuous adaptation of the granules, at pH of 4.5 and HRT of 5.5 h, produced hydrogen and successfully suppressed the methanogenic activity. An even distribution of  $\beta$ -polysaccharides and cells was observed when the adapted granules were stained with fluorescent molecular tags. The reactor productivity was  $71 \pm 6 \text{ mL-H}_2 \text{ L}_{\text{reactor}}^{-1} \text{ h}^{-1}$  with a yield of  $2.6 \pm 0.2 \text{ mol H}_2 \text{ mol}^{-1} \text{ glucose}$ .

**CONCLUSION:** Hydrogen was produced from methanogenic granules. The continuous strategy successfully suppressed the methanogenic activity of the seeded granules and the hydrogen production was stable. The high yield and even distribution of cells suggests that the continuous adaptation generated a more active biomass due to an improved mass transfer present in the process.

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**Keywords:** biohydrogen; methanogenic activity; anaerobic granules; continuous operation; selection pressure; fluorescent molecular tags

## INTRODUCTION

Hydrogen production by dark fermentation is affected by many parameters, including pH,<sup>1–3</sup> temperature,<sup>4,5</sup> substrate,<sup>6,7</sup> inoculum applied,<sup>8,9</sup> packing material<sup>10,11</sup> and hydraulic retention time (HRT).<sup>12–14</sup> The enrichment of hydrogen-producing bacteria is a critical step in the start-up process. A major disadvantage in using mixed bacterial cultures is the presence of hydrogen-consuming microorganisms and the subsequent methane production.<sup>15</sup>

The type of packing material of the reactor has also been shown to have an important role in (hydrogen-consuming) methanogens development. Activated carbon has already been reported to favor colonization by methane-producing bacteria,<sup>10</sup> contrary to what has been observed with ceramic cubes.<sup>11</sup> In particular, the porosity of the packing material adopted should play a role in such speciation, as it most likely affects layer formation. Nevertheless, even in the presence or absence of the packing material itself (immobilized biomass versus liquid suspension), the processes operated with the same physical parameters and wastewater have been shown to favor the development of hydrogen-consuming bacteria. It has been observed that by allowing the development of biofilm on a support, hydrogen-consuming activities can be more important than the suspended cultures.<sup>16</sup>

Several pretreatment methods have been developed to inactivate these hydrogen-consuming microorganisms. The most

common pretreatments use heat-shock,<sup>15</sup> chemical substances,<sup>17</sup> or strong acid/base application.<sup>18</sup> Some of them are based on the premise that hydrogen-producing bacteria (*Clostridium*) can sporulate under adverse environments and can be reactivated with adequate environmental conditions.<sup>19,20</sup> In the case of chemical treatments (2-bromoethanesulfonic acid salt and iodopropane), these inhibit the methyl-coenzyme M reductase, key component for methanogenesis.<sup>17,21</sup>

These methods can be used at lab-scale but may be limiting and impractical for large-scale use.<sup>22</sup> Also, the use of some of these pretreatments has affected the structure and the microbiology of the granular sludge seeded.<sup>23</sup> Therefore, the need to obtain a hydrogen-producing inoculum by a more practical method is necessary.

One option is to wash out the hydrogen-consuming microorganisms (e.g. methanogenic and propionic acid-producing

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