Analysis of Bacterial DNA from Bioreactor Effluent Samples for Hydrogen Production by Dark Fermentation

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Abstract

Hydrogen (H_2) production from biomass using bacteria is a cost-effective approach. The bacterial DNA from effluent samples were extracted, amplified and analysed, and taxonomic trees of all the species present were obtained. HRTs and synthetic matrix influenced bacterial growth. *Clostridium butyricum* was prominent in the samples indicating its versatile growth conditions.

Introduction

The biological production of H_2 is attracting more attention due to the utilisation of biomass from waste such as effluents. The efficient H_2 production depends on the bacteria and their ability to degrade the substrate [1]. Hydraulic retention time (HRT) is one of the critical factors affecting the long-term operation of H_2 productivity. Shorter HRT could enhance bioreactor performance but frequently leads to failure due to shortened microbial substrate contact time and biomass loss [2]. The study aims to optimize HRTs to enhance bacterial growth and its interaction with the substrate to increase the H_2 yields.

Aims and methods

The research aims to determine the bacteria prominent in effluent samples with high H_2-producing ability for dark fermentation using sugars as a substrate. Four powdered commercial inoculums (R1 to R4) were used in the bioreactors for dark fermentation. The bacteria present in the inoculums are listed in Table 1. A substrate of mixed sugars (lactose, fructose, and sucrose) was used in all four bioreactors with three HRT conditions of 10 h, 7 h and 7 h using plastic hollow balls as synthetic matrices to immobilise microbes. At 10 and 7 h of HRT, the bioreactors were operated with 30 min "on" periods while introducing the feed and then 30 min "off". When balls were, the samples were collected by opening the bioreactor, taking the balls and sonicating them in distilled water.

The bacterial extraction was carried out by filtering the effluent samples, collecting residues, and extracting genomic bacterial DNA using the NZYtech soil DNA kit (Portugal). The extracted DNA was amplified using a 16S barcoding KIT 1-24 (SQK-16S024, Oxford Nanopore Technologies) and sequenced using MinION technology (Oxford). The sequences were obtained by MinKNOW software (Oxford), demultiplexed and analysed in EPI2ME agent software (Oxford). The taxonomic tree of all the bacterial species was obtained which enabled the identification of H_2-producing bacteria.

Results

The results showed *Clostridium butyricum* in all the effluents under HRTs of 7 and 10 h. This bacterium has been extensively studied for its ability to produce H_2 [3] and, therefore could be a potential microbe in the research, followed by *Clostridium saccharoperbutylacetonicum* [4], *Clostridium saccharobutylicum* [5], *Clostridium chromiireducens* and *Raoultella ornithinolytica* species, not included in commercial inoculums data but present in all the samples and are known for producing H_2.

In the analyses with inoculum R1, at HRTs of 10 h, 7 h and 7 h with balls, *Huaxiibacter chinensis* was observed. *Clostridium beijerinckii, Leuconostoc mesenteroides, Klebsiella pasteurii, Janthinobacterium rivuli, Janthinobacterium lividum, Duganella levis* were observed at 10 h but when HRT was reduced these bacteria disappeared. Additionally, *Pseudomonas fluorescens, Pseudomonas putida, and Raoultella planticola* were observed at 10 and 7 h with balls indicating that to reduce HRT, they require synthetic matrices to grow.

In studies with inoculum R2, at 10 h, 7 h and 7 h with balls, *Raoultella planticola* has been observed in all the bioreactors. *Pseudomonas fluorescens, Pseudomonas veronii, Comamonas testosteroni, Pseudomonas fildesensis, Clostridium acidisoli,*

Clostridium beijerinckii were observed only at 10 h and disappeared when the HRT was reduced to 7 h. *Pseudomonas extremaustralis, Janthinobacterium rivuli* appeared at 10 h, and disappeared when the HRT was reduced to 7 h but reappeared with balls.

When analysing inoculum R3, *Pseudomonas fluorescens, Janthinobacterium rivuli, Clostridium chromiireducens, Clostridium puniceum, Lactococcus lactis* were observed in all three conditions showing no impact of HRT on their growth. Additionally, *Pseudomonas putida, Huaxiibacter chinensis, Klebsiella aerogenes, Raoultella planticola, Klebsiella pasteurii, Citrobacter murliniae, Leuconostoc mesenteroides* were only observed at 10 h indicating it as optimal HRT. *Janthinobacterium lividum* was observed only at 7 h, with and without the balls and *Pseudomonas veronii* at 10 h and 7 h with balls indicating the requirement of a synthetic matrix to reduce HRT.

In inoculum R4 analysis, 7 h HRT with balls didn't provide enough DNA to be analysed, so only two conditions were observed. *Clostridium chromiireducens, Clostridium puniceum, Pseudomonas veronii, Lactococcus lactis, Pseudomonas extremaustralis* were observed at both HRTs, indicating no influence of HRT on these bacteria. *Huaxiibacter chinensis, Klebsiella aerogenes, Raoultella planticola, Klebsiella pasteurii, Lactococcus chungangensis, Clostridium acidisoli, Leuconostoc mesenteroides, Bacillus velezensis* were observed only at 10 h. Additionally, *Janthinobacterium lividum, Janthinobacterium rivuli* were observed only at 7 h indicating it as optimal HRT for the growth of these bacteria.

Conclusion

The study has demonstrated the ability of commercial inoculums to grow under diverse environmental conditions at different HRTs to produce H_2. Some bacteria disappeared when the HRTs were reduced, but some new bacteria were observed too. It was also observed that to reduce the HRTs, synthetic matrices can be used, as several species of bacteria which were observed at 10 h, disappeared at 7 h HRT, but when plastic balls were introduced, the bacteria reappeared. The most prominent bacteria include *Clostridium species* followed by *Pseudomonas species*. The growth of these bacteria indicates their ability to grow under diverse environmental conditions proving to be an efficient microbe for H_2 production under varying conditions.

References

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Table 1. Bacteria present in Commercial Inoculums

Inoculum R1	Inoculum R2	Inoculum R3	Inoculum R4
B. subtilis	*B. subtilis*	*B. subtilis*	*C. butyricum*
B. megaterium	*B. megaterium*	*B. megaterium*	*B. amyloliquefaciens*
B. amyloliquefaciens	*B. amyloliquefaciens*	*B. amyloliquefaciens*	*Lactobacillus acidophilus*
B. licheniformis	*B. licheniformis*	*B. licheniformis*	
P. putida	*P. putida*	*P. putida*	
P. fluorescens	*P. fluorescens*	*P. fluorescens*	
C. butyricum	*C. butyricum*	*C. butyricum*	
	Lactobacillus acidophilus		