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XIII JORNADA DE JÓVENES INVESTIGADORES/AS DEL I3A

Analysis of bacterial DNA from effluent samples for hydrogen production by dark fermentation

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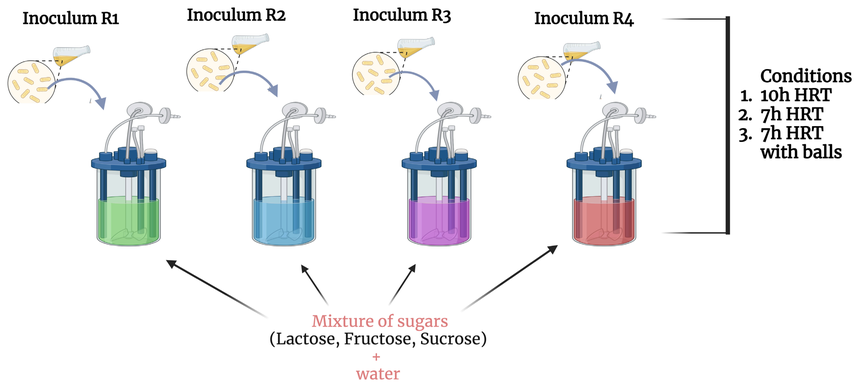
INTRODUCTION

The biological production of hydrogen is attracting more attention due to the utilization of biomass from waste such as effluents. The efficient production of hydrogen highly depends on the bacteria and their ability to degrade organic substrates used as raw material in the bioreactor. Hydraulic retention time (HRT) is one of the critical factors affecting the long-term operation of hydrogen productivity and substrate conversion efficiency of the bacteria under dark fermentation conditions. The study aims to analyse the bacteria involved in the production of hydrogen and optimize the HRT to enhance bacterial growth and its interaction with the substrate to increase hydrogen yields.

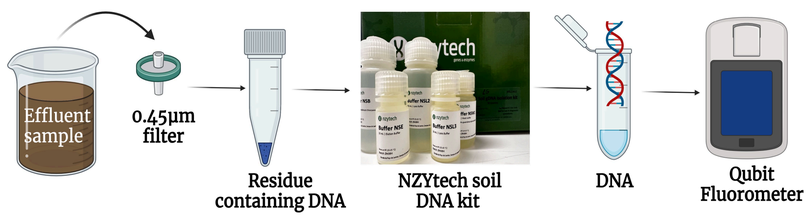
METHODOLOGY

Dark Fermentation

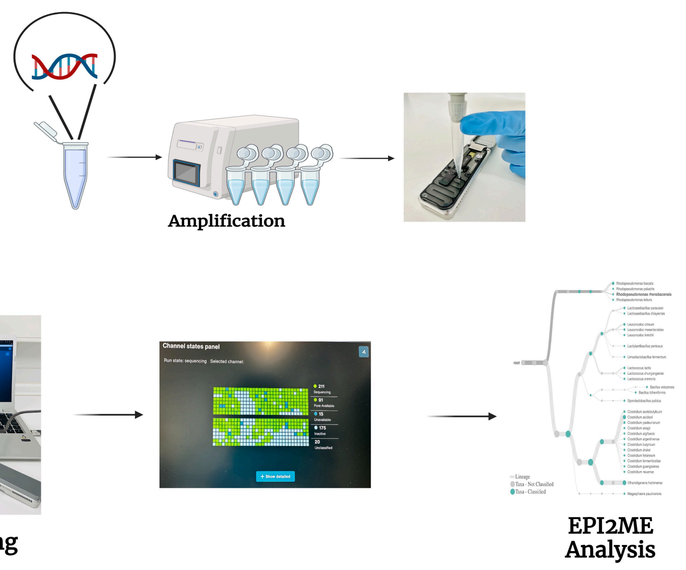


Four powdered inoculums (R1 to R4) with known microbes (see table 1, ✓) were used in the bioreactors for dark fermentation. A substrate of mixed sugars (lactose, fructose, and sucrose) was used in all four bioreactors with all three HRT conditions of 10 h, 7 h and 7 h using plastic hollow balls as synthetic matrices to immobilise microbes.

Analysis of Bacteria



The bacterial extraction was carried out by filtering the effluent samples, with a 0.45 µm filter. The residues were collected, and genomic bacterial DNA was extracted using the NZYtech soil DNA kit. The quantification of DNA was performed using a Qubit fluorometer.



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) and demultiplexed into .fastq files to be analysed in EPI2ME agent software (Oxford). The taxonomic tree with all the bacterial species in the samples was obtained. This enabled the identification of hydrogen-producing bacteria present in the samples.

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RESULTS

The results obtained showed the dominance of ***Clostridium butyricum*** in all the effluent samples under HRTs of both 7 and 10 h in dark fermentation followed by ***Clostridium saccharoperbutylacetonicum***, ***Clostridium saccharobutylicum***, ***Clostridium chromiireducens*** and ***Raoultella ornithinolytica*** species.

***Table 1**: Prominent species observed in the original inoculums and in dark fermentation bioreactors*

	R1	10 h	7 h	7 h + balls	R2	10 h	7 h	7 h + balls	R3	10 h	7 h	7 h + balls	R4	10 h	7 h
Bacillus amyloliquefaciens									✓✓				✓✓		
Bacillus licheniformis	✓✓				✓✓				✓✓				✓		
Bacillus megaterium	✓				✓				✓						
Bacillus subtilis	✓✓			✓	✓✓			✓	✓✓				✓		
Bacillus velezensis	✓				✓				✓				✓	✓	
Citrobacter murliniae										✓					
Clostridium acidisoli						✓			✓					✓	
Clostridium beijerinckii	✓	✓			✓	✓			✓				✓		
Clostridium butyricum	✓✓	✓	✓	✓	✓✓	✓	✓	✓	✓✓	✓	✓	✓	✓✓	✓	✓
Clostridium chromiireducens	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clostridium puniceum	✓		✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓
Clostridium saccharobutylicum	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clostridium saccharoperbutylacetonicum	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Comamonas testosteroni					✓	✓							✓		
Duganella levis		✓													
Huaxiibacter chinensis		✓	✓	✓	✓	✓	✓			✓				✓	
Janthinobacterium lividum		✓			✓			✓			✓	✓			✓
Janthinobacterium rivuli	✓	✓			✓	✓		✓		✓	✓	✓	✓		✓
Janthinobacterium violaceinigrum	✓				✓			✓							
Klebsiella aerogenes		✓	✓		✓	✓	✓			✓				✓	
Klebsiella pasteurii		✓			✓					✓				✓	
Lactobacillus acidophilus					✓								✓		
Lactococcus chungangensis	✓		✓	✓	✓		✓	✓	✓						
Lactococcus cremoris				✓	✓										
Lactococcus lactis			✓	✓	✓					✓	✓	✓	✓	✓	✓
Leuconostoc mesenteroides		✓			✓	✓	✓		✓	✓			✓	✓	
Propionispira arcuata			✓		✓						✓				
Pseudomonas extremaustralis	✓				✓	✓		✓		✓	✓	✓	✓	✓	✓
Pseudomonas fildesensis					✓	✓									
Pseudomonas fluorescens	✓	✓		✓	✓	✓			✓	✓	✓	✓			✓
Pseudomonas putida	✓	✓		✓	✓				✓	✓					
Pseudomonas veronii					✓	✓				✓		✓	✓	✓	✓
Raoultella ornithinolytica	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Raoultella planticola		✓		✓	✓	✓	✓	✓		✓			✓	✓	

✓ *Stated by the producer*
✓ *Identified by analysis*

In samples analysis with **inoculum R1**, ***Clostridium beijerinckii***, ***Leuconostoc mesenteroides***, ***Klebsiella pasteurii***, ***Janthinobacterium rivuli***, ***Janthinobacterium lividum***, ***Duganella levis*** were obtained only at 10 h HRT. ***Pseudomonas fluorescens***, ***Pseudomonas putida***, *and* ***Raoultella planticola*** were observed at 10 h HRT and 7 h HRT with balls indicating that to reduce the retention time, which is the major objective of the analysis, these microbes require synthetic matrices to interact more with the substrate.

In samples analysis with **inoculum R2**, ***Pseudomonas extremaustralis***, ***Pseudomonas fluorescens***, ***Pseudomonas veronii***, ***Comamonas testosteroni***, ***Pseudomonas fildesensis***, ***Clostridium acidisoli***, ***Clostridium beijerinckii*** were observed only at 10 h HRT. ***Janthinobacterium rivuli*** were obtained at 10 h HRT and disappeared from the samples when the HRT was reduced to 7 h, but it was observed that in a bioreactor with 7 h HRT and balls, these bacteria reappeared.

In dark fermentation studies with **inoculum R3**, ***Pseudomonas putida***, ***Huaxiibacter chinensis***, ***Klebsiella aerogenes***, ***Raoultella planticola***, ***Klebsiella pasteurii***, ***Citrobacter murliniae***, ***Leuconostoc mesenteroides*** were observed only at an HRT of 10 h indicating it as their optimal retention time. ***Pseudomonas veronii***, at 10 h and 7 h HRT with balls indicating that to reduce the retention time a synthetic matrix is required to immobilize the bacteria.

In dark fermentation studies with **inoculum R4,** the effluent sample obtained from the bioreactor with 7 h HRT with balls for immobilization didn't provide enough DNA to be analysed. ***Huaxiibacter chinensis***, ***Klebsiella aerogenes***, ***Raoultella planticola***, ***Klebsiella pasteurii***, ***Lactococcus chungangensis***, ***Clostridium acidisoli***, ***Leuconostoc mesenteroides***, ***Bacillus velezensis*** were observed only at an HRT of 10 h and disappeared when the HRT was reduced. ***Janthinobacterium lividum***, and ***Janthinobacterium rivuli*** were observed only at 7 h HRT indicating it as an optimal retention time for the growth of these bacteria.

CONCLUSIONS

The study has demonstrated the growth pattern of commercial inoculums under diverse environmental conditions at different HRTs. The bacteria observed were either obligate anaerobes or facultative anaerobes except *Duganella levis* and *Comamonas testosteroni*. Some bacteria disappeared when HRT was reduced but some new bacteria were found in the samples. It was also observed that to reduce the HRTs, synthetic matrices like plastic balls can be introduced. The most prominent bacteria observed in the samples include *Clostridium species* followed by *Pseudomonas species*.

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