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Kinetics and microbial community analysis for hydrogen production using raw grass inoculated with different pretreated mixed culture

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1 **Kinetics and microbial community analysis for hydrogen production**  
2 **using raw grass inoculated with different pretreated mixed culture**

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10 **Abstract:** In this study, five pretreatment methods (heat shock, acid, base, aeration  
11 and gamma radiation) were applied for enriching hydrogen producers from  
12 anaerobically digested sludge, aiming to compare their hydrogen fermentation  
13 performance using raw ryegrass as substrate. Results showed that various  
14 pretreatment methods caused great variations on grass hydrogen fermentation  
15 performance. Acid pretreatment was most efficient compared with other tested  
16 pretreatment methods, with relevant hydrogen yield of 64.4 mL/g dry grass and  
17 organics removal of 31.4%. Kinetics results showed that the first-order kinetic model  
18 fitted hydrogen evolution better than the modified Gompertz model. Microbiological  
19 analysis showed that various pretreatment methods caused great variations on  
20 microbial activity and microbial community composition. *Clostridium* and  
21 *Enterococcus* were two dominant genera, while relative abundances of these two  
22 genera varied greatly for different pretreated samples. Difference in microbial activity  
23 and microbial community distribution induced by the pretreatment methods might  
24 directly cause different ryegrass fermentation performance.

25 **Keywords:** Inoculum pretreatment; fermentative hydrogen production; grass; kinetic  
26 model; microbial community

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