TITLE: CHARACTERIZATION OF KINETICS AND HYDROGEN-PRODUCING ACTIVITY OF *ENTEROCOCCUS* SP. ISOLATED FROM CITRUS PEEL WASTE

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ABSTRACT:

This study aimed at evaluating the kinetics of *Enterococcus casseliflavus* isolated from Citrus Peel Waste (CPW) and its application on biodigestion of lignocellulosic biomass, with concomitant production of H_2 and organic acids. The strain was isolated from an anaerobic consortium obtained through self-fermentation of *in natura* CPW by serial dilution (10^{-1} to 10^{-30}) in PCS medium (peptone 5 $g.L^{-1}$, yeast extract 1 $g.L^{-1}$, $CaCO_3$ 5 $g.L^{-1}$, NaCl 5 $g.L^{-1}$; pH 7.0) for 48 hours under static and mesophilic conditions (37°C). The flask with the higher dilution in which H_2 was still being produced was plated (PCS medium plus 1.5% agar) and incubated in an anaerobe Gas-Pack Jar at the same conditions. The hydrogen-producing strains were identified through 16S rRNA gene sequencing by Sanger method. The sequence obtained was similar to *E. casseliflavus* (99% similarity). The kinetics parameters estimated after the isolation step (glucose 3 $g.L^{-1}$) were specific growth rate (μ 0.35 h), generation time (*Tg*= 1.98 h^{-1}), production (*P*= 9.1 mmol $H_2.L^{-1}$), yield (*Rm*= 1.99 $mmol.h^{-1}$), and fermentation starting period (λ= 4.08 h), as well as 61.34% cellulose (filter paper) degradation rate. It was possible to observe assimilation and production of hydrogen from various carbon sources (3 $g.L^{-1}$) evaluated (glucose, fructose, sucrose, xylose, starch, glycerol, cellobiose, cellulose and lactose), especially from xylose, with *P*= 10.3 mmol $H_2.L^{-1}$. The main metabolite was acetic acid (365 $mg.L^{-1}$), which indicates prevalence of the acetogenic metabolic pathway. The use of CPW (15 $g.L^{-1}$) as substrate, with addition of the isolated strain, resulted in higher hydrogen production (*P*= 13.9 mmol $H_2.L^{-1}$; *Rm*= 1.09 $mmol.h^{-1}$ and λ= 2.12 h), making it possible to infer that the application of *E. casseliflavus* has great potential in biodigestion of complex substrates to obtain products of biotechnological interest.

Keywords: lignocellulosic biomass, autochthonous consortia, kinetic parameters, agro-industrial waste, nutritional evaluation

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