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# Biomass hydrolysis during Solid State Dark Fermentation: Effect of total solids contents and hydrogen partial pressure

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Solid State Dark Fermentation (SSDF) is emerging as a valuable technology for producing biohydrogen as well as organic acids and other value-added by-products from waste biomass (Motte et al., 2014; Motte et al., 2015). Besides to the advantages in operation, i.e. high substrate loading rates and low water addition, this technology offers economic benefits by reducing the reactor volume and specific energy requirements, an efficient handling of digestate and a higher technical simplicity. The commercial high solids processes are generally operated at a total solids (TS) content of >20% TS (Motte et al., 2014). However, the biogas yields at certain TS content is compromised due to the mass and energy transfer limitations driven by system's low water content (Motte et al., 2014; Valdez-Vazquez & Poggi-Varaldo, 2009). Thus, microbial activity can be impacted by the transport of soluble components (i.e. substrates, intermediate and end-metabolites). Recently, Motte et al. (2014) showed the dependency of biohydrogen production on TS content. At the same time, Cazier et al. (2015) showed an inhibition of biomass hydrolysis in (SS-AD) anaerobic digestion due to a high hydrogen partial pressure. However, it is rather unknown how acidogenesis and biohydrogen yields could be impacted by high TS content with probable subsequent inhibition of substrate hydrolysis by high local H<sub>2</sub> partial pressure in SSDF process.

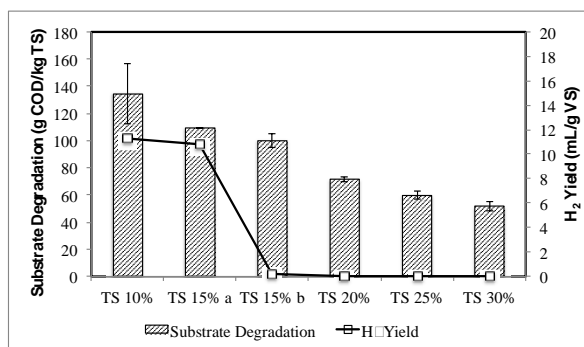
The present study aimed at investigating the total solids content on organic waste conversion in SSDF and the particular effect of hydrogen partial pressure (p<sub>H<sub>2</sub></sub>).

Experiments were designed to study the effect of p<sub>H<sub>2</sub></sub> and TS content on substrate degradation and biochemical pathways in batch SSDF tests. Food waste prepared in the laboratory and heat shocked waste activated sludge with a substrate to microorganism ratio of 10 (g volatile solids (VS) substrate/g VS inoculum) was used. Digestate at different TS content was prepared to investigate the effect of TS. To study the effect of p<sub>H<sub>2</sub></sub>, H<sub>2</sub> was initially added in the headspace of the 600 ml serum bottles in two sets of tests; in one p<sub>H<sub>2</sub></sub> equivalent to 550 mbar (≈35% H<sub>2</sub> in the headspace) and 1080 mbar (≈66% H<sub>2</sub> in the headspace) in other. A control with only N<sub>2</sub> in headspace carried and the final total pressure at the start of the tests was 1500 mbar. Culture pH was maintained around 5.5 with MES buffer and was incubated at the bottles were incubated at mesophilic temperature (37 °C). Substrate degradation was estimated theoretically calculating the difference in metabolic end-products (accumulated in both gaseous and liquid phase), at the initial and final state.

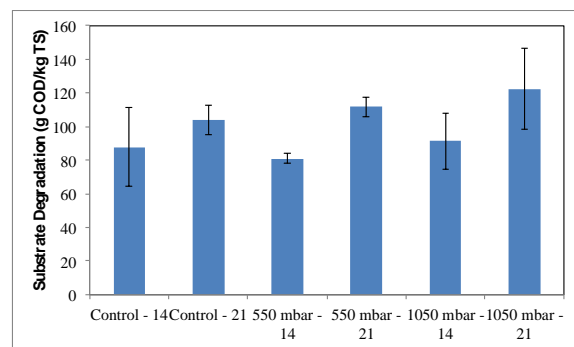
Figure 1 shows effect of TS content on substrate degradation after 14 days. The level of substrate conversion showed decreasing trend and there was significant shift in the metabolic pathways (Results not shown here). At higher TS content, the metabolic pathways shifted to lactic acid formation, which does not yield any H<sub>2</sub>. H<sub>2</sub> was produced only in the TS content 10%, while two behaviors were observed at 15% TS, only one of the three replicates at TS 15%

produced H<sub>2</sub> (15% a shown in Figure 2). This might suggest that the limiting effect of TS content might starts between 15 and 20 % as in agreement with Motte et al. (2014) which reported a metabolic shift at 19% TS with wheat straw as substrate. Abbasi et al. (2012) showed significant inhibition of methane yields at 30 % TS due to accumulation of intermediates such as organic acids, dissolved hydrogen. Thus, p<sub>H<sub>2</sub></sub> might have impact on substrate conversion in SSDF.

Figure 2 shows the total substrate degradation values (expressed as g COD/kg TS) after 14 and 21 days of DF at different p<sub>H<sub>2</sub></sub>. The level of inhibition of p<sub>H<sub>2</sub></sub> on substrate hydrolysis was determined based on substrate degradation (Figure 1), which does not showed any significant effect on hydrolysis of biomass. These results are in contrast with the study of Cazier et al., (2015), where inhibition on hydrolysis started at p<sub>H<sub>2</sub></sub> > 750 mbars. The difference in results might be attributed to lower operational pH (less than 5) in SSDF than SS-AD (higher than pH 7), which might determines the metabolic shift.



**Figure 1** Substrate degradation (in g COD per kg of initial TS) at the end of two fermentation times at different TS content



**Figure 2** Substrate degradation (in g COD per kg of initial TS) at the end of two fermentation times at different p<sub>H<sub>2</sub></sub>

TS content has effect on the substrate conversion and metabolic pathways. Therefore, the TS content in the SSDF has to be maintained less than 15 %, for the process aimed for biohydrogen production. The results from the study showed that accumulation of H<sub>2</sub> as gaseous products does not have inhibitory effect on hydrolysis of organic biomass in SSDF.

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