Production of bio-based VFAs by acidogenic fermentation of protein hydrolysates from agriculture and tannery wastes

M. Menini[1], S. Lupinelli[2], P. Critelli[1], G. Pesante[1], F. Battista[1], P. Girelli [2], N. Frison[1], D. Bolzonella[1]

[1]Department of Biotechnology, University of Verona, Verona, 37134, Italy
[2]ILSA Spa Via Quinta Strada 28, 36071 Arzignano (Italy)
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Presenting author email: miriam.menini@univr.it

Every year, food and leather industries produce billions of tons of waste, most of which are disposed by landfill or incineration (Gendebien *et al*, 2001; Dhayalan *et al*, 2007). Novel recycling alternatives aim at the implementation of biotechnologies that convert such wastes into added value and bio-based compounds such as green fuels for energy production (e.g., methane), building blocks (e.g., volatile fatty acids VFAs) and others (e.g., bone flour). A mixed culture is usually used to produce the up mentioned bio-based products by means of acidogenic fermentation (Atasoy *et al*, 2019). Waste and wastewaters coming from tanneries and food industries are rich in protein (around 40-60% in COD, Duong *et al*, 2019), which can be hydrolysed under anaerobic digestion conditions, to produce peptides and amino acids; these can subsequently be fermented into VFAs and finally converted to CH_4 and CO_2 by an association of microorganisms (Tang *et al*, 2004). Previous work on such methods was published by Bashkar *et al* (2007), who assessed the potential of fermentation of waste derived from a tannery, by using a mixed culture of lactic bacteria to obtain animal feed and H_2S. Duong *et al* (2019) investigated the hydrolysis and degradation of gelatine as a model of dissolved protein that undergoes anaerobic digestion process.

The aim of this study was to investigate the potential for the acidogenic fermentation of three different substrates rich in proteins, obtained from the hydrolysation of tanneries and agricultural wastes (soyabeans, Alfaalfa (*Medicago sativa*) and animal epithelia). During the study we assessed the production of VFAs, with particular focus on the yields and on the pH conditions that gave better results. The concomitant production of polyhydroxyalkanoates (PHAs) was also tested, since they are value-added products that can be used as bioplastic (Beccari *et al*, 2009; Valentino *et al*, 2014) and therefore have high market value.

The experimental set-up l consisted of 2 serum bottles of 1 L of volume for each pH tested, filled up to 250 mL; the overall experiment time was seven days, the substrate-inoculum ratio (S/I) was set at 10 gCOD/gTVS, the temperature was kept at 37°C (mesophilic condition) and the pH condition tested were 4: not buffered, 8, 9 and 10 (7 for the Alfalfa hydrolysate, due to the initial pH of 5). The pH was adjusted by the addiction of NaOH 30% (w/v) or sulfuric acid 2.5 M. A sample was collected daily from every bottle, including the control, for the analysis of COD (total and soluble), total solids, pH and of solid, ammonia and phosphorus content (using the standard methods, APHA, AWWA, WEF, 2007). VFAs production was monitored by HPLC (Dionex 1100, Thermofisher, USA).

The characterisation of the three different substrates is reported in **Errore. L'origine riferimento non è stata trovata.**. Noticeable is the high ammonia content of the soybean hydrolysate and the low solid and COD content of the Alfaalfa waste.

Table 1. Substrates' characteristics before acidogenic fermentation. TS=total solids, TVS=total volatile solids, ww =wet weight, COD=chemical oxygen demand, N-NH_4=Nitrogen-Ammonium, P=Phosphorus.

Characteristic	Unit	Soybeans	Alfalfa	Animal epithelia
Total solids	gTS/kg (ww)	544	352	641
Volatile solids	gTVS/kg (ww)	491	256	582
pH	-	6	5	6
Total COD	gCOD/L	638	357	679
Soluble COD	gCOD/L	411	214	602
Ammonia	mgN-NH_4/L	6310	105	44.6
Phosphorus	mgP/g (ww)	1793	2446	-

At the end of the experimental period the production yields (calculated as $gCOD_{VFAs}$/gCOD) were observed to be higher for the animal epithelia at the non-buffered pH. Moreover, the best yields for the agricultural waste hydrolysates were obtained by buffering the pH at 9; pH10 was generally the one producing the worst results (**Errore. L'origine riferimento non è stata trovata.**).

VFAs production was observed for all the three substrates, when comparing the amounts at the end of the experiment (TF) with the beginning (T0). The detected VFAs include mainly acetic and propionic acid, with

butyric, isobutyric, pentanoic e isopentanoic also found in lower amounts depending on the substrate and pH (data not presented).
The best performing substrate was the animal epithelia, with VFAS yields almost double than the other substrates. Overall, the non-buffered pH produced the highest amount of VFAs than all the other pHs, for all the different substrates (Figure 2).

The production of PHAs is still under investigation, with the results expected in the coming weeks.

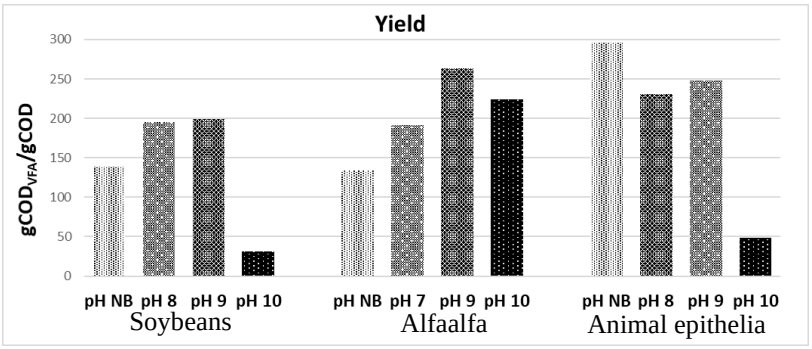

Figure 1. Production yields for the substrates at the tested pHs.

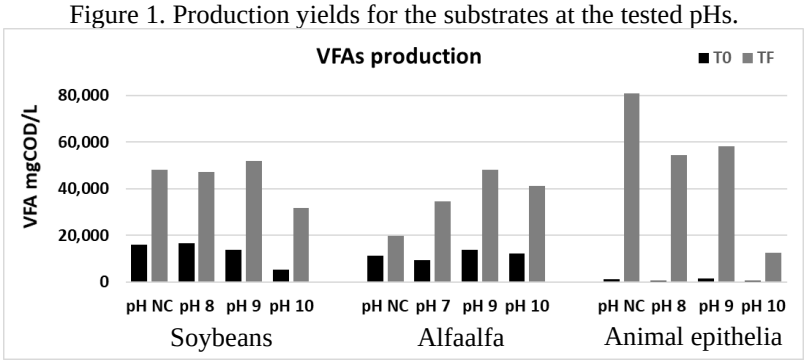

Figure 2. VFA production for the three substrates at the tested pHs. T0=start and TF=end of the experiment.

These initial experiments suggest that the best working conditions for the plant hydrolysates are at pH 9 or non-buffered (which settles at around 8) for the animal epithelia. The next phases of the experimental set-up include the identification of the ideal HRT (hydraulic retention time) with a fed-batch reactor, and subsequently a continuous fermentation system at the identified best pH and HRT. The animal epithelia appear to be the preferrable substrate to use for obtaining the best yields.

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