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ACIDOGENIC DEGRADATION OF THE NITROGEN FRACTION IN VINASSE

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SUMMARY

Degradation of the nitrogen fraction in sugar beet vinasse during continuous acidogenesis was studied. The total nitrogen content in the vinasse was approximately 4 $g \cdot L^{-1}$, with only traces of ammonia nitrogen. During acidogenesis $N\text{-}NH_4^+$ increased proportionately to the breakdown of betaine and amino acids.

INTRODUCTION

In an earlier paper (Gil-Peña *et al.*, 1986), 60-70 % of the chemical oxygen demand (COD) in vinasse was found to be broken down to volatile fatty acids (VFAs) during acidogenesis. Thus, from 30 to 40 % of the COD may be unaltered at the end of the process. The ammonia nitrogen ($N\text{-}NH_4^+$) concentration in the effluent was also reported to be about one-third of the initial total nitrogen in the vinasse. It was therefore concluded that approximately two-thirds of the total nitrogen did not undergo degradation during the optimum hydraulic retention time (HRT) for acidogenesis, which was found to be eight hours.

Ammonia is produced by anaerobic bacteria as a metabolite during the anaerobic digestion of organic matter containing nitrogen compounds such as proteins, amino acids, etc. In an aqueous medium, there is a balance between ammonia and ammonium. At the same time, the CO_2 produced during digestion affects the amount of dissolved CO_2 and the buffering capacity of the system (Pfeffer, 1979), giving rise to a carbonate-ammonium bicarbonate balance. Because of the high $N\text{-}NH_4^+$ concentration in the effluent, in protein-rich culture media the pH must be adjusted by adding a strong acid (Trosch *et al.*, 1983). $N\text{-}NH_4^+$ levels higher than 1.7 $g \cdot L^{-1}$ may be toxic to methanogenic bacteria that have not been previously adapted to high concentrations of ammonium (Koster and Lettinga, 1983).

At the start of acidogenesis in sugar beet vinasse, the pH rises, stabilizing at around 6.8-7.0 throughout the continuous process (Gil-Peña *et al.*, 1986). As a result, the acids produced are ionized. The $N\text{-}NH_4^+$ concentration in the effluent was 1.2 $g \cdot L^{-1}$ at an HRT of eight hours. As already pointed out, this is indicative of incomplete breakdown of the nitrogen compounds present initially.

The present study analyzes the nitrogen fraction and the residual products following acidogenesis. An HRT of 24 hours was employed, similar to the longer HRTs reported in the literature.

Table 1. Mean initial composition of the vinasse used in this study

	$\bar{x}$	σ (n=5)	
pH	4.45	0.35	
COD ($g\ O_2 \cdot L^{-1}$)	70.68	5.24	
Total solids ($g \cdot L^{-1}$)	83.75	12.68	
Mineral solids ($g \cdot L^{-1}$)	21.30	3.30	
Volatile solids ($g \cdot L^{-1}$)	62.45	9.36	
Total nitrogen ($g \cdot L^{-1}$)	4.20	0.41	
$N\text{-}NH_4^+$ ($g \cdot L^{-1}$)		Max. 0.28	Min. 0.03
Protein nitrogen (N x 6.25)	27.31		

Table 2. Acidogenesis conditions

Flow rate (ml/h)	HRT (h)	Loading (Kg $COD \cdot m^{-3} \cdot d^{-1}$)	Total VFA ($g \cdot L^{-1}$)
370	24	52	15.7

Table 3. Betaine concentration in the vinasse

Vinasse	$\bar{x}$ ($g \cdot L^{-1}$)	σ (n=5)	% total solids	% volatile solids	Nitrogen content ($g \cdot L^{-1}$)
Initial	18.60	3.37	22.2	29.8	2.52
After acidogenesis	6.77	0.86	-	-	0.92

Table 4. Total nitrogen balance before and after sugar beet vinasse acidogenesis

	Initial vinasse	Acidified vinasse
Total nitrogen	4.20	4.30
$N\text{-}NH_3$	0.17	2.23
Betaine nitrogen	2.52	0.92
Amino acid nitrogen (total amino acids/6.25)	0.60	0.07

MATERIAL AND METHODS

Fermentation conditions: Acidogenesis was carried out in a fermentor described by Gil-Peña et al. (1986) at a temperature of 30 °C and with an HRT of 24 h. Working volume was approximately 9 L, the support medium was expanded clay (5 mm in diameter), fixed-bed volume was 0.5 L, recycling was 9 $L \cdot min^{-1}$. The vinasse was dissolved in water (3:1) and enriched with nutrient salts (2.5 $g \cdot L^{-1}$ $(NH_4)_2SO_4$; 0.4 $g \cdot L^{-1}$ $MgSO_4 7H_2O$; and 0.1 $g \cdot L^{-1}$ KH_2PO_4). An inoculum of bacteria from rumen fluid previously adapted to the vinasse was used.

Analytical methods: Quantification of the VFAs was performed by gas chromatography using a column packed with Porapak QS. Column temperature was 200 °C, injector temperature 250 °C, injection volume 1 µl. The COD and total nitrogen were determined by the Tecator method (1981). Separation and quantification of the amino acids was performed using an amino acid autoanalyzer. Betaine determination was carried out by HPLC, with an amine-bonded silica column (Spherisob-NH_2, 25 x 0.4 Supelco). Analysis conditions were: acetonitrile/water (75:25) solvent at a flow rate of 0.9 $ml \cdot min^{-1}$. A Waters model R-401 differential refractometer was employed as the dectector. Prior to analysis the samples were passed through a Waters Sep-pak C^{18} primed with 1 ml of methanol and 2 ml of water. One ml of vinasse was eluted through the Sep-pak with 2 ml of water. Final sample volume was 3 ml. Injection volume was 20 µl.

RESULTS AND DISCUSSION

Table 1 gives the characteristics of the vinasse used in this experiment. Bearing in mind the standard deviation, the values were similar to those for the vinasses used in previous studies (Gil-Peña et al., 1987). Total nitrogen was 4.37 $g \cdot L^{-1}$, equivalent to 27.3 $g \cdot L^{-1}$ of protein. The $N\text{-}NH_4^+$ content was quite low, between 0.03 and 0.28 $g \cdot L^{-1}$, and for this reason 0.64 $g \cdot L^{-1}$ $(NH_4)_2SO_4$ was added at the outset to facilitate bacterial growth.

Acidogenic fermentation took place normally, as in the past, yielding a VFA concentration of 15.7 $g \cdot L^{-1}$ (Table 2). Fermentor operation was continuous for one month in the conditions set forth in Table 2.

Initally the vinasse contained approximately 4 $g \cdot L^{-1}$ of free amino acids (Figure 1), with aspartic acid as the major component with a mean value of 773 $mg \cdot L^{-1}$ and a very low standard deviation of 5 $mg \cdot L^{-1}$. This component was followed, in terms of quantity, by alanine and isoleucine, although the concentration of these two amino acids, like that of tyrosine and the phenylalanine+tryptophane pair, was

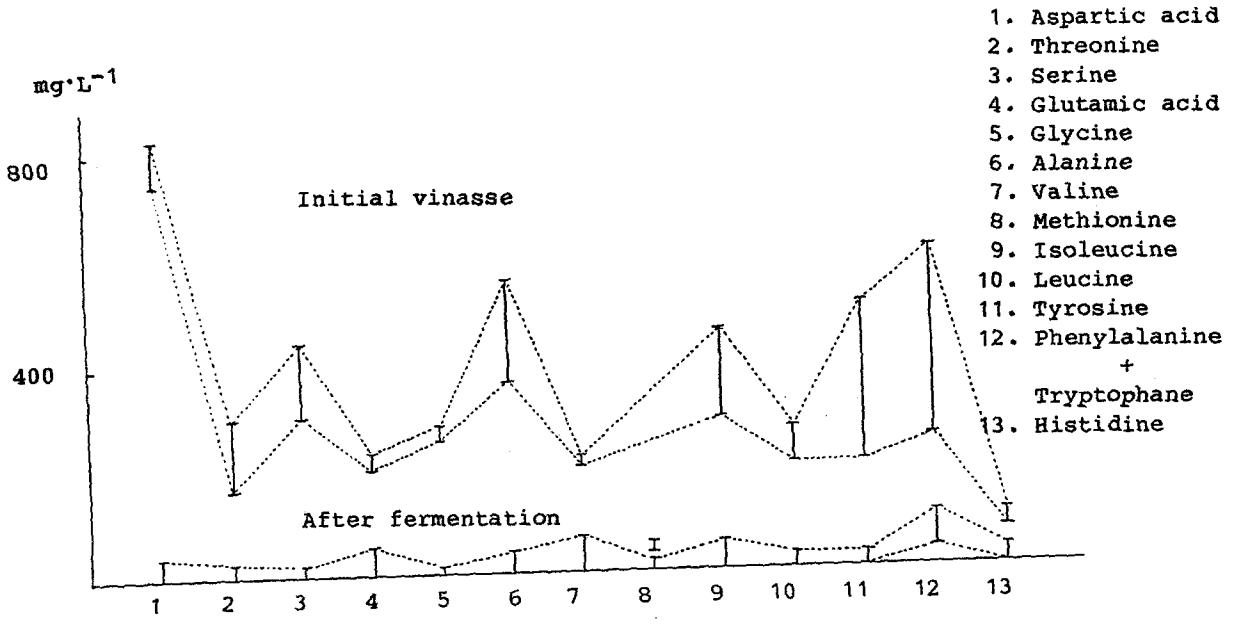


Figure 1. Maximum and minimum amino acid contents in the initial vinasse and after acidogenesis

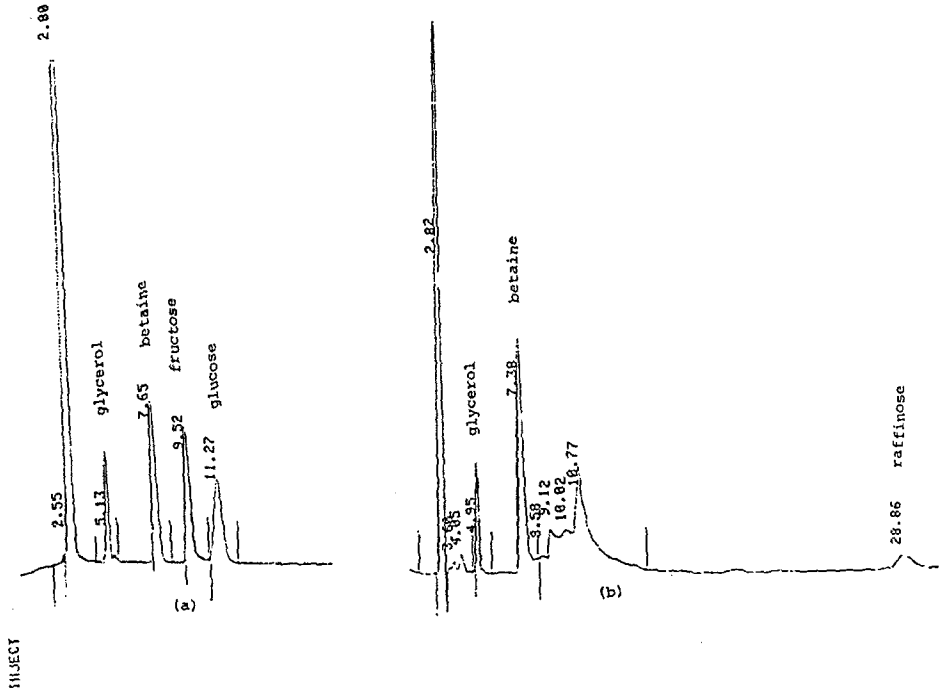
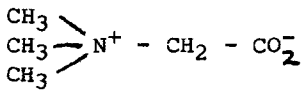


Figure 2. HPLC separation of betaine, sugar, and glycerol (a) standard solution; (b) initial vinasse

quite variable in the vinasse. Considerable amounts of glutamic acid, glycine, valine, and leucine were also present, with low standard deviations. Traces (5 $mg \cdot L^{-1}$) of proline and cystine were also present, while methionine did not exceed 50 $mg \cdot L^{-1}$.

The main component of the vinasse was betaine:



Betaine is thus a source of methyl groups, and its behaviour is similar to that of choline and methionine.

The method of analysis employed gave good separation of betaine from the sugars and glycerol in the chromatogram (Figure 2). Similar chromatographic conditions were used following extraction and concentration by Vialle et al. (1981) to determine the betaine in wines. The separation obtained was quite similar to ours. In contrast, Steinle and Fischer (1978), using an acetonitrile:water mixture (83:17) at a pH of 5.5, reported that betaine eluted after the sugars.

Table 3 presents the betaine concentration in the vinasse and its contribution to the total nitrogen. The vinasse contained 18.6 $g \cdot L^{-1}$ of betaine, corresponding to 22 % of the total solids and 33 % of the organic matter (volatile solids), as reported by Ferrando and Theodosiades (1960).

By the end of acidogenesis, the amino acids were almost entirely absent from the medium (Figure 1), with approximately 0.4 $g \cdot L^{-1}$ of total amino acids remaining in the acidified vinasse.

The betaine fell by approximately two-thirds (Table 3), and the HRT of 24 h proved to be too short for complete degradation. No data on the anaerobic degradation of betaine have been found in the literature. Glanser-Soljan et al. (1985) studied aerobic betaine breakdown by Trichosporum yeasts. They reported 72 % betaine degradation in 21 h using a mixed yeast culture.

Table 4 shows the nitrogen balance before and after acidogenesis. $N\text{-}NH_4^+$ had increased by 2.06 $g \cdot L^{-1}$ in the acidified vinasse, corresponding to the decrease in amino acids and betaine that occurred during fermentation. After 8 h of HRT, the $N\text{-}NH_4^+$ was 1.2 $g \cdot L^{-1}$. Calculating nitrogen fraction degradation on the basis of the amount of $N\text{-}NH_4^+$ produced gives a nitrogen fraction breakdown of 30 % after 8 h and 50 % after 24 h. Betaine degradation was 65 % and amino acid breakdown 90 % after 24 h.

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