

M. BECCARI*, L. CAMPANELLA*, M. MAJONE*, E. ROLLE*

BIOCONVERSION OF HIGHLY POLLUTED WASTES TO ORGANIC ACIDS

Acidogenic fermentation of two wastes from food industry (whey and olive oil mill wastewaters) has been investigated. Production of volatile fatty acids was determined in batch tests for both substrates and compared to an easily biodegradable substrate (glucose). Whey showed high conversion yield (80%) to be reached and butyric acid was the main organic acid produced. Olive oil mill wastewater showed lower conversion yield than whey, and acetic acid was mainly produced. The presence or absence of a methanogenesis inhibitor and different levels of phosphate did not significantly affect conversion yield. In addition, preliminary results of kinetic tests are presented.

SYMBOLS LIST

- b – specific biomass loss rate (T^{-1}),
- K – Michaelis-Menten half velocity coefficient ($M dm^{-3}$),
- P – total volatile fatty acids concentration ($M dm^{-3}$),
- q – maximum specific rate of substrate utilization (T^{-1}),
- S – soluble substrate concentration ($M dm^{-3}$),
- t – time (T),
- Y – net biomass yield coefficient (MM^{-1}),
- X – biomass concentration ($M dm^{-3}$),
- μ – maximum specific biomass growth rate (T^{-1}).

1. INTRODUCTION

Declining reserves of fossil fuels (especially crude oils and natural gas) have stimulated great interest in liquid fuels and production of valuable chemicals from ubiquitous and renewable feedstocks by biotechnological processes. Sugar and starch

* Department of Chemistry, University "La Sapienza", Rome, Italy.

are easily converted in ethanol using current fermentation and separation technologies. However, this process has some inherent drawbacks: the feedstocks are expensive and have competing use as food, and effluents are highly polluted.

The acidogenic fermentation may be an attractive alternative. In fact, this process allows obtaining volatile fatty acids (VFAs) from a large variety of wastes. The acids are recoverable by solvent extraction and then decarboxylated and dimerized by electrochemical processes to obtain alkanes and olefins. Esterification using novel techniques could be another important method for recovering these acids and simultaneously obtaining valuable chemicals [1]–[3]. Hence, acidogenic fermentation seems to be suitable for obtaining valuable products as well as for disposing wastes.

The purpose of this research was to examine the capability of producing VFAs by acidogenic fermentation of two typical high organic content wastes from food industry: whey and olive oil mill wastewater. The primary objective of the work was to determine the ability to fermentation of these substrates by comparing the yields of their bioconversion to VFAs with the behaviour of an easily biodegradable substrate (glucose). Other important objectives of this study were aimed at evaluation of the effects of pH, phosphate availability and presence of a methanogenesis inhibitor on the extent of VFA production. In addition, preliminary kinetic analysis of the process is presented.

2. EXPERIMENTAL

The composition of the wastes considered is shown in the table; for reference substrate (glucose) the COD was calculated on theoretical basis. Biotreatability tests

Table

Waste characteristics

Parameter	Waste composition	
	Whey	Olive oil mill wastewater
pH	3.4	5.1
COD (g/dm ³)	41.4	93.6
Total solids (g/dm ³)	47.4	87.7
Total volatit solids (g/dm ³)	41.6	69.6

were performed on 200 cm³ assay bottles submerged in thermostatic water baths and periodically stirred; incubation temperature was 25°C. Assay bottles were filled in the following way:

first, aliquot (20–40 cm³) of substrate diluted with distilled water in order to obtain a final COD concentration of 10 g/dm³;

second, seed inoculum (no acclimated) formed by anaerobically digested sewage sludge; the COD added with the inoculum was 10% of the substrate COD;

third, nutrient solution (prepared according to OWEN [4]), in order to obtain a final volume of 150 cm³ in the assay bottle.

The anaerobic conditions during filling operation were assured by continuous flushing of nitrogen gas. The initial pH ranged from 6 to 7.

At regular time intervals, samples of assay liquid were collected taking care to maintain anaerobic conditions in test bottles. Samples were centrifuged for a period of 30 minutes at 4000 rpm and the liquid phase was filtered on 0.45 µm cellulosic filters. Both pH and VFAs contents were determined in the filtrate. The analysis of the VFAs was conducted by gas chromatography with a flame ionization detector [5].

Tests were performed both in presence ($5.0 \cdot 10^{-4}$ mol/dm³) and in absence of sodium salt of bromoetansulfonic acid (BES) that inhibits methanogenesis [2]. The influence of pH and phosphorous availability were also investigated. Acid production yield for total or single acid was calculated by taking into account the disproportionation reactions for the acid formation, according to [1], and was expressed in terms of grams of acetic acid equivalent produced per gram of COD of substrate.

Kinetic tests were performed only on the whey. Filling procedure was similar but seed inoculum was previously grown on 0.45 µm filtrate whey before to be anaerobically added at high concentration (about 20 mg/dm³) to the fresh substrate. No nutrient solution was added. Sampling and analytical determinations were the same as the above mentioned.

3. RESULTS AND DISCUSSION

3.1. BIOTREATABILITY

Excepting the tests performed at the highest phosphate concentration (0.6 mol/dm³) the assay pH quickly decreased and final pH ranged from 5.0 to 5.5. In this case, only acetic and butyric acids were produced from the tested substrates.

The data obtained in the absence of BES are summarized in figures 1, 2, and 3. From the figures the following suggestions can be drawn:

conversion of whey to total VFAs is high (about 80%), the same order of magnitude is found for glucose; olive oil mill wastewater is more recalcitrant (conversion about 40%);

butyric acid was the main VFA produced from whey, as well as from glucose; acetic was, on the contrary, in the greatest concentration when olive oil mill wastewater was treated.

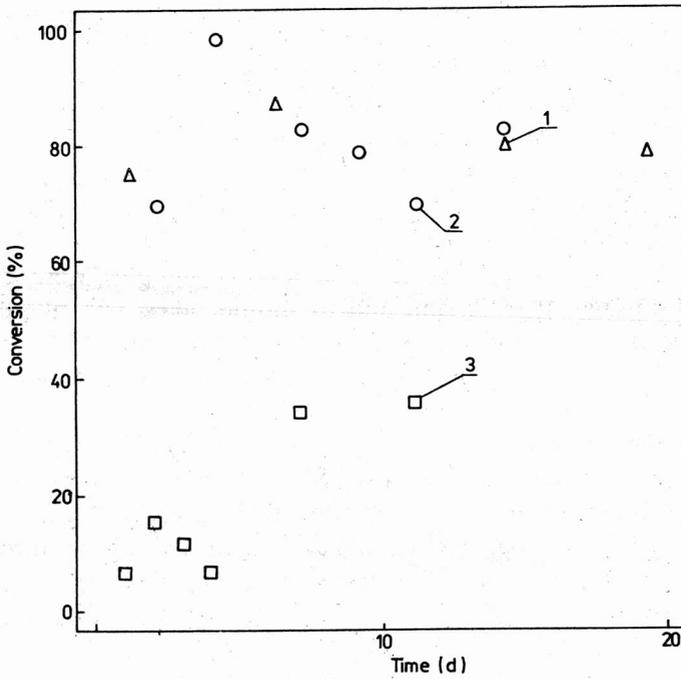


Fig. 1. Conversion percentage to total VFA vs. time; BES absent, no addition of phosphate
1 - glucose, 2 - whey, 3 - olive oil mill wastewater

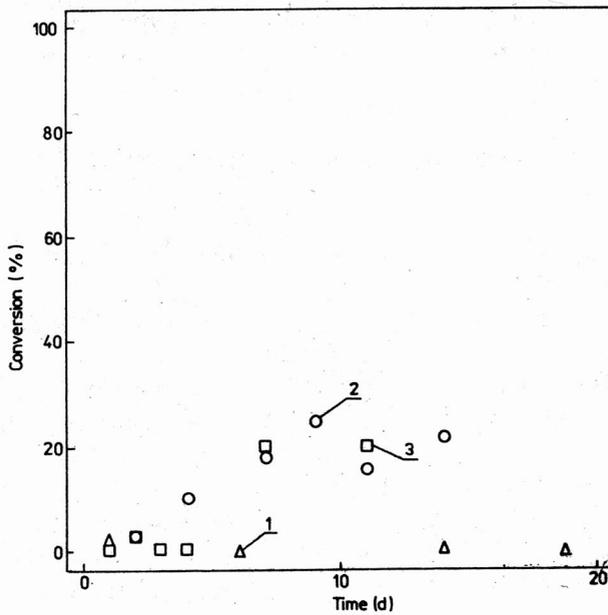


Fig. 2. Conversion percentage to acetic acid vs. time; BES absent, no addition of phosphate
1 - glucose, 2 - whey, 3 - olive oil mill wastewater

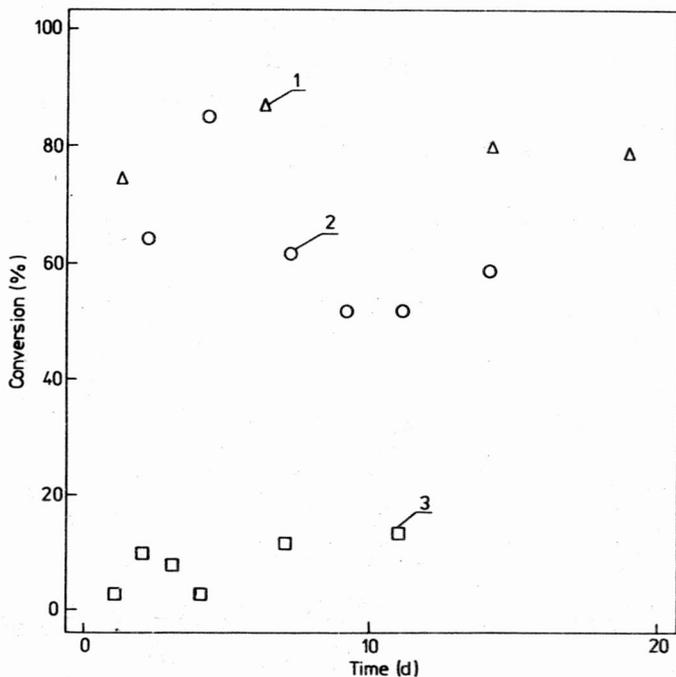


Fig. 3. Conversion percentage to butyric acid vs. time; BES absent, no addition of phosphate
1 - glucose, 2 - whey, 3 - olive oil mill wastewater

Figures 4 and 5 show the total acid production obtained when phosphate was added at high concentration (0.06 mol/dm^3), with or without BES addition. Comparing these results with the data in figure 1, it seems that final acid production is not significantly affected by phosphate (at least at 0.06 mol/dm^3) and BES.

The good reproducibility of data is confirmed by figure 6.

In the tests performed with the addition of phosphate to a level of 0.6 mol/dm^3 , the pH remained above 6. The steady state yields were comparable to those obtained in the runs at pH below 6 but significant amounts of propionic, valeric and caproic acids were determined (figures 7 and 8).

3.2. KINETIC ANALYSIS

In some previous works performed in order to evaluate the most suitable substrate for the acidogenic fermentation, the choice among a large variety of feedstocks was accomplished by assuming a first-order kinetics and comparing the values of the substrate utilization constant \hat{q} obtained from fill and draw semi-continuous experiments [6]. The chief drawback of this method, however, is that no distinction is made between soluble and particulate organics from a kinetic point of view.

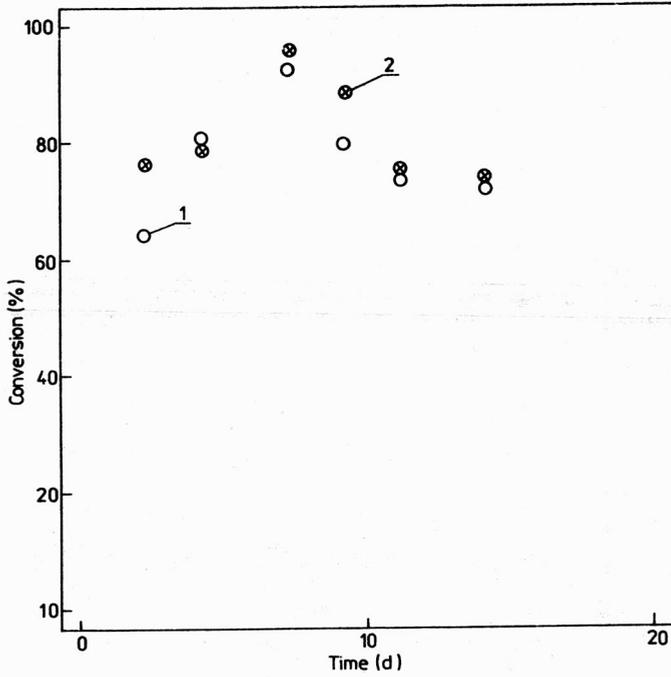


Fig. 4. Whey conversion percentage to total VFA vs. time; 0.6 mol/dm³ phosphate
 1 - without BES, 2 - with 5·10⁻⁴ mol/dm³ BES

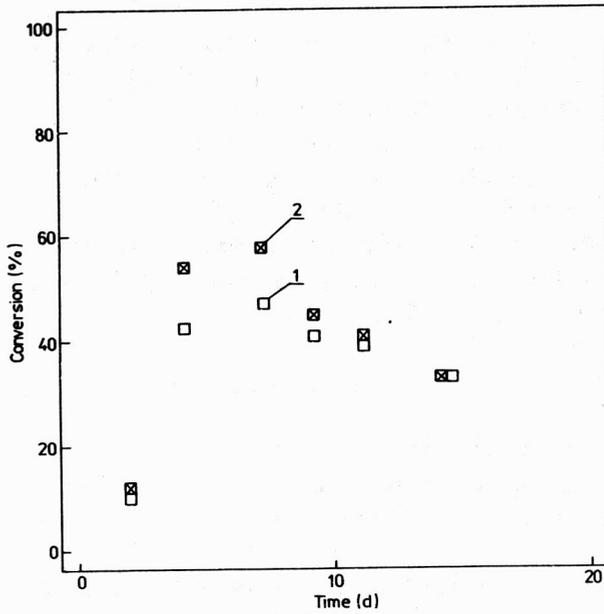


Fig. 5. Olive oil mill wastewater conversion percentage to total VFA vs. time; 0.06 mol/dm³ phosphate
 1 - without BES, 2 - with 5·10⁻⁴ mol/dm³ BES

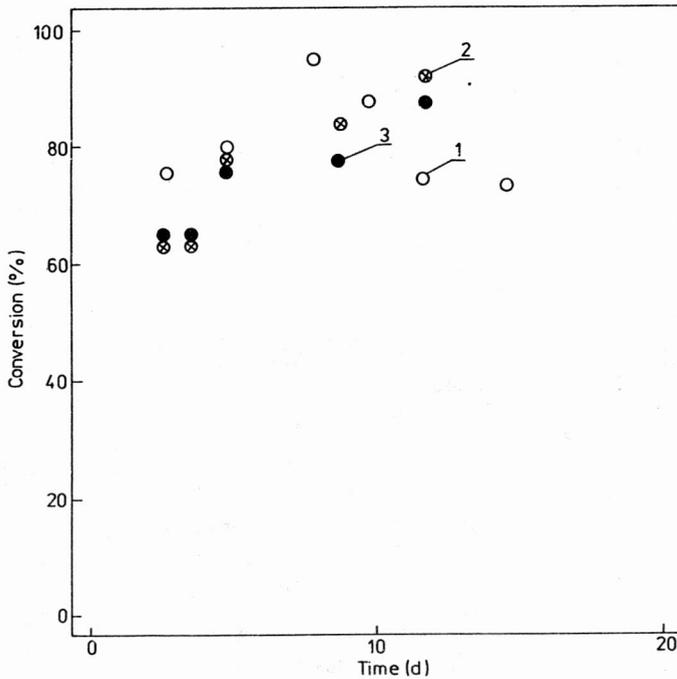


Fig. 6. Reproducibility of whey conversion percentage to total VFA vs. time: 0.06 mol/dm^3 phosphate, $5 \cdot 10^{-4} \text{ mol/dm}^3$ BES

1 - run 1, 2 - run 2, 3 - run 3

GHOSH [7] pointed out that there was a lack of information on the kinetics of particulate substrates; however, no use was made of other equations except Monod's to express the utilization rate even in the case of particulate substrate (the volatile suspended solids of activated sludges).

According to the kinetic modelling suggested by EASTMAN and FERGUSON [8]: all the components of the system are measured in terms of COD, so that reactants and products can be compared directly;

Monod's equation is applied only to express soluble substrate utilization rate; a first-order kinetics is assumed to evaluate the hydrolysis rate of biodegradable particulate substrate.

The constants needed for the acidogenic fermentation modelling can be found from the slopes and intercepts of plotted experimental data using linearized versions of the steady state equations describing the process that occurs in a chemostat (a completely mixed, continuous flow laboratory reactor without solids recycle).

Preliminary kinetic analysis can also be performed in a batch apparatus; this technique is less time consuming in comparison to chemostat; moreover, by properly choosing experimental conditions, the biomass concentration can be assumed to be nearly constant during the run.

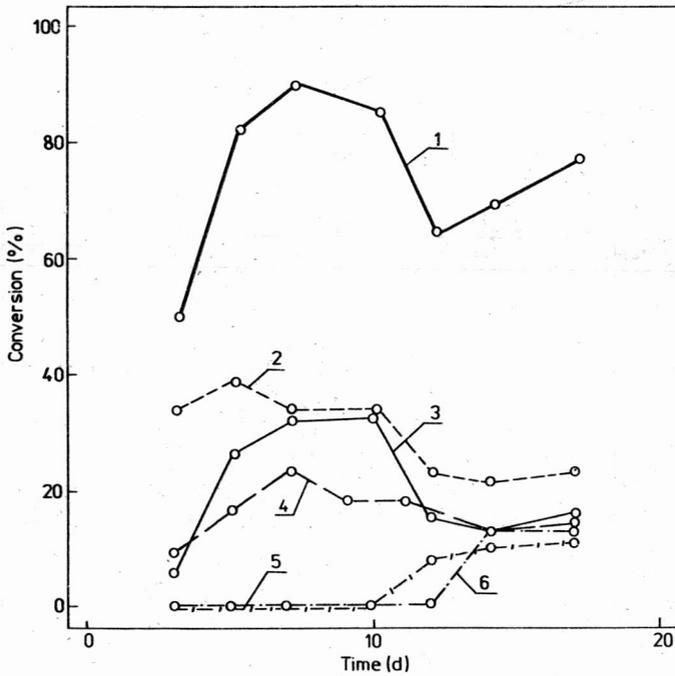


Fig. 7. Whely conversion percentage vs. time; 0.6 mol/dm³ phosphate

1 - total conversion, 2 - conversion to acetic acid, 3 - conversion to propionic acid, 4 - conversion to butyric acid, 5 - conversion to valeric acid, 6 - conversion to caproic acid

In this work, kinetic batch tests on whely were carried out on the basis of the following equations:

soluble substrate utilization rate (the particulate COD fraction of whely being neglectable):

$$-\frac{dS}{dt} = \frac{\hat{\mu}}{Y} \cdot \frac{S}{K+S} \cdot X$$

or (by using the differential method of analysis of data):

$$-\frac{1}{ds/dt} = \frac{Y}{\hat{\mu}X} + \frac{KY}{\hat{\mu}X} \cdot \frac{1}{S} \quad (1)$$

total VFAs production rate:

$$\frac{dP}{dt} = \left(\frac{1}{Y} - 1 \right) \cdot \hat{\mu} \cdot \frac{S}{K+S} \cdot X + bX$$

or (by neglecting endogenous respiration):

$$\frac{1}{dP/dt} = \frac{Y}{(1-Y)\hat{\mu}X} + \frac{KY}{(1-Y)\hat{\mu}X} \cdot \frac{1}{S} \quad (2)$$

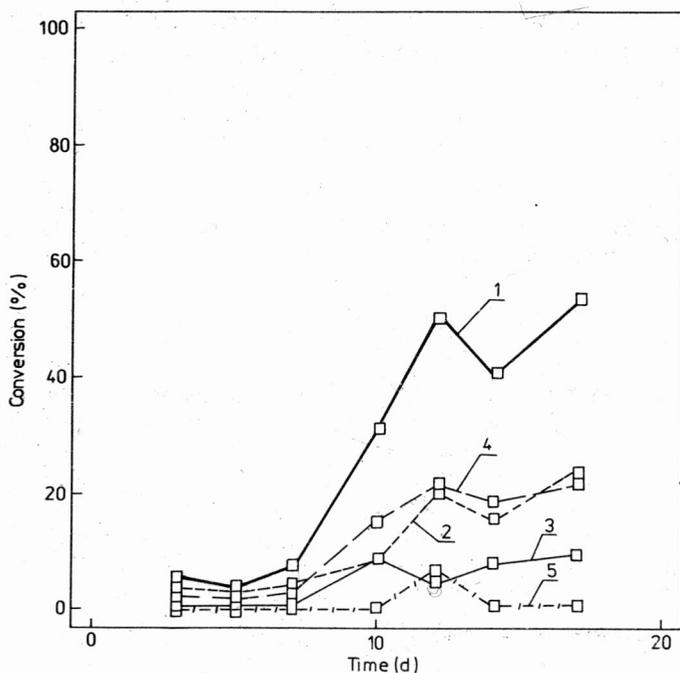


Fig. 8. Olive oil mill wastewater conversion percentage vs. time; 0.6 mol/dm³ phosphate
 1 - total conversion, 2 - conversion to acetic acid, 3 - conversion to propionic acid, 4 - conversion to butyric acid,
 5 - conversion to caproic acid

From the slopes and intercepts of plotted experimental data using equations (1) and (2), the constants K , $\hat{\mu}$ and Y can be found.

Preliminary tests performed at high biomass concentration show that substrate consumption rate follows a zero-order kinetics even when residual substrate concentrations are in the region of 5 g/dm³; the maximum specific substrate-utilization rate \hat{q} (that equals $\hat{\mu}/Y$) is 0.026 h⁻¹.

4. CONCLUSIONS

They seems to be suitable to undergo acidogenic fermentation because of high yield to be reached and butyric acid oriented production, while olive oil mill wastewater appears to be more refractory. The process can be controlled by proper adjustment of operating parameters with no necessity of an expensive chemical suppression. At pH above 6, higher acids than butyric are also produced: because of their good behaviour in the next phases of liquid fuel production, this feature needs to be examined more deeply. More research is also going on to test the effect of other conditions such as temperature, substrate initial concentration and product ac-

cumulation. Preliminary tests give a maximum specific rate of whey utilization of 0.026 h^{-1} and a half velocity coefficient less than 5 g/dm^3 .

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BIOKONWERSJA SILNIE ZANIECZYSZCZONYCH ŚCIEKÓW DO KWASÓW ORGANICZNYCH

Zbadano proces kwaśnej fermentacji dwóch rodzajów ścieków z przemysłu spożywczego (serwatki i ścieków pochodzących z produkcji oliwy). Wykonano testy okresowe dla obu rodzajów ścieków i produkcję lotnych kwasów tłuszczowych porównano z wynikami fermentacji glukozy (substrat łatwo ulegający biologicznemu rozkładowi). Dla serwatki uzyskano wysoki stopień konwersji (80%), kwas masłowy zaś był głównym organicznym produktem fermentacji. Dla ścieków pochodzących z produkcji oliwy uzyskano mniejszy stopień konwersji, głównym produktem fermentacji był natomiast kwas octowy. Stwierdzono, że obecność (lub brak) związku hamującego fermentację metanową oraz różne stężenia fosforanu mają nieznaczny wpływ na stopień konwersji. Dodatkowo przedstawiono wstępne wyniki badań kinetycznych.

БИОКОНВЕРСИЯ СИЛЬНО ЗАГРЯЗНЕННЫХ СТОЧНЫХ ВОД ДО ОРГАНИЧЕСКИХ КИСЛОТ

Исследован процесс кислого брожения двух видов сточных вод из пищевой промышленности (сыворотки и сточных вод, происходящих из производства масла). Проведены временные тесты для обоих видов сточных вод и производство летучих жирных кислот сравнено с результатами брожения глюкозы (субстрат легко подвергающийся биологическому разложению). Для сыворотки получена большая степень конверсии (80%), а масляная кислота была главным органическим продуктом брожения. Для сточных вод, происходящих из производства масла, полученная степень конверсии была меньше и главным продуктом брожения была уксусная кислота. Было установлено, что наличие (или отсутствие) соединения, тормозящего метанное брожение, а также разные концентрации фосфата, имеют незначительное влияние на степень конверсии. Добавочно представлены предварительные результаты кинетических исследований.