

Solids and COD Balances to Describe Fed-Batch Codigestion of Sludge and Completely Prehydrolyzed Lignocellulosics

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Material balances of volatile solids, total dry solids and COD in an anaerobic digester fed with mixtures of domestic sludges and prehydrolyzed lignocellulosics are proposed to describe fed-batch operation. Separate complete prehydrolysis of the feed performed to increase its contents of soluble organic substances proves to strongly affect the methanogenic phase, probably due to the formation of toxic inhibitors during lignin hydrolysis. This suggests that the removal of the residue remaining after hemicellulosic sugars solubilization is necessary to offer acceptable methane yields in a poorly-mixed digester as that used in this study. Although the proposed approach seems to apply satisfactorily to COD only at relatively low organic load, it can be proposed as a useful tool to follow the solids breakdown during fed-batch operation.

1 Introduction

Anaerobic processes are increasingly considered for the treatment of wastes with high organic strength mainly because of their valuable products and of the energy necessary to run aerobic processes [1]. The main residues submitted to anaerobic digestion are zootechnical sludges [2], nontoxic organic sludges from meat packing, brewing, pharmaceutical, chemical and food processing industries [3,4], and sludges from municipal wastewater treatment plants [5,6].

The peculiar advantages of this technology have recently been extended with success to the digestion of the organic fraction of municipal solid wastes (MSWOF) [7]. Improvements have been obtained by sourting the refuses at source or mechanically [8], shredding their organic fraction [9], separating the acidogenic phase from the methanogenic one [10], or recirculating a fraction of the effluent [11]. MSWOF and sludges can also be codigested to produce biogas. Cecchi et al. demonstrated that the biogas yield linearly increases with increasing the proportion of MSWOF with respect to the sludge, but also carbon dioxide content considerably increases [12].

Only a few attempts were made to improve anaerobic digestion efficiency by increasing the organic strength of the feed by bioaugmentation [13] or by preliminary hydrolysis [14,15]. Great difficulties are met when woody materials are contained in the waste because of their strong lignocellulosic and hemicellulosic structures, which need hydrolysis under drastic conditions before digestion. Dilute acid treatment [16,17] is effective to hydrolyze hemicellulosic fraction, but it produces some inhibitors of cell metabolism [18]. On the contrary, lignin and cellulose of the remaining residue can be only partially solubilized by separate alkaline hydrolysis at high temperature, but the resulting hydrolysate is strongly recalcitrant [19].

Preliminary steady-state results of fed-batch codigestion of separately prehydrolyzed cellulosic, hemicellulosic and ligninic materials mixed with settled sludge were presented in a previous paper [20]. A maximum methane production rate of only 5.6 mmol/l/d at organic load of 4.6 g_{COD}/l/d suggested that satisfactory efficiency of the system could exclusively be ensured by removing the ligninic fraction from the waste to be digested.

Lastly, although the kinetic study of fed-batch digestion of similar materials was well developed for the period of time between one feed and the next [21], no attempt was made to describe the approach to pseudostationary conditions from the beginning of the fermentation. A contribution is provided in this study where mass balances to describe the behaviors of COD, volatile solids and total dry solids are proposed.

2 Experimental

2.1 Experimental Setup

Fed-batch runs were carried out simultaneously using 3 liter and 2 liter digesters, both filled with active mesophilic sludges supplied by the municipal anaerobic digester of Volpara (Genoa, Italy). Given volumes of anaerobic sludges were daily replaced by the same quantity of feed and subsequently the reacting mass was gently mixed for 2 minutes. Time interval between two consecutive feeds was 24 hours. Digestion temperature was kept at 37 ± 0.1 °C by means of a water bath.

2.2 Feed Preparation

The procedures for both enzymatic hydrolysis of starchy residues and caustic lignin treatment were previously described [20]. The hydrolysis of the hemicellulosic fraction was performed according to the dilute acid T.V.A. procedure [17] followed by countercurrent operation.

To ensure a relatively constant composition of the feed, the sludge was centrifuged for 5 min at 2,000 rpm and subsequently diluted up to 91.6 g/l total dry solids. Suspensions

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prepared by mixing 1.00 liter dilute domestic sewage sludges, 0.682 liters wood hydrolysate, and 0.301 liters corn starch hydrolysate were diluted with tap water up to the selected COD. The average compositions of this suspension before dilution is listed in Tab. 1.

Table 1. Composition of the feed.

Component	Concentration	
Glucose	[g/l]	77,7
Maltose	[g/l]	2,32
Pentoses	[g/l]	37,6
Hexosic oligosaccharides	[g/l]	6,70
Acetic acid	[g/l]	10,7
Proteins	[g/l]	2,35
Furfural	[g/l]	1,00
Hydroxymethylfurfural	[g/l]	0,41

2.3 Analytical Procedures

COD, total dry solids, and volatile solids concentrations were determined as described in APHA Standard Methods [22]. Biogas production was measured by liquid level displacement. The fractions of methane, carbon dioxide, and nitrogen were determined by gas chromatography.

Amounts of the main components of biogas were calculated assuming that a) biogas can roughly be considered a perfect gas; b) biogas pressure can be considered coincident with the atmospheric pressure; c) molar fractions of secondary components of biogas (H₂S, H₂, NH₃, etc.) can be considered constant (4–5% of total biogas volume); d) vapor pressure of water at 37 °C is about 0.062 atm.

3 Theoretical

Although mixing in the digester was not ideal, the reactor can be modeled using the typical equations of a continuous stirred tank reactor (CSTR).

3.1 COD Balance

During the period of time between one feed and the next ($\Delta t = 1$ day), the reactor can be considered a closed system; so, the total oxidation number of all substances contained in the reactor before feeding should equal that of sludges plus biogas produced after a given time. The following COD balance can then be written:¹⁾

$$COD_{S(j)} (V_r - q \Delta t) + COD^\circ q \Delta t = COD_{S(j+1)} V_r + COD_{BG} V_r \quad (1)$$

where $COD_{S(j)}$ and $COD_{S(j+1)}$ are the CODs of the digesting mass at the “j-th” and “j+1-th” days, COD° the feed COD, COD_{BG} the concentration of biogas oxidizable components, V_r the working volume of the reactor, and q the flow rate.

Considering the following average composition for the secondary oxidizable components of biogas: 1.5% H₂S, 1.0% H₂, 3.0% N₂, and 0.5% NH₃ [5] and making reference to a biogas methane content of 50.0%, COD_{BG} can approximately be calculated by the equation

$$COD_{BG} = M_{O_2} (2 n'_{CH_4} + 1.5 n'_{H_2S} + 0.5 n'_{H_2} + n'_{N_2} + 1.25 n'_{NH_3}) \Delta t = \chi n'_{CH_4} \Delta t \quad (2)$$

where n'_{CH_4} is the daily molar production of methane per unit reactor volume, M_{O_2} the oxygen molecular weight, and $\chi \sim 0.068 \text{ gCOD/mmole}_{CH_4}$ the consumption of organic substances per produced millimole of methane.

COD balance between the “j-th” and “j+1-th” days can then be written as:

$$COD_{S(j+1)} = A \cdot COD_{S(j)} + (OLR - \chi \cdot n'_{CH_4(j)}) \Delta t \quad (3)$$

where

$$A = (V_r - q \Delta t)/V_r = 1 - \Delta t/\theta \quad (4)$$

OLR and θ being the organic load and the residence time, respectively.

Developing the balance from the beginning of fed-batch digestion, the COD of the digesting mass at the “j-th” day can be linked to both methane production and starting COD:

$$COD_{S(j)} = A^j COD_{S(0)} + \{OLR (1 + A + \dots + A^{j-1}) - \chi [A^{j-1} n'_{CH_4(1)} + A^{j-2} n'_{CH_4(2)} + \dots + n'_{CH_4(j)}]\} \Delta t \quad (5)$$

Since methane production achieves a constant value after about 10–15 days, (5) becomes:

$$COD_{S(j)} = A^j COD_{S(0)} + [(OLR - \chi n'_{CH_4})(1 + A + \dots + A^{j-1})] \Delta t \quad (6)$$

For $j \rightarrow \infty$ and $A < 1$ (pseudostationary conditions), is:

$$1 + A + \dots + A^{j-1} = 1/(1 - A) \quad (7)$$

which means that the COD in the digester approaches that of CSTR:

$$COD_S = (OLR - \chi n'_{CH_4}) \theta \quad (8)$$

3.2 Solids Balances

The solids balances can be developed taking in mind that anaerobic digestion includes many transformations and a lot

1) List of symbols at the end of the paper.

of intermediates. A simplified scheme of the reactions supposed to take place in the process is illustrated in Fig. 1.

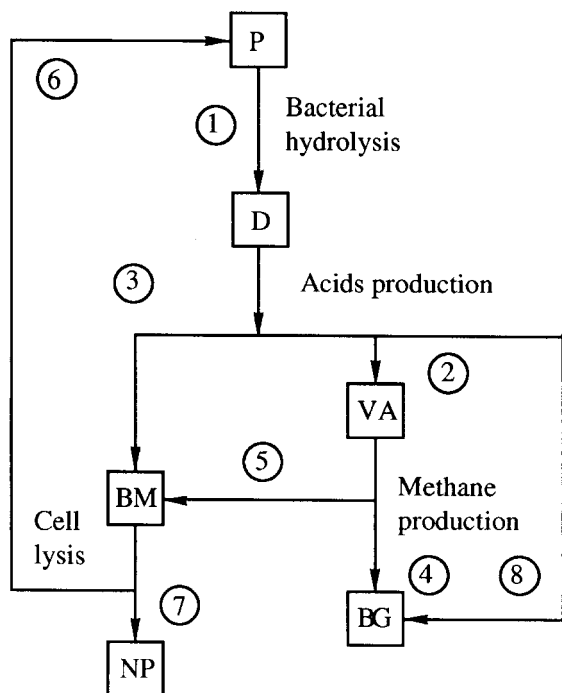


Figure 1. Scheme of the reactions taking place during the anaerobic codigestion of sludges and prehydrolyzed lignocellulosics. P = organic polymers directly hydrolyzable by microorganisms; D = simple organic substances either present in the feed or produced by bacterial hydrolysis; NP = hardly digestible organic polymers; BM = biomass; BG = biogas; VA = volatile acids.

Compounds can also be subdivided, according to their behavior when submitted to thermal tests, into total dry solids (TDS), volatile solids (VS), and inorganic compounds (I). Mass balance for total dry solids can then be written as:

$$W_{TDS} = W_D + W_{BM} + W_P + W_{NP} + W_I \quad (9)$$

Considering the rate, r_n , of a general “n-th” reaction of the scheme in Fig. 1 roughly constant within each time interval between one feed and the next, this balance becomes:

$$\frac{W_{TDS(j+1)} - W_{TDS(j)}}{\Delta t} = -\frac{W_{TDS(j)}}{\theta} + \frac{W_{TDS}^\circ}{\theta} - r_{2(j)} + r_{5(j)} - r_{8(j)} \quad (10)$$

Applying this equation from the beginning of the fermentation up to the “j-th” day, and defining:

$$r_{TDS} = r_8 + r_2 - r_5 \quad (11)$$

one can obtain:

$$W_{TDS(j)} = W_{TDS(0)} (1 - \Delta t/\theta)^j + W_{TDS}^\circ (\Delta t/\theta) \sum_{k=0}^{j-1} (1 - \Delta t/\theta)^k - \sum_{k=0}^{j-1} (1 - \Delta t/\theta)^k r_{TDS(j-k-1)} \quad (12)$$

It is also possible to express this balance through another iterative form which introduces the number of feeds (or days in the case under consideration) elapsing between two experimental data (m):

$$W_{TDS(j+m)} = W_{TDS(j)} (1 - \Delta t/\theta)^m + W_{TDS}^\circ (\Delta t/\theta) \sum_{k=0}^{m-1} (1 - \Delta t/\theta)^k - \sum_{k=0}^{m-1} (1 - \Delta t/\theta)^k r_{TDS(j+m-k-1)} \quad (13)$$

Since methane yield on consumed total dry solids depends on the feed composition and because of the lack of data on the digestion of woody materials, an empirical approach is followed here: the theoretical behavior of W_{TDS} is simulated without considering any degradation reaction and then r_{TDS} is calculated as the difference between theoretical and experimental values of this quantity. The absolute average consumption rate for a time interval of m days is then defined as:

$$R_{TDS} = \frac{\sum_{k=0}^{m-1} (1 - \Delta t/\theta)^k r_{TDS(j+m-k-1)}}{\sum_{k=0}^{m-1} (1 - \Delta t/\theta)^k} \quad (14)$$

On the analogy of (12), the following balance can be written for volatile solids:

$$W_{VS(j)} = W_{VS(0)} (1 - \Delta t/\theta)^j + W_{VS}^\circ (\Delta t/\theta) \sum_{k=0}^{j-1} (1 - \Delta t/\theta)^k - \sum_{k=0}^{j-1} (1 - \Delta t/\theta)^k r_{VS(j-k-1)} \quad (15)$$

According to the scheme of Fig. 1, it must be that:

$$r_{TDS} = r_{VS} = r_8 + r_2 - r_5 \equiv r, \quad R_{TDS} = R_{VS} \equiv R \quad (16)$$

For $j \rightarrow \infty$, the digester performance approaches that of CSTR under steady-state conditions:

$$R = (W^\circ - W)/\theta \quad (17)$$

4 Results

4.1 Previous Results

A previous set of batch digestion tests demonstrated [20] that maximum final methane and biogas specific productions (11.5 mmol/l and 0.620 l/l) were obtained with this feed using a starting COD of 3.8 g/l. The corresponding maximum ultimate methane yield (0.103 l/g_{VS}) was comparable with that reported for collected paper (0.084–0.100 l/g_{VS}) [23], but lower than for hardwoods excluding oak (0.22–0.32 l/g_{VS}) [24] and MSWOF (0.27 l/g_{VS}) [25] and considerably higher than for softwoods or yard wastes (0.01–0.06 and 0.05–0.09 l/g_{VS}, respectively) [23,26].

The pseudosteady-state results of fed-batch tests, also presented in the above work, evidenced an optimum organic load for methane yield on fed COD of 2.2 g/l [20] as well as a maximum load threshold over which the breakdown of solids could not be sustained (4.2 g_{TDS}/ld and 3.4 g_{VS}/ld, respectively).

The mean methane yield (52%) was little lower than that reported for presorted MSWOF (59.5%) [8], while maximum biogas and methane production rates (0.319 and 0.142 d⁻¹) were probably affected by the inhibiting role of ligninic byproducts. Corresponding maximum rate and yield of volatile solids breakdown were only 0.424 g_{VS}/ld and 14.7%, respectively, that are comparable with those reported for municipal sewage sludge (0.425 g_{VS}/ld and 25%) [8].

4.2 Evaluation of Mass Balances During Fed-batch Operation

The behavior of COD transformation into methane versus time (Fig. 2) shows that pseudosteady-state conditions were achieved more slowly at high rather than at low organic load, probably due to the faster metabolism of the acidogenic bacteria with respect to the methanogenic ones. In addition, the relative amplitude of oscillations generated by fed-batch operation were more marked at low rather than at high load. It was proposed that sludge under starvation conditions (low *OLR*), when subjected to new feed, produces biogas more quickly than well-fed sludge (high *OLR*) [20]. This “excess biogas production” was ascribed to an excess revival of activity of the biological system to the restored substrate availability after a starvation period, analogous to the so-called phosphorus “overplus accumulation” by activated sludges previously submitted to stress conditions [27].

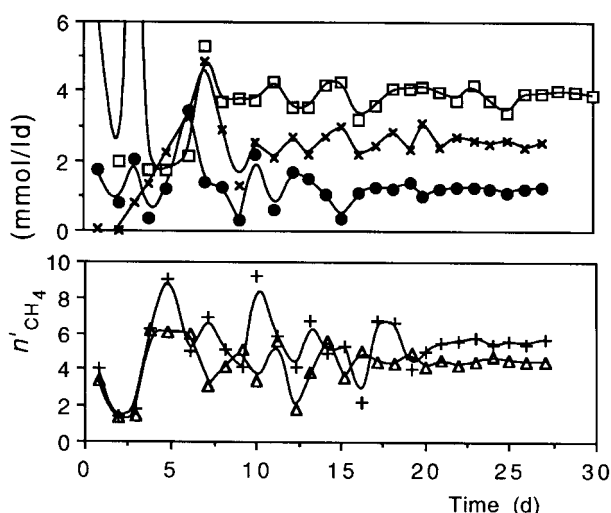


Figure 2. Methane daily production per unit reactor volume during the fed-batch codigestion of sludges and prehydrolyzed lignocellulosics at different organic loads. *OLR* (g_{COD}/ld): (●) 0.8; (×) 1.4; (Δ) 2.2; (+) 4.6; (□) 6.1.

Fig. 3 shows a comparison between COD data experimentally determined in the digester before the achievement of

steady state and those calculated by (3). Assuming a constant methane production after about 10–15 days, this estimation can be made by using the values of methane production rates obtained under steady-state conditions at the organic load under consideration (Tab. 2). Satisfactory agreement between calculated and experimental results was observed only at relatively low organic load (*OLR* ≤ 2.2 g_{COD}/ld). This means that daily consumption of digestible organic compounds was almost complete under this threshold, while at higher loads they tended to accumulate in the digester mainly in the form of VA. This was confirmed by consistent pH decreases, which provoked a deterioration of the fermentation system.

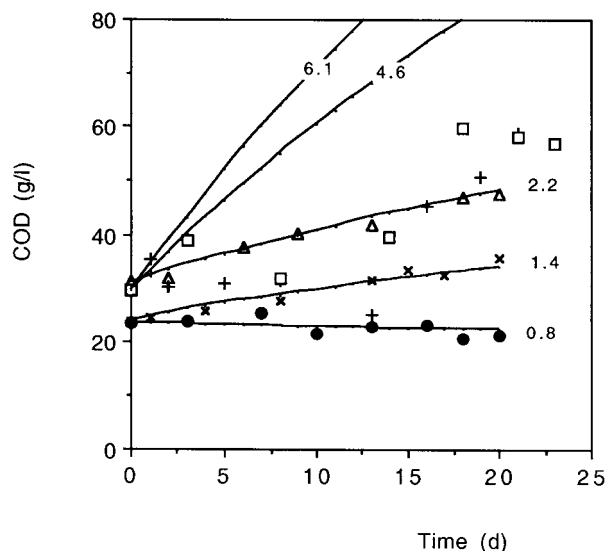


Figure 3. Comparison between theoretical (continuous curves) and experimental values (symbols) of COD in the digester during the fed-batch codigestion of sludges and prehydrolyzed lignocellulosics. Parameter = *OLR* (g_{COD}/ld): (●) 0.8; (×) 1.4; (Δ) 2.2; (+) 4.6; (□) 6.1.

Table 2. Parameter values used in the COD balance proposed for fed-batch codigestion of sludges and prehydrolyzed lignocellulosics. $\Delta t = 1$ day; $\chi = 0.068$ g_{COD}/mmol_{CH4}

<i>OLR</i> (g _{COD} /ld)	0,8	1,4	2,2	3,4	4,6	6,1
Residence time (d)	20	20	20	30	20	30
V_r (l)	3,0	2,0	2,0	3,0	2,0	3,0
A (-)	0,95	0,95	0,95	0,97	0,95	0,97
n'_{CH4} (mmol _{CH4} /ld)	1,21	2,54	4,47	5,24	5,57	3,96

Fig. 4 shows, as an example, the behaviors of both volatile and total dry solids concentrations versus time during the start-up of anaerobic digestion carried out at residence time of 20 days and at volatile solids loading rate of 4.60 g_{COD}/ld. In particular, the experimental data of solids concentrations are compared with the respective theoretical values which would be obtained without considering any degradation reaction

(W_{VS}^{th} and W_{TDS}^{th}). The corresponding behaviors of the absolute average consumption rates (R_{VS} and R_{TDS}) as well as the experimental ratio of volatile solids to total dry solids (ω) are shown in Fig. 5. Since both R_{VS} and R_{TDS} were found to reach constant values after a certain time from the beginning of fermentation, one can reasonably think that both solids consumption rates tend to go together with actual daily consumption rate of organic substances. In addition, as a comparison between the values calculated for these parameters shows, equalities described by (16) are satisfactorily verified, thus confirming the validity of the assumption that all dry solids consumption is due to volatile solids degradation and that the proposed method for reaction rate calculation could reliably be applied to different feeds.

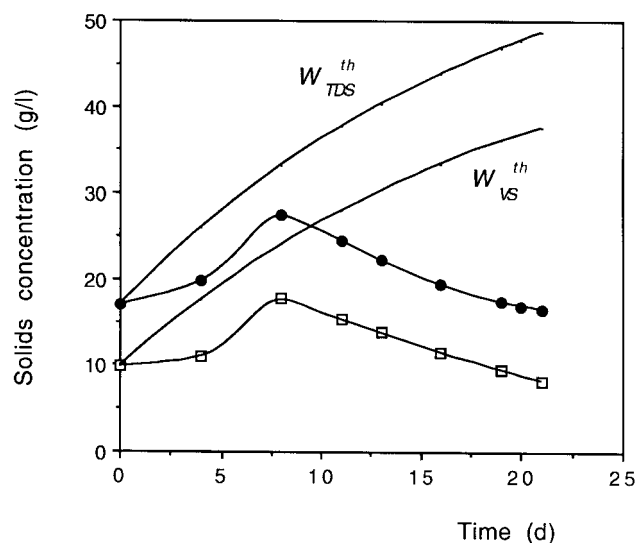


Figure 4. Behaviors of volatile and total dry solids concentrations during the fed-batch codigestion of sludges and prehydrolyzed lignocellulosics. $\theta = 20$ days. $OLR = 4.6 \text{ g}_{COD}/\text{ld}$. Theoretical values calculated without considering breakdown: W_{VS}^{th} , volatile solids; W_{TDS}^{th} , total dry solids. Experimental values: (\square) volatile solids; (\bullet) total dry solids.

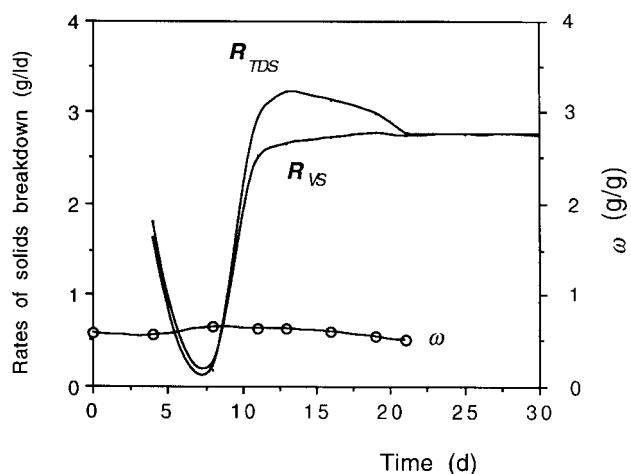


Figure 5. Comparison of the absolute average rates of volatile solids (R_{VS}) and total dry solids (R_{TDS}) breakdown during the fed-batch codigestion of sludges and prehydrolyzed lignocellulosics. $\theta = 20$ days. $OLR = 4.6 \text{ g}_{COD}/\text{ld}$. (\circ) Experimental ratio of volatile solids to total dry solids.

It is worth noticing that, although the prehydrolysis of woody wastes tested in this study (including solubilized lignin fraction) allowed to work at organic loads of volatile solids about 30% higher than the traditional digestion of municipal sewage sludges, most of the production parameters were substantially lower. This unsatisfactory result, which can reasonably be ascribed to the formation of toxic inhibitors released by the hydrolysis of lignin [28], suggests to remove this fraction just after hemicellulose hydrolysis before digestion.

5 Conclusions

Sludges from municipal wastewater treatment have been mixed with starch, hemicellulose and lignin hydrolysates, in proportions simulating the organic strength of hydrolyzed forest residues rich in woody materials, and subsequently digested in fed-batch operation. Hydrolysis has proved useful to increase the total amount of polluting substances to digest, but has certainly affected the yield of transformation of the organic substances into methane. It is possible that lignin degradation byproducts have strongly inhibited the anaerobic metabolism. It seems suitable to look for best operative conditions for a partial chemical hydrolysis of woody wastes in order to mildly quicken volatile acid production and so to optimize the digestion. The next attempt will be to only recover and digest hemicellulose and (eventually) cellulose fractions and to remove the toxic ligninic fraction.

The investigation on the start-up phase has allowed to propose material balances describing either COD or solids behaviors in such a digester, utilising feeds more concentrated, in terms of both COD and volatile solids, than the materials usually feeding traditional anaerobic digesters. COD balance described satisfactorily the behavior of organic substances only at relatively low organic load at which they are completely consumed by the microbial consortium. Solids balances, on the other hand, can be considered as a useful tool to follow the digester performances.

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Symbols used

A	$[-]$	bulk constant, defined in (4)
COD	$[\text{g}_{O_2}/\text{l}]$	chemical oxygen demand
M	$[\text{g}/\text{mmol}]$	molecular weight
n	$[\text{mmol}/\text{l}]$	molar production of gas per unit reactor volume
OLR	$[\text{g}_{COD}/\text{ld}]$	organic loading rate
q	$[\text{l}/\text{d}]$	feed flow rate
r	$[\text{g}/\text{ld}]$	reaction rate
\bar{r}	$[\text{g}/\text{ld}]$	average reaction rate
R	$[\text{g}/\text{ld}]$	absolute average rate of solids consumption
t	$[\text{d}]$	fermentation time

V [l] volume
W [g/l] mass concentration

VA volatile acids
VS volatile solids

Greek symbols

θ [d] residence time
 λ [g/mmol_{CH₄}] equivalent COD ascribable to biogas per produced millimole of methane
 ω [g_{VS}/g_{TDS}] ratio of volatile solids to total dry solids in the sludge

Subscripts

j value referred to the “j-th” day of fermentation
m number of days or feeds elapsing between two experimental data
n value referred to the “n-th” reaction
r reactor

Superscripts

' value referred to the time unit
° value referred to the feed

Abbreviations

BG biogas
BM living biomass
D simple organic substances, except VA, directly metabolizable by microorganisms
I inorganic compounds
MSW municipal solid wastes
MSWOF organic fraction of municipal solid wastes
NP organic polymers which cannot be directly hydrolyzed by microorganisms
P organic polymers directly hydrolyzable by microorganisms
S sludges
TDS total dry solids

References

- [1] Sixt, H.; Sahm, H., *Biomethanation*, in: *Biotechnology of Waste Treatment and Exploitation* (J. M. Sidwich, R. S. Holdom, Eds.), Ellis Horwood Ltd, Chichester 1987, pp. 147–172.
- [2] Hobson, P. N.; Shaw, B. G., *Wat. Res.* 8 (1974) pp. 507–516.
- [3] Naveau, H. P.; Nyns, E. J.; Binot, R.; Delafontaine, M., *Recycling of Effluents and Organic Residues into Methane by Anaerobic Digestion. New Perspectives*, in: *Recycling Berlin '79* (K. J. Thomé-Kozmiensky, Ed.), Vol. 2, E. Freitag, Berlin 1979, pp. 783–788.
- [4] Di Bernardino, S.; Bersi, R.; Converti, A.; Rovatti, M., *Bioproc. Eng.* 16 (1997) pp. 65–70.
- [5] Del Borghi, M.; Solisio, C.; Ferraiolo, G., *La Chimica e l'Industria (Milan)* 66 (1984) pp. 90–96.
- [6] Del Borghi, M.; Solisio, C.; Ferraiolo, G., *La Chimica e l'Industria (Milan)* 66 (1984) pp. 519–526.
- [7] Cecchi, F.; Mata-Alvarez, J., *Anaerobic Digestion of Municipal Solid Waste. An Up-to-date Review*, in: *Solid State Cultivation* (H. W. Doelle, D. A. Mitchell, C. E. Rolz, Eds.), Elsevier, Essex (UK) 1992, pp. 369–389.
- [8] Mata-Alvarez, J.; Cecchi, F.; Pavan, P.; Llabrés, P., *Biological Wastes* 33 (1990) pp. 181–199.
- [9] Traverso, P. G.; Cecchi, F., *Biomass* 16 (1988) pp. 97–106.
- [10] Wise, D. L.; Wentworth, R. L.; Augenstein, D. C.; Cooney, C. J., *AIChE Symp. Series* 181 74 (1978) pp. 47–55.
- [11] Cecchi, F.; Marcomini, A.; Pavan, P.; Fazzini, G.; Mata-Alvarez, J., *Ingegneria Sanitaria* 38(4) (1990) pp. 20–24.
- [12] Cecchi, F.; Traverso, P. G., *La Chimica e l'Industria (Milan)* 67 (1985) pp. 609–616.
- [13] Gijzen, H. J.; Zwart, K. B.; Teunissen, M. J.; Vogels, G. D., *Biotechnol. Bioeng.* 32 (1988) pp. 749–755.
- [14] Novelli, A.; Ottonello, F.; Converti, A.; Lodi, A.; Rovatti, M.; Del Borghi, M., *Chem. Biochem. Eng. Q.* 9 (1995) pp. 195–199.
- [15] Grethlein, H. E., *Biotechnol. Bioeng. Symp.* 5 (1975) pp. 303–318.
- [16] Parajó, J. C.; Vasquez, D.; Alonso, J. L.; Santos, V.; Dominguez, H., *Holz als Roh- und Werkstoff* 51 (1993) pp. 357–363.
- [17] Beck, M.J.; Strickland, R.C., *Biomass* 6 (1984) pp. 101–110.
- [18] Azhar, A. F.; Bery, M. K.; Colcord, A. R.; Roberts, R. S.; Corbitt, G. V., *Biotechnol. Bioeng. Symp.* 11 (1981) pp. 293–300.
- [19] Pfeffer, J. T.; Khan, K. A., *Biotechnol. Bioeng.* 18 (1976) pp. 1179–1191.
- [20] Converti, A.; Drago, F.; Ghiazza, G.; Del Borghi, M.; Macchiavello, A., *J. Chem. Tech. Biotechnol.* 69 (1997) pp. 231–239.
- [21] Cecchi, F.; Mata-Alvarez, J.; Traverso, P. G.; Medici, F.; Fazzini, G., *Biomass* 23 (1990) pp. 79–102.
- [22] APHA, *Standard Methods for the Examination of Water and Wastewater*, 14th edition, APHA, AWWA, WPCF, Washington DC 1975.
- [23] Owens, J. M.; Chynoweth, D. P., *Wat. Sci. Technol.* 27(2) (1993) pp. 1–14.
- [24] Chynoweth, D. P.; Jerger, D. E., *Devel. Indust. Microbiol.* 26 (1985) pp. 235–246.
- [25] Cecchi, F.; Pavan, P.; Mata-Alvarez, J.; Bassetti, A.; Cozzolino, C., *Waste Management & Research* 9 (1991) pp. 305–315.
- [26] O' Keefe, D. M.; Chynoweth, D. P.; Barkdoll, A. W.; Nordstedt, R. A.; Owens, J. M.; Sifontes, J., *Wat. Sci. Technol.* 27(2) (1993) pp. 77–86.
- [27] Converti, A.; Zilli, M.; Poloniecki, R. H.; Del Borghi, M.; Ferraiolo, G., *Wat. Res.* 27 (1993) pp. 791–798.
- [28] Watson-Craick, I. A.; Sinclair, K. J.; James, A. G.; Sulisti, Senior, E., *Wat. Sci. Technol.* 27(2) (1993) pp. 15–24.