Thermodynamic considerations on the performance of two-staged anaerobic digesters

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Abstract

An upflow acidogenic phase hybrid digester (combining a sludge blanket and fixed film) was sampled at different digester levels (200, 400, 600 and 800 mℓ) in order to evaluate thermodynamically the theoretical amount of energy of the metabolites present for use as substrate by the syntrophic methanogenic bacteria in a second stage digester. The distribution of metabolite concentrations along the digester axis was evaluated at different temperatures (29 to 40°C). The major metabolites detected were ethanol, acetic, propionic, n-butyric and n-caproic acid. Results showed that most of the metabolites were markedly influenced by changes in temperature and concentrations varied at the different digester levels. Calculations indicated that at a temperature of 35°C and at all digester levels the composition of metabolites in the effluent was optimal with respect to the thermodynamic energy theoretically available to the organisms in a second acetogenic/methanogenic stage.

Introduction

The physical separation of the anaerobic digestion process into the acid production (acidogenic) phase and the methane production (methanogenic) phase has been proposed as a means of increased control over substrate flow (Cohen *et al.*, 1979; 1980) and subsequent energy conservation (Van Andel and Breure, 1984). However, two-staged digester systems are not used much in practice due to increased capital costs and the necessity for more sophisticated control. In practice many full-scale plants make use of a feed balancing tank because of fluctuations in feed composition (Ross, 1989). This feed balancing tank could be used as an acidogenic stage pre-digester so as to manipulate the composition of the metabolites. It has been shown that methanogenesis can be enhanced in a two-stage process if certain energetically favourable acidogenic metabolites can be produced which are then used as substrate by the methanogenic population (Pipyn and Verstraete, 1981). However, controlled substrate flow in anaerobic digesters is difficult to ensure and as a result little control can be exerted on energy yields in these digesters.

Various researchers have shown that substrate pH and digester temperature are two external parameters which have a marked influence on the composition of acidogenic phase metabolites (Breure *et al.*, 1985; Cohen *et al.*, 1984; Zoetemeyer *et al.*, 1982). It was also shown that control over substrate flow by manipulation of the substrate pH and digester temperature is possible (Joubert and Britz, 1986). Since mixed bacterial populations have hitherto been used in acidogenic phase digesters, little effective control can be exerted on substrate flow by means other than the manipulation of external parameters. If insight can be gained into the metabolite composition inside the acidogenic phase digester, it might be possible to predict whether these external parameters can be manipulated to increase two-stage digester efficiencies. The aim of this study was thus to determine the extent of variation in metabolite composition at the different levels in an upflow acidogenic phase digester resulting from changes in temperature and whether these variations can be used effectively as an efficiency-forecast parameter for a two-stage digester system.

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Experimental

Digester

An upflow hybrid reactor (Fig. 1), as previously described by Joubert and Britz (1986), combining an upflow sludge blanket and

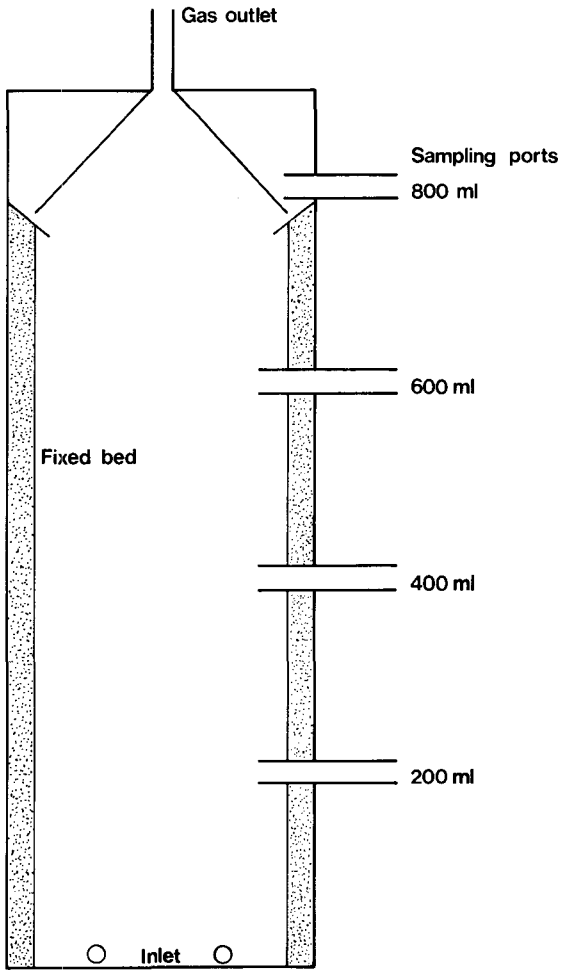

Figure 1
Diagram of the acidogenic phase hybrid digester

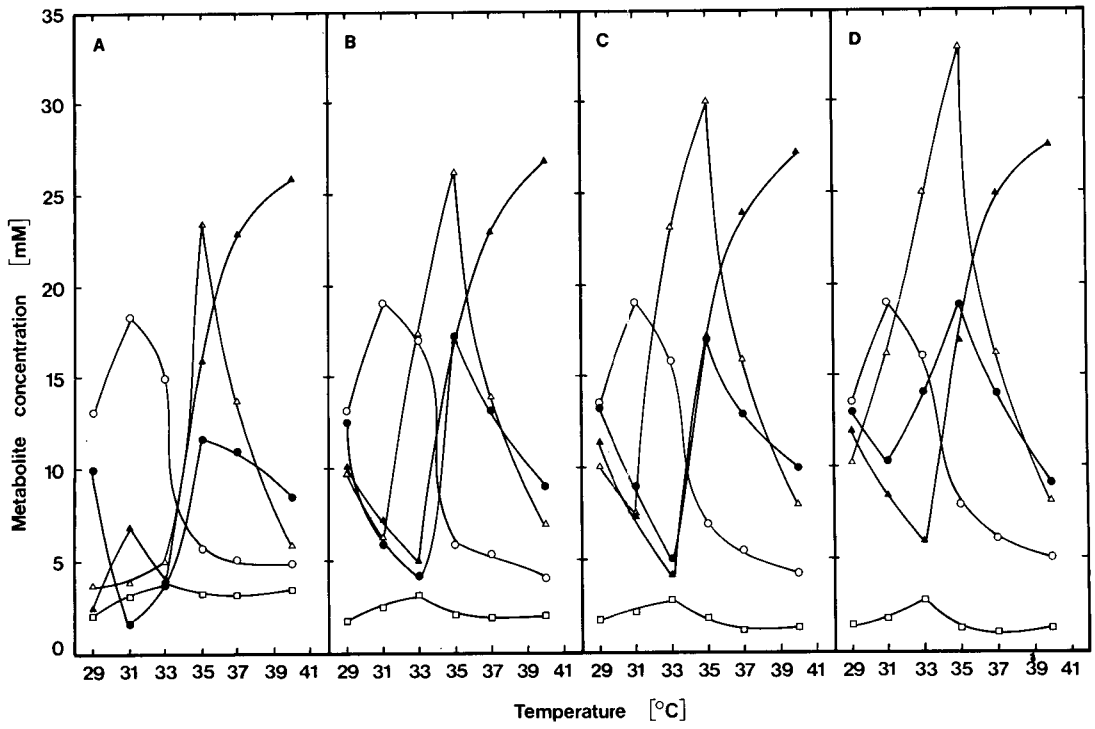

Figure 2
Influence of temperature on metabolite formation and concentration at the different digester levels. A=200 mℓ sampling port. B=400 mℓ sampling port. C=600 mℓ sampling port. D=800 mℓ sampling port (● = ethanol; Δ=acetic acid; O = propionic acid; ▲ = butyric acid; □ = caproic acid)

fixed film digester, was used as an acidogenic phase digester. Biogas exited at the top via a gas-solid separator. Gas production was measured by displacement of a brine solution and volumes corrected for standard temperature and pressure (STP). The reactor was originally seeded using a mixture of active laboratory sludge and municipal sewage digester sludge. Sampling ports, representing reactor volumes of 200, 400, 600 and 800 mℓ, were fitted to the side of the digester. The reactor was continuously fed with a sterile synthetic sucrose substrate (COD = 9 480 mg/ℓ) consisting of: sucrose, 9,0 g/ℓ; KH_2PO_4, 0,5 g/ℓ; urea, 0,5 g/ℓ and trace elements (Nel *et al.*, 1985). The substrate was thoroughly boiled and allowed to cool before use. The substrate pH (pH 5,9) was not adjusted and no continuous neutralisation was used in the reactor. Constant reactor temperatures (29°, 31°, 33°, 35°, 37° and 40°C) were maintained using the method of Meyer *et al.* (1983). The digester was operated under stable state conditions at a loading rate of 25,9 kg COD/m³.d and a hydraulic retention time of 11 h. In this study steady state conditions were assumed when, after 10 volume turnovers, after each of the individual temperatures had been set, parameters showed a variation of less than 5%. This was repeated three times.

Gas chromatography

All metabolites were identified using dual columns (Joubert and Britz, 1986). Volatile fatty acids (VFA) were determined using a Hewlett Packard gas chromatograph (GC), equipped with a flame ionisation detector. A 30 m x 0,75 mm i.d. SP1000 capillary column was used. The chromatograph was programmed at an initial temperature of 120°C, then increased at a rate of 6°C/min and finally held at 160°C. The detector and the inlet temperatures were 250°C and 160°C respectively and nitrogen used as carrier at a flow rate of 3 mℓ/min. Sample volumes were 2 μℓ.

A Hewlett Packard 5710A GC equipped with a 1,5 m x 1,2 mm stainless steel column packed with Porapak N, 100-120 mesh, was used to determine ethanol. Nitrogen at a rate of 50 mℓ/min was used as carrier gas. The inlet temperature was 200°C, column temperature 165°C and the FID temperature 250°C. Sample volumes of 3 μℓ were used.

Biogas composition was determined using a Perkin Elmer 820 GC equipped with a thermal conductivity detector and column (4,0 m x 0,3 mm i.d.) packed with Porapak N, 80-100 mesh. The oven temperature was set at 55°C and hydrogen used as carrier gas.

Results and discussion

Metabolites

The influence of temperature on metabolite formation and concentration, detected at the different digester levels, is illustrated in Fig. 2. The major metabolites detected include ethanol, acetic, propionic, n-butyric and n-caproic acid. From Fig. 2 it can be seen that the maximum concentrations of ethanol (18,4 mM), acetic acid (32,8 mM), propionic acid (19,6 mM), n-butyric acid (28,2 mM) and n-caproic acid (4,1 mM) were respectively obtained at 35°, 35°, 31°, 40° and 33°C. Results illustrated in Fig. 2 clearly show that the concentrations of all the metabolites detected in the digester, excluding caproic acid, were markedly influenced by temperature. It is also clear that, excluding propionic and caproic acids, metabolite compositions varied markedly in the digester and

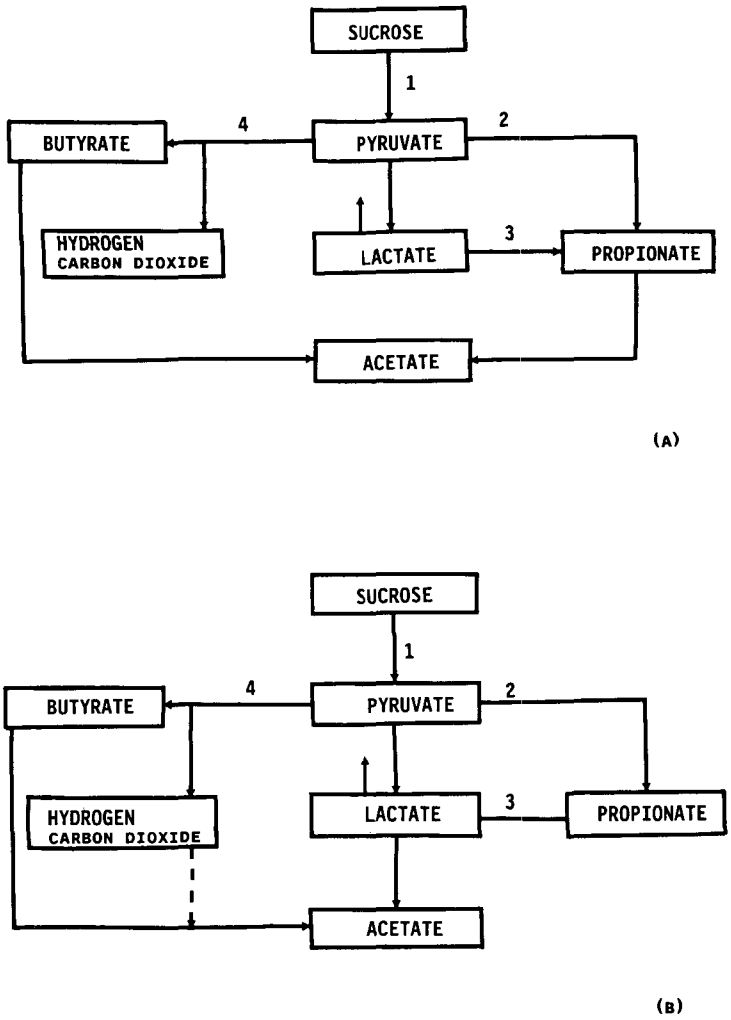

Figure 3
Fermentation modes of sucrose to pyruvate and pyruvate to various metabolic end-products (1 = Embden-Meyerhof-Parnas pathway; 2 = succinate-propionate pathway; 3 = acrylate pathway; 4 = butyrate pathway)

were greatly influenced by the different digester levels. This marked influence of the digester level on the metabolite composition strongly suggests that the liquid flow within the digester was primarily of the plug flow type. If the digester had been operated at a lower retention time, liquid flow within the digester was primarily of the plug flow type. If the digester had been operated at a lower retention time, liquid flow within the digester would have approached a mixed flow pattern (Joubert and Britz, 1987), thereby reducing the effect of digester level on the metabolite composition. Since the various metabolite concentrations were not influenced by the digester level to the same extent, the homogeneity of the bacteria within the digester may, to some extent, be suspect. It can be theorised that if the various types of bacteria had been dispersed homogeneously inside the digester, the influence of digester level on metabolite composition would have been more equal.

In a previous study (Joubert and Britz, 1986) it was found that both temperature and substrate pH had a marked effect on acidogenic phase metabolite composition and concentrations. In addition it can also be seen (Fig. 2) that digester level (in this upflow digester configuration) influenced metabolite concentrations. It would therefore be advantageous to obtain an analysis of the acidogenic phase effluent or if this phase is seen as an acidogenic stage pre-digester, to enable one to possibly improve the performance of a second phase methanogenic digester by directing the substrate to an energetically favourable effluent composition to be used as substrate for the acetogenic/methanogenic phase digester. Pipyn and Verstraete (1981) reported that the order of preference for formation of energetically favourable, acidogenic phase metabolites are lactic acid, ethanol, butyric acid, acetic acid and propionic acid. It should, however, be kept in mind that this order of preference was derived for a digester set-up where the gas, produced in the acidogenic phase digester, was directly fed to the methanogenic phase digester. This was to conserve the large amount of energy as hydrogen. If, from a practical point of view, no gas circulation is used, as was the case in this study, the energy quantum available for the acetogenic bacteria in a methanogenic phase digester will only be available as aqueous acidogenic phase metabolites. Since a mixed bacterial population was used in this study it is not possible to predict the precise substrate flow, but two possible fermentation modes to various metabolic end-products, as illustrated in Fig. 3, can be postulated (Kisaalita *et al.*, 1989). If the available aqueous metabolites can be converted quantitatively to methane gas by the combined activities of the

TABLE 1
ENERGY AVAILABLE FOR THE SYNTROPHIC BACTERIA AT THE DIFFERENT ACIDOGENIC PHASE DIGESTER LEVELS (VALUES GIVEN IN JOULE (J) PER MOL SUBSTRATE CONVERTED)

Sampling port (mℓ)	Temp. (°C)	EtOH	Acetic acid	Propionic	n-Butyric	n-Caproic	Total
200	29°C	-927,7	-177,8	-747,1	-228,8	-264,8	-2 286,2
400		-1 141,8	-288,3	-747,2	-817,0	-198,6	-3 192,8
600		-1 159,6	-310,0	-764,1	-947,7	-158,9	-3 340,3
800		-1 159,6	-319,3	-781,1	-964,1	-102,4	-3 326,5
200	31°C	-80,3	-133,3	-1 052,8	-571,9	-410,4	-2 248,7
400		-544,1	-186,0	-1 086,7	-580,1	-317,8	-2 714,7
600		-856,3	-326,3	-1 086,7	-712,8	-267,2	-3 249,4
800		-877,7	-296,0	-1 098,0	-579,1	-115,7	-3 233,7
200	33°C	-267,6	-155,0	-826,4	-351,3	-542,8	-2 143,1
400		-383,6	-546,3	-837,7	-384,0	-410,4	-2 552,0
600		-463,8	-691,3	-849,0	-326,8	-357,5	-2 688,4
800		-1 240,0	-775,0	-888,6	-408,5	-344,2	-3 656,3
200	35°C	-1 240,0	-744,0	-322,6	-1 282,7	-450,2	-4 039,5
400		-1 552,1	-815,3	-350,9	-1 405,2	-251,6	-4 375,1
600		-1 507,5	-939,3	-396,2	-1 405,2	-198,6	-4 446,8
800		-1 623,4	-1 010,6	-430,2	-1 388,9	-158,9	-4 612,0
200	37°C	-1 008,0	-421,6	-288,7	-1 870,9	-450,2	-4 039,4
400		-1 204,2	-443,3	-305,6	-1 911,8	-225,1	-4 090,0
600		-1 088,2	-474,3	-333,9	-1 928,1	-185,4	-4 009,9
800		-1 240,0	-396,0	-333,9	-1 951,7	-108,9	-4 030,5
200	40°C	-515,5	-186,0	-181,1	-2 116,0	-476,6	-3 575,2
400		-811,7	-217,0	-226,4	-2 189,6	-278,0	-3 722,7
600		-811,7	-226,0	-254,7	-2 222,2	-198,0	-3 713,5
800		-816,3	-238,7	-254,7	-2 263,1	-132,4	-3 705,2

*It was assumed that the values for the Gibbs free energy of the reactions, $\Delta G^{0'}$, calculated by using a standard temperature of 25°C, were not significantly influenced by the different temperatures used (29 to 40°C)

acetogenic and methanogenic bacteria, calculations can be made to ascertain which acidogenic phase effluent, produced under the prevailing conditions, would provide the most energy for a second phase methanogenic digester. When the energetics of the biochemical reactions leading to methane are, therefore, compared for all the different acidogenic phase metabolites, it should theoretically be possible to forecast whether the current operating conditions can be altered to increase digester performance. However, since the production of acids cannot be restricted to the acidogenic phase digester alone, one must assume that thermodynamic considerations alone are of limited value in improving reactor performance.

Thermodynamics

During the mineralisation of the acidogenic phase effluent, in the methanogenic digester, hydrogen is an obligate product during the degradation of intermediates such as alcohols and fatty acids. High *in situ* concentrations of hydrogen, however, limit the metabolic reactions of hydrogen-producing bacteria. The individual reactions within the digester, therefore, occur under non-standard conditions. Thermodynamic evaluations of the different acidogenic phase effluents should therefore be calculated using the Gibbs free energy (ΔG) which is based on the *in situ* concentrations of the various reactants and products. Recent studies (Conrad *et al.*, 1986) have, however, shown that even if *in situ* reactions were endergonic, cluster formation and close contact between bacteria may eventually result in the degradation of such intermediates. Since analytical difficulties exist in determining the hydrogen partial pressures in methanogenic environments (Conrad *et al.*, 1986) actual Gibbs free energies (ΔG) of reactions under these conditions are difficult to obtain. However, under such circumstances the standard Gibbs free energy values, at pH 7 ($\Delta G^{0'}$), will at least provide some indication of energy favourable effluent compositions which will provide the maximum available energy for the most delicate and rate-determining bacterial groups. As part of this study, using sucrose as carbon source, the conversion of the various detected metabolites (ethanol, acetic, propionic, butyric and caproic acid) was evaluated thermodynamically using the standard free energy changes ($\Delta G^{0'}$) associated with the partial reac-

tions leading to methane formation. The energy values and partial reactions reported by Thauer *et al.* (1977) relate to the standard physiological conditions of pH 7,0, temperature of 25°C, substrate and product concentrations of 1,0 mol/kg in aqueous solution, and hydrogen in the gaseous state.

If the acidogenic phase effluent is directly fed to a second phase methanogenic digester, the methane bacteria can produce methane from acetic acid as shown in Eq. 1:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (1)$$

The energy derived from Eq. (1) is -31,0 kJ/mol acetic acid.

When propionic acid is used as substrate, it is firstly converted to acetic acid and hydrogen and these are then, in turn, converted to methane. The sum reaction for the conversion of propionic acid to methane yields -56,6 kJ/mol propionic acid. The butyric acid detected in the acidogenic phase effluent is converted to methane via acetic and hydrogen gas formation by syntrophy of acetogenic and methanogenic bacteria. The sum reaction for this conversion provides -81,7 kJ/mol n-butyric acid. The n-caproic acid formed by the acidogenic phase microorganism is converted to acetic acid and hydrogen in the second phase digester. Methane is formed from these substrates and the sum reaction provides -132,4 kJ/mol n-caproic acid for the syntrophic interaction of acetogenic and methanogenic bacteria. An alcoholic fermentation of the sucrose in the acidogenic phase digester was probably responsible for the ethanol detected in the first phase effluent. A conversion of ethanol to acetic acid and hydrogen is performed before methane can be produced from these substrates. The sum reaction yields 1,5 mol methane per mol ethanol and provides -89,2 kJ/mol ethanol for the acetogenic-methanogenic consortium.

From the sum reactions of methane production it can be calculated for the various acidogenic metabolites that the order or preference for metabolite formation, for this specific case where no gas circulation is used, is caproic acid, ethanol, butyric acid, propionic acid and finally, acetic acid. The amount of energy which can thus be obtained from the metabolites detected in Fig. 2 is calculated using these sum reactions (Table 1). From the energy values given in Table 1 and from Fig. 2 it can be seen that the amount of energy which can be obtained for the syntrophic acetogenic and methanogenic bacteria is markedly influenced by both the concentration and composition of the metabolites in the acidogenic digester effluent. From Table 1 it can be calculated that digester level increased the total available energy by ca. 45,5%, 43,9%, 70,6%, 14,2% and 3,6% at operating temperatures of 29°C, 31°C, 33°C, 35°C and 40°C respectively. At an operating temperature of 37°C the total avialable energy was hardly influenced by the digester levels. It is interesting to find that the best energy values were obtained at 35°C which is similar to results found with the manipulation of temperature and substrate pH (Joubert and Britz, 1986). The variation in available energy indicates that an operational acidogenic phase digester would not necessarily, under existing operating temperatures, provide the rate-determining bacterial groups of the methanogenic phase digester with a substrate composition enabling the highest energy output. If a sub-optimal energy situation is found, as is the case at operating temperatures of 29°C, 31°C, 37°C and 40°C (Table 1), alterations may be introduced to enhance two-stage digester efficiency. External operational parameters, such as loading rate or substrate pH, may be altered to favour the production of higher energy value products. From the partial reactions given by Thauer *et al.* (1977) and the data presented in Table 1 it can be seen that for the free energy changes of a methanogenic conversion of one mol substrate, more exergonic energy exists for the conversion of the more complex substrates to methane. However, when these energy values are normalised to one mol methane formed, the free energies for conversion of acetic, propionic, butyric and caproic acid are nearly equal (ca. -31 to -33 kJ/mol methane). The normalised free energy change of methane production from ethanol is, in comparison, -59,5 kJ/mol methane produced. From these energy values it may thus be concluded that although the free energy of formation of one mol methane remains nearly the same for the fatty acid substrates, a methanogen has to split a relatively small molecule, in the case of acetate, for the conversion to methane. If caproic acid is, however, used as substrate at least three microbes have to interact to accomplish the conversion to methane. The conversion of the more complex fatty acid substrate in the methanogenic phase digester therefore increases the necessity for very close interaction amongst the relevant microbes. This important prerequisite for mineralisation of the substrate and the fact that certain intermediary reaction rates may become rate limiting demands that future consideration should be given to the microbial groups which are actively involved in metabolite degradation if control over substrate flow is to be augmented.

Conclusions

Analysis of metabolite composition and concentrations inside the acidogenic phase digester may therefore be a valuable parameter to monitor two-stage digester performance. From the thermodynamic analysis it is also clear that the determination of the exact *in situ* concentrations of the various reactants, products and external parameters in the digester is very difficult to obtain; the standard Gibbs free energy changes, at pH 7,0, may provide some indication of thermodynamically favourable acidogenic phase digester effluents; and that thermodynamic analyses alone will only provide border conditions and cannot be used, as such, to evaluate digester performance. Yet these border conditions have to be met in the first place. How actual kinetics and gain in metabolic energy (ATP) of the organisms will influence the performance of a second stage methanogenic reactor merits further investigations.

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