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MICROCALORIMETRIC MONITORING OF ANAEROBIC DIGESTION PROCESSES

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Abstract

Microcalorimetry was used for monitoring anaerobic digestion processes of heavily polluted industrial waste waters (from cheese industry, distilleries, yeast plant). Interpreting the thermal power–time curves by HPLC, some sub-processes in batch cultures were tentatively identified as acidogenic, acetogenic and methanogenic. Processes underlying power–time curves up to 10 h were different for different wastes. In the case of cheese whey and distillery waste it was acidogenesis, in the case of sulfate containing waste – presumably reduction of sulfates. The effect of Biotreat 100 (BimKemi Eesti Ltd.), a preparation for removing H_2S from waste water, was observed for these processes.

Keywords: activated sludge, anaerobic digestion, microcalorimetry, monitoring, sulfate, sulfide, volatile fatty acids

Introduction

The tests to monitor the effects of inhibitory compounds on anaerobic bacteria, in particular on methanogens can be done directly with reactor sludge or with pure cultures of anaerobic bacteria. The parameters that indicate inhibition are the decrease of gas (methane) yield, accumulation of volatile fatty acids (VFA), the decrease of chemical oxygen demand (COD) removal efficiency [1]. Specific sludge activities are usually analysed using batch experiments that generate acute toxicity data on the effect of single dose of an inhibitor. Microcalorimetry has been widely used to study the growth of microorganisms, e.g. various bacteria and yeasts [2–5]. As heat production during microbial growth is characteristic of an organism under controlled growth conditions microcalorimetry can be also used for monitoring processes in anaerobic reactors [4]. Lately several studies on microbial growth kinetics, based on microcalorimetry, have been published, e.g. on environmental effects on bacterial growth [6–8] and determination of acute cellular toxicity [9]. The idea to use microcalorimetry for routine microbial testing first appeared in 1970s [10] but was put aside as other analytical techniques turned out to be cheaper and easier to handle [11]. At present isothermal microcalorimetric techniques are well developed and possibly this application area will soon become of practical importance [12]. In our previous study

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the power–time curves of reactor samples were used, in addition to traditional reactor parameters (pH, gas production, VFAs) as indicators for the performance of anaerobic digester fed with cheese industry waste [13, 14]. The aim of this study was to extend the possibilities of microcalorimetric monitoring to other types of substrate (distillery and yeast waste) and to use biological oxidation as means to cope with the inhibitory effect of sulfate containing wastes.

Materials and methods

Laboratory-scale anaerobic digester (W8, Armfield, UK) of 4.8 dm^{-3} working volume with biogas collection and measuring units was used at the temperature 35–37°C. It consisted of two independently operating units – upflow anaerobic sludge blanket reactor (UASB) and upflow anaerobic fixed film reactor (UAFF). Sludge seed for the reactors originated from sugar industry waste anaerobic treatment plant, plastic 'Bioball' filter elements were used as carrier for biomass in UAFF [15]. The reactors were used in parallel (cheese whey) or as a two-stage setup (distillery and yeast waste).

For monitoring the process, samples were taken anaerobically from the reactors and placed immediately into the 2277 Thermal Activity Monitor (Thermometric, Järfälla, Sweden) – an isothermal heat conduction microcalorimeter. Batch experiments were run in standard mode with 3 mL glass ampoules at 25 or 35°C. To initiate the experiment, substrate (distillery waste or cheese whey diluted with water to 3.6%, yeast waste to 36.6%), was added to the aliquot of sample taken from the reactor. Four parallel experiments, run simultaneously on the microcalorimeter were stopped at different time moments by removing the ampoule from the calorimeter and adding 2-propanol [13, 14]. Power–time curves were dynamically corrected and registered using data acquisition program Digitam 3.0 (Thermometric, Järfälla, Sweden).

HPLC analyses of the samples from microcalorimeter were performed on Waters HPLC 600 with RI detector (512x), using column HPX 87H and 0.0045 M H_2SO_4 as eluent [16]. Analyses of chemical oxygen demand (COD) and volatile fatty acids (VFA) content in the anaerobic reactors were performed separately, by standard methods for waste water examination [17]. COD determination corresponded to DIN 38409-H41-1, VFAs were isolated from waste water by distillation and titrated further with NaOH. Sulfate concentration was determined by turbidimetric method (Standard Method 4500-SO_4^{2-} for waste water [18]).

Results and discussion

Monitoring of anaerobic digestion processes

Microcalorimetric monitoring combined with HPLC analysis revealed the dependence of the shape of thermal power–time curve on the operational efficiency of the anaerobic reactor. With laboratory-scale UASB reactor processing cheese whey, three characteristic types of power–time curves were registered depending on organic

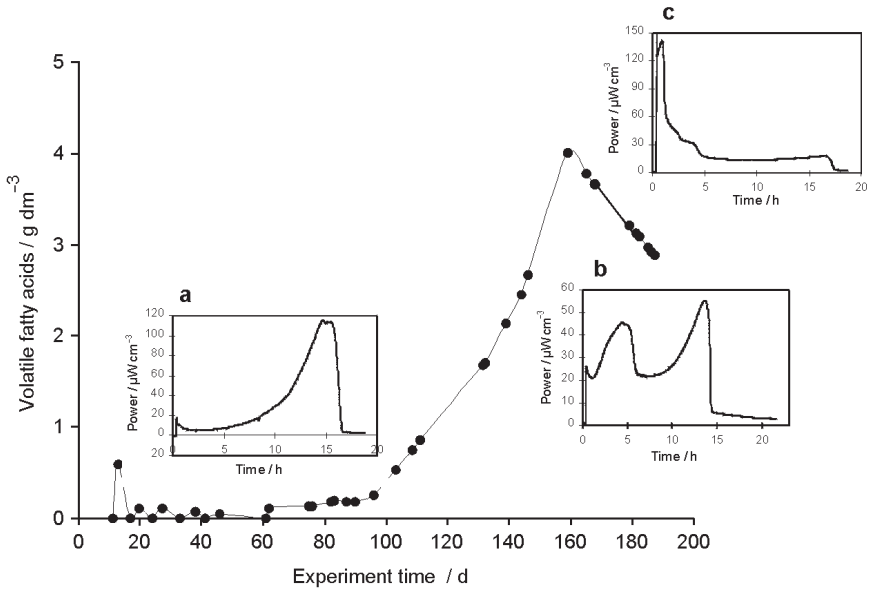

Fig. 1 Three types of power–time curves depending on the content of volatile fatty acids; a – experiment days 0 to 60, concentration of VFAs 50 to 100 mg cm^{-3}, b – experiment days 61 to 103, concentration of VFAs 137 to 530 mg cm^{-3}, c – experiment days 104 to 187, concentration of VFAs up to 4000 mg cm^{-3}. Organic loading rates cf. Fig. 3

loading rate (OLR) and volatile fatty acids content in the outlet. In the high efficiency stage of the bioprocess at low OLRs a single maximum (70 to 120 µW cm^{-3}) at 14 to 15 h was observed on the power–time curve (Fig. 1). Increasing OLR over 3.9 kg COD m^{-3} d^{-1} another maximum at 3 to 4 h appeared. The values of thermal power were 40 to 50 and 50 to 60 µW cm^{-3} correspondingly. The third type of power–time curve corresponding to serious process instability (at OLR over 7.8 kg COD m^{-3} d^{-1} was characterized by one sharp maximum in the region of 1 to 4 h. Describing the processes in bioreactors by microcalorimetry, it should be considered that growth of many species is proceeding simultaneously, as well as different substrates could be used depending on the reactor conditions (multiauxic growth). Therefore alternative analyses (HPLC, outplating) are inevitable. According to HPLC analysis, hydrolytic and acidogenic bacteria were most probably responsible for the first maximum on the power–time curve, as during the first 3 to 4 h characteristic products to this type of fermentation (lactic, propionic, acetic acid) were formed, simultaneously with the decrease of lactose concentration (Fig. 2a). The second maximum corresponds apparently to bacteria using lactate as a substrate, i.e. primarily acetogenic and acetoclastic bacteria with low K_s (limiting substrate concentration) values. The inhibitory factor turned out to be VFAs, especially acetate. If the speed of acetate formation exceeds its utilization speed by methanogens (third type of power–time curves), the accumu-

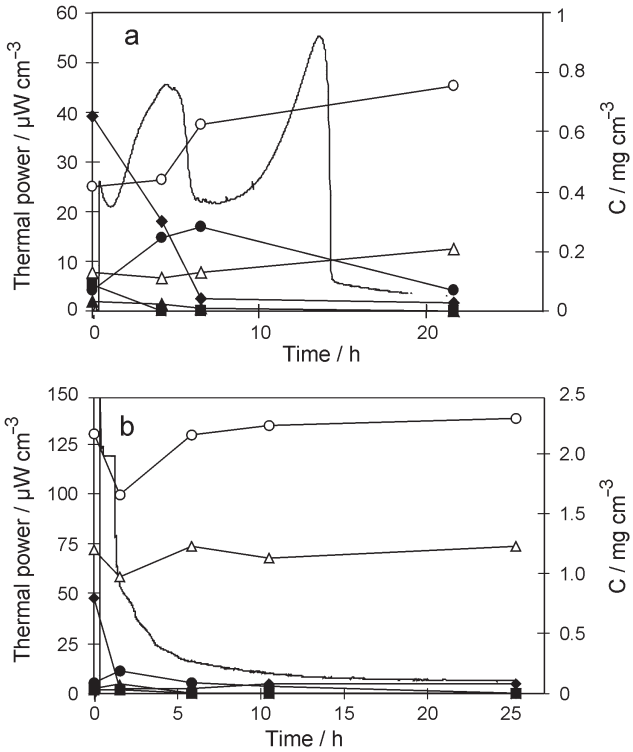

Fig. 2 Power–time curves and sugars and acids content in the UASB reactor with cheese whey a – at medium loadings; b – at high loadings. ◆ – lactose, ■ – glucose, ▲ – galactose, ● – lactate, o – acetate, and Δ – propionate concentrations were determined as described in methods

lation of the latter leads to the drop of pH together with the inhibiting effect to the whole system (Fig. 2b). Determination of VFAs is often used for establishing the danger of operational instability of the anaerobic reactor as acetic acid is a weak acid and the increase of VFAs is noticeable before extensive decrease of pH. The VFAs concentration indicating the danger of process instability is >5 g dm^{-3} (as total VFAs), and 2 g dm^{-3} (as acetate) [19].

Determination of quantitative characteristics of growth

Power–time curves can be used for the determination of the quantitative characteristics of growth [4–9]. In exponential growth phase the relationship between the concentration of biomass X and specific growth rate μ is described by the first order kinetic equation

$$dX/dt=\mu X \quad (1)$$

μ is usually determined from the measurement of biomass, i.e. from the plot of ln$X$ *vs*. time t. If the stoichiometry of biomass growth does not change during the growth, the rate and amount of biomass formation, (dX/dt) and (X–X_0), are proportional to the rate and amount of heat production, dQ/dt and Q. Assuming that the initial concentration of biomass is low, $X \cong X - X_0$, μ can be determined from calorimetric measurements, i.e. plot of ln$Q$ *vs*. time t [4, 9]. The rate of biomass increase is proportional to the rate of increase in the heat production (where Y_Q is the proportionality factor):

$$\mathrm{d}X/\mathrm{d}t = Y_Q \mathrm{d}Q/\mathrm{d}t \tag{2}$$

Using the definition of specific growth rate (Eq. (1)) and replacing it into Eq. (2) we get the relationship between μ and dQ/dt:

$$\mu X = Y_Q \mathrm{d}Q/\mathrm{d}t \tag{3}$$

On the other hand, the increase of biomass in the exponential growth phase is an exponential function:

$$X = X_0 e^{\mu t} \tag{4}$$

Replacing the expression for biomass from Eq. (4) into Eq. (3), the expression for the heat production rate is obtained:

$$\mu X_0 e^{\mu t} = Y_Q \mathrm{d}Q/\mathrm{d}t \tag{5}$$

$$\mathrm{d}Q/\mathrm{d}t = 1/Y_Q \mu X_0 e^{\mu t} \tag{6}$$

After integrating we get the direct relationship between heat production rate and specific growth rate underlying the proportionality between these two parameters:

$$\ln(\mathrm{d}Q/\mathrm{d}t) = \ln(\mathrm{d}Q/\mathrm{d}t)_{t=0} + \mu t \tag{7}$$

where $\ln(\mathrm{d}Q/\mathrm{d}t)_{t=0} = \ln(1/Y_Q \mu X_0 e^{\mu})$.

Equation (7) is the simplest equation for calculating specific growth rate. More sophisticated methods recommend considering also the microbial death rate constant [6–8] or time constant of calorimeter [5]. However, automatic dynamic correction of the instrument with Tian equation, used by us excluded the need for additional calculation of the latter [12]. Using Eq. (7), the specific growth rates were determined for basic bacterial consortia in the reactor – acidogenic and acetogenic/methanogenic bacteria. In the case of cheese whey the maximum specific growth rates for acetogenic/methanogenic bacteria in the UASB reactor in the stationary state were determined to be 0.3 h^{-1}. At VFAs concentration 1 g dm^{-3}, μ_{max} of acetogenic/methanogenic populations started to decrease (down to 0.05 h^{-1}) indicating that accumulation of acids in the reactor impedes the growth of this type of microflora (Fig. 3). μ_{max} of acidogenic bacteria did not decrease substantially and was in the region of 0.25 to 0.5 h^{-1} (data not shown). In the second stage of the distillery waste reactor, specific growth rates for acetogenic/methanogenic microflora were high (μ_{max}=0.2 to 0.5 h^{-1}), in good agreement with pH and gas production values (Fig. 4). In acidogenesis, mainly CO_2 is formed. In the acetogenic/methanogenic stage, in addition to that CH_4 is generated (55–75% of total gas volume), the increased gas production being an in-

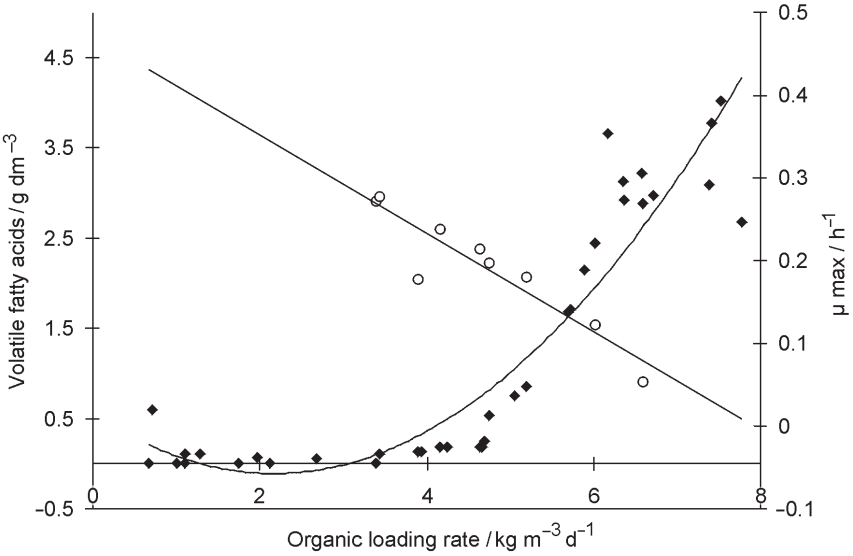

Fig. 3 Volatile fatty acids content and growth rate of acetogenic/methanogenic bacteria in the UASB reactor with cheese whey depending on the organic loading rate. ◆ – VFAs, o – μ_{max}

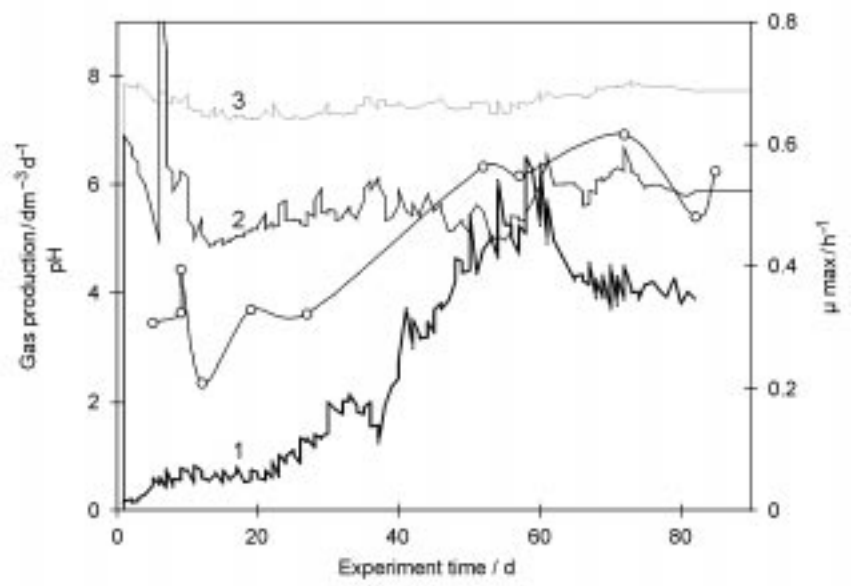

Fig. 4 Gas production (data from biogas collection and measuring unit), pH and specific growth rate of acetogenic/methanogenic bacteria (methanogenic stage in the two-stage anaerobic digester with distillery waste). 1 – gas, 2 – inlet pH, 3 – outlet pH, o – μ_{max}

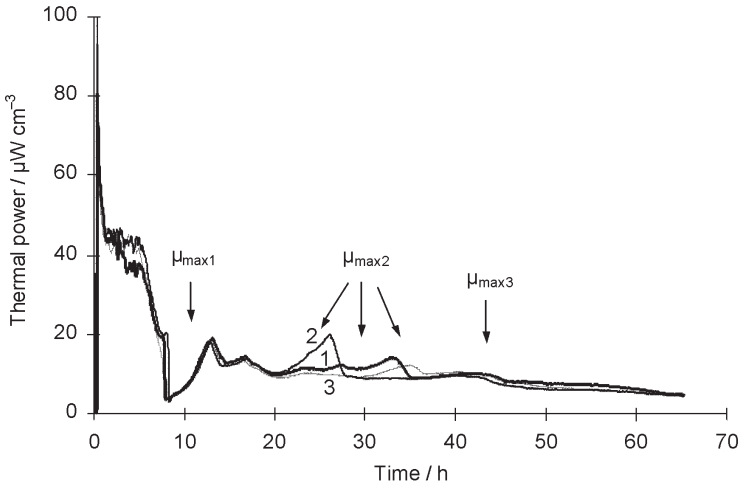

Fig. 5 Specific growth rate μ_{max} as an indicator on the influence of Biotreat on acetogenesis and methanogenesis in the first stage of yeast waste water digester. Substrate (yeast waste), diluted with water or Biotreat solution to 36.6%, was added to the sample taken from the reactor. Concentrations of Biotreat, 1 – 0 mg dm^{-3}, 2 – 50 mg dm^{-3}, 3 – 500 mg dm^{-3}

direct evidence of methanogenic activity. Anaerobic treatment of yeast industry waste water results in formation of sulfide as the main product of sulfate respiration. Depending on pH, hydrogen disulfide can be in the form of H_2S (gas), HS^-, S^{2-}. In microcalorimetric experiments, yeast waste, mixed with aqueous solutions of Biotreat 100, was added to the samples from the digester. Power–time curves show probable evolution of gas, followed by peaks of different consortia of bacteria (Fig. 5). Chemical analyses of these samples indicated rapid decrease of sulfate concentration during the first hours of experiment (from 2 to 1.3 g dm^{-3}). pH in these samples was below 7.5, indicating that at least 30% of H_2S+HS^- should have been in the form of H_2S (gas). Bacteria responsible for these processes were obviously sulfate reducing bacteria. Three more consortia of bacteria can be observed with μ_{max1}=0.38, μ_{max2}=0.06 to 0.13 and μ_{max3}=0.02.

Identification of subprocesses in anaerobic digester

On low organic loading rates with low VFAs concentrations, growth of bacteria with low values of K_s (acetogenic and acetoclastic (methanogenic) bacteria) is favoured in the batch sample in microcalorimeter (type *a* of power–time curve, Fig. 1). If the concentration of VFAs increases the conditions for the growth of hydrolytic and acidogenic microflora (with higher K_s values) will improve, expressed by the corresponding peak (type *b* of power–time curve, Fig. 1). In the conditions of overloading the quick start-up of acetoclastic processes is explained by high concentrations of acetate and propionate (over 1 g dm^{-3}) already at the beginning of experiment (type *c* of

power–time curve, Fig. 1). Due to high concentration of VFAs, growth of all groups of bacteria is inhibited. In the absence of sufficient oxygen (e.g. anaerobic microorganisms or aerobic cells at high rates of glycolysis), cells use substrate phosphorylation to provide the necessary adenosine triphosphate (ATP) by reducing pyruvate to lactate. The latter and products of its further metabolism (propionate, acetate, formate) are harmful to cells as their excretion lowers the pH of culture medium [11].

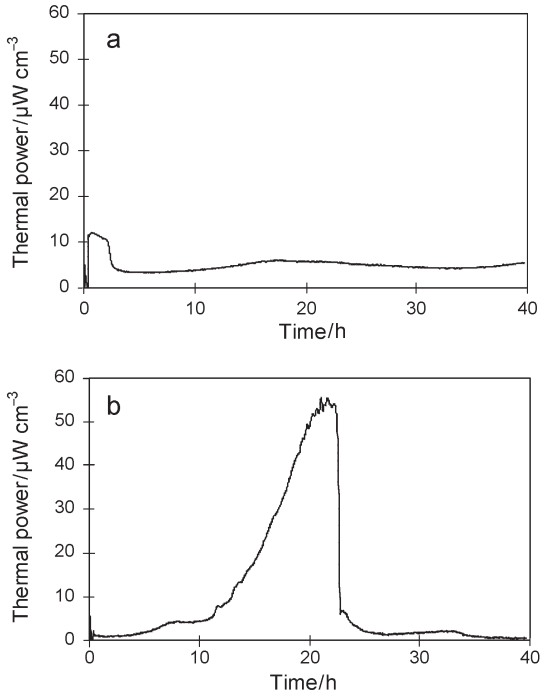

Fig. 6 Typical power–time curves for the a – acidogenic phase and b – acetogenic/methanogenic phase in the two-stage anaerobic digester with distillery waste

By power–time curves, the processes in the two-stage set-up (acidogenic and methanogenic stage) of distillery waste reactor could be clearly distinguished with maximums being in the range of 0 to10 h and 10 to 25 h, respectively (Fig. 6). To suppress the growth of hydrolytic and acidogenic bacteria, 0.025 g dm^{-3} Biotreat 100 was added to the first stage, guaranteeing the level of acetate below the inhibiting concentration (2 g dm^{-3}) up to the OLR 4.1 kg COD m^{-3} d^{-1}. By HPLC analysis, the plateau on power–time curve in this reactor was also generated by acidogenic microorganisms with typical products of metabolism lactic, acetic and propionic acid (data not shown). The VFAs produced in the acidogenic stage were readily used as substrate in the second stage, the methanogenic reactor. Concentration of acetate and other VFAs

remained below 400 mg cm^{-3} and the maximum on the power–time curve was in the region for acetogenic/acetoclastic bacteria (Fig. 7).

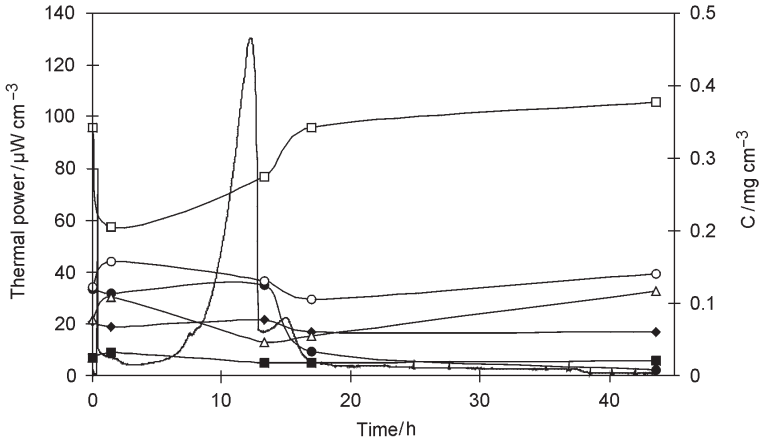

Fig. 7 Power–time curve and sugars and acids content in the acetogenic/methanogenic stage of anaerobic reactor with distillery waste. ◆ – maltose, ■ – glucose, ● – lactate, o – acetate, Δ – propionate and ❑ – butyrate concentrations were determined as described in methods

The processes in the anaerobic reactor treating yeast industry waste are different from the bioprocesses of cheese whey and distillery waste. Due to low content of sugars and acids and high content of betain and sulfates, yeast industry waste is biodegradable with difficulty. Two-stage scheme of anaerobic reactors was used again,

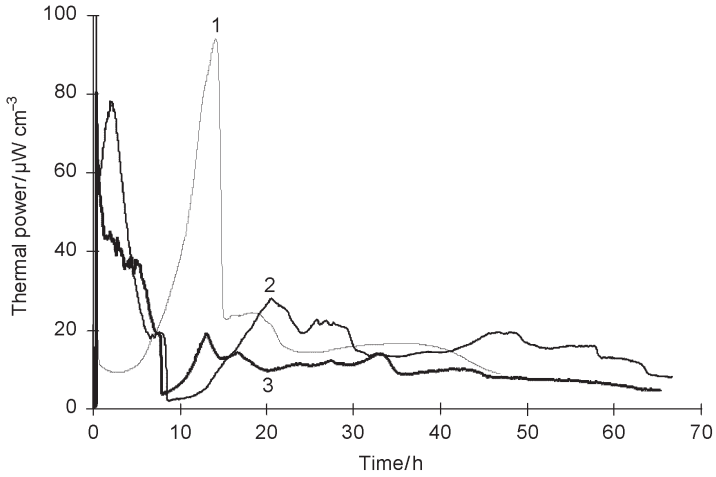

Fig. 8 Adaptation of granular sludge to yeast industry waste; 1 – day 61, 2 – day 86, 3 – day 94

with UAFF reactor in the first, sulfate reduction stage and UASB reactor in the second, methanogenic stage. The most important prerequisite for stable work of the digester appeared to be adaptation of sludge, well followed in microcalorimetric (batch) experiments of samples taken from the second stage, UASB reactor on days 61–94 (Fig. 8). At the end of the experiment the shape of power–time curves resembled that of the well-adapted USFF reactor samples (Fig. 5). Use of granulated sludge for the treatment of sulfate rich waste is not so much limited by the concentration of sulfides but by the slow increase of methanogenic biomass as compared to acetogens and sulfidogens which have lower values of K_s for their common substrates (acetate, formate, H_2) [20].

Influence of Biotreat 100 on anaerobic digestion process

Sulfate reducing bacteria (SRB) will competitively interact with other anaerobic bacteria involved in methanogenesis, resulting in the formation of H_2S rather than methane. One measure to reduce sulfide concentration is biological sulfide oxidation. Preparation Biotreat 100 is able to altering the dynamics of mixed anaerobic populations. Favouring the growth of sulfur oxidizing bacteria results in oxidizing hydrogen sulfide (H_2S) to more oxidized compounds, favouring the growth of methanogens leads to the increase in producing methane. Biotreat 100 consists of dried biomass of green microalgae *Chlorococcum* sp. and was isolated in BimKemi Eesti Ltd. [21]. It is unicellular eucaryotic microalga with spherical or ellipsoidal cells belonging to the phylum *Chlorophyta* (green algae). Small doses (0.02 g dm^{-3}), sufficient to maintain sulfide concentration in desired limits, indicate that these cells contain growth factor-like substances. Biotreat was originally designed to function in aerobic conditions at pH=6.0 to 9.0, temperature <45°C and H_2S concentration <0.08 g dm^{-3}. In this study we could prove that Biotreat was equally well usable in completely anaerobic conditions. Addition of it to all reactors (0.025 to 0.05 g dm^{-3}) resulted in increased biogas production and specific growth rate of acetogenic/methanogenic bacteria (Fig. 4). In microcalorimetric experiments with yeast waste, Biotreat was added supplementary to the samples taken from the digester. Bacteria with specific growth rate μ_{max2} were sensitive to various Biotreat concentrations and might be sulfur oxidizing bacteria (Fig. 5). The possible mechanism could be partial biological oxidation of sulfide to solid S^0. Under oxygen-limited conditions, i.e. dissolved oxygen concentrations below 0.1 mg dm^{-3}, S^0 is the main end product of sulfide oxidation [22].

Conclusions

Microcalorimetric monitoring combined with HPLC analysis revealed the dependence of power–time curves on the operational efficiency of the anaerobic reactor. Hydrolytic and acidogenic bacteria were most probably responsible for the first maximum on the power–time curve, as typical products to this type of fermentation were formed. The second maximum corresponds to bacteria using lactate as a substrate.

Biotreat 100, a preparation from green algae was well usable in completely anaerobic conditions and influenced the state of processes, as reflected in changes of power–time curves. The stable work of reactors was expressed in the increased biogas production and increased specific growth rates of acetogenic/methanogenic bacteria.

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