Treatment of high-strength dairy wastewater in an anaerobic deep reservoir: Analysis of the methanogenic fermentation pathway and the rate-limiting step

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ABSTRACT

The wastewater of the largest dairy factory in Israel (Tnuva, Tel-Yosef), discharging approximately 6000 tons BOD per year, is treated in two serial, deep reservoirs (anaerobic/facultative). In this study, which focused on the anaerobic reservoir, we combined in situ measurements (over 18 months) and supporting lab experiments, in order to evaluate its efficiency and to identify the rate-limiting step of the methanogenic fermentation pathway. The anaerobic reservoir could remove above 75% of the BOD and COD all year round, but this was not enough to prevent malodors during the winter. Acetate and propionate, products of lactose fermentation, were the predominant intermediate metabolites in the reservoir and their concentrations were strongly dependent on the temperature and the organic load. The combined effects of colder winter temperatures and seasonal increase of organic load, resulted in a decreased rate of propionate oxidation and a consequent accumulation of soluble BOD and COD. Laboratory batch experiments, conducted during this season, found propionate oxidation to be the rate-limiting step in the process, characterized by a lag period preceding its degradation.

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1. Introduction

Wastewater storage and treatment reservoirs were developed in Israel in the early 1970s. They were first used to accumulate treated effluent for irrigation purposes, but since their stabilization potentials have been realized, they are also used to treat raw wastewater (Mara and Pearson, 1992; Juanico and Shelef, 1994). Reservoirs are similar to wastewater stabilization ponds, but since they are much deeper and their volume is much higher, they are also similar to small lakes; therefore, limnological phenomena might become important (Friedler et al., 2003). Anaerobic stabilization ponds offer a cost effective way to treat wastewater with high organic content, since they are mechanically simple, easy to operate by non-professionals and require less land than facultative and aerobic ponds. However, this simplicity belies the high complexity of the physical, chemical and the microbial process. As a consequence, the microbial process within...
lagoons systems is less well understood than in biological reactors, while effluent quality is less predictable (Grady et al., 1999). Anaerobic reservoirs may have advantages and disadvantages similar to anaerobic ponds, although their efficiency has never been studied in detail.

Mineralization of organic matter under methanogenic conditions proceeds in a number of steps, carried out by different groups of bacteria (Harper and Pohland, 1986). Each of these steps and the balance between them is important for the overall process. Oxidation of VFAs and methanogenesis are low energy yielding and are only the rate-limiting steps in anaerobic reactors (Pavlostathis and Giraldo-Gomez, 1991).

In an anaerobic environment, oxidation of VFAs to acetate is thermodynamically favorable, only when hydrogen concentration is low enough. Therefore, VFA oxidation will take place, only if hydrogen is consumed by other bacteria (i.e., the methanogens) (Harper and Pohland, 1986).

Methanogenic fermentation has been studied predominantly in intensive anaerobic reactors, or in a natural environment and, to our knowledge, has not been carefully described in wastewater stabilization ponds or reservoirs. Intensive reactors are usually operated under constant conditions (temperature, pH, etc.), with much lower retention time and higher organic loads than anaerobic lagoons.

Furthermore, most of the bacteria in intensive anaerobic reactors are associated in flocs and the internal conditions within the floc can be very different from the conditions in the liquor (Arcand et al., 1994). In lagoons, most of the bacteria in the liquor are free living and the diffusion rate of hydrogen between the hydrogen-producing and the hydrogen-consuming bacteria might limit hydrogen consumption (McCarty and Smith, 1986).

The wastewaters of the largest dairy factory in Israel (Tnuva, Tel-Yosef) are treated in two serial, deep reservoirs. This study focuses on the first anaerobic reservoir, which was designed according to a unique concept. It is extremely deep (13 m), having a volume of 330,000 m$^3$ that results in a retention time of approximately 150 days and is operated as a continuous flow system, under high and fluctuating organic loads. In this study, we combined in situ measurements (over 18 months) and supporting lab experiments, in order to: 1) analyze the performance of an anaerobic reservoir under different groups of bacteria (Harper and Pohland, 1986).

In batch experiments, the gas and the water phases were sampled (Wildco auto-sampler) every hour (40 ml) into a tank (20 l) stored at 4 °C before inoculation. The liquor or sediment samples were dispensed into the serum bottles that were sealed thereafter with gas-tight rubber stoppers. In addition, the activity of the samples, inoculated in a mineral medium containing a single carbon source, was tested using 3 ml samples in 27 ml of medium. The mineral medium was composed of (in g/l): K$_2$HPO$_4$ 3(H$_2$O) (0.4), NaHCO$_3$ (5), NH$_4$Cl (1), MgCl$_2$ 6(H$_2$O) (1), CaCl$_2$ 2(H$_2$O) (0.4), resazurin (0.001), cysteine/hydrochloride (0.5), Na$_2$ SO$_4$ 9(H$_2$O) (0.5), and supplemented with lactose (14.6 mM) or propionate (32.8 mM) as a sole carbon source. Vitamin and trace elements were added, according to Whitman et al. (1991). An additional carbon source was added according to the experiment and pH was adjusted to 7. The medium was prepared according to Whitman et al. (1991), inserted into the anaerobic chamber (in the serum bottles), opened for inoculation and immediately resealed. All cultures were incubated at 28 °C without shaking.

2.2. Sampling

Samples from depths of 0, 6, and 12 m were taken once a week. Temperature, oxygen (YSI model 58) and pH (Radiometer, PHM 80) were measured at the site. liquor and sediment samples for chemical analysis were transported to the lab at 4 °C. For the batch experiments, the samples were carried in BOD bottles to avoid oxygen diffusion and were inoculated on the same day. The influent was automatically sampled (Wildco auto-sampler) every hour (40 ml) into a tank (20 l) stored at 4 °C. Once a week, the tank was well mixed and sampled for chemical analysis. This sample represents an average of the weekly influent. After sampling, the tank content was discharged, the tank was cleaned and replaced in the auto sampler. The volume of influent entering the reservoir was measured and noted once a week.

2.3. Batch experiments

All experiments were conducted under anaerobic conditions in serum bottles (125 ml), with a minimum of two replications. The kinetics of methane production and of acetate and propionate consumption by liquor (6 m) or sediment were followed in two types of experiment. The activity of 100% samples (microcosms experiment) was investigated with serum bottles that were placed in an anaerobic chamber (Forma Scientific, Anaerobic System Model 1025/1029: atmosphere of 94% N$_2$ and 6% H$_2$) 24 h before inoculation. The liquor or sediment samples were dispensed into the serum bottles that were sealed thereafter with gas-tight rubber stoppers. In addition, the activity of the samples, inoculated in a mineral medium containing a single carbon source, was tested using 3 ml samples in 27 ml of medium. The mineral medium was composed of (in g/l): K$_2$HPO$_4$ 3(H$_2$O) (0.4), NaHCO$_3$ (5), NH$_4$Cl (1), MgCl$_2$ 6(H$_2$O) (1), CaCl$_2$ 2(H$_2$O) (0.4), resazurin (0.001), cysteine/hydrochloride (0.5), Na$_2$ SO$_4$ 9(H$_2$O) (0.5), and supplemented with lactose (14.6 mM) or propionate (32.8 mM) as a sole carbon source. Vitamin and trace elements were added, according to Whitman et al. (1991). An additional carbon source was added according to the experiment and pH was adjusted to 7. The medium was prepared according to Whitman et al. (1991), inserted into the anaerobic chamber (in the serum bottles), opened for inoculation and immediately resealed. All cultures were incubated at 28 °C without shaking.

2.4. Analysis

Total suspended solids (TSS, 105 °C), fixed suspended solids (TSS, 550 °C), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total kjeldhal nitrogen (TKN), oil & grease; ammonia (NH$_4$), nitrate (NO$_3$); and nitrite (NO$_2$) were determined according to standard methods (APHA, 1992). Major ions (including Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Cl$^-$, PO$_4^{3-}$) were analyzed by an atomic absorption system (AAnalyst 200; Perkin-Elmer, CT, USA).

In batch experiments, the gas and the water phases were sampled through the septa by syringe. Methane (in the gas phase) and VFA (in the water phase) concentrations were determined by a gas chromatograph H.P. 5890, equipped with
a packed column (80/120 carbopack B-DA/4% CARBOWAX 20M) and flame ionization detector. Lactose concentration was measured with reagent Antron (Dische, 1962).

3. Results

3.1. Field observations

The reservoir was always anaerobic ($O_2 < 0.2 \text{ mg/l}$ all year round). Because of the seasonal fluctuations in milk production, the BOD and COD organic loads varied from 14 and 40 g m$^{-3}$ day$^{-1}$ in the summer, to 80 and 160 g m$^{-3}$ day$^{-1}$ in the winter, respectively.

The higher organic loads occur in the winter, when the temperature is low (Fig 1A) and microbial metabolism slows down, resulting in accumulation of BOD, COD and VFAs, a drop in the pH (Fig. 1B,C), and emission of odors. Based on the GC analysis, we tentatively identified the presence of acetate, propionate, butyrate, $\gamma$-hydroxy butyrate, lactate, and valerate. Acetate and propionate were the most significant VFAs that were identified: they were the first to be detected at the beginning of the winter and their concentrations were higher during the winter than those of other VFAs (maximum concentrations of propionate, acetate and the sum of all the rest were 13, 7.8 and 4 mM, respectively) and they were last to disappear at the end of the winter. For this reason, we focused on the metabolism of acetate and propionate.

At the beginning of the winter, the concentrations of propionate and acetate increased almost stoichiometrically with the increase in BOD and COD (Fig 1). In February, when the temperature dropped to a minimum ($15 \degree C$) and the organic load reached a maximum (165 g COD m$^{-3}$ day$^{-1}$), accumulation of propionate was accelerated, while the accumulation of acetate, a product of propionate oxidation, ceased. Concentrations of VFAs, BOD and COD began to decrease gradually in the middle of May, when the temperature increased (above $22 \degree C$) and organic load decreased (around $110 \text{ g COD m}^{-3} \text{ day}^{-1}$). Propionate concentration only started to drop at the beginning of August, concurrently with a sharp decrease of BOD and COD. At this time, most of the acetate was already consumed, the temperature reached its maximum (ca. $29 \degree C$) and organic load dropped to around $50 \text{ g COD m}^{-3} \text{ day}^{-1}$. During this whole period, the pH seemed to be a sensitive indicator of the performance of the reservoir (Fig 1C). At the beginning of the winter, pH started to drop with temperature decrease and organic load increase. At the beginning of spring, pH started to rise when the organic load decreased and temperature increased. Despite the accumulation of organic matter in the reservoir during the winter, the removal percentages of total COD, total BOD and TSS were always above 75.7%, 77.9% and 66.5%, respectively (Fig 1B).

3.2. Laboratory batch experiment

VFA concentrations depend on their formation rate and consumption rate. The in vivo controlled laboratory experiments, however, reinforce field observation. When sediment or liquor samples were inoculated in a defined mineral medium containing a single carbon source, the predominant products of lactose fermentation were acetate and propionate (Fig. 2) and acetate was the product of propionate oxidation (Fig. 3). While lactose fermentation was fast, both in sediment or liquor experiments, the degradation of acetate and propionate were much faster in the sediment experiments. Additionally, in the sediment experiment, acetate and propionate were utilized at similar rates, while in the liquor experiment, the oxidation of propionate was much slower than acetate cleavage. Other intermediates detected in the lactose fermentation experiments included ethanol, propa-
nol, butyric acid, lactic acid and valeric acid. Of these, ethanol had the highest maximal concentration (about 6 mM) and its degradation rate was relatively fast (it disappeared after 3 and 6 days in the sediment and liquor samples, respectively). Thus, ethanol might also be an important intermediate metabolite, although it is rapidly oxidized and does not accumulate in the reservoir.

In the sediment microcosms, methane production was much faster and to a higher quantity than in the liquor microcosms. In a similar way to the defined medium experiments, microcosms of the sediment layer consumed acetate and propionate at comparable rates and faster than microcosms of liquor, while in the liquor microcosms, the oxidation of propionate was much slower than acetate cleavage (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first report concerning treatment of high-strength wastewater in a deep anaerobic reservoir. Moreover, detailed investigations on the subject of the methanogenic fermentation pathway in anaerobic lagoons are scarce and the results presented here might therefore also be extrapolated for anaerobic stabilization ponds.

During the course of this study, organic load in the reservoir (Fig. 1A) was always below the design criteria for the treatment of domestic wastewater in anaerobic stabilization ponds in the Mediterranean climate (200 g BOD m⁻³ day⁻¹ at 15 °C and 100 g BOD m⁻³ day⁻¹ at above 25 °C). BOD removal
Fig. 1B was always higher than predicted by these criteria (50% at 15°C and above 25°C) (Mara and Pearson, 1998). However, due to the high-strength wastewater and its specific content (e.g., lactose, proteins and fats) it was not enough to prevent the accumulation of soluble organic matter (Fig. 1B,C) and odor emissions in the winter. In these months, we could detect accumulation of sludge at the bottom of the reservoir (estimated at 30–40 cm) and it seems that a high fraction of the organic load (the particulate matter) was removed by sedimentation. The sludge was further degraded during the summer and no sludge accumulation was observed over a period of 8 years.

VFAs, mainly acetate and propionate, together with pH, are good indicators of the performance of anaerobic reactors (Harper and Pohland, 1986). This study suggests that they are also good indicators of the performance of anaerobic lagoons. Acetate and propionate started to accumulate as soon as COD started to increase and their accumulation was strongly dependent on the temperature and on the organic load. At the end of the winter, it appeared that acetate degradation continued, while propionate oxidation virtually stopped. Laboratory batch experiments, which were conducted in this season, confirmed the field observations that: 1) the main fermentation products of lactose were acetate and propionate, both by liquor and sediment samples; and 2) propionate degradation by liquor samples was much slower than acetate degradation and it started only after a long lag.

As expected, methane production by the sediment samples was faster and reached higher levels than in the liquor samples. At the same time, acetate and propionate were consumed faster in the sediment, with no observed lag in
propionate oxidation. The faster microbial activity in the sediment layer is probably due to a higher microbial concentration and diversity. Additionally, the redox potential in the sediment layer was probably lower and the proximity of hydrogen producer to hydrogen consumer might help to maintain a low hydrogen concentration, probably contributing to the faster degradation of propionate (Gonzalez-Gil et al., 2001). Unfortunately, we were unable to measure redox potential and hydrogen concentration.

Low organic load does not guarantee better performance of anaerobic ponds. For example, Pearson et al. (1996) observed similar BOD removal rates (82–86%) for organic loads, between 25% and 100% of the maximal permissible organic loading, at a constant temperature of 17°C. However, when the organic load increases abruptly, fermentative bacteria, which grow quickly, are likely to proliferate and produce more VFAs and hydrogen. On the other hand, methanogens and VFA oxidizers that grow more slowly, may not be able to remove the excess fermentation products fast enough (Harper and Pohland, 1986). This may result in the accumulation of VFAs and hydrogen. Thermodynamic calculations and experimental results suggest that when the temperature drops from 28 to 15°C, a hydrogen concentration favorable to VFA oxidizers is 10 times lower (Conard and Wetter, 1990; Lee and Zinder, 1988). Accordingly, it may be assumed that during the winter, due to the combined effect of increased organic load and decreased temperature, the activity of propionate oxidizers may be dramatically reduced, as evidenced by the results obtained. In agreement with our results, Lettinga et al. (1999) showed that propionate degradation in a UASB reactor is the most sensitive to low temperatures (3–8°C). Similarly, samples from tundra wetlands (Polar Ural, ambient temperature 10–15°C) could degrade acetate but not propionate (Kotsyurbenco et al., 1996).

During the winter, the elevated levels of VFAs were associated with a drop in the pH (Fig 1C). High concentration of VFAs, especially at low pH (6.5 in the winter), is toxic to bacteria, especially to propionate oxidizers and methanogens (Beaty and Mclnerny, 1989; van den Heuvel, 1988), while a pH of 6.5 is more favorable for fermentative bacteria than for propionate oxidizers and methanogens (Kissalita et al., 1987; Boone and Xun, 1987). Such activity could act as a positive feedback, exacerbating the imbalance between the different bacterial groups and, thereby reducing the efficiency of organic matter removal in the reservoir.

The type of fermentation might also play an important role in the performance of the reservoir. Propionate-forming bacteria are unaffected by hydrogen concentrations and grow rapidly in high substrate concentrations, but they are poor scavengers (McCarty and Mosey, 1991). These bacteria are therefore likely to proliferate in the highly loaded reservoir during the winter, and thus contribute to accelerated propionate accumulation.

5. Summary and conclusions

This paper summarizes a study aimed at checking the efficiency and limitations of a deep anaerobic reservoir with an extended retention time, designed for the treatment of concentrated dairy wastewater. The main conclusions drawn from this study are:

1. The anaerobic reservoir can remove above 75% of the incoming organic matter all year round, by both sedimentation and degradation. However, due to the unique composition of the dairy wastewater and its high particulate organic content, organic load design criteria should be adjusted to more conservative values than those applied for conventional anaerobic stabilization ponds systems, to prevent nuisance odors, especially during winter.
2. VFAs and pH are good indicators for the performance of anaerobic lagoons. Acetate and propionate, products of lactose fermentation, were found to be the predominant intermediate metabolites in the reservoir, and their concentrations were strongly dependent on the temperature and the organic load.
3. The combined effect of colder winter temperatures and seasonal increase of organic load resulted in a decreased rate of propionate oxidation and a consequent accumulation of soluble BOD and COD.
4. Laboratory batch experiments, conducted during this season with liquor samples from the reservoir, found propionate oxidation to be the rate-limiting step in the process, characterized by a lag period preceding its degradation. The propionate oxidation rate in the sediment layer was as high as acetate degradation, even during the winter, suggesting that the sediment might have an important role as a shelter for the propionate oxidizers when conditions in the liquor are unfavorable.

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References


