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КИНЕТИКА ОБРАЗОВАНИЯ И РАСХОДА ЛЕТУЧИХ ЖИРНЫХ КИСЛОТ КАК МЕТОД ИССЛЕДОВАНИЯ ЛЕГКО ОКИСЛЯЕМЫХ СТОЧНЫХ ВОД ПО БИОДЕГРАДАЦИИ ХПК

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Исследована кинетика образования и расхода летучих жирных кислот (ЛЖК) и спиртов в ходе анаэробного разложения модельных сточных вод и гранулированного ила (ГИ) при разных концентрации сорбитола, pH и температуре. Максимальное удаление ХПК и максимальное образование метана наблюдались при концентрации сорбитола 3.5–7.5 г/л, pH 9.0–9.5 и температуре 30–37°С в присутствии 4.18–5.02 г/л взвешенных летучих твердых веществ. При этих условиях расход ЛЖК и спиртов был наибольшим, и образование метана было в 3 раза выше, чем в присутствии 1.7 г/л гранулированного ила в качестве инокулята. Наблюдалась хорошая корреляция между метаногенной активностью и эффективностью удаления ХПК. В присутствии промежуточных продуктов, таких как избыток ЛЖК, происходит уменьшение pH и ингибирование стадии образования метана (метаногенеза), что свидетельствует о важности мониторинга образования и расхода ЛЖК как прямого индикатора при определении физиологического состояния реактора.

INTRODUCTION

Most microorganisms grow best under neutral pH conditions, since other pH values may adversely affect metabolism by altering the chemical equilibrium of enzymatic reactions, or by actually destroying the enzymes. In order to avoid an inhibition due to the presence of intermediary products of biodegradation, it is an important to understand the complex process of anaerobic digestion [11]. Anaerobic degradation has various steps. In the hydrolysis the influent matter is broken down to smaller molecules, suitable for microorganisms. Fermentation or acidogenesis results in the formation of carbon dioxide, hydrogen and VFA, with acetate, propionate and butyrate as the major VFA´s. Propionate and butyrate are oxidized to acetate during acetogenesis and finally, in the methanogenic process, acetate, hydrogen and carbon dioxide are converted into methane. The methanogens are most sensitive to pH variations. Low pH affects the chain of biological reactions in the anaerobic digestion and the reactor cease to function optimally [11]. The acetic acid producing bacteria grow on substrates that contain alcohol, sugar, organic nitrogen, vitamins. They have three main characteristics: *first* they are highly acidophilic, grow under pH as low as 4 and 5–6 is the optimum pH; *second,* they have high capability to oxidize organic substrates into partially oxidized products; *third,* the capability to oxidize ethanol into acetic acid is one of the most notorious properties of these group of bacteria [17]. They can also oxidize other mono and polyatomic alcohols in their corresponding acid. For industrial production of acetic acid, *Acetobacter aceti, Acetobacter pasterianus* or *Acetobacter peroxidans, Gluconobacter oxydans* are widely used. There are also other acetic acid producing bacteria, such as; *Clostridium thermoaceticum, Clostridium formicoaceticum*, etc., this bacteria in particular produce acetate from CO_2+H_2, using methanol and formate [17]. The propionic acid producing bacteria such as; *Propionibacterium shermanii* and *Propionibacterium technicum*, synthesize high quantities of propionic acid, and also acetic acid. They can use glucose, lactose and other sugars as substrate. The propionic acid is a strong inhibitor of the anaerobic digestion in general and of the propionic acid producing bacteria itself; and the maximum production of propionic acid is 15 g/l and 3 g/l of acetic acid at pH of 6.0. *Clostridium butyricum* and *Clostridium pasteurianum* are butyric acid producing bacteria and they ferment sugar to butyric acid, acetic acid, CO_2 and H_2 [17]. Environmental factors which influence biological reactions, such as pH, temperature, nutrients and inhibitors concentrations, are amenable to external control in the anaerobic process. In recent years many mathematical models to describe such combined factors have been developed for use as a strategy to solve problems concerning anaerobic wastewater treatment [4, 6, 10, 14, 20]. One of the most common strategies to overcome this problem is the addition of alkali such as sodium hydroxide, which increased the cost of treatment and made it expensive. Inspite of the problem described above, anaerobic treatment of industrial wastewater can be considered as a well established technology with a wide range of applications. So far, practically all full scale applications of anaerobic treatment are restricted to concentrated wastewaters with temperatures exceeding 18°C [8], and no proper study has been reported yet on the anaerobic treatment of easy acidifying wastewater. In this study sorbitol was chosen as carbon source, which is widely used in the food, pharmaceutical, cosmetic and chemical industries.

MATERIALS AND METHODS

Wastewater and inoculum

For all experiments in this study, synthetic *was*tewater containing sorbitol as carbon source was used. The inoculum used in this study was granular sludge from an UASB reactor treating brewery wastewater.

Reactors and the experimental set-up

The influence of CO (sorbitol), pH, temperature and granular sludge as inoculum (VSS, g/l) on anaerobic biodegradation was studied. Anaerobic batch reactors (120 ml volume) with 45 ml of working volume, including 5 ml of granular sludge was used. Various CO (gl^{-1}) concentrations in this study were; 4.9, 6.7, 9.3, 11.9, 13.6 and 19.8, that were equivalent to 3.5, 5, 7.5, 9, 12, 15 g of sorbitol/l. For the experiment to determine the optimum pH, the digesters were adjusted to various pH levels (7.0, 7.5, 8.0, 8.5, 9.0, 10, 10.5 and 11) with 1 N sodium hydroxide. The temperature influence were defined at different temperatures (20, 30, 37, 45, 50 and 60°C) using thermostatic incubators. In addition, the influence of VSS on anaerobic biodegradation of sorbitol was studied with various levels of VSS, *viz.*, 0.83, 1.7, 2.17, 3.35, 4.18 and 5.02 g/l.

Analytical Methods

CO, TSS, VSS were determined according to standard methods [1], unless otherwise indicated. The methane and VFA were determined by a Varian gas chromatography (GC) equipped with athermal conductivity detector and flame ionization detector (FI) respectively with helium as carrier gas. The pH was analyzed in a potentiometer (VWR Model 8000). All experiments were maintained at five replications.

Results and discussion

1. Study of the influence of the CO concentration over the methanogenic process and biodegradation.

The results obtained in the first stage of this work showed that the maximum methane production was at 4.9 g CO/l, which was equivalent to 3.5 g sorbitol/l under optimum pH conditions (pH 9.5). A clear decrease in the methane production and low CO removal was observed at higher CO concentrations. The methane production in terms of CO concentration is given in Fig. 1. This indicated that there was a strong relationship between the methanogenic activity, CO removal efficiency and the initial CO concentration [7]. A clear inhibition due to the high CO concentration resulted in higher production of VFA, and less consumption of acetate, which indicated that the methanogenic process was significantly influenced by the substrate concentration [20]. Methanogenesis is a complex process of multiple stages, and every stage are carried out by different group of microorganisms (Hydrolytic, fermentative, acetogenic and methanogenic), and in this study. the methanogenic stage was inhibited by the excess of VFA produced during previous stages, which caused a drop in pH. This showed that it is important to monitor closely the pattern of the production and consumption of VFA [7, 10, 14] because this is a direct indicator of the physiological condition of the reactor. The relationship between consumption of VFA and methane production with sorbitol at 3.5 g/l, pH of 9.5, are shown in Fig. 2. It was observed that the volatile fatty acids were almost totally consumed, which avoided their accumulation and also maintained the pH at near neutral values favoring the methanogenesis process.

The kinetics of the present study was analyzed by following equation developed by Fiestas et al. [5]:

$$G = G_m [1 - e^{-kat}] \ . \qquad (1)$$

Where G_m is the maximum volume of methane accumulated at an infinite digestion time and is the prod-

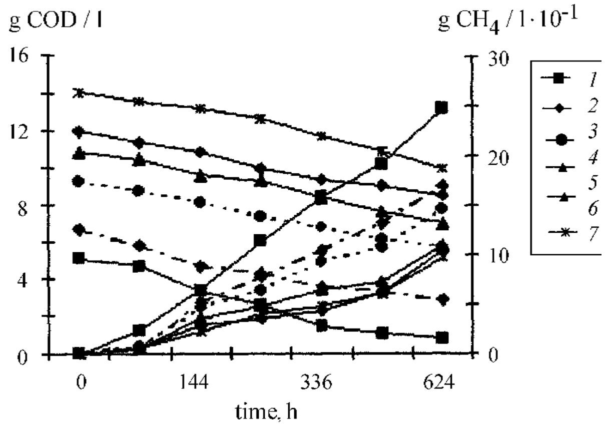


Fig 1 Methane production and COD removal at different COD concentrations

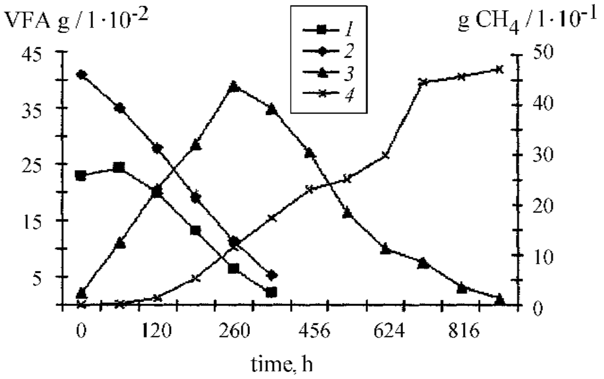
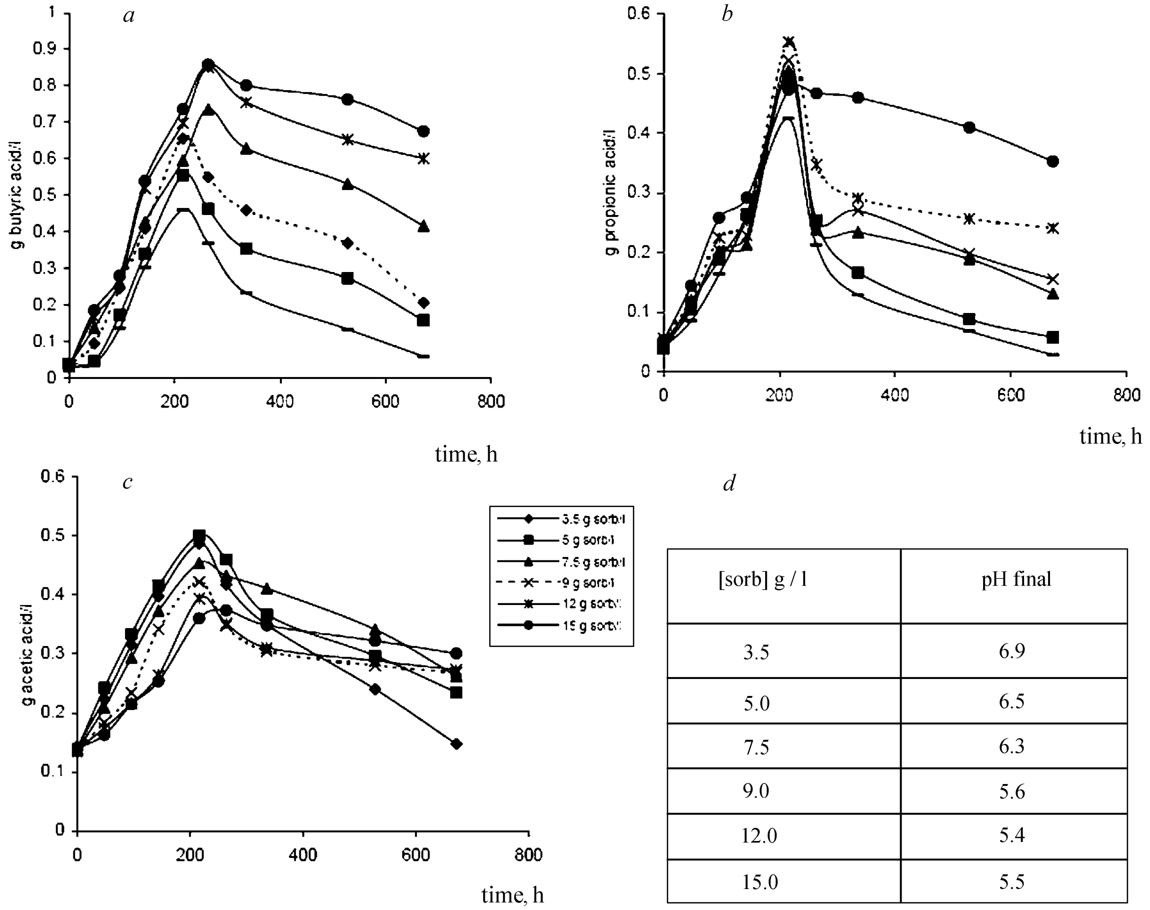


Fig. 2 Production and consumption of VFA and methane production at an optimum concentration of sorbitol and at optimum pH 9.5

uct of the initial substrate concentration (S_0) and the yield coefficient of the product (Y_p): $G_m = S_0Y_p$; and k_a is apparent kinetic constant that includes the biomass concentration (x): $k_a = kx$.

The kinetics for the butyric, propionic and acetic acid formation and consumption are given in Table 1. It was observed that production of propionic acid was more than butyric and acetic acid in all cases. This indicated that the reaction is of second order kinetics. In the case of consumption of different VFA, propionic acid was consumed more, followed by acetic acid. Propionic acid consumption can be related to the formation of acetic acid and the consumption of acetic acid can be related to methane formation.

Results obtained on the influence of pH showed that pH 9.5 was optimum for the anaerobic biodegradation of sorbitol, as there was a higher production of methane at this pH. Hence, this pH was used in all further experiments in this study. Batch and UASB reactors under initial non-favorable conditions resulted in less CO removal [19]. Fig. 3, *a–d* showed the kinetics of VFA consumption, which demonstrated that the physiological conditions in the reactor were better when the initial pH was 9.5 with 3.5 g/l of CO. uring the start-up period at pH 9.5, an acidification occured which decreased it to neutral conditions and thereby prevented an inhibition due to high initial pH. Later the pH in the reactor was stabilized around 6.3–6.9, which did not inhibit the methanogenic process. In addition, it was also observed that an initial pH of 9.5 enhanced the performance of the reactors at higher CO concentrations



[sorb] g / l	pH final
3.5	6.9
5.0	6.5
7.5	6.3
9.0	5.6
12.0	5.4
15.0	5.5

Fig. 3. Production and consumption of VFA at different concentrations of sorbitol and at an initial pH 9.5: *a* – butyric acid, *b* – propionic acid, *c* – acetic acid, *d* – final pH in relation to different initial pH

Table 1

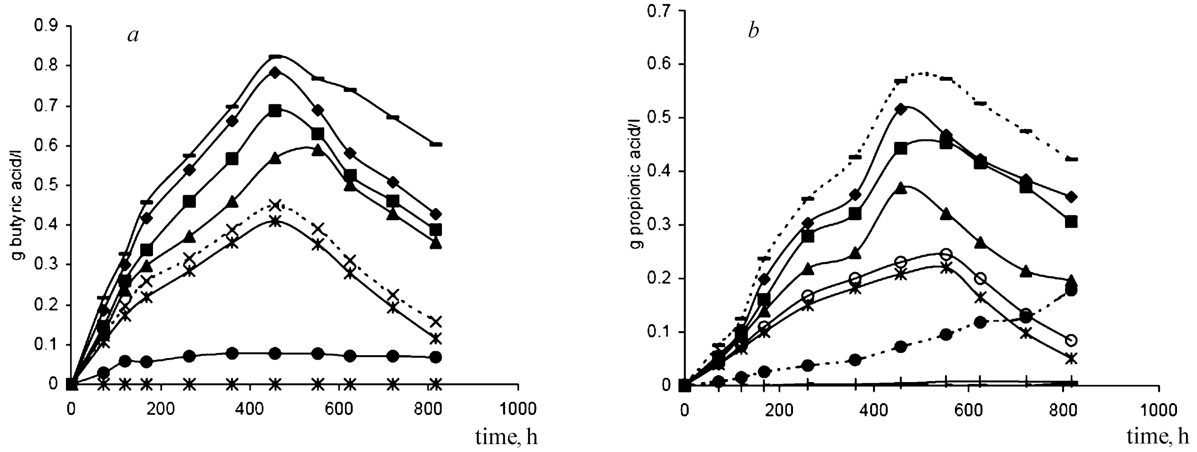
Kinetic parameters for formation and consumption of VFA

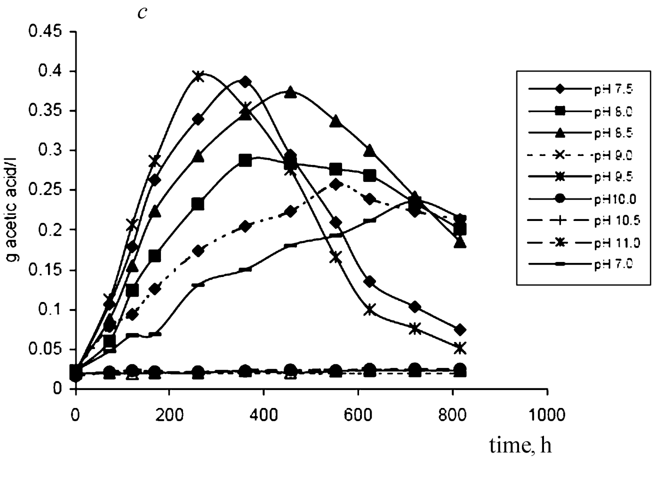
VFA	$K_f \times 10^3$ h^{-1}	$K_c \times 10^{-3}$ h^{-1}	n_f	n_c
Propionic acid	16	361	1	1
Butyric acid	4		1	
Acetic acid	16	2	1	1

(≥7.5 g sorbitol/l), in comparison to the reactors maintained at a pH lesser or higher than 9.5 (Fig. 4). It was observed that at pH below 9.0 and higher than 9.5, the VFA′s tend to accumulate or the process does not even start up and this indicated that pH management is an important issue in the case of easily acidifying wastewater. It was further observed that production of butyric and propionic acids increased with increasing concentration of sorbitol and the accumulation of these acids was related to the drop in pH. However, in the case of acetic acid, a continuous utilization was observed with an initial pH of 9.5 and with 3.5 g of sorbitol/l.

The effect of temperature on the pattern of methane production and the kinetics of the generation and consumption of VFA is given in Fig. 5. The results showed that the performance of the reactors deteriorated at temperatures higher than 37°C. It was reported earlier that high. temperatures caused granule disintegration and as a result the methane production was affected [4, 19]. The performance of an acidogenic reactor can be evaluated by determination of the degree of acidification, which can be quantified by measuring the CO equivalent of intermediary products, such as VFA. A comparison of the latter form of the Arrhenius equation to the equation for a straight line, $y = mx + b$, where it is obvious that if we plot $\ln(V_0)$*vs.* $1/T$, we will get a plot where the slope is $-E_a/R$ and the intercept in ln(A) is given in Fig. 6.

The influence of the different temperature on the degree of acidification, on the rate of product formation in terms of VFA′s was assessed. The degree of acidification was quantified using the percentage of initial substrate concentration converted to VFA′s. The initial substrate concentration (S_0) was measured in g CO/l and fermentation products was converted to theoretical equivalent in g CO/l (S_p), as shown in Table 2.





d

pH initial	pH final
7.0	4.53
7.5	4.98
8.0	5.21
8.5	6.12
9.0	6.47
9.5	6.89
10.0	9.75
10.5	10.25
11.0	10.89

Fig. 4. Production and consumption of VFA at different initial pH and at an optimum concentration of 3.5 g/l of sorbitol: *a* – butyric acid, *b* – propionic acid, *c* – acetic acid, *d* – final pH in relation to different initial pH

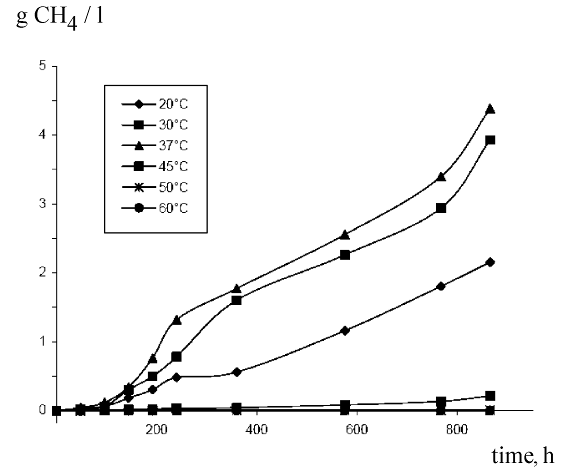


Fig. 5. Methane production at different temperatures; 3.5 g sorbitol/ l; pH 9.5

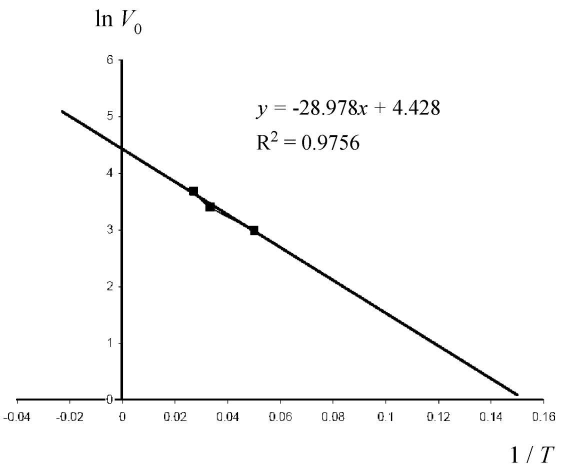


Fig. 6. Arrhenius equation to the equation for a straight line, $y = mx + b$, $\ln(V_0)$ *vs*; $1/T$

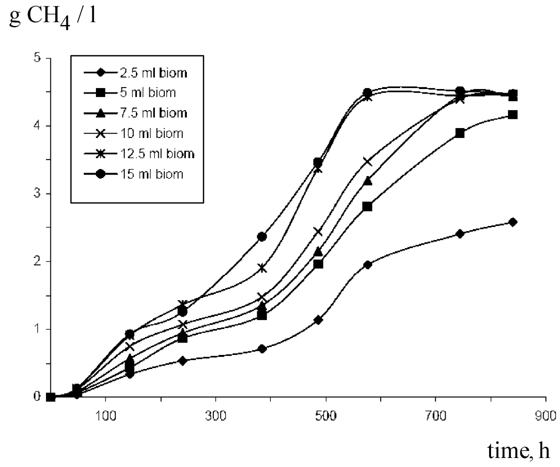


Fig. 7. Methane production at different concentrations of initial biomass; 37°C; 3.5 g sorbitol/ l; pH 9.5 Figure captions

In case of the experiment with different concentrations of biomass, it was observed that the initial quantity of biomass had a positive effect on the methanogenic process and the CO removal. Biodegradation efficiency was improved when the initial biomass concentration was increased from 0.83 to 5.02 g/l VSS. ata in Fig. 7 showed that the difference in the efficiency of biodegradation of reactors with 4.18 and 5.02 g/l of VSS was insignificant. Therefore it is recommended to use inoculum of 25% of the working volume of the reactor for easily acidifying substrate containing wastewater. Higher initial biomass concentrations over this range are unnecessary and will not increase the CO removal and further it would decrease the effective working volume.

Table 2

Operational parameters and Percent of acidification

Temperature (ºC)	Initial COD concentration (g/l)	Final COD concentration (g/l)	Maximum Total volatile fatty acids concentration (g/l)	Percent of acidification (%)*
20	5.07	1.81	2.06	40.61
30	5.09	1.29	1.82	35.89
37	5.1	0.89	1.62	31.95
45	5.09	3.79	0.68	13.41
50	5.09	4.81	0.53	10.41
60	5.09	4.97	0.16	0

*% acidification = $(S_0 \backslash S_P) \cdot 100$; S_0 = Initial COD concentration; S_P = Fermentation products (VFA).

CONCLUSIONS

Optimum conditions necessary for an efficient anaerobic biodegradation of wastewater polluted with easily acidifying wastewater was demonstrated in this study. In this study, anaerobic biodegradation of sorbitol was achieved with a detailed study on the kinetics of production and consumption of intermediary products such as VFA.

An initial pH (9.0–9.5), an ambient temperature in the range of 28–38°C increased the biodegradation efficiency. An inoculum-medium ratio of 1:4 was optimum to accomplish a satisfactory CO removal and higher production of biogas.

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KINETIC ASSESSMENT OF THE VOLATILE FATTY ACIDS AS STRATEGY TO MAINTAIN THE SUSTAINABILITY OF THE BIODEGRADATION OF CHEMICAL OXYGEN DEMAND OF EASILY ACIDIFYING WASTEWATER

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Kinetics of the production and consumption of volatile fatty acids (VFA) such as acetic-, propionic-, butyric- acid and alcohols during anaerobic digestion of model wastewater was studied under different concentrations of sorbitol (g/l) as carbon source, pH, temperature and granular sludge (GS). Methane production was measured. Higher COD removal and methane production were obtained at 3.5–7.5 g sorbitol/l, with an initial pH of 9–9.5, and at 30–37°C with 4.18–5.02 g/l of VSS. At these conditions VFA and alcohols were consumed more and produced three times more methane than with 1.7 g/l VSS of granular sludge as inoculum. A strong relation between methanogenic activity, COD removal efficiency was observed for all the initial COD concentrations studied. The methanogenic stage was inhibited by the presence intermediary products such as VFA in excess, which caused a drop in pH and inhibited methanogenesis. This fact demonstrated the importance of monitoring the production and consumption of VFA as a direct indicator for the physiological condition of the reactor. The treatment of easily acidifying wastewater like used in this case can be achieved with a detailed kinetic study on the production and consumption of intermediary products to establish the optimum parameters for higher efficiency of COD removal and methane production.