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Microbial activity and process kinetics of the Upflow Anaerobic Sludge Blanket reactor for decomposition of selected chlorinated substances

NIVA - REPORT

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Abstract:
Biological, anaerobic treatment of industrial wastewater was studied with use of the Upflow Anaerobic Sludge Blanket. Degradation of some chlorinated substances (6-chlorvanillin(6CV), trichloroacetic acid(TCA)) which are found in bleaching wastewater, was followed. The rate of degradation of 6CV was determined. The rates of 6-chlorovanillin degradation obtained are in the range from 0.76 to 3.63 mg6CV/gVSS*d. Respectively methane gas production was determined at about 0.22 gCOD-CH4/gVSS*d. Experiences on the UASB reactors performance is also presented. We have experienced that addition of 6-chlorovanillin to both : synthetic as well as bleachingwastewaters, causes problems. This was observed as loss of bacterial activity and low methane gas production. Reactors fed with the synthetic medium kept up activity longer than those fed with bleaching wastewater.
Chlorinated compounds in bleaching wastewaters may be degraded anaerobically. However, the performance of UASB reactors fed with bleaching wastewater needs to be studied further due to problems arising from constituents other than chlorinated compounds.

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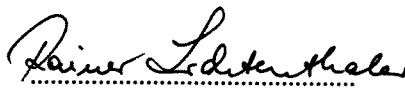1. anaerob nedbrytning
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Final report

Contents

Authors´ Preface

The project was initiated within the Umbrella Nordic Environmental Biotechnology Programme: "Detoxification and Regeneration of Wastewater and Waste of Industrial Origin" (1990-1993) supported by the Nordic Industrial Funds (NI) and was started by April 1990. Four main groups of projects were formed:
1. Pulp and paper industry wastewaters
2. Biodegradation of priority chemicals
3. Biofixation of heavy metals
4. Solid waste treatment.

The Norwegian Institute for Water Research (NIVA) participated with projects in groups 1, 2 and 3. The project described in this report was placed in group 2.

There was coordination between the projects in groups 1 and 2 such as analytical methodology and chemicals to be tested.
This project's industrial partner was NORSK HYDRO giving financial support. Furthermore NIVA contributed substantially.

In addition Norsk Hydro has performed studies the following problems connected to the Biotechnology Programme:
- microbial degradation of chlorinated dioxines and dibenzofuranes
- biological treatment of wastewater from pulp and paper industry, including use of macro and micro nutrients
- use of nitrate as electron acceptor within microbial anaerobic degradation of wastewater
- effects of chemical precipitation on the performance of the anaerobic digestors

Personnel participating in the project at NIVA:

- **Bente Wathne** — project leader from the start of the project from April 1990 to September 1991

- **Grazyna Englund** — project leader from September 1991 to August 1992; research scientist, responsible for the research management during the whole project

- **Rainer Lichtenthaler** — project leader from August 1992

- **Åse Bakketun** — research assistant, from the start of the project

- **Helle J. Rasmussen** — analysis GC, VFA and chemicals, from August 1992

- **Åse K. Rogne** — research assistant, partly involved from November 1992

- **Katja Grolle** — visiting researcher from the Netherlands, 1 month in the period of April to June 1992

- **Thomas Hellsten** — student of the Engineering High School in Sarpsborg, involved in this project from March to June 1993

- **Frode Nipen** — student of the Engineering High School in Sarpsborg, involved in the literature study on PCB-degradation, from March to June 1993

- **Morten Laake** — research manager of the biotechnology group at NIVA, consulting the performance of the project, until August 1992

Industrial partner   Norsk Hydro A/S :
- **Knut Osnes** - head of the Department of Biotechnology
- **Jon Hovland** - Department of Biotechnology
- **Henrik Høyvik** - Department of Biotechnology

Summary

The first years of the project were mostly dedicated to broaden the competence on the anaerobic wastewater treatment the with use of the UASB reactors, their start-up, as well as to get in function the neccessary analytical methods.

Further activities were concentrated on the degradation of the selected chlorinated compounds. As discussed with the part project A.2.2 ("Biological degradation of specific organochlorine substances in wastewater treatment") it was of interest to follow common wastewater and chlorinated compounds occurring there, in order to gain information about the two different anaerobic systems studied in the two projects (UASB and biofilm anaerobic filter). Because of budget limitation it was decided to concentrate first on 2 compounds, namely: 6-chlorovanillin (6CV) and trichloroacetic acid (TCA) and to use bleaching wastewater from the same source as A.2.2 for investigations. The same analytical methods were used for determination of chemical concentrations.

Four UASB reactors run on the synthetic wastewater were adapted gradually to the 100% bleaching wastewater and consequently three of them were fed additionally with the two organochloric chemicals (separate and in combination). Chemicals were pumped from the separate stock solution bottles (of low pH). UASB 1 functioned as a control and was corrected for the extra carbon (with acetic acid -HAc), because the 6CV was dissolved in $HAc+H_2O$ (50:50) and caused extra COD.

Two new reactors were started-up after loosing bacterial methanogenic activity.They were fed with synthetic wastewater only. After reaching the steady state, one was additionally fed with 6CV while the other was used as a control. The chemical was added directly into the medium. Batch pre-tests with sludge (1.5 gVSS/l)(VSS-volatile suspended solids) adapted and unadapted to 6CV were carried out (with 7.5 mg 6CV/l) in order to obtain perliminary information on the kinetics of the 6CV degradation. VFA (volatile fatty acid) substrate (C2:C3:C4=75:20:05) (acetic:propionic:butyric acids) was used at concentration of 4 gCOD/l. Temperature was held constant at 26±2 °C.

The specific rate of degradation of 6CV was found to be **1.94 mg/gVSS/d**.

Addition of 6CV and acetic acid caused problems again (presumably as a result of too high contribution of the acetic acid fraction). Therefore the following degradation tests with 30 mg/l and the series of concentrations (0, 10, 20 and 40 mg 6CV/l) were conducted with the sludge from a new reactor,at pseudo steady-state, fed with the synthetic medium only.

The rates of 6-chlorovanillin degradation obtained were in the range from **0.76 to 3.63 mg6CV/gVSS/d** and were in agreement with the previous obtained value. The specific methane gas production rate was measured from 0.22 to 0.25 gCOD-CH4/gVSS*d independent on the 6CV concentration.

From the experimental work performed in this project it can be concluded:

- addition of 6-chlorovanillin to both synthetic as well as bleaching wastewaters, causes problems for the performance of the continuous reactors. This was observed as loss of bacterial activity and low methane gas production. Reactors fed with synthetic medium kept up activity longer than those fed with bleaching wastewater.

- extra acetic acid added, necessary to keep 6CV in solution, influenced performance negatively also of the control reactor. Loss of activity in the reactors may also be the result of shifting the $C_2:C_3:C_4$ ratio from 75:20:05 in favor of increased C_2 in relation to C_3 and C_4. Additional

precautions have to be taken to compensate for this (as for example more accurate pH adjustment having in mind the 50% inhibition concentration of C_2, C_3 and C_4 and/or use of more concentrated stock solution of 6CV). This phenomenon did not occur in the batch tests, as COD conditions and pH are more stringent and the chemical was dosed once.

• chlorinated compounds in bleaching wastewaters may be degraded anaerobically. However, the performance of UASB reactors fed with bleaching wastewater needs to be studied further due to problems arising from constituents other than the added chlorinated compounds.

1. Introduction

The objectives of the project were:
- to contribute to the understanding of the anaerobic treatment technologies, especially Upflow Anaerobic Sludge Blanket (UASB) reactors

and
. - to study the microbial activity and the process kinetics of the UASB reactor for decomposition of chlorinated substances (especially those present in run-off from bleaching processes in pulp and paper industry).

1.1. Anaerobic treatment

The anaerobic wastewater treatment which was first developed in the sugar industry, has now been successfully scaled up and introduced in many industrial areas, such as the paper industry, starch industry, potato processing industry, brewery, distillery etc. The recent developments include the application of anaerobic systems for the treatment of complicated wastewaters from chemical industries. In principal, anaerobic treatment is a pre-treatment method, requiring a post-treatment step for removing remaining pollutants, mainly the biodegradable organic matter and ammonium.

The conversion of organic material into biogas is the result of the metabolism of microorganisms. In the absence of oxygen the organic matter is degraded in a sequence of steps: hydrolysis, acid formation and, finally, methane formation. A general scheme of this process is shown below (in Figure 1.1)(2):
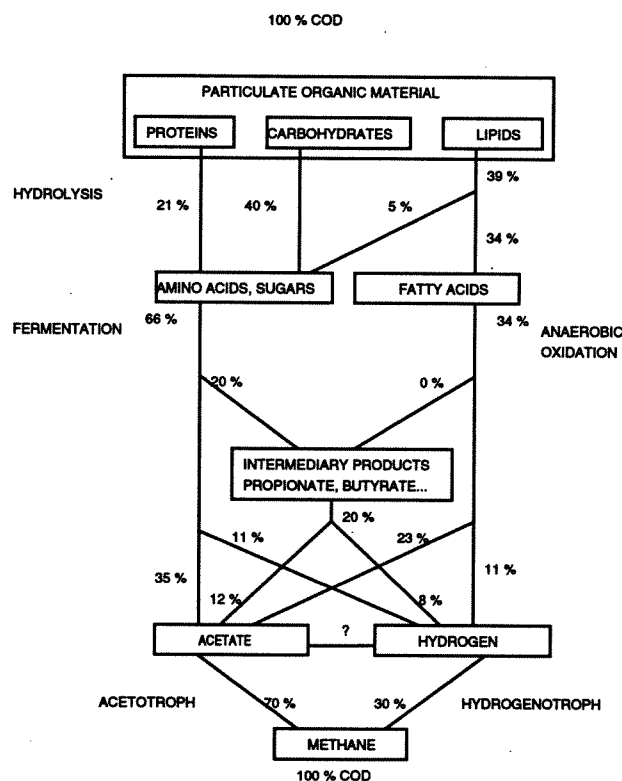
Figure 1.1   General scheme of the anaerobic degradation of the organic matter.

Among the most important advantages of anaerobic treatment are:
- with high level biomass concentration, less space is required than in aerobic treatment
- energy is saved due to biogas production
- less bacterial sludge is produced than in aerobic systems

The table below compares the growth parameters of aerobic and anaerobic bacteria (2).

Table 1.   Growth parameters for aerobic and anaerobic organisms.

type bacteria	doubling time (days)	Y (gvss/gCOD)	k_{max} (gCOD.gvss^{-1}d^{-1})	K_s (mM)
aerobic(sugar)	0.03	0.40	58	0.25
acidogens	0.125	0.14	39.6	?
acetogens	3.5	0.03	6.6	0.4
methanogens				
H$_2$	0.5	0.07	19.6	0.004
C$_2$ (meth.trix)	7.0	0.02	5.0	0.30
C$_2$ (sarcina)	1.5	0.04	11.6	5.0

Min. doubling time $= \ln 2 / k_{max}$. $Y = \ln 2 / \mu_{max}$

Y - cell yield (gVSS produced/gCOD consumed)
k_{max} - reaction rate (gCOD converted/gVSS/d)
μ_{max} - maximum growth rate (d^{-1})
K_s - substrate concentration at which μ is egual to μ_{max} /2 (mM)

1.2. Bleaching process

The principal aim of bleaching of pulps is to increase the paper brightnes, purity and strength. The chromophoric compounds in unbleached pulps are predominantly functional groups of degraded and altered residual lignin. Bleaching can be performed by converting and stabilizing chromophoric groups without loss of substances ("lignin-preserving bleaching") or by removing the lignin ("lignin-removing bleaching")(3).

Chlorine and chlorine compounds are the best established and widely used bleaching agents for chemical pulps. Other used agents are peroxide and oxygen. The development of new chlorine-free bleaching processes offers interesting perspectives to reduce the environmental impact of bleachery effluents.
When concerning the total toxicity in effluents generated at a typical kraft pulp and paper mill, bleaching effluents are not the most important source of aquatic toxicity. However, bleaching effluents have received the highest attention due to the growing concern about the release of toxic chlorinated aromatic compounds in the environment. Many of these compounds have been shown to be considerably resistant to biological degradation and are persistent in to the environment (4,5). Bleaching plant effluents excert strong toxic effects on fish (6,7), invertebrates (8,9), and algae (9,10).

2. Experimental set-up and methods

The anaerobic degradation experiments are performed in the following order:
1. Performance of batch specific methanogenic activity tests of the granular sludge
2. Performance of toxicity tests
3. Start up of continuous dosing of wastewater/chemicals to the UASB reactors
4. Batch-wise kinetic studies of the degradation of chemicals at steady state.

All the experiments were performed in a thermostated room. The air temperature was kept at 26 ± 2 °C. The methodology for the tests is based on the experiences from the Agricultural University of Wageningen, Dept. of Environmental Technologies.(1,2)

Granular sludge was collected from full-scale treatment plant with UASB reactors at the maize factory, Latestain, in the Netherlands. It was further stored in the cooling room under nitrogen gas (approx. 4 °C and flushed with nitrogen gas occasionally).

2.1. Methanogenic activity assay

The experimental set-up for the batch method to determine the specific methanogenic activity of the granular sludge is shown in Fig 2.1 (2). The system is based on the so called "serum bottle liquid displacement system". The gas outlet is made of tubing connected to a syringe and a needle. The tests were performed at constant temperature (26 ± 2 °C).
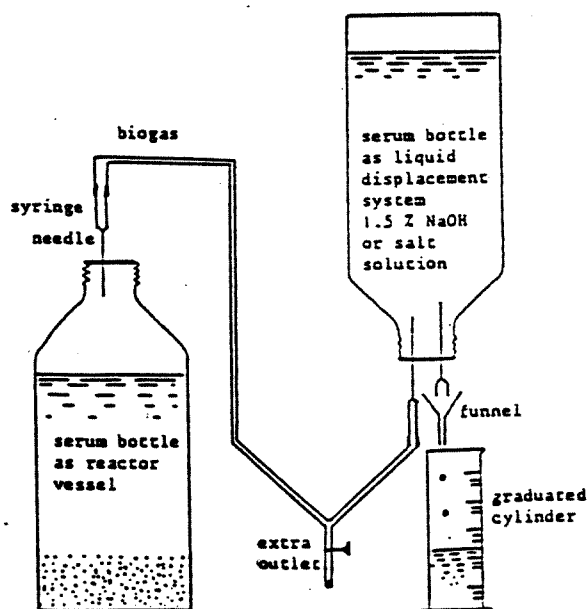
Figure 2.1.   The experimental set-up for the batch methanogenic activity assays.

Optimal conditions have to be assured (temperature, concentration of the substrate and the sludge, composition of the VFA substrate, pH etc.) The granular sludge concentration should not exceed 2 gVSS/l (used: 1.5 gVSS/l). A mixture of acetate (C_2), propionate (C_3) and butyrate (C_4) in the ratio of: 75:20:05 with a total concentration of 4 gCOD/l was used as substrate during the tests. Additonally, minerals and nutrients needed for optimal growth, were also supplied. $NaHCO_3$ was

added to give buffering capacity (1 gNaHCO$_3$ /1 g biodegradable COD of wastewater). pH was adjusted to 7.0 with NaOH. Tables 2 and 3 in Appendix 1 present the composition of stock solutions and trace elements as well as the recommended sludge and VFA substrate concentrations (2). As the substrate anaerobically degrades biogas is produced. The volume of methane gas (CH$_4$) produced was measured by liquid displacement. The liquid used is a strong solution of NaOH (1.5-3 %). As the biogas passes through these high pH solutions, the CO$_2$ of the biogas is converted to carbonate and absorbed into the liquid. Only the methane passes through the solution and the equivalent volume is pushed out of the flask. Using a control bottle the gas production can be corrected for the air pressure and temperature changes.

The COD convertion to methane gas can be calculated using the standard gCOD to mL CH$_4$ conversion factors. At 0 °C 1gCOD is equal to 350 mL of dry methane gas. The theoretical yield of gas at different temperatures can be calculated as following (assuming an elevation of sea level):

$$1 \text{ gCOD} = [350 * (273 + \text{temperature in °C}) / 273] \text{ mL CH}_4$$

The specific methanogenic activity is also related to the amount of time the sludge has to adapt to the VFA substrate used. During the first VFA feeding, the sludge is usually adapting itself to the VFA substrate. After the initial adaptation, a continued increase in activity will be observed per additional feeding. However these increases are small due to growth of new methanogenic bacteria in the sludge. In the tests performed at NIVA the first and second feedings were performed. When about 80% of the substrate was utilized in the first feeding, second VFA feeding was performed by adding more VFA stock solution at the same concentration as the first feeding.

The results of the tests are presented as a function of gas production in time.
The average slope during the activity period (where the gas production is almost linear) represents the rate of methane production in time (R). This value is converted to the specific methanogenic activity (ACT) in g CH$_4$-COD/gVSS/d according to the following:

$$ACT = (R^*24) / (CF*V*VSS)$$

where:
R = rate in mL CH$_4$/h
CF = conversion factor in mL CH$_4$ / gCOD
V = effective liquid volume in l
VSS = volatile suspended solids, sludge concentration in gVSS/l

2.2. Methanogenic toxicity assay

The purpose of the toxicity assay is to determine the reduction in methanogenic activity due to inhibiting compounds. The toxicity is determined by comparing the activity of the sludge fed only with substrate, to the sludge fed the same substrate added suspected toxic compound. There is differentiation between the toxicity determination methods (2,3):
   - toxicity of non-substrate inhibitors (where the inhibiting compound does not supply substrate to the medium); the experimental set-up is the same as the activity test. The "control" receives only VFA substrate, and the "treatments" receive in addition to the VFA substrate also variable concentrations of the inhibitor. pH must be adjusted.
   - toxicity of substrate inhibitors (where the inhibiting compounds or the wastewater with inhibiting compounds supply substrate to the medium, but less than 50% of the COD supplied by the VFA control); the experimental set-up is similar as the activity test. The "control" receives only VFA substrate. The "treatments" receive the same VFA as "control" and the substrate containing inhibitor. pH must be adjusted.

- toxicity of substrate inhibitors (where the inhibitors or the inhibiting wastewater supply more than 50% of the COD supplied by the VFA control); the experimental set-up is like an activity test. Each treatment concentration of inhibitors or inhibiting wastewater must have a separate VFA control. The performance of the tests (first feeding or exposure feeding and second feeding or recovery feeding) are according to dutch methods (1,2,3).

In order to calculate the inhibition the methanogenic activities (ACT) of the control (C) and treatments (T) are determined according to the calculations for the activity test. The percentage activity (%ACT) of the treatment compared to substrate control is calculated as follows:

$$\%ACT = (ACT_t / ACT_c) * 100$$

The percentage inhibition (%INHIB) is:

$$\%INHIB = 100 - \%ACT$$

2.2.1. Batch tests with synthetic wastewater and chemicals

Prior to addition of chemicals into the reactors batch tests were performed in order to observe the influence of the chemicals on the methanogenic activity of the granular sludge. If a toxicity is observed the applicable concentration of the chemicals would be chosen as the start concentration in case of continuous dosing into the reactors. The chemicals used were: 6-chlorovanillin (6CV) and trichloroacetic acid (TCA), in concentrations 0, 2.5 and 5.0 mg/l in three combinations: only 6CV, only TCA and the mixture of both in 50:50 ratio. Because the real concentration of the chemicals in wastewater is very low, the 10 times values were used in the experiments. Synthetic wastewater (VFA wastewater) was used ($C_2:C_3:C_4=75:20:05$) with the concentration of 4 and 1 gCOD/l. 4 gCOD/l is the standard concentration for tests and 1 gCOD/l was selected since the COD of bleaching wastewater was around this value. Macro and micro elements were added as described previously. pH was adjusted with NaOH, and $NaHCO_3$ was added (1 gNaHCO$_3$ / 1 gCOD) to increase the buffer capacity.

2.2.2. Batch tests with bleaching wastewater and chemicals

Bleaching wastewater was collected from Stora Billerud, Division Paper in Grums, Sweden with the following characteristics before the treatment in the aerated basin :

flow	around 40000 m^3/d
COD	1000-1300 mg/l
BOD	265-420 mg/l
suspended solids	70-75 mg/l
AOX	4-30 mg/l
chlorate	80-105 mg/l
N_{tot}	3-6 mg/l
P_{tot}	1-3 mg/l
pH	6-7

For bleaching purposes ClO_2 and H_2O_2 were used.

Similar experiments as with the synthetic wastewater was performed, adding accordingly 3 gCOD

from the VFA wastewater in the test with 4 gCOD/l, and using only bleaching wastewater in the test with 1 gCOD/l.

2.3. Continuous runs in the UASB reactors

After characterization of sludge and substrate continuous runs with 4 UASB reactors (each of 2 l volume) were started in order to adapt the whole system until the steady state was obtained. The hydraulic retention time was held about 10 hours. The temperature of air was kept constant at the level of 26 ± 2 °C, while the wastewater temperature in the reactors was around 26 ± 1 °C.

Scheme of the laboratory scale UASB reactor is shown below in Fig 2.2:

1 influent , medium and test-compound	6 effluent
2 stones , to insure fine distribution of medium	7 gas sampler
3 sludge blanket	8 NaOH-flask
4 liquid phase	9 soda lime pellets
5 gas collector	10 to gasmeter
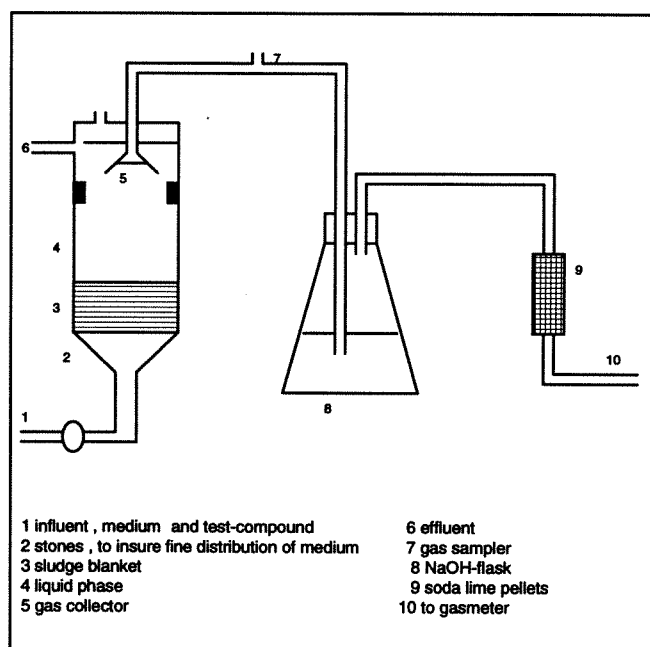
Figure 2.2.   Schematical presentation of laboratory-scale UASB reactor.

Performance of the reactors was controlled regulary. Measurements of: temperature, pH of the influent and the effluent and amount of gas produced were conducted almost every day. GC analyses for VFA (volatile fatty acids)/COD (chemical oxygen demand) were made every second/third day.

2.3.1. Analyses

Samples for (VFA) determination were filtered (Millex-GS filter, 0.22 μm), conserved with formic acid and determined by gas chromatography (GC) using a fused silica capillary tubing, Supelco and WCOT fused silica, Chrompack no 7680, as pre-column and column respectively. The temperature of the column, the injection and the flame ionization detector were (FID) 80 (initial) to 140 (end), 260 and 270, respectively. Nitrogen gas was used as a carrier gas at a flow rate of 2.5 ml/min. TSS and VSS were determined according to Standard Methods (11). The pH was determined with a pH meter JENWAY Model 3070 immediately after sampling in order to avoid a pH rise due to loss of carbon dioxide from the liquid.

Analyses of chemicals in bleaching effluents were established and the procedures included extraction, cleanup, derivatization followed by GC/ECD (column-Rtx-5, Restek no 10208,30m, 0.25mm id) to analyse the compounds concentration (12,13,14). The gas chromatographic methods give very low detection limits (15), for the chlorinated compounds.

6CV analyses showed to be problematic. Firstly analysed samples showed values lower than expected. It was found that addition of the inner standard (tribromphenol) and hexane in addition to the H_2SO_4 (applied usually) was of primary importance for stability of the samples.

2.3.2. UASB with the synthetic medium

The synthetic medium used in the experiments was a mixture of volatile fatty acid: acetic(C_2), propionic (C_3) and butyric (C_4) in a ratio of 75:20:05 on COD basis, respectively. The medium contained macronutrients (N, P and S) and trace elements required for bacterial growth (2,3). pH was adjusted to neutral.

2.3.3. Adaptation of the sludge to bleaching wastewater

Four UASB reactors with the granular sludge were run first on the synthetic wastewater (mixture C_2:C_3:C_4 - 75:20:05). Afterwards they were adapted gradually to the 100% bleaching wastewater by diminishing the percentage of the synthetic wastewater and substituted with the real wastewater. Consequently the COD concentration of the influent decreased from about 2.6 gCOD/l to the wastewater COD of about 1gCOD/l at the 100% bleaching wastewater. The adaptation procedure is shown in table 4 in the Appendix 2.

2.3.4. Start of dosing of the chosen chemicals

Once adaptation was reached, three of the reactors were fed additionally with the organochlorine chemicals (6CV, TCA and 6CV+TCA). Chemicals were pumped from separate stock solutions bottles (of very low pH). UASB 1 functioned as the control and was corrected for the extra carbon (with acetic acid -(C_2)). This was needed because the 6CV was dissolved in HAc+H_2O (50:50) and caused extra COD. Unfortunately, pumps supplying wastewater were often blocked because of the fiber particles, even though precautions were made to remove the largest particles. This resulted in lost activity of the bacterial consortium due to pH schock. This part of the investigations was supported by the student of the Engineering High School in Sarpsborg, Thomas Hellsten, and is presented in his thesis (19).

2.3.5. New start of the UASB reactors with a new granular sludge

After trying to recover the activity (by stopping the dosing of the chemicals and feeding the reactors with only the simplest substrate (C_2)) without success, two new reactors (reactor 1 and 2) were started-up with the granular sludge and fed with the synthetic wastewater. The methanogenic activity of the granular sludge (prior the start-up) showed the specific activity of the stored sludge to be similar as before. After achieving the pseudo steady state, one reactor was fed with 6CV while the other was used as a control.

2.3.6. Dosing of 6-chlorovanillin (6CV)

Once the adaptation was reached one of the reactors was fed additionally with 5 mg/l of 6CV. UASB 1 functioned as control and was corrected for the extra carbon (with acetic acid) as the 6CV was dissolved in HAc+H$_2$O (50:50) and caused extra COD. The chemical was added directly into the medium and pH was adjusted. The reactors were followed daily in order to control their performance. In addition the determination of the 6CV concentration in the influent and the effluent were made regularly. 6CV concentration was also determined in the sludge. This way sludge got adapted in both reactors to VFA substrate and additionally to the one adapted to 6CV. First, preliminary batch test with 7.5 mg 6CV/l was performed with the sludge from both reactors. In the meantime deterioration of the reactor activity was noticed. Attempts were made to gain back the optimal bacterial activity by decreasing the concentration of the carbon in the medium, or stopping periodically the feeding and using only recirculation of the effluent, followed by gradually increased carbon loading.

2.3.7. New start of the UASB reactor

While working on the two reactors improvement, a new reactor was started up (number 3) and adapted to the synthetic VFA medium. Temperature was held at 26±2 °C, as previously. This was made in order to save time and to be able to perform kinetic studies of 6CV degradation.

2.4. Batch tests - determination of the rate of degradation of 6-chlorovanillin

Batch tests with 6CV were performed in order to determine the chemical's degradation kinetics. Tests were carried out in three steps:

1. preliminary batch test with the adapted and unadapted sludge (reactor 1 and 2) and 7.5 mg 6CV/l (somewhat higher than the reactor concentration) to determine the sampling intervals proportional to degradation

2. preliminary batch test with sludge adapted to VFA substrate only (reactor 3) and 30 mg 6CV/l in order to observe weather there is inhibition of process reaction and to determine the sampling intervals proportional to degradation

3. batch test with sludge adapted to VFA substrate only (reactor 3) and the range of concentrations of 6CV (0, 10, 20 and 40 mg/l). The additional precautions were made in order to hold the C_2:C_3:C_4 ratio around 75:20:05. Tests were performed as duplicates. For one of the parallels the experiment ended after the chemicals were removed. Sludge was analysed for 6CV content, in order to observe whether there was any adsorption in the sludge. For the other of the parallels second dose followed the first in order to learn about the adaptation of the sludge to the chemicals.

The concentrations of the chemical were determined in time and the specific degradation rates (related to gVSS, to gCOD-CH_4 and gCOD-VFA) were calculated. The start and the end concentration of the 6CV in the sludge itself was also determined. Further, the removal rate against the chemical's concentration was plotted to determine the order of the reaction.

3. Results and Discussion

3.1. Methanogenic activity assay

The results of the first batch test (first and second feeding) on the specific methanogenic activity of the 3 types of the granular sludge are presented in the Figure 3.1. Tests were performed at 26±2 °C.
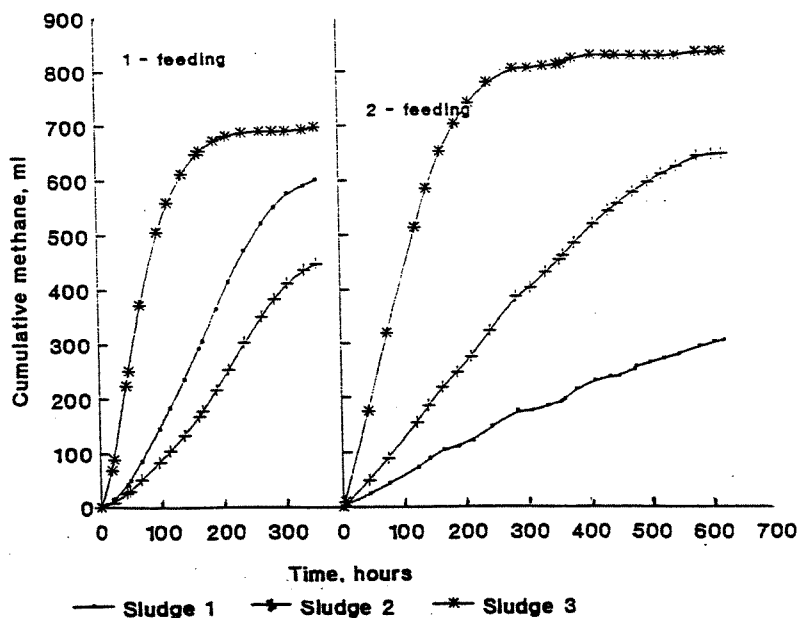
Figure 3.1.   Cumulative methane gas production from 3 sludges:
1: from the full scale UASB reactor treating paper wastewater,
2: from the full scale UASB reactor treating brewery wastewater, Bawaria,
and 3: from the full scale UASB reactor treating maize processing wastewater, Latestein.
1.5 gVSS/l, substrate: VFA (75:20:05) 4 gCOD/l, temperature 25°C.

The specific methanogenic activity was calculated as a slope of the exponential part of the cumulative gas production curve ("active period") and refered to the biomass expressed in g VSS/l (Fig.3.1). The highest activity was obtained for the granular sludge from the maize processing factory and was 0.15 g CH4-COD/gVSS*d.Other sludges showed relatively smaller activities. Sludge with the best performance (sludge 3) was chosen for all further experimental tests.

After the methanogenic activity of the bacteria in the UASB reactors was lost new batch test was performed in order to determine the specific methanogenic activity. Sludges stored in the refrigerator were used for this purpose. Latestain sludge was shown to be still "active" with similar activity of 0.15 g CH4-COD/gVSS/d as before (Figure 3.2)
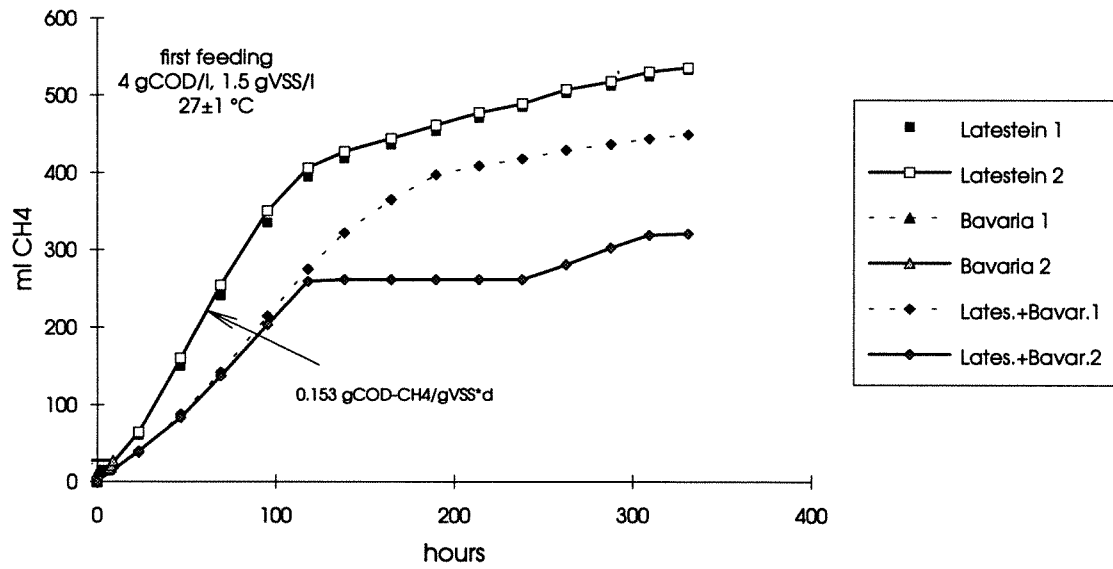
Figure 3.2.   Cumulative methane gas production from the full scale UASB reactor treating
maize processing wastewater, May 1993.

Tables with the experimental results are enclosed in the Annex 3.

3.2. Methanogenic toxicity assay

3.2.1. Batch tests with synthetic wastewater and chemicals

Figure 3.3 shows the results from the exposure and the recovery periods of granular sludge to chemicals (6CV and TCA) at the VFA substrate concentration of 4 and 1 gCOD/l. Doses of the chemicals were 0, 2.5 and 5.0 mg/l. 6CV and TCA were tested individually and mixed.
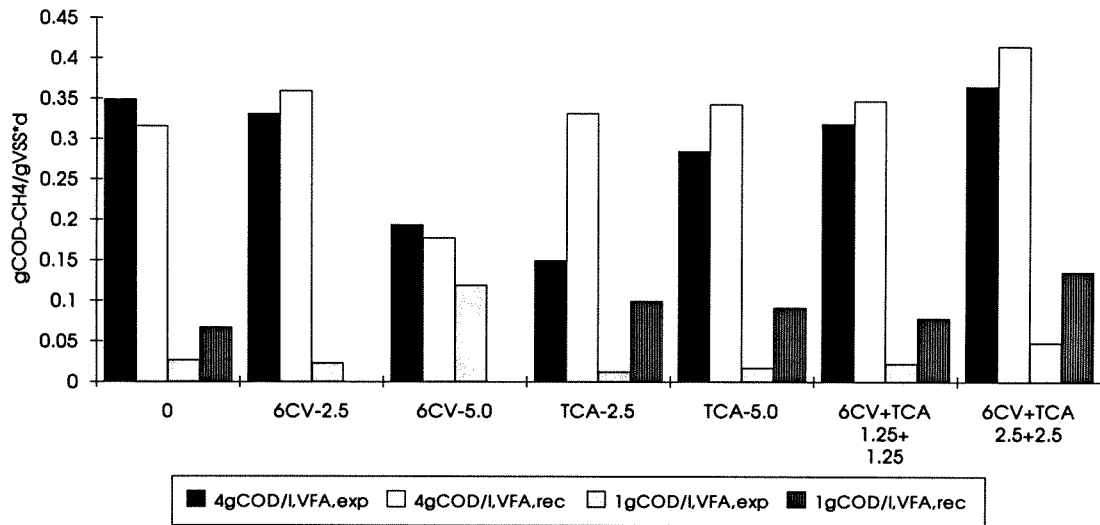
Figure 3.3.   Activity (gCOD-CH4/gVSS*d) from the batch tests with synthetic wastewater (4 and 1 gCOD/l) and the chemicals.

All the results are summerized and concluded in the next chapter.
Other results are enclosed in Annex 4.

3.2.2. Batch tests with bleaching wastewater and chemicals
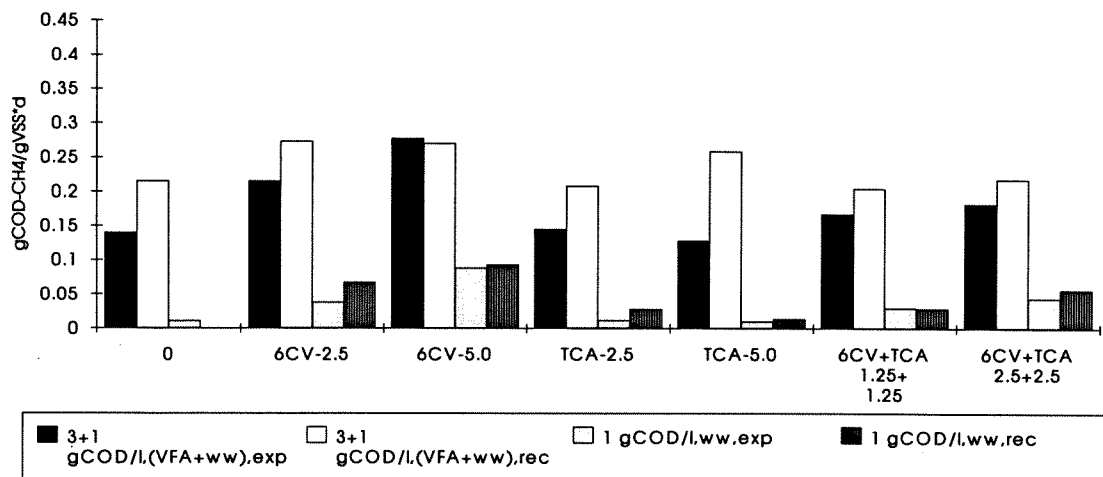
Figure 3.4   Activity (gCOD-CH4/gVSS*d) from the batch tests with bleaching wastewater (3+1 and 1 gCOD/l) and the chemicals.

Figure 3.4 shows the results from the exposure and the recovery periods of granular sludge to chemicals at the substrate concentration of 4 (1 gCOD/l from bleaching wastewater and 3 gCOD/l- from the VFA wastewater) and 1 gCOD/l.
Other results are enclosed in Annex 4.

All the results are summerized in the Table 5.

Table 5. The specific methanogenic activites based on the methane gas production.

Activity gCOD-CH4/gVSS*d

Test	Substrate			6CV		TCA		6CV+TCA	
name	name	conc.	0	6CV-2.5	6CV-5.0	TCA-2.5	TCA-5.0	6CV+TCA 1.25+ 1.25	6CV+TCA 2.5+2.5
		gCOD/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
TOX-1	VFAexpos	4	0.349	0.331	0.193	0.149	0.284	0.318	0.364
	VFArecov	4	0.316	0.359	0.177	0.331	0.342	0.346	0.414
	VFAexpos	1	0.027	0.023	0.119	0.012	0.017	0.022	0.048
	VFArecov	1	0.067	0.237*	0.246	0.099	0.091	0.078	0.135
TOX-2	VFA+ww	3+1,exp	0.14	0.215	0.277	0.145	0.128	0.167	0.182
	VFA+ww	3+1,recov	0.215	0.273	0.27	0.208	0.259	0.204	0.218
	ww-expos	1	0.011	0.038	0.088	0.012	0.01	0.03	0.044
	ww-recov	1		0.067	0.093	0.028	0.014	0.029	0.056

It seems that when TCA was used activity was lower than in the control. 6CV and the mixture of 6CV and TCA caused increase in activity. It can also be observed that the activities of the recovery tests (where chemicals are not present) are higher (with exception for 6CV, 4 g COD/l, VFA substrate) than the activities of exposure period (where sludge samples were exposed to the chemicals).

In most cases it could be noticed that:
- the specific methanogenic activities of the recovery tests are higher than those obtained during the exposure tests
- in the tests where the substrate was synthetic (VFA mixture) activity values are reasonable higher than those with the mixture of VFA and bleaching wastewater or plain bleaching wastewater
- no inhibition of methane gas production at tested concentrations (2.5 and 5.0 mg/l) with 6CV and TCA was observed. In contrary the addition of chemicals dissolved in acetic acid resulted in increased carbon concentration and led respectively to extended gas production.

From these tests it can be concluded that the concentration of 5 mg/l of the chemicals is safe for start-up in the continuous reactors.

3.3. Continuous runs in the UASB reactors

Because of the problems with continuous pumping of bleaching wastewater two additional start-ups of the reactors were necessary. Firstly, four reactors were adapted to the VFA wastewater and consequently adapted to bleaching wastewater (started in August 1992, "rested" during the Christmas 1992, and run until April 1993). Stop in April was caused by loosing the methanogenic activity. This was a result of pH-shock from the pumping of the very low pH chemicals stock solution while bleaching wastewater pumping was blocked by the large particles. In May and June 1993 trials were made to recover the bacterial activity, but without success. Secondly, two new reactors were adapted to the VFA wastewater (started July 1993). Performance of the reactors based on the gas production is shown in Figure 3.5.
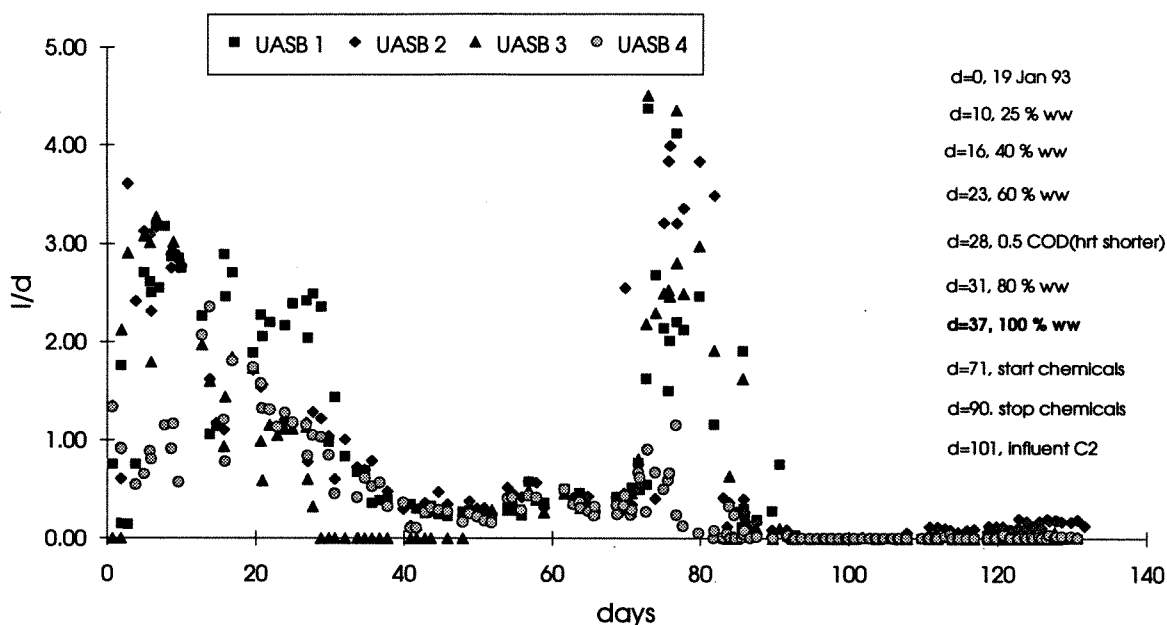
Figure 3.5.   Gas production - performance of the four UASB reactors. Gradual adaptation to bleaching wastewater and dosing of chemicals (control, 6CV, TCA, 6CV+TCA).

After reactors were adapted to synthetic wastewater with the carbon concentration of 2.6 gCOD/l (VFA (75:20:05)) gradual adaptation to bleaching wastewater took place. Synthetic wastewater was replaced in about one week intervals with 25, 40, 60, 80 % and finally at day 37 of run of reactors with 100 % bleaching wastewater. Carbon concentration was 1 gCOD/l. Reactors were run at this concentration for the next three weeks and showed relative stable performance. Zero gas production shown for reactor 3 was caused by misfunctioning of the gas-meter. At day 71 dosing of the chemicals started (UASB1-control, UASB2-5 mg6CV/l, UASB3-5 mgTCA/l and UASB4-2.5 mg6CV/l+2.5 mgTCA/l). Increase of carbon concentration was caused by that the 6CV stock solution was prepared with pure acetic acid. After a few days a drastic pH decrease to 3 was noticed as a result of blocked pumps and therby not delivering wastewater to the reactors. This finally resulted in total loss of the methanogenic activity. Recovery of activity was attempted by stopping the dosing of the chemicals and feeding the reactors with carbon in form of C_2 only.
Additional information on the reactors performance is enclosed in Appendixes 2 and 5.

3.3.1. UASB with the synthetic medium

Results are presented in the main paragraph 3.3.

3.3.2. Adaptation of the sludge to bleaching wastewater

Results are presented in the main paragraph 3.3.

3.3.3. Start of dosing of the chosen chemicals

Two chemicals were continuously dosed to the reactors. 6-chlorovanillin and trichloroacetic acid. Reactor 1 worked as control, 5 mg 6CV/l was added to the reactor 2, 5 mg TCA/l was added to the reactor 3 and a mixture of 2.5 mg 6CV/l + 2.5 mg TCA/l - to the reactor 4. Results are included in 3.3 and Appendix 5.

3.3.4. New start of the UASB reactors with a new granular sludge

Results of the two reactors performance are presented below. Room temperature was of 26±2 C. Figure 3.6 shows the performance of the reactors based on the COD removal and Figure 3.7 - efficiency of COD removal.
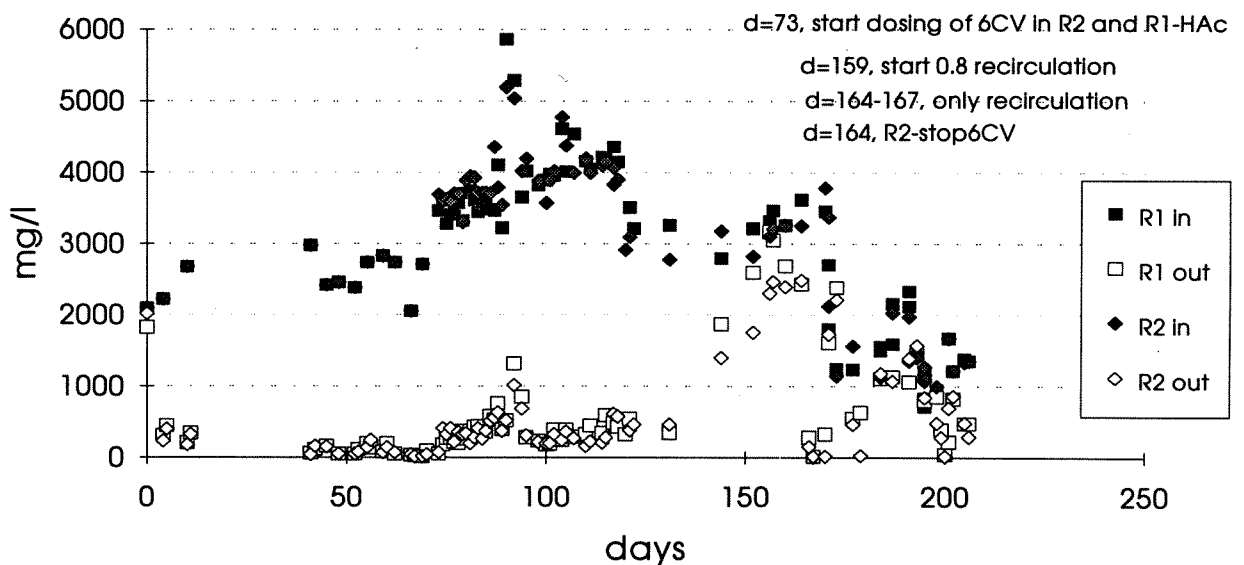
Figure 3.6.   UASB 1 and 2, COD in and out, temp.±27 °C.
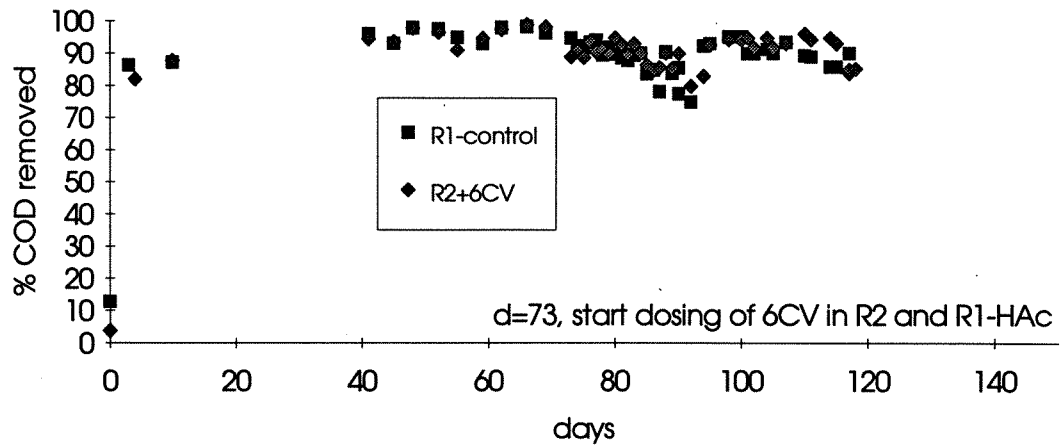
Figure 3.7.   Efficiency (COD removed) of the UASB 1 and 2, temp.±27 °C.

Additional results are enclosed in Appendix 6.

3.3.5. Dosing of 6-chlorovanillin

At day 73 of the continuous run of these two reactors addition of the 6CV (5mg/l) was started to the one of the reactors. Figure 3.8 show the reactors performance and their efficiency based on the COD, after the dosing was started.
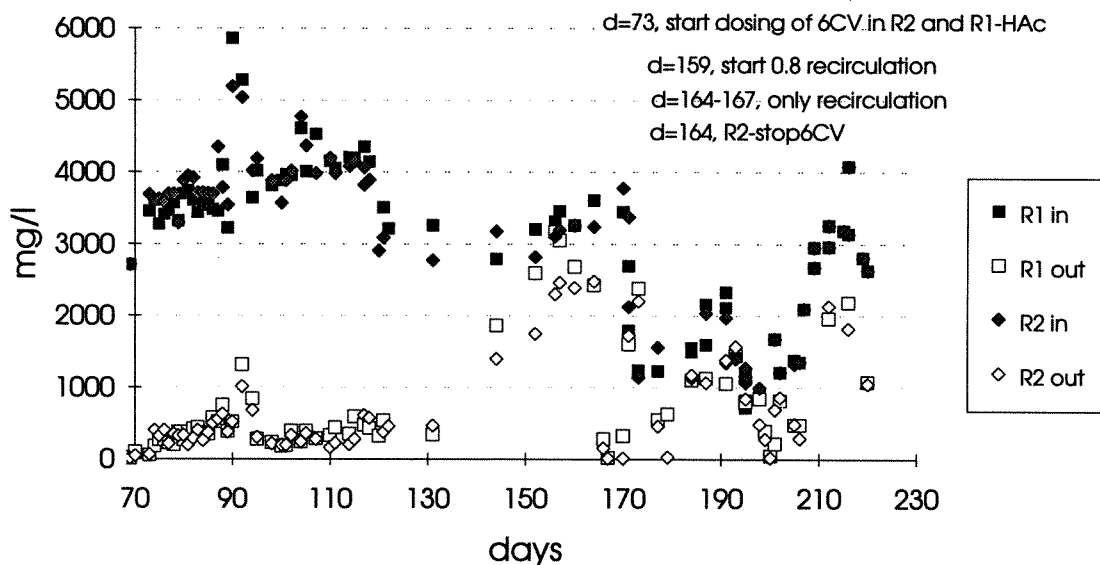
Figure 3.8.   UASB 1 and 2, COD in and out from 6CV was dosed, temp.±27 °C.

No inhibition of the gas production in the reactor with the 6CV compared to the control reactor is observed at the initial stage of the performance, hovewer, somewhat higher COD/VFA concentrations are noticed in the effluent of the reactor with 6CV. Preliminary batch tests with

sludge withdrawn from the reactors at pseudo steady-state and with 7.5 mg 6CV/l were conducted. Loss of the methanogenic activity in both reactors was noticed after the next 6 weeks period. High C2 concentration was due to that 6CV was dissolved in acetic acid. Loss of activity in the reactors may be the result of shifting the $C_2:C_3:C_4$ ratio from 75:20:05 in favour of increased C_2 in relation to C_3 and C_4 and that the C_2 fraction exeeded the 50% inhibition limit.

Figure 3.9 shows the efficiency of the anaerobically removed 6-chlorovanillin.
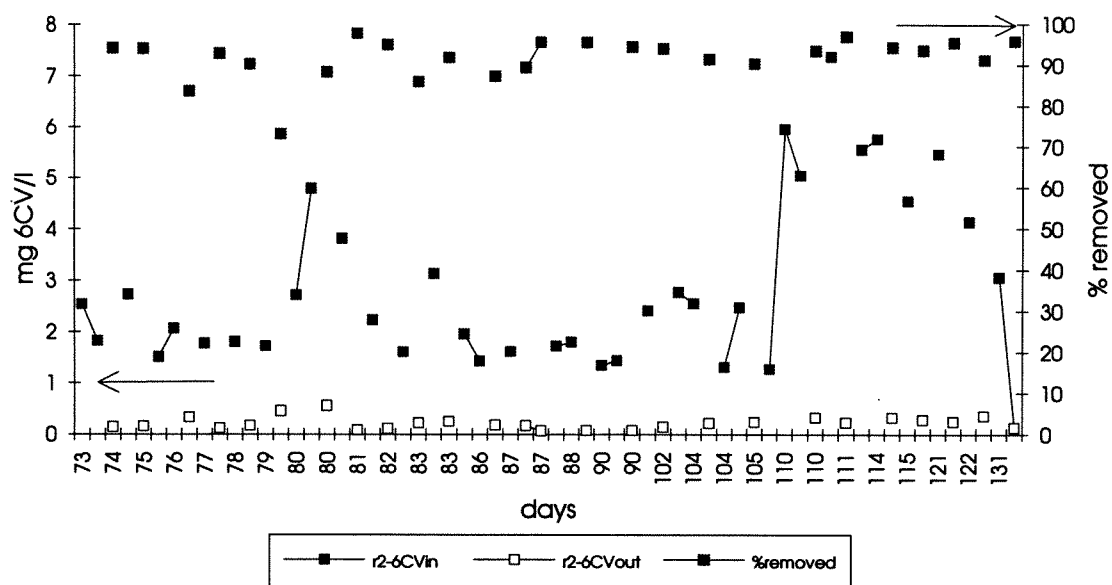
Figure 3.9.   UASB 2, dosing of 6CV and it's removal efficiency, temp.±27 °C.
6CV analyses were problematic mainly due to unknown problems with sample storage. Firstly analysed samples showed values lower than expected. It was found that addition of the inner standard (tribromphenol) and hexane with tert-Butylmethyl ether in addition to the H_2SO_4 (applied usually) was of primary importance for stability of the samples.

Dosage of 5 mg 6CV/l was likely over the total period (day 73 - 130) giving removal efficiency of higher than 95 % over the total dosage period.

3.3.6. New start of the UASB reactor

Performance of the reactor is presented in Figure 3.10. At day 4 part of sludge was withdrawn in order to test it with 30 mg6CV/l, At day 15, next test was set-up, with the range of the 6-chlorovanillin concentrations (0=blank, 10, 20 and 40 mg/l). Figure 3.11 shows the gas production.
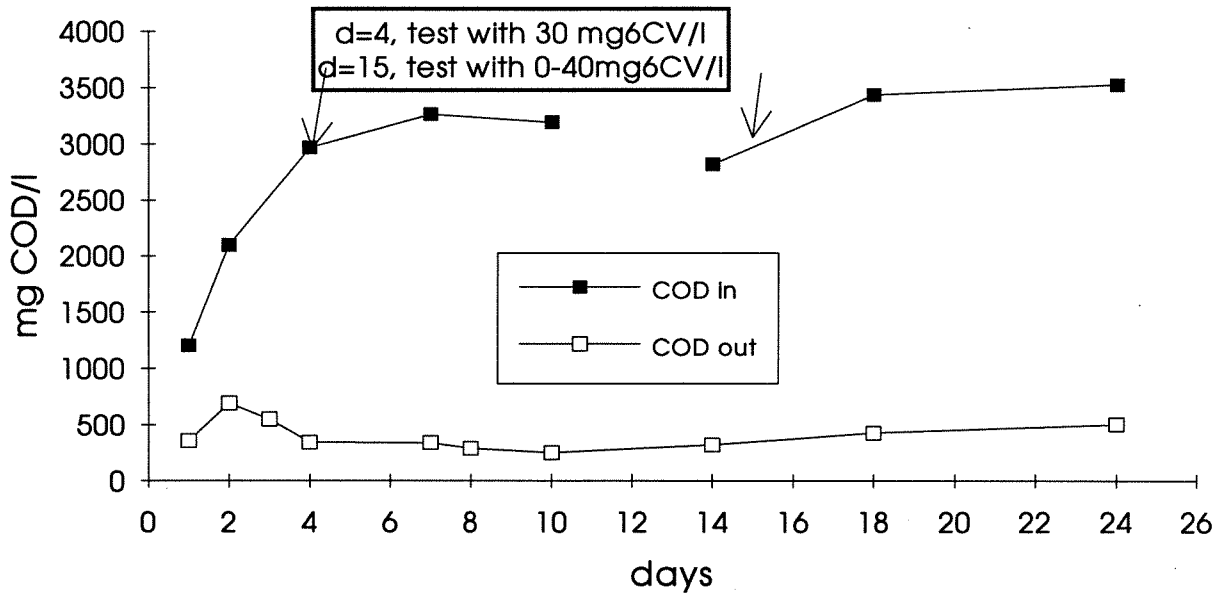
Figure 3.10.   UASB 3, COD in and out, temp.26±1 °C.
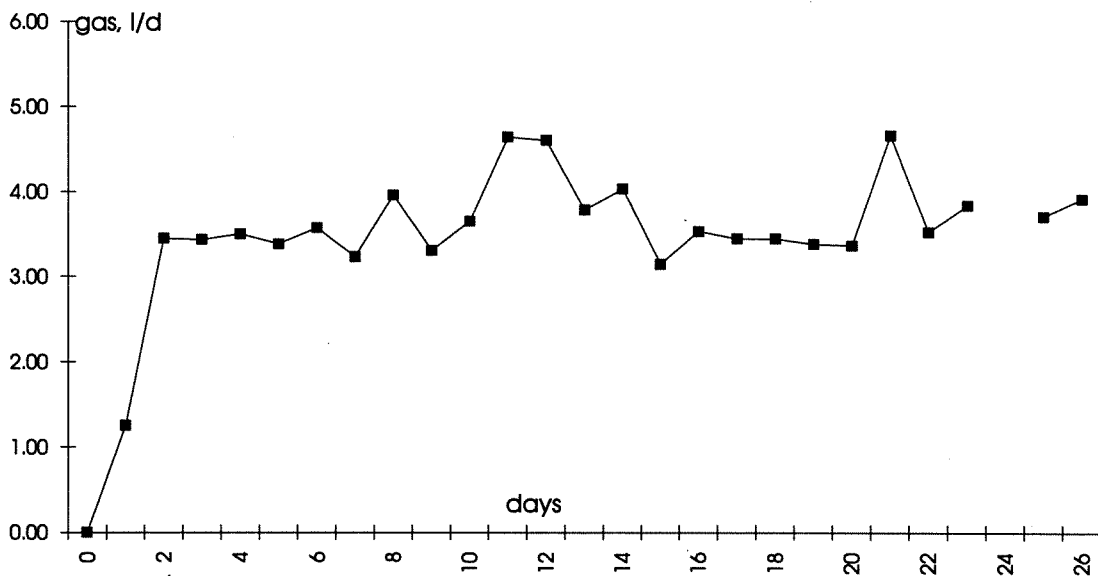
Figure 3.11.   UASB 3, gas production in time, temp.26±1 °C.

Additional results of the reactor 3 are enclosed in Appendix 7.

3.4. Batch tests - determination of the rate of degradation of 6-chlorovanillin

Results of the batch test on 6CV degradation for the 1st (with 2 samples parallel) and the 2nd dosing, where 6CV concentration was 0, 10, 20 and 40 mg/l, the VFA substrate concentration was of 4 gCOD/l and the sludge concentration of 1.5 gVSS/l are shown in the Figure 3.12 below. Temperature was held at 26±2°C.
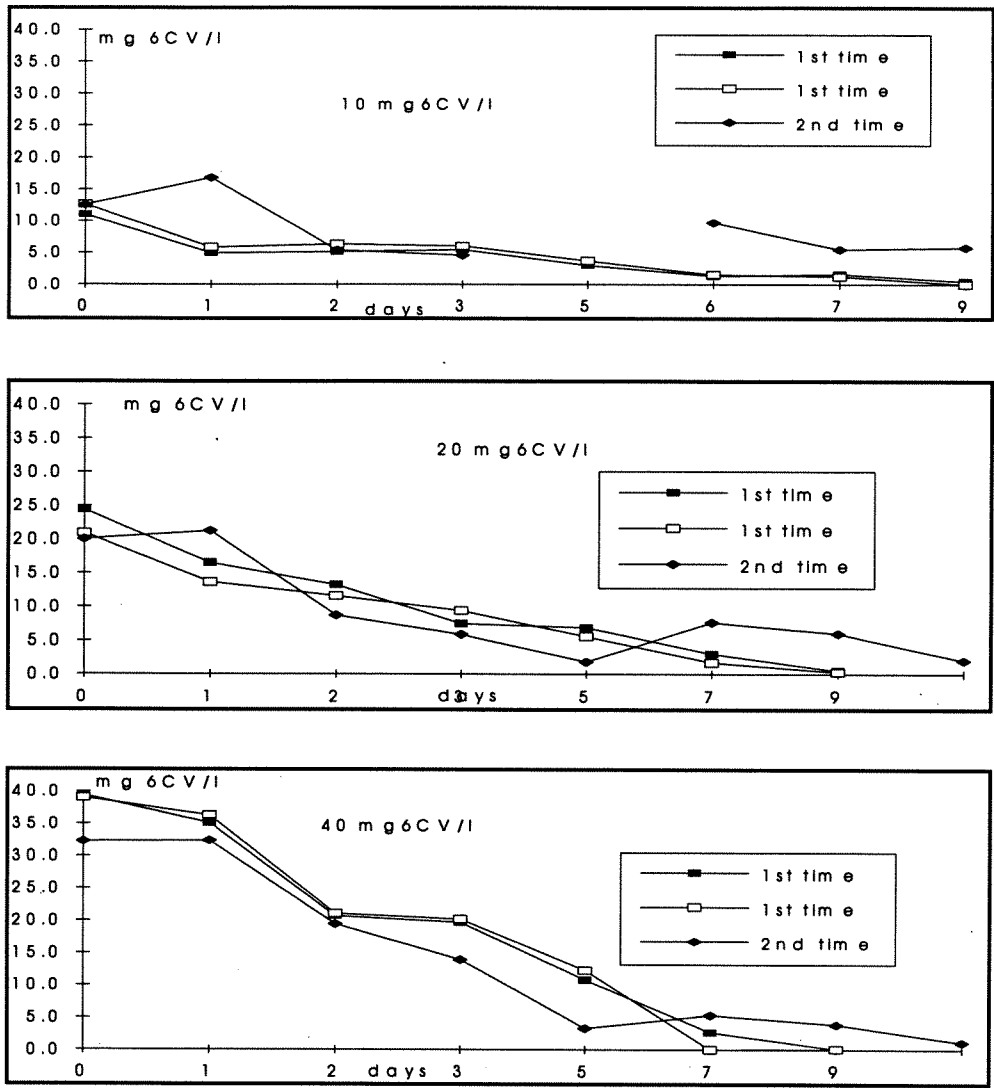
Figure 3.12.   6 chlorovanillin degradation in time, temperature 26±2 °C.

One of the parallels from the first dosing was continued with the second dose of VFA and 6CV. No tendency was shown for the adaptation of sludge to the 6 chlorovanillin. Degradation curves from the 2nd dosing show similar trends as those from the first parallels.

Respectively, Figure 3.13 shows COD-VFA degradation over time for the same 1st and 2nd tests at different 6CV concentrations.
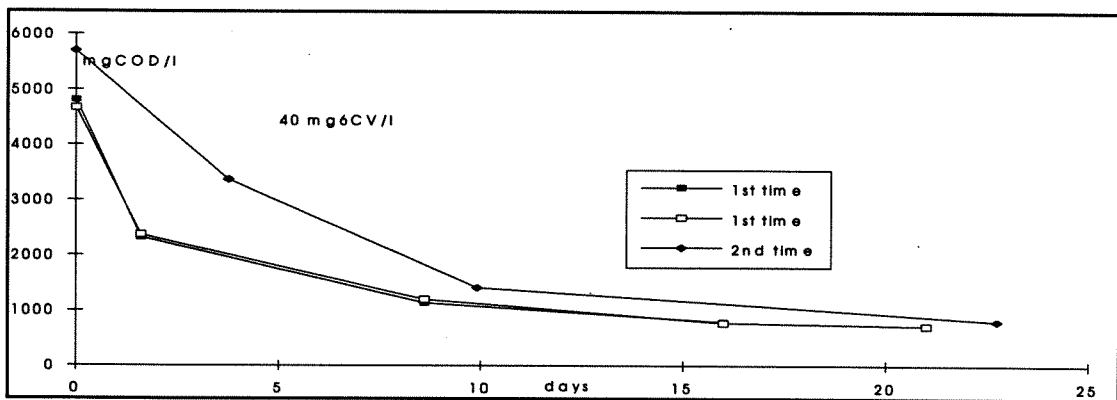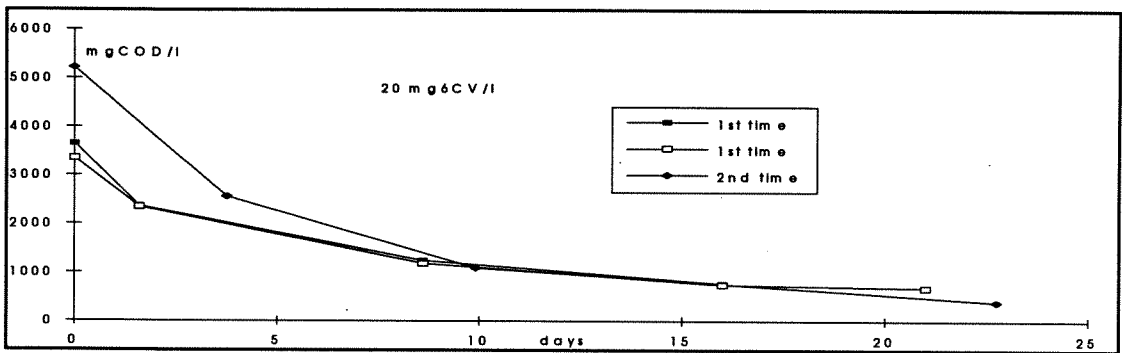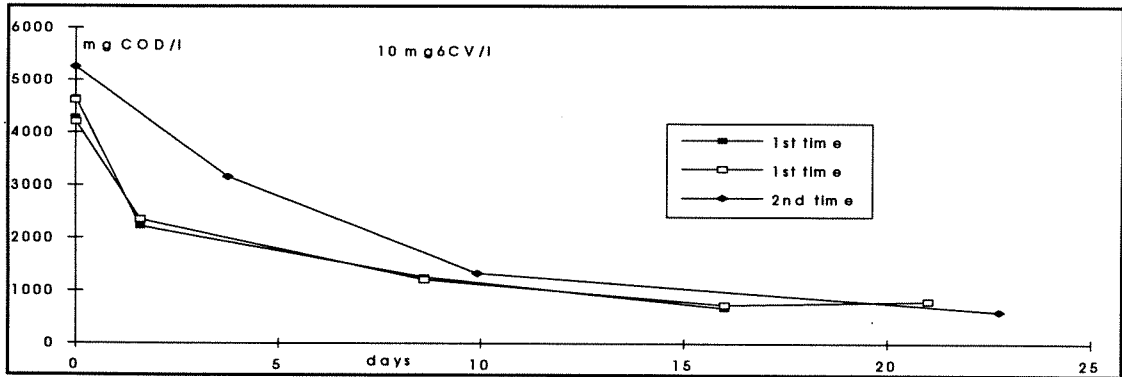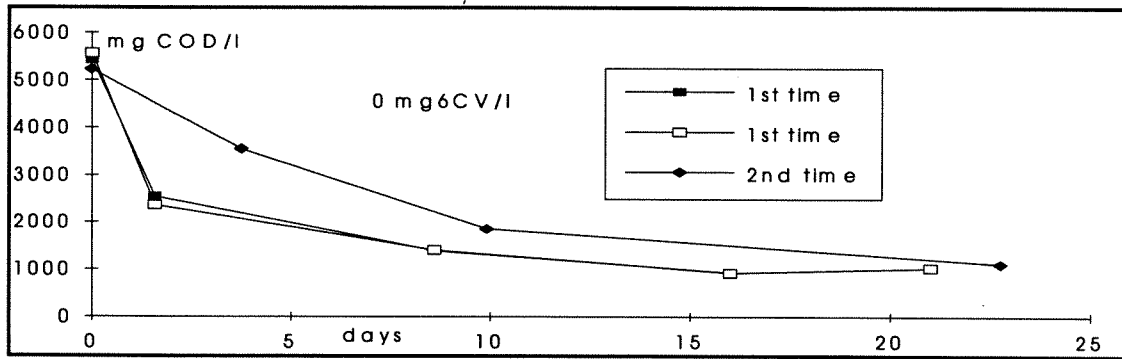
Figure 3.13. COD degradation in time at different 6CV concentrations.

No significant differences were observed between the different concentrations of 6 CV or the parallels (1st time). For the 2nd time runs it can be noticed that the initial degradation slopes were less steep than during the first time dosing of the chemicals.

Figure 3.14 presents the relation of the 6-chlorovanillin concentration versus the specific degradation rates and Figure 3.15 shows the gas production in time.
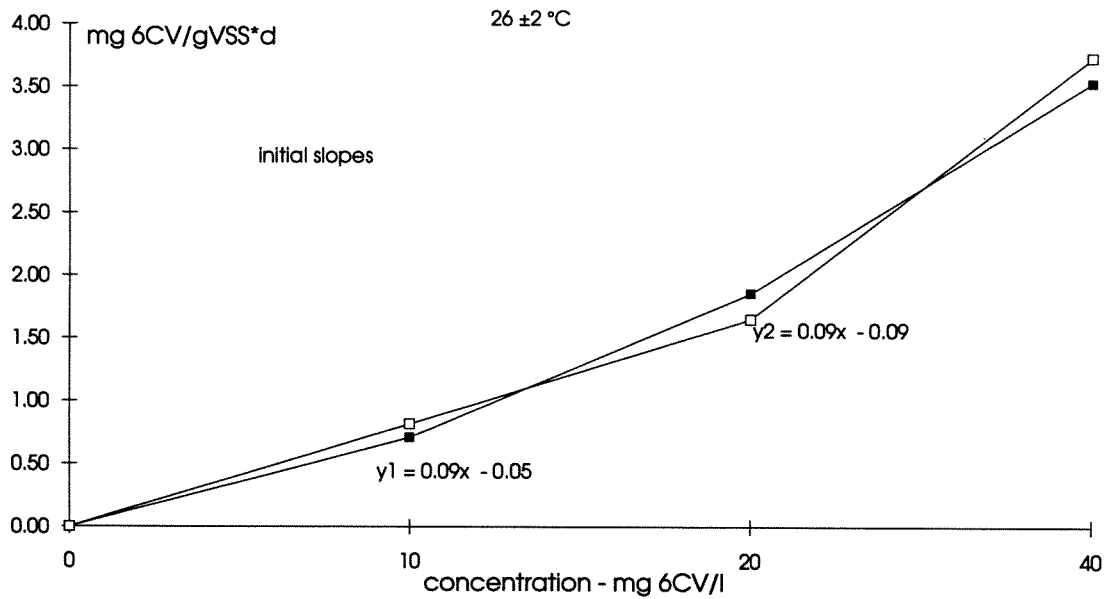
Figure 3.14. Specific degradation rates of 6cv versus concentration of 6CV.
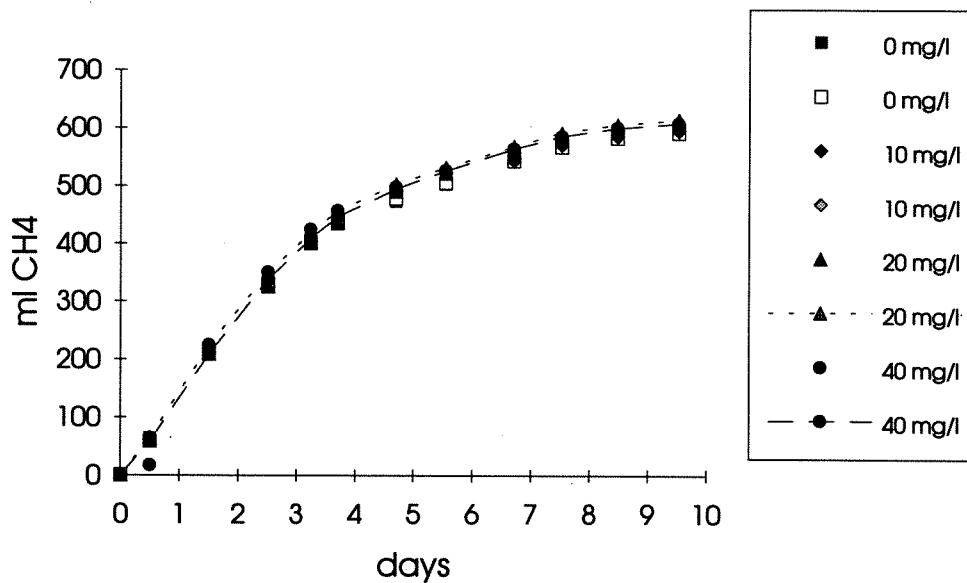
Figure 3.15. Gas production in the test with the range of 6CV concentrations, temp.25±1 °C.

Results of the experiments on degradation of 6CV are summerized in Table 6 below:

Reaction rates from the kinetics of 6CV test

mg 6CV/l	0	0	10	10	20	20	40	40
mg6CV/gVSS*d			0.71	0.81	1.85	1.65	3.53	3.73
mg6CV/gCOD			3.35	4.18	11.40	10.95	15.09	16.81
gCOD-CH4/gVSS*d	0.22	0.22	0.25	0.22	0.21	0.23	0.25	0.22
gCOD-VFA/gVSS*d (bad corellation)	0.15	0.15	0.21	0.19	0.16	0.15	0.23	0.22

Based on the overall results it can be concluded that:
• no toxicity was observed regarding COD degradation
• ther is no correlation between COD and 6CV degradation

Taking into consideration that the gas production was approximately equal for all tested cases, one can assume that the relationship between the degradation rate and the concentration of 6CV (in the range of tested concentration and the tested temperature) is linear.

Other results are enclosed in Appendix 8.

4. Conclusions

The rate of degradation of 6CV (in the batch pre-tests with sludge adapted and unadapted to 6CV (1.5 gVSS/l) and 7.5 mg 6CV/l)was found to be 1.94 mg/gVSS*d.

The following degradation tests with 30 mg/l and the series of concentrations (0, 10, 20 and 40 mg 6CV/l) were conducted with the sludge from a new reactor, fed with the synthetic medium only, at pseudo steady-state. The rates of 6-chlorovanillin degradation obtained are in the range from 0.76 to 3.63 mg6CV/gVSS*d. The specific methane gas production rate was measured from 0.22 to 0.25 gCOD-CH4/gVSS*d independent on the 6CV concentration.

From the experimental work performed in this project it can be concluded:
- addition of 6-chlorovanillin to both : synthetic as well as bleaching wastewaters, causes problems. This was observed as loss of bacterial activity and low methane gas production, presumabely as a result of too high participation of the acetic acid fraction. Reactors fed with synthetic medium kept up activity longer than those fed with bleaching wastewater
- extra acetic acid added, necessary to keep 6CV in solution influenced performance negatively also of the control reactor
- chlorinated compounds in bleaching wastewaters may be degraded anaerobically. However, the performance of UASB reactors fed with bleaching wastewater needs to be studied further due to problems arising from constituents other than chlorinated compounds.

Loss of activity in the reactors may also be the result of shifting the C_2:C_3:C_4 ratio from 75:20:05 in favor of increased C_2 in relation to C_3 and C_4. Additional precautions have to be taken to compensate for this (as for example more accurate pH adjustment having in mind the 50% inhibition concentration of C_2, C_3 and C_4 and/or use of more concentrated stock solution of 6CV). This phenomenon did not occur in the batch tests, as COD conditions and pH are more stringent and the chemical was dosed once.

In case of the application of bleaching effluent additional precautions must be insured (as removal of the fibers and other large particles). Proper knowledge of the wastewater composition is of importance.

5. General remarks and advice on UASB reactor operation

5.1. Application for kraft bleach effluent

UASB reactors are known to be successfully applied worldwide in wastewater treatment. However, the lack of detailed knowledge on how the chemical composition influences the bacterial activity prevents so far the application of UASB-technology for the treatment of bleaching wastewaters.

Moreover, the problem of chlorinated compounds in bleaching effluents seem to become less pronounced in the future due to efforts to minimize the use of the chlorine dioxide and substitute with other agents. The toxicity of pulping wastewaters was found to depend strongly on the pulping conditions used. The development of new chlorine-free bleaching processes offers perspectives to reduce the environmental impact of bleachers effluents. Bleaching sequences including an oxygen bleaching stage appear to be the most promising for the future. The main advantage of oxygen bleaching is that the resulting effluents do not contain chlorinated lignin fragments and they can be processed within the normal kraft liquor incineration systems. The BOD load can be reduced by

25-50% and the colour in the effluent by 65-80% compared to conventional bleaching (3,12,13).

5.2. Advice on reactor performance

• start-up conditions:

The granulation of anaerobic sludge (the transformation of flocculent anaerobic biomass into stable aggregates) can take place by many ways (22, 23) and can be affected by:
   - different seed matherials used and their amount (digested sewage sludge, flocculent sludge, aerobic activated sludge, primary sludge from an aerobic plant etc.)
   - type of wastewater used (VFA (volatile fatty acid) mixtures, different types of wastewater, sucrose-containing substrates etc.)
   - the avaibility of essential nutrients for optimal growth conditions
   - the pH at the optimal range (6.5 - 7.8)
   - presence of some cations at desired concentration (for example: Ca^{2+} ions at concentrations of 100-200 mg/l stimulate the granulation process, and become inhibitory at concentrations exceeding 2500 mg/l according to McCarty (24) while Wu et al (25) found that at 800 mgCa^{2+}/l granulation will not proceed satisfactorily)
   - the sludge selection pressure which originate from both the hydraulic loading rate (or superficial upflow velocity) (0.1 to 1.0 m/d in the first week of an initial start-up, which will gradually increase, ultimately to values up to 30 to 50 m/d, which are frequently applied in full-scale UASB installations) and the gas loading rate.

Both the sludge characteristics themselves and the sludge characteristics needed for proper operation are closely related to the process conditions applied and the composition of the wastewater treated.

In general, the initial start-up of UASB reactor using digested sewage sludge as innoculum may last several month before a distinct granular sludge bed develops (22).
Figure 5.1 below demonstrates the extreme complexity of the relations between the many microbiological and physical factors involved in sludge granulation (23):

SUBSTRATE COMPOSITION
- CONCENTRATION
- COMPOSITION
- pH

PROCESS CONDITIONS
- SLUDGE LOADING RATE
- UPWARD VELOCITY LIQUID
- PROCESS TEMPERATURE

REACTOR DIMENSIONS
- HEIGHT
- THREE PHASE SEPARATOR
- INFLUENT INLET SYSTEM

BIOLOGY LEVEL
- COMPOSITION
- KINETICS
- STABILITY
- MORPHOLOGY
- BACTERIAL DENSITY

GRANULE LEVEL
- DIFFUSION/POROSITY
- (SUBSTRATE) TRANSPORT
- SPECIFIC GRAVITY
- ASH CONTENT
- DIAMETER

REACTOR LEVEL
- MIXING CHARACTERISTICS
- SHEAR FORCES
- SLUDGE WASH-OUT
- PRECIPITATION / INCORPORATION

BIOLOGICAL SLUDGE CHARACTERISTICS
- ACTIVITY
- DEGRADATION PATHWAY

FYSICAL SLUDGE CHARACTERISTICS
- STRENGTH
- SEDIMENTATION VELOCITY

NET SLUDGE GROWTH

TREATMENT EFFICIENCY
PROCESS STABILITY

Figure5.1.   Factors affecting sludge granulation (23).

Today however, almost all full-scale reactors are started-up using granular seed sludge instead. In this case, the start-up period for full-scale UASB reactors now takes only a few days. This implies a need for new knowledge of factors affecting the quality and growth of anaerobic granular sludge. Some process conditions and/or types of wastewaters may cause a serious decrease in granule quality or an insufficient growth of granular sludge. Also changes in process conditions (as: loading rate, influent concentration, substrate composition, process temperature etc.) may cause an instability in the treatment process(23).

The best way to know that the biomass has been adopted to the new conditions is to observe the biogass formation and compare to the expexted level. Batch methanogenic activity tests may be carried out, in regular interval of time, to observe the trend of bacterial activity.

- continuous operation:

    - For optimal continuous operation following parameters should be checked:
        - COD concentration in the influent and the effluent, (by VFA determination by GC concentrations of the fractions of C_2, C_3 C_4 etc can be determinated (recalculated to COD with the COD factors: 1.067, 1.514 and 1.818 gCOD/gVFA; for C_2, C3 and C_4, respectively))
        - biogas production should be compared to the expected value
        - pH in the reactor
        - flow rate into the reactor (consequently, retention time, organic volumetric rate $(kg/m^3.d)$
        - oxygen concentration (ocasionally)

- Some recommended values for operating conditions are listed in Appendix 9, based on the literature.

• problems experienced during this study:

- The UASB-reactors operated satisfactorily while synthetic wastewater was applied. Necessary macro- and micronutrients, the optimal conditions of pH, temperature and the carbon concentration in desired form were assured. Here, the general recommendations for the reactor operations (given in literature reference 2) were followed. Continuous and stable gas production was obtained as a result of carbon degradation.
- Operational problems arised when bleaching wastewater was dosed. To some extent this could be expected because the wastewater has a lower carbon concentration (COD ca. 1 g/l) than recommended for UASB reactors (> 2 gCOD/l). Even if gas production was lower than calculated, stable gas production was observed over longer periods of time.
Beside the necessity of removal of larger particles from the wastewater (blocking of pumps) also the total chemical composition of the wastewater appeared to be of great importance for the optimal performance of the reactor. Likely not only halogenated compounds may influence the different bacterial processes but also other unknown organic and inorganic constituents.
As experienced later in the project (batch tests), the selected chlorinated compounds did not inhibit bacterial activity at the chosen concentrations.
- Other operational problems appeared when selected chemicals were dosed continuously. The necessity of low pH to keep the test-chemical in solution and the increase of the C_2-fraction (acetic acid) in the influent to the UASB reactors led to inhibition and finally loss of the methanogenic activity.

Most of the problems arised in connection to the chemicals are not relevant for field application, where the chemicals are as a mixture and in general at lower concentrations.

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Appendix 1. Composition of stock solutions and nutrient and trace elements.

Table 2 Composition of Stock Solutions of Nutrients and Trace Elements, Used in the Laboratory*

Solution 1. Macro-nutrients

NH_4Cl	170 g L^{-1}	
KH_2PO_4	37 g L^{-1}	

$CaCl_2.2H_2O$	8 g L^{-1}
$MgSO4.4H_2O$	9 g L^{-1}

Solution 2. Trace elements

$FeCl_3.4H_2O$	2000 mg L^{-1}
$CoCl_2.6H_2O$	2000 mg L^{-1}
$MnCl_2.4H_2O$	500 mg L^{-1}
$CuCl_2.2H_2O$	30 mg L^{-1}
$ZnCl_2$	50 mg L^{-1}
H_3BO_3	50 mg L^{-1}

$(NH_4)_6Mo_7O_24.4H_2O$	90 mg L^{-1}
$Na_2SeO_3.5H_2O$	100 mg L^{-1}
$NiCl_2.6H_2O$	50 mg L^{-1}
EDTA	1000 mg L^{-1}
HCl 36 %	1 mL L^{-1}
✝ Resazurin	500 mg L^{-1}

Solution 3. Sulfide. This solution has to be made freshly. Make 25 mL.

$Na_2S.9H_2O$ 100 g L^{-1}

4. Yeast Extract**

* These solutions are added to the (synthetic) wastewater in a concentration of 1 mL L^{-1}. When the COD of the (synthetic) wastewater exceeds 3000 mg L^{-1}, then the macro-nutrient solution concentration is doubled.
** add 0.2 g yeast extract L^{-1} (synthetic) wastewater

Table 3 Recommended Sludge and VFA Substrate Concentrations for the Methanogenic Activity Assay

Experiment System	Sludge g VSS L^{-1}	VFA* g COD L^{-1}
Stirred**	2.0 to 5.0	2.0 to 4.0
Unstirred	1.0 to 1.5	3.5 to 4.5

* neutralized to pH 7
** do you expect the activity of your sludge to be less than 0.1 g COD_{CH4} g^{-1} VSS d^{-1} ? Than use stirred system with 5 g VSS L^{-1} sludge and 2.0 g COD L^{-1} VFA, the digestor volume should also be greater than 2 L.

Appendix 2. The adaptation procedure of synthetic wastewater to bleaching effluent.

Table 4

UASB-reactors
performance from January 1993

No. day	Date	R1	R2	R3	R4	Total COD g//l
0	19Jan.	◄——————VFA(75:20:05) ——————►				2.6
10	29 Jan.	VFA	◄—— 25 % ww ——►			2.6/2.2
16	4 Feb.	VFA	40 % ww			2.6/1.96
23	11 Feb.	VFA	60 % ww			2.6/1.64
27	15 Feb.	◄——	50 % VFA concentration ►			1.3/1.31
31	19 Feb.	◄—— 80 % ww ——————►				1.06
37	25 Feb.	◄—— 100 % ww ——————►				1.00
56	17 Mar.	◄—— mixed medium manually ►				1.00
71	31 Mar.	control	◄— start chemicals —►			4.70
76-85	5-14 Apr.	◄—— mixed continuously ►				4.70
90	19 Apr.	◄— stop chemicals —►				1.00
94	23 Apr.	C2	◄—— 100 % ww ——►			1.00
97	26 Apr.		↓	C2	↓	1.00
101	30 Apr.		↓	C2	↓	1.50
106	04-May		▼	C2	▼	2.00
111	10-May			C2		3.00

UASB 1+2+3+4, general performance,1993

Appendix 3. Methanogenic activity results.

METHANOGENIC ACTIVITY TEST

FEED NO: 1

<--------------------------------- 4 g COD --------------------------------->

DATE:	TIME:	RUNTIME (days)	days	hours	TEMP	R1 latestein L1	R2 latestein L2	R3 bavaria B1	R4 bavaria B2	R5 late.+ba L1+B1	R6 late.+bav L2+B2	R7 control pr. day
11/05/93	16:00	0.00	0	0	26.9	0	0	0	0	0	0	0
12/05/93	15:15	0.97	1	23	27.4	60.22	63.8	14.16	14.78	37.5	39.47	8.09
13/05/93	15:00	1.96	2	47	27.1	149.95	159.94	14.43	14.78	87.65	83.56	3.21
14/05/93	13:15	2.89	3	69	26.6	241	254.49	14.86	14.78	141.79	137.09	0.43
15/05/93	15:25	3.98	4	95	27.2	334.36	349.91	14.88	14.78	214.42	203.31	0.45
16/05/93	14:10	4.92	5	118	27.0	394.51	406.06	14.88	14.78	275.05	259.35	-0.3
17/05/93	10:25	5.77	6	138	27.1	418.41	427.16	14.88	14.78	321.45	261.45	-0.3
18/05/93	12:25	6.85	7	164	27.1	436.51	444.16	15.97	17.36	364.75	261.45	-0.3
19/05/93	13:35	7.90	8	190	27.3	453.61	461.26	18.57	21.26	397.05	261.45	-0.4
20/05/93	14:00	8.92	9	214	27.4	470.51	477.16	22.97	27.56	408.85	261.45	-0.2
21/05/92	14:00	-355.08	10	238	27.1	484.71	488.86	27.17	33.06	418.25	261.45	-0.4
22/05/92	14:30	-354.06	11	262	27.6	502.51	507.46	35.37	43.06	429.35	280.45	-0.2
23/05/92	15:30	-353.02	12	288	27.5	512.21	517.96	40.87	51.06	436.75	302.25	-0.3
24/05/92	13:05	-352.12	13	309	27.5	524.21	530.06	48.27	60.96	443.95	318.55	-0.1
25/05/92	10:50	-351.22	14	331	25.9	532.71	535.76	52.77	67.56	449.35	320.35	

Activity	mlCH4/d slope-reg.line1	86.1	90.24	57.53	53.32
	mgCOD-CH4/d.	223.64	234.39	149.43	138.49
Activity(1.5gVS gCOD-CH4/d*gVSS		0.149	0.156	0.100	0.092
	avg.	0.153		0.096	
CODfactor	384.61538				
	385ml $\gg$ 1gCOD=1000mgCOD				

METHANOGENIC ACTIVITY TEST

FEED 2

<--------------------------- 4 gCOD --------------------------->

DATE:	TIME:	RUNTIME (days)			TEMP.	R1 latestein L1	R2 latestein L2	R3 bavaria B1	R4 bavaria B2	R5 late.+bav L1+B1	R6 late.+bav L2+B2	R7 control pr. day
25/05/93	14:25:00	13.93	14	1	25.9	0	0	0	0	0	0	0
26/05/93	16:05:00	15.00	15	2	26.5	19.07	17.84	0.93	1.63	5.86	19.42	0.4
27/05/93	15:20:00	15.97	16	3	26.6	37.99	36.47	0.93	1.63	13.18	29.7	0.3
28/05/93	17:10:00	17.05	17	4	26.6	56.87	55.37	0.93	1.63	17.88	35.77	0.4
29/05/93	16:05:00	19.00	19	5	26.9	74.05	72.55	0.93	1.63	21.82	40.78	0.3
30/05/93	11:05:00	19.80	20	6	26.8	89.05	88.03	0.93	1.63	21.82	43.3	0.3
31/05/93	12:45:00	19.86	20	7	27.1	106.39	105.3	0.93	1.63	26.21	43.3	0.2
01/06/93	12:20:00	20.85	21	8	26.8	121.92	121.99	1.03	1.73	26.24	43.3	0.5
02/06/93	13:10:00	21.88	22	9	27.0	135.97	136.84	1.03	1.73	26.24	49.75	0.3
04/06/93	10:00:00	23.75	24	10	27.2	168	171.53	1.13	1.83	26.24	58.36	0.6
06/06/93	15:30:00	25.98	26	11	28.1	213.82	220.21	1.23	1.93	31.94	64.42	0.7
07/06/93	16:00:00	27.00	27	12	28.1	229.53	238.67	1.23	1.93	31.94	73.79	0.2
08/06/93	15:30:00	27.98	28	13	28.2	246.83	255.17	1.23	1.93	31.94	73.79	0.3
10/06/93	09:30:00	29.73	30	14	28.2	277.78	288.3	1.23	1.93	36.65	76.14	0.5
14/06/93	09:30:00	33.73	34	15	27.7	361.13	375.07	2.13	2.83	36.65	76.14	1.6
18/06/93	15:15:00	37.97	38	17	28.2	434.53	453.57	2.13	2.83	42.73	95.47	0.8
22/06/93	16:20:00	42.01	42	18	27.9	490.63	518.87	2.13	2.83	42.73	95.47	0.5
23/06/93	12:45:00	42.86	43	19	28.2	501.13	531.37	2.13	2.83	42.73	95.47	0.3
24/06/93	10:25:00	43.77	44	20	27.9	511.03	542	2.13	2.83	42.73	95.47	

Activity	mlCH4/d	18.95	18.47
	mgCOD-CH4/d.	49.22	47.97

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Appendix 4. Tests with 6CV and TCA (exposure and recovery, 4 and 1 gCOD/l)

exposure
4 gCOD/l, 1.5 gVSS/l
27±2 °C

recovery
4 gCOD/l, 1.5 gVSS/l
27±2 °C

exposure
1 gCOD/l, 1.5 gVSS/l
27±2 °C

recovery
1gCOD/l, 1.5 gVSS/l
27±2 °C

ml CH4

days

Legend:
- 0
- 2.5 mg6CV/l
- 5 mg6CV/l
- 2.5 mgTCA/l
- 5 mgTCA/l
- 1.25+1.25 mg6CV+TCA/l
- 2.5+2.5 mgCV+TCA/l
- regres.0mg/l
- reg.2.5 mg6CV/l
- reg.5 mg6CV/l
- reg.2.5 mgTCA/l
- reg.5 mgTCA/l
- reg.1.25+1.25

Legend:
- 0 mg/l
- 2.5 mg6CV/l
- 5 mg6CV/l
- 2.5 mgTCA/l
- 5 mgTCA/l
- 1.25+1.25 mg6CV+TCA/l
- 2.5+2.5 mg6CV+TCA/l

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TOX 1
FEED 1   **Exposure**
27.7 gra. C

<-------- -------- -------- 4 g COD/l -------- -------- -------->

DATE:	TIME:	RUNTIME days	R 1 ——	R 2 2.5 mg/l 6 CV	R 3 5 mg/l 6 CV	R 4 2.5 mg/l TCAc	R 5 5 mg/l TCAc	R 6 1.25+1.25 6CV+TCAc	R 7 2.5+2.5 6CV+TCAc
16/10/92	18:30:00	0	0	0	0	0	0	0	0
17/10/92	13:15:00	1	33.8	109.15	30.43	2.03	32.44	33.18	37.05
17/10/92	15:25:00	1	38.36	125.77	35.33	23.44	37.4	38.74	43.57
18/10/92	16:00:00	2	184.7	224	98.82	81.83	123.6	131.08	146
19/10/92	12:45:00	3	226.27	325.3	150.28	123.32	209.02	225.42	251.71
20/10/92	11:00:00	4	244.36	431.6	202.58	159.82	293.32	322.12	361.21
21/10/92	13:00:00	5	246.26	531.7	260.98	191.02	373.92	415.12	468.51
22/10/92	14:00:00	6	246.55	615.2	310.08	214.02	433.42	479.32	549.01
23/10/92	15:00:00	7	248.4	682.65	350.88	228.36	467.52	511.12	597.9
24/10/92	13:35:00	8	249.8	710.67	370.68	231.52	482.72	537.32	620.01
25/10/92	12:40:00	9	251.37	733.14	384.48	236.82	498.42	553.92	641.21
26/10/92	14:50:00	10	251.7	748.8	387.08	238.92	509.52	574.02	659.61
27/10/92	13:30:00	11	303.4	763.15	386.88	243.42	520.82	588.62	678.21
28/10/92	14:30:00	12	303.2	778.34	395.58	250.72	533.62	603.72	696.51
29/10/92	11:00:00	13	302.6	789.88	400.78	255.62	543.42	616.12	708.71
30/10/92	14:50:00	14	302.25	800.13	400.48	255.32	551.82	626.82	709.51
31/10/92	10:45:00	15	301.99	808.65	400.18	254.82	559.02	635.82	710.01
01/11/92	14:30:00	16	300.9	825.1	414.08	267.32	572.72	651.82	709.51
02/11/92	14:10:00	17	300.95	838.98	426.98	274.82	585.42	666.22	709.51
03/11/92	14:10:00	18	315.9	853	441.18	287.72	598.42	680.42	709.21
04/11/92	14:40:00	19	315.2	858.4	441.48	287.22	604.12	688.62	709.01
05/11/92	10:00:00	20	314.9	860	441.28	287.02	606.72	691.72	708.81
06/11/92	11:20:00	21	383	871.4	450.38	296.82	621.22	704.22	708.51
07/11/92	14:30:00	22	390.1	884.1	461.38	304.72	635.12	718.02	708.31
09/11/92	10:30:00	24	400.7	894	460.58	312.22	644.62	729.52	707.91
11/11/92	09:45:00	26	422.5	916.2	495.08	339.52	668.12	756.12	707.31
	CODtot, m		4180.08	5233.64	5763.5	4464.28	4456.52	4621	4998.2

mlCH4/d	slope,ml/d	77.6688905 (or 103.0)	102.378622	50.6061684	41.5565117	82.5658841	92.18121417	105.163611
mgCOD-CH4/d		201.737378	252.03397	131.444593	107.938991	214.456842	239.4317251	273.1522378
mgCOD-CH4/gVSS*d		155.182598	193.872285	101.111226	83.0299933	164.966801	184.1782501	210.117106
mgCOD-CH4/d*gCOD		0.04826161	0.04815654	0.02280638	0.02417837	0.04812204	0.051813834	0.054650122

TOX 1
FEED 1   **Exposure**
27.7 gra. C

<-------- -------- -------- 1 gCOD/l -------- -------- -------->

DATE:	TIME:	RUNTIME	R 8 ——	R 9 2.5 mg/l 6 CV	R 10 5 mg/l 6 CV	R 11 2.5 mg/l TCAc	R 12 5 mg/l TCAc	R 13 1.25+1.25 6CV+TCAc	R 14 2.5+2.5 6CV+TCAc	R 15 VANN
16/10/92	18:30:00	0	0	0	0	0	0	0	0	0
17/10/92	13:15:00	1	14.2	15.06	28.4	13.99	13.58	25.8	28.69	4.15
17/10/92	15:25:00	1	14.62	29.67	32.21	13.99	13.95	27.18	30.49	4.15
18/10/92	16:00:00	2	23.72	44.84	79.16	19.31	21.02	40.54	47.63	4.25
19/10/92	12:45:00	3	31.93	52.82	107.72	23.89	27.64	47.69	58.48	4.59
20/10/92	11:00:00	4	38.43	57.67	128.92	26.39	30.94	52.11	67.09	4.3
21/10/92	13:00:00	5	46.13	63.22	140.42	29.28	34.84	56.89	86.38	3.89
22/10/92	14:00:00	6	53.43	67.22	147.02	32.19	38.94	61.79	87.28	3.49
23/10/92	15:00:00	7	62.23	72.02	155.32	35.79	44.34	67.59	89.52	3.19
24/10/92	13:35:00	8	65.83	74.02	160.52	36.39	46.64	67.29	92.68	2.79
25/10/92	12:40:00	9	70.91	76.09	163.74	37.43	48.18	66.99	94.63	2.55
26/10/92	14:50:00	10	74.63	75.72	163.12	38.19	48.04	71.59	94.38	2.19
27/10/92	13:30:00	11	83.03	75.32	162.62	36.89	49.84	75.89	94.08	1.79
28/10/92	14:30:00	12	90.73	74.92	161.92	36.49	51.74	79.39	93.78	1.49
29/10/92	11:00:00	13	97.73	74.62	161.42	36.19	52.84	81.49	98.98	1.19
30/10/92	14:50:00	14	99.73	74.22	165.92	35.79	52.84	81.09	98.58	0.79
31/10/92	10:45:00	15	102.93	73.92	171.02	35.69	54.34	80.89	100.28	0.39
01/11/92	14:30:00	16	110.93	81.12	178.62	35.09	58.44	80.29	105.68	-0.21
02/11/92	14:10:00	17	117.33	86.62	186.42	40.89	63.68	80.25	110.58	-0.26
03/11/92	14:10:00	18	123.53	95.82	196.32	40.39	70.04	93.69	118.78	-0.61
04/11/92	14:40:00	19	123.13	95.52	196.02	40.19	70.54	93.39	118.48	-0.91
05/11/92	10:00:00	20	123.03	95.32	195.82	39.99	72.14	93.19	118.38	-1.21
06/11/92	11:20:00	21	122.73	94.92	201.02	39.59	77.09	92.8	123.04	-0.21
07/11/92	14:30:00	22	128.13	94.62	205.02	39.39	82.14	102.29	129.18	0.41
09/11/92	10:30:00	24	127.63	94.02	207.12	38.89	87.14	102.09	134.18	-0.21
11/11/92	09:45:00	26	141.53	118.92	221.42	38.29	99.04	113.29	151.38	5.09
	CODtot, m		1206.08	1909	2512.94	1350.91	1289.0*	1416.32	1754.19	59.43

mlCH4/d	slope	7.77955176	12.3337763	34.3560603	3.46220657	4.81339419	6.196392229	11.91119072
mgCOD-CH4/d	1gCOD=385ml	20.206628	32.0357825	89.2365202	8.99274433	12.5023226	16.09452527	30.93815771
mgCOD-CH4/gVSS*d		13.4710853	21.3571884	59.4910135	5.99516289	8.33488171	10.72968351	20.62543847
mgCOD-CH4/gCOD*d		0.01675397	0.01678145	0.0355108	0.0066568	0.00969924	0.011363622	0.01763672

TOX 1
RECOVERY
27.7 gra. C

			R 1	R 2	R 3	R 4	R 5	R 6	R 7
						<-- 4 g COD/l -->			
DATE:	TIME:	RUNTIME days	———	2.5 mg/l 6 CV	5 mg/l 6 CV	2.5 mg/l ICAc	5 mg/l ICAc	1.25+1.25 6CV+ICAc	2.5+2.5 6CV+ICAc
11/11/92	17:00	0	0	0	0	0	0	0	0
12/11/92	08:01	1	24.27	31.11	1.59	24.8	21.9	30.86	34.9
13/11/92	11:15	2	153.21	162.66	3.39	156.99	141.2	180.89	199.78
14/11/92	13:30	3	272.71	288.33	3.05	283.14	260.25	317.31	348.06
15/11/92	15:15	4	373.31	400.97	4.89	389.59	364.21	428.71	469.86
16/11/92	10:35	5	445.21	487.77	4.49	465.19	440.11	505.81	555.06
17/11/92	08:15	6	515.21	574.27	51.59	536.19	512.11	576.21	630.36
18/11/92	10:45	7	574.11	654.57	97.09	598.99	516.21	631.91	697.96
19/11/92	15:45	8	618.41	739.67	172.49	661.19	516.01	675.01	741.36
20/11/92	12:50	9	622.81	769.17	211.79	670.29	515.71	681.11	741.56
20/11/92	13:15	9		777.37		679.29		689.11	752.76
21/11/92	15:10	10	622.71	797.87	242.49	689.29	521.91	704.21	752.46
22/11/92	16:30	11	622.31	805.27	254.29	689.69	521.61	706.63	752.14
23/11/92	10:00	12	622.21	812.37	263.89	689.89	528.81	713.41	751.96
24/11/92	14:30	13	647.81	829.07	285.29	712.09	559.81	733.21	760.26
25/11/92	14:45	14	671.21	843.37	303.89	731.49	581.71	753.51	774.16
26/11/92	10:45	15	678.01	856.62	313.06	737.88	591.21	761.11	803.9
27/11/92	13:45	16	678.11	856.87	313.29	737.29	591.21	764.31	834.46
28/11/92	14:20	17	696.41	868.68	326.09	752.19	600.81	779.41	852.06
30/11/92	09:00	19	705.11	874.29	338.89	758.91	600.21	778.86	851.64
30/11/92	14:00	19	708.11	879.27	333.89	763.39	600.31	778.87	851.63
	slope	mlCH4/d	82.73813	97.98803	51.07467	87.89443	98.79127	89.274282	109.77357
	mgCOD-CH4/d		214.9042	254.5144	132.6615	228.2972	256.6007	231.88125	285.12616
Activity1.5	mgCOD-CH4/d*gVSS		143.2695	169.6762	88.44098	152.1981	171.0671	154.5875	190.08411

CODfactc 384.6ca.1gCOD=1000mgCOD

TOX 1
RECOVERY
27.7 gra. C

			R 8	R 9	R 10	R 11	R 12	R 13	R 14	R 15
						<-- 1 gCOD/l -->				
DATE:	TIME:	RUNTIME (	———	2.5 mg/l 6 CV	5 mg/l 6 CV	2.5 mg/l ICAc	5 mg/l ICAc	1.25+1.25 6CV+ICAc	2.5+2.5 6CV+ICAc	VANN
11/11/92	17:00:00	0	0	0	0	0	0	0	0	0
12/11/92	08:01:00	1	12.95	1.89	24.27	17.31	13.73	16.16	20.61	3.17
13/11/92	11:15:00	2	45.87	5.81	117.98	56.54	50.98	49.59	79	4.41
14/11/92	13:30:00	3	56.07	5.91	181.93	80.51	71.75	66.13	106.97	7.15
15/11/92	15:15:00	4	60.57	5.61	205.73	96.81	89.15	76.43	118.37	
16/11/92	10:35:00	5	67.57	6.61	227.13	109.91	102.15	88.43	129.07	7.86
17/11/92	08:15:00	6	73.97	7.71	247.23	119.61	111.65	99.03	139.17	7.45
18/11/92	10:45:00	7	77.87	9.01	262.53	122.91	117.25	107.33	146.67	7.15
19/11/92	15:45:00	8	88.07	8.61	287.73	139.71	130.05	123.93	161.07	6.75
20/11/92	12:50:00	9	88.67	8.41	292.13	140.41	131.75	124.53	163.17	6.44
21/11/92	15:10:00	10	88.37	8.11	293.73	140.11	131.65	124.33	164.37	6.15
22/11/92	16:30:00	11	88.05	7.74	293.86	139.81	131.25	123.99	164.44	
23/11/92	10:00:00	12	92.07	7.51	293.63	139.61	131.15	123.83	164.77	
24/11/92	14:30:00	13	96.87	88.91	312.03	156.41	139.55	123.53	164.81	5.05
25/11/92	14:45:00	14	103.47	94.11	311.73	168.71	150.15	123.23	164.31	4.85
26/11/92	10:45:00	15	105.53	95.79	311.52	173.53	152.1	148.52	176.07	12.5
27/11/92	13:45:00	16	105.17	95.41	311.01	173.11	153.15	148.23	175.91	12.5
28/11/92	14:20:00	17	108.47	97.81	336.01	172.91	160.45	155.93	175.61	17.9
30/11/92	09:00:00	19	107.98	97.3	341.18	172.36	160.07	155.41	175.18	20.6
30/11/92	14:00:00	19	107.96	97.25	345.15	172.36	160.04	155.42	175.17	21.9
	slope	mlCH4/c	19.40988			19.84596	19.03438	15.57694		
	mgCOD-CH4/d		50.41526	0		51.54796	49.43994	40.459585		
Activity1.5gV	mgCOD-CH4/d*gVSS		33.61017	0		34.3653	32.95996	26.973057		
	if	4gCOd	134.4407	0		137.4612	131.8398	107.89223		
with4gCOD	mgCOD-CH4/d*g\		143.269	169.6762	88.44098	152.1981	171.0671	154.5875	190.08411	

44

TOX 2
FEED 1
27.7 gra. (Wastewater + chemicals   <------ ---------------4 g COD/l -------- -------- ------->

DATE:	TIME:	RUNTIME days	MICRO MACRO	R 1 ———	R 2 2.5 mg/l 6 CV	R 3 5 mg/l 6 CV	R 4 2.5 mg/l TCAc	R 5 5 mg/l TCAc	R 6 1.25+1.25 6CV+TCAc	R 7 2.5+2.5 6CV+TCAc
01/12/92	17:00:00	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02/12/92	09:25:00	1	2.12	30.27	29.81	30.27	28.57	31.94	28.20	26.51
03/12/92	14:50:00	2	6.92	123.43	133.30	134.54	111.39	130.93	128.95	125.25
04/12/92	10:00:00	3	7.32	175.80	196.60	206.32	156.14	184.79	190.25	188.91
05/12/92	14:00:00	4	7.00	240.35	281.72	312.54	211.23	243.51	266.31	274.87
06/12/92	15:15:00	5	6.75	284.99	353.10	409.04	258.86	284.24	322.31	222.47
07/12/92	09:00:00	6	6.57	291.74	385.30	456.14	281.89	290.04	331.61	345.87
08/12/92	09:30:00	7	6.33	316.85	423.80	505.24	308.63	307.63	357.81	380.37
09/12/92	09:30:00	8	6.28	337.05	455.70	546.94	321.33	320.81	357.41	398.77
10/12/92	14:40:00	9	6.65	355.05	482.30	580.74	337.33	339.31	357.31	419.37
11/12/92	12:30:00	10	8.38	376.65	505.10	606.84	356.53	363.01	386.61	427.97
12/12/92	14:15:00	11	9.29	404.35	529.10	633.24	381.43	393.31	404.31	434.57
13/12/92	19:10:00	12	10.78	428.65	551.90	655.44	407.23	420.31	404.11	434.17
14/12/92	10:05:00	13	11.72	450.25	575.80	681.04	428.73	429.61	466.21	439.57
15/12/92	09:30:00	14	12.08	471.45	598.50	705.84	450.93	457.41	507.51	440.37

	slope mlCH4/d -			40.4326	64.78872	85.75048	43.11309	36.736014	49.384102	40.323947
	mgCOD-CH4/d			105.0197	168.2824	222.7285	111.9821	95.418218	128.2704	104.73752
	mgCOD-CH4/gVSSd			70.013	112.188	148.486	74.655	63.612	85.514	69.825

TOX 2
FEED 1
27.7 gra. C   <------ ---------------1 gCOD/l -------- -------- ------->

DATE:	TIME:	RUNTIME days	R 8 ———	R 9 2.5 mg/l 6 CV	R 10 5 mg/l 6 CV	R 11 2.5 mg/l TCAc	R 12 5 mg/l TCAc	R 13 1.25+1.25 6CV+TCAc	R 14 2.5+2.5 6CV+TCAc	R 15 MICRO MACRC
01/12/92	17:00:00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0C
02/12/92	09:25:00	1	3.40	1.17	24.84	4.54	4.22	9.68	13.79	6.28
03/12/92	14:50:00	2	9.54	3.69	85.76	12.48	11.77	27.29	42.25	16.05
04/12/92	10:00:00	3	9.39	3.86	115.17	15.20	14.46	35.72	53.81	18.48
05/12/92	14:00:00	4	9.04	5.59	143.33	17.71	15.52	43.92	64.49	20.05
06/12/92	15:15:00	5	8.76	49.06	153.27	19.55	16.84	47.27	69.72	21.52
07/12/92	09:00:00	6	8.59	51.42	154.12	19.30	16.47	46.92	69.51	21.32
08/12/92	09:30:00	7	8.38	51.11	166.63	19.02	15.92	47.28	77.23	21.12
09/12/92	09:30:00	8	8.11	47.87	178.39	18.80	15.53	51.71	83.07	20.56
10/12/92	14:40:00	9	7.86	50.44	198.88	18.50	18.86	60.07	91.39	25.96
11/12/92	12:30:00	10	7.74	50.19	218.13	29.51	24.62	69.92	100.49	32.15
12/12/92	14:15:00	11	7.48	49.80	234.40	33.28	28.72	78.12	108.84	36.95
13/12/92	19:10:00	12	7.18	49.41	237.78	33.53	28.00	79.23	114.38	36.92
14/12/92	10:05:00	13	26.14	49.29	237.53	35.31	30.92	83.52	121.59	39.25
15/12/92	09:30:00	14	29.54	48.99	252.43	37.01	32.12	85.32	127.79	40.65

	CODtot, n	1206.08		1909	2512.94	1350.91	1289	1416.32	1754.19	59.43
	slope mlCH4/d -	-			22.29896	2.30046	1.558537	6.5928442	9.0062243	
	mgCOD-CH4/d				57.91937	5.975221	4.0481481	17.124271	23.39279	
	gCOD-CH4/gVSSd				0.039	0.004	0.003	0.011	0.016	
	mgCOD-CH4/gCODd				0.023	0.004	0.003	0.012	0.013	

TOX2
RECOVERY
27.7 gra. (Wastewaters-ch) <------- --------- --------4 g COD/l -------- -------- ---------------->

DATE:	TIME:	RUNTIME days	R 1 -MICRO/ -MACRO	R 2 KONTROL	R 3 -6CV	R 4 -6CV	R 5 -TCAc	R 6 -TCAc	R 7 -6CV/TCAc	R 8
15/12/92	12:00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16/12/92	09:45	1	-0.40	52.58	57.98	59.52	43.21	54.22	53.46	55.52
17/12/92	12:25	2	-0.36	140.10	164.42	169.13	121.18	151.38	143.36	149.57
18/12/92	09:00	3	0.18	201.11	240.00	241.76	175.25	218.55	202.86	212.44
19/12/92	16:30	4	2.51	285.61	344.72	341.48	248.74	313.49	276.98	303.43
21/12/92	10:15	6	2.17	364.38	454.92	454.28	310.64	403.59	352.88	370.93
22/12/92	08:10	7	2.15	396.68	500.82	503.38	333.74	439.79	382.58	397.43
23/12/92	09:00	8	2.05	424.98	541.02	543.98	355.14	468.99	403.88	416.83
24/12/92	12:25	9	5.89	464.68	593.32	592.08	393.24	511.39	444.78	455.73
25/12/92	12:12	10	6.65	476.58	606.92	604.98	406.04	523.59	460.68	470.03
26/12/92	14:00	11	6.35	484.08	615.52	616.38	418.04	533.09	469.48	480.33
27/12/92	14:30	12	6.05	483.68	619.32	615.88	417.74	532.49	469.18	480.03
28/12/92	12:00	13	7.15	489.98	629.22	622.42	427.74	544.79	483.18	486.23
29/12/92	09:45	14	7.51	502.18	641.62	626.08	436.14	553.49	495.28	498.93
30/12/92	17:35	15	7.70	513.68	654.02	630.68	446.14	562.69	507.48	509.33
31/12/92	16:00	16	7.47	513.88	655.02	630.28	447.84	563.59	509.78	512.33
01/01/93	15:10	17	7.22	513.58	654.62	639.08	447.54	564.19	510.08	512.63
02/01/93	15:45	18	7.65	513.48	664.52	652.58	447.24	563.89	512.68	522.73
03/01/93	15:35	19	7.43	513.08	664.42	652.28	446.94	563.39	512.38	522.33
04/01/93	09:30	20	7.25	512.88	674.12	658.48	446.84	563.19	525.28	522.23
05/01/93	11:15	21	6.95	512.58	689.02	668.18	446.44	562.69	537.38	540.63
06/01/93	14:30	22	11.72	552.18	709.62	682.88	487.54	609.19	555.08	560.63
08/01/93	16:30	24	14.04	567.38	732.82	698.18	503.94	611.09	554.48	578.93
09/01/93	17:00	25	13.75	567.28	757.12	708.28	503.74	638.49	554.38	596.93
11/01/93	08:40	27	20.15	566.78	759.22	758.28	544.74	667.69	553.78	624.83
12/01/93	12:45	28	20.65	602.78	783.52	767.88	544.44	677.69	601.98	633.33
14/01/93	09:10	30	20.15	615.98	783.12	777.48	558.04	676.69	601.48	648.63
15/01/93	14:20	31	19.85	615.68	782.72	789.08	573.64	676.19	601.18	649.03
18/01/93	09:35	34	19.10	644.38	808.42	814.88	595.64	674.99	600.58	694.13
20/01/93	11:00	36	18.57	643.68	821.32	829.68	594.94	674.29	599.88	695.13
22/01/93	14:30	38	18.03	642.98	859.72	879.38	630.14	673.29	599.38	694.53
25/01/93	10:15	41	17.17	642.18	882.67	907.99	673.44	672.19	598.48	693.53
27/01/93	13:15	43	16.56	641.28	890.32	920.38	688.94	671.39	597.28	692.73
01/02/93	16:15	48	15.45	639.68	889.52	934.29	694.54	669.29	596.68	761.13
02/02/93	10:30	49	15.22	639.48	889.18	941.08	710.24	668.89	596.58	760.93

TOX 2
RECOVERY Wastewater-chemicals
27.7 gra. C <------- ---------- --------1 gCOD/l -------- --------- -------->

DATE:	TIME:	RUNTIME days	R 9 -6CV	R 10 -6CV	R 11 -TCAc	R 12 -TCAc	R 13 -6CV/TCAc	R 14	R 15 KONTROL	R 16 -MICRO/ -MACRC
15/12/92	12:00:00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16/12/92	09:45:00	0.91	17.82	26.01	4.33	0.59	8.14	13.59	15.59	-0.21
17/12/92	12:25:00	2.02	49.52	72.42	14.65	3.81	20.50	36.13	39.63	-0.48
18/12/92	09:00:00	2.88	70.70	102.49	25.27	9.69	30.72	54.01	56.96	4.36
19/12/92	16:30:00	4.19	95.53	139.02	37.43	19.50	47.14	75.57	78.12	11.37
21/12/92	10:15:00	5.93	115.91	161.42	42.97	19.01	47.74	95.01	96.84	11.00
22/12/92	08:10:00	6.84	127.71	175.52	48.97	18.81	51.14	107.11	108.74	10.80
23/12/92	09:00:00	7.88	137.51	186.32	54.57	18.61	54.74	118.71	120.84	10.50
24/12/92	12:25:00	9.02	161.51	212.02	72.07	18.21	75.44	144.21	146.84	24.67
25/12/92	12:18:00	10.01	171.31	220.72	77.67	18.11	80.94	154.11	157.24	29.00
26/12/92	14:05:00	11.09	177.11	224.72	81.17	38.11	86.54	161.61	162.64	31.70
27/12/92	14:30:00	12.10	177.51	224.32	80.87	37.81	86.24	163.51	162.34	31.50
28/12/92	12:00	13.00	182.21	226.02	84.47	39.51	88.84	167.61	165.74	34.20
29/12/92	09:45	13.91	187.51	236.32	88.37	45.01	97.34	173.11	174.14	38.60
30/12/92	17:35	15.23	191.71	241.72	92.17	49.41	102.64	177.71	179.14	41.90
31/12/92	16:00	16.17	191.51	241.52	92.07	49.71	102.24	177.91	179.34	42.00
01/01/93	15:10	17.13	191.21	240.82	91.77	49.51	102.04	177.71	179.24	41.80
02/01/93	15:45	18.16	190.91	240.42	93.57	52.31	104.74	182.21	178.94	44.10
03/01/93	15:35	19.15	190.51	240.02	93.37	52.01	104.44	181.81	178.64	43.90
04/01/93	09:30	19.90	193.41	239.62	93.17	55.21	104.24	181.71	178.44	43.70
05/01/93	11:15	20.97	194.61	239.22	92.77	60.51	114.14	171.41	178.14	43.40
06/01/93	14:30	22.10	211.06	266.17	105.07	68.39	122.71	200.34	199.01	56.85
08/01/93	16:30	24.19	217.89	277.15	111.73	76.77	132.33	199.27	206.07	63.00
09/01/93	17:00	25.21	224.51	287.43	118.87	76.41	169.74	199.11	205.84	68.30
11/01/93	08:40	26.86	239.61	306.62	130.87	97.31	156.74	219.51	218.94	79.50
12/01/93	12:45	28.03	242.01	305.92	130.47	99.21	159.44	222.41	224.14	79.20
14/01/93	09:10	29.88	251.68	307.38	129.91	98.71	165.25	221.45	223.56	78.57
15/01/93	14:20	31.10	265.51	306.72	135.77	98.41	172.94	225.51	223.14	86.60
18/01/93	09:35	33.90	290.31	305.82	149.37	97.61	187.84	248.21	222.34	96.70
20/01/93	11:00	35.96	304.11	304.92	148.67	97.01	187.24	247.71	221.84	96.00
22/01/93	14:30	38.10	335.01	303.92	172.77	96.41	186.54	247.11	242.94	95.30
25/01/93	10:15	40.93	357.81	302.72	187.06	95.47	185.50	246.11	257.62	112.02
27/01/93	13:15	43.05	371.61	317.22	191.97	94.81	184.78	245.41	257.03	111.38
01/02/93	16:15	48.18	385.81	314.02	190.77	93.31	183.14	244.01	255.24	109.50
02/02/93	10:30	48.94	390.61	313.62	190.57	93.21	182.94	243.81	255.14	109.30

Appendix 5. 4 UASB - performance.

1234PHIO.XLC

UASB 1+2+3+4, pH inn + ut

d=0,19 jan 1993

Legend:
- ■ UASB 1, pH inn   □ UASB 1, pH ut   ◆ UASB 2, pH inn   ◇ UASB 2, pH ut   ▲ UASB 3, pH inn   △ UASB 3, pH ut
- ● UASB 4, pH inn   ○ UASB 4, pH ut

d=71, start kjemikalier
d=90, stopp kjemikalier
d=101, medium C2

dager

pH

49

1234HRT.XLC

UASB 1+2+3+4, ±28°C., HRT

dag=0, 19 jan 93

Legend:
- UASB1 (■)
- UASB 2 (♦)
- UASB 3 (▲)
- UASB 4 (◉)

x-axis: dager (0 to 130)
y-axis: timer (0 to 30)

UASB 1+2+3+4, air temperature ±28 ° C

d=0, 19 jan 1993

dager

grader Celsius

| ■ UASB 1 | ♦ UASB 2 | ▲ UASB 3 | ⊙ UASB 4 |

Appendix 6. Two new UASB (1 and 2).

12NYPH.XLC

R1+R2, ca. 28 deg.Celsius, pH in + out

d=73, start dosing of 6CV in R2

days

pH

| ■ UASB 1 pH in | ♦ UASB 1 pH out | ▲ UASB 2 pH out | ⊙ UASB 2 pH in |

VFA12INU.XLC

UASB reactors, new start (3.7.93), ±28°C

VFA in and out

mg COD/l

5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0

0   50   100   150   200   250

days

R1in-new   R1out   R2in-new   R2out

d=73, start dosing of 6CV in R2 and R1-HAc

54

Efficiency (COD removal) of the UASB reactors, 28±1 °C

Legend:
- R1-control
- R2+6CV

% COD removed vs days

d=73, start dosing of 6CV in R2 and R1-HAc

Appendix 7. UASB 3.

UASB 3

UASB 3,HRT, 26±1°C

% COD removed

Appendix 8. Tests on the determination of the 6CV kinetics.

Test with 16 (30) mg 6CV/l, from UASB3

	days	analysed	mg6CV/l
0 mg-check 28.	0	03/02/94	0.02
30 mg/l 28.1	0	03/02/94	16.26
30 mg/l 1.2	5	02/02/94	9.69
30 mg/l 2.2	6	02/02/94	8.26
30 mg/l 7.2	11	08/02/94	0.37

r^2:	0.996586	slope	-1.44391
		mg6CV/d	1.44
	mg6CV/gVSS*d		0.963

Test with 16 mg 6CV/l

UASB 3, new
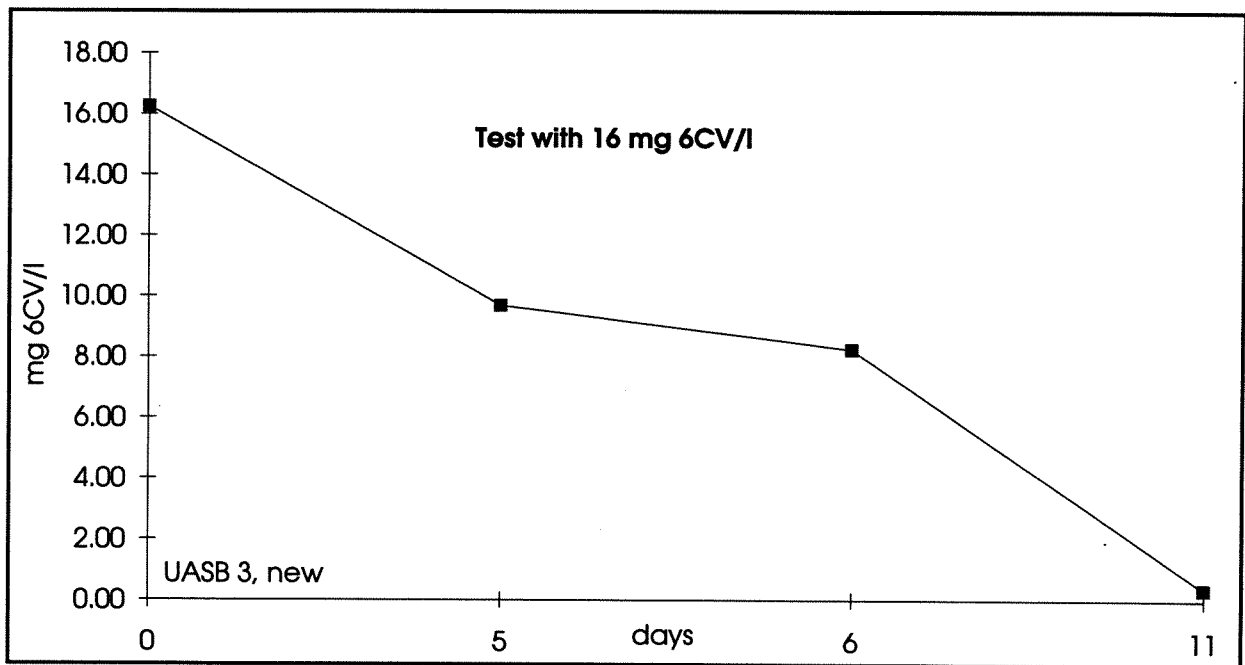
Test with 16 mg6CV/l, gas production

±26 °C

mg NaOH

DATE:	TIME:	RUNTIME	R 1	R2	temp,
		days	0	16mg6cv/l	
28/01/94	11:30:00	0	0	0	26.4
28/01/94	17:30:00	0	23.31	21.94	
29/01/94	14:30:00	1	113.93	106.76	
30/01/94	13:50:00	2	235.06	203.13	
31/01/94	15:45:00	3	364.5	302.43	25.8
01/02/94	16:25:00	4	392.7	378.63	25.8
02/02/94	09:25:00	5	423.9	433.43	25.8
02/02/94	17:15:00	5	428.1	449.7	
03/02/94	13:30:00	6	444	467.8	26.2
04/02/94	08:45:00	7	453.1	479.33	25.4
04/02/94	16:30:00	7	456.2	484.63	
07/02/94	09:05:00	10	491.7	546.8	
08/02/94	10:20:00	11	503.1	572.63	28.7
09/02/94	09:50:00	12	528.9	590.43	26.4
11/02/94	02:40:00	14	540.3	612.43	25.6
18/02/94	10:05:00	21	573.6	660.73	27.5
22/02/94	15:45:00	25	601	658.83	27.2

gas production in the test with 16 mg6CV/l and 4gCOD/l(75:20:05)

26±1°C

from UASB 3

Legend:
- 0mg 6CV/L
- 16mg 6CV/l

x-axis: days
y-axis: ml CH4

TESTREPO.XLS
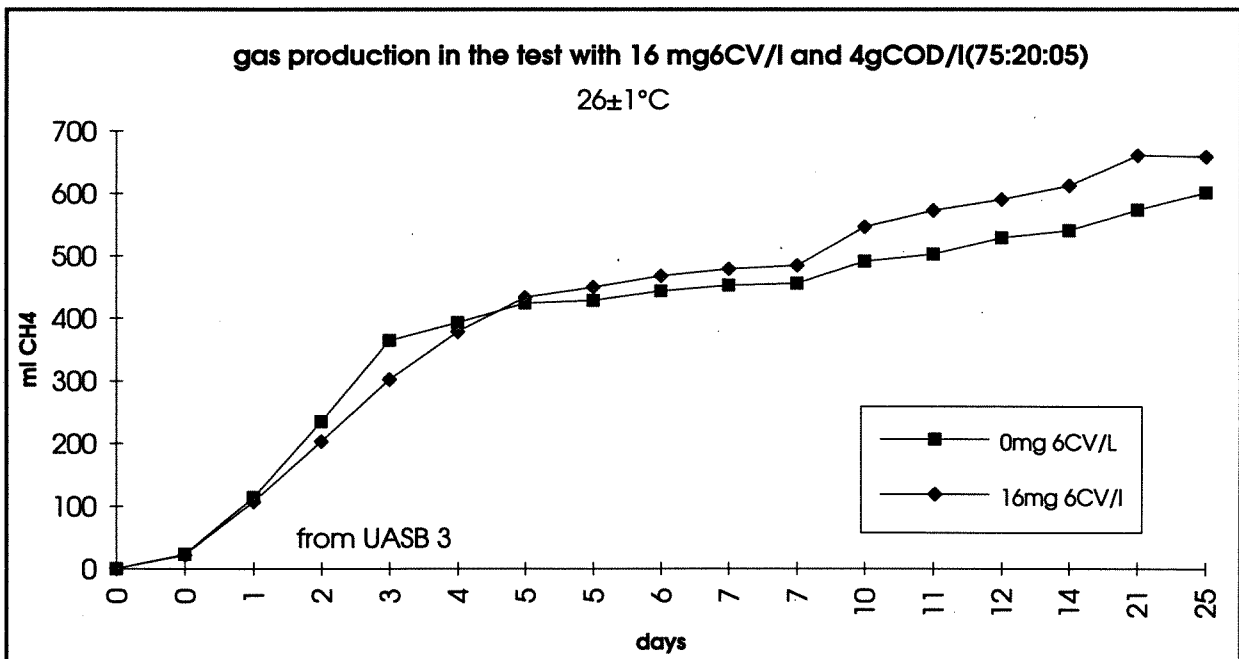
Test with 0.10.20 and 40 mg6CV/l
sludge from UASB3
temp.26±2°C

6CV

days	10 3		10 4	20 5		20 6	20		6	40 7		40 8	40
	6cv		vfa	6cv		vfa	vfa		6cv	6cv		vfa	vfa
0	11.1		14.3	21.4		5384	5384		24.0	51.5		3188	3429
0	11.0		12.6	24.4		3652	3652		20.9	39.4		4810	4665
1	4.9		5.8	16.5			2362		13.6	35.1			
2	5.1		6.3	13.2		2362			11.6	20.7		2325	2371
3	5.4		6.0	7.5					9.4	19.7			
5	3.0		3.7	6.9					5.6	10.8			
6	1.3		1.5	3.0					1.7	2.7			
7	1.6		1.2			1244	1244					1130	1196
9	0.5		0.0	0.5		775	750		0.3	0.1		793	774
16							686						719
21													

	6cv-sist			6cv-sist		6cv-sist	6cv-sist			6cv-sist	6cv-sist	6cv-2siste
mg6CV/d	0.9380	1.0620	1.2230	1.2223	2.3800	2.7790	2.1190	2.4740	5.2910	5.5910
mg6CV/gVSS*d	0.63	0.71	0.82	0.81	1.59	1.85	1.41	1.65	3.53	3.73
mg6CV/gCOD(VFA	2.96	3.35	4.18	4.18	9.76	11.40	9.38	10.95	15.09	16.81372
mg6CV/gCOD*d	0.20	0.29	0.29	0.29	0.51	0.66	0.58	0.68	1.58	1.16237

VFA

days time	0	0	10	VFAmg/l 10	20	20	VFA 40	40 mg6CV/l
0	4966	4833	4282	4613	5384	3352	3188	3429
0	5425	5560	4646	4202	3652	3348	4810	4665
2	2546	2366	2224	2353	2362	2345	2325	2371
9	1393	1408	1256	1211	1244	1184	1130	1196
16	929	924	671	732	775	750	793	774
21		1030		802		686		719

mgCOD-VFA/d	226.92	226.97	317.17	292.27	243.8748	225.9	350.5	332.5 all bad correlation
gCOD-VFA/gVSS*d	0.15	0.15	0.21	0.19	0.16	0.15	0.23	0.22

6CVALLE.XLC

test on degradation of 6CV

0, 10, 20 and 40 mg6CV/l

temp. 26±2°C

Legend:
- 10 mg6CV/l
- 10 mg6CV/l
- 20 mg6CV/l
- 20 mg6CV/l
- 40 mg6CV/l
- 40 mg6CV/l

y-axis: mg/l — 0, 10, 20, 30, 40, 50

x-axis: days — 0, 1, 2, 3, 4, 5, 6, 7, 8, 9

GAS6CVKI.XLS
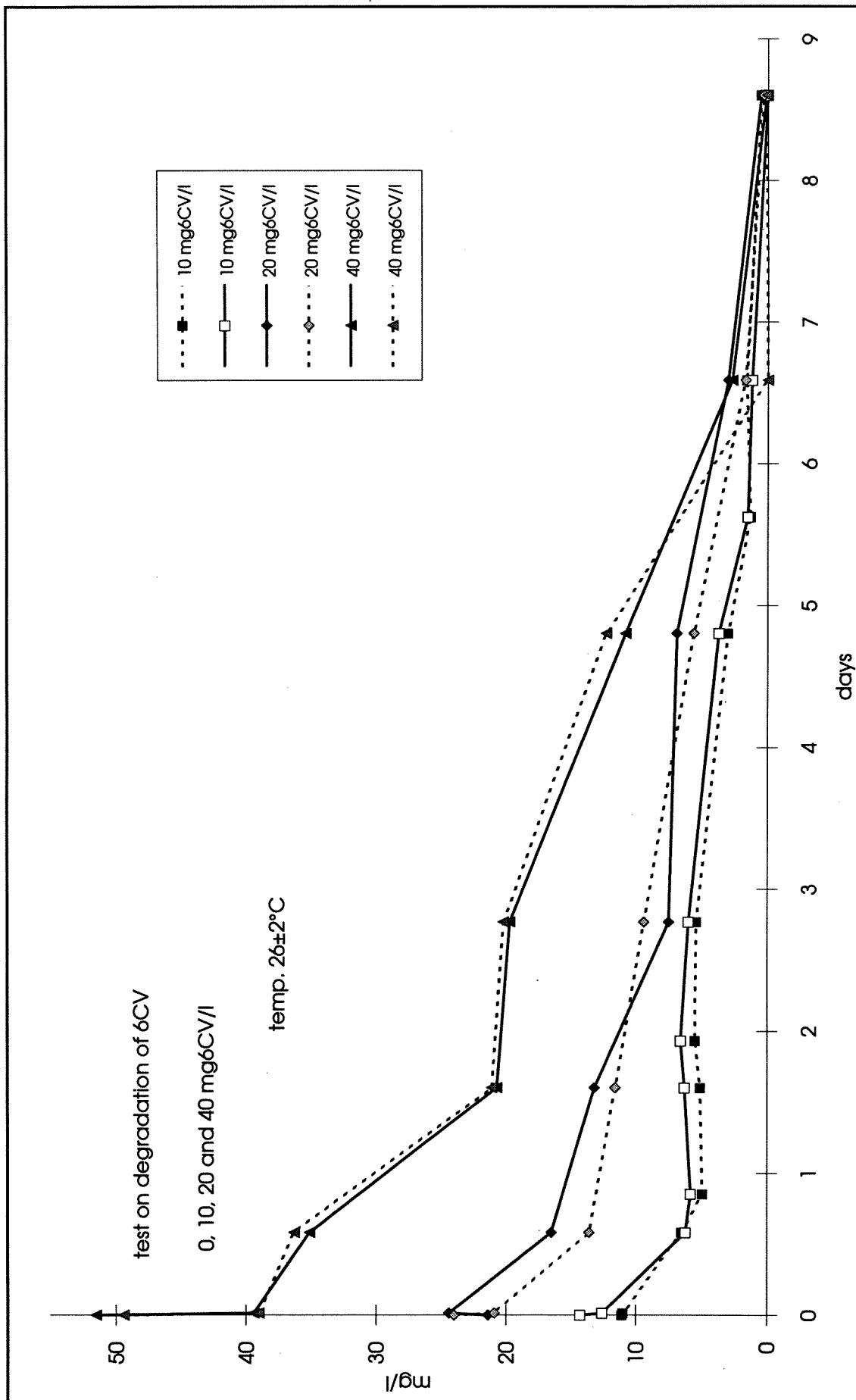
6CV

Test with 0+10+20+40 mg6CV/l

±26 °C

mg NaOH

DATE:	TIME:	RUNTIME days	R1 ——— 0	R2 ----- 0	3 10	4 10	5 20	6 20	7 40	8 40	9 only slam
08/02/94	21:20:00	0.0	0	0	0	0	0	0	0	0	0
09/02/94	09:15:00	0.5	63.3	57.95	69.1	65.26	61.42	63.59	17.19	57.97	5.9
10/02/94	09:35:00	1.5	217.07	210.06	321.11	219.42	209.66	223.9	224.5	209.86	9.33
11/02/94	09:40:00	2.5	335.32	326.42	365.33	339.82	325.97	349.76	350.35	337.8	8.57
12/02/94	03:10:00	3.2	408.72	400.02		413.42	402.97	419.56	424.35	409.3	
12/02/94	14:10:00	3.7	439.32	434.52	424.88	448.32	436.57	456.06	456.45	446.2	6.78
13/02/94	14:10:00	4.7	473.5	477.32	435.60	493.32	491.07	503.16	497.85	495	5.68
14/02/94	10:25:00	5.5	503.72	505.92	441.50	522.52	522.37	531.56	524.25	526.1	4.55
15/02/94	14:15:00	6.7	543.32	543.62	447.30	543.72	558.07	568.06	559.95	564.2	1.58
16/02/94	09:55:00	7.5	571.32	567.42	578.50	570.82	581.92	592.36	580.65	585.6	
17/02/94	08:50:00	8.5	587.62	583.02	592.60	584.72	597.32	606.46	595.05	600.5	-0.22
18/02/94	09:55:00	9.5	596.52	591.62	600.20	594.92	608.17	614.76	598.2	608.4	-0.82
21/02/94	08:30:00	12.5	594.70	605.82	614.70	606.82	623.27	627.36	596.05	620.2	-3.42
22/02/94	08:35:00	13.5	606.22	612.52	620.00	615	631.97	635.16	622.25	626.8	-4.42

30
26
388 lgCOD
383.2 r^2:

	slope-3h	ml CH44/.	0.992267	0.992516	0.861167	0.992552	0.994631	0.9900911	0.9755232	0.993268	
	gCOD-CH4/.d		125.58	124.25	147.02	126.71	123.96	130.01	147.08	128.60	
			326.17	322.74	381.86	329.11	321.98	337.68	382.02	334.02	
fra CH4gas	gCOD-CH4/gVSS.d		0.217	0.215	0.255	0.219	0.215	0.225	0.255	0.223	
fraVFAdegrc	mgCOD-VFA/gVSS.d		0.151	0.151	0.211	0.195	0.163	0.151	0.234	0.222	all bad co

sludge from UASB 3
Test with 0, 10, 20 and 40 mg6CV/l

mg6CV/l conc.	mg6CV/gVSS*d initial slopes	
0	0.00	0.00
10	0.71	0.81
20	1.85	1.65
40	3.53	3.73

y = 0.09x - 0.05   y = 0.09x - 0.09
r^2:   0.995263 r^2:   0.995835

reg.line   0.093   0.098
r^2:corell.   0.993   0.997

          16   0.96   other test

sludge rom UASB 3

Legend:
■ test 3 (0,10,20,40 mg6CV/l)
□ test 3 (0,10,20,40 mg6CV/l)
◆ test 2 (16 mg6CV/l)

y-axis: mg6CV/gVSS*d
x-axis: concentration mg6CV/l
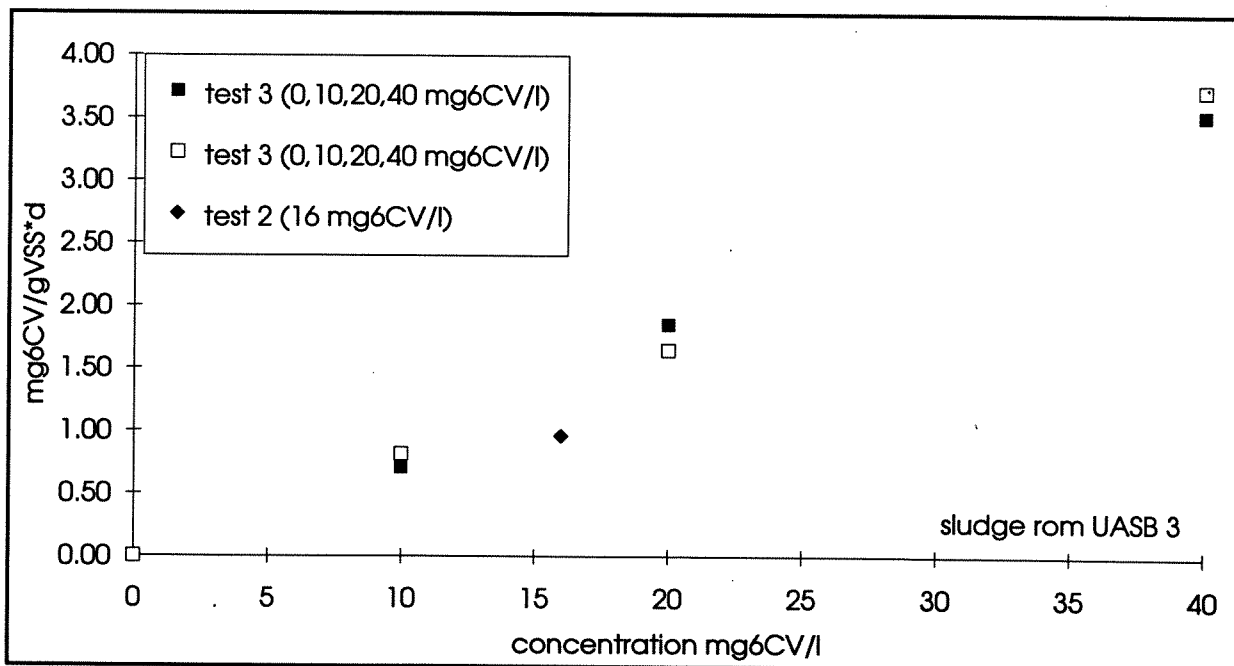
Appendix 9. Some criteria, guidlines and design parametrs for optimal operation of UASB reactor.

Enumeration of references refer to Chapter 6.

TABLE 1 — Applicable space loads in granular sludge and flocculent sludge UASB-reactors in relation to the strength of the wastewater and the height of the insoluble COD-fraction in the wastewater.

Strength of waste (mg COD/l)	fraction insoluble COD (%)	applicable loading rates at 30 °C (kg COD/m^3.d)		
		granular sludge UASB	flocculent sludge UASB little TSS-removal	significant TSS-removal
up to 2000	10 to 30%	2 – 4	8 – 12	2 – 4
	30 to 60%	2 – 4	8 – 14	2 – 4
	60 to 100%	*	*	*
2000 – 6000	10 to 30%	3 – 5	12 – 18	3 – 5
	30 to 60%	4 – 6	12 – 24	2 – 6
	60 to 100%	4 – 8	*	2 – 6
6000 – 9000	10 to 30%	4 – 6	15 – 20	4 – 6
	30 to 60%	5 – 7	15 – 24	3 – 7
	60 to 100%	6 – 8	*	3 – 8
9000 – 18000	10 to 30%	5 – 8	15 – 24	4 – 6
	30 to 60%	doubtful at TSS>6-8 g/l	doubtful at TSS>6-8 g/l	3 – 7
	60 to 100%	*	*	3 – 7

* application of the UASB-process makes no sense under these conditions

The 50% Inhibitory Concentration of Organic Acids for Methanogenesis at Various pH Values.

pH	50% Inhibitory Concentration* mg COD L^{-1}	
	Acetate (C_2)	Propionate (C_3)
5.0	44	13
5.5	106	30
6.0	300	80
6.5	912	241
7.0	2851	745
7.5	8976	2358
8.0	28368	7398

* The 50% inhibitory concentrations were calculated based on the inhibition of the unionized organic acid and the acid dissociation constants of C_2 and C_3.

66

TABLE 2   Maximal admissible hydraulic retention time at various reactor heights at a maximum admissible average daily liquid surface load ($V_{0,max}$) of 3 m/hr and 1 m/hr, assuming that merely the hydraulic load is restrictive.

reactor height (m)	minimum admissible hydraulic retention time (hrs)	
	at $V_{0,max}$ = 3m/hr	at $V_{0,max}$ = 3m/hr
3	1	3
4	1.3	4.9
5	1.7	5.1
6	2	6
9	3	9
12	4	12

However, for sub-optimal mesophilic and psychrophilic temperature conditions, generally the admissible organic space load is the limiting factor. This is illustrated in Table 3.

TABLE 3.   Tentative design criteria for granular sludge bed UASB-reactors with respect to applicable hydraulic Retention Times (HRT's) at different operational temperatures in 4 m and 8 m tall UASB-reactors for dilute (i.e. up to 1000 mg COD/l), mainly soluble wastewaters. (Admissible average daily surface load: 3 m/hr; see for admissible organic space loads Table 3)

Temperature range (°C)	HRT-values (hrs)			
	8 m tall reactor		4 m tall reactor	
	daily average	peak during 2-6 hrs	daily average	peak during 2-6 hrs
16 - 19	4 - 6	3 - 4	4 - 5	2.5 - 4
22 - 26	3 - 4	2 - 3	2.5 - 4	1.5 - 3
> 26	2 - 3	1.5 - 2	1.5 - 3	1.25 - 2

TABLE 4.   Applicable organic volumetric loading rates in relation to operational temperatures for a soluble VFA, a non-VFA soluble wastewater and a wastewater containing 30% settleable SS-COD, in granular sludge UASB-reactors containing an average sludge concentration of 25 kg VSS/m³ (hydraulic load not restrictive).

temperature (°C)	organic volumetric loading rate (kg.m^{-3}.day^{-1})		
	VFA-wastewater	non-VFA wastewater	30% SS-COD
15	2 - 4	1.5 - 3	1.5 - 2 (satisfactory SS-removal)
20	4 - 6	2 - 4	2 - 3 (satisfactory SS-removal)
25	6 - 12	4 - 8	3 - 6 (reasonable SS-removal)
30	10 - 18	8 - 12	6 - 9 (moderate SS-removal)
35	15 - 24	12 - 18	9 - 14 (fairly poor SS-removal)
40	20 - 32	15 - 24	14 - 18 (poor SS-removal)

An important wastewater in the category "very low-strength" wastewaters is raw and/or pre-settled domestic sewage. The safe applicable HRT's for sewage depend very strongly on the temperature. Table 5 gives some guidelines.

TABLE 5   Applicable Hydraulic Retention Times (HRT's) for raw domestic sewage in a 4 m tall UASB-reactor at various temperature ranges.

| Temperature range (°C) | daily average | HRT-values (hrs) | |
		maximum during 4-6 hrs	acceptable peak during 2-6 hrs
16 - 19	> 10 - 14	> 7 - 9	> 3 - 5
22 - 26	> 7 - 9	> 5 - 7	> ± 3
> 26	> 6	> 4	> 2.5

TABLE 6   Rough guidelines for the number of feed-inlet points required in a UASB-reactor.

type of sludge present	area per feed inlet point (m²)
Dense flocculent sludge (> 40 kg TSS/m³)	0.5 - 1 at loads < 1 kg COD/m³.day 1 - 2 at loads 1-2 kg COD/m³.day 2 - 3 at loads > 2 kg COD/m³.day
Medium thick flocculent sludge (20-40 kg TSS/m³)	1 - 2 at loads < 1-2 kg COD/m³.day 2 - 5 at loads > 3 kg COD/m³.day
Granular sludge	0.5 - 1 at loads up to 2 kg COD/m³.day 0.5 - 2 at loads 2-4 kg COD/m³.day >2 at loads > 4 kg COD/m³.day

TABLE 7   Main objectives of the GSS-device for UASB-systems treating soluble types of wastewaters.

1. To separate and discharge biogas from the reactor

2. To prevent as efficiently as possible the wash-out of viable bacterial matter

3. To enable the sludge to slide back into the digester compartment

4. To serve as a kind of barrier (stopper) for rapid excessive expansions of a sludge blanket (which is mainly composed of flocculent sludge) into the settler

5. To provide a polishing effect.

6. To prevent the washout of floating granular sludge

TABLE 8   Summary of tentative guidelines for the design of the gas-solids separator device.

1. The slope of the settler bottom (i.e. inclined wall of the gascollector) should be between 45-60°.

2. The surface area of the apertures between the gascollectors should be 15-20% of the reactor surface area.

3. The height of the gascollector should be between 1.5-2 m at reactor heights of 5-7 m.

4. A liquid gas interface should be maintained in the gascollector in order to facilitate the release and collection of gas bubbles and to combat scumlayer formation.

5. The overlap of the baffles installed beneath the apertures should be 10-20 cm in order to avoid upward flowing gas bubbles to enter the settler compartment.

6. Generally scum layer baffles should be installed in front of the effluent weirs.

7. The diameter of the gas exhaust pipes should be sufficient to guarantee the easy removal of the biogas from the gas collection cap, particularly also in the case of foaming.

8. In the upper part of the gas-cap anti-foam spray-nozzles should be installed in the case the treatment of the wastewater is accompanied with heavy foaming.

Table 1 Schematic overview of the problems observed during our study with regard to the functioning of granular sludge in UASB reactors.

	Problem		Cause		Solution
1	Insufficient sludge growth	1.1a	Trace-element- or nutrient limitation.	1.2a	Raising the nutrient- and/or trace-element concentration in the UASB influent
		1.1b	Too high a degree of influent pre-acidification	1.2b	Reducing the degree of pre-acidification.
		1.1c	Too low a sludge loading rate	1.2c	Increasing the loading rate (removing sludge)
		1.1d	Granular sludge wash-out (see 4, 5)		
		1.1e	Wash-out flocculant sludge, granule disintegration (see 6)		
2	Insufficient methanogenic capacity, (reactor overloaded).	2.1a	Not enough sludge in the reactor	2.2a	Reducing the loading rate. Raising the amount of sludge. Using external seed sludge. Promoting sludge growth (see 1), reducing wash-out (see 3-6)
		2.1b	Insufficient methanogenic activity (see 3).	2.2b	Decreasing sludge loading rate, increasing (methanogenic) activity of the sludge (see 3.2)
3	Insufficient methanogenic activity	3.1a	Nutrient- or trace-element deficiency (see 1.1a)		
		3.1b	An abundant growth of acidogenic bacteria	3.2b	Increasing the degree of wastewater pre-acidification. Reducing the loading rate.
		3.1c	Accumulation of organic suspended material in the sludge bed	3.2c	Ensuring that the influent does not contain suspended material.
		3.1d	A too low process temperature	3.2d	Increasing temperature
		3.1e	Toxic compounds in the wastewater fed or activity inhibiting conditions (see 6.1d).		
4	Granule wash-out.	4.1a	Gas trapped in hollow granules. formation of too big granules due to insufficient forces: low temperature, low loading rate, low influent concentration (see also 6.1a,b)		Increasing forces on granules, reducing the granule size.
		4.1b	Gas entrapment due the the formation of a layered structure, covering the granules with (acidogenic) biomass.	4.2b	Applying more stable process conditions, increasing the degree of wastewater pre-acidification.
5	Sludge wash-out, formation of bulking sludge and fluffy granules	5.1a	The conglomeration of individual sludge granules, related to suspended acidogenic bacteria in the influent.	5.2a	Withdrawing suspended matter from the influent. Diminishing the degree of pre-acidification.
		5.1b	Extensive growth of suspended or more or less at the granule surface attached acidogenic bacteria.	5.2b	Increasing the degree of pre-acidification. Intensifying the mixing applied.
		5.1c	Formation of very fluffy granules, strong growth of attached acidogenic bacteria.	5.2c	Increasing the degree of pre-acidification, Decreasing the sludge loading rate.
6	Granule disintegration.	6.1a	"Delayed" start-up problems, see 6.1 b-d.	6.2a	Applying other start-up strategy (faster increase of sludge loading rate), choosing another type of seed sludge.
		6.1b	Sudden variations in loading rate and/or influent concentration.	6.2b	Applying a more stable process conditions.
		6.1c	Sudden increase in the degree of pre-acidification. Starvation of acidogenic bacteria.	6.2c	Applying a more constant pre-acidification; (at start-up: choosing another type of seed sludge).
		6.1d	(Periodically) exposure to toxic compounds harmful conditions.	6.2d	Removing or detoxifying the toxic compound. Keeping longer .aption periods. Using a larger hydraulic buffer.
		6.1e	Too strong mechanical forces	6.2e	Preventing too strong mechanical forces, decreasing sludge loading rate.
		6.1f	Formation flocculant sludge due to an insufficient selection pressure.	6.2f	No problem if the process is stable. Otherwise increasing the selection pressure (effluent recirculation).

Table 1. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength ethanol wastewater without oxygen supply (R1) at 30°C. Inoculation with 20 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]							
		1	2	3	4	5	6	7 [c]	8
Operational:									
COD_{in}	(mg COD/l)	637	943	670	637	822	722	690	2402
HRT	(h)	28.3	16.2	10.2	4.1	10.0	2.6	10.3	2.6
OLR	(g COD/l·d)	0.5	1.3	1.6	3.7	2.0	6.8	1.6	22.7
SLR	(g COD/g VSS·d)	0.03	0.07	0.08	0.19	0.10	0.34	0.16	2.27
O_2LR	(g O_2/l·d)	0	0	0	0	0	0	0	0
Efficiency ($% COD_{in}$):									
E		99	99	99	99	99	97	99	77
CELLS		24	32	22	25	13	25	19	37
M_{total}		75	67	77	74	86	72	80	40
$M_{dissolved}$		12	8	11	12	9	10	11	3
A		76	68	78	75	87	75	81	53

[a] Abbreviations are defined in Materials and Methods
[b] Periods: 1 (day 0-9), 2 (day 10-24), 3 (day 25-44), 4 (day 45-47), 5 (day 48-51), 6 (day 52-54), 7 (day 78-85), 8 (day 85-88)
[c] Sludge concentration adjusted to 10 g VSS/l

Table 2. Operational conditions and treatment efficiency of the lab-scale UASB reactor fed low strength ethanol wastewater with oxygen supply (R2) at 30 °C. Inoculation with 20 g VSS per liter reactor. Average values.

Parameter [a]		Experimental periods [b]							
		1	2	3	4	5	6	7 [c]	8
Operational:									
COD_{in}	(mg COD/l)	422	673	720	693	914	671	846	2102
HRT	(h)	29.0	15.0	10.6	3.9	9.9	2.7	11.1	2.4
OLR	(g COD/l·d)	0.3	1.1	1.6	4.2	2.2	5.9	1.8	20.6
SLR	(g COD/g VSS·d)	0.02	0.05	0.08	0.21	0.11	0.30	0.17	2.06
O_2LR	(g O_2/l·d)	0.004	0.008	0.011	0.031	0.012	0.044	0.011	0.050
Efficiency ($% COD_{in}$):									
E		99	99	99	97	99	95	99	66
CELLS		12	11	18	26	15	24	23	26
M_{total}		87	88	81	71	84	71	76	40
$M_{dissolved}$		18	11	10	11	8	11	9	4
A		88	89	82	74	85	76	77	57

[a] Abbreviations are defined in Materials and Methods
[b] Periods: 1 (day 0-9), 2 (day 10-24), 3 (day 25-44), 4 (day 45-47), 5 (day 48-51), 6 (day 52-54), 7 (day 78-85), 8 (day 85-88)
[c] Sludge concentration adjusted to 10 g VSS/l

NIVA