

Combined biological treatment of high-sulphate wastewater from yeast production

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Keywords

anaerobic; anoxic; baker's yeast; betaine (trimethylglycine); sludge; sulphate-rich wastewater.

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doi:10.1111/j.1747-6593.2007.00097.x

Abstract

The wastewater from baker's yeast production contains above-average concentrations of organic pollutants (25 000 mg/L total chemical oxygen demand, TCOD), nutrients (1500 mg/L N_{tot} , 100 mg/L P_{tot}) and sulphate (2900 mg/L SO_4^{2-}). Baker's yeast wastewater with a flow rate of 190 m³/day was treated in a mesophilic anaerobic/anoxic continuous stirred tank reactor (CSTR) system. At the expense of the reduction of trimethylglycine (or betaine-component of sugar-beet molasses) to other nitrogen-containing compounds, it was possible to re-oxidize the sulphides to elemental sulphur, remove them from the wastewater and increase biogas production. Therefore, the average removal efficiency in the anaerobic/anoxic system was 79% by TCOD, 100% by SO_4^{2-} in which the concentration of sulphides in the effluent did not exceed 50 mg/L. The application of this combined anaerobic/anoxic system to a full-scale treatment plant supported biogas production up to 1300 m³/day, and the purification of wastewater was feasible without the use of granular sludge.

List of symbols

BOD	biological oxygen demand (mg/L)
COD	chemical oxygen demand (mg/L)
DS	dry solids (%)
HELCOM	Helsinki Commission or Baltic Marine Environment Protection Commission
N_{tot}	total concentration of nitrogen (mg/L)
P_{tot}	total concentration of phosphorus (mg/L)
SBR	sequenced batch reactor
SCOD	solubilized chemical oxygen demand (mg/L)
SRB	sulphate-reducing bacteria
SS	suspended solids (mg/L)
t	time (h)
TCOD	total chemical oxygen demand (mg/L)
TKN	total Kjeldahl nitrogen (mg/L)
UASB	up-flow anaerobic sludge blanket reactor
VFAs	concentration of volatile fatty acids (meq/dm ³)
VSS	volatile suspended solids (mg/L)
WWTP	wastewater treatment plant

Introduction

Sources of sulphate and sulphide pollution

Many industrial processes, including the food and fermentation industries, generate wastewaters containing high levels of organic matter and sulphate. Yeast industry wastewater contains low levels of readily degradable sugars and acids and high levels of trimethylglycine and sulphate. Sulphate-reducing bacteria (SRB) compete with methane-producing micro-organisms for the available organic carbon, resulting in the formation of hydrogen sulphide. When treating high-sulphate wastewater, high concentrations of sulphur compounds hinder wastewater treatment and the production of methane gas. This phenomenon results from the microbiological reduction of sulphates into sulphides. The stability of the treatment process is dependent on the pH value as well as the concentration of the sulphides formed. Sulphides formed during the treatment process inhibit the growth of methanogens as well as the SRB in the pH range of 7.2–8.5 (O'Flaherty *et al.* 1998).

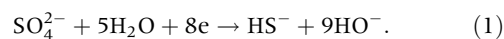
Strategies to avoid high sulphide concentrations in wastewater

The dilution of wastewater is an efficient method for the reduction of pollutant concentrations but it is not consistent with environmental protection strategies of the HELCOM (*Helsinki Commission* or *Baltic Marine Environment Protection Commission*) (Versprille 2000). The HELCOM convention contracted in 1974 for protection of the marine environment of the Baltic Sea Area includes tasks that cannot effectively be accomplished by national efforts alone but by close regional co-operation. One of its main tasks is to restrict pollution from land-based sources to the sea by point or diffuse inputs from all sources on land reaching the sea waterborne, airborne or directly from the coast (<http://www.helcom.fi/stc/files/Convention/Conv0704.pdf>).

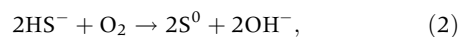
Fox & Venkatasubbiah (1996) and Janssen *et al.* (1997) found that it is also possible to remove the hydrogen sulphide produced in the anaerobic reactor from sulphate by partly oxidizing it into elemental sulphur. This process can be performed in an anoxic reactor where the concentration of oxygen is below 0.1 mg O₂/L. The elemental sulphur formed can be removed in the sedimentation tank. The wastewater circulates from the anaerobic reactor to the subsequent aerobic reactor and from that point back to the anaerobic reactor. This method enabled 95% removal of the sulphate, and the residual concentration of sulphides in the outlet of the treatment system was below 20 mg/L, while also facilitating stable pH conditions. In the Chinese patent N°1144782, 1997, the removal of sulphides from an anaerobic reactor has been solved by feeding the reactor with a controlled concentration of O₂ or air (Shan & Xiong 1997). A similar method has also been used in the Netherlands (Lens *et al.* 2000) and United States (Zitomer & Shrout 2000) without observing any inhibiting effect on the methanogens. Industrial (Buisman 1996) as well as laboratory experiments have shown that sulphide-containing wastewater leaving anaerobic reactors does not inhibit the processes in the aerobic reactor.

Biological methods for the removal of sulphur-containing compounds from wastewater

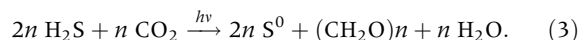
In the case of biological treatment, sulphate, sulphite and other sulphur compounds are reduced in an anaerobic step to sulphide, which in turn can be oxidized to elemental sulphur by way of limited oxidation (Buisman 1996; Lens *et al.* 1998). For reducing sulphur compounds to sulphide, an electron donor is necessary, as follows from the reaction:



Biotechnological processes for sulphide removal consist in the conversion of sulphide into elemental sulphur by colourless sulphur bacteria (*Thiobacilli*), (Buisman *et al.* 1990; Janssen *et al.* 1997) according to the following reaction:



or by genera of anaerobic photosynthetic bacteria from the families *Chlorobiaceae* and *Chromatiaceae* that catalyse the photosynthetic van Niel reaction (Henshaw *et al.* 1998):



In the latter case, light radiated to a photosynthetic reactor is coupled to the conversion of sulphide to elemental sulphur using the reverse citric acid cycle (Arnon cycle). The advantage of such a method is that only small waste streams remain because the sulphur, that is, formed can be reused. However, the disadvantage is that, especially when the effluent contains little organic matter, electron donors (methanol, ethanol, glucose and other saccharides, organic acids, H₂ and CO) have to be added in order to provide sufficient reducing equivalents for the SRB. This, as a result, increases the costs of this method substantially (Buisman 1996). Organic compounds that have more than two carbon atoms that degrade under anaerobic conditions give H₂ and acetate. H₂ can be used as an electron donor for the reduction of sulphate and sulphite.

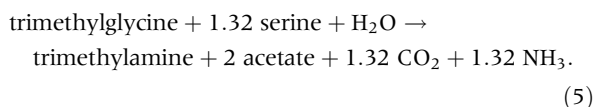
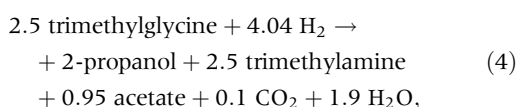
Role of trimethylglycine in anaerobic processes

Anaerobic granular sludge bed technology with upward-flow anaerobic sludge blanket (UASB) reactors is used for high-rate anaerobic treatment of wastewater. However, the UASB reactor is often inapplicable for the treatment of high sulphate-containing wastewaters (Blonskaja *et al.* 2001). The instability and increased washout of sludge granules observed can be explained by the fact that under stress conditions, all energy gained by bacteria from dissimilation is used for the generation of metabolic products, and not for the growth of cells (Weijma *et al.* 2000).

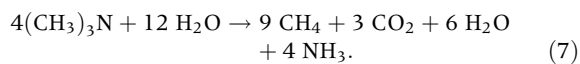
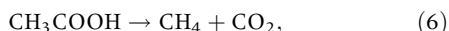
Sugar-beet molasses used as a growth medium for yeast contains large amounts [up to 6% dry solids (DS)] of betaine also known as *N,N,N*-trimethyl glycine, a soluble nitrogenous compound. Molasses is used as a substrate in a wide range of industrial fermentations, for example, alcohol, acid and yeast cell production. Trimethylglycine is not significantly consumed during these fermentations, passes the

subsequent processing stages and becomes a significant constituent of wastewater (Thalasso *et al.* 1999).

Trimethylglycine is a compatible solute, which is able to restore and maintain the osmotic balance of living cells. It is synthesized and accumulated in response to abiotic stress. Trimethylglycine also acts as a methyl group donor and has a number of important applications including its use as a feed additive (Nyssola *et al.* 2000). In anaerobic treatment plants, trimethylglycine can be nearly entirely degraded by a multistep process with the nitrogen-containing intermediates trimethylamine and other methylated amines, which are further degraded by methanogens, yielding CO₂, ammonium and methane (Thalasso *et al.* 1999). The ammonium formed buffers the treatment system and enables its stable function. The cleavage of trimethylglycine into trimethylamine and acetate is characteristic of some halophilic fermentative bacteria (Moune *et al.* 1999).



A similar cleavage mechanism for trimethylglycine under anaerobic conditions has also been reported for *Clostridium sporogenes* (Naumann *et al.* 1983; von Zumbusch *et al.* 1994) while the fermentation products of *Eubacterium limosum* are *N,N*-dimethylglycine, acetic acid and butyric acid (Müller *et al.* 1981; von Zumbusch *et al.* 1994). The acetate and trimethylamine can be readily used as carbon and energy sources by acetotrophic (e.g. *Methanobacterium soehngenii*) and methylotrophic methanogens (e.g. *Methanosarcina barkeri*), respectively (Tchobanoglous & Burton 1991):



Because trimethylglycine is undetected by a chemical oxygen demand (COD) dichromate assay, its concentration can be underestimated, which in turn leads to the significant overloading of wastewater treatment plants (WWTPs). Furthermore, trimethylglycine is a nitrogenous compound, in which its complete anaerobic degradation can result in the increase of effluent ammonia concentration. This will raise the risk of the ammonia

inhibition of the anaerobic stage by free ammonia (Thalasso *et al.* 1999).

Thalasso *et al.* suggest that trimethylglycine degradation does not appear to be coupled to sulphate reduction during the treatment of high-sulphate wastewaters (Thalasso *et al.* 1999).

The wastewater treatment plant (including a biological purification facility) for the treatment of the separation of residues of baker's yeast at Salutaguse Yeast Factory has been in operation since 1991, but has never performed satisfactorily. Thus, the aim of this work was to achieve the optimal set-up and operational parameters for removing sulphate and avoiding the inhibitory effects of sulphides in the anaerobic treatment of yeast industry wastewaters.

Materials and methods

Experimental set-up

The original treatment facility of OY Tampella Ab (Finland) consisted of an anaerobic pretreatment stage (mixing tank of 180 m³ with a stirrer and two UASB reactors each of 180 m³ volume), followed by an aerobic stage (activated sludge with a 360 m³ aeration tank) and a secondary sedimentation tank (45 m³) for final treatment before discharge Fig. 1(a). The biological treatment of wastewater was intended to be performed first in the anaerobic stage with granules forming methanogens, followed by an aerobic treatment with activated sludge. After the initial start-up of the plant in 1991, increased disintegration of sludge granules accompanied by their flow-out was observed at the plant. Instability of the anaerobic stage resulted in noncompliance of the whole process with the requirements of environmental inspection. The average concentrations of pollutants as well as environmental standards are shown in Table 1.

The new technological scheme (Fig. 1b) differed from the originally designed set-up in the following:

- The two parallel reactors of the anaerobic digestion unit were inoculated with anaerobic sludge, brought from the Tallinn Municipal WWTP.
- The aerobic stage was replaced by the anoxic stage. The concentration of oxygen was kept at a level of 0.1 mg/L with an on-line oxygen analyser Marvet OxyMat 99-1 (Elke Sensor LLC, Tallinn, Estonia).
- The temperature was automatically controlled at $+35 \pm 2^\circ\text{C}$ with contact steam injection to the incoming streams of the mixing tank and both anaerobic reactors. Temperature-monitoring electrodes were installed directly into the reactor wall (PT-100) and connected with the controller, which regulated steam injection pneumatic valves.

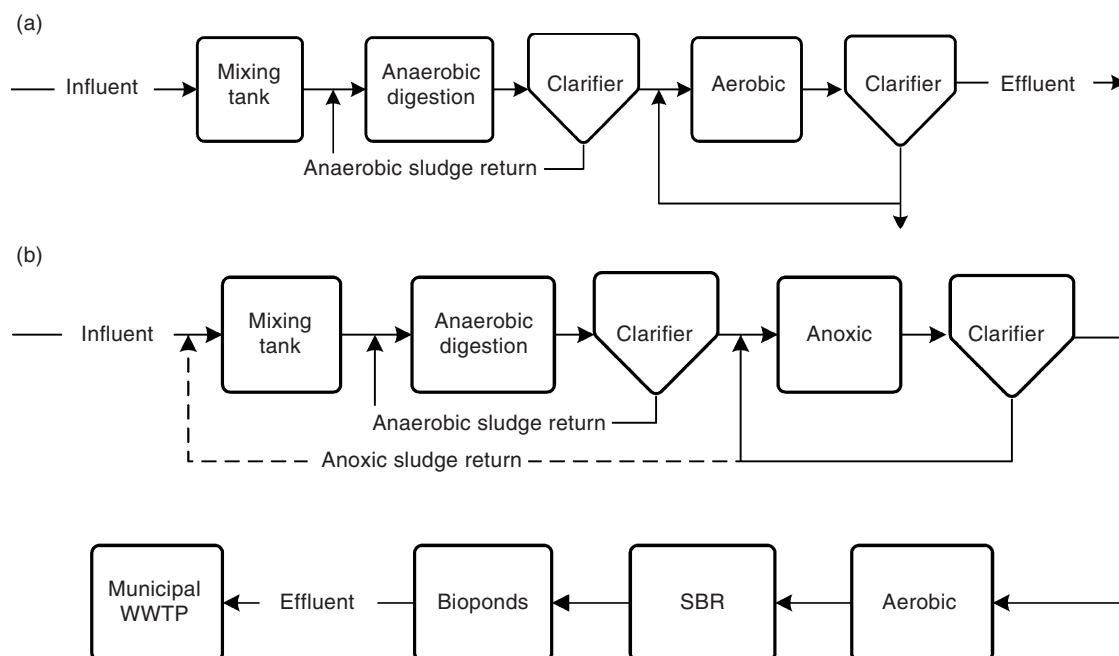


Fig. 1. (a) The original treatment facility of OY Tampella Ab (Finland); (b) the technological set-up used in the Salutaguse Yeast Factory with recirculation of the effluent from anoxic reactor back to the inlet. SBR, sequencing batch reactor; WWTP, wastewater treatment plant.

Table 1 Average concentrations of pollutants in the mixing tank, in the anaerobic reactors and in aerobic reactor in the original configuration

Sample	COD ^a (mg O ₂ /L)	BOD (mg O ₂ /L)	Sedimentable solids (SS)	Total Kjeldahl nitrogen (TKN)	Phosphorus (P)	SO ₄ ²⁻ (mg/L)	S ²⁻ (mg/L)
Mixing tank	14 916	7383	10 518	1453	36	3781	1249
Anaerobic reactor 1	10 463	5897	2744	1460	45	1751	543
Anaerobic reactor 2	10 040	5350	2776	1391	46	1243	402
Aerobic reactor	3124	2036	1487	664	30	1087	334
Effluent outflow ^b	1300	300	200	200	10	100	50
Environmental standard	125 ^c	15 ^c	15 ^c	5 ^d	1 ^c	250 ^{e,f}	–

^aHidden contamination load at the expense of trimethylglycine has not been taken into account. Because trimethylglycine is undetected by a COD dichromate assay, its concentration can be underestimated.

^bThe effluent was diluted with cooling water before final disposal to the local river.

^cPermission for special use of water No. L.VV.RA-35029 (in Estonian) http://klis.envir.ee/klis/per/view_doc?doc_id=35029.

^dOrder of directing wastewater to bodies of water and soil, Regulation No. 269 of the Government of the Estonian Republic of 31 July 2001, State Gazette 2001, 69, 424 (in Estonian).

^eRequirements for quality and control of drinking water and methods of analysis. Regulation No. 82 of Minister of Social Affairs of 31 July 2001, State Gazette 2001, 100, 1369 (in Estonian).

^fThe standard value for sulphate concentration in drinking water was taken into account as the plant is located in the area open to ground water. COD, chemical oxygen demand; BOD, biological oxygen demand.

- Part of the wastewater leaving the secondary settler was recirculated back to the inlet, that is, to the mixing tank.
- Before disposal, anoxic effluent was treated by an aerobic sequencing batch reactor (SBR).

Characteristics of wastewater

The Salutaguse Yeast Factory (a subsidiary of Lallemand Inc., Ontario, Canada) generated 271 m³/day of waste-

water originating 100% from beet molasses. The wastewater is characterized by high biological oxygen demand (BOD) (up to 12 000 mg/L) and COD (up to 25 700 mg/L by dichromate method) values. Sulphur is present in the wastewater as sulphate ions (up to 5700 mg/L).

The wastewater streams of Salutaguse Yeast Factory consist of high-strength wastewater (Table 2, Fig. 2):

- first separation (high concentrated wastewater, $t=+40^{\circ}\text{C}$, pH between 4 and 5);

Table 2 Average wastewater characteristics of Salu-taguse Yeast Plant, Estonia

Waste stream	COD excluding betaine			COD including betaine (+20%)			BOD			Sedimentable solids (SS)			Total Kjeldahl nitrogen (TKN)			Phosphorus (P)			SO ₄ ²⁻		
	Flow (m ³ /day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)
High strength wastewater in the holding tank	190	20000	1400	24000	1680	8000	560	500	35	2000	140	30	2.1	4000	280						
Wash water	100	3000	300			2000	200	500	50	100	10	20	2	400	40						
Cooling water	800																				
Municipal water	No data, limited amount																				

COD, chemical oxygen demand; BOD, biological oxygen demand.

- molasses clarification/cleaning (limited amounts, depends on the type/quality of molasses; this stream is included in the high concentrated wastewater);
- yeast wash water ($t=+14^{\circ}\text{C}$, pH between 6 and 10);
- 20% of floor and equipment wash water;

and low-strength wastewater

- 80% of floor and equipment wash water;
- cooling water ($t=+28$ to $+30^{\circ}\text{C}$, pH 7); and
- municipal wastewater (limited amount, directly to the anoxic stage).

High-strength wastewater was pumped into a mixing tank and low-strength wastewater was sent directly to an anoxic reactor (Fig. 2). The next stage was SBR, which was also used as a bypass. The anaerobic reactors were fed with a mixture of high-strength wastewater and recycled anoxic sludge. The temperature of wastewater from the incoming yeast production was $+28 \div +33^{\circ}\text{C}$. The flow rate of the incoming wastewater was measured by flow meters (MAG-XM, ABB, Zürich, Switzerland). Reactor feed and internal recycling flow rate measurements were conducted using Danfoss MAG1000/1100 electromagnetic flow metres (Danfoss, NordBorg, Denmark).

Chemical analyses

The volatile suspended solids (VSS) content of anaerobic sludge samples and settled sludge volume were analysed, as described in Standard Methods for the Examination of Water and Wastewater, 1989. Influent and effluent liquid samples were sampled and analysed 3 days/week. Analyses of COD, total nitrogen, sulphates and dissolved sulphides were conducted using HACH reagents and equipment according to the standard methods: COD – Reactor Digestion Method, US EPA approved for reporting wastewater analysis; sulphate – SulfaVer 4 Method, US EPA approved for reporting wastewater analysis; and sulphide – Methylene Blue Method, US EPA accepted for reporting wastewater analysis, total nitrogen by the Persulfate Digestion Method (American Public Health Association 1995).

Results and discussion

Change of process parameters during the treatment process

In the modified anaerobic/anoxic reactor system, a stable, buffered system was observed with a pH between 7.2 and 7.5, self-regulated by the biological process (without neutralization) and with good purification efficiency.

Recirculation of a residual sludge from the anoxic stage back to the anaerobic stage guaranteed rapid changes in

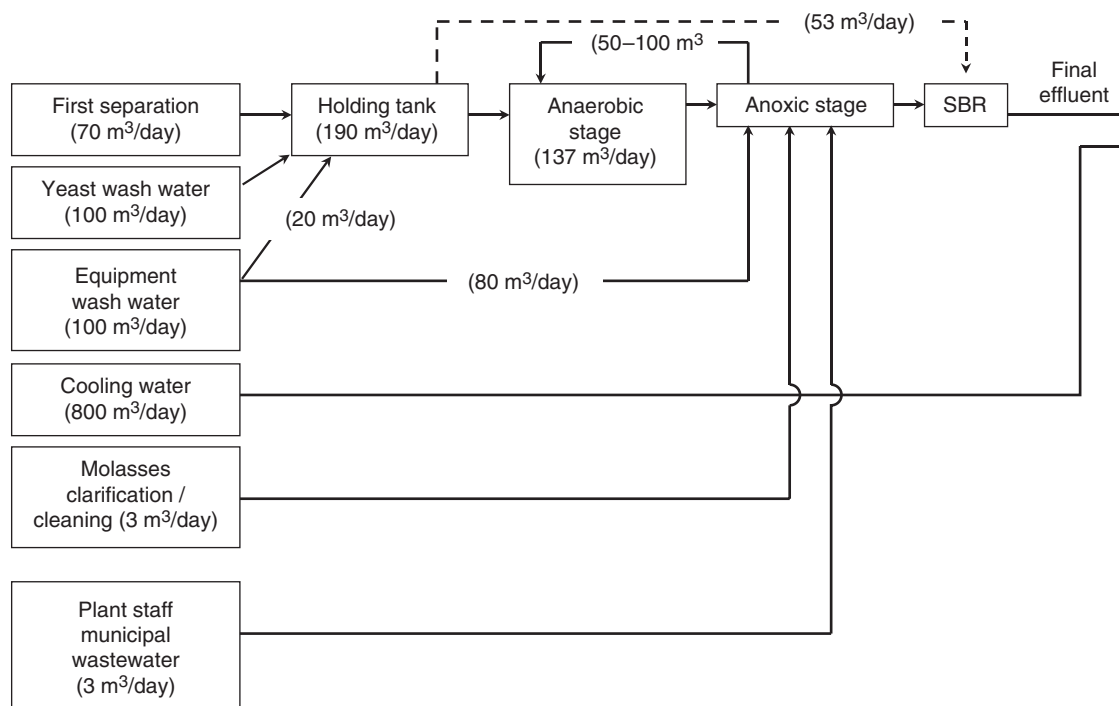


Fig. 2. Scheme of wastewater streams at Salutaguse Yeast Factory. SBR, sequencing batch reactor.

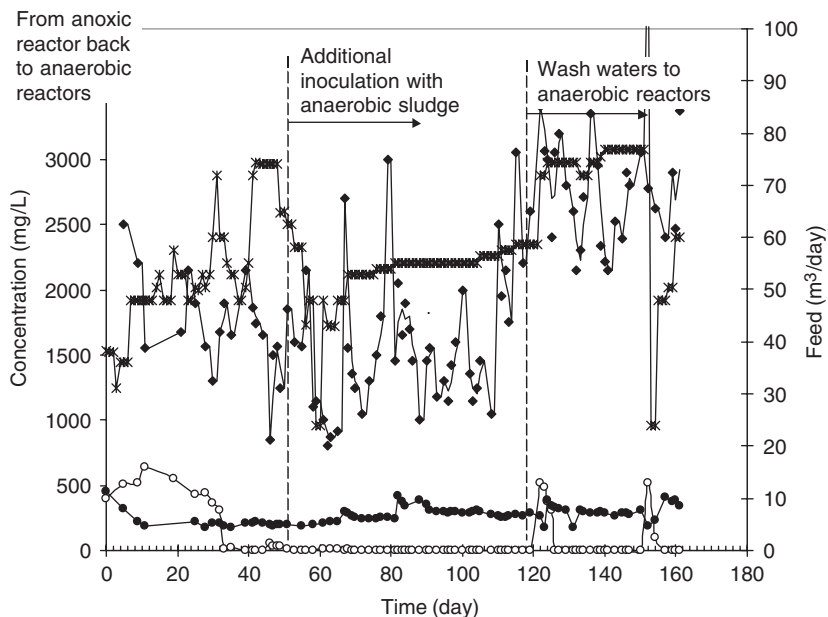


Fig. 3. Hydraulic loading rate (feed, m^3/day) and the content of sulphates and sulphides (mg/L) in the effluent from anaerobic reactor 2: $\blacklozenge$, sulphates inlet; $\circ$, sulphates outlet; $\bullet$, sulphides; $\times$, feed.

the sulphate and sulphide contents. Initially, the concentration of sulphates in the outlet of anaerobic reactors increased but the concentration of sulphides did not change much (Fig. 3). Despite the origin of the inoculation sludge (residual sludge from the municipal WWTP),

after a slight initial increase, the concentration of sulphates started to decrease constantly, reaching zero in 35 days (acclimatization effect). The concentration of sulphides in the anaerobic reactor 1 increased to some extent on account of increasing feed, while there was no evident

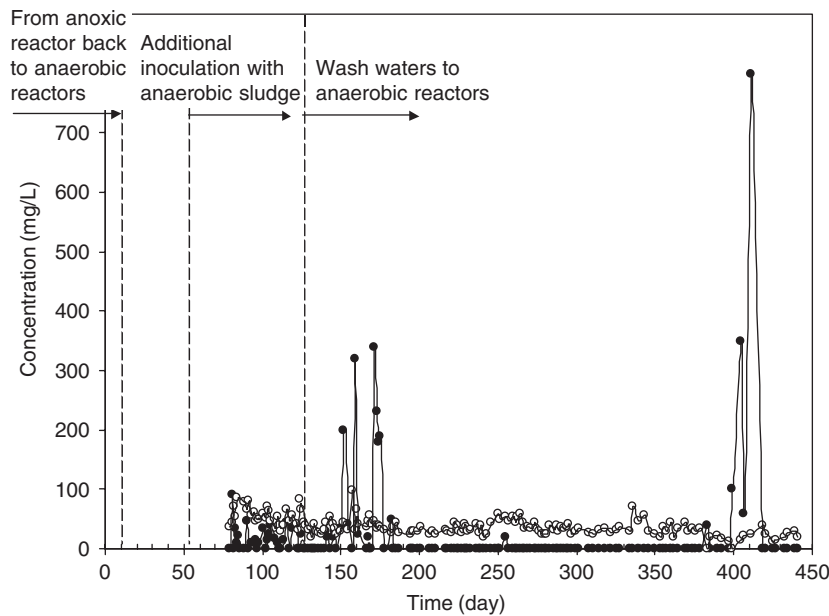


Fig. 4. Influence of recirculation on the concentration of sulphates and sulphides in anoxic reactor: ●, sulphates (mg/L); ○, sulphides (mg/L).

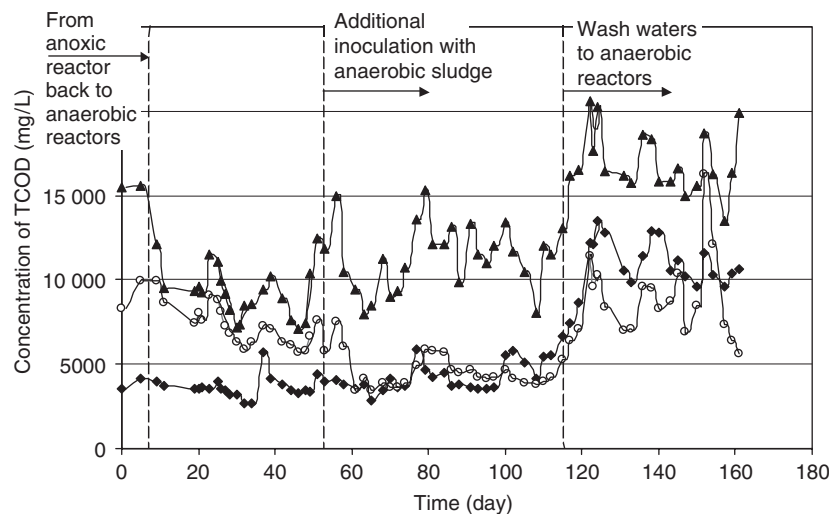


Fig. 5. Concentration of total chemical oxygen demand (TCOD, mg/L) in anaerobic reactors: ▲, TCOD of the inflow to the mixing tank; ◆, TCOD of reactor 1; ○, TCOD of reactor 2.

correlation between the concentration of sulphides and the hydraulic loading rate in reactor 2. In the anoxic tank, the concentration of sulphates also remained close to zero while the concentration of sulphides did not exceed 50 mg/L in most of the cases (Fig. 4). The anoxic reactor as well as anaerobic reactors recovered from fluctuations of sulphate concentration (due to various reasons) in a very short time (Figs 3 and 4).

Simultaneous with the decline in the concentration of sulphates, the COD value of wastewater in the mixing

tank (inlet) also decreased, caused by the dilution effect resulting from recirculation from the sedimentation tank (Fig. 5).

On the 51st day of the experiments, reactor 2 was supplemented with an additional amount (50 m^3) of residual sludge, collected from the anoxic reactor. Thus, its transportation from the Tallinn Municipal WWTP as well as the possible contamination of the reactor with fine particles of sand was avoided. This supplementary inoculation of reactor 2 reduced its effluent contamination

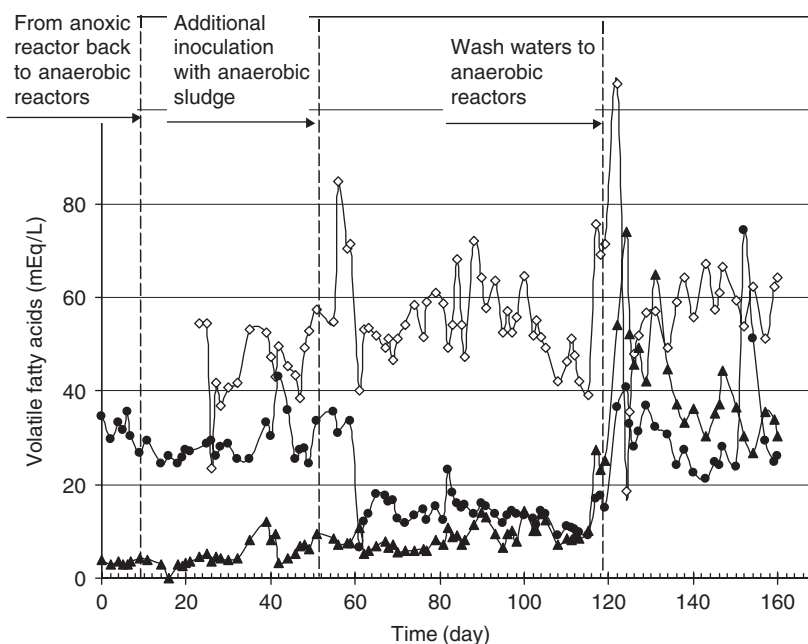


Fig. 6. Concentration of volatile fatty acids (mg/L): ◇, in the mixing tank; ▲, in the effluent from anaerobic reactor 1; ●, in the effluent from anaerobic reactor 2.

Table 3 Concentrations of pollutants in the mixing tank, in anaerobic reactors, in the inlet to the anoxic tank and in the effluent from the anoxic tank at the end of the experiment (day 166)

Sample	Feed (m ³ /day)	TCOD (mg O ₂ /L)	Decrease of TCOD (%)	SCOD (mg O ₂ /L)	Decrease of SCOD (%)	SO ₄ ²⁻ (mg/L)	Decrease of SO ₄ ²⁻ (%)	S ²⁻ (mg/L)
Holding tank ^a +20% from betaine	190	19 020		18 260		4200		
		22 824		21 912				
Mixing tank	137	15 540	23.2	12 980	40.8	2900	31	36.2
Anaerobic reactor 1	72	11 380	35.1	8250	36.4	400	86.2	360
Anaerobic reactor 2	65	4850	72.3	4080	68.6	0	100	390
Influent to anoxic reactor	190	12 338		10 634				
Effluent from anoxic reactor	190	4890	60.4	4360	59	0	100	37.2
Total treatment efficiency			78.6		80.1			

^aNot shown in the technological scheme in Fig. 1(b).

TCOD, total chemical oxygen demand; SCOD, supernatant COD.

(expressed as COD), in spite of the increasing contamination of the influent from the mixing tank (Fig. 5). Simultaneously, there was a sharp decrease in the volatile fatty acids (VFAs) content in the effluent from reactor 2 (Fig. 6).

Because of the use of a combined anaerobic/aerobic reactor system, the biological purification process had already started in the mixing tank. This phenomenon is illustrated with the data for chemical analyses presented in Table 3. These data demonstrate that the supernatant COD (SCOD) of the sample from the mixing tank was decreased up to 40% and the sulphide content up to 31% compared with the corresponding values in

the holding tank. In the mixing tank, the concentration of sulphides that originated from sulphates was 36.2 mg/L. These results confirm that the elaboration of the modified technological set-up of wastewater treatment also converted the mixing tank into a biological reactor.

The removal of sulphates from wastewater is not as complicated as guaranteeing the stability of this system. The purification scheme under study can be distinguished from other similar ones by returning anoxic residual sludge back to the mixing tank. The above-mentioned technology enables the ability to work at a high loading rate without using granular sludge. As a result

Table 4 Results of chemical analysis on the laboratory SBR reactor

Duration of experiment (day)	Flow (mL/day)	COD of influent (mg/L)	COD of effluent (mg/L)	Δ COD (mg/L)	COD removed (g)	Theoretical biogas production from COD [L/(g COD*day)] ^a	Real biogas production by COD removed (L/g)	N _{tot} of influent (mg/L)	N _{tot} of effluent (mg/L)	Betaine concentration (g/L)	Biogas from betaine (L/day)	Total theoretical biogas (L/day)	Real biogas production (L/day)
22	105	23 660	15 840	7820	0.821	0.287	1.729	255	690	4.5233	0.203	0.490	1.420
39	175	20 280	17 200	3080	0.539	0.189	2.059	250	270	1.7700	0.132	0.321	1.110
47	200	20 280	13 960	6320	1.264	0.442	0.736	475	875	5.7361	0.490	0.932	0.930
68	200	20 540	5030	15 510	3.102	1.086	0.484	325	650	4.2611	0.364	1.450	1.500
75	200	20 540	3970	16 570	3.314	1.160	0.772	345	550	3.6056	0.308	1.468	2.560
88	245	22 890	3670	19 220	4.709	1.648	0.656	255	690	4.5233	0.473	2.121	3.090
100	280	22 890	11 040	11 850	3.318	1.161	0.452	250	270	1.7700	0.212	1.373	1.500
Average	201	21 583	10 101	11 481	2.438	0.853	0.710	308	571	3.741	0.312	1.165	1.730

^aTheoretical biogas production from COD was calculated on the assumption that on the degradation of carbonaceous organic material 0.35 m³ of methane per kg of COD converted is produced. COD, chemical oxygen demand; SBR, sequenced batch reactor.

- better stability as to washout of the sludge was achieved;
- for controlling pH, there was no need to use chemicals; and
- anoxic sludge retained methanogenic activity, and for methane gas production there was no need to re-inoculate the reactors.

Estimation of trimethylglycine content in wastewater

The HPLC analyses (data not presented) of molasses, separation residue, samples from the holding tank, mixing tank, anaerobic reactors and anoxic reactor have shown that trimethylglycine possibly present in wastewater is degraded in the mixing tank. Regarding the literature data, using sugar-beet molasses as a growth medium, after cultivation of yeasts up to 4.5 g/L trimethylglycine could remain in the separation residue (high-strength wastewater) (Thalasso *et al.* 1999). However, this amount might be omitted from the COD analysis by the bichromate method. The latter is performed as acid hydrolysis at elevated temperatures. Depending on the presence of methyl groups linked to nitrogen atom, gaseous products could be formed that will not be recorded. Therefore, it would be reasonable to add to the COD concentration in the holding tank an additional 20% of a hidden contamination load at the expense of trimethylglycine (Versprille 2000) (Table 2). Betaine as some other nitrogen-containing compounds (e.g. pyridine Thalasso *et al.* 1999) resists oxidation during the standard dichromate method for COD determination. Thus, the treatment efficiency of the entire system appeared to be 79%. During the set-up period, the values of total COD (TCOD) in the effluent of anaerobic reactor 1 were in the range of 3520–13 520 mg O₂/L and in the range of 3830–16 270 mg O₂/L in anaerobic reactor 2.

Anaerobic micro-organisms degrade trimethylglycine completely into trimethylamine, acetate and other compounds [Eqs (4) and (5)]. Trimethylamine is further degraded into methane, CO₂ and ammonia [Eq. (7)], while the ratios between trimethylglycine and trimethylamine and trimethylamine and ammonia always remain equimolar. Assuming that nitrogen compounds produced during the microbiological degradation of trimethylglycine practically do not volatilize (in an anaerobic reactor), based on their apparently increased values an approximate estimation of trimethylglycine content in wastewater can be given. To prove the above-presented assumption, a separate experiment on a laboratory SBR was conducted, using wastewater from the Salutaguse Yeast Factory. The analysis of N_{tot} was performed by the Persulfate Digestion Method that mostly considers the

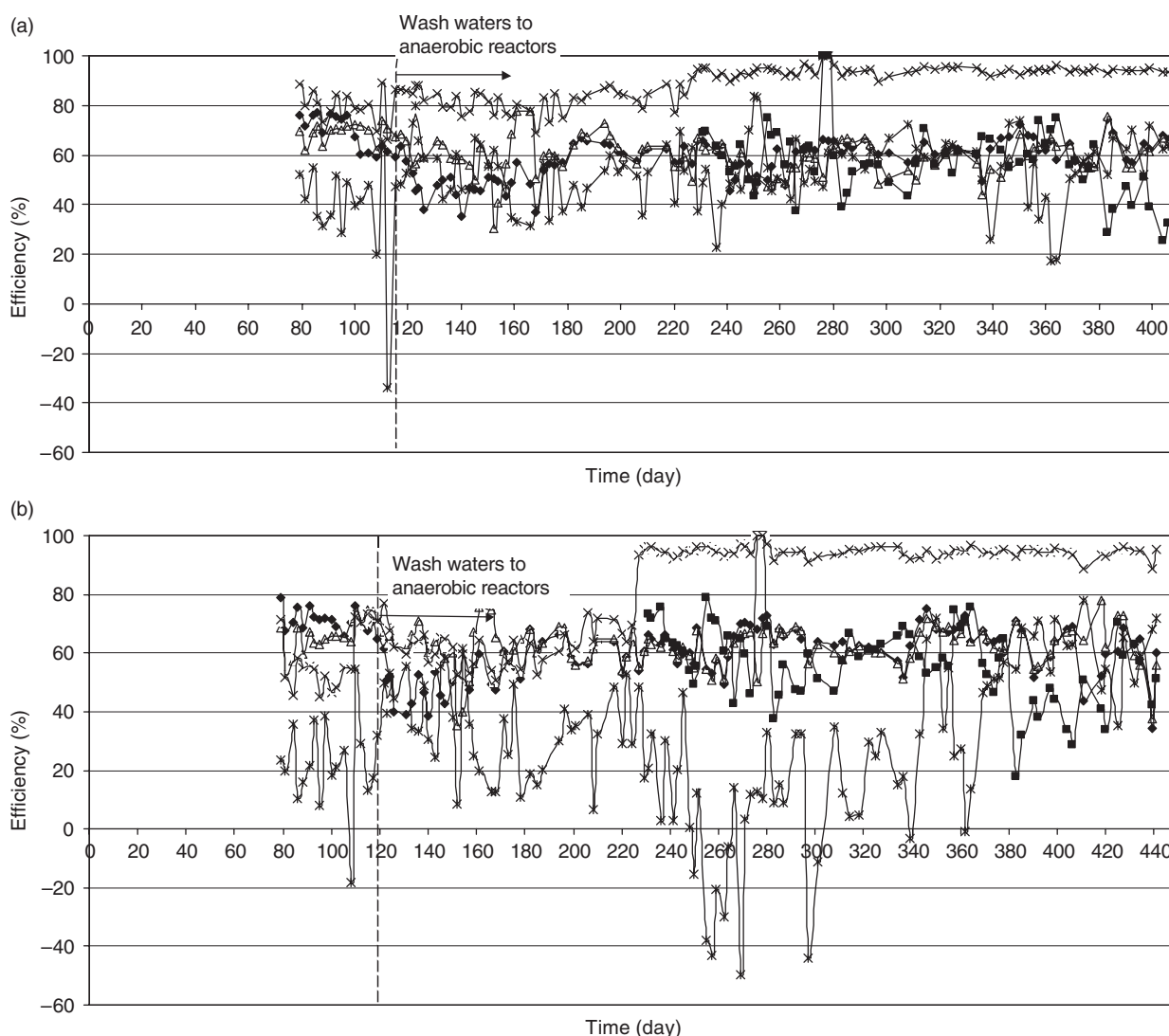


Fig. 7. The treatment efficiency: (a) by the total chemical oxygen demand (TCOD, %) value, (b) by the supernatant COD value (SCOD, %). ♦, anaerobic reactor 1; △, anaerobic reactor 2; *, anoxic reactor; ■, sequenced batch reactor (SBR); ×, the whole system.

nitrogen present as amino groups (in proteins and amino acids) as well as NH_4 . The concentrations of NO_3^- and NO_2^- in the influent were practically zero. The concentration of N_{tot} in the influent was 250–475 mg/L and 270–875 mg/L in the effluent (average 571 mg/L) (Table 4). Therefore, according to Eqs (4) and (5), from trimethylglycine, trimethylamine in a ratio of 1:1 (and further NH_4 with the same ratio) could be obtained. Taking $\text{MW}_{\text{NH}_4} = 18$ and $\text{MW}_{\text{trimethylglycine}} = 118$, we obtain $0.571/18 = 0.032$ mol, corresponding to $0.032 \times 118 = 3.74$ g/L trimethylglycine. This is the concentration of trimethylglycine in the industrial wastewater of Salutaguse Yeast Factory by theoretical calculations.

From trimethylamine, in turn, we can obtain methane in a ratio of 4:9 [Eq. (7)], e.g. from 1 mol trimethylamine (trimethylglycine) 2.25 mol methane. Thus, from 1 mol (118 g) trimethylglycine $9/4 \times 22.4 = 50.4$ L methane can be formed and from 3.74 g/L trimethylglycine in the reactor the formation of $3.74/118 \times 50.4 = 1.60$ L methane is possible. The degradation of carbonaceous organic material by anaerobic bacteria leads to the production of methane at the theoretical stoichiometric conversion rate of 0.35 m^3 of methane per kg of COD converted (Sax & Lusk 1995). Adding 1.60 to the 0.35 L methane produced from the rest of COD, we obtained 1.95 L, which was almost the same amount of methane (per g COD removed) as observed in our experiments (Table 4).

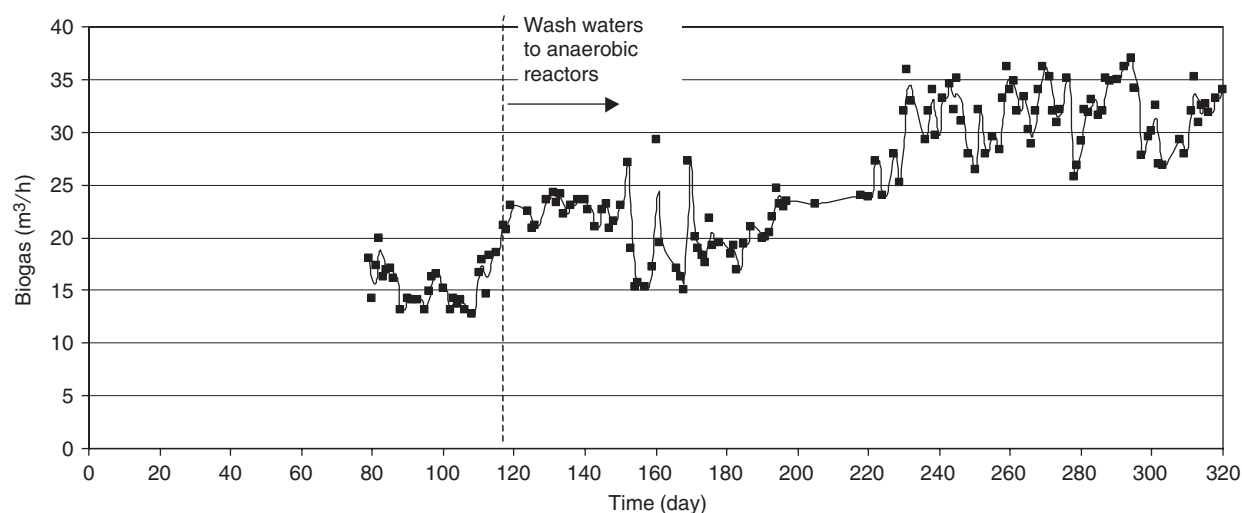
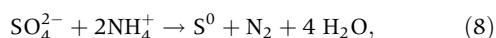


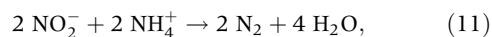
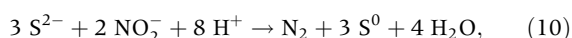
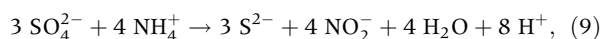
Fig. 8. Production of biogas in the anaerobic reactor 2 (m³/h).

Decrease of sulphide concentration at the expense of trimethylglycine

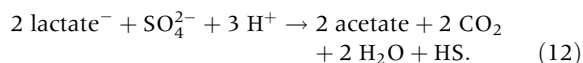
In redox reactions, the nitrogen (oxidation state +5) contained in trimethylglycine can be an electron acceptor for two electrons. The sulphide ion can donate two electrons, and thus, can be converted into elemental sulphur. Considering the stoichiometric ratio in the chemical reaction between trimethylglycine (ammonia) and the sulphide ion (sulphate ion) (Fdž-Polanco *et al.* 2001)



consisting of the following reactions:



the concentration of sulphide ions can be decreased by 1026 mg (0.032 mol) at the expense of 3.74 mg (0.032 mol) trimethylglycine. The concentration of sulphates in the holding tank was 4200 mg/L, from which the SRB are able to produce 1400 mg/L sulphides (Widdel & Hansen 1991) [Eq. (12), Table 3].



If the concentration of sulphides in wastewater can be reduced at the expense of trimethylglycine, then the residual concentration of sulphide ions should be 1400–1026=376 mg/L. At the end of the experiment, the concentration of sulphides in the reactor 1 of the anaero-

bic digestion unit (Fig. 1b) was measured as 360 mg/L and as 390 mg/L, in reactor 2 giving an average of 375 mg/L. The fluctuations in the sulphide concentration during the experiment were 176–410 (average 296) mg/L in reactor 1 and 176–417 (average 307) mg/L in reactor 2.

According to the technological set-up presented in Fig. 1, the biological processes in the anaerobic reactors and in the anoxic reactor are inter-related by the returned sludge from the secondary sedimentation tanks. The effluent from the settler (by anoxic reactor) is recircled to the inlet of the mixing tank. The evaluation of the efficiency of anaerobic and anoxic stages as well as the total efficiency of the system has demonstrated that leading the sludge back to the holding tank improved the efficiency of the anoxic stage. The performances of the anaerobic stage and the anoxic stage counterbalance each other, guaranteeing the relative stability of the entire system (Fig. 7a and b). After the start-up research, the wastewater loading was increased to 400 m³/day, up to 40% more than the initial loading. Aerobic polishing was commenced on day 220. The final treatment efficiency of the entire system consisting of two anaerobic reactors, anoxic reactor and SBR for aerobic polishing appeared to be up to 98% (by TCOD) and over 90% (by SCOD).

Because of additional inoculation of reactor 2 with adapted anaerobic sludge from the anoxic reactor (on day 51), the biogas production from the former was more intensive. Exact biogas measurement was commenced on day 76. Leading returned sludge to the mixing tank increased the production of biogas up to 25 m³/h (Fig. 8). The maximum biogas production achieved was up to 37 m³/h in both reactors because of increased loading (up to 16 kg COD/m³/day).

Conclusions

(1) Different from other similar biological wastewater purification schemes, residual sludge from the anoxic stage was returned to the beginning of the purification scheme (mixing tank). The main results of our combined anaerobic/anoxic reactors system were as follows:

- The purification of baker's yeast wastewater (produced from sugar-beet molasses, having a high SO_4^{2-} content) was feasible without the use of granular sludge.
- There was no need to use chemicals for pH control.
- The returned anoxic sludge retained methanogenic activity; no additional inoculation of anaerobic reactors was needed.

• COD reduction efficiency (by TCOD) in the anaerobic+anoxic stage was up to 80%, and up to 98% in the anaerobic+anoxic+SBR stage, supporting an average biogas production of $1300 \text{ m}^3/\text{day}$ (with two anaerobic reactors). The system operated in a stable manner, without sludge washout. The complete purification of wastewaters from sulphates (with 100% efficiency) accompanied by moderate production of sulphides (up to 50 mg/L) was obviously possible at the expense of reduction of trimethylglycine to other nitrogen-containing compounds.

(2) Therefore, considering the above-mentioned latest achievements in the treatment of high-sulphate-containing wastewaters, it was decided that there was no need for the urgent change of molasses preparation technology or of the chemical composition of mineral salts solution used for the cultivation of the yeast culture based on sugar-beet molasses. This set-up supported the creation of more favourable conditions for the methane-producing microorganisms and avoided their takeover by the SRB. The concentration of dissolved oxygen in the anoxic reactor was kept strictly below $0.1 \text{ mg O}_2/\text{L}$, enabling a continuous decrease in sulphide content.

(3) The increase of the daily volume of biogas production in an anaerobic reactor was explained by the presence of trimethylglycine in sugar-beet molasses. Considering the positive effect of trimethylglycine on biogas production, studies on the precise analytical measurement of intermediate products of anaerobic digestion continue. Furthermore, trimethylglycine can be used as an osmolyte to treat wastewaters with high concentrations of salts.

Acknowledgements

The authors would like to thank Guy Marleau, Zhigen Zhang (Lallemand Inc., Canada) for technical and financial support, and Andres Mets, Tiina Valk and Olev Kroon (Salutaguse Yeast Factory) for their excellent collaboration. The financial support from the Estonian Science

Foundation (Grants Nos. 5653 and 5889) is gratefully acknowledged.

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