THESIS ON CIVIL AND ENVIRONMENTAL ENGINEERING F8

Combined treatment of sulfate-rich molasses wastewater from yeast industry

Technology optimization

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PRESS

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Declaration

I declare that the current thesis is my original and unaided work. It is being submitted for the Degree of Doctor of Philosophy in Engineering Sciences at the Tallinn University of Technology, Tallinn, Estonia. It has not been submitted for any degree or examination in any other university.

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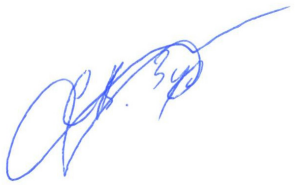

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KOKKUVÕTE

Pärmitööstuse sulfaatiderikka melassijäägi kombineeritud töötlemine

Käesoleva uurimuse põhiline eesmärk oli edasi arendada optimaalne skeem ja tööparameetrid sulfaatide eemaldamiseks ja sulfiidide inhibeeriva mõju vältimiseks AS Salutaguse Pärmitehase pärmireovee anaeroobsel töötlemisel. Käesolev töö viidi läbi, kasutades uuringuid AS Salutaguse Pärmitehase sulfaatiderikka reovee anaeroobse/aeroobse töötluse tööstusliku skeemi väljaarendamise ja juurutamise kohta, uuringuid pagaripärmi reovett töötleva anaeroobse laboratoorse annuspuhasti kohta, uuringuid anaeroobse bioreaktori modifikatsiooni kohta, parandamaks biomassi viibeaega muda degaseerimise tehnoloogia juurutamise teel ning uuringuid osooni ja koagulantide kasutusvõimaluste kohta bioloogiliselt eelkäideldud pärmireovee järeltöötlusel. Tehti kindlaks, et annuspuhasti protsessiga võrreldes on kombineeritud anaeroobsel/anoksilisel protsessil paremad käidutingimused ja parem sulfaatiderikka reovee puhastusefektiivsus. Laboratoorse annuspuhasti skeemiga saavutati stabiilne KHT eemaldamise efektiivsus (umbes 80%), kui rakendatav orgaaniline koormus varieerus piirides 7,7 kuni 8,0 kg KHT m^{-3} päev^{-1}. Mõlema puhastusskeemiga saadud katsetuste tulemusena leiti sulfaatide eemaldamise ja sulfiidide inhibeeriva toime vältimise tööparameetrid anaeroobsel töötlusel ning kohandati need tööstuslikule reoveepuhastile, kasutades olemasolevat aparatuuri. Melassi töötlemise reovesi sisaldab suures koguses värvilisi ühendeid (melanoidiine jms), mis annavad reoveele tumepruuni värvuse ja suure orgaanilise koormuse. Bioloogiliselt eelkäideldud pagaripärmi reovee järeltöötluse katsetes uuriti koagulatsiooni astet ja osoneerimise mõju reovee kvaliteedile. Katsetuste käigus tehti kindlaks, et bioloogiliselt eelkäideldud pärmi reovee järelosoneerimise tulemuseks oli KHT alanemine 30-49% ning tarbitud osooni annus (mg osooni mg eemaldatud KHT kohta) oli vahemikus 1,2 kuni 2,5. Reovee bioloogiline lagundadatus, väljendatuna BHT ja KHT vahelise suhtega, osoneerimiskatsete vältel üldiselt suurenes. Koagulatsiooni astmes (Fe^{3+} ja Al^{3+}) oli võimalik saavutada märkimisväärne värviliste ühendite sisalduse vähenemine (kuni 90%). Lisaks värvuse eemaldamisele oli raua (200 mg L^{-1}) ja alumiiniumi (400 mg^{-1}) abil võimalik oluliselt eemaldada ka KHT (40-61%), lämmastikku (74-76%) ja fosforit (kuni 78%). Üritati parandada anaeroobse reaktori toimimist ja vältida kõrgetest tipukoormustest tingitud sette väljapesemist. Arendati välja sobiv meetod, mis aitab säilitada biomassi anaeroobses reaktoris ja katsetati seda tööstuslikus süsteemis. Anaeroobne muda degaseerimise seade võimaldas tõsta orgaanilist tipukoormust kuni 17,8 kg KHT$m^{-3}d^{-1}$ ilma metanogeenset protsessi häirimata.

Märksõnad: aktiivmuda vaakumtöötlus, anaeroobne, betaiin, biogaas, järelpuhastus, koaguleerimine, melanoidiinid, melassijääk, osoneerimine, suhkrupeedi melass, sulfaate redutseerivad bakterid.

ABSTRACT

Combined treatment of sulfate-rich molasses wastewater from yeast industry

The main purpose of the present study was to develop further optimal setup and operational parameters for removing of sulfates and avoiding inhibitory effects of sulfides in anaerobic treatment of yeast wastewaters at Salutaguse Yeast factory, Estonia. Present work was carried out using the studies on development and introducing of full-scale anaerobic/anoxic treatment scheme of sulfate-rich wastewater at Salutaguse Yeast Factory, Estonia, lab-scale anaerobic Sequence Batch Reactor (SBR) treating baker's yeast effluent, modification of anaerobic biorector to improve biomass retention by introducing sludge de-gassing technology and possibilities of using ozone and coagulants for post-treatment of biologically pre-treated yeast wastewater. It was established that compared to SBR process, the combined anaerobic/anoxic process indicates better operation results and treatment efficiency for purification sulfate rich wastewater. Lab-scale anaerobic SBR scheme demonstrated stable COD removal efficiency (about 80%) when applied organic loading rate varied between 7.7 and 8.0 $kgCODm^{-3}d^{-1}$. Full scale combined anaerobic/anoxic demonstrated similar COD removal efficiency with applied loading of 12.0-16.0 $kgCODm^{-3}d^{-1}$. As the result of experimental studies with both purification schemes the operational parameters for removing of sulfates and avoiding inhibitory effects of sulfides in anaerobic treatment were found and adopted at full-scale wastewater treatment plant using existing equipment. Wastewater from molasses processing presents a large amount of colored substances (melanoidins, etc.) that give dark brown color and high organic load to the effluents. In the experiments with post-treatment of biologically pre-treated baker's yeast wastewater effluent, coagulation step and ozonation impact on effluent quality were studied. During experimental studies it was established that the post-ozonation of biologically treated yeast wastewater resulted in the reduction of COD by 30–49%, and the consumed ozone dosage (mg ozone per mg of COD removed) ranged from 1.2 to 2.5. The biodegradability of the wastewater, expressed as BOD/COD ratio, generally increased during the ozonation tests. During coagulation step (Fe^{3+} and Al^{3+}) significant decrease of color substances could be achieved (up to 90%). In addition to color removal application of iron (200 mgL^{-1}) and aluminium (400 mgL^{-1}) showed the significant removal of COD (40-61%), nitrogen (74-76%) and phousphorous (up to 78%). Attempts to impove anaerobic reactor performance and prevent sludge washing out caused by applied high peak loads were done. The suitable techniques which helps to retain biomass in anaerobic digester was developed and tested at full-scale system. Anaerobic sludge de-gaasing unit allowed to increase peak organic loading rate up to 17,8 kg $CODm^{3}d^{-1}$ without methanogenic process failure.

Keywords: anaerobic, beet molasses, betaine, biogas, coagulation, melanoidins, post-ozonation, sulfate reducing bacteria, vacuum sludge treatment, yeast wastewater.

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CONTENTS

LIST OF PUBLICATIONS INCLUDED IN THE THESIS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Zub, S., Kurissoo, T., Menert, A., Blonskaja, V. (2002). The anaerobic/anoxic treatment of sulphate-rich wastewater. In. Proc.*2nd Biennial Conference on Management of Wastewaters: Edinburgh, UK, 15-17 April 2002*, p. 285-294

II. Krapivina, M., Kurissoo, T., Blonskaja, V., Zub, S. and Vilu, R. (2007). Treatment of sulphate containing yeast wastewater in an anaerobic sequence batch reactor. Proceedings *of the Estonian Academy of Sciences. Chemistry*, 56(1), 38-52.

III. Zub, S., Blonskaja, V., Kamenev, I. (2006). Possibilities of using ozone for the treatment of wastewater from the yeast industry. Proceedings *of the Estonian Academy of Sciences. Chemistry*, 55(1), 29-39.

IV. Zub, S., Kurissoo, T. and Mets, A. (16.07.2007). Device for the anaerobic purification of wastewater with obtaining of biogas. *Utility Model* EE 00665 U1, The Estonian Utility Model Gazette 3/2007, p.9. ISSN 1023-6546.

V. Blonskaja, V., Zub, S., Krapivina, M. (2005). Anaerobic treatment of yeast industry wastes-years of industrial experience. In: Conference proceedings. Vilnius, 2005. Vol.1, *Environmental Engineering The 6th International Conference, Lithuania, May 26-27*, p. 331-336. SBN 9986-05-858-9.

Other publications:

[1] Kurissoo, T., Menert, A., Zub, S. (2002). Monitoring of anaerobic/anoxic treatment of sulphate-rich wastewaters from yeast industry. –In: Abstracts: Xth International Congress of Bacteriology and Applied Microbiology, Paris, 27 July-1 August, Paris: EDK, 159-159.

[2] Zub, S. (2002). Water-soluble vitamins determination in inactive yeast products by HPLC. Food and nutrition, 108-109.

[3] Zub, S., Kurissoo, T., Menert, A., Blonskaja, V. (2007). The anaerobic/anoxic treatment of sulphate-rich wastewater. **to be submitted in the Water and Environmental Journal**

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The author's contribution in the appended publications:

Publication **I**: The study was initiated by the author. He is principally responsible for the data analysis and in the preparation and writing of the manuscript in cooperation with other authors.

Publication **II**: The author is responsible for the data analyses, and participated in the general design of the study and writing the manuscript.

Publication **III**: The study was initiated by the author. He is partly responsible for the data collection and analysis. The author participated together with BSc-students in the experiments.

Publication **IV**: The author carried out the design and construction of prototype equipment, interpreted the obtained data and prepared necessary documentation together with co-authors for *Utility Model*.

Publication **V**: The author is responsible for the data collection and analysis. He participated in the preparation of manuscript as the leading author.

1. INTRODUCTION

The level of pollution in waste water and the amount of waste produced by the FDM (Food, Drink and Milk Industries) sector can represent a significant load in some countries or regions. While most emissions from the FDM industry are biodegradable, some sectors use raw materials such as sugar cane or beet molasses which are resistant to conventional treatment methods and can introduce serious difficulties, e.g. non-biodegradable color substances which remain in waste water.

Traditionally, in many European countries, the sector has not been heavily regulated by environmental legislation. The impetus for the sector to improve its environmental performance has therefore been based on efficiency improvements, e.g. maximizing the utilization of materials, which subsequently leads to a minimization of waste.

There is now a trend towards focusing on proactive environmental management systems, natural resource conservation and the improvements of waste minimization techniques. To ensure sustainability, the effects of the raw material supply, food processing, transport, distribution, preparation, and disposal must be considered and controlled. Both primary production and processing are critically dependent upon a reliable water supply and adequate water quality, in conformity with legal requirements.

Water consumption is one of the key environmental issues for the FDM sector. Most of the water which is not used as an ingredient ultimately appears in the waste water stream. Typically, untreated FDM waste water is high in both COD and BOD. Levels can be 10-100 times higher than in domestic waste water. The SS concentration varies from negligible to as high as 120000 mg L^{-1}. Generally the fermentation processes are the biggest contributors of wastewater pollution in FDM sector, e.g. alcohol and baker's yeast production and breweries.

Fermentation is the process of using microorganisms to produce valuable products such as antibiotics, industrial enzymes, food, and chemicals. Baker's yeast (*Saccharomyces cerevesiae*) is one of the oldest products of industrial fermentation. It is still one of the most important fermentation products based on volume of sales and its use for bread-making, a stable food for large section of world's population. Yeasts are the most important and the most extensively used microorganisms in food industry. They are cultured for the cell mass, cell components, and products that they produce during the fermentation.

Yeast production wastewater is a complex mixture. Most of the contaminants in the wastewater are due to the use of molasses as a main raw material. During yeast fermentation process, the sugars contained in the molasses are utilized as carbon and energy source. The major part of the non-sugar substances in the molasses (molasses residuals), however, is not assimilable by the yeast and releases unchanged to the processing wastewater, which represents the principal

waste in the yeast production process. Besides molasses residuals, yeast production wastewater also contains chemicals added during fermentation (e.g. various salts, antifoams, propionic acids, brine, etc), yeast metabolites and residual yeast cells.

Since the main substrate for baker's yeast production in Europe is sugar beet molasses, these wastewaters are high strength (10000-80000 mg L^{-1} by COD), strong nitrogenous (1500-2500 mg L^{-1} total N), sulfate (2000-10000 mg L^{-1}), phosphorus (30-60 mg L^{-1}), recalcitrant for biodegradation and highly coloured (melanoidins etc.) substances. For each ton of the end product, about 17-25 m^3 of wastewater is discharged.

Anaerobic treatment of wastewaters containing high amount of sulfate has been studied extensively in recent years. One of the main industries producing sulfate rich wastewaters are the fermentation industries – distilleries and yeast production plants. Technological production of citric acid, monosodium glutamate and nucleic acid also leads to formation of sulfate rich wastewaters. For the treatment of high strength wastewaters anaerobic digestion appears to be economically more attractive than the aerobic processes. Two important goals are achieved in anaerobic processes simultaneously: removal of organic matter and sulfates. Advantages of anaerobic digestion include also relatively low sludge production and low energy needs compared with aerobic treatment. However, high sulfate content can lead to destabilization of treatment processes due to the hydrogen sulfide formation. Biological sulfide oxidation to elemental sulphur seems to be the best solution for this problem, because elemental sulphur is inert solid material, which could be easily removed from the treatment system.

Currently many yeast factories are faced with heavy trade-effluent charges. Land disposal options generate problems with ground water pollution and are prohibited in majority of the European regions. Many local municipal sewage treatment plants are now insisting on pre-treatment of such effluents before discharge into their sewerage.

2. AIM OF THE STUDY

The aim of this study was, first of all develop further optimal setup and operational parameters for removing of sulfates and avoiding inhibitory effects of sulfides in anaerobic treatment of yeast wastewaters at Salutaguse Yeast factory, Estonia. The next aim was adaptation of optimal technological setup parameters at full scale wastewater treatment plant using existing equipment.

One of the most difficult aspects of the anaerobic system operation is the retention of the sludge in the reactor, due to the low density of the sludge and the rising of generated biogas bubbles. In order to keep the sludge in the reactor, comparatively difficult aim was targeted in this investigation – to develop

technology and techniques which helps to retain necessary biomass in anaerobic digester at Salutaguse Yeast Factory WWTP.

Wastewater from molasses processing presents a large amount of colored substances that give dark brown color and high organic load to the effluents. After a multistage biological treatment most of the organic load is removed. However, the brown color does not disappear and it can even increase because of the repolymerization of colored compounds. The main colored compounds are known as melanoidins. Therefore, the last aim of present work was to study additional treatments to remove color substances from biologically pre-treated effluent by ozonation and coagulation.

3. LITERATURE REVIEW

3.1. Anaerobic digestion processes

The anaerobic microbial conversion of organic substrates to methane is a complex biogenic process involving a number of microbial populations, often linked by their individual substrate and product specificities. As shown in Figure 1, the overall conversion process may be described as involving both direct and indirect symbiotic associations between different groups of microorganisms. Although these associations have been illustrated in various ways, nine recognizable steps, each mediated by a specific group of microorganisms and their enzyme complements, can be identified, including:

1) Enzymatic hydrolysis of organic polymers to intermediate organic monomers such as sugars, fatty acids, and amino acids
2) Fermentation of organic monomers to hydrogen (or formate), bicarbonate, pyruvate, alcohols, and lower fatty acids (acetate, butyrate, and propionate)
3) Oxidation of reduced organic products to hydrogen (formate), bicarbonate, and acetate by obligate hydrogen-producing acetogens (OHPA)
4) Acetogenic respiration of bicarbonate by homoacetogens (HA)
5) Oxidation of reduced organic products (alcohols, butyric and propionic acids) to bicarbonate and acetate by nitrate-reducing bacteria (NRB) and sulfate-reducing bacteria (SRB)
6) Oxidation of acetate to bicarbonate by nitrate-reducing bacteria (NRB) and sulfate-reducing bacteria (SRB)
7) Oxidation of hydrogen (or formate) by nitrate-reducing bacteria and sulfate-reducing bacteria (SRB)
8) Aceticlastic methane fermentation
9) Methanogenic respiration of bicarbonate

These conversion possibilities can serve as a convenient basis for emphasizing some important biochemical and environmental requirements of the anaerobic microbial treatment of organic substrates and for directing the development or selection of substrate-linked process configurations.

3.2. Substrate specificities

The methanogenic bacteria are crucial to the anaerobic stabilization of a variety of substrates, since they constitute a major final step in the transfer of electrons from the various donor species. Unfortunately, known methanogens utilize only a narrow array of relatively simple substrates for growth and metabolism, the most familiar and frequently acknowledged of which are the hydrogen-mediated reduction of carbon dioxide and the aceticlastic cleavage of acetic acid. However, it is known that many methanogens may also utilize formate, and to a lesser degree, alcohols or carbon monodioxide, as electron donors. Therefore, in the presence of an abundant source of organic substrate, approximately two-thirds of the methane produced during anaerobic microbial conversion is derived from the methyl moiety of acetate, and about one-third is derived from dioxide reduction.

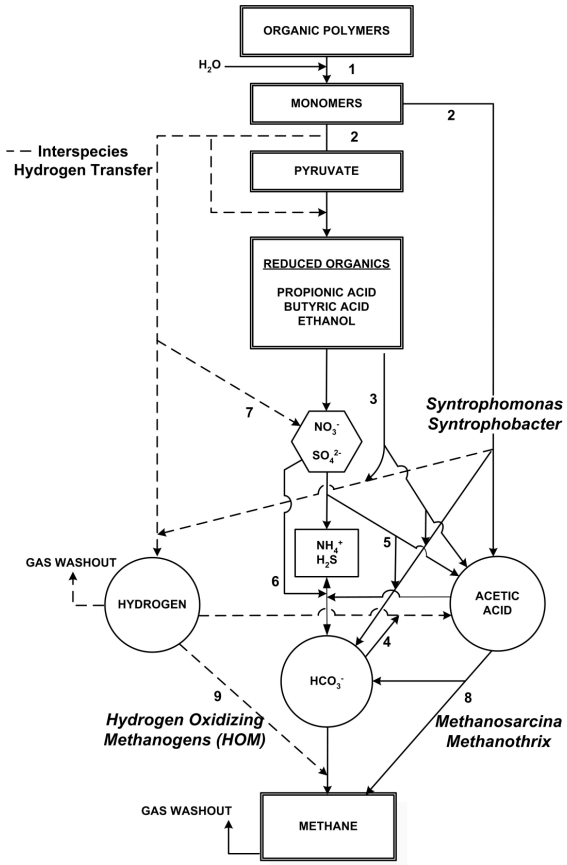

Figure 1. Substrate conversion patterns associated with the anaerobic treatment of wastewaters. Legend: (1) Hydrolysis of organic polymers; (2) Fermentation of organic monomers; (3) Oxidation of propionic and butyric acids and alcohols by OHPA; (4) Acetogenic respiration of bicarbonate; (5) Oxidation of propionic and butyric acids and alcohols by SRB and NRB; (6) Oxidation of acetic acid by SRB and NRB; (7) Oxidation of hydrogen by SRB and NRB; (8) Aceticlastic methane fermentation; and (9) Methanogenic respiration of bicarbonate (Harper and Pohland, 1987)

As indicated in Figure 1, the primary aceticlastic methangens, *Methanosarcina* and *Methanosaeta* (*Methanothrix*), are relatively slow-growing genera with 24 hour doubling times. Therefore, they are vulnerable to competition from the more rapidly-growing hydrogenotrophs (hydrogen-oxidizing methanogens) with 1 to 4 hour doubling times (Jones, 1991). Moreover, the former methanogenic bacteria can be adversely affected by the accumulation of hydrogen, and maintenance of low hydrogen (or formate) levels is important if these species are to contribute effectively to overall substrate conversion and mineralization.

It has also been recognized that methane may be derived from the methyl moieties of a variey of 1-carbon compounds, including methanol, dimethyl sulfide, and mono-, di- and trimethylamines. In this cases, methane may be formed by a "disproportionation reaction" (Jones, 1991), whereby some of the substrate is oxidized to generate reducing equivalents for subsequent methyl group reduction. In addition, secondary alcohols, including 2-propanol and 2-butanol, as well as the primary alcohols ethanol, 1-propanol, and 1-butanol, are partially oxidized and serve as electron donors for reduction of carbon dioxide to methane (see Table 1).

Table 1. Selected substrates and methane-producing reactions*

Reactions	
Hydrogenotrophic reactions:	
4 H_2 + CO_2	**→ CH_4 + 2H_2O**
4 Formate	**→ CH_4 + 3CO_2 + 2H_2O**
4 (2-propanol) + CO_2	**→ CH_4 + 4 acetone + 2H_2O**
Aceticlastic reaction:	
Acetate	**→ CH_4 + CO_2**
Disproportionation reactions:	
4 Methanol	**→ 3CH_4 + CO_2 + 2H_2O**
4 Methylamine + 3H_2O	**→ 3CH_4 + CO_2 + 4NH_4^+**
2 Dimethyl sulfide + 2H_2	**→ 3CH_4 + CO_2 + H_2S**

* Adapted from reference (Jones, 1991)

As also indicated in Figure 1, the presence of alternative electron acceptors such as nitrate or sulfate may inhibit methanogenesis, since sulfate-reducing bacteria (SRB) can outcompete methanogens for available substrates (H_2, acetate), and hydrogen sulfide production can predominate over methanogenesis. Accordingly, in those cases, organic carbon is oxidized to carbon dioxide with a concomitant reduction of sulfate to hydrogen sulfide. With the exception of methylamines and possibly dimethyl sulfide, the major methanogenic substrates, acetate, formate, alcohols, and hydrogen plus carbon dioxide, also serve as substrates for sulfate-reducing bacteria. Thus, methane production from sulfate-rich substrates may be limited by such substrate preference.

3.3. Biochemical interactions

The methanogens and their biochemical interactions during methane fermentation have been reviewed by several authors (Jones *et al*., 1987, Rouviere *et al*., 1988, DiMarco *et al*., 1990). The major biochemical interactions for acetogenic and methanogenic conversion of the principal precursor substrates illustrated in Figure 1 are further defined in Table 2 in terms of associated redox half-reactions and biochemical standard free energy levels (Harper and Pohland, 1986).

Accordingly, when a particular metabolic pathway dominates a particular substrate conversion sequence, it is frequently regulated by the intensity of hydrogen (or formate) production and its potential for accumulation to inhibiting levels. Therefore, a lack of syntropy between the hydrogen-producing acidogens and the hydrogen-consuming methanogens, sulfate-reducing bacteria (SRB) or nitrate-reducing bacteria (NRB) can result in excessive accumulation of hydrogen or intermediate conversion products unless other hydrogen sinks (Fe^{3+}, Mn^{+4}, oxygen, unsaturated compounds, etc.) are available. Indeed, he thermodynamic energy yield of the oxidation of organic compounds, coupled to reduction of various electron acceptors, decreases in the order $O_2 > NO_3 > MnO_2 > Fe^{3+} > SO_4 > CO_2$ (Kiene, 1991). Theoretically, greater energy is available and, hence, greater growth yield of the microorganisms that can use the most favorable electron acceptors (Thauer *et al*., 1997).

Anaerobic microbial conversion systems are most efficient when operating in the absence of inhibition. In the case of potential inhibition of hydrogen, this usually requires both ultimate cleavage of acetate and reduction of carbon dioxide. Conversion of the higher organic fatty acid homologues (butyric and propionic acids) to acetate and hydrogen is accomplished by organisms that grow only when hydrogen is used by the hydrogenotrophs, a process termed "interspecies hydrogen transfer" (Iannotti *et al*., 1973, Wolin,1977). In case of obligate interspecies hydrogen (or formate) transfer, syntrophy between the production of hydrogen from the acids and its utilization by methanogens is necessary to permit reactions that yield energy for the growth of both species. Hence, a common characteristic of such syntrophic associations is the "thermodynamic barrier" to the reduction of protons to hydrogen, a barrier that can be overcome by coupling the formation of hydrogen to the reduction of carbon dioxide to methane.

The microorganisms that oxidize butyrate and propionate to acetate and hydrogen grow very slowly, even in the presence of methnogens. The generation time for *Syntrophomonas wolfei* on butyrate is about 3 days (McInerney *et al.,* 1981), and that *Syntrophobacter wolinii* growing on propionate is about 7 days (Boone and Bryant, 1980). Therefore, the complete conversion of these substrates may require processes with longer solids retention times (SRT), a future of many conventional anaerobic microbial treatment systems providing enhanced biomass containment.

Table 2. Some Redox Half-reactions Responsible for Anaerobic Microbial Conversion of Selected Substrates [*]

Reactions				
Oxidations (electron donating reactions)				**ΔG_0, KJ**
Propionate → Acetate:	**$CH_3CH_2COO^-$ + $3H_2O$**	**→ CH_3COO^- + H^+ + HCO_3^- + $3H_2$**		**+76.1**
Butyrate → Acetate:	**$CH_3CH_2CH_2COO^-$ + $2H_2O$**	**→ $2CH_3COO^-$ + H^+ + $2H_2$**		**+48.1**
Ethanol → Acetate:	**CH_3CH_2OH + H_2O**	**→ CH_3COO^- + H^+ + $2H_2$**		**+9.6**
Lactate → Acetate:	**$CH_3CHOHCOO^-$ + $2H_2O$**	**→ CH_3COO^- + HCO_3^- + H^+ + $2H_2$**		**-4.2**
Acetate → Methane:	**CH_3COO^- + H_2O**	**→ HCO_3^- + CH_4**		**-31.0**
Respirative (electron accepting reactions)				
HCO_3^- → Acetate:	**$2HCO_3^-$ + $4H_2$ + H^+**	**→ CH_3COO^- + $4H_2O$**		**-104.6**
HCO_3^- → Methane:	**HCO_3^- + $4H_2$ + H^+**	**→ CH_4 + $3H_2O$**		**-135.6**
Sulfate → Sulfide:	**SO_4^{2-} + $4H_2$ + H^+**	**→ HS^- + $4H_2O$**		**-151.9**
	CH_3COO^- + SO_4^{2-} + H^+	**→ $2HCO_3^-$ + H_2S**		**-59.9**
Nitrate → Ammonia:	**NO_3^- + $4H_2$ + $2H^+$**	**→ NH_4^+ + $3H_2O$**		**-599.6**

	$CH_3COO^- + NO_3^- + H^+ + H_2O \rightarrow 2HCO_3^- + NH_4^+$	-511.4
Nitrate → Nitrogen gas:	$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$	-1120.5

* Adapted from reference (Harper and Pohland, 1986)

The activities of fatty acid-oxidizing bacteria are crucial for complete methanogenic fermentation, since low molecular weight fatty acids such as propionate and butyrate are common intermediate products and cannot be catabolized by methanogens. Butyrate catabolism by *Syntrophomonas wolfei* proceeds by β-oxidation with the release of acetate and hydrogen (McInerney *et al.,* 1981) or formate (Boone *et al.,*1989). Propionate oxidation is less well understood, but is accomplished by *Syntrophoacter wolinii* (Boone and Bryant, 1980) with the production of acetate and carbon dioxide, again with concomitant production of hydrogen of formate. These oxidation are thermodynamically possible only when the reduced product (hydrogen or formate) is maintained at low concentrations by the "scavenging" activities of the methanogens. The growth of the fatty acid-oxidizing bacteria in the consortia with methanogens is very slow and may limit methanogenesis in some systems. Accordingly, the accumulation of fatty acids, particularly propionic acid, is a common indicator of stress in anaerobic digestion systems.

3.4. Environmental factors

Besides the necessary of available substrate and viable microbial populations, the principal environmental factors affecting the rates of methanogenesis in anaerobic microbial conversion processes include pH, temperature, ionic strength or salinity, nutrients, and toxic or inhibitory substances.

3.4.1. pH

Most anaerobic conversion processes operate best at near neutral pH. Deviations from this optimum, if not introduced with the influent substrate, are usually consequenced by excess production and accumulation of acidic or basic conversion products such as organic fatty acids or ammonia, respectively. Moreover, the intensity of pH will affect the solubility and reaction behavior of other potentially influencing substances, including both organic and inorganic species.

Low pH and excessive acid production and accumulation, which displaces the more neutral pH bicarbonate buffer system (Pohland and Bloodgood, 1963, Pohland and Suidan, 1987), are considered conditions more inhibitory to methanogens than fermentative bacteria. These latter species can also continue to produce fatty acids, despite pH depression, thereby aggravating the environmental condition further. However, methanogenesis is known to occur in both acidic and alkaline environments, suggesting that methane production is not exclusively limited to a neutral pH. The effect is apparently manifested differently for the various anaerobic consortia, since *Methanosarcina barkeri* and *Methanosarcina vacuolata*, two well-known acetate-degrading methanogens, grow well at low pH with an optimum pH of 5 when cultured on hydrogen and methanol as the catabolic substrate (Maestrojuan and Boone, 1991). Similarly, hydrogen-oxidizing methanogens (Boone *at al.,* 1986) and

methylotrophic methanogens (Liu *et al.,*1990, Mathrani *et al.,*1988) have been found at very alkaline pH values, but no aceticlastic methanogens have been found (Boone, 1991). Therefore, it seems that some biochemical interactions and degradation pathways may be influenced by pH, including the possible inhibition of hydrogen production (Conrad *et al.,* 1987), which may then explain the lesser importance of hydrogen to methanogenic process applications at other than neutral pH have not been exhausted.

3.4.2. Temperature

As with most microbially mediated processes, methanogenesis has been shown to be strongly temperature-dependent, with reaction rates generally increasing with temperature up to 60^{o}C. Two optimal temperature ranges, mesophilic (near 35^{o}C) and thermophilic (55 to 60^{o}C), with decreased rates between these optima, have been often been cited. However, it has been suggested that low rates between these optima may have been due to a lack of adaptation (Macki and Bryant, 1981). With temperatures at or above 70^{o}C, methanogenic rates have been reported to decrease (Zinder *et al*., 1984), although a larger pool of substrate may be available for conversion when higher temperatures are present (Westrich and Berner, 1988). Moreover, when inhabited by complex microbial consortia, including sulfate and nitrate reducers, temperature influences may be more significant and advantageous to certain species, to the detriment of others.

3.4.3. Ionic strength and Salinity

As already indicated, sulfate exerts a significant control on the viability of methanogenesis in the presence of certain substrates, primarily because of the competition between sulfate-reducing bacteria (SRB) and methanogens. Salinity effects on methane fluxes have been examined in marine systems (Capone and Kiene, 1988, DeLaune *et al*., 1983, Bartlett *et al*., 1987), and fluxes have generally been greater in freshwater regions of marshes and estuaries. Salinities up to 0.2 M NaCl have been reported to have minimal effects on mixed methanogenic populations, but higher salinities are inhibitory (Boone, 1991). The total ionic strength would also affect chemical activity and, therefore, the possible effect of other chemical species in terms of inhibition.

3.4.4. Nutrients

In addition to the fundamental requirements for micronutrients such as carbon and nitrogen, the inability of many anaerobes to synthesize some essential vitamin or amino acid often necessitates supplementation of the culture medium with specific nutrients for growth and metabolism. Generally, the gross level of essential nutrients can be evaluated if the biomass yield is known, and the C:N ratio is frequently utilized to describe this micronutrient requirement. On occasion, this ratio will be affected by substrate specificity, but if measured as chemical oxygen demand (COD), COD:N ratios of about 400:7 and 1000:7

have been estimated as required at high and low substrates loadings, respectively (Henze and Harremoes, 1983). Similarly, a N:P ratio of approximately 7:1 has been reported as required (Stronach *et al.,*1986), although establishing specific nutrient requirements in mixed substrate and population systems can be elusive and better determined separately for each circumstance.

Other trace elements considered as necessary for various conditions of active methanogenesis include iron, nickel, magnesium, calcium, sodium, barium, tungsten, molybdate, selenium and cobalt. In the case of selenium, tungsten and nickel, these elements are implicated in the enzyme systems of acetogenic and methanogenic bacteria (Stronach *et al.*, 1986). For example, the formate dehydrogenase and hydrogenase of *Methanococcus vanielii*, the formate dehydrogenase of *Clostridium thermoaceticum*, and the hydrogenase of *Desulfovibrio desulfuricans* require the presence of selenium, tungsten and nickel respectively. Normally, mixed substrate systems, particularly those involving waste discharges, have an abundance of essential nutrients, unless the waste is from a process that disallows such introduction.

3.4.5. Toxicity and inhibition

Toxicity or inhibition of methanogenic processes can be consequenced by a variety of circumstances, including the generation of intermediary products such as the volatile fatty acids, which may also manifest an adverse pH effect. Whereas methanogenic microbial growth has been often shown to be restricted in the presence of excessive amounts of VFAs, particularly when propionate accumulates, sudden increases in concentration of either acetate or butyric have also exhibited stimulation of the process (Iannotti and Fischer, 1984).

In terms of the volatile fatty acids, the effects manifested are often related to other environmental conditions, particularly pH and buffer capacity (alkalinity) as originally introduced in terms of “volatile acid alkalinity” (Pohland and Bloodgood, 1963) and later operationally modified (Pohland and Suidan, 1987). Therefore, the overall inhibitory effect of the volatile fatty acids is related to the pH established by the prevailing buffer system, and may involve elevation of the concentration of unionized or un-dissociated species, with a greater internal cellular effect as they more readily migrate across the bacterial cell membrane (Pohland and Martin, 1969). Accordingly, volatile fatty acids may accumulate due to other stresses mentioned previously and can thereby not only function as weak acid buffers to lower the pH, but can then exert an inhibitory effect with pH on the microbial consortia present.

As with the volatile fatty acids, hydrogen sulfide and ammonia, such as from reduction of sulfate and nitrate by sulfate- and nitrogen-reducing bacteria, are also capable of forming weak acid and weak base buffer systems (see Figure 2). Although these systems are usually less intensive and less likely to exert

principal control on the pH, unless the influent substrate contains high levels, both sulfide and ammonia have been implicated in exerting toxic effects on methanogenesis. Here again, the concentration of sulfide or ammonium species present would be pH-dependent, and the former could be rendered insoluble by association with other cations.

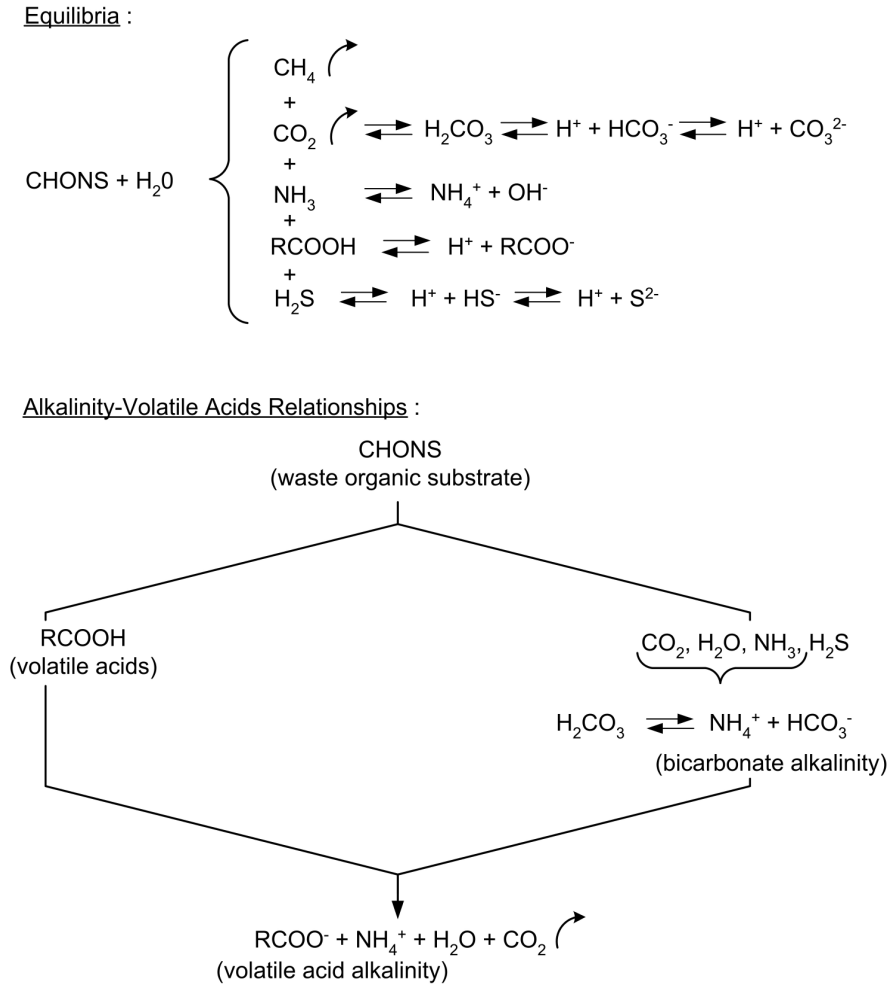

Figure 2. Major acid-base equilibria and their influence on buffer capacity during anaerobic microbial conversion of waste organic substrates (Pohland and Suidan, 1987)

Hence, sulfide precipitates can form, as is the case with iron sulfide, thereby effectively eliminating potential toxic effects of either the precipitate metal or the uncomplexed sulfide. Similarly, at elevated pH levels, free ammonia might exist at concentrations sufficient to exert a toxic or inhibitory effect. In either case, whether or not such inhibition occurs is again dependent on other

environmental factors and the ability of the microbial consortia to accumulate or adapt to the imposed stress.

Sulfide toxicity has been oserved at concentrations ranging from 200 to 1500 mg L^{-1} (Stronach *et al.,* 1986) until acclimation occurred or the sulfide concentrations could be reduced by precipitation or release into the gas phase. Therefore, the potential toxic effects of sulfide, normally present in solution as a weak acid, would be a function of pH as well as the presence of precipitants such as most of the heavy metals. Similarly, the associated heavy metal toxicity would also be mediated by the presence of sulfides and the propensity to contribute to precipitation of the sparingly soluble metal sulfides to be discussed subsequently.

In the case of the weak base, ammonia, microbial acclimation is particularly important and is often linked to the presence of volatile fatty acids and the effect of the acid-neutralization capacity of ammonia on pH. Hence, the "inhibitory concentration" of ammonia may vary depending on other environmental factors and the type of exposure expressed on the more sensitive methanogenic populations, with free ammonia being generally considered more toxic than the ionized ammonium species. This may account for the difference between observed results and the potential for reversibility of toxic effects even at short exposures with high ammonia nitrogen loadings. The methanogen, *Methanobacterium formicium* has been reported to be partially inhibited at a total ammonia concentration of 3000 mg L^{-1} and a pH of 7.1, whereas 4000 mg L^{-1} caused complete inhibition (Stronach *et al.,* 1986). In contrast, non-methanogenic populations have been reported to be functional at ammonia concentrations in excess of 6000 mg L^{-1} and at a pH of 8 (Cross *et al*., 1983).

Heavy metal toxicity has been often implicated as acause for failure of anaerobic microbial conversion processes, as influenced by oxidation-reduction potential (ORP), pH and ionic strength and the resultant speciation of the metals or metal complexes. It is generally accepted that free metals exert a toxic threshold, above which inhibition or failure of the process occurs. The difficulty is establishing that threshold in recognition of the moderating effects that can be imposed by the availability of complexes or precipitants such as sulfides. Moreover, the reducing potential (negative ORP) present in anaerobic methanogenic systems can alter the valence of some of the metals from a more oxidized to a more reduced state (e.g., iron and copper), thus affecting the potential requirements for detoxification.

Often associated with heavy metals as a consequence of source, cyanide can also adversely affect anaerobic conversion processes, depending on concentration and exposure time (Stronach *et al*., 1986). However, rapid acclimation is known to be possible, even under the stress expressed by multiple toxic effects of the other system components (Cross *et al.,* 1983). It has also

been reported that whereas cyanide prevents methane formation from acetate, it does not prevent formation from either carbon dioxide or methanol in cultures of *Methanosarcina barkeri*, a methanogen whose carbon monoxide dehydrogenase enzyme is inhibited by cyanide (Stronach *et al*., 1986).

Besides the effects of inorganic heavy metals and cyanide, anaerobic microbial conversion processes are known to be adversely affected by a vast array of anthropogenic and recalcitrant compounds, some of which may be ultimately susceptible to bioconversation if appropriate conditions of acclimation and process selection are provided. Notwithstanding the generally accepted notion that these compounds persist unchanged for extended periods of time, considerable evidence is appearing that suggests potential for microbial conversion of many of the compounds heretofore categorized as recalcitrant.

3.5. Anaerobic treatment of sulfate-rich wastewaters

3.5.1. Sulfate reduction by sulfate reducing bacteria

The reducing conditions prevailing in anaerobic processes of sulfate rich wastewaters result in the increase of hydrogen sulfide content. Although part of the sulfides originates from the sulfur containing amino acids, probably most of them are formed during the reduction of sulfates present in the raw wastewater. The main reactions of sulfate reduction performed by sulfate reducing bacteria (SRB) are the following reaction (Widdel *et al.*, 1991, Menert, 2001):

$$2\ \text{lactate}^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ \text{acetate}^- + 2\ CO_2 + 2\ H_2O + HS^- \qquad (1)$$
$$\Delta G'_0 = -74.5\ \text{kJ/ 2 lactate}$$

$$\text{acetate}^- + SO_4^{2-} + 3\ H^+ \rightarrow 2CO_2 + H_2S + 2\ H_2O \quad \Delta G'_0 = -57\ \text{kJ/ reaction} \qquad (2)$$

By generation times SRB can be classified as fast growers ($\tau \approx 3$ h) represented by the genera *Desulfovibrio, Desulfomicrobium, Desulfobotulus, Desulfobulbus, Desulfotomaculum* and *Thermodesulfobacterium*. They utilize H_2, formate, lactate, ethanol, pyruvate, and convert it to acetate, which accumulates. Slow growers with $\tau \approx 15$ h are represented by the genera *Desulfobacter, Desulfobacterium, Desulfococcus, Desulfosarcina, Desulfonema* and some species of *Desulfotomaculum*. They utilize acetate, lactate, pyruvate, fatty acids, fumarate, malate, formate and H_2 and perform their complete oxidation to CO_2, H_2O and H_2S.

Sulfate metabolism comprises several individual stages (Menert, 2001).
1. Sulfate transport that is accomplished by active symport with $3H^+$ or Na^+ *(Desulfococcus).* Strong inhibitors of transport are molybdate, chromate and selenate.

2. Activation of sulfate by ATP sulfurylase.

$$SO_4^{2-} + 2\ H^+\ ATP \rightarrow APS\ [AMP\text{-}SO_4] + PPi \qquad \Delta G'_0 = +46\ kJ \quad (3)$$

This is the first reaction in the reductive assimilation of sulfate for biosynthesis:

$$ATP + SO_4^{2-} \rightarrow\rightarrow\rightarrow\rightarrow HS^- \quad (4)$$

$$HS^- + serine \rightarrow cysteine + H_2O \quad (5)$$

as well as in dissimilative reduction (reactions 1, 2 and 8). APS is referred to as adenosylsulfate or adenosyl phosphosulfonate or adenosine-5'-phosphosulfate. In the same way molybdate and selenate can be converted by ATP sulfurylase into adenosine phospho-molybdate and adenosine.phosphoselenate leading thus to the toxic effects.

3. Formation of bisulfite:

$$APS\ [AMP\text{-}SO_4] \xrightarrow{\text{APS reductase [4Fe4S], } FADH_2} HSO_3^- + AMP + H^+ \quad \Delta G'_0 = -69\ kJ \quad (6)$$

4. Reduction of bisulfite:

$$HSO_3^- \xrightarrow[(6e^-)]{3\ H_2} HS^- + 3\ H_2O \qquad \Delta G'_0 = -171\ kJ \quad (7)$$

Bisulfite reductase is a $\alpha 2\beta 2$ (167 - 215 kDa) protein containing desulfoviridin, desulforubidin or desulfofuscidin. The actual reaction route to sulfide is still in doubt and may involve either direct reduction or participation of intermediates such as trithionate (S_3O_6) or thiosulfite (S_2O_3). From the point of view of energetics two high-energy bonds were used to activate sulfate and two ATPs per mole of lactate oxidized to acetate are generated by substrate level phosphorylation. Acetotrophic SRB obtain ATP via citric acid cycle at the level of citrate lyase (Widdel and Hansen, 1991).

Both fast growing (e.g. *Desulfovibrio*) and slow growing (e.g. *Desulfococcus*) sulfate reducing bacteria are able to partially oxidize some organic compounds into acetate and CO_2 that in turn can be metabolized by methane producing bacteria or the SRB themselves (Widdel and Hansen, 1991, Traore *et al.,*1983, Menert, 2001). Interactions between these two groups of microorganisms can be very diverse - trophic complementary, inhibition, etc. Of particular importance

is the competitive relationship towards the use of hydrogen since it can be the energy source for the both groups of bacteria.

3.5.2. Alternative electron acceptors for sulfate reducing bacteria

Some colourless sulfur bacteria (*Thiobacillus denitrificans*) use sulfur compounds such as thiosulfate, elemental sulfur and sulfide as electron donors, and in the absence of oxygen they use nitrate as electron acceptor (Montgomery *et al*., 1990). A sulfide resistent strain of *T. denitrificans* was successfully grown in a coculture with the sulfate reducing bacteria *Desulfovibrio desulfuricans* without accumulation of sulfide (Montgomery *et al*., 1990). As *T. denitrificans* strain F is a chemoautotrophic bacterium, no additional nutrients are needed to support its growth. For long ago it has been noticed that addition of nitrate inhibits sulfide production in many environments. Environments such as sewage digester sludge contain large populations of denitrifiers that can use the sulfide produced by sulfate reducing bacteria, or compete for electron donors required for sulfate reduction. The first explanation to this was that products of NO_3^- reduction (NO, N_2O) might inhibit the growth of SRB (Montgomery *et al*., 1990).

The common understanding is that the order of electron acceptor utilization by SRB is $O_2 > H^+ > NO_3^- > Mn > Fe^{3+} > SO_4^{2-}$, followed by methanogenesis as dictated by thermodynamics (Mathews *et al.*, 1995, Kennedy *et al.*, 2001). Though microbial O_2 and NO_3^- respiration are thermodynamically more favoured, concentrations of these electron acceptors in ground water are usually small, and thus not available to SRB. In addition to that, O_2 solubility in water is limited and its addition can result in fouling of injection equipment of treatment plants. Though nitrate is soluble, its concentration in drinking water is restricted by regulation to 10 mg L^{-1} (as N) (Kennedy *et al.*, 2001). On the other hand, comparatively large amounts of Fe^{3+} (> 1000 mg kg^{-1}) and dissolved SO_4^{2-} (50 - 1000 mg L^{-1}) can occur naturally and have been used as electron acceptors to stimulate bioremediation of organic contaminants in landfills (Kennedy *et al.*, 2001).

The use of H^+ as electron acceptor is limited by excess production of H_2 that is inhibitory to both methanogenic bacteria (MB) and sulfate reducing bacteria (SRB). The obligate proton reducing acetogenic bacteria or obligate hydrogen producing anaerobes (OHPA) include those that beta-oxidize short-chain fatty acids, decarboxylate propionate and oxidize ethanol and similar alcohols. Their growth occurs only in the presence of either a methanogen or any other microorganism using hydrogen. H_2 is a stringent feedback inhibitor of hydrogenase and prevents the conversion of NADH to NAD^+, which is essential for the growth of these bacteria. Some species of *Desulfovibrio* utilize both sulfate and H^+ as electron acceptors and produce H_2 if grown in syntrophic

association with an H_2 utilizing methanogen (McInerney *et al*., 1981, Traore *et al*., 1981):

$$\text{lactate}^- + 0.37\ SO_4^{2-} + 0.56\ H^+ \rightarrow$$
$$CO_2 + 0.98\ \text{acetate}^- + 0.02\ \text{ethanol} + 0.16\ H_2S + 0.215\ HS^- + 0.5\ H_2O + 0.48\ H_2 \quad (8)$$
$$\Delta H_{met} = -36.6\ \text{kJ mol}^{-1}$$

Sulfate reducing bacteria which contain hydrogenase (hydrogen: ferricytochrome c_3 oxidoreductase EC. 1.12.2.1) and cytochrome c_3 can either produce or consume molecular hydrogen. H_2 is consumed if they grow by anaerobic oxidation of hydrogen with concomitant reduction of sulfate (Traore *et al*., 1981, Oude Elferink *et al*., 1998):

$$H_2 + 0.25\ SO_4^{2-} + 0.25\ H^+ \rightarrow 0.25\ HS^- + H_2O \quad (9)$$

H_2 is produced by some species of *Desulfovibrio* during growth on pyruvate (Traore *et al*., 1983, Traore *et al*., 1981) or any other volatile fatty acid medium, lacking sulfate (Oude Elferink *et al*., 1998):

$$\text{pyruvate}^- + H_2O \rightarrow \text{acetate}^- + CO_2 + H_2 \qquad \Delta H_{cat} = -1.64\ \text{kJ mol}^{-1} \quad (10)$$
$$\text{lactate}^- + H_2O \rightarrow \text{acetate}^- + CO_2 + 2\ H_2 \qquad \Delta H_{cat} = +73.30\ \text{kJ mol}^{-1} \quad (11)$$
$$\text{propionate}^- + 3H_2O \rightarrow \text{acetate} + HCO_3^- + H^+ + 3\ H_2 \quad (12)$$
$$\text{butyrate}^- + 2\ H_2O \rightarrow 2\ \text{acetate}^- + H^+ + 2\ H_2 \quad (13)$$

Already at low concentrations (1 mM) sulfate turned out to be a better electron acceptor than methanogen *Methanosarcina barkeri* or H^+ for SRB *Desulfovibrio vulgaris* Hildenborough (lactate and pyruvate as electron donors) (Traore *et al*., 1983). Molecular hydrogen was produced by the bacteria in a relatively high proportion at high (36 mM) sulfate concentrations (0.5 mol H_2 per mol of lactate as the energy source (Eq. 11)) (Traore *et al*., 1981). This H_2 production was interpreted as a device to minimize H_2S production in the culture media. H_2 production seemed to precede sulfate reduction since a significant amount of H_2 was accumulated in the culture medium before H_2S could be detected (Traore *et al*., 1981). The greater "lack" of energy observed during the growth on lactate (ΔH_{met}= -36.36 kJ mol^{-1}) as compared to that on pyruvate (ΔH_{met} = -70.22 kJ mol^{-1}) could be attributed to the greater amount of H_2 produced by the former substrate. In natural conditions the loss of energy induced by H_2 production is counterbalanced by utilization of H_2 and CO_2 from SRB by MB. Hydrogen accumulation is inhibitory because of thermodynamic reasons - H^0_f = -4.19 kJ mol^{-1} at t = 25°C (Brown, 1969).

In the absence of sulfate, *Desulfovibrio* species degraded little lactate to acetate, CO_2 and H_2, due to the relatively positive change in free energy, unless H_2 using bacteria (H_2-using methanogens) were present to maintain low concentration of H_2 (McInerney *et al*., 1981).

3.5.3. Methods for avoiding the inhibitory effect of sulfides

Anaerobic treatment of sulfate rich wastewaters, e.g. from food industry, fermentation industry, paper and pulp industry etc. may be accompanied by production of sulfides (Buismann *et al*., 1990). In addition to their toxicity and corrosive properties, sulfides have also inhibitory effect on methanogenesis. To prevent or decrease the sulfide inhibition in anaerobic reactors, different process schemes have been proposed to integrate sulfate reduction, methanogenesis and sulfide removal in order to achieve the removal of both organic matter and sulfurous compounds. More information in (Colleran *et al*., 1995).

3.5.3.1. Physicochemical sulfide removal

Physicochemical oxidation processes for sulfide removal are follows (Lens *et al*., 2000):

- Electrochemical oxidation,
- Chlorination, ozonation,
- Potassium permanganate ($KMnO_4$), or hydrogen peroxide (H_2O_2) treatment.

In all these oxidation processes, elemental sulfur, thiosulfate and sulfate are the final products in a varying ratio depending on the pH. The sulfide removal process can be placed after the anaerobic step, an option that implies installation of an extra unit in the treatment system.

Other nowadays methods commonly used to remove sulfide from the wastewater are:

- Dilution of the influent – in some cases sulfide concentration can be reduced below inhibitory level in anaerobic reactors by diluting the influent, for example, with sulfate-free potable water. Utilization of unpolluted water to meet this target is not preferred (Lens *et al*., 2000).
- Sulfide stripping – sulfide can be stripped directly from anaerobic reactor. Sulfide can be stripped from liquid in the ANTRIC filter by gas that is passed through a recirculation system in which the H_2S in the gas had beeb removed in a scrubber. Alternatively, sulfide can be stripped in a separate stripping column with either biogas or a stripping gas (N_2) from which the effluent is recycled to the anaerobic reactor (Särner, 1990).
- The reactor pH – operating the reactor at an elevated pH it is possible to reduce the H_2S concentration. The pH reducing (>7.5) will lead to the

decreasing of the undissociated H_2S in liquid which decreases its toxicity.

- The temperature control. Sulfide level is normally inhibitory to anaerobic organisms at levels of above 100 mg L^{-1} (HS^-) and completely inhibits at levels above 200 mg L^{-1} (HS^-). The associated form (H_2S) is thought to be inhibitory agent at 50-100 mg L^{-1} H_2S-S. H_2S-HS^- is in equilibrium and H_2S solubility is strongly affected by temperature. Higher temperatures decrease H_2S solubility and equilibria pK ad each degree of temperature change around 35^0C changes effective H_2S concentration by approximately 2%. Therefore, operating at higher temperatures is often a sulfide control strategy. More information is in (Speece, 1996).
- Precipitation of sulfide. This method includes dosing with metal salts to anaerobic digester (especially Fe^{2+} and Fe^{3+}).

3.5.3.2 Biological sulfide removal

Biotechnological processes for sulfide removal consist in conversion of sulfide into elemental sulfur by colourless sulfur bacteria (*Thiobacilli*) (Buisman *et al*., 1990, Janssen *et al*., 1997, Janssen *et al*., 1999, Menert, 2001) according to the reaction (14):

$$2\ HS^- + O_2 \rightarrow 2\ S^0 + 2\ OH^- \qquad \Delta G_0 = -169.35\ \text{kJ mol}^{-1} \quad (14)$$

or by genera of anaerobic photosynthetic bacteria from the families *Chlorobiaceae* and *Chromaticeae* that catalyze the photosynthetic van Niel reaction (Cork, 1985, Henshaw *et al*., 1998):

$$2n\ H_2S + n\ CO_2 \xrightarrow{n\nu} 2n\ S^0 + (CH_2O)_n + n\ H_2O \quad (15)$$

In the latter case light radiated to a photosynthetic reactor is coupled to the conversion of sulfide to elemental sulfur using the reverse citric acid cycle (Arnon cycle). According to experimental results, photoautotrophic bacteria produce a higher percentage conversion of S^{2-} to S^0 as compared to chemoautotrophic bacteria. There is also a thermodynamic advantage of using electrons from S^{2-} in comparison to those from S^0 or $S_2O_3^{2-}$: energy change per mole of electrons released from the oxidation of S^{2-} to S^0 is the highest (-26 kJ $mole^{-1}$) as compared to other sulfur species (Henshaw *et al*., 1998). The greatest drawbacks of the system with the phostosynthetic bacteria remain its low cost effectiveness due to the use of a light source and use of suspended-growth reactors to ensure the transparency of medium.

For the growth of colourless sulfur bacteria producing S^o (chemoautotrophs, see above), it is important for microaerophilic conditions to prevail, e.g. in the presence of sulfide concentrations up to 80 mg L^{-1} only less than 10% sulfate

was produced if O_2 concentration remained below 6 mg L^{-1}. Sulfide was concluded to be inhibitory to sulfate producing microorganisms or a more preferred electron donor than sulfur (Buisman *et al*., 1990). Later Janssen (Janssen *et al*., 1997) found the optimal concentration of oxygen to be 0.1 mg L^{-1}, guaranteeing the 92% conversion of sulfide into elemental sulfur. The optimal molar ratio of oxygen to sulfide consumption was 0.7 (autotrophic conditions) and 1.0 - 1.6 (heterotrophic conditions).

3.6. Characteristics of baker's yeast wastewater

Yeast production wastewater is a complex mixture. Most of the contaminants in the wastewater are due to the use of molasses as a main raw material. As a by-product of sugar manufacturing, molasses has 45 to 50% of residual sugars, 15 to 20% of non-sugar organic substances, 10 to 15% of ash (minerals) and about 20% of water. During yeast fermentation process, the sugars contained in the molasses were utilized as carbon and energy source. The major part of the non-sugar substances in the molasses (molasses residuals), however, is not assimilable by the yeast and releases unchanged to the processing wastewater, which represents the principal waste in the yeast production process. Besides molasses residuals, yeast production wastewater also contains chemicals added during fermentation (e.g. various salts, antifoams, propionic acids, brine, etc), yeast metabolites and residual yeast cells.

Depending on type of yeast fermented (commercial baker's yeast, seed yeast or yeast for special products), the yeast fermentation wastewaters are characterized by high content of COD (10000-80000 mg L^{-1}), strong nitrogenous (1500-2500 mg L^{-1} total N), sulfate-rich (2000-10000 mg L^{-1}), phosphorus (30-60 mg L^{-1}), recalcitrant for biodegradation and highly coloured (melanoidins etc.) substances.

3.6.1. Beet molasses – main raw material for baker's yeast fermentation

The principal raw materials used in producing baker's yeast are the pure yeast culture and molasses. The yeast strain used in producing compressed yeast is *Saccharomyces cerevisiae.* Other yeast strains are required to produce dry yeast products. Several types of dry yeast are produced, including active dry yeast (ADY) and instant dry yeast (IDY). Instant dry yeast is produced from a faster-reacting yeast strain than that used for ADY. The main difference between ADY and IDY is that ADY has to be dissolved in warm water before usage, but IDY does not. Another product is inactive dry yeast. This is yeast which has been inactivated and dried. It should have no diastase activity.

Cane molasses and beet molasses are the principal carbon sources to promote yeast growth. Molasses contains 45 to 55 % fermentable sugars, in the forms of sucrose, glucose, and fructose.

The amount and type of used cane and beet molasses depend on the availability of the molasses types, costs, and the presence of inhibitors and toxins. Usually, a blend consisting of both cane and beet molasses is used in the fermentations. Once the molasses mixture is blended, the pH is adjusted to between 4.5 and 5.0 because more alkaline mixture promotes bacteria growth. As a rule concentrated sulfuric acid is utilized for pH adjustment because of price, hadling simplity and availability. Bacteria growth occurs under the same conditions as yeast growth, making pH monitoring very important. The molasses mixture is clarified to remove any sludge and is then sterilized with high-pressure steam. After sterilization it is diluted with water and held in holding tanks until it is needed for the fermentation process.

A variety of essential nutrients and vitamins is also required for yeast production. The nutrient and mineral requirements include nitrogen, potassium, phosphate, magnesium, and calcium, with traces of iron, zinc, copper, manganese, and molybdenum. Normally, nitrogen is supplied by adding ammonium salts, aqueous ammonia, or anhydrous ammonia to the feedstock. Phosphates and magnesium are added, in form of phosphoric acid or phosphate salts and magnesium salts. Vitamins are also required for yeast growth (biotin, inositol, pantothenic acid, and thiamine). Thiamine is added to the feedstock. Most of the other vitamins and nutrients are already present in sufficient amounts in the molasses. The composition of molasses is shown in Table 3.

Table 3. An average composition of beet and cane molasses at 75% dry matter (Olbrich, 1973)

Constituent	Beet molasses	Cane molasses
Total sugar (%)	48-52	48-56
Non-sugar organic matter (%)	12-17	9-12
Protein (Nx6.25)(%)	6-10	2-4
Potassium (%)	2.0-7.0	1.5-5.0
Calcium (%)	0.1-0.5	0.4-0.8
Magnesium (%)	ca. 0.09	0.06
Phosphorus (%)	0.02-0.07	0.6-2.0
Biotin (%)	0.02-0.15	1.0-3.0
Pantothenic acid (mg kg^{-1})	50-100	15-55
Inositol (mg kg^{-1})	5000-8000	2500-6000
Thiamine (mg kg^{-1})	ca. 1.3	1.8

3.6.2. Beet molasses components in wastewater

During yeast fermentation process, the sugars contained in the molasses were utilized as carbon and energy source. The major part of the non-sugar substances in the molasses (molasses residuals), however, is not assimilable by the yeast and releases unchanged to the processing wastewater, which represents the principal waste in the yeast production process.

3.6.2.1. Betaine and its behavior in anaerobic treatment processes

Betaine ($(CH_3)_3N^+CH_2COO^-$), also known as N,N,N-trimethyl glycine, is one of the main soluble nitrogenous compounds in sugar-beet. Sugar-beet molasses used as growth medium for yeast contains large amounts of trimethylglycine or betaine (up to 6% DS) (Thalasso *et al*., 1999). This highly water-soluble compound (maximum solubility of 1600 g dm^{-3} of water) is extracted with sucrose from sugar-beet pulp and is carried through the subsequent processing stages into the molasses fraction.

Betaine is not consumed to any signifcant extent during baker's yeast fermentation and appears to largely pass through the subsequent processing stages, becoming a signifcant constituent of wastewaters produced by yeast industry processing beet molasses. The presence of betaine in wastewaters from fermentation plants using sugar-beet molasses as substrate has been confrmed by Derycke et al. (Derycke *et al*., 1993).

In anaerobic treatment plants, trimethylglycine can be almost totally degraded by a multi-step process with nitrogen-containing intermediates trimethylamine and other methylated amines, which are further degraded by methanogens, yielding CO_2, ammonium and methane (Thalasso *et al*., 1999). The ammonium formed buffers the treatment system and enables its stable work. Cleavage of trimethylglycine into trimethylamine and acetate is characteristic of some halophilic fermentative bacteria (Moune *et al*., 1999).

$$2.5 \text{ trimethylglycine} + 4.04\ H_2 \rightarrow 2\text{-propanol} + 2.5 \text{ trimethylamine} + 0.95 \text{ acetate} + 0.1\ CO_2 + 1.9\ H_2O \quad (16)$$

$$\text{trimethylglycine} + 1.32 \text{ serine} + H_2O \rightarrow \text{trimethylamine} + 2 \text{ acetate} + 1.32\ CO_2 + 1.32\ NH_3 \quad (17)$$

The similar cleavage mechanism for trimethylglycine under anaerobic conditions has also been reported for *Clostridium sporogenes* (Naumann *et al*., 1983, Zumbusch *et al*., 1994) while the fermentation products of *Eubacterium limosum* are *N,N*-dimethylglycine, acetic acid and butyric acid (Müller *et al*., 1981, Tchobanoglous *et al*., 1991). 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 (Sax and Lusk, 1995):

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (18)$$

$$4\,(CH_3)_3N + 12\,H_2O \rightarrow 9\,CH_4 + 3\,CO_2 + 6\,H_2O + 4\,NH_3 \quad (19)$$

Since betaine is not detected by COD dichromate assay its concentration can be underestimated that leads to significant overloading of WWTPs. Furthermore, betaine is a nitrogenous compound, its complete anaerobic degradation can result in the increase of effluent ammonia concentration. This will raise the risk of ammonia inhibition of the anaerobic stage by free ammonia (Thalasso *et al*., 1999, Menert, 2001).

Considering that betaine is not present at significant concentrations in sugar-cane molasses, these potential problems do not apply to wastewaters plants treating effluents from sugar-cane molasses fermentation plants.

3.6.2.2. Colour substances

Sugar beets contain no colouring materials, but they do contain colour-forming substances. Sugar and nonsugars participate in the production of colour (caramel substances, melanoidines) or nonsugars as such (phenol-iron complexes, melanins) are responsible. The degree of discoloration is related primarily to pH and temperature. The discoloration in sugar juices increases threefold for each 10°C rise in temperature of processing. Colouring matter is formed not only when bases or acids react, but also, to some extent, from decomposition of saccharose. These mixtures of colour-conferring materials are referred to in the literature under various names, such as caramelan, caramelene, carameline, saccharan and fuscazinic acid. In addition, furfural derivatives are formed simultaneously together with volatile compounds (aldehydes, such as acrolein) and also carbon dioxide.

The colouring matters that appear in the course of sugar manufacture can be divided into the following groups (Olbrich, 1973):

A) ***Caramel materials***. These substances are the results of thermal decomposition (including loss of water) of saccharose; they contain no nitrogen. At constant pH the formation of caramel is directly proportional to the effective temperature.
B) ***Polyphenol-iron complexes***. Pyrocatechol (plant pigment), which occurs in the epidermis and the head of beets (in amounts around 0.02%) leads to a yellow-greenish discoloration of the sugar juices resulting from the formation of a pyrocatechol-iron complex. This is not entirely removed during the defecation of the juice and can be found in molasses.

C) ***Melanoidines.*** Melanoidines are high molecular weight polymers. The formation of melanoidins comprises a set of consecutive and parallel chemical reactions taking place between amino compounds and carbohydrates during a Maillard reaction (Cämmerer and Kroh, 1995).

D) ***Melanins***. Beet tyrosinase, which belongs to the polyphenol-oxidases, contains copper in its active group. On access of air it introduces the oxidation of various aromatic compounds (pyrocatechol, tyrosine) and products blackish-grey discolorations. This reaction, known as melanin-formation, requires only the enzymatically catalyzed oxidation for its initiation and then proceeds as a chain reaction passing through red and red-brown intermediate stages to orthoquinone-like compounds. Since this discoloration can be removed almost completely in the predefecation of beet-sugar juice, melanins seldom appear in molasses.

Phenolic compounds responsible for beet molasses wastewater color are partly removed (63% removal) during aerobic-anoxic treatment process, but color removal accounted for only 8-23% (Kalyuzhnyi *et al*., 2005). This is in accordance with other literature data (Francisca Kalavathi *et al*., 2001) that the visible color is mainly asociated with other substances than phenolic compounds namely, with persistent to biodegradation melanoidins. Conventional anaerobic-aerobic treatment processes can accomplish the degradation of melanoidins only up to 6% or 7% (Gonzàlez *et al*., 1999, Guimaraes *et al*., 1999). Therefore, it is necessary to study additional treatments to remove color from molasses effluents and prevent the serious environmental problems that colored wastewaters can promote in river courses such as the reduction of both photosynthetic activity and dissolved oxygen concentration.

Melanoidins can be removed by physico-chemical treatments. These methods require high reagent dosages and generate a large amount of sludge (Gonzàlez *et al*., 1999). It was reported (Gladchenko *et al*., 2004) that application of iron and aluminium coagulation for molasses wastewater post-treatment of biofilter effluents showed that color, COD, nitrogen and phosphate decreased with incresing acting Fe and Al concentrations and the discharge limits were already archievable under iron concentrations around 200 mg L^{-1} and auminium around 540 mg L^{-1}.

Biological treatments with certain bacteria and fungi have also been applied, leading to lower color removal efficiency (Miyata *et al*., 2000). Many reseachers have tried to isolate microorganisms, which have the ability to decolorize melanoidins. Melanoidins have antioxidant properties and are toxic to many microorganisms in wastewater treatment (Frankel *et al*., 1978). It has been reported, however, that basidiomycetes including *Corius* sp. No.20 (Watanabe *et al*., 1982), dueteromycestes including *Aspergillus fumigatus* G-2-6 (Ohmomo *et al*., 1987), *Aspergillus oryzae* Y-2-32 (Ohmomo *et al*., 1988 b)

and bacteria including *Lactobacillus hilgardi* (Ohmomo *et al*., 1988 a) showed melanoidin-decolorization activity. The potential of these microorganisms to remove melanoidin from molasses wastewater are clear, but their actual use for molasses wastewater treatment processes might be difficult from the viewpoints of the stability and maintanace of color removal activity. Operation of proposed biological process will be difficult due to contamination with competetive microorganisms.

Ozone has been used in many countries for the treatment of drinking water. Ozonation processes are particularly attractive because ozone can destroy hazardous organic contaminants. In the last years ozone has been applied successfully to the treatment of specific compounds such as dyes, phenolic compounds, pesticides and organochlorides (Wu and Masten, 2002, Kamenev, 2003). Neverless, the oxidation of molasses wastewater with ozone is rather limited to few investigations. In these studies synthetic solutions or very diluted wastewater were oxidized with ozone (Kim *et al*., 1985, Gehringer *et al*., 1997).

Currently many yeast factories are faced with heavy trade-effluent charges. Land disposal options generate problems with ground water pollution and are prohibited in majority of the Europian regions. Many local municipal sewage treatment plants are now insisting on pre-treatment of such effluents before discharge into their sewerage.

4. EXPERIMENTAL STUDY

The experimental work was conducted using arrangements and procedures typical for anaerobic wastewater treatment studies.

4.1. Materials and methods

4.1.1. Experimental methods and procedure

Purification of wastewater was studied according to the following procedure. First, the anaerobic/anoxic treatment scheme was studied at full-scale equipment decribed below. Next, the lab-scale anaerobic Sequence Batch Reactor various schemes were tested. Lastly, post-ozonation and coagulation step of biologically treated wastewater was conducted.

4.1.1.1. Full-scale wastewater treatment equipment (PAPERS I and V)

The original treatment facility of OY Tampella AB (Finland) consisted of anaerobic pre-treatment stage (stainless steel mixing tank of 180 m³ with a stirrer and two up-flow anaerobic sludge blanket (UASB) reactors each of 180 m³ volume), followed by an aerobic stage (activated sludge with a 300 m³aeration tank) and a secondary sedimentation tank (45 m³) for final treatment before discharge.

At the beginning of present investigation new technological scheme was introduced which differed from the originally designed set-up mainly in the following (Figure 3):

- The reactors 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^{-1} with an on-line oxygen analyzer Marvet OxyMat 99-1.
- The temperature was automatically controlled at +35±2°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.
- Part of the anoxic stage sludge was re-circulated back to the inlet, i.e. to the mixing tank (acidification stage)
- Before disposal, anoxic effluent was treated by an aerobic sequencing batch reactor (SBR).

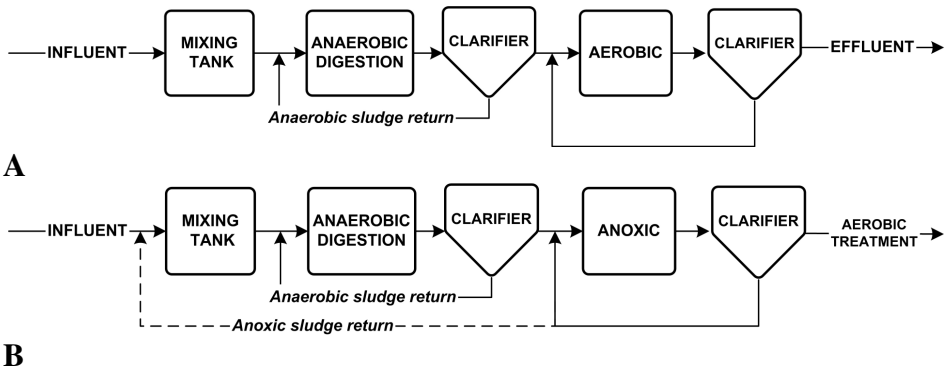

Figure 3. Initial and modified treatment schemes. **A** - original scheme for baker's yeast wastewater treatment (OY Tampella AB, Finland), **B** - modified anaerobic/anoxic schme proposed and adapted by author at the beginning of experiments

Wastewater from the yeast production plant processing beet sugar molasses was divided into high strength wastewater and wash water. High strength wastewater was pumped to mixing tank and wash water was sent directly to anoxic stage. The anaerobic reactors were fed with mixture of high strength wastewater and recycled anoxic sludge. The temperature of wastewater incoming from yeast production was 28-33 ^{o}C. Flow rate of incoming wastewater was measured by Baily Fisher Porter MAG-XM (CM) flow meters. Reactors feed and internal recycling flow rates measurements were conducted using Danfoss MAG1000/1100 electromagnetic flow meters.

4.1.1.2. Lab-scale wastewater treatment equipment (PAPER II)

Three different schemes of laboratory-scale experimental set-ups of ASBR (Anaerobic Sequence Batch Reactor) were used.

In the first experimental set-up stand-alone ASBR was used (Figure 4). ASBR with an active liquid volume of 0.7 L was made of glass tubing 0,145m × 0,075m (diameter). Plastic tubes were attached to the filling and drawing port. Peristaltic inflow pumps (Zalimp, Poland) were used at rates 0,51-0,48 L h^{-1} to fill the reactor and draw off the effluent, and to mix the suspension during the treatment process. The constant temperature during the operation was maintained by thermostat (35°±2°C).

In the second scheme ASBR was loaded with polymeric filler (Water group, Germany): 0.8cm x 1.0cm diameter, with a conditional surface area of 640 m^2m^{-3}. The volume of carriers was 0.5 L. Otherwise the experimental set-up was as in the first case.

In the third set-up, coupled sequence batch reactor (CSBR), where anaerobic effluent from the ASBR was recycled through a microaerophilic system (Figure5). Mixing in microaerophilic reactor was carried out using a magnetic stirrer with regulated stirring speed (Beco, MM-5, 220W). Biogas from anaerobic reactor was passed to the microaerophilic reactor with recycling effluent. Anoxic reactor was open and the temperature of the water was same as a temperature of air in the room (20±2°C). The oxygen concentration was kept on the level 0.1-0.15 mgL^{-1}.

Methane gas production was measured using a wet gas meter after adsorption of CO2 and H2S in a scrubber with a 10% NaOH solution.

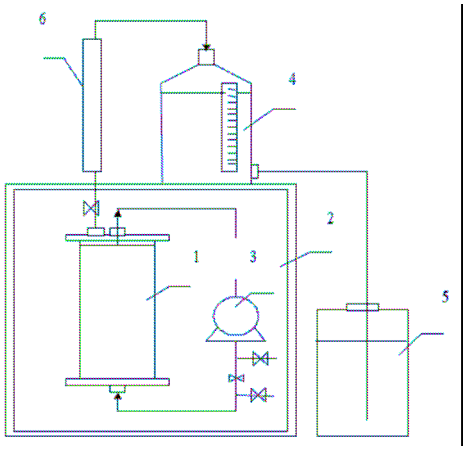

Figure 4. Laboratory set-up for anaerobic sequencing batch reactor (ASBR). 1-anaerobic reactor, 2-thermostat, 3-peristaltic pump, 4-wet gas meter, 5-water collector, 6 – alkali lock

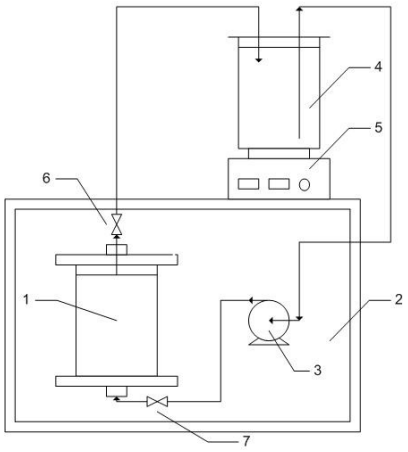

Figure 5. Laboratory set-up for coupled aerobic/anaerobic SBR (CSBR). 1-anaerobic reactor, 2-thermostat, 3-peristaltic pump, 4-aerobic reactor, 5-magnetic stirrer, 6 and 7 - valves

4.1.1.3. Post-ozonation of biologically treated wastewater (PAPER III)

A laboratory set-up shown in Figure 6 was used in the experiments of post-ozonation. Ozonation of wastewaters was conducted in a 0.9-L semi-batch glass reactor with a continuous gas flow through the liquid. Ozone-air gaseous mixture was generated in the ozone generator OZON-2M. The ozone-air mixture was introduced to the bottom of the reactor through a ceramic diffuser. Wastewater was ozonated until an ozone breakthrough was observed after a period of complete ozone absorption by the wastewater. The ozonation experiments were carried out without pH adjusting.

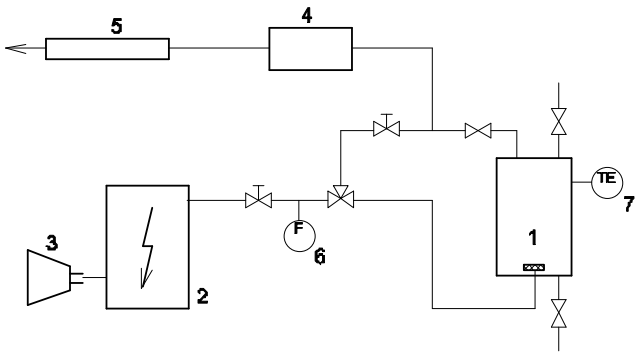

Figure 6. Laboratory set-up for batch ozonation. 1 – semi-batch reactor, 2- ozone generator Ozon-2M, 3 – air compressor, 4 – ozone analyzer Anseros GM-6040, 5 – residual ozone destruction unit, 6 – flow meter, 7 - thermometer

In the experiments, the absorption of ozone into pure water was studied. To minimize the ozone self-decomposition, pH of water was adjusted to 2. The ozone-air gaseous mixture was led through the water in the reactor. The concentrations of ozone in the liquid in the reactor and in the outlet gas from the reactor were monitored and recorded.

4.1.1.4. Coagulation assays

Tests were performed with 200 ml of anaerobicly/aerobicly pre-treated effluent in a laboratory glass under continuous stirring and pH control. Addition of coagulants ($FeCl_3 \cdot 6H_2O$ and $Al_2(SO_4)_3$) was carried out under 200 rpm, then intensity of stirring was reduced to 40 rpm to complete a flocculation process during which pH was maintained at 7.2-7.5 by addition of sodium hydroxide.

4.1.2. Substrates

In all lab-scale experiments only real wastewaters from baker's yeast production processing 100% sugar beet molasses were used. Real wastewater was obtained from Salutaguse Yeast Factory, Estonia. The wastewaters were collected from factory 1-2 times per week and subsequently refrigerated in the laboratory.

4.1.3. Seed sludges

At the beginning of full-scale experiments at Salutaguse Yeast Factory both anaerobic reactors were inoculated with digested sewage sludge from the mesophilic digester, Tallinn treatment plant. (PAPER I and V)

In case of lab-scale experiments two types of seed sludge were used for the comparison of the efficiency of the process. Anaerobic sludge from the anaerobic digester of the municipal WWTP, Tallinn, Estonia, which was not adapted for the treatment of sulfates, was used in the first two lab-scale experimental set-ups, and sulfate adapted anaerobic sludge from full-scale anaerobic digesters of the Salutaguse Yeast Factory, Estonia, was used in the case of CSBR. (PAPER II)

4.1.4. Analyses

This section describes the methods used for regular samples analyzing throughout the research. These included total nitrogen, ammonia, phosphorus, sulfate and total sulfide, chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) and biological oxygen demand (BOD). BOD was analysed according to procedure 5210 of the *Standard Methods* (APHA, 1998). Total and volatile solids were analysed as for method 2540D and E in *Standard Methods* (APHA, 1998).

Chemical analyses were conducted using HACH reagents and equipment according to the *Hach Standard Methods*: COD – Reactor Digestion Method, US EPA approved for reporting wastewater analysis; sulfate – SulfaVer 4 Method, US EPA approved for reporting wastewater analysis; sulfide – Methylene Blue Method, US EPA accepted for reporting wastewater analysis, total nitrogen by Persulfate Digestion Method.

Soluble content of pollutants was determined for the samples obtained through filtration with GF/A (Whatman) or for samples centrifuged at 5000 rpm for 20 minutes. The pH measurements were conducted using portatible pH-meter HACH HQ-30.

The most commonly used type of analysis was colorimetry. A HACH DR2010 and DR2800 spectro-photometers were used in conjunction with 16mm circular cuvettes. Samples were diluted using a Biohit 250µl and 1000µl pipettes depending on the expected concentration.

Other analyses such as biogas content (CH_4, CO_2, H_2S), wastewater liquid color, phenolic compounds content were ordered from accriditated laboratory.

4.2. Results and discussion

Present work was carried out using the studies on development and introducing of full-scale anaerobic/anoxic treatment scheme of sulfate-rich wastewater at Salutaguse Yeast Factory, Estonia (PAPER I and V), lab-scale anaerobic Sequence Batch Reactor (SBR) treating baker's yeast effluent (PAPER II), modification of anaerobic biorector to improve biomass retention by introducing sludge de-gassing technology (PAPER IV) and possibilities of using ozone (PAPER III) and coagulants for post-treatment of biologically pre-treated yeast wastewater.

4.2.1. Combined anaerobic/anoxic treatment of sulfate-rich wastewater from yeast (PAPER I and V)

All experiments with anaerobic/anoxic treatment scheme were conducted at Salutaguse Yeast Factory, Estonia using existing full-scale equipment by the reason of time limitation established by local environmental inspection.

Dealing with yeast industry wastewater it must be considered that wastewater contains low levels of readily degradable sugars and acids and high levels of trimethylglycine and sulfate. Sulfate reducing bacteria (SRB) compete with methane producing microorganisms for the available organic carbon resulting in the formation of hydrogen sulfide. When treating high-sulfate wastewater, high concentrations of sulphur compounds hinder wastewater treatment and the production of methane gas. This phenomenon results from the microbiological reduction of sulfates into sulfides. The stability of the treatment process is

dependent on the pH value as well as the concentration of sulfides formed. Sulfides 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). All mentioned above could be summarized as a main key point when designing anaerobic treatment process for sulfate-rich baker's yeast wastewater.

The wastewater treatment plant (incl. a biological purification facility) for the treatment of the separation of residues of baker's yeast at Salutaguse (Estonia) was constructed by the Finnish contractor OY Tampella AB. It has been in operation since 1991, but has never performed satisfactorily. Thus, the aim of this study was to achieve the optimal set-up and operational parameters for removing sulfate and avoiding the inhibitory effects of sulfides in the anaerobic treatment of yeast industry wastewaters.

It is possible to remove the hydrogen sulfide produced in the anaerobic reactor from sulfate 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 O_2/L. The elemental sulphur formed can be removed in the sedimentation tank (Janssen *et al.,* 1997, Fox *et al.,* 1996). The wastewater circulates from the anaerobic reactor to the subsequent aerobic reactor and from that point back to the anaerobic reactor.

Considering the specific character of baker's yeast wastewater suitable technological treatment scheme was developed and introduced to existing equipment (Figure 7). The main difference between original designs is that new scheme contained anoxic sludge recirculation back to anaerobic stage (acidification tank). This industrial experiment has shown good results and process stability within short period of acclimation and long period of operation without surplus investments for wastewater treatment plant. All existing equipments were utilized in this experiment. Before disposal, anoxic effluent was treated by an aerobic sequencing batch reactor (SBR).

Wastewater from the yeast production plant, processing sugar-beet molasses was divided into high strength wastewater (first separation, yeast wash water, 20% of the equipment wash water) and wash water (80% of equipment wash water, molasses clarification/cleaning, plant staff municipal waste water). High strength wastewater was pumped into a mixing tank and wash water was sent directly to an anoxic reactor. The next stage was SBR, which was used also as a bypass. The anaerobic reactors were fed with a mixture of high strength wastewater and recycled anoxic sludge. The temperature of the incoming wastewater from yeast production was $+28 \div +33^{\circ}C$.

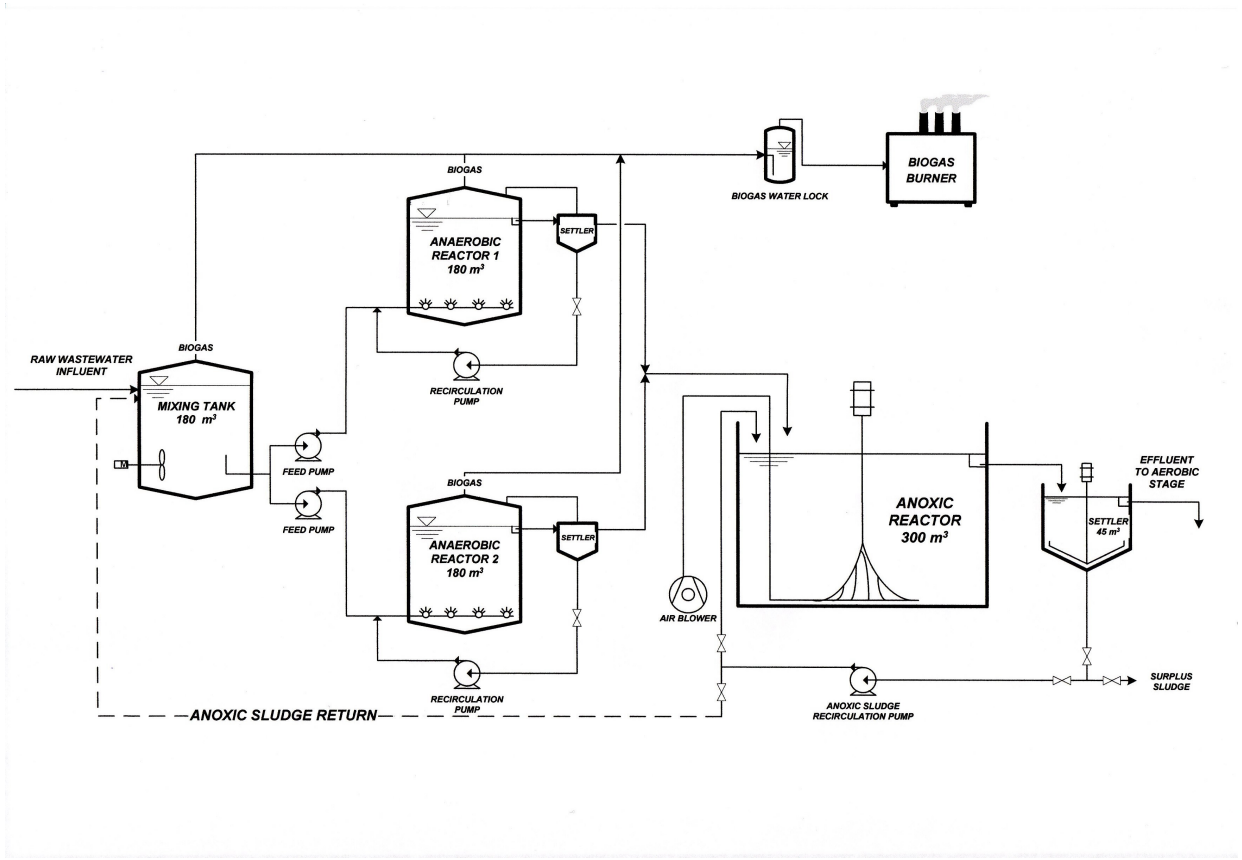

Figure 7. The technological set-up used in the Salutaguse Yeast Factory with re-circulation of the sludge from anoxic reactor back to the inlet, i.e. to the mixing tank

4.2.1.1. Characteristics of wastewater

The Salutaguse Yeast Factory (a subsidiary of Lallemand Inc.) generated 270 m^3 d^{-1} of wastewater originating 100% from beet molasses. The yeast wastewater to be treated in anaerobic/anoxic stage is characterized by high BOD (up to 14550 mg L^{-1}) and COD (up to 26155 mg L^{-1} by dichromate method) values. Sulphur is present in the wastewater as sulfate ions (up to 4060 mg L^{-1}).

The wastewater streams of Salutaguse Factory consist of (Table 4, Figure 8):

- first separation (high concentrated wastewater, t=+40°C, pH between 4 and 5),
- yeast wash water (t=+14°C, pH between 6 and 10),
- floor and equipment wash water;
- cooling water (t=+28 to +30°C, pH 7);
- molasses clarification/cleaning (limited amounts, depends on type/quality of molasses, this stream is included in the high concentrated wastewater);
- municipal wastewater (limited amount, directly to anoxic stage).

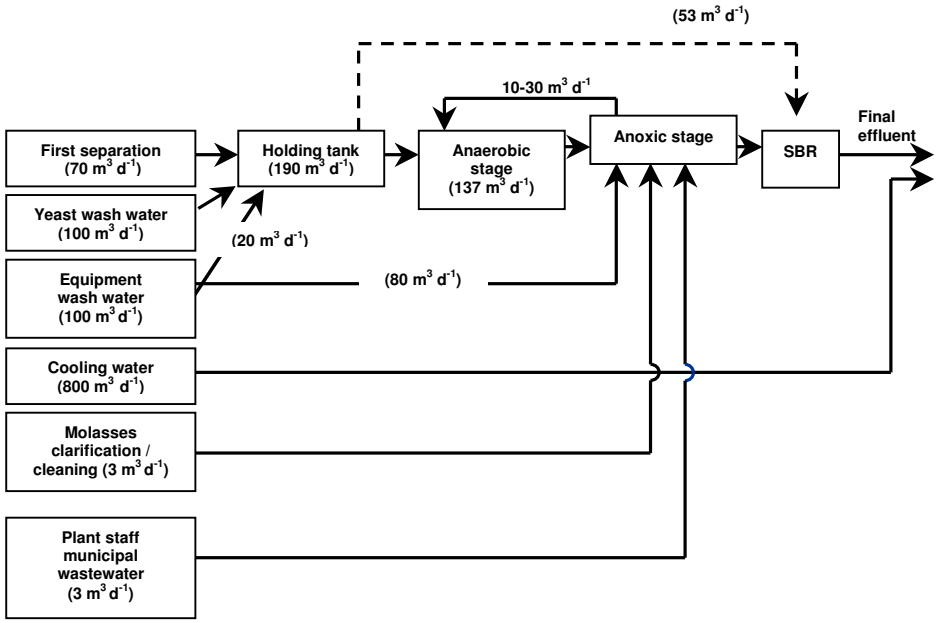

Figure 8. Scheme of wastewater streams at Salutaguse Yeast Factory

Table 4. The average wastewater characteristics of Salutaguse Yeast Plant, Estonia

Type of wastewater		COD excluding betaine		COD including betaine (+20%)		BOD		Suspended Solids (SS)		Total Nitrogen		Total Phosphorus (P)		SO_4^{2-}	
	Hydraulic loading ($m^3 d^{-1}$)	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})	Concentration (mg L^{-1})	Loading (kg d^{-1})
First separation beer	70	55000	3850	66000	4620	30500	2135	1500	105	3500	245	60	4,2	8000	560
Yeast cream wash water	100	10600	1060	12720	1272	5890	589	300	30	700	70	10	1,0	2100	210
Equipment washing	100	3000	300			2000	200	500	50	100	10	20	2,0	400	40
Total influent average	**270**	**19295**	**5210**	**21820**	**5892**	**10830**	**2924**	**685**	**185**	**1204**	**325**	**26,7**	**7,2**	**3000**	**810**
Influent to anaerobic/ anoxic stage	190	26155	4970	31010	5892	14550	2764	716	136	1660	315,2	27,6	5,24	4057	771
Cooling water (directly to creek)	800	-	-	-	-	-	-	-	-	-	-	-	-	-	-

In the modified anaerobic/anoxic reactors system a stabile, 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. Therefore, considering the above-mentioned latest achievements in the treatment of high sulfate 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 favorable conditions for the methane-producing microorganisms and avoided their takeover by the sulfate reducing bacteria. The concentration of dissolved oxygen in anoxic reactor was kept strictly below 0.1 mgO_2 L^{-1}, enabling continuing decreases in sulfide content.

4.2.1.2. Change of process parameters during the treatment process

Recirculation of a part of the wastewater/sludge from the anoxic stage back to the anaerobic stage guaranteed rapid changes in the sulfate and sulfide content. Initially the concentration of sulfates in the outlet of anaerobic reactors increased but the concentration of sulfides did not change much (Figure 9). Despite the origin of the inoculation sludge (residual sludge from the municipal WWTP), after a slight initial increase, the concentration of sulfates started to decrease constantly reaching zero in 35 days (acclimatization effect). The concentration of sulfides in the anaerobic reactor 1 increased to some extent on the account of increasing feed, while there was no evident correlation between the concentration of sulfides and the hydraulic loading rate in the reactor 2. In the anoxic tank, the concentration of sulfates also remained close to zero while the concentration of sulfides did not exceed 50 mg L^{-1} in most of the cases (Figure 10). An anoxic reactor as well as anaerobic reactors recovered from fluctuations of sulfate concentration (evoked by various reasons) in a very short time (Figure 9 and Figure 10).

Simultaneously with the drop in the concentration of sulfates the COD value of wastewater in the mixing tank (inlet) also decreased, caused by the dilution effect resulting from re-circulation from the sedimentation tank (Figure 11).

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, the necessity of the transportation of it from the Tallinn Municipal WWTP was avoided as well as the possible contamination of the reactor with fine particles of sand. This supplementary inoculation of reactor 2 reduced its effluent contamination (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 (Figure 12).

Because of the use of combined anaerobic/aerobic reactors system, the biological purification process had started already in the mixing tank. Results from analyses demonstrated that supernatant COD (SCOD) of the sample from the mixing tank was decreased up to 40% and the sulfide content up to 31% compared to the corresponding values in the holding tank. In the mixing tank, the concentration of sulfides originated from sulfates was 36.2 mg L^{-1}. 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 sulfates 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 granulated sludge.

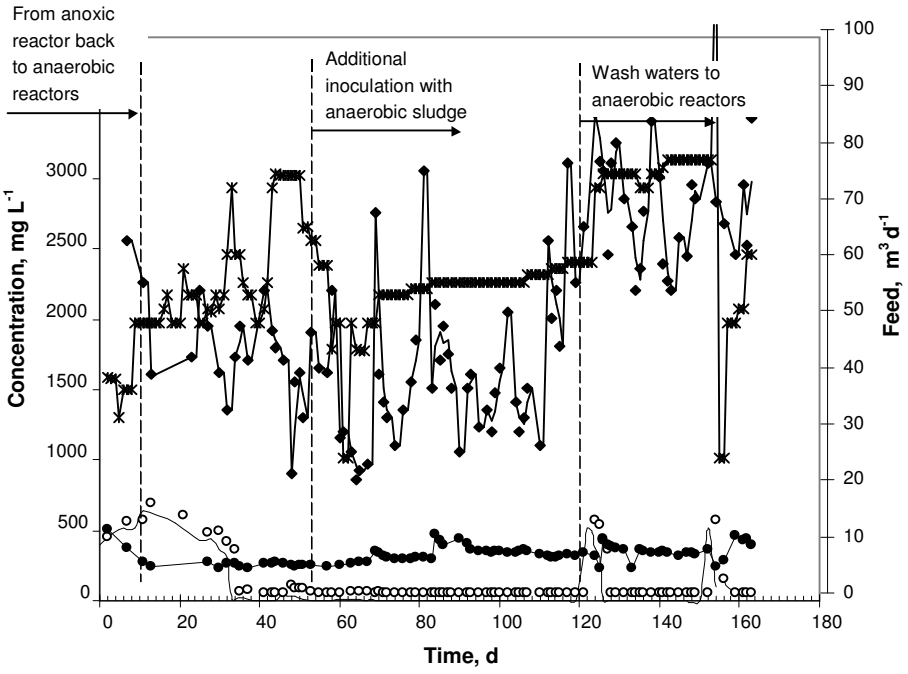

Figure 9. Hydraulic loading rate (feed, m^3 d^{-1}) and the content of sulfates and sulfides (mg L^{-1}) in the effluent from anaerobic reactor 2: ◆- sulfates inlet, ○ - sulfates outlet, ● - sulfides, × - feed.

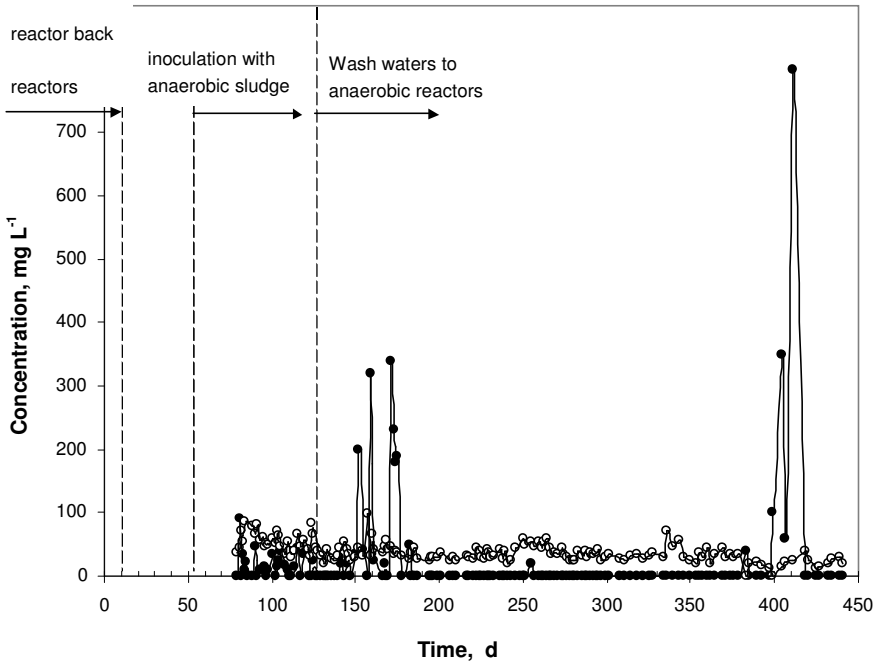

Figure 10. Influence of re-circulation on the concentration of sulfates and sulfides in anoxic reactor: ● - sulfates (mg L^{-1}), ○ - sulfides (mg L^{-1})

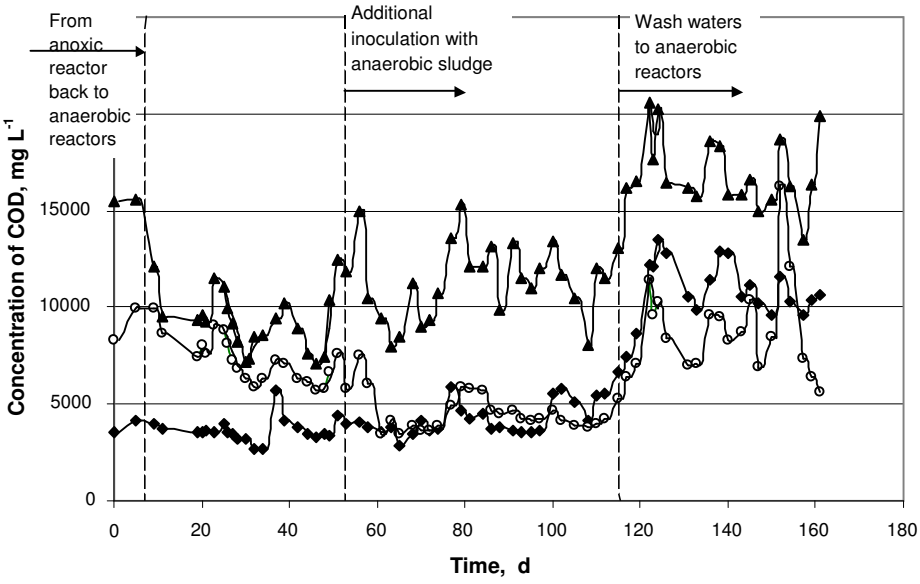

Figure 11. Concentration of total chemical oxygen demand (TCOD, mg L^{-1}) in anaerobic reactors: ▲ - TCOD of the inflow to the mixing tank, ◆- TCOD of reactor 1, ○ - TCOD of reactor 2.

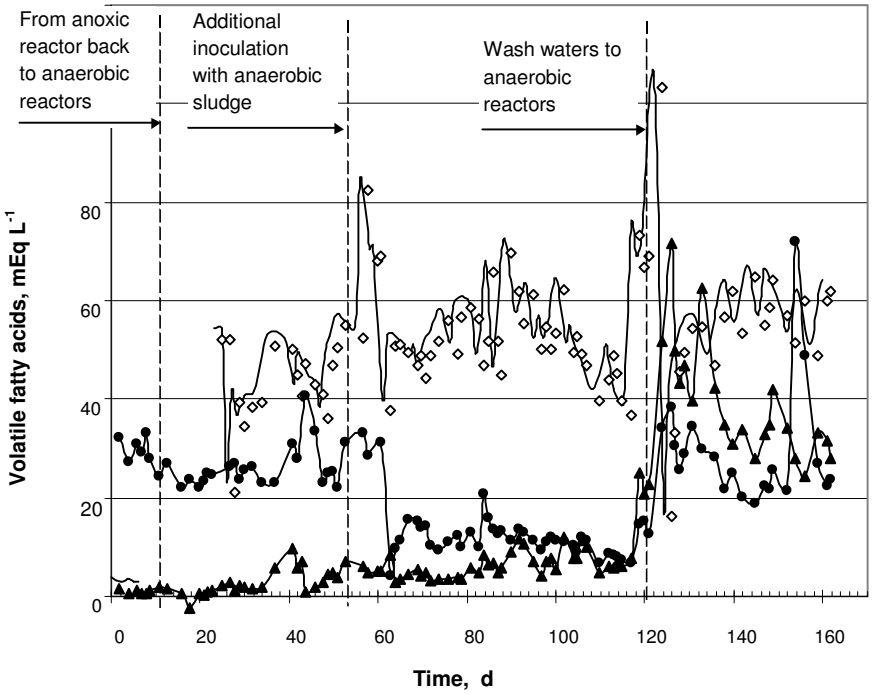

Figure 12. Concentration of volatile fatty acids (VFAs, mg L^{-1}): ◇ - in the mixing tank, ▲- in the effluent from anaerobic reactor 1, ●- in the effluent from anaerobic reactor 2.

4.2.1.3. Estimation of trimethylglycine content in wastewater

Regarding the literature data, using sugar-beet molasses as a growth medium, after cultivation of yeasts up to 4.5 g L^{-1} trimethylglycine could remain in the separation residue (high strength wastewater) (Thalasso *et al*., 1999). However, that 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 N-atom, gaseous products could be formed that will not be recorded (Menert, 2001). Therefore, it would be reasonable to add to the COD concentration in the holding tank an additional 20% of hidden contamination load at the expense of trimethylglycine (Table 4). Thus, the treatment efficiency of the entire anaerobic/anoxic system appeared to be 79%.

Anaerobic microorganisms degrade trimethylglycine completely into trimethylamine, acetate, and other compounds (Eq. 20 and 21).

2.5 trimethylglycine + 4.04 H_2 →
2-propanol + 2.5 trimethylamine + 0.95 acetate + 0.1 CO_2 +1.9 H_2O (20)

trimethylglycine + 1.32 serine + $H_2O \rightarrow$

trimethylamine + 2 acetate + 1.32 CO_2 + 1.32 NH_3 (21)

Trimethylamine is further degraded into methane, CO_2 and ammonia (Eq. 22), while the ratios between trimethylglycine and trimethylamine and trimethylamine and ammonia always remain equimolar.

$$4\ (CH_3)_3N + 12\ H_2O \rightarrow 9\ CH_4 + 3\ CO_2 + 6\ H_2O + 4\ NH_3 \quad (22)$$

Assuming that N-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 laboratory sequencing batch reactor (SBR) was conducted, using wastewater from the Salutaguse Plant. The analyses of N_{tot} were performed by the persulfate digestion method that mostly considers the 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^{-1} and in the effluent 270-875 mg L^{-1} (average 571 mg L^{-1}) (Table 5). Therefore, according to equations 20 and 21, from trimethylglycine trimethylamine in the ratio of 1:1 (and further NH_4 with the same ratio) could be obtained. Taking MW_{NH4}=18 and $MW_{trimethylglycine}$=118, we get 0.571/18=0.032 mol, corresponding to 0.032*118=3.74 g L^{-1} 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 (equation 22), e.g. from 1 mol trimethylamine (trimethylglycine) 2.25 mol methane. Thus, from 1 mol (118 g) trimethylglycine 9/4*22.4=50.4 L methane can be formed and from 3.74 g L^{-1} trimethylglycine in the reactor the formation of 3.74/118*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 *et al*., 1995). Adding 1.60 L to 0,35 L methane produced from the rest of COD we get 1.95 L that was almost the same amount of methane (per g COD removed) as observed in our experiments (Table 5).

Table 5. Results of chemical analysis on laboratory SBR reactor

Duration of experiment (d)	Flow (ml d^{-1})	COD of influent (mg L^{-1})	COD of effluent (mg L^{-1})	ΔCOD (mg L^{-1})	COD removed (g)	Theoretical biogas production from COD (L (g COD*d)$^{-1}$)*	Real biogas production by COD removed (L g^{-1})	N_{tot} of influent (mg L^{-1})	N_{tot} of effluent (mg L^{-1})	Betaine concentration (g L^{-1})	Biogas from betaine (L d^{-1})	Total theoretical biogas (L d^{-1})	Real biogas production (L d^{-1})
22	105	23660	15840	7820	0.821	0.287	1.729	255	690	4.5233	0.203	0.490	1.420
39	175	20280	17200	3080	0.539	0.189	2.059	250	270	1.7700	0.132	0.321	1.110
47	200	20280	13960	6320	1.264	0.442	0.736	475	875	5.7361	0.490	0.932	0.930
68	200	20540	5030	15510	3.102	1.086	0.484	325	650	4.2611	0.364	1.450	1.500
75	200	20540	3970	16570	3.314	1.160	0.772	345	550	3.6056	0.308	1.468	2.560
88	245	22890	3670	19220	4.709	1.648	0.656	255	690	4.5233	0.473	2.121	3.090
100	280	22890	11040	11850	3.318	1.161	0.452	250	270	1.7700	0.212	1.373	1.500
Average	**201**	**21583**	**10101**	**11481**	**2.438**	**0.853**	**0.710**	**308**	**571**	**3.741**	**0.312**	**1.165**	**1.730**

*Theoretical biogas production from COD was calculated on the assumption that on the degradation of carbonaceous organic material 0.35 m^3 of methane per kg of COD converted is produced.

4.2.1.4. Decrease of sulfide 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 sulfide ion can donate two electrons, and thus, be converted into elemental sulphur. Considering the stoichometric ratio in the chemical reaction between trimethylglycine (ammonia) and the sulfide ion (sulfate ion) (Fdz-Polanco *et al*., 2001).

$$SO_4^{2-} + 2\ NH_4^+ \rightarrow S^0 + N_2 + 4\ H_2O \quad (23)$$

consisting of the following reactions:

$$3\ SO_4^{2-} + 4\ NH_4^+ \rightarrow 3\ S^{2-} + 4\ NO_2^- + 4\ H_2O + 8\ H^+ \quad (24)$$

$$3\ S^{2-} + 2\ NO_2^- + 8\ H^+ \rightarrow N_2 + 3\ S^0 + 4\ H_2O \quad (25)$$

$$2\ NO_2^- + 2\ NH_4^+ \rightarrow 2\ N_2 + 4\ H_2O \quad (26)$$

the concentration of sulfide ions can be decreased by 1,026 mg (0.032 mol) at the expense of 3,74 mg (0.032 mol) trimethylglycine. The concentration of sulfates in the holding tank at the end of the experiment (day 166) was 4200 mg/L from which the sulfate reducing bacteria are able to produce 1,400 mg/L sulfides (Widdel *et al.,* 1991),(Eq. 27).

$$2\ \text{lactate}^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ \text{acetate}^- + 2\ CO_2 + 2\ H_2O + HS^- \quad (27)$$

If the concentration of sulfides in the wastewater can be reduced at the expense of trimethylglycine, then the residual concentration of sulfide ions should be 1,400-1,026=376 mg L^{-1}. At the end of the experiment, the concentration of sulfides in the anaerobic reactor 1 was measured as 360 mg L^{-1} and in the reactor 2 as 390 mg L^{-1} giving an average of 375 mg L^{-1}. The fluctuations of sulfide concentration during the experiment were 176 - 410 (average 296) mg L^{-1} in reactor 1 and 176-417 (average 307) mg L^{-1} in reactor 2.

According to the technological set-up presented in Figure 3, 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 settler (by anoxic reactor) is re-circled 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 wash waters 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. 13A and 13B). After the start-up research, the wastewater loading was increased to 400 $m^3\ d^{-1}$, 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 BOD) and over 90% (by COD).

A

B

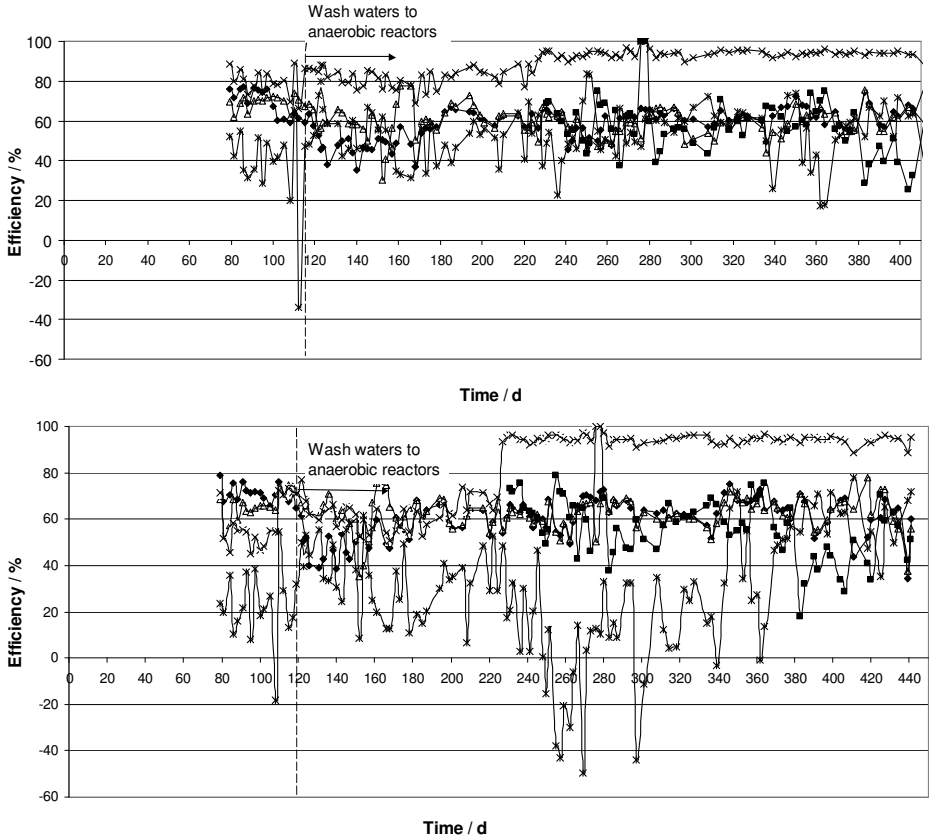

Figure 13. The treatment efficiency: **A**) by the total COD value (TCOD, %), **B**) by the soluble COD value (SCOD, %). ◆- anaerobic reactor 1, △ - anaerobic reactor 2, ✳ - anoxic reactor, - sequenced batch reactor, ✕ - the whole system.

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 wash waters to the mixing tank increased the production of biogas up to 25m^3 h^{-1}. The maximum biogas production achieved was up to 37m^3 h^{-1} in both reactors because of increased loading (up to 16 kg COD m^{-3} d^{-1}).

4.2.1.5. Energy balance

Energy is produced in form of biogas during anaerobic fermentation. During experiment taken biogas samples indicated that average CH_4 content was between 65% and 70% by volume. Considering actual loading (5210 kgCOD day^{-1}) the expected theoretical methane production will be in the range of 1094-1188 Nm^3CH_4. These values correspond to biogas volumes of 1683-1827 Nm^3 day^{-1}.

For Salutaguse Yeast Factory is more attractive electrical energy needed to decrease expanses for aeration (second stage of wastewater treatment process).

Considering expected biogas production of 1683-1827 Nm^3 day^{-1} with methane content of 65% it agrees with an energy capacity of 10612-11523 kWh day^{-1}. Combined (electricity/heat) microturbine could produce about 133-144 kWh (~30% efficiency) of electrical energy and 221-240 kWh (~50% efficiency) heat energy simultaneously.

4.2.1.6. Conclusions

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

- The purification of baker's yeast wastewater (produced from sugar beet molasses, having high SO_4^{2-} content) was feasible without the use of granulated 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 in anaerobic + anoxic stage was up to 80%, in anaerobic + anoxic + SBR stage up to 90% supporting average biogas production 1800 m^3 /d (with two anaerobic reactors). The system operated stabile, without sludge washout. The complete purification of wastewaters from sulfates (with 100% efficiency) accompanied by moderate production of sulfides (up to 50 mg L^{-1}) was obviously possible at the expense of reduction of trimethylglycine to other nitrogen-containing compounds.

4.2.2. Treatment of sulfate containing yeast wastewater in an ASBR (PAPER II)

The main aim of the experiments of Anaerobic Sequencing Batch Reactor (ASBR) with baker's yeast wastewater was to establish the application possibilities and expected maximum efficiency.

4.2.2.1. Study 1 - Anaerobic Sequencing Batch Reactor (ASBR)

The sludge adaptation and process steady phase achieving a constant OLR value (7.7 $kgCODm^{-3}d^{-1}$) was applied. During the operation at a constant OLR of 7.7 $kgCODm^{-3}d^{-1}$ a significant increase in the removal efficiency to over 80% was observed. The maximum treatment efficiency (84% by COD) and the maximum biogas production of 3.79 Ld^{-1} was reached at OLR values between 7.7 and 8.0 $kgCODm^{-3}d^{-1}$. At higher OLR (over 8.01 $kgCODm^{-3}d^{-1}$) treatment efficiency decreased.

The average sulfate removal efficiency was 95% in the experiment. Sulfate conversion to sulfide was greater than 80% during the start-up period. Then during the days 39-89 while the OLR was constant no inhibition was detected and approximately 100% removal efficiency was observed, furthermore the concentration of sulfates in effluent did not exceed 40 mgL^{-1}.

The composition of biogas was measured on the 65th day of experiment and was as follows: 60% CH_4, 35% CO_2 and 2.7% H_2S. This composition indicated that mainly the methanogenic mineralization of organic matter in the ASBR was taking place.

4.2.2.2. Study II – ASBR with a polymeric filler

Experiments with polymeric filler used as a support material for microorganisms were performed in order to study the influence of artificial filler on the efficiency of process. Previous studies had shown that using of support material favored the adherence of methanogenic bacteria and accelerated washout of SRB. The authors (Isa *et al*, 1986) demonstrated a poor attachment ability of SRB. They have concluded on the basis of the results of their experiments that in the presence of filler SRB were washed out of the reactor providing acetotrophic methanogenic bacteria with a sufficient growth advantage. These data suggested that artificial carrier could stimulate methanogenic activity in anaerobic digester, and increase the efficiency and stability of the treatment processes.

One of two reactors was filled with polymeric filler and another was operated like in *Study I* without filler for comparison. The COD and sulfate removal efficiency was not significantly different between the two reactors studied, however, in reactor without carrier slightly higher average treatment efficiency was observed, sulfate removal efficiencies varied from 85% to 100%. The

sulfide concentrations in effluents of both reactors did not exceed inhibitory levels and were not higher than 123 mg L^{-1} and 110 mg L^{-1} respectively.

The efficiency of phosphorous removal in the reactor with carrier was significantly higher (up to 79%) than in the control reactor (57%). It could be assumed that the carrier promoted deposition of insoluble materials, for example precipitation of $Ca_3(PO4)_2$. This conclusion was supported by the observation that scaling of the carrier beads was observed in the experiment. The fast clogging of the system with a carrier treating sulfate rich wastewaters has been described also in other study (Thalasso *et al*, 1999). In addition to facilitating scaling, carrier could hamper the equal distribution of wastewater over the sections of the reactor, which could result in lower COD and sulfate treatment efficiency. Therefore it can be concluded that the application of the carrier for the given treatment system was not effective and can't be recommended.

4.2.2.3. Study III – Coupled microaerophilic/anaerobic system (CSBR)

In the CSBR the effluent from the anaerobic reactor was recycled through an aeration system. The content of oxygen in microaerophilic reservoir was kept at the level of 0.1-0.15 mg L^{-1} to prevent sulfate formation in the oxidation of the sulfide formed in the anaerobic stage of process leaving sulphur in the form of elemental sulphur (S^0) (Buisman *et al*, 1990). The formation of elemental sulphur is an advantage because sulphur is a colloid, inert solid and can be removed from the wastewater for example by gravity sedimentation. Anaerobic reactor was seeded with sulfate adapted anaerobic sludge, and microaerophilic reactor was seeded with activated sludge obtained from the full-scale aerobic reactor of the Salutaguse yeast plant, Estonia.

The maximum OLR achieved was 7.74 kgCOD $m^{-3}d^{-1}$. The average pH value of the final effluent was 8.2 and the alkalinity always remained up to 177 mEq L^{-1} at average pH of influent 4.2. There were no attempts to adjust pH of the influent. High pH values could be explained by formation of hydroxide ion during the following biological overall reaction, taking place in a microaerophilic sulfide removal system.

The results obtained allowed to conclude that rather good COD treatment efficiency (50%-70%) was observed during the experiment. Since the sludge had been well adapted to the wastewater very quick start up was observed. Only a few days after seeding the COD removal was significantly increased and reached 70%.

Sulfates removal efficiency achieved in our experiments was excellent - more than 98%. Due to low dissolved oxygen concentration (0.1-0.15 mg L^{-1}) there were almost no sulfides and sulfates in the effluent. Only approximately 0.5 mg L^{-1} H_2S and 0-30 mg L^{-1} of $SO4^{2-}$ were present in the effluent while up to 3.6 g L^{-1} sulfate had been reduced.

4.2.2.4. Conclusions

The results of the study carried out demonstrated that the anaerobic sequencing batch reactor (ASBR) is a suitable and effective method for anaerobic treatment of sulfate rich wastewaters from baker's yeast production plant. Optimal parameters of the process were determined. However, the problems of sulfide formation caused significant malodour problems and corrosion of equipment during the experiment.

Experiments with two additional schemes developed for solving the sulfide formation problem showed that using of plastic carriers in the reactor led to the decrease of the treatment efficiency which was the result of the accumulation of insoluble sediment (presumably $CaCO_3$ and $Ca_3(PO4)_2$) on the surface of the carriers, so this technology can not be recommended for large scale application.

Combining anaerobic sulfate reduction with biological oxidation of sulfide in coupled microaerophilic/anaerobic SBR (CSBR) showed the best results and might be preferable for the treatment of sulfate rich yeast wastewaters. Since the scaling of biomass and the fast accumulation of inorganic compounds were observed also in this case, successful application of the CSBR technology requires finding a solution for removal of the inorganic precipitate from the reactor.

4.2.3. Post-treatment of biologically pre-treated wastewater effluent

Wastewater from molasses processing presents a large amount of colored substances that give dark brown color and high organic load to the effluents. After a multistage biological treatment most of the organic load is removed. However, the brown color does not disappear and it can even increase because of the repolymerization of colored compounds. The main colored compounds are known as melanoidins (Gonzàlez *et al.,* 1999).

Melanoidins are high molecular weight polymers. The formation of melanoidins comprises a set of consecutive and parallel chemical reactions taking place between amino compounds and carbohydrates during a Maillard reaction (Cämmerer and Kroh, 1995). Conventional anaerobic-aerobic treatment processes can accomplish the degradation of melanoidins only up to 6% or 7% (Guimaraes *et al.,* 1999). Therefore, it was necessary to study additional treatments to remove color from molasses effluents and prevent the serious environmental problems that colored wastewaters can promote in river courses such as the reduction of both photosynthetic activity and dissolved oxygen concentration.

Regarding the literature data melanoidins can be removed by:

1) Physico-chemical treatments. These methods require high reagent dosages and generate a large amount of sludge.
2) Chemical oxidation with ozone.
3) Biological treatments with certain bacteria and fungi have also been applied, leading to lower color removal efficiencies. (Miyata *et al.*, 2000)

This study aims to investigate the precipitation of colored substances with coagulation and the oxydation with ozone of highly polluted biologically pre-treated wastewater from Salutaguse Yeast Factory processing beet molasses.

In the experiments of coagulation and ozonation, biologically treated yeast wastewater from Salutaguse Yeast Factory was used. Samples of wastewater were taken over a period of two months from aerobic Sequencing Batch Reactor effluent. This biologically treated wastewater had a relatively high residual COD and brown colour. The values of the most important parameters were: COD – 1500–2200 mg L^{-1}, BOD – 160–310 mg L^{-1}, N-total – 250-350 mg L^{-1}, P-tot – 15-25 mg/L^{-1}, pH – about 8.

4.2.3.1. Coagulation - performance of iron and aluminium coagulation step

Some results on efficiency of Fe^{3+} and Al^{+3} coagulation step for treatment of aerobic SBR effluent are presented in Table 6 and Table 7. It is seen that all parameters (total COD, N-total, P-total and color) decreased with increasing acting Fe and Al concentrations and the disharge limits are already achieveble under iron concentrations around **200** mg L^{-1} and aluminium around **400** mg L^{-1}. The color of wastewater underwent dramatic changes from deep brown to pastel yellow after coagulation. These results are superior (with regard to coagulant added) to those reported in the literature for anaerobically treated baker's yeast wastewater (Kalyuzhnyi *et al*., 2003).

Table 6. Performance of iron coagulation step

	Acting Fe concentration, mg L^{-1}					
	0	50	100	150	200	*500*
COD_{tot},mg L^{-1}	2120	1750	1350	1040	830	*710*
Phenols, mg L^{-1}	288	260	224	178	125	*99*
P_{tot}, mg L^{-1}	21	18,3	15,2	10,2	4,5	*2,1*
N_{tot}, mg L^{-1}	310	245	125	101	78	*61*
OD_{580}	0.414	0,380	0,331	0,250	0,165	*0,067*
Sludge percentage, % vol*.	-	ND	ND	5,4	15,8	*63,4*
SVI, ml g^{-1} TSS	-	ND	ND	210	270	*420*
Sludge VSS/TSS, %	-	*ND*	*ND*	*90*	*88,9*	*33,5*

*after 30 min of settling

ND-not detected

Table 7. Performance of aluminium coagulation step

	Acting Al concentration, mg L^{-1}					
	0	100	200	400	600	*800*
COD_{tot},mg L^{-1}	2120	1910	1690	1270	800	*760*
Phenols, mg L^{-1}	288	144	82	67	64	*59*
P_{tot}, mg L^{-1}	21	15,7	7,2	4,5	3,4	*1,5*
N_{tot}, mg L^{-1}	310	180	112	74	65	*54*
OD_{580}	0.414	0,361	0,139	0,112	0,076	*0,065*
Sludge percentage, % vol*.	-	35,5	91,4	93,5	94,4	*96,3*
SVI, ml g^{-1} TSS	-	655	615	545	355	*341*
Sludge VSS/TSS, %	-	*42*	*52,4*	*56,5*	*52,2*	*39,8*

*after 30 min of settling

The sludge formed under an acting Fe concentration of 200 mg L^{-1} was relatively large (SVI_{Fe} = 270 ml g^{-1} TSS). An acting Al concentration of 400 mg L^{-1} produced much more sludge (SVI_{Al}=545 ml g^{-1} TSS). The iron sludge had high (~90%) VSS content compared to sludge obtained during Al coagulation step (56,5% VSS) showing the significant removal of organic COD and nitrogen during the iron coagulation step.

Figure 14 shows the evolution of color removal and COD decrease for experiments carried out at different Fe^{3+} and Al^{3+} dosages.

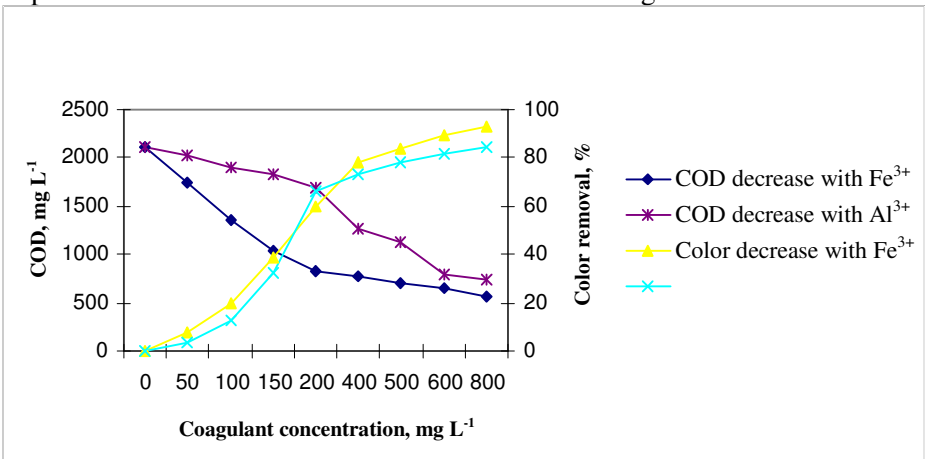

Figure 14. Effect of Fe^{3+} and Al^{+3} dosages on color and COD removal during coagulation experiments

As the result of coagulation, absorbance at 580 nm decreased significantly. Applied concentration of Fe^{3+} and Al^{3+} had positive effect not only on color

removal but also on COD removal. Melanoidins are responsible for the brown color and included in COD.

Conclusion for coagulation

- The application of iron chloride and aluminium sulfate coagulation for post-treatment of anaerobic-aerobic effluents may fulfill the discharge limits to the sewer under iron concentrations around 200 mg L^{-1} and aluminium concentrations around 400 mg L^{-1}.
- Significant decrease of color substances could be achieved using coagulation. It is very important when final effluent is treated by ultraviolet radiation to meet biological limits.
- Generated during coagulation sludge could be the problem when full scale process applied. The iron sludge had much better SVI which is important parameter for sludge further handling (separation, de-watering).
- Considering that Salutaguse Yeast Factory has daily 270 m^3 d^{-1} of wastewater to be treated using coagulation process it could be proposed that up to 348 kg TSS d^{-1} will be generated additionally if Fe^{3+} is used and up to 230 kg TSS d^{-1} in case of Al^{3+}. It corresponds to amount of sludge 1,05-1,58 m^3 d^{-1} (at 22%DS after sludge dewatering).
- Installation of flotation unit is needed before sludge de-watering.

4.2.3.2. Post-ozonation of biologically treated wastewater

The results of the post-ozonation of biologically treated yeast wastewater are presented in Table 8. The experiments indicated that the efficiency of postozonation in terms of COD (COD removal) ranged from 30% to 49%, and the ratio dn/COD, consumed ozone dosage mg of ozone per mg of COD removed, ranged from 1.2 to 2.5.

Figures 15 to 19 express the dependence of CODtot, CODsol, BOD, and BOD/COD, the biodegradability of the wastewater, on the consumed ozone dose dn (mg of ozone per litre of treated wastewater). The ozone dosage required to decrease the residual COD noticeably was about 1000–1500 mg L^{-1}, and both CODtot and CODsol decreased. This indicates that during post-ozonation the particulated organic matter in the wastewater was partly solubilized and the soluble matter was being oxidized.

Table 8. Results of post-ozonation of biologically treated wastewater

Run	Parameters of biologically treated yeast wastewater			Consumed ozone dosage, dn/ΔCOD, mgO_3 $mgCOD^{-1}$	Efficiency of post-ozonation ΔCOD, %	Parameters of wastewater after post-ozonation		
	COD_{tot}, mg L^{-1}	BOD, mg L^{-1}	BOD/ COD			COD_{tot}, mg L^{-1}	BOD, mg L^{-1}	BOD/ COD
1	2055	161	0.08	2.45	30	1460	317	0.22
2	2120	579	0.27	2.47	31	1470	381	0.26
3	1480	204	0.14	2.2	34	970	310	0.32
4	1860	308	0.17	1.2	49	940	297	0.32
5	1940	147	0.08	1.6	30	1430	250	0.17

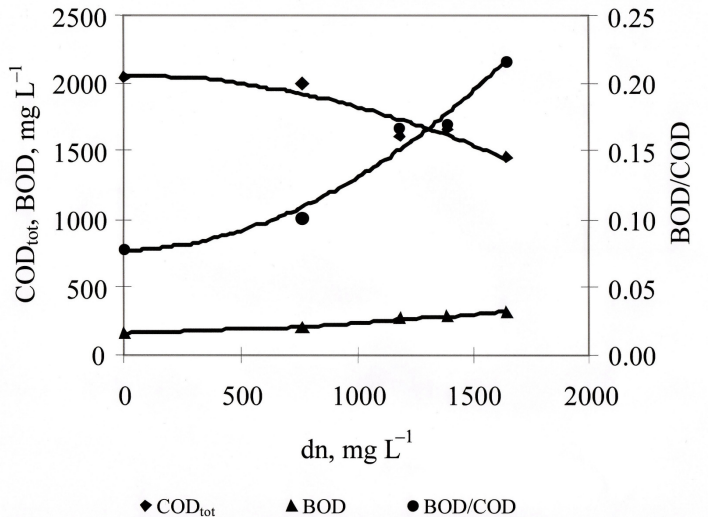

Figure 15. The effect of ozone dosage (dn) on CODtot, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 1). The pH rose from 7.3 to 7.9

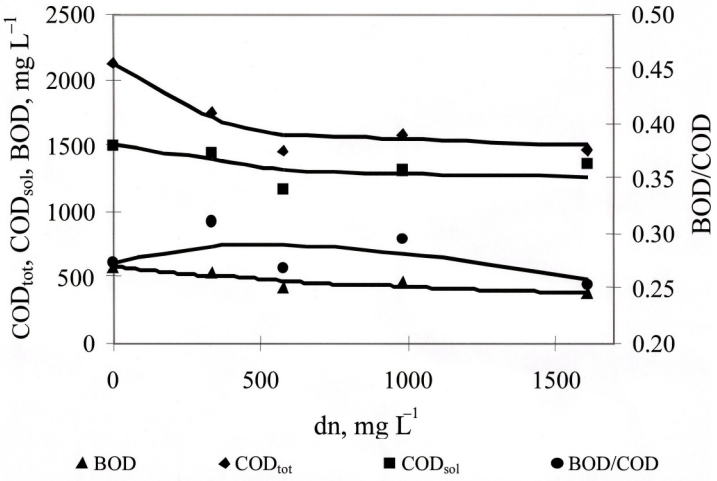

Figure 16. The effect of ozone dosage dn on CODtot, CODsol, BOD, and BOD/COD of biologically treated yeast wastewater (run 2). The pH fell from 8.3 to 8.1

As a rule, the results of the post-ozonation of yeast wastewater depend on the wastewater composition and consequently on the previous process of biological purification. BOD and biodegradability (BOD/COD) of the wastewater increased during ozonation, as shown in Figures 15, 17, and 19. In run 2, illustrated in Figure 16, BOD decreased. This can be explained by the low efficiency of the biological treatment before ozonation. The biologically treated water contained a large amount of biodegradable compounds – CODtot was 2120 mg L^{-1} and BOD was 580 mg L^{-1}. Even in this case, the biodegradability was initially enhanced by ozonation (at ozone dose of 300 mg L^{-1}) followed by

a decrease. In run 4 (Figure 18), BOD decreased slightly, and the biodegradability increased in this case due to a faster decrease in COD.

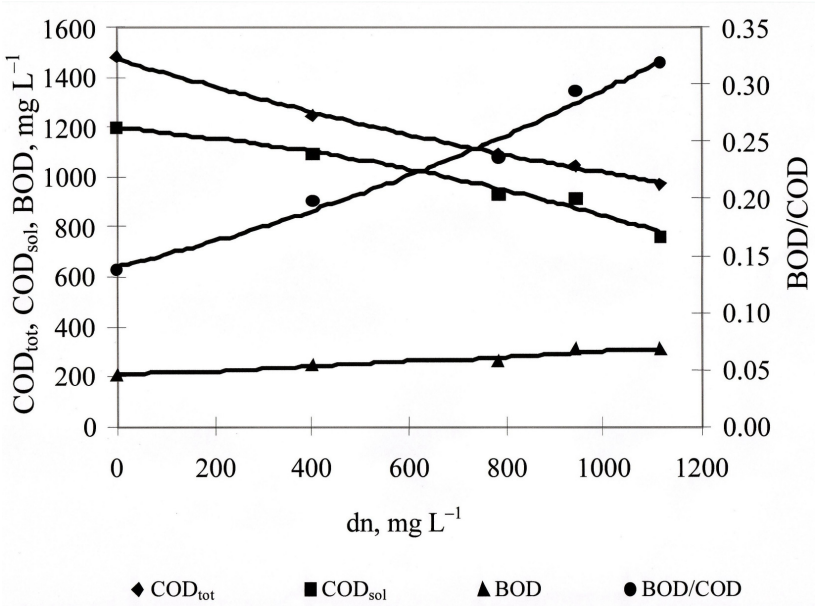

Figure 17. The effect of ozone dosage dn on CODtot, CODsol, BOD, and BOD/COD of biologically treated yeast wastewater (run 3). The pH rose from 7.3 to 7.9.

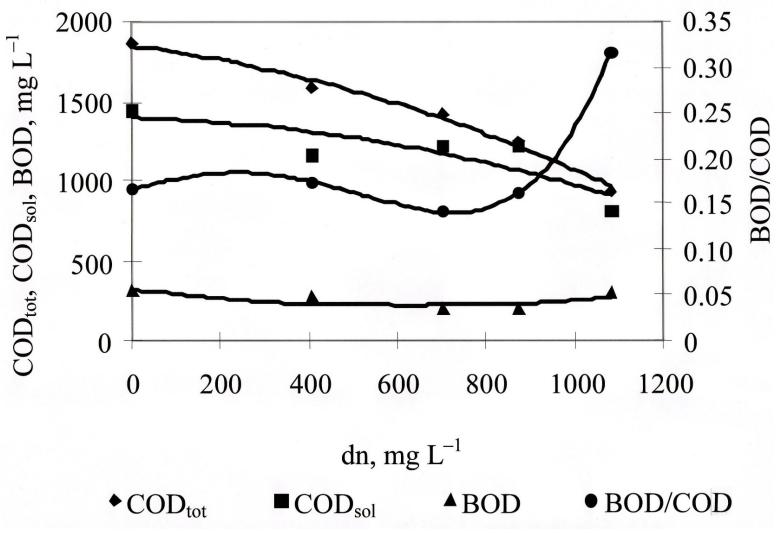

Figure 18. The effect of ozone dosage dn on CODtot, CODsol, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 4). The pH rose from 7.3 to 7.9.

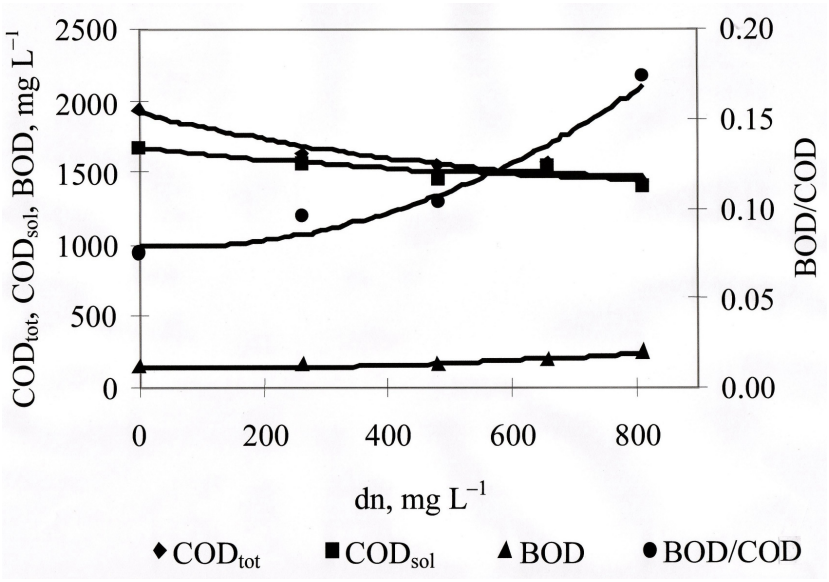

Figure 19. The effect of ozone dosage (dn) on CODtot, CODsol, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 5). The pH rose from 7.9 to 8.4.

Generally, during ozonation the pH decreases as a result of the formation of carboxylic acids. However, in the experiments of post-ozonation of the yeast wastewater, the pH decreased only in run 2. In all other runs the pH increased. The reason could be that acidic products of biochemical oxidation had been degraded by ozone.

In all cases, ozonation removed the colour and distinct odour of the treated wastewater. Initially, the biologically treated wastewater was dark brown and had a distinct odour, and after ozonation it was practically transparent and colourless with no odour specific to this wastewater.

The post-ozonation experiments indicate that ozonation can be used in the tertiary treatment of yeast wastewater for the reduction of colour, odour, and overall concentration of organic contaminants and matter. Since the biodegradability of the yeast wastewater increased during the ozonation, or at least at the beginning of post-ozonation, it is possible to include ozonation into the combined purification process simultaneously with anaerobic and aerobic bio-oxidation. In the combined process, the goal of ozonation is enhancement of biodegradability and removal of colour and odour. Taking into account that ozonation is still an expensive technology, the last option – application of ozonation in combinations with biological methods – may be more economical for yeast wastewater purification than ozonation alone.

Conclusion for ozonation

It was established that the post-ozonation of biologically treated yeast wastewater resulted in the reduction of COD by 30–49%, and the consumed ozone dosage (mg ozone per mg of COD removed) ranged from 1.2 to 2.5. Compareteyly low COD removal could be explained by the fact that ozone oxidizes unsaturated bonds and leads to partial oxidation products, causing wastewater decolorization. However, ozonation generates organic compounds that also contribute to COD and rarely produces complete mineralization of organic matter to carbon dioxide and water. The biodegradability of the wastewater, expressed as BOD/COD ratio, generally increased during the ozonation. Also, the odour problem of the yeast wastewater was eliminated. Thus, it is possible to use ozonation in the tertiary treatment of yeast wastewater or to include ozonation into the combined purification process simultaneously with anaerobic and aerobic bio-oxidation.

Application of ozonation in a combined process seems to be promising for yeast wastewater purification; in this case, the target of ozonation is the enhancement of biodegradability and removal of colour and odour.

Ozonation cost estimation for yeast factory effluent

The ozone dosage required to decrease the residual COD noticeably was about 1000–1500 mg L^{-1}, and both CODtot and CODsol decreased. It was reported that electical power consumption to produce pure ozone from air is 17-30 kWh kgO_3^{-1} (Haapea *et al.,* 2002)

Considering that Salutaguse Yeast Factory has daily 270 m^3 d^{-1} of wastewater to be treated using ozonation process it could be proposed that up to 405 kgO_3 d^{-1} is required. It corresponds to 6885-12150 kWh day^{-1} electrical energy consumption. In addition to mentioned above installation of modern ozone generator is needed.

4.2.4. Modification of anaerobic bioreactor to improve biomass retention

The most difficult aspect of the anaerobic system operation is the retention of the sludge in the reactor, due to the low density of the sludge and the rising of generated biogas bubbles. In order to keep the sludge in the reactor, the biomass has to grow in the form of dense flocs or preferably granules.

When dealing with high peak organic loadings to be treated it is very often take place the biomass washout from reactor. Biomass losses lead to organic removal efficiency dramatic decrease and overall process failure.

There are various types of microbial conglomerates – granules, pellets, flocs. Flocs and flocular sludge have a loose undefined structure. They often settle as a homogeneous layer with a settling velocity of 0.1-1m h^{-1}. As such they are

generally unsuitable for high rate upflow anaerobic rectors unless a separate solid separation phase is included (Lettinga and Hulfhoff Pol, 1991). There are several factors that may adversely effect settling velocity and cause biomass washout. These include excessive growth of extracellular polymeric substances and acidogenic bacteria (causing a fluffy surface) (Alphenaar, 1994), attachment of flocs or granules by biogas bubbles produced (Thaveesri, et al., 1995). Extracellular polymeric substances consist mainly of protein and polysaccharides in ratio of 2:1 – 6:1 (Schmidt and Ahring, 1996).

However, anaerobic wastewater treatment system at Salutaguse Yeast Factory is utilizing regular flocular anaerobic sludge in both reactors (initially UASB) with relatively high loading (12.0-16.0 kgCOD $m^{-3}day^{-1}$). The main explanation for this is recently introduced novel technology for treatment of anaerobic sludge with vacuum. Following text is the summary of investigation.

4.2.4.1. Development of device for wastewater anaerobic treatment with biogas production.

As the result of anaerobic degradation of organic compounds of wastewater the anaerobic bacterial biomass and the biogas are formed. Obtained biogas composition depends on the type of the bacteria utilized in the treatment process and on the composition of wastewater to be treated. Besides non-soluble gases like methane and hydrogen, some soluble gases (H_2S and NH_3) and poorly soluble gases (CO_2) are formed during anaerobic process of organic substrate degradation.

Resultant gases CO_2, NH_3 and other partially are dissolved in the water, ionizing it with the formation of acidic or alkaline environment and participating subsequently in the process of the metabolism of anaerobic sludge. The acidic environment (pH lower than 6,2) suppresses the process of methane formation, therefore prevention the appearance of acidic environmental condition contributes to the more complete decomposition of organic substances by the anaerobic methane producing microorganisms.

Not dissolved in the water methane, hydrogen and poorly soluble gases like carbon dioxide (CO_2) form the small gas bubbles, which remain inside of sludge flocs and granules or are attached to their surface. All this decreases the general specific density of flakes and granules and their capability for precipitation, contributing to the washing out of active sludge from the bioreactor. As a result of the washing out of active sludge necessary microbial biomass decreases in reactor, this in turn leads to decreasing of wastewater treatment efficiency.

The different methods for increasing sludge specific density are used to prevent the washing out of active biomass from bioreactor. One of such methods is an increase of sludge specific density by the dosing of the special additives to influent, which improve the process of granular sludge formation. Proposed bio-supplement „GRANDOS“ promotes the different bacterial groups involved in

the anaerobic digestion to grow in granules. GRANDOS is composed of readily available carbohydrates which favour the growth of acidogenic bacteria; the latter ensures the formation of sufficiently strong sludge flocs or granules. GRANDOS also contains a surface tension active compound which facilitates the detachment of the gas bubbles from the microbial flocs and granules so that the latter are retained more effectively in the reactor. In other words it could be concluded that declared product prevents the washing out of active sludge flakes (granules) from the anaerobic bioreactor.

More information about proposed product is available at GRANDOS official webpage (http://www.iol.ie/~metrotec/quikload/avecom/avecom.htm).

A negative side of this method includes the supplementation of extra pollution coming with proposed additive. According to product specification bio-supplement contains COD/N/P in ratio of 100/5/2. Overdosing of additive could easily cause digester overloading and bacterial consortium composition change – acidogenic bacteria will dominate. Regular use of additive also increases anaerobic system maintenance and operation costs.

The washing out of active sludge from the anaerobic reactor can be avoided by maintenance of the layer of active sludge in the pseudoliquified state with the use of artificial granules. Such technique was proposed by Yoda et al.(patent US 4 762 612; Motoyuki Yoda Et Al; 09.08.1988). Equipment for the anaerobic treatment of waste water with production of biogas contains the anaerobic bioreactor, and the system of recirculation. Anaerobic sludge is located in the lower part of reactor together with influent distribution system. Outlet of pre-treated wastewater and the release of the generated biogas are located in the upper part of the bioreactor. The system of recirculation is connected between the outlet of the pre-treated effluent and the lower part of the bioreactor - influent distribution. Part of pre-treated effluent and raw wastewater influent are directed to the lower part of the bioreactor where through feed distribution system passed to reactor and directed upward, penetrating the layer of active sludge and creating in it the pseudoliquified state, or fluidized bed. The layer of active sludge contains the artificial granules of the specific size and the specific density, which serve as the carriers of active sludge.

Proposed method has the following deficiencies:

- In practice it is not easy to keep fluidized bed in full-scale reactor especially when dealing with non constant loadings (biogas production varies also).
- The usage of artificial granules as biomass carrier decreases the working volume of anaerobic reactor.
- Artificial granules in the course of time excessively outgrow by mineral and organic precipitation. Activity of granular biomass decreases. In practice it means that working reactor have to be stopped for cleaning or artificial granules replacement. Re-start of anaerobic process after cleaning takes long period of time to reach steady phase again.

4.2.4.2. Essence of the invention

The aim of present invention was the elimination of mentioned above deficiencies and the creation of the device for wastewater anaerobic treatment, which ensures an increase of anaerobic bioreactor operation effectiveness with simultaneous simplification in the operation of device and decrease of its operating costs.

Object of the invention was reached by the fact that, according to present invention, the anaerobic treatment of wastewater with production of biogas occurs in the device, which contains anaerobic bioreactor, system of recirculation, device of vacuum degassing in the form of the inverted letter U and if necessary second sedimentation tank and/or the collector for surplus active sludge, where in the lower part of the bioreactor anaerobic active sludge and the distribution of influent are located, in the upper part of the bioreactor are located the outlet of the pre-treated liquid and gas cavity with the outlet of the biogas, where the system of recirculation is connected between the outlet of the recirculated liquid and the lower part of the bioreactor, and where, in contrast to the known device (patent US 4 762 612; Motoyuki Yoda Et Al; 09.08.1988), the outlet of the recirculated liquid is placed into the location of anaerobic active sludge. Therefore the recirculated liquid contains more active sludge, than the recirculated liquid in the known device, where the outlet of the recirculated liquid is located in the upper part of the bioreactor, near the outlet of the purified liquid, where a quantity of active sludge is small; in this case the device of vacuum degassing in the form of the inverted letter U is located in the system of recirculation, the lower end of supply line is connected with the outlet of the recirculated liquid, upper end of supply line is connected to the degassing chamber of higher than liquid level in the bioreactor, and the lower end of outlet pipe has a connection with the lower part of the bioreactor. As a result of this the recirculated liquid enters the degassing chamber, where occurs removal of gases, which are contained in the active sludge.

In the totality, this ensures the entering to bioreactor of degassed recycled liquid together with degassed recycled sludge which has higher capability for precipitation than sludge before degassing treatment. The degassed active sludge is much better retained in bioreactor, contributing to an increase of the mass of active sludge. This ensures a substantial increase in effectiveness and wastewater treatment efficiency, which is manifested in an increase in the yield of methane and an improvement in the quality of purified wastewater, in comparison with the nearest known device.

Furthermore, vacuum degassing decreases also a quantity of dissociated carbon and hydrosulfuric acids and increases their isolation from the water phase in the form of carbon dioxide (CO_2) and hydrogen sulfide H_2S, which leads to an increase in pH of the bioreactor medium and strengthens its alkaline reaction. This creates the favourable conditions (optimum values of pH and the

elimination of toxic hydrogen sulfide) for the vital activity of the methane-forming bacteria, which, in turn, increases the purifying ability of anaerobic bioreactor.

The device of purification of wastewater, according to invention, has following construction (Figure 20).

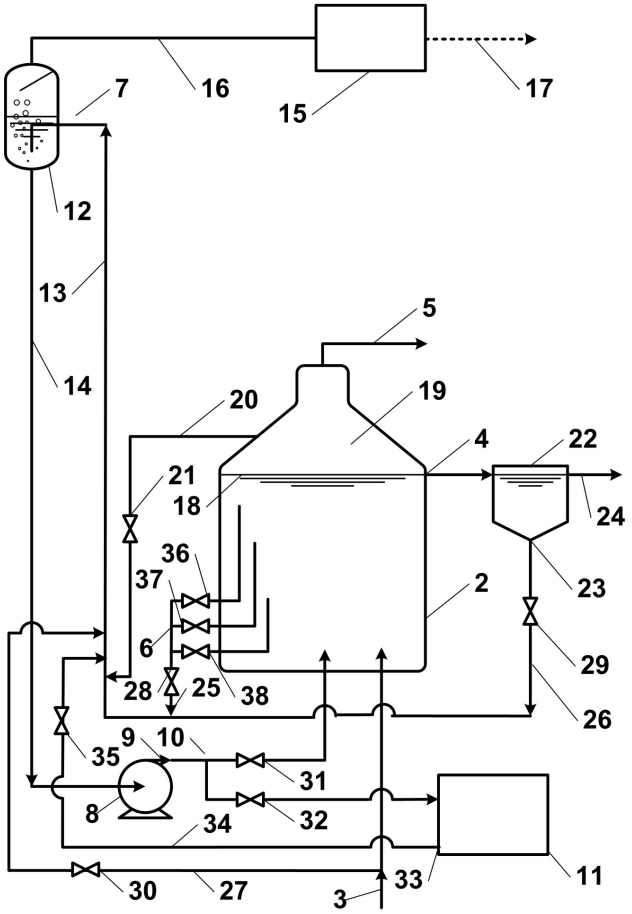

Figure 20. Device of anaerobic purification of wastewater with obtaining of biogas.
2 - anaerobic bioreactor (methane reactor); 3 - inlet of raw wastewater; 4 - outlet of pre-treated liquid; 5 – biogas release; 6 - outlet of the recirculated liquid; 7 - equipment of vacuum degassing; 8 - recirculation pump; 9 - output of recirculation pump; 10 – pipe divider ; 11 - collector of the surplus sludge; 12- degassing chamber; 13 - supply line; 14 - drain pipe; 15 - source of vacuum, vacuum pump; 16 - vacuum pipeline; 17 - release of biogas; 18 - liquid level; 19 - gas cavity; 20 - biogas pipeline, 21 – gate valve; 22 - second sedimentation tank; 23 - outlet of the recirculated liquid; 24 - pipeline of the purified liquid outlet (effluent) ; 25-27 - conduit, 28-32 - valves, 33 – outlet ftom sludge collector; 34 - conduit, 35-37 – gate valves.

Purification of wastewater is accomplished in methane reactor **2** in the continuous operation regime. Raw wastewater is given into methane reactor continuously or with the portions through the inlet **3** located in the bottom and, getting mixed with the located in the reactor suspension of active sludge, they rise upward. The decomposition of organic pollution in methane reactor is accompanied by the formation of new anaerobic microbial biomass and gaseous substances, including of CH_4, CO_2, small quantity of H_2 and toxic H_2S. All these gases are separated from the liquid and they are gathered above, in the gas cavity **19** of methane reactor, from where they are derived through the biogas release pipe **5**. But the part of smaller gas bubbles is not detached off from the flakes of active sludge, which reduces the specific density of active sludge flocs. Additionally certain quantity of CO_2 and H_2S, that appear in the process of anaerobic treatment process, interacts with the water with the formation of the dissociated carbon acid and the hydrosulfuric acid, which increases the acidic reaction (it reduces pH value) in the methane reactor, decreasing the purifying ability of anaerobic active sludge. Furthermore, resultant hydrogen sulfide H_2S is toxic to methanogens. An increase in H_2S concentration decreases their viability.

For an improvement of anaerobic treatment process, the part of the mixture (sludge and pre-treated effluent) is derived from methane reactor through the outlet of recirculated liquids **6** and rises along suction supply line **13** into the degassing chamber **12** of vacuum degassing device **7**. Under the action of vacuum small gas bubbles are enlarged and are torn off from the flocs of active sludge, increasing the specific density of active sludge. In this case biogas release from the water phase occurs also. The part of the active sludge, which is located in second sedimentation tank **22** and in the collector of surplus sludge **11**, also can be directed through the outlets of **23** and **33** into vacuum degassing device **7**.

Vacuum pump **15** and recirculation pump **8** support the liquid flow in the recirculation system, which creates possibility for an increase of the gas bubbles size in the supply line of degassing chamber **13** and gas release from the liquid in degassing chamber **12**. With a sufficient power of vacuum pump it is possible to support the circulation of liquid in the system of recirculation without application of a recirculation pump. Vacuum pump **15** ensures the suction of the recirculated liquid into supply line **13** and into degassing chamber **12**.

Upper end of supply line **13** is located not less than on 5 m higher than the bioreactor liquid level **18** and it is connected to degassing chamber **12** on 0,3-2 m lower than place of attachment to it of the vacuum pipe **16** opening, which connects vacuum pump with the degassing chamber. In operating conditions in the upper part of degassing chamber **12** the space is free from the liquid, where is created the negative pressure, which varies from -0,05 MPa (-0,5 atm) to -0, 098 MPa (-0,97 atm). The height of the part of the degassing chamber free from

the liquid and applied negative pressure appearing to be in dependence on the mutual arrangement of device elements (methane bioreactor, the device of vacuum degassing and vacuum pump, their elements, conduits) and on the relationship of the pipelines dimensions, and also on the rise velocity of the liquid flow. The totality of these factors provides the rise of the recirculated liquid into the degassing chamber, the intensive degassing of the liquid entering, and also the removal of the isolated gas from the chamber with the action of vacuum pump **15**, preventing in this case the entry of liquid and active sludge into vacuum pump. The separated gas is derived from the vacuum pump through the biogas outlet **17**.

Degasified active sludge gets down from degassing chamber **12** on drain pipe **14** to the entrance of recirculation pump **8** and further will be pumped into the lower part of methane bioreactor **2**. Thus, pre-treated in vacuum chamber sludge is freed from gas bubbles, which possesses large specific weight and higher purifying ability than sludge before degasification. Furthermore, under the conditions of vacuum degassing the concentration of dissociated in the water phase carbon acid and hydrosulfuric acid is reduced, as a result of which rises the alkaline reaction and the value of pH of the liquid, which is returned from the system of recirculation to methane bioreactor. This creates in methane reactor optimal environment conditions for the vital activity of the methane-forming bacteria, this in turn, increases the possibility of organic substances decomposition in methane reactor.

Recirculation pump **8** is supplied with pipe divider **10** for the adjustable distribution of the flow between its two branches. Through one branch the recirculated liquid will be given into the lower part of methane bioreactor, through another branch - into the collector of the surpluses of active sludge **11**. This ensures maintenance in the bioreactor of an optimum concentration of microbial biomass.

In the device according to present invention it is possible send the part of the biogas from the bioreactor gas cavity **19** (through the biogas pipe **20**) into the lower part of the supply line **13** of degassing device **7**. This increases the speed of the recirculation flow through degassing chamber **12** and intensifies the detachment and release of gas bubbles. For guaranteeing the continuously variable control of the system operation regime of waste-water treatment following gate-valves are used: **21, 28 - 32, 35 – 38**.

4.2.4.3. Device test in practice

To evaluate the influence of vacuum degassing technology on anaerobic wastewater treatment system following tests were conducted in practise. The co-work of methane bioreactor together sludge de-gassing unit depending on hydraulic load and operating time was investigated. One of two in parallel working identical methane reactors with a capacity of 180 m^3 (R1) was

equipped with the device of degassing active sludge. Second methane reactor (R2) left to work in the previous regime, without the device of degassing active sludge. The results of tests are represented in the following table:

Table 9. Comparison of reactors operation performance

	Reactor 1 (equipped with sludge vacuum treatment)	Reactor 2 (without vacuum treatment)
Prior to the beginning of the tests	Hydraulic loading: 3 m^3 h^{-1} Organic loading: 15,8 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 51% Reactor content pH=7,0	Hydraulic loading: 3 m^3 h^{-1} Organic loading: 15,8 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 51% Reactor content pH=7,0
7 days after the beginning of the tests	Pressure in vacuum de-gassing chamber: –0,85 Bar Hydraulic loading: 3 m^3 h^{-1} Organic loading: 16,3 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 57% Reactor content pH=7,2	– Hydraulic loading: 3 m^3 h^{-1} Organic loading: 16,3 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 53% Reactor content pH=7,0
14 days after the beginning of the tests	Pressure in vacuum de-gassing chamber: –0,85 Bar Hydraulic loading: 5 m^3 h^{-1} Organic loading: 17,8 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 60,8 % Reactor content pH=7,5	– Hydraulic loading: 4 m^3 h^{-1} Organic loading: 15,2 kgCOD $m^{-3}day^{-1}$ COD removal efficiency: 52,2 % Reactor content pH=7,0

An increase in the hydraulic load of the methane reactor 2 up to 4 m^{-3} h^{-1} caused in 14 days the partial washing out of active sludge and reduction in the effectiveness of COD removal efficiency down to 52,2%. However, bioreactor equipped with degassing system indicated an increase of COD removal efficiency up to 60,8% even if operated under higher hydraulic and COD loading (5 m^3 h^{-1}, 17,8 kgCOD$m^{-3}day^{-1}$). In the methane reactor 1, equipped with the device of degassing, an increase in the hydraulic load and increased biogas production did not cause any sludge washing out from bioreactor. In spite of an increase in the hydraulic and organic load it was noted a substantial improvement in the effect of purification.

Introduced degassing system for anaerobic bioreactor indicated comparatively good results during exploitation guaranteeing stable operation of anaerobic wastewater treatment stage.

Present invention was protected as intellectual property in form of *Utility Model* EE 00665 U1 “Device for the anaerobic purification of wastewater with obtaining of biogas” starting from 16 July 2007.

5. CONCLUSIONS

Purification of baker's yeast wastewater was experimentally studied. Both lab-scale and full-scale purification equipment were utilized in this study to clarify the most suitable technological setup for anaerobic treatment of yeast wastewaters at Salutaguse Yeast Factory. As the result of experimental study operational parameters for removing of sulfates and avoiding inhibitory effects of sulfides in anaerobic treatment were found and adopted at full-scale wastewater treatment plant using existing equipment.

Combining anaerobic sulfate reduction with biological oxidation of sulfide in studied anaerobic/anoxic treatment scheme showed the best results and might be preferable for the treatment of sulfate rich yeast wastewaters. The main results obtained during experiments with full-scale anaerobic/anoxic reactors system were:

- the purification of baker's yeast wastewater (produced from sugar beet molasses, having high SO_4^{2-} content) was feasible without the use of granulated sludge.
- for controlling pH there was no need to use chemicals.
- the returned anoxic sludge retained methanogenic activity, for methane gas production there was no need to re-inoculate anaerobic reactors.
- during experiments combined anaerobic/anoxic system operated under organic loading rate varied between 12.0-16.0 $kgCODm^{-3}d^{-1}$ with stable COD removal efficiency. COD reduction efficiency (by TCOD) in anaerobic + anoxic stage was up to 80%, in anaerobic + anoxic + SBR stage up to 90% supporting average biogas production 1800 m^3/d (with two anaerobic reactors).

The results of the study carried out with lab-scale equipment demonstrated that the anaerobic sequencing batch reactor (ASBR) is a suitable and effective method for anaerobic treatment of sulfate rich wastewaters from baker's yeast production plant. ASBR scheme demonstrated stable COD removal efficiency (about 80%) when applied organic loading rate varied between 7.7 and 8.0 $kgCODm^{-3}d^{-1}$. At higher organic loading rate (over 8.01 $kgCODm^{-3}d^{-1}$) treatment efficiency decreased.

In the experiments with post-treatment of biologically pre-treated baker's yeast wastewater effluent, coagulation step and ozonation impact on effluent quality were studied.

The application of iron chloride and aluminium sulfate coagulation for post-treatment of anaerobic-aerobic effluents may fulfill the discharge limits to the sewer under iron concentrations around 200 mg L^{-1} and aluminium concentrations around 400 mg L^{-1}. Significant decrease of color substances could be achieved using coagulation (up to 90%). Both iron and aluminium

showed the significant removal of organic COD (40-61%), nitrogen (74-76%) and phousphorous (up to 78%) during coagulation step. Generated during coagulation sludge could be the problem when full-scale process applied. Awaited sludge amount will be 1,05-1,58 m^3 d^{-1} (at 22%DS after sludge dewatering).

Application of ozonation as post-treatment of effluent seems to be promising for yeast wastewater purification. During experimental studies it was established that the post-ozonation of biologically treated yeast wastewater resulted in the reduction of COD by 30–49%, and the consumed ozone dosage (mg ozone per mg of COD removed) ranged from 1.2 to 2.5. The biodegradability of the wastewater, expressed as BOD/COD ratio, generally increased during the ozonation tests. In case of full-scale ozonation system (Salutaguse Yeast Factory) up to 405 kgO_3 d^{-1} is required to decrease the residual COD noticeably. It corresponds to electrical energy of 6885-12150 kWh day^{-1}. There were no significant changes in nitrogen and phosphorous content when ozonation applied.

After the start-up research the wastewater loading was increased to 400 m^3 d^{-1} that was up to 40 % more than the initial loading. Aerobic polishing was started on day 220. The final treatment efficiency of the whole system consisting of two anaerobic reactors, anoxic reactor and 2xSBR for aerobic polishing appeared to be up to 98 % (by TCOD) and over 90 % (by SCOD).

One of the most difficult aspects of the anaerobic system operation is the retention of the sludge in the reactor when high organic loading applied. The influence of vacuum degassing technology was studied. The suitable techniques which helpes to retain biomass in anaerobic digester was developed and tested at full-scale system. Introduced degassing system for anaerobic bioreactor indicated comparatively good results during exploitation guaranteeing stable operation of anaerobic wastewater treatment stage. Anaerobic sludge de-gaasing unit allowed to increase organic loading rate up to 17,8 $kgCODm^{-3}d^{-1}$. In experiments, anaerobic reactor equipped with the device of degassing, an increase in the hydraulic load and increased biogas production did not cause any sludge washing out from bioreactor. In spite of an increase in the hydraulic and organic load it was noted a substantial improvement in the effect of purification. Present invention was protected as intellectual property in form of *Utility Model* EE 00665 U1 “Device for the anaerobic purification of wastewater with obtaining of biogas” starting from 16 July 2007.

Due to the use of molasses as the only economically acceptable carbon source for large scale food biomass production, the effluent of yeast industry is characterized by high COD and nitrogen loads originating from not assimilated residual compounds in the fermentation broth. As these substances are difficult to degrade by any micro-organism, full biological treatment, whether on site in specialized installations or by co-treatment with urban waste waters in

municipal plants, will only remove about 90% of the original COD. Considering this fact and stricter environmental politics in future it is reasonable to look for other alternatives to decrease pollution discharge from yeast industry processing beet or cane molasses. One possible way to decrease COD and nitrogen loads is replacement of raw material with other carbon source for yeast fermentation, for example corn syrup or hydrolyzed starch. These sources are completely utilized by yeast during fermentation and do not contribute pollution. More efficient but energy consuming technologies like evaporation and anaerobic treatment of distillates reveal a COD removal of over 98% of the original load, with the benefits, that products from evaporation can be recycled in the agrarian sector and that biogas may be used for partial energy coverage.

As a result of the performed improvements during full-scale experiments, the 4-year permission from local environmental inspection for further continuing of yeast production at Salutaguse Yeast Factory was achieved in year 2002.

As the result of good suitability of combined anaerobic/anoxic treatment scheme for purify sulfate rich wastewater it was planned to introduce this technology to other yeast factories belonging to Lallemand Inc. For example in year 2007/2008 it was planned to modify and improve existing wastewater treatment plant in Passau, Germany. The first stage of purification will be combined anaerobic/anoxic treatment process.

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List of symbols and abbreviations

ADY	active dry yeast
Al	aluminium
AMP	adenosine monophospate
ANTRIC	anaerobic trickling filter
APS	adenosylsulfate or adenosyl phosphosulfonate or adenosine-5'-phosphosulfate.
ASBR	anaerobic sequencing batch reactor
ATP	adenosine triphospate
BOD	biochemical oxygen demand ($mgO_2\ L^{-1}$)
CM	conduct meter
COD	chemical oxygen demand ($mgO_2\ L^{-1}$)
CSBR	coupled sequencing batsh reactor
dn	ozone dosage mg per mg COD removed
DS	dry solids (%)
$FADH_2$	flavin adenine dinucleotide (reduced form)
FDM	food, drink and milk industries
Fe	iron
G	Gibbs free energy
G'_0	Gibbs free energy at standard conditions
H	enthalpy (J, kJ)
HA	homoacetogens
HOM	hydrogen oxidizing methanogens
HPLC	high-pressure (or high-performance) liquid chromatography
IDY	instant dry yeast
M	molar concentration unit
MB	methanogenic bacteria
MW	molecular weight of compound
N	nitrogen
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide (reduced form)
NRB	nitrate-reducing bacteria
Ntot	total nitrogen
OD	optical density
OHPA	obligatory hydrogen producing anaerobes
OLR	organic loading rate (kg COD $m^{-3}\ d^{-1}$)
ORP	oxidation-reduction potential
P	phosphorous
PP_i	pyrophosphate
Ptot	total phosphorus

rpm	rotations per minute
SBR	sequencing batch reactor
SCOD (COD_{sol})	soluble chemical oxygen demand ($mgO_2\ L^{-1}$)
SRB	sulfate reducing bacteria
SRT	solids retention time
SS	suspended solids (mgL^{-1})
SVI	sludge volumetric index (ml g^{-1} TSS)
TCOD (COD_{tot})	total chemical oxygen demand ($mgO_2\ L^{-1}$)
TSS	total suspended solids (mg L^{-1})
UASB	Upflow Anaerobic Sludge Blanket (reactor)
VFAs	volatile fatty acids
VSS	volatile suspended solids (mg L^{-1})
WWTP	wastewater treatment plant

Greek letters

μ	specific growth rate of microorganisms (d^{-1}, h^{-1})
Δ	difference
τ	generation time of microflora (d^{-1})

Subscripts and superscripts

tot	total
cat	catabolism
met	metabolism
f	fusion
sol	soluble
O_2	oxygen
O_3	ozone

PAPER I

S. Zub, T. Kurissoo, A. Menert, V. Blonskaja.
The anaerobic/anoxic treatment of sulphate-rich wastewater.

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PAPER II

M. Krapivina, T. Kurissoo, V. Blonskaja, S. Zub, R. Vilu. Treatment of sulphate containing yeast wastewater in an anaerobic sequence batch reactor.

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PAPER III

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PAPER IV

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Utility Model EE 00665 U1, The Estonian Utility Model Gazette 3/2007, p.9. ISSN 1023-6546

PAPER V

V. Blonskaja, S. Zub, M. Krapivina. Anaerobic treatment of yeast industry wastes-years of industrial experience.

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10.7
The Anaerobic/Anoxic Treatment of Sulphate-Rich Wastewater

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ABSTRACT

The undiluted wastewater from baker's yeast production contains high average concentrations of organic pollutants (25,020 mg/l total COD; 23,420 mg/l soluble COD), nutrients (1470 mg/l total N, 100 mg/l total P) and sulphates (2940 mg/l SO_4^{2-}). Earlier studies using UASB, SBR and anaerobic/anoxic treatment have shown the latter to be the best scheme for purification of this type of wastewater. Baker's yeast wastewater with a volume of 190 m^3/d was treated with ungranulated anaerobic sludge in a modified anaerobic/anoxic reactors system. The temperature of the anaerobic stage was kept between 30 and 36 ^oC; the pH of between 7.2 and 7.5 was self-regulated by the biological process without neutralization. Analyses of sugar-beet molasses and Baker's yeast wastewater has indicated the presence of betaine and its removal in anaerobic reactors. The average removal efficiency in the anaerobic/anoxic system was: total COD 78.6%; soluble COD, 80.1% and SO_4^{2-} 100%. Application of the modified anaerobic/anoxic system to a full-scale treatment plant supported biogas production up to 1200 m^3/d. Thus it was demonstrated that this scheme is suitable for purification of sulphate rich wastewaters also in full scale.

INTRODUCTION

Many industrial processes, including food and fermentation industry, generate wastewaters containing organic matter and sulphates. Yeast industry wastewaters is low in easily degradable sugars and acids and with high content of betaine and sulphates and is difficult to biodegrade. Sulphate reducing bacteria (SRB) interact competitively with other anaerobic bacteria involved in methanogenesis, resulting in the formation of H_2S, CO_2 and organic acids rather than methane. If discharged into a receiving watercourse this wastewater can cause depletion of oxygen, acceleration of eutrophication and formation of H_2S in the bottom sediments.

When treating high sulphate containing wastewaters, high concentrations of sulphur compounds hinder wastewater treatment and production of methane gas. This phenomenon results from microbiological reduction of sulphates into sulphides and the stability of the treatment process is dependent on the pH value as well as the concentration of sulphides formed. At pH values in the range 7.0 to 7.5 the growth rates of methanogenic bacteria (MB) and SRB are approximately equal. Below pH 7.0 the SRB start to dominate and above pH 7.5 the methanogenic bacteria dominate. Sulphides formed during the treatment process inhibit the growth of MB as well as the SRB in the pH range of 7.2-8.5[1].

Versprille[2] proposed a method for reducing the sulphide content of the Salutaguse Yeast Plant wastewaters by dilution with the non-contaminated cooling water. Although an efficient method for reduction of pollutant concentration, it does not harmonize with the environmental protection strategies of the HELCOM recommendations that prohibit achieving the established limit value for pollutants at the expense of dilution.

It is possible to remove hydrogen sulphide produced in the anaerobic reactor from the sulphates by partly oxidizing it into elemental sulphur. This process can be performed in an anaerobic reactor where the concentration of oxygen is below 0.1 mg O_2/l. The elemental sulphur formed can be removed in the sedimentation tank[3]. The wastewater circulates from anaerobic reactor to the subsequent aerobic reactor and from there back to the anaerobic reactor. This method allowed 95% removal of sulphates and the residual concentration of sulphides in the outlet of the treatment system was below 20 mg/l while also facilitating stable pH conditions[4]. In the Chinese patent N°1144782, 1997 the removal of sulphides from anaerobic reactor has been solved by feeding the reactor with a controlled concentration of O_2 or air[5]. A similar method has been used also in the Netherlands[6] and United States[7] without observing any inhibiting effect on the M B. Industrial[8] as well as laboratory

experiments[9] have shown that sulphide containing wastewater leaving anaerobic reactors does not inhibit the activated sludge process in the aerobic reactor. During the aerobic purification process the COD decreased and pH of the wastewater increased indicating the decomposition of organic compounds (including organic acids), formation of ammonia from amine compounds, formation of elementary sulfphur S^0 from sulphide ions and evaporation of H_2S into the atmosphere during ventilation.

The equipment for biological purification of separation residues of baker's yeast at Salutaguse (built by a Finnish Contractor, Tampela) has been in operation since 1991, but has never performed satisfactorily. Thus the aim of this work was to achieve the optimal setup and operational parameters for removing of sulphates and avoiding inhibitory effects of sulphides in anaerobic treatment of yeast industry wastewaters.

THEORY

There are generally two types of methods available for the removal of sulphur containing compounds: physicochemical methods and biological methods[10]. Physicochemical treatment methods include precipitation, ion exchange and membrane filtration (electrodialysis and reverse osmosis). The disadvantages of these methods are high costs and the large stream of waste that results.

In the case of biological treatment, sulphate, sulphite and other sulphur compounds are reduced in an anaerobic step to give sulphide, which in tum can be oxidized to elemental sulphur[8]. For reducing sulphur compounds to sulphide an electron donor is necessary, as follows from the reaction:

$$SO_4^{2-} + 5\ H_2O + 8\ e \rightarrow HS^- + 9\ HO^- \qquad (1)$$

Biotechnological processes for sulphide removal consist in conversion of sulphide into elemental sulphur by colourless sulfur bacteria (*Thiobacilli*) [3,11], according to reaction (2):

$$2\ HS^- + O_2 \rightarrow 2\ S^0 + 2\ OH^- \qquad (2)$$

or by genera of anaerobic photosynthetic bacteria from the families *Chlorobiaceae* and *Chromaticeae* that catalyze the photosynthetic van Niel reaction [12]:

$$2n\ H_2S + n\ CO_2 \xrightarrow{nv} 2n\ S^0 + (CH_2O)_n + n\ H_2O \qquad (3)$$

In the latter case light radiated to a photosynthetic reactor is coupled to the conversion of sulphide to elemental sulfur using the reverse citric acid cycle (Amon cycle). The advantage of such method is that only small waste streams remain because the sulphur 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, H_2 and CO) have to be added in order to provide sufficient reduction equivalents for the SRB. Organic compounds having two or more carbon atoms degrade under anaerobic conditions to give H_2 and acetate. The H_2 can be used as an electron donor for reduction of sulphate and sulphite and the like, but under normal conditions, about 50% of the acetate is converted to methane by M B. Thus an additional electron donor has to be added[8] which increases the costs of this method substantially.

Sugar-beet molasses used as growth medium for yeast contains large amounts of betaine (up to 6% w/w). In anaerobic treatment plants, betaine is almost totally degraded by a multistep degradation process with the nitrogen-containing intermediates trimethylamine and other methylated amines, which are further degraded by methanogenic bacteria, yielding CO_2, ammonium and methane[13]. Ammonium produced during the digestion leads to an increase in alkalinity.

MATERIALS AND METHODS

Experimental setup

Wastewater from the yeast production plant, processing beet sugar molasses was divided into high strength wastewater and wash water. High strength wastewater was pumped to mixing tank and wash water was sent directly to anoxic stage. The anaerobic reactors were fed with mixture of high strength wastewater and recycled anoxic sludge. The temperature of wastewater from the incoming yeast production was 28 to 33°C. The flow rate of the incoming wastewater was measured by Baily Fisher Porter MAG-XM (CM) flow meters. Reactors feed and internal recycling flow rates measurements were conducted using Danfoss MAG1000/1100 electromagnetic flow meters. The equipment for biological purification of separation residues of baker's yeast at Salutaguse of Tampela consisted of an anaerobic pre-treatment stage (mixing tank of 180 m^3 with a stirrer and two upflow anaerobic sludge blanket (UASB) reactors each of 180 m^3 volume), followed by aerobic stage (activated sludge with a 300 m^3 aeration tank) and a secondary sedimentation tank (45 m^3) for final treatment before discharge via a ditch to river. The novel technological scheme used in this study is presented in Figure 1. It differs from the originally designed set-up in the following: a) the anaerobic stage is followed by the anoxic stage; b) part of wastewater finally leaving the secondary sedimentation tank is re-circulated back to the inlet, i.e. the to the mixing tank; c) for final purification of the effluent the aerobic sequencing batch reactor is used.

For the experimental set-up, two anaerobic UASB reactors of 180 m^3 of reaction volume were operated during an experimental period of more than 166 days. All the experiments were performed on the working treatment system without any stop in the technological process. The reactors were inoculated with ungranulated anaerobic sludge. Reactor 1 was

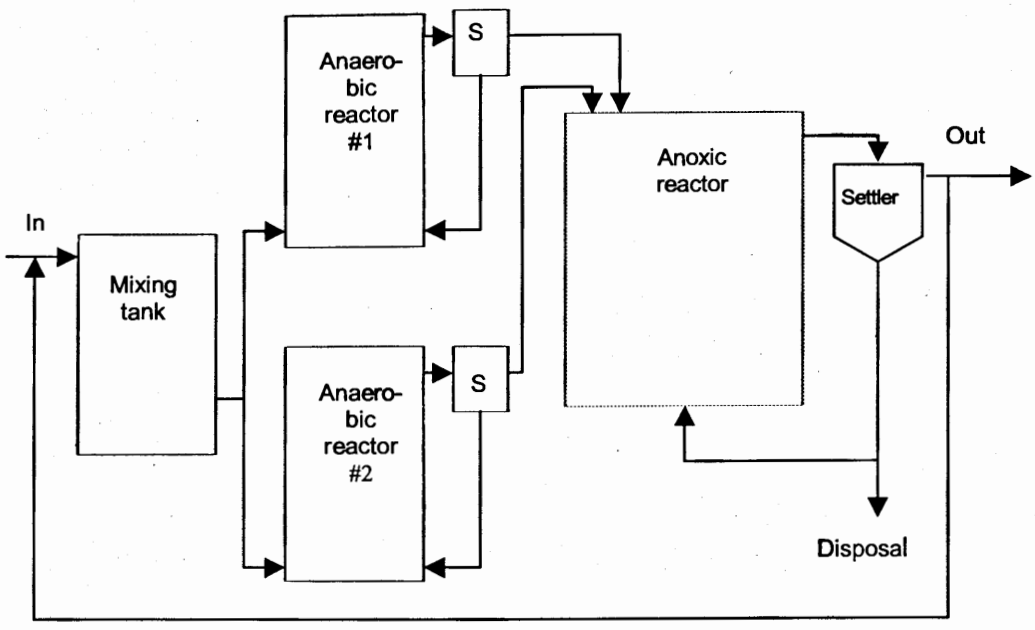


Figure 1: *The technological set-up used in the Salutaguse Yeast Factory (Kohila, Estonia) with the re-circulation of the outlet flow from the anoxic reactor back to the anaerobic reactors. S- settler.*

inoculated with anaerobically digested excess sludge, brought from the Tallinn Municipal WWTP, reactor 2 was also inoculated with excess activated sludge from the factory itself (collected from the anoxic reactor). Temperature was maintained at 30 to 36^0 C by contact steam injection. Temperature monitoring electrodes were installed direct to the reactor wall (PT-100) and connected with the controller, which controls the steam injection pneumatic valves.

Characteristics of wastewater

The production capacity of wastewater from the Salutaguse Yeast Factory (a subsidiary of Lallemand Inc.) is 99000 m^3/y originating for 100% from beet molasses. The separation residue is characterized by high BOD (up to 12,000 mg O_2/l) and COD (up to 25,700 mg O_2/l by dichromate method) values. Sulphur is present in the separation residue basically as sulphate ions (up to 5700 mg/l). The equipment for biological purification of separation residues of baker's yeast at Saluatguse was constructed in 1991. Due to the high sulphate concentration and lack of appropriate technological instruction for purification of high sulphate containing wastewater, the anaerobic digesters were only put into continuous operation in September 2000. The wastewaters of the Salutaguse Plant (Table 1) consist of five wastewater streams: first separation (high concentrated wastewater), wash water, cooling water, molasses clarification / cleaning (limited amounts, depends on type / quality of molasses, this stream is included in the high concentrated wastewater), municipal wastewater (limited amount, directly to anoxic stage).

Chemical analyses

The total COD, sulphate and total sulphide, the volatile suspended solids (VSS) content of anaerobic sludge samples and settled sludge volume were analyzed as described in Standard Methods for the Examination of Water and Wastewater, 1989. Influent and effluent liquid samples were analysed 3 days per week. Analyses of COD, sulphates and 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; Sulphide – Methylene Blue Method, US EPA accepted for reporting wastewater analysis

RESULTS AND DISCUSSION

The UASB reactor is often not applicable for the treatment of high sulphate containing wastewaters [14]. The observed instability and increased wash-out of sludge granules can be explained by the fact that under stress conditions, all energy gained by bacteria from dissimilation is used for generation of metabolic products, not for the growth of cells[15]. However, in the modified anaerobic/anoxic reactors 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 a good purification efficiency.

One explanation of the high values and stability of pH in the reactors is the fact that sugar-beet molasses used as growth medium for yeast contains large amounts of betaine. The ammonium buffering the treatment system has been evidently formed from betaine. Cleavage of betaine into trimethylamine and acetate is characteristic of some halophilic fermentative bacteria [16]. The strain *Haloanaerobacter salinarus* sp. nov. grows by fermenting carbohydrates or by using the Stickland

Table 1: *The average wastewater characterisitcs of Salutaguse Yeast Plant, Estonia*

Waste stream	Flow ($m^3 d^{-1}$)	COD excl. betaine		COD incl. betaine (+20%)		BOD		Sedimentable solids (SS)		Total Kjeldahl nitrogen (TKN)		Phospho-rous (P)		SO_4^{2-}		t (°C)	pH
		Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)	Concentration ($mg L^{-1}$)	Loading ($kg day^{-1}$)		
High strength wastewater in holding tank	70	20 000	1 400	24 000	1 680	8 000	560	500	35	2 000	140	30	2.1	4000	280	40	4 - 5
Wash water	100	3 000	300			2 000	200	500	50	100	10	20	2	400	40	14	6 - 10
Cooling water	800															28 - 30	7
Municipal water	No data, limited amount																

**Not shown in the technical scheme on figure 1*

Table 2: *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 ($m^3 d^{-1}$)	TCOD ($mg O_2 L^{-1}$)	Decrease of TCOD (%)	SCOD ($mg O_2 L^{-1}$)	Decrease of SCOD (%)	SO_4^{2-} ($mg L^{-1}$)	Decrease of SO_4^{2-} (%)	S^{2-} ($mg L^{-1}$)
Holding tank*	190	19 020		18 260		4 200		
+ 20% from betaine		22 824		21 912				
Mixing tank	137	15 540	23.2	12 980	40.8	2 900	31	36.2
Anaerobic reactor #1	72	11 380	35.1	8 250	36.4	400	86.2	360
Anaerobic reactor #2	65	4 850	72.3	4 080	68.6	0	100	390
Influent to anoxic reactor (theoretical)	190	12 338		10 634				
Effluent from anoxic reactor	190	4 890	60.4	4 360	59	0	100	37.2
Total treatment efficiency			78.6		80.1			

reaction with either serine or H_2 as electron donors and glycine-betaine as acceptor, which is reduced to trimethylamine:

2.5 glycine-betaine + 4.04 H_2 →

2-propanol + 2.5 trimethylamine + 0.95 acetate + 0.1 CO_2 +1.9 H_2O (4)

glycine-betaine + 1.32 serine + H_2O →

trimethylamine + 2 acetate + 1.32 CO_2 + 1.32 NH_3 (5)

A similar cleavage mechanism for glycine-betaine under anaerobic conditions has also been reported for *Clostridium sporogenes* [17,18], while the fermentation products of *Eubacterium limosum* are *N,N*-dimethylglycine, acetic acid and butyric acid [18,19]. The resulting trimethylamine and other methylated amines are further degraded by methanogenic bacteria, yielding CO_2, ammonium and methane. 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 [20].

$CH_3COOH \rightarrow CH_4 + CO_2$ (6)

$4\ (CH_3)_3N + H_2O \rightarrow 9\ CH_4 + 3\ CO_2 + 6\ H_2O + 4\ NH_3$ (7)

Thus considering the previously mentioned latest achievements in treatment of high sulphate containing wastewaters, it was decided that there was no need for the urgent change of molasses preparation technology nor of the chemical composition of mineral salts solution used for the cultivation of the yeast culture based on sugar beet molasses. Instead the scheme presented in Figure 1 was used. This set-up supported the creation of more favourable conditions for the methane-producing bacteria and avoided their takeover by the sulphate reducing bacteria. The concentration of dissolved oxygen in anoxic reactor was kept strictly below 0.1 mg O_2 /l, enabling a continuing decrease in the sulphide content.

Change of process parameters during the treatment process

The previously described setup for the purification system guaranteed rapid changes in the sulphate and sulphide content of the anaerobic reactors. Recirculation of part of the wastewater leaving the sedimentation tank back to the inlet, i.e. to the mixing tank initially increased the concentration of sulphates in the outlet of anaerobic reactors. However the concentration of sulphides did not change much (Figures 2a and 2b). Despite the origin of the inoculation sludge (excess sludge from the municipal WWTP), after a slight initial increase, the concentration of sulphates started to decrease constantly, reacing zero after 35 days. The concentration of sulphides in the anaerobic reactor 1 increased to some extent on the account of increasing feed, while there was no evident correlation between the concentration of sulphides and the hydraulic loading rate in the reactor 2. Simultaneously with the drop in the concentration of sulphates the COD value of wastewater in the mixing tank (inlet) also decreased, caused by the dilution effect resulting from re-circulation from the sedimentation tank (Figure 3).

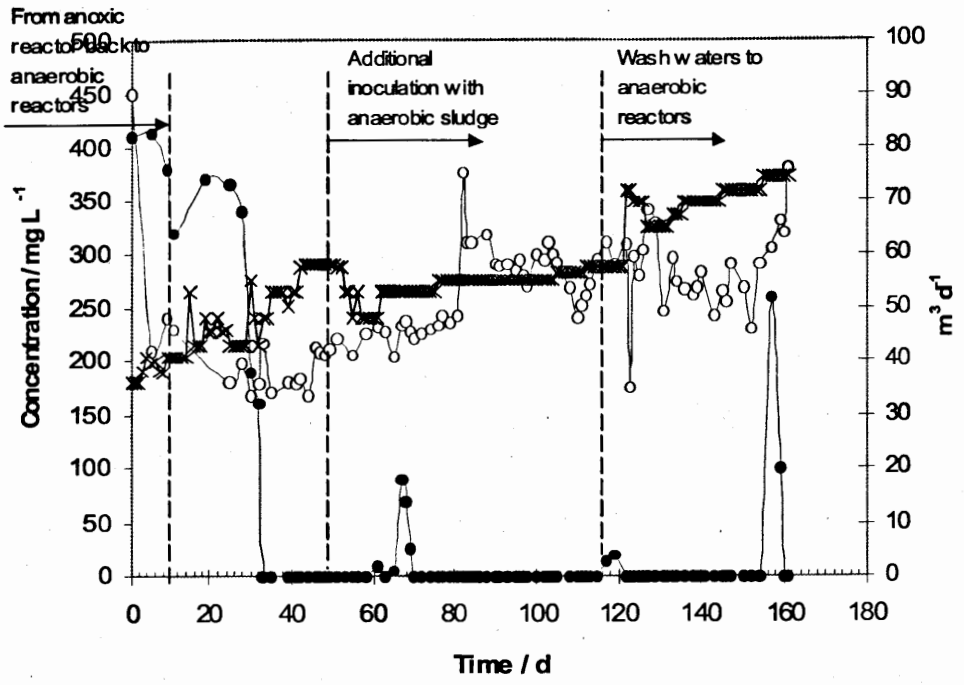
On the 51 day of experiment reactor 2 was supplemented with an additional amount (50 m^3) of activated sludge, collected from the anoxic reactor. Thus the necessity of transportation of it from the Tallinn Municipal WWTP was avoided as well as the possible contamination of the reactor with fine particles of sand. This supplementary inoculation of reactor 2 reduced its effluent contamination (expressed as COD), in spite of the increasing contamination of the influent from the mixing tank (Figure 3). Simultaneously there was a sharp decrease in the VFAs content in the effluent from reactor 2 (Figure 4).

As a result of the use of the modified anaerobic/anoxic reactors system the biological purification process had already started in the mixing tank. This phenomenon is illustrated with data for chemical analyses presented in Table 2. The data presented in Table 2 demonstrates that the COD of the supernatant of the sample from the mixing tank (SCOD) has been decreased up to 40% and the sulphide content up to 31% as compared to the corresponding values in the holding tank. In the mixing tank the concentration of sulphides originating from sulphates is 36.2 mg/l. These results confirm that elaboration of the novel technological setup of wastewater treatment has converted also the mixing tank into a biological reactor.

The HPLC analyses (data not presented) of molasses, separation residue, samples from holding tank, mixing tank, anaerobic reactors and anoxic reactor have shown that the betaine present in the wastewater is already degraded in the mixing tank. Therefore it would be reasonable to add to the COD concentration in the holding tank an additional 20% of hidden contamination load at the expense of betaine[2]. Thus the treatment efficiency of the whole system appeared to be 78.6%. Achieving this better treatment efficiency turned out to be directly dependent on the operation of the anaerobic reactors. During the setup period the values of total COD (TCOD) in the anaerobic reactor 1 were in the range of 3,520 to 13,520 mg O_2/l and in the anaerobic reactor 2 in the range of 3,830 to 16,270 mg O_2/l.

The concentration of betaine in the industrial wastewater of the Salutaguse Yeast Factory may be up to 3,750 mg/l. In redox reactions the nitrogen (oxidation state +5) contained in betaine can be an electron acceptor for two electrons. On the other hand, the sulphide ion can donate two electrons and thus be converted into elemental sulphur. Considering the stoichometric ratio in the chemical reaction between betaine (ammonia)

a)



b)

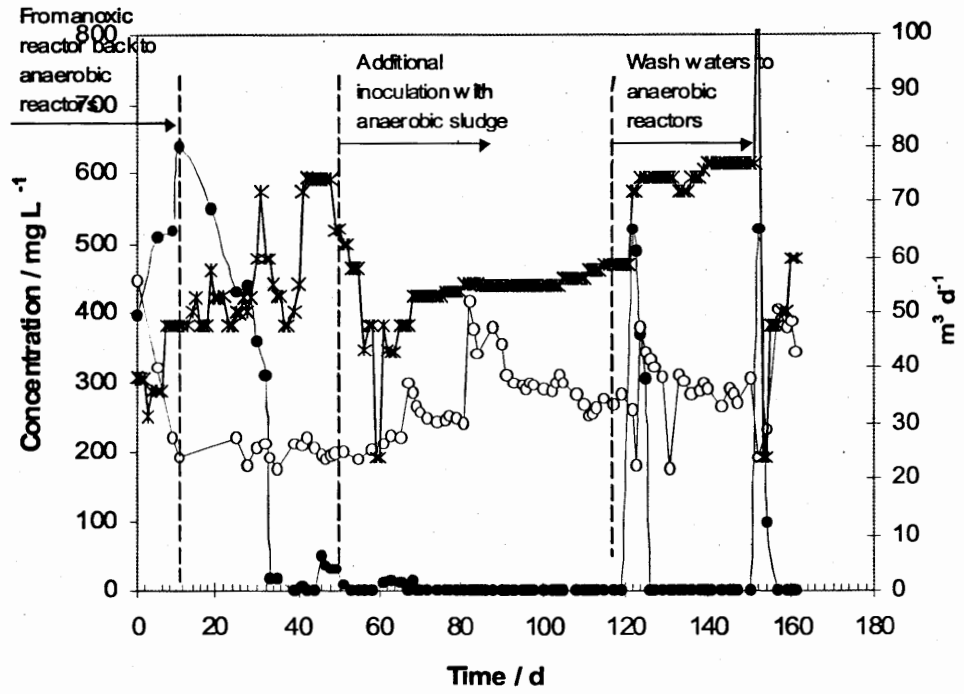


Figure 2: *Hydraulic loading rate (feed, $m^3 d^{-1}$) and the content of sulfates and sulfides (mg L^{-1}) in the effluent from a) anaerobic reactor #1, b) anaerobic reactor #2. • - sulfates, o - sulfides, x - feed.*

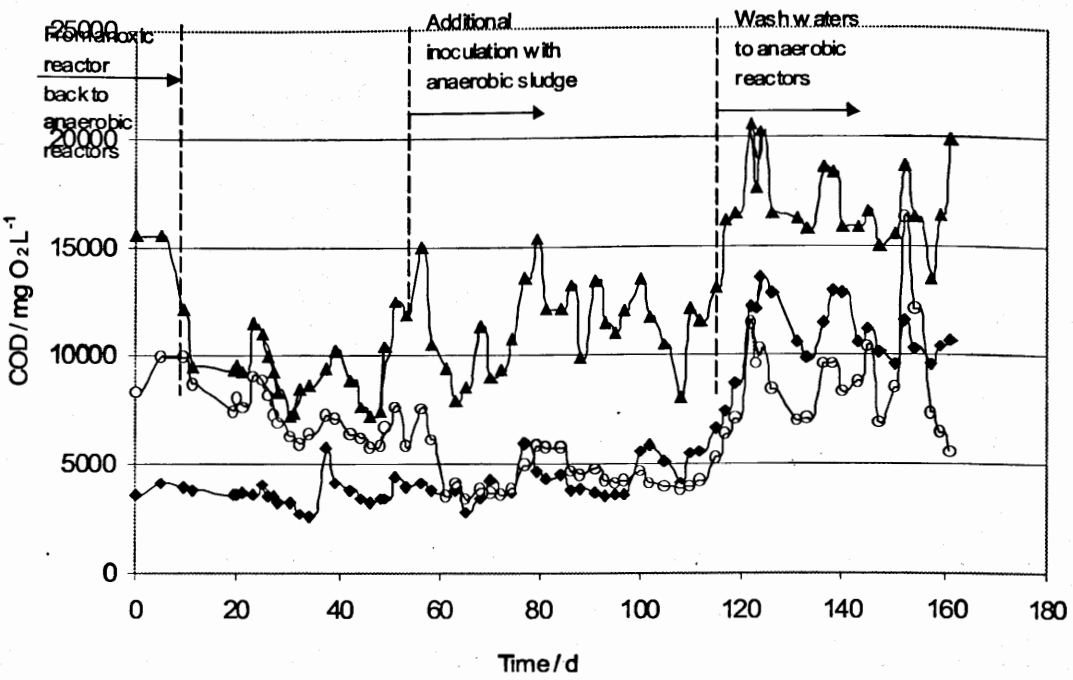


Figure 3: *Total chemical oxygen demand (TCOD) values of anaerobic reactors: ▲ - TCOD of the inflow to the mixing tank, ◆ - TCOD of reactor #1, O - TCOD of reactor #2.*

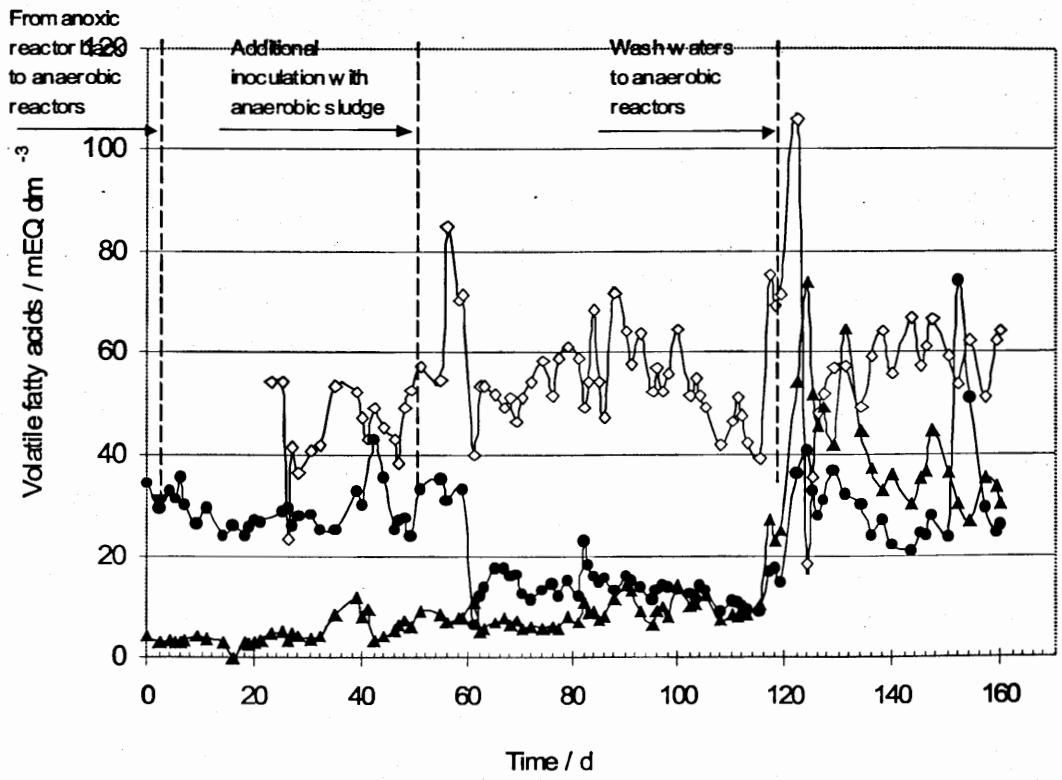


Figure 4: Concentration of volatile fatty acids (VFAs) ◇ - in the mixing tank, ▲ - in the effluent from anaerobic reactor #1, ● - in the effluent from anaerobic reactor #2.

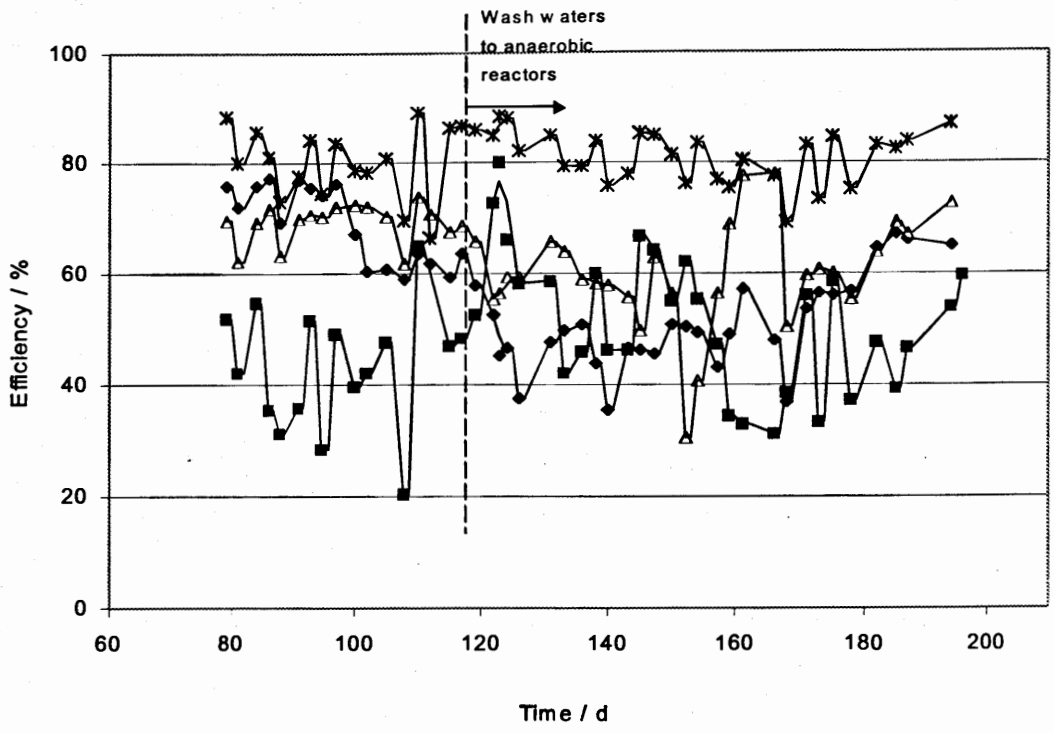


Figure 5: *The treatment efficiency by the total COD value (TCOD): ◆ - anaerobic reactor #1, Δ - anaerobic reactor #2, ■ - anoxic reactor, × - the whole system.*

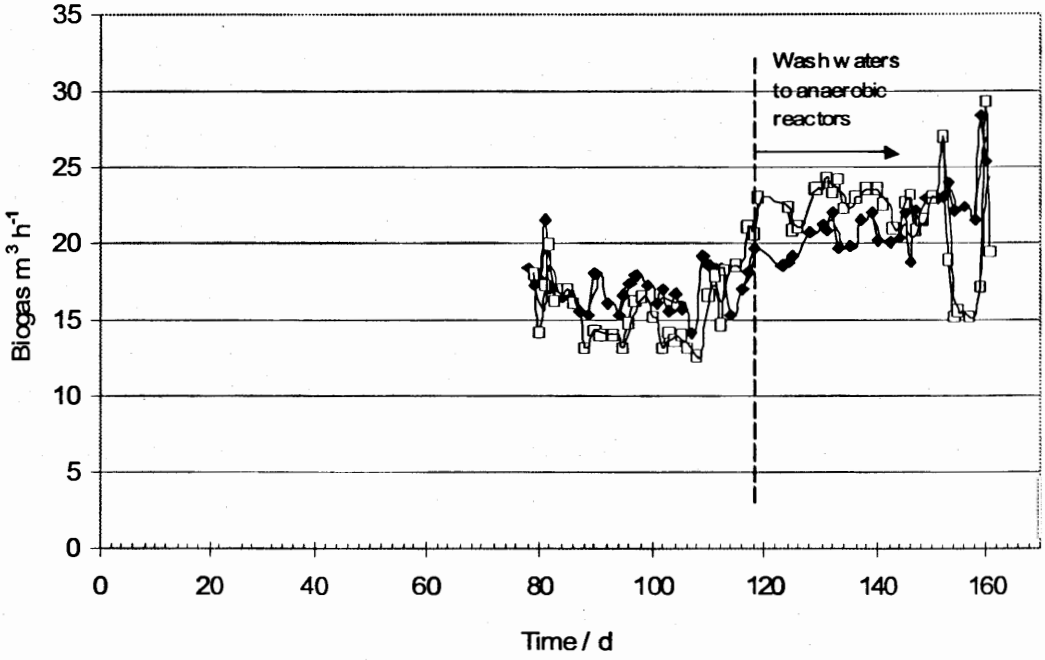


Figure 6: *Production of biogas in the anaerobic reactors: ◆- reactor #1; □ - reactor #2.*

and the sulphide ion (sulphate ion)[20],

$$SO_4^{2-} + 2\ NH_4^+ \rightarrow S^0 + N_2 + 4\ H_2O \quad (8)$$

consisting of the following reactions:

$$SO_4^{2-} + 4\ NH_4^+ \rightarrow S^{2-} + 4\ NO_2^- + 4\ H_2O + 8\ H^+ \quad (9)$$

$$3\ S^{2-} + 2\ NO_2^- + 8\ H^+ \rightarrow N_2 + 3\ S^0 + 4\ H_2O \quad (10)$$

$$NO_2^- + 2\ NH_4^+ \rightarrow 2\ N_2 + 4\ H_2O \quad (11)$$

the concentration of sulphide ions can be decreased by 1,026 mg at the expense of 3,750 mg betaine. The concentration of sulphates in the holding tank was 4,200 mg/l from which the sulphate reducing bacteria are able to produce 1,400 mg/l sulphides (Table 2). If the concentration of sulphides in the wastewater can be reduced at the expense of betaine, then the residual concentration of sulphide ions should be 1,400 – 1,026=374 mg/l. At the end of the experiment the concentration of sulphides in the anaerobic reactor 1 was measured as 360 mg/l and in the reactor 2 as 390 mg/l giving an average of 375 mg/l. The fluctuations of 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.

The inter-relation of anerobic and anoxic stages

According to the technological set-up presented in Figure 1, the biological processes in the anaerobic reactors and in the anoxic reactor are inter-related by the returned sludge from the secondary sedimentation tank (by the anoxic reactor) to the inlet of the mixing tank. Evaluation of the efficiency of anaerobic and anoxic stages as well as the total efficiency of the system has demonstrated that leading the wash waters back to the holding tank improved the efficiency of the anoxic stage (Figure 5). One possible reason may lay in the resulting increased concentration of VFAs in the effluent from anaerobic reactors entering the anoxic reactor. This increased concentration of VFAs forces the bacterial consortium in anoxic reactor to metabolize more substrate using more oxygen for it and thus increasing the efficiency of this stage. Thus the performances of the anaerobic stage and the anoxic stage counterbalance each other guaranteeing the relative stability of the whole system (Figure 5).

Biogas production

Biogas measurement was started on day 76. Leading wash waters to the mixing tank increased the production of biogas slightly (Figure 6). As a result of an additional inoculation with adapted anaerobic sludge from anoxic reactor the biogas production from reactor 2 was more intensive. The maximum biogas production achieved was up to 25 m^3 /h in both reactors.

CONCLUSIONS

The existing technological set-up is still not optimal as the total treatment efficiency based on COD (about 80%) is still not comparable with the efficiency of sulphate reduction (100%). The reason might lay in sedimentation properties of the sludge and in hydraulic loading to the reactors, which is too high. As stated before, the reactors were initially inoculated with anaerobically digested excess sludge from the municipal WWTP, instead of granulated sludge or plastic carriers with fixed film. As a result the proportion of sludge carried away from the anaerobic reactors was high and part of the sludge in reactors was not sufficient for the acetogenic / methanogenic consortia to completely metabolize the organic acids. The intensive carrying away of sludge started directly after leading the wash waters to the mixing tank. The latter was in good correlation with the COD values of the effluent from both anaerobic reactors (data not shown). Comparing the data on VFAs and COD in the effluent of the anaerobic reactors and in the effluent of the anoxic reactor it was concluded that the success of this novel type of purification process, based on the recirculation system with the use of returned sludge depends most critically on avoiding the loss of sludge from the reactors.

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Treatment of sulphate containing yeast wastewater in an anaerobic sequence batch reactor

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Abstract. Anaerobic mesophilic fermentation of sulphate containing yeast industry wastewaters at laboratory scale with anaerobic sequence batch reactors (ASBR) was studied. Three different treatment schemes were investigated – ASBR with and without a polymeric filler and coupled microaerophilic/anaerobic SBR (CSBR). The optimal concentration of sludge (total solids 17.3 g L^{-1}) in the reactor and the optimal reaction time (22 h) were determined. It was shown that in the case of ASBR efficient treatment characterized by chemical oxygen demand (COD) removal of 75–82% took place at volume loading rates up to 7.7–8.0 kgCOD m^{-3} d^{-1} and at COD/$(SO_4)^{2-}$ ratio 8.0. In optimal conditions the methane content of the biogas was 60%. The best results for sulphate removal (99%) were achieved in the CSBR with the concentration of sulphide in the reactor effluent being about 10 mg L^{-1}. Decreasing treatment efficiency after a long-time exploitation of these reactors occurred as a result of the formation of insoluble sediment (presumably $CaCO_3$ and $Ca_3(PO_4)_2$).

Key words: anaerobic sequence batch reactor (ASBR), calcium precipitates, high strength wastewater, sulphate reduction, yeast industry.

INTRODUCTION

Wastewater from the yeast industry contains extremely high concentrations of COD (up to 30 g L^{-1}) and sulphate (up to 4.5 g L^{-1}). For the treatment of high strength wastewaters anaerobic digestion appears to be economically more

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attractive than aerobic processes. Two important goals are achieved simultaneously in anaerobic processes: removal of organic matter and sulphates.

Advantages of anaerobic digestion include also relatively low sludge production and low energy need compared with aerobic treatment. However, a high sulphate content can lead to the destabilization of the anaerobic treatment processes due to the hydrogen sulphide formation [1], especially if COD/$(SO_4)^{2-}$ is below 10 [2]. Despite these difficulties anaerobic digestion has been successfully applied for the treatment of a variety of sulphate-rich wastewaters both at laboratory and full-scale levels [1]. In comparison with continuous anaerobic methods, anaerobic digestion is a more flexible and cost-effective treatment technology [3]. However, there are no reports in the literature on the treatment of sulphate-rich wastewaters using anaerobic sequence batch reactors (ASBR).

The main aim of this research work was to study the treatment process of sulphate-rich high strength wastewaters from a yeast production plant using ASBR technologies.

MATERIALS AND METHODS

Experimental set-ups

Three different schemes of laboratory-scale experimental set-ups of ASBR were used. In the first experimental set-up (Fig. 1) a stand-alone ASBR was used. The ASBR with an active liquid volume of 0.7 L was made of glass tubing of 0.145 m × 0.075 m (diameter). Plastic tubes were attached to the filling and

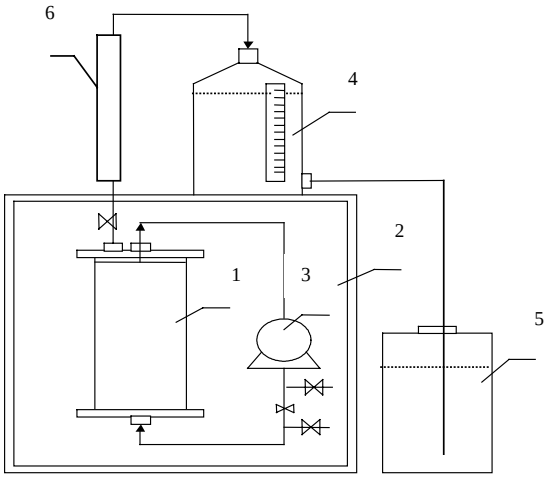

Fig. 1. Laboratory set-up for an anaerobic sequencing batch reactor. 1 – ASBR, 2 – thermostat, 3 – peristaltic pump, 4 – wet gas meter, 5 – water collector, 6 – alkali lock.

drawing ports. Peristaltic inflow pumps (Zalimp, Poland) were used at rates of 0.48–0.51 L h^{-1} to fill the reactor, draw off the effluent, and to mix the suspension during the treatment process. The temperature was maintained constant (35 ± 2 °C) during the operation by a thermostat. Methane gas production was measured using a wet gas meter after absorption of CO_2 and H_2S in a scrubber with 10% NaOH solution.

In the second scheme the ASBR was loaded with a polymeric filler (Water Group, Germany): 0.8 cm × 1.0 cm diameter, with a conditional surface area of 640 m^2 m^{-3}. The volume of carriers was 0.5 L. Otherwise the experimental set-up was as in the first case.

In the third set-up, a coupled sequence batch reactor (CSBR) where the anaerobic effluent from the ASBR was recycled through a microaerophilic system was applied. Mixing in the microaerophilic reactor was carried out using a magnetic stirrer with regulated stirring speed (Beco, MM-5, 220 W). The biogas from the anaerobic reactor was passed to the microaerophilic reactor with the recycling effluent. The anoxic reactor was open and the temperature of the water was the same as the temperature of the air in the room (20 ± 2 °C). The oxygen concentration was kept at 0.1–0.15 mg L^{-1}.

Operating cycle parameters

The operating cycles of the ASBRs in all three set-ups consisted of three stages: (1) filling and decanting stage – this was accomplished by replacing the upper layer of the liquid in the reactors (effluent) with the lower layer adding influent to the bottom of the reactor, (2) reaction stage with uninterrupted agitation (by suspension recycling), and (3) sludge settling stage. The total cycle length was 24 h made up of 23 h of reaction–agitation, 0.5 h at rest for settling, and 0.5 h for filling and drawing (Fig. 2).

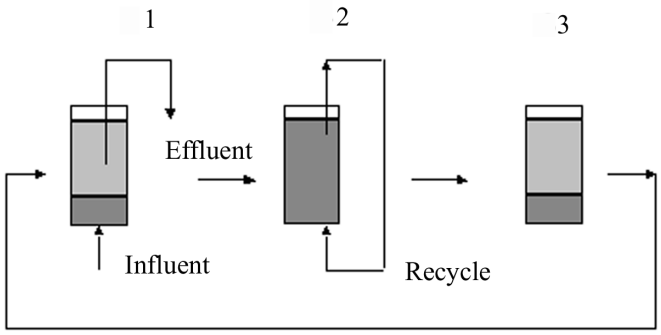

Fig. 2. Operation cycles of an ASBR. 1 – filling and decanting cycle, 2 – reaction cycle, 3 – sludge settling.

Sludge used

Two types of seed sludge were used for comparing the efficiencies of the processes. Anaerobic sludge from the anaerobic digester of the municipal wastewater treatment plant (WWTP), Tallinn, Estonia, which was not adapted for the treatment of sulphates, was used in the first two experimental set-ups, and sulphate adapted anaerobic sludge from full-scale anaerobic digesters of a yeast plant (AS Salutaguse Pärmitehas, Estonia) was used in the case of the CSBR.

Morphology of the sludge

The morphology of the seed sludge and of the sludge at the end of the experiments was investigated using microscopy. Sludge samples of 10 mL were washed with 10 mL of distilled water and allowed to settle while the turbid layer was drained. The procedure was repeated until the water became transparent. The washed sludge samples were placed into a 3.5 cm Petry dish and studied.

Microscopy of the structure of the seed anaerobic sludge from Tallinn WWTP showed that the sludge was of granulated type. The approximate size of granules was 1.7–2.0 mm. The sludge was mixed with sand, which seemed to be a good carrier of the sludge granules.

Investigation of the structure of the adapted to the sulphates seed anaerobic sludge from the Salutaguse yeast plant showed that the sludge was of flocculated type with a small percentage of single granules. The approximate size of granules was 0.5 mm. The activated sludge used in the CSBR experiment for seeding the microaerophilic reactor was completely flocculated.

Sulphate-rich high strength yeast production wastewaters

The reactors were fed with wastewater from the full-scale yeast production plant of Salutaguse (Estonia). The chemical composition of the wastewater was as follows: total COD 14.4–25.7 g L^{-1}, SO_4^{2-} 3.5–5.3 g L^{-1}, COD/SO_4^{2-} 2.71–7.63, total solids 12.9–21.6 g L^{-1}, total N 250–350 mg L^{-1}, total P 17.3–48.2 mg L^{-1}, trimethylglycine 3.7–4.0 g L^{-1}. Prior to treatment the wastewater was stored at 4 °C to prevent premature denaturation.

Sampling and monitoring

The production of biogas in anaerobic reactors, the influent and effluent pH, and the temperature of the sludge were measured daily. For the pH determinations a pH meter (E6121, Evicon) was used. Dissolved oxygen concentration in the microaerophilic reservoir was controlled twice a day by a conductivity and dissolved-oxygen meter (WTW.GMBH, M325/Oxi-L5). The COD, total solids (TS), sulphate, and total sulphides concentrations in the effluent were measured weekly, dissolved phosphorous and total nitrogen contents were analysed twice a

month. In all cases standard procedures described in standard methods [4] for wastewater examination were used. Effluent samples were drawn from the ASBR upon completion of the 30 min decant cycle. Sulphide and sulphate contents were determined immediately. The COD and phosphorous samples were frozen before analysis. Completely mixed samples were taken from the ASBR reactor before and after the end of the experiments and used for TS determination. The biogas composition was determined with gas chromatography.

RESULTS AND DISCUSSIONS

The optimal concentration of sludge for the start-up in usual anaerobic processes is 30–40% of the volume of the reactor, about 15 g L^{-1} of the sludge [5].

The start-up experiments with three different amounts of seed sludge were carried out during 47 days. Three identical reactors (first scheme) were seeded with 30% (TS 12.9 g L^{-1}) of anaerobic sludge obtained from the anaerobic digester (municipal WWTP, Tallinn, Estonia), 40% (TS 17.3 g L^{-1}), and 50% (TS 21.6 g L^{-1}), respectively. To allow biomass to adapt to sulphate-rich wastewater the sludge load was increased step-by-step (5% weekly). During the start-up period the organic load rate (OLR) was gradually increased from 1.4 kgCOD m^{-3} d^{-1} to 7.1 kgCOD m^{-3} d^{-1} (Fig. 3) and hydraulic retention time (HRT) was changed from 10 and to 2.5 days, respectively.

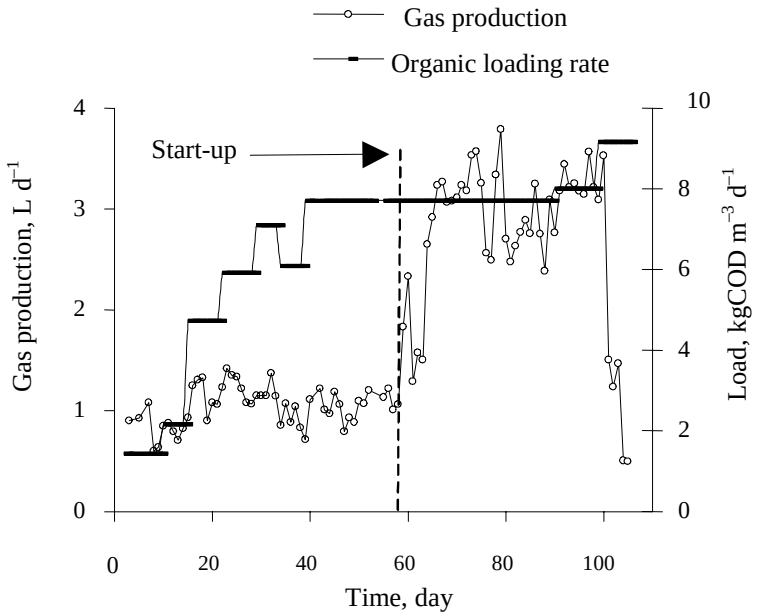

Fig. 3. Organic load and gas production during the experiment.

During a month following seeding the OLR of 7.7 kgCOD m^{-3} d^{-1} was maintained and then the reactors were operated at a constant OLR value. The efficiency of the treatment process during the start-up was monitored by biogas production (Fig. 4). A faster start-up of the reactor inoculated with 40% of sludge than of those inoculated with 30% and 50% of sludge was observed. The average gas production rates were 0.96, 0.6, and 0.4 L d^{-1}, respectively. Maximum gas production of 1.6 L d^{-1} was detected for the reactor with 40% sludge on the 27th day of experiments. On the basis of these results the reactor with the sludge concentration of 17.3 g L^{-1} was selected for the subsequent experiments.

As seen from Table 1, during the start-up period the COD removal efficiency in the reactor was rather low – 10–33%, but it increased toward the end of the

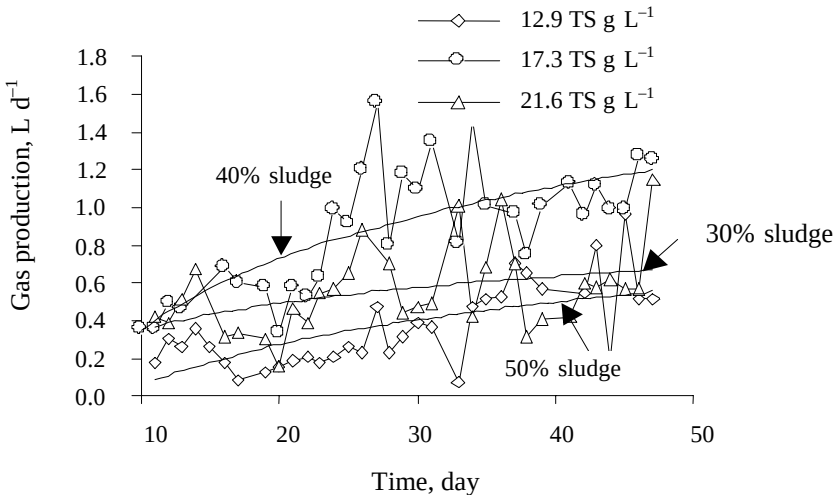

Fig. 4. Gas production at different seed sludge contents.

Table 1. Results of the experiment with the organic load TS 17.3 g L^{-1}

Day	COD in influent, mg L^{-1}	COD in effluent, mg L^{-1}	Organics removal efficiency, %	Gas production, m^3 kg^{-1} COD removal	Sulphate in influent, mgSO_4^{2-} L^{-1}	Sulphate in effluent, mgSO_4^{2-} L^{-1}	Sulphate removal efficiency, %	Sulphide in effluent, mg S^{2-} L^{-1}
Start-up								
11	14 380	12 880	10	0.243	5 300	840	84	6.2
22	23 660	15 840	33	0.157	5 300	40	99	41.4
39	20 280	17 200	15	0.178	3 100	320	90	7.3
47	20 280	13 960	31	0.232	3 600	10	100	18.3
Steady state								
68	20 540	5 030	80	0.126	4 800	40	99	10.5
75	20 540	3 970	81	0.165	3 000	20	99	2.3
88	22 890	3 670	84	0.187	3 000	10	100	36.7
100	22 890	11 040	52	0.161	3 500	340	90	28.1

start-up period. The change of the operational parameters during the start-up could be explained by the process of adaptation of bacteria to a gradual increase of OLR (from 2.16 to 7.7 kgCOD m^{-3} d^{-1}).

Study of the ASBR process in the treatment of high strength sulphate containing yeast production wastewaters

The selected reactor with 40% sludge was operated during the start-up stage until day 39 and then a constant OLR value (7.7 kgCOD m^{-3} d^{-1}) was applied from 39th to 89th day of the experiment (see Fig. 3). On the 89th–105th days of operation the amount of the feedstock was increased to 0.35 L in order to check the maximum possible loading rate. The maximum OLR applied during this phase was 9.16 kgCOD m^{-3} d^{-1} on the 98th day of operation. At this OLR inhibition of the treatment process was observed. Gas production decreased from 3.5 to 0.4 L d^{-1} and the pH of the effluent fell to 6.01. The experiment was stopped after the process was destabilized (see data in Table 1).

Stabilization of the pH in the ASBR during the operations is shown in Fig. 5. During the first month of the ASBR experiment the pH of the influent was adjusted using 10% NaOH solution. Afterwards the reactor was operated without any adjustment and the average pH value of the reactor effluent was 7.4, which indicated a high efficiency of the anaerobic digestion process. Alkalinity did not vary much during the study, the average values always remained above 118 mEq L^{-1}. Alkalinity was presumably produced as a result of the reduction of sulphates to H_2S in the presence of organic carbon sources, which supplied the necessary energy in accordance with the following equation [6]:

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + H_2S. \quad (1)$$

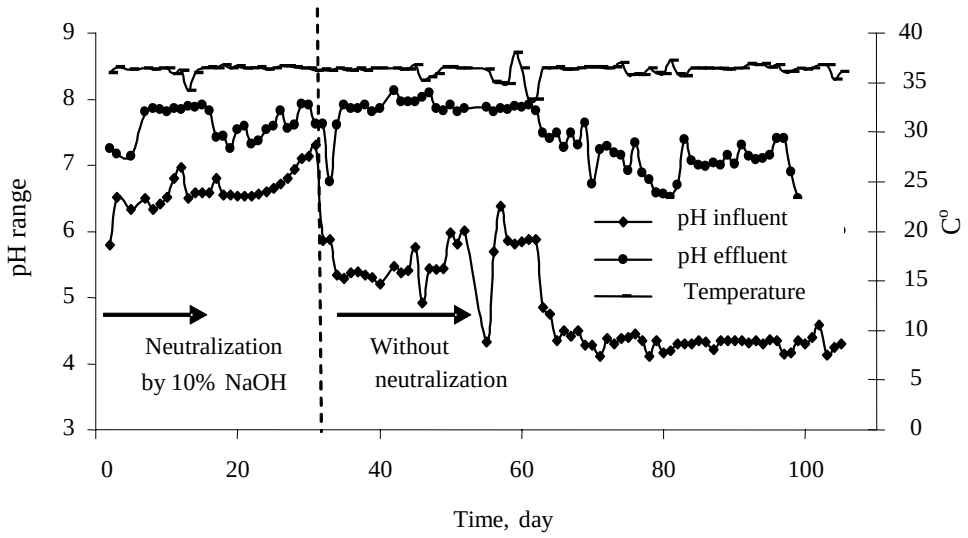

Fig. 5. Differences in the pH of the influent and effluent.

During the operation at a constant OLR of 7.7 kgCOD m^{-3} d^{-1} a significant increase in the removal efficiency to over 80% was observed. The maximum treatment efficiency (removal of 84% of COD) and the maximum biogas production of 3.79 L d^{-1} was reached at OLR values between 7.7 and 8.0 kgCOD m^{-3} d^{-1}. At higher OLR (over 8.01 kgCOD m^{-3} d^{-1}, days 89–105) the treatment efficiency decreased.

The data obtained on the treatment efficiency in COD removal were in agreement with the data published for yeast wastewater treatment process in [7].

The average sulphate removal efficiency was 95% in the experiment. Sulphate conversion to sulphide was greater than 80% during the start-up period. Then during days 39–89 when the OLR was constant no inhibition was detected and a nearly 100% removal efficiency was observed. Furthermore, the concentration of sulphates in the effluent did not exceed 40 mg L^{-1}. Due to the high OLR (9.2 kgCOD m^{-3} d^{-1}, day 98) the conversion efficiency decreased after day 100 to 90%. The data indicated that sulphate reduction was limited at higher OLR, and higher sulphate concentrations were observed in the influent. In fact, it has been supposed that for a successful anaerobic treatment a COD/SO_4^{2-} ratio higher than 10 is necessary [8]. Lower ratios were thought to be detrimental to methanogenesis because they led to the production of excessive sulphide concentrations (>150–200 mg L^{-1}). In the experiment the sulphide concentrations in the effluents of neither ASBR nor CSBR exceeded the inhibitory levels (150 mg L^{-1}, [9]) despite the fact that the COD/SO_4^{2-} ratio of the influent was always lower than 8. The effluent sulphide concentration was lower than 41.4 mg S_2L^{-1}.

During the steady state period of operation (days 39–89) the rate of biogas production varied between 2.30 and 3.85 L d^{-1}. This indicated that the performance and functioning of the reactor were rather variable. The variability observed was caused most probably by competition between sulphidogens and methanogens and possible inhibitory influence of sulphides (average value 18.85 mg S_2 L^{-1}), although they did not exceed the inhibitory level.

The composition of biogas was measured on the 68th day of the experiment and was as follows: 60% CH_4, 35% CO_2, 2.7% H_2S. This composition indicated that mainly methanogenic mineralization of organic matter was taking place in the ASBR. The biogas production rate during the operation cycle was measured on the 50th day of the experiment. The data obtained showed that the rate of biogas production was the greatest at the start of the cycle (during the first 7 h after the period of raw water input), and then slowly decreased with time, reaching very low and relatively stable levels at the end of the reaction stage. Biogas production completely stopped in the reactor on the 22nd–23rd hour of the cycle. The data showed that the lengths of the stages of the treatment cycle had been chosen correctly.

Previous results have indicated that inoculation of UASB with non-sulphate-adapted sludge could lead to complete inhibition of the treatment process [2] because bacterial groups, especially methanogens, could not adapt to the high levels of sulphide present in the influent. However, in our research full inhibition

of the process did not take place. This could be explained by the presence of non-competitive substrates for methanogens (trimethylglycine) in yeast wastewater. Since trimethylglycine remains undetected by a COD dichromate assay, its concentration can be underestimated, which in turn may lead to a significant overloading of WWTPs. It is known that sugarbeet molasses used as a component of the growth medium for baker's yeast [5] in the Salutaguse yeast plant contains up to 6% w/w trimethylglycine. In anaerobic treatment plants, trimethylglycine is practically totally degraded through a multistep degradation process with the formation of nitrogen-containing intermediates – trimethylamine and other methylated amines [10]. These intermediates are further degraded by methanogenic bacteria, yielding CO_2, ammonium, and methane. The presence of trimethylglycine could allow methanogens to maintain a significant population in a sulphate containing environment, which stimulates the growth of sulphate reducing bacteria (SRB), competitors of methanogens for the same substrates in the anaerobic treatment processes. Degradation of trimethylglycine (trimethyl-glycine is a nitrogenous compound, whose complete anaerobic degradation can result in an increase of the effluent ammonia concentration) and formation of amines can explain also accumulation of N_{tot} during the experiments carried out by us (see Table 2).

As seen from Table 2, the effluent concentrations of N_{tot} increased on average from 236 to 570 mgN L^{-1}. It should be noted that removal of all nitrogen compounds would require anaerobic, microaerophilic, and aerobic conditions established simultaneously in different locations of the anaerobic reactor, which is highly improbable in the case of the small-scale laboratory vessels used in our experiments.

The results of the present study (Table 2) demonstrate the ability of the ASBR process to achieve a good phosphorus removal efficiency – up to 61%. As calcium chloride is used in the technological process of yeast production, wastewaters are characterized by a rather high content of calcium ions. Under these conditions the high phosphorous removal efficiency could be explained by precipitation as a result of the formation of insoluble $Ca_3(PO_4)_2$.

Table 2. Change of phosphorus and nitrogen content in the ASBR during the steady-state period

Day	N_{tot} in influent, mg L^{-1}	N_{tot} in effluent, mg L^{-1}	N accumulation, %	P_{tot} in influent, PO_4^{3-} mg L^{-1}	P_{tot} in effluent, PO_4^{3-} mg L^{-1}	P removal efficiency, %
39	245	275	12	32.2	13.9	56.9
47	475	870	83	48.2	24.3	49.6
68	325	650	100	28.5	22.2	22.1
75	345	550	59	17.3	15.3	10.7
88	255	690	170	32.6	19.2	41.1
100	250	270	8	34.2	13.3	61.1

Despite the high sulphate treatment efficiency achieved in the ASBR, sulphide production during the process was significant, and this led to the observed instability of the process. In the large-scale experiments the instability of the processes could create significant difficulties in applying the ASBR technology for the treatment of yeast wastewaters. In addition to the inhibition of the process, sulphide formation caused also major malodour problems and corrosion of equipment during the experiment. In the further experimental work two modifications of the ASBR technique were investigated to reduce the problems noted. Accumulation of sulphides was an indication that competition between methanogens and SRB was won by the latter.

The aim of the further investigation was to find experimental conditions where methanogens would prevail, and the reduction of sulphate would stop at the level of elemental sulphur. An ASBR with a polymeric filler and coupled microaerophilic/anaerobic sequence batch reactor (CSBR) were investigated.

ASBR with a polymeric filler

Experiments with a polymeric filler used as a support material for micro-organisms were performed in order to study the influence of an artificial filler on the efficiency of the process. Previous studies [11] had shown that the use of a support material favours the adherence of methanogenic bacteria and accelerates the washout of SRB. A poor attachment ability of SRB was demonstrated. It was concluded on the basis of the experimental results that in the presence of a filler SRB are washed out of the reactor providing acetotrophic methanogenic bacteria with a sufficient growth advantage. These data suggest that an artificial carrier could stimulate methanogenic activity in the anaerobic digester and increase the efficiency and stability of the treatment processes.

Two reactors were operated in our series of experiments during 68 days. One was loaded with a polymeric filler and the other was operated like the first one but without the filler. The operational conditions were the same as described in the previous experiments. The COD and sulphate removal efficiencies were not significantly different between the two reactors studied; however, in the reactor without the carrier a slightly higher average treatment efficiency was observed, sulphate removal efficiencies varied from 85% to 100%. The sulphide concentrations in the effluents of either reactors did not exceed inhibitory levels and were not higher than 123 and 110 mg L^{-1}, respectively. These data are in agreement with the results of our previous experiment. The efficiency of phosphorous removal in the reactor with the carrier was significantly higher (up to 79%) than in the control reactor (57%). It can be assumed that the carrier promoted deposition of insoluble materials, for example precipitation of $Ca_3(PO_4)_2$. This conclusion was supported by the observation that scaling of the carrier beads was observed in the experiment. The fast clogging of the system with a carrier when treating sulphate-rich wastewaters has been described also in several other studies [12, 13]. In addition to facilitating scaling, a carrier could hamper equal

distribution of wastewater over the sections of the reactor, which could result in a lower COD and sulphate treatment efficiency. Therefore it can be concluded that the application of the carrier for the given treatment system was not effective and cannot be recommended.

Coupled microaerophilic/anaerobic system (CSBR)

In the CSBR the effluent from the anaerobic reactor was recycled through an aeration system. The content of oxygen in the microaerophilic reservoir was kept at the level of 0.1–0.15 mg L^{-1} to prevent sulphate formation in the oxidation of the sulphide formed in the anaerobic stage of the process leaving sulphur in the form of elemental sulphur (S^0) [14]. It was assumed to be the best for simultaneous solution of two problems: sulphate and sulphide removal. The formation of elemental sulphur is an advantage because sulphur is a colloid, inert solid and can be removed from the wastewater for example by gravity sedimentation. The anaerobic reactor was seeded with sulphate adapted anaerobic sludge, and the micro-aerophilic reactor was seeded with activated sludge obtained from the full-scale aerobic reactor of the Salutaguse yeast plant, Estonia.

The CSBR was operated during 68 days under the operational conditions described in previous experiments. The maximum OLR achieved was 7.74 kgCOD m^{-3} d^{-1}. The average pH value of the final effluent was 8.2 and the alkalinity always remained up to 177 mEq L^{-1} at the average pH of the influent 4.2. No attempts were made to adjust the pH of the influent. High pH values could be explained by the formation of hydroxide ion during the following biological overall reaction, taking place in a microaerophilic sulphide removal system [15]:

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-. \quad (2)$$

The results obtained allow us to conclude that a rather good COD removal efficiency (50–70%) was achieved during the experiment. Since the sludge had been well adapted to wastewater a very quick start-up was observed. Only a few days after seeding, the COD removal significantly increased and reached 70%.

The optimal COD loading found for the ASBR was 6–8 kgCOD m^{-3} d^{-1}. The highest COD removal efficiencies, exceeding 65% in the CSBR, were observed at the same (from 6 to 8 kgCOD m^{-3} d^{-1}) OLR values (Fig. 6).

Taking into consideration that the optimal ORL value reported in the literature [1] for different methanogenic reactors varies remarkably – from 4 to 12 kgCOD m^{-3} d^{-1} – the results obtained in our experiments were quite good for the treatment of high strength sulphate-rich wastewaters. The sulphate removal efficiency achieved in our experiments was excellent – more than 98%. Due to the low dissolved oxygen concentration (0.1–0.15 mg L^{-1}) there were almost no sulphides and sulphates in the effluent (Fig. 7). Only approximately 0.5 mg L^{-1} of H_2S and 0–30 mg L^{-1} of SO_4^{2-} were present in the effluent while up to 3.6 g L^{-1} of sulphate had been reduced.

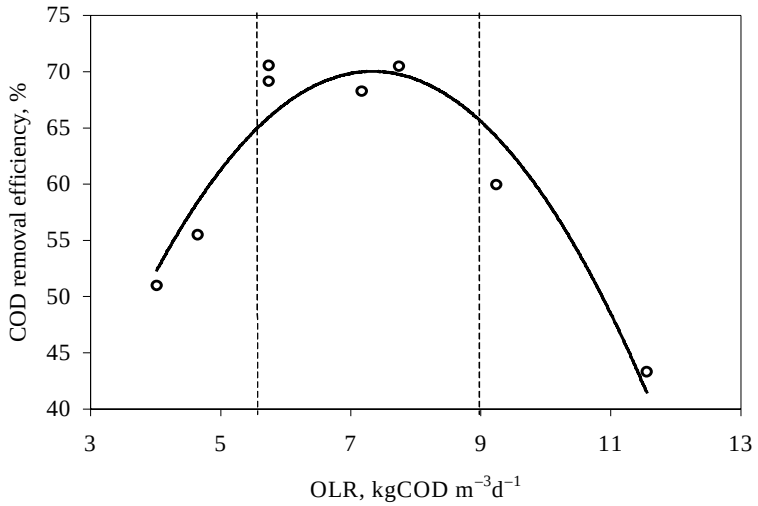

Fig. 6. Relationship between OLR and the efficiency of COD removal.

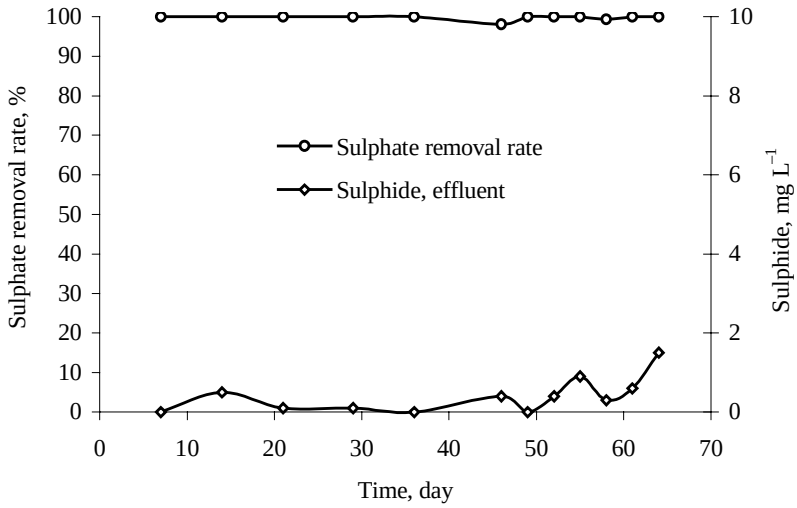

Fig. 7. Concentration of sulphide and the rate of sulphate removal.

Our data suggest that keeping a low level of the dissolved oxygen concentration in the microaerophilic part of the treatment system helps to poise the treatment process towards the formation of elemental sulphur and that the coupled microaerophilic/anaerobic treatment processes of sulphate-containing wastewaters were effective in alleviating sulphide inhibition of both methanogenesis and sulphate reduction. Last but not least, the exceptional stability of the CSBR process should be noted. The operational conditions worked out in the

laboratory-scale experiments were successfully applied at full scale in the Salutaguse yeast plant, where the process has been applied by now for more than a year.

Final sludge tests

Microscopic examination of the sludge and of the biomass concentration were performed at the beginning and at the end of each experiment. In none of our experiments granulation was detected. However, significant changes in the structure of the sludge were recorded.

Serious scaling of biomass by inorganic precipitation was observed already during 3.5 months of operation. Measurements of biomass concentration showed that the density of the sludge had also significantly changed since the start of the experiments. The sludge concentration varied between 43.2 g TS L^{-1} at the beginning of the experiment and 62 g TS L^{-1} in the ASBR and 65.2 g TS L^{-1} in the CSBR at the end of the study (Fig. 8). The difference between the values of total solids and volatile suspended solids indicated the presence of inorganic salts in suspension, possibly calcium carbonate and phosphates. Due to the formation of elemental sulphur in the CSBR a faster accumulation rate of inorganic compounds was observed than in the ASBR. With all advantages of this type of reactor the fast accumulation of inorganic compounds is an essential disadvantage. Precipitation of inorganic salts, as for example calcium carbonate, can indirectly upset the reactor performance by scaling [6, 16], which interferes with a good mass transport of substrate and reaction products. Scaling of biomass by Ca precipitates ($CaCO_3$ and/or $Ca_3(PO_4)_2$) may already occur at Ca^{2+} concentrations of 400 mg L^{-1} [17]. Also clogging problems can arise from precipitates in the piping system. Unfortunately, the concentration of calcium in the influent and effluent was not measured in the present study and the problem of the formation and removal of inorganic precipitate requires more detailed study in the future.

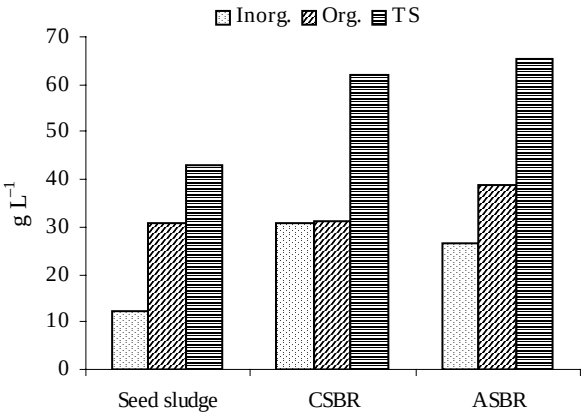

Fig. 8. Changes in the sludge composition during the CSBR and the ASBR processes.

CONCLUSIONS

The results of the study carried out demonstrated that the anaerobic sequencing batch reactor (ASBR) is a suitable and effective tool for anaerobic treatment of sulphate-rich wastewaters from baker's yeast production plants. Optimal parameters of the process were determined. However, sulphide formation caused significant malodour problems and corrosion of equipment during the experiment.

Experiments with two additional schemes developed for solving the sulphide formation problem showed that use of plastic carriers in the reactor led to a decrease of the treatment efficiency due to the accumulation of insoluble sediment (presumably $CaCO_3$ and $Ca_3(PO_4)_2$) on the surface of the carriers. So this technology cannot be recommended for large-scale application.

Combination of anaerobic sulphate reduction with biological oxidation of sulphide in a coupled microaerophilic/anaerobic SBR (CSBR) showed the best results and might be preferable for the treatment of sulphate-rich yeast wastewaters. As the scaling of biomass and fast accumulation of inorganic compounds were observed also in this case, successful application of the CSBR technology requires finding a solution for the removal of the inorganic precipitate from the reactor. The data obtained by us will be useful in designing full-scale ASBR and CSBR processes.

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Pärmitööstuse sulfaate sisaldavate reovete töötlemine anaeroobse annuspuhasti reaktoris

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Laboratoorse annuspuhastina töötava anaeroobse reaktori (ASBR) vahendusel on uuritud pärmitööstuse mesofiilsel temperatuurirežiimil sulfaate sisaldavate reovete puhastusprotsessi. On kasutatud kolme erinevat režiimi: puhastusprotsessi läbiviimist vabalt elunevate anaeroobsete bakterite suspensiooniga, polümeerse täidise pinnale kinnitunud anaeroobsete bakteritega ja mikroaerofiilset/anaeroobset töötlust annuspuhastis (CSBR). Anaeroobsete bakterite suspensiooni optimaalseks kontsentratsiooniks on leitud 17,3 g L^{-1} kuivaine järgi ja reaktsiooniajaks 22 tundi. ASBR-i režiimis töötavas seadmes alaneb KHT 75–82% mahukoormuse 7,7–8,0 kgKHT m^{-3} d^{-1} ja KHT/$(SO_4)^{2-}$ suhte 8,0 korral. Eralduvas biogaasis on metaanisisaldus optimaalsete tingimuste korral 60%. Parim tulemus (99%) sulfaatioonide sisalduse alandamisel saavutatakse CSBR-i režiimil, kusjuures reaktorist väljunud reovees on sulfiidioonide sisaldus 10 mg L^{-1} piires. Annuspuhastina töötava anaeroobse reaktori pikaajalisel töös hoidmisel väheneb puhastusprotsessi efektiivsus, mis on autorite hinnangul tingitud lahustumatu mineraalse sette moodustumisest, mis võib koosneda kaltsiumkarbonaadist ($CaCO_3$) ja kaltsiumfosfaadist ($Ca_3(PO_4)_2$).

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Possibilities of using ozone for the treatment of wastewater from the yeast industry

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Abstract. The purpose of the study was to establish if ozonation can be used to enhance the process of purifying wastewater from the yeast industry. The results of the experiments indicate that ozonation can be used in the tertiary treatment of yeast wastewater for the reduction of colour, odour, and the overall concentration of contaminants. During ozonation, the biodegradability of the wastewater increased; therefore it is possible to include ozonation into the combined purification process simultaneously with anaerobic and aerobic bio-oxidation. In addition, the application of ozonation as a pre-treatment method for anaerobic digestion of excess sludge from wastewater treatment plants was studied. Ozonation of the excess sludge resulted in a reduction of the sludge amount and increased the solubility of sludge organic matter. Although the solubility of sludge increased, the process of anaerobic mesophilic digestion was not improved.

Key words: ozonation, yeast wastewater, excess sludge, anaerobic digestion.

INTRODUCTION

The food industry is one of the contributors of wastewater pollution. The total amount of the wastewater is not large but the level of its contamination is very high. Also, the composition of wastewater varies considerably with each branch and mill type of the industry.

The wastewater from the yeast industry is characterized by a high chemical oxygen demand (COD), dark colour, and high concentrations of total nitrogen (N_{tot}) and non-biodegradable organic pollutants. Most of the contaminants in the

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wastewater are due to the use of molasses as a main raw material. As a by-product of sugar manufacturing, molasses contains 45–50% residual sugars, 15–20% non-sugar organic substances, 10–15% ash (minerals), and about 20% water. During yeast fermentation, the sugars contained in the molasses are a source of carbon and energy. However, a major part of the non-sugar substances in the molasses are not assimilable by the yeast and are released unchanged to the processing wastewater. These compounds represent the principal waste from the yeast production process. In addition, the chemicals added during fermentation (e.g. antifoams, propionic acids, brine, etc.), yeast metabolites, and residual yeast cells are in the wastewater.

In Estonia, Salutaguse Yeast Factory produces about 330 m^3 of wastewater per day, which is currently treated with a combination of anaerobic/anoxic biological oxidation followed by aerobic stages with activated sludge (Fig. 1). The raw wastewater contains high concentrations of organic pollutants (25 020 mg L^{-1} total chemical oxygen demand, COD_{tot}; 23 420 mg L^{-1} soluble chemical oxygen demand, COD_{sol}), nutrients (1470 mg L^{-1} of total nitrogen, N_{tot}; 100 mg L^{-1} of total phosphorus, P_{tot}), and sulphates (2940 mg L^{-1} SO_4^{2-}).

Salutaguse Yeast Factory has developed and improved its biological wastewater treatment process during the last years to reach effluent targets. However, the current technology is still not optimal as the total treatment efficiency in terms of COD is only about 80%. Even after multi-step biological treatment, the wastewater contains a relatively high amount of pollutants – mainly slowly biodegradable or non-biodegradable compounds such as melanoidins. Melanoidins are the high molecular weight polymers responsible for the brown colour, residual COD, and nitrogen in baker's yeast effluent [1].

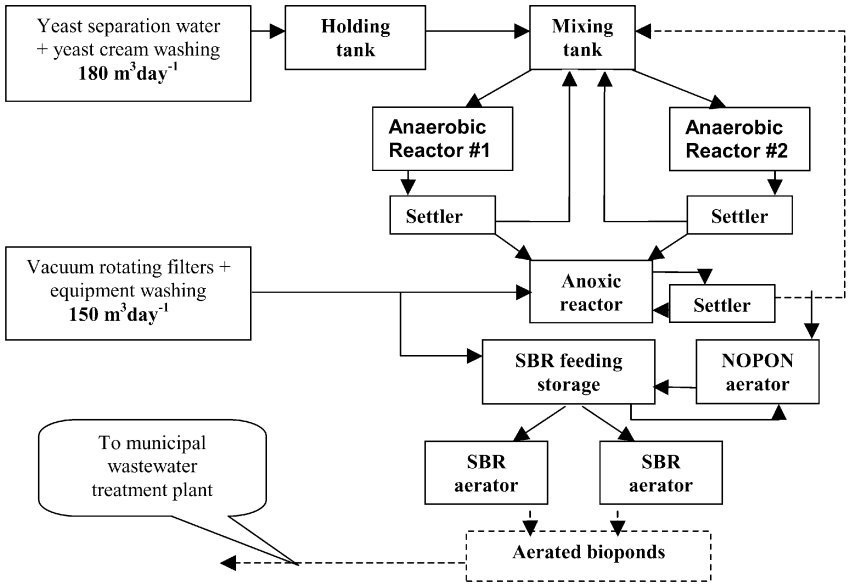

Fig. 1. The technological set-up used in Salutaguse Yeast Factory (Kohila, Estonia).

In summary, the main problems in the treatment of yeast wastewater are high concentration of COD in the effluent, colour, odour, and a high amount of excess sludge generated in the wastewater treatment process.

If a higher degree of purification is required, biological purification can be used in combination with other processes such as physico-chemical, chemical, or advanced oxidation processes [2]. Advantages of ozonation in treatment applications are removal of toxicity, destruction of organic matter, and enhancement of the biodegradability of recalcitrant wastewaters [3]. Ozone pre-treatment has been used to improve subsequent biodegradation [4–6]. In addition, ozonation can also increase the biodegradability of previously biologically treated wastewaters [7], thus enabling the use of an additional biological treatment stage, and repetitive treatment schemes. Presently, special attention is paid to the recycling combinations of biological treatment and ozonation [8, 9] where increased discharge quality at decreased oxidant consumption was obtained. It has been shown that a combined method, aerobic bio-oxidation with ozonation of recycled biologically treated wastewater, enabled improvement in purification efficiency at low ozone dosages [10, 11].

Another concern is the reduction of the excess sludge. According to new regulations, in effect since 2001, the priority is to develop new ways to reduce the amount of waste (excess sludge) on-site and to recycle biomass as much as possible [12]. Anaerobic digestion is an economical and environmentally friendly strategy for solving this problem. At present, a million tonnes of organic wastes are digested per year [13]. These are converted to biogas and to a stabilized residual matter. Today the anaerobic digestion method for sludge stabilization is used in Estonia too.

Anaerobic digestion of excess sludge is a multi-stage process and it is generally limited by the rate of hydrolysis of suspended matter and organic solids [13]. This stage of digestion is very important and therefore it is reasonable to carry it out in a separate reactor as a pre-treatment process. There are several methods that can be used: mechanical methods, ultrasonic disintegration, chemical methods, thermal pre-treatment, and aerobic and anaerobic pre-treatment [14]. Most of these methods have an influence on mesophilic anaerobic digestion and result in a better supply of soluble substrate to methanogenic bacteria.

The aim of this study was to establish if the application of ozone can enhance the purification of yeast wastewater, and improve the pre-treatment of excess sludge.

MATERIALS AND METHODS

Post-ozonation of biologically treated wastewater

In the experiments of post-ozonation, biologically treated yeast wastewater from Salutaguse Yeast Factory was used. Samples of wastewater were taken over a period of two months. This biologically treated wastewater had a relatively

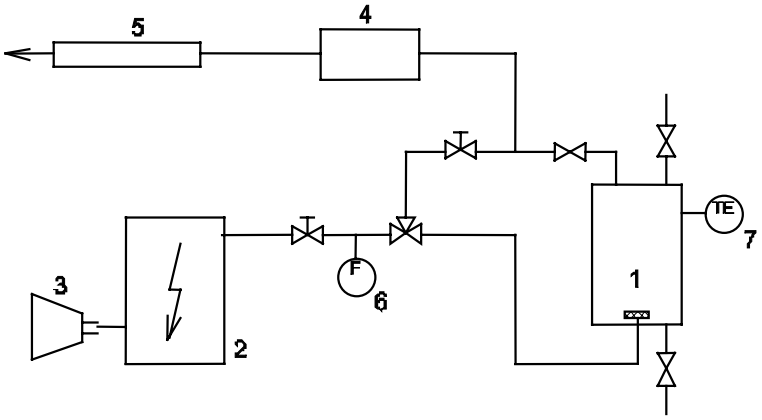

Fig. 2. Laboratory set-up for batch ozonation. 1 – semi-batch reactor, 2 – ozone generator Ozon-2M, 3 – air compressor, 4 – ozone analyser Anseros GM-6040, 5 – residual ozone destruction unit, 6 – flow meter, 7 – thermometer.

high residual COD and brown colour. The values of the most important parameters were: COD – 1500–2200 mg L^{-1}, BOD – 160–310 mg L^{-1}, pH – about 8.

Figure 2 illutrates the laboratory set-up used in the experiments of post-ozonation. Ozonation of wastewater was conducted in a 0.9-L semi-batch glass reactor with a continuous gas flow through the liquid. This ozone–air gaseous mixture was generated in the ozone generator OZON-2M. The ozone–air mixture was introduced to the bottom of the reactor through a ceramic diffuser. Wastewater was ozonated until an ozone breakthrough was observed after a period of complete ozone absorption by the wastewater. The experiments were carried out without adjusting the pH.

Pre-ozonation of excess sludge

In this part of the study, the influence of pre-ozonation of excess sludge on its anaerobic mesophilic digestion was tested.

The ozonation of the sludge was carried out in a laboratory set-up analogous to that shown in Fig. 2. The batch reactor was a 1.3-L glass reactor supplied with a foam destructor. The sludge was ozonated for 20, 105, and 240 min, with the corresponding consumed ozone doses of 23.8, 89.0, and 313.3 mgO_3 L^{-1}.

In the following tests of anaerobic digestion, mesophilic anaerobic inoculation sludge (10% of the total volume of the reactor) was added to the pre-ozonated sludge to initiate a mesophilic anaerobic digestion process. Flasks (volume 1.0 L) containing sludge mixture were then placed into an air-thermostat at mesophilic temperature conditions (35 ± 1 °C). Anaerobic digestion was carried out for 27 days. The flux of the generated biogas, a criterion for the evaluation of the process efficiency, was measured continuously.

Analyses

The amount of total organic matter in the wastewater was determined as chemical oxygen demand of unfiltered sample COD_{tot}, the amount of soluble organic matter as COD of filtered samples COD_{sol}, and the amount of biodegradable organic matter as seven-day bio-chemical oxygen demand BOD. In addition, the pH of the wastewater was measured.

For sludge, the following concentrations were measured: COD of sludge mixture (COD_{tot}) and its supernatant, the solubilized fraction (COD_{sol}), and total suspended solids (TSS).

Analyses of COD were conducted using HACH reagents and equipment according to the standard methods. BOD was determined using procedure 5210 of the Standard Methods for the Examination of Water and Wastewater. The pH was measured with an Evicon E6121 pH-meter. Ozone concentration in gaseous phase was measured with an ozone analyser Anseros GM-6040.

RESULTS AND DISCUSSION

Post-ozonation of biologically treated wastewater

The results of the post-ozonation of biologically treated yeast wastewater are presented in Table 1. The experiments indicated that the efficiency of post-ozonation in terms of COD (COD removal) ranged from 30% to 49%, and the ratio dn/ΔCOD, consumed ozone dosage mg of ozone per mg of COD removed, ranged from 1.2 to 2.5.

Figures 3 to 7 express the dependence of COD_{tot}, COD_{sol}, BOD, and BOD/COD, the biodegradability of the wastewater, on the consumed ozone dose dn (mg of ozone per litre of treated wastewater). The ozone dosage required to decrease the residual COD noticeably was about 1000–1500 mg L^{-1}, and both COD_{tot} and COD_{sol} decreased. This indicates that during post-ozonation the particulated organic matter in the wastewater was partly solubilized and the soluble matter was being oxidized.

Table 1. Results of post-ozonation of biologically treated wastewater

Run	Parameters of biologically treated yeast wastewater			Consumed ozone dosage, dn/ΔCOD, $mgO_3\ mgCOD^{-1}$	Efficiency of post-ozonation ΔCOD, %	Parameters of wastewater after post-ozonation		
	COD_{tot}, mg L^{-1}	BOD, mg L^{-1}	BOD/COD			COD_{tot}, mg L^{-1}	BOD, mg L^{-1}	BOD/COD
1	2055	161	0.08	2.45	30	1460	317	0.22
2	2120	579	0.27	2.47	31	1470	381	0.26
3	1480	204	0.14	2.2	34	970	310	0.32
4	1860	308	0.17	1.2	49	940	297	0.32
5	1940	147	0.08	1.6	30	1430	250	0.17

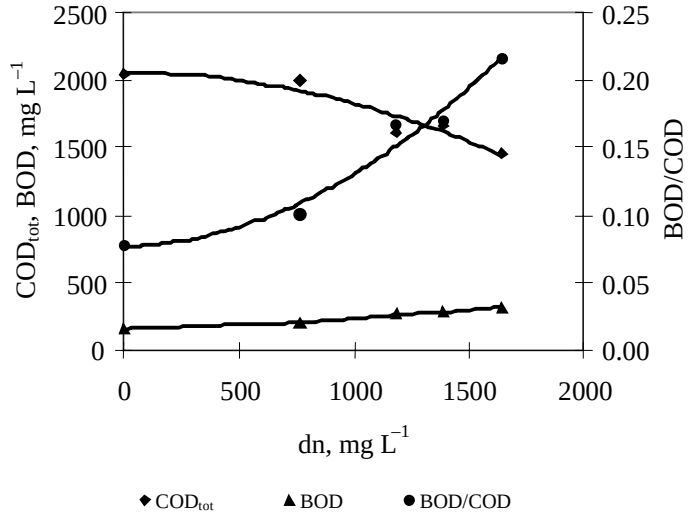

Fig. 3. The effect of ozone dosage (dn) on COD_{tot}, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 1). The pH rose from 7.3 to 7.9.

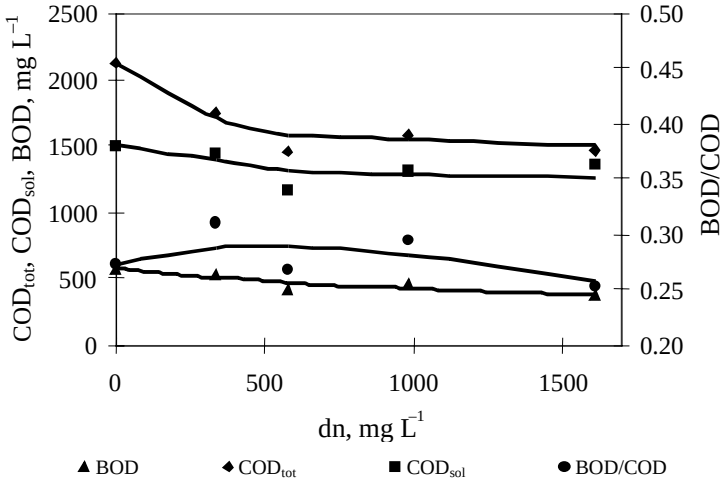

Fig. 4. The effect of ozone dosage dn on COD_{tot}, COD_{sol}, BOD, and BOD/COD of biologically treated yeast wastewater (run 2). The pH fell from 8.3 to 8.1.

As a rule, the results of the post-ozonation of yeast wastewater depend on the wastewater composition and consequently on the previous process of biological purification. BOD and biodegradability (BOD/COD) of the wastewater increased during ozonation, as shown in Figs 3, 5, and 7. In run 2, illustrated in Fig. 4, BOD decreased. This can be explained by the low efficiency of the biological treatment before ozonation. The biologically treated water contained a large

amount of biodegradable compounds – COD_{tot} was 2120 mg L^{-1} and BOD was 580 mg L^{-1}. Even in this case, the biodegradability was initially enhanced by ozonation (at ozone dose of 300 mg L^{-1}) followed by a decrease. In run 4 (Fig. 6), BOD decreased slightly, and the biodegradability increased in this case due to a faster decrease in COD.

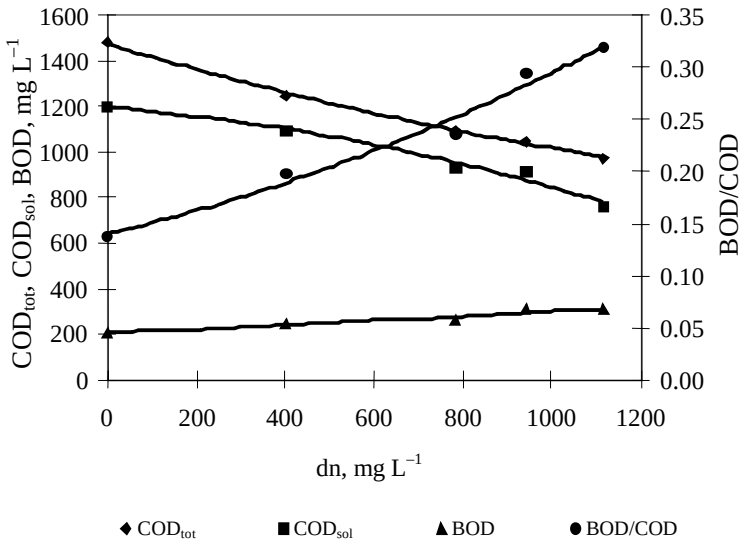

Fig. 5. The effect of ozone dosage dn on COD_{tot}, COD_{sol}, BOD, and BOD/COD of biologically treated yeast wastewater (run 3). The pH rose from 7.3 to 7.9.

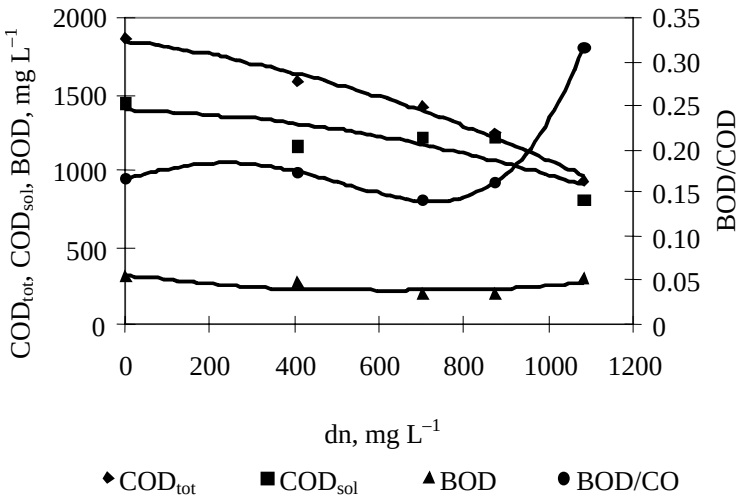

Fig. 6. The effect of ozone dosage dn on COD_{tot}, COD_{sol}, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 4). The pH rose from 7.3 to 7.9.

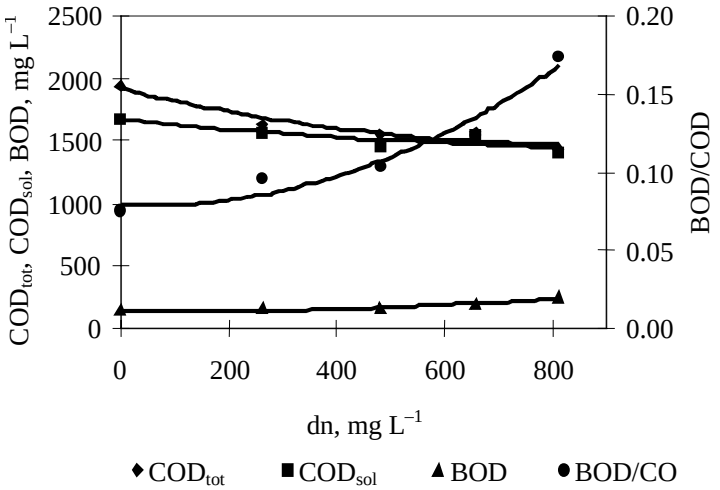

Fig. 7. The effect of ozone dosage (dn) on COD_{tot}, COD_{sol}, BOD, and the ratio BOD/COD of biologically treated yeast wastewater (run 5). The pH rose from 7.9 to 8.4.

Generally, during ozonation the pH decreases as a result of the formation of carboxylic acids. However, in the experiments of post-ozonation of the yeast wastewater, the pH decreased only in run 2. In all other runs the pH increased. The reason could be that acidic products of biochemical oxidation had been degraded by ozone.

In all cases, ozonation removed the colour and distinct odour of the treated wastewater. Initially, the biologically treated wastewater was dark brown and had a distinct odour, and after ozonation it was practically transparent and colourless with no odour specific to this wastewater.

The post-ozonation experiments indicate that ozonation can be used in the tertiary treatment of yeast wastewater for the reduction of colour, odour, and overall concentration of organic contaminants and matter. Since the biodegradability of the yeast wastewater increased during the ozonation, or at least at the beginning of post-ozonation, it is possible to include ozonation into the combined purification process simultaneously with anaerobic and aerobic bio-oxidation. In the combined process, the goal of ozonation is enhancement of biodegradability and removal of colour and odour. Taking into account that ozonation is still an expensive technology, the last option – application of ozonation in combinations with biological methods – may be more economical for yeast wastewater purification than ozonation alone.

Pre-ozonation of excess sludge

During the experiments raw sludge (COD_{tot} 85 000 mg L^{-1}, COD_{sol} 5300 mg L^{-1}, and TSS 39 200 mg L^{-1}), sludge after pre-treatment and after anaerobic mesophilic digestion process were analysed.

Table 2. Main results of sludge pre-treatment with ozone

Consumed ozone dosage, $mgO_3\ L^{-1}$	Sludge characteristics								
	COD_{tot}, $mg\ L^{-1}$			COD_{sol}, $mg\ L^{-1}$			TSS, $mg\ L^{-1}$		
	Raw sludge	After pre-treat-ment	ΔCOD, %	Raw sludge	After pre-treat-ment	ΔCOD, %	Raw sludge	After pre-treat ment	ΔTSS, %
0	85 000			5300			39 200		
23.8	85 000	64 000	25	5300	7000	−32	39 200	34 100	13
89.0	85 000	70 000	18	5300	6500	−23	39 200	35 900	8
313.3	85 000	73 000	14	5300	7500	−42	39 200	36 200	8

The main results of the pre-ozonation of excess sludge are shown in Tables 2 and 3. These tables contain a control experiment without ozonation for direct comparison.

During sludge pre-treatment COD_{tot} decreased by up to 25% as a result of the oxidation of organic materials. COD_{sol} of the pretreated sludge was higher than that in the control reactor because the products of ozonation (alcohols, aldehydes, organic acids) are more soluble than the initial organic material. Thus ozonation enabled us to transfer more organic matter into soluble form (the COD_{sol} increase 42%) and as a result the amount of sludge (as TSS) decreased by up to 13%.

The results of the anaerobic digestion process are shown in Table 3.

During the mesophilic anaerobic stage of digestion, the best results were achieved in the control sample without ozonation. The decomposition of organic matter was 70%, the amount of total suspended solids decreased by up to 35%, and as a result, the best biogas production occurred (up to 0.01 $m^3\ kg^{-1}$ COD_{tot} removed).

The pre-treatment has to be evaluated by its effects on the overall process. The ozone pre-treatment did not improve the mesophilic anaerobic digestion process (COD_{tot} and TSS removal and biogas production were lower than in the control experiment) and therefore cannot be used for this purpose.

Table 3. Main results of the anaerobic sludge digestion process

Consumed ozone dosage in sludge pre-treatment, $mgO_3\ L^{-1}$	Sludge characteristics								
	COD_{tot}, $mg\ L^{-1}$			COD_{sol}, $mg\ L^{-1}$			TSS, $mg\ L^{-1}$		
	Before MAD	After MAD	ΔCOD, %	Before MAD	After MAD	ΔCOD, %	Before MAD	After MAD	ΔTSS, %
0	85 000	25 400	70	5300	3470	35	39 200	25 400	35
23.8	64 000	30 700	52	7000	8220	17	34 100	28 800	15
89.0	70 000	26 900	62	6500	5030	23	35 900	27 400	24
313.3	73 000	25 800	65	7500	3920	48	36 200	27 000	25

MAD – mesophilic anaerobic digestion of the sludge.

CONCLUSIONS

It was established that the post-ozonation of biologically treated yeast wastewater resulted in the reduction of COD by 30–49%, and the consumed ozone dosage (mg ozone per mg of COD removed) ranged from 1.2 to 2.5. The biodegradability of the wastewater, expressed as BOD/COD ratio, generally increased during the ozonation. Also, the colour and odour problems of the yeast wastewater were eliminated. Thus, it is possible to use ozonation in the tertiary treatment of yeast wastewater or to include ozonation into the combined purification process simultaneously with anaerobic and aerobic bio-oxidation. Application of ozonation in a combined process seems to be promising for yeast wastewater purification; in this case, the target of ozonation is the enhancement of biodegradability and removal of colour and odour.

Although the pre-treatment of the excess sludge with ozone resulted in a reduction of the sludge amount and increased the solubility of sludge organic matter, the following process of mesophilic anaerobic digestion was not improved.

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Osooni kasutamise võimalustest pärmitööstuse reovee töötlemisel

Viktoria Blonskaja, Inna Kamenev ja Sergei Zub

Töö eesmärgiks on hinnata osoonimise rakendamise võimalikkust pärmitööstuse reovee puhastusprotsessi parandamiseks.

Katsetest on selgunud, et osoonimist on võimalik kasutada bioloogiliselt puhastatud reovee süvapuhastuses värvuse ning lõhna kõrvaldamiseks ja reoainete kontsentratsiooni vähendamiseks. Kuna osoonimisel suureneb reovee biolagundatavus, on otstarbekas kasutada osoonimist reovee puhastamisel kombineeritult koos anaeroobse ja aeroobse biooksüdatsiooniga.

Samuti on uuritud reovee puhastusprotsessis tekkinud jääkmuda osoonimist enne selle anaeroobset kääritamist. Jääkmuda eeltöötlemine osooniga vähendab muda hulka ja suurendab selle orgaanilise aine lahustuvust. Muda lahustuvus küll suureneb, kuid sellele järgneva muda mesofiilse kääritamise protsessi efektiivsus ei parane.

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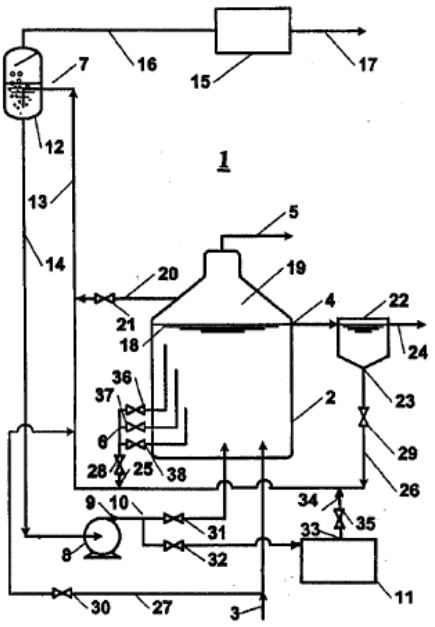
(54) Seade reovee anaeroobseks puhastamiseks biogaasi saamisega

(57) Leiutis käsitleb seadet reovee anaeroobseks puhastamiseks koos biogaasi saamisega. Pidevrežiimis töötav seade (1) reoainete kahjutustamiseks koosneb anaeroobsest bioreaktorist (2), retsirkulatsioonisüsteemist ja vajaduse korral sekundaarsest selitist (22) ja/või liigmuda kollektorist (11). Bioreaktori alaosas paikneb anaeroobne aktiivmuda ja toimub heitvee sissevool (3), ülaosas paiknevad puhastatud reovee väljundtoru (4) ja biogaasi sisaldav ruumiosa (19) biogaasi väljundtoruga (5). Retsirkulatsioonisüsteem on ühendatud retsirkuleeriva vedeliku väljavõtu koha (6,23,33) ning metaantanki alaosa vahele ning sisaldab vaakumdegaseerimisseadet (7). Retsirkuleeriva vedeliku väljavõtu koht asub kõrge kontsentratsiooniga aktiivmuda sisaldavas bioreaktori alaosas, võib ka asuda sekundaarses selitis, liigmuda kollektoris. Degaseerimiskambrist (12) väljuv retsirkuleeriv vedelik suunatakse väljavoolutoru (14) kaudu harutorustikku (10), kus see jagatakse kaheks osaks, millest üks saabub bioreaktori alaossa, teine aga liigmuda kollektorisse. Alarõhk degaseerimiskambri ülaosas on -0,05 MPa kuni -0,098 MPa. Uudsus on selles, et koos retsirkuleeriva vedelikuga viiakse anaeroobne aktiivmuda välja kõrge kontsentratsiooniga tsoonist (bioreaktori alaosa, sekundaarne seliti, liigmuda kollektor) ning degaseeritakse vaakumdegaseerimisseadmes. Leiutis tagab reovee puhastamise efektiivsuse ja tootlikkuse suurendamise.



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ANAEROBIC TREATMENT OF YEAST INDUSTRY WASTES - YEARS OF INDUSTRAL EXPERIENCE

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Abstract. This paper described some aspects of the operation of the local treatment plant of bakers industry. The operation factors of stable treatment performance were optimized. Included are results of the lab-scale experiments with ozone and results of one or two stage anaerobic mesophilic treatment process with different types of reactors. During ozonation, the biodegradability of the wastewater increased, therefore, the ozonation can be used in the post treatment of yeast wastewater for the reduction of colour, odour, and the overall concentration of contaminants.

Keywords: anaerobic mesophilic fermentation, organic matter, post-ozonation, sulphate rich wastewaters, yeast industry.

1. Introduction

Food industry is one of the major contributors for wastewater pollution. Baker's yeast industry effluents are characterized with high chemical oxygen demand (COD), sulphate, total nitrogen (N_{tot}), dark colour, and non-biodegradable organic compounds (melanoidins). Slow or non-biogradable substances melanoidins are the high molecular weight polymers responsible for the brown colour, residual COD, and nitrogen in baker's yeast effluent. Melanoidins are formed in a set of consecutive and parallel chemical reactions between amino compounds and carbohydrates during a Maillard reaction [1].

Most of the contaminants in the wastewater are due to the use of molasses as a main raw material.

Summarizing, the main problems for the treatment of yeast wastewater are high concentration of COD, sulphate, colour and odour.

The article presents a summary of the important aspects of the operation of the anaerobic treatment process.

2. Materials and methods

Anaerobic mesophilic (36 ±1°C) treatment of highly concentrated wastewater of the yeast industry was studied at the laboratory and pilot plant scales. The biological oxygen demand (BOD_7), chemical oxygen demand (COD), sulphate, sulphide, Suspended Solids (SS), N_{tot}, P_{tot}, dry matter, alkalinity and volatile fatty acids (VFA) of wastewater and effluent were measured according to the Standard Methods for Water and Wastewater Examination [2]. Flow rates, biogas production rate, temperature and effluent pH were measured daily.

3. Theory

Anaerobic wastewater treatment technologies are used throughout the world for effective treatment of this type of wastewaters.

One or two reactors (stages) can be used for the anaerobic digestion process. As known, methanogens were more active in the phase-separated set-up than in the single-phase system [3, 4]. As fermentation proceeds at a much greater speed than acetogenesis/methanogenesis, the former is carried out in acidic conditions in separate reactor.

A two-phase system of anaerobic digestion process has many advantages, such as optimizing the potential rate limiting steps (hydrolysis and methanogenesis), improving the reaction kinetics and stability.

During the anaerobic processes two important goals are achieved simultaneously: removal of organic matter and of sulphates (yeast industry

wastewater). However, high sulphate content can lead to destabilization of the process due to hydrogen sulphide formation [5].

Sulphate reducing bacteria (SBR) interact competitively with other anaerobic bacteria involved in methanogenesis, resulting in formation of H_2S rather than methane (Figure 1).

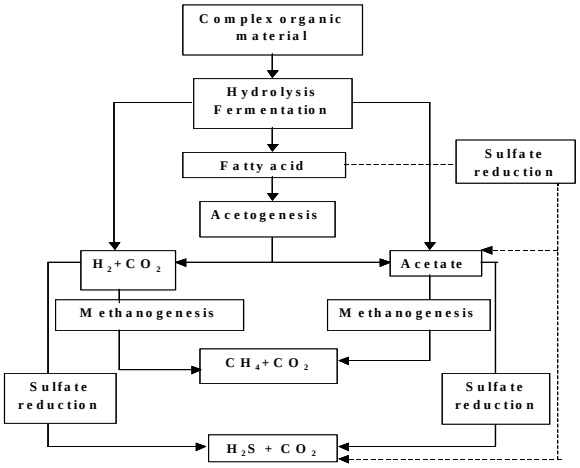

Fig. 1. Anaerobic degradation of sulphate containing wastewater [3].

Sulphate reducing bacteria product sulphide, which is higher concentration toxic to methanogenic bacteria [6]. On the other hand sulphide is the major sulfur source of methanogenic bacteria. Sulphide toxicity is highly dependents on pH. This means that low pH (<6.5) increases toxicity because unionized hydrogen sulphide is able to pass through the cell membranes[7].

The anaerobic mesophilic digestion processes responsible for the formation of odour-causing substances produce inorganic gases. The malodorous inorganic gases are primarily ammonia (NH_3) and hydrogen sulphide (H_2S). In addition to odour, hydrogen sulphide in the gas phase causes both human health and corrosion problems [8].

The main problems with formation of H_2S during the anaerobic digestion process are [5]:

1. the inhibitory effect of H_2S on many bacterial groups,
2. a reduction of methane yield and thus less energy recovery,
3. a odour, corrosion of piping and pumps,
4. the production of sulphide and formation of sulphide precipitates.

The strategies consist of the determination of the optimal pH to maintain the concentration of both the unionized H_2S and unionized NH_3 as low as possible. Since the inhibitory effects of both compounds determine the overall efficiency.

Successful strategies include dilution of the wastewater, decreasing the ionized sulphide concentration (by scrubbing or precipitation) and employing a multi step anaerobic digestion process [5]:

1. Anaerobic digestion in two stages (pre-acidification step with sulphate reduction followed by a methanogenic stage). The sulphide can be removed in the first stage or between the two stages,
2. The precipitation of sulphide in the anaerobic digester by the use of iron is the accumulation of FeS precipitate in the reactor.
3. Removal of sulphide from the effluent of the reactor combined with recirculation of the effluent. The removal of the sulphide can be conducted by chemical precipitation or by chemical or biological oxidation.

4. Results and discussion

The undiluted wastewater from baker's yeast production contains high average concentrations of organic pollutants (25,020 mg L^{-1} total COD; 23,420 mg L^{-1} soluble COD), nutrients (1,470 mg L^{-1} N_{tot}, 100 mg L^{-1} P_{tot}) and sulphates (2,940 mg L^{-1} SO_4^{2-}).

The anaerobic mesophilic process was investigated for COD and sulphate removal.

Two–stage system with anaerobic filter (AF) on the first stage and upflow anaerobic sludge bed (UASB) reactor on the second stage respectively and single stage system with – sequence batch reactor (SBR) were analysed.

Two-stage system [9]

Experiments were conducted with a hydraulic retention time (HRT) of 5 to 16 days and organic load rate (OLR) of 0.6 to 4.3kgCOD·m^{-3}·d^{-1} in the first stage and 9 to 15 days at ORL 0.1 to 1.4 kgCOD·m^{-3}·d^{-1} in the second stage. COD removal efficiency was 88% and 70% in the first and second stage respectively. The main results of the experiment are shown at Table 1.

Summarising the results obtained under steady state conditions one can conclude for the investigated system, that COD reduction decreased with decrease of HRT, as well as with decrease of influent waste.

Sulphate removal efficiency was 98% of which up to 91% was removed (converted to sulphides) in the first reactor. The content of H_2S in biogas was 2,8%.

Experiments shown, that high pH (>8) value together with high sulphide concentrations inhibit the growth of methanogenes and in lesser extend biogas production decrease rapidly.

Anaerobic treatment is considered as a feasible treatment method for sulphate rich wastewaters. However, a high sulphate content can cause major trouble to the process, mainly because in interactions between sulphate reducers and methanogens.

Table 1. Summary of the results during the experimental run period

Parameters	Unit	Influent		Effluent			
				AF		UASB	
		Min	Max	Min	Max	Min	Max
COD	mgL^{-1}	3000	27000	973	13000	1080	9720
Sulphate	mgL^{-1}	883	4900	390	575	118	370
Alkalinity	mgL^{-1}			1900	11410	184	2720
Dry matter	mgL^{-1}			2.9	15.0	3.1	14.0
VFAs,	mgL^{-1}			130	2840	184	2720
Gas production	$L\ d^{-1}$			0,174	7,544	0,024	0,642
pH				5.6	8.3	7.8	8.6

The sulphate reduction, as well as methanogenesis took place predominantly in the first stage of the two-stage digestion scheme. In the case of two-stage scheme the total sulphate removal was 98%, and COD removal was up to 88%.

Loss of methanogenesis on the second stages of the digestion processes (UASB) can result in the formation of high concentrations of VFAs and sulphides with a significant risk of creating odour problems.

The UASB reactor is often not applicable for the treatment of high sulphate containing wastewaters in mezophilic conditions. The observed instability and increased wash-out of sludge granules observed can be explained by the fact that under stress conditions, all energy gained by bacteria from dissimilation is used for generation of metabolic products, not for the growth of cells [10]. However, in the modified anaerobic/anoxic reactors system a stabile, 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.

The AF can be useful for suitability for treatment of soluble organic, but UASB system better for well settling sludge.

Single stage-SBR [11].

Three different treatment schemes were investigated – anaerobic sequence batch reactor (ASBR), ASBR with the polymeric filler and coupled microaerophilic/anaerobic SBR (CSBR). The optimal contents of sludge - 40% (S S 17.3 $g{\cdot}L^{-1}$) in the reactor, and the optimal reaction time - 22 hours were determined by monitoring the rate of biogas production. Optimal time for sludge settling cycle – 0.5-1.0 hours was established on the basis of the study of the kinetics of sludge settling processes.

It was shown that in the case of ASBR efficient treatment characterized by COD removal of 75%-82% took place at COD loading rates up to 7.7-8.0 $kgCOD{\cdot}m^{-3}{\cdot}d^{-1}$ and at COD/ SO_4^{2-} ratio 8.0. Sulphate removal efficiency was up to 95% (Figure 2). The temperature and pH changes during the experiments are shown in Figure 3. Methane content of the biogas in optimal conditions was 60%.

In the case of CSBR anaerobic effluent from the reactor was recycled through an aeration system, and the content of oxygen in microaerophilic reservoir was kept on the level 0.1-0.15 $mg{\cdot}L^{-1}$ to direct the process of sulphur reduction towards the formation of elemental sulphur, and to prevent the formation of toxic H_2S. Sulphate removal efficiency in CSBR was 99%, and the concentration of sulphide in the reactor effluent was about 10 $mg{\cdot}L^{-1}$. Decreasing of treatment efficiency after a long-time exploitation of these reactors studied were the result of formation of insoluble sediment (presumably $CaCO_3$ and $Ca_3(PO_4)_2$).

The high cation (both Na and Ca) concentrations present in sulphate-rich wastewater can inhibit anaerobic bacteria. The calcium ion does not exert a severe direct toxic effect, $CaCO_3$ and $Ca_3(PO_4)_2$ precipitates can indirectly upset the reactor performance by scaling.

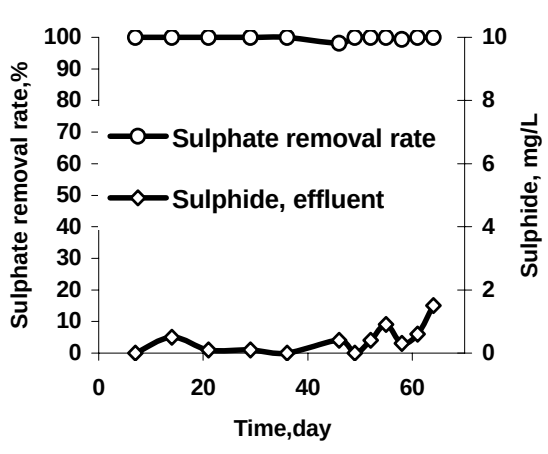

Fig. 2. Sulphate removal efficiency during the experiment

These precipitates are entrapped in the reactor biomass and results in a complete loss of the activity of the sludge granules owing the calcium layer which can completely block substrate transport [12]. Clogging problems can also arise from precipitates in the piping system.

Biomass scaling and the fast accumulation of inorganic compounds were detected during the

experiment. For successful application of this technology it is necessary to find a way to remove the inorganic precipitate from the reactor.

The results of the study carried out demonstrated that the anaerobic sequencing batch reactor (ASBR) is a suitable and effective method for anaerobic treatment of sulphate rich wastewaters from baker's yeast production plant. However, the problems of sulphide formation caused significant malodour problems and corrosion of equipment during the experiment.

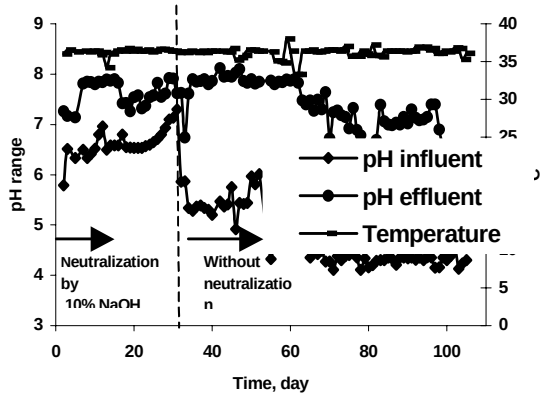

Fig. 3. Temperature and pH changes time

The temperature, pH control, acidity, alkalinity, sulphate and gas production were main factors for improving to anaerobic process.

The lab-scale experiments with one and two-stage process shown, that the combination of anaerobic digestion process with AF or ASBR with anoxic-anaerobic treatment can be useful for COD and sulphate removal from yeast wastewaters.

Ozonation

The application of ozonation in combined process may be more economical and prospective for yeast wastewater purification; in this case, the target of ozonation is the enhancement of biodegradability and removal of colour and odour. During the lab-scale experiments with post-ozonation of biologically treated yeast wastewater were received that, the reduction of COD by 30 to 49%, and the consumed ozone dosage per mg of COD removed ranged from 1.2 to 2.5. The biodegradability of the wastewater, expressed as BOD/COD ratio, generally increased during the ozonation. Also, the colour and odour problems of the yeast wastewater were eliminated.

The local treatment plant.

The equipment for biological purification of separation residues of baker's yeast at Salutaguse Yeast Plant (built by a Finnish Contractor, Tampela) is in operation since 1991, but has never performed satisfactory.

The main results from lab-scale experiments were adopted at Salutaguse wastewater treatment plant. After introducing modified anaerobic/anoxic system local treatment plant has been in stable operation more than 5 years (Figure 4).

Wastewater from the yeast production plant processing beet sugar molasses with a volume of 190 $m^3\ d^{-1}$ (after year 2003 average daily volume is 350 $m^3 d^{-1}$) was treated with anaerobic sludge in modified anaerobic/anoxic reactors system. The anaerobic reactors were fed with mixture of raw wastewater and recycled anoxic sludge. The temperature of the anaerobic stage has been kept between 30 and 36 °C; the pH of between 7.2 and 7.5 was self-regulated by the biological process without neutralization. Both anaerobic reactors are operating with COD loading of 12 – 16 $kgCOD \cdot m^{-3} \cdot d^{-1}$.

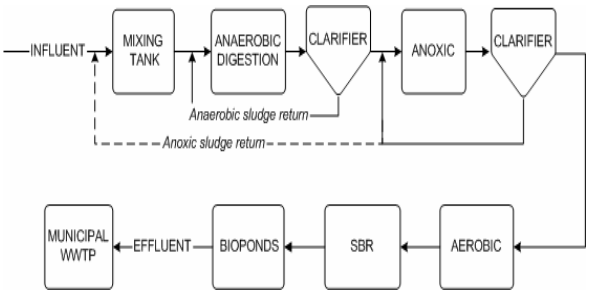

Fig. 4. The principal scheme of wastewater treatment in Yeast Factory (Estonia)

Principal technological set-up differs from the originally designed set-up in the following:

a) the anaerobic stage is followed by the anoxic stage;
b) part of anoxic sludge from secondary sedimentation tank is re-circulated back to the inlet, i.e. the to the mixing tank;
c) for final purification of the effluent the aerobic sequencing batch reactors and aerated bio ponds are used.

According to the technological set-up presented in Figure 4, the biological processes in the anaerobic reactors and in the anoxic reactor are inter-related by the returned sludge from the secondary sedimentation tank (by the anoxic reactor) to the inlet of the mixing tank. Evaluation of the efficiency of anaerobic and anoxic stages as well as the total efficiency of the system has demonstrated that leading the wash waters back to the holding tank improved the efficiency of the anoxic stage. The possible reason might lay in the resulting increased concentration of VFAs in the effluent from anaerobic reactors entering the anoxic reactor. This increased concentration of VFAs forces the bacterial consortium in anoxic reactor to metabolize more substrate spending more oxygen for it and thus increasing the efficiency of this stage. Thus the performances of the anaerobic stage and the anoxic stage counterbalance each other guaranteeing the relative stability of the whole system.

At (present) moment COD removal efficiency is about 80% and sulphate removal is up 100% correspondingly.

It was concluded that the success of this type of purification process, based on the recirculation system with the use of returned sludge depends most critically on avoiding the loss of sludge from the anaerobic reactors.

The main results of treatment wastewater from yeast industry are given at Table 2.

Table 2. The main results of the treatment process.

Removal,%	Years		
	1993*	2000**	2003***
COD	30	80	85
BOD_7	45	90	98
Sulphate	15	100	100
N tot	10	65	75
P tot	5	50	60

WWTP status:

** Initial process design. Anaerobic/aerobic stages only*

*** After introducing of modified anaerobic/anoxic reactors system.*

**** Raw wastewater volume increased up to 350 m^3 d^{-1}. Expanding of aerobic stage*

5. Conclusions

During the anaerobic digestion processes two important goals are achieved simultaneously: removal of organic matter and of sulphates (yeast industry wastewater).

The UASB reactor is often not applicable for the anaerobic mesophilic treatment of high sulphate containing wastewaters. Loss of methanogenesis on the second stages of the digestion processes (UASB) can result in the formation of high concentrations of VFAs and sulphides with a significant risk of creating odour problems.

The results of the study carried out demonstrated that the anaerobic sequencing batch reactor (ASBR) is a suitable and effective method for anaerobic treatment of sulphate rich wastewaters from baker's yeast production plant. For successful application of this technology it is necessary to find a way to remove the inorganic precipitate sediments from the reactor.

During ozonation, the biodegradability of the wastewater increased, therefore, the ozonation can be used in the post- treatment of yeast wastewater for the reduction of colour, odour, and the overall concentration of contaminants.

The main results from lab-scale experiments were adopted at Salutaguse wastewater treatment plant and results of this-high effisiency of treatment process.

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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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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 m^3/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 m^3/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 O_2/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 O_2 or air (Shan & Xiong 1998). 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:

$$SO_4^{2-} + 5H_2O + 8e \rightarrow HS^- + 9HO^-. \quad (1)$$

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:

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-, \quad (2)$$

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

$$2n\ H_2S + n\ CO_2 \xrightarrow{h\nu} 2n\ S^0 + (CH_2O)n + n\ H_2O. \quad (3)$$

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, H_2 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 H_2 and acetate. H_2 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 (Nyyssola *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 CO_2, ammonium and methane (Thalasso *et al*. 1999). The ammonium formed buffers the treatment system and Q2 enables its stable function. The cleavage of trimethylglycine into trimethylamine and acetate is characteristic of some halophilic fermentative bacteria (Moune *et al*. 1999).

$$\begin{aligned}2.5\ \text{trimethylglycine} + 4.04\ H_2 \rightarrow \\ + \text{2-propanol2.5 trimethylamine} \\ + 0.95\ \text{acetate} + 0.1\ CO_2 + 1.9\ H_2O,\end{aligned} \quad (4)$$

$$\begin{aligned}\text{trimethylglycine} + 1.32\ \text{serine} + H_2O \rightarrow \\ \text{trimethylamine} + 2\ \text{acetate} + 1.32\ CO_2 + 1.32\ NH_3.\end{aligned} \quad (5)$$

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):

$$CH_3COOH \rightarrow CH_4 + CO_2, \quad (6)$$

$$\begin{aligned}4(CH_3)_3N + 12\ H_2O \rightarrow 9\ CH_4 + 3\ CO_2 + 6\ H_2O \\ + 4\ NH_3.\end{aligned} \quad (7)$$

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 m^3 with a stirrer and two UASB reactors each of 180 m^3 volume), followed by an aerobic stage (activated sludge with a 360 m^3 aeration tank) and a secondary sedimentation tank (45 m^3) 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. Q3
- The temperature was automatically controlled at $+35 \pm 2\ ^\circ C$ 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.

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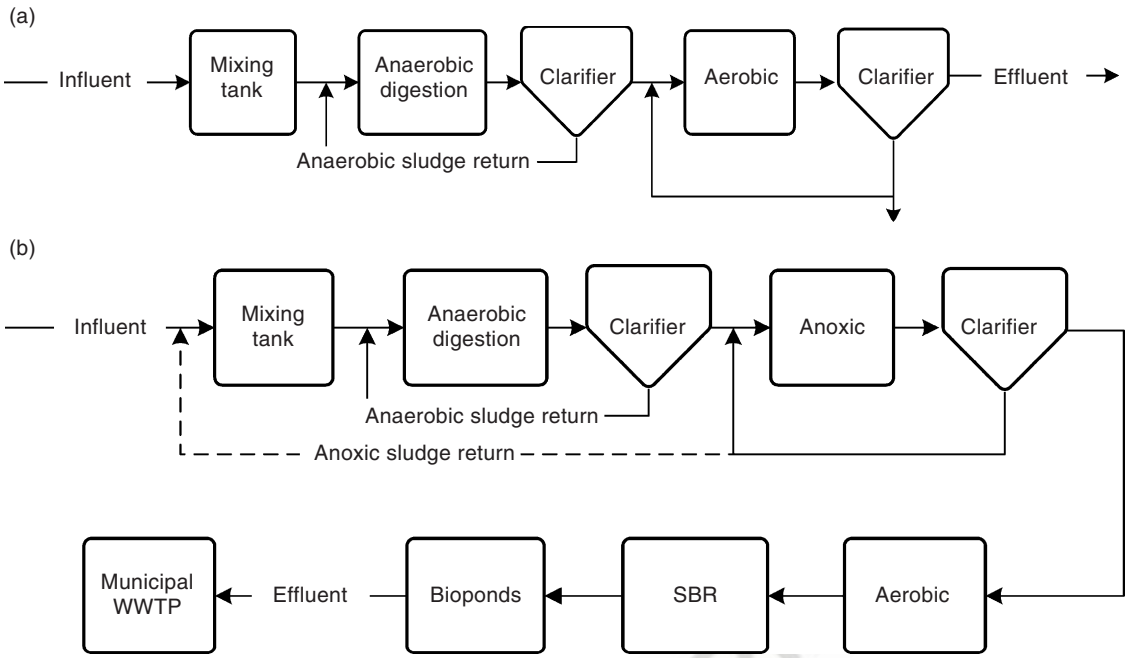

Fig. 1. (a) The original treatment facility of OY Tampella Ab (Finland); (b) the technological set-up used in the Salutaguse Yeast Factory with recirculation of the effluent from anoxic reactor back to the inlet. SBR, sequencing batch reactor; WWTP, wastewater treatment plant.

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]	–

[a]Hidden 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.
[b]The effluent was diluted with cooling water before final disposal to the local river.
[c]Permission for special use of water No. L.VV.RA-35029 (in Estonian) http://klis.envir.ee/klis/per/view_doc?doc_id=35029.
[d]Order 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).
[e]Requirements 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).
[f]The 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.) generated 271 m^3/day of wastewater 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 °C, pH between 4 and 5);

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Table 2 Average wastewater characteristics of Salutaguse Yeast Plant, Estonia

Waste stream	Flow (m^3/day)	COD excluding betaine		COD including betaine (+20%)		BOD		Sedimentable solids (SS)		Total Kjeldahl nitrogen (TKN)		Phosphorus (P)		SO_4^{2-}	
		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)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)	Concentration (mg/L)	Loading (kg/day)
High strength wastewater in the holding tank	190	20 000	1400	24 000	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 °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 °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÷+33 °C. The flow rate of the incoming wastewater was measured by flow meters (MAG-XM, Baily Fisher Porter). Reactor feed and internal recycling flow rate measurements were conducted using Danfoss MAG1000/1100 electromagnetic flow metres.

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

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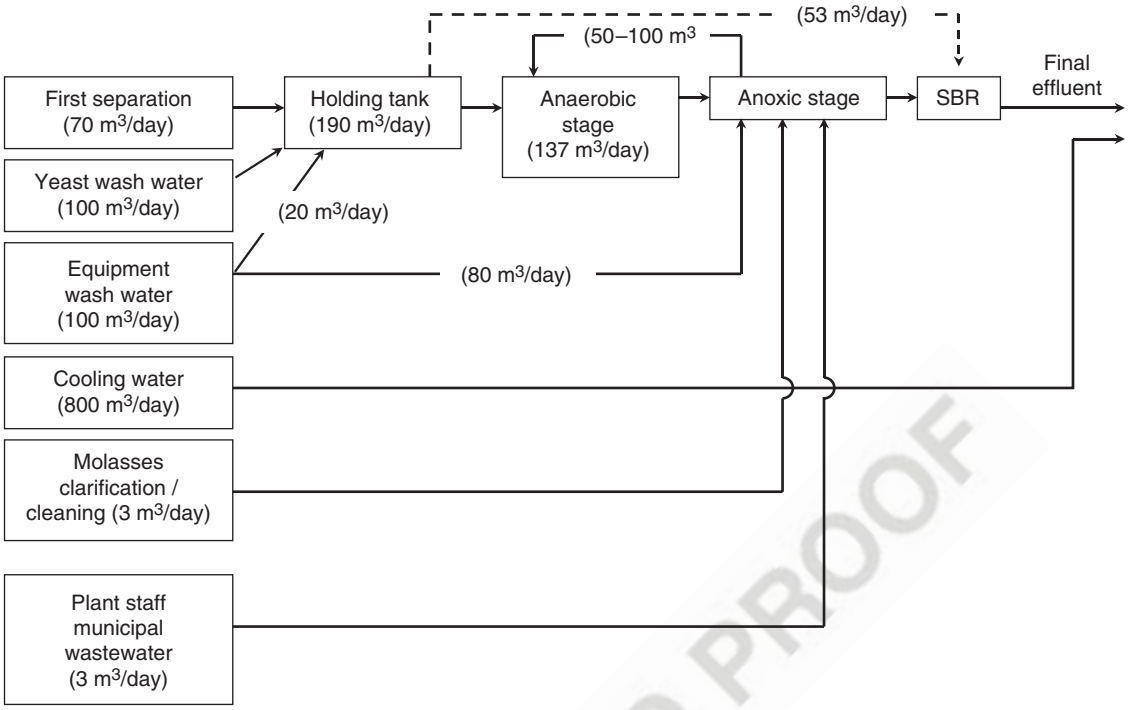

Fig. 2. Scheme of wastewater streams at Salutaguse Yeast Factory. SBR, sequencing batch reactor.

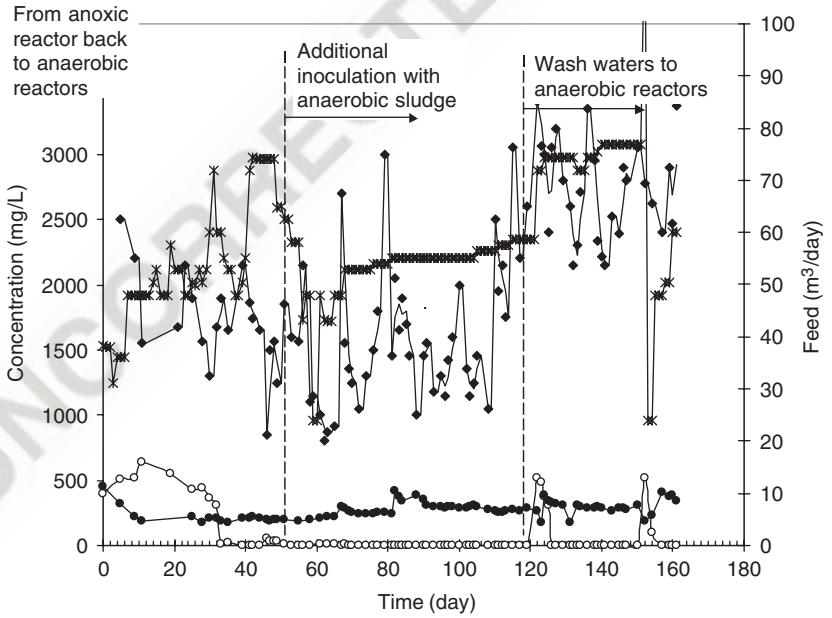

Fig. 3. Hydraulic loading rate (feed, m^3/day) and the content of sulphates and sulphides (mg/L) in the effluent from anaerobic reactor 2: ♦, sulphates inlet; ○, sulphates outlet; ●, sulphides; ×, feed.

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

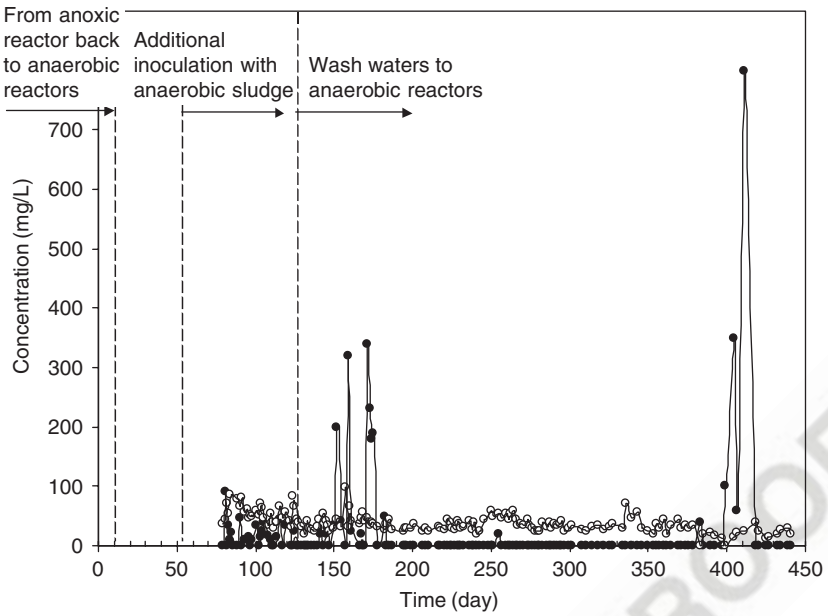

Fig. 4. Influence of recirculation on the concentration of sulphates and sulphides in anoxic reactor: ●, sulphates (mg/L); ○, sulphides (mg/L).

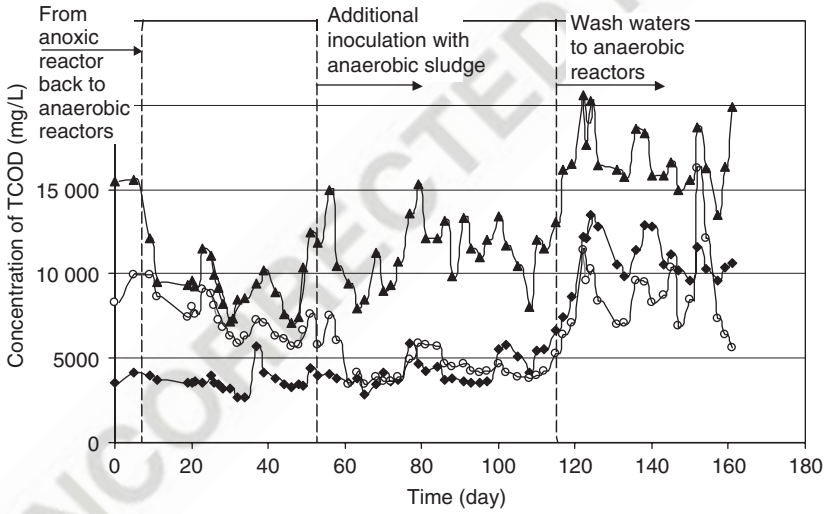

Fig. 5. Concentration of total chemical oxygen demand (TCOD, mg/L) in anaerobic reactors: ▲, TCOD of the inflow to the mixing tank; ♦, TCOD of reactor 1; ○, TCOD of reactor 2.

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

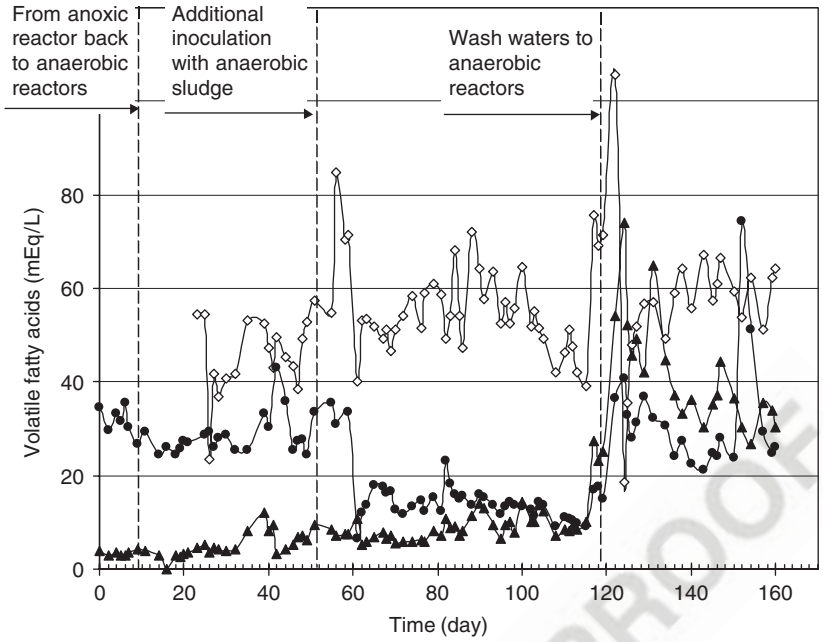

Fig. 6. Concentration of volatile fatty acids (mg/L): ◊, in the mixing tank;, in the effluent from anaerobic reactor 1; •, in the effluent from anaerobic reactor 2.

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	190	19 020		18 260		4200		
betaine		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			

[a]Not 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)	ΔCOD (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

[a]Theoretical biogas production from COD was calculated on the assumption that on the degradation of carbonaceous organic material 0.35 m^3 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 metanogenic activity, and for methane gas production there was no need to re-inoculate the reactors. Q7

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 O_2/L and in the range of 3830–16 270 mg O_2/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, CO_2 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

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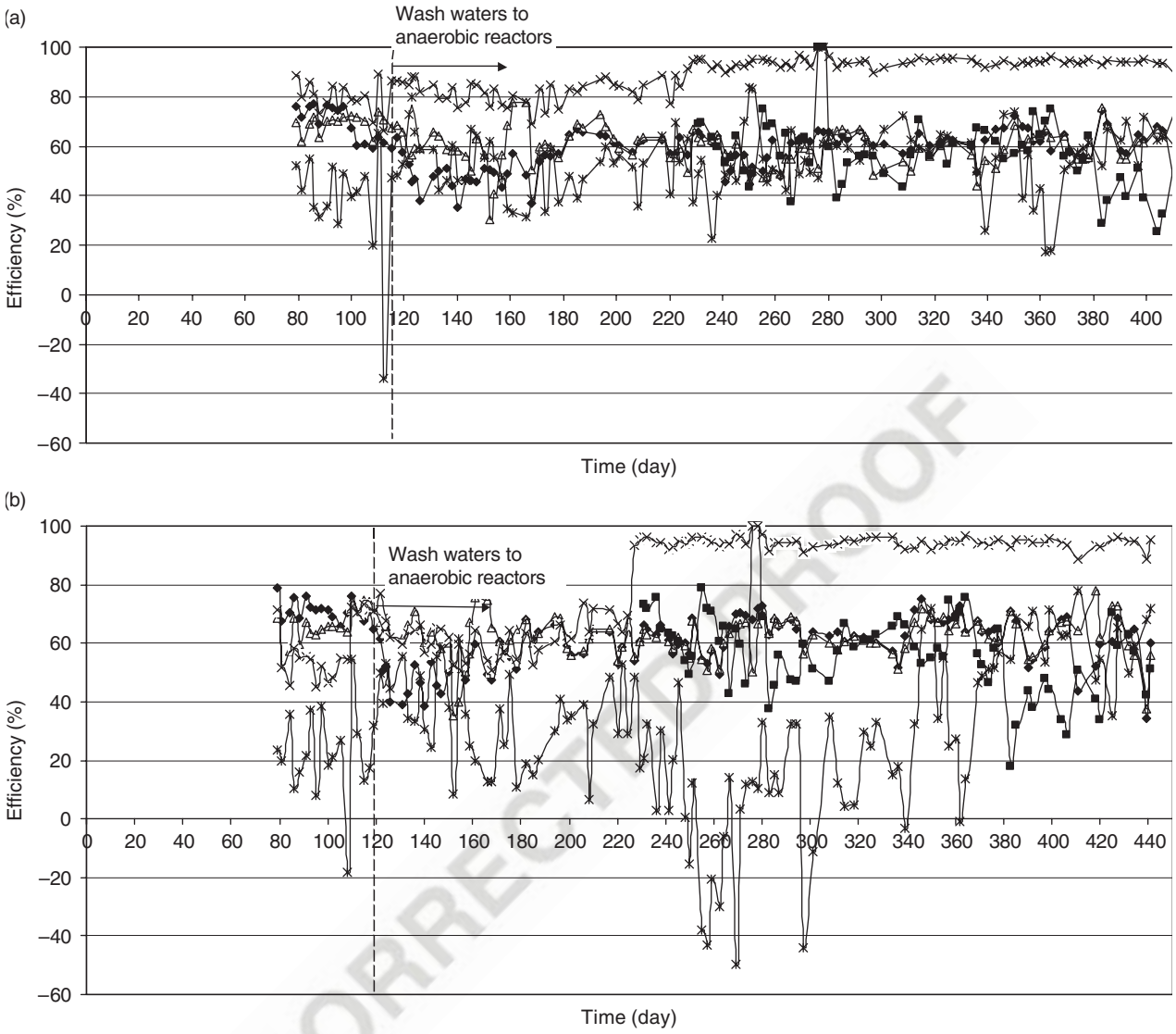

Q16 **Fig. 7.** The treatment efficiency: (a) by the total chemical oxygen demand (TCOD, %) value, (b) by the supernatant COD value (SCOD, %). ◆, anaerobic reactor 1; △, anaerobic reactor 2; ∗, anoxic reactor; ■, sequenced batch reactor (SBR); ×, the whole system.

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 $MW_{NH_4} = 18$ and $MW_{trimethylglycine} = 118$, 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 1.60–0.35 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).

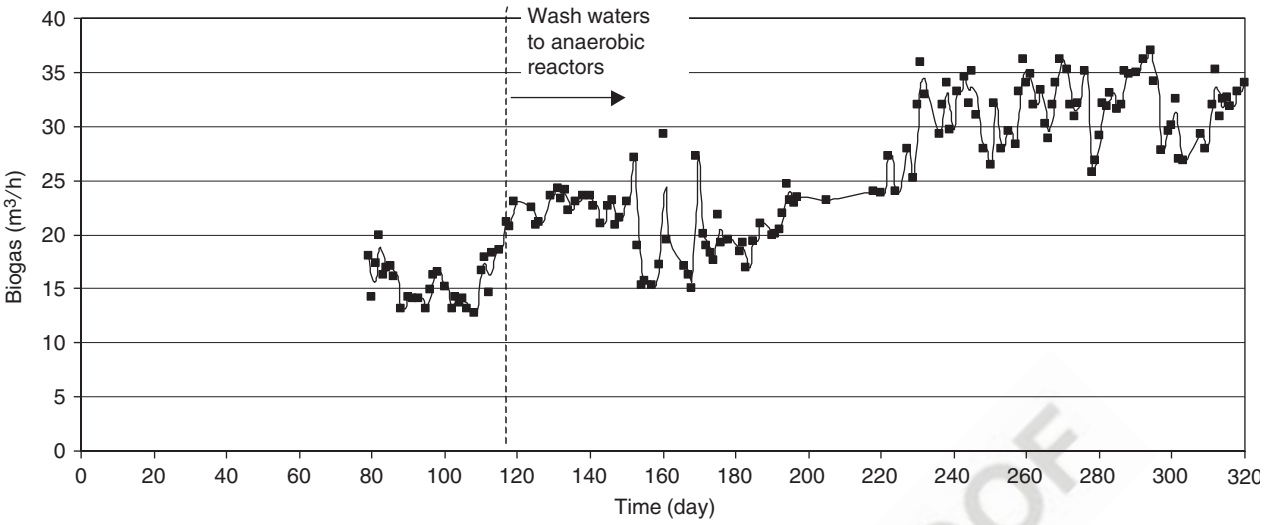

Fig. 8. Production of biogas in the anaerobic reactor 2 (m^3/h).

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 stoichometric ratio in the chemical reaction between trimethylglycine (ammonia) and the sulphide ion (sulphate ion) (Fdz-Polanco *et al*. 2001)

$$SO_4^{2-} + 2NH_4^+ \rightarrow S^0 + N_2 + 4\ H_2O, \quad (8)$$

consisting of the following reactions:

$$3\ SO_4^{2-} + 4\ NH_4^+ \rightarrow 3\ S^{2-} + 4\ NO_2^- + 4\ H_2O + 8\ H^+, \quad (9)$$

$$3\ S^{2-} + 2\ NO_2^- + 8\ H^+ \rightarrow N_2 + 3\ S^0 + 4\ H_2O, \quad (10)$$

$$2\ NO_2^- + 2\ NH_4^+ \rightarrow 2\ N_2 + 4\ H_2O, \quad (11)$$

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].

$$2\ \text{lactate}^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ \text{acetate} + 2\ CO_2 + 2\ H_2O + HS. \quad (12)$$

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 anaerobic 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 m^3/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 m^3/h (Fig. 8). The maximum biogas production achieved was up to 37 m^3/h in both reactors because of increased loading (up to 16 kg COD/m^3/day).

Conclusions

Q8 **(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 m^3/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 mg O_2/L, enabling a continuing 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, thrimethylglycine can be used as an osmolyte to treat wastewaters with high concentrations of salts.

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