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WASTEWATER TREATMENT FOR NITRATE AND HALOGENATED ACIDS

Tsvetomila Parvanova-Mancheva, Evgenia Vasileva, Venko Beschkov
Institute of Chemical Engineering, Bulgarian Academy of Sciences,
Acad. G. Bonchev str., bl. 103, Sofia, Bulgaria,
E-mail: mila_parvanova@abv.bg

Abstract

Important hazardous pollutants of underground water are nitrate ions. Their content in natural water may be due to the use of nitrogen fertilizers as well as of the inadequate treatment of wastewater from households, industry and agriculture. The most appropriate and progressive method for practical denitrification is the biological, combined with the stimulating effect of constant electric field. They are characterized by speed and lack of secondary and waste products.

Since all carboxylic acids are still in use, much scientific interest has focused on their biodegradation. The halogenated acids are classified as hazardous substances, contaminating surface and underground water. The strain Xanthobacter autotrophicus GJ 10 is known for its ability to degrade severe chemical pollutants and to use them as a sole carbon source. In the present study the degradation kinetics by entrapped in polyacrylamide gel immobilized cells was compared to those of the free culture. The gels were used in repeated-batch processes and in fed batch ones. High amounds of Monochloroacetic acid (MCA) and Dichloroacetic acid (DCA) were degradated.

Key words: wastewater, denitrification, nitrate ions, halogenated acids

1. INTRODUCTION

The urbanization, industrialization and different agricultural activities of the people lead to the environment pollution. When the water used up for drinking, industrial and other purposes is contaminated by various substances and alters its natural physical properties and chemical composition, it becomes wastewater. In recent years, scientists are searching for more novel approaches for wastewater treatment. The advantages of biotechnological methods are: ability to separate the majority of organic pollutants, simple apparatus design and relatively low operation costs.

Nitrogen-containing and halogenated compounds are an example for pollutants, which can cause the serious problems at their throwing out into the environment, such as eutrophication of rivers [1], deterioration of water quality and potential health risk of the humans and animals. Sources of waste and pollution of natural waters with the nitrogen-containing compounds are the manufactures of fertilizers, explosives and electronics. Halogenated compounds are widely used as herbicides, insecticides, antibiotics, medicines and so on. At the same time nitrate ions and halogenated compounds are considered as major pollutants. The increasing their concentration in water sources has led to serious harm to living organisms.

Nitrate ions are reduced in stomach to nitro-amines, which are a prerequisite for ~~receipt of~~ gastritis and cancer [2]. Risk is their reduced form (nitrites), which is the result of biochemical reduction into the human body [3]. When the nitrates enter the body of pregnant women, they are reduced to nitrites in

the stomach after you pass into the embryo. The nitrites convert hemoglobin to methemoglobin in the blood, which can no longer carry oxygen to cell tissues. As a results, it is obtained the so-called in medicine blue baby syndrome [4].

$$\text{Hemoglobin } (Fe2^{+}) \rightarrow \text{Methemoglobin}$$
$$\text{(reacts with } O_2) \qquad \text{(does not react with } O_2) \qquad (1)$$

Water sources can become contaminated with nitrates in several ways. Most of the studies indicate that the main contamination of groundwater with nitrates are due to the agricultural sources [5]. Ever-increasing worldwide sing(??) of fertilizers in agriculture is the cause of soil pollution by nitrates. Nitrate ions penetrate the soil and they fall into the groundwater. Another way for surface water contamination by nitrate is the wastewater from households, industry, animal farms [6, 7]. The wastewater from farms contains ammonia, which due to decomposition of waste of domestic animals. This ammonia in natural conditions is partially oxidized to nitrate.

Direct consumption of groundwater is impossible due to the presence of nitrate. In order to protect consumers from the effects of high concentrations of nitrates are introduced standards-limit values of nitrate in drinking water 50 mg dm^{-3} [8]. The requirements concerning drinking water are more stringent and the maximum allowable concentration of nitrogen ion nitraten is 10 mg dm^{-3} [9]. In some European countries the maximum allowable concentration of nitrate in drinking water is 12 mg dm^{-3} [10]. In Bulgaria, the limit values of nitrates and nitrites in water drinking were higher, namely 50 $mgNO^{3-}$ dm^{-3} and 0.5 $mgNO^{2-}$ dm^{-3} [11].

The halogenated acids are also classified as hazard substances, contaminating surface and underground waters. The pollutants have been found also in rainwater, drinking water and in snow. Typical concentrations of monochloroacetic acid (MCA) in the aquatic systems have not exceeded μicrograms per liter but in treated wastewaters levels of mg/liter have been found. Numerous studies have demonstrated that mono- and dichloroacetic acids are toxic to aquatic life, in particular to algae [12].

Monohalogenated acetic acids have been widely used in industry and agriculture. The most widespread of this class of chemicals, monochloroacetate has been used in the production of thioglycolic acid, herbicides (metolachlor, alachlor, propachlor and metazachlor), carboxymethylcellulose, preservatives for beverages and other chemicals [13]. Monobromoacetic acid (MBA) has been used as a chemical intermediate for the manufacturing of various chemicals with application in agriculture and pharmacy; it is also formed as a side-product during water disinfection [14]. Dichloroacetic acid (DCA) is an important intermediate in the synthesis of antibiotics, sulphonamides and plant protectives [15].

It is known the various methods for purification of water, which give a nearly complete elimination, but the production of secondary pollutants remain. The exception is the biological denitrification, where nitrate is reduced to harmless nitrogen gas and by-waste products virtually receive. The potential of this method is great. Its flexibility makes it applicable to wastewater from households, agriculture and industry of nitrate at different concentrations. Restrictions on its wider practical application are the slow growth of microorganisms and their wash out of continuous digesters for industrial processes.

The biological treatment is the conversion of the contaminants contained in the organic wastewater to simpler compounds through the vital activity of particular cultivated microorganisms under certain

conditions. Some of them degrade halogenated aliphatic hydrocarbons through specific enzyme systems that break or abate the carbon-halogen bands. These microorganisms use contaminants rather as carbon source and energy, and have the capacity to transform the intermediate products in digestible substrates.

There is a variety of bacterial strains that are able to break the carbon-halogen band of the fatty acid molecules [16, 17]. One of them is *Xanthobacter autotrophicus GJ10*. Cells of the strain are able to decompose ethylene chloride to 2-dichloroetanol, MCA and glycolic acid (GA).

A microorganism what is capable to degrade all monohaloacetates is *Moraxella* sp. B [18]. This strain can grow satisfactorily on mono-fluoroacetic acid as well as on its chloro- and bromo-analogues. High specific growth rates and relatively high inhibitory concentrations for monohaloacetates were found. The growth of the strain on DCA was very slow as compared to other substrates and other strains accompanied with a very long lag-phase. No growth on di-fluoro-acetic acid was observed [19].

Biodegradation of low substrate concentrations of xenobiotic has been neglected field of research. *Aminobacter* sp. has great potential in degrading substrate at low substrate concentration. This bacterium was capable in degrading 2, 2 - dichloropropionic acid.

2. METHODS FOR DENITRIFICATION

Traditional methods to remove nitrates

The traditional processes for water treatment are coagulation, filtration and disinfection (to be required for drinking water). However, all these processes no effectively remove nitrate ions from water. Therefore, the application of complementary methods is required. Two major groups of processes carry out the removal of nitrate ions from natural and wastewaters: physicochemical and biological. The most common traditional physicochemical processes include - reverse osmosis, ion extraction (ion exchange), electro-dialysis and adsorption on activated carbon with pH adjustment [4, 5, 6, 20]. These are convection methods for reduction of nitrate [21].

The ion exchange is limited by several problems: poor selectivity for nitrate and regeneration of the resin [22].

Some drawbacks of these methods are convection(?) reason for their limited use. They are very expensive, and they separate waste streams with high nitrate or byproducts. For example, in ion extraction, the removal of both nitrate and sulfate ions from the treated water is happened, but after that it is necessity to use an additional method for regenerating the resin [4, 22]. The problem with reverse osmosis is that the membranes used are not suitable for nitrates. The degree of directly removal of salts depends on the valency of their ions. Moreover, reverse osmosis remove many kinds of ions, thus considerably reducing the mineral components in the water, making it unsuitable for drinking purposes [23]. Using this method is limited by the fact that it is very expensive and the resulting concentrated effluents pose a major problem with its subsequent processing [1].

Generally, in physico-chemical methods to produce small volume waste streams, but with a high concentration of nitrate ions, which require further disposal.

Biological denitrification

The most suitable method is the biological denitrification. For years, this process is used for sewage treatment. Biological denitrification is highly selective to nitrate ions. Process efficiency is very high and can reach 100%, which was not observed in any of the other methods used to purify waste water from nitrates.

In comparison with traditional methods, which fail to remove nitrate, but separate them into new high-density flows, accompanied by products, biological methods ensure complete elimination of pollutants through the full reduction of nitrate. Removal of nitrates has been reported by aerobic denitrification [24] most of the tests were conducted with denitrification facultative anaerobic organisms in the absence of oxygen [21]. In the biological denitrification, denitrifying bacteria use nitrate as final electron acceptor in their respiration process in the absence of oxygen.

Denitrifying bacteria reduce inorganic nitrogen compounds such as nitrate and nitrite to gaseous molecular nitrogen. Also, contrary to certain contaminants for which treatment is necessary the introduction of a certain type of bacteria, denitrifying bacteria are found everywhere in nature [25]. Moreover, microbiological elimination of nitrates can be very economical strategy for the treatment of polluted and wastewaters [26]. As a result, there has been great interest in biological denitrification, because it is inexpensive, although very slow process and requires time for it to go completely, especially when treating wastewater with very high concentrations of nitrates [3]. To increase the rate of denitrification treatment is proposed to be conducted by applying a new method that can lead to a better contact between nitrate ions and microorganisms in the water flow. Also, many efforts are focused on creating new methods, including convection and biological methods for treatment of contaminated waters. This is a membrane biofilm reactor, which was recently reported [7].

Bio-electrochemical denitrification

Especially progressive approach is a bio-electrochemical decomposition of nitrate ions. This process is kept under constant potential at the cathode, ie in potentiostatic mode. In a previous work Beschkov et al. ([27]) has shown that bio-denitrification stimulated by constant electric field in the potentiostatic mode runs faster by avoiding the lag-phase growth in culture from Pseudomonas denitrificans [27].

Their results are shown on Fig. 1 and Fig. 2

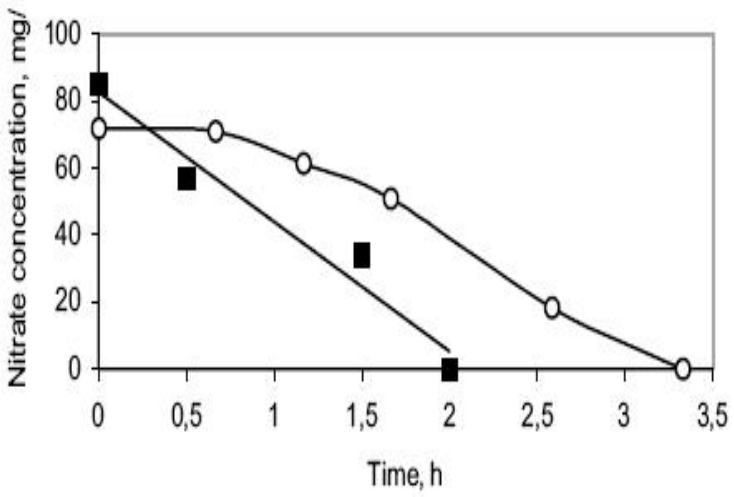


Fig. 1. Comparison of microbial denitrification with and without application of electric field: ○ - without electric field, ■ - with electric field (Fig. 2 of that article [27]).

It was found that the maximum rate of denitrification was observed in cathode potential of 0.01 volts against the saturated hydrogen electrode – Fig. 2.(Fig. 5 of that article) [27].

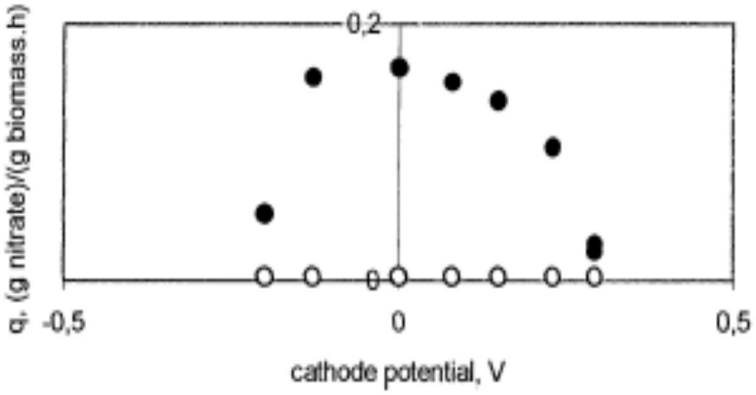


Fig.2. Dependence of the rate of denitrification potential of the cathode:
○ - without electric field, ■ - with electric field (Fig. 5 of the cited article [27]).

Measurements of the voltage applied to show that in this case the effect of accelerated denitrification is not due to electrochemical processes: direct reduction of nitrate, hydrogen extraction or electro-chemical regeneration of cofactors, as measured amperage is very small (of the order of micro-amps), and measured a current yield is orders of magnitude lower (about 0.01 Coulombs) than the stoichiometric (180 coulombs)

A disadvantage of biological denitrification is the risk of bacterial contamination of the treated water. This risk is expected and disinfection is absolutely essential [23]. Another disadvantage of biological methods is the low growth rate of microorganisms.

3. HALOGENATED POLLUTANTS AND THEIR BIODEGRADATION

Halogenated aliphatic compounds are prevalent groundwater contaminants and are significant components of hazardous wastes and landfill leachates. Many hazardous halogenated aliphatic compounds released from industrial, commercial, and agricultural sources are chlorinated or brominated alkanes and alkenes that contain one to three carbon atoms, such as halogenated alkanoic acids (HAA), haloalkanes, trichloroethylene, trichloroethane and ethylene di-bromide. Chlorinated ethanes and ethers have been commonly used as refrigerants in manufacturing, as solvents in the dry-cleaning (metal and plastic) and lacquer industries, and in semiconductor manufacturing.

Chlorinated compounds are the most extensively studied because of the highly publicized problems associated with 1,1,1–trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), other pesticides, and numerous industrial solvents. Hence, chlorinated compounds serve as the basis for most of the information available on the biotransformation of synthetic compounds. Several excellent reviews have been published [28, 29, 30, 31, 32, 33].

Most of the information available on the biodegradation of chlorinated compounds is on oxidative degradation, since aerobic culture techniques are relatively simple compared with anaerobic culture methods. Also, aerobic processes are considered the most efficient and generally applicable. Recently, anaerobic microbial communities have shown the ability to degrade a variety of groundwater

pollutants, including chlorinated aromatic compounds and trichloroethylene [34, 35, 36, 37, 38, 39, 40, 41].

For convenience, the chlorinated hydrocarbons degraded by microorganisms (bacteria and fungi) are grouped into three classes: aliphatic, polycyclic and aromatic [42]. Some of the chlorinated hydrocarbons and the microorganisms capable of degrading them are listed in Table 1. This table list chlorinated compounds that are degraded by some soil and aquatic microorganisms. They also illustrate the wide variety of microorganisms that participate in environmentally significant biodegradation reactions.

Table 1. Biodegradation of chlorinated aliphatic compounds

Compound	Microorganism	Reference
2-Monochloropropionic acid	*Pseudomonas sp.*	43
2-Monochloroacetic acid	*Alcaligenes sp.*	44
1,2-Dichloroethane	Anaerobes	34
	Xanthobacter autotrophicus (GJ10)	45
	Methane-utilizing bacteria	46
	P. fluorescens	47
1,1,1-Trichloroethylene	Anaerobes	34
	Methanotrophs	48
Perchloroethane	*Methanosarcina sp.*	36
1-Chlorobutane	Aerobes и Anaerobes	49
1,2-Dichloropropane	*P. fluorescens*	47
1,3-Dochloropropane	Aerobes	46
1,9-Dichlorononane	Anaerobes	46
Trichloroethylene	Anaerobes	34
	Anaerobes	37
	Aerobes	50

	Aerobes	51
	Methane-utilizing bacteria	52
	P. putida	53
	Methane-oxidizing bacteria	38
	P. fluorescens	47
3-Chloro-4-hydroxybenzaldehyde	Anaerobes	39
Chlorolignin	*Sporotrichum pulverulentum*	54
Monochloro-, bromo-, iodoalkanes	*Arthrobacter sp.*	55

With the exception of 2-monochloropropionic acid and monochloroacetic acid, detailed studies of the biochemical and genetic basis for the biodegradations of chlorinated aliphatic compounds are lacking. A number of soil microorganisms which synthesize dehalogenase are capable of utilizing Halogenated alkanoic acids [43, 44]. Hardman et al. [43] examined four *Pseudomonas* and two *Alcaligenes* species capable of growth on 2-monochloropropionic acid and monochloroacetic acid.

Haloalkanoic acids such as 2, 2–dichloropropionic acid D, L-2-chloropropionic acid are not well studied. These halogenated compounds are commonly used as herbicides. Among all kind of herbicides, dalapon (2, 2–dichloropropionic acid) and glyphosate were widely used to kill unwanted, sparing non-target type of plants and perennial grasses [56, 57]. The isolation and identification of dehalogenase producing bacteria were well studied. The isolated bacteria possess more than one dehalogenases for example in *Rhizobium* sp. [58].

During cultivation of strain *Aminobacter* sp. only haloalkanoic acids were able to support growth of the organism as the sole carbon and energy source. *Aminobacter* species were isolated from diverse terrestrial and marine environments. Some of these bacteria were known as methyl halide-degrading bacteria which capable of degradation of methyl-halide compound. The following halogenated compounds such as 3-chloropropionic acid, monochloroacetate, dichloroacetate and trichloroacete did not support growth of the *Aminobacter* sp. [59].

4. DEGRADATION OF HALOACETIC ACIDS

The haloacetic acids are one of the most important by-products of chlorination treatment. They are formed when organic matters in the water react with the chlorine used to treat the water. Chlorination is the most common water disinfection method all over the world due to the fact that it's effective even in low concentration, accessible, cost-effective and has residual. Secondary reactions and the formation of organic chlorinated and pathogenic compounds have intensified the need of removing disinfection by-products and also have raised the importance of world wide researches. Disinfection by-products such as trihalomethanes and haloacetic acids, which are the most important, are considered carcinogenic and pathogenic [60].

There are some methods used to alleviate disinfection by-products [61]:

1) Minimize precursors and secondary reactions. Keeping organic matter away from water is one way of preventing disinfection by-products. Absorption with the granular activated carbon can reduce organic matter levels.

2) Reducing disinfectants dosage. In 2005, Singer et al. studied disinfection by-products reducing methods and came to this conclusion that dividing the overall disinfection process into several sequences and lowering the dosage of disinfectants used in each time [62]. In order to reach higher results, feeding granular activated carbon before each new disinfection stages can considerably minimize natural organic matters. Chlorination can then be performed later.

3) Using alternative disinfectants. Disinfectants which can substitute chlorine are UV light, potassium permanganate, ozone and chloramines or chlorine dioxide compounds.

4) Removing and separating disinfection by-products by photodegradation, electrochemical reduction and reductive dehalogenation of Haloacetic acids. Other removing of Haloacetic acids is by biologically active carbon and granular activated carbon adsorption. It has long been assumed that granular activated carbon could not remove acids effectively due to their hydrophilic nature and their high ionization in aquatic systems [63]. This is one of the best methods because firstly the disinfection process is safely done and in the second place the disinfection by-products removal process takes place with highest performance.

5) Biodegradation of haloacetic acids. The role of microorganisms on the degradation of Haloacetic acids has been well cited in the literature. The fact that low concentrations of acids have been observed in the presence of low levels of chlorine or chloramine confirms the involvement of microorganisms in the degradation [62]. Several other studies have reported the biodegradation of haloacetic acids in the absence of a disinfectant residual [64, 65]. Baribeau et al. [65] demonstrated the biodegradation of Haloacetic acids in annular reactors at both cold and warm water conditions and in the presence and absence of chlorine and chloramine residual. Degradation was observed for the dihalogenated Haloacetic acid species under warm water conditions. The trihalogenated species, in turn, showed no degradation in the absence of the disinfectant residual. It was suggested that longer retention times are required for degradation of the trihalogenated species to occur. A much greater decrease of Dihaloacetic acid at high temperature than at low temperature is evidence of biologically mediated degradation of haloacetic acids because at higher temperatures, the growth rate of bacteria is enhanced [66]. Some microorganisms have ability to utilize Haloacetic acids [67, 68, 69].

Significant contaminant of wastewater is ethylene chloride [16]. Intermediate product of its degradation is monochloroacetic acid (MCA), which is also very toxic halogenated compound. This acid has been produced in amounts exceeding 300 000 t annually. The main destinations of the compound include thioglycolic acid (intermediate in the manufacture of organotin stabilizers for PVC), herbicides (metolachlor, alachlor, propachlor and metazachlor), carboxymethylcellulose, preservatives for beverages and other chemicals. From the whole class of dihaloacetates, only dichloroacetic acid (DCA) has been produced in large quantities. This compound is an important intermediate in the synthesis of antibiotics, sulphonamides and plant protectives.

The strain *Xanthobacter autotrophicus GJ 10* is known for its ability to degrade severe chemical pollutants and to use them as a sole carbon source. *Xanthobacter autotrophicus GJ 10* is an aerobic strain and we expect to accelerate the process delivering sufficiently amounts of oxygen to the cells. There are two manners to increase the quantity of dissolved oxygen in the media – to increase the stirring rate and to raise the airflow supply. The highest conversion of MCA was achieved in fermentor with a magnetic stirrer at airflow 1.0 l/min (2.2 vvm) and agitation rate of 500 rpm [70].

The degradation kinetics of Monochloroacetic acid by immobilized in polyacrylamide gel and the free cells were compared. Fermentation by immobilized cells were conducted in repeated-batch processes

(six consecutive runs in flasks) and in fed batch processes. The highest degradation rate was achieved using fed batch processes in fermentor in full aerobic conditions. Fed batch process was applied with the aim to increase the final volume of consumed MCA and to overcome the substrate inhibition [71].

The amount of the consumed dissolved oxygen correspondence to the level of acid consumption. The immobilized culture was used not only for direct MCA utilization, but also as a reservoir of new bacterial cells. The immobilized cells leakage was because of the gel particles exhaustion. Better results were obtained with free cells of *Xanthobacter autotrophicus GJ 10*. In the same bioreactor, the biodegradation of MCA and DCA was compared at equal conditions. The kinetic model of the process was developed and high amounts of mono- and dichloroacetic acids were degradated [72].

Results are shown in Table 2, where the most important indexes are the amount of consumed MCA and DCA, and the time for biodegradation of the both acids.

Table 2 – Biodegradation of MCA and DCA (processes and results)

Results	Repeated - batch processes in flasks	Fed batch processes in fermentor				
	Immobilized cells	Immobilized cells	Free cells 400 rpm 0,25 l/min	Free cells 500 rpm 0,25 l/min	Free cells 500 rpm 1,00 l/min	Free cells 500 rpm 1,00 l/min
Consumed MCA (mM)	50	150	155	155	300	-
Time for biodegradation of MCA (h)	450	330	280	190	240	-
Consumed DCA (mM)	-	-	-	-	-	80
Time for biodegradation of DCA (h)	-	-	-	-	-	190

5. CONCLUSION

From the known methods for practical denitrification most appropriate and progressive are the biological, combined with the stimulating effect of constant electric field. They are characterized by high velocity and lack of secondary and waste products. There are two ways for its realization: at a constant current (galvanostatichen) and a constant cathode potential (potentiostatic). It was established that at the galvanostatic approach the denitrification is realized for expense of the reduction with separated hydrogen by the electrolysis or by direct cathodic reduction of nitrates and nitrites. In this group of processes has high-energy consumption due to the relatively strong currents (between 1 and 200 milliamps). In potentiostatic method is observed a significant acceleration of nitrate and nitrite reduction, as this effect can not be explained by electro-chemical electrode processes.

There are many microorganisms for biodegradation of chlorinated aliphatic compounds. A microorganism which is capable to degrade all monohaloacetates is *Moraxella* sp. *Aminobacter* sp. has great potential in degrading 2, 2 - dichloropropionic acid. Haloalkanoic acid such as D, L-2-chloropropionic acid could be degrade with *Rhizobium* sp. The biodegradation of Monochloroacetic acid and dichloroacetic acid was able with X*anthobacter autotrophicus GJ 10* strain. These carboxylic acids were used as carbon substrates of inoculate in fed batch processes. The best results were obtained with free cells of *Xanthobacter autotrophicus GJ 10*. It was found, that the current quantity of the dissolved oxygen corresponds to the current quantity of the consumed acid. After substrate addition the amount of the available dissolved oxygen in the bioreactor is decreasing, because to have larger consummation of acid, the bacterial cells need more dissolved oxygen. Total quantity of degraded acids in fermentor was 300 mm MCA for 10 days and 80 mm DCA for 8 days.

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