Nitrification of Ammonia (Sulphate) by Means of Mixed Culture of Microorganisms: (Part I.)

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This paper discusses oxidation of ammonia-*N* from ammonium sulphate in a synthetic medium by means of mixed culture of microorganisms. The members of mixed culture were isolated from wastewaters from chemical and cellulose industry. Mixed culture of microorganisms was adapted on ammonium sulphate through period more than one year. After adaptation, 1.9 g L^{-1} of wet biomass was used in batch experiments. The glucose, 3-Na-citrate, Na–acetate or methanol was added as a source of carbon (C:N = 6:1), initial pH value was 5.5 or 7.5, and mass concentration of ammonium sulphate in synthetic medium was 0.2 g L^{-1} and 0.6 g L^{-1}, respectively. Oxidation lasted 18 and 48 h and ambient temperature was 23 °C. Results of these experiments showed that initial mass concentration of ammonia-*N* had been changed and in the mean time accumulation of nitrate-*N* had slightly increased.

Key words:
Nitrification, ammonium sulphate, mixed culture of microorganisms, carbon source

Introduction

Nitrogen is one of the essential elements that can be found in living, beings, mainly in proteins and nucleic acids. In general, humans and animals utilize ammonia nitrogen; plants prefer nitrate nitrogen (although they utilize ammonia too), while microorganisms use all types of nitrogen compounds and close the nitrogen cycle in the nature.[1,2] Nitrification can positively or negatively affect nitrogen retention in system, depending on the environmental conditions.[3]

Not so long ago it was thought that autotrophic nitrification is a relatively simple reaction and ammonia in environment is oxidized to nitrate by acting two groups of autotrophic nitrifying bacteria. The first group of bacteria supplies itself with energy for growth by oxidation ammonia to nitrite (genus *Nitrosomonas*) and second group obtain energy by oxidizing nitrite to nitrate (genus *Nitrobacter*). Both groups of bacteria utilize CO_2 as a source of carbon and energy during reductive Calvin's pentose phosphate cycle. The knowledge of nitrifying bacteria has evolved since the modern molecular techniques had been developed and ammonia oxidizing bacteria have been divided into five genera: *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio and Nitrosolobus*. The nitrite oxidizing bacteria have been assigned to four genera: *Nitrobacter, Nitrococcus, Nitrospina and Nitrospira*.[4,5]

Many heterotrophic bacteria[6] and fungi[7] as well can transform ammonia-*N* to element nitrogen (N_2) under condition that carbon source and oxygen are disposable. Biochemical pathways of heterotrophic nitrification are relatively well studied and it is clearly documented that two different pathways of ammonia-*N* oxidation exist, depending if it is organic or inorganic source. In both pathways nitrate-*N* is produced.[8] Many heterotrophic nitrifying bacteria perform a denitrification simultaneously accumulating a little of nitrate-*N*, or nitrate-*N* is not produced. The research has shown that nitrification and denitrification can occur at the same time in mixed culture (e.g. activated sludge) as well as in monoculture cultivated in synthetic medium or in wastewater.[9–11]

The aim of this work was to study aerobic oxidation of ammonia-*N* in synthetic medium by means of mixed culture of microorganisms.

Materials and methods

Microorganisms

Mixed culture of microorganisms, used in this work, was composed of two different microbial populations: a) bacteria isolated from activated sludge from chemical industry, and b) yeast isolated from

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black liquor from cellulose industry. These two cultures were randomly selected because of their diversity and availability. Each sample was inoculated to the nutrient medium, bacterial and yeast one,[12] and after incubation all grown colonies were collected and inoculated to the artificial medium (Table 1) with ammonium sulphate as a source of ammonia-*N*. After 48 h of aeration grown biomass was centrifuged and inoculated to the higher concentration of ammonium sulphate. At the end of that process of adaptation, which lasted more than one year, the remaining cultures were isolated and identified.[13–17]

The usual identification tests as well as API tests[17] (Analytical Profile Index) were applied for identification of microorganisms isolated from mixed culture. The genera of bacteria and yeast were identified observing the morphological and cultural characteristics of organisms and biochemical reactions. In total, five genera of bacteria (*Curtobacterium* sp*., Bacillus* sp*., Micrococcus* sp*., Klebsiella* sp*. and Arthrobacter* sp.) and one genus of yeast (*Geotrichum* sp.) were isolated from adapted mixed culture of microorganisms.

Synthetic medium

Adaptation of mixed culture of microorganisms and aerobic removal of ammonium sulphate was carried out in a synthetic medium consisting of ingredients as it is shown in Table 1. Carbon sources were: glucose, or 3–Na–citrate, or Na–acetate or methanol. Initial pH values were 5.5 or 7.5 due to the composition of mixed culture. Yeast prefers lower and bacteria higher pH values.

Methods

Oxidation was performed in aerobic reactor of 1 L with 0.5 L of synthetic medium (Table 1) containing one of carbon source: glucose, or 3–Na–citrate, or Na–acetate or methanol, and the ratio of carbon to nitrogen (C:N) in all experiments was 6:1. The oxidation of two different mass concentration of ammonium sulphate was investigated: 0.2 g L^{-1} during 18 h and 0.6 g L^{-1} during 48 h at two initial pH value 5.5 or 7.5. During all experiments pH value was not corrected. Concentration of dissolved oxygen was 3.5 mg L^{-1} and the same flow through air remained during the whole oxidation process. The temperature in the reactor was maintained at 23 °C during the process.

Table 1 – *Composition of synthetic medium*

Ingredient	Mass concentration γ/g L^{-1}
$(NH_4)_2SO_4$	0.2 or 0.6
K_2HPO_4	0.85
$MgSO_4x7H_2O$	0.2
Distilled water (L)	1.0
pH value*	5.5 or 7.5

*pH value was adjusted by adding sodium hydroxide solution concentration of 2 mol L^{-1}, or by diluted sulphur acid 20 %.

Analytical procedures

Analytical procedures were carried out according the APHA standards[18] (1998). In synthetic medium the changing of ammonia–*N*, accumulation of nitrate–*N* and changing of pH value were measured. Biomass of microorganisms were measured as wet biomass to speed up obtaining the results.

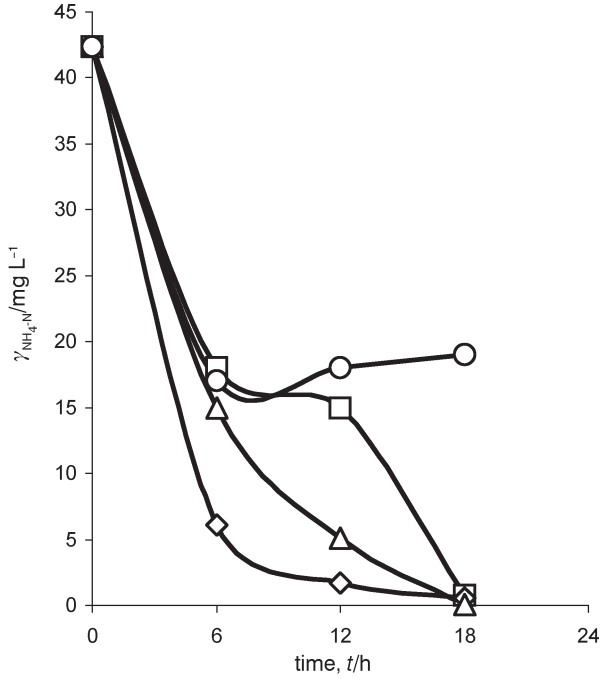
Results and discussion

Ammonia oxidation is the primary step in the oxidation of ammonium sulphate to nitrate–*N* and parameters for nitrification are temperature, pH value, dissolved oxygen, retention time and mixed culture of microorganisms present in the system.

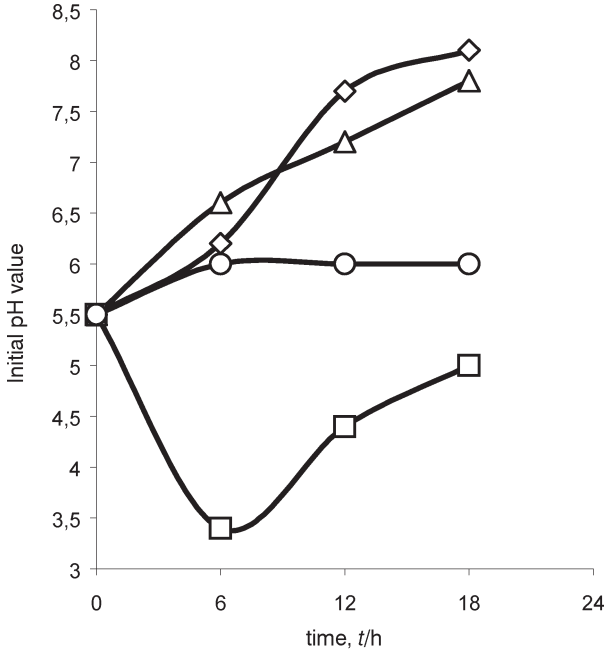
Mixed culture of microorganisms, consisted of facultative heterotrophic bacteria *Curtobacterium, Bacillus, Micrococcus, Klebsiella, Arthrobacter* and yeast *Geotrichum*, differently eliminate in synthetic medium (Table 1) ammonia–*N* from ammonium sulphate mass concentration of 0.2 g L^{-1} during 18 h (Fig. 1 to 6) and 0.6 g L^{-1} during 48 h (Fig. 7 to 12).

At initial pH value 5.5 (Fig. 1) the oxidation of ammonia-*N* is very similar in samples with glucose, 3-Na-citrate and Na-acetate (the degree of oxidation was 98.2 %; 98.6 % and 99.8 %, respectively). However, in sample with methanol, the ammonia–*N* oxidation was only 55.2 % (Fig. 1). The reason for that may be in poor substrate or in insufficient ratio of carbon to nitrogen.[8] In samples of initial pH value 7.5 there were similarity of curves with glucose, Na-acetate and 3-Na-citrate (Fig. 4), and oxidation were 97.9 %, 97.6 % and 99.9 %, respectively. The difference was again in the sample with methanol, where concentration of ammonia-*N* remained at 17.8 mg L^{-1}, or oxidation was only 59.9 % (Fig. 4). The oxidation of methanol does not take place through three carboxylic acids cycle.[8,9]

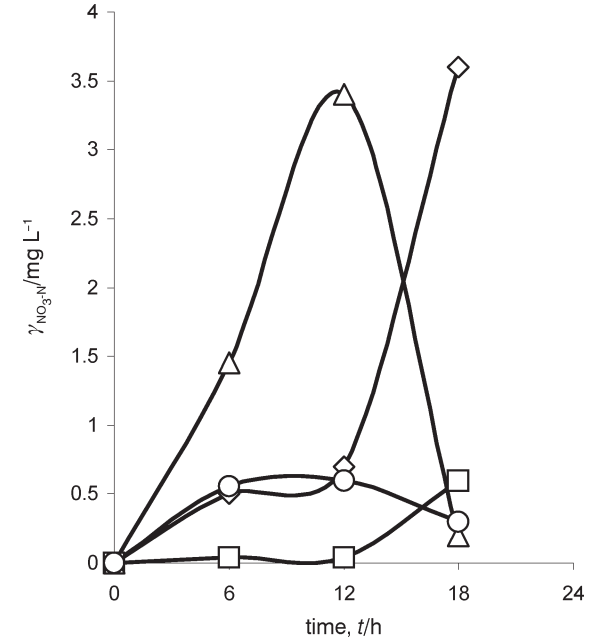
The same procedure was applied for oxidation of ammonia-*N* (127.4 mg L^{-1} NH_4–*N*) from ammonium sulphate mass concentration of 0.6 g L^{-1}, and that time oxidation lasted not 18, but 48 h (Fig. 7 to 12). Oxidation of ammonia-*N* (Fig. 7 and 10) in samples with 3-Na-citrate, at initial pH value 5.5 or



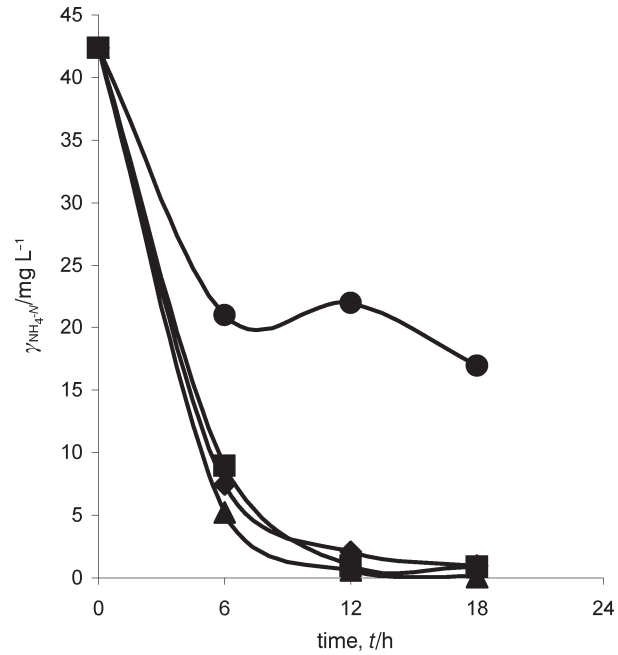
F i g . 1 – *Oxidation of ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–□–), 3–Na–citrate (–◊–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



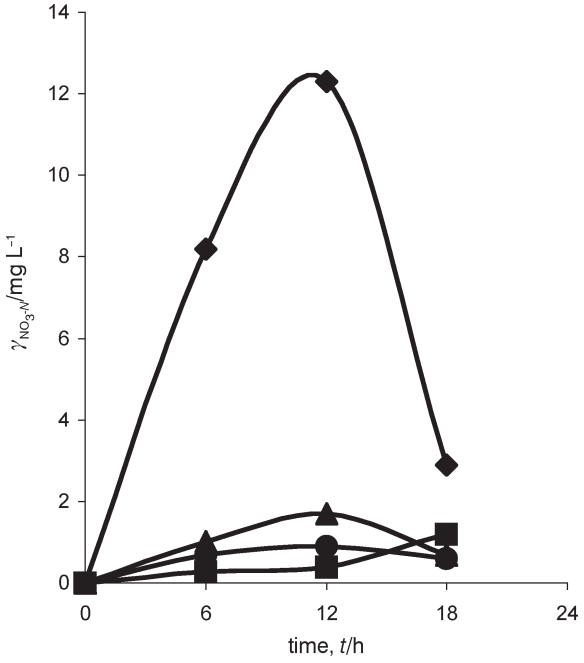
F i g . 3 – *Changing of initial pH value of 5.5 during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–□–), 3–Na–citrate (–◊–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



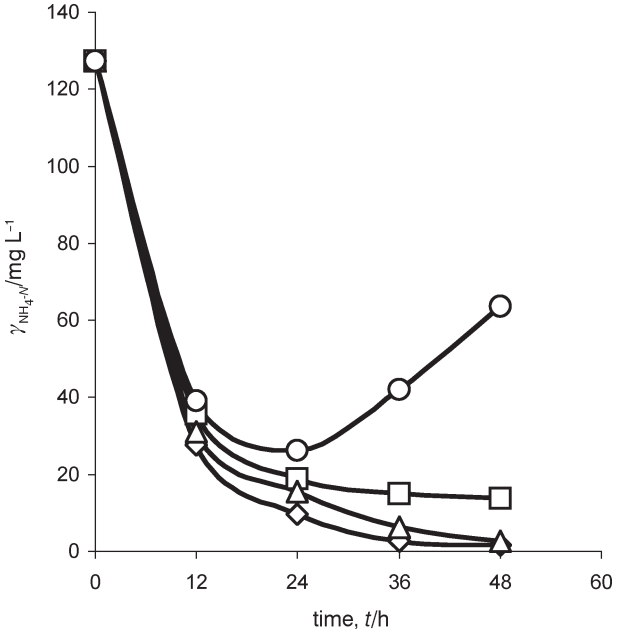
F i g . 2 – *Accumulation of nitrate-N during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–□–), 3–Na–citrate (–◊–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



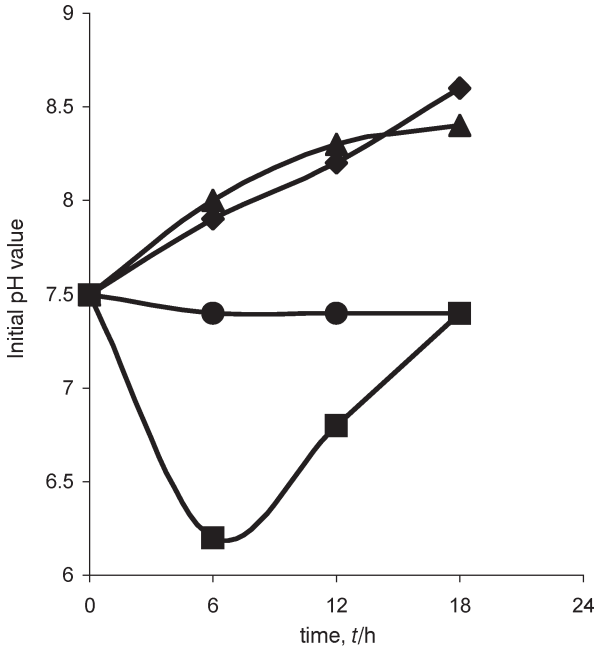
F i g . 4 – *Oxidation ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



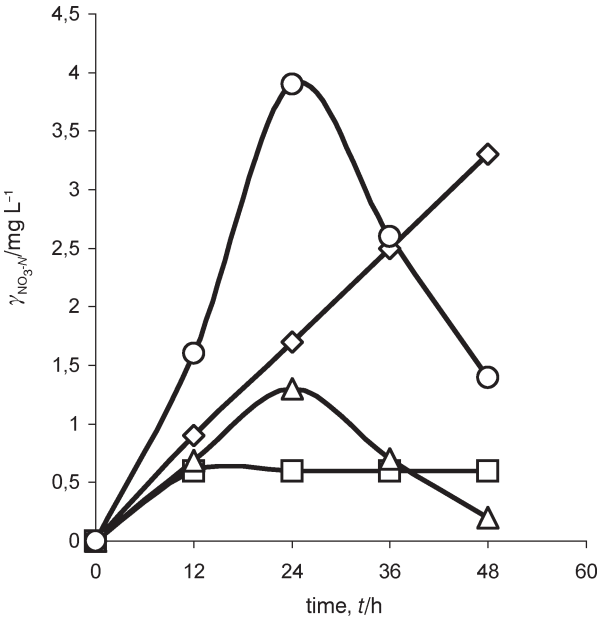
F i g . 5 – *Accumulation of nitrate-N during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



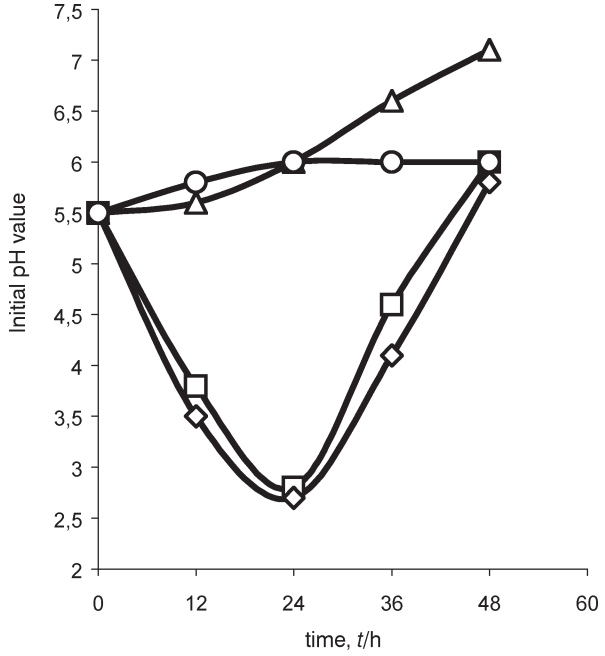
F i g . 7 – *Oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–□–), 3–Na–citrate (–◇–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 48 h at 23 °C.*



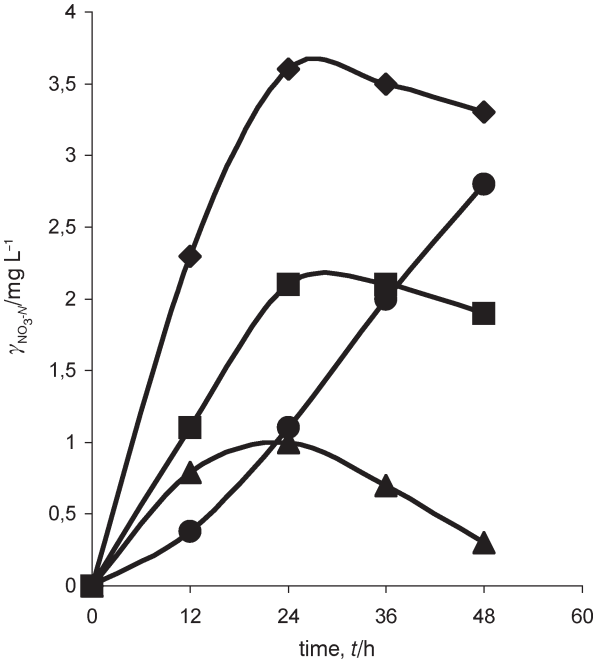
F i g . 6 – *Changing of initial pH value of 7.5 during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.2 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



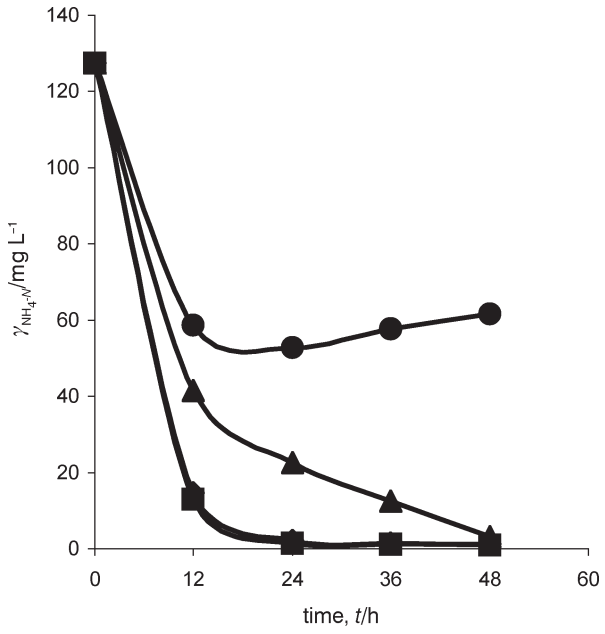
F i g . 8 – *Accumulation of nitrate-N during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–□–), 3–Na–citrate (–◇–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 48 h at 23 °C.*



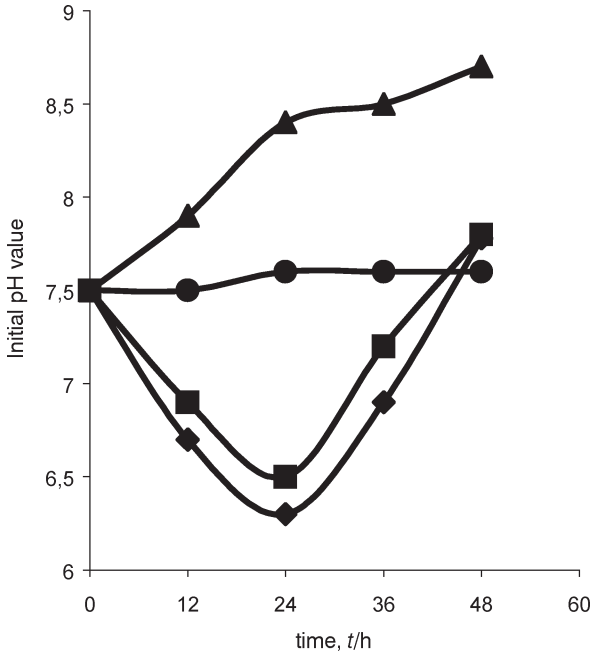
F i g . 9 – *Changing of initial pH value of 5.5 during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–□–), 3–Na–citrate (–◊–), Na–acetate (–△–) or methanol (–○–) in synthetic medium at initial pH value 5.5 by means of mixed culture of microorganisms during 48 h at 23 °C.*



F i g . 1 1 – *Accumulation of nitrate-N during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



F i g . 1 0 – *Oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*



F i g . 1 2 – *Changing of initial pH value of 7.5 during oxidation of ammonia-N from ammonium sulphate mass concentration of 0.6 g L^{-1} with glucose (–■–), 3–Na–citrate (–♦–), Na–acetate (–▲–) or methanol (–●–) in synthetic medium at initial pH value 7.5 by means of mixed culture of microorganisms during 18 h at 23 °C.*

7.5, was again in high percentage: from 89.2 to 99.9 %, respectively. However, when methanol was added the oxidation during first 24 h was almost similar as in previous two experiments (Fig. 1 and 4). Still, as the oxidation is in progress, a mixed culture of microorganisms re-establish formed nitrate-*N* back to the ammonia-*N*. According to literature[3–7] the cause of that could be whether in diminishing of symbiotic activity of higher number of different bacteria and yeast, or in unfavourable ratio of carbon to nitrogen (C:N), or formed intermediate product inhibit removal of ammonium sulphate.

The fluctuations in removal of ammonium sulphate in synthetic medium show that each change of carbon source probably influences the balance among the members of mixed culture of microorganisms. According to the literature[3,4] it seems that the initial pH value of 5.5 or 7.5 are low for nitrification process. In mixed culture of microorganisms different genera of bacteria prevail and consequently they prefer pH value between 6 and 9. However, the yeast prefers some lower pH value, about 5.5. This variety of members of mixed culture, as well as different carbon sources, influence the intensity of change of initial pH value 5.5 (Fig. 3 and 9) or 7.5 (Fig. 6 and 12), to the degree of ammonia-*N* oxidation from ammonium sulphate and to the oxidation of organic matter (as COD value; chemical oxygen demand), from 29 to 82 %. At the same time the relatively low accumulation of nitrate-*N* (Fig. 2, 5, 8 and 11) occurs in synthetic medium as well. The most intensive change of pH value can be seen in sample with glucose and 3-Na-citrate (Fig. 3 and 6), while the permanent growth was in samples with 3-Na-citrate and Na-acetate. Similarly, change of pH value was noticed during oxidation of higher concentration of ammonium sulphate (Fig. 9 and 12).

Conclusion

Mixed culture of microorganisms has shown ability of aerobic removal of ammonium sulphate concentration of 0.2 and 0.6 g L^{-1} with different carbon source: glucose, or 3-Na-citrate, or Na-acetate or methanol.

In most samples, except with methanol, initial pH value significantly influences the aerobic removal of ammonium sulphate.

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