INVESTIGATION OF NITRIFICATION CHARACTERISTICS OF A NITROGENOUS INDUSTRIAL WASTEWATER

by

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INVESTIGATION OF NITRIFICATION CHARACTERISTICS OF A NITROGENOUS INDUSTRIAL WASTEWATER

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ABSTRACT

Treatment of wastewaters containing very high amounts of nitrogen is a problem for industries like the fertilizer industry. Up to date methods like air stripping or ion exchange can also be used in removal of nitrogen but they have certain disadvantages.

The main purpose of this thesis was to present another known process used in nitrogen removal. The general concern of the study is to determine if nitrification process could tolerate industrial wastewaters containing high concentrations of nitrogen.

In the beginning of the experimental part of the study, sludge previously acclimated to nitrification was introduced to increasing concentrations of ammonium nitrogen. In the second part of the study, wastewater from a fertilizer industry was used in order to detect the removal capacity of the system. Throughout the study effects of pH on the system and the system's tolerance was observed. Data obtained during the study proved that nitrification is a process that behaves according to the zero order kinetics. Finally, the results indicated that a nitrification process can surmount very high amounts of nitrogen if the environmental conditions are idealized.

ÖZET

AZOTLU ENDÜSTRİYEL BİR ATIKSUYUN NİTRİFİKASYON KARAKTERİSTİĞİNİN İNCELENMESİ

Yüksek konsantrasyonlarda azot içeren, gübre endüstrisi gibi kimi endüstrilerin atıksularının arıtımı bir problem oluşturmaktadır. Bugüne kadar kullanılmakta olan amonyak sıyırma veya iyon değiştirme gibi metodların düşük verime sahip olduğu gözlenmiştir.

Bu çalışmanın amacı , bir başka azot giderim metodu olan nitrifikasyonu incelemektir. Nitrifikasyon prosesinin yüksek konsantrasyonda azot içeren endüstriel atıksuların arıtımında uygulanabilirliği tartışılmaktadır

Çalışmanin basında, önceden nitrifikasyona alıştırılan çamura giderek artan konsantrasyonlarda amonyum azotu yüklenmiştir. Çalışmanın bir sonraki bölümünde ise, bir gübre endüstrisinden temin edilen atıksu kullanılmıştır. Sistemin giderme verimi incelenmiştir. Çalışma süresince pH'in sisteme etkisi ve sistemin buna karşı toleransı gözönünde bulundurulmuştur. Elde edilen deneysel sonuçlara dayanarak, nitrifikasyon kinetiğinin sıfırıncı dereceden olduğu ve uygun şartlar sağlandığında yüksek miktarda azotu giderebildigi gözlemlenmiştir.

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LIST OF SYMBOLS

k = reaction rate coefficient

K_s = half-velocity constant

Q = flow

r = rate of reaction

S = concentration of substrate at any give time

S_o = concentration of initial substrate

t = time

V = volume

X = biomass concentration at any given time

X_a = average biomass concentration ($(X_a = X_1+X_2+...+X_n)/n$)

Y = yield coefficient

μ = growth rate of nitrifiers

μ_{max} = maximum growth rate of nitrifiers

1. INTRODUCTION

A growing awareness of the dangers of excess amounts of nutrients in the environment especially in water has led to the establishment of different water quality criteria. These criteria indicate the maximum permissible concentrations that are consistent with the protection of aquatic and human life.

Excess nutrients present a problem to biological waste treatment because usually high concentrations of a nutrient may result in process disorders. If these nutrients are to be successfully removed, information concerning acclimation and inhibitory levels must be also known.

One of the most important of these nutrients is nitrogen, which is found in many forms in soil, water and air environments. Nitrogen found in domestic wastewaters can be removed by the activated sludge and further treatment is seldom needed. On the other hand some industries like fertilizer, fermentation, meat and milk, discharge high amounts of nitrogen that must be treated by one of the nitrogen removal methods. Since fertilizer industry is one of the most important industries in Turkey, treating its wastewaters is a current problem. Wastewater from a fertilizer industry is used in this study. In literature, there are very few studies about the treatability of such a wastewater by biological processes.

The most commonly used method for such industrial wastewaters containing very high concentrations of nitrogen is the air stripping process. This process has many disadvantages so recent studies are concerned with the nitrification and denitrification methods. Nitrification and denitrification or the biological treatment of nitrogen is a well known and widely used process but it is not usually used in treating high concentrations. One main reason is that nitrification is said to be inhibited by its own substrate.

The purpose of this study is to investigate the treatability of high nitrogen concentrations by nitrification. Laboratory work is performed in batch reactors with ammonium nitrogen

concentrations reaching up to 1000 mg per liter. Some of the experiments are run with wastewater from a fertilizer industry.

Another discussion in the study is the high initial pH of the fertilizer industry wastewater that is stated to be inhibitory to the process. Also nitrite nitrogen may build up in the system and the joined effects of high nitrite, ammonium concentrations and pH result in lower removal efficiencies. In all of the runs effect of high pH and nitrogen concentrations are examined.

In earlier studies discussing the degree of reaction, there is a disagreement about the degree of nitrification, whether it's zero or first order.A study about the determination of the behavioral kinetics of the nitrification process by different approaches is another concern of the study.

2. THEORY REVIEW

2.1 Nitrogen in the Environment

Nitrogen is an element widely found in soil, water or air environments. As well being in element form it also exists in many compounds because of the high number of oxidation states it can assume. Nitrogen can exist in seven oxidation states as given below:

NH_3 [-3] , N_2 [0] , N_2O[1] , NO [2] , N_2O_3 [3] , NO_2 [4] , N_2O_5 [5]

In biological processes compounds of nitrogen in one, two and four oxidation states have little or no significance whereas all the other forms are important.

The relationship between the various compounds and the transformations which can occur are often presented in a diagram known as nitrogen cycle. Figure 2.1 shows a common nitrogen cycle.

The atmosphere serves as a reservoir of N_2 gas from which nitrogen is removed naturally by electrical discharge and nitrogen giving organisms. During electrical storms large amounts of nitrogen are oxidized to N_2O_5 and its anion with water produces HNO_3 which is carried to the earth in the rain. Nitrates are also produced by direct oxidation of nitrogen or of ammonia in the production of commercial fertilizers. The nitrates serve to fertilize plant life and are converted to proteins.

remaining in the bodies of dead animals and plants are converted in large measure to ammonia by the action of saprophytic bacteria, under aerobic or anaerobic conditions.

$$\text{Protein} + \text{bacteria} \Rightarrow NH_3$$

Some nitrogen always remains in nondigestible matter and becomes part of the nondigestible residue. The ammonia released by bacterial action on urea and proteins may be used by plants directly to produce plant protein. If it is released in excess of plant requirements, the excess is oxidized by autotrophic nitrifying bacteria by nitrification. The nitrates formed may serve as fertilizer for plants. Nitrates produced in excess of the needs of plant life are carried away in water because the soil can't hold them. Under anaerobic conditions nitrates and nitrites are both reduced to nitrogen gas by denitrification.

2.2 Effects of Nitrogen Discharge

Nitrogen discharged from a source has adverse effects on environment that it is exposed to. A well known effect is that nitrogen in the fixed forms of ammonium and nitrate has ability for algal growth which in turn leads to eutrophication which could be expressed as excessive plant growth and/or algae growth resulting from excess nutrients in the receiving water.

Unionized ammonia is toxic to fish and other aquatic life forms. The amount of unionized ammonia is based on the pH of water, since ammonia is converted to the non-toxic ammonium ion with decreasing pH. For example, the criterion for salamonid fish is 0.02 mg per liter of unionized ammonia and for tolerant species is 0.08 mg per liter. These values are equivalent to total ammonia-nitrogen concentrations of approximately 0.5 mg per liter and 5 mg per liter at pH 8, respectively.

In receiving waters, hypochlorous acid reacts with ammonia to form chloramines that are much less effective. The major reactions are as follows:

$$NH_4^+ + HOCl \Rightarrow NH_2Cl \text{ (monochloroamine)} + H_2O + H^+$$

$$NH_2Cl + HOCl \Rightarrow NHCl_2 \text{ (dichloroamine)} + H_2O$$

$$NHCl_2 + HOCl \Rightarrow NCl_3 \text{ (nitrogen trichloride)} + H_2$$

Only after the addition of large quantities of chlorine does free chlorine exist (10 mg per liter chlorine per 1 mg per liter ammonia conc.). When ammonia is discharged to environment the depletion of receiving water oxygen sources can occur as ammonia is oxidized to nitrite and furthermore nitrite is oxidized to nitrate. Theoretically 1.0 mg of ammonia nitrogen can exert an oxygen demand of 4.6 mg per liter when converted to nitrate nitrogen. Nitrification from a discharged wastewater rarely occurs because of competing reactions, such as algal photosynthesis and environmental conditions adverse to nitrifying bacteria.

A major effect of nitrogen is associated with the nitrate form. Nitrate in drinking water causes methemoglobinemia, a sometimes fatal blood disorder which affects infants less than three months old. When water high in nitrate is used for preparing infant formulas, nitrate is reduced to nitrite in the stomach after ingestion. The nitrites react with hemoglobin in the blood to form methemoglobin, which is incapable of carrying oxygen which as a result causes suffocation, accompanied by a bluish tinge to the skin.

2.3 Significance of Nitrogen and Treatment Methods

In an average sanitary waste water most of the nitrogen is in soluble and colloidal organic forms, the amount of nitrogen removed by primary sedimentation is limited to about 15 per

cent. Uptake of nitrogen in a conventional biological treatment is only another 10 per cent. In general, the amount of nitrogen in biological floc produced in activated sludge treatment of a wastewater is equal to about 4 per cent of the BOD applied. For example, with a total reduction of only 25 per cent, the effluent contains 26 mg per liter of an influent value of 35 mg per liter. In the example, approximately 2 mg per liter is organic nitrogen bound in the effluent suspended solids. The remaining 24 mg per liter is in the form of ammonia, except when nitrification occurs during aeration. Oxidation of a portion of the nitrogen content of the wastewater treatment methods, nitrogen removal in conventional biological treatment systems ranges from nearly zero up to 40 per cent. Control and treatment of ammonia and other forms of nitrogen for the above listed reasons can be accomplished by several different methods.

2.3.1 Biological Treatment of Ammonia (nitrification and denitrification processes)

Since the topic of this thesis considers nitrification and denitrification this will be explained later on in Sections 3.1 and 3.2.

2.3.2 Ammonia Stripping

In wastewater, either ammonium ions (NH_4^+), or dissolved ammonia gas (NH_3), or both may be present. At pH 7, only ammonium ions in true solutions are present. At pH 12, only dissolved ammonia gas is present, and this gas can be liberated from wastewater under proper conditions. The equilibrium is presented by the equation (Culp and Wesner, 1978):

$$NH_4^+ \Leftrightarrow NH_3 + H^+$$

As the pH is increased above 7.0 the reaction proceeds to the right. Free ammonia in the system may inhibit nitrification. This is further discussed in Section 3.4.4

The ammonia stripping process consists of:

(a) raising the pH of water to the values in the range of 10.8-11.5;

(b) formation and reformation of water droplets in a stripping tower;

(c) providing air-water contact and droplet agitation by circulation of large quantities of air through the tower.

Although this process is very easy to control and simple it has two limitations:

(a) the practical inability to operate the process at ambient air temperatures below 0°C;

(b) the deposition of calcium carbonate scale from the water onto the stripping tower fill, which results in loss of efficiency from reduced air circulation and droplet formation and may eventually plug the tower.

2.3.3 Selective Ion Exchange

Use of conventional ion exchange resin for removal of nitrogenous material from wastewater is based on using an ion exchanger which is selective for ammonium nitrogen. An exchanger favored is clinoptilolite, a zeolite which occurs naturally in deposits. It is selective for ammonium relative to calcium, magnesium and sodium. The removal of ammonium from the spent regenerant permits regenerant reuse. The ammonium may be removed from the regenerant and released to the atmosphere as ammonia or nitrogen gas, or it may be recovered as an ammonium solution for use as a fertilizer. The wastewater is passed downward through a bed of clinoptilolite during the normal service cycle (typically 1.2-1.5 meters of mesh particles). The clinoptilolite can be regenerated by passing a concentrated salt solution through the bed. Design factors may be listed as (Culp and Wesner, 1978):

(a) pH: 4-8 optimum;

(b) hydraulic loading rate: 7.5-20 bed volumes/hr;

(c) clinoptilolite size;

(d) pretreatment of suspended solids (max. 35 mg/lt);

(e) wastewater composition;

(f) length of service cycle;

(g) bed depth;

(h) determination of ion exchanger size.

Although this process has some disadvantages because of the preference of exchangers for ions other than ammonium or nitrate, there current studies. In addition , the regeneration of conventional ion exchange resins results in regenerant wastes hard to handle. The process is not significantly impaired at temperatures usually encountered, and ion exchange equipment can be automatically controlled, requiring only occasional monitoring inspection and maintenance.

2.3.4 Breakpoint Chlorination

Breakpoint chlorination is accomplished by the addition of chlorine to wastewater to oxidize ammonia nitrogen in solution to nitrogen gas and other stable compounds. Although its very effective in the removal of NH_4^+-N; NO_2^--N and NO_3^--N can not be removed by this method. Since space requirements and capital costs are low and the process is not sensitive to toxic substances and temperature, the method can be preferred in problem areas. On the other hand its effluent may produce high chlorine residuals which is itself toxic. Method is sensitive to pH and needs skilled operators with a considerably high operating cost. The overall theoretical reaction can be written as follows:

$$3Cl_2 + 2NH_4^+ \Rightarrow N_2 + 6HCl + 2H^+$$

The mass ratio of chlorine as Cl_2 to ammonia as N is about 8 to 1. In addition acidity produced by the reaction must be neutralized by the addition of caustic soda or lime.

3. PROCESS DESCRIPTION

3.1 Nitrification

Nitrification is a biological process where ammonia nitrogen is converted to nitrate nitrogen. The process has two steps :

$$NH_4^+ + 3O_2 \Rightarrow NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + 1/2O_2 \Rightarrow NO_3^-$$

The first step , conversion of ammonia to nitrite and the second step, conversion of nitrite to nitrate is held out by generas *Nitrosomonas* and *Nitrobacter*, respectively. Both of these groups are autotrophic, deriving energy for growth from the oxidation of inorganic nitrogen compounds. Also inorganic carbon (as carbondioxide) is used for synthesis.

Energy and synthesis relationships:

The stoichiometric oxidation reaction of ammonium to nitrite by *Nitrosomonas* is:

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \Rightarrow NO_2^- + 2H_2CO_3 + H_2O$$

The loss of free energy has been estimated to be between 58 and 84 kcal per mol of ammonia.

The stoichiometric oxidation reaction of nitrite to nitrate by *Nitrobacter* is:

$$NO_2^- + 0.5O_2 \Rightarrow NO_3^-$$

The loss of free energy for this reaction is between 15.4 to 20.9 kcal per mole of nitrite. The overall oxidation of ammonium:

$$NH_4^+ + 2O_2 \Rightarrow NO_3^- + 2H^+ + H_2O$$

In the above equations the production of free acid (H^+) and the consumption of carbondioxide gas is noticed. Actually these reactions take place at pH levels less than 8.5 so that the products of acid results in immediate reaction with bicarbonate ion (HCO_3^-) with the production of carbonic acid (H_2CO_3). This phenomenon is the reason of pH decrease during nitrification process.

If it is assumed that the empirical formulation of bacterial cells is $C_5H_7NO_2$, the equations for growth of *Nitrosomonas* and *Nitrobacter* are shown below:

$$15CO_2 + 13NH_4^+ \Rightarrow 10NO_2^- + 3C_5H_7NO_2 + 23H^+ + 4H_20$$

$$5CO_2 + NH_4^+ + 10NO_2^- + 2H_20 \Rightarrow 10NO3^- + C_5H_7NO_2 + H^+$$

Experimental yield values for *Nitrosomonas* and *Nitrobacter* range from 0.04 to 0.13 mg VSS grown per mg of ammonia nitrogen oxidized and 0.02 to 0.07 mg VSS grown per mg of nitrite nitrogen oxidized.

The overall synthesis and oxidation reactions for *Nitrosomonas* and *Nitrobacter* can be given as follows:

$$55NH_4^+ + 76O_2 + 109HCO_3^- \Rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3$$

$$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195O_2 \Rightarrow C_5H_7NO_2 + 3H_2O + 400NO_3^-$$

Finally , the overall synthesis and oxidation reaction for nitrification is written as:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \Rightarrow 0.021C_5H_7NO_2 + 1.041H_2O + 0.98NO_3^- + 1.88H_2CO_3$$

Factors that are effective and kinetics of the process will be further explained in Section 3.3.

3.2 Denitrification

Denitrification is the process where the nitrogen in the forms of nitrite and nitrate is converted into nitrogen gas. If in the raw water there is also ammonia nitrogen then a nitrification process must be held prior to denitrification so that ammonia nitrogen is converted into an oxidized form.

Using methanol as a carbon source, the stoichiometry of the denitrification process can be described as follows.

Energy reaction:

$$6NO_3^- + 2CH_3OH \Rightarrow 6NO_2^- + 2CO_2 + 4H_2O$$

$$6NO_2^- + 3CH_3OH \Rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^-$$

Overall energy reaction:

$$6NO_3^- + 5CH_3OH \Rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$$

Synthesis reaction:

$$3NO_3^- + 14CH_3OH + CO_2 + 3H^+ \Rightarrow 3C_5H_7NO_2 + H_2O$$

Overall reaction for denitrification:

$$NO_3^- + 1.08CH_3OH + H^+ \Rightarrow 0.065C_5H_7NO_2 + 0.47N_2 + 0.76CO_2 + 2.44H_2O$$

In the denitrification process, nitrate is reduced to nitrogen gas by the same facultative, heterotrophic bacteria of a relatively broad range including *Pseudomonas, Micrococus* and

Bacillus involved in the oxidation of carbonaceous material. For reduction to occur, the dissolved oxygen level must be available to the bacteria. The most commonly used external carbon source is methanol, CH_3OH. Theoretically , each mg per lt of nitrate should require 1.9 mg per liter of methanol. Under treatment plant conditions this value can raise up to 3 mg per liter which makes the process an expensive one.

It has been observed that the concentration of nitrate will affect the maximum growth of the organisms responsible for denitrification. Carbon concentration, temperature and pH are the most significant factors.

3.3 Classification of Nitrification-Denitrification Systems

Nitrification processes can be roughly classified as separate stage or single stage. In a single stage nitrification system organic matter removal and nitrogen removal take place in one reactor whereas in a separate stage system an aeration tank is followed by a nitrification tank in which organic matter and nitrogen is removed, sequencially. It has been found that when the BOD_5 over TKN ratio of a system is greater than about 5, the process can be classified as a single stage system or in other words a combined carbon oxidation and nitrification process, and when the ratio is less than 3, it can be classified as a separate stage nitrification process, (Metcalf and Eddy, 1991).

According to the type of microorganisms the nitrification process can be further divided as suspended growth or attached growth , both in the case of the single and separate stage nitrogen removal. The principal attached growth processes for nitrification are rotating biological contactors (RBC's) and trickling filters, and the most commonly used suspended growth process is the activated sludge of various kinds.

Although single stage systems have quite low capital and operational cost requirements, they are not protected against toxicants and there is only moderate stability of operation whereas in separate stage nitrification there is a good protection against most toxicants and operation is relatively stable.

Since nitrification and denitrification processes often follow each other , there exist two different types of systems. The first one is the combined nitrification/denitrification system (single sludge) where internal and endogenous carbon sources are used. There are specific advantages to the process including the reduction in the volume of air needed to achieve nitrification and BOD_5 removal, elimination of the need for supplemental organic carbon sources, and elimination of the of intermediate clarifiers and return sludge systems required in a staged system. The second kind is the separate stage (separate sludge) system where denitrification is in a separate reactor using methanol or another external source of organic carbon and the sludge is generated separately in each reactor. Since denitrification is held at in a separate reactor various processes have been developed which can be grouped as attached growth or suspended growth. The principal attached growth processes are packed bed reactors (gas filled or liquid filled), fluid-bed reactors, and rotating biological contactors. Activated sludge systems are also used in the case of nitrification.

3.4 Parameters Affecting Nitrification Efficiency

When designing treatment systems parameters such as temperature, pH, dissolved oxygen concentration, aeration characteristics and many others are significant in the removal efficiency of the nitrification process. These parameters are effective in the growth rate of nitrifiers in the system. In literature , the optimum value for such factors are given meaning the range in which the maximum growth rate reaches maximum. Although it is relatively easy to stay in this range in lab-scale work, in all practical systems these parameters act to affect the nitrification rate. It has been showed that the combined effect of several limiting

factors on biological growth can be introduced into the Monod equation as a product of many factors(EPA, 1975).

$$\mu = \mu_{max}(L/K_L+L)(N/K_N+N)(P/K_P+P)$$

where μ = growth rate of nitrifiers, day^{-1}

μ_{max} = max. growth rate of nitrifiers, day^{-1}

L, P, N = concentration of growth limiting substance, mg/l

K_L, K_P, K_N = half saturation constants for the corresponding substances, mg/l

In this section most significant parameters affecting nitrification rate is very briefly summarized.

3.4.1. Temperature

Nitrification reactions follow the Van't Hoff-Arrhenius law up to 30°C and proceeds better in warmer seasons or climates. The overall optimum temperature for the growth of nitrifying bacteria appears to be in the range 28°C - 36 °C, although optimum temperatures up to 42°C have been reported for *Nitrobacter*. The growth constants of nitrifying bacteria are affected greatly by temperature. Knowles, Downing and Barrett (1965) estimated that the temperature coefficient (increase in minimum specific growth rate constant) for *Nitrosomonas* was 9.5 per cent per degree centigrade. The temperature coefficient for *Nitrobacter* is found to be about 5.9 per cent per °C. However literature values vary considerably, EPA (1975).

Quinlan (1985) stated that optimum temperature is very closely related to ambient dissolved oxygen and nitrogen concentrations. At the concentrations occurring in natural waters and domestic wastewaters, the rate of NO_2-N oxidation by *NitroWinogradskyl* should have an

optimum temperature below 15°C and be thermally inhibited at higher temperatures disagreeing with many other researchers.(Charley,et al.; 1980)

Antoniou et al (1990) worked on an equation for the temperature and pH dependence of *Nitrosomonas* growth in activated sludge. This expression is useful in establishing the capacity of activated sludge facilities that are required to nitrify wastewaters, too. They also studied the maximum specific growth rate of *Nitrosomonas* for a particular wastewater as a function of temperature for different mixed liquor temperatures and pH. Poduska and Andrews (1974) and other researchers performed laboratory work under different temperatures considering the strong influence of temperature on nitrifiers.

3.4.2. pH

Much work has been done on the effect of pH on the activity of nitrifiers and it seems that there is a considerable agreement on the results. The overall optimum pH for nitrification process seems to be in the range of 7.2-8.6 and it appears to be slightly on the alkaline side. Antoniou et al (1990) studied the maximum specific growth rate of *Nitrosomonas* for a particular wastewater with varying pH following the theoretical procedures given before.

One mechanism by which pH affects the rate of nitrification has been proposed by Anthonisen (1976). His hypothesis is based on the fact that ammonia-ammonium and nitrite-nitric acid equilibria depend on pH. He postulated that, when the intracellular pH of a nitrifying organism is lower than the pH of the extracellular environment, free ammonia (FA) will penetrate the cell membrane. Ionized ammonia is postulated to remain in the extracellular environment. Similarly, when intracellular pH is higher than that of the extracellular environment, free nitrous acid (FNA) permeates the cell, not nitrite ion. Later on Suzuki et al (1974) presented work on the effect of pH on nitrification process as a result of Nitrobacter inhibition. This will be furthermore be examined in Section 3.3.4.

3.4.3. Dissolved Oxygen

Oxygen is utilized in the oxidation reactions carried out by nitrifying bacteria. The stoichiometric quantities of oxygen required are 3.43 mg for nitrification of 1mg NH3-N and 1.14 mg for nitratification of 1 mg NO_2-N.

The theoretical nitrogenous oxygen demand (NOD) is 4.57 mg per milligram of ammonia nitrogen. A factor found interesting by a number of workers is the actual ratio of oxygen consumed to nitrogen oxidized, which is often different than predicted stoichiometrically. The mass of oxygen transfer utilized per unit mass of ammonia nitrogen nitrified was slightly less than the theoretical value in an other case, where oxygen utilization was determined by correlation of data, (Adams,1974). Results from a number of studies on the effect of dissolved oxygen concentration show that nitrification can occur at dissolved oxygen concentrations as low as 0.5 mg per lt but still achieving high efficiencies only after 4.5-5 mg per liter, (Knowles et al, 1965: Beccari et al, 1992).

Most studies are done in suspended-growth systems. In the case of attached growth, oxygen availability to a nitrifying slime is subject to diffusion limitations to a greater degree, as the bulk dissolved oxygen concentrations can be significantly different from that within the slime.

3.4.4 Nitrogen Concentrations

The initial conversion of ammonium to nitrite by *Nitrosomonas* has traditionally been regarded as the rate limiting step for nitrification metabolism. This perspective implicitly assumes that subsequent oxidation of nitrite by *Nitrobacter* occurs more rapidly, and that NO_2-N concentrations are consequently maintained at low values. However, numerous

bench and full scale nitrification systems have reportedly encountered elevated nitrite concentrations. Several concerns are generated by this circumstance, including:

(a) an increased chlorine demand;
(b) an increased effluent nitrogenous oxygen demand;
(c) potential nitrite toxicity;
(d) possible nitrosamine formation.

Both *Nitrosomonas* and *Nitrobacter* are sensitive to their own substrate, ammonium nitrogen and nitrite nitrogen respectively and more so to the substrate of other. According to Anthonisen, Loehr, Prakasam (1976), the degree of inhibition depends upon the ammonia-ammonium and the nitrite nitrous acid equilibrium. Other researchers support the suggestion that inhibition is due to free ammonia and undissociated nitrous acid; concentrations of these species have significance in inhibition of nitrification. Boon (1962) agree that the nitrate nitrogen non-competitively inhibits oxidation of 224 mg per liter NO_2-N, with 50 per cent inhibition at 2800 mg per liter nitrate nitrogen.

Anthonisen, Loehr, Prakasam (1976) studied the inhibition of nitrification by ammonia and nitrous acid. Amount of free ammonia (FA) in the environment may result in the inhibition of the activity of *Nitrobacters* resulting in nitrite build-up. As well as, free ammonia, nitrite concentration as unionized nitrous acid (FNA) and pH are also effective on this phenomenon. They stated that as nitrite oxidation occurs, there is a release of hydrogen ions that decreases the pH to an extent related to the buffering capacity of the system. The nitrite formed will exist in equilibrium with unionized nitrous acid. As the pH decreases, the concentration of FNA will increase. Two processes work to reduce FA inhibition. As the pH decreases, the ammonia equilibrium will adjust and the concentration of FA will decrease. In addition , the total ammonia concentration will decrease as is it oxidized to nitrite. These reductions tend to relieve inhibition of the *Nitrobacters* caused by FA, promoting oxidation of nitrite to nitrate.

These conditions may be portrayed graphically. Figure 3.1 can be used to indicate the factors that are involved and to identify situations in which nitrification will or will not occur. Zone 1 represents the condition when the FA concentration is high enough to inhibit

both *Nitrosomonas* and *Nitrobacters*. No nitrification will occur and ammonia will accumulate in the system. At lower concentrations of FA, only Nitrobacters may be inhibited and nitrite accumulation will occur. This condition is represented by Zone 2. At still lower FA concentrations, neither *Nitrobacters* nor *Nitrosomonas* will be inhibited and complete nitrification will occur, (Zone 3). In the absence of any FNA inhibition, complete nitrification by FNA may occur, and Zone 4 represents this condition.

The boundaries of the zones are noted as [A], [B], [C] as sharp separations. However because of factors such as acclimation, numbers of active organisms, and the effect of temperature on reaction rates, it is likely that boundary conditions will consist of ranges rather than sharp separations.

Turk (1989),(1,2), Alleman (1984), Balmelle et al. (1992) studied the process changes that could be used to maintain nitrite build-up and overcome the effects of acclimation to free ammonia so that the use of a shortened nitrification/denitrification pathway for nitrogen removal. However, it is stated that nitrite oxidizers appear to be capable of tolerating ever increasing levels of FA, causing a decline in nitrite accumulation. Nitrite build-up could be maintained for an extended period of time but it is accepted that this not stable due to apparent eventual acclimation of the nitrite oxidizers to FA. None of these studies employ high nitrogen concentration. In literature there is a lack on information how nitrifying systems would behave in case of high strength nitrogenous wastes.

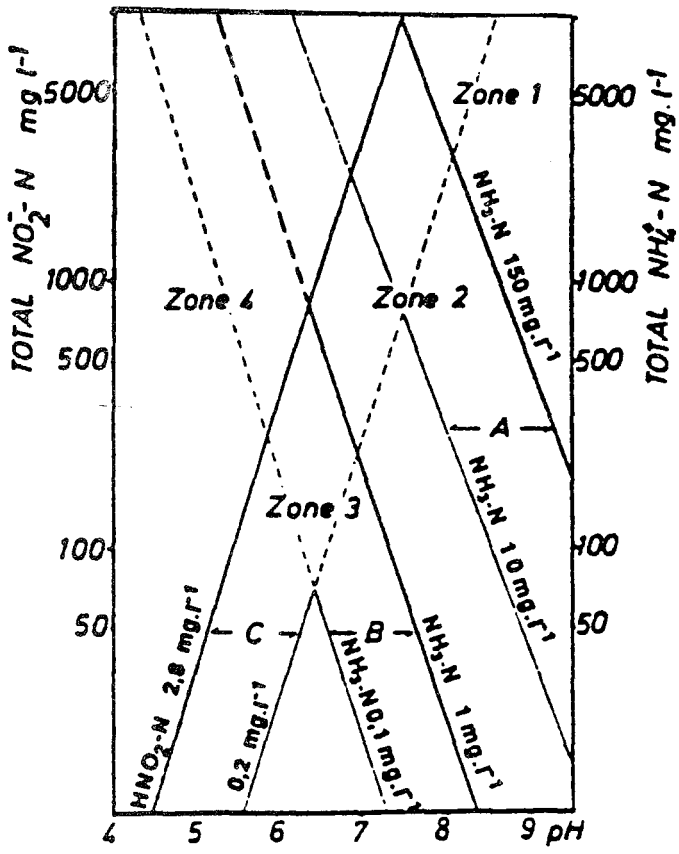


FIGURE 3.1: Nitrification Tolerance Graph, (Anthonisen, et al., 1976)

3.4.5 Other Factors

There are other important and effective factors to nitrification which must not be underestimated. (Hanaki et al.,1990; Tam et al., 1992; Azimi and Horan, 1991; Brenner and Argaman, 1990)These can be listed as :

(a) concentration of nitrifiers;

(b) sludge age, organic loading and detention time;

(c) turbulence;

(d) light;

(e) micronutrients;

(f) organic matter;

(g) adaptability and microbial interactions;

(h) toxic elements.

3.5. Modeling in Nitrification

A process can be translated into mathematical models in different forms. One form concerns the mass or volume of living material (distributed models); another takes into account the concentrations of structural and functional units, such as population density (segregated models). All biomass may be considered the same (unstructured models) or differences in composition of biomass may be allowed (structured models).

As might be expected modeling in the field of nitrification has followed the path of modeling in microbial and enzyme kinetics. The empirical expression after Monod (1949), analogous to Langmuir adsorption isotherms and Michaelis-Menten (single enzyme, single substrate) kinetics, has been employed to describe the growth of nitrifying bacteria:

$$(1/X) * (dX/dt) = \mu = \mu_{max} * S / (Ks+S)$$

where: X = biomass concentration, mg/lt

μ = growth rate of nitrifiers, day^{-1}

μ_{max} = maximum growth rate of nitrifiers, day^{-1}

S =substrate concentration, mg/lt

K_s = half velocity constant, mg/lt

The Monod expression has also been used to model ammonia or nitrite utilization, (Knowles et al, 1965; Williamson and Mc Carty, 1975).

$$-dS / dt = (1/Y) * \mu_{max} * X * S / (Ks+S)$$

where: Y = yield coefficient

It was stated that the biokinetic constants μ_{max}, K_s and Y had to be considered as constants valid only for the respective experimental conditions. For batch reactors, two different

approaches were developed, (Braha and Hafner, 1987). In the first approach the change in substrate and biomass concentrations with time were studied. Graphs plotted by (So-S)/(t*Xa) vs ln(So/S)/(t*Xa) resulted in the determination of the constants although regression coefficients were considerably low.

The second approach gives the kinetic constants by plotting rate biomass change versus the rate of substrate change per biomass. By this method Y and K_d were easily found. However, the biokinetic constants μ_{max} and K_s were still unknown.

Braha and Hafner (1987) studied with batch cultures and proposed that if the K_s value is very high and the maximum growth rate likewise appears at very high substrate concentrations, it should be possible to model the substrate elimination also via application of a first order reaction.

4. HIGH STRENGTH NITROGENOUS INDUSTRIAL WASTES

Industries containing high nitrogen concentrations can be found in a wide range some of which are fertilizer, fermentation, milk,poultry and meat industries. In this thesis, the concerned industry is the fertilizer industry in Turkey with various products that are widely used in agriculture. Discharge limitations for the industry is 50 mg per liter of ammonium nitrogen and the same amount for nitrate nitrogen. There are no limitations stated for nitrite nitrogen. In many of the fertilizer plants air stripping method is being used in the treatment of industries wastes although it seems to be an inefficient removal method.

4.1. Fertilizer Industry

Fertilizer industry is a branch of the chemical industry that produces essential material - nutrients which are mainly composed of phosphorus, potassium, and nitrogen - for agriculture. This industry identifies two kinds of products -non-mixed and mixed. Non-mixed or straight fertilizers are defined as those which contain only a single plant nutrient. Mixed fertilizers are defined as those which contain two or more primary plant nutrients . Mixed fertilizers can be produced by chemically reacting different ingredients and utilizing the chemical reaction as the binding force, or simply by mechanically blending together straight fertilizers. Some straight fertilizers are : Ammonia, Urea, Ammonium Nitrate , Ammonium Sulfate, Phosphoric Acid, Normal Superphosphate, Triple Superphosphate. Ammonium Phosphates are examples of mixed fertilizers.

The fertilizer industry may be divided into three main categories, which are: (1) fertilizer raw materials

(2) fertilizer intermediates

(3) fertilizer products

Fertilizer raw materials are mostly elemental phosphorus, potash, and sulfur. The other two categories are:

(1) fertilizer intermediates
    * sulfuric acid
    * phosphoric acid
    * nitric acid
(2) fertilizer products
    * solid fertilizers
        1) N-fertilizers
            a) ammonium nitrate
            b) urea
            c) ammonium sulfate
        2) P-fertilizers
            a) superphosphates
        3) NP- fertilizers
            a) monoammonium phosphate
            b) diammonium phosphate
        4) NPK-fertilizers
        5) blended fertilizers
    * liquid fertilizers
        1) ammonia
        2) liquid formations
        3) slurry formations

In fertilizer industry raw materials used in processes differentiate to get different kinds of fertilizers. Some of the outcomes may be reused as a raw material for another product. For example, monoammonium phosphate is at the same time the raw material for blend fertilizers. In industry a part of the raw material is brought from outside whereas others are produced as by-products.

Process steps of phosphate fertilizers are: sulfuric acid production, phosphate rock grinding, wet finishing of phosphoric acid, phosphoric acid concentration and cleaning, normal superphosphate, triple superphosphate, ammonium phosphate. The most important steps are sulfuric acid and phosphoric acid processes.

Process steps of nitrogen fertilizers are : ammonia, urea, ammonium nitrate, and nitric acid. Ammonia is the raw material of the other three processes as well as being a fertilizer alone.

4.1.1 Wastewater Characteristics of Fertilizer Industry and Treatment Options

The effluent streams can be characterized as either phosphoric acid effluent or an ammonia effluent. The phosphoric acid effluent is high in fluoride concentration, low in pH, and high in phosphate and suspended solids concentrations. Usually water is contained for reuse but a more sophisticated method is the two stage liming process. The first stage of lime treatment brings the pH up to three or four and reduces the fluoride concentration to 20-25 mg per liter and the phosphorus concentration to 50-60 mg per liter. The CaF_2 precipitate is settled out and the effluent is treated again with lime to raise the pH to six or seven. The F and P concentrations are reduced to about 10 mg per liter. The water is clarified and released to a receiving system.

Concern of this study is the other effluent type that is characteristic of ammonia production and ammonia containing products. Most of the contamination comes from ammonia production itself. It is characteristically high in ammonia from effluent gas-scrubbing and gas cleaning operations and high in sodium hydroxide or carbonate from gas cleaning processes. Common methods to remove ammonia such as ion exchange or air stripping may be used. Studies concerning nitrification and denitrification methods are very limited since effluent ammonia nitrogen concentration may be as high as 4000 mg per liter and this level is usually inhibitory to the method.

4.2. Gemlik Fertilizer Industry

The wastewater used in this study was obtained from one of the largest fertilizer industries in Turkey named Gemlik Fertilizer Industry (Gemlik Azot Sanayii) in Gemlik, Bursa. The industry is a nitrogen fertilizer one, consisting of four units which can be listed as:

(a) HNO_3 unit: oxidation of ammonia;
oxidation of nitrogen oxides under high pressure;
acidification;
production of nitric acid;

(b) Calcium ammonium nitrate unit: preparation of limestone;
ammonium nitrate production;

(c) Cooling water unit: preparation of cooling water;

(d) Packaging and storage unit.

The wastewater characteristics of the industry will be explained in Section 5.2.

5. EXPERIMENTAL WORK

5.1 Apparatus

Acclimation was done in six liter beakers while experiments were performed in three liter beakers with a working volume of two and a half liters. (Figure 5.1) For aeration, aquarium pumps and air diffusers were used and dissolved air concentration in none of the reactors was allowed to fall below six mg per liter which was sufficient for the biomass within reactors to carry out biochemical oxidation to maintain life.Since the concentration of dissolved oxygen is effective on nitrification process, maintaining high concentrations of it, makes the system independent of this factor. The reactors were placed in a water bath at 25°C. Heating was supplied by an aquarium heater. During all experiments, temperature was continuously monitored at small time intervals whereby the range was 23-26°C. The pH of the mixed liquor was continuously controlled and adjusted except two of the runs.

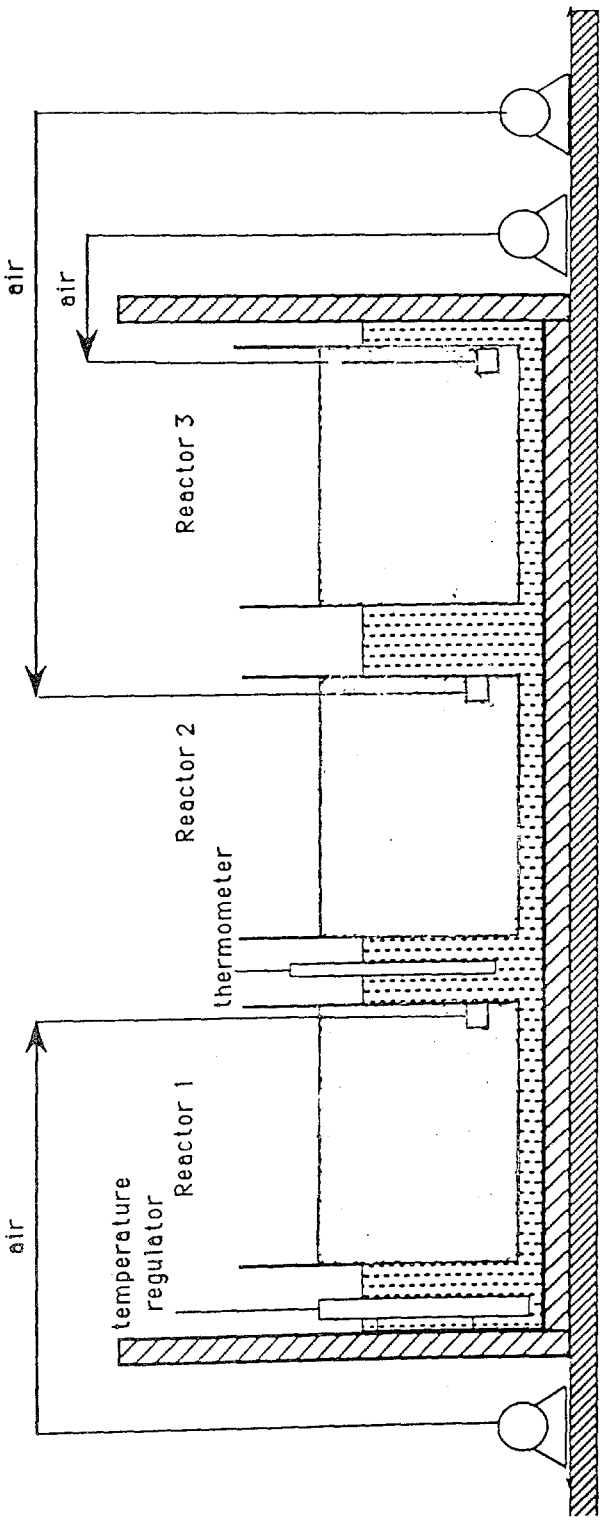


FIGURE 5.1: Experimental Set-up

5.2 Composition of Wastewater

5.2.1 Synthetic Feed

The synthetic feed was composed of two parts as a nutrient solution and a mineral solution. The nutrient solution was prepared as a nitrogen and phosphorus source. In some of the runs NH_4Cl was replaced by $(NH_4)_2SO_4$ keeping ammonium concentration the same, since excess chloride causes interference in some of the analyses. The mineral solution was for the supply of trace elements. The composition of the medium is given in Table 5.1. Amount of feed added varied with different runs, ammonium nitrogen concentration ranging from 100 to 1000 mg per liter. In the initial period of the acclimation studies, low amounts of glucose were added to the feed to give a low COD. For 100 mg per liter of COD, 0.1 gr per liter of glucose was added to the feed. After a certain period of time glucose addition was neglected. As the inorganic carbon source, $CaCO_3$ was used throughout the research.

5.2.2 Industrial Wastewater

In the last three runs wastewater from a nitrogen fertilizer industry was used as the nitrogen source in the medium. About 200 liters of grab samples were taken from the discharge end of the industry. Samples are preserved in a cool place (about 4^0C) after decreasing their pH to about two. Preparation of the feed was similar to the earlier runs (Table 5.1) except this time there was no addition of any form of a nitrogen compound. Ammonium nitrogen was supplied by the industrial wastewater itself The composition of the wastewater analyzed in

the laboratory is given in Table 5.2. In Table 5.3 some of the analyses results given by the industry are listed. The properties of each run are given in Section 5.5.

TABLE 5.1: Composition of Synthetic Feed Solution

Constituents	Concentration (g/lt)
K_2HPO_4	320
KH_2PO_4	160
NH_4Cl	120
$MgSO_4$	15
$Fe.SO_4.7H_2O$	0.5
$ZnSO_4.7H_2O$	0.5
$MnSO_4.H_2O$	0.4
$CaCl_2$	2
$CaCO_3$	220

TABLE 5.2 : Laboratory analysis of composite samples of the industrial wastewater

NH_4-N (mg/lt)	576
pH	9.6
(NO_3+NO_2)-N (mg/lt)	306
COD (mg/lt)	<30
MLSS (mg/lt)	30

TABLE 5.3: Previous wastewater composition reported by Gemlik Fertilizer Industry

	unit	Day 1	Day 2	Day 3	Day 4	Average
NH_4-N	(mg/lt)	620	655	350	535	540
pH		9.66	9.74	9.16	9.49	9.51
NO_3-N	(mg/lt)	406.3	297	327.7	426	364.25
Chloride	(mg/lt)					135
MLSS	(mg/lt)	-	-	-	-	-
COD	(mg/lt)	-	-	-	-	-

5.3 Biomass

The activated sludge was taken from the treatment plant of a meat processing industry. Since nitrogen is considerably high in such industries, in the sludge of the treatment plants nitrifiers are to be found. In sewage, heterotrophs are the dominating organisms with small amounts of nitrifiers. Aim was to enrich the nitrifier population. During acclimation period COD:N ratio was changed from 1:1 to 1:2 in a decreasing order. After these runs were completed, the addition of COD was omitted for some time in order to maximize the growth of nitrifiers and the ratio of nitrifiers to heterotrophs. Throughout the runs MLVSS to MLSS ratio was ranging in from 0.83 to 0.87.

5.4 Analyses

Samples taken just before analyses were filtered by a microfilter in order to minimize the suspended solids concentration which could affect the results of the measurements. If immediate analyses could not be done samples were preserved by acidification to a pH of about two.

All the forms of nitrogen considered were determined by preliminary distillation followed by titration. In preliminary distillation, a known volume of sample with an adjusted pH of about 9.5 is distillated for 15 minutes, collecting the distillate into an indicating boric acid solution. The solution is then back titrated with an acid. Distillation was done by Gerhardt Vapodest 12 distillation apparatus. For nitrate and nitrite nitrogen determination, Devarda alloy method was used. This alloy was added to the same sample previously used in determining NH_4^+-N and the procedure was repeated. Also spectrophotometric methods were used to analyze nitrite and nitrate separately by Hach DR/3 Spectrophotometer using Nitriver 2 and Nitraver 5 test kits.

Mixed liquor suspended solids concentration was determined by using dry weight measurements. The filter paper used was a microfilter paper with pores 0.45 μm in diameter.

For COD analysis, the dichromate closed reflux method was applied photometrically.

Dissolved oxygen was measured by Hach Portable D.O. apparatus and the pH of the samples were measured with an Orion SAS20 pH meter.

All measurements done are according to those given in Standard Methods.

5.5 Experimental Procedure

Acclimation of the sludge was performed in order to observe higher efficiencies when working with industrial wastewater containing high ammonium nitrogen concentrations. Three runs with varying initial characteristics were studied. The purpose of the first three runs was to enrich the nitrifiers in the sludge meanwhile getting accustomed to the analyses methods. Enrichment is done by adjusting varying biomass concentrations, while staying in the optimum level for pH to achieve the highest rate for nitrification. In all of the runs it was observed that there was no change in biomass concentration, so an average concentration was used throughout the calculations. Chudoba et. al.(1992), stated that in batch cultivation at low initial substrate to initial biomass concentrations, there is a very low or no observed change in biomass concentration and this was noticed in all of the experimental runs of the study.

In Run 1, influent ammonium nitrogen concentration was about 100 mg per liter with a 100 mg per liter of COD addition so that the theoretical COD/NH_4-N ratio was about one to one.

Purpose of Run 2 was to increase the ammonium nitrogen and COD ratio (NH_4-N/COD) to two. Run 2 has a significant approach since when pH is high, removal of ammonium nitrogen by air stripping is likely to occur as explained in Section 2.3.2. Two reactors were ran parallel to each other, one with sludge and one without named as the control unit. It was observed that a little amount of influent ammonium nitrogen was air stripped because of the controlled pH.

Finally in Run 3, the amount of COD was neglected and only a calculated influent of 100 mg per liter of ammonium nitrogen was added to the system.

In Table 5.4, properties of the acclimation runs are given.

After the acclimation period of sludge to ammonium nitrogen was over, five different runs were performed. In all the runs, temperature and dissolved air concentrations were in the optimum range stated for nitrification. In Table 5.5 initial ammonium nitrogen concentrations and average biomass concentrations of the five runs are given.

TABLE 5.4: Properties of the Acclimation Runs

Run number	Initial NH_4-N conc. (measured)	COD conc. (calculated)	MLSS conc. (average)
	(mg/lt)	(mg/lt)	(mg/lt)
Run 1	69.97	100	1575
Run 2	57.96	50	1055
Run 3	93.24	-	945

TABLE 5.5: Properties of the experimental runs

Run number	Initial NH_4-N conc.	MLSS conc.
	(mg/lt)(measured)	(mg/lt)(average)
Run 4	97.2	285
Run 5a	187.2	480
Run 5b	392	355
Run 5c	525.3	357
Run 6a	948.9	1390
Run 6b	873.5	1520
Run 7a	333.06	1215
Run 7b	364.06	1370
Run 8	372.12	354

In Run 4, the purpose was to obtain dependable and accurate data from a newly acclimated sludge.

In Run 5, three reactors with volumes of two and a half liters were used to examine the effects of increased initial ammonium nitrogen concentration.

With the results of Run 5 being encouraging, the initial ammonium nitrogen was increased to near 1000 mg per liter. In Run 6, two parallel reactors were established. The main difference of the two reactors was that in Run 6b, there was no pH adjustment. The initial substrate concentrations being very high, the effect of decreasing pH on the system was observed. As stated before pH decreases as nitrification process takes place. The pH decrease, if left unadjusted, results in decrease in the substrate removal rate.

Runs 4, 5, and 6 were performed with the synthetic feed given in Table 5.1.

In Run 7, wastewater from the nitrogenous fertilizer industry in Gemlik was used in order to determine the system performance for a real industrial wastewater high in nitrogen content. This run was established in two parallel reactors. In Run 7b, there was no pH adjustment in order to observe any inhibition that would result from the decrease in pH.

Run 8 was established in order to control if nitrogen removal is carried out by nitrification or air stripping because in the last two runs the high initial pH favors the air stripping process. Two parallel reactors with similar properties were used. A control reactor was established in order to realize the amount of air stripping in the system and this control reactor contained no biomass.

6. RESULTS AND CONCLUSION

6.1 Results

6.1.1. Results of the Acclimation Runs

As discussed earlier in Section 5 first three runs were held out in order to acclimate sludge to nitrification. For each of the three runs of acclimation, Run 1, 2, and 3, substrate removal, product formation, change in MLSS concentration and pH are given throughout Figures 6.1 to 6.12. In Section 5, it was stated that in Run 2 two parallel reactors were established in order to realize the effects of ammonia stripping that might be the cause of the substrate removal observed in Run 1. Figure 6.13 gives the change in substrate for the control reactor.

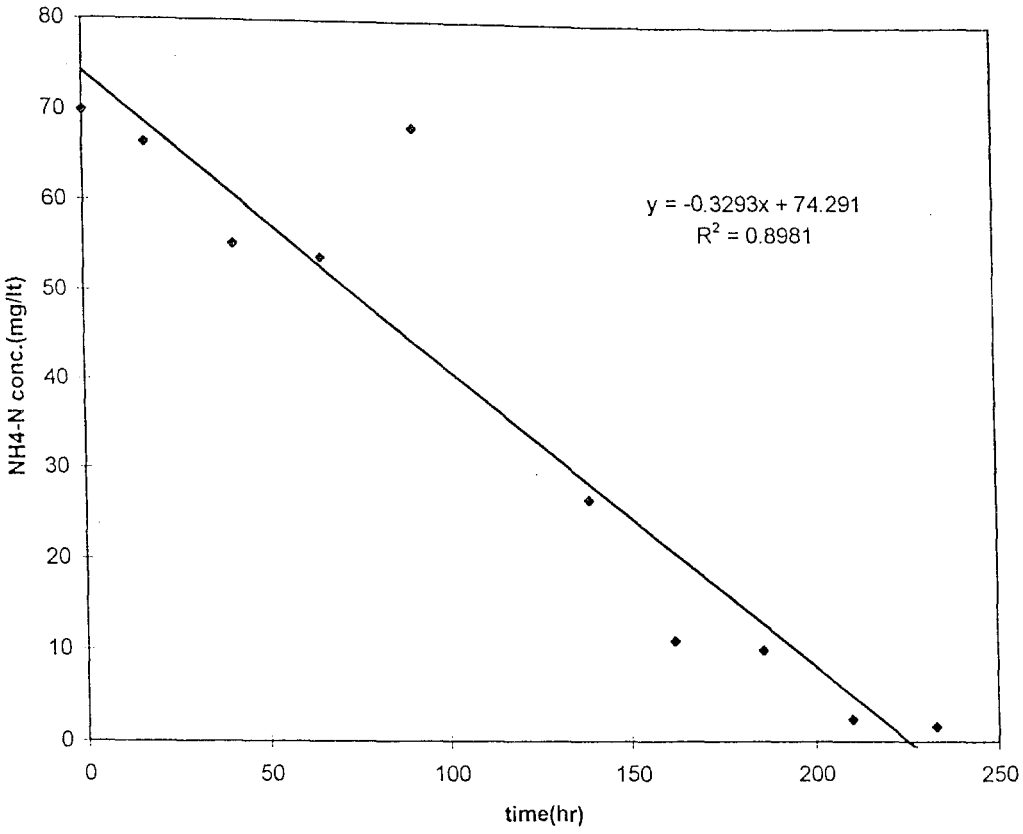


Figure 6.1: Change in substrate concentration of Acclimation Run 1

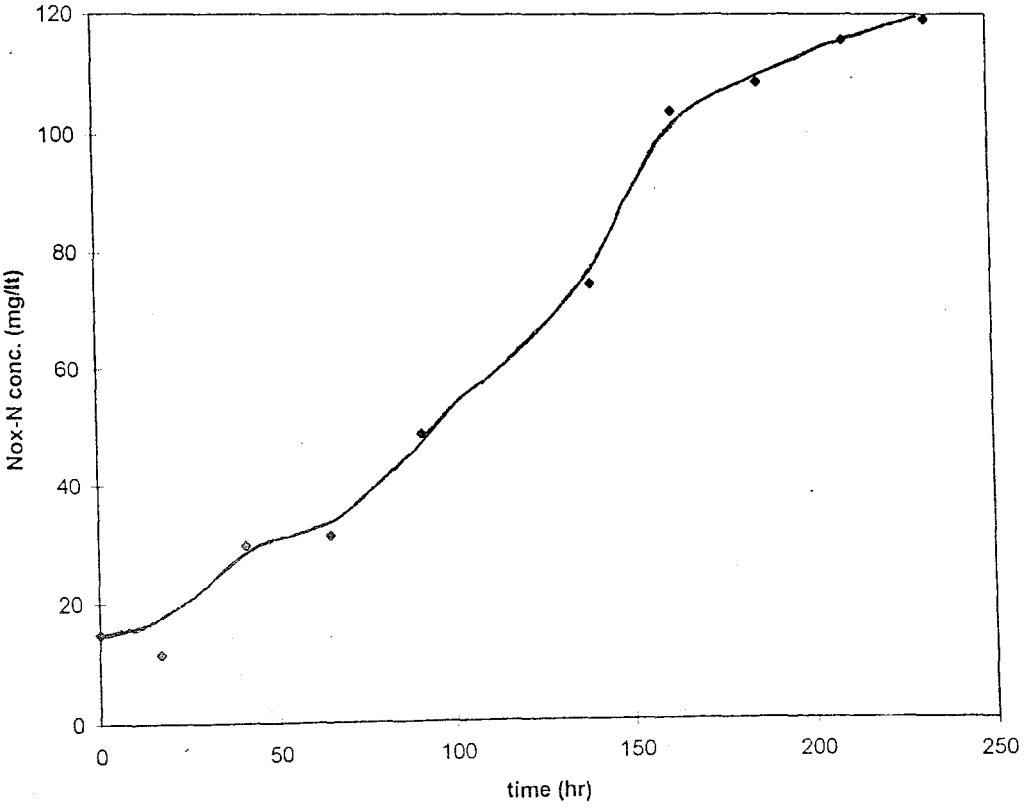


Figure 6.2: Change in product concentration of Acclimation Run 1

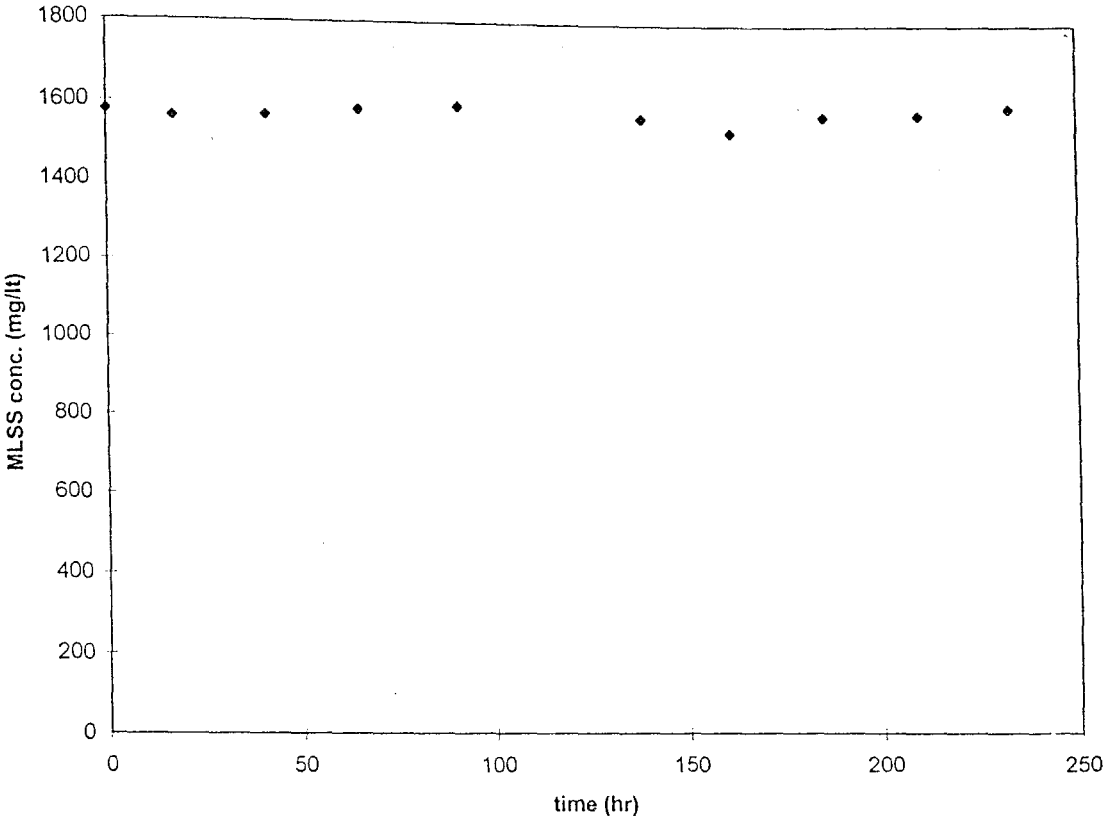


Figure 6.3: Change in MLSS Concentration of Acclimation Run 1

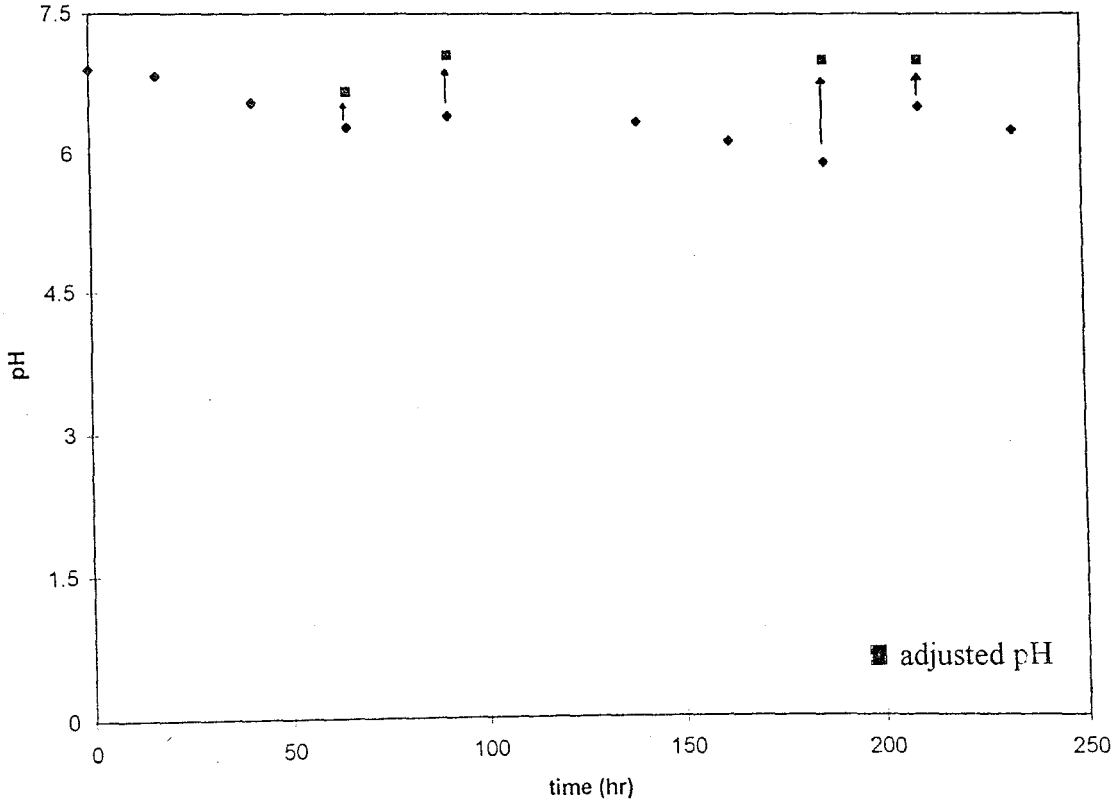


Figure 6.4: Change in pH of Acclimation Run 1

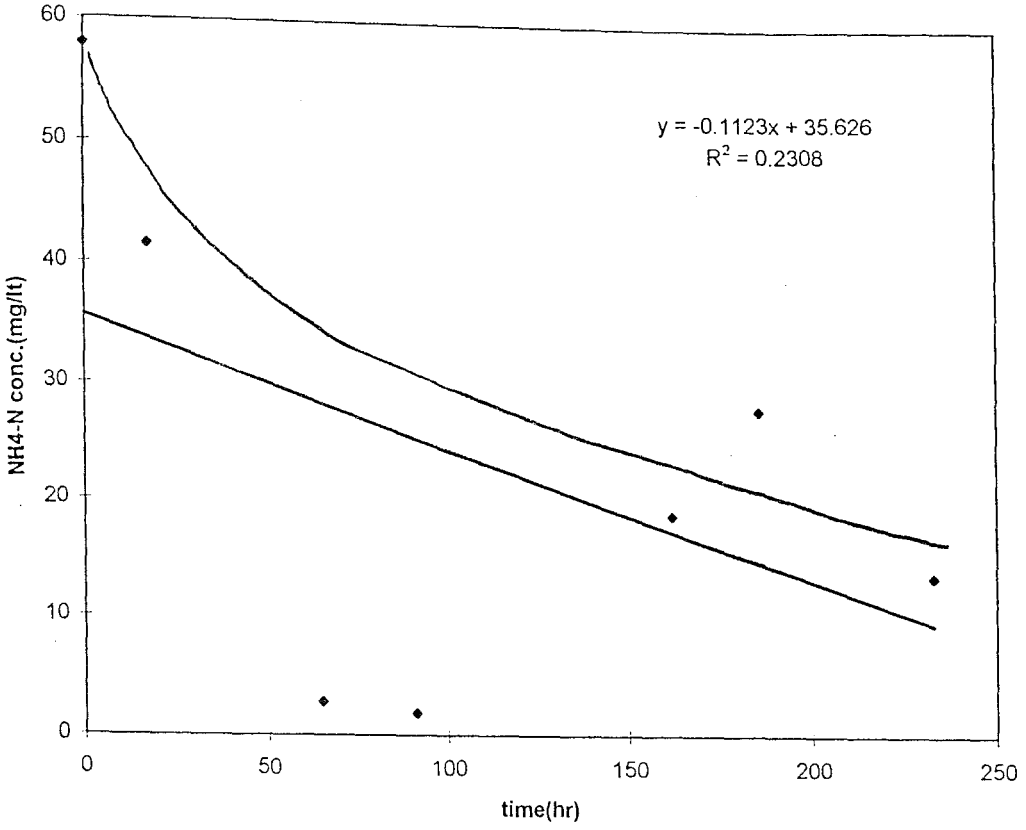


Figure 6.5: Change in substrate concentration of Acclimation Run 2

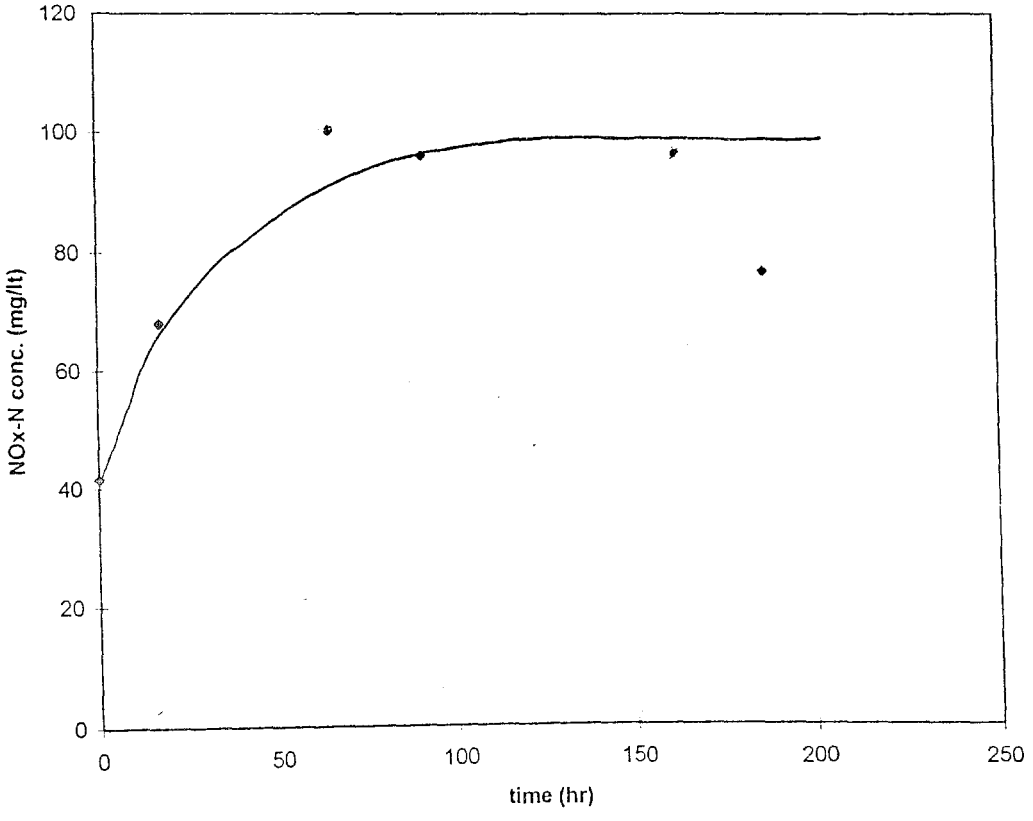


Figure 6.6: Change in product concentration of Acclimation Run 2

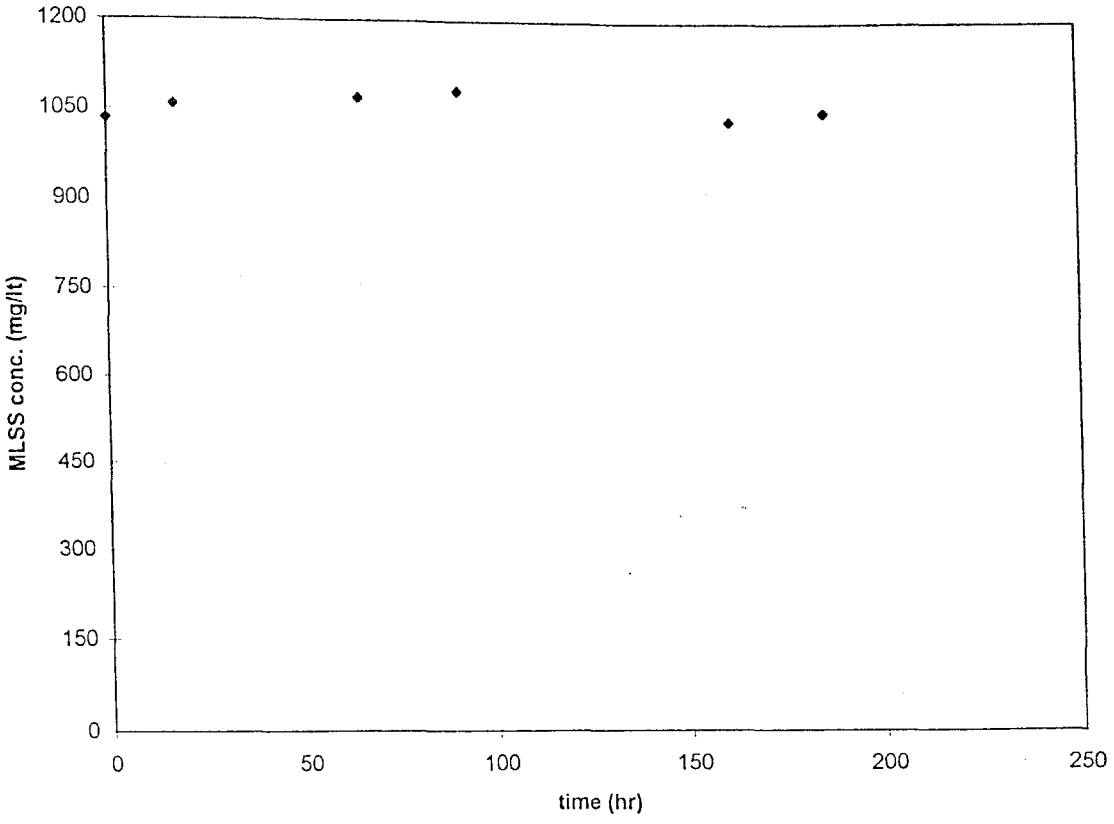


Figure 6.7: Change in MLSS Concentration of Acclimation Run 2

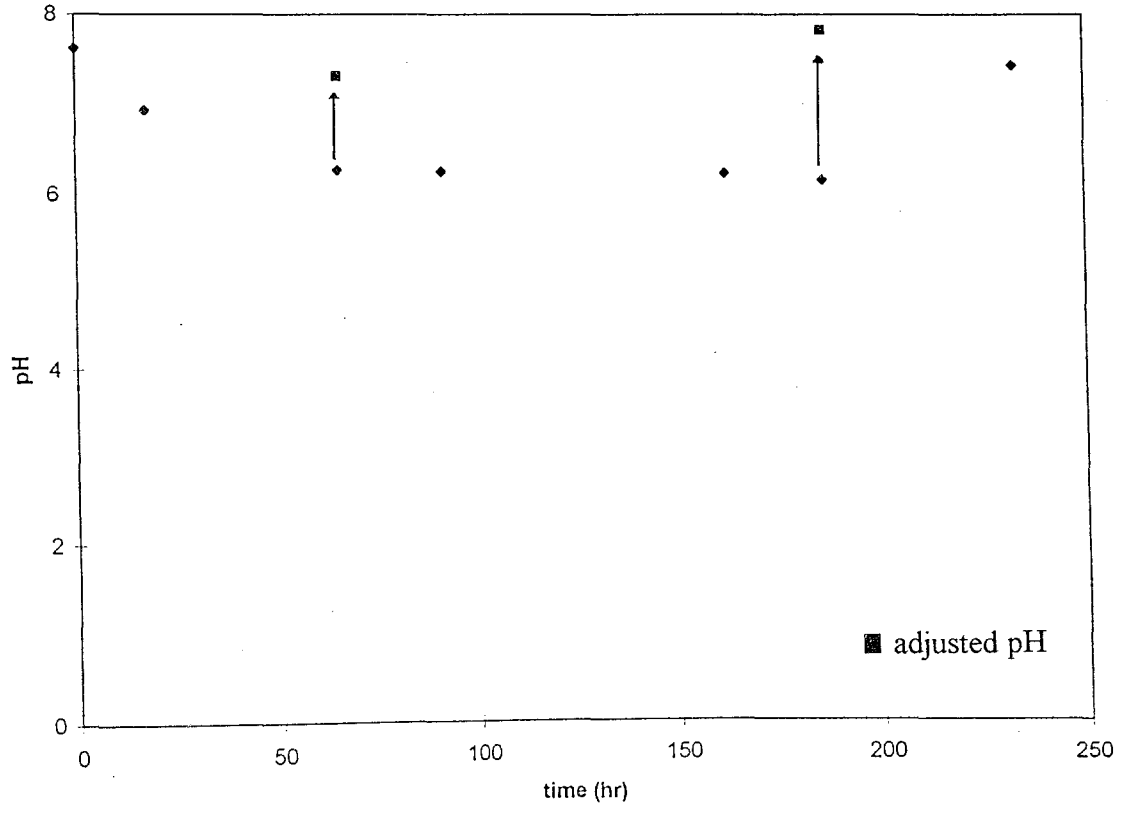


Figure 6.8: Change in pH of Acclimation Run 2

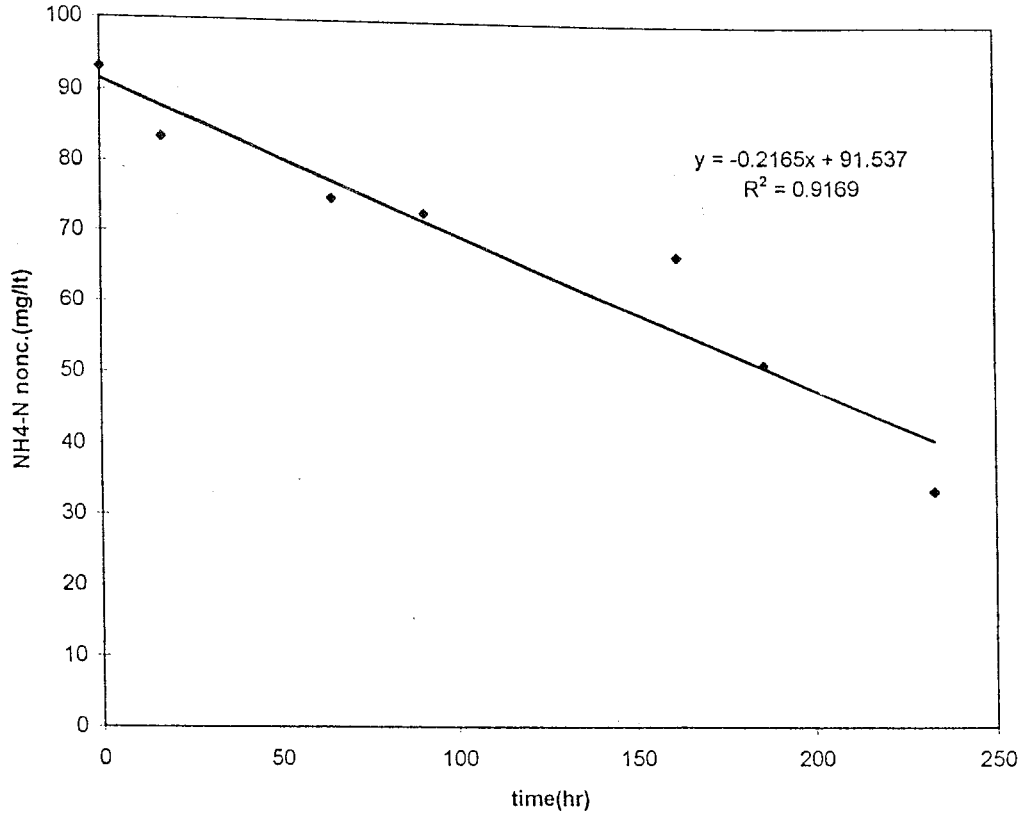


Figure 6.9: Change in substrate concentration of Acclimation Run 3

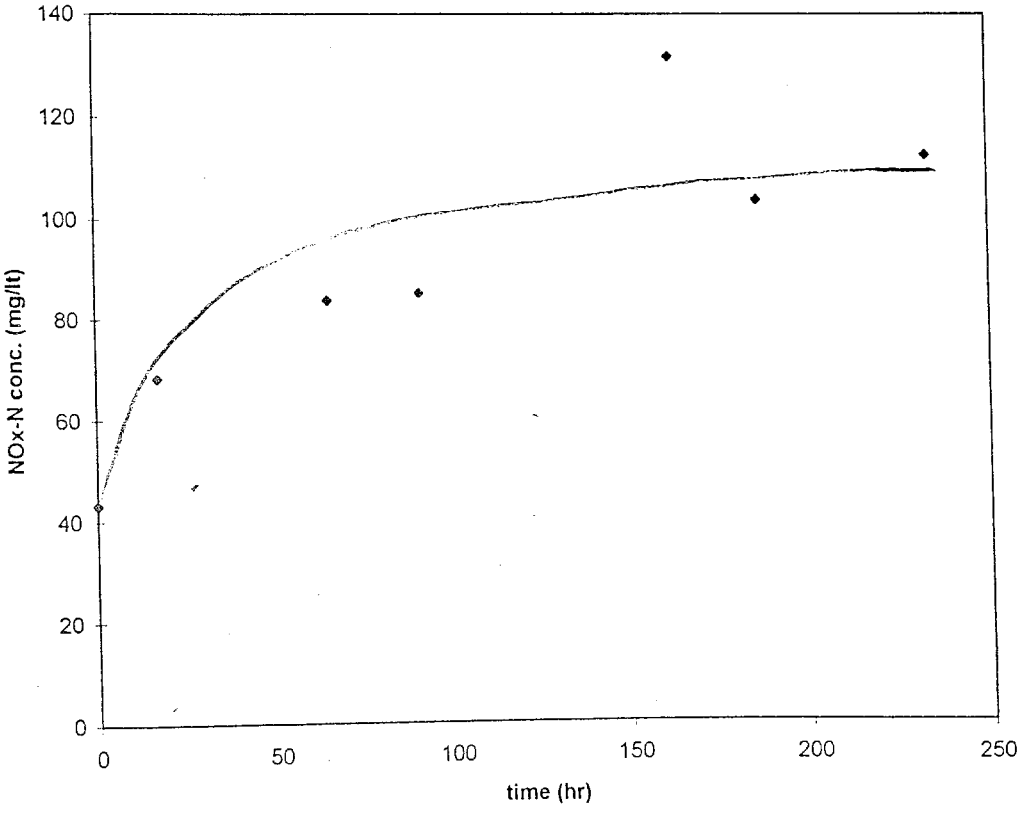


Figure 6.10: Change in product of Acclimation Run 3

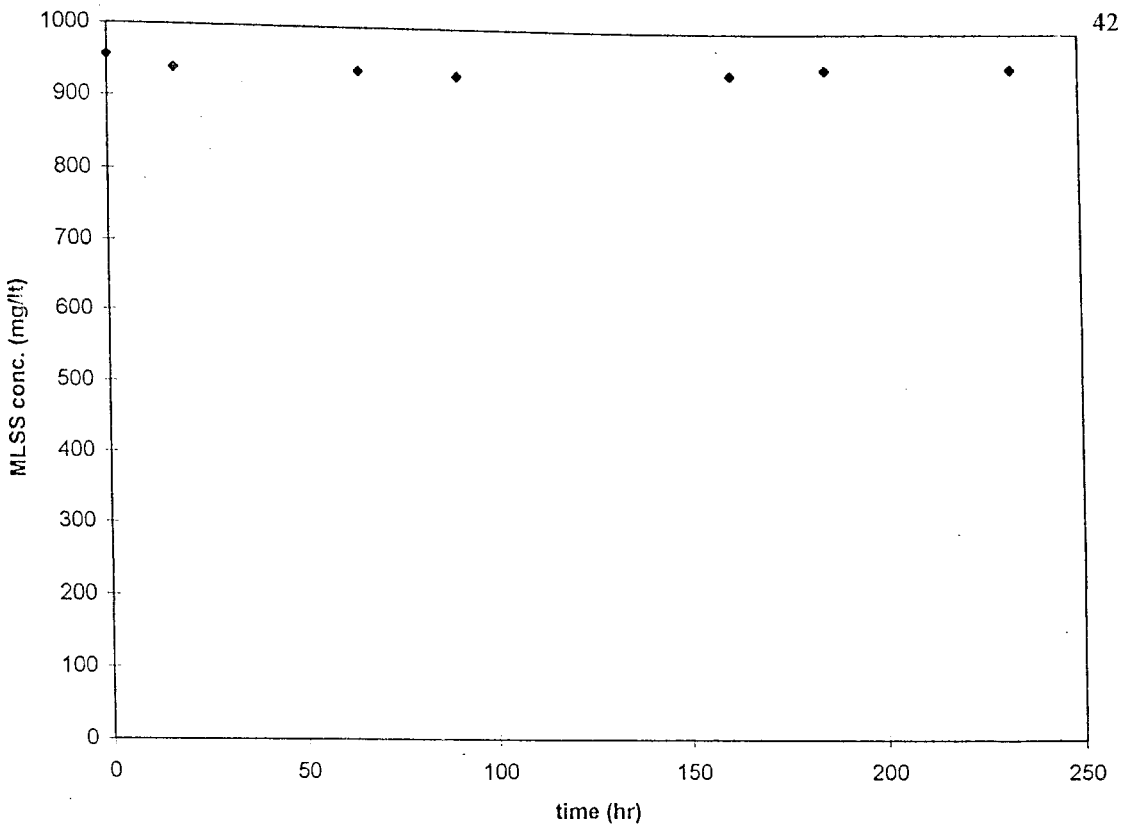


Figure 6.11: Change in MLSS Concentration of Acclimation Run 3

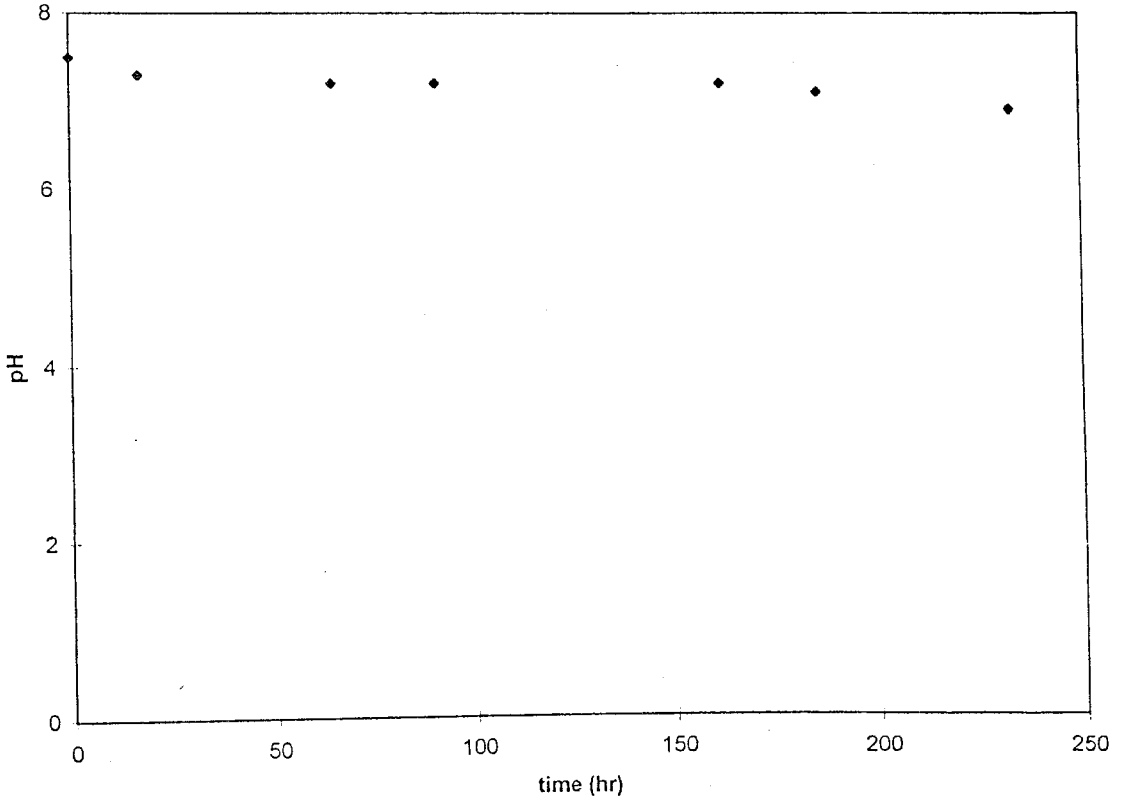


Figure 6.12: Change in pH of Acclimation Run 3

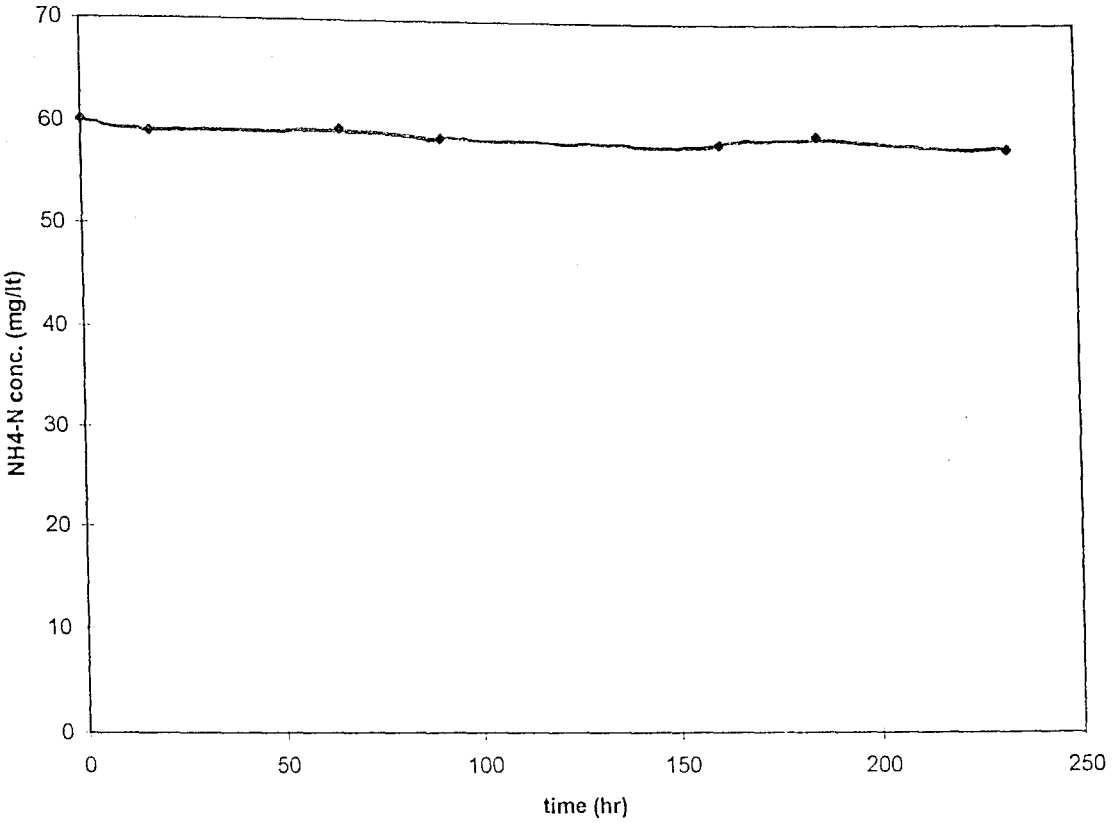


Figure 6.13: Change in substrate concentration in the control reactor of Acclimation Run 2

6.1.2 Results of the Experimental Runs

After the acclimation of sludge to nitrification or in other words enrichment of nitrifiers in the culture was over, a new set of runs were established. Results obtained from laboratory work are plotted in Figures 6.14 through 6.59. For each run change in substrate, product, MLSS, pH , and nitrite nitrogen concentrations are given.

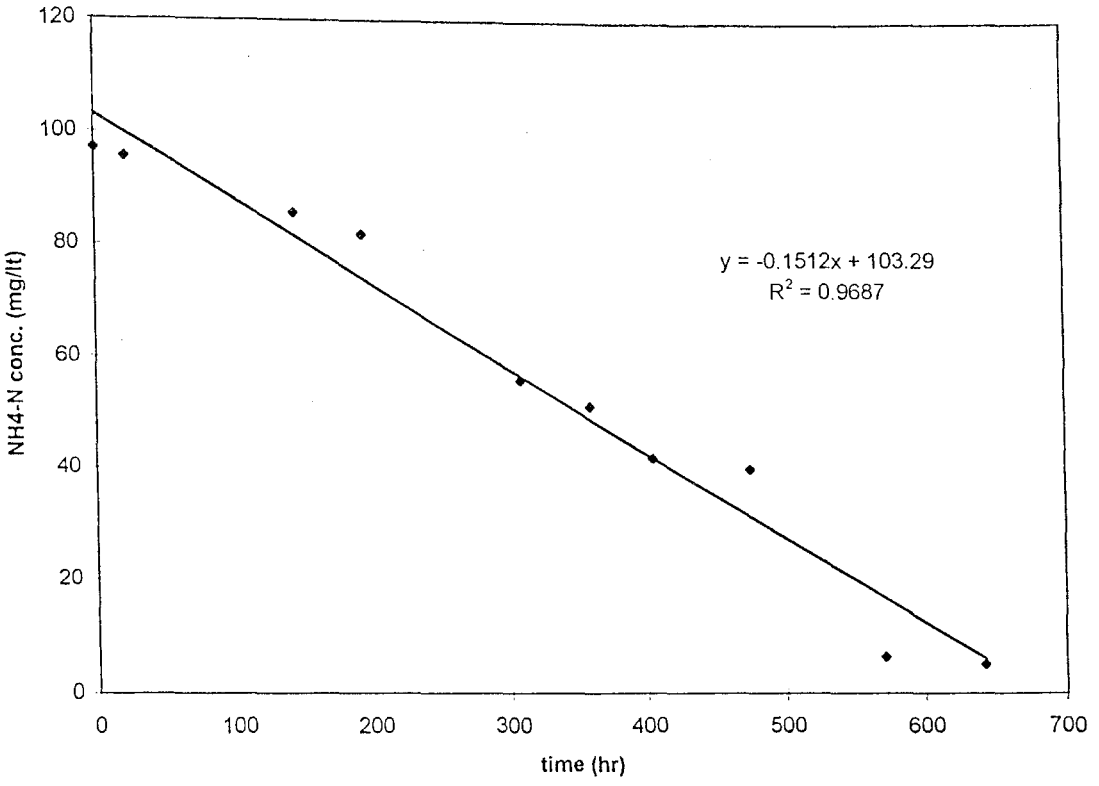


Figure 6.14: Change in substrate concentration of Run 4

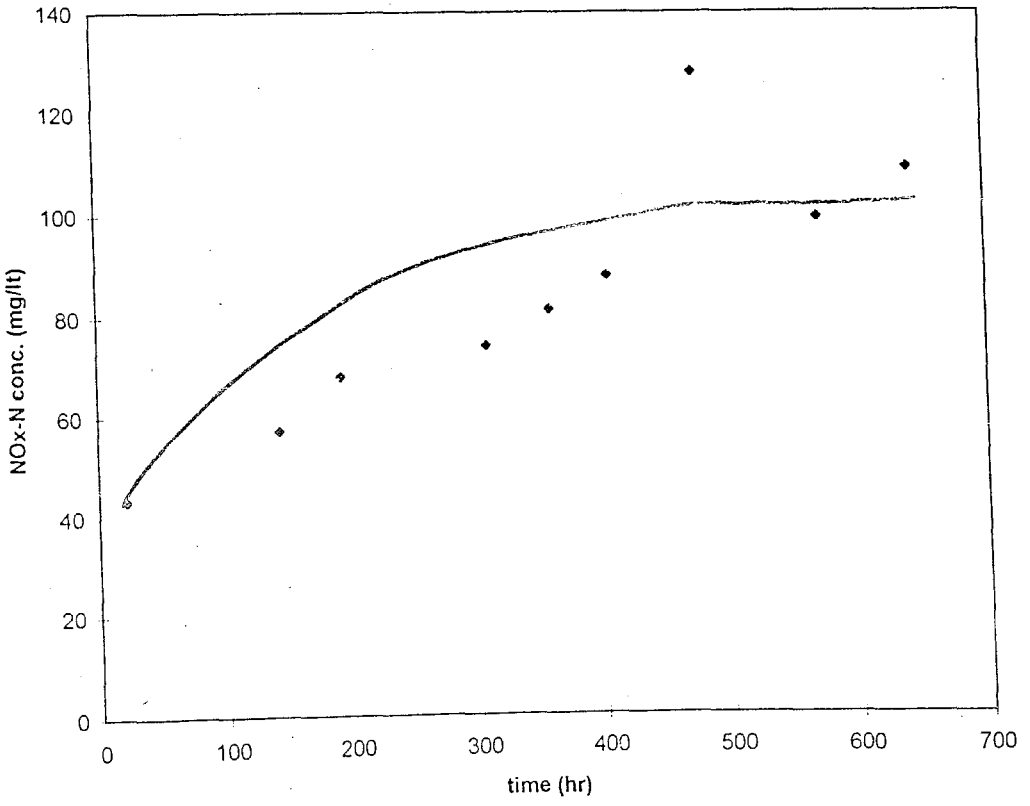


Figure 6.15: Change in product concentration of Run 4

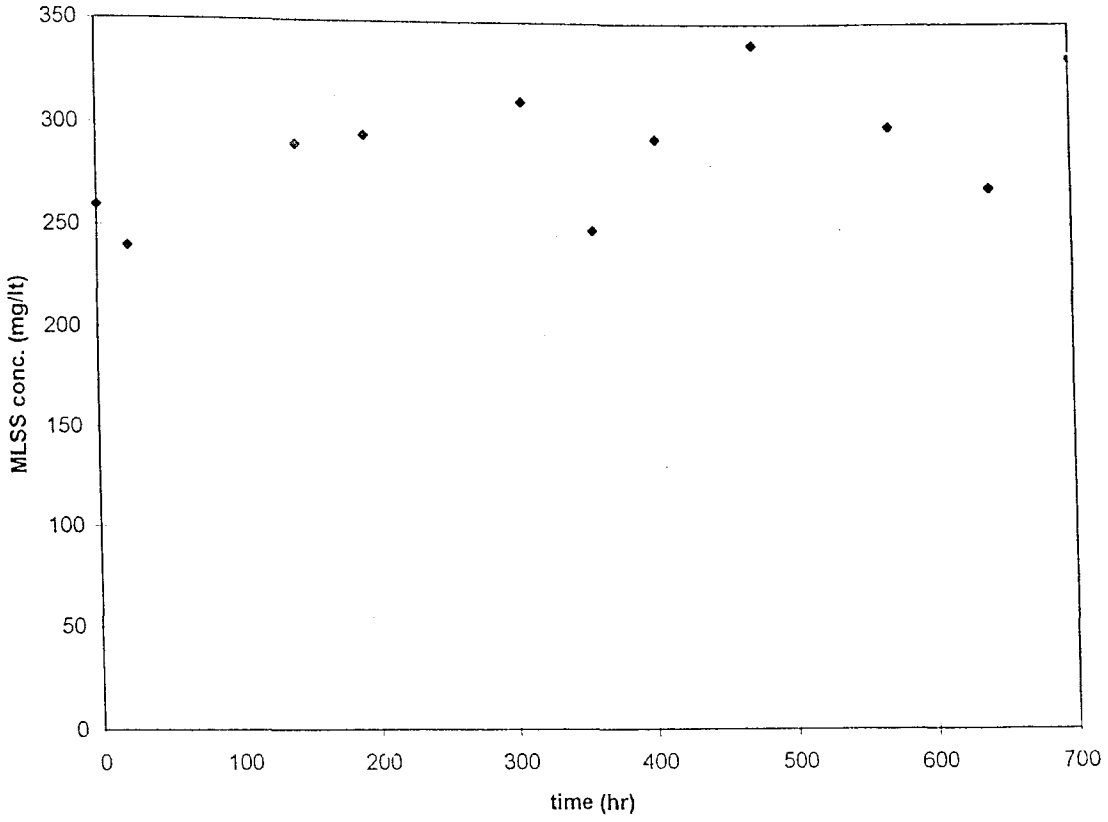


Figure 6.16: Change in MLSS Concentration of Run 4

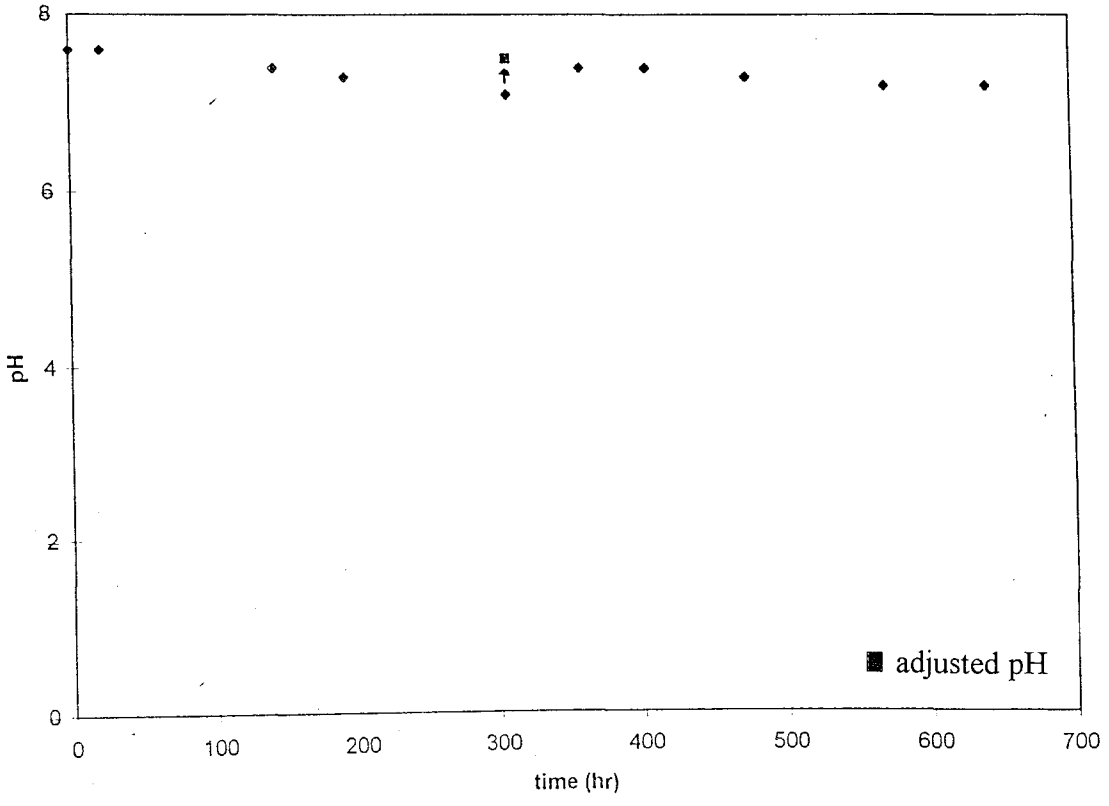


Figure 6.17: Change in pH of Run 4

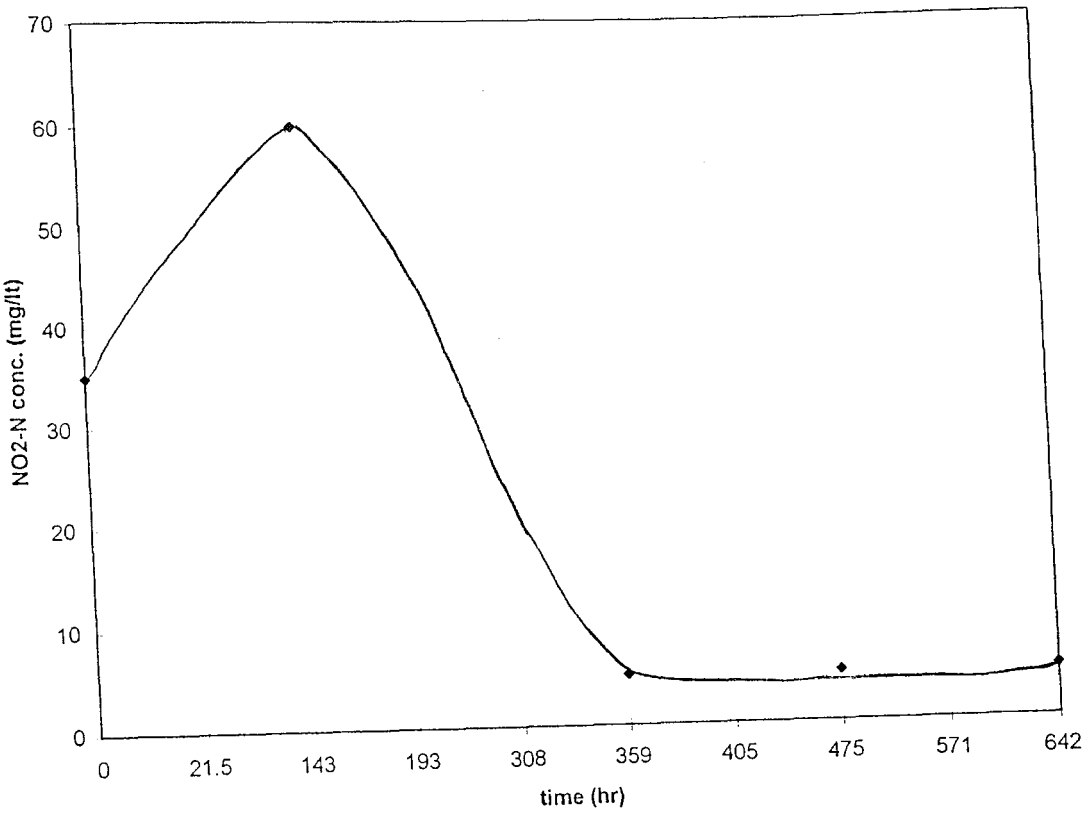


Figure 6.18: NO_2-N formation of Run 4

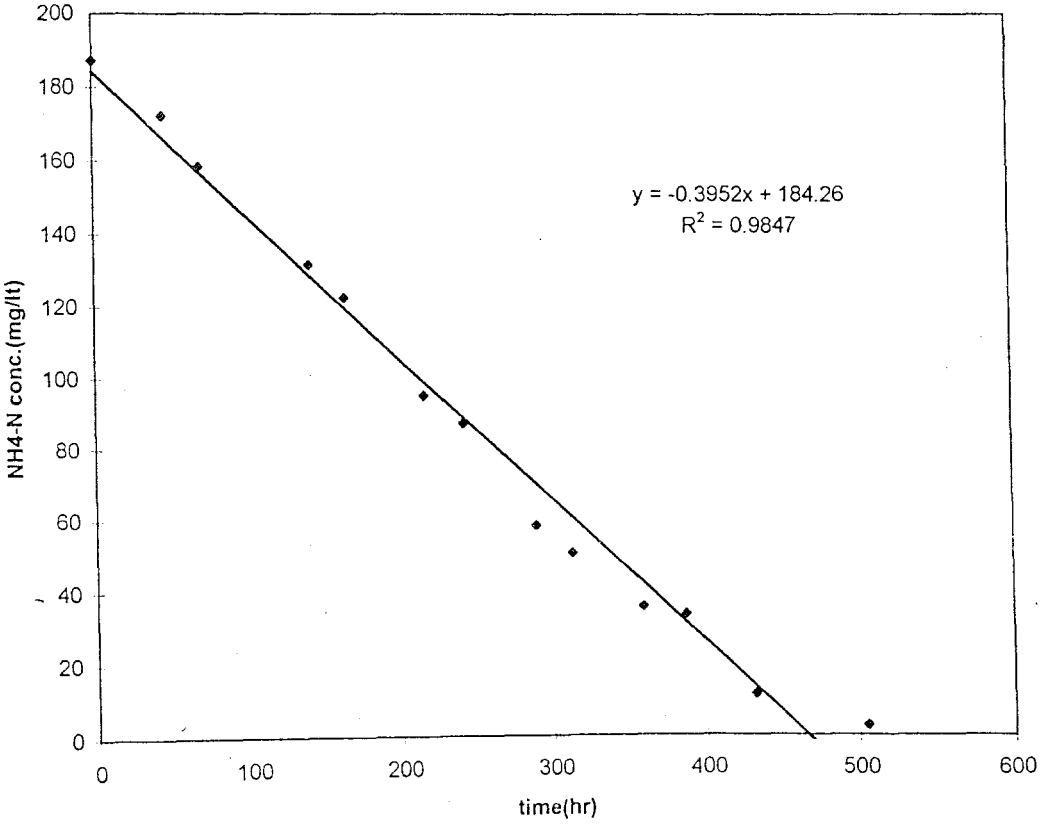


Figure 6.19: Change in substrate concentration of Run 5a

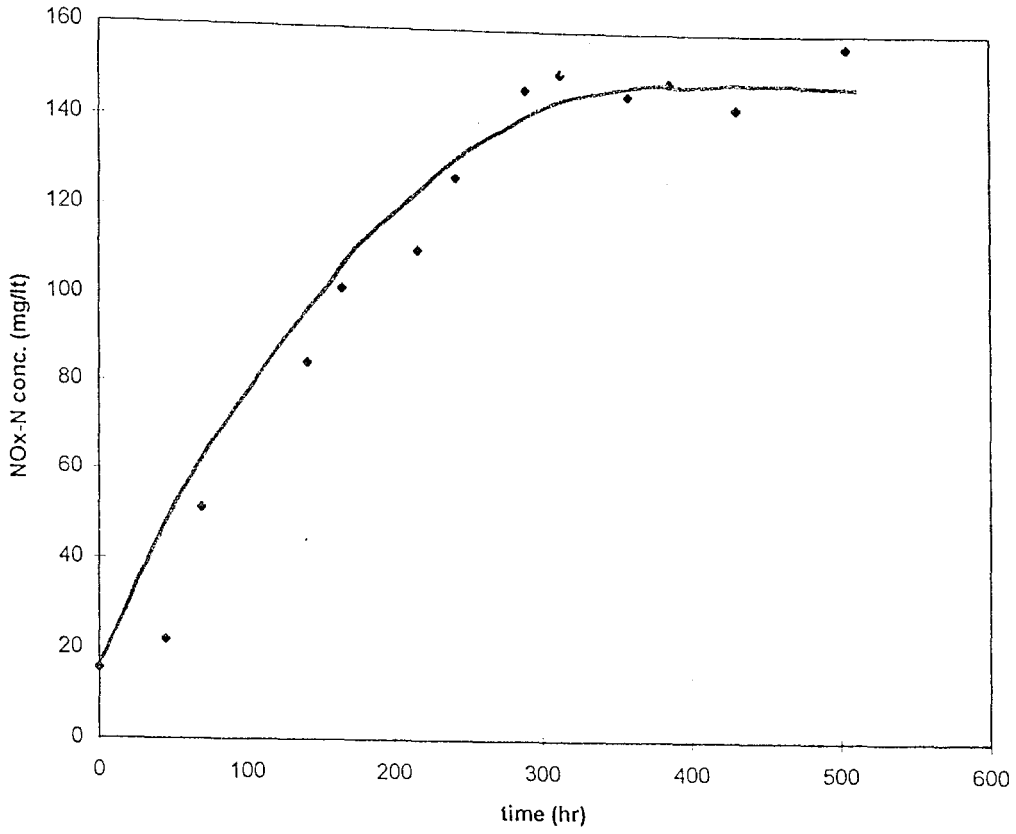


Figure 6.20: Change in product concentration of Run 5a

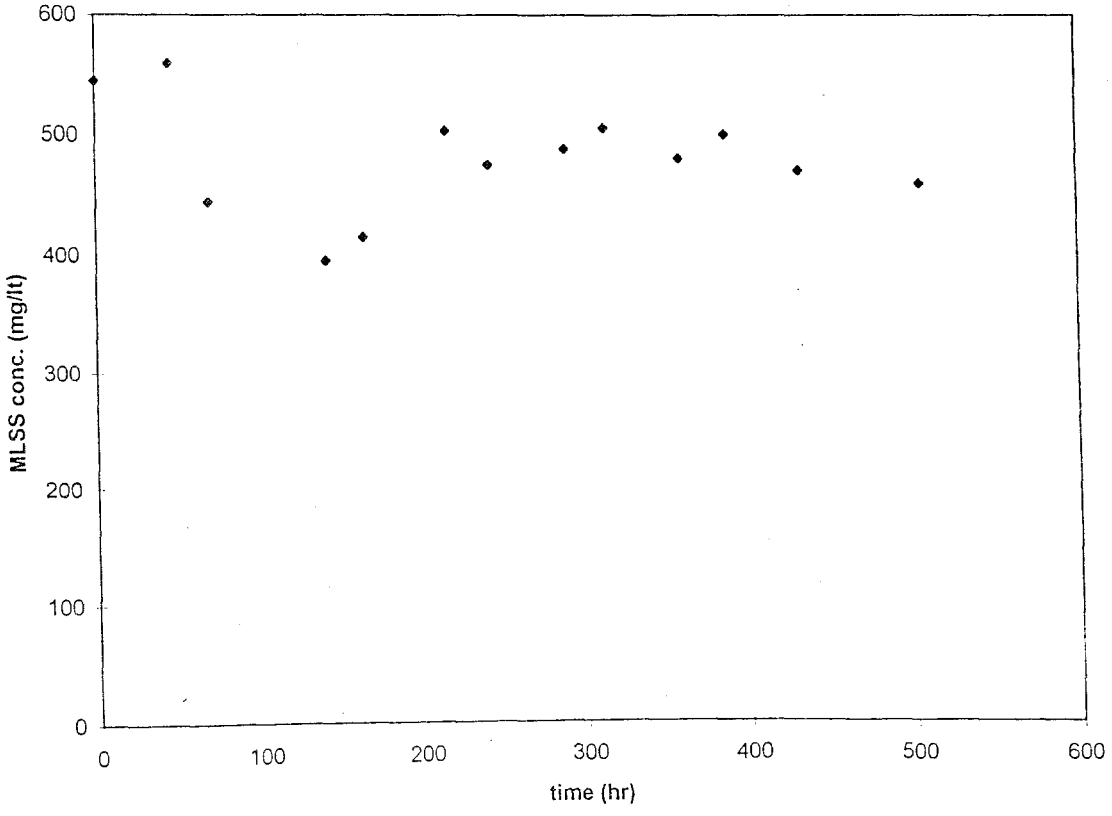


Figure 6.21: Change in MLSS Concentration of Run 5a

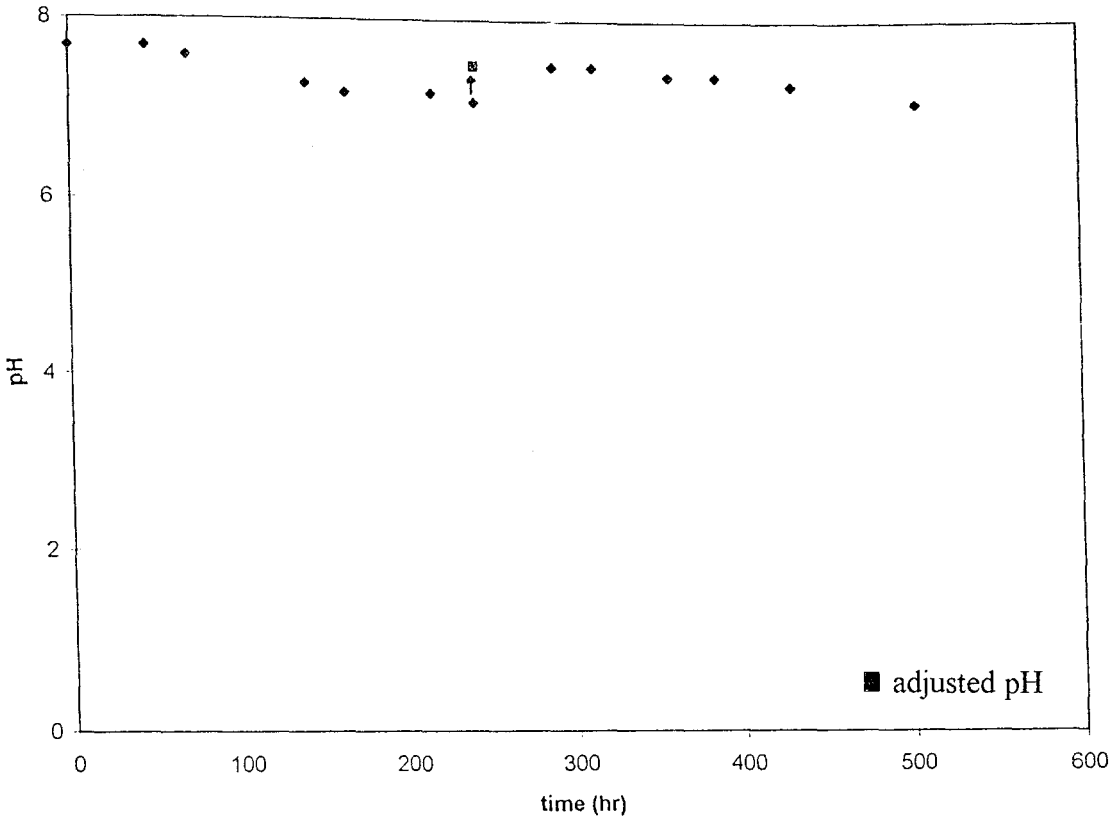


Figure 6.22: Change in pH of Run 5a

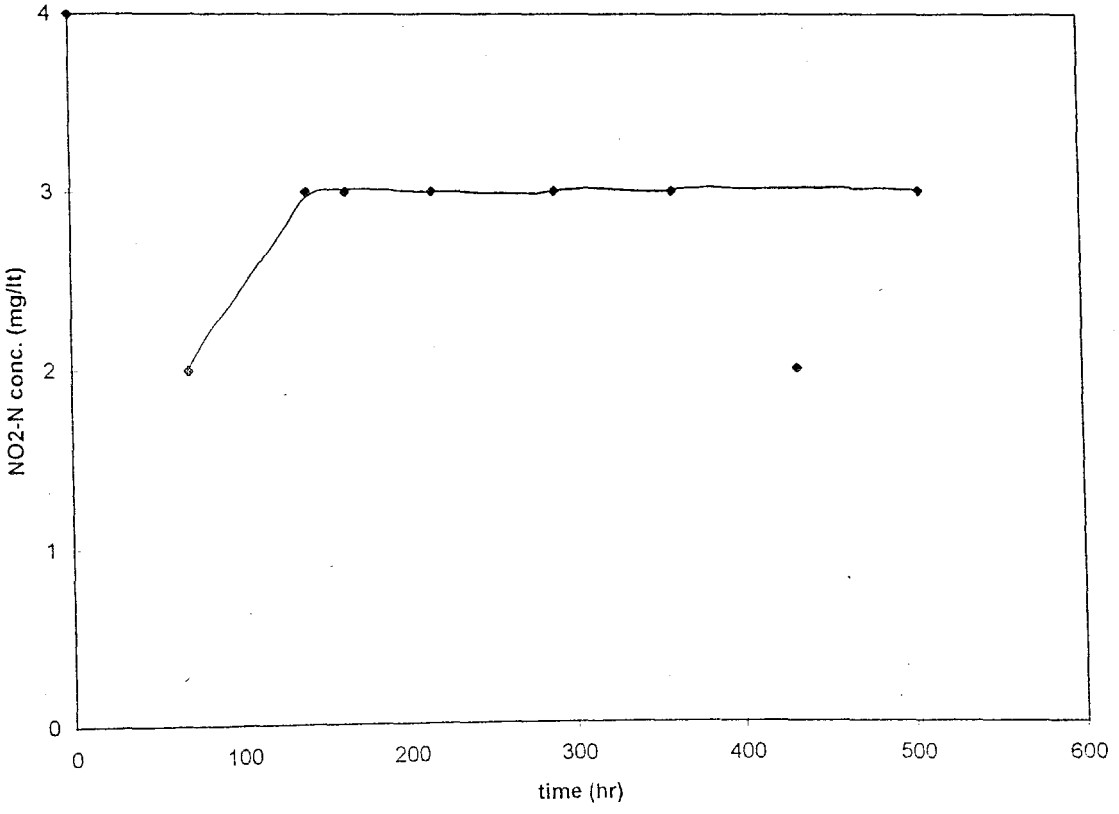


Figure 6.23: NO_2-N formation of Run 5a

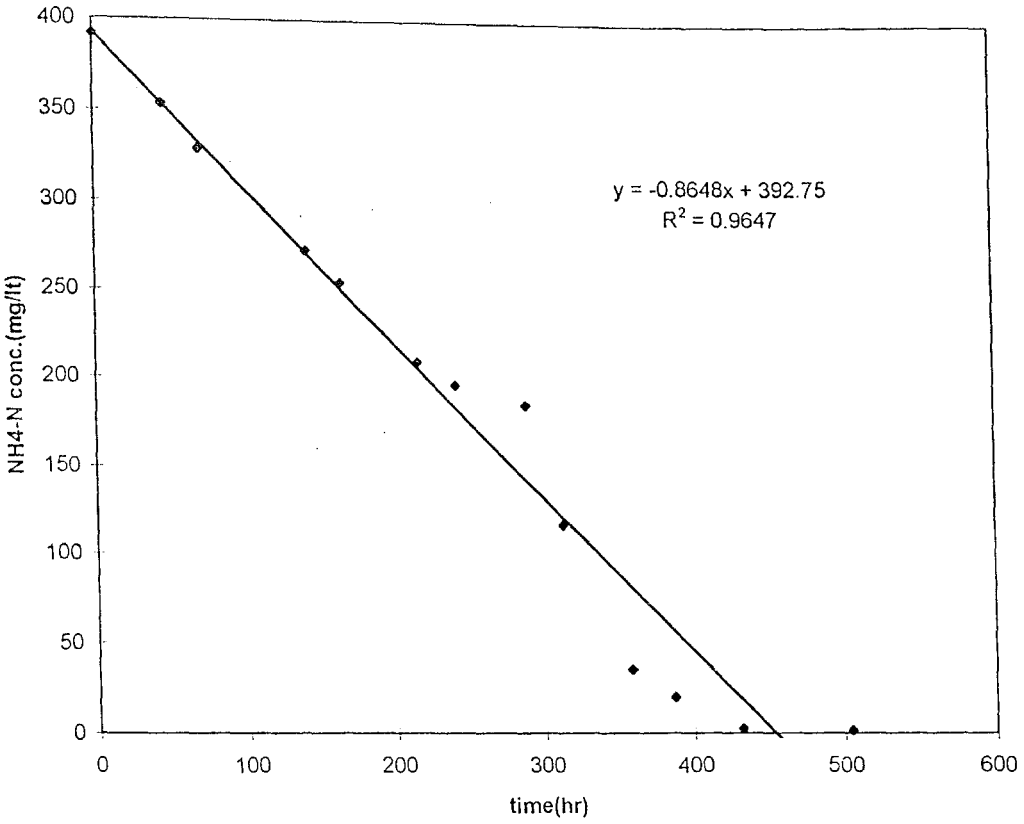


Figure 6.24: Change in substrate concentration of Run 5b

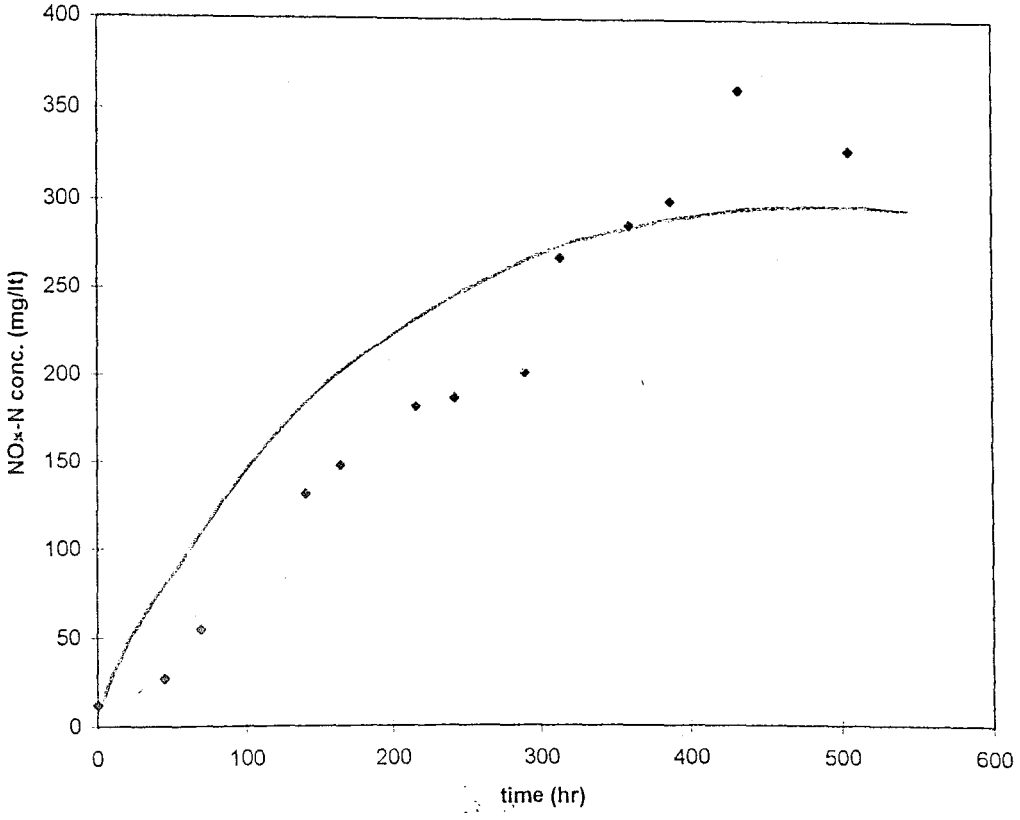


Figure 6.25: Change in product concentration of Run 5b

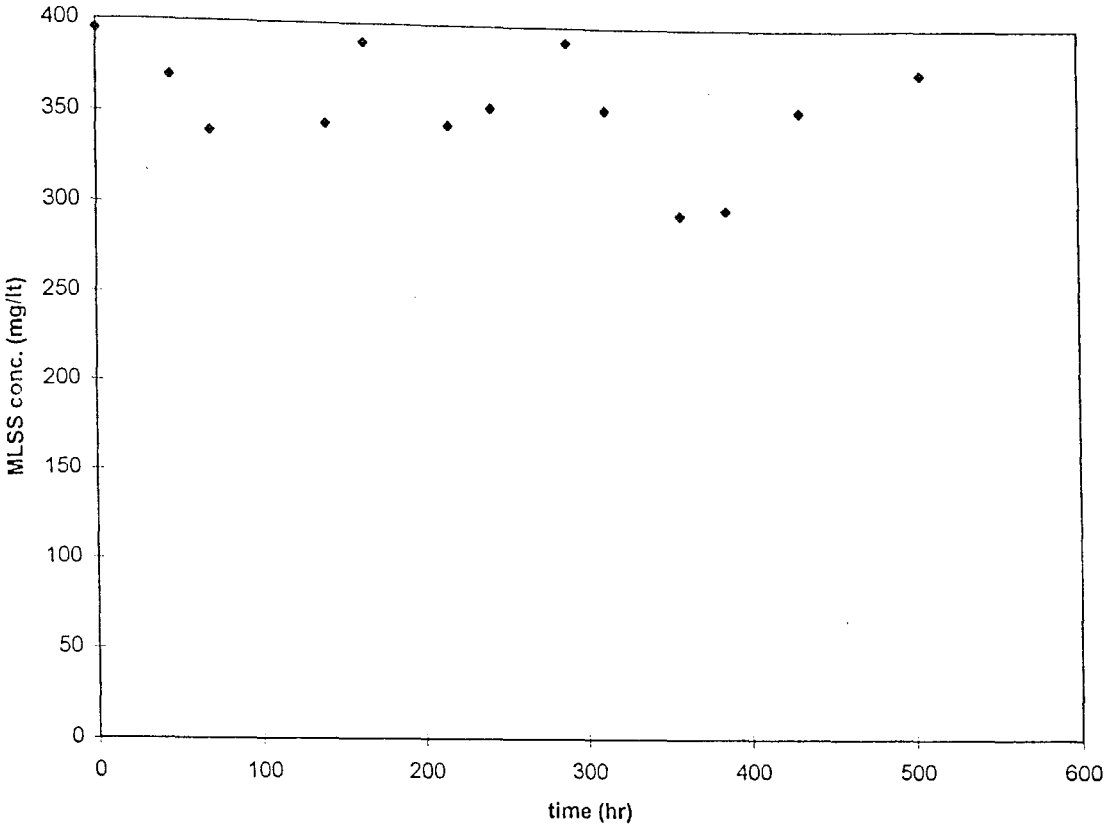


Figure 6.26: Change in MLSS Concentration of Run 5b

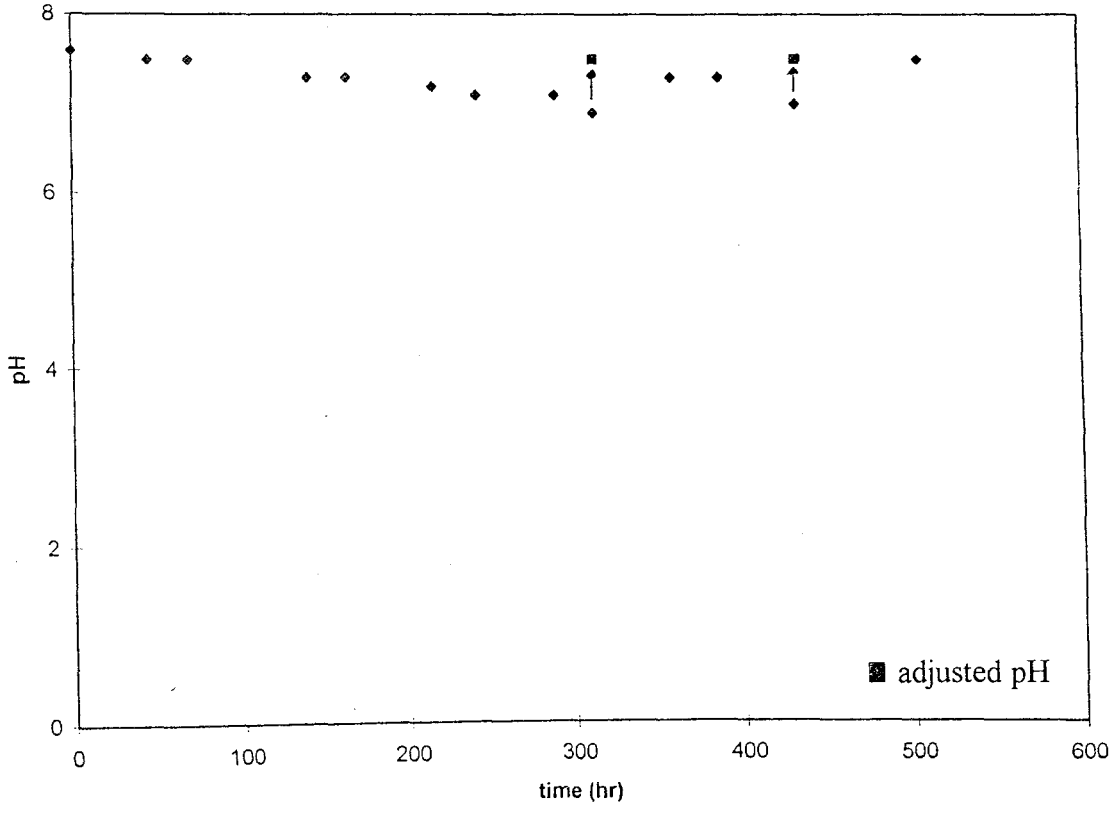


Figure 6.27: Change in pH of Run 5b

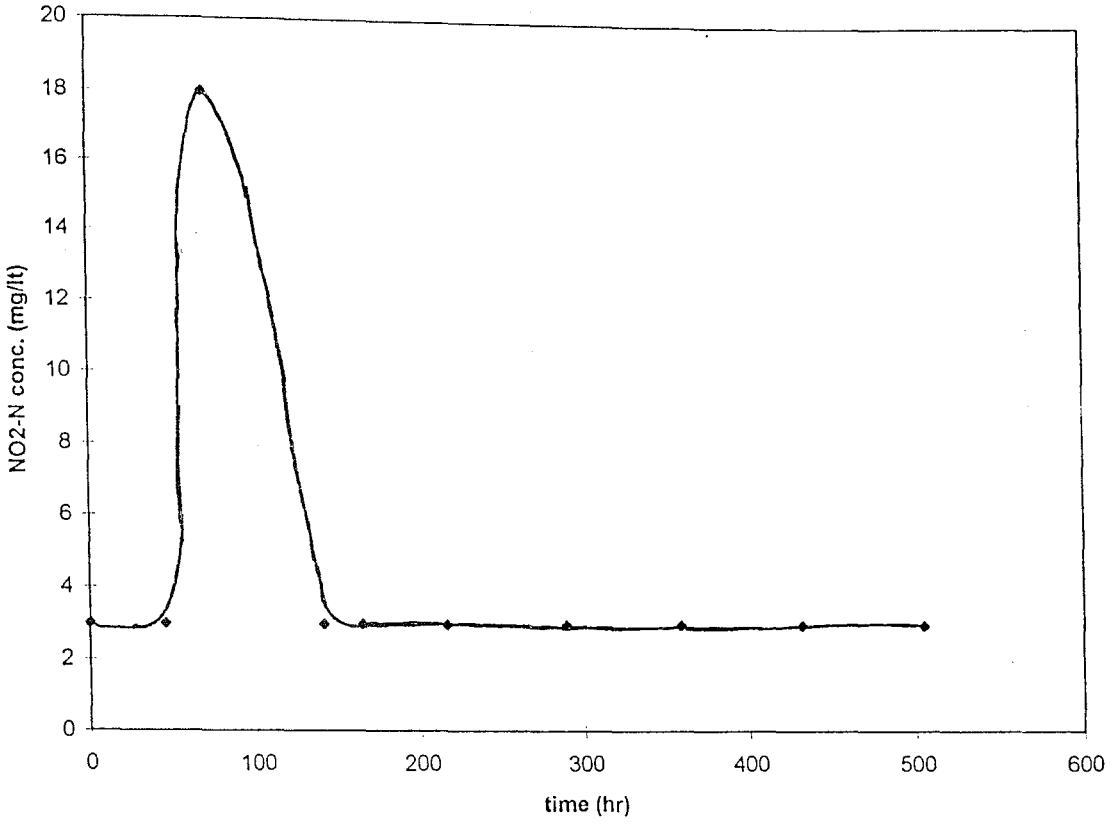


Figure 6.28: NO_2-N formation of Run 5b

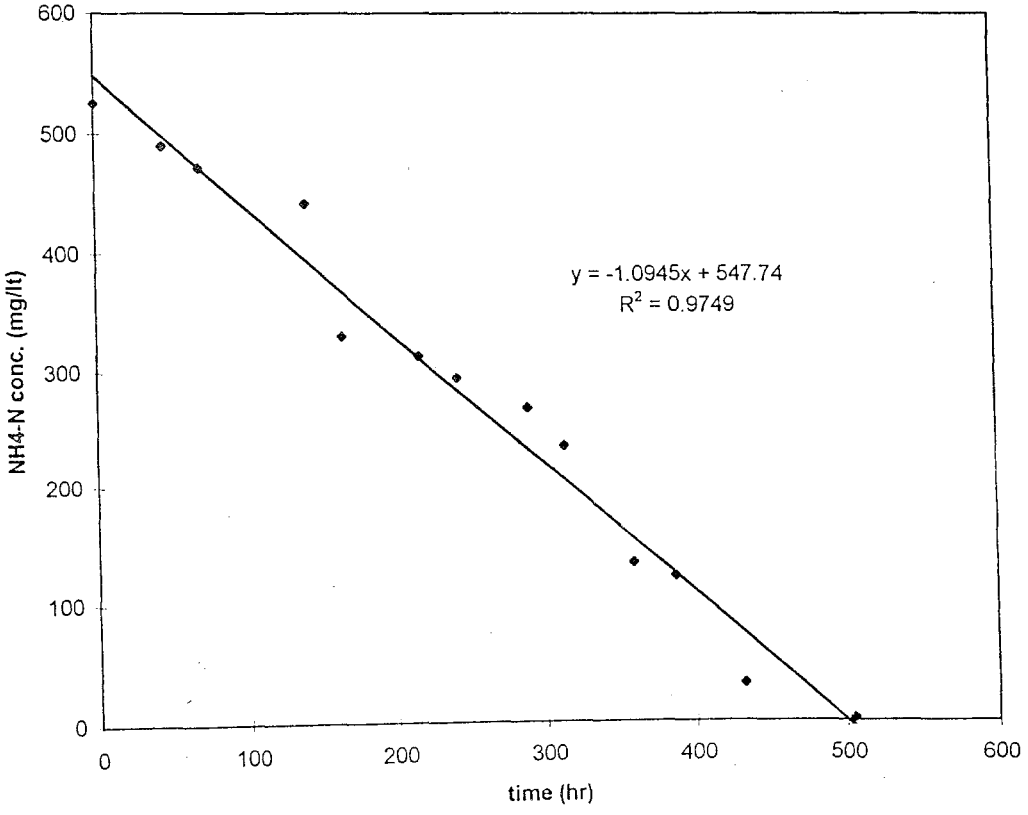


Figure 6.29: Change in substrate concentration of Run 5c

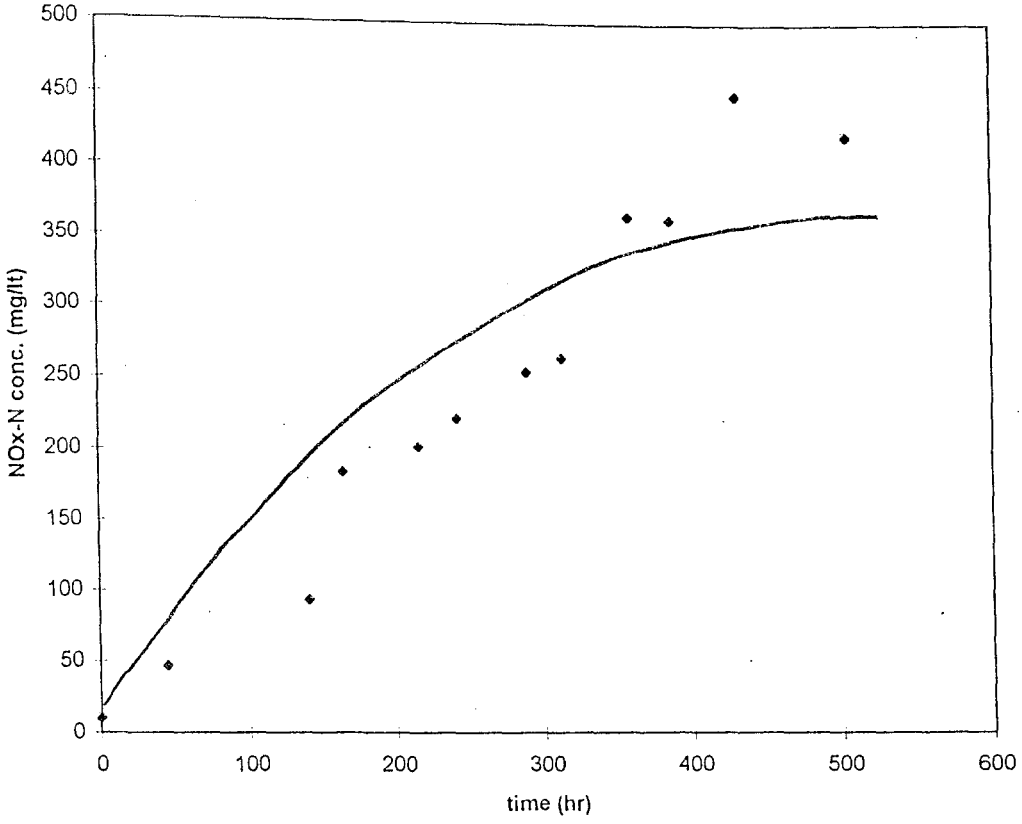


Figure 6.30: Change in product concentration of Run 5c

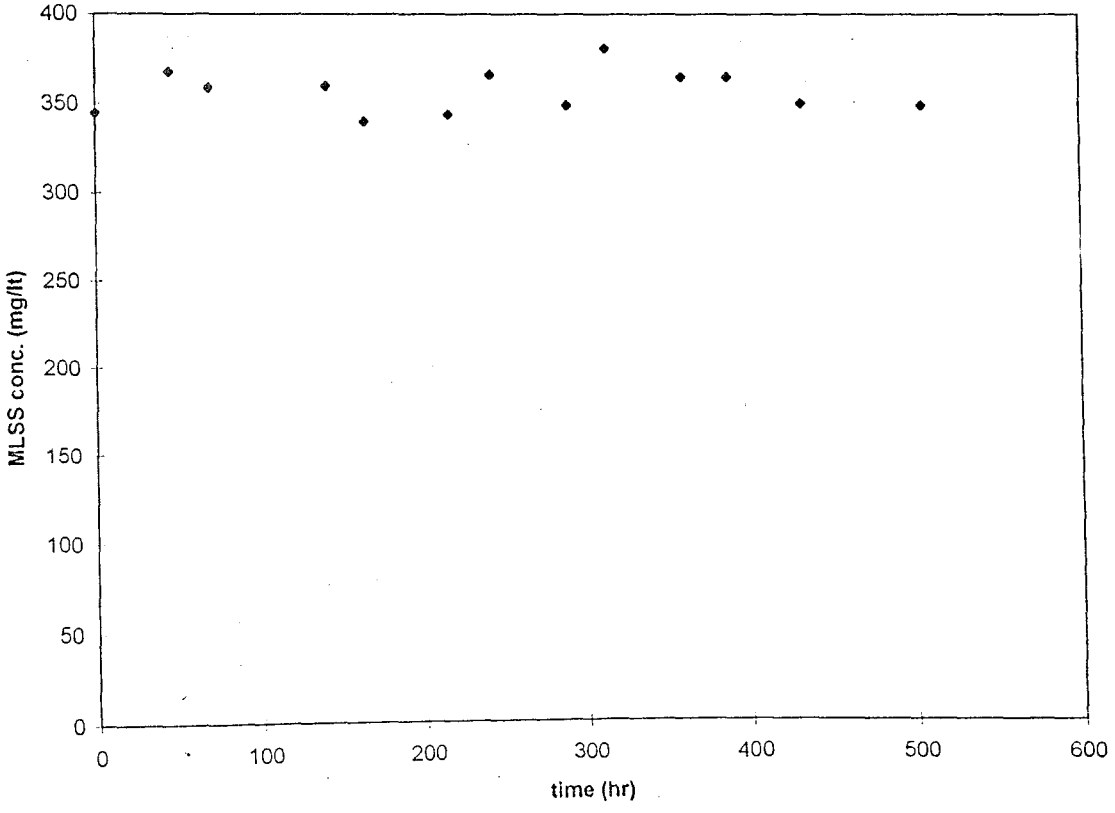


Figure 6.31: Change in MLSS Concentration of Run 5c

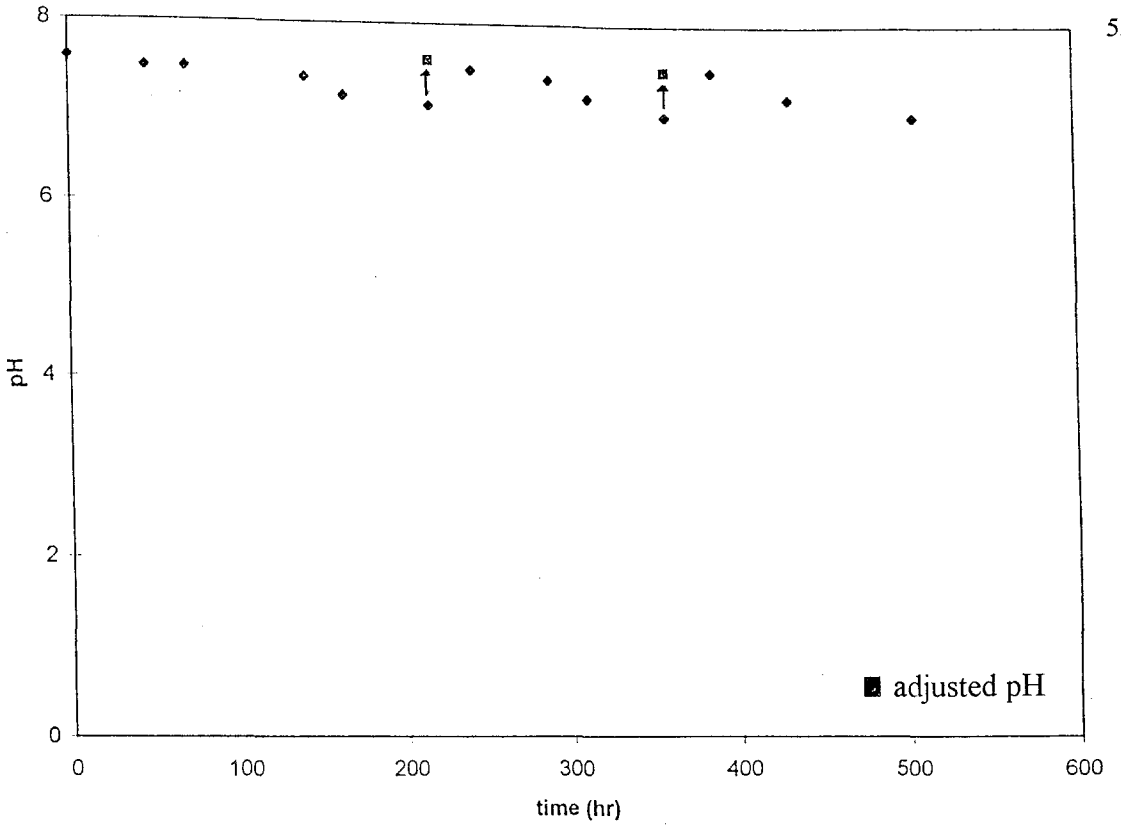


Figure 6.32: Change in pH of Run 5c

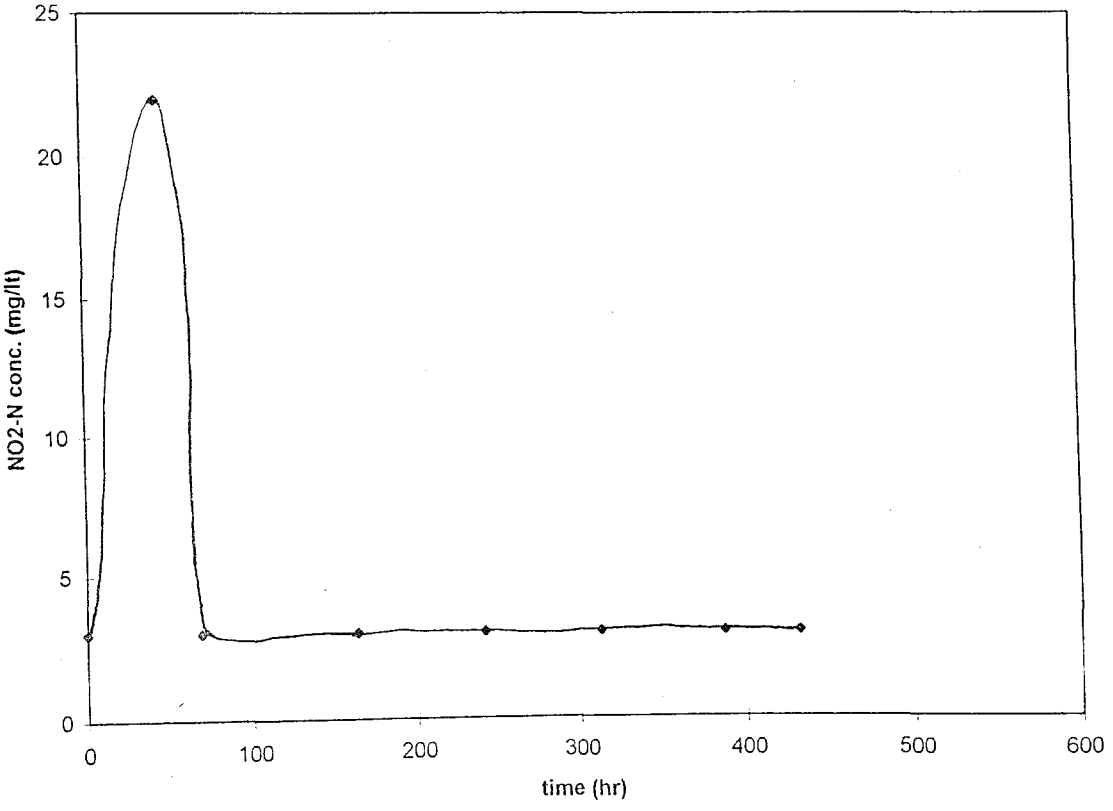


Figure 6.33: NO_2-N formation of Run 5c

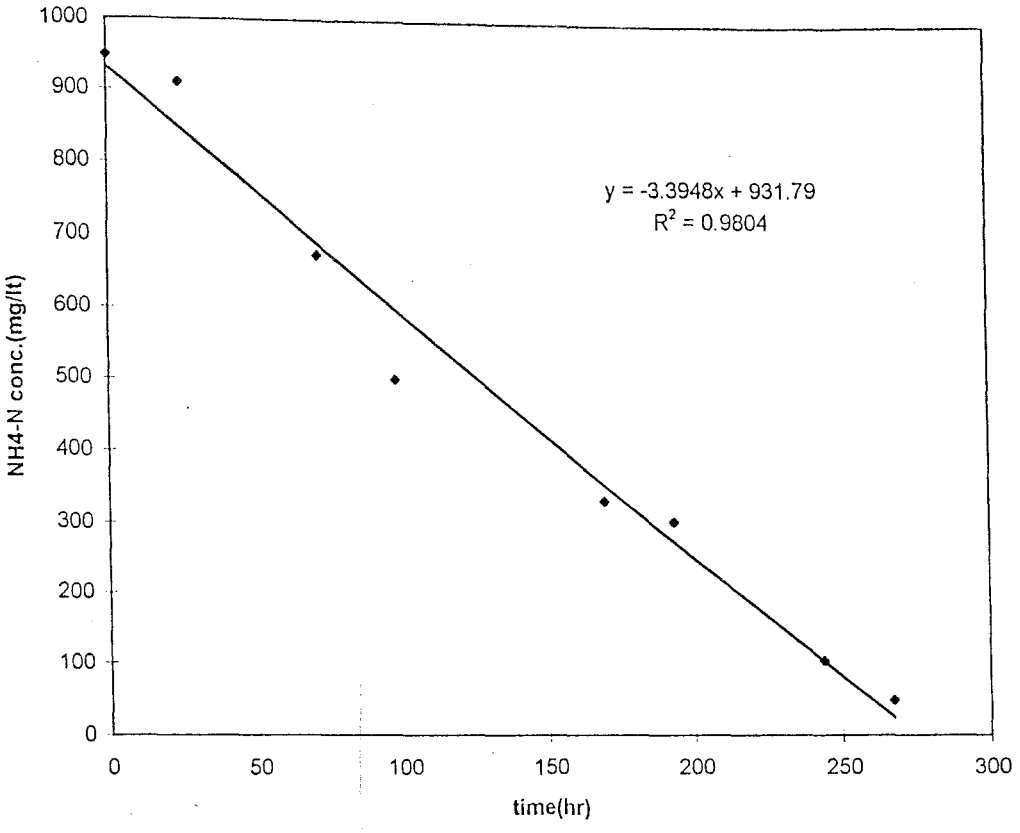


Figure 6.34: Change in substrate concentration of Run 6a

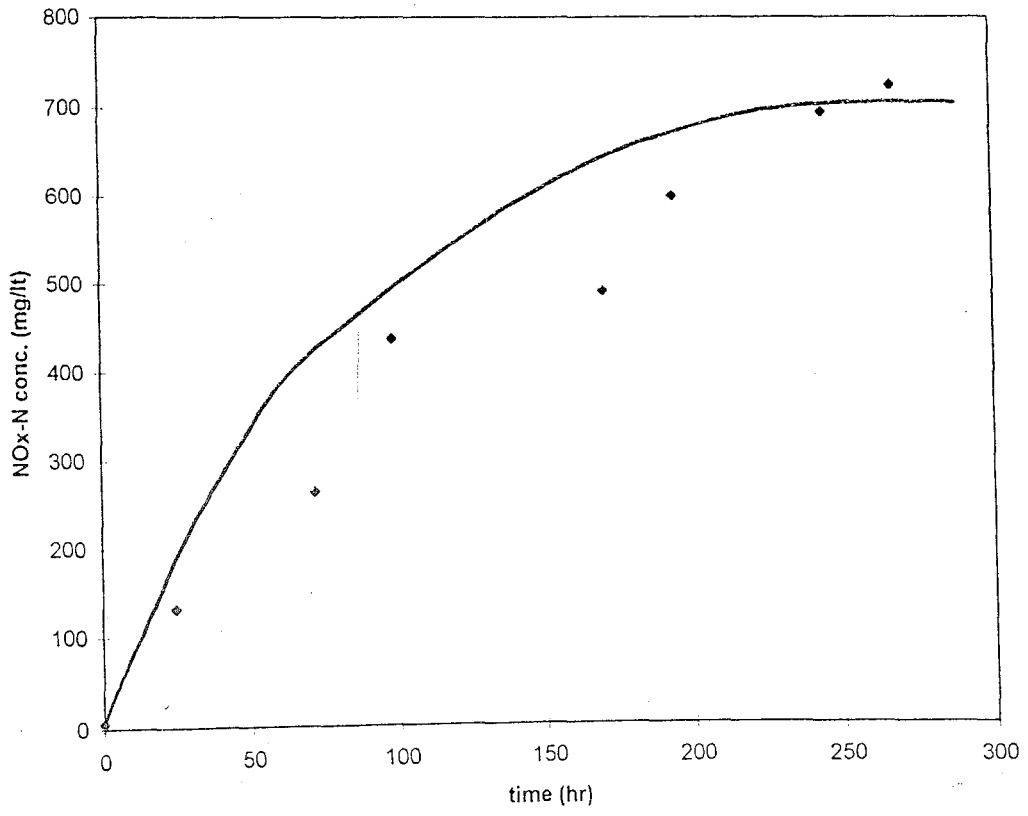


Figure 6.35: Change in product concentration of Run 6a

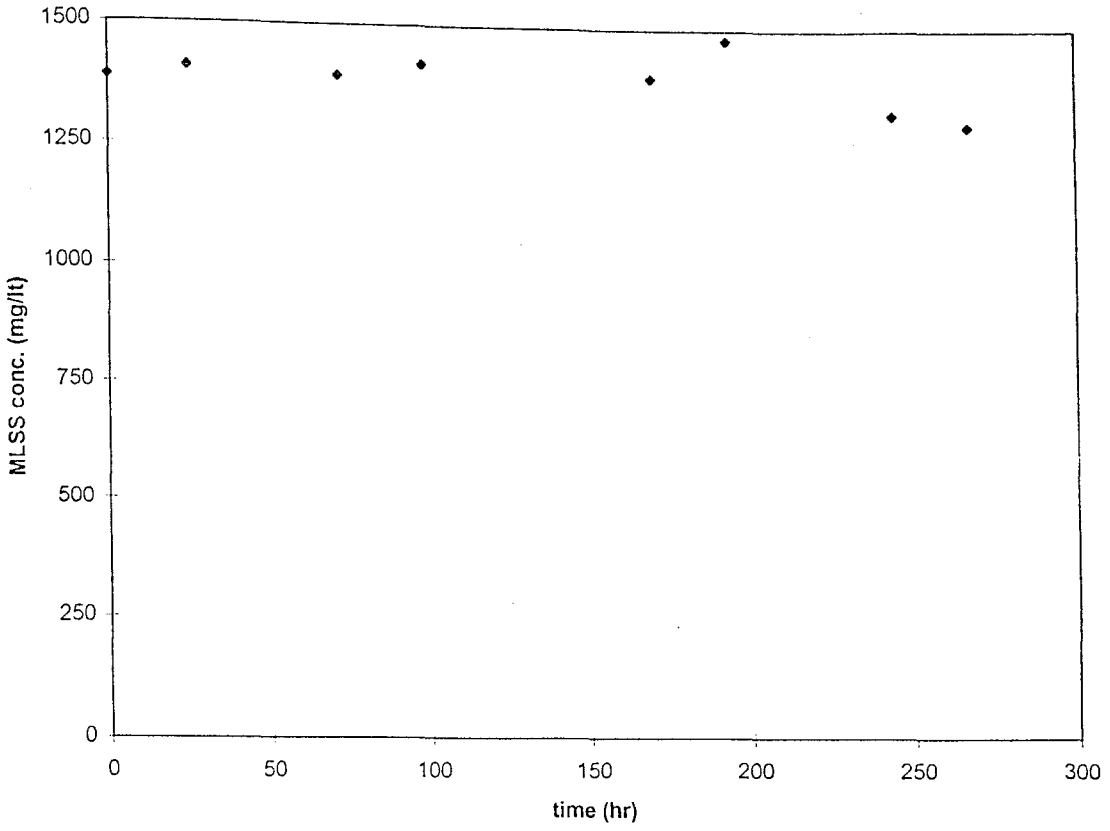


Figure 6.36: Change in MLSS Concentration of Run 6a

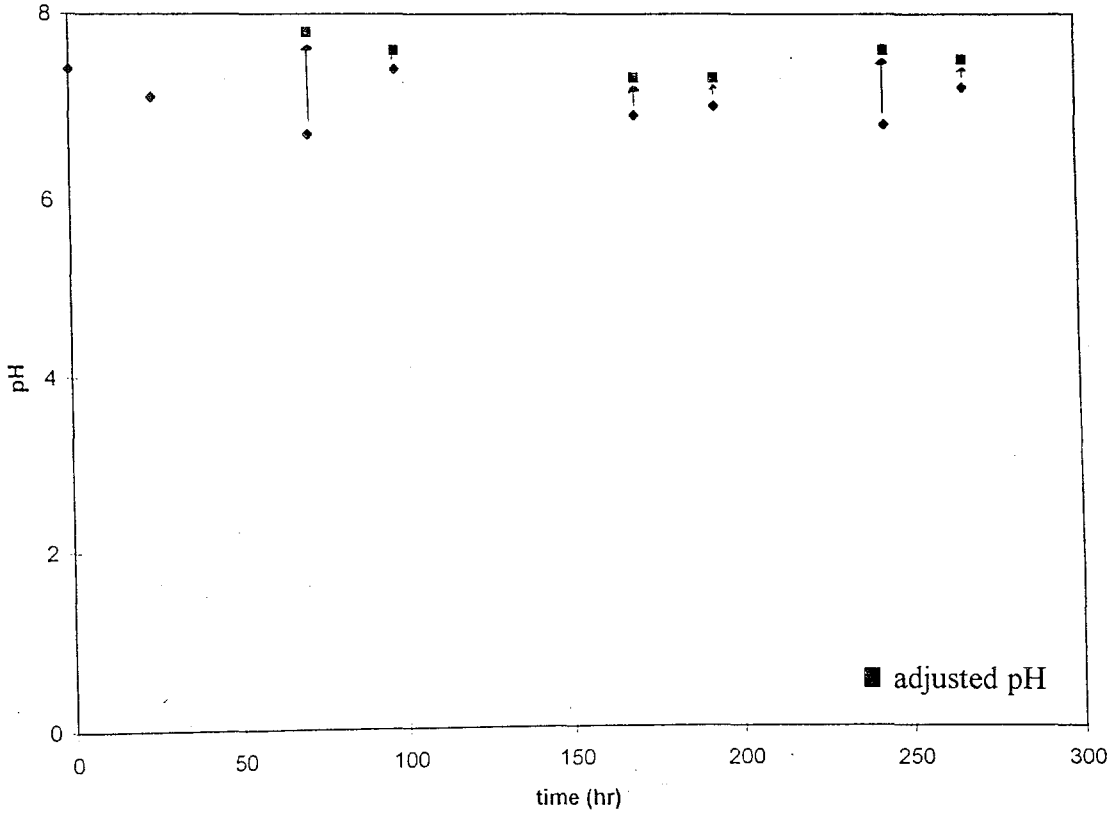


Figure 6.37: Change in pH of Run 6a

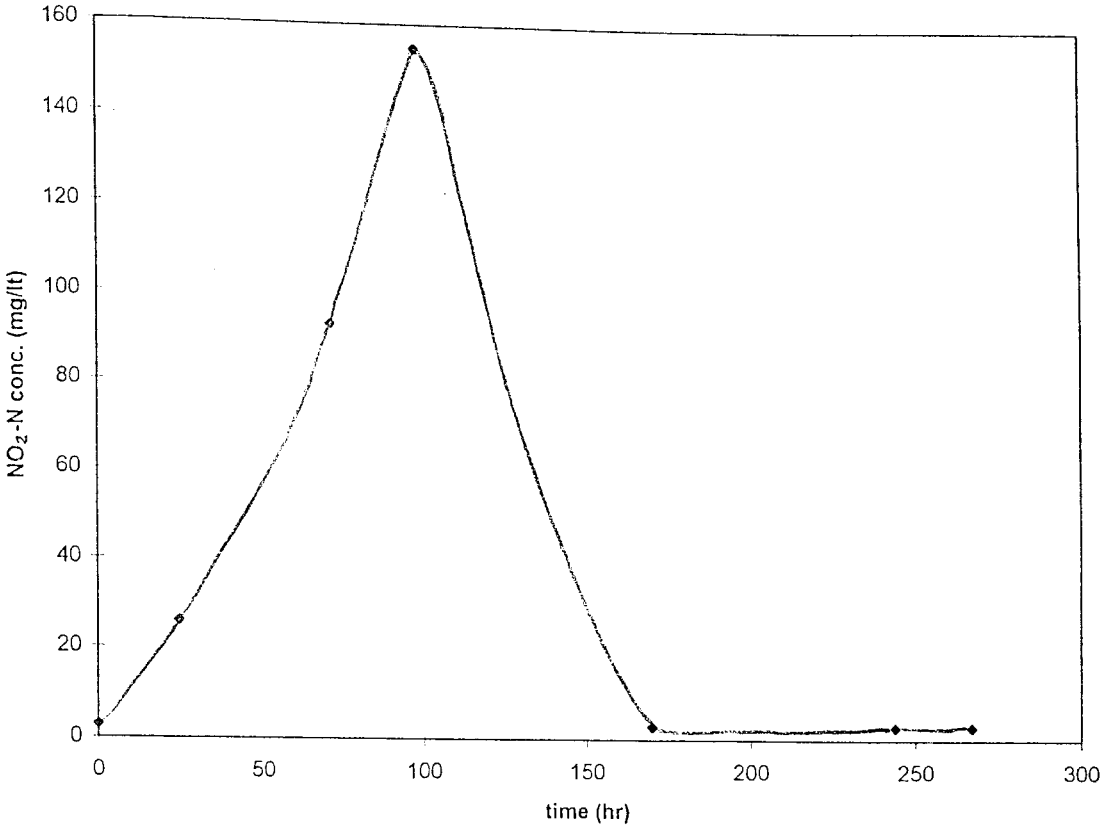


Figure 6.38: NO_2-N formation of Run 6a

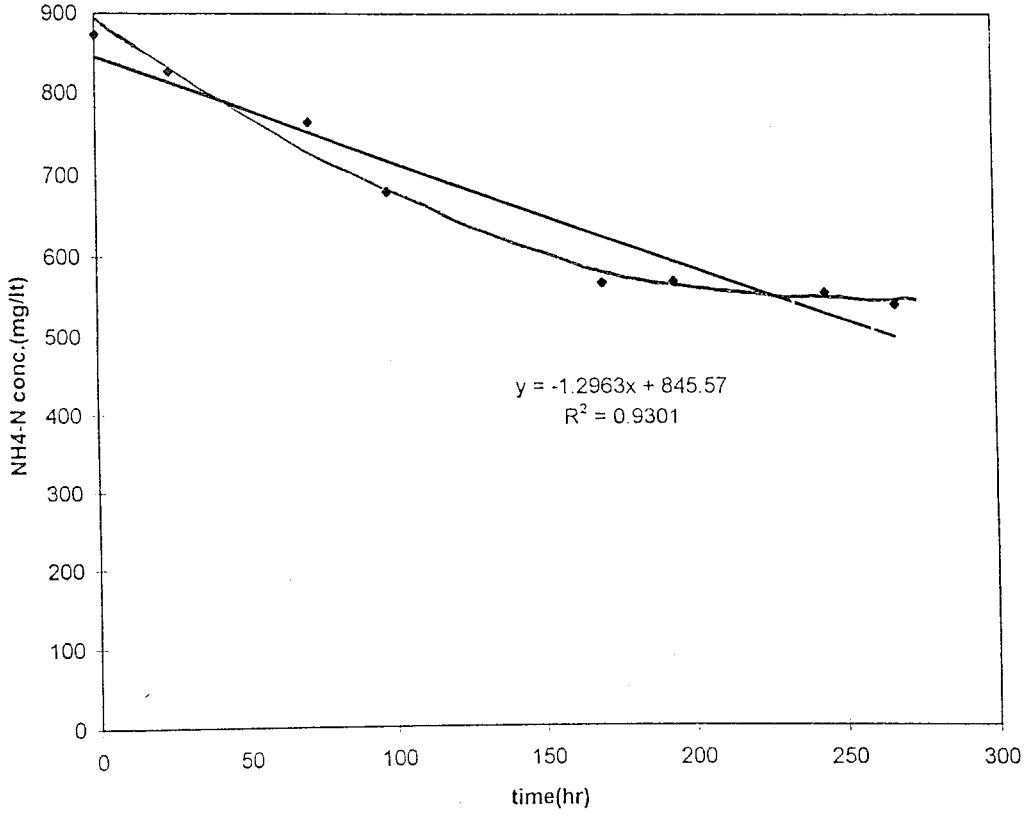


Figure 6.39: Change in substrate concentration of Run 6b

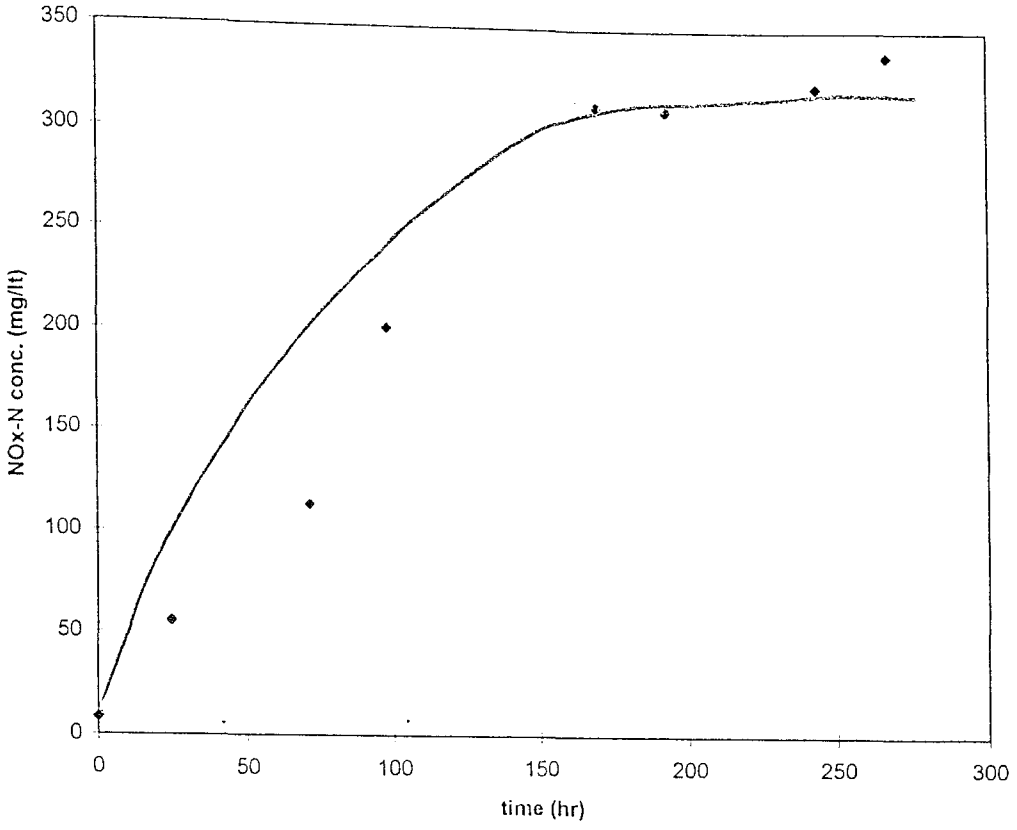


Figure 6.40: Change in product concentration of Run 6b

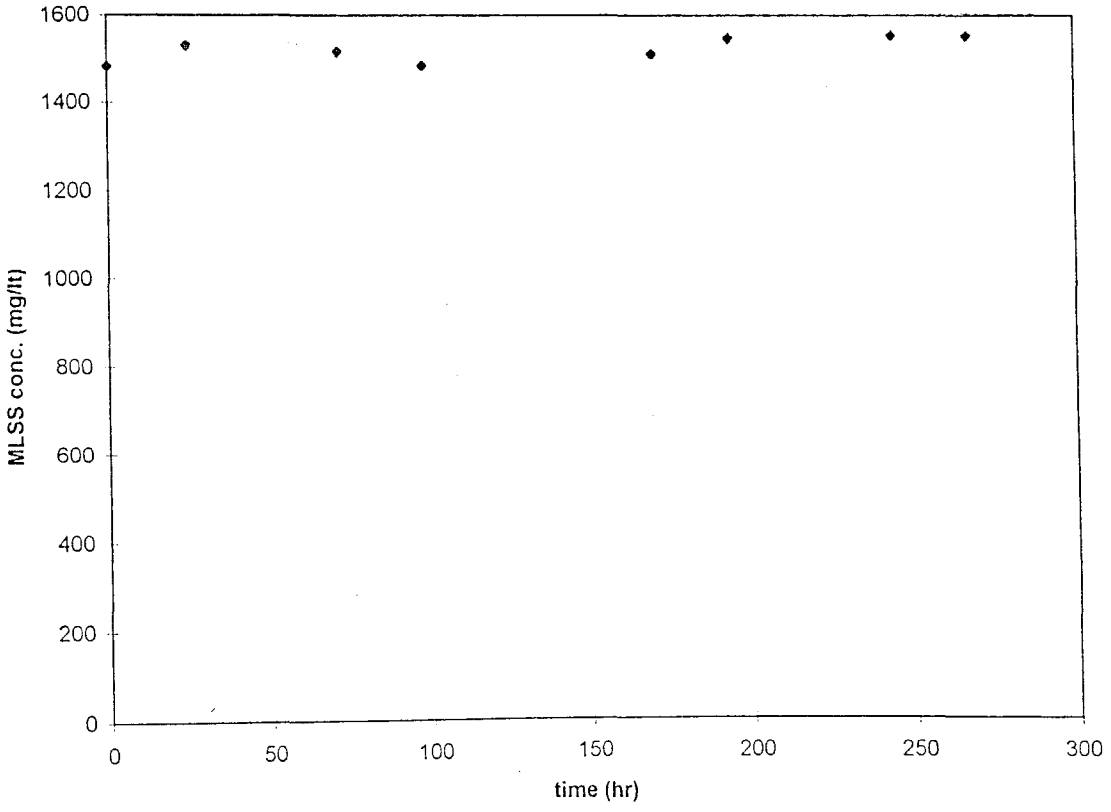


Figure 6.41: Change in MLSS Concentration of Run 6b

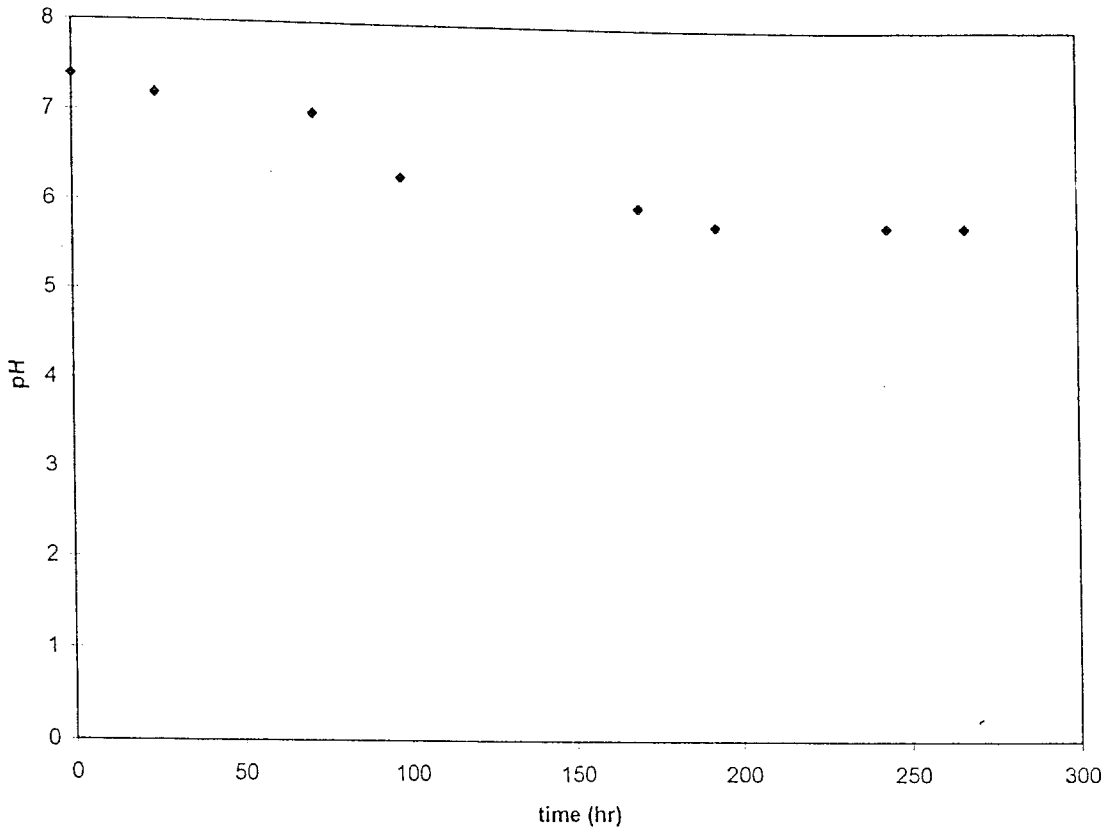


Figure 6.42: Change in pH of Run 6b

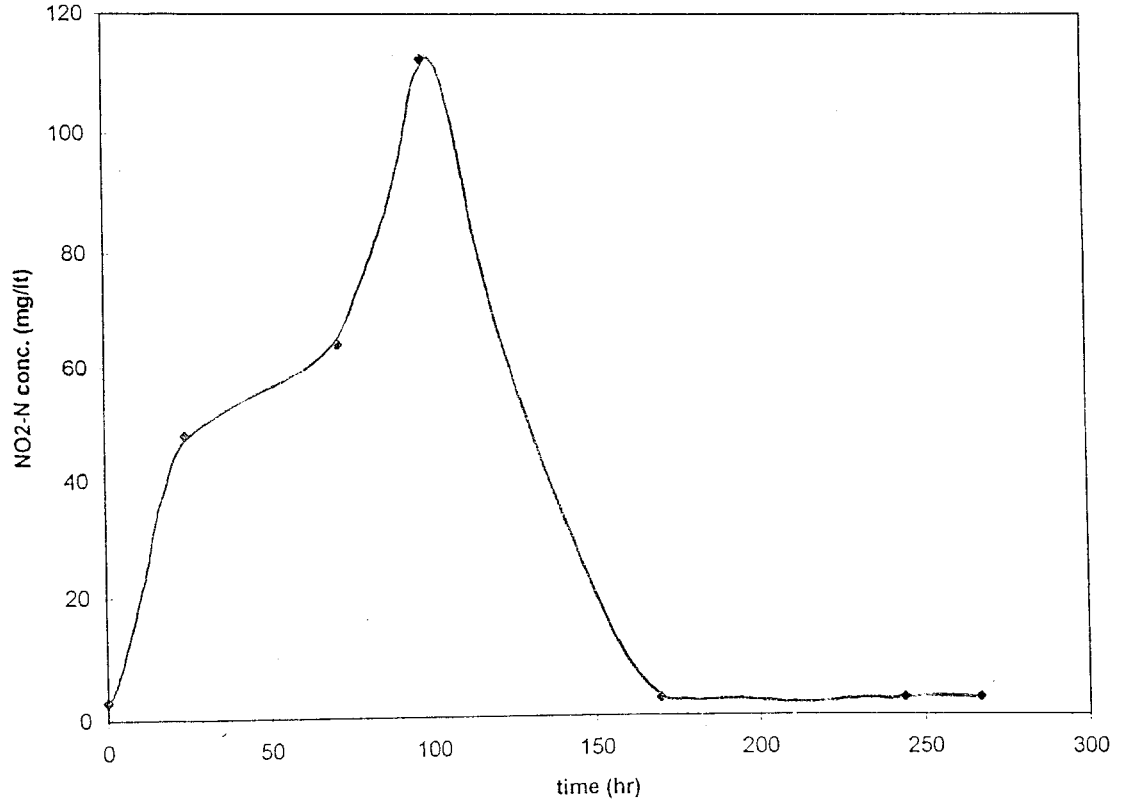


Figure 6.43: NO_2-N formation of Run 6b

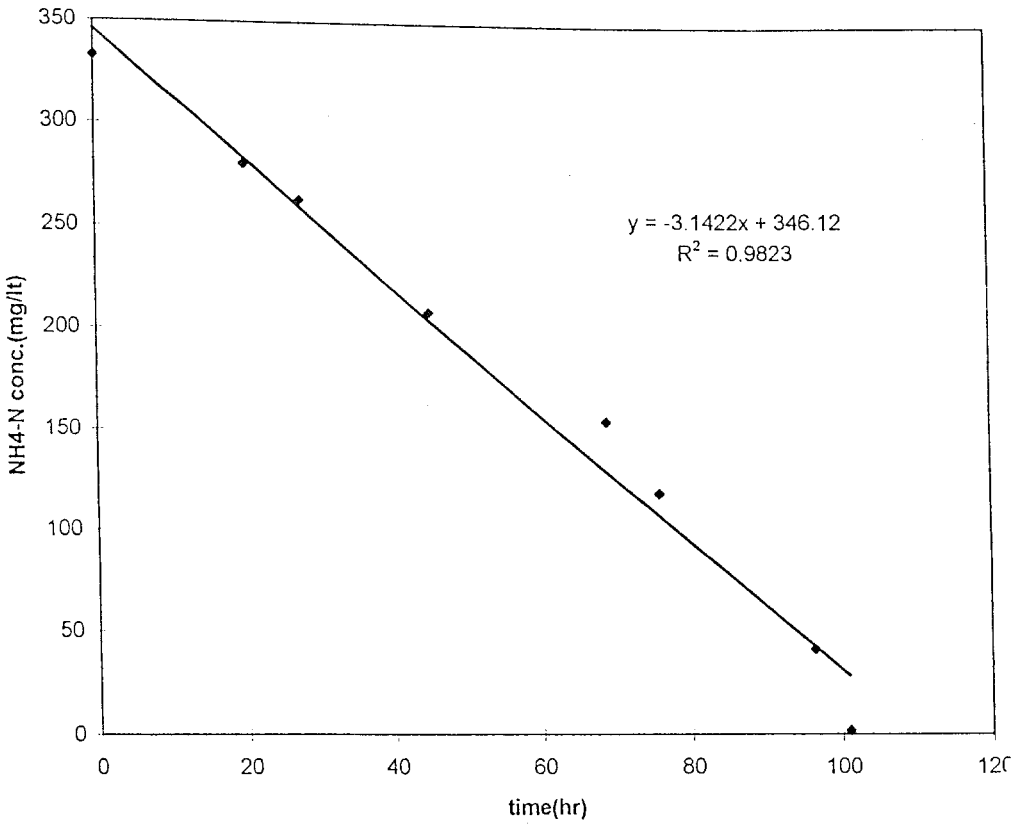


Figure 6.44: Change in substrate concentration of Run 7a

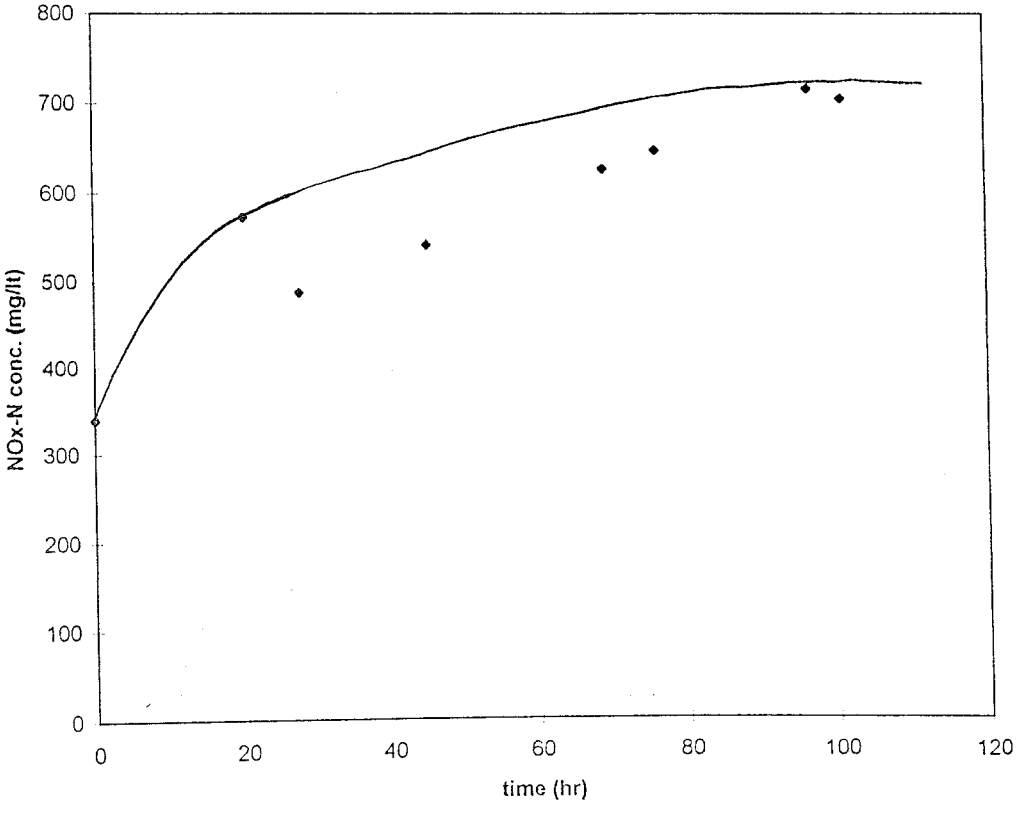


Figure 6.45: Change in product concentration of Run 7a

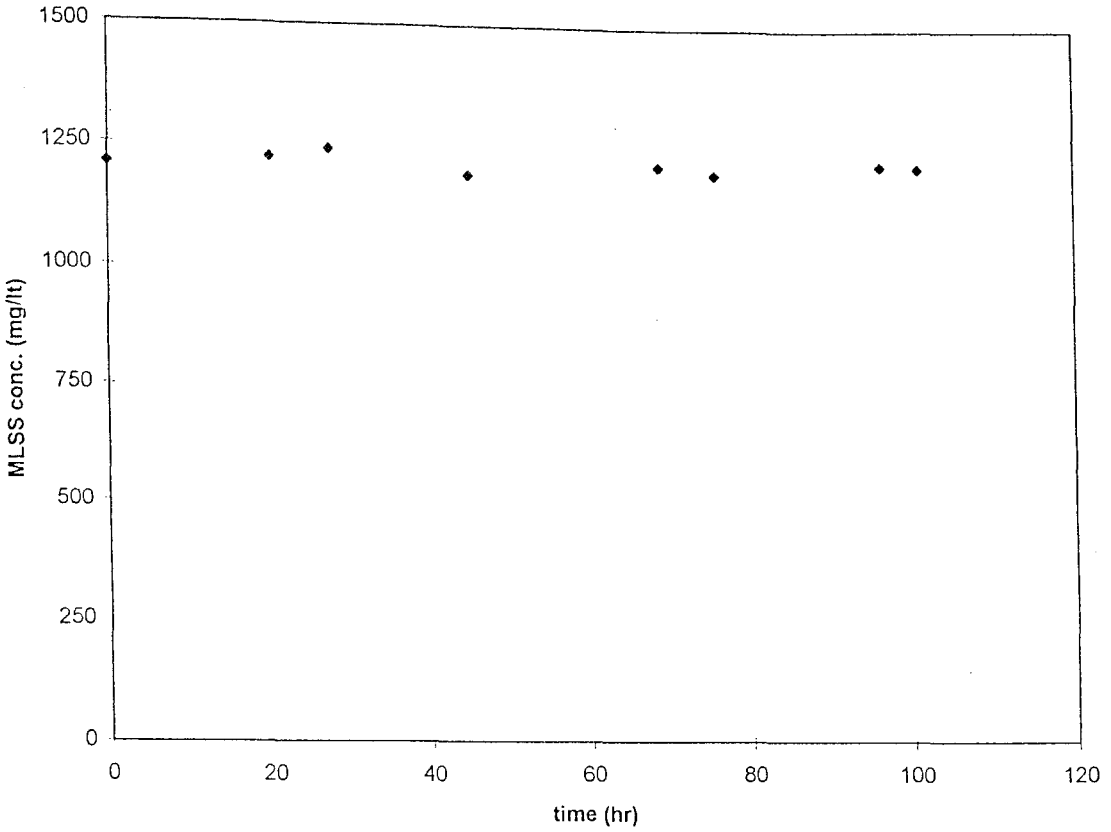


Figure 6.46: Change in MLSS Concentration of Run 7a

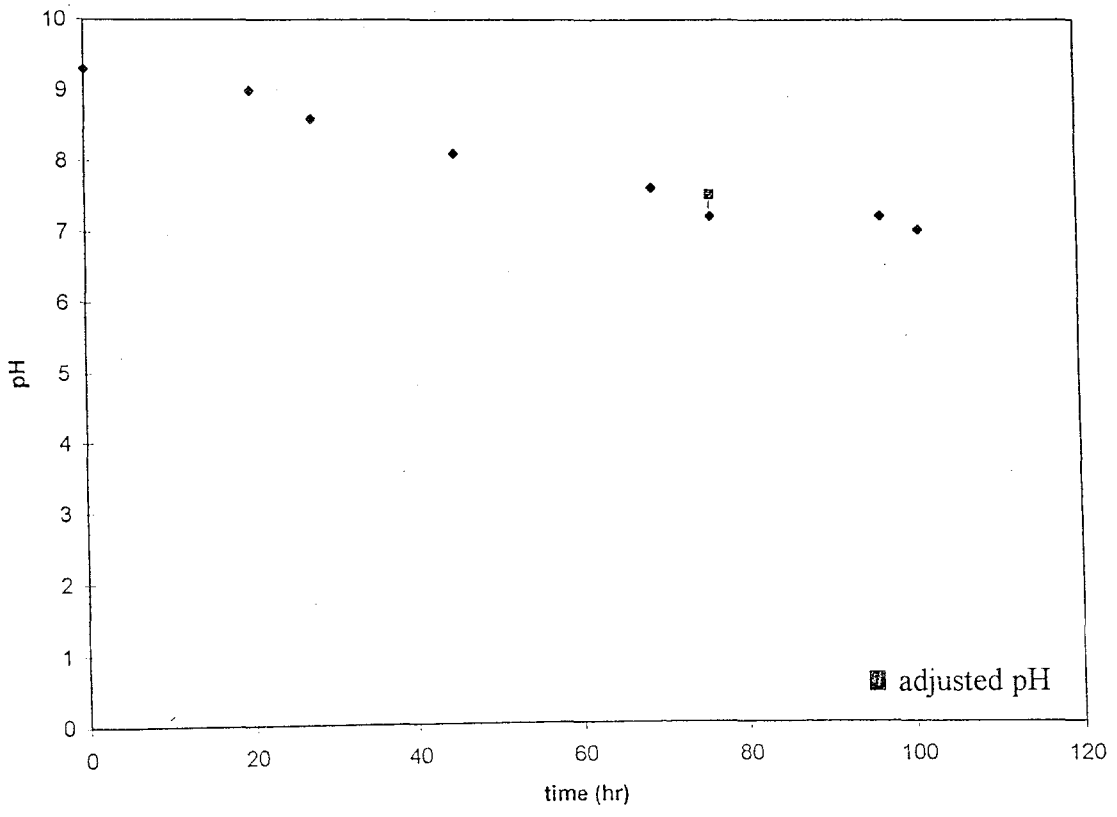


Figure 6.47: Change in pH of Run 7a

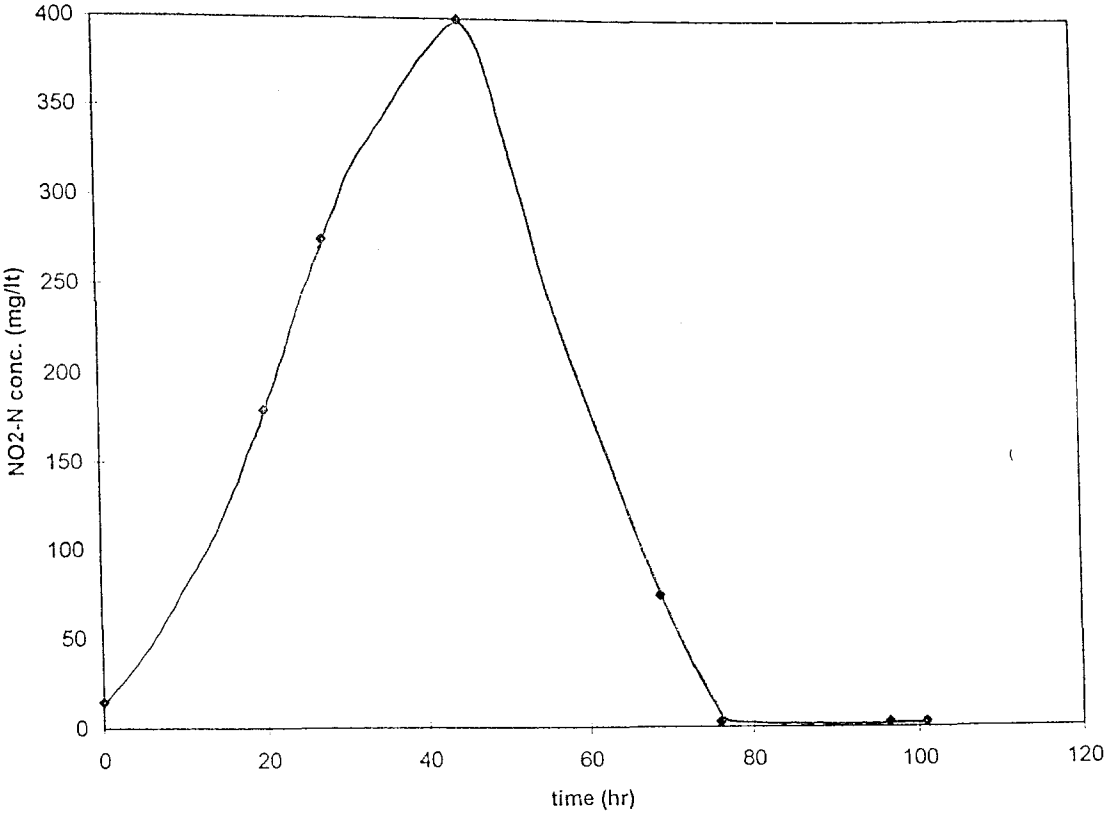


Figure 6.48: NO_2-N formation of Run 7a

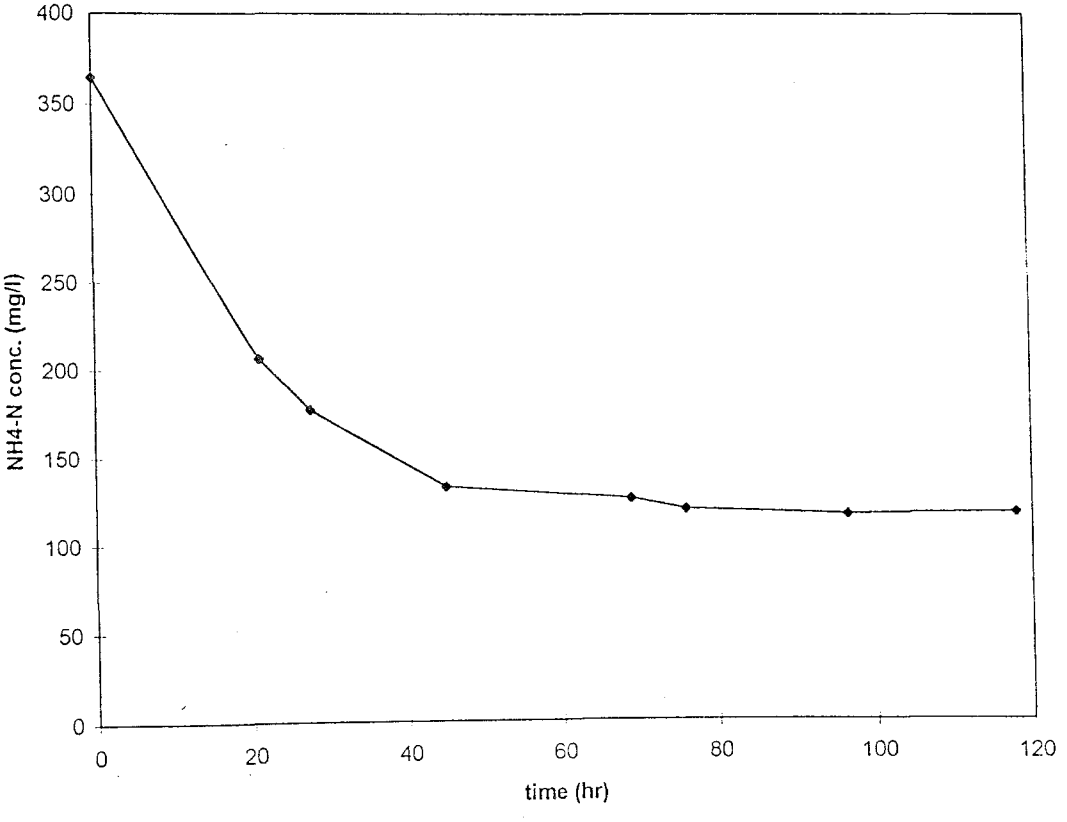


Figure 6.49: Change in substrate concentration of Run 7b

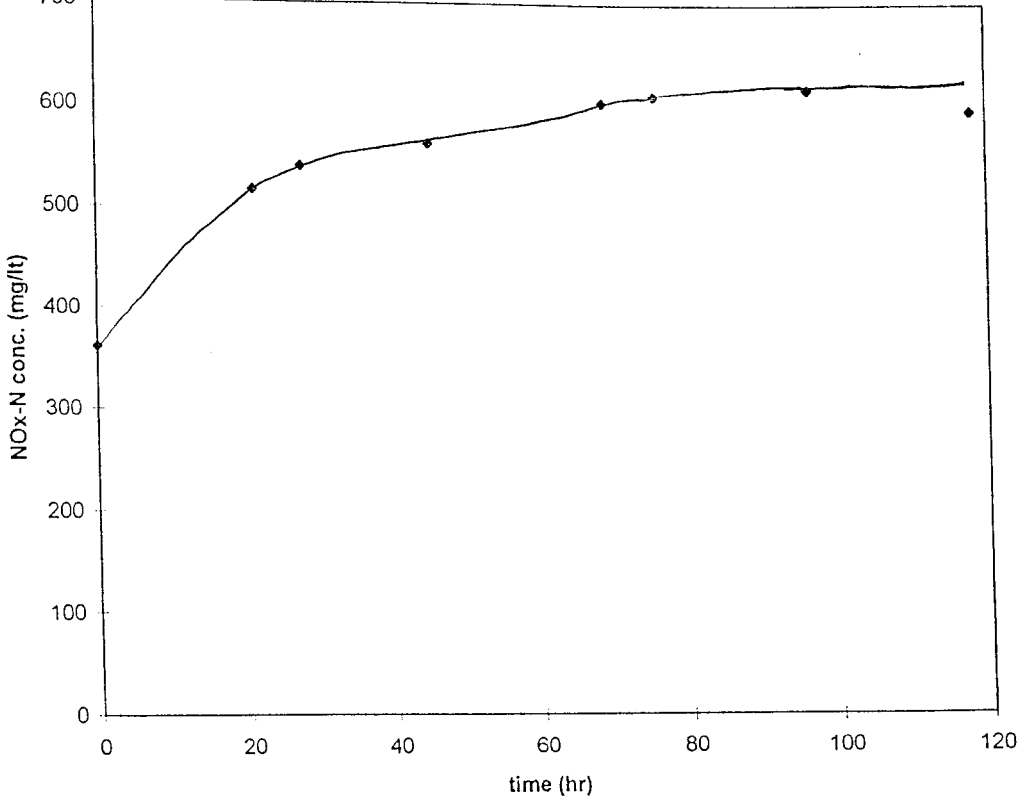


Figure 6.50: Change in product concentration of Run 7b

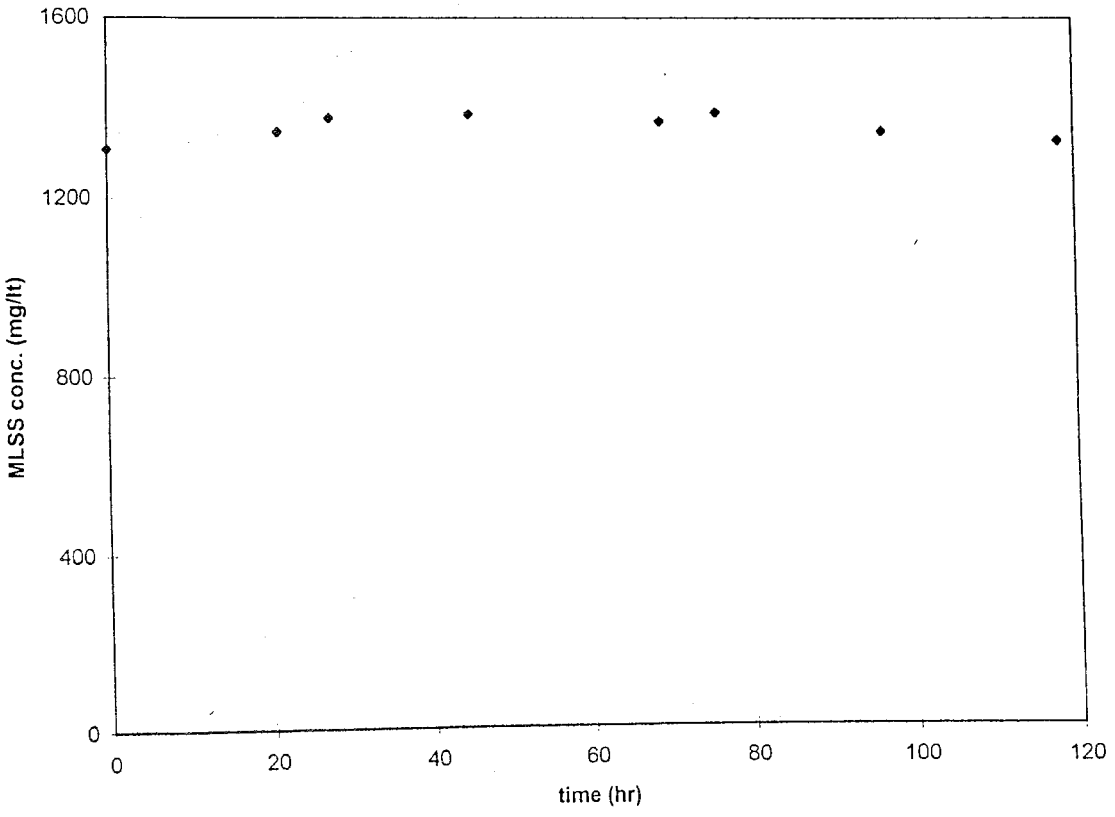


Figure 6.51: Change in MLSS Concentration of Run 7b

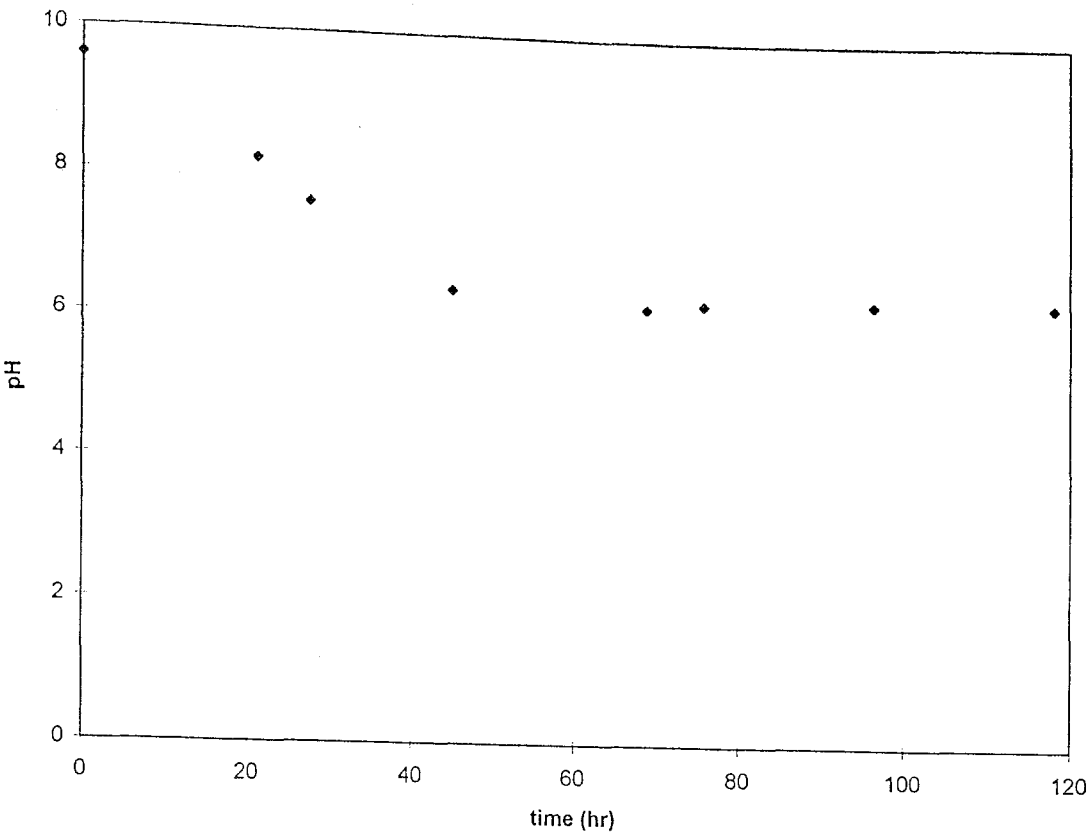


Figure 6.52: Change in pH of Run 7b

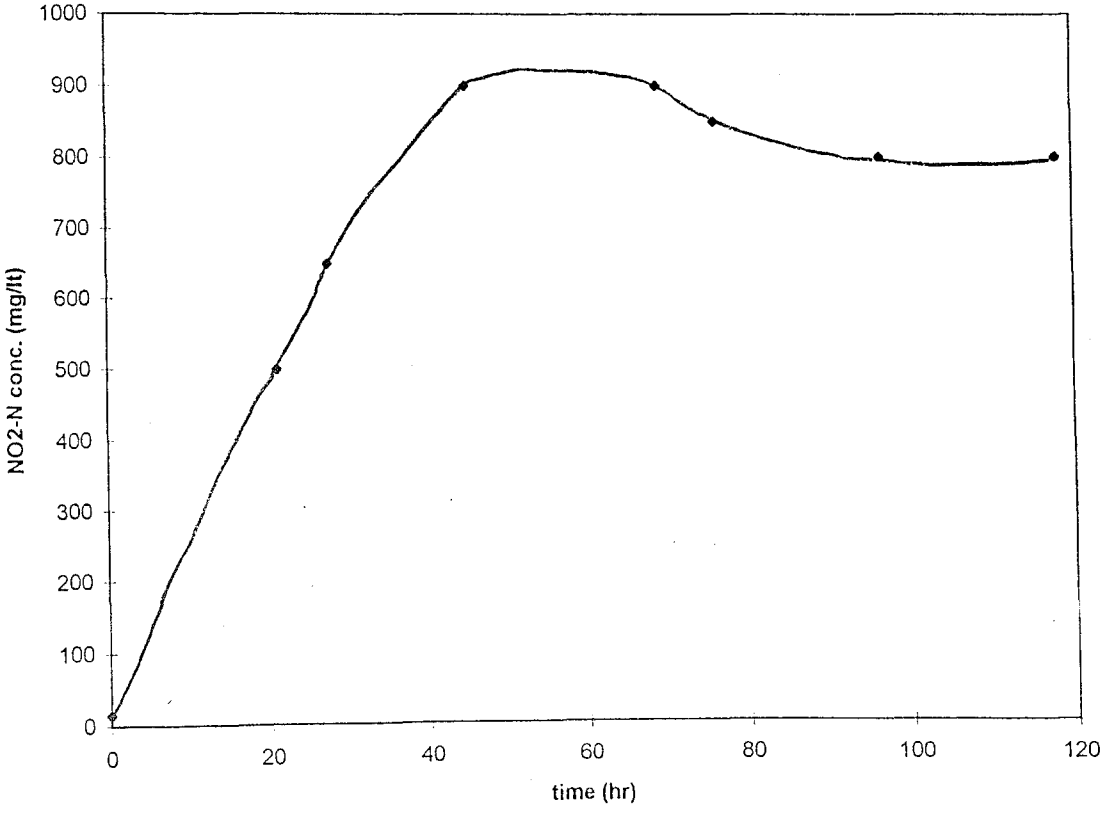


Figure 6.53: NO_2-N formation of Run 7b

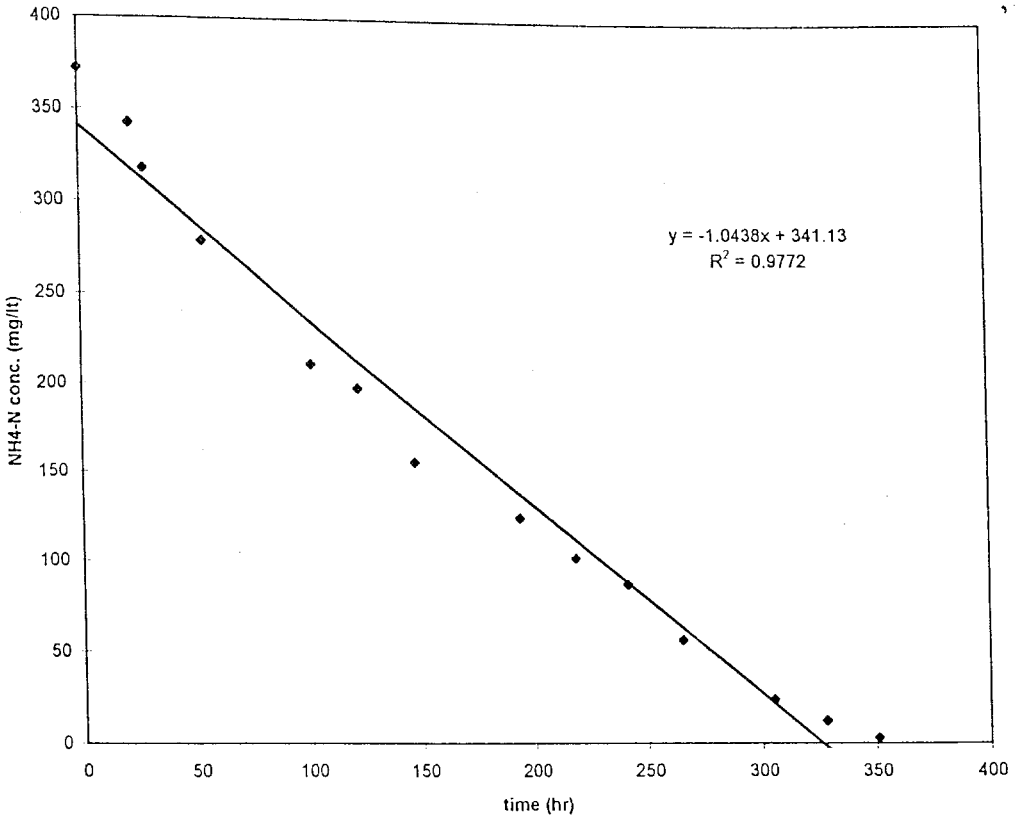


Figure 6.54: Change in substrate concentration of Run 8

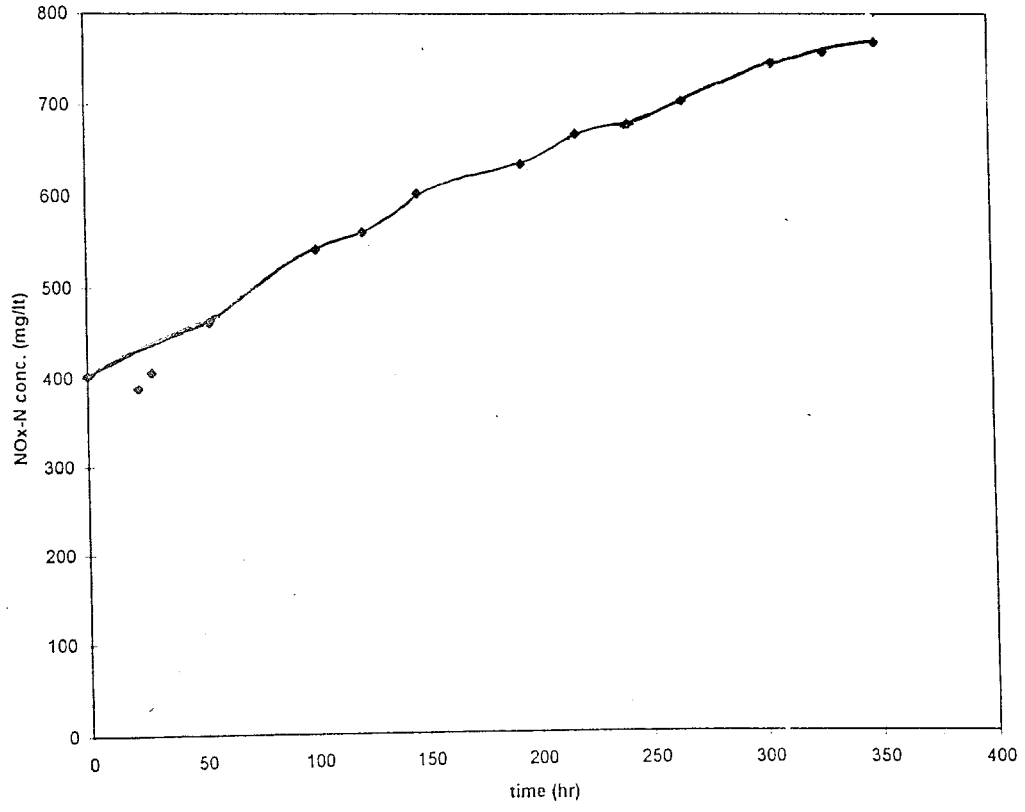


Figure 6.55: Change in product concentration of Run 8

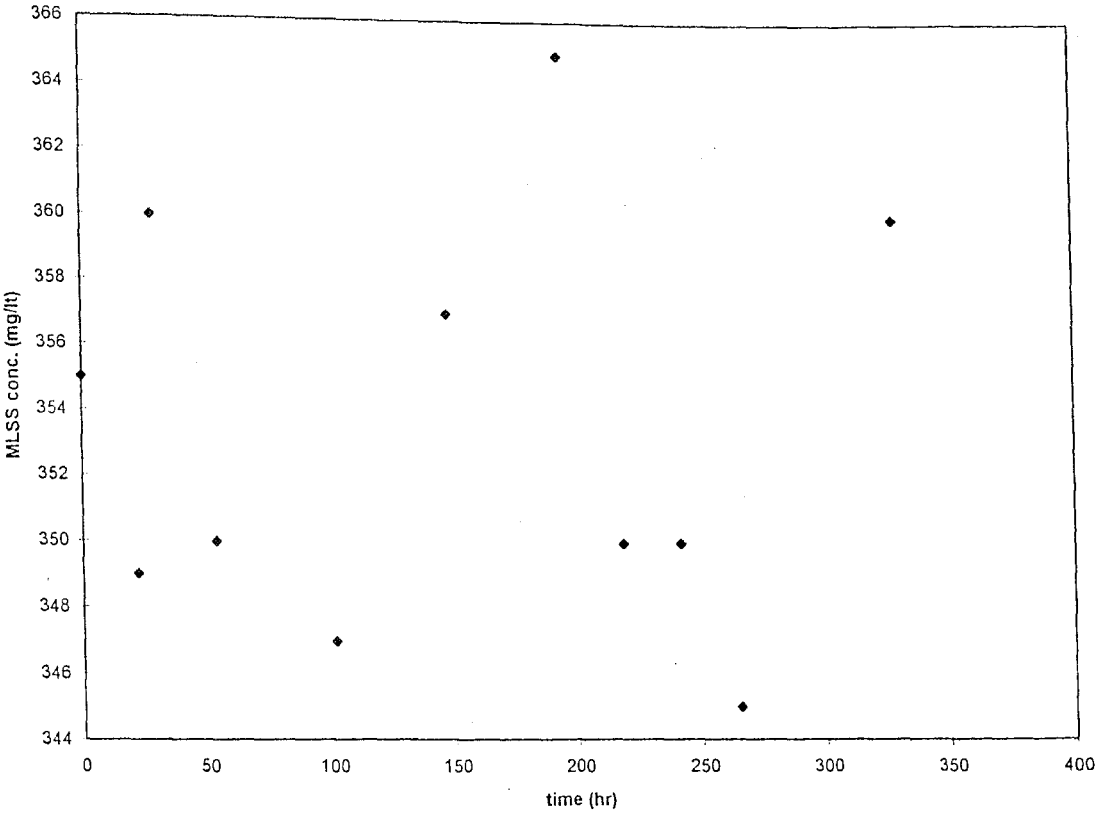


Figure 6.56: Change in MLSS Concentration of Run 8

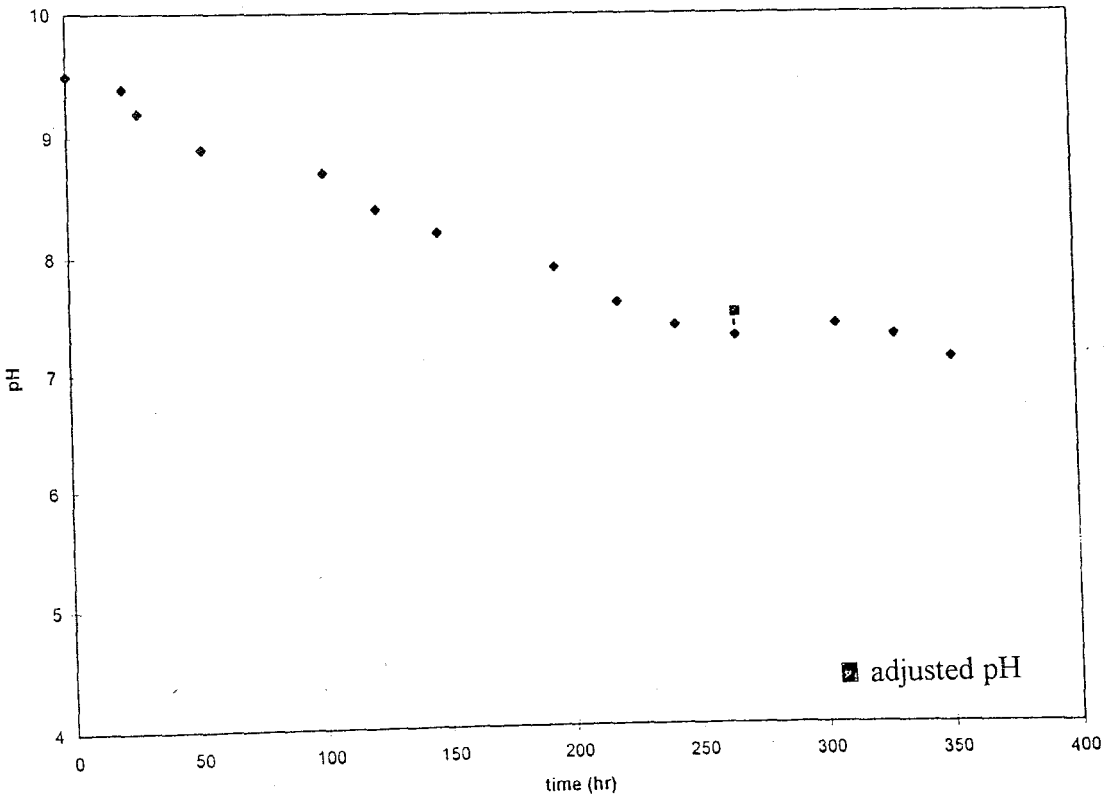


Figure 6.57: Change in pH of Run 8

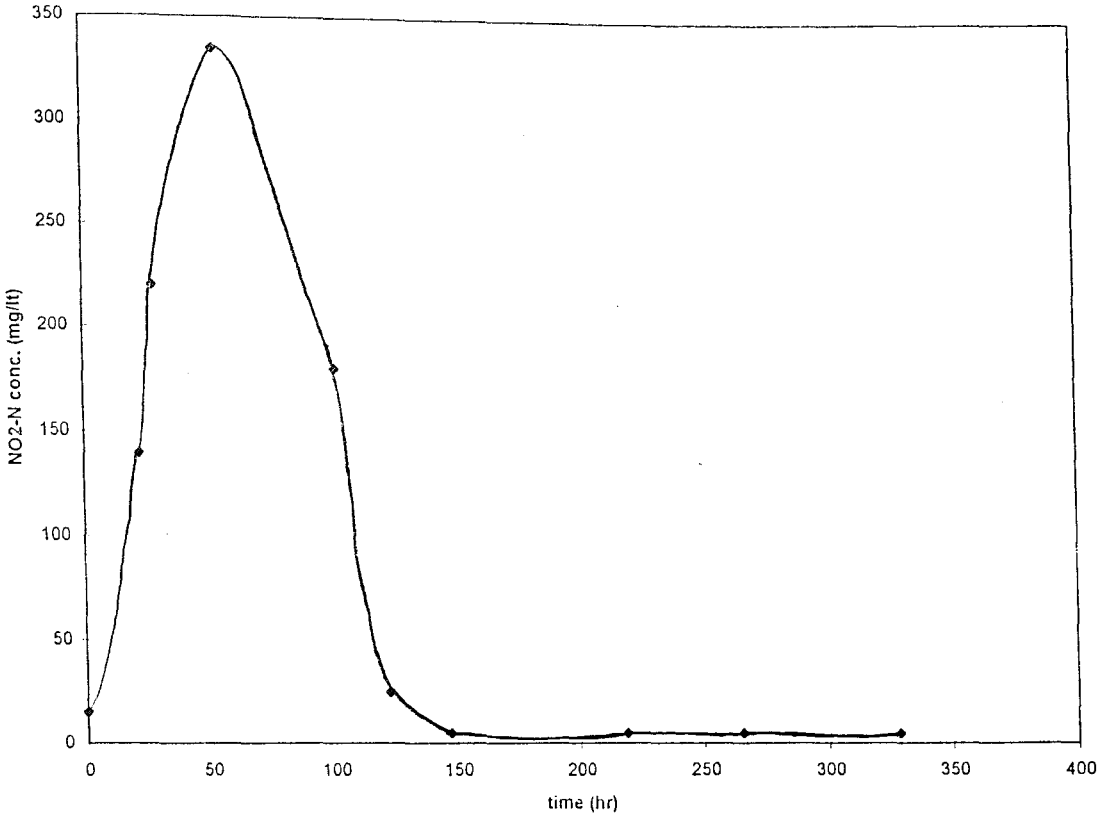


Figure 6.58: NO_2-N formation of Run 8

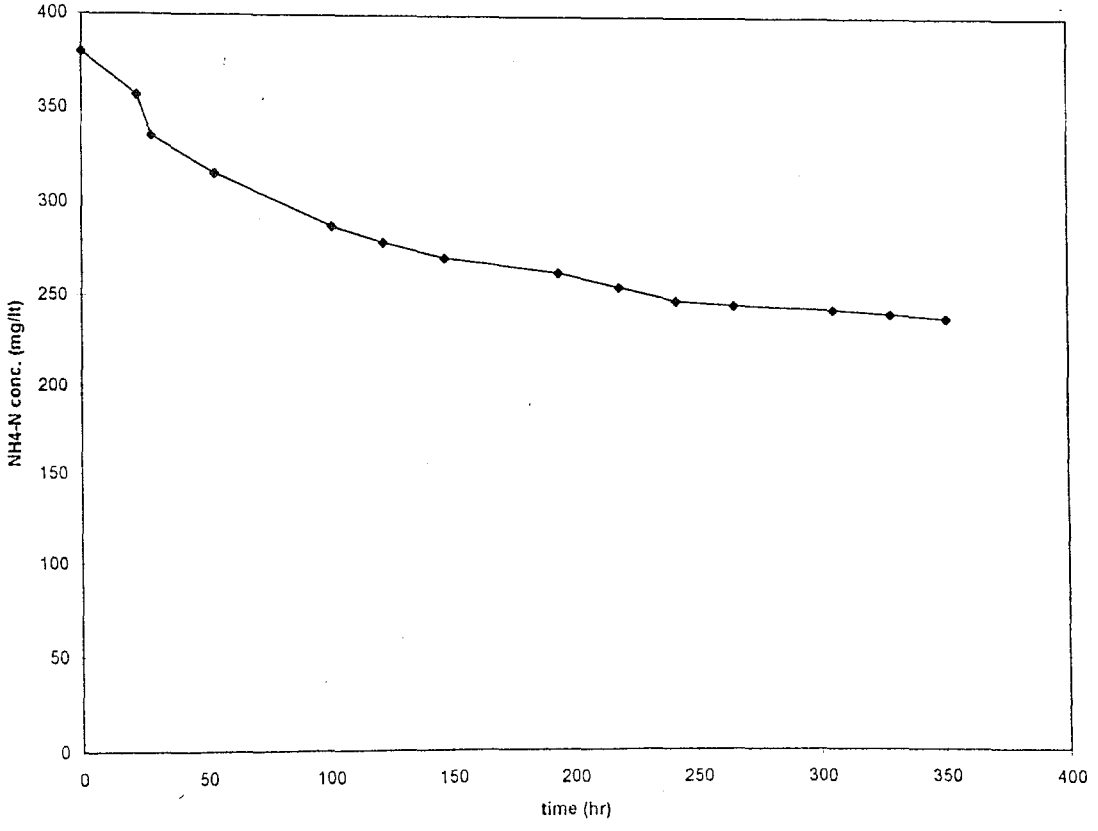


Figure 6.59: Change in substrate concentration in the Control Reactor of Run 8

Since the initial pH of fertilizer wastewater is high, in Run 8 a control reactor is used in order to determine the effect of any ammonia stripping that might happen. Figure 6.59 gives the change in substrate removal of the control reactor for Run 8. In Runs 4, 5a, 5b, 5c, and 6a there was a considerable difference between the change in substrate and product concentrations, which would be expected to be equal according to the mass balance of a system. Experimental errors are the main sources of this difference.

6.2 Discussion and Conclusion

6.2.1. Substrate Removal Efficiencies

In all of the acclimation runs the substrate was totally removed. Only in Runs 6b and 7b nitrification process was inhibited and all the ammonium nitrogen was not converted to nitrate nitrogen. For two runs, Run 2 and 8, control reactors were established to see if there was any ammonia stripping taking place. In Run 2, there was almost no air stripping (Figures 6.5 and 6.13). However, in Run 8, about 10 per cent of the ammonium nitrogen removed was by ammonia stripping because of the high pH in the wastewater,(Figures 6.54 and 6.59).

Many researchers considered initial ammonium nitrogen concentrations only up to 200 mg per liter although very few worked with higher influent concentrations. Almost in all the systems removal efficiencies were more than 90 per cent. It may be concluded that nitrification process under optimum conditions can be applied to wastewaters containing high amounts of nitrogen even up to 1000 mg per liter of ammonia nitrogen.

6.2.2. Substrate Removal Kinetics

One concern of this section is to determine the kinetic behavior of nitrification. Most of the previously done studies state that nitrification behaves as first order kinetics. On the other hand, some disagree and give it as zero order meaning that the substrate removal rate is independent of the substrate concentration. For completely mixed batch reactors since there is no inflow or outflow, rate of change in the mass of a substrate within the reactor is equal to the rate of the reaction in the reactor. This can be expressed as:

$$V * (dS / dt) = Q * S_0 - Q * S - V * r$$

$$V * (dS / dt) = -V * r$$

$$dS / dt = -r$$

where: V = volume of the reactor, liters
S = substrate concentration, mg/lt
Q = flow, lt/sec
S_0 = initial substrate concentration, mg/lt
r = rate of the reaction

On the other hand using the Monod equation, which is:

$$\mu = \mu_{max} * S / (K_s + S)$$

where: μ = growth rate of nitrifiers, day^{-1}
μ_{max} = maximum growth rate of nitrifiers, day^{-1}
K_s = half velocity constant, mg/lt

Multiplying each side of the above equation by X:

$$\mu * X = \mu_{max} * X * S / (K_S + S) = r_x$$

where: X= biomass concentration, mg/lt

r_x = biomass formation rate, mg/(lt.day)

If substrate concentration is much higher than the half saturation constant ($S \gg K_s$) , then:

$$r_x = \mu_{max} * X$$

$$r_x = Y * r_s$$

$$k = \mu_{max} / Y$$

where: r_s = substrate utilization rate, mg/(lt.day)

Y = yield coefficient

k = reaction rate coefficient, day^{-1}

From the three equations above:

$$dS / dt = -k * X$$

Slopes of the equations of the substrate removal versus time graphs, give k*X, and with the help of this product, k value can be easily found by dividing the product to the average biomass concentration in each run.

The first order kinetics are compared to the zero order kinetics. In the first order kinetics, substrate removal rate is affected by the substrate concentration as well as the biomass concentration. Removal rate can be expressed as:

$$dS / dt = -k_1 * X * S$$

By integrating above equation:

$$\ln (So / S) = k_1 * X * t$$

Figures 6.60 through 6.68 are plotted with the laboratory data according to the first order kinetics of substrate removal described above.

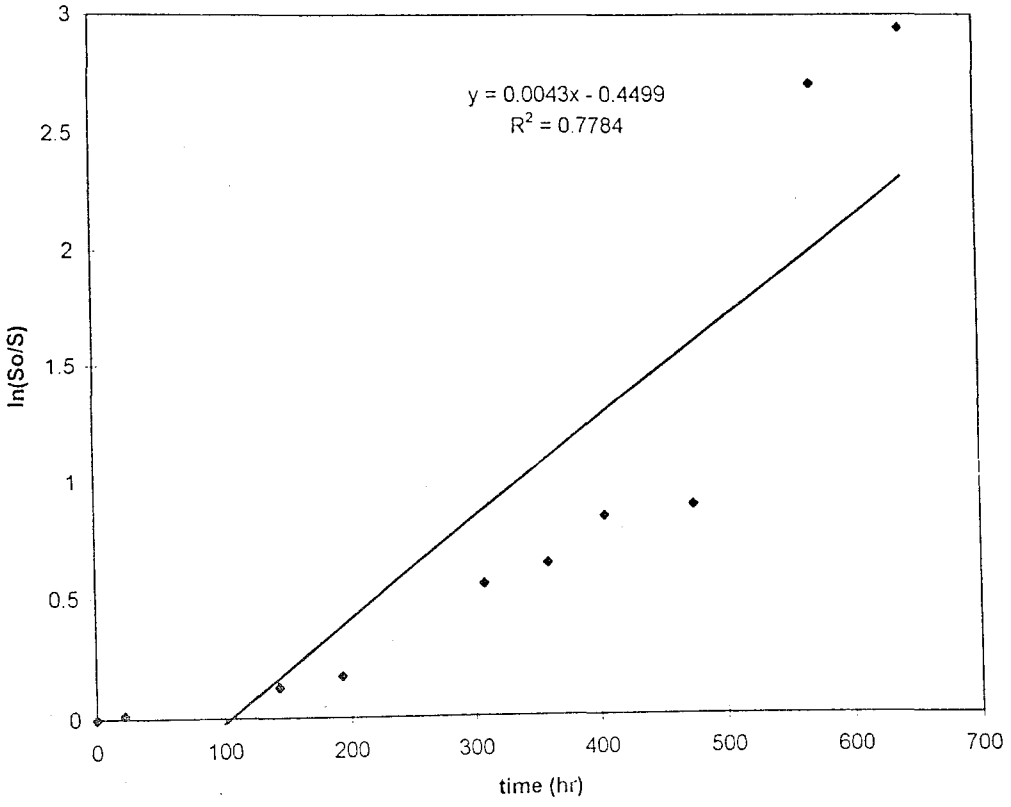


Figure 6.60: Substrate Removal According to First Order Kinetics for Run 4

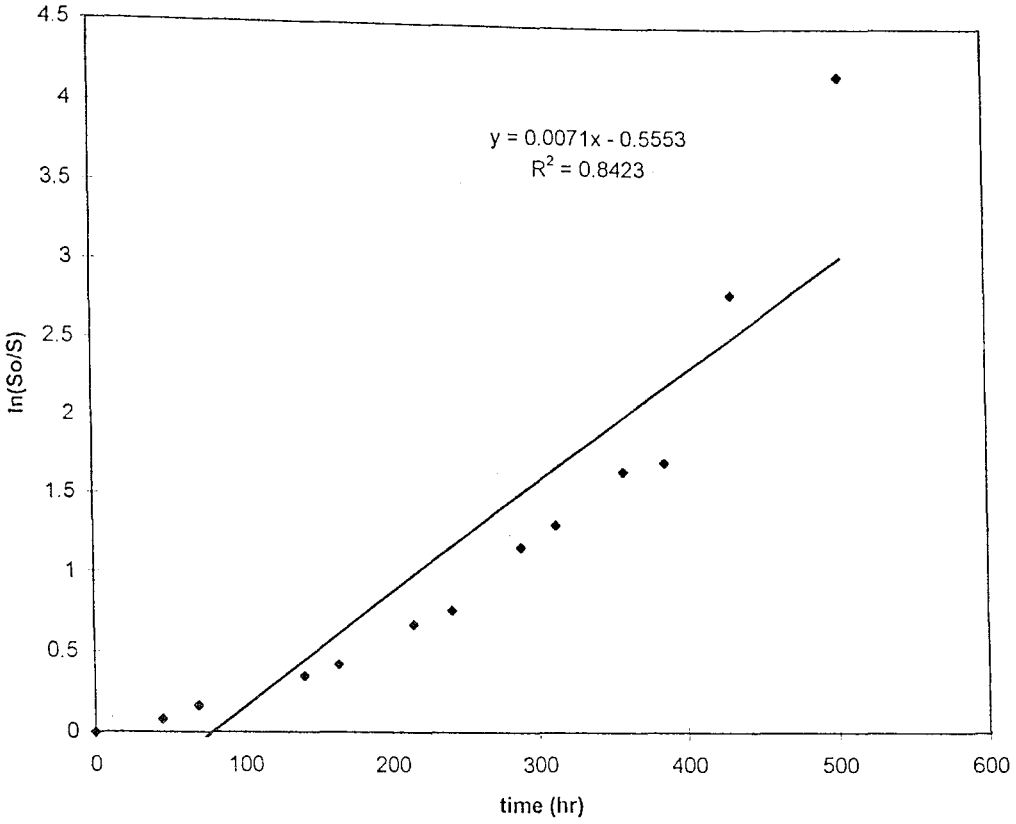


Figure 6.61: Substrate Removal According to First Order Kinetics for Run 5a

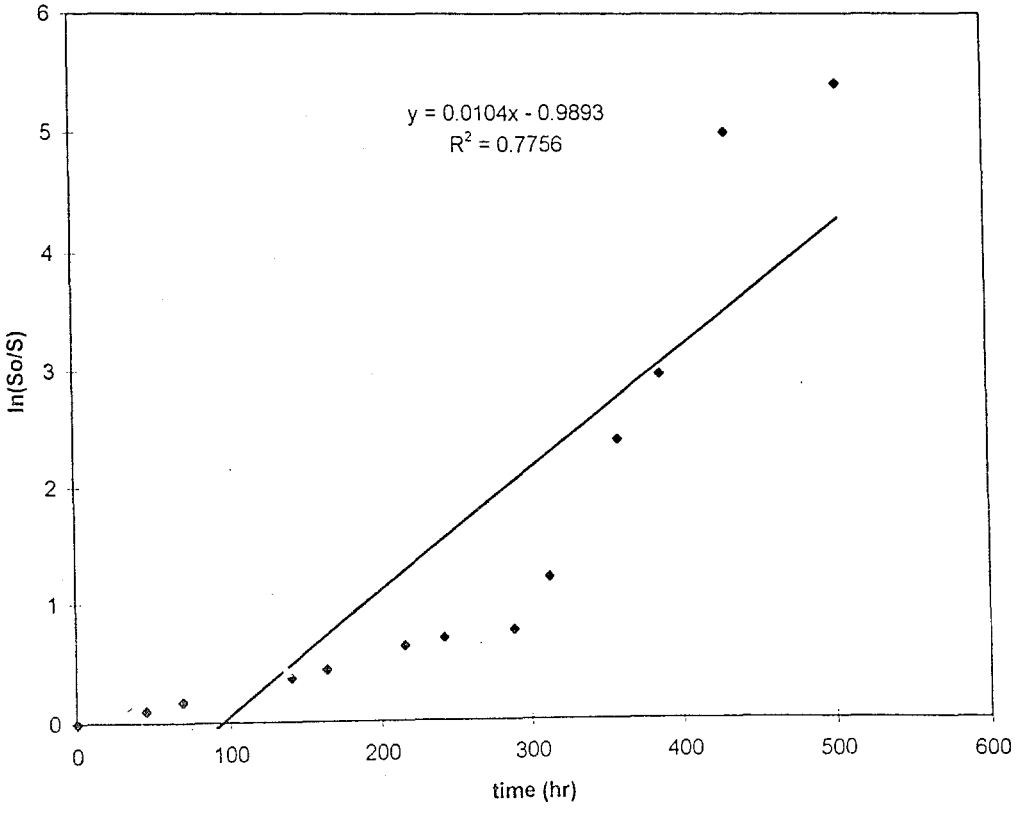


Figure 6.62: Substrate Removal According to First Order Kinetics for Run 5b

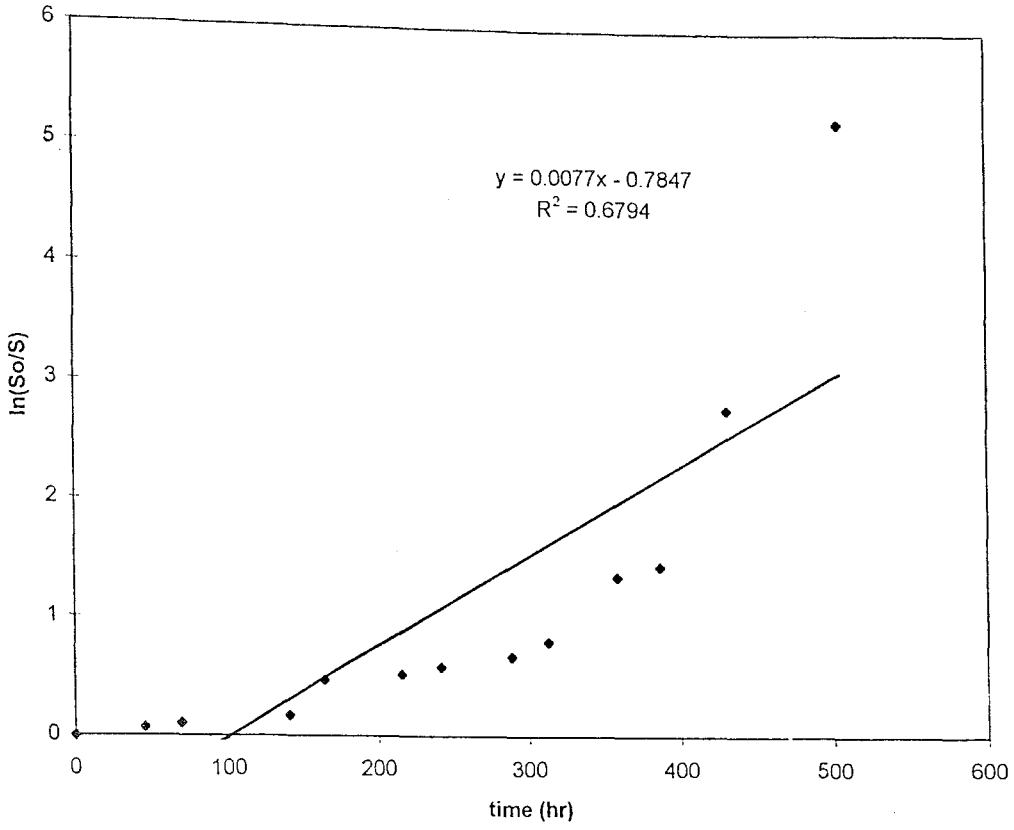


Figure 6.63: Substrate Removal According to First Order Kinetics for Run 5c

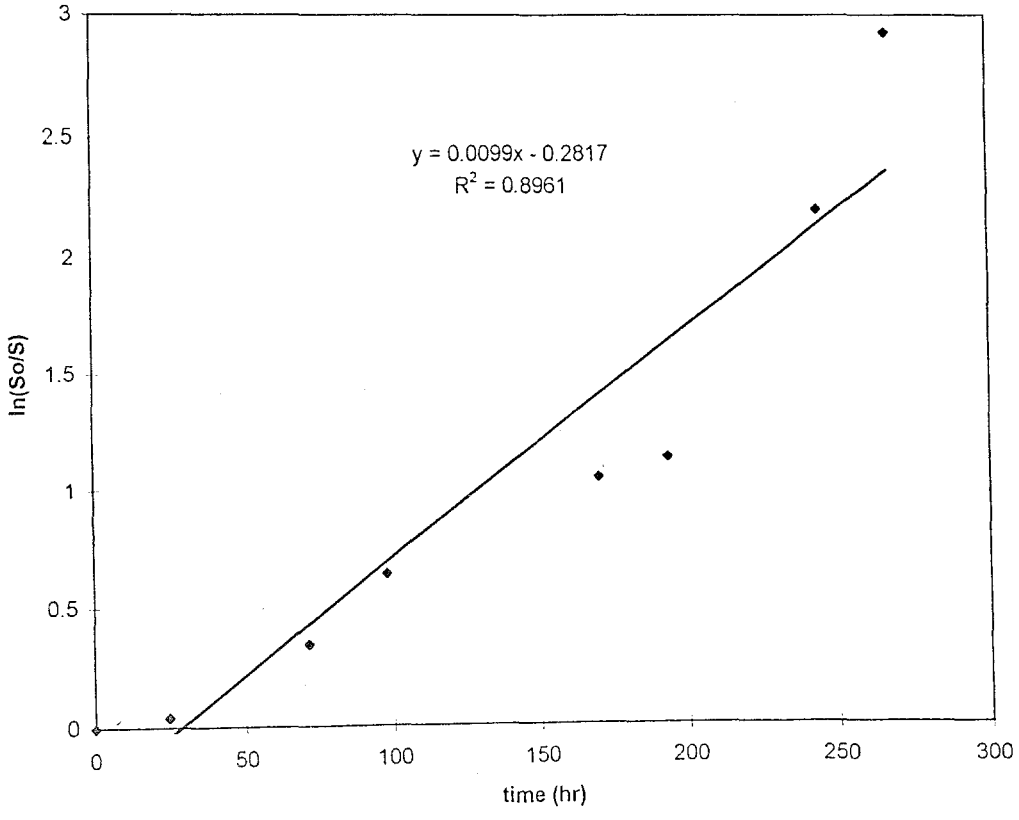


Figure 6.64: Substrate Removal According to First Order Kinetics for Run 6a

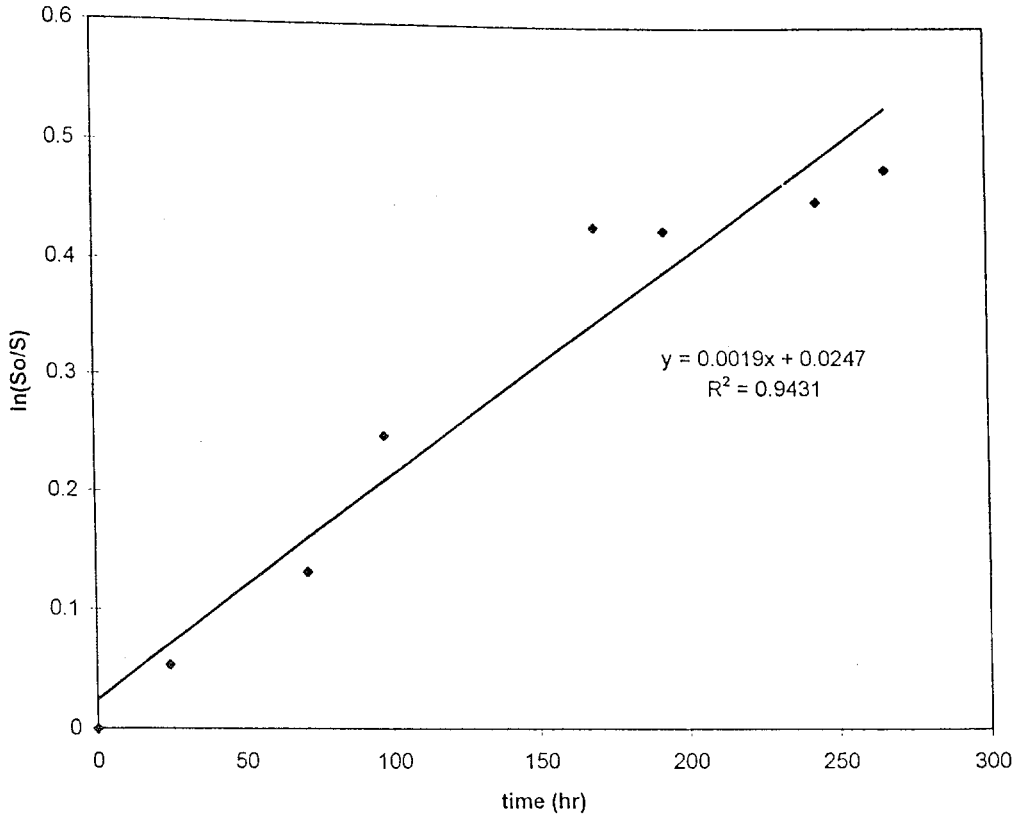


Figure 6.65: Substrate Removal According to First Order Kinetics for Run 6b

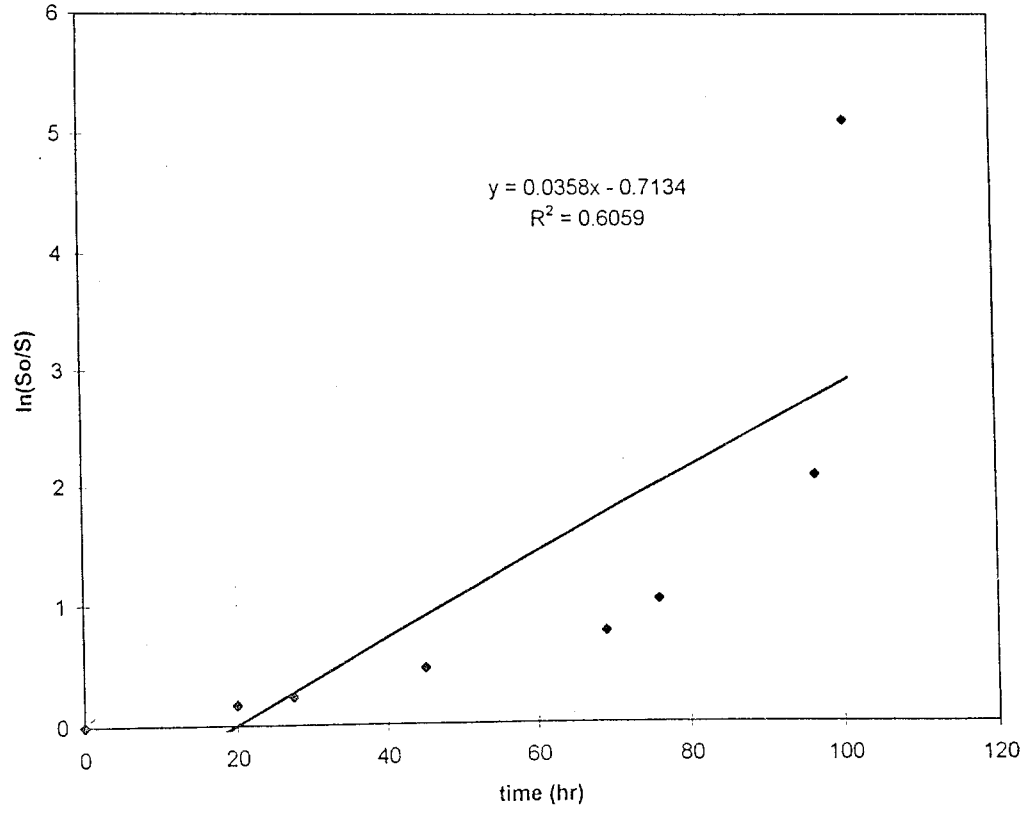


Figure 6.66: Substrate Removal According to First Order Kinetics for Run 7a

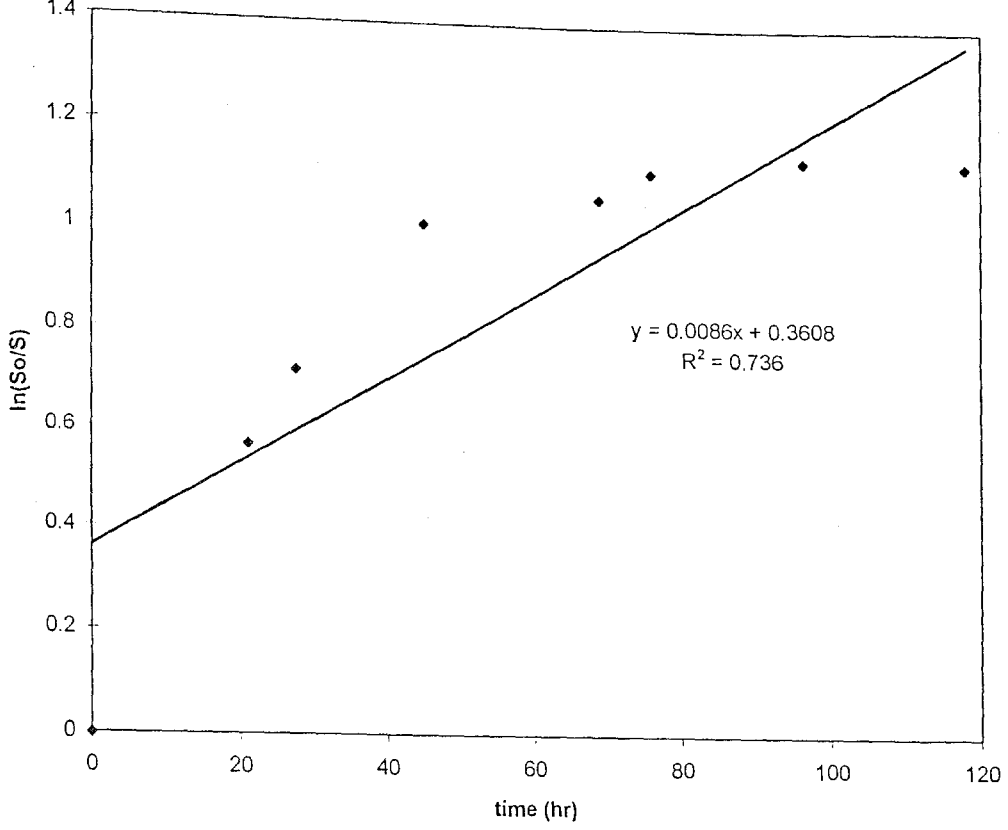


Figure 6.67: Substrate Removal According to First Order Kinetics for Run 7b

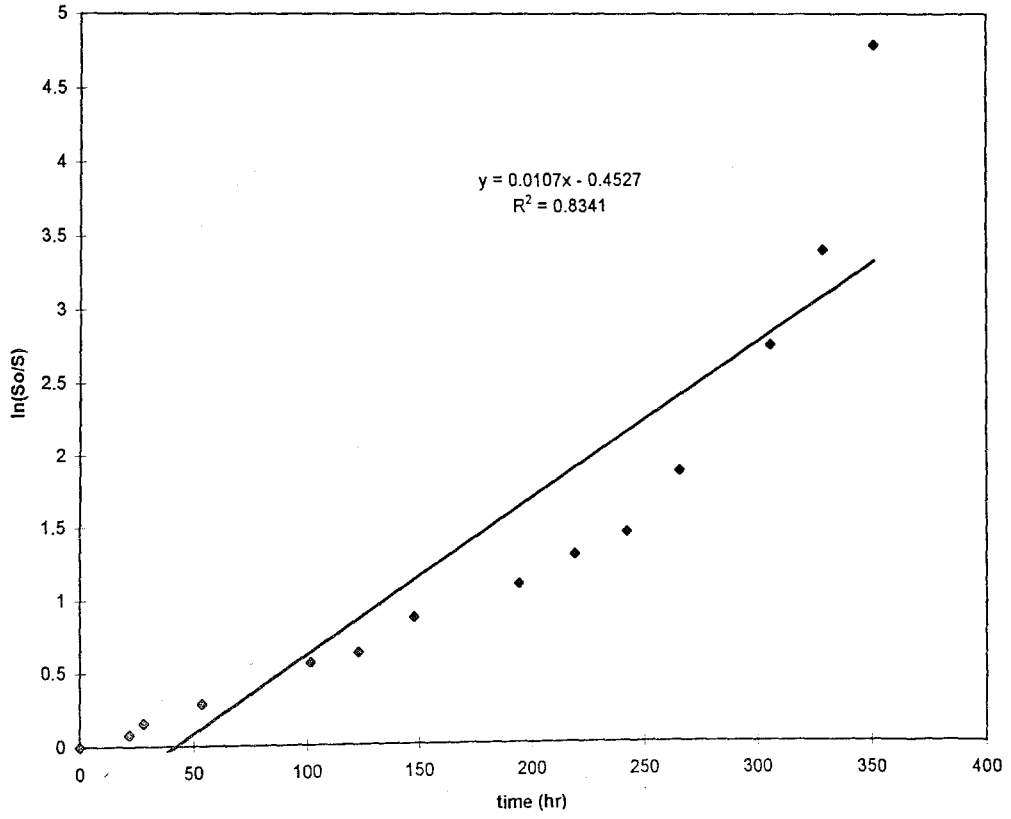


Figure 6.68: Substrate Removal According to First Order Kinetics for Run 8

Similar to the zero order kinetics, k can be found from the slope of the equations of the lines in Figures 6.60 through 6.68. In Table 6.1, k and k_1 values obtained from all the runs are given. In the table the regression coefficients of each line are given as r^2.

Table 6.1: Rate Coefficients of Substrate Removal

	zero order		first order	
	k (mg NH_4-N/ mgMLSS.hr)	r^2	k_1 (l.mgMLSS/hr)	r^2
Run 1	0.00021	0.8981	0.00001	0.8555
Run 2	0.00011	0.2308	0.000002	0.0141
Run 3	0.00023	0.9169	0.000006	0.8501
Run 4	0.00053	0.9687	0.0000151	0.7784
Run 5a	0.00082	0.9847	0.0000148	0.8423
Run 5b	0.00244	0.9647	0.00003	0.7756
Run 5c	0.00263	0.9775	0.000014	0.6377
Run 6a	0.00244	0.9804	0.0000071	0.8961
Run 6b	0.00085	0.9301	0.0000013	0.9431
Run 7a	0.00259	0.9823	0.0000295	0.6059
Run 7b	0.00123	0.6288	0.000004	0.7479
Run 8	0.003	0.9772	0.00003	0.8341

Actually for the last three runs where industrial wastewater was used a certain amount of the removal was done by air stripping since the initial pH is well above nine. The values in the tables are the results considering this effect.

When the rate values for zero order and first order kinetics are compared from the table above. k values of the zero order kinetics are much closer to the various values given in literature which vary considerably from 0.001 to $7.5hr^{-1}$ with much higher regression coefficients. It can be stated that process is closer to zero order kinetics. Results of the first three runs must not be taken into consideration since they are acclimation runs and data can not be relied upon.

As discussed earlier in Section 3.5, a model proposed by Braha and Hafner (1987) for the calculation of kinetic constants in batch reactors will be applied in order to determine the kinetic constants of nitrification. Studies in batch reactors about the determination of these constants are very few. Since there is no observed change in the biomass concentration in this study, the model proposed by Braha and Hafner (1987) will be applied. According to this method, by plotting (So-S)/(t*Xa) versus ln(So/S)/(t*Xa), the slope gives Ks and the y-intercept gives k. The results of data from laboratory research is given in Figures 6.69 through 6.77.

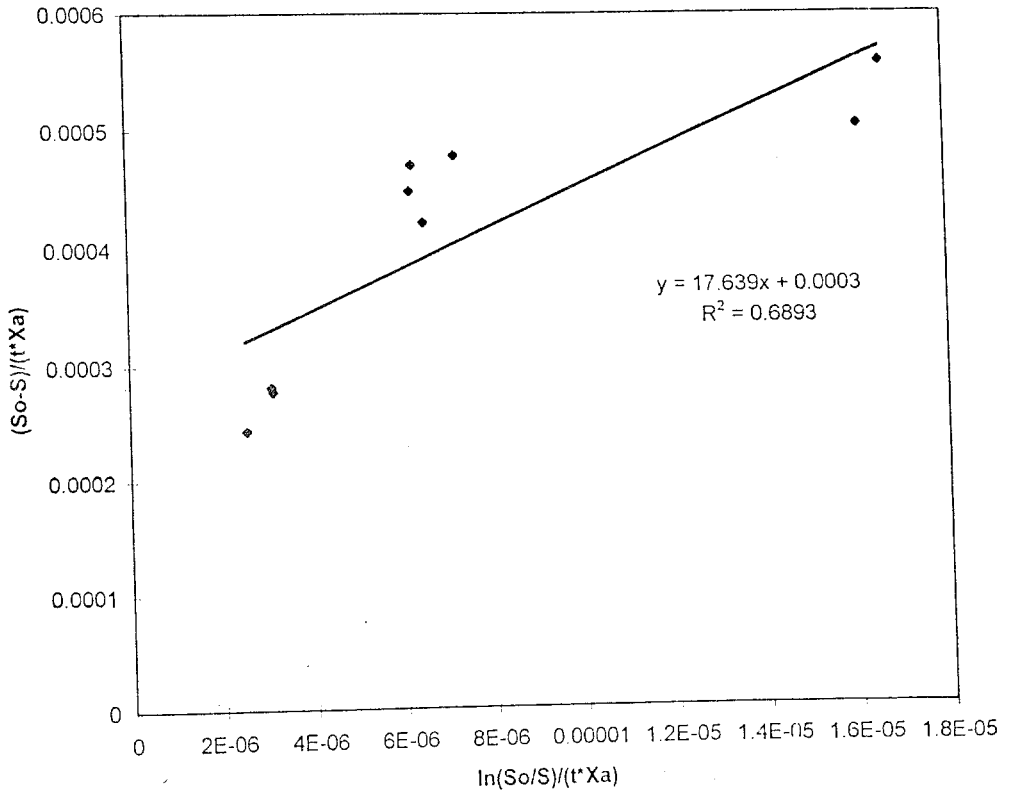


Figure 6.69: Application of the Model for Run 4

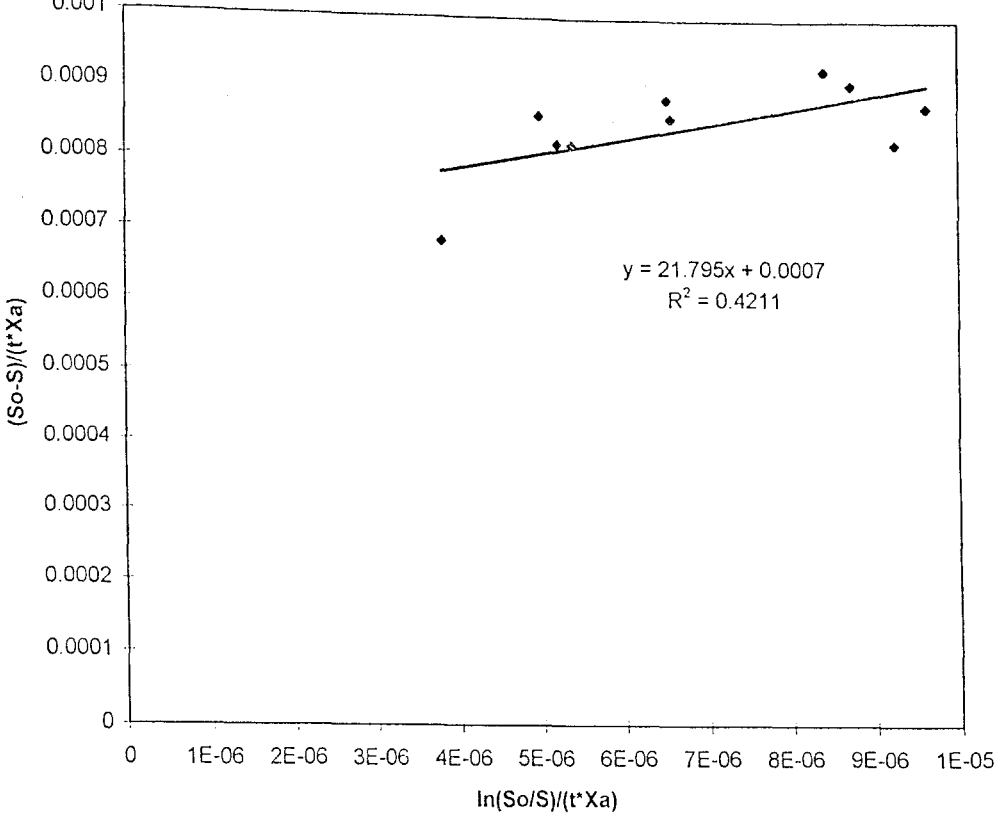


Figure 6.70: Application of the Model for Run 5a

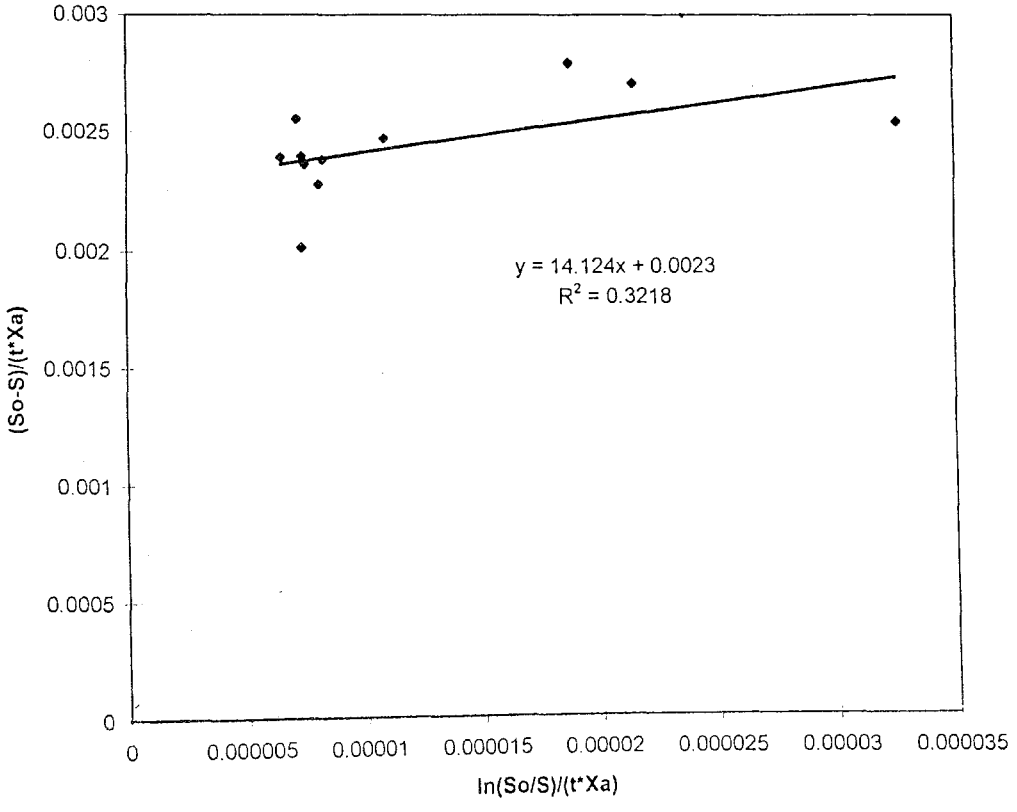


Figure 6.71: Application of the Model for Run 5b

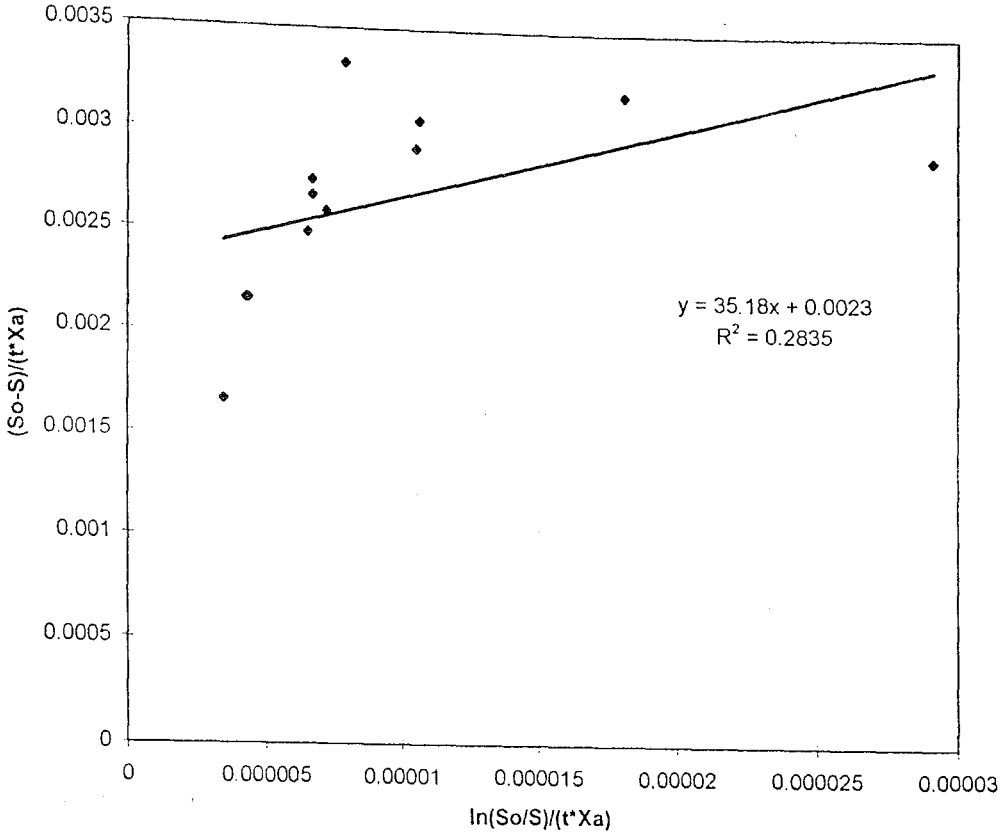


Figure 6.72: Application of the Model for Run 5c

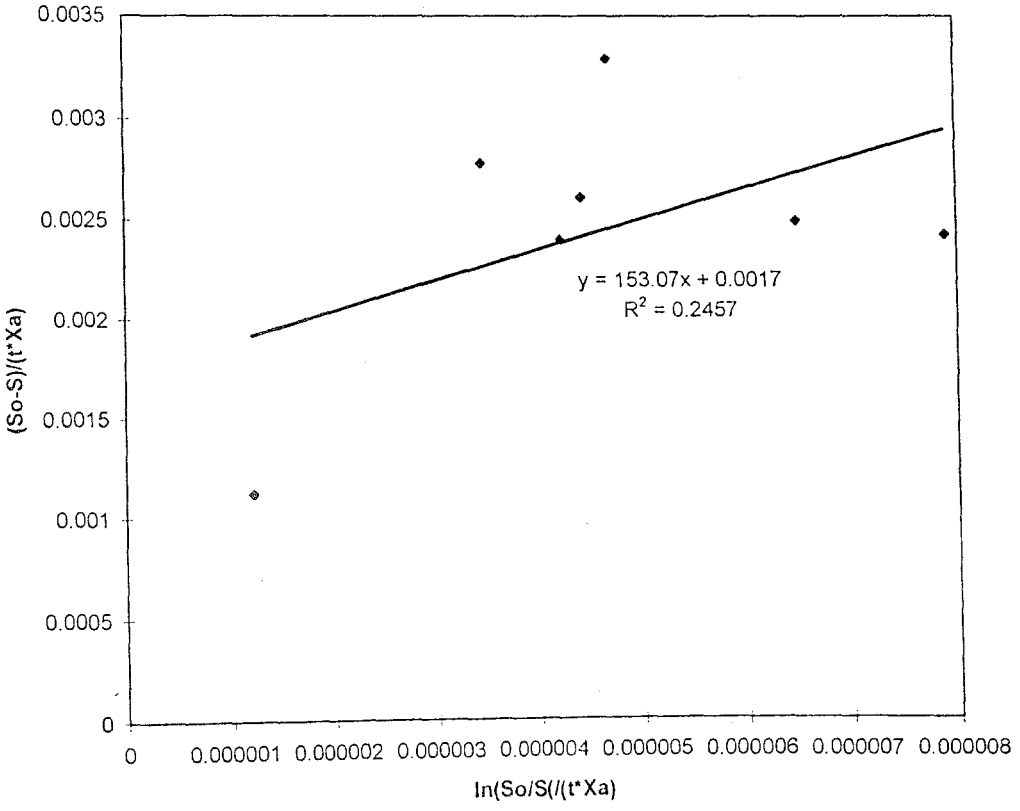


Figure 6.73: Application of the Model for Run 6a

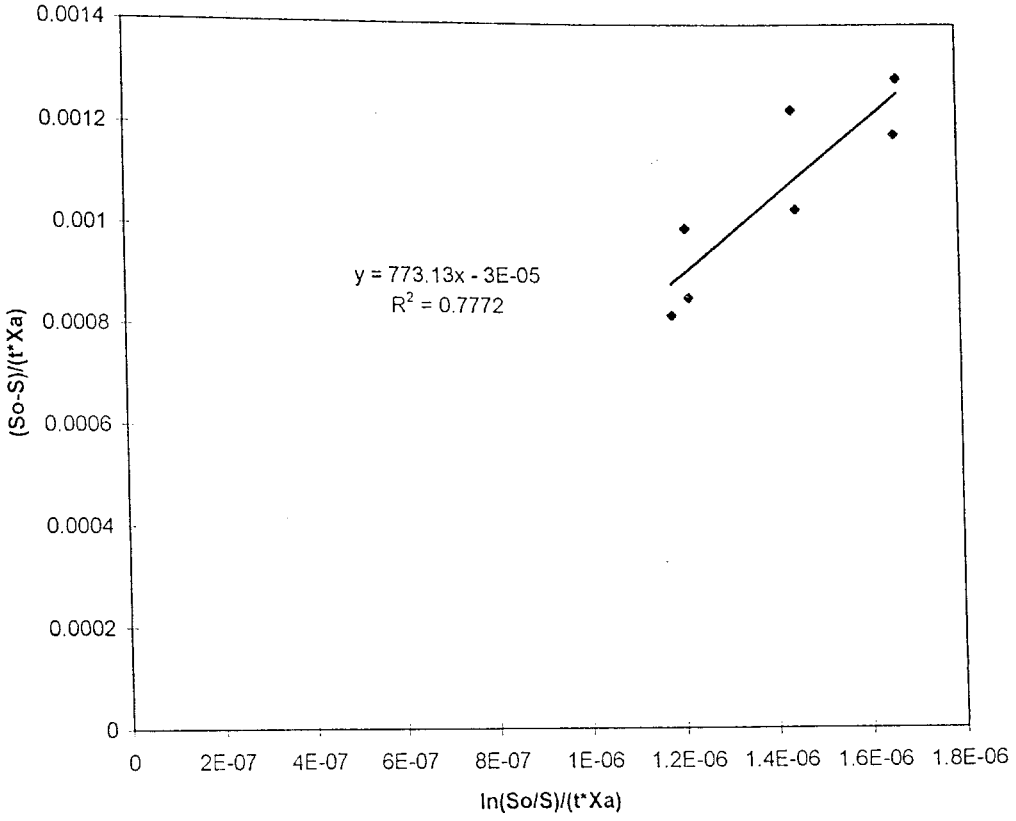


Figure 6.74: Application of the Model for Run 6b

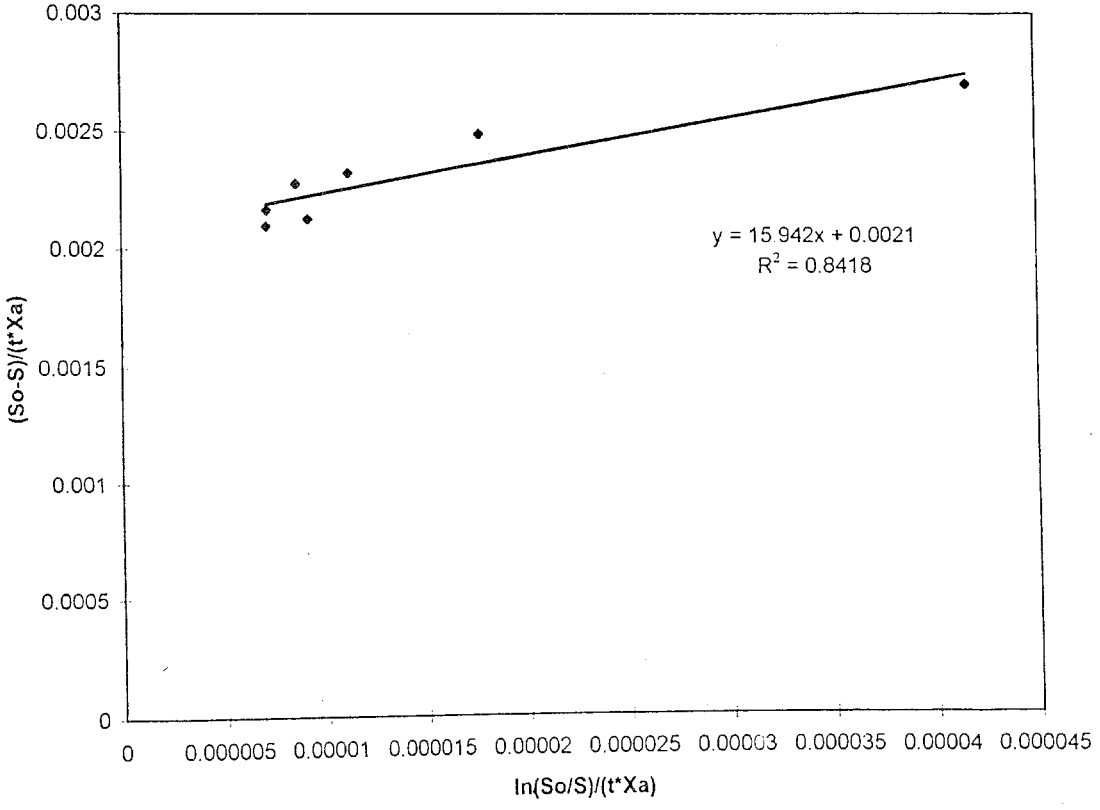


Figure 6.75: Application of the Model for Run 7a

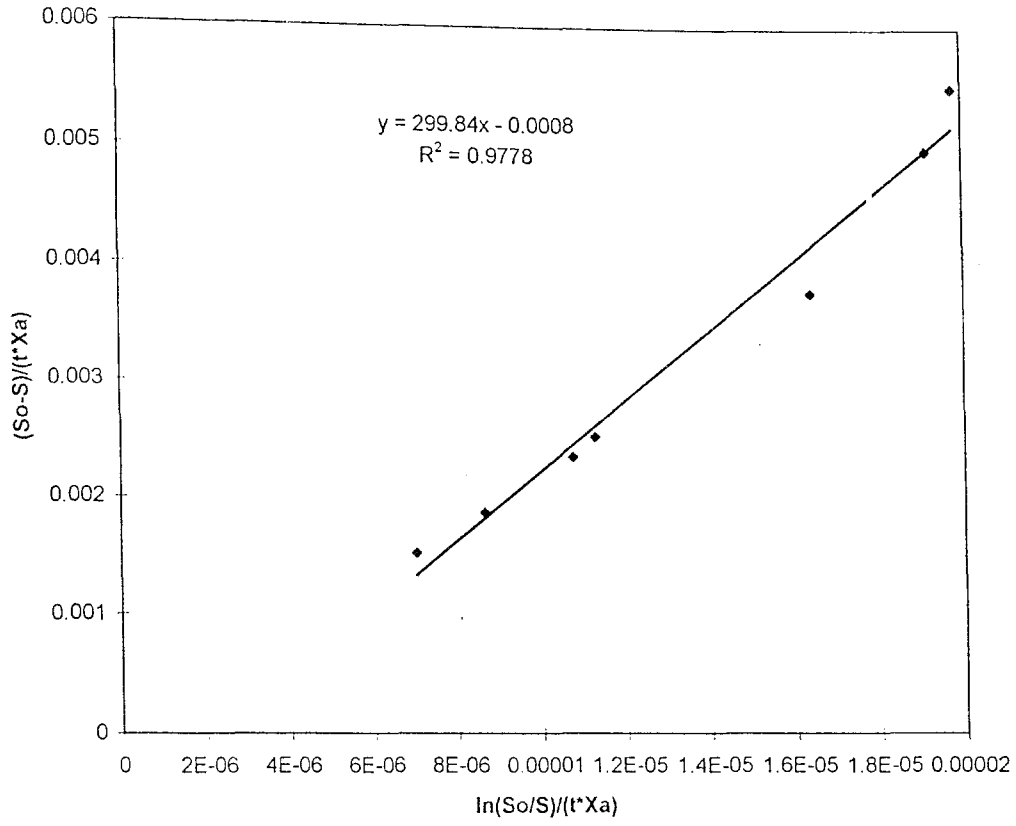


Figure 6.76: Application of the Model for Run 7b

Table 6.2: The results of the applied model according to Braha and Hafner (1987).

Run number	k (mg NH_4-N/mg MLSS.hr)	K_s ($mgNH_4$-N/l)	r^2
Run 4	0.0003	17.64	0.6893
Run 5a	0.0007	21.79	0.4211
Run 5b	0.0023	14.12	0.3218
Run 5c	0.0023	35.18	0.2835
Run 6a	0.0017	153.07	0.2457
Run 6b	0.00003	773.13	0.7772
Run 7a	0.0021	15.94	0.8418
Run 7b	0.0008	299.8	0.9778

Reaction rate coefficients are quite similar to the coefficients determined by zero order kinetics in Section 6.2.2, in Runs 4, 5a, 5b, 6a, and 7a. However, in Runs 5c, 6b, and 7b, k values obtained by zero order kinetics are much lower than the new k values. The most probable reason is that in Runs 6b and 7b complete nitrification was not achieved because of the inhibition effects and this affected reaction rate values.

On the other hand the half saturation constants are much higher than values given in literature. In the inhibition runs 6b and 7b when pH was unadjusted, Ks values reach 700 mg per liter. Except these two runs Ks values are considerably lower. The regression coefficients are not high enough to confirm the method. In order to apply this method and obtain high regression coefficients, the data should not be interpreted as a whole. These results are close to the results obtained by Braha and Hafner, (1987).

6.2.3 Nitrification Inhibition

In Section 3.4.4 nitrogen forms that are affective in the inhibition were discussed. The nitrification tolerance graph according to Anthonisen et al. (1976) and accepted by many others, (Figure 3.1), will be used in determining the nitrification characteristics for the four runs. It must be remembered that the borders of the zones are not strict and many other factors like acclimation or the biomass concentration may have effects. To be able to state the zone in each run free ammonia and nitrous acid concentrations are calculated. Free ammonia,(FA), in the system is calculated from the following equation with the measured NH_4-N concentrations and pH, (Alleman, 1984).

$$FA = ([NH^+_4\text{-}N][10^{pH}]) / ((Kb/Kw)+10^{pH})$$

where: FA: concentration of the free form of ammonium nitrogen in solution, mgN/l.

$[NH^+_4\text{-}N]$: concentration of ammonium nitrogen, mgN/l.

K_b : ionization constant for ammonium which is $10^{-9.24}$.

K_w : ionization constant for water which is $0.69x10^{-14}$.

Free nitrous acid (FNA) is calculated by the following equation, (Alleman, 1984):

$$FNA = [NO_2\text{-}N] / (K_a * 10^{pH})$$

where: FNA: concentration of the free form of nitric nitrogen in solution, mgN/l.

[NO2-N] : nitrite concentration, mgN/l.

K_a : ionization constant for NO^-_2 which is $10^{-3.4}$.

In Table 6.3, the calculated FA and FNA values are given.

Table 6.3 : Calculated values of FA and FNA for each run.

Run no	FA concentration (mgN/l)	FNA concentration (mgN/l)
Run 4	2.2217	0.0007
Run 5a	5.3549	0.0005
Run 5b	8.9599	0.0013
Run 5c	10.3658	0.0011
Run 6a	13.8011	0.0011
Run 6b	12.0705	0.0106
Run 7a	12.0067	0.0004
Run 7b	255.6624	0.8910
Run 8	11.0688	0.0074

In Runs 4, 5a, 5b, and 5c although there is an increase in the initial ammonium concentration, total nitrite nitrogen, free ammonia, and free nitrous acid concentrations are low so all the three runs are in Zone 3 where complete nitrification occurs.

Runs 6a and 6b have a very high influent NH_4-N concentration resulting in high NH_3-N concentrations. Run 6a has low NO_2-N and FNA concentrations and pH of the run is about seven, letting the run stay in Zone 3. Although Run 6a is in Zone 3, Run 6b is on the border of Zone 3 and Zone 2. This is because the higher nitrite and FNA concentrations.

Although Run 7a does not have a very high NH_4-N concentration, some nitrite accumulation occurred for a short period of time. The system recovered this build-up quickly and complete nitrification once more occurred in Zone 3. Run 7b is significantly different from the other runs because all the factors considered in this section are high in this run and this resulted in the total inhibition of *Nitrobacter* and *Nitrosomonas* occurring by FA.

7. CONCLUSION AND RECOMMENDATIONS

A series of batch studies utilizing biomass were performed using synthetic feed and also industrial wastewater with high a nitrogen content. Dissolved oxygen and temperature were in the optimum ranges for all the runs. Industrial wastewater was obtained from a nitrogenous fertilizer industry.

Examination of the kinetic behavior of the process showed that the process behaves according to zero order kinetics. This was an expected result although in literature nitrification is usually treated as first order. Initial substrate concentration is too high in the runs so half-velocity coefficient would be much smaller than the substrate giving a zero order reaction. The differences in the reaction rate coefficients can be explained by the different feeds used during the runs. Also first order kinetics were applied for substrate removal but rate and regression coefficients were much more lower than the literature values. It can be concluded that the kinetics of nitrification process is behaving according to the zero order kinetics for the concentration ranges studied.

Once steady state is reached, determination of the kinetic constants of many processes including nitrification are stated to be much more easier in continuous flow reactors than batch reactors. Models for batch reactors have been developed in order to determine these constants since experiments done in batch reactors are less time consuming. Application of such a model which bases on Monod kinetics has been performed giving reaction rate coefficients closer to the ones given by zero order reaction kinetics, although the regression coefficients were low.

Another important environmental factor is the pH of the solution. Low pH results in inhibition while at high pH values ammonia stripping process may happen instead of nitrification. In some of the runs pH was deliberately left uncontrolled so there was a decrease for some time. At values of pH of about five there is a total inhibition in nitrification. Also a joined effect of high initial ammonium nitrogen and pH observed. Very

high concentrations of initial free NH_3 which can be easily air stripped under certain conditions seemed to be inhibitory to nitrification as well.

A continuation of this study can be accomplished by using continuous flow reactors for the determination of the process kinetics in the case of treatment of wastewaters with high nitrogen concentrations.

A further study can be done by developing a model appropriate for such conditions in batch reactors which can be applied in determining the kinetic constants of nitrification.

Another research can be based on the inhibition effects of pH and ammonia concentrations. Inhibitory levels may be more strictly determined for batch reactors as well as continuous flow reactors.

Nitrite build-up is probably one of the most important problems and at the same time depending on the conditions can be a solution for a shortened pathway of nitrification and denitrification processes. High amounts of initial ammonium nitrogen results in a build up of nitrite nitrogen in the system. This build-up is not permanent and after a period of time system acclimates itself and conversion to nitrate happens. If this build-up can be made to last, then the product of nitrification as nitrite could be used immediately in denitrification. By this method , the second step of nitrification and the first step of denitrification would be removed and the process be much more advantageous.

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