ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

UNCERTAINTY ANALYSIS IN THE MEASUREMENT OF NITRIFICATION KINETICS IN URBAN WASTEWATER TREATMENT PLANTS IN TÜRKİYE

M.Sc. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

TÜRKİYE'DE KENTSEL ATIKSU ARITMA TESİSLERİNDE NİTRİFİKASYON KİNETİĞİ ÖLÇÜMÜNDE BELİRSİZLİK ANALİZİ

YÜKSEK LİSANS TEZİ

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HAZIRAN 2024

Berker Leblebici, a M.Sc. student of ITU Graduate School student ID 501211801, successfully defended the thesis/dissertation entitled "UNCERTAINTY ANALYSIS IN THE MEASUREMENT OF NITRIFICATION KINETICS IN URBAN WASTEWATER TREATMENT PLANTS IN TÜRKİYE", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 24.06.2024
Date of Defense : 28.06.2024

To my loved ones,

FOREWORD

I would like to seize this moment to express my profound gratitude to the individuals whose invaluable contributions have been crucial in the successful conclusion of this scientific study. Your assistance and encouragement have been priceless, and I sincerely acknowledge your contributions.

First and foremost, I would like to express my heartfelt gratitude of the priceless guidance, patience, unwavering support, and expert knowledge of my mentor, Prof. Dr. Emine ÇOKGÖR, who has been instrumental in the development of the progress of the study. Her consistent encouragement and thoughtful feedback have provided a strong foundation for my research, allowing me to overcome challenges and achieve a deeper understanding.

I am a deeply grateful to Prof. Dr. Güçlü İNSEL, his inspiring vision, profound knowledge, and thoughtful guidance have significantly shaped my approach to environmental modeling and influenced my academic journey. I am sincerely thankful for the wisdom and perspective he has shared, which have enriched my understanding and motivated my research efforts.

I am greatly appreciative of the unwavering support of my family, whose unconditional love and empathetic understanding have been essential in guiding me through the ups and downs of this academic journey.

I am deeply appreciative of the invaluable contributions made to this thesis at every level. The encouraging remarks and useful criticisms from my colleagues and friends have significantly enhanced my understanding and elevated the quality of this work.

It is a privilege to present this thesis, which is the result of diligent effort. I am most excited about the prospect of this work contributing to the existing body of knowledge in the field of environmental biotechnology.

June 2024

Berker LEBLEBİCİ
Environmental Engineer

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ABBREVIATIONS

ASM1	**:** Activated Sludge Model No. 1
ASM2	**:** Activated Sludge Model No. 2
ASM2d	**:** Activated Sludge Model No. 2d
ASM3	**:** Activated Sludge Model No. 3
AOB	**:** Ammonia:Oxidizing Bacteria
DPAOs	**:** Denitrifying Phosphorus Accumulating Organisms
MS-Excel	**:** Microsoft Excel
NOB	**:** Nitrite Oxidizing Bacteria
NOX	**:** Nitrogen Oxides
VBA	**:** Visual Basic for Applications
WWTP	**:** Wastewater Treatment Plant
SSE	**:** Sum of Squared Errors

SYMBOLS

α	: Significance level
μ_A	: Specific growth rate of autotrophs [day^{-1}]
$\hat{\mu}_A$	: Maximum specific growth rate of autotrophs [day^{-1}]
N	: Rate-limiting substrate concentration [mg/l]
K_N	: Half-saturation constant of the rate-limiting substratei [mg/1]
X_A	: Biomass concentration of the autotrophs [mgVSS/l].
b_A	: Endogenous decay rate for autotrophs [day^{-1}].
Y_A	: Autotrophic yield coefficient [g cells synthesized per g NH_4-N oxidized]
q_A	: Specific ammonium removal rate [g NH_3-N/g VSS·day]
S_c	: Critical value of SSE at the boundry of the confidence contour
S_{min}	: Minimum value of the SSE
p	: Number of parameters
n	: Number of observations
$F_{p,n-p}$	: Critical value form the Fisher's Table (F-Distribution Table)

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UNCERTAINITY ANALYSIS IN THE MEASUREMENT OF NITRIFICATION KINETICS IN URBAN WASTEWATER TREATMENT PLANTS IN TÜRKİYE

SUMMARY

In this study, relation between the maximum growth rate of nitrifiers and the initial amount of active biomass placed at the beginning of the nitrification experiment was investigated by using parameter estimation and uncertainity analysis for four different wastewater treatment plants, taking into account local conditions. By focusing on these parameters, critical importance of the effects of initial conditions on the nitfication experiment is investigated.

To designing and optimising the performance of wastewater treatment plants, nitrification kinetics play a decisive role. Nitrification directly affects aerobic sludge age, which is one of the most important parameters to obtain volume calculations. Understanding of nitrification kinetics will enable predict and control the rates of these biochemical transformations with greater sensitivity and, in turn, increase the effectiveness of future nitrogen removal processes. Nitrification is important for the alleviation of aquatic eutrophication and the minimization of the toxic ammonia concentration to meet the ever-increasing stringent environmental discharge regulations. In addition, it has significant impacts on designed advanced treatment technologies and sustainable practices along the pathways to ensure minimal anthropogenic impact on the natural ecosystems.

Four different advanced biological wastewater treatment plants across the Marmara Region of Türkiye are taken into account to highlight the importance of the local conditions. From these plants daily samples were taken and nitrification experiment was carried on, and with the results of the experiments and the data generated by the model are used in this study.

Some assumptions such as assuming the respiration and growth as constant are made in order to reduce the complexity of the initial conditions and to improve practical identifiability and lesser uncertainity between the focused parameters.

The nitrification experiment results of the plants, that were modeled using Microsoft Excel program, used and with the help of sum of squared method, parameter estimations are performed, and the effect of the initial conditions of the experiment, and uncertainties are investigated.

According to the results, approximate confidence intervals were visualized as contour plots based on critical values calculated at a 95% confidence level for each plant. The effects of local conditions on nitrification kinetics experiments were discussed based on observed similar trends in approximate confidence intervals.

Nitrifiers growth rates in Türkiye found to be lower than that of standard values, and errors in maximum growth rate of nitrifiers are calculated to be in the order of 5-10%, which is acceptable according to the general design margins which is typically range between 20% to 25%. Also, for the practical applications overdesign is very general

approach for construction of wastewater treathment plant design and oftenly varies between 10-30%. Thus, the importance of factors that may arise from the initial conditions of the experiment was emphasized, and the most suitable experimental conditions were examined.

In many studies, the significance test of the data can be overlooked; however, this study focuses on the uncertainty analysis between the maximum growth rate of nitrifiers and the initial active biomass placed at the beginning of the experiment. Meanwhile, it is important to acknowledge that this study may be one of the first investigations to explore this specific subject.

TÜRKİYE'DE KENTSEL ATIKSU ARITMA TESİSLERİNDE NİTRİFİKASYON KİNETİĞİ ÖLÇÜMÜNDE BELİRSİZLİK ANALİZİ

ÖZET

Bu çalışmada, nitrifikasyon deneyinin başlangıcında yerleştirilen aktif biyokütlenin başlangıç miktarı (X_A) ile nitrifiyerlerin maksimum büyüme hızı ($\hat{\mu}_A$) arasındaki ilişki, yerel koşullar dikkate alınarak dört farklı atık su arıtma tesisi için parametre tahmini ve belirsizlik analizi kullanılarak araştırılmıştır. Bu parametrelere odaklanarak, başlangıç koşullarının nitrifikasyon deneyine olan etkilerinin kritik önemi incelenmiştir. Genelde gözardı edilen verilerin güven testi bu çalışmanın temelini oluşturmaktadır ve nitrifikasyon deneyinin başlangıç koşullarının önemi vurgulanmaktadır. Bu yönüyle tez çalışması, ilgli alanda gerçekleştirilen ilk araştırmalardan biri olma özelliğini taşımaktadır.

Nitrifikasyon deneyinin başlangıcında beslenen aktif biyokütlenin başlangıç miktarı, nitrifikasyon kinetiğini ve dolayısıyla atıksu arıtma süreçlerinin verimliliği etkilemektedir. Başlangıç biyokütle miktarının yeterli olması, nitrifiyerlerin hızlı bir şekilde büyümesini ve amonyağı nitrata oksitleyen enzimatik aktivitelerin daha optimum düzeyde gerçekleşmesine yardımcı olur. Bu durum, biyokimyasal reaksiyonların kinetiğini etkileyerek, sürecin verimli bir şekilde ilerlemesini mümkün kılar. Başlangıçta yerleştirilen biyokütlenin miktarı, aynı zamanda mikroorganizma popülasyonunun çeşitliliğini ve yoğunluğunu da belirler. Dolayısıyla, başlangıç biyokütle miktarının optimize edilmesi, süreç içi belirsizliklerin ve potansiyel operasyonel aksaklıkların minimize edilmesine yardımcı olur.

Nitrifiyerlerin maksimum büyüme hızı, nitrifiyerlerin optimum koşullarda ulaşabileceği en yüksek çoğalma hızını ifade eder ve bu hız, nitrifikasyon kinetiklerinin temel dinamiklerinden biridir. Bu parametre, sistemin amonyağı ne kadar hızlı işleyebileceğini, dolayısıyla arıtma kapasitesini ve etkinliğini doğrudan etkiler. Yüksek maksimum büyüme hızına sahip nitrifiyerler, daha hızlı nitrifikasyon süreçleri sağlar ve bu da daha düşük biyoreaktör hacimleri ve daha kısa hidrolik bekleme süreleri ile yüksek verimlilikte arıtma gerçekleştirilebilmesine olanak sağlar. Nitrifiyerlerin maksimum büyüme hızı birçok çevresel değişkene ve atıksu karakterizasyonuna bağlı olarak değişmektedir, bu nedenle yerel koşulların göz önüne alınması literatürdeki farklı coğrafyalarda ve toplumlarda kullanılan genel kabullerin kullanılmasındansa daha isabetli olacaktır.

Optimum başlangıç koşullarının sağlanması, biyolojik reaksiyonların daha öngörülebilir ve kontrollü bir şekilde gerçekleşmesine olanak tanır. Bu da, proses kontrolü ve yönetimi açısından önemli bir avantaj sağlar. Başlangıç biyokütle miktarının doğru bir şekilde ayarlanması, aynı zamanda enerji tüketimi ve işletme maliyetlerinin düşürülmesine katkıda bulunur, çünkü optimum miktarda biyokütle ile daha düşük enerji girdileriyle yüksek verimlilik elde edilebilir.

Atık su arıtma tesislerinin tasarımı ve performansının optimize edilmesi için nitrifikasyon kinetikleri belirleyici bir rol oynamaktadır. Nitrifikasyon, hacim hesaplamalarını elde etmek için en önemli parametrelerden biri olan aerobik çamur yaşını doğrudan etkilemektedir. Aslında, nitrifikasyon, amonyanın nitrifiyerlar aracılığıyla nitratlara biyolojik oksidasyonu sürecidir ve hem mühendislik

sistemlerinde hem de doğal sistemlerde azot döngüsünün önemli bir adımıdır. Nitrifikasyon kinetiklerinin anlaşılması, bu biyokimyasal dönüşüm hızlarının daha hassas bir şekilde tahmin edilmesini ve kontrol edilmesini sağlayacak ve böylece gelecekteki azot giderme süreçlerinin etkinliğini artıracaktır. Nitrifikasyon, sucul ötrofikasyonun hafifletilmesi ve giderek daha sıkı hale gelen çevresel deşarj yönetmeliklerine uymak için toksik amonyak konsantrasyonunun minimize edilmesi açısından önemlidir. Ayrıca, ileri arıtma teknolojileri ve sürdürülebilir uygulamalar üzerinde önemli etkileri vardır ve doğal ekosistemler üzerindeki antropojenik etkilerin en aza indirilmesini sağlamak için önemli bir yoldur.

Çalışmada kullanılan deneysel veriler Marmara Bölgesi'nde bulunan dört farklı ileri biyolojik atık su arıtma tesisiden alınan örneklere dayanmaktadır. Böylece yerel koşulların nitrifikasyon kinetiği üzerine etkilerinin önemini vurgulamak amaçlanmıştır. Bu atıksu arıtma tesisleri kapasiteleri popülasyon eşdeğeri atıksu olarak 1.6 milyondan 2.6 milyona kadar değişmekte olup, herbiri ileri biyolojik arıtma tesisleridir. Tesislerden günlük örnekler 8 günlük bir periyotta alınmış ve alınan örneklerle nitrifikasyon deneyleri gerçekleştirilmiştir. Anlamlı sonuçlar elde edebilmek adına numune alımları yaklaşık 7.5 saat arayla ve günde en az iki kere olacak şekilde gerçekleştirilmiştir. Deney sonuçları kaydedilerek Nitrijen Oksit (NO_X) artışlarını nitrifikasyon veriminin bir göstergesi olduğu düşünülmüştür. Tez çalışmasında kullanılan ilk veri girdisi olarak, ölçülen NO_X değerleri bu deneylerin sonuçlarından alınmıştır ve oluşturulmuş olan model ile birlikte çalışmanın yapı taşını oluşturmuştur.

Başlangıç koşullarının karmaşıklığını azaltmak ve odaklanılan parametreler arasındaki pratik tanımlanabilirliği ve belirsizliği iyileştirmek için, büyüme ve ölüm hızlarının sabit olduğu kabul edilmiştir. Ayrıca ototrofların ölüm hızı (b_A) için literatürde sıklıkla kullanılan 0.1, 0.15, 0.17 ve 0.2 değerleri esas alınmıştır. Çalışmanın sonuçlarına bu kabuller doğrultusunda ulaşılmış ve belirtilen her b_A değerleri için ayrı olarak hesaplanmıştır. Ayrıca detaylı gösterimleri sağlamak ve karşılaştırmaları gerçekleştirmek için b_A 0.15 değeri seçilmiş ve gerekli olduğunda ilgili hesaplamalar bu değer üzerinden yapılmıştır.

Tesislerin nitrifikasyon deney sonuçları, MS-Excel programı kullanılarak modellenmiştir. Tez çalışmasında girdi olarak kullanılan ikinci set veriler oluşturulmuş olan modellerin çıktılarıdır. MS-Excel'in eklentileri arasında yer alan Solver programı ile minimum hatayı veren $\hat{\mu}_A$ ve X_A değerleri her tesis için tespit edilmiş ve modellerin kalibrasyonları tespit edilen değerler ile en az hatayı verecek şekilde yapılmıştır. Solver'ın çalışma prensibi yine MS-Excel'in bir eklentisi olan Visual Basic for Applications (VBA) uygulaması üzerinden çalıştırılarak belirli sınırlarda, istenilen sıklıkla parametreleri değiştirerek değişen her noktadaki hesaplanan kareler toplamı hatalarını not alacak şekilde bir makro oluşturulmuş ve parametre tahminleri daha sonra çizilecek grafikler üzerinden net bir şekilde görülecek kadar detaylandırılarak büyük veri setleri elde edilmiştir. Oluşturulmuş olan veri setlerindeki tesis ve ototrofların ölüm hızları ile kategorize edilmiştir.

Sonuçlar arasındaki belirsizliğin incelenmesi ve daha kolay anlaşılabilmesi için verilerin görselleştirilmesi elzem kabul edilerek Sigma-Plot uygulaması üzerinden parametrelerin birbirlerine göre durumlarını ve bu durumlardaki hataları gösterecek grafikler çizdirilmiştir. Grafiklerde x ekseni X_A, y ekseni $\hat{\mu}_A$ ve yüksekliği belirten z ekseni olarak da kareler toplamı hataları yer alacak şekilde kontur grafikleri iki eksenli koordinat sistemi üzerine çizdirilmiştir.

Yaklaşık güven aralıkları literatürde de genel kabul görmüş bir değer olarak her tesis için %95 güven seviyesinde hesaplanmasına karar verilmiştir. Parametre sayıları ve veri sayıları göz önüne alınarak α:0.05 önem seviyesi için Fisher Tablosu kullanılarak belirlenen güven aralığındaki kritik değerler hesaplanmıştır. Hesaplanan kritik değerler göz önüne alınarak her grafik sadece çözüm alanını gösterecek şekilde kesilmiş ve rahatça gözlemlenebilir hale getirilmiştir. Elde edilen grafikler parametrelerin birbirlerine göre değişim sıklıkları azaltıralrak daha detaylı bir hale getirilmiştir ve gözlemlenen çözüm aralıklarının vurgulanması için sınırlar belirlenmiştir. Böylece oluşturulan nihai parametre tahminleri istenilen çözüm alanını daha detaylı gösterecek şekilde optimize edilmiştir.

Sonuç olarak her tesisin, belirlenmiş olan b_A değerleri göz önüne alınarak %95 güven aralığı ile anlamlı sonuç veren X_A ve $\hat{\mu}_A$ kombinasyonları; merkezde minimum hata olmakla birlikte, merkezden uzaklaştıkça eş-hata çizgileri oranında artarak yine de kabul edilebilir olan son değerlerine (S_{crit}) doğru artmakta olacak şekilde grafikler çizilmiştir. Yerel koşullar dikkate alıarak çizilmiş olan grafikler incelenerek deneyin başlangıç koşullarının nitrifikasyon kinetiğine etkisi ve parametreler arasındaki belirsizlikler yaklaşık güven aralıklarında gözlemlenen eğilimlere ve hata dağılımlarına dayanarak tartışılmıştır.

Yapılmış olan belirsizlik analizi neticesinde tesisler için hesaplanan nitrifiyerlerin maksimum büyüme hızındaki ve başlangıçta beslenen biyokütle oranları arasındaki belirsizlikler %5-15 mertebesinde, genel olarak %10'dan küçük olarak yüzde hata oranları değişse de kabul edilebilir sınırlar dahilinde kaldığı bulunmuştur. Tasarım kriterlerinde literatürde yer alan emniyet faktörü değerleri de genellikle %20 ile %25 arasında değişmekte ve bazı durumlarda %50 mertebesine kadar çıkabilmektedir. Bu hata toleransı da dikkate alındığında , sonuç olarak bulunan değerler tolere edilebilir olarak görülmüştür ve kabul edilebilir olarak değerlendirilmiştir. Ayrıca b_A değerleri göz önüne alındığında $\hat{\mu}_A$ değerlerindeki değişim ($\hat{\mu}_A - b_A$) değerinin beklendiği gibi sabit kaldığı görülmektedir. Bu ifadenin değişmemesi çamur yaşı hesaplarının doğru bir şekilde belirlenebileceğini ortaya koymaktadır. Ayrıca için literatürdeki iki uç değer bile karşılaştırılsa, $\hat{\mu}_A$ değerlerindeki paralel değişme beklenildiği gibi olmuştur.

Pratik uygulamalarda yapılan hesaplamaların üzerine tolerans payı eklenmesi ile teorik hesapların pratikte sorun teşkil etmemesi ve gelecek risklerin bertaraf edilmesinin sağlanması atık su arıtma tesisi tasarımı ve inşaatında çok yaygın bir yaklaşımdır. Eklenen bu torens payı da göz önüne alındığında bulunan konsantrasyonlar kabul edilebilir olarak değerlendirilebilir. Elde edilen sonuçlara bakılarak optimum nitrifikasyon deneyi tasarımına bir adım da daha yaklaşılmasına yardımcı olacağı görülmektedir. Bu nedenle, deneyin başlangıç koşullarından kaynaklanabilecek faktörlerin önemi vurgulanmış ve en uygun deney koşulları incelenmiştir.

Türkiye'deki nitrifiyerlerin büyüme hızlarının literatürde kullanılan dünya genelindeki standart değerlerden, yaklaşık yarısı kadar, daha düşük olduğu bulunmuştur. Bu farkın, arıtma tesislerine deşarj edilen atıksu kaynaklarına ve dolayısıyla atıksu karakterlerindeki farklılıklardan kaynaklandığı düşünülmektedir. Çalışmanın sonucunda, özellikle ototrofların büyüme hızlarındaki dramatik farklar yerel yaklaşımın önemini vurgulamıştır.

1. INTRODUCTION

In wastewater treatment plants (WWTPs), nitrification is a crucial process where ammonia is converted from oxidized to nitrate by the metabolic processes of nitrifying bacteria. The reduction of nitrogen compounds in effluent waters' negative environmental effects is largely dependent on this bioconversion process.

Nitrification kinetics are affected by several parameters; however, two key parameters influencing nitrification kinetics are found to be more outstanding among the others, which are the growth rate of nitrifiers and the initial active biomass due to their insights about the critical importance of the initial conditions. Understanding the uncertainties associated with these parameters is essential for optimizing nitrification processes and improving the reliability of wastewater treatment systems.

At the beginning of the nitrification experiment, the initial amount of active biomass supplied significantly influences nitrification kinetics and, consequently, the efficiency of wastewater treatment processes. Adequate initial biomass ensures the rapid growth of nitrifiers and facilitates the enzymatic activities that oxidize ammonia to nitrate at an optimal level. This condition affects the kinetics of biochemical reactions, enabling the process to proceed efficiently. The amount of biomass introduced initially also determines the diversity and density of the microbial population. Therefore, optimizing the initial biomass amount helps minimize process uncertainties and potential operational disruptions.

The maximum growth rate of nitrifiers represents the highest reproduction rate they can achieve under optimal conditions and is a fundamental dynamic of nitrification kinetics. This parameter directly affects how quickly the system can process ammonia, thus influencing the treatment capacity and efficiency. Nitrifiers with a high maximum growth rate enable faster nitrification processes, allowing for high-efficiency treatment with smaller bioreactor volumes and shorter hydraulic retention times. The maximum growth rate of nitrifiers varies with numerous environmental variables and wastewater characteristics, making it more accurate to consider local conditions rather than relying on general assumptions used in different geographical and societal contexts.

Ensuring optimal initial conditions allows biological reactions to occur in a more predictable and controlled manner, providing a significant advantage in process control and management. Proper adjustment of the initial biomass amount also contributes to reducing energy consumption and operational costs, as high efficiency can be achieved with lower energy inputs when the biomass is at an optimal level.

Design and optimization of wastewater treatment plant is strongly related to the nitrification kinetics. Nitrification directly affects the aerobic sludge age, which is one of the most critical parameters for volume calculations. allows for more precise prediction and control of these biochemical reaction rates, thereby enhancing the effectiveness of future nitrogen removal processes. Nitrification is essential for mitigating aquatic eutrophication and complying with increasingly stringent environmental discharge regulations by minimizing toxic ammonia concentrations. Additionally, it has significant implications for advanced treatment technologies and sustainable practices, contributing to minimizing anthropogenic impacts on natural ecosystems.

To understand a complex processes and optimise them, modeling becomes a remarkably useful tool, and with the right approach, model presicion can be improved. In the thesis, generated model is calibrated to get the best fit to the measured data and have enhanced insgiht. Properly calibrated models have the ability to predict system behavior and simulate various scenarios, which helps with decision-making and system planning. Furthermore, precise models help achieve higher treatment efficiencies while limiting energy consumption, chemical usage, and operational expenses. Thus, by conducting the uncertainity analysis, the model is enabled to assess more reliable predictions and improvised performance, while getting clearer sense of concepts such as optimum experimental design.

In environmental science, engineering, economics, and, among many other disciplines; identifyability, parameter estimation, and uncertainty analysis are crucial elements of modeling and data analysis. Such concepts are essential to improving model predictions, deepening the understanding of complex systems, and helping individuals make informed decisions.

The capacity to independently ascertain the values of model parameters from empirical data is known as identificability. Large numbers of parameters are frequently used in mathematical modeling to reflect underlying behaviors or characteristics of the system. Different combinations of parameters, however, may produce comparable model predictions because not all parameters may be recognized. Identifiability is a crucial component of trustworthy and understandable model construction.

Finding the values of model parameters that best suit observable data is known as parameter estimation. Usually, this procedure entails optimizing a selected objective function, like decreasing the discrepancy between experimental data and model predictions. For models to be calibrated to real-world data and to increase their prediction power, accurate parameter estimate is essential. Researchers can verify model assumptions, improve model structures, and learn more about the underlying mechanisms influencing system behavior by estimating parameters from observable data.

The goal of uncertainty analysis is to put a number on the level of uncertainty surrounding parameter estimations and model predictions. It entails determining the sources of uncertainty that could result from measurement mistakes, model assumptions, or intrinsic variability in the system, as well as evaluating how sensitive the model outputs are to changes in the input parameters. Decision-makers can evaluate the risks involved in various courses of action by using the useful information that uncertainty analysis offers regarding the robustness and reliability of model predictions. Researchers can improve their decision-making, pinpoint regions in need of more data collection or model improvement, and effectively express the constraints of their findings by quantifying uncertainty.

To achieve the objectives of this thesis, the parameter estimation process is conducted, followed by the calculation of contour regions representing a 95% confidence level. These contour plots, with the sum of square error (SSE) on the z-axis and the parameters on the x and y axis, were used to visualize the uncertainty and identify acceptable parameter ranges. The acceptable regions were shown to facilitate a clear understanding of the parameter interactions and their impact on model accuracy.

1.1 Purpose of Thesis

In many studies, the significance test of the data can be overlooked; however, in this study, uncertainty analysis between the maximum specific growth rate of nitrifiers and the initial active biomass fed at the beginning of the experiment is the main focus. This feature makes the study unique and forms the basis of this study and emphasizes the importance of the initial conditions of the nitrification experiment. In this respect, the thesis has the distinction of being one of the first researches carried out in the relevant field.

The purpose of this thesis is to perform an in-depth uncertainty analysis of the specific growth rate of nitrifiers and the initial active biomass, which are fundamental to the nitrification kinetics in WWTPs. This analysis will provide insights into how these uncertainties affect model predictions and, consequently, the performance of the treatment process. By utilizing local WWTP data to estimate parameters and validate the model, this study emphasizes the significance of local conditions in shaping nitrification dynamics. Also selected parameters help to highlight the importance of the initial conditions and give comprehensive idea of optimum experimental design.

1.2 Scope of Thesis

The scope of this thesis encompasses several key components. It begins with a literature review that provides an overview of nitrification processes, their significance in wastewater treatment, also explains the nitrification kinetics, and the key parameters affecting nitrification with their typical ranges. Also enviromental modeling and some model samples are given, its importance of parameter identifiability and uncertainty analysis are highligted and the concept of optimal experimental design is explained. Following this, the methodology section details the description of the local WWTPs, experimental setup and the initial conditions from which data were collected, the parameter estimation process, and the calculation and interpretation of contour regions for uncertainty analysis. The results and discussion section presents the optimum points that have minimum error values for each plant for the growth rate of nitrifiers and the initial active biomass, along with contour plots illustrating the acceptable parameter regions with respect to the sum of squared error. Moreover, this section interprets these results in the context of local WWTP conditions, examines the impact of parameter uncertainties on nitrification model predictions, and compares the findings with other studies to underscore the influence of local environmental factors. The conclusion summarizes the key findings, discusses the implications for the design and operation of nitrification processes in WWTPs, and offers recommendations for future research to further reduce uncertainties in nitrification kinetics.

2. LITERATURE REVIEW

2.1 Wastewater Treatment

Wastewater treatment is the process of eliminating pollutants from both municipal and industrial wastewater in order to generate a treated effluent that may either be released into the environment or reused. Treatments consist of a combination of physical, chemical, and biological methods aimed at eliminating organic matter, nutrients, pathogens, and other pollutants from water in order to prevent harm to the environment. From the most basic and primitive methods for waste management adopted by the ancient civilizations to the most sophisticated and advanced treatment systems in use today, a lot of development has taken place in the treatment of wastewaters over the years. The Indus Valley Civilization and the Romans adopted rudimentary techniques for waste disposal and water management in their respective eras. It therefore shows that during the 19th century, when the onset of the Industrial Revolution and rapid growth of urbanization and industrialization took place, some major developments concerning the wastewater treatment techniques began. In addition, techniques for primary treatment, such as sedimentation and screening, were initially adopted for water pollution control. It wasn't until the early 20th century that the main breakthrough, the activated sludge process, was discovered by Edward Ardern and W.T. Lockett in 1914. This was the application of aerobic microorganisms to decompose organic pollutants in wastewater that paved the way for the modernization of wastewater treatment; it is still being used to this day and is considered by many as one of the most significant breakthroughs in the advancement of the industry.

In these days, the process of treating both domestic and industrial wastewater prior to discharging it into natural ecosystems has gained significance in protecting water resources (Sözen S. et al., 2009). Discharge standards of countries are becoming more comprehensive and stringent.

2.2 Biological Nitrogen Removal

The biological process represents the more common method for treatment, and among others, the activated sludge process is the most widely applied procedure. Conceived initially to remove organic carbon and prevent deficiencies of oxygen in receiving waters, practical design of these systems was based on empirical notions until the 1970s.

Substantial progress has been achieved in the field of activated sludge modeling. It has been acknowledged that in addition to organic carbon, the regulation of nitrogen and phosphorus is also essential. Unregulated nitrogen inputs can result in detrimental consequences such as oxygen depletion, mass fish mortality, and eutrophication in the surrounding ecosystems. A substantial quantity of ammonia nitrogen undergoes oxidation to form nitrate. This process depletes the oxygen in the water, hence reducing the amount of oxygen accessible for aquatic life. Moreover, under normal circumstances, ammonia nitrogen has the ability to transform into free ammonia, which can be harmful to fish even when present in small amounts. Phytoplankton and aquatic plants depend on ammonia as a vital nutrient for their survival. If the amount is insufficient, it ultimately causes eutrophication in the water bodies. Regarding the research that was conducted on processes, the activated sludge system is considered the most effective biological approach for nitrogen removal from wastewater, and it is also one of the most cost-effective options.

The first step of biological nitrogen removal is nitrification, which occurs in aerobic environments and involves autotrophic species converting ammonia nitrogen into nitrate. In anoxic conditions, denitrification occurs and involves heterotrophic organisms converting nitrate nitrogen into nitrogen gas, which is the second step of nitrogen removal. Activated sludge systems and models have been created to effectively eliminate both carbon and nitrogen components by building upon the widely utilized system for these elements (Sözen, 1995).

Biological nitrification is the enzymatic conversion of ammonia to nitrite and ultimately to nitrate by microorganisms in an oxygen-rich environment. Autotrophic bacteria perform this process by utilizing inorganic carbon as their carbon source and inorganic nitrogen as their energy source. The objective is to convert ammonia nitrogen into nitrate nitrogen through the sequence stated below:

$$NH^{4+} - N \rightarrow NO^{2-} - N \rightarrow NO^{3-} - N \qquad (2.1)$$

Oxidation processes take place in a two-step process involving distinct types of autotrophic bacteria. There are a total of five recognized types of organisms capable of oxidizing ammonia to nitrite. The organisms mentioned are Nitrosomonas europaea, monocella, Nitrococcus, Nitrospira, Nitrosocystis, and Nitrosgloea. The second phase involves the oxidation of nitrite to nitrate, a process performed by Nitrobacter and Nitrocystis species (Sözen, 1995). Nitrosomonas is the predominant species in the initial stage, while Nitrobacter takes precedence in the subsequent stage (Painter, 1970).

2.3 Nitrification Kinetics

The growth of Nitrosomonas is typically the rate limiting stage in the process of nitrification. Assuming the dissolved oxygen is not a limiting factor, the growth rate of the nitrifiying bacteria can be stated as:

$$\mu_A = \frac{\widehat{\mu}_A N}{K_N + N} \qquad (2.2)$$

according to Monod kinetics. Where,

μ_A: specific growth rate of autotrophs [day^{-1}],

$\widehat{\mu}_A$: maximum specific growth rate of autotrophs [day^{-1}],

N : rate-limiting substrate concentration [mg/l],

K_N : half-saturation constant of the rate-limiting substratei [mg/1].

Nitrosomonas is primarily limited by ammonia nitrogen, while Nitrobacter is primarily limited by nitrite nitrogen. Research indicates that the pace at which Nitrobacter grows is higher compared to Nitrosomonas. Therefore, it is believed that the reaction in which Nitrosomonas converts ammonia nitrogen to nitrite nitrogen, known as the "oxidation of ammonia nitrogen to nitrite nitrogen" reaction, is the phase that determines the overall speed of the nitrification process. The Monod phrase can be used in the above case as:

$$\mu_A = \frac{\hat{\mu}_A S_{NH}}{K_{NH} + S_{NH}} \tag{2.3}$$

Where,

S_{NH}: ammonia concentration [mg/l],

K_{NH}: half-saturation constant of the ammonia concentration [mg/l].

Changes in the nitrification organisms can be expresses as below:

$$\frac{dX_A}{dt} = \mu_A X_A - b_A X_A = \frac{\hat{\mu}_A S_{NH}}{K_{NH} + S_{NH}} X_A - b_A X_A \tag{2.4}$$

Where,

X_A: biomass concentration of the autotrophs [mgVSS/l].

b_A: endogenous decay rate for autotrophs [mg/l].

The reaction stated below represents the endegenous respiration mechanism:

$$\frac{dX_A}{dt} = -b_A X_A \tag{2.5}$$

Also, that equation can be stated based on the assumption that substrate removal only happens during the proliferation process:

$$\frac{dS_{NH}}{dt} = -\frac{1}{Y_A}\frac{dX_A}{dt} = -\frac{1}{Y_A}\frac{\hat{\mu}_A S_{NH}}{K_{NH} + S_{NH}} X_A \tag{2.6}$$

Where,

Y_A: autotrophic yield coefficient [g cells synthesized per g NH4-N oxidized].

With the following equation:

$$q_A = \frac{\mu_A}{Y_A} = \frac{1}{Y_A}\frac{\hat{\mu}_A S_{NH}}{K_{NH} + S_{NH}} \tag{2.7}$$

where,

q_A: specific ammonium removal rate [g NH_3-N/g VSS·day],

this equation can be written:

$$\frac{dS_{NH}}{dt} = -q_A X_A \tag{2.8}$$

By considering the energy equations, it becomes apparent that the oxidation of 1 mole of ammonia nitrogen results in the production of 1 mole of nitrate nitrogen (Sözen, 1995). Thus,

$$\frac{dS_{NO}}{dt} = -\frac{dS_{NH}}{dt} \tag{2.9}$$

can present the relation.

Heterotrophic organisms are less responsive to environmental variables compared to autotrophic species (Sözen, 1995). The nitrification process is influenced by environmental conditions such as oxygen levels, pH levels, temperature, the presence of hazardous substances, and the C/N ratio. In the following sub-sections, some of the most important conditions are reviewed.

The variations in these conditions can lead to significant differences in observed nitrification rates, highlighting the importance of precise experimental design and parameter estimation.

2.3.1 Effects of oxygen levels

The process of nitrification is extremely responsive to changes in the concentration of dissolved oxygen. Research conducted in small-scale facilities with oxygen concentrations of 2, 4, and 8 mg/l highlights that the nitrification rate only reduces by 10% at low oxygen levels (Sözen, 1995). By maintaining a concentration of 2 mg/l, it becomes possible to efficiently operate large-scale facilities, resulting in substantial energy conservation (Painter, 1970). The rate of nitrification is influenced by the dissolved oxygen (DO) content in activated sludge. Unlike the findings regarding the breakdown of organic compounds by aerobic heterotrophic bacteria, the rates of nitrification reflect a rise until DO concentrations reach 3 to 4 mg/l (Metcalf & Eddy et al., 2014).

2.3.2 Effects of pH

Sözen (1995) states that, experimental investigations indicate that the ideal pH range for growth is between 7.5 and 8.6. There is no notable decline in the rate of nitrification until a pH of 9.5 is reached, but it ceases entirely below a pH of 6.3. According to the James & Vijayanandan (2023), the optimum pH range for conventional nitrification process is 8–8.4. Due to the reseach in simultaneous nitrification and denitrification, Wang et al. (2020) claims that the neutral pH around 7.5–8 was the optimum condition.

The lower threshold for pH has been determined to be between 6 and 6.5, while the optimal pH for growth is around 8. There are only minor variations in growth rate between pH 7.5 and 8.5. Unfortunately, due to the significant variability in the data obtained from several studies, it was not feasible to determine a factor that accurately represents the impact of pH on the growth rate constants of ammonia- and nitrite-oxidizing organisms (Painter & Loveless, 1983).

Metcalf & Eddy et al. (2014) states that, the most efficient rates of nitrification are observed when the pH levels are between 7.5 and 8.0. Ammonia oxidation rates decrease substantially when pH values drop below 7.0. The decrease in the rate of ammonia oxidation at lower pH may be attributed to the decline in the concentration of free ammonia (NH_3). Previous studies have suggested that NH_3-N may be the actual substrate for ammonia-oxidizing bacteria (AOB). At pH levels ranging from around 5.8 to 6.0, the rates of ammonia oxidation can be as low as 10 to 20 percent of the rate observed at pH 7.0.

2.3.3 Effects of temperature

In activated sludge systems operating below 25°C, the modeling of the process typically focuses on the AOB rather than the nitrite-oxidizing bacteria (NOB). This is because the NOB have a faster ability to utilize nitrite, resulting in little presence of nitrite-nitrogen (NO_2-N). Nevertheless, when the temperature exceeds 28°C or when the dissolved oxygen concentrations are low (below 0.50 mg/L), it is crucial to take into account the kinetics of both groups. This is because higher temperatures and low DO levels promote the growth of ammonia-oxidizing bacteria over nitrite-oxidizing bacteria, resulting in a lower concentration of NH4-N compared to NO2-N (Metcalf & Eddy et al., 2014). According to the James & Vijayanandan (2023), the optimum temperature for conventional nitrification is 22–27 °C.

Several research in the literature have investigated the correlation between maximal growth rate and temperature fluctuations, and it has been noted that the findings exhibit considerable variability. research indicate that the rate of proliferation between 7 and 30 °C follows the Arrhenius relationship. It is recommended that pilot-scale research be conducted outside this temperature range. Therefore, it is crucial to conduct repeated experimental studies for every type of wastewater and temperature being investigated in order to ensure accurate assessments (Sözen, 1995).

2.3.4 Effects of C/N ratio

The carbon-to-nitrogen ratio is a crucial factor that influences the rate of nitrification. The nitrification rate is the quantity of ammonia that is converted into nitrate nitrogen by a specific quantity of microorganisms within a specific time period. Only autotrophic organisms are responsible for this process, and the rates are reported based on total biomass because it is not feasible to assess autotrophic and heterotrophic organisms individually.

The quantity of autotrophs is determined based on the TKN/BOD (Total Kjeldahl Nitrogen to Biochemical Oxygen Demand over 5 days) ratio. Augmenting this ratio at any sludge age enhances the proportion of autotrophs and hence raises the nitrification rate. When nitrification rates are measured based on total biomass instead of active biomass, the results vary greatly. Hence, it is imperative to consider that the nitrification rate lacks significance as a criterion (Sözen, 1995).

In literature, threre are several values for $\hat{\mu}_A$, and b_A that are used to be able to properly study with various different wastewater chracteristics. Some of them are shown in the Table 2.1.

Table 2.1: $\hat{\mu}_A$, and b_A values in literature.

$\hat{\mu}_A$ 20 °C	15 °C	10 °C	b_A	Sources
0.8	-	0.3	0.15-0.2	Henze, Grady, et al., 1987
0.34-0.65	-	-	0.05-0.15	Henze, Grady L. Jr., et al., 1987
0.69	-	0.4	0.05-0.15	Antoniou et al., 1990
1	-	-	0.1	Gujer & Henze, 1991
0.5	0.44	0.14	-	Doğruel & Orhon, 2021
1	-	0.35	0.15	Henze et al., 1999
0.2-1	-	-	0.04	Barker & Dold, 1997
0.65	-	-	-	Henze et al., 1999
0.42	0.27	-	-	Sözen et al., 1996
1	-	0.35	0.17	Gujer et al., 1999
-	-	-	0.17	Melcer, 2003
-	-	-	0.15	Sözen S. et al., 2009
1	-	-	0.1	Katipoglu-Yazan et al., 2012
0.7	-	-	0.1	Katipoglu-Yazan et al., 2013
-	-	-	0.15, 0.17, 0.2	Metcalf & Eddy et al., 2014
0.65	-	-	0.12	Katipoglu-Yazan et al., 2015
0.5	0.36	-	-	İnsel et al., 2021
0.2-0.9	-	-	0.05-0.15	Öztürk et al., 2017
0.28-1.44	-	-	-	Sözen, 1995
0.768	-	-	-	Grady L. Jr. et al., 2011

2.3.5 Growth rate of autotrophs

The kinetic constants of nitrifying bacteria are being determined through the implementation of studies in both batch and continuous systems. These investigations contain both mixed cultures including autotrophs and heterotrophs, as well as pure cultures consisting solely of autotrophs. In a controlled environment with only one type of microorganism, the values for kinetic constants can be calculated by linearizing the Monod expression and directly measuring the rate at which the substrate is used. In heterogeneous cultures, due to the inability to quantify the nitrifying bacteria present in the community of organisms, it is not feasible to ascertain the precise rate of proliferation or the rate at which certain substrates are removed.

The growth rate of autotrophic organisms is the crucial parameter in the modeling and design of systems for nitrogen removal. Insufficient knowledge of this rate prevents adequate time for organisms to proliferate inside the system, resulting in their removal from the system. The measurement techniques established for this purpose focus on determining the quantity of autotrophic organisms in a mixed culture, as well as their net proliferation rates or particular substrate removal rates.

Despite the fact that only NO_2-N is produced in the initial stage, the presence of Nitrobacters immediately converts this product to NO_3-N. Given that the contribution of NO_3-N in the incoming stream may be ignored it can be inferred that the quantity of NO_2-N transformed into NO_3-N is also a result of the Nitrosomonas activity. Consequently, NO_3-N + NO_3-N measurements are made in the activity measures of this organism community, indicating the total oxidized nitrogen type, or NO_X-N. Activity studies provide results in the form of g NO_X-N/g biomass·time for Nitrosomonas activity, and g NO_3-N/g biomass·time for Nitrobacter. Growth rates in mixed cultures are typically expressed in terms of Nitrosomonas, which is a group of organisms that are limiting the maximum rate of growth.

From the equation below:

$$lnS_{NO} = ln\frac{1}{Y_A}\frac{\hat{\mu}_A}{\hat{\mu}_A - b_A}X_{A0} + (\hat{\mu}_A - b_A)t \qquad (2.10)$$

The slope of the line, derived by plotting the logarithm of the quantity of oxidized nitrogen (NO_X) against time, indicates the net growth rate of Nitrosomonas. The line seems to have a slope that is not affected by the initial concentration of active autotrophic biomass X_A. in real-life scenarios, there is a noticeable rise in NO_X levels in batch systems when using tiny amounts of inoculum from nitrifying systems, or in fill-discharge systems with low F/M (based on organic matter such as COD, BOD) ratios and a non-nitrifying inoculum sludge (Sözen, 1995).

Since that substrate production is directly proportional to the quantity of microorganisms, $\hat{\mu}_A$ can be determined by analyzing the graph representing the quantity of oxidized nitrogen generated over time in the system.

2.4 Environmental Modeling

The reasons for understanding biological kinetics are far more applied since the knowledge helps optimize designs and operations of wastewater treatment processes. It will not only impact reactor sizing but also aeration requirements and sludge handling strategies. Proper prediction of the nitrification rate will help engineers design systems that ensure both cost efficiency and meeting environmental regulations. By understanding the kinetics, process conditions can be optimized by engineers to reduce operational costs and increase overall system efficiency in wastewater treatment systems. A comprehension of this is also essential for improving and meeting rigorous discharge standards that are necessary to safeguard aquatic ecosystems from the overloading of nutrients, and this underscores the practical importance of such knowledge. (Henze, 2008)

In the optimization of the design and operation of activated sludge systems, modeling has dramatically enhanced the process. Activated Sludge Model No. 1 (ASM1) was developed in the 1980s and presents a detailed description of the biological system in activated sludge treatment, which includes carbon oxidation, nitrification, denitrification, and endogenous processes. The addition of phosphorus removal to that model created Activated Sludge Model 2 (ASM2). In Activated Sludge Model 2d (ASM2d), the modeling of denitrifying phosphorus-accumulating organisms (DPAOs) was included for systems seeking simultaneous nitrogen and phosphorus removal. The third model with more modifications is Activated Sludge Model 3 (ASM3), which includes the detailed representation of biological processes, especially hydrolysis of particulate organic matter, and adds storage of compounds. (Gujer et al., 1999; Henze, 1995; Henze et al., 1999; Henze, Grady, et al., 1987)

To simulate nitrification kinetics and investigate the model accuracy, there are many studies that uses traditional models their modified model as well. In senior studies, such as Marsili-Libelli, (1989), simplified SML model is introduced, and it was demonstrated to compare effectively with the well-known Monod kinetics, but it has the structural simplification and numerical consistency that make it more identifiable.

The model employed by Barker & Dold, (1997), which investigated respiration and growth are presumed to be constant, while decay rate is disregarded despite its significance. Nevertheless, this approach results in a reduction in the complexity of the initial conditions and model structure, which in turn facilitates the investigation of identifiability applications.

Moreover, some of the studies are focused into modification of existing models, since the representation of unique conditions would be improved. An example can be given as Zhao et al. (2013), that used a traditional model Qual2E as Model A and modified model as Model B. In summary, the validation results demonstrate that, when compared to the traditional Qual2E nitrification model, the nitrification model modified to incorporate bacterial growth functions based upon basic VSS measurements and nitrate-nitrogen assimilation was able to better predict the concentrations of ammonia, nitrate, and organic nitrogen constituents. Specifically, Model B's capacity to replicate the variations in NO_3-N and Org-N highlights the importance of bacterial growth functions (Zhao et al., 2013).

In the study, used model was generated from Monod Equations accordingly to the Melcer et al. (2003) to avoid over-parameterization and provide more identifiable structure to have proper uncertainty analysis between selected parameters.

2.5 Uncertainity Analysis

Uncertainty analysis is a systematic approach to quantify the uncertainty in model parameters and predictions. It is crucial for identifying the sources of variability and their impact on model outcomes. In the context of nitrification kinetics, uncertainty analysis helps examine how variations in parameters, such as the maximum growth rate of nitrifiers and the initial active biomass, affect the accuracy of parameter estimation and model predictions.

The relationship between the maximum growth rate of nitrifiers and the initial active biomass is a critical focus in nitrification studies. At the beginning of an experiment, the initial conditions can significantly influence the observed kinetics and the subsequent parameter estimation results. For instance, if the initial biomass concentration is too low, it may lead to underestimation of the maximum growth rate, while a high initial biomass concentration might result in overestimation. Uncertainty analysis allows researchers to systematically vary these initial conditions and assess their impact on the estimated parameters.

Several studies have employed uncertainty analysis to investigate nitrification kinetics. For example, a study by Moradkhani et al. (2005) applied Bayesian recursive estimation to assess the uncertainty in hydrologic model states and parameters. This approach enabled the researchers to incorporate prior knowledge and quantify the uncertainty in model predictions, which is particularly useful in dealing with complex environmental systems. Similarly, Renard et al. (2010) highlighted the challenge of understanding predictive uncertainty in hydrologic modeling and emphasized the importance of robust uncertainty analysis frameworks for accurate model predictions.

The necessity of a comprehensive uncertainty analysis framework in evaluating nitrification kinetics cannot be overstated. Such a framework enables systematically quantifying the impact of various sources of uncertainty on model predictions. This is crucial for optimizing the design and operation of urban wastewater treatment plants, as it helps ensure that the treatment processes are robust and reliable under varying conditions.

Key considerations for optimizing the design and operation of WWTPs through uncertainty analysis include the identification of key parameters. This involves determining which parameters have the most significant impact on model predictions and focusing efforts on accurately estimating these parameters. By pinpointing these crucial elements, the accuracy and reliability of the models can be significantly promoted.

2.6 Optimal Experimental Design

Parameter estimation is a crucial procedure in developing mathematical models that precisely depict real-world systems. The process entails ascertaining the values of unidentified parameters within a model by utilizing experimental or observational data. Precise parameter estimation is essential for the predictive efficacy and dependability of the model.

Simulations with different scenarios, it is possible to understand how variations in environmental conditions and operational parameters affect nitrification kinetics and overall WWTP performance. This analysis helps in designing robust systems capable of performing well under a range of conditions, ensuring consistent and reliable treatment outcomes. (Brun et al., 2001; Draper & Smith, 1998; Jacquez, 1991; Petersen et al., 2001)

Prior to determining the most suitable model structure and optimal parameter values based on experimental data, it is essential to first test the identifiability and distinguishability of nonlinear parametric model structures to ensure meaningful conclusions about significant parameters or state variables. These strategies can be applied even before data collection. If model structures are indistinguishable or not uniquely recognizable, additional tests or hypotheses can be designed to resolve structural ambiguities, known as qualitative experimentation design. Once structures are clearly identified and differentiated, specific experiments should be selected to optimize input shapes and measurement schedules, thereby providing the most pertinent information and achieving high precision in parameter estimation, a process referred to as designing experiments with quantitative data (Walter & Pronzato, 1996).

The process of designing an experiment for parameter estimation consists of two distinct parts. The first method is qualitative and involves choosing an appropriate arrangement of the input/output ports in order to identify, if feasible, all the relevant characteristics. The second step involves a quantitative approach that focuses on optimizing a suitable criterion, taking into account factors such as input shapes and sample schedule. The goal is to extract the maximum amount of information from the data that will be collected. When the parameters of the model are nonlinear, which is often the case with phenomenological models, both steps of the analysis provide unique challenges. (Walter & Pronzato, 1990).

Grady et al. (1996) emphasises variation even in the pure cultures in the literature regarding the values of the kinetic parameters and gives three factors seem to be particularly significant as: cultural history, parameter identifiability, and the methodology used to quantify the parameters.

Such as the maximnum growth rate of nitrifiers and initial active biomass concentration studied in the thesis. The mathematical relationship between two parameters is such that they are interdependent, it will be unattainable to produce separate estimates for them, regardless of the level of precision in conducting the test. Furthermore, the characteristics of the test significantly affect the capacity to determine specific parameters. (L. Grady et al., 1996).

A method for optimal experimental design in parameter estimation for unstructured growth models is examined by Baltes et al. (1994). This method used for fed-batch processes, underscores the significance of model validity, as unstructured growth models are frequently rendered invalid under transitory conditions. As a result, a composite objective function has been implemented to evaluate the accuracy of the estimated kinetic parameters and the validity of the model. The results obtained from the application of this method to fed-batch processes have been satisfactory. Various fed-batch strategies are examined in terms of the quality of parameter estimation and the validity of the model. Furthermore, yeast fermentations were implemented to execute experimental verification.

Optimal experimental design is crucial for improving the accuracy and efficiency of model-based studies, particularly in fields such as engineering and biochemistry. A state-space model, which is linear and time-invariant, is a common choice for these applications. Duarte et al., (2021) highlight the systematic approach to optimal experimental design for this model category. Their method involves generating locally D-optimal designs by integrating the calculation of the Fisher Information Matrix's determinant and parametric sensitivity computation into a Nonlinear Programming formulation. A global optimization solver addresses the numerical problem that arises, ensuring that the model is identifiable through the Fisher Information Matrix when the model converges.

A recent study from Gutiérrez et al. (2024), investigated the effects of varying sludge age and temperature on nitrification performance using optimal experimental design. By systematically altering these parameters, they developed a robust model that accurately predicts nitrification under different operational scenarios. This study underscores the importance of considering environmental and operational factors in the design of nitrification experiments to ensure the reliability and applicability of the model predictions.

In the literature, similar studies are carried on by focusing on OUR batch tests. For example, Insel et al. (2003) investigated the kinetics of hydrolysis in surface-saturation conditions using respirometric measurements. It considered the identifiability of mathematical models both in theory and in practice. The identifiable parameters of a selected model were obtained from respirograms. In addition, the data obtained from the experiments was analyzed using OED approach, considering several initial circumstances of the batch respirometric experiment.

Another study by Hidalgo & Ayesa, (2001) et al. presents a mathematical tool for analyzing local identifiability in high-order state-space nonlinear systems. This tool may be simply applied and implemented in simulators using a discrete-time method. The concept relies on recursively evaluating a reduced information matrix while simulating a calibration experiment and establishing a set of information parameters based on geometric interpretations of this matrix. For instance, the suggested methodology has been applied to analyze an OUR batch test in terms of ASM No. 1 calibration.

Also, one of the key studies that was taken as an example for this study is Insel (2005). Similarly to Insel (2005), this study based on the empirical data collected from laboratory-scale setups, and the dependability of the produced solutions was evaluated by employing uncertainity analysis. The examination of the impact of starting experimental conditions on the estimate of model parameters was done to demonstrate the experiment's information content. However, the thesis focuses on nitrification kinetics by the means of maximum specific growth rate and initial active biomass concentration. Thus makes the study unique in the way that execution of the uncertainity analysis into these parameters with the OED perspective.

These approaches demonstrate the significance of optimal experimental design in enhancing the accuracy and reliability of environmental models. By considering OED concept and uncertainity analysis; parameter estimation, model identifiability, and validity can be improved. Therefore, by considering local conditions, the estimation of the kinetic constants, which are crucial for understanding and predicting the behavior of biochemical systems, can be investigated more precisely.

3. MATERIAL & METHODS

The experimental procedures and model creation are carried out accordinly to (Melcer et al., 2003). The study based on data from the nitrification experiment's results as measured NO_X, and modeled data that is generated by the model.

In the sections 3.1 and 3.2 related information about the used data is given. Starting from the section 3.3, following sections that constitute the main subjects of the thesis begin.

3.1 Wastewater Treatment Plants

Sampling for the nitrification experiments were carried on four different advanced biological treatment plants. They are all located in Marmara Region Türkiye, and have comperabily higher treatment capacities from the other treatment plants in the region. They have capacities ranging between 1.6 million to 2.6 million population equivalent wastewater.

Brief information about the WWTPs are stated below:

Plant 1 has a capacity of 400,000 m^3; It treats the wastewater of approximately 1,600,000 people in With the facility, 54,750 tons of sludge is prevented from being dumped into the sea and 146,000,000 m^3 of wastewater is purified annually.

Plant 2 has advanced biological treatment capacity of 600,000 m^3/day m^3/day. It serves to the population of 2,400,000 people. In principle, the facility has an advanced biological process with Nitrogen - Phosphorus removal.Plant 3

Plant 3 has a daily treatment capacity of 200,000 m^3; there is also an MBR Recovery Unit that carries out advanced treatment with a capacity of 20,000 m^3/day. At its final capacity, the treatment plant, together with other facilities in the planning stage, will treat wastewater from a population of 800,000 people with a flow rate of 500,000 m^3/day.

Plant 4 is constructed with the capacity is 150,000 m^3/day as the 1st and was put into service in 1998, the capacity of the 2nd stage is 100,000 m^3/day in 2009, and the capacity of the 3rd stage is 400,000 m³/day and was put into service in 2022. The facility, whose total capacity reaches 650,000 m³/day, treats wastewater from a population equivalent to approximately 2.6 million.

3.2 Experimental Design and Modeling Approach

Experimetal setup that is used each and every nitrification experiment shown as below as Figure 3.1.

Figure 3.1 : Experimental setup.

Initial conditions of the nitrification experiment are given in the Table 3.2 below.

Table 3.1: Initial conditions of the nitrification experiments for each WWTPs.

	Plant 1	Plant 2	Plant 3	Plant 4
MLSS	8870	9540	7030	5180
pH	7.2	7.2	7.2	7.2
DO	8.6	8.6	8.6	8.6
Volume	4	4	4	4
NO_2-N +NO_3-N	5	1	2.6	2.6
NH_4-N	0.3	0.3	0.3	0.3
Added NH4	70 mg/L	70 mg/L	70 mg/L	70 mg/L
Added Biomass	70 mL	60 mL	70 mL	100 ml
Added Alkalinity	3.363gr $NaHCO_3$	3.363gr $NaHCO_3$	3.363gr $NaHCO_3$	3.363gr $NaHCO_3$

In seven-eight days period several samples were taken from the WWTPs and after the experiment measured NO_X concentrations are noted. As mentioned in the equation 2.10, nitrification rates can be measured by observing the NO_X concentrations.

After the experiment's results are noted and used as inputs, a simple model is created accordingly to the Melcer et al. (2003) to mimic the process. Then, with the model results, sum of squared error (SSE) is calculated in each point. As an example, Plant 3's measured and model data is shown in the Table 3.2 with the sum of the squared errors.

Table 3.2: Data used for the Plant 3 with SSEs.

Time Days	NO_X measured mgN/L	NO_X model mgN/L	SSE
0.00	5.3	5.00	0.1
0.69	7	7.75	0.57
1.00	9	9.15	0.02
1.69	11.6	12.56	0.92
2.00	13.8	14.29	0.24
2.69	17.8	18.52	0.52
3.00	19.8	20.66	0.74
3.69	25	25.91	0.83
4.00	28.7	28.57	0.02
4.69	35.5	35.08	0.18
5.00	39.5	38.37	1.27
5.69	47.1	46.45	0.43
6.00	52.2	50.53	2.78
6.69	60.4	60.54	0.02
7.00	64.1	65.61	2.28

From the literature, many endogenous decay rate coefficients are found for autotrophs, and some of the b_A values that are used in the environmental models shown in the Table 3.3.

Table 3.3: Endogenous decay rate values in several models.

Models	b_A value	Temperature °C	Source
ASM1 Activated Sludge Model No. 1	0.15 - 0.20	20	Henze, Grady, et al., 1987
ASM2d Activated Sludge Model No. 2d	0.15	20	Henze et al., 1999
ASM3 Activated Sludge Model No. 3	0.17	20	Gujer et al., 1999
IWA STR Model	0.2	20	Orhon et al., 1999
Methods for wastewater characterization in activated sludge modeling	0.17	20	Melcer, 2003
Model based process optimization of enhanced wastewater treatment plants	0.15	20	Sözen S. et al., 2009

After considering the values; b_A values are selected as 0.1, 0.15, 0.17, and 0.2 to be able to give an extended point of view. These values are used in the following sections.

3.3 Parameter Estimation

Daily samples taken from each facility were examined under the same conditions and nitrogen concentrations were measured. To perform parameter estimation, the sum of squared error (SSE) method was chosen as the appropriate method considering the current conditions and the measured data and model generated data were compared. Although automatic methods for model calibration have become widespread (Wagener et al., 2003), manual calibration for the created model has been deemed sufficient due to the simplicity of the model and curve-fitting method is used.

To calibrate the model, Solver Add-in is used to determine at which concentrations of $\hat{\mu}_A$ and X_A results with the minimum error. Firstly, from Excel Options, “Add-ins” is selected and Solver Add-in should be enabled. After that, from “Developer” section, Solver should appear in the list of “Excel Add-ins”. By doing that Solver can be found under the “Data” section. In Solver, objective function is selected as sum of squared error cell to minimum, by changing variable cells $\hat{\mu}_A$ and X_A. After running the Solver final values of $\hat{\mu}_A$ and X_A are set to the model, so model calibration is done as achieving the minimum possible error.

At that point, the Solver's working principle is found to be suitable to generate a macro to perform larger volume of parameter estimations, which enables to higher resolution in the result graphs. Its default principle includes series of trials by using variable cells within the fixed constraint precision to reach the objective function's determined destination. With the help of the Visual Basic for Applications (VBA) in Microsoft Excel (MS-Excel), working principle of the Solver can be integrated into a macro and the parameter estimation process is automatized. In VBA, the Solver can be modified to set ranges and step size to each variable and note every single result as objective function which is selected as sum of squared error cell. Thus, each and every combination between variables can be seen and a three-dimensional graph can be drawn by using the parameter estimation.

VBA is a tool in Visual Studio for Excel, it needs to be enabled in the Excel Options same as the Solver, and after that it can be found under the "Developer" section. To integrate Solver to the VBA, "References" should be opened, which is under the "Tools" section in VBA. Figure 3.2 can be seen.

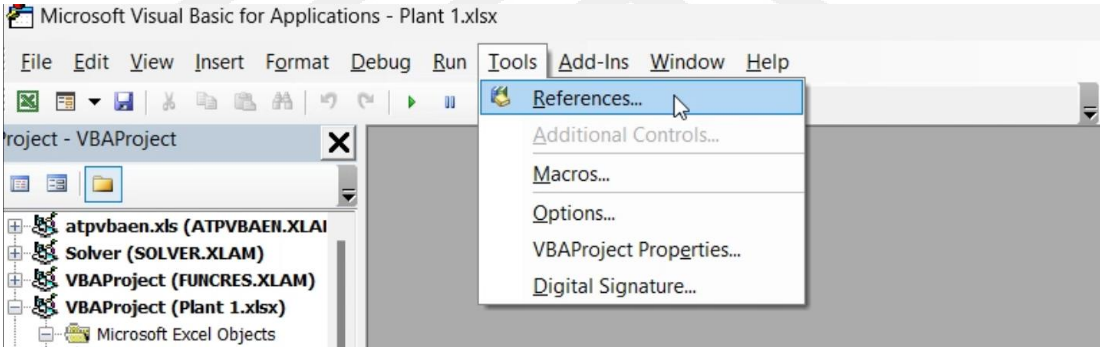

Figure 3.2 : Demonstration the location of references in VBA.

In the "References", the Solver should be enabled as shown in the Figure 3.3 below.

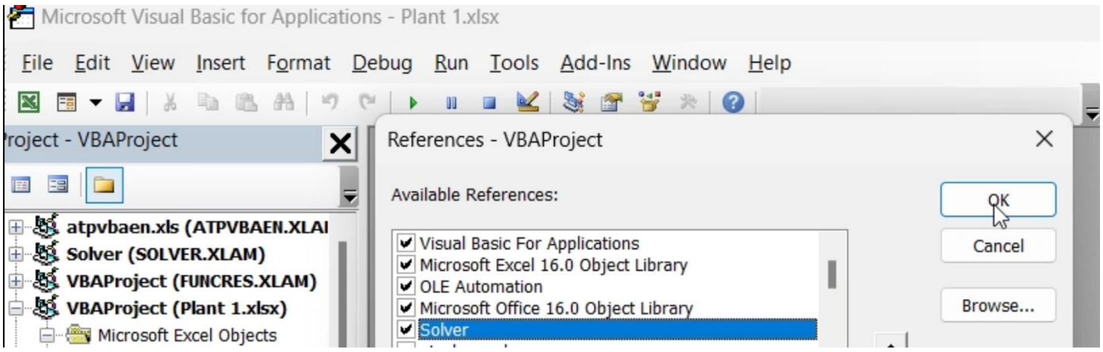

Figure 3.3 : Demonstration of enabling the Solver in VBA.

After that from “Insert” section, a new module should be added. Then, macro is generated. The objective function is selected as the sum of squared error and parameters are set to change in determined step size and range, and examination of the parameters in certain ranges are proceed relative to each other and the outputs can be seen together for each value calculated sequentially. The sum of squared errors given by the changing values for each step were are noted next to the relevant parameter values. After a series of trials, the parameter ranges and step size were determined.

As an example, generated macro for Plant 1 is given in the Figure 3.4 below with the necessary explanations:

```
Sub RunSolverWithRecording()
    Dim ws As Worksheet
    Dim var1Cell As Range, var2Cell As Range
    Dim var1Value As Double, var2Value As Double
    Dim rowNum As Long ' Change from Integer to Long
    Application.ScreenUpdating = False ' To increase the process speed
    ' Set your worksheet
    Set ws = ThisWorkbook.Sheets("Plant1") ' Change "TheSheetName" to the actual sheet name

    ' Set ranges for variables
    Set var1Cell = ws.Range("K2") ' Change "K2" to the first variable cell
    Set var2Cell = ws.Range("K4") ' Change "K4" to the second variable cell

    ' Set ranges for recording variables and results
    Dim var1RecordCell As Range, var2RecordCell As Range, objValueCell As Range, objRecordCell As
Range
    Set var1RecordCell = ws.Range("C40") ' Change "C40" to the first variable record cell
    Set var2RecordCell = ws.Range("D40") ' Change "D40" to the second variable record cell
    Set objValueCell = ws.Range("G22") ' Change "G26" to the specific objective value cell
    Set objRecordCell = ws.Range("E40") ' Change "E40" to the objective value record cell

    ' Set step size for variables
    Dim stepSize1 As Double, stepSize2 As Double
    stepSize1 = 0.0004 ' Change the step size for the first variable
    stepSize2 = 0.005 ' Change the step size for the second variable
    rowNum = 0 ' Start recording in row 40

    ' Loop through variable ranges and perform calculations
    For var1Value = 0.33 To 0.4 Step stepSize1
        For var2Value = 1.96 To 3.04 Step stepSize2
            ' Set variable values
            var1Cell.Value = var1Value
            var2Cell.Value = var2Value

            ' Record variables and objective value in specified columns
            var1RecordCell.Offset(rowNum, 0).Value = var1Value
            var2RecordCell.Offset(rowNum, 0).Value = var2Value
            objRecordCell.Offset(rowNum, 0).Value = objValueCell.Value ' Record the objective value
            rowNum = rowNum + 1
        Next var2Value
    Next var1Value
End Sub
```

Figure 3.4: Generated macro for the parameter estimation for Plan1 at b_A=0.15.

Firstly, the variables are introduced. “ws” is the worksheet object, “var1Cell” and “var2Cell” are the cells where the variables will be set, “var1Value” and “var2Value” are the values for those variables, and “rowNum” is used to keep track of the current row for recording the results. After that, according to the aimed data’s properties, the most suitable definition should be selected. For instance, if all of the data consists of integers and the volume of the dataset considerably small (number of iterations are low) “integer” can be selected, because it needs lesser memory usage. However, the data is limited and in longer datasets it may cause “overflow” error. As in the study’s case, since the dataset is planned to be in high volumes (number of iterations are large), “long” is selected to avoid errors. Then, screen updating is turned off to increase the macro execution speed. If it is not turned of, each and every change in the parameters and objective function can be seen via related excel sheet. Selecing worksheet is done by “ws” command and “Plant1” is selected. The worksheet should be entered based on the exact spelling. To proceed, variable cells are introduced due to their location in the related worksheet. Until now inputs are mainly defined, so outputs need to be set. After the entering the record cells, macro is now able to note the outcomes beginning with the introduced cells, and along with the step size and ranges it will continue to record the results. Thus, after defining the record cells, step size and ranges are selected. Here, considering the basics of parameter estimation and the confidence regions (see eq. 3.1), several trials are executed to find the most suitable ranges and step sizes that gives the proper resolution in the contour plots. Smaller volumes of data is preferred in this step, so the visualization and optimization processes can be done conveniently. After obvering the proper ranges, step size can be lowered to have more detailed results and final parameter estimates can be generated.

In conclusion, this nested loop iterates across the ranges of “var1Value” and “var2Value” using the supplied step sizes. For each combination of values; the variable values are assigned in the “var1Cell” and “var2Cell”, the values and the output from “objValueCell” are recorded in the designated columns, and the value of the row counter “rowNum” is increased.

Final data ranges and quantities are shown in the Tables 3.4 and 3.5.

Table 3.4: Ranges of parameters and approximate data quantitities generated for Plant 1 and Plant 2.

	Plant 1					Plant 2			
Graph Limits	bA 0.1	bA 0.15	bA 0.17	bA 0.2	Graph Limits	bA 0.1	bA 0.15	bA 0.17	bA 0.2
X_A	2.26	1.98	1.88	1.76	X_A	0.158	0.137	0.13	0.12
	3.55	3.01	2.84	2.62		0.333	0.284	0.268	0.247
$\hat{\mu}_A$	0.282	0.332	0.352	0.382	$\hat{\mu}_A$	0.284	0.334	0.354	0.384
	0.348	0.398	0.418	0.448		0.326	0.376	0.396	0.426
Data	50k	38k	35k	29k	Data	53k	42k	49k	55k

Table 3.5: Ranges of parameters and approximate data quantitities generated for Plant 3 and Plant 4.

	Plant 3					Plant 4			
Graph Limits	bA 0.1	bA 0.15	bA 0.17	bA 0.2	Graph Limits	bA 0.1	bA 0.15	bA 0.17	bA 0.2
X_A	1.58	1.4	1.338	1.25	X_A	1.28	1.12	1.06	0.99
	2.5	2.16	2.04	1.892		2.22	1.87	1.76	1.62
$\hat{\mu}_A$	0.312	0.362	0.382	0.412	$\hat{\mu}_A$	0.27	0.32	0.34	0.37
	0.376	0.426	0.446	0.476		0.343	0.393	0.413	0.443
Data	30k	30k	28k	35k	Data	69k	88k	94k	74k

3.4 Confidence Contours

To visualize the results, contour plot graphs were created using the "Sigma Plot" application. Critical values were obtained according to the Draper & Smith (1998) at α=0.05 significance level. From the following equation:

$$S_c = S_{min}[1 + \left(\frac{p}{n-p}\right) F_{p,n-p}] \tag{3.1}$$

Where,

S_c: is the critical value of SSE at the boundry of the confidence contour,

S_{min}: is the minimum value of the SSE,

p: number of parameters,

n: number of observations,

$F_{p,n-p}$: is the critical value form the Fisher's Table (for α:0.05)

Confidence regions are calculated for each WWTP. Related calculations can be seen below:

For Plant 1;

S_{min}: 10.9057,

p: 2,

n: 15

$F_{2,12}$: 3.885 [at α: 0.05]

$$S_c = 10.9057 \quad [1 + \left(\frac{2}{15-2}\right) 3.885]$$

$$S_c = 17.424$$

For Plant 2;

S_{min}: 168.0869,

p: 2,

n: 28

$F_{2,25}$: 3.39 [at α: 0.05]

$$S_c = 168.0869 \quad [1 + \left(\frac{2}{28-2}\right) 3.39]$$

$$S_c = 211.919$$

For Plant 3;

S_{min}: 16.9487

p: 2,

n: 16,

$F_{2,13}$: 3.806 [at α: 0.05]

$$S_c = 16.9487 \quad [1 + \left(\frac{2}{16-2}\right) 3.806]$$

$$S_c = 26.164$$

For Plant 4;

S_{min}: 12.0820

p: 2,

n: 17,

$F_{2,14}$: 3.682 [at α: 0.05]

$$S_c = 12.0820 \quad [1 + \left(\frac{2}{17-2}\right) 3.682]$$

$$S_c = 18.014$$

Since the effect of changes in the b_A values on the minimum SSE values is remarkably minor, S_{min} of the plants are calculated by assuming b_A= 0.1 and further calculations for 0.15, 0.17 and 0.2 are neglected. For example, at b_A= 0.1, S_{min} is calculated as 12.0820512, which is 12.0820505 at b_A= 0.2. Thus, difference between S_{min} values is considered as negligible.

The calculated critical values were used to cut the error level-based graphs, and as a result, solution areas for 95% confidence intervals were obtained. These procedures done for each WWTP with determined b_A values.

4. RESULTS & DISCUSSION

Contour plots are generated by using sum squared errors of $\hat{\mu}_A$ and X_A and illustrated in the two dimentional-coordinate system. SSE is at lowest in the center of the contours and gradually increases.

Center of the graphs shows higher accuracy estimations; since the contour plots are limited by setting the error range S_{min} to S_{crit} to show only the intervals higher than the acceptable error margin as the confidence region. In this way, all of the drawn parts of the graphs illustrates the accurate estimates with 95% confidence level. However, in order to have the most optimum values that could be obtained the middle of the inner circles should be observed where the SSE is at its lowest, S_{min}.

In the Tables 4.1 and 4.2, the optimum $\hat{\mu}_A$ and X_A values of the WWTPs are shown at the minimum SSE points with respect to their b_A.

Table 4.1: $\hat{\mu}_A$ and X_A values at minimum SEEs for Plant 1 and Plant 2.

Plant 1				Plant 2			
Min Error Points			Min Error	Min Error Points			Min Error
b 0.1	$\hat{\mu}_A$	0.3152	10.9057	bA 0.1	$\hat{\mu}_A$	0.3045	168.0869
	X_A	2.8291			X_A	0.2334	
b 0.15	$\hat{\mu}_A$	0.3652	10.9057	bA 0.15	$\hat{\mu}_A$	0.3545	168.0869
	X_A	2.4417			X_A	0.2004	
b 0.17	$\hat{\mu}_A$	0.3852	10.9057	bA 0.17	$\hat{\mu}_A$	0.3745	168.0869
	X_A	2.3150			X_A	0.1897	
b.0.2	$\hat{\mu}_A$	0.4152	10.9057	bA.0.2	$\hat{\mu}_A$	0.4045	168.0869
	X_A	2.1477			X_A	0.1756	

Table 4.2: $\hat{\mu}_A$ and X_A values at minimum SEEs for Plant 3 and Plant 4.

Plant 3				Plant 4			
Min Error Points			Min Error	Min Error Points			Min Error
bA 0.1	$\hat{\mu}_A$	0.3445	16.9487	bA 0.1	$\hat{\mu}_A$	0.3068	12.0821
	X_A	1.9876			X_A	1.6843	
bA 0.15	$\hat{\mu}_A$	0.3945	16.9487	bA 0.15	$\hat{\mu}_A$	0.3568	12.0821
	X_A	1.7356			X_A	1.4482	
bA 0.17	$\hat{\mu}_A$	0.4145	16.9487	bA 0.17	$\hat{\mu}_A$	0.3768	12.0821
	X_A	1.6520			X_A	1.3714	
bA.0.2	$\hat{\mu}_A$	0.4445	16.9487	bA.0.2	$\hat{\mu}_A$	0.4068	12.0821
	X_A	1.5404			X_A	1.2702	

On the following pages, contour plots are presented. At first, in the Figures 4.1, 4.2, 4.3, .4., 4.5, 4.6, 4.7, and 4.8 are given in order according to plant numbers and b_A values. In this part, plots are shown separately to provide more detailed visualization. Thus, the changes in the shapes and trends of the contour plots can be seen more efficiently. After that, in the Figures 4.9, 4.10, 4.11, and 4.12, plots are placed in the same Plant-wised two dimensioned-coordinate systems to see the effects of variations in b_A values. Furthermore, it has become more convenient to detect alterations in the entire contours.

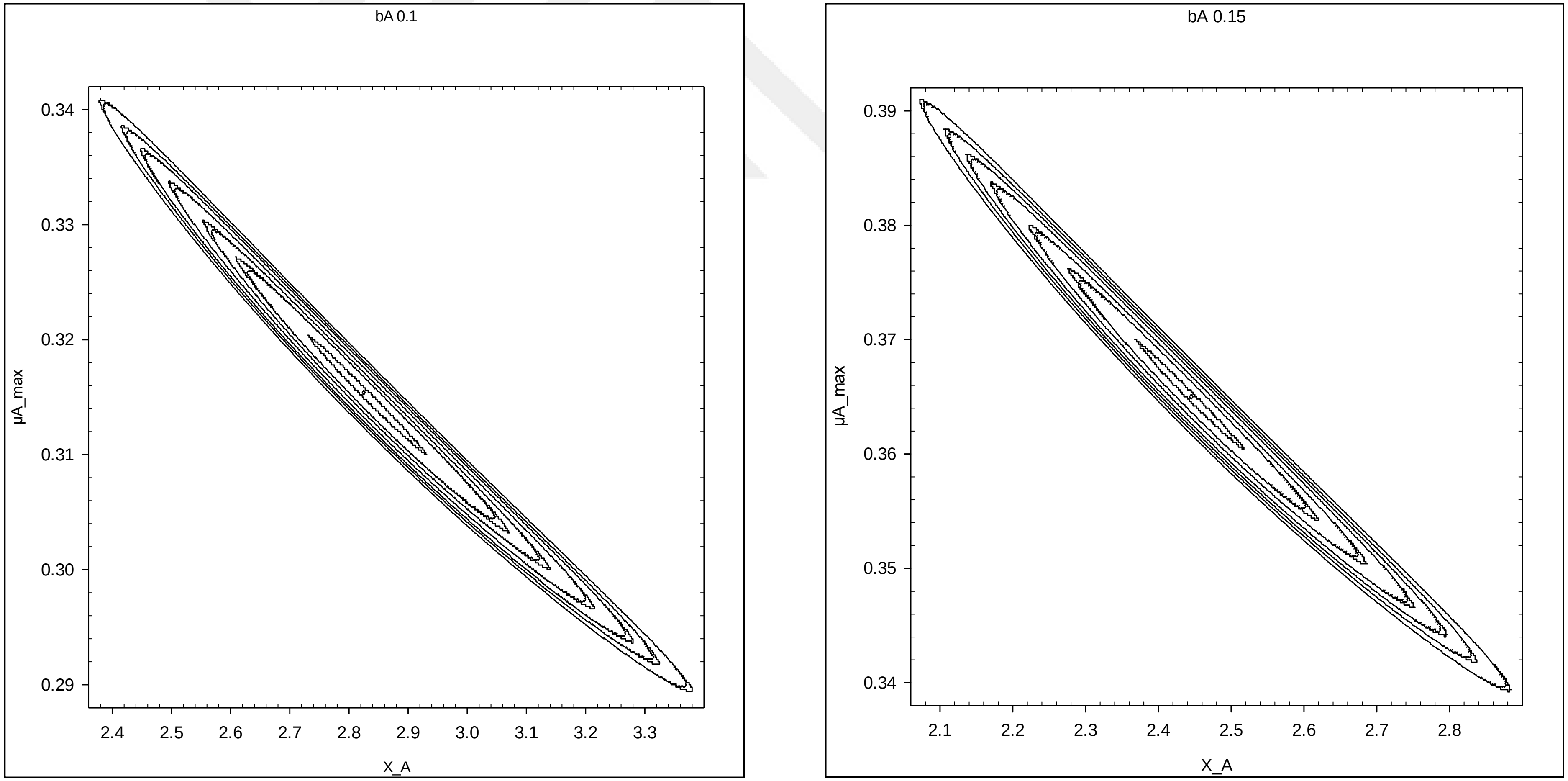

Figure 4.1: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 1 are shown; where the b_A: 0.1 and 0.15 (only acceptable region is shown as the confidence region, S_{min}-S_c).

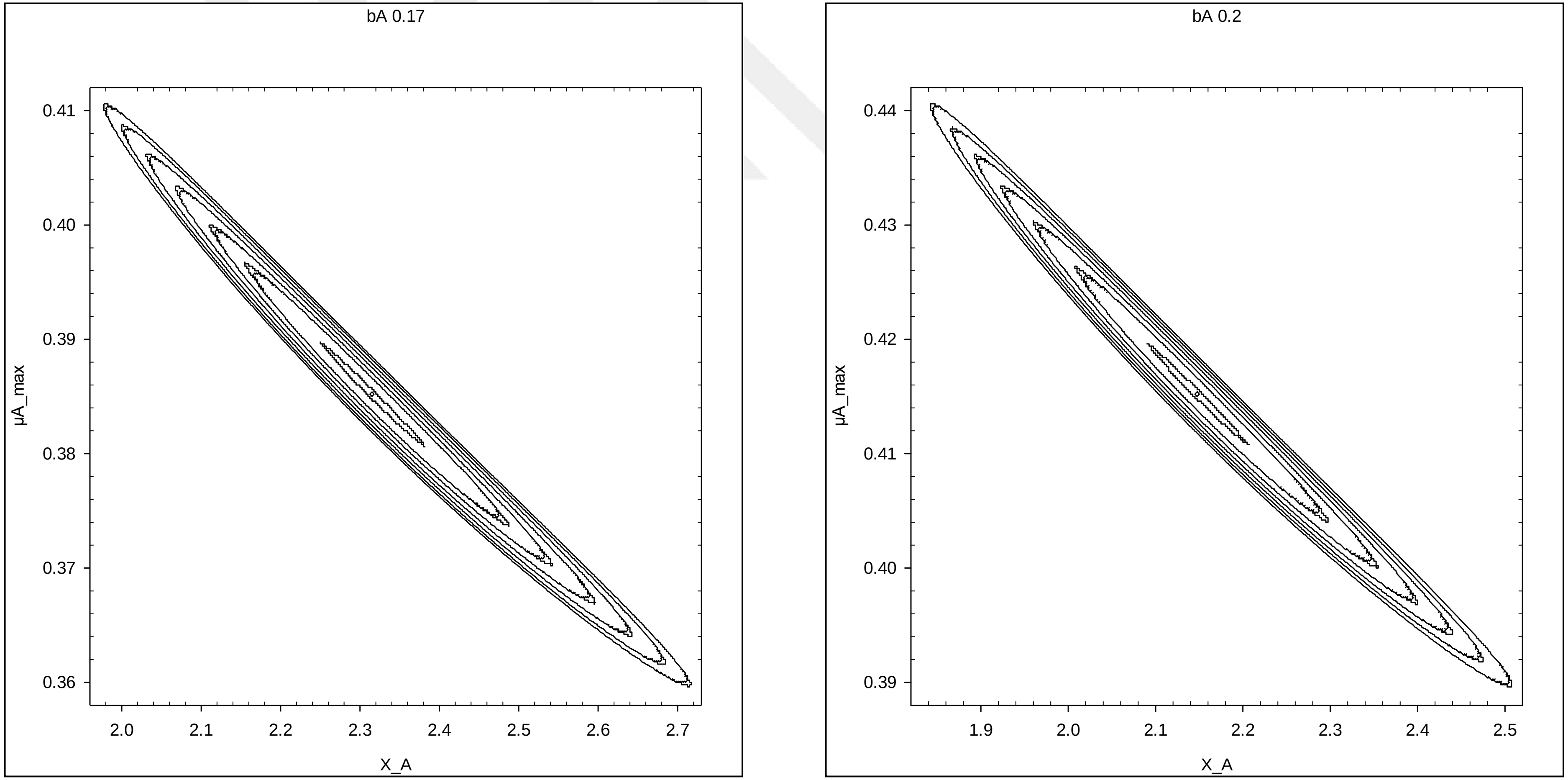

Figure 4.2: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 1 are shown; where the b_A: 0.17 and 0.2 (only acceptable region is shown as the confidence region, S_{min}-S_c).

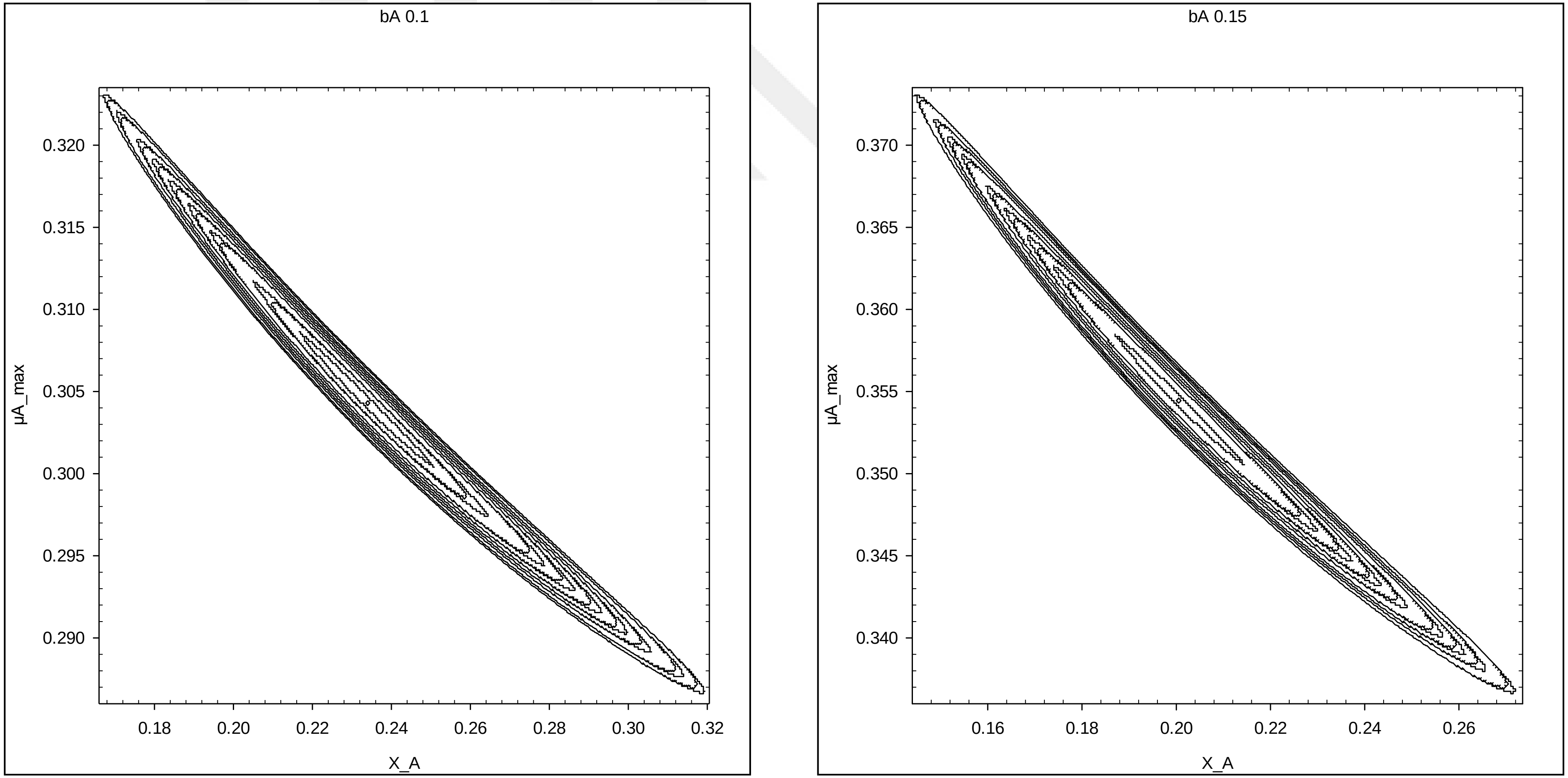

Figure 4.3: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 2 are shown; where the b_A: 0.1 and 0.15 (only acceptable region is shown as the confidence region, S_{min}-S_c).

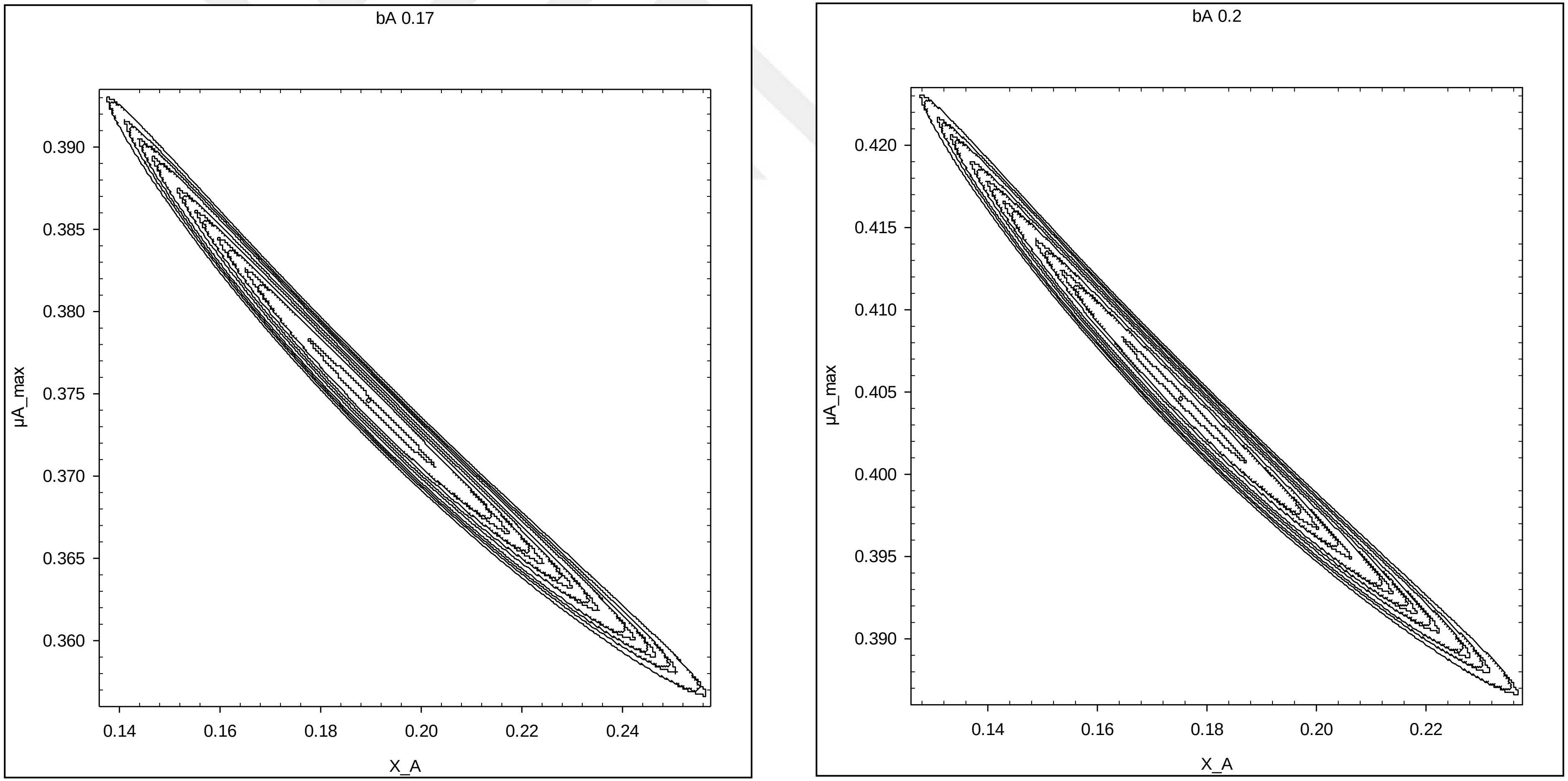

Figure 4.4: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 2 are shown; where the b_A: 0.17 and 0.2 (only acceptable region is shown as the confidence region, S_{min}-S_c).

In Figures 4.1 and 4.2, contour plots for Plant 1 can be seen. Although the data ranges that are used to plot the graph varies from 60 000 to 120 000, graphs resolution seems similar and can be considered as smooth enough to show the ellipsoid shapes of the contour plots. The direct impact of bA values on maximum specific growth rate can be seen easily between the plots. Also, X_A values changing similarly in the opposite direction. Despite the fact that confidence region covers all of the area of solution in 95% probability, the center (minimum error points) is not symetrical. As the observed minumum SSE area closer to the top-left of the graphs, it indicates for the optimal design perspective slightly higher $\hat{\mu}_A$ values and slightly lower X_A concentrations may result with lower errors in the confidence region.

In Figure 4.3 and 4.4, contour plots for Plant 2 are are shown. Among the advanced wastewater treatment plants that are examined in the study, Plant 2 has the highest measurement number. It was expected to see lower SSE errors and wider confidence region; however, Plant 2 has the maximum SSE. It may be caused by the quality of the data. Also, another reason can be the initial active biomass concentration, which is quite low comparing to the other WWTPs. Thus, as can be seen in the Figure 4.17, increase in the NO_X concentration is slower than the others.

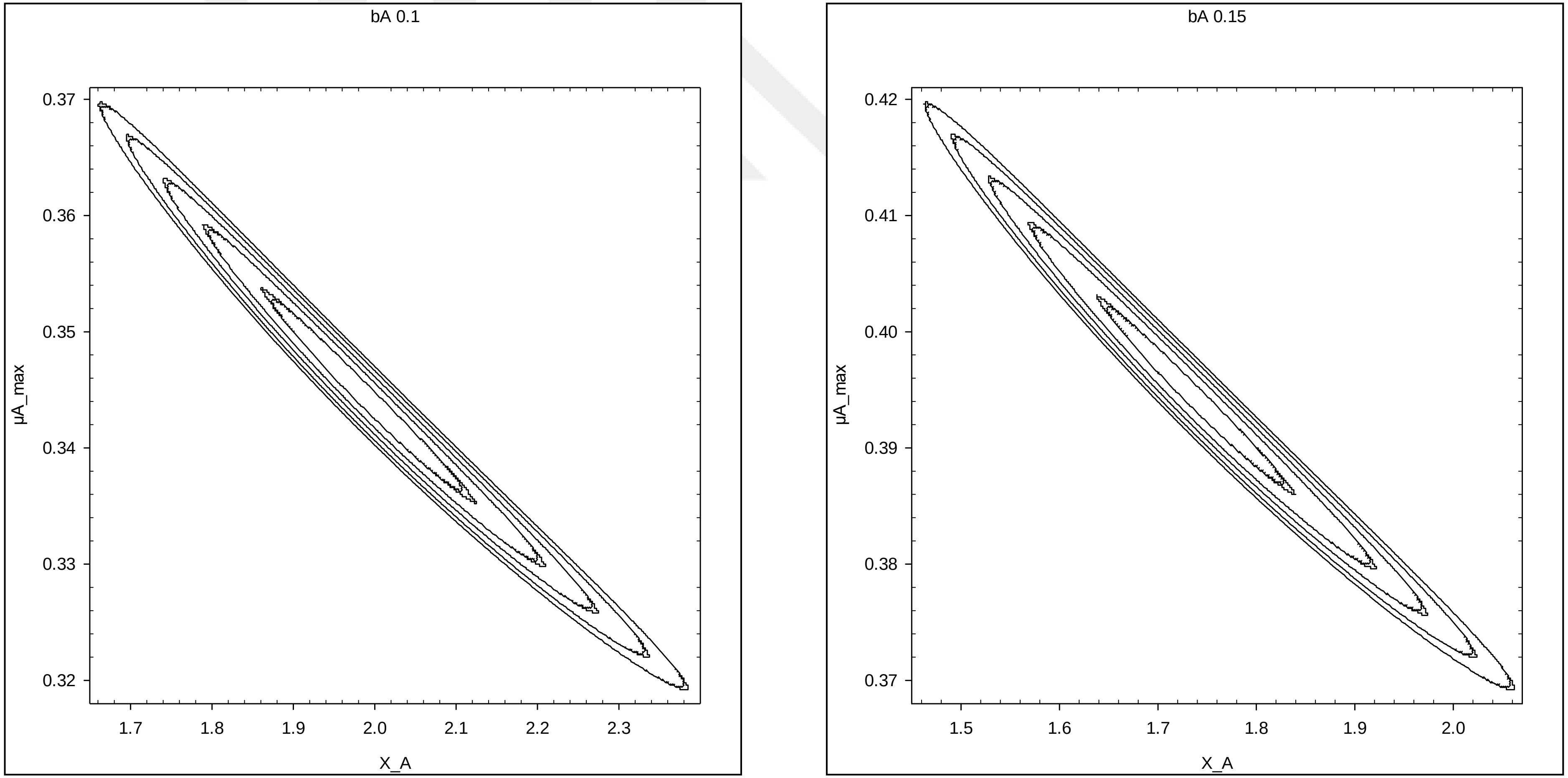

Figure 4.5: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 3 are shown; where the b_A: 0.1 and 0.15 (only acceptable region is shown as the confidence region, S_{min}-S_c).

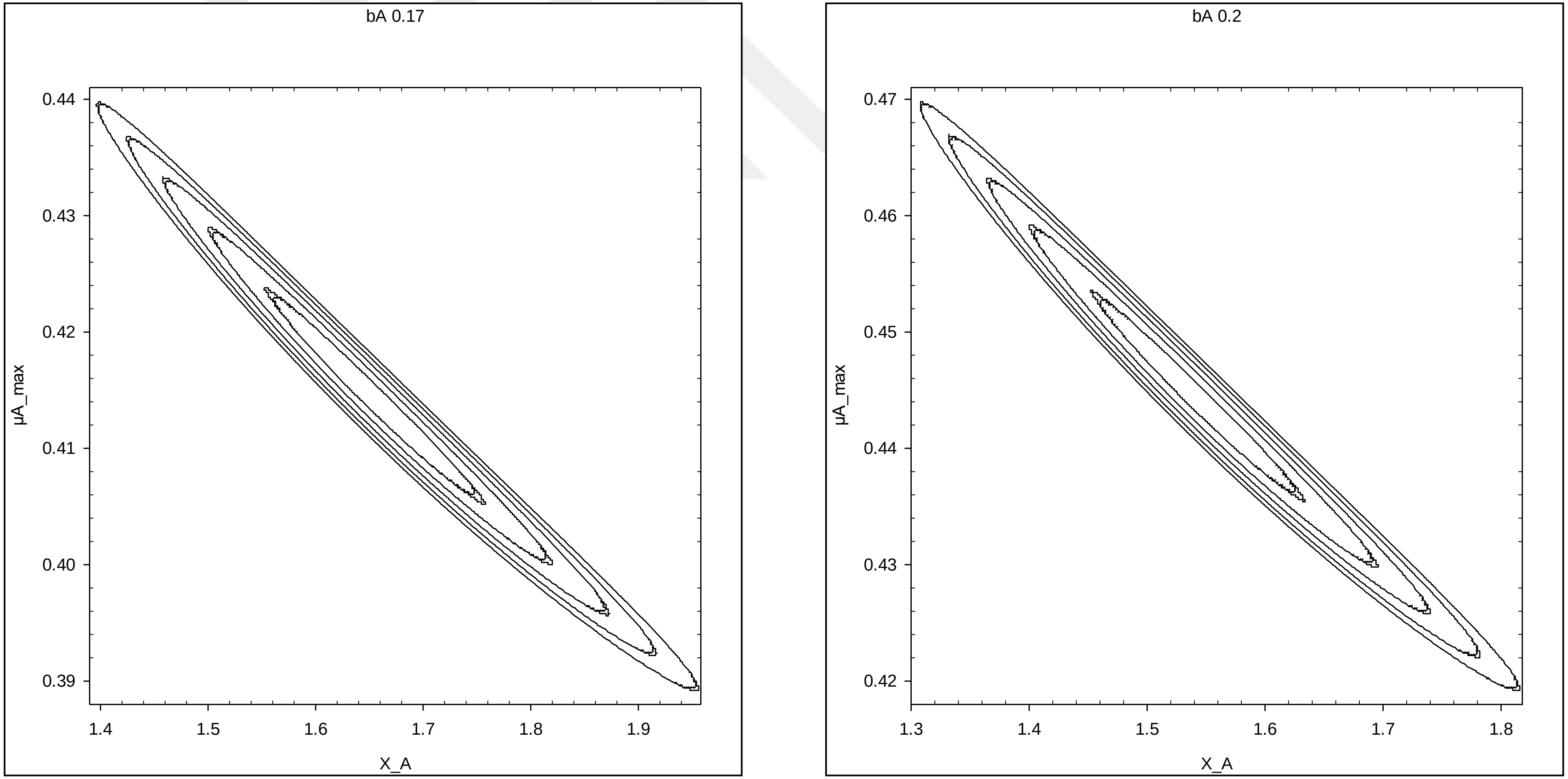

Figure 4.6: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 3 are shown; where the b_A: 0.17 and 0.2 (only acceptable region is shown as the confidence region, S_{min}-S_c).

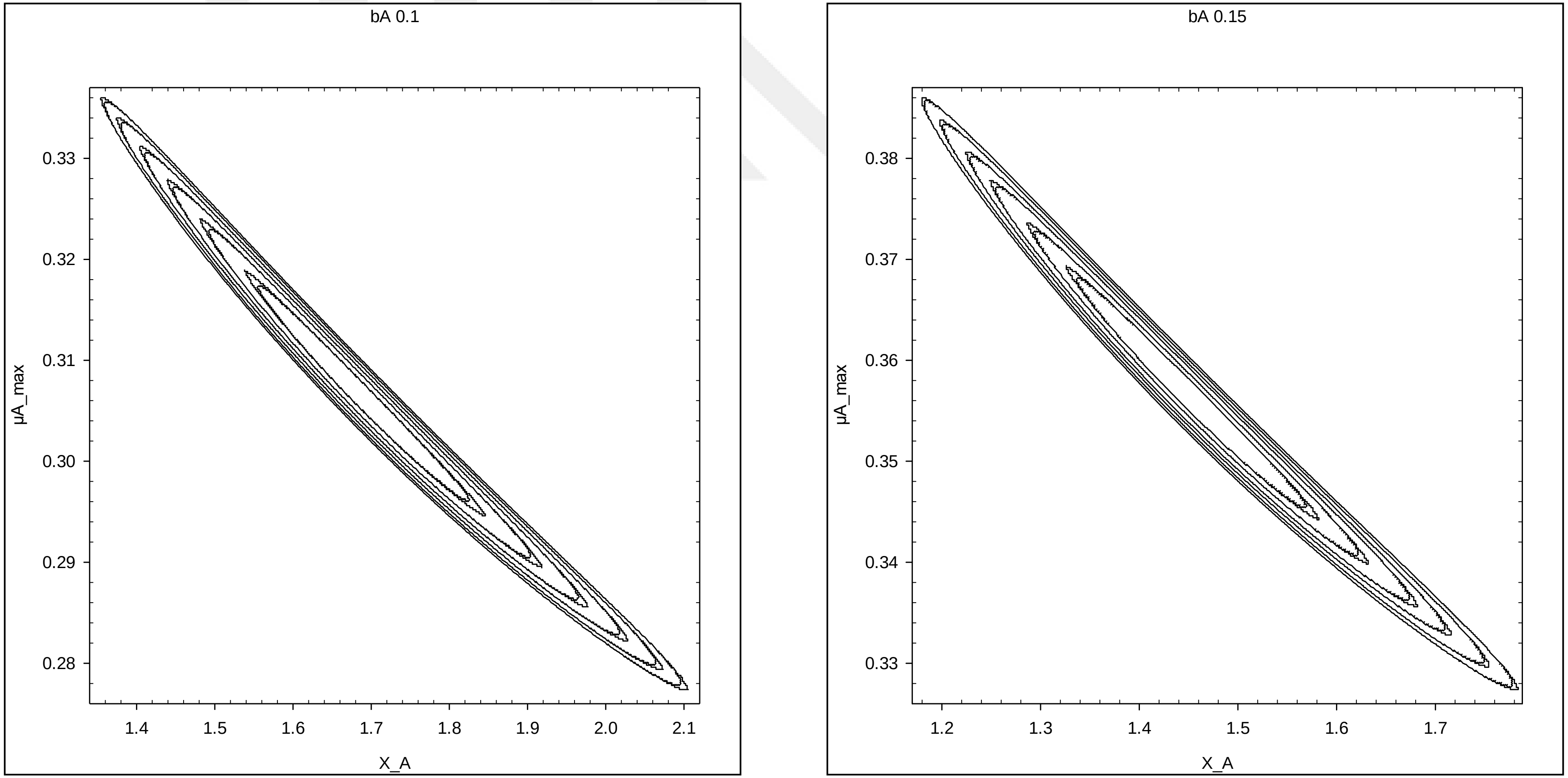

Figure 4.7: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 4 are shown; where the b_A: 0.1 and 0.15 (only acceptable region is shown as the confidence region, S_{min}-S_c).

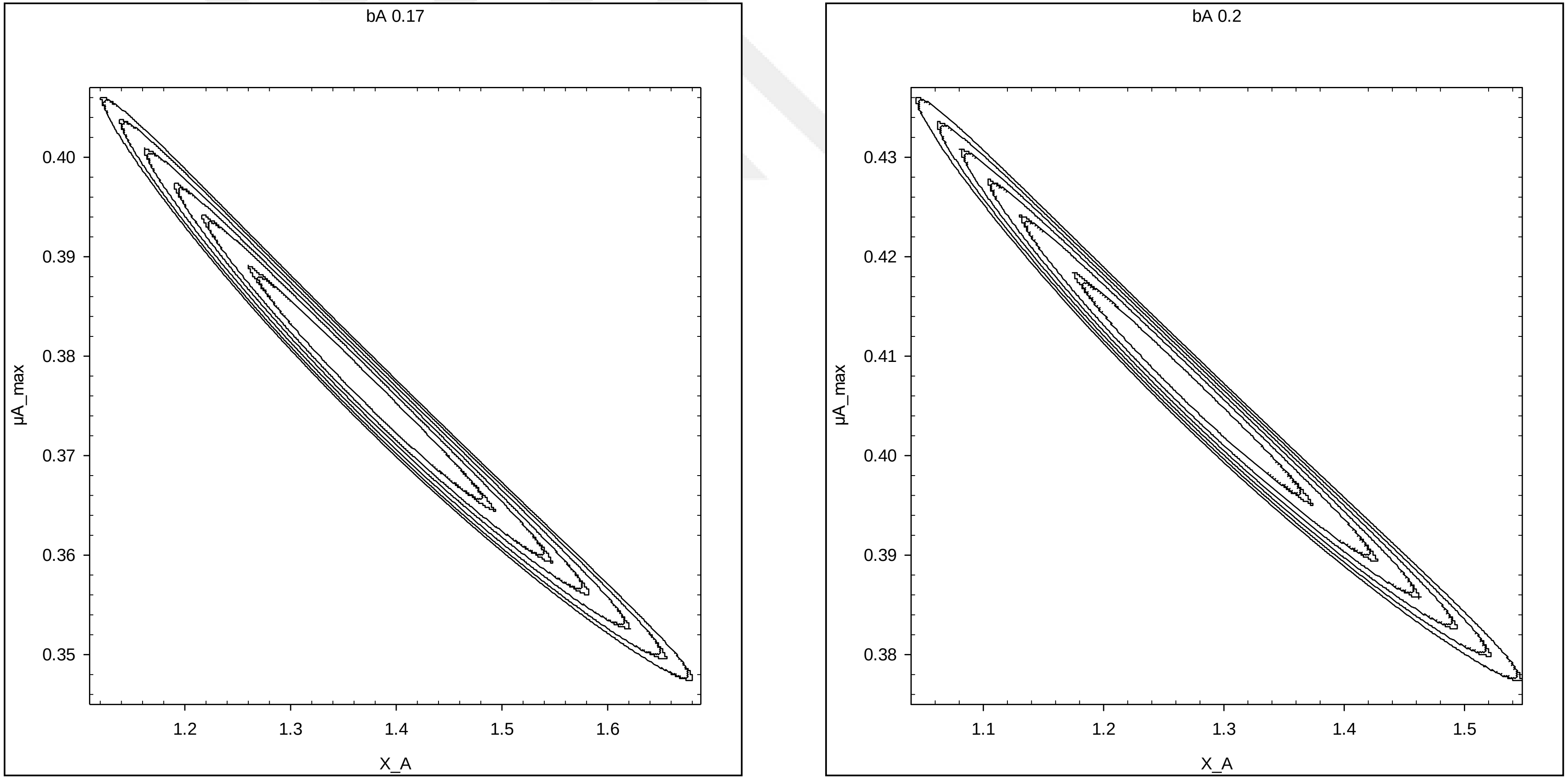

Figure 4.8: Contour plots of the initial active biomass (x-axis), the maximum specific growth rate of autotrophs (y-axis), and SSE values (z-axis) for Plant 4 are shown; where the b_A: 0.17 and 0.2 (only acceptable region is shown as the confidence region, S_{min}-S_c).

The shape of the graphs indicates that the uncertainy analysis process is not problematic, because the results are obtained as expected and similar to the literature. Figures 4.5, 4.6, 4.7, and 4.8 demonstrate that the contour plots of Plants 3 and 4 exhibit similar features to those of Plant 1. Also, for the Plant 1, 3, and 4, the center points that are found to be closer to the upper left which means that the lowest error values are found slightly less than the middle value of X_A, and lightly higher than the middle value of $\hat{\mu}_A$. Despite the plot center's similar location to the other plants for X_A, the minimum error was slightly lower than the middle value of $\hat{\mu}_A$ in Plant 2.

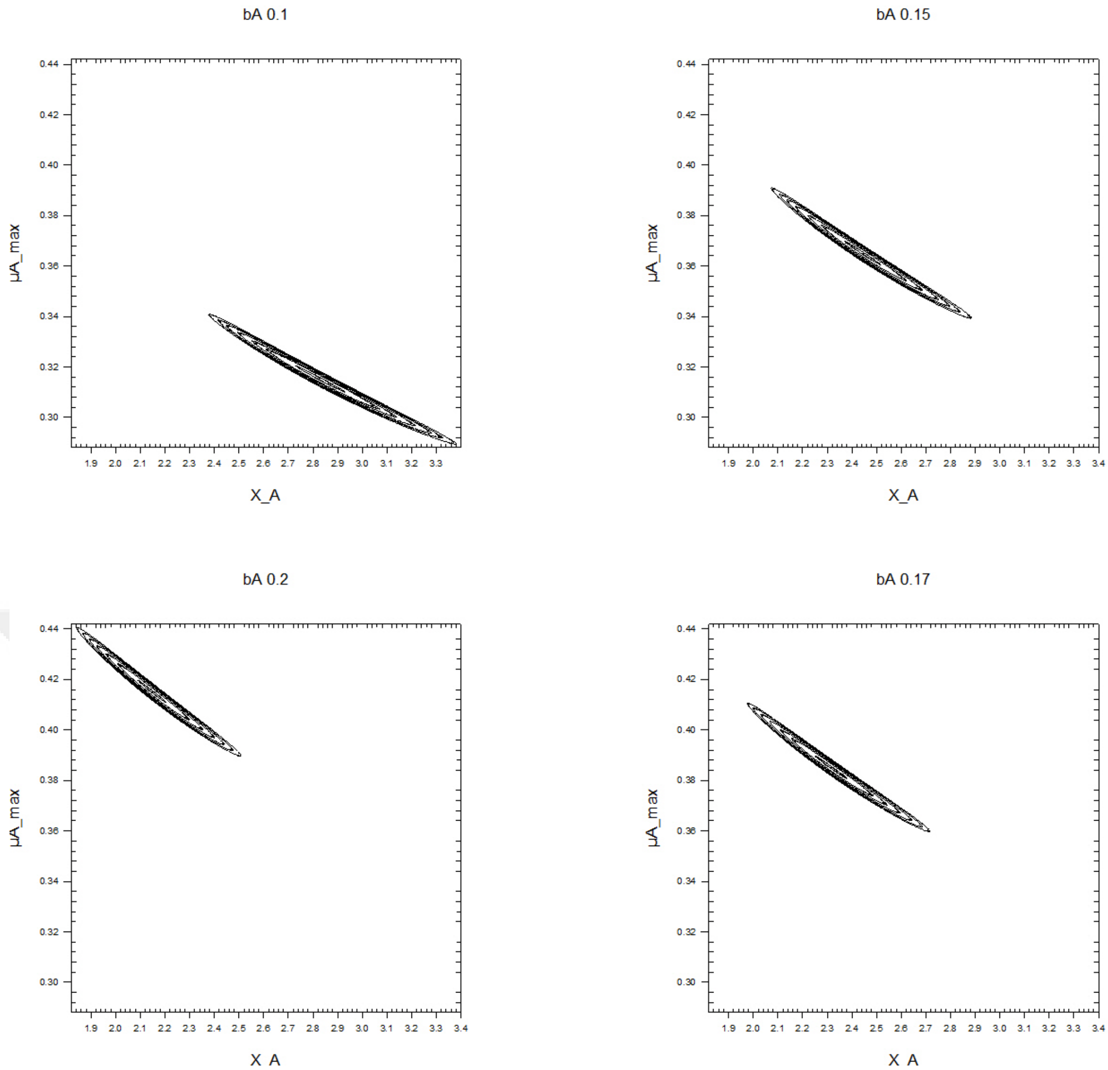

Figure 4.9: Contour graphs of Plant 1 are shown in the same coordinate system.

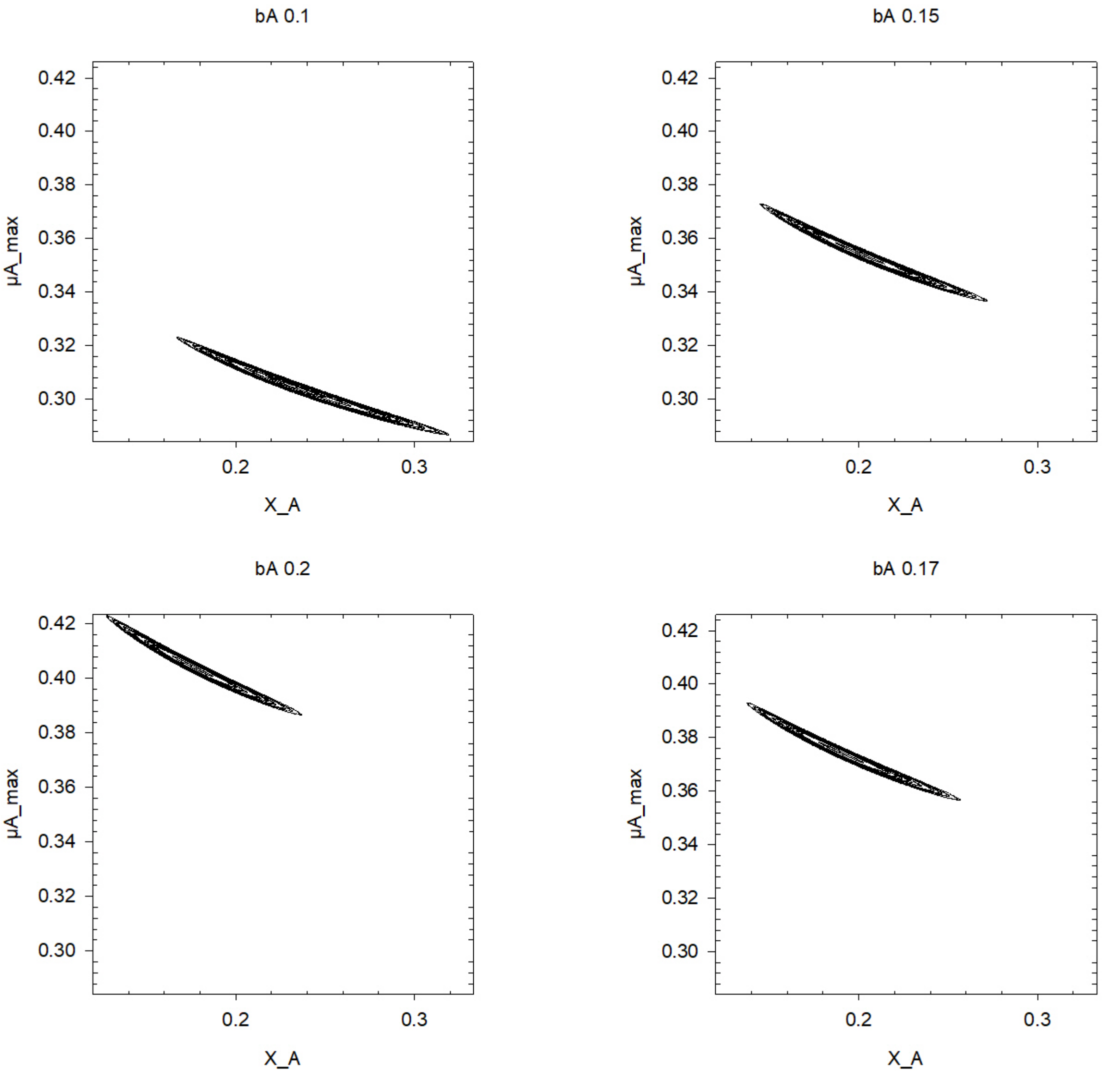

Figure 4.10: Contour graphs of Plant 2 are shown in the same coordinate system.

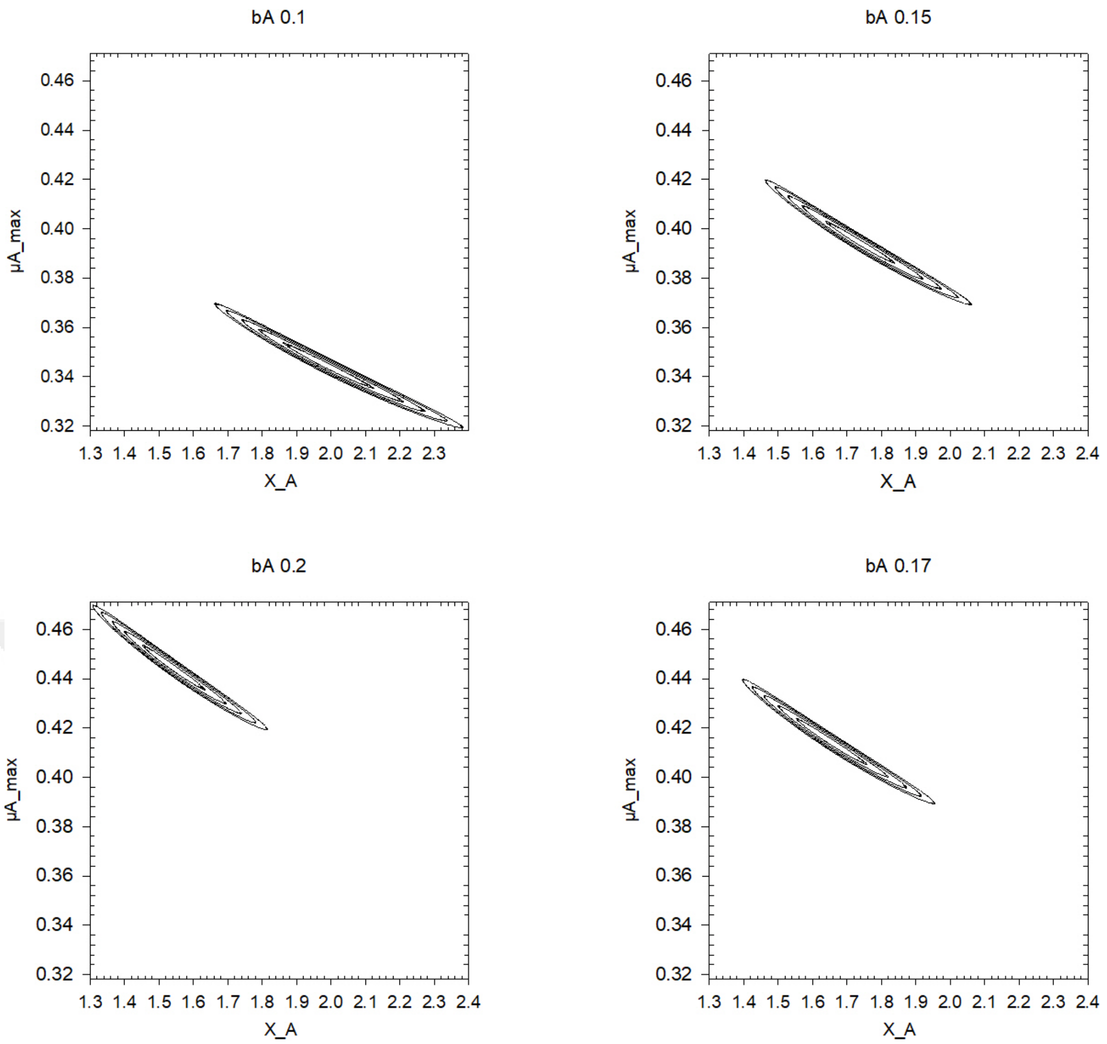

Figure 4.11: Contour graphs of Plant 3 are shown in the same coordinate system.

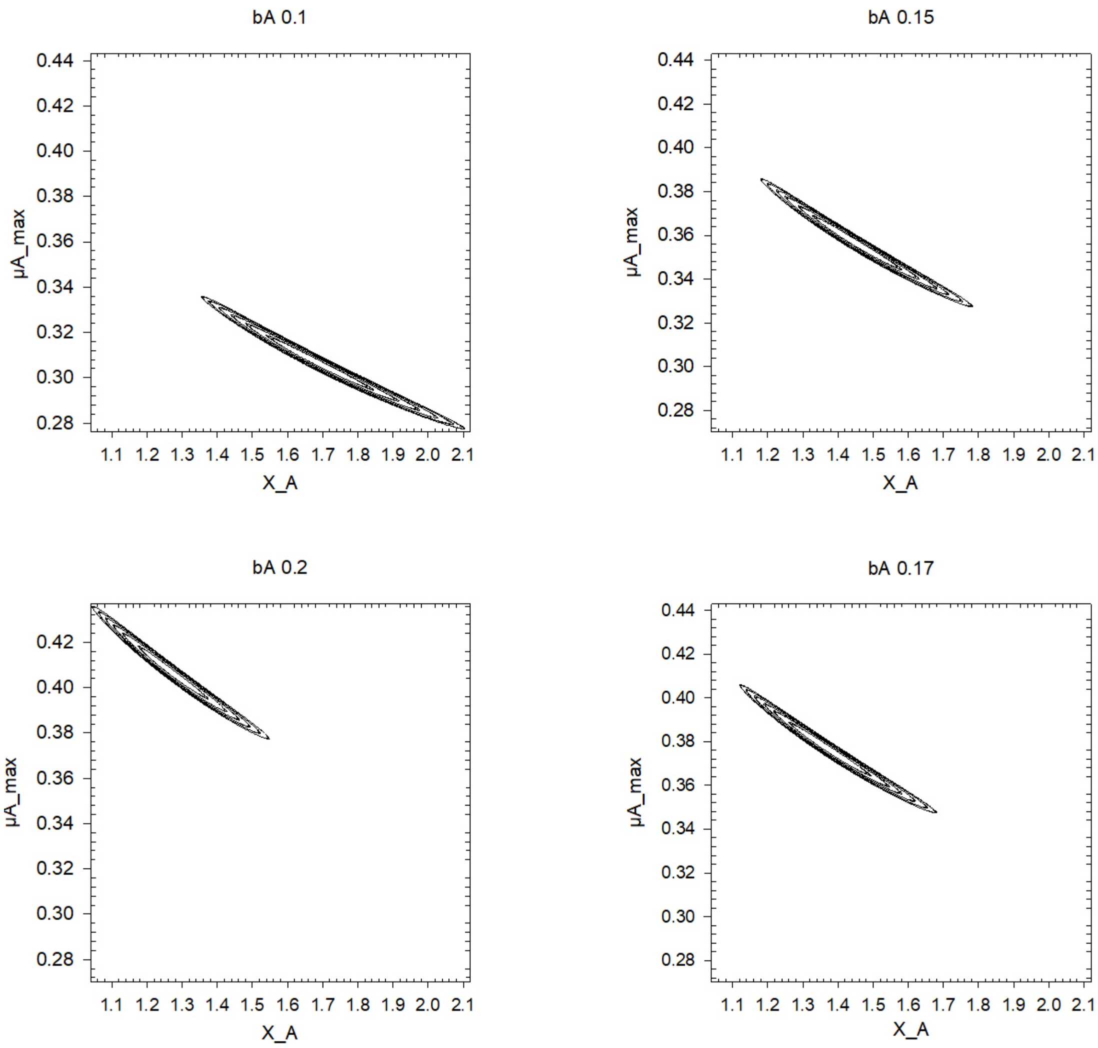

Figure 4.12: Contour graphs of Plant 4 are shown in the same coordinate system.

From the Figures 4.9, 4.10, 4.11, and 4.12; effects of b_A values can be seen clearly. Furthermore, the sizes of the confidence regions can be compared. After examination of the graphs, the largest confidence regions of WWTPs are observed at b_A 0.1. Plants 1, 3 and 4 looks similar in many ways: however, the slope of the Plant 2 seems different.

To be able to see the possible outcomes by the difference in the data quantities, some detailed contour plots are generated with lower step sizes at b_A 0.15. Figures 4.13, 4.14, 4.15, and 4.16 can be found on this purpose. These contours have smoother curves and more circular ellipsoid lines, so they can reflect the boundaries, trends and details of the graphs more clearly.

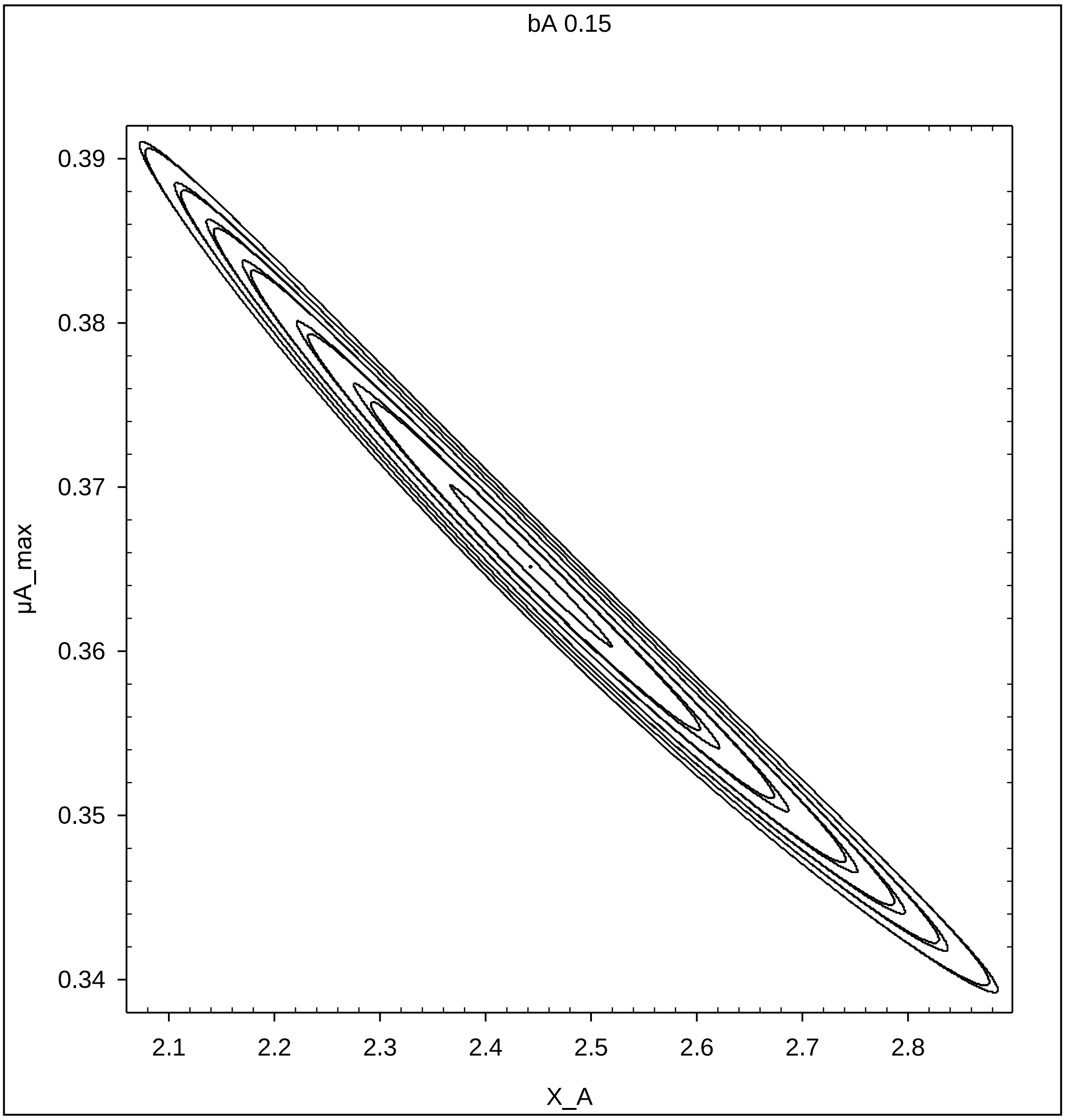

Figure 4.13: SSE contour plots of Plant 1.

For the Plant 1, around 485 thousand steps included to be able to obtain sufficient point of view. Minimum error value is found to be 10.9 where the critical value is 17.42. Within the acceptable confidence region with 95% confidence $\hat{\mu_A}$ values are observed to change approximately 7% for the both sides where X_A values varied nearly 15% around the minimum error point.

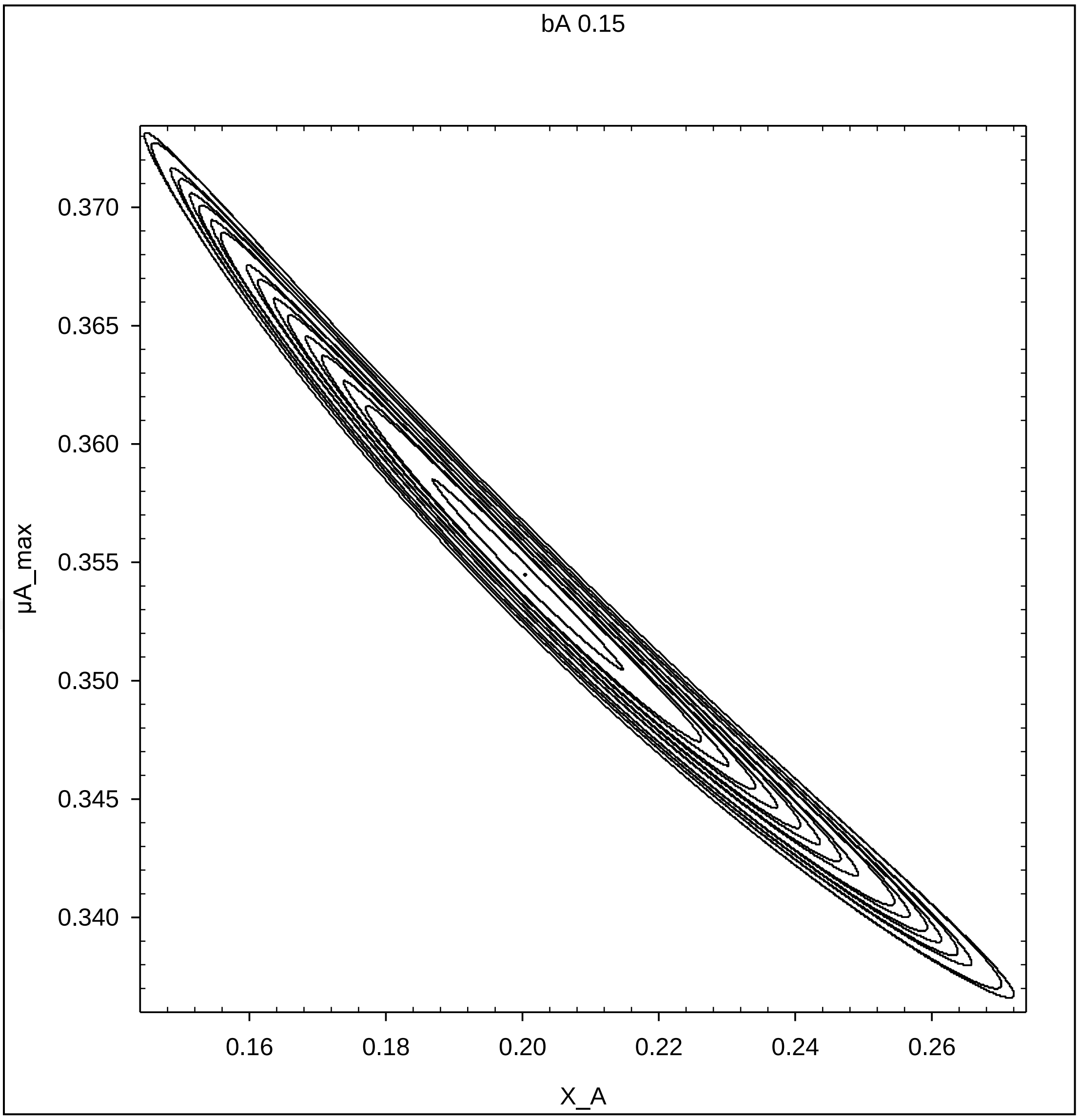

Figure 4.14: SSE contour plots of Plant 2.

In the Figure 4.13, detailed contour plot of Plant 2 can be seen. More than 400 thousand different iterations are made with the estimated points, and taken into consideration to plot the contours. Minimum SSE value is found to be 168.09 and the critical value is 211.92. In this particular figure, higher SSE values are observed in comparison to the other plants.

In the Plant 3, wider intervals are observed, it can be seen in the Figure 4.15. The optimum value is close but lower than the 2 mg/L of initial active biomass and near to the $\hat{\mu_A}$ 0.345 day^{-1}.

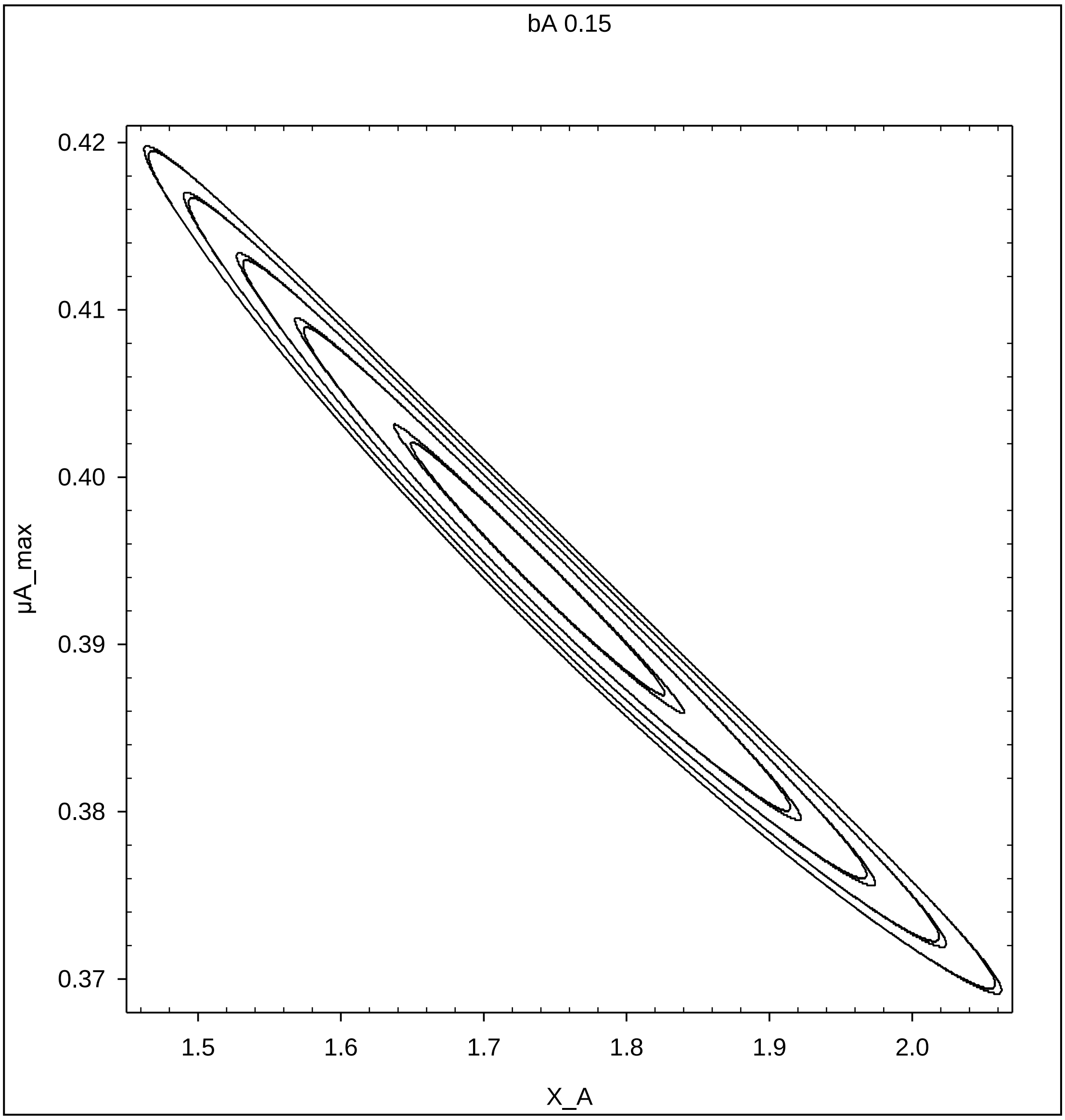

Figure 4.15: SSE contour plots of Plant 3.

To be able to conclude with a sufficient results, more than 410 thousand of parameter estimation combinations are compared and errors in each points are noted. According to the calculations, the minimum SSE is found as 16.95 where the critical SSE is 26.16.

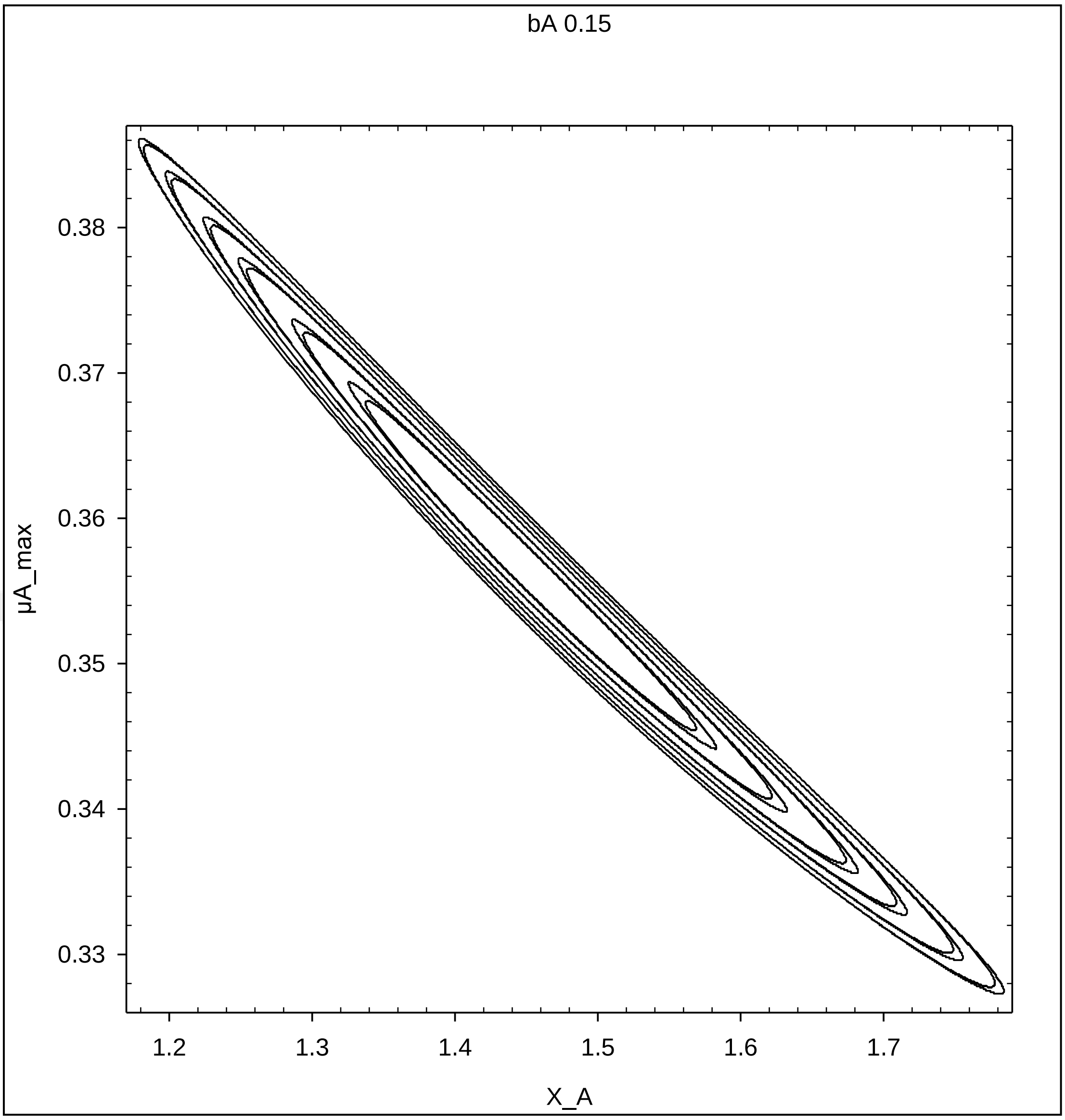

Figure 4.16: SSE contour plots of Plant 4.

Figure 4.16 illustrates the contour graph of the Plant 4. Over 390 thousands of calculated steps are generated for the figure and S_{min} is found to be 12.08 where the Scrit is 18.01. Approximately, between the 0.385 - 0.325 for $\hat{\mu}_A$ and between 1.2 – 1.8 for X_A values, the region in the figure can be considered as acceptable with 95% confidence.

In general the figures shows similar trends and the shapes of the contour plots are ellipsoid shaped. These shapes of contour plots are preferable condition that indicates the parameter estimations are seems to be accurate.

Using the S_{min} values of each plant, the models are calibrated, and best fits to the measured data are acquired. As in the Eq. 3.1, changes in the b_A values are considered to be insignificant and graphs are drawn at b_A= 0.15.

In the Figure 4.17, calibrated models of the WWTPs can be seen with respect to the measured data from the nitrification experiments.

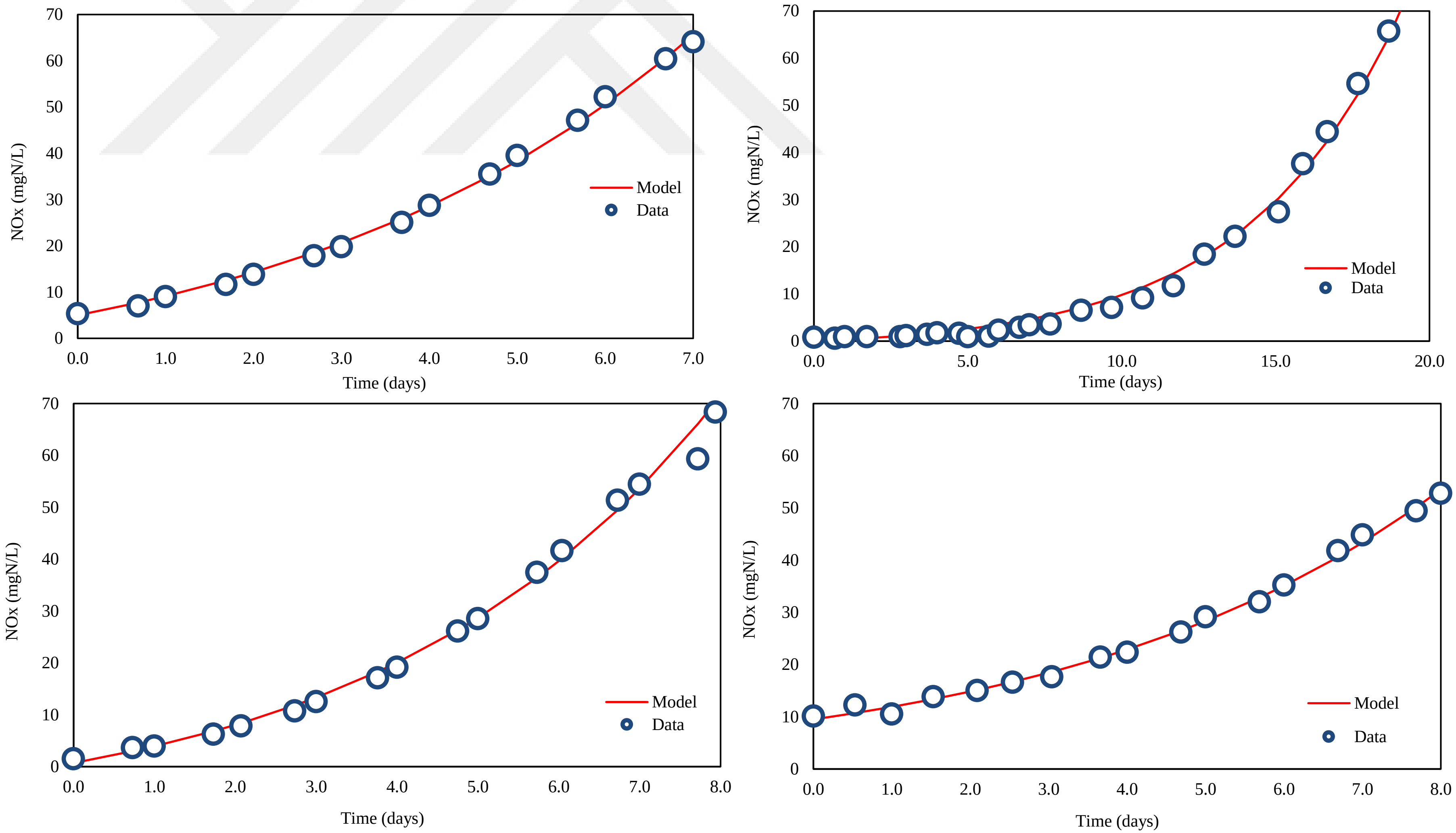

Figure 4.17: Calibrated model graphs of Plant 1 (top-left), Plant 2 (top-right), Plant 3 (bottom-left), and Plant 4 (bottom-right).

Figure 4.17 demonstrates that the measured data points and the model created match each other. In addition, it can be seen how the initial low Xa values for the Plant 2 affect the NO_X output. After a preparation phase of approximately one week, nitrification started more effectively. These conditions can be considered as a reason for the high error values calculated for the Plant 2.

After the model calibration, the difference between the kinetic coefficients determined for sample facilities in Türkiye and the values accepted in the literature ASM1, ASM2d (Henze et al., 1999; Henze, Grady, et al., 1987) and world-renowned design methods from the literature (Grady L. Jr. et al., 2011; Wichern et al., 2003; Gujer et al., 1999; Barker & Dold, 1997) have been demonstrated. Comparison can be seen for the growth rate of nitrifiers in the following Figure 4.18. The related calculations are done by using 0.15 as b_A.

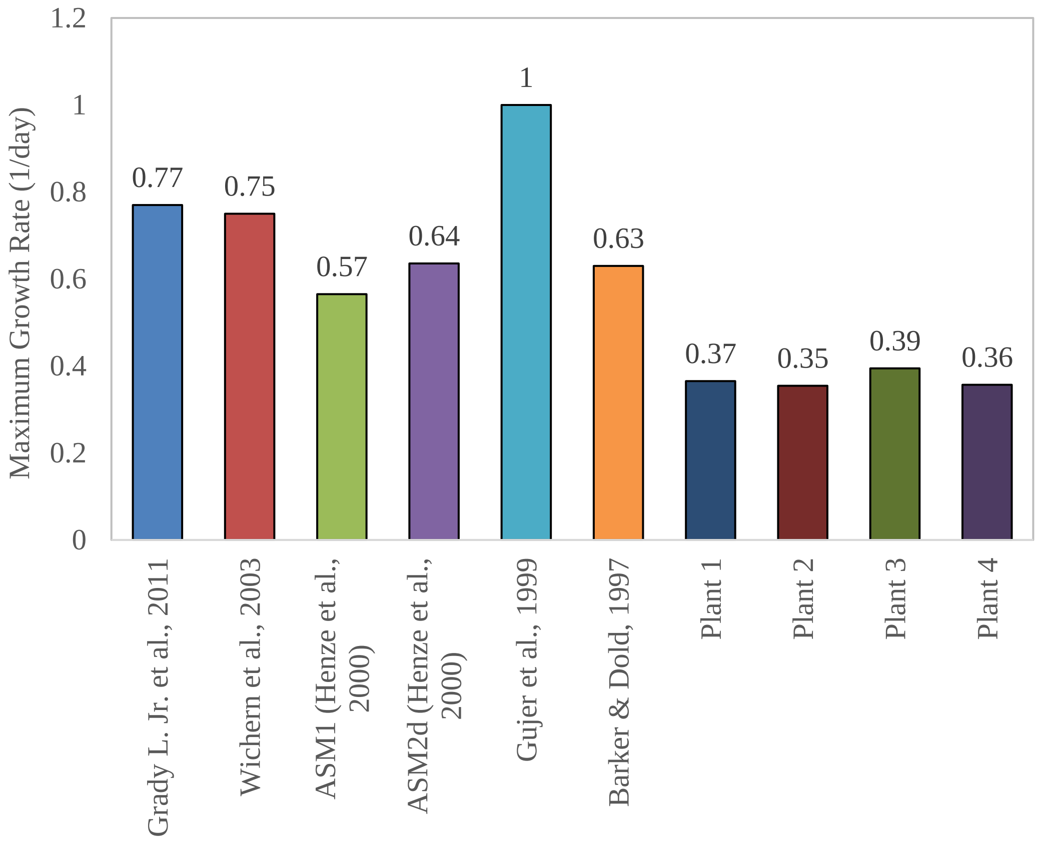

Figure 4.18: Comparison of the maximum specific growth rates of nitrifiers.

The maximum specific growth rates of nitrifiers in Türkiye are found to be lower than that of standard values in each plant nearly by half.

As a result, considering the determined bA values of each facility, X_A and $\hat{\mu}_A$ combinations that give significant results with a 95% confidence interval are visualized.

Consequently, the calculated and visualized combinations of X_A and $\hat{\mu}_A$ that yielded significant findings with a 95% confidence interval were determined based on the given bA values of each facility. Upon examination of the contour plot graphs, it is observed that the graphs are not perfectly symmetrical, the SSEs tent to decrease and reach the minimum towards their center. As one moves away from the center, the error increases in proportion to the iso-error lines and continues to increase towards the acceptable final drawn values (S_c). Outside of the S_c is not visualized. The study analyzed the graphs that considered local conditions to investigate how the initial conditions of the experiment influenced the nitrification kinetics. It also examined the uncertainties in the parameters by analyzing the observed trends and error distributions within approximate confidence intervals.Furthermore, it has become more convenient to detect alterations in the entire graph.

By examining the graphs drawn taking into account local conditions, the effect of the initial conditions of the experiment on the nitrification kinetics and the uncertainties between the parameters were discussed based on the observed trends and error distributions in approximate confidence intervals.

As a result of the uncertainty analysis, it was found that the uncertainties between the maximum growth rate of the nitrifiers calculated for the facilities and the initially fed biomass rates remained within acceptable limits, although the percentage error rates varied between 5-15%. The safety factor values in the design criteria and literature generally vary between 20% and 25%, and in some cases can go up to 50%. Considering this error tolerance, the resulting values were deemed tolerable and acceptable. Moreover, considering the bA values, it is seen that the change in $\hat{\mu}_A$ values ($\hat{\mu}_A$-b_A) remains constant as expected. The fact that this expression does not change reveals that mud age calculations can be determined accurately. Moreover, even if the two extreme values in the literature are compared, the parallel change in $\hat{\mu}_A$ values is as expected.

Adding a safety margin to the calculations made in practical applications, ensuring that theoretical calculations do not pose problems in practice and future risks are eliminated is a very common approach in wastewater treatment plant design and construction. This margin can vary from 10% to 50% in some cases. İnsel et al. (2021) gives safety factors of 1.2 and 1.25 to avoid risks, also according to the Öztürk et al. (2017), margin can be 50% for the aerobic sludge age calculations with the factor of 1.5.

Considering this added tolerance levels, the concentrations found can be considered acceptable. Looking at the results obtained, it is seen that it will help to get one step closer to the optimum nitrification experiment design. For this reason, the importance of factors that may arise from the initial conditions of the experiment was emphasized and the most suitable experimental conditions were examined.

It was found that the growth rates of nitrifiers in Türkiye were approximately half lower than the worldwide standard values used in the literature. It is thought that this difference is due to the differences in the wastewater sources discharged to the treatment plants and therefore the wastewater characters. As a result of the study, the dramatic differences in the growth rates of autotrophs emphasized the importance of the local approach.

5. CONCLUSION

In the study, the importance of factors that may arise from the initial conditions of the experiment was emphasized and the most suitable experimental conditions were examined.

The contour plots give reliable insights into the uncertainties of the selected parameters realite to each other. Error in maximum growth rate of nitrifiers is in the order of 5-15% among the plants. From the circular shape of the contour intervals, parameter estimation processes may considered as accured.

Results show that, consideration of initial conditions and performing significance test in the implentation of the experimental approaches, the model accurancy can be improved and error can be lowered by almost 15%.

Considering the added safety margins that are used in environmental design applications, the concentrations found can be considered acceptable. Looking at the results obtained, it is seen that it will help to get one step closer to the optimum nitrification experiment design.

Error could be minimized by optimizing sampling points and initial biomass concentrations. Moreover, increasing number of samples could assist to error minimization as well. Also, starting with lower X_A concentrations can result with higher errors.

That should be noted, to increase the identifiability of the model parameters several assumptions are made. Thus the results are not extensive for general use, but simplicity may also result with more comprehensive point of view about the uncertainty.

It was found that the growth rates of nitrifiers in Türkiye were approximately half lower than the worldwide standard values used in the literature. It is thought that this difference is due to the differences in the wastewater sources discharged to the treatment plants and therefore the wastewater characters. As a result of the study, the dramatic differences in the growth rates of autotrophs emphasized the importance of the local approach.

Many studies tend to overlook the significance test of the data, but this study concentrates on analyzing the uncertainty between the maximum growth rate of nitrifiers and the initial active biomass at the start of the experiment bu considering local conditions. Nevertheless, it is worth noting that this study could potentially be one of the first investigations to examine this particular subject.

For the future applications, considering the optimum experimental design may help to improve local design standard of the WWTP's design and reducing the parameter uncertainty.

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