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YAHYA

AMMONIA RECOVERY
FROM WASTEWATER
CONTAINING UREA USING
AN ENZYME MEMBRANE
REACTOR (EMR)

PhD THESIS

YEAR 2022

NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF CIVIL ENGINEERING

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Approval.

We certify that we have read the thesis submitted by MUKHTAR NUHU YAHYA titled **"Ammonia Recovery from Wastewater Containing Urea Using an Enzyme Membrane Reactor (EMR)"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy in Civil Engineering.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Mukhtar Nuhu YAHYA
11/03/2022

Acknowledgments

I would like to thank my supervisor Prof. Dr. Hüseyin GÖKÇEKUŞfor his patience, motivation, enthusiasm, and immense contribution, toward achieving this success. Moreover, I will like to appreciate my two other supervisors from Gebze Technical University, Kocaeli, Turkey, Prof. Dr. Bulent Keskinler and Prof. Dr. Ahmet Karagunduz for their help throughout my stay in their university and beyond. I also want to appreciate Prof. Dr. Derin Orhonfor his help and guidance.

Besides my advisors, I would like to thank the rest of my thesis committee: Prof.Dr. Hatice Erkurt (Committee Chairlady, Department of Enironmental Engineering, CIU), the rest of the committee members: Prof. Dr. Umut Turker (Dean of Civil and Environmental Engineering, EMU) Prof. Dr. Rana Kidak (Department of Environmental Engineering, CIU), Prof. Dr. Vahid Nourani (University of Tebriz, Iran) and other respected committee members for their encouragement and knowledgeable comments.

I am very grateful to my parent for their endless support throughout my academic years and my life in general. Also, my appreciation goes to my wife, and the rest of my family members for their encouragement and contributions.

My profound gratitude goes to all my friends, and colleagues who have always been source of strength and encouragement

I must express my gratitude to Kano State Government and Near East University for giving me the opportunity to obtain this valuable Certificate.

Mukhtar Nuhu YAHYA

DEDICATED TO MY PARENTS

Abstract

Ammonia Recovery from Wastewater Containing Urea Using an Enzyme Membrane Reactor (EMR)

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PhD, Department of Civil and Environmental Engineering

March,2022,115 pages

Recovering useful nutrients from wastewaters represents a novel approach for clean water production with significant environmental benefits. This thesis describes the use of an Enzyme Membrane Reactor (EMR) system for the recovery of ammonia from wastewater containing urea. The aim of this thesis is to improve the understanding on the transport of total ammonia-nitrogen as well as to optimize the ammonia recovery in an EMR system. In animals, urea is the major nitrogenous breakdown product of protein metabolism. The process intensification of urea enzymatic hydrolysis by urease (Jack bean urease) in a membrane reactor was investigated in this study. The study focused on the recovery of ammonia from wastewater containing urea. To assess the digestibility and affinity of the substrate for the enzyme, batch and continuous enzymatic hydrolysis operations were carried out at varied concentrations of the substrate. It was achieved by using an improved continuous enzyme membrane reactor (EMR) in conjunction with an ultra filtration membrane (250kDa) to collect the hydrolysate samples. The Ammonium nitrogen concentration of the feed was changed between 100mg/L and 500mg/L. The studies were carried out in the laboratory at a room temperature of 22°C, a flow rate of 25mL/min, a urease concentration of 67ug/mL, ionic strength (I=0, 0.01, 0.05), and ammonium nitrogen addition (0, 100mg/L, 200mg/L, 500mg/L) at various concentrations. Further investigation was carried out to determine the influence of ionic strength, ammonium nitrogen concentration, feed concentration, and enzyme concentration on the hydrolysis of urea. The elemental composition of the Ultra-Filtration (UF) membrane utilized in this work was determined using SEM and EDX. This study indicates that the higher ionic strength, higher concentrations of NH_4SO_2, and Ammonium nitrogen (NH_3-N) additions in any system containing urea inhibits hydrolysis of urea by reducing the urease enzyme activity in the system over time. Herein, we conclude that this system when put in place will be a sustainable alternative for conversion of urea to ammonia in any wastewater containing urea by utilizing urease in an EMR.

Key Words: Urease, Hydrolysis of Urea, Ultra-Filtration, Enzymatic membrane reactor.

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List of Abbreviations

TAN	Total Ammonium Nitrogen
COD	Chemical Oxygen Demand
TKN	Total Kjeldahl's Nitrogen
WWTP	Wastewater Treatment Plant
WRRF	Water Resource Recovery Facility
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance
UF	Ultra-Filtration
RO	Reverse Osmosis
FO	Forward Osmosis
MD	Membrane Distillation
ED	Electrodialysis
BES	Bio-Electrochemical System
ES	Electrochemical System
SEM	Scanning Electron Microscopy
EDAX	Energy Dispersive X-ray spectroscopy
EMR	Enzyme Membrane Reactor

NPK	Nitrogen Phosphorus Potassium
DI Water	Deionized water
UWWTD	Urban Wastewater Treatment Directives
EU	European Union
EFMA	European Fertilizer Manufacturer's Association
CSTR	Continous Stirred-Tank Reactor
PFR	Plug Flow Reactors
US	United States
MWCO	Molecular weight cut-off
PVDF	Poly-vinylidene fluoride
MFCs	Microbial Fuel Cells
MECs	Microbial Electrolysis Cells
I	Ionic Strength
CO_2	Carbon dioxide
CO	Carbon monoxide
NO_2	Nitrogen dioxide
NO	Nitrogen oxide
AN	Ammonia Nitrogen

L	Litres
NH4-N	Ammonium nitrogen
K_m	Michealis constant
V_{max}	Maximum reaction rate
HCl	Hydrochloric acid
NaOH	Sodium Hydroxide
H_2SO_4	Sulphuric acid
N	Normality
(NH4)2SO2	Ammonium sulphate
GHG	Green House Gas
Mg	Magnesium
H_2	Hydrogen
HF-LLMC	Hollow Fiber Liquid-Liquid Membrane Contactors
MM	Michaelis Menten
ACA	Aminocephalosporanic Acid
UBE	Urea Bioreactor Electrochemical
HTL	Hydrothermal Liquefaction
MLR	Multilinear Regression

ANN	Artificial Neural Network
NSE	Nash Sutcliffe efficiency
RMSE	Root mean square error
RRMSE	Relative root mean square error
R	Correlation
GPR	Gaussian Process Regression
AI	Artificial Intelligent
SVR	Support vector regression
PBIAS	Percent mean bias
MAE	Mean Absolute Error
Fe	Iron
Ca	Calcium
Ni	Nickel
Al	Aluminium
F	Florine
BP	Back-Propagation

CHAPTER I

Introduction

General Background

Resource (nutrient) recovery is the practice of recovering resources such as nitrogen and phosphorus from waste streams that would otherwise be discarded or converted into an environmentally friendly fertilizer to be used for agricultural production. Fertilizers are generally made up of one or more of these essential elements or nutrients (NPK). These fertilizers are classified into two classes' organic and inorganic fertilizers according to their production sources. Organic fertilizers are produced from organic substances i.e., plant and animals, while inorganic fertilizer is made from synthetic raw materials in the industry. Most of these inorganic fertilizer production industries discharge their effluent into our water ways thereby affecting aquatic life ecosystems directly or indirectly (Ayilara et al., 2020).

Industrial and domestic wastewater contains significant volumes of nitrogenous compounds, urea and ammonia inclusive. Urea is a nitrogen fertilizer widely used in agriculture. Annually, 100 million tons of urea are produced, of which abundant quantities are used as mineral fertilizer (Sigurdarson et al., 2018). Both urea and ammonia are found in enormous concentrations in wastewater, mostly through runoffs that cause more harm than good to the environment and discharge large amounts of pollutants into receiving water bodies. The NH3-N level in this discharged wastewater from the industrial facilities can be around 125 mg/L, while the urea level can be around 750 mg/L (Latkar & Chakrabarti, 1994). According to estimates, the ammonium ion (NH4+) accounts for around 40-50 percent of the total nitrogen in municipal wastewater treatment plants (Y. Qin & Cabral, 2002). Ammonia is a toxic pollutant in wastewaters that both affects fish species and is also undesirable for humans as it is oxidized by nitrifying microorganisms to form nitrite and nitrate (Hasanoĝlu et al., 2010).

Excessive plant growth in the ecosystem is generally caused by the overload of these two most harmful environmental problems common species, which are ammonia and ammonium NH_3/NH_4. These two contributes to the well-known eutrophication phenomenon as well as a reduction in the overall quality of the water supply. Its negative influence on human health as well as its role in climate change makes ammonia and CO_2 hazardous air contaminants. Therefore, ammonia manufacturing is seen as an industrial source of carbon dioxide emissions that contribute to global warming (Vecino, 2019). Several chemical processes exist for manufacturing of ammonia. The three most well-known and widely used methods are the Haber-Bosch process, indirect electrochemical process and urea decomposition. The Haber-Bosch process was developed a century ago and is implemented at a very high temperature of between 400-500^{O}C and a pressure of between 130-170bar. In this process, under high temperature and pressure hydrogen in gaseous form react with nitrogen under the influence of a catalyst (Kyriakou et al., 2019). This process is very costly as it requires higher temperatures and pressures.

Apart from human activities, other large amounts of wastewaters containing urea are generated from urea production processes. Moreover, coastal systems have been facing tremendous attacks from urea as a result of the anthropogenic activities of many industries in recent decades, which has attracted the attention of numerous researchers in order to devise a solution to this problem (Lilong et al., 2013).

As the demand for Urea is increasing significantly with the rapid development of chemical industries and population growth as food security is needed by all countries. Urea is not only an important nitrogen fertilizer per se, but is also a good raw material for other chemical producing industries. Currently, there is worldwide attention on achieving food security, which has made urea and other forms of agricultural fertilizers increasingly scarce (Lilong et al., 2013). Urea is the world’s most widely used N fertilizer, having approximately 46% N content, available in most markets, highly soluble, high foliar uptake and a low corrosion capacity among other advantages when use in agriculture (Modolo et al., 2018). In agriculture, nitrogen-containing fertilizers are applied to the field regularly for good growth of plants and maximization of crop yields and most of it ends in wastewater stream through surface runoffs.

The most known method for converting urea to ammonia is through hydrolysis process. Urea, a non-toxic chemical substance that provides little risk when in contact with the environment, animals, plant life, or humans when used for a small requirement (such as up to 50 kilogram per hour). Under normal temperature and pressure, urea is a solid and can be moved in bulk or even be stored safely for a long period of time unless if converted to ammonia. Urea can be transported safely, as it does not leak or explode but requires extensive safety when stored.

Many methods have been developed and are applied for urea recovery or removal from wastewater, and many more are either at the development level or are being newly introduced. These include: hydrolysis of urea, enzymatic hydrolysis, catalytic decomposition of urea, decomposition by strong oxidants, removal by adsorbents, electrochemical urea removal method, etc (Urbańczyk et al., 2016; Wojciech Simka, Jerzy Piotrowski, Robak & Nawrat, 2009). Moreover, several ongoing researches are being funded by many industries on urea recovery and conversion to ammonia. Urea discharge is becoming a challenge for many industries because of environmental laws and regulations. This study intends to provide an alternative separation technique for recovering ammonia from urea, which, if recovered, can be used by farmers in ammonia-based fertilizers and contribute to wastewater treatment cost reduction and environmental sustainability (Guo et al., 2019).

In the first stage of this study, maximum rate of reaction Vmax and Michaelis constant Km values was demonstrated using simple linearized graphical methods developed by Lineweaver-Bulk and Langmuir. As for every enzymatic reaction, Km and Vmax can be evaluated by detecting the variation of the reaction rate under substrate concentration. This will support a successful application of MM kinetics and will serve as an essential tool for analysing substrate-enzyme interactions in batch system as well as a guide for the second stage of the study which is the continous EMR system.

In the second stage of the study, urea hydrolysis was analysed in synthetic wastewater prepared in a laboratory. We also investigated the dependence of the enzyme (urease) on ionic strength, ammonium nitrogen addition and on different feed concentrations of urea as well as how all these features affect the hydrodynamic, design and performance of the system. An effective EMR system was set up for the recovery of ammonia that will convert the problematic pollutant urea that is mostly found in industrial as well as agricultural runoffs into an attractive economical commodity.

Ammonia Recovery

Rapid population growth and urbanization are driving up demand for food and increasing pressure on resource availability; at the same time, legal and environmental drivers have promoted the recovery and conversion of resources found in wastewater and the conversion of such resources into valuable products, resulting in a win-win situation. Wastewater contains variety of nutrients that can be used for human food production as organic manure. Mineral fertilizers (NPK fertilizers) are the main fertilizers used in modern agriculture. The mining of phosphorus rocks and Haber-Bosch process have been the most commonly used approaches used for the manufacturing of phosphorus-based and nitrogen-based fertilizers respectively (Shaddel, Bakhtiary-davijany, et al., 2019).

Because of the lesser operational necessity and economic motivation, nitrogen (ammonia) recovery has gotten less attention than phosphorus recovery. When recovered nitrogen (ammonia) as an immediate application on site, the cost incurred in the extraction or recovery process is reduced (Hasanoĝlu et al., 2010). However, in years to come the most research will focus on ammonia/nitrogen recovery especially for the case of manure usage on farms and for good environmental sustainability.

Importance of Recovery of resources from wastewater

Wastewater was originally considered a waste product, but it is now regarded far too valuable to be wasted. Wastewater has abundant of energy in it and contains other nutrients such as nitrogen, phosphorus and other promising materials, talk less of other increasingly valuable resources that are found in the water itself. If not harnessed, these nutrients particularly phosphorus and nitrogen can pose a lot of danger to the environment and to the infrastructure associated with transporting wastewaters. This makes the Wastewater Treatment Plant (WWTP) models to be shifted to a more efficient model called the Water Resource Recovery Facility (WRRF) (Schaubroeck et al., 2015). The WRRF extracts valuable nutrient that are previously unexploited from wastewaters using biochemical, physical, physicochemical and biological conversion processes. These days, nutrient cycle sustainability and recycling approaches are important measures of considerations when developing any recovery approach. Nitrogen and phosphorus are some valuable resources found in wastewater that can be good components of fertilizer used in agriculture when recovered. These substances are regarded as pollutants, and they have the potential to promote eutrophication in water bodies. Following the introduction of the European Council's Urban Wastewater Treatment Directive (UWWTD) in 1991, significant modifications in wastewater treatment infrastructure were also observed. Furthermore, the restriction on dumping sludge into the sea, as well as the phosphorus and nitrogen discharge limit, creates a need for more effective treatment technologies and infrastructure to deal with the increased amount of sludge being released.

Many wastewater treatment operators are retrofitting existing plants to integrate new technology for energy, nutrient, and carbon recovery in order to reduce greenhouse gas emissions and enhance resource recovery. Therefore, reuse and recovery of resources found in wastewaters are becoming more attractive, nutrient recovery from all wastewater sources is receiving increasing attention by researchers in recent years and more researches are now focusing on recovery of resources (Damgaard et al., 2021). Approximately 65-70% of the nutrients are now being recovered from wastewater, especially those that can be reuse or can be use as fertilizers or as raw materials in other productions (Gonza, 2019).Wastewaters can be exploited as a resource since they include organic matter, phosphate, nitrogen, heavy metals, and other valuable materials (Peter et al., 2016).

Many researchers published in the recent decade included thorough information on the mechanics of nutrient recovery from wastewater, other influential factors affecting recovery, future direction of recovered nutrients and so on. Only few out of these researchers focus on the economic as well as technical feasibilities of recovery which is of a great importance. Estimations from researches conducted shows that an average of 15-30% of the nitrogen from fertilizer applied in agricultural lands ends up in wastewater (Javier Mateo-Sagasta & Turral, 2017). In order to reduce the risk associated with the eutrophication factors, recovery/removal is very crucial.

Ammonia recovery directly from wastewater would create a shortcut in the nitrogen cycle, while energy and resources would be saved by avoiding the conversion of NH_3 to N_2 and then back to NH_3. Another relevance of ammonia arises from its ease of conversion into a wide range of nitrogen-containing compounds. This would result in reducing the amount of energy consumption as well as the greenhouse gas (GHG) emission in both the production and removal processes.

Economic value of Recovery

Nutrient recovery not only provides additional fertilizers for food production, but it also lowers the cost of wastewater treatment. Recovery of fertilizer-used nutrients from wastewater is a long-term solution for wastewater management that also contributes to social sustainability. Substantial amount of nutrients is contained in wastewater such as nitrogen and phosphorus, and these nutrients when recovered can be used as raw materials for other applications or in producing other bio-products. Thus, providing an alternative for the generation of revenue when the recovery system is put in place. Moreover, minimizing the danger of discharging these wastewaters containing nutrients and making the water ecosystem free from hazardous chemicals (Damgaard et al., 2021)**.**

Advantages of the Recovery

Nutrient recovery can help utilities reduce treatment costs while also allowing valuable resources to be reused as slow-release fertilizers in the agricultural sector. This recovery also helps in the reduction of the amount of sludge being generated as well as the effect of scaling nuisance associated with wastewaters. Another advantage of the recovery is that it minimizes the effect of scaling caused by precipitation and this also helps in increasing the pump capacity as well as other pipe and tank problems associated with wastewater treatment facilities. It also helps in the reduction of the operating and maintenance costs by selling of recovered product. Another benefit of nutrient recovery is that it helps in lowering the nutrients contents in wastewater. Also, the nutrient recovery reduces the amount of water in treated sludge and decrease the wastewater flow rate, both improving wastewater management and environmental sustainability (Ye et al., 2020).

Limitations of the Recovery

- Energy intensive process
- Large amount of sludge generation
- Ammonia conversion back to N_2

- N_2O emission increasing pollution in the form of greenhouse gas emission (GHG) (Ye, Hao, et al., 2018).

Major Resources Being Recovered from Wastewater

Nitrogen

Nitrogen is an essential nutrient for plants and animals. The major contributors of nitrogen are human activities. The most common forms of nitrogen in wastewaters are ammonia, ammonium, nitrate, nitrite and organic nitrogen. Nitrogen in domestic wastewater consists of approximately 60-70% ammonia nitrogen and 30-40% organic nitrogen (Kumar et al., 2013). Most of the ammonia nitrogen is derived from urea, which breaks down rapidly to ammonia in wastewater influents.

Increased in food production and world population growth focused of 10 billion people by 2050, these and many more reasons makes the demand for nitrogen and phosphorus increasing (Shaddel, Bakhtiary-davijany, et al., 2019). Nitrogen is one of the major recovered resources recovered from wastewaters. On average around 15-30% of the nitrogen from fertilizer applications ends up in wastewaters, which are usually through sewer overflows, effluent from wastewater treatment plants or from discharges of untreated sewage. This nitrogen and phosphorus that are found in wastewaters through sewages through discharges if properly recovered can plays a critical role in plant growth in agricultural activities. Ammonia is a major species of N nutrients which is commercially produced through the Haber-Bosch process and it is the sixth most produced chemical in the United States (Shaddel & Viak, 2019). Haber-Bosch process is a very expensive process of ammonia production and emits a lot of CO_2 gas (Tao et al., 2019). In a chemical process, recovering nitrogen from wastewater as ammonia can be done at a high and consistent efficiency at a low cost. The pH and temperature of the wastewater regulate the equilibrium of the ammonium ion and dissolved ammonia gas. At a pH above 11, all ammonia appears as NH_3 gas with a little effect of temperature (Shaddel & Viak, 2019).However, increased awareness of nitrogen recovery is required, since many studies now focus on phosphorus recovery because to its lower operational demand and economic motive. Nitrogen recovery's major goal is to shorten the nitrogen cycle by turning nitrogen from WWTP side streams into synthetic fertilizer. In this way, nitrogen fertilizer production might be lowered while also having a good impact on the environment (Shaddel, Viak, et al., 2019).

Phosphorus

Phosphorus is a finite resource that cannot be replenished and one of the three most important nutrients that are required for plant growth (Howorth et al., 2014).The most prevalent type of phosphorus is found in igneous and sedimentary deposits on Earth, with mining being the most feasible method of extraction. These "abundantly exploitable" phosphorus sources will be exhausted in 45–100 years if extraction and consumption rates continue at current levels. Phosphorus resources are declining day by day in both quality and accessibility and Phosphate been added to the EU's list of important raw minerals, and its reserve is shrinking every day. There are three main ways in which phosphate enters into wastewater as well as leached into the ground water; these can be through leaching from the natural mineral deposit, agricultural activities, and liquid urban waste (i.e. domestic and industrial) (Panasiuk, 2010).

Wastewater is now undergoing a paradigm change from being viewed as a waste to be treated to a proactive interest in recovering materials and energy within wastewaters. With the current shift and many more, a lot of studies are being carried out on how phosphorus can be recovered from wastewater and new innovations are now applied at full scale (Cie & Konieczka, 2017)

Urea

Urea (NH_2CONH_2), the most widely used produced fertilizer globally and is cheaper than any form of nitrogen fertilizer including ammonium nitrogen and ammonium sulfate. Very reactive, urea fertilizer emits less N_2O gas than ammonium nitrogen as the source of N provide by urea needs to pass through two different conversion processes before the nitrification or denitrification for the N_2O production. In this process, AN provides NO_3^- and NH_4^+ directly to the soil leaving it exposed to nitrification and denitrification processes, whichunder suitable conditions leads to rapid loss of N as N_2O. However, when urea is applied directly to the soil, nitrogen is lost from the applied urea as a result of volatilization and is hydrolyzed by the action of enzyme urease present in the soil. This enzyme urease is mostly produced by the soil bacteria. When urea is hydrolyzed, it immediately become unstable forming NH_3 and carbamate. Volatilization of NH_3 have grate impact on the environment and affect quality, which can negatively affect human health, cause lot of environmental pollution, and affect farmers economically(Byrne et al., 2020).

The basic ingredients for the production of urea with a capacity of 1500 tons per day are liquid ammonium and gaseous carbon dioxide (Dejanovi, 2010). Urea ($(NH_2)_2CO$) is the predominant constituent in the nitrogen excretion from the body and representing over 50% of the total solutes in the urine (Rose et al., 2015). Pure urea is a colorless, odorless substance that may be degraded by acids, bases, and urease. It's a weak base, but it's more powerful than most amides (Urbańczyk et al., 2016).As a foliar fertilizer, urea in an aqueous solution containing magnesium sulfate or ammonium nitrate can be used. Urea enters the environment not only as a result of fertilizer leaching from fields and agro-breeding farms, in effluents from plants that use it as a raw material, but also from the byproduct of mammalian protein metabolism, (A Zaher, 2021). The amount of urea in our coastal systems can be attributed to protein metabolism. The major problem of urea as a pollutant in wastewater is that even after being hydrolyzed, it still generates ammonia gas (another toxic pollutant) (A Zaher, 2021). Urea in coastal systems can generate either through an autochthonous source, which produce urea within the system, or through an allochthonous, which supplies urea from outside the system. Recent studies have showed that growth of marine phytoplankton is promoted as a result of the amount of urea in the atmosphere and is one of the important factors affecting biological communities these days. Runoffs from lands that include those from industrial wastewater, domestic sewages, agricultural fertilizers etc. are potentially the main sources of urea in coastal waters. Urea is a widespread compound in the ecosystem these days, as human demand for grains has increases due to population growth, so be it increased in the application of urea as fertilizer. Because of the widespread use of urea in many industries, some amount are found in waste (Lilong et al., 2013; Urbańczyk et al., 2016). A study conducted by European Fertilizer Manufacturer’s Association (EFMA) in 2000 shows the amount of urea and ammonium released into wastewater by urea plants as 20-320mg/L and 30-230mg/L respectively (Dejanovi, 2010). The goal of urea plant wastewater treatment is to eliminate urea, ammonia, and carbon dioxide from the process condensate.

Another source of urea found in wastewater is from urea production plants itself. As for every ton of urea produced, 0.3 ton of wastewater containing urea, ammonia and carbon dioxide is being discharged from the urea concentration and evaporation section of the plant. This wastewater need to be treated prior to discharge from the plant or if it is to be reused for proper environmental monitoring and going with the laws of environmental discharge standards (Rahimpour et al., 2010).

Treating the wastewater with high level of urea and ammonia-nitrogen is one of the problems faced by different plant globally (A Zaher, 2021). Industrial and domestic wastewaters contain a huge number of nitrogenous compounds, including urea and ammonia. In agriculture, urea is a common nitrogen fertilizer. A total of 100 million tons of urea are produced each year, with large proportions being used as mineral fertilizer (Dejanovi, 2010; Seitzinger et al., 2006). Urea and ammonia in large proportions enter wastewater primarily through runoffs, causing more harm than benefit to the ecosystem and dumping a large amount of contaminant into receiving water bodies. The NH3-N level in this discharged wastewater from the production facilities can be around 125mg/L, while the urea level can be around 750mg/L.

Due the current need for environmental protection and the possibility of upgrading this waste stream to valuable high-pressure boiler feed water or cooling water, several procedures for treating these urea-containing streams have been proposed. While 100 parts per million of urea was originally regarded acceptable for plant effluent, today's standards generally demand for a maximum concentration of 10 parts per million (Rahimpour et al., 2010).

Urea demand increases significantly with the rapid development of chemical industries, population growth, climate change, urbanization as well as the stressful need for food security by any country. Urea is not only an important nitrogen fertilizer per se, but also a good raw material for chemical producing industries. Around 85% of global ammonia production is used as fertilizer, with the remaining 15% employed in other industrial applications such as fibers, plastics, and explosives, among other things (Vecino, 2019).Currently, there is worldwide attention to achieve food security, which makes urea and other forms of agricultural fertilizers scarce (Lilong et al., 2013).

Urea fertilizer factories produce thousands of cubic meters of effluent per day, which contains a very large amount of urea and ammonia-nitrogen 1800mg/L and 800mg/L respectively (Shukla et al., 2019). A research conducted by (Prasetya et al., 2019) shows urea concentration in wastewater range to be between 1500-10,000 mg/L while that of ammonia ranges from 400-3000 mg/L. Discharging such toxic and concentrated effluents causes serious damage to the environmental ecosystem and water bodies. Excessive plant growth in the ecosystem is generally caused by the overload of the two most harmful environmental problems common species that is ammonia and ammonium (NH_3/NH_4). Moreover, because of its negative effects on human health and climate change, ammonia is a dangerous air pollutant. Ammonia manufacturing is thus seen as an industrial source of carbon dioxide emissions that contribute to the greenhouse effect (Vecino, 2019). A part from human activities, other large amounts of urea containing wastewaters is generated from urea production processes. Moreover, coastal systems are facing tremendous attacks of urea by anthropogenic activities by many industries over the last decades, which attracted the attention of many researchers on how to come with a solution to this problem (Lilong et al., 2013).

Different technologies do exist that helps in reducing the amount nitrogen to concentrations below discharge levels set by the U.S Environmental Protection Agency (EPA). These techniques are very efficient and can be used for treatment of effluents with ammonia, phosphorus and urea concentrations of different range.

As part of the ongoing paradigm shift from traditional end-of-pipe wastewater treatment to modern development of new technologies focusing on reuse and recovery of resources from wastewater, this research set out to propose an alternative separation technique for the recovery of ammonia from wastewater containing urea, a major pollutant in agricultural and industrial wastewaters. The recovered ammonia from the system, if properly recovered, can be used as ammonia-based fertilizers, contributing not only to environmental sustainability but also as an extra source of profit that can assist lower the cost of wastewater treatment (Guo et al., 2019).

Another objective of the process is to recover as much ammonia from wastewater containing urea as possible at minimal cost, and risk while maintaining maximum urease activity inside the reactor.

Statement of the problem

- The Haber-Bosch method, which is costly and energy-intensive, is used to produce ammonia, which is utilized in fertilizer manufacture.
- Different technologies of separating urea from wastewater do exist, complicated and energy intensive.
- This process also produces GHG, which has direct health consequences for people, societies, and the environment (2.6 metric tons of GHG per metric ton of ammonia produced).
- Urea is also present in several types of industrial wastewater and need to be pre-treated prior to discharge.

Aim and Objectives of the study

Aim

The aim of this thesis is to study the hydrolysis of urea contained in wastewater as well as the continous recovery of ammonia contained in wastewater containing urea in an Enzymatic Membrane reactor (EMR)

Objectives

- To analyze the effect of Enzyme (urease) concentration on urea hydrolysis.
- To analyze the effect of pH, feed concentration, ionic strength and ammonium sulphate $(NH_4)_2SO_4$ addition on Enzyme (urease) activity.
- To analyze the effect of temperature on the ammonia recovery
- To analyze clean and used membrane surface using SEM and EDAX analysis technique.
- To evaluate the efficiency of machine learning models in estimating the NH4-N recovery

Significance of the Study

In the prospect of achieving the complete recovery of urea in wastewater by an enzymatic process, this work presents an appropriate example of coupling a chemical/biological reaction and a membrane separation technology. In addition, integrating membrane technology with biological process will also help in enhancing organic removal and thereby reducing the fouling effects in membrane (Zarebska, 2014). This system is proposed because of its uniqueness in terms of enriching the ammonium ion inside the reactor and separating it from foreign matter with less energy input. Moreover, the system will also have an advantage over the previous enzymatic systems in terms of conversion, enzyme stability and efficiency (Grimrath et al., 2011). The retention of enzymes in the system will make the slurry recycling economically sound and enhance the cleaning of the membrane. When implemented, the system will have the unique advantage of enzyme recovery, which is lacking in other conventional batch reactors. The membrane system will also help in the separation or selectivity of components in the reactor according to their molecular weights. Furthermore, the results of this research will be useful for any wastewater containing urea.

Chapter I summary

This chapter gives a general background on the recovery of ammonia from wastewater as well. The study problem statement, define the important terms used in the study as well as discuss the need for the study. The aims and objectives of the study, purpose of study as well as significance of the research are all included in this chapter.

CHAPTER II

Literature Review

Ammonia production

Drawbacks and environmental impacts of Ammonia production

Nitrogen is form through a process called nitrogen fixation which can be carried out naturally or artificially. Nitrogen is made available to plant by the conversion of inert atmospheric nitrogen (N_2) to a more reactive form such as ammonia (NH_3) or nitrate (NO_3^-). Naturally, nitrogen fixation takes place when atmospheric N_2 is reduced to NH_3 by certain prokaryotes nitrogenase, an enzyme complex. While on the other hand artificial nitrogen fixation occurs mostly through the Haber-Bosch process.

Haber-Bosch process is a collaborative work between Fritz Haber and Corl Bosch. In 1908 Haber synthesizes ammonia from N_2 and hydrogen (H_2) and four years later his laboratory setup was up scaled to an industrial level by Bosch. For decades, the Haber-Bosch process for manufacturing ammonia from a 3:1 combination of hydrogen and nitrogen has been in use. As a result, the cost-cutting potential for ammonia production is restricted to lowering feedstock costs. Clean hydrogen production at a low cost is thus critical for manufacturing economically feasible clean ammonia. This process is the currently used method for fertilizer production. In the process, a lot of energy is required to break the triple bond of molecular nitrogen ion so as to convert it to two molecules of ammonia (Gonza, 2019).

Around 80% of the globally used synthetic fertilizer in agricultural production is produced through Haber-Bosch process. For great efficiency, such a plant must have a large number of process units and considerable heat integration, but still remains the most economical pathway for ammonia production (Cloete et al., 2021). This makes the role played by Haber- Bosch process crucial in modern agricultural production. Haber-Bosch process is an energy demanding process consuming around 1-2% of the global energy supply and accounting for 3-5% of the global natural gas consumption. The natural gas consumption is as a result of the H_2 usage in the process and the factor that makes the process an energy-intensive one, thereby resulting to a large amount of CO_2 emission (Ye, Ngo, *et al.*, 2018; Arpita Iddya, Dianxun Hou, Chia Miang Khor, Zhiyong Ren, Jefferson Tester, Posmanike and Grossf, Amit, 2020). This energy usage increases not only the cost of fertilizer, but also the cost of food processing and distribution globally. Moreover, energy used in fertilizer production worsens other environmental impacts such as the air quality as well as climate change.

Ammonia removal from wastewater

In agricultural productivity, nitrogen is a crucial nutritional ingredient. If nitrogen gas is extracted from the atmosphere and converted into nitrogen compounds for use, it will cost a significant quantity of energy (Kitano et al., 2012). Estimations from researches conducted show that in average 15-30% of the nitrogen from fertilizers applied in agricultural lands ends up in wastewater (Matassa, S., Batstone, D. J., Hülsen, T., Schnoor, J. and Verstraete, 2015; Mulder, 2018) Sewer overflows, discharges from untreated sewage and effluents from wastewater treatment plants containing nitrogen enters into surface waters and add up to the nitrogen content in the surface water. In order to decrease the risk associated to eutrophication of receiving water bodies, it is of crucial importance to remove nitrogen from wastewater.

Why ammonia recovery instead of total removal of ammonia

Recovering total ammonium nitrogen directly from wastewater would create a shortcut in the nitrogen cycle, while energy and resources would be saved by circumventing the conversion of NH3 to N2 and then back to NH3. Therefore, this would result in reduced energy consumption and greenhouse gas emissions associated with both its production and removal processes. It is important in any wastewater treatment plant to look into the mechanism of recovery of ammonia.

Current technologies for ammonia recovery

The most recent technologies used for ammonia recovery include;

- Air stripping
- Struvite precipitation
- Ion exchange
- Electrochemical systems

- Membrane processes

This thesis involves the use of membrane process incorporated with enzyme inside a reactor for the recovery of ammonia from urea containing wastewater.

Recovery for Ammonia

It is important in any wastewater treatment plant to look into the mechanism of recovery of ammonia. This will provide useful information on optimizing the recovery processes as well as the subsequent application of recovered nutrients. The only known ammonia recovery system that is installed and working presently is the one at VEAS WWTP in Oslo, Norway. VEAS WWTP is the largest wastewater treatment plant in Norway with the objective of ammonia recovery (Sagberg et al., 2006).

Table 1

Ammonium-Nitrogen content in the most common kinds of wastewater

Wastewater	NH4-N concentrations (mg/L)	References
Municipal	100	(Ye, Hao, et al., 2018)
Human urine	9000	(Kuntke et al., 2011)
Landfill leachate	2000	(Ye, Hao, et al., 2018)
Reject water	1000	(Lilong et al., 2013)
Hydrolysate of anaerobically digested sludge	1000	(Yu et al., 2017)
Hydrolysate of food waste	1081	(Kwan et al., 2016)

Bio-solids:

The term "bio-solids" refers to any sludge that receives one or more treatments, or any stabilized organic solid derived from sewage treatment processes that can be managed safely to be used beneficially for their nutrient, energy, or other values. This is the most common way to reuse nitrogen from waste water in agriculture. Bio-solids are sprayed at rates that ensure adequate nitrogen supply to crops (N). They also use fewer nutrients because of the additional nutrients they contain. The sludge adsorbs numerous potentially harmful substances, such as heavy metals, which is an issue with this strategy. This road is becoming increasingly problematic in Europe, and it is likely that bio-solids will no longer be allowed to be used as a fertilizer in the near future.

Struvite precipitation:

The most prevalent method for recovering ammonia from wastewater is the Struvite process. In struvite precipitation process ammonia in form of struvite is recovered simultaneously with phosphate at optimum pH of 9. Struvite is a naturally occurring mineral comprised of magnesium, ammonium, and phosphate ($MgNH_4PO_4.6H_2O$). Struvite precipitation is also an effective process where fertilizers having high Mg, N, and P content can be used for recovered (Ye, Ngo, et al., 2018). However, it is constrained by a number of variables, including pH, temperature, and the presence of magnesium, phosphorus, and calcium (Pawlak-kruczek & Urbanowska, 2019). With Struvite precipitation, it is very easy to remove ammonium and phosphate content from any water or wastewater to produce a solid compound with little traces of impurities.

A study conducted shows how Struvite precipitation is being increased worldwide due to its economic feasibility. A lot of US dollars are being saved or generated per year when WWTPs are run at lower flow rates, as this will increase the capacity of Struvite being recovered (Ahmed et al., 2015). This nutrient removed or recovered (NH4-N) can further be used as slow-release fertilizer for crops and vegetables. Struvite production could help with sludge dewatering while also preventing scaling.

Another research conducted by (Ye, Ngo, et al., 2018) stated that Pressure-related circumstances, including water hammers and filtration systems having high ionic concentrations, can also cause Struvite precipitation.

Another study conducted by (Li et al., 2012) shows how chemical precipitation is used as a technology in the removal of ammonium from 7-Aminocephalosporanic acid (ACA) wastewater at an optimum pH of 9. In the study nine different combinations of chemicals were used in order to determine the most efficient combination for NH4-N removal. The research concludes how efficient Struvite precipitation is in terms of ammonia removal in wastewater containing 7-ACA.

Ammonia stripping

Ammonia stripping is among the various methods used in removing ammoniacal nitrogen from solutions. Ammonia stripping is found to be an efficient method among all the various methods used for the recovery or removal of ammonia from highly concentrated wastewater. In this method, ammoniacal nitrogen is removed as a gas by supplying gas such as air or steam. Its only limitation is its inefficiency in treating wastewater containing low concentrations of NH_3 (Brennan *et al.*, 2020; Li *et al.*, 2020). The reaction can be described as;

$$NH3 + H2O \leftrightharpoons NH_4^+ + OH - \qquad (1)$$

Ammonia stripping is usually carried out in a cooling tower by the action of stripping air into wastewater containing ammonia from beneath. This construction enhances the transfer of ammonia from wastewater to air. The ammonia in the air can later be recovered by reacting with an acid solution. Scaling problem in the cooling towers faced due to lime addition in order to increase the pH and freezing action of the wastewater during cold weather are the two most faced problems encountered by ammonia stripping process. In the last decade, the use of lime addition is replaced by NaOH to increase the pH of the process thereby eliminating the scaling problem encountered by this system. The freezing problems are also eliminated through the re-circulated aspect of air instead of cold air from the surrounding as ammonia recovery now is taken place using acid and in a closed loop circulation (Shaddel & Viak, 2019).

High gas flow rates, elevated temperatures, and high pH (10 to 12) are required for a complete TAN recovery, which is achieved by the addition of a significant amount of base, resulting in a high energy demand for conventional stripping. The formation of ammonia gas is favored by an increase in pH, which helps in shifting the chemical equilibrium to the right, thus inducing the formation of ammonia gas. Lime and caustic soda are commonly used in this pH-dependent process to maintain a pH range of 10.8-11.5 (Sengupta et al., 2017; Shaddel & Viak, 2019). Ammonia stripping is a particularly efficient method used for ammonia recovery from highly concentrated waste water.

In a study conducted by (Ferraz et al., 2013) ammonia stripping was performed in a land fill leachate using a packed tower at room temperature and at an elevated pH of 11, and a removal efficiency of around 98% was recorded after 24 hours of stripping.

A study conducted by (El-bourawi et al., 2007) shows the importance of pH over reaction temperature in the ammonia recovery process using stripping. Other factors looked into by this study after pH, include feed temperature and concentration polarizations within the feed boundary layer. The result of the experiment shows how high feed temperature, low downstream pressure and high initial feed concentrations and pH levels enhance ammonia removal efficiency.

Adsorption (Ion Exchange)

Zeolites and other minerals like clay or tuff that possess the ion exchange characteristics can be employed for ammonium recovery in wastewaters. This process is done by pumping the wastewater via zeolite filled columns. This system has an approximate of 99% efficiency in ammonia concentration removal. The major problem encountered when using an ion exchange process in wastewater is clogging, especially when dealing with animal wastewater (Shaddel & Viak, 2019). A study conducted by (M.Buday, 1994) were ammonia are absorbed and reused from an ammonium nitrate fertilizer plant's wastewater using cation exchange resins. With nitric acid, the depleted resins were regenerated, yielding an ammonium nitrate solution that was recycled into fertilizer manufacturing.

One ion exchanger with a high affinity for ammonium ion is the clinoptilolite, a naturally occurring zeolite. Studies have shown how clinoptilolite can be used effectively in removing ammonia in wastewater. Clinoptilolite is also reported to have a classical aluminosilicate cage like structure and therefore exhibits significant macro-porosity similar to those possess by macro-net resins. As such both can be used as good and effective ion exchangers in wastewater having higher molecular weight contaminants such as proteins (Jorgensen & Weatherley, 2003).

Membrane Processes

Membrane processes are quite remarkable approaches for good separation technologies. Having several advantages such as decreasing the amount of sludge generation in WWTP, the superiority of permeate, and the possibility of totally recycle the water generated. Compared to other conventional technologies used, membrane separation is easier and user friendly than any other process, require less energy, less space, and has a more manageable operation cost (Gul, A.; Hruza, J.; Yalcinkaya, 2021).

Membrane processes are widely accepted as a key stage in a variety of processes in a wide range of industries, including water purification. Membrane filtration has produced very specific separations at room temperature with no phase change, making it a more cost-effective separation method than vacuum filtration or spray drying. The most common driving force is hydrostatic pressure, but other factors such as electrical potential, concentration gradient, and temperature can also play a role (Alcaraz & Segura. Valderrama, 2012).

Membranes have the ability to enrich ammonia and aid in its separation from other pollutants in wastewater. As such, membrane concentration for the recovery of ammonium in wastewater seems to be one of the most economical approaches in recovery processes. For example, membrane processes such as reverse osmosis (RO), forward osmosis (FO), membrane distillation (MD), and electrodialysis (ED), for example, mainly reduce or concentrate the volume of waste in water streams and are the predominant technologies used in ammonia recovery (Sengupta et al., 2017). Natural osmotic pressure is utilized in FO to drive water from the feed to the draw side of the system; hydraulic forces are used in RO to offset osmotic pressure between the feed and draw solutions. These are all processes that help in recovery process as they are mostly used for separations of pollutant or nutrient in wastewater.

Another study by (Hasanoĝlu et al., 2010) shows ammonia removal was performed using a hollow fiber flat sheet membrane. Diluted solution of sulfuric acid was used as a receiving solution to accelerate the removal of ammonia by means of a reaction that will be converting ammonia into ammonium sulfate $(NH_4)_2SO_4$. This study conducted under different operational configurations using commercial hollow fiber has high extraction percentage of ammonia up to 99.83%.

Table 2

Comparison between different techniques used in ammonia recovery

S/N	Ammonia recovery technologies	Energy usage	Surface area	Limitations	Flow rates	Temperature	Optimum pH	Recovery Efficiency	Economic feasibility
1.	Air Stripping	High	More space needed	1.Scalling problem 2. In efficiency in treating WW containing low conc. of NH3. 3. Higher maintenance	Higher flow rate	Elevated temperature	10-12	98%	Not economical
2.	Adsorption (Ion Exchange)	Low energy consumption	Less space	Clogging of the resins	Low flow rate	Room temperature		99%	Economical
3.	Struvite precipitation	Low energy	-	1. Presence of magnesium, phosphorus and calcium in the output.	-	Room temperature	9		Less economical
4.	Electrochemical systems	Less energy	Less space	1.High internal resistance 2. Limited conductivity of electrodes.	Larger flow rate	Room temperature	9-12		Not economical
5.	Membrane processes	Less energy	Less space	1. Fouling effect. 2.Reverse salt flux	Low flow rate	Room temperature	7-7.4	99.8%	Most economical approach

Ammonia Manufacturing Processes

The three most known methods of ammonia manufacturing are:

- The Haber-Bosch process.
- Indirect electrochemical dissociation.
- Urea decomposition process.

Haber-Bosch Ammonia Production Process

Several chemical processes for ammonia manufacturing do exist. The three most well-known and widely used methods are the Haber-Bosch process, indirect electrochemical process, and urea decomposition. The Haber-Bosch process, developed centuries ago, is employed at a very high temperature of between 400-500^{O}C and 500 ^{O}C and a pressure of between 130 and 170 bars. In this process, gaseous hydrogen and nitrogen react over a metal catalyst at high temperatures and pressures. A large-scale industrial process, Haber-Bosch is mostly used for the manufacturing of a large tone/hour range of ammonia, though not economically feasible for a small amount of production (Kyriakou et al., 2019; Sahu et al., 2011). This process is very costly as it requires higher temperatures and pressures. Another great challenge of the Haber-Bosch process centers is the high carbon dioxide emissions associated with them. The CO_2 byproduct of ammonia production is one of the most significant single sources of industrial carbon pollution, making it a critical target for global warming mitigation (Ojha et al., 2019). Secondly, the electrochemical process is also used in ammonia and other pollutant removal in wastewater. Electrochemical process is a dependable wastewater treatment process with minimum sludge generation, excellent operational efficiency, and the ability to operate at a wide range of ambient temperatures. Limitations to this process include; chlorine ion concentration in wastewater, pH, current density, the electric voltage applied, and the type of anode used. Higher current densities also affect this system, as it will lead to more electric power consumption and directly increase the operational cost (Ghimire et al., 2019). Urea decomposition being it catalytic, strong oxidant, or hydrolysis, is another process used in ammonia removal and recovery. All these processes listed above have their respective limits and costs incurred as all processes are conducted at high temperatures and pressures, which limits their usage by many industries (Urbańczyk et al., 2016).

Ammonia Recovery from Wastewater Using an Electro-Chemical System

This is another method for producing ammonia that requires high temperatures close to 400°C as well as an ambient pressure. Both the Haber-Bosch process and the electrochemical dissociation require a very large area for operation, and a large amount of hydrogen is also needed (Sahu et al., 2011). These are systems that use electrodes for oxidation and reduction to take place. In electrochemical systems (ES), purely electrochemical reactions take place, usually catalyzed by noble metal electrodes, whereas in bio-electrochemical systems (BES), as the name implies, one or both of the electrodes used are catalyzed by electrochemically active microorganisms.

In recent years, BES has gained more attention from experts and has emerged as an energy-efficient system because of its potential for the recovery of energy and valuable products in wastewater such as TAN. ES is also attracting the attention of researchers and wastewater experts due to its ability to degrade a wide range of contaminants in wastewater at ambient temperature and pressure, with minimal or sludge generation, small equipment size, and efficiency. ES is also used to remove COD (Chemical Oxygen Demand) and color from wastewaters from many industries, though multiple parameters influence the system, including Cl^- concentration, pH, current density, electric voltage application, and the type of electrode utilized, notably on the anode side (Bukhari, 2008).

. However, the comparatively expensive costs of electrodes and worries about the existence of harmful byproducts in the treated water have hindered the adoption of electrochemical therapy. Electrochemical methods may be tailored to a wide range of applications and readily integrated with other technologies. They are especially appealing for decentralized wastewater treatment because of their modular architecture and compact size (Radjenovic & Sedlak, 2015).

Various electrochemical techniques have recently been utilized to recover ammonium nitrogen from various types of waste water, including bio-electrochemical systems (BES), Membrane Electro-Sorption (MES), Electro-chemical Stripping (ECS), and Electrodialysis (ED) (e.g., urine, livestock wastewater, and synthesized waste water).

One of the advantages of the electrochemical systems is that energy is also recovered, in addition to ammonia recovery (Yang, 2021).

Monica et al. found that ammonium and other organic contaminants may be efficiently removed from wastewater mixed with sea water using an electrochemical technique. Many ES methods have been successfully employed to remove ammonium and organics from wastewater since then (Ghimire et al., 2019).

Another study by (Kuntke et al., 2011; M. Qin & He, 2014) shows how several kinds of BES are used in ammonia recovery from wastewater. These systems include microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), though the MEC system works at a lesser efficiency than the MFC.

Another study conducted by (Nicolau, Fonseca, States, et al., 2014) that uses a Urea Bioreactor Electrochemical (UBE) unit which is a combined system showed how feasible an electrochemical system can be used to achieve more than 80% organic carbon removal as well as converting 86% of urea to ammonia in wastewater.

Another study conducted by (Zhang et al., 2021) where an integrated system was made in order to recover ammonia from wastewater. This system called electrochemical membrane-absorbed ammonia system is used to recover (NH4)2SO4 in an acid solution. 97% and 69% removal and recovery efficiencies were recorded with a low initial feed concentration respectively. This method has the benefit of using less energy than previous documented processes, and it also has the ability to recover ammonia from wastewater, which saves energy.

Ammonia Recovery from Wastewater Using Membrane Technology

The recovery of ammonia from wastewater is seriously affected by the presence of organic compounds, heavy metals, and other poisonous chemicals. For the separation of these foreign substances from wastewater, a biological process coupled with membrane technology inside a reactor can help. This is because biological processes are commonly utilized for wastewater treatment and can minimize the number of foreign particles in the wastewater. More efforts and expertise are needed to enhance ammonium recovery or separation from foreign substances using membrane technology. For this reason, a less energy-efficient membrane system or technology is proposed in order to convert or hydrolyze the urea inside the reactor and separate it from any foreign matter. In addition to that, incorporating membrane technology with a biological process (use of urease) can improve organic removal while simultaneously reducing the effect of fouling, which has a negative impact on the use of membranes in wastewater treatment plants. As demonstrated by a study done by (Ye, Hao, et al., 2018) membrane fouling can weaken the electrical conductivity of the electrodialysis (ED) process, which will result in an increase in energy consumption and has negative impact on subsequent recovery of ammonia.

Another study conducted by (Sayegh, A., Shylaja Prakash, N., Pedersen, T. H., Horn, H., & Saravia, 2021) that evaluate the application of ultra-filtration mechanism for the separation of particles and oil droplets and the recovery of ammonia from hydrothermal liquefaction (HTL) wastewater. In this study a 100KDa molecular weight cutoff UF membrane was used for the experiment in combination with air stripping process and an acid and base trap for the recovery. The study application achieves up to 88% recovery in the acid traps and proves that membrane technology can be successful in the treatment of complex real HTC wastewater.

Membrane Reactors for Hydrolysis

According to published sources, the word “membrane reactor” was first mentioned in the chemical engineering literature in around the year 1980. The term "membrane reactor" does not have a recognized definition at this time because there is no acceptable term for it. When used in this context, the term refers to any membrane device that performs chemical conversion under specific conditions and that makes use of the membrane's distinct contacting and separation properties (Vladisavljevi, 2016). Membrane-assisted biocatalysis with synthetic membranes is a hot issue in engineering circles, which is unsurprising. The structure and activities of any biological membrane, as stated by numerous specialists in the area of membrane technology, reveals that nature has already built a highly integrated chemical plant that professionals in the field of treatment replicate for membrane separations, as (Matson & Quinn, 1992) pointed out.

Membrane reactors are categorized as ultra-filtration (UF) or microfiltration (MF). Membrane reactors, biphasic (organic or aqueous) membrane reactors, membrane aeration reactors, or extractive membrane reactors, depending on the technique of operation. When the product has a lower molecular weight than the substrate and both the product and the substrate are soluble in the same solvents, UF membrane reactors are commonly utilized. In this case, enzymes are added to the substrate solution or are attached to the membrane but the enzymes will be trapped by the membrane and will not be able to pass through it; nevertheless, the product may easily flow through and be collected on the permeate side.

The idea of using membrane reactors for cellulose hydrolysis via enzymatic hydrolysis has piqued interest in a number of studies and research projects focusing on various parts of the process. Membrane reactors provide the distinct advantage of simultaneously recovering enzymes and removing glucose, which is not possible in conventional batch or continuous reactors (Nguyenhuynh et al., 2017). Therefore, membrane filtration can be easily used in separating glucose or ammonia from the hydrolysis process to minimize product inhibition while at the same time retaining the enzyme inside the reactor to be used for a longer time. All these are advantages of using membrane filtration.

When using a membrane separation method in conjunction with a hydrolysis reactor, component selectivity based on molecular weight is critical. The usage of a membrane would efficiently contain big molecular weight enzymes while permitting glucose or ammonia permeability with a sufficient molecular weight cutoff. Ultra-filtration membranes are often used in the filtration process to selectively fractionate glucose and separate enzyme cellulases and cellulose in most research because the molecular weight cutoff is in the range of 5 to 50 kDa. Several studies using various enzyme systems with various cellulose substrates have confirmed the suitability of membrane filtration in the enzymatic hydrolysis process, with the conclusion that ultra-filtration membrane separation results in complete rejection of cellulose and enzyme cellulases, but zero rejection of glucose.

A study conducted by (Gan et al., 2002) concluded that the removal of glucose using a membrane reactor results from higher conversion of cellulose as compared to same conversion using a batch reactor.

Another study also show how batch enzymatic hydrolysis with a lower substrate concentration experienced product glucose inhibition on enzyme, whereas product inhibition was not observed in a system consisting of a reactor coupled with ultra-filtration running at a high substrate concentration (Mameri et al., 2000).

The main purpose behind using a UF membrane is for the membrane to contain the dissolved enzyme molecules in the reaction vessel (reactor), while the products are allowed to leave or are collected in another vessel outside the system i.e., a size-selective separation. This method can be carried out in a batch or continuous stirred tank reactor. The solution is constantly delivered to the vessel in a continuous flow process, while the products, as well as any surplus solvents, are removed in a feed-and-bleed operation. The procedure removes the product while leaving the biocatalyst and high molecular weight substrate intact and making it possible to reuse and to recover the enzyme used. This is another reason why we are using a continuous system in this study.

Enzymatic Membrane Reactors

For centuries, the existence of enzymes has been known. Since then, various studies have been conducted, with the first being done by a Swedish chemist, John Jacob Berzelius, in 1835. This chemist termed the enzyme's action catalytic. The first enzyme was identified in its pure state in the year 1926 by James B. Summer of Cornell University with a great work of isolating and crystallizing this important catalyst urease from Jack bean, and this work has earned him a Nobel prize in the year 1947 (Enzymes & Processes, 1972).

Enzymes are catalysts that speeds up the rate of a chemical reaction without itself (the enzyme) suffering from any irreversible chemical change. Enzymes usually are neither been used up in reactions, nor are they seen in the product.

Reactions containing enzyme are considered green and efficient method with mild reaction conditions, fewer side effects, and better cleanliness and environmental safety processes. However, industrial applications are limited due to their unstable properties and difficulties in the process to recycle enzyme used (Cen et al., 2019).

The transformation of a substrate into products in any reaction mainly involves the addition of enzymes to speed the reaction. It is either a homogenous or heterogeneous system, which is called bio-catalysis. As a matter of fact, the separation of the reactant from the product is a very crucial task when it comes to chemical processes. And is crucial when it involves processes that involves recovery or reuse of the catalyst used in the reaction. Membrane reactors constitute an attempt to integrate all these problems into a single operation, such as separating different soluble products of different molecular weights and concentrations, catalytic conversion, , and catalyst recovery, which will go a long way towards reducing the cost of recovery and reuse (Prazeres & Cabral, 1994).

The basic concept of any membrane reactor is based on the enzyme-substrate separation by a semi-permeable membrane, which forms a selective barrier. A driving pressure or force is usually used in this kind of separation of solutes from the reaction mixture. Complete enzyme retention is the number one and one of the most important criteria for the any successful continuous operation that took place inside a reactor. Upon this retention, the enzyme becomes confined to a defined region of the membrane reactor where the reaction with the substrate occurs, and this will make the enzyme to have be more useful for reuse (Nguyenhuynh et al., 2017).

According to a research conducted by (Nguyenhuynh et al., 2017) membrane reactors have the distinct advantage of simultaneously recovering enzymes and removing glucose, which is something that other batch reactors and continuous reactors like CSTR and PFR lack.

Size of biocatalyst, substrates, and products are the most important factors that are needed for the selection of a membrane material in addition to the chemical components in the solution. Other harsh conditions such as pH and temperature are also things to consider as membrane material has to be resilient to some operation conditions. while membranes are being stable in the presence of a strong oxidant, its material should be chosen in order to reach a minimum fouling formation at its surface. Moreover, the fouling effect of a membrane is strongly dependent on the material used in its manufacturing, pore size of the membrane as well as its surface characteristics (H, Arash., F, 2020).

Different industries have given attention to membrane bioreactors (MBR) in the past decades. When an enzyme is incorporated into the MBR, it is called an "enzymatic membrane bioreactor" (EMR) (Aghababaie et al., 2019).

The EMRs work with a selective membrane, allowing the products to be continuously been separated from the reaction system and, as such, helping to move the equilibrium of the reactant to the product side. The system increases the productivity of the entire process, thereby decreasing production costs, making the reaction environment stable and allowing for high catalytic yields.

Developed in the late 1960’s, EMR was first proposed by Zydeny et al. and, after that, a series of such EMR systems have been used in the production cycle. In the 1980's, an EMR was developed by Wichmann and Wandrey for the continuous production of leucine dehydrogenase from *B. stearothermophilus*. Moreover, in the 1990's, (Liese & Zelinski, T. Kula M R,.Kierkels C,. Karutz M,.Kragl U, 1998) established a free enzyme membrane reactor system for the production of N-acetylneuraminic acid. From the 1990’s to date, a lot of EMR’s systems have been successfully used in food, pharmaceuticals, and industrial wastewater treatments. This type of reactor is quite simple to use at all scale, but it has a number of drawbacks, particularly when dealing with large volumes of real wastewater, as is frequent in case of industry: low in terms of efficiency, high operation and maintenance costs, loss of catalytic activity (inhibition effect), significant product quality fluctuation, and so on (Rios et al., 2004).

Until recently, EMR's most prevalent and widespread applications have been in the hydrolysis of macro-molecules such as proteins, lipids, and other similar compounds. They have just recently begun to be successfully in the removal of numerous pollutants contained in wastewater both in food and pharmaceutical applications. Research conducted by Rios et al. demonstrates the removal of phenol and cyanide from coke factory wastewater using enzymes. Despite extensive contact durations, very minor biodegradation rates of real wastewater were obtained as a result of this study (Rios et al., 2004).

Batch and continuous ammonia recovery from wastewater

Several techniques do exist for the recovery of ammonia from wastewater, and many more are still in the developmental stage. Recognizing the potential of recycled nutrients and the value of wastewater as a resource, researchers have begun to look into nutrient recovery from wastewater. This nutrient recovery from wastewater bodies will benefit not only wastewater management but also environmental sustainability.

A study conducted by (Rahimpour et al., 2010) to remove urea, ammonia, and carbon dioxide from wastewater from a conventional urea plant. The study was conducted at high and low substrate concentrations using a cascade of hydrolyser-desorber systems. The result of the research shows how an increase in both the inlet temperature of wastewater from the plant, stream flow rate, and reflux ratio improves the removal efficiencies of both the urea and ammonia.

Another study conducted by (Prasetya et al., 2019) in which urea recovery was conducted by adsorption using porous carbon. The purpose of the study was to uptake urea from fertilizer manufacturing wastewater by adsorption using porous carbon and uses it as fertilizer. The research was conducted at room temperature and used a urea concentration range of 500–20,000 mg/L. The study also shows that the urea concentration in wastewater is generally between the ranges of 2000–4000 mg/L.

A sorption pre-treatment approach was employed to remove any leftover DOM and decrease membrane fouling before processing with hollow fiber liquid-liquid membrane contactors (HF-LLMC). The findings of this study reveal that high ammonia removal rates of 76 percent and 94 percent were achieved using one-step and two-step HF-LLMC procedures, respectively (Vecino, 2019).

Another research conducted by (Arpita Iddya, Dianxun Hou, Chia Miang Khor, Zhiyong Ren, Jefferson Tester, Posmanike & Grossf, 2020), showed that ammonia was recovered using an electrically conductive gas-stripping membrane immersed in a reactor. The membrane is used to recover ammonia from wastewater efficiently, and it has been shown to be more energy efficient than other existing treatment methods. This technology also has the potential to address one of anaerobic wastewater treatment's fundamental flaws: its inability to properly remove nitrogen contamination.

Properties and applications of urease

Urea amidohydrolase (urease) is a nickel-dependent enzyme found in a variety of plants and microorganisms that catalyzes the conversion of urea to ammonia and carbon dioxide, resulting in a pH rise (Estiu et al., 2004). The use of urease in urea hydrolysis has been explored as one of the best strategies for converting urea to two moles of ammonia (NH_3) and carbon dioxide (CO_2) (Nicolau, Fonseca, States, et al., 2014). Urease can be found in plants, algae, yeast, bacteria, as well as in soils as soil enzyme. The best studied urease is the one from Jack Bean, which was also identified as the first nickel enzyme and the first to be crystallized. As shown below, urea catalyzes the hydrolysis of urea to produce ammonia and carbamate, which then hydrolyzes spontaneously to produce carbonic acid and another molecule of ammonia.

$$NH2\text{-}CO\text{-}NH2+H2O \longrightarrow NH3+NH2\text{-}CO\text{-}OH \tag{2}$$

$$NH2\text{-}CO\text{-}OH + H2O \longrightarrow NH3 + H2CO3 \tag{3}$$

This is due to the fact that in the conversion process, a substantial amount of nitrogen is transferred to ammonia gas, yielding up to 70% of the nitrogen lost to the environment. For better environmental sustainability and risk reduction, it is critical to develop a technology, system, or strategy that allows for more efficient urea hydrolysis with less loss to the atmosphere and with a decrease or suppression of the urea negative effect (Modolo et al., 2018)

The most challenging process in any enzymatic process is the determination of kinetic parameters, where the enzymatic activity is determined in terms of what are called "initial rates" at different substrate concentrations and time-course reaction analysis. This involves running a series of enzyme assays at multiple substrate concentrations and measuring their initial rates of reactions (Tomczak & Węglarz-Tomczak, 2019).

In bioreactor engineering, one of the most challenging and most difficult tasks is modeling of enzymatic hydrolysis of lignocellulose materials. This is due to the complexity of substrates, the action of enzymes, and the interaction between enzymes and substrates (Bäuerle et al., 2017; Carvalho et al., 2013). Another technical problem and economic obstacle that affect enzymatic hydrolysis is the high selectivity and mild reaction conditions of cellulose, its high cost, and low efficiency (Lou et al., 2018).

The urease enzyme is found in significant levels in jack beans and other seeds, and it has an amino acid composition. Urease, a nickel-dependent enzyme, has strong catalytic activity in the hydrolysis reaction, which decomposes urea into ammonia under mild conditions. Urease has long been utilized as a catalyst in the hydrolysis of urea, at a rate of approximately 10^4 times that of the un-catalyzed reaction. Temperature, water content, and nitrogen application rate all have an impact on urease activity (Lou et al., 2018), and urease is also dependent on ionic strength, pH, and storage time (Lacy, 1988). Urease is being used directly in a lot of research conducted for the treatment of wastewater containing urea as well as other organisms with the same activity as urease (Tong & Xu, 2012). Urea-bearing wastewater is subjected to high heat and pressures for hydrolysis in more recent urea manufacturing units. This is one of the shortcomings of urea hydrolysis, and this makes urea hydrolysis of wastewater containing urea using urease an alternative these days.

Urease can either be in an immobilized form supported by a rigid suitable material or be applied in a batch system. In the batch system, it is necessary to tackle the challenge of separating urease from an ammonia-carbon dioxide solution and reusing the urease. This need may be met by the use of ultra-filtration technology. Another important factor to consider in the separation is molecular weight differences between these elements, as this will help in finding a suitable ultra-filtration membrane for the task.

Urea hydrolysis in wastewater using urease

Urea hydrolysis is a chemical reaction that occurs in soils, the human body, and in wastewater urine diversion systems. The hydrolysis of urea in soils begins as soon as urea fertilizer is applied or cow pee is deposited on grazed soil. This hydrolysis is usually completed within 1 to 2 days. Urea hydrolysis as a chemical reaction is catalyzed by the action of urease enzyme (urea amiodrolase) to form what is called ammonium carbonate, which is unstable, dissociates into ammonium (NH_4^+) and carbonate (CO_3^{2-}).

(4)

Urease activities are generally increase by the following factors, which include; soil temperature greater than 5^0C, and soil pH above 6.5. (Lacy, 1988) showed recovery of ammonia from a nitrogen fertilizer plant with the help of a urease enzyme after concentrating the ammonia to about 11% on a weak acidic cation exchanger. This study divided the whole process into four different units: the hydrolysis of urea by enzyme urease, the separation of the enzyme urease from the effluent using an ultra-filtration membrane, the removal of CO_2, and finally the removal of ammonia on a weak acidic cation exchanger. The paper also showed the positive correlation between the increase in urease and increase the hydrolysis process and vice versa.

In another study, (Lou et al., 2018) aimed at revealing the coupling effects of three factors on urease activity, which were temperature, moisture content, and nitrogen application rate on soils. This study showed how temperature increases were increasing the urease activity in soils, which goes in line with research conducted by (Moyo, 1989). Although moisture content and nitrogen application increased the rate of urea hydrolysis on soils, there was a significant negative correlation between increases in water content, nitrogen application and hydrolysis rate.

A study conducted by (Revilla et al., 2005) compared two different methods of urea removal, the enzymatic and direct methods, for estuaries and some coastal waters. In the study, to assess the amount of urea obtained in the streams and coastal waters, jack bean urease was used for the hydrolysis process. At first stage, enzymatic method was addressed using a single reagent. Secondly, the effect of humic acid and salinity on the accuracy of both methods in synthetic samples made in the laboratory and with real natural samples from the coastal waters. Th study concluded that the direct method is more accurate compared to the enzymatic method when the sample has less salinity, but recommend other factors to be considered such as laboratory conditions, effect of time on the hydrolysis, and other crucial factors like the complete water system under study when the method to measure dissolved urea has to be decided in future research.

Other disadvantages of this process include; productivity, operating, maintenance costs and technical knowledge, issues related to inactivation of the enzyme, significant product quality fluctuation, and so on. These issues are normally faced particularly when dealing with huge volumes of real wastewater, as is commonly the case in industry: Feed concentrations, temperature, pH, ionic strength, and a variety of other parameters all influence the hydrolysis of urea in wastewater.

Enzyme concentration

The rate of the hydrolysis process is influenced by the concentration of enzymes. As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. Increasing enzyme concentration will speed up the reaction, as long as there is substrate available to bind to. Once all of the substrate is bound, the reaction will no longer speed up, since there will be nothing for additional enzymes to bind to.

A study conducted by (Tunay & Tatli, 2006) shows how at the beginning of the experiment 50% of urea conversion followed the same pattern using 5mg/L urease. Increasing the urease from 5mg/L to 10mg/L the process becomes rapid as urease concentration was increased to 25mg/L. in the study all system fit well the zero-order kinetics and the slope increases with increase urease concentration. The results of the study indicated that enzyme concentration was as a very effective factor that affects the rate of hydrolysis. Furthermore, he more the urine is been diluted, the less the hydrolysis rate. This means dilution is directly proportional to the rate of hydrolysis. The study also concludes pH as an important parameter when it comes to hydrolysis, as change in pH has inhibits the hydrolysis rate.

Effects of Inhibitors or Activators on Enzyme Activity

Enzymes catalyze virtually every process in a cell. This catalysis is usually altered by certain organic or inorganic molecules called modifiers. These molecules, when they speed the rate of a reaction, are called activators, while inhibitors are those molecules that reduce or decrease the rate of a reaction. Enzyme inhibitors are substances that usually affect the enzyme catalytic activity, sometimes slows the reaction rate or sometimes stops the reaction completely. Different chemical additives that normally impact the action of the enzyme urease are used to inhibit urea hydrolysis (Ray & Saetta, 2017).

Competitive, non-competitive, and substrate inhibition are the three most common kinds of enzyme inhibition. The existence of the enzyme substrate complex ES is at the heart of most inhibition theories. As previously stated, the existence of transient ES structures has been confirmed in the laboratory. Competitive inhibition occurs when the enzyme is given both the substrate and a compound that looks similar to the substrate. The "lock-key theory" of enzyme catalysis can be applied to explain why enzyme inhibition occur. According to a recent study, adding dilute acids, bases, or alkali to lower the pH of the substrate between 4-4.5 or raise it over 11 slows the hydrolysis process (Ray et al., 2019).

Effects of temperature on enzyme activity

Temperature has an impact on enzyme activity, just as it does on other chemical reactions. Although denaturation of the enzyme protein reduces product synthesis at high temperatures, rates rise by 4 to 8% per degree centigrade. Like other chemical processes, the rate of an enzyme-catalyzed reaction increases as the temperature rises. When the temperature is raised by ten degrees Celsius, most enzymes increase their activity by 50 to 100 percent. Small changes in reaction temperature such as 1 or 2°C might cause results to differ by 10 to 20%. Most of the research conducted shows how temperature plays an important role in most chemical reactions and is an important parameter to consider in ammonia production from urea (Sahu et al., 2011).

In another study conducted by (Moyo, 1989) that evaluated the effect of temperature on urea hydrolysis rate at temperatures of 5, 15, 25, 35, and 45°C on soil samples, and concluded that increasing temperature from 5 to 45°C as increasing the rate of hydrolysis of urea. The fact that many enzymes are impacted negatively at high temperatures complicates enzymatic processes, because majority of mammalian enzymes are deactivated at temperatures above 40°C. This is the reason why most enzyme tests are performed at temps below 40°C. At even modest temperatures, enzymes deactivate over time. The best temperature to store enzymes is 5°C or lower. When enzymes are frozen or are stored at negative temperature, there activity is lost. This is the reason why, in this research, all enzymes used were stored at a temperature below 5°C. The effect of temperature on any reaction is an important factor to be considered in every study. Thus, it is very important when carrying out any experiment that involves enzyme addition to ensure that the temperature remains constant and that it is also recorded for reference for better comparison with other studies conducted at other temperatures.

Effects of pH on enzyme activity

Each enzyme has an optimum pH range that is most favorable. Changing the pH outside of this range will surely slow the enzymatic activity. Most enzymes are affected by changes in pH. The most favorable pH is the pH value where the enzyme is most active, generally called the optimum pH. Extremely high or low pH values generally result in a complete loss of activity for most enzymes. Most enzymes work at a neutral pH value of 7.4. This agrees with the research conducted by (Nicolau, Fonseca, States, et al., 2014) were the optimum urease pH of 7.4 was seen as the optimum pH level were the activity of the enzyme is seen as maximum. pH of enzymes plays a role in their stability as well. There is a pH optimal stability area for each enzyme, just as there is for activity. The optimum pH value for each enzyme varies substantially, as seen in the table below:

Table 3

pH levels for optimum activity of enzymes

Enzyme name	pH optimum
Lipase (Pancreas)	8.0
Lipase (stomach)	4.0-5.0
Lipase (castor oil)	4.7
Pepsin	1.5-1.6
Trypsin	7.8-8.7
Urease	7.0
Invertase	4.5

Maltase	6.1-6.8
Amylase (Pancreas)	6.7-7.0
Amylase (malt)	4.6-5.2
Catalase	7.0

Other parameters to be considered apart from pH and temperature when studying enzymatic processes include; ionic strength, feed concentration difference, salts actions and many more, all the above mentioned can serve as either inhibitor or activator in any reaction and can have influence on enzymatic processes. In order for an enzymatic reaction or process to be accurate and repeatable, each of these physical and chemical characteristics must be examined and tuned.

Effects of ionic strength on enzyme activity

The ionic strength (I) of a solution is the measure of the concentration of ions in a solution and is a key factor to consider in an enzymatic reaction. Denoted as I and It is defined as the;

$$I = \frac{1}{2}\sum(v)^2\,[i] \qquad (5)$$

Where $[i]$ is the molar concentration of the ion's species and $(v)^2$ is the net charge of the ion squared.

Ionic strength may also affect the activity of an enzyme by changing the stability and solubility of the enzyme as well as that of the substrate. Some enzymes require low ionic strength for maximum activity, while others require a substantial level of salts for significant activity. For most enzymes, there is a variation in activity with ionic strength, so the value of the optimum enzymatic activity should be fixed and recorded. The ionic strength effect also becomes more important with hydrophilic enzyme.

Chapter II Summary

In this chapter II, we present an overview of the ammonia recovery and removal mechanisms, and review state-of-the-art research on all the mechanisms involved. Insight of the most used and the most important mechanisms for ammonia recovery and their limitations and main challenges faced when using the mechanisms. Moreover, we discuss and show how economically feasible an enzyme membrane technology can help in the recovery of ammonia. We also explain the need for technologies that focus on ammonia recovery instead of removing it from wastewater containing urea, in order to close the nitrogen cycle in a more sustainable and more efficient way. Herein, we proposed the use of an EMR system for ammonia recovery from urea rich wastewater.

CHAPTER III

Methodology

Materials and Methods

Phase I: Batch hydrolysis

Batch enzymatic hydrolysis experiments were carried out at various substrate concentrations in order to determine the enzyme-substrate affinity and digestibility. The synthetic urea containing wastewater was mixed with some urease enzyme in the batch system in the quantities. Consequently, rate model parameters were determined to provide a basis for comparison between the batch and the continuous-mode enzymatic hydrolysis. A batch system was used to establish the kinetic parameters (V_{max} and K_m), which are essential in analyzing substrate-enzyme interaction as in table 3 below;

The determinations of the value of Km and $Vmax$ for the activity of urease enzyme at different pH values were done using both Lineweaver-Bulk and Hanes Woolf plot. For the Lineweaver-Bulk plot, the plot of inverse initial velocity 1/V was done versus the inverse of substrate concentration 1/[S]. While for the Hanes Woolf plot [S]/V was plotted against the substrate concentration [S].

Table 4

Kinetic Parameters showing Lineweaver-Bulk and Hanes Woolf plots

Model	Equation	Slope and intercept
Lineweaver-Bulk	$\frac{1}{v} = \frac{Km}{Vmax} + 1/Vmax$	$slope: Km/Vmax$ $intercept = 1/Vmax$
Hanes Woolf plot	$\frac{[S]}{V} = \frac{Km}{Vmax} + s/Vmax$	$slope: 1/Vmax$ $intercept = Km/Vmax$

The continuous operation will serve as a proof-of-concept for the development of an EMR system to be used in the conversion of urea to ammonia in wastewater treatment systems. The system will consist of a reactor coupled with a UF membrane. In the batch experiments, 100mg/L urea- N was mixed with different concentrations of urease enzyme in a batch reactor. Jack bean Urease (EC.3.5.1.5 Lyophilized, KGaA 64271, Darmstadt Germany) was used as the source of urease throughout the experiment and at a dose of 0.067g/L. This was based on preliminary experiment conducted where four different concentrations of urease were used to stimulate the hydrolysis of urea (0.20, 0.15, 0.10 and 0.067g/L) as shown in the result section in Figure 3. 0.067g/L was used in the system in order to minimize cost and to reduce the effect of membrane fouling. Moreover, the time taken for urea hydrolysis depends on the amount of urease added or present in the surrounding environment and varies in different environment. All the experiments were conducted at room temperature and an effluent pH of between 7.0 and 7.5. The pH and conductivity of each sample was measured using CRISON 2000pH electrode (HachLange Spain, S.L.U., Spain).

Materials used

The Urea used for this research was purchased from Merck KGaA 64271 (Darmstadt, Germany) with physico-chemical properties as follows; purity of 99.5%; pH value of 9.0; a molar mass of 60.06g/mol; density 1.34g/cm^3; melting point of 132-134°C. 100mg/l and 500mg/l of substrate concentration where used for all the analysis at a room temperature of 20±1°C and a fixed enzyme concentration of 0.067g/l. Urease enzyme (EC.3.5.1.5 Lyophilized) purchased from Merck (KGaA 64271, Darmstadt Germany).The enzyme has a specific activity at 5U/mg. After the enzyme solution was prepared it was kept refrigerated at -5°C for further use (Standard Methods for the Examination of Water and Wastewater, 1998). DI water used for the experiments was produced by the MilliQ SP Ultra-pure-water purification system (a registered trademark of Merck KGaA, Darmstadt, Germany). Chemicals used for this experiment include sodium chloride (NaCl) for different ionic strength measurements, ammonium sulfate $(NH_4)_2SO_4$, Sodium hydroxide (NaOH), Boric acid indicator, Borate buffer, Sulphuric acid (H_2SO_4). All chemicals and other reagents used were extra pure and are made as indicated in the standard methods and are used as received.

Phase II: Continuous Enzymatic Hydrolysis of Urea (configuration).

This study was mainly performed at the water and wastewater laboratory of Gebze Technical University. In this lab a continuous mode experiments for the ammonia recovery were carried out in a 4L Bioreactor (BIOFLO 3000) coupled with an Ultra Filtration membrane and stirred at 200rpm. Two high pressure automated peristaltic pumps (Watson Marlow Peristaltic Pump Hydra cell, Falmouth, Cornwall, United Kingdom) equipped with a flow regulator were used to maintain the wastewater level inside the reactor (Figure 1). To keep the wastewater level inside the reactor constant, the feed (synthetic wastewater) and permeate solutions were pumped in opposite directions and at the same flow rate (25mL/min). The synthetic wastewater level inside the reactor was maintained at 4L, no pH correction was made during the experiment and the operating temperature maintained at room temperature. The UF membrane used in this study was purchased from Sterlitech (Auburn, WA 98001, USA). The active layer of the UF membrane is made of Polyvinylidene fluoride (PVDF) of 250KDa molecular weight cut-off (MWCO). The UF membrane has a length of 6cm to 8cm and having a diameter range from 0.5 to 1.4 cm. Moreover, it has the advantage of resisting maximum temperature of up to 98°C when need to be sterilized with hot water. The UF membrane was used in order not to allow the enzyme urease from passing to the permeate side of the membrane.

Membrane Cleaning Procedure

Used membrane can be washed and clean by the backwashed process using Deionized (DI) water. Backwashing of the membrane was done with Deionized (DI) water for one hour after each experimental run-in order to keep the membrane clean and reduce the effect of membrane fouling. DI water is an extremely pure water used in the laboratory that is contaminant free and had all ions removed. To further reduce some risk associated with contamination and mixing, all equipment's used were thoroughly cleaned before use and the reactor vessel were washed and sterilized. The samples to be analyzed for the NH_3 concentration were taken from a tank at the permeate side of the membrane.

Table 5

Reactor operation Parameters of the continous system.

Parameter	Value	Units
Reactor Volume	4	L
Reactor temperature	20	℃
pH	6.5-7.0	-
Water flow rate	25	mL/min

Figure 1

Schematic diagram of submerged membrane reactor attached to a Bench top BIOSTAT system.

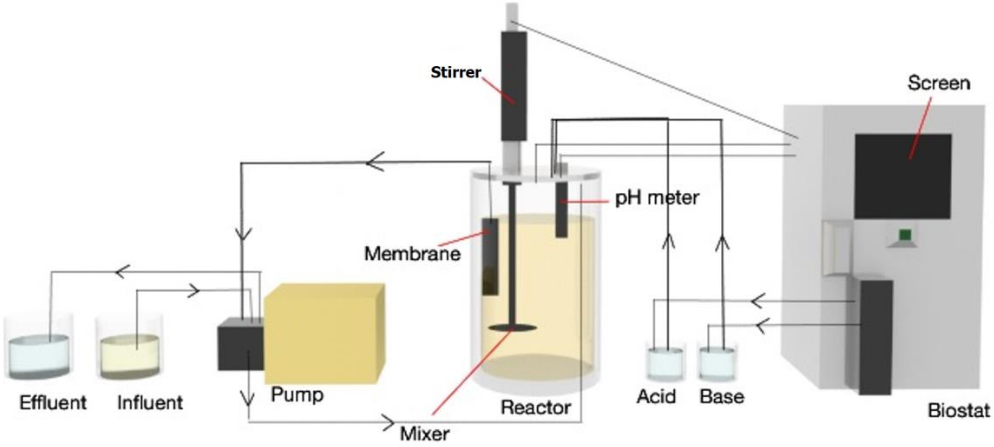
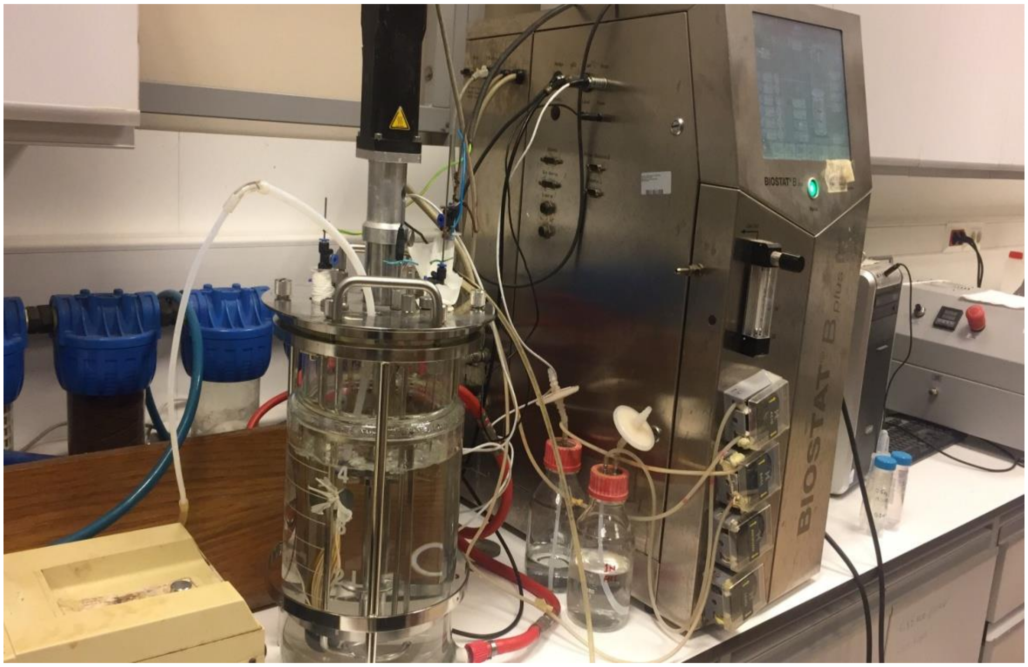


Figure 2

Real image of the submerged membrane reactor attached to a bench top BIOSTAT system



Total Kjeldahl Nitrogen and Ammonia Nitrogen

Total Kjeldahl Nitrogen (TKN) measures the sum of organic nitrogen, ammonia, and ammonium. The method analyzes all the three (3) negative states of nitrogen present in sample, but fails to account for nitrogen in the form of azide, azine, azo, hydrazine, nitrate, nitrite, etc. TKN determinations followed the procedure described in Section 4500-Norg B of Standard Methods (American Public Health Association-American Water Works Association-Water Environment Federation, 1998), and the equipment used was the Kjeldatherm block-digestion-system and a Vapodest 20s distillation system. The method to determine TKN consists of heating the sample with Sulphuric acid, which later decomposes the organic substance by oxidation to liberate and then reduced nitrogen as ammonium sulphate. In this step, potassium sulphate is added to increase the boiling point of the medium and cupric sulphate as catalyst. After samples digestion, a three (3) drops of sodium hydroxide (NaOH) was added, and the resulting ammonia solution is distilled from an alkaline medium. After drops of NaOH addition, the addition of boric acid to the solution containing mixtures of indicators (methyl red and methylene blue) was made. The ammonia presents in the sample, corresponding to the amount of nitrogen contained in the initial sample, reacts with the boric acid, and is determined indirectly by the titration of the borate ion formed with a H_2SO_4 standard solution. The determination of ammonia nitrogen followed the same procedure described in Section 4500-NH3 B and Section 4500-NH3 C of Standard Methods, using the same distillation and titration systems used to determine TKN. The determination of ammonia nitrogen is like the determination of Kjeldahl nitrogen, although in this case the sample does not undergo digestion. For the Ammonia Nitrogen (AN) analysis, samples were buffered at pH 9.5 level and borate buffer is then used to decrease the hydrolysis of cyanides and organic nitrogen compounds. Then, they were also distilled into a solution containing boric acid, and a mixture of indicators used in TKN determination, and later titrated with a standard H_2SO_4 0.2 N solutions. The distillation system was automatically operated for 4min and the collected sample was titrated with 0.02N sulfuric acid (H_2SO_4) and the titer value is used to calculate the NH4-N concentration using the formula below as defined in standard method(American Public Health Association-American Water Works Association-Water Environment Federation, 1998).

$$NH4 - N = \frac{\text{Titre value}*\text{N}*14000}{\text{Volume of sample (mL)}} \tag{6}$$

Data Analysis and experimental reproducibility

The ammonium nitrogen analysis was determined using the Kjeldahl's method (American Public Health Association-American Water Works Association-Water Environment Federation, 1998). The initial reaction velocity v was determined from equation below.

$$v = \frac{dC_{t}.}{\text{dt}} \tag{7}$$

Where $\text{dC}_{t}. =$ concentration of NH4-N in mg/L, $d\text{t} =$ time taken for the conversion

Experiments were performed in duplicate, to ascertain the reproducibility of the process. For the statistical analysis, SPSS version 21.0 was used and for the analysis of significance in comparison of multiple experimental results a One-way ANOVA (with Turkey post hoc test) was used. The student’s t-test was used in order to evaluate statistical differences between the results.

pH and conductivity measurement

In this work, the pH of the samples, thermostatised at 20 ºC, was measured using a pH meter CRISON 2000pH Electrode. Conductivity is a measure of the ability of an aqueous solution to carry an electric current. The ability depends on the presence of ions, on the total concentration and mobility, and on the temperature. In this work, samples conductivity was measured using a conductivity meter. For the batch hydrolysis, the pH of each batch sample was measured using CRISON 2000pH electrode (HachLange Spain, S.L.U.,Spain). The continuous system's pH levels were maintained by automatic dosing of sulfuric acid or sodium hydroxide solution.

SEM and EDAX Technique

SEM and EDX or EDAX are two chemical micro-analysis techniques that are performed together in order to analyse or measure the atomic composition in a membrane before and after use in this study, for proper monitoring of the membrane and to minimize the fouling effect in the membrane used. It is also used as an excellent tool to study membrane failure as well as the issue of membrane fouling. SEM and EDX analysis are very important instrumental techniques in identifying two important causes of reduced membrane performance and restricted membrane lifetime. The membranes used in this study were washed thoroughly with distilled water before being inserted into the reactor. After the system (EMR) was run, the used membranes were removed, weighed with an analytical balance mettler (weighing balance), and dried at 60^{O}C before being taken for EDAX analysis. The membranes were analysed by an analyser of dispersed energy, model EDAX marks Dxprime, with a CDU leap detector. Topographic analysis was also conducted using a JSM-5800LV microscope (JEOL). Morphology image are formed when the primary electron beams interact with the sample secondary electrons, while information on the sample composition is formed as a result of the back-scattering of the electrons. To obtain the elemental composition, we analysed the topography images as well as the secondary X-rays emitted.

Using other AI models to predict the effect of some input variables on urease activity in urea hydrolysis.

Material and methods

Data collection

The data used for this study was obtained from the real experiment conducted in the laboratory at the wastewater laboratory, Gebze Technical University Kocaeli, Turkey. Laboratory culture experiments were conducted at different ionic strength (I) measures (0, 0.01, 0.05), different ammonium sulphate $(NH_4)_2SO_4$ addition (0, 100, 200, and 500mg/L), and different feed concentration of urea as N (100, 500mg/L) inside a continous Enzymatic Membrane Reactor (EMR) system. The data used for the predictions was the same real data used for the experiment used in the result figure (5, 6, and 7). Pearson correlation were used for the selection of the relevance of each of the potential input parameters for estimating the Ammonium nitrogen recovery, and lastly evaluation and comparing the result of the model with that of the experimental analysis.

Performance Evaluation and data normalization

Before feeding data to AI models, data normalization is conducted where the data is normalized by putting all predictor and regressor variables into the same range in order to avoid higher numeric range data to be overshadowed the lower numeric range data. (Vahid Nourani, Gökçekuş, et al., 2020). Another advantage of normalizing data is to simplify the numerical calculations of the model which reduces the time taken for convergence as well as increases model's accuracy. In this study, the data collected were normalized between the range of 0 and 1 using the equation below.

$$x_{norm} = \frac{x - x_{min}}{x_{max} - x_{min}} \qquad (8)$$

Where x_{norm} is the normalized data value x, x_{min} *and* x_{max} are the observed, minimum and maximum data, respectively.

After the normalization, this study's experimental and simulated outcomes were assessed using five distinct performance efficiency criteria, including; the Nash Sutcliffe efficiency (NSE), root mean square error (RMSE), correlation coefficient (R), mean square error

(MSE) and mean absolute percentage error (MAPE) as shown in the table 4 below; The NSE values ranges from -∞ to 1 and is a parameter that indicates how well the model fits. A model is said to be perfect, if it has NSE value of 1 and the model efficiency decreases as the value of NSE moves far from 1 and vice versa Nourani et al., (2020a). The accuracy of the model can be interpreted based on the R^2 values as very good when ($0.75 < NSE \leq 1$), good when ($0.65 < NSE \leq 0.75$), satisfactory when ($0.50 \leq NSE \leq 0.65$) and unsatisfactory when ($NSE< 0.50$) (Moriasi et al., 2007). RMSE is used for measuring the average error produced by the models and one of the best used measures for computing the model performance. The RMSE value ranged between 0 and +∞ and is zero in the best model (Nourani and Sayyah 2012). MSE is the sum of the square of the differences between the observed values and predicted, although residual show proportionality effect at an individual level in MAE, the consequence of square raise MSE always higher than the MAE and the effect of outliers can easily get recognize (Pham et al., 2019). Moreover, MAPE is robust like MAE and provide clear understanding since percentages are easily conceptualized. However, applications of MAPE are reported to have many short-coming resulting in the use of division process, the data value might be higher when the actual value is remarkably small, also the biasness of MAPE towards predictions is analytically lower than the actual values. Additionally, MAPE become undefined when the data contains zero value. Finally, RRMSE was also used, which could be evaluated based on the defined ranges: Excellent for RRMSE values less than10%, Good for values between 10% and20%, Fair for RRMSE values between 20% and30%, and Poor if RRMSE value is greater than30% (Rabehi et al., 2020).

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(N_{obs_i} - N_{pre_i}\right)^2}{\sum_{i=1}^{n} \left(N_{obs_i} - \overline{N_{pre_\iota}}\right)^2} \qquad (9)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(N_{obs_i} - N_{pre_i}\right)^2}{n}} \qquad (10)$$

$$R = \frac{\sum_{i=1}^{n}\left(N_{obs_i} - \overline{N_{obs_\imath}}\right)\left(N_{pre_i} - \overline{N_{pre_\imath}}\right)}{\sqrt{\sum_{i=1}^{n}\left(N_{obs_i} - N_{obs_i}\right)^2 \sum_{i=1}^{n}\left(N_{pre_i} - N_{pre_i}\right)^2}} \tag{11}$$

$$MAPE = \frac{100\%}{N}\sum_{i=1}^{N}\left|\frac{y_i - \hat{y}_i}{y_i}\right| \tag{12}$$

$$i = 1,2,3 \ldots \ldots \ldots \ldots . N$$

Wherey_i , $\hat{y}_i$, $\bar{y}_i$ and N stands for experimental data, predicted data, average value of experimental data and total number of data instances respectively.

Artificial intelligence models

Artificial Neural Network (ANN)

ANN is a machine learning information- processing approach that is used to analyse data using a network of decision layers. It mimics the human brain as a data processing and analysing software, and works through try and error process. The process consists of neurons which are processing elements that are connected by synaptic. Generally, ANN consist of input layers, hidden layers and output layer, where data are introduced into the ANN, processed and results are produced respectively. The input layer sent the values it collected to the available neurons present in the hidden layer and then will be transformed it into a valuable information by the hidden neurons as output. All neurons involved received information from a neuron with which they are connected to. A function called the activation function is then used to transform the total number of weighted inputs plus that of the bias. Equation 15 and 16 are the logical tangent and hyperbolic tangent and are the most common used activation functions. When the activation functions change the weighted sum of the input, the outputs get closer to 1 and when the input get larger, and smaller when the input is 0. Because of its resilience and the well-known and readily accessible software to that can be applied. ANN has been used in a great number of research in chemical, transportation and other fields of engineering in general in recent years. Two-layered feed-forward network with sigmoid hidden neurons and linear output neurons using the Levenberg-Marquardt backpropagation method as the training algorithm are the conventional ANN model topology.Despite the fact that it is considered a "black box" technology, several researchers have employed a variety of ways to address this problem and give in-depth analysis and interpretation of ANN models. Equation 17 and 18 below are used for calculating the output z_j of the neuron j in the ith layer.

$$f(x) = \frac{1}{1+e^{-x}} \tag{13}$$

$$f(x) = \frac{e^{x}-e^{-x}}{e^{x}+e^{-x}} \tag{14}$$

$$Q_j = \sum_{i=1}^{n}(w_{ij}x_i + b_i) \tag{15}$$

$$z_j = f(Q_j) \tag{16}$$

Where Q_j is the activation value in the neuron j in the ith layer, x_i is the ith input vector of the n input, $f(x)$ is the activation function and w_{ij} is the weight of the ith input, and the neuron j and b_i are the bias in the ith bias term. To achieve the desired output, weight that represents the connection strength between neuron and bias are adjusted using different training inputs and corresponding output values. For the training of a neural network, Back-propagation (BP) algorithm are mostly used (Vahid Nourani, Gökçekus, et al., 2020).

Multi linear regression (MLR)

In engineering sciences, linear regression analysis is a typical approach for modeling and analyzing many variables. Regression analysis is particularly useful for understanding how typical the value of a dependent variable changes when one, two or more of the independent variables (inputs) is changed while the other independent variables remain constant, as well as for exploring the interactions that describe the relationship between these variables (Doğan & Akgüngör, 2013). The dependent variable and regressor variables may be used for the input parameters using the expression below (Elkiran et al., 2018):

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \cdots + b_ix_i + \xi \tag{17}$$

Where x_i represents the value of the i^{th} predictor, b_0 s the regression constant, b_i as the coefficient of the i^{th} predictor and ξ as the error term.

Gaussian Process Regression (GPR)

Gaussian process regression can be referred to as any robust non-linear prediction model, probabilistic, nonparametric, supervised, and unsupervised learning method that generalizes the non-linear and complex function mapping hidden in data sets. Recently, GPR has increasingly attracted the attention of researchers from different engineering fields (Cheng et al., 2013; Omran et al., 2016). GPR is capable of handling non-linear data due to the use of kernel functions. Moreover, one of the meritsof a GPR model is that the model can provide a reliable response to input data (Pal & Deswal, 2010).

For a training set M = $\{(x_i, y_i) \mid i = 1, \ldots, n\}$, the inputdata $X \in R^{M\times n}$is known as the design matrix and $y \in R^n$is thevector of the target output. The primary assumption of the GPR model is that the output y is evaluated as (Williams & Rasmussen, 2006).

$$y = f(x)+\varepsilon \qquad (18)$$

where, $\varepsilon \sim N(0,\sigma_n^2)$, ϵ R is the homoscedastic noise of the all samples x_i

Support Vector Regression (SVR)

SVR was designed based on the Support Vector Machine (SVM) conception, which is normally used for non-linear classifications and regression of problems (Vahid Nourani, Gökçekus, et al., 2020).SVR is a well-known engineering regression problem with several benefits such as high learning speed, superior generalization ability, and good noise-tolerating (Moriyama et al., 2016; Muthukumar et al., 2003). SVR used a kernel function for mapping the data from the sample space into a higher dimensional characteristic space. This regression model can transform a non-linear problem into a linear problem by learning the complex relationships between dependent and independent variables. SVR models have been employed in several regression analyses in the engineering field (Ahmad et al., 2020).

Consider training dataset with n points. $\left\{(x_1, y_1), (x_2, y_2)(x_3, y_3 .... (x_n, y_n)\right\}$ Where $x_i \in R^n$ are the input variables, $y_i \in R^n$ are the target variables of x_i, N is the number of datasets, determine the mapping function $f(x) \in R^n$ to explain the correlation of independent values $x = \{x_1, x_2, x_3 ...... x_i, x_N\}$ and dependent values $y = \{y_1, y_2, y_3 ...... y_i, y_N\}$ is the regression problem. Therefore, linear SVR regression function can be expressed as in Eq. (4).

$$f(x) = w\phi.(x) + b \qquad (19)$$

Where $\phi(x)$ defined the non-linear mapping function, ***w*** is the weight vector, *b* is the bias.

CHAPTER III Summary

First, Batch enzymatic hydrolysis experiments were carried out at various substrate concentrations in order to determine the enzyme-substrate affinity and digestibility. Secondly, a continous system was run and the hydrolysate samples were obtained from an optimized continuous EMR system coupled with a UF membrane of 250 KDa MWCO. Two different feed concentrations (urea as N) of 100mg/L and 500 mg/L were used to analyze the effect of feed concentration on urea hydrolysis. Laboratory experiments were conducted at room temperature of 22^{O}C, a flow rate of 25 mL/min, urease concentration of 0.067 g/L, ionic strength of I = 0, 0.01, and 0.05, and ammonium nitrogen addition of 0, 100 mg/L, 200 mg/L, 500 mg/L. This chapter also provides the theoretical background of the different models used for the temperature effect on urea hydrolysis. The chapter also described the different evaluation criteria used for assessing the performance of the models as well as the validation method used. The summary of the observed data as well as explanatory details of the measured data were discussed in this chapter.

CHAPTER IV

Results and Findings

This chapter provides information about the result and findings based on the collected data conducted on the research and the analysis conducted throughout the study.

Effect of pH on enzyme (urease) activity

To study the effect of pH on urease activity over a range of substrate concentrations, sodium hydroxide (NaOH) and Hydrochloric acid (HCl) were used to raise and drop the pH values. Enzyme activity was evaluated using the Michaelis-Menten Equation (see in table 3) at different influent pH levels (4.0, 6.0, 7.0, 8.0, and at no pH control) and at room temperature (20±2^{O}C). The effects of reaction pH on enzyme (urease) activity at different pH values are presented in Table 4 and Figure 3 below. The results indicated higher enzyme activity at no control pH, (Km =223.65mg/L andVmax =13.83), which are lower compared to all other pH values even though the regression coefficient is not the highest. Based on the results shown below, the values of the enzyme kinetic parameters determined from the Lineweaver-Bulk Plot were selected as the best for urea hydrolysis.

Figure 3

Lineweaver-Burk plot.

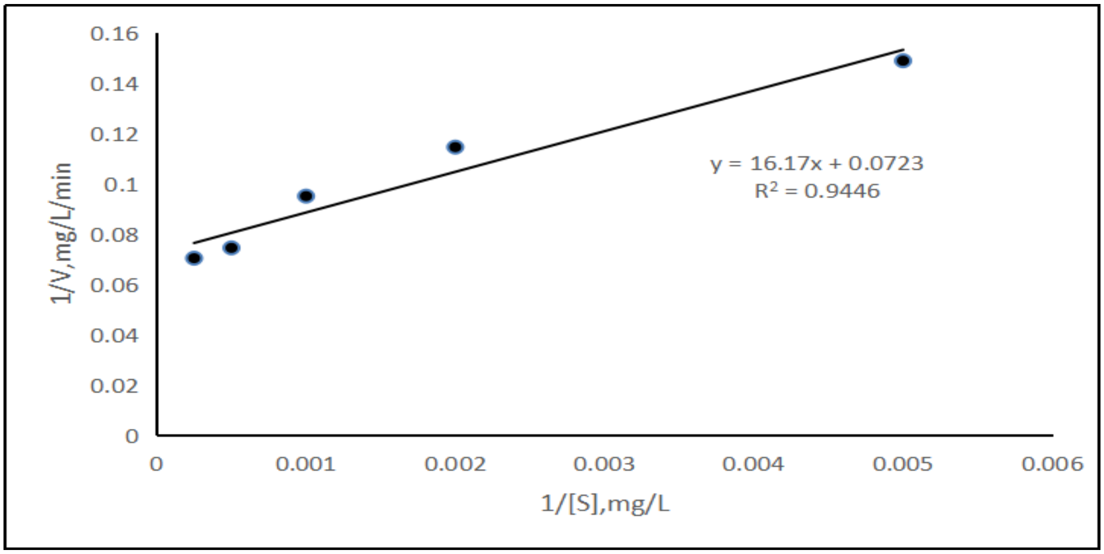


Table 6

Enzyme kinetic values for Km and V_{max} at different pH values by using the linearized model

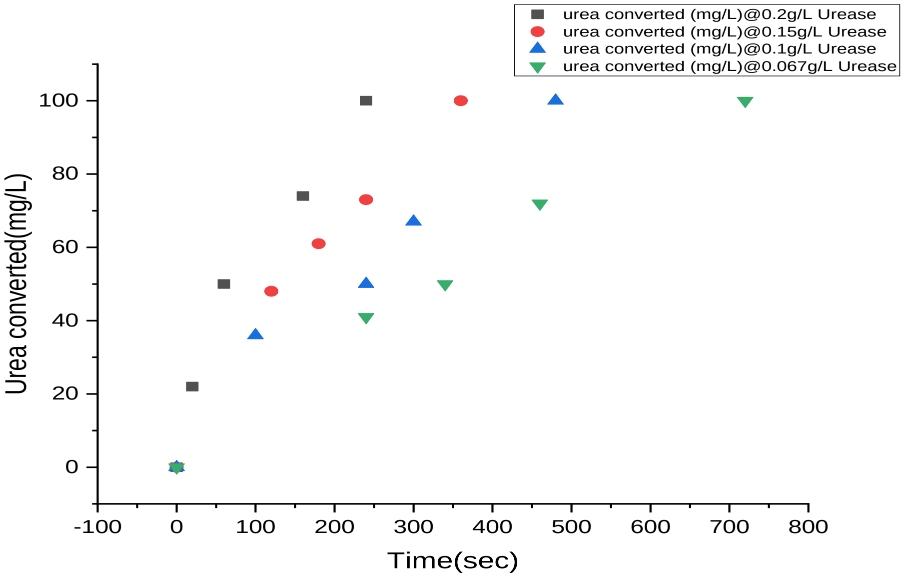
pH	Kinetic parameters	Lineweaver-Bulk	Hanes Woolf plot
No control pH	Km (mg/L)	223.65	354.15
	Vmax (mg/L.min)	13.83	15.48
	R^2	0.9446	0.9973
pH 4	Km (mg/L)	334.73	724.69
	Vmax (mg/L.min)	15.77	20.2
	R^2	0.9601	0.9824
pH 6	Km (mg/L)	353.35	675.26
	Vmax (mg/L.min)	12.37	15.8
	R^2	0.8066	0.9854
pH 7	Km (mg/L)	418.47	525.23
	Vmax (mg/L.min)	15.1	16
	R^2	0.9724	0.9873
pH 8	Km (mg/L)	630.25	1042
	Vmax (mg/L.min)	15.43	16.81
	R^2	0.9084	0.9078

Effect of Enzyme Concentration on batch hydrolysis of urea

In this study, a sample containing 100mg/L urea as N was mixed with different concentrations of urease enzyme in a batch reactor. Urease (EC.3.5.1.5 Lyophilized) at 0.2, 0.15, 0.1 and 0.067g/L were being poured into the batch system in order to measure the activity of the urease at different concentration and the time taken for the hydrolysis was measured and recorded. All this is done at room temperature and an effluent pH of between 7-7.5.

Figure 4

Rate of Urea Hydrolysis by Urease Activity.

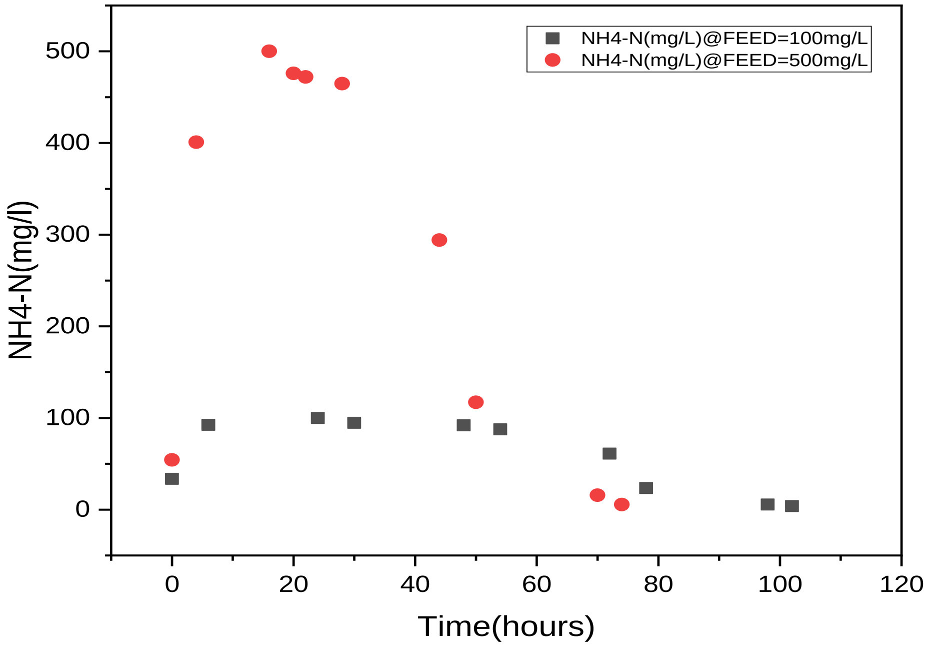


Feed concentration effect on urease activity.

The effect of feed concentration on urease activity as well as the maximum urea conversion rate was studied at 100mg/L and 500mg/L feed concentration of urea-N with an enzyme concentration of 0.067g/L and a flow rate of 25mL/min. The experiments were performed at room temperature (20±2°C) with no pH control, as the activity of the urease can be affected by both pH and temperature variations.

Figure 5

Effect of feed concentration on urease activity in urea conversion.

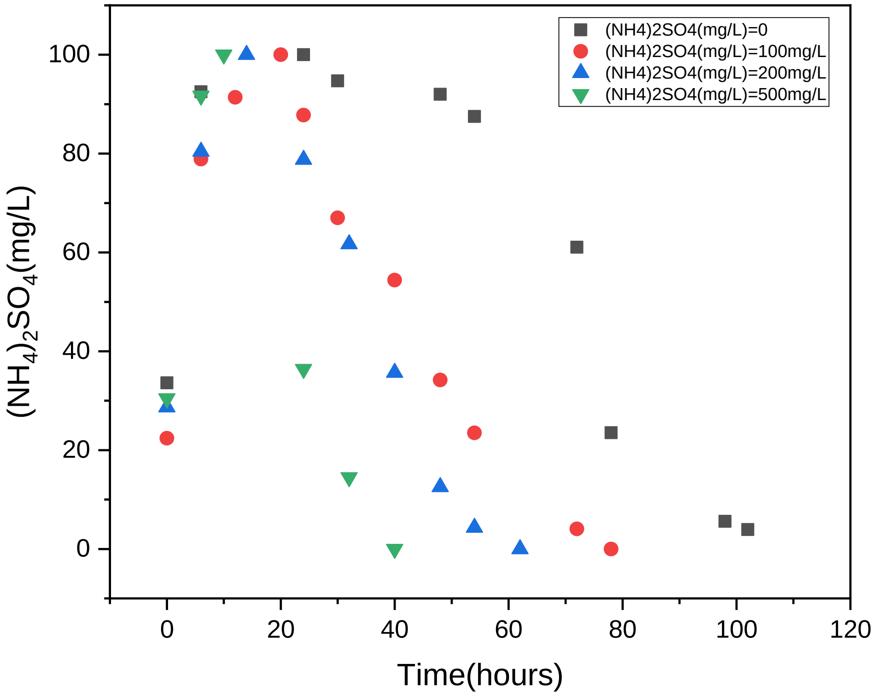


Effect of Ammonium sulphate $(NH_4)_2SO_4$ addition on urease activity.

In this case, ammonium sulphate $(NH_4)_2SO_4$ was added to the continuous reactor in order to determine its effect on urea hydrolysis. Concentrations of $(NH_4)_2SO_4$ were varied from 0mg/L, 100mg/L, 200mg/L and 500mg/L in a continuous reactor containing urea (100mg/L) and urease (concentration of 0.067g/L) at a stirring speed of 200rpm and at room temperature (20±2°C). The pH of the substrate was kept the same without being altered and all analyses were performed at room temperature.

Figure 6

Effect of ammonium-nitrogen addition on urease activity in urea conversion.



Effect of feed concentration pH on urease activity.

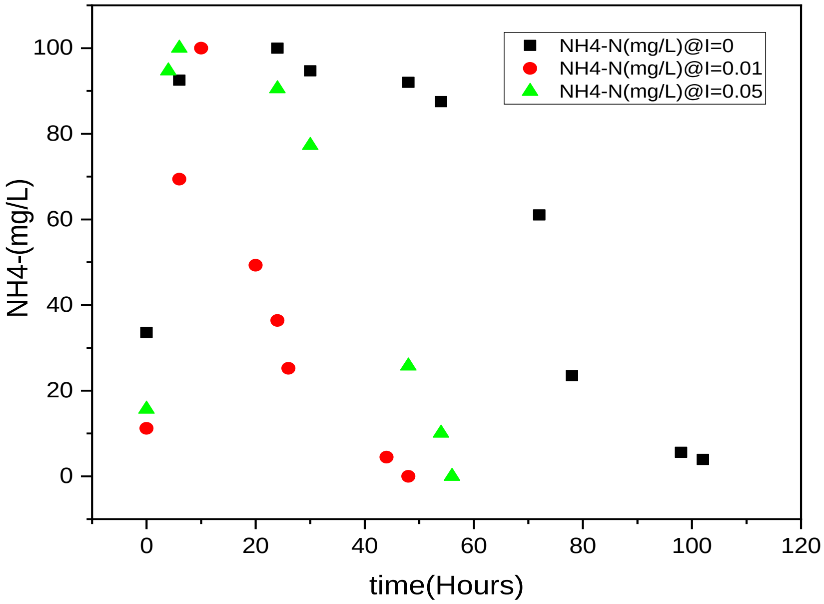
ThepH effect on urease activity was also analyzed in the range of pH 4-9 and at no pH control in a batch system experiment in this study; Lineweaver andHanes Woolf plot were used to evaluate the enzyme activity at different pH values and at room temperature of 20±2^{o}C, and the results were presented in Table 2 above. The results indicate higher enzyme activity at no pH control with K_m=223.65mg/L and a V_{max}=13.83. Lower the value of K_m, the higher the enzyme-substrate affinity shows good relationship between substrate and enzyme and vice versa. The batch system results serve as a reference for the continuous system.

Effect of Ionic Strength on urease activity.

In this study, ionic strength effect was also analyzed by the addition of sodium chloride (NaCl) to the feed concentration. This was done in different ionic strength concentrations of I=0, 0.01, and 0.05. Enzymes can be precipitated or denatured by the action of salts, solvents and other reagents.

Figure 7

Effect of ionic strength on urease activity in urea conversion



Analysis of membrane surface and fouling

Membrane autopsy was performed by Scanning Electron Microscopy (SEM) for analysis of membrane morphology which was equipped with an energy-dispersive system of X-ray (EDS). The physicochemical or elemental features of the membrane were investigated using (EDAX) coupled with the SEM. The EDAX of the secondary X-ray emitted by the sample exposed to the electron beam showed the presence of some component on the surface of the membrane.

Figure 8

SEM images of the used UF membrane after being used inside the reactor.

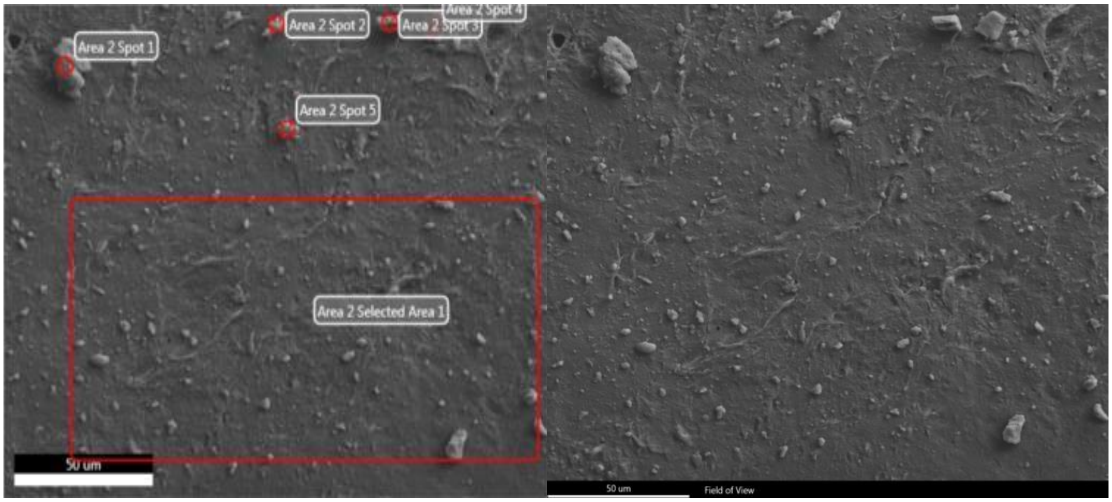
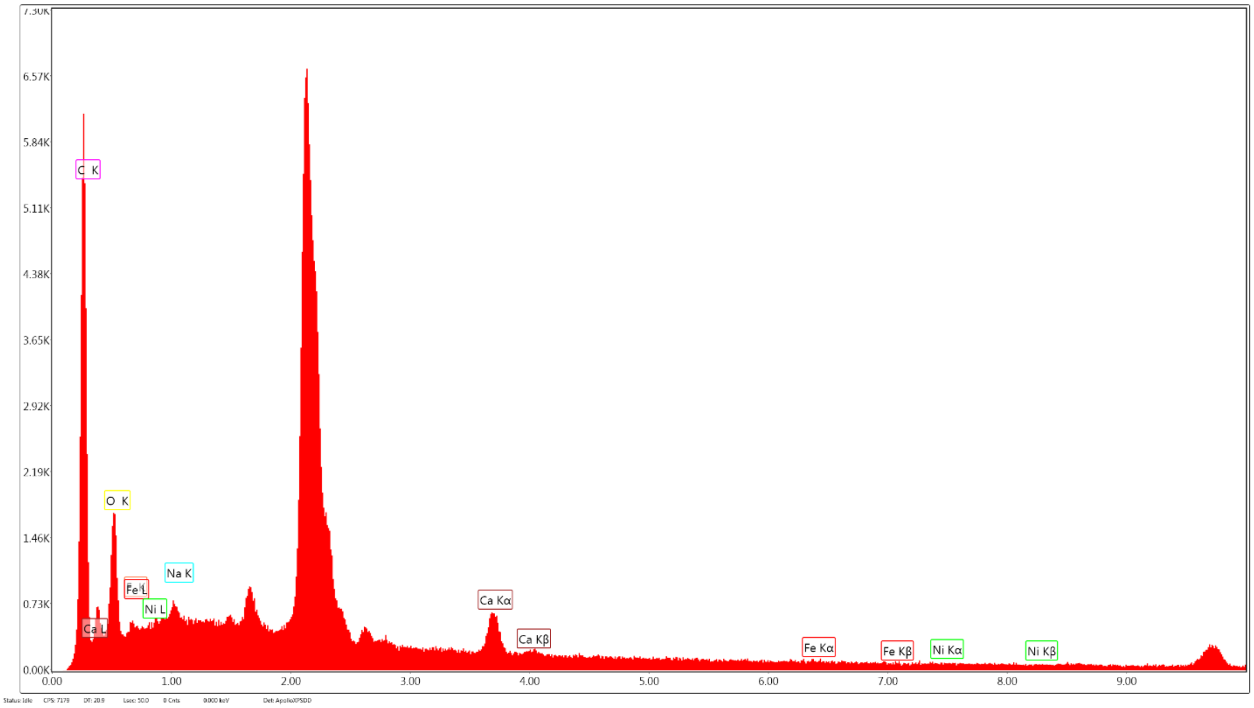


Figure 9

Chemical composition of Ultra filtration membrane surface determined by the EDAX Technique (spot 3 and that of selected area 1).



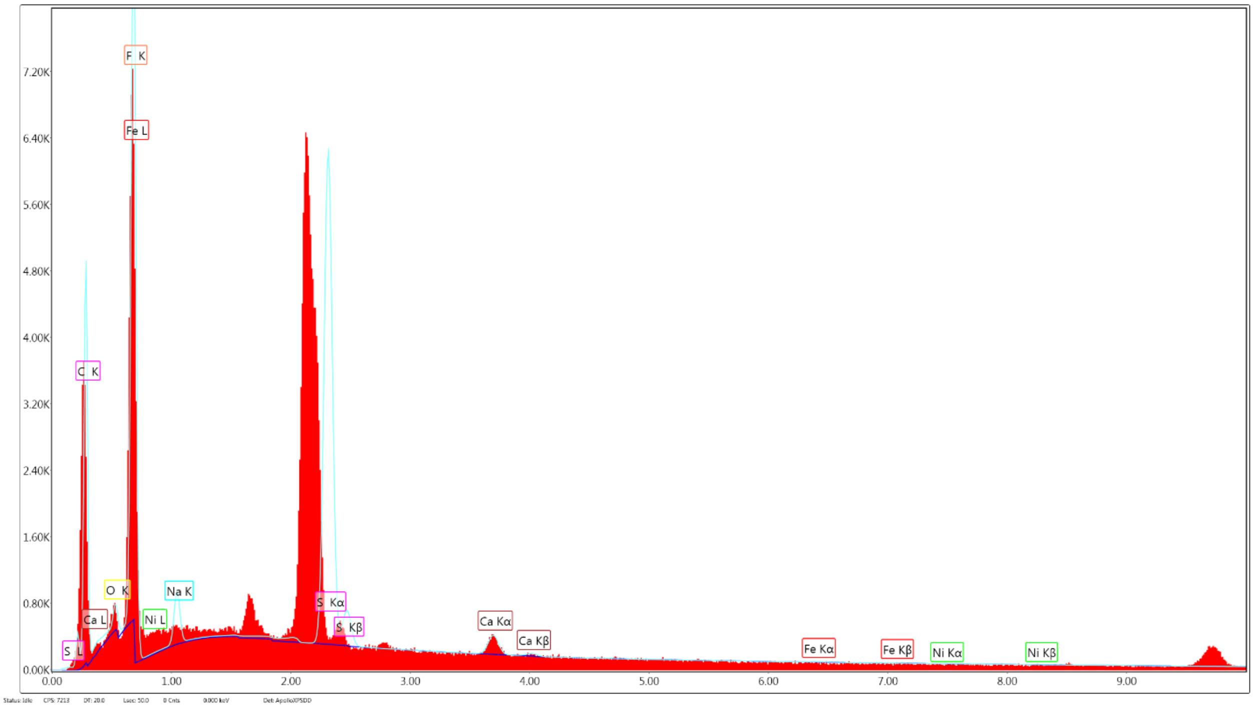


Table 7

Elemental composition of the used membrane surface determined by the EDAX technique at different spots.

Spot 1	Element	Conc.(%mass)	Net Int. Error	Spot 2	Element	Conc.(%mass)	Net Int. Error
	C	58.37	0.01		C	18.57	0.01
	O	26.93	0.01		O	42.16	0.01
	F	3.12	0.06		F	8.48	0.01
	Na	3	0.04		Na	2.78	0.02
	Ca	4.76	0.05		Mg	1.48	0.03
	Fe	1.61	0.32		Al	0.78	0.03
	Ni	2.21	0.21		Ca	25.48	0.01
	Total	100	-		Fe	0.13	0.57
					Ni	0.12	0.56
					Total	100	-

Table 8

Elemental composition of the used membrane surface determined by the EDAX technique

Spot 3	Element	Conc.(%mass)	Net Int. Error	Spot 4	Element	Conc.(%mass)	Net Int. Error
	C	13.8	0.01		C	10.96	0.01
	O	40.32	0.01		O	46.67	0.01
	F	7.98	0.02		Al	0.79	0.02
	Na	2.9	0.03		Ca	41.58	0
	Al	1.18	0.02				
	Ca	33.52	0.01				
	Fe	0.18	0.58				
	Ni	0.13	0.58				
	Total	100			Total	100	

Table 9

Elemental composition of area 2 (selected area 1) membrane surface determined by the EDAX technique

Spot 3	Element	Conc.(%mass)	Net Int. Error
	C	40.26	0.01
	O	5.29	0.03
	F	30.26	0.01
	Na	0.82	0.01
	S	15.74	0.03
	Ca	1.45	0.06
	Fe	0.12	0.59
	Ni	0.07	0.57
	Total	100	

AI Model results

Table 10

Result of the AI Models with temperature as input variable

Models		NSE	RMSE	MAE	PBIAS	R
ANN	Training	0.9916	0.0261	0.0179	0.0555	0.9957
	Testing	0.9716	0.0310	0.0235	0.1241	0.9799
MLR	Training	0.7036	0.1002	0.0693	0.3605	0.8256
	Testing	0.6554	0.1674	0.1160	0.3605	0.8075
SVR	Training	0.9744	0.0456	0.0350	0.1089	0.9870
	Testing	0.8197	0.0781	0.0498	0.2635	0.8900
GPR	Training	0.9999	0.0001	0.0000	0.0002	1.0000
	Testing	0.9999	0.0005	0.0003	0.0016	1.0000

Table 11

Result of the AI Models without temperature as input variable

Models		NSE	RMSE	MAE	PBIAS	R
ANN	Training	0.9960	0.0117	0.0080	0.0424	0.9973
	Testing	0.9621	0.0555	0.0314	0.0977	0.9806
MLR	Training	0.5070	0.2002	0.1410	0.4384	0.7238
	Testing	0.3291	0.1507	0.0996	0.5266	0.8244
SVR	Training	0.9485	0.0647	0.0440	0.1367	0.9763
	Testing	0.7463	0.0927	0.0553	0.2924	0.8348
GPR	Training	0.9999	0.0001	0.0001	0.0002	1.0000
	Testing	0.9999	0.0004	0.0002	0.0013	1.0000

Figure 10

Multi-scatter plot of both training and testing for SVR, GPR, ANN and MLR with temperature as a dependent variable

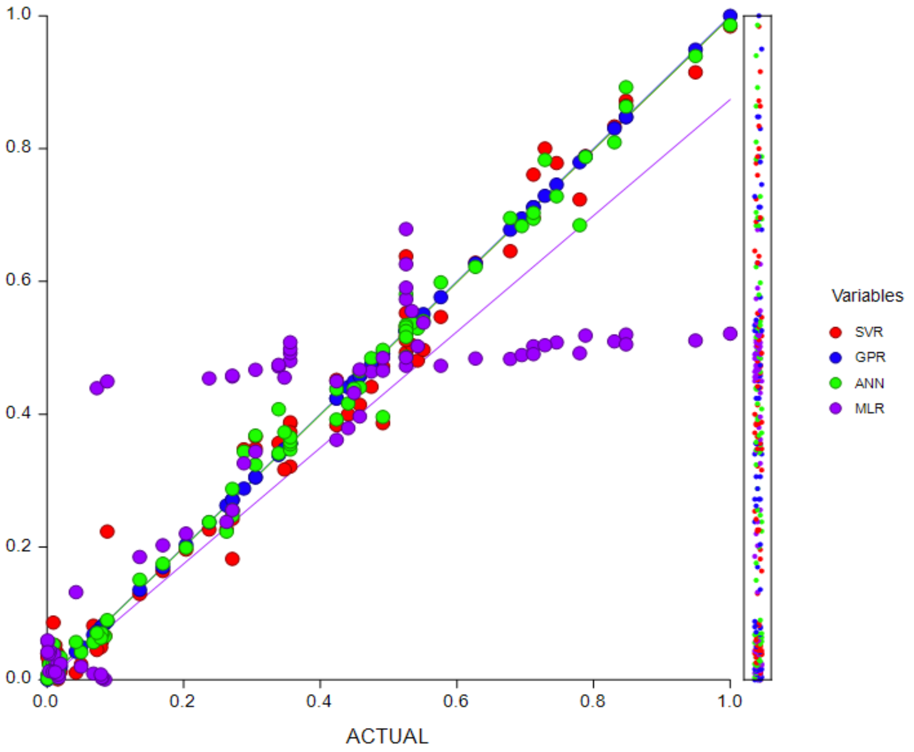


Figure 11

Taylor diagram comparing the performance of the developed models in the prediction of Ammonia recovery with temperature as a dependent variable.

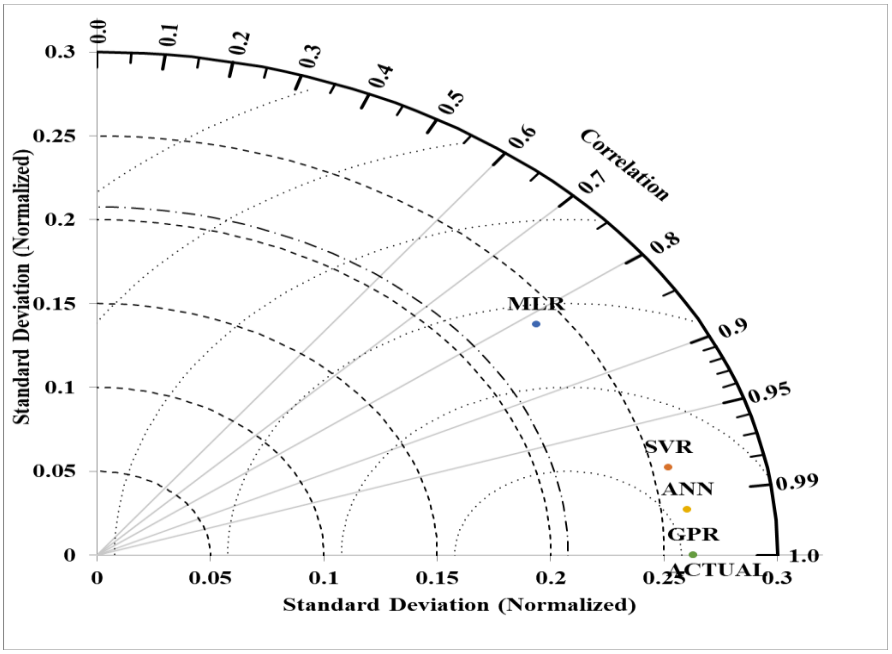


Figure 12

Multi-scatter plot of both training and testing for SVR, GPR, ANN and MLR without temperature as a dependent variable

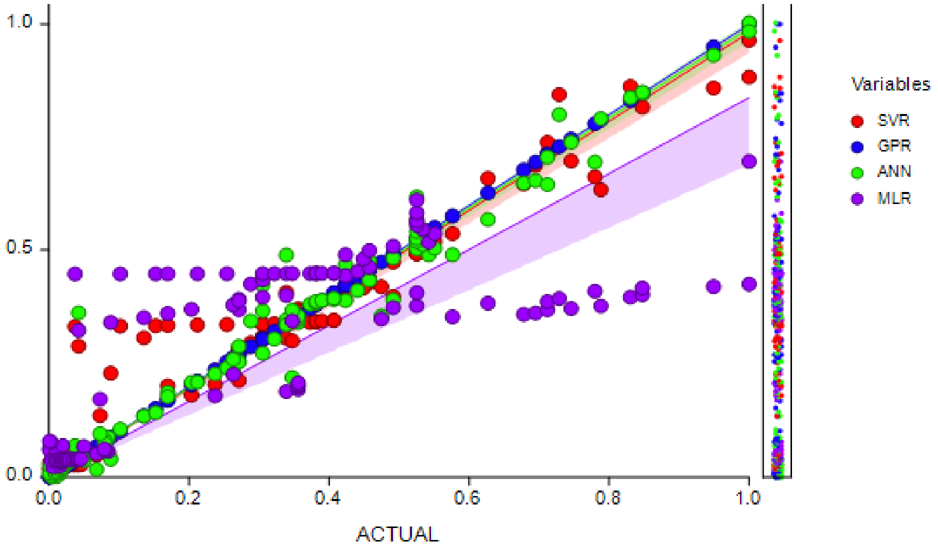
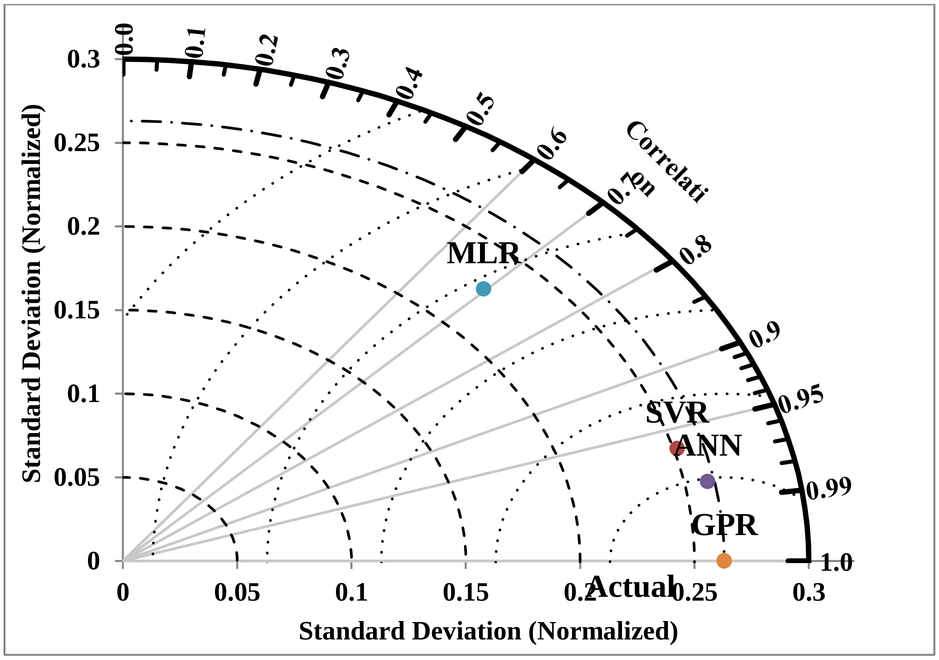


Figure 13

Taylor diagram comparing the performance of the developed models in the prediction of Ammonia recovery without temperature as a dependent variable.



CHAPTER IV Summary

This chapter provide the results as well as the findings of the study. The ionic strength effect, NH4-N concentration effect, feed concentration effect, and enzyme concentration effect on urea hydrolysis were determined in the continuous Enzyme membrane rector (EMR). Moreover, (SEM) and (EDAX) analysis were also used in identifying and examining the surface morphology, physicochemical properties and other elemental composition of the UF membrane used in order to determine the changes in the pore sizes of the membrane. Furthermore, AI model results were also presented in this chapter for the prediction of effect of temperature on urea hydrolysis. The models were evaluated using the NSE, RMSE, MAPE, RRMSE and R.

CHAPTER V

Discussion

This chapter presents the discussion of these findings in comparison to the studies in the literature.

Effect of pH on enzyme (urease) activity

The kinetic data were analysed at different pH values using the initial rate method. Both Km and Vmax values were calculated using linear equations and the results are presented in Table 4 above. From the results obtained, it shows different values of Km and Vmax calculated at no pH control value to a pH value of 8. The Km and Vmax values at no pH control for the Lineweaver-bulk and Langmuir models were calculated to be 223.65 and 354.15m/L, and 13.83 and 15.48mg/L/min, respectively. The lower the Km value reveals higher the affinity of enzyme for the substrate and vice versa. Normally, the effect or influence of pH on activity is usually caused by perturbations of enzyme distributed among differently protonated forms (Range 4-9).

A study conducted by (M.S.Cabral, 1994) found sharp dependence of both Km and Vmax on pH when analysing urease in a pH range of 4 to 9. Other researches made have proved that urea hydrolysis follows a simple M-M Kinetics, were the reaction rate increases with increase substrate concentration to the extent that the saturation point is reached where the substrate concentration is sufficient to saturate the amount of enzyme used (Cabrera, 1991).

Another study by (Rachhpal-Singh, 1984) showed that the pH effect may also depend on different urea concentrations; this implies that the kinetic parameters of urease activity can be affected by soil pH. Another research by (Ray & Saetta, 2017) found how controlling the urea hydrolysis reaction can also be inhibited by lowering the pH to 4-4.5 by adding a dilute acid such as acetic acid or increasing the pH to above 11 by adding base.

Effect of Enzyme Concentration on batch hydrolysis of urea

In Figure 3, the graph shows that the time taken for urea to hydrolyse in any wastewater containing urea depends on the amount of urease present or added. This also implies that the higher the enzyme urease concentration, the greater the conversion/hydrolysis. For this study, we will be using a urease concentration of 0.067g/L. This result agrees with a study conducted by (Tunay & Tatli, 2006) where urea hydrolysis is studied on human urine samples and the result of the hydrolysis shows enzyme concentration as being very efficient measure to consider when studying rate of hydrolysis.

Moreover, the lifetime of a membrane used in an enzymatic process is prolonged by the action of the enzyme, and most enzymatic activities take place at lower temperatures and mild pH ranges. In a study by Munoz-Aguado in1996, it was reported that the use of lower amounts of enzymes in the membrane process can result in lower cleaning efficiency and higher cleaning duration, whereas higher enzymatic concentrations do not necessarily increase the cleaning action, thus increasing membrane fouling (Shi et al., 2014; Wiley & Fane, 1996).

Effect of Feed concentration on urease activity

Here, the effect of different feed concentrations of urea as N (urea as-N) on urease in the hydrolysis efficiency has also been studied to determine how much influent ammonia concentration the EMR can accept without affecting the hydrolysis efficiency as well as the urease activity inside the reactor. From the graph it can be seen from Figure 4 in the previous chapter shows the NH3-N concentration in the permeate side as a function of time. For a feed concentration of 100mg/L urea as-N, the ammonia concentration in the permeate increases gradually from an initial value of 33mg/L to a maximum conversion value of 100mg/L at 22hrs. Total conversion of urea to ammonia was achieved after 22 hrs and the maximum time taken for the urease activity to elapse was 102hrs. For the feed concentration of 500mg/L urea as N, the urea conversion to ammonia was shown to be around 50mg/L at 0 hours and reached a peak (500mg/L) at around 18 hours and the urease lost all its activity after 74 hours. It can be concluded from the graph that for any increase in initial concentration of urea, the conversion increases marginally at room temperature (20±2). From the results, it can also be concluded that the amount of NH_4-N produced by the system is directly proportional to the initial concentration of the urea in wastewater; however, urease (enzyme) activity was adversely affected by high feed concentration. These findings are in agreement with a research conducted by (Bremner & Chai, 1989), in which hydrolysis rate was affected by high urea concentration due to uncompetitive substrate inhibition or denaturation of enzymes at high urea concentrations. Though many researchers have demonstrated that urea hydrolysis follows M-M Kinetics, where increase in substrate concentration increases the rate of reaction or hydrolysis until a saturation point is reached. Another research by (Sahu et al., 2011) also goes in line with this result of this study, where the conversion or hydrolysis of urea was slightly affected by the initial amount of urea in the feed tank. The result shows increase in the initial concentration of urea decrease the hydrolysis action marginally. Hence, during the decomposition the solution, having high urea content, will give more ammonia, carbon dioxide and water vapor as product from the reactor, but decrease the urease activity.

A study by (Cabrera, 1991) determined the hydrolysis of urea concentrations ranging from 8.01 to 10 M and discovered that there may be two reactions for urea, the first reaction having higher affinity and the other lower affinity as a result of difference in feed concentrations. At urea concentrations less than 0.1 M, higher affinity is noticed and was responsible for the majority of the hydrolysis.But at 8 M, was only a tiny contributor is noticed. In general, the urea-N concentration at which both low and high affinity enzyme processes equally contribute is 0.5 M. the study also discovered that when the urea concentration is more than 6 M, the rate of urea hydrolysis slows down, possibly due to enzyme denaturation or substrate inhibition.

Effect of Ammonium sulphate $(NH_4)_2SO_4$ addition on urease activity.

As can be seen in Figure 5, urea hydrolysis to ammonia is the best with no addition of NH4-N, and this is achieved at around 10 hrs. Moreover, a drastic increase in the time for the urea to hydrolyse occurs as the ammonium nitrogen concentration is reduced, and this in turn increases the urease activity. The enzyme activity is higher with no NH_4-N addition [0mg/L $(NH_4)_2SO_4$)], although it takes more time to attain the maximum 100mg/L urea hydrolysis (28 hours). The results suggest that urease is inhibited by higher $(NH_4)_2SO_4$) concentrations. This concurs with a research conducted by (Tong & Xu, 2012). In a similar study by Kumar and Wagenet, urease activity was reported to be lowest in alkaline and saline (R.S.Dharmakeerthi, 1996). This research backs up a previous study in which divalent salts (Na_2SO_4 and $(NH_4)_2SO_4$) were added to an enzyme-containing solution. Salt action influenced the effectiveness of polyelectrolyte-protein complex formation; at low salt concentrations, the enzyme was dramatically stimulated, whereas at greater salt concentrations, it was inhibited. (Tikhonenko et al., 2009). These data can be used in order to evaluate the best working conditions for any urea hydrolysis process attached to wastewater treatment either on a pilot-scale or in big plants.

Effect of feed concentration pH on urease activity

The stability of enzymes is usually affected by the level of pH of the feed. Most enzyme activities are lost as a result of extremely high or low pH values. Optimum pH is achieved where the enzyme activity is the highest (7.0-7.4) as a rapid increase in pH may have a greater impact on urea hydrolysis (Engelhardt et al., 2020). For this study, a continuous system experiment was conducted using a UF membrane, but no pH adjustment was made as the feed solution pH was already at the optimum level for urea hydrolysis (from batch system experiment result presented in table 2.). this goes with the research conducted by (Nicolau, Fonseca, Rodríguez-Martínez, et al., 2014) that shows only slight differences were observed with a change in pH in the immobilization of urease. In another research conducted by Nelson and Cox (Nelson, D. L., 2004), the effect of pH values results on the variations of enzymatic activity and alteration in charges of the protein and substrates in the reaction were investigated. The optimum pH for the activity of jack bean urease was reported to be between 7 and 8 (Revilla et al., 2005).

Effect of Ionic Strength on urease activity

Catalytic activity is the key performing parameter of any enzymatic process and depends solely on the concentrations of the substrates and the product, as well as on the pH and ionic strength (Bolivar & Nidetzky, 2019). Many studies have reported that an increase in ionic strength reduces enzyme activity (Bosco et al., 2002; M. Casteneda-Agullo & J. R. Whitaker, 1961). Moreover, the stability and solubility of the enzyme as well as that of the substrate is found to be affected by increased ionic strength. Thus, the effect of salts on stability becomes a more important issue of concern. In a study by (Bosco et al., 2002), the ionic strength influence on the activity of lignin peroxides showed an S-shaped dependence of the activity of Lip Isoenzyme Mixture (LIM) with respect to ionic strength. All these were done to predict the behaviour of enzymes, ionic strength and the surrounding environment associated to the enzyme molecule. Figure 6, in the previous chapter shows the effect of ionic strength on enzyme (urease) activity for different ionic strength values of I=0, 0.01 and 0.05. The graph shows a substantial decrease in the activity of urease with an increase in ionic strength over time. The graph also shows how ionic strength serves as an inhibitor in urea hydrolysis. This research agrees with the research conducted by (Tikhonenko et al., 2009) in which the effects of different types of inorganic mono-valent salts were studied and the result shows that the solution of the mono-valent salts NaCl, KCl, and NH_4Cl decreases the enzyme urease activity drastically with an increase in ionic strength.(Tikhonenko et al., 2009).

This research also agrees with a recent of a research conducted by (Arqué et al., 2020) that shows how the presence of PBS, NaOH, NaCl, and HEPES reduced self-propulsion of a urease-powered micromotor pointing towards ion-dependent mechanisms of motion. Furthermore, the research concludes that the presence of ionic species (NaCl) clearly affects the motion of the enzymatic micromotors regardless of enzyme activity and pH.

Moreover, study conducted by (Gul, A.; Hruza, J.; Yalcinkaya, 2021) shows how ionic strength, pH, and particle electric charges can also cause fouling in membranes. Charge on the particles, charges on the membrane, stability of particles as well as the cake formation on used membrane all have are affected by ionic strength and the pH of the feed.

Analysis of membrane surface and fouling

The results from the SEM analysis show different elementary compositions at different spots and the selected area, and how these components are evenly distributed across all spots on the membrane as shown in Figure 7. The resolution in the images provided can be used to distinguish between a clean membrane and a membrane used in the reactor for easy identification. The EDAX results presented in Tables 3 and 4 below show the elements that are attached or get stacked to the membrane and the percentage of each element. For this study, the elements attached to the ultra-filtration membrane being used included a high percentage of oxygen, carbon, calcium fluorine, sodium and some traces of iron, nickel and aluminium.

The result obtained from Table 4. Indicates that carbon (58%), and oxygen (27%) were the major constituents in the spot 1 of the membrane, while calcium (Ca), Florine (F), Sodium (Na), Nickel (Ni), and Iron (Fe) were most abundant inorganic constituents at 4.8%, 3.12%, 3%, 2.2%, 1.6% respectively at the same spot. In spot 2, the major constituents in the membrane are Carbon (18.6%), oxygen (42%) and calcium (25%), while Florine (F), Sodium (Na), and magnesium (Mg) were the most abundant inorganic constituents at 8%, 2.8%, and 1.5% respectively. Other elements with trace composition were aluminium (Al), Iron (Fe), and Nickel (Ni).

From table 5. The result indicated major elements attached to the membrane at spot 3 as Carbon (13.8%), Oxygen (40%), and calcium (33%). Most abundant inorganic elements are; Florine (F), sodium (Na), and Aluminium (Al) with mass concentration percentages of 8, 2.9, and 1.18 respectively. While a trace of iron (Fe) and Nickel were also found at the spot. In spot 4, it is indicated that the major constituents were carbon (11%), Oxygen (47%) and calcium (42%) at that spot while the only trace element was aluminium with only 0.8% mass concentration percentage.

For the area 2 in the selected area 1as shown in figure 7 above, indicated that carbon (46.26%), Florine (30.26%), and sulphur (15.74%) are the major elements attached to the membrane in this whole area. While the most abundant inorganic are oxygen and calcium with mass concentration 5.3% and 1.5% respectively. Traces of sodium (Na), iron (Fe), and Nickel (Ni) were found in the area.

The presence of sodium (Na), carbon (C), and oxygen (O) in the membrane was as a result of the nature of the manufacturing process of the membrane, this is due to the fact that membrane used in this study were fabricated from cellulose that of calcium was due to the surface contamination effect, while the traces of Nickel was found to be from the urease enzyme used for the conversion of urea to ammonia. All these chemical compositions on the membrane are of great importance in determining the potential fouling, which depends on the chemical nature of the membrane, effluent and the membrane pores.

Toxicity of ammonia and urea

Ammonia gas is extremely corrosive and irritating to the skin, eyes, nose, and respiratory tract. Exposure by inhalation causes irritation of the nose, throat, and mucous membranes. Lacrimation and irritation generally begin at 130 to 200 ppm, although symptoms of eye and upper tract respiratory tract irritation have been reported at 30-50ppm. The maximum short exposure tolerance has been reported as being 300-500 ppm for 0.5 to 1 hour (Noael, 2004) At 400-700 ppm severe eye and respiratory irritation can occur, with the potential for permanent damage. At 1700 ppm convulsive coughing and bronchial spasms occur, and half hour exposure to this concentration is potentially fatal. Exposure at 3000 ppm is intolerable and exposure to high concentrations (above approximately 2500 ppm) is life threatening, causing severe damage to the respiratory tract, resulting in bronchitis, chemical pneumonitis, and pulmonary edema (build-up of fluid in the lungs), which can be fatal. At 5000-10000 ppm, death can occur from suffocation (Holness et al., 2010)

Ammonia is a naturally occurring compound though it is usually present in low concentrations in uncontaminated sites. Ammonia is toxic to a wide range of aquatic organisms at elevated concentrations. Fish are the most sensitive species and cold-water, oxygen-sensitive, fish such as trout are the most vulnerable (Wade et al., 2010).

Unlike ammonia, urea is classified as a non-toxic compound and non-comparable to ammonia in terms of toxicity (Blanca, 2000). Moreover, ammonia is more useful than urea and can be converted to more useful nutrient than urea.

Recovered ammonium

The recovered ammonium will be evaluated through an analysis of crop yield in an agricultural field. The effect of Struvite on maize grown in the field was examined by Rahman et al in 2011. During the plant growing season, the study's findings revealed a lower rate of Struvite leaching and nutrient release. According to another study, both ammonium salts and liquid ammonia recovered have strong potential to supplement fertilizer usage in agriculture. Moreover, ammonium sulfate $(NH_4)_2SO_4$ can also be employed in other industries for the manufacturing of nitrogen polymers as well as in food production industries (Ye, Hao, et al., 2018).

Discussion of AI results for analysing the effect of temperature on urea hydrolysis

In this study, AI based models were used to analyzed the effect of temperature on urea hydrolysis. Four different AI models were used for this prediction. The AI model perform based learning process, recognition of data set both linear and no-linear. The data set was used to develop AI-based models which include MLR, GPR, SVR and ANN models. For all the above listed models, we compare all with;

1. Temperature (T), different feed concentration of urea as N, and time taken for the urea to hydrolyze. All as input variables
2. Different feed concentration of urea as N, and time taken for the urea to hydrolyze. All as input variables

These are done to analyze the effect of temperature on urea hydrolysis.

This research looks into the feasibility and efficacy of using ML to predict ammonia recovery from urea-containing wastewater and compares different model results. A total of a 110 values dataset were used in this study, out of which 22 are from the experimental work conducted by myself in the lab and 88 datasets collected from published researches from literature. The input variables used for this study are feed concentrations, time taken for urea hydrolysis and temperature. The 110 dataset was initially divided into two portions; seventy percent (70%) of the data was employed for training while thirty percent (30%) was utilized for the testing.

Table 9 and 10 shows the statistical indicators used in checking the prediction efficiency of an AI-based models (GPR, ANN, MLR and SVR), which are sufficient to assist the efficiency of the developed models as describing errors and goodness of fit. The model with the highest NSE value among the models in both scenarios is the GPR model with 99.99% NSE value in both training and testing for both scenarios.

Fig. 11 and 13 demonstrates the Taylor diagrams, which were used to compare the stability as well as to evaluate the model's performance among the models for the prediction of ammonium recovery with and without temperature. Taylor diagram defines several statistical parameters such as NSE, RMSE and correlation coefficient (R). in this diagram the correlation between the actual and predicted values were translated using the azimuthal position, and the value of the GPR model indicated higher correlation value of approximately 99% nearest to the actual value as shown in both Fig 11 and 13. The MLR has the least in prediction accuracy with the lowest NSE and R values in both scenarios as shown in table 9 and 10 above. This showed that the GPR model in both scenarios as the best compared to the other three models and is having highest fitness. Thus, the diagram confirmed that the developed models revealed good performance, which could be applied in modeling of any ammonia recovery system.

Sensitivity analysis

The selection of the main input parameters in AI modeling is a critical issue for achieving appropriate results. In this study, non-linear sensitivity analysis was conducted in order to determine the importance ammonia recovery from wastewater using predictors; temperature, time of recovery and urea concentrations as input variables. This are used in show the relationship between inputs parameters and the recovered ammonia in this study. Previous studies have employed the use of the conventional linear correlation coefficients for the selection of dominant input parameters and the determination of relative importance of the individual parameters in AI models (e.g. Elkiran et al., 2018). However, the method has already been criticized for selecting input parameters in modeling nonlinear problems, since a nonlinear relationship may exist between the input and the target parameters (Nourani et al., 2014). Due to the criticism of the correlation method for selecting the dominant parameters, researchers have shifted to nonlinear sensitivity analysis methods such as the single-input single-output neural sensitivity analysis (Nourani et al., 2019b; Nourani et al., 2019c), feature removal/R^2 metric sensitivity analysis (Giam & Olden, 2015; Hamad et al., 2017), partial derivative sensitivity analysis (Shaghaghi et al., 2017), and mutual information (MI) (V. Nourani et al., 2015), etc. In this study, the sensitivity analysis was applied in order to determine the effect of temperature on the recovery being temperature having a relative importance as an input variable. This application has been used in many studies and was successful (e.g. Giam and Olden, 2015; Hamad et al., 2017; Nourani et al., 2019c). Based on the correlation result it shows that recovery is sensitive to change in temperature.

Cost analysis

The major cost of ammonia recovery system is the cost of the chemicals used in the process which include an acid (H_2SO_4) and an alkali (NaOH) addition for the pH control and for the stripping of an acid for the final collection of ammonia in the form of $(NH_4)_2SO_4$. According to the results of the experiment obtained the total cost of this ammonia recovery system depends on the energy usage (pumping and stirring), material used (membrane), chemicals used (urease enzyme), and time taken for the recovery. A part from the cost incurred; other benefit associated with this kind of treatment is the materials or recovered materials can be used to supplements fertilizer and reduces the expense required for the production of fertilizer using the industrial Haber-Bosch process as well as the amount of sludge disposal to the environment. Though most pilot studies show negative economic analysis results, but when recovered the ammonium sulphate $(NH_4)_2SO_4$ can successfully replace the commercial fertilizer use in agricultural production. This will help reduce the demand for commercial fertilizers, protection of the natural water courses, as well as the reduction in the greenhouse gas emissions resulting from the high temperature and pressure Haber-Bosch process.

Moreover, integrating this process with other nutrients recovery such as phosphorus and WWTP will also help in the cost reduction of the process. Furthermore, this process can only be cost efficient when applied to large wastewater treatment plants that contains high concentration of urea and ammonium ion.

Chapter V Summary

Herein, the result of all the research objectives were discussed as well as comparing the results with other literatures related to this research. This study revealed that higher ionic strength and higher concentrations of $(NH_4)_2SO_2$ and higher feed concentration (urea as-N) inhibits hydrolysis of urea, thereby reducing the urease enzyme activity in the system over time inside the reactor. AI models used in the study provide higher prediction accuracy and agree with the result of the experiment done in the lab. And other studies from literature. Moreover, cost analysis was also performed in order to compare the total cost incurred with the well-known conventional Haber-Bosch process.

CHAPTER VI

Conclusion and Recommendations

Conclusion

In this study, urea hydrolysis process was investigated experimentally on urea in both batch and continous system. Enzymatic urea hydrolysis itself is a complex process and the rate depends on several factors which vary during the conversion process. This work presented a concept of an EMR system to reach maximum ammonia recovery from water containing urea. This EMR system when put in place can be used to hydrolyse urea to ammonia in wastewater. The system will not only reduce the cost of ammonia recovery, but also reduce the environmental footprint associated with the removal process. More particularly, it has been shown that ensuring entire enzyme rejection inside the reacting volume can result in higher productivity, reduced activity loss, lower end-product quality variability, and so on. From the results obtained, it can be concluded that the system provides an alternative targeting the conversion of urea to ammonia, and also the effect of inhibitors as well as activators in the conversion process. Moreover, another advantage of the proposed system is the continuous use of enzyme in the reactor, which is economical and will be an innovation that will attract stakeholders' attention in wastewater reuse.Another advantage of the recovered product is that it may be utilized to supplement fertilizer production, reducing the cost of the Haber-Bosch process in the industrial setting. Furthermore, cost, energy and environmental footprint associated with ammonia/ammonium recovery from wastewater are also reduced by this process as less urease is used. The results from this experiment show how the ionic strength effect and ammonium nitrogen addition serve as inhibitors in urea hydrolysis to ammonia and carbon dioxide and also reduce the urease activity. Moreover, the experiment also explored how wastewaters containing higher concentrations of urea can be hydrolysed to ammonia and carbon dioxide using urease as an enzyme. This was done by varying the feed concentration from 100mg/L to 500mg/L as most wastewater containing urea contains approximately 750mg/L of urea. However, the results showed that the system could be used in any wastewater treatment system containing urea or ammonia and will be a more economical and sustainable system over a batch process. On the hand, the SEM and EDAX analysis this can be used in determining the relationship that exists between the surface membrane topography and bio fouling processes. This will also help to analyse the type, elemental structure and topography of the membrane which can help users to increase their efficiency during operation and to enlarge their application range as well as to monitor any change in structure of the membrane. Moreover, the current study explored the use of different data-driven models which include three non-linear and one linear models; the models used are; ANN, MLR, SVR and GPR for the modeling and optimization of the urease activities in urea hydrolysis with and without temperature as an input variable. Five different performance evaluation models were used for the evaluation of the performance namely; NSE, MAE, RMSE,R and PBAIS. Comparison was also made between the result of the models that include temperature as input variable and that without temperature as input variable in order to analyze the effect of temperature on urea hydrolysis. Result obtained showed GPR model outperforming all the other three models in both scenarios. The NSE was used to check the performance of each model, and the result obtained showed the GPR model as the best model for the prediction of the effect of temperature on urea hydrolysis and with the highest accuracy of an NSE-value of 0.9999 in both the training and testing phase. Additionally, this finding was supported with other statistical indicators; namely; MAE, RMSE, and PBIAS values of 0.0000, 0.0001, and 0.0002 in training and 0.0003,0.0005 and 0.0016 in testing respectively. The stability analysis results show higher stability of the GPR algorithm, and the higher prediction accuracy was achieved. The result in this study could offer valuable insight into using these proposed models for the ammonia recovery prediction from wastewater containing urea.

The result of the study can also be used as a prediction guide for ammonia recovery from wastewater containing urea using EMR under different experimental conditions.

Recommendations

Considering the result obtained from this study, future recommendations made are;

1. Future research for ammonia recovery should also focus more on issues related to membrane fouling, which is still a big challenge issue in most EMR system as it decreases the filtration efficiency, energy usage as well as the membrane lifetime.
2. Future research could also consider another pilot-scale or full-scale system of EMR using real urea-rich wastewater from WWTP.
3. Future studies could also imply other AI-models for comparing for the prediction of urea hydrolysis in real wastewaters.
4. Effect of other inhibitors or activators should also be looked into in future work.
5. Future research could also use real urea-rich wastewater and focus on different temperature, so as to examine the performance of the system at different temperatures.

Chapter VI Summary

In this chapter, we discuss why EMR systems for ammonia recovery from wastewater containing urea have not been applied on a larger scale, but still in pilot scale. Based on the findings of this thesis and other literature, we discuss the future perspectives and propose some future recommendations to be followed to bring this technology close to application with little modifications.

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https://doi.org/10.1016/j.jclepro.2021.126554

APPENDIX 1

CURRICULUM VITAE

APPENDIX 1

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Yahya, Mukhtar Nuhu

Nationality: Nigerian

Date and Place of Birth: 8th August, 1987, Kano-Nigeria

Marital Status: Married

EDUCATION

Degree	Institution	Year of Graduation
M.Sc.	Brunel University, London. (UK). Department of Civil Engineering	2014
B.Sc.	BUK Department of Civil Engineering	2012

WORK EXPERIENCE

Year	Place	Enrollment
2015 – Present	Bayero University, Kano-Nigeria.	Lecturer I

LANGUAGES

Hausa and English

MEMBERSHIP OF PROFESSIONAL ORGANISATIONS

Member, Nigerian Society of Engineers (NSE), Nigeria No.46582

Member, Nigerian Institute of Agricultural Engineers (NIAE), Nigeria No.M1938

Registered Engineer, Council for the Regulation of Engineering in Nigeria (COREN- R58899).

PUBLICATIONS IN INTERNATIONAL REFEREED JOURNALS (IN COVERAGE OF SCI/SCI-EXPANDED):

- **Yahya, M.N**.; Gökçekus, H.; Orhon, D.; Keskinler, B.; Karagunduz, A.; Omwene, P.I. A Study on the Hydrolysis of Urea Contained in Wastewater and Continuous Recovery of Ammonia by an Enzymatic Membrane Reactor. *Processes* 2021, 9, 1703.
- **M.N.Yahya,** H. Gokcekus., P.Paul., D.Orhon.,(2019) Experimental basis of an emergency filtration systems treating river flood water for rural areas in developing countries.*Desalination and Water Treatment* Pg 133-138.**(Q3)**
- **Yahya, M.N**, Umar, I.K**.,** Ibrahim, A., Gokcekus, H. (2020). Water quality evaluation of some boreholes and dug-wells in Dorayi. *Desalination and Water Treatment,* 26819, 1–7. **(Q3).**
- **Yahya**, **M.N**., H. Gokcekus., Uzun, D, Uzun, B. (2020) Evaluation of wastewater treatment technologies using TOPSIS.*Desalination and Water Treatment* Pg 416–422. **(Q3).**

PUBLICATIONS IN INTERNATIONAL REFEREED JOURNALS (IN COVERAGE OF British Education Index, ESCI, Science Direct, Scopus, IEEE):

- **M.N.Yahya**, H. Gokcekus., Uzun, D.(2020) Comparative Analysis of Wastewater Treatment Technologies. Jurnal Kejuruteraan (Journal of Engineering) Online First https://doi.org/10.17576/jkukm-2020-32(2)-xx 11 pages ISSN:0128-0198 e-ISSN:2289-7526
- Nura J. Shanono, Habibu Isma'il, Nuraddeen M. Nasidi, **Mukhtar N. Yahya**, Shehu I. Umar, Aminu Y. Nuradeen, Al-Ameen M. Musa, Zakariya Mustapha, Muazu D. Zakari (2021). Assessing the Operational Performance and Stakeholders' Perceptions on the Management of Irrigation Projects in Kano, Nigeria. 9 (2): 317–325. ISSN: 2147–8384 / e-ISSN: 2564–6826 doi: 10.33202/comuagri.936306

PAPERS PUBLISHED IN OTHER INTERNATIONAL INDEXED JOURNALS

- Musa Alhaji Ibrahim, **Mukhtar Nuhu Yahya**, Yusuf Sahin (2020) Predicting the mass loss of Polytetraflouroethylene-filled composites using artificial intelligence techniques. *Bayero J. of Eng. and Tech.* Vol.16 No.3, August, 2021 PP 80 – 93.

Book and Book Chapter published

- **M.N.Yahya,** I.M.T.Usman, H. Gokcekus, D.U.Ozsahin, B.Uzun(2021). Comparative Analysis for Irrigation Water Application Methods Using TOPSIS. *Under exclusive license to Springer Nature Switzerland AG 2021*.
- Musa Alhaji Ibrahim, Yusuf Şahin, Auwal Ibrahim, Auwalu Yusuf Gidado and **Mukhtar Nuhu Yahya**. Specific Wear Rate Modeling of Polytetraflouroethylene Composites Via Artificial Neural Network (ANN) and Adaptive Neuro Fuzzy Inference System (ANFIS) Tools.

International Conferences Attended

- A.Ibrahim, S.K.Lary., M.D.Zakari, N.M.Nasidi, **M.N.Yahya**.(2019) Performance evaluation of some selected infiltration models at Bayero University farm, Kano-Nigeria**.** Materials Science and Engineering 527 (2019) 012013 IOP Publishing doi:10.1088/1757-899X/527/1/012013.

- **Yahya, M.N**., Umar, I. K**.,** Ibrahim, A., & Gokcekus, H. (2019). Water quality evaluation of some boreholes and dug wells in Kano-Nigeria. 2nd International Conference on "Water Problems in the Mediterranean Countries". Nicosia, North Cyprus.

- **Yahya M.N**.,Paul, P., Tadda M.A., Ibrahim A.(2018) Effects of tree barks on water treatment. Proceedings of iSTEAMS Multidisciplinary Research Nuxus Conference, Celeb University, Lagos, Nigeria, May, 2017.

- Tadda M.A., Ahsan A., Maina M.M., **Yahya M.N.,** Muhammad A.I. (2016) Low-cost Leachate treatment technology using Electrolysis and Activated Carbon. Presented at the NIAE 37th Annual Conference and General Meeting, Minna 2016. pp.464 – 470

Conferences Organized

- Member, Organization Committee, 2nd International conference on the environment survival and sustainability, 7 – 11 October 2019. Near East University, Nicosia, Cyprus.
- Member, Organization Committee, 2nd International conference on Water problems in the Mediterranean Countries (WPMC 2019), 06 – 10 May, 2019. Near East University, Nicosia, Cyprus.
- Member, Organization Committee, 2nd International conference on the Cyprus Issue: Past, Present and the Vision for the Future, 1 – 3 April, 2019. Near East University, Nicosia, Cyprus

THESISES

Master

Yahya, M.N (2014) *Design and development of an emergency river flood water filtration system for rural areas in developing countries*. Department of Civil Engineering. Bayero University, Kano-Nigeria.

Project

Yahya, M.N (2012) *Design of distributory and field canals for Ganuwar kuka sector, Hadejia valley irrigation project, Auyo Local Govt. Jigawa State-Nigeria*.Unpublished Undergraduate project (B.SC.), Bayero University Kano, Department of Civil Engineering, Faculty of Engineering, Kano, Nigeria.

COURSES GIVEN (2015-2021)

Undergraduate:

- AGR4201 - Introduction to Agricultural Mechanization
- AGE3201 - Introduction to Agricultural Engineering
- AGE3210 - Introduction to Agricultural Engineering (SAFE).
- EGR3101 - Engineer in Society II

SPORTS

Football and Table tennis

HOBBIES

Reading, movies

YAKIN DOĞU ÜNİVERSİTESİ

Appendix 2

Ethical Approval Letter

Date:11th March, 2022

To the Graduate School of Applied Sciences

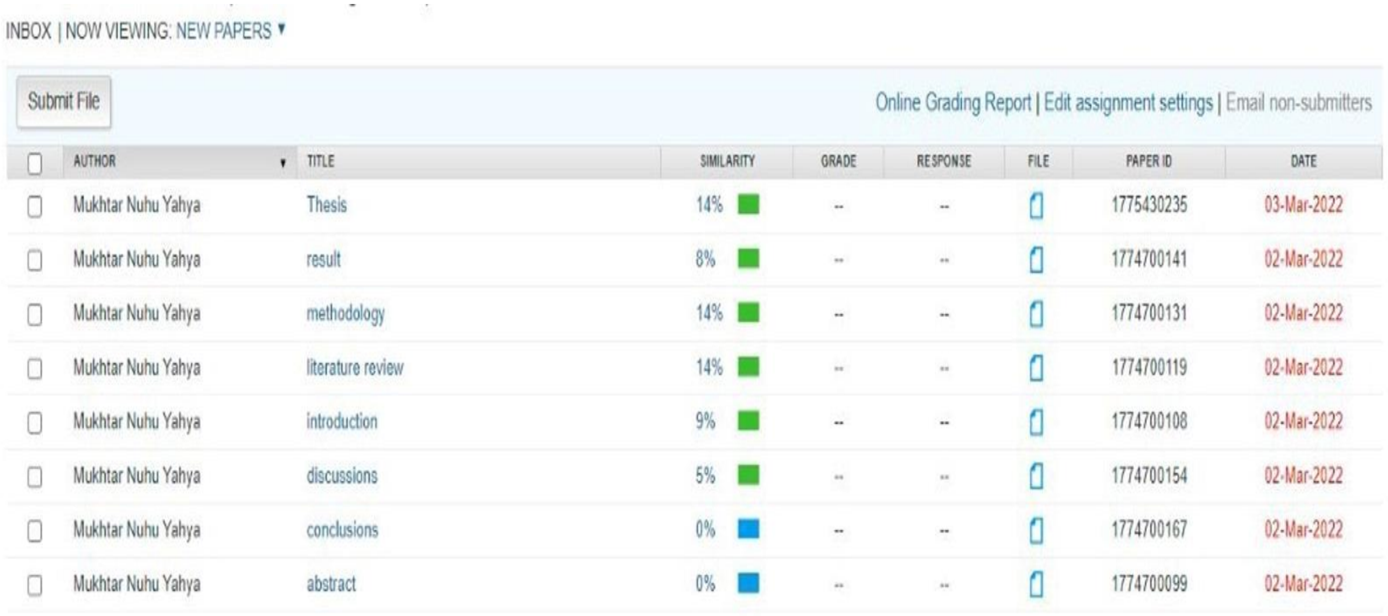
REFERENCE: MUKHTAR NUHU YAHYA (20178447)

The research project titled “**Ammonia Recovery from Wastewater Containing Urea using an Enzyme Membrane Reactor (EMR).**” has been evaluated. Since the researcher(s) will not collect primary data from humans, animals, plants or earth, this project does not need to go through the ethics committee.

Title: Dean, Faculty of Civil and Environmental Engineering
Name: Prof. Dr. Huseyin Gokcekus
Role in the Research Project: Supervisor

Appendix 3

Similarity Report



☐	AUTHOR	TITLE	SIMILARITY	GRADE	RESPONSE	FILE	PAPER ID	DATE
☐	Mukhtar Nuhu Yahya	Thesis	14% 🟩	--	--	🗋	1775430235	03-Mar-2022
☐	Mukhtar Nuhu Yahya	result	8% 🟩	--	--	🗋	1774700141	02-Mar-2022
☐	Mukhtar Nuhu Yahya	methodology	14% 🟩	--	--	🗋	1774700131	02-Mar-2022
☐	Mukhtar Nuhu Yahya	literature review	14% 🟩	--	--	🗋	1774700119	02-Mar-2022
☐	Mukhtar Nuhu Yahya	introduction	9% 🟩	--	--	🗋	1774700108	02-Mar-2022
☐	Mukhtar Nuhu Yahya	discussions	5% 🟩	--	--	🗋	1774700154	02-Mar-2022
☐	Mukhtar Nuhu Yahya	conclusions	0% 🟦	--	--	🗋	1774700167	02-Mar-2022
☐	Mukhtar Nuhu Yahya	abstract	0% 🟦	--	--	🗋	1774700099	02-Mar-2022

Prof. Dr. Hüseyin Gökçekuş

Approval.

We certify that we have read the thesis submitted by MUKHTAR NUHU YAHYA titled **"Ammonia Recovery from Wastewater Containing Urea Using an Enzyme Membrane Reactor (EMR)"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy in Civil Engineering.

Examining Committee	Name-Surname	Signature
Head of the Committee:	Prof. Dr. Hatice Erkurt	…………………
Committee Member:	Prof. Dr Rana Kidak	…………………
Committee Member:	Prof. Dr. Umut Turker	…………………
Committee Member:	Prof. Dr. Vahid Nourani	…………………
Supervisor:	Prof. Dr. Huseyin Gokcekus	…………………

Approved by the Head of the Department

12 /12 /2022

…………………………

Prof. Dr. Kabir SADEGHI

Head of Department

Approved by the Institute of Graduate Studies

Prof. Dr.K. Hüsnü Can Başer

Head of the Institute