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ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CIVIL AND ENVIRONMENTAL
ENGINEERING

Dissertation on:

**Optimization of Up-flow Anaerobic Sludge Blanket Reactor:
A case of Kality Wastewater Treatment Plant, Ethiopia.**

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DECLARATION

I declare this Ph.D. research dissertation is my bona fide work that all sources of materials used for this dissertation have been duly acknowledged. The research work is not submitted to any other University, college or any other institution to get degree, diploma or certificate. This research dissertation is submitted in partial fulfillment of the requirements for Ph.D. Degree in Civil and Environmental Engineering (Water Supply and Environmental Engineering) at Addis Ababa University.

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ABSTRACT

Enhancing the performance of an up-flow anaerobic sludge blanket reactor (UASB) for unregulated sewage treatment by optimising its operating parameters at a moderate temperature is contingent upon controlling variables like temperature, pH, organic loading rate (OLR), and hydraulic retention time (HRT). The primary goal of the study was to improve the performance of the Kality Wastewater Treatment Plant (WWTP), which uses a UASB reactor through optimization of operational parameters. To do this, controllable operational parameters were optimised, toxic heavy metals concentrations were measured in the unit processes like UASB reactor, Trickling filter, and secondary clarifier. Samples were also tested at downstream irrigated soil and plants for toxic heavy metals concentration. Eventually, the UASB reactor was modelled using general process simulation (GPS-X). Sixty experimental data sets were collected during the rainy seasons (June, July and August) and dry seasons (February, March and April). Central composite design-response surface method (CCD-RSM) tool was used for optimization of operational parameters. Using this CCD-RSM, a temperature of 21.58°C, OLR of 2.99kg COD/m³.d, HRT of 4.37hrs and pH of 6.3 operational parameters were achieved during the rainy seasons. Experimental triplications were conducted using the rainy seasons optimized parameters and about 92.70% Chemical Oxygen Demand(COD), 99.06% Total Suspended Solid(TSS), and 94.50% volatile suspended solid (VSS) were achieved. For dry season's data, CCD-RSM gave raise optimized operational parameters of temperature (23.04°C), OLR (2.23kg COD/m³.d), pH (7.5), and HRT (11.43hrs). Using optimized operational parameters, experimental triplications were conducted and achieved a removal efficiencies 84.07%COD, 99.89%TSS, and 100%VSS which is much higher than the removal efficiency set by Kality WWTP manual (55% COD, 55%BOD₅ and 70%TSS). At the halfway point of the HRT, the reactor's process wellbeing was assessed by measuring the VFA to alkalinity ratio, VSS to TSS ratio, mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS). Moreover, because of point and non-point sources of wastewater to the treatment plant multiple issues that have public and environmental health significance could be observed. One of the issue is the evaluation of the WWTP's unit processes for toxic heavy metals. They were evaluated for toxic heavy metals removal since the existence of higher concentration of these metals in WWTP's unit processes

significantly affects the wellbeing of microorganisms through significant change of pH and the formation of complex compound which precipitate and corrodes the distribution pipes. The second issue is associated with the prevalent use of the treated effluent for irrigation by local farmers. Data were collected to identify and quantify toxic heavy metals in treatment plant's effluent, irrigated soil, and vegetables. The laboratory tests revealed that majority of the toxic heavy metals were present in the samples analyzed (Chromium, Cadmium, Arsenic, Copper, Lead, and Manganese) and the unit processes were inefficient to remove these metals. Furthermore, a model describing the main biological processes occurring in the UASB reactors was developed. General Process Simulation-X (GPS-X) was used in this experimental research since it is the best tool for simulation and optimization of WWTP processes. Before simulation, influent characterization was done for mass balance around the secondary clarifier which was helpful for calibrating the GPS-X model. The sensitivity analysis result showed that model parameters like kinetic (Maximum Specific Growth Rate on Substrate, Maximum Growth Rate for Ammonia Oxidizer, Maximum Growth Rate for nitrite Oxidizer, Maximum growth rate of PAO) and stoichiometric (Ammonia-oxidizer yield, and Nitrite-Oxidizer yield) values were the most sensitive parameters to calibrate the UASB reactor but the most influential model parameter was kinetic value (heterotrophic maximum specific growth rate). Average absolute relative error (ARE) was used to evaluate the best fit between the measured and simulated data sets. ARE values for COD, TSS and ammonia-nitrogen being less than 7-15% showed that the model was calibrated. Finally the calibrated model was validated based on actual dry seasons data sets collected experimentally. As the simulated results showed acceptable model performances, the developed model can be used by operators, designers and researchers for further studies.

Keywords: GPS-X, mathematical model, operational parameters, optimization, moderate temperature, up-flow sludge blanket reactor, toxic heavy metal, and wastewater.

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LIST OF ACRONYMS

ACP	:	Anaerobic Contact Process
AD	:	Anaerobic Digester
AFB	:	Anaerobic Fluidized Bed
ASBR	:	Anaerobic Sequential Batch Reactor
As	:	Arsenic
AAWSA	:	Addis Ababa Water and Sewerage Authority
BIOWIN	:	Biological Wastewater Treatment in Window
BOD	:	Biochemical Oxygen Demand
BOD ₅	:	Biochemical Oxygen Demand after five days
CCD-RSM	:	Central Composite Design – Response Surface Method
Cd	:	Cadmium
COD	:	Chemical Oxygen Demand
CSTR	:	Continuously Stirred Tank Reactor
Cu	:	Copper
DNA	:	Di ammonium Acid
DO	:	Dissolved Oxygen
EGSB	:	Expanded Granular Sludge Blanket
FAO	:	Food Authority Organization
F/M	:	Food to Microorganisms ratio
GF-AAS	:	Graphite Furnace Atomic Absorption Spectroscopy
GPS-X	:	Generalized Process Simulation for water and wastewater treatment
HM	:	Food to Microorganisms ratio
HRT	:	Hydraulic Retention Time
ICAR	:	Internal Circulation anaerobic reactor
ICP-AAS	:	Coupled Plasma –Optical Emission spectrometry

Mn	:	Manganese
MWW	:	Municipal Wastewater
NH ₃	:	Ammonia
NH ₄ ⁺	:	Ammonia Ion
OLR	:	Organic Loading Rate
pH	:	Power of Hydrogen
PID	:	Process Integrated Derivative
Pb	:	Lead
SBR	:	Sequential Batch Reactor
SGSB	:	Static Granular Sludge Blanket
SMA	:	Specific Methanogenic Activity
SRT	:	Solid Retention Time
STOAT	:	Sewage Treatment Operation and Analysis over Time
SUMO	:	Simulation of Urban Mobility
TCOD	:	Total Chemical Oxygen Demand
TDS	:	Total Dissolved Solid
TP	:	Total Phosphorus
TN	:	Total Nitrogen
TOC	:	Total Organic Carbon
TSS	:	Total Suspended Solid
VFA	:	Volatile Fatty Acid
VSS	:	Volatile Suspended Solid
Vup	:	Up-flow Velocity
WHO	:	World Health Organization
WWTP	:	Wastewater Treatment Plant
Zn	:	Zinc

1. INTRODUCTION

1.1 Back ground of the study

There are two types of biological processes with a high content of organic materials in wastewater treatment plants: aerobic and anaerobic processes (Xii, 2011; Aswir & Misbah, 2018). One of several anaerobic wastewater treatment processes is the up-flow anaerobic sludge blanket (UASB) reactor. The UASB reactor is a popular method for removing organic matter from wastewater. It is an anaerobic process in which microorganisms remove organic matter. The UASB reactor can treat both domestic and industrial wastewater because it performs better in mesophilic conditions (Rodríguez-Gómez et al., 2014, Schultz et al., 2015). Because it does not require any media to grow microorganisms and has lower operation and maintenance costs than aerobic treatment, it may be the best method for treating domestic and industrial wastewater in developing countries (Chong et al., 2012, Lomte & Bobade, 2015a). The primary goals of wastewater treatment plants are to reduce organic and inorganic pollutants in wastewater while also improving treatment efficiency. The treatment efficiency of the UASB reactor is evaluated using the removal efficiencies of the biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), and total dissolved solid (TDS) (Parihar et al., 2017, Wijaya & Soedjono, 2018, Pahal et al., 2020).

Microorganisms can be classified into different groups based on the temperature range that supports their optimal growth and metabolic activity. This classification is important for understanding how various environmental conditions can affect microbial communities in systems such as UASB reactors. Psychrophilic Temperature Range for Growth (Typically -20°C to 20°C) Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. :2014). Mesophilic temperature (Range for Growth: 20°C to 45°C) Ray, B., & Bhunia, A. 2013: Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. ,2014). Thermophile temperature (45°C to 75°C) (Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. ,2014).

In recent years, UASB reactors have become a popular choice for wastewater treatment due to their efficiency in removing organic pollutants and generating biogas. However, the presence of toxic heavy metals in wastewater poses significant

challenges to the optimal performance of these reactors. Even though the UASB reactor was employed for biological processes it should be evaluated for toxic heavy metal removal efficiency for the following reasons. **Inhibition of Microbial Activity:** Heavy metals such as cadmium, lead, mercury, and others can inhibit the metabolic activities of the microbial communities within the UASB reactor. These metals can disrupt enzyme function and cellular processes, leading to reduced efficiency in the breakdown of organic matter (Wang, Q., Du, G., & Chen, J., 2004; Chen, Y., Cheng, J. J., & Creamer, K. S., 2008). **Disruption of Granule Formation:** UASB reactors rely on the formation of granular sludge to maintain high biomass concentrations. Heavy metals can interfere with the aggregation of microbial cells, affecting granule stability and formation, which can result in poor sludge retention and reactor performance (Fang, H. H., & Liu, H., 2002; Liu, Y., Xu, H. L., Yang, S. F., & Tay, J. H., 2003). **Toxicity to Specific Microbial Groups:** Different microbial groups within the UASB reactor are responsible for various stages of the anaerobic digestion process. Heavy metals can be toxic to specific groups, such as methanogens, which are crucial for methane production. This can lead to reduced biogas production and lower overall treatment efficiency (Demirel, B., & Scherer, P.; 2008). **Competition for Nutrients:** Heavy metals can bind to essential nutrients and trace elements, making them less available to the microbial communities. This can limit the growth and activity of microorganisms, further reducing the reactor's performance (Koki, K., & Boonkerd, N., 2006; Bhattacharyya, P., & Singh, D. P., 2010). **Alteration of Reactor pH:** The presence of heavy metals can lead to changes in the pH of the reactor environment. Extreme pH levels can further inhibit microbial activity and disrupt the balance of the anaerobic digestion process (Wieland, P., 2010). **Formation of Insoluble Compounds:** Some heavy metals can form insoluble compounds with other elements in the reactor, which can precipitate and accumulate in the sludge. This can reduce the effective volume of the reactor and require more frequent de-sludge operations (Baek, K., Kim, H. S., Oh, S. K., & Kim, J. O. (2003).

The practice of using treated effluent for irrigation will boost food production, which will support Addis Ababa's formal food production. Due to the rapid growth of the population in Addis Ababa and the expansion of sub cities, residents are increasingly using treated wastewater from domestic treatment plants for irrigation to help sustain their livelihoods. An increase in population should result in an increase in

sectors such as urban agricultural production and irrigated lands, which rely on water resources for irrigation. These conditions have compelled farmers to irrigate with alternative water sources (i.e., blackish water, wastewater, and effluent water), thereby closing the gap between freshwater availability and crop demand (Gray & Becker, 2002; Amer, 2010; Intriago et al., 2018). Irrigation with treated municipal wastewater is an important component of global environmental strategies. The scientific community is focusing more on it (Pasquale et al., 2015; Elgallal et al., 2016; Intriago et al., 2018). In a number of countries, treated wastewater is already regarded as a suitable water source for irrigation. Primarily in agriculture and landscaping (Kaizer & Osakwe, 2010; Edwin et al., 2014; Yeshiwas & Tadele, 2017). Despite the potential economic, social and environmental benefits of irrigation with treated municipal wastewater, the effects of reuse must be continuously monitored to ensure resource protection, soil health and particularly human health. Irrigation with treated wastewater produces a significant amount of biodegradable organic material (carbon and nitrogen). Mineral such as macro and micronutrients (phosphorous, potassium, and magnesium) and toxic heavy metals (Molybdenum, Selenium, Boron, Chromium, Manganese, Lead, Arsenic, Cadmium and Copper) required for crop growth. But they affect human health beyond the recommended concentration (Woldetsadik et al., 2017; Intriago et al., 2018; Mohanty et al., 2021). To maintain soil quality, however, the possibility of trace pollutants, heavy metals, and salts accumulating in the soil must be considered. Heavy metal content in effluents can vary depending on treatment method and wastewater source (industrial, municipal, etc.). Their concentration in the receiving system (soil, plant) must be monitored (Prabu, 2009; Gameda et al., 2021). In contrast, long-term application of fully, partially treated or untreated wastewater may result in toxic heavy metal deposition in the soil (Elgallal et al., 2016; Vivaldi et al., 2019). Residential and commercial effluents, agricultural wastes, atmospheric deposition, and industrial emissions carried by storm water into WWTP systems carry a variety of pollutants. These pollutants enrich urban wastewater with toxic heavy metals (Kaizer & Osakwe, 2010); Khan et al., 2016; Yeshiwas & Tadele, 2017). This study was conducted to identify and quantify the types of toxic heavy metals that exist in Kality domestic wastewater treatment plant. The identification and quantification was made in effluent, downstream irrigated soil, and local cabbages. The study further investigated their effect on human health, and comparing the magnitude with the Ethiopian Guideline

Ambient Environmental Standards, FAO/WHO. Eventually, the determination of the treatment efficiency of UASB reactor, trickling filter, and secondary clarifier in removing toxic and heavy metals were assessed.

Despite the fact that UASB reactors have been utilized for more than 10 years, there is no model that describes the processes that occur in the UASB reactor for the Kality WWTP. Anaerobic treatment like UASB reactor is widely used to treat wastewater with high organic matter concentrations. Many environmental studies focus on the degradation of wastewater in anaerobic reactors because this type of process not only degrades organic matter but also produces fuel in the form of biogas and produces low quantities of sludge, reducing the frequency of reactor maintenance (Rodriguez & Moreno, 2010). The main processes that occur in UASB reactors are Reaction model (used to model the reaction inside the reactor), Hydraulic model (used to describe the flow inside the reactor), Solid-liquid separation model (used to model the top part of the reactor), Gas- liquid separation model (used to model the gas produced inside the model), Fluidization model (used to model the solids coming from the UASB reactor and its relationship with the height of the sludge blanket) and substrate modeling. GPS-X was the tool used to model the UASB reactor. The GPS-X model's components are separated into three categories. This includes user inputs, state variables, and composite variables. The first are referred to as the basic variables since they are in charge of system characterization (GPS- X's dynamic model of differential equations) and are continually integrated over time. Composite variables, on the other hand, are derived from the matrix product of state variables and stoichiometric constants. The parameters that characterize a WWTP model are state and composite variables, and they provide an overview of how the WWTP is acting at any given time. When GPS-X creates a layout model, it calculates the corresponding composite variables after establishing a material balance for each state variable in the unit process (Pereira, 2014). Both of these variables can be classified into two types of matter based on their physical properties: soluble matter (S components) and particulate matter (X components). During the treatment process, soluble components are carried with the water and may contain an ionic charge, whereas particulate components (electrically neutral) are connected with the activated sludge and can thus be concentrated sedimentation/thickening (<https://www.hatch.com/hydromantis/gps-x/index.html>).

The purpose of this research was to improve the performance of the UASB reactor under moderate temperature by optimizing operational parameters. Generally, fixing one or two parameters for moderate temperature will not result in efficient removal for UASB reactor. Since the Kality's UASB reactor's removal efficiency was set at 55% for BOD₅, COD and 70% for TSS, the effects of operational parameters such as temperature, OLR, HRT, and pH on the removal efficiency were investigated. The central composite design-surface response methodologies was used to analyze data for COD, TSS, and VSS. Furthermore, the influent and effluent concentrations of sulfate ion (SO₄²⁻), VFA, and alkalinity were taken for UASB reactor performance analysis.

1.2 STATEMENT OF THE PROBLEMS

The objective of wastewater treatment plants is to efficiently reduce organic and inorganic pollutants in effluent wastewater. To achieve this, researchers have considered various parameters to enhance the performance of Up-flow Anaerobic Sludge Blanket (UASB) reactors. For instance, Yetilmezsoy and Sakar focused on hydraulic retention time (HRT) and found that optimal chemical oxygen demand (COD) removals ranged from 86.3% to 90.6% (Yetilmezsoy and Sakar, 2008). However, other studies have shown that HRT is not the sole factor affecting UASB performance. Tendulkar et al. demonstrated the significance of temperature, achieving a COD removal efficiency of 70% with a fixed HRT of 4.7 days at 13-25°C (Tendulkar et al., 2006). Zhang and Vrieze conducted a pilot-scale investigation and found that COD removal efficiency decreased from 60% at 12.5-20°C to 51.5% at 10°C due to insufficient methanogenic capacity (Zhang and Vrieze, 2018). Similarly, Rizvi et al. reported COD removal efficiencies of 82%, 70%, 68%, and 44% at temperatures of 28°C, 20°C, 14°C, and 10°C, respectively, highlighting the impact of temperature (Rizvi et al., 2015). Nugroho and Santoso examined the effects of organic loading rate (OLR) alongside temperature and HRT in a mesophilic UASB reactor, finding that COD removal exceeded 90% at an OLR of 0.4 g/L d but dropped below 50% at an OLR of 15 g/L d (Nugroho and Santoso, 2019). A study in Ghana reported COD and BOD removal efficiencies of 93% and 98%, respectively, at mesophilic temperatures but did not investigate performance at moderate temperatures or during varying seasonal conditions (Arthur et al., 2022). Currently, the Kality Wastewater Treatment Plant (WWTP) in Addis Ababa shows estimated removal efficiencies of 55% for BOD₅, 55% for COD, and 70% for total suspended solids (TSS) at room temperature (20°C). This highlights the need to enhance UASB reactor performance by considering multiple operational parameters. Unlike previous studies, this research will focus on optimizing independent parameters like temperature, OLR, and pH, and dependent parameters like HRT, specifically at moderate temperatures.

Optimization is crucial for Kality WWTP due to discrepancies between design and actual influent concentrations. The plant was designed for an influent BOD₅ of 470 mg/L, while the actual influent BOD₅ ranges from 628 to 2950 mg/L. Similarly, the design ammonia-nitrogen concentration was 45 mg/L, but the actual concentration

ranges from 33.9 to 65.88 mg/L. The design TSS concentration was 600 mg/L, whereas actual TSS ranges from 3 to 970 mg/L. Additionally, the design temperature of 20°C does not account for lower temperatures during the night and rainy seasons or higher temperatures during the day and dry seasons.

Moreover, the unit processes of Kality WWTP need evaluation for toxic heavy metals, which affect microorganism health and hinder wastewater distribution in the UASB reactor. The use of treated or untreated sewage for irrigation in Addis Ababa poses public health risks due to the presence of toxic heavy metals like chromium, cadmium, arsenic, copper, lead, and manganese in vegetables. These concentrations should be checked against EPA and FAO/WHO standards to ensure safety. In this research, efforts will be made to determine the optimal operating parameters of the UASB reactor, including temperature, OLR, pH, and HRT. Additionally, the concentrations of toxic heavy metals in the treatment plant effluent, downstream irrigated soil, and cultivated vegetables were analysed. Furthermore, the full-scale UASB reactor of Kality WWTP will be modelled using the GPS-X model.

In general, this experimental research, the determination of toxic heavy metal concentrations and their effects in the unit processes, modelling of the UASB reactor using GPS-X, optimisation of four operational parameters (Temperature, OLR, pH, and HRT) with the design and construction of experimental reactor set up for effluent data collection, were the noble ideas that other researchers around the world did not attempt.

1.3 RESEARCH QUESTIONS

1. Is optimization of UASB reactor operational parameters effective in removing COD, BOD₅, and TSS, among other things, at moderate temperatures?
2. Is the concentration of sulfate ions at the outlet greater than the inlet concentration showing that there is more CH₄ and less H₂S productions?
3. Is the VFA /alkalinity ratio, MLSS, and MLVSS with in the acceptable range which eventually confirms the normal working condition of the pilot reactor set-up?
4. To what extent does the Kality wastewater treatment plant's UASB reactor, trickling filter (TF), remove Toxic Heavy Metals (THM)?
5. Is the concentration of toxic heavy metals in the downstream cultivated

vegetables grown by the effluent higher than the EPA and WHO/FAO standards?

6. Do optimizing operational parameters such as temperature, organic loading, HRT, and pH improve the performance of UASB reactor working at a moderate temperature?
7. Is modeling UASB reactor helpful for WWTP operators, designers and researchers for further studies?

1.4 OBJECTIVES OF THE DISSERTATION

1.4.1 General Objective

The general objective of this research is Performance enhancement of UASB reactor for moderate temperature through optimization of operational parameters such as Temperature, OLR, pH, HRT, and determination of toxic heavy metals concentrations in unit processes and modeling the UASB reactor using GPS-X: in case of Kality wastewater treatment plant.

1.4.2 Specific objectives

- To evaluate physico-chemical characteristics of the wastewater at UASB reactor and determination of the existence and concentration of toxic heavy metals in the WWTP effluent, irrigated soil, and irrigated plants.
- To optimize the operational parameters of the reactor and to predict UASB reactor performance.
- Modeling UASB reactor using GPS-X which helps the WWTP operators, designers and researchers to create different scenarios.

1.5 SIGNIFICANCE OF THE STUDY

The study work will have the following significances for different parties:

1.5.1 Academia:

The research findings will be utilized as a reference and starting point for future studies in domestic wastewater treatment by researchers.

1.5.2 Policy makers/regulatory bodies:

It is an alarm for policymakers and regulatory bodies, particularly environmental

policymakers, that the aquatic environment is at its worst due to poor performance of domestic wastewater treatment plants and that development plans must be revised or modified, as well as corrective actions taken based on the findings.

1.5.3 Industrialists/operators:

It demonstrates their firm's environmental and societal status, as well as gaps in the implementation of the adopted environmental management technologies, so that they can take appropriate action or make necessary process adjustments to act in an environmentally friendly and socially acceptable manner, without which their business and/or development activity will be unable to continue.

1.5.4 Community:

The community living downstream of the treatment plant and the residents of Addis Ababa should be informed of the presence of hazardous heavy metals in plants such as local cabbage and Kosta that are grown using the WWTP's effluent.

1.6 SCOPE OF THE RESEARCH

This research focused on identifying, quantifying the physical and chemical characteristics of wastewater, optimization of operational parameters and modeling of UASB reactor of Kaliti wastewater treatment plant for moderate temperature. The samples were analyzed at laboratory for the concentration of COD, TSS, VSS, alkalinity, VFA, SO_4^{2-} , $\text{NH}_4^+\text{-N}$, total nitrogen (TN), total phosphorus (TP), MLSS, MLVSS and pH at the influent and effluent of the UASB reactor set up. The performance of the UASB reactor was evaluated for different combinations of operational parameters like temperature, OLR, pH and HRT using CCD-RSM. Moreover the existence and concentration of toxic heavy metals in the irrigate soil, plant samples and effluents were examined. The removal efficiency of UASB reactor, Trickling filter, and secondary clarifiers were examined for toxic heavy metals. Finally, Modeling of the UASB reactor was done by using GPS-X software.

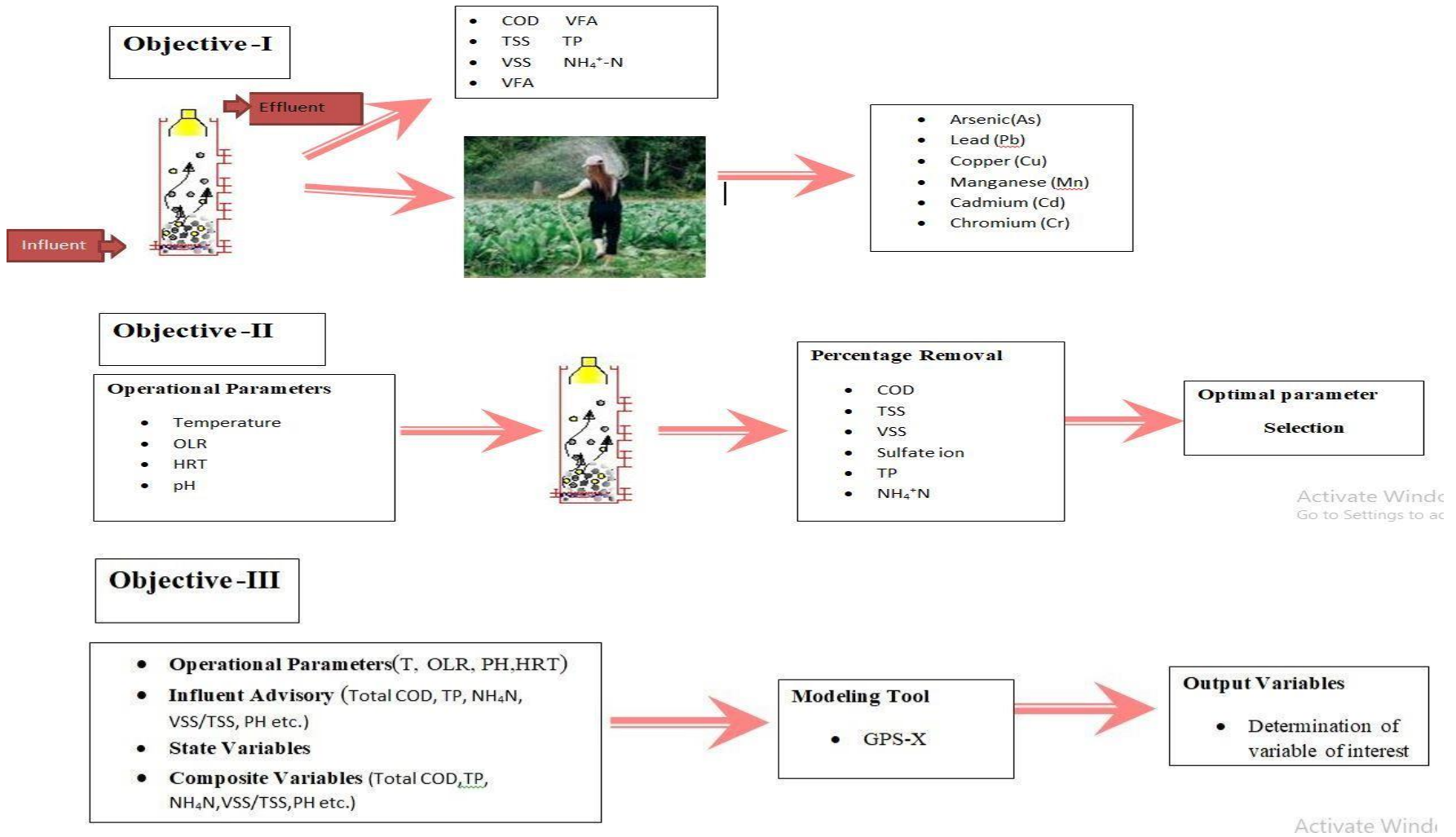


Figure 1 Schematic diagram to show the scope of the research

1.7. LIMITATION AND DELIMITATION

The experimental reactor configuration includes a cooling system that has a compressor. The cooling system's compressor had a lower capacity and could only cool 48.6L of wastewater. This was due to the inability to get a larger capacity compressor locally and their sky rocketing cost which was much greater than the budget allocated for this research. Moreover, since UASB reactor operational parameters such as granulation and mixing were uncontrollable in this study, only four controllable operational parameters like temperature, OLR, pH and HRT were considered.

GPS-X educational software was used to model the UASB reactor of Kality wastewater treatment plant. Educational version of GPS-X 8.0.1 was used since the cost of the GPS-X was 20 fold of the budge allocated by the University for Ph.D. study. If original software were used for modeling the UASB reactor, better model parameters were achieved for modeling the UASB reactor.

1.8. CONCEPTUAL FRAMEWORK

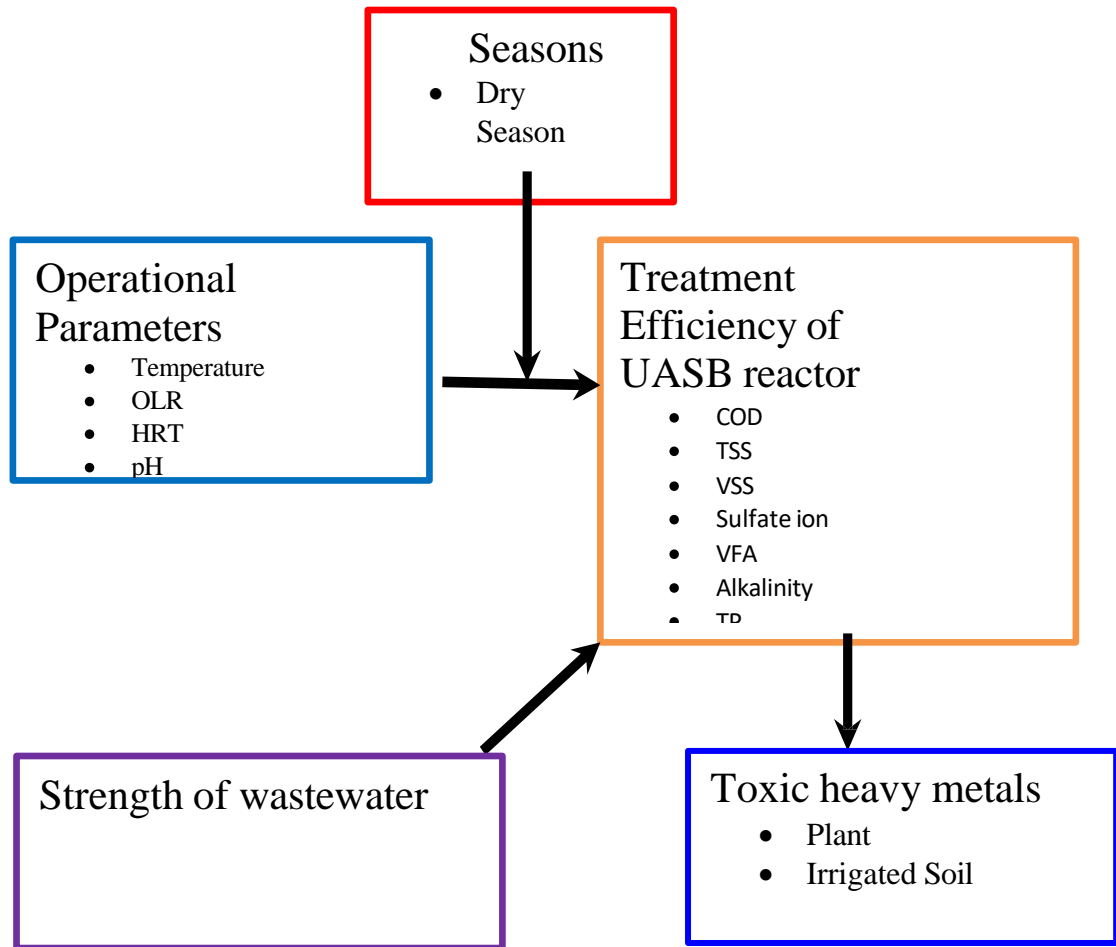


Figure 2 Conceptual Frame Works of the research

1.9. DISSERTATION STRUCTURE

This Ph.D. dissertation contains about seven chapters where the first chapter covers the general introduction which covers the background of the study, problem of the statement, objectives, and research questions, scope of the study and significance of the study. The second chapter is addressing the literature review related to the dissertation works. Materials and methods are covered under chapter three to achieve the formulated objectives. The fourth chapter addressed the results obtained and discussion of the results relative to other studies. Moreover, chapter six is all about discussion of the results and furthermore, conclusion, recommendation and future research directions were addressed in the seventh chapter.

2. LITERATURE REVIEW

2.1. General Introduction

Wastewater treatment has long been recognized as potential problem both in the developing and developed countries. As far back as 1970, Lattinga and his co-workers devised a new mechanism by which wastewater is treated. They innovated up-flow anaerobic sludge blanket reactor (UASB) which became rapidly widespread due to its great performances (de Lemos Chernicharo, 2015). Now days there are Expanded Granular Sludge Blanket (EGSB) and Static Granular Sludge Blanket (SGSB) reactors that are applicable in the treatment of domestic and industrial wastewater (M. Zheng et al., 2014; Lomte & Bobade, 2015a). With granules, UASB and EGSB reactor are able to treat high-strength domestic and industrial wastewaters. The difference between UASB, EGSB and SGSB reactors is both UASB and EGSB reactors are an up-flow type reactors on the other hand SGSB is down flow reactor. EGSB is the modification of conventional UASB and it works at higher up-flow velocity and higher loading rate than UASB (Lomte & Bobade, 2015a). In this reactor the up-flow velocity is greater than 4m/h and the sludge tends to expand and eliminates the dead zone. It also best works at lower temperature (4-20°C) (Lucas Seghezze et al., 1998; Saravanan & Sreekrishnan, 2006). EGSB is a combination of UASB and dewatering. It consists of two or more UASB reactor situated on the top of each other .The EGSB system has been reported to successfully treat wastewaters with high lipid content which causes foaming and scum as well as handling organic loading rates three to six times greater than that of conventional UASB system with similar efficiency (Zheng et al., 2012; Lomte & Bobade, 2015b). Anaerobic contact process is similar to the activated sludge process in many aspects .The system consists of a completely mixed anaerobic reactor with gas collection, followed by a clarifier for solid- liquid separation (Lier et al., 2015). Part of the settled sludge is recycled to the reactor to increase the solids retention time (SRT) .The SRT is usually greater than the hydraulic retention time(HRT) (Services et al., 2002). Anaerobic Sequencing Batch Reactor (ASBR) was developed by Dague and co-workers in the late 1980s at Iowa State University in Ames, Iowa. It is a suspended growth process where biological conversions and solid –liquid separation all takes place in the same reactor. Gas is collected on a continuous basis .One of the advantage of the process is the formation of dense, granular sludge that has a high settling (Treatment &

Management, 2013; Musa et al., 2018).

In the study conducted by Kinetics of Bacterial Growth Relationship between Population Density and Specific Growth Rate of Continuous Cultures, Some characteristics of UASB, EGSB, and SGBR were investigated in order to compare their performances. The researchers found that good performances were observed in SGSB like UASB and EGSB but sludge granulations were yet not understood (Lomte & Bobade, 2015b; Sun et al., 2016). The researchers presented the suitability of the UASB reactors for tropical developing country. They underlined that, due to minimum energy consumption and lesser sludge formation UASB reactor is an obvious choice nowadays. The researchers further showed the important uses of UASB over anaerobic wastewater treatment methods like septic tank, anaerobic fluidized bed (AFB), anaerobic fixed bed, sequencing batch reactor (SBR), expanded granular sludge blanket (EGSB). They expanded their research in solving the existing challenges in design as well as operation of UASB.

2.2. Up-flow anaerobic sludge blanket reactor

2.2.1. UASB reactor zone

Up-Flow Anaerobic Sludge Blanket Reactor has four different zones these are: The inlet, sludge blanket, gas-liquid separator and settling zones (Vahid & Karyab, 2006; Aswir & Misbah, 2018). Inlet is region where wastewater after grit chamber enters to the UASB reactor (Girma et al., 2023). Sludge blanket zone is middle zone and it is regions where organic compound is digested by micro-organisms and digested product forms Biogas and biomass (sludge, it is 5% the total organic matter means 95% efficiency of the UASB reactor). (Bhatti et al., 2014, Kapumbe et al., 2019). Gas-solid separator zone is region where the gas is fleered out and it is separated from the solid and slides down and gets settled at the bottom (Lomte & Bobade, 2015a; Ali, 2020). Settling zone is region where the solid separated from the liquid slides and settle (Brito et al., 2020).

2.2.2. The working principle of UASB reactor

Wastewater enters at bottom of the UASB reactor and it up-flows and passes through anaerobic sludge blanket. Then the micro-organisms in the sludge blanket get in contact with the organic compounds. When organic compound is broken down by anaerobic digestion, biogas such as methane gets removed. Eventually, the liquid –

solid separator separates liquid and solid. UASB best works at mesophilic temperature (30-35°C) this is because methanogenesis results in greatest removal of COD and BOD but if the temperature is lowered methanogenesis growth results in poor soluble COD and BOD removal and deterioration of the sludge stability (Álvarez et al., 2004; Li & Yu, 2016;Girma et al., 2023).

2.2.3. Effect of Different Parameters on the Removal Efficiency of UASB

Reactor

2.2.3.1. Effect of Temperature

Anaerobic treatment of wastewater can take place at all three temperature ranges (psychrophilic, mesophilic, and thermophilic) (Rizvi et al., 2015; Girma et al., 2023). However, low temperature generally leads to a decline in the growth and survival of micro- organism (maximum specific growth rate), decrease in methanogenic activity, decrease in diffusivity of gases, an increase in viscosity of liquids which leads to decrease in the gas- liquid transfer, smallest granule size formation and in the greatest amount of wash out of sludge (Luostarinen et al., 2007; Khanh et al., 2011; Daud et al., 2018). Methanogenic at lower temperature range is 10 to 20 times lower than the activity at 35°C which requires the an increase in the biomass in the reactor (10 to 20 times) or operating at higher sludge retention time (SRT) and hydraulic retention time (HRT) in order to achieve the same COD removal efficiency as that obtained at 35°C (Lew et al., 2003; Khanh et al., 2011; Rizvi et al., 2015).

2.2.3.2. Effect of Hydraulic Retention Time (HRT)

In the municipal wastewater treatment, hydraulic retention time is the most important parameter that affects the performance of the UASB reactor (Pandya et al., 2011; Rizvi et al., 2015; Daud et al., 2018) but some scientists reported HRT has no distinct effect on the performance efficiency of UASB (Ladu & Lü, 2014). The difference in idea in theses researchers is the difference in the reactor design, operating procedures, and range of HRT (Seghezze et al., 2002; Farajzadehha et al., 2012; Rizvi et al., 2015). Flow rate is another important operational parameter that affects hydraulic retention time. If the diameter of the reactor is too large in the UASB process, it may cause liquid channeling in the reactor, resulting in insufficient contact between the substrate and biomass (Zeng et al., 2005). As a result of insufficient mixing within the reactor, large reactors result in decreased biogas production and sludge washout. In

contrast, comparatively more height may promote substrate mixing leading to proper contact of substrate with microorganisms resulting in more organic matter degradation and formation of biogas (Kalyuzhnyi et al., 2006 ; Farajzadehha et al., 2012; Trivedi et al., 2015) . The up-flow velocity (V_{up}) is helpful in providing adequate mixing of the substrate and biomass without channeling and maintaining the hydraulic retention time (Zheng et al., 2012; Rate et al., 2022).

2.2.3.3. Effect of Organic Loading Rate (OLR)

Organic loading rate is the primary parameter that has a significant impact on microbial ecology and the operation of the UASB process (Azbar et al., 2009). UASB reactor is preferred for its potential to treat wastewater having low content of suspended solids and gives higher methane yield (Jijai et al., 2015; Daud et al., 2018; Kapumbe et al., 2019). The reactors seeded with granular activated sludge can give high performance within a brief startup period and can also adapt quickly to increase OLR (Daud et al., 2018; Kapumbe et al., 2019) .The effect of OLR on the performance of a UASB reactor depends on a number of factors which sometimes have a dissimilar effect, mostly contradictory, on the performance of UASB reactor (Azbar et al., 2009; Sushma & Pal, 2013; Musa et al., 2018).

2.2.3.4. Effect of pH

The pH of an anaerobic reactor is especially important because methanogenesis process can proceed at a high rate only when the pH is maintained in the range of 6.3-7.8 (Azbar et al., 2009; Arthur et al., 2022). In the case of domestic sewage, pH naturally remains in this range because of the buffering capacity of the acid -base system (carbonate system), and addition of chemical is not required (Leitão et al., 2005; Khanh et al., 2011; Musa et al., 2018). The pH and buffering capacity of UASB reactors used for sewage treatment in tropical and subtropical countries have been reported to be extremely stable. When treating domestic wastewater with an anaerobic reactor, both hydrolysis and acidogenesis rates improve, and pH 7 provides an optimal working environment for anaerobic digestion, resulting in more than 80% total organic carbon and COD removal (Lester et al., 2009; Daud et al., 2018; L. Zhang et al., 2018).

2.3. Challenges of the UASB reactor

UASB reactors are sensitive to sudden changes in influent parameters such as flow rate, organic load, and temperature (Renato Carrhá Leitão et al., 2006). Shock loads can disrupt the microbial community and impact the reactor's performance (Meinzinger & Oldenburg, 2009; Lohani et al., 2016). Anaerobic processes are temperature dependent, and UASB reactors perform best within a specific temperature range. Cold temperatures can slow microbial activity, resulting in lower treatment efficiency (Aswir & Misbah, 2018). UASB reactor performance relies heavily on effective sludge retention. Ensuring proper sludge settlement and preventing the carryover of suspended solids in the treated effluent can be challenging (Kripa Shankar Singh & Viraraghavan, 2002; L. Seghezzi et al., 2002). The start-up of UASB reactors can be time-consuming. It requires the formation and establishment of an anaerobic microbial consortium. During this time, the reactor may not operate at peak efficiency (Bhatti et al., 2014; Kaviyarasan, 2014). UASB reactors frequently rely on the formation and maintenance of granular sludge for effective treatment. Hydraulic shear forces, high organic loading rates, and microbial disturbances can all have an impact on the stability of granular sludge structures (Lomte & Bobade, 2015a; Montes et al., 2019). UASB reactors are primarily intended for organic matter removal and are less effective at removing nutrients like nitrogen and phosphorus. Additional treatment steps may be required to address nutrient removal if necessary (Kaviyarasan, 2014; Montes et al., 2019). It can be difficult to scale up UASB reactors from laboratory or pilot scale to full-scale applications. When dealing with larger volumes, factors like hydraulic distribution, mixing, and reactor geometry become increasingly important (Lester et al., 2009; Enitan et al., 2015). Adequate monitoring and control systems are essential for maintaining optimal reactor performance. However, continuous monitoring of key parameters and implementation of control strategies can be demanding (O'Flaherty et al., 2006; Schultz et al., 2015).

2.4. Optimization of UASB reactor operational parameters

Researchers tried to demonstrate the efficiency of the UASB system, at 6-8 h of HRT, 15- 16°C of temperature and 330-360 mg/l of influent (Álvarez et al., 2004). A removal of about 79% for total chemical oxygen demand (TCOD), 52% for total

suspended solids (TSS and 60% for biological oxygen demand (BOD₅) were achieved. On the other hand de Graaf et al. in their research entitled anaerobic treatment of concentrated black water in a UASB reactor at a short HRT, an experimental investigation was conducted for the removal of COD using concentrated black (toilet) water at 25°C and achieved 78% COD at an HRT of 8.7 days (de Graaff et al., 2010). But L. Zhang et al. conducted a pilot scale UASB-digester to treat domestic wastewater at temperatures of 10-20°C and an HRT of 6 h and the researchers found that COD removal efficiency of $60 \pm 4.6\%$ (L. Zhang et al., 2018a). K. Singh and Vashya studied about biodegradation based kinetic of UASB reactor in treating municipal wastewater through various models. They studied on microbial growth kinetics using six different HRTs (22, 18, 14, 10, 6, 3 h). In their experimental work they found a removal efficiency ranging from 91 to 72% of COD with decrease of HRT (K. Singh & Valshya, 2017). But other researchers used five different HRT (1-5day) values for WWTP employing Up-flow Anaerobic Sludge Blanket (UASB) Reactors. They conducted a laboratory-scale UASB reactor. They concluded that as HRT decreased from 5 to 1 day, the organic removal efficiencies decreased from 91.49 to 43.23 %, from 89.36 to 45.13 % and from 87.23 to 32.69 %. In this study they selected mathematical models including Monod, Contois, Grau second-order and Modified Stover-Kicannon kinetic models (Jijai et al., 2015).

On the other hand, with startup of 120days and with sludge age stabilization of 80days, the performance of UASB reactor was enhanced with sludge age(60days) and temperature(17°C), the COD removal rate was observed to be 57-62%.The researcher concluded the removal efficiency of UASB was elevated to the range of 79-81% at the sludge age of 150days ,and temperature of 30°C .Finally the researcher concluded that the removal efficiency of UASB is optimum at sludge age of 120-150days and temperature of 25-30°C.The feasibility of sewage treatment by UASB reactor was studied by (Shigeki Uemura and Hideki Harada , 2000) using actual sewage at a fixed hydraulic retention time of 4.7 h, and at temperatures range of 25-13°C, for six months. They described the average total COD removals and solid COD removals achieved were 70% and 80%, respectively. They concluded that the Total COD removal rate depend on influent strength, especially solid COD concentration, rather than operational temperature. But in the research topic, anaerobic treatment of raw domestic wastewater in a UASB-digester at 10°C and microbial community dynamics

(L. Zhang et al., 2018b) they emphasized that temperature greatly affects the performance of UASB reactor. To show this, they conducted a pilot scale of UASB-digester to treat domestic wastewater at operational temperatures of 10-20°C and an HRT of 6 h. They reported that stable chemical oxygen demand (COD) removal efficiency of $60 \pm 4.6\%$ was obtained during the operation at 12.5-20°C. They concluded that the removal efficiency of COD decreased to $51.5 \pm 5.5\%$ when the temperature is reduced to 10°C. They reasoned out that this decline in removal efficiency is due to insufficient methanogenic capacity caused by low temperature. Similarly in the research done by Lew et al. (Lew et al., 2003), the performance of domestic wastewater at four different operational temperatures (28, 20, 14 and 10°C) were used to investigate the removal efficiency of UASB for COD. In their research, they found 82%, 70%, 68%, and 44% respectively as removal efficiency of UASB. The researchers concluded that the removal efficiency was declined as an operational temperature of the UASB reactor gets reduced (L. Zhang et al., 2018a). On the other hand Alvarez et al. in their research, performance of a UASB-digester system treating domestic wastewater at steady state, the efficiency of the UASB system reactor, at 6-8 h of HRT, and 15-16°C of temperature, the chemical oxygen demand removal was reported to be 79% (Álvarez et al., 2004). In the research topic Performance of an on-site UASB reactor treating leachate at low temperature (K. S. Singh & Viraraghavan, 2003; L. Zhang et al., 2018b; L. Zhang et al., 2018a) a pilot-scale UASB reactor was used to study the treatment of municipal landfill leachate (COD 1.5-3.2 g L⁻¹) at low temperatures (13-23°C) and in on-site conditions for 226 days. The reactor was successfully operated by lowering the process temperature as the temperature of the leachate decreased in winter.

Despite changes in leachate quality, 65- 75% COD removals were achieved at 18-23°C but as temperature got declined from 13-14°C the removal efficiency of UASB reactor was found to be 50-55%. The researchers also found that the specific methanogenic activity (SMA) of the mesophilic (37°C) inoculum used in the study increased 7 fold at 11°C and 3 fold at 22-24°C during the run. But in the research topic Low-strength wastewater treatment by a UASB reactor, the researchers described that the feasibility of anaerobic treatment of a low-strength synthetic wastewater using a semi-pilot- scale up-flow anaerobic sludge blanket (UASB) reactor was used at temperature conditions (20-35°C) with a hydraulic retention time

(HRT) of 3 h about 90-92% COD and 94-96% BOD removal were achieved (Klesyk, 2017).

2.5. Sources of toxic heavy metals

Unregulated sewage refers to wastewater that is not adequately treated, monitored, or controlled before being discharged into the environment. This can include domestic sewage, industrial effluents, or agricultural runoff that bypasses treatment facilities, or it can result from inadequate or malfunctioning treatment systems that fail to meet regulatory standards.

2.5.1. Illegal connections and Industrial Effluents

2.5.2. Metal Finishing and Electroplating

Electroplating and metal finishing industries often discharge wastewater containing heavy metals like chromium, nickel, cadmium, and zinc. These metals are used in various plating processes and can be toxic to the microbial community in UASB reactors (F. Ismail, et al., 2015).

2.5.2.1. Mining Operations

Mining operations can introduce heavy metals such as mercury, arsenic, and lead into wastewater, which can be challenging to treat in UASB reactors due to their toxic effects on anaerobic bacteria (M. Mudhoo, et al., 2011).

2.5.2.2. Textile and Dyeing Industries

Wastewater from textile and dyeing industries may contain metals like copper and chromium, used in dyes and pigments, which can be inhibitory to the microbial communities in UASB reactors (K. Malik, et al., 2004)

2.5.2.3. Battery Manufacturing

Battery manufacturing processes discharge effluents containing lead, cadmium, and mercury, which are known for their toxicity and potential to disrupt anaerobic digestion in UASB reactors (R. W. Peters, et al., 1991.).

2.5.3. Agricultural Runoff

2.5.3.1. Fertilizers and Pesticides

Agricultural runoff, especially from the use of fertilizers and pesticides, introduces heavy metals like arsenic, lead, and cadmium into water bodies, which can enter wastewater treatment systems (L. H. Pivetz, et al., 2001).

2.5.4. Municipal Wastewater

2.5.4.1. Domestic Waste

Household products like batteries, paints, and electronic waste contribute to the presence of heavy metals like mercury, lead, and cadmium in municipal wastewater, which can inhibit microbial activity in UASB reactors (J.R. Mihelcic, et al. (2008).

2.5.5. Storm water Runoff

Urban runoff can carry heavy metals from road surfaces, vehicles, and building materials into wastewater systems, posing a challenge for treatment in UASB reactors (D. Butler, et al., 2001).

2.5.4. Electronic Waste Recycling

Improper disposal or recycling of electronic waste results in wastewater containing heavy metals such as lead, mercury, and cadmium, which are toxic to the anaerobic bacteria in UASB reactors (H. Wang, et al., 2012).

2.5.5. Landfill Leachate

Leachate from landfills can contain various heavy metals, including lead, mercury, arsenic, and cadmium, depending on the types of waste disposed of. These metals can be difficult to treat in UASB reactors (S. Renou, et al. (2008).

2.6. Effect of toxic heavy metal on human health

Eating food crops grown in wastewater-irrigated areas is one of the most important factors contributing to human pathogen exposure. Furthermore, growing crops for human consumption on wastewater-irrigated soil may result in trace metal uptake and buildup in edible plant parts, posing a risk to humans (A. Singh et al., 2010; Zia et al., 2017 ; Intriago et al., 2018). Heavy metals are extremely dangerous due to their non-

biodegradability, long half- lives, and high bioaccumulation potential (Fitamo et al., 2007).According to several researchers, excessive accumulation of heavy metals and even important trace elements like Copper (Cu) ,Cadmium (Cd), Manganese(Mn) , Arsenic(As) ,Lead(Pb), and Zinc(Zn) in the human body can cause serious health problems (He et al., 2005; Duruibe et al., 2007 ; Yeshiwas & Tadele, 2017) . Some heavy metals are required for human biological processes, but their consumption can have unanticipated negative effects on health and the physiological system, depending on the dosage (concentration) (Tchounwou et al., 2012).Excessive heavy metal accumulation in agricultural soils as a result of wastewater irrigation may result in soil contamination as well as increased heavy metal uptake by crops (Muchuweti et al., 2006). J.J.Kim et al. studies showed that, heavy metals, despite their beneficial health effects, act as carcinogenic agents (J. J. Kim et al., 2019). Dissolved forms of these metals enter the food chain via various means, including soil pollutants, water and air pollutants, and eventually end up in humans, causing severe damage to the cellular system. Toxic metals pose health risks based on the concentrations of these metals in specific media and the length of exposure. Long-term and chronic exposure to hazardous metals, even at low levels, can cause health problems (Mahalakshmi, 2012).

2.7. Diseases caused by toxic heavy metals

Heavy metal toxicity, according to A. Singh et al. , can be classified into the following categories: Nephrotoxicity, Neurotoxicity, Hepatotoxicity, Carcinogenicity, Cardiovascular toxicity, Immunological toxicity, Skin toxicity, Genotoxicity, and Reproductive and developmental toxicity (A. Singh et al., 2010).

2.7.4. Nephrotoxicity

Lead has toxic effects on various organ systems, but those in the kidney are the most difficult. Acute lead nephropathy causes proximal tubular dysfunction, resulting in Fanconi-like syndrome (Tchounwou et al., 2012). Cadmium can also cause glucosuria, Fanconi-like syndrome, Phosphaturia , and aminoaciduria (Reyes et al., 2013).

2.7.5. Neurotoxicity

Manganese is a necessary element that is involved in a number of physiological functions in the body. Acute exposure may have a neuro-protective effect by lowering apoptotic cellular death, but chronic exposure can result in dangerous illnesses such as Alzheimer's and Parkinson's disease (Goldhaber, 2003).When arsenic is consumed, it causes central nervous system cognitive impairment. It has also been linked to a variety of neurological disorders, including neurodevelopmental changes, and is associated with an increase in neurodegenerative diseases (Garza-Lombó et al., 2019). Arsenic exposure also has an impact on synaptic transmission and neurotransmitter balance (Tolins et al., 2014).

2.7.6. Hepatotoxicity

Cadmium affects two human tissues: the renal cortex and the liver (Bernard, 2004).It accumulates in the liver during acute exposure and has been linked to a variety of hepatic dysfunctions. Cadmium alters the redox balance of cells, causing oxidative stress and hepatocellular damage (Zalups, 2000). Cadmium induced hepatotoxicity, both acute and chronic, causes liver failure and thus increases the risk of cancer (Hyder et al., 2013).Arsenic poisoning increases the risk of cancer by binding to DNA binding proteins and slowing the repair process (Uter et al., 2018).

2.7.7. Carcinogenicity

Lead is a carcinogenic chemical that causes the DNA repair mechanism, cellular tumor- regulating genes, and chromosomal structure and sequence to be damaged. It interferes with transcription by removing zinc from certain regulatory proteins (Silbergeld et al., 2000).

2.7.8. Cardiovascular toxicity

Cadmium is a carcinogenic and toxic metal (Services et al., 2002). Exposure to low to moderate levels of cadmium causes hypertension (Tellez-Plaza et al., 2008), diabetes (Urinary Cadmium, Impaired Fasting Glucose, and Diabetes in the (Schwartz et al., 2003), carotid atherosclerosis (Messner et al., 2009), peripheral arterial disease (Navas-Acien et al., 2004), myocardial infarction (Peters et al., 2010). Cadmium has been linked to an increased risk of cardiovascular death in the general population of the United States in prospective studies (Tellez-Plaza et al., 2013).

2.8. Effect of toxic heavy metals in UASB reactor

The presence of toxic heavy metals can significantly impact the performance of Up-flow Anaerobic Sludge Blanket (UASB) reactors in several ways: **Inhibition of Microbial Activity:** Heavy metals such as cadmium, lead, mercury, and others can inhibit the metabolic activities of the microbial communities within the UASB reactor. These metals can disrupt enzyme function and cellular processes, leading to reduced efficiency in the breakdown of organic matter (Wang, Q., Du, G., & Chen, J., 2004; Chen, Y., Cheng, J. J., & Creamer, K. S., 2008). **Disruption of Granule Formation:** UASB reactors rely on the formation of granular sludge to maintain high biomass concentrations. Heavy metals can interfere with the aggregation of microbial cells, affecting granule stability and formation, which can result in poor sludge retention and reactor performance (Fang, H. H., & Liu, H. (2002; Liu, Y., Xu, H. L., Yang, S. F., & Tay, J. H. ,2003). **Toxicity to Specific Microbial Groups:** Different microbial groups within the UASB reactor are responsible for various stages of the anaerobic digestion process. Heavy metals can be toxic to specific groups, such as methanogens, which are crucial for methane production. This can lead to reduced biogas production and lower overall treatment efficiency (Zayed, G., & winter, J. (2000; Demirel, B., & Scherer, P., 2008).

Competition for Nutrients: Heavy metals can bind to essential nutrients and trace elements, making them less available to the microbial communities. This can limit the growth and activity of microorganisms, further reducing the reactor's performance (Koki, K., & Boonkerd, N., 2006; Bhattacharyya, P., & Singh, D. P., 2010).

Alteration of Reactor pH: The presence of heavy metals can lead to changes in the pH of the reactor environment. Extreme pH levels can further inhibit microbial activity and disrupt the balance of the anaerobic digestion process (Weiland, P., 2010).

Formation of Insoluble Compounds: Some heavy metals can form insoluble compounds with other elements in the reactor, which can precipitate and accumulate in the sludge. This can reduce the effective volume of the reactor and require more frequent desludging operations (Baek, K., Kim, H. S., Oh, S. K., & Kim, J. O., 2003).

2.8.4. Mitigation Strategies

To mitigate the negative effects of heavy metals on UASB reactor performance, several strategies can be employed: **Pre-treatment:** Remove heavy metals from the wastewater before it enters the UASB reactor through chemical precipitation, adsorption, ion exchange, or membrane filtration. **Bioremediation:** Use specific microorganisms or plants that can accumulate and detoxify heavy metals, thereby reducing their concentrations in the wastewater. **pH Control:** Maintain optimal pH levels in the reactor to minimize the solubility and bioavailability of heavy metals. **Nutrient Supplementation:** Add essential nutrients and trace elements to counteract the inhibitory effects of heavy metals and support microbial activity. **Operational Adjustments:** Adjust hydraulic retention time (HRT), organic loading rate (OLR), and other operational parameters to optimize conditions for microbial communities and minimize the impact of heavy metals.

2.9. Modeling WWTP's processes

Model is a set of equations that are solved with in the simulator or a model is a simplified representation of what occurs in the reality. It is defined by a series of mathematical equations and procedures, constituted by different variables and parameters dependent on time. A model allows studying and analyzing engineering questions in a shorter period of time, reducing the costs associated with laboratory analysis (Yetilmezsoy & Sakar, 2008). In a WWTP, models can be used to evaluate the system response to a variety of perturbations, thus allowing the implementation of strategies that guarantee a better performance (Serdarevic & Dzibur, 2016). Models

are also useful for process optimization and control, for example to evaluate several scenarios that might lead to improved operation of existing WWTPs and design alternatives for new WWTP installations via simulation (Reactors, 2013; Serdarevic & Dzubur, 2016). If we can create models that are accurate representations of real world systems, we will be able to conduct experiments that would otherwise be impossible. There are essentially two approaches when constructing an analytical model: mechanistic modeling and empirical modeling (Batstone et al., 2015). Mechanistic models are based on an understanding of the behavior of a system's components (white box approach) while empirical models are based on direct observation, cause-effect relations between the input and the output variables and extensive data records regression modeling or neural networks (black-box approach). Empirical models are very simple but low on strength, since they are specific for a certain situation to which the relations were estimated (Yetilmezsoy & Sakar, 2008; Młyński et al., 2019). Mechanistic models, on the other hand, are more complex than empirical models because they try to describe all the processes that involve a certain phenomenon based on a collection of differential equations, such as continuity equations, momentum and energy conservation, mass transport and biological reactions and thus they require a careful parameterization (Batstone et al., 2015). While empirical models are typically easier to build, they provide less insight than mechanistic models. The latter provides more realistic predictions and more can be done with it in terms of analyses. Nowadays for the design, control, predict system behavior and all, modeling using computer simulations are increasingly used. Different Modeling software packages such as Solids Separation Process (SSSP), Sewage Treatment Operation and Analysis over Time (STOAT), aquatic simulation (AQUASIM), Generalized Process Simulation for water and wastewater treatment (GPS-X) and wastewater evaluation and simulation tool (WEST) are available on the market. The quality of simulation studies can vary strongly depending on the goal set, expertise available and resources spent (Manea, 2016). Different approaches and insufficient documentation make the quality assessment and comparability of this study difficult. To overcome these obstacles a general guideline for the implementation of a simulation study is needed. This would help to remove the current barriers to the treatment models. A WWTP simulator environment can be described as software that allows the modeler to simulate a waste water treatment plant.

2.9.4. Wastewater Treatment Plant modeling tools

Wastewater treatment plant modeling tools are used to simulate and optimize the process in the WWTPs. Some common ones include Sewage Treatment Operation and Analysis over Time (STOAT), SIMBA, Biological Wastewater Treatment in Window (BIOWIN), Generalized Process Simulation (*GPS-X*) etc. They allow Engineers to predict how changes in parameters like flow rate, chemical dosing, operational parameters. Optimization and materials upgrades will affect plant performance.

2.9.4.1. Sewage Treatment Operation and Analysis over Time (STOAT)

It is a useful and reliable tool for the prediction and optimization of unit processes of wastewater treatment. But its most drawback is it overestimates the results but which may be adjusted after several iterations (Jaiswal et al., 2019). It is economical and practical simulation software that is used to solve the existing problems of small WWTPs.

2.9.4.2. SIMBA

It is the more advanced software in terms of control logic modeling. It is used to model small wastewater treatment plant processes. It can model Biological, gravity settling model, aeration and chemical precipitation processes (Erbe et al., 2002; Alex, 2008).

2.9.4.3. Biological Wastewater Treatment in Window (BIOWIN)

It is used for design, up grading, optimizing wastewater treatment plants. It is also used to predict the biodegradability of organic chemicals in water. While it not specifically designed for modeling WWTPs, it can still be useful in assessing the potential for organic compound to degrade with in treatment systems. It can help in understanding the fate of various pollutants in wastewater treatment process and their potential impact on the environment. Each of the software has different advantages and disadvantages. There are also software like Simulation of Urban Mobility (SUMO). It is a Microsoft Excel based model, which makes importing datasets easier. But in comparison with SIMBA, SIMBA is the more advanced software in terms of control logic modeling. However, the two most widely used wastewater modeling

tools are BIOWIN and GPS-X (Serdarevic & Dzibur, 2016). The modeling interface and set-up for the software is similar as they have “configuration” menus to place modules or model units to create a model representation of water resource recovery facilities (WRRF). The biological models available in GPS-X is Mantis2 (Pereira, 2014; Asmah, 2018).

2.10. GPS-X

Generalized Process Simulation (GPS-X) for water and wastewater treatment model components are basically divided into state and composite variables. The first ones are known as the basic variables that are responsible for the system’s characterization (GPS-X’s dynamic model of differential equations) and they are continuously integrated over time. On the other hand composite variables are obtained from the matrix product of state variables and stoichiometric constants. State and composite variables are the parameters that characterize a WWTP plant model and they give an overlook of how the WWTP is behaving at a certain moment. When GPS-X builds a model of a layout, it establishes a material balance for each state variable in the unit process and then calculates the corresponding composite variables (Hydromantis_GPS-X_v8, n.d., 2013). Both of these variables can be characterized according to the two physical types of matter: soluble matter (S components) and particulate matter (X components). The soluble components are transported with the water during the treatment process and may carry ionic charge, whereas particulate components (electrically neutral) are associated with the activated sludge and therefore can be concentrated by sedimentation/thickening (Pereira, 2014).

2.10.4. Advantage of GPS-X

Modelers are using GPS-X for modeling WWTP since the tool has many advantages. Some of these advantages are: the flexibilities available, used for WWTP optimization and simulator, used to model large wastewater treatment plants, it is user-friendly drag and drop options for unit operations and unit processes and comprehensive database of unit processes allows users to quickly assemble a treatment plant model, enter characterization data, and run simulations (Hydromantis_GPS-X_v7, n.d., 2013). Studies were conducted using GPS-X software for modification of existing process in order to improve the efficiency of the current plants. Studies conducted in Portcel at an industrial complex in which the existing plant is modeled using a new scenario for

improving efficiency using GPS-X, showed that process modifications and model calibration can be easily done using this software (Lester et al., 2009; Vicentin et al., 2019 ; Ghawi & Tisti, 2021). Other studies by (Zhang et al., 2018; Nazif et al., 2023) using GPS-X suggest that this software can be used to improve the capacity, operating efficiency and optimization of existing facility. A study by Mare Andre and his colloquies shows the application of GPS-X for plant optimization. They reviewed the role of dynamic model in plant capacity performance assessment using different what if scenarios. They suggested that due to increased operating and energy costs, several upgrades were needed for the existing facility in order to meet the constraints which were analyzed and calibrated using GPS-X (Mare Andre, Amrutha & Haseena, 2020).

2.10.5. Approaches in GPS-X modeling

2.10.5.1. Simple approaches

This type of approach usually used anaerobic digester (AD) and solid- liquid separator. The practice is it separated the solid and feed it back to the reactor (Hydromantis_GPS-X_v7, n.d., 2013). Typically consist of a closed tank or container where organic waste is mixed and allowed to ferment anaerobically. The digestion process occurs in a mixed or suspended growth environment.

Generally it provides effective treatment for organic waste but may have lower efficiency compared to UASB reactors, especially for high-strength wastewater.

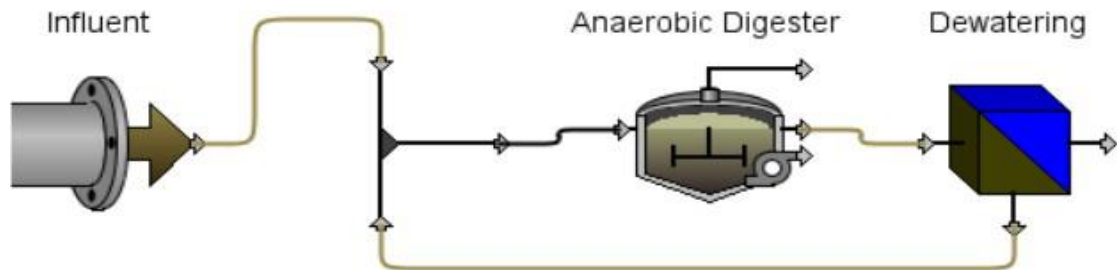


Figure 3 Simple approaches in modeling UASB reactor

2.10.5.2. Advanced approaches

This type of modeling involves the use of UASB reactor. While both anaerobic digesters and UASB reactors are utilized for the treatment of organic waste and the creation of biogas, the advanced approach uses the UASB reactor since it has a greater efficiency and works well with more difficult wastewater streams. The selection of these technologies is contingent upon various criteria, including the wastewater's composition, treatment goals, and available resources. Utilize an upward flow of wastewater through a bed of granular sludge, forming a dense sludge blanket where anaerobic digestion takes place. The design promotes the separation of biomass and wastewater, allowing for more efficient treatment. It is known for their high treatment efficiency, particularly for wastewater with high organic loads. The design and operation of UASB reactors promote the retention of biomass and enhance the degradation of organic matter.

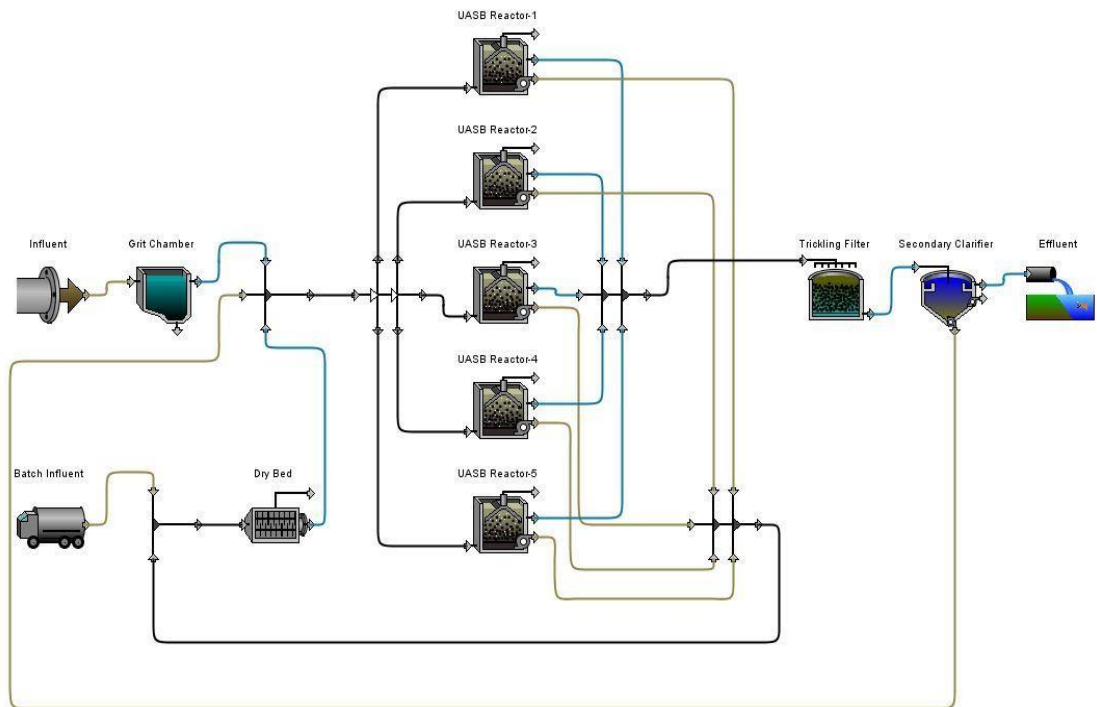


Figure 4 Advanced approaches in modeling UASB reactor

2.10.6. Modeling UASB reactor in GPS-X

The main processes that occur in UASB reactors are substrate degradation, microorganism growth, and biogas production (Hydromantis_GPS-X_v7, n.d., 2013). Reaction model (Used to model the reaction going on inside the reactor), Hydraulic model (Used to describe the flow inside the reactor), Solid- liquid separation model (Used to model the top part of the reactor), Gas- liquid separation model (Used to model the gas produced inside the model), Fluidization model (Used to model the solids coming from the UASB reactor and its relationship with the height of the sludge blanket) and Substrate transport model (Kalyuzhnyi et al., 2006; Rodríguez-Gómez, R.1 & , Moreno, L.1 and Liu, 2013; Rodríguez-Gómez et al., 2014).

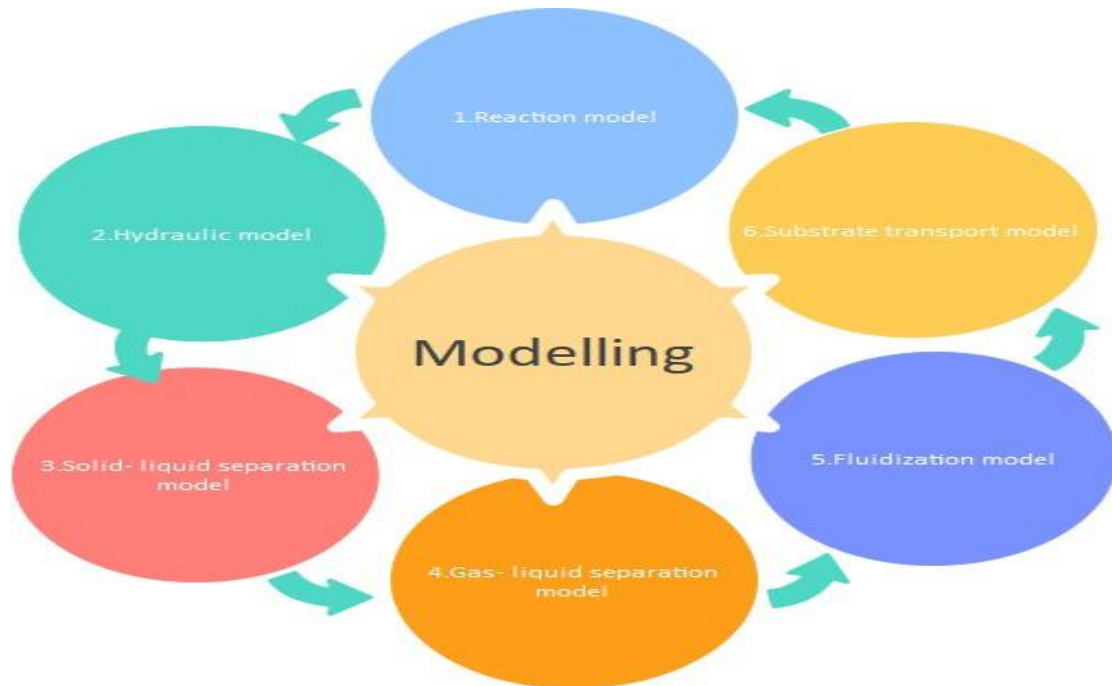


Figure 5 Model components in UASB reactor modeling

2.10.6.1. Start up in UASB and Mass balance in modeling

2.10.6.2. Start up in UASB

In the research done on Start-up alternatives and performance of UASB pilot plant treating diluted municipal wastewater at low temperature, different start-up procedures of UASB digester were carried out (Bhatti et al., 2014; Pererva et al., 2020). Start-up without inoculum was delayed for about 120 day. The digester reached 75-85% total suspended solids (TSS) removal, 54-58% total chemical oxygen demand (TCOD) removal and 63-73% biological oxygen demand (BOD₅) (Álvarez et al., 2006).

In the other experiment, the researchers found the removal at influent concentrations of 240-340 mg TCOD_i, temperatures of 13.5-15°C and hydraulic retention times (HRT) of 10-11h. When the digested sludge was used as inoculum after the start-up period of 75 days, digester efficiencies were 58%, 41% and 54% for TSS, TCOD and BOD₅ respectively. Working at 169 mg TCOD_i, temperature of 14°C and HRT of 11h the sludge bed developed and stabilized quickly. When using a hydraulically adapted inoculum, but TCOD and BOD₅ removals remained low and there was volatile fatty acids (VFA) accumulation in the effluent (Liu et al., 2021; Arthur et al., 2022). In

their other research, the researchers used digester to enhance the biodegradation of influent solids retained in the UASB reactor at low temperatures, to increase its specific methanogenic activity. The sludge drawn from the UASB's middle zone entered the digester's upper zone and then circulated from the digester's bottom to the UASB's bottom. Hydraulic retention time 16-27day was used in the digester. The digester temperature was set at an optimum value ranging from 25 to 35°C.

The steady state efficiency of the UASB system, were set at 6-8 h of HRT, 15-16°C of temperature, the researchers found that the removal efficiency of chemical oxygen demand was 79%, and 60% of biological oxygen demand (BOD₅)(Arthur et al., 2022). In the other research topic, UASB reactor startup for the treatment of municipal wastewater followed by advanced oxidation process reactor (Bhatti et al., 2014) the researcher used two different nutrients during the UASB start-up period, which was designed to decrease the hydraulic retention time (HRT) from 48 to 24 and 12 to 6 h at average temperatures of 25- 34°C. In the first stage, start-up was with glucose for 14 days and then the reactor was also fed with macro and micronutrients as a synthetic nutrient influent from 15 to 45 days as the second stage. The researcher found out the removal efficiencies of the chemical oxygen demand was 80% and 98% on the 6th and 32nd day of the first and second stage, respectively. The maximum substrate removal rate of 0.08 mg COD mg⁻¹ VSS d⁻¹ was observed for glucose and synthetic nutrient influent on the 8th and 40th days, respectively. When the reactor's COD removal efficiency reached its maximum, it was switched to municipal wastewater (MWW) mixed with industrial wastewater. While treating MWW, the HRT was gradually reduced with a one week interval. For further cleaning, the UASB effluent was treated with 40% waste hydrogen peroxide. The whole integrated treatment process was successful to reduce the COD by 99%, total suspended solids (TSS) by 73%, total nitrogen (TN) by 84% and turbidity by 67% (Bhatti et al., 2014). Up-flow anaerobic sludge blanket (UASB) reactor has been employed in industrial and municipal wastewater treatment for decades. However, the lengthy development time required for anaerobic granules severely limits the technology's application.

Understanding the mechanisms of anaerobic granulation is necessary for developing a strategy for rapid UASB startup. But in this experimental research, 10L of sludge was inoculated from sludge blanket zone to the reactor set up to speed up the rate of startup.

2.10.6.3. Mass balance in modeling

In the research done by Del Castillo et al. first order kinetic models were used in order to determine the outlet pollutant concentration based on the inlet concentration (Del Castillo et al., 2020). On the other hand, Bhunia & Ghangrekar in their research three kinetic models namely, Monod, Grau second-order, and Haldane model were considered for the analysis. Both linear and nonlinear regressions have been performed to examine the best fit among the kinetic models. In this process, five error analysis methods have been used to analyze the data.

In addition to optimizing kinetic coefficients and minimizing associated errors, prediction of effluent COD has been undertaken to validate the applicability of kinetic models. In both cases, Grau second order model is found to be the best class of fit for wide range of data sets in UASB reactor (Bhunia & Ghangrekar, 2008). In the same way, nonlinear modeling study was carried out to evaluate the performance of UASB reactors treating poultry manure wastewater under different organic and hydraulic loading conditions. Similarly, K. Singh and Valshya used kinetic models like Grau second-order, followed by first order method and Monod method in order to determine the kinetic parameters using line regression on the basis of experimental results. The results indicated that Grau second order; first order and Monod models were most suitable for estimation of kinetic coefficients in UASB with values of coefficient of correlation 0.9958, 0.8809, and 0.9677 respectively. They concluded that Grau second order is the best model to determine the kinetic parameters (K. Singh & Valshya, 2017).

On the other hand, on the research topic effect of temperature on low-strength wastewater treatment by UASB reactor using poly vinyl alcohol gel carrier, the researcher used the following hydraulic retention times (HRT) adopted for the UASB reactor, (8.0; 9.6; 8.4; 6.0 and 4.8 h). The researcher also used operational temperature $23.4^{\circ}\text{C}\pm 1.5^{\circ}\text{C}$. The analyzed physical-chemical parameters were temperature, COD, BOD, total volatile solids (influent, effluent and of the reactor's profile sludge), flow

rate and nutrients (N and P). In this research kinetic coefficients $Y=0.091 \text{ mg t COD mg TVS}^{-1} \cdot \text{d}^{-1}$, decay coefficient $K_d=0.01 \text{ d}^{-1}$ and concentration of limiting substrate $K_s=282.5 \text{ t COD mg L}^{-1}$ and maximum growth rate $\mu_{\text{max}} = 0.051 \text{ d}^{-1}$ were achieved. The temperature was decreased from 35°C to 25°C and then to 15°C. HRT was reduced from 2.0 to 0.22 hours. The COD removal rate reached 28 kg COD $\text{m}^3 \text{ d}^{-1}$ at 35°C, 16 kg COD $\text{m}^3 \text{ d}^{-1}$ at 25°C, and 6 kg COD $\text{m}^3 \text{ d}^{-1}$ at 15°C. The COD removal rate was reduced by half for each temperature reduction of 10°C (Khanh et al., 2011).

In this research, two identical pilot scale up-flow anaerobic sludge blanket (UASB) reactors (15.7L) were run at mesophilic conditions (30-35°C) in a temperature-controlled environment with three hydraulic retention times (θ) of 15.7, 12 and 8.0 days. Imposed volumetric organic loading rates (LV) ranged from 0.65 to 4.257 kg COD/ ($\text{m}^3 \cdot \text{day}$). On the basis of experimental results, two empirical models having a satisfactory correlation coefficient of about 0.9954 and 0.9416 were developed to predict daily biogas production (Q_g) and effluent COD concentration (Se), respectively. Findings of this modeling study showed that optimal COD removals ranging from 86.3% to 90.6% were predicted with HRTs of 7.9, 9.5, 11.2, 12.6, and 13.7 and volumetric Organic loading rate of 1.27, 1.58, 1.78, 1.99, 2.20 and 2.45 kg COD/ ($\text{m}^3 \cdot \text{day}$) (Yetilmezsoy & Sakar, 2008).

However, GPS-X was used to model the UASB reactor in this experimental study. The tool also eliminates the need to collect data in order to generate constants for drawing mass balance curves. The tool only needs to collect influent and effluent data from the reactor setup. The tool determines the inlet and outlet mass balance around the secondary clarifier. The only thing the modeler should do is precisely characterizing the influent and effluent for influent advisory, UASB reactor, Trickling filter, secondary clarifier, and effluent. Eventually, the modeler should draw the cumulative mass of TSS out of the clarifier(X) to the cumulative mass TSS in the clarifier(Y).

2.10.6.4. Biological Processes in UASB reactor

There are several key biological processes taking place in the UASB reactor. These are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In hydrolysis carbohydrates, proteins and fats will be broken down to glucose, amino acid and fatty acids respectively. In acidogenesis, the simple organic compound produced by

hydrolysis will be converted to volatile fatty acids (VFA) and alcohols. In turn the VFA and alcohols will decompose to produce acetates, carbon dioxide, and hydrogen by acetogenesis. In methanogenesis, acetates, CO₂ and hydrogen will decompose to produce CO₂ and methane gas.

2.10.6.4.1. Monod Kinetics for substrate utilization

$$\frac{dS}{dt} = \frac{\mu_{\max} \cdot S}{K_s + S}$$

$\frac{dS}{dt}$ - is the rate change of substrate with time

S - is the substrate concentration

K_s - is the Monod constant, representing the substrate concentration at which the growth rate is half of μ_{\max}

2.10.6.4.2. Contois Kinetics for biomass Growth

$$\frac{dX}{dt} = \frac{\mu_{\max} \cdot S}{K_s + S} \cdot \frac{X}{K_x + X}$$

Where:

$\frac{dX}{dt}$ - is the rate change of biomass concentration over time

X - is the biomass concentration

K_x - is the Contois constant representing the biomass concentration at which the growth rate is half of μ_{\max} .

2.10.6.4.3. Methane production by Methanogenesis

$$\frac{dCH_4}{dt} = \frac{V_{\max} \cdot S}{K_s + S + \frac{S^2}{K_1}}$$

Where: $\frac{dCH_4}{dt}$ is the rate of methane production for methane production time

V_{max} - is the maximum methane production rate

K_s - is the Monod constant for methane production

K₁- is the inhibition constant for intermediate products

2.10.6.4.4. Ammonia Oxidation

$$\frac{dNH_3}{dt} = Y \quad \frac{\mu_{\max-NH_3} \cdot NH_3}{K_{NH_3} + NH_3} = X$$

Where: Y_{AOB} - is the yield of ammonia oxidizing biomass (AOB) converting ammonia to biomass (ammonia oxidizer yield)

$\mu_{\max-NH_3}$ - is maximum growth rate for ammonia oxidizer yield

2.10.6.4.5. Nitrite Oxidation

$$\frac{dNO_2}{dt} = Y \quad \frac{\mu_{\max-NO_2} \cdot NO_2}{K_{NO_2} + NO_2} = X$$

Where: Y_{NOB} - is the yield of nitrite oxidizing biomass (NOB) converting nitrite to biomass (nitrite oxidizer yield)

$\mu_{\max-NO_2}$ - is maximum growth rate for nitrite oxidizer yield

2.10.6.4.6. Phosphate oxidizer

$$\frac{dp}{dt} = \frac{\mu_{\max-PAO} \cdot P}{K_p + P}$$

Where: $\frac{dp}{dt}$ - is the rate of change of phosphorus concentration overtime.

Y_{PAO} - is the yield coefficient of PAO indicating the amount of biomass produced per unit of phosphorus consumed

$\mu_{\max-PAO}$ - is the maximum specific growth rate of PAO on phosphorus

K_p - is the Monod constant for phosphorus, representing the phosphorus concentration at which the growth rate half of $\mu_{\max-PAO}$

3.1. MATERIALS AND METHODS

3.1.1. Description of the study area

Kality domestic wastewater treatment plant is owned by the Addis Ababa City Water and Sewerage Authority (AAWSA). It is located in Addis Ababa, Ethiopia, at 8°55' 11"N and 38°45'19"E. It is capable of handling a maximum flow rate of 100,000m³/day. The treatment processes include pretreatment units UASB (Up-flow Anaerobic Sludge Blanket) reactor, trickling filters, secondary clarifiers, and chlorination/dechlorination for disinfection before discharging the effluent into the nearby river. Kality wastewater treatment plant accounts for approximately 29% of the city's wastewater treatment coverage. The plant receives sewage from the northern, western, and central areas of the city. The wastewater is collected in two ways: first, through sewer lines/trunk lines (800mm and 1500mm pipes), and second, through trucks. The wastewater treatment plant is the biggest of its kind in Ethiopia employing UASB reactor. The wastewater was designed to treat the wastewater coming from seven sub cities with an approximate population equivalent of 600,000. After mechanical screening, the wastewater goes to grit chamber where grits are removed. After grit chamber, the wastewater was divided in twenty UASB reactors of dimension 4.9mx20mx20m. After the wastewater is treated in the UASB reactor, it is divided between four trickling filters where the system decomposes the organic matter by attached system by the use of micro-organisms. Again after trickling filter, there are four secondary clarifiers where majority of the materials get settled before joining the tertiary treatment. After disinfection through the use of chlorination, the effluent is discharged to the nearby river little Akaki. The general layout of Kality Wastewater Treatment Plant is shown in the figure-6.



Figure 6 kality wastewater treatment plant satellite location

3.1.2. Experimental reactor set up

The configuration of the pilot-scale reactor used is shown in Figure 7. The UASB (Up- flow Anaerobic Sludge Blanket) reactor was inserted in to 100L water-filled barrel and a refrigerator's evaporator mounted on opposite sides of the barrel. The evaporators were attached to compressors, radiators, and a supply fan fixed outside the setup to lower the temperature to the appropriate value. A 2500W heater was fitted inside the water-filled barrel and used to elevate the temperature when needed. The control board panel included a timer, thermostat, and temperature sensors. The cooling system, heating system, sensors, and power sources were all linked to the Process Integrated Derivatives (PID) control board. The PID control board was the central control unit of the pilot-scale UASB reactor, managing temperature, Hydraulic Retention Time (HRT), heating, and cooling systems. Measuring the influent (initial) COD concentration of the sample wastewater assisted in determining the HRT, which was later input into the timer. The PID thermostat was used to set the desired temperature, estimated from the CCD-RSM model, and the reactor sensor measured the temperature of the wastewater in the barrel. Inside the barrel, a 0.0486 m³ square reactor was submerged and filled with wastewater. This reactor volume was chosen because it was not possible to obtain a larger capacity compressor for cooling, and to enable easy management of influent and effluent. The barrel and pilot-scale UASB reactor were wrapped with sponge and later by aluminum foil to help maintain a

stable wastewater temperature inside the reactor and the water temperature in the barrel. One advantage of the system used was that, it allowed the incorporation of the PID control board for data collection through temperature management via heating and cooling systems, as well as HRT. The reactor with the PID control board could operate independently, but the influent for the reactor setup was sourced from the WWTP employing a UASB reactor.

3.1.3. Chemicals and materials

The influent and effluent COD (Chemical Oxygen Demand) concentrations were determined according to the Dichromate Methods for the examination of Water and Wastewater, using medium (0-1500 mg/L) and high range (0-15,000 mg/L) COD kits provided by Hach. NaOH and H₂SO₄ were used to adjust the pH of the raw wastewater during the experiments. A Hach DR890 Colorimetric instrument and relevant medium kits (0-700 mg/L) were used to measure the influent and effluent concentrations of sulfate ions. Alkalinity was measured using Alkaphot tablets and a Palin test 7100 photometer. The pH was measured using a pH meter purchased from Hach. During the experimental investigation, instruments such as the DR 5000 Spectrometer (HACH-USA), the DR890 Colorimetric (HACH-USA), the Palin test photometer (England), the oven and furnace (Germany), the pH meter (HACH-USA), and filter paper 45μ were used.

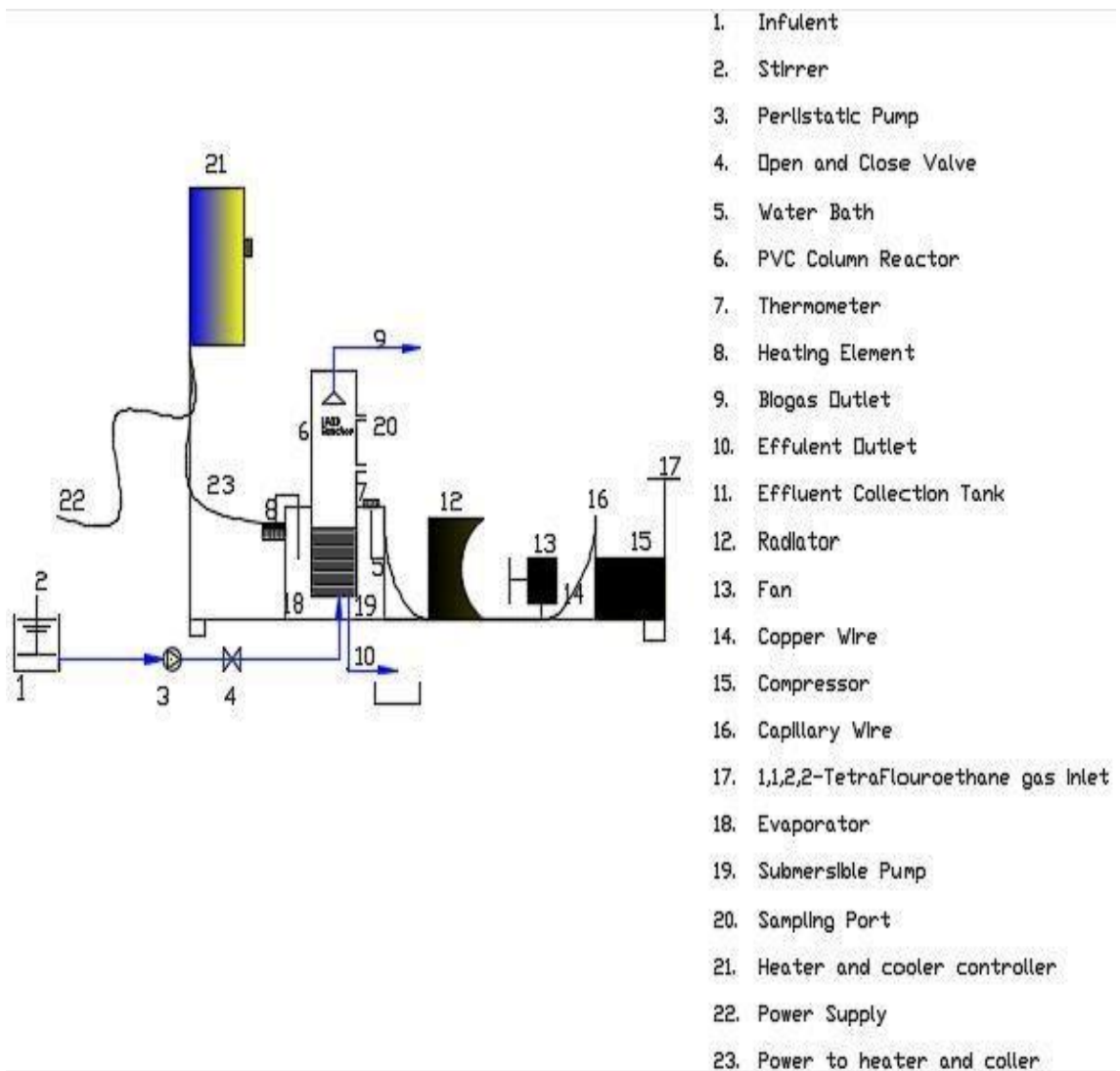


Figure 7 Schematic diagram of PID control board with pilot scale UASB reactor

3.1.4. Sampling Techniques

The grab sampling method was utilized to determine influent and effluent concentrations. For all runs, influent samples were collected every day early in the morning from the inlet of WWTP employing UASB reactor. The effluent concentration for each run was determined from the pilot- scale UASB reactor after the wastewater had remained for the specified HRT, OLR, pH, and temperature. Water samples were taken at UASB reactor inlet and outlet, Trickling filter and secondary clarifier outlet for the determination of toxic heavy metals concentration. Soil and vegetables samples were also taken from different plots of land for toxic heavy metals concentration determination.

3.1.5. Reactor set up sampling ports

There are sampling ports on the UASB reactor set up. Four sampling ports were located at 0.32m, 0.38m, 1.18m and 1.5m on the reactor set up. Samples such as sulfate ions, ammonia-nitrogen, mixed liquor suspended solids, mixed liquor volatile suspended solid were taken at 0.38m. But samples such as COD, TSS and VSS were taken at 1.5m sampling port.

3.2. Data collection methods using pilot scale UASB

3.2.1. Experimental Procedure for data collection

Data were collected during the rainy (June, July and August) and dry seasons (February, March and April) for performance enhancement of UASB reactor through optimization of operational parameters. Samples were taken every day in the morning from the inlet of the existing UASB reactor. 2mL of the raw sample was put in one COD vial of high range (0mg/L-15,000mg/L) Hach and distilled water in another. The vials were turned up and down several times for mixing. The one with distilled water was used as a control. Adjust the temperature and timer of the digester to 150°C and for 120 minutes. As you heard Vep sound from the digester to mean it was the right time to insert both vials. At the end of 2hrs the vials were taken out and turned up and down to mix, and then they were put for 30 minutes to settle. After 30 minutes, the reading was taken using COD viro spectrophotometer of high range. The blank was used to make the spectrophotometer zero measurement. Then the viro with sample was inserted and the reading was taken. The same procedure was applied for final COD concentration determination but low rage vials 0mg/L to 1,500mg/L was used 0.2mL of sample and distilled water were used .

Calorimetric determination was used to determine the initial and final concentration of sulfate ions. Initially, two cuvettes one for control and the other for sample were used. The sample was filled with two cuvettes to 10mL mark. In one of the cuvette add sulfate powder pillow and put them for 5 minutes to mix. Then the blank was put to the calorimetric DR890 and set to zero (mg/L).The second vial with powdered pillow was put in to the instrument and the reading was taken. The same procedure was iterated for the determination of the out let concentration of sulfate ions. For the determination of alkalinity, Palin test photometer 7100 was used with alkalinity tablet. Raw sample was filled to 10mL mark in one test tube and distilled water in the other.

In the test tube with wastewater sample, one alkalinity tablet was added to the test tube and crashed to powder. The test tubes were put for 10 minutes to mix. The blank was put in the photometer to make the reading to zero. Then put the second test tube in the photometer and the reading was taken in mg/L as CaCO₃. The same procedure was iterated to determine the final concentration of alkalinity. Titration was used to determine the VFA inlet and outlet concentration.

The performance efficiency of the reactor set up was measured using equation-1 below and the highest performance efficiency showed the parameters combinations were operational parameters to be selected (Kim et al., 2023).

$$\% \text{Performance efficiency of UASB Reactor} = \left[\frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \right] * 100 \quad (1)$$

where: C influent is the concentration of raw sewage in mg/L

C effluent is the concentration of sewage leaving the reactor set up in mg/L

TSS and VSS were determined by using Gravimetric methods. Inlet and out let concentration of alkalinity were measured by using Palin test photometer Phot002 using Alkaphot reagent. VFA was determined using titration methods. Influent and effluent concentrations of Sulfate ion was determined by Colorimetric determination method using sulfate pillow reagent.

3.2.2. HRT Determination

After the determination of the initial COD, the amount of time the wastewater should remained in the reactor set up for each test before taking the effluent sample should be determined. To do so the following approach was used (Resolutions taken at the World Conference on Lung Health, 2003).

$$HRT = \frac{S_o}{OLR} * 24 * 10^{-3} \quad (2)$$

Where Q-is discharge (m³/d), S_o –is initial COD concentrations in mg/L, OLR is organic loading rate (kg COD/m³.d).

3.2.3. Operational Parameters

The experimental study took into account several operational factors, including temperature (0 to 30°C), since Kality WWTP operates at room temperature. Data obtained during the night-time rainy seasons ranged from 10 to 12°C, while during the daytime dry seasons, data were gathered between 25 and 27°C.

HRT (4 to 12 hours) was taken into consideration. If the HRT was shorter than 4 hours, there would be microbial washout, which would decrease the activity of microorganisms on organic compounds and lower UASB reactor performance. Conversely, if the HRT exceeded 12 hours, an excessive amount of sludge would accumulate at the sludge blanket zone, which would lower the UASB reactor's performance. OLR (1 to 3 kg COD/m³.d) was taken into consideration because, in the event that the OLR is higher than three, the sludge bed produces biogas, which in turn generates gas pockets that lift the sludge bed to the gas-liquid-solid separator zone, where it forms excessive foams, hence decreasing the UASB reactor's performance. OLR is directly correlated with food, thus if it falls below 1 kg COD/m³.d, the microbes will perish. The pH range of 6.3 to 7.8 was used in this experimental study because a pH higher than 7.8 interferes with the processes of acidogenesis and methanogenesis in anaerobic digestion, which lowers methane generation. Once more, lowering the pH below 6.3 will result in an acidic environment, which will increase the generation of hydrogen sulphide and smaller chains of carboxylic acid (acetic, propionic, and butyric acid) which eventually reduce the performance of UASB reactor. In general, operational parameters of temperature 0 to 30°C, OLR of 1 to 3 kg COD/m³.d, HRT 4 to 12 hrs, pH of 6.3 to 7.8 were used to improve the performance of the UASB reactor at moderate temperatures for unregulated sewage treatment.

3.2.4. Statistical Analysis

The CCD-RSM tool was utilized for optimization, performance enhancement, and data analysis (Alkhatib et al., 2015; Rahman et al., 2022). This tool has five levels for every operational parameter. The coded form is from $-\alpha$ to $+\alpha$ ($-\alpha, -1, 0, +1, +\alpha$). Each parameter was coded as follows. Temperature (A), OLR (B), pH(C) and HRT (D) (Zhao et al., 2008). The following formula was used to determine the total number of pilot scale experimental runs (Chollom et al., 2020; Ansari et al., 2022).

$$N = K^2 + 2K + C_0 = 4^2 + 2*4 + 6 = 30 \quad (3)$$

Where N- represents the total number of experimental runs, K- is the number of operational parameters considered; Co- is the center point.

Table 1 Five levels used by RSM-CCD and coded operational parameters (Bui, 2018)

Operational Parameters	Code with unit	Five levels used in CCD-RSM				
		$-\alpha$	-1	0	+1	$+\alpha$
Temperature	A(°C)	-15	0	15	30	45
OLR	B(kg COD/m ³ .d)	0	1	2	3	4
pH	C	5.55	6.3	7.05	7.8	8.55
HRT	D (hrs.)	0	4	8	12	16

3.2.5. Modeling COD, TSS and VSS performance efficiency using RSM-CCD

Response surface method-central composite design was used to model the performance of COD, TSS, and VSS. The model equations' appropriateness was assessed using analysis of variance (ANOVA) (Rahman et al., 2022). The Coefficient of variation, coefficient of determination (R²), adjusted coefficient of determination (adj-R²), and prediction coefficient of determination (pred.R²) were used to examine the quality of the model developed. RSM- CCD developed the following general mathematical model for %COD, %TSS and %VSS removal (Bashir et al., 2012; Bashir et al., 2015).

$$Y = \beta_0 + \sum_{i=1}^k (\beta_i x_i) + \sum_{i=1}^k (\beta_{ii} x_i^2) + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

Where; Y is the experimental response variable, β_0 is the intercept β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linear effect, double interaction and quadratic effects respectively. X_i , x_j are the independent variables (experimental variables) and ε is random error.

3.2.6. Data inputs in to CCD-RSM

The experimental design software produced 30 experimental runs for four operational parameters. Data were collected and entered into CCD-RSM to determine the percentage removal, optimize the operational parameters, and to model COD, TSS, and VSS removal.

3.2.7. Methods for heavy and toxic heavy metals determination (Water, plant and soil)

3.2.7.1. Survey of Reconnaissance

Before the official survey began, a reconnaissance survey was conducted. The preliminary data needed for sampling and sample transportation was assessed. The site description, vegetable varieties grown downstream of the wastewater treatment plant, and irrigated areas using the treatment plant's effluent were all documented.

3.2.7.2. Procedure for collecting and preparing samples

Six specific sampling areas were selected based on vegetable availability, irrigation soil, and wastewater. These sampling locations can be found at the Kaliti WWTP. For all samples, grab sampling techniques was employed. Four water samples were taken from various treatment plant unit processing regions (sample-1: UASB reactor inlet, sample-2: UASB reactor outlet, sample-3: trickling filter outlet, and sample-4: secondary clarifier outlet). Three representative soil samples were collected from the irrigation areas. Two types of plants were collected from the area where treatment plant effluent was used as a source for water. These points were chosen because samples would produce a better result.

3.2.7.3. Water Sampling Points

Frequency: Water samples should be collected at regular intervals, such as weekly or monthly, depending on the expected variability of heavy metal concentrations and the dynamics of the water source.

Duration: Sampling should ideally be carried out over a period of at least one year to account for seasonal variations and any other temporal changes in water quality. But since the frequency of testing and duration of sampling too long grab sampling were taken at various unit process locations. Four water samples were collected from the treatment plant. The sampling locations are as follows: (sample-1: UASB reactor inlet, sample-2: UASB reactor outlet, Sample-3: trickling filter outlet, sample -4: secondary clarifier outlet).

3.2.7.4. Water sample collection

To avoid cross-contamination, at each sampling station, three different samples at different intervals were collected. For each sampling location, 1000mL of wastewater using pre- cleaned amber colored bottles were taken. The locations of the samples were labeled. The water samples were kept cool in an icebox, and the time between sampling and analysis was kept to a bare minimum of 2hrs.

3.2.7.5. Water digestion procedure

The Optimum Procedure for Digestion of Water and Sediment Samples was carried out for three hours at 250°C using 50 mL of water, 4 mL of HNO₃, and 1 mL HCl. The digested volume was remained at 25 mL after digestion, and was filtered in a 50 mL Erlenmeyer flask.

3.2.8. Soil Sample Collection

Frequency: Soil samples are typically collected less frequently than water samples. Quarterly sampling (every three months) can be a good starting point.

Duration: Similar to water sampling, soil sampling should ideally cover at least one year to account for seasonal variations and agricultural practices that may affect heavy metal concentrations. But since the frequency of testing and duration of sampling too long grab sampling were taken at various irrigational plots. Three kilograms of soil from three different areas were taken from the irrigation area. The samples were taken during the dry season to allow for easier soil-water mixing and to avoid inaccurate results caused by urea fertilizer and lime. To obtain an accurate result, the samples were made leaf-free. To avoid mixing with metal containers, they were stored in non-reactive containers in plastic bags. To avoid mixing of plant leaves and other materials; the sampling depth was set between 10 and 15 cm.

3.2.8.1. Soil sample preparation

The soil sample was dried in an oven set to 30-40°C until it reached a constant weight. The dried sample was sieved at 2mm sieve size. The sample was gridded to 9µm. The Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument was used to collect the sample for heavy metal analysis.

3.2.8.2. Soil Digestion Procedure

The sample (1g) was weighed into a 50 mL crucible and treated with 10mL of concentrated HNO₃. To allow oxidation, the solution was placed on a hot plate for 30-

45 minutes. Following cooling, 4 ml of 20% H₂O₂ was added, and the solution was reheated on a hot plate until the digest became clear and semi-dried. Before Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) analysis, the suspension was filtered into a 50mL volumetric flask and diluted with deionized distilled water to the mark.

3.2.9. Plant Samples

Frequency: Vegetable samples can be collected at each harvest. If multiple crops are grown in a year, samples should be taken for each crop cycle.

Duration: Sampling should cover at least one full growing season for each type of vegetable being studied. Ideally, this should be extended to multiple seasons to understand any longer-term trends. But since the frequency of testing and duration of sampling were too long grab sampling was taken at various irrigational plots. Farmers irrigate two types of widely consumed fresh vegetables grown with Kaliti wastewater treatment effluent during the dry seasons: Ethiopian Kale (Habesha Gommen) and Swiss Chard(Kosta).

3.2.9.1. Plant sample collection

Two different plant samples were collected (each 500gram). Three samples for each plant samples were collected from every corner of the plots to ensure that the samples were representative. The samples were carefully collected to avoid damaging, dead, or dying plant tissue. Before placing the samples in the bag, the extra soil from the plant material was brushed off. To avoid cross-contamination, samples were collected in clean plastic bags separately. The plant samples were kept cool in an icebox.

3.2.9.2. Dry ashing procedures

In a "high form" porcelain crucible, 1.25g of sample was weighed. The sample was placed in the furnace, which gradually raised the temperature to 540°C. The sample was ashed for 6 hours and then wet with distilled water before being dried on a hot plate with 5-10 mL of 6N HCl. To dissolve the ash, 10 mL of 1N HCl was added to the sample before the ash was dissolved. The sample was transferred to the inductive coupled Plasma (ICP) test tube.

3.3. Methods for UASB reactor modeling

3.3.1. Input data

The input data utilized for calibration was gathered from the UASB reactor at the wastewater treatment plant. For the calibration of GPS-X, thirty data sets collected by AAWSA were employed, and the data were validated using Python to eliminate any missing or outlier values. Additionally, thirty validation data points were obtained from the reactor during the dry period, as the strength was notably higher at that time, and all data points from the rainy seasons fell within the range established during the dry seasons.

3.3.2. GPS-X modeling steps

The following are steps taken to model the UASB reactor.

Step-1: Collection of data for calibration and validation

Step-2: Construction of the UASB reactor layout

Step-3: Data Validation

Step-4: Running the default values and drawing the mass balance around the secondary clarifier. That is assumed to be the actual cumulative mass balance.

Step-5: Characterization of the influent advisory (inserting the values of Total COD, Total TKN, TP, Ammonia- nitrogen, VSS/TSS ratio, pH, Carbonate alkalinity etc.) and iterating the values of organic fractions until the composite variables TSS and VSS attains the mass balance (equal to the measured values).

Step-6: Running the model

Step-7: Preliminary sensitivity analysis to reduce the number of model parameters

Step-8: Time series type optimization of the model. This can be done by adjusting kinetic, stoichiometric and settling parameters. This helps for the best fit between the data set and the simulated model.

Step-9: Drag and put the parameter you are interested in from the influent characterization and put as a control, insert the minimum and maximum range and insert the data points for each parameter. Do the same for other unit processes you think affect your model.

Step-10: Outputs

Drag and put the type of graph you're interested in under the outputs. For example: For influent-right click on influent –output variables (the select every parameter you're interested in and you think affects your model under the outputs creating new layer). Insert the minimum and maximum output values.

Step-11: Run the model for 30 data points

Step-12: The fit between the simulated data and the measured data will be evaluated from the plotted graph.

Step-13: If the simulation and the measured data were not fit, you need to go to the fine tuning of the kinetic, stoichiometric and settling parameters for the best fit.

3.3.3. Assumptions adopted to develop the model

1. The pH of the UASB reactor is neutral
2. The biological reactions in the UASB reactor take place at constant temperature
3. There is uniform mixing in the UASB reactor
4. For any influent characters the model parameters are kept constant
5. There are enough organic and inorganic nutrients to ensure the growth of microorganisms.

3.3.4. Sensitivity Analysis

Sensitivity analysis evaluates how the parameters used in model calibration affect outputs. During calibration, settling, kinetic and stoichiometric parameters were used for sensitivity analysis to reduce the number of iteration and calibration of the GPS-X. The sensitivity analysis of the model was done by changing one model parameter at a time and observing the over lay of the simulation and the measured data sets. The performance of sensitivity analysis decreases the number of model parameters hence increases the predictive capacity of the model. The following formula is used to determine normalized sensitivity coefficient (Vitanza et al., 2016; Sadri Moghaddam & Pirali, 2021). Two sensitivity measures are calculated: the normalized sensitivity coefficient ($S_{i,j}$) and the mean square sensitivity measure (δj_{msqr}).

$$S_{i,j} = \left| \frac{\frac{\Delta y}{y_i}}{\frac{\Delta x}{x_i}} \right| \quad (5)$$

The normalized sensitivity coefficient ($S_{i,j}$) is calculated by comparing the percentage change in the output variable (Y_i) to a 10% change in the input variable (X_i) and 'n' is the number of data collected (Liwarska-Bizukojc et al., 2011). The impact of a parameter on the model output can be classified as

$S_{i,j} < 0.25$ indicates that a parameter has no significant influence on the model output; if $0.25 < S_{i,j} < 1$ means that a parameter is influential; $1 < S_{i,j} < 2$ means that a parameter is very influential : $S_{i,j} > 2$ means that a parameter is extremely influential. This work applied a 10% increase to the input variable for $S_{i,j}$ calculations. Each input variable and model parameter was calculated separately. A parameter is considered influential if its $S_{i,j}$ value equals or exceeds 0.25 (Liwarska-Bizukojc et al., 2011; Hu et al., 2014; Vitanza et al., 2016). The sensitivity measure evaluates the importance of individual parameters in least squares parameter estimation. It is defined as:

$$\delta_j^{msqr} = \sqrt{\frac{1}{n} \sum_{i=1}^n S_{i,j}^2} \quad (6)$$

A high δ_j^{msqr} value indicates that a parameter has a significant impact on simulation results, while a zero value indicates that the results are unaffected by the parameter.

3.3.5. Calibration and Validation

Model calibration involves determination of model parameters to fit specific data collected from a full-scale WWTP under study (Gernaey et al., 2004; Małkinia & Zaborowska, 2018; Dutta et al., 2018). When calibrating the WWTP model, it's important to carefully check and enter the collected measured data as an input (Meijer et al., 2002). Based on the sensitivity analysis made, some selected settling, kinetic, and stoichiometric parameters were used for calibration of the GPS-X. For validation of the model, different independent data sets were collected. To evaluate the best fit between the simulation and the measured data sets, average absolute relative error (ARE) was used. The following formula was used to evaluate the agreement between the simulation and measured data sets (Bhunia & Ghangrekar, 2008; Liwarska-Bizukojc et al., 2011; Vitanza et al., 2016).

$$\text{ARE} = \frac{1}{n} \sum \frac{|X_i - Y_i|}{X_i} * 100 \quad (7)$$

Where X_i - is the measured values of the output variables Y_i - is the simulated values of the output variables n - is the number of variables (data)

4. RESULT AND DISCUSSION

4.1. Characterization of wastewater parameters

Wastewater is characterized based on origin, composition and flow variation. In practice inlet and outlet samples were collected for 30 different runs for rainy seasons and dry seasons. Parameters such as COD, SO_4^{2-} , VFA, Alkalinity, TSS, TP, TN, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$ and VSS were measured. The parameters load showed variation of (pH: 5.55 - 8.55), (SO_4^{2-} 16 - 87.5mg/L), VFA to alkalinity ratio (0.06 - 0.058), alkalinity (170 - 260mg/L as CaCO_3), TSS (3 - 235.44mg/L) and VSS (10 - 260mg/L) for rainy seasons. The operational parameters load for dry seasons were with a variation of TN (4.3 - 66mgTN/L), $\text{NH}_4^+ \text{- N}$ (32.8 - 69.2mg $\text{NH}_4^+ \text{ N/L}$), TP (1.19 - 11.9mgTP/L), (pH:5.55 - 8.55), SO_4^{2-} (6 - 80mg SO_4^{2-} /L), VFA to alkalinity ratio (0.055 - 0.15), alkalinity (155 - 370mg CaCO_3 /L), TSS (3 - 770mg/L) and VSS (0 - 880mg/L). The collected data showed that the wastewater strength comply with the standard set for the WWTP. The inlet and outlet WW data collected during the rainy season was tabulated in appendix-A, B, C.

4.2. RAINY (June ,July and August) SEASONS

The average daily inflow and outflow during the rainy seasons is shown in the table 2.

Table 2 Average daily inflow and out flow on June, July and August (during rainy seasons)

Date	Average daily Inflow	Average Daily Outflow	Date	Average daily Inflow	Average Daily Outflow	Date	Average daily Inflow	Average Daily Outflow
	(m3/d)	(m3/d)		(m3/d)	(m3/d)		(m3/d)	(m3/d)
01-Jun-22	104736	90512	01-Jul-22	109864	106600	01-Aug-22	102688	99288
02-Jun-22	95436	90536	02-Jul-22	102544	101704	02-Aug-22	99248	96904
03-Jun-22	97880	93128	03-Jul-22	104984	104616	03-Aug-22	99240	94696
04-Jun-22	10920	91880	04-Jul-22	101246	97104	04-Aug-22	97360	91816
05-Jun-22	97232	92672	05-Jul-22	100000	91822	05-Aug-22	90936	86256
06-Jun-22	108432	104568	06-Jul-22	104224	101360	06-Aug-22	91648	93736
07-Jun-22	101432	99512	07-Jul-22	107048	106916	07-Aug-22	96152	97304
08-Jun-22	109872	94544	08-Jul-22	102704	102160	08-Aug-22	95456	94488
09-Jun-22	104256	99000	09-Jul-22	103856	101920	09-Aug-22	91352	91136
10-Jun-22	101552	94792	10-Jul-22	109608	101880	10-Aug-22	91768	90824
11-Jun-22	104120	93256	11-Jul-22	107216	105152	11-Aug-22	98048	97600
12-Jun-22	95168	99768	12-Jul-22	103712	102176	12-Aug-22	99776	98656
13-Jun-22	97352	93488	13-Jul-22	101192	100776	13-Aug-22	93744	92304
14-Jun-22	109296	94616	14-Jul-22	107648	107192	14-Aug-22	97072	93160
15-Jun-22	100160	91208	15-Jul-22	102264	101952	15-Aug-22	94400	93696
16-Jun-22	104440	98640	16-Jul-22	107600	104912	16-Aug-22	97136	95808
17-Jun-22	105120	98256	17-Jul-22	103624	100464	17-Aug-22	96592	95912
18-Jun-22	107880	97776	18-Jul-22	104672	101120	18-Aug-22	84912	84016
19-Jun-22	108464	98488	19-Jul-22	102616	101512	19-Aug-22	80304	79904
20-Jun-22	109656	92168	20-Jul-22	100000	97500	20-Aug-22	73608	64848
21-Jun-22	103488	91072	21-Jul-22	100000	97500	21-Aug-22	78432	69096
22-Jun-22	102432	95312	22-Jul-22	108324	104480	22-Aug-22	73560	64464
23-Jun-22	108648	92328	23-Jul-22	108568	108054	23-Aug-22	85920	79104
24-Jun-22	109664	95944	24-Jul-22	100600	100456	24-Aug-22	90216	85320
25-Jun-22	94440	96112	25-Jul-22	108944	105736	25-Aug-22	90640	72456
26-Jun-22	104104	94192	26-Jul-22	101168	101304	26-Aug-22	92768	64224
27-Jun-22	107848	92328	27-Jul-22	106112	104448	27-Aug-22	90104	80216
28-Jun-22	104272	94144	28-Jul-22	109040	104384	28-Aug-22	95520	84336
29-Jun-22	106120	99720	29-Jul-22	107192	105952	29-Aug-22	92016	81984
30-Jun-22	105376	99040	30-Jul-22	102560	102704	30-Aug-22	97416	99904
Average	100660	95300	Average	104638	102462	Average	91934.4	87115.2
Min	10920	90512	Min	100000	91822	Min	73560	64224
Max	109872	104568	Max	109864	108054	Max	102688	99904
Total Sum	3019796	2859000	Total Sum	3139130	3073856	Total Sum	2758032	2613456

4.2.1. Experimental Validation

In order to verify the optimization results, triple experiments were performed under

predicted conditions by the developed model. The model predicted 91.06% removal of COD, 98.33% TSS and 93.26% VSS at an optimal temperature of 21.58°C, OLR of 2.99kgCOD/m³.d, pH of 6.3, and HRT of 4.37 hrs. The experimental values obtained at these operational conditions were 92.70 %, 99.06%, 94.5% for COD, TSS and VSS respectively and were in close agreement with the result obtained from the model(<5%) and hence the model was validated. These removal efficiencies COD, TSS and VSS were achieved since the wastewater was diluted with run-off (Table-2).

4.2.2. Performance enhancement modeling and model analysis

Performance enhancement experiments were carried out using the reactor set up according to CCD-RSM of the response surface methodology. The results (percentage COD, TSS and VSS) from the wastewater on the interaction of four operational parameters such as temperature, OLR, pH, and HRT were used for the model generation. Quadratic model was suggested because its adjusted R-Squared (0.9269), predicted R-Squared (0.6350), and R squared (0.9622) values were greater than linear adjusted R-Squared (0.4068), predicted R-Squared (0.6350), and R squared (0.4886) values. A second order quadratic regression was performed to estimate the response function as a second order polynomial. The examination of the model fit summary revealed that quadratic model was statistically significant for the response percentage COD, TSS and of COD, TSS, and VSS, were fitted using regression analysis.

The second order polynomial equation developed represents responses as functions of temperature (A), OLR (B), pH (C) and HRT (D). The response functions, namely the percentage removal of COD, TSS, and VSS, were fitted using regression analysis:

$$\% \text{COD Removal} = 76.8045 + 12.3033A + 7.0157B - 10.6719AB - 15.7041AD - 8.9942A^2 + 2.8596C^2 \quad (8)$$

$$\% \text{TSS Removal} = 75.5637 + 6.2977A - 8.9878B - 4.5348C - 14.9922D - 9.0683AB + 9.5147AD + 7.8825BC + -28.7737BD + 13.1374CD - 6.4923A^2 - 6.1265B^2 \quad (9)$$

$$\% \text{VSS Removal} = 56.0954 + 9.1897A - 16.3308B - 18.1609D - 11.4775AB + 10.6264AC + 17.9508AD + 11.9614BC - 34.054BD + 21.9357CD - 6.8842A^2 + 7.3882C^2 + 7.3529D^2 \quad (10)$$

To the best of the researcher knowledge no work has been done to develop similar model using CCD -RSM. But other researchers had done models with different software tools like MATLAB2011a Monod models, and Contois model (Işık &

Sponza, 2005; Jijai et al., 2015) and got successful performance prediction and optimizations of parameters. The above equations (equation 8, 9 and 10) describe how the removal efficiency of the UASB reactor (%COD, %TSS & %VSS) was affected by the individual variables (linear and quadratic or double interaction). The analysis of variance supported the quadratic model's adequacy and significance (ANOVA). The ANOVA analysis was done by means of Fisher's F-test. The model F-value observed was 27.29 showing that the model was significant. In the %COD removal coded parameters like A, B, AB AD, BD, A², C² were statistically significant (P<0.05) model terms at the 95 % confidence level. The non-significant value of lack of fit (F-value of 1.11) for the model showed that the developed model was valid. Furthermore the fitting of the experimental data to the regression model was checked and suitably explained by the value of the adjusted determination coefficient (R² Adj. = 0.927). This result means that 92.7 % of the total variation on percentage removal of COD data can be explained by the selected model. The adequate precision ratio of 22.22 indicated an appropriate signal to noise ratio. Again in Figure 8, the plot of studentized residuals versus run order was tested and the residuals were scattered randomly around ±4.00. This was an indication of better fitness of the model with the experimental data. Adjusted coefficient of determination and plot of studentized residuals for percentage removal of TSS and VSS are shown in appendix-D and E.

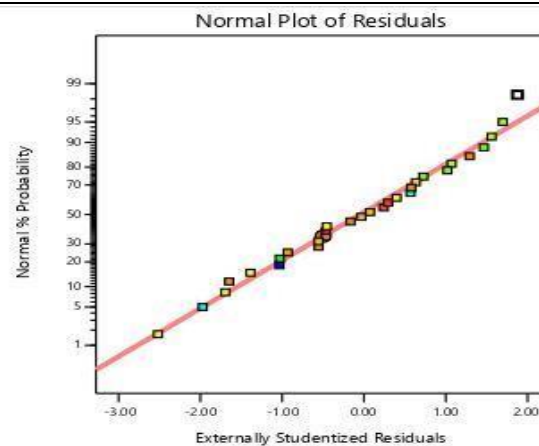


Figure 8 plot of studentized residuals for rainy season's data

4.2.3. Quantitative effects of the factors during the rainy season

Figure 9 (a) shows the combined effect of temperature and OLR on removal

efficiency of COD. The percentage removal of COD increased with increasing temperature and OLR; the removal efficiency increased with increasing OLR from 1 to 3kgCOD/m³.d. This can be explained by the fact that the activities of microorganisms increases with in the range of mesophilic temperature (Daija et al., 2016).In addition, temperature has a significant impact on the performance of a UASB reactor because it affects the hydrolysis process, substrate utilization rate, solids settling, and gas transfer rates (Chou et al., 2004; Álvarez et al., 2004; Hernández Leal et al., 2011).

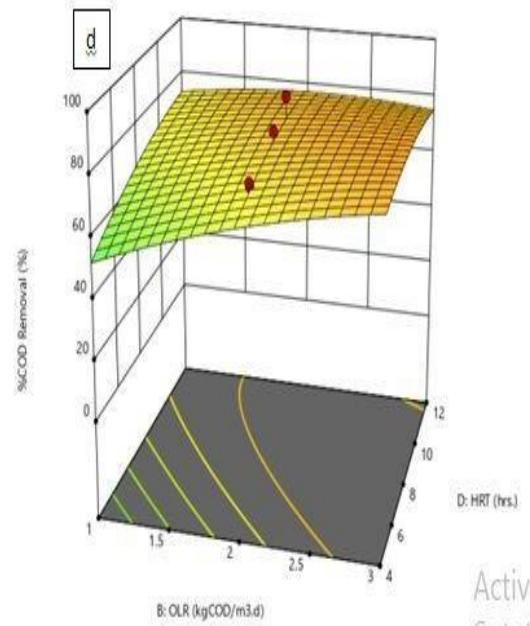
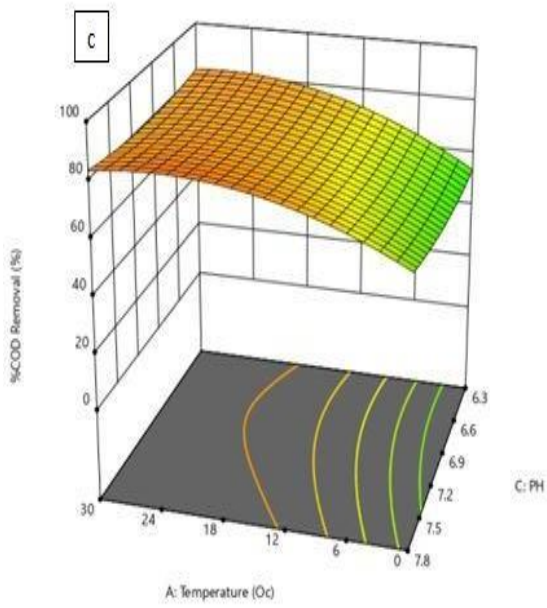
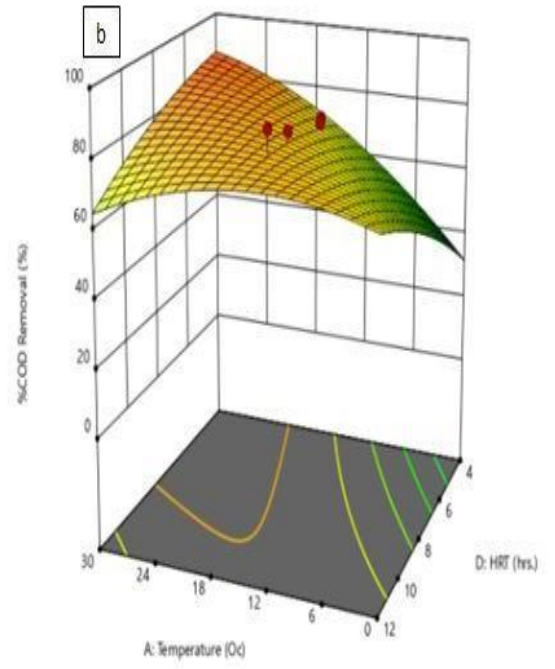
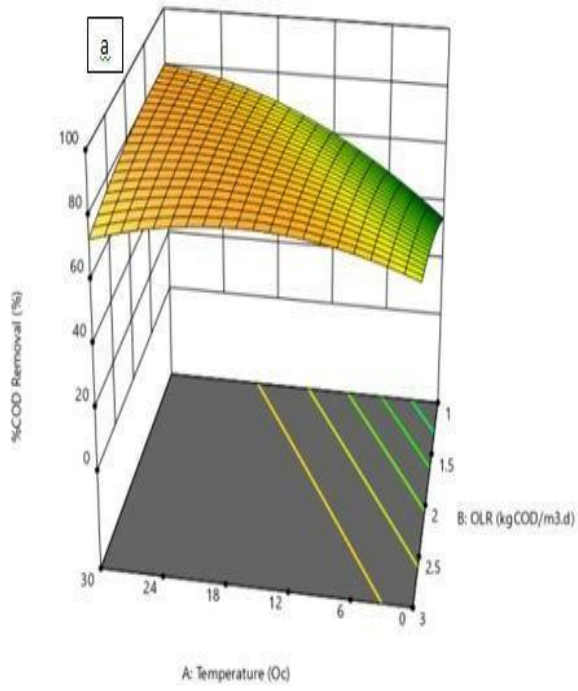
Figure 9 (b) depicts that the interactional effects of temperature and HRT on the percent removal of COD. From Figure 9 it can be seen that at lower temperature and lower HRT the removal efficiency of COD reached minimum. This is because as temperature and HRT get reduced the contact time between microorganisms and substrate get reduced and insufficient methanogens occurs (Ladu & Lü, 2014; Besharati Fard et al., 2019). But as temperature increases with HRT the removal efficiency of COD get increased since the activities of microorganism's increases and the chance in contact between the microorganisms and substrate in wastewater get increases (Hernández Leal et al., 2011; Verma & Kumar, 2013a).

Again in figure 9 (c) the combined effect of temperature and pH on the percentage removals of COD was shown. From the plot it was seen that, temperature and pH were increased at about 7 resulted in to an increase in percent removal of COD this was because an increase in pH in the range of 6.3 to 7.8 increase methanogenesis (S. J. Zhang et al., 2013; Casserly & Erijman, 2003). The plot also showed that at lower pH value, the percentage removal of COD was seen to be maximum at a temperature of 23.5°C beyond this temperature the percentage removal of COD was decreased this is because methanogenesis process is hindered(A. Ahmad et al., 2012; Nugroho & Santoso, 2019).

In figure 9 (d) the combined effect of OLR and HRT on percentage removals of COD was shown. Increasing both OLR and HRT at a time, increased the COD removal efficiency. The reason might be as substrate concentration get increased with HRT, the probability of the microorganisms to get in contact with the substrate will be enhanced and hence the removal efficiency of the COD get increases (Verma & Kumar, 2013b; Farajzadehha et al., 2012;de Graaff et al., 2010). The combined effect of OLR and pH on percentage removal of COD was shown in figure 9(e). It was

observed that a sharp increase in the COD removal occurred when the pH value of the solutions changed from 6.3 to 7.0 this was occurred up to 2.6kgCOD/m³.d but as OLR increased the percentage removal of COD was seen decreasing. The decrease in COD removal efficiency at higher pH might be due to the methanogenesis process hindrance (Wang et al., 2021; Arthur et al., 2022).

Figure 9 (f) depicted the combined effect of HRT and pH on percentage removal of COD. From the plot it was seen that, increasing in both HRT and pH up to certain limit but beyond the concentrations the percentage removal was decreased, due to weak degradation of organic compounds and hence this was due to the hindrance in methanogenesis process (O'Flaherty et al., 2006; Sushma & Pal, 2013; Ghawi & Tisti, 2021). Appendix D and E show the combined effects of temperature against OLR, temperature against HRT and OLR against HRT, temperature against pH, OLR against pH, and HRT against pH for percentage removal of TSS and VSS.



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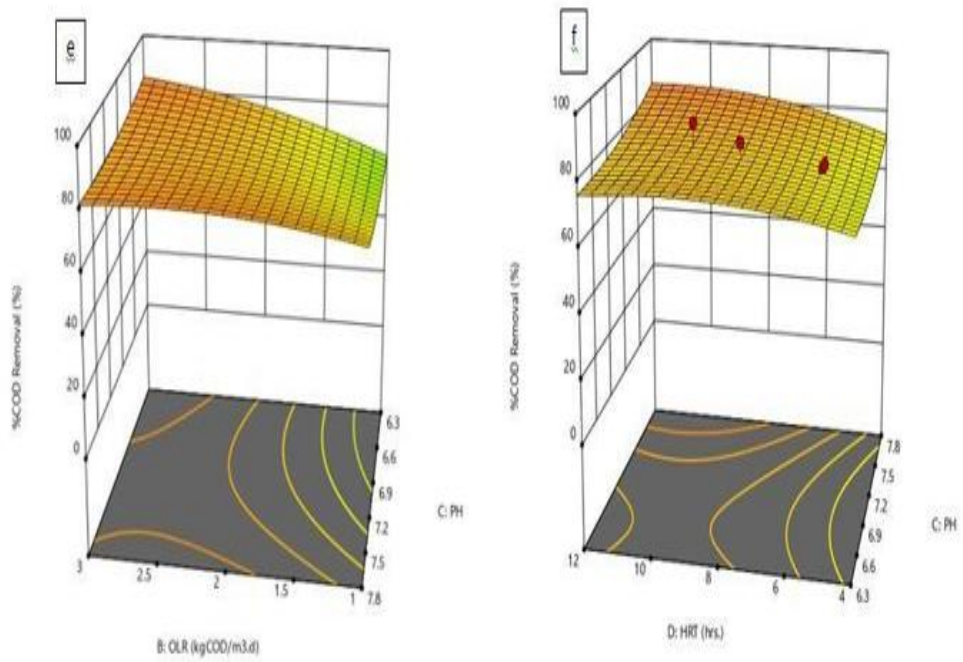


Figure 9 Response 3D plot surface for %COD removal (a) Temperature vs. OLR (b) Temperature vs. HRT (c) Temperature vs. pH (d) OLR vs. HRT (e) OLR vs. pH (f) HRT vs. pH during the rainy seasons

Table 3 Percentage COD removal and HRT determined based on equation (1&2) during rainy seasons

No	Temperature (°C)	pH	OLR (kg COD/m ³ .d)	HRT (hrs.)	Inlet COD (mg/l)	% COD removal
1	30	6.30	1	1.92	80±255	87.1±4.400
2	30	6.30	3	4.64	580±0.00	82.8±6.000
3	30	7.80	3	5.84	730±119.5	93.6±7.192
4	30	7.80	3	6.72	840±119.5	78.1±7.192
5	30	6.3	3	4.64	580±0.00	94.8±6.000
6	30	7.80	3	4.25	550±119.5	93.1±7.192
7	30	6.30	1	5.52	590±255	78.3±4.400
8	30	7.05	2	8.17	690±0.00	84.2±0.000
9	15	8.55	2	7.08	490±0.00	74.3±0.000
10	15	7.05	2	5.53	790±0.00	86.1±0.000
11	15	7.05	4	4.45	480±0.00	75.6±0.000
12	15	7.05	2	5.46	830±101.7	81.1±9.792
13	15	7.05	2	9.57	530±101.7	73.2±9.792
14	15	7.05	2	6.22	540±101.7	65.6±9.792
15	15	7.05	2	6.29	740±101.7	74.9±9.792
16	15	7.05	2	8.53	700±101.7	83.4±9.792
17	15	7.05	2	8.24	640±101.7	68.8±9.792
18	15	8.55	2	7.41	800±0.00	64.4±0.000
19	15	7.05	0	0.00	990±0.00	67.4±0.000
20	15	7.05	2	0.00	730±101.7	58.9±9.792
21	0	6.30	3	6.40	540±80.0	54.8±2.050
22	0	6.3	3	7.55	700±80.0	58.9±2.050
23	0	6.30	1	17.30	610±185	62.9±1.900
24	0	6.30	1	12.60	980±185	66.7±1.900
25	0	7.80	3	5.36	830±0.00	72.9±43.700
26	0	7.80	3	4.53	830±0.00	-14.5±43.700
27	0	7.80	1	23.30	810±20.0	56.7±15.200
28	0	7.80	1	19.50	850±20.0	87.1±15.200
29	-5	7.05	2	9.58	980±0.00	82.8±00.000
30	45	7.05	2	9.44	720±0.00	93.6±00.000

4.2.4. Sulfate ion Concentration

As shown in table 3 the effluent concentration of sulfate ion was seen increasing relative to the influent. This is due to high methanogenesis process which hinders sulfur reducing bacterial which produces H₂S (Sun et al., 2016; Li & Yu, 2016; Aziz et al., 2011). At lower temperatures like -5°C methanogenesis process was hindered much amount of hydrogen sulfide gas was produced causing global warming, causes acid rains, and causes corrosion of wastewater treatment plant. If the HRT in the UASB

reactor is in the range of 4-12hrs elevated sulfate concentrations may be observed in the effluent. Temperature can influence the metabolic activity of microorganisms involved in sulfate reduction during lower temperature. At the range of 15-30°C, the activity of bacteria may be enhanced, leading to increased sulfate ion production rates.

Table 4 Influent and effluent sulfate ion concentrations collected for thirty experimental runs during the rainy seasons

No	Temperature (°C)	pH	OLR(kg COD/m ³ .d)	HRT (hrs.)	In let SO ₄ ²⁻ (mg/l)	Out let SO ₄ ²⁻ (mg/l)
1	30	6.30	1	1.92	16±16.5	40±8.00
2	30	6.30	3	4.64	63±6.00	82±1.00
3	30	7.80	3	5.84	52±2.36	75±6.98
4	30	7.80	3	6.72	47±2.36	65±6.98
5	30	6.30	3	4.64	51±6.00	80±1.00
6	30	7.80	3	4.25	52±2.36	82±6.98
7	30	6.30	1	5.52	49±16.5	56±8.00
8	30	7.05	2	8.17	53±0.00	80±0.00
9	15	8.55	2	7.08	55±0.00	80±0.00
10	15	7.05	2	5.53	43±8.05	80±26.46
11	15	7.05	4	4.45	30±0.00	80±0.00
12	15	7.05	2	5.46	52±8.05	80±26.46
13	15	7.05	2	9.57	38±8.05	80±26.46
14	15	7.05	2	6.22	46±8.05	80±26.46
15	15	7.05	2	6.29	31±8.05	80±26.46
16	15	7.05	2	8.53	34±8.05	80±26.46
17	15	7.05	2	8.24	39±8.05	80±26.46
18	15	8.55	2	7.41	34±0.00	80±0.00
19	15	7.05	0	0.00	0±0.00	0±0.00
20	15	7.05	2	0.00	25±8.05	0±26.46
21	0	6.30	3	6.40	30±2.50	80±3.75
22	0	6.30	3	7.55	25±2.50	87.5±3.75
23	0	6.30	1	17.3	17±4.50	67±17.5
24	0	6.30	1	12.6	26±4.50	32±17.5
25	0	7.80	3	5.36	22±2.50	37±15.0
26	0	7.80	3	4.53	17±2.50	67±15.0
27	0	7.80	1	23.3	24±1.00	50±6.50
28	0	7.80	1	19.53	22±1.00	37±6.50
29	-5	7.05	2	9.58	31±0.00	4±0.00
30	45	7.05	2	9.44	24±0.00	50±0.00

4.2.5. VFA to Alkalinity ratio for the rainy seasons

As presented in Table 4, 5 and 6 the VFA to alkalinity ratio ranges from 0.05 to 0.15, along with the mixed liquor suspended solids (MLSS) (10,000mg/L to 30,000mg/L) and mixed liquor volatile suspended solids (MLVSS) (7,000mg/L to 12,000mg/L), indicates that the UASB reactor is operating efficiently. As saprophytes break down complex molecules acids are produced in the system (acetic, propionic and butyric acids) (Mu & Yu, 2007; Klesyk, 2017). The alkalinity in the system accepts the hydrogen ion released liquor suspended solid by acids, and methanogenesis takes over to maintain the pH.

Table 5 VFA to alkalinity ratio collected for thirty experimental for rainy seasons

No	Temperature (°C)	pH	OLR(kg COD/m ³ .d)	HRT (hrs.)	Influent VFA to alkalinity ratio	Effluent VFA to alkalinity ratio
1	30	6.30	1	1.92	0.100±0.005	0.075±0.003
2	30	6.30	3	4.64	0.070±0.025	0.150±0.015
3	30	7.80	3	5.84	0.060±0.029	0.069±0.017
4	30	7.80	3	6.72	0.090±0.029	0.100±0.017
5	30	6.30	3	4.64	0.120±0.025	0.120±0.015
6	30	7.80	3	4.25	0.130±0.029	0.110±0.017
7	30	6.30	1	5.52	0.090±0.005	0.080±0.003
8	30	7.05	2	8.17	0.070±0.000	0.140±0.000
9	15	8.55	2	7.08	0.080±0.000	0.090±0.000
10	15	7.05	2	5.53	0.090±0.019	0.120±0.000
11	15	7.05	4	4.45	0.100±0.000	0.120±0.000
12	15	7.05	2	5.46	0.130±0.019	0.150±0.044
13	15	7.05	2	9.57	0.120±0.019	0.090±0.044
14	15	7.05	2	6.22	0.140±0.019	0.100±0.044
15	15	7.05	2	6.29	0.090±0.019	0.140±0.044
16	15	7.05	2	8.53	0.110±0.019	0.130±0.044
17	15	7.05	2	8.24	0.090±0.019	0.120±0.044
18	15	8.55	2	7.41	0.080±0.000	0.140±0.000
19	15	7.05	0	0.00	0.080±0.000	0.000±0.000
20	15	7.05	2	0.00	0.091±0.019	0.000±0.044
21	0	6.30	3	6.40	0.077±0.004	0.087±0.012
22	0	6.30	3	7.55	0.085±0.004	0.110±0.012
23	0	6.30	1	17.3	0.100±0.012	0.150±0.015
24	0	6.30	1	12.6	0.097±0.012	0.120±0.015
25	0	7.80	3	5.36	0.120±0.005	0.090±0.001
26	0	7.80	3	4.53	0.130±0.005	0.089±0.001
27	0	7.80	1	23.3	0.096±0.002	0.110±0.026
28	0	7.80	1	19.53	0.100±0.002	0.058±0.026
29	-5	7.05	2	9.58	0.120±0.000	0.120±0.000
30	45	7.05	2	9.44	0.100±0.000	0.100±0.000

Table 6 MLSS collected at half HRT to control the reactor set up

dry Filter paper mass	dry flier paper mass +dry sludge	MLSS(mg/L)
at 105°C,60mL		
0.0677	1.3337	21,100
0.0651	1.4631	23,300
0.0669	1.2369	19,500
0.6521	1.8521	20,000
0.0651	1.7211	27,600
0.0751	1.1071	17,200
0.0699	1.1859	18,600
0.0698	1.4318	22,700
0.0641	1.5941	25,500
0.0699	1.6299	26,000
0.0716	1.5236	24,200
0.0712	1.2892	20,300
0	0	0
0	0	0
0.0761	1.2641	19,800
0.0768	1.1388	17,700
0.0709	1.2109	19,000
0.0722	1.4222	22,500
0.0752	1.4792	23,400
0.0758	1.7018	27,100
0.0708	1.6608	26,500
0.0733	1.7293	27,600
0.0723	1.4703	23,300
0.0655	1.2655	20,000
0.0652	1.1512	18,100
0.0655	1.2475	19,700
0.0655	1.4035	22,300
0.0706	1.5466	24,600
0.0655	0.4945	7150 for -5°C
0.0721	1.1605	11100 for 45°C

Table 7 MLVSS collected at half HRT to control the reactor set up

Weight of dry dish +ignited paper	Weight of dry dish	MLVSS(mg/L)
70	68.7397	15,000
70	68.7396	13,700
75	73.9948	18,200
75	73.9958	13,600
75	73.9957	16,000
72	71.3191	12,800
75	73.3191	21,700
73	71.9024	17,000
83	81.6353	15,600
73	71.5518	16,100
71	69.4789	22,100
71	69.968	10,400
0	0	0
0	0	0
73	72.1193	17,100
73	72.1131	13,400
73	72.2051	20,500
73	72.205	12,000
74	72.8765	13,500
74	72.5926	21,100
70	69.34	16,100
73	72.1984	17,000
74	73.1488	13,200
74	72.7675	21,600
73	71.9748	19,800
73	72.1133	14,300
74	73.1494	20,100
73	72.3346	14,100
73	71.5434	1000 for -5°C
72	72.5931	3000for 45°C

4.3.DRY (FEBRUARY, MARCH AND APRIL) SEASON

The average daily inflow and outflow during the dry seasons is shown in the table 8.

Table 8 Average daily inflow and out flow on February, March and April
(during dry seasons)

Date	Average daily Inflow	Average Daily Outflow	Date	Average daily Inflow	Average Daily Outflow	Date	Average daily Inflow	Average Daily Outflow
	(m3/d)	(m3/d)		(m3/d)	(m3/d)		(m3/d)	(m3/d)
01-Feb-22	73389	68976	01-Mar-22	69144	66888	01-Apr-22	64848	62760
02-Feb-22	70760	69024	02-Mar-22	60144	55296	02-Apr-22	73464	66552
03-Feb-22	73256	68520	03-Mar-22	59352	55344	03-Apr-22	65712	58704
04-Feb-22	72628	68808	04-Mar-22	60480	59712	04-Apr-22	64848	64632
05-Feb-22	75012	66912	05-Mar-22	62208	62928	05-Apr-22	63576	62712
06-Feb-22	67899	65088	06-Mar-22	61896	61584	06-Apr-22	72072	65304
07-Feb-22	67669	64320	07-Mar-22	66576	66600	07-Apr-22	67704	64800
08-Feb-22	67322	64800	08-Mar-22	64104	64920	08-Apr-22	67008	63672
09-Feb-22	66456	65280	09-Mar-22	61920	61632	09-Apr-22	65496	49604
10-Feb-22	66518	64896	10-Mar-22	63144	63552	10-Apr-22	66600	63312
11-Feb-22	68765	64488	11-Mar-22	61728	62592	11-Apr-22	69168	66552
12-Feb-22	68326	65554	12-Mar-22	62736	63912	12-Apr-22	69048	66312
13-Feb-22	60987	67032	13-Mar-22	63888	65664	13-Apr-22	66648	62664
14-Feb-22	72341	68208	14-Mar-22	69424	68664	14-Apr-22	70392	69408
15-Feb-22	64536	66432	15-Mar-22	66816	65184	15-Apr-22	73560	73560
16-Feb-22	65643	65400	16-Mar-22	65832	62784	16-Apr-22	69648	68784
17-Feb-22	65432	65496	17-Mar-22	67344	62568	17-Apr-22	65136	58968
18-Feb-22	69876	65304	18-Mar-22	66120	61824	18-Apr-22	68688	64776
19-Feb-22	65467	65256	19-Mar-22	63792	60912	19-Apr-22	69000	62880
20-Feb-22	69000	66624	20-Mar-22	61392	59496	20-Apr-22	66984	60768
21-Feb-22	70321	68952	21-Mar-22	61776	60360	21-Apr-22	67512	61176
22-Feb-22	66543	65760	22-Mar-22	71568	71208	22-Apr-22	64584	59064
23-Feb-22	64563	64824	23-Mar-22	75168	75168	23-Apr-22	65328	61728
24-Feb-22	66798	64968	24-Mar-22	67104	62088	24-Apr-22	66696	64200
25-Feb-22	66687	68472	25-Mar-22	58352	51288	25-Apr-22	68376	60960
26-Feb-22	67891	63912	26-Mar-22	66960	55872	26-Apr-22	68184	68184
27-Feb-22	67324	66432	27-Mar-22	64680	64480	27-Apr-22	72624	62568
28-Feb-22	72288	71328	28-Mar-22	67008	66984	28-Apr-22	71952	69744
29/02/2022	75123	65789	29-Mar-22	67128	63816	29-Apr-22	69336	63864
30/02/2022	67632	64789	30-Mar-22	63216	60096	30-Apr-22	77400	73848
Average	68548.4	66388.13	Average	64700	62780.5	Average	68386.4	64068.7
Min	60987	63912	Min	58352	51288	Min	63576	49604
Max	75123	71328	Max	75168	75168	Max	77400	73848
Total Sum	1913697	1861066	Total Sum	1941000	1883416	Total Sum	2051592	1922060

4.3.1. Experimental Validation

The RSM-CCD model estimated the removal efficiencies to be approximately 83.4% COD, 100% TSS and 99% VSS. At 23.04°C, 2.23 kg COD/m³.d of OLR, 7.50 pH, and 11.43 hours of HRT, these removal efficiencies were attained. In the experiment, less than 5% precision was achieved for 84.1% COD, 99.89% TSS, and 100% VSS. As a result, the model was confirmed. The removal efficiencies during the rainy seasons (92.70 %COD, 99.06%TSS, and 94.5%VSS) were much higher than the removal during the dry seasons. This was because the influent to the plant increased by about 35% due to entry of stormwater runoff to the sewer system which causes dilution of the wastewater (refer table 2 and table 8).

4.3.2. Parameter Characterization

Parameters such as Total Nitrogen (TN), Total Phosphorous or Ortho-phosphate (TP), COD, TSS, pH, VSS, SO₄²⁻, VFA, and Alkalinity, were measured. The dry season operational parameters load were with a variation of TN (4.3mgTN/L - 66mgTN/L), NH₄⁺ - N(32.8mg NH₄⁺/L - 69.2mg NH₄⁺/L), TP (1.19mgTP/L - 11.9mgTP/L), (pH:5.55 - 8.55), SO₄²⁻ (6mgSO₄²⁻/L - 80mg SO₄²⁻/L), VFA to alkalinity ratio (0.055 - 0.15), alkalinity (155- 370mgCaCO₃/L), TSS (3-770mg/L) and VSS (0-880mg/L).

4.3.3. Performance enhancement modeling and model analysis

The overall removal efficiency of a model's predicted capacity is determined by the value coefficient of determination R² but it is not the only parameter to determine the efficiency. For the model with best predictive efficiency the value of R² is nearly close to one (Nair et al., 2014; Rahman et al., 2022).

Table 9 Quadratic and linear models R² comparison for the dry seasons

Quadratic		Linear	
R ²	0.9977	R ²	0.5259
Adjusted R ²	0.9956	Adjusted R ²	0.4719
Predicted R ²	0.9863	Predicted R ²	0.2704

The RSM-CCD determined that the quadratic model was the best model because its adjusted R² and predicted R² were greater than the linear values. Quadratic regression was done for the prediction of the responses as a second order polynomial model was statically significant. The quadratic model developed showed a response with coded

operational parameters like temperature (A), OLR (B), pH(C) and HRT (D).

4.3.4. COD Model output by RSM – CCD

The %COD removal model shown in equation (12) was obtained from the CCD-RSM.

$$\begin{aligned} \% \text{COD} = & 70.5749 + 18.0757A - 7.9972B + 6.9780C + 15.2942D + 12.5759AB + \\ & 6.9919AD - 3.1719BC - 11.1343BD - 7.8575CD - 19.5888A^2 + 6.5013B^2 + 4.8995C^2 - \\ & 9.4604D^2 \end{aligned} \quad (11)$$

The %COD in equation (12) was obtained from RSM-CCD. The model coded operational parameters A, B, C, D, AB, AD, BC, BD, CD, A², B², C², D² were statistically significant with p-value less than 0.05 at 95 % confidence level. The percentage removal of COD was affected by single parameter or the interaction of operational parameters. They can affect the model negatively or positively. Fisher's F-test was used for ANOVA analysis. The model was significant since the model's F-value was obtained to be 465.21. There was only 0.01% chance that an F-value this large could occur due to noise. For this model the probability of P statistics obtained were < 0.0001. The lack of fit F-value 232.69 implied the lack of fit was not significant relative to the pure error. There was a 5.13% chance that a large lack of fit F-value could occur due to noise. We needed the model to fit, so a non-significant lack of fit was acceptable. To explain the fit between experimental and model data, the adjusted R² determination coefficient value (0.9956) was used. The adjusted R² = 0.9956 indicates that the developed model could describe approximately 99.56% of the total percentage removal of COD data. The signal-to-noise ratio was calculated using the fit statistics with sufficient precision. A ratio of more than four was preferred. The adequate precision ratio of 68.89 in this model indicated an adequate signal. This model could thus be used to navigate the design space.

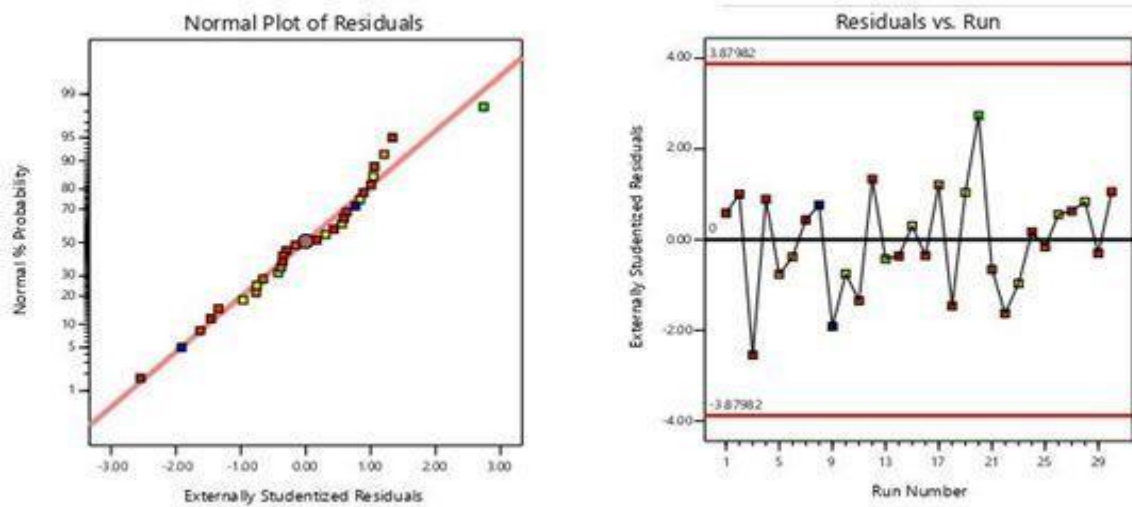


Figure 10 plot of studentized residuals vs. collected experimental data for dry seasons. To further elaborate the model, a plot was made between predicted percentages removals and studentized residual, with the values ranging between ± 3.00 . The plots showed that the model fit the experimental data.

4.3.5. TSS Model output by RSM-CCD

Equation (13) shows the TSS removal model achieved from RSM-CCD. From the model developed one can understand that the percentage removal of TSS was affected by linear or combined interactions of operational parameters. They affect the model negatively or positively.

$$\%TSS = 94.9013 - 5.5261A - 8.8758C + 18.3182D + 5.6324AC + 7.2205AD - 3.9458BC - 3.3562BD + 6.3232CD - 6.4446A^2 - 12.7649D^2 \quad (12)$$

4.3.6. VSS Model outputs by RSM-CCD

Equation (14) shows the VSS removal model obtained from RSM-CCD tool. Single or double interactions of operational parameters had a negative or positive effect on the percentage removal of VSS.

$$\%VSS = 88.1682 - 7.0277A - 4.4879B + 22.4402D + 7.3832AD - 3.2492A^2 - 9.7178D^2 \quad (13)$$

4.3.7. Model generated Results

The model generated a percentage removal for COD ranging from 0 to 98.6%, TSS ranging from 0 to 100%, and VSS ranging from 0 to 99.9%. From all of the model's outputs, an optimal temperature of 23.04°C, OLR of 2.23kgCOD/m³.d, pH of 7.5, and HRT of 11.43hrs were chosen. This optimal condition was chosen because the goal of the research was to find a way to improve the performance efficiency of the UASB reactor in relation to the surrounding temperature. However, if a mechanism for increasing the temperature of the wastewater is developed, the percentage removal of COD, TSS, and VSS could be increased to 98.6%, 100%, and 100%, respectively.

4.3.8. Interactional effects of operational parameters on %COD removal

Figure 11 (a) shows the concurrent effects of OLR and temperature on the removal efficiency of COD which is obtained from equation 12. The removal efficiency of COD increases from 20 to 80% as the organic load increases from 1 to 3kgCOD/m³.d and the temperature rises to 30°C (Besharati Fard et al., 2019). At constant OLR of 1kgCOD/m³.d and as the temperature rises from 0 to 30°C the COD removal efficiency decreases from 80 to 70%. This drop in COD removal is due to a drop in OLR not due to temperature. Microorganisms consume available substrates more efficiently, increasing COD removal efficiency. Keeping OLR 3kgCOD/m³.d and decreasing the temperature from 30 to 0°C will alter the working mechanism of WWTP which eventually needs acclimatization and start up (Daija et al., 2016; Zwain et al., 2022). At a constant OLR of 3kgCOD/m³.d and a temperature of 0°C, COD removal efficiency decreases from 85 to 20%. This decrease in COD removal efficiency is due to decrease in temperature (L. Zhang et al., 2018a).

The interactional effect of HRT and temperature is shown in figure 11(b). As HRT goes from 4 to 12hrs and the temperature rises from 0 to 30°C the COD removal efficiency increases from 30 to 85%. Keeping the HRT at 4hrs and increasing the temperature from 0 to 30°C the COD removal efficiency decreases from 85 to 38%. This decrease is due to a decrease in HRT (Bhatti et al., 2014; Ma et al., 2013). Furthermore keeping the HRT at 12hrs and the temperature at 0°C the efficiency of COD removal decreases from 85 to 30%. This decrease in %COD removal is due to a decrease temperature.

The interactional effect of pH and temperature is shown in figure 11(c). As one increases pH from 6.3 to 7.8 and the temperature from 0 to 30°C the removal efficiency of the COD increases from 45 to 80%. Keep the pH constant at 6.3 and increasing the temperature from 0 to 30°C the removal efficiency of the COD decreases from 80 to 65%. This decrease in %COD removal efficiency is due to a decrease in pH (Paltahé et al., 2019). If the temperature is kept constant at 0°C and the pH is increased from 6.3 to 7.8 the removal efficiency of the COD decreases from 85 to 45%. This decrease in removal efficiency of COD is due to decrease in temperature.

The interactional effect of OLR and pH is shown in figure 11(d). As one increases OLR from 1 to 3kgCOD/m³.d and the pH from 6.3 to 7.8 the removal efficiency of COD decreases from 100 to 78% (Girma et al., 2023). Keeping the OLR at farthest distance 3kgCOD/m³.d and decreasing the pH from 7.8 to 6.3 will decrease the COD removal efficiency from 78 to 69%. The reason in decrease in COD removal efficiency goes to the decrease in pH.

The interactional effects of HRT and OLR on %COD removal is shown in figure 11(e). As one increases the value of OLR from 1 to 3kgCOD/m³.d and HRT from 4 to 12hrs the removal efficiency of COD decreases from 100 to 64%. Keeping the OLR at farthest distance 3kgCOD/m³.d and the HRT at 4hrs the removal efficiency of the COD decreases from 64 to 58%. This decrease in removal efficiency of the COD is due to a decrease in HRT (Cunha et al., 2019).

The interactional effect between HRT and pH on %COD removal is shown in figure 11(f).

Increasing the pH from 6.3 to 7.8 and the HRT from 4 to 12hrs increases the removal efficiency of COD from 80 to 82%. Keeping the HRT at 4hrs and increasing the pH from 6.3 to 7.8 decreases the COD removal efficiency from 82 to 65%. This decrease in COD removal efficiency goes to a decrease in HRT (Leitão et al., 2005).

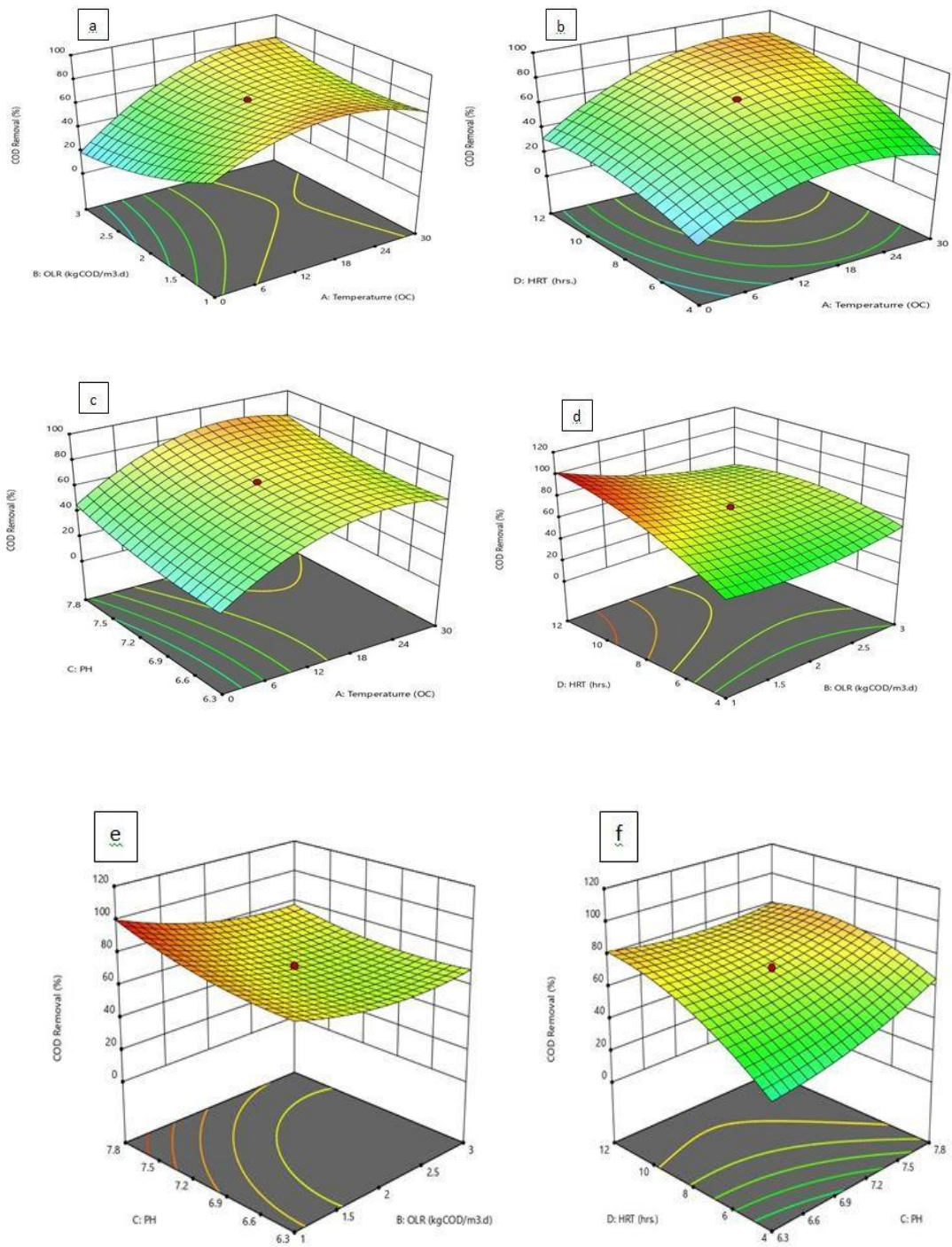


Figure 11 Response 3D surface for % COD removal (a) OLR vs. Temperature (b) HRT vs. Temperature (c) pH vs. Temperature (d) pH vs. OLR (e) HRT vs. OLR (e) HRT vs. OLR (f) HRT vs. pH

Table 10 Operational parameters with their respective inlet, outlet COD concentration &%COD removal during the dry seasons

No	Temperature (°C)	OLR (kg COD/m ³ .d)	pH	HRT (hrs.)	Inlet COD (mg/L)	Outlet COD(mg/L)	%COD Removal
1	30	1	6.3	12.00	310.0	08.029±128.951	97.41±17.70
2	30	1	7.8	15.6	1720	00.000±6.552	100±2.34
3	30	3	6.3	12.00	2950	181.13±27.235	93.86±6.43
4	30	3	7.8	5.84	960.0	167.52±36.821	82.55±2.29
5	30	1	6.3	7.44	700.0	265.93±128.951	62.01±17.70
6	30	3	6.3	7.68	1240	235.60±27.235	81.00±6.43
7	30	3	7.8	6.72	730.0	93.878±36.821	87.14±2.29
8	30	1	7.8	10.08	280.0	13.104±6.552	95.32±2.34
9	15	2	7.05	8.00	1240	341.62±130.060	72.45±23.79
10	15	4	7.05	8.00	1200	261.60±0.000	78.20±0.00
11	15	2	7.05	8.00	800.0	222.00±130.060	72.25±23.79
12	15	2	7.05	16.00	1260	470.61±130.060	62.65±23.79
13	15	0	7.05	0.00	1120	00.00±0.000	00.00±0.00
14	15	2	7.05	0.00	1100	00.00±0.000	00.00±0.00
15	15	2	7.05	10.2	1240	326.86±130.060	73.64±23.79
16	15	2	7.05	11.52	960.0	230.40±130.060	76.00±23.79
17	15	2	8.55	11.52	1200	47.04±0.000	96.08±0.00
18	15	2	7.05	15.12	1120	336.00±130.060	70.00±23.79
19	15	2	5.55	13.44	800.0	06.00±0.000	99.25±0.00
20	15	2	7.05	14.40	1210	356.71±130.060	70.52±23.79
21	0	1	6.3	12.00	420.0	116.13±55.620	72.35±0.75
22	0	3	7.8	6.24	350.0	244.16±200.830	30.24±1.93
23	0	1	6.3	16.00	780.0	227.37±55.620	70.85±0.75
24	0	3	7.8	4.56	980.0	645.82±200.830	34.10±1.930
25	0	1	7.8	13.2	570.0	147.57±60.405	74.11±5.96
26	0	1	7.8	16.00	710.0	268.38±60.405	62.20±5.96
27	0	3	6.3	3.22	1200	1200±126.910	00.00±7.76
28	0	3	6.3	12.00	1120	946.18±126.910	15.52±7.76
29	-5	2	7.05	9.6	800.0	720.00±0.000	10.00±0.00
30	45	2	7.05	14.7	1120	560.00±0.000	50.00±0.00

4.3.9. Sulfate ion Concentration

In this experimental research the effluent sulfate ion concentration was found to be greater than the influent. This is due to high methanogenesis process which hinders sulfur reducing bacteria (Sun et al., 2016; Li & Yu, 2016; Aziz et al., 2011).

Table 11 influent and effluent sulfate concentrations for thirty experimental runs during the dry seasons

No	Temperature (°C)	OLR (Kg COD /m ³ .d)	pH	HRT (hrs.)	Inlet Sulfate ion (mg/L)	Outlet Sulfate ion(mg/L)
1	30	1	6.30	12.00	6.0±13.500	80±0.000
2	30	1	7.80	15.60	30±3.500	20±16.00
3	30	3	6.30	12.00	43±11.000	80±0.000
4	30	3	7.80	5.84	70±20.500	80±5.000
5	30	1	6.30	7.44	33±13.500	80±0.000
6	30	3	6.30	7.68	21±11.000	80±0.00
7	30	3	7.80	6.72	29±20.500	70±5.000
8	30	1	7.80	10.08	23±3.500	52±16,00
9	15	2	7.05	8.00	22±4.473	56±20.949
10	15	4	7.05	8.00	19±0.000	72±0.000
11	15	2	7.05	8.00	22±4.473	76±20.949
12	15	2	7.05	16.00	28±4.473	61±20.949
13	15	0	7.05	0.00	0±0.000	0±0.000
14	15	2	7.05	0.00	28±4.473	0±20.949
15	15	2	7.05	10. 20	26±4.473	66±20.949
16	15	2	7.05	11.52	26±4.473	67±20.949
17	15	2	7.05	15.52	20±4.473	54±20.949
18	15	2	8.55	11.52	37±4.473	80±20.949
19	15	2	5.55	13.44	26±4.473	54±20.949
20	15	2	7.05	14.40	28±4.473	51±20.949
21	0	1	6.30	12.00	59±7.000	80±10.500
22	0	3	7.80	6.24	25±12.500	75±2.500
23	0	1	6.30	16.00	45±7.000	59±10.500
24	0	3	7.80	4.56	50±12.500	80±2.500
25	0	1	7.80	13.20	53±12.500	80±3.500
26	0	1	7.80	16.00	28±12.500	73±3.500
27	0	3	6.30	3.22	70±24.500	80±0,000
28	0	3	6.30	12.00	21±24.500	80±0,000
29	-5	2	7.05	9.60	45±0.000	5.0±0.000
30	45	2	7.05	14.47	27±0.000	22±0.000

At -5°C, pH 7.05, OLR 2kgCOD/m³.d, and HRT 9.6 hours, the effluent SO₄⁻² concentration was found to be extremely decreasing. This is due to the fact that as the temperature drops to extremely to low levels, the activity of anaerobic microorganisms decreases (O’Flaherty et al., 2006; Liu et al., 2021; Luostarinen et al., 2007).

4.3.10. VFA to Alkalinity ratio

In this research, VFA to alkalinity ratio was seen in the range of 0.055-0.15. This showed that the UASB reactor was operating at normal condition. As saprophytes break down complex molecules acids are produced in the system (acetic, propionic and butyric acids) (Khanh et al., 2011; V. Singh et al., 2016). The alkalinity in the system accepts the hydrogen ion released by acids, and methanogenesis takes over to maintain the pH (Mu & Yu, 2007).

Table 12 VFA to alkalinity ratio for thirty experimental runs during the dry seasons

Expt. no	Temperature (°C)	OLR (kg COD/m ³ .d)	HRT (Hrs.)	pH	Inlet VFA to alkalinity Ratio	Outlet VFA to alkalinity Ratio
1	30	1	6.30	12.00	0.030±0.030	0.050±0.045
2	30	1	7.80	15.60	0.090±0.000	0.060±0.003
3	30	3	6.30	12.00	0.098±0.017	0.080±0.010
4	30	3	7.80	5.84	0.060±0.015	0.050±0.008
5	30	1	6.30	7.44	0.090±0.030	0.140±0.045
6	30	3	6.30	7.68	0.065±0.017	0.060±0.010
7	30	3	7.80	6.72	0.090±0.015	0.065±0.008
8	30	1	7.80	10.08	0.090±0.000	0.065±0.003
9	15	2	7.05	8.00	0.080±0.031	0.060±0.029
10	15	4	7.05	8.00	0.088±0.000	0.060±0.000
11	15	2	7.05	8.00	0.055±0.031	0.080±0.029
12	15	2	7.05	16.00	0.090±0.031	0.088±0.029
13	15	0	7.05	0.00	0.00±0.000	0.000±0.000
14	15	2	7.05	0.00	0.000±0.031	0.000±0.029
15	15	2	7.05	10.20	0.100±0.031	0.080±0.029
16	15	2	7.05	11.52	0.099±0.031	0.054±0.029
17	15	2	7.05	15.12	0.080±0.031	0.090±0.029
18	15	2	8.55	11.52	0.100±0.000	0.150±0.000
19	15	2	5.55	13.44	0.100±0.000	0.067±0.000
20	15	2	7.05	14.40	0.060±0.031	0.090±0.029
21	0	1	6.30	12.00	0.086±0.016	0.050±0.002
22	0	3	7.80	6.24	0.080±0.005	0.053±0.011
23	0	1	7.80	16.00	0.060±0.030	0.050±0.000
24	0	3	7.80	4.56	0.089±0.005	0.075±0.011
25	0	1	7.80	13.20	0.120±0.030	0.050±0.000
26	0	1	6.30	16.00	0.055±0.016	0.053±0.002
27	0	3	6.30	3.22	0.090±0.024	0.060±0.020
28	0	3	6.30	12.00	0.043±0.024	0.020±0.020
29	-5	2	7.05	9.60	0.110±0.000	0.095±0.000
30	45	2	7.05	14.47	0.106±0.000	0.103±0.000

At a temperature of -5°C , pH 7.05, OLR $2\text{kgCOD}/\text{m}^3\cdot\text{d}$, and HRT 9.58hrs the outlet VFA to alkalinity ratio was seen out of the range since acidogenesis and methanogenesis were hindered with decrease in temperature (Montes et al., 2019; Mrunalini M. Powar* et al., 2013).

4.4. Heavy metals in wastewater samples

The major heavy metals found during the analysis of Kality Domestic Wastewater Treatment Plant effluent, irrigated soil, and plants were Iron, Manganese, Nickel, Cobalt, Copper, Zinc, Cadmium, Mercury, Lead, Arsenic, Boron, and Chromium. Toxic heavy metals like Arsenic, Cadmium, Chromium, Copper, Manganese, and Lead were also discovered in the effluent, as shown in table 9.

The values in the table are the average value of the triplicates done during the laboratory test. Water samples were collected at the UASB reactor's inlet and outlet, as well as the trickling filter and secondary clarifier's outlets. Heavy metals and toxic heavy metals were identified in the samples. Heavy metals such as Iron, Manganese, Nickel, Cobalt, Copper, Zinc, Cadmium, Mercury, Lead, Arsenic, Boron, and Chromium were found in all of the samples (table 9). The samples were analyzed again for the presence of toxic heavy metals. Arsenic, Cadmium, Chromium, Copper, Manganese, and Lead were found in all of them, as shown in the table 10. Point sources like illegal connections of ink factory, and leather industries and non-point sources such as construction sites, agricultural farms and garages were assumed to be the possible sources of toxic heavy metals.

Table 13 Toxic heavy metals and their mean concentration in the water samples

Toxic Heavy metal	(Sample-1) Concentration at UASB inlet (mg/L)	(Sample-2) Concentration at UASB outlet (mg/L)	(Sample-3) Concentration at Trickling filter outlet (mg/L)	(Sample-4) Concentration at Secondary clarifier outlet (mg/L)	Surface water quality standard by Guideline Ambient Environment Standard for Ethiopia(mg/L)	FAO/WHO standard for irrigation water (mg/L)
Arsenic (As)	0.07±0.01	0.048±0.009	0.034±0.034	0.059±0.059	0.05mg/l	0.10
Cadmium (Cd)	0.05±.001	0.053±0.005	0.036±0.002	0.056±0.004	5µg/l	0.01
Chromium (Cr)	0.15±.002	0.103±0.011	0.075±0.012	0.073±0.055	50µg/l	0.55
Copper (Cu)	0.058±.01	0.014±0.01	0.006±0.002	0.08±0.003	5mg/l	0.017
Manganese (Mn)	1.215±.01	0.8±0.003	0.578±0.005	0.441±0.003	0.3mg/l	0.20
Lead (Pb)	0.490±.08	0.436±0.041	0.219±0.005	0.334±0.001	0.05mg/l	0.065

As shown in the table 9, the UASB reactor has a removal efficiency of 31.43 % for As, 6 % for Cd, 31.33 % for Cr, 75.86 % for Cu, 34.15 % for Manganese, and 11.02 % for Lead. The UASB reactor removal efficiency for toxic heavy metals such as Arsenic is higher than the surface water quality standard set by Surface water quality standard by Guideline Ambient Environment Standard for Ethiopia but it is lower for Cd, Cr, Cu, Mn, and Lead. Since the UASB reactor's primary use is for biological processes, it is inefficient in removing toxic heavy metals. Based on FAO/WHO standards, the UASB reactor is effective at removing toxic heavy metals such as Arsenic, Cr, and Cu, but it is ineffective at removing toxic heavy metals such as Cadmium, Manganese, and Lead. The removal efficiency of toxic heavy metals (As, Cr, Cd, Mn, Cu, Pb) by trickling filters and secondary clarifiers is below the standard for both surface water quality guidelines and FAO/WHO. Despite the fact that the treatment plant's primary purpose is for biological processes, the removal of toxic

heavy metals from domestic wastewater must be taken into account for the following reasons.

Inhibition of Microbial Activity: Heavy metals such as cadmium, lead, mercury, and others can inhibit the metabolic activities of the microbial communities within the UASB reactor. These metals can disrupt enzyme function and cellular processes, leading to reduced efficiency in the breakdown of organic matter (Wang, Q., Du, G., & Chen, J., 2004; Chen, Y., Cheng, J. J., & Creamer, K. S., 2008).

Disruption of Granule Formation: UASB reactors rely on the formation of granular sludge to maintain high biomass concentrations. Heavy metals can interfere with the aggregation of microbial cells, affecting granule stability and formation, which can result in poor sludge retention and reactor performance (Fang, H. H., & Liu, H. (2002; Liu, Y., Xu, H. L., Yang, S. F., & Tay, J. H. ,2003).

Toxicity to Specific Microbial Groups: Different microbial groups within the UASB reactor are responsible for various stages of the anaerobic digestion process. Heavy metals can be toxic to specific groups, such as methanogens, which are crucial for methane production. This can lead to reduced biogas production and lower overall treatment efficiency (Zayed, G., & winter, J. (2000; Demirel, B., & Scherer, P., 2008).

Competition for Nutrients: Heavy metals can bind to essential nutrients and trace elements, making them less available to the microbial communities. This can limit the growth and activity of microorganisms, further reducing the reactor's performance (Koki, K., & Boonkerd, N., 2006; Bhattacharyya, P., & Singh, D. P., 2010).

Alteration of Reactor pH: The presence of heavy metals can lead to changes in the pH of the reactor environment. Extreme pH levels can further inhibit microbial activity and disrupt the balance of the anaerobic digestion process (Weiland, P., 2010).

4.5. Heavy metals in soil samples

The values in the table 10 were the average values for triplicates done during the laboratory test. The soil sample contained toxic heavy metals such as Arsenic, Cadmium, Chromium, Copper, Manganese, and Lead. Unless otherwise these elements are removed by increasing the treatment efficiency of the wastewater treatment plant they will result in altering the human tissues (Järup, 2003; He et al., 2005). Lead has toxic effects on various organ systems, but those in the kidney are the most difficult. Acute Lead nephropathy causes proximal tubular dysfunction, resulting in Fanconi-like syndrome. Cadmium can also cause glucosuria, Fanconi-like syndrome, Phosphaturia, and aminoaciduria (Reyes et al., 2013). The main source of human exposure is drinking arsenic-contaminated water. Arsenic exposure causes cancers in the integumentary, respiratory, hepatobiliary, and urinary systems. Cutaneous lesions are common side effects of long-term arsenic exposure. Arsenical skin cancers frequently precede the development of other internal cancers, making arsenic-induced skin carcinogenesis an excellent model for studying the progression of chemical carcinogenesis (Huang et al., 2019). Cadmium exposure has been linked to a history of stroke and heart failure (Peters et al., 2010). Chromium is declared as a potent carcinogenic. It has significant importance in altering the immune response (Shrivastava et al., 2002).

Table 14 Soil sample's mean toxic heavy metals concentration

Name	Arsenic (As)(mg/L)	Cadmium (Cd)(mg/L)	Chromium (Cr)(mg/L)	Copper (Cu)(mg/L)	Manganese (Mn)(mg/L)	Lead (Pb)(mg/L)
Concentration	8.76±0.14	7.63±0.09	41.287±0.87	21.209±0.41	929.67±12.3	119.6±3.4
EPA Standard	≤20	≤0.5	≤20	≤500	N/A	≤40

4.6. Heavy metals in plant samples

The values in the table 11 were the average values for triplication done during the laboratory test. Toxic heavy metals As, Cd, Cr, Cu, Mn, and Pb were found in local cabbage (Habesha Gommen) and Swiss chard (kosta). Human consumption of unsafe concentrations of toxic heavy metals accumulated in plants taken as a food may

disrupt numerous biological processes. Accumulation of toxic heavy metals in the kidney and liver of humans, disrupt numerous human metabolism leading to cardiovascular diseases, nervous diseases, kidney, and bone diseases (Yeshiwas & Tadele, 2017; Chaoua et al., 2019). Prolonged exposure and higher intake of toxic heavy metals through edible plants can be harmful to aquatic biota and human being (Dixit et al., 2015).

The fact that Ethiopia lacks established criteria makes determining the concentration of hazardous heavy metals in plant samples extremely disadvantageous. Ethiopia's Food and Drug Authority should prepare the permissible concentration for toxic heavy metal in the plant samples.

Table 15 plant samples mean toxic heavy metal concentration

Heavy metals	Ethiopian Local Cabbage concentration (mg/l)	Swiss Chord concentration (mg/l)	WHO mg/kg
Arsenic (As)	0.766±0.038	0.272±0.027	0.2
Cadmium (Cd)	0.189±0.014	0.008±0.046	0.1-0.4
Chromium (Cr)	1.193±0.092	0.327±0.042	N/A
Copper (Cu)	4.724±0.420	4.349±0.040	N/A
Manganese (Mn)	23.618±1.288	54.32±0.286	N/A
Lead (Pb)	2.784±0.373	0.559±0.045	0.1-0.2

4.7. UASB reactor modeling

4.7.1. Influent Characterization

Influent characterization is the most important step in modeling the WWTP using GPS-X. Before one goes to modeling, influent should be characterized in the influent advisory, state variables and composite variables of the GPS-X. After the modeler has entered the measured concentration of Total COD, Total TKN, TP, Ammonia – nitrogen, VSS to TSS ratio, pH, etc. of the measured data, the modeler has to check under the composite variable the magnitude of TSS and VSS. Their magnitudes should be equal to the measured value. If the values of TSS and VSS are not equal to the measured values, so the modeler has to go to the organic fractions and change the

values iteratively until the values of TSS and VSS are equal to the measured value. For an influent, the model selected should be codstates. It is selected since state and composite variables fractions were determined based on COD (Pereira, 2014; Małkinia & Zaborowska, 2018; Vicentin et al., 2019; Jasim, 2020). The values of the organic fractions and stoichiometric ratios used during influent characterization are tabulated in table-16.

Table 16 Organic fractions and stoichiometric ratio used during characterization of influent

Parameter	Symbol	Unit	Default Values	Calibrated Value
1. Organic Fractions				
Soluble inert fraction of total COD	frsi	—	Raw:0.05 Primary:0.075	0.011
Readily biodegradable fraction of total COD	frss	—	Raw:0.2 Primary:0.3	0.11
Particulate inert fraction of total COD	frsi	—	Raw:0.13 Primary:0.1	0.01
Colloidal fraction of slowly biodegradable COD	frscol	—	Raw:0.13 Primary:0.26	0.28
2. Stoichiometric ratio				
VSS/TSS	ivsstotss	—	Raw:0.6 Primary:0.85	0.75
BOD ₅ /COD	—	gO ₂ /gCOD	Raw:0.4 Primary:0.75	0.616
NH ₄ -N/TKN	—	—	Raw:0.65 Primary:0.9	0.729

4.7.2. Steady-state

The default values of the GPS-X were run after the model lay out was constructed. The actual total cumulative mass balance around the secondary clarifier was drawn which later on compared with the simulated graph after the input and output values were entered in to GPS-X. Compared with the simulated graph after the input and output data entry, if the simulated graph is slightly identical with the actual cumulative mass graph is around the secondary clarifier , possibly one can say the model is calibrated but that is not the essential way to say a given model is calibrated(Meijer et al., 2002; Kalyuzhnyi et al., 2006). The second way to say the model is calibrated is by the determination of mean average relative error (ARE). To evaluate the best fit between the simulation and the measured data sets, average absolute relative error (ARE) was used. It was used to evaluate the agreement between the simulation and measured data sets (Bhunja & Ghangrekar, 2008; Liwarska-Bizukojc et al., 2011; Vitanza et al., 2016).

4.8. GPS-X default values and simulated results

Base model scenario simulation was performed to assess the ARE results. ARE values of 94.73%, 84.48%, 1.72%, 25.55%, 96.27% for COD, TKN, NH₄-N, TP and TSS respectively were achieved during the default run shown in Appendix J. These percentage of ARE showed that calibration of the GPS-X is crucial before using the tool for design , predictions of different scenarios and further studies.

Table 17 Normalized sensitivity coefficient for model targets of kinetic and stoichiometric parameters

Model Parameters	Default value	Simulated values	S _{i,j}			
			COD	TSS	NH ₄ -N	TP
Kinetic						
1. Ammonia oxidizing biomass						
Maximum growth rate for ammonia oxidizer	0.91/d	5.75 1/d	0.935	5	27.75	120.96
2. Nitrite oxidizing biomass						
Maximum growth rate for nitrite oxidizer	1 1/d	5.75 1/d	0.935	5	27.75	120.96
3. Poly-phosphate oxidizing biomass(PAO)						
Maximum growth rate of PAO	1 1/d	0.7 1/d	0.935	5	27.75	120.96
4. heterotrophic maximum specific growth rate($\mu_{max,H}$)	3.2 1/d	6.5 1/d	-	5	27.75	120.96
Stoichiometric						
1. Ammonia oxidizing biomass						
Maximum growth rate for ammonia oxidizer	0.18 1/d	2.4 1/d	0.935	5	27.75	120.96
2. Nitrite oxidizing biomass						
Maximum growth rate for nitrite oxidizer	0.06 1/d	2.4 1/d	0.935	5	27.75	120.96
Double exponential parameters						
SVI	150	150	-	-		
Clarification	0.5	0.6	0.935	5	27.75	120.96
Hindered zone settling parameter	0.0004	0.0003	0.935	5	27.75	120.96
Flocculants zone settling parameter	0.0025	0.002	0.935	5	27.75	120.96

The table shows selected model parameters with higher $\delta jmsqr$ values, which have a greater impact on simulation results. Model parameters with zero or less than one $\delta jmsqr$ values have less impact on simulation performance.

4.9. Calibration and validation of the model

4.9.1. Calibration

Wastewater treatment plants simulation involves mainly two steps, model calibration and model validation. The model was gone to simulation with the default values which help for the draw of the actual total cumulative mass balance (Total mass inflow Vs. Total mass outflow). The flow and the type of flow were also given due attention. After in-depth influent characterization, the next step was to select the best model for each unit process. Codstate, mantis2, simple 1d models were selected for influent, anaerobic digester, trickling filter and secondary clarifier respectively. Input parameters (physical, operational, model stoichiometric, kinetic and consistency) were inserted for the respective unit processes. The settling, model stoichiometric and kinetic parameters were set in such a way that there is best fit between the simulated result and measured data sets. Input data were placed as a control, and output data were inserted and placed under output for each GPS-X parameter that the modeler was interested in. Model calibration was carried out using a total of sixty data sets (30 for each). Finally the simulation was run for thirty days. The model calibration results are shown in the figure-3, which shows the measured data sets and the simulated values of COD, TSS, and NH₄-N. Model calibration was required since ARE results between the default simulated values and measured values were extremely high. ARE was used to evaluate the agreement between the simulated and measured values. ARE of less than 7-15% is sufficient to show the agreement between calibrated - simulated results (Liwarska-Bizukoje et al., 2011; Sadri Moghaddam & Pirali, 2021). After fine tuning the settling, kinetic and stoichiometric model parameters, the simulated result and measured data have the ARE values as shown in the table 14. The model was well calibrated as ARE values of 0.17%, 1.1%, 3.13% for COD, TSS, and NH₄-N respectively were much better than the default values.

Table 17 ARE values obtained during model Calibration

Parameters	Effluent value	Simulated value	%ARE
COD	32.4	25.54	0.71%
TSS	15.8	10.54	1.10%
NH ₄ -N	2.33	0.14	3.13%

Table 18 kinetic and stoichiometric parameters values used during calibration

Model Parameters	Default value	Simulated values
Kinetic		
1. Ammonia oxidizing biomass		
Maximum growth rate for ammonia oxidizer	0.9 1/d	5.75 1/d
2. Nitrite oxidizing biomass		
Maximum growth rate for nitrite oxidizer	1 1/d	5.75 1/d
3. Poly-phosphate oxidizing biomass (PAO)		
Maximum growth rate of PAO	1 1/d	0.7 1/d
4. heterotrophic maximum specific growth rate ($\mu_{\max,H}$)	3.2 1/d	6.5 1/d
Stoichiometric		
1. Ammonia oxidizing biomass		
Maximum growth rate for ammonia oxidizer	0.18 1/d	2.4 1/d
2. Nitrite oxidizing biomass		
Maximum growth rate for nitrite oxidizer	0.06 1/d	2.4 1/d
Double exponential parameters		
SVI	150	150
Clarification	0.5	0.6
Hindered zone settling parameter	0.0004	0.0003
Flocculants zone settling parameter	0.0025	0.002

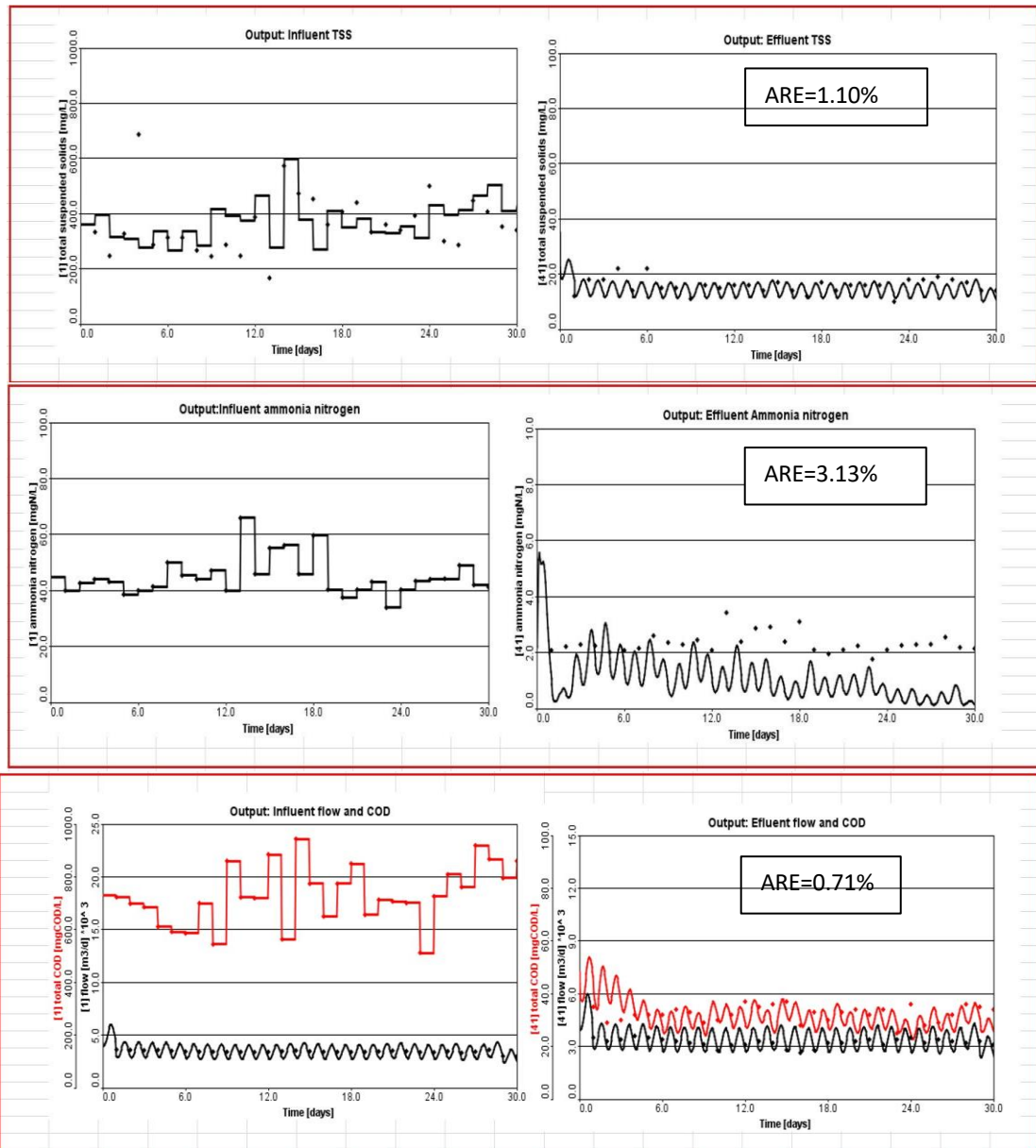
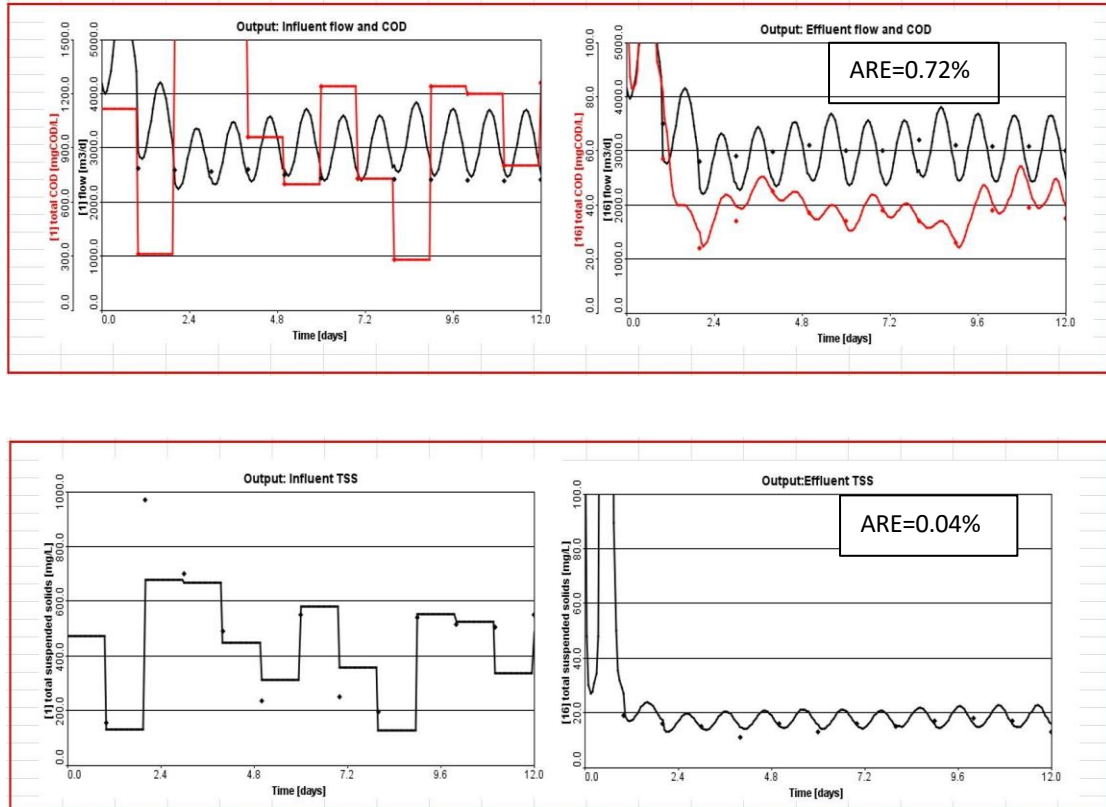


Figure 12 Measured Vs. simulated graph for COD, TSS and NH₄-N during calibration

Figure 12 depicts the measured and the simulated values of COD, TSS and NH₄-N. ARE values showed that there is agreement between the simulated and measured values of COD, TSS and ammonia-nitrogen this depicting that the kinetic and stoichiometric parameter values used for calibration of the model were good.

VALIDATION

For validation of the model, independent experimentally collected data sets were used. The data were collected using the UASB reactor set up. The data used for simulation were collected during the dry season since the wastewater was concentrated. These dry seasons data were used for simulation since the magnitude of the measured parameters were very high compared with the rainy seasons. Operational parameters such as OLR (0-4kgCOD/m³.d), Temperature (-5- 45°C), HRT (0-16hrs.), and pH (5.55-8.55) were used for data collection. At OLR of 0kgCOD/m³.d and HRT of 0hrs, the concentration of TSS, and ammonia-nitrogen were considered Zero. Due to this reason only twelve data sets were used for validation of the model. ARE values of 0.72%, 0.04%, 2.19% were obtained for COD, TSS and NH₄-N respectively.



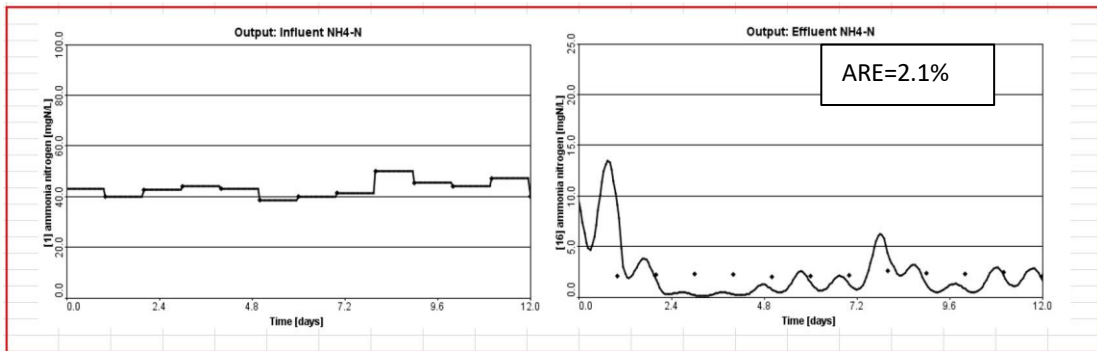


Figure 13 measured vs. simulated graph for COD, TSS and NH₄-N during validation

Figure 13 depicts that ARE values were much less than 7-15% showing the model parameters resulted in good performance during calibration and validation of the model. Therefore, it is possible to conclude that the model developed can be used to predict the performance of the UASB reactor for different scenarios.

7. CONCLUSION AND RECOMMENDATION

7.1. CONCLUSION

The goal of the research was to optimize operational parameters in unregulated wastewater treatment plant using a UASB reactor at a moderate temperature in order to increase performance efficiency of UASB reactor. Moreover the concentration of toxic heavy metals at downstream irrigated soil, vegetable, effluent were determined and the UASB reactor was modeled using GPS-X. The effect of selected operational parameters such as temperature (0-30°C), OLR (1-3kgCOD/m³.d), pH (6.3-7.8), and HRT (4-12hrs.) were investigated and optimized for higher removal efficiency of COD, TSS and VSS using CCD-RSM. The optimal parameters found were temperature = 21.58°C, OLR = 2.99kgCOD/m³.d, pH= 6.3 and HRT = 4.37hrs. Experimental triplication were done and a maximum removal efficiency of 92.70% COD, 99.06% TSS and 94.5%VSS during the rainy seasons were achieved. At these optimum conditions CCD-RSM predicted about 91.06%COD, 98.35% TSS and 93.26%VSS removal efficiency. These higher removal efficiencies were achieved during the rainy seasons since the sewage was diluted by the run-off. Moreover, the influent and effluent concentrations of VFA, alkalinity, sulfate ion, TP, ammonia-nitrogen, VFA to alkalinity ratio, MLSS, MLVSS and pH were measured. The reactor's health was assessed at half-life using VFA to alkalinity, MLSS and MLVSS measurements. The magnitude of the measured values indicated that the reactor was functioning normally. Outlet concentration of VFA and alkalinity were found to be decreasing. This is because as saprophytes break down complex molecules acids are produced in the system (acetic, propionic and butyric acids). The alkalinity in the system accepts the hydrogen ion released by acids, and methanogenesis takes over to maintain the pH. The sulfate ion effluent concentration was found to be increasing with respect to the influent concentration. The reason is that the existence of sulfur-reducing bacteria is hampered by an anaerobic condition of VFA and alkalinity at a pH less than 7.8. This in turn increases the production of CH₄ than H₂S gas.

During the dry seasons, temperature of 23.04°C, OLR (2.3kgCOD/m³.d), pH (7.5), and HRT (11.43h) were optimized using RSM-CCD. At these optimum conditions CCD-RSM predicted about 83.4%COD, 100% TSS and 99%VSS removal efficiencies. Experimental triplication was conducted and a removals of 84.1, 99.89 and 100% for chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) respectively were achieved. In comparison with the

dry seasons, higher removal efficiencies for COD, TSS and VSS were achieved during the rainy seasons since there was no run off for dilution of concentrated wastewater for the ease of microorganism to act on organic compounds. Furthermore during this experimental research, the influent and effluent concentration of sulfate ions, total nitrogen, alkalinity, total phosphorous, VFA, VFA to alkalinity ratio, MLSS, MLVSS and pH were measured. Relative to the influent concentration, the effluent concentration of VFA and alkalinity were seen decreasing. The reason is as microorganisms break the organic compounds, acids like acetic, propionic and butyric acids are produced and release hydrogen which later accepted by the alkalinity to maintain the pH by methanogenesis. The effluent concentration of sulfate ion was seen increasing since the activities of sulfur-reducing bacteria were hindered by the anaerobic conditions created by acid, and alkalinity. This creates higher production of methane gas than hydrogen sulfide.

The presence of heavy metals, toxic heavy metals, and their concentrations in treatment plant effluent, irrigated soil, and vegetable plants were investigated during dry seasons. To advance the research, wastewater samples were collected at the UASB reactor's inlet and outlet, the trickling filter and secondary clarifier outlet. Again soil samples from a cultivated field, and irrigated local cabbages and Swiss chard grown by the effluent were collected and tested. The presence and concentration of heavy metals and toxic heavy metals were determined in the samples. Furthermore, the ability of unit processes such as the UASB reactor, trickling filter, and secondary clarifier to remove toxic heavy metals was determined. Heavy metals such as Iron, Nickel, Cobalt, Zinc, Mercury, and Boron were found in all of the samples tested. Majority of toxic heavy metals such as Chromium, Cadmium, Arsenic, Copper, Lead, and Manganese were also found in the samples. Moreover, the mean concentrations of these chemicals in effluent, soil, and cabbage plants were compared to Ethiopian Ambient Environmental Standards and FAO/WHO Standards. Majority of the mean concentrations of toxic heavy metals found in the effluent, irrigated soil samples, and vegetable (local cabbage and Swiss) plants were found to be above the standards set by Ethiopian Ambient Environmental Standards and FAO/WHO. The UASB reactor was the first unit process investigated. Though the UASB reactor removed some toxic heavy metals, its removal efficiency was determined to be less than the standards. Likewise, laboratory results showed that, trickling filters and secondary clarifiers were ineffective in removing toxic heavy metals like Manganese, Chromium, Copper,

Cadmium, Lead, and Arsenic. The unit processes such as UASB reactors were evaluated for the removal efficiency of toxic heavy metals since the metabolic activities of the microbial communities inside the UASB reactor can be inhibited by heavy metals like Cadmium, Lead, Mercury, and others. The breakdown of organic matter can be less efficiently accomplished by these metals because they can interfere with cellular processes and enzyme function (Wang, Q., Du, G., & Chen, J., 2004; Chen, Y., Cheng, J. J., & Creamer, K. S., 2008). The anaerobic digestion process is carried out in stages by distinct microbial groups in the UASB reactor. Certain organisms, such as methanogens, which are essential for the synthesis of methane, may be toxic to heavy metals. Lower biogas output and overall treatment efficiency may result from this (Zayed, G., & Winter, J. (2000); Demirel, B., & Scherer, P., 2008). The ability of heavy metals to bind to trace elements and vital nutrients reduces their availability to microbial communities. As a result, the performance of the reactor may be further compromised (Koki, K., & Boonkerd, N., 2006; Bhattacharyya, P., & Singh, D. P., 2010). PH alteration in the Reactor: The pH of the reactor environment can vary when heavy metals are present. Because the concentrations of toxic heavy metals were above the standards and inefficiency of the unit processes in removing them, individuals exposed to the consumption of vegetables irrigated by the plant's effluent have a high risk of developing diseases.

In this experimental research, the GPS-X software was used for modeling of the UASB reactor of Kality Wastewater Treatment Plant. Some influent characterization, kinetic and stoichiometric default values were adjusted for the calibration of GPS-X. With the use of sensitivity analysis, kinetic parameters like heterotrophic biomass (maximum specific growth rate on substrate, maximum growth rate for ammonia-oxidizer, maximum growth rate for nitrite oxidizer), and model stoichiometry such as (ammonia oxidizer yield, nitrite oxidizer yield, ammonia-oxidizer yield) were influential model parameters but maximum specific growth rate on substrate was the most influential model parameter. ARE was employed to show the agreement between the measured and the simulated results. The simulated GPS-X results fitted well the measured data sets with ARE of less than 15%. Using GPS-X, about 95.56% COD and about 95.64% TSS were achieved, which were much higher than the removal efficiency set by the Kality wastewater treatment plant's manual. Generally, the simulated results implied that the model could accurately predict the changes in COD, TSS and ammonia-nitrogen during calibration and validation. Therefore, the

model can be used for scenarios prediction, optimization and further researches.

7.2. RECOMMENDATION

For Policy makers

- The relevant entities ought to devote sufficient time and resources in utilising the optimal operating parameters in order to enhance the UASB reactor's performance efficiency.
- Addis Ababa Water and Sewerage Authority(AAWSA) used to employ the optimized operational parameters(temperature ,OLR ,HRT and pH) to increase the performance efficiency of the UASB reactor
- In order to improve the performance efficiency of the UASB reactor and the effluent of the WWTP to satisfy EPA standards, Addis Ababa Water and Sewerage Authority (AAWSA) should be required to use the effluent after secondary clarifier for dilution.

For researchers

- Controllable operational parameters like up- flow velocity and other parameters like granulation and mixing should further be considered for performance enhancement of the UASB reactor since four operational parameters like temperature, OLR, pH and HRT.
- Further experimental investigations using other reactor configurations like continuous stirred tank reactors should be used to mimic the UASB reactor
- Larger reactor size of UASB reactor should be considered to mimic the actual UASB reactor in flow distribution and sludge return to dry bed.
- There should be more research done on the ammonia-nitrogen concentration in the UASB reactor.
- Pretreatment techniques should be investigated for the removal of toxic heavy metals that hinders the wellbeing of the microorganisms.
- The amount of infiltration to the sewer line during the rainy seasons exceeds the maximum threshold, so care should be taken to avoid shock loads to the UASB reactor

Reference

- AAWSA. (2019). *Kaliti Wastewater Treatment Plant and Sanitary Sewer Trunk*.
- Ahmad, A., Ghufran, R., & Abd. Wahid, Z. (2012). Effect of cod loading rate on an upflow anaerobic sludge blanket reactor during anaerobic digestion of palm oil mill effluent with butyrate. *Journal of Environmental Engineering and Landscape*, 20(4), 256–264. <https://doi.org/10.3846/16486897.2012.656647>.
- Alex, J. (2008). *Wastewater process modelling in Simulink and SIMBA process library*. 22–22. <https://doi.org/10.1049/ic:20040636>.
- Ali, M. A. G. (2020). Problems encountered during the start-up of up- flow anaerobic sludge blanket reactors (UASB) at 20 °C & 15 °C. *Advances in Food Science, Sustainable Agriculture and A groindustrial Engineering* 3(1).
- Alkhatib, M. F., Mamun, A. A., & Akbar, I. (2015). Application of response surface methodology (RSM) for optimization of color removal from POME by granular activated carbon. *International Journal of Environmental Science and Technology*, 12(4), 1295–1302. <https://doi.org/10.1007/s13762-014-0504-4>
- Álvarez, J. A., Armstrong, E., Presas, J., Gómez, M., & Soto, M. (2004). Performance of a UASB-digester system treating domestic wastewater. *Environmental*, 25(10), 1189–1199.
- Álvarez, J. A., Ruiz, I., Gómez, M., Presas, J., & Soto, M. (2006). Start-up alternatives and performance of an UASB pilot plant treating diluted municipal wastewater at low temperature. *Bioresource Technology*, 97(14), 1640–1649. <https://doi.org/10.1016/j.biortech.2005.07.033>
- Amer, K. H. (2010). Corn crop response under managing different irrigation and salinity levels. *Agricultural Water Management*, 97(10), 1553–1563. <https://doi.org/10.1016/j.agwat.2010.05.010>.
- Amrutha, M. C. V, & Haseena, P. V. (2020). *EasyChair Preprint Waste water treatment plant analysis and simulation using computational tools : A review Waste water treatment plant analysis and simulation using computational tools : A review*.
- Ansari, K., Shrikhande, A., Malik, M. A., Alahmadi, A. A., Alwetaishi, M., Alzaed,

- A. N., & Elbeltagi, A. (2022). Optimization and Operational Analysis of Domestic Greywater Treatment by Electrocoagulation Filtration Using Response Surface Methodology. *Sustainability (Switzerland)*, 14(22). <https://doi.org/10.3390/su142215230>.
- Arthur, P. M. A., Konaté, Y., Sawadogo, B., Sagoe, G., Dwumfour-Asare, B., Ahmed, I., & Williams, M. N. V. (2022). Performance evaluation of a full-scale upflow anaerobic sludge blanket reactor coupled with trickling filters for municipal wastewater treatment in a developing country. *Heliyon*, 8(8). <https://doi.org/10.1016/j.heliyon.2022.e10129>.
- Asmah, Q. (2018). Sustainable Improvements for Jenin Wastewater Treatment Plant. *Sustainable Development Strategies of Agricultural Land in the West Bank : Tubas Governorate as a Case Study*, 1–6.
- Aswir, & Misbah, H. (2018). No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title.
- Azbar, N., Tutuk, F., & Keskin, T. (2009). Effect of organic loading rate on the performance of an up-flow anaerobic sludge blanket reactor treating olive mill effluent. *Biotechnology and Bioprocess Engineering*, 14(1), 99–104.
- Aziz, A., Latiff, A., Tun, U., Onn, H., Daud, Z., Tun, U., Onn, H., Efficiency, A., & Limestone, C. U. (2011). *formation process. June 2014*.
- Barampouti, E. M. P., Mai, S. T., & Vlyssides, A. G. (2005). Dynamic modeling of biogas production in an UASB reactor for potato processing wastewater treatment. *Chemical Engineering Journal*, 106(1), 53–58. <https://doi.org/10.1016/j.cej.2004.06.010>.
- Bashir, M. J. K., Amr, S. S. A., Aziz, S. Q., Aun, N. C., Sethupathi, S., Technology, G., Tunku, U., & Rahman, A. (2015). Wastewater Treatment Processes Optimization Using Response Surface Methodology (RSM) Compared with Conventional Methods : Review and Comparative Study Department of Environmental Engineering , Faculty of Engineering School of Civil Engineering , Enginee. *Middle-East Journal of Scientific Research*, 23(2), 244–252. <https://doi.org/10.5829/idosi.mejsr.2015.23.02.52>.
- Bashir, M. J. K., Aziz, H. A., Aziz, S. Q., & Amr, S. A. (2012). An overview of

- wastewater treatment processes optimization using response surface methodology (RSM). *The 4th International Engineering Conference*.
- Batstone, D. J., Puyol, D., Flores-Alsina, X., & Rodríguez, J. (2015). Mathematical modelling of anaerobic digestion processes: applications and future needs. *Reviews in Environmental Science and Biotechnology*, *14*(4), 595–613. <https://doi.org/10.1007/s11157-015-9376-4>.
- Bernard, A. (2004). Renal dysfunction induced by cadmium: Biomarkers of critical effects. *BioMetals*, *17*(5), 519–523. <https://doi.org/10.1023/B:BIOM.0000045731.75602.b9>.
- Besharati Fard, M., Mirbagheri, S. A., Pendashteh, A., & Alavi, J. (2019). Biological treatment of slaughterhouse wastewater: Kinetic modeling and prediction of effluent. *Journal of Environmental Health Science and Engineering*, *17*(2), 731–741. <https://doi.org/10.1007/s40201-019-00389-4>.
- Bhatti, Z. A., Maqbool, F., Malik, A. H., & Mehmood, Q. (2014). UASB reactor startup for the treatment of municipal wastewater followed by advanced oxidation process. *Brazilian Journal of Chemical Engineering*, *31*(3), 715–726. <https://doi.org/10.1590/0104>.
- Bhunia, P., & Ghangrekar, M. M. (2008). Analysis, evaluation, and optimization of kinetic parameters for performance appraisal and design of UASB reactors. *Bioresource Technology*, *99*(7), 2132–2140.
- Brito, M. G. S. L., Nunes, F. C. B., Magalhães, H. L. F., Lima, W. M. P. B., Moura, F. L. C., Neto, S. R. F., & Lima, A. G. B. (2020). Hydrodynamics of uasb reactor treating domestic wastewater: A three-dimensional numerical study. *Water (Switzerland)*, *12*(1). <https://doi.org/10.3390/w12010279>.
- Bui, H. M. (2018). Applying response surface methodology to optimize the treatment of swine slaughterhouse wastewater by electrocoagulation. *Polish Journal of Environmental Studies*, *27*(5), 1975–1981.
- Casserly, C., & Erijman, L. (2003). Molecular monitoring of microbial diversity in an UASB reactor. *International Biodeterioration and Biodegradation*, *52*(1), 7–12. [https://doi.org/10.1016/S0964-8305\(02\)00094-X](https://doi.org/10.1016/S0964-8305(02)00094-X).
- Chaoua, S., Boussaa, S., El Gharmali, A., & Boumezzough, A. (2019). Impact of

irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in Morocco. *Journal of the Saudi Society of Agricultural Sciences*, 18(4), 429–436.

Chollom, M. N., Rathilal, S., Swalaha, F. M., Bakare, B. F., & Tetteh, E. K. (2020). Comparison of response surface methods for the optimization of an upflow anaerobic sludge blanket for the treatment of slaughterhouse wastewater. *Environmental Engineering Research*, 25(1), 114–122.

Chong, S., Sen, T. K., Kayaalp, A., & Ang, H. M. (2012). The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment - A State- of-the-art review. *Water Research*, 46(11), 3434–3470. <https://doi.org/10.1016/j.watres.2012.03.066>.

Chou, H. H., Huang, J. S., & Hong, W. F. (2004). Temperature dependency of granule characteristics and kinetic behavior in UASB reactors. *Journal of Chemical Technology and Biotechnology*, 79(8), 797–808. <https://doi.org/10.1002/jctb.999>.

Cunha, M. P., Ferraz, R. M., Sancinetti, G. P., & Rodriguez, R. P. (2019). Long-term performance of a UASB reactor treating acid mine drainage: effects of sulfate loading rate, hydraulic retention time, and COD/SO₄²⁻ ratio. *Biodegradation*, 30(1), 47–58. <https://doi.org/10.1007/s10532-018-9863-8>

Daija, L., Selberg, A., Rikmann, E., Zekker, I., Tenno, T., & Tenno, T. (2016). The influence of lower temperature, influent fluctuations and long retention time on the performance of an upflow mode laboratory-scale septic tank. *Desalination and Water Treatment*, 57(40), 18679–18687. <https://doi.org/10.1080/19443994.2015.1094421>.

Daud, M. K., Rizvi, H., Akram, M. F., Ali, S., Rizwan, M., Nafees, M., & Jin, Z. S. (2018a). Review of upflow anaerobic sludge blanket reactor technology: Effect of different parameters and developments for domestic wastewater treatment. *Journal of Chemistry*, 2018. <https://doi.org/10.1155/2018/1596319>.

Daud, M. K., Rizvi, H., Akram, M. F., Ali, S., Rizwan, M., Nafees, M., & Jin, Z. S. (2018b). *Journal of Chemistry*, 2018. <https://doi.org/10.1155/2018/1596319> de Graaff, M. S., Temmink, H., Zeeman,

- G., & Buisman, C. J. N. (2010). Anaerobic treatment of concentrated black water in a UASB reactor at a short HRT. *Water (Switzerland)*, 2(1), 101–119. <https://doi.org/10.3390/w2010101>
- de Lemos Chernicharo, C. A. (2015). Anaerobic Reactors. In *Water Intelligence Online* (Vol. 6, Issue 0). <https://doi.org/10.2166/9781780402116>.
- Del Castillo, A. F., Garibay, M. V., Senés-Guerrero, C., Yebra-Montes, C., de Anda, J., & Gradilla-Hernández, M. S. (2020). Mathematical modeling of a domestic wastewater treatment system combining a septic tank, an up-flow anaerobic filter, and a constructed wetland. *Water (Switzerland)*, 12(11), 1–20. <https://doi.org/10.3390/w12113019>.
- Dixit, R., Wasiullah, Malaviya, D., Pandiyan, K., Singh, U. B., Sahu, A., Shukla, R., Singh, B. P., Rai, J. P., Sharma, P. K., Lade, H., & Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability (Switzerland)*, 7(2), 2189–2212. <https://doi.org/10.3390/su7022189>.
- Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, 2(5), 112–118. <https://doi.org/10.1016/j.proenv.2011.09.146>.
- Edwin, G. A., Gopalsamy, P., & Muthu, N. (2014). Characterization of domestic gray water from point source to determine the potential for urban residential reuse: a short review. *Applied Water Science*, 4(1), 39–49. <https://doi.org/10.1007/s13201-013-0128-8>.
- Elgallal, M., Fletcher, L., & Evans, B. (2016). Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: A review. *Agricultural Water Management*, 177.
- ENGINEERING, C. C. C. E. (2019). *Kaliti Wastewater Treatment Plant and Sanitary Sewer Trunk Main*.
- Enitan, A. M., Adeyemo, J., Swalaha, F. M., & Bux, F. (2015). Anaerobic Digestion Model to Enhance Treatment of Brewery Wastewater for Biogas Production Using UASB Reactor. *Environmental Modeling and Assessment*, 20(6), 673–685. <https://doi.org/10.1007/s10666-015-9457-3>.

- Erbe, V., Risholt, L. P., Schilling, W., & Londong, J. (2002). Integrated modelling for analysis and optimisation of wastewater systems - The Odenthal case. *Urban Water*, 4(1), 63–71. [https://doi.org/10.1016/S1462-0758\(01\)00060-7](https://doi.org/10.1016/S1462-0758(01)00060-7).
- Farajzadehha, S., Mirbagheri, S. A., Farajzadehha, S., & Shayegan, J. (2012). Lab Scale Study of HRT and OLR Optimization in UASB Reactor for Pretreating Fortified Wastewater in Various Operational Temperatures. *APCBEE Procedia*, 1(January), 90–95. <https://doi.org/10.1016/j.apcbee.2012.03.016>.
- Fitamo, D., Itana, F., & Olsson, M. (2007). Total contents and sequential extraction of heavy metals in soils irrigated with wastewater, Akaki, Ethiopia. *Environmental Management*, 39(2), 178–193. <https://doi.org/10.1007/s00267>.
- Garza-Lombó, C., Pappa, A., Panayiotidis, M. I., Gonsebatt, M. E., & Franco, R. (2019). Arsenic-induced neurotoxicity: a mechanistic appraisal. *Journal of Biological Inorganic Chemistry*, 24(8), 1305–1316. <https://doi.org/10.1007/s00775-019-01740-8>.
- Gemeda, F. T., Guta, D. D., Wakjira, F. S., & Gebresenbet, G. (2021). Occurrence of heavy metal in water, soil, and plants in fields irrigated with industrial wastewater in Sabata town, Ethiopia. *Environmental Science and Pollution Research*, 28(10), 12382–12396. <https://doi.org/10.1007/s11356-020-10621-6>.
- Ghawi, A. H., & Tisti, H. J. M. (2021). Performance of temperature changes and dosage for enhancement of nitrogen and phosphorus removal from wastewater: A comparative study. *Journal of Physics: Conference Series*, 1895(1). <https://doi.org/10.1088/1742-6596/1895/1/012033>.
- Girma, J., Kemal, A., & Nigussie, A. (2023). Performance enhancement of up-flow anaerobic sludge blanket reactor for psychrophilic temperature during the dry season: Kality wastewater treatment plant. *Heliyon*, 9(9).
- Goldhaber, S. B. (2003). Trace element risk assessment: Essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, 38(2), 232–242. [https://doi.org/10.1016/S0273-2300\(02\)00020-X](https://doi.org/10.1016/S0273-2300(02)00020-X).
- Gray, S. R., & Becker, N. S. C. (2002). Contaminant flows in urban residential water systems. *Urban Water*, 4(4), 331–346. <https://doi.org/10.1016/S1462>

- He, Z. L., Yang, X. E., & Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *Journal of Trace Elements in Medicine and Biology*, *19*(2–3), 125–140. <https://doi.org/10.1016/j.jtemb.2005.02.010>.
- Hernández Leal, L., Temmink, H., Zeeman, G., & Buisman, C. J. N. (2011). Characterization and anaerobic biodegradability of grey water. *Desalination*, *270*(1–3), 111–115. <https://doi.org/10.1016/j.desal.2010.11.029>.
- Huang, H. W., Lee, C. H., & Yu, H. S. (2019). Arsenic-induced carcinogenesis and immune dysregulation. *International Journal of Environmental Research and Public Health*, *16*(15). <https://doi.org/10.3390/ijerph16152746>
- Hyder, O., Chung, M., Cosgrove, D., Herman, J. M., Li, Z., Firoozmand, A., Gurakar, A.
- Koteish, A., & Pawlik, T. M. (2013). Cadmium Exposure and Liver Disease among US Adults. *Journal of Gastrointestinal Surgery*, *17*(7), 1265–1273. <https://doi.org/10.1007/s11605-013-2210-9> *Hydromantis_GPS-X_v7*. (n.d.).
- Intriago, J. C., López-Gálvez, F., Allende, A., Vivaldi, G. A., Camposeo, S., Nicolás Nicolás, E., Alarcón, J. J., & Pedrero Salcedo, F. (2018). Agricultural reuse of municipal wastewater through an integral water reclamation management. *Journal of Environmental Management*, *213*, 135–141. <https://doi.org/10.1016/j.jenvman.2018.02.011>.
- Işık, M., & Sponza, D. T. (2005). Substrate removal kinetics in an upflow anaerobic sludge blanket reactor decolorising simulated textile wastewater. *Process Biochemistry*, *40*(3–4), 1189–1199. <https://doi.org/10.1016/j.procbio.2004.04.014>
- Jaiswal, A., Kharade, S., Mahale, V., Mane, M., & Salunke, P. J. (2019). *Analysis and Design of Advanced Sewage Treatment Plant*. 3821–3825. <https://doi.org/10.15680/IJIRSET.2019.0804031>.
- Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*, 167–182. <https://doi.org/10.1093/bmb/ldg032>.
- Jijai, S., Srisuwan, G., O-Thong, S., Ismail, N., & Siripatana, C. (2015). Effect of Granule Sizes on the Performance of Upflow Anaerobic Sludge Blanket (UASB) Reactors for Cassava Wastewater Treatment. In *Energy Procedia* (Vol. 79). Elsevier B.V. <https://doi.org/10.1016/j.egypro.2015.11.482>.

- Kaizer, A., & Osakwe, S. (2010). Physicochemical Characteristics and Heavy Metal Levels in Water Samples from Five River Systems in Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management*.
- Kalyuzhnyi, S. V., Fedorovich, V. V., & Lens, P. (2006). Dispersed plug flow model for upflow anaerobic sludge bed reactors with focus on granular sludge dynamics. *Journal of Industrial Microbiology and Biotechnology*, 33(3), 221–237. <https://doi.org/10.1007/s10295-005-0217-2>.
- Kapumbe, D. J., Min, L., Kisoholo, M. A., Sun, C. Y., & Yongfeng, L. (2019). Effect of organic loading rate on coproduction of hydrogen and methane from molasses wastewater and energy conversion by two-phase anaerobic fermentation. *Applied Ecology and Environmental Research*, 17(5),
- Kaviyarasan, K. (2014). Application of UASB Reactor in Industrial Wastewater Treatment—A Review. *International Journal of Scientific & Engineering Research*, 5(1), 584–589. <http://www.ijser.org/researchpaper/Application-of-UASB-Reactor-in-IndustrialWastewater>.
- Khan, M. U., Muhammad, S., Malik, R. N., Khan, S. A., & Tariq, M. (2016). Heavy metals potential health risk assessment through consumption of wastewater irrigated wild plants: A case study. *Human and Ecological Risk Assessment*, 22(1), 141–152.
- Khanh, D., Quan, L., Zhang, W., Hira, D., & Furukawa, K. (2011). Effect of temperature on low-strength wastewater treatment by UASB reactor using poly (vinyl alcohol)-gel carrier. <https://doi.org/10.1016/j.biortech.2011.09.108>
- Kim, Y., Cho, H., Choi, Y., Koo, J., & Lee, S. (2023). Optimization and Evaluation for the Capacitive Deionization Process of Wastewater Reuse in Combined Cycle Power Plants. *Membranes*, 13(3), 1–20.
- Klesyk, D. (2017). *Strategies of improvement of UASB reactor operation (Thesis)*.
- Ladu, J. L. C., & Lü, X. W. (2014). Effects of hydraulic retention time, temperature, and effluent recycling on efficiency of anaerobic filter in treating rural domestic wastewater. *Water Science and Engineering*, 7(2), 168–182. <https://doi.org/10.3882/j.issn.1674>.
- Leitão, R. C., Silva-Filho, J. A., Sanders, W., van Haandel, A. C., Zeeman, G., &

- Lettinga, G. (2005). The effect of operational conditions on the performance of UASB reactors for domestic wastewater treatment. *Water Science and Technology*, 52(1–2), 299–305. <https://doi.org/10.2166/wst.2005.0531>.
- Lester, J. N., Soares, A., San Martin, D., Harper, P., Jefferson, B., Brigg, J., Wood, E., & Cartmell, E. (2009). A novel approach to the anaerobic treatment of municipal wastewater in temperate climates through primary sludge fortification. *Environmental Technology*, 30(10), 985–994.
- Lew, B., Belavski, M., Admon, S., Tarre, S., & Green, M. (2003). Temperature effect on UASB reactor operation for domestic wastewater treatment in temperate climate regions. *Water Science and Technology*, 48(3), 25–30.
- Li, W. W., & Yu, H. Q. (2016). Advances in Energy-Producing Anaerobic Biotechnologies for Municipal Wastewater Treatment. *Engineering*, 2(4), 438–446. <https://doi.org/10.1016/J.ENG.2016.04.017>.
- Lier, J. B., Zee, F. P., Frijters, C. T. M. J., & Ersahin, M. E. (2015). Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Reviews in Environmental Science and Bio/Technology*, 14(4), 681–702. <https://doi.org/10.1007/s11157-015-9375-5>.
- Lohani, S. P., Wang, S., Lackner, S., Horn, H., Khanal, S. N., & Bakke, R. (2016). ADM1 modeling of UASB treating domestic wastewater in Nepal. *Renewable Energy*, 95, 263–268. <https://doi.org/10.1016/j.renene.2016.04.014>
- Lomte, A. T., & Bobade, V. V. (2015a). Challenges in UASB reactor system design: A Review. *International Journal of Science and Research*, 4(4), 802–807.
- Lomte, A. T., & Bobade, V. V. (2015b). Suitability of UASB Reactor System in Tropical Developing Countries like India. *International Journal of Science and Research(IJSR)*, 4(4), 801–807.
- Luostarinen, S., Sanders, W., Kujawa-Roeleveld, K., & Zeeman, G. (2007). Effect of temperature on anaerobic treatment of black water in UASB-septic tank systems. *Bioresource Technology*, 98(5), 980–986.
- Ma, B., Peng, Y., Zhang, S., Wang, J., Gan, Y., Chang, J., Wang, S., Wang, S., & Zhu, G. (2013). Performance of anammox UASB reactor treating low strength wastewater under moderate and low temperatures. *Bioresource Technology*.

- Mahalakshmi, M. (2012). Characteristic levels of heavy metals in canned tuna fish. *Journal of Toxicology and Environmental Health Sciences*, 4(2), 43–45.
- Manea, E. E. (2016). *Simulation of Full-Scale Wastewater Treatment Biological Processes*. October 2016, 422–426.
- Messner, B., Knoflach, M., Seubert, A., Ritsch, A., Pfaller, K., Henderson, B., Shen, Y. H., Zeller, I., Willeit, J., Laufer, G., Wick, G., Kiechl, S., & Bernhard, D. (2009). Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 29(9), 1392–1398.
- Młyński, D., Bugajski, P., & Młyńska, A. (2019). Application of the mathematical simulation methods for the assessment of the waste water treatment plant operation work reliability. *Water (Switzerland)*, 11(5). <https://doi.org/10.3390/w11050873>.
- Mohanty, B., Das, A., Mandal, R., Banerji, U., & Acharyya, S. (2021). Heavy Metals in Soils and Vegetation from Wastewater Irrigated Croplands near Ahmedabad, Gujarat: Risk to Human Health. *Nature Environment and Pollution Technology*, 20(1), 163–175.
- Montes, J. A., Leivas, R., Martínez-Prieto, D., & Rico, C. (2019). Biogas production from the liquid waste of distilled gin production: Optimization of UASB reactor performance with increasing organic loading rate for co-digestion with swine wastewater. *Bioresource Technology*, 274(November 2018), 43–47. <https://doi.org/10.1016/j.biortech.2018.11.060>.
- Mrunalini M. Powar*, Vijay S. Kore, Sunanda V. Kore, & Girish S. Kulkarni. (2013). Review on Applications of Uasb Technology for Wastewater Treatment. *International Journal of Advanced Science, Engineering*
- Mu, Y., & Yu, H. Q. (2007). Simulation of biological hydrogen production in a UASB reactor using neural network and genetic algorithm. *International Journal of Hydrogen Energy*, 32(15 SPEC. ISS.), 3308–3314. <https://doi.org/10.1016/j.ijhydene.2007.05.021>.
- Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., & Lester, J. N. (2006). Heavy metal content of vegetables irrigated with mixtures

- of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agriculture, Ecosystems and Environment*, 112(1), 41–48. <https://doi.org/10.1016/j.agee.2005.04.028>.
- Musa, M. A., Idrus, S., Hasfalina, C. M., & Daud, N. N. N. (2018). Effect of organic loading rate on anaerobic digestion performance of mesophilic (UASB) reactor using cattle slaughterhouse wastewater as substrate. *International Journal of Environmental Research and Public Health*, 15(10).
- Nair, A. T., Makwana, A. R., & Ahammed, M. M. (2014). The use of response surface methodology for modelling and analysis of water and wastewater treatment processes: A review. *Water Science and Technology*, 69(3),
- Narnoli, S. K., & Mehrotra, I. (1997). Sludge blanket of UASB reactor: Mathematical simulation. *Water Research*, 31(4), 715–726.
- Navas-Acien, A., Selvin, E., Sharrett, A. R., Calderon-Aranda, E., Silbergeld, E., & Guallar, E. (2004). Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation*, 109(25), 3196–3201.
- Nazif, S., Forouzanmehr, F., & Khatibi, Y. (2023). Developing a practical model for the optimal operation of wastewater treatment plant considering influent characteristics. *Environmental Science and Pollution Research*, 39764–39782. <https://doi.org/10.1007/s11356-022-24981>.
- Nugroho, G., & Santoso, S. A. (2019). Dynamical modeling of substrate and biomass effluents in up-flow anaerobic sludge blanket (UASB) biogas reactor. *International Journal of Industrial Chemistry*, 10(4), 311–319. <https://doi.org/10.1007/s40090-019-00194-w>.
- O’Flaherty, V., Collins, G., & Mahony, T. (2006). The microbiology and biochemistry of anaerobic bioreactors with relevance to domestic sewage treatment. *Reviews in Environmental Science and Biotechnology*, 5(1), 39–55. <https://doi.org/10.1007/s11157-005-5478-8>.
- Pahal, V., Rana, R., Singh, G., & Dadhich, K. S. (2020). ASSESSMENT OF VARIOUS PHYSICO – CHEMICAL PARAMETERS OF TREATED AND UNTREATED WASTE WATER FROM DISTRICT. 39(1), 123–129.
- Paltahe, A., Cornelius, T., Sambo, B., Christian, D., Téri, T., Rallet, D., &

- Wahabou, A. (2019). Physico-Chemical Characterization of Local Tannery Waste Water Before and After Flocculation Treatment. *International Journal*
- Pandya, P., Sharma, A. K., Sharma, S., & Verma, S. (2011). Effect of operational and design parameters on removal efficiency of a pilot-scale uasb reactor treating dairy wastewater. *Journal of Industrial Pollution Control*, 27(2), 103
- Parihar, R. S., Baredar, P., Sharma, A. K., & Rajput, R. L. (2017). Performance of uasb reactor as a common effluent treatment plant: A case study. *International Journal of Civil Engineering and Technology*, 8(9), 279–285.
- Pasquale, Navarro, A., Domenico Palumbo, A., Mastrangelo, M., Lonigro, A., & Mastroilli, M. (2015). Bioenergy productivity of sugar beet irrigated with reclaimed wastewaters. *Italian Journal of Agronomy*, 10(3), 155–159.
- Pereira, S. (2014). *Modelling Of A Wastewater Treatment Plant Using GPS-X*. September. https://run.unl.pt/bitstream/10362/13621/1/Pereira_2014.pdf
- Pererva, Y., Miller, C. D., & Sims, R. C. (2020). Approaches in design of laboratory-scale uasb reactors. *Processes*, 8(6), 1–26.
- Peters, J. L., Perlstein, T. S., Perry, M. J., McNeely, E., & Weuve, J. (2010). Cadmium exposure in association with history of stroke and heart failure. *Environmental Research*, 110(2), 199–206.
- Prabu, P. C. (2009). Impact of heavy metal contamination of Akaki river of Ethiopia on soil and metal toxicity on cultivated vegetable crops. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 8(9), 818–827.
- Rahman, S. M. A., Sharma, P., & Said, Z. (2022). Application of Response Surface Methodology based D-optimal Design for Modeling and Optimization of Osmotic dehydration of Zucchini. *Digital Chemical Engineering*, 4(June), 100039. <https://doi.org/10.1016/j.dche.2022.100039>.
- Rate, O. L., Time, R., & Velocity, U. (2022). *Local Capacity Building by experimental and modeling-based performance enhancement for domestic Wastewater Treatment Plant at moderate temperature*. Reactors, U. (2013). *Archive of SID* Archive of SID. (3), 605–614. Resolutions taken at the World Conference on Lung Health, B. 1990. (1990). Resolutions taken at the World

- Conference on Lung Health, Boston 1990. In *Bulletin of the International Union Against Tuberculosis and Lung Disease* (Vol. 65, Issues 2–3, pp. 7–9).
- Reyes, J. L., Molina-Jijón, E., Rodríguez-Muñoz, R., Bautista-García, P., Debray-García, Y., & Namorado, M. D. C. (2013). Tight junction proteins and oxidative stress in heavy metals- induced nephrotoxicity. *BioMed*
- Rizvi, H., Ahmad, N., Abbas, F., Bukhari, I. H., Yasar, A., Ali, S., Yasmeen, T., & Riaz, M. (2015). Start-up of UASB reactors treating municipal wastewater and effect of temperature/sludge age and hydraulic retention time (HRT) on its performance. *Arabian Journal of Chemistry*, 8(6), 780–786.
- Rodríguez-Gómez, R.1, 2*, & , Moreno, L.1 and Liu, L.... 1. (2013). *International Journal of Environmental Research*, 7(3), 605–614.
- Rodríguez-Gómez, R., Renman, G., Moreno, L., & Liu, L. (2014). A model to describe the performance of the UASB reactor. *Biodegradation*, 25(2), 239–251. <https://doi.org/10.1007/s10532-013-9656-z>.
- Rodriguez, R., & Moreno, L. (2010). Modelling of an Upflow Anaerobic Sludge Blanket reactor. *WIT Transactions on Ecology and the Environment*, 135, 301–310. <https://doi.org/10.2495/WP100261>.
- Saravanan, V., & Sreekrishnan, T. R. (2006). Modelling anaerobic biofilm reactors- A review. *Journal of Environmental Management*, 81(1), 1–18.
- Schultz, J., Jensen, A. L., Pinheiro, A., & Da Silva, J. D. (2015). m. *Proceedings - 2015 9th International Conference on Complex, Intelligent, and Software Intensive Systems, CISIS 2015*, 398–401.
- Schwartz, G. G., Il'Yasova, D., & Ivanova, A. (2003). Urinary cadmium, impaired fasting glucose, and diabetes in the NHANES III. *Diabetes Care*, 26(2), 468–470. <https://doi.org/10.2337/diacare.26.2.468>.
- Seghezzi, L., Guerra, R. G., González, S. M., Trupiano, A. P., Figueroa, M. E., Cuevas, C. M., Zeeman, G., & Lettinga, G. (2002). Removal efficiency and methanogenic activity profiles in a pilot-scale UASB reactor treating settled sewage at moderate temperatures. *Water Science and Technology: A Journal of the International Association on Water Pollution Research*, 45(10), 243

- Seghezzeo, Lucas, Zeeman, G., Van Lier, J. B., Hamelers, H. V. M., & Lettinga, G. (1998). A review: The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology*, *65*(3), 175–190.
- Serdarevic, A., & Dzibur, A. (2016). Wastewater process modeling. *Coupled Systems Mechanics*, *5*(1), 21–39. <https://doi.org/10.12989/csm.2016.5.1.021>.
- Services, D. S., Sanitation, A. A., & Project, I. (2002). *Wastewater Masterplan Volume 1 Executive Summary Improvement Project*.
- Shrivastava, R., Upreti, R. K., Seth, P. K., & Chaturvedi, U. C. (2002). Effects of chromium on the immune system. *FEMS Immunology and Medical Microbiology*, *34*(1), 1–7. [https://doi.org/10.1016/S0928-8244\(02\)00345-0](https://doi.org/10.1016/S0928-8244(02)00345-0).
- Silbergeld, E. K., Waalkes, M., & Rice, J. M. (2000). Lead as a carcinogen: Experimental evidence and mechanisms of action. *American Journal of Industrial Medicine*, *38*(3), 316–323. <https://doi.org/10.1002/1097-0274>.
- Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology*, *48*(2), 611–619. <https://doi.org/10.1016/j.fct.2009.11.041>.
- Singh, K. S., & Viraraghavan, T. (2003). Impact of temperature on performance, microbiological, and hydrodynamic aspects of UASB reactors treating municipal wastewater. *Water Science and Technology*, *48*(6), 211–217.
- Singh, K., & Valshya, R. (2017). A biodegradation based kinetic study of UASB Reactor in treating municipal wastewater through various models. *49th Annual Convention of IWWA on “Smart Water Management,” January*, 1–8.
- Singh, Kripa Shankar, Harada, H., & Viraraghavan, T. (1996). Low-strength wastewater treatment by a UASB reactor. *Bioresource Technology*, *55*(3), 187–194. [https://doi.org/10.1016/0960-8524\(96\)86817-9](https://doi.org/10.1016/0960-8524(96)86817-9).
- Singh, Kripa Shankar, & Viraraghavan, T. (2002). Effect of temperature on bio-kinetic coefficients in UASB treatment of municipal wastewater. *Water, Air, and Soil Pollution*, *136* (1–4), 243–254.
- Singh, V., Singh, R. P., & Pandey, N. D. (2016). *Dynamic Performance of UASB Reactor Treating Municipal Wastewater*.

- Start-up and performance evaluation of upflow anaerobic sludge blanket reactor treating supernatant of hydrothermally treated municipal sludge: Effect of initial organic loading rate Liu, S., Wang, Y., Guo, J., Wang, W., & Dong, R. (2021). Start-up and performance evaluation of upflow anaerobic sludge blanket reactor treating supernatant of hydrothermally treated municipal sludge: Effect of initial organic loading rate. *Biochemical Engineering Journal*, 166(August), 107843. <https://doi.org/10.1016/j.bej.2020.107843>
- Sun, J., Dai, X., Wang, Q., Pan, Y., & Ni, B. J. (2016). Modelling Methane Production and Sulfate Reduction in Anaerobic Granular Sludge Reactor with Ethanol as Electron Donor. *Scientific Reports*, 6(September), 1–11.
- Sushma, & Pal, J. (2013). Performance of UASB reactor at different flow rate treating sewage wastewater. *International Journal of ChemTech Research*,
- Tandukar, M., Machdar, I., Uemura, S., Ohashi, A., & Harada, H. (2006). Potential of a Combination of UASB and DHS Reactor as a Novel Sewage Treatment System for Developing Countries: Long-Term Evaluation. *Journal of Environmental Engineering*, 132(2), 166–172.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Molecular, clinical and environmental toxicology Volume 3: Environmental Toxicology. In *Molecular, Clinical and Environmental Toxicology* (Vol. 101). <https://doi.org/10.1007/978-3-7643-8340-4>.
- Tellez-Plaza, M., Guallar, E., Howard, B. V., Umans, J. G., Francesconi, K. A., Goessler, W., Silbergeld, E. K., Devereux, R. B., & Navas-Acien, A. (2013). Cadmium exposure and incident cardiovascular disease. *Epidemiology*, 24(3), 421–429. <https://doi.org/10.1097/EDE.0b013e31828b0631>.
- Tellez-Plaza, M., Navas-Acien, A., Crainiceanu, C. M., & Guallar, E. (2008). Cadmium exposure and hypertension in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives*, 116(1), 51–56. <https://doi.org/10.1289/ehp.10764>
- Tolins, M., Ruchirawat, M., & Landrigan, P. (2014). The developmental neurotoxicity of arsenic: Cognitive and behavioral consequences of early life exposure. *Annals of Global Health*, 80(4), 303–314. <https://doi.org/10.1016/j.aogh.2014.09.005> Treatment, W., & Management, I.

W. W. (2013). *Biological Treatment Processes 3.1*. 1–46.

- Tripathi, B. D., Sikandar, M., & Shukla, S. C. (1991). Physico-chemical characterization of city sewage discharged into river Ganga at Varanasi, India. *Environment International*, *17*(5), 469–478. <https://doi.org/10.1016/0160>
- Trivedi, R. D., Patel, R. B., Vyas, D. D. S., & Patel, S. M. (2015). Performance Evaluation of Hybrid UASB Reactor for Treatment of Pharma Industry Wastewater. *International Journal for Scientific Research and Development*, <http://www.ijserd.com/articles/IJSRDV3I1425.pdf>
- Uter, W., Werfel, T., White, I. R., & Johansen, J. D. (2018). Contact allergy: A review of current problems from a clinical perspective. *International Journal of Environmental Research and Public Health*, *15*(6).
- Vahid, H., & Karyab, H. (2006). Performance Evaluation of Full Scale Uasb Reactor in. *Evaluation*, *3*(2), 79–84.
- Verma, A. K., & Kumar, S. (2013a). *Characterization of Sewage and Design of a Uasb Reactor for Its Treatment*. *V(V)*, 37–43.
- Verma, A. K., & Kumar, S. (2013b). *Characterization of Sewage and Design of a Uasb Reactor for Its Treatment*.
- Vicentin, R., Fdz-Polanco, F., & Fdz-Polanco, M. (2019). Energy integration in wastewater treatment plants by anaerobic digestion of urban waste: A process design and simulation study. *International Journal of Chemical Engineering*, *2019*. <https://doi.org/10.1155/2019/2621048>.
- Vivaldi, G. A., Camposeo, S., Lopriore, G., Romero-Trigueros, C., & Salcedo, F. P. (2019). Using saline reclaimed water on almond grown in Mediterranean conditions: Deficit irrigation strategies and salinity effects. *Water Science and Technology: WaterSupply*, *19*(5), 1413–1421.
- Wang, Z., Sun, Y., Yao, W., Ba, Q., & Wang, H. (2021). Effects of Cadmium Exposure on the Immune System and Immunoregulation. *Frontiers in Immunology*, *12*(July), 1–15. <https://doi.org/10.3389/fimmu.2021.695484>.
- Wijaya, I. M. W., & Soedjono, E. S. (2018). Physicochemical Characteristic of

- Municipal Wastewater in Tropical Area: Case Study of Surabaya City, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 135(1). <https://doi.org/10.1088/1755>.
- Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F., & Gebrekidan, H. (2017). Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa, Ethiopia. *International Journal of Food Contamination*, 4(1). <https://doi.org/10.1186/s40550-017-0053-y>
- Xii, P. N. (2011). *Raúl Rodríguez Gómez Licentiate Thesis in Chemical Engineering Department*.
- Yeshiwas, Y., & Tadele, E. (2017). Review on Heavy Metal Contamination in Vegetables Grown in Ethiopia and Its Economic Welfare Implications. *Journal of Biology, Agriculture and Healthcare*, 7(17), 31–44.
- Yetilmezsoy, K., & Sakar, S. (2008). Development of empirical models for performance evaluation of UASB reactors treating poultry manure wastewater under different operational conditions. *Journal of Hazardous Materials*, 153(1–2), 532–543. <https://doi.org/10.1016/j.jhazmat.2007.08.087>.
- Zalups, R. K. (2000). Evidence for basolateral uptake of cadmium in the kidneys of rats. *Toxicology and Applied Pharmacology*, 164(1), 15–23.
- Zhang, L., De Vrieze, J., Hendrickx, T. L. G., Wei, W., Temmink, H., Rijnaarts, H., & Zeeman, G. (2018a). Anaerobic treatment of raw domestic wastewater in a UASB-digester at 10 °C and microbial community dynamics. *Chemical Engineering Journal*, 334(June2017), 2088–2097.
- Zhang, L., De Vrieze, J., Hendrickx, T. L. G., Wei, W., Temmink, H., Rijnaarts, H., & Zeeman, G. (2018b). Anaerobic treatment of raw domestic wastewater in a UASB-digester at 10 °C and microbial community dynamics. In *Chemical Engineering Journal* (Vol. 334, pp. 2088–2097). <https://doi.org/10.1016/j.cej.2017.11.073>.
- Zhang, S. J., Liu, N. R., & Zhang, C. X. (2013). Study on the performance of modified UASB process treating sewage. *Advanced Materials Research*, 610–613, 2174–2178. <https://doi.org/10.4028/www.scientific.net/AMR.610>.
- Zhao, B. H., Yue, Z. B., Zhao, Q. B., Mu, Y., Yu, H. Q., Harada, H., & Li,

- Y. Y. (2008). Optimization of hydrogen production in a granule-based UASB reactor. *International Journal of Hydrogen Energy*, 33(10), 2454–2461. <https://doi.org/10.1016/j.ijhydene.2008.03.008>.
- Zheng, M. X., Wang, K. J., Zuo, J. E., Yan, Z., Fang, H., & Yu, J. W. (2012). Flow pattern analysis of a full-scale expanded granular sludge bed-type reactor under different organic loading rates. *Bioresource Technology*, 107, 33–40. <https://doi.org/10.1016/j.biortech.2011.11.102>.
- Zheng, M., Yan, Z., Zuo, J., & Wang, K. (2014). Concept and application of anaerobic suspended granular sludge bed (SGSB) reactor for wastewater treatment. *Frontiers of Environmental Science and Engineering*, 8(5), 797–804. <https://doi.org/10.1007/s11783-013-0597-x>.
- Zia, M. H., Watts, M. J., Niaz, A., Middleton, D. R. S., & Kim, A. W. (2017). Health risk assessment of potentially harmful elements and dietary minerals from vegetables irrigated with untreated wastewater, Pakistan. *Environmental Geochemistry and Health*, 39(4), 707–728. <https://doi.org/10.1007/s10653>.
- Zwain, H. M., Barghash, H., Vakili, M., Majdi, H. S., & Dahlan, I. (2022). Modeling and optimization of process parametric interaction during high-rate anaerobic digestion of recycled paper mill wastewater using the response surface methodology. *Water Reuse*, 12(1), 78–91. <https://doi.org/10.2166/wrd.2022.088>.

APPENDIXES

Appendix A – Operational Parameters distribution during rainy seasons

Temperature, PH, OLR and HRT distribution by CCD-RSM for experimental data collection during the rainy seasons.

No	Temperature (°C)	pH	OLR (kg COD/m ³ .d)	HRT (hrs.)
1	30	6.30	1	1.92
2	30	6.30	3	4.64
3	30	7.80	3	5.84
4	30	7.80	3	6.72
5	30	6.3	3	4.64
6	30	7.80	3	4.25
7	30	6.30	1	5.52
8	30	7.05	2	8.17
9	15	8.55	2	7.08
10	15	7.05	2	5.53
11	15	7.05	4	4.45
12	15	7.05	2	5.46
13	15	7.05	2	9.57
14	15	7.05	2	6.22
15	15	7.05	2	6.29
16	15	7.05	2	8.53
17	15	7.05	2	8.24
18	15	8.55	2	7.41
19	15	7.05	0	0.00

20	15	7.05	2	0.00
21	0	6.30	3	6.40
22	0	6.3	3	7.55
23	0	6.30	1	17.30
24	0	6.30	1	12.60
25	0	7.80	3	5.36
26	0	7.80	3	4.53
27	0	7.80	1	23.30
28	0	7.80	1	19.50
29	-5	7.05	2	9.58
30	45	7.05	2	9.44

Appendix B- Inlet and outlet COD data collected during the rainy seasons

Inlet and outlet COD collected for thirty experimental data during the rainy seasons

No	Temperature (°C)	pH	OLR (kg COD/m ³ .d)	HRT (hrs.)	Inlet COD (mg/l)	%COD removal
1	30	6.30	1	1.92	80±255	87.1±4.40
2	30	6.30	3	4.64	580±0.00	82.8±6.00
3	30	7.80	3	5.84	730±119.5	93.6±7.192
4	30	7.80	3	6.72	840±119.5	78.1±7.192
5	30	6.3	3	4.64	580±0.00	94.8±6.00
6	30	7.80	3	4.25	550±119.5	93.1±7.192
7	30	6.30	1	5.52	590±255	78.3±4.40
8	30	7.05	2	8.17	690±0.00	84.2±0.00
9	15	8.55	2	7.08	490±0.00	74.3±0.00
10	15	7.05	2	5.53	790±0.00	86.1±0.00
11	15	7.05	4	4.45	480±0.00	75.6±0.00
12	15	7.05	2	5.46	830±101.7	81.1±9.792
13	15	7.05	2	9.57	530±101.7	73.2±9.792
14	15	7.05	2	6.22	540±101.7	65.6±9.792
15	15	7.05	2	6.29	740±101.7	74.9±9.792
16	15	7.05	2	8.53	700±101.7	83.4±9.792
17	15	7.05	2	8.24	640±101.7	68.8±9.792
18	15	8.55	2	7.41	800±0.00	64.4±0.00
19	15	7.05	0	0.00	990±0.00	67.4±0.00
20	15	7.05	2	0.00	730±101.7	58.9±9.792

21	0	6.30	3	6.40	540±80.0	54.8±2.050
22	0	6.3	3	7.55	700±80.0	58.9±2.050
23	0	6.30	1	17.30	610±185	62.9±1.900
24	0	6.30	1	12.60	980±185	66.7±1.900
25	0	7.80	3	5.36	830±0.00	72.9±43.700
26	0	7.80	3	4.53	830±0.00	-14.5±43.700
27	0	7.80	1	23.30	810±20.0	56.7±15.200
28	0	7.80	1	19.50	850±20.0	87.1±15.200
29	-5	7.05	2	9.58	980±0.00	82.8±0.000
30	45	7.05	2	9.44	720±0.00	93.6±0.000

Appendix C –Percentage Removal of COD, TSS and VSS during the rainy seasons

Experimental data and predicted responses for COD, TSS and VSS during rainy seasons using coding

R u n O r d e r	Coded Value				% Removal					
	A	B	C	D	COD		TSS		VSS	
					Observed	Predicted	Observed	Predicted	Observed	Predicted
					1	0	1	6.30	12	54.81
2	30	3	6.30	4.64	90.36	92.92	80.29	83.91	65.71	66.65
3	15	2	7.05	8.08	80.35	76.89	78.35	75.27	61.23	55.74
4	15	0	7.05	0.00	0.00	1.90	0.00	1.98	0.00	1.26
5	30	3	7.80	6.43	78.09	78.22	80.96	67.71	66.17	59.98
6	15	2	7.05	7.41	68.75	76.08	73.94	77.87	60.00	58.93
7	15	2	7.05	8.53	74.86	77.31	75.00	73.66	40.00	53.82
8	30	1	7.80	5.51	93.56	88.99	60.57	58.14	50.43	53.57
9	0	1	7.80	12.0	72.89	70.01	60.89	59.15	50.11	50.50
10	45	2	7.05	9.44	56.57	55.20	60.96	64.23	50.77	54.28
11	0	3	6.30	6.24	64.38	66.44	85.35	87.67	80.00	81.93
12	0	3	6.30	7.55	75.87	71.13	66.78	65.10	58.65	50.36
13	0	3	7.80	5.36	58.86	61.02	82.57	81.62	75.35	72.43
14	15	2	8.55	7.05	87.63	91.26	50.35	58.62	70.00	74.64
15	30	3	6.30	4.39	94.83	93.71	90.67	87.37	76.63	70.98
16	0	1	6.30	16.0	58.90	62.42	75.35	73.49	55.00	55.43
17	15	2	7.05	6.29	65.56	74.26	75.87	82.80	60.53	65.20
18	0	1	7.80	4.53	20.89	18.95	28.75	25.53	15.25	11.09
19	30	1	7.80	8.17	84.20	88.07	83.49	80.63	93.26	88.00
20	15	2	7.05	9.57	85.78	77.92	65.35	70.37	59.59	50.10

21	15	2	5.55	8.24	83.43	83.18	80.35	76.72	83.43	87.27
22	15	2	7.05	6.22	73.21	74.13	85.69	83.13	60.30	65.63
23	30	1	6.30	2.00	87.05	80.70	90.69	87.72	92.86	91.89
24	15	2	7.05	5.53	74.29	72.69	92.35	86.54	70.00	70.11
25	30	1	6.3	4.5	75.35	81.87	84.35	87.36	90.25	88.58
26	-15	2	7.05	9.58	25.00	29.75	22.89	24.26	-15.00	-10.03
27	30	3	7.8	4.25	85.69	84.00	75.49	82.49	72.00	72.16
28	15	2	7.05	5.46	75.63	72.53	85.35	86.90	70.00	70.59
29	15	4	7.05	4.45	80.29	81.77	98.35	101.02	83.00	90.22
30	0	3	7.8	5.36	66.73	61.02	85.35	81.62	75.35	72.43

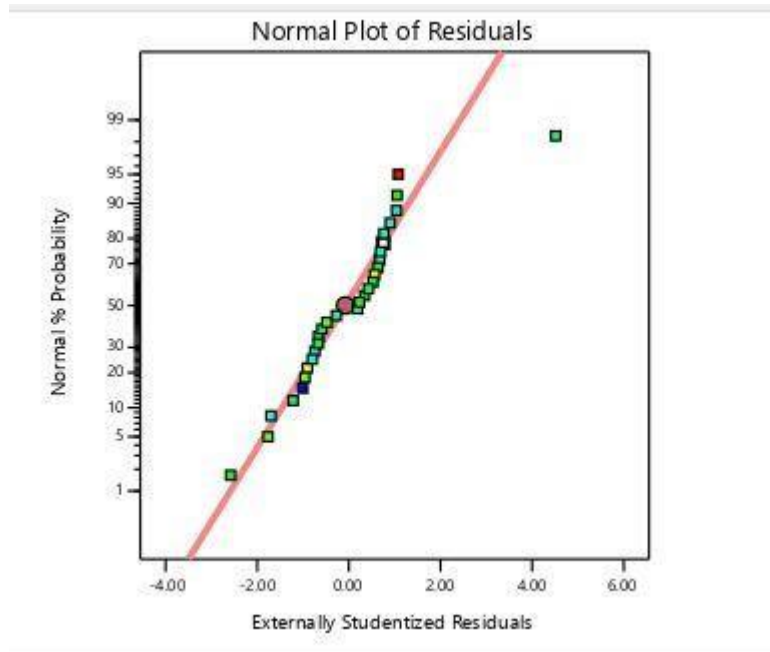
i. Fit Statistics for COD during the rainy season

Std.Dev.	6.22	R ²	0.9575
Mean	71.39	Adjusted R ²	0.9178
CV%	8.71	Predicted R ²	0.6728
		Adeq. Precision	23.5173

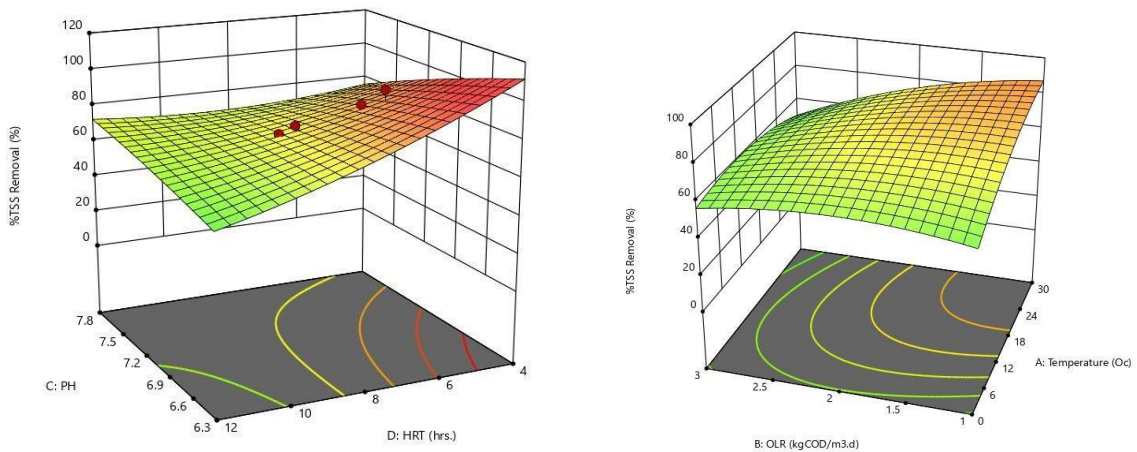
ii. Triplication during the Rainy seasons

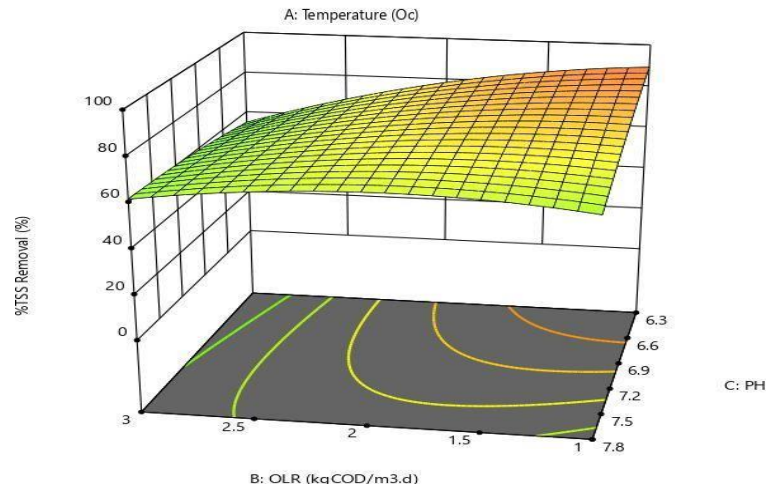
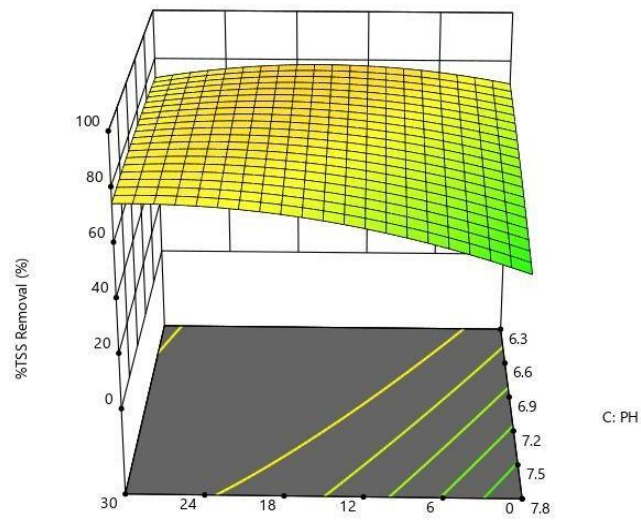
Expt. number	Optimized Operational Parameters (Temperature =24.51°C, OLR=2.99kg COD/m ³ .d, PH=6.3, HRT=4hrs.)		
	COD Removal	TSS Removal	VSS Removal
1	92.5%	98.91%	94.49
2	93.8%	98.89%	94.42
3	91.7%	99.38%	94.49
Avg.	92.70%	99.06%	94.5%

iii. Plot of studentized Residual during the rainy seasons



iv. 3D graph for %TSS removal during the rainy season pH vs. Temperature; HRT vs. Temperature; OLR vs. pH; HRT vs. pH; OLR vs. Temperature



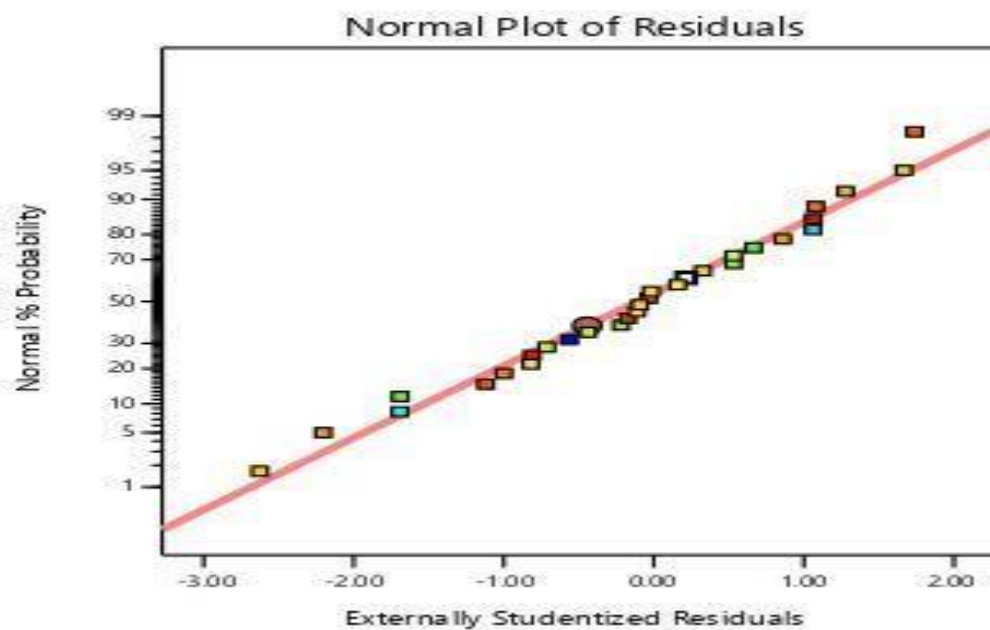


Appendix D- Percentage removal of VSS during the rainy season

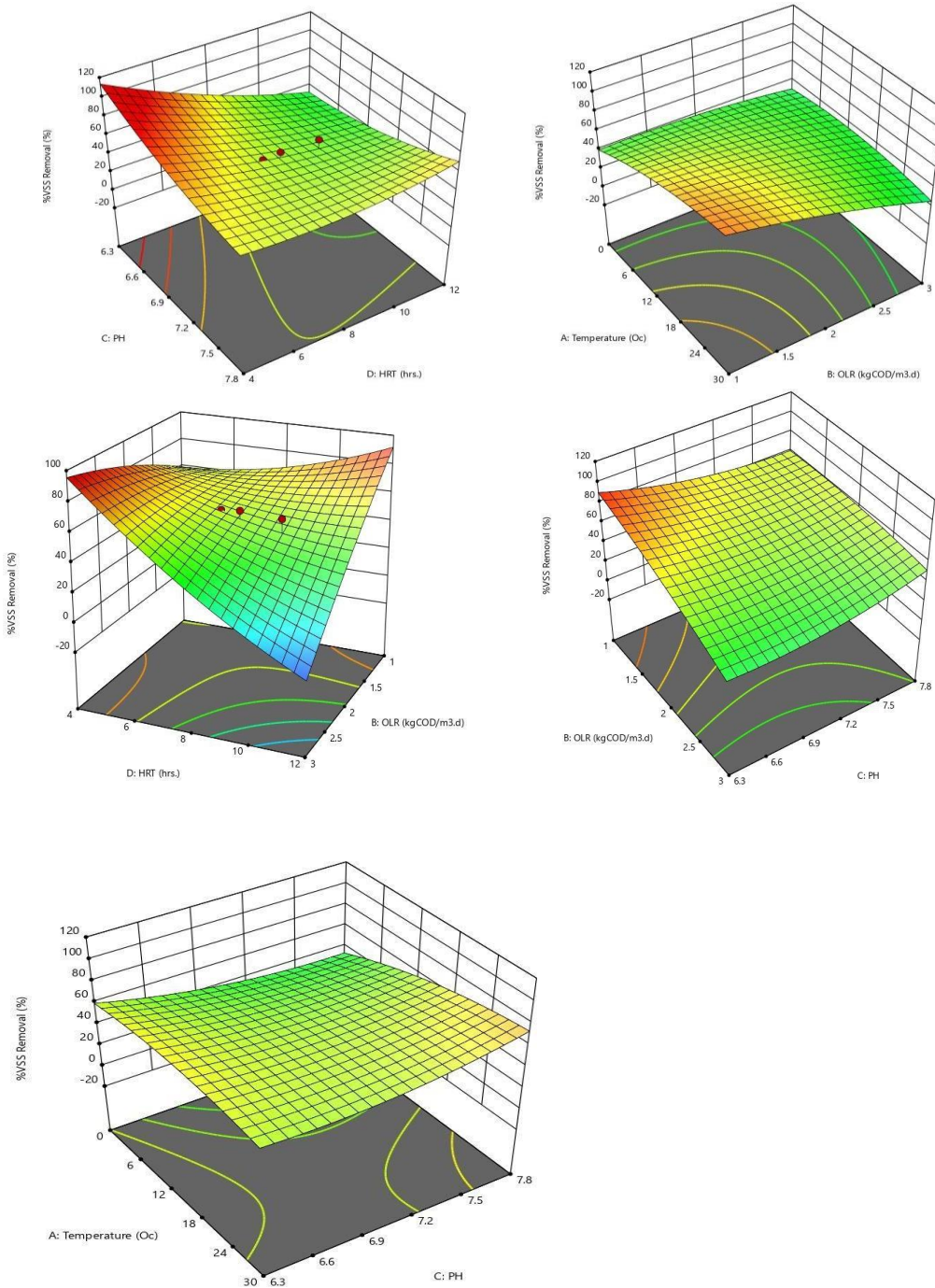
i. Fit Statistics for VSS during the rainy season

Std.Dev.	6.92	R ²	0.9595
Mean	61.03	Adjusted R ²	0.9218
CV%	11.34	Predicted R ²	0.7447
Adeq. Precision	20.8334		

ii. Plot of Studentized residual for VSS



**iii. 3D graph for %VSS removal during the rainy seasons,
pH vs. Temperature; HRT vs. Temperature; OLR vs. pH;
HRT vs. pH; OLR vs. Temperature**



Appendix E-Percentage Removal of COD during the dry season

i. Fit Statistics

Std. Dev.	2.08	R²	0.9977
Mean	62.99	Adjusted R²	0.9956
C.V. %	3.31	Predicted R²	0.9863
		Adeq Precision	68.8956

- ✓ The **Predicted R²** of 0.9863 is in reasonable agreement with the **Adjusted R²** of 0.9956; i.e. the difference is less than 0.2.
- ✓ **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 68.896 indicates an adequate signal. This model can be used to navigate the design space.

ii. Experimental data and predicted responses for COD during dry season

Run Order	Coded Value				% Removal	
	A	B	C	D	COD	
					Observed	Predicted
1	0	1	6.30	12	54.81	49.46
2	30	3	6.30	4.64	90.36	92.92
3	15	2	7.05	8.08	80.35	76.89
4	15	0	7.05	0.00	0.00	1.90
5	30	3	7.80	6.43	78.09	78.22
6	15	2	7.05	7.41	68.75	76.08
7	15	2	7.05	8.53	74.86	77.31
8	30	1	7.80	5.51	93.56	88.99
9	0	1	7.80	12.0	72.89	70.01
10	45	2	7.05	9.44	56.57	55.20
11	0	3	6.30	6.24	64.38	66.44
12	0	3	6.30	7.55	75.87	71.13
13	0	3	7.80	5.36	58.86	61.02
14	15	2	8.55	7.05	87.63	91.26
15	30	3	6.30	4.39	94.83	93.71
16	0	1	6.30	16.0	58.90	62.42
17	15	2	7.05	6.29	65.56	74.26
18	0	1	7.80	4.53	20.89	18.95
19	30	1	7.80	8.17	84.20	88.07
20	15	2	7.05	9.57	85.78	77.92

21	15	2	5.55	8.24	83.43	83.18
22	15	2	7.05	6.22	73.21	74.13
23	30	1	6.30	2.00	87.05	80.70
24	15	2	7.05	5.53	74.29	72.69
25	30	1	6.3	4.5	75.35	81.87
26	-15	2	7.05	9.58	25.00	29.75
27	30	3	7.8	4.25	85.69	84.00
28	15	2	7.05	5.46	75.63	72.53
29	15	4	7.05	4.45	80.29	81.77
30	0	3	7.8	5.36	66.73	61.02

iii. Fit Summary Response on COD Removal during the dry season

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0004	0.0048	0.4719	0.2704	
Quadratic	< 0.0001	0.0513	0.9956	0.9863	Suggested

iv. Model Summary Statistics for COD during dry season

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	22.73	0.5447	0.4719	0.2704	20694.29	
Quadratic	2.08	0.9977	0.9956	0.9863	389.85	Suggested

v. ANOVA for Quadratic model Response on COD Removal during the dry season

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	28297.75	14	2021.27	465.21	< 0.0001	significant
A-Temperature	3197.66	1	3197.66	735.97	< 0.0001	
B-OLR	843.04	1	843.04	194.03	< 0.0001	
C-PH	494.82	1	494.82	113.89	< 0.0001	
D-HRT	4670.81	1	4670.81	1075.03	< 0.0001	
AB	1267.88	1	1267.88	291.81	< 0.0001	
AC	17.39	1	17.39	4.00	0.0639	
AD	403.63	1	403.63	92.90	< 0.0001	
BC	71.41	1	71.41	16.44	0.0010	
BD	2310.58	1	2310.58	531.80	< 0.0001	
CD	485.21	1	485.21	111.67	< 0.0001	
A ²	4796.78	1	4796.78	1104.02	< 0.0001	
B ²	933.28	1	933.28	214.80	< 0.0001	
C ²	602.02	1	602.02	138.56	< 0.0001	
D ²	3478.37	1	3478.37	800.58	< 0.0001	
Residual	65.17	15	4.34			
Lack of Fit	65.15	14	4.65	232.69	0.0513	not significant
Pure Error	0.0200	1	0.0200			
Cor Total	28362.92	29				

Factor coding is **coded**.

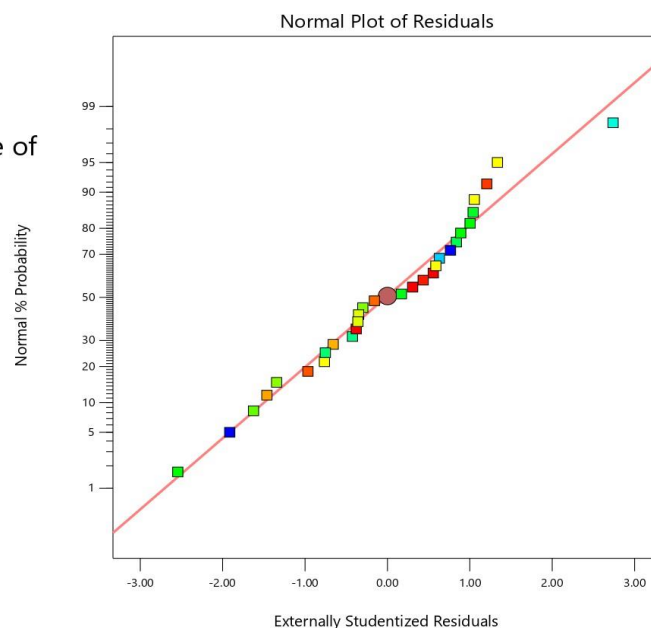
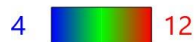
Sum of squares is **Type III - Partial**

- The **Model F-value** of 465.21 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.
- **P-values** less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AB, AD, BC, BD, CD, A², B², C², D² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
- The **Lack of Fit F-value** of 232.69 implies there is a 5.13% chance that a Lack of Fit F- value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.
- Focus on the model maximizing the **Adjusted R²** and the **Predicted R²**.

vi. Normal Plot of Residuals for COD removal during dry season

COD Removal

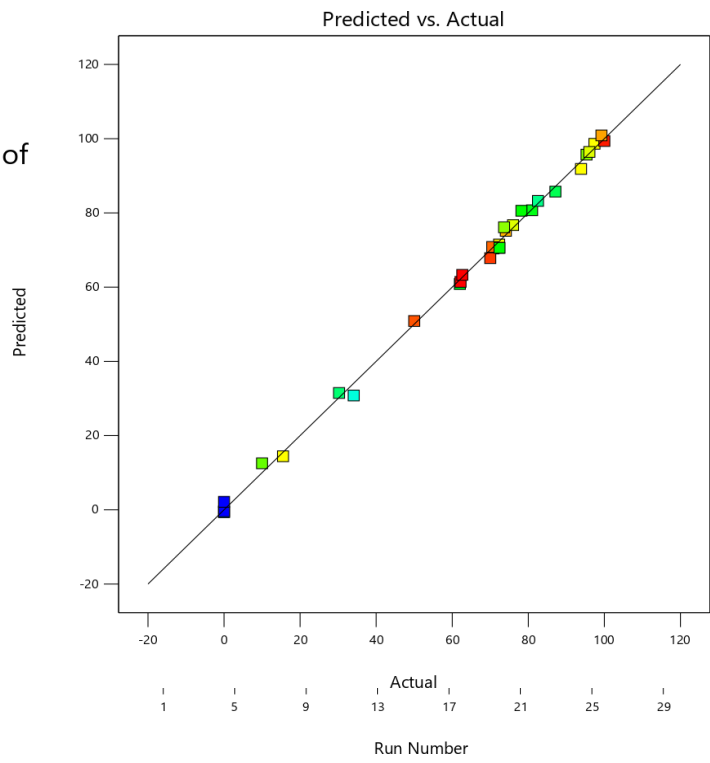
Color points by value of HRT:



vii. Predicted vs. actual data for COD removal during the dry season

COD Removal

Color points by value of HRT:



Appendix F- Percentage Removal of TSS during the dry season

i. Actual vs. predicted TSS removal during the dry season

Run Order	Coded Value				TSS	
	A	B	C	D	Observed	Predicated
1	0	1	6.30	12	65.35	68.82
2	30	3	6.30	4.64	80.29	83.91
3	15	2	7.05	8.08	78.35	75.27
4	15	0	7.05	0.00	0.00	1.98
5	30	3	7.80	6.43	80.96	67.71
6	15	2	7.05	7.41	73.94	77.87
7	15	2	7.05	8.53	75.00	73.66
8	30	1	7.80	5.51	60.57	58.14
9	0	1	7.80	12.0	60.89	59.15
10	45	2	7.05	9.44	60.96	64.23
11	0	3	6.30	6.24	85.35	87.67
12	0	3	6.30	7.55	66.78	65.10
13	0	3	7.80	5.36	82.57	81.62
14	15	2	8.55	7.05	50.35	58.62
15	30	3	6.30	4.39	90.67	87.37
16	0	1	6.30	16.0	75.35	73.49
17	15	2	7.05	6.29	75.87	82.80
18	0	1	7.80	4.53	28.75	25.53
19	30	1	7.80	8.17	83.49	80.63
20	15	2	7.05	9.57	65.35	70.37

21	15	2	5.55	8.24	80.35	76.72
22	15	2	7.05	6.22	85.69	83.13
23	30	1	6.30	2.00	90.69	87.72
24	15	2	7.05	5.53	92.35	86.54
25	30	1	6.3	4.5	84.35	87.36
26	-15	2	7.05	9.58	22.89	24.26
27	30	3	7.8	4.25	75.49	82.49
28	15	2	7.05	5.46	85.35	86.90
29	15	4	7.05	4.45	98.35	101.02
30	0	3	7.8	5.36	85.35	81.62

ii. Triplication during the dry season

Expt. number	Optimized Operational Parameters (Temperature =23.0°C, OLR=2.23kg COD/m ³ .d, PH=7.5, HRT=11.5hrs.)		
	COD Removal	TSS Removal	VSS Removal
1	84.22%	99.78	100.02%
2	83.92%	99.99	99.99%
3	84.02%	299.77	100.00%
Avg.	84.1%	99.9%	100.00%

iii. Fit Statistics for TSS during the dry season

Std. Dev.	4.45	R ²	0.9844
Mean	82.65	Adjusted R ²	0.9699
C.V. %	5.39	Predicted R ²	0.8151
		Adeq Precision	34.0104

- The **Predicted R²** of 0.8151 is in reasonable agreement with the **Adjusted R²** of 0.9699; i.e. the difference is less than 0.2.
- **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 34.010 indicates an adequate signal. This model can be used to navigate the design space.

iv. Fit Summary Response for TSS Removal during the dry season

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0005	0.0241	0.4628	0.2713	
Quadratic	< 0.0001	0.0996	0.9699	0.8151	Suggested

v. Model Summary Statistics

Focus on the model maximizing the Adjusted R² and the Predicted R².

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	18.81	0.5369	0.4628	0.2713	13910.50	
Quadratic	4.45	0.9844	0.9699	0.8151	3530.22	Suggested

vi. Lack of Fit Tests for TSS during the dry season

The selected model should have insignificant lack-of-fit.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	8840.45	24	368.35	1069.39	0.0241	
Quadratic	296.94	14	21.21	61.58	0.0996	Suggested

vii. ANOVA for Quadratic model Response TSS Removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	18791.80	14	1342.27	67.73	< 0.0001	significant
A-Temperature	298.87	1	298.87	15.08	0.0015	
B-OLR	12.71	1	12.71	0.6412	0.4358	
C-PH	800.56	1	800.56	40.39	< 0.0001	
D-HRT	6700.40	1	6700.40	338.08	< 0.0001	
AB	111.00	1	111.00	5.60	0.0318	
AC	479.99	1	479.99	24.22	0.0002	
AD	430.46	1	430.46	21.72	0.0003	
BC	110.52	1	110.52	5.58	0.0322	
BD	209.94	1	209.94	10.59	0.0053	
CD	314.22	1	314.22	15.85	0.0012	
A ²	519.21	1	519.21	26.20	0.0001	
B ²	45.44	1	45.44	2.29	0.1508	
C ²	7.14	1	7.14	0.3602	0.5573	
D ²	6332.80	1	6332.80	319.53	< 0.0001	
Residual	297.29	15	19.82			
Lack of Fit	296.94	14	21.21	61.58	0.0996	not significant
Pure Error	0.3444	1	0.3444			
Cor Total	19089.09	29				
Cor Total	19089.	29				

	09	
--	----	--

Factor coding is **coded**.

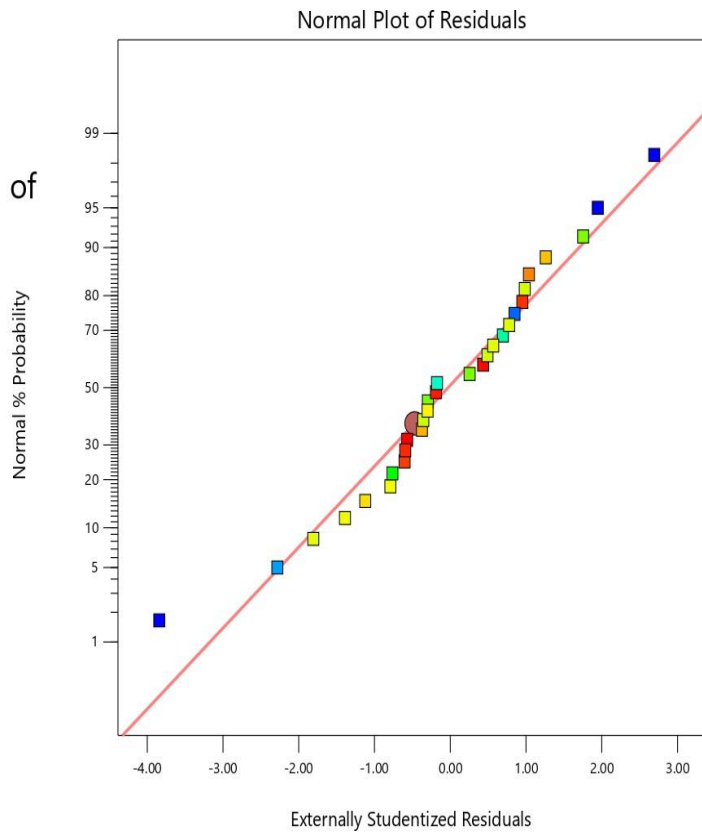
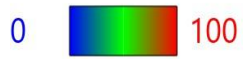
Sum of squares is **Type III - Partial**

- The **Model F-value** of 67.73 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.
- **P-values** less than 0.0500 indicate model terms are significant. In this case A, C, D, AB, AC, AD, BC, BD, CD, A², D² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
- The **Lack of Fit F-value** of 61.58 implies there is a 9.96% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.

viii. Studentized residuals of TSS for dry season

TSS Removal

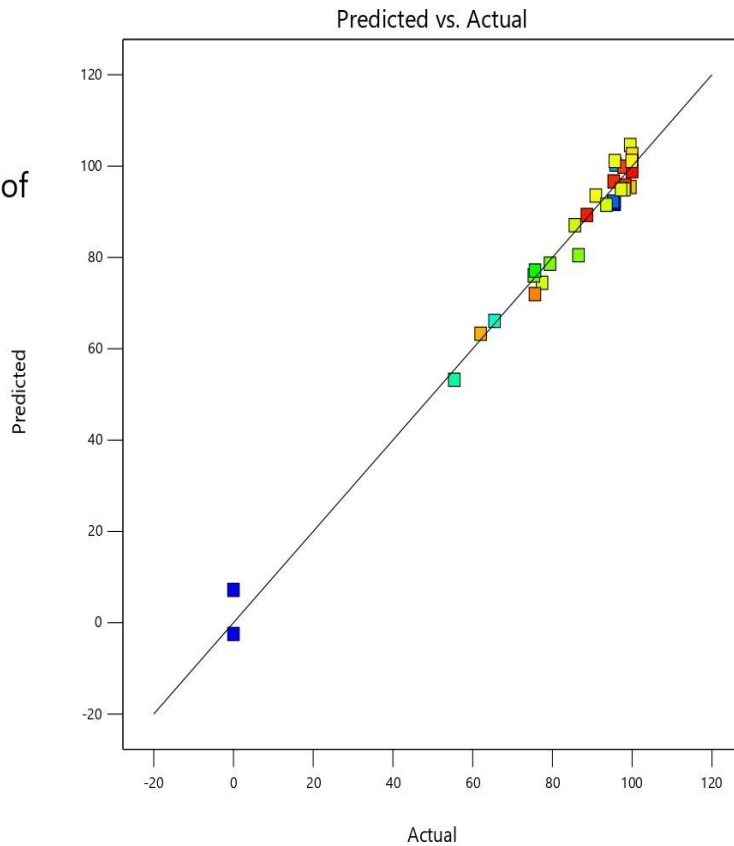
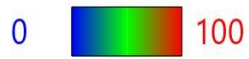
Color points by value of
COD Removal:



ix. Predicted vs. Actual removal for TSS during the dry season

TSS Removal

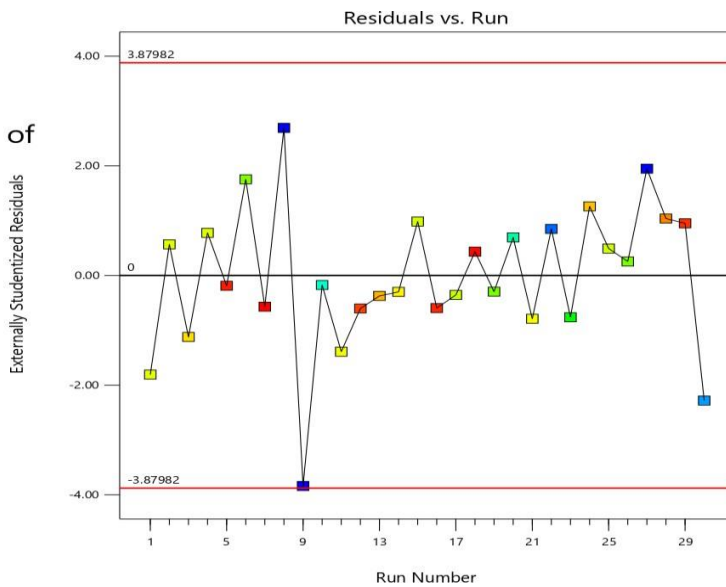
Color points by value of
COD Removal:



Residuals vs. Run for TSS during the dry season

TSS Removal

Color points by value of
COD Removal:



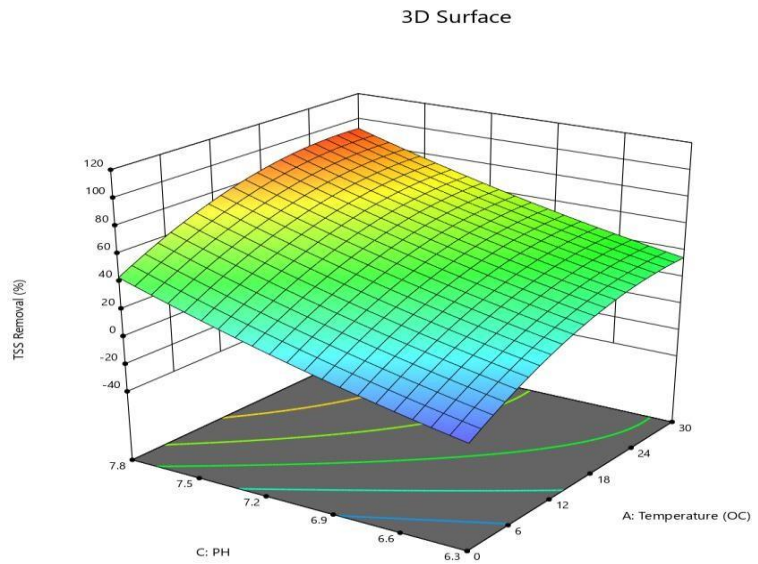
x. 3D graph for %TSS removal pH vs. Temperature; HRT vs. Temperature; OLR vs. pH; HRT vs. pH; OLR vs. Temperature

Factor Coding: Actual

TSS Removal (%)
-15.74  99.66

X1 = A
X2 = C

Actual Factors
B = 2
D = 32

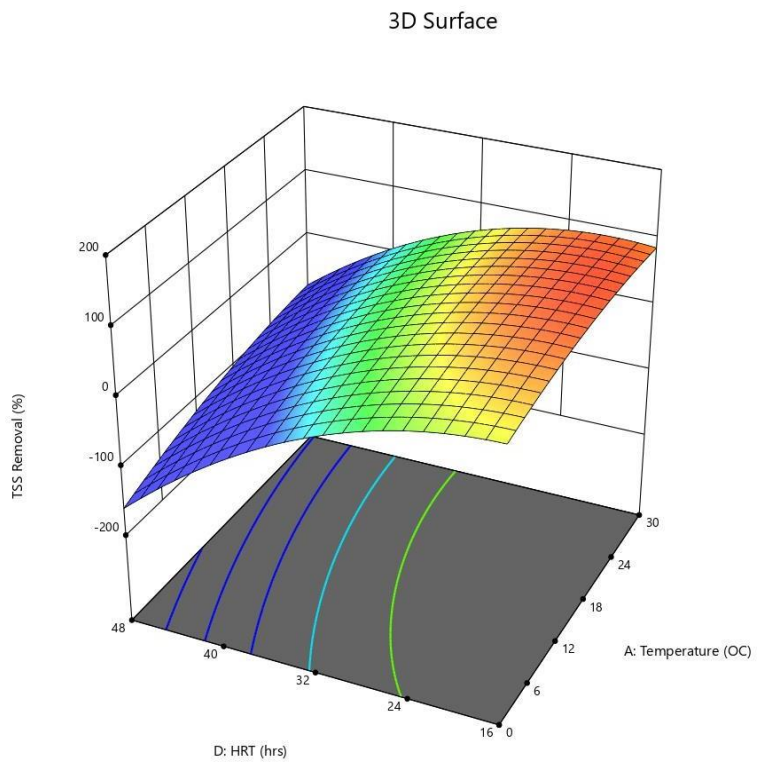


Factor Coding: Actual

TSS Removal (%)
-15.74  99.66

X1 = A
X2 = D

Actual Factors
B = 2
C = 7.05

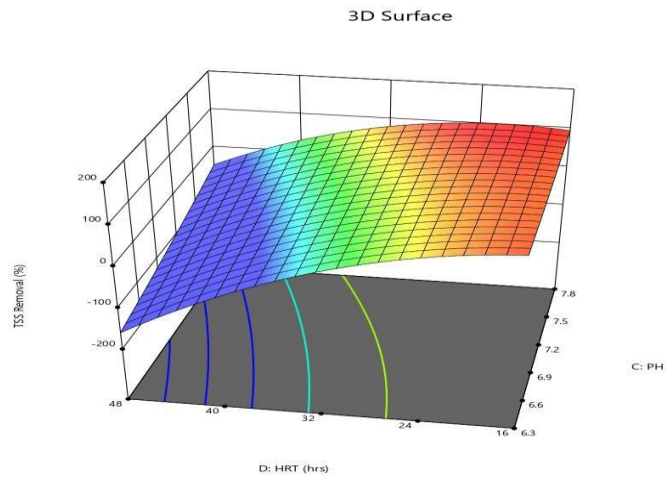


Factor Coding: Actual

TSS Removal (%)
-15.74  99.66

X1 = C
X2 = D

Actual Factors
A = 15
B = 2

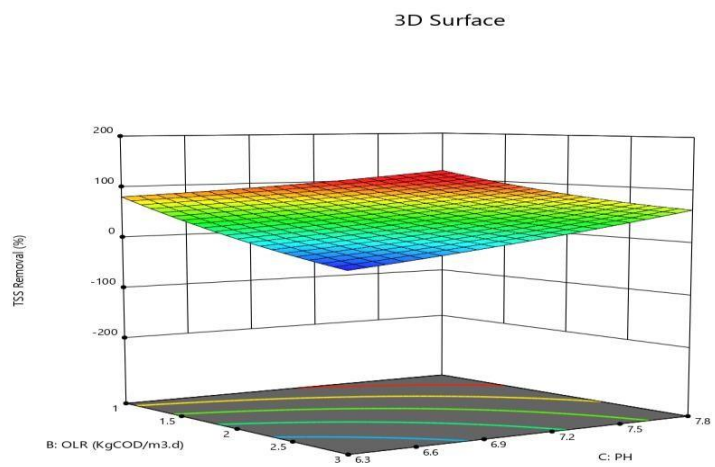


Factor Coding: Actual

TSS Removal (%)
-15.74  99.66

X1 = B
X2 = C

Actual Factors
A = 15
D = 32

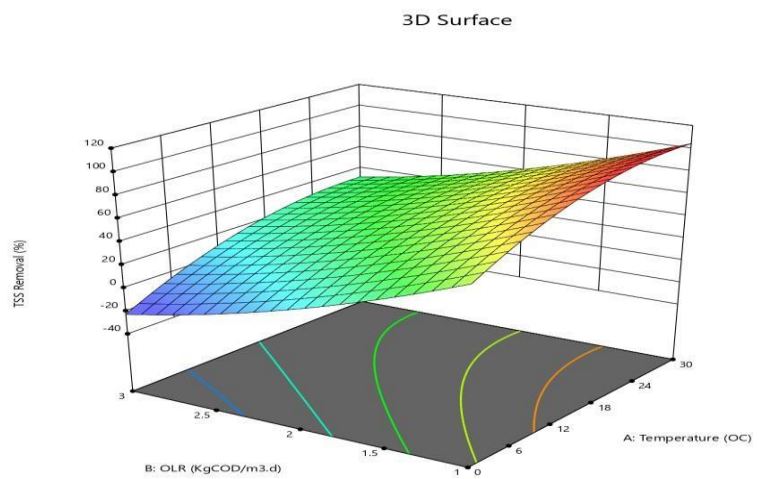


Factor Coding: Actual

TSS Removal (%)
-15.74  99.66

X1 = A
X2 = B

Actual Factors
C = 7.05
D = 32



Appendix G- Percentage Removal of VSS during the dry season

i. Actual vs. Predicted %removal for VSS during the dry season

Run Order	Coded Value				VSS	
	A	B	C	D	Observed	Predicated
	1	0	1	6.30	12	60.00
2	30	3	6.30	4.64	65.71	66.65
3	15	2	7.05	8.08	61.23	55.74
4	15	0	7.05	0.00	0.00	1.26
5	30	3	7.80	6.43	66.17	59.98
6	15	2	7.05	7.41	60.00	58.93
7	15	2	7.05	8.53	40.00	53.82
8	30	1	7.80	5.51	50.43	53.57
9	0	1	7.80	12.0	50.11	50.50
10	45	2	7.05	9.44	50.77	54.28
11	0	3	6.30	6.24	80.00	81.93
12	0	3	6.30	7.55	58.65	50.36
13	0	3	7.80	5.36	75.35	72.43
14	15	2	8.55	7.05	70.00	74.64
15	30	3	6.30	4.39	76.63	70.98
16	0	1	6.30	16.0	55.00	55.43
17	15	2	7.05	6.29	60.53	65.20
18	0	1	7.80	4.53	15.25	11.09
19	30	1	7.80	8.17	93.26	88.00
20	15	2	7.05	9.57	59.59	50.10

21	15	2	5.55	8.24	83.43	87.27
22	15	2	7.05	6.22	60.30	65.63
23	30	1	6.30	2.00	92.86	91.89
24	15	2	7.05	5.53	70.00	70.11
25	30	1	6.3	4.5	90.25	88.58
26	-15	2	7.05	9.58	-15.00	-10.03
27	30	3	7.8	4.25	72.00	72.16
28	15	2	7.05	5.46	70.00	70.59
29	15	4	7.05	4.45	83.00	90.22
30	0	3	7.8	5.36	75.35	72.43

ii. Fit Statistics

Std. Dev.	20.11	R ²	0.7800
Mean	79.92	Adjusted R ²	0.5747
C.V. %	25.16	Predicted R ²	-0.9012
		Adeq Precision	9.1495

- A negative **Predicted R²** implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.
- **Adeq. Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 9.149 indicates an adequate signal. This model can be used to navigate the design space.

iii. Fit Summary Response VSS Removal during the dry season

Source	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0290	0.0225	0.2348	-0.0043	
Quadratic	0.0026	0.0296	0.5747	-0.9012	Suggested

- M Case(s) with leverage of 1.0000: PRESS statistic not defined.
- Focus on the model maximizing the **Adjusted R²** and the **Predicted R²**.

iv. Model Summary Statistics

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	26.97	0.3404	0.2348	-0.0043	27688.07	
Quadratic	20.11	0.7800	0.5747	-0.9012	52414.08	Suggested

v. Lack of Fit Tests

- The selected model should have insignificant lack-of-fit.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	18185.37	24	757.72	1229.97	0.0225	
Quadratic	6063.85	14	433.13	703.08	0.0296	Suggested

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	21505.13	14	1536.08	3.80	0.0074	significant
A-Temperature	908.38	1	908.38	2.25	0.1546	
B-OLR	537.81	1	537.81	1.33	0.2668	
C-PH	1617.91	1	1617.91	4.00	0.0639	
D-HRT	2605.05	1	2605.05	6.44	0.0227	
AB	17.69	1	17.69	0.0438	0.8371	
AC	4.16	1	4.16	0.0103	0.9205	
AD	595.71	1	595.71	1.47	0.2436	
BC	804.47	1	804.47	1.99	0.1788	
BD	1250.82	1	1250.82	3.09	0.0990	
CD	2160.42	1	2160.42	5.34	0.0354	
A ²	5155.38	1	5155.38	12.75	0.0028	
B ²	1005.80	1	1005.80	2.49	0.1356	
C ²	280.81	1	280.81	0.6946	0.4177	
D ²	6335.06	1	6335.06	15.67	0.0013	
Residual	6064.46	15	404.30			
Lack of Fit	6063.85	14	433.13	703.08	0.0296	significant
Pure Error	0.6160	1	0.6160			
Cor Total	27569.59	29				

vi. ANOVA for Quadratic model Response VSS Removal

Factor coding is **coded**.

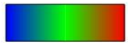
Sum of squares is **Type III - Partial**

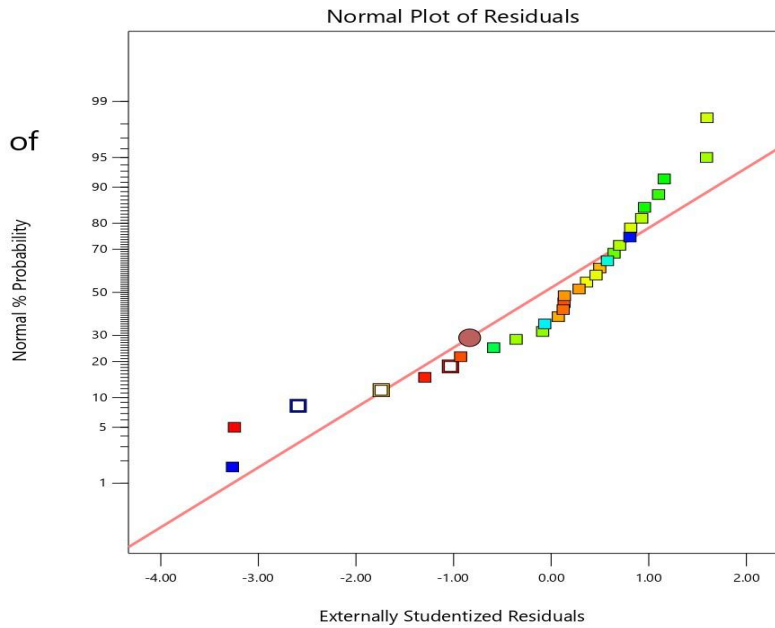
- The **Model F-value** of 3.80 implies the model is significant. There is only a 0.74% chance that an F-value this large could occur due to noise.
- **P-values** less than 0.0500 indicate model terms are significant. In this case D, CD, A², D² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
- The **Lack of Fit F-value** of 703.08 implies the Lack of Fit is significant. There is only a 2.96% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.

vii. Studentized Residual for VSS removal during the dry season

VSS Removal

Color points by value of COD Removal:

-1.52  92.57

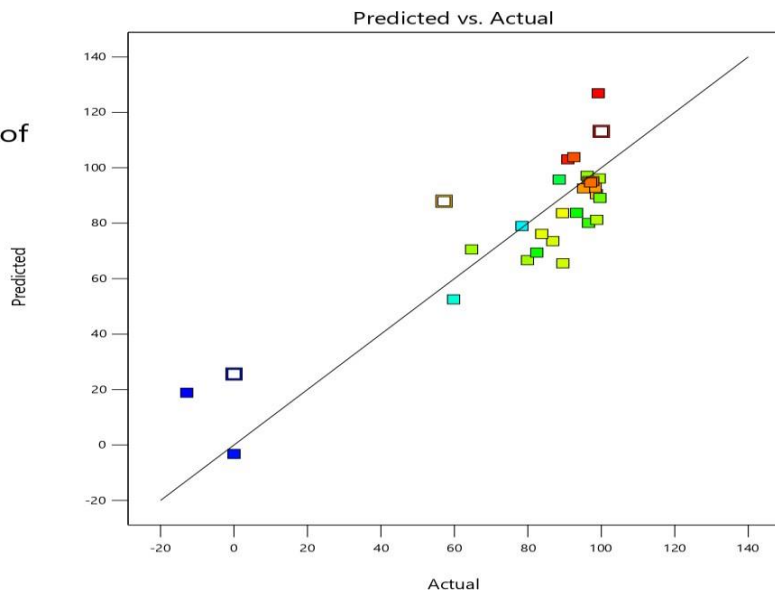


viii. Predicted Vs. Actual for VSS removal during the dry season

VSS Removal

Color points by value of COD Removal:

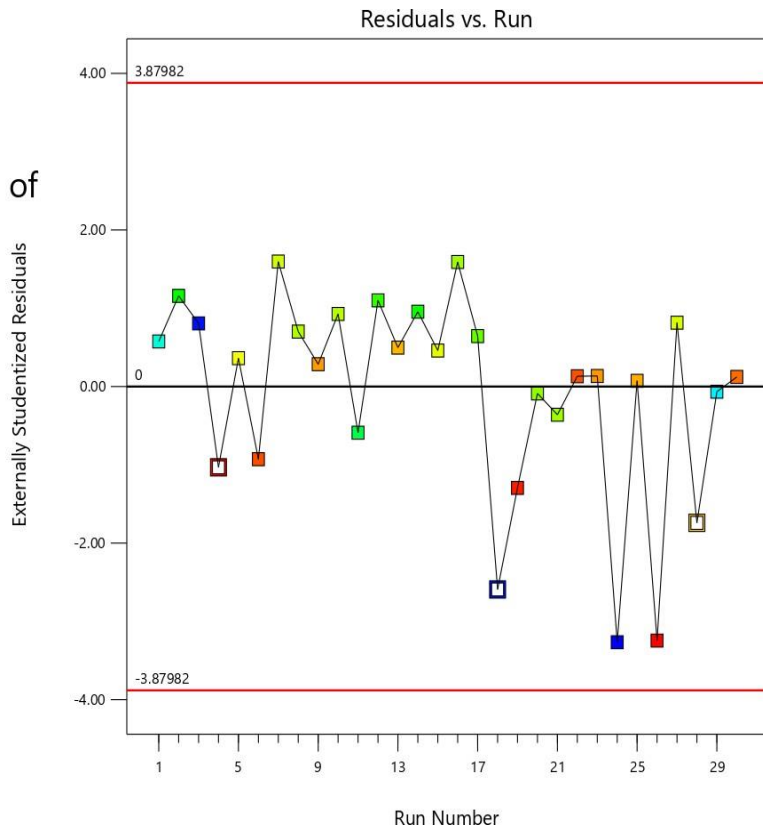
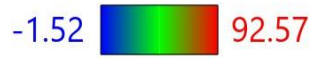
-1.52  92.57



ix. Residuals vs. Run for VSS removal during the dry season

VSS Removal

Color points by value of
COD Removal:



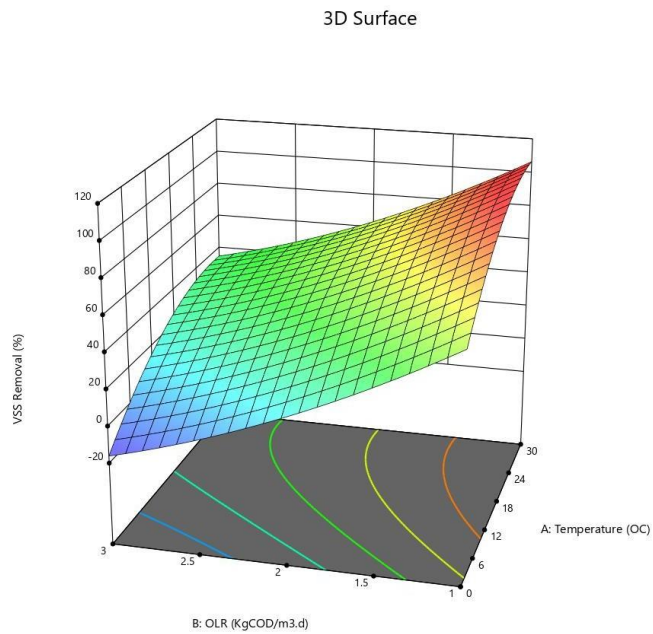
x. 3D graph for %VSS removal pH vs. Temperature; HRT vs. Temperature; OLR vs. pH; HRT vs. pH; OLR vs. Temperature

Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = A
X2 = B

Actual Factors
C = 7.05
D = 32

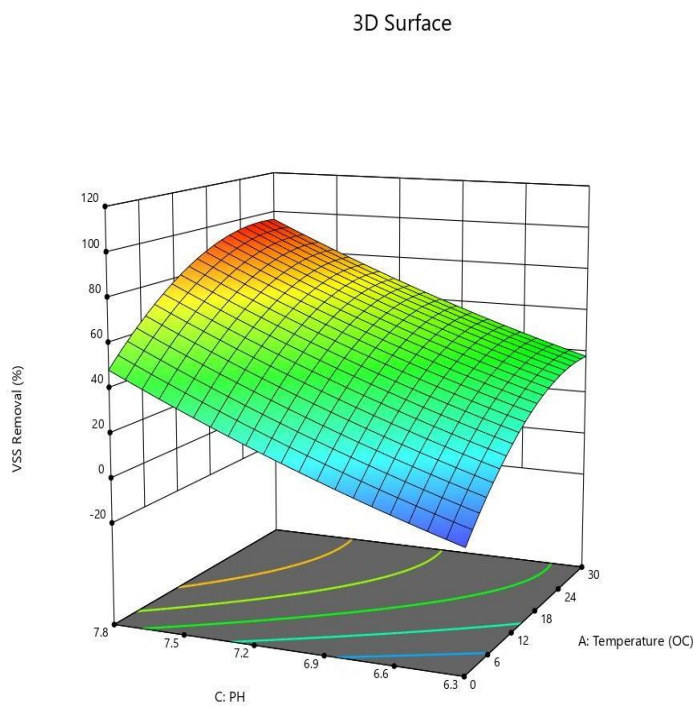


Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = A
X2 = C

Actual Factors
B = 2
D = 32



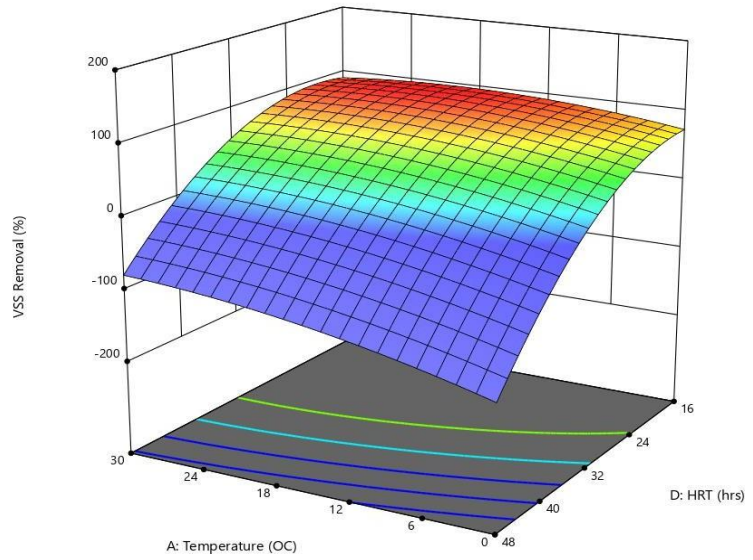
Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = A
X2 = D

Actual Factors
B = 2
C = 7.05

3D Surface



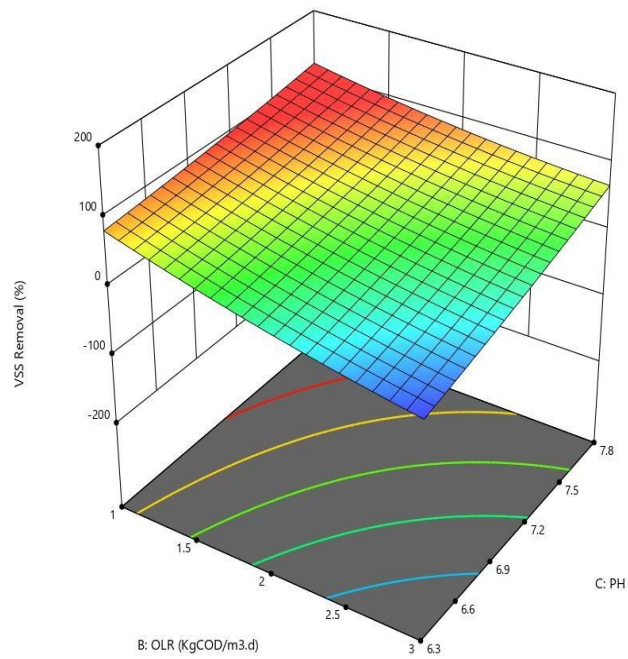
Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = B
X2 = C

Actual Factors
A = 15
D = 32

3D Surface

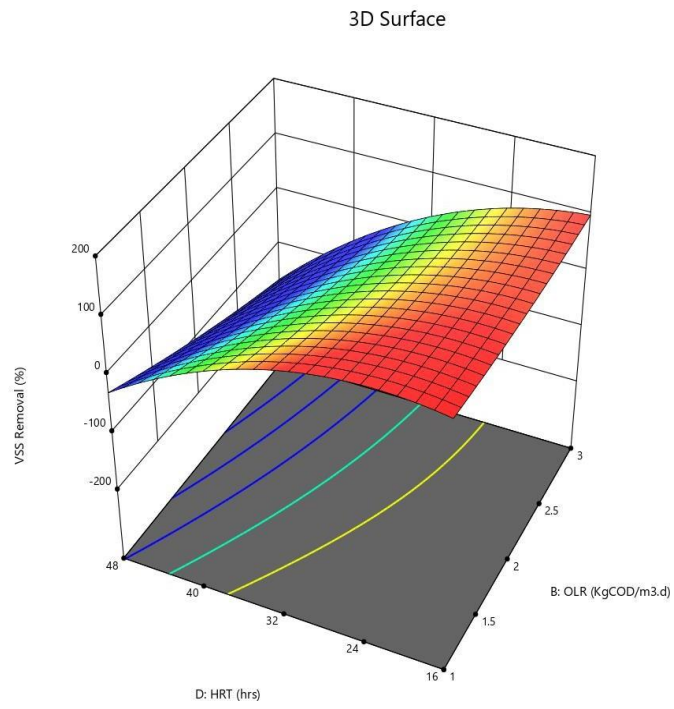


Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = B
X2 = D

Actual Factors
A = 15
C = 7.05

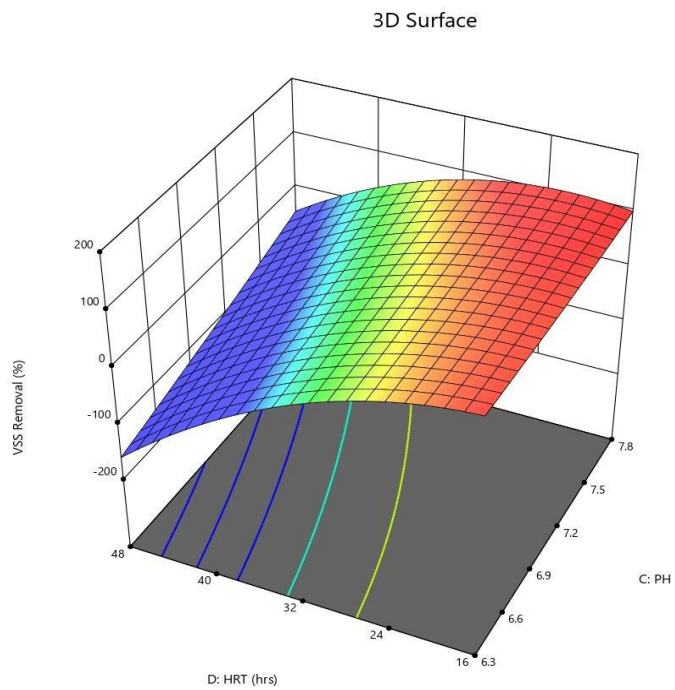


Factor Coding: Actual

VSS Removal (%)
-12.86  100

X1 = C
X2 = D

Actual Factors
A = 15
B = 2



Appendix –H Toxic heavy metals concentration in water, soil and plant samples

Lab Code	Description	Concentration	Fe	Mn	Ni	Co	Cu	Zn	Cd	Hg	Pb	As	B	Cr
			mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Blank 2	Blank 2	Sample-1	0.097	< 0.01	0.167	0.077	0.030	0.024	0.059	0.009	0.526	0.068	< 0.05	0.157
		Sample-2	0.084	< 0.01	0.135	0.068	0.036	0.022	0.058	0.008	0.510	0.049	< 0.05	0.114
		Sample-3	0.101	< 0.01	0.149	0.061	0.033	0.019	0.053	0.007	0.614	0.063	< 0.05	0.119
		Mean	0.094	< 0.01	0.150	0.069	0.033	0.022	0.057	0.008	0.550	0.060	< 0.05	0.130
		Stdv	0.009	-	0.016	0.008	0.003	0.002	0.003	0.001	0.056	0.010	-	0.024
21HWA 1083	UASB In-let	Sample-1	3.171	1.212	0.137	0.133	0.050	0.354	0.053	< 0.01	0.429	0.065	< 0.05	0.143
		Sample-2	3.214	1.213	0.167	0.137	0.056	0.363	0.051	< 0.01	0.465	0.076	< 0.05	0.146
		Sample-3	3.216	1.221	0.133	0.144	0.067	0.359	0.054	< 0.01	0.578	0.076	< 0.05	0.146
		Mean	3.200	1.215	0.146	0.138	0.058	0.359	0.053	< 0.01	0.490	0.072	< 0.05	0.145
		Stdv	0.025	0.005	0.018	0.006	0.009	0.004	0.001	-	0.078	0.006	-	0.002
21HWA 1084	UASB Out-let	Sample-1	0.572	0.800	0.114	0.044	0.023	0.073	0.052	< 0.01	0.479	0.055	< 0.05	0.094
		Sample-2	0.569	0.803	0.104	0.035	0.015	0.074	0.058	< 0.01	0.431	0.052	< 0.05	0.115
		Sample-3	0.568	0.797	0.114	0.042	0.003	0.074	0.049	< 0.01	0.398	0.038	< 0.05	0.101
		Mean	0.570	0.800	0.111	0.040	0.014	0.074	0.053	< 0.01	0.436	0.048	< 0.05	0.103
		Stdv	0.003	0.003	0.006	0.005	0.010	0.001	0.005	-	0.041	0.009	-	0.011
21HWA 1085	TF Out let	Sample-1	0.718	0.583	0.078	0.031	0.008	0.094	0.035	< 0.01	0.184	0.032	< 0.05	0.080
		Sample-2	0.713	0.579	0.071	0.062	0.005	0.097	0.038	< 0.01	0.234	0.037	< 0.05	0.083
		Sample-3	0.706	0.572	0.070	0.083	0.005	0.094	0.037	< 0.01	0.240	0.034	< 0.05	0.062
		Mean	0.712	0.578	0.073	0.059	0.006	0.095	0.036	< 0.01	0.219	0.034	< 0.05	0.075
		Stdv	0.006	0.005	0.004	0.026	0.002	0.002	0.002	-	0.031	0.003	-	0.012
21HWA 1086	S.Clarifire Out-let	Sample-1	0.377	0.444	0.123	0.134	0.078	0.11	0.06	0.06	0.29	0.061	< 0.05	0.103
		Sample-2	0.365	0.441	0.111	0.127	0.084	0.09	0.05	0.06	0.39	0.055	< 0.05	0.107
		Sample-3	0.366	0.438	0.085	0.130	0.078	0.08	0.05	0.06	0.31	0.060	< 0.05	0.010
		Mean	0.369	0.441	0.106	0.130	0.080	0.092	0.056	0.063	0.334	0.059	< 0.05	0.073
		Stdv	0.007	0.003	0.019	0.004	0.003	0.013	0.004	0.002	0.053	0.003	-	0.055
BK	Blank Soil	Sample-1	9.084	1.279	1.694	2.224	0.621	1.462	0.988	1.084	31.372	1.086	0.671	2.620
		Sample-2	10.020	1.300	1.185	2.240	0.671	1.407	1.077	1.019	26.285	1.174	0.650	2.981
		Sample-3	11.230	1.269	1.190	2.004	0.523	1.374	1.058	1.200	30.811	1.801	0.670	2.712
		Mean	10.111	1.283	1.356	2.156	0.605	1.414	1.041	1.101	29.489	1.354	0.664	2.771
		Stdv	1.076	0.016	0.292	0.132	0.075	0.044	0.047	0.092	2.789	0.390	0.012	0.188
22HST 1590	Soil sample	Sample-1	15810.400	928.601	41.989	53.937	20.789	115.883	7.517	5.870	123.039	8.900	15.274	40.288
		Sample-2	15874.800	918.022	42.212	55.138	21.608	118.185	7.692	5.426	116.453	8.751	15.820	41.743
		Sample-3	16380.300	942.372	42.328	55.952	21.229	119.811	7.673	6.132	119.339	8.623	16.041	41.829
		Mean	16021.833	929.665	42.176	55.009	21.209	117.960	7.627	5.809	119.610	8.758	15.712	41.287
		Stdv	312.107	12.210	0.172	1.014	0.410	1.974	0.096	0.357	3.301	0.139	0.395	0.866
BK	Blank for plant	Sample-1	24.959	1.549	0.500	0.637	0.637	3.633	0.383	0.448	7.443	< 0.04	< 0.05	0.361
		Sample-2	25.181	1.679	0.596	0.506	0.543	3.239	0.348	0.419	7.730	< 0.04	< 0.05	0.379
		Sample-3	25.605	1.821	0.505	0.504	0.561	3.177	0.357	0.408	7.064	< 0.04	< 0.05	0.347
		Mean	25.248	1.683	0.534	0.549	0.580	3.350	0.363	0.425	7.412	0.000	0.000	0.362
		Stdv	0.328	0.136	0.054	0.076	0.050	0.247	0.018	0.021	0.334	#DIV/0!	#DIV/0!	0.016

22HPA 1176	Local Cabbage	Sample-1	165.054	24.816	< 0.02	< 0.07	5.111	32.439	0.176	0.976	3.004	0.753	14.964	1.285
		Sample-2	158.562	23.781	< 0.02	< 0.07	4.784	30.940	0.203	0.993	2.353	0.809	14.096	1.193
		Sample-3	148.296	22.256	< 0.02	< 0.07	4.277	28.878	0.189	0.967	2.995	0.737	13.225	1.101
		Mean	157.304	23.618	< 0.02	< 0.07	4.724	30.752	0.189	0.979	2.784	0.766	14.095	1.193
		Stdv	8.450	1.288	-	-	0.420	1.788	0.014	0.013	0.373	0.038	0.870	0.092
		Sample-1	114.457	54.025	< 0.02	< 0.07	4.346	15.464	< 0.008	0.525	0.519	0.304	19.107	0.324
22HPA 1176'	Swiss Chard	Sample-2	115.269	54.340	< 0.02	< 0.07	4.310	15.595	< 0.008	0.449	0.550	0.257	19.410	0.373
		Sample-3	116.379	54.596	< 0.02	< 0.07	4.390	15.650	< 0.008	0.441	0.608	0.256	19.337	0.283
		Mean	115.368	54.320	0.000	0.000	4.349	15.570	0.000	0.472	0.559	0.272	19.285	0.327
		Stdv	0.965	0.286	#DIV/0!	#DIV/0!	0.040	0.096	#DIV/0!	0.046	0.045	0.027	0.158	0.045

Appendix- I ARE of COD, TSS, TKN, TP and NH4-N

Raw input data		Wastewater Outfall default run	ARE
Total COD	730.2 mg/L	38.45mgCOD/L	94.73%
TKN	61.46mgN/L	44.95mgN/L	84.48%
NH ₄ -N	44.80mgN/L	44.03mgN/L	1.72%
TP	17.73mgP/L	13.2mg/L	25.55%
TSS	362.4mg/L	13.05 mg/L	96.27%