Final report to the Willy Hager Foundation
for the project

Investigation on system conditions for the biodegradation of polyvinyl alcohol

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List of abbreviations

ACE	Abundance-based Coverage Estimator
CC	Calibration curve
DH	Degree of hydrolysis
DOC	Dissolved organic carbon
DOE	Design of experiment
DP	Degree of polymerization
EPS	extracellular polymeric substances
F/M	Food to Microorganisms ratio
MW	Molecular weight
OD	Optical density
OTUs	Operational taxonomic unit
PCR	Polymerase Chain Reaction
PSI	Pounds per square inch
PVA	Polyvinyl alochol
SVI	Sludge volume index
TOC	Total organic carbon
WWTP	Wastewater treatment plant
ZWT	Zahn-Wellens method (test)

1 Introduction

1.1 Motivation

Based on United Nations Sustainable Development Goals 2015 (SDGs), as stated in goal 14, is to reduce the environmental impacts from plastics and existing polymers (Walker, 2021). There are continuous and increasing efforts from all over the world to develop new synthetic sustainable material which is harmless to the environment based on different end-of-life scenarios. This includes the EU initiative (Directive 2019/904) for the disposal of single-use plastics. Biodegradable options are considered as alternatives to conventional polymers.

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer which is applied on huge scale in industry with utilization that exceeds one megaton due to its specific properties. The increasing application of PVA in multiple areas such as textiles, foods, medicine, industry, construction, and chemicals has led to high levels of emissions to the environment. The existence of PVA in the environment at high concentrations has different consequences starting from its ability of plastic particles to adsorb dangerous contaminants, to altering gas exchange and oxygen transfer in affecting the aqua life and ecosystem, to causing pollution when it leaks to groundwater, aside with long existence without biodegradation in marine environments.

Therefore, it is significant to investigate and develop degradation methods, including physical-chemical methods such as chemical oxidation (for instance Fenton process or photocatalytic degradation processes), which still requires more investigations to reduce the application costs and increase their efficiency to ensure complete degradation and avoid producing toxic or intermediate compounds. Biodegradation of PVA is considered a very promising field with respect to classify PVA as a fully sustainable material, since it proves that is completely biodegradable with considering the presence of certain conditions and procedures. Related organisms with their enzymatic activities are proven to be able to degrade PVA as mentioned in previous studies.

To ensure practical application of PVA biodegradation, certain system conditions in wastewater treatment plant under aerobic/anaerobic conditions must be met to ensure the occurrence of biodegradation. These conditions including temperature (18 -33 °C), adaptation of organisms which has certain limitations considering the conditions in municipal WWTP, low F/M ratio (0.15 kg BOD_5/ kg SS.d) pH, solubility of PVA, dissolved oxygen and others.

In textile industries, which are responsible of high wastewater volumes due to its necessity in the production process, these conditions are not met, where high COD loads including high PVA concentrations (500 mg/L) which is responsible for 30 to 70% of COD loads,

temperatures above 33°C and non or partial adaptation of organisms occur, with the availability of other easy biodegradable compounds as carbon source. Which all led to discharging wastewater without meeting the requirements of water quality parameters. Also, temperature range which is considered a crucial factor in biodegradation process, is either difficult to be met since these industries apply high temperatures in the manufacturing process, or due to high climate temperature in manufacturing countries, such as Bangladesh and China.

The aim of this project is to investigate the system conditions which is necessary to achieve high PVA biodegradation, considering low – high temperature ranges, pH, adaptation to high temperatures, type and specification of PVA and its effects in biodegradation process, and specify the related strains which is able to degrade PVA, and their effects in increasing the kinetic rates of biodegradation.

1.2 Objective

1.2.1 General aspects

The majority of the available specialist literature related to PVA biodegradation under aerobic conditions lacks on investigation of biodegradation aspects at high temperatures, adaptation aspects and combination between biodegradation capacity of the sludge represented in kinetic rates, related strains and enzymatic activities and applicability aspects. Previous investigations (Sträßner,1995; Schönberger, 1997; Rolsky & Kelkar, 2021) showed that is there is no degradation possible at 40°C and initial investigations confirm these aspects. There are also missing details about the specifications of system conditions necessary for biodegradation using different microbiocoenosis. There is also a lack of information regarding to the main strains that could degrade PVA under high temperatures, with technical applicability of the cultures containing these strains. For all of these mentioned aspects, there are a considerable need for research here to help in the implementation of the application of process development.

1.2.2 PVA spectrometric measurement method

To ensure high accuracy of the planned experiments, PVA method was investigated by testing different factors; including specifications of PVA, absorbance wavelengths, types of water, reaction time, age of applied solutions, and types of filtration.

1.2.3 Optimum system conditions for PVA biodegradation

Without meeting certain system conditions, PVA is not biodegradable under aerobic conditions. Therefore, specific parameters were tested depending mainly in F/M ratio and

temperature and applying municipal microbiocoenosis using batch tests to specify the optimum system conditions based on kinetics.

1.2.4 Adaptation effect in biodegradability of PVA

In the batch test, the effect of adaptation of municipal sludge is tested to be applied for different aspects. Firstly, after adaptation phase at optimum temperature, the potential of microbiocoenosis is tested to degrade PVA at higher temperature range where it was not possible to degrade before (36-42°C). secondly, investigation whether the adaptation of degrading organisms is a concentration dependant phase or there are other factors have an essential role in adaptation. Low concentrations of PVA should specify the minimum concentration which is necessary for full adaptation of PVA.

1.2.5 Industrial sludge potential in PVA biodegradation at high temperature

Adapted sludge to PVA biodegradation is investigated. This includes a series of biodegradation experiments to specify the temperature, concentration, and pH ranges, with testing the degradation as a proof of biological effect. Also, parametric and process comparisons between the different sludge types were included with microscopic analysis to provide more details and specifications.

1.3 Detailed work program

1.3.1 Step 1: Literature research/procurement of materials

First of all, an extensive literature search should complete the overview of existing aspects related to PVA biodegradation. The focus was on information on their reference, including PVA properties and applications, effect of PVA on health and environment, Physical and chemical PVA degradation approaches, biodegradation aspects including PVA biodegradation in treatment plants, specific microbial communities and the enzymatic activity including different conditions (aerobic, anaerobic, nitrifying conditions) and hybrid systems. Materials and equipment that are interesting for the project should also be obtained in this phase.

1.3.2 Step 2: Design of Zahn-Wellens method

Also, to minimize the number of experiments that will be carried out in this study, a design of experiments (DoE) technique were used to plan experiments. Therefore, it is a technique of great importance for the industry. It enables obtaining of more reliable results while saving

time and resources, based in potential factors whose variation might impact the response variable (process output) (DOE). The planning was used to obtain valid results and introduce objective conclusions. The planning must maximize the quantity of information that can be obtained for each variation performed. This step also includes the design of the single experiment duration and sampling plan.

1.3.3 Step 3: Laboratory tests for PVA biodegradability

Biodegradability tests were carried out in this step under specific ranges. Firstly, initial series of experiments to specify system conditions including kinetic estimation and mathematical representation of results. Secondly, two stage experiment by application of adaptation phase and increase the temperature to higher ranges and testing the biodegradability. Third part is related to explore the effects of specification of PVA and threshold concentration which is necessary for the adaptation of organisms in the applied microbiocoenosis.

In the other part of research, different microbiocoenosis were tested (municipal and adapted sludge). The sludge with best biodegradation kinetics were investigated in depth; by implementing liquid culture and plate techniques to identify and isolate the strains, and to specify the conditions that these strains can survive with the ability to grow and degrade PVA. For this purpose, viability testing of samples was performed after freezing. Also, different inspections including microscope, 16s amplicon sequencing analysis were performed to bring all related details.

1.3.4 Step 4: final report

A detailed report on the results of all work steps should be prepared. At the end of the project, the Willy Hager Foundation will be presented with a comprehensive written report, in which all relevant results of the project are listed in a comprehensible manner.

1.3.5 Temporal progress of the project

The chronological progress of the work was planned as shown in Figure 1.1

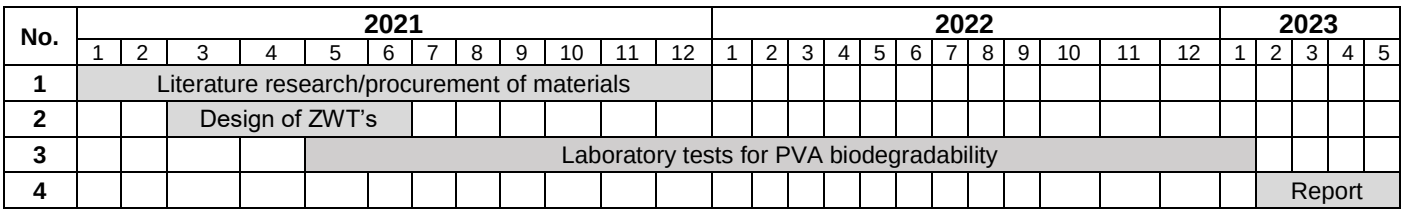


Figure 1.1: Time schedule of the steps of the projects.

1.4 Thesis

Several student theses were performed as part or related to this project, so some parts of the results of this final report come from these theses. Diagrams and text modules of these theses can therefore be part of this report. These are the following theses:

Sachit Dhakal (2021): Study of biodegradation of polyvinyl alcohol (PVA) as a function of Food-To-Mass ratio. Master thesis.

Md Shahriar Kabir (2022): Investigation on system conditions for the biodegradation of polyvinyl alcohol. Master thesis.

Jian Zhang (2022): Korrelation des biologischen Abbaus von PVA in verschiedenen Testsystemen. Master thesis.

2 State of knowledge

2.1 Polyvinyl alcohol and its properties

Polyvinyl alcohol (PVA) is a hydrophilic synthetic polymer in the form of colorless amorphous powder. It can be produced by the hydrolysis of poly (vinyl acetate) (PVAc); since vinyl alcohol tends to convert spontaneously into the enol form of acetaldehyde, polymerization can't be applied directly from the corresponding monomer ((Chiellini et al., 2003), Ben Halima, 2016)

PVA has a backbone composed only of carbon atoms, including head-to-tail 1,3-diol units. However, PVA has a minor percentage (usually under 1–2%) of head-to-head 1,2-diol units. PVA can be molded in various shapes, such as containers and films.

As shown in figure 2.1, the synthesis cycle starts with ethylene, which is converted to acetic acid by oxidation. In addition to this method, carbonylation of methanol (Monsanto Process) is the most applied internationally to produce acetic acid. With catalysis in the gas phase, acetic acid is converted to vinyl acetate monomer (VAM) and finally to PVAc and PVA. The molecular weight and different properties of PVAc and PVA are based on the applied polymerization production method. If the whole cycle is closed by biodegradation, a practical, sustainable material cycle can be achieved (Rieger et al., 2012).

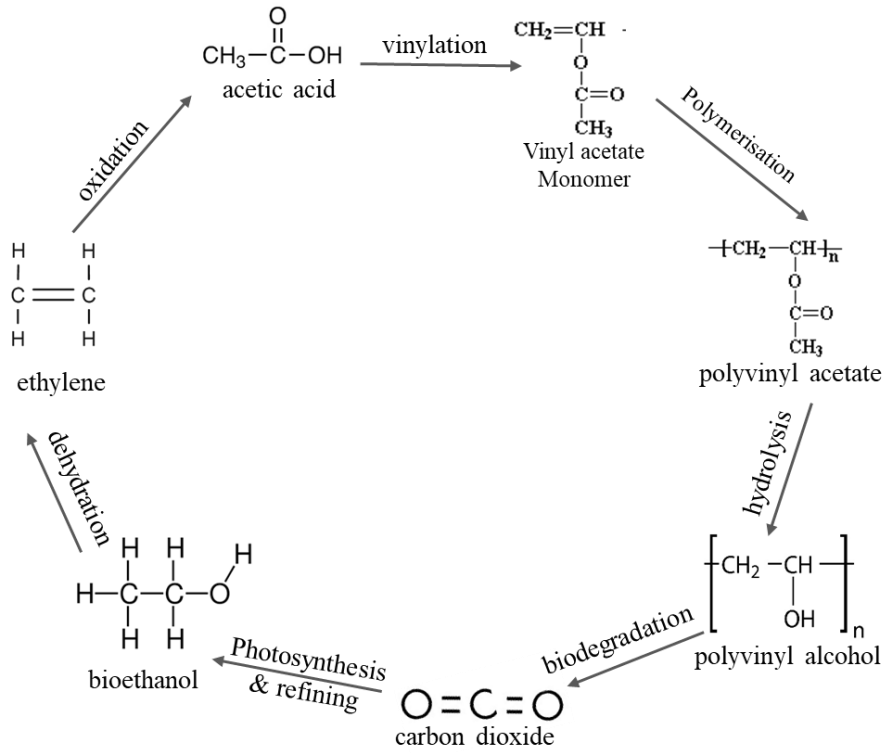
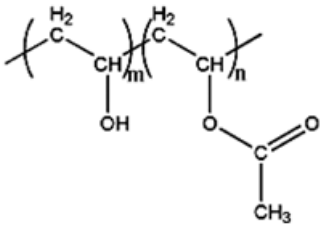


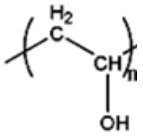
Figure 2.1: Vinyl acetate circle represents the manufacturing of PVA (Rieger et al., 2012).

2.2 Physical and chemical properties

PVA is categorized based on its structure to fully (with 1% to 3% residual acetyl groups) and partially hydrolysed structure (with 10 % to 20% of residual acetyl groups), as shown in figure 2.2. The category and number of monomers, i.e., the chain lengths, significantly impact application properties.



Partially hydrolysed PVA



Fully hydrolysed PVA

Figure 2.2: Structural formula for PVA: fully hydrolysed; partially hydrolysed (Lacasse, 2004)

Acetate groups next to alcohol groups are more readily hydrolysed than acetate groups having only other acetate groups, which makes partially hydrolysed PVA looks like a block structure rather than a random structure. With the degree of hydrolysis 87-89 % for MW between 25,000 and 100,000, DP, 600–2,400, PVA is soluble in cold water. While with higher DH the solubility decreases. Fully hydrolysed PVA is only soluble in water at temperatures above 80°C. This is due to the building up of intermolecular hydrogen bonds, which results in a high crystallization compared to partially hydrolysed PVA (Rieger et al., 2012;).

The CAS number and other information on PVA, such as the range of molecular weight, the empirical formula, the physical appearance, specific gravity, and solubility, are compiled in Table 2.1.

Table 2.1: Chemical Identity and physical properties of PVA (Rowe et al., 2009)

CAS no.	9002-89-5
Molecular Weight	20 000–200 000
Structural Formula	(–CH2CHOH–)–n–(–CH2CHOCOCH3–)–m
Empirical Formula	(C2H4O)n(C4H6O2)m
Physical Appearance	Odourless, white to cream-colored granular powder
Specific Gravity	1.19–1.31
Solubility	Insoluble in aliphatic and aromatic hydrocarbons, esters, ketones, and oils; slightly soluble in ethanol (95%), water-soluble.
Boiling point	228 °C
Melting point	150-190 °C
Viscosity at 20°C (mPa.s)	Low: 4-7, Medium:21-33, Hard:40-65
Physical properties	Tensile strength, thermostable, adhesive, emulsifying.

PVA foaming properties are affected mainly by their structures. The degree of hydrolysis has the main effect, where low degree increases the surface tension and water solubility, which is reflected interms of bubble size and foam stability. Higher degrees increase water fastness and gelling tendency (Rosen, Song, 1996). It is also found that the volume of foaming is also related to the viscosity (higher leads to higher foaming volumes), where it is generated even with mixing at room temperature (25°C), with direct effect of stirring or aeration (Hou, Wang, 2017).

2.3 Applications of PVA in industry

The global PVA market was valued in 2021 with USD 791.7 million in 2021, with expected growth of 6.3% from 2022-2030 (Polaris, 2023). Furthermore, global production is reaching one megaton. The increasing need for bio-based PVA products elevates the polyvinyl alcohol market growth, especially in the Southeast of Asia and Pacific regions.

PVA is applied for many different polymer dispersions used in the construction industry. Also, it is applied as a binder in the paper industry, including fiber coating and packing. also in farming, in latex paints, and in the production of disposable plastic articles (particularly PVA films). (Chiellini et al., 2003). Furthermore, PVA is used in emulsion polymerization, producing poly (vinyl butyral). In medical applications, PVA is applied in composite membrane manufacturing, hydrogels for biomedical uses, drug delivery microplastics. It is also applied in the treatment of dry eye drops and contact lenses. In field of biotechnology, it is applied in enzyme entrapment and electrospinning for biomedical applications (Kawai, Hu, 2009). Its application is particularly important in the textile industry as described below.

2.3.1 Application of PVA in textile industry

Textile is one of the largest industries present globally, especially in Asian countries like China, Bangladesh, India, Pakistan, Vietnam, and Sri Lanka. It is a high-consumption water industry; using large freshwater quantities for textile wet processing, also called textile finishing. The specific water consumption varies between 20 to 300 L/kg of fabric. The specific water consumption is higher for cotton than for polyester finishing ((ZDHC), 2018).

The textile process includes desizing, , scouring, bleaching, mercerising, dyeing, printing, and final finishing, including different washing operations. The wastewater effluents resulting from these processes differ significantly in composition, because of different make-ups, textile

substrates, chemicals applied, and machinery (Schoenberger, 2018). Table 2.2. Summarize the main processes and the chemicals applied with wastewater characteristics.

Table 2.2: Textile processes and the chemicals applied with wastewater characteristics (Bisschops & Spanjers, 2003), ((ZDHC), 2018).

Process	Details	Chemicals added / Wastewater Characteristics
Sizing	Cotton and yarns are sized before weaving to gain strength and avoid breaking the fibers.	Starch, PVA, carboxyl methyl cellulose (CMC), polyacrylates.
Desizing	Sizing agents are removed by washing with detergents (in case of PVA or acrylates), or using enzymes to remove starch. After desizing, the fabric is intensively washed.	High BOD and COD due to starch, CMC, PVA, fats, waxes, surfactants, enzymes, High in BOD, SS, and TDS.
Scouring	Removing of natural impurities	Strong alkali (NaOH), electrolyte resistant surfactants (fatty alcohols ethloxlyates, alkane sulfonates), detergents. High COD, SS, BOD
Mercerisation	Treatment of cotton fabric with high-strength caustic soda solution under tension to improve dye uptake and tear strength and to obtain a silk-like luster.	Washing water with very high caustic soda content which can be recovered
Dyeing	Applied to textiles in dye solutions or dispersions. Also, dye accelerators may be added in case of polyester fibers.	Dyes, urea, reducing agents, oxidising agents, acetic acid, surfactants, nitrobenzene sulfonate and other substances. Strongly colored, can contain heavy metals if heavy metal containing dyestuffs are applied, high BOD, TDS, and low in SS, can contain sulphide when sulphur dyes are applied.
Bleaching	Removing natural coloring from cotton, blend fabrics, wool, synthetic fibers	Hydrogen peroxide, sodium hypochlorite, , NaOH, surfactants, sodium silicate, sodium sulfite, sodium phosphate, enzymes to remove surplus peroxide, short cotton fibre. High in alkalinity, TDS, SS and fibres
Printing	Dyes and auxiliaries are applied in specific part of the fabric, including print pastes.	Dyes, urea, gums, binders, thickeners, alkalis, reducing agents, film-forming substances (styrene butadiene co-polymers), polyacrylates, mineral oils, Highly coloured, high BOD, oily appearance, heavy metals, high SS, and slightly alkaline
Finishing	Last step applied to ensure the comfort, durability or human safety with the finished product.	High in BOD, COD, possibly toxic compounds, possibly small volume

In the textile industry, PVA is used as a sizing agent worldwide, which is necessary for controlling friction and electrostatic charges and thus protecting the warp yarns against the mechanical stress of the weaving process. Depending on its high strength, low moisture absorption, good rot resistance, waterproofness, and no need for bleaching, it is applied to form films with ease and with high bond strength to provide a coating which can protect spun

and filament yarn (Lacasse, 2004). However, after weaving, sizing agents have done their job and have to be removed entirely prior to subsequent finishing processes that are dyeing and/or printing. PVA can be wahed off with hot water and detergent. As a consequence, they are contained in wastewater where they represent between 30 and 70 % of the overall COD load of a textile finishing industry (EC Textile BREF, 2003). COD and BOD_5 values of sizing agents are shown in table 2.3.

Table 2.3: COD and BOD_5 values for the most common sizing agents (Textile BREF, 2003)

Sizing Agent	COD- Value (mg O_2/g)	BOD_5 – Value (mg O_2/g)
Starch	900 - 1000	500-600
Carboxymethyl cellulose (CMC)	800-1000	50-90
PVA	**1700**	**30-80**
Polyacrylates	900-1650	<50
Galactomannans	1000-1150	400
PES – dispersions	1450-1700	<50
Protein sizing agents	1200	700-800

ZDHC program (global industry initiative for implementing safer chemical management practices) set three levels of parametric limits of textile wastewater (foundational, progressive, and aspirational levels) to specify the quality of the effluents and level treatments to be applied as shown in Appendix A.2.

2.4 Effect of PVA on ecosystem and health

For application of PVA in pharmaceutical and dietary supplement product, the oral toxicity of PVA is very low, with lethal dosage (LD50) in range of 15-20 g/kg, where it is considered at least concern category, and don’t accumulate in the human body when it is orally administrated, tested in range of 5000 mg/kg bodyweight/day for 90 day dietary study (DeMerlis, Schoneker, 2003). Also, PVA is not mutagenic and/or clastogenic (Schweikl et al., 1996).

For the existence of PVA in environment at high concentrations have different consequences. Among the various synthetic/semisynthetic polymeric materials, PVA's high production,

utilization, and emission to natural water can be associated with significant pollution of rivers, lakes, and other aquatic ecosystems. Initial studies proved that PVA could alter gas exchanges, such as carbon dioxide exchange, affecting aquatic ecosystems (Julinová, 2018). It also can cause pollution through leaching into the groundwater. With high ability to mobilize heavy metals and accumulate on all environmental media, PVA can cause pollution through sediments to water resources and soil (Chowdhury et al., 2015).

Wastewater and stormwater include hydrophilic compounds, such as pharmaceuticals, biocides, herbicides, personal care products, …etc. If PVA exists in wastewater at higher concentration, it can transport up these compounds to the food chain. During such phenomena, the toxicity levels increase due to higher the contaminant concentrations (Spahr et al., 2020). Also, PVA has the ability to foam due to its surface properties (Shan et al., 2009). This has negative effect either to the environment through inhibition of oxygen transfer in water bodies which leads to irreparable harm to aquatic life, or to the operation of the biological treatment process, which makes it runs unsteadily and affects its performance (Zhang & Yu, 2004).

In case of plastic incineration, airborne pollutants and contaminants are released. With incomplete PVA breakdown after conventional treatment, a material fraction being sequestered within biosolids can be present, which negatively affects microbial activity, bulk density, and water-holding capacity of the soils (Venkatesan & Halden, 2014).

Considering the marine environment, PVA-based polymers which are existing in marine environments; are negligibly biodegradable without acclimated inoculum (assessed by theoretical oxygen demand percent) , and improve slightly when it is combined with glycerol (Alonso-López et al., 2021).

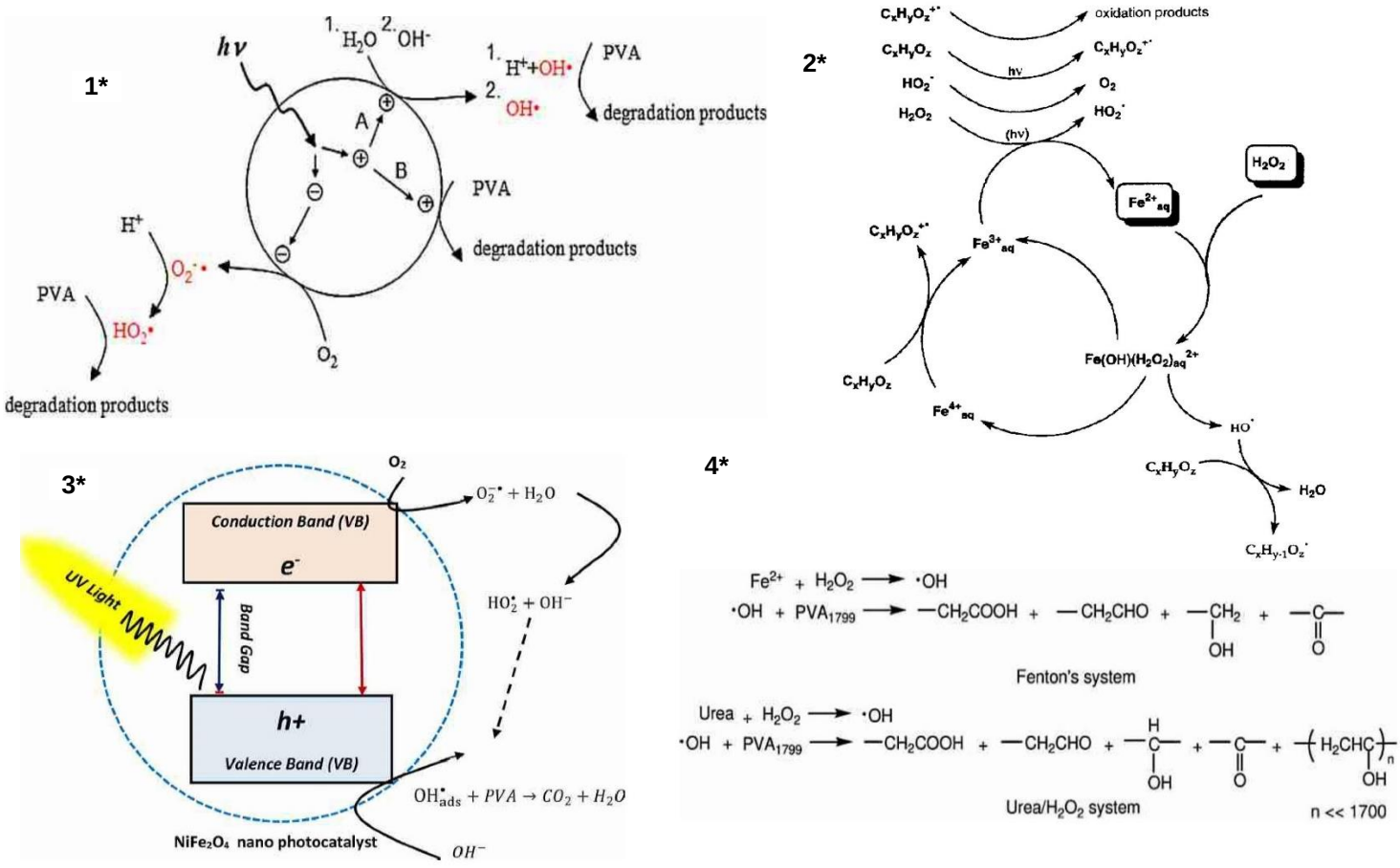
All of the mentioned and related studies confirm harmful effects and significant pollution of PVA in ecosystems and it consequences in affecting the food chains and reflected to human health, which signifies the importance of finding sufficient solutions for PVA degradation and bioremediation.

2.5 Degradation of PVA by physical-chemical methods

Due to high application in industry and its existence in huge amounts, the elimination of PVA, which is resistant to degradation (Corti et al., 2002), becomes more interesting to be investigated. Several studies investigated the physical-chemical methods and the biological methods.

Physical-chemical methods such as electro-coagulation, chemical oxidation processes like the Fenton process, photocatalytic degradation, and advanced oxidation process were investigated. Some of these methods were either low in removal efficiency or with high application costs, and could produce toxic matter (Ajmi et al., 2018). Table 2.4. Summarizes the relevant studies and its results, and Figure 2.3 summerize the main schemes of chemical and photooxidative reactions for PVA degradation.

Figure 2.3: Main Scheme of reactions in PVA degradation using chemical – Physical Methods



1*: Photocataylic PVA degradation reaction scheme using P-25 TiO2 (Hsu et al., 2011).
2*: Thermal and photochemical reactions involved in application of fenton processes (Lei et al., 1998).
3*: Photocataylic PVA degradation reaction scheme using NiFe2O4. (Shokri, 2021).
4*: Urea/H2O2 Activation-Oxidation System in Degradation of PVA (Shan et al., 2009).

Table 2.4: Main investigated physical-chemical methods and application results for PVA degradation

Technology	Methodology	Conditions & Parameters	Results	Reference
Chemical oxidation	UV-365 nm/S_2O_8 process	pH [3 – 11], $Na_2S_2O_8$ [0.13 –1.0 mM], UV, PVA [10-30mg/L] Temperature [25-60 °C] Time [30-120 min]	The degradation efficiency is higher under acidic conditions than alkaline conditions. Higher $Na_2S_2O_8$ dosage and a higher temperature were correlated with a higher PVA degradation efficiency. At UV-365 nm, pH 3, an $Na_2S_2O_8$ dosage of 0.06 g/L, a temperature of 55 °C, and an initial PVA of 20 mg/L, around 100 % of PVA was degraded.	(Lin et al., 2014) (Lin et al., 2013)
Chemical oxidation	Urea/H2O2 Activation-Oxidation	Fenton's reagent:$Fe(II)/H_2O_2$ [0.1 -1ml/l]	the degradation products contained aldehyde, ketone groups, and carbon dioxide. In the application of desizing and scouring process of polyester/cotton 65/35 fabric, the degradation rate of PVA was 94.7%, desizing rate of PVA was 99%.	(Shan et al., 2009)
Chemical oxidation	UV/chlorine oxidation	UV [0- 3120 MJ/cm_2], Cl [0- 20mg/L] PVA [2-16 mg/L, 95000 MW.]. time [0-20 min], pH [4 -10]	UV irradiation or chlorination alone did not degrade PVA. UV/chlorine oxidation showed good efficiency, The rate constant k increased linearly from 0/min to 0.3/ min with increasing chlorine dosage in range of 0 mg/L to 20 mg/L.	(Ye et al., 2017)
Chemical oxidation and irradiation	$Na_2S_2O_8$ activation LED visible irradiation	Fe-O-PAN [5 g/L] , PVA [50 mg/L], $Na_2S_2O_8$, LED, temperature [25°C], pH [4-6]	Fe complex (Fe-O-PAN) with LED irradiation activated effectively $Na_2S_2O_8$ for PVA degradation. The main reactive oxygen source were hydroxyl radicals which are related to PVA degradation. Highest efficiency at pH 7 and concentration of 50 mg/l PVA.	(Dong et al., 2019)
Co-precipitation	Co-precipitation /nano photocatalyst	UV, $NiFe_2O_4$ [0.3 g/L] PVA [25 -70 mg/L], Time [0 -140 min]	Removal of PVA was 94.3%, COD removal 41.3%, time was 140 min. Optimum conditions were (catalyst concentration 0.3 g/l, PVA concentration 25 mg/l and pH of 6. Degradation $Kapp = 2.06 \times 10^{-2}\ min^{-1}$ half-life ($t_{1/2}$ =33.6 min), followed pseudo first-order reaction.	(Shokri, 2021)
Chemical oxidation with Fenton process	oxidation and coagulation	iron salts [5-50 mg/L]. H_2O_2 [10-100 mg/L] oxidation time [0-30 min], speed of mixing [40-100 rpm]	90 % of color removal at 5 min reaction using low dosages of H_2O_2 and Fe(II). Removal ratio efficiency of 5.6: 1.2 for colour removal & COD removal. This method is not preferred for COD removal	(Kang et al., 2002)
Chemical oxidation with Fenton Process	H_2O_2 oxidation	pH [2-7] , time [10- 80 min] , T [30 -80 °C] , H_2O_2 [0.4 -1.6 d/g/mL] H_2O_2/ $Fe(NO_3)_2$ [7-12 d/g/mL]	The optimum factors are: H_2O_2 dosage is 650 g, the molar ratio of H_2O_2/$Fe(NO_3)_2$ is 10, initial pH value is 4, the reaction temperature is 50 °C, and reaction time 30 min. The degrading efficiency & TOC removal was 99%	(Zhu & Ge, 2021)
Electro-coagulation	iron electro-coagulation.	Pairs, current densities [1.25 – 7.5 mAcm-2] , electrolytes, T [288-318 K] initial electrolyte concentration [0.004 -0.016 N]. PVA [100 mg/L]	Fe/Al electrode pair is optimum. The optimum parameters are: current density of 5mAcm−2, supporting electrolyte concentration of 0.008N NaCl, and temperature of 298 K. The PVA removal efficiency decreased with increasing in the initial concentrations.	(Chou, 2010)

		Time [0-120 min], agititation speed [300 rpm]		
Technology	**Methodology**	**Conditions/ Parameters**	**Results**	**Reference**
Photocatalytic degradation	Adsorption and photocatalytic degradation	pH [3-10], P-25 TiO_2 dosage [0.12-1 g/L] PVA [10-30 mg/L, 22000 MW.] , Anions [$NaNO_3$, Na_2SO_4] time [0-160 min].	Lower PVA concentration and pH, with higher P-25 Ti O_2 dosage, achieved high removal efficiency of PVA during 150 min of operation. Cl and SO_4 ions reduced the removal efficiency of PVA, while NO_3 ions did not affect it. Wth a 0.12 dosage of P-25 TiO_2 of 0.12 g /L, and no inorganic anions, PVA removal rate was 100%, at pH 5, 20 mg/ L PVA, and an air flow rate of 400 mL/ min, as measured after 150 min	(Hsu et al., 2011)
Photocatalytic degradation	photooxidative degradation	UV/ H_2O_2, Time [0-150 min], pH [2.5 -5] PVA [50 - 500 mg/L, 130 kg/mol]	With UV/ H_2O_2, TOC removal of 87% in 2hrs was achieved in stepwise operation (semi-batch), with reduction in polymer to 91%. With H_2O_2 only, 43% TOC removal and 21% and reduction of polymer molecular weights was obtained. With UV only, no PVA polymer degradation is achieved.	(Hamad et al., 2014)
Adsorption	Adsorption on powdered activated carbon (PAC).	PAC dosage [1-10 g/L], PVA [25-100 mg/L], pH [6.3], Temp. [293-313 K] Contact time [0-60 min]	Spontaneous process was indicated from the negative free energy of adsorption at all temperatures. Optimum parameters: pH of 6.3, time of contact 30 min, adsorbent dose of 5 g/L. 92% PVA removal obtained	(Behera et al., 2008)
Advanced oxidation processes	Ozonation, Synergic oxidation, ionizing radiation	O_3/ultrasonic, O_3/ultraviolet, Time [0-30 min], pH [1.0-13.0] Absorbed dose [0-12 kGy]	For ozonation, TOC values of PVA solutions showed a decrease in pH values of 7.0 and 13.0, where PVA mineralization was 13% and 50% after 30 min. For Ionizing radiation, TOC showed a decrease at pH values of 1.0 and pH 3.0, where PVA mineralization was 94% and 97%. Sediments were found in the bottom of the radiation-proof glass tubes at pH 1.0 and pH 3.0. Applying ozonation and ionizing radiation together can acheive a synergistic effect on PVA mineralization at initial pH 3.0–9.4. Under strongly alkaline conditions (initial pH 13.0), excess OH^- dominated the reaction process. Most Ozone was decomposed to OH, which decreased the possibility of a synergistic effect.	(Sun et al., 2015)

2.6 Biodegradation of PVA

Biodegradation is a complex natural procedure where organic matter is converted to simpler compounds; then, the break-down products, as part of the carbon cycle, can be used for the formation of new organic compounds Polymers with hydrolysable backbones, reduced crystallinity and additives are susceptible to biodegradation (Lipsa et al., 2015). biodegradation of PVA relies mainly on the surrounding conditions that PVA encounters when it is released into the environment. Solubility of PVA in water (which is favorable for biodegradation) or PVA in solid state and the occurrence of biotic or abiotic structures that can interact the surface of the polymer is essential.

2.6.1 Microbial communities and enzymatic degradation

The biological degradation of PVA is an interesting biological aspect received a high attention in the last years. It relies mainly on the ability of adaptation of certain microbes and the activity of their enzymes. Therefore, several microorganisms and enzymes were isolated and tested for biodgeradation. This includes bacteria, fungi, and algae.

Nord (1936) proved that PVA sustained biodegradation the action of *Fusarium lini* were investigated, a phytopathogenic fungus, which using extracellular activity by a 'dehydratase' and produce carbon dioxide and water as a result of. Suzuki (1937) showed that PVA is completely degraded and utilized by a bacterial strain, belonging to *Pseudomonas,* as a sole source of carbon and energy. After an extensive screening of the PVA-degrading microorganisms from environmental samples.

PVA-degrading microorganisms are not ubiquitous within the environment. These microorganisms are: *Pseudomonas O-3*, *Pseudomonas vesicularis PD, Pseudomonas sp. VM15C, Pseudomonas vesicularis var. povalyticus PH, Pseudomonas sp. strain A-41, Alcaligenes faecalis KK314, Bacilllus megaterium BX1*, strain *PN19, Geotrichum sp. WF9101, Sphingomonas sp. TJ7 and γ-proteobacteria TK-2, Penicillium sp. WSH02-21, Microbacterium barkeri KCCM10507 and Paenibacillus, amylolyticus KCCM, Sphingopyxis sp. PVA3, Streptomyces venezuelae GY1, Stenotrophomonas rhizophila QL-P4* (Kawai & Hu, 2009). Also, *Povalibacter uvarum* which where isolated from grapes (Nogi et al., 2014a), *Thalassospira povalilytica* were found in marine (Nogi et al., 2014b) proved to be able to degrade PVA.

Finally, fungai PVA-degraders which are of *Aspergilus, Penicillium* (Ben Halima, 2016), *Fomitopsis* (Tsujiyama & Okada, 2013) and *Phanerochaete chrysoporium* (Chiellini et al., 2003).

Watanabe (1976) studied the properties of enzyme that is produced from *Pseudomonas* that can degrade PVA. Under certain conditions, including pH (7 to 9), 40 degrees' temperature, and oxygen availability, the enzyme oxidized PVA. Tang (2010) found that 1,4-butanediol could improve PVA enzyme production by mixed microbial culture using controlled two-stage fermentation by adding additional carbon source and PVA.

Symbiotic behaviors were identified in many studies between strains to degrade PVA. Shimao (1984) and Sakazawa (1982) proved the utilization of PVA by pairs of bacterial symbionts in mixed cultures. Pseudomonas sp. VM15A and VM15C formed a potent PVA mixed culture, with PQQ as main growth factor. Hashimoto and Fujita (1985) found that Pseudomonas vesicularis var. povalolyticus PH, required thiamine, cystine, isoleucine and tyrosine as growth factors which are supplied by *Flavobacterium sp* during degradation activity. Vaclavkova (2007) found a symbiotic behavior between *Sphingomonas sp*. and *Rhodococcus erythropolis* strain, which supplied PQQ during the degradation process. Furthermore, Mori (1996) found symbiotic microorganisms identified as *Bacillus megaterium BX1* could degrade PVA with cocultivation of *PN19*, without the need of PQQ. Kwangken (2004) identified a mixture of *Microbacterium barkeri KCCM* 10507 and *Paenibacillus amylolyticus KCCM* 10508 that could degrade 90% of PVA at 750 mg/l concentration. Kim (2003) discovered a new PVA degrader strain *Sphingomonas sp. SA3* in the form of yellow colonies that needs *Sphingomonas sp. SA2* as symbiote strain as growth factor producer. Marusincová (2013) proved that it is possible to degrade PVA under denitrifying and aerobic conditions with a microbial culture from a municipal treatment plant, where the organism was identified as *Steroidobacter sp. PD*.

Previous studies that are related to enzyme activities in PVA biodegradation are receiving more attention. There are two types mainly: exo- enzymes that are working in the extracellular space and periplasmatic volume, and there are membrane-associated enzymes that connected to the cytochrome based electron transport chains in the cell.

Basically, degradation happens by oxidation of the carbon backbone followed by a random endocleavage of the polymer chains. Enzymes are responsible of breaking down the polymers

into monomers, oligomeric structures, and other metabolites. The hydroxyl group is oxidized by extracellular secondary alcohol oxidase (SAO), PVA oxidase or PVA dehydrogenase (PVA-DH) detected in the membrane (Exogenous pyrroloquinoline quinone (PQQ) dependent). Depending on the number of hydroxyl groups, one hydroxyl group leads to monoketone structures, and two hydroxyl group leads to β-diketone structures. After that either hydrolsis of β-diketone structures (by β-diketone hydrolase (BDH)) or aldolase reaction of monoketone structures (by aldolase) do occur (Kawai & Hu, 2009).

The random cleavage of C-C bonds along polymer chain formings carboxylic acids indicating a pH drop in the reaction mixture. SAO mainly catalyze the oxidation of vinyl oligomers with MW. of 220-1500 (Chiellini et al., 2003).

It is also noted that β-diketone of more than five carbon chain length was necessary for activity of hydrolase, and hydrolytic cleavage happens at the shorter side if the chain length is different in β-diketone structures. For intercellular cell activity, the estrase is targeting low molecular weight of PVA fractions. Following intercellular metabolism, esterases transfer acetic acid and remaining molecules are passing through β-oxidation pathway and utilitzed as energy source, before it finally oxidised to carbon dioxide and water (Klomklang et al., 2005). Table 2.5 summerize the enzymes that are directly involved in PVA biodegradation with the related registered genes as sited in literature. Genetic informations are grouped using EC classfication (Enzyme Commission number) are registered in uniprotein database, but not all details are registered yet because it still in process (including SADH and involved genes) (Rieger et al., 2012).

Table 2.5: list of registered enzymes and corresponding genes related to PVA biodegradation.

EC number	Name	Synonyms	Gene
1.1.3.30	Poly(vinyl alcohol) oxidase	PVA oxidase, poly(vinyl alcohol) oxidoreductase, poly(vinyl alcohol) dehydrogenase	-
1.1.3.18	Secondary-alcohol oxidoreductase	Polyvinyl alcohol oxidase, PVA oxidase, SAO	-
1.1.1.x (no entry)	Secondary-alcohol dehydrogenase	SADH (NAD)	-
1.1.2.6	Polyvinyl alcohol dehydrogenase (cytochrome)	Poly(vinyl alcohol) dehydrogenase, PVA dehydrogenase, PVADH, PVADH-S, PQQ dependent PVA-DH, EC 1.1.99.23 (from 2010), apoenzyme acts on oxiPVA as specific aldolase	pvaA, cytC
3.7.1.7	Beta-diketone hydrolase	Oxidised poly(vinyl alcohol) hydrolase, oxiPVA hydrolase, OPH, OPH hydrolase, BDH	pvaB, bdh, oph
3.1.1.1	Acetylesterase (PVA)	Poly (vinylalcohol-co-vinylacetat) esterase, P(VA-co-VAc) esterase	-

There are different pathways suggested by fungi. For example, *Phanerochaete chrysoporium*, where the ligninolitic peroxidase (LiP) start to degrade PVA by carbonyl groups and double bonds formation, thus increasing the macromolecule unsaturation, and leads to a decrease in average molecular weight by 80%. Also, fungal strain Geotrichum fermentans WF9101 was recognized as an (NAD+) dependent secondary alcohol dehydrogenase, which is not very stable and have a major rule in cleavage of C-C PVA chain (Chiellini et al., 2003). PVA biodegradation pathways are explained in detail in figure 2.4, 2.5 and 2.6.

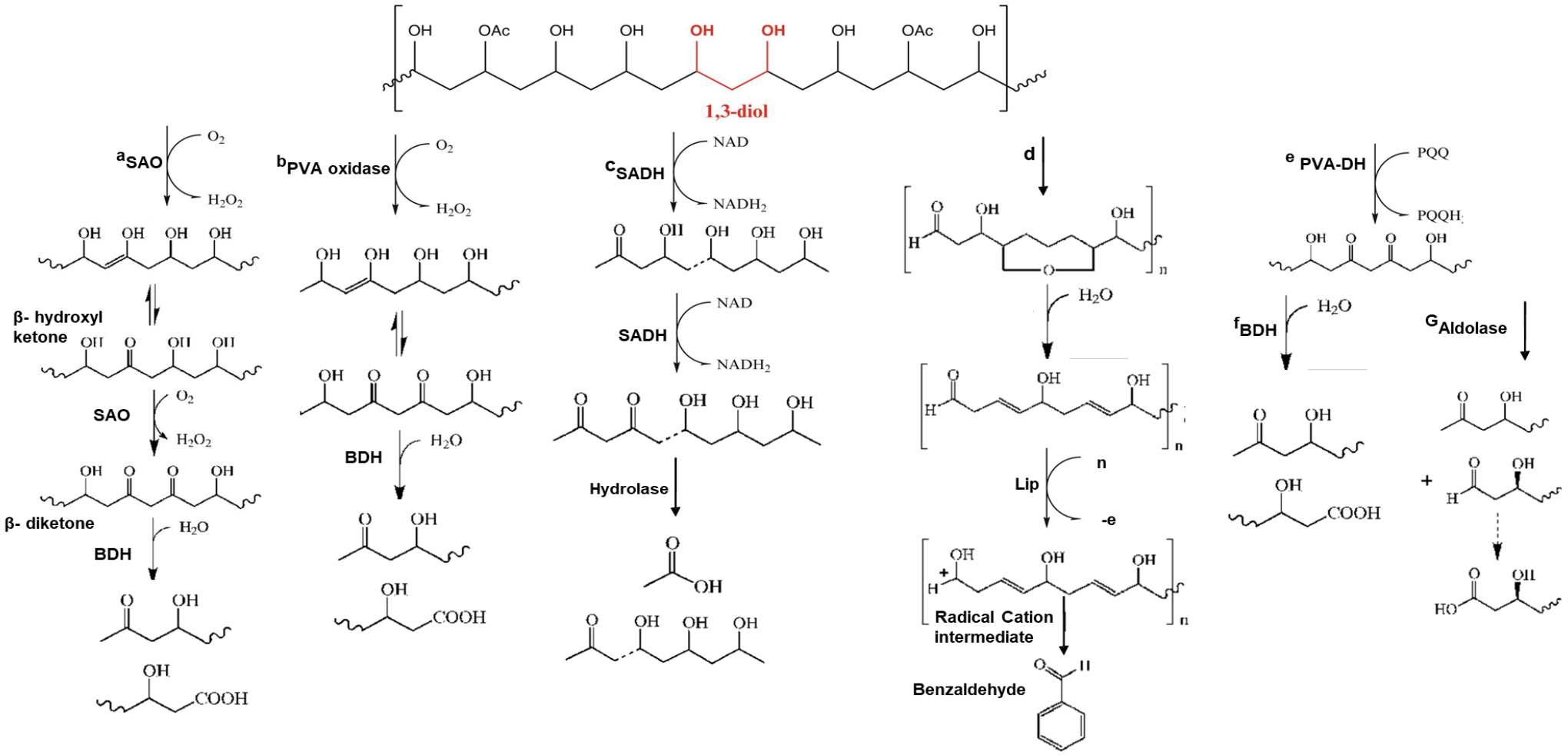


Figure 2.4: PVA Biodegradation pathways mentioned in literature involving extracellular enzymatic activities by different group of strains.

a: Biodegradation ᵃD, and also for Brevibacterium incertum (Sakai et al., 1986)

b: Biodegradation pathway of PVA as mediated by a specific PVA-oxidase and b-diketone hydrolase (BDH), for Pseudomonas borealis O3 strain (Suzuki, 1976).

c: Biodegradation pathway of oligomeric PVA as mediated by secondary alcohol dehydrogenase for Geothricum fermentans (Mori et al., 1996).

d: Biodegradation pathway of PVA as mediated for lignine peroxidase by Phanerochaete chrysosporium (Mejía et al., 1999)

e: Biodegradation pathway of PVA as mediated for different bacterial cultures as PQQ dependent

f: Biodegradation pathway of PVA as mediated by a PVAdehydrogenase PQQ-dependent in symbiotic bacterial culture for Pseudomonas sp. VM15A and VM15C (Hashimato, Fujita, 1985).

G: Biodegradation pathway of PVA as mediated by PVAdehydrogenase from Alcaligenes faecalis KK314 by an aldolase type reaction (Matsumura, Tanaka, 1994).

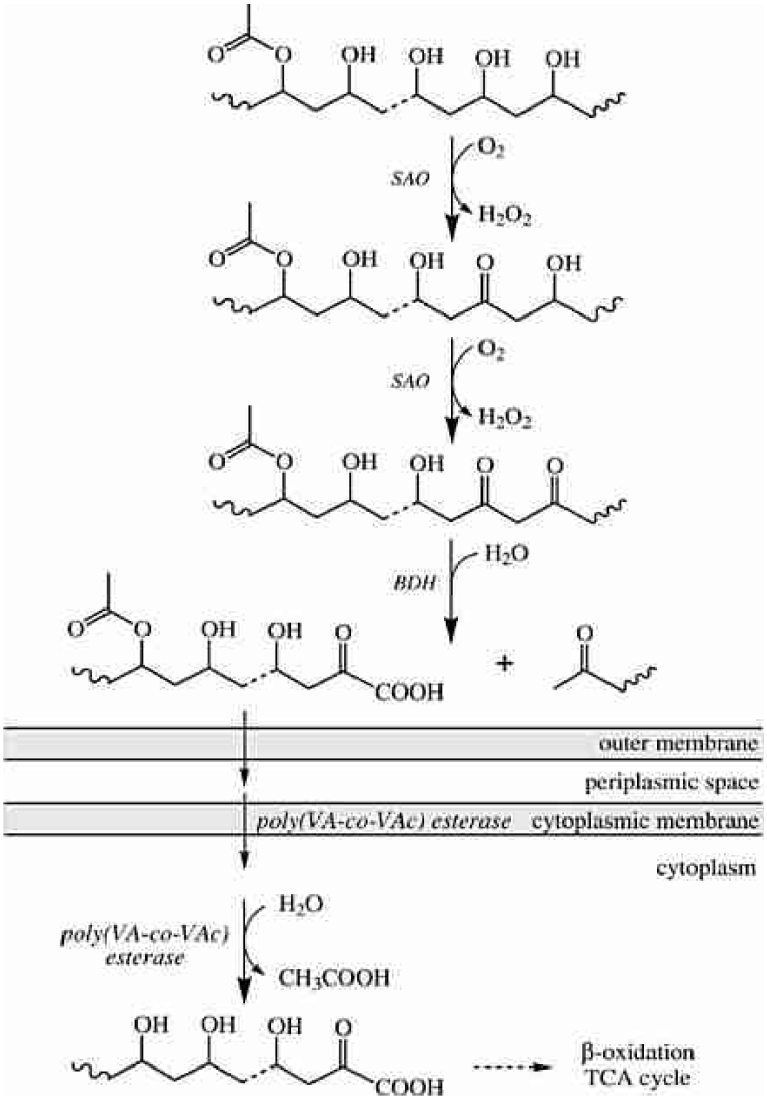


Figure 2.5: PVA Biodegradation pathways for partially acetylated PVA (Sakai et al., 1986).

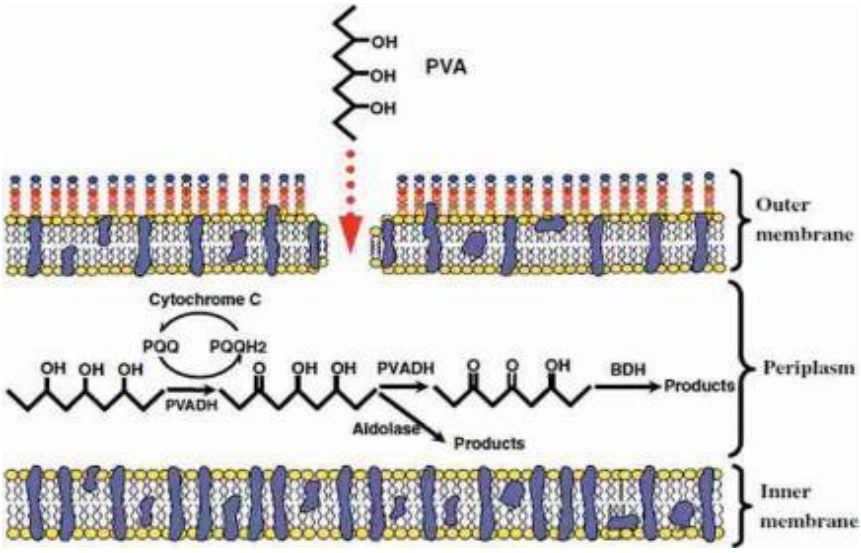


Figure 2.6: PVA Biodegradation include PVA dehydrogenase in the periplasmic space (Kawai, Hu, 2009).

2.6.2 Effects of polymer properties on PVA biodegradability

The properties of polymer structures of PVA affect its biodegradability. These factors effect summarised as following (Chiellini et al., 2003;(Chiellini et al., 2006):

- Degree of polymerization (DP) and degree of hydrolysis (DH) did not significantly influence PVA biodegradation for samples having 20% residual acetyl groups and DP in the approximate range of 10–2,000 and DH of 0.5- 100KDa.
- Degree of hydrolysis (72%) delayed the growth of microorganisms compared with other higher DH. As lower DH means the presence of more acetyl groups esterified with alcohol groups, microorganisms must have a hydrolase for acetyl groups to utilize low DH-PVA.
- The highest hydrolysis rate was recorded in the presence of PVA samples having the lowest molecular weight (MW) and (DH).
- Some results suggested that PVA with acetyl groups was depolymerized to low MW, which was taken up into the cytoplasm and then acetyl groups were removed by the esterase.
- Several research groups have suggested that isotactic PVA is more readily degraded than atactic PVA.
- Increased 1,2-glycol content decreased the catalytic reaction rate of PVA dehydrogenase. This is related to β-ketone formation leading to the split of a carbon chain.
- The syndiotactic portions of PVA were initially depolymerized rather than isotactic portions by *Flammulina velutipes*. (Tsujiyama et al., 2011).

2.6.3 Removal of PVA in wastewater treatment plants

Wastewater treatment plants receive PVA from domestic, commercial or industrial sources. Generally, wastewater passes through primary treatment, biological treatment, and secondary and tertiary treatment before leaving WWTP.

So far, PVA removal has not been widely investigated in primary treatments through bar screen and grit removal and primary sedimentation tank. Sorption to the suspended solids might not be significant (Katsoyiannis et al., 2006), because PVA is a hydrophilic polymer and tends to remain in the aqueous phase more than in solid phase. In some cases, PVA may form gelatinous matters with fats and lipids which might be removed from the aqueous phase, but there is no detailed study explaining the fate of PVA in the primary sedimentation tank.

In Activated sludge systems (ASP), there are several factors controlling the treatment performance. These factors, also called system conditions, including F/M ratio, pH, O_2,

adaptation of microbial communities in ASP, hydraulic retention time (HRT) and others. Since PVA is staying in aqueous phase, HRT have a major rule in degradation.

Schönberger (1997) proved that low F/M ratio, (0.2 to 0.4 kg BOD_5/kg MLSS), the degradation is more that 90% at temperatures above 18 °C. Kumar (2014) investigated the removal of COD, MLVSS, and HRT using steady-state ASP. The colour and COD removal reached 90% and 91% after 36 hr with MLVSS 5000 mg/l at 30°C. Hoffmann (2003) found that unadapted mixed culture was able to degrade PVA after 10 days' lag phase; with PVA degradation of 88 % in 187 ± 25 hours for unadapted sludge, and (20% was degraded in 25 h). For adapted sludge, 90 % was degraded in 29 ± 2 hours.

Sträßner (1995) mentioned the effect of temperature on PVA biodegradation. The optimal temperature was found to be in a range of 26 °C to 30 °C. Taking into consideration the previous studies, it shows that degradation happens by the pure strains within the range of 30-35°C.

Chamoun (2018a/b) investigated the biodegradability of PVA under high temperatures (33-41°C), applying Zahn-Wellens method using sludge from ISWA-LFKW. She found that the optimum degradation was achieved at 31 °C with low or no degradation at temperatures higher than 35°C. She carried out one test which is not sufficient for ultimate conclusions. She also used only one activated sludge.

In secondary sedimentation tank, the studies didn't investigate the evidence of biodegradation, adsorption or volatilization. However, due to the density of PVA, a percent of PVA might settle down and leave the treatment plant. For sand filtration, and comparing PVA to other hydrophilic materials, it is unlikely that sand filtration would have a significant effect on PVA concentrations (Rolsky & Kelkar, 2021).

For anaerobic degradability, Matsmura (1993) proved PVA biodegradability of 60% efficiency using PVA (MW. 2000 and 14000) Rongrong (2011) applied lab-scale hybrid anaerobic baffled reactor to treat textile wastewater (from desizing procedure). The optimum conditions are HRT 5 of days, alkalinity of 500 mg/l, and effluent cycle ratio is 94, the PVA removal was 18%. Russo (2009) tested starch/ PVA blends (90:10, 75:25, 50:50, and 0:100) to measure the extent and rate of plastic solubilisation. At 90:10 ratios, the extent of solubilisation based on COD was 60%, with lower COD for high PVA percentages. He also found that the total solid remained was higher with higher PVA portions, concluding that PVA inhibits the degradation process. Yu (2010) investigated PVA degradation using sequencing batch reactors (SBR).

The SBR mixing had no effect on degradation, while adding nitrate led to 91.8 % PVA reduction by forming intermediates, with very low COD removal. Pseji (2006) investigated biodegradation of PVA blends (PVA/Starch/glycerol) with anaerobic bacterial cultures based on carbon and biogas. The degradation range was 4.1 to 19.8%. High carbon difference was related to biodegradation of starch not PVA. New investigation by Song (2021) showed that PVA could be degraded using symmetrical stream anaerobic bioreactor. This batch bioreacter was used for wastewater containing PVA with concentrations of COD of 3014 mg/l and of PVA of 413 mg/l. The PVA and COD removal efficiencies are 90.7% and 89.4% respectively. In general, anaerobic conditions show low PVA biodegradability compared to aerobic conditions.

He (2013) applied a combined process (integrated ozone biological aerated filters) and membrane filtration for textile wastewater. Results showed that average COD reduced from 100 to 45 mg/l, average BOD_5 from 20 to 7.6 mg/l, average color 50° to 7°, with removal efficiency of 100% for PVA (concentration of 35 mg/l) and 73.4% for UV254.

2.7 Summary

The specialist articles published to date lack investigations on the biodegradation of PVA of under high temperatures; considering ranges (31-49°C). Initial investigations at ISWA showed that it is possible to achieve biodegradation at high temperatures if the process is opitimised. Also, it is very essential from practical prospects (especially in textile industry) to identify in depth the optimum system conditions to biodegrade PVA in activated sludge systems. It is also worth mentioning that many of previous studies related to strains and enzymatic activities but did not investigate applicable aspects and results with respect to real treatment plants with biodegradation aspects.

Therefore, investigating different microbiocoenosis will bring detailed information about optimisation options for the design and operation of wastewater treatment plants, which all were investigated in detail in this project.

3 Materials and methods

3.1 Chemicals & materials

3.1.1 Chemicals

All the solutions used in the experiments were made with water produced from drinking water in the laboratory using an ion exchanger (Seradest SD 2000) and a downstream filter unit (Seralpur PRO 90 CN). Polyvinyl alcohol (PVA) types are according to table 3.1, and in the form of white-creamy powder. Since PVA type 1 was supplied by a textile company, it was applied mainly in all experiments to present the real situation in this study. Other types were applied in PVA spectrometric measurements and biodegradation experiments for molecular weight effect comparisons.

Table 3.1: Polyvinyl alcohol types applied in this research.

PVA Type	Molecular Weight (MW)	Degree of Hydrolysis (%) (DH)	Degree of Polymerisation (DP)	Manufacturer/ Supplier
1	60,000	100%, Fully	-	Lauffenmühle
2	9,000-10,000	80%	-	AIDRICH
3	22,000	97.5-99.5%	500	FlukaChemika
4	88,000-97,000	98-99%	-	AlfaAesar
5	195,000	98-98.8%	4300	FlukaChemika

For PVA measurements, boric acid (H_3BO_3) (100%, EMSURE), iodine (I) (0.05 mol/l, VWR).

For Zahn-Wellens methods, anhydrous potassium dihydrogenphosphate (KH_2PO_4)(>99%, EMSURE), anhydrous dipotassium hydrogen phosphate (K_2HPO_4)(99%, CHEMSOLUTE), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)(99.5, EMSURE), ammonium chloride (NH_4Cl) (>99%, Supelco) , magnesium sulfate heptahydrate ($MgSO_4 \cdot 7\ H_2O$) (>99%, EMSURE), calcium chloride dihydrate: 36.4 g ($CaCl_2 \cdot 2\ H_2O$) (>99%, EMSURE), iron(III) chloride hexahydrate: ($FeCl_3 \cdot 6\ H_2O$) (>99%, EMSURE).

Sludge was obtained from activated sludge basin from ISWA wastewater treatment plant (Bändtale 2, 70569, Stuttgart), and from Cilander (Swiss textile finishing industry) (Cilanderstrasse 19, 9100 Herisau).

3.1.2 Equipment and supplies

All of the following supplies were applied in the biodegradation investigation. Membrane filters (0.45 µm pore size, nylon, Sartorius), 2000 mL bottles (reactors) aerated with Pasteur pipettes, length 150 mm (Assistant No. 40567001), Diaphragm pump with hoses (company: Schego), water bathes (five folds) (company: Memmert, GFL, Louda), desiccator, pipettes, 10 mL and 50 mL for taking samples (company: Hirschmann), paper filter 597 1/2 (company: Whatman), cellulose filter, 45 µm (company: Sartorius), balance (company: Sartorius), drying oven (company: VWR). Also, PVA was determined photometrically (by rectangular cuvette) using a V-550 spectrophotometer from (company: Jasco Deutschland GmbH).

3.2 Preparation of materials

3.2.1 Solutions preparation

For PVA measurements, a solution of 40 g/l boric acid (4%) was prepared using a stirrer with a heating function at 50°C. Also, (10%) iodine solution (0.05 M) was prepared and kept in a refrigerator to ensure the iodine stays fresh and maintains its potency.

For Zahn-Wellens- method, solutions A, B, C, D. Solution A was prepared as in Table 3.2, and pH should be around 7.4. Solution B contains 22.5 g of Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7\ H_2O$) dissolved in one litre of deionized water, and Solution C is made by dissolving 36.4 g calcium chloride dihydrate ($CaCl_2 \cdot 2\ H_2O$) in one litre deionized water. Solution D is made by dissolving 0.25 g iron(III) chloride hexahydrate ($FeCl_3 \cdot 6\ H_2O$) in one liter deionized water.

Table 3.2: Solution (A) applied in Zahn-Wellens method

Anhydrous potassium dihydrogen phosphate (KH_2PO_4)	8.50 g;
Anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	21.75 g;
Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	33.40 g
Ammonium chloride (NH_4Cl)	0.50 g.
Distilled water (filled to)	1000 mL

3.2.2 Sludge preparation

For Zahn-Wellens method (ISO 9888), sludge has to be washed and prepared. After sludge sampling (grab sample from the activated sludge tank of ISWA wastewater treatment plant), the sludge remained for 30 min without stirring/aeration to settle down the solids. After that, the suspended solid and water phases are separated, the water is removed from the bucket using a vacuum tube, and tap water (including minerals) is added to the solid phase. The air pump and stirrer is then turned on for mixing at least 30 min, then the mixing is stopped for 15 min, and water is removed. This procedure was repeated three times at least to remove impurities from the sludge. After the last mixing part, the sludge is left to settle down for 45 minutes, before removing the water. In the end, a sample for measurements of suspended solids is taken and the sludge remained under mixing and aeration until it is used in the tests. This procedure is applied only if the suspended solids is settable (municipal sludge), which was not applicable in the sludge sample from Cilander (sludge from a textile wastewater treatment plant). Cilander sample was not settling down even after more than 120 min, when it is applied directly in biodegradation tests.

3.3 Analytics

3.3.1 Polyvinyl alcohol spectrometric measurements

PVA measurements were according to the PVA method (Finley, 1961) as a basic colorimetric reaction. For 50 mL flasks, 20 mL of boric acid (40 mg/L), PVA fully solubilized solution sample (amount is dependent on concentration), 1 mL of iodine (10%), and filled up to 50 mL with distilled water. After 20 min, the absorption of the formed coloured compounds is photometrically measured, as shown in Figure 3.1. The colours gradually went from light yellow-orange to dark blue as the PVA concentrations increased. The complex is related to the formation of cyclic acetal and cyclic borate groups of PVA (Pritchard, 1972). Cyclic groups help in forming helical crystallization by reducing the flexibility of PVA chain by acetalation or boration, which all lead to form helix conjunction with iodine. Iodine in aqueous solution according to the mentioned reaction generates a quantity of its iodine ions, which provide the matrix to form PVA helix. The mentioned method does not include many details related to absorbance readings related to wavelengths, PVA types, time of reaction, types of water. Therefore, further tests were applied to characterize and clarify these points.

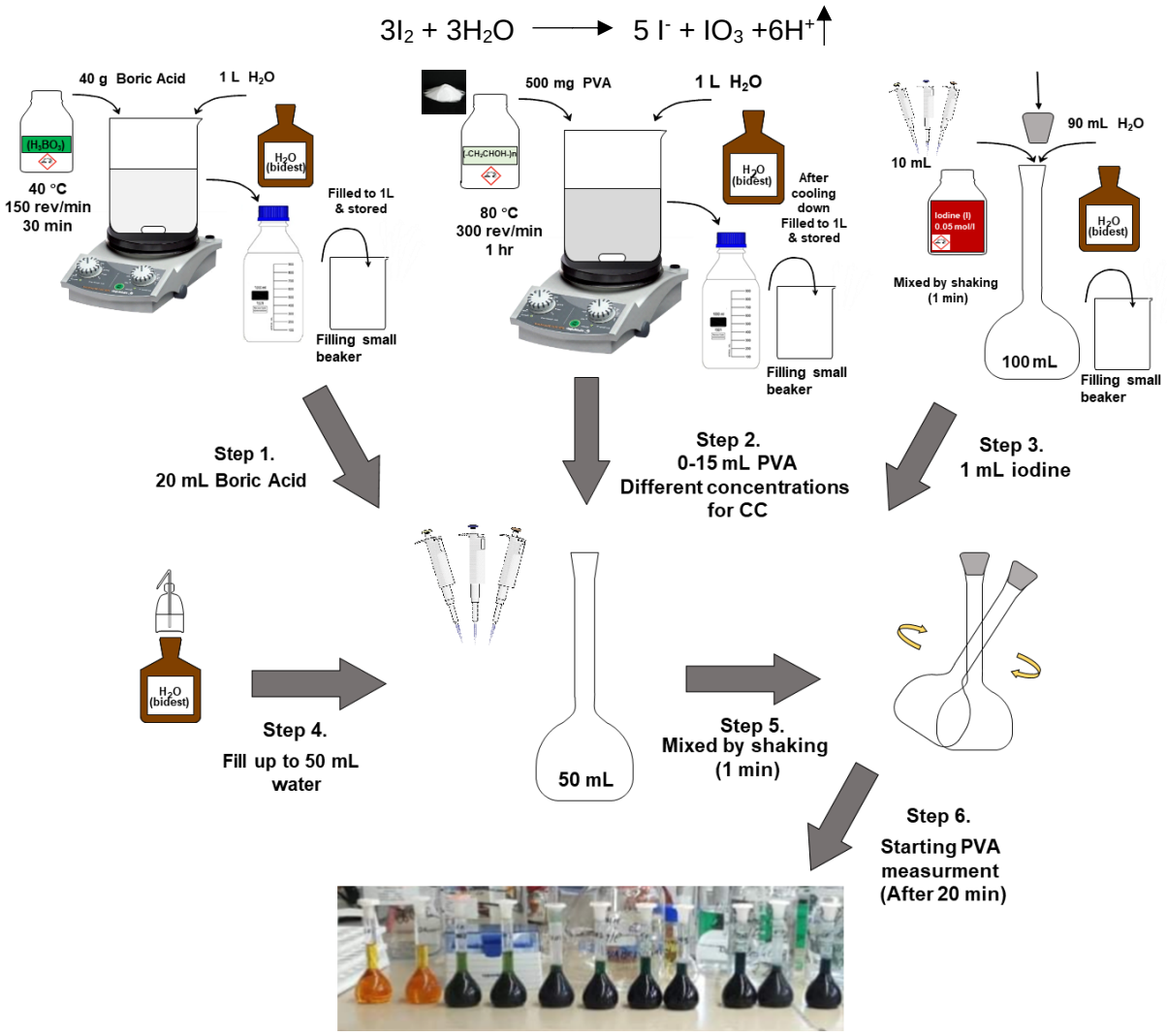


Figure 3.1: Preparation of solutions and PVA sample preparation method.

Because of the existence of the linear relationship between the output value (absorbance) and the sample content (PVA concentration) in many measurement methods, linear regression is usually used. Therefore, Calibration curves (C.C) was applied at different ranges. The measurements based on absorbance using a spectrometer were done according to (DIN 38402-51). The calibration function is as in Equation 1, the slope of C.C as in Equation 2 and the confidence interval as in Equation 3.

$$y = a + b.x \qquad \text{(Equation 1)}$$

$$b = \frac{\sum_{i=1}^{N}(x_i - \bar{x}).(y_i - \bar{y})}{\sum_{i=1}^{N}(x_i - \bar{x})^2} \qquad \text{(Equation 2)}$$

$$\widehat{x_{1,2}} = \frac{\widehat{y_{1,2}} - a}{b} \pm \frac{S_y . t}{b} . \sqrt{\frac{1}{N} + \frac{1}{\hat{n}} + \frac{(\hat{\bar{y}} - \bar{y})^2}{b^2 * \sum_{i=1}^{N} (x_i - \bar{x})^2}} \qquad \text{(Equation 3)}$$

Where,

a **:** calculated blank (ordinate intercept of the calibration straight line).
b **:** sensitivity of the method (slope of the calibration line; coefficient of regression)
x**:** concentration of the standard sample.
$\bar{x}$**:** mean of the standard concentrations x_i
y_i: information value for the concentration x_i
$\bar{y}$: mean of the information values y_i
$\widehat{x_{1,2}}$: concentration of the analytical sample, calculated from the mean of the information values *y.*
$S_y . t$: residual standard deviation obtained by linear regression calculation.
$\widehat{y_{1,2}}$: mean of information values resulting from *n* replicates.
$\hat{n}$: number of replicates.

To ensure the quality of the measurements, the confidence interval is narrowed down by the middle of C.C as shown in graph (Figure 3.2). Therefore, many calibration curves were obtained with ranges (0-4), (0-10), (0-20), (0-50), (0-100), and (0-150). For spectrometric measurements, usually 1 cm cuvette is applied for these measurements. But for the low ranges (4-10-20), due to very low absorbance values, 5 cm cuvette was applied.

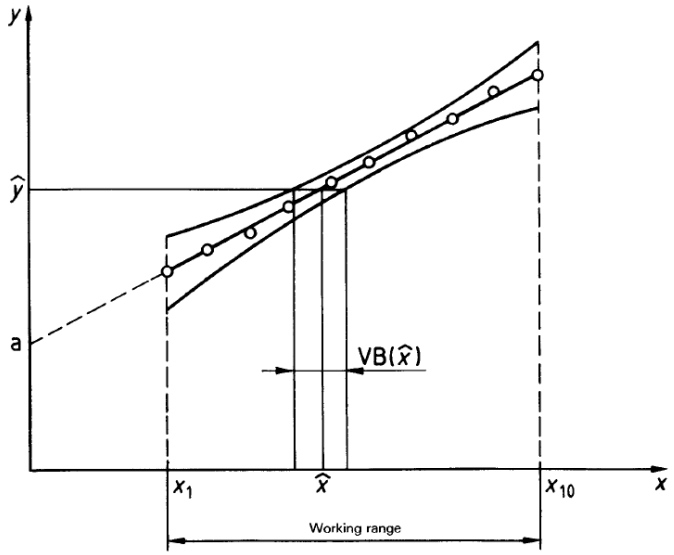


Figure 3.2: confidence interval and working range applied in C.C.

To check the validity of the C.C, an internal quality standard (I.Q.S) is performed. Usually, an independent control sample with known concentration is measured, whereby the

concentration must be within the working range (normally in the middle of C.C) before each series of measurements. The measurement result must not exceed a specified tolerance range (E< 5%) as in Equation 4, otherwise, the control calibration curve or the measurements must be prepared again.

$$\text{Error } (\%) = \left(\frac{X_{IQS} - X_{C.C}}{X_{C.C}}\right) * 100\% \qquad \text{(Equation 4)}$$

Where,

X_{IQS}: the measured concentration of the control sample.

$X_{C.C}$: the concentration in the C.C for the same control sample concentration.

3.3.2 Suspended solids measurements

Suspended solids in the sludge were measured according to ISO 11923. To do so, 0.45 µm membrane filters were dried in the oven for at least 3 hrs at 110°C, stayed in the desiccator for 30 min and finally weighed. Sludge sample (10-20 mL) was pipetted with a sludge pipette from the sludge bucket after washing and placed in the filter on a gas pressing machine. The gas is turned on to force the water to leave the filter and then the solids are retained. After two minutes, the pressing machine is switched off, and the filter is returned again to the oven for at least three hours to be dried, and then the weight is measured as shown in Figure 3.3. Calculation of suspended solids concentration is measured according to Equation 5, and the volume of sludge that would be added to the vessels would be as in Equation 6.

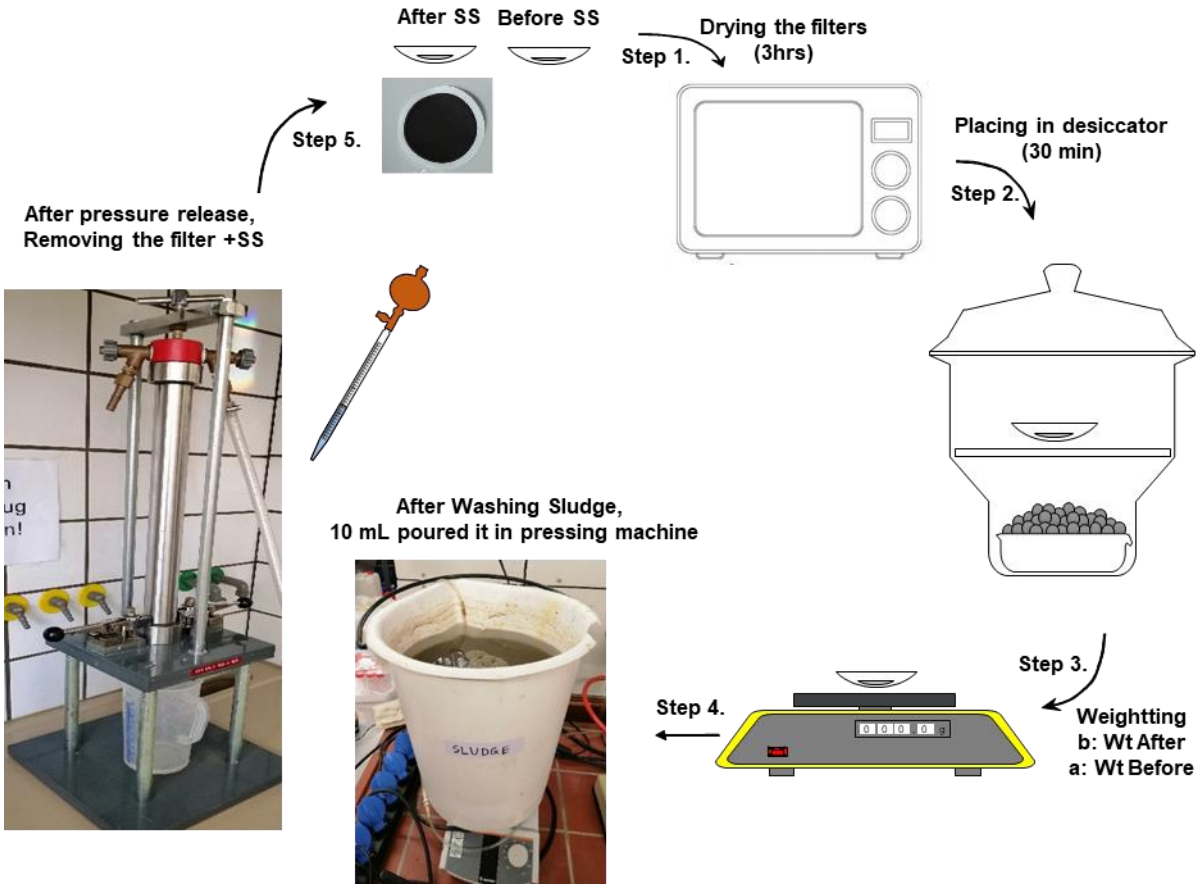


Figure 3.3: Sludge washing procedure.

$$\rho = \frac{1000\,(b-a)}{V} \qquad \text{(Equation 5)}$$

Where,

b: is the mass of the filter after the filtration, in milligrams;
a: is the mass of the filter before the filtration, in milligrams;
V: is the volume of the sample in millilitres. If the sample has been weighed, consider 1 g of mass as equivalent to 1 ml.

$$C_1V_1 = C_2V_2 \qquad \text{(Equation 6)}$$

C_1: measured concentration of SS in the sludge sample.
C_2: applied concentration of SS in the biodegradation test vessels.
V_1: required sludge sample volume to be calculated
V_2: volume of vessels applied in the test (1000 mL, 2000 mL).

3.3.3 Other measurements

DOC measurements were done in accordance with the Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) in Water (DIN EN 1484 – 1997-08). These measurements were performed at Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft (ISWA), and Eurofins Institut Jäger GmbH (located in Tübingen-Germany).

pH is measured using pH meter 315i with pH electrode, WTW 3430 (company: WTW Sentix), and controlled using hydrochloric acid (HCl) and sodium hydroxide (NaOH) solutions. Temperature is measured using a temperature monitoring device which is connected to the laptop, and oxygen is measured using oxygen meter WTW 3430 (company: WTW Sentix). Nitrate, nitrite, ammonium, ortho-, and total phosphate were measured using a rapid test (NANOCOLOR), sulfate (LCK 153), Starch (LCK 357) were determined using Hach quick cuvette tests (thermostat: Hach Lange HT200S, photometer: Hach Lange DR2800). Also, optical density was measured using a spectrometer at 546 nm wavelength.

3.4 Investigation of polyvinyl alcohol spectrometric measurements

To understand the dependencies and improve the accuracy regarding to the determination of PVA, the effects of different parameters were investigated.

Firstly, multiple samples for different PVA types were prepared (molecular weights: 9,000, 22,000, 88,000-97,000, 60,000, 195,000). Also, samples for different types of wastewater (wastewater, sludge water, distilled water) centrifuged and filtered were tested without PVA and without reagents. In addition to that, samples with membrane filter, paper filter, and without filtration were prepared and tested. Also, samples from old PVA stock solution (more than six months old), and fresh PVA solution were compared.

All the samples were prepared using fresh boric acid and iodine, and the spectrometric measurements were performed using a V-550 spectrophotometer as shown in Figure 3.4.

Figure 3.4: Spectrometer used for PVA measurements

The investigated effects of the following parameters on the absorbance are:

- The effect of different PVA molecular weights on the absorbance: many calibration curves were prepared with a working range (0 – 20 mg/L) using 5 cm cuvettes. The equations, slopes, and R-square were determined using linear regression.
- The effect of different spectrometric wavelengths: wavelength range (400-920 nm) was tested for the same concentration of PVA by applying different PVA molecular weight stock solutions, to identify the optimum wavelength for each type. This comparison was also performed for different types of wastewater.
- Reaction time for PVA complex: reaction times (1, 2, 5, 8, 10, 12, 15, 20, 30, 40, 60 min) were tested using individual samples with the same concentration.
- Different types of filtration: different concentrations with range (0-100 mg/L) were prepared for paper and membrane filtrated samples and compared to the sample without filtration.
- Old and new PVA solutions were tested through range (0-100 mg/L) to compare the stability of PVA regarding to time.

3.5 Design of experiments (DoE) for biodegradability tests.

Multi-variable experiments, where the investigations involve the study of the effects of different parameters on the objective or response functions, are a time, cost and efforts consuming procedure. Therefore, the statistical design of experiments is applied.
For the objective of testing the parameters by reducing the number of performed trials and maximizing the information output with minimum experimental effort, understanding of the interactions among the process variables is required (Cox & Reid, 2000). These methods provide efficient solution strategies that significantly increase the effectiveness of the experiments and the quality of the results. It is the mathematical modeling of multi-parameter problems based on empirically determined data.

The aim of statistical experimental design and modelling is to determine the best possible relationship ($z = f(x_1\ x_2,\ \ldots\ .\ x_k)$) between a target variable z (regression point) and several ($k \geq 2$) possible influencing variables x_i (factors) and thus also a significance test of the previously created working hypothesis. For this purpose, the D-optimal design was applied to reduce the number of experiments.

D-optimal plans guarantee the minimization of the forecast or confidence interval of the calculated estimation parameters (regression coefficients, standard errors, etc.). Essential advantages of the D-optimal design of experiments compared to the largely orthogonal Plackett- Burmann or Box-Behnken designs described in the literature are the free choice of the test levels and the independence of the number of tests from the number of levels of the factors. This also reduces the test efforts and increases the extraction of various information from the tests.

From another point of view, the information content of the individual tests depends essentially on their position in the variable range. If the position is unsuitable, parameter and reaction modeling in the chemical and chemical-technical industry specification or model discrimination is not possible. Therefore, the assessment of experimental design is evaluated using specific criteria before and after implementation of the data. Standard factorial or fractional factorial designs need too many attempts for the resources or time necessary for experiments. The design space is constrained (the process space contains factor settings that are not feasible or are impossible to run).

As shown in Table 3.5, the experimental plan for the first phase of the experiments is to specify the optimum conditions for PVA biodegradation using municipal microbiocoenosis; The

variables ($x_1 \ldots x_5$) are considered. x_1 and x_2 are varied and x_3, x_4, x_5 are fixed. The modelling y is carried out with a complete quadratic polynomial with the linear, quadratic and mixed terms that describe the interactions of the setting variables (Equation 7). The results of the planned tests are evaluated using regression analysis methods. The resulting model equations describe the behavior of the target variables in the examined parameter space with a defined accuracy, which allows a scientific interpretation of the estimated values.

Table 3.3: Parameters investigated in the first phase of experiments

Variables (k)	Unit	dimension	Range					No. of values
x_1= Temperature	T	°C	18	23		28	33	4
x_2= F_{PVA}/M	F/M	-	0.01	0.03	0.1	0.2	0.5	5
x_3 Sludge type	S_{ISWA}	-	Constant					1
x_4 pH (mg/L)	pH	-	Constant					1
x_5 Reactor volume	V	-	Constant					1

$$y = a_0 + a_1x_1 + a_2x_2 + a_{11}x_{12} + a_{22}x_{22} + a_{12}x_1x_2 \quad \text{(Equation 7)}$$

$$NV = 1 + 2k\frac{k(k-1)}{2} + FG \quad \text{(Equation 8)}$$

The proposed number of experiments NV results from the number of variables (k = 2), and the degrees of freedom FG required for error estimation as shown in (Equation 8). For lower error expectations, the degree of freedom was assumed to be 6.

To represent the model in the matrix form, as in Equations 7 and 8 was applied using matrix X which includes the coefficient of each variable. X' is the transpose matrix of X and $(X'X)^{-1}$ is the dispersion matrix (or correlation matrix in some references) resulting from the inverse of the multiplication of the two matrices, which is a symmetrical diagonal matrix (Equations. 9 & 10) and applied to calculate the precision of the method (Aguiar et al., 1995). The precision increases with lower $(X'X)^{-1}$.

$$y = X * a + e \quad \text{(Equation 9)}$$

$$a = (X'X)^{-1}X'y \text{ (Equation 10)}$$

For evaluation of the design, many evaluation indicators were calculated to estimate the error and quality of output data. These indicators are summarized in Table 3.4. Also, a regression analysis was applied for the assessment of results.

Table 3.4: Main applied evaluation indicators of DOE (Cox, Reid, 2000),Montgomery, 1997, (Aguiar et al., 1995).

Evaluation Indicators	Description	Value Range	Optimum Values
Condition number	The degree of orthogonality or collinearity of the setting variables, the ratio between smaller and higher singular values of *X*	0-1.0	1.0
D- Criterion/ optimality	maximizing the determinant of the design matrix. This criterion leads to the minimization of the volume of the confidence ellipsoid for the unknown parameters of the regression model. Used to compare different DOE	0-1.0	0.8 - 0.99
G- Criterion/ optimality	minimizing the maximum possible variance of the forecast in a specified forecast range.	0-1.0	0.6 - 0.7
Level occupancy	shows the homogeneity of the test point distribution on the levels of the setting variables, which, in addition to the condition number and the correlation matrix evaluation, represents a strong evaluation criterion of the design matrix.	0-1.0	0.6 - 0.8
Spatial distribution	the desired number of interior points and the number of realized points. The quality of quadratic regression models depends largely on the number and location of the test points in the model space.	0-1.0	1.0
Euclidean distance	The function which is applied for optimization for calculating the best possible test plan based on the planning concept	0-1.0	1.0

The software “DAPO" (design analysis & process optimization) is applied in order to perform the DOE. It is a software for statistical test planning, mathematical modeling and optimization of scientific and technical processes.

3.6 Laboratory test for investigation of polyvinyl alcohol biodegradability

This International Standard (DIN EN ISO 9888; OECD 302B) is applied to determine the aerobic biodegradability of organic compounds or wastewater containing manifold organic compounds using inocula from biological wastewater treatment plants. It is a static aqueous test system, where the test contains an activated sludge as inoculum and the organic test compound or the wastewater as the sole carbon source. The concentration of the test compound added can be determined both substance-specific or by means of DOC. The test period is 28 days for testing a single organic compound and can be adjusted to a shorter testing period for wastewater (in order to simulate the wastewater treatment plant considered). However, for special investigations such as the determination of adaptation times, the Zahn-Wellens method is run longer.

To set the experiment, four vessels are used in each water bath with a specific temperature; two test vessels include the test compound, one reference vessel, and one blank vessel according to DIN procedure. In some experiments, Each vessel contains a certain amount of washed sludge (calculated based on Equation 5). Also, solutions (A, B, C, D) (as in section 3.2.1) which are added as nutrients to avoid any restriction due to nutrient deficiency; For each 1000 mL: 10 mL of solution A, 1 mL of solution B, C, D. Finally, PVA is added in test vessels (with specific concentration), ethylene glycol in reference vessel (known biodegradable water-soluble organic compound) and water is added in blank vessels. In some experiments, additional vessels are added in check the adsorption of PVA to test vessels glass. It contains the same content as test vessels but without inoculum. The experiments were performed in the dark.

According to the DIN procedure, the sludge concentration should be adjusted based on the DOC measurement for the test vessel (for 50 mg/L, 0.2 g/L SS should be applied, and for 400 mg/L DOC, 1 g/L should be applied). This condition was changed and the performed experiments were based on different concentrations of PVA to the fixed concentration of sludge (1 mg/L SS).

The lab was mobilized with all necessary equipment as shown in Figure 3.5, including water baths to control specific temperatures and reduce temperature fluctuation, a thermal sensor to measure temperature for each vessel through time of the experiment, pH and oxygen meters are used to measure them in order to keep them within the range (pH between 6.5 and 7.5 and oxygen between 6 to 8) through the experiment. Also, flasks, aeration pumps sample tubes, vessels, centrifuge, Eppendorf pipettes, hydrochloric acid and sodium hydroxide solutions were present in the lab.

Figure 3.5: Mobilizing the lab for experiments

To start each experiment, the water baths were filled one day before with distilled water to set the temperature precisely and monitor variations, and each of the vessels was filled as mentioned previously, connected to aeration pumps and a thermal sensor. The final set is as in Figure 3.6.

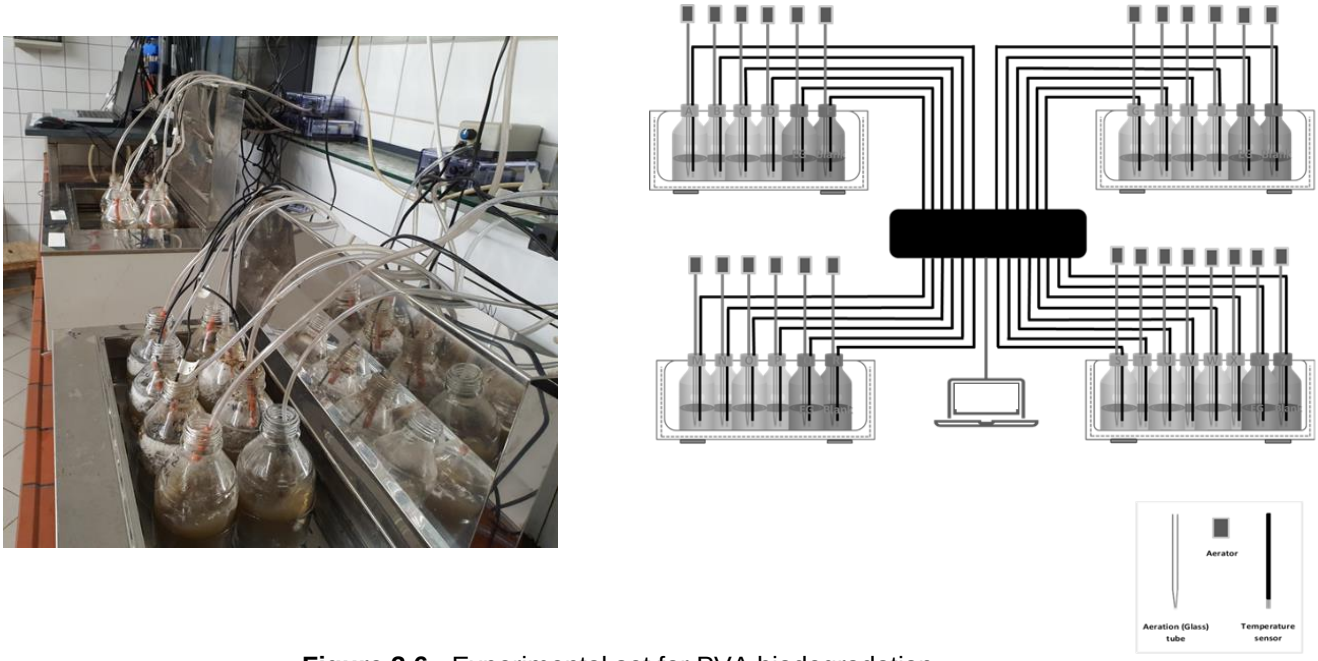


Figure 3.6: Experimental set for PVA biodegradation

Before sampling, the weight is measured for each vessel and the evaporated volume is refilled with distilled water. As shown in Figure 3.7, samples from each vessel were taken in the beginning of the experiment and after three hours, and every day. Each sample was taken to centrifuge (4000 rev/min) for 10 min, filtered with 0.45 µm filter and stored in 100 mL glass vessels for PVA and DOC measurements. In some experiments, three samples per day were taken during the degradation phase to calculate the degradation kinetics precisely.

Figure 3.7: Centrifuge and sample preparation

To represent the results, PVA and DOC degradation curves based on sample measurements throughout the days of the experiment is calculated and drawn according to Equation 11.

$$D_t = \left(1 - \frac{\rho_{cTt} - \rho_{cBt}}{\rho_{cT1} - \rho_{cB1}}\right) . 100 \qquad \text{(Equation 11)}$$

Where,

ρ_{cT1} : is the DOC concentration, in milligrams per liter, at time t1 in the test vessel.

ρ_{cB1} : is the DOC concentration, in milligrams per liter, at time t1 in the blank vessel.

ρ_{cTt} : is the DOC concentration, in milligrams per liter, at time t in the test vessel.

ρ_{cBt} : is the DOC concentration, in milligrams per liter, at time t in the blank vessel.

There are other equations for cases of high adsorbing substances, where DOC concentration can be less (> 20%) after three hours. Since PVA is non/slightly absorbed to sludge, this case was not considered.

3.6.1 Investigation of optimum system conditions for PVA biodegradation (Phase I)

The first phase of experiments includes the investigation on optimum system conditions for the PVA biodegradation based on municipal activated sludge from LFKW treatment plant. Samples from the influent of the primary sedimentation tank, activated sludge, and effluent of the treatment plant were checked several times, and the PVA concentration was less than 2 mg/L.

The investigated parameters are based on temperature and concentration of PVA and corresponding (F/M ratio), as shown in Table 3.5. The sludge applied in this experiment was from LFKW treatment plant- ISWA.

Table 3.5: Parameters investigated in the first phase of the experiments

Parameter	Range	Fixed / varied
pH	6.5 – 7.5	Fixed
O_2 (mg/L)	5 – 8.5	Fixed
Sludge type	1 (non-adapted)	Fixed
Temperature (°C)	18 – 23 – 28 – 33	Varied
F_{PVA}/M	0.01 – 0.03 – 0.1 – 0.2 – 0.5	Varied

For this purpose, four water baths were set for the tested temperatures (18-33°C) one day before starting the experiments. All the temperatures were set with ± 0.5 °C based on the temperature monitoring system. Also, fresh solutions of PVA (molecular weight 60,000, fully hydrolyzed) at concentrations of 500 mg/L, and nutrient solutions (A, B, C, D) were prepared and applied in this experiment.

The number of experiments was reduced according to the design of experiments from 20 different sets to 12 sets. Each set was prepared in duplicates (two test vessels according to DIN- not including reference and blank vessel). Each set of experiments took different time periods, depending in temperature mainly. In general, the time for each experiment was 15 - 20 days.

3.6.2 Estimation of degradation kinetics

Biodegradation kinetics refers to the study of how organisms break down and transform complex organic compounds into simpler molecules, ultimately leading to their complete mineralization into carbon dioxide, water, and other inorganic compounds. The kinetics of biodegradation involves the measurement of the rate at which biodegradable compounds are transformed by microorganisms in different environmental conditions. It is estimated by different mathematical expressions, which gain more complexity with including more variables that can affect the biological removal of organic compounds in the aquatic environment.

The biodegradation kinetics of PVA typically follows first-order kinetics, which means that the rate of degradation is proportional to the concentration of PVA remaining. The biodegradation in the batch test can be described mathematically as a pseudo-first-order degradation (Abegglen, Siegrist H., 2012) as shown in Equation 12.

$$\frac{dCi}{dt} = K_{bio} * SS * C_i \qquad \text{(Equation 12)}$$

Where,

$\frac{dCi}{dt}$: degradation rate of substance (mg/(l·d)).
K_{bio}: biological degradation constant for substance i (L/(gSS d)).
SS : sludge concentration in the reactor (gSS/L).
C_i : concentration of substance i (mg/L).

Also, The Michaelis-Menten model can be used to estimate the biodegradation rate of organic compounds in different environments, such as soils, sediments, and wastewater treatment systems. It is used to describe enzyme-catalyzed reactions, while the pseudo-first-order kinetics model describes the degradation of a substance by a first-order reaction. These two models are distinct and describe different types of reactions, but it is possible to apply the pseudo-first-order kinetics model to enzyme-catalyzed reactions to obtain a pseudo-first-order rate constant (Boyer, 2012)

The following equation can express the pseudo-first-order kinetics model:

$$\ln(C_t) = \ln(C_0) - kt \qquad \text{(Equation 13)}$$

Where,
C_t: is the concentration of the substance at time t (mg/L), C_0 is the initial concentration of the substance (mg/L). k: is the pseudo-first-order rate constant (d^{-1}). t: is time (d). As shown in Figure (3.9), k is calculated based on the slope of the natural logarithm of concentration.

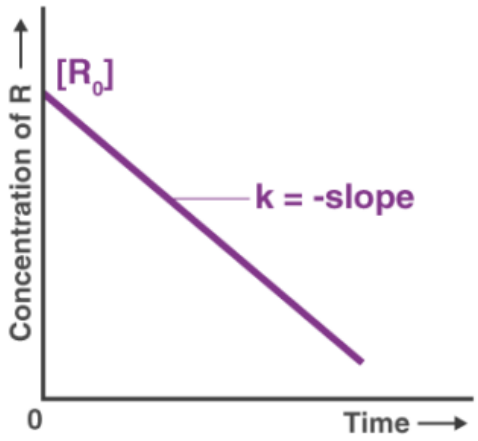


Figure 3.8: Graphical representation of Michalis Menten relationships

First-order rate constants can be used to calculate a half-life ($t_{1/2}$) for the test substance using the relationship:

$$t_{1/2} = \frac{\ln(2)}{k} \qquad \text{(Equation 14)}$$

However, it should be considered that when the half-life concept is applied in biodegradation, the rate can be limited by the rate of entry of substrate into the cell.

The calculations were The degradation constants were calculated according to the three methods and summarized in the results and discussion part.

3.6.3 Regression analysis for kinetic results

Regression analysis is a widely applied statistical technique to identify and predict the relationship between variables; a dependent variable and other (one or more) independent variables. One popular approach to performing regression analysis is the least squares regression analysis, which involves minimizing the squared differences sum between the observed and predicted values. However, this method is sensitive to outliers, which can significantly affect the model's accuracy. In contrast, robust least squares regression analysis is designed to handle outliers and provide a more accurate model.

The goal of this method is to find the line of best fit. The method assumes that the errors are normally distributed and have constant variance. Simple linear regression involves only one independent variable, while multiple linear regression involves two or more independent variables. The model is typically represented as in equation 17:

$$y = b_0 + b_1x_1 + b_2x_2 + \cdots b_kx_k + e \quad \text{(Equation 17)}$$

where y is the dependent variable, x_1, x_2, …, x_k are the independent variables e is the error term and b_0, b_1, b_2,.. b_k are the regression coefficients.

Geometrically the regression coefficients have the same interpretation as in the bivariate case - slopes with respect to the corresponding variable. When there are two predictor variables, the linear regression is geometrically a plane in 3-space, as shown in Figure 3.10.

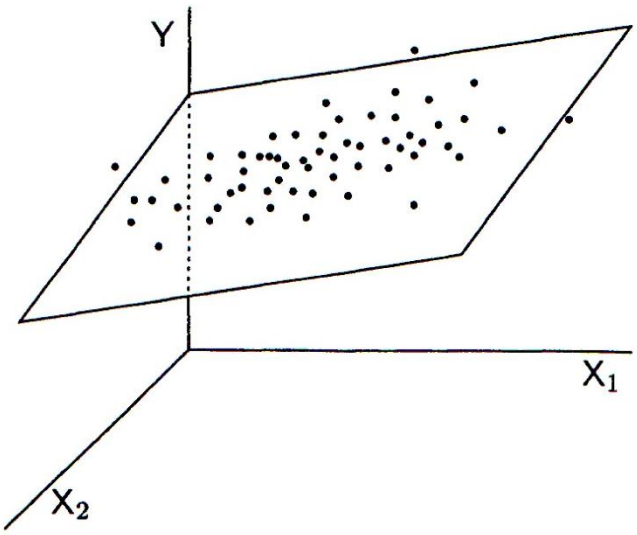


Figure 3.9: 3-D Regression of y on two variables (x1 and x2) in variable space (Magwene, 2020).

In most cases of regression, standard bivariate and multiple regression assumes that the predictor variables (x_1, x_2,,…) are observed without error. That is, uncertainty in the regression model is only associated with the outcome variable, not the predictors (Magwene, 2020). Also, If the explanatory variables (x_1, x_2,… x_k) are highly correlated, then the regression solution can be "unstable" – a small change in the data could lead to a large change in the regression model.

Both least squares and robust least squares methods were applied to assess and represent the results input regarding to k values in terms of temperature and concentration and to localize the optimum system conditions range.

3.6.4 Investigation of the effect of adaptation on PVA degradation under high temperatures (Phase II)

Based on the first phase of experiments, the optimum temperature and concentration were applied in the second experiment. This experiment is based on two stages; the first stage is based on starting five water baths with the same temperature and concentration for the test vessels, waiting for adaptation and first degradation phases to occur. After that, the suspended solids are measured again for each vessel, PVA is added based on suspended solids, to keep F_{PVA}/M ratio fixed for all testing vessels. The experiment in the second stage were reset again to determine biodegradation behavior. The temperatures are raised from optimum temperature four higher temperatures and one with the same temperature, as explained in Table 3.8.

Table 3.6: Parameters investigated in the second phase of experiments

Parameter	Range	Fixed / varied
pH	6.5 – 7.5	Fixed
O_2 (mg/L)	5 – 8.5	Fixed
Sludge type	1 (non-adapted)	Fixed
Temperature (°C) (1st stage)	28	Fixed
Temperature (°C) (2nd Stage)	28-36-38-40-42	Varied
F_{PVA}/M	0.1	Fixed

As applied in previous phase, samples were collected in the beginning, after 3 hours and at each day of the experiment. Additional samples were also collected every eight hours in the

degradation phase of first stage, after starting the second stage after 3hrs. The number of sets tested for this experiment were 5; each set was in duplicates. Each set of experiments lasted for 30 days.

3.6.5 Investigation on effect of adapted sludge to biodegradation of PVA

In this set of experiments, sludge was collected from Cilander AG (textile finishing industry in Herisau/Switzerland). The aeration pool receives PVA frequently and in high concentrations (300-500 mg/L), and the temperature range (35-40°C). Parameters were measured, including pH, O_2, SVI, and suspended solids. Because the sludge wasn't settling down (tested after 30 min, 1 hr, 2 hrs), washing the sludge were not possible to avoid losing the solids.

The same parameters were investigated as mentioned in table 3.3, but there was only one stage to be applied, since the sludge is adapted to PVA degrading and there is lag phase. The number of sets were 5, each set was in duplicates, and the experiment lasted for 3-4 days.

3.6.6 Investigation on effect of molecular weight of PVA in biodegradation

For this set of experiments, different types of PVA as mentioned in table 3.1 were applied to investigate the effect of different molecular weights in the adaptation and kinetic of biodegradation of PVA. For this purpose, 500 mg/L of fresh stock solution was applied for each type was prepared. With higher molecular weights, the temperature required to make the solutions were higher (>80 °C). for each type of PVA a certain calibration curve was prepared for measurements.

Five water baths were run at a temperature of 28°C, and PVA concentration in test vessels was 100 mg/L, SS of 1 g/L, and all parameters were measured as in the previous experiment. The number of sets were 6, and the experiment lasted for 10 days.

3.6.7 Investigation on threshold concentration for adaptation of sludge to PVA biodegradation

Threshold concentration is the concentration of a substance that initiates a response. In case of PVA biodegradation, it is the minimum concentration that is necessary for the microbiocoenosis to adapt and initiate the biodegradation. For this purpose, minimum

concentrations of PVA were applied in this experiment, as shown in Table 3.7. For this purpose, different PVA calibration curve was applied with a specific very low range. Also, higher samples volumes (100 mL for each vessel were collected), since higher sample amount is applied to PVA measurements (25 mL for each flask). The number of sets of experiment were 4 and the experiment lasted for 10 days.

Table 3.7: Parameters investigated in the threshold experiments

Parameter	Range	Fixed / varied
Temperature (°C)	28	Fixed
PVA concentration (mg/L)	1 – 2 – 5 - 10	Fixed

3.7 Further investigations for characterisation of adapted sludge

After initial evaluation of the adapted sludge (Cilander – Switzerland), and based on high biodegradation kinetics under high temperatures, further investigations were performed in order to obtain more specifications about the microbiosnosis. Preliminary investigation includes SVI test, O_2, pH, wastewater characteristics, microscopic examination and Solids concentration.

3.7.1 Proof of biological activity

This aspect was investigated based on the examination of the inoculum at different conditions. For this purpose, three biodegradation tests were performed. For the first one, 500 mL in 1 L vessel of Inoculum was autoclaved for 20 min at 121 °C in a pressure of 15 psi. Also, another 500 mL in 1 L container of inoculum was centrifuged (4000 rev/min for 10 min), then filtered using a 0.45 µm cellulose filter and autoclaved at the same conditions. The third inoculum was applied as fresh active inoculum. The applied temperature was 40°C, and the concentration of PVA was 100 mg/L.

First inoculum was applied to prepare abiotic sludge based on heat sterilization to verify if the biological activity is inhibited and to estimate PVA mineralization by the change in concentration due to sorption to dead sludge. Second sample was applied to prepare sterilized supernatant and to estimate PVA concentration change due to chemical reactions (oxidation, hydrolysis, etc). Third inoculum was applied directly as fresh sludge to estimate normal biodegradation of PVA at 40°C. The test lasted for 6-7 days and final comparison was made between the three samples.

3.7.2 pH range experiment

In this set of experiments, pH range (5,6,7,7.2,7.5,7.75,8,9) was applied in experiments set. To perform these tests, 8 sets (16 test vessels were prepared). Each set has a pH value close to the value in the range. pH was controlled using HCl and NaOH. Since the sludge is adapted, the PVA degradation started at the beginning of the test. For this purpose, pH was continuously monitored for the first 3 hours.

During the experiment, the pH values were always shifting toward 7.7 ??, and to achieve accurate results, only three hours was considered based on the degradation rate. The applied temperature was 40 °C, and the PVA concentration was 100 mg/L.

3.7.3 Limits of temperature

In this experiment, 45 and 49 °C were tested for PVA biodegradation to verify the temperature limits this sludge can achieve degradation. Consequently, 2 sets were applied with 100 mg/L, and this experiment lasted for 14 days.

3.7.4 Limits of concentration

To test the limits of concentrations that this sludge could achieve biodegradation in, a range of concentrations (60, 100, 200, 500, 1000, 1500, 2000 mg/L) were applied with 1 g/L SS. To perform it, 7 sets of experiments were investigated at 40 °C. for PVA measurement, different calibration curves and different sample volumes were applied to achieve accuracy in measurements. Samples were taken three times in the first day to determine biodegradation kinetics precisely. This experiment lasted for 4-13 days, depending in concentration.

4 Results and discussion

4.1 Investigation of polyvinyl alcohol spectrometric measurements

The determination of polyvinyl alcohol (PVA) by the formation of the yellow orange to dark green complex method were tested statistically based on DIN38402-51 as mentioned in materials and methods. To achieve accuracy, different calibration curves (C.C) were applied using 1 cm and 5 cm cuvettes, with minimum of five calibration points which is evenly distributed over the working area, and meeting the calculation of the confidence interval by applying values in the middle of C.C and considering the limit of detection and limit of quantification in the executed measurements. In general, fresh solutions of boric acid and iodine are applied to test the validity and accuracy of measurements, as in the following sections.

4.1.1 Effect of different molecular weights in absorbance

Correlation between the concentration of PVA and absorbance was found to be linear in different ranges (tested between 0 till 200 mg/L) and using different types of PVA. For this comparison, a range of 0-20 mg/L using 5cm cuvette were applied for different PVA calibration curves. It was found the PVA with lower MW. (9000-10,000) shows the highest absorbance compared to other PVA types. Also, the higher MW, the lower extinctions found. PVA with MW. 60,000, 195,000 and 88,000-97,000 show slight differences with lowest extinctions to last type as shown in Figure 4.1. variations in concentration by applying certain extinction value reach more than 60%; which explains the considerable effect of MW. for PVA types in measurements. From other point of view, this method proves that when PVA is degraded using different degradation methods, it can only detect PVA and not intermediate products after cleavage of C-C bond to shorter monomers chains, since lower MW. PVA shows remarkably higher extinction the higher MW.

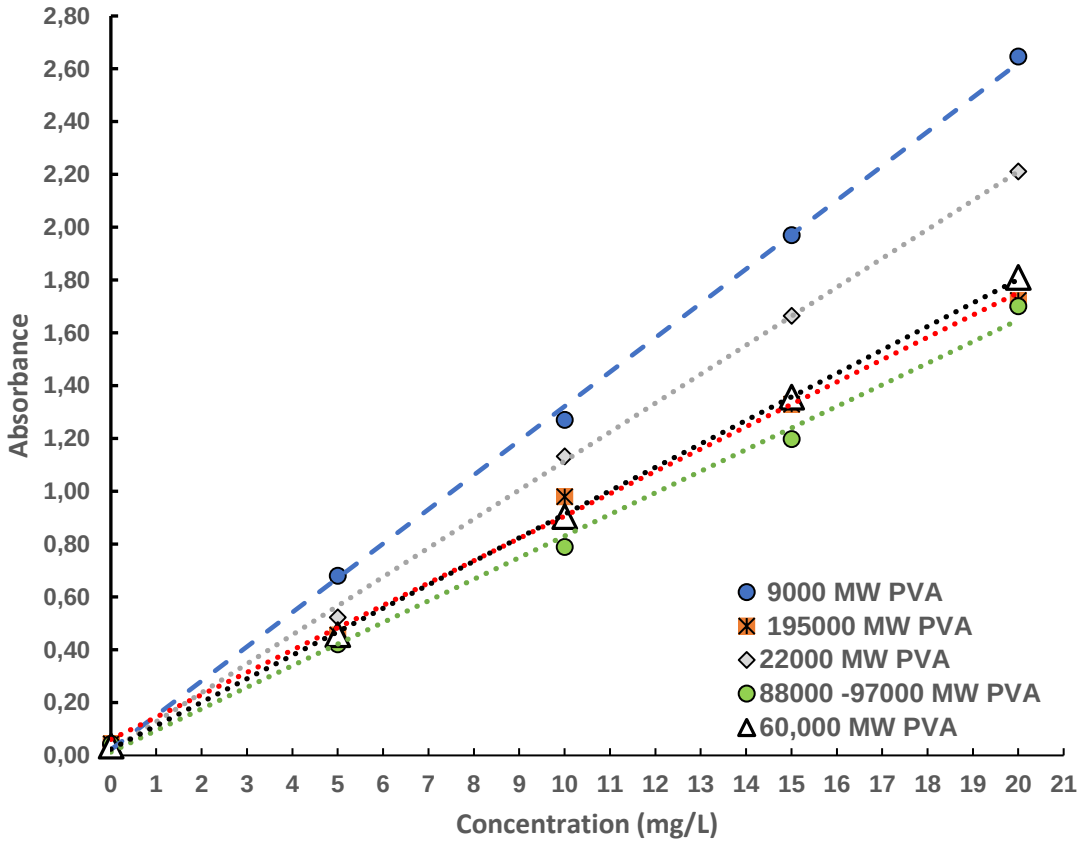


Figure 4.1: Absorption Spectra of different types (MW) of PVA complex.

As shown in Table 4.1, calibration curve parameters including the slope and y intercept was determined. The slope decreases with increase in MW. The coefficient of determination (R^2) shows goodness of fitting of observed points to the linear regression. Also, Limit of detection (LOD) and limit of quantification (LOQ) were estimated based on (Kromidas, 2011). It shows different values based on MW. between 0.03 and 0.67. Compared to Joshi (1979) , LOD were between 0.14 and 0.47, and Pitchard (1972) were 0.4 mg/L. Further details and Anova statistical tests are in Appendix (1)

Table 4.1: summary of parametric details of calibration curves for different types of PVA.

Analysis Parameters	PVA (MW. 9,000)	PVA (MW.22,000)	PVA (MW.60,000)	PVA (MW.88000)	PVA (MW.195,000)
Y- intercept (a)	0.0219	0.0180	0.0235	0.0126	0.0606
Slope (b)	1.2999	0.1097	0.0889	0.0818	0.0846
R^2	0.9991	0.992	0.9998	0.9995	0.991
Standard error (SE)	0.0272	0.0232	0.0088	0.0376	0.0381
Standard deviation (SD)	0.0122	0.0104	0.0039	0.0168	0.0171
Limit of Detection (LOD)	0.0309	0.3128	0.1463	0.6785	0.6653
Limit of Quantification (LOQ)	0.0935	0.9479	0.4432	2.0562	2.0160

4.1.2 Optimum wavelength for different types of PVA.

PVA complexes were prepared at certain absorbance (1.4) and corresponding PVA solutions applied (1.4 – 1.6 mL of 500 mg/L PVA solutions). Since alcohol groups of partly hydrolysed polymer (residual acetate groups) affects the absorbance, wavelengths that shows higher absorbance correlate approximately with their stability (Joshi et al., 1979).
The Absorbance spectra of different types of PVA shows in general similar shape. The peaks were found at 630 for PVA with MW. 60,000, 660 for PVA with MW. 88000 and 680 for PVA with MW. 195,000 and 22,000, as shown in Figure 4.2. The difference between the maximum found are 10, 30, and 60 nm compared to Finley (1961) and Pitchard (1979). In order to minimise interference of excess iodine and with regard to width of peaks, and to produce comparable results, 680 nm wavelength was applied in experimental measurements.

In addition to that, different types of water (without reagents) were applied. It was found that no peak in any matrix spectrum in the same ranges (620-700 nm). Only sludge water shows higher extinctions via different wavelengths as shown in Figure 4.3, because the sludge water has higher extinction without reagents.

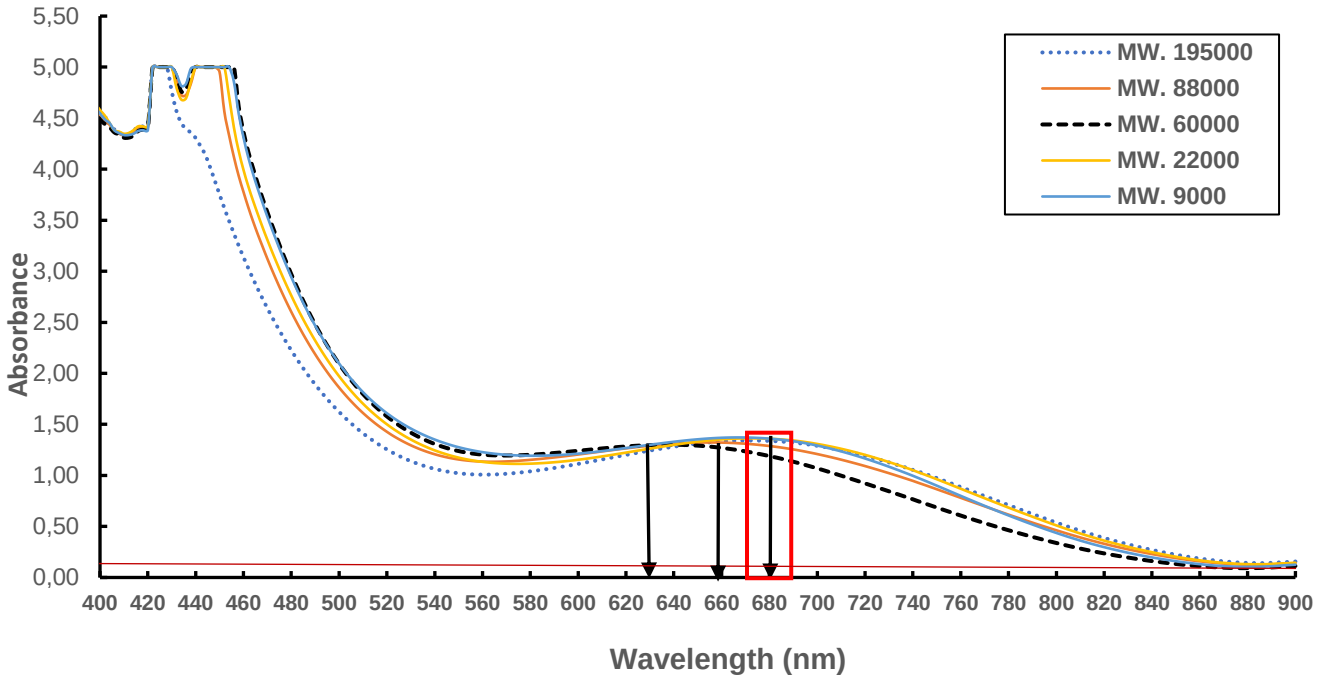


Figure 4.2: Absorption spectra of different types (MW) of PVA complex.

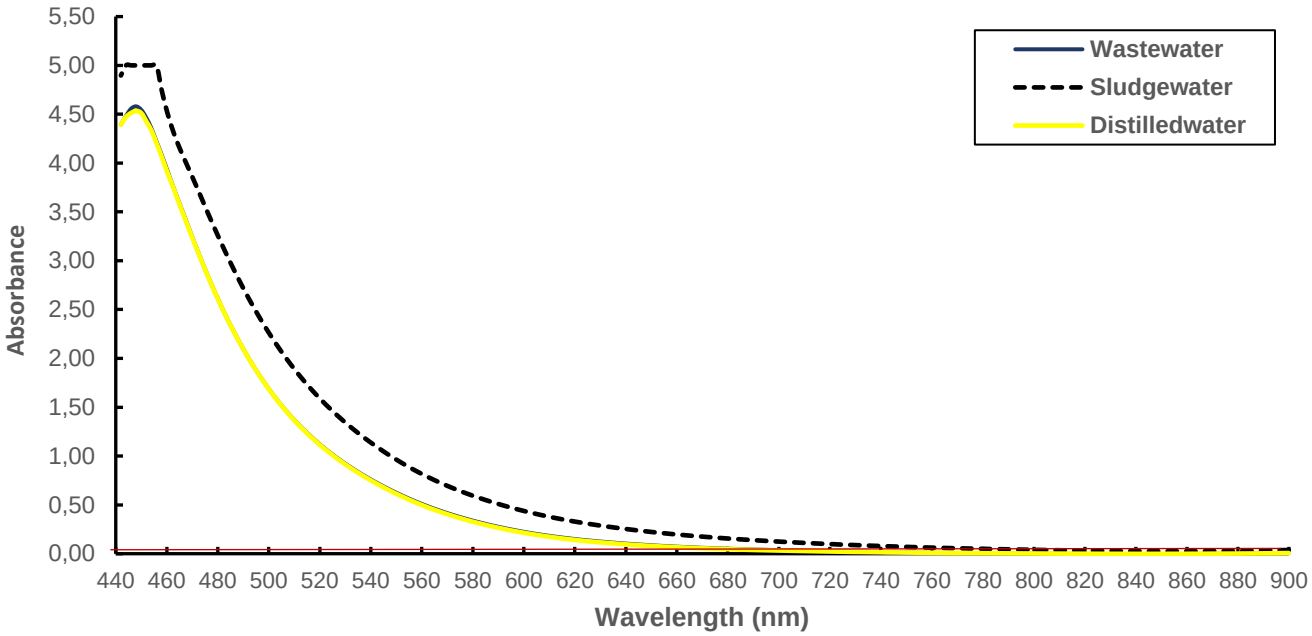


Figure 4.3: Absorption spectra of different types water applied.

4.1.3 Effect of reaction time on absorbance

After mixing reagents with samples using same concentration of PVA, several measurements were made based on time after mixing. After 1min, more than 87% were observed with higher increase between 10 and 20 min. Maximum absorbance appeared after 20 min of the reaction as shown in Figure 4.4. compared to Procházková (2014), 20 min reaction time was chosen to be applied in measurements.

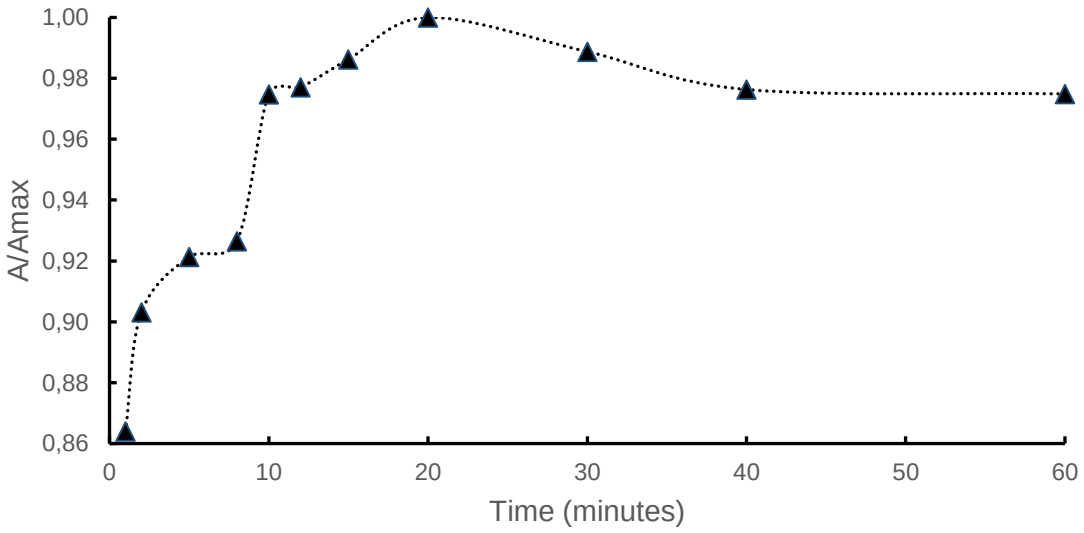


Figure 4.4: Absorbance ratio of PVA at same concentration through time.

4.1.4 Effect of different filtration methods

Filtration effect were tested in the range of 0 -100 mg/L for 60,000 MW. PVA. Variations between filtered and non-filtered PVA samples were found to be negligible; taking into consideration solubilizing PVA under high temperature (above 80°C). the test was performed in duplicates and the results are matching. Differences between non-filtered and paper filtered are between 3-8%, and between non-filtered and membrane filtered samples are between 2-4.5 %. Procházková (2014) found the same with paper filter, but more differences in membrane filter (20%), which is related to sorption of PVA to the membrane. This difference is related either to the solubility of PVA applied in that case, or due to higher molecular weight (130,000 compared to 60,000).

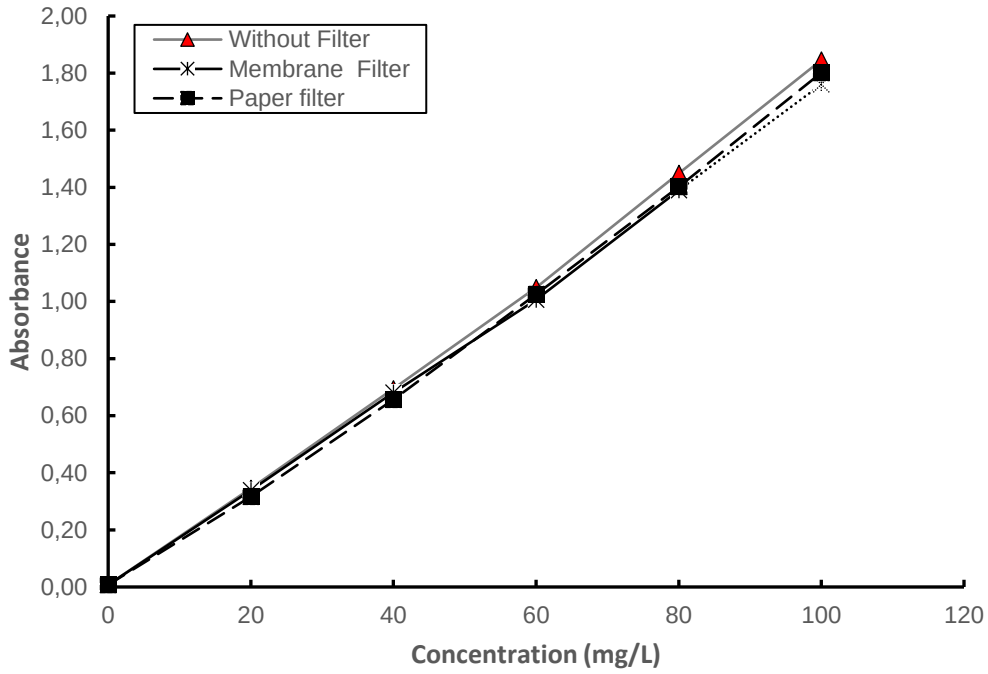


Figure 4.5: Absorbance measured for different filtration types applied to PVA solution.

4.1.5 Effect of PVA age on absorbance

PVA solutions (60,000 MW - 6 months old and 1-day fresh solution) in range 0 -100 were applied to determine if there are any reduction in PVA concentration overtime. It was found that there is not significant difference between old and new PVA solution, which concludes that fully soluble PVA is stable (non-volatile) over time as shown in Figure 4.6.

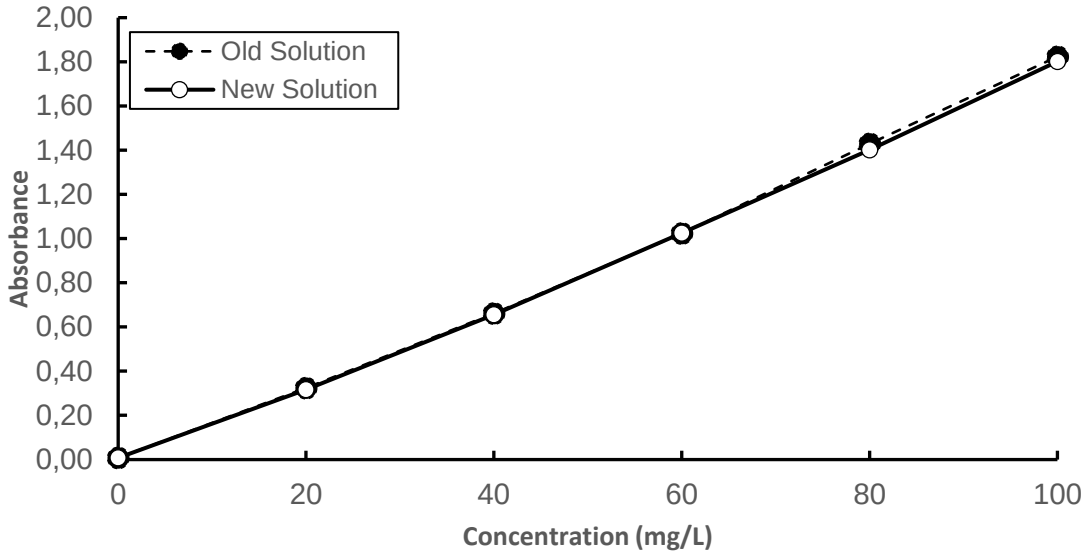


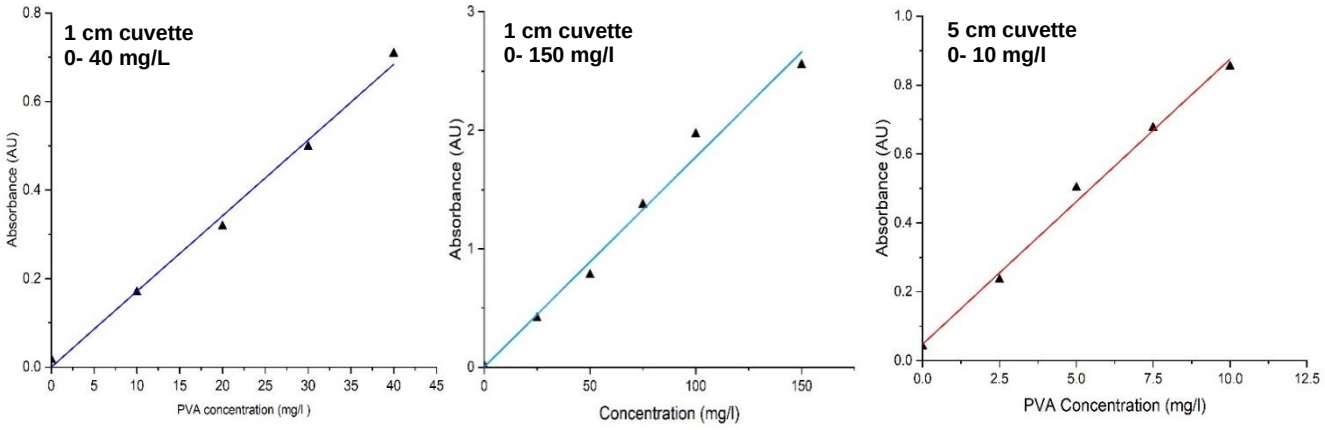
Figure 4.6: Old and new PVA solutions absorbance.

4.1.6 Summary

Optimum wavelength for PVA measurements were varied between 630 and 680 nm depending on PVA type. It was also found that sludge water shows higher extinctions compared to wastewater or distilled water. To locate optimum reaction time, peak was found after 20 min. ot was also proved that there is minimum effect of filtration using different types of PVA, if it is fully soluble in water. PVA solution is also stable over time, since there is no change in concentration over 8 month testing period.

To ensure highest accuracy with minimum error, different ranges of CC applied as shown in Figure 4.7. IQS check was performed before and after each measurement and was less than 5%.

Since there are different concentrations of PVA (either same type of different types), various calibration curves were applied with different ranges and different cuvettes as shown in figure 4.7. Internal quality standard was applied before and after all measurements and it ranges was less than 5%. Since DOC method couldn't detect very low concentrations related to PVA, this method can detect till 1 mg/L PVA concentration in water.



Equation	y = a + b*x
Plot	Absorbance
Weight	No Weighting
Intercept	-1.5E-4 ± 0.01837
Slope	0.01711 ± 7.49978E-4
Residual Sum of Squares	0.00169
Pearson's r	0.99713
R-Square (COD)	0.99427
Adj. R-Square	0.99235

Equation	y = a + b*x
Plot	Absorbance
Weight	No Weighting
Intercept	0.00241 ± 0.08702
Slope	0.01773 ± 0.00105
Residual Sum of Squares	0.06425
Pearson's r	0.99306
R-Square (COD)	0.98617
Adj. R-Square	0.98272

Equation	y = a + b*x
Plot	Absorbance
Weight	No Weighting
Intercept	0.04799 ± 0.02259
Slope	0.08272 ± 0.00369
Residual Sum of Squares	0.00255
Pearson's r	0.99703
R-Square (COD)	0.99407
Adj. R-Square	0.99209

Figure 4.7: Different calibration curves applied in PVA measurements.

4.2 Design of experiments (DoE) for biodegradability tests.

D-optimal design of experiments was applied to reduce the number of experiments for the first phase, to determine the optimum system conditions for PVA biodegradation using municipal sludge. The total number of sets of experiments was 20 and reduced to 12 as shown in Table 4.2.

The diagnostic parameters, in particular the condition number, level occupancy and the spatial point distribution confirms a very good planning quality (all values are above 95%, The G-efficiency is close to optimum values (0.6-0.7)) as an essential prerequisite for an equally good quality of the regression result as shown in Figure 4.7.

Table 4.2: D-optimal design of experiments result.

Nr	F/M-ratio x_1 []	Temper. x_2 [°C]	Plan diagnostics
1	0.01	23	Condition number HK = 0.9997
2	0.01	18	
3	0.03	23	Defficiency D = 97.8 %
4	0.03	28	G-efficiency G = 56.1 %
5	0.10	28	
6	0.10	33	Interior points IP = 4
7	0.20	28	
8	0.20	18	
9	0.20	33	Level allocation [2 2 2 3 3]
10	0.50	33	3 3 3 3
11	0.50	18	Correlations matrix.
12	0.50	23	[1 0.0119]
			0.0119 1

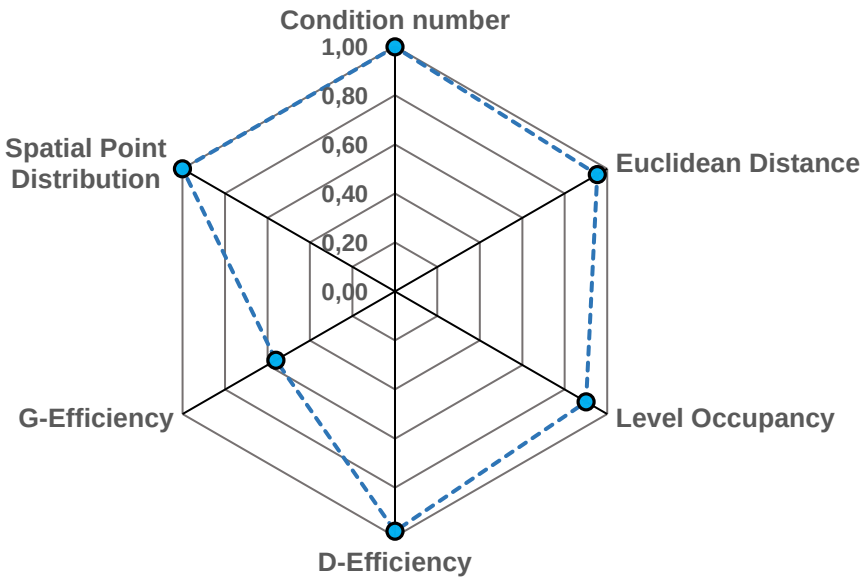
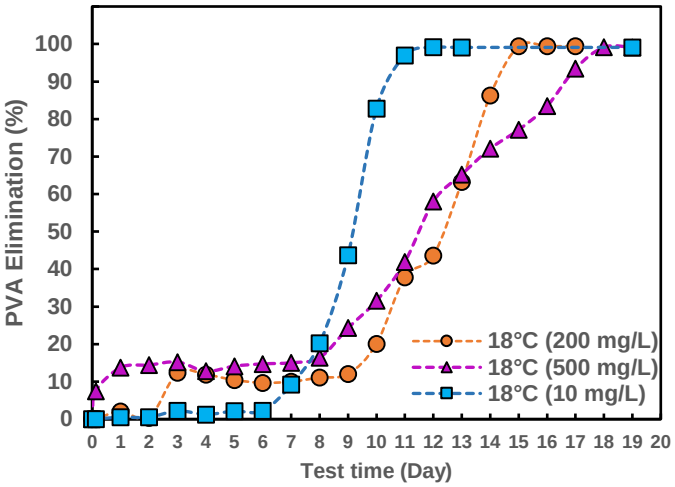


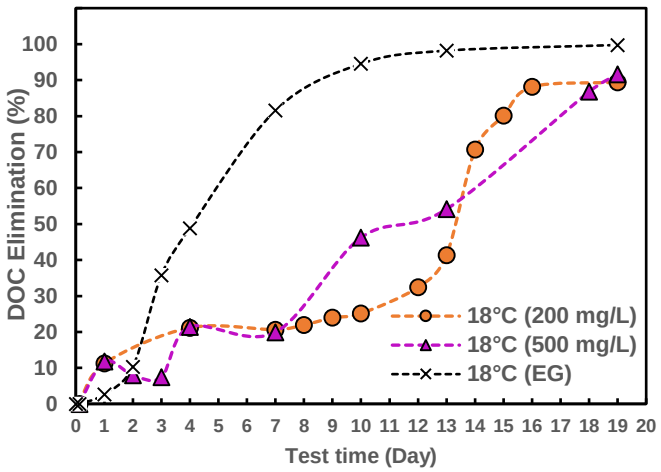
Figure 4.8: Diagnostic parameters for assessment of design of experiment.

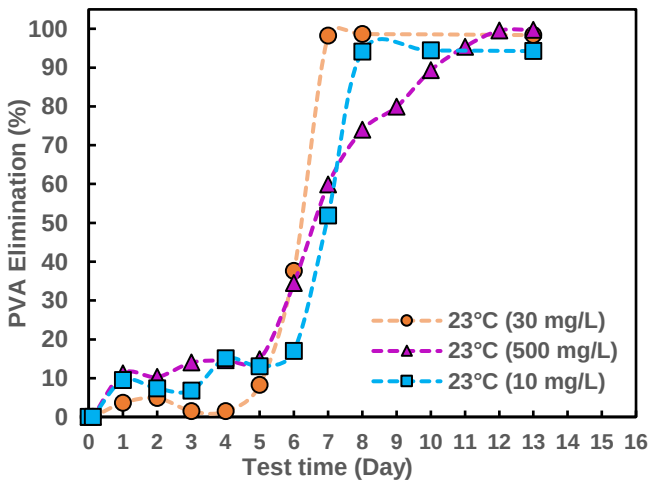
4.3 Laboratory test for investigation of polyvinyl alcohol biodegradability

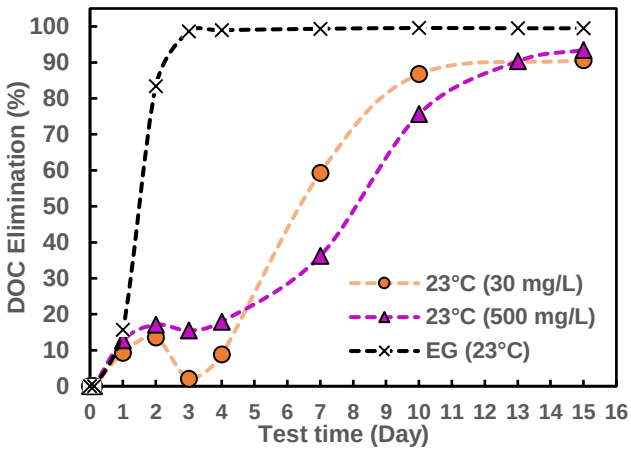
4.3.1 Investigation on optimum system conditions for PVA biodegradation (Phase I)

To determine the biodegradation of PVA by investigation the concentration and temperature ranges, degradation percentages were calculated based on measured values of PVA and DOC filtered samples taken from experiments based in Equation 11. In Figure 4.9 summary graph for PVA and DOC biodegradation curve corresponding to each temperature. Temperature measurements through these experiments are in appendix A4.1.









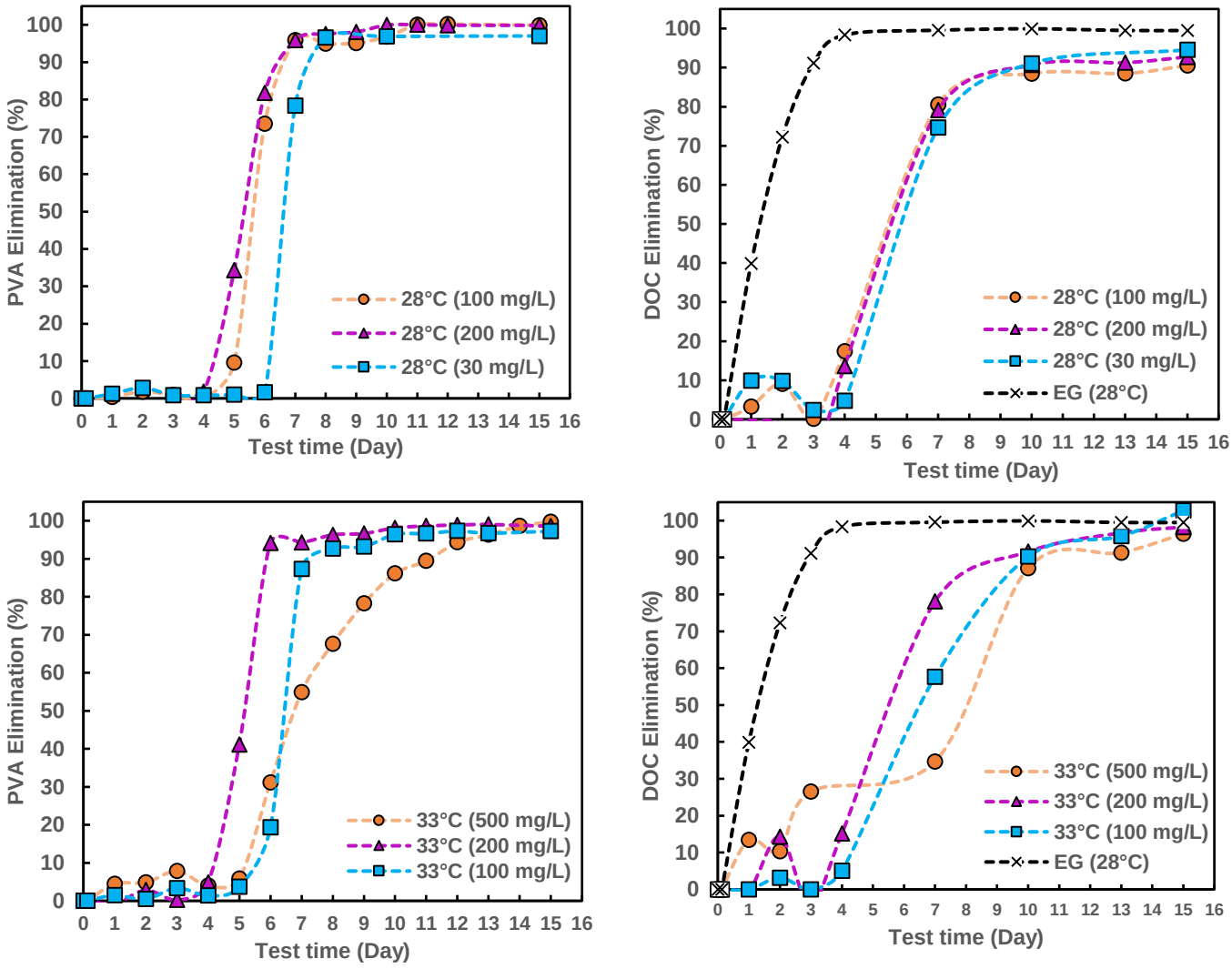


Figure 4.9: Summary of PVA and DOC elimination curves for the first phase of experiments.

Suspended solids were measured before and after washing the sludge. Before washing the average was between 4-5 mg/L and after washing ranges between 10- 11 mg/L. The added concentration of test solution (PVA) was based to reach planned F/M ratio and 1 g/L of SS was applied.

As shown in Figure 4.9, there is a very good matching between PVA and DOC curves. In some concentrations (200 and 500mg/L) there were drop in initial days of the experiments, due to high foaming which is generated after starting the aeration in test vessels. The foaming also led to a drop in weight in the first three days of the experiment. There were several attempts to control it, but average foaming losses were found and can't be controlled to a certain extent even with repetitions of these experiments.

Since there is no significant difference between DOC values of tested and blank vessels at PVA concentration of 10 mg/L, DOC curves were not included and biodegradation can only be determined based on PVA curves. It is also noted that at 30mg/L concentration DOC

elimination curve reaches 77%, even the last day measurements show very close values to the blank and that's because the difference between 1st day DOC measurements and blank measurements is not so high compared to higher concentrations.

At 18°C, adaptation phase took 10-11 days before degradation started compared to 7 days at 23°C, 5 days at 28°C and 33°C. this conclude that 28 and 33°C shows less time needed for adaptation for sludge to initiate the degradation. Adaptation and degradation were compared in Table 4.3.

Table 4.3: Summary of adaptation and PVA degradation for 1st phase of experiments.

Analysis parameters	Adaptation (d)	Concentration (mg/L)	%PVA deg.	End of deg.phase (d)	% loss due to foaming
18°C	10 -11	10	90	10	<2
		200	99	16	<10
		500	94	19	<30
23°C	7	10	95	8	< 1
		30	98	8	<1
		500	95	12	30
28°C	5	30	97	7	<5
		100	96	7	0
		200	95	8	0
33°C	5	100	88	8	1
		200	95	8	1
		500	90	12	9

28 and 33°C showed higher degradation kinetics compared to 18 and 23 °C, explained by the number of days needed for degradation, where 100 mg/L showed more than 95% degradation in less time (7 days). Kinetics will be compared as in the following sections.

Abiotic elimination checks by applying abiotic vessel containing test medium and PVA (at two concentrations 10 mg/L and 100 mg/L) without inoculum. It was found that there is no drop in PVA concentration at these concentrations due to adsorption or air stripping.

4.3.2 Estimation of degradation kinetics

Based on first series of experiments to specify the optimum system conditions for PVA biodegradation using municipal sludge, Kbio and k were calculated based on equation 12 and 13 and half-life as in equation 14 as shown in Table 4.4. adaptation phase was excluded with

losses due to foaming, and calculations were applied in the biodegradation phase only. The differences between Michalis Menten constant (k) values are not so high, where it showed maximum value at 28°C at 100 mg/L with 1.95 d^{-1}, with minimum half-life of 0.35 d.

It is also noted that K_{bio} at 28°C showed almost constant value for different concentrations, which also conclude that 28°C is the optimum temperature for PVA biodegradation using municipal microbiocoenosis.

The highest degradation rate using this sludge is 70 mg PVA/g SS found at higher concentrations (200 mg/L and 500 mg/L because the remaining substrate concentration is still high even after first day degradation).

Table 4.4: Summary of kinetic calculations for optimum system conditions of PVA biodegradation.

Temperature	Concentration (mg/L)	$\frac{dCi}{dt}$ (mg/(l·d))	K_{bio} L/(g SS d)	k (d^{-1})	$t_{1/2}$ (d)
18°C	10	1.95	0.19	0.76	0.91
	200	26.49	0.16	0.31	1.42
	500	31.63	0.10	0.21	3.56
23°C	10	2.22	0.29	1.00	0.69
	30	14.1	0.35	1.33	1.80
	500	60.4	0.17	0.41	1.67
28°C	30	7.70	0.3	1.64	0.61
	100	38.49	0.33	1.95	0.35
	200	70.30	0.33	1.23	0.46
33°C	100	40.13	0.33	1.08	0.76
	200	51.73	0.24	1.16	0.64
	500	70.36	0.14	0.38	1.83

4.3.3 Assessment of k values using regression analysis

The assessment using regression analysis was carried out for summerised k and Kbio values. The assessment of representation of the model data is based on the indicators including significance, Vinf and coefficient of determination.

For Kbio, the model diagnostic assessment shows high significance of Kbio values (90%) and very good variance inflation factor (Vif) with value of 1.089 (optimum values is between 1 and 1.5, with higher representation of the model around 1.0) as shown in Table 4.5 and Figure 4.10.

Table 4.5:Assessment of kbio data and model representation.

Indicator	Value	Polynomial definition
Significance	90.00	Kbio: Regressionspolynom Kbio = -7.036667e-01 +7.756667e-02*x1 -3.059655e-04*x2 -1.413940e-03*x1*x1
Coefficient of determination	85.67	
Normal distribution of residuals	96.65	
R adjusted	80.29	
Variance inflation factor	1.089	
Parameter rating:		
x2	82.8	
x1	17.2	

Kbio: Regressionskoeffizienten

Term	Koeffizient
a[x0]	-0.703667
a[x1]	0.077567
a[x2]	-0.000306
a[x1*x1]	-0.001414

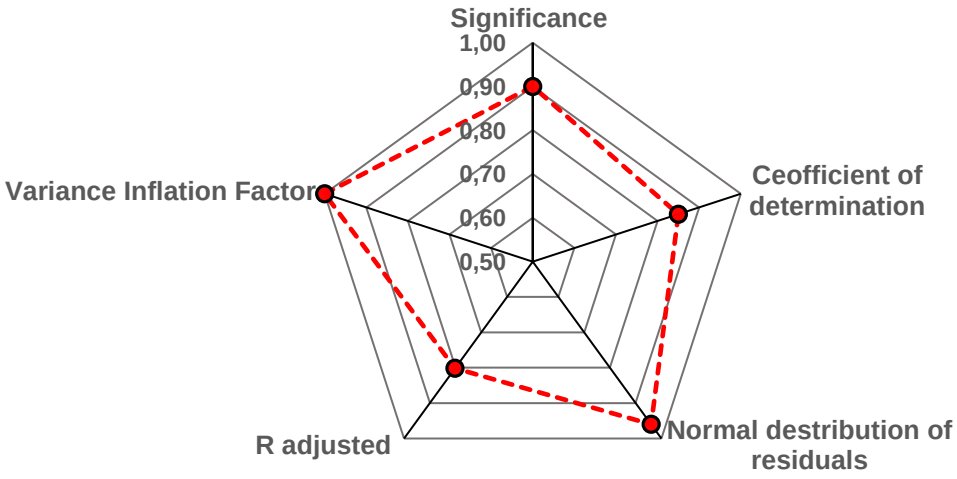


Figure 4.10: model variable diagnostics of Kbio values.

The results of regression as shown in Figure 4.11 and 4.12 represents that optimum values around 28°C at concentration approaching 100 mg/L. very low standard deviation residual values (close to 0) proves very low dispersions of modeled values.

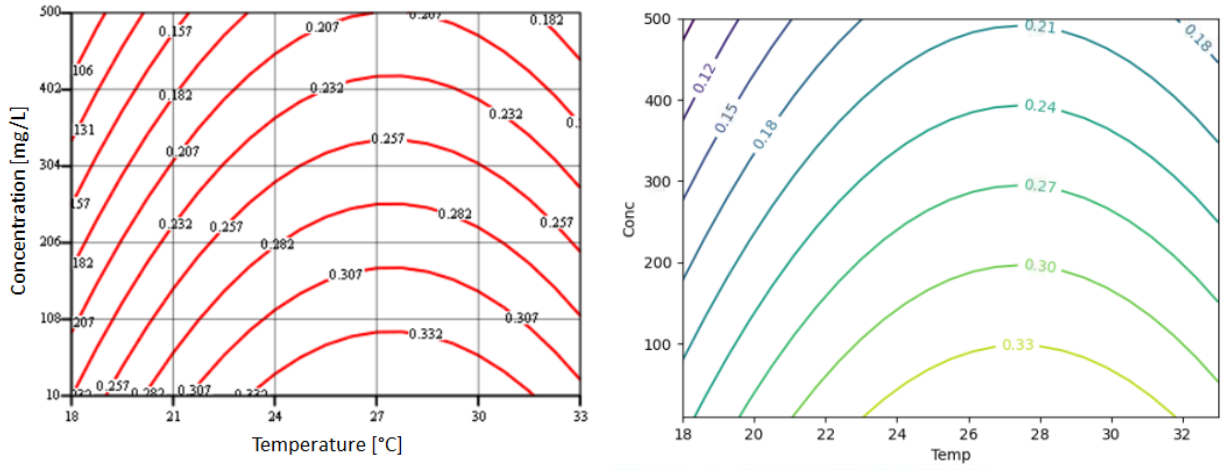


Figure 4.11: Kbio value before and after optimisation using the model.

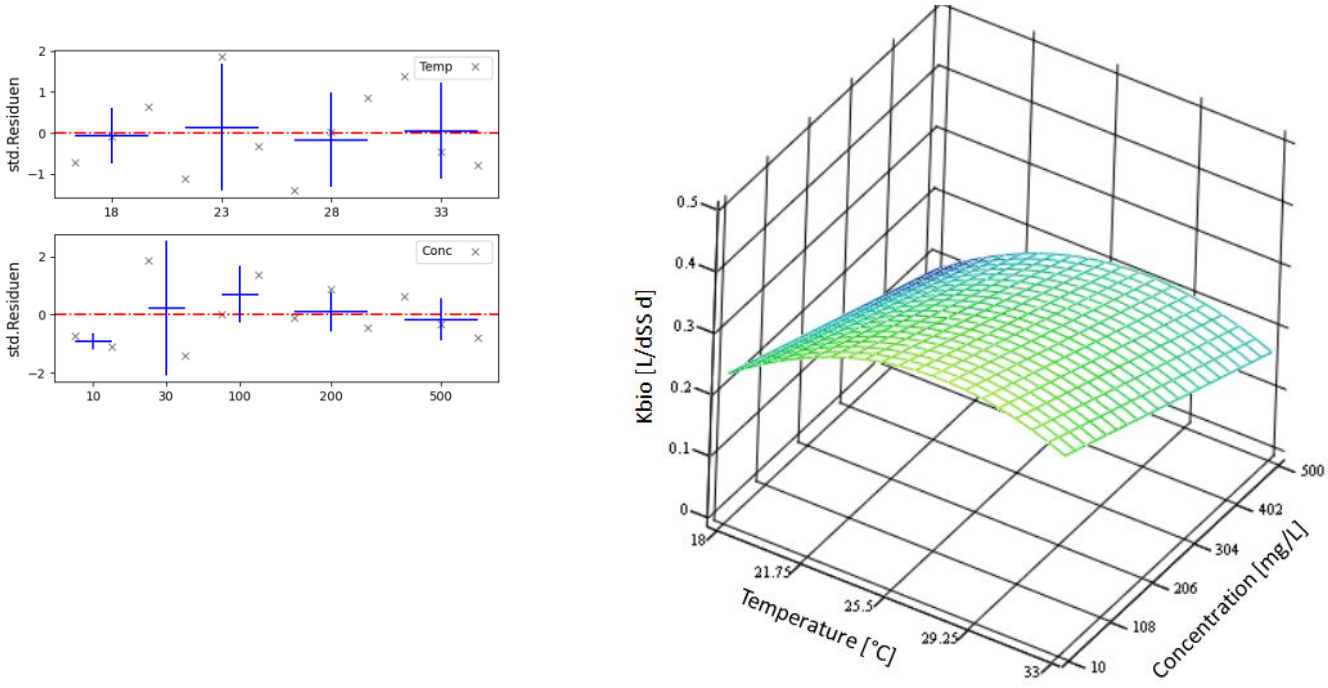


Figure 4.12: Regression 3-D model (temperature, concentration and Kbio) with standard deviations.

For k (d-1) values, the model diagnostic assessment shows high significance of Kbio values (90%) and very good variance inflation factor (Vif) with value of 1.07 as shown in Table 4.6 and Figure 4.13.

Table 4.6: diagnostic assessment of k data and model representation.

Indicator	Value	Polynomial definition
Significance	90.00	k: Regressionspolynom k = -5.126085e+00 +4.827292e-01*x1 -1.648132e-03*x2 -8.657319e-03*x1*x1
Coefficient of determination	82.28	
Normal distribution of residuals	95.05	
R adjusted	75.64	k: Regressionskoeffizienten
Variance inflation factor	1.07	Term Koeffizient a[x0] -5.126085 a[x1] 0.482729 a[x2] -0.001648 a[x1*x1] -0.008657
Parameter rating:		
x2	58.3	
x1	41.7	

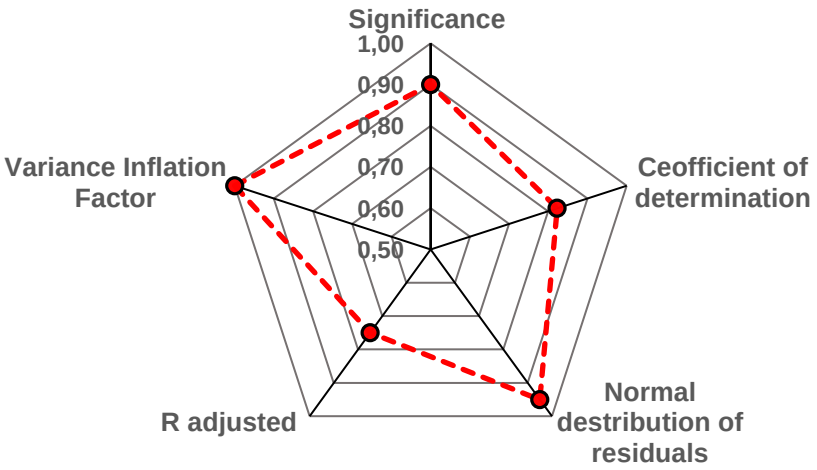


Figure 4.13: Model variable diagnostics of Kbio values.

The results of regression as shown in Figure 4.14 and 4.15 represents that optimum values at 27.8 at concentration 100 mg/L.

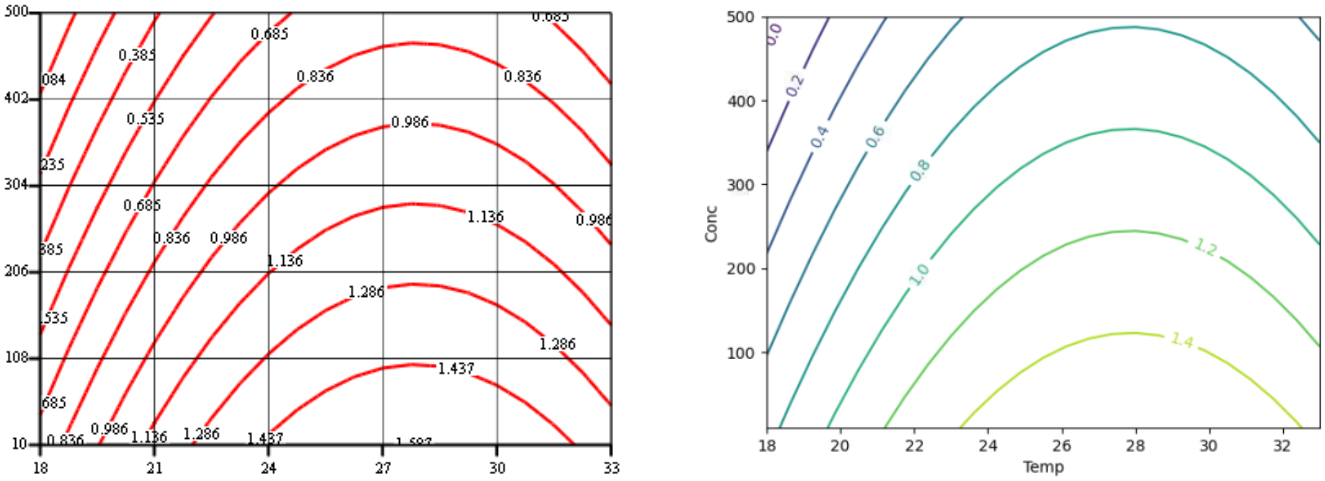


Figure 4.14: k values before and after optimisation using the model.

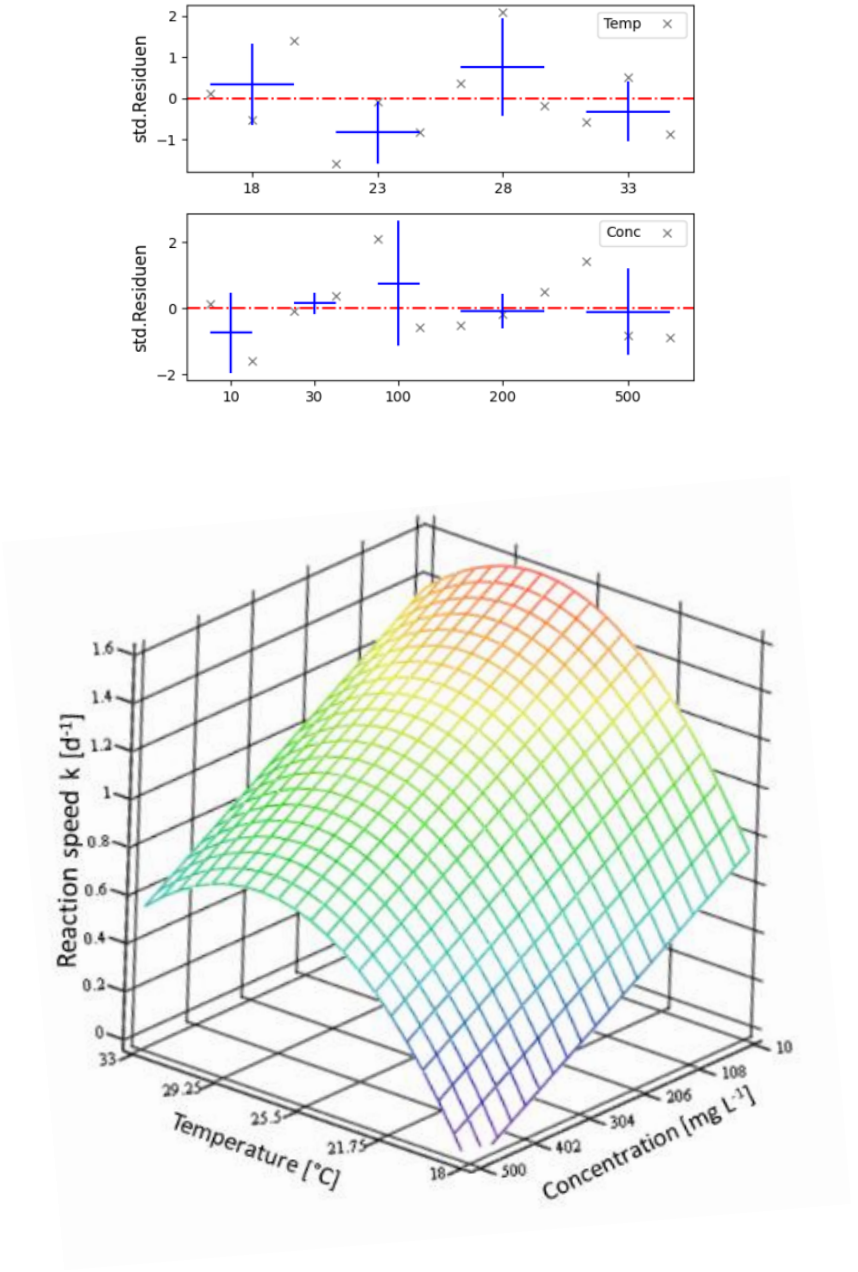


Figure 4.15: Regression 3-D model (temperature, concentration and k) with standard deviations.

4.3.4 Investigation of the effect of adaptation on PVA degradation under high temperatures (Phase II)

After specification of 28°C and 100 mg/L based on first phase results and regression analysis as optimum system conditions using municipal sludge, it was applied to adapt the sludge to give it the ability to degrade PVA under higher temperature range (36-42°C). After degradation in the first stage of experiment, samples from each of the vessels were taken for remeasurements of SS; to make the Fpva/M ratio fixed. Also, nutrient solutions (A,B,C,D) were added after the 25th day of experiment to ensure there is enough test medium is existing for organisms to remain active.

For the first stage of experiment (28°C), degradation shows typical kinetics in 10 individual test vessels that is in the first experiment (5 adaptation days, 2 days for more than 95% degradation), even though different seasonal variations (Winter and Summer seasons), degradation was not affected. Summary of these details are shown in Figure 4.16. Temperarture measurements through these experiments are in appendix A4.2.

For the 2nd stage of experiments, where the temperature raised into 28-42°C and test reset (added PVA), the degradation showed different patterns. For 28°C and 36°C degradation (presented in more than 95% PVA degradation and more than 85% DOC Elimination) happened in two days, representing high adaptation for sludge to degrade PVA at these temperatures. At 38°C, only it lasted 2 days till the degradation stopped with 40% in PVA and DOC elimination curves. For 40°C and 42 °C the degradation stopped in the next day, concluding that there is no enzymatic activity at these temperatures. Decrease in DOC elimination values and appearance of negative values due to increase in DOC values compared to the start of the second phase, which represent that oxidation of organisms some of their own cellular mass instead of PVA, or cells lysis, and rupture, which results in releasing the intracellular organic matter from cells leading to an increase in the dissolved organic carbon content.

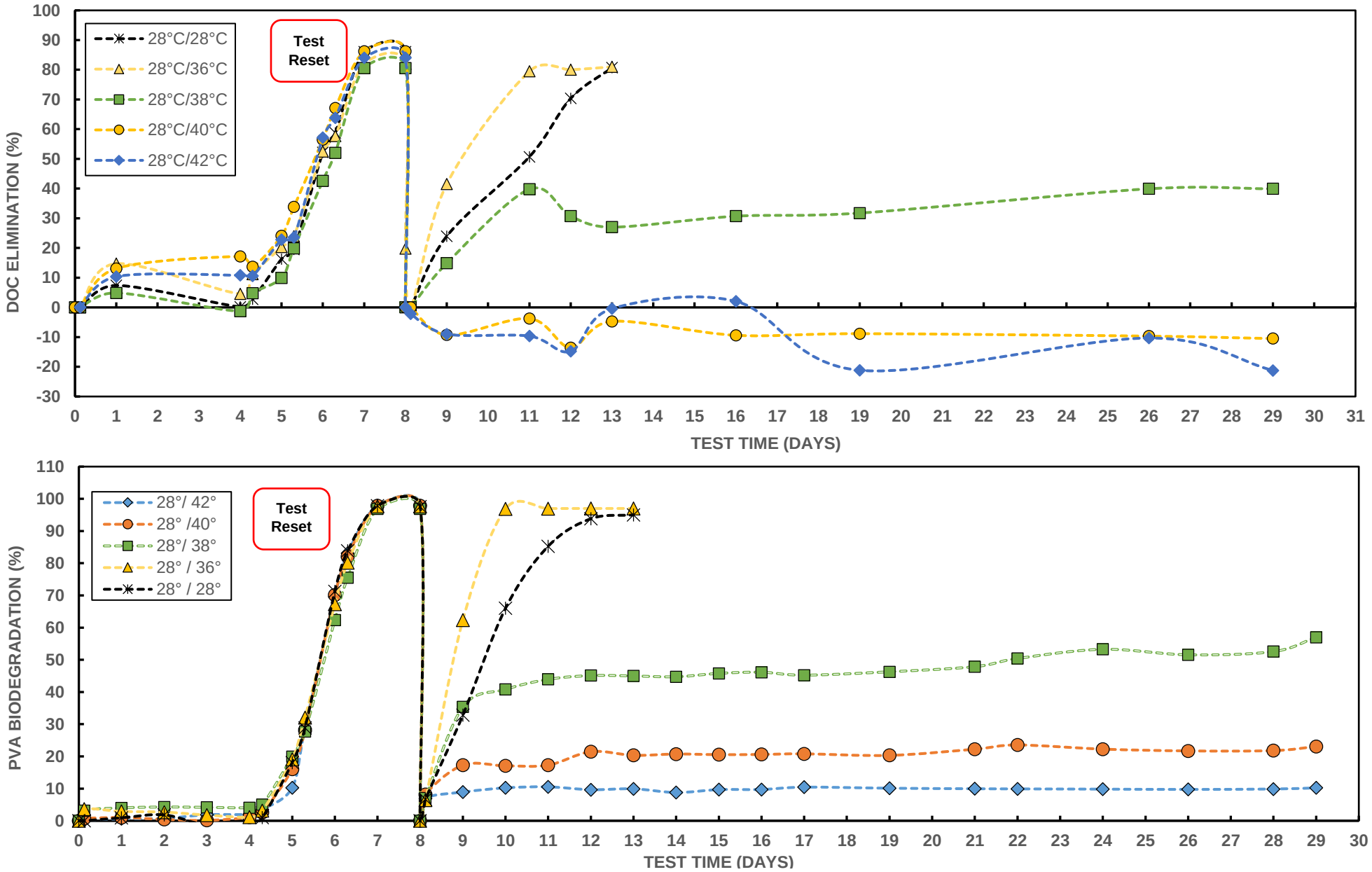


Figure 4.16: DOC and PVA eliminations curves of two stage experiment.

4.3.5 Investigation on effect of molecular weight of PVA in biodegradation

In this experiment, at concentration of 100 mg/L at 28°C, it was noted that high foaming in low MW PVA (9000, 80% hydrolysis degree) compared to other types which also affected the concentration drop (>10%). Foaming is related to PVA properties, where the surface activity and water solubility would increase with lower degrees of hydrolysis, and water fastness and gelling tendency is related to higher degrees (Jia et al., 2017). Other types showed less foaming rates (22,000 almost no foaming, low at 88,000 and 195,000 l).

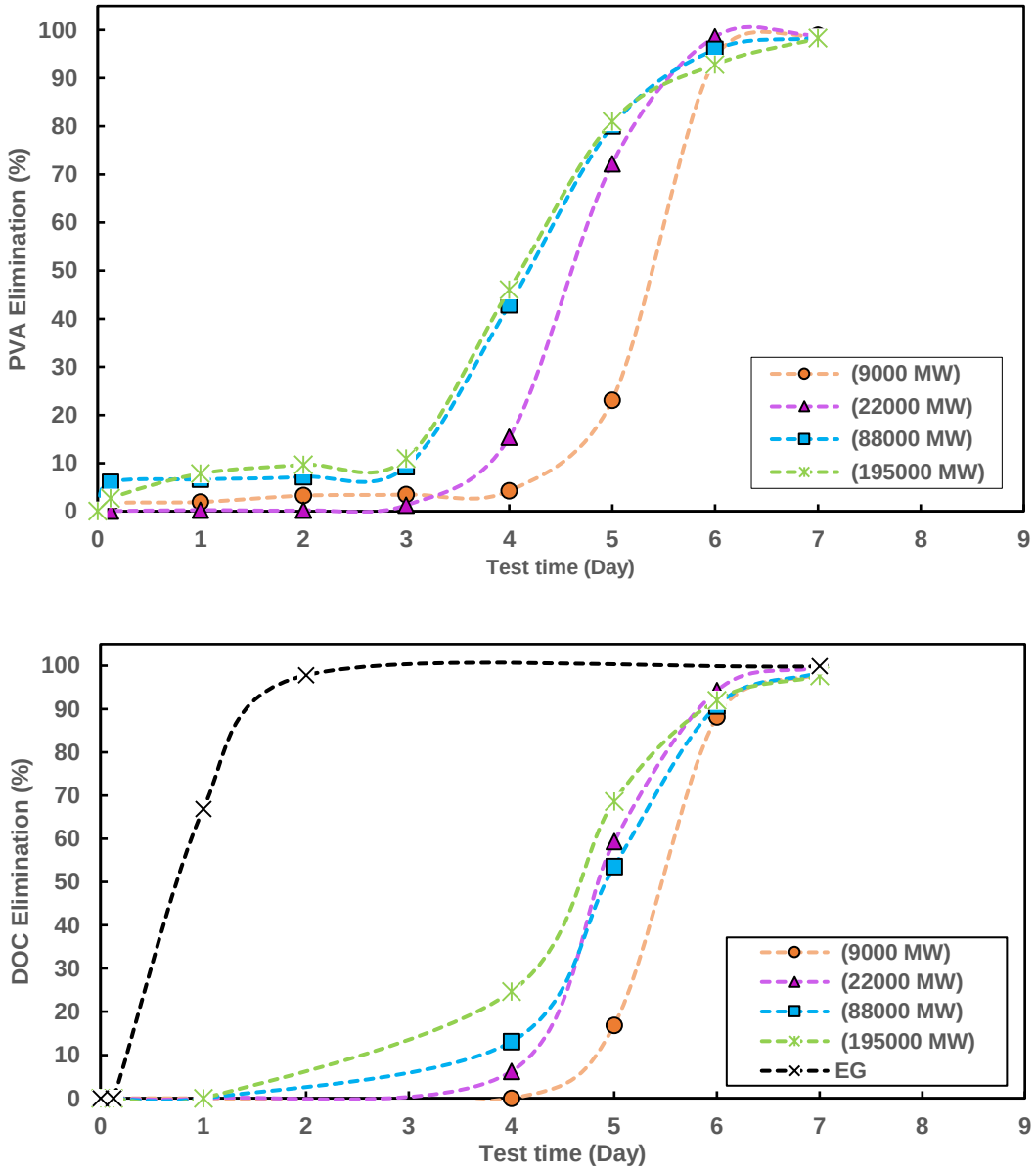


Figure 4.17: PVA and DOC elimination curves for different types of PVA experiments.

As shown in Figure 4.17, the PVA and DOC elimination curve shows slight differences between the different types. For 9000 MW, PVA required more time (1 day) for adaptation before starting the degradation. This proofs that there is no significant difference in biodegradation related to effect of molecular weight of PVA at optimum conditions.

4.3.6 Investigation on threshold concentration for adaptation of sludge to PVA biodegradation

According to very low concentrations applied in this experiment, higher PVA variations are found in measurements compared to higher concentrations. Higher sample volumes were applied to reach highest accuracy even at 1 mg/L.

As applied before in two stage experiments, test was reset after more than 95% degradation at all testing vessels, 10 mg/L PVA were added to observe the degradation of PVA. It was found that the degradation started in the first day after reset, with different degradation rates presented in the ninth day. As shown in Figure 4.18; For test vessels which started with 1mg/L, the PVA degradation reached 55%, 2 mg/L reached 80%, 5 mg/L and 10 mg/L reached 99%.

It can be concluded that adaptation happens even at 1 mg/L, due to immediate degradation in the second stage, and the adaptation is not only a concentration dependent, but also temperature factor has significant influence in adaptation of microoganisms. Also, it was found that full adaptation happens at 5 mg/L as more than 99% degradation happened at 5 and 10 mg/L.

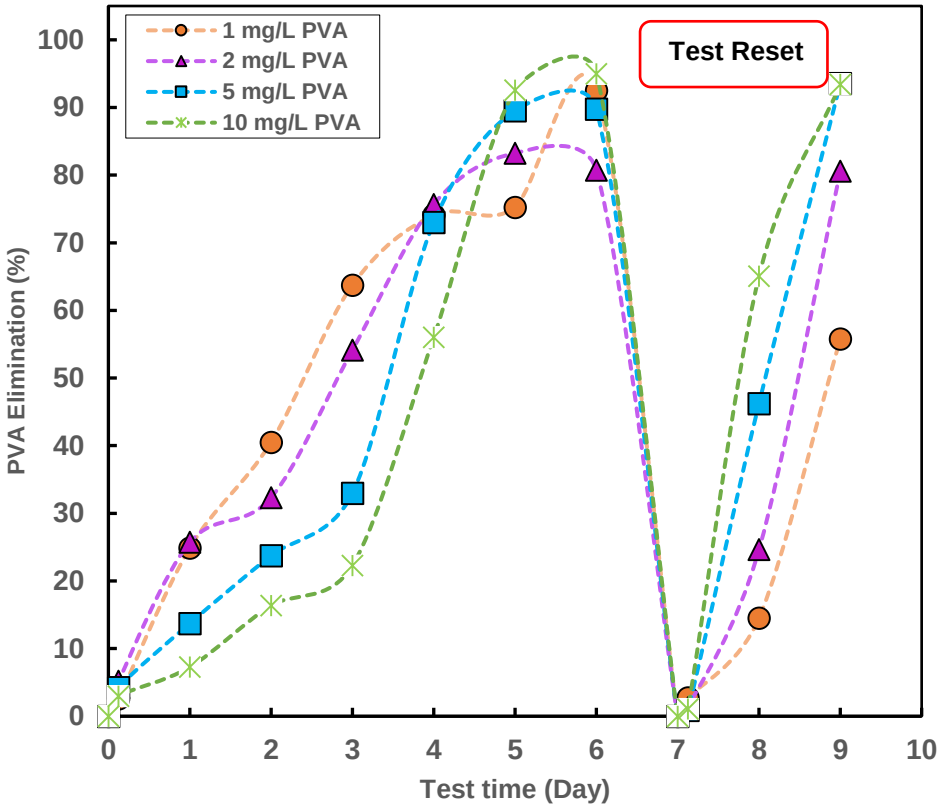


Figure 4.18: PVA Elimination curve based on minimum concentration of PVA applied.

4.3.7 Summary

- Temperature 18 and 23°C showed higher adaptation and degradation periods compared to 28 and 33°C.
- Optimum system conditions were found at 28°C where the adaptation phase is 5-6 days and the kinetics are highest at 100 mg/L as explained in tables 4.3 and 4.4. Kbio L/(g SS d) values show similar rates (0.3 -0.33 L/(gSS d) which is highest compared to other temperatures, and k (d^{-1}) were maximum at 28°C with 1.95.
- The assessment of kinetics using an optimizing regression model proved that the model is representative and the measured data is adequately described, since the coefficients of determination are higher than 80% which is acceptable for biological models. Vif around 1 and significance of 90% show that applied values in creating the regression model is showing the ranges of optimum conditions using Kbio and k values.
- Two stage experiments proved that adaptation is applicable to adapt the sludge to 36 and 28°C with higher degradation rates at 36°C, and not adequate for higher temperatures (38-42°C).
- No significant effect was found using different molecular weight of PVA, since degradation kinetics and adaptation is similar except that the sludge required 1 day more for adaptation at PVA 9000. MW.
- Foaming rates are higher based on MW and DH, where lower DH shows higher foaming rates, and higher concentrations (200 and 500 mg/L) shows continuous foaming in the first day of tests compared to other concentrations.
- At the presence of higher temperatures (28°°C), the adaptation happens at minimum measured concentration of 1 mg/L PVA, and full adaptation happens at 5 and 10 mg/L.

4.4 Investigations for characterisation of adapted sludge

4.4.1 Investigation on effect of adapted sludge to biodegradation of PVA

To apply same planned procedure, as in section 4.3.3. the experiment was initiated with temperatures 28 - 42°C without adaptation phase. The sludge was collected from Cilander-Switzerland. There was no ability to wash the sludge due to poor settleability, the average SS concentration 4 – 4.5 mg/L and dissolved oxygen concentration of 2-2.5 mg/L. other parameters were measured from different places in the treatment plant; the effluent of primary sedimentation tank (inflow to activated sludge), the flow after membrane separation, and effluent of treatment plant as summerised in Table 4.5. high COD and DOC values are related to high PVA concentrations in the manufacturing process. It also can be noted that high sulphate, low nitrate and nitrite concentrations, alkaline conditions and high temperature were determined.

Table 4.5: Parameters measured from different treatment stages at Cilander.

Parameters	Inflow to activated sludge	Flow after membrane separation	Effluent of treatment plant
Nitrate (NO_3-N)	2.47	1.57	-
Nitrite (NO_2-N)	0.05	1.17	0.007
Ammounium (NH_4-N)	7.12	10.9	-
Phosphate (P_{total}l)	11.9	11.4	4.83
ortho-phosphate (PO_4-P)	7.72	9.24	3.82
Sulfate (SO_4)	1830	1780	-
Starch	11.9	9.76	-
DOC	354	190	45
COD	1502	266	106
PVA	513	-	-
Temperature	36	-	-
pH	7.9	-	-

According to PVA and DOC elimination curves as in Fig. 4.19. the degradation started in the first day at all temperature with almost same kinetics; except at 28°C which shows slightly less degradation than other high temperature, indicating that the inoculum is adapted to higher range temperatures than 28°C. More than 94% of degradation happened till the second day

of experiment. PVA and DOC elimination curves shows good matching, but DOC elimination curve at 42°C showed less than 80% elimination. Temperarture measurments through these experiments are in appendix A4.3.

DOC values in blank vessels at all temperature shows close range (25-37 mg/L) at the end of experiment. Ethylene glycol DOC elimination also showed same as PVA degradation at these temperatures.

The pH values are showing more alkaline conditions (always shifting to 7.6-7.8), even with frequent adding of HCl to keep it around 7.3-7.5. The foaming effect were not noticed using this sludge even with high concentrations (500 mg/L); represented with no decrease in concentration or weight.

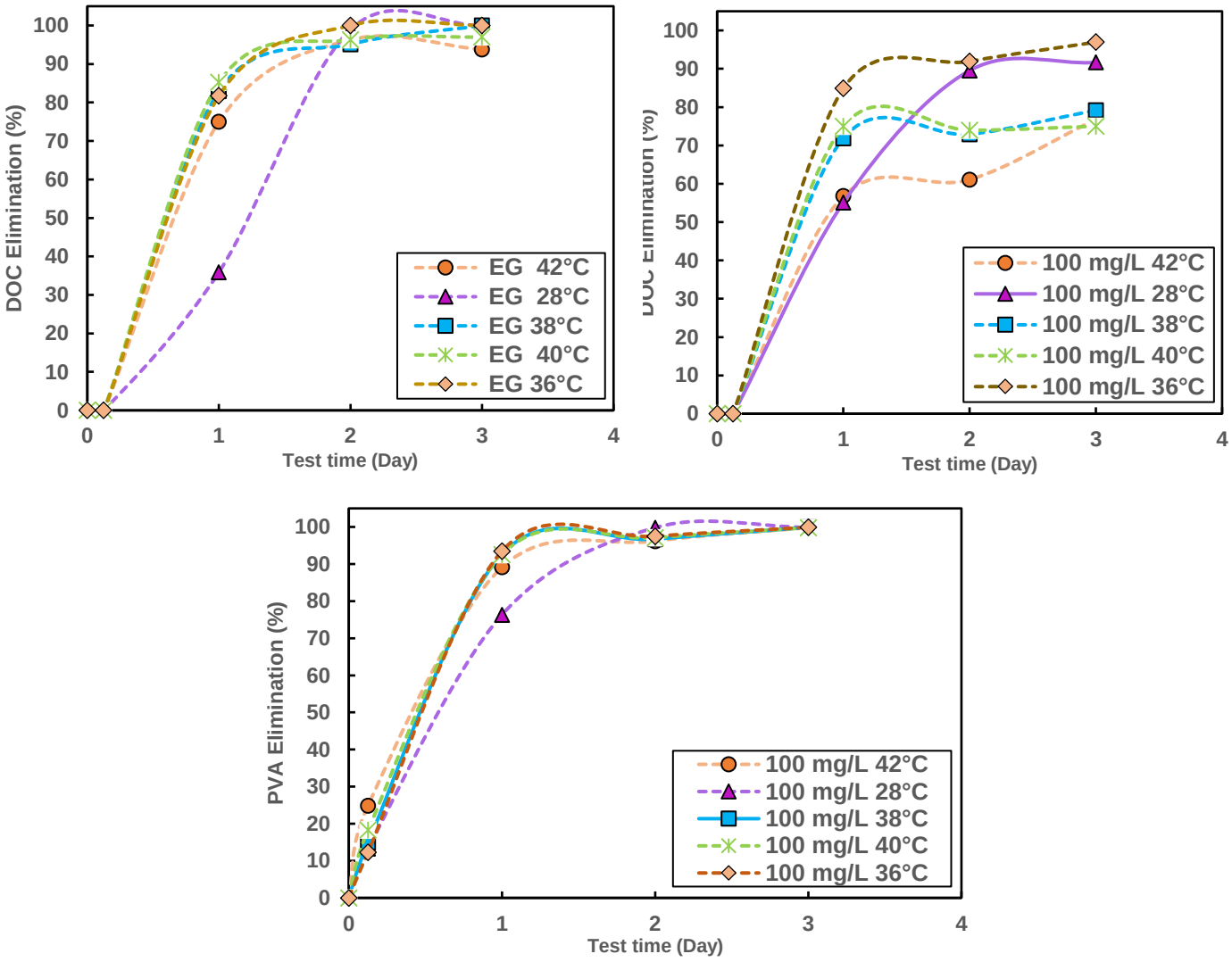


Figure 4.19: PVA and DOC elimination curves using adapted sludge.

4.4.2 Proof of biological activity

In this approach at 40 °C, active sludge and autoclaved sludge were prepared according to DIN ISO 9888. The concentration of PVA was 100 mg/L in these sets. For autoclaved supernatant sets, to simulate the real treatment plant case, only filtered supernatant was applied with test medium and added concentration of PVA is 100 mg/L. Because the supernatant already contains high concentration of PVA, the total PVA concentration was 206 mg/L.

A shown in Figure 4.20, the variations in concentrations of PVA in autoclaved sludge and supernatant were less than 10%, with 97% degradation in active sludge. It can be concluded that more than 90% of PVA is biologically degraded, with almost no adsorption to dead sludge (<5%) and very low decrease in PVA that can be related to chemical reaction.

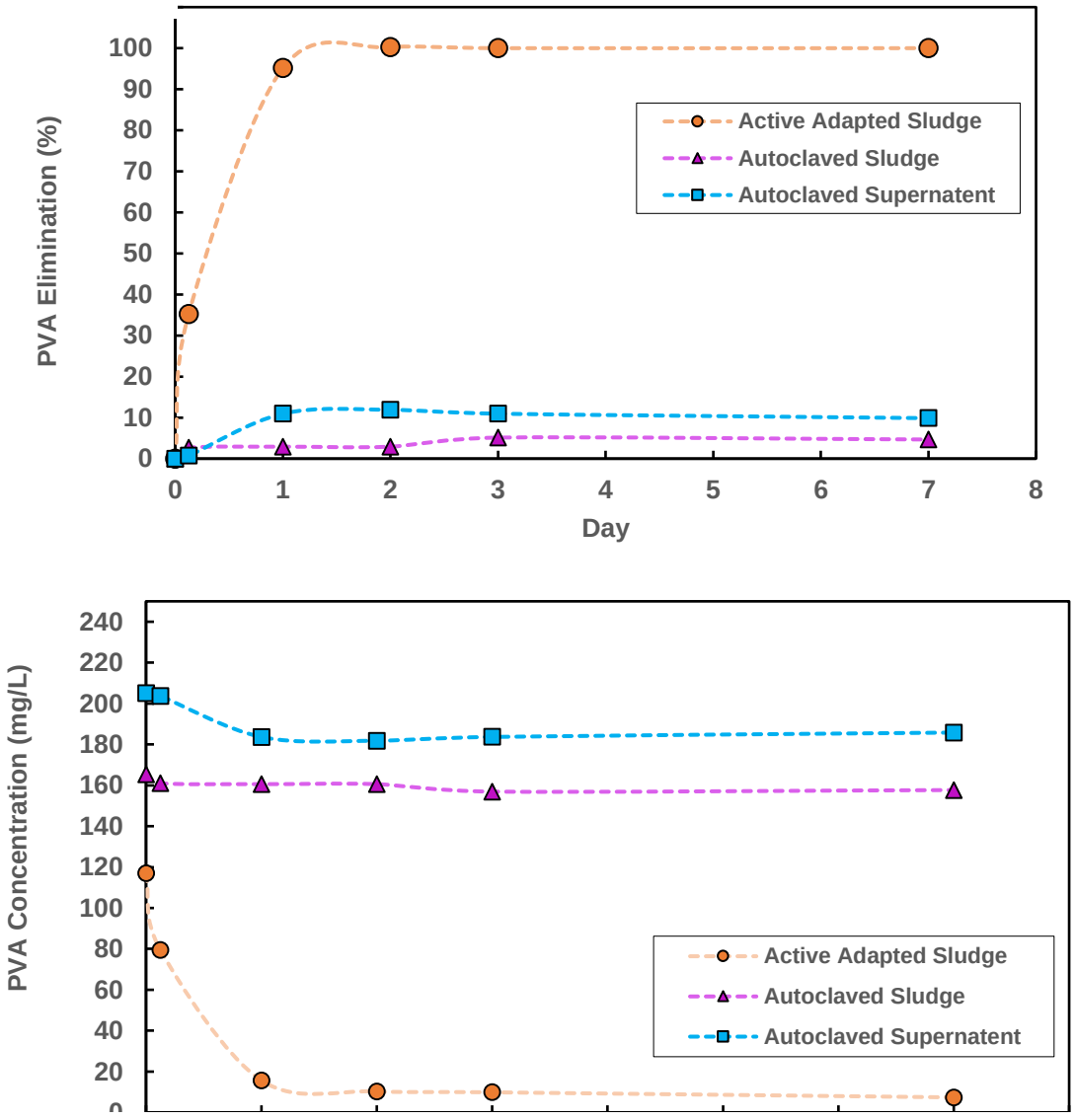


Figure 4.20: PVA Elimination and concentration curves in abiotic testing experiment.

4.4.3 pH range experiment

PVA elimination was determined in this approach for the first three hours at 40°C. It was found that there is no change in concentration at pH of 5, 17% elimination at pH of 6, with highest range between 7 and 7.75 with maximum at 7.5 of 55% elimination as shown in Figure 4.21. This concludes that the inoculum is more active at alkaline conditions compared to neutral – acidic pH values.

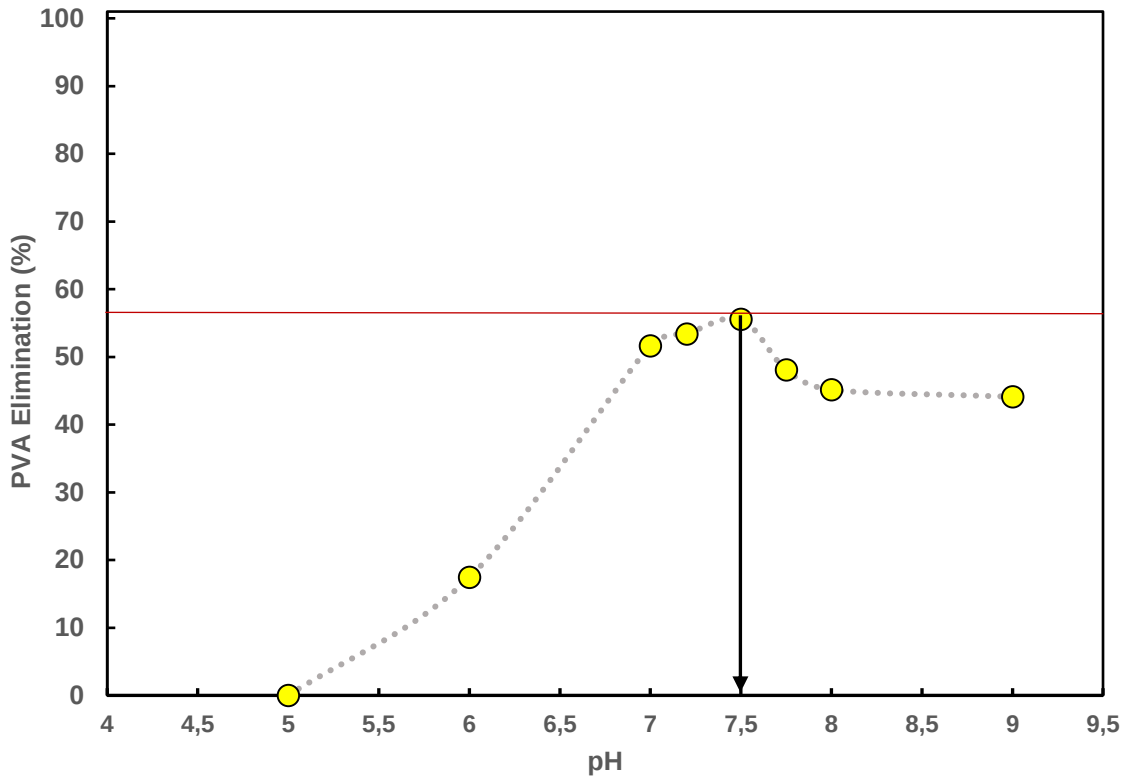


Figure 4.21: PVA elimination rates in percent after 3 hours with different pH values.

4.4.4 Limits of temperature

The degradation at high temperatures (45 and 49°C) was observed only in first day and then stopped. According to PVA concentrations measurements, about 70% of PVA was degraded at 45 °C and 27% at 49°C.

DOC values show increasing concentrations over time in both temperatures. At 45 °C, the test vessels showed values of 88 and 83 mgC/L in the first day and then increased to 100 and 110

mgC/L by the end of experiments. Also DOC values for reference vessel (EG) show increasing values from 76 to 110 mgC/L as in test vessel. At 49 °C, the values increased from 80 to 150 mgC/L in test vessels and 120 mgC/L at EG which confirms that even after stopping the degradation of PVA, there is high thermal death of organisms due to high temperature.

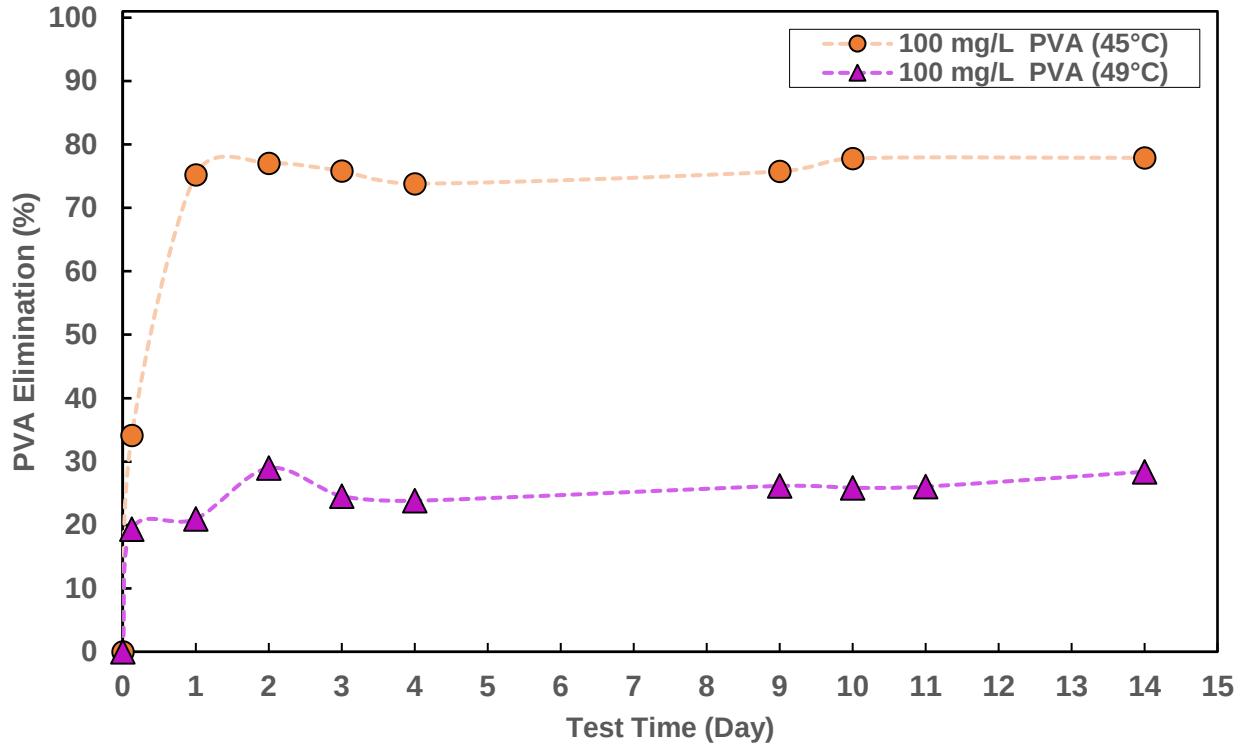


Figure 4.22: PVA elimination curves for high-temperature test vessels.

4.4.5 Limits of concentration

A concentration range of 60 mg/L till 2000 mg/L of PVA were tested at 40 °C, as shown in Figure 4.23, 4.24, and 4.25 each set of duplicate showed same range of degradation over time. More than 95% PVA degradation happened in the first day of experiment for 60 and 100 mg/L, 88% for 200 mg/L, 77% for 500 mg/L, and 64% for 100 mg/L. For 1500 mg/L and 2000 mg/L, and due to very high dilution factor, the uncertainty increased in measurements. Even though, it was noted that for 1500 mg/L, more than 90% degradation happened at 5th day, and for 2000 mg/L the degradation of same percentage reached at day 8 of the experiment.

It also can be noted that maximum degradation rate happened at concentration 1000, 1500 and 2000 mg/L at initial day with highest rate of 450-600 mgPVA /gSS. Day. This high degradation rate can be represented with high elimination.

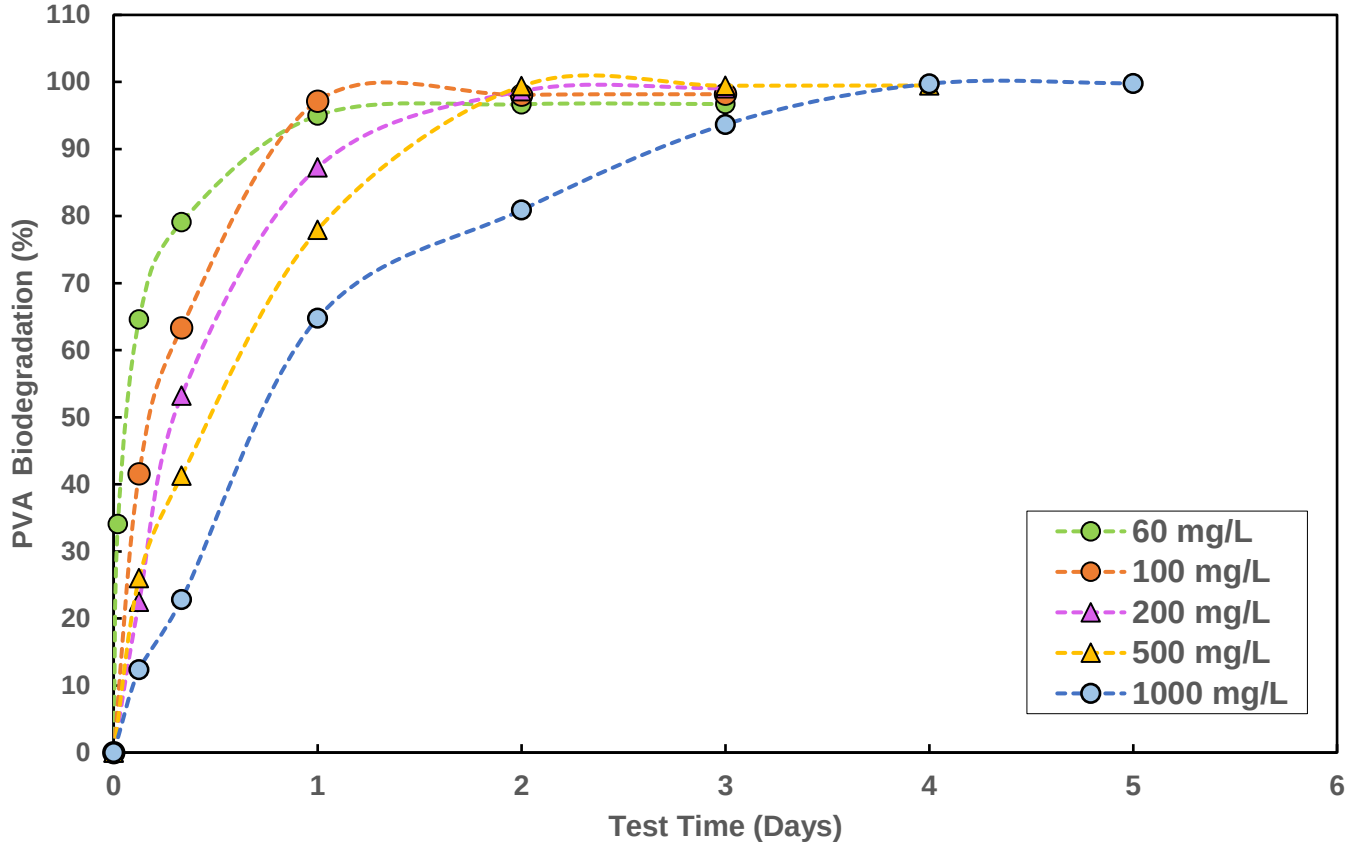


Figure 4.23: PVA elimination curves based on PVA concentration using adapted sludge.

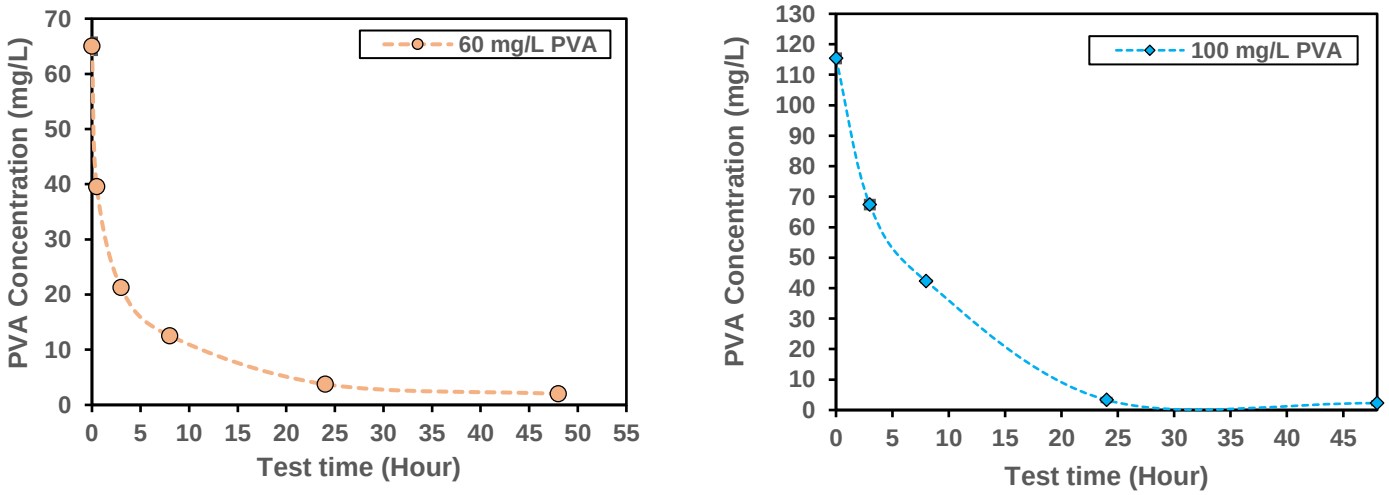


Figure 4.24: PVA concentration change (60 -100 mg/L) using adapted sludge at 40°C.

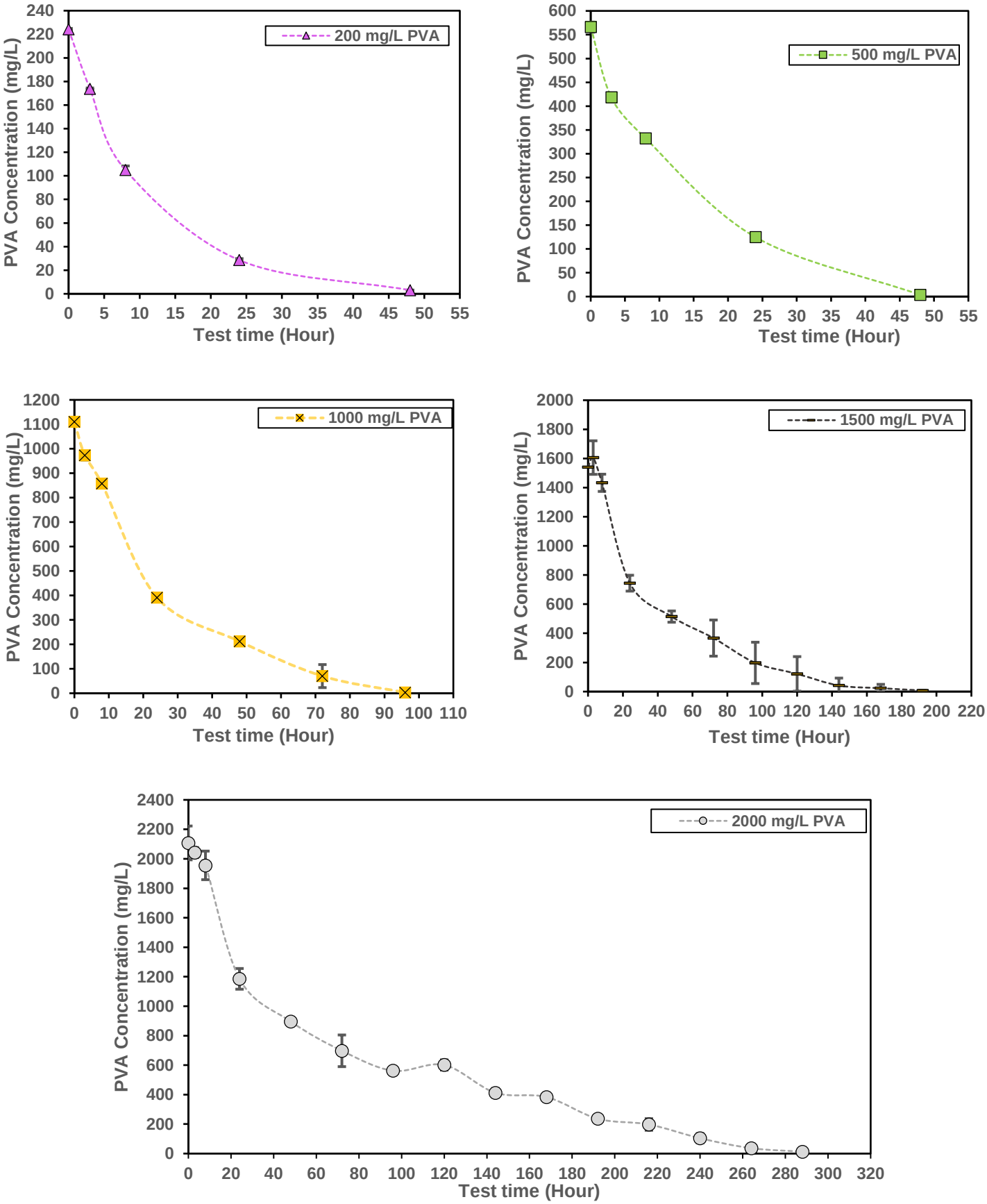


Figure 4.25: PVA concentration change (200 – 200 mg/L) using adapted sludge at 40°C.

4.4.6 Summary

- For Cilander treatment plant, the aeration tank have different parametric ranges compared to the municipal treatment plant, where high COD, sulphate, PVA rates with the existence of starch. It is also noted that oxygen concentrations are low which might affect the microbial activity and settlability of sludge.
- Biodegradation tests showed complete degradation (more than 90%) in the first day, at all temperatures (36-42°C).
- Autoclaved sludge and autoclaved supernatant showed very low decrease in PVA degradation over 7 days of experiment (<10%), compared to full degradation using active sludge, tested at 40°C.
- Optimum pH value at 40°C for PVA biodegradation was found at 7.5 (slightly alkaline conditions).
- At temperatures between 45 and 49°C the degradation stopped in the first day after 70% and 10% degradation. Higher DOC values showed that significantly reduced and activity of microbial communities at such high temperatures.
- 60 -2000 mg/L PVA concentration range was tested and proved to be degraded. High degradation rates (450-600 mg PVA/gSS) were found in first day, with more than 90% degradation happening at 5^{th} day at 1500, and for 2000 mg/L the degradation of same percentage reached at day 8 of the experiment.

4.5 Comparison summary between municipal and PVA adapted sludge

4.5.1 Yearly variations of measured parameters

There are major differences between the two sludges. This includes parameters (P, SO_4, N, SVI, PVA, DOC, COD), different microbiocoenosis (different strains appearance by microscope), different oxygen consumption, and different PVA biodegradation kinetics.

Parameters comparison as in Table 4.7 shows high differences that explain the difference in wastewater characteristics between COD values of both sludges. High COD values in Cilander sludge show high organic loads entering the biological unit compared to municipal wastewater as shown in Figure 4.26, with low ammonium concentrations, high sulphate and PVA concentrations and higher temperature in operation. Also, as mentioned before, adapted sludge is not settleable (SVI fixed) which was tested over 30 min, 1 hour, and 2 hours as shown in Figure 4.28.

Table 4.7 : Summary of measured parameters for municipal and adapted microbiocoenosis.

	Inflow to Cilander activated sludge (mg/L)	Inflow to LFKW activated sludge (mg/L)
COD	1800-10000 (varied)	350-450
Nitrogen (Ntot)	340-550	15-45
Ammonium (NH_4-N)	2.5-3.5	25-35
Nitrate (NO_3-N)	2-3	0-1
Nitrite (NO_2-N)	0-0.05	0-0.3
Phosphate (P_{total})	25-50 (varied)	4.5-6
pH	7.6-8.0	6.5-7.2
Sulfate (SO_4)	1700-2200	100-400
Starch	10-20	< 5
DOC	350-1050	100-200
PVA	200-500	< 2
Temperature	**25-42 (varied)**	**8-19**
SVI	250	100-120

Comparisons for certain parameters are shown in Figure 4.27. Low ammonium concentrations in activated sludge may lead to lower nitrification levels with limitation of growth and activity of nitrifying bacteria. It is also documented that low ammonium concentration might be related to

susceptibility to foaming and sludge poor settleability due to low production of extracellular polymeric substances (EPS) which has a role in stabilizing the sludge.

Also, high sulphate concentration can lead to formation of hydrogen sulphide (H_2S) which has odour and is toxic to microorganisms at high concentrations. It can be also a reason of poor settleability, since sulfate reducing bacteria can cause operational problems through inhibition of biological process.

It is also noted that Cilander records of wastewater composition indicate high variations in COD, temperature, and other parameters. This has an adverse effect on process stability and performance, might cause biomass washout/loss, nutrient imbalance, foaming, and bulking of sludge.

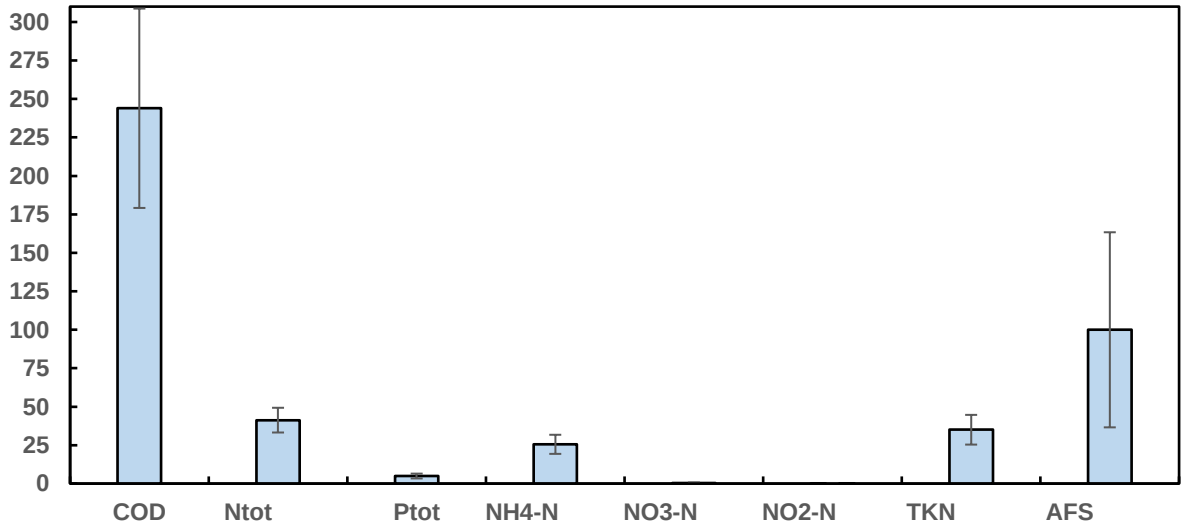


Figure 4.26: Measured parameters and ranges of LFKW activated sludge inflow (2021).

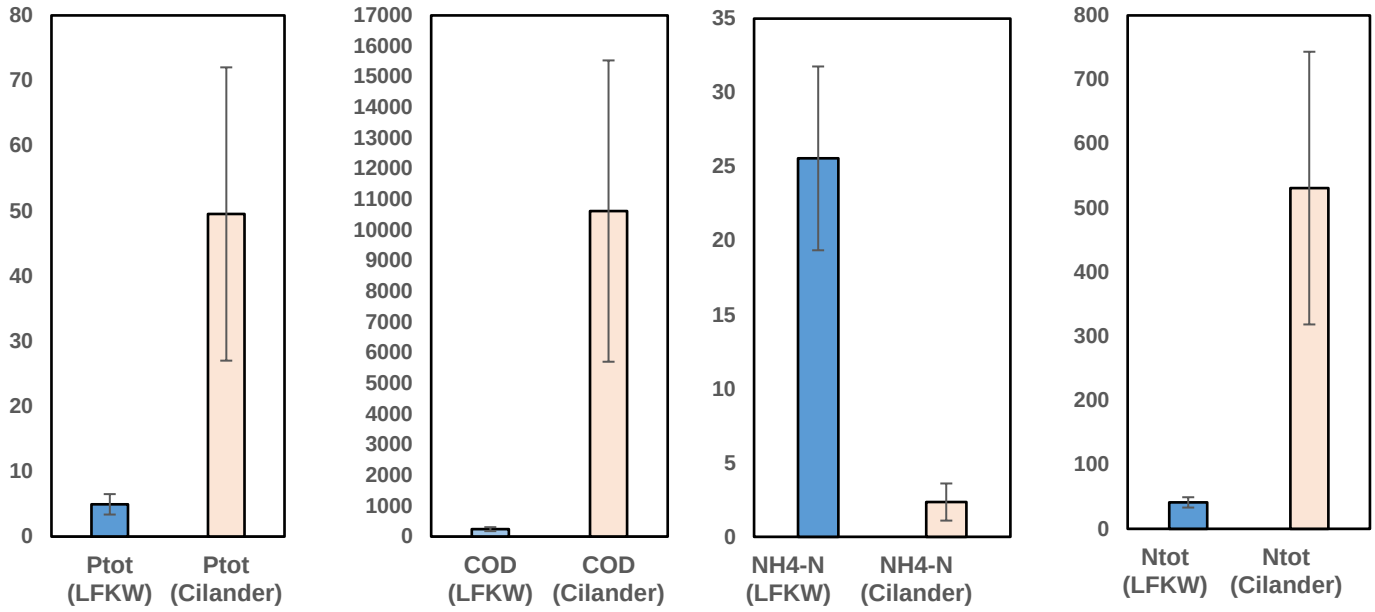


Figure 4.27: Comparison between two types of sludge parameters (NH4-N, Ntot, COD, Ptot).

Figure 4.28: Measured SVI for LFKW (left) and for Cilander (right).

Poor settlability has many reasons including: high organic loading which could lead to suffering of microorganisms to break down and assimilate the organic matter, or nutrient imbalance (specifically phosphorus and nitrogen) which might lead to overgrowth of specific organisms over others, poor aeration and mixing, or presence of toxic materials

(Tchobanoglous et al., 2014). It is expected as found in parameters measurement, that adapted sludge has partially the existence of some of these reasons. High SVI also can be caused by the existence of filamentous bacteria or insufficient retention time might cause this problem.

4.5.2 Oxygen consumption rate

Oxygen consumption was tested on-site and lab for both types of sludges, as shown in Figure 4.29. It was found that oxygen concentration at Cilander sample (after many repetitions) does not reach higher concentrations (6 mg/L compared to 10 mg/L for LFKW), even with application of same aerators in the lab. This can be related to high organic matter existing in Cilander samples, which can surpass the available oxygen supply and result lower oxygen during measurements. Also, the decease in both samples were exponential, with less steep change in Cilander samples, and this could be related to high mortality percent of microorganisms in the sample. This could be related to many factors, including endogenous decay of organisms since they have a limited lifespan, and with application of long sludge age in Cilander, it could be the main factor. Other factors might have effect is high changes in temperature, pH, nutrient availability, or toxic effect that could present (Henze et al., 2008).

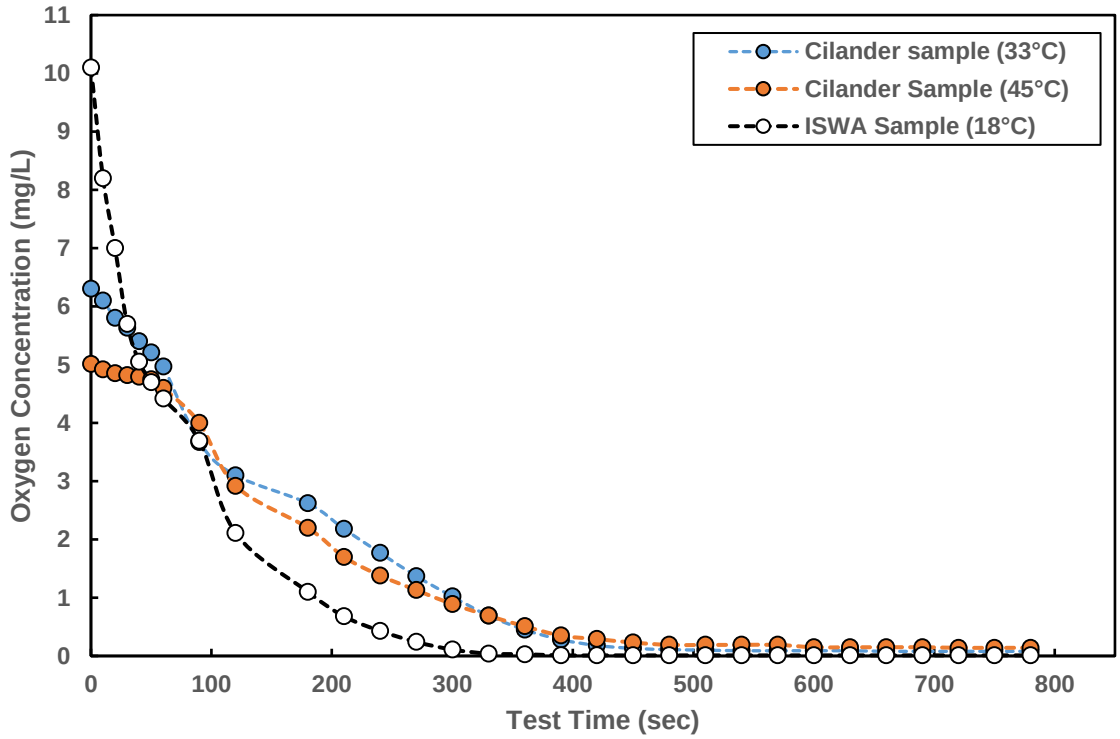


Figure 4.29: Oxygen measurements over time for sludge types.

It is also noted that adapted sludge tested at 45°C has lower oxygen consumption than same sludge at 33 °C. This indicate higher mortality rate of organisms as it also concluded from higher DOC rates for samples taken from 45°C experiment.

4.5.3 Microscopic Images comparison

For microscopic photos, Cilander sludge photos include existence of large flocks and long strains in the shape of coccus and streptococcus (gram-positive cocci bacteria that typically arrange themselves in chains or pairs) which is not present in LFKW sample (mixed culture) with existence of different organisms including protozoa, fungi, and different types of bacteria without existence of large flocks as shown in Figure 4.30.

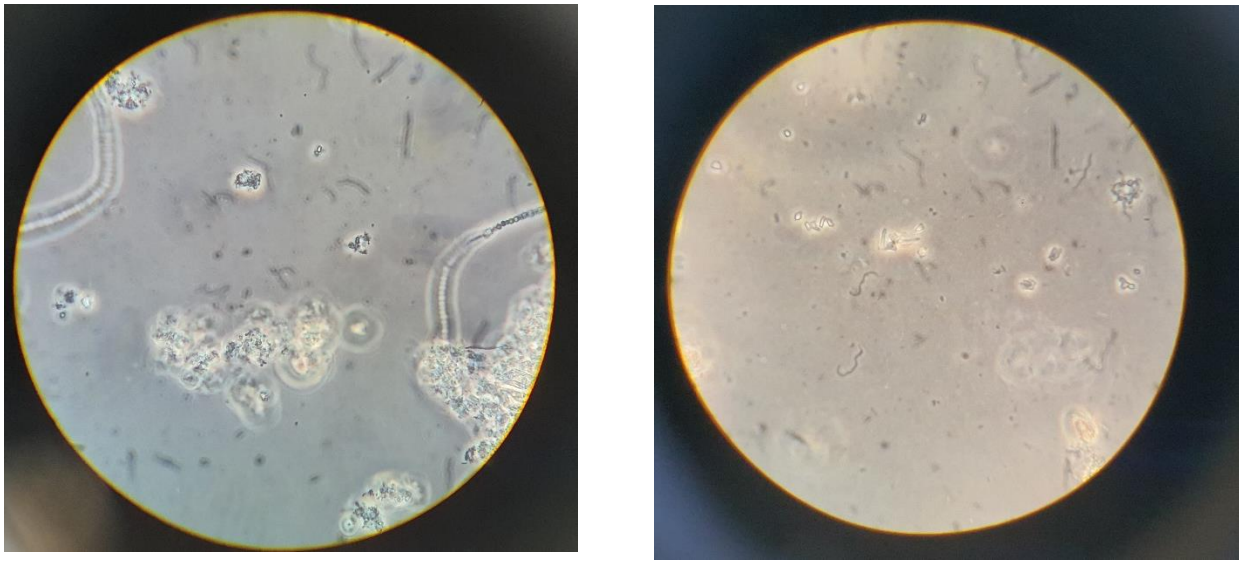


Figure 4.30: Microscopic photos for adapted sludge (left) and municipal sludge (right) magnified 100 times.

4.5.4 Summary

- The yearly variations are higher in Cilander compared to LFKW. These variations can affect the C:N:P ratio in weekly and monthly basis. Also different parametric ranges high COD (around 10,000 mg/L at specific days), sulphate, PVA rates with existance of starch, with low ammonium rates.

- Oxygen consumption rates showed exponential trend in both types, with less values at cilander compared to LFKW (initial values 6 and 5 mg/L at cilander compared to 10 mg/L at LFKW). This proves high mortality of the organisms due to many factors, the major one is long sludge age.
- Temperature ranges with PVA elimination percentages for the different types are demonstrated in Figure 4.31. the organisms at adapted sludge not only are able to degrade at higher temperatures, but also with higher kinetics (k values of 3.5 compared to 1.95 at optimum conditions for municipal sludge).

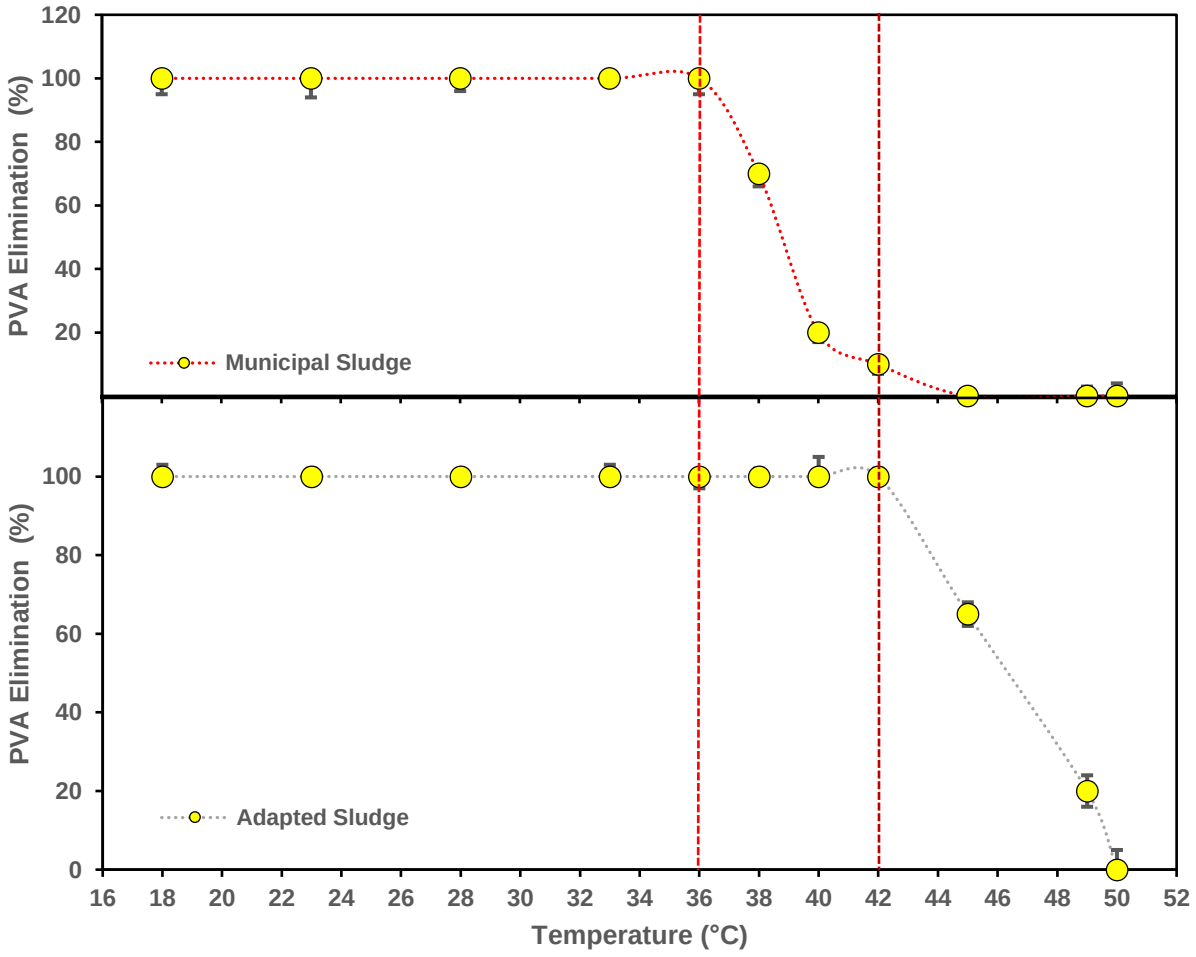


Figure 4.31: Comparison of two sludges on temperature range and PVA elimination

5 Summary

Step 1: Literature research/procurement of materials

The specialist articles published to date lack investigations on the biodegradation of PVA of under high temperatures; considering ranges (31-49°C). Initial investigations at ISWA showed that it is possible to achieve biodegradation at high temperature if the process is optimised. Also, it is very essential from practical prospects (especially in textile industry) to identify in depth the optimum system conditions to biodegrade PVA in activated sludge systems. It is also worth mentioning that many of previous studies related to strains and enzymatic activities but did not investigate applicable aspects and results with respect to real treatment plants with biodegradation aspects.

Therefore, investigating different microbiocoenosis will bring detailed information about optimisation options for the design and operation of wastewater treatment plants, which all were investigated in detail in this project.

The lab was mobilized with all necessary equipments materials and chemicals necessary for experimental sets and analytics, including water baths to control specific temperature and reduce temperature fluctuation, a thermal sensor to measure temperature for each vessel through time of the experiment, pH and oxygen meters.

Step 2: Design of Zahn-Wellens method

D-optimal design guarantees the minimization of the forecast or confidence interval of the calculated estimation parameters (regression coefficients, standard errors, etc.). D-optimal advantage compared to the other methods is the free choice of the test levels and the independence of the number of tests from the number of levels of the factors. This also reduces the test efforts and increases the extraction of various information from the tests.
D-optimal design of experiment diagnostic show very good planning quality based on statistical indicators. The number of experiments were reduced from 20 to 12 sets, with high D-efficiency of 97.8% and G-efficiency of 56.1%.

Also, DOC sampling plan were made to minimize DOC samples with the conservation of tracking the values through the experiments and have comparable results with PVA measurements.

Step 3: Laboratory tests for PVA biodegradability

PVA spectrometric method

- It was found that molecular weight of PVA has high effect on absorbance values with variations up to 60-70%, with higher absorbance values for lower PVA MWs.
- Different optimum wavelengths found between 630 and 680 nm according to the MWs of PVA, and 680 nm was applied for all types to generate comparable results.
- For reaction times, peak value was found after 20 min.
- Minimum effect of filtration was found using membrane and paper filter for fully solubilised PVA with differences between 2-4.5%. Also, no significant differences were observed between old and new PVA solutions, which confirms that PVA is stable over time.

Optimum system conditions

- Abiotic elimination checks by applying abiotic vessel containing test medium and PVA (at two concentrations 10 mg/L and 100 mg/L) without inoculum. It was found that there is no drop in PVA concentration at these concentrations due to adsorption or air stripping.
- 200 and 500 mg/L had high concentration losses due to foaming varied between 10 and 30%. 18°C showed lower degradation kinetics. The adaptation phase was highest; it took 9-11 days depending on concentration. The degradation lasted till day 11-19 depending on concentration with highest degradation rate of 31 mgPVA/ gSS.day.
- 23 °C showed less adaptation (7 days) with better degradation kinetics (7 days for 10 and 30 mg/L, 12 days for 500 mg/L), the highest degradation rate was around 60 mgPVA/ gSS.day.
- 28 °C and 33°C showed the least adaptation days (5-6 days) with minimum degradation phase at 28 °C (2 days at 30 and 100 mg/L). The highest degradation rate was 70 mgPVA/ gSS.day.
- Determination of kinetics showed that Kbio at 28 °C had values between (0.3 -0.33 L/(gSS d)), with the highest k(d^{-1}) at 28 °C of 100 mg/L. Optimised regression analysis showed high representation of the values to the investigated range of parameters with a significance of 90% and optimum variance inflation factor of 1.089.

Two phases experiments

- Optimum conditions of 100 mg/L at 28°C were applied in this experiment. The adaptation phase showed matching degradation rates for all test vessels. After test reset at 9th day, the sludge was adapted to achieve full degradation at 28 and 36 °C. The degradation stopped at 38-42°C, and DOC showed higher values than in the test reset, which indicates cell lysis or rupture of microbial cells.

Different types of PVA and threshold concentration experiments

- No significant difference in biodegradation of different molecular weights of PVA was determined and same kinetics were found.
- It is found that adaptation happens even at minimum tested concentration of 1 mg/L, which proves that adaptation is not only a concentration dependant, but also temperature has its effect. Full adaptation was found at 5 mg/L and 10 mg/L since full degradation witnessed after test reset.

Adapted Sludge investigations

- Parameters measurements showed high COD, sulphate, PVA and temperature values with existence of starch; also pH values proved that the sludge is functioning under alkaline conditions.

- No adaptation phase was observed. The degradation started immediately from the first hours of experiments. High kinetics were witnessed since more 90% degradation of substrate 100 mg/L PVA happened in the first day at all high temperatures (36-42 °C). DOC values showed same kinetics as PVA with lower DOC eliminations with more than 90% for 36, 38, and 80% for 40 and 42°C.

- Proof of biological activity showed that less than 10% of PVA elimination was due to adsorption to the dead sludge or a chemical reaction using autoclaved sludge and autoclaved supernatant, where 95% degradation happened using normal active sludge in the first day at same test conditions (40°C, 100 mg/L PVA, 1 g/L SS).

- pH testing range showed highest degradation of 55% which happened after 3 hrs at pH of 7.5, which represents the optimum value for this microbiocoenosis.

- The degradation reached 70% and 30% for 45 and 49°C and stopped at the first day of experiment which confirms the limits of temperature for this type of sludge. It is also noted that DOC values were increasing through the days of experiment with ratios 30% to 100% at 45 and 49 °C.

- PVA Concentration range (60 -2000 mg/L) at 40 °C were tested and kinetics shows high degradation rates varied between 450 and 650 mg PVA/gSS.day, which is 7-9 times higher

than municipal sludge (70 mg PVA/gSS.day at optimum conditions) , and high k(d-1) of 3.5 which is twice higher compared to municipal sludge with 1.95 at optimum conditions.

-

- Comparison of yearly variations of parameters showed high variations in COD, Ntot, temperature in Cilander treatment plant, with low ammonium concentrations and high SVI.

- Comparison of calculated kinetics based on the performed experiments are summerised in Table 4.8. It can be concluded that the sludge used did not only start the biodegradation immediately, but also showed around double values in modelled k and Kbio. The degradation rates are higher up to 7-8 times than municipal sludge even after adaptation, which demonstrates the potential of adapted sludge in PVA biodegradation even at high temperatures which was never proved before in specialised articles and previous PVA biodegradation studies.

Table 5.1: Kinetics comparison for the different types of sludges.

Parameter	Adapted sludge (40°C)	Municipal sludge (opt.C.) (28°C)
kbio (L/(gSS d))	0.7	0.32
k (d^{-1})	3.52	1.9
dc/dt (mg/(l·d. gSS))	500- 600	60- 70
Maximum tested PVA concentration (mg/L) without toxic effect	2000	1000
Foaming effect	Very low	high
Adaptation (days)	0	5-11

6 Exploitation activities

A patent related to this research project is submitted for registration at The Technologie License Büro (TLB).

Also, publications are going to be submitted soon in international, peer-reviewed journals and conferences and the use of the results in a dissertation are planned.

- Polyvinyl Alcohol: Overview in production, properties, applications and impacts on the environment, with current and future degradation potential to achieve sustainability.
  (This publication as demonstrated in the report includes **all** references related to PVA which are published till 2022).
- improved spectrometric method for high-accuracy measurement of different types of Polyvinyl alcohol
- Identifying the optimum system conditions for biodegradation of Polyvinyl alcohol under aerobic conditions using batch reactors.
- Kinetic study and statistical analysis of system conditions for biodegradation of polyvinyl alcohol.
- Effect of Polyvinyl alcohol properties and threshold concentration on biodegradation of polyvinyl alcohol using batch reactors.
- A detailed comparison of Municipal and adapted sludge for degradation potential of PVA under high temperatures.

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Appendix

A1. Regression Statistics and Anova Analysis for different types of PVA

- For PVA (MW. 9,000):

Regression Statistics	
Multiple R	0.999563115
R Square	0.999126421
Adjusted R Square	0.998835228
Standard Error	0.035087049
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.224090049	4.224090049	3431.148256	1.09611E-05
Residual	3	0.003693303	0.001231101		
Total	4	4.227783352			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.02185	0.027178311	0.803949879	0.480230076	-0.064643516	0.108344	-0.064643516	0.108343516
X Variable 1	1.29986	0.022190998	58.5760041	1.09611E-05	1.22923834	1.370482	1.22923834	1.37048166

- For PVA (MW. 195,000):

Regression Statistics	
Multiple R	0.997974005
R Square	0.995952114
Adjusted R Square	0.994602819
Standard Error	0.049225769
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	1.788613264	1.788613264	738.1275722	0.000109436
Residual	3	0.007269529	0.002423176		
Total	4	1.795882793			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.06058	0.038130117	1.58877038	0.210323658	-0.060767049	0.181927	-0.060767049	0.181927049
X Variable 1	0.084584	0.003113311	27.16850331	0.000109436	0.074676055	0.094492	0.074676055	0.094491945

- For PVA (MW. 22,000):

Regression Statistics	
Multiple R	0.999551015
R Square	0.999102232
Adjusted R Square	0.998802976
Standard Error	0.030006476
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	3.006054756	3.006054756	3338.61936	1.14196E-05
Residual	3	0.002701166	0.000900389		
Total	4	3.008755922			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%
Intercept	0.01796	0.023242916	0.772708549	0.495989995	-0.056009333	0.091929	-0.056009333
X Variable 1	0.109655	0.001897776	57.78078712	1.14196E-05	0.103615429	0.115695	0.103615429

- For PVA (MW. 88,000):

Regression Statistics	
Multiple R	0.99789279
R Square	0.99579002
Adjusted R Square	0.994386694
Standard Error	0.048554492
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	1.672891801	1.672891801	709.5925189	0.00011608
Residual	3	0.007072616	0.002357539		
Total	4	1.679964417			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%
Intercept	0.01264	0.037610148	0.336079511	0.758944373	-0.107052275	0.132332	-0.107052275
X Variable 1	0.081802	0.003070856	26.63817785	0.00011608	0.072029167	0.091575	0.072029167

- For PVA (MW. 60,000):

Regression Statistics	
Multiple R	0.999901794
R Square	0.999803597
Adjusted R Square	0.999738129
Standard Error	0.011379763
Observations	5

	df	SS	MS	F	Significance F
Regression	1	1.977669841	1.977669841	15271.69971	1.16825E-06
Residual	3	0.000388497	0.000129499		
Total	4	1.978058338			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.02345	0.008814726	2.660320827	0.076317766	-0.004602393	0.051502	-0.004602393	0.051502393
X Variable 1	0.088942	0.000719719	123.5787187	1.16825E-06	0.086651532	0.091232	0.086651532	0.091232468

A2. ZDHC wastewater guidelines limits & effluent charachteristics

Comparison of ZDHC reporting limits with Chinese and Bangladesh effluent standards (ZDHC), 2018)

Parameter	Foundational	Progressive	Aspirational	China direct discharge/ indirect discharge GB 4287-2012 with 2015 amendment	Bangladesh
Temperature (°C)	Max 35	30	25		45 max
Colour (m-1) (λ -436nm;525;620 nm)	7;5;3	5;3;2	2;1;1	50/80[a]	NA
pH	6.0 - 9.0			6.0 - 9.0	6.5 - 9.0
TSS mg/L	50	15	5	50/100	100
TDS mg/L					2,100
BOD_5 mg/L	30	15	5	20/50	150
COD mg/L	150	80	40	80/200	200[b]
Total Nitrogen mg/L	20	10	5	15/30	NA
Ammonium-N mg/L	10	1	0.5	10/20	50[b]
Total Phosphorous (P) mg/L	3	0.5	0.1	0.5/1.5	8[b]
Oil and grease mg/L	10	2	0.5	10/15/100[d]	10
Phenol mg/L	0.5	0.01	0.001	0.5/0.5/2.0[d]	5
Adsorbable Organic Halogen (AOX) mg/L	5	1	0.1	12/12	NA
Coliform bacteria (bacteria/100 mL)	400	100	25		
Persistent foam	Not visible				
Anions					
Cyanide (CN) mg/L	0.2	0.1	0.05	0.5/0.5/0.1[d]	0.1[b]
Sulphide (S^{2-}) mg/L	0.5	0.05	0.01	0.5/0.5	2.0
Sulphite (SO_3^{2-}) mg/L	2	0.5	0.2		

Metals					
Antimony (Sb) mg/L	0.1	0.05	0.01	0.1/0.1	
Arsenic (As) mg/L	0.05	0.01	0.005	0.5[d]	0.2[b]
Cadmium (Cd) mg/L	0.1	0.05	0.01	0.1[d]	0.5[b]
Chromium Total, mg/L	0.2	0.1	0.05	1.5[d]	2.0
Chromium (VI) (mg/L	0.05	0.005	0.001	Not detectable	0.1[b]
Cobalt (Co) mg/L	0.05	0.02	0.01		
Copper (Cu) mg/L	1.0	0.5	0.25	0.5/1.0/2.0[d]	0.5[b]
Lead (Pb) mg/L	0.1	0.05	0.01	1.0[d]	0.1[b]
Mercury (Hg) mg/L	0.01	0.005	0.001	0.05[d]	0.1[b]
Nickel (Ni) mg/L	0.1	0.02	0.05	1.0[d]	1.0[b]
Silver (Ag) mg/L	0.1	0.05	0.005	0.5[d]	
Zince (Zn) mg/L	5.0	1.0	0.5	2.0/5/5[d]	5[b]
Alkylphenol (AP) and Alkylphenol Ethoxylates (APEOs): Including all isomers (e)	Reporting limit 5 µg/L				
Chlorophenols	Reporting limit 0.5 µg/L				
Dyes (carcinogenic or equivalent concern (e)	Reporting limit 500 µg/L				
Flame retardents (e)	Reporting limit 5.0 µg/L				
Glycols (e)	Reporting limit 50.0 µg/L				
Otho-Phthalates – Including all ortho esters of phthalic acid (e	Reporting limit 10.0 µg/L				

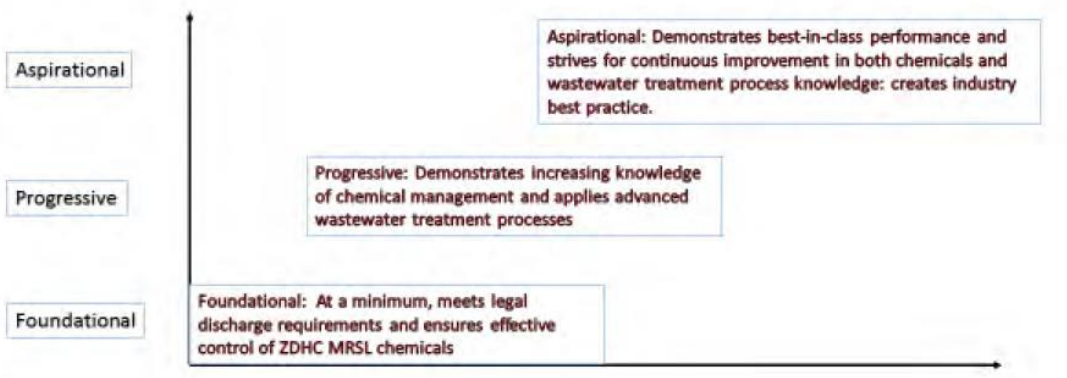
(a) – Colour method is multiple dilution.

(b) – Not specific to textile industry but applies to industrial units discharging water to inland water.

(d) – Not specific to textile industry. Values are for integrated wastewater standard GB 8978 – 1996 for Tier I/II/III.

(e) – For a full description of the relevant compounds please refer to the ZDHC Wastewater Guidelines.

ZDHC 3 Levels



- Levels of pH, BOD_5, COD In textile processes (ZDHC), 2018).

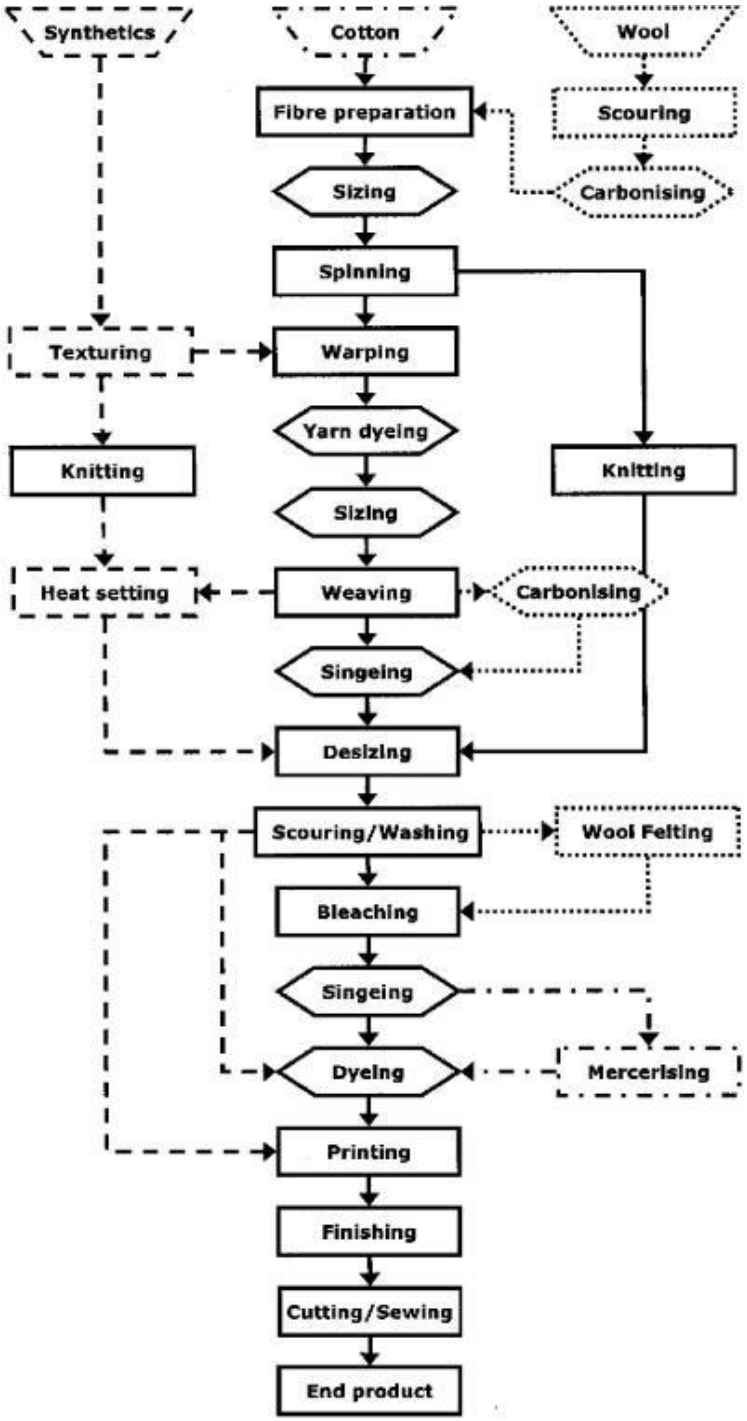
Source of effluent	pH	BOD (mg/L)	COD mg/L	BOD/COD
Process effluent	5.8 – 6.5	1,700 – 5,200	10,000 – 15,000	0.17 – 0.34
Scouring	10 – 13	260 – 400	1,200 – 3,300	0.22 – 0.12
Bleaching	8.5 – 9.6	50 – 100	150 – 500	0.3 – 0.2
Mercerising	8.0 – 10.0	20 – 50	100 – 200	0.20 – 0.25
Dyeing	7 – 10	400 – 1,200	1,000 – 3,000	0.4
Wash Effluent				
After bleaching	8.0 – 9.0	10 – 20	50 – 100	0.2
After acid rinsing	6.5 – 7.6	25 – 50	120 – 250	0.2
After dyeing (hot wash)	7.5 – 8.5	100 – 200	300 -500	0.3 – 0.4
After dyeing (acid and soap wash)	7.5 – 8.64	25 – 50	50 – 100	0.5
After dyeing (final wash)	7.0 – 7.8		25 – 50	
Printing washing	8.0 – 9.0	115 – 150	250 – 450	0.46 – 0.33
Blanket washing of rotary printer	7.0 – 8.0	25 – 50	100 – 150	0.25 – 0.3

- Typical charachteristics of untreated effluents (ZDHC), 2018)

Parameter	Range
pH	6 – 10
Temperature ºC	35 – 45
Total dissolved solids mg/L	1,000 – 12,000
Biological Oxygen Demand (BOD) mg/L	80 – 6,000
Chemical Oxygen Demand (COD) mg/L	150 – 12,000
Total suspended solids (TSS), mg/L	15 – 8,000
Chloride, mg/L	1,000 – 6,000
Free chlorine, mg/L	<10
Oil & Grease, mg/L	10 – 30
Total Kjeldahl Nitrogen (TKN) mg/L	70 – 80
Nitrate (NO3) mg/L	<15
Free ammonia, mg/L	<10
Colour (Pt-Co)	50 – 2,500
Sulphate, (SO4) mg/L	600 – 1,000
Heavy metals, mg/L	<10
ZDHC MRSL chemicals*	Not typically regulated or measured in influent water

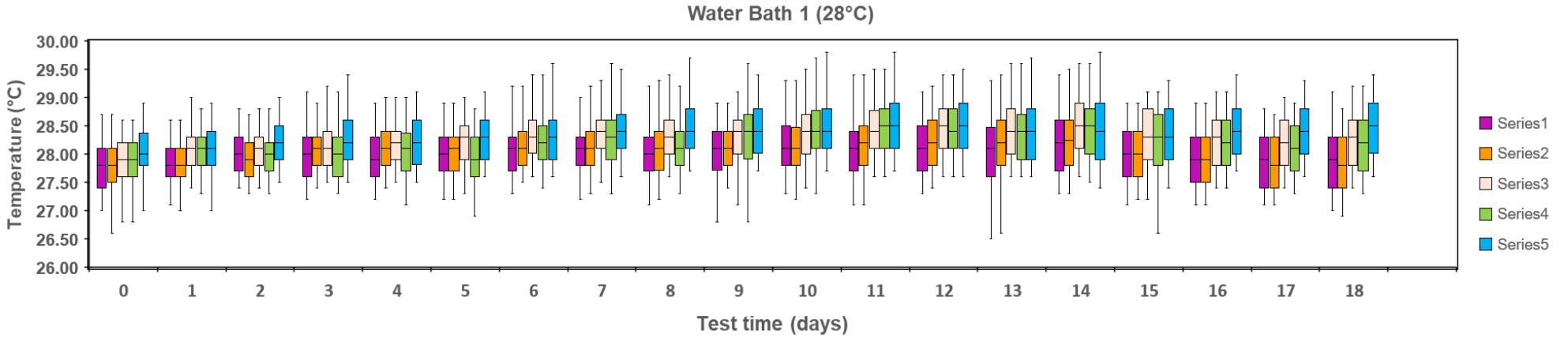
A3. Textile manufacturing process scheme.

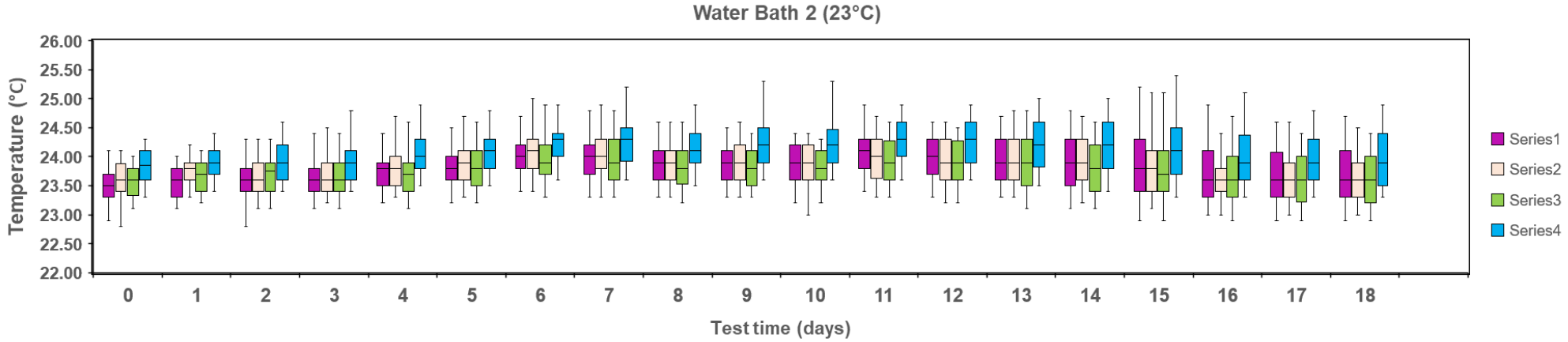
- flowchart for processes taking place in textile manufacturing. Square boxes represent processes that occur in same order, and 'diamond' shaped boxes represent processes that can occur at different places in the chain. The line style the fabric types related to these processes (Bisschops & Spanjers, 2003).

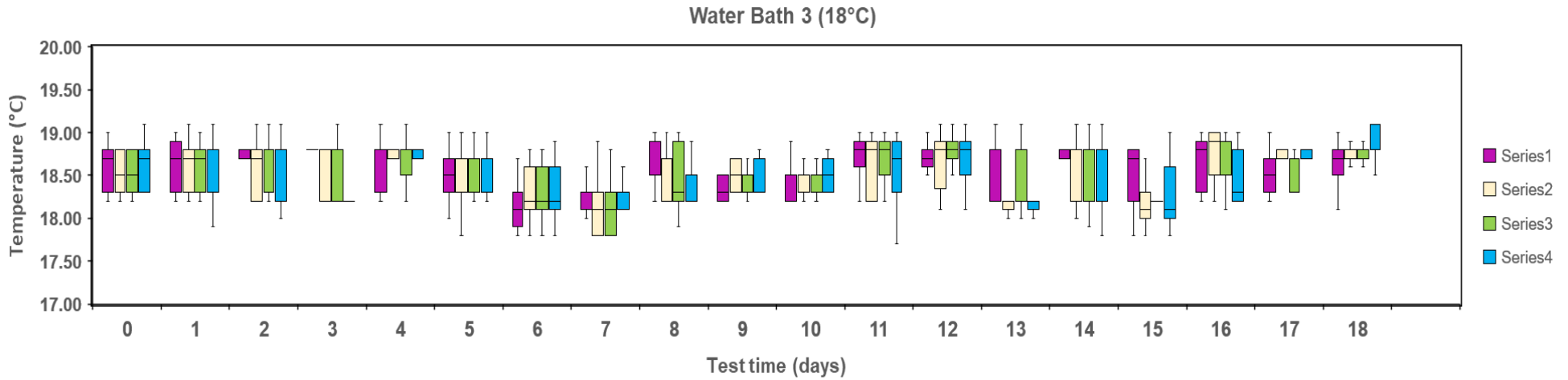


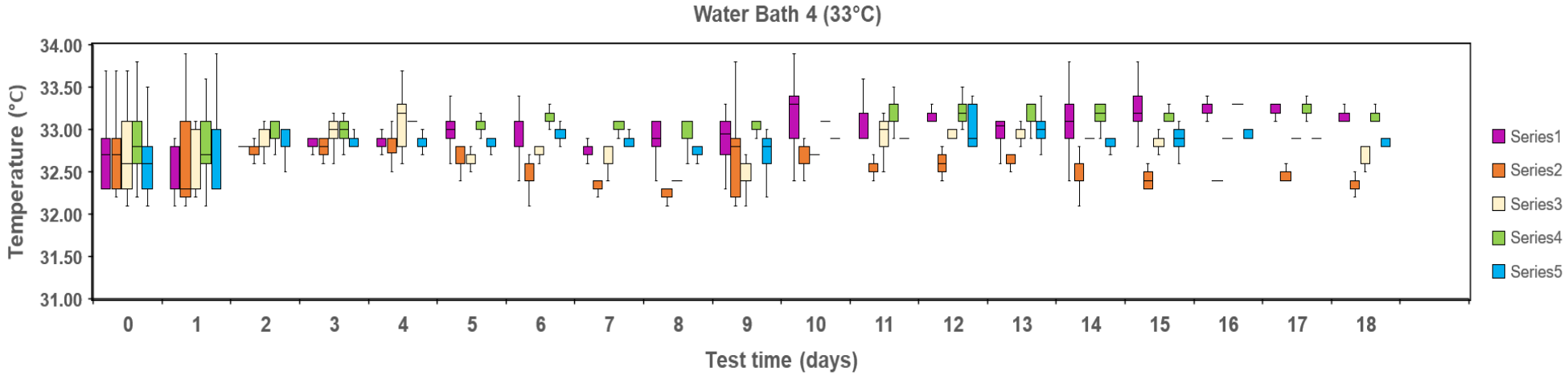
A.4 Temperature Fluctuations in biodegradation experiments

A. 4.1 Optimum System Conditions experiments

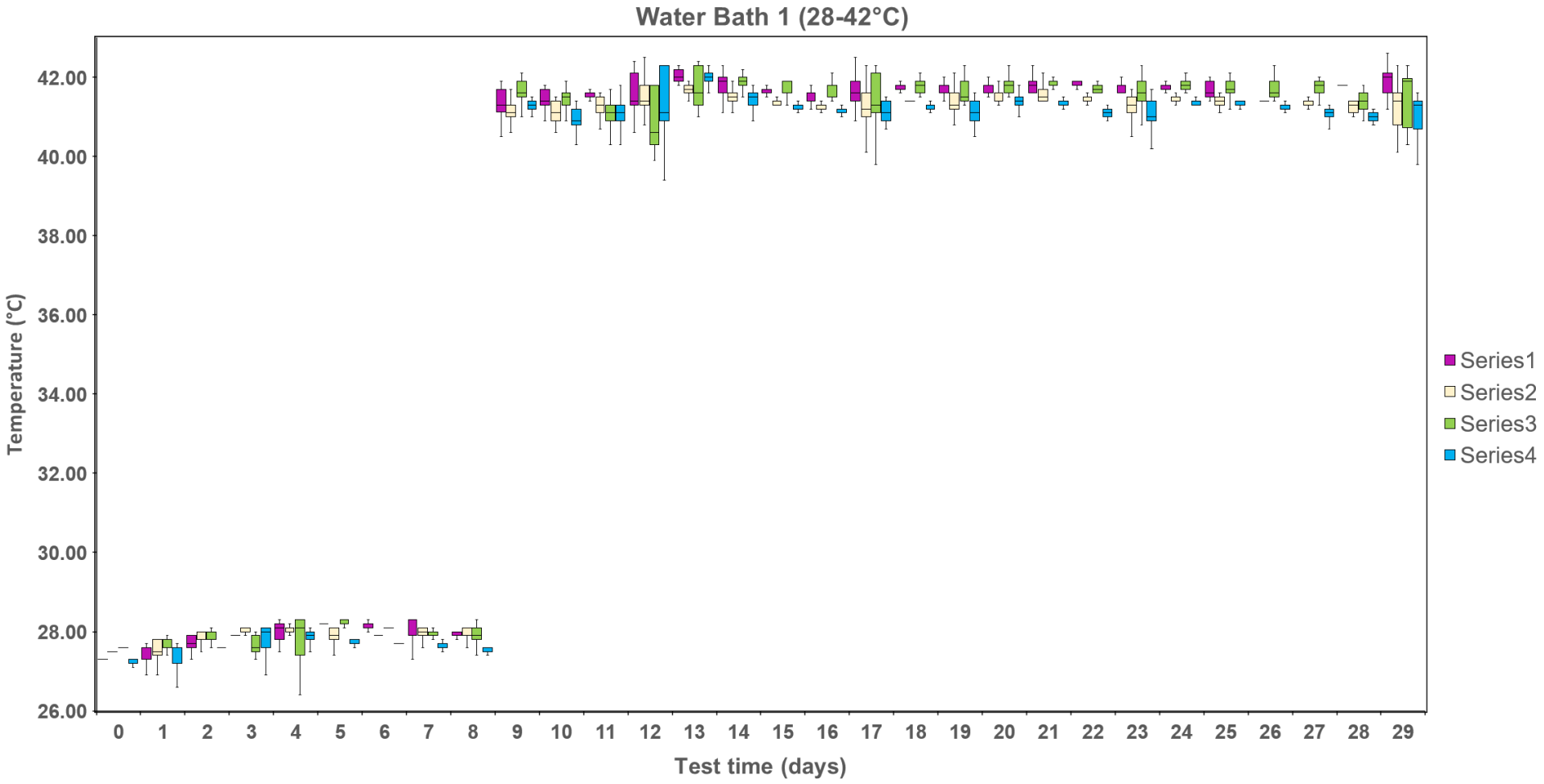


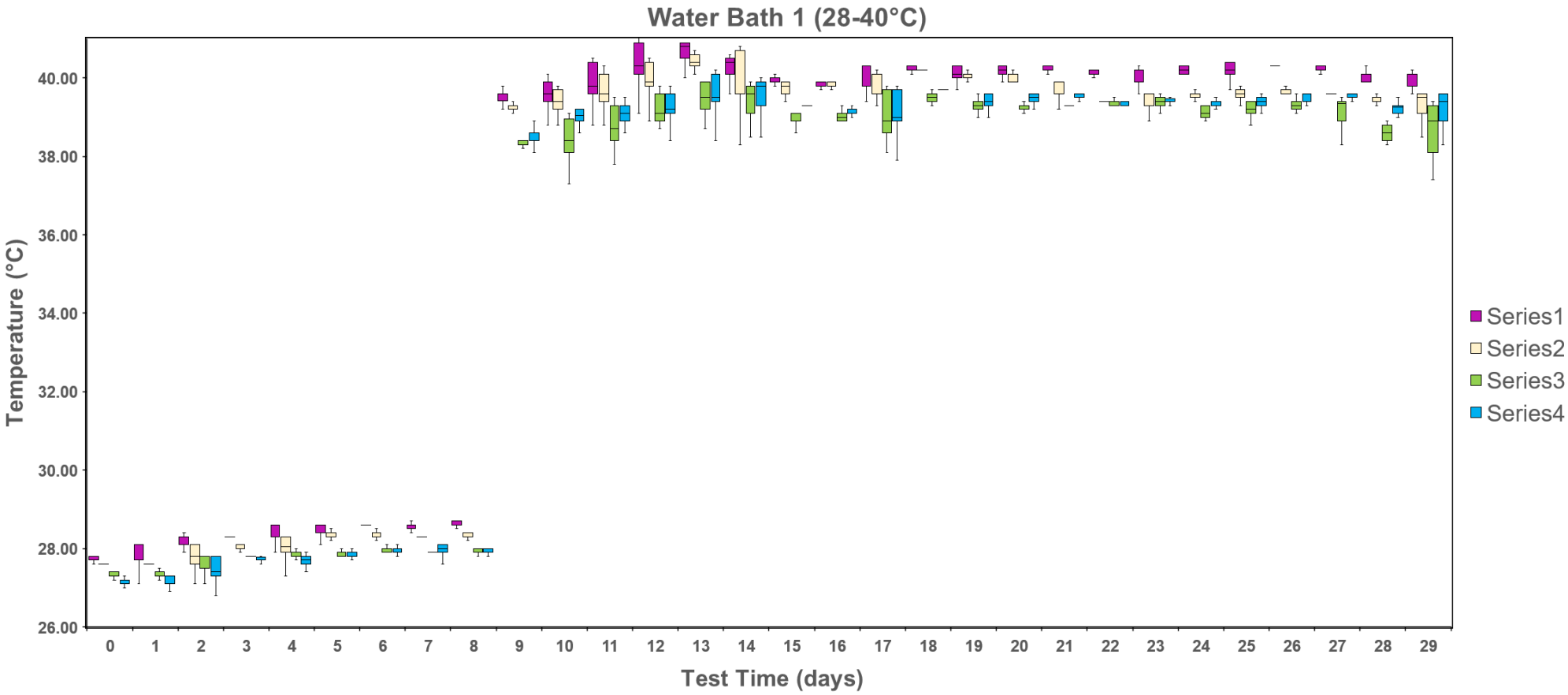


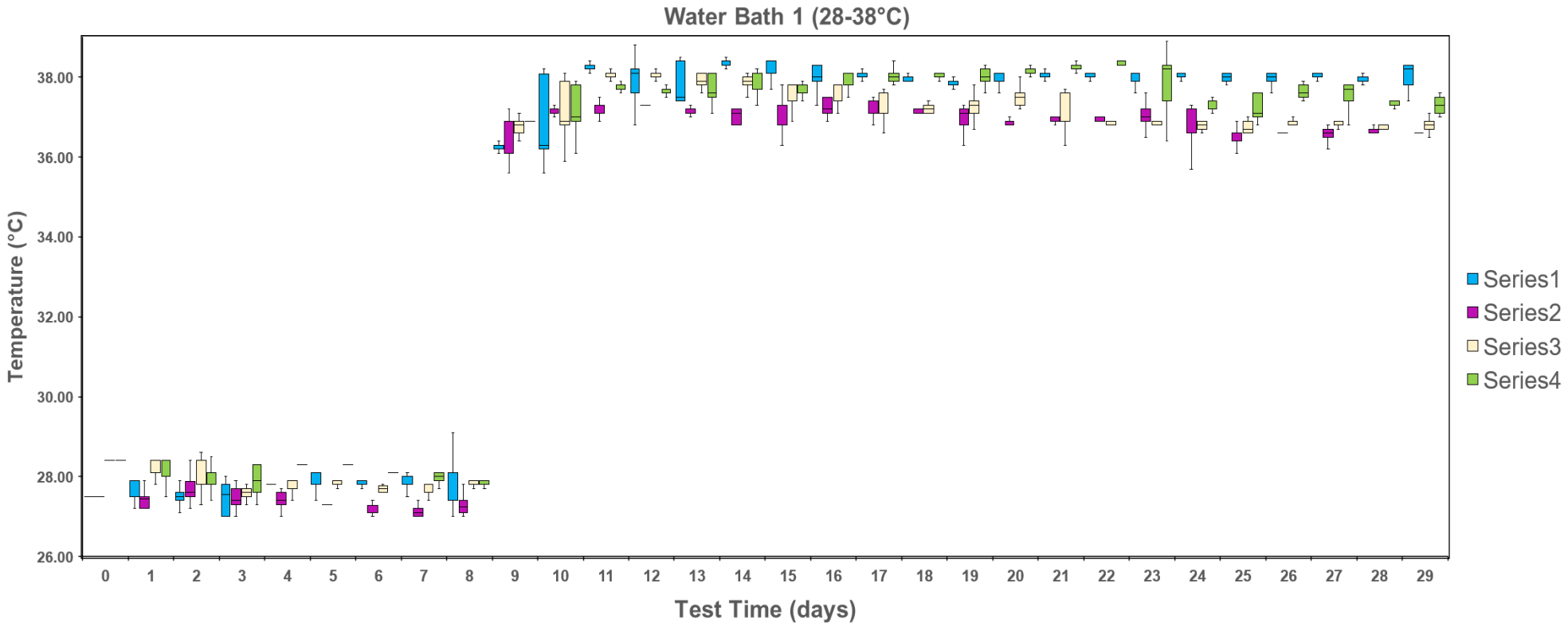

Water Bath 3 (18°C)
Temperature (°C)
20.00
19.50
19.00
18.50
18.00
17.50
17.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Test time (days)
Series1
Series2
Series3
Series4

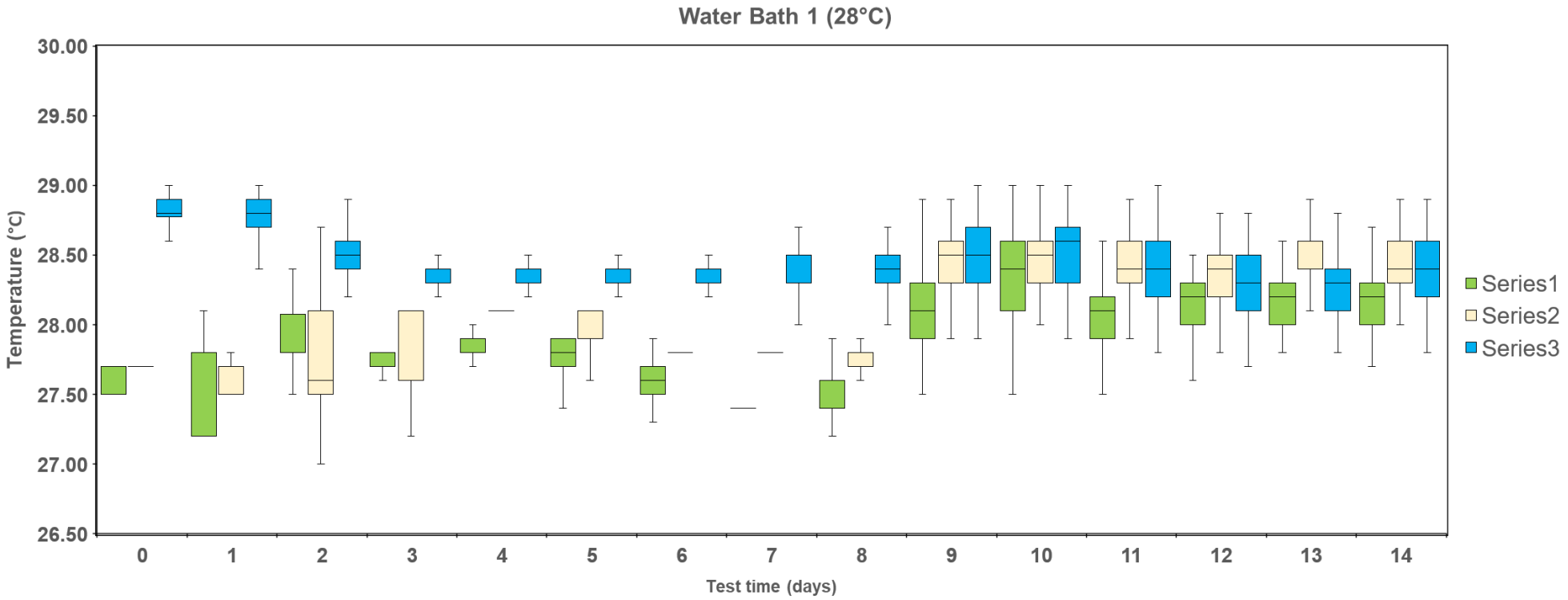

Water Bath 4 (33°C)
Temperature (°C)
34.00
33.50
33.00
32.50
32.00
31.50
31.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Test time (days)
Series1
Series2
Series3
Series4
Series5

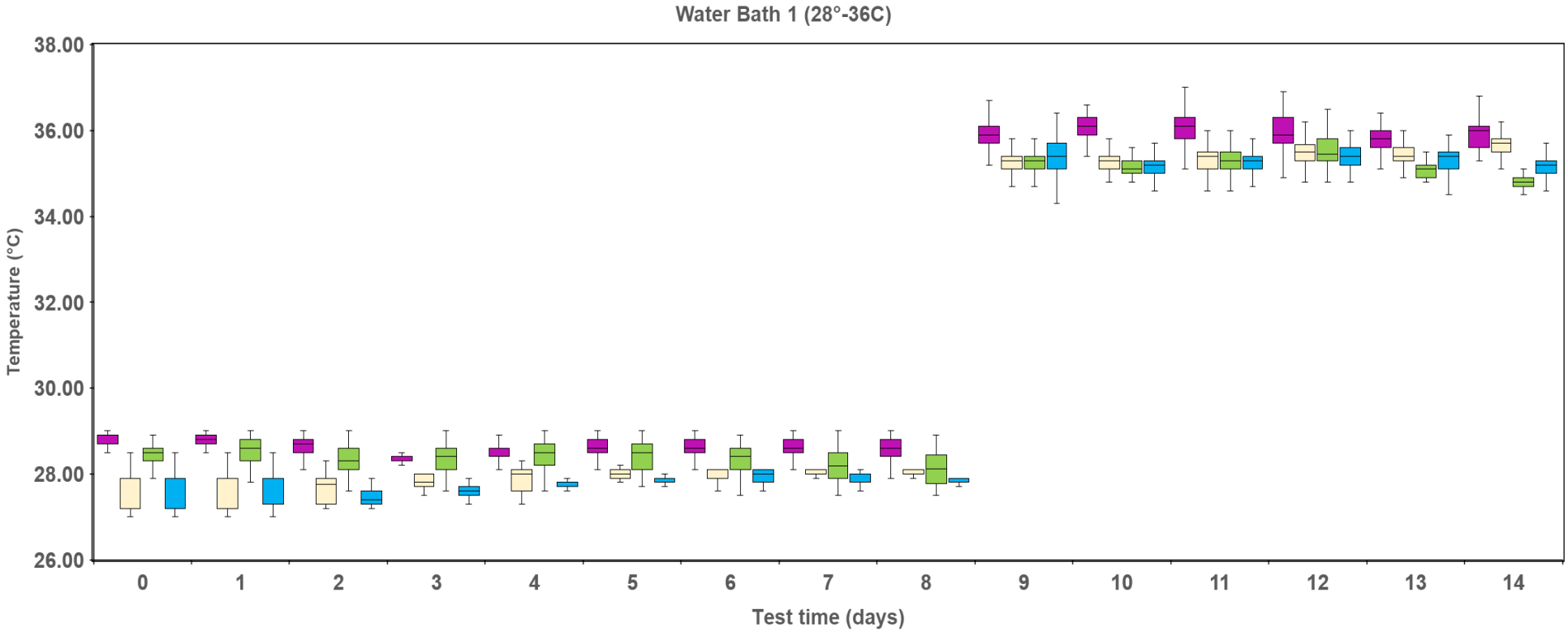
A. 4.2 Two Phases experiments



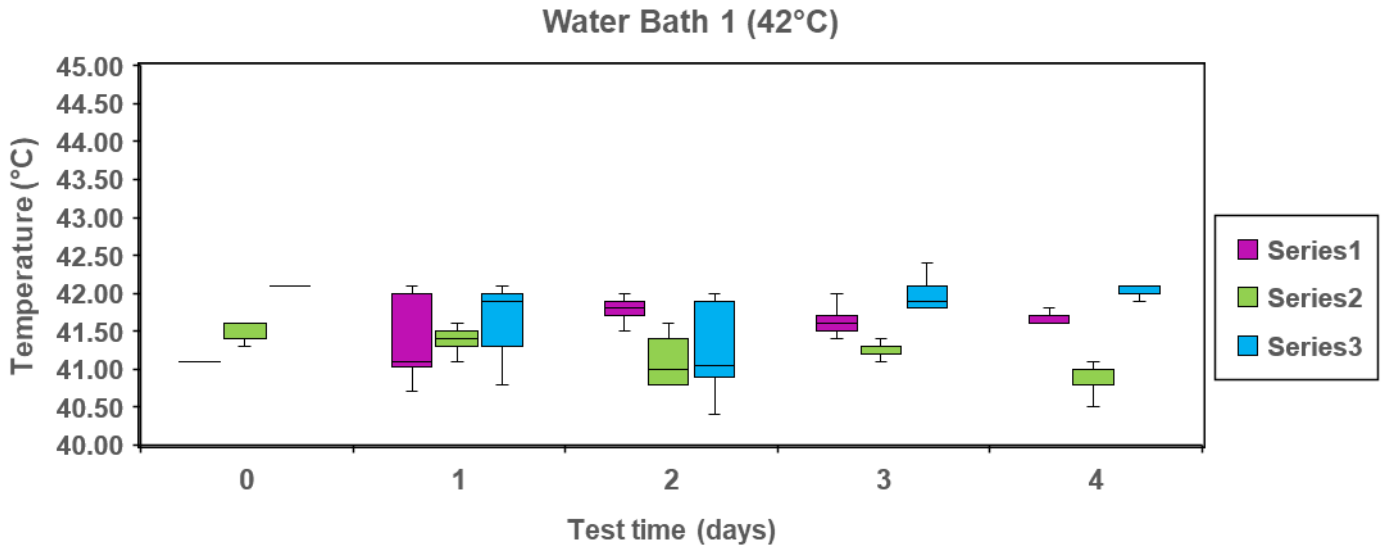

Water Bath 1 (28-40°C)
Temperature (°C)
40.00
38.00
36.00
34.00
32.00
30.00
28.00
26.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Test Time (days)
Series1
Series2
Series3
Series4

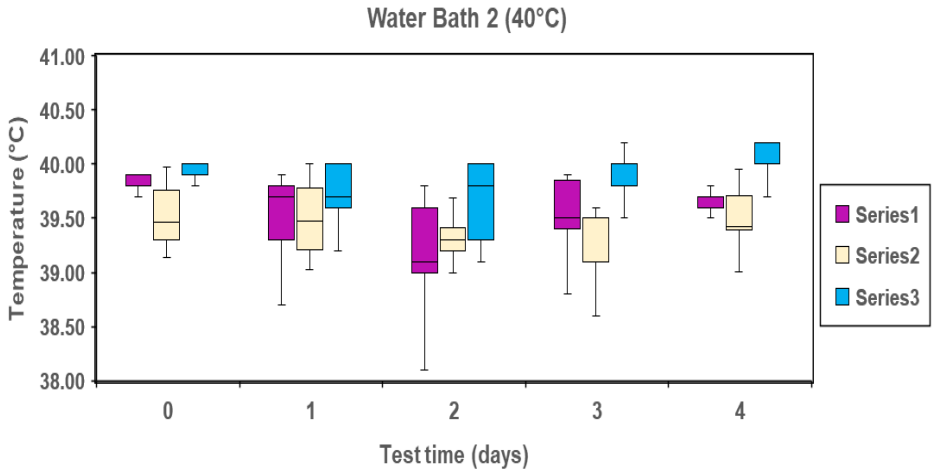

Water Bath 1 (28-38°C)
Temperature (°C)
38.00
36.00
34.00
32.00
30.00
28.00
26.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Test Time (days)
Series1
Series2
Series3
Series4

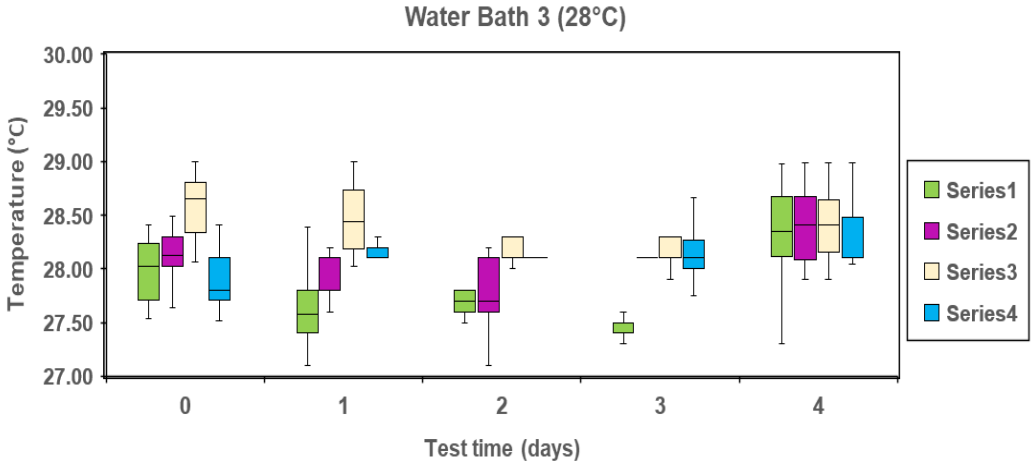

Water Bath 1 (28°C)
Temperature (°C)
30.00
29.50
29.00
28.50
28.00
27.50
27.00
26.50
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Test time (days)
Series1
Series2
Series3

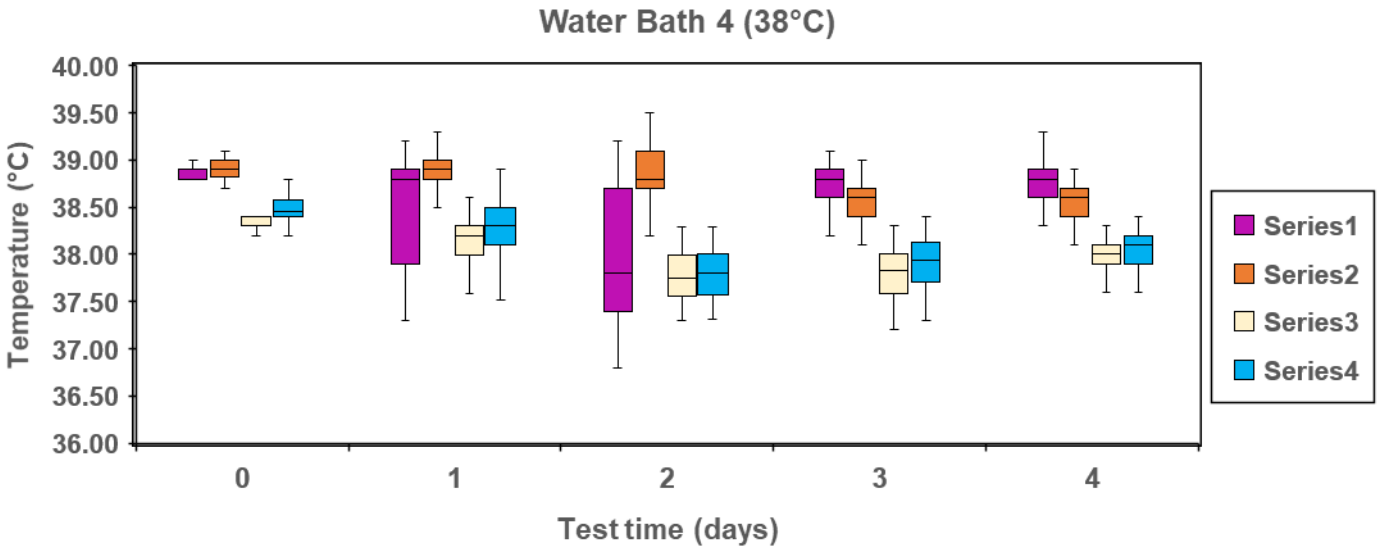

Water Bath 1 (28°-36C)
38.00
36.00
34.00
32.00
30.00
28.00
26.00
Temperature (°C)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Test time (days)

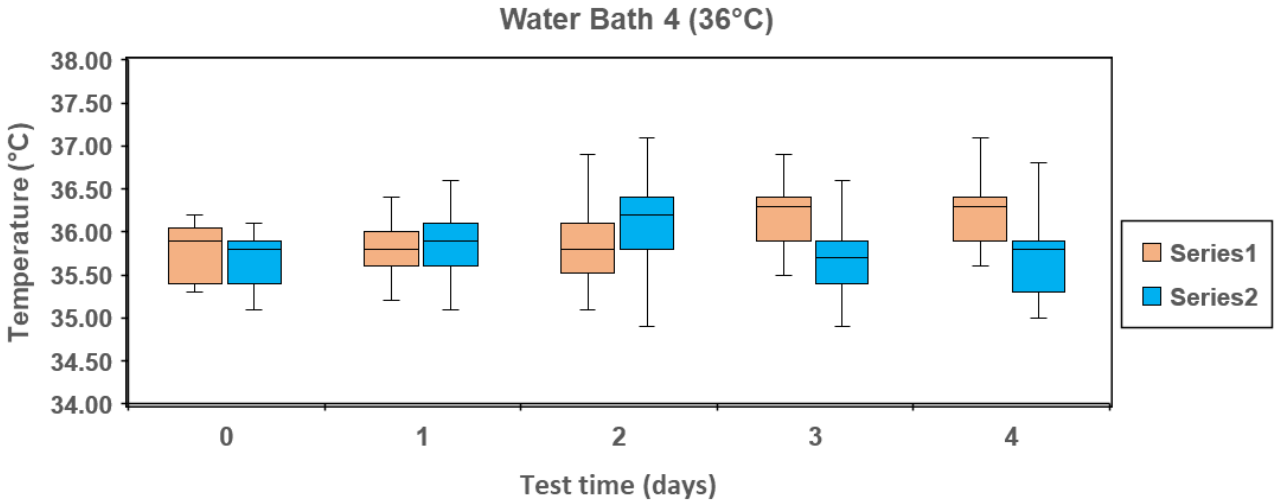
A 4.3 Cilander experiments (Adapted sludge)








Water Bath 4 (38°C)
Temperature (°C)
40.00
39.50
39.00
38.50
38.00
37.50
37.00
36.50
36.00
0
1
2
3
4
Test time (days)
Series1
Series2
Series3
Series4


Water Bath 4 (36°C)
Temperature (°C)
38.00
37.50
37.00
36.50
36.00
35.50
35.00
34.50
34.00
0
1
2
3
4
Test time (days)
Series1
Series2