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**http://dx.doi.org/10.1016/j.scp.2023.101127**

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# Developing a Smart and Clean Technology for Bioremediation of Antibiotic Contamination in Arable Lands

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# Developing a Smart and Clean Technology for Bioremediation of Antibiotic Contamination in Arable Lands


## Abstract

This study presents a smart technological framework to efficiently remove azithromycin from natural soil resources using bioremediation techniques. The framework consists of several modules, each with different models such as *Penicillium Simplicissimum* (PS) bioactivity, soft computing models, statistical optimisation, Machine Learning (ML) algorithms, and Decision Tree (DT) control system based on Removal Percentage (RP). The first module involves designing experiments using a literature review and the Taguchi Orthogonal design method for cultural conditions. The RP is predicted as a function of cultural parameters using Response Surface Methodology (RSM) and three ML algorithms: Instance-Based K (IBK), KStar, and Locally Weighted Learning (LWL). The sensitivity analysis shows that pH is the most important factor among all parameters, including pH, Aeration Intensity (AI), Temperature, Microbial/Food (M/F) ratio, and Retention Time (RT), with a p-value of $< 0.0001$. AI is the next most significant parameter, also with a p-value of $< 0.0001$. The optimal biological conditions for removing azithromycin from soil resources are a temperature of 32°C, pH of 5.5, M/F ratio of 1.59 mg/g, and AI of 8.59 $m^3/h$. During the 100-day bioremediation process, RP was found to be an insignificant factor for more than 25 days, which simplifies the conditions. Among the ML algorithms, the IBK model provided the most accurate prediction of RT, with a correlation coefficient of over 95%.



**Keywords:** Azithromycin; bioremediation; machine learning; *penicillium simplicissimum*; Taguchi design.

## 1. Introduction

Managing soil health is a significant challenge in agriculture. Moreover, it is a complex issue that is difficult to address using traditional methods (Mohammad et al., 2021). With the increasing prevalence of new diseases worldwide, people especially in low-income countries are resorting to antibiotics to treat infections (Klein et al., 2021). This trend can have adverse health effects due to the carcinogenic properties of antibiotics (Llor and Bjerrum, 2014). Recent studies have also shown that the widespread use of antibiotics in natural resources such as air, soil, water, and humans can lead to health risks as microorganisms develop drug resistance (Zhang et al., 2015).

Azithromycin (AZ) is one of the most widely used antibiotics for treating bacterial infections. According to the Food and Drug Administration (USFDA), AZ (Zithromax or Zmax) can disrupt the electrical processes of the heart, leading to a potentially fatal irregular heartbeat (Patel et al., 2020). Due to the persistent nature of antibiotics, their recalcitrance, and the emergence of resistance genes, their widespread presence in natural resources can cause a global environmental problem, making their remediation essential (Cycoń et al., 2019).

Azithromycin is classified as an Endocrine Disrupting Compound (EDC), which can pose unprecedented health risks due to pollutant emissions in soil and water (Lau et al., 2020). Soil pollution by EDCs can occur when pharmaceutical and industrial solid wastes are released into the environment without adhering to regulations and landfill standards (Hu et al., 2010). According to a survey conducted by SOM-institute[1] in Sweden in 2020, environmental pollution and antibiotic resistance are considered significant concerns by the public. The release of azithromycin in soil

[1] Source(s): SOM-institute; ID 909223

exacerbates both issues and increases public concerns. Therefore, reducing the concentration of azithromycin in soil would alleviate these problems and increase public satisfaction.

Addressing antimicrobial resistance (AMR) requires clear global guidelines and regulatory options, but this has been challenging due to the diversity and dynamic nature of healthcare and regulatory systems across different countries (Chokshi et al., 2019). As a result, the World Health Organisation (WHO) has made policies and recommendations, but it is up to each nation to regulate antibiotic resistance. To tackle AMR, individuals, healthcare professionals, policymakers, the healthcare industry, and the agricultural sector all need to act.

The WHO recommends that individuals use antibiotics only with a prescription, follow infection prevention guidelines, and practice safe food preparation according to the WHO Five Keys to Safer Food (FKSF). Policymakers can develop a national action plan to reduce antibiotic resistance, improve surveillance systems of antibiotic use, regulate the proper use and disposal of medicine, and educate the public on antibiotic resistance. Healthcare professionals can prevent infections by maintaining a clean and sterile working environment, prescribing antibiotics only when necessary, reporting antibiotic-resistant infections to surveillance systems, and educating patients on the risks of antibiotic misuse and how to prevent infections. The healthcare industry should invest in research and development of new antibiotics, diagnostics, and vaccines. The agricultural sector must use antibiotics in animals only under the supervision of a veterinarian, avoid using antibiotics to prevent diseases, and use vaccines instead. Fine practices can help reduce infections and improve biosecurity on farms (WHO, 2019).

In 2015, the World Health Assembly established a global action plan on AMR, consisting of 5 key policies: 1) enhancing public awareness about AMR, 2) promoting stewardship of antibiotics, 3) reducing and preventing infection, 4) improving surveillance systems and research, and 5)

ensuring sustainable investment in combating AMR. The Assembly also urged each country to develop its own national action plan (NAP) to combat AMR (Anderson, 2020). In response, Iran created its own NAP (IRI-NAP) in 2016 in five sections (summarised in Table 1) aligned with the main policies outlined in the global action plan (Moradi et al., 2018).

Among the most common types of antibiotics (e.g., *β-lactams*, *Macrolides*, *Fluoroquinolones*, *Tetracyclines*, *Sulfonamides*, *Diaminopyrimidines*, *Lincosamides*, and their degradation products), according to the reports, Fluoroquinolones, Tetracyclines, and Sulfonamides have the highest concentration in soil samples mostly caused by manure and wastewater irrigation (Yang et al., 2021). Table 2 shows the concentration data of some of the frequently found antibiotics in soil.

**Table 1. Selected policies of the national action plan in Iran against Antimicrobial resistance**

| Goal | Policy |
|---|---|
| **Enhance public awareness of AMR** | -Conduct education courses for specific groups such as children and elders<br>-Run awareness campaigns for people working in related fields<br>-Initiate targeted activities |
| **Optimise the use of antibiotics** | -Strategic purchases of antimicrobial medicines to improve the quality<br>-Empowering medical institutions to create guidelines and manuals for antimicrobial stewardship of their own |
| **Prevent and control infections** | -Promotion of vaccines<br>-Support NGO activities in coordination with hospitals to prevent and control infection<br>-Control antimicrobial residue in food productions<br>-Sending AMR experts all over the country to respond quickly while outbreaks happen |
| **Improve surveillance system** | -Create a monitoring system of prescriptions and antibiotics<br>-Update and monitor prescription criteria for antibiotics<br>-Increase the capacity of laboratories dealing with AMR<br>-Research the AMR surveillance systems |
| **Guarantee sustainable investment and research in combating AMR** | -Promote research to clarify the necessity of AMR<br>-Create a database of resistant genes<br>-Conduct more research to deeply investigate the impact of AMR on health<br>-Reconsidering the microbial diagnosis, treatment, and prevention approaches<br>-Promote research and industry with the international collaboration |

79 **Table 2. The highest concentrations of some major antibiotics reported in the soil environment.**

| Type | Antibiotic | Concentration (ng/g) | Reference |
|---|---|---|---|
| *β-lactams* | *Amoxicillin* | 200 | Braschi et al., 2013 |
| *Fluoroquinolones* | Ciprofloxacin | 350 | Al Masud et al., 2023; Martínez-Carballo et al., 2007; Karci and Balcioglu, 2009; Hu et al., 2010; Van Doorslaer et al., 2014; Pan and Chu, 2017b |
| | *Difloxacin* | 21.5 | |
| | *Enrofloxacin* | 1,347.60 | |
| | *Norfloxacin* | 5,610 | |
| | *Ofloxacin* | 898 | |
| *Quinolone* | *Sarafloxacin* | 5.92 | Rashid et al., 2023 |
| *Macrolides* | *Enrofloxacin* | 22.93 | Thiele-Bruhn, 2003; Leal et al., 2012; Tasho and Cho, 2016; Pan and Chu, 2017, 55 |
| | *Erythromycin* | 100 | |
| | *Tylosin* | 1,250 | |
| | *Azithromycin* | 1,000 | Topp et al., 2016 |
| | Total macrolides | 1.471 | Li et al., 2023 |
| *Sulfonamides* | *Sulfachloropyridazine* | 52.9 | Thiele-Bruhn, 2003; Dolliver et al., 2007; Karci and Balcioglu, 2009; Hu et al., 2010; Carter et al., 2014; Pan and Chu, 2017 |
| | *Sulfadiazine* | 85.5 | |
| | *Sulfadimethoxine* | 40.4 | |
| | *Sulfadoxine* | 9.1 | |
| | *Sulfamethoxazole* | 54.5 | |
| | *Sulfamethazine* | 200–25,000 | |
| | *Sulfamonomethoxine* | 5.37 | |
| | *Sulfapyridine* | 5.11 | |
| | Total sulfonamides | 18.497 | Li et al., 2023 |
| *Tetracyclines* | *Chlortetracycline* | 12,900 | Hamscher et al., 2002; Thiele-Bruhn, 2003; Karci and Balcioglu, 2009; Hu et al., 2010; Liu et al., 2016; Tasho and Cho, 2016; Pan and Chu, 2017; Łukaszewicz et al., 2018 |
| | *Doxycycline* | 728 | |
| | *Oxytetracycline* | 50,000 | |
| | *Minocycline* | 32 | |
| | *Tetracycline* | 2,683 | |

80

81 Various techniques are available for degrading antibiotics in different environments such as water,
82 wastewater, soil, and solid waste. These techniques include adsorption (Gheibi et al., 2023),
83 integrated biological treatment with membranes (Zhao et al., 2021), permeable reactive barriers

(Zhao et al., 2018), fungus-based bioremediation (Mohammadi et al., 2021), and electrochemical systems (Bicudo et al., 2017). However, each technique has its strengths and weaknesses and is applicable in specific real field situations. For example, permeable reactive barriers, membranes, biofilm membranes, and adsorption processes provide an acceptable efficiency of more than 95%, but they have limited capacity for decontamination and may require regeneration or recovery of the system in a short time (Zhang et al., 2020c). On the other hand, coagulation and electrocoagulation procedures have high efficiency but involve chemical material addition and unusual energy consumption (Bicudo et al., 2021). Bioremediation, which involves living organisms such as fungi, algae, bacteria, plants, and animals, is a process that removes or detoxifies pollutants in the environment (Jagtap, 2020). It is environmentally friendly, requires low capital investment, and has minimal energy consumption, making it a popular method for degradation (Irshad et al., 2021). Bioremediation is particularly suitable for removing antibiotic compounds that are sensitive to pH and temperature (Liu et al., 2017).

Mycoremediation is a type of bioremediation that involves the use of fungi as the decomposer of pollutants (Schmit and Mueller, 2007). Fungi are a diverse group of microorganisms with unique characteristics, including the ability to form extensive mycelial networks, low specificity of their catabolic enzymes, and their independence from using pollutants as a growth substrate (Harms et al., 2011). Fungi are known to be capable of degrading and mineralising recalcitrant antibiotics due to their non-specific, non-stereoselective enzymatic systems based on free-radical levels (Čvančarová et al., 2015). Over the past two decades, fungi have been widely used for the treatment of waste and wastewater as well as for the degradation of hazardous compounds (Khatoon et al., 2021). Fungi have multiple strategies to cope with toxic compounds, such as antibiotics, including *bioadsorption*, *biomineralisation* (bio-precipitation) as

well as biotransformation and biodegradation mediated by enzymatic systems (Olicón-Hernández et al., 2017).

Fungi have been found to play a critical role in removing heavy metals and mineralising various types of pollutants, including phenols, halogenated phenolic compounds, petroleum hydrocarbons, and polycyclic aromatic compounds (Sing, 2006). They are known to excrete enzymes for the decomposition of carbohydrates without prior hydrolysis, making them highly effective in degrading a vast number of pollutants (Bellaouchi et al., 2021). Fungi have several advantages such as being easy to grow in fermenters and having a filamentous structure that allows for easy separation of fungal biomass (Akhtar and Abdullah, 2014). *Penicillium* strains are popular among all other fungi species as they can live in saline environments and have been reported to treat heavy metals, polycyclic aromatic hydrocarbons, phenol and its derivatives, wastewater, and wastes (Leitão et al., 2007). Among these species, *Penicillium simplicissimum* (PS) has been selected as the decomposer in this study to learn the effectiveness of this specie in the removal of AZ in soil (Sowmya et al., 2015).

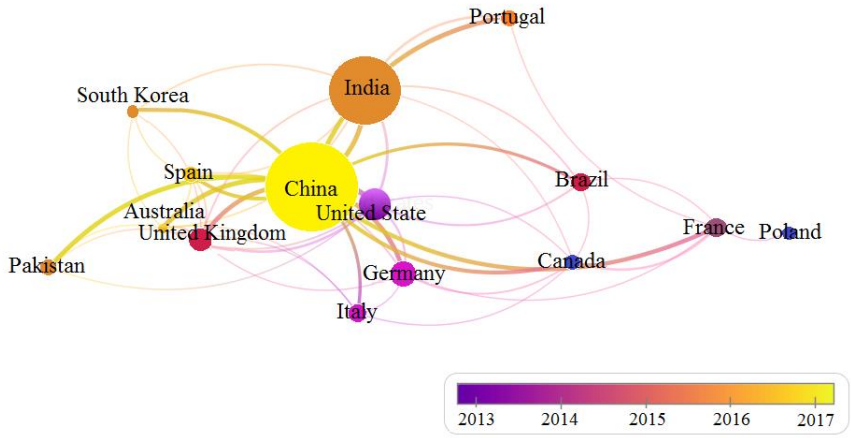
The performance of microorganisms is mainly dependent on the cultural conditions (Khayati and Barati, 2017). The efficiency of biodegradation processes relies on several factors such as pH, temperature, soil properties and substrate (Oliveira et al., 2020). Conventional optimisation methods take a lot of time and cost due to numerous variables, which can be overcome by using multi-factor methods such as Taguchi orthogonal design (Carraro et al., 2022). This approach helps to investigate parameters, obtain more data, and reduce time and cost by suggesting effective adjustments to control factors. Taguchi has been used for myco-synthesis of nano-silver, wastewater treatment processes, and bioremediation and biodegradation process optimisation.

The use of Machine Learning (ML) approaches has significantly helped in modelling biodegradation procedures with high accuracy and non-limited applicability (Sodhi and Singh, 2022). Several studies have been conducted on the bioremediation of various pollutants by fungi, and ML algorithms such as Random Forest (RF), Adaptive Neuro-Fuzzy Inference System (ANFIS), Random Tree (Mohammadi et al., 2021), Support Vector Machine (SVM) (Liu et al., 2022), M5 Pruned model tree, Gaussian Processes (GP), and Sequential Minimal Optimisation (SMOreg) (Akbarian et al., 2022) have been applied to predict the biodegradation efficiency and cultural conditions.

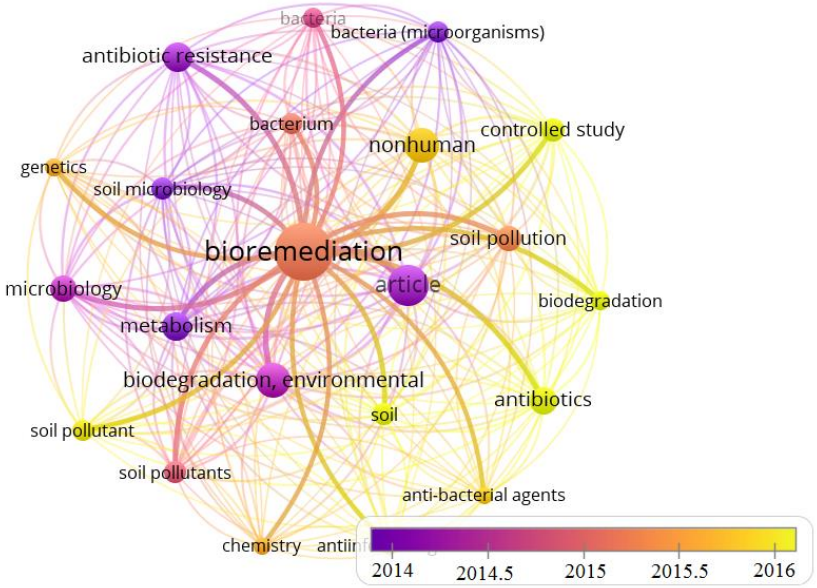
The fate and remediation of antibiotic pollutants in the environment have been extensively researched. Vermillion and Tjeerdema (2017) studied the degradation kinetics of AZ under aerobic and anaerobic conditions. Sidhu et al. (2019) used Continuous Stirred-Tank Reactors (CSTRs) to remediate *ciprofloxacin* and AZ with soil-based biomaterials, while Li et al. (2019) used electrokinetic remediation for tetracycline-polluted soil. Zhan et al. (2021) evaluated heat treatment for the decontamination of *tetracycline* and *roxarsone*. Mohammadi et al. (2021) proposed a fungus-based purification method for *amoxicillin*, while Sidhu et al. (2021) assessed AZ resistance in biosolids. Zelt et al. (2021) emphasised the importance of purifying antibiotics from agricultural soil to ensure food chain health.

To evaluate the research on bioremediation process for decontamination of antibiotics from soil, the library assessment was carried out using the VOSviewer software, where more than ten documents (Fig. 1a) and over 100 repetitions of keywords (Fig. 1b) related to soil, bioremediation, and antibiotics were filtered (Fig. 1). The results showed that China, India, and the United States have conducted most of the investigations on this topic. The research on this subject in Iran was challenging due to the limitation of facilities and equipment. The bioremediation subject is

associated with soil pollution, antibiotics, soil microbiology, and biodegradation issues, and it is regarded as a pressing issue, essential for scientific communities, which is the aim of this study. The figures generated by the software represent different ranges of time series and show the accumulated published documents based on different subjects and the focus of the country's contributions.




(a)




(b)

**Fig. 1. Scientometry analysis of antibiotic bioremediation from soil resources through (a) country, and (b) occurrence of keywords aspects**

Table 3 provides an overview of research that has used fungi for the removal of antibiotics. The authors argue that a sustainable system for soil quality management is necessary to reduce the impact of biomagnification, epidemiological disease, and immunological issues (Fasihi et al., 2021), but previous studies have not considered a smart system for controlling bioremediation structures. Therefore, this study aims to develop a smart framework for optimal control and degradation of AZ in soil using *Penicillium Simplicissimum* (PS) bioactivities, the Taguchi design method for optimisation, and three lazy machine learning models to predict bioremediation behaviour. The study also aims to design a control system for the bioremediation system based on decision tree modelling and evaluate a sustainable industry using conceptual models. The authors argue that this study will address a knowledge gap related to optimising decontamination of AZ by considering optimisation, system performance prediction, and process control through conceptual modeling at the same time.

**Table 3. Bioremediation studies using fungus for antibiotic removal.**

| Fungal name | Antibiotic contamination | Mechanism | Reference |
|---|---|---|---|
| ***Trametes versicolor*** | Azithromycin | Bio-oxidation | Del Álamo et al., (2022) |
| ***Ganoderma lucidum*** | 20 different antibiotics | Biodegradation | Salandez et al., (2022) |
| ***Penicillium oxalicum RJJ-2*** | Erythromycin | Biodegradation | Ren et al., (2021) |
| ***Penicillium commune*** ***Epicoccum nigrum*** ***Trichoderma harzianum*** ***Aspergillus terreus*** ***Beauveria bassiana*** | Oxytetracycline | Biodegradation | Ahumada-Rudolph et al., (2021) |

| Fungal name | Antibiotic contamination | Mechanism | Reference |
|---|---|---|---|
| ***Penicillium restrictum*** | Sulfamethoxazole, Erythromycin, Tetracycline | Biodegradation | Fakhri et al., (2021) |
| ***Aspergillus flavus*** | Amoxicillin | Biodegradation | Mohammadi et al., (2021) |
| ***Trametes versicolor*** | Azithromycin | Biodegradation | Tormo-Budowski et al., (2021) |
| ***Pleurotus ostreatus*** | Sulfonamides Tetracyclines | Biodegradation | Camacho-Arévalo et al., (2021) |
| ***Trametes polyzona*** | Amoxicillin | Biodegradation | Lueangjaroenkit et al., (2019) |
| ***Pycnoporus sanguineus, Phanerochaete chrysosporium*** | Ciprofloxacin | Biodegradation | Gao et al., (2018) |
| ***Pleurotus ostreatus*** | Ciprofloxacin | Biodegradation | Singh et al., (2017) |
| ***Aspergillus terreus FZC3*** | Gentamicin | Biosorption and biodegradation | Liu et al., (2016) |
| ***Trichoderma harzianum*** | Clarithromycin | Biodegradation | Buchicchio et al., (2016) |
| ***Trametes versicolor*** | *Cefalexin, Ciprofloxacin, Etracycline* | Biodegradation | Badia-Fabregat et al., (2016) |
| ***Irpex lacteusb, Trametes versicolor*** | *Ciprofloxacin, Norfloxacin, Ofloxacin* | Biodegradation | Čvančarová et al., (2015) |
| ***Trametes versicolor*** | *Ofloxacin* | Biodegradation | Gros et al., (2014) |
| ***Trametes versicolor*** | *Erythromycin, Ciprofloxacine, Azithromycin, Cefalexine* | Biodegradation | Cruz-Morató et al., (2013) |
| ***Trametes versicolor*** | *Norfloxacin Ciprofloxacin* | Biodegradation | Prieto et al., (2011) |
| ***Cunninghamella elegans*** | *Flumequine* | Biotransformation | Williams et al., (2007) |

| Fungal name | Antibiotic contamination | Mechanism | Reference |
|---|---|---|---|
| ***Trichoderma viride*** | *Ciprofloxacin* *Norfloxacin* | Identification of degraded products | Parshikov et al., (2002) |
| ***Pestalotiopsis guepini*** | *Ciprofloxacin* *Norfloxacin* | Biotransformation | Parshikov et al., (2001) |
| ***Mucor ramannianus*** | *Enrofloxacin* | Biotransformation | Parshikov et al., (2000) |
| ***Gloeophyllum striatum*** | *Ciprofloxacin,* *Enrofloxacin* | Biodegradation and metabolite identification | Wetzstein et al., (1997, 1999) |

## 2. Materials and Methods

### 2.1. Methodology

This investigation proposes a new smart framework for the bioremediation of AZ in soil, which includes lab-scale tests and a prediction system. The study highlights the need for sustainable and nature-based approaches to manage Emerging pollutants (EPs) such as AZ. The proposed framework involves setting up a lab-scale bioremediation system and optimising control factors and designing a prediction system for AZ bioremediation. The study also presents a flow cycle of AZ in the environment as illustrated in Fig. 2 that emphasises the carcinogenic effects of EPs on human health, highlighting the urgency of managing them with sustainable and nature-based approaches (Abubakr et al., 2020). The study emphasises the importance of smart, sustainable systems for environmental purification and highlights the use of bioremediation techniques with a decision support system as a no-chemical and sustainable method for the decontamination of soil from AZ. The soil samples were collected in the Industrial Centre of

Mashhad, Iran for the purpose of investigating the soil properties and preparing the experimental setup.

**Fig. 2. Schematic plan of sustainable antibiotic bioremediation cycle in the study**
***PS*: *Penicillium Simplicissimum***

## 2.2. Experimental techniques

Fig. 3 provides a list of materials and reagents used in the experiments, including measurement procedures, soil evaluation, and field practices. The PS seed was obtained from the Iranian Genetic Bank (IGB), while all other materials were purchased from Merck, Darmstadt, Germany. Distilled water was used throughout the experiments, and the soil sample was collected from the Quchan road region in Mashhad, Iran, where a pharmaceutical complex is located. The purpose of the experiments was to mitigate the adverse effects of polluted effluent on the surrounding soil. As the AZ concentration was synthesised in the laboratory, all soil samples were planted deep to ensure the absence of AZ.

The study collected soil samples from three points in a polluted district for lab-scale experiments and field performance assessment. AZ solutions were prepared using a 1000 mg/g stock standard solution from Merck, Germany, and the concentrations of AZ in the samples were measured at five points (12, 9, 3, 7, and 1.5 mg/g) with a mean amount of 6.5 mg/g, representing the simulated concentrations of the real accumulated pollution condition in the field.

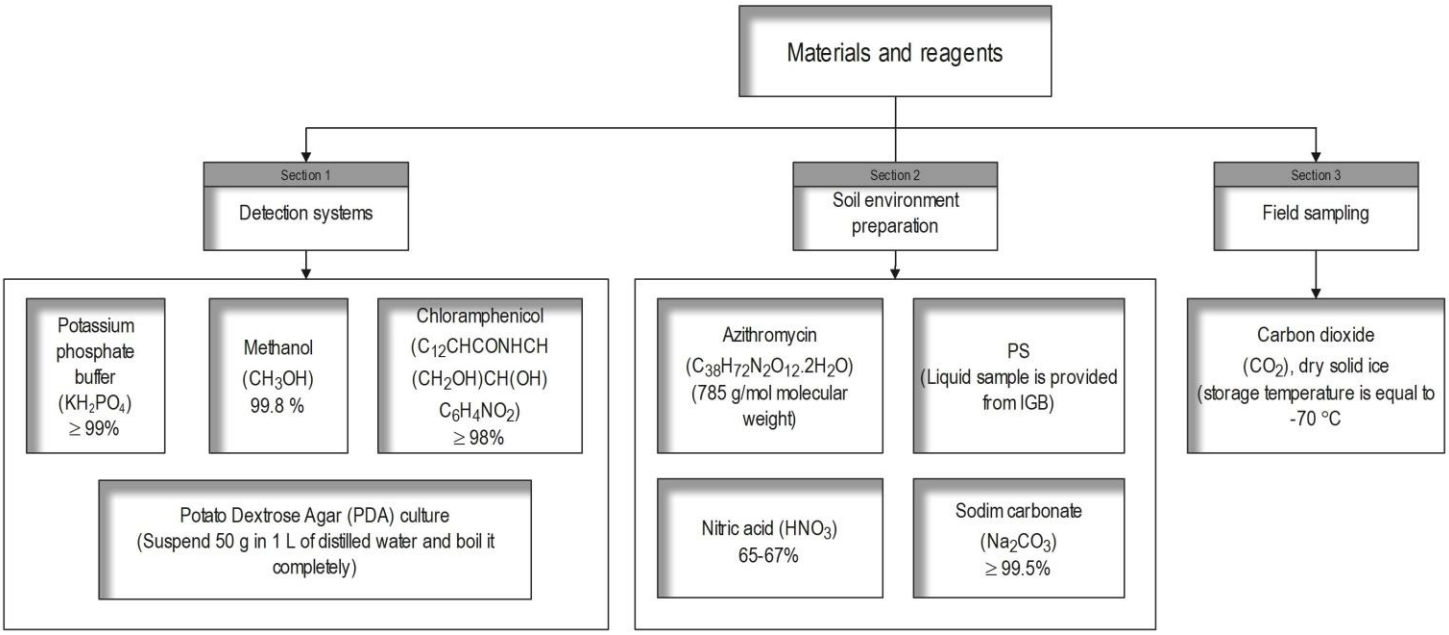



**Fig. 3. Materials and regents used in the experiments.**

The detection system used in the experiment includes several instruments from different manufacturers, such as the Waters Alliance 2695 HPLC from the USA, a pH and EC meter from Switzerland, an autoclave, and an incubator by WTW from Germany, and a temperature meter by HTC Instrument from India. Other equipment used includes a belt-driven air compressor1.5-7.5 kW made by REMEZA Co. from Germany, a plastic batch reactor by Kisker Co. from Germany, steel electrothermal elements by Element Co. from Iran, a sterile steel vessel by Kisker Co., from Germany, and a steel soil sampling device by Gilson Co. from USA. The study's controlling system

uses Arduino hardware and adjusts temperature by coordinating thermal sensors and elements. The mobile phase for AZ measurement is adjusted with a concentration on isocratic flow, and the AZ detector is set on UV (210 nm) and fluorescence with corresponding emission (435nm) and excitation wavelengths (365nm). Fig. 4 elaborates on the information (including model numbers) of the instruments used to detect AZ, preparation of the soil medium, and samples from different points of the soil (Hussain et al., 2021).

Selective isolates of PS in Petri dishes with three repetitions for each sample containing Potato Dextrose Agar (PDA) were placed in a growth chamber at 28°C and under light cycle conditions (12:12) for two weeks for insemination and transfer. After inoculation, spore suspension using distilled water sterile containing 0.1% Tween 80 was prepared. Purification of isolates was done by single spore method on a water-agar culture medium (WA) (Babaahmadi et al., 2018).

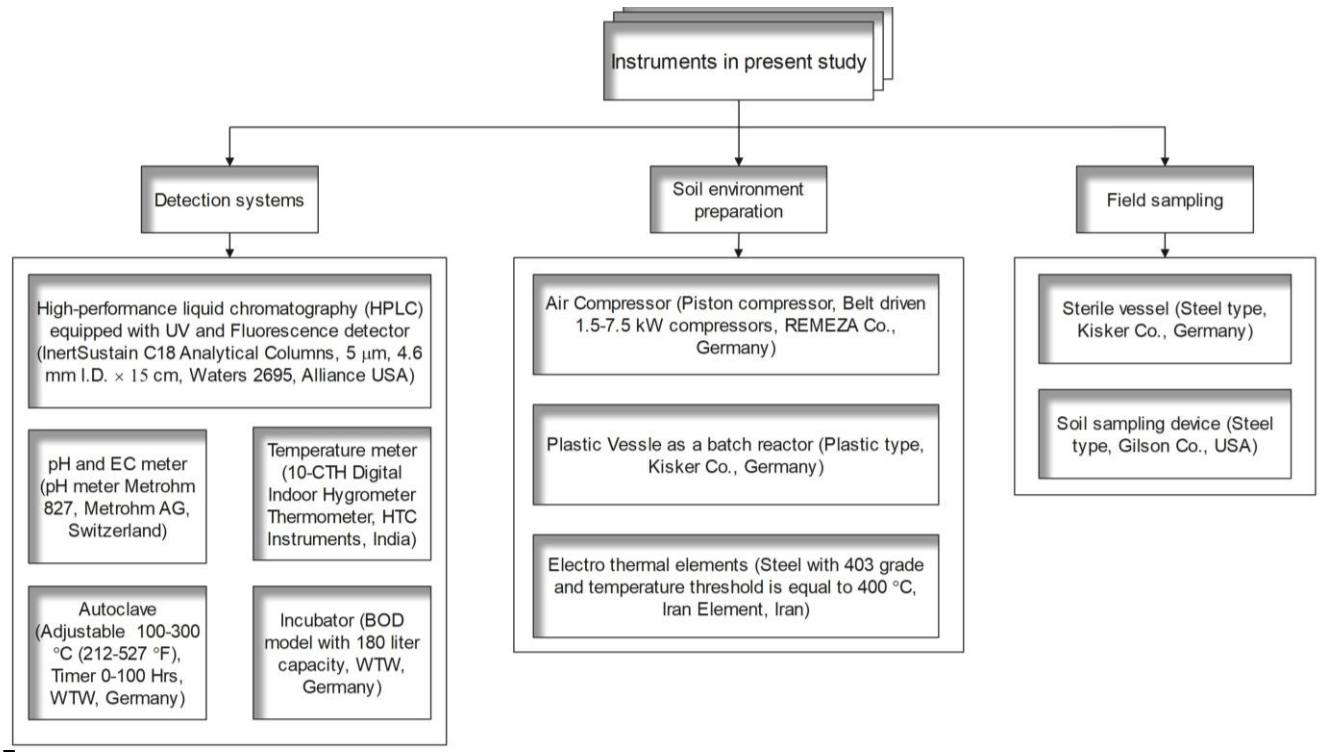
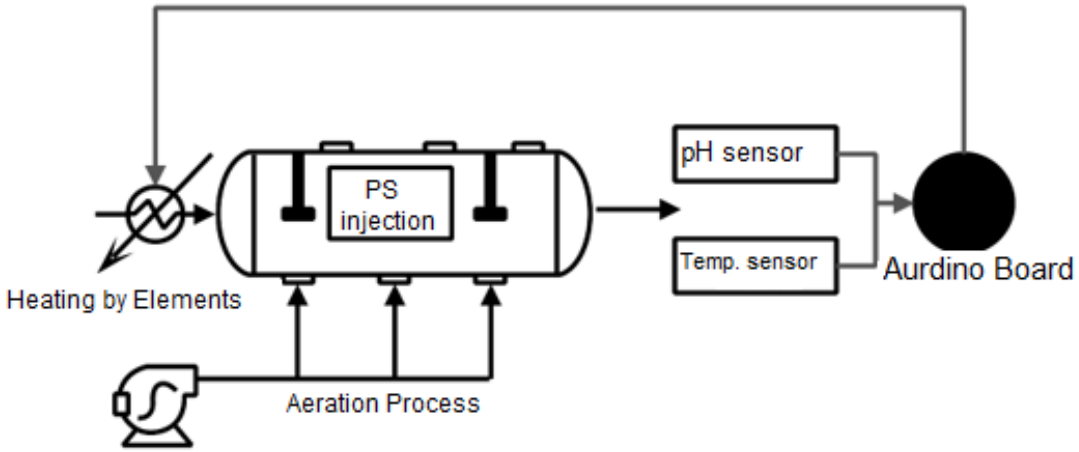



**Fig. 4. Instruments and their specifications used in the experiments.**

234 Fig. 5 provides a diagram of the key elements and sensors utilised in the laboratory-scale
235 experiments, which were based on bioreactor design principles and environmental regulations. The
236 lab-scale setup consists of two parts: online and offline systems. The offline system includes a
237 fungal seedling, a pH meter (Metrohm 827, Metrohm AG, Switzerland), and manually adjusted
238 air pressure (using piston compressor, Belt driven 1.5-7.5 kW, REMEZA Co., Germany). The soil
239 layers used in the experiment are uniform and have the same characteristics. Heating is controlled
240 by solid elements and the temperature is monitored using an Arduino controller. The experiment
241 package size 10×15×30 cm with a soil mass of 6.750 kg.




242

243 **Fig. 5. Schematic layout of the lab-scale set up in the study.**

244

245 This study involves measuring AZ and culturing PS. The stages for the determination of
246 AZ and PS culturing are described by Mohammadi et al. (2021). To measure AZ, the HPLC
247 instrument (InterSustain C1 Analytical Columns, 5µm, 4.6mm I.D. × 15cm, Waters 2695,
248 Alliance, USA) must be adjusted with specific settings for the detector wavelengths, oven column
249 temperature, and flow rate equal to 210 nm, 40 ºC, and 0.8 mL/min, respectively and adding 50
250 mm Methanol-phosphate buffer 0.02 M (90:10, v/v).

To reach pH 8, phosphate acid was used, and the injection volume was set to 50 µL. A calibration curve was plotted with 5 data points resulting in an $R^2$ greater than 0.96. By 1 mg/g spiked concentration, the value of AZ is equal to 0.005 mg/g (the limit of AZ is equal to 0.005mg/g). To culture the PS for seeding into the bio-engine, several steps are required. These include obtaining PS seeds from the IGB, creating a suspension of initial seeds with sterilised water under laboratory conditions, mixing the provided suspension with WA culturing environment in a sterilised hood, transferring the created seedlings to normal laboratory conditions after 12-18 hours, and charging the seedlings onto PDA Petri dishes with suitable slops for complete growth in the incubator for 4-5 days. Finally, the cultivated PS can be stored in the laboratory's refrigerator at 4°C for future experimental tests.

## 2.3. Optimisation and numerical models

### 2.3.1. Taguchi design

Taguchi is a useful tool for designing complex systems and finding the best set of designs for quality parameters, despite noise factors. Experiments are conducted using an experimental matrix known as the orthogonal array, and quality loss values are calculated for each quality characteristic. The quality loss function is categorised into three types, and the values are transformed into a signal-to-noise (*S/N*) ratio that shows the dispersion around the desired value. The larger *S/N* ratio indicates better quality, and it is calculated using Eq. 1 as:

$$S/N = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad \textbf{Eq. 1}$$

where $y_i = i^{th}$ observed response value, $n$ = the number of observations in a trial. The *S/N* ratio is a measure of the effect of control factor levels on the response quality. A higher S/N ratio corresponds to

272 better performance of the response. Therefore, the optimal levels of parameters can be obtained by
273 identifying the levels that result in the highest *S/N* ratios.

274 The Taguchi model is used to design experiments by determining parameter characteristics,
275 defining levels, designing the orthogonal matrix, and inspecting results by desirability and S/N
276 ratio (Koilraj et al., 2012). This study's experimental parameters include Temperature (*T*) (ºC),
277 Retention Time (*RT*) (day), pH, Aeration Intensity (*AI*) ($m^3$/h), and Microorganism to Food weight
278 ratio (*M/F*) (mg/g) as influential parameters according to the literature. The Taguchi design method
279 in MiniTab16 software is used to obtain the most efficient range of parameters, and the primary
280 levels are set in Table 4. All experiments are initially conducted for 20 days, and AZ concentration
281 is measured in both influent and effluent every five days from day five to day 100. The PS used in
282 the bio-engine for AZ decontamination is fed by local agricultural waste from the municipal waste
283 centre in Mashhad.

284

285 **Table 4. The primary data of Taguchi design for being optimised.**

| Parameter | 1 | 2 | 3 |
|---|---|---|---|
| **Temperature (ºC)** | 24 | 28 | 32 |
| **Retention time (day)** | 20 | 40 | 60 |
| **pH** | 3 | 6 | 9 |
| **Aeration intensity ($m^3$/h)** | 8 | 14 | 20 |
| **Microbial/Food ratio (mg/g)** | 1 | 4 | 7 |

286

287

288

### 2.3.2. Response Surface Methodology (RSM)

RSM is a statistical tool used to predict the interactions between factors that may be difficult to observe or too complex to test experimentally. RSM provides a way to investigate the relationship between variables and a response variable or performance characteristic of a system under control. The relationship between the controlling variables ($X_1$, $X_2$, ..., $X_n$) and the response ($Z$) is represented by a function $f$, as shown in Eq. 2. In this equation, $\varepsilon$ represents other sources of variables that are not included in the function $f$, which is assumed to have a normal distribution with a mean of zero and variance of $\sigma^2$. Therefore, the expected value of the response function is the $f$ function.

$$Z = f(X_1, X_2, \ldots, X_n) + \varepsilon \qquad \textbf{Eq. 2}$$

To simplify the estimation of the complicated $f$ function, a polynomial equation based on experimental data can be used. This polynomial equation can estimate the response of the variables in points where there is insufficient experimental data. A second-order polynomial function, represented by Eq. (3) is commonly used as an estimation of the actual response surface around a desired point. This approach is known as response surface methodology and can help researchers understand the relationship between variables and the response (Sarabia and Ortiz, 2009).

$$Z = a_0 + a_1X_1 + a_2X_2 + \ldots + a_nX_n + a_{12}X_1X_2 + a_{13}X_1X_3 + \ldots + a_{1n}X_1X_n + a_{23}X_2X_3 + a_{24}X_2X_4 + \ldots + a_{2n}X_2X_n + \ldots + a_{(n-1)\,n}X_{n-1}X_n + a_{11}X^2_1 + \ldots + a_{nn}X^2_n \qquad \textbf{Eq. 3}$$

The ANOVA test is used to determine the significance of the regression (Sarabia and Ortiz 2009). Using RSM, researchers can predict the values of culture parameters at points where experimental data is lacking. In this study, the efficiency of the reactor in Fig. 5 is evaluated on a lab-scale setup using optimal conditions, and the resulting data is used in Design Expert 7 software

311 to predict data for other points. These predicted values can be considered as the desired set of
312 control variables based on the operating situation.

313 **2.3.3. Machine learning algorithms**

314 Prediction models based on ML are used in the study to estimate AZ degradation in soil.
315 The performance of ML models can vary depending on the dataset and the specific problem being
316 addressed. Therefore, it's recommended to evaluate multiple algorithms and compare their
317 performance before selecting the most appropriate one for a specific application. As such, several
318 factors were considered for selecting the most effective ML algorithms for this study, including
319 the nature of the problem, available data, and prior experience. After careful consideration, IBK,
320 Kstar, and LWL were selected for their suitability for classification and regression tasks with
321 complex decision boundaries and non-linear relationships. The prediction models of these
322 algorithms are developed using WEKA 3.9 software by training based on 70% of the experimental
323 data and then validating based on the remaining 30% of the data. The main objective is to compare
324 the performance of these methods for application to bioremediation processes. More details of
325 modelling with these algorithms are outlined below.

326 **2.3.3.1 Instance-Based K (IBK) algorithm**

327 The IBK algorithm with the *K* parameter falls into the category of regression and
328 classification lazy algorithms. The IBK algorithm works by identifying similarities between
329 instances and specifying the number of nearest neighbours to use when classifying a test instance.
330 It can select the most suitable value of *K* by using cross-validation and distance weighting,
331 (Moayedi et al., 2019). Note that in WEKA software, IBK is based on cross-validation, which
332 helps find the best value for K's nearest neighbour.

333

### 2.3.3.2 K-Star algorithm

Kstar, another lazy learning algorithm, selects the most relevant features for classification using statistical methods based on the K nearest neighbour method and is suitable for datasets with many features. Unlike other instance-based learners, K-Star attempts to divide *n* data points into *k* clusters using an entropic distance measure. This involves computing the probability of transforming one instance into another, which requires measuring the distance between instances. To achieve this, the algorithm determines a finite set of transformations that map an instance into another and transforms an instance using a limited series of transformations starting at point 'a' and ending at 'b'. The K-Star computation is as follows:

$$K(y_i, n) = -\ln \acute{P}(y_i, n) \quad \textbf{Eq. 4}$$

where $n$ = new data points attached to the most expected class $y_i$ and $P'$ = the probability of the point $i$ reaching point $j$ through a random path.

### 2.3.3.2 Locally Weighted Learning algorithm

LWL, a non-parametric regression algorithm, assigns weights to training instances based on their distance from the query point and is suitable for regression tasks with non-linear relationships. prediction in LWL is based on local functions using a subset of data to replace a global function that result in faster predictions. More specifically, a local model is created for each point of interest based on the neighbouring data of the query point instead of a global model for the entire dataset. To satisfy this, each data point becomes a weighting factor that represents its influence on the prediction. This means that the closer a point is to the query point, the more weight it receives, making this method very accurate and allowing new training points to be added easily. If there is a continuous function $f$ with noise $\varepsilon$, then the LWL cost function is as follows:

$$y = f(x) + \varepsilon \quad \textbf{Eq. 5}$$

$$G = \frac{1}{2}\sum w_i x_q (y_i - x_i \beta_q)^2 \quad \textbf{Eq. 6}$$

where $x_q$ is the point of interest (or query point) which is the point where we want the prediction $y_q$. Labelled training data $D = \{(x_i, y_i) | i = 1, 2, ..., n\}$ where each data point of $x_i$ belongs to a corresponding $y_i$. $w_i$ represents the weight of the corresponding set of $(x_i, y_i)$ for the current prediction which is computed through a weighting function. $\beta$ describes the regression coefficient of the linear model.

The algorithm aims to find the $\beta$ in a way that minimises the $G$ function for the current point of interest $x_q$. It is one of the main differences of this method to global least functions where $\beta$ is dependent on the $x_q$ and finding $w_i$ through two these steps: (1) The distance function $d(x_i, x_q)$; $d = \sqrt{(x - q)D(x - q)}$, measures the relevance of the training points in the current prediction. This function receives two inputs and gives one number. $D$ describes the distance metric which is an important parameter expressing the size and shape of the receptive field; and (2) The Kernel function $K(d)$; $K(d) = \exp(-d^2)$ gives out a weight for each distance value.

These three algorithms (IBK, Kstar, and LWL) are also specifically suitable for the AZ decontamination process due to a range of factors that are difficult to capture in a simple model. For example, the process may have many factors influencing the performance of fungi for which K-star is a suitable algorithm while other algorithms such as SVM and RF may be inappropriate for problems with complex decision boundaries or many input features.

### 2.3.4. Decision Tree

After identifying the optimal conditions for the experiments, a decision tree (DT) is created to develop a smart control system (Amini et al., 2021; Gheibi et al., 2019). In this context, the DT

serves as a dashboard for the decision support system. DTs are a simple modelling technique that represents a sequence of interventions over a period as a graphical tree-like structure. The tree has branches and leaves representing options between alternatives and outcomes, respectively. It consists of three parts (Fig. 6): the root node, which is the starting point of the tree; branches, which represent possible answers; and leaf nodes, where each branch ends and are shown in three types: decision nodes, chance nodes, and terminal nodes.

The DT structure produces a series of rules that explain the path from the root to a leaf of the DT. Each path represents a rule, and the leaf is labelled with the class in which the correct value of records is assigned. DTs have limitations in modelling decision problems but provide several advantages, such as visual aid that requires no further explanation and is easy to understand. They can also cover both quantitative and qualitative data and consider data sets that may contain errors or missing values. DTs are also convenient to draw and free of complicated computations.

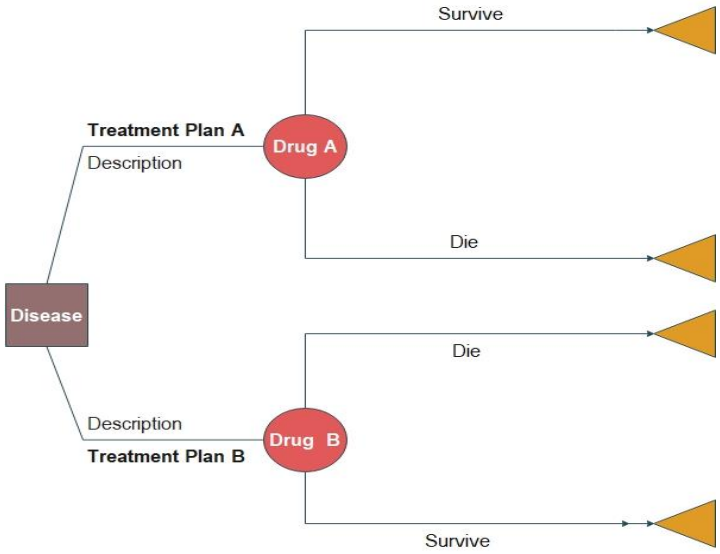



*Fig. 6. A simple example of a decision tree*

## 3. Results and discussion

### 3.1. Mechanism of the bioremediation process

Fig. 7 illustrates the biodegradation mechanism of AZ through the *Penicillium Simplicissimum* (PS) bioremediation process and other fundamental concepts. The PS fungal cell walls are negatively charged due to the presence of various functional groups, such as carboxylic, phosphate, amine, or sulfhydryl, in different wall components such as hemicelluloses, pectin, and lignin (Fomina et al., 2007). The decomposition process is carried out through the transfer of electrons between $O_2$ and organic matter by fungus activities (Bell et al., 2011). The PS decomposes AZ compounds using hydrolase and free radical enzymes via the oxidation-reduction process (Dias et al., 2021).

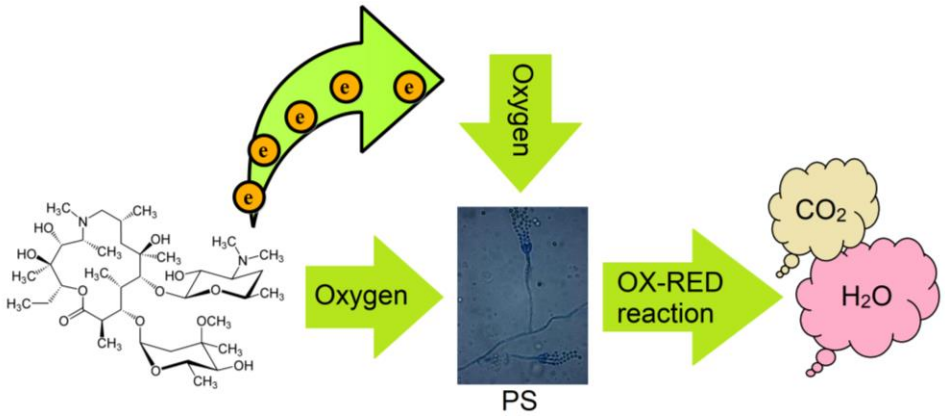



**Fig. 7. The mechanism of azithromycin biodegradation by fungus activities in this study**

The degradation process of AZ involves a reduction half-reaction and an oxidation half-reaction catalysed by enzymes secreted by PS. The reduction half-reaction involves the transfer of a hydride from the substrate to the reactant, resulting in a binary complex between the two-electron reduced enzyme and the p-quinone methide of the substrate. In the oxidation half-reaction, the reduced AZ is oxidised by molecular oxygen with the concomitant hydration of the quinone methide intermediate. This process continues until $CO_2$ and $H_2O$ are the final products of the

reaction, which is called mineralisation. The main compounds generated during the degradation process of AZ are safe intermediate materials containing $CO_2$ and $H_2O$ (Deblonde et al., 2011) while some degradation products are also potential to be created under aerobic conditions (Fig. 8).

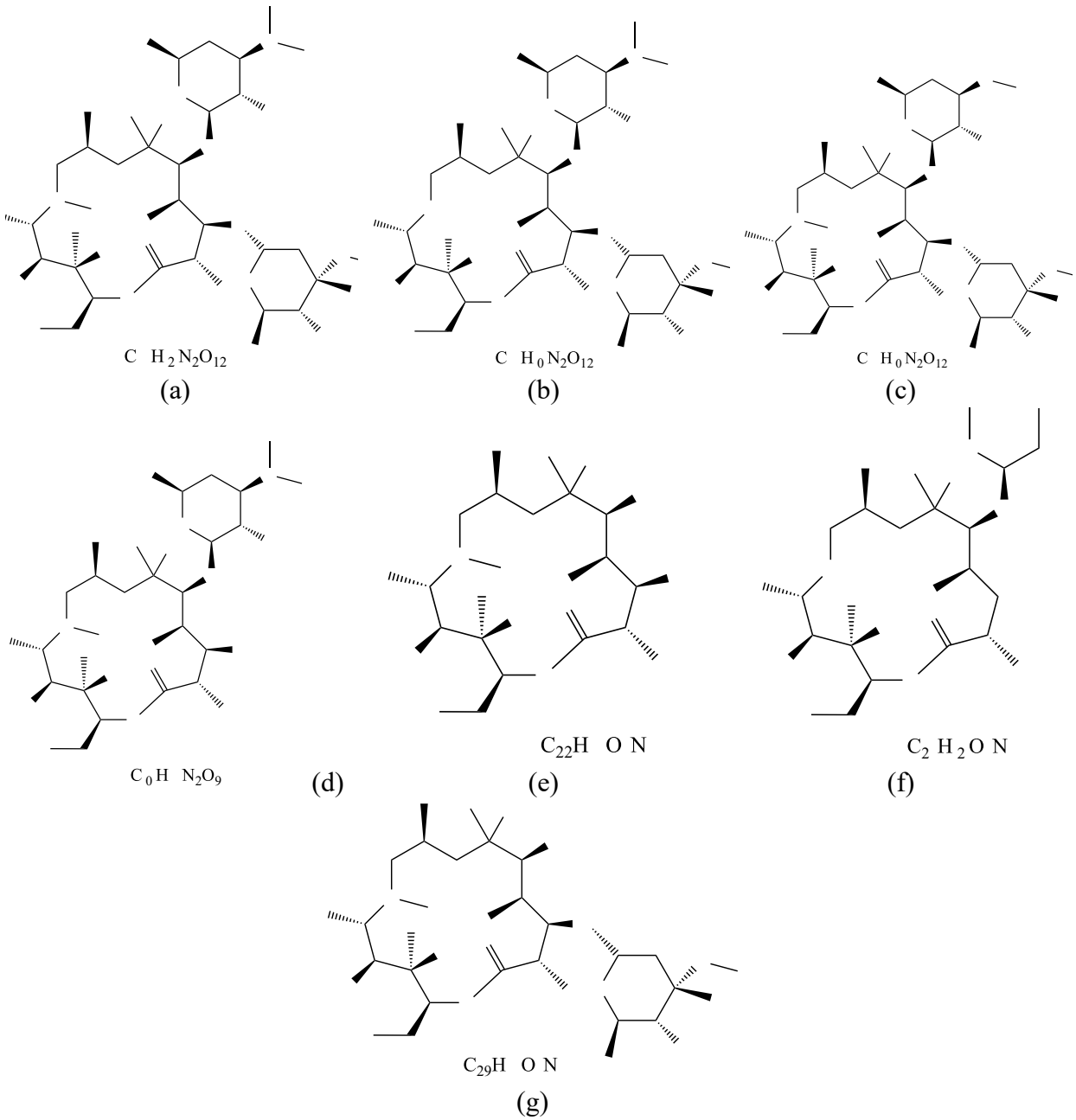



**Fig. 8. (a) Azithromycin and the potential degradation products, (b) 9a-N-desmethyl azithromycin, (c) Desclandose azithromycin, (d, e, f) resulted from the removal of some other groups from azithromycin, (g) N-desmethyl azithromycin**

The bioremediation of AZ by PS involves the formation of a binary complex between the enzyme and the substrate, which could be an important step in breaking down the antibiotic into less harmful forms (Fleming, 1946). Enzymes play a critical role in bioremediation, and PS may use them to break down AZ which is an antibiotic that can accumulate in the environment and potentially lead to negative effects on ecosystems (Clarke, 2015). Therefore, the input of the clean technology was AZ as a hazardous material in nature, and the outputs were $CO_2$ and $H_2O$ as safe materials for the environment. Hence, the bioremediation process of AZ by PS can be assumed as a clean and green technology for soil protection.

## 3.2. Optimisation and mathematical modelling

### 3.3.2. Taguchi design analysis

The Taguchi model was used to determine the optimal parameters for bioremediation of AZ by PS. The result of the experiments designed by Taguchi method as illustrated in Fig. 9 and Table 5 shows that the best temperature range was 28°C, and lower temperatures resulted in a steeper reduction in RP due to the increase in the hydrolysis rate of AZ. Retention time also affected RP, with a RT of 40 days resulting in the highest RP. The pH of the soil was critical for both antibiotic stability and fungus activity, with an acidic environment being better for removing AZ from the soil. Aeration intensity performed optimally at around 14 $m^3/h$, while the degradation rate improved as the M/F ratio increased.

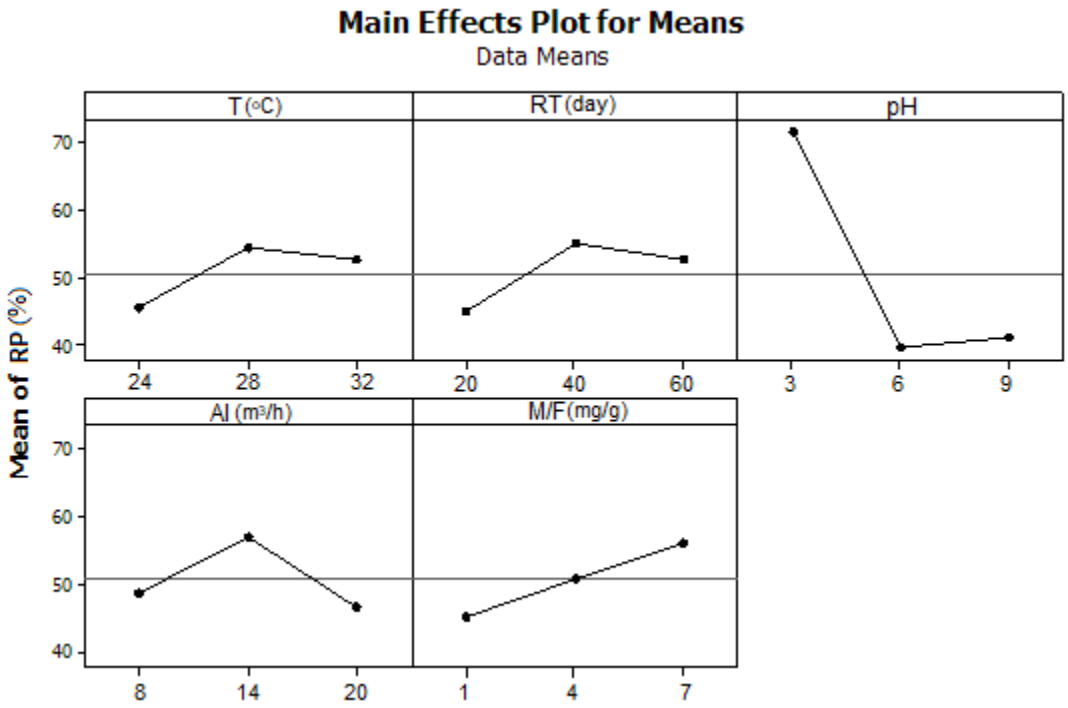



**Fig 9. Results of Taguchi design optimisation for Temperature (T), Retention time (RT), pH, Aeration intensity (AI) and Microbial/Food (M/F) ratio**

The Taguchi Orthogonal Array approach is designed to isolate the effects of selected variables, and adding extraneous variables outside the orthogonal array can introduce confounding effects that bias the results. Careful selection of variables is important, and if additional variables are included, a larger orthogonal array can be used for comparison. In such cases, a comparison can be made between the original orthogonal array and the expanded orthogonal array to assess the impact of the additional variables on the prediction model. However, adding variables outside the orthogonal array can make the process more complex and may not lead to better results. In this study, important features were extracted from the literature review (Mohammadi et al., 2021), and the optimisation process is done as per the operational optimum condition.

453 **Table 5. Results of experimental trials based on the Taguchi design method.**

| T (°C) | RT (day) | pH | AI ($m^3$/h) | M/F (mg/g) | Performance RP (%) |
|---|---|---|---|---|---|
| **24** | 20 | 3 | 8 | 1 | 48 |
| **24** | 20 | 3 | 8 | 4 | 57 |
| **24** | 20 | 3 | 8 | 7 | 69 |
| **24** | 40 | 6 | 14 | 1 | 38 |
| **24** | 40 | 6 | 14 | 4 | 45 |
| **24** | 40 | 6 | 14 | 7 | 51 |
| **24** | 60 | 9 | 20 | 1 | 27 |
| **24** | 60 | 9 | 20 | 4 | 34 |
| **24** | 60 | 9 | 20 | 7 | 39 |
| **28** | 20 | 6 | 20 | 1 | 31 |
| **28** | 20 | 6 | 20 | 4 | 32 |
| **28** | 20 | 6 | 20 | 7 | 36 |
| **28** | 40 | 9 | 8 | 1 | 42 |
| **28** | 40 | 9 | 8 | 4 | 48 |
| **28** | 40 | 9 | 8 | 7 | 50 |
| **28** | 60 | 3 | 14 | 1 | 76 |
| **28** | 60 | 3 | 14 | 4 | 84 |
| **28** | 60 | 3 | 14 | 7 | 89 |
| **32** | 20 | 9 | 14 | 1 | 40 |
| **32** | 20 | 9 | 14 | 4 | 44 |
| **32** | 20 | 9 | 14 | 7 | 45 |
| **32** | 40 | 3 | 20 | 1 | 66 |
| **32** | 40 | 3 | 20 | 4 | 73 |
| **32** | 40 | 3 | 20 | 7 | 81 |
| **32** | 60 | 6 | 8 | 1 | 38 |
| **32** | 60 | 6 | 8 | 4 | 41 |
| **32** | 60 | 6 | 8 | 7 | 45 |

454

455 ### 3.3.3. Response surface methodology

456 The RSM used the historical data analysis to determine the maximum degradation rate of
457 AZ and the impact of various cultural conditions on the rate of degradation. As such, the Design-
458 Expert 7.0.0 software was used to predict the response of AZ degradation rate to cultural conditions
459 including temperature, retention time, aeration intensity, pH, and M/F ratio. Therefore, a quadratic
460 polynomial equation was obtained based on the above parameters to predict the response of the

AZ degradation rate. This equation considers the interactions between the different cultural conditions to provide a more accurate prediction of the degradation rate as follows:

$$P = 54.70 + 4.08A - 2.39B - 31.83C - 23.94D + 5.5E - 33.22AB - 12.61AC - 1.58AE + 1BC + 0.083BE - 2CE - 0.33DE - 5.81A^2 - 0.22E^2 \quad \textbf{Eq. 7}$$

Where $P$ = the predicted value of AZ removal percentage and $A$, $B$, $C$, $D$, and $E$ = coded factors of temperature, retention time, pH, aeration intensity, and *M/F* ratio, respectively. The statistical data of the quadratic polynomial equation indicates this is a significant equation ($p<0.0001$ and $R^2 = 0.9887$). The regression data also demonstrate that temperature ($A$), pH ($C$), aeration intensity ($D$), *M/F* ratio ($E$), and the square term of temperature ($A^2$) were significant ($p<0.0002$), whereas the square term of *M/F* and retention time ($B$) were insignificant. It is also evident that pH with the $p<0.0001$ and the highest F-value is the most influential factor followed by aeration intensity. Table 6 also shows the result of the ANOVA analysis including interactions and the squared effects.

In other words, Eq. 7 represents a mathematical formula that can be used to predict the removal percentage (RP) of AZ in a bioremediation process using PS, based on certain operational factors. These factors include the initial concentration of AZ, the size of the fungi inoculum, and the duration of the process. This equation can be particularly useful for optimising bioremediation processes for AZ or similar pollutants. By incorporating the variables described in the equation, it can help predict the removal percentage of AZ without having to conduct time-consuming and expensive experimental trials. Moreover, this equation can be applied in various settings beyond the bioremediation process discussed in this study. It can be adapted to fit different experimental designs or operational factors, or it can be used as a foundation for developing more sophisticated models of bioremediation processes.

484 **Table 6. Results of ANOVA analysis in this study**

| Source | *Sum of Squares* | *Mean Square* | *F-Value* | *P-value Prob > F* |
|---|---|---|---|---|
| **Model** | 7904.03 | 564.57 | 163.10 | < 0.0001 |
| **Temperature (T)** | 92.34 | 92.34 | 26.67 | 0.0002 |
| **Retention time (RT)** | 25.68 | 25.68 | 7.42 | 0.0185 |
| **pH** | 4560.12 | 4560.12 | 1317.41 | < 0.0001 |
| **Aeration intensity (AI)** | 1474.29 | 1474.29 | 425.92 | < 0.0001 |
| **Microbial/Food ratio (M/F)** | 544.50 | 544.5 | 157.30 | < 0.0001 |
| **T × RT** | 1655.57 | 1655.57 | 478.29 | < 0.0001 |
| **T × pH** | 238.56 | 238.56 | 68.92 | < 0.0001 |
| **T × AI** | 0.00 | | | |
| **T × M/F** | 30.08 | 30.08 | 8.69 | 0.0122 |
| **RT × pH** | 2.00 | 2.00 | 0.58 | 0.4619 |
| **RT × AI** | 0.00 | | | |
| **RT × M/F** | 0.08 | 0.08 | 0.02 | 0.8793 |
| **pH × AI** | 0.00 | | | |
| **pH × M/F** | 48.00 | 48.00 | 13.87 | 0.0029 |
| **AI × M/F** | 1.33 | 1.33 | 0.39 | 0.5464 |
| **$T^2$** | 115.56 | 115.56 | 33.38 | < 0.0001 |
| **$(RT)^2$** | 0.00 | | | |
| **$(pH)^2$** | 0.00 | | | |
| **$(AI)^2$** | 0.00 | | | |
| **$(M/F)^2$** | 0.30 | 0.30 | 0.09 | 0.7748 |

485

486 Fig. 10 depicts the results of some statistical tests according to the ANOVA computation
487 as variance analysis for effective factors used in the prediction model. The sensitivity analysis

reveals that the maximum efficiency occurs at a pH of approximately 3 when the retention time (RT), aeration intensity (AI), and M/F ratio are fixed. The plots show that the slope of pH and AI are higher than the other parameters, indicating that the removal percentage (RP) is more sensitive to changes in these factors. These results are consistent with the outcome predicted by the RSM analysis. Table 7 provides some of the data resulting from the prediction model by the RSM analysis. Based on Fig. 10, it is evident that the aeration volume and pH control should receive constant attention during the operation of the system, and the operator should adjust them to optimal conditions with high precision under different conditions.

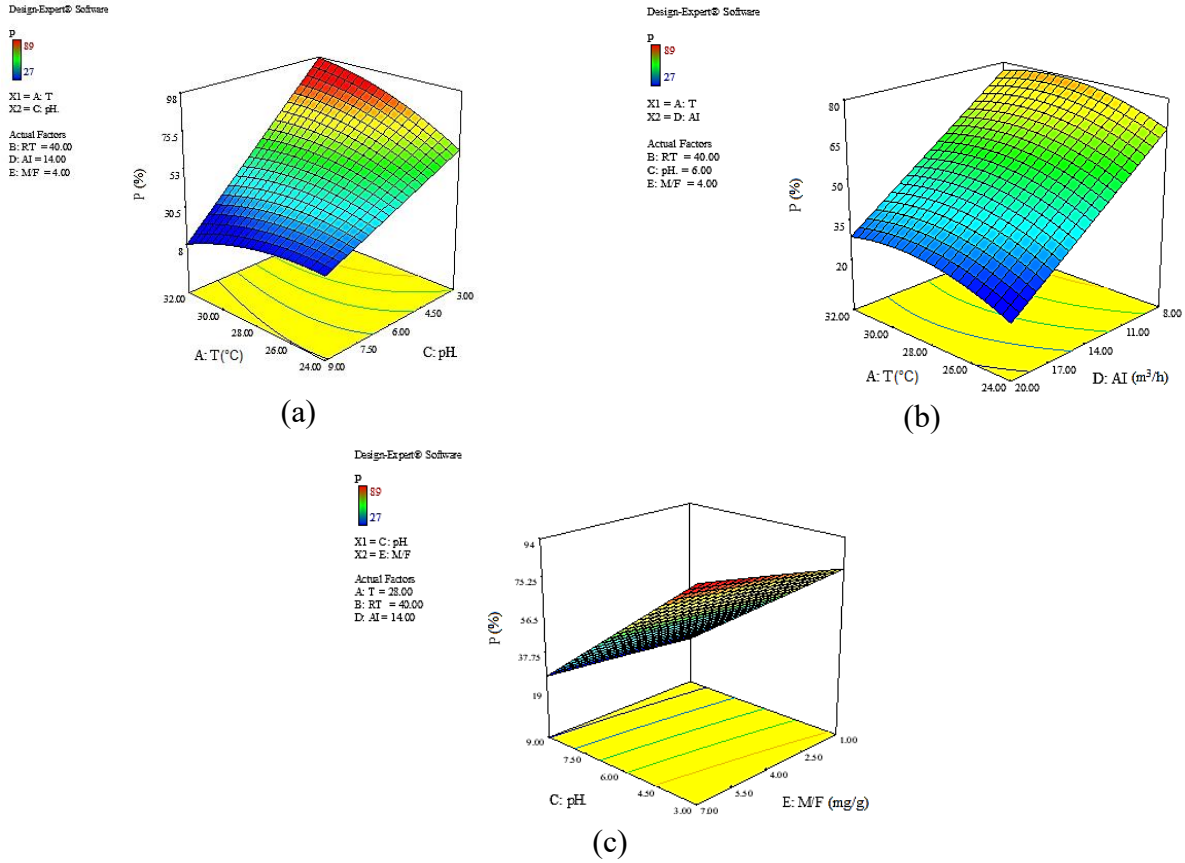



**Fig 10. The dual sensitive analysis of AZ bioremediation factors in this study a) Temperature (T) against pH, b) T against Aeration Intensity (AI) c) pH against Microbial/Food (M/F) ratio**

500 **Table 7. The optimisation results obtained from the RSM analysis.**

| No. | T (°C) | RT (day) | pH | AI ($m^3$/h) | M/F (mg/g) | RP (%) |
|---|---|---|---|---|---|---|
| **1** | 29.44 | 25.88 | 3.03 | 13.64 | 4.63 | 100 |
| **2** | 31.53 | 25.83 | 4.02 | 14.47 | 2.13 | 99.79 |
| **3** | 28.98 | 45.1 | 3.42 | 8.73 | 1.74 | 97.96 |
| **4** | 30.95 | 41.78 | 3.1 | 13.76 | 5.91 | 96.67 |
| **5** | 31.1 | 28.21 | 5.52 | 8.55 | 1.59 | 95.39 |
| **6** | 31.98 | 25.71 | 6.39 | 8.04 | 1.09 | 92.28 |

501

502 The data in Table 7 indicates that the best performance for AZ removal is achieved at a pH
503 lower than 4. However, it is also possible to achieve high efficiency at higher pH levels, which are
504 more practical to achieve in real-world settings due to the complexity of the test for reaching the
505 pH of 3. Studies on AZ biodegradation in soil environments are limited, but Li et al., (2012) found
506 that microbiomes in soil are most efficient at pH levels between 6.5-8.5 and temperatures between
507 25-45 °C. They also noted that the optimum pH for tetracycline degradation was around 6.5. Ding
508 et al., (2016) investigated the simultaneous removal of 14 different antibiotics from soil using
509 laccase oxidation and soil adsorption processes. They achieved over 70% efficiency in the first 15
510 minutes at pH 6 and a temperature of approximately 25 °C.

511 The optimum temperature range for AZ bioremediation under fungal activity is between
512 28 and 32 °C, which is consistent with expectations from Taguchi modelling. Retention time (RT)
513 does not significantly affect the optimum cultural conditions and is effective for periods of more
514 than 25 days. Anaerobic degradation of AZ is ineffective, according to Vermillion and Tjeerdema
515 (2017), who found that biotic degradation in aerobic environments leads to higher levels of AZ
516 removal.

Based on the research findings, the suggested operating conditions for AZ bioremediation under fungal activity are outlined in the fifth row of Table 7. While the removal efficiency may not be the highest, it is at a desirable level, and the recommended cultural conditions, such as pH, are more practical to achieve. The pH level is closer to the natural pH of the soil, and AI is at a low level, reducing power usage and associated costs including the energy cost and depreciation/operating costs. The low M/F ratio means that cheap and abundant agricultural waste can be used as a food source, reducing waste and costs (El-Ramady et al., 2022). Finally, the low RT range reduces waiting time and allows for early response of the system.

The degradation of AZ by fungi in the soil can be represented by the following:

$$AZ + H_2O \rightarrow \text{Degradation products}$$

It should be mentioned that the moisture percentage of the examined soil in this study is kept between 30%-45% which is enough for completing the reactions. The range of moisture percentage is related to the type of agriculture and land curing process in the case study. To accurately evaluate the effect of increasing the concentration of AZ on the rate of degradation, a comprehensive experimental study should be conducted that considers all the relevant factors and monitors the degradation process over time. This would involve measuring the concentration of AZ and its degradation products at different time intervals and under different operational conditions. The results of such a study could be used to optimise the bioremediation process and develop more efficient strategies for treating contaminated soils.

If the reaction is first order concerning AZ, the rate of the reaction is given by $rate = k[AZ]$, where $k$ is the rate constant, and $[AZ]$ is the concentration of AZ. If the concentration of AZ is increased, the rate of the reaction will also increase proportionally, because the new $rate = k[2AZ] = 2k[AZ] = 2(old\ rate)$. This shows that the reaction rate is directly proportional to the

concentration of AZ when the reaction is first ordered with respect to AZ. If the reaction is second order concerning water, the rate of the reaction is given by $rate = k\,[H_2O]^2$. If water concentration is increased, the rate of the reaction will increase proportionally to the square of the concentration of water, because the new $rate = k\,[(2H_2O)^2] = 4k\,[H_2O]^2 = 4(old\ rate)$. This shows that the reaction rate is proportional to the square of the concentration of water when the reaction is second order concerning water. Changes in the concentration of azithromycin and water can have a significant effect on the rate of degradation of azithromycin by fungi in soil. In this reaction, the rate of degradation is directly proportional to the concentration of azithromycin and proportional to the square of the concentration of water. However, it should be noted that the degradation of AZ by fungi is a complex process that may involve multiple reactions and intermediates. Therefore, the kinetics of the degradation process may not always follow a simple first-order or second-order rate law. The actual rate of degradation may depend on various factors, including the type of fungus used, the initial concentration of AZ, the pH, temperature, moisture content, and the presence of other pollutants or organic matter in the soil. It is important to note that the specific reaction mechanism and the environmental conditions in the soil may also play a role in determining the rate of the reaction. Therefore, changing in concentrations of different elements in the reaction, the rate is changed, and operational features are considered constant approximately because they are related to the origin of the applied fungi and its interactions with AZ.

### 3.3. Machine learning prediction modelling

The results of the ML models (IBK, KStar, and LWL algorithms) for predicting the bioremediation of AZ under PS degradation are presented in Table 8. All the ML models have correlation coefficients above the acceptance range. However, the IBK with a correlation coefficient of 0.95 outperforms the other two models with high accuracy and more confidence. Furthermore, a correlation coefficient of 0.94 has been achieved through the KStar model which

means a close accuracy to the IBK simulation. This suggests that IBK and KStar algorithms may be more suitable for predicting the bioremediation of AZ under PS degradation, while LWL may not be as accurate with a correlation coefficient of 0.89. The value of these prediction models is better understood by looking at the complexity, cost, and time of conducting the experiments. However, it is important to conduct further testing and validation to confirm the reliability and generalisability of these models as well as considering other factors such as interpretability, scalability, and computational efficiency when selecting an ML algorithm for a specific application.

**Table 8. The outcomes of prediction models through IBK, KStar, and LWL algorithms**

| Parameters of ML algorithm | IBK | KStar | LWL |
|---|---|---|---|
| ***Correlation coefficient*** | 0.95 | 0.94 | 0.89 |
| ***Mean absolute error*** | 4.07 | 4.45 | 6.28 |
| ***Root mean squared error*** | 5.099 | 5.55 | 8.04 |
| ***Relative absolute error (%)*** | 28.23 | 30.83 | 43.50 |
| ***Root relative squared error (%)*** | 28.85 | 31.40 | 45.46 |

Several studies have used various ML algorithms to predict the behaviour of bio-engine systems. For example, Mohammadi et al., (2021) used RF, ANFIS, and RT algorithms to predict amoxicillin removal efficiency from soil, achieving correlation coefficients of 0.97, 0.95, and 0.99, respectively. Amiri et al., (2022) employed an M5 tree model to predict AZ removal efficiency from aqueous solutions, achieving a correlation coefficient of 0.946 and RMSE of 9.89%. Mojiri et al., (2020) used an artificial neural network to predict the removal efficiency of ciprofloxacin in wastewater, achieving $R^2 > 0.99$. Zhu et al., (2021) investigated the adsorption capacity of antibiotics on carbon-based adsorbents, finding that random forest-based algorithms performed

582 better than other models, with the specific surface area of adsorbents being highly important.
583 Lastly, Arab et al., (2022) used ANN and ANFIS approaches to predict the experimental data for
584 removing cephalexin from the water and found these models with high accuracy, achieving
585 accuracy of 88.21% and 93.87%, respectively, at a pH of 6.14.

586 The study highlights the benefits of using AI in predicting the performance of a system
587 accurately without conducting costly and time-consuming tests. In other words, if this system
588 undergoes changes in operation for any reason; Using the methods of *AI* and the performance of
589 the operator to adjust the parameters, the soft ML-based system forecasted the removal efficiency
590 of AZ. The ML algorithms used in this study collected data via the results of optimised solutions
591 from Taguchi runs. The size of the data entered in ML modelling is the same as the data derived
592 from Taguchi simulations. However, the ML algorithms may not perform well when used to make
593 predictions outside of that range mainly because the model may not be able to accurately capture
594 the underlying relationships between the variables being modelled when applied to data within the
595 training range. This should be considered as a constraint of *ML* models. Note that as experiments
596 are based on Taguchi's design, the optimal situation is necessarily among the experiments that
597 have taken place.

598 Furthermore, RSM prediction modelling may outperform ML algorithms due to its ability to model
599 complex relationships between variables and response variables, and its capacity to optimise the
600 system being studied. RSM can also provide a clear understanding of the underlying mechanisms
601 driving the system, making it easier to identify the most important variables and interactions. In
602 contrast, ML algorithms may struggle to capture complex interactions and can be opaque, making
603 it difficult to understand why a particular prediction is made.
604

### 3.4. Bioremediation control system

The DT modelling in Fig. 11 shows the influential parameters for the bioremediation process and how they should be controlled. The bioremediation process can be maintained at a high-efficiency level by adjusting the parameters to their optimised values using the dashboard. The four most important parameters are M/F, pH, T, and AI, which are adjusted in parallel based on the ANOVA analysis. Reaction time is then controlled at the optimum value of 25 days, followed by other effective factors organised in the DT. The DT is based on a linear control system and can help maintain the process at high efficiency.

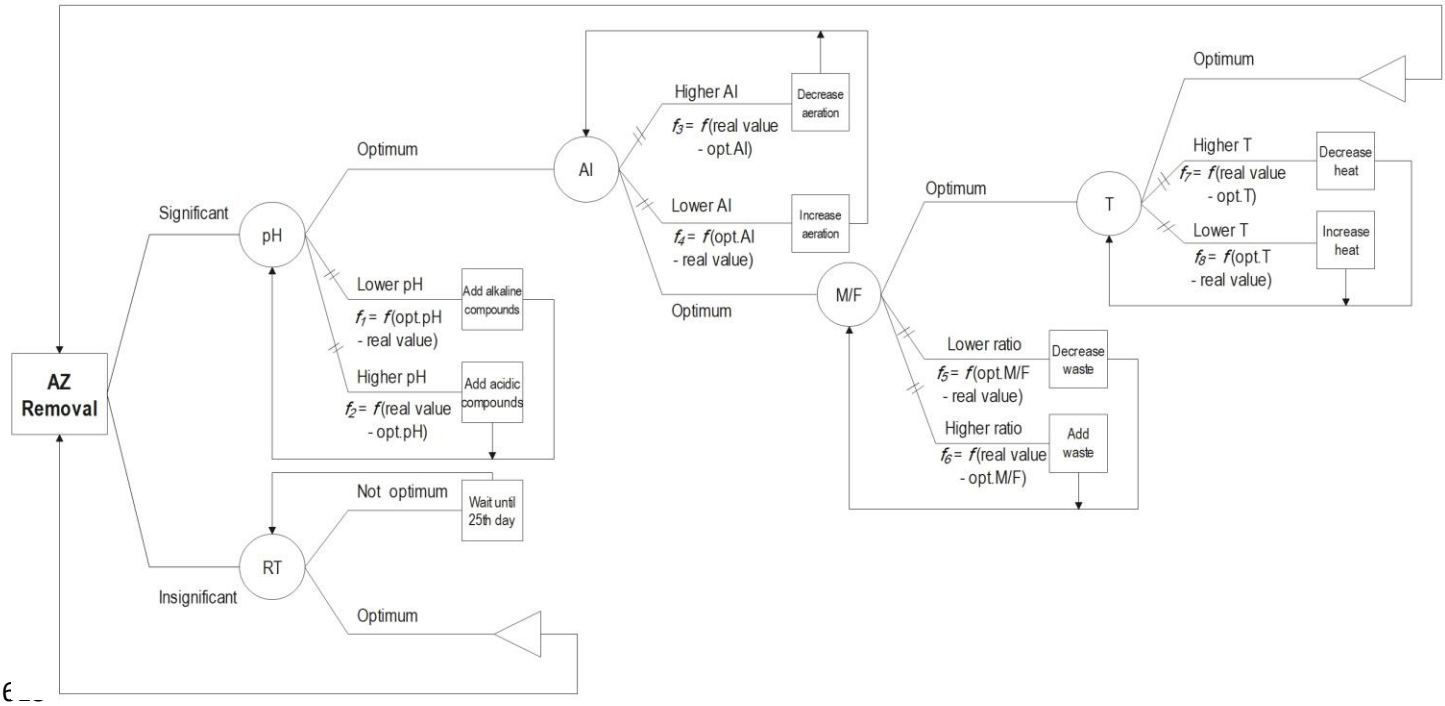



**Fig 11. The decision tree based on RSM analysis.**

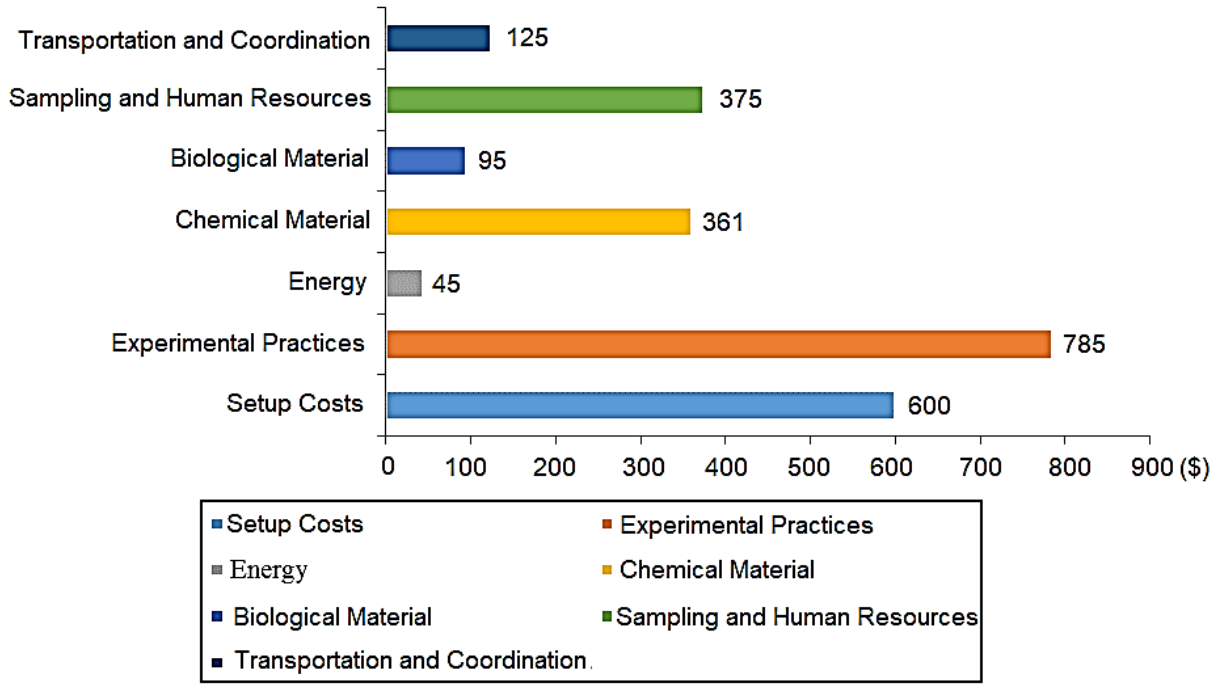
The process of AZ bioremediation involves two types of factors: significant and insignificant. The insignificant factor, which is RT, does not require specific optimisation and only needs to be more than 25 days. On the other hand, the significant factors need to be adjusted accurately. According to ANOVA analysis, pH is the most influential factor that needs to be monitored consistently. The optimised range for pH is 5.5, and if the pH is below this level, alkaline compounds should be added to the soil. Conversely,

620 acidic compounds should be added to raise the pH if it is too high. The modification can be calculated
621 through the error functions $f_1$ and $f_2$. Once the optimum pH is achieved, the system moves on to
622 adjust AI. Functions $f_3$ and $f_4$ are used to calculate the difference in current aeration intensity and
623 adjust AI to achieve optimum degradation. The condition is being constantly checking and further
624 passes to the next cultural parameter which is the M/F ratio. The same process is repeated for M/F
625 ratio and temperature, and functions $f_5$, $f_6$, $f_7$, and $f_8$ are used to amend the specified parameters
626 respectively. This whole process is constantly repeated to ensure that the optimum condition is
627 maintained throughout the project.

628 **3.5. Economic evaluation of the system**

629 To implement the bioremediation process for the degradation of AZ in soil in real-world
630 scenarios, it is important to consider its economic evaluation. The process can be assessed using
631 frameworks for smart sustainable operation of systems, although there is no specific framework
632 for the exact economic assessment of biological methods. Scientific knowledge and experiences
633 can be employed to help with the real field operation of the process.

634 The economic evaluation of the bioremediation process for AZ degradation in soil as
635 illustrated in Fig. 12 indicates that operational costs (37%) are more significant than investment
636 costs (63%). Therefore, the bioremediation approach is appropriate for treating antibiotic
637 contamination in soil, with a focus on operational costs. It should be noted that the main challenge
638 of biological decontamination is related to operational costs, and this challenge is addressed by the
639 investigated bioremediation process, as shown by the outcomes of other studies (Ghadami et al.,
640 2021; Gheibi et al., 2021; Gheibi et al., 2021; Mirabi et al., 2019). The investment costs for the
641 bioremediation process include a fee for transference, cost of experimental tests, setup preparation,
642 and organisational costs. On the other hand, the operational costs consist of the cost of human
643 resources, sampling practices, energy price, and material consumption.




644

645 **Fig. 12. Cost-effective analysis Economic value in $ for different stages of the experiment in this study**

646

647 By using the obtained costs provided for lab scale and assuming a scale-up factor of 10, an
648 estimation of the costs involved in the full-scale implementation of the bioremediation technology
649 can be obtained based on calculations given in the appendix. Based on these calculations, the total
650 estimated cost for full-scale implementation is $23,865. However, it is important to note that these
651 costs are based on the financial conditions of the case study in Iran, where human resource and
652 energy costs are much lower than in similar cases in Western countries.

653 According to fundamental equations and models, the bioremediation project is expected to
654 generate an annual profit of $237,163 and a total revenue of $250,000 per year. The net present
655 value (NPV) and internal rate of return (IRR) of the project will depend on the discount rate used
656 and the actual costs and revenues incurred during the project's lifetime. It is worth noting that the
657 total annual cost of the project is higher than the annual operational costs alone, which supports
658 the notion that investment costs are higher than operational costs in environmental bioprocesses.

659 Finally, it should be mentioned that a comparison with another research on the
660 decontamination of *amoxicillin* by *Aspergillus Flavus* fungi in the soil environment (Mohammadi
661 et al., 2021) reveals differences in energy values (AI difference), experimental practices
662 (difference in protocols), and chemical materials (difference in roadmap of both studies), which
663 contribute to cost differences.

664 Maier and Tjeerdema, (2018) evaluated AZ biodegradation and sorption by considering
665 the effects of operational parameters. The study conveyed that aerobic bioremediation is the best
666 option between aerobic degradation, anaerobic decomposition, and sorption procedures. However,
667 the bioremediation reaction time in their study is much slower (about 150 days) than PS fungi (at
668 least 25 days) as per the achievements of this study. Another study by Hanamoto and Ogawa
669 (2019) presented a smart system for predicting the sorption of AZ onto organic and inorganic
670 compounds in sediments. They evaluated both ion exchange and adsorption processes. However,
671 the main disadvantage of their achievements is related to regeneration essentially after completing
672 the surface capacity. However, this study continuously conducts antibiotic decontamination with
673 the application of bioremediation.

674 The bioactivities of various strains of *Penicillium* in the process of decontamination are
675 similar, and their characteristics and decontamination mechanisms summarised in Table 9. It is
676 worth noting that all strains can produce enzymes such as proteases and lipases, which break down
677 the complex organic molecules in azithromycin, and they also produce extracellular enzymes that
678 can degrade the molecule. However, the biodegradation mechanism of *Penicillium chrysogenum*
679 differs slightly. Therefore, based on theoretical evaluations, the ML computations, and optimum
680 operational conditions developed in this study, it is possible to apply them to other strains.
681 However, experimental practices and verification are necessary to confirm this hypothesis.

682 **Table 9. The mechanism of azithromycin contamination decomposition by different strains of Penicillium**

| Strain type | Description | Decontamination mechanism | Reference |
|---|---|---|---|
| *Penicillium chrysogenum* | It is commonly found in soil, and it is applied as a source of penicillin. | Breaking down the β-lactam ring structure of azithromycin by β-lactamases enzyme. It also decomposes molecules by extracellular enzymes, such as proteases and lipases. | Leitão et al., 2007 |
| *Penicillium roqueforti* | It is used to produce different types of cheese widely. | Breaking down the complex organic molecules in azithromycin | Chang et al., 1996 |
| *Penicillium notatum* | It is the main source of penicillin and, it is used for the production | Breaking down the complex organic molecules in azithromycin by enzymes | Bujacz et al., 1995 |
| *Penicillium camemberti* | It is used to produce soft cheeses. | Changing the structure of azithromycin by using proteases and lipases enzymes | Lessard et al., 2014 |
| *Penicillium glaucum* | It is utilised due to the product's hard cheeses. | Applying proteases and lipases enzymes due to the degraded structure of azithromycin | Hugo, 1991 |
| *Penicillium candidum* | It is applied to produce blue cheese | Using both proteases and lipases and extracellular enzymes for changing the formulation of azithromycin | Lessard et al., 2014 |

683

684 ## 4. Practical applications and prospects

685 To improve the efficiency of decontaminating antibiotics from soil, the study recommends a
686 combination of adsorption and bioremediation techniques, with bio-adsorption being considered
687 as a potential alternative to conventional adsorption methods. Further research could be conducted
688 to determine the individual contributions of biodegradation, adsorption, aeration, and heating in
689 the decontamination process. Furthermore, studying the kinetics of fungal activity could provide
690 more precise insights into the bioremediation mechanism. Although the study focused on the
691 decontamination of azithromycin by *Penicillium* fungus, future research should also evaluate the
692 competitive effects of co-existing antibiotics such as *amoxicillin* and *azithromycin*, to determine
693 which antibiotic exhibits greater affinity for degradation in soil. In other words, these measures
694 would improve the sustainability of decontaminating antibiotics from soil resources.

## 5. Conclusions

This study focused on developing a sustainable and eco-friendly approach for removing antibiotics from soil samples using bioactivities, without the need for any hazardous chemicals. The study also explored the potential of ISWM as a novel approach for purifying hazardous materials in the environment. The study found that the M/F ratio was the most significant factor in removing AZ from soil samples and identified the optimal temperature, pH, AI, and M/F ratio for AZ removal. The study found that the IBK model had the highest accuracy in predicting optimal conditions for AZ removal. The study also conducted an economic evaluation of the system and found that 63% of the cost was associated with the investment, while 37% was associated with the operation. The study recommended integrating adsorption and bioremediation techniques for the purification of antibiotics from soil resources, with bio-adsorption being evaluated as an alternative to simple adsorption processes.

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