

REMOVAL OF ANTIBIOTICS FROM MILK BY ADSORPTION METHOD

Submitted in partial fulfillment of the requirements of the award of

Bachelor of Science Degree in Biotechnology

by

K. ANITHA (41770004)



DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO & CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY -1 UNIVERSITY BY UGC

Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ANITHA K (41770004)** who carried out the Project Report entitled "**REMOVAL OF ANTIBIOTICS FROM MILK BY ADSORPTION METHOD**" under my supervision from November 2023 to April 2024.



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DECLARATION

I, **Anitha K (41770004)** hereby declare that the Project report entitled “**REMOVAL OF ANTIBIOTICS FROM MILK BY ADSORPTION METHOD**” done by me under the guidance of **Dr. P. PRAKASH**, at **Department of Biotechnology, Sathyabama Institute of Science and Technology** is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

Antibiotics play a pivotal role in human medicine, animal husbandry, veterinary practices, and aquaculture. However, their widespread use has led to the contamination of water bodies, including milk. The presence of antibiotic residues in milk poses health risks and environmental concerns. In recent years, adsorption methods have gained prominence as effective techniques for removing antibiotics from milk. This research article provides an elaborate overview of adsorptive removal methods, focusing on natural and modified adsorbents. We explore the adsorption mechanisms relevant to antibiotic sorption. Additionally, we discuss the future prospects and challenges associated with adsorption techniques for treating antibiotic-contaminated wastewater.

Keywords: *Antibiotics, Penicillin, milk, activated carbon, adsorption*

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LIST OF ABBREVIATION

ABBREVIATION	DESCRIPTION
AC	Activated carbon.
AMX	Amoxicillin
API	Active Pharmaceutical Ingredients.
APO	Advanced Oxidation Process
ARG	Antibiotic Resistance Gene
CHAC	Corn Husk Activated Carbon.
COOH	Carboxylic acid
FTIR	Fourier-Transform Infrared Spectroscopy
HNO ₃	Nitric acid
NaOH	Sodium Hydroxide
NH ₄ OH	Ammonium Hydroxide
P	Penicillin
SEM	Scanning Electron Microscope

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CHAPTER 1

INTRODUCTION

Milk, as a biofluid, undergoes various processes from production to consumption, creating a dynamic matrix that requires sophisticated analytical techniques for precise detection and quantification of antibiotic residues. The presence of these residues in milk is often associated with the administration of antibiotics to dairy animals for therapeutic purposes or disease prevention. Understanding and controlling the levels of antibiotics in milk are critical not only for ensuring food safety but also for addressing broader public health and environmental concerns.

Antibiotics have transformed medicine and agriculture since Sir Alexander Fleming's 1928 discovery of penicillin. They are used in aquaculture, human health, and animal production. Unfortunately, due to overuse, antibiotics are now present in milk and other environmental matrices. Since antibiotics have special qualities, it is imperative that they be removed from milk. This article explores the use of different adsorbents in the adsorptive removal of antibiotics from milk.

1.1 ADSORBENTS FOR ELIMINATING ANTIBIOTICS

The effectiveness of several adsorbents in eliminating antibiotics from water has been studied. Among them are:

1. Adsorbents derived from agricultural waste: Using agricultural byproducts as adsorbents is a greener method.
2. Nanomaterials: Highly reactive and surface aread engineered nanoparticles.
3. Layered double hydroxides (LDHs): These substances have exceptional adsorption capabilities and adjustable characteristics.

1.2 ANTIBIOTIC ADSORPTION MECHANISMS

Several mechanisms are involved in the adsorption process:

1. Electrostatic attraction: Charged spots on the adsorbent surface interact with antibiotics. Antibiotics interact with charged sites on the adsorbent surface. Electrostatic forces play a significant role in the adsorption process. Positively charged antibiotics are attracted to negatively charged adsorbents and vice versa (Alsager et al., 2018)
2. π - π interaction: Antibiotics' aromatic rings create π connections with the adsorbent. Aromatic rings present in antibiotics form π bonds with the adsorbent. This interaction contributes to the stability of the adsorption complex. π – π interactions enhance the affinity between antibiotics and adsorbents (Abd Halim et al., 2024)
3. Hydrogen bonding: Antibiotics' hydrogen atoms form bonds with the adsorbent. Hydrogen atoms in antibiotics engage in bonding with the adsorbent. These hydrogen bonds strengthen the association between antibiotics and adsorbents. Hydrogen bonding is particularly relevant in polar solvents.

1.3 CRUCIAL ELEMENTS OF ADSORBENT CHOICE SELECTING APPROPRIATE ADSORBENTS

Constitution and attributes: The physical and chemical features of the adsorbent.

Temperature and pH: These affect the ability of adsorption.

Models and isotherms of adsorption kinetics: Crucial to comprehending the behavior of sorption.

1.4 NOTABLE ANTIBIOTICS AND ADSORBENTS

Several noteworthy adsorbents have been covered in the literature, such as: -

Adsorbents based on graphene - Adsorbents layered with double hydroxides -

Magnetic nanoparticles Removing prominent antibiotics like Sulfamethoxazole,

Tetracycline, and Metronidazole

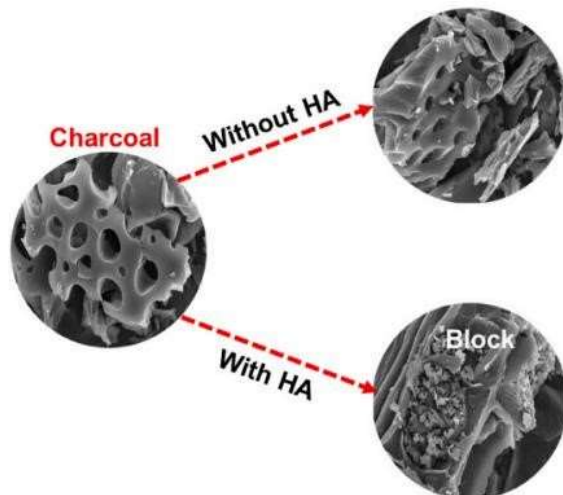


Figure 1.1 Adsorption of antibiotics using charcoal (Lee et al., 2024)

Prospects and Difficulties for the Future Adsorption methods have potential, yet problems still exist.

The problems of cost-effectiveness, scalability, and regeneration need to be addressed by researchers. Novel adsorbents and hybrid materials could be used in future developments.

This thorough analysis highlights the applicability of these developments to milk contamination while illuminating current advances in the adsorptive removal of antibiotics from water. We can lessen the effects of antibiotic residues in milk and protect the public's health by comprehending the mechanisms and choosing the right adsorbents.

In human health, animal agriculture, veterinary care, and aquaculture, antibiotics are essential. But the extensive use of them has contaminated milk and other water sources. Milk with leftover antibiotics can be harmful to human health and the environment. Adsorption techniques have become more popular as efficient ways to get rid of antibiotics from milk in recent years. With an emphasis on natural and modified adsorbents, this research paper offers a thorough review of adsorptive removal techniques. In relation to antibiotic sorption, we investigate the adsorption processes, kinetics models, and isotherms. We also go over the potential and

difficulties that adsorption methods for cleaning wastewater tainted with antibiotics may present in the future.

The necessity to provide reliable analytical techniques for the methodical examination of antibiotics in milk is the driving force behind this research. The detection, measurement, and characterisation of antibiotic residues in milk samples are the main goals. We seek to offer a thorough evaluation of the antibiotic landscape in milk, including a wide variety of antibiotic classes, by utilizing cutting-edge analytical techniques (Aldoori et al., 2023).

1.5 METHODS TO ESTIMATE ANTIBIOTICS

Antibiotics have a significant role in the treatment of disorders caused by bacterial infections. Due to a lack of complete information, antibiotics are seen as “panacea”, leading in inappropriate use even abuse in animal husbandry and medical industries. Improper use of antibiotics in clinical practice might disrupt the function of some organs and harm the nerve, renal and blood system. It is particularly dangerous for youngsters, the elderly and individuals with renal failure. The overuse of antibiotics can potentially trigger the formation of drug-resistant super bacteria, presenting a serious danger to world health. In addition, the misuse in aquaculture business may lead to residues in animal-derived meals. Therefore, it is vital and urgent to reliably identify trace antibiotics in complex matrices such as food and biological materials. So far, many analytical techniques for antibiotics detection have been published, such as high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA) and sensor (Zhou et al., 2021).

The techniques for identifying trace ABX in milk include chromatographic methods, microbiological approaches, immunochemical techniques and biosensors, etc. with high pressure liquid chromatography (HPLC) finding widespread application (Butovskaya et al., 2023). As milk includes complex constituents, such as protein, lactose, fat and inorganic ions, the detection of ABX might be affected by milk contents during HPLC analysis. This indicates that sample pretreatment is the most crucial technique as it may minimize interference from milk constituents (cleanup) and enrich

ABX in magnitude (preconcentration) in order to accomplish accurate ABX measurement (Ahmadijokani et al., 2022).

Several pretreatment procedures, including solvent extraction, solid phase extraction (SPE) and centrifugal ultrafiltration, etc., have been developed. SPE is an improved pretreatment technology that is based on low-pressure liquid chromatography and liquid-solid phase equilibrium, and has been widely used to separate target analytes from samples, preconcentrate ABX and clean samples for HPLC analysis. Compared to solvent extraction, SPE is more convenient and efficient. However, deproteinization and the follow-up of centrifugation, sample loading, evaporation, reconstitution and filtering are still essential. If the deproteinization step of milk is missed, excessive throughput of milk in the SPE column might produce obstruction, a unstable flowrate and variable adsorption periods. In addition, either MeOH or H₂O are commonly used to clean up SPE columns following sample loading to further reduce the interference of contaminants (Liu et al., 2022).

1.6 INSTRUMENT-BASED DETECTION TECHNIQUES

Instrument-based detection methods are commonly applied for the analysis of quinolone antibiotics in milk. These procedures employ modern technology to examine milk samples and determine the presence of quinolone residues. Common instrument-based approaches include chromatographic procedures such as chromatography paired with various detectors and fluorescence spectroscopy techniques. These instrument-based procedures offer outstanding sensitivity, selectivity, and accuracy in measuring quinolone antibiotics, enabling accurate detection even at trace doses. However, successful adoption of these procedures involves the use of precise laboratory apparatus and meticulous sample preparation to provide accurate and repeatable findings. The combination of instrumental analysis and proper sample preparation procedures plays a critical role in assuring the reliability and quality of quinolone antibiotic detection in milk samples (Liu et al., 2023)

The present study comprises of the evaluation of Penicillin in the milk and its removal strategies.

CHAPTER 2

LITERATURE SURVEY

2.1 EFFECT OF ANTIBIOTICS IN ECOSYSTEM

Antibiotics endanger human health and harm a variety of ecosystems. Antibiotics are found in minuscule amounts relative to naturally occurring organic components in ecosystems that comprise soil, water, and sediment. Antibiotics are becoming more and more prevalent worldwide, endangering both human health and aquatic ecosystems (Kumar et al., 2018). The main purpose of antibiotics is to prevent illness in humans and animals. They are frequently eliminated from the body and released into the environment, nevertheless, because they are difficult for the body to absorb and metabolize. Antibiotic-resistant genes (ARG) have been observed as a result of the constant release of antibiotics into the environment, while residual concentrations of antibiotics normally range from a few ng/L to µg/L. Antibiotics' capacity to treat infectious illnesses is diminished by ARG. Thus, limiting the quantity of antibiotics in the environment is crucial. (Lee et al., 2024)

Regulation is therefore required, and the appropriate use of antibiotics is imperative. However, the routine use of antibiotics on healthy animals in nations such as India to prevent illness and infection and to encourage growth creates a pathway for the influx of antibiotics into dairy products and milk (Zhou et al., 2021). Consumers' health may be adversely affected by leftover antibiotics in milk, which can lead to allergic reactions and the emergence of resistant strains. The presence of antibiotic contamination in milk might result in substantial financial losses for the companies that produce and make milk and milk products. Even though antimicrobial medications are helpful in treating human illnesses, their presence in milk has a negative impact on public health, increasing the risk of drug resistance and hypersensitivity. (Devakumari, et al., 2021)

2.2 WORLD SCENARIO

Antibiotics have been widely employed in people and animals to combat microbial diseases and enhance the development of farmed animals as feed additives. Antibiotics that are manufactured worldwide can enter the environment following their use and are detected as remnants in municipal sewage, and even food. While the amounts may be small, the ongoing introduction of antibiotics poses a possible

longterm threat to aquatic ecosystems. Moreover, there exists a significant problem in the field of veterinary medicine, namely in relation to cows, where the milk derived from treated animals becomes contaminated with antibiotics (Yang et al., 2019). For instance, marb Penicillin (MAR) is a recently developed fluoroquinolone antibiotic. After the initial administration, a maximum concentration of MAR in milk was found to be $1.024 \mu\text{g mL}^{-1}$, which is significantly above the maximum residue limit (MRL) of $0.075 \mu\text{g mL}^{-1}$ specified by the European Union Council Regulation 2377/90.

In order to minimize wastage of milk resources and mitigate environmental concerns, it is imperative to collect and treat milk contaminated with antibiotics, ensuring that it meets the necessary quality standards or is completely free from antibiotics. Consequently, it is anticipated that purified milk will be recycled for the purpose of nourishing calves (Ge et al., 2021).

2.3 USE OF CHARCOAL FOR REMOVAL OF ANTIBIOTICS

Biochar is a more cost-effective and non-toxic material that can be used to remove antibiotics from tainted milk in an economically viable manner (Wu and Hu, 2021). One of the best methods for reducing antibiotic contamination is adsorption, which has the benefits of being inexpensive, having a straightforward design, and being simple to use. Because these variables affect the properties of the biochar, such as surface area, surface functional groups, and porosity, and because they are easily changed to eliminate particular antibiotics found in milk, the adsorption capacity of biochar is dependent on the biomass feedstock and the pyrolysis method. In this work, samples of raw milk were checked for antibiotic residues, and the contaminated samples were treated with biochar to adsorb the antibiotics out of them (Chun et al., 2004) (Devi et al., 2014).

Ammonium, phosphate, and antibiotics were all eliminated at the same time by applying the pellet. Pellet strength was reduced by the charcoal, and bentonite reduced the overall adsorption efficiency (Wafy, 2024). A 10 weight percent binder and charcoal dose were suggested in consideration of the adsorption performance and pellet strength. The reaction media used to heat treat the pellet had a significant impact on its characteristics. Comparing the pellets calcined in nitrogen against those calcined in air, the latter demonstrated weaker adsorption performance. Pelletization, however, resulted in a decrease in surface area and obstruction of adsorption sites, which

reduced adsorption capacity. Indicating that the particle maintained its shape during adsorption, the pelletized adsorbent showed compressive strength with minimal mass loss. Using low-grade charcoal, bentonite, and alum sludge, respectively, the pellet concurrently eliminated phosphate, ammonium, and antibiotics. This indicates that the adsorbent can be treated with contaminated groundwater by using it as a reactive medium in a permeable reactive barrier (Jo et al., 2021).

2.4 TYPES OF ABSORBANTS

Chitosan and activated charcoal are eco-friendly ingredients, which might be employed in dairy wastewater treatment. The surface morphology of chitosan was discovered to be rough with some fiber type structures, whereas, the activated charcoal displayed primarily rough surface with nanopores. This study proved the influence of adsorbent addition on coagulation system for wastewater treatment. The maximum removal capacity of COD was 90%, while, the TN and TP removal capacity amounted to 66% and 65% correspondingly with the addition of the adsorbent. According to FTIR and NMR spectroscopic examinations of treated wastewater the pollutants including lipids, proteins and carbohydrates were decreased. The affordable and easy-to-reach coagulant and adsorbent surely make this hybrid coagulation-adsorption-based technique a practical alternative for the treatment of dairy effluent (D'íez et al., 2021).

To date, TCs in water have been detected using a wide range of analytical methods, such as gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), liquid chromatography-equipped mass spectroscopy (LC-MS), field-effect transistor, ion mobility spectroscopy (IMS), thin-layer chromatography (TLC), Raman spectroscopy (RS), electrochemical biosensing, and paper-based microfluidics. While these techniques can detect TCs with high precision and sensitivity, they suffer from drawbacks such as capital and operating expenses, time-consuming and complex testing settings, and expert operation requirements. In contrast to the approaches discussed above, optical analysis has become popular for detecting TCs in water due to its rapid reaction time, high sensitivity, convenience of use, and facile construction.

For optical detection, conventional sensors are grouped into five categories, including colorimetry, fluorescence, surface plasmon resonance (SPR), surface-enhanced Raman scattering (SERS), and chemiluminescence (CL) (Ulloa et al., 2024). Among the optical sensors, fluorescence probes have attracted great interest for TCs detection in water due to their excellent selectivity and sensitivity, quick response, low cost, and good repeatability. To date, several fluorescent probes, such as quantum (QD) and carbon (CD) dots, europium ions (Eu³⁺), carbon nanomaterials, metallic nanoclusters (MNC), fluorescent metal–organic frameworks (FMOFs), among others, have been utilized for the detection of TCs in water. Among the various family members of fluorescent-based probes, FMOFs have been recently introduced as excellent candidates for the detection of TCs, mainly due to their superior fluorescence emission/quenching ability, infinite tenability, simple surface modification, tunable pore size, and functionality, and good thermal and chemical equilibrium. The present study covers the recent progress in MOFs-based fluorescent sensors for TCs identification (Ahmadijokani et al., 2022)

2.5 PENICILLIN

Residual prescription antibiotics have been identified in milk since early national and regional studies showing a prevalence of 7 to 15% (7, 8, 13, 14, 16, New York State Food Laboratory Staff, 1984, personal communication). Increased testing for antibiotics and the implementation of restrictions surrounding their entrance into milk have not removed the problem (Sharma et al., 2024).

One cause has been the better sensitivity of new or improved testing to identify trace penicillin and other antibiotics in milk. The *Bacillus stearothermophilus* test, now official in New York and other states, can detect around 0.002 IU penicillin/mL milk. Public health professionals are worried that even little doses of antibiotics, particularly penicillin, absorbed by the human through the food chain is potentially hazardous. This is largely because their presence may promote loss of antibiotic sensitivity in pathogenic or food poisoning bacteria such as *Staphylococci* with subsequent ineffectiveness of medical therapy if antibiotics are provided (Sahoo et al., 2023).

Economic costs to the milk producer, handler, and processor can be considerable since, in addition to legal fines, contemporary procedures for salvaging tainted milks

operate poorly and may merely shift the antibiotic residue into other dairy products. Hill claimed that milk was being rejected by handlers because antibiotics were present and load losses were too severe. Disposal may be accomplished by selling to veal firms at distress prices, dumping onto open fields, where permitted, or by centrifuging out the fat component. Penicillinase, an enzyme specialized for penicillin, successfully cancels the bacteriostatic function of penicillin but retains the altered penicillin molecule in milk while introducing another component, the enzyme, whose effect on human health is unknown. For this reason, usage of penicillinase in this nation and abroad is forbidden. Charm recently patented a technique to remove beta-lactam antibiotics such as penicillin from milk by its transit through an activated charcoal column with adsorption of the antibiotic (Roba, 2023) (Mohan et al., 2023).

The notion is modeled after an established procedure by pharmaceutical corporations to eliminate contaminants in commercial antibiotic production. Charm reported that the US Food and Drug Administration has raised reservations regarding his approach associated with microbiology and the physical characteristics of fat. In the dairy sector, large scale ultrafiltration of milk and whey at low temperature is becoming a regular procedure. Ultrafiltration employing polysulfone membranes of molecular weight ranging from 10,000 to 20,000 daltons retains fat, protein, insoluble salts, bacteria, viruses, and enzymes. Antibiotics may penetrate molecular membranes, a property utilized by the pharmaceutical industry to isolate and harvest significant amounts of antibiotics from liquid substrate. However, removal of trace antibiotics from milk by ultrafiltration and their efficiency have not been established. Objectives of the present investigation were to eliminate penicillin G to undetectable levels (Rivera-Utrilla et al 2017).

The Food and Drug Administration has expressed concerns regarding this process that deals with microbiology and the physical properties of fat. Large scale ultrafiltration of milk and whey at low temperatures is becoming a common practice in the dairy industry. Antimicrobial agents can permeate biological membranes, a characteristic that is used by the pharmaceutical industry to separate and harvest large concentrations of antibiotics from liquid substrate; however, separation of trace drugs from milk by ultrafiltration and its effectiveness have not been shown. (Kosikowski and Jimenez-Flores, 1985).

All age groups across the world drink a lot of milk, but babies, kids, and the elderly drink it the most. More than 6 billion people drink milk and milk-derived products worldwide. Milk is considered an essential element of many human diets and a complete meal. Because it offers a well-balanced supply of vital nutrients, such as minerals, fat, and protein. There are reports that milk is tainted with a range of adulterants, chemicals, and medicinal substances despite its great nutritional value and widespread use. Drug residues have an adverse effect on milk quality, and when concentrations exceed allowable limits, they pose serious health risks to consumers (Niu et al., 2023).

According to the Food Safety and Standards Act of 2006, residues of veterinary pharmaceuticals are defined as the parent compounds, their metabolites, or both in any edible part of any animal product. They also include residues of the veterinary drugs' related impurities. Food quality that poses a risk to human health, such as zoonotic illnesses and unfavorable xenobiotic effects, is referred to as "food safety." Reliability for residues in milk and dairy products and their effect on public health have recently come to light. According to Ge, antimicrobial residues have the potential to reach the human food chain and have toxicological consequences on people. Because they consume more milk and milk products than other age groups, infants and growing children are more vulnerable to residues. Human health is also at risk from antimicrobial resistance (AMR), as people gradually become resistant to antibiotics that may be required to treat medical conditions. It is therefore vitally necessary to guarantee that milk and milk products are free of antibacterial residues. The ecology and milk production are intrinsically intertwined (Ge et al., 2021).

2.6 TECHNIQUES FOR DETECTION OF DRUG RESIDUES IN MILK

As stated below, a number of methods are available for identifying various drug residue types in milk and dairy products. Antibiotic residue detection techniques fall into two categories: confirmatory methods and screening approaches. The first approach is globally qualitative or semi-quantitative, which sets it apart from the other two. On the other hand, the latter guarantees a very accurate identification of the kind and amount of residue under investigation (Forouzani et al., 2024).

CHAPTER 3

AIM AND SCOPE

3.1 AIM

To investigate the effectiveness of activated carbon derived in removing Penicillin from milk.

3.2 OBJECTIVES

- To Synthesize and characterize carbon particles
- To evaluate the efficiency of the carbon particles for Penicillin adsorption.
- To characterize the results using instrumentation such as FTIR and SEM.

CHAPTER 4

MATERIALS AND METHODS

4.1 COLLECTION OF ADSORBENT AND ADSORBATE

The milk was collected from the local vegetable market and Penicillin was purchased from the medical shop in Chennai, Tamilnadu. The collected sample were packed in the polythene bag and processed for further studies.

4.1.1 Standard graph of Penicillin

A stock solution of Penicillin was prepared by dissolving a known amount of Penicillin in a suitable solvent, such as water, to achieve a concentration range that covered the expected concentrations in the samples. From the stock solution, a series of working standard solutions with different concentrations (100 ppm to 1000 ppm) were prepared. The absorbance of each standard solution was measured at a specific wavelength using a UV-Visible spectrophotometer at 295 nm. A graph of the absorbance values versus the corresponding concentrations of the standard solutions was plotted. This graph served as the calibration curve (Liu et al., 2022).

4.2 PROPERTY ANALYSIS OF THE ACTIVATED CARBON

4.2.1 Pore volume

The adsorbent (1g) was transferred into a 10ml measuring cylinder in order to get the total volume of the sample. The sample was then poured into a beaker containing 20ml of deionized water and boiled for 5min. The content in the beaker was then filtered, superficially dried, and weighed. The pore volume of the sample was determined by dividing the increase in weight of the sample by the density of water.

4.2.2 Moisture content determination:

50g of the adsorbent (corn husk) was collected and dried in an oven for 4 hours at 150°C.

The moisture content was calculated from the following formula:

$$X_o = \frac{W_1 - W_2}{W_1} \times 100$$

X_o = Moisture content on wet basis
 W_1 = Initial weight of sample
 W_2 = Final weight of sample after drying

4.3 ADSORPTION STUDIES

Adsorption studies of Penicillin was done by Activated carbon. Absorbance was measured by UV-visible spectroscopy at 295nm (Labuto et al., 2022).

4.3.1 Adsorption using Activated carbon (Ekka et al., 2022).

Penicillin absorbs UV light at specific wavelengths. By measuring the UV absorbance of a solution before and after contact with activated carbon, the amount of adsorbed Penicillin is determined.

- ❖ A stock solution of Penicillin was prepared at a known concentration.
- ❖ Activated carbon(100mg,250mg,500mg,750mg,1000mg) were added to each Erlenmeyer flask.
- ❖ known volume(1000ppm) of each Penicillin solution was added to the flasks. Cap the flasks and shake them at a constant speed for a predetermined time (e.g., 1 hour, 2 hours, etc.). This allows for the adsorption of Penicillin onto the activated carbon.
- ❖ After the shaking time, separate the activated carbon from the solution by filtration.
- ❖ Using a filter paper separate the solution from the activated carbon.
- ❖ Absorbance was measured by the remaining Penicillin solution (supernatant) using a UV-Vis spectrophotometer at a specific wavelength where Penicillin has a strong absorbance peak at 295nm.
- ❖ The absorbance of the Penicillin solutions before and after contact with the activated carbon. A decrease in absorbance indicates adsorption of Penicillin onto the activated carbon.
- ❖ The amount of Penicillin adsorbed can be calculated based on the initial and final concentrations of the Penicillin solution.
- ❖ The calibration curve was constructed by plotting the absorbance of known Penicillin concentrations against their respective concentrations.

4.4. CHARACTERIZATION OF ADSORBENT

4.4.1 SEM

Scanning Electron Microscopy (SEM) is a type of electron microscope that creates pictures of solid surfaces by scanning them with electron beams. Electron beams impact the atoms on the sample surface, producing a variety of signals. Electron detectors detect these impulses. These signals include information about the sample's adsorption in surface structure (Lee et al., 2024). Activated carbon adsorption morphology was studied.

4.4.2 FTIR

Surface functional group analysis of the Adsorbed adsorbate as well as of the raw material was done before and after adsorption, using an FTIR model NICOLET 6700 (Thermo Fisher Scientific, U.S.A.). FTIR analysis of the adsorbent was done to find and analyse functional groups present on the surface of the adsorbate (Li et al., 2020).

CHAPTER 5

RESULTS AND DISCUSSION

Antibiotics are a growing environmental concern, impacting ecosystems globally and posing risks to both human health and the environment. Their presence can lead to the development of antibiotic-resistant bacteria. To combat this issue, various methods are used to remove antibiotics from the environment, including advanced oxidation processes, activated sludge treatment, vermicomposting, and adsorption. Among these, adsorption is highly effective.

This study found that activated carbon Hi-media, is a promising approach to removing antibiotics like Penicillin by Adsorption.

5.1 STANDARD GRAPH OF PENICILLIN

Preparation of standard graph for penicillin. First take 1ml of Penicillin in 90ml of water mix it well. After a few hours take a reading about 280 to 300nm.

The standard graph for Penicillin concentration showed a linear relationship between concentration and absorbance, as shown in the fig 5.1.

Table 5.1: Concentration and Absorbance of Penicillin

S.NO	Penicillin(ml)	Water(ml)	Readings(nm)
1.	1	99	0.300
2.	2	98	0.320
3.	3	97	0.332
4.	4	96	0.340
5.	5	95	0.350
6.	6	94	0.360
7.	7	93	0.370
8.	8	92	0.385
9.	9	91	0.405
10.	10	90	0.410

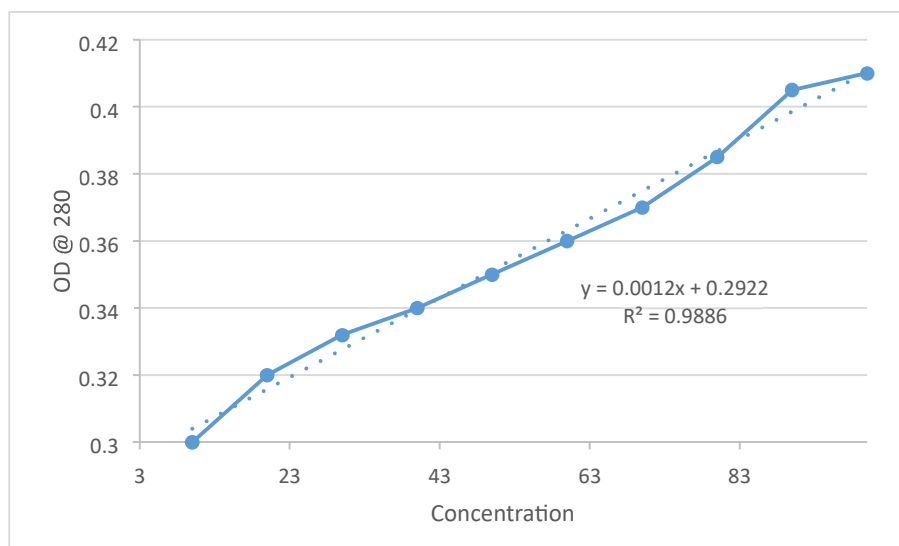


Figure: 5.1: Optimization of Activated carbon concentration

Table 5.2: Adsorption of Penicillin using activated carbon (250 mg)

Penicillin (ml)	Milk(ml)	Water(ml)	Activated Carbon	Initial Adsorption	Final Adsorption
1	25	24	250	10%	80%
2	25	23	250	12%	90%
3	25	22	250	11%	90%
4	25	21	250	10%	80%
5	25	20	250	12%	80%
6	25	19	250	12%	90%
7	25	18	250	10%	90%
8	25	17	250	12%	80%
9	25	16	250	11%	90%
10	25	15	250	13%	90%
Control	25	--	--	10%	100%

Table 5.3: Adsorption of Penicillin using activated carbon (500 mg)

Penicillin (ml)	Milk (ml)	Water (ml)	Activated Carbon	Initial Adsorption	Final Adsorption
1	25	24	500	10%	90%
2	25	23	500	12%	90%
3	25	22	500	11%	80%
4	25	21	500	12%	80%
5	25	20	500	12%	90%
6	25	19	500	12%	90%
7	25	18	500	11%	80%
8	25	17	500	12%	80%
9	25	16	500	13%	90%
10	25	15	500	12%	90%
Control	25	--	500	10%	100%

Table 5.4: Adsorption of Penicillin using activated carbon (750 g)

Penicillin (ml)	Milk(ml)	Water(ml)	Activated carbon(mg)	Initial Adsorption	Final Adsorption
1	25	24	750	10%	80%
2	25	23	750	10%	80%
3	25	22	750	11%	90%
4	25	21	750	12%	90%
5	25	20	750	11%	90%
6	25	19	750	10%	80%
7	25	18	750	11%	90%
8	25	17	750	12%	80%
9	25	16	750	13%	90%
10	25	15	750	14%	90%
control	25	--	--	10%	100%

1ml of Penicillin in 90ml of water mix it well.

Table 5.5: Adsorption of Penicillin using activated carbon (1000 g)

Penicillin (ml)	Milk (ml)	Water (ml)	Activated carbon (mg)	Initial Adsorption	Final Adsorption
1	25	24	1000	10%	80%
2	25	23	1000	11%	80%
3	25	22	1000	13%	90%
4	25	21	1000	10%	90%
5	25	20	1000	10%	80%
6	25	19	1000	11%	80%
7	25	18	1000	10%	90%
8	25	17	1000	12%	80%
9	25	16	1000	13%	90%
10	25	15	1000	14%	90%
control	25	--		10%	100%

Table 5.6: Adsorption of optimized Penicillin using activated carbon

1g of penicillin	Milk (ml)	Water (ml)	Activated carbon (g)	Initial Adsorption	Final Adsorption
5	25	20	1	10%	90%
5	25	20	1	10%	90%
5	25	20	1	10%	90%
5	25	20	1	10%	92%
5	25	20	1	9%	92%
5	25	20	1	9%	94%
5	25	20	1	9%	95%
5	25	20	1	8	94%
5	25	20	1	8%	95%
5	25	20	1	9%	95%

control	25	--	--	8%	100%
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5.2 FTIR ANALYSIS

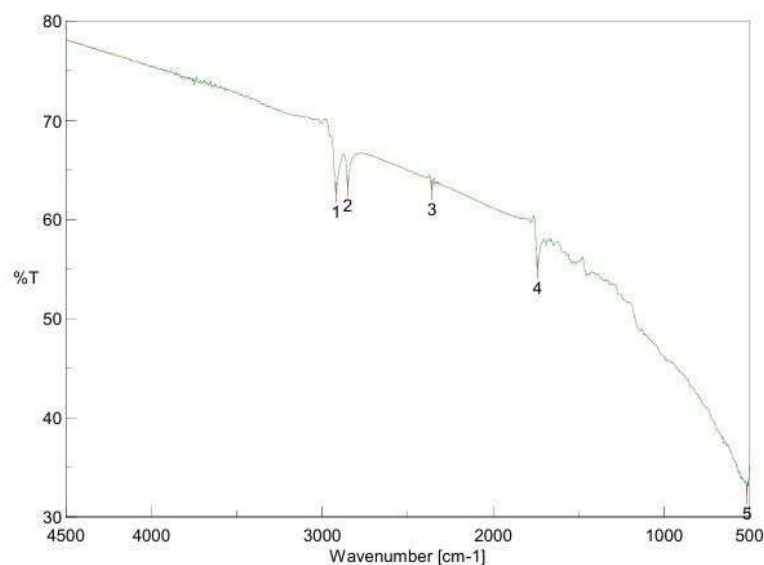


Figure 5.2: FTIR result of initial adsorption of 500ppm at 0 hours.

These peaks show that the final adsorption of Penicillin contains a variety of functional groups that are typical of the carbonaceous material made from activated carbon. Organic compounds frequently exhibit O-H stretching vibrations, which are represented by the peak at 3203.18 cm^{-1} . It's possible that other functional groups or C-C triple bonds are responsible for the peak at 2362.37 cm^{-1} . (lu et al., 2019) Peaks at lower wavenumbers, like 519.722 cm^{-1} , 509.115 cm^{-1} , and 1206.26 cm^{-1} , might represent various kinds of vibrations in the carbon structure. As reported by Ge et al. (2021), For this samples, the O-H stretching vibration in phenolic hydroxyl groups was detected at a bandwidth of approximately $3446.18\text{--}3414.08\text{ cm}^{-1}$. Oxygen is lost during the activation and surface modification process, which is why this region's intensity has decreased. Indicating the existence of C-H stretching connected to alkane groups, the other identical peaks were discovered at bandwidths between 2926.22 and 2913.15 cm^{-1} . They found that their C-C multiple bond stretching occurred at wavelengths of approximately 2100 and 2300 cm^{-1} , and their CH₂ bending vibrations were detected at $1465.92\text{--}1429.33\text{ cm}^{-1}$.

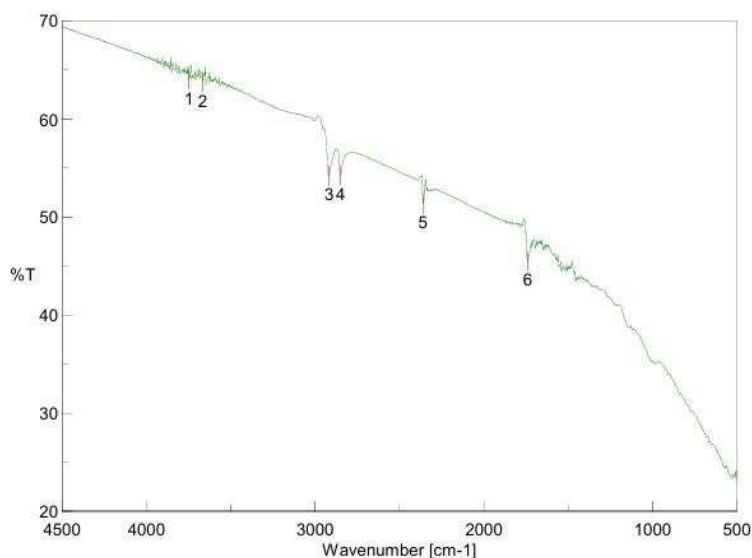


Figure:5.3: FTIR result of final adsorption.

These peaks show that the activated carbon contains a variety of functional groups, each of which is related to a particular chemical bond. For instance, the peak at 2360.44 cm^{-1} may suggest the presence of C-C triple bonds or possibly a combination of functional groups, whereas the peak at 3195.47 cm^{-1} suggests the presence of O-H stretching vibrations. The peaks at lower wavenumbers, like 505.258 cm^{-1} and 1208.18 cm^{-1} , might represent various kinds of vibrations in the carbon structure. Amin et al., 2017 investigated into the adsorption of penicillin using a magnetic graphene oxide composite in their study. Functional groups including hydroxyl (-OH), carboxyl (-COOH), and amino (-NH₂) groups were present in the composite, according to the FTIR analysis, and they contributed to the adsorption of penicillin through hydrogen bonding and electrostatic interactions. This study shows that the initial adsorption of Penicillin using the AC composite is superior through a thorough comparison of the FTIR analysis and adsorption capacity of the two composites (Jo et al., 2021).

5.3 SEM ANALYSIS:

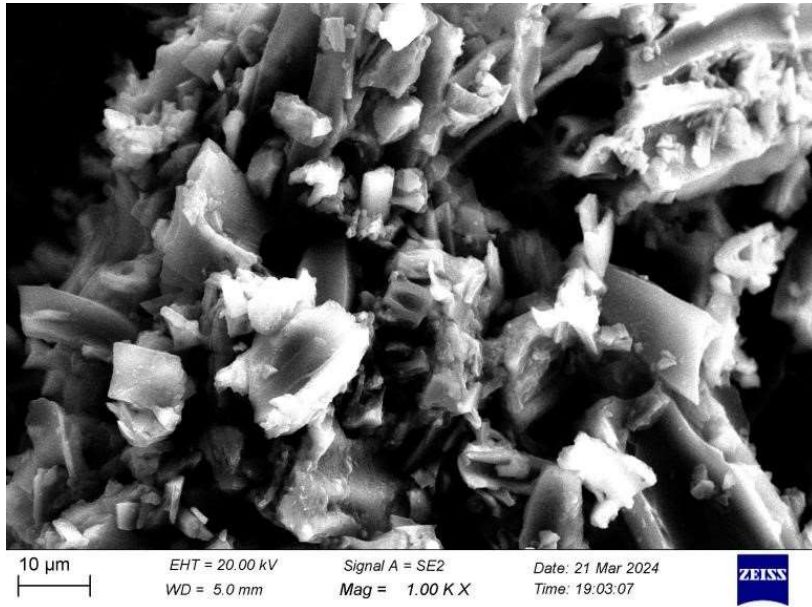


Figure:5.4: SEM images of activated carbon

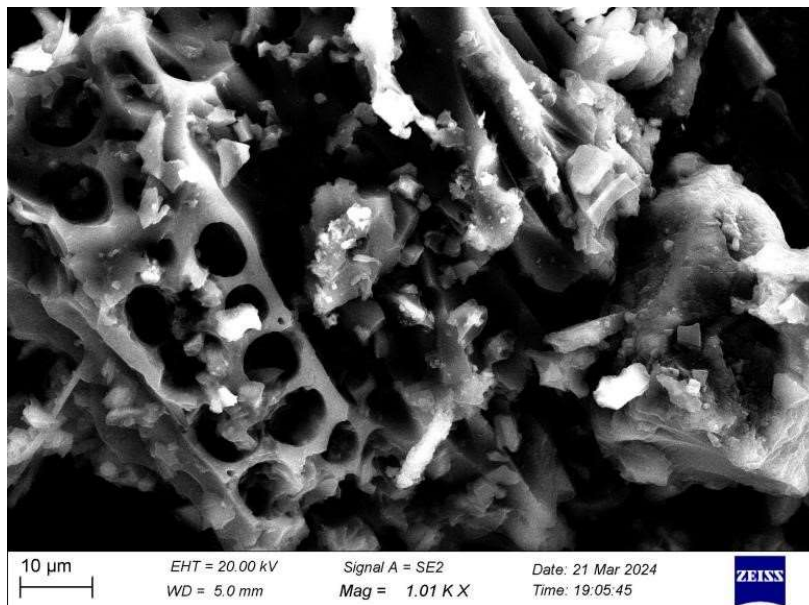


Figure:5.5: SEM images of activated carbon with penicillin

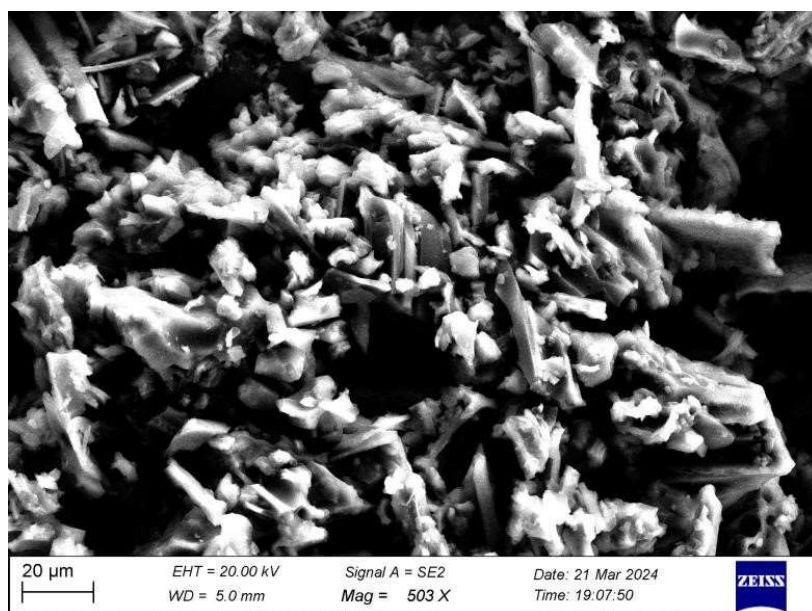


Figure:5.6: SEM images of activated carbon with penicillin @ 20 µm

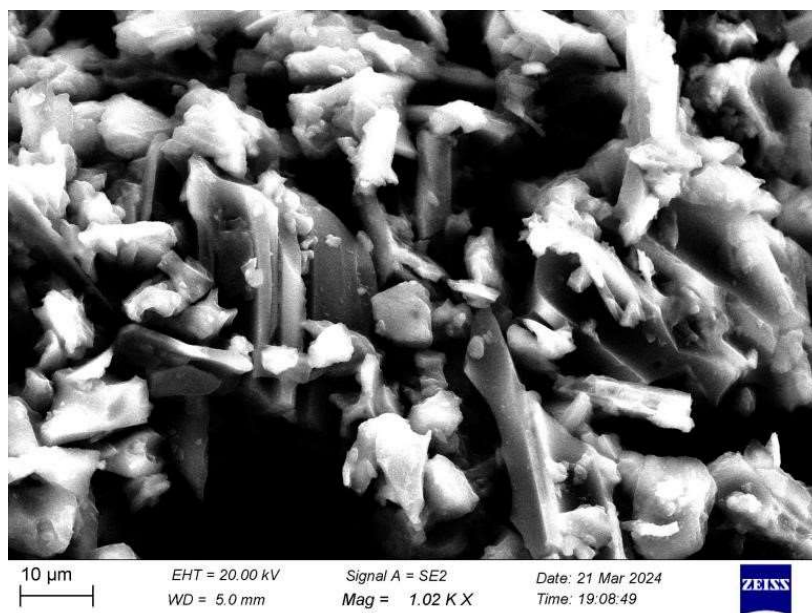


Figure:5.7: SEM images of initial adsorption in 30 min

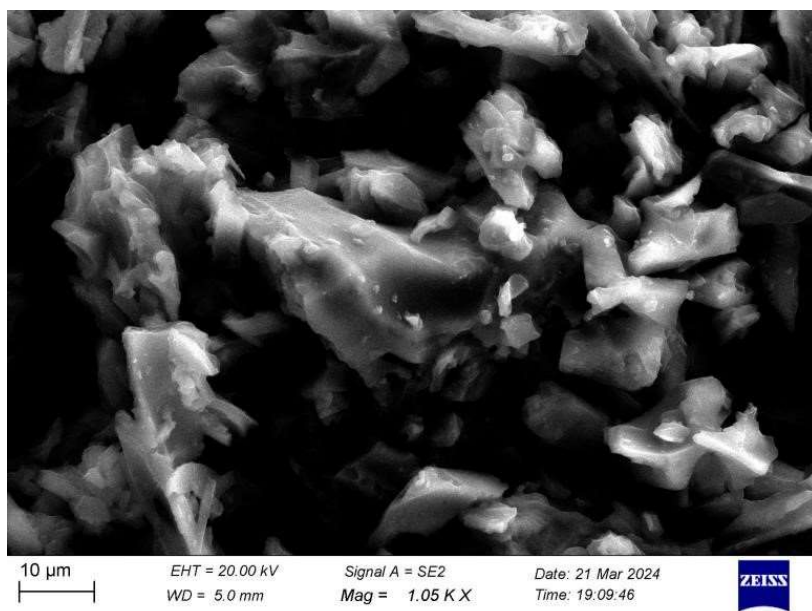


Figure:5.8: SEM images of initial adsorption in 1 hour

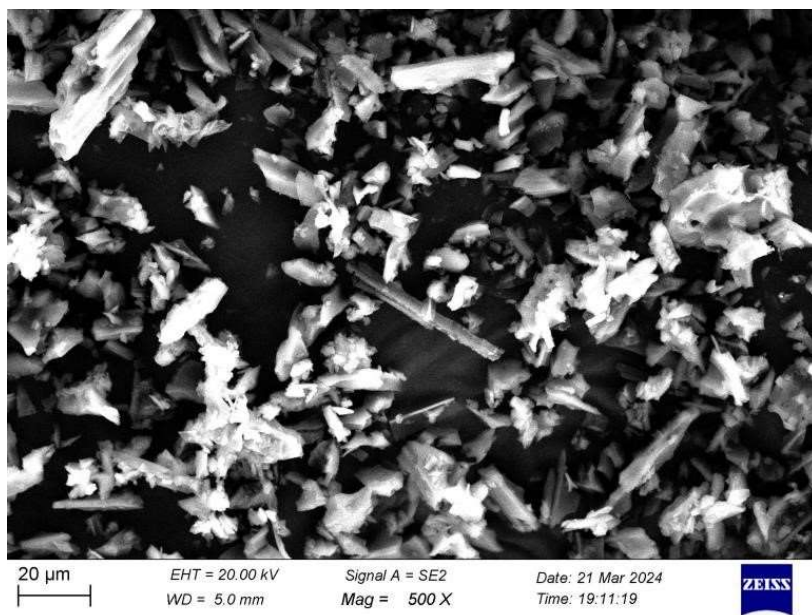


Figure:5.9: SEM images of adsorption in 1.5 hour

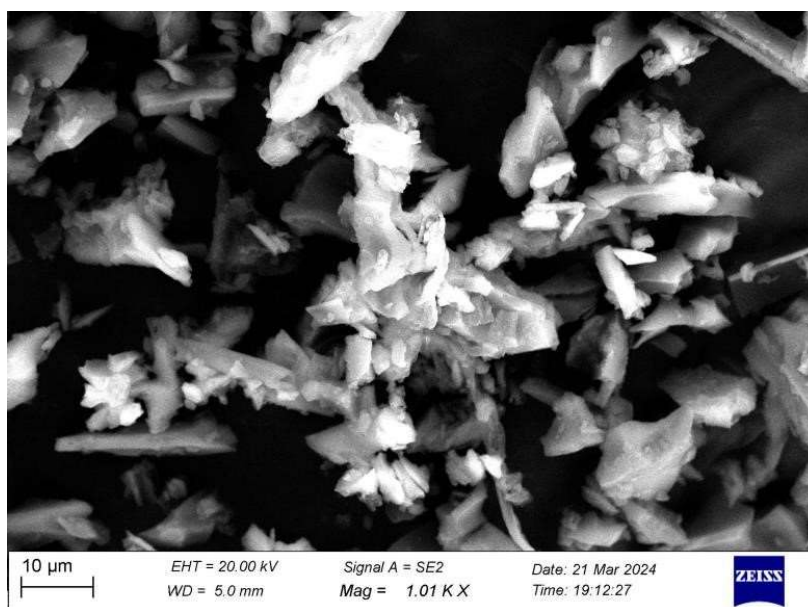


Figure:5.10: SEM images of adsorption in 2 hour

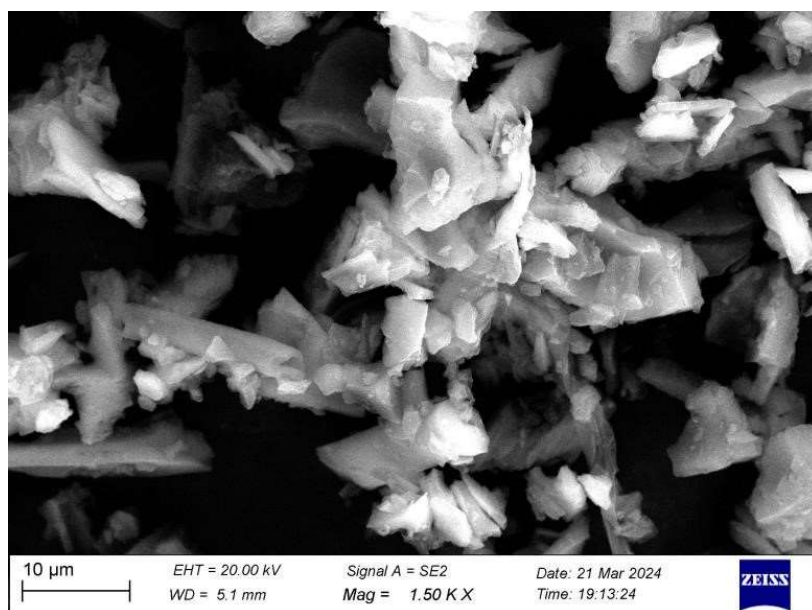


Figure:5.11: SEM images of adsorption in 3 hour

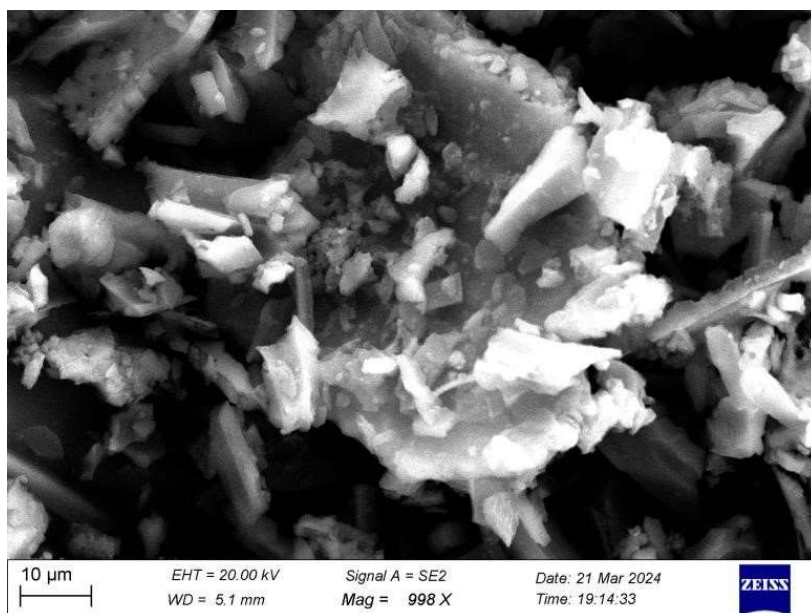


Figure:5.12: SEM images of adsorption in 4 hour

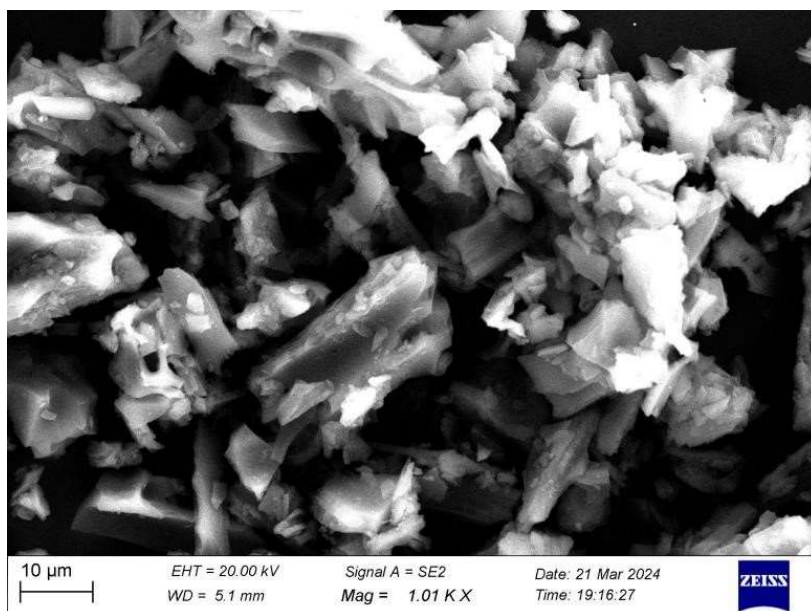


Figure:5.13: SEM images of final adsorption in 5 hour

The morphology and surface properties of the activated carbon-was examined using scanning electron microscopy (SEM) to the initial adsorption of Penicillin. The composite shows up as a heterogeneous mixture of different-sized and shaped particles at lower magnifications.

Particles of activated carbon with a porous structure are visible at higher magnifications (500x, 1kx, and 5kx). Throughout the carbon matrix are scattered and show up as bright, nodular features (size range: 10-50 nm) that contrast with the darker carbon matrix. When examining the composite's surface texture at the highest magnification, surface irregularities and the presence of functional groups that may be involved in Penicillin adsorption may be seen.

The observed adsorption performance was correlated with the modifications made to WPAC's morphology, textural characteristics, and surface content using various modification techniques through a thorough evaluation. Less BET surface area and porosity were surprisingly found in the best-performing samples, indicating that these properties do not predominate antibiotic adsorption. Comprehensive decomposition/reconstruction events were seen at the surface of carbon, particularly during Microwave irradiation, as demonstrated by UHR FESEM measurements, EDS analysis, and DRIFT spectra. Involved in the adsorption process were newly created or converted oxygen-containing surface groups that were identified by XPS.

CHAPTER 6

SUMMARY AND CONCLUSION

Antibiotics are frequently used on dairy farms to treat a variety of illnesses, including bacterial infections like mastitis and cystitis. In order to expedite the food production process and enhance product quality, they are also employed as growth promoters. The bulk of the administered antibiotics are eliminated unaltered, with very little of them being ingested. Because of this, animal farm effluents are a significant source of antibiotics in the environment.

One of the main strategies for controlling mastitis in many nations is the use of antibiotic therapy to treat and prevent udder infections in cows. A lot of attention and worry has been focused on the appropriate management and monitoring of antibiotic usage in treatments in order to minimize contamination of raw milk, as a result of the extensive use of antibiotics for the treatment of mastitis in dairy cows. Since the public's consumption of milk and milk products may be affected by antibiotic residues, the extensive usage of antibiotics has produced possible issues. Antibiotic-contaminated milk and milk products that contain more than a certain amount

of residue are deemed inappropriate for human consumption due to the significant impact on public health. Global health is at risk from the complicated problem of antibiotic resistance.

The key advantages of the hybrid techniques include reduced time consumption and low instrumental consumption for wastewater treatment, which makes it economically doable technology. It is predicted that the current study will shed new insights on the milk treatment.

These findings imply that, in addition to target pollutants like Penicillin and other members of antibiotics, developed adsorption methods should take into account the impacts of high quantities of naturally occurring organic matter.

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