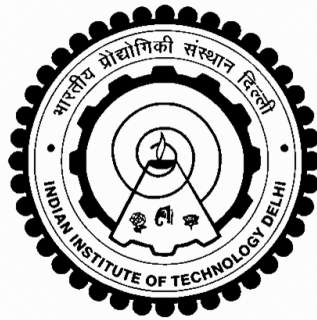


**DEVELOPMENT OF ADSORPTION BASED TREATMENT
TECHNOLOGY FOR THE REMOVAL OF ANTIBIOTIC RESISTANCE
GENES FROM WASTEWATER**

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SCHOOL OF INTERDISCIPLINARY RESEARCH (SIRe)

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2024

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TECHNOLOGY FOR THE REMOVAL OF ANTIBIOTIC RESISTANCE
GENES FROM WASTEWATER**

by

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Submitted

In fulfilment of the requirements of the degree of

Doctor of philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2024

Certificate

This is to certify that the thesis entitled “**Development of adsorption based treatment technology for the removal of antibiotic resistance genes from wastewater**” being submitted by **Abhilasha Pant** is worthy of consideration for the award of the degree of Doctor of philosophy. The thesis has been prepared by her under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi and is a record of the original bonafide research work. The results presented in this thesis have not been submitted in part or full to any other universities or institutes for the award of any degree or diploma.



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Abhilasha Pant

Abstract

Antibiotic resistance is a global health issue that poses a significant threat to public health. Antibiotic resistance genes (ARGs) can be transferred from bacteria in wastewater to human pathogens, leading to the spread of antibiotic resistance. As a result, there is an urgent need to develop effective wastewater treatment technologies to remove ARGs. Adsorption-based treatment technologies have shown great promise in the removal of ARGs from wastewater, due to their high efficiency and low cost.

The aim of the current research work was first to select an effective adsorbent material among five different adsorbent materials on the basis of their performance, regeneration capability and cost effectivity. Bark, Pyrolysed bark (PB), CTAB-modified pyrolysed bark (CTAB-PB), Chitosan (CS), Chitosan nanoparticle (CNP) and diethyl aminoethyl cellulose (DEAE-C) were the studied materials. Among all the adsorbent materials DEAE-C exhibited great removal efficiency and regeneration capacity. Various factors affecting the adsorption performance of adsorbent materials, including pH, temperature, contact time, initial concentration of ARGs, and the presence of other contaminants were then analysed for DEAE-C in batch. The adsorption process followed the pseudo-second-order process kinetics, and the Langmuir model gave the best fitting of the isotherm model. The maximum adsorption capacity of the

adsorbent was found to be 65.40 $\mu\text{g}/\text{mg}$ at pH 7 ± 0.5 . A detailed desorption study of the material revealed that DEAE-C could be recycled for at least ten adsorption cycles and the desorption of the adsorbed ARG was brought down to 20% of the reaction volume of the adsorption. We thank the reviewer for their comment. Additionally, the concentrated ARGs from the desorption process can be effectively treated using advanced oxidation techniques for complete degradation, and the regenerant can be recycled back using unit operation techniques like evaporation.

A detailed column study for the continuous adsorption of ARGs was also examined for the process. As, DEAE-C as a material possesses the gelation property leading to the swelling of the material in water. Therefore, the DEAE was immobilised on mechanically strong and inert material silica. Different parameters like packing shape, packing size, packing height, and flowrate were studied to check their effect on the removal efficiency of the studied DNA. The adsorption capacity of the column varied in range of ~ 4.5 to $7.8 \mu\text{g}/\text{g}$ of DNA adsorbed. The breakthrough curves were fitted to the Thomas and Yoon Nelson model to estimate the maximum adsorption capacity and run time before the regeneration required for the column. The adsorbent was regenerated using 0.5 M NaCl solution at pH around 8.5 ± 0.5 and could be reused upto 4 regeneration cycles. Furthermore, a larger volume of column of constant empty bed contact time was developed which demonstrated the treatability of 17.1 l volumes of water and adsorption capacity of $4.65 \mu\text{g}/\text{g}$. In real secondary treated wastewater matrix, the developed large volume column exhibited 2.05, 1.67, and 1.47 log gene copies/ml removal of bla_{CTXM}, 16S rDNA, and int 1 respectively on maintaining a low flowrate.

Saar (Hindi)

प्रतिवाहित औषधिक सुरक्षा एक वैश्विक स्वास्थ्य समस्या है जो सार्वजनिक स्वास्थ्य के लिए एक महत्वपूर्ण खतरा पैदा करती है। प्रतिवाहित औषधिक सुरक्षा जीन (ARGs) वास्तविकतः जीवाणु से मानव पथोजनों में स्थानांतरित हो सकते हैं, जो औषधिक सुरक्षा का प्रसार करता है। इस परिणामस्वरूप, ARGs को हटाने के लिए प्रभावी प्रवाही जल उपचय प्रौद्योगिकियों के विकास की आपातता है। विज्ञापन-आधारित उपचय प्रौद्योगिकियों ने जल उपचय से ARGs को हटाने में उच्च प्रदर्शन और कम लागत के कारण महत्वपूर्ण प्रमाण दिखाया है।

वर्तमान अनुसंधान कार्य का उद्देश्य पहले पांच विभिन्न विज्ञापक सामग्रियों में से एक प्रभावी विज्ञापक सामग्री का चयन करना था, जिसे उनके प्रदर्शन, पुनर्जीवन क्षमता और लागत प्रभावीता के आधार पर किया गया। छाल, पायरोलाइज्ड छाल (PB), सीटैब-संशोधित पायरोलाइज्ड छाल (CTAB-PB), काइटोसन (CS), काइटोसन नैनोपार्टिकल (CNP) और डायथिल ऐमिनोइथिल सेल्युलोज (DEAE-C) अध्ययन की गई सामग्रियों थीं। सभी विज्ञापक सामग्रियों में DEAE-C ने श्रेष्ठ हटाने क्षमता और पुनर्जीवन क्षमता दिखाई। DEAE-C के

लिए विभिन्न कारकों का अध्ययन किया गया जो विज्ञापन सामग्रियों की प्रदर्शन क्षमता को प्रभावित करते हैं, जैसे pH, तापमान, संपर्क समय, ARGs की प्रारंभिक उच्चतम आकृति, और अन्य प्रदूषकों की मौजूदगी। विज्ञापन प्रक्रिया ने अनुकरणीय द्वितीय क्रम प्रक्रिया की प्रक्रिया का पालन किया, और लैंगमुइर मॉडल ने इसोथर्म मॉडल का सर्वश्रेष्ठ मिलान दिया। विज्ञापन सामग्री की अधिकतम विज्ञापन क्षमता का पता pH 7 ± 0.5 पर लगभग $65.40 \mu\text{g}/\text{mg}$ पाया गया। सामग्री का विस्तृत मोछन अध्ययन ने दिखाया कि DEAE-C को कम से कम दस विज्ञापन चक्रों के लिए पुनःचक्रित किया जा सकता है और विज्ञापित ARG का विज्ञापन प्रतिक्रिया के रिएक्शन आयत के 20% तक घटा दिया गया। प्रतिस्थायी विज्ञापन के लिए आरजी की निरंतर विज्ञापन अध्ययन का विवरण भी प्रक्रिया के लिए जांचा गया। क्योंकि जल में सामग्री का सुइने के गुण प्राप्त करने के लिए DEAE-C एक सामग्री के रूप में है। इसलिए, DEAE को यांत्रिक रूप से मजबूत और असक्रिय सामग्री सिलिका पर आबंधित किया गया। पैकिंग आकार, पैकिंग ऊंचाई, और फ्लो दर की भिन्न पैरामीटरों का अध्ययन किया गया था जो अध्ययन की जाने वाली डीएनए की हटाने क्षमता पर उनका प्रभाव देखने के लिए किया गया था। कॉलम की विज्ञापन क्षमता विभिन्न रेंज में थी, जो

लगभग ~ 4.5 से $7.8 \mu\text{g/g}$ डीएनए के विज्ञापित होती थी। थॉमस और यून नेलसन मॉडल के लिए प्रवेश गतिकाओं को फिट करने के लिए पारग्रह विकरण और संचय की आवश्यकता का मूल्यांकन किया गया। विज्ञापक को 0.5 M एनाCl और pH लगभग 8.5 ± 0.5 पर पुनःसंशोधित किया गया और इसे 4 पुनःउत्पादन चक्रों तक पुनःप्रयोग किया जा सकता था। इसके अतिरिक्त, स्थिर खाली बेड संपर्क समय के स्थिति में विकसित एक अधिक आयामी कॉलम था जिसने 17.1 लीटर जल और $4.65 \mu\text{g/g}$ की विज्ञापन क्षमता के उपचय की क्षमता का प्रदर्शन किया। वास्तविक द्वितीय संशोधित जलवायु मैट्रिक्स में, विकसित बड़े आयामी कॉलम ने एक निम्न फ्लोरेट बनाए रखते हुए bla CTXM, 16S rDNA, और int 1 के 2.05 , 1.67 , और 1.47 लॉग जीन कॉपियों/मिलीटर का सफल निकालन किया।

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Nomenclature

AMR	Antimicrobial resistance
ARB	Antibiotic resistance bacteria
ARG	Antibiotic resistance genes
ASP	Activated sludge process
bla CTXM	beta-lactamase gene
CNP	Chitosan nanoparticles
CS	Chitosan
CTAB	Cetyltrimethylammonium Bromide
CTAB-PB	Cetyltrimethylammonium Bromide modified pyrolyzed bark
DEAE-C	Diethylaminoethyl cellulose
DEAE-Si	Diethylaminoethyl coated silica gel
<i>E. coli</i>	<i>Escherichia coli</i>
GPTMS	Glycidoxypropyltrimethoxysilane
GC	Gene copies
Int 1	Class 1 Integron
MoEFCC	Ministry of Environment, Forest and Climate Change
PB	Pyrolyzed bar
ROS	Reactive oxygen species
WHO	World Health Organisation
WWTP	Wastewater treatment plant