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**DEVELOPMENT OF A NOVEL FILTER-INCUBATION SYSTEM  
ENCOMPASSING OIL PALM EMPTY FRUIT BUNCH BIOSORBENT AND  
ANTARTIC MICROALGAE FOR DIESEL REMOVAL**

By

**NURUL AINI BINTI PUASA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of  
Philosophy**

**November 2023**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**Chair : Associate Professor Siti Aqlima Ahmad, PhD**  
**Faculty : Biotechnology and Biomolecular Sciences**

Diesel is commonly used as a fuel in Antarctica to enable human activities. However, the increasing demand for diesel raises the risk of oil contamination due to accidental events, which can threaten the Antarctic environment. Through the provisions of the Antarctic Treaty, the environment in Antarctica is protected by the implementation of steps to combat and minimise fuel pollution, as required under the Protocol on Environmental Protection to the Antarctic Treaty. Across the continent of Antarctica, chronically low temperatures can reduce the rate of hydrocarbon attenuation, making pollution management challenging in isolated and extreme regions. The current oil remediation technologies have the potential to generate secondary pollution as they are unable to make use of the biomass. New bioremediation approaches adopted for removal of diesel in Antarctic that are environmentally friendly are required. Therefore, there has been increasing interest in the potential for using agricultural waste as a biosorbent. The empty fruit bunch (EFB) produced from oil palms, a cost-effective material, contains cellulose-related materials that have shown good results in trial pollution treatments. In parallel, studies of the potential for remediation of diesel pollution in the Antarctic using indigenous microalgae remain limited to date, while the optimised conditions required are yet to be determined. This study's central goal was to combine evaluation of the diesel-sorption capabilities of EFB and diesel-degrading capabilities of native Antarctic microalgae in a filter-incubation system designed to remove diesel and contribute to potential bioremediation efforts in Antarctica. In this study, EFB was screened using conventional analysis, one-factor-at-a-time (OFAT) and statistical analysis, response surface methodology (RSM), whereby the effects of EFB on diesel biosorption were characterised. The optimum conditions for EFB diesel biosorption were determined to be 172°C with 19.4 min heating time, 0.096 g/cm<sup>3</sup> packing density and 10% initial diesel concentration. The predicted model was highly significant ( $R^2 = 0.9987$ ) and four interactions, between temperature and packing density, temperature and diesel concentration, time and packing density, and packing density and diesel

concentration, were confirmed to significantly affect the diesel biosorption. Using pre-heat treated EFB, 25.33 mL of oil was successfully absorbed from an initial volume of 40 mL. The EFB sample was subjected to morphological and chemical content analysis relating to diesel biosorption. For bioremediation of diesel hydrocarbons, an Antarctic microalgal (strain WCY\_AQ5\_2) was screened, identified and characterised using conventional OFAT and statistical RSM analysis. The optimal degradation conditions were 1% diesel concentration, 11.17 mg/L NaCl concentration, pH 6.22, 0.415 g/L NaNO<sub>3</sub> and photoperiod of 12:12 h L/D. The predicted model was highly significant ( $R^2 = 0.9848$ ) and confirmed that the parameters of salinity and pH significantly influenced diesel biodegradation. Diesel biodegradation of 66.08% was achieved within 7 d incubation period. Morphological and molecular examination supported that strain WCY\_AQ5\_2 represents the species *Chlorella vulgaris*. The effect of heavy metal contamination (Ag, Al, As, Cd, Co, Cr, Cu, Ni, Pb and Zn) on the degradation of diesel was further assessed and analysed by assessing their half-maximal inhibition concentration (IC<sub>50</sub>). The findings demonstrated that Cr, Cu and Ag inhibited the degradation of diesel by more than 50%, with IC<sub>50</sub> values of 3.165, 3.371 and 4.205 ppm, respectively. These findings were then integrated into the development of a filter-incubation system that uses EFB as the sorbent with optimum packing density (0.1 g/cm<sup>3</sup>) in the filter press machine in combination with the native Antarctic microalgal strain WCY\_AQ5\_2 in a light incubation tank illuminated with optimum red light that boosted the alga's ability to degrade diesel to 70.7%. The novelty of this study is the used of the bioremediatory agents, EFB as biosorbent and native Antarctic microalgal as diesel degrader integrated in a novel filter-incubation system for diesel removal. The findings of this study help advance the diesel biosorption and bioremediation techniques currently available in Antarctica.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGUNAN SISTEM INKUBASI PENAPIS BAHARU MELIPUTI  
BIOPENYERAP TANDAN KOSONG MINYAK KELAPA SAWIT DAN  
MIKROALGA ANTARTIK UNTUK PEMBUANGAN DIESEL**

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Diesel biasanya digunakan sebagai bahan api di Antartika untuk menjalankan aktiviti manusia. Walau bagaimanapun, peningkatan permintaan untuk diesel meningkatkan risiko pencemaran minyak akibat kejadian tidak disengajakan, yang boleh mengancam persekitaran Antartika. Melalui peruntukan Perjanjian Antartika, alam sekitar di Antartika dilindungi oleh pelaksanaan langkah-langkah untuk memerangi dan meminimumkan pencemaran bahan api, seperti yang termaktub di bawah Protokol Perlindungan Alam Sekitar kepada Perjanjian Antartika. Di seluruh benua Antartika, suhu rendah yang kritikal boleh mengurangkan kadar penguraian hidrokarbon dan menyebabkan pengurusan pencemaran mencabar di kawasan terpencil dan ekstrem. Teknologi sedia ada bagi penyingkiran minyak mempunyai potensi untuk menjana pencemaran sekunder kerana tidak dapat menggunakan semula biojisim. Pendekatan bioremediasi baharu yang mesra alam dan dapat diterima pakai diperlukan untuk penyingkiran diesel di Antartika. Justeru, terdapat peningkatan minat terhadap potensi untuk menggunakan sisa pertanian sebagai biopenyerapan. Tandan kosong kelapa sawit (EFB) yang dihasilkan daripada kelapa sawit dapat menjimatkan kos dan mengandungi bahan berkaitan selulosa yang telah menunjukkan hasil yang baik dalam percubaan proses rawatan pencemaran. Secara selari, kajian tentang potensi pemulihan pencemaran diesel di Antartika menggunakan mikroalga asli masih terhad sehingga kini, manakala keadaan optimum yang diperlukan masih belum dapat ditentukan. Matlamat utama kajian ini adalah untuk menggabungkan penilaian keupayaan penyerapan diesel oleh EFB dan keupayaan mengurangkan diesel mikroalga asli Antartika dalam sistem penapis-pengeraman yang direka untuk menyingkirkan diesel dan menyumbang kepada usaha biopemulihan yang berpotensi di Antartika. Dalam kajian ini, EFB telah disaring dengan menggunakan analisis konvensional, satu-faktor-pada-satu-masa (OFAT) dan analisis statistik, kaedah permukaan tindak balas (RSM), di mana kesan EFB ke atas penyerapan diesel telah dicirikan. Keadaan EFB optimum untuk penyerapan diesel ditentukan dengan suhu 172°C dan 19.4 min

masa pemanasan, ketumpatan pembungkusan 0.096 g/cm<sup>3</sup> dan kepekatan awal diesel sebanyak 10%. Model yang diramalkan adalah sangat ketara ( $R^2 = 0.9987$ ) dan empat parameter, antara suhu dan ketumpatan pembungkusan, suhu dan kepekatan diesel, masa dan ketumpatan pembungkusan, dan ketumpatan pembungkusan dan kepekatan diesel, telah disahkan memberi kesan ketara kepada penyerapan diesel. Dengan menggunakan EFB yang dirawat dengan pembakaran, 25.33 mL minyak berjaya diserap daripada isipadu awal 40 mL. Sampel EFB telah tertakluk kepada analisis kandungan morfologi dan kimia yang berkaitan dengan penyerapan diesel. Untuk bioremediasi hidrokarbon diesel, mikroalga Antartika (strain WCY\_AQ5\_2) telah disaring, dikenal pasti dan dicirikan menggunakan analisis OFAT dan RSM. Keadaan degradasi optimum ialah dengan 1% kepekatan diesel, 11.17 mg/L kepekatan NaCl, pH 6.22, 0.415 g/L NaNO<sub>3</sub> dan tempoh fotokala 12:12 h L/D. Model yang diramalkan adalah sangat ketara ( $R^2 = 0.9848$ ) dan mengesahkan bahawa parameter kemasinan dan pH mempengaruhi biodegradasi diesel dengan ketara. Biodegradasi diesel sebanyak 66.08% telah dicapai dalam tempoh penderaman 7 hari. Pemeriksaan morfologi dan molekul menyokong bahawa strain WCY\_AQ5\_2 mewakili spesies *Chlorella vulgaris*. Kesan pencemaran logam berat (Ag, Al, As, Cd, Co, Cr, Cu, Ni, Pb dan Zn) terhadap degradasi diesel dinilai dan dianalisis dengan menilai kepekatan perencatan separuh maksimum (IC<sub>50</sub>). Penemuan menunjukkan bahawa Cr, Cu dan Ag menghalang degradasi diesel lebih daripada 50%, dengan nilai IC<sub>50</sub> sebanyak 3.165, 3.371 dan 4.205 ppm. Penemuan ini kemudiannya disepadukan ke dalam pembangunan sistem penderaman penapis yang menggunakan EFB sebagai penyerap dengan ketumpatan pembungkusan optimum (0.1 g/cm<sup>3</sup>) dalam mesin penekan penapis bersama kombinasi dengan strain mikroalga Antartika asli WCY\_AQ5\_2 di dalam tangki penderaman cahaya yang diterangi dengan lampu merah optimum yang meningkatkan keupayaan alga untuk mengurangkan diesel sebanyak 70.7%. Kebaharuan kajian ini adalah dengan penggunaan agen bioremediasi, EFB sebagai biopenyeras dan mikroalga Antartika asli sebagai agen penguraian diesel yang disepadukan dalam sistem penapis-penderaman untuk penyingkiran diesel. Penemuan kajian ini membantu memajukan teknik penyerapan dan bioremediasi diesel yang kini tersedia di Antartika.

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## LIST OF ABBREVIATIONS

$\beta$	Beta
%	Percentage
$^{\circ}\text{C}$	Degree celsius
<	Less than
$\pm$	Plus–minus sign
$\leq$	Equal or less than
$\mu$	Specific growth rate
$\mu\text{A}$	Micro-Ampere
$\mu\text{L}$	Microlitre
$\mu\text{M}$	Micromolar
18S rRNA	18S ribosomal nucleic acid
3D	Three-dimensional
$(\text{NH}_4)_2\text{SO}_4$	Ammonium sulphate
$(\text{NH}_4)_2\text{CO}_3$	Ammonium carbonate
Ag	Silver
Al	Aluminium
ANZ	Antarctic New Zealand
ANOVA	Analysis of variance
As	Arsenic
ATP	Adenosine triphosphate
ATS	Antarctic Treaty System
BAS	British Antarctic Survey
BE	Biodegradation efficiency
BLAST	Basic Local Alignment Search Tool

BLASTN	Basic Local Alignment Search Tool: Nucleotide
BOD	Biological oxygen demand
bp	Base pair
C	Carbon
Car	Carotenoids
C-H	Alkyl group
C-O	Carboxyl group
C-O-C	Ether
C=C	Alkene group
C=O	Carbonyl group
C≡C	Alkynes group
CaCl <sub>2</sub>	Calcium chloride
CCD	Central composite design
Cd	Cadmium
Chl- <i>a</i>	Chlorophyll <i>a</i>
Chl- <i>b</i>	Chlorophyll <i>b</i>
Cl	Chlorine
cm	Centimetre
cm <sup>-1</sup>	Reciprocal centimetre
cm <sup>3</sup>	Cubic centimetre
Co	Cobalt
COD	Chemical oxygen demand
CO <sub>2</sub>	Carbon dioxide
Co(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	Cobalt nitrate
Cr	Chromium
Cu	Copper

CuSO <sub>4</sub> .5H <sub>2</sub> O	Copper(II) sulphate
CYP	Cytochrome P450
d	Day
dH <sub>2</sub> O	Distilled water
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DoE	Design of experiment
e.g	exempli gratia (for example)
ECA	Emission control area
EFB	Empty fruit bunch
EDTA.Na <sub>2</sub>	Ethylenediaminetetracetic acid disodium
EPA	Environmental Protection Agency
et al.	and others
F	Fischer value
FeSO <sub>4</sub>	Ferrous sulphate
FTIR	Fourier transform infrared spectroscopy
g	Gram
gDNA	Genomic deoxyribonucleic acid
h	Hour
HCl	Hydrochloric acid
HFO	Heavy fuel oil
Hg	Mercury
H <sub>3</sub> BO <sub>4</sub>	Hydrogen perborate
IC <sub>50</sub>	Half-maximal inhibitory concentration
IFO 180	Intermediate fuel oil
IMO	International Maritime Organisation

Inc.	Incorporated
kb	Kilobase
KCl	Potassium chloride
kg	Kilogram
$\text{KH}_2\text{PO}_4$	Potassium dihydrogen phosphate
$\text{K}_2\text{HPO}_4$	Dipotassium hydrogen phosphate
kJ	KiloJoule
km	Kilometre
$\text{KNO}_3$	Pottasium nitrate
KOH	Potassium hydroxide
KOPRI	Korea Polar Research Institute
kV	Kilovolt
L	Litre
L/D	Light and dark cycle
m	Metre
M	Molar
MARPOL	International Convention for the Prevention of Pollution from Ships
MFF	Mesocarp fruit fibres
Mg	Magnesium
mg	Milligram
MGO	Marine gas oil
$\text{MgSO}_4$	Magnesium sulphate
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	Magnesium sulphate heptahydrate
min	Minute
MJ	Mega-Joule

mL	Millilitre
mm	Millimetre
mM	Millimolar
Mn	Manganese
MnCl <sub>2</sub>	Manganese(II) chloride
Mo	Molybdenum
MoO <sub>3</sub>	Molybdenum trioxide
N	Nitrogen
Na	Sodium
NaCl	Sodium chloride
NaNO <sub>3</sub>	Sodium nitrate
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
NH <sub>4</sub> <sup>+</sup>	Ammonium
NH <sub>4</sub> Cl	Ammonium chloride
NH <sub>4</sub> NO <sub>3</sub>	Ammonium nitrate
Ni	Nickel
nm	Nanometre
NO <sub>x</sub>	Nitrogen oxide
NO <sub>3</sub> <sup>-</sup>	Nitrate
O	Oxygen
O-H	Hydroxyl
OD <sub>620</sub>	Optical density at 620 nm
OFAT	One-factor-at-a-time
P	Phosphate
<i>P</i>	Probability value

PacBio	Pacific Biosciences
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PBD	Plackett-Burman design
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pH	$-\log H^+$ concentration
PKS	Palm kernel shells
pM	picoMolar
POME	Palm oil mill effluent
POPs	Persistent organic pollutants
ppb	Part per billion
ppm	Part per million
ppt	Part per thousand
PVC	Polyvinyl chloride
R <sup>2</sup>	Coefficient determination
RNA	Ribonucleic acid
RNase A	Ribonuclease A
ROS	Reactive oxygen species
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RSM	Response surface methodology
SAB	Special Antarctic Blend
Se	Selenium
SC	Sorption capacity
sec	Second

SEM	Scanning electron microscopy
SO <sub>x</sub>	Sulphur oxide
sp.	Species (singular)
spp.	Species (plural)
TAE	Tris-Acetate-EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
TN93+G	Tamura-Nei with a gamma-distributed model
TPH	Total petroleum hydrocarbon
UV	Ultra-violet
v	Volume
ver.	Version
WAF	Water accommodated fraction
YPASM	Sultan Mizan Antarctic Research Foundation
Zn	Zinc
ZnSO <sub>4</sub>	Zinc sulphate

## CHAPTER 1

### INTRODUCTION

Antarctica is the world's most remote continent, located in the southernmost part of the globe. It is a popular target for researchers, explorers and travellers owing to its extreme and exceptional environments. Between 2012–2013 and 2019–2020, the numbers of tourist visitors to Antarctica increased by 52% (Kariminia et al., 2013; IAATO, 2020), before the hiatus created by the global COVID-19 pandemic (Hughes and Convey, 2020), but recovered rapidly to a new record of over 100,000 in the 2022/23 season (Antarctic Treaty Consultative Meeting, 2022). As a result, the demand for diesel in Antarctica has also increased to provide fuel for vehicles and electricity generation in research stations and other activities concentrated (Ruberto et al., 2005; Brown et al., 2023). Large quantities of fuel are transported across the Southern Ocean to facilitate activities in Antarctica (Australian Antarctic Division, 2012), raising the danger of contamination from oil spill incidents (Errington et al., 2018). Accidental oil leaks can be more serious in Antarctica's severe environments, since there can often be no containment of the spilled fuel, making cleaning more challenging. As the rate of natural degradation is reduced under the severe environmental conditions, oil-derived pollutants are challenging to eliminate (Brown et al., 2016; Zakaria et al., 2019a; Abdulrasheed et al., 2020a; Zahri et al., 2020).

Diesel fuel formulations used in Antarctica are adapted to the continent's extreme temperatures. Larger vessels in Antarctica are fueled by Intermediate Grade Fuel Oil (IFO 180) and Marine Gas Oil (MGO) (Wong et al., 2021). Special Antarctic Blend (SAB), a cold-climate diesel formulation with low density and viscosity, is commonly used in Antarctic and sub-Antarctic regions to avoid wax formation (Lim et al., 2017; Bennett, 2014). The composition of hydrocarbons in SAB, MGO and IFO 180 differ, resulting in varying levels of environmental toxicity. Depending on the hydrocarbon composition, diesel can have varied water-accommodated fraction (WAF) characteristics when interacting with water (Brown et al., 2016). The bioavailability of WAF to marine organisms determines how an oil spill will affect them. According to Lim et al. (2021), SAB diesel has the largest magnitude of acute toxicity after brief exposure, although MGO and IFO 180 are typically more hazardous than SAB following extended exposure. These risks have become more prominent in recent years as on average two million litres of SAB and MGO are consumed annually per major facility, sustaining over 100 stations in Antarctica (IAATO, 2020).

Pollution from diesel presents an ongoing threat to the ecosystems of Antarctica. The species involved, level of exposure, as well as types of oil, have distinct impacts on how severe the consequences of any oil spill are. Seabirds from the Haswell archipelago, including Adélie penguins, south polar skuas, brown skuas and Wilson's storm petrels were reported to have oil contamination on their plumage and legs following an oil spill (Golubev, 2021). Birds with light oil contamination need to work harder to fly owing to the plumage's reduced lift and

thrust, which makes them more prone to stress injury and limits their ability to search for food, while heavily oiled birds are immobilised because of plumage collapse (King et al., 2021). Due to their higher metabolic rate, oil-coated penguins exhibit a considerable reduction in mass, with serious implications for survival (Culik et al., 1991). Surface-dwelling animals and birds may be killed when exposed to an oil spill. The challenges of pollution in Antarctica is also a global concern since it also holds the largest freshwater reserve in the globe (90%) and plays significant roles in the control of global climate and ocean circulation (Turner et al., 2009; Convey and Peck, 2019; Subramaniam et al., 2020). Hence, human intervention is required in these areas to accelerate the remediation process of polluted sites.

Heavy metals are present naturally in the environment but are also released through human activities. In Antarctica, high levels of heavy metals have been detected in biotic samples from terrestrial and aquatic organisms, as well as in abiotic samples from soil, snow and the atmosphere (Chu et al., 2019a). Parts of the northern Antarctic Peninsula and South Shetland Islands, such as Deception Island, host areas of volcanic activity that can naturally release heavy metals (De Ferro et al., 2014). Anthropogenic sources such as sewage, mining, oil spills, abandoned dump sites and pesticides can also pollute the environment with heavy metals. Evidence of heavy metal contamination has been reported around Russian Antarctic stations due to human activities (Aleksseev and Abakumov, 2020). Increased levels of copper (Cu), lead (Pb) and zinc (Zn) have been detected at an abandoned waste dumping site in Antarctica (Snape et al., 2001). Studies have also shown that heavy metals can travel large distances through the atmosphere and arrive in Antarctica (Bargagli, 2008). Heavy metals present in contaminated sites can influence the bioremediation of other contaminants (Tengku-Mazuki et al., 2020; Zakaria et al., 2020; Zahri et al., 2021b). Heavy metal co-contamination is investigated in order to assess the effects of heavy metals on bioremediation processes. This co-contamination could both hinder or accelerate the process rate depending on the resistance of microorganisms to heavy metals at particular concentrations.

Bioremediation is an important tool for environmental clean-up that uses biological mechanisms to reduce pollutant concentrations. In principle, bioremediation can be maximised by precisely controlling abiotic factors such as temperature, pH, medium formulation and substrate concentration (Lee et al., 2018; Sharma, 2021). Biodegradation can be more dependable, cost-saving and environmentally friendly than other currently more widely used chemical or physical approaches (Malla et al., 2018; Yadav et al., 2022). Pollutants can be converted into biodegradable compounds, improving ecosystem long-term viability in the affected areas. Recently, there has been increasing interest in using agricultural biosorbents that are cheap, effective, non-toxic and easily available (Zamparas et al., 2020). The potential use of this biosorbent technology presents an opportunity for increased efficiency of oil removal from water without additional contamination. The use of empty fruit bunch (EFB) agricultural waste as a biosorbent is promising owing to its efficient disposal and cost-effectiveness (Abdullah and Sulaiman, 2013a).

In parallel, microalgae can remove some pollutants by integrating them into their metabolism (Markou and Georgakakis, 2011). For instance, some microalgae have the ability to progressively break off the rings in polycyclic aromatic hydrocarbons (PAHs), leading to conversion to non-toxic products, ultimately carbon dioxide. Given this background, the development of a combination such as an EFB filter-incubation system approach supplemented with diesel removal by Antarctic microalgae has become more viable (Markou and Georgakakis, 2011; Jahi et al., 2020). This is in accordance with the Antarctic Treaty's requirements, which prohibit the introduction of non-local microorganisms to the continent to ensure the protection of the Antarctic environment (Jesus et al., 2015), meaning that any bioremediation effort in Antarctica must use native microorganisms to reduce the chance of further adverse environmental impacts. The current approaches to address oil pollution in Antarctica use biological resources, such as bacteria. Compared to utilising bacteria, employing microalgae species for diesel degradation is more favourable since microalgae biomass potentially be reused as a filter, biofuel, and feedstock.

The importance of studying hydrocarbon degradation in Antarctic ecosystems has been widely recognised although, as yet, there has been very limited research addressing how local microalgal species respond to the presence of hydrocarbons and heavy metals. Consequently, adopting the microalgal incubation tank approach to support bioremediation may be advantageous since it provides microalgae with a regulated and contained environment to carry out the bioremediation process (Yusoff et al., 2019). A large-scale light-incubation tank may increase the efficiency of bioremediation when combined with the biosorption technology of the filter press machine. This is consistent with earlier research that found that employing physical filtration and bioremediation technologies together increased the effectiveness of cleaning up oil spills in Antarctica (Cappello et al., 2014). Even though synthetic sorbents are currently commonly used, their disposal can be challenging. To develop a synergistic strategy, first, remove diesel from the environment and then undergo biodegradation, the deployment of an eco-friendly biosorbent employing EFB in a filter press machine paired with a light-incubation tank technique is examined in this study. The novelty of this study is the use of the bioremediatory agents, EFB as biosorbent and native Antarctic microalgal as diesel degrader integrated in a novel filter-incubation system for diesel removal. The eventual implementation of a comprehensive strategy to deal with oil spills could be facilitated by the development of such a system. Hence, the findings of this study will advance the currently-available diesel biosorption and bioremediation approaches available in Antarctica.

This study's primary goal is to evaluate the diesel-sorption capabilities of EFB and the diesel-degrading capabilities of a native Antarctic microalgal in a filter-incubation system intended to enable diesel removal and bioremediation in Antarctica. The study was implemented with four objectives:

1. To screen and characterise the effects of EFB with different pretreatments on diesel biosorption.

2. To screen and identify an effective diesel-degrading native Antarctic microalgal.
3. To investigate the effects of co-contamination with selected heavy metals on microalgal diesel degradation.
4. To assess the most effective EFB biosorbent and microalgal in a newly-designed filter-incubation system for diesel degradation.



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