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Biosorption of heavy metals using permeable pavement biofilms
Quantification, optimization, modelling and sustainability evaluation

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Biosorption of heavy metals using permeable pavement biofilms: Quantification, modelling, optimization and sustainability evaluation

By

Alireza Fathollahi

A thesis submitted in partial fulfilment of the requirements of Coventry University
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Abstract

The present critical overview document is comprised of a background, methodological approach and discussion of a portfolio of peer reviewed journal publications, on the remediation of contaminated urban runoff containing soluble heavy metals by permeable pavement living biofilms. The biosorption efficiencies of different bacterial strains for heavy metals, were evaluated using a meta-analysis technique. The experimental biosorption studies were carried out to examine the equilibrium, thermodynamics and kinetics of heavy metals removal, using permeable pavement living biofilms, incubated on a nonwoven polypropylene and polyethylene geotextile. In sustainability studies, Life Cycle Assessment and Life Cycle Costing methodologies were used to investigate the life cycle impacts of permeable pavements and compare the results to alternative drainage systems. The results of the meta-analysis study, revealed that Firmicute phyla had the highest overall (living and dead) biosorption efficiencies for heavy metals. Living bacterial biomass was more efficient in biosorption of Cu(II), Zn(II) and Pb(II) than dead biomass. High temperatures (>35 °C) were less efficient in Cu(II) and Zn(II) removal and the maximum biosorption efficiency for non-essential heavy metals occurred at short contact times (<2 h). With 20 mg L^{-1} initial concentration of Hg (II), living permeable pavement biofilms with incubation times of 1, 7, 14, 21 and 28 days, showed biosorption efficiencies of 55.72, 67.12, 61.37, 62.57 and 65.38%, respectively. Thermodynamic and kinetic modelling of data revealed that a physical, feasible, spontaneous and exothermic process controlled the biosorption, with a diffusion process observed in external layers of the biofilm, fitting a pseudo-second order kinetic model. Results of sustainability studies showed that permeable pavements had lower life cycle impacts on human health than kerb and gully, infiltration basin and infiltration trench systems. Transportation of materials and construction civil works had large contributions in life cycle inventories and associated environmental impacts of the drainage systems.

Keywords: *Bacteria, Heavy metal, Meta-analysis, Biosorption, Kinetic, Permeable Pavement, Biofilm, Thermodynamics, LCA, LCC.*

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Chapter One

Introduction

1.1. Background

The present critical overview document presents a portfolio of peer reviewed journal publications on the under-investigated role of permeable pavement biofilms in the uptake of soluble heavy metals in urban runoff. The author of this manuscript believes that the selected portfolio of research outputs makes an original significant contribution to the field of contaminated water treatment and advances research into sustainable drainage systems (SuDS).

The rapid urbanization seen across the world in recent decades has resulted in a significant increase in impervious land ratio in urban areas (McGrane, 2016). The increased impervious surfaces caused by the construction of buildings and roads has reformed the natural flow pathways of rain and stormwater (Walsh et al., 2012). The alteration in waterflow paths has led to accumulation of various organic and inorganic compounds including heavy metals and hydrocarbons (Ali et al., 2019). In addition to the vehicular sources of contaminations, leached out compounds from pavement surfaces and atmospheric pollutants that have been accumulated on the urban roads, are collected by runoff flows during rainfall events and conveyed to the receiving systems (Muller et al., 2020). Major vehicular sources of urban runoff contamination are presented in Table 1.1. The contaminated water eventually finds its way to waterbodies and underground water reservoirs triggering serious negative effects on the environmental and ecological components (Bhateria and Jain, 2016).

Table 1.1. Vehicular sources of contamination to roads (Fathollahi et al., 2020).

Source	Contaminant
Vehicle use	Hydrocarbons
Exhaust gases and particulates	PAHs, NOx, Ni, BTEX
Catalytic converters	Rh, Pd, Pt
Tyre wear	TSS, Cd, Cu, Zn, PAHs, microplastics
Wear of tyre studs	TSS, Cd, Cu, Ni, Pb, Sb, Zn, PAHs
Brake wear	Cr, Ni
Engine and vehicle body wear	Pb
Bodywork paint	Pb, Fe (steel), Zn
Wheel balancing weights Vehicle washing	Pb, Cd, Cr, Zn, phthalates, NPs, NPEOs
Road abrasion	TSS, PAHs, microplastics

BTEX: benzene series, NPEOs: nonylphenol ethoxylates, NPs: nonylphenols; TSS: total suspended solids.

Heavy metals are reported to accumulate in higher rates than other chemicals in the environment, with a possible pathway to the food chain causing negative effects on human health (Tchounwou et al., 2012). A list of priority pollutants in water systems and their associated toxic thresholds for receiving waters, are provided by EU Water Framework Directive (Directive 2000/60/EC) and priority pollutant directive (Directive 2013/39/EU), to mitigate the negative effects of water and environmental contamination by diffuse pollution. According to previous studies, the concentrations of heavy metals in urban runoff exceeds the EU Water Framework Directive guideline limitations (Wiering et al., 2020). Therefore, urban runoff is considered as a significant source of heavy metal diffuse pollution (Sakson et al., 2018).

In recent years, Sustainable Drainage System (SuDS) have been used to manage the urban stormwater as quickly as possible with the minimum environmental impacts to design the urban pavements and drainage systems to function as similarly as possible to the natural conditions before urban development (Charlesworth et al., 2003). SuDS devices are designed to reduce the amount of produced runoff and to control water pollution. Previous studies have shown that bioretention systems such as SuDS devices use physical, chemical and biological processes including filtration, sedimentation and biosorption to remediate contaminated runoff and increase the water quality (Fathollahi et al., 2020). However, the underlying science of the bioremediation occurring withing SuDS devices, such as permeable pavements, have been under investigated. The present thesis has begun to unravel some of the unknown bioremediation processes, including biosorption of heavy metals taking place in SuDS.

1.2. Research Context

This work is based on a novel research project supported by the European Union through the Marie Sklodowska-Curie grant No 765057, project name SAFERUP. This project aimed to provide the European community with innovative solutions to form the urban paved environment of the future. Footpaths, bike lanes, roads, intersections, squares and all other walkable surfaces that are used every day were designed, constructed and managed to meet context-sensitive criteria including not only safety, mobility and costs (construction and maintenance), but also sustainability, environmental impacts, accessibility, aesthetics, circular economy and local economy preservation. In this project, the author was committed to finding *in-situ* remediation strategies to prevent contamination of water by the use of new materials in landscaping. Many new innovative paving materials use nanoparticles, metals (e.g., for heat conductive frost resistant paving) microplastics and admixtures for increasing recycled content. The environmental risk from these materials being mobilised in runoff must be understood and the need to design-in countermeasures to prevent non-point source pollution, installing source control, is great.

In this portfolio of research outputs, author have examined a long-neglected area of stormwater management, the underlying science behind the removal of soluble metals by biological agents in the form of bacterial biofilms. Stormwater management papers that focus on soluble metals do not consider the contribution of microbes to possible biosorption of contaminants. Equally, biosorption papers routinely use recycled organic material or non-living microbial biomass to remove soluble metals rather than intact, membrane grown bacterial biofilms that can remove large amounts of heavy metal concentrations as shown in the presented work. Results from the project have demonstrated some key scientific principles including the benefits of using live rather than dead microbial biofilms for soluble heavy metal removal and the robustness of mature bacterial biofilms subjected to concentrations of metals as high as 200 mg L^{-1}, showing less than 0.1 order of magnitude decrease in numbers. Moreover, the underlying physical, chemical and biological mechanisms of biosorption for heavy metals including physisorption with a physical process which is spontaneous, feasible and exothermic under the present research experimental conditions and the speed of assembly of a strongly biosorbent bacterial biofilm have been evaluated.

The present work has opened a new area of research to investigate the specific role of microbes in the removal of metals by stormwater systems, by focusing on the densities of bacteria, their survival, their speed of accumulation and their efficacy at different pH conditions. The method to investigate the biological, physical and chemical processes controlling the metal removal by a living bacterial system is novel, original and has long been missing in the literature.

In order to advance this study, the author assembled evidence from the existing literature on the biological and environmental variables surrounding biosorption. The goal was to reveal the way the likes of pH, metal concentration and microbial biofilms affect the efficiency with which bacteria adsorb cadmium, copper, lead and zinc. This was done by a justified, methodological and scientifically credible method, known as meta-analysis.

The use of meta-analysis is a robust way to examine existing literature in a new way, pursuing a different goal in the relevant scientific field. Meta-analysis is a trusted and valued method for background studies and focusing on existing evidence in the biomedical sciences, typically to assist researchers in evaluating the strength of a case for a new health intervention. Although meta-analysis is not common in the chemical sciences, the author believes that this work has added significant relevant information to an emerging environmental remediation methodology. The author also believes this work has shown that meta-analysis is a viable technique for re-evaluation of historic data in this field.

The lack of a meta-analysis in the biosorption field of research, motivated the author to carry out the current study to identify beneficial bacterial strains and optimum operational conditions, reported in the past decade. The meta-analysis will help researchers and practitioners to choose the optimum operational conditions and the most beneficial bacterial phyla, to achieve the highest biosorption efficiency for heavy metals. Researchers hoping to perform primary investigation on biosorption, will be able to consult this paper to orientate their studies, in terms of what conditions will bring most success to their investigations. This study brings a new and informative angle to the science of biosorption of metals for application to diffuse pollution.

Costs and environmental impacts of permeable pavement were carried out using Life Cycle Costing (LCC) and Life Cycle Assessment (LCA) methodologies. LCA and LCC studies help to evaluate the sustainability of heavy metal removal using living biofilms within the structure of permeable pavement and compare the results with other common drainage systems, including filter drains, swales and wetlands. This holistic approach will help the decision makers to choose a drainage system with the lowest environmental and cost impacts, considering the role of permeable pavement biofilms in remediation of contaminated runoff.

The present sustainability study has a focus on a more complete analysis of a number of aspects of drainage asset performance, including ongoing maintenance, water quality and carbon release aspects. Concrete use is also responsible for making drainage options, even those described as sustainable devices, more impactful on receiving environments due to emissions. The results contained are inherently transferrable, being relevant to future water management strategies and leading to more effective budgeting and with a degree of extra capacity planning for future weather and climate.

1.3. Research objectives

Previous studies on removal of heavy metals using biosorbents were mainly focused on using non-living powdered biomasses developed by a single species culture of bacteria, fungi or algae. There were limited studies in the literature that investigated the efficiencies of living microbial biofilms in removal of heavy metals from contaminated water and compare the results with non-living biosorbents. The lack of such studies was more noticeable in the area of metal biosorption in storm water treatment devices, including permeable pavement systems (PPS) with no study recorded on efficiency of living biofilms in biosorption of heavy metals from contaminated runoff. Therefore, the papers included in the present critical overview document were focused on developing a mixed culture living biofilm on a conventional geotextile widely used in PPS for biosorption of heavy metals from contaminated runoff. The biological, physical and chemical processes involved in the heavy metal biosorption by the developed biofilms in sustainable drainage system (SuDS) devices, were investigated using mathematical modelling of the biosorption data to achieve an in-depth understanding of the removal mechanisms. Additionally, the effect of different factors including pH, contact time, initial metal concentration, temperature, biomass dosage and metal type on the biosorption efficiencies, were aimed to be evaluated. Therefore, the overall aims and objectives of the present work were:

- To understand the efficiencies of living and non-living bacterial biosorbents in biosorption of different heavy metals using meta-analysis.
- To identify the optimal biosorption conditions and biosorbents for removal of heavy metals from contaminated runoff.
- To examine the effects of factors including pH, temperature and concentrations of heavy metals on the development of bacterial biofilms.
- To assess the effect nutritional and environmental conditions on the bacterial attachment and biofilm formation.
- To propose and carry out a comprehensive LCA and LCC study to evaluate sustainability of contaminated runoff remediation using living permeable pavement biofilms.

1.4. Portfolio outline

This portfolio consists of 5 peer-reviewed Q1 journal papers and is organized as presented in Table 1.2. The papers in this portfolio are selected based on their novelty and significant contribution to knowledge. The publications in the portfolio present a coherent body of work based on a research project that the author has been the leading researcher of, supported by the European Union through the Marie Sklodowska-Curie grant No 765057, project name SAFERUP! This critical overview document aims to link the 5 selected publications using 5 main themes on biosorption of heavy metals using permeable pavement biofilms: chemical evaluation, biological evaluation, sustainability evaluation, modelling and optimization of the process. Figure 1.1 presents the coherence and interrelationship between the publications included in the portfolio.

The present chapter provides a general introduction to road drainage systems, sources and scales of urban runoff contamination and the context of this research. Chapter two of this critical overview document discusses the fundamental theories of 5 main themes of the papers in portfolio. Chapter three includes the methodologies used in the research and presents an evaluation of the adequacy of methods and controls. Chapter four includes a discussion of important findings of each theme. Chapter 5 of this document argues the originality and novelty of the research findings to highlight the significant contribution to knowledge. Chapter 6 consists of a conclusion, opportunities for future research and the claim for this work being at the required standard for a PhD.

In paper number 1 of the portfolio, the effect of temperature, contact time, initial metal concentration, biomass dosage, pH, bacterial state, heavy metal type and bacterial phyla on the biosorption of heavy metals using living and non-living bacterial biomass were investigated using statistical methods such as meta-analysis. Paper number 2 includes the detailed analysis of living biofilms efficiencies in the removal of heavy metals from contaminated water within the structure of drainage systems, for which their sustainability was evaluated in paper number 5. The chemical, isothermal, biological and physical processes involved in the heavy metal removal using living biofilms were evaluated in paper number 3. The effects of nutritional and environmental conditions on living biofilms development and contaminated runoff remediation were presented in paper number 4. In paper number 5, the environmental and cost impacts of SuDS devices were carried out using LCC and LCA methodologies. LCA and LCC studies help to evaluate the sustainability of heavy metal removal using living biofilms within the structure road drainage systems.

Table 1.2. Portfolio of research outputs.

Output No	Citation detail	Journal*
1	**Fathollahi, A.**, Khasteganan, N., Coupe, S.J., Newman, A.P., 2021. A meta-analysis of metal biosorption by suspended bacteria from three phyla. Chemosphere. 268.	Chemosphere (impact factor: 7.086 ; h-index:248)
2	**Fathollahi, A.**, Coupe, S.J., El-Sheikh, A.H., Sañudo-Fontaneda, L.A., 2020. The biosorption of mercury by permeable pavement biofilms in stormwater attenuation. Sci. Total Environ. 741.	Science of the Total Environmental (impact factor: 8.013 ; h-index:244)
3	**Fathollahi, A.**, Coupe, S.J., El-Sheikh, A.H., Nnadi, E., 2021. Cu(II) Biosorption by Living Biofilms: Isothermal, Chemical, Physical and Biological Evaluation. Journal of Environmental Management. 282.	Journal of Environmental Management (impact factor: 6.789 ; h-index:179)
4	**Fathollahi, A.,** Coupe, S.J., 2021. Effect of environmental and nutritional conditions on the formation of single and mixed-species biofilms and their efficiency in cadmium removal. Chemosphere. 283.	Chemosphere (impact factor: 7.086 ; h-index:248)
5	**Fathollahi, A.**, Coupe, S.J., 2021. Life cycle assessment and life cycle costing of road drainage systems for sustainability evaluation: Quantifying the contribution of different life cycle phases. Sci. Total Environ. 776. https://doi.org/10.1016/j.scitotenv.2021.145937	Science of the Total Environmental (impact factor: 8.013 ; h-index:244)

*Journal statistics date: Jan 2022

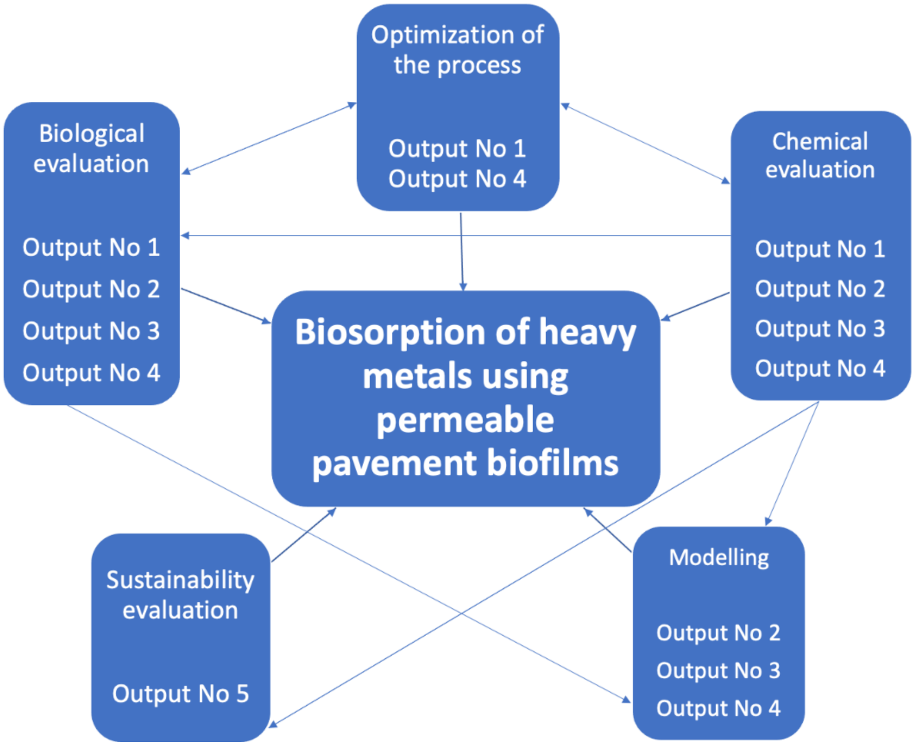

Figure 1.1. Coherence and interrelationship between the publications included in the portfolio.

1.5. Collaborative Work

The candidate was the first and corresponding author of all the papers selected in this portfolio. Conceptualisation and methodology of the experimental phases was achieved by working with the director of studies, with laboratory effort performed by the candidate. The candidate led the analysis of results with support from DOS and co-authors and produced the first draft of papers before working with co-authors to edit before final submission by the candidate. Formal letters from DOS and co-authors are included in the appendices stating their contributions and the leading role of the candidate in research resulting in papers.

Chapter Two

Biosorption of Heavy Metals from Aqueous Solution

2.1. Biosorption

Biosorption is a metabolic passive physicochemical process of chemical compounds binding to a biosorbent surface. The biosorbent is of biological origin, such as microorganisms and is used to adsorb or absorb target pollutants including metal ions (Torres, 2020). The biosorption process depends on the affinity of the biosorbent cells to the metal species, using mechanisms such as adsorption, absorption, precipitation, complexation and ion exchange (Redha, 2020). In one of most important biosorption processes, target metal species are adsorbed to the functional groups available on the extracellular polymeric substances (EPS) on the surface of the biosorbent (Wei et al., 2016). The metal binding to the EPS layer occurs through various routes depending on the type of available functional groups. For instance, when charged carboxyl, phosphate and sulphate groups are available on the surface of the biosorbent, an electrostatic interaction bonds the metal species to the functional groups (Saravanan and Kumar, 2020). Anionic functional groups of the membrane lipids and proteins are other biosorbent agents for binding with chemical compounds (Gupta and Diwan, 2017).

The present study aimed to understand the efficiency of living biofilms as biosorbent in the removal of heavy metals from contaminated water. Biofilm is an assemblage of surface-associated microbial cells that is enclosed in an EPS matrix (Donlan, 2002). Biofilm is made of different living micro-organisms, including bacteria, algae, fungi and communities of protists evolving and growing as a collective (Muhammad et al., 2020). Micro-organisms in the biofilm are protected from adverse environmental factors such as temperature, pH, toxic chemical compounds and immune response by the EPS layer (Yin et al., 2019). The EPS layer of biofilms can act an as active binding sites for the attachment of heavy metal ions and consequently, their removal from contaminated water.

The main advantages of biosorbents are their low cost, less chemical release and sludge formation, simple operational stages and higher metal adsorption capacities in comparison with other adsorbents, such as activated carbons and ion-exchange resins (De Gisi et al., 2016). These advantages of the biosorption technique have made this technology attractive for researchers and industry. As a result, various types of biosorbents have been studied and used for the polluted water and wastewater remediation (Munoz et al., 2015; Mwandira et al., 2020; Ghaed et al., 2013). Bacteria, algae and fungal derived biomasses are amongst the most popular biosorbents for removing heavy metal ions from contaminated water (Vahabisani and An, 2021).

2.1.1. Algal biosorbents

Algae species are classified as two autotrophic categories of microalgae and macroalgae. Microalgae are unicellular species that use photosynthesis and grow in fresh and salt water (Khan et al., 2018). Diatoms, blue algae, green algae and golden algae are four main microalgae groups. Macroalgae are multicellular species including brown, red and green algae. All three groups of macroalgae species have optimum growth in fresh or salt water (Singh and Singh, 2015). Algal biomass can increase in low nutritional media which lowers the costs of biosorbent production (Randrianarison and Ashraf, 2017). In addition to low industrial costs, algal biomasses provide high adsorption capacities. Minimal toxic substances are produced during the biosorbent development (Kumar et al., 2020). The high metal uptake capacities of algae biomasses are due to the structure of cell walls consisting of chitin, polysaccharides, proteins and lipids (Spain et al., 2021). The composition of algae cell walls provides active binding sites including sulfhydryl, hydroxyl, phosphate, sulfate, imidazole, amine and carboxyl functional groups (Bilal et al., 2018; Spain et al., 2021).

The biosorption of metals by algal biomasses mainly consists of ion exchange and complexation mechanisms (Redha et al., 2020). Studies have reported that brown algae species have the highest metal biosorption capacities (Pennesi et al., 2012). The availability, low price, limited sludge production and high surface to volume ratio, has made brown algae a favourite biosorbent for industry (Mohsenpour et al., 2021). Researchers have studied metal biosorption capacities for various algal biomasses. *Sargassum sp.* (brown algae), *Cladophora fascicularis* and *Spirogyra hyaline* biomasses have shown mercury biosorption capacities of 14.8, 20.0 and 39.2 mg L^{-1}, respectively (Subhashini et al., 2011; Viraraghavan and Srinivasan, 2011). Algal biomasses have also shown high biosorption capacities for copper ions: *Calotropis procera* (14.5 mg L^{-1}), Sargassum sp. (18.6 mg L^{-1}) and Ascophyllum nodosum (70.9 mg L^{-1}) (Lodeiro et al., 2002; Subhashini et al., 2011; Kumar et al., 2012). High cadmium biosorption capacities of 95.0 and 114.9 mg L^{-1} have been reported for *Bifurcaria bifurcate* and *Ascophyllum nodosum*, respectively (Gin et al., 2002; Kumar et al., 2012).

2.1.2. Fungal biosorbents

Fungi are eukaryotic organisms including moulds, yeasts and mushrooms with cell structures consisting of functional groups proper for biosorption processes in both living and non-living states.

Fungi biomasses adsorb the metal species using two main mechanisms of active or passive uptake. In the active uptake process, fungi cells accumulate target metal species in the intercellular matrix (Bellion et al., 2006). The active uptake mechanism of biosorption by fungi-based biomasses is only associated with living cells. However, the passive uptake mechanism of fungi biomasses, consists of interaction between metal ions and active binding sites such as functional groups existing on fungi cells outer layer and does not involve cell metabolism (Dhankhar and Hooda, 2011).

Fungal biomass can be cultivated at large scale using a simple fermentation process with low costs, which makes fungal biosorbents attractive for industries (Dhankhar and Hooda, 2011). The FTIR analysis of fungal biosorbents have shown that amine and phosphate functional groups are the main agents in the heavy metal binding process (Legorreta-Castañeda et al., 2020). In recent years, numerous researches have approved the high efficiencies of algal biomasses in adsorption of heavy metals. Mamisahebei et al. (2007) reported an As(III) adsorption capacity of 24.5 mg L^{-1} for fungal specie of *Penicillium chrysogenum*. *Aspergillus cristatus, Aspergillus niger* and *Hydrilla verticillata* fungal biosorbents showed adsorption capacities of 23.3, 13.1 and 15.5 mg L^{-1} for cadmium ions, respectively (Martínez-Júarez et al., 2012; Rodriguez et al., 2013). Various experimental investigations have observed high efficiencies of fungal biomasses in bioremediation of other heavy metals such as chromium, copper, nickel, mercury and lead (Tay et al., 2012; Hassan et al., 2012).

2.1.3. Bacterial biosorbents

Bacteria are considered as the most abundant and diverse microorganisms (Zilber-Rosenberg and Rosenberg, 2008). Based on morphology, cocci, rod and spirillum are some the of the most common shapes. Based on cell wall composition and thickness, bacteria are classified as gram positive and gram negative. The cell walls of gram-positive bacteria are thicker and consist of 90% peptidoglycan layers connected with amino acid bridges which results in a higher affinity to metal cations and consequently a higher biosorption capacity for metals (Waldemar et al., 2008). However, gram negative bacteria cell walls consist of just 20% peptidoglycan and the rest of membranes are made of phospholipids and lipopolysaccharides (Silhavy et al., 2010). The peptidoglycan cell layers contain carboxyl, amino, phosphate and sulphate functional groups which provide active binding sites for metal ion chelation (Thomas and Rice, 2014).

The metal uptake by bacterial biomasses generally consists of passive attachment of metal ions to the functional groups available on the bacterial cell walls, followed by active internalization of the ions by cells (Kapahi and Sachdeva, 2019). Studies have shown high metal biosorption capacities for bacterial biomasses. Hemambika et al. (2011) have reported a mercury biosorption capacity of 43.2 mg L^{-1} for bacterial species of *Enterobacter cloacae. Geobacillus thermodenitrificans, Bacillus cereus, Thiobacillus thiooxidans* and *Enterobacter cloacae* have shown copper biosorption capacities of 51.0, 50.3, 39.8 and 78.9 mg L^{-1}, respectively (Samarth et al., 2012; Babak et al., 2012). The cadmium biosorption capacity of *Actinomycetes sp.* was reported as 32.6 mg L^{-1} (Congeevaram et al., 2007).

2.2. Biosorption mechanisms

Biosorbents employ various mechanisms for remediation of heavy metal ions depending on the type, state and classification of biomass (Torres, 2020). In addition, the complex biological processes of most biosorbents have made the biosorption processes difficult to comprehend. The biosorption process is based on two means: i) active cell metabolism which is only associated with living biosorbents, and ii) passive uptake of target metal species by biosorbent cells (Ayangbenro and Babalola, 2017). The active cell metabolism of metals is also known as bioaccumulation. The bioaccumulation process involves an active movement of metal ions through the microorganism cell wall and accumulation in the living cell (Igiri et al., 2018). There are three routes for passive uptake of metal ions by biosorbents, including cell surface adsorption, extracellular and intracellular accumulation (Ayangbenro and Babalola, 2017). Unlike bioaccumulation, passive uptake of metal ions by biosorbent involves processes such as ion exchange, physical adsorption and precipitation (Ayele et al., 2021). The two types of passive metal biosorption have different uptake mechanisms. The extracellular accumulation process involves metal ion transport through a cell membrane. In addition to the type and active or passive uptake process of the biosorbent, the metal speciation and characteristics are also considered as influential factors that regulate the biosorption process (Igiri et al., 2018). Oxidation state, molecular weight and ratio are amongst the target metal characteristics controlling the active and passive uptake by biosorbents (Ayangbenro and Babalola, 2017). Another factor that influences the biosorption process is the type of biosorbent. The type of biosorbent defines the key properties of biomass surface, including forms of functional groups and their availability for metal ions (Redha, 2020). Moreover, type of biosorbent and its associated active binding sites, control the affinity between the biomass cell walls and target metal species (El Seyed and El-Seyed, 2020). The role of factors such as pH, temperature, initial metal concentration and biosorbent dosage on the efficiency of the biosorbent process, will be discussed later in the present document. In summary, biosorption processes involve four mechanisms of ion exchange, physical adsorption, precipitation and complexation.

2.2.1. Ion exchange

Ion exchange is the most important process in most water and wastewater treatment plants. Ion exchange is also utilized in separation techniques in agricultural and medical research. Ion exchange consists of an interchange of ions between a solid phase (biosorbent) and a liquid phase (solution containing metal ions). In other words, ion exchange involves the binary exchange of the target metal with the counter ion available on the surface of biomass. The biosorbents are designed in a way to exchange their ions with targeted metal ions as quickly as possible. In the case of bacterial biosorbents, microorganism cell walls contain polysaccharides which are involved in ion exchange processes with metal ions (Muthu et al., 2017). It has been observed that after the ion exchange process using bacterial biomasses, concentration of some non-target ions such as calcium and magnesium increased, due to the release of exchanged ions in the solution (Ding et al., 2012). Liu et al., (2012) reported the removal of Cu (II), Zn (II), and Pb (II) ions by ion exchange with K^+, Na^+, Mg^+, and Ca^+ using rice straw derived biosorbents.

Ion exchange can take place by anion or cation exchange. The most active functional groups present on the biosorbents surface which participate in the ions exchange processes are carboxyl, phosphate and hydroxyl groups (Bilal et al., 2018). Several studies on biosorbents have reported ion exchange as the primary biosorption mechanism. A study that used *Spirulina* for biosorption of Cr, Cd and Cu ions, reported carboxyl, phosphate and hydroxyl functional groups as ion exchange agents on the biosorbent surface (Chojnacka et al., 2005).

2.2.2. Complexation

Complexation is a process of two or more species forming a complex using covalent bonds or electrostatic attraction, taking place on a functional group, with available electron for donation (Schneckenburger et al., 2018). Mononuclear (monodentate) and Polynuclear (multidentate) are two types of formed complexes. In mononuclear complexes, the target metal specie occupies the central position of complex, formed with the functional group available on the surface of the biosorbent (Dostalek, 2011). However, during formation of the polynuclear complex, two or more metal ions are involved in forming the complex with functional groups (Galan Mascaros et al., 2018). The metal species associated with polynuclear complexes can be neutral or have a positive or negative charge depending on the type and number of functional groups involved in the complexation mechanism. In general, mononuclear complexes have proven to be more efficient than polynuclear complexes in metal ion removal from aqueous solution, since the availability of multiple functional groups may result in multiple metal ion attachment (Pal and Hanan, 2014). Studies have reported that amine, phosphonate hydroxyl and carboxyl functional groups are the most abundant functional groups involved in surface complexation of heavy metal ions (Chakravarty et al., 2012; Hu et al., 2012; Han et al., 2006). The most stable form of complexation mechanism is called chelation, in which the metal specie bonds with the functional groups on the biosorbent surface in more than one position (Flora and Pachauri, 2010). The two most influential factors in the complexation process are the degree of affinity between metal specie and functional group, as well as the availability and accessibility of binding sites for the donation of electrons.

2.2.3. Physical adsorption

The process of interaction between subcritical fluid species and solid phase surface is called physical adsorption or physiosorption. Physical adsorption is the simplest biosorption mechanism which consists of metal species attaching to the biosorbent surface using weak forces of Van der Waals, hydrogen bonding and hydrophobic interactions (Torres et al., 2020). One of the advantages of physical adsorption in comparison with other mechanisms of biosorption, are its simple and low-cost replication into manufacturing process which does not involve sophisticated surface functionalization. However, a lack of special functionalization of the biosorbent surface may result in desorption of functional groups and attached target specie, during or after the biosorption process (Wu et al., 2013). In other words, adsorption is easily implemented but there is a possibility of reversibility during the adsorption process (Horber, 2002). Moreover, physical adsorption mechanisms are affected by the pH of the target specie solution, which can decrease the affinity of metal ions and functional groups on the biosorbent (Lucaci et al., 2020). Physical adsorption normally occurs within a monolayer on the biosorbent surface, which results in a lower biosorption capacity, in comparison to mechanisms with multilayer attachment (Kamga, 2019). Physical adsorption mechanisms using nonbiological molecular sieves such as zeolites and activated carbon have been investigated by researchers and used widely by water and wastewater treatment facilities (Abdel Salam et al., 2011; Karnib et al., 2014).

2.2.4. Precipitation

The precipitation of target metal ions by biosorbent, occurs through the surface of bacterial biomass or transport into the microorganism cells. The latter mechanism occurs only in the metabolism dependent biosorption, but the former process can take place in both metabolism dependent and in independent metal uptake. The metabolism dependent precipitation is a natural mechanism of the microorganisms to protect the cells in harsh environmental conditions including high metal ion concentrations (Rampelotto, 2010). However, the metabolism independent precipitation, is a chemical reaction which involves the affinity and bonding between functional group and target metal ion (Jahanbakht et al., 2014). The main agent involved in the precipitation process of metal ions are functional groups available on the extracellular polymeric substances (EPS) layer of microbial biomass. Many studies have reported precipitation as the main uptake mechanism of metal ions using biosorbents (Farhan and Khadom, 2015; Redha 2020).

2.3. Influential factors on biosorption process

Biosorption processes are influenced by various operational factors including temperature, pH, contact time, biosorbent dosage and initial metal concentration.

2.3.1. Effect of temperature

Temperature has a dual effect on the biosorption process efficiency. Firstly, temperature can change the surface structure of the biosorbent including the active binding site such as functional groups (Fathollahi et al., 2020). As a result, the availability of functional groups and affinity between biosorbent surface and target metal specie, reduces. On the other hand, temperature is an influential factor that controls the thermodynamics and kinetics of the biosorption process. In endothermic biosorption reactions, a decrease in the temperature will result in an increased metal uptake by the biosorbent and the opposite will occur with increasing the temperature (El-Naggar et al., 2018). However, in the case of an exothermic biosorption mechanism, increasing the temperature will decrease the overall metal uptake efficiency. Depending on the type of the biosorbent and target metal specie, the optimum biosorption temperature should be evaluated to achieve maximum interaction between metal ions and biosorbent surface. Previous research has shown that temperatures between 20 to 35℃ provide the optimum biosorption efficiency for most uptake processes (Fadel et al., 2017; Redha 2020).

2.3.2. Effect of pH

Target metal characteristics such as speciation, charge and solubility are affected by the pH of the solution (Duwiejuah et al., 2020). pH also affects the chemistry and activity of binding sites existing on the exterior layers of biosorbents such as functional groups. At lower pH, binding sites of the biosorbent will be occupied by an excess concentrations of hydronium ions. The competition between hydronium and target metal ions in binding with functional groups results in a lower biosorption efficiency on low pH (Redha, 2020). With increasing pH of the solution, the number of hydronium ions decreases, and consequently metal ions have more active binding site available to occupy which results in a higher biosorption efficiency (Redha, 2020). Further increase in the pH, depending on the metal ion type, will result in the formation of metal hydroxide followed by precipitation of metals, which alters the biosorption efficiency. In other words, biosorption efficiency normally increases with an increase in pH value until the optimum pH is reached, any further increase in the pH value from this point will cause metal precipitation and consequently decrease in the biosorption efficiency. Increasing pH also causes the functional groups to achieve negative charges because of deprotonation. This phenomenon eliminates the repulsive forces between positively charged functional groups and metal cations and as a result, kinetics and the efficiency of biosorption, increases. The increase in pH negatively charges the carboxyl, phosphate and hydroxyl groups which are the most abundant functional groups in the biosorption process

(Liu et al., 2017). For the same reason described earlier, further increases in the pH, although in favour of negatively charging the functional groups, will cause the metal ions to precipitate and decrease the biosorption efficiency. Many studies have reported the effect of pH on biosorption efficiency and recommended optimum pH for metal uptake by different biosorbents (Kilic et al., 2014; Farnane et al., 2018).

2.3.3. Effect of contact time

The time that it takes for the target metal ions to interact with active binding sites existing on the exterior layers of biosorbents and attain the maximum biosorption, is known as contact time. The biosorption capacity of biosorbents are not altered by the contact time, but can cause limitation of the biosorption efficiency if the duration of the process is not enough for the maximum for metal ions to interact with active binding sites. Therefore, increasing the contact time results in an increase in biosorption capacity until the saturation of active binding sites by target metal ions (Arshadi et al., 2014). A further increase in contact time does not have any effect on the biosorption process efficiency. In general, depending on the biosorbent type, more than 90% of the metal uptake by biosorbents occur with a rapid rate within the first hour of contact time due to the vacancy and availability of active binding sites (Kanamarlapudi et al., 2018). However, during the remaining time of the biosorption process, due to a low percentage of binding site availability, a decrease in biosorption rate will be observed. Previous studies have reported the optimum contact time for biosorption of different metal species using various biosorbents (Gupta and Kumar 2019; Isma et al., 2019).

2.3.4. Effect of initial metal concentration

Initial metal concentration does not directly influence the biosorption capacity of the biosorbent. However, the initial metal concentration helps the metal ions to overcome the mass transfer resistance between liquid target specie and biosorbent phase (Kanamarlapudi et al., 2018). Increasing the initial metal concentration leads to an increase in biosorption capacity of the biosorbent. After reaching the initial metal concentration that saturates the binding sites and functional group existing on the exterior layers of biosorbents, a further increase in the initial concentration will result in a biosorption efficiency decline. In other words, at lower initial concentration of metals than saturation point, a complete and effective interaction between target metal ions and active binding sites occurs. At higher initial concentrations than the saturation point, metal ions remain in the solution with no active binding site available for interaction which leads to a lower biosorption efficiency (Wang et al., 2018). Many studies have evaluated the saturation initial concentration of metals for various biosorbents and have reported the role of initial concentration on biosorption efficiencies (Jamoussi et al., 2020; Hu et al., 2020).

In addition to their role on biosorption efficiencies, pH, temperature and initial metal concentrations have a critical influence on the number of viable cells and the attachment of micro-organisms on surfaces to form living biofilms. A comprehensive analysis of the effect of pH, temperature and metal concentrations on biofilm formation is available in publication number 4 of this critical overview document.

2.3.5. Effect of biosorbent dosage

As functional groups and binding sites exist on the exterior layers of biosorbents, the biosorbent dosage is an influential factor in the efficiency of biosorption process. Increasing the biosorption dosage is equivalent to increasing the surface area of the biosorbent which provides more binding sites for target metal ions resulting in a more efficient biosorption. At lower dosages of biosorbent, the number of target metal ions adsorbed by the unit weight of biosorbent, is higher than increased quantities of biosorbent. This phenomenon is due to a lower ratio of metal ion concentrations to biosorbent surface, which results in higher number of unoccupied binding sites (Lucaci et al., 2020). Previous reports have showed the effect of biosorbent dosage on heavy metal removal from aqueous solution and have suggested the optimum dosages for various biomasses (Al-Senani and Al-Fawzan, 2018; Ugwu et al., 2020).

2.4. Modelling the biosorption of heavy metals from aqueous solution

2.4.1. Biosorption equilibrium models

Biosorption equilibrium models are used to predict the biosorption capacity of biosorbents and metal concentrations of treated wastewater. Langmuir models, Freundlich model and Dubnin-Radushkevich (D-R) models are used to describe the distribution of metal ions at different biosorption stages. Langmuir and Freundlich models are the most popular models among researchers in the field of biosorption.

The main assumption of the Langmuir model is that the sorption takes place on a monolayer of the adsorbent surface and biosorption occurs on a homogeneous biosorbent surface (Din et al., 2013).

The Langmuir model has been successful in evaluating the biosorption capacity of various biosorbents for many years. The main equation of Langmuir model is as follows:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} K_L}$$

where q_e is the amount of adsorbed molecules on the surface of the adsorbent at any time (mg g^{-1}), C_e is equilibrium aqueous-phase concentration of adsorbate (mg L^{-1}), q_{max} shows the maximum adsorption capacity (mg g^{-1}) and K_L is the Langmuir constant (L mg^{-1}). K_L and q_{max} are calculated by plotting C_e/q_e against C_e. The slope and intercept of resulted straight line are $1/(q_{max}K_L)$ and $1/q_{max}$, respectively.

The Freundlich model assumes that the biosorption occurs on a heterogeneous surface with a multilayer adsorption on the adsorbent surface (Ayawei et al., 2017). The linear equation of Freundlich model is:

$$\log q_e = (\frac{1}{n}) \log C_e + \log K_F$$

Where K_F and n are biosorption extension and nonlinearity indicators which are evaluated by plotting log q_e vs. log C_e and calculating slope and intercept.

The value of q_e is evaluated using:

$$q_e = (C_0 - C_e)(\frac{V}{M})$$

where V is the volume of biosorbate solution applied to biosorbent (L), C_o is the initial concentration of target metal ion (mg L^{-1}), C_e is the concentration of metal ion in solution at equilibrium (mg L^{-1}) and M is the dry weight of the biosorbent (g).

The Dubnin-Radushkevich (D-R) model uses a Gaussian energy distribution on a heterogeneous surface to describe the sorption process. The D-R model is used to calculate the energy of biosorption and does not assume that the surface of the biosorbent is homogeneous (Abdel-Aty et al., 2012). The calculated energy of biosorption is used to understand the physical or chemical nature of the adsorption. D-R model equation is as follows:

$$\ln q_e = \ln q_s - \beta\, \varepsilon^2$$

where q_e is the amount of metal ion biosorbed at equilibrium (mg g^{-1}), q_s is a theoretical value that describes the isotherm saturation capacity (mg g^{-1}), β is the Dubinin-Radushkevich isotherm constant (mol^2 kJ^{-2}), ε is the Polanyi potential and calculated using the equation below:

$$\varepsilon = RT \ln \left(1 + \frac{1}{Ce}\right)$$

Where R is the universal gas constant (8.314 J mol^{-1} K^{-1}). The mean free energy (kJ mol^{-1}) of adsorption is calculated as:

$$E = (-2B)^{-1/2}$$

where B is a constant related to the mean free energy of adsorption (mol^2 J^{-2}).

2.4.2. Kinetic models

Kinetic models help to understand the effect of contact time on the rate of biosorption process and provide the optimum contact time for the uptake of target species by the biosorbent under investigation. Pseudo-first order model and pseudo-second order models are among the most important and widely used to describe biosorption kinetics. The equation for the pseudo-first order model is as follows (Lagergeren 1898):

$$\frac{dq_e}{dt} = k_1(q_e - q_t)$$

where q_e (mg g^{-1}) is the adsorbate amount adsorbed at equilibrium, q_t (mg g^{-1}) the adsorbate amount adsorbed at time t, and k_1 is the rate constant of the pseudo-first order model equation (min^{-1}). The $k_1$value is calculated using:

$$\log\left(\frac{q_e - q_t}{q_e}\right) = -\frac{k_1}{2.303}t$$

The straight-line plot of log ($q_e - q_t$) against t gives log (q_e) as slope and intercept equal to $k_1/2.303$. Therefore, the amount of solute adsorbed per gram of sorbent at equilibrium (q_e) and the first-order sorption rate constant (k_1) can be evaluated from the slope and the intercept.

The pseudo-second order kinetic model equation is (Ho and McKay 1998):

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$$

in which k_2 (g mg^{-1} min^{-1}) is the pseudo-second order kinetic rate constant. k_2 equation is:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e}t$$

$$h = k_2\,{q_e}^2$$

where h is the rate of adsorption (mg g^{-1} min^{-1}), q_e and k_2 are calculated by plotting t/q_t against t. The slope and intercept of the resulting line are q_e and k_2 , respectively.

The Weber and Morris (1963) intra-particle diffusion model, is used to evaluate the kinetics of the biosorption and model the diffusion processes controlling the target metal specie uptake, using the biosorbent. Intraparticle diffusion is assumed to be the controlling stage in the Weber and Morris model and hence control the overall rate of the adsorption process. The equation for Weber and Morris intraparticle diffusion model is as follows:

$$q_t = k_{id}\, t^{0.5} + C$$

where k_{id} (mg $g^{-1/2}$ $min^{-1/2}$) is the intraparticle diffusion rate constant and C (mg g^{-1}) is a constant related to the thickness of the diffusion boundary layer.

2.4.3. Thermodynamic model

The Gibbs free energy model is the main model used to study the thermodynamic of the heavy metal biosorption process. Thermodynamic parameters including free energy charge (**Δ**G, classic Vant Hoff equation), entropy (ΔS) and enthalpy (ΔH) using the following equations (Gibbs free energy change):

$$\Delta G = -RT \ln K$$

$$K = Q_{max} K_L$$

$$\Delta G = \Delta H - T.\Delta S$$

Where R (8.314 J mol^{-1} K^{-1}) is the universal gas constant and T is the sorption temperature in Kelvin. Values of ΔH and ΔS are the intercept and slope of the graph of ΔG versus T.

2.5. Sustainability evaluation of bioremediation systems

To achieve sustainability in future cities, the environmental, economic and social impacts of any strategy or system to manage urban runoff should be evaluated. This necessity is due to the emerging concerns of climate change and its direct and indirect influence on human life (Ebi 2021). Any alteration in the approach to manage urban runoff may result in a significant change in life cycle environmental impacts and life cycle costing of a system (McGrane 2016). Considering the importance of mitigating the negative effects of emerging diffuse contaminants in coping with problems such as climate change, understanding the life cycle environmental and cost impacts, is essential.

Sustainability assessment techniques are continuously evolving and results eventually lead to improvements of the available tools. Life cycle sustainability assessment (LCSA) is considered as a sustainability evaluation technique based on life cycle basis. The LCSA technique integrates the three pillars of sustainability (environmental, economic and social) as tools to evaluate and compare alternative techniques to prevent diffuse pollution in urban area (Guinée 2015). The sustainability evaluation (LCSA) technique is as follows:

$$\text{LCSA} = \text{LCA} + \text{LCC} + \text{SLCA}$$

where: LCSA, life cycle sustainability assessment; LCA, life cycle assessment (environmental); LCC, life cycle costing (economic); SLCA, social life cycle assessment (social). This kind of approach has been used to assess drainage systems including SuDS (Fathallohi and Coupe 2021).

This holistic approach will bring a dual benefit to the bioremediation system outputs. Firstly, environmental, economic and social sustainability of the proposed mitigation and prevention strategies will be evaluated. Secondly, the outcomes of LCSA sustainability studies will be easy and ready-made to aid practitioners, decision makers and policy makers choose the most sustainable strategies. Moreover, the outputs of the sustainability analysis will be more practical and easier to use for stakeholders and water quality industries.

Life Cycle Assessment (LCA) is considered as an accepted and successful methodology to assess the environmental impacts of products, systems and any strategy related to the urban environment. LCA is also known as the “from cradle to grave” method. The ISO 14044 LCA method is a global standard for LCA studies that considers the environmental impacts of the system under investigation in all stages of project development, including extraction of virgin construction aggregates, transporting to the construction site, civil works period and construction, end of service, demolition and removal (Sandanayake et al., 2021). This standard LCA method has been widely used by researchers and industries to model the environmental impacts of products, systems and urban infrastructure (Khasreen et al., 2009).

Chapter Three

Methodological Approach

The present critical overview document consists of 5 projects on quantification, optimization, modelling and sustainability evaluation of heavy metal biosorption using permeable pavement biofilms. A complete description of the methodologies used by each project has been elaborated in the materials and method section of the publications. The present chapter briefly mentions some of the main methodologies used in each project.

3.1. Statistical analysis of biosorption data for process optimization

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to extract the published metal biosorption data between January 1, 2010 and January 1, 2020. A searched strategy based on Medical Subject Headings (MeSH) was designed including keywords of ‘Biosorption’ OR ‘Biosorptions’ OR ‘Biosorptive’ OR ‘Removal’ AND ‘Bacteria’ OR ‘Bacterias’ OR ‘Bacteriae’ OR ‘Microbiology’ AND ‘Heavy metal’ OR ‘Metal’ OR ‘Ion’ to search Elsevier, PubMed and Wiley databases. An inclusion and exclusion criteria were designed based on PRISMA guideline to select original journal papers with adequate experimental data out of all documents, found in database search stage. In the next stage, a system was designed to extract the data from selected studies. Data including 1) type of heavy metal, 2) bacterial species, 3) bacterial state, (living or dead organisms) 4) biomass dosage, 5) initial heavy metal concentration, 6) temperature, 7) contact time, 8) pH and 9) equilibrium concentration or biosorption capacity, were extracted prior to quality assessment. The quality assessment of the selected papers consisted of excluding papers with reported data of less than 5 out of 9 criteria mentioned above. Figure 2.1

presents the flow chart of the method used for the selection of studies included in the meta-analysis based on PRISMA guideline.

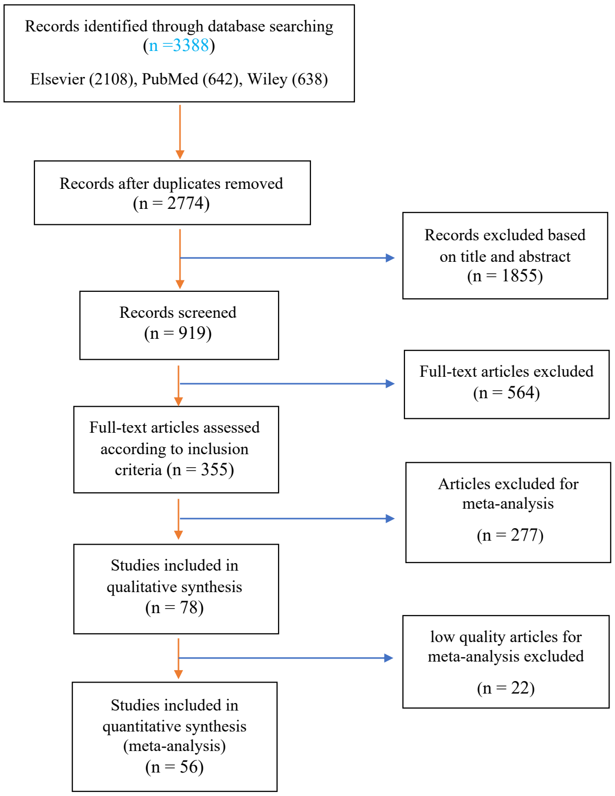

Figure 2.1. PRISMA guided flow chart of study selection process.

RevMan 5 meta-analysis software (Cochrane, London) was used to carry out the statistical analysis for the extracted data from the 56 final studies. The R-parameter was calculated for each single study to eliminate the methodological differences of studies (Hedges et al., 1999). The R-parameter equation is as follows:

$$R = \mathrm{Ln}\ (X_e/X_c)$$

in which X_e and X_c are the concentration of adsorbed target metal ion at equilibrium and the initial metal concentration prior to the start of the process, respectively.

The meta-analysis was carried out using the calculated R parameter, sample size and biosorption capacities of bacterial strains. The heterogeneity of the statistically analysed data from selected studies were evaluated using I^2 and Tau^2 parameters. Statistical significance of the data was evaluated by calculation of 95% confidence intervals (CI) and mean effect sizes. Publication bias was evaluated using R software (4.0) and Egger's and Begg's tests.

3.2. Bioreactor for biofilm growth

A nonwoven geotextile sheet commonly used as a layer in PPS was utilized as a surface for the development of living biofilms in all the biofilm quantification, optimization and biosorption assays in the present critical overview document. The selected geotextile manufactured by Terram company, Maldon, UK, had a composition of 70% polypropylene and 30% polyethylene (product commercial name: Inbitex). Circle sheets of the geotextile with surface area of 65 cm^2 were prepared, preceding addition to the incubation stage in the bioreactor. The lab scale experimental bioreactor was a 12-litre plastic container with air pumps at the base to keep the dissolved oxygen between 4-6 mg L^{-1} to maintain an aerobic condition for the microbial development with a pH ranging from 7-8 and the bioreactor temperature was kept between 20-25°C. The bioreactor was kept in a dark place to stop the algal biofilm formation.

3.3. Quantification of heavy metals

All the metal ion concentrations in samples taken after the biosorption assays, were quantified using ICP-OES instrumentation. The instrument used for the quantification of metals in the present study was a Perkin-Elmer optima 5300 DV ICP-OES.

3.4. Quantification of biofilm growth

3.4.1. Colony Forming Units

Counting colony forming units (CFU) is one of the widely used methodologies to quantify the biofilm formation. Geotextile circles were harvested from bioreactor prior to their agitation in a 10 mL saline solution for the detachment of biofilms from geotextile circles. Samples were taken from each geotextile circle detached biofilm for serial dilution, inoculation onto nutrient agar petri dishes and incubation at 37°C for 48 hours, prior to counting CFUs per 1 mL of sample.

3.4.2 Fourier Transform Infrared (FTIR) spectroscopy

The FTIR analysis of functional groups on the surface of biofilms developed on the geotextiles in the present study was carried out using a Nicolet iN10 Infrared Microscope (Thermo Fisher Scientific) instrument using a cooled detector technique. Reflection FTIR technique was acquired to analyse the absorption spectra of biofilms using wavelengths in the range of 400 to 4000 cm^{-1}.

3.5. Life cycle assessment (LCA) and life cycle costing (LCC)

LCA and LCC study of the permeable pavement system was carried out according to the ISO 14040 (2006) and ISO 14044 (2006) standards. The design specifications of permeable pavement for the LCA and LCC studies were extracted from UK standard: reservoir pavements for drainage attenuation, formerly HD 221/18 (DMRB CD 531, 2020). Life cycle inventories (LCI) of the permeable pavement system were evaluated using SimaPro 9.0 software package, developed by Pre-Consultants (2018) and the ecoinvent 3.5 database. OFFROAD2011 software was used to modify the ecoinvent LCI database where appropriate. The ReCiPe model (version 1.1) was used to investigate the environmental impacts of permeable pavement inventories extracted using the ecoinvent database. The PlanSwift database was used for the LCC studies of the permeable pavement system.

3.6. Statistical analysis

SPSS software (Version 21.0) was used for the statistical analysis in all assays in this study. A p-value < 0.05 was assumed to be statistically significant in all assays related to biosorption efficiency evaluation and quantifying the formation of living biofilms (for more information see publications number 1 and 2).

Chapter Four

Discussion

4.1. Optimization of biosorption process

According to the results from publication number 1, the process of heavy metal biosorption using bacterial biomasses are affected by factors including type of heavy metal, temperature, phylum and species of bacteria, pH, contact time, biofilm state (living, non-living), initial heavy metal concentration and biomass dosage. Publication number 1 aimed to report the optimum condition for the factors above to reach the highest heavy metal biosorption efficiencies for bacterial based biosorbents.

4.1.1. Effect of type of metal

Results of the present study revealed that the order of bacterial biomasses efficiency in heavy metal biosorption was Cd(II) > Cr(III) > Pb(II) > Zn(II) > Cu(II) > Ni(II) > Mn(II). This observation can be explained by characteristics of heavy metals such as molecular weight, ionic radius and the oxidation state. In the process of complexation, functional groups and active binding sites existing on the surface bacterial biosorbents, show higher affinity with heavy metal ions (Redha, 2020). This observation was consistent with results described earlier, where bacterial functional groups had the lowest biosorption efficiencies for lighter metals such as Ni (II) and Mn (II). The heaviest metal (Cd) showed the highest uptake among the metals.

4.1.2. Effect of bacterial state and phyla

The type of bacteria is known as an influential factor in the process of complexation of heavy metals (Presentato et al., 2020; Fathollahi et al., 2021). The difference in complexation processes occurs because each bacterial species makes its exclusive EPS layer with a particular functional group configuration (Gupta and Diwan, 2017). The results of the study revealed that the phylum proteobacteria (living + non-living) had the highest biosorption capacity, followed by phylum firmicutes and then phylum actinobacteria based biosorbents. Results also showed that living proteobacteria biomasses had higher biosorption capacities in comparison with non-living biomasses of proteobacteria phyla. In case of only non-living biosorbents, the firmicute phyla showed the highest biosorption capacities for heavy metal ions. These observations may be due to the difference in cell structure of bacteria from different phyla. The higher biosorption efficiency of living proteobacteria phyla, may be due to the lesser thickness of gram-negative phyla cell wall, in comparison with gram-positive phyla (Vollmer et al., 2008; Fathollahi et al., 2021). As a result, gram-negative proteobacteria based biosorbents provide a quicker and easier route for heavy metal ions into the bacterial cell which results in higher biosorption efficiency.

4.1.3. Dual effect of bacterial phyla and heavy metal type

The role of bacterial phyla on metal removal capacities of biosorbents for different heavy metal ions was evaluated. According to the results, the effect of bacterial phyla on the removal of Cd(II) ions by bacterial biosorbents was not statistically significant. Moreover, as Cd(II) is a non-essential heavy metal and bacterial cells do not actively use Cd(II) ions in metabolic processes, the bacterial state did not have any effect on the biosorption efficiencies. However, for essential Cu(II) metal ions, phylum and state showed a significant effect on the biosorption efficiencies with highest and lowest efficiencies associated to proteobacteria and actinobacteria, respectively. For Pb(II) ions, firmicutes, actinobacteria and proteobacteria based biosorbents showed the highest to lowest biosorption efficiencies. Amongst all 7 heavy metals under investigation in this study, Cr(III) ions showed the highest dependency on the type of bacterial phyla with the maximum efficiency associated with firmicutes phyla and the lowest with proteobacteria.

In general, firmicute based biosorbents showed the following order in efficiencies in biosorption of heavy metals: Cr(III) > Cd(II) > Cu(II) > Pb(II) > Ni(II) > Mn(II). The order efficiency for actinobacteria and proteobacteria based biosorbents was Ni(II) > Cr(III) > Pb(II) > Cu(II) > Zn(II) and Cu(II) > Cd(II) > Zn(II) > Pb(II) > Mn(II), respectively.

4.1.4. Effect of pH

The pH of a microbial solution has been reported to alter the electrostatic charges, active binding sites and functional groups associated with the bacterial biosorbents (Hong and Brown, 2008). The speciation of the target metal ion has been reported to change in different pH (Namieśnik and Rabajczyk, 2010). Therefore, the present study aimed to report the optimum pH to increase the efficiencies of living bacterial biofilms in removal of different heavy metals. Results of the study revealed that Cr(III) was the only heavy metal in the study that showed higher biosorption efficiency in low pH (<6). This observation was in line with existing data in the literature (Rasheed et al., 2020, Nguema et al., 2014, Sethuraman and Balasubramanian, 2010). The lower biosorption efficiencies in higher pH can be because of the shift of monovalent $HCrO_4^-$ to divalent $Cr_2O_7^{-2}$ and CrO_4^{-2} ions in metal solutions (Palmer et al., 1994). A pH between 6 and 7.5 was observed to be favourable for biosorption of Cd(II), Cu(II), Ni(II), Pb(II), Mn(II) and Zn(II) ions by bacterial biosorbents.

4.1.5. Effect of temperature

Temperature effects the thermodynamics of the metal biosorption by biosorbents and causes change in the structure of active binding sites resulting in alteration in biosorption efficiency (Maiti and Drohat, 2012). The optimum temperature for the heavy metal removal by bacterial biosorbents was investigated in the present study. According to the results, the highest Cd(II) and Cr(III) ions removal efficiency was achieved at temperatures higher than 35°C. For Ni(II) ions, bacterial biosorbents had the maximum uptake efficiency at medium temperatures ($27\ °C \leq T \leq 35\ °C$). Zn(II) and Cu(II) biosorption efficiencies of bacterial biomasses decreased with increase in temperature. This observation was due to the fact that living bacterial biomasses actively uptake the essential Zn(II) and Cu(II) ions in cellular metabolism. However, with increasing temperature, the number of viable cells decreases and consequently lower metabolism results in lower metal uptake efficiency (Huete-Stauffer et al., 2015; Fathollahi et al., 2021).

4.1.6. Effect of contact time

Many studies have reported contact time as a major contributor in biosorption efficiency, where the biosorption rate is fast in the beginning of the process and slows down because of the occupation of binding sites by metal ions (Lucaci et al., 2020; Redha 2020). The present study evaluated the effect of contact time on the efficiency of bacterial biomasses on different heavy metals and reported the optimum biosorption durations. According to the results, the highest Cd(II) removal efficiency was observed at medium contact time ($2\ h < T < 24\ h$). At higher contact times the Cd(II) removal efficiencies of biosorbents were decreased. This may be due to the changes in the solution pH as a result of living bacterial metabolism processes, which alters the speciation of metal ions and consequently causes their precipitation (Liu et al., 2018, Ratzke et al., 2018, Xie et al., 2018). However, bacterial biomasses showed the maximum removal efficiencies for Zn(II) and Cu(II) ions at long contact time (>24 h). This observation may be due to two main reasons. Firstly, number of viable cells increases at longer contact times and as a result the number of active binding sites existing on the surface of bacterial cells increases. Secondly, at longer contact time bacterial cells actively used essential Cu(II) and Cu(II) ions in the process of metabolism, which leads to a higher biosorption efficiency (Stewart et al., 2019, Buracco et al., 2018).

4.2. Biosorption efficiency of permeable pavement biofilms

One of the objectives of publication number 2, 3 and 4 of the present critical overview document, was to evaluate the efficiency of permeable pavement living bacterial biofilms in the removal of Cu(II), Hg(II) and Cd(II) ions from contaminated runoff. In the following section the efficiencies and influential factors on biosorption using permeable pavement biofilms will be discussed.

4.2.1. Biosorption of Cu(II) by permeable pavement biofilms

According to the results, the maximum removal efficiency of 92% and biosorption capacity of 16.8 mg g^{-1} for Cu(II) ions by living permeable pavement biofilms was observed at pH 6. The FTIR results revealed that the carboxyl groups were the dominant functional groups available on the surface of the living biofilms under investigation. At $pH<6$, the biosorption efficiencies of living biofilms decreased. This may be due to the fact that carboxyl functional groups are in non-ionic -COOH form at low pH which results in lower interaction between functional group and Cu(II) ions (Madeira et al., 2020).

Results of the present study revealed that increasing the initial Cu(II) initial concentration resulted in an increase in the final removal capacity of the living biofilms. The opposite was observed for biosorption efficiencies. The maximum biosorption efficiency (97%) was observed on day 21 of biofilm formation for initial concentration of 1 mg L^{-1} Cu(II). The minimum Cu(II) biosorption efficiency (7%) was measured on day 1 of biofilm incubation (200 mg L^{-1} Cu(II) ions). The decrease in Cu(II) biosorption efficiencies at higher initial metal concentrations was due to the decrease in number of available binding sites.

The Cu(II) removal efficiency of permeable pavement biofilms increased with the increase in incubation time. The minimum and maximum biosorption efficiencies were observed for 1 day and 21-day incubated biofilms, respectively. However, the biosorption capacities decreased with maturation of the biofilm as the maximum Cu(II) biosorption capacity was observed in day 1 and the minimum was associated with day 21. The same trend was observed for Cd(II) and Hg(II) ions. This was due to the fact that the biosorption took place on a monolayer (according to modelling data), meaning that due to maturation of the biofilm, the mass of the biofilm increased. However, as only the monolayer was active in the Cu(II) biosorption process, the observed overall biosorption efficiencies decreased.

4.2.2. Biosorption of Hg(II) by permeable pavement biofilms

The 1-day incubated biofilms showed 81% and 56% removal efficiencies for 1 mg L^{-1} and 20 mg L^{-1} concentrations of Hg(II) ions, respectively. The maximum Hg (II) biosorption efficiency (85%) for 1 mg L^{-1} concentration of Hg(II) was observed on day 28 of incubation time. The biofilm removal efficiency (day 1 of incubation) for Hg(II) increased from 7 mg g^{-1} to 101 mg g^{-1} with an increase in initial metal concentration from 1 mg L^{-1} to 20 mg L^{-1}. The increase in biosorption capacity at higher concentrations of metal is due to the increase in ratio of occupied to non-occupied binding site with increase in number of available metal ions (Ghorbani et al., 2008).

In a similar pattern as the biosorption of Cu(II), the Hg(II) uptake capacity of the living biofilms decreased with incubation time. This was due to inactivity of inner layers of biofilm in the biosorption process. In other words, the ratio of surface area to mass decreased with maturation of the biofilm which resulted in a lower biosorption capacity. The biosorption data revealed that the Hg(II) uptake capacity of living biofilms decreases with an increase in temperature. This observation may be due to the Hg(II) ions detaching from the biosorbent surface at high temperatures. Moreover, higher temperatures have been reported by previous researchers as a binding site disruption agent (Meena et al., 2005). The FTIR analysis showed that amine, hydroxyl and carboxyl groups were the main functional groups involved in the Hg(II) biosorption by permeable pavement biofilms.

4.2.3. Biosorption of Cd(II) by permeable pavement biofilms

The batch biosorption experiments indicated that with the maturation of permeable pavement biofilms, the Cd(II) biosorption efficiencies increased, with the maximum efficiency observed on day 35 of biofilm growth. The biosorption efficiencies constantly increased from day 1 to day 21 of maturation. However, a decrease in biosorption efficiency was observed on day 28 of incubation. The FTIR and mass analysis of the biofilms incubated on day 1 to 35 revealed that the biofilm mass decreased on day 28 in comparison with day 21 of the incubation. This observation was consistent with the decrease in biosorpion efficiency on day 28 of biofilm growth. These observations were associated with the dispersion stage of biofilm formation where the biomass reaches its maximum growth and releases parts of the biofilm to the bulk solution. The highest Cd(II) removal capacity was observed on the first day of biofilm development. The biosorption capacities constantly reduced with maturation of the biofilm until day 28 of incubation where an increase in biosorption capacity was observed. This increase was due to the dispersion stage of living biofilm formation which resulted in an increase in surface to mass ratio of biosorbent. This phenomenon resulted in more Cd(II) biosorption per unit weight of the biosorbent and was consistent with Hg(II) and Cu(II) biosorption results.

4.3. Optimization of permeable pavement biofilms development

Output number 4 of present critical overview document aimed to investigate the effect of environmental conditions such as pH and temperature, and nutritional conditions on the permeable pavement bacterial attachment and growth. Different types and concentrations of nutrition were evaluated to optimize the single and mixed-species biofilms.

4.3.1. Effect of type and amount of nutrition on biofilm formation

Single and mixed-species culture of *A. meyeri*, *B. cereus, E. coli*, *P. fluorescens* strains were inoculated into bioreactors with different amount of nutrients including phosphate (0-50 g L^{-1}), glucose (0-40 g L^{-1}), amino acid (0-50 g L^{-1}), nitrate (0-3 g L^{-1}), calcium (0-10 g L^{-1}) and magnesium (0-10 g L^{-1}). Incubated geotextile circles were harvested a prior to biofilm quantification assays including direct mass measurement, counting viable cells and FTIR analysis.

The biofilm quantification assays revealed that increasing the phosphate content up to 50 mg L^{-1} resulted in an increase in biofilm formation. However, further increasing the phosphate concentration showed a reverse effect on biofilm growth. This observation may be due to shrinkage of bacterial cells caused by hypertonic environment initiated by high content of phosphate (Brocker et al., 2012). According to the results, the optimum concentration of glucose for biofilm growth was 10 g L^{-1}. By increasing the glucose content to 20 gL^{-1}, the osmotic shock reduced the biofilm development which resulted in lower number of viable cells (Chang et al., 2014). 1 gL^{-1} of amino acid was observed to be the optimum concentration for biofilm formation. Higher concentrations of amino acid resulted in lower attachment of the bacterial cells to the geotextile surface and consequently lower biofilm growth. This observation has been previously reported by other researchers (Warraich et al., 2020). Results of the present study showed that 0.2 mg L^{-1}, 5 mg L^{-1} and 0.5 mg L^{-1} were optimum content of nitrate, calcium and magnesium for biofilm formation, respectively.

4.3.2. Effect of temperature and pH on biofilm formation

Mixed species bacterial biofilms were developed at operational temperatures of 15, 25, 35 and 45 °C for 21 days to examine the influence of bioreactor temperature on biofilm formation. The biofilm quantification results revealed that 25 °C was the optimum bioreactor temperature for the attachment of bacteria on geotextile fibres and biofilm formation. Further increase in the temperature resulted in lower biofilm growth. Similar observations have been reported by previous studies (Mizan et al., 2018; Mathlouthi et al., 2018). The reduction in biofilm growth may be due to the destructive effect of high temperatures on the structure of bacterial cells and their attachment to the geotextile surface (Russell, 2003).

To study the influence of bioreactor operational pH on biofilm formation, a mixed bacterial inoculum was added to the bioreactor containing geotextile circles at different pH of 4, 5, 6, 7, 8 and 9. Biofilm formation quantification assays revealed that increasing the pH from 5 to 7 constantly promoted the biofilm formation. However, increasing the pH from 7 to 9 resulted in considerable reduction in viable cells. This may be due to negative effects of high and low pH on the integrity of cytoplasmic proteins in bacterial cells (Cendra et al., 2019; Krulwich et al., 2011).

4.4. Modelling the biosorption process

One of the aims of outputs number 2, 3 and 4 was to mathematically model the metal biosorption data for permeable pavement biofilms using various equilibrium, kinetic and thermodynamic models. Mathematical modelling of the data helps to better understand the nature of the biosorption process and factors controlling its rate.

4.4.1. Modelling of equilibrium metal biosorption data

Biosorption isotherm modelling reveals the distribution of active binding sites on the surface of the biosorbents and provides information on the affinity of metal ions and binding sites of biosorbents (Kyzas and Matis, 2015). Cu (II), Hg(II) and Cd(II) biosorption data were modelled using Langmuir, Freundlich and Dubnin Radushkevich (D-R) mathematical models. Results from Langmuir model showed that biosorpion of Cu(II), Hg(II) and Cd(II) by permeable pavement biofilms takes place on a monolayer at all stages of biofilm formation (day 1 to 28). The biosorption data did not fully fit the Freundlich isotherm which indicated that multilayer action was less important in the metal uptake process. However, multilayer action played a role in biosorption process of more mature biofilms including day 14 and day 21. According to the models, the maximum Cu(II) and Hg(II) biosorption capacity on day 1 was 132 and 154 mg g^{-1}, respectively. Cu(II) and Hg(II) isotherms had an L shape which confirms the biosorption taking place on monolayer and high affinity between metal ions and binding site of the biofilm (Giles and Smith, 1974). Moreover, the L-shaped isotherms showed that as initial metal concentration increased, the vacant active binding sites on the biofilms surface decreased. This observation was as a result of metal ions covering the biosorption monolayer sites.

The free energy of metal biosorption (E) by permeable pavement biofilms was calculated using a D-R model. The value of E was less than 8 kJ mol^{-1} for all stages of biofilm formation (day 1 to 28). This observation indicated that the biosorption of metals by permeable pavement biofilms under investigation in this study, had a physical adsorption nature (Batool et al., 2018).

4.4.2. Kinetic modelling of the biosorption

Pseudo-first-order and pseudo-second-order kinetic models were used to evaluate the kinetic experimental data for metal biosorption by permeable pavement biofilms, to understand the mechanisms of the process and identify the rate-controlling phases. According to the results, the experimental data of Cu(II) biosorption followed the pseudo-second kinetic model, which indicated the role of diffusion in the external layer of biofilm. However, the Hg(II) biosorption data fitted the first pseudo-first-order model. This may be due to the fact that Hg(II) is a non-essential metal and not involved in bacterial metabolism.

The Weber and Morris mathematical model was used to understand the intra-particle diffusion processes involved in the Cu(II) and Hg(II) removal by living biofilms. According to the results, at concentrations less than 50 mg L^{-1}, the removal process of Cu(II) ions by living biofilms took place by a rate-limiting single stage intraparticle diffusion process. However, at higher concentrations a biphasic process with two linear lines occurred. Kinetic modelling of the data revealed that more than 80% of the removal capacity of permeable pavement biofilms for Cu(II) ions occurred by film diffusion (surface biosorption). The extra 20% of the biosorption was an intra-particle diffusion process taking place through pore diffusion of metal ions. Pore diffusion was only observed at Cu(II) concentrations ranging between 100-200 mg L^{-1}. This result may be a result of two phenomena: Firstly, the high metal concentrations may have pushed the Cu(II) ions to inner layers of the bacterial biofilms. Secondly, bacterial cells may actively uptake the essential Cu(II) ions for cellular metabolism (Festa and Thiele, 2011).

4.5. Sustainability evaluation of biosorption using permeable pavement biofilms

Outputs number 1 to 4 were focused on the quantification, modelling and optimization of the metal biosorption using permeable pavement biofilms. However, evaluating the biosorption capacities of a systems alone does not necessarily suggests it is the best practical strategy. Therefore, output number 5 aimed to evaluate the sustainability of metal biosorption using permeable pavement biofilms, using LCA and LCC methodologies and compare the environmental and cost impacts to other drainage systems available.

Normalised cost and normalised climate change potential, ozone layer depletion potential, acidification potential of soil and water, eutrophication potential, photochemical oxidant formation, depletion of abiotic resources-elements and depletion of abiotic resources-fossil fuels were evaluated for permeable pavement and 9 other drainage system options. These included filter drain, infiltration trench, soakaways, infiltration basin, wetland, retention pond, swale, filter strip and kerb and gully systems. All stages in a life cycle of drainage systems such as aggregate extraction, transportation, construction, civils work, maintenance and end-of-life phases were included in the inventory analysis, to evaluate emissions to air, water and soil.

According to the results, when the environmental impacts were normalised by flow or storage capacities, permeable pavement systems showed lower environmental impacts compared with drainage systems that were designed to retain the urban stormwater rather than convey it. This result was due to the design nature of permeable pavements dealing with higher volumes of runoff in the longer term. However, normalising the environmental impacts by size of the drainage systems, resulted in higher negative environmental impacts for permeable pavements. This is a result of high material demand for permeable pavement, which leads to more material extraction, transport and construction inventories. It is important to note permeable pavement was the only drainage component in this research that served as a load bearing pavement, the rest of the drainage systems were designed to only convey or store water.

The endpoint indicators of the ReCiPe model, showed that permeable pavement has lower impacts on human health than kerb and gully, infiltration basin and infiltration trench systems. However, due to high demand for material, permeable pavement showed the highest resource depletion rate. The overall environmental impacts of permeable pavement on climate change, ozone layer depletion potential, acidification potential of soil and water, eutrophication potential, photochemical ozone creation potential and depletion of abiotic resources-elements were 1.16E+02 kg CO_2 eq. m^{-2}, 3.29E+01 kg CFC11-eq. m^{-2}, 7.16E+04 kg SO_2 eq. m^{-2}, 2.95E+05 kg PO_4 eq. m^{-2}, 3.42E+05 kg C_2H_2 eq. m^{-2} and 6.28E+02 kg Sb eq. m^{-2}, respectively.

Chapter Five

Contribution to Knowledge

The present chapter highlights the objectives, findings and contribution to knowledge of each output included in this critical overview document.

5.1. Output number 1

In this paper we investigated the effect of the type of bacterial biomass from different phyla, bacterial state (living or non-living), environmental factors such as temperature and pH, type of heavy metal (e.g., Cr, Cd, Pb), initial metal concentration and biosorbent dosage on the biosorption process. The research objectives of this study were as follows:

- To identify the heavy metal removal efficiencies of various bacterial biosorbents using statistical analysis tools such as meta-analysis.
- To evaluate the effect of factors such as contact time, temperature, metal type, initial metal concentration, pH, bacterial phyla and dosage of biosorbent on the metal uptake efficiencies.
- To select a set of biosorption studies published in the last decade according to PRISMA guidelines, prior to comparatively and descriptively analysis of the data to understand the principal biological, physical and chemical processes.
- To identify the optimum environmental conditions for the heavy metal biosorption process using living and non-living bacterial biofilms.
- To identify and report the knowledge gap in the literature and call for studies in the missing heavy metal biosorption areas.

The results of this study could help researchers and wastewater treatment industry to select the most appropriate bacterial biosorbents and biosorption conditions according to their needs. This study was the first to use meta-analysis technique in the field of heavy metal biosorption and prepare a valid statistical overview of the biosorption research field. In this output, data from previous studies were subjected to a meta-analysis. Results of the present study were outputs of the meta-analysis study on the selected studies based on PRISMA guideline and designed search strategy. This study provided missing overall understanding of heavy metal removal processes using bacterial adsorbents.

Main findings and original contribution to knowledge of the present study were as follows:

- The order of heavy metal removal efficiencies of biosorbents was as follows: Cd > Cr > Pb > Zn > Cu > Ni > Mn.
- Bacterial biosorbents (living and non-living) from Firmicutes phyla had the highest heavy metal removal efficiencies.
- Living biomasses from Proteobacteria phyla showed the highest heavy metal removal performance considering living and non-living biomasses from different phyla. Non-living biosorbents from Proteobacteria phyla had lower metal removal efficiencies in comparison with living Proteobacteria biomasses.
- The maximum heavy metal efficiencies for Cd, Pb and Zn was observed at pH ranging between 6-7.5. However, a more acidic medium resulted in higher biosorption efficiencies for other heavy metals.

- Increasing the temperature to higher than 35°C led to a reduction in the Cu and Zn biosorption efficiencies and to an increase in Cd and Cr ions removal. Pb ions removal by bacterial biomasses was not dependant on the temperature of the biosorption process that were studied in this research.
- The highest removal efficiency of non-essential metals by bacterial biomasses took place during a contact times less than 2 hours. Living organisms showed the maximum Zn and Cu ions (essential metals) removal efficiencies at biosorption durations longer than 24 hours.

5.2. Output number 2

This study was focused on evaluating the feasibility of developing a microbial biofilm on the surface of a geotextile layer within the structure of permeable pavement in a bench-scale prototype, using a microbial consortium isolated from SuDS devices in Coventry. After developing the biofilm on the nonwoven geotextile layer, the efficiency and capacity of the biosorbent in removal of soluble mercury was evaluated using batch biosorption experiments and mathematical modelling. Different mathematical models including Freundlich, Langmuir and D-R equations were utilized to investigate the underlying mechanisms of Hg(II) removal by the living PPS biofilms. The Hg(II) equilibrium biosorption data fitted the Freundlich model. Mercury was selected as the target metal ion, as it is a non-essential metal and bacterial cells do not uptake the metal as a part of their metabolism. Furthermore, mercury is not present in the natural environment unless an anthropogenic activity originates its presence. Mercury is also an element of importance in SuDS due to its toxicity for all living organisms and its presence in soil and stormwater (Kondo et al., 2016).

The main objectives of output number 2 were:

- To evaluate the biosorption efficiencies of different stages of biofilm formation on geotextiles.
- To evaluate the factors controlling the biosorption of mercury using PPS living biofilms.
- To evaluate the physical and chemical processes of mercury biosorption using permeable pavement biofilms.
- Modelling the equilibrium, kinetics and thermodynamic of the biosorption process.

This study is the first of its type in the research area of SuDS. Previous studies have reported the degradation of oil by permeable pavements, but there were no studies to look for efficiencies of PPS biofilms in removal of heavy metals from contaminated water. The present study, for the first time, investigated the physical, chemical and biological processes occurring during the biosorption of metals by PPS biofilms.

The main findings and original contribution to knowledge of the output number 2 were:

- With an initial concentration of 20 mg L^{-1} Hg(II), living PPS biofilm developed on geotextiles for 1, 7, 14, 21 and 28 days, showed Hg(II) removal capacities (efficiencies) of 101.3 (55.7%), 24.4 (67.1%), 16.8 (61.4%), 9.7 (62.6%) and 13.3 (65.4%) mg g^{-1}, respectively.
- The thermodynamic modelling of the data revealed the spontaneous, feasible and exothermic nature of Hg(II) removal by living PPS biofilms.
- Hg(II) biosorption using living PPS biofilms showed a better correlation with pseudo second order kinetic models in comparison with pseudo first order model.
- FTIR analysis indicated that amine, hydroxyl and carboxyl groups were the main functional groups involved in the process of Hg(II) removal using living PPS biofilms.

5.3. Output number 3

A survey of the literature revealed that the majority of biosorption studies use non-living biosorbents for the uptake of heavy metals from contaminated water and wastewater. Limited studies have been conducted to investigate the mechanisms of biosorption using living mixed species biofilms. Output number 3 aimed to continue the subjects of output number 2 and look for efficiency of permeable pavement biofilms in heavy metal removal. This study investigated the Cu(II) uptake efficiencies by living biofilm. Cu(II) was chosen because of it is an essential metal for biological life and metabolism. Moreover, high concentrations of Cu(II) ions have reported in urban stormwater. The role of permeable pavement systems in removal of soluble Cu(II) was absent in the literature. In addition to physical and chemical characterisation of the biosorption process, output number 3 aimed to investigate the biological processes involved in the uptake process and measured the toxicity of high concentration of Cu(II) for PPS biofilms.

The main objectives of the study were:

- Studying the biological processes taking place during the removal of Cu(II) ions using PPS biofilms.
- Evaluating the negative effects of pH and different metal ion concentrations on bacterial populations in living PPS biofilms at all stages of biofilm formation.
- Examining the potential protective role of EPS layer of biofilms and geotextile fibres in shielding the bacterial cells from metal ions.
- Evaluating the Cu(II) uptake efficiencies of permeable pavement biofilms using batch biosorption experiments.
- Modelling the thermodynamics, kinetic and equilibrium biosorption data

- Examining the influence of pH, temperature and initial metal concentrations on bacterial communities within PPS and their Cu(II) biosorption efficiencies.

Output number 3 was the first published paper on quantification of permeable pavement biofilm development patterns, effect of Cu(II) concentrations on number of microorganisms. This study aimed to correlate the toxicity of Cu(II) ions for permeable pavement biofilms with their metal removal efficiencies. Moreover, this study revealed the role of geotextile fibres and EPS layer in shielding the bacterial cells in harsh conditions, including low pH and high concentrations of heavy metals.

The main findings and original contribution to knowledge of the output number 3 were:
- The maximum CFU number was observed on day 21 of PPS living biofilm formation where the maximum resistance against 200 mg L^{-1} Cu(II) was observed that was due to the development of a more mature EPS layer.
- The maximum number of viable cells in the PPS biofilms was observed after 21 days from the start of living biofilm formation.
- More developed EPS layer of 21 days incubated biofilms showed the highest protection rate against pH 2 and 200 mg L^{-1} Cu(II) concentration.
- Living biofilms on the geotextile fibres had a higher survival rate in contact with Cu(II) ions with initial concentration of 200 mg L^{-1} than biofilms which were detached from geotextile fibres.
- Thermodynamic studies revealed that the removal of Cu(II) ions using the PPS biofilms took place by a physical, feasible, spontaneous and exothermic process.

- Modelling the kinetic of the biosorption data using pseudo-second order model indicated that a blend intraparticle and film diffusion occurred during the biosorption process.
- Isothermal modelling of the Cu(II) removal by PPS biofilms data showed that the uptake process took place on a monolayer.
- The maximum Cu(II) removal efficiency (97%) by the permeable pavement biofilms occurred on day 21 of biofilm formation.
- Carboxyl, hydroxyl, phosphate and amino functional groups contributed in Cu(II) complexation.

5.4. Output number 4

The study for output number 4 was carried out to examine the formation patterns of single and mixed-species bacterial biofilms on a geotextile layer, within the structure of permeable pavements and to optimize the biofilm development conditions. This study hypothesised that environmental conditions such as pH, temperature and nutritional contents including amino acid and glucose are influential on bacterial attachment to the surface of geotextiles. Moreover, environmental and nutritional conditions can promote or prevent the maturation of attached biofilm. The metal biosorption efficiencies of living biofilms developed in optimum nutritional and environmental conditions were evaluated.

To examine the hypothesis of the study following goals were set:

- To evaluate and quantify the effect of the type of bacteria on the attachment and biofilm development on the geotextile.
- To assess the effect of pH and temperature on the bacterial attachment to geotextile sheets and biofilm formation.

- To assess the effect of different nutrients and their concentrations on the bacterial attachment to geotextile sheets and biofilm formation.
- To evaluate the toxicity of different concentrations of Cd(II) on different species of bacteria and to examine the role of biofilm in the protection of bacterial cells.
- To evaluate the Cd(II) removal capacities and efficiencies of optimized biofilms formed on a geotextile sheet.
- To model biosorption data and identify the underlying physical and chemical processes taking place during Cd(II) removal using PPS living biofilms.

The results from output number 4 of this critical overview document were the first published work reporting the optimum nutritional and environmental conditions for developing the novel single and mixed species bacteria strains. Moreover, the toxicity of low, medium and high concentrations of Cd(II) on planktonic and biofilm growth, of single and mixed-species of bacteria was examined and reported. The results of this study helped to better understand the mechanisms of development and biological role of permeable pavement biofilms in bioremediation of stormwater.

The main findings and original contribution to knowledge of the output number 4 were:
- The maximum biofilm growth was observed at phosphate, glucose, amino acid, nitrate, calcium and magnesium concentrations of 25, 10, 1, 1.5, 5 and 0.5 gL^{-1}, respectively.
- Both single and multi-species permeable pavement biofilms showed the best development in 25-35°C.
- Low and high pH values significantly reduced the biofilm formation. The most efficient pH for single and multispecies biofilms was 7.

- Permeable pavement biofilms grown in optimized environmental and nutritional conditions showed maximum Cd(II) biosorption efficiency of 99% at day 35 of biofilm development for 0.1 mg L^{-1} of Cd(II). This observation was higher than the highest biosorption efficiency (85%) recorded for biofilms developed in normal conditions.

5.5. Output number 5

Output number 5 aimed to evaluate the sustainability of metal biosorption using permeable pavement biofilms. This study investigated the environmental and cost impacts of the permeable pavement and compared the results with alternative urban drainage systems. This study was the first application of comparative LCA and LCC methodologies, for permeable pavement and 9 other urban drainage systems.

The original contribution to knowledge of output number 5 was evaluating the normalised cost and normalised climate change potential, ozone layer depletion potential, acidification potential of soil and water, eutrophication potential, photochemical oxidant formation, depletion of abiotic resources-elements and depletion of abiotic resources-fossil fuels for permeable pavement and 9 other drainage system options. All stages in a life cycle of drainage systems including aggregate extraction, transportation of construction materials, civil work and construction phases, maintenance and end of life phase were included in the inventory analysis to evaluate emissions to air, water and soil. The results of the output number 5 complete the other 4 outputs in the document. Therefore, this critical overview document contains the physical, chemical, biological, isothermal and sustainability evaluation of metal biosorption using permeable pavement biofilms.

Chapter Six

Conclusion, Future Research and Claim for PhD

6.1. Conclusions

The role of permeable pavement biofilms in bioremediation of contaminated stormwater has been under-investigated for many years. Although some studies have reported the lower concentrations of some compounds after filtration through permeable pavements, no robust laboratory investigation of the biosorption process was available in the literature. Therefore, the selection of outputs in the present critical overview document aimed to quantify, model and optimize the heavy metal biosorption process by permeable pavement biofilms. Some of the key conclusions of the present work are:

- The permeable pavement biofilms grown in optimized environmental and nutritional conditions showed a biosorption efficiency of 99% for an initial metal concentration of 0.1 mg L^{-1}.
- Thermodynamic studies revealed that target metal biosorption by the permeable pavement biofilms had a physical nature and was exothermic, feasible and spontaneous.
- Kinetic modelling of the of the biosorption data using pseudo-second order model indicated that a blend intraparticle and film diffusion occurred during the metal biosorption process.
- Carboxyl, hydroxyl, phosphate and amino functional groups were the main binding sites contributing to target metal complexation using PPS biofilms.
- The optimum nutritional condition for biofilm growth was observed at phosphate, glucose, amino acid, nitrate, calcium and magnesium concentrations of 25, 10, 1, 1.5, 5 and 0.5 g L^{-1}, respectively.
- The optimum environmental condition for biofilm formation was observed at pH 7 and 25-35°C.

- Maximum number of viable cells in the PPS biofilms was observed on day 21 of living biofilm formation. The EPS layer of 21 days incubated biofilms showed the highest bacterial protection rate against harsh environmental conditions.
- Sustainability evaluation of the proposed biosorption system showed that permeable pavement had lower impacts on human health than kerb and gully, infiltration basin and infiltration trench drainage systems.

6.2. Future research

Due to the high number of publications available in the literature, the meta-analysis in this study was only focused on papers published on bacterial biosorbents published between 2010 and 2020. More meta-analyses are needed on algal and fungal biosorbents, and to include publications dated before 2010. The biosorption studies in the present work were limited to heavy metals. Further lab-scaled investigations are needed to understand the efficiency of permeable pavement biofilms in degradation of organic compounds with high concentrations in the environment including pesticides, pharmaceuticals and PAHs. In addition to biosorption experiments, mathematical modelling of the data is necessary to better understand the biosorption mechanisms. The role of permeable pavement biofilms in microplastic uptake needs to be investigated. Larger scale (rig scale, m^2 size) biosorption experiments are recommended for future research. The present work was limited in using a single conventional geotextile sheet which is widely used in PPS. Due to instrumental limitations, manufacturing optimum geotextile for the growth of the biofilm was not possible in this study. Further work is needed on optimizing the geotextile manufacturing process to produce a geotextile sheet with optimum morphology, webbing and substance. Finally, there is a need for further work on an updated life cycle inventory database for LCA studies related to permeable pavements, considering its efficiency in removal of organic and inorganic compounds.

6.3. Claim for PhD

The author of this critical overview document believes that the present document and the portfolio of research outputs validate the fact that the required academic standard, originality and significant contribution to knowledge has been achieved for the award of PhD. The claim for PhD is based on following reasoning:

1. The selected portfolio presents a significant, continuous and coherent contribution to knowledge and explored a completely unknown subject of biosorption using permeable pavement biofilms.
2. The selected outputs in the portfolio address all aspects of the subject including physical, chemical, biological, isothermal and sustainability evaluation of the system under investigation.
3. Selected outputs in the portfolio have been published in top 5 Elsevier journals in environmental chemistry with impact factors higher than 6.7 and h-index higher than 179 (Table 1.2). Therefore, selected outputs meet the standards of the UK Research Excellence Framework (REF) 5-year research quality assessment.
4. Selected outputs have been cited more than 50 times in less than 10 months from the publication date in 2021.
5. The portfolio presents an original and independent contribution to knowledge on the removal of metals using permeable pavement biofilms.
6. The analytical methods, control and experimental design were appropriate with adequate quality control measures.

7. The selected outputs show the author’s expertise and understanding of the field and published literature.

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