SELECTED OPERATING PROCESS VARIABLES FOR A BIOFLOCCULANT SUPPORTED COLUMN FLOTATION SYSTEM

by

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DECLARATION

I, **Melody Ruvimbo Mukandi**, declare that the contents of this thesis represent my own unaided work, except where specifically acknowledged in the text, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology and funders.

All intellectual concepts, theories, methodologies, and material derivations used in this thesis and published in various scientific journals (except those that the candidate is not the first author in) were derived solely by the candidate and first author of the published manuscripts. Were appropriate intellectual property of others was acknowledged by using appropriate references. The contribution of co-authors for the conference(s) and the published manuscript(s) was in a training capacity, research assistance and supervisory capacity.

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ABSTRACT

The poultry industry generates significant volumes of slaughterhouse wastewater, which is laden with numerous pollutants, thus requiring treatment prior to discharge. However, the current typical water and wastewater treatment technologies have reached their limits due to the concentration of the pollutants therein and the large volumes of the wastewater to be treated, which influence the resultant water quality. Hence, there is a need for new technologies, re-engineering of the existing wastewater treatment equipment, and incorporating new unit designs to improve the treatment processes or system performance. Flotation is a well-known separation technology with the potential to be applied in wastewater pretreatment. Flotation is highly dependent on bubble size generated by air diffusers. However, inherent drawbacks underscore the significance of air diffusers design, making their design and study important. Currently, chemical flocculants, although widely used, are discouraged in flotation systems as some are considered harmful to humans and the environment. Meanwhile, the use of bioflocculants is considered eco-friendly, albeit their application requires further studies at an industrial scale. For the current study, the main aim was to evaluate the effect of selected operating process variables, i.e., diffuser type, bioflocculant form, and feed flow rate, on the performance of a bioflocculant-supported column flotation system for poultry slaughterhouse wastewater (PSW) pretreatment.

Firstly, the design and production of laboratory-scale 3D-printed spargers using the Laser-Powder Bed Fusion, a part of additive manufacturing, was explored to determine their applicability in a flotation system for wastewater pretreatment. Furthermore, they were compared to conventionally sintered/molded diffusers through microstructural analysis employing optical microscopy, tested for Vickers hardness, and analyzed for surface topography, including composition, using a scanning electron microscope and energy-dispersive spectroscopy. The application of 3D-printed spargers was proven feasible, revealing their porous nature, albeit with fewer pores than molded diffusers. Notably, the latter's, dense pores and better microstructure was thought to significantly enhance their suitability for optimizing the column flotation process. To overcome limitations related to pore properties, there is a need to explore new-generation alloys and optimize the 3D-printing process to make the final product more competitive and efficient than molded diffusers.

Secondly, the study went on to focus on the isolation of bioflocculant-producing microorganisms from PSW. Characteristics of the produced bioflocculant were determined, including the optimum storage conditions and the flocculation mechanism. Twenty microorganisms were isolated, and the D2 isolate had maximum flocculation activity. It was identified using 16S rDNA to be a *Bacillus* species and using RpoD to be a *Bacillus megaterium*. The bioflocculant was composed of mainly polysaccharides and proteins and was better stored in a crude form under frozen conditions.

Thirdly, the flocculation mechanism was assessed by Response Surface Methodology (RSM) at pH 4 (min) to 9 (max); bioflocculant dosage of 1% (min) to 3% (max) v/v with an assessment of changes in zeta potential as a measure of the changes in the electrostatic potential of the bulk solution. Zeta potential results confirmed that the bioflocculant was ionic, albeit charge neutralization was not the primary mechanism. These results were inconclusive in determining optimum conditions for flocculation activity; hence, flocs were viewed under a microscope, showing the optimum conditions for flocculation activity at pH 6.5 with a bioflocculant dosage of 2% (v/v). A bonding type test was carried out, and hydrogen bonding was identified as predominant, suggesting a bridging mechanism. This assertion was supported by the type of functional groups present in the structure of the bioflocculant produced by the D2 isolate.

Fourthly, three variables, i.e., diffuser design/type, bioflocculant form, and influent flow rate, were evaluated to determine their effect on the performance of a bioflocculant-supported column flotation system. It was found that diffuser type and feed flow rate were influenced by bioflocculant efficacy, thus affecting the overall column flotation system performance. In addition, it was determined that 3D-printed air diffusers and cell-free bioflocculants were a superior type and form, respectively, compared to their counterparts, i.e., molded diffusers and cell-bound bioflocculants. Combining 3D-printed air diffusers and cell-free bioflocculants at a feed flow rate of 1 ml/min resulted in relatively high pollutant removal (COD, TSS, protein, and turbidity reduction).

The study laid a foundation for exploring 3D-printed air diffusers, a relatively new technology in conjunction with bioflocculants usage that are regarded as eco-friendly, for application in wastewater pretreatment to enhance the performance of column flotation systems.

Keywords: Additive manufacturing; Bioflocculant; Column flotation; Diffuser design; Moulded diffusers; Flocculation mechanism, Microstructure; Poultry slaughterhouse wastewater; Wastewater treatment, 3D printed diffusers

DEDICATION

I dedicate this thesis to my mom TAMBUDZAI CONCILIA MUKANDI (you are and will always be my Hero!!!), to my daughter AZIELLA TANYARADZWA take after your mom, you are the best thing that happened to me during all the difficulties associated with this journey and to the GIRL CHILD out there (I am saying to you, you can make it, please don't let the societal norms suppress you!!!).

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RESEARCH OUTPUTS

The following research outputs represent the contributions of the candidate to scientific knowledge and development during the doctoral studies:

- **Mukandi, M.R**., Basitere, M., Okeleye, B.I., Chidi, B.S., Ntwampe, S.K.O. and Thole, A. 2021 Influence of diffuser design on selected operating variables for wastewater flotation systems: a review. *Water Pract. Technol*. **16**(4), 1049-1066.
- Mukandi, M.R., Basitere, M., Ntwampe, S.K., Njoya, M., Chidi, B.S., Dlangamandla, C. and Mpongwana, N., 2024. Evaluation of Selected Operating Process Variables for a Bioflocculant Supported Column Flotation System. *Water*, ***16***(2), 329.

The following research articles, based on research studies reported in this thesis, are under review:

- **M. R. Mukandi[1]**, M. Basitere[2^], S. K. O. Ntwampe[3] B. S. Chidi[1] 2023 3D printed vis-à-vis molded spargers for application in air flotation systems: An exploratory study.
- **M. R. Mukandi[1]**, M. Basitere[2^], S. K. O. Ntwampe[3] B. S. Chidi[1] 2023 A Bioflocculant Producing Microorganism from Poultry Slaughterhouse Wastewater: Elucidation of Flocculation Mechanism and Efficacy on Pollutant Removal.

The nature of a science laboratory means that part of the research intersects with other researchers so in that regard these are the publications that are came as a result of collaborative effort during the course of the doctoral studies:

- Dlangamandla, C., Ntwampe, S.K., Basitere, M., Chidi, B.S., Okeleye, B.I. and **Mukandi, M.R.** 2023 Production, Application, and Efficacy of Biodefoamers from *Bacillus*, *Aeromonas*, *Klebsiella*, *Comamonas* spp. Consortium for the Defoamation of Poultry Slaughterhouse Wastewater. *Water*. **15**(4), 655.
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- Dlangamandla C., **Mukandi M**., Basitere M. and Ntwampe S. K. O. 2018 Bioflocculants Producing Microorganisms from Poultry Slaughterhouse Wastewater for Application in a Biological Dissolved Air Flotation System. *Section Industrial Wastewater Treatment And Other Topics. In 10th Eastern European Young Water Professionals Conference IWA YWP,* Zagreb, Croatia, 7-12 May, 313 – 314. ISBN: 978-953-8168-23-9.

LAYOUT OF THESIS

The aim of this study was to evaluate the impact of selected operating process variables on the performance of a bioflocculant supported column flotation system (BioCF) which uses microbial flocculants (bioflocculants) for poultry slaughterhouse wastewater (PSW) pretreatment. Furthermore, the design and manufacture of novel 3D-printed air diffusers is reported. The thesis is divided into the following chapters, each written in a form of a publication (except for Chapter 1):

Chapter 1: Introduction. The chapter provides a background on different operational parameters including their limitations and the need for new innovative technological treatment methods for wastewater. Furthermore, it provides aims and objectives, hypothesis, including the significance and delineation of the study.

Chapter 2: This chapter focuses on diffuser design for a column flotation system. It discusses various aspects pertaining diffuser design which includes advances in diffuser design, effect of diffuser design on operational parameters and introduces 3D-printing of diffusers.

Chapter 3: In this chapter, the additive manufacturing technology is introduced. The feasibility of manufacturing air diffusers using the additive manufacturing technology is explored. The resultant 3D-printed air diffusers are compared against the moulded air diffusers in terms of their microstructure, mainly focussing on the pore characteristics.

Chapter 4: This chapter provides the materials and methods used for isolation and characterization of bioflocculant producing microorganisms (BPMs) including analysis of storage conditions of the crude bioflocculant. Furthermore, the flocculation mechanism was elucidated.

Chapter 5: The chapter focuses on the pretreatment of PSW using a BioCF system. The process variables considered were feed flow rate, bioflocculant form and diffuser type, for optimizing or improving the BioCF performance.

Chapter 6: This chapter gives the overall conclusions of the study and recommendations for future studies.

Appendices: Lists auxiliary information (methods; tables; images) which was deemed supplementary thus not needed for the body of the thesis.

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Nomenclature

Symbol	Description	Units
$\frac{A}{S}$	Air solid ratio	-
f	Pressurization system efficiency at pressure	0.8
P	Operating pressure	Kg/cm^2
S_a	Air solubility	mL/L
$sCOD$	Soluble chemical oxygen demand	mg/L
$tCOD$	Total chemical oxygen demand	mg/L
X	Influent solids concentration	mg/L

GLOSSARY

Basic Terms and Concepts

Term	**Definition/Explanation**
Additive manufacturing and 3D printing	Is a process whereby a three-dimensional object is made from a digital file (Bobyr *et al.,* 2019) by randomized addition of material during the manufacturing of a product with 3D printing being the orderly/sequential layering of material to manufacture a product.
Air diffuser/ sparger	It is a device that distributes air bubbles into the liquid through pores by creating dispersed air bubbles (Ham *et al.* 2021)
Bioflocculants	Are extra-polymeric substances of high molecular weight that are produced by microorganisms during cell lysis and hydrolysis of macromolecules which are usually composed of lipids, proteins, polysaccharides, nucleic acids and other substances (Sheng *et al.,* 2010),
Column flotation	Is a relatively simple physical separation process whereby microbubbles attach with particles and rise to the top where they are subsequently removed (Shukla *et al.* 2010).
Flocculants	Are substances that facilitate agglomeration of suspended and colloidal particles thus forming flocs. (Macczak *et al.,* 2020)
Flotation	Is a separation technology based on the principle of bubble attachment to particle matter attachment to form flocs (Naghdi & Schenk, 2016),
Molding/sintering process	Is basically the application of heat just below the melting point of the metal such that the particles bond and hold thus forming the new shape (Hobert 2015).
Wastewater	Water containing waste, or water that has been in contact with waste material. South African National Water Act (NWA) of 1998 (Act. 36 of 1998).
Wastewater treatment	Is the process of removing pollutants from water, which are of concern to the ecosystem, mainly, humans and the environment (Khantaee & Kasiri, 2011).

Abbreviations	**Description**
AM:	Additive manufacturing
BioCF:	Bioflocculant supported column flotation
BPM:	Bioflocculant producing microorganism

CB:	Cell-bound
CF:	Cell-free
DAF:	Dissolved air flotation
DiAFs	Dispersed air flotation systems
FOG:	Fats, oil and grease
FTIR:	Fourier transform infrared
pH:	Potential of hydrogen
LPBF:	Laser powder bed fusion
PSW:	Poultry slaughterhouse wastewater
rpm:	Revolutions per minute (rev/min)
RSM:	Response surface methodology
sCOD:	Soluble chemical oxygen demand
tCOD:	Total chemical oxygen demand
TDS:	Total dissolved solids
TSS:	Total suspended solids
3D:	Three dimensional

CHAPTER 1

INTRODUCTION

CHAPTER 1

1. INTRODUCTION

1.1 General background

Water is crucial for economic development and the sustainability of human life. However, the increasing water shortages and indiscriminate wastewater disposal into local freshwater sources have made the development and improvement of wastewater treatment technologies a necessity, thus leading many nations to adopt diverse wastewater treatment techniques (Bustillo-Lecompte & Mehrvar, 2015), including enforcement of penalties through legislation (Yetilmezsoy & Sapci-Zengin, 2009). According to the National Water Act (NWA 36) 1998, "wastewater means water containing waste or water that has been in contact with waste material." The wastewater treatment systems currently in place are a way of remediating water for reuse and safe disposal to the environment to prevent serious health challenges or clinical outcomes for humans and to avoid the non-availability of water (Al-Sabagh et al., 2015). Moreover, there is an increase in the incidence of freshwater contamination of water systems due to the inadequate remediation of wastewater discharge, particularly in developing countries. This has resulted from global growth, urbanization, and increased demand for food supply, particularly for poultry products, which are mainly affordable to impoverished communities (Bustillo-Lecompte & Mehrvar, 2015). This has caused the poultry industry to proliferate due to increased demand for poultry products as a cheap source of protein, which increases potable water usage and consequently generates an increased quantity of poultry slaughterhouse wastewater (PSW). PSW is characterized by a complex composition of pollutants such as blood, feces, fats, oil and grease (FOG) from the slaughtering of birds, and detergents from the sanitation of equipment and surfaces, resulting in high COD, suspended solids, and other parameters being high in the effluent (Del Nery et al., 2016; De Nardi et al., 2008). This calls for the pretreatment of PSW prior to consequent treatment and/or discharge.

Selection of the best treatment method for a particular type of industrial wastewater is usually complex. It depends on several factors, including but not limited to the level of contaminant concentration, removal of the primary contaminants, treatment methods available, flexibility of the methods, and the efficiency of the treatment system. The performance of a water treatment system can be monitored by assessing the water quality parameters prior to and post-treatment (Oller et al., 2011). Flotation, which is a well-known separation technology with the potential to be applied in wastewater pretreatment from various industries (AmaralFiljo et al., 2016), is considered the best separation technology for the pretreatment of FOG-containing wastewater such as PSW; hence, the application of a flotation system and the use of chemicals to improve particle removal efficiency (Zouboulis & Avranas, 2000). However, the use of chemicals in environmental engineering systems is unappealing. In the present study, the efficiency of a bioflocculant-supported column flotation (BioCF) system, which makes use of bioflocculants of microbial origin as a support mechanism, will be evaluated for its potential to replace the chemical flotation systems currently in use. The utilization of cell-free and cell-bound flocculants will be assessed to determine its effect on BioCF performance. Moreover, process efficiency is affected by numerous other factors, including sparging rate, hydraulic retention time, and flow rate; hence, controlling such parameters is of utmost importance (Dassey & Theegala, 2011). In the present study, the effect of diffuser design and feed flow rate on the performance of a BioCF system, which in turn determines pollutant removal efficiency, will be evaluated for effective performance monitoring.

1.2 Research problem

Water usage for domestic and industrial activities is on the rise, mainly in countries experiencing rapid economic growth, and as the water demand is increasing, so is the quantity of wastewater being produced. The poultry industry is one of the growing industries in the agricultural sector. It has become a generator of a high quantity of poultry slaughterhouse wastewater (PSW) with high levels of BOD, COD, suspended solids, and oil/grease/fats (FOG). All these are detrimental to humans and the environment; hence, there is a need for the pretreatment of the PSW before discharge using appropriate technologies. A column flotation (CF) system can be used for pretreating wastewater. In the application of eco-friendly systems, chemical flocculants are discouraged as some of them are considered harmful to both humans and the environment. Meanwhile, using flocculants of microbial origin is considered benign and thus requires further studies to be incorporated into pretreatment processes at an industrial scale. Wastewater treatment technologies currently in use have reached their limits due to the quantity and high strength quality of the wastewater to be treated; hence, technological improvements and innovation are needed. The BioCF system that utilizes flocculants of microbial origin has never been fully explored to improve flocculant performance by using cell-bound or cell-free bioflocculants. Moreover, the BioCF has never had some of its operational parameters and diffuser design improved for some time. It is, therefore, necessary to adequately determine the effect of its operational parameters to describe the performance and efficiency of the BioCF system with a novel diffuser design for PSW pretreatment.

1.3 Novelty

This study was motivated by the need for improved pretreatment technologies due to increased potable water consumption within the poultry industry and water shortages in the Western Cape, South Africa. The study focused on the parameters that affect the performance of a BioCF system that has never been studied before. Additive manufacturing of 3D-printed air diffusers was explored and is still a new technology.

Furthermore, a BioCF with 3D-printed air diffusers, which is a reasonably new competing technology for pretreatment of wastewater that makes use of flocculants produced by microorganisms, was studied - with bioflocculants free of cells in comparison to the ones with cells having never been previously investigated. The BioCF with 3D-printed air diffusers is still in its developmental stages and has yet to be implemented on an industrial scale; hence, it is still an exciting area of research that needs more attention.

1.4 Research rationale

According to the UN (2015), SDG goal 6.3 states that "By 2030, improved water quality must be achieved by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing treated water recycling and safe reuse globally". Moreover, due to the scarcity and shortage of water, the objective of wastewater treatment plant activities has changed from treatment for disposal to treatment for recycling; thus, a high level of treatment efficiency is required. Hence, improved technologies in wastewater management need not be emphasized as they are a must.

1.5 Hypothesis

H0: Operational parameters that are diffuser design, bioflocculant form, and feed flow rate will affect the performance of a BioCF for the pretreatment of PSW.

H1: Operational parameters that are diffuser design, bioflocculant form, and feed flow rate will have minimal impact on the performance of a BioCF for the pretreatment of PSW.

1.6 Research questions

- Is the 3D printing of air diffusers using additive manufacturing technology feasible?
- What effect will different forms of bioflocculants have on the overall performance of the BioCF system?
- Will the use of bioflocculants in a CF influence other system operational parameters?
- Which diffusers between 3D-printed and molded diffusers perform better when used in a BioCF, provided that other operational parameters are steady?

Which combination of selected operating process variables best affects overall BioCF efficiency?

1.7 Research aims and objectives

The study aimed to evaluate selected operating process variables for a bioflocculant-supported column flotation system with novel 3D-printed diffusers. This was achieved by dividing the research into four phases, each with its objectives. Phase 1: To identify significant gaps in flotation systems and air diffuser designs for wastewater pretreatment; Phase 2: To design air diffusers and manufacture them using additive manufacturing and 3D printing for application in a bench-scale column flotation system; Phase 3: To isolate bioflocculant producing microorganisms from the PSW to be pretreated and characterize the bioflocculant having a high flocculation efficiency from an identified strain; and Phase 4: To examine the effect of parameters (feed flow rate, bioflocculant form, and diffuser type) on the performance of a BioCF system for the pretreatment of PSW.

Phase 1: To identify significant gaps in flotation systems and air diffuser designs for wastewater pretreatment.
Objective 1: Assess historical standing, influential parameters, and challenges in diffuser design for column flotation systems and
Objective 2: Suggest exploration of new technology for diffuser manufacturing for diffuser pore structure control, thus air bubble formation.

Phase 2: To design and manufacture air diffusers using additive manufacturing and 3D printing for application in a bench-scale BioCF system.
Objective 1: To ascertain the feasibility of manufacturing diffusers using additive manufacturing technology and
Objective 2: To evaluate the properties of the manufactured diffusers compared to existing diffusers (molded/sintered steel air diffusers).

Phase 3: To isolate bioflocculant-producing microorganisms and characterize the bioflocculants produced.
Objective 1: To isolate and identify a microorganism that produces bioflocculants to be applied in a BioCF for particle flocculation purpose,
Objective 2: To assess and characterize the bioflocculants, including their storage conditions and
Objective 3: To elucidate the flocculation mechanism of the bioflocculants.

Phase 4: To examine the effect of parameters (feed flow rate, bioflocculant form, and diffusers design) on the performance of a BioCF system:
Objective 1: To evaluate whether feed flow rate affects pollutant removal,
Objective 2: To determine the parameter/s that significantly affect the overall BioCF efficiency and
Objective 3: To determine the best type of diffuser design and Bioflocculant form that results in higher removal efficiency.

1.8 Significance of the research

Recently, there has been an increased need to develop improved alternative water treatment technologies, mainly in industry and agriculture. The poultry industry has grown due to population increase, globalization, and industrialization, thus consuming a large quantity of fresh water and producing a large quantity of wastewater with complex pollutants. Suffice it to say that the water and wastewater treatment technologies that are currently in use have reached their limit, caused by the quantity and quality of pollution and the large volumes of water to be treated; hence, the need for technological innovation to produce adequate water and wastewater quality for a particular purpose. Therefore, this study explored the use of 3D-printed air diffusers- manufactured using additive manufacturing, a relatively new technology- in a BioCF. Furthermore, other parameters that include using different bioflocculant forms and various feed flow rates during wastewater pretreatment were evaluated to optimize the BioCF system. Overall, using such an optimized system would result in improved system performance, efficient pretreatment of wastewater, and better understanding, use, and manipulation of the additive manufacturing technology in system build-ups.

1.9 Delineation of the research

- The scope of the research is solely focused on wastewater from one poultry slaughterhouse operation in Cape Town, South Africa; and the use of air diffusers (whether 3D printed or moulded).
- Data was gathered around what transpired prior and post poultry wastewater treatment using the BioCF system.
- The BioCF system's long-term stability and scalability in an industrial setting was not investigated, along with a detailed assessment of its overall environmental impact, including the lifecycle analysis of the 3D-printed diffusers and bioflocculants.
- The study focussed on PSW case study, however it can be applied to other different case studies.

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CHAPTER 2

LITERATURE REVIEW

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CHAPTER 2

2. Influence of diffuser design on selected operating variables for wastewater flotation systems: a review

2.1 Objectives

Objective 1: Assess historical standing, influential parameters, and challenges in diffuser design for column flotation systems and

Objective 2: Suggest exploration of new technology for diffuser manufacturing for diffuser pore structure control, thus air bubble formation.

2.2 Historical perspective of diffuser design and applications

The use of gas or air as a carrier or buoyant medium in separation processes was discovered more than a century ago (Boyle 2003). Initially, it was due to chemical reactions in a liquid medium, which were later harnessed into pneumatical mixing; that is, the direct introduction of air through diffusion by submerged pipes and porous media (Rohlich 1954). The aeration of wastewater began as early as 1882 in England using perforated pipes and tubes. A perforated metal plate diffuser was patented as early as 1904 (Martin 1927). There are different processes in wastewater treatment where bubbles play a role, which include coagulation, flocculation, flotation, oxidation, and aerobic biological wastewater treatment (Atkinson et al. 2019). However, the addition of chemical agents has improved processes such as coagulation and flocculation. In most cases, for biological treatment methods, aeration is used to meet the dissolved oxygen demand of microorganisms mainly in activated sludge (AS) processes, whereas in other treatment methods, such as flotation, it is used to separate suspended particles from wastewater (Behnisch et al. 2018).

At the beginning of the 1900s, air flotation technology use and early development were for froth flotation systems whereby milled mineral ore mixed with water was agitated together with air and aggregate in foam at the top of the water while the gangue settled at the bottom of the flotation vessel. Other bubble generation processes, such as electrolytic and vacuum flotation, were also invented around the same period (Chow 2007). A vacuum air flotation was also used around the 1920s as a water clarification system, with more research being conducted in the 1960s on this technology (Edzwald 2010). Dissolved air flotation (DAF) development in Scandinavia was conducted in the 1960s and has since been well established for use in the pulp and paper industry, drinking water production and wastewater treatment (Crossley & Valade 2006). In Southern Africa during the 1960s, flotation was mainly applied in the mineral industry with little application in municipal or industrial wastewater treatment (Offringa 1995). Although most of the flotation types and cells were designed and developed for various applications including column flotation, some were patented in the first two decades of the 20th century. Column flotation was invented late around 1919 with most research and its extensive introduction in large-scale operations being in the mid-1960s (Rubinstein 1995). These developments might have been achieved as a result of advancement in bubble generation technology or aeration techniques.

In wastewater treatment, perforated pipes were the first to be used for aeration and the first accepted means of aeration diffusers were horizontal porous plates. The theory that small bubbles result in sufficient dissolved oxygen mass transfer led to the use of porous plate diffusers. Moreover, this resulted in the replacement of perforated pipes that produced big bubbles with porous diffusers that improved aeration. The fabrication of porous plates was an advancement and a part of the early development of diffusers whereby porous ceramic diffusers were used due to their ease of operation (Roe 1945; Boyle & Redmon 1983). Overall, air diffusers come in different shapes, designs and materials of construction, ranging from porous wood, perforated metal pipes, to ceramic plates with the latter being deemed the most efficient especially for industrial applications prior to 1945 (Roe 1945). As an attempt to advance diffuser usage, new shapes emerged, which included plates, domes and recently discs. Meanwhile, early investigations used diffusers ranging from basswood plates, perforated iron pipes and even air jets. There is ample evidence that their design influences process operability and efficiency. Most research conducted was based on air permeability, which is a measure of how easily air passes through a liquid (Ernest 1994; Boyle 2003). The reason for improvements of diffusers was to increase their efficiency, and to have simplified servicing, which is cleaning and ease of parts disassembly (Roe 1945). However, to improve diffusers, their designs need to be understood as they exist in different forms or types and such designs can be influenced by the quality characteristics of the liquid in which they operate.

This review seeks to discuss and consolidate information on air diffusers, although other air releasing devices are mentioned and discussed for differentiation purposes. Furthermore, the elucidation of column flotation operations under dispersed air flotation bubble generation method and influences in wastewater treatment diffuser design are also highlighted. This is a neglected area in wastewater technology practice and development.

2.3 Flotation in wastewater treatment

Flotation is made understandable by the Archimedes principle, which is also known as the buoyancy principle whereby the flotation process utilizes microbubbles to form and lift flocs or particles in a fluid to the top for skimming (Gopalratnam et al. 1988). The bubble rise rate can be described by the Stokes' principles and for better flotability, the particles are supposed to be hydrophobic as this determines the contact angle of the particles with the wastewater (Gopalratnam et al. 1988; Oliveira et al. 2010).

Flotation originated from the mineral industry and has gained widespread application in the fresh water and wastewater industry (Ndikubwimana et al. 2016). It is a well-known that separation technology with the potential of being applied in wastewater pre-treatment from various industries is a must. Industrial wastewater requires pretreatment before disposal or reuse to reduce the high level of contaminants including suspended solids (Peleka et al. 2018). Flotation in wastewater has been used for the removal of fibre, solids, macromolecule ions and other materials (Rubio et al. 2002). Its main advantage over settling techniques is such that high separation kinetics as well as the ability to separate particles that have a low density that cannot be removed through settling can be achieved (Amaral Filjo et al. 2016). This also makes it a very useful process for the pre-treatment of industrial wastewater containing fats, grease and oil (FOG), such as those from meat processing plants and petrochemical refineries (Bennett & Shammas 2010). Other advantages include shorter flotation periods even under high solids loading rates (Tian et al. 2018).

Flotation is classified based on the microbubble generation technique and is divided into three classes covering DAFs, which is based on Henry's law, a broad range of Dispersed Air Flotation systems (DiAFs), and electrolysis, which sometimes falls under DiAFs (Lu et al. 2016; Kyzas & Matis 2018). The DAF depends on the solubility of air in water and is operated such that the water is air saturated at high pressures, albeit with the air being introduced into the tank at atmospheric pressure (Deliyanni et al. 2017). Whereas, in electrolysis, bubbles are generated through the electrolysis of the carrier to create the microbubbles. In DiAFs, microbubbles are generated by compressing air directly as it passes through porous material including diffusion discs or by the use of an impeller or hydraulic injectors to get air into the water (Lu et al. 2016). In larger agitated cells, the air is introduced through the bottom of the agitator while bubbles are formed as a result of the impellor shearing effect (Deliyanni et al. 2017), whereas air diffusers or spargers are used in smaller cells including column flotation. This then results in different flotations under DiAFs based on the equipment used; that is, jet flotation, impeller air flotation, disc/sparger air flotation and pump suction pipe air flotation (Lu et al. 2016).

Rubio et al. (2002) and Kundu & Mishra (2018) discussed various types of flotation systems and different bubble generation techniques and processes that show variations in bubble generation technology (see Table 1). Many researchers have modified the currently existing flotation processes whereas others have even given the flotation processes different names according to the notable features of the processes. However, designers must determine whether the same classification remains if the method for bubble generation produces a bubble size out of known specifications.

Table 1: Different types of flotation and bubble generation methods (Rubio *et al.* 2002; Kundu & Mishra 2018).

Name of the flotation system	**Method of bubble generation**	**Range of bubble size (nm)**
Electro flotation	Uses electric field in between electrodes	~20
Dispersed/induced air flotation	Uses mechanical agitation or air injection system	700-1500
Dissolved air flotation	Dissolve air in water	30-100
Nozzle flotation	Use a gas aspiration nozzle that draws air into recycled water that is discharged in the flotation tank	400-800
Column flotation	Use of many bubble generation techniques that includes sparging through porous media, static or mechanical shear contacting and jetting	100-1500
Centrifugal flotation	Generates bubbles through air suctioning by static mixers/nozzles	100-1000
Jet flotation	Entraps air into the liquid by a vacuum effect	100-600
Cavitation air flotation	Bubbles formed as a result of the rotation of the cavitation aerator (impeller)	30-70

Flotation is affected by different factors, which include bubble distribution and velocity, including shape, size, specific gravity and surface charge of the suspended solids (Matis 1995). The efficiency of a flotation process is affected by the type of wastewater to be treated, the nature of the contaminants present in the wastewater, the design of the flotation system and its operational parameters (Temesgen et al. 2017). Overall, the control of bubble generation to obtain an optimum size range during the flotation process is of paramount importance as it enhances process efficiency (Seger et al. 2019). Bubble size produced in a column flotation can range between 50 and 1,000 µm, while DAF produces smaller bubbles between 30 and 100 µm, which in turn leads to higher removal/separation efficiencies (de Sena et al. 2008), especially when treating wastewater with minute particles that require fine bubbles for particle separation. However, DAF has several disadvantages that include high consumption of power, the requirement for a separate flotation tank, and higher service cost, which leads to higher operational costs, and the mechanical complexity of the system due to the use of a saturator and compressor (Li & Tsuge 2006; Painmanakul et al. 2010; Tao et al. 2019). Equally, electro-flotation is characterized by good bubble controllability, but it is power intensive and has low treated wastewater throughput; hence, it is not suitable for small-scale applications (Lu et al. 2016; Tao et al. 2019). Thus, the development of a system that is easy to operate and cheaper is necessary. Therefore, DiAFs can be an attractive alternative as they have a lower capital investment and lower operational costs (Gu & Chiang 1999).

2.4 Column flotation system as a preferred pre-treatment method for wastewater

Flotation is used mainly where sedimentation is not applicable due to the nature and presence of dispersed solids, and their settling rates, and thus becomes an efficient and economical process to apply (Ahmadi & Mostafapour 2017). For wastewater pre-treatment, the removal of particulate matter, macromolecules, microplastics and fibres results in reduced chemical oxygen demand (COD) and biological oxygen demand (BOD) of the pre-treated wastewater (Ives & Bernhardt 1995). To achieve high treated wastewater quality, treatment technologies that are economical, durable and robust are a necessity as the influent varies in terms of quality and quantity from different industries (Frank et al. 2017).

A flotation method combined with a coagulation/flocculation system has been deemed to be an effective pretreatment method for industrial wastewater (Liu et al. 2010). Challenges of a flotation system include the usage of a high dosage of flocculants and breakage of flocs by big bubbles (Ndikubwimana et al. 2016). However, the use of synthetic flocculants in flotation processes continues to be criticized due to their toxicity potential during application as their residue ends up in the treated water. Hence, the development of alternative flocculants that are biodegradable to reduce these toxicity concerns and perhaps acceptance or application on an industrial scale in suspended solids separation processes (Silva et al. 2019). The usage of a bioflocculant was investigated extensively by Dlangamandla et al. (2018) and Mukandi (2017), whereby bioflocculants were used instead of chemical flocculants.

Column flotation is a relatively simple physical separation process whereby microbubbles attach with particles and rise to the top where they are subsequently removed (Shukla et al. 2010). Flotation columns are classified based on various reasons that include the column number, bubble generator location, relative motion of solidswastewater to be separated and whether there is a packed bed or not (Cheng et al. 2016a). Column flotation came about as a way of mitigating problems that arise when using conventional mechanical cells such as several cleaning stages required, maintenance requirements and high size to capacity ratio (Al-Thyabat et al. 2011). It has been used in various industries that include oil recovery, mineral beneficiation, paper de-inking, food industry and wastewater treatment. Column flotation is still not well understood despite all the advancement in process knowledge over time; hence, scaling up is still a major concern. However, there are three approaches that are employed for column flotation scale-up and these are based on maintaining a constant recovery, column area and column volume (Vashisth et al. 2011) through usage of a bench-scale system. Bench-scale testing is an important characterisation tool that is a cheap, simple and quick way of obtaining key information. Figure 1 shows a typical column flotation bench-scale setup for wastewater treatment (Mukandi 2017). The use of such a system (through bench scale testing) would give a better understanding of any new design and its feasibility.

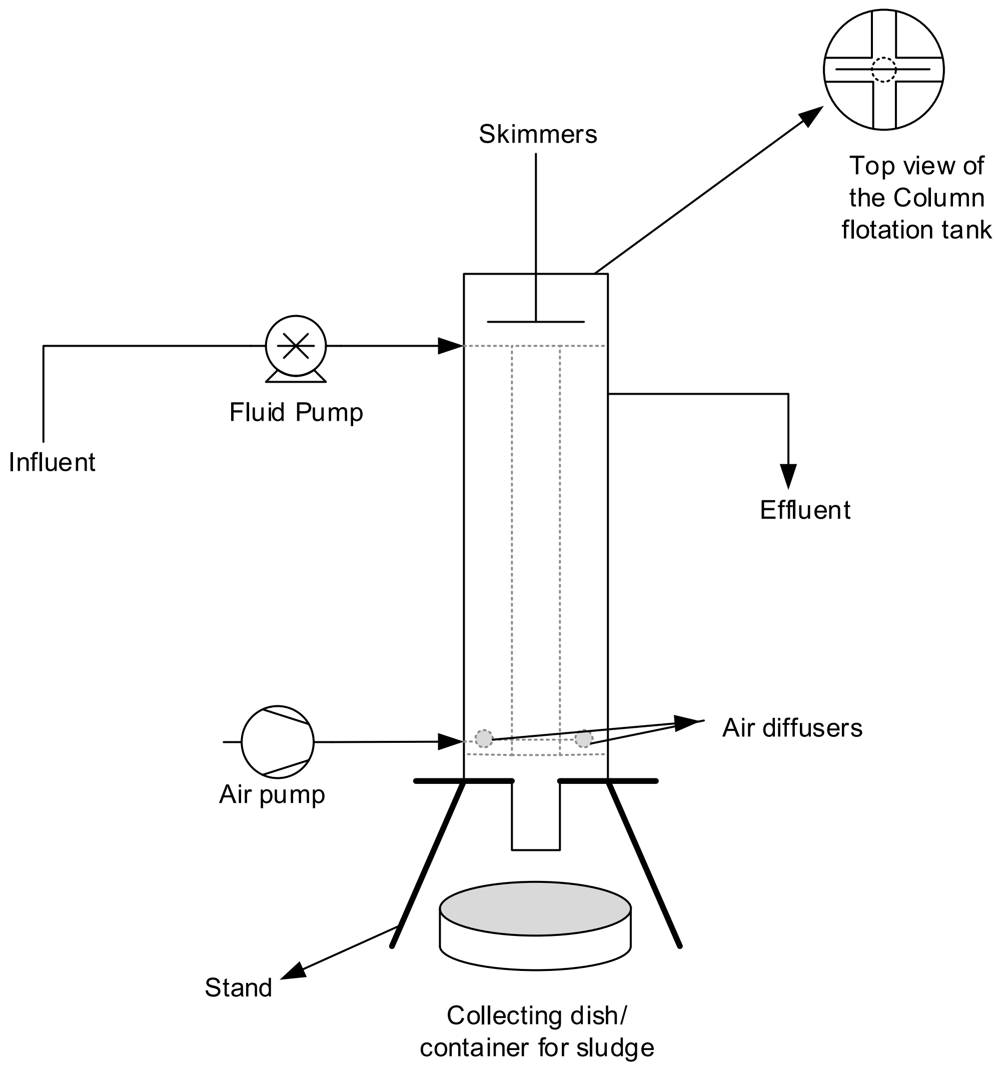

Figure 1: Schematic illustration of the column flotation system, bench-scale set-up.

Advantages of a column flotation over others include lower operating costs due to the absence of movable parts, less energy consumption due to the absence of a compressor or rotating parts (agitators), and lower floor space requirements due to its vertical construction, as compared to conventional flotation systems (Rubinstein 1995; Chaiarrekij et al. 2000), and reasonable flexibility for automatic controls (Wang et al. 2019). Due to the recurring expansion of flotation technology application in varying industries, the use of flotation columns has pioneered the development and improvement of sparger systems (Finch 1995). Lu et al. (2016) studied the influence of a flotation device for the pre-treatment of oilfield wastewater. They discovered that the use of a bubbling device which was a microporous metallic disc simplified the bubble generation process, thus laying a foundation for microbubble flotation application. Overall, it was found that the device, which included a flotation column, was more reliable, yielding 90% removal efficiency, and was easy to assemble.

Microbubbles for a column flotation under DiAFs are produced using a sparger or mechanical mixer (Tao et al. 2019). It is a prerequisite for the system to have sufficient production of microbubbles for high flotation efficiency and to also generate bubble volume concentration that is stable (Haarhoff 2008). Air bubbles must be small to maximize the gas/liquid/particle interfacial area as this can lead to improved particle removal even when flocculants are not used (Offringa 1995). Large bubbles do not easily attach to the particles whereas minutely smaller bubbles lead to breakage of formed flocs. In addressing these challenges, the costs related to bubble generation and breakage of flocs needs critical considerations (Féris et al. 2001). Consequently, the average bubble size of 100 microns is preferred (Gopalratnam et al. 1988); hence, flotation systems that use this bubble size can remove many pollutants that are found in wastewater (Dupre et al. 1998). Moreover, the bubble size in a flotation column is dependent on the generation method and device used, which are the key research points of a flotation column (Zhekun et al. 2010). Bubbles generated in column flotation are usually produced through two main types of spargers; that is, internal porous spargers (filter cloth sparger and perforated rubbers) and external generators such as turbo air spargers (Cheng et al. 2016a).

Optimization of flotation system operating process variables must focus on sparging rate, solid loading rate, hydraulic loading rate, air-solid ratio (Gopalratnam et al. 1988) and the addition of suitable flocculants. In this case, bioflocculants are suitable to impart environmental benignity (Mukandi 2017). There are various other factors apart from process operating variables that are considered when designing a flotation system, which include the type and quality of wastewater, the extent of the contamination, the level of treatment required and the type of diffusers to be used (Dassey & Theegala 2011). Most existing studies are vague in terms of disclosing the geometry or properties of air releasing devices used in flotation systems. Although the investigations focus on improving the bubble properties in a flotation system, the type of diffusers or nozzles used are rarely mentioned. Mostly rudimentary mentions of diffusers or nozzles design properties are used. This creates a gap that needs to be researched for the treatment of a particular type of wastewater using a specified type of diffuser with well-defined design properties.

2.5 Different types of air diffusers

Bubbles are obtained through usage or application of air releasing devices and are generated when the pressure is suddenly changed in the aperture points of the devices. These devices produce bubbles in various ways and come in different forms with the most commonly used being porous air diffusers/spargers, nozzles, gate, fixed orifices and needle valves, amongst others (Zabel 1985). Porous air diffusers generate bubbles by breaking up air through liquid displacement. This is achieved through the injection of air under pressure below the liquid surface. Nozzles are used to release air bubbles through the discharge of air-saturated water (Nadayil et al. 2015). The bubbles are formed because of a pressure drop on the narrowing of the nozzle whereas a flow constrictor increases the flow velocity, thus inducing bubble nucleation (Etchepare et al. 2017). In nozzle flow constriction, bubbles formed travel through the tank at pressures greater than atmospheric pressures but the difference with nozzles is mainly their configuration (Rodrigues & Rubio 2003). For needle valves and other orifices, air-saturated water is passed through constricted areas and clouds of bubbles are formed downstream of the constriction into the wastewater (Rubio et al. 2016; Azevedo et al. 2018) while on the other hand, injectors provide a jet of saturated water (Nadayil et al. 2015).

Some of the nozzles or valves are adjustable, whereas others are fixed (Haarhoff & Van vuuren 1995), with others such as needle valves being adjustable and having a self-cleaning ability. A large number of different nozzles and diffusers were patented between 1970 and 1980 and are continuously being improved under their initial or first patent registration (Rykaart & Haarhoff 1995). Table 2 enlists some of the air releasing devices that were patented, and they offer numerous advantages in comparison to others.

Table 2: Examples of patented air releasing devices and their notable attributes.

Patent specification number	Name of air release device/ description	Notable properties/attributes	Reference
US2294973A	Fluid treatment diffuser element	Strong, efficient, simple and easy to manufacture as a way of withstanding shocks and avoiding bottom coalesces that result in big bubbles	Ford (1942)
US2639131A	Diffuser for gases	Facilitates cleaning of the air supply orifice thus no need for removal; hence, saving labour and time	Procter (1953)
US2815943A	Diffuser tube	The tube inflates quickly thus removing any lodged solid matter and rapidly deflate to avoid clogging when air is supplied or cut off respectively. Cheap and ease of manufacturing.	Lamb (1957)

US4338192A	Clarifier bubble generation and distribution nozzle	For the production of bubbles with a diameter less than 100 micrometres	Krasnoff & Luthi (1982)
US4477341A	Injector apparatus having a constriction in a following adjoining mixing pipe	High airflow rate with economical/reasonable energy consumption	Schweiss & Dorflinger (1984)
US4842777A	Pressurized mixing injector	Inject high airflow rate, Strong and normal aeration which in turn minimize clogging problems	Lamort (1989)
US4981623A	Diffuser for aeration basin	Improved diaphragm diffuser that will keep the membrane in place to prevent rupture or dislodging	Ryan (1991)
US5139663A	Discharge valve for dissolved air flotation	Nozzle assembly that is improved and capable of self-cleaning, and a Nozzle assembly that can have its flow rates changed from a remote location	Mapples (1992)
US5154351A	Dispersion water nozzle	Reduction of pressure in dispersion water flow, and To produce bubbles of about 100 micrometres with Sufficient distribution of air	Takko (1992)
US6367783B1	Fine bubble diffusers	A diffuser that cannot easily be clogged by organic matter, and that Prevent backflow into the diffuser air supply source	Raftis (2002)
US9138752B2	Dissolved gas flotation pressure reduction nozzle	Increased turbulences that favours microbubble production, Uniform bubble size, Corrosive resistant material used, and Simple manufacturing and installation of the nozzles	Amato *et al.* (2015)
US9808810B2	Nozzle for dissolved air flotation system	To produce microbubbles even at low pressures, To produce uniform sized microbubbles with extended existence time in fluid, Nozzles that are easy to manufacture and have a simple structure	Park *et al.* (2017)

Diffusers that can be removed and serviced are more advantageous than those that are fixed as they can be easily removed without dewatering the tanks or stopping the aeration process (Roe 1945). Diffuser designs determine bubble size, distribution and rise velocity, with diffuser functionality being dependent on pore diameter, number and orientation in the device. Inadequate diffuser designs, for a particular process, result in challenges such as (i) weeping (slow discharge of air bubbles), which may cause undesirable or longer residence time, and result in poor performance of the system, (ii) blockage of pores or clogging, especially when there is a high concentration of solid particulate matter in the wastewater, (iii) fouling and scaling by biofilms, which result in increased dynamic wet pressure (DWP), which lead to bigger bubbles, culminating in a negative effect on the air supply which will require more energy to pump, (iv) dead zones due to non-uniform sparging or redundant placement of diffusers, and (v) high cost of operation due to high energy consumption (Kulkarni & Joshi 2011; Odize et al. 2017), and coalesce issues (Rodrigues & Rubio 2003). However, their application can be improved by addressing known challenges; hence, the need to improve existing diffuser designs.

2.6 Diffuser design: An engineering approach

In diffuser design, it is important to consider internal hydraulic parameters that include: (1) uniform discharge of air along the diffuser to ensure sufficient distribution of bubbles to minimize operational challenges; (2) use of simple geometries to minimize manufacturing and operational costs; (3) cater for no or low flow of air to prevent particle deposition including backflow to alleviate frequent out of place cleaning and maintenance challenges and (4) to minimize effects of the unsteady-state performance of compressors supplying the pressurized air (Bleninger et al. 2005). Moreover, various factors determine the performance of air diffusers which include the depth at which they are placed in the wastewater, and thus the external pressure that the diffuser will experience (Cheng et al. 2016b).

Additionally, DWP which is defined as the head loss across a diffuser being operated under submerged conditions, is an important parameter to consider when designing air diffusers. It increases with time due to fouling and is material dependent, thus different materials have different DWP. This is attributed to different material properties, with the pressure factor indicating the performance of the diffuser (Rosso & Stenstrom 2006). The DWP ratio of old to new diffusers must be quantifiable to assess the diffuser deterioration over time, although this is dependent on foulant (wastewater) characteristics and cleaning frequency. It is, however, also influenced by process operational conditions. Furthermore, pore geometry has an influence on DWP with round pores being rigid and acting as a control opening that can withstand high pressure, whereas membrane pores/slits can expand when the pressure increases. However, a low DWP does not guarantee the best performance as there is a minimum pressure required for the proper distribution of the air (Rosso et al. 2012). Such analysis is scantily reported and there is minimal guidelines or information available for the design of diffusers in published literature.

2.6.1 Diffuser, bubble formation and dynamics: efficacy in flotation systems

For flotation, the air is introduced into the tank in the form of bubbles. Bubbles exist in different forms; that is, as nanobubbles with a diameter of less than 0.2 microns, while microbubbles have a diameter of 10–100 microns and lastly, macro bubbles that have a diameter of greater than 100 microns, which tend to rise faster than the rest. Moreover, microbubbles are of interest in water and wastewater treatment as nanobubbles take longer to rise to the top while macro bubbles are inefficient due to their high rising velocity (Sadatomi et al. 2005; Basso et al. 2018). They have adsorbent properties as they can adsorb and agglomerate particulate matter due to their bigger surface area and their negative charge (Lee et al. 2019), which neutralizes repulsion forces between suspended matter. When bubbles and suspended particles are similar in size, maximum collision efficiency is achieved (Han 2002). Compared to bigger bubbles, microbubbles have an increased interfacial area and thus more interaction area, which improves bubble-particle collision, hence increasing flotation efficiency. In column flotation, the collision probability is higher due to large aerated volumes of the column and long passage that bubbles and particles travel along the height (Rubinstein 1995). Overall, bubble size measurements are conducted using laser-based methods and by image analysis techniques (Zhang et al. 2015).

Bubble size is influenced by diffuser design and affects bubble particle collision, and thus attachment of suspended solids including the rise velocity, which affects the efficiency of the system (Edzwald 2010). The size is governed by a sparging rate, pore size and also the wastewater parameter-volumetric quality and mixing velocity (Basso et al. 2018). Most researchers have focused their work on micro and nanobubbles in a bid to improve the efficiency of flotation systems. They do this by investigating bubble generation methods, characterization of bubbles and their categories as well as the development of bubble measurement methods (Temesgen et al. 2017). Moreover, bubble hydrodynamics have been extensively researched to assess bubble size influences on fluid dynamic behaviour using Computational Fluid Dynamics (CFD) (Chen et al. 2016), bubble surface modifications by surfactants (Henderson et al. 2008) and pressure effects on bubble size (Han et al. 2002). However, most researchers just mention that they used nozzles, needle valves or diffusers without sharing much-needed information about the air releasing devices and design features. Much less attention has also been paid to the type, design or configuration of air releasing devices that are being used to date, thus making it difficult to compare results from researchers using different applications.

Bubble generation is affected by; (1) operating conditions, which includes air flow rate and the sparging rate that is influenced by the operational pressure, (2) structural design that includes pore size (or diameter), pore geometrical configuration (e.g. contact angle), the device's materials of construction and submergence attributes and (3) physical properties, which include the quality characteristics of the water as it comes into contact with the diffusers (Xiao et al. 2019). Microbubbles have been known to be suitable for flotation systems and are generated mainly through three ways; that is, (a) dissolving air in a liquid and subsequently releasing it through air releasing devices that lead to nucleation of small bubbles, (b) cavitation induction through the usage of power ultrasound and (c) air delivered under low pressure forming bubbles with the aid of additional features such as mechanical vibrations, flow focussing and pneumatic fluid oscillation. Dissolving air in wastewater is widely or commonly used and the latter has the lowest energy consumption amongst the three (Zimmerman et al. 2008; Zimmerman et al. 2009). Bubble formation through cavitation, by forming nuclei first followed by growth as the bubble rises through wastewater, is desirable. However, in bubble growth, coalescence is a major challenge that affects flotation systems (Edzwald 2010).

Generation of bubbles through the saturation of water with air has limitations that include the high pressure needed for injecting the saturated liquid, the mixing of air and the recycling of gas/liquid, which can lead to equipment damage or contamination due to the nature of contaminants present in the water being treated (Ahmed et al. 2018). These disadvantages might be overcome by using diffusers without a recycle stream provided they generate the required bubble size for the particular or specified process.

2.6.2 Effect of diffuser shape

There are many different shapes of air diffusers that have evolved and emerged in accordance with different industry needs. These include domes, which are usually mounted at the bottom of a tank, panels that usually covers the flotation tank floor, flexible membranes that come in many shapes- from tubes, discs to flat surfaces, and flat stripes that usually covers the floor of a flotation tank as well (Ovezea 2009).

The shape of the diffusers can influence the air-water contact volume of rising air/microbubbles such that the greater the contact volume, the higher the flotation efficiency (Roe 1945), which can also affect the formation of flocs. In an attempt to optimise flotation systems, Féris et al. (2000) investigated the use of two types of diffusers, traditional, which were longitudinal (Figure 2(a)), and newly developed mushroom type diffusers (Figure 2(b)). The results showed that with the use of surfactants (sodium oleate), the mushroom type had high removal efficiency as compared to the former, thus showing that the shape of diffusers has an effect on the formation and breakage of flocs under turbulent conditions (Féris et al. 2000).

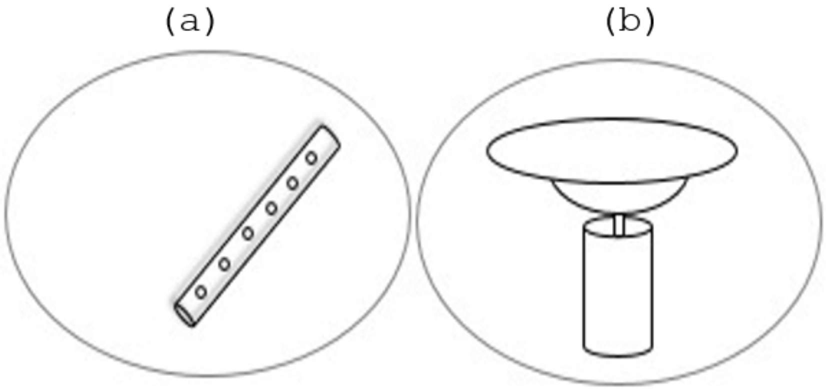

Figure 2: Illustration of (a) longitudinal and (b) mushroom type diffuser (Féris et al. 2000).

Hence, diffuser shape also influences the aeration efficiency, which in turn affects the overall system efficiency. Cheng et al. 2016b) investigated the effect of different microporous diffuser shapes – I, C, S and disc shape, on aeration performance and discovered that the I shape had optimal aeration whereas the disc shape had the poorest performance amongst the shapes investigated. Hence, understanding the design of air diffusers for improved flotation efficiency and low energy consumption by maximizing the design shape need not be understated as there is minimal information available on the design of diffuser shape and the shape influences on performance.

2.6.3 Influence of aperture/pore size on diffuser functionality

Fixed orifice nozzles and manual needle valves tend to limit the recycle flow to a small range under different operating pressures. With fixed nozzles, the recycling system must be designed to two or three operating points by having each orifice with its pressure inlet that can be controlled by varying pressure control valves. The orifices should be able to be switched on and off with minimized backflow when not in operation (Crossley & Valade 2006). Diffuser pores can be varied in size to affect energy consumption. Coarse bubble diffusers have higher energy consumption as compared to fine pore diffusers, which are energy efficient (Noble et al. 2016). Furthermore, pore size determines bubble size, which in turn affects the type of bubbles formed, with bigger bubbles having lower efficiency while smaller bubbles are determined to have a higher process efficiency (Rosso et al. 2008). Moreover, blockage of pores results in increased pressure on diffusers and reduced air supply channels, which is counteracted by increased airflow resulting in high energy cost with the consequential effect being the deformation of pore structure as well as breakage of diffusers. This can however be countered using a diverse pore structure and suitable materials of construction such as blended material containing additives for enhancement of chemical and mechanical properties (Eusebi & Battistoni 2014).

Microbubble generation has its own challenges as most would expect that the smaller the pore size of diffusers, the smaller the bubble size would be; however, this is not the case, as other factors such as the wetting force exerted by the liquid surrounding the diffuser surface acts as an anchor and as a result the bubbles keep growing unless the force is disrupted. This can affect the bubble size apart from the air releasing device aperture size (Zimmerman et al. 2009). The other challenge is the spacing between adjacent bubbles or pores in the diffusers, which lead to coalescence of bubbles or channelling (Zimmerman et al. 2008). Behnisch et al. (2018) investigated different designs of fine aperture pore membrane diffusers, focusing mainly on the slit length and density to improve the aeration system treating water. Diffusers with a dense slit pattern and smaller length were recommended for saline water, whereas in tap water a dense slit resulted in bigger bubbles due to coalescence. This observation showed that diffuser design affects the wastewater being treated and this is also dependent on the contaminants present in the wastewater (Behnisch et al. 2018). Bubble size generation by air diffusers cannot only be varied by use of different types of diffusers but also by other means that include the application of surface tension reducing chemicals, use of alternative pressurised air generation techniques and diffusers made of different materials of construction (Kyzas & Matis 2018); hence, it is important to improve the design and functionality of air diffusers as a way of mitigating a variety of challenges as mentioned above.

2.6.4 Materials of construction used in diffuser manufacturing

Materials of construction used early in the century were bonded silica sands but sometimes could be mixed material such as sand-cement plates. To improve aeration, early investigators focussed on the development and generation of fine bubbles. The materials of construction that they based their investigation on included porous volcanic rock (pumice), firebrick, sandstone, and mixtures of glass and sand. However, many of the mentioned materials were dense thus leading to increased pressure head loss and reduced efficiency (Boyle 2003).

Nowadays, air diffusers are mainly made from ceramics, porous plastics, and perforated membranes. Ceramic diffusers are made from a combination of silica, alumina, and aluminium silicates. The media consist of sized material particles that are mixed with bonding materials and are moulded into various shapes at a high temperature. This results in an interconnected network of passages whereby the air flows through the diffuser. Porous plastics for diffusers are made from propylene, polytetrafluoroethylene, and polyethylene for the rigid diffusers while high density polyethylene and rubber are used for non-rigid diffusers. They are fabricated in such a way that they all have a network of channels through which air travels. Similarly, perforated membranes are made from elastomers, and these are normally referred to as flexible diffusers (Shammas 2007). Other materials can be used but the above mentioned are cost-effective and can be produced in different sizes and specifications. However, they are prone to chemical degradation and biological fouling (Nadayil et al. 2015). Several factors determine or contribute to fouling, which include the suitable properties of the materials of construction of diffusers, the nature of the contaminants or the type of wastewater and operating conditions (Odize et al. 2017). The air diffusers are currently being coated using different coating materials that include polyurethanes and silicones that serve as antimicrobials, which however degrade with time (Garrido-Baserba et al. 2016); hence, finding materials that minimize biofilms attachment is a necessity.

The physical and chemical characteristics of construction materials used to produce diffusers each influence the workability of apertures in air diffusers, and thus bubble formation (Eusebi & Battistoni 2014). One of the challenges of bubble generation in relation to the material of construction is that the liquid surrounding the pore on the diffuser surface acts as an anchor, which aids the wetting force in the attachment of the growing bubble to the surface of the diffuser. The bubble will continually grow unless the anchoring force is disrupted or the buoyant force exceeds the anchoring force (Zimmerman et al. 2008; Zimmerman et al. 2009). Additionally, the mechanical properties of diffuser materials are characterized by parameters such as Young's modulus of elasticity and hardness, which affect the deformation of pore shape. This is used to predict breakage of diffusers, and this is further affected by the presence of additive and corrosive pollutants in the water being treated as they affect the material's stiffness and strength over time (Eusebi et al. 2017), thus making it important to select a suitable combination of materials of construction for a particular type of application.

Recently, Lee et al. (2019) investigated bubble generation at different entry and exit angles. Notably, the nozzles were synthesized using 3D printing specifically using acrylonitrile butadiene styrene (ABS) resin with an aluminium cover casing. Interestingly, can 3D printing be used to print air diffusers which are strong, durable, made with material that affects biofilm formation thus reducing biofilm attachment and formation while having properties to effectively design aperture sites for desired bubble size? These are pertinent questions that must be addressed in future diffuser designs. Thus, this is another niche area for future research.

2.7 Operating factors affecting diffuser design

2.7.1 Impact on microbial community removal and attachment

Constituents of wastewater pose dangers to both humans and the ecosystem. A greater number of organisms in wastewater are pathogens and viruses that can disrupt nature and make humans sick (Nadayil et al. 2015). Edzwald (2010) indicated that an effective flotation system can remove microorganisms such as *Giardia* cysts. Similarly, Andreoli & Sabogal-Paz (2017) investigated the removal of *Giardia* spp. and *Cryptosporidium* spp. using a combination of flotation and filtration systems. They discovered a higher removal of cysts and oocysts from the wastewater with the flotation system compared to the filtration method. Santos & Daniels (2017) previously used a bench-scale DAF to remove *Giardia* cysts from wastewater, but this was an ineffective method as it was dependent on flocculant supplementation. However, whether diffusers aid in the removal of microorganisms due to their materials of construction possessing antimicrobial properties still needs some further investigations.

Diffuser surfaces are a unique environment for biofilm formation due to the provision of an adherence surface, sufficient oxygen supply from the incoming air and adequate nutrients from the wastewater being treated. Additionally, operating process variables play a role in the formation of biofilms on diffusers. This includes levels of dissolved oxygen, the concentration of ions in the wastewater to be treated as well as the soluble substrate availability, which collectively promote biofilm growth (Garrido-Baserba et al. 2018). Consequently, sparger systems' attributes can favour fouling, which can be grouped into three categories that are biological, organic and inorganic fouling with biofouling or the development of biofilm on diffusers being of great concern (Meng et al. 2009). Organic matter can also contribute to the deposition of biopolymers (Odize et al. 2017) and inorganics can lead to the deposition of different cationic and anionic precipitates of chemical or biological nature, which could further exacerbate the fouling challenges (Wang et al. 2008). Additionally, biofouling is a result of deposition of small particles onto the diffusers leading to the formation of a cake layer, which is composed of particles built-up, thereby leading to flow resistance, thus loss of bubble generation efficiency (Harun & Zimmerman 2019). This leads to an increase of back pressure on the air pump or compressor (Rosso & Shaw 2015), resulting in decreased aeration efficiency. DWP increases with increasing time in operation (Zhao et al. 2004). This also results in an increase in bubble size because of material property alteration and leads to increased energy costs (Garrido-Baserba et al. 2016). Fouling can either be reversible or irreversible. Reversible fouling is because of loosely bound materials, which can be easily removed by physical cleaning processes, whereas irreversible fouling is a more complicated phenomenon and can cause clogging, which requires chemical cleaning for its removal (Odize et al. 2017).

Researchers have tried to chemically clean diffusers such that they are less vulnerable to fouling. However, challenges arise since diffusers are porous hence a continuous supply of cleaning chemicals is a challenge. Moreover, by using some coatings on the diffuser material of construction as having been used previously, some of these coatings are not resistant to cleaning chemicals, have poor durability and easily leach (Hamza et al. 1997). Zhao et al. (2004) investigated the effect of free energy of diffusers surfaces to minimize microbial adhesion. They used a nickel polytetrafluoroethylene (PTFE) coating and achieved a reduced microbial adhesion of 68–94%. To mitigate these challenges, routine cleaning-in-place of diffusers must be implemented. However, diffuser maintenance can delay or take operator's time, thus increasing downtime (Boyle & Redmon 1983); hence, innovative diffusers that require very minimal maintenance are needed. During disinfection, operations using oxidative micro and nanobubbles is deemed effective when compared to normal air bubbles produced through a porous material (Sung et al. 2017), as diffusers can be designed in such a way that they produce oxidative bubbles that will affect the microbial community in the wastewater while carrying out their normal flotation process. However, future research needs to investigate if additives such as silver nanoparticles that can be incorporated during diffuser production reduce the microbial load in wastewater being treated due to their antimicrobial properties.

2.7.2 Sparging rate influences

Sparging rate is important in the production of suitable microbubbles. It determines the bubble size, which influences residence time in the form of throughput rates of treated wastewater and rising bubble velocity with bigger bubbles having lesser residence time, and thus less bubble particle collision times than smaller bubbles, which leads to inefficiencies (Edzwald et al. 1992). Gas flow rate determines the air bubble quantity that goes into a flotation system, whilst affecting the bubble size and the flow pattern in column flotation; hence, an averaged flow rate is advised (Liu et al. 1999).

Also, the pressure loss of a system determines the energy consumption, especially for diffusive aeration. This is usually an indicator of clogging and fouling that further causes increased back pressure on the diffusers, thus indicating reductions in the performance of the system. The pressure loss can be estimated using Equation (1) (Krampe 2011).

$$\Delta P = P_T - P_D - P_P \tag{1}$$

Where:

ΔP = Diffuser pressure loss,

P_T = Pressure of the total air supply,

P_D = Hydrostatic pressure resultant from injection depth, and

P_P = Pressure loss of valves and/or pipes.

Hence, when designing diffusers, properties that reduce chances of clogging and fouling thus minimising pressure loss and the need for increased gas flow rate should be considered.

2.7.3 Suspended solids loading rate influences

The suspended solid loading rate is an important factor in flotation system operation as it determines the quantity and concentration of suspended solids (flocs) to be removed. For example, suspended solids being fed into the tank per minute (Al-Sabagh et al. 2015) can affect the performance of the flotation system, which in turn affects the air-solid ratio, which can be estimated by Equation (2).

$$\frac{A}{S} = \frac{1.3S_a(fP-1)}{X} \tag{2}$$

Where:

$\frac{A}{S}$ = air-solid ratio (Kg air/Kg suspended solids in the feed),

S_a = air solubility (mL/L),

P = operating pressure (Kg/ cm^2 or Pa),

f = pressurisation system efficiency at pressure 0.8, and

X = influent solids concentration (mg/L).

A/S ratio is the measure of the released quantity of air per solid mass available in a flotation cell. Air-solid ratio affects the collision frequency and buoyancy velocity of particle-bubble formations, including the suspended solids removal rates, which influences the overall performance of a flotation system. Various A/S ratios have been used in different applications (see Table 3).

Table 3: Examples of the required A/S ratios used in different applications.

A/S ratios	Applications	References
0.05–0.22 kg air/kg solids	Iron hydroxide precipitation	Féris et al. (2001)
0.027–0.066 kg air/kg solids	Wastewater treatment (milk industry)	dos Santos Pereira et al. (2018)
0.015 kg air/kg solids	Textile effluent treatment	Pioltine & Reali (2011)
0.054–0.078 kg air/kg solids	Poultry slaughterhouse wastewater treatment	de Nardi et al. (2008)

Removal efficiencies will be negatively affected if less than the required amount of air is supplied whereas energy will be wasted if too much air is supplied; hence, an optimum supply should be employed thus making the A/S ratio an important parameter to be considered when designing air diffusers and flotation systems (Othman et al. 2021). Flotation systems have no tool that satisfactorily expresses it due to system complexity and many unknowns (Tai & Doo 1997; El-Gohary et al. 2010); therefore, designing of diffusers that distributes sufficient air is a necessity especially with the increased quantity and reduced quality of wastewater needing treatment.

2.7.4 Hydraulic loading rate

Flotation systems are limited by operating costs, complex operating process variables, maintenance, and optimisation. However, diffuser design has led to technological developments, which in turn resulted in increased hydraulic loading rate (HLR). HLR of up to 30 mh1 for high-rate flotation systems have been reported (Edzwald 2010; Azevedo et al. 2017). However, with such design improvements over the years, it is important to consider improvements in relation to the rising velocity of bubbles when designing the diffusers to avoid bubble floc aggregate disintegration (Maeng et al. 2017).

2.8 Overall remarks on diffuser design and its use in a column flotation

This review allowed for identification of the gaps within diffuser design literature in relation to their physical design aspect and how operational parameters affect it. Importantly, at some instances, questions have been raised and suggestions have been mentioned. However, there is minimal information on the design of air diffusers as most research is focused on hydrodynamic properties rather than the air releasing device’s properties and configurations including design. The review of diffuser design has highlighted that:

- Significant progress has been made to improve the efficiency of the diffusers; that is, new shapes have been introduced and materials used for construction has been varied including being mixed, but the diffusers are still inadequate to meet the process efficiency for purposes due to increased quantity and reduced quality of wastewater to be treated; hence, the need for improvement need not be understated.
- The challenges associated with air diffusers and their applications are known, and these include fouling, clogging, poor system efficiency and breaking, amongst others, thus reflecting the need to improve their design.
- The importance of understanding the effect of the diffuser design on operational parameters and the factors that are considered important when designing diffusers; that is, cost, durability and robustness using appropriate materials of construction.
- Process variables (sparging rate, suspended solid loading rate and hydraulic loading rate) should be considered when designing diffusers as they have an effect on the efficiency of the diffusers.

- Addition of additives to the diffusers can give them extra properties such as antimicrobial activities while carrying out their normal aeration/sparging processes.

Therefore, future work can:

- Explore whether the application of new technology such as 3D printing of air diffusers for use in a column flotation system can provide for suitable bubble size production that can lead to reduced energy consumption and a simplified system.
- Designing air diffusers that have oxidative air generating properties, which might affect the microbial community present in the wastewater being treated, thus reducing the rate of fouling and improving the quality of treated water in terms of reduced microbial load while carrying out the normal flotation process.

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CHAPTER 3

3D printed vis-à-vis molded spargers for application in air flotation systems: An exploratory study

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CHAPTER 3

3 3D printed vis-à-vis molded spargers for application in air flotation systems: An exploratory study.

3.1 Introduction

Air flotation is a separation method based on gravity (Al-Dulaimi & Al-Yaqoobi 2021). Air flotation systems had their origin in mineral processing field; however, they are now being applied in other fields such as recycling of organic compounds, oil water separation and wastewater treatment (Li *et al.* 2016). Air bubbles are a key element in the effective functioning of a flotation process. The rising air bubbles are responsible for the capture and suspension of colloidal particles, lifting the bubble-particle aggregates to the top of the flotation system to form a concentrate, which will then be subsequently scrapped off (Ding *et al.* 2022). Hence, sparging is important for this process. Overall, sparging has advanced over time, from traditional/ancient mechanical ways such as mechanical surface scrappers to modern fine bubble spargers (Moga *et al.* 2019), and so have the methods of production of these sparging devices.

A sparger is a device that distributes air bubbles into the liquid through pores by creating dispersed air bubbles (Ham *et al.* 2021). Spargers are devices that produce air bubbles when compressed air is forced through their pores under submergence in a liquid (Kyzas *et al.* 2021). Some of the reasons why spargers are used in flotation processes is due to their controllable bubble size and availability. Spargers do have drawbacks, which include energy consumption, clogging, pressure loss, and many others (Fagkaew *et al.* 2022). Hence, the design and study of spargers is of paramount importance to overcome some of the drawbacks. This has led to the exploration of new sparger manufacturing methods such as 3D printing.

3D printing is a technology that is currently emerging or on the rise. It is a process whereby a three-dimensional object is made from a digital file (Bobyr *et al.* 2019). It uses the layer-by-layer fabrication method to create objects. Various 3D printing techniques are used to process different kinds of materials. However, these are grouped into seven process categories namely, photo-polymerization, material jetting, material extrusion, direct energy deposition, sheet lamination, powder bed fusion, and binder jetting. They are also grouped according to the form of feed material used, i.e., solid, liquid, and powder-based printers. 3D printing is being applied in various industries which include biomedical, building and construction, food and water, and wastewater industries (Lee *et al.* 2016). Direct energy deposition and powder bed fusion are the two most commonly used methods in 3D printing of metallic materials (Krakhmalev *et al.* 2018). For the present study, Laser Powder Bed Fusion (LPBF) was used for the manufacture of 3D-printed spargers. LPBF makes use of laser beams to melt and fuse metal powder layer by layer to form parts (Li *et al.* 2022). The sintering technique of metal powders is also considered to be versatile and efficient when fabricating metal elements such as membranes and spargers (Allioux *et al.* 2017). Sintered stainless steel metal spargers are fabricated using powdered metal, which is fused because of subjection to heat just below the melting point of the metal (Hobert 2015).

In this study, the application of 3D-printed spargers was explored to determine their field applicability assessing pore complexity and structure in comparison to sintered/molded spargers. Microstructures and pore orientation were studied through metallurgical analysis.

3.2 Objectives

Objective 1: To ascertain the feasibility of manufacturing diffusers using additive manufacturing technology,

Objective 2: To evaluate the properties of the manufactured diffusers compared to existing diffusers (molded/sintered steel air diffusers).

3.3 Materials and Methods

3.3.1 Production of 3D printed spargers

The additive manufacturing system type used in the manufacture of spargers in this project is Laser-Powder Bed Fusion (LPBF). It operates by printing parts through melting a fine layer of metal powder using a laser beam under an inert gas atmosphere. This powder is first melted onto a solid substrate made of the same material. After melting of the selected regions of the powder- driven by a ‘sliced’ CAD model of the sparger, the next layer is then deposited onto the substrate. This process keeps repeating layer by layer until the full height of the sparger is built. Once complete, the un-melted powder is removed, and the sparger is cut from the substrate for cleaning, inspection and other post processing procedures. The 3D printed spargers were made from 316 stainless steel powder which was gas atomized and spherical with a specific particle size distribution.

3.3.2 Production of moulded spargers

The moulded spargers were made using 0.2 of 316L porous micron stainless steel powder. The powder was added to a cup with the shape of the desired spargers and pressed. This was followed by a sintering process which is basically the application of heat just below the melting point of the metal such that the particles bond and hold thus forming the new shape (Hobert 2015).

3.3.3 Specifications of spargers

The spargers had a porous part, which was 1 inch long in length, 0.5 inch in width, stainless steel connecting tube that was 1 inch long as well with an opening of 0.125-inch diameter. The spargers were made of 316 and 316L stainless steel with a media grade of 0.2 for 3D printed and moulded spargers respectively. Moulded spargers had a shiny smooth final finish whereas the 3D printed spargers had a rough finish.

3.3.4 Air sparger property analysis

3.3.4.1 Cutting of the spargers

Both the 3D printed and moulded spargers were held by a bench vice and were cut into several sized pieces using a hacksaw. They were also cut using a Mac-Afric Bench Lathe (MacAfric Manufacturing, Johannesburg, South Africa). The connector and the welded part were discarded, and the other pieces were used for further evaluations. The cut pieces were polished using a Mac-Afric Bench Lathe and a metal filler.

3.3.4.2 Mounting of the spargers

The cut pieces were mounted (formed into a mould) using the hot mount machine (Struers Labopress-3, Struers, Copenhagen, Denmark) for ease of grinding. Mount stick powder was spread onto the holder to avoid the mould from sticking to the stage. Thereafter, the cut pieces were laid onto the holder with the sides protruding horizontally and vertically. The ram was lowered to 40mm down and black phenolic resins were carefully added ensuring the samples do not topple. After filling, the ram was dropped all the way down and the opening was closed. The hot mount machine was set at a force of 20kN with heat treatment at 180 ℃ for 7 mins and cooling for 7 mins. The ram was pushed up until it stopped and then the start button was pressed. When the whole process was completed, the moulds were removed and labelled.

3.3.4.3 Grinding and polishing of the sparger samples

The embedded sparger samples were grinded and polished using a grinding machine, i.e., the Struers LaboPol-5. Grinding was performed using Rhaco grit grinding discs (Akasel) with abrasive grain size of P800 and P1200, respectively. Water was applied after every 30 seconds to act as a coolant to avoid the overheating of the samples and to ensure a constant surface finish. Post completion of the grinding process, the grinding paper was removed and the platen was cleaned thoroughly with water so that the polishing step can follow. For polishing a Moran pad with a flocked woven cloth was used along with polycrystalline diamond suspension with the following grain sizes; 6µm, 3µm, 1µm (Akasel) and a MasterPrep polishing suspension of 0.05 µm grain size (Buehler), respectively. Ethanol was used as the lubricant. In between steps the grinded or polished samples were checked under a Zeiss Stemi DV4 stereo microscope (Carl Zeiss AG, Oberkochen, Germany) at a low magnification for scratches and also to ensure that the desired surface finish has been achieved. Scratches must be of the same size and same direction. After every grinding or polishing step, the samples were sonicated in an ultrasonic bath for 2 mins (with water for grinding step and with ethanol for polishing step) to remove grit and also to avoid cross contamination. After sonication, the samples were rinsed and dried using a dryer.

3.3.4.4 Etching of the sparger samples

The 3D printed sparger samples were etched using aqua regia solution and the moulded sparger samples were etched using glyceregia. The whole surface of the resin was immersed with the etchant. After a set period of time the etchant was removed, and the samples were rinsed with distilled water and under running water from the tap. Samples were further rinsed with ethanol and blow dried using a dryer.

3.3.4.5 Sparger property analysis

After the samples were embedded into black phenolic resins, they were then grinded, polished and etched. The resultant samples were viewed under an AE2000 Trinocular 100W-Motic Inverted Metallurgical microscope (MoticEurope, Barcelona, Spain) for microstructure analysis, tested for vicker's hardness using an Innovatest Falcon 500 (Innovatest, Maastricht, The Netherlands) and were also analysed for surface topography and composition using Nova NanoSEM 230 scanning electron microscope (FEI Company, Hillsboro, United States) and energy-dispersive spectroscopy.

3.4 Results and Discussion

3.4.1 Feasibility of air sparger manufacturing

The methods and materials used for the manufacture of spargers have been through numerous modifications and development over the years. However, the main goal has been to improve the quantity and characteristics of pores (Atwater *et al.* 2018) so as to improve performance. The results of manufacturing spargers using 3D printing turned out to be positive and the spargers were porous, hence, a success. However, there were some shortfalls or limitations which needs to be worked on in future studies to refine them.

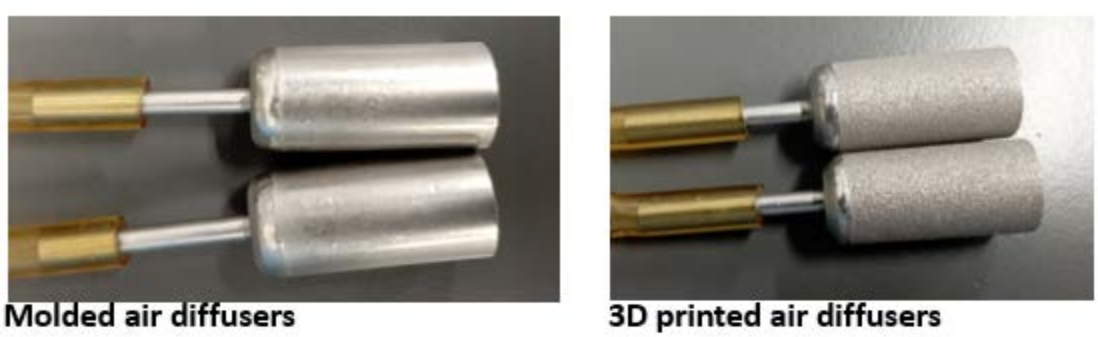


Figure 3: Photographic illustration of 3D printed spargers vs moulded spargers.

Overall, both the 3D printed, and the molded spargers had similar specifications. These included the dimensions of the spargers and the particle size of the different material powders used. It was observed that the finished product (molded spargers) had a shiny finish whereas, the 3D printed ones had a rough finish (refer to Figure 3). This could have been attributed to the different methods of manufacturing.

This is in agreement with (Chen *et al.* 2021), who found out that the surface finish of the 3D printed spargers was rough, which was proven to be a limitation of the LPBF process used.

For preparation of spargers for physicochemical property analysis, it was found that 3D printed air spargers were hard to cut as compared to the sintered ones. However, this was theoretical, and it contradicted with the experimental results from the hardness test as shown in Figure 4. The Vickers hardness results for 3D printed spargers was 139.15HV0.5/10 whereas for sintered, it was 207.75HV0.5/10. This could have resulted from porosity and due to the heat treatment effects involved in the different manufacturing methods used.

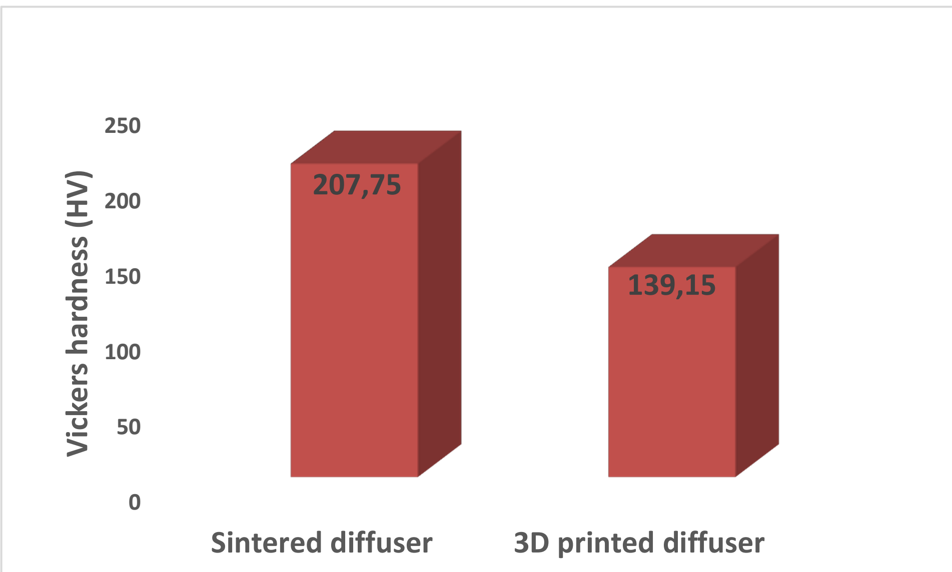


Figure 4: Micro hardness test results for the spargers.

The type of stainless steel used for the molded spargers was 316L and the 3D printed ones were manufactured using 316 stainless steel. This steel had different chemical composition as shown by the EDS results - Table 4. This means the steel involved is an austenitic stainless steel.

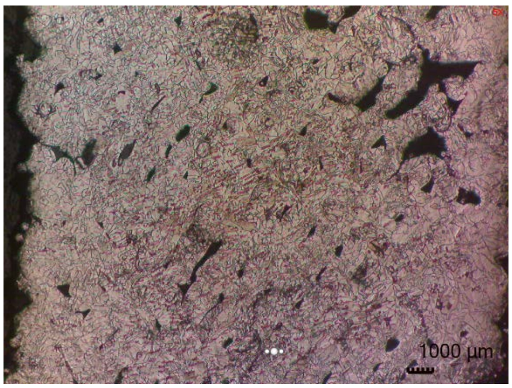
Table 4: Chemical composition of the 316 and 316L sintered steel and 3D printed spargers (wt%).

Air sparger	**Spectrum**	**C**	**Si**	**Cr**	**Mn**	**Ni**	**Mo**	**Fe**	**Total**
316L sintered steel	Mean	≤0.03	1.05	16.44	-	9.8	2.51	bal	100
316 3D printed		≤0.08	0.55	17.57	0.94	11.72	2.77	bal	100

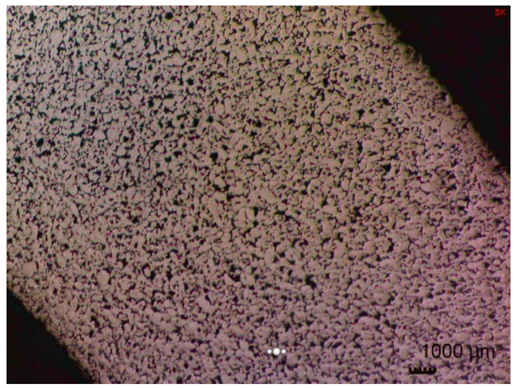
NB: All results in weight %. C=carbon, Si=Silicon, Cr=Chromium, Mn=Manganese, Fe=Iron, Ni=Nickel, and Mo=Molybdenum

3.4.2 Microstructure Characterization

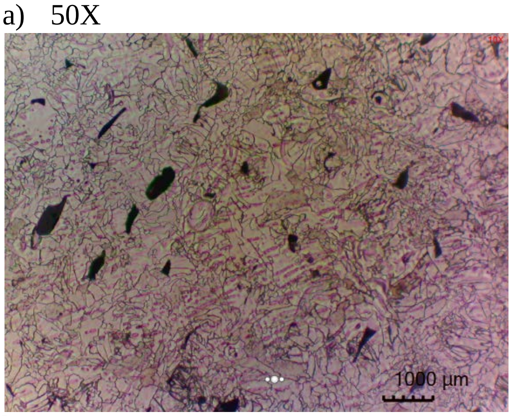
Recently 3D printing has been showing considerable progress in the fabrication of complex shaped parts/items for use in industries (Zhang & Zhang 2019). The spargers that were produced were supposed to be porous thus making them complex. The micrographs from the optical microscopy shown in Figure 5(a-d) depict that the 3D printed spargers were indeed porous.



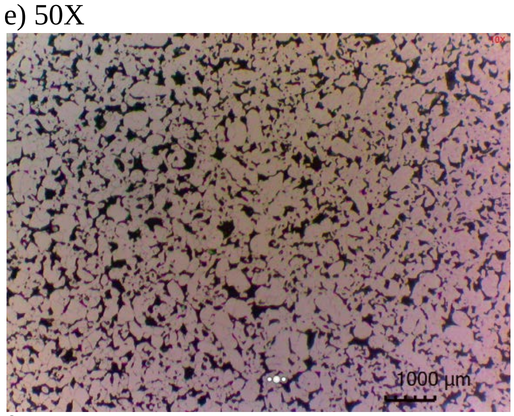
a) 50X



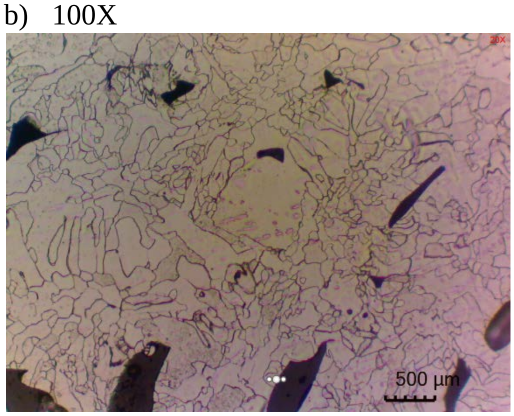
e) 50X



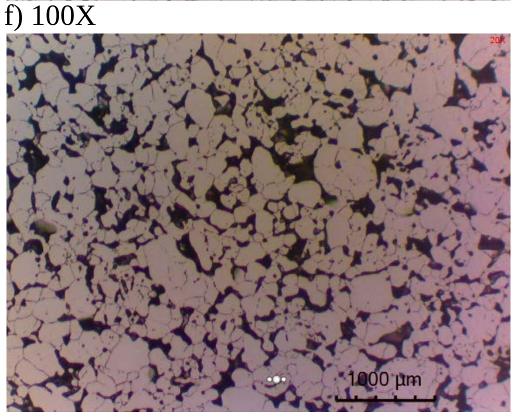
b) 100X



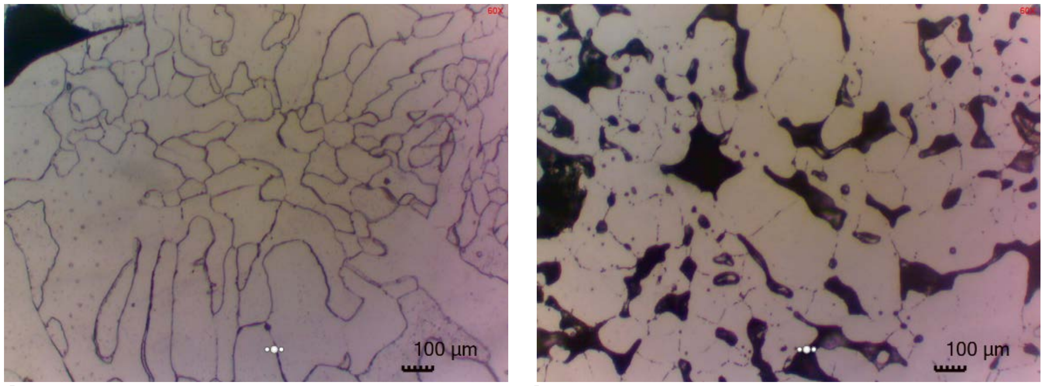
f) 100X



c) 200X



g) 200X

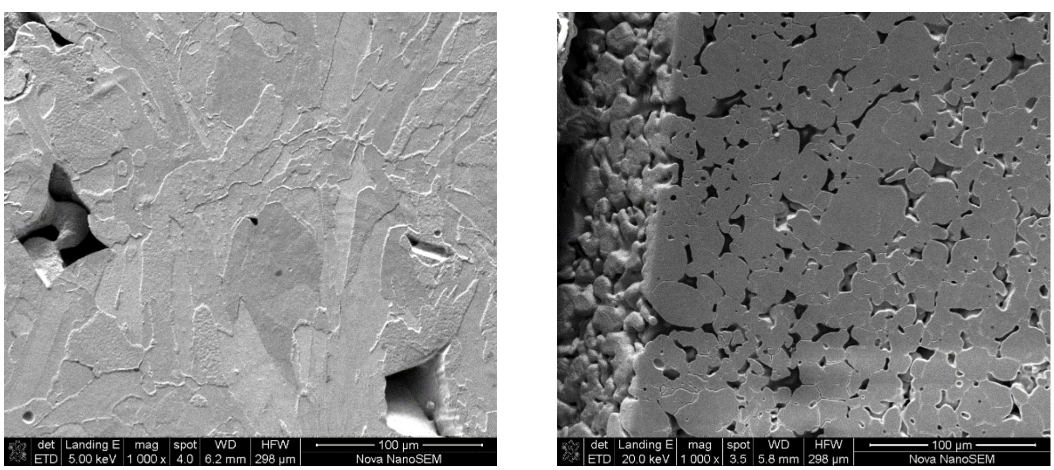


d) 500X h) 500X

Figure 5: Micrographs 3D printed spargers (a, b, c, and d) and molded spargers (e, f, g and h) viewed using a Motic AE2000MET Inverted Metallurgical Microscope under various magnifications.

The pores for the 3D printed spargers were irregularly shaped, big and not evenly dispersed. Their size varied. However, when compared to sintered spargers, the spargers were very porous. The pores were small, and some were irregular whilst others were spherical. The optical micrographs for 3D printed spargers also show that a columnar dendritic structure was present. Dendrites form as the layers are being added and the object is growing during the solidification process which takes place along the heat gradient within a melt pool as part of the LPBF manufacturing process (Sabzi 2022).

SEM micrograph in Figure 6 further confirmed that the 3D printed spargers had fewer pores which are significantly larger in size as compared to the molded spargers which had a higher concentration of smaller pores. This will have an effect on the performance of the spargers as it affects the size of the air bubbles produced and ultimately the rising velocity.



a) d)

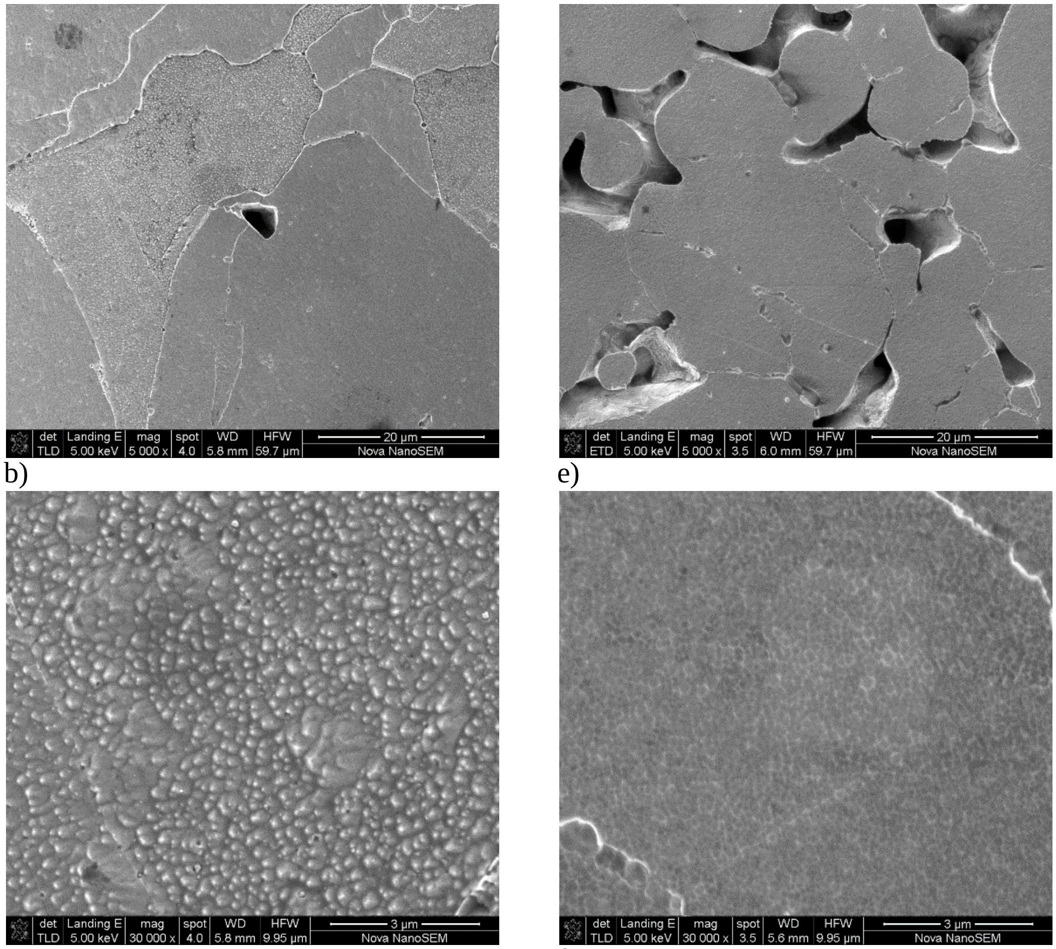


Figure 6: SEM micrographs of 3D printed spargers (a-c) and sintered spargers (d-f) at various magnifications.

Furthermore, the 3D printed air spargers had a bigger grain structure as shown in Figure 4.c which were not uniform. The molded spargers had small equiaxed grains as depicted by Figure 4.f. This is attributed to the method of manufacture. The difference in microstructure was attributed to the different processing conditions used in the manufacturing methods. Microstructure control has been proven to be difficult during LPBF as a result of a high thermal gradient and the high cooling rate of the process as compared to molding whereby subsequent thermo-mechanical processing is applied to carefully control the microstructure (Sabzi 2022). Considering that the major function of spargers is to release air bubbles (Cohen *et al.* 2017), the design and manufacture of air spargers is very important as this influences their microstructure which in turn influences air distribution and control of bubble size thus having an effect on their overall performance.

3.5 Summary

The application of 3D printing in the manufacture of spargers has proven to be feasible as the resultant spargers were porous which is the major property of spargers as their function is to produce air bubbles. On the other hand, the sintered steel spargers had a better microstructure as it had dense pores which are favorable for a flotation process. To overcome certain limitations such as limited number of pores and pore size consistency, new generation alloys need to be explored. Furthermore, there is a need for optimization of 3D printing such that resultant spargers are effective than those produced by current conventional methods.

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CHAPTER 4

Bioflocculant Producing *Bacillus megaterium* from Poultry Slaughterhouse Wastewater: Elucidation of Flocculation Efficacy and Mechanism

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CHAPTER 4

Bioflocculant Producing *Bacillus megaterium* from Poultry Slaughterhouse Wastewater: Elucidation of Flocculation Efficacy and Mechanism

4.1 Introduction

Flocculation is a method that promotes solid-liquid separation. It is a cheap and efficient way of aggregating colloidal particles to form bigger flocs that can be separated from wastewater (Jiang et al., 2021; Liu et al., 2020). Flocculation is widely used as a separation technique in various floc removal facilities. Flocculation is achieved by using flocculants to extract insoluble substances from a suspension (Muhammad et al., 2023), as they enable dispersed particles/colloids to aggregate. They aggregate pollutants that are fine and subtle and form bigger flocs. Such pollutants are usually challenging to get rid of as they tend to be suspended in the liquid medium instead of settling or floating at the top; hence, the application of flocculants to make them heavier and more prominent, thus easier to separate (Bakar et al., 2021).

Flocculants are characterized based on their chemical composition and are grouped into three categories, namely i) inorganic, ii) organic, and iii) natural occurring (Selepe et al., 2022). Inorganic flocculants are mainly salts such as alum. They are cheap but highly dependent on operating conditions. Additionally, chemical flocculants produce by-products with high metal concentrations that are hazardous to both the environment and humans, leading to the divergence of attention to flocculants of microbial origin (Okaiyeto et al., 2020), referred to as bioflocculants. On the other hand, organic synthetics are suitable in low dosages but have degradability problems (Jiang et al., 2021). Thus, organic and inorganic flocculants are harmful. In contrast, plant-based flocculants in the natural flocculant group are inconvenient to produce due to climate and ecological changes, leaving bioflocculants as a promising alternative (Selepe et al., 2022). Bioflocculants have excellent properties, which include low to non-toxicity, low concentration usage, and adaptability to a wide range of pH levels (Jiang et al., 2021).

Bioflocculants are extracellular polymeric substances (EPS) naturally synthesized by microorganisms (Ma et al., 2020). These metabolites are secreted during the growth of microorganisms and cell lysis. Many microorganisms such as fungi, algae and bacteria have shown the ability to produce bioflocculants (Muhammad et al., 2023) that are produced through microbial fermentation. This process is affected by various factors, including nutrient availability and composition, physicochemical parameters, and culture conditions (Mohammed & Dagang, 2019). The polymeric characterization of bioflocculants varies in terms of their composition of proteins, polysaccharides, polyamino acids, and sugars, with the polysaccharides and proteins receiving the most attention, as they constitute the most significant proportion of the flocculants and consequently influence their flocculation activity (Bakar et al., 2021; Gosai & Nardkar, 2022). Various bioflocculation mechanisms are currently accepted, including sweeping, adsorption bridging, chemical reactions, and change neutralization (Pagliaro et al., 2021). The flocculation mechanisms of chemical flocculants are well known, but the mechanism related to bioflocculants needs to be fully explored (Artifon et al., 2021).

According to the green chemistry analogy, chemical flocculants are unfavorable because they are environmentally hazardous; therefore, the need for safer eco-friendly alternatives does not need to be emphasized but is a necessity; consequently, bioflocculants are gaining popularity. Despite this, bioflocculants are deemed not ideal for use on an industrial scale because of their high production costs, low yields, and poor flocculation efficiency (Liu et al., 2023). Therefore, it is of the utmost importance to screen for novel bioflocculant-producing microorganisms that produce bioflocculants with high flocculating capabilities and higher yields and understand how they function, including their optimal conditions so that they can be applied on an industrial scale. The main aim of this study was to isolate a bioflocculant-producing strain from poultry slaughterhouse wastewater, focusing on selecting the strain with the highest flocculation activity level. The inquiry included an analysis of many characteristics associated with the bioflocculant produced, including its storage conditions and the ideal parameters for flocculation.

Furthermore, a comprehensive understanding of the flocculation mechanism has been achieved, shedding light on the operational principles of bioflocculants obtained from microorganisms in large-scale industrial applications. This knowledge is expected to provide significant benefits in terms of cost reduction in downstream bioflocculant processing. This study was conducted with the following specific objectives: (1) To isolate and identify microorganisms that produce bioflocculants that can be utilized for particle flocculation (2) To assess and characterize the flocculants including storage conditions of cell-free bioflocculants, free and bound bioflocculant, and (3) To elucidate the flocculation mechanism of the bioflocculants.

4.2 Objectives

Objective 1: To isolate and identify a microorganism that produces bioflocculants to be applied in a BioCF for particle flocculation purpose.

Objective 2: To assess and characterize the bioflocculants including their storage conditions.

Objective 3: To elucidate the flocculation mechanism of the bioflocculants.

4.3 Materials and Methods

4.3.1 Microbial isolation

Twenty (n = 20) morphologically different bacterial microorganisms were isolated from swabs and the poultry slaughterhouse wastewater (PSW) collected from the drainage port of a poultry slaughterhouse in Cape Town, Western Cape, South Africa, which slaughters about 1 million birds per week, and then stored at 4 ℃ before use. The samples were subjected to repeated dilution, resulting in a dilution factor of $\times 10^8$. This approach was used to enhance the prospect of obtaining distinct colonies. A volume of 100 µL obtained from the preceding four dilutions were used for spread plating onto nutrient agar (31 g/L, Biolab, Diagnostics Laboratory Inc Budapest, Hungary). The plates were inverted and incubated at 36 ℃ for 24 hr. After the incubation period, colonies were distinguished based on their physical appearance, which included color, appearance, texture, and form. Different and single colonies were picked and subcultured onto fresh nutrient agar plates using a streak plating technique. Continued sub-culturing occurred until pure cultures were obtained (Kurniawan et al., 2021). The pure culture plates were coded in alphabetical order and numbered. Bacterial isolates were screened for bioflocculant production using a standardized method by determining flocculation activity (Maliehe et al. 2019). The isolate with high flocculation activity was characterized using morphological features (structure, color, Gram reaction) with an Olympus light microscope (Olympus CX21 FS1, Tudortech Pty Ltd, Bryanston, South Africa) at a ×100 magnification. Isolate D2 was the selected strain and was used for further experiments.

DNA was isolated from the bacteria (isolate D2) using Zymo's Bacterial DNA extraction kit (Zymo Research LLC, California, USA) and a Thermo Fisher Scientific (Johannesburg, South Africa) DreamTaq™ DNA polymerase and universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' CGGTTACCTTGTTACGACTT 3') for forward and reverse reactions, respectively, were then used for amplification of the target 16S region. The amplicons were gel extracted using Zymo's Zymoclean™ Gel DNA recovery kit (Zymo Research LLC, California, USA) and sequenced by the ABI PRISM™ 3500xl Genetic Analyzer (Thermo Fisher Scientific, Johannesburg, South Africa). The DNA was cleaned to remove post-cycle sequencing reaction contaminants using Zymo's ZR-96 DNA Sequencing clean-up kit (Zymo Research LLC, California, USA), followed by sequence analysis using a QIAGEN CLC Main Workbench v.7 (QIAGEN Sciences LCL, Maryland, USA). The resultant 16S rDNA was used to determine most similar sequences using the Basic Local Alignment Search Tool (BLAST) via the National Centre for Biotechnology Information (NCBI) databases (https://www.ncbi.nlm.nih.gov/). For RpoD, the primer sequence for the targeted region was as follows: RpoD-F 5'ATCGCAAAACGGTATGTCG3' and RpoD-R 5'TCTTGTACGRCCRTCATCAAG3' (Zymo's Bacterial DNA extraction kit, Zymo Research LLC, California, USA), and the same procedure was followed.

4.3.2 Bioflocculant production

The bioflocculant production medium was similar to the one used by Humudat et al. (2014). It consisted of 3.5 g $CaCl_2$, 0.5 g glucose, 0.5 g K_2HPO_4, 0.1 g $MgSO_4.7H_2O$, 0.05 g NaCl, 1 g $(NH_4)_2SO_4$, 2.5 g peptone powder and 0.5 g yeast extract in 500 ml distilled sterile water. The medium was filter sterilized using 0.20μm sterile membrane syringe filters (Merck Millipore, Burlington, Massachusetts, USA) as it contained some heat-sensitive compounds. A loopful of bacteria was inoculated into 50 ml of bioflocculant production medium and incubated in a shaking incubator (Labwit ZWYR-240 shaking incubator, Labwit Scientific, Australia) at 36.5 ℃ under 121 rpm for 24 hours. These culture conditions were based on the previous work by Mukandi (2017). After the incubation period, 5 ml of the bacterial suspension was used as an inoculum to inoculate 45 ml of the bioflocculant production medium, which was further incubated in a shaker incubator under the same conditions as the inoculum. The resultant fermentation culture broth was used for further experiments.

4.3.3 Flocculation activity

Flocculation activity was quantified according to Maliehe et al. (2019) using a kaolin clay suspension of 4 g/L. Kaolin suspension (50 ml) was aliquoted into 250 ml Erlenmeyer flasks and mixed with 1.5 ml of $CaCl_2$ (1% w/v) and 1 ml of crude (centrifuged) bioflocculant sample. A control was prepared the same way but with the bioflocculant being replaced by a sterile bioflocculant production medium. The mixture was swirled and transferred into a 50 ml glass measuring cylinder. This mixture was left standing for 5 minutes at ambient temperature to settle. A sample of the top layer was withdrawn, and its optical density was read at 550 nm using a spectrophotometer (Jenway 7305 Spectrophotometer, Bibby Scientific Ltd, Staffordshire, United Kingdom). The flocculation activity was calculated using Eq. 1, with the quantification being done in duplicates, and the average was used for reporting results.

$$\% \, \boldsymbol{Flocculation\ Activity} = \frac{\boldsymbol{A-B}}{\boldsymbol{A}} * \mathbf{100} \tag{1}$$

Where:

A = absorbance of control, and

B = absorbance of sample.

4.3.4 Bioflocculant extraction and purification

The fermentation broth from above was centrifuged at 4000 rpm using a megafuge (Heraeus megafuge 1.0, Gemini bv, Apeldoorn, Netherlands) for 30 minutes to separate the cells from the supernatant. After that, the supernatant was mixed with chilled (4 °C) ethanol in a ratio of 1:2. The supernatant-ethanol mixture was swirled and then centrifuged further at 4000 rpm for 30 min to precipitate the crude bioflocculant. The supernatant was discarded, and the pellet was dialyzed against distilled sterile water overnight. After dialysis, water was discarded, and the pellet was vacuum-dried using a desiccator. The dried crude bioflocculant was used for functional group analysis using a Fourier transform infrared (FTIR) spectrometer.

4.3.5 Characterization of bioflocculant produced by FTIR

The main functional groups of the bioflocculant produced by Isolate D2 were identified using FTIR (Spectrum Two FTIR spectrometer, PerkinElmer Inc., Waltham, USA). The absorption spectrum was recorded in the range of 4000 – 500 cm^{-1}. An IR spectrum database was used to analyze the organic compound.

4.3.6 Bioflocculants stability analysis

The effect of bioflocculant stability under different storage conditions over time was studied on a kaolin flocculation system by evaluating the flocculation activity of samples stored at room temperature, chilled samples, and frozen samples every 5 days for one month. Crude bioflocculant samples were aliquoted into Eppendorf and stored in their respective storage conditions (bench for room temperature, fridge at 4 ℃ for the chilled sample and freezer at -18 ℃ for the frozen one). The samples from the freezer were thawed at room temperature prior to use, after which the flocculation activity method was implemented.

4.3.7 Response surface methodology (RSM) experimental design

As part of elucidating the flocculation mechanism of the bioflocculant produced by D2, charge neutralization, and floc formation were studied. Design-Expert (v. 11) was used to generate the statistical design of experiments. Central Composite Design (CCD), a widely adopted experimental design technique that allows researchers to efficiently explore the response surface and identify the optimal combination of factors, was used to optimize parameters (pH and bioflocculant concentration, i.e., dosage) being evaluated. Two numerical factors [pH (4 - 9) and bioflocculant dosage (1 – 3% (v/v))] were optimized. Experimental runs (n = 13, see Table1 in results section) were generated, and the generated conditions were used for zeta potential experiments. Zeta potential (mV), the response variable (Y), was fitted into a second-order model in the form of a quadratic polynomial equation to correlate it to the independent variables. The 3D graphs were generated directly from the software.

4.3.8 Zeta potential

The surface charge/zeta potential of the particles was measured at pH, and bioflocculant dosage values determined by RSM during the experimental design. The procedure for flocculation activity was followed with minor changes. Kaolin clay suspension was prepared, and 50 ml was aliquoted into 250 ml Erlenmeyer flasks. The pH of each suspension was adjusted using 1 M of NaOH or 1 M of HCl to the values predetermined by CCD. 1.5 ml of 1% (w/v) of $CaCl_2$ was added to the suspensions, excluding those tested without $CaCl_2$. Predetermined dosages of bioflocculants were added to their respective flasks with the mixture of kaolin suspension and $CaCl_2$. After that, the flasks were gently swirled, and the contents were poured into 50 ml measuring cylinders and left to settle for 8 min. After settling, the top layer of the supernatant was carefully withdrawn for zeta potential measurements using a Zetasizer Nano ZS (Malvern Panalytical Ltd, Malvern, United Kingdom). The measurements made were for:

1. Kaolin clay before pH adjustments at various pHs,
2. Kaolin clay suspension with $CaCl_2$ at various pHs, and
3. Kaolin clay suspension with $CaCl_2$ and bioflocculants at various pHs and dosages.

Additionally, a loopful of the suspension was quickly recovered after addition into measuring cylinders and fixed onto slides for visual observations of floc sizes and formation under an electron Olympus CX21 FS1 microscope.

4.3.9 Bonding type determination

The bonding type of bioflocculant produced by isolate D2 was carried out using three types of chemical treatments, i.e., urea, HCl, and EDTA-Na_2. The bacterial flocculation activity described previously was carried out. However, the absorbance was measured after the settling period, and the supernatant was carefully removed. Chemical solutions of 5M urea, 0.5M HCl, and 10 mM EDTA-Na_2 were each added to a measuring cylinder with flocs in a manner that disturbs the flocs that had settled. The measuring cylinders were then slightly swirled and left to settle for a further 5 min. The absorbance (A_{550nm}) was measured, and qualitative observations were made (He et al., 2010).

4.4 Results and Discussion

4.4.1 Identification and characterization of the microbes

Twenty microorganisms were isolated from PSW and were screened for bioflocculant production using a kaolin clay suspension for flocculation activity assessments. Isolate D2 produced bioflocculants with the highest activity compared to the flocculants produced by other isolates. Its morphology was circular with cream-white colonies. A gram test showed a rod-shaped, arranged in linear sequences, and tested Gram-positive. The 16S rDNA results predicted that it was a Bacillus subspecies, *Bacillus aryabhattai*, or *Bacillus megaterium* with 100% identity. For a group of Bacillus subspecies, sequencing the universal 16S rRNA region, which is usually expected of all bacteria, does not discriminate between them, hence making it difficult to identify them (Blackwood et al., 2004). However, since the 16S gene did not sufficiently distinguish between the different species of *Bacillus*, for better resolution, RpoD was employed, which was able to distinguish well between the different *Bacillus* species. The organism was identified as *Bacillus megaterium* (98.71%), an identity with an accession number CP001983.1 which was deposited into the GenBank. Therefore, the strain and the bioflocculants were named *Bacillus* sp. D2 and D2, respectively.

It is worth noting that several researchers (Karthiga et al., 2015; Luo et al., 2016; Selepe et al., 2022; Pu et al., 2020; Yuan et al., 2011) have studied or characterized bioflocculant produced by *Bacillus megaterium*, which was either isolated from various niches or taken from a culture collection. The results or characteristics of the bioflocculants in these studies vary, which can be attributed to the origin of the microorganism, the methods used for flocculants recovery, and other factors. In addition, because bioflocculants are produced through microbial fermentation, this process is influenced by several variables, such as the availability and composition of nutrients, physicochemical parameters, and culture conditions, resulting in bioflocculants with varying properties (Mohammed & Dagang, 2019). Therefore, intensive research is required to understand their properties better if microbial flocculants are to be implemented on an industrial scale.

4.4.2 Characterization of the produced bioflocculant

The flocculation characteristics of bioflocculants are contingent upon the presence of functional groups (Tawila et al., 2019). Diverse microorganisms can create bioflocculants that exhibit distinct compositions and chemical structures (Oyewole et al., 2023). FTIR was used to analyze the functional groups of the bioflocculant (D2). The resultant spectrum shown in Figure 7 had a broad polymeric stretch of the hydroxyl (O-H) group at 3296.94 cm^{-1}. A peak identified at 1631.88 cm^{-1} is related to the carbonyl group from amides, which represents the presence of proteins (Artifon et al., 2022). This was followed by a weak stretch at 1406.04 cm^{-1}, indicating the presence of the carboxylate group. Hydroxyl and carboxyl groups are favorable for flocculation, as they are known to participate in hydrogen bonding with the particles or pollutants (Zheng et al., 2008). The peaks at 1083.65 and 990.76 cm^{-1} depict ethers, typically sugar derivatives. Overall, it was evident from the FTIR analysis that bioflocculant D2 is made up of polysaccharides and proteins. This agrees with the literature reviewed that proteins and polysaccharides make up the highest composition of EPS, and they indirectly affect flocculation activity (Bakar et al., 2021).

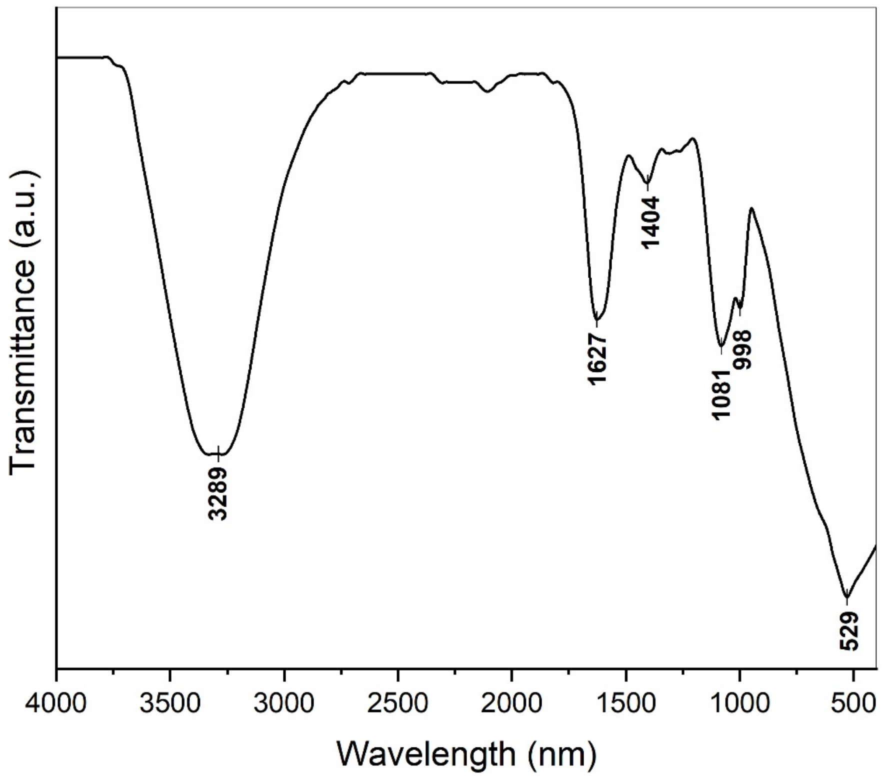


Figure 7: FTIR spectrum of bioflocculant produced by *Bacillus* sp D2 (from *Bacillus megaterium*).

4.4.3 Effect of storage conditions

According to Liu et al. (2023), the manufacturing cost has been identified as a significant obstacle to the widespread implementation of bioflocculants in industrial settings. Consequently, there is a pressing need to explore strategies to mitigate these costs. The current study examined crude bioflocculant as a cost-effective alternative in downstream solid-liquid separation processes. These processes often include using chemicals, resulting in additional processes thus expenses that may be avoided. Numerous methods are employed for the downstream processing of bioflocculant, i.e., the purification step, which utilizes various chemicals. Chemicals such as chloroform and n-butyl-alcohol in proportion of 5:2 (v/v) (Abu Tawila et al., 2018), 2% hexadecyltrimethylammonium bromide (Muthulakshmi et al., 2023), chloroform and methanol at a ratio of 2:1 (v/v) (Maliehe et al., 2016) and 32% (w/v) sulfuric acid (Yin et al., 2014), have been used for bioflocculation purification processes. When manufacturing bioflocculants, it is necessary to avoid using an excessive amount of these chemicals, mainly if the production is to be scaled up for wastewater treatment. Therefore, the storage conditions of the crude bioflocculant were evaluated to determine the bioflocculants' viability.

Figure 8 shows the differences in flocculation activity of the bioflocculant when stored under chilled, frozen, and room temperature conditions over twenty days. The results show a reduced efficiency as the days progressed for the various conditions. The worst form of preservation was observed at room temperature, with activity above 80% on day 0 and down to below 30% within five days of storage.

The flocculants lost their efficiency as the days progressed to days 10 and 15. This may have been caused by the proliferation of microorganisms in the crude bioflocculant, as centrifugation may not have eliminated all cells. Overall, ambient temperature is conducive to the growth of various microorganisms. It is close to the optimal temperature for cultivating the microorganisms used in the experiments, thereby increasing the likelihood of survival and growth. In addition, *Bacillus* spp. are known to be spore-forming (Setlow, 2014), so they may have resurrected and produced EPS in response to the conditions they were exposed to, rendering the bioflocculant ineffective. The chilled flocculant lost efficiency gradually over time until there was no activity. This could be because the temperature (4 °C) was unfavorable for the rapid revival of spores and the rapid growth of microorganisms if there were any remaining cells. It was noted that contamination risks exist at ambient temperature and in chilled environments. However, the frozen bioflocculants maintained a reasonable flocculation efficacy over time (n = 20 days). The minor decrease in flocculation efficiency may have been due to water accumulation under frigid conditions, as the chilling temperature used was 0 °C or during thawing. The results indicate that crude bioflocculant can be retained for future use under frozen conditions because it does not lose much of its effectiveness.

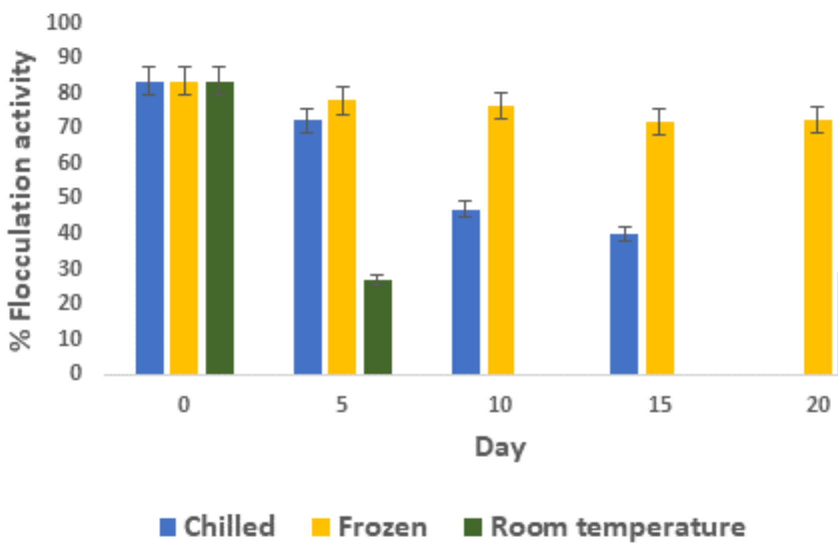


Figure 8: A graphical illustration of changes in flocculation activity of bioflocculant, *Bacillus* sp. D2 (from *Bacillus megaterium*), stored at various conditions over twenty days.

4.4.4 Zeta potential

Zeta potential measures the force of repulsion between suspended particles and the distance that must be covered/overlapped for agglomeration (Maliene et al., 2016). The zeta potential measurements were used to determine if charge neutralization was the responsible mechanism and to determine the optimal conditions for flocculation using central composite design (CCD). Table 5 shows the zeta potential results under different physicochemical conditions. The zeta potential of kaolin clay decreased with an increase in pH values, i.e., it was -19.8 at pH 2.96 and decreased to -48.3 at pH 10.04. This resulted from increased electrostatic repulsion force on the negatively charged flocs due to raised charged densities from pH increases. However, the zeta potential increased after the addition of $CaCl_2$ for all pH conditions because Ca^{2+}, which is a divalent cation, enhances flocculation activity through charge neutralization and stabilization of negative charges (Wang *et al.,* 2015). It was further noted that adding bioflocculant D2 to a suspension of kaolin clay and $CaCl_2$ decreased the zeta potential's negative charge. This observation confirms that the bioflocculant has a negative charge, namely an anionic charge, which, therefore, leads to the observed drop. The negative zeta potential of the bioflocculant may also be attributed to the presence of functional groups possessing negative charges, as shown by the FTIR analysis. The reduction observed may be attributed to the electrical repulsion resulting from the presence of similarly charged layers between the bioflocculants and kaolin clay (Guo et al., 2015). This implied that the primary mechanism of flocculation is not charged neutralization since it was shown to occur only after the introduction of a cation. This indicates the involvement of a bridging mechanism, therefore necessitating further investigation by a bonding-type test.

Table 5: Zeta potential results.

Conditions		**Zeta potential (mV)**		
pH	**Bioflocculant (% v/v)**	**Kaolin/$CaCl_2$/Bioflocculants**	**Kaolin**	**Kaolin/$CaCl_2$**
2.96	2	-16.9	-19.8	-4.64
4	1	-20.4	-31.7	-11.1
4	3	-21.6		
6.5	0.59	-21.0	-41.2	-20.3
6.5	2	-22.6		
6.5	3.41	-21.4		
9	1	-21.1	-44.0	-17.9
9	3	-20.6		
10.04	2	-21.2	-48.3	-19.7

NB: In kaolin clay samples and Kaolin clay $CaCl_2$, bioflocculants were not added.

Additionally, it should be noted that the zeta potential results do not provide a direct means of determining the optimal conditions for achieving maximal flocculation activity. The results underwent Analysis of Variance (ANOVA) to statistically assess the suitability and significance of the model generated to describe the association between zeta potential, pH and bioflocculant dosage. However, it was determined that the model needed to be improved. The results from the ANOVA are shown in Table 6. According to Mohammed and Dagang (2019), a p-value of less than 0.05 indicates statistical significance for the analyzed model or component. The current model had a significance level of 0.0281, indicating its significance.

Nevertheless, when evaluating the model's dependability using the coefficient of determination (R^2), it was determined that there was an insufficient correlation between the projected R^2 and the experimental response. The corrected R^2 value was determined to be 0.6277, while the anticipated R^2 value was found to be -0.5442. This discrepancy exceeds the established threshold of 0.2, as Agunbiade et al. (2022) specified, indicating that the model was inadequate.

Table 6: Analysis of variance (ANOVA).

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	22.97	5	4.59	5.05	0.0281	significant
A-pH	4.18	1	4.18	4.59	0.0694	
B-Biofloculant dosage	0.2002	1	0.2002	0.2200	0.6533	
AB	0.7225	1	0.7225	0.7937	0.4026	
A^2	17.26	1	17.26	18.96	0.0033	
B^2	1.74	1	1.74	1.91	0.2094	
Residual	6.37	7	0.9103			
Lack of Fit	6.37	3	2.12			
Pure Error	0.0000	4	0.0000			
Corr. Total	29.34	12				

$R^2 = 0.7828$, adjusted $R^2 = 0.6277$, predicted $R^2 = -0.5442$.

The second order model was established in terms of coded factors expressing the relationship between zeta potential, pH and bioflocculant dosage. This is shown by Eq. 2, while the depiction of the response is shown in Figure 9.

$$Zeta\ potential\ (mV) = -22.60 - 0.7226A - 0.1582B + 0.4250AB + 1.57A^2 + 0.5000B^2 \quad (2)$$

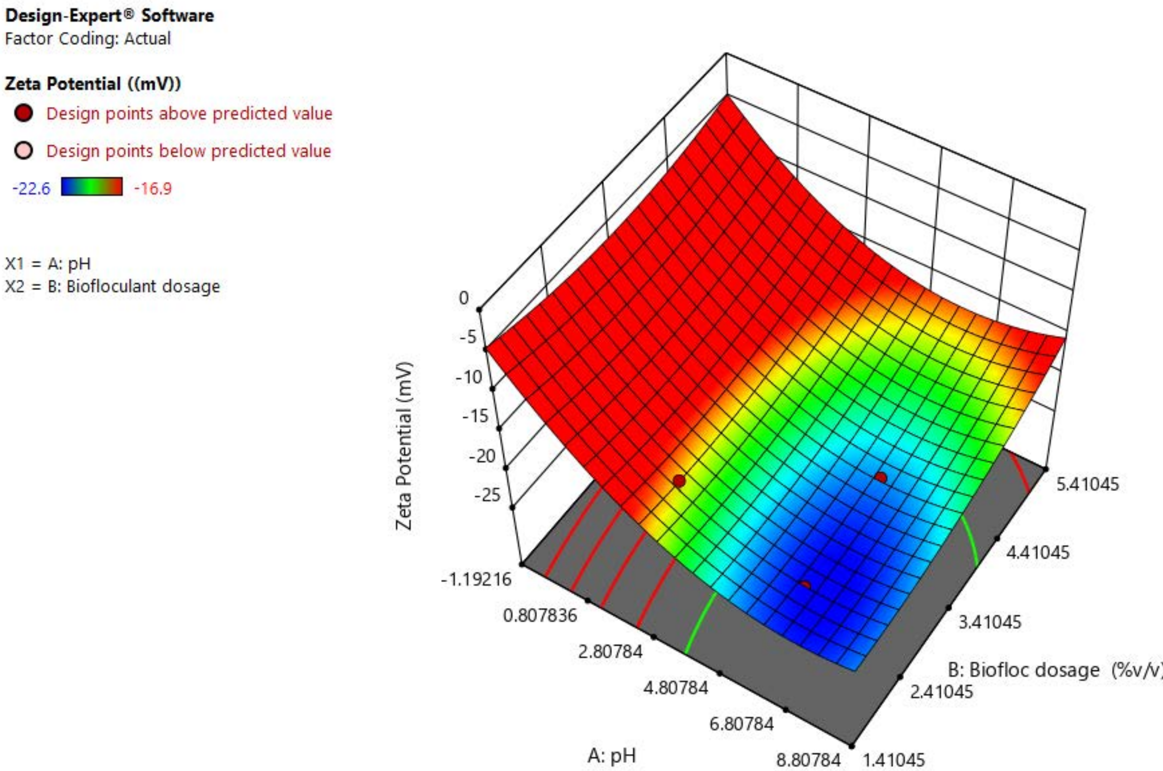
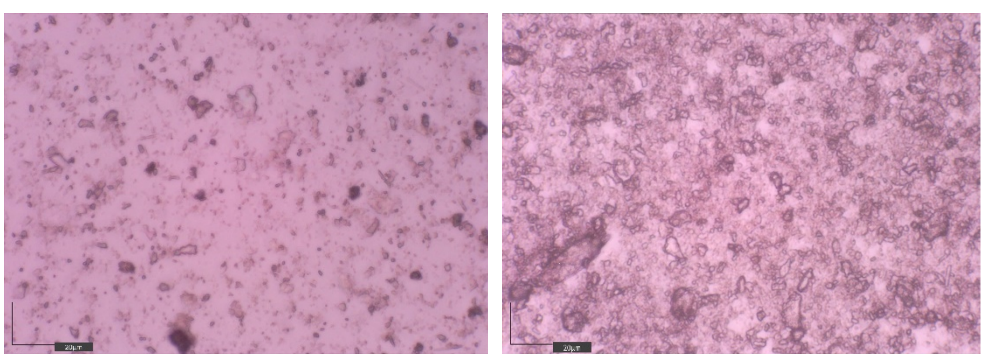
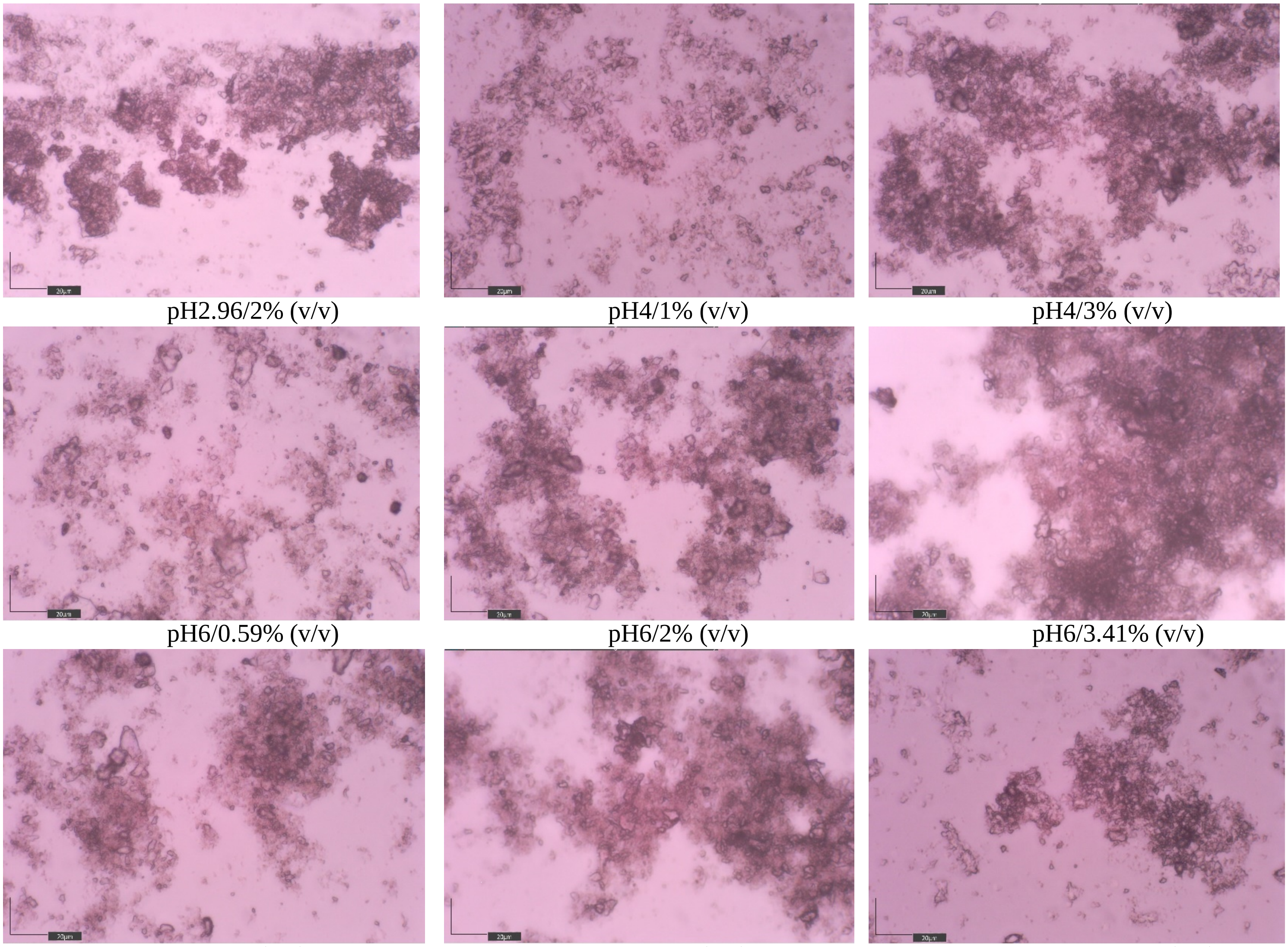


Figure 9: Graphical illustration of zeta potentials' relationship with changes in pH and bioflocculant dosage.

In addition, the three-dimensional quadratic model surface plot underscores the significance of pH as a determining factor in the modulation of zeta potential, as seen by the asymmetrical distribution observed in the graph. Given the unsatisfactory findings of the zeta potential analysis in establishing the optimal circumstances for maximal flocculation activity, an alternative approach was taken by observing formed flocs under a light microscope, as seen in Figure 10.



Kaolin clay Kaolin clay/$CaCl_2$



pH2.96/2% (v/v) pH4/1% (v/v) pH4/3% (v/v)

pH6/0.59% (v/v) pH6/2% (v/v) pH6/3.41% (v/v)

pH9/1% (v/v) pH9/3% (v/v) pH10.04/2% (v/v)

Figure 10: Microscopic images of floc formation at various pHs and bioflocculant dosage (20µm scale bar).

The optical microscope images revealed that the kaolin clay particles had a minimum and dispersed distribution before introducing any other substances. The suspension or dispersion of kaolin clay particles in a solution is due to their negative charge, leading to an electronic double layer (Liu et al., 2015). The addition of $CaCl_2$ resulted in a modest increase in density, which may be attributed to the process of charge neutralization. The occurrence of flocculation is evident in the subsequent images after the introduction of the D2 bioflocculant. Larger, more compact aggregates were seen to have developed. The flocs exhibited a discernible shape in contrast to the aggregation of particles induced by $CaCl_2$, providing more evidence that the flocculation process involves charge neutralization followed by bridging (Xia et al., 2022).

Furthermore, it was apparent from the visual representations that the most favorable aggregation of flocs occurred at a pH level of 6.5, accompanied by a dose of 2% (v/v). Under such circumstances, the flocs exhibit a moderate size while the dose remains within an optimal range, avoiding excessive or insufficient amounts. Upon examination of the minimum dose, namely 0.59 at pH 6.5, it became evident that the flocs exhibited a diminutive size and poor density. According to Pu et al. (2020), the flocculation activity is diminished at lower doses due to reduced surface area available for adsorption. The floc exhibited a higher density than the other samples when the bioflocculant was administered at its maximum dose of 3.41% (v/v) under pH 6.5. However, it was not chosen as the most suitable option because its dosage was about 1.5 times higher than the optimal level. This indicated that the dosage was too high. At a dosage of 2% (v/v), the bioflocculant exhibited effective flocculation activity.

Consequently, using the maximum dosage would result in wastage. Generally, the bioflocculant D2 exhibited favorable performance throughout a broad pH spectrum when used at an optimal dose. Moreover, its application for suspended solid elimination is supported by its beneficial influence on the aggregation of suspended particles into flocs.

4.4.5 Confirmation of flocculation mechanism through bonding type test

The flocs underwent three chemical treatments, namely 5M Urea, 0.5M HCl, and 10 mM EDTA-Na_2. According to Liu et al. (2015), EDTA-Na_2 and HCl have been seen to disrupt ionic connections, whereas urea has been found to disrupt hydrogen bonds. The observation results indicated that the flocs treated with urea had a significant impact. This phenomenon may be attributed to the interaction between urea and kaolin clay particles, disrupting hydrogen bonds between the particles and the bioflocculant. Consequently, the flocs created experienced a collapse, resulting in the turbidity seen in the reaction system. The flocs treated with EDTA-Na_2 exhibited a minor disruption level, indicating weak ionic connections between the bioflocculant and the kaolin particles. In addition, applying hydrochloric acid (HCl) to the flocs resulted in little or insignificant impact. This implies that hydrogen bonding is mostly the primary bonding mechanism between D2 flocculants and kaolin particles. Hydrogen bonding occurs when hydroxyl and amide functional groups are present (Hatta, 2021). The chemical structure of bioflocculants contains functional groups that facilitate the bridging process by offering binding sites to attach contaminants or particles (Ayangbenro et al., 2019).

The results contrast those of Pu et al. (2020), who discovered that the bioflocculant generated by *B. megaterium* exhibited an ionic bonding nature in the bonding type test. This observation underscores the need for a more extensive comprehension of the behavioural dynamics of bioflocculant producing microorganisms to effectively leverage their economic potential, given their susceptibility to multifarious circumstances and situations. In summary, the underlying mechanism of action for bioflocculant D2 involves a two-stage process. The first phase involves the neutralization of charges via the interaction with Ca^{2+} ions, forming Ca^{2+} and kaolin complexes. This interaction serves to stabilize and neutralize the overall charge. Subsequently, a bridging process ensues, whereby this mechanism gives rise to a three-dimensional configuration, wherein the functional groups present in the bioflocculant, mostly polysaccharides, serve as the bridging component connecting the kaolin particles. The presence of these functional groups facilitates two forms of interaction, namely ionic and hydrogen bonding, resulting in the adsorption of bioflocculants onto the surface of kaolin clay particles (Bisht & Lal, 2019).

4.5 Summary

The study identified a microorganism capable of producing a bioflocculant with strong flocculating properties from PSW. Additionally, the study focused on finding the best conditions for flocculation and understanding the mechanism behind the flocculation process. Isolate D2, identified as *Bacillus megaterium,* exhibited the highest level of flocculation activity. Analysis of the bioflocculant indicated the presence of hydroxyl, amide, and carboxyl functional groups, indicating that it is mainly made of polysaccharides and proteins. The optimal storage conditions for the crude bioflocculant were found to be freezing temperatures, as opposed to room temperature or refrigeration. In addition, the RSM was used to identify the optimal flocculation conditions and investigate the underlying floc formation mechanism. This was achieved by assessing the zeta potential as the response variable while considering pH and bioflocculant dose as the independent factors. It has been determined that charged neutralization is not the fundamental mechanism for flocculation. The zeta potential findings yielded equivocal outcomes concerning the identification of optimal flocculation settings.

Consequently, the flocs were examined using a microscope. The best flocculation conditions were determined based on microscopy observations, with a proposed pH of 6.5 and a bioflocculant dose of 2% (v/v). Based on the results of a bonding type test, hydrogen bonding was the prevailing bonding mechanism; albeit the flocculation process was postulated to proceed in two distinct stages. The first stage involving neutralizing charges by introducing Ca^{2+} ions, while the second stage is facilitated by the bridging action of functional groups, providing more support for this hypothesis. It is advisable to do thorough research on bioflocculants to enhance our understanding of their suitability for industrial implementation, given their susceptibility to many influencing circumstances.

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CHAPTER 5

Evaluation of Selected Operating Process Variables for a Bioflocculant Supported Column Flotation System

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CHAPTER 5

5 Evaluation of Selected Operating Process Variables for a Bioflocculant Supported Column Flotation System

5.1 Introduction

The poultry industry is experiencing significant growth due to the increasing demand for poultry products, which can be attributed to urbanization and burgeoning population growth. However, the industry faces various challenges, with one of the most pressing being the production of large quantities of wastewater due to the high consumption of fresh water [1]. Poultry slaughterhouse wastewater (PSW) is generated during bird slaughter, scald, bleeding, cutting, and packaging, including washing and cleaning equipment and facilities. Disinfectants are also present in the wastewater, leading to the water being classified as polluted [2]. The primary pollutant in PSW is organic matter, including blood, fats, oil and grease (FOG), unprocessed food, and soluble proteins. This results in a high level of CODs and BODs, necessitating the pretreatment of onsite wastewater prior to discharge [3], which has been further complicated by stricter regulations regarding wastewater discharge, as well as the increasing cost of fines [4]. Due to fluctuations in the composition of PSW and influent flow rate, based on the processing stage, the treatment method must be adapted to cater to these variations [5]. However, the inherent quantity and quality of PSW from the poultry industry requires the improvement of the treatment processes. Therefore, developing new technologies, re-engineering existing wastewater treatment equipment, and incorporating new designs are essential to enhance the treatment processes or system performance [6].

PSW has been treated with a variety of techniques, including flotation. The mineral-processing industry gave rise to this gravity separation technique, which is today extensively used in water and wastewater treatment. Industrial wastewater is often laden with contaminants and must be remediated before discharge or used for any other purpose [7]. Flotation has been used in wastewater treatment to remove difficult-to-separate particles, FOG, and residual compounds [8,9].

On the other hand, flotation is a separation process whereby particles with a low density either float to the top of the medium or settle at the bottom and are separated. This is facilitated by air bubbles forming bubble-particle aggregates that rise to the top, where they are subsequently scraped off [8]. This implies that the flotation process is based on the particles' attachment to air bubbles. Bubbles serve as a transport medium for flotation particles. Hence, bubble creation or generators are an important component of a flotation system. Bubbles are primarily generated through two broad categories, i.e., dispersed and dissolved air [7]. In dispersed air, bubbles are generated by directly supplying compressed air into bubble generation devices, including microporous tubes, diffusion discs, and hydraulic injectors. In contrast, dissolved air involves dissolving air in water under pressure and then releasing it at atmospheric pressure. The generation cost for dissolved air is higher than that of dispersed as it requires high energy and rigid operating conditions. Hence, there is a need for an easy operation development [10].

Various forms of flotation are used in separation processes, including induced air flotation (IAF), dissolved air flotation (DAF), Jet, Ion flotation, and column flotation. Notably, DAF has superior separation efficiency in wastewater treatment but is expensive due to high water saturation costs [9]. For this study, column flotation was explored as it is relatively cheap. The advantages of a column flotation are higher separation efficiency, lower capital required due to the simplicity of the system setup, and lower operating cost due to the lack of complexity of the process [11].

A column flotation is a vessel that is at least twice as tall as it is wide. It is a separation process driven by variations in surface properties, and air-sparging devices are employed to generate the necessary air bubbles that enable separation and removal. Air bubbles are introduced near the bottom, and these bubbles adhere to chemically altered and naturally hydrophobic particles, forming bubble-particle aggregates [12,13]. The flocculation step, which involves flocculants, is employed to improve the process efficiency for bubble particle capture. Flocculation has long been widely used to remove pollutants in wastewater and water treatment due to its effectiveness and convenience [14]. Flocculants are used to aid particle aggregation and facilitate floc formation as they act as bridging compounds. This is typically due to ionic and hydrogen bond formation and electrostatic interactions [14,15]. According to green chemistry, chemical flocculants are disadvantageous as they produce toxic sludge and are non-biodegradable. Moreover, bioflocculants are considered safe to handle, biodegradable, and environmentally benign. This is why bioflocculants are becoming increasingly popular [16]. Bioflocculants of microbial origin are basically non-toxic extra polymeric substances formed by the microbe or its metabolites, which include proteins, polysaccharides, DNA, glycoproteins, and others. In other words, bioflocculants are macromolecules and these molecules contain hydrophilic groups such as carboxyl and hydroxyl groups that favour floc formation by adsorption bridging [17]. Bioflocculants originate from lysis or the metabolism of microorganisms. They are subdivided into soluble and bound flocculants, with bound being attached to the cell whereas soluble flocculants are dissolved in a solution or are weakly bound with cells [18]. These forms can be separated by centrifugation [19]. However, information regarding these two types of bioflocculants is limited [20]. Bioflocculants of microbial origin will be employed in this investigation.

Various factors, including operating variables, equipment, and chemicals, influence flotation processes. This, in turn, influences the removal efficiency [21]. Despite being developed in the 20th century, it still needs to be better understood and could be more efficient [22]. As research on the mechanism and processes of flotation has intensified, its drawbacks have been observed in high cost, large equipment, intricate processes, and bubble sizes, which can lead to variations in removal efficiencies [10]. Furthermore, studies conducted in the past have indicated that aeration devices may only sometimes be optimized [12], and recent attention has been paid to new technologies that enhance aeration processes [23] and, in this case, 3D printing will be considered.

Three-dimensional printing, also known as additive manufacturing or rapid prototyping, produces an object through layer-by-layer fabrication using computer-aided design drawings (CADs). Three-dimensional printing offers flexibility in terms of design specifications. The technology can produce an object using a wide range of materials including hybrid combinations [24]. Materials that include polymers, pure metals, metal alloys, composites, ceramics, and thermoplastics can be used in 3D printing [25]. The primary difference between 3D printing and traditional methods is that the former involves an additive approach producing minimal to no waste whilst the latter involves a subtractive approach, meaning additional waste is produced [26,27]. The conventional/traditional techniques include a combination of bending, moulding, cutting, gluing, welding grinding, and assembling. Three-dimensional printing has led to the reduction in build time as compared to some of the traditional manufacturing techniques, which take longer due to the steps/processes involved [26]. Another benefit of 3D printing is that it fosters innovation as it has the ability to print using a wide range of materials with limited restrictions in the production of complex structures as compared to the traditional methods in which other materials cannot be utilized [27,28]. Another distinction is that 3D printing has a competitive advantage due to its ease of customisation and also ease of manufacturing geometrically complex parts [29], e.g., irregular shapes, variable thickness, hollow interiors, etc., which can be produced based on CAD. This leads to multimaterial, lightweight, ergonomic products, etc. Though 3D printing has advantages over traditional methods, it will not replace them completely, but rather revolutionize the production methods [30].

The production or use of 3D-printed air spargers has yet to be explored. Hence, the current research focused on the design of diffusers as a parameter for comparing 3D-printed and moulded diffusers. Additionally, bioflocculation forms and feed flow rates were also considered as key parameters to assess their impact on overall system performance for a bioflocculation-supported column flotation for pretreatment of PSW. The study lays a foundation for the exploration of 3D-printed air diffusers, a relatively new technology in conjunction with microbial flocculants for application in industry to enhance the performance of column flotation systems.

5.2 Objectives

To evaluate whether feed flow rate has an effect on pollutant removal,

To determine the parameter/s that significantly affect the overall BioCF efficiency, To determine the best type of diffuser design and Bioflocculant form that result in higher removal efficiency.

5.3 Materials and Methods

5.3.1 Column air flotation bench scale setup

This study employed a column flotation tank similar to the one previously designed by [31] (2016), albeit with minor modifications. Figures 11 and 12 depict the schematic and photographic illustrations of the column flotation tank. The column flotation tank's design featured a cross-sectional shape to maximize the surface area, an inlet near the top, and one sampling point (outlet) positioned just below the inlet but on the opposite side of the column. This configuration facilitated the separation of formed flocs from PSW and the pretreated wastewater. The components of the column flotation system were connected using silicon tubing. PSW from a 2 L holding tank was continuously fed into the plexiglass column flotation tank with an adequate volume capacity of 1.13 L via a Gilson peristaltic pump, with the flow rate being varied based on response surface methodology (RSM). *B. megaterium*-derived D2 flocculants were utilized for the flocculation process. Compressed air, regulated by pressure gauges and an airflow meter, was injected into the column flotation tank through air diffusers, resulting in a bubbling stream. Microbubbles produced at the tank's base attach to the flocs/solids, forming bubble-particle aggregates that rise to the top of the tank, where they are subsequently skimmed. Samples were collected at predetermined intervals and were analysed for quality water parameters. All experiments and tests were conducted at room temperature.

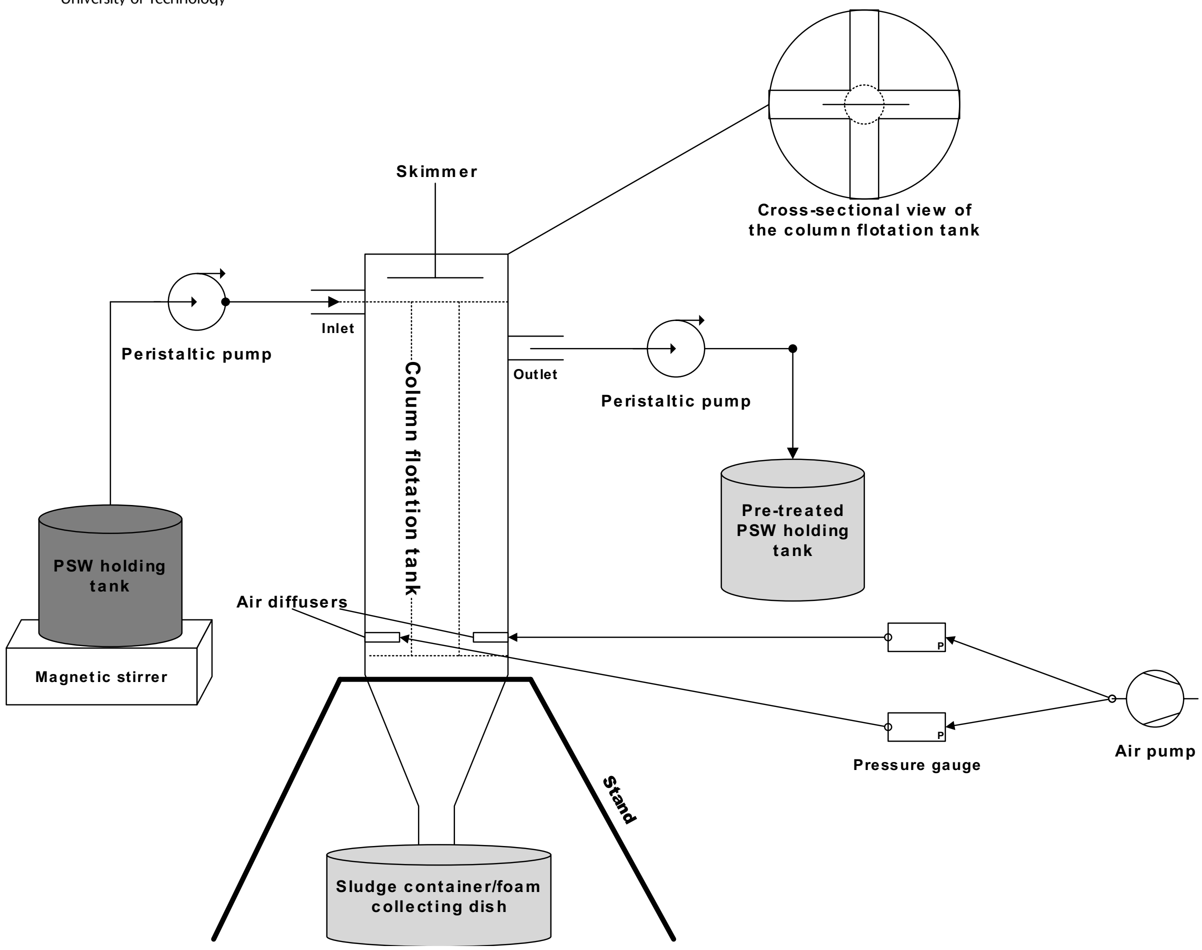

Figure 11: Schematic illustration of a column flotation system setup.

Figure 12: Photographic illustration of a column flotation.

5.3.2 Wastewater source

PSW was obtained from a poultry slaughterhouse in Cape Town, Western Cape, South Africa. The water was explicitly collected from the slaughtering plant, whereby PSW generated was from the slaughtering and washing of birds, cleaning of surfaces, and processing of by-products [32]. The wastewater was collected in 25 L polypropylene containers and stored at 4 °C before use to inhibit/lessen any biological activity. The PSW characteristics were analysed using standard methods prior to and post-pretreatment. Table 7 lists the average initial PSW parameters before running the system.

Table 7. Average PSW parameters prior to pretreatment.

Parameter	Average
pH	6.64
COD	2017.62 mg/L
Turbidity	449.87 NTU
Suspended solids	836.15 mg/L
Protein	370.68 μg/mL

5.3.3 Bioflocculant Production and Flocculation Activity Confirmation

B. Megaterium, previously isolated from PSW, was used for bioflocculant production. A loopful of the bacteria from nutrient agar plates was transferred into 50 mL bioflocculant production media as formulated by [33] (2017), and was incubated in a shaker incubator (Labwit ZWYR-240 shaking incubator, Labwit Scientific, Burwood East, VIC, Australia) at 36.5 °C under 121 rpm for 24 h. Following the incubation period, 5 mL of the fermentation broth was further transferred into 45 mL of bioflocculant production media and was incubated under the same conditions as the inoculum. The resultant fermentation culture broth was used for cell-bound bioflocculants (as is) and cell-free bioflocculants (supernatant after centrifugation to remove cells). The flocculation activity was then quantified using 4 g/L kaolin clay suspension whereby 50 mL of the suspension was aliquoted into 250 mL conical flasks and 1.5 mL of $CaCl_2$ (1% *w*/*v*) and 1 mL of the bioflocculant for sample or 1 mL of distilled water for control were added to the suspension. The mixture was swirled and transferred into 50 mL measuring cylinders where it was allowed to settle for 5 min. A sample was then withdrawn from the top layer and its optical density was read at 550 nm using a spectrophotometer (Jenway 7305 Spectrophotometer, Bibby Scientific Ltd., Staffordshire, United Kingdom). The flocculation activity was calculated using Equation (1), with the quantification being carried out in duplicate, and this served as confirmation that indeed the bioflocculants were effective.

$$\%\, Flocculation\ Activity = \frac{A - B}{A} \times 100 \qquad (1)$$

where:

A = absorbance of control, and

B = absorbance of sample.

5.3.4 Using Design Expert Software for Performance Analysis of the Bioflocculant Column Flotation System

Response surface methodology in Design Expert is an assemblage of statistical and mathematical tools used in process optimization. Furthermore, it can be used to evaluate the significance of multiple parameters in intricate interactions [34], and it was used to optimize the input parameters. The effect of different operating parameters in removing pollutants (COD, TSS, turbidity, and protein) was assessed for experimental design. This involved varying the influent flow rate, use of different bioflocculant forms (including those with and without cells), and utilizing different diffusers (moulded vs. 3D printed diffusers), which had an effect on bubble size and formation, thus ultimately having an impact on pollutant removal. The range of the flow rate values used was based on the trial run. Chemical oxygen demand reduction, suspended solids removal, turbidity, and protein reduction rate were analysed as responses (Y), and the yields were calculated as a percentage removal using Equation (2).

$$Y_n\,\% = \frac{Y_a - Y_b}{Y_a} \times 100 \quad (2)$$

where:

Y_n % is the yield (COD or tSS or turbidity) percentage removal,

Y_a is the response variable initial value, and

Y_b is the response variable final value.

Randomized optimal design (custom) was used as there was one numerical factor and two categorical factors. However, based on the design matrix, 18 experimental runs were conducted. Table 8 presents the randomly optimized (custom) 18 generated runs for one numerical factor and two categorical factors, the conditions used in this study.

Table 8. Experimental design for the independent variables using central composite design matrix.

	Factor 1	Factor 2	Factor 3
Run	A: Feed Flow Rate (mL/min)	B: Diffuser (Type)	C: Bioflocculants (Appendage Type)
1	1.26	Moulded	Cell bound
2	1.37	3D printed	Cell bound
3	1.26	3D printed	Cell free
4	1.00	3D printed	Cell bound
5	1.00	Moulded	Cell bound
6	1.00	Moulded	Cell free
7	1.00	3D printed	Cell free
8	1.74	Moulded	Cell free

9	2.00	Moulded	Cell bound
10	1.74	Moulded	Cell free
11	2.00	3D printed	Cell free
12	1.73	3D printed	Cell bound
13	2.00	3D printed	Cell free
14	1.37	Moulded	Cell free
15	1.26	Moulded	Cell bound
16	2.00	3D printed	Cell bound
17	2.00	Moulded	Cell bound
18	1.26	3D printed	Cell free

5.3.5 Analytical methods

PSW samples collected before and after pretreatment using a column flotation system were tested for water quality parameters. pH, COD, TSS, turbidity, and protein concentration were analysed. The pH of the wastewater was measured using a pH meter (Crison PH 25 plus, Crison Instruments s.a., Barcelona, Spain). Turbidity measurements were performed using a portable TURB 355 IR turbidimeter. SS concentration was determined according to the EPA Method 160.2. COD was determined according to the U.S. Environmental Protection Agency (EPA) 410.4 procedure for COD determination of surface and wastewater 33]. This involved utilizing HANNA high range (HI93754C-25 HR, 0–15,000 mg/L) COD test kits. Protein concentration was determined using the Bradford Assay (BIO-RAD Quick Start™ Bradford protein assay kit, CA, USA), and the correlation coefficient was used to check the accuracy of the line obtained from plotting the absorbance against the known concentrations of a protein.

5.3.6 Statistical analysis

Statistical analysis was performed using Microsoft Excel, Origin 2018 graphing, and Python libraries such as Pandas, Matplotlib, Scipy, and Seaborn. Furthermore, the results were presented regarding the average of at least duplicates.

5.4 Results and discussion

It was previously highlighted that equipment, various chemicals, and other operational parameters [21] influence flotation processes. Therefore, this study's selected operating process variables were sparger design, bioflocculant form, and feed flow rate. Design Expert version 11 generated the conditions through RSM. This statistical and mathematical approach allows the simultaneous analysis of multiple factors at various levels, including their combined effect on the response [35].

5.4.1 Influence of bioflocculants on poultry slaughterhouse wastewater pretreatment

Bioflocculants, used in water and wastewater treatment, destabilize particulate matter and act as a bridging agent. This leads to the aggregation of particles, thus forming flocs, which are easily separable [36]. The flocculation efficacy of the bioflocculant D2 was first validated using kaolin suspension; therefore, its potential in the treatment of PSW was evaluated by analysing the TSS before and immediately after adding bioflocculants at time 0. The impact is illustrated in Figure 13.

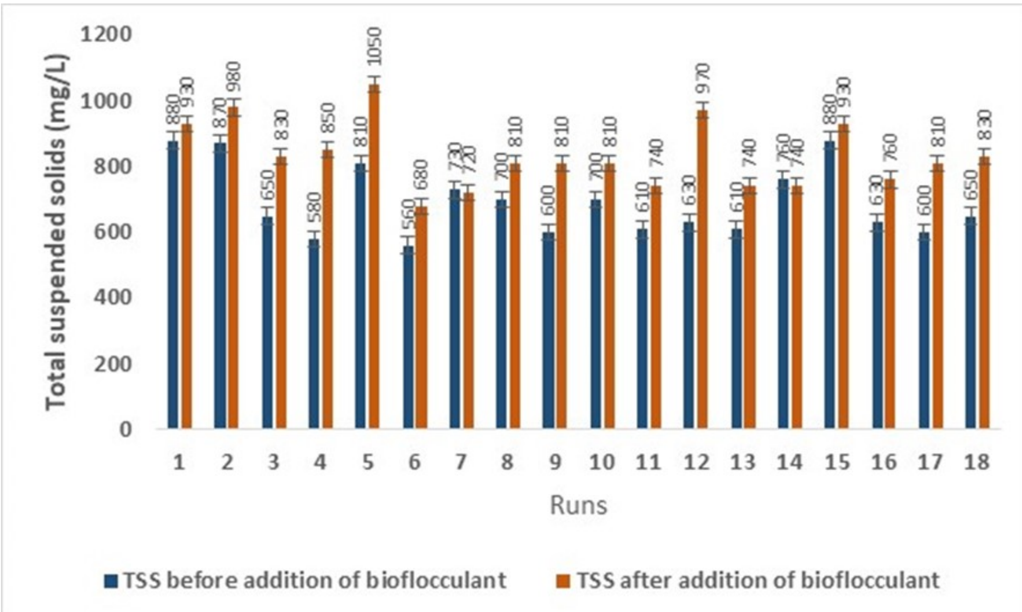

Figure 13. TSS before and after the addition of bioflocculants with run 3, 6, 7, 8, 10, 11, 13, 14, and 18 having cell-free bioflocculants and run 1, 2, 4, 5, 9, 12, 15, 16, and 17 having bioflocculants with cells.

The effect of different forms of flocculants, i.e., between cell-free compared to bound flocculants, was exhibited by the variation in TSS reduction. The results also demonstrated that supplementing flocculants to the PSW increases the turbidity of the resultant PSW-bioflocculant water. This distinction could also be attributed to the aggregation of particles, thus resulting in the formation of flocs, as evidenced in the withdrawn samples. The results concur with those of [37] (2023), which confirmed the flocculation of particulate matter in natural water apart from kaolin clay suspensions. Its results indicated that the suspended solids were flocculated efficiently when the flocculants were added alone. Interestingly, its bioflocculant was from Bacillus sp., although the strain was not mentioned.

Furthermore, run numbers 4, 5, 9, 12, and 17 significantly changed after adding cell-bound bioflocculants, resulting in increments for TSS between 210 and 340 mg/L, i.e., the difference between before addition of bioflocculants and after the addition of bioflocculants. In contrast, runs supplemented with cell-free bioflocculants had a TSS range between 110 and 180 mg/L, i.e., the difference between before and after addition of bioflocculants. This difference was initially attributed to bacterial cells. Additionally, flocculants convert dissolved solids/soluble matter into tiny particles that form insoluble complexes and become part of the flocs [38], contributing to the increase in TSS. The findings suggest that supplementing bioflocculant D2 positively affected the PSW and subsequently impacted the flotation system’s performance by increasing pollutant removal efficiency. This underscores their potential applicability as a substitute for chemical flocculants.

5.4.2 Comparative analysis of comparable variables

A comparative analysis was conducted to determine the effect of the bioflocculant form (cell free vs. cell bound) and diffuser type (3D printed vs. moulded) to find a combination that performs better. This was accomplished by analysing results when the system was operated under the same operational conditions. Figure 14 illustrates pollutant removal efficiency.

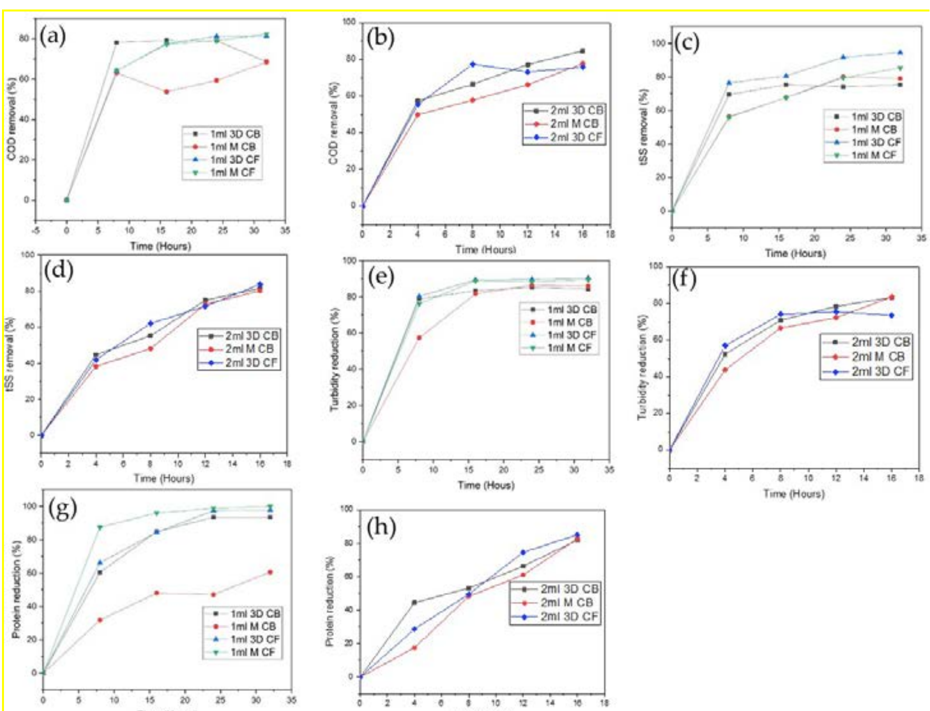

Figure 14: Graphical representation of pollutant removal, i.e., COD (a,b), TSS (c,d), turbidity (e,f), and protein under specific operational conditions (g,h). *NB: 3D = 3D-printed diffusers, M = Moulded diffusers, CB = Cell-bound bioflocculants, CF = Cell-free bioflocculants, 1 mL = 1 mL/min and 2 mL = 2 mL/min.*

5.4.2.1 Comparison of bioflocculant forms

The effect of the bioflocculant form (cell-free vs. cell-bound bioflocculant) was evaluated concerning pollutant removal while maintaining a consistent inflow rate and diffuser type. Bioflocculants generally exist in two forms, i.e., cell-bound bioflocculants, which are affixed and within bacterial cells, and soluble bioflocculants, which are dissolved in a solution as extracellular by-products [39]. Crude bioflocculants obtained after centrifugation were used as cell-free flocculants, and the fermentation broth without centrifugation, which had both soluble and bound bioflocculants, was used as cell-bound flocculants. Figure 14a,c,e,g shows that when 3D-printed diffusers when used at a feed flow rate of 1 mL/min with either cell-free or cell-bound flocculants, the pollutant removal was high for the cell-free flocculation system compared to when cell-bound flocculants were used. Similarly, when moulded diffusers are employed at a similar 1 mL/min inflow rate, the cell-free flocculation system had higher pollutant removal than the cell-bound flocculant system.

Overall, the trend demonstrated that cell-bound flocculants had an inadequate pollutant removal rate, with protein reduction at its lowest at only 60%. However, increasing the inflow rate when using 3D-printed diffusers to 2 mL/min showed that cell-bound flocculants were more effective in reducing COD and turbidity than when cell-free flocculants were used. On the other hand, the reduction in proteins and TSS was only marginally more significant for cell-free flocculants.

Based on our findings, it was deduced that cell-free flocculants were superior to cell-bound flocculants because they yield better overall pollutant removal efficiencies. The lower removal efficiencies with cell-bound flocculants may be attributed to the proliferation of microorganisms during flocculation or the effect of other constituents used in the broth. Non-settleable microorganism growth increases turbidity and reduces pollutant removal [40], as bacterial cellular membrane functional groups can bind some flocculants, reducing flocculation activity [41].

5.4.2.2 Comparison of Diffuser Types

The effect of diffuser design was assessed, with pollutant removal being the outcome. The difference in the microporous structure is mainly dependent on the fabrication method. The 3D-printed air diffusers were manufactured using the laser-powder bed fusion technology, while the diffusers used for comparative analysis were fabricated using the traditional method of moulding/sintering. Pollutant removal for 3D-printed diffusers, when compared to moulded diffusers under similar operational parameters (flow rate and bioflocculant form), is displayed in Figure 14a–h.

Three-dimensional printed air diffusers had better performance when compared to the moulded variety. While COD removal and turbidity reduction were roughly similar for both diffuser types, TSS removal was higher for 3D-printed air diffusers. However, protein reduction was slightly higher for moulded diffusers using cell-free flocculants at 1 mL/min (Figure 14a,c,e,g). However, under the same circumstances, the moulded diffusers' TSS removal and turbidity reduction were marginally more significant than those of 3D-printed diffusers. A closer examination reveals that 3D-printed air diffusers had high removal efficiencies of above 70% just after the beginning of the experiments and continued to rise till the end at operating parameters of 1 mL/min with cell-bound bioflocculants, whereas the moulded diffuser had 60% removal rates, which dropped and subsequently rose although below that of the 3D-printed diffuser experiments. At 2 mL/min with cell-bound bioflocculant (Figure 14b,d,f,h), COD removal was higher for 3D-printed diffusers than for moulded diffusers. However, TSS removal, protein, and turbidity reduction at the end were more or less the same. However, it is noteworthy that the trend indicates that the performance of 3D-printed diffusers started higher than that of moulded diffusers.

These results demonstrate that the type of diffusers affected the performance of the flotation system, with 3D-printed air diffusers outperforming moulded ones in terms of performance. Three-dimensional printed air diffusers had a rough finish compared to the moulded ones, which had a smooth finish. With that said, the surface finish of the 3D-printed air diffusers might have contributed to keeping bubble sizes favourable for flocculation by preventing bubbles from coalescing. This, however, supports the idea that the 3D-printing of diffusers has the potential to enhance column air flocculation system performance. Hence, further exploration is needed to determine the effect of 3D-printed diffusers with dense pores when used for flocculation.

5.4.3 Correlation of the variables

Pearson correlation coefficient serves as a measure of the linear association between two sets of variables. To investigate the relationship between the physicochemical parameters, that is, the pollutant removal efficiencies based on operational parameters, the assessment of correlation coefficient was applied. The matrices presented in Figure 15 showed that the correlation coefficients were generally very high, indicating a strong correlation between the physicochemical parameters. Upon closer inspection, turbidity and TSS strongly correlated for systems in which 3D-printed diffusers and cell-free bioflocculant were used (Figures 13b and c, respectively). The least, albeit still high, correlation was between turbidity and protein removal at 0.78 (Figure 15a).

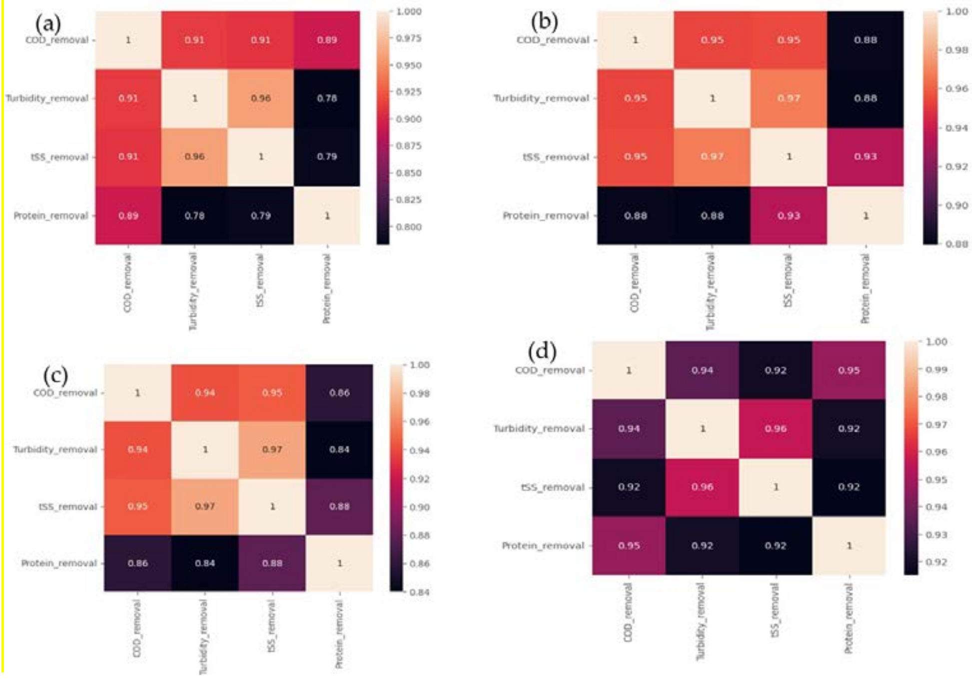

Figure 15. Pearson correlation coefficient of different physiochemical parameters based on different variables showing correlation matrix of removal percentages for moulded diffusers (a), 3D-printed diffusers (b), cell-bound bioflocculants (c), and cell-free bioflocculants (d).

Regarding all variables assessed, 3D-printed air diffusers and cell-free flocculants constitute an amenable combination that may improve column air flocculation system performance on a large scale.

5.4.4 Surface Plots of the Comparable Variables based on pollutant removal.

The surface plots show pollutant removals for various variables based on the flow rate and time interaction. The pollutant removals for cell-bound bioflocculant, cell-free bioflocculant, moulded diffusers, and 3D-printed air diffusers are displayed in Figures 16–19.

For cell-bound bioflocculants (Figure 16), the high pollutant removal rates of between 70% and 80% were attained within 10 to 15 h of operating the system except for protein removal, which extended between 15 h and 20 h. Overall, protein removal was unsatisfactory, as indicated by hue, which denotes low removal efficiencies. Furthermore, the plots demonstrated that turbidity removal was relatively high for the range of flow rates studied.

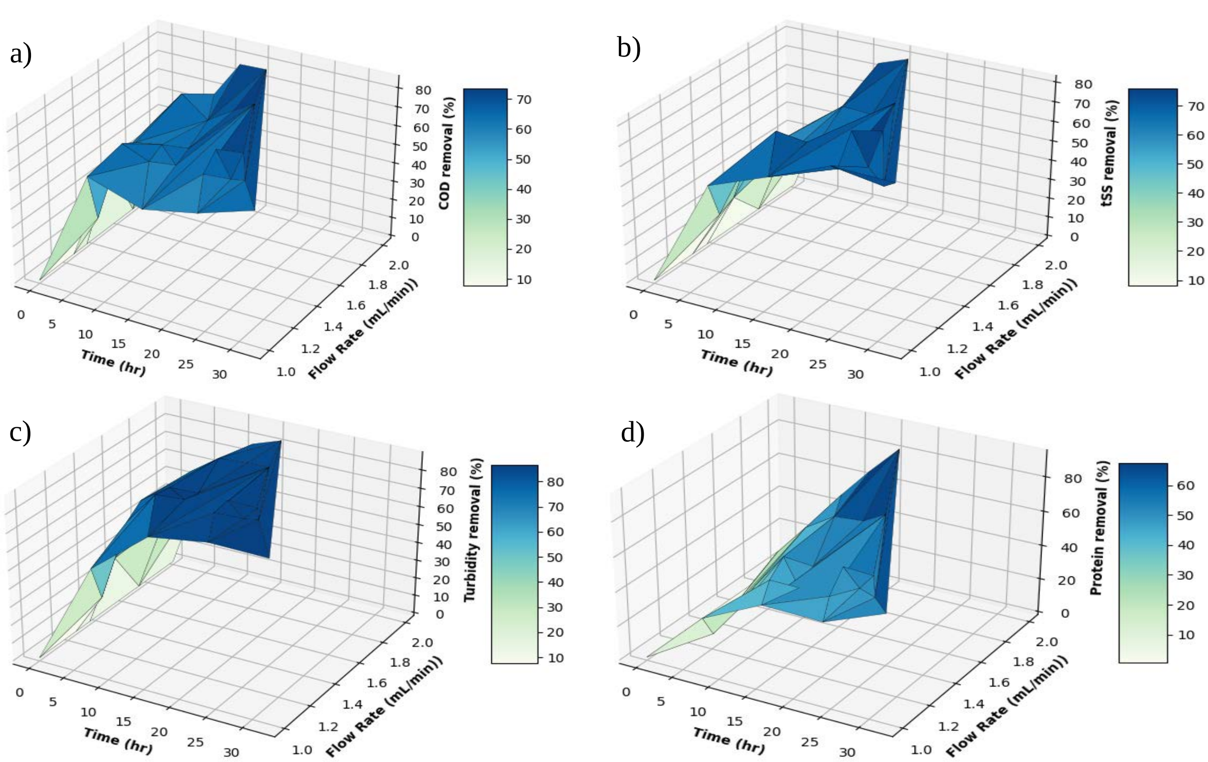

Figure 16: Surface plots of (a) COD, (b) TSS, (c) turbidity, and (d) protein removal from cell-bound bioflocculants.

The surface plots for cell-free bioflocculants (Figure 17) indicate that the protein removal was comparatively more significant than that of cell-bound bioflocculants. There was high COD, turbidity, and protein removal from 5 h, with TSS removal occurring after 10 h.

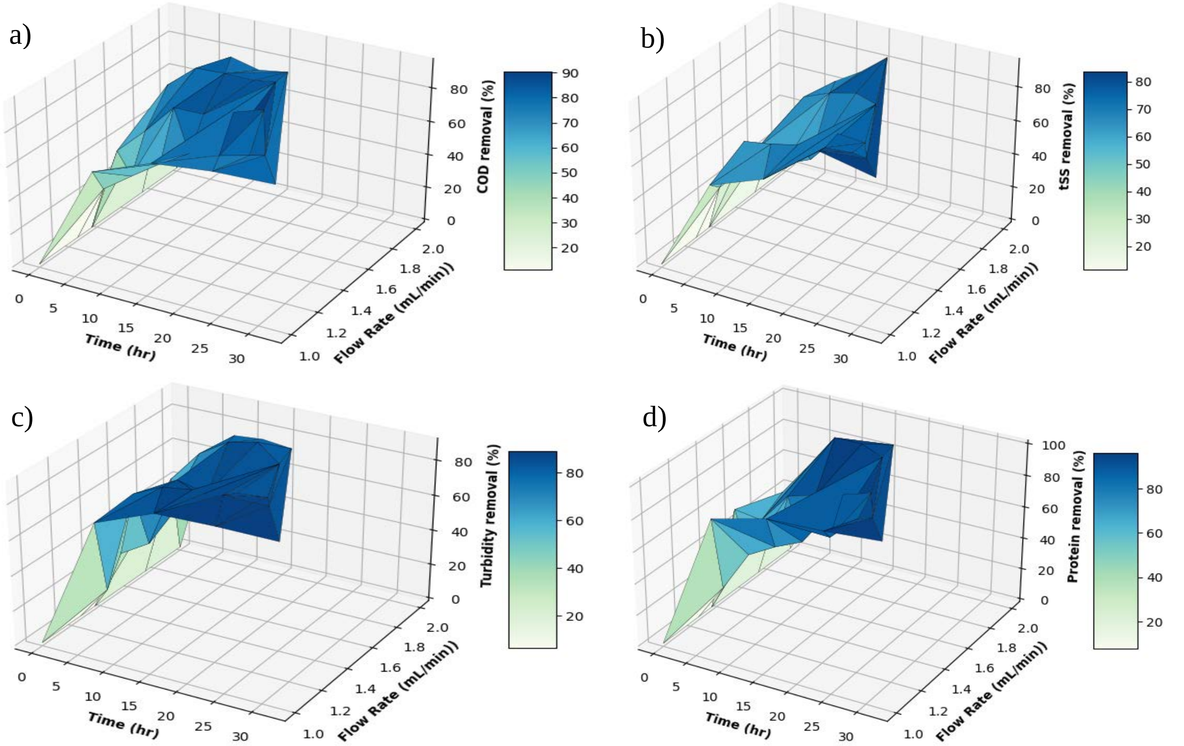

Figure 17: Surface plots of (a) COD, (b) TSS, (c) turbidity, and (d) protein removal from cell-free bioflocculants.

High removal rates were seen in the Figure 18 plots for moulded diffusers after 10 to 15 h of system operation. However, protein removal fluctuated and had significantly low removals at nearly 20 h.

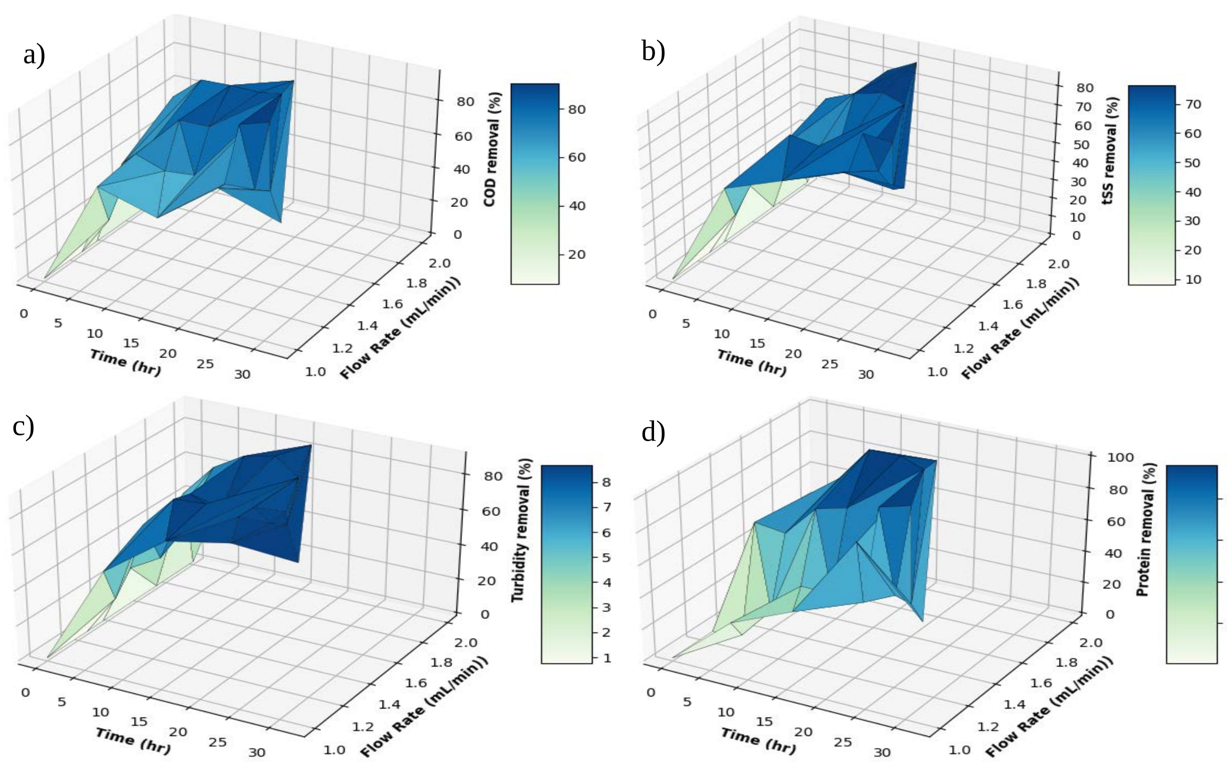

Figure 18: Surface plots of (a) COD, (b) TSS, (c) turbidity, and (d) protein removal for moulded diffusers.

In line with the findings of other variables, the surface plots (Figure 19) for 3D-printed air diffusers demonstrated low removal efficiencies for protein. Increased removal rates were attained only after operating the flocculation system for 10 to 15 h, albeit turbidity removal was comparatively high across a broad range of flow rates.

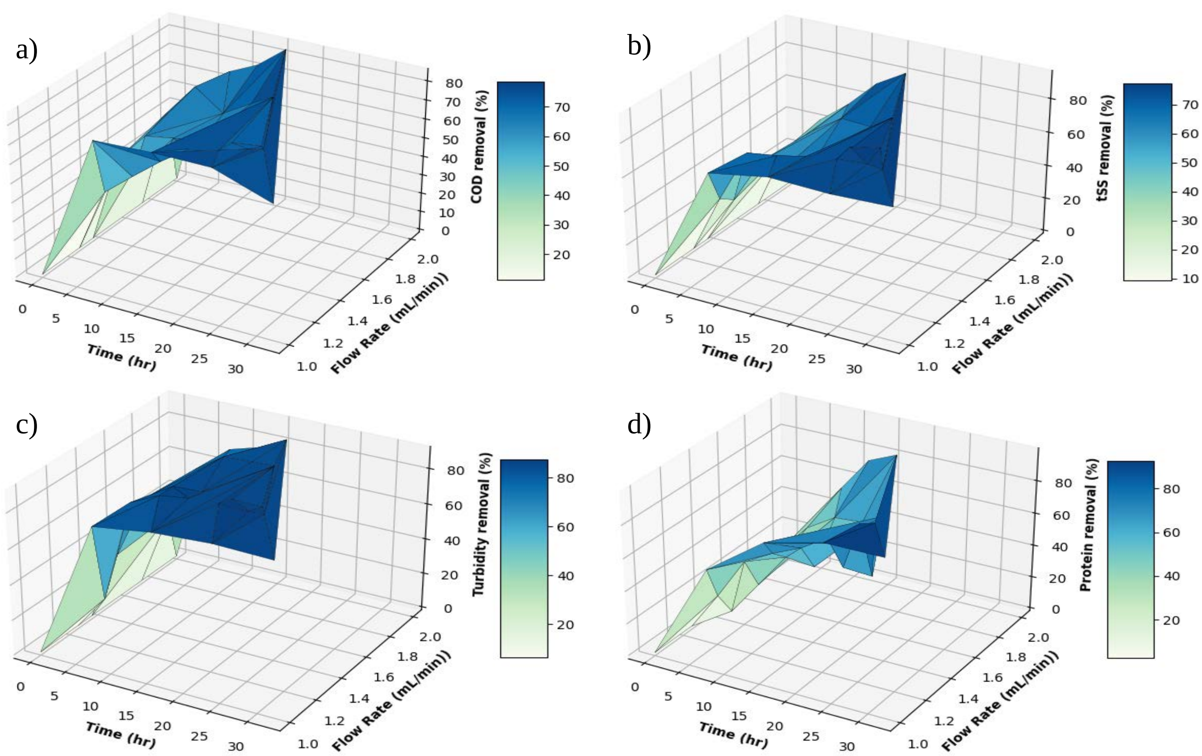

Figure 19: Surface plots of (a) COD, (b) TSS, (c) turbidity, and (d) protein removal for 3D-printed air diffusers.

5.4.5 Overall bioflocculant column flotation (BioCF) system performance

The flotation method is a pretreatment technology employed to remove organic matter, different types of suspended solids, oils, and other pollutants from wastewater [42]. The PSW was subjected to physicochemical analysis before the pretreatment process was initiated. Generally, the wastewater exhibited a substantial organic load (COD, TSS, and proteins), which also led to the wastewater's turbidity. The variation in process parameters regarding feed flow rate, bioflocculant form, and diffuser type resulted in varying system performance regarding pollutant removal. The performance of the flotation system was evaluated based on pollutant removal efficiencies for COD, TSS, turbidity, and protein reduction, as illustrated in Figures 20–23.

The COD removal rate is typically used to measure the strength and treatability of wastewater [43], thus, organic matter content in the wastewater [44]. The graphs in Figure 10 depict moulded diffusers' performance with cell-free flocculants, indicating higher COD removal rates, with removal efficiencies of over 80% for all three-feed flow rates. The removal was the least, ranging between 60% and 80% for moulded diffusers with cell-bound bioflocculant. Additionally, the maximum removal of COD was observed at a flow rate of 1.365 mL/min.

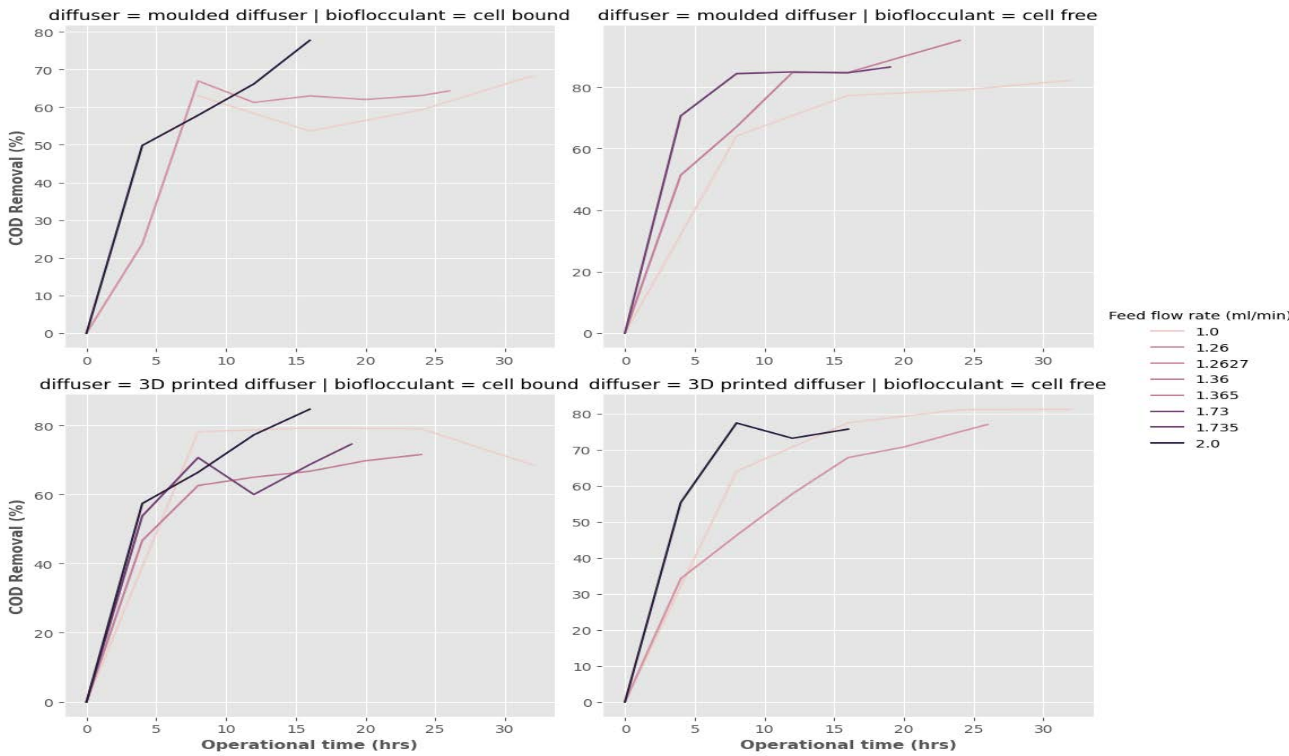

Figure 20: Graphical representation of COD removal under various conditions (diffuser type, bioflocculant form, and feed flow rate).

Solids such as soft tissue, excrement, feathers, etc. are responsible for high TSS values in PSW [44]. The graph in Figure 21 shows that TSS removal was high for 3D-printed diffusers with cell-free flocculants. It can be noticed that at a flow rate of 2 mL/min, the TSS removal efficiency was just above 80%, as well as at the flow rate of 1 mL/min, where TSS removal rates were the highest except for a combination of cell-bound flocculants with 3D-printed air diffusers.

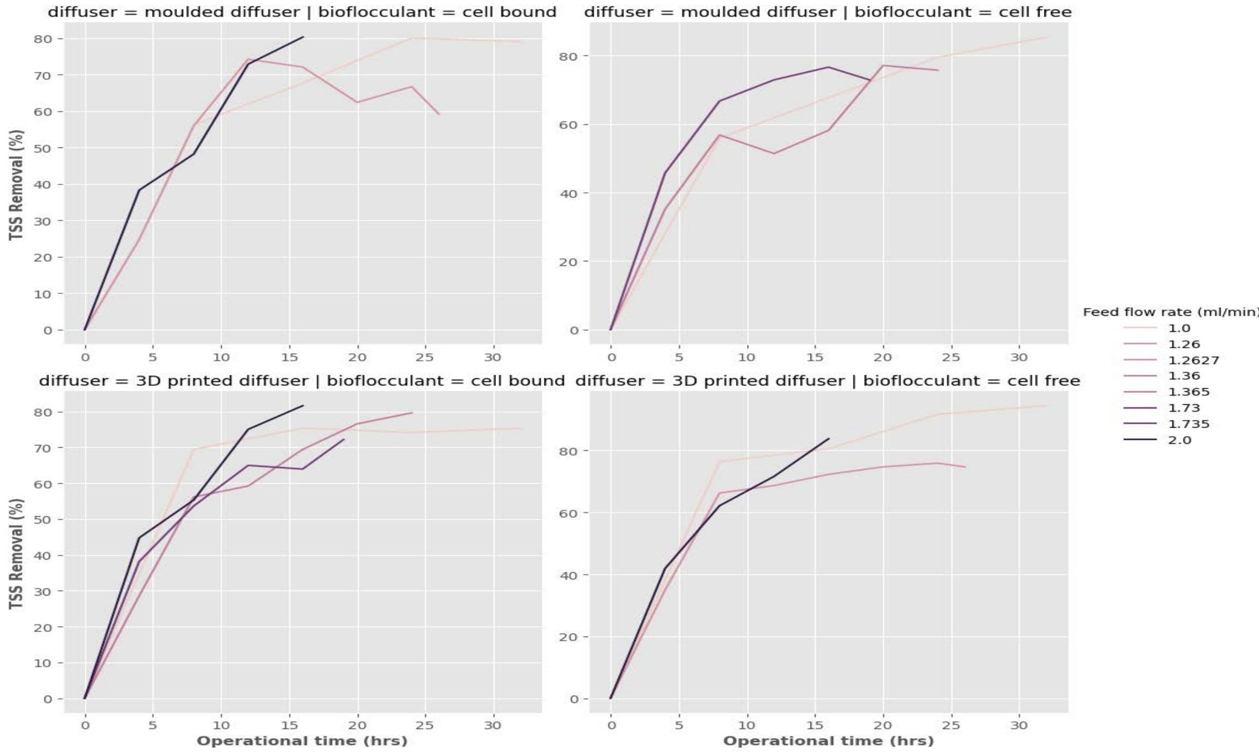

Figure 21: Graphical representation of TSS removal under various conditions (diffuser type, bioflocculant form, and feed flow rate).

Turbidity in PSW is also elevated by blood and urine apart from suspended solids. Regarding turbidity reduction (Figure 22), the flotation process proved effective as most results were above an 80% removal efficiency across a range of flow rates assessed.

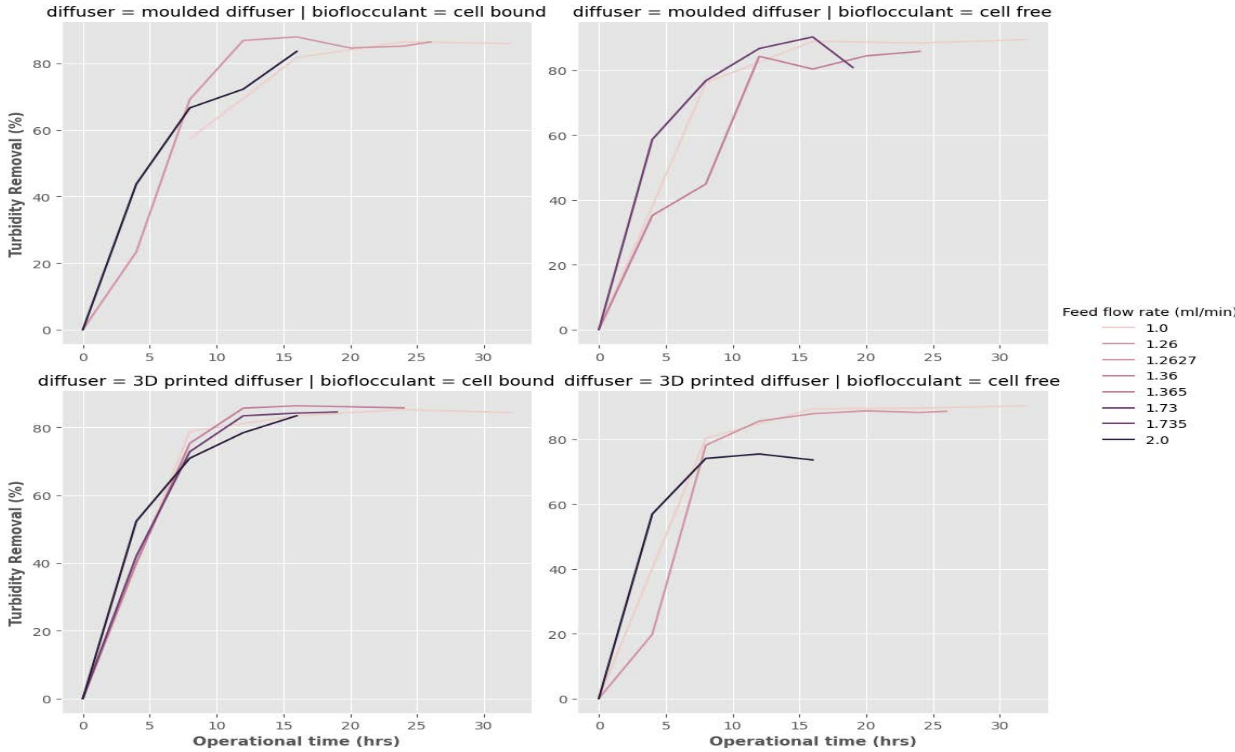

Figure 22: Graphical representation of turbidity reduction under various conditions (diffuser type, bioflocculant form, and feed flow rate).

Figure 23 shows that the system was not too effective in reducing the protein content of the PSW, mainly where cell-bound flocculants were used; an attribute also associated with both 3D-printed and moulded diffusers. This could have been attributed to increased microbial community proliferation as the PSW. However, the protein removal efficiency was relatively high for a flow rate of 1 mL/min, as this would have resulted in increased hydraulic retention time for the system.

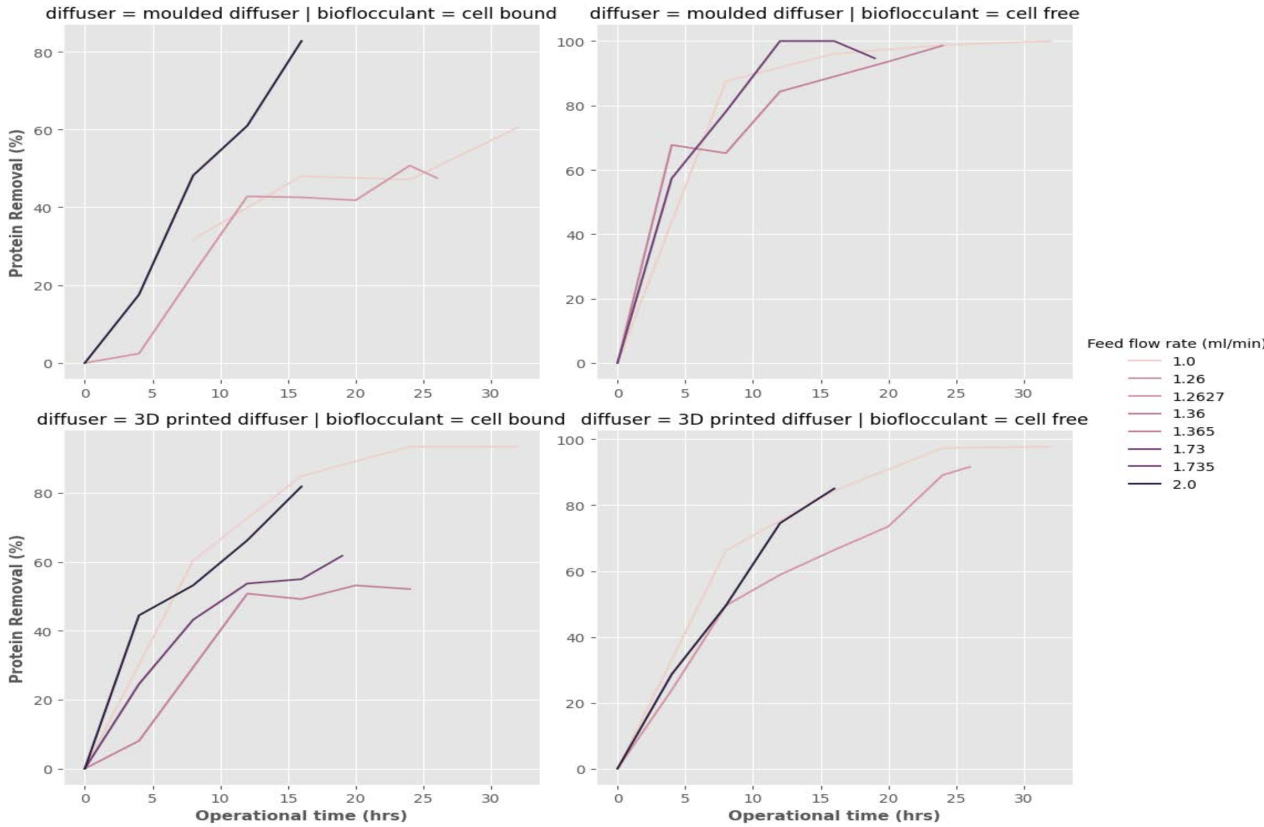

Figure 23: Graphical representation of protein reduction under various conditions (diffuser type, bioflocculant form, and feed flow rate).

Ref. [38] (2008) used a column flotation to treat meat processing wastewater. The authors found that it had acceptable removal efficiencies of organic matter and, thus, recommended that it be used as a cheap alternative to dissolved air-flotation systems, provided the right flocculants are used. Although DAF systems are known for high removal efficiencies, according to most reports, column flotation can achieve such high removal efficiencies at relatively low costs with minimal maintenance, provided that parameters affecting the flotation process are optimized.

Based on the findings, it is evident that the system successfully pretreated PSW. Furthermore, it shows that the selected variables affected the flotation system performance. On average, a 1 mL/min flow rate combination with 3D-printed diffusers and cell-free bioflocculants yielded high pollutant removal. Overall, treatment with cell-free flocculants achieved relatively high removal efficiencies compared to cell-bound flocculants. When cell-bound flocculants were used, it is apparent that protein removal efficiencies were poor and there were lower COD removal rates as opposed to when cell-free flocculants were used. This further confirms that the form of bioflocculant affects other parameters, especially on diffusers, as they will be linked to poor pollutant-removal efficiencies. The form of bioflocculants and type of diffusers affect the performance of a bioflocculant-supported column flotation. The flow rate did not exclusively affect the removal efficiencies as the results varied.

5.5 Summary

Removing pollutants from PSW using a bioflocculant-supported column flotation proved an effective pretreatment method. The performance of the system was affected by various parameters, and the following conclusions stand out: the best bioflocculant form was cell-free flocculants, and the best diffuser type was the 3D-printed air diffuser as high removal rates were attained when these variables were employed as compared to cell-bound flocculants and moulded diffusers. There is a need to explore more manufacturing 3D-printed diffusers in terms of improving the diffusers themselves and employing them in different types of wastewaters as the results reflected that they can improve the flotation system performance. It is further recommended that the BioCF system's long-term stability and scalability in an industrial setting be investigated, along with a detailed assessment of its overall environmental impact, including the lifecycle analysis of the 3D-printed diffusers and bioflocculants.

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CHAPTER 6

OVERALL CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 6

6. OVERALL CONCLUSIONS AND RECOMMENDATIONS

6.1 Overall Conclusions

The bioflocculant-supported column flotation (BioCF) system was successful in pre-treating PSW, and its performance was evaluated based on the three variables (bioflocculant form, diffuser type, and feed flow rate) and the resultant pollutant removal. Improving system performance favors the poultry industry as freshwater usage increases due to increased poultry product production demands.

This study laid a foundation for exploring the application of 3D-printed diffusers manufactured using additive manufacturing, a reasonably new technology. Despite the 3D-printed diffusers having fewer pores, which seemed enormous, and a rough finish, their performance proved superior to molded diffusers, which were observed to have dense pores deemed favorable for flotation processes. The pollutant removal efficiency of the 3D-printed air diffusers was higher than that of those manufactured using molding.

Moreover, the bioflocculants produced by B. megaterium, isolated from PSW to be treated, were employed in their crude form. The bioflocculant was first optimized, i.e., before being applied in PSW pre-treatment, using kaolin clay suspension such that its physicochemical properties are known, and an optimum dosage may be used to avoid negative interferences with the BioCF system. Cell-bound bioflocculants yielded poor results compared to cell-free flocculants, which were thought to have resulted from the proliferation of microorganisms, thus contributing to an altered structure thus, functional groups. Notably, the bioflocculant form had an impact on other variables, thus affecting the BioCF system performance as the cell-bound were associated with more removal rate fluctuations than cell-free flocculants. The activity of cell-free bioflocculants can be retained when they are kept frozen.

Flow rate did not impact the system much, as the removal efficiency was not influenced much by it. The lesser impact posed by flow rate indicates that the system can potentially cater to fluctuations in influent going into the BioCF system. The BioCF is a promising separation method because it uses environmentally friendly flocculants and utilizes a column, which requires a reduced footprint because the tank is longitudinal and can be operated vertically. Additionally, it uses dispersed air, which is less expensive than the water saturation used in other types of flotation systems and 3D-printed air diffusers.

Overall the results agree with the hypothesis that operational parameters that are diffuser design, bioflocculant form, and feed flow rate affected the performance of a BioCF for the pretreatment of PSW.

6.2 Highlights

- Manufacturing of 3D-printing of diffusers proved to be feasible and is a promising technique for the improvement of diffusers, hence, enhancement of the flotation processes,
- Cell-free bioflocculants utilization in PSW pre-treatment was successful and, thus, a potential substitute for chemical flocculants,
- 3D-printed diffusers outperformed molded diffusers, thus making them a better type for improving flotation performance,
- Cell-free bioflocculants were superior to cell-bound bioflocculants, and
- The combination of cell-free bioflocculants and 3D-printed diffusers increases column flotation system efficiency at moderate pH.

6.3 Recommendations for future studies

Future studies should focus on optimizing the laser powder bed fusion process to optimize the production of 3D-printed diffusers. Investigating the feasibility of embedding materials with antimicrobial properties in 3D-printed air diffusers would be beneficial. Additionally, research should concentrate on the airflow rate requirements of the 3D-printed diffusers, including the formation, size, and distribution of bubbles. Finally, yet importantly, since the feed flow rate did not affect the system used for this research, the impact of suspended solid loading rates must be examined in applying the BioCF.

APPENDICES

APPENDICES

Appendix A: Analytical methods

Appendix A1: COD analysis

- The block Hanna reactor which was pre-set to 150℃ for two hours was switched on and was allowed to heat up to the desired temperature,
- Using the hi93754c-25 cod high range (0 to 15000) solution 0.2mls of sample was added to the vial with the supplied syringes,
- The caps were tightly screwed on and the mixtures were inverted several times to mix,
- The cells were then heated in the Hanna reactor at 150℃ for two hours,
- After the digestion period the Hanna reactor switched off automatically and the vials were allowed to cool down for 20 mins
- Thereafter the vials were inverted several times and were then placed in a rack to cool down to room temperature,
- The COD concentration was read after cooling off in a Hannah multiparameter.

Appendix A2: Total suspended solids determination

- A clamp funnel and a glass fiber disk were placed onto the base and attached to a suction flask,
- Following the application of vacuum, three consecutive 20ml volumes of milli-Q water were used to cleanse the filter.
- After removing the filter with a twizzer, it was put in an aluminum dish and ignited in the muffle furnace for 30 minutes at 550°C,
- Thereafter the filter was further washed three times with 20ml washes of milli-Q water, and the filter was dried in an oven set to 103°C for an hour,
- The filter was then allowed to cool in a desiccator before being weighed,
- A small amount of milli-Q water was added to the filer paper so that it attaches to the base,
- A certain volume of the samples was transeferd onto the filter paper after mixing and vacuum was applied even after water has passed through,
- The filter paper was then put in the aluminium dish and was heated at 103℃ in a drying oven for 1hour,
- it was then put into a desiccator to cool off and was then weighed afterwards,
- TSS concentration was calculated based on the equation below,

$$TSS\left(\frac{mg}{L}\right) = (A - B) * 1000/C$$

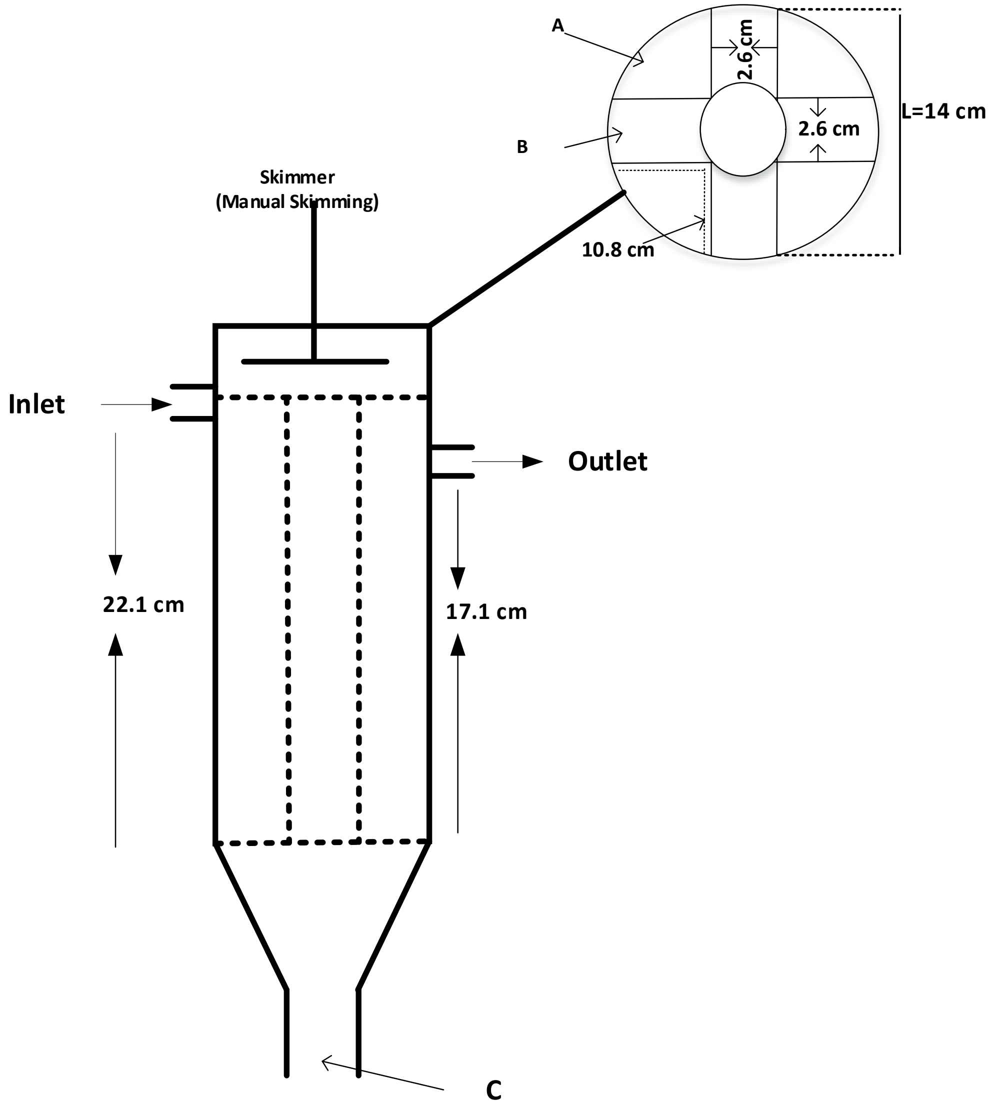
- Where: A= Filter and dish+ residue weight in mg
    - B=Filter and dish weight in mg
    - C= Sample volume in mL

Appendix A3: Protein concentration determination

- The Bradford reagents (1X dye) were allowed to warm to room temperature and were repeatedly inverted to mix,
- 2mg/ml of Bovine serum albumin was diluted to yield standard concentrations of 2000, 1500, 1000, 750, 500, 250, and 125µg/ml.
- 60 µl of each standard, blank water, and the unknown sample were pipetted into individual cuvettes and 3ml of 1X reagent dye were then added to each cuvette and mixed,
- The solutions were incubated at room temperature for 5 mins, after which the absorbance was measured using a spectrophotometer at 595 nm.
- A standard curve was created by plotting absorbance vs. concentration, and the unknown sample concentration was ascertained using the standard curve.

Appendices B: Column flotation system

Appendices B1: Bench scale column flotation system



NB
A:Opening leading to the cone , where the scrapped off waste flow through
B: Wastewater holding Area
C: Exit leading to waste collection tank

Figure B1: Schematic illustration of the column tank with measurements

Appendices B2: Column flotation system calculations

- Assumptions:
  - All quarters are the same
  - Quarters can be flattened
  - If the quarter is flattened the width is 10.8cm

- **Wastewater holding area**

Cross sectional area

$$L * W = 14 * 2.6 = 36cm^2 * 2 = 72.8cm^2$$

$$L * W = 2.6 * 2.6 = 6.74cm^2$$

Therefore $72.8 - 6.76 = 66.04cm^2$

- **Volume**

$$Area\ of\ B * H = 66.04 * 17.1 = 1129.28cm^3$$

- **Surface area**

$$L * H = 2.6 * 17.1 = 44.46cm^2$$

$$L * H\ 10.8 * 17.1 = 184.68cm^2$$

Therefore $4(184.68 + 44.46) = 916.56cm^2$