Laboratory-Scale Production and Testing of a Bionanomaterial Technology for Large-Scale Wastewater Treatment

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Wastewater treatment remains a challenge in South Africa (SA) and many other African countries for several reasons, including inefficient treatment processes that result to poor quality of treated wastewater discharged to the environment. In some cases, especially dense informal settlements, domestic wastewater is not treated altogether, and it is discharged directly to the environment into nearby streams and rivers. Poorly managed mine tailings affect the quality of water in the mine area and in its surroundings and changes hydrological and topographical conditions, sometimes drastically.

The impact of water pollution is enormous for several reasons, including the following:

- Freshwater is already a scarce resource in South Africa and many other countries due to climate change and increase in consumption, thus the detrimental effect of polluting an already scarce resource is a serious threat to life in general.
- Pollution of freshwater sources directly affects the livelihood and health of humans, animals and sometimes plant-life that depend on freshwater for survival.
- The treatment of wastewater is becoming more complex as new contaminants find their way into wastewater, e.g. pharmaceutical drugs and new chemicals and materials such as nanostructured materials, etc. Conventional treatment processes are not always efficient and this results to discharge of non-compliant quality water into the environment.
- The economy is negatively impacted because many industries need clean water for many of their production processes and treating or purifying wastewater is a costly process for any organization whether private or public.

With all these challenges that already exist, it is imperative to invest in how efficiently we treat our wastewater. The goal should be to treat the wastewater to a level that it can be re-used or recycled or to at least meet all the criteria prescribed for compliance. For example, the South African guidelines for effluent quality compliance as given in the Green Drop Report 2022 stipulates that the effluent quality must comply to 90% (in total) with the authorised limits for the respective categories: (a) 90% microbiological compliance, (b) 90% chemical compliance, and (c) 90% physical compliance. Many wastewater treatment plants (WWTPs) in SA fail significantly to meet this requirement.

AIM OF THE PROJECT

The aim of this project was to investigate the potential use of nanostructured biopolymer materials (also known as bionanocoils – BNCs) as flocculant materials in wastewater treatment in a joint R&D partnership with Biopolynet Inc, a cleantech company based in Canada that focuses on designing novel bio-polymeric network solutions. The materials were derived from agricultural biomass feedstocks using a biophysical process at a laboratory scale. The choice of the nanostructured biopolymer materials was due to their unique properties, such as being environmentally friendly or biodegradable, abundance of feedstocks and nanosized resulting to relatively high number of active sites for flocculation chemistry, ability to control chemical composition and charge, regeneration efficiency, non-toxicity and low cost. Due to the large volumes of wastewater that must be treated at a given time, it is imperative to have flocculants that require low dosages, i.e. flocculants that allow a lot to be done with less.

METHOD

The BNCs were successfully synthesized following a procedure developed by Biopolynet Inc. The goal was to customize the technology using local feedstocks and perform laboratory scale test work using standard jar test experiments. Tests were performed on different types of samples including municipal wastewater (sewage), mine tailings, and wastewater from a chemical manufacturing company.

SUMMARY OF FINDINGS AND CONCLUSION

In all tests the turbidity of wastewater was reduced by at least 90% after treatment with the BNCs over a wide pH range (pH 4-9) depending on the type of wastewater. The rate of flocculation (sedimentation/settling) was controlled by the dosages, which had an impact on the size of the flocs formed. Sedimentation was achieved within a minute in most of the tests conducted. The results of the project have demonstrated the potential for mass production and application of the BNC technology at a commercial scale in South Africa. Thus, while the aim at this stage was to investigate the viability, the next stage will focus on locally producing the BNCs at large-scale, optimization, and commercialization.

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

South Africa (SA) and many developing countries worldwide face serious problems that relate to poor water quality, scarcity, and management. Recently (2017-2021), Southern Africa and some parts of sub-Saharan Africa experienced severe droughts which left many areas without water. Some areas such as the Nelson Mandela Bay, Gqeberha in South Africa are still facing water scarcity in 2022. Also, the region still faces a daunting assignment for sustainable production and distribution of clean water to all households. Many rural communities have no access to clean drinking water [1-6]. Many informal settlements have poor domestic wastewater management, resulting to poor sanitation. Many drinking and wastewater treatment plants (WWTPs) require large volumes of costly chemicals that are added to the water to purify it; thus, the price of water remains high and unaffordable to poor people [7-11].

Many WWTPs and industries do not treat their wastewater to levels that could be re-used, as shown in Green Drop reports for WWTPs in South Africa (2014 and 2022) [12]. The wastewater produced is discharged to the environment, usually into rivers. According to the Water Research Commission of South Africa, the environmental discharge of saline water is contributed by mining sector (33%), petroleum processing (28%), power production (15%), paper (8%) and metal processing (7%) [13]. Based on the National Environmental Management Waste Act of 2013, the industrial wastewater discharge should not exceed 100 000 ppm TDS. Inappropriate discharge of high saline effluent presented triple ecological and socio-economic effects. Nonetheless, it is allowed in the South African coast to cater for economic considerations rather than environmental and social considerations. Despite this, the evidence of discharging the treated effluent to the estuaries should be provided to determine discharge suitability.

Attaining the Sustainable Development Goal 6 remains a challenge for many people living in poor communities in the region. Moreover, water, energy and food are essential for human well-being, poverty reduction and sustainable development [14]. Each of the three sources is inter-related in a complex system, which has been referred to as the water-energy-food (WEF) nexus. This nexus suggests that if any one of these resources is not available or generates harmful waste, then one or both the others are negatively affected [15]. Therefore, solutions must be crafted in a way that does not negatively affect the W-E-F nexus.

SA is generally doing well in producing clean drinking water to its citizens especially in urban areas and metros, but not so well in wastewater treatment. For example, the latest Blue and Green-Drop reports on SAs municipal WWTPs show that besides poor management of the WWTPs, the treatment processes used are not efficient for the removal of microbial and chemical contaminants from discharged wastewaters. The result is that nearby communities, especially in rural and informal settlements that use river water for domestic purposes, are exposed to contaminated water. Thus, contamination by inadequately treated wastewater discharge remains a serious problem affecting the lives of people [16]. Bacteria and viruses in contaminated water are known to grow and accumulate on either biotic or abiotic surfaces. The growth of bacteria on surfaces results to biofilm formation, which is largely attributed to the accumulation of soluble microbial products (SMP) and extracellular polymeric substances (EPS) produced by bacteria on material surfaces [17,18]. This has detrimental effects to consumers who must collect and store the untreated water before use.

Besides microbial water contamination, chemical water contaminants such as phenols and polycyclic aromatic hydrocarbons (PAHs), pharmaceutically discharged endocrine disruptors, and toxic metals such as lead, chromium and mercury have been reported as contaminants often present in the wastewater due to activities within the proximity of the water sources [1,19].

The use and disposal of phenols and its derivative products such as resins are the primary sources of phenols in water bodies. Notably, the health effects of phenols and PAHs on fauna and flora differ. Although phenols and PAHs are moderately persistent in water bodies [19,20], they are also absorbed by plant roots and be translocated to other parts of the plants. However, plants possess mechanisms that protect them against the effects of the organic contaminants [21]. Nonetheless, phenols and PAHs potentially bio-accumulate in fish and other aquatic animals living in water and in human beings. Depending on their concentration levels and exposure times, PAHs and phenols are known to cause tumours, affect the reproductive system and result in the development of reduced immunity. Their acute toxicity effects include irritation of skin, eyes, nervous system, vomiting, diarrhoea, confusion, and nausea [21,22] while the long-term effects are skin inflammation, liver damage, decreased immune system function, eye cataract damage and the destruction of the red blood cells [21,23,24].

Toxic metals are known to be sources of health problems such as reduced growth and development, destruction of organs, improper working of the nervous system, cancer, and fatal at elevated concentrations [25,26]. For instance, lead (Pb) and mercury (Hg) are known to cause kidney malfunctioning and miscarriages in adults [25,27]. They are also known to cause inadequate growth of the brain grey matter in children, which leads to poor intelligence [1,25,28]. Also, tailings from mining activities contribute significantly to poor quality of both ground and surface water and this does not only affect the pH of the water but also contributes to pollution of toxic metals to aquatic environment [29]. Acidified water leaching from the landfills of mine tailings lowers the water pH and contributes to increase in metal concentrations, thus, impacting negatively on aquatic life [29,30]. The soil layer affected by the leachates from the mine tailing dumps is concentrated with heavy metals and fails to support vegetation [29]. The iron present in the mine water leachate undergoes oxidation and controls the pH of the affected water sources. This oxidation process is very slow and affects areas kilometres away from the mine tailing landfills [29,31].

Clearly there is a need to consider other solutions or technologies for water and wastewater treatment. This project proposes the use of nanostructured biomass-derived polymers or bionanomaterials as an alternative to chemical-based materials for wastewater treatment, e.g. synthetic polymers used as flocculant materials. Chemical flocculant materials are currently used in tonnes in SA [32,33]. Technically, biomass-derived flocculants are produced from cellulose-based materials such as wood dust or plant materials. However, the synthesis of nanocellulose flocculants is costly and their flocculation properties are limited to a specific range [34]. For instance, the amount of energy (~30,000 kWh/ton) is required to delaminate fibres for production of nanocellulose flocculants [32-34]. Biomass materials have not been explored to a commercial level in SA. Some work has been done on nanocellulose, e.g. by researchers at UKZN but the research outcomes remain scholarly work. Elsewhere in the world, these materials are being produced at a commercial scale, e.g. pilot tested in Brazil and Canada [35-37].

This project was focussed on the treatment of various wastewater samples obtained from various South African sectors such as the mining industry, private companies, and municipality. The project involved conducting applied research for the laboratory scale synthesis, modification and testing of the bionanoflocullants; hereinafter represented as the bionanocoils (BNCs). After completion of this project, it is recommended to construct a scale-up, piloting and commercialization strategy for the bionanomaterials as flocculants for large scale wastewater treatment. Ultimately, the modified BNC technology will allow industries to produce water that can be recycled (efficient use of water) or safe to release to the environment. The technology can be customised for each application, i.e. type of wastewater (e.g. municipal sewerage, oil contaminated, mine tailings, etc.). In other applications, it can be used as a cost-effective pre-treatment step for membrane-based filtration technologies such as ultra and nanofiltration systems used in wastewater treatment. Thus, the technology could be beneficial to research entities such as the DSI/Mintek Nanotechnology Innovation Centre-Water Development focus area that produces such membranes.

For this project, the wastewater related problems that were be addressed by our innovative BNC technology are the following:

(i) Mine wastewater treatment: Efficient, environmentally friendly, and low-cost technologies for mine wastewater treatment and recovery are needed. New statistics released in the SA Parliament (2019), according to news articles (Mail & Guardian, May 2019), show that 118 mines around SA are polluting rivers and inadequately test for contamination, thus otherwise polluting SA's waterways. Much of the mining activities are negatively affecting water in the coalfields of Mpumalanga and Limpopo, which ranked first and second, respectively, in the number of mines not compliant with their water-use licences. Our research has shown that the cost of current technologies is a major hurdle to mining companies that produce megalitres of wastewater a day.

(ii) Municipal wastewater treatment: Efficient, environment friendly and low-cost technologies for wastewater treatment (including sewerage and sludge dewatering) are needed. Many wastewater bodies/treatment plants in SA do not purify wastewater to desired levels required for discharge, let alone for re-use. Informal and rural settlements near WWTPs still struggle with domestic wastewater management, and this causes a health risk to people living in these areas. With successful field-tests with private clients (e.g. mining companies), SabiNano and partners will further deploy the technology to municipalities.

(iii) Sludge generated from wastewater treatment: The use of chemical-based flocculants for sludge dewatering is not ideal for the environment since these contain components that are not compatible with the environment. Our customised BNC technology is an ideal solution that will be produced locally, piloted, and field-tested in industry.

1.2 CONTEXTUALISATION

The pollution of freshwater resources by industrial waste effluent and untreated sewerage is a big environmental concern globally. The spill-over effect of water pollution is enormous for several reasons including:

(i) freshwater is already a scarce resource in South Africa (SA) and many other countries due to climate change and rise in consumption, thus the detrimental effect of polluting an already scarce resource cannot be taken lightly,

(ii) pollution of freshwater directly affects the livelihood and health of humans, animals and sometimes plant-life that depend on freshwater for survival. For example, mining influences the quality and quantity of water in the mine area and in its surroundings and changes hydrological and topographical conditions, sometimes drastically,

(iii) the economy is negatively impacted because many industries need clean water for many of their production processes and treating or purifying wastewater is a costly process for any organization whether private or government owned. Wastewater re-use is almost non-existent in SA.

To contextualize the magnitude of the problem in SA, some recent news articles about the wastewater situation in SA can be found in these links.

(i) https://www.sowetanlive.co.za/news/south-africa/2020-01-02-south-africas-sewage-system-collapse-a-time-bomb/

(ii) https://www.dailymaverick.co.za/article/2019-04-07-department-of-water-and-sanitation-signals-renewed-efforts-to-manage-sas-water-and-sewerage-systems/

(ii) https://www.dailymaverick.co.za/article/2020-02-20-province-raps-city-of-cape-towns-knuckles-over-state-of-rivers/

(iv) https://mg.co.za/article/2019-05-17-00-big-increase-in-mine-water-pollution/.

From an industry (e.g. mining industry) perspective, environmental pollution caused by wastewater discharge and emission of greenhouse gases has become a critical component for the sustainability of their businesses. In fact, it is compulsory to have a strategy that will deal with such problems. A good example is Rainbow Minerals' Climate and Water Sustainability Strategy which includes a policy for water stewardship that considers environmental impact and profitability of the Group's mining activities [38]. The main operational water applications include dewatering, dust suppression, ore processing and tailings management. All these applications need technologies that can minimize the loss of water and so far, currently used products/technologies do not give optimal results especially for less established mining companies.

Municipal wastewater discharge by wastewater treatment plants (WWTPs) is also another major contributor to freshwater pollution in SA. Many municipal operated WWTPs fail to treat wastewater to levels that are suitable for discharge to the environment, let alone for reuse [13,39]. Sludge, dewatering is another problem that is often not given much attention, yet a lot of water is lost. Through strategic partnership with Biopolynet Inc., the project team led by SabiNano identified an opportunity to help mining companies to solve these problems at an affordable cost by locally developing and supplying an environmentally friendly flocculant technology/product based on nanoscale biomaterials (bionanomaterials) for industrial-scale use. Thus, the project can directly contribute to the economic and environmental sustainability of our economy and societies and in way a meaningful effort to Sustainable Development Goal 6: Ensure access to water and sanitation for all [40].

There are several conventional flocculation and coagulation treatment chemicals that are used in effluent wastewater treatment processes for solids removal, water clarification, lime softening, sludge thickening, and solids dewatering. Flocculation and sedimentation are widely employed in the purification of drinking water as well as in sewage treatment, storm-water treatment, and treatment of industrial wastewater streams [41,42]. Chemical flocculants are also classified as organic, such as polyelectrolyte polymers carrying a charge (e.g. polyacrylamides and polysaccharides cationic starch and chitosan) or inorganic (e.g. aluminum sulfate, aluminum chloride, sodium aluminate, aluminum chlorohydrate, polyaluminum chloride, ferric chloride, ferric sulfate, ferrous sulfate, and ferric chloride sulfate).

For use in large-scale operations, as anticipated for wastewater treatment and other applications such as production of microalgae biomass for fuels, a flocculant must meet certain key requirements: it must be effective, economical, environmentally benign, and readily available. Only certain inorganic flocculants (e.g. aluminum sulfate, or alum, and ferric chloride) meet all these criteria. Many other effective organic flocculants have been developed [34,43-45], but none is as cheap as the commonly used inorganic salts [41,42,46]. Moreover, these flocculants are based on chemicals that have some toxic elements and they are not biocompatible with the environment. Besides toxicological implications, the use of chemical flocculant agents for water purification is associated with high costs that may not even be met by many communities in the developing world. In recent years, these challenges have been advocated to be addressed using natural and rather safe alternative methods of water purification. SA has an abundance of unused waste biomass materials (raw materials) that form a major feedstock for the project.

On the bright side of things, the outcomes of the project will not only have an element of providing an alternative technological solution to wastewater treatment, but it will bring about other desired elements such as local manufacturing, thus creating new job opportunities and skills (through human capital development), especially to previously disadvantaged groups of people. The public-private partnership (PPP) approach enables the funder (Water Research Commission) to sit at the same table with the private companies developing the technology (SabiNano and Biopolynet), the university/science council research partners (University of the Witwatersrand and Mintek) and commercial partners for implementation of the solution/technology (Water Technovation SA). Moreover, the technology requires low capital cost, exhibits high performance, feedstocks are locally available, and it is environmentally friendly.

Finally, post the scientific research phase, the skills-sets of the personnel are critical for the success of the project as it influences productivity and the quality of the products and services rendered. To ensure that the workers and researchers are capacitated with the essential skills for the project; higher education, technical/on-the-job training and business etiquette programs will be put in place. This will create an environment in which employees will learn better and apply innovative ideas, acquire new competencies, develop skills, behaviours, and attitudes to advance the project and open new income streams for the company.

1.3 AIM OF THE STUDY

The aim of the project was to produce and test bionanomaterial-based flocculant materials (also known as bionanocoils – BNCs) as nanostructured biopolymers for wastewater treatment solution at a laboratory scale.

1.4 OUTCOME AND EXPECTED IMPACT

The envisaged outcome of the project is a lab-scale tested BNC flocculant technology for large-scale wastewater treatment, applicable in sewage treatment, sludge dewatering, mine tailings treatment and dust suppression.

Impact: Positive contribution to a greener wastewater treatment approach produced in SA with a potential to export to other African and BRICS countries. The technology will also present SA with a competitive edge to the existing conventional wastewater treatment methods due to its low cost and affordability. This will have a positive bearing on environmental sustainability and wastewater treatment in general.

1.5 REFERENCES

1. F. Fernández-luqueño, F. López-valdez, P. Gamero-melo, S. Luna-Suarez, E.N. Aguilera-gonzález, A.I. Martínez, M.S. García-Guilermo, G. Hernández-martínez, R. Herrera-mendoza, M.A. Álvarez-Garza, I.R. Perez-Velazquez, Heavy metal pollution in drinking water – a global risk for human health: A review, African J. Eviron. Sci. Technol. 7 (2013) 567-584. doi:10.5897/AJEST12.197.
2. F.R. Rijsberman, Water scarcity: Fact or fiction?, Agric. Water Manag. 80 (2006) 5-22. doi:10.1016/j.agwat.2005.07.001.
3. I.E. Ahaneku, P.A. Adeoye, Impact of pit latrines on groundwater quality of Fokoslum, Ibadan, Southwestern Nigeria, Br. J. Appl. Sci. Technol. 4 (2014) 440-449.
4. J.R. Gumbo, R.A. Dzaga, N.S. Nethengwe, Impact on water quality of Nandoni water reservoir downstream of municipal sewage plants in Vhembe district, South Africa, Sustain. 8 (2016) 1-16. doi:10.3390/su8070597.
5. L.N. Nthunya, N.P. Khumalo, A.R. Verliefde, B.B. Mamba, S.D. Mhlanga, Quantitative analysis of phenols and PAHs in the Nandoni Dam in Limpopo Province, South Africa: A preliminary study for dam water quality management, Phys. Chem. Earth, Parts A/B/C. (2019) 1-9. doi:10.1016/j.pce.2019.02.003.
6. L.N. Nthunya, M.L. Masheane, S.P. Malinga, E.N. Nxumalo, B.B. Mamba, S.D. Mhlanga, Determination of toxic metals in drinking water sources in the Chief Albert Luthuli Local Municipality in Mpumalanga, South Africa, Phys. Chem. Earth. 100 (2017) 94-100. doi:10.1016/j.pce.2017.04.006.
7. K.G. Linden, M. Mohseni, Advanced oxidation processes: Applications in drinking water treatment, in: Compr. Water Qual. Purif., Elsevier Ltd., 2014: pp. 148-172. doi:10.1016/B978-0-12-382182-9.00031-1.
8. E. Okoniewska, J. Lach, M. Kacprzak, E. Neczaj, The removal of manganese, iron and ammonium nitrogen on impregnated activated carbon, Desalination 206 (2007) 251-258. doi:10.1016/j.desal.2006.04.055.

9. I. Shah, R. Adnan, W.S.W. Ngah, N. Mohamed, Iron impregnated activated carbon as an efficient adsorbent for the removal of methylene blue: Regeneration and kinetics studies, PLoS One 10 (2015) 1-23. doi:10.1371/journal.pone.0122603.
10. WHO, Manganese in Drinking-water. Background document for development of WHO guidelines for drinking water quality, World Heal. Organ. Guidel. (2011) 1-21. http://www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf (accessed October 4, 2018).
11. M.A. Khadre, A. E. Yousef, J.-G. Kim, Microbiological aspects of ozone applications in food : a review, 66 (2001)1242-1252. doi.org/10.1111/j.1365-2621.2001.tb15196.x.
12. (a) S.A. Mitchell, M.P. de Wit, J.N. Blignaut, D. Crookes, Wastewater treatment plants: the financing mechanisms associated with achieving green drop rating, WRC, Pretoria, 2014; (b) Green Drop Report 2022 https://ws.dws.gov.za/iris/releases/Report_DPW_Rev02_29Mar22_MN%20web.pdf
13. I. Van der Merwe, A. Lourens, C. Waygood, An Investigation of Innovative Approaches to Brine Handling., Johannesburg, 2009.
14. O. Dubois, J.-M. Faurès, E. Felix, A. Flammini, J. Hoogeveen, L. Pluschke, M. Puri, O. Ünver, The Water-Energy-Food Nexus: A new approach in support of food security and sustainable agriculture, FAO, United Nations, 2014.
15. P. Andrews-Speed, S. Zhang, The Water-Energy-Food Nexus, in: China as a Glob. Clean Energy Champion, Palgrave Macmillan, Singapore, 2019: pp. 215-243. doi:10.1007/978-981-13-3492-4_9.
16. B. Ye, L. Yang, Y. Li, W. Wang, H. Li, Water sources and their protection from the impact of microbial contamination in rural areas of Beijing, China, Int. J. Environ. Res. Public Health. 10 (2013) 879-891. doi:10.3390/ijerph10030879.
17. K.R. Zodrow, E. Bar-Zeev, M.J. Giannetto, M. Elimelech, Biofouling and microbial communities in Membrane Distillation and Reverse Osmosis, Environ. Sci. Technol. 48 (2014) 13155-13164. doi:10.1021/es503051t.
18. T. Nguyen, F.A. Roddick, L. Fan, Biofouling of water treatment membranes: A review of the underlying causes, monitoring techniques and control measures, Membranes (Basel). 2 (2012) 804-840. doi:10.3390/membranes2040804.
19. D. Del Rio, A. Rodriguez-Mateos, J.P.E. Spencer, M. Tognolini, G. Borges, A. Crozier, Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases., Antioxid. Redox Signal. 18 (2013) 1818-92. doi:10.1089/ars.2012.4581.
20. D. de A. Azevedo, E. Gerchon, E.O. dos Reis, Monitoring of pesticides and polycyclic aromatic hydrocarbons in water from Paraiba do Sul River, Brazil, J. Braz. Chem. Soc. 15 (2004) 292-299.
21. Agency for Toxic Substances and Disease Registry, Toxicological profile for styrene, (2010) 1-236. doi:http://dx.doi.org/10.1155/2013/286524.
22. H. Babich, D.L. Davis, Phenol : A review of environmental and health risks, Regul. Toxicol. Pharmacol. 1 (1981) 90-109.
23. G. Grimmer, H. Brune, G. Dettbarn, K.W. Naujack, U. Mohr, R. Wenzel-Hartung, Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream smoke of cigarettes evaluated by implantation into the lungs of rats, Cancer Lett. 43 (1988) 173-177. doi:10.1016/0304-3835(88)90167-X.
24. J.F. Collins, J.P. Brown, G. V Alexeeff, A.G. Salmon, Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives., Regul. Toxicol. Pharmacol. 28 (1998) 45-54. doi:10.1006/rtph.1998.1235.
25. J.O. Duruibe, M.O.C. Ogwuegbu, J.N. Egwurugwu, Heavy metal pollution and human biotoxic effects, Int. J. Phys. Sci. 2 (2007) 112-118.
26. P.B. Tchounwou, C.G. Yedjou, A.K. Patlolla, D.J. Sutton, Heavy Metals Toxicity and the Environment, Natl. Institutes Heal. Public Access. 101 (2012) 1-30. doi:10.1007/978-3-7643-8340-4.
27. K. Sarder, S. Ali, S. Hameed, S. Afzal, M.B. Shakoor, S.A. Bharwana, H.M. Tauqeer, Heavy Metals Contamination and what are the Impacts on Living Organisms, Greener J. Environ. Manag. Public Saf. 2 (2013) 172-179.

28. L. Jarup, Hazards of heavy metal contamination, Br. Med. Bull. 68 (2003) 167-182. doi:10.1093/bmb/ldg032.
29. K. Naicker, E. Cukrowska, T.S. McCarthy, Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs, Environ. Pollut. 122 (2003) 29-40. doi:10.1016/S0269-7491(02)00281-6.
30. T.S. McCarthy, The impact of acid mine drainage in South Africa, S. Afr. J. Sci. 107 (2011) 1-7. doi:10.4102/sajs.v107i5/6.712.
31. A.S. Rogowski, Acid mine drainage from reclaimed coal strip mines: model description, Water Resources 20 (1984) 233-242.
32. A. Nkalane, O.A. Oyewo, T. Leswifi, M.S. Onyango, Application of coagulant obtained through charge reversal of sawdust-derived cellulose nanocrystals in the enhancement of water turbidity removal, Mater. Res. Express. 6 (2019) 1-10. doi:10.1088/2053-1591/ab3b49.
33. B. Zulu, O.A. Oyewo, B. Sithole, T.Y. Leswifi, M.S. Onyango, Functionalized sawdust-derived cellulose nanocrystalline adsorbent for efficient removal of vanadium from aqueous solution, Front. Environ. Sci. 8 (2020) 1-12. doi:10.3389/fenvs.2020.00056.
34. D.K. Patel, S.D. Dutta, K.T. Lim, Nanocellulose-based polymer hybrids and their emerging applications in biomedical engineering and water purification, RSC Adv. 9 (2019) 19143-19162. doi:10.1039/c9ra03261d.
35. E.S. Tarleton, R.J. Wakeman, Pretreatment of suspensions, in: Solid/Liquid Sep. Equip. Sel. Process Des., 2007: pp. 126-151. doi:10.1016/b978-185617421-3/50003-1.
36. J. Huang, S. Fu, L. Gan, Lignin chemicals and their applications, in: Lignin Chemicals Applications, Elsevier, 2019: pp. 79-134. doi:10.1016/b978-0-12-813941-7.00004-7.
37. T. Chatsungnoen, Y. Chisti, Flocculation and electroflocculation for algal biomass recovery, in: Biofuels from Algae, 2nd ed., Elsevier B.V., 2019: pp. 257-286. doi:10.1016/b978-0-444-64192-2.00011-1.
38. T.M. Askham, H.M. Van der Poll, Water sustainability of selected mining companies in South Africa, Sustainability 9 (2017) 1-16. doi:10.3390/su9060957.
39. Sershen, N. Rodda, T.A. Stenström, S. Schmidt, M. Dent, F. Bux, N. Hanke, C.A. Buckley, C. Fennemore, Water security in South Africa: Perceptions on public expectations and municipal obligations, governance and water re-use, Water SA 42 (2016) 456-465. doi:10.4314/wsa.v42i3.11.
40. T.L. Cumming, R.T. Shackleton, J. Förster, J. Dini, A. Khan, M. Gumula, I. Kubiszewski, Achieving the national development agenda and the Sustainable Development Goals (SDGs) through investment in ecological infrastructure: A case study of South Africa, Ecosyst. Serv. 27 (2017) 253-260. doi:10.1016/j.ecoser.2017.05.005.
41. G.S. Simate, The treatment of brewery wastewater for reuse by integration of coagulation/flocculation and sedimentation with carbon nanotubes “sandwiched” in a granular filter bed, J. Ind. Eng. Chem. 21 (2015) 1277-1285. doi:10.1016/j.jiec.2014.06.001.
42. X. Guo, Z. Wu, M. He, Removal of antimony(V) and antimony(III) from drinking water by coagulation-flocculation-sedimentation (CFS), Water Res. 43 (2009) 4327-4335. doi:10.1016/j.watres.2009.06.033.
43. N.H. Kim, T. Imai, M. Wada, J. Sugiyama, Molecular directionality in cellulose polymorphs, Biomacromolecules 7 (2006) 274-280. doi:10.1021/bm0506391.
44. F.M. Pelissari, P.J.D.A. Sobral, F.C. Menegalli, Isolation and characterization of cellulose nanofibers from banana peels, Cellulose 21 (2014) 417-432. doi:10.1007/s10570-013-0138-6.
45. R.K. Mishra, S.K. Ha, K. Verma, S.K. Tiwari, Recent progress in selected bio-nanomaterials and their engineering applications: An overview, J. Sci. Adv. Mater. Devices. 3 (2018) 263-288. doi:10.1016/j.jsamd.2018.05.003.
46. C.S. Fitzpatrick, J. Gregory, Coagulation and filtration, in: Handbook of Water Wastewater Microbiology, Elsevier, 2003: pp. 633-655. doi:10.1016/B978-012470100-7/50039-X.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Water is the main driver for industrial operations and a need for survival of all living organisms. However, the quality of existing freshwater resources is poor in many parts of the world [1,2]. The quality of water depends on various characteristics linked to geological and ecological features of the water basin, as well as the rate of water pollution. The increase in economic activities and improper sanitation have contributed to the degradation in quality of freshwater sources [3,4]. Water pollution is defined as an increase in chemical, physical or biological contaminants that cannot be controlled by the natural activities [5,6]. Water pollution induced by the refineries, mining, tanneries, pharmaceuticals, pulp mills, sugar production and other industrial discharges has led to the disappearance of biodiversity and aquatic ecosystems as well as the spreading of diseases to humans and animals [2,7-9].

Additionally, resource mismanagement of integrated water supply has brought devastating challenges in water sectors [10]. This problem is exacerbated by poor management of large-scale physical infrastructure and centralized water management systems, particularly in wastewater. Although environmental problems caused by discharge of poorly treated wastewater could be minimised through decentralisation of water management, lack of knowledge within water sector where solutions are targeted towards the end-of-the-pipe product remains key [10,11]. Regions where water supply is increasingly being constrained, the demand-side management is remodelled with the focus to manage water demand and also calling for efficient water use practices [12,13]. A typical example is municipal control of water use in Western Cape Province of SA, where the level of the water in the main supply dams falls below 30% of its capacity, thus threatening domestic use of water. In this regard, cost-effective and efficient treatment of wastewater for secondary use is imperative. Although conventional treatment of wastewater has been existing since the 19th century, their successes were associated with their safe discharge to the environmental [14,15]. This project sought to provide an alternative solution for wastewater treatment sector. This involves the application of biomass-derived biopolymers as flocculants for removal of unwanted colloidal, dissolved solids and toxic contaminants. The design of the technology also makes it ideal for recovery of precious metals by flocculation.

2.2 BIOMASS BIOPOLYMERS AS FLOCCULANT MATERIALS

The rampant interest in greener technologies has drawn attention towards the use of biomass materials for nano-based materials synthesis due to their remarkable properties [16]. To define the suitability for high performing treatment processes, nanotechnology has offered suitable platform to control morphologies and chemical functionalities of these biomass-based materials [17]. Consequently, prospects accompanying a new class of bionanoflocculants with exceptional functionalities such as adsorptive capabilities have been presented over the last few years [18,19]. Among these, nanoflocculants produced from naturally occurring biomass have received remarkable attention in research [20]. Their tuneable structures and properties, including high surface area, thermal stability, and reduced ecological footprint, make them suitable for a large variety of applications in the wastewater treatment industry [21,22].

Biomass-derived flocculants or simply bioflocculants are produced *via* mechanical disintegration, biological or chemical treatment [16,17]. These bioflocculants have gained popularity due to their cost-effectiveness, non-toxicity, biodegradability, minimisation of secondary pollution and their capability to coagulate trace elements [18-20]. Evaluation of bioflocculants was prompted by the challenges of current industrially dominating chemical flocculants which are costly, hazardous and often need to be imported from abroad to supplement local supply [21-23].

Bioflocculants demonstrating efficient coagulation of colloidal particles resulting in accelerated floc sedimentation are increasingly becoming common in drinking and wastewater purification. Notably, these flocking agents have shown capabilities to remove water impurities at their lowest dosages within a short space of time [18,24]. Currently, inorganic flocculants including aluminium sulphate and iron chloride are extensively used for drinking and wastewater treatment, particularly because of their high availability and low costs [22,25]. However, these inorganic flocculants are pH sensitive where low pollutant removal is observed at lower pH values [23]. Also, inorganic flocculants produce large amounts of sludge which cause secondary pollution, specifically polluting the underground water caused by leaching metals from the sludge [26]. Because of this, scientists are advancing water purification processes addressing modern problems *via* increased use of biomass-based flocculants [3,27,28].

Bioflocculants can form large flocs that quickly settle in the solution. These flocculants do not only show stability to shear stress, but also high effectiveness at low dosages [29]. The effects of bioflocculation depends on the size of structure, i.e. the particle rotational radius which conforms to movement privileges in solution. Added advantages of biomass, specifically polysaccharides include availability, biodegradability, and high flocculating effects. These compounds are characterised by glucosidic (–C-O-C–), hydroxyl (–OH , amines (R–NH_2) and carbonyl (R_2–C=O) groups [30-32]. These functional groups play the greatest role in the flocculation processes.

Polysaccharides are naturally occurring products sourced from cellulose, starch, pectin, chitin (a product of crustaceans). Also, bioflocculants are derived from algae, bacteria, plants, tunicates and agro-waste [17]. Among other sources, plants are naturally occurring and available in abundance thus making isolated bioflocculants cost-effective. These include rice starch, leaves, jackfruit seed starch, *cassia obtusifolia* seed gum, [3]. This technology does not only utilise the plant products for removal of toxic pollutants from water but also saves the environment from land and air pollution caused by the rotten waste heaps-produced toxins. The raw materials used in the synthesis of bioflocculants are renewable, which is an added advantage for continuous development and supply [29]. Importantly, the use of biomass opens further research directions towards development of coagulants from natural materials. To date, several studies have reported successful use of bioflocculants to treat wastewater.

Liu *et al.* [33] synthesised a novel bioflocculant MBF-C9 produced by a salt-tolerant, alkaliphilic *Bacillus agaradhaerens C9*. The effects of culture conditions such as initial pH, carbon source, nitrogen source, C/N ratio, and NaCl concentrations on MBF-C9 production were studied. Reportedly, 4.65 g/L purified MBF-C9 was extracted under the following optimized conditions: 10 g/L glucose as carbon source, 10 g/L yeast extract as nitrogen source and initial pH 10.2. The MBF-C9 contained 65.4% polysaccharides, 4.7% proteins, and 1.7% nucleic acids. The highest flocculating rate of 95.3% for kaolin suspension was achieved at a dosage of 1.5 mg/L [33]. Also, bioflocculants produced through microbial fermentation, extraction and refining have been developed since 1970s. These flocculants remove pollutants at low dosages, they possess high flocculating effect, biodegradable and are easily handled. For instance,

Agunbiade *et al*. [28] produced bioflocculant from *Streptomyces platensis* and evaluated its potential for treatment of river and wastewater. This bioflocculant removed chemical oxygen demand (COD) in river water and meat processing wastewater at efficiencies of 63.1 and 46.6% respectively. Also, the turbidity was reduced by 84.3 and 75.6% respectively [28]. However, microbial polysaccharides are costly in large scale applications. This may hinder their economic applications in wastewater treatment. This has prompted research gearing towards production of biopolymers characterised by coiled nanostructures [34,35]. These coiled nanostructured biopolymers (BNCs) are produced through a biophysical process in which the macromolecular chains of the materials are altered. These nanostructured flocculating materials are also cross-linked to enhance their preparatory methods and the application in wastewater purification. Also, incorporation of the nanoparticles to improved bioflocculants properties against external stress is imperative.

2.3 NANOSTRUCTURED BIOMATERIALS

The biomass used for synthesis of nanocoiled (BNC) bioflocculants contains at least one of a hemicellulose, starch, and an alginate biopolymer macromolecules [21,36,37]. The biomass raw material comprises of a mixture of different biopolymers for specific applications. On the other hand, biomass raw material may comprise both a hemicellulose biopolymer macromolecule and a starch biopolymer macromolecule. This mixed biomass raw material is prepared, by combining a source of hemicellulose biopolymer macromolecules, such as pulp mill biomass, with a source of starch biopolymer macromolecules, such as potato starch or corn starch [38,39]. Furthermore, the biomass raw material contains any number of additional materials, which may serve different roles in the preparation of the biopolymer nanocoils. The biomass-based flocculants are designed to meet specific properties for applications in wastewater [16,40,41]. These materials can bind particles and form an interlocked complex web. Owing to their ability to change application rates, nanocoiled bioflocculants provide a user interface enabling precise control, stabilization, and durability to be tailored as required. When added to the wastewater, nanocoiled bioflocculants form networks with metals, thus facilitating their removal from water. These biomass-based nanocoiled flocculants have been extracted and evaluated for their potential application in wastewater treatment.

2.4 MECHANISM OF FLOCCULATION FOR HARVESTING OF WATER POLLUTANTS

Bioflocculants consists of different compositions of polysaccharides, proteins, nucleic acids, uronic acids (i.e. sugars where the CH_2OH group has been oxidised to form a carboxylic acid group). These components afford bioflocculants with carbonyl, glucosidic, hydroxyl, and amines groups that act as active sites for particle adsorption [16]. Mechanism of bioflocculants follows two processes, namely (a) charge neutralisation (b) and polymer bridging (**Figure 2-1**).

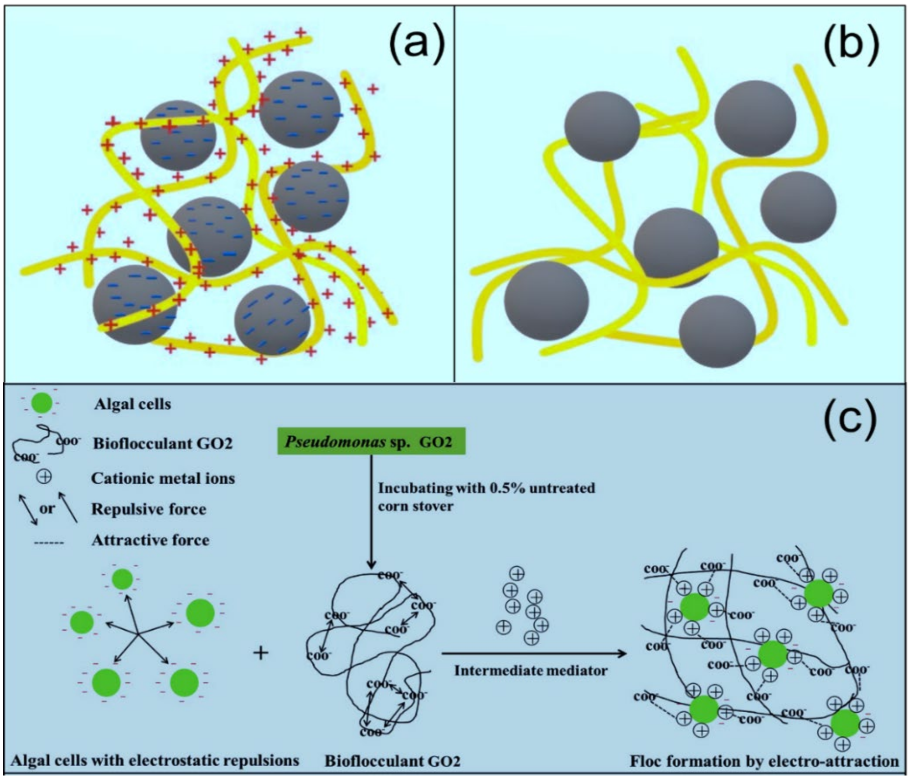


Figure 2-1: Mechanism of flocculation during harvesting: (a) charge neutralization; (b) polymer bridging; and (c) floc formation through electrostatic interaction [16,29].

The flocculation process is activated by electrostatic and hydrophobic interactions, complexation, hydrogen bonding and bridging of macromolecules [35]. Notably, flocculation is a multi-stage process that involves physico-chemical interactions between molecules [3]. When dosing a flocculant in the wastewater, the minimum concentration required to form settleable flocs (flocculation window) should be established.

(a) Charge neutralisation

During flocculation, the process of charge neutralisation occurs when the flocculant and colloidal particles possess opposite charges. During this process, the charge density of the particles is reduced causing destabilisation due to repulsive interactions [16]. This neutralisation effect reduces zeta potential of the colloids, thus an interaction reduction between the colloids and the dispersing medium. Reduced zeta potential promotes creation of van der Waals forces which facilitate the aggregation of the flocs.

Polyelectrolytic bioflocculants carrying ionic charge in their chain induce partial neutralisation of the colloids charge [40]. However, neutralisation emanating from these flocculants is incomplete and this leads to formation of positive and negative charge on the colloids. These differences in charges facilitate attraction of neighbouring particles, thus flocs formation [40]. The flocs formed through a partial neutralisation are strongly bonded compared to complete neutralisation process [3].

(b) Bioflocculation bridging

During polymer bridging, the colloidal particles are adsorbed on the flocculant causing formation of coils and tails suspended in solution. Adsorption occurs because of hydrogen bond formation, van der Waals forces and electrostatic interaction between the bioflocculant and colloidal particles [16]. The formed coils of the adjacent particles are intertwined during stirring to form large flocs [27]. For this mechanism to occur, the molecular weight of the biopolymer must be relatively high (10^6 Da or above). Also, biopolymers form entangled microchains and coiled structures facilitating entrapment of colloidal particles within networks [28]. Classically, the flocs formed by this process are stronger and larger compared to those formed through the use of inorganic flocculants. Other advantages of biopolymeric flocculants is their simplicity and ease of modification to introduce functional groups responsible for binding of impurities [19].

2.5 PARAMETERS AFFECTING FLOCCULATION

The process of flocculation and its effectiveness is affected by several factors including surface charge of the colloidal particle of the bioflocculant, the molecular weight and concentration of the bioflocculant, pH of the medium, ionic strength, temperature, agitation speed and the flocculation mechanism. However, most factors particularly dominating the reported literature are briefly discussed below.

2.5.1 Flocculant concentration

The optimum concentration/dose of the flocculant at which flocs can settle should be established. Insufficient or high doses of the flocculants affect the flocculation performance. When flocculant is dosed at higher concentration, it destabilises the floc formation [29]. At higher doses, colloidal particles become over clouded resulting in saturation of their surfaces. This saturation reduces particle stability, hence their difficulty to flocculate. Agunbiade *et al.* evaluated the jar test dosing of the bioflocculant required to clarify kaolin clay suspension (4 g/L) following a standard protocol [28]. This protocol involved rapid mixing at 160 rpm for 2 min, followed by gradual flocculation of 40 rpm for 2 min and 5 min sedimentation. To measure the flocculating activity, 2 mL was withdrawn from the clarifying phase after sedimentation. The flocculant dosage ranged from 0.1 to 1.0 mg/mL. 0.2 mg/L of the flocculant resulted in 94.8% flocculating activity. An increase in flocculant dose beyond 0.2 mg/L led to the decrease in turbidity reduction and flocculating activity. Therefore, 0.2 mg/L was reported as the optimum dose for clarification of kaolin clay suspension.

2.5.2 Effect of pH

The pH is one of the most important parameters influencing the efficiency of flocculation. An increase in solution pH beyond alkaline environment hinders flocculation activity even when flocculant dose is increased. Notably, increasing the dose before optimum concentration increases flocculating activity. However, this phenomenon is not possible at higher pH values. Therefore, the solution requires acidification to improve activity. Therefore, different types of flocculants require pH optimisation. Solution pH influences the zeta potential of the solution, and subsequently the mechanism pathway. Dlamini *et al.* evaluated the optimum pH required to produce highest flocculating activity during treatment of coal mine wastewater. The pH range of 3-11 was adjusted using NaOH (1.0 M) or HCl (1.0 M). The bioflocculant was subjected to high temperatures (50-100°C) in a water bath for 30 min to determine thermostability, after which the flocculation activity was. The maximum flocculating activity of the bioflocculant was reported at pH 7. The optimum pH can also be established by determining the effect of charge on the bioflocculant. This is achieved through determination of the point of zero charge (pzc). At this point, the charge of the flocculant is zero. Above this pH, the flocculant become negatively charged, thus facilitating the electrostatic attraction of positively charged ions. This phenomenon was reported by Guo *et al.* [16] where pH values below 8 achieved more than 90% flocculating efficiency. It was reported that, the pH affected the electronic states of the bioflocculant resulting in improved flocculation efficiency. The increase in pH above 8 reduced flocculating activity due to weakening of spatila charge arrangements.

2.5.3 Effect of electrolyte

The electrolyte plays an important role in destabilizing colloids to facilitate aggregation. The electrical charge of the particles determines the mutual repulsions resulting in solution stabilization. Addition of inorganic salts in the flocculation media reduces the electric layer leading to formation and settlement of the flocs [16]. Upon addition of the electrolyte, the ionic strength of the solution changes leading to increased rate of flocs settlement. Notably, the flocculating activity is more pronounced upon addition of divalent cations compared to monovalent cations. The effect of electrolytic salts has been reported in several studies. In their study, Agunbiade *et al.* [42] investigated the effects of monovalent, divalent and trivalent cations sources (Na^+,K^+,Mg^{2+},Mn^{2+}, Al^{3+} and Fe^{3+} were also investigated for optimum bioflocculating activity of *Arthrobacter humicola*-produced bioflocculant. The flocculating activity upon addition of Fe^{3+}, Al^{3+}, K^+, Mg^{2+}, Na^+, Mn^{2+}, Ca^{2+} were 57.90 ± 0., 65.30 ± 0.6%, 33.10 ± 1.6%, 88.70 ± 0.9%, 41.70 ± 1.5%, 75.70 ± 2.25%, 89.00 ± 0.70% respectively. Based on these results, the tested cations enhanced the flocculating activity of the bioflocculant though that of the monovalent ions was minimal. The flocculating activity was highest upon addition of divalent cations (Ca^{2+} and Mg^{2+}) [42]. This implies that neutralization of the negative charge of the flocculation medium was enhanced by addition of divalent cations, thus improving the adsorption of pollutants onto the surface of the bioflocculant. This facilitated agglomeration of the formed aggregates and sedimentation. Also, these cations promote formation of bridges between particles which in turn improves flocculating efficiency.

2.5.4 Effecting of shear rate

The formation, stability and settling ability of the flocs depends on frequency of particle interactions between aggregates. During this process, weakly bound or bonded aggregates break apart and remain suspended in solution. When shear forces increase during intensive mixing, the formed flocs crumble into small fragments. Although high shear rate disintegrates flocs, the fragmented particles can be regrown upon reduction in shear stress. The reversibility of the disintegration and regrowth of the flocs depends on the type of flocculant used.

2.6 SLUDGE DEWATERING

2.6.1 Overview

The disposal of the sludge forms an integral part of water and wastewater treatment. This process covers more than 50% of the operational costs during wastewater treatment. Dewatering of the sludge should be conducted to minimize waste prior to disposal. Also, the cost of disposal could be reduced after dewatering. Sludge consists of highly complicated components including water and particulate matter. The presence of various types of solids within the sludge ensure existence of water characterised by various states and thermodynamic properties including vapour pressure, enthalpy, entropy, viscosity, and density. The sludge water is classified according to difficulties encountered in its separation from the solid materials. About 70% of water occupying the voids of the sludge, which is not controlled by the capillary forces forms part of the first classification. The interstitial water trapped inside the sludge cavities forms part of the second classification. The sludge adsorbs part of the water on the surface of its particles which forms part of the third classification. The last one is called water of hydration which is bound on the surface of the solids. These classified waters are the key drivers determining the rate of sludge dewatering.

Conventional methods such as include sonication, centrifugation, and sonication have been applied in the dewatering processes. These processes can only remove free water and part of the bound water leaving the water occupying the voids and the interstitial positions. To improve dewatering efficiency, flocculation has been applied in sludge dewatering process.

2.6.2 Flocculation

Flocculation is cost-effective, user-friendly, and relatively a mature sludge dewatering technology. As indicated earlier, efficiency of flocculation depends on several flocculant properties including ionic properties, molecular weight and functional groups. Due to their high molecular weight leading to bridging potential, polymer-based flocculants have received remarkable interest in sludge dewatering. Mechanism of sludge dewatering is similar to that of wastewater treatment, thus making the bioflocculants more applicable in this process. However, it is worth noting that the fundamental objective of sludge dewatering is to remove water from the solid materials.

The flocculants initiate formation of aggregates and subsequently the sludge cake. Therefore, this sludge dewatering is more complication than a normal flocculation process during wastewater treatment. In this process, flocculants collide with suspended colloids to form large flocs. Like in wastewater treatment, flocculation during sludge dewatering is affected by various factors including the conditioner dose, pH, temperature.

2.6.2.1 Conditioner dose

Flocculant dose is the most important parameter affecting flocculation process during sludge dewatering. The optimum dose should be established to minimised unnecessary addition of the flocculant. Optimal dosing leads to stabilization of the flocs due to sufficient coverage of the sludge particles by the flocculant [43]. Dewaterability is affected by flocculant overdose due to fluctuation of the soluble extracellular polymeric substances (EPS) caused by the increased amounts of biomatters [44]. Also, underdosing causes weak charge neutralisation and low bridging effects [44]. Similarly, the compactness of the sludge is reduced at high flocculant doses. Therefore, overdosing of the flocculant deteriorate the dewatering performance. Specifically, the flocculant overdose increases the viscosity of the sludge water resulting in reduced water separation from the solid particles [45].

2.6.2.2 pH

During sludge dewatering, the pH of the medium should be optimised. It has been previously indicated that charge neutralisation in the presence of ionic flocculants changes at various pH levels [46]. A similar phenomenon is realised in sludge dewatering where negative charge of flocculants increases with increase in medium pH [46]. Nonetheless, bioflocculants with neutral and strong ionic properties are able to sustain a wide pH during sludge dewatering. Similarly, flocculant characterised by weak ionic strength are strongly affected by pH variation [47]. Similar challenge is seen in bioflocculants characterised by weak acidic groups such as carboxyl groups. During sludge dewatering, the pH variation affects the leakage of EPS. At acidic conditions, the EPS are reduced and destroyed, leading to good dewaterability and floc settleability.

2.6.2.3 Temperature

Like other flocculation processes, temperature affects flocculation during sludge dewatering. At high temperature, bioflocculants dissolve resulting in configuration of macromolecules which initiates bridging [48]. However, elevated temperature can cause instability in floc formation [48]. The increase in temperature can also cause a decrease in shear rate and weaken the network of the formed flocs [49]. The rate of dewaterability could be changes by altering the temperature, which changes the sludge properties. For instance, at low temperatures, the flocs become weaker, thus increasing the concentration of the suspended solids [49].

2.7 REFERENCES

1. [1] K.G. Zuurbier, P.J. Stuyfzand, Consequences and mitigation of saltwater intrusion induced by short-circuiting during aquifer storage and recovery in a coastal subsurface, Hydrol. Earth Syst. Sci. 21 (2017) 1173-1188. https://doi.org/10.5194/hess-21-1173-2017.
2. [2] J.R. Gumbo, R.A. Dzaga, N.S. Nethengwe, Impact on water quality of Nandoni water reservoir downstream of municipal sewage plants in Vhembe district, South Africa, Sustain. 8 (2016) 1-16. https://doi.org/10.3390/su8070597.
3. [3] S.N.A. Mohd-Salleh, N.S. Mohd-Zin, N. Othman, A review of wastewater treatment using natural material and its potential as aid and composite coagulant, Sains Malaysiana. 48 (2019) 155-164. https://doi.org/10.17576/jsm-2019-4801-18.
4. [4] R.K. Mishra, S.C. Dubey, Fresh water availability and it’s global challenge, Int. J. Eng. Sci. Invent. Res. Dev. 2 (2015) 351-407.
5. [5] A.M. Sulej, Ż. Polkowska, J. Namieśnik, Contaminants in airport runoff water in the vicinities of two international airports in Poland, Polish J. Environ. Stud. 21 (2012) 725-739.
6. [6] L.N. Nthunya, S. Maifadi, B. Mamba, Bhekie, A.R. Verliefde, S.D. Mhlanga, Spectroscopic determination of water salinity in brackish surface water in Nandoni Dam, at Vhembe District, Limpopo Province, South Africa, Water. 10 (2018) 1-13. https://doi.org/10.3390/w10080990.
7. [7] T. Rajaram, A. Das, Water pollution by industrial effluents in India: Discharge scenarios and case for participatory ecosystem specific local regulation, Futures. 40 (2008) 56-69. https://doi.org/10.1016/j.futures.2007.06.002.
8. [8] Y. Nergis, M. Sharif, A.F. Choudhry, A. Hussain, J.A. Butt, Impact of Industrial and Sewage Effluents on Karachi Coastal Water and Sediment Quality, Middle-East J. Sci. Res. 11 (2012) 1443-1454. https://doi.org/10.5829/idosi.mejsr.2012.11.10.63209.
9. [9] F. Fernández-luqueño, F. López-valdez, P. Gamero-melo, S. Luna-Suarez, E.N. Aguilera-gonzález, A.I. Martínez, M.S. García-Guilermo, G. Hernández-martínez, R. Herrera-mendoza, M.A. Álvarez-Garza, I.R. Perez-Velazquez, Heavy metal pollution in drinking water – a global risk for human health: A review, African J. Emvironmental Sci. Technol. 7 (2013) 567-584. https://doi.org/10.5897/AJEST12.197.
10. [10] V. Srinivasan, E.F. Lambin, S.M. Gorelick, B.H. Thompson, S. Rozelle, The nature and causes of the global water crisis: Syndromes from a meta-analysis of coupled human-water studies, Water Resour. Res. 48 (2012) 1-16. https://doi.org/10.1029/2011WR011087.

11. [11] Y. Bettini, R. Brown, F.J. De Haan, Water scarcity and institutional change: Lessons in adaptive governance from the drought experience of Perth, Western Australia, Water Sci. Technol. 67 (2013) 2160-2168. https://doi.org/10.2166/wst.2013.127.
12. [12] F.R. Rijsberman, Water scarcity: Fact or fiction?, Agric. Water Manag. 80 (2006) 5-22. https://doi.org/10.1016/j.agwat.2005.07.001.
13. [13] P.H. Gleick, M. Palaniappan, Peak water limits to freshwater withdrawal and use, Proc. Natl. Acad. Sci. 107 (2010) 11155-11162. https://doi.org/10.1073/pnas.1004812107.
14. [14] M.R. Awual, M.M. Hasan, M.A. Khaleque, M.C. Sheikh, Treatment of copper(II) containing wastewater by a newly developed ligand based facial conjugate materials, Chem. Eng. J. 288 (2016) 368-376. https://doi.org/10.1016/j.cej.2015.11.108.
15. [15] C. V Vedavyasan, Pretreatment trends — an overview, Desalination. 203 (2007) 296-299. https://doi.org/10.1016/j.desal.2006.04.012.
16. [16] H. Guo, C. Hong, B. Zheng, F. Lu, D. Jiang, W. Qin, Bioflocculants' production in a biomass-degrading bacterium using untreated corn stover as carbon source and use of bioflocculants for microalgae harvest, Biotechnol. Biofuels. 10 (2017) 1-12. https://doi.org/10.1186/s13068-017-0987-6.
17. [17] K.P.Y. Shak, Y.L. Pang, S.K. Mah, Nanocellulose: Recent advances and its prospects in environmental remediation, Beilstein J. Nanotechnol. 9 (2018) 2479-2498. https://doi.org/10.3762/bjnano.9.232.
18. [18] Y. Li, Y. Xu, L. Liu, X. Jiang, K. Zhang, T. Zheng, H. Wang, First evidence of bioflocculant from Shinella albus with flocculation activity on harvesting of Chlorella vulgaris biomass, Bioresour. Technol. 218 (2016) 807-815. https://doi.org/10.1016/j.biortech.2016.07.034.
19. [19] Y. Yin, Z. Tian, W. Tang, L. Li, L. Song, S.P. Mcelmurry, Production and characterization of high efficiency bioflocculant isolated from Klebsiella sp . ZZ-3, Bioresour. Technol. 171 (2014) 336-342. https://doi.org/10.1016/j.biortech.2014.08.094.
20. [20] N.G. Dlamini, A.K. Basson, Wastewater Treatment by a Polymeric Bioflocculant and Iron Nanoparticles Synthesized from a Bioflocculant, Polymers (Basel). 12 (2020) 1-14. https://doi.org/10.3390/polym12071618.
21. [21] H. Jung, D. Pauly, Water in the Pulp and Paper Industry, in: Treatise Water Sci., Elsevier B.V., Netherlands, 2011: pp. 667-683. https://doi.org/10.1016/B978-0-444-53199-5.00100-7.
22. [22] R. Valavala, J. Sohn, J. Han, N. Her, Y. Yoon, Pretreatment in reverse osmosis seawater desalination: A short review, Environ. Eng. Res. 16 (2011) 205-212. https://doi.org/10.4491/eer.2011.16.4.205.
23. [23] T. Abitbol, A. Rivkin, Y. Cao, Y. Nevo, E. Abraham, T. Ben-shalom, S. Lapidot, O. Shoseyov, Nanocellulose , a tiny fiber with huge applications, Curr. Opin. Biotechnol. 39 (2016) 76-88. https://doi.org/10.1016/j.copbio.2016.01.002.
24. [24] Z.M.A. Tawila, S. Ismail, A. Dadrasnia, Production and Characterization of a Bioflocculant Produced by Bacillus salmalaya 139SI-7 and Its Applications in Wastewater Treatment, Molecules. 23 (2018) 1-17. https://doi.org/10.3390/molecules23102689.
25. [25] H. Huang, K. Schwab, J.G. Jacangelo, Pretreatment for low pressure membranes in water treatment: A review, Environ. Sci. Technol. 43 (2009) 3011-3019. https://doi.org/10.1021/es802473r.
26. [26] M. Sillanpää, A. Matilainen, NOM Removal by Coagulation, in: Nat. Org. Matter Water Charact. Treat. Methods, Elsevier Inc., 2015: pp. 55-80. https://doi.org/10.1016/B978-0-12-801503-2.00003-3.
27. [27] J. Liu, J. Ma, Y. Liu, Y. Yang, D. Yue, H. Wang, Optimized production of a novel bioflocculant M-C11 by Klebsiella sp . and its application in sludge dewatering, J. Environ. Sci. 26 (2014) 2076-2083. https://doi.org/10.1016/j.jes.2014.08.007.
28. [28] M. Agunbiade, C. Pohl, O. Ashafa, Bioflocculant production from Streptomyces platensis and its potential for river and waste water treatment, Brazilian J. Microbiol. 49 (2018) 731-741. https://doi.org/10.1016/j.bjm.2017.02.013.
29. [29] P. Maćczak, H. Kaczmarek, M. Ziegler-Borowska, Recent achievements in polymer bio-based flocculants for water treatment, Materials (Basel). 13 (2020) 1-41. https://doi.org/10.3390/ma13183951.
30. [30] J. Puls, S. a. Wilson, D. Holter, Degradation of Cellulose Acetate-Based Materials: A Review,

J. Polym. Environ. 19 (2010) 152-165. https://doi.org/10.1007/s10924-010-0258-0.
31. [31] B. Voncina, V. Vivod, Cyclodextrins in Textile Finishing, (n.d.).
32. [32] A.M. Abdelgawad, S.M. Hudson, O.J. Rojas, Antimicrobial wound dressing nanofiber mats from multicomponent (chitosan/silver-NPs/polyvinyl alcohol) systems, Carbohydr. Polym. 100 (2014) 166-178. https://doi.org/10.1016/j.carbpol.2012.12.043.
33. [33] C. Liu, K. Wang, J. Jiang, W. Liu, J. Wang, A novel bioflocculant produced by a salt-tolerant , alkaliphilic and biofilm-forming strain Bacillus agaradhaerens C9 and its application in harvesting Chlorella minutissima UTEX2341, Biochem. Eng. J. 93 (2015) 166-172. https://doi.org/10.1016/j.bej.2014.10.006.
34. [34] H. Wu, S. Nagarajan, J. Shu, T. Zhang, L. Zhou, Y. Duan, Green and facile surface modi fi cation of cellulose nanocrystal as the route to produce poly (lactic acid) nanocomposites with improved properties, Carbohydr. Polym. 197 (2018) 204-214. https://doi.org/10.1016/j.carbpol.2018.05.087.
35. [35] N. Das, N. Ojha, S.K. Mandal, Wastewater treatment using plant-derived bioflocculants: Green chemistry approach for safe environment, Water Sci. Technol. 83 (2021) 1797-1812. https://doi.org/10.2166/wst.2021.100.
36. [36] A. Mautner, Nanocellulose water treatment membranes and filters: a review, Polym. Int. 69 (2020) 741-751. https://doi.org/10.1002/pi.5993.
37. [37] R.K. Mishra, S.K. Ha, K. Verma, S.K. Tiwari, Recent progress in selected bio-nanomaterials and their engineering applications: An overview, J. Sci. Adv. Mater. Devices. 3 (2018) 263-288. https://doi.org/10.1016/j.jsamd.2018.05.003.
38. [38] D. O'Connell, William, C. Birkinshaw, T. O'Dwyer, Francis, Heavy metal adsorbents prepared from the modification of cellulose: A review, Bioresour. Technol. 99 (2008) 6709-6724. https://doi.org/10.1016/j.biortech.2008.01.036.
39. [39] R.A. Festucci-Buselli, W.C. Otoni, C.P. Joshi, Structure, organization, and functions of cellulose synthase complexes in higher plants, Brazilian J. Plant Physiol. 19 (2007) 1-13. https://doi.org/10.1590/s1677-04202007000100001.
40. [40] K. Muylaert, L. Bastiaens, D. Vandamme, L. Gouveia, Harvesting of microalgae: Overview of process options and their strengths and drawbacks, in: Microalgae-Based Biofuels Bioprod. From Feed. Cultiv. to End-Products, Elsevier Ltd., 2017: pp. 113-132. https://doi.org/10.1016/B978-0-08-101023-5.00005-4.
41. [41] T. Chatsungnoen, Y. Chisti, Flocculation and electroflocculation for algal biomass recovery, in: Biofuels from Algae, 2nd ed., Elsevier B.V., 2019: pp. 257-286. https://doi.org/10.1016/b978-0-444-64192-2.00011-1.
42. [42] M.O. Agunbiade, E. Van Heerden, C.H. Pohl, A.T. Ashafa, Flocculating performance of a bioflocculant produced by Arthrobacter humicola in sewage waste water treatment, BMC Biotechnol. 17 (2017) 1-9. https://doi.org/10.1186/s12896-017-0375-0.
43. [43] Q. Lin, H. Peng, S. Zhong, J. Xiang, Synthesis, characterization, and secondary sludge dewatering performance of a novel combined silicon-aluminum-iron-starch flocculant, J. Hazard. Mater. 285 (2015) 199-206. https://doi.org/10.1016/j.jhazmat.2014.12.005.
44. [44] W. Zhang, P. Xiao, Y. Liu, S. Xu, F. Xiao, D. Wang, C.W.K. Chow, Understanding the impact of chemical conditioning with inorganic polymer flocculants on soluble extracellular polymeric substances in relation to the sludge dewaterability, Sep. Purif. Technol. 132 (2014) 430-437. https://doi.org/10.1016/j.seppur.2014.05.034.
45. [45] Y.J. Dong, Y.L. Wang, J. Feng, Rheological and fractal characteristics of unconditioned and conditioned water treatment residuals, Water Res. 45 (2011) 3871-3882. https://doi.org/10.1016/j.watres.2011.04.042.
46. [46] E. Neyens, J. Baeyens, A review of thermal sludge pre-treatment processes to improve dewaterability, J. Hazard. Mater. 98 (2003) 51-67. https://doi.org/10.1016/S0304-3894(02)00320-5.
47. [47] X. Wang, M. Li, X. Song, Z. Chen, B. Wu, S. Zhang, Preparation and evaluation of titanium-based xerogel as a promising coagulant for water/wastewater treatment, Environ. Sci. Technol. 50 (2016) 9619-9626. https://doi.org/10.1021/acs.est.6b03321.
48. [48] W.Y. Lu, T. Zhang, D.Y. Zhang, C.H. Li, J.P. Wen, L.X. Du, A novel bioflocculant produced by Enterobacter aerogenes and its use in defecating the trona suspension, Biochem. Eng. J. 27 (2005) 1-7.

https://doi.org/10.1016/j.bej.2005.04.026.
49. [49] B.M. Wilén, D. Lumley, A. Mattsson, T. Mino, Relationship between floc composition and flocculation and settling properties studied at a full scale activated sludge plant, Water Res. 42 (2008) 4404-4418. https://doi.org/10.1016/j.watres.2008.07.033.

CHAPTER 3: EXPERIMENTAL METHODOLOGY

3.1 SELECTION AND CLASSIFICATION OF BIOMASS MATERIALS

Replenishable biomass was identified as a sustainable source for extracting the biopolymers required for this project. The criteria for selection was influenced by abundance and ease of replenishing used biomass, i.e. preferably be available at no cost (e.g. waste type of biomass), ease processibility (e.g. ability to prepare in fine powder) and ease of extraction of the desired biopolymers (i.e. considering mass production). Also, biomass waste produced in abundance from various farming processes requires special handling which is often costly to dispose. However, waste produced from these sources could be exploited as the feedstock towards production of bioflocculants. Thus, several biomass feedstocks were identified from agro and food waste sources (e.g. maize husks and stalks), wood (pulp) and commercially available starch (**Figure 3-1**).

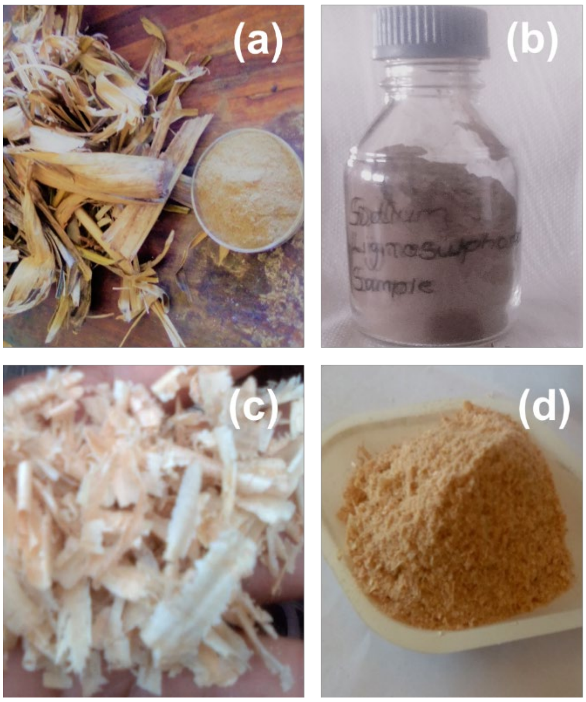


Figure 3-1: Pictorial representation of biomass materials use in this study (a) maize stalk (agro waste), (b) lignosulfonate, (c) wood shavings, and (d) wood sawdust.

Based on their compositions, the collected biomass and raw materials were classified as presented in **Table 3-1**. Maize plant stalks were obtained from a maize garden in Johannesburg (Gauteng Province) and a maize farm in Delmas in the Mpumalanga Province. Sodium lignosulfonate was obtained from SAPPI, a pulp company based in the Mpumalanga Province. Wood dust was supplied by a sawdust producer in Johannesburg South, and it was a residue of a merchant timber palm trees. Trisodium phosphate or sodium orthophosphate, bentonite, biochar, glycerol solution, oil, chloroacetic acid or monochloroacetic acid, boric acid, starch, calcium carbonate, monocalcium phosphate and other solvents were supplied by SabiNano laboratories in Randburg and were used as received. All experiments were carried out in SabiNano laboratories.

Table 3-1: Classification of biomass and raw materials used in this project.

Raw material	Biopolymer composition	Percentage of biopolymer composition
Wood dust	Cellulose, Lignin Hemicellulose	Cellulose 40.3% Lignin 28% Hemicellulose 28.7%
Lignosulfonate powder	Lignin	Lignin 70-75%
Maize plant stalks	Starch Cellulose Lignin Hemicellulose	Starch 70% Cellulose 45.7% Lignin 4.03% Hemicellulose 35.8%
Carboxymethyl cellulose	Cellulose	Cellulose 100%

3.2 BIONANOCOIL (BNC) SYNTHESIS: EXTRACTION AND MODIFICATION OF BIOPOLYMERS

The process used to synthesis the BNCs was a biophysical process that involved a simple laboratory reactor setup, mainly made from glassware. The bionanocoil (BNC) biopolymeric flocculant materials were synthesised following a modified procedure patented by Moghadam and Zangeneh [1]. Firstly, the collected biomass was dried and ground into fine particles using crushing and grinding equipment. To synthesize the biopolymer nanocoils, varying amounts of starch were dispersed in water to produce an aqueous mixture. The operating temperatures and pH were adjusted to determine the best working conditions for the synthesis of the BNCs. In these prepared mixtures, the varying amounts of glycerol were added to create appropriate conditions (prevention of cross-linking *via* induced repulsive forces) for the formation of nanocoils.

To give the starch nanocoils a highly negative charge and reduce the radius of gyration of the polymer chains, the biomass was added to the solution. The biomass contained, among other things, prepared from hemicellulose, phosphate, boron, and lignin. The carboxylic groups were bound to the surface of the starch polymer chains to change the shape of the starch macromolecules from substantially linear to coiled.

An oil was then added to the solution followed by continuous mixing until the coiled structures achieved a desired size distribution. Borax or boric acid and a calcium salt were then added to the mixture to stabilize the nanocoil size and form a stable suspension. The synthesised nanocoiled structures were separated from the solutions and dried. Microscopic and spectroscopic techniques were used to characterise the produced nanocoils, i.e. Transmission Electron Microscope (TEM), Fourier Transform Infrared (FTIR) and Thermogravimetric Analysis (TGA). The synthesized BNCs were evaluated for their potential use in a wide range of wastewater samples obtained from various industries including mining, fisheries, and others. **Figure 3-2** presents a graphical sketch of the procedure used to prepare the BNCs.

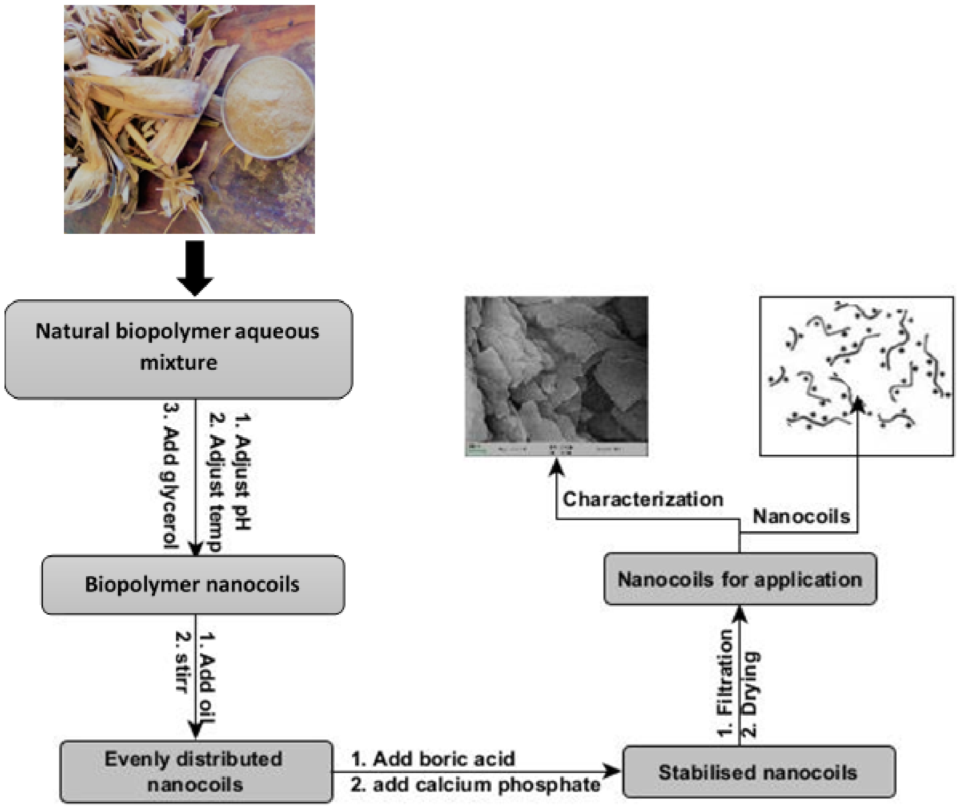


Figure 3-2: A graphical sketch for extraction and conditioning of biopolymer nanocoils used as flocculants.

A summarised step-by-step protocol of the steps involved in the synthesis of BNCs is presented below. For a start, the preparation involved 50 mL total volume of the solution (i.e. when adding contents, the final volume of the entire mixture was 50 mL). The process was increased by multiplying the feedstocks by a factor of 10 and repeated until at least 10 kg BNCs of the produced. Mixing was done using an overhead stirrer in a 5-litre glass cylinder. The protocol is as follows:

(i) Dissolve varying amounts (2-10 wt%) of biomass in water (~ 42 mL) to produce an aqueous solution.

(ii) To increase solution alkalinity, add 1-5 wt% trisodium phosphate. Adjust the solution pH to 8-10 and the reaction temperature to 25-50°C.

(iii) Add varying amounts of 5-10 wt% glycerol to create the right conditions for formation of the BNCs (i.e. repulsive forces to prevent unnecessary cross-linking).

(iv) To give the nanocoils a highly negative charge and reduce the radius of gyration of the polymer chains, add 1-5 wt% biomass to the solution. The biomass contained, among other things cellulose or lignin.

(v) Bind carboxylic acid groups from 2-10 wt% monochloroacetic acid to the surface of the starch polymer chains to change the shape of the starch macromolecules from substantially linear to coiled. It should be noted that the biomass also contains different concentrations of acetic acid.

(vi) Add 1% of oil to the solution followed by continuous mixing until the BNCs achieved a desired size distribution (Add 1 mL to overall 50 mL solution if the density of oil is 0.93 g/mL).

(vii) Add 2-3 wt% boric acid and 2-3 wt% calcium salt to stabilize the nanocoil size and form a stable suspension.

Several BNC formulations were synthesised. For this work, maize husks and stalks were selected because they are a common waste product of maize harvesting and fine powders could readily be produced using a standard electric grinder. The maize plant husks and stalks contain natural fibres composed primarily of carbohydrate polymers (cellulose and hemicellulose as main components), aromatic polymers (lignin), and ash (**Figure 3-3**) [2]. The quality, stability and charge of the BNCs could be adjusted by adding small percentages of starch, lignin, and chitosan, natural calcium.

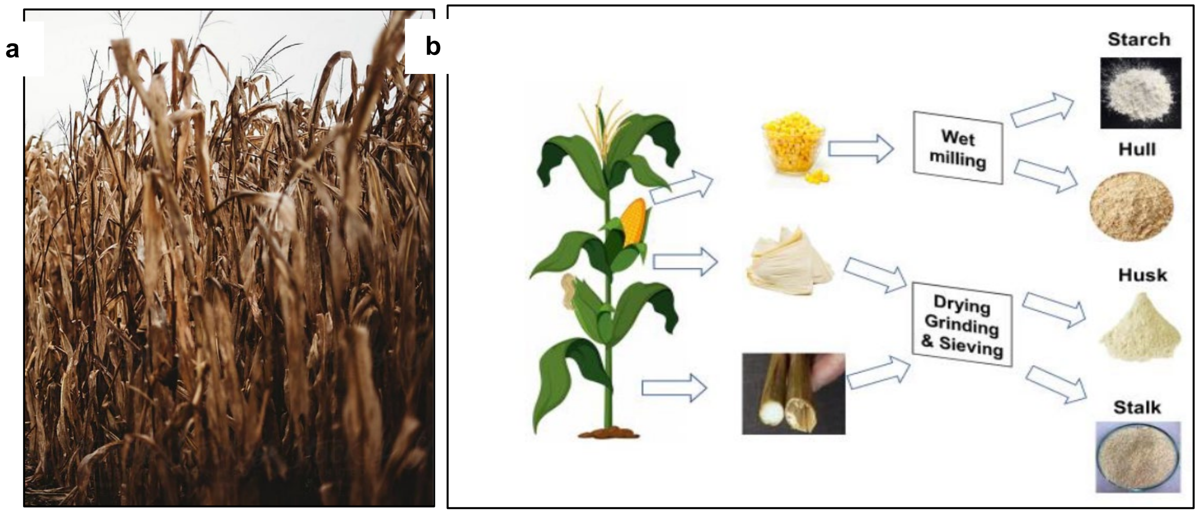


Figure 3-3: Extraction and preparation of maize biomass: (a) dry maize husks and stalks, (b) preparation of fine biomass powder by wet milling or grinding and sieving. In this project, fine powders were obtained by drying and sieving [2].

3.3 WASTEWATER SAMPLES AND COMPOSITION

Wastewater samples used in this project were supplied by various companies including the mining industry, sewerage treatment plants and other wastewater producing industries (private companies) (**Table 3-2**).

Table 3-2: A list of all the wastewater samples obtained and treated using BNCs in this project.

Sample	Sample type
Iron ore tailings wastewater	Mine wastewater
Mine effluent (hydraulic contaminated wastewater)	Mine wastewater
Mixed mine effluent (hydraulic contaminated wastewater)	Mine wastewater
Fisheries effluent wastewater	Fisheries wastewater
Milk-of-magnesia wastewater	Chemicals wastewater
Coal mine wastewater	Mine wastewater
Sewerage treatment works wastewater	Municipal wastewater

No pH adjustment was made on the samples provided. Test work was done within 12 h of receipt of samples and were kept in a cool place in the lab to avoid changes in the integrity of the samples. Physical parameters such as pH, turbidity, conductivity, total dissolved solids, and dissolved oxygen were determined from point of sample collection.

The quality of wastewater samples differed for each source and the names of each source are not given for ethical research reasons. While the aim was to remove suspended solids from wastewater by aggregating contaminants into flakes or “flocs” that settle at the bottom, in mine wastewater samples for example, it was desirable that the customized BNCs needed to achieve the following results:

- Reduce the concentrations of dissolved and total metals to below acceptable standards (e.g. Fe concentration).
- Achieve desirable pH levels (between 6-9) after application.
- Reduce turbidity, total suspended solids, total silica, free cyanide, ammonia as N, total dissolved solids, and conductivity to below acceptable standards.

3.4 JAR TESTS TO DETERMINE FLOCCULATION EFFICACY

A jar test procedure was used to determine the flocculant dose in the wastewater samples (see an example on **Figure 3-4**). During the test, the measured volumes of the wastewater sample were transferred to the different jars. Both rapid and slow mixing speed were set to run for 25 min each. Medicine droppers were used to sample the water at the depth of 3 cm to determine the initial and final turbidity of the water samples. The initial and final turbidity measurements were used to evaluate the residual turbidity.

Figure 3-4: Jar test apparatus used for BNC flocculation test work.

3.5 BIONANOFLOCCULANT CHARACTERIZATION

The synthesised bioflocculant materials are characterised using a range of techniques including Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy, (Perkin Elmer FTIR spectrometer, Frontier Optica, USA) to ascertain the surface functional groups on the bionanomaterials. Firstly, a background run is undertaken prior to the analysis of the materials. During the ATR running of the background, the infrared light scans the sample holder and produces the Bernstein wave. The Bernstein wave scans the material outside the sample holder to a distance known as depth of penetration (dp) [3-7]. After running a systems background, the sample is analysed within the specified wavelength range to obtain the FTIR spectra.

The surface morphology of the modified BNCs was studied using a scanning electron microscope (SEM, JEOL 7800F). The samples were coated with carbon under vacuum using a carbon coater (Quadrum Q150TE). The SEM images were acquired at an operating voltage of 20 kV. The JSM-7800F highest performance makes it possible to observe the finest structural morphology of nanomaterials at 1,000,000X magnification with 1 nm resolution [8]. Also, it collects large area mapping at low magnification without distortion. Importantly, SEM performs low KV imaging analysis of highly magnetic samples and also image thin electron transparent samples with 0.8 nm resolution using an optical Scanning Transmission Electron Microscopy (STEM) detector [9].

Microscopic images of the BNCs were obtained using FEI Tecnai T12 field emission electron microscope (TEM) equipped with CCD cameras. The TEM analysis was performed at an acceleration voltage of 200 kV. The samples were prepared by depositing a small amount of synthesized nanocoils onto a TEM grid (200 mesh size Cu-grid) coated with a lacy carbon film. The TEM uses a high voltage electron beam to generate an image. Electrons emitted from the gun travel through the microscope's vacuum tube. These electrons are focused by electromagnetic lens into a very fine beam. This beam then passes through thin specimen followed by scattering or capturing by a fluorescent screen. For such reasons, the samples were spread on the Cu-grid as thin layer. The identification of specific areas of an image, or pixels with specified characteristics, were used to visually interpret the obtained image.

An auto Sorb physisorption technique is used to determine the pore volume, surface area, adsorption, and desorption capabilities of the prepared BNCs. These properties provides important physical information relating to flocculation functions [10,11]. Differences in surface area and porosity of the BNCs possessing similar physical dimension can greatly influence its performance. Therefore, accurate determination of these properties is imperative [12]. The samples are degassed while heating to remove weakly adsorbed molecules. The analysis was typically performed at the liquefaction temperature of the gas [13]. When a gas molecule randomly encounters the surface of the molecule, it is attracted to the surface by the intrinsic surface energy of the sample, and later comes down at least momentarily to the surface [14]. As the pressure is increased, the number of molecules strikes the surface and becomes adsorbed. The number of molecules adsorbed increases as a function of pressure [15]. As pressure increases, multiple layers of molecules are produced on the free surface and on pore walls. Ultimately the surface is completely covered, and pores are filled, thus defining the principle of physisorption (pore volume, surface area, adsorption and desorption capabilities) were determined by BET method [16].

For determination of ammonia concentration and total nitrogen in wastewater samples, a Merck Spectroquant® SQ Pharo 300 with test kits was used to analyse ammonium-nitrogen (NH_4-N). The quantification of NH_4-N using the Spectroquant® NH_4-N cell test kit method measures dissolved ammonia and ammonia ions hence the concentration is reported mg/L NH_4-N. A solution containing no NH_4-N is labelled as a blank and analysed for NH_4-N. The measured pH of the blank and NH_4-N containing samples should be between 7 and 8, which is within the acceptable pH of the method (pH 4-13). When the sample is added into the reaction cell and mixed with the provided indicators in the test kit, it is allowed to completely react. Samples that do not contain ammonium-nitrogen when reaction is complete turn yellow and samples that contain ammonium-nitrogen turn yellow-green to green. After 15 min reaction time has lapsed and no colour change observed, the cell is inserted in the Spectoquant® measuring chamber and concentration is measured.

3.6 REFERENCES

[1] A. Moghadam, A. Zangeneh, Biopolymers having coiled nanostructures and processes incorporating the bopolymers, US 2016/0237174 A1, 2016. https://patentscope.wipo.int/search/en/detail.jsf;jsessionid=A942E422BFF04FDC874B0922B51B0046.wapp1nA?docId=US241661885&_cid=P10-K6N3FO-47215-1.

[2] M.I.J. Ibrahim, S.M. Sapuan, E.S. Zainudin, M.Y.M Zuhri, Extraction, chemical composition and characterization of potential lignocellulosic biomasses and polymers from corn plant parts, Bioresources. 14 (2019) 6485-6500.

[3] A.A. Gabrienko, E. V Morozov, V. Subramani, O.N. Martyanov, S.G. Kazarian, Chemical Visualization of Asphaltenes Aggregation Processes Studied in Situ with ATR-FTIR Spectroscopic Imaging and NMR Imaging, J. Phys. Chem. C. 119 (2015) 2646-2660. https://doi.org/10.1021/jp511891f.

[4] A.R. Hind, S.K. Bhargava, A. McKinnon, At the solid / liquid interface : FTIR / ATR – the tool of choice, Adv. Colloids Interface Sci. 93 (2001) 91-114.

[5] R.C. Thomas, A. Hierlemann, A.W. Staton, M. Hill, A.J. Ricco, Reflectance Infrared Spectroscopy on Operating Surface Acoustic Wave Chemical Sensors during Exposure to Gas-Phase Analytes, Anal. Chem. 1425 (1991) 1-7.

[6] O. Anjos, M.G. Campos, P.C. Ruiz, P. Antunes, Application of FTIR-ATR spectroscopy to the quantification of sugar in honey, Food Chem. 169 (2015) 218-223. https://doi.org/10.1016/j.foodchem.2014.07.138.

[7] M. Bass, V. Freger, Facile evaluation of coating thickness on membranes using ATR-FTIR Facile evaluation of coating thickness on membranes using ATR-FTIR, J. Memb. Sci. 492 (2018) 348-354. https://doi.org/10.1016/j.memsci.2015.05.059.

[8] G.E. Lloyd, Atomic number and crystallographic contrast images with the SEM: a review of backscattered electron techniques, Mineral. Mag. 51 (1987) 3-19. https://doi.org/10.1180/minmag.1987.051.359.02.

[9] S. Zaefferer, A critical review of orientation microscopy in SEM and TEM, Cryst. Res. Technol. 46 (2011) 607-628. https://doi.org/10.1002/crat.201100125.

[10] Y. Liu, Y. Su, J. Cao, J. Guan, R. Zhang, M. He, Antifouling , high-flux oil / water separation carbon nanotube membranes by polymer-mediated surface charging and hydrophilization, J. Memb. Sci. 542 (2017) 254-263. https://doi.org/10.1016/j.memsci.2017.08.018.

[11] S. Manjula, R. Subramanian, Membrane technology in degumming, dewaxing, deacidifying, and decolorizing edible oils., Crit. Rev. Food Sci. Nutr. 46 (2006) 569-592. https://doi.org/10.1080/10408390500357746.

[12] N. Singh, S.M. Husson, B. Zdyrko, I. Luzinov, Surface modification of microporous PVDF membranes by ATRP, J. Memb. Sci. 262 (2005) 81-90. https://doi.org/10.1016/j.memsci.2005.03.053.

[13] E. Yuliwati, A.F. Ismail, T. Matsuura, M.A. Kassim, M.S. Abdullah, Effect of modified pvdf hollow fiber submerged ultrafiltration membrane for refinery wastewater treatment, Desalination. 283 (2011) 214-220. https://doi.org/10.1016/j.desal.2011.03.049.

[14] S. Zhou, G. Hao, X. Zhou, W. Jiang, T. Wang, N. Zhang, L. Yu, One-pot synthesis of robust superhydrophobic, functionalized graphene/polyurethane sponge for effective continuous oil-water separation, Chem. Eng. J. 302 (2016) 155-162. https://doi.org/10.1016/j.cej.2016.05.051.

[15] J. Wu, N. Wang, L. Wang, H. Dong, Y. Zhao, L. Jiang, Electrospun porous structure fibrous film with high oil adsorption capacity, ACS Appl. Mater. Interfaces. 4 (2012) 3207-3212. https://doi.org/10.1021/am300544d.

[16] B.J.F. Chizmar, J.C. Soper, American Economic Association Specification and Development of New Pre-College Tests : BET and TEL Author (s): John F . Chizmar and John C . Soper Source : The American Economic Review , Vol . 71 , No . 2 , Papers and Proceedings of the Ninety-Third Ann, Am. Econ. Assoc. 71 (2020) 194-199.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 INTRODUCTION

The results produced in this study were obtained in collaboration with Biopolynet Inc under a R&D agreement that exists between Biopolynet and SabiNano. Biopolynet Inc. is a Canadian clean-tech company that develops and provides novel bio-based polymeric network solutions to help industries such as agriculture, mining, and oil and gas execute on their climate adoption strategies and boost their circular economy goals.

Biopolynet is the creator of BioNanoCoil™ (BNC) technology. BNC is a U.S. and Canada patented platform technology (CA 2920860; US 62/116,007) where bioscience, nanotechnology, and coiled polymerization techniques are combined to deliver a novel network formation method. BNC is a coiled nanostructure agri-based biopolymer that is specifically designed and configured to incorporate solid particles, thereby enabling these solid particles to be held and trapped together against external stresses, solvents, movements, etc. In this way, BNC represents a significant technology step-change toward sustainable effluent dewatering and nutrient recovery goals in different market segments.

4.2 PRELIMINARY PROJECT ACTIVITIES AND RESULTS

During this phase (Phase 1) of the project, the aim was to customize Biopolynet's platform proprietary technology (the BNC) toward solid and nutrient removal from different type of wastewater provided by mining and sewerage wastewater treatment industries in South Africa. Three stages of preliminary experiment and testing were designed and followed to reach the abovementioned objective.

Stage 1: Laboratory work for customization of BNC formula based on local raw materials (presented in **Section 3.1**).
Stage 2: Laboratory work for optimization of customized – BNC for the removal of constituent of concern in wastewater collected in different industries.
Stage 3: Pilot testing, process optimization. This part will be done at a later stage and falls outside of the WRC project scope.

4.3 STAGE 1 – LABORATORY WORK FOR CUSTOMIZATION OF BNC FORMULA BASED ON LOCAL RAW MATERIALS

4.3.1 Experimental work

Biopolynet and Sabinano performed experimental design and laboratory scale experimental tests to produce and optimize the BioNanoCoil (BNC)-based solution for wastewater treatment. In this work, local materials including lignin, starch, and bentonite were used to produce BNC for local application. The modified BNC was used for solid-liquid separation from a wastewater sample, which was an animal waste slurry with high ammonia concentration. The primary goals of the preliminary laboratory experiments were to:

1) Modify the BNC formula based on locally available raw ingredients.
2) Optimize the modified BNC formula to increase solid removal and efficient solid-liquid separation of wastewater.
3) Optimize the BNC formula to maximize element of concern removal from wastewater.

4) Optimize the concentration of a binder (cross-linking agent) and the mixing time for modified BNC to increase the setting time of the reaction in the dewatering process.

Different BNC formulas were designed based on the protocol presented in Chapter 3. These experiments included the use of different BNC formulas and various synthesis conditions to achieve a unique formula and process to run a stable constituent of concerned removal and dewatering process while minimizing fresh water and using locally made BNC materials.

The first set of BNC samples was prepared based on ten different concentrations of ingredients and mixing time to identify optimum parameters. The experimental matrix in **Table 4-1** below represents the pattern of conducting experiments. Depending on the selection of ingredients, the proportions, and the mixing time, the solid-liquid separation rate and nutrient removal was measured.

Table 4-1: Experimental matrix to test different parameters to optimize the BNC formula to improve the nutrient removal and solid separation.

	BNC 1	BNC2	BNC3	BNC4	BNC5	BNC6	BNC7	BNC8	BNC9	BNC10
A (%)	1	1	0.75	0.75	1	0.5	0.2	0.2	0.1	0.1
B (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
C (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D (%)	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
E (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mixing time (min)	1	1	1	1	1	1	1	1	1	1

BNC^{1-10} = Bionanocoil

Based on previous experience, the percentage ammonia removal (measured as ammonium, NH_4^+) is a good indicator of BNC activity and its efficiency for removal of other constituent of concern. **Figure 4-1** provides the percentage reduction of ammonia in the liquid phase using different natural resources [1,2]. Bentonite gave the highest reduction between 40-50%. Perlite and starch give the lowest reduction between 10-20%. A small percentage of such materials combined with our BNCs (nanostructured form of biopolymers) and a binder provides a much higher percentage removal of ammonia and total nitrogen (mg/L) of up to 68% and 98% respectively on animal slurry wastewater (See case study in **Figure 4-4**). Also, it is worth mentioning that materials in **Figure 4-1** do not have the capacity to remove both the solids and nutrients in the wastewater as it can be done with our modified BNCs. The goal was to achieve an efficient cost-effective single step treatment solution for the wastewater treatment and sludge dewatering.

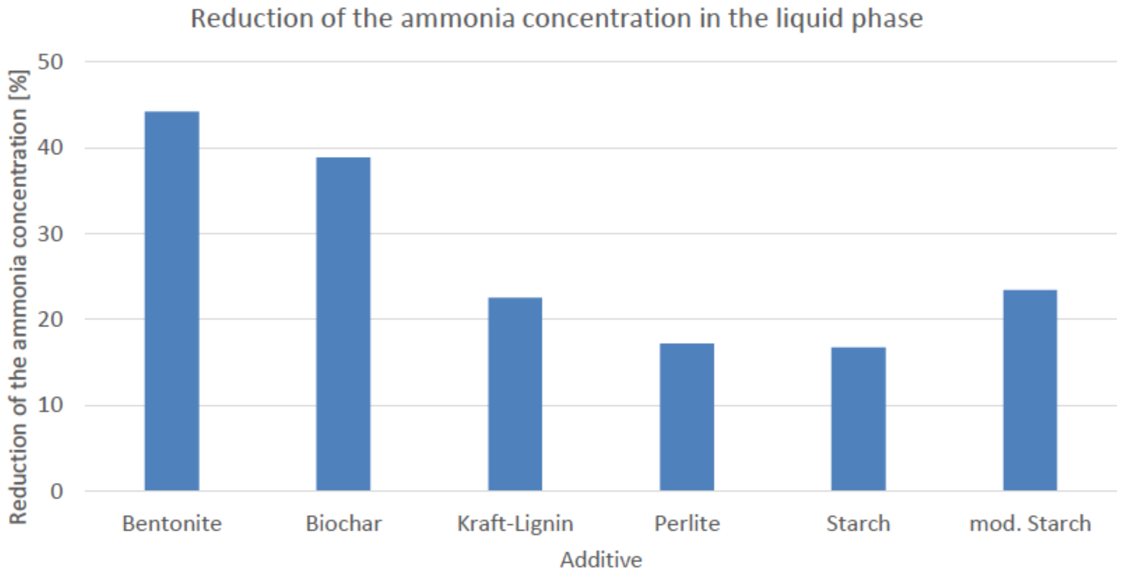


Figure 4-1: Reduction of ammonia concentration in the liquid phase by different materials.

Figure 4-2 presents the enrichment factor of ammonium (NH_4^+) in a solid sludge after addition of different doses of BNC 1 (5, 10, 15, 20 ml) in 1 litre of sewerage wastewater. A higher enrichment factor of approximately 2.2 could be achieved with addition of 10 ml of BNC. Similarly, a higher reduction of about 27% was observed with addition of 10 ml BNC. Further investigations are necessary to determine the optimum dosages and to ascertain why the enrichment factor data does to correspond with the ammonia reductions observed. The reduction of ammonia concentration (and other nutrients or dissolved substances) in the liquid phase can be improved by mixing a locally available binder with the modified BNC1. The preliminary experiments involved three different cheap readily available materials. The binders were labelled B1, B2 and B3 to avoid disclosure of potential IP. In **Figure 4-3**, the binders themselves showed some capacity to reduce the ammonia concentration in the liquid phase. However, it was observed that a combination of the binders, in this case B2 and B3 in equal amounts improves the performance of the binder in reducing ammonia concentration in solution. This combination is further used in the final BNC formulation for the best performance.

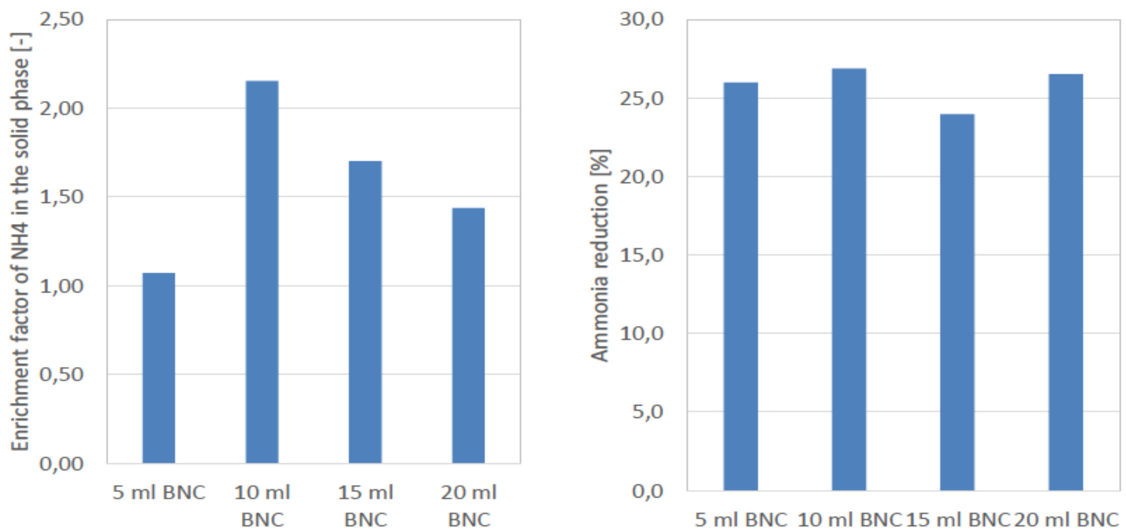


Figure 4-2: The effect of adding different dosages of BNC1 on a wastewater sample. Data on the left show enrichment factors for ammonia. Data on the right show ammonia reduction.

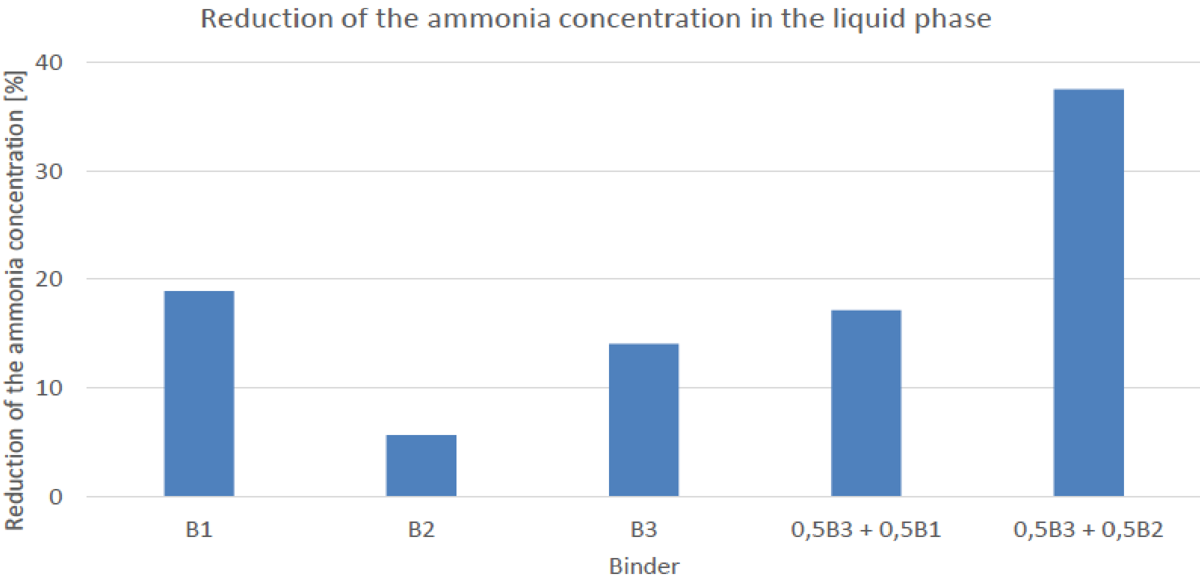


Figure 4-3: Reduction of ammonia in the liquid phase using locally available binder materials.

The best performing BNC made from local biomass feedstocks is a combination of the modified BNC, which is a combination of biopolymers composing of a binder material. As a benchmark, results from a case study by Biopolynet are presented in Figure 4-4. The results clearly show the potential of the BNC in removing ammonia, nitrogen, phosphorus, potassium, and total solids from animal slurry wastewater. In this project, the goal was improving such performance by producing formulations of the BNC using local feedstocks that had been identified. The preliminary results obtained are a good indication of the performance of the BNC polymers.

Parameter	Raw Digetsed Hog Slurry	Hog Slurry Liquid *	Hog Slurry Solid **	% Reduction
Ammonium (mg/L) [1]	1710	558	4448 mg/Kg	67
pH	6.3	6.68	7.38	N/A
Potassium (mg/L)	2490	1,090	5090 mg/Kg	56
Total Solids (%)	15.4	7.1	20.3	54
Total Nitrogen (mg/L) [2]	7360	953	29800 mg/Kg	87
Total Phorphorus (mg/L) [3]	2540	39	12500 mg/Kg	98

** This is the supernatant after concentrating the solids using the BNC.*

***This is the thickened solids.*

1. The pupose of this experiment had been to lower the concentration of Ammonium ion (NH_4^+) in the liquid to around 500 mg/L.
This has been 67% reduction in this compound which ended up in the separated solids.

2. Total Nitrogen was reduced in the supernantant by 87%.

3. Total Phosphorus (TP) content was reduced by 98.5% to 39 mg/L.

BOD reduction had not been a focus of this experiment although BOD must have been reduced by several folds after thickening of solids.

Figure 4-4: Reduction in hog manure ammonia and total phosphorus using BNC polymer – data supplied by Biopolynet for reference.

Figure 4-5 presents some images of BNC test work conducted in our laboratories (SabiNano). The images clearly show the efficiency of the solution towards wastewater treatment, especially slurry/sludge dewatering.

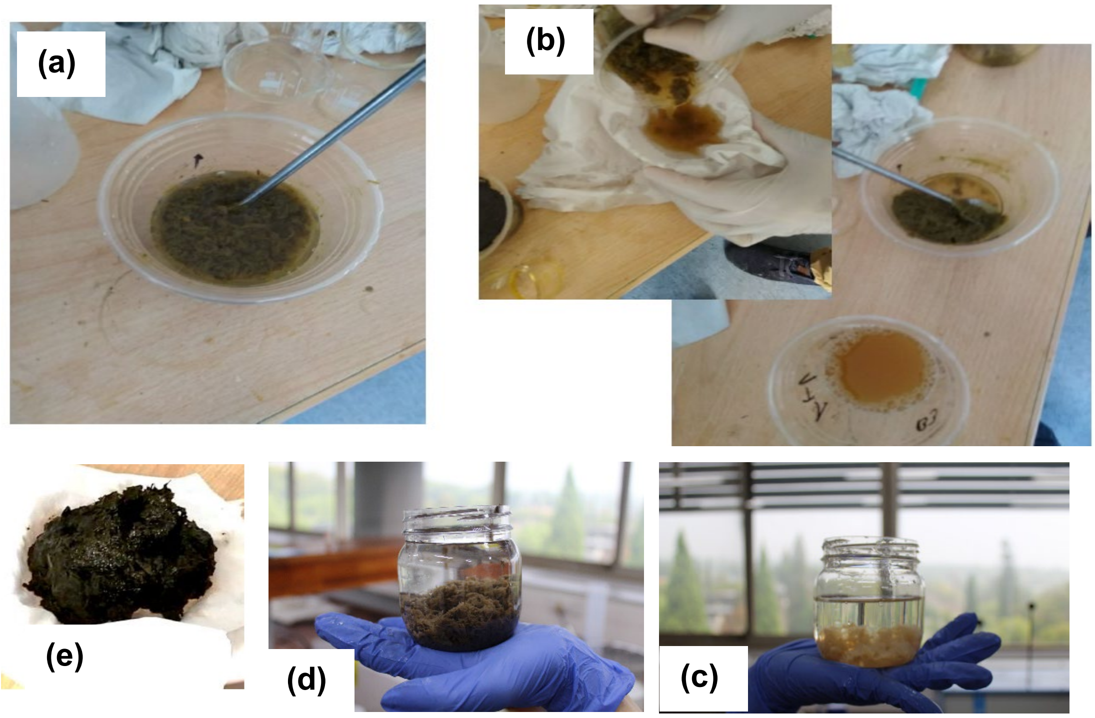


Figure 4-5: Pictures showing de-watering (solid removal) performance of the modified BNC: (a) sewage samples with small quantities of BNCs, (b) separation of sludge from residual water through filtration and decantation, (c, d) images showing how solids separate from solution after addition of BNCs, (d) sludge after de-watering with BNCs.

4.3.2 Characterisation of the BNCs

4.3.2.1 Physical appearance

Pictures of BNC1 and BNC2 master batches are shown in **Figure 4-6**. The biopolymers appeared like a paste that was brown (BNC1) and light brown (BNC2). These were labelled as master batches or concentrates. The difference was due to the addition of starch to BNC2.

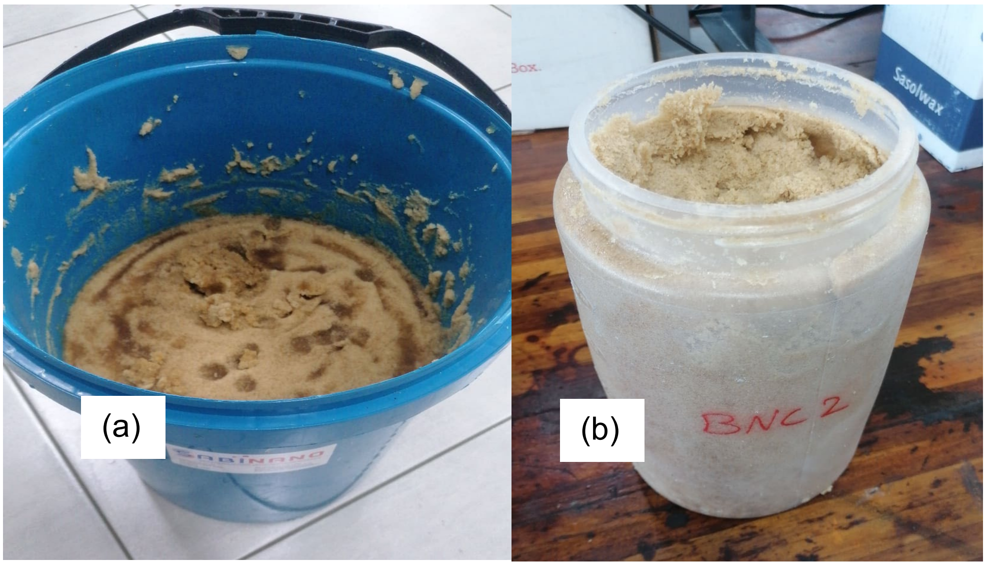


Figure 4-6: Pictures of (a) BNC1 and (b) BNC2.

From these biopolymers small quantities were dissolved in water and used in solution form, as they are typically used during flocculation in an industrial scale plant (**Figure 4-7**). Typically, in a flocculation tank, the BNCs (solid) are dissolved in dedicated tanks to form dilute solutions of the flocculants followed by pumping to the flocculation tanks. There are usually two or more dosing pipes used to feed the flocculant materials in a liquid form.

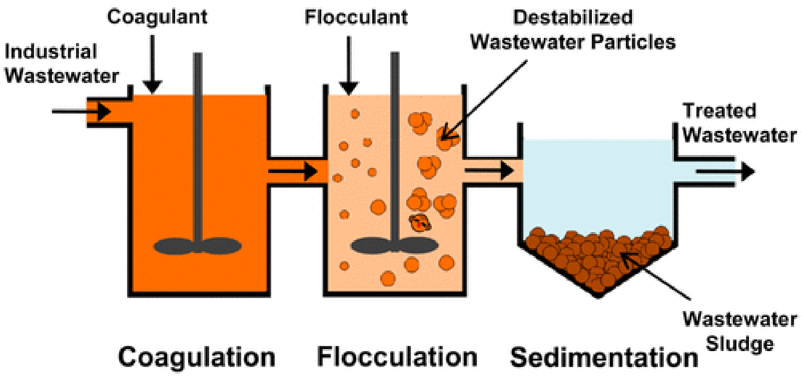


Figure 4-7: A typical coagulation-flocculation setup for industrial wastewater treatment.

4.3.2.2 TEM analysis

To evaluate the morphological structure of the BNCs, TEM micrographs were obtained using a FEI Tecnai T12 TEM (**Figure 4-8 and Figure 4-9**). As expected, the BNCs presented nano structures that were either rod-like (BNC1) or round-like (BNC2) shapes forming intertwined networks. The average size (diameter) of each nanocoil was approximately 5-10 nm. Although the BNCs presented to some extent a degree of gelation, the hemicellulose and lignin reduced it significantly. Also, **Figure 4-8** showed less cross-linking of the BNC1 coils compared to BNC2 coils.

TEM images of a binder (food grade) were also taken (**Figure 4-10**). It could be seen that the binder itself consists small nanostructures that were spherical in shape. The binder itself is soluble in water and readily interacts with the coiled structures of the BNC to form flocs. The BNCs are used together with the binder for good results.

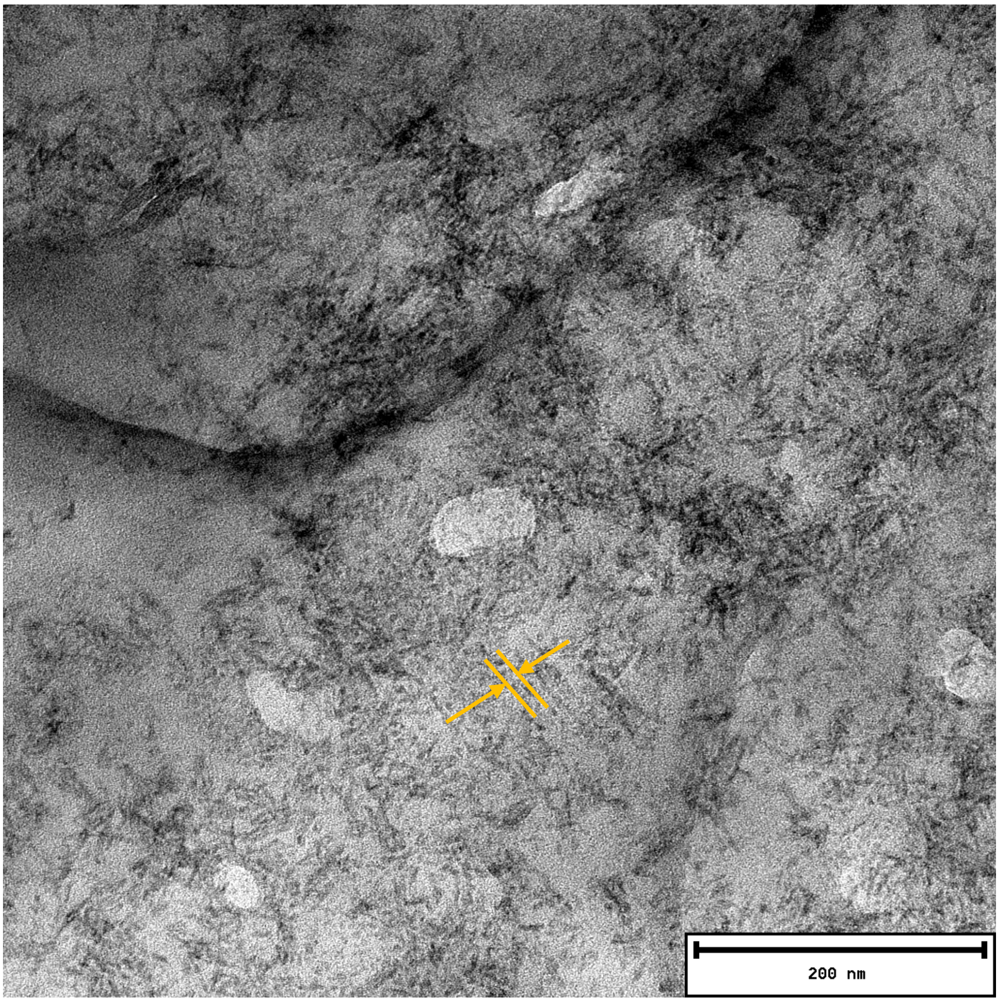


Figure 4-8: TEM micrograph of BNC1. Orange arrows show the diameter of a rod-shaped nanocoil.

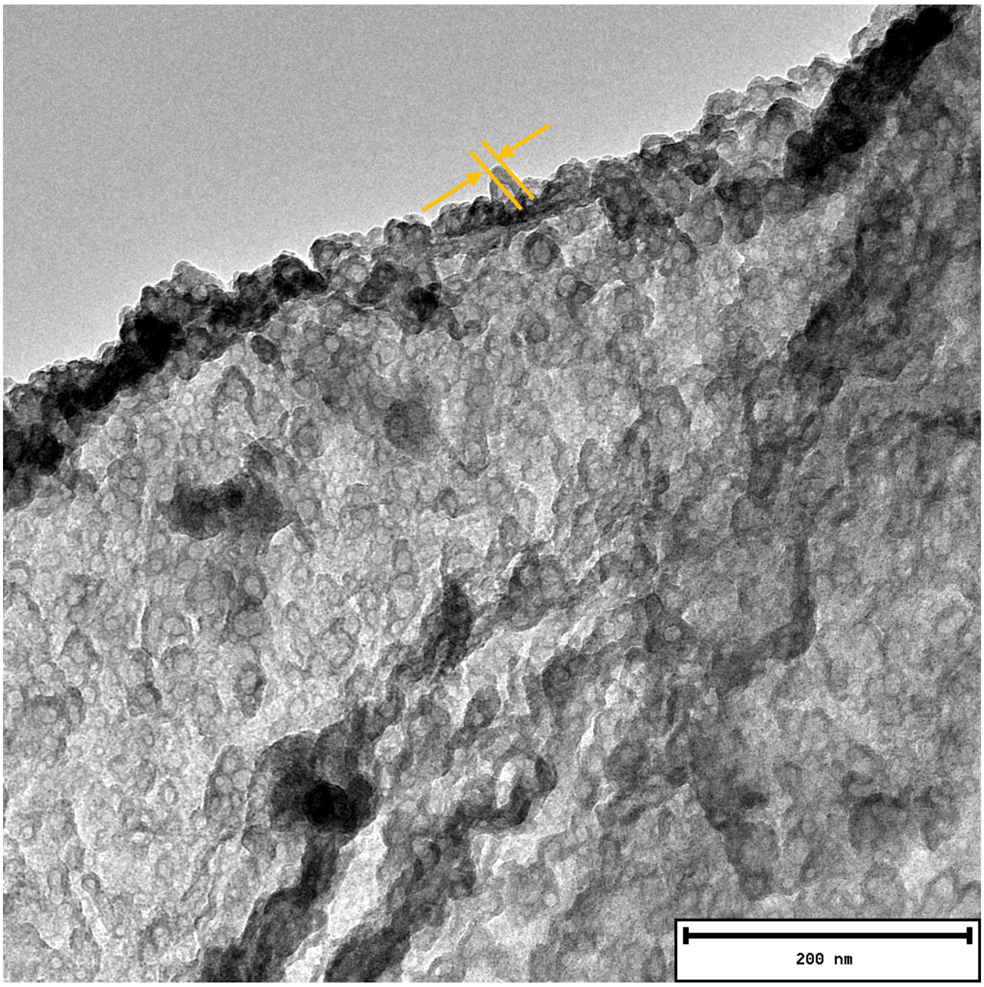


Figure 4-9: TEM micrograph of BNC2. Orange arrows show the diameter of a round-shaped nanocoil.

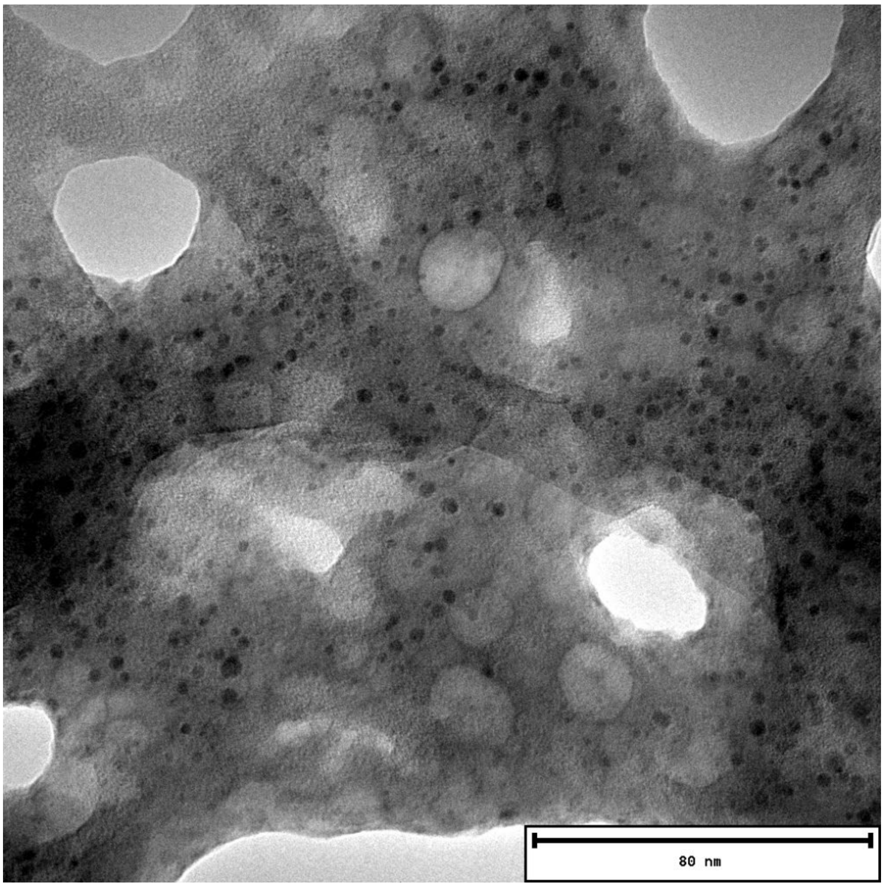


Figure 4-10: TEM micrograph of Binder 1. The fine nanoparticles of the binder (binder 1) are black dots in the image.

4.3.2.3 FTIR Spectroscopy Analysis

The structural and chemical make-up of the synthesized BNCs was confirmed by FTIR spectroscopy. BNC1 and BNC2 presented similar FTIR spectra indicating no change in chemical composition upon the addition of starch (**Figure 4-11)**. Characteristic absorption bands of BNCs were observed at 3293 cm^{-1}, 2935 cm^{-1}, 2880 cm^{-1}, 922 cm^{-1} correspond to O-H vibrations and stretching, C-H stretching, C-H vibration, and C-O stretching respectively. Cellulosic b-glycosidic linkage band was recorded at 851 cm^{-1}. Furthermore, C-O-C pyranose ring band was recorded at 1032 cm^{-1}. The sharp peak at 1609 cm^{-1} signals the oxidation of polysaccharides during nanocoil formation.

4.3.2.4 TGA analysis

Thermal decay profiles of the BNCs and binder 1 are presented in **Figure 4-12** indicating the mass losses at various stages. The thermal analysis was carried out at the temperature range of 30-900°C in air. An approximate 20% mass loss between 43-129 °C was associated with the moisture content from the BNCs. Due to their hygroscopic nature, the BNCs absorbed and retained high amount of moisture. The BNCs presented a slight thermal stability at 145 °C. Following this, hemicellulose decomposition began at 155-261 °C. Varying levels of mass losses for BNC1 and BNC2 were recorded at 348-414°C and 540-93°C corresponding to decomposition of cellulose with different degree of volatility. The final mass loss at 594-700°C was attributed to complete pyrolysis of lignin. A thermal stability of the binder presented 20% mass loss at 41-128°C associated with the bound water molecules (**Figure 4-12c**). Afterwards, the binder remained thermally stable until 900°C.

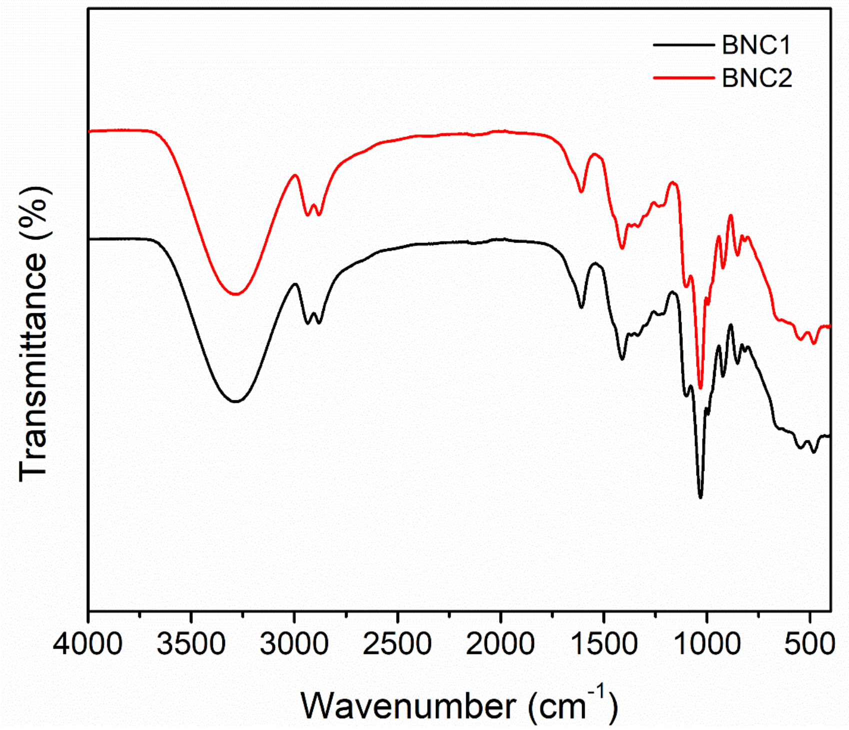


Figure 4-11: The FTIR spectra of BNC1 and BNC2.

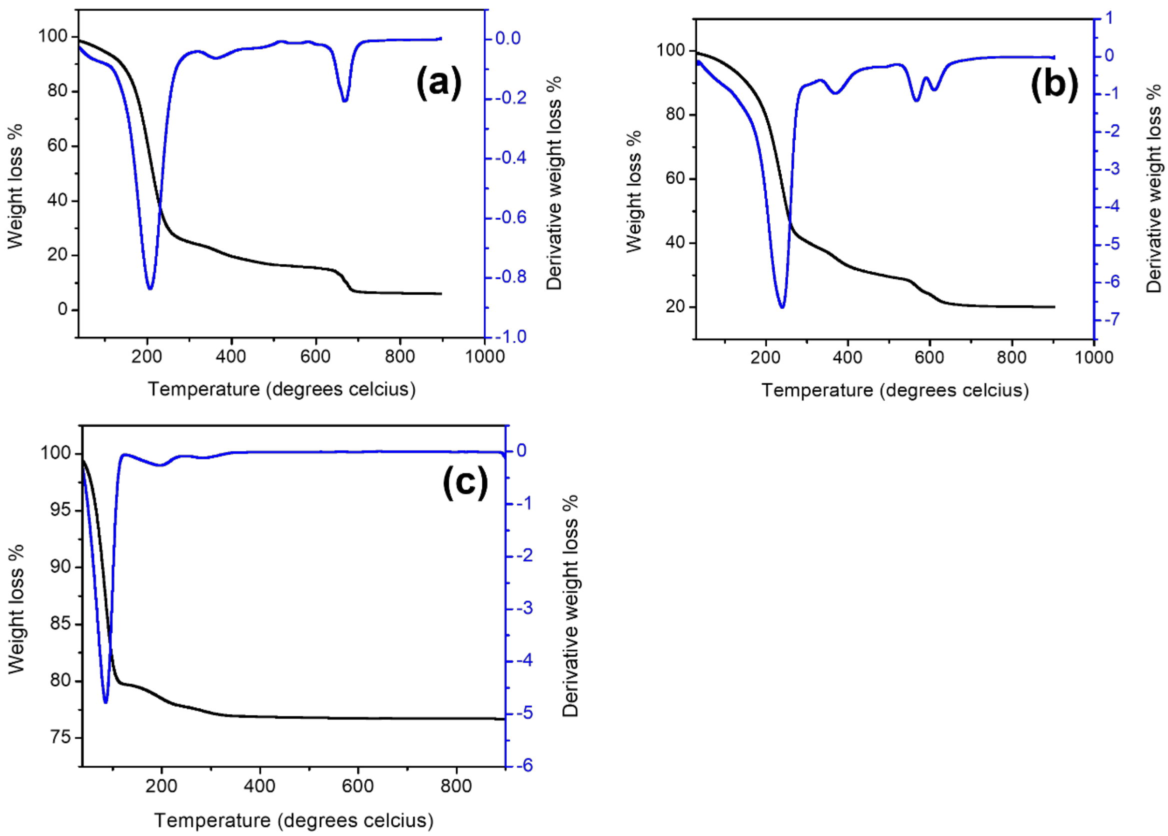


Figure 4-12: The TGA curves of (a) BNC1, (b) BNC2 and (c) binder.

4.4 LABORATORY-SCALE TEST WORK USING BNC1 AND BNC2

Numerous flocculation tests were conducted using wastewater samples obtained from different sources as presented in Chapter 3:

(a) Iron ore mine tailings
(b) Mine effluent (hydraulic oil contaminated water)
(c) Mixed mine effluent (hydraulic oil contaminated water)
(d) Fisheries effluent wastewater
(e) Milk of magnesia wastewater
(f) Coal wastewater

4.4.1 Preparation of BNC and binder solutions

Solutions of the synthesized BNCs and binder samples were prepared by dissolving calculated amounts of the BNCs in tap water as presented in **Table 4-2**. The BNC solutions were mixed for approximately 5 minutes to ensure that the BNCs are well dissolved in the solution. **Figure 4-12** presents images of the BNC and binder solutions prepared in the lab. The BNC samples are distinguishable by their brown colour, whereas the binder samples were clear due to their compositions.

Table 4-2: Preparation of BNC and binder solutions in water.

BNC name	Mass weighed (g)	Vol. of tap water used (ml)	Concentration
BNC1	50	1000	5%
BNC2	50	1000	5%
Binder 1	100	1000	10%
Binder 2	100	1000	10%

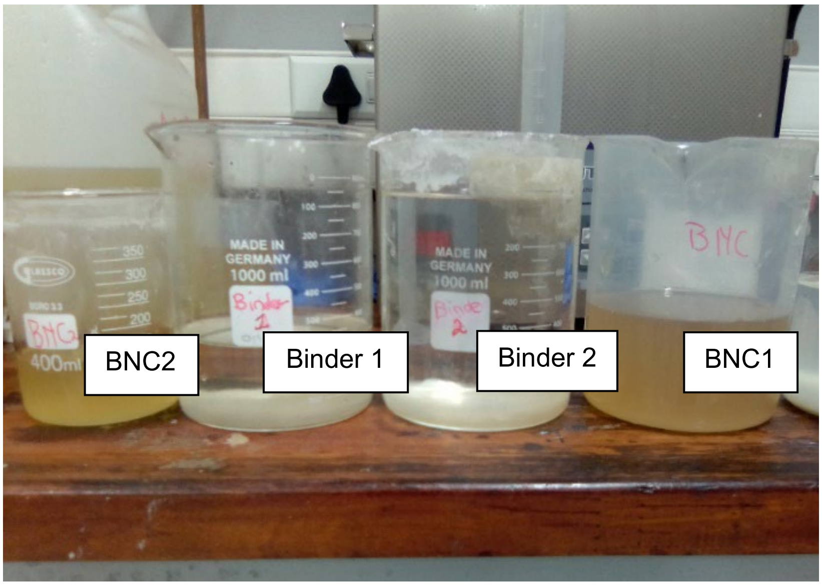


Figure 4-13: BNC and binder solutions prepared in the laboratory.

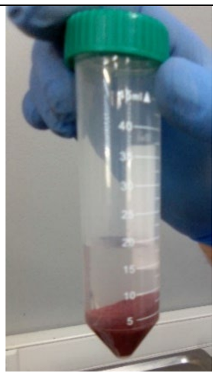
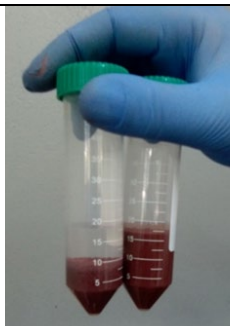
4.4.2 Flocculation tests of real wastewater samples

Laboratory-scale flocculation test experiments were conducted in a series of multiple-tests starting with small samples using 50 ml capped graduated centrifuge tubes as well as 100 ml, and 500 ml measuring cylinders with a stopper. Comparative flocculation tests were further conducted using a flocculator for selected wastewater samples (see section 3.4). The main parameter monitored or observed in this study was the turbidity (physical parameter) since the aim was to establish the efficiency of the BNCs in flocculation and sedimentation of suspended solids and turbidity. The efficiency of the BNCs on removal of heavy metals and other chemical pollutants has been demonstrated in several studies by Biopolynet Inc.

(a) Iron ore tailings experiment

An iron ore mine tailings sample (5 litres) was obtained from an iron ore mine in the Northern Cape Province. The sample was kept in a cool place prior to use. **Table 4-3** presents the compositions of BNC and binder materials used and the results that were obtained. The clarity of the treated solutions and the rate at which the flocs were formed and settled to the bottom on the measuring container were the main determining factors. In some tests, the concentration of the main ions and some physical parameters were determined.

Table 4-3: Treatment of iron ore wastewater using synthesized BNC materials.

Volume of iron ore wastewater sample treated	Volume of BNC and binder	Picture of treated sample and observation
20 ml	➢ 3x drops of BNC 1 ➢ 5x drops of Binder 1 (NB: 10 drops = 1 ml). The BNC solution was transferred using a 10 ml dropper.	 • Small flocs formed with slow settling rate.
20 ml	➢ 5x drops of BNC 1 ➢ 5x drops of prepared Binder 1	 • Increase in size of flocs formed with slight increase in settling rate.

Volume of iron ore wastewater sample treated	Volume of BNC and binder	Picture of treated sample and observation
100 ml	➢ 10x drops of Binder 2 ➢ 25x drops of prepared BNC 1 ➢ 40x drops of prepared Binder 1	 • Bigger size of flocs formed with increased settling rate.
500 ml	➢ 40x drops of prepared Binder 2 ➢ 7 ml of prepared BNC 1 ➢ 25 drops of prepared Binder 1	 • Bigger size of flocs formed with fast settling rate.

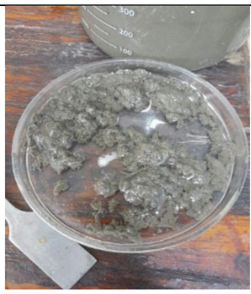
Figure 4-14 shows pictures of the treated iron ore wastewater using BNC1. The raw iron ore wastewater contained 320 mg/L of iron (Fe). After the flocculation, the treated solutions contained 0.5 ml/L iron. The BNC demonstrated high separation efficiency that is desired in the industry.

Figure 4-14: Treated iron ore wastewater samples after filtration. The concentration of Fe was 0.5 mg/L after treatment/flocculation.

(b) Mine effluent (hydraulic oil contaminated water)

Two 5 litre samples obtained from the mine that contained mainly water contaminated with hydraulic oil were supplied and was tested using the prepared BNC solutions. The results are presented in **Table 4-4**.

Table 4-4: Treatment of hydraulic oil contaminated mine wastewater using BNC materials.

Volume of mine effluent sample (A) treated	Volume of BNC and binder	Picture of treated sample
100 ml	➢ 1 ml binder 2 ➢ 1.5 ml BNC1 ➢ 3 ml binder 1	 • Small flocs were obtained with slow settling time.
100 ml	➢ 2 ml Binder 2 ➢ 5 ml BNC 1 ➢ 8 ml binder 1	 • Big flocs were obtained with fast setting time.
100 ml	➢ 1.5 ml BNC2 ➢ 1 ml binder 1	 • Medium size flocs with slow settling time.
100 ml	➢ 1.5 ml drops BNC2 ➢ 5x drops binder 1	 • Medium size flocs with fast settling time and excellent clarity.

(c) Mixed mine effluent (hydraulic oil contaminated water)

To increase the complexity of the hydraulic oil contaminated water, the two samples supplied were mixed in one container and flocculation tests were carried using BNC1 and BNC2. The best results were obtained using BNC2. Some of the volumes of BNC2 and binder 1 used are given below. **Figure 4-15** presents images of the untreated and treated wastewater.

(i) 100 ml of A+B wastewater
- 3 ml of prepared BNC 2
- 10 ml of prepared Binder 1

(ii) 100 ml of A+B wastewater
- 5 ml of prepared BNC 2
- 10 ml of prepared Binder 1

(iii) 500 ml of A+B wastewater
- 25 ml of prepared BNC 2
- 50 ml of prepared Binder1

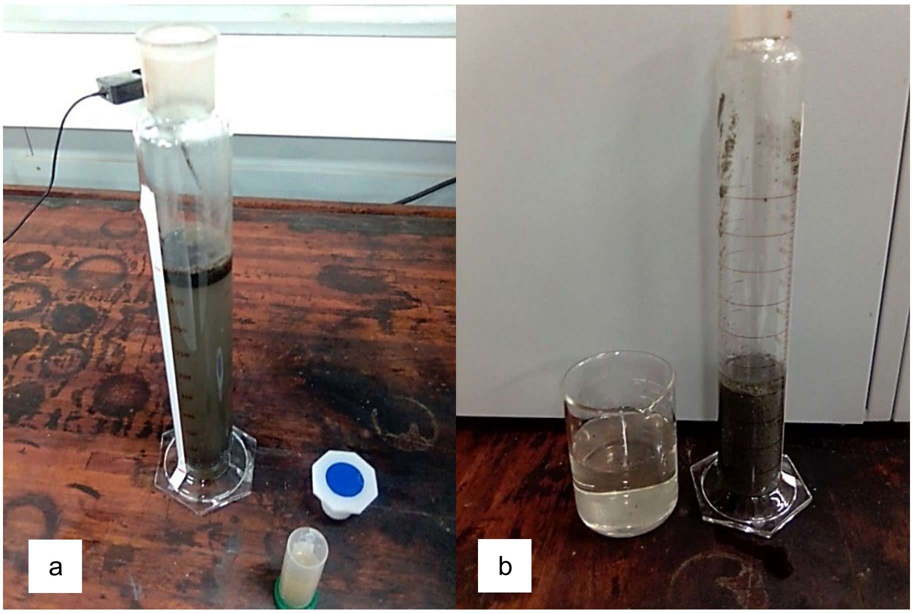


Figure 4-15: (a) A mixture of A+B untreated wastewater, and (b) clear water removed from the cylinder after flocculation and filtration.

(d) Fisheries effluent wastewater

A 5 litres sample of wastewater from a fisheries company in the Western Cape was obtained and flocculation tests were conducted. This sample contained less suspended solids compared to the mine wastewater. Several optimisation experiments were carried out using BNC1 and BNC2. BNC1 was found to be the polymer giving the best results. The composition of polymer and binder were:
- 4x drops binder 2
- 2 ml BNC1
- 10 ml binder 1

The flocs were small, but the settling time was satisfactory. The resulting solutions were clear, suggesting that the BNC1 was working efficiently (**Figure 4-16**).

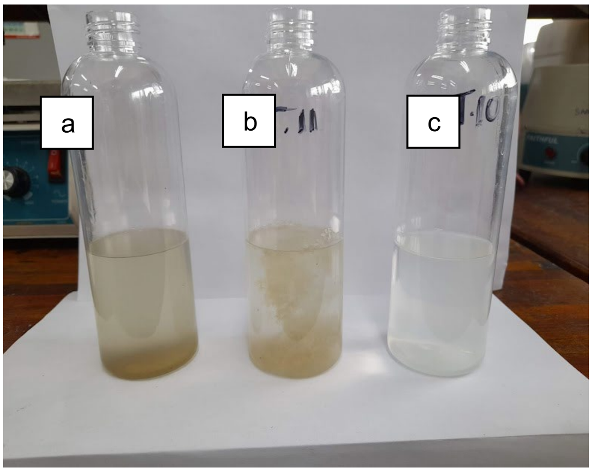


Figure 4-16: Pictures of fisheries wastewater treated with (a) BNC2, (b) BNC1 and (c) a clear filtered solution of treated fisheries using BNC1.

Flocculation experiments were further conducted by mixing binder 1 and binder 2 first before adding to the solution. This was done to keep dosing components to 2 as it is typically in the flocculation plants. The result can be seen in **Figure 4-17** as positive. Technically, the mix caused formation of intertwined/cross-linked nanocoil which is undesirable in flocculation processes. To achieve successful flocculation, the BNC should form stable flocs upon interacting with the pollutant of interest in the presence of a binder.

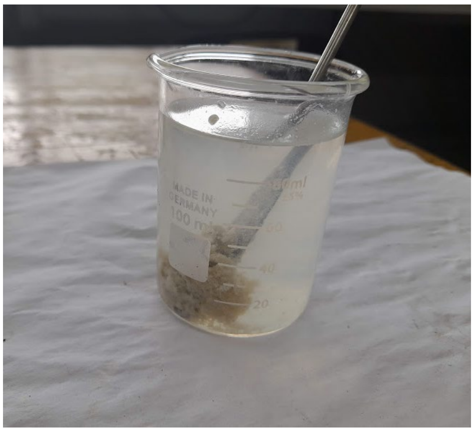


Figure 4-17: A picture showing the outcome of mixing binders before adding to wastewater.

(e) Milk of magnesia wastewater

A wastewater sample was also obtained from a company that produces milk of magnesia (white solution of dissolved solids). Several experiments were carried out using BNC1 and the results are presented in **Figures 4-18** and **4-19**. Separation was obtained using 5 ml of BNC 1 and 10 ml of Binder1. However, the size of the flocs was small and took time to settle. To increase the size of the flocs, the volumes of BNC1 and binder used were reduced (an inverse effect). For example, 1 ml of BNC1 and 2.5 ml of binder 1 gave good results.

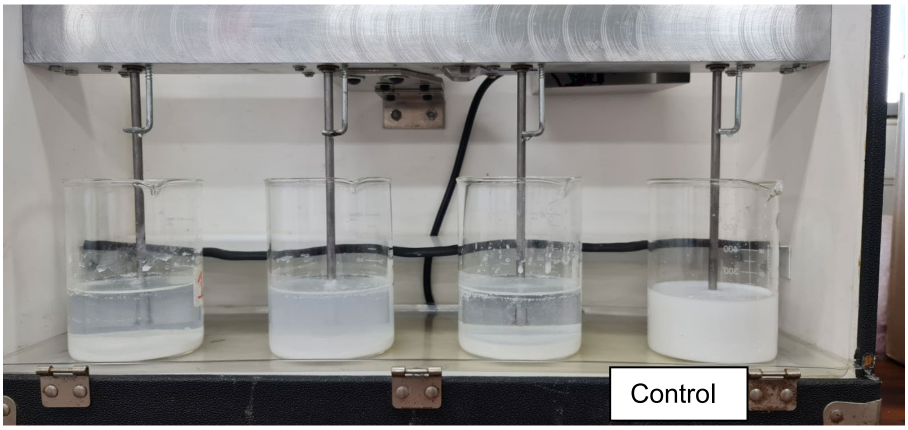


Figure 4-18: Images of milk of magnesia wastewater (250 ml) treated with different concentrations of BNC1. Smaller flocs were formed.

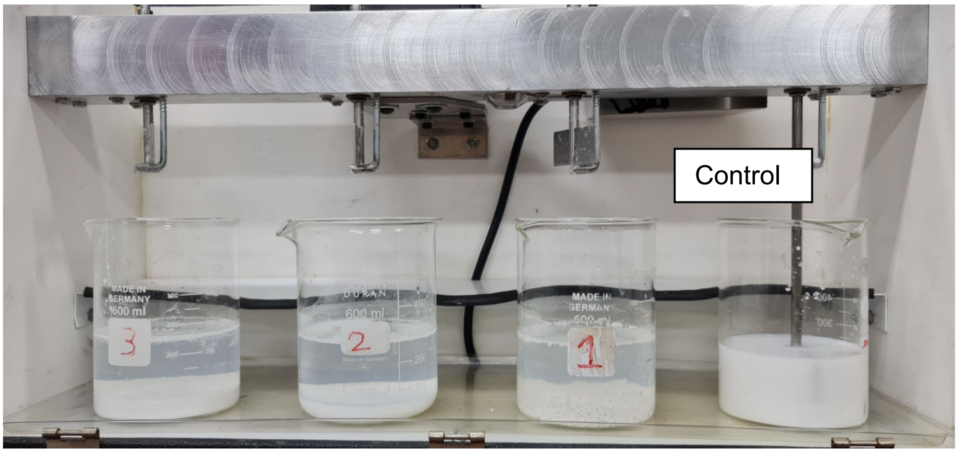


Figure 4-19: Images of milk of magnesia wastewater (250 ml) treated with different concentrations of BNC1. Bigger flocs were formed.

(f) Coal wastewater

A wastewater sample obtained from a coal mine was obtained and flocculation experiments were conducted. BNC1 was also found to be the biopolymer that gave the best results. Flocculation was achieved by 25 ml of binder 1 followed by a mixture of binder 1 and 2 (50 ml) (**Figure 4-20**). Here, comparative tests using a chemical-based flocculant currently used in the industry. The BNC1 gave a much clear solution and the settling time of the flocs was faster.

Figure 4-20: Comparison of the coal wastewater treated with BNC1 (beaker) and with conventional chemical flocculant.

4.5 SUMMARY OF FINDINGS

The synthesis of BNC biopolymeric materials was successful. These were characterised by TEM, FTIR and TGA. The prepared BNCs were tested in a series of experiments including wastewater samples from 6 sites (lab scale) and 1 site (test work at the mine labs). Excellent results were obtained with the BNCs as demonstrated by their ability to separate a wide range of suspended solids in wastewater samples with at least 90% reduction in turbidity. The synthesized BNCs are clearly applicable at a large scale as demonstrated by a field trial and jar tests that were conducted. The dosages of BNCs were varied and optimised for the various wastewaters at laboratory scale. Calculation of estimation based on the dosages used indicated that the cost can vary depending on the quality of the wastewater. For example, less BNC dosages were required to treat municipal sewage and milk of magnesia wastewater *vs.* hydraulic oil contaminated wastewater from the mine. Production at large scale is expected to result to lower cost of production due to economies of scale. Thus, the BNC technology is a viable approach to wastewater treatment in South Africa. Some investment in setting up a scaled-up production facility is required through strategic partnerships.

4.6 REFERENCES

1. Przemysław Seruga, Małgorzata Krzywonos, Justyna Pyżanowska, Agnieszka Urbanowska, Halina Pawlak-Kruczek, Łukasz Niedźwiecki, Removal of Ammonia from the Municipal Waste Treatment Effluents using Natural Minerals, Molecules 2019; 24(20): 3633.
2. Fei Gao, Yingwen Xue, Pinya Deng, Xiaoru Cheng & Kai Yang (2015) Removal of aqueous ammonium by biochars derived from agricultural residuals at different pyrolysis temperatures, Chemical Speciation & Bioavailability, 27:2, 92-97, DOI: 10.1080/09542299.2015.108716

CHAPTER 5: GENERAL CONCULSION AND RECOMMENDATIONS

5.1 GENERAL CONCLUSION

The laboratory-scale production and testing of a bionanomaterial technology (BNCs) for use in large-scale wastewater treatment was successfully completed in the project. The main goal was to convert readily available biomass waste materials, particularly agricultural waste such as maize stalk and husks, into nanostructured materials that were used as flocculants. The performance and efficiency of the materials is presented in Chapter 4. As a general conclusion to the project, several other important aspects were considered, and these are summarized below.

(i) Oil contaminant removal efficiency

The BNCs demonstrated a 98% percentage removal efficiency of oils that were present in mine wastewater samples that were contaminated with hydraulic oil (section 4.7.2). The treated wastewater was left to stand for 6 months EPA and the presence of oil was tested using Method 1664A-Extraction of Oil and Grease from Water Samples Using Solid-Phase Extraction (SPE) Disk Configuration. The results demonstrate the multifunctional performance of the BNCs in wastewater treatment, thus can be extended to oil/water separations at an industrial scale.

(ii) Environmental safety

While the BNCs are made from environmentally friendly materials (i.e. waste from mainly materials), analysis performed showed that there were no toxic components in the materials themselves. However, it was necessary to check if there was no leaching or disintegration of the BNCs in the treated wastewater. The analysis done using UV-Vis and fluorescence measurements demonstrated that the BNCs did not leach and could only be found in the flocs or sludge.

(iii) Cost analysis of the BNCs

The determination of the cost of production at lab-scale is completely different from industrial scale. The cost elements include direct (e.g. raw materials, equipment, energy, labour, etc.) and indirect costs (e.g. quality control, building space, overhead labour, marketing, etc.). In this project, the main cost elements considered were the raw materials as presented in chapter 3 such as the maize husks/stalks, solvents, and characterization. After all the materials inputs were considered, it was concluded that the cost of the BNCs could be in the significantly be lower than that of flocculants currently in the market. This could be achieved by considering the following key elements:

- The raw materials (agricultural feedstocks) are readily available for free or at negligible cost. There is also a wide variety of biomass materials to select from.
- Solvents are reagents used are cheap and can be industrial grade. For example. There is no need to use deionised water in the production process.
- Economies of scale can readily be achieved, i.e. the phenomenon where the average costs per unit of output decrease with the increase in the scale or magnitude of the output being produced by a firm. Automation can contribute to lowering the cost of production.
- Continuous research and applying the principles of green chemistry and engineering can help to replace costly chemicals with less expensive and environmentally friendly ones.

5.2 RECOMMENDATIONS FOR FUTURE WORK

Since the project was focussed on a laboratory-scale production, the following recommendations can be made for future work.

(i) Resource recovery application

The BNCs have demonstrated the potential for the recovery of precious metals like gold and can be tested for other metals such as the platinum group metals (PGMs). This application can assist the mining industry to recover small traces of lost metals to their benefit. Additionally, since the BNCs are biocompatible, they can be reused in manure for agricultural applications. Treated wastewater can be reused to lower the costs associated with water used in industry.

(ii) Detailed comparison of performance

A detailed tabled comparison (for wastewater) with existing adsorbents with respect to performance with respect to other parameters such as carbon oxygen demand in wastewater and reduction of pathogens and chemicals of concern (CEC) that are reduced is necessary. High-performance of the BNCs could potentially also reduce the requirement for disinfectant in wastewater treatment.

(iii) Environmental degradation

Studies need to be done to understand what needs to be done with the adsorbed contaminated BNC after use. Questions that need to be answered include knowing how degradable are the BNCs; what triggers degradation besides temperature? For example, the BNCs with contaminants can then be treated in a lined dam, triggered to degrade and then contaminants collected. This could be extended to the nuclear industry.

(iv) Local industrial-scale production

The next phase of the project is to develop an industrial-scale production plan of the BNC technology and to carry out a full-market research study. This will entail a process-based cost modelling and providing a complete business model canvas for the technology.