

NEXT GENERATION NANOCHITOSAN

Applications in Animal Husbandry, Aquaculture and Food Conservation

Edited by
Charles Oluwaseun Adetunji
Daniel Ingo Hefft
Jaison Jeevanandam
Michael K. Danquah

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Dr. Danquah's research findings are well published and cited with over 230 peer-reviewed journal articles, book chapters, conference publications, and technical reports, and a current H-index of 33. His research has also resulted in intellectual properties and patent applications, large-scale manufacturing plants, and commercialized products and formulations.

Foreword

Nanotechnology is a fairly new branch of discipline of science and ever since its creation has caused a lot of traction.

With technological advances, we are now able to produce nano-sized materials at a large scale. Nanomaterials are such materials with particle sizes ranging from 1 nm to 1000 nm.

Due to their size, nanomaterials often bring advantages when compared to macromaterials of millimeter scale. This is mainly due to the more attractive surface-to-volume ratio, making nanomaterials more potent. Nanotechnology has already penetrated many industries, be it textile industries, pharmaceutical industries, agricultural industries, and food industries (just to name a very few prominent sectors).

There are still many unknowns around nanomaterials. For example, titanium dioxide nanoparticle, a common food additive, recently has been classified by the European Food Safety Authority (EFSA) as not safe due to the small particle size causing adverse health effects (genotoxicity). However, looking at the current COVID-19 pandemic, nanotechnology has been a key contributor to the vaccine rollout with the Pfizer-BioNTech and Moderna vaccines, both using nanoparticles in their formulations. Also, textiles that incorporate nanoparticles have higher tensile strength and have better water repellency making these textiles more durable and functional.

Next Generation Nanochitosan: Applications in Animal Husbandry, Aquaculture and Food Conservation is a book designed for scientists and practitioners to gain valuable insights on the application of nanochitosan, an abundant biopolymer and waste product from the fishing industry. This book is divided into four themes. The first theme is titled *Green synthesis and characterization of nanochitosan from aquatic organisms and animal products* allowing the reader the different sources chitin can be obtained from. This will also include the processes required to turn chitosan into a nanomaterial (nanochitosan).

From there the reader will explore three application fields where nanochitosan is applied in. This will include blended content from lab research to large-scale applications. The three themes are:

(i) Application in animal husbandry,
(ii) Application in aquaculture, and
(iii) Application of nanochitosan in the protection and packaging of aquatic and animal-based food.

We hope this book encourages young researchers to utilize waste products in new ways and we believe that the exploitation of nanotechnology along with waste product utilization will become an important factor to produce in the future more sustainable, less environmental harmful, and smarter products and solutions.

CHAPTER 23

Application of nanochitosan in the control of biofouling in aquatic ponds

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Introduction

Surface-associated organismal-mediated biofouling have been attributed to the activities of several micro and macro sized organisms such as bacteria and mussels, respectively (Melo and Bott, 1997). Archana et al. (2019) also described biofouling as a process which entailed the colonization of natural or artificial surfaces or substances in direct contact with any form of aqueous media by aquatic micro- or macro-organisms. The authors stated that nutritional conditioning process usually occurs immediately a surface is submerged in water and this conditioning encompass the macromolecular adsorption of water borne moieties such as peptides and carbohydrates. It has been observed that several microorganisms such as bacteria, unicellular fungi, and algae are able to effect the colonization of the conditioned surface/substance thus creating a biofilm layer which can be described as gathering of attached organisms within a time period of hours (Archana et al., 2019).

The microbial biofilm which causes the microfouling or slime formation can invariably serve as viable base for the growth and proliferation of macrofouling macrobiota whose composition can range from aquatic plants such as seaweed to aquatic fauna such as; Polychaetes, bryozoans, mussels, ascidians, and barnacles (Archana et al., 2019). Durr and Watson (2010) opined that in the aquaculture industry, biofouling is known to affect various types of equipment, infrastructure, and even faunal stock respectively. The authors gave specific examples of aquaculture related equipment and infrastructure impacted by biofouling to include; Boats and barges, buoys and ropes respectively. Melo and Bott (1997) described a detailed summary of a cascade of events with regards to biofilm layer development and also listed several environmental factors which have a direct impact on the microbial biofilm development. Some of these factors included;

Next Generation Nanochitosan
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availability of nutrients, condition of surface, and particle and temperature effects (Melo and Bott, 1997). Archana et al. (2019) stated that a wide array of antifouling approaches are available from the commercial perspective with some procedures which include biological, electrical, and surface microtexture respectively.

Chitosan has been described as a hydrocolloid biopolymer, which at very low pH, is positively charged due to the presence of very reactive amino moieties (Kumar et al., 2019). Chemically, chitosan, which can be derived directly from the alkaline deacetylation of chitin, is a partially deacetylated polymer of N-acetyl glucosamine (Zhao et al., 2011). The biopolymer is known to comprise of a β- (1, 4)-linked-D-glucosamine residue harboring scattered acetylated amine molecules (Zhao et al., 2011). The chitosan-associated amine and –OH groups confer unique properties on the biopolymer making the moiety readily available for a range of chemical reactions (Zhao et al., 2011).

Chitosan can be utilized in a plethora of applications based on some of its inherent properties which include; nontoxicity, biodegradability and antimicrobial qualities (Divya and Jisha, 2018). The biopolymer can be utilized in a wide range of anthropogenic activities such as biomedical industrial sector, agro-allied and food industries, genetic engineering, mitigation of environmental contamination, water reuse, and treatment, respectively (Divya and Jisha, 2018). Chitosan production from chitin has been regarded as a sustainable approach that could be applied in the reduction of environmental challenges from the shell solid waste emanating from the sea food processing industry (Divya and Jisha, 2018).

It has been estimated that on a yearly basis, about 60,000–80,000 tons of shell waste are generated worldwide and the high volume of this waste stream makes biodegradation a relatively slow process and a source of environmental concern (Divya and Jisha, 2018). Chitin production from shell waste is an appropriate solution to this issue as chitin has many applications and can also be deacetylated to create chitosan which has a plethora of applications (Divya and Jisha, 2018). The antimicrobial properties of chitosan would make the biopolymer an ideal antifouling agent in the elimination of biofouling in aquatic ponds. Specifically, the antibacterial activities associated with chitosan have been attributed to several molecular associations existing between the membrane of the bacteria and the moieties of the amino acids that are positively charged (Wang et al., 2018). Some of the vital physicochemical characteristics of chitosan include; degree of deacetylation (DD), molecular weight (MW), and solubility respectively (Kumar et al., 2019).

The extent of deacetylation has been described as an essential chemical quality of chitosan which can principally determine the free amino compound profile ($-NH_2$) emanating from the incomplete substitution of acetyl functional groups which results in the formation of a copolymer of both D-glucosamine and *N*-acetyl-glucosamine respectively (Kumar et al., 2019). Copolymers created and possessing a higher than 50% content of D-glucosamine units are usually regarded as chitosan, whereas copolymers harboring

more than 50% of N-acetyl-glucosamine units are regarded as being chitin (Kumar et al., 2019). The relative percentage composition of D-glucosamine known as the degree of deacetylation (DD), whilst the percentage content of N-acetyl-glucosamine groups are indicated as the degree of acetylation (DA) with reference to chitin. This is with regards to chitosan (Kumar et al., 2019). A variety of procedures which include; UV–visible spectrophotometry, infrared radiation, gel permeation chromatography, and potentiometric titration can be utilized to evaluate the degree of deacetylation for a chitosan biopolymer (Divya and Jisha, 2018). Deacetylation can be conducted utilizing a variety of enzymatic preparations or chemical procedures, with the latter being the most utilized process based on its comparative affordability and the inherent capacity to produce large volumes of chitosan (Moutinho et al., 2019). Different types of acid and alkali can be utilized in the process of chemical deacetylation procedure (Moutinho et al., 2019). However, alkalis such as sodium hydroxide are more frequently utilized based on the trend that the glycosidic bonds have much higher susceptibility to acid attack (Moutinho et al., 2019). In the course of chemical deacetylation, parameters such as; amounts of NaOH applied, concentration, reaction time, and temperature would have a direct effect on some characteristics which include; DD and MW of the chitosan end product (Moutinho et al., 2019). In enzymatic-mediated deacetylation procedure, chitin deacetylases are utilized to conduct a highly regulated, nondegradable process that would ensure the formation of a pure, well-defined chitosan (Moutinho et al., 2019). These enzymes are known to be glycoproteins and are present in numerous species of fungi and insects (Moutinho et al., 2019). Younes and Rinaudo (2015) described several examined fungal derived chitin deacetylases from some fungal species; *Mucor rouxii, Absidia coerulea*, *Aspergillus nidulans,* and specific *Colletotrichum lindemuthianum* strains respectively. Chitin deacetylases are known to be very stable at a high temperature of 50°C and also display a substrate specificity for β- [1,4]-linked N-acetyl-D-glucosamine moiety (Moutinho et al., 2019). However prior to enzyme treatment, the chitin moiety has to be pretreated (Moutinho et al., 2019). The pretreatment process would enhance the accessibility of the acetyl compounds present in the chitin substrate to the enzyme (Moutinho et al., 2019).

The production of chitosan nanoparticles (ChNPs) has gained considerable interest over time primarily as a consequence of the successful application of ChNPs in a variety of biomedical fields ranging from utilization as antimicrobial agents, carriers for drug delivery, and tissue engineering respectively (Rizeq et al., 2019). The ability of ChNPs to inhibit bacteria from producing biofilms has also been documented (Moutinho et al., 2019). The inhibitory activity of antifouling paint containing chitosan-zinc oxide hybrid nanoparticle against bacterial and diatom fouling was documented to be higher in comparison with antifouling paint which contained just chitosan alone (Kumar et al., 2019). Several approaches such as polyelectrolyte complex method, ionotropic gelation procedure, reverse micellar procedure, microemulsion technique, and emulsification solvent diffusion procedure have been utilized in the synthesis and production of various

types of ChNPs (Divya and Jisha, 2018). Both ionotropic gelation and polyelectrolyte complex procedures have been documented; have been the most widely utilized techniques for the synthesis of ChNPs (Divya and Jisha, 2018).

Therefore, this chapter intends to provide comprehensive information on the application of nanochitosan in the control of biofouling in aquatic ponds.

Role of biological and physical measured in the regulation of biofouling on aquaculture

Several studies have been conducted with respect to the impacts of biofouling on marine aquaculture. Fitridge et al. (2012) examined the overall impacts on biofouling on aquaculture and its operations. The authors also evaluated the effectiveness of methods used for the control of biofouling. In addition to a vast range of organisms involved, they outlined some impacts of biofouling in shellfish aquaculture which include; physical damage by biofouling organisms, mechanical interference of shell which affects nutritional features and vulnerability to predators, struggle for the limited space and other resources, environmental modification leading to a reduction in the flow of water, a reduction in the volume of dissolved oxygen, and a fall in the amount of food available and rise in mass from biofouling biomass on apparatus, which leads to increased cost of production that might be linked to the requirement of extra maintenance. Effects of biofouling in fish aquaculture included restriction of water exchange as a result of the growth of fouling organisms, increased disease risk when biofouling organisms act as stores for disease-causing microorganisms and cage deformation (structural fatigue) as a result of the extra weight by fouling. They concluded that while there were a few cases where biofouling did not lead to a decrease in production (at times it even led to an increase, as the biofouling species could serve as a food source for the reared species), they, however, pointed out that these cases were mere exceptions and that biofouling was generally harmful to the growth and cultivation of fish and shell fish.

Nys and Guenther (2009) reviewed the effect and control of biofouling on finfish aquaculture. It was highlighted that biofouling of finfish nettings and cages was carried out by nearly every invertebrate phylum. It was noted that microalgae spores, diatoms, and protozoa brought about the colonization of the surface within an interval of days. Subsequently, other biofouling fauna, such as mussels, bryozoans, tunicates, and barnacles where able to settle in. This wide variety in species was noted as a reason for difficulties in controlling the process. Among the impacts of biofouling on finfish aquaculture were restriction of water exchange (transmission of water through nets reducing from 57.5% to 13.1% after 120 days), alteration of water quality, increase in disease risk (when biofouling species act as reservoir for pathogens), cage deformation, and structural damage. It was concluded that while there were numerous impacts of biofouling on finfish aquaculture, better control procedures should be implemented to ensure maintenance of the environment required for the growth of aquafarmed species.

Cornejo et al. (2019) further evaluated the varying impacts of biofouling in aquaculture netting within fish cage based on hydrodynamics. The authors had performed the modeling of the effects of the fish cage hydrodynamics with varying biofouling magnitudes. They placed several measuring points at different depths and distances away from the cages to measure the velocity fluctuation associated with the different levels of biofouling. They also investigated biofouling effects on water availability inside the cages. Their results revealed the highest percentage reduction (48%) with respect to mean flow velocity inside the cage at maximal level of net biofouling. The results also indicated a maximal reduction; 36% with respect to volumetric flow rate. Both the mean flow velocity and water availability showed an inverse relationship with the quantity of biofouling in the net cage. It was further reported that water retention in biofouled cage netting could be up to 10–100 times higher than for clean cage netting depending on the location. The authors concluded that an increase in water retention could lead to accumulation of particulate waste products and consequently, a reduction in oxygen availability which could adversely affect the growth of reared species.

Nakano and Strayer (2014) examined biofouling organisms in fresh water with a focus on their biology, economic impacts, and ecological roles. The authors observed that the accumulation of biofouling species biomasses on nets and solid surfaces could reach a wet mass of hundreds of grams to tens of kilograms per square meter. Organisms enumerated as prominent biofouling organisms include; bivalves, hydroids, bryozoans, sponges, hydropsychid caddisflies, and tubebuilding amphipods. They further reported that the death of large assemblages of these biofouling fauna could lead to an adverse effect on the taste, color, and odor of drinking water. It was also reported that these freshwater biofouling organisms could also cause damage to aquacultural nets and cages.

Large depositions of biofouling organisms in water bodies can have significant ecological effects as demonstrated by Lacoste and Gaertner-Mazouni (2014), who evaluated biofouling associations, their effect on aquaculture and their impact on the ecosystem. The authors estimated a biofouling cover of 40–60% in aquafarms, with a total biomass of about 10–20% of reared species after 1 year. They revealed that biofouling species was primarily composed of sponges, barnacles, ascidians, bryozoans including other related groups such as algae, bivalve, and hydroids. On the potential effect of biofouling on aquaculture, the authors indicated that algal fouling could lead to a hindrance of water flow which prevented the continuous closing and opening of valves among the bivalves, leading to a decrease in availability of food and oxygen to the bivalves. Other effects attributed to biofouling species include; an increase in oxygen demand and competition for food with reared bivalves. The authors concluded that the presence of biofouling organisms might not have as much of a negative effect on the cultivated species as had been envisaged however, their influence on ecosystem functioning had been underestimated.

More emphasis on the effect of biofouling on the ecosystem was described by Atalah et al. (2020) who evaluated the impacts of biofouling as a form of trophic footprints

on the ecosystem. They evaluated the broad scale geographical distributions of such organisms in the sea scape of aquaculture. Species of biofouling organisms which had previously shown high abundance were the main focus. Some of these biofouling organisms considered included *Ciona robusta, Styela clava, Didemnum vexillum, Mytilus galloprovincialis,* specie of red algal (*C. apiculatum* and *P. abscissoides*), filamentous green algae (*Cladophora ruchingeri* and *Pylaiella littoralis*), and *Codium fragile*, which is a green alga comprised of several subspecies. They evaluated relationships between these biofouling species and mussel farm structures and examined likely drivers of species distributions. The results showed a regional distribution of biofouling organisms that varied among different habitats and seasons. They reported that the proliferation of the organisms had a severe "trophic footprint" on the ecosystem and that their presence in substantial numbers led to a significant decrease in the availability of suspended organic matter and phytoplankton. They also conducted an assessment which revealed that biofouling waste deposition (of up to 600 kg) beneath the aquafarms led to organic enrichment of sediments, a consequent reduction in oxygen levels and in general, changes to the biodiversity of an area.

Despite many studies revealing the impact of biofouling on aquafarmed species, only few have represented their findings in the form of numerical data, detailing the actual effect of these biofouling organisms on the yield, and growth of fish and shell fish. Among these is a study by Sievers et al. (2013) which investigated the possibility that biofouling by *Ectopleura crocea, Ciona intestinalis,* and *Styela clava* had any effect on shell growth and flesh weight of cultivated mussel *Mytilus galloprovincialis.* Mussels were collected from different aquafarms and the sizes of their shells as well as their weight when dried were studied. Then mussel ropes were inoculated using biofouling species of low medium densities so to ascertain whether the organisms were able to affect the flesh weight or shell growth of mussels. In addition, they examined if the biofouling had any effect on food consumption of mussels which would be indicative of exploitative or interference competition. The results revealed that large mussels were 2.1–3.2% shorter and with flesh yield 9–14% less than the control. Smaller mussels were more affected by biofouling as these organisms were 4% shorter and with a flesh yield 13–21% lower than the control. The authors concluded that the obvious impact of these biofouling species on mussel growth rate and flesh yield led to a decrease in the quantity of mussels produced, an increase in the time until harvesting and eventually resulted in apperciable economic losses.

Fletcher et al. (2013) investigated the effect of biofouling by the colonial ascidian *Didemnum vexillum* on the culture green mussel of New Zealand. This was achieved by the experimental assessment of the effect of different levels of biofouling by *D. vexillum* on *Perna canaliculus* density. Small, medium, and large size groups of the mussels were attached to ropes and submerged in 5 replicates. Ambient (natural) and enhanced biofouling was allowed to occur over a 15-month period. The results revealed an

appreciable impact of biofouling treatment on the weight and density of mussels in the minute size group but not in the moderate or big classes. The average density of the small sized mussels varied from 83% to 121% which was higher in the untreated group relative to the ambient and enhanced biofouling treatment groups. Similarly, in the control group, the average weight of mussels in the small size class was between 42% and 77% which was higher compared to ambient and enhanced biofouling treatment groups. They hypothesized that "higher filtration rates and a superior competition for resources in the larger mussels were the reason for the less pronounced reductions in flesh weights" in the bigger mussels. The authors concluded that a more in-depth understanding of the effect of biofouling organisms across diverse phases of mussel production would be required.

The relationship between biofouling accumulation and losses of juvenile mussels (*Perna canaliculus*) during two different nursery stages was evaluated by South et al. (2019). The mussels were attached to ropes and submerged in water. At the end of the experimental period a total of 89 different taxa of biofouling species were identified. The most dominant of them included amphipods, ascidians, bryozoans, mussels, and algae. In the first experiment, the results revealed a decline of 22.5% in the number of mussels after the first 15 days. The decline continued at a rate of 0.3% per day until the end of the experiment. In the second experiment, a 38.4% decline was observed after the first 8 days and continued at a rate of 0.5% until the 77th day and at a slower rate of 0.2% per day till the 138th day of the experiment. For experiment one and two, a total of 64.4% and 78.3% of *Perna canaliculus* were observed over the duration of the experiment. The richness of biofouling species was observed to increase during this period.

The economic impact of biofouling has also been examined. Forrest and Atalah (2017) investigated the economic implications of *Mytilus* biofouling in the regional mussel industry with a focus on the effect of *Mytilus* on *Perna* crop yield. The authors studied also studied the regional prevalence and distribution of *Mytilus* and calculations on the economic implications of biofouling by *Mytilus* across two regions: Kaitaia and TOS. They reported a mean *Mytilus* cover on crops from Kaitaia spat of $10.74 \pm 0.77\%$ and a mean cover of $(7.77 \pm 0.64\%)$ from the TOS spat. The worst-case Mytilus cover was 32.80% for Kaitaia and 24.89% for TOS. For TOS and Kaitaia, in the absence of *Mytilus*, a Perna yield of 3.91 $kgm^{-1}yr^{-1}$ and 2.03 $kgm^{-1}yr^{-1}$ respectively was predicted. The results showed a significant decrease in annual *Perna* yield with an increase in *Mytilus* cover. This decrease was more evident in TOS than for Kaitaia spat. A decrease of 0.284 $kgm^{-1}yr^{-1}$ and 0.196 $kgm^{-1}yr^{-1}$ for TOS and Kaitaia spat were predicted by GLS model implemented. Losses as a result of biofouling by *Mytilus galloprovincialis* were reported to be up to USD $11.4 million annually.

Bouwman (2020) investigated the impacts of biofouling abundance on cleaner fish behavior among other things including the effect of spatial factors on biofouling and biofouling conditions at the time of net cleaning. To determine the effect of

biofouling on fish distribution in the net cage, the "maximum" biofouling abundance (the abundance on nets at the day of cleaning) was determined. Then, the number of cleaner fish in close proximity to the net before and after cleaning was measured. The author revealed that, to a significant level, more cleaner fish were observed be close to net walls before cleaning (biofouling cover >30%) than after net cleaning. The author attributed this difference to the disturbance of the habitat which made the fish reluctant to move close to the nets, the release of cleaning waste into the water and the removal of the biofouling (which fish usually forage on). It was concluded that the effect of net fouling on cleaner fish location in the cage may not be as profound as has been previously suspected by the fish farmers. These findings were in line with an earlier research by Lacoste et al. (2014), which examined the impact of biofouling on the aquafarmed pearl oyster, *Pinctada margaritifera*. The initial pearl oyster population was divided into two groups. In one group, according to the usual practice, the pearl oysters were washed every 3 months to prevent biofouling. The other group was kept without being washed to allow biofouling to take place so as to assess the efficiency of the removal of biofouling on growth and proliferation of pearl oysters over a 14-month period. The growth of the oysters was determined by measuring the size of their shells. Gonad development among species of the oysters was also measured. The results obtained showed no significant difference between increases in shell size of cleaned and covered groups of oysters at any given date. Furthermore, it was observed that biofouling did not have any influence on the reproductive cycle of pearl oysters. It was concluded that under the conditions considered, biofouling epibionts did not have any adverse effect on growth of the pearl oysters.

Many studies have been conducted to determine the potential impacts of biofouling on aquafarmed species. While some authors are convinced of major negative effects of biofouling, some concluded that the effects are insignificant, with some others convinced that the effects on the ecosystem are underestimated compared to the actual effect on the farmed species.

Applications of nanochitosan as antibiofouling agents

The antibioadhesive properties of nanochitosan have been examined in different studies, one of which was by El-Ghaffar et al. (2020). The authors evaluated the impact of nanoparticles based on chitosan associated with cellulose acetate membranes. The work was aimed at improving the resistance of cellulose acetate membranes to biofouling through the introduction of nanoparticles made of chitosan by a process described as the phase-inversion technique. The performance of the fabricated nanochitosan-incorporated membrane was tested as a measurement of the water flux permeated across it when supplied with 35,000 mg/L NaCl solution at room temperature under pressure. To assess the antifouling properties of the membrane, a static adhesion test was conducted. In addition to an increase in pure water permeability during desalination, the results revealed

that the nanochitosan-containing membrane had less number of adhered microorganisms compared to the blank membrane. The number of adhered microorganisms was shown to be in direct proportion with the concentration of nanochitosan incorporated into the membrane. It was concluded that nanochitosan-incorporated membranes had better resistance to bacterial adhesion than membranes without nanochitosan.

With respect to bioadhesion prevention, effective methods of preventing the colonization of submerged surfaces by biofouling organisms have been investigated. El-saieda and Ibrahim (2020) evaluated the antibiofouling properties of nanochitosan (prepared from shrimp shell) and nanoparticle of zinc oxide capped with chitosan. Nanochitosan and nanochitosan-capped ZnO nanoparticles were mixed with marine paint and applied on PVC panels and immersed in water over a 65-day period, after which observations were made on the organism diversity and populations present on the surface of the panels. After 16 days of immersion, 90% of the uncoated panel surface was surrounded in mucous slime film and within a span of 31 days there was appearance of tubeworms. The panels coated with nanochitosan were less affected (a 50% cover) after the same period of time while the panels coated with nanoparticles based on chitosan capping were least affected, having only a small amount of silt on its surface and a general antifouling of 90%. They concluded that coating immersed surfaces in nanochitosan and the capped zinc oxide nanoparticle inhibited the colonization of the surfaces by organisms (including marine bacteria, green algae, tubeworms, and barnacles) without any environmental risk.

Also, Al-Naamani et al. (2017) investigated the antifouling activities of chitosan and hybrid coating of ZnO-chitosan nanocomposite. In addition to developing and characterizing chitosan and ZnO-chitosan coatings, the study assessed the action of the coatings on the diatom *Navicula incerta* (known to be dominant in marine biofilms) and the marine bacterium *Pseudoalteromonas nigrifaciens*. The results showed that the density of the diatoms declined by 16% in comparison to the uncoated samples used as control due to the chitosan coating. On the other hand, the ZnO coatings brought about a total inhibition of diatoms. The chitosan-ZnO coatings resulted to greater inhibition of the growth of the bacteria when compared to the chitosan alone. It was reported that the smoothness of the surface of the chitosan was a major factor that led to the better anti-fouling performance. It was concluded that, ZnO nanoparticles incorporated into chitosan for coating of underwater surfaces was a promising method of preventing marine biofouling.

Nanochitosan has been discovered to improve the hydrophilicity of surfaces which in turn prevent adhesion by biofouling organisms as illustrated by Ghaemi et al. (2018). They carried out a study on the preparation and characterization of chitosan nanoparticles. Fabrication of pristine PES (polyethersulfone) and nanocomposite membrane (casting solution) was done by phase inversion method. The results showed an increase in hydrophilicity and water permeability, hence an increase in the antifouling properties

of the nanoparticles. It was concluded that the ideas developed in the research had commerical potentials based on its high nitrate elimination, permeability, and environmentally friendly biocompatible nanoadditive.

Bagheripour et al. (2018) synthesized an activated carbon/chitosan nanoparticle utilized in the preparation of a nanofiltration (NF) membrane with enhanced selectivity and antifouling performance. After fabrication, the membrane was characterized for its hydrophilicity, surface morphology, filtration, and antifouling performance (flux recovery ratio). The results revealed an 80.9% flux recovery ratio for the activated nanochitosan membrane compared to polyethersulfone membrane with a much reduced flux recovery value of 40.4%. This was indicative of an easier removal of biofoulants from the membrane. The reason attributed for this trend was a decrease in membrane hydrophobicity by the addition of the activated nanochitosan membrane. It was concluded that the antifouling activity of the casting solution was improved significantly by utilizing activated nanochitosan membranes.

A large percentage of biofouling organisms are microbes such as algae, protozoans, and bacteria. The ability of nanochitosan particles to inhibit the growth of these microorganisms is fundamental in their overall effectiveness as antibiofouling agents. Sangeetha et al. (2018) investigated the fabrication of novel electrospun nanochitosan through reactive electrospinning process. The antimicrobial activity of the novel electrospun nanochitosan was tested on two bacterial and fungal species; *S. aureus*, *E. coli*, *P. notatum* and *A. flavus* using disk diffusion and tetracycline as control antibiotic. The plates were incubated and the zones of inhibition were recorded. The results revealed the potent antimicrobial activity of the electrospun nanochitosan.

In a similar research, Wang et al. (2018) prepared pH responsive chitosan nanocapsules abbreviated as CAP@CS using microemulsion technique and assessed its antifouling effect on selected bacterial species. A strong bacteriostatic effect varying from 95.37% to 95.37% for *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* respectively was documented. This antibacterial activity of CAP@CS increased with decreasing pH as a result of the release of capsaicin coupled with a spike in the acidity of the environment as a result of bacterial reproduction.

In addition to its antimicrobial performance, Abiraman and Balasubramanian (2017) investigated the effect of smoothness or roughness of surfaces in aiding or inhibiting biofouling in aquatic ecosystems. They assessed the large scale synthesis of copper nanoparticle decorated with chitosan (size <2 nm) in aqueous medium. In evaluating its antifouling performance, panels coated with chitosan decorated copper nanoparticles (CDC NP) and other panels uncoated were dipped in a tank containing algal species *Arthrospira*, *Chlorella,* and *Amphora*. Samples were collected at regular intervals from the tanks for analysis. Furthermore, the antibacterial properties of the CDCNP were assessed using agar well diffusion method. Water contact angle images revealed

that surfaces coated with CDCNP were more hydrophilic while uncoated surfaces were hydrophobic. This was highly relevant because an increase in hydrophilicity led to a proportional increase in the antifouling of the surface. It was further reported that rough surfaces (such as the uncoated surfaces) were easily more impacted by biofouling organisms than smooth surfaces (such as the CDCNP-coated surfaces). The results from the panels dipped in the tanks revealed partial algal growth on the CDCNP surfaces coated with panel. This trend contrasted with observations recorded for the uncoated panels that were thoroughly impacted by the algae. The antibacterial testing results revealed significant CDCNP antimicrobial activity against both Gram positive and negative organisms in a concentration-dependent manner. It was concluded that CDCNP-coated surfaces was an environmentally friendly approach that could be utilized in controlling biofouling in both fresh and marine environments.

A review of applications of chitosan in composite coatings in food, paint, water treatment and as an antifouling agent was conducted by Kumar et al. (2019). It was reported that coating plastic surfaces with 2.5% chitosan remarkably decreased colonization by bryozoan *Bugula neritina* compared to untreated surfaces. It was also reported that a non-toxic paint base mixed with chitosan and applied to plastic surfaces protected the surfaces from biofouling for over 1 week of laboratory studies. Also, chitosan-based paints considerably reduced populations of microfouling on the surface of a sea glider exposed to periodical oscillations of environmental condition. The antifouling activity of chitosan nanocoating was discovered to be dependent on its thickness. The use of chitosan to create membranes with antifouling characteristics was also documented. Some of these membranes had strong antibacterial properties, inhibiting growth of both Gram-positive bacteria (more considerably) and Gram negative bacteria.

More researches focusing on the preventive action of nanochitosan on biofouling encompassed a study by Natarajan et al. (2018), who examined the antialgal and antifouling effect of TiO_2-, Ag-incorporated nanocomposites and pristine chitosan films on the marine algae; *Dunaliella salina* under UV-C irradiation and dark conditions. The results showed a reduction in the viability of *Dunaliella salina* with increase in concentration of pristine TiO_2 films with a viability of $54.75 \pm 0.09\%$ and $49.40 \pm 0.44\%$ ($P < 0.001$) compared to control for dark and UV-C exposure conditions respectively. Evaluation of the antifouling effect of chitosan, pristine (TiO_2 and Ag), and nanocomposite (TiO_2/Ag) coatings were done by assessing slime formation and mass measurement with significant inhibition. The basis of their action was outlined as decrease in cell viability, cell membrane damage and prevention of algal biomass formation.

Additionally, Pounraj et al. (2018) developed several nanocomposites using graphene oxide nanosheets (GOns), chitosan (Ch), and doped silver nanoparticles (AgNP) and their ability to prevent biofilm formation by *Escherichia coli* and *Bacillus subtilis* was

assessed. The different nanocomposites were prepared by a process known as the drop casting method and their antibiofouling and antiadhesion properties were evaluated. The authors revealed that *Escherichia coli* and *Bacillus subtilis* were unable to grow on the surface of the nanocomposite films unlike the control plate where growth was observed all over the plate surface. Little biofilm cover was observed on the nanocomposite surfaces compared to the control which had significant cover as revealed by Field Emission Scanning Electron Microscopy (FESEM).

Abiraman et al. (2016) highlighted the preparation of Chitosan Adorned Zinc Oxide Nano Rod (CAZO-NR) panels and evaluated their antifouling action on different biofouling algae; *Arthrospira, Chlorella,* and *Amphora* in fresh and marine environments. The organisms were cultured in tanks which contained CAZO-NR-painted surfaces. Unlike the control where a steady and rapid increase in cell count and chlorophyll concentration was documented, the tanks harboring CAZO-NR nanopaint-coated panels showed a decrease in those parameters by 75–90% as a result of destruction of algal cells. The authors indicated that the smoothness and homogeneity of the CAZO-NR-painted surfaces in comparison to the uncoated surfaces was one of the primary factors which caused algal growth inhibition.

Conclusion and future recommendation

This chapter has provided detailed information on the application of nanochitosan as antifouling agents. This review also established that biofouling is a natural phenomenon and it has a negative impact on the aesthetics and appearance of impacted fishing equipment, materials and ponds. CS-NPs and chitosan-containing nanocomposites generally present an environmentally-friendly method for the control of biofouling in natural and agricultural aquatic environments respectively. There is a need to increase the awareness about the benefical uses of nanochitosan as a antifouling agent. It is reommended that national Governments should implement policies promoting the utilization of nanochitosan as bioremediation and antifouling agents. There is also a need for more exploratory studies focusing on the use of diverse chitin sources for nanochitosan production purposes. These recommendations when implemented could invariably address issues pertaining to accumulation and disposal of aquacultural shell wastes in the environment.

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NEXT GENERATION NANOCHITOSAN

Applications in Animal Husbandry, Aquaculture and Food Conservation

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A practical reference of novel applications of nanochitosan to improve the production of a healthy and abundant food supply

Next Generation Nanochitosan: Applications in Animal Husbandry, Aquaculture and Food Conservation provides a comprehensive and state-of-the-art information on the application of nanochitosan for improving products, especially for the evaluation of biological active molecules, disease therapeutics, transport vehicles for DNA, targeted drug delivery, gene therapy, developing towards high performance fish yields, preservation of foods, tissue engineering, and improving the taste of aquatic and animal feeds as **fish growth promoter**.

The reference is for postgraduate and graduate students for industry professionals in the agri-food sector, dairy sector, and the aquaculture sector. It is especially useful for industrial fisheries that deal with wild capture fishing and aquafarming, and scientists and engineers working on post-capture processing stages. Additionally, environmentalists may find this as an essential reference because it reveals challenges around farming techniques in a world facing climate change, escalating population growth, and an ever-increasing scarcity of water. Details on the application of nanochitosan as an effective additive for the delivery of vaccines, hormones, vitamins, nutrients and antioxidants, biological active constituents and their wider application for the protection and management of farm animals and fishes against disease-causing pathogens are provided.

Key Features

- Provides applications for the protection and management of farmed animals and fish against disease-causing pathogens
- Includes relevant information, commercialized products, and innovative technologies on nanochitosan with industrial perspectives
- Highlights the application potentials of nanochitosan across the entire value chain of food production.

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