Microbial biopolymers as innovative, exploitable 'green tools' for sustainable treatment of water

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Abstract

Affording water quality and safety is an important national criterion and sustainable option advocated for water treatment and may be envisaged as a scientific function of innovations, parallel technology development and policy decision. Over the past decade our focus have been to develop *green polymers* as alternatives of currently used chemical water treatment agents and apply them as biosensors for rapid and effective detection of waterborne bacterial pathogens and parasites to facilitate timely intervention. To this end, we have demonstrated the potential prospects of several microbial biopolymers as flocculating biopolymers, and surface engineered selected biopolymers for effective binding and killing of prominent waterborne bacterial pathogens. High throughput detection using selected protozoal binding biopolymers have been designed for rapid detection of oocysts in water, a persistent threat to human life. The specific ability of some biopolymers to sequester phosphate have led us to develop these class of biopolymers as novel phosphate removal agents apt in aquatic bodies with high phosphate concentration; their abilities were exploited to design biosensors for rapid detection of phosphate in water. Given the enormous functional attributes, non toxicity and robustness of microbial biopolymers, the cumulative profile of their applicability is highlighted through our studies. A significant technological application of these green polymers in water safety and quality for application for benefitting national health is proposed.

Introduction

India offers a huge potential for optimal utilization of its water resources. With extensive river and canal system of about 195.210 km, comprising of 14 major rivers, 44 medium rivers and numerous small rivers and streams, the pond and tank resources are estimated at 2.36 million. In spite of this abundance, source sustainability is a serious challenge for India which is heavily dependent of groundwater. About 80% of the schemes are groundwater-based where recharge of the source has lacked persistently. Since groundwater-based water service delivery is increasingly challenging, there is a shift to surface water. However, the sustainability of surface water sources fluctuates significantly by climate variability, snow melting, insufficient flow in rivers and scanty rainfall. Therefore, water conservation, recharge and source security are critical for the Government of India to reach its ambitious target to cover 55% of households by piped water connections by 2017 and also to reach everyone by 2025 (Water aid, 2017)

Contamination of water sources, both bacteriological and chemical, has emerged as a critical problem over the years. Water treatment technologies need to be sustainable and institutions nearer to the users to develop quick and sustainable solutions. In fact, environmental un-sustainability, specifically arising from improper solid and liquid waste disposal, calls for linking sanitation into the whole water delivery chain. Whereas, new National Rural Drinking Water Programme (NRDWP 2010) guidelines and the Government of India XII Five Year Plan (2012–17) are anticipated to provide a paradigm shift in approach to the right direction, innovation led research for providing safety and affordability of water quality is clearly the need of the hour.

'Green polymers' have occupied the global center-stage as an enabling and transformative technology with a strong biotechnological and interdisciplinary involvement leading to platforms, which can be drivers of new value-chains, industries and employment opportunities. Moreover, the creative use of biopolymers can offer solutions for sustenance in agriculture, medical, health, water and environmental management. The growing relevance of the indispensable role of biopolymers in sustainable development, has spurred significant interest amongst scientists for translation of concerted ideas and inventions to technology for economic development and global competitiveness.

Our research efforts have concentrated on developing sustainable approaches using biotechnological means to achieve solutions to conventional chemicals used for treating water. Over the past decade, our laboratory have consistently investigated polymers of biogenic origin particularly those elaborated by microorganisms. Several biopolymers have been characterized with unique structural and functional attributes apt for human applications; being completely biodegradable and non toxic, they pose no threat or problems for applications. A major objective of our research has being translation of functional properties of these biopolymers for water safety and quality. We used two specific approaches in efforts to contribute to the existing water problems: (a) Development of sustainable microbial polymers which can act as flocculants and inactivation agents for water treatment and phosphate binding biopolymers; and (b) Microbial biopolymers which can be used for specific binding to bacterial pathogens and protozoal parasites in water and can be used as novel biosensors for accurate detection of microbial risk.

Case studies

(a) Microbial biopolymers as green flocculants for source water treatment

Microorganisms represent the nature's greatest library of sustainable resource. Extensive prospecting for potential microorganisms capable of producing biopolymers enabled creation of a library comprised of biopolymer producers. Exhaustive screening and characterization of the biopolymers as flocculating agents (Khaira et al., 2014) led us to select *Klebsiella terrigens* biopolymer which was subsequently shown to bind and remove water borne pathogens *Salmonella typhimurium, Shigella flexneri* 2a, *Escherichia coli* O157:H7 in addition to other water contaminants. Performance of this microbial flocculant was extensively trialled in poultry effluent and surface waters with comparable and better results with their chemical counterparts. These studies initiated through research grants from the University Grants Commission (UGC) and Water Technology Initiative of the Department of Science and Technology (DST–WTI) inspired us to surface engineer the flocculant targeted to inactivate the water borne pathogens simultaneously. In subsequent studies, we introduced chemical moieties onto surfaces of the biopolymer. These new class of surface engineered biopolymers demonstrated effective binding and killing of waterborne pathogens possessed excellent shelf, pH stability and specifically inactivated *Salmonella, Shigella* flexneri 2a *Aeromonas hydrophila* and *Escherichia coli* O157:H7(Khaira et al., 2014; Khaira and Ghosh, 2016).

Established to be completely nontoxic and biodegradable, we envisage potential applicability of the biopolymer for imparting high degree source safety to water as well as prime candidates for ensuring water bio-security.

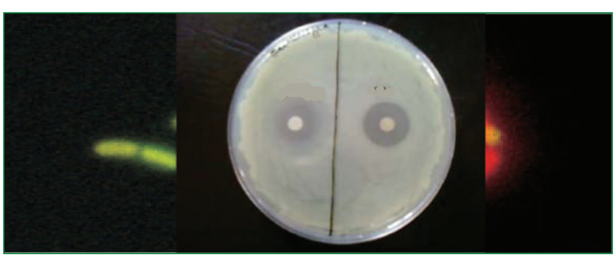

Figure 1: The inactivation of water borne pathogens by surface engineered microbial biopolymers. Live pathogens are stained yellow and dead orange.

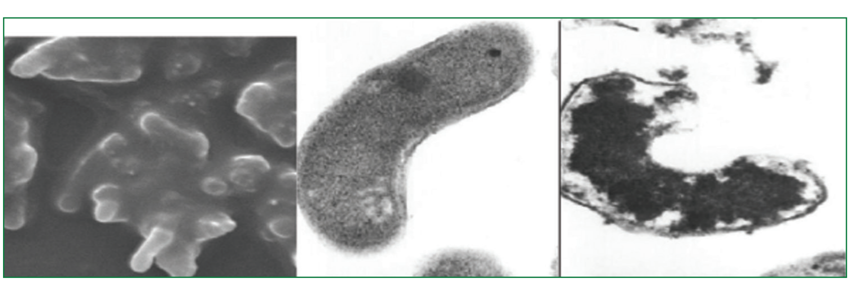

Figure 2: Electron micrographs of water borne pathogens: *Salmonella* typhimurium and *Shigella flexneri* 2a treated with surface engineered microbial biopolymer. The complete damage leading to cell death is evident from the morphology of treated cells.

(b) Harnessing microbial biopolymers as biosensors

As a protozoan parasite, *Cryptosporidium* presents a unique threat to human health and continues to be a concern with significant morbidity and mortality across the globe. Its resistance to both environmental stress and standard water treatment chlorination disinfection procedures enable it to survive for up to 16 months in water. Additionally, these organisms are ubiquitous in the environment and have an extremely low infectious dose. For some *C. parvum* isolates, one of the human pathogenic species, less than ten oocysts can be required to cause infection. This number should be compared against the billions of oocysts that an infected host could shed during an episode of infection. (During a clinical infection a calf may shed around ten thousand millions oocysts, which would provide enough parasites to infect the whole human population of Europe.) The greatest challenge, lie in its early and accurate and detection so as to initiate immediate intervention. Using earlier leads from our research (Ghosh et al., 2009) and financial grant from DST-UKIERI bilateral project, we (University of Edinburgh, Heriot Watts University, UK) carried out extensive experiments on evaluating *Cryptosporidium* oocyst binding to multitude of bacterial biopolymers. Our ultimate aim was to design a rapid, biological detection system for the oocysts in water using the biopolymer array. The selected biopolymers, proven to be non toxic and with exceptionally high degree of oocyst binding were incorporated into detection chips. The system was able to bind and thereby detect oocysts in various water samples. We are in a process of commercializing this novel approach (Sharma et al., 2015, Bridle et al., 2014). In future we expect rapid, accurate detection platforms through lab-on-chip systems, which will incorporate these biopolymers for both detection of *Cryptosporidium* oocysts and water borne bacterial pathogens.

Phosphates in water pose an important problem for water reuse and aquatic life. An environmentally isolated bacterial strain of *Acinetobacter* showed remarkable

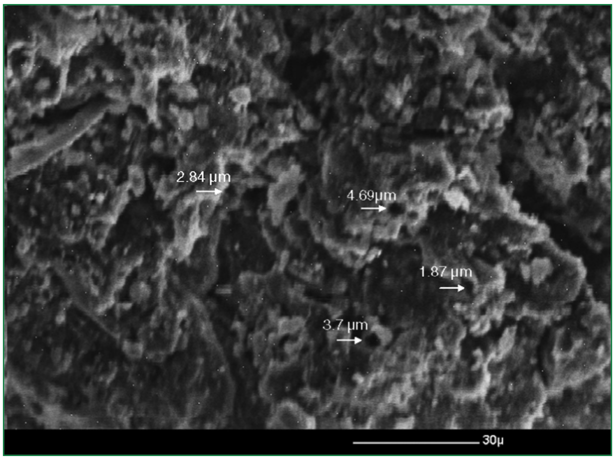

Figure 3: Scanning electron micrograph of *Cryptosporidium* binding biopolymer produced by bacteria

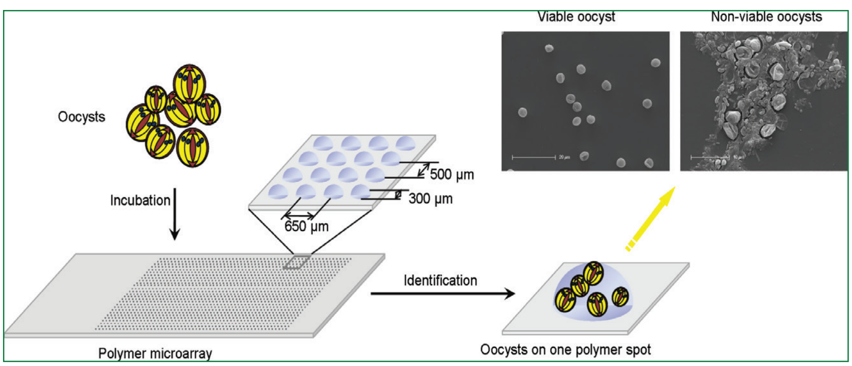

Figure 4: A biopolymer microarray developed for high throughput detection of *Cryptosporidium* oocysts. Inset: Left - shows viable oocysts and right: oocysts bound to biopolymer as detected in array.

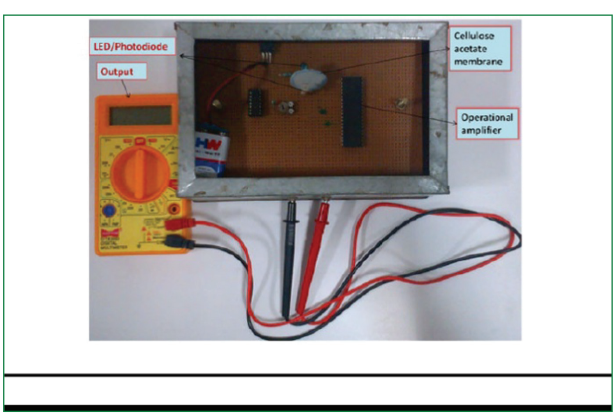

Figure 5: Biosensor developed with microbial phosphate binding biopolymer. The biosensor is capable of detecting phosphate concentrations of 1 μgL^{-1} in aquatic systems.

ability to sequester phosphate reversibly. This raised hope for double use of this biopolymer in phosphate removal and replenishing them through biopolymer itself in phosphate depleted soil (Kaur et al., 2014).

To monitor phosphate levels, a biopolymer based sensor utilizing simple colorimetric detection was devised. The biosensor could detect phosphate concentrations over a wide range and could be beneficial for analyzing phosphate in aquatic bodies as routine measures; it is worth mentioning that existing detection devices usually do not possess sensitivity to either high or low levels of phosphate or are complicated and costly.

Conclusion and future direction

With the use of globally available knowledge and technological advancements, India would be able to address some of its major problems related to water—lacking awareness of total ground water resources, wasted water due to leakages and flooding during monsoon or tropical storms. Judicious actions made regarding the mentioned challenges, will enable smarter infrastructural decisions and thereby provide water to a larger segment of the population, who currently receive intermittent or no supply. The challenges of water safety at source and water reuse require more critical approaches. Sustainable options have become most important in addressing this issue. Exploiting the abundant and diverse microorganisms for biopolymers with unique capabilities especially for water safety, require technological partnering between institutions, groups or individuals for scale up applications. Needless to say, a multifaceted approach in resolving the complex issues of water quality and safety is most desirable with close partnership of government agencies. Besides, value driven research in this area, national and international technology transfers and formulation of pockets of excellence catering to business models and national policies in water will be extremely important in this regard.

Acknowledgement

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Conflict of Interest

The author declares that there are no conflict of interest.

Disclaimer

The views, process, products described herein, do not necessarily endorse them for any specific application.

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South Asia Water Initiative (SAWI)

The South Asia Water Initiative (SWAI) is a World Bank programme that aims to increase regional cooperation in the management of the major Himalayan river systems in South Asia to deliver sustainable, fair and inclusive development and climate resilience. The program is funded by Governments of United Kingdom, Australia and Norway.

The major Himalayan river systems — the Indus, Ganges and Brahmaputra — span multiple countries (Afghanistan, Bangladesh, Bhutan, China, India, Nepal and Pakistan), landscapes (mountains, valleys, lowlands and deltas) and cultures. The program therefore works across basins and countries to support knowledge generation and sharing, capacity development, dialogue, participatory decision processes, and investment designs. In the context of water resources planning and management, the program promotes poverty alleviation, economic development, gender inclusion and climate change adaptation.

The program is structured into five Focus Areas (FAs): the three major Himalayan river basins of South Asia (Indus, Ganges and Brahmaputra), the Sundarbans Landscape and Regional Cross-Cutting activities. The program aims to achieve the following results:

- Increases in trust and confidence in regional or basin water management as a result of dialogue processes.
- Strengthening of stakeholder inputs to government decisions as a result of participatory processes that facilitate transboundary knowledge generation and sharing.
- Strengthening of the capacity of water resources organizations in areas relevant to transboundary cooperation
- Increases in accessible regional basin or sub-basin-level knowledge
- Design of regional, basin or sub-basin-level interventions that improve livelihoods and ecosystem sustainability

SAWI supports activities across multiple sectors such as Water, Environment and Energy in order to achieve the above-mentioned results.

For more information, access:

http://www.worldbank.org/en/programs/sawi#1