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Website: www.mutagens.co.in

E-mail: submit@mutagens.co.in

researchsubmission@hotmail.com

Research Paper

BIOFILMS AND BIOFOULING: COST AND EFFECT ON DRINKING WATER QUALITY FOR HUMAN DEVELOPMENT

E J Nya

Biotechnology & Genetics Centre,
Akwa Ibom State University,
P. M. B 1167, Uyo, Nigeria.

Abstract

It is microbial cells attached to surfaces in moist environments that form biofilms. Biofouling implies their accumulation on both living and non-living surfaces. Biofilm-associated cells are known to cost nations billions of US Dollars every year in equipment and machine maintenance, product contamination, medical device-related infections and energy loss; with attendant adverse effects on drinking water quality, causing disinfectant decay, taste, odour and spread of pathogenic diseases. However, biofouling reduces the efficiency of hydropower turbine and clog up pipes, channels and membranes, thus increasing the pumping costs incurred in the water distributions. Principally, these costs are goaded with those expended in continuous battle against biofilms in public health (medical) and domestic settings. While biofilms causes diverse array of problems, they can be of advantage. Biofilms function as biofilters, releasing metabolites such as organic and inorganic acids, ammonia, hydrogen sulphide and displaying bioaccumulation properties. On this note, this paper reviews the challenges and various methods adopted to prevent and inhibit the formation of biofilms, managing biofilms, advances in biofilm research for quality drinking water, public health implications of biofilms and economic cost of biofilms.

Key words: Biofilms, biofouling, microbial cells and extracellular polymeric substance (EPS).

INTRODUCTION

Biofilms are aggregates of microbial cells embedded in a self-produced matrix of extracellular polymeric substance (EPS) and adhering to non-living or living surfaces [1]. They are clusters of microbial cells occurring as slime on a surface of nearly every moist environment with sufficient nutrient flow and where surface attachment can be established. Within these clusters, the matrix protects the cells from harsh environment and facilitates communication among them through chemical and physical signals or quorum sensing. With this they have a greater chance of survival. Microbial cells within biofilms seem to have been transformed and had acquired different properties as they are more resistant to antimicrobial chemicals [2] and are capable of

degrading toxic chemicals released into the environment [3]. Therefore, biofilms are both harmful and beneficial and are currently of great interest in both medical and industrial fields.

Biofilm formations have a distinct order of event and developmental stages. Single bacterium is capable of conditioning the surface. Usually, within seconds of inoculation began the excretion of slimy film that covers the surface; and soon the colonizing species becomes permanently attached to the surface. More microbes become embedded into the slimy surface; and after several days, a cluster of substance composing of several species of microbes referred to biofilm is formed. With time, as the biofilm grows large, small portions break off and are carried to other area by the flowing water to begin colonizing new surfaces [4]. The major factors affecting the formation of biofilm on surfaces are electro-chemical properties of the surface, nutrient availability and water flow [5]. The chemical modification of surfaces affects the rate and extent of microbial attachment. By attaching itself to a surface, the cells increase the amount of nutrients available and thus, increase their chance for growth and survival [6]. Some microbes secrete and degrade substances that are essential for the growth of other microbial cells in the formation.

The growth of microorganisms in water distribution system can be affected by both biological factors and interaction of various physico-chemical factors such as pipe material, water temperature, pH, hydraulic conditions, nutrients, disinfectant concentration in water [7] [8] [9] [10]. Certain pipe materials can modify and degrade disinfectant residuals, leading to increased microbial growth in water distribution system [11] [12]. Different types of pipe materials can also affect microbial growth by releasing chemical compounds such as phosphorus ions, copper, iron and organic compounds. Formation of biofilm has been shown to be slower in copper pipes than in polyethylene (PE) pipes, and that copper ions released by the pipe leads to lower microbial numbers in the water distribution system. Whereas plastic pipes such as PE, which most people uses as the best replacement for conventional metal plumbing pipe because of their cost effectiveness have been known to biodegrade organic compounds and trigger off microbial growth with attendant biofilm formation [12].

There is increasing concern in many quarters about the present of some specks and particulates in water surfaces with respect to their relationship with microorganisms. This suspicion is not erroneous as many micro-organisms (bacteria, fungi, algae and cyanobacteria) have been known to colonize water bodies playing important role in degradation of available nutrients in the water. Therefore, this review attempts to examine the cost effect of microbial growth on drinking water, attendant declining water quality, taste and odour, disinfectant decay and spread of pathogenic diseases [13]. Accurate understanding of the microbial community present in biofilms in water distribution systems is critical to ensuring safe drinking water for human development.

2. Advances in Biofilms Research for Quality Drinking Water.

Current research on the concept of biofilms and biofouling centered on the investigation of biofilms occurring on inert and living surfaces, processing and drinking water distribution systems, giving new insights into the bacterial composition and also on the dynamics of microbial activity. The assessment of the potential effect of biofilms in water quality deterioration as well as their impact on the infrastructure, erosion of structural materials and the environment. With the use of state-of-the-art scientific analysis, characterization of bacterial distribution on surfaces has been made easy through the use of an array of instruments, particularly environmental scanning electron microscope (ESEM) and Raman microscopy. Also, the scientific community has shown keen interest in the investigation of enzymatic and physiological activities of biofilms consisting in the detection of metabolic products with desirable or undesirable properties. The influence of temperature and nutrient concentration on biofouling process; the effects of different disinfectants against bacterial diversity embedded in the biofilm matrix and the emergence and dispersion of genes encoding resistance to antibiotics and disinfectants through utilization of molecular approach have been achieved. Other areas where advances has been made are in the use of High throughput screening for chemical compounds capable of inhibiting biofilm formation and growth [14], evaluation of

disinfection efficacy of novel steam vapor system [15] and the development of small molecules that inhibit and/or disperse bacterial biofilms through non-microbicidal mechanisms [16]. Moreover, the potential impact of biofilms on biosafety practices and health risk of infectious diseases from biofilms in public healthcare facilities are not left out. Future research focus in biofilms are likely to be on: (i) imaging of biofilms in situ, (ii) in vitro and in vivo models of biofilms, (iii) genetic, metabolic, and immunologic probes for real-time polymerase chain reaction PCR analysis, (iv) antimicrobial resistance in biofilms associated pathogens, and (v) identification and phenotypic characterization of microbial cells colonizing surfaces, among others.

3. Biofilms impact on drinking water quality

The occurrences of biofilms in water distribution systems and storage have been shown to induce water quality deterioration [17] [18] and biocorrosion of structural surfaces [19] [20] [21]. Other consequences include water treatment yield loss, efficiency reduction in cooling or heating exchange transport, as well as in membrane processes.

Biofouling in drinking and industrial water systems induce residual disinfectants depletion and may cause aesthetic problems such as changes in colour, odour and taste caused by biodegradation of nutrients due to chemical compounds released. More important, by hosting pathogenic organisms as bacteria, viruses, protozoa, algae, fungi and other invertebrates, it poses a threat to human and animal health. Many authors have emphasized that great majority of water related health problems are the result of microbial contamination [22]. In line with this, naturally

occurring biofilms and biofouling in drinking water is known to constitute microbial reservoirs for opportunistic and emerging pathogens to furthering dissemination and infestation of other surfaces [23] [18]. Costerton (1994) refer to them as primary colonizers but more often as secondary colonizers in ecological surfaces promoting the adhesion at the interface [24].

Emerging pathogens have been seen as those pathogens that invade human population for the first time, or have occurred with increasing incidence in areas where they have not been reported previously, usually for over the last 20 years. They include: bacteria (pathogenic *E. coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Mycobacterium avium* complex), parasitic protozoa (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Toxoplasma gondii*), viruses (noroviruses, hepatitis E) and toxic cyanobacteria [25]. Whereas, opportunistic pathogens are mostly those pathogens that capitalizes on the weakness of their host immunity such as immune compromised individual, to cause harm but would be harmless to a healthy individual [26] [27]. These two categories of pathogens have been detected worldwide in drinking water associated biofilms, in raw water and water sediments.

Impact of biofilms and biofouling in drinking water distribution systems may also include the detrimental effects of chemicals, principally, toxins and other inorganic compounds produced by the microorganisms inhabiting the biofilms matrix and water sediments. Different volatile compounds, secreted as a result of microbial metabolism or particulates decay such as organic and inorganic acids, enzymes and metallic oxides may cause aesthetic and organoleptic characteristics problems in water, mostly colour change, odours and taste degradation which may have great impact on the acceptance of the water by the consumers and end-users. Peter (2008), investigated the sources of taste and odour in drinking water in order to fashion out acceptable mitigation strategies, he discovered that low chlorine residuals, stagnant water, plastic pipes and particles accumulation in distribution systems causes increase generation of taste and odour compounds, consequent upon high microbial activities [28]. In line with this, UK EPA (2004), also assert that other sources of aesthetic and organoleptic problems in drinking water may be as a result of activities of bacteria involved in sulphur cycle, thus producing sulphur associated odours and yellow discoloration [26]. Cerrato et al, (2006), in his work showed that oxidation and reduction of soluble metals by biofilms associated microorganisms may produce metal oxides, leading to metallic taste and colour stained water. However, access to safe drinking water continues to be essential for human development especially in developing countries [29].

4. Biofilms Implications in Public Health

Biofilms have been implicated in public health care and home setting. Considerable evidences exists in this respect, depicting biofilms as being responsible for a variety of nosocomial infections associated with medical devices, hospital equipment, household wet surfaces such as sink, toilets cutting tray or board and other hard surfaces, which can act as reservoirs. [30]. Microorganisms associated biofilms on health facilities may compose of bacteria transmitted from the body of hospital patients, the public health workers, tap waters or it can be even airborne. Bacteria have been isolated from catheter biofilms. Catheters are used generally for administration of fluid, nutrition solution, blood products and drips. Biofilms have been detected in urinary catheter, contact lenses, intrauterine devices (IUDs) [31] among others. Kokare et. al. (2009), in their work reported evidence implicating biofilms in diseases such as otitis media (common ear infection involving inflammation of mucoperiosteal lining), bacterial endocarditis, and Legionnaire's disease [31]. Other examples of diseases caused by biofilms associated bacteria are those found in patients with cystic fibrosis, chronic bacterial prostatitis of the prostate gland, and periodontitis disease involving the supporting tissues of teeth, gums, periodontal tissues, ligament which may cause complete loosening of teeth. Donlan (2001) also showed a spectrum of medical devices and others used in the healthcare setting to have harbor biofilms, resulting in health care device-associated infections [32].

Table 1 provides a list of microorganisms commonly associated with biofilms on indwelling medical devices.

Table 1. Microorganisms commonly associated with biofilms on indwelling medical devices

Microorganism	Isolated from biofilms on
<i>Candida albicans</i>	Central venous catheter, Intrauterine device, artificial voice prosthesis
Coagulase-negative staphylococci	Central venous catheter Intrauterine device Prosthetic heart valve, urinary catheter.
<i>Enterococcus</i> spp.	Central venous catheter Intrauterine device Prosthetic heart valve urinary catheter
<i>Klebsiella pneumoniae</i>	Central venous catheter, urinary catheter
<i>Pseudomonas aeruginosa</i>	Central venous catheter, urinary catheter
<i>Staphylococcus aureus</i>	Artificial hip prosthesis, Central venous catheter, Intrauterine device. Prosthetic heart valve

In a recent study, researchers have shown that biofilms attaching itself to activated carbon particles in water filters have the potential to seed bacteria into the drinking water supply system, which are then distributed throughout the hospital network. Thus furthering the growth of microbial contents and associated extra-polymeric substances in hospital tap water supplies. Biofilms in potable water distribution systems have the potential to harbor enteric pathogens, *L. pneumophila*, nontuberculous mycobacteria, and possibly *Helicobacter pylori* [33]. These can impose heavy toll on public health, affect water safety and principally drive up the cost of potable water production..

5. Economic Cost of Biofilms

Access to safe drinking-water is essential to health, and is a basic human right and should be seen as a major component of any government policy on health care [34]. Biofouling in water systems generates economic cost vis-a-vis technical problems, such as biocorrosion of structural surfaces, water quality deterioration. as well as water aesthetic values such as taste, odour and water colour. The most alarming consequences of biofouling in drinking water distribution systems consist on the economic burden incurred by both government and

industries in waste water treatment, head lose filtration, and energy depletion. Specifically, biofilms cost the UK billions of pounds every year in energy losses, product contamination, equipment damage, and medical infections. The costs from energy losses alone are said to be staggering. For instance, fuel consumption of large ships with clean hulls have been found to be low as compare to those whose hulls are contaminated by biofouling with colonies of microbial biofilms. However, as fuel prices increases, the cost impact of biofilms increases. More importantly, it has been alleged in some quarters that approximately 5% of the UK's carbon emissions come from shipping, so greater fuel usage will definitely translates to climate change. This in itself has an environmental price as well.

Similarly, biofouling can reduce hydropower turbine efficiency by 40% and clog up pipes and membranes, thus significantly increasing the pumping costs incurred in the water and petroleum industries. However, these costs are worsened with those expended in battle against biofilms on medical and domestic settings. From tourism and hospitality organization working to preserve the nation's heritage, biofilms have been known to cause significant erosion of structural decor, used in decorating many historic buildings and monuments. Thus, preventing and managing biofilms is a big business, and individual or companies with a deeper understanding of biofilms' interactions with surfaces and their impact on water flow will have a competitive advantage in developing products that enhance biofilm control.

Many third world countries are concerned with reduced efficacy of antimicrobial agents occasioned by biofouling of surfaces. Hitherto, antimicrobial agents that have been effective in controlling and inhibiting bacterial growth in vivo are less effective on infection associated with biofilms [35] [36]. Accordingly, O'Toole (2002) estimated that the economic burden of infections arising from biofilms is \$6 billion per year in the United States [37]. The scientific communities and health care industries are only beginning to realize the magnitude of the impact of biofilms on healthcare costs. According to a report by the U.S. Government Accounting Office (GAO), available evidence on biofilms and attendant microbial resistance is inadequate to assess costs to public health systems. [38]

6. Challenge of Removing and Preventing Biofilms

Biofilm formation is a universal phenomenon. Its formation is a part of microbial strategies adopted to survive in harsh, hostile environments. Deepa and Rajendran (2013) reported that they occur widely on ships hulls, boats, non compatible contact lenses, implanted medical devices, showers pipes, cooling pipes in nuclear reactors, and water distribution systems [39]. However, biofilms can occur in natural environments like oceans, seas, lakes, river beds, streams and in controlled environments like aquaculture ponds, wastewater treatment plants systems. Interestingly, biofilms has been known to increase the costs of production, machineries and equipment maintenance, created public health and environmental concerns all over the world.

The challenge of removing biofilms and preventing biofouling has been studied extensively and research is increasingly being focused on addressing the issue. So far natural and synthetic compounds have been used to prevent and inhibit the formation of biofilms. Various solutions and methods have also been recommended by research scientists ranging from simple antifouling paints containing compounds like copper and tributyltin (TBT) to mechanical methods like scraping the fouling organisms off the ship surfaces [40] [41]. However, Champ (2003), has asserted that TBT has damaging effects on the marine environment as it is not biodegradable and has recently been banned under the International maritime organization treaty (IMO) [42]. Feng et. al., (2009) reported several chemicals they studied in addressing the biofilm challenge to include benzalkonium chloride [43], chlorine [44], sodium hypochlorite [45], hydrogen peroxide[46], pyrethroids [43] among others. In the same line, Rittschof (2000) had previously reported from his research outcome the use of natural compounds from plants and other organisms like phytochemicals and marine microbes, for the prevention of the biofilm [47]. These natural compounds have been assessed to be less toxic and environmental friendly. Zhen Zeng, (2013) reported how Marine sponges have been shown to have potential anti-

microbial activity [48]. Bhattarai, (2007) affirmed the use of traditional methods ranging from scraping and applying animal fat to the uses of organic synthetic compounds as traditional anti fouling agents [49]. In another study by Hazan et. al., (2006) the use of ultra violet radiation, ozone, reverse osmosis technique, low energy surface acoustic waves and lasers was demonstrated to be successful [50]. A group of research scientists in the University of Glasgow, Scotland, UK, had use specific nanoparticles, such as silver, on desalination membranes to prevent biofouling. They demonstrated from their study how a bacteria cell absorbing a nanoparticles, was destroyed, through high resolution field-emission environmental scanning electron microscope (ESEM). Their discovery had led to scientists achieving greater success in preventing biofouling by attaching silver nanoparticles to desalination membranes [51] [52].

However, to achieve effective biofilms control and prevention, for improvement in water quality for human development, a spectrum of highly specialized equipment such as the Imaging Spectroscopy and Raman microscopy are needed. These instruments are capable of characterizing bacterial distribution and analyzing the effectiveness of biocides, nanoparticles, surface chemistry solutions, and other treatment systems on biofilms. With this the task of cost reduction for improved water quality production for human development could be in sight.

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REFERENCES

- [1] J. W Costerton; P. S. Stwearl and E. P. Greenberg. Bac- terial Biofilms: A Common Cause of Persistent Infec- tion," *Science*, Vol. 284, pp. 1318-1322. (1999).
- [2] R. M. Atlas and R. Bartha. *Microbial Ecology: Fundamentals and Applications*, Addison Wesley Longman, NY 4th ed. pp. 66-67; 179-181. 1998
- [3] M. R. Fries, L.J. Forney and J.M. Tiedje.. Phenol-and toluene-degrading microbial populations from an aquifer in which successful trichloroethene co-metabolism occurred. *Appl. Environ. Microbiol.* 63:1523-1530. (1997).
- [4] P. Bottner. Response of Microbial Biomass to Alternate Moist and Dry Conditions in a Soil Incubated with 14C and 15N-Labeled Plant Material," *Soil Biology & Bio- chemistry*, Vol. 17, pp. 329-337. (1985).
- [5] K. Mattila. *Biofilms on Stainless Steels Exposed to Process Waters*," Ph.D. Dissertation, University of Hel- sinki, Finland, 2002.
- [6] K. C. Marshall. Colonization, Adhesion, and Biofilms. In: Hurst J.C. (ed.) *Manual of Environmental Microbiology*. ASM Press, Washington DC . pp. 358-365. (1997).
- [7] M. W. Le Chevallier, C. D. Lowry & R. G. Lee. Disinfecting biofilms in a model distribution system. *J. Am. Water Works Assoc.* 82(7), 87-99. (1990).
- [8] J. C. Block, K. Haudidier, J. L. Aquin., J. Miaza. & Y. Levi. Biofilm accumulation in drinking water distribution systems. *J. Am. Water Works Assoc.* 6(4), 333-343. (1993).
- [9] P. H. Piriou, S. Dukan, Y. Levi, & P. A Jarrige. Prevention of bacterial growth in drinking water distribution. *Water Sci. Technol.* 35(11-12), 283-287. (1997).
- [10] S. Srinivasan, G. W. Harrington, I. Xagorarakis & G. Ramesh. Factors affecting bulk to total bacteria ratio in drinking water distribution systems. *Water Res.* 42(14), 3393-3404. (2008).
- [11] N. B. Hallam, J. R. West, C. F. Forster & J. Simms. The potential for biofilm growth in water distribution systems. *Water Res.* 35(17), 4063-4071. (2001).
- [12] M. J. Lehtola, I. T. Miettinenena, M. M. Keinanen, T. K. Kekkia, O. Laineb, A. Hirvonen, T. Vartiainen & P. J. Martikainen. Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res.* 38, 3769-3779. (2004).

- [13] J. Rubulis & T. Juhna. Evaluating the potential of biofilm control in water supply systems by removal of phosphorus from drinking water. *Water Sci. Technol.* 55 (8-9), 211-217. (2007).
- [14] L. Song; J. Wu and C. Xi. High throughput screening for chemical compounds inhibiting biofilm formation and growth. *Am J Infect Control.* 40 (10): pp 926-30. doi: 10.1016 / j.ajic. 2011.11.013. (2012).
- [15] W. Cai; J. Wu; C. Xi and M. E. Meyerhoff. Biofilms on environmental surfaces: evaluation of the disinfection efficacy of a novel steam vapor system, *biomaterials.* 33(32):7933-44. doi: 10.1016. (2012).
- [16] Justin J. Richards and Christian Melander. Small Molecule Approaches toward the Non-Microbicidal Modulation of Bacterial Biofilm Growth and Maintenance, *Anti-Infective Agents in Medicinal Chemistry, 8*, 295-314. (2009).
- [17] D. G. Lee; S. J. Kim & S. J. Park. Effect of reservoirs on microbiological water qualities in a drinking water distribution system. *J Microbiol Biotechn*, Vol.16, pp. 1060-1067. (2006).
- [18] J. Wingender & H. C. Flemming. Biofilms in drinking water and their role as reservoir for pathogens. *Int J Hyg Environ Heal*, Vol.213, pp. 190-197, (2011).
- [19] H. A. Videla and W. G. Characklis. Biofouling and microbially influenced corrosion. *International Biodegradation and Biodeterioration*, 29: 195-207. (1992).
- [20] I. B Beech and H. C. Flemming. Microbiological fundamentals. In: Beech, I., Bergel, A., Mollica, A., Flemming, H.C., Scotto, V., Sand, W. (Eds.) Simple methods for the investigation of the role of biofilms in corrosion. *Biocorrosion*, 00-02: 3-15. (2000).
- [21] S. E. Coetser & T. E. Cloete. Biofouling and biocorrosion in industrial water systems. *Crit Rev Microbiol*, Vol.31, No.4, pp. 213-232, (2005).
- [22] M.R. Riley; C. P. Gerba & M. Elimelech. Biological approaches for addressing the grand challenge of providing access to clean drinking water. *J Biol Eng*, Vol.5, No.2. (2011).
- [23] U. Szewzik; R. Szewzyk; W. Manz & K. H. Schleifer. Microbiological safety of drinking water. *Annu Rev Microbiol*, Vol.54, pp. 81-127. (2000).
- [24] J. W. Costerton. Structure of biofilms, In: *Biofouling and biocorrosion in industrial water systems*, Geesey, G.G.; Lewandowski, Z. & Flemming, H.C. (Ed.) CRC Press, ISBN 0 87371 928 X, USA. (1994).
- [25] P. R. Hunter; P. Payment; N. Ashbolt & J. Bartram. Assessment of risk, In: *Assessing microbial safety of drinking water*, IWA Publishing, ISBN 1 84339 036 1, London, UK. (2003).
- [26] UK EPA- Environment Protection Agency. *The microbiology of drinking water (2004) - Part 11 - Taste, odour and related aesthetic problems*, London, UK, http://www.environmentagency.gov.uk/static/documents/Research/mdwpart112004_859972.pdf. (2004).
- [27] US Environmental Protection Agency. Control of biofilm growth in drinking water distribution systems, Seminar publication, Washington, USA, www.epa.gov/nrmrl/pubs/625r92001/625r92001.html. (1992).
- [28] A. Peter. Taste and odor in drinking water: Sources and mitigation strategies, PhD Thesis, Swiss Federal Institute of Technology Zurich, Switzerland, <http://e-collection.ethbib.ethz.ch/eserv/eth:30628/eth-30628-02.pdf>. (2008).
- [29] J.M. Cerrato; L. P. Reyes; C. N. Alvarado & A. M. Dietrich. Effect of PVC and iron materials on Mn (II) deposition in drinking water distribution systems. *Water Res*, Vol.40, pp. 2720-2726. (2006).
- [30] Venkat Rao; Rashmi Ghei and Yildiz Chambers. Biofilms Research Implications to Biosafety and Public Health. *Applied Biosafety*, 10 (2) pp. 83-90. (2005).
- [31] C. R. Kokare; S. Chakraborty; A. N. Khopade and K. R. Mahadik. Biofilm: Importance and application. *Indian Journal of Biotechnology*, Vol. 8, Pp 159 - 168. (2009).
- [32] R.M. Donlan. Biofilms and device-associated infections. *Emerg Infect Dis.*7: 277-81. (2001).
- [33] R.M. Donlan. Biofilms: Microbial Life on Surfaces, *Emerging Infectious Diseases*, Vol. 8, pp. 881-890. doi:10.3201/eid0809.020063. (2002).

- [34] WHO. Guidelines for drinking-water quality, Vol. 1: 3rd Ed., Recommendations, Geneva, pp. 1- 294. (2008).
- [35] T. F. Mah & G. A. O'Toole. Mechanism of biofilm resistance to antimicrobial agents. *Trends in Microbiology*, 9, 34-39. (2001).
- [36] P. S. Stewart; J. Rayner; F. Roe & W. M. Rees. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. *Journal of Applied Microbiology*, 91, 525-532. (2001).
- [37] G. A. O'Toole. A resistance switch. *Nature*, 416, 695-696. (2002).
- [38] GAO- Government Accounting Office. Antimicrobial resistance: Data to assess public health threat from resistant bacteria are limited. *GAO Report*. Washington, DC: GAO/ HEHS/ NSIAD/ RCED-99/132. (1999).
- [39] Deepa Madathil and N. Rajendran. Prevention and inhibition of environmental biofilms: future perspectives. *Int J Pharm Bio Sci*. 4(3): pp 1398 – 1409. (2013).
- [40] G. Borkow & J. Gabbay. Copper as a biocidal tool. *Curr Med Chem*, 12(18), Pp 2163-2175. (2005).
- [41] K. A. Dafforn; T. M. Glasby & E. L. Johnston. Differential effects of tributyltin and copper antifoulants on recruitment of non-indigenous species. *Biofouling*, 24(1), 23-33. (2008).
- [42] M. A. Champ. Economic and environmental impacts on ports and harbors from the convention to ban harmful marine anti-fouling systems. *Mar Pollut Bull*, 46(8), 935-940. (2003).
- [43] D. Feng; C. Ke; S. Li; C. Lu & F. Guo Pyrethroids as promising marine antifoulants: laboratory and field studies. *Mar Biotechnol (NY)*, 11(2), 153-160. (2009).
- [44] K. J. Grobe; J. Zahller & P.S. Stewart. Role of dose concentration in biocide efficacy against *Pseudomonas aeruginosa* biofilms. *J Ind Microbiol Biotechnol*, 29(1), 10-15. (2002)
- [45] S. B. Luppens; M. W. Reij; R. W. van der Heijden; F. M. Rombouts & T. Abee. Development of a standard test to assess the resistance of *Staphylococcus aureus* biofilm cells to disinfectants. *Appl Environ Microbiol*, 68(9), 4194-4200. (2002).
- [46] E. Bardouniotis, H. Ceri & M. E. Olson. Biofilm formation and biocide susceptibility testing of *Mycobacterium fortuitum* and *Mycobacterium marinum*. *Curr Microbiol*, 46(1), 28-32. (2003).
- [47] D. Rittschof. Natural product antifoulants: One perspective on the challenges related to coatings development. *Biofouling*, 15(1-3), 119-127. (2000).
- [48] Zhen Zeng; Jing Zhao; Caihuan Ke & Dexiang Wang. Antimicrobial activities of novel cultivable bacteria isolated from marine sponge *Tedania anhelan*. *Chinese J Oceanology and Limnology*, 31(3), 581-590. (2013).
- [49] H. D. Bhattarai; B. Paudel; N. S. Park; K. S. Lee & H. W. Shin Evaluation of antifouling activity of eight commercially available organic chemicals against the early foulers marine bacteria and *Ulva* spores. *J Environ Biol*, 28(4), 857-863. (2007).
- [50] Z. Hazan; J. Zumeris; H. Jacob; H. Raskin; G. Kratysh; M. Vishnia & G. Lavie. Effective prevention of microbial biofilm formation on medical devices by low-energy surface acoustic waves. *Antimicrob Agents Chemother*, 50(12), 4144-4152. (2006).
- [51] R. C. Pratik; A. M. Shalaka; B. Vrishali B. Shidore & Suresh P. Kamble. Effect of biosynthesized silver nanoparticles on *Staphylococcus aureus* biofilm quenching and prevention of biofilm formation, *Int J Pharm Bio Sci* 3(1): 222- 229. (2013).
- [52] S. K. Das; M. M. Khan; T. Parandhaman; F. Laffir; A. K. Guha; G. Sekaran & A. B. Mandal. Nano-silica fabricated with silver nanoparticles: antifouling adsorbent for efficient dye removal, effective water disinfection and biofouling control. *Nanoscale*. 5(12): 5549-60. (2013).