A Review on Current Strategies for Biofilm Control in Food Industry

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Abstract. Biofilms are still a serious threat to the world. Biofilms are formed due to the natural tendency of microorganisms according to environmental factors. And they are solicitude in many fields counting food, medical and environmental. Biofilms are hard to exterminate due to their resistant phenotype. Since biofilms is a surface episode it develops on the different surfaces in food industry which can be very severe for the consumers, because it can cause serious illness to the consumers as well as monetary loss. In the current scenario to prevent biofilm formation the basic protocols that are used are cleaning and disinfection which cannot remove biofilms properly. Consequently, the new strategies are developing along with improving conventional control methods. Use of enzymes, biosurfactants, electrostatic interactions, essential oils to prevent biofilm formation.

This review intent on the present strategies that are in use or is developing for controlling biofilms. Which can offer statistics about major concerns in food industries.

Keywords: Biofilm · Control Methods · Food Industries · Essential oil · Biosurfactants

1 Introduction

Biofilms is a serious issue that are occupying most industries including Medical and Food Corporation or business. Biofilms are major cause of antibiotic resistance, nosocomial infection and food borne illnesses. Biofilms are rigid and dynamic in nature and is quite beneficial to the microbes itself as it helps in adhesion, metabolite exchange, quorum sensing and resistance to drugs [1]. But are detrimental to the humans, as biofilms is a concerning issue than it is on research articles or news causing enormous amount of diseases and illness. Biofilms are dynamic structure consisting of single or multi species that are enclosed in extracellular polymeric matrix (EPS) [2]. Biofilm development starts with the binding of single cell to the distinctive surfaces, accompanied by growth and maturation which give rise to microcolony. Later, enlarges to form large colonies in which microbial community communicate and entrap nutrients [3]. Detachment and dispersal is followed afterwards. Which gets attached to the new point as a new source of contamination [4]. Microbial biofilms are the inhabitant in the food processing equipment's. These biofilms are menace to both mankind and animals. Consuming food

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produced from food manufacturing unit that has biofilms of pathogenic bacteria and toxins that can easily contaminate food which can cause food borne illness and outbreaks. The economical aspect has to be considered, since food products contamination with spoilage microorganisms leads to shelf life reduction, with economic losses. In addition, deterioration of pipes, bioreactors may arise in the presence of biofilms. Control and prevention are the preferable choice to avoid major food borne illness outbreak which can be achieve by hygiene and disinfection in food industries. To combat biofilms in the food industries, use of enzymes and disruption of quorum sensing, use of bacteriophage, use of bacteriocins and use of essential oils is being considered [4, 5]. Therefore, the aim of this review is to enlist all the data on disinfection and biofilm removal methods and to discuss about it. This paper focus on control strategies of biofilm in food units.

2 Biofilms a Threat to Food Industries

Biofilms are complex of the same species or different species of microorganisms. Which is confined in an extracellular polymeric matrix. Biofilms consisting of different species aids in better attachment. Biofilms can form immediately in food industries the steps are the binding of the cells to the surface. Next, the coupling to the surface that is followed by microcolony formation. Finally, the biofilm's 3D structure is formed, giving rise to a complexes of ecological communities after dispersal [6]. These biofilms can bind on any surfaces of food industry gadgets which include stainless steel, plastics, wood, glass etc. [7]. Major health issues can rise with either intoxication or infections major food borne illnesses are caused by *E coli, Enterobacteriaceae* family. And some VBNC (viable but non culturable) like *Listeria monocytogenes* [5] Dairy, fish processing, meat, poultry and also ready to eat manufacturing industries are the worst hit industry with the biofilm havoc. It is very hard to maintain a sterile environment because somehow biofilms can form if proper cleaning of vessels or equipment's are not done. In the dairy industry milk is the most perishable food item and the spoilage is result of either poor cleaning or poor hygiene [8]. And major microorganisms that are being encountered in dairy industries are from the genus *Enterobacter* [9]. While major biofilm formers in the fish processing industries are *Vibrio* species [10] and also presence of *Salmonella spp. Aeromonas spp. or Bacillus spp.* [11]. *Salmonella* or *Campylobacter Spp* are major biofilm formers in poultry industry [12]. Talking about ready to eat industries, RTE usually follows strict guidelines and there are few contaminations, biofilms are usually formed by *E. coli* and *Listeria Spp.* Only in perishable food items [13, 14].

3 Biofilm Control Strategies

Precisely, prevention is a better concept than controlling the biofilm formation. So, it can be achieved by the regular disinfection of the equipment and the surfaces to prevent firm attachment of biofilm formers [15].

Biofilm formation takes place depend upon microbial community present and type of material the equipment. Considerable approaches have been made to control the biofilms are chemical methods biological method and physical methods Fig. 1 enlisting all the biofilm control methods. Along with the growing techniques use of conventional cleaning and disinfection shall be used along with these methods to prevent any diseases to both human and animals.

3.1 Chemical Control Methods

3.1.1 Biosurfactants

Biosurfactants are natural components of microbial origin and it alters the adhesion and binding capacity. Lichenysin is a biosurfactant that is formed by *B. licheniformis.* This biosurfactant can lessen the binding of microbes including MRSA, *C. albicans*, *Y. enterocolitica* or *C. jejuni* [16]. *B. amyloliquefaciens* forms biosurfactant named fengycin, iturin and surfactin. This alters the membrane permeability, eventually disrupting it and causing cell swelling and death [17].

3.1.2 Steel Coatings

The use of nanoparticles are in recent trends and shows promising results. Nanoparticles have antimicrobial properties so they are less prone to the resistance. These nanoparticles can be mixed with new nanoparticles to form nanocomposite that will exhibit new properties [18]. For example use of sulfhydryl compounds were able to reduce *Staphylococcus aureus* biofilms on polystyrene polymer by inhibition of extracellular matrix genes (ica) [19], another example of coating stainless steel surfaces with Ni-*P*-polytetrafluoroethylene. This compound was able to reduce *Bacillus licheniformis* and able to reduce milk deposition [20]. Of the uses of nanoparticles with liposomal formulations with bacteriocin nisin this showed antimicrobial activity against *S. aureus* biofilms [21].

3.1.3 Enzymes Based Detergents

Enzymes are considered to be a green approach as it is biodegradable and have low toxicity. Chiefly used enzymes are either proteases or glycosidases for biofilm removal [22]. For instance application of pectin methyltransferase can reduce biofilms from the bioreactors and pipes [23]. Cetyltrimethyl ammonium bromide with cellulase can efficaciously take away Salmonella enterica biofilms [24]. Whereas, *Bacillus spp.* are producer of subtilisin, which is used against *Pseudomonas aeruginosa* and *L. monocytogenes* these are serine proteases that inhibit adhesins, crucial for biofilms attachment [25].

3.1.4 Disinfection

Disinfection is being used for the cleaning purpose since are very long period but it may be effective against free cells but not on the biofilm. The disinfection is used in combination with enzyme or biosurfactant. Quaternary ammonium compounds, sodium hypochlorite favoured choice of disinfections. Table 1 Depicts the efficacy of each disinfectant and biofilm on which they act.

Table 1. List of disinfections and kind of biofilm they show their effect.

Disinfection/treatment	Biofilm type
Hydrogen peroxide; sodium dichloroisocyanurate; peracetic acid [26]	*Staph. Aureus*
Peroxides; quaternary ammonium compounds; chlorine [27]	*L. monocytogenes*
Sodium hypochlorite [28]	*S. typhimurium*
Chlorine; chlorine dioxide; commercial detergent [29]	*B. cereus and Pseudomonas spp.*
Sodium hydroxide; nitric acid [30]	*Mixed species*

3.2 Biological Control Methods

3.2.1 Bacteriocins

A protective mechanism in which a bacteria produces peptides that are lethal and can kill the same species or closely related members are called as bacteriocins. For instance nisin bacteriocin is produced by *Lactococcus lactis* which has shown activity against *L. monocytogenes* on stainless steel surfaces [31]. Bacteriocins are usually produced by lactic acid bacteria (LAB) such as pediocin produced by *Enterococcus spp.* Which inhibits *L. monocytogenes*. Another one *lactococcus spp.* functioning against *Brochothrix thermosphacta*. Lastly another bacteriocin garvicin produced by lactococcus garvieae which is active against the same pathogenic strain of same bacterium [32] as these do not cause any harm to animals and humans it is commercial safe.

3.2.2 Quorum Sensing Inhibition

Quorum sensing is a bacterial way of communicating with each other. They communicate via sensing molecule called as autoinducers. There are ways in which one can block autoinducers to stop the quorum sensing. Extensive research has been going on the compounds that specifically blocks autoinducers including AHL (acyl homoserine lactones). one such study is on halogenated furanones derived from red alga (*Delisea pulchra*). This compound interfere with the protein receptors and treatment with this compound has shown reduction in the virulenece factor and prevent biofilm formation by *Pseudomonas aeruginosa* and *Serratia liquefaciens* [33, 34] another way of QS inhibition is quorum quenching another strategy involves the use of paraoxonases. Paraoxonases are a type of quorum quenching enzyme. These enzymes cause the hydrolysis of the lactone ring of AHL causing biofilm inhibition of *P. aeruginosa* [35]. Some natural sources are also used for quorum quenching like grapefruit extract that showed inhibition of biofilm formation in *E.coli, P.aeruginosa, Vibrio harveyi* and *S. enterica* [36]. This affects the regulators in quorum sensing. Although in literature there are several QQ molecules mentioned like green tea extract or seed extract.

3.2.3 Essential Oils

Essential oil are the plant derived compound that show antimicrobial activity. Major essential oils are derived from monoterpenoids, flavonoids and sesquiterpenoids. Reduction in the microbial load from 10^7 CFU/ml to 10^3 CFU/ml of *S. aureus* biofilm from the steel with the use of *Cinnamomum cassia* essential oil [37]. Three of the major pathogens *E.coli, S. enterica* and *P. aeruginosa* biofilm was reduced by 80% with the essential oil derived from the medicinal plants *Holarrhena antidysenterica* and *Andrographis paniculata* [38]. Citral an essential oil from lemon grass has antimicrobial activity, antiadhesion and antibiofilm activity.

3.3 Physical Control Methods

Physical forces are sometimes used for the biofilm removal when biological and chemical methods didn't give promising results. For example use of high hydrostatic pressure (<300 MPa) is effective in reducing microbial load but not efficient in removing

Table 2. Physical treatment and their effects on biofilms

PHYSICAL TREATMENT	BIOFILM TYPE	RESULT
Ultrasound	*S. aureus, Salmonella spp.*	Combination of acidic electrolyzed water (AEW) and ultrasound showed biofilm reduction. [41]
	S. epidermidis	A long US irradiation (24 h) can lower the biofilm amount [42]
Electrical field	*MRSA, E. coli, P. aeruginosa*	The motility of bacteria is greatly affected. 80–100% of bacteria were disintegrated with 300 pulses. [43]
Magnetic field	*S. aureus*	Biofilm (4–6-fold) decreased by the antibiotic penetration through channels.[44]
	S. aureus, P. aeruginosa	Antimicrobials resulted in fifty percent decrease in biofilm growth. [45]
	P. aeruginosa	Biofilm reduction.[46]
Irradiation	*S. typhimurium*	Combined X-ray/NaOCl treatment showed the highest reduction of biofilms.[47]
	Listeria monocytogenes	UV-C and NaOCl positive effect on L. monocytogenes biofilms formed on the stainless steel surface [48].

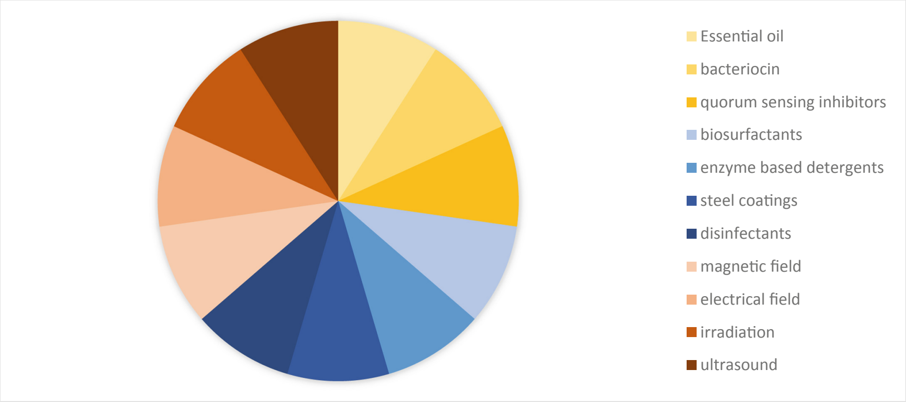

Fig. 1. Methods used for biofilm control in a food industry. (Portions in the pie chart doesn't represent any data.)

endospores [39]. Another method non thermal plasma is a partially ionised gas with low temperature and with antimicrobial activity. In this method the atmospheric pressure is mixed with UVlight with oxygen nitrogen ozone and water with an electrical discharge that can inhibit the biofilms of both gram positive and gram negative bacteria but is highly costly so less preferred method [40].

Other physical methods involve ultrasound, electrical field, magnetic field and irradiation. And they have shown actual effect on biofilm removal to some extent. Table 2 depicts these physical forces and their action on the biofilms.

4 Conclusion

Microbial contamination in the food industry is a serious issue as it adversely affects the health and also the economy. The major problem that causes food contamination is a buildup of biofilms on the working table, in the bioreactors, presence of pathogenic biofilms can actually be hazardous as it can easily cause food borne disease, gastritis or intoxication. Earlier biofilms were handled with either cleaning or disinfection but sooner it started developing resistance. But with the novel researches the development of various methods for biofilm removal for instance chemical disinfection is used because it is cheap and affordable. Also physical methods of biofilm control were used and also are in still use like high jet water spray or scraping but as the world advances so is the techniques development of several techniques for example ultrasound or irradiation etc. but these are costly. Physical methods are great in dealing with the biofilm removal but one thing they lack is penetration within the biofilm which doesn't cause proper disintegration.

With the novel approaches biological control methods are developing but are less in use. Use of essential oils are excellent technique to combat biofilms naturally also study is there about bacteriophages disrupting the biofilms but are usually lab scale.

My major focus is on quorum sensing inhibition because it is a game changer and it can disassembly biofilms easily with the help of quorum quenching. Another big success can be achieved in the field of nanotechnology involved with the surface that prevent biofilm attachment. One thing that is can be a disadvantage in biological method is that application of essential oil or QSI can cost industries their time. With the advancement in research I believe novel practices will be soon available that will be effective and cheap and will reduce food borne disease.

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