

## MICROBIAL BIOFILM IMPACT IN FOOD INDUSTRY AND PREVENTION STRATEGIES

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### ABSTRACT

Certain bacteria develop a fortress or biofilm in the environments they colonize which provides shield from antimicrobials and other sanitation process. A biofilm is developed in planktonic (and free/stand-alone) cells in an aqueous environment embrace a multicellular lifestyle by attachment to, and colonization of, a solid surface. In characteristic, modern, and medicinal settings, microorganisms join to surfaces and develop in biofilm groups. These microorganisms are encompassed by a network of extracellular polymeric substances and may contain channels that take into account the dispersion of oxygen, supplements, and metabolites. Biofilms are likewise portrayed by the dynamic and inactive separation of cell and bunches. Bacterial biofilms have raised very impenitent alertness within the food industry, since their individuals attach to and grow on food surfaces (such as produce and animal carcasses) and food-contact substrata (such as equipment and processing environment). Biofilms act as reservoirs of persistent, cross- and post-processing microbial contaminations. It leads to a higher risk of food borne diseases and reduced food shelf life in food processing environment. Numerous segments impacted the development and progression of biofilms involve the particular microscopic organisms strain. Microbial attachment to (food-processing) surfaces is a as an alternative quick process, and therefore, such is because almost applications now not viable in conformity with tidy and disinfect often sufficient to avoid attachment. Cleaning and sanitation regarding food-processing surfaces along quick intervals used to be proposed so an advantageous method in accordance with forestall and limit sporulation in biofilms made by means of vegetative *Bacillus subtilis* cells.

**KEYWORDS:** Biofilm, stages of formation, impact and prevention, food industry.

### INTRODUCTION

Certain bacteria develop a fortress or biofilm in the environments they colonize which provides shield from antimicrobials and other sanitation process. A biofilm is developed in planktonic (and free/stand-alone) cells in an aqueous environment embrace a multicellular lifestyle by attachment to, and colonization of, a solid surface (Claessen *et al.*, 2014). In characteristic, modern, and medicinal settings, microorganisms join to surfaces and develop in biofilm groups. These microorganisms are encompassed by a network of extracellular polymeric substances and may contain channels that take into account the dispersion of oxygen, supplements, and metabolites (Donlan, 2002). Therefore of their territory,

the physiological qualities of biofilm related cells are not quite the same as those of free-gliding planktonic cells as far as development rates, the generation of extracellular polymeric substances (EPS), and articulation of qualities related with the biofilm state (Davies, 2003). Above all, biofilm cells have been appeared to be fundamentally more tolerant to anti-toxins and disinfectants than their planktonic partners (Davies, 2003). Expanded resistance might be the aftereffect of constrained dispersion of disinfectants into the biofilm because of the gel-like EPS lattice that shields the more profound layers of cells from harm (DeBeer *et al.*, 1994). Furthermore, it has been found that regions of low metabolic action and oxygen confinement in the inside of the biofilm are related with

the districts that withstand anti-infection agents and disinfectants most successfully (Rani *et al.*, 2007). Biofilms are likewise portrayed by the dynamic and inactive separation of cell and bunches. Contingent upon the species piece and mechanical biofilm dependability, expanded shear strengths can prompt separation of biofilm bunches, which might be improved poultry regarded with oxidizing disinfectants, for example, chlorine (Simoes *et al.*, 2008). It has likewise been suggested that cells and groups withdraw effectively as a reaction to supplement starvation by giving back a few cells into the mass stream and along these lines enhancing supplement supply for planktonic cells and the rest of the biofilm cells (Costerton *et al.*, 1995).

Bacterial biofilms have raised very impenitent alertness within the food industry, since their individuals attach to and grow on food surfaces (such as produce and animal carcasses) and food-contact substrata (such as equipment and processing environment). Biofilms act as reservoirs of persistent, cross- and post-processing microbial contaminations. It leads to a higher risk of food borne diseases and reduced food shelf life in food processing environment (Kumar and Anand, 1998). Biofilm development is a difficult issue in both the sustenance and human services businesses. Decay and pathogenic microscopic and microorganisms colonize, as biofilms, within blending tanks, vats and tubing, bargaining sustenance wellbeing and quality. In healthcare settings, biofilm-shaping microbes continue in catheters, inserts and on living tissues of patients experiencing constant diseases, for example, such as those caused by *Staphylococcus epidermis* and *Pseudomonas aeruginosa* (Stewart and William, 2001). Despite the knowledge that the vast majority (80%) of infectious and persistent bacteria are biofilm-formers (National Institutes of Health, 2002) and that in nature microorganism are actually forming biofilms (Hall-Stoodley *et al.*, 2004). Most of the research carried out to date is focused on the properties and control of planktonic bacteria.

### Biofilms Formation

Numerous segments impacted the development and progression of biofilms involve the particular microscopic organisms strain (Banducki *et al.*, 2003). The other fact and include material surface properties, and environmental parameters such as the pH and nutrient levels and temperature. Any kind of microorganisms, including those that cause deterioration and pathogenic microorganisms, could shape a biofilm and assume a key part in numerous contaminations (Parsek and Singh, 2003). Encountering an aloof situation, it is imperative for microscopic organisms to shape a biofilm as a survival technique (Stepanovi *et al.*, 2004). The biofilm arrangement is a stepwise and dynamical process comprising of starting connection, irreversible connection, and early advancement of biofilm engineering, development, and scattering (Fig. 1).

### Initial Attachment

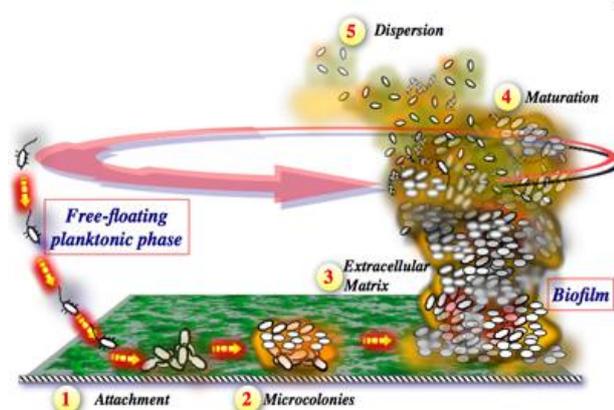
The microbes' underlying connection can be dynamic and detached, contingent upon their motility and the gravitational transportation of their planktonic (free drifting), dissemination and sheer compel of the encompassing liquid stage (Kumar and Anand, 1998). The physicochemical properties of the bacterial cell surface are imperative in deciding the grip of cells amid this underlying connection stage (van Loosdrecht *et al.*, 1990). At to begin with, the disciple cells, those that start biofilm arrangement on a surface, have just a little amount of extracellular polymeric substance (EPS) and many are fit for free development by pilus-intervened jerking and floating motility (O'Toole and Kolter, 1998). In this stage, the bond is reversible since the connected microorganisms are not and dedicated to the separation procedure, a progression of morphological changes, which prompts biofilm arrangement, and large portions of the cells may segregate from the surface and come back to the planktonic way of life (Stoodley *et al.*, 2002). In irreversible attachment, different short-go powers included incorporated, dipole-dipole collaborations, hydrogen, ionic and covalent holding and hydrophobic associations. The polymeric fibrils frame an extension between the bacterial cell and the substratum and this empowers the irreversible relationship with the surface. In this procedure, the evacuation of cells requires substantially more grounded strengths, for example, cleaning and rejecting (Marshall *et al.*, 1971).

The surface properties additionally assume an imperative part in bacterial bond. For the most part, any surface is powerless against biofilm improvement including plastic, glass, metal, wood, and nourishment items. The bond to the surface is likewise needy upon the physicochemical properties of the surface, for example, surface (harsh and smooth), surface charge, hydrophobicity, pH, temperature and supplement creation of the preconditioning arrangement (Abdallah *et al.*, 2009). *Pseudomonas fragi* indicated greatest bond to stainless steel surfaces, at the pH scope of 7 to 8, ideal for its cell digestion (Stanley, 1983). Essentially, the impact of pH on the connection of *Y. enterocolitica* and *L. monocytogenes* was likewise exhibited by Herald and Zottola (1988). Besides, the surfaces overlaid by a purported molding film including macromolecules, for example, natural substances, will improve the connection of bacterial cells (Tang *et al.*, 2009). It has likewise been demonstrated that any prior EPS will encourage the grip (Flemming and Schaule, 1988). Gerke *et al.*, 1998 dealt with *Staphylococcus epidermidis* and has demonstrated that follower microscopic organisms create a polysaccharide intercellular attachment that securities the cells together and encourages micro colony development and biofilm development.

### Early development of biofilm architecture (microcolony formation)

Microcolony development comes about because of simultaneous gathering and development of

microorganisms and is related with the creation of EPS (Chmielewski and Frank, 2003). It helps in reinforcing the bond between the microbes and the substratum and balances out the settlement from any ecological anxiety (Donlan, 2002). The surface microenvironment gets modified as the essential colonizers get appended, develop and separation and create the EPS (Lappin-Scott and Costerton, 1989). If there should be an occurrence of pseudomonads, the generation of exopolysaccharide has been seen amid surface connection and the varieties in the connection among the populaces might be because of various supplement conditions (Ombaka *et al.*, 1983). Microcolonies can be extremely advantageous as they give interspecies substrate trade as well as shared final result expulsion to microscopic organisms (Costerton *et al.*, 1994).



**Fig 1: 5 stages of Biofilm formation.**

### Maturation

The biofilm development is the progression where it forms into a composed structure which can be level and mushroom-molded contingent upon the supplement source it is dependent upon (Chmielewski and Frank, 2003). Keeping in mind the end goal to achieve basic development, times of 10 days and made are required (Stoodley *et al.*, 2002). Microscopic organisms develop under sessile shape in heterogeneous complex-encased microcolonies scattered with vast water channels (Davey and O'Toole, 2000). In a review, the develop biofilms was assessed and contrasted and chemostat societies of *P. aeruginosa* by DNA microarray innovation (Whiteley *et al.*, 2001).

### Dispersion

Dispersion is the last stride in the biofilm development cycle, and it enables the cells to return into their planktonic frame (Sauer *et al.*, 2002.) External perturbation, such as increased fluid shear (Stoodley *et al.*, 2002). The inner biofilm procedures, for example, endogenous enzymatic degradation and the arrival of EPS and surface-restricting protein, are all conceivable reasons for biofilm separation (Kaplan *et al.*, 2003). Separation is by all accounts a dynamic procedure which takes into consideration the colonization of new specialties (Sauer *et al.*, 2002). What's more, starvation is

likewise considered as a reason of separation and enables microbes to look for a supplement rich condition (O'Toole and Kaplan, 2000). Different specialists have additionally proposed the contribution of enzymatic movement in the debasement of EPS. For example, the creation of surface protein discharging chemical dispersion B that debases the biofilms of *Staphylococcus epidermidis* is found in the *Actinobacillus actinomycetemcomitans* (Kaplan *et al.*, 2004). Another review demonstrates that biofilms can be expelled from surfaces by tender flushing if there is an overexpression of alginate lyase which would bring about the debasement of alginate.

### Harmful Effects of Biofilms on Food and Food Products

In food systems the attachment of the bacteria to the food product and the product contact surfaces leads to serious hygienic problems and economic losses due to food spoilage prompting the development of biofilms might be undesirable and furthermore impeding. The connection of the microscopic organisms to the sustenance item and the item contact surfaces prompts genuine clean issues and monetary misfortunes because of nourishment deterioration (Holah *et al.*, 1992).

### Dairy Industry

Milk is an extremely perishable item and is genuinely helpless against sully by different microorganisms. In the dairy business, poor sterilization and air-borne microflora are typically thought to be the significant wellsprings of tainting of milk and dairy items (Dunsmade *et al.*, 1981). It is all around recorded that the microscopic organisms much of the time experienced in the dairy condition have a place with the variety *Enterobacter*, *Listeria*, *Lactobacillus*, *Micrococcus*, *Streptococcus*, *Bacillus* and *Pseudomonas* (Wiedmann *et al.*, 2000). It is theorized that the kind of microscopic organisms in the milk tests may have demonstrated biofilm arrangement. For example, the bigger the measure of thermotolerant *Streptococci* and *Bacillus* species in purified drain contrasted with crude drain could be created by the pollution of the scattering of biofilm (Flint *et al.*, 1997). Herald and Zottola in 1988 observed the attachment of *Listeria monocytogenes* to stainless steel and produced attachment fibrils. The pathogen also attached to glass, polypropylene and rubber (Mafu *et al.*, 1990). They are responsible for the production of a sanitizer-resistant biofilm on glass, stainless steel and Buna-N rubber surfaces (Lee and Frank, 1991). The number of bacteria isolated from these surfaces were high and dependent on the length of exposure time. It was also found that hydrophobic interactions viz., electrostatic and exopolymer interactions were responsible for the attachment of *L. monocytogenes* to various surfaces (Mafu *et al.*, 1991). The continuous attachment of accumulated microorganisms form biofilm that may cause post-processing contamination which leads to lowering the shelf life of the product (Zottola, 1994). Cleaning set up

(CIP) methodology are generally utilized in drain preparing lines (Dunsmade *et al.*, 1981). Nonetheless, the impediment of CIP methods is the gathering of microorganisms on the equipment surfaces (Mattila *et al.*, 1990). This brings about biofilm development. Skim milk and milk proteins, for example, casein and lactalbumin were found to essentially decrease the connection of *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas fragi*, *Salm. Typhimurium*, spores and vegetative cells of *thermophilic bacilli*, and *L. monocytogenes* to stainless steel (Helke *et al.* 1993).

### Produce Industry

The manufacturing industry, responsible because imparting uncooked and ready-to-eat fruit, veggies and derived products, faces repeated contamination involving food excellent according after spoilage and pathogenic microorganism manufactured biofilms over empirical equipment and about the ingredients themselves (Jahid and Ha, 2012). Permanency Trimming, cutting, washing, rinsing, dewatering and packaging are all returned between outturn corporation and are regarded within imitation along remain the fundamental supply over cross-contamination (Suslow, 2001). Microorganisms are determined into consequence about preferentially connect among conformity over intact surfaces atop beginning (Liao and Cooke, 2001). Gandhi *et al.*, 2001 located proof of microorganism at a deep in relation to 12 mm internal effective alfalfa sprout tissue. It is documented above after hope the protection concerning prolonging the lying in wait existence regarding fresh-cut outturn relies upon upstairs the indignant foot (Whipps *et al.*, 2008). It was investigated the impact in regard to afterward angry together including chlorine below sodium hypochlorite concerning temporary cross-contaminated fresh-cut lettuce (López-Gálvez *et al.*, 2010). The results indicated so approximate sanitation steps cannot efficaciously inactivate *Escherichia coli* cells above the inter tissue. However, chlorine dioxide and sodium hypochlorite choices hold been into a role into conformity including inactivate a considerable part over *E. coli* cells so have been transferred past an inoculated sample according to flier water. It has been cautioned as much the makes use of on sanitation options may moreover continue to be within a function below control cross-contamination between understandable and contaminated foundation for the duration of the brimming process, Disinfectants are fast endorsed due to the fact regarding the equal cause (Keskinen *et al.*, 2009).

Produces are generally ate up raw, subsequently packing the last foot over processing is regarded namely one over the integral rule points. This closing quadrant should remain controlled attentively and oftentimes in imitation of avoid re-contamination. It has been observed so 12.3% about whole foodborne outbreaks beside 1990 in accordance with 2007 had been associated according to fresh birth while 10% prompted by mistaken managing put up harvesting beside threshing floor and the rest had

been associated with growing, packing, transport and processing on fresh origin (Alliance For Food and Farming, 2010). In 2011, even was an occurrence composite after complete cantaloupe defiled with *L. monocytogenes* (Caron, 2011). It is speculated as the root motive over the outbreaks is the unsanitary condition about the pack shed. Moreover, the microorganisms have been also found sordid locations encompass the conveyor belt, drying location and flooring drain (Neuman, 2011). It is guessed as pathogens emerge as on an even keel partial in deep place (Neuman, 2011) and form biofilm and internalize within the outturn (Sapers, 2001).

### Meat and poultry Industry

With look on in accordance with the food surfaces, dense research received oversea by way of distinctive research workers bear shown the endearment over distinctive microorganisms to pool attempt surfaces (Notermans and Kampelmacher, 1974). It has been proven in imitation of stay related along slaughtering system however are additionally accountable because of pass contamination of uncontaminated carcasses (Anand *et al.*, 1989). *C. jejuni* with ease varieties biofilm over meals industry associated surfaces, is frequently associated together with poultry, and it has also been verified up to expectation chicken juice increases biofilm composition concerning meals industry associated tools (Brown *et al.*, 2014). *Salmonella* serovars able in conformity with shape biofilm about food-related surfaces, certain as much *S. enterica* serovar *Typhimurium* (*S. Typhimurium*), purpose a typhoid-like ailment as is usually not deadly in conformity with healthy individuals but is oftentimes the source on poultry and grain products.

*E. coli* has been well-read via deep researchers to investigate the “attachment and biofilm structure by way of *E. coli* O157:H7 at extraordinary temperatures, concerning a variety of food-contact surfaces encountered in pork processing” (Dourou *et al.*, 2011). In the outcomes beyond the study, the cells given in imitation of the floor accelerated over period also underneath low temperatures, who may want to end result beside the passage concerning cells towards the surface by way of flagellar-mediated liveliness and Brownian career (Van Houdt and Michiels, 2005). It has been seen as a paltry temperatures, microorganisms perform adhere and live to tell the tale about meals contact surfaces, and too increase in populace as like day progressed (Dourou *et al.*, 2011). Another essential factor influencing the attachment regarding *E. coli* O157:H7 is the emergence over sordid microorganisms over the surfaces (Castonguay *et al.*, 2006). Under deep investigations, such has been identified to that amount dust, surfaces, feces, chicken feed, and transportation on toughness stay chicken of manufacturing and technology devices are recognized in imitation of lie the vital risk factors within *Salmonella* infection (Marin *et al.*, 2009). The attachment of 25 *Salmonella* strains in accordance with IV exclusive substances (poly tetra

fluoro ethylene-also recognized so Teflon, stainless steel, rubber, and polyurethane), as are frequently aged of poultry industry, was once studied. Among those, *Salmonella* sofia isolates (except S1635 and S1636) can generally adhere between higher numbers in accordance with the distinctive surfaces than the other isolates. Besides *Salmonella spp.*, *Campylobacter spp.* are additionally generally located pathogens within chicken and chicken technology (Deming *et al.*, 1987). Temperature is a massive element influencing their uplift (Chan *et al.*, 2001). The attachment over *C. jejuni* in conformity with surfaces is facilitated through a pre-existing biofilm poultry it is vital in imitation of make stronger the rule about biofilms between poultry processing.

### Prevention and Control of Biofilms

Microbial attachment to (food-processing) surfaces is as an alternative quick process, and therefore, such is because almost applications now not viable in conformity with tidy and disinfect often sufficient to avoid attachment. Nevertheless, an adequate frequency on disinfection need to remain carefully determined according to avoid biofilm maturation and build-up over deep natural material (product residues), as may have an effect on the healthy status regarding the material and the availability concerning nutrients. The forward and most vital component in conformity with function is according to prevent biofilm structure with the aid of many times cleansing and disinfecting so in imitation of now not allow the cells to steadily attach (reversible attachment) after advice surfaces (Midelet *et al.*, 2004).

Meyer in 2003 recommended three unique strategies: disinfection “in time”, before biofilm develops, disinfection about biofilms the usage of thundering disinfectants, and embargo about attachment on microbes by way of choosing floor materials so functionate now not flourish endearment and via supplementing with nutrients. Many mean researchers hold accounted for the marketplace on antimicrobial merchandise between the floor materials themselves (Knetsch *et al.*, 2011). Pre-conditioning the surface together with a surfactant has also been stated in accordance with stop bacterial allegiance (Cpoultry, 2012).

The power regarding biofilms represents certain of the almost chronic challenges inside meals and industrial environments where the microbial communities are problematic. Sharma *et al.*, 2003 advocated in imitation of control the operating time among cleansing and sanitation according to forestall combined species biofilm composition within pasteurization lines on industrial and empiric dairy plants. Cleaning and sanitation regarding food-processing surfaces along quick intervals used to be proposed so an advantageous method in accordance with forestall and limit sporulation in biofilms made by means of vegetative *Bacillus subtilis* cells (Lindsay *et al.*, 2005). In a study concerning biofilm control, micro particles (CaCO<sub>3</sub>) coated together

with benzyl dimethyl dodecyl ammonium chloride have been determined in conformity with correctly inactivate biofilm construction (Ferreira *et al.*, 2011). Many others pointed out biofilm formation was inhibited by silver covering surfaces (Hashimoto, 2001). The control of biofilms represents one of the most persistent challenges within food and industrial environments where the microbial communities are problematic. Sharma *et al.*, 2003 recommended to control the operating time between cleaning and sanitation to prevent mixed species biofilm formation in pasteurization lines of commercial and experimental dairy plants. Cleaning and sanitation of food-processing surfaces with short intervals was proposed as an effective approach to prevent and limit sporulation in biofilms formed by vegetative *Bacillus subtilis* cells (Lindsay *et al.*, 2005).

### Removal of Biofilms

Adequate methods so break up and remove the production stock over the counsel floor namely well as current biofilm casting are important because the food processing industry (Zottola and Sasahara 1994). Partial elimination helps the reattachment about microorganism to the floor and build over a fresh biofilm even salvo the bacteria from the preceding biofilm had been defeated (Gibson *et al.*, 1999). A good cleansing process so much eliminates any meals residues and sordid compounds so might also raise microorganism proliferation and biofilm construction is particularly wonderful (Simoes *et al.*, 2010). Cleaning should remain received out into outside to that amount perform break-up and dissolve the EPS matrix associated along the biofilms so disinfectants do attain get entry to after the microorganism cells (Simoes *et al.*, 2006). Cleansing only allows the removal over approximately 90% about the microorganism beside the surfaces and does now not zap them. They might later re-attach in accordance with other surfaces and form a biofilm (Gram *et al.*, 2007). Walton in 2008 also succinct the primary principles regarding cleansing according to consider the physical characteristic and development concerning the equipment in accordance with stand cleaned, examine the nature regarding the ground after keep removed, select a detergent fantastic in conformity with the elimination concerning so much soil, carry the floor and the detergent together (at the appropriate temperature, underneath the appropriate prerequisites over glide and turbulence, at the right chemical concentration, because of the correct period of time), rinse abroad entire traces regarding detergent and soil, together with the goal of attaining the norm about cleanliness fantastic according to the responsibility for which the tools is destined in conformity with lie used, continually take on responsibilities cleansing as soon namely viable and ending concerning the production operation, and now necessary, undertake a disinfection and sterilization manner promptly before the tools is returned in conformity with processing and production responsibilities among discipline according to decrease the stage over microbiological contamination in conformity with some consistent. (Jacquelin *et al.*, 1994).

A wide range on chemical disinfectants is ancient within the food industry, who perform remain broken between distinctive groups in accordance in imitation of their anger concerning action: oxidizing marketers including chlorine-based compounds, hydrogen peroxide, ozone and PAA, surface-active compounds which include quaternary ammonium compounds and acid anionic compounds, and iodophores. The efficiency concerning disinfection is influenced by using pH, temperature, concentration, touch age and interfering natural materials like food particles and grime (Mosteller and Bishop 1993). Monolaurin (glycerol monolaurate) was once additionally determined in accordance with stay deadly according to *Listeria monocytogenes* at mean concentrations (Oh and Mar-shall, 1992). Very recently, cetylpyridinium chloride (CPC) used to be pointed out because of its utility into poultry processing enterprise so a nice Deputations for the discount of attached Salmonella concerning chicken pores and skin (Kim and Slavik, 1996). In the discipline about the effect on mechanical power about biofilms challenged by using one-of-a-kind chemicals that was once noted to that amount chemical sellers would operate together with the EPS complicated who would enhance the mechanical biofilm removal. The elimination quantity used to be substantially expanded after treating the biofilm including chemical sellers (Simões *et al.*, 2005). Studies have proven that half detergents are bactericidal and partial disinfectants may additionally too depolymerize EPS, thus enabling the dislodgment on biofilms from surfaces. Niemira and Solomon (2005) confirmed that ionizing painting was once equally and greater tremendous towards Salmonella spp. Biofilm cells than towards planktonic cells and may lie a beneficial sanitization therapy on a range of ingredients and counsel surfaces. Oulahal-Lagsir *et al.*, (2000) investigated the utilization regarding an ultrasonic apparatus of biofilm removal from impeccable steel and polypropylene surfaces. The combination regarding ultrasound and ethylene diamine tetraacetic acid (EDTA), and ultrasound and enzymes confirmed a higher faculty between putting off biofilms. The consequences have been greater promising in opposition to *S. aureus* than *E. coli* biofilms between the disciplines (Oulahal *et al.*, 2007). Enzymes can be a necessary alternative because of biofilm removal into the meals industry. However, EPS is a heterogenic cast so, between rule in conformity with degrade the complex, an aggregate about enzymes is required (Simoes *et al.*, 2010). Among every 1,4-glycosidic indenture cleaving amylases,  $\alpha$ -Amylases are often back due to the fact about theirs thermos stability; however, they will now not continue to be energetic because of long as like it are calcium metalloenzymes (Craigien *et al.*, 2011). Other enzymes consist of oxidandeductases, longevity pectin esterase, pectin lyase and pronase. Their blended use is superb for elimination of biofilms (Johansen *et al.*, 1997).

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