



Novel technologies to eradicate microbial biofilms

TEAGASC research has shown a number of new technologies with the potential to help fight microbial biofilms.

Microbial biofilms

Biofilms are significantly important in environmental, industrial, and clinical contexts. They are complex microbial multicellular communities formed on most biotic and abiotic surfaces, and are responsible for 80% of chronic human infections leading to hospitalisation and death. Microbial biofilm has been an active form of research since the first definition of biofilm in 1968 by Bill Costerton and co-workers. Bacterial biofilms (Figure 1) are defined as: "A microbial derived sessile community characterised by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription" (Donlan and Costerton, 2002).

They are one of the most persistent states for bacteria, which can withstand severe environmental conditions and have significantly elevated tolerance to antimicrobial agents. The resistance of the bacterial biofilms is mainly attributed to multiple mechanisms: inability of antimicrobial agents to penetrate the biofilm matrix; ability of the bacteria to survive starvation conditions; the physical and genetic heterogeneity of biofilm microenvironment; and, the presence of the non-active persister cells within the biofilm community. To counteract these challenges, there is need for an advanced disinfection technique/methodology that could help eradicate microbial biofilms and help control food- and healthcare-associated human infections. Non-thermal plasma (Figure 2) and acoustic airborne are emergent technologies, which have attracted attention for their enhanced microbial safety, non-thermal nature and fast processing. The Ultrafilm project will offer an innovative solution to eradicate biofilms, which could help develop a new sustainable technology to solve serious economic and health problems. The project's importance is emphasised in the current

climate of increasing tolerance and resistance of biofilms to standard decontamination methods and agents.

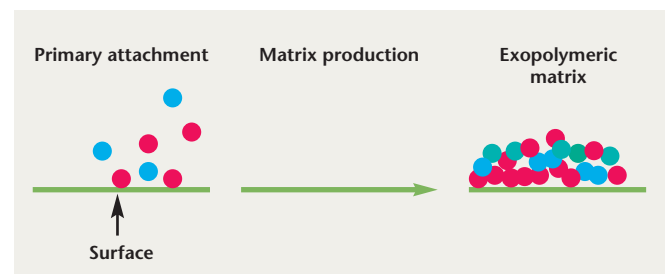


FIGURE 1: Bacterial biofilm on surface.

Atmospheric cold plasma

A wide range of applications of atmospheric cold plasma have emerged in the last two decades, which offer the possibility of inactivating a range of microbial populations and are the subject of intensive research. Atmospheric cold plasma generates a diverse array of reactive species, UV, high electric fields and charged particles, which individually or in synergy are capable of contributing significantly to bacterial inactivation. The results from bacterial biofilm populations inoculated on stainless steel coupons treated with high-voltage (240V) atmospheric cold plasma are presented in Figure 3. Plasma treatments for three minutes considerably reduced *Listeria innocua* and *Escherichia coli* by 2.8 and 3.6 Log₁₀ CFU/ml, respectively. Further, treatment for five and ten minutes reduced the bacterial population below the detection limit (1 Log₁₀ cfu/ml).

Ultrasound

Ultrasound is well known to have a significant effect on various processes in the food industry. Substantial literature is available

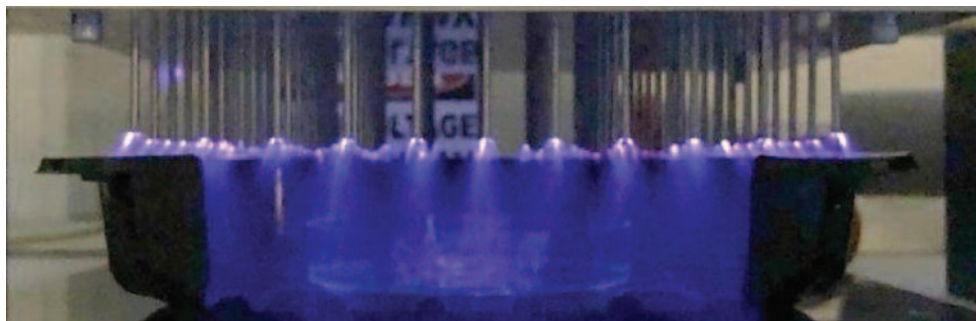


FIGURE 2: Plasma leap system, with the system voltage of 240V, 2500Hz frequency treatment, with biofilm coupon placed in a petri dish at the centre of the system.

describing the application of contact type ultrasound for drying, defoaming and decontamination of various microorganisms, whereas relatively little is known about the acoustic non-contact ultrasound and its application against microbial biofilms. Application of high ultrasonic stresses along with acoustic pressure is known to induce a rapid series of contractions and expansions in bacterial cells, leading to various morphological changes such as formation of pores, thinning and disruption of cell membranes. Airborne acoustics operated at 35kHz for 15 minutes has demonstrated 1.6-2.4 log reductions in bacterial biofilm populations formed on stainless steel coupons (Figure 3). Bacterial biofilms have complex structures and chemical compositions, which may vary among bacterial strains. It is necessary to optimise the treatment parameters to enhance treatment efficacy. A number of significant gaps, such as understanding the mechanism of action, characterisation and in-depth interaction to treat bacterial biofilms are elusive and still remain to be fully addressed. For biofilms, the study will focus on the synergistic effects of airborne acoustics in combination with cold plasma technologies to accelerate and eradicate microbial biofilms more effectively.

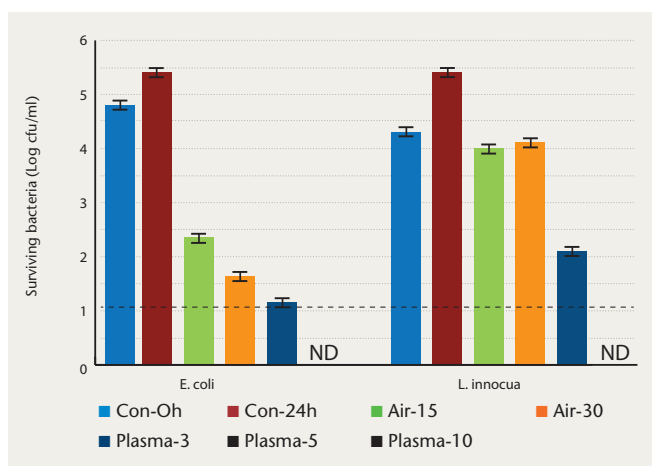


FIGURE 3: Surviving populations of *E. coli* and *L. innocua* 96h biofilm after airborne acoustics (15 and 30 minutes) and plasma treatments (three, five and ten minutes) estimated by colony count assay. The dotted line indicates a detection limit (1 Log₁₀ cfu/ml). ND: Not detected (below detection limit). Vertical lines on the column indicate standard deviation.

Conclusion

Many traditional food processing techniques are reaching their optimum performance, while consumer demands grow, and food and environmental regulations tighten. The unique physics and chemistry found with atmospheric cold plasma and airborne acoustics could offer agri-food industries a new technology-driven tool for supporting a sustainable food industry. Further studies on synergistic microbial effects using these technologies could help develop effective bio-decontamination technology to solve serious economic and health issues associated with biofilms.

Acknowledgment

This study has been supported by Science Foundation Ireland (SFI) under Grant Number 17/CDA/4653.

Reference

Donlan, R.M. and Costerton, J.W. (2002). 'Biofilms: survival mechanisms of clinically relevant microorganisms'. *Clinical Microbiology Reviews*, 15(2): 167-193.

Authors

Apurva Patange

Postdoctoral Research Fellow, Food Chemistry and Technology Department, Teagasc Food Research Centre, Ashtown, Dublin 15
Correspondence: apurva.patange@teagasc.ie

Kaye Burgess

Senior Research Officer, Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin 15

James Curtin

Professor and Head, School of Food Science and Environmental Health, Technological University Dublin

Brijesh Tiwari

Principal Research Officer, Food Chemistry and Technology Department, Teagasc Food Research Centre, Ashtown, Dublin 15

