



## Disparity of Biofilm Formation in *Salmonella typhimurium* on Glass and Wood Surfaces at Diverse Incubation Temperatures

Sani Aliyu<sup>1</sup> and Bello Usman<sup>2</sup>

<sup>1</sup>Department of Microbiology, Umaru Musa Yar'adua University Katsina, Katsina, PMB 2218 Katsina State.

<sup>2</sup>Department of Biochemistry Umaru Musa Yaradua University, PMB 2218 Katsina State Katsina  
Corresponding author email: [aliyu.sani@umyu.edu.ng](mailto:aliyu.sani@umyu.edu.ng); Phone number: 08068986670

### Abstract

*Salmonella Typhimurium* is a pathogenic gram-negative bacterium that commonly causes food-borne diseases known as gastroenteritis. Biofilm can exist on many surfaces in food preparation e.g glass, wood, plastic, metal and other food items. The aim of this study was to evaluate and quantify the biofilm formation of *S. typhimurium* on food contact surfaces (glass and wood) at different intervals (24, 48 and 72 hours) and different incubation temperatures (10, 28 and 37 °C). The inoculum used was  $10^8$  CFU ml<sup>-1</sup> of *S. typhimurium*. Formation of biofilm was quantified using beads vortex and agar plate count method. Biofilm of *S. typhimurium* was formed on both surfaces with preference more to glass surfaces, but all the densities of biofilm formed can cause infections. On Glass surfaces a total of  $1.62 \times 10^9$  CFU ml<sup>-1</sup> *S. typhimurium* was achieved as the highest biofilm density. Thirty seven degree was the most preferred incubation temperature of *S. typhimurium* cells, through which the highest biofilm cells were formed and 10°C incubation temperature supported the least biofilm densities. The result showed that, the biofilm formation of *S. typhimurium* was influenced by incubation temperatures and increases with time.

**Key words:** *Salmonella typhimurium*, biofilm, temperature, surface.

### INTRODUCTION

*Salmonella typhimurium* is an important socio-economic problem in several countries, mainly in developing countries where it is reported as the main agent responsible for the food-borne diseases outbreaks (Autune, *et al.*, 2003). It is one of the most problematic diseases in terms of public health all over the world because of its high endemicity, mainly because of the difficulty in controlling it and the significant morbidity rate (CDC, 2013). The agent is frequently transmitted to humans by means of food that are of animal's origin, such as meat, eggs, and milk (Donlan, 2002). Rapid and rampant transmission of *S. typhimurium* to humans, generally occurs by means of contaminated food and water. *S. typhimurium* has also become a global public health concern because of its resistance to a certain group of antibiotics and extensive host range. Although for it to be more virulent to date, factors responsible are not yet fully explain. It had been proven that, the disease occurs in many human cases worldwide every year and resulted to death (WHO, 2014).

*Salmonella* biofilms are formed on different surfaces in the environment that possibly lead to the constant spreading of this bacterium through contact (WHO, 2014). A biofilm is a gathering of microorganism in which cells stick

to one another on a surface. These colonies of cells always confine together within a matrix of self-created extracellular polymeric substance (EPS). This substances of biofilm which are alluded to as slime (in spite of the fact that not everything portrayed slime as biofilms), is a polymeric combination of polysaccharides, and protein (Donlan, 2002). Biofilms can form structures on living or nonliving surfaces and can be found common in nature, mechanically and in clinical settings. Microorganisms (Biofilm) can possibly survive in either of the two phenotypes: planktonic or sessile (Donlan, 2002). The sessile and phenotype are from an old or mature biofilm. While a planktonic are free living-coasting microorganism which sessile biofilm communities can offer climb to non-sessile cells, planktonic are microscopic organisms that can quickly duplicate and arrange it self under favourable condition (Donlan, 2002). Formation of biofilm by this strain (14028) of *S. typhimurium* on different surfaces need to be undertaken in order to understand its mechanisms, so that a possible solution can be put in place to the spread of this pathogenic bacteria (Donlan, 2002). The aim of this study was to observe the biofilm formation of *S. typhimurium* on glass and wood surfaces at different intervals and incubation temperatures.

**MATERIALS AND METHODS****Bacterial Strain and Culture Condition**

Strain (14028 ATCC) of *S. typhimurium* was used in this study which was obtained from the Microbiology Laboratory, Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA), Kuala Terengganu, Malaysia. It was sub cultured in tryptic soy agar (TSA) and incubated at 37°C.

**Preparation of Inoculum**

The overnight cultured of *S. typhimurium* was inoculated by taking a single colony into 5 ml tryptic soy broth (TSB) and incubated at 37°C for 1 h. The culture was then put into a conical flask containing 200 ml of TSB, and incubated at 37°C for 17 h inside a rotary shaker. The cells were harvested by centrifugation at 6000 x g, 5 minutes, 10°C. The pellet were washed twice with phosphate buffer saline (PBS) and re-suspended in 10 ml of TSB. One millilitre of the suspension was used to measure the optical density (OD) which was 0.1 at 600 nm and concluded to be 10<sup>8</sup> CFU ml<sup>-1</sup>. This suspension was used in this study as the inoculum (Van Merode *et al.*, 2006 and Kostaki *et al.*, 2012).

**Development of Biofilm On Glass Surfaces**

Glass slides were used for the development biofilm in this study. The glass surfaces were washed with commercial detergent to remove grease. The clean glass surfaces were sterilized by autoclave at 121°C for 15 minutes. Each piece of glass slide was placed in a petri dish and inoculated with 200 µl of the inoculum and allowed for 3 h. Thereafter, 10 ml of TSB was dispensed in the petri dish. Plates were incubated at (10, 28 and 37)°C and after 24, 48 and 72 h of incubation respectively, the set of surfaces were aseptically removed and washed twice with 2 ml of PBS in order to remove the unattached cells. The bacterial cells were quantified using beads vortexed and plate count methods (Giaouris and Nychas, 2006).

**Development of Biofilm On Wood Surfaces**

In this study wooden stick were used in biofilm development. The wood surfaces were

sterilized by autoclave at 121°C for 15 min. Each piece of wood was placed in petri dish and inoculated with 200 µl of the inoculum and allowed for 3 h. After that 10 ml of TSB was dispensed in the petri dish. Plates were incubated at 10, 28, and 37°C respectively. After 24, 48 and 72 hours of incubation, the set of surfaces were aseptically removed and washed twice with 2 ml of PBS in order to remove the unattached cells. The bacterial cells were again quantified using beads vortexed method and plates count methods (Giaouris and Nychas, 2006).

**Quantification of Biofilm**

The quantification of biofilm formed after incubation was conducted using bead vortexed method with some modification. Each surface was gently and aseptically removed from the petri dish after it reached the incubation time and carefully washed by rinsing through the pipetting of 10 ml PBS on each surface twice. Between the two rinse steps each surface was immersed on 5 ml of the PBS to remove the unattached cells. Each surface was put inside a 50 ml plastic tube containing 10 ml of TSB and 3 glass beads. The tubes were vortexed for 2 minutes each at maximum speed which detached all the biofilms on the surface. The detached bacterial cells in the TSB were subsequently enumerated by agar plating after 6-fold serial dilutions and the plates were incubated at 37°C for 24 h. Then the viable *S. typhimurium* colonies were taken as CFU ml<sup>-1</sup> (Giaouris and Nychas, 2006).

**RESULTS****Development of Biofilm On Surfaces**

Biofilmformation was achieved on glass and wood surfaces. The biofilm cell were quantified by beads vortex method and agar plated on nutrient agar (NA). After 24 hours colonies were counted and converted to colony forming units (CFU ml<sup>-1</sup>) and the result is shown in the table below:

Table 1: Biofilm of *S. typhimurium* after 24 hours

Temperatures (°C)	Surfaces	
	Glass (CFUml <sup>-1</sup> )	Wood (CFUml <sup>-1</sup> )
10	3.00x10 <sup>8</sup>	3.10x10 <sup>8</sup>
28	1.01x10 <sup>9</sup>	8.50x10 <sup>8</sup>
37	1.25x10 <sup>9</sup>	1.00x10 <sup>9</sup>

Table 2: Biofilm of *S. typhimurium* after 48 hours

Temperature (°C)	Surfaces	
	Glass (CFUml <sup>-1</sup> )	Wood (CFUml <sup>-1</sup> )
10	6.20x10 <sup>8</sup>	4.30x10 <sup>8</sup>
28	1.18x10 <sup>9</sup>	1.02x10 <sup>9</sup>
37	1.32x10 <sup>9</sup>	1.17x10 <sup>9</sup>

Table 3: Biofilm of *S. typhimurium* after 72 hours

Temperature (°C)	Surfaces	
	Glass (CFUml <sup>-1</sup> )	Wood (CFUml <sup>-1</sup> )
10	8.30x10 <sup>8</sup>	5.40x10 <sup>8</sup>
28	1.28x10 <sup>9</sup>	1.13x10 <sup>9</sup>
37	1.62x10 <sup>9</sup>	1.45x10 <sup>9</sup>

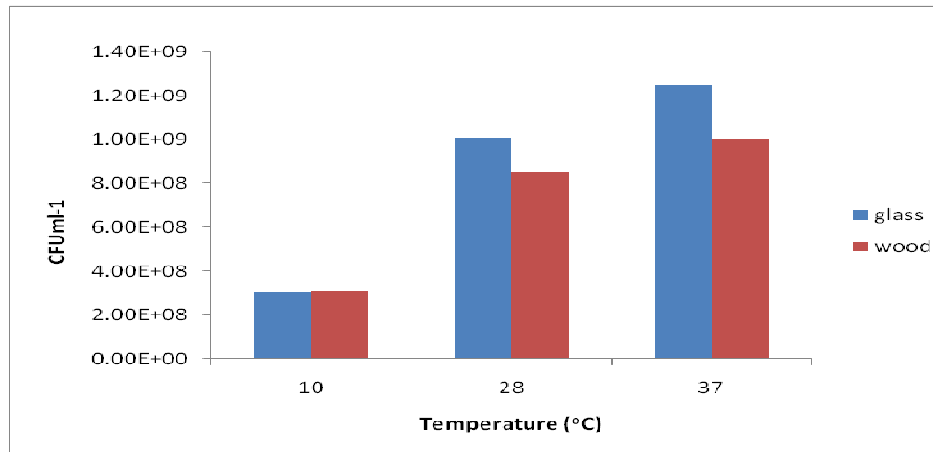


Figure 1: Biofilm Formation on Glass and Wood Surfaces at 24 h

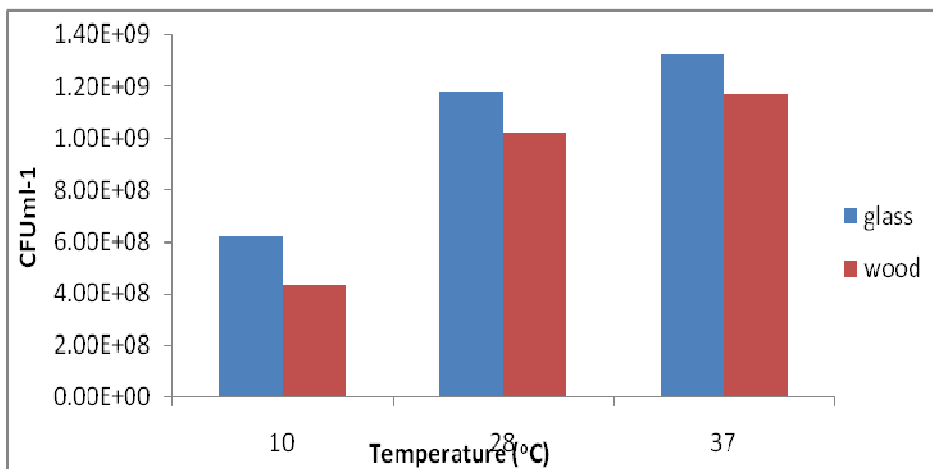


Figure 2: Biofilm Formation on Glass and Wood Surfaces at 48

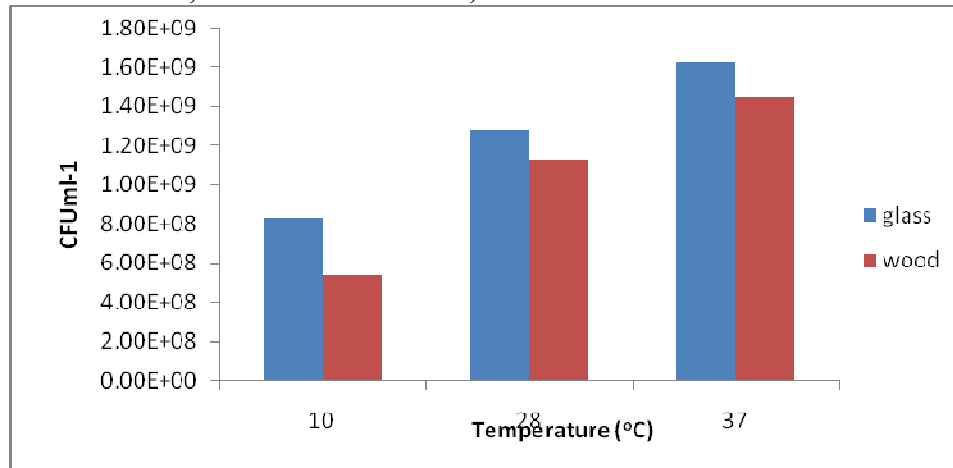


Figure 3: Biofilm Formation on Glass and Wood Surfaces at 72 h

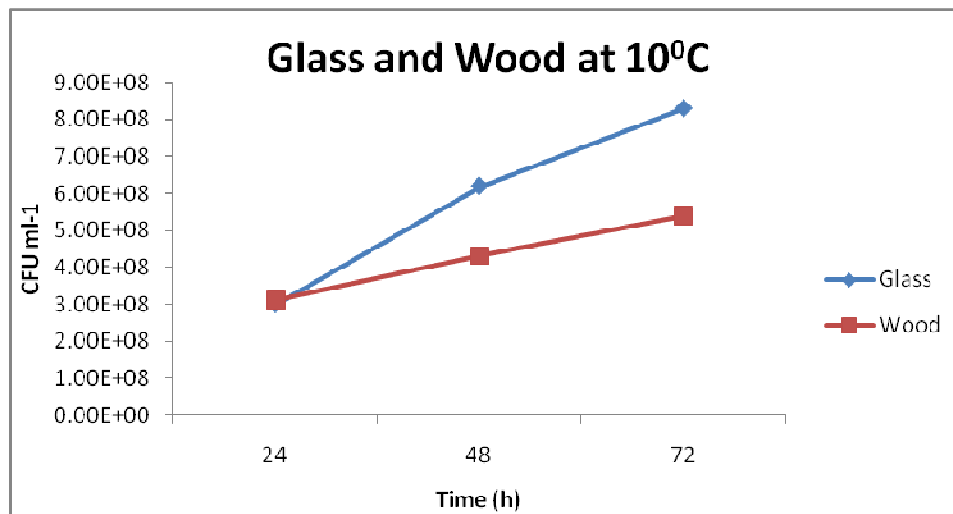


Figure 4: Biofilm formation and adhesion time on glass and wood Surfaces at 10°C

**DICUSSION**

Bacteria generally require a very suitable incubation temperature to grow. This bacterial cells usually modify themselves and the substratum within, in order to take advantage of the new conditions likely to favor them so that, they are able to initiate an exponential growth. Factors that favours bacterial growth particularly temperature usually affects the growth condition of bacteria in many ways. Therefore, in order to determine the quantity of bacterial cells under a set of incubation temperature time rate is very important (Zotolla and Sasahara 1994). The concentration of *S. typhimurium* used in this study was 10<sup>8</sup> (CFU ml<sup>-1</sup>), in order to form a mature biofilm and further explained that, for a mature biofilm development to occur the range of the concentration of bacterial inoculum should not be lower than 10<sup>6</sup> or 10<sup>7</sup> (CFU ml<sup>-1</sup>). It was observed in this study at different incubation

time and temperature the glass surfaces produced the maximum density of *S. typhimurium* biofilm cells with a single log number difference compared with the biofilm formed on wood surface (Van Houdt and Michiels 2010).

Development of biofilm on glass and wood surfaces at different incubation temperatures is shown in Table 1, 2 and 3. Formation of biofilm on both wood and glass surfaces by *S. typhimurium* at 24, 48 and 72 h indicated that, a higher biofilm was formed on wood surfaces than glass surfaces under 10°C incubation temperature however, at 28°C and 37°C incubation temperature glass surfaces were more favored for the biofilm formation of *S. typhimurium* than wood surfaces. Fig 1. shows that, after 24 h the highest biofilm was formed on glass surface in all the three sets of incubation temperatures used. This result outcome may be due to differences in surface

characteristics nature (the glass surface is smooth and wood is rough). The previous study also produced similar result where glass surfaces allowed more biofilm adherence than wood surfaces (Van Houdt and Michiels 2010).

At 48 h interval, biofilm formation on both wood and glass surfaces by *S. typhimurium* at all the three set of incubation temperatures was observed (Fig. 2). In all the three incubation temperatures glass surfaces revealed higher biofilm formation compared to wood surfaces. Similar result was observed at 24, 48, and 72 h of incubation under 10, 28 and 37°C incubation temperature respectively for both surfaces as indicated in Fig. 3. At all the three incubation temperatures used in this study, biofilm formation on glass surfaces was higher than on wood surfaces. The formation of biofilm of *S. typhimurium* was highest at 37°C at all intervals on both glass and wood surfaces. Fig. 4 shows the relationship between biofilm formation on both surfaces (glass and wood) with time. This study revealed that biofilm formation *S. typhimurium* increases with time at all three sets of incubation temperatures. In this study three different temperatures were used in the incubation of *S. typhimurium* (10, 28 and 37) °C. This was conducted in order to mimic the process of contamination which usually takes place in different foods industries and foods cafes. Foods or raw foods materials are kept in different levels of incubations that lead to the development of planktonic to a sessile community (biofilm) which will be directly or indirectly transmitted from one individual to another through contamination and possibly cause disease. All the surfaces (glass and wood) used on the biofilm formation of *S. typhimurium* indicated a remarkable biofilm growth at different incubation time and temperature. Formation of biofilm begins from the zero level of biofilm, which continue from little at zero hour to complex at different hours. This process follows the normal bacterial growth curve model and also allow us to understand that, each of this surfaces used initially there were very less biofilm cells, but after the inoculation, the cells take some time to get use to the new environment and also get adapted to all the environmental conditions. Then later cells get attach to each surface before starting the process of replications (Pui *et al.*, 2011).

At the early stage of attachment, bacterial cells coordinate themselves by the process of

communication called quorum sensing which lead them to the formation of polysaccharidessugars. These sugars help bacterial cells to become well fixed in the new environment or the new surface. Also this coordination exhibited by these bacteria is a physiological and physico-chemical process that was observed by Vander Waals Lewis acid-base and electric interactions (Van Houdt and Michiels 2010). The rate at which *S. typhimurium* attached to a surfaces at the initial state is related to the rate at which *S. typhimurium* coordinate and replicate to form more and more biofilm because, when incubation time increase under a fixed incubation temperature more bacteria cells have enough time and suitable condition to double and form biofilm on a surface (Ukuku and Feit, 2002). As long as the incubation time increases, the surface biofilm will continue to be formed until a mature biofilm is formed which will not be easy to be washed away.

In a study carried out by Pui *et al.*, (2011), it was observed that, the amount of attached bacterial cells formed on a plastic surface depend on incubation time and temperature.

The storage facilities like a fridge and a lot of kitchen utensils in the food industries and restaurant usually are made up of glass, wood, plastics, metals. Indeed all the materials used today in the kitchen are made of one of this surface and all those materials are capable of supporting or providing good incubation conditions for microbes that may either come along with the food materials or introduced from outside to form biofilm.

#### CONCLUSION

Conclusively biofilms can lead the public in to a persistent source of contamination which causes serious out break of diseases. From the outcome of this study we can conclude that, both wood and glass surfaces favor biofilms formations of *S. typhimurium* strain cells, and these surfaces are extensively available in our environment and hence through these surfaces transmission of disease like gastroenteritis and enteric fever can be possible. Therefore both the food industries and individuals have to pay more attention to the sanitation of the utensils and should consider a constant application of effective disinfectants on surfaces associated with food. The optimum incubation temperature and time for food preparation and packaging also need to be considered in order to prevent food borne diseases.

## REFERENCES

- Antunes, Patrícia, et al. (2003) "Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents." *International journal of food microbiology* 82.2 (: 97-103.)
- Center for Disease Control and Prevention (2013). Surveillance for food borne disease outbreaks- United States, 2009-2010. *MMWR Morb. Mortal. Wkly. Rep.* 62:41-47>>Medline.
- Donlan, R.M. (2002) Biofilms: microbial life on surfaces. *Emerging Infectious Diseases* 8, 881-890.
- Giaouris, D. and E. Nychas, 2006. The adherence of Salmonella enteritidis PT4 to stainless steel: The importance of the air-liquid interface and nutrient availability. *Food Microbiol.*, 23 (8): 747-752.
- Gilbert, P.,J.R. Das, M.V. Jones and D.G. Allison, (2001). Assessment of resistance towards biocides following the attachment of microorganisms to and growth on, surfaces. *J. Applied Microbiol.*, 91:248-254.
- Kostaki, M., Chorianopoulos, N., Braxou, E., Nychas, G. J., & Giaouris, E. (2012). Differential biofilm formation and chemical disinfection resistance of sessile cells of *Listeria monocytogenes* strains under monospecies and dual-species (with *Salmonella enterica*) conditions. *Applied and environmental microbiology*, 78(8), 2586-2595.
- Pui, C. H., Carroll, W. L., Meshinchi, S., & Arceci, R. J. (2011). Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *Journal of Clinical Oncology*, 29(5), 551-565.
- Ukuku. D. O.. & Feit. W. (2002). Behavior of *Listeria ,nonocvlogoes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *A Journal of rood Protection.* 65. 924-930.
- Van Houdt, R., and Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology*, 109(4), 1117-1131.
- World Health Organization 2014.
- Zottola, E. A., & Sasahara, K. C. (1994). Microbial biofilms in the food processing industry—Should they be a concern?. *International journal of food microbiology*, 23(2), 125-148.