Review

Interaction between Biofilm Formation, Surface Material and Cleanability Considering Different Materials Used in Pig Facilities—An Overview

Erika Yukari Nakanishi [1,2,*], Joahnn H. Palacios [2], Stéphane Godbout [1,2] and Sébastien Fournel [1]

1   Department of Soil and Agri-Food Engineering, Faculty of Agriculture and Food Sciences, Laval University, 2425, Rue de l'Agriculture, Quebec City, QC G1V 0A6, Canada; stephane.godbout@irda.qc.ca (S.G.); sebastien.fournel@fsaa.ulaval.ca (S.F.)
2   Research and Development Institute for the Agri-Environment, 2700, Rue Einstein, Quebec City, QC G1P 3W8, Canada; joahnn.palacios@irda.qc.ca
*   Correspondence: erika-yukari.nakanishi.1@ulaval.ca

Abstract: Sometimes the contamination in pig facilities can persist even after the washing and disinfection procedure. Some factors could influence this persistence, such as bacteria type, biofilm formation, material type and washing parameters. Therefore, this review summarizes how the type of surface can influence bacteria colonization and how the washing procedure can impact sanitary aspects, considering the different materials used in pig facilities. Studies have shown that biofilm formation on the surface of different materials is a complex system influenced by environmental conditions and the characteristics of each material's surface and group of bacteria. These parameters, along with the washing parameters, are the main factors having an impact on the removal or persistence of biofilm in pig facilities even after the cleaning and disinfection processes. Some options are available for proper removal of biofilms, such as chemical treatments (i.e., detergent application), the use of hot water (which is indicated for some materials) and a longer washing time.

1. Introduction

Nowadays, beef, pork and chicken are the main sources of animal protein consumed on a global scale. Typically, as for many livestock production systems, pigs are raised under controlled environments (i.e., closed facilities) and high animal density. This combination is one of the main reasons leading to massive deaths of animals when a herd is stricken by a disease. The economic consequences can be disastrous, as they also lead to slower animal growth as well as to higher costs associated with the treatment and management of sick animals [1,2].

Several pathogens can cause swine diseases worldwide, such as bacteria including *Streptococcus* ssp., *Salmonella* spp., *Campylobacter* spp. and *Escherichia* ssp. [3–5] or viruses including *Coronaviridae* spp. and *Kobuvirus* spp. [2,6]. The most common diseases in pigs caused by bacteria are respiratory and enteric infections. The most prominent bacteria causing these infections are *Mycoplasma hyopneumoniae*, with secondary bacterial bronchopneumonia, which is responsible for the main cases of pneumonia, and *Escherichia coli*, resulting in neonatal and post-weaning diarrhea in pigs [7]. Many of these microorganisms can also cause diseases in humans because they are often transmitted through contaminated food [5].

An example of a severe outbreak of diarrhea and deaths in pigs was noticed in 2019 in China, caused by *Escherichia coli*, which resulted in huge economic losses. These strains of bacteria demonstrated resistance to several antibiotics, such as cefalexin, cefazolin, amikacin, gentamicin, penicillin, kanamycin, ampicillin, piperacillin, minocycline, tetracycline, doxycycline, streptomycin, lincomycin, vancomycin and erythromycin [8]. In

addition, *Porcine epidemic diarrhea virus* (PEDV) is another disease reported worldwide, which causes diarrhea and piglet deaths, consequently resulting in economic loss. It was first reported in the U.K. in 1971, and later in other countries in Europe, Asia, the USA and, in 2014, in Canada. The virus entry in Canadian herds may have occurred through contaminated food [6,9]. In 2019, when the *African swine fever* (ASF) outbreaks occurred mainly in China, more than 1 million pigs had been culled by 23 April 2019, which resulted in a decline of 18.8% of the hog inventory and 21% of the breeding sows of the country [10]. The ASF virus showed great capacity to spread on a global scale, affecting several countries in Europe, Africa and Asia [11]. The main factor that allowed the virus to spread to other continents was the informal shipment of infected pork products [12].

Different drugs can be found on the market to treat these diseases, and the most widely used are antibiotics; in the swine industry, penicillin and tetracyclines are the most common. However, with the case of an indiscriminate antibiotics use to prevent and control diseases, it could cause continuous antibiotics exposure to microorganisms. Consequently, this could trigger a substantial selection pressure resulting in the emergence of resistant strains to these agents (i.e., antimicrobial resistance—AMR) [13]. For example, the strains of *E. coli* [8] and methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 [14] have been reported in the literature as highly resistant strains to antibiotics. In addition, for certain diseases, such as ASF, there are no vaccines or treatments available. Therefore, the only measure to contain and cease contamination is prevention and control, such as the implementation of appropriate surveillance and strict sanitary measures [11,12].

Since pig facilities present a high microbial charge, control measures such as washing procedures are used to prevent most of the direct and indirect contamination between animals. However, pig producers do not always pay enough attention to the efficiency of the washing procedures.

Bacteria are able to resist and attach on surfaces due to the formation of a complex structure known as biofilm [15], making it difficult to achieve proper washing. Consequently, contamination can persist. In addition, some pathogens can survive for days and even for months in the environment as well as on the surface of material such as plastics, metals, objects and, fabrics [4,16–18].

The means used by microorganisms to survive and persist will depend on the type of surface/material. In addition, washing procedure efficiency can be influenced by material type. However, as previously mentioned, until now not much attention has been given to investigating the influence of the washing procedure on the persistence of microorganisms on the materials' surface livestock facilities. Therefore, the main goal of this review was to study the interaction between biofilm, surface material and cleanability on sanitation, while considering different materials usually employed in pig facilities. To achieve this, two sub-topics were investigated: (i) the influence of the material surface on bacterial colonization and (ii) the impact of the washing procedures on the sanitary conditions of the material, i.e., capability of removing the biofilm on the surface material.

This review is divided in three parts, the first introduces the main materials used in pig facilities. The second presents the bacteria colonization process and how different surfaces can influence the colonization process and even the bacteria's persistence. In the last part, this review explores the impact of washing procedures on the sanitary conditions of the materials.

To conduct this review, the principle of content analysis was used [19]. This method focuses on accurately making valid inferences on the collected data with the aim of disclosing central aspects of previous studies. The search, selection and collection of the data used peer-reviewed articles, pertinent books and technical reports available on Science Direct and Engineering Village databases.

2. Material Types in Pig Facilities

In industrialized countries, pig production is realized in different types of facilities, typically organized according to the animals' life stage [20,21]: (i) gestation; (ii) farrowing;

(iii) nursery and (iv) grow-finish. In breeding and gestation buildings, sows are bred and kept individually in stalls or in group pen for the gestation period (16–20 weeks). Farrowing facilities house lactating sows and their offspring until the piglets are weaned (3–4 weeks of age). The weaned piglets are kept in nursery barns for about 6–9 weeks in pens comprising 20–30 animals. In grow-finish barns, pigs are kept in pens containing 30 to 50 animals until they reach their market weight (16–18 weeks).

Each pig facility needs different types of construction materials for the building itself and for the equipment and components (crates, partition, feeders, etc.). The choice of material used inside the building depends on its purpose (e.g., wall, floor, crates) and particularly on whether or not the animals are in contact with it [22]. Firstly, the material must have physical, mechanical and thermal properties that meet the standard requirements for their use, such as floor, pen partitions, walls or other [23]. The surface must be easily cleanable and must not get damaged by the cleaning methods used [22–24]. Moreover, the material should not cause injury to the animals [25]. Table 1 presents the main materials used for each pig facility type, with a focus on those materials that are in direct contact with the animals or that undergo cleaning during the washing process.

Table 1. Main indoor material according to its location, equipment or component composition for each type of swine facility.

Type of Facility	Location/Equipment/Component	Material	Reference
Gestation	Gestation crate	Galvanized steel	[26]
	Pens' slatted floor	Precast concrete slats	[23,24]
Farrowing	Slatted floor in farrowing crates	Plastisol or high-density polyethylene (HDPE)	[26]
		Plastic-coated steel slats or plastic slotted flooring	[24]
		Rough expanded metal, plastic coated, fiberglass reinforced t-slats, welded wire, woven wire or cast-iron grid	[23,26]
	Central part of the slatted floor in farrowing crates	Cast-iron grid	[23,26]
	Farrowing crates (partition)	Galvanized steel	[26]
		Plywood, steel or concrete panels	[23]
Nursery	Slatted floor in farrowing crates	Plastisol or high-density polyethylene (HDPE)	[26]
		Plastic-coated steel slats or plastic slotted flooring	[24]
		Rough expanded metal, plastic coated, molded plastics, perforated metal planks, fiberglass reinforced t-slats, flattened expanded metal, woven wire or cast-iron grid	[23]
Grow-Finish	Walls	Concrete panels or blockwork or pre-stressed concrete panels	[25]
	Solid floor	Concrete	
	Slatted floor	Precast concrete products (BS EN 12737:2004+A1:2007)	[23–25]
	Door	Polyvinyl chloride (PVC)	[26]
		PVC or galvanized steel	
	Pen partition	Plastic panel bolted to metal (stainless steel) posts or concrete blocks or concrete panel	[25]
		Steel fence or concrete (cast in place or prefabricated panel)	[23]
		High-density polyethylene (HDPE)	[24]
	Feeder	Black plastic	[25]

The materials can be separated into four main groups: cement-based materials, plastic, metals and engineered wood materials [26]. Cement-based materials such as concrete, cement or brick with different qualities are used in large scale for walls, plain floors, slatted floors or even partitions in the pig facilities. Plastics are used for slatted floors, doors, feeders, partitions, walls and ceilings.

3. Influence of the Surface Type in the Bacteria Colonization

3.1. Biofilm and Formation Phases

The characterization of the material's surface helps to provide a better understanding of how microorganisms interact and colonize in different materials, i.e., bio-receptivity of the material [27]. Bacterial colonization on a surface is a complex process that depends on the interplay of three main factors: (i) the material surface characteristics, such as specific surface roughness, surface porosity or topography, chemical composition and wettability or surface-free energy (SFE); (ii) the bacterial properties, i.e., surface charge, surface energy, shape and size, appendages, adhesins, etc. and (iii) the environmental conditions, such as the flow conditions around the surface (temperature, humidity, viscosity, hydrodynamics, pH, surface tension, ionic strength and dielectric properties) [22,27–31].

The pathogenicity of some bacteria, such as *S. epidermidis*, is attributed to its ability to adhere to surfaces, form a biofilm and remain on the surface [30]. The adhesion of micro-organisms to surfaces is an important parameter for assessing cleanability and thus minimizing transmission to animals in pig facilities [22]. The biofilms provide structural stability and protection from stressful conditions. The biofilms are composed of complex microbial structures, derived from extracellular polymeric substance (EPS). EPSs are formed mainly of proteins and carbohydrates. Biofilm formation depends mainly on microorganism type and density, temperature, pH, nutrient availability and type of materials [32].

To the best of our knowledge, no research has linked biofilm stages to ongoing infections in pig facilities. However, the phases and factors leading to biofilm formation are presented below. These do not explain any ongoing infection in pig facilities, but they describe at which stage the material plays a role in the bacterial attachment and the complexity of the biofilm that allow bacteria to remain on the surface even after cleaning and disinfection processes.

Figure 1 shows a schematic model of the phases and factors leading to biofilm formation. The first step in the process of bacterial adhesion to a biotic or abiotic surface consists of the initial attraction of the cells to the surface. The initial attraction involves locating, approaching, and sensing the proximity of the surface, followed by attachment [30]. This first step of bacteria attachment is reversible primarily through so-called non-specific interactions associated with physicochemical properties [33]. This early stage of biofilm formation is closely related to the properties of materials, which means that surface roughness, surface-free energy (SFE) and chemical composition have a large role in bacteria attachment.

The physical properties of the attachment process are a function of the distance and SFE. The main forces in play are Van der Waals attraction forces, the effect of surface electrostatic charge, hydrophobic interactions, Brownian motion and gravitational forces. Chemical interactions are linked to the surface-associated chemical gradients (chemotaxis) and adhesion sites' surface-bound chemoattractants ("haptotaxis"). These gradients are formed from the presence of various chemical stimuli or the degradation of certain surface components [28–30,34,35].
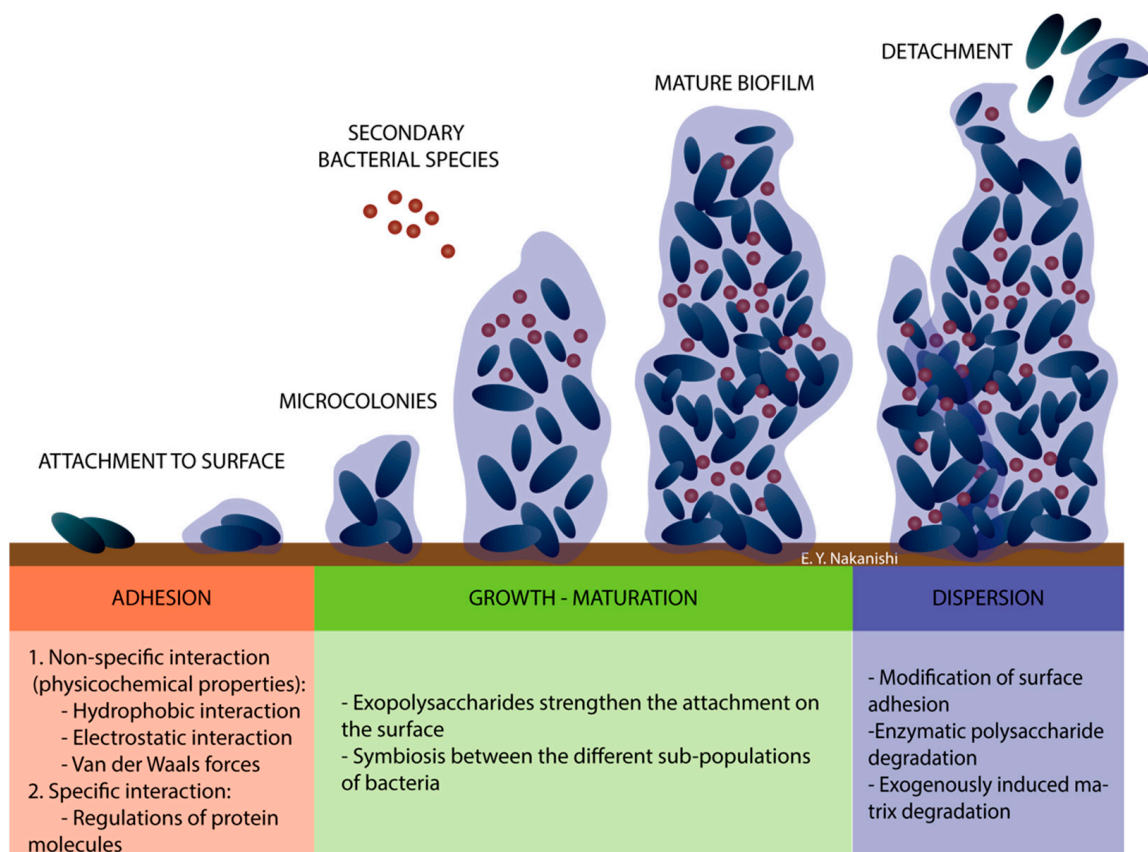
Figure 1. Schematic model of the phases and factors involved in biofilm formation.

Bacterial attachment makes adhesion possible in the second step of biofilm formation. Bacteria attach themselves irreversibly by specific interactions that involve distinct recognition, such as the regulation of protein molecule binding on the interacting surfaces [33,35,36]. This suggests a firmer adhesion of the bacteria to a surface by the selective-bridging function of bacterial surface structures, which include lipopolysaccharides, capsules, fimbriae, or pili and slime [30,37].

After the adhesion process, the biofilm is formed, i.e., the bacteria multiply rapidly and achieve a high cell density and grow in structures called microcolonies. These microcolonies produce one or more exopolysaccharides self-secreted by bacteria, helping to strengthen the attachment to the surface [33,38]. The biofilm maturation allows the transport of nutrients and metabolic waste, oxygenation, communication between the different sub-populations of bacteria within the biofilm and the synchronization of their behavior by the signal molecule exchanges [33]. In addition, mature biofilms can provide extra protection for the bacteria against environmental stressors, such as desiccation or UV rays, disinfectant or sanitizing agents as well as antibiotics [39–41]. This is possible due to the exopolymer matrix that prevents the diffusion of harmful chemical and environmental influences inside the biofilm [39].

However, many factors such as cellular density, the lack of nutrients, oxygen, extracellular signaling molecules, and environmental conditions can incite the detachment and dispersion process. The bacteria or specific subpopulation detached will spread to new locations [30,33,42,43]. An extensive review of the biofilm dispersion process by Rumbaugh and Sauer [43] considered the main mechanisms to be: (i) modification of surface adhesions; (ii) enzymatic polysaccharide degradation and (iii) exogenously induced matrix degradation.

3.2. Surface Roughness Properties

3.2.1. Importance of Surface Roughness in Pig Facilities

In pig facilities, surface roughness is an important selection criterion for materials, mainly for floors. Rough floors are more abrasive to the feet and knees and thus can cause injuries to nursing pigs. Materials with high porosity or roughness can also retain moisture and manure, which will subsequently facilitate bacteria growth and make cleaning more difficult [24]. Materials with smooth surfaces can also cause hoof and leg injuries, as they can be slippery [23,24]. However, smooth surfaces drain and dry more rapidly, facilitating cleaning and disinfection.

Therefore, surface roughness significantly impacts bacterial attachment and subsequent biofilm formation in pig facilities. The pits, grooves and irregularities of rougher surfaces provide protection to the bacteria from removal forces, i.e., cleaning processes, and provide a larger global area available for colonization [28]. In fact, attachment can be established more easily on rough materials where bacteria are sheltered [36].

3.2.2. Surface Roughness of Materials

Different techniques have been used to analyze specific surface roughness and surface porosity, such as scanning electron microscopy (SEM), atomic force microscopy (AFM), lateral force microscopy (LFM), optical microscopy, coherence scanning interferometry (CSI) and confocal microscopy. The standard roughness parameters can be established by bidimensional (R) or tridimensional (S) analysis. The roughness parameters most used are the arithmetic mean of roughness (Ra) and the mean square deviation of roughness (Rq) [44,45]. Ra is defined as the average deviation of the profile in relation to its mean line and Rq is considered as an amplitude parameter that describes the variation of the surface topography in relation to an average plane [46,47]. Therefore, in order to take into account the location and the spacing between peaks and valleys, other roughness parameters can be used, such as the mean peak height (Rpm), the mean valley depth (Rvm), the mean peak-to-valley height (Rz), the ten points height (Rz) and the maximum valley depth (Rv) [45].

Table 2 presents the roughness parameter values obtained in the literature for the four main groups of materials found within a pig facility (cement-based materials, plastic, metals and engineered wood materials). Cement-based materials, in general, present a complex structure with a high surface roughness. Engineered wood materials, like cement, have a high surface roughness mainly due to the anatomic characteristic of the wood fibers [48–50]. Consequently, ordinary and prefabricated concrete as well as plywood and particleboard are more difficult to clean and keep in adequate sanitary condition.

In the case of the HDPE-coated steel slats found in pig facilities, the surface roughness is higher compared to other plastic materials. This comes from the thermal spray coating used in the production process. The coating is formed by the deposition of fine particles in a molten or semi-molten condition or even in fully solid state on the metal surface [51,52]. Overall, plastics and metals have a smoother roughness profile than the cement-based and engineered wood materials. Consequently, plastics and metals materials are easier to clean and maintain.

Table 2. Roughness parameters values of cement-based materials, plastics, metals and engineered wood materials.

Group	Material	Ra (μm)	Rq (nm)	Rp (μm)	Rv (μm)	Rz (μm)	Reference
Cement	Mortar	4.32	-	10.5	10.16	-	[53]
	Mortar + hydrophobization	1.0–2.5	-	3.0–5.5	4.2–7.9	-	
	Lightweight mortar	4.29	-	10.3	10.6	-	[54]
Plastics	High-density polyethylene (HDPE)	0.02	24.2	-	-	-	[55]
	Ethylene vinyl acetate (EVA)/poly-vinylidene dichloride film	-	13.86	-	-	-	[56]
	Polyvinyl chloride (PVC)	0.017	27	-	-	-	[57]
	HDPE-coated steel (by thermal spraying)	0.22 ± 0.02	-	-	-	-	[51]
Metals	316 Stainless	0.26	8	-	-	-	[31,58]
	Stainless steel 304	-	19	-	-	-	[59]
Engineered wood materials	Particleboard	7.33–9.14	-	-	-	51–55	[60]
	Medium density fiberboard (MDF)	2.57–3.81	-	-	-	27–34	[48,60]
	Plywood	4.30–8.59	-	-	-	-	[61]

Ra—average roughness; Rq—mean square deviation of roughness; Rp—maximum peak height; Rv—maximum valley depth; Rz—mean peak-to-valley high.

3.2.3. The Impact of Roughness on Bacteria Colonization

In the first step of biofilm formation, the manner in which bacteria locate, approach and sense the proximity of the surface or, in other words, how the bacteria view the surface, can be influenced by the roughness scale and the roughness geometry. The geometric roughness parameters provide the link between the microorganisms' size and the roughness geometry [27].

Apedo et al. [27] studied the geometric roughness parameters of cement pastes with CSI analysis and the "window resizing" technique to calculate the sampling and the convolution. Sampling is the parameter related to the measuring tool, while the convolution is the parameter related to the roughness viewed by a bacterium of a given size, as shown in Figure 2. When the convolution sphere (probe radius, Rp) is ≤ 8, the surface measured with the sampling is the same for the convolution (Figure 2a), i.e., the perception of bacteria occurs on all surfaces. However, when the Rp > 8, the discrepancy between sampling and convolution becomes large enough because many parts of the sampled surface become inaccessible to the convolution sphere (Figure 2b). Furthermore, the surface viewed by the bacteria (convolution) is substantially smaller than the surface viewed by the measuring tool (sampling). Therefore, in these inaccessible parts, bacterial attachment is impossible.
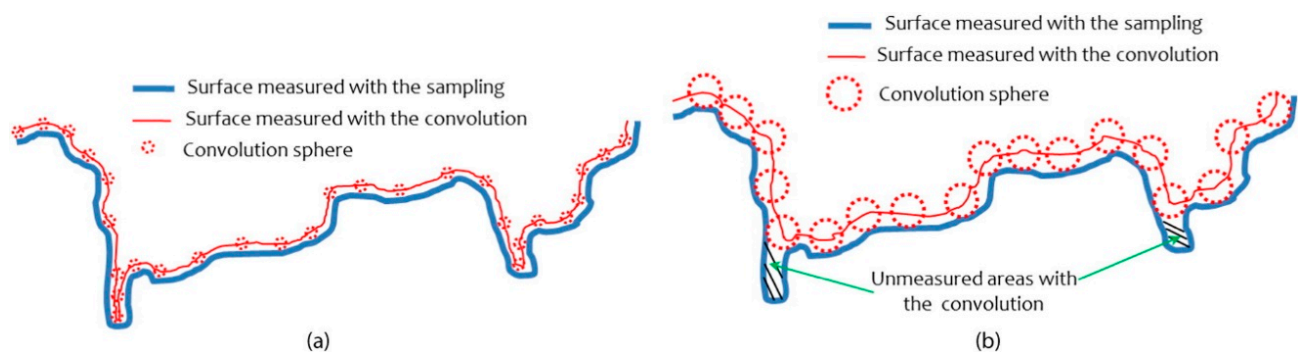

Figure 2. Measured surfaces using CSI (sampling and convolution): (**a**) Rp (probe radius) ≤ 8 (**b**) Rp > 8 [27].

Bacteria attachment factors (e.g., hydrodynamics, surface wettability, air entrapment, topography-induced cell ordering and segregation, physicochemical forces, cell membrane deformation and chemical gradient) could be influenced by the roughness scale, which includes the micrometric and nanometric scales. An extensive review on the influence of the roughness scale in the factors of attachment by Cheng et al. [29] considered for example that a surface with large pores presents a lower energy barrier for a bacterium to overcome in order to attach compared with the smaller pore surfaces, as the bacteria have a lower contact with the surface. In other words, there is an electrostatic and an acid–base interaction having lower forces against bacteria. Another example is the film's conditioning and chemical gradients, which could affect attachment: (i) masking or changing surface properties; (ii) modifying the surface topography and (iii) providing sites for specific bacteria–surface interactions. Therefore, as presented by the authors, the film's conditioning and chemical gradient factors have more effects on nanoscale roughness when compared to microscale.

3.3. Surface-Free Energy Properties

3.3.1. Surface-Free Energy Materials

Materials with surface roughness in the nanoscale, such as some plastics and metals, present pits, grooves and irregularities inaccessible to the attachment of bacteria. For these types of surfaces, physical properties such as SFE can influence and improve bacterial attachment [28].

Surface-free energy has a definite impact on the wettability of materials. Surface-free energy is characterized by the interaction between the forces of cohesion and adhesion that determine whether or not wetting occurs [28]. This property is strongly dependent on the chemical composition of the surface and its roughness [57]. It can be established by contact angle measurement of the angle of the drop formed on the surface. Surface-free energy can be calculated by the three liquid phase methods based on the VanOss–Chaundhury–Good theory of wettability. The smaller the angle of the drop formed on the surface (less than 90°), the greater the wettability or SFE, i.e., it is considered a hydrophilic surface. On the other hand, an angle greater or equal to 90° represents a hydrophobic surface [62], as shown in Figure 3.
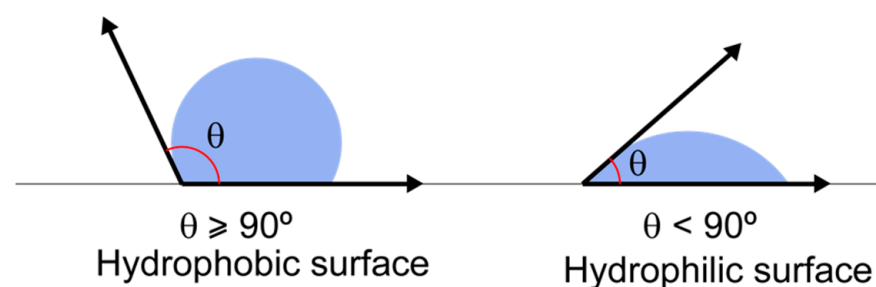

θ ≥ 90°
Hydrophobic surface

θ < 90°
Hydrophilic surface

Figure 3. Illustration of the angle measurement (θ) formed by a drop on hydrophobic and hydrophilic surfaces.

Cement-based materials present high surface-free energy, which means that cement is a hydrophilic material. Plastics exhibit different surface characteristics; for example, HPDE is considered a hydrophobic material, while PVC is considered a hydrophilic material [57,63]. Stainless surfaces are inherently hydrophilic with a nearly 90° contact angle [59] (Table 3).

Table 3. Contact angle and surface-free energy values of cement-based materials, plastics, metals and engineered wood materials.

Group	Material	CA (°)	SFE (mJ·m^{-2})	Reference
Cement base	Mortar	39.7 [a]	59.64	[53]
	Mortar + hydrophobization	103–113 [a]	15–21	
	Lightweight mortar	12.1 [a]; 29.5 [b]	81.1	[54]
	Lightweight mortar + hydrophobization	38–107 [a]; 42–98 [b]	18–70	
Plastics	High-density polyethylene (HDPE)	97 [a]; 46 [b]; 60 [c]	37	[63]
	Ethylene vinyl acetate (EVA)/poly-vinylidene dichloride film	88 [a]	-	[56]
	Polyvinyl chloride (PVC)	66 [a]	-	[57]
Metals	Stainless steel 316	86.8	26.9	[31,35]
	Stainless steel 304	80–85		[59]
Engineered wood materials	Plywood	33.8 [a]		[61]

CA—contact angle with [a] distilled/deionized water, [b] diiodomethane and [c] ethylene glycol; SFE—surface-free energy.

3.3.2. Influence of SFE on Bacterial Colonization

Microbial surface hydrophobicity has been noted to be a dominant factor in influencing adhesion on surfaces [35]. Generally, hydrophobic surfaces, or surfaces with a low SFE, are preferred for attachment by a hydrophobic bacterium or a bacterium with a low SFE. However, bacteria with hydrophilic properties (high SFE) prefer to adhere to hydrophilic surfaces or surfaces with high SFE [28,38]. For example, *Staphylococcus aureus* presents a hydrophilic character, and thus this bacterium favors metal alloys (titanium-aluminum (6%) vanadium (4%)), over polymers (ultra-high molecular weight polyethylene) that present a more hydrophobic surface [64]. On the other hand, *Mycobacterium avium* is characterized by its outermost surface containing glycopeptidolipids and hydrophobic mycolic acids that give it a hydrophobic property [65]. Norton et al. [66] evaluated higher levels of *M. avium* biofilm on iron and galvanized pipe surfaces compared with copper or PVC surfaces.

According to Daffonchio et al. [67], bacteria are considered hydrophobic when presenting a contact angle greater than 45° and hydrophilic when presenting a contact angle less than 45°. Therefore, most bacteria shown in Table 4 have a contact angle lower than 45°, which means that they have a hydrophilic character, except *Clostridium proteolyticum* and *Pseudomonas aeruginosa* that present a hydrophobic character (CA > 45°). Considering the materials used in pig facilities (Table 3), these bacteria with a hydrophilic property will prefer to colonize first on the cement and engineered wood materials and then on polyvinyl chloride (PVC), stainless steel and high-density polyethylene (HDPE).

Moreover, the intensity of adhesion forces and the accumulation velocity in the colonization process are influenced by bacterial hydrophobicity [31,35]. Harimawan et al. [35] explored the nature of the adhesion mechanisms of two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Massilia timoniae*) and one Gram-positive bacteria (*Bacillus subtilis*) on stainless steel. The authors observed that the Gram-negative bacteria presented higher adhesion forces compared with the Gram-positive bacteria. The main reason for this is because *P. aeruginosa* (CA = 47.9°) and *M. timonae* (CA = 39.6°) have a higher hydrophobicity than *B. subtilis* (CA = 31.1°), as their contact angles (*P. aeruginosa* and *M.timonae*) are closer to the hydrophobicity of the stainless steel, with a contact angle of 86.8°. Further, in a study carried out by Mueller et al. [31], the authors demonstrated that *P. aeruginosa* is more hydrophobic than *P. fluorescens*, and consequently the process of cellular accumulation of *P. aeruginosa* was 5 times faster than *P. fluorescens* on stainless steel, copper, silicon and glass materials. It shows that when comparing bacteria with different hydrophobicity on the same surface, the cell accumulation is faster when the bacteria's hydrophobicity is closer to the one of the surfaces, which is P. aeruginosa in this case.

Table 4. Contact angle and surface energy values of several bacteria.

Classification	Bacteria	CA (°)	γ_{BV} (mN/m)	Reference
Gram-positive aerobic to facultatively anaerobic cocci	*Staphylococcus aureus*	25.3 ± 2.9 [a]	-	[68]
		18.5–26.4 [b]	69.1 ± 0.6	[69,70]
	Staphylococcus epidermidis	18–33 [a]	-	[71]
		23.4 ± 0.5 [b]	67.1 ± 0.3	[70]
	Enterococcus faecalis ATCC 6055	12.7 ± 1.1 [b]	73	[67]
	Streptococcus thermophilus ST69	28.0 ± 1.5 [b]	64	
Gram-positive aerobic to microaerophilic non-spore-forming bacilli	*Listeria monocytogenes*	26.1 ± 1.2 [b]	66.3 ± 0.6	[70]
Gram-positive aerobic spore-forming bacilli	*Bacillus subtilis*	31.1 ± 9.6 [a]	-	[35]
	Bacillus cereus	8.1 ± 0.8 [b]	76	[67]
Gram-positive anaerobic spore-forming bacilli	*Clostridium proteolyticum* DSM 3090T	94.4 ± 0.9 [b]	26	
Gram-negative aerobic to facultatively anaerobic bacilli	*Escherichia coli*	16.7–22.2 [b]	67.9–69.7	[67,70]
Gram-negative	*Pseudomonas aeruginosa*	47.9 ± 8.7 [a]	-	[35]
	Massilia timonae	39.6 ± 3.7 [a]	-	

CA—contact angle with [a] water, [b] saline water (0.1 M NaCl); γ_{BV}—surface energy.

Even though a difference exists between the hydrophobicity of the surface and of the bacteria, the colonization process can still occur [71]. In this case, the non-specific chemical interaction with the material surface is an important mechanism for bacterial adhesion. An example of this is the capacity of *Staphylococcus epidermidis*, a bacterium that has a hydrophilic property, to colonize on polyethylene, which is a hydrophobic surface. It is possible that because *S. epidermidis* produces the protein adhesin (e.g., exopolysaccharide) it can adhere to a hydrophobic surface [64,72]. In addition, the non-specific chemical interaction is influenced by the chemical composition of the surface material. Therefore, the level of corrosion or decomposition of the material can influence adhesion and biofilm formation [66]. The materials used in pig facilities are exposed to several different conditions, for example, water from cleaning, drinking water, food residues, high humidity inside the building and chemical compounds from manure (i.e., ammonium and hydrogen sulfate), boosting corrosion and/or decomposition of the material [23].

As observed, the materials' surface has a great impact on the bacterial colonization process. One method to prevent bacterial adhesion is changing the material surface characteristics [28]. The literature has suggested different methods for modifying the hydrophobicity of different types of materials, such as chemical change of hardened cement pastes (HCP) [73], mortar [53], low-density polyethylene (LDPE) [74], thermal change (termorretification) of plywood [61] and the addition of metal particles on plastics [56,57].

4. Impact of Washing Procedures on the Materials' Sanitary Characteristics

4.1. Material Cleanability Evaluation Methods and Parameters

Although cleaning and disinfection processes are not able to totally eliminate the risk of disease, they can help minimize the negative impact of most endemic pigs' infections [75]. Actually, proper cleaning and disinfection before stocking new pigs in a facility are very important for preventing the spread of infectious agents [76]. As mentioned before, bacteria could be sheltered in pits, grooves and irregularities of rougher surfaces [28] as well as inside the biofilm [15,39,40].

Many methods are available for evaluating the effect of cleaning and disinfection processes on materials' surfaces, i.e., the presence or absence of bacteria or biofilm. These methods for evaluating cleanability are based on a measurement principle, which includes microbiological analysis [77], chemical analysis (i.e., chromatography and spectrometry) [78], biochemical analysis (i.e., ATP (adenosine triphosphate) bioluminescence) [76], physical analysis (i.e., colorimetry) [79,80], visual methods and radiochemical methods [80,81].

Luyckx et al. [77] evaluated the presence of some bacteria before and after the classical cleaning and disinfection protocol for nursery units. This protocol consisted of removing the manure with cold water, then after 24 h, the pen was soaked for 30 min with degreaser detergent. Subsequently, the pen was washed with high pressure (150 bar) cold water and finally, it was disinfected and kept empty for two weeks. With this washing protocol, the authors observed a 3.54 log CFU/sampling area reduction of *Enterococcus* spp. However, the presence of these bacteria was observed on floors and drinking nipples even after the protocol was used. For *E. coli* and *fecal coliforms*, reductions of 41% and 51% were observed, respectively; however, they were still found on floors, drinking nipples and feeding troughs after the washing protocol. Methicillin-resistant *Staphylococcus aureus* (MRSA) was reduced by 81% after the cleaning protocol, but drinking nipples were still the most contaminated by MRSA after disinfection. Therefore, according to Luyckx et al. [77], the area where bacteria still remain, even after the cleaning and disinfection protocols, are grid floors and drinking nipples.

Yi et al. [76] carried out the method using ATP bioluminescence in an empty pig farrowing unit. The evaluation was carried out before and after cleaning and at different times after disinfection (i.e., 1 h, 3 h, 6 h, 24 h, 48 h, 5 days and 7 days) in three locations: two on the polypropylene grid floor (center and corner) and one in feeders. The authors observed that the mean ATP bioluminescence levels of the floor corner and feeders measured after cleaning were not significantly different from those measured before cleaning. Moreover, the mean ATP bioluminescence levels increased 1 and 3 h after disinfection for floor corners and feeders, respectively. For the floor center, a significant decrease of the mean ATP bioluminescence levels occurred after cleaning and persisted up to 48 h after disinfection. After this time, the levels measured exceeded those measured immediately after cleaning.

The type of surface and biofilm are important elements of bacterial prevalence. For example, bacteria such as *Haemophilus influenza*, *Bordetella pertussis*, *Proteus vulgaris* and others can remain for days. On the other hand, bacteria such as *Enterococcus* spp., *Staphylococcus aureus*, *Streptococcus*, *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa* can survive for months on dry surfaces [18]. Hurnik [82] describes some survival times of common pig pathogens in different environments:

- *Mycoplasma Hyopneumoniae*: Up to 7 days in organic matter
- Actinobacillus Pleuropneumoniae: Few days in organic matter
- *Pasteurella Multocida*: 8 days in water or 6 days in liquid manure
- *Streptococcus suis*: 25 days (9 °C) or 100 days (0 °C)
- *Salmonella s*sp.: Years in manure, 115 days in water and 120 days in soil
- *Escherichia coli*: 11 weeks in manure

In addition, some flaws in cleaning and disinfection processes are pointed out by Dias et al. [75], and they are related to the prevalence of bacteria even after cleaning and disinfecting. Those flaws are the incomplete removal of wastes before cleaning procedures, the lack of wall and ceiling disinfection and inadequate water quantity and pressure.

4.2. Effect of the Washing Procedure on Biofilm Destruction on Different Materials

No standardized washing procedure (i.e., following the same washing parameters and conditions) exists and, even when checking recommendations, the parameters are not standardized. Furthermore, each producer has his own washing procedure (parameters). Because many washing parameters are used, it raises the following question: Which are the proper measures to adequately remove the biofilm and eliminate bacteria?

Some washing parameters such as water jet pressure, water temperature, washing time and distance between the water jet and the material surface can be adjusted optimally to ensure that the biofilm is destroyed and that the cleaning process is more efficient. Although cleaning and disinfection processes are highly important in pig facilities, little attention is given to the relationship between the parameters influencing biofilm destruction and the cleanability of the surface materials in pig facilities. Moreover, from the perspective of the pig producers, there is a certain skepticism regarding the value of hygiene and disinfection.

Burfoot and Middleton [58] studied the impact of water pressure on the removal of biofilms of *P. aeruginosa* and *S. aureus* from stainless steel surfaces. A 15° flat nozzle with two nozzle pressures (2.5 and 9.5 MPa) and different distances between the nozzle and targeted surface (20 to 120 cm) was used to vary the impact pressure. The authors highlighted that the impact pressure did not have a significant effect on the removal of the biofilm. Furthermore, *P. aeruginosa* biofilm was the bacteria most difficult to remove.

The authors also investigated other cleaning parameters, such as water temperature (8 °C and 60 °C), washing time (5, 30 and 60 s), detergent and nozzle angle (45° and 90°) relative to the sample surface. The results showed that these parameters impacted the microbial reduction. The use of hot water resulted in a greater microbial reduction than cold water. When cold water was used, the microbial removal increased with the cleaning time, whereas the same trend was not observed with hot water. The application of detergent produced a 5.6-log^{10} microbial reduction for both hot and cold water at a pressure of 9.5 MPa. No clear relationship was found between the angle of the nozzle and microbial removal. However, it is important to highlight that this trend could be different with other surface materials.

Kymäläinen et al. [80] evaluated the impact of water temperature (10, 40 and 70 °C) on the surface cleanability of a cement-based material using colorimetric measurements. In this study, pig manure and synthetic pig manure were used to soil surfaces. The cleaning was carried out with high pressure (12 MPa), a passage speed of 0.9 m·s^{-1}, a distance of 18 cm between the nozzle and the surface and a nozzle angle of 45° from the perpendicular. The results showed that cleanability was not influenced by the water temperatures used. However, according to Böhm [83], for concrete as well as for wood-based material surfaces, the optimum water temperature for cleaning is about 40 °C. On the other hand, for metal surfaces, higher water temperatures may provide better results, as long as the wash time is sufficient.

In addition, as mentioned by Hurnik [82], using hot water might reduce the washing time by about 22% when a presoak is not performed. However, there is no reduction on the washing time, between hot or cold water, when the presoak is used. An important point highlighted by the authors is that hot water is more efficient to realize the cleaning. However, it can create a fog that affects visualization during the cleaning procedure. Further, the application of a detergent can results in a reduction of 12% of the washing time.

When considering the type of flooring (fully or partial slatted), a pre-soak step decreases the washing time of partial slatted flooring but not of fully slatted flooring. One reason pre-soaking does not impact the washing time of fully slatted flooring is because there is no accumulation of manure like on partial slatted flooring [84]. However, there is no information about the impact pre-soaking has on biofilm removal.

Other parameters such as nozzle type can have important effects on cleaning processes. For example, Predicala et al. [84] observed that the use of a conventional nozzle (i.e., rotating nozzle) results in a 62% reduction of microbial ATP, while in other nozzles (Y-nozzle, water broom and 4-in nozzle) this reduction was 16–34% on concrete surfaces (floor). In this case, the effect of the impact force caused by a conventional nozzle allowed for a better cleaning on a rougher surface.

From these results, it appears that bacteria are still present even after the cleaning and disinfection processes. Therefore, the biofilm is not completely removed, bacterial transmission will continue and, incidentally, pigs may be infected. Consequently, further studies focused on washing parameters and their impact on biofilm removal on different

surfaces are necessary for developing more efficient cleaning and disinfection processes and standards.

5. Conclusions

This review summarizes the main effects of surface material on bacterial colonization as well as the impacts of washing procedures on the sanitary factors related to the different materials used in pig facilities.

Firstly, biofilm formation on the surface of different types of materials is a complex system affected by the characteristics of each material's surface, of each group of bacteria as well as the environmental conditions within the pig facility. The material properties (roughness and free-energy surface) play an important role in the early stage of bacterial attachment and consequently biofilm formation. Secondly, the use of common washing procedures does not allow the complete removal of the biofilm on surfaces.

In fact, the relationship between the surface material, bacteria characteristics and washing parameters can explain why some organisms are easily removed or why sometimes some bacteria persist in pig facilities even after the cleaning and disinfection process. The bacteria persistence comes from the fact that the biofilm is not removed completely; as a result, certain bacteria survive inside the biofilm and continue spreading diseases. This demonstrates the importance of not just removing bacteria but also removing the biofilm on the surface in order to prevent the spreading of diseases.

To increase biofilm removal, some parameters such as chemical treatment (i.e., detergent application), higher temperature (for some materials), or longer treatment/washing times have to be considered.

Therefore, this overview may serve to clarify the importance of the following three factors for improving sanitary conditions in pig facilities: biofilm, surface material and washing parameters.

However, it is important to emphasize here that more studies are required to better understand the relationships between the previously discussed factors. For example, studies should determine the best combination of washing parameters as a function of material type and bacteria that can be harmful to pigs. Since several studies conducted in pig facilities have shown that the feeders, drinkers and floors are the sites where bacteria persist the most even after the cleaning and disinfection process, future research should focus on metals, plastics and cement-based materials.

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