Summary

A common survival strategy for microorganisms on surfaces is the development of a biofilm. Biofilms can be defined as organizations of microorganisms enclosed in a gel-like matrix, which are adherent to surfaces. The biofilm functions to both distribute nutrients to the biofilm cells and to protect the cells from environmental threats. In many cases, microbial biofilms are of a mixed nature, consisting of more than one strain and species of microorganism.

Yeasts and bacteria in mixed biofilms on medical prostheses are responsible for diseases and a loss of function of the device. Mixed biofilms on denture surfaces result in denture stomatitis, a disease characterized by redness and pain where the gums contact the denture surface. Furthermore, mixed biofilms on silicone rubber voice prostheses interfere with the prosthesis valve, causing it to remain open and allowing the passage of food and liquid to the trachea, or causing increased air-flow resistance.

Initial development of mixed biofilms occurs through interactions between microorganisms. When different microorganisms, which are suspended in a fluid, come in contact with each other and stick together, these microorganisms are said to coaggregate. Conversely, if one of the microorganisms is affixed to a surface, and interactions take place which lead to their sticking together, then coadhesion occurs. In biofilm formation, it is theorized that initially adhering microbial strains, the early colonizers, which are especially adept at attaching to bare surfaces or surfaces with conditioning films, adhere to the surface. Through coadhesion events, other microorganisms, the late colonizers, which can only adhere to other microorganisms, then adhere.

To date, most research has focused on coaggregation and coadhesion between bacterial strains, and there is little known about the coadhesion of yeasts and bacteria at surfaces. Therefore, this thesis has as its aims to firstly, develop an efficient method to investigate adhesive interactions between yeasts and bacteria at surfaces, secondly to determine whether bacterial adhesion is a prerequisite for yeast adhesion at a surface, and thirdly to identify the role of the microorganism's surface physico-chemical properties in these adhesive interactions.

The adhesive interactions between bacteria and yeasts have been studied employing several of the methods originally developed for studying adhesive interactions between bacteria. However, in many of the methods employed the larger size of the yeasts as compared with bacteria results in strong sedimentation of the yeasts, often invalidating the method adapted as concluded from the literature review, presented in **Chapter 1**. In addition, most methods are semi-quantitative and do not properly control mass transport. Consequently, adhesive interaction mechanisms between yeasts and bacteria identified hitherto, including lectin binding and protein-protein interactions, must be regarded with

caution. Extensive physico-chemical characteristics of yeast cell surfaces are not available and a physico-chemical mechanism has not yet been put forth. A new method for quantifying adhesive interactions between yeasts and bacteria is proposed in **Chapter 2**, based on the use of a parallel plate flow chamber, in which the influence of adhering bacteria upon the kinetics of yeast adhesion and aggregation of the adhering yeasts is quantitatively evaluated, under carefully controlled mass transport.

The extended DLVO (Derjaguin-Landau-Verwey-Overbeek) approach has been applied to explain adhesive interactions between *Candida albicans* ATCC 10261 and *Streptococcus gordonii* NCTC 7869 adhering on glass in **Chapter 3**. Contact angles with different liquids and the zeta potentials of both the yeasts and bacteria were determined and their adhesive interactions were measured in a parallel plate flow chamber. Streptococci were first allowed to adhere to the bottom glass plate of the flow chamber to different seeding densities, and subsequently deposition of yeasts was monitored with an image analysis system, yielding the degree of initial surface aggregation of the adhering yeasts and their spatial arrangement in a stationary end point. Irrespective of growth temperature, the yeast cells appeared uncharged in TNMC buffer, but yeasts grown at 37°C were intrinsically more hydrophilic and had an increased electron-donating character than cells grown at 30°C. All yeasts showed surface aggregation due to attractive Lifshitz-Van der Waals forces. In addition, acid-base interactions between yeasts, yeasts and the glass substratum, and yeasts and the streptococci were attractive for yeasts grown at 30°C, but yeasts grown at 37°C only had favourable acid-base interactions with the bacteria, explaining the positive relationship between the surface coverage of the glass by streptococci and the surface aggregation of the yeasts.

In **Chapter 4** the adhesion to glass of aerated and non-aerated *C. albicans* ATCC 10261 in the presence and absence of adhering *S. gordonii* NCTC 7869 was determined in a parallel plate flow chamber. In addition, the influence of aeration on the yeast cell surface hydrophobicity, surface charge, and elemental cell surface composition was measured. *S. gordonii* adhering at the glass surface caused a reduction in the initial deposition rate of *C. albicans*, regardless of aeration. In a stationary end-point, only adhesion of non-aerated *C. albicans* was suppressed by the adhering *S. gordonii*. Non-aerated yeasts had a higher O/C elemental surface concentration ratio, indicative of cell surface polysaccharides, than aerated yeasts, at the expense of nitrogen-rich cell surface proteins. Summarizing, this study suggests that highly localized, hydrophobic cell surface proteins on *C. albicans* are a prerequisite for their interaction with adhering streptococci.

Chapter 5 investigated the influence of saliva on the adhesive interactions between *C. albicans* and *Streptococcus sanguis* or *Actinomyces naeslundii* on denture acrylic. First, bacteria were allowed to adhere to the acrylic surface from a flowing suspension, and subsequently yeasts were flowed over the acrylic surface. The organisms were assayed in the presence or absence of human whole saliva. All experiments were carried out in a parallel plate flow chamber and enumeration was done in situ with an image analysis system. In the absence of adhering bacteria, adhesion of *C. albicans* from buffer was more extensive than from saliva. However, in the presence of adhering bacteria, yeast adhesion from saliva was increased with respect to adhesion of yeasts from buffer, indicating that specific salivary components constitute a bridge between bacteria and yeasts. In all cases, yeast aggregates consisting of 3 to 5 yeast cells were observed adhering to the surface. A surface physico-chemical analysis of the microbial cell surfaces prior to and after bathing the microorganisms in saliva, suggests that this bridging is mediated by acid-base interactions since all strains show a major increase in electron-donating surface free energy parameters upon bathing in saliva, with no change in their zeta potentials. The surface physico-chemical analysis furthermore suggests that *S. sanguis* and *A. naeslundii* may use a different mechanism for adhesive interactions with *C. albicans* in saliva.

A new assay for the simultaneous determination of the adhesive interactions between yeasts and different bacterial strains on a surface under controlled hydrodynamic conditions is described in **Chapter 6**. On an acrylic surface, the presence of adhering bacteria suppressed adhesion of *C. albicans* ATCC 10261 to various degrees, depending on the bacterial strain involved. Suppression of *C. albicans* ATCC 10261 adhesion was strongest by *A. naeslundii* T14V-J1, while adhering *S. gordonii* NCTC 7869 caused the weakest suppression of yeast adhesion. When adhering yeasts and bacteria were challenged with the high detachment force of a passing air-liquid interface, the majority of the yeasts detached, while *C. albicans* adhering on the control, bare polymethylmethacrylate surface formed aggregates. Summarizing, this study presents a new method to determine suggested adhesive interactions between yeasts and adhering bacteria under controlled hydrodynamic conditions. However, the results seem to indicate that these adhesive interactions may well not exist, but that instead different bacterial strains have varying abilities to discourage yeast adhesion.

In **Chapter 7** adhesive interactions between *C. albicans*, *Candida krusei*, and *Candida tropicalis* and eight bacterial strains, all isolated from explanted voice prostheses, were investigated in a parallel plate flow chamber from saliva and buffer. Bacteria were first allowed to adhere to silicone rubber, after which the flow chamber was perfused with a yeast suspension. Generally, the presence of adhering bacteria suppressed adhesion of the yeasts. In saliva, however, *Staphylococcus aureus* GB 2/1 enhanced adhesion of both *C. tropicalis* and

C. albicans, while *Stomatococcus mucilaginosus* GB 24/2 enhanced the adhesion of *C. tropicalis* to the silicone rubber surface. In buffer, the passage of an air-liquid interface caused 100% detachment of *C. albicans* and *C. krusei*, regardless of the presence or absence of adhering bacteria, while detachment of *C. tropicalis* was dependent on the adhering bacterial strain. In saliva, all three yeast strains fully detached upon passage of an air-liquid interface. This study shows that bacterial adhesion mostly reduces subsequent adhesion of yeasts. Only a few bacterial strains stimulate adhesion of yeasts, provided salivary adhesion mediators are present.

Chapter 8, the general discussion of this thesis, describes the development of the methodology, and a method to improve the system i.e. working at 37°C, for investigating adhesive interactions between yeasts and bacteria in the parallel plate flow chamber. Adhesive interactions between yeasts and bacteria can be better characterised by investigating the role of specific adhesion and anti-adhesion molecules using the dot assay method as described here. Furthermore, mechanisms for yeast suppression by the bacteria were discussed. Finally, the clinical relevance of interactions between yeasts and bacteria at surfaces is described, for example, the potential selective removal of specific bacterial species to prevent the enhancement of yeast adhesion, or the stimulation of specific bacterial strains to suppress yeast adhesion.

In summary, this thesis presents an improved method for the investigation of adhesive interactions between yeasts and bacteria at surfaces. The method controls mass transport to the surface, and avoids the passage of an air-liquid interface which can cause adhesion artifacts. In general, in the absence of specific adhesion mediators, such as saliva, adhering bacteria suppressed the adhesion of yeasts on a substratum. The mechanism of adhesive interactions is not yet clear. However, with further experimentation on the specific components of the adhesive interactions and adhesion mediators, the mechanisms should be identified in due course.