The Adhesion Force Study of Dairy Thermophile *Anoxybacillus flavithermus* CM with Atomic Force Microscopy

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Engineering
in
Chemical & Nanotechnology

at Massey University, Manawatu,
New Zealand

Mohd Salihin Mohd Saidi

2014

Abstract

Anoxybacillus flavithermus is a common species of thermophilic bacteria discovered in most milk powder manufacturing plants through out New Zealand. The contamination of it's spores into the finished milk powder is an on-going problem as these spores are able to survive the sterilization process. Cheating death, *A. flavithermus* spores were then believed to attached on the stainless steel surface piping of the production line and germinate into a mature bacteria. A single surviving spore could grow to produce more spores that eventually dislodged from the colony and deposited together with the packaged milk powder. Over the storage time, the contaminated product will gives an off flavor as it deteriorates from bacterial action within.

Currently, the applied cleaning method is by rinsing the target section with 1% sodium hydroxide & acid solutions before being flushed out to remove any microorganisms attached on the interior surfaces. However, it is not very effective in removing spores and there is very little information on the value of the spore's adhesion force on a stainless steel surface. With that in mind, the aim of this study is to determine a proper adhesion force value between a dairy strain spore, *A.flavithermus* CM and stainless steel surface using the Atomic Force Microscopy (AFM) system. Meanwhile, *Geobacillus strearothermophilus* ATCC 2641 which is also a thermophilic organism was used over the study for comparison purpose.

To measure the adhesion force under an Atomic Force Microscopy (AFM), the crude suspension was first purified using two-phase separation method. Polyethylene glycol (PEG) and phosphate buffer were used as the phase separation chemicals while 0.1% polysorbate 20 was added to the freshly purified spores' suspension to aid the imaging sequence under the AFM. All AFM imaging and force measurements were done in air and conducted using the silicon type CSG 11/Au cantilever. The crucial Force-Volume imaging was done on a 32x32 grid scan size (1024 samples) on a scan rate of 0.5 Hz.

It was calculated that a single *A. flavithermus* CM spore has an adhesive force value of 16.8 μN when attached on a stainless steel surface. It has a stronger localize adhesive value of 3.9 nN than a *G.stearothermophilus* ATCC 2641 spore with just 3.6 nN. However, *G.stearothermophilus* ATCC 2641 has a larger adhesive force of 21.1 μN on a stainless steel surface due to it's larger spore size. It was also found that spore's hydrophobicity does not dictates the magnitude of it's adhesion on any surface.

The results from this study have provide the dairy industry an extra sight on the quantitative value of the adhesion force of thermophilic spores, particularly *A.flavithermus* CM. This will help the dairy industry to design strategies in preventing spores from adhering to its production lines.

Contents

Table of Figures