CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This research demonstrated the development of micro-scale cultivation and digital microscopy-assisted technique to perform total plate count (TPC) yeast and mold detection. To develop the micro inoculation culture (MIC), extensive literature survey was performed to compare the advantages and disadvantages of the proposed method to the existing protocols (e.g., pour plate and spread plate techniques) as well as the commercialized rapid method that is the Petrifilms^{TM}. The engineering and microbiological aspects of these methodologies were critically analyzed to reduce analytical cost per sample and simplify the procedures and related equipment. The goal was to establish a simple-to-use technique and low capital investment and testing supplies. An application of visual aid can further lessen the detection time owing to higher magnification power using a series of magnifying lenses. The prototype digital microscopy was able to shorten the TPC colony detection time to approximately 6 - 8 h. In addition, the colony images were automatically converted to a digital format for future references and further analysis using digital image analytical tools. Such technique allows the evaluation of colony size and color.

A novel application of digital image analysis helped magnify the colony image and expedite rapid enumeration and identification. The proposed protocol was streamlined by improving protocol to detect industrial hygienic microbial contaminants. The standard calibration was also performed to validate the micro-inoculation technique (MIC) against the spread and pour plate techniques. This technology was successfully implemented to determine the total plate counts (TPC), yeast and mold of various types of factory samples, including finished frozen food products, production line swabs, plasticine and dough clay samples. For industrial samples, the MIC resulted in equivalent numbers of aerobic plate counts to the conventional techniques within 12-15 h as opposed to 24-48 h from the spread and pour plate techniques.

The size and characteristic of TPC colonies were observed using the prototype system of image analysis. The systematic improvement of colony detectability was

demonstrated. This digital microscope enabled fast colony enumeration, which was linearly correlated with the reduction of colony incubations from generally 24 h to only 8 h. Agar color (i.e., green, red and yellow dye) of the media was used to enhance the contrast of the colony and the background. The contrast represents by delta E in the RGB system, which is the root-mean-square error of R, G and B indices. The red background provided the best color contrast over 60% larger delta E value compare to the traditional agar color.

The study of *E. coli* colony growth on CCA was facilitated by the use of a prototype digital imagery system. The highest colony expansion on CCA was obtained at a significantly higher incubation temperature setting (40^{o}C) than typical setting (35^{o}C) recommended by most microbiological standards and handbooks. The development of blue color attribute also followed the kinetics of colony area growth. The use of medium magnification digital microscopy allowed the detection of both colony and blue chroma within 8 hours suggesting the detection time of *E. coli* colony can be substantially reduced from overnight with human visual detection to 8 hours by microscopy-assisted inspection. The logistic model was very useful to extract important kinetic parameters of *E.coli* colony expansion. And the effect of CCA concentrations on *E. coli* color the size and purplish *E. coli* colonies was gradually changed as a result of the CCA dilution. At lower dilutions, the images of the *E. coli* colonies were very faint affecting colony numbers and the distinction of colony itself. For colony count purposes, the CCA preparation according to manufacturer's recommendation was able to reduce to 60-70% without compromising the colony detectivity. The tracking of the RGB color attributes as a function of culturing time indicated that the blue color attributes developed from beta-glucuronidase activity only differed when the CCA concentration went below 40%. The uses of the developed digital microscope were able to extent the dilution further and minimize the concentration of the CCA medium.

The optimization of the kinetics and growth characteristic of yeast and mold (i.e., *S. cereviseae* and *A. niger*) and the nutrient requirements (e.g., carbon and nitrogen prerequisites) were extensively scrutinized using Tukey test to differentiate the statistical differences. Mold growth kinetic and mathematical simulation showed no significant different when carbon and nitrogen sources were altered. Any nutrient broths from standard recipes were equivalently effective to resuscitate and encompassed

sufficient nutrients for mold growth. The yeast growth, the dextrose solution returned fast growth in liquid media. Therefore, to grow yeast and mold in liquid culture, the cost of broth media can be drastically reduced using local carbon and nitrogen alternatives.

5.2 Recommendations

The results from this study showed an opportunity TPC yeast and mold detection by using the micro inoculation culture (MIC). However, the optimal temperature incubation and substrate dilution of CCA of this proposed detection procedure suggested from this study were performed on pure culture. The actual wild types or native strains may require longer incubation. The results from this study were only guideline for detection TPC, yeast and mold in food, plasticine, and dough clay samples. This research only included *Escherichia coli*, *Saccharomyces cerevisiae* and *Aspergillus niger*. More relevant strains of pathogenic and non-pathogenic need to be included in future studies. The implementation in real industrial application was required to gather more growth data and adjust the analytical procedure for practicality.

In term of mathematical method, though sigmoid model is suitable for detect maximum growth rate in this experiment but it cannot compare the result between sigmoid and others model to make more precise and accurate for predict the maximum growth rate next.