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Abstract

Ethanol, bleach and anti-bacterial hand soap are three kinds of disinfectant which have been widely used in common laboratory. In this study, a comparing experiment on these three disinfectants' efficiency was conducted against Escherichia coli (E.coli) and mixed culture obtained for bacterial reactor. Contact time was considered as influence parameter, and spread plating-plate counting was used as numeration method for bacteria concentration. The results showed that 10% bleach had the best efficiency against both the two cultures and 70% ethanol had a better effect on pure culture than mixed culture, which revealed its sterilizing selectivity. For anti-bacterial hand soap, almost no disinfection effect was detected, which made it not be recommended for using as sterilization.

Key word: disinfectant, ethanol, bleach, hand soap, E.coli.

Introduction

Bacteria are a major cause of disease and even human death. Disinfectant as an effective agent to kill or eliminate bacteria is widely used in varies ways, especially in microbial laboratory. Disinfectants can be mainly divided into five agents: alkylating, sulfhydryl combining, oxidizing, dehydrating and permeable.

The most commonly used disinfectants in lab are ethanol, bleach and hand soup. Bleach, with a main constituent of sodium hypochlorite, effects by oxidizing the cell of microorganisms and attacking essential cell components including lipid, protein, and DNA (Ho-Hyuk Jang et al, 2008). Ethanol, as a dehydrating agent, causes the cell membrane damage, rapid denaturalization of proteins with subsequent metabolism interference and cell lyses (Larson and Morton, 1991). Hand soap, as a daily used disinfectant, normally works by stripping away the outer layer of oil on the skin and prevents bacteria present in the body from coming to the surface of the hand. Many studies have been done on comparison of disinfectant efficiency, and ethanol and bleach are believed have immediate effect against most organisms (Carly N. Jordan, et al, 2006). For bacterial strains, *E.coli* has been used widely in disinfectant test as a pathogen indicator. In this study, a disinfectant experiment was conducted using 70% laboratory ethanol, 10% commercial bleach and 50% commercial antibacterial hand soap against pure *E.coli* strain and mixed culture collected from aerobic batch reactor.

The efficiency of disinfectant was compared under 4 different contact time. Spread plating and plate counting were introduced in the study as the numeration method.

Materials and Methods

Bacteria

Pure *E.coli* isolate was obtained from ATCC and incubated in nutrient broth liquid for 48 h at 37 ^{o}C in a shaker table. Mixed culture was directly collected from an aerobic sludge sequencing batch reactor in Environmental Laboratory, University of Kansas. Before the experiment, in order to determine the initial cell concentration, for both kinds of culture, spread plating (Adam Driks, 2002) was performed on dilutions 10^{-4}-10^{-6}. After that, all the plates were incubated in an incubator for 24h at 37 ^{o}C and counted by colonies for numeration.

Disinfectants and culturing mediate

Three kinds of disinfectants were tested: ethanol (Ethanol, Anhydrous, Fisher Scientific), diluted by deionized water to 70%; commercial bleach (Ultra Bleach, Target Corporation), which contains 6% sodium hypochlorite, diluted by deionized water to 10% and anti-microbial hand soap (Softsoap®, Colgate-Palmolive Company), which contains 0.15% triclosan, diluted by distilled water to 50%. The mediate for E.coli culturing containing 0.8% nutrient broth (Difco^{TM} Nutrient Broth, Becton, Dickinson and Company) was prepared in deionized water. The mediate for spread plates containing 0.8% nutrient broth (Difco^{TM} Nutrient Broth, Becton, Dickinson and Company) and 1.5% soy agar (Tryptic soy agar, Fisher Scientific) was also prepared in deionized water. Both kinds of mediate were autoclaved to sterilize before use.

Disinfectant experiment

The experiment was performed in 1.5ml tubes, using three kinds of disinfectant mentioned above. Four different contact time: 30sec, 1min, 5min, 10min were also tested. For each tube, 0.1ml of culture solution was added into 0.9ml of disinfectant. After certain contact time, a 5000rpm centrifuge was performed for 5min to separate the culture from the solution. Supernatant was discarded and then the tube was refilled by deionized water, followed by spread plating on each tube. After the experiment, all the result tubes were stored in refrigerator at 4 ^{o}C. The next day, plate counting was performed on each spread plate after 24h culturing at 37 ^{o}C in the incubator.

Result

As aforementioned, original concentration of both kinds of culture was obtained by viable plate counts, which is shown in Table 1. The plates for both pure culture and mix culture with dilution rates form 10^{-4} thru 10^{-8} were counted.

Table 1 Original cell concentration

	Pure culture	Mixed culture
original concentration (cfu/mL)	1.0×10^{8}	2.3×10^{6}

The results of disinfectant test were also measured by visible plate counting, which is shown in Figure 1 and 2.

Figure 1 Mixed culture disinfectant test result (cell concentration after disinfectant test)

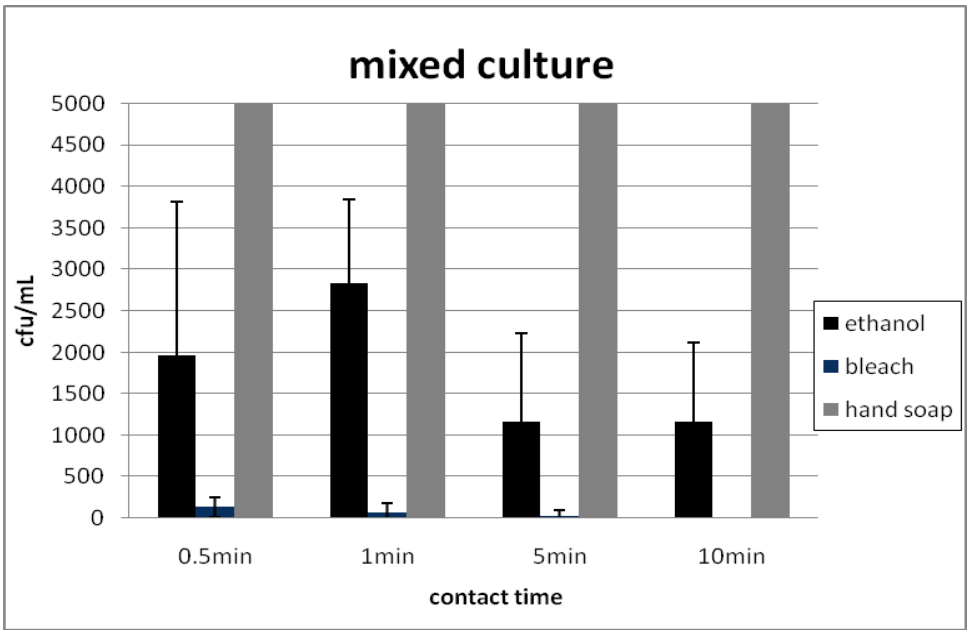
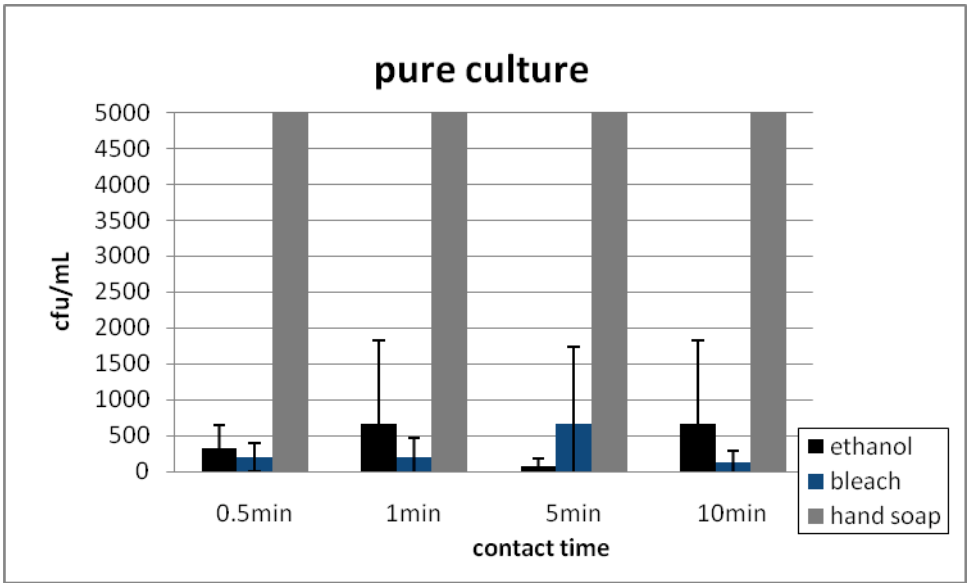


Figure 2 Pure E.coli culture disinfectant test result (cell concentration after disinfectant test)

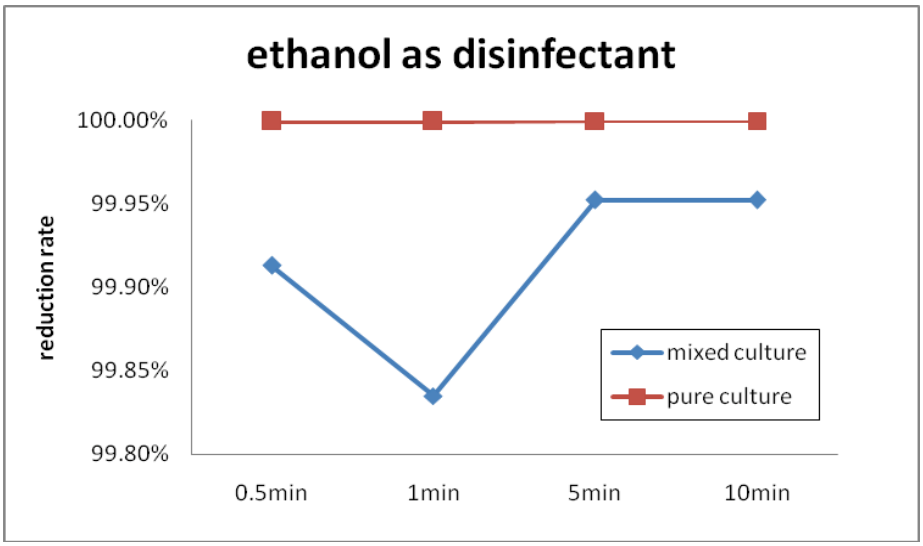


As figure 1 and 2 showed, it is relatively significant that bleach has the best efficiency against both pure *E.coli* culture and mixed culture, which had the lowest number of colonies in most of tests, with a reduction rate generally above 99.99%. For ethanol, which also had an acceptable reduction of generally above 99.9%, it has a better efficiency against pure *E.coli* culture than mixed culture. To the contrary, extremely

high cell concentration of hand soap treated samples was obtained, which will be shown with more detail below.

The efficiency of ethanol against pure *E.coli* culture and mixed culture is further compared in Figure 3.

Figure 3 Ethanol kill rate against pure *E.coli* culture and mixed culture



According to the figure above, pure *E.coli* culture had a much higher reduction than mixed culture after ethanol treated, and mixed culture reduction was more time related than *E.coli* reduction.

A further cell concentration measurement of hand soap treated samples was conducted by plate counting on sample dilutions 10^{-4}-10^{-6}. The results were shown in Figure 4 and 5, compared with blank samples using deionized water as disinfectant.

Figure 4 Pure culture disinfectant result using hand soap (cell concentration after disinfectant test)

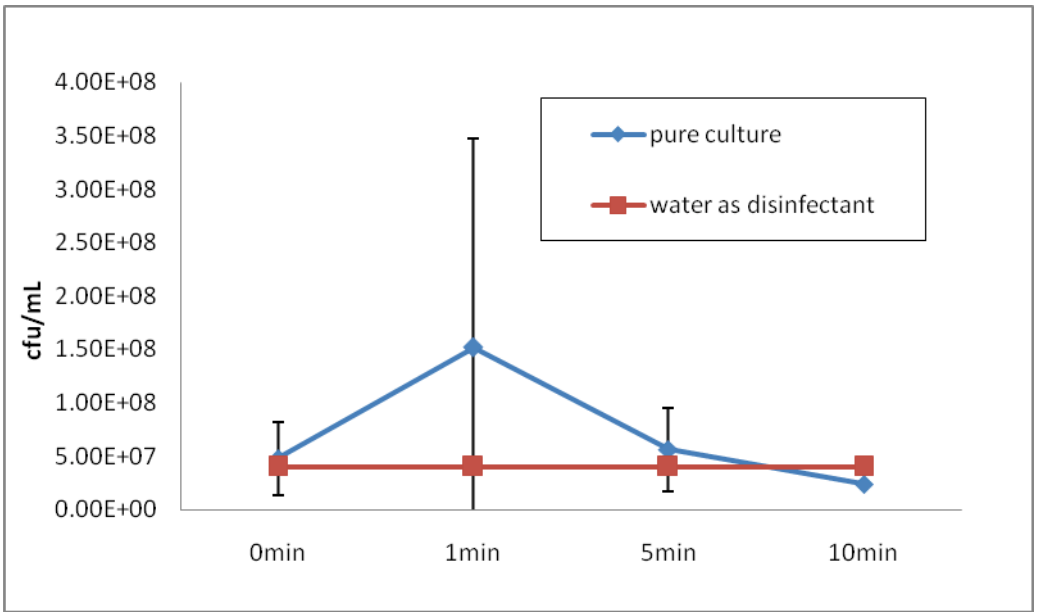


Figure 5 Mixed culture disinfectant result using hand soap (cell concentration after disinfectant test)

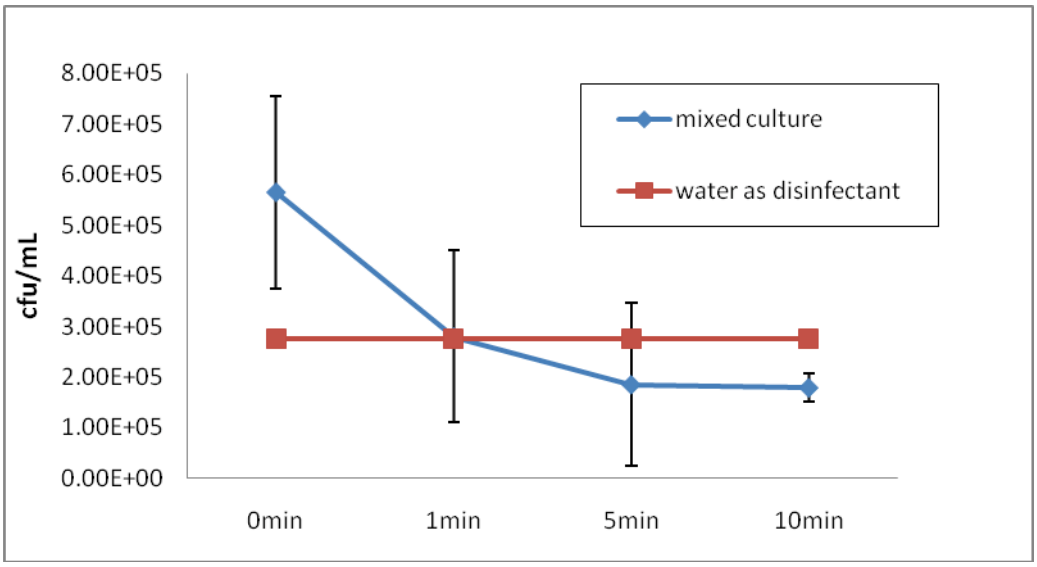


Figure 4 and 5 indicates that in the first 5 minutes, the values of the colonies did not change a lot with the origin ones; also it did not deviate significantly from the ones using water as disinfectant. In 10 minutes, the counts began to decrease slightly. It implied that hand soap had no disinfection effect on the sample in a short time and the long time effect needs a further analysis.

Discussion

The data generally showed an immediate efficiency of bleach and ethanol against both E. coli and mixed bacteria culture, which supported former studies (T.A. Gaonkar, et al., 2006). And after comparison, the efficiency of bleach is the best of the three disinfectants against mixed culture, which had a killing rate of 99.99% in 30S, compared to 99.9% of ethanol. On the other hand, for pure *E.coli* culture, bleach and ethanol had similar efficiency, with a reduction of more than 99.999% in 30S. However, to the contrary, an extremely low, nearly null efficiency of antimicrobial hand-soap against both pure *E.coli* culture and mixed culture was also revealed in the test. From the results, it is easy to indicate bleach had an ideal bactericidal capability against both pure *E.coli* culture and mixed culture, and ethanol had a better capability against pure *E.coli* culture than mixed culture, which revealed a selective pattern. The reason for this result mainly is the mechanism of bleach and ethanol's sterilizing. A former study found that oxidation reactions will occur when the bleach is dissolved in water, which can destroy organisms' fold structure, leading to sterilization (Barindra Sana et al, 2006). And for ethanol, sterilization is mainly due to dehydration of protein and the enzyme to deactivate and prevent bacteria growing (James R. Cronmiller et al, 1999). It is reasonable to explain that most protein have generally similar chemical characters for bleach to oxidize and deconstruct, but different protein

has different biological characters, which cause a selectivity for ethanol to deactivate. Former researches also found that some kinds of bacteria can't be killed easily and have some characteristics of resistance on ethanol, some of them existing in wastewater can alive even in very high concentration of ethanol (Yi Hsing Lin et al, 2002) (Stephen B. Pruett et, 2004), which supported this explanation. Also, some researchers found that ethanol, as a form of alcohols, is rapidly bactericidal against vegetative organisms as well as being tuberculocidal, viricidal and fungicidal but has no activity against bacterial spores (Ayliffe et al, 1993), which is also a possible reason for ethanol's sterilizing selectivity.

For contact time, the immediate efficiency of bleach and ethanol was revealed by the high reduction rate in the 30S reaction. However, compared to bleach, ethanol still showed a relatively significant decreasing tendency on reduction against mixed culture (from 99.9% in 30S to about 99.99% in 10min), which can also be described by ethanol's sterilizing selectivity that some bacteria in the mixed culture probably have characteristics of resistance on ethanol (Stephen B. Pruett ect, 2004). On the other hand, the immediate killing pattern of bleach can be explained by its oxidizing mechanism. Another study also found similar result that bleach is rapidly bactericidal achieving a 5 $\log_{10}$ kill of *P. aeruginosa* and other vegetative organisms in one minute (A.P.Fraise, 1999).

The low efficiency of hand soap can also be explained by its disinfectant mechanism that it normally works by stripping away the outer layer of oil on the skin to sweep the bacteria from the surface of hands. As a result, its capability to sterilize is significantly limited. Another possible reason for the low efficiency is the low contact time. The active ingredient of the hand soap is triclosan, which is a well known effective antibiotic (Herbert P. Schweizer, 2001). However, a study revealed that triclosan's antibacterial effect is time related, and the ideal time is more than 20min (H. Wisplinghoff, et al., 2007). As a result, in a test with a contact time of 10min or shorter, it cannot effect well. The decreasing tendency of treated bacteria concentration from 5min to 10min for hand soap also supported this explanation.

Overall, bleach and ethanol are both effective disinfectants for sterilization against most kinds of bacteria, but bleach is slightly better in general cases. Anti-bacterial hand soap is not an ideal disinfection, which has nearly null effect on killing bacteria.

Conclusion

The main goal of this study is to compare the efficiency of three disinfectants (10% commercial bleach, 70% ethanol and 50% commercial anti-bacterial hand soap) and investigate their differences of sterilizing pattern. From the results, the following conclusions were obtained.

1. Among the three common disinfectants tested in this project, 10% bleach had the best efficiency against both pure *E.coli* and mixed culture due to its oxidization

mechanism.
2. 70% ethanol had better disinfection effect on pure *E.coli* culture than mixed culture, which revealed its sterilizing selectivity.
3. Hand soap had almost null efficiency of against both pure *E.coli* and mixed culture. As a result, it is not recommended to use in experimental disinfection or sterilization.
Further work can be done on DNA analysis to determine the certain strains of bacteria that have ethanol resistance and to investigate the efficiency of DNA damaging on bacteria by disinfectants.

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