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RESEARCH ARTICLE

COMBINED EFFECT OF TEMPERATURE AND pH ON *PSEUDOMONAS AERUGINOSA* ISOLATED FROM A COSMETIC PRODUCT

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ABSTRACT

Environmental factors have a profound effect on the microbial quality of cosmetic products. Research works have been done investigating effects of the factors singly. In this work, the microbial quality of the samples and the effect of environmental factors such as temperature and pH with change in time on the growth of *Pseudomonas aeruginosa* obtained by culture were examined. Our findings indicate that the effects of environmental factors such as temperature and pH on the growth of *Pseudomonas aeruginosa* with change in time increased with increase in temperature and time while effect of pH revealed that growth of the organism was highest at pH 7. The effect of pH varied across time and growth increased with increase in time. The combined effect of different temperatures and different pH with change in time on the growth of *Pseudomonas aeruginosa* showed that growth is affected when the microorganism was exposed to more than one factor. The combined effect produced a growth pattern that is different when the factors are acting independently. Environmental factors influence significantly the condition of products during in-use by consumers and storage.

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INTRODUCTION

The Federal Food and Drug Cosmetic Act criteria defined cosmetics as substances or objects intended to be sprinkled, rubbed, sprayed on, poured, or incorporated into, or otherwise used on the human body or any part thereof for beautifying, cleansing, increasing attractiveness, or changing the appearance, and objects purposed for application as a component of any such objects; except that soap shall not be included in such term (US FDA, 2004).Immediately a cosmetic product is opened till the moment a consumer discards it, it is subject to variable and constant microbial contamination arising from the domestic environment and the consumers' hands and body fluids. Microorganisms usually found in cosmetic preparations include *Klebsiella, Staphylococcus, Enterobacter, Pseudomonas aeruginosa, Bacillus* species, *Penicillium* and *Candida albicans* (Perry, 2001). Contaminated cosmetic formulations are relatively infrequent but certain of the products cause a health danger because of their incapability to prevent the growth of microbes of possible faecal origin during in-use (Okeke and Lamikanra, 2001). Also, Haft-baradaran *et al*. (2014) reported 40% of the Iranian sunscreen creams, 73.3% of the imported products, and 43.3% of the formulated cosmetic products at the point of purchase contained at least one of the undesirable microorganisms and also showed contamination with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Razooki *et al*. (2009) analyzed several brands of cosmetic samples which includes body and hand lotion, facial cleanser, shampoo, and liquid soaps. In their study, 26.4% of 60 cosmetic products analyzed were contaminated. Majority of the contaminations were due to bacteria and no fungal contaminant was found. Keshavarz *et al*. (2014) showed microbial contaminations in 46% of the 135 moisturizing cream. The highest contamination was caused by *Pseudomonas aeruginosa*. Microorganisms that are not required to be seen in cosmetic products are;

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Staphylococcus aureus, Salmonella spp., *Escherichia coli, Candida albicans, Pseudomonas aeruginosa,* and *Clostridium* spp (Onurday, *et al*., 2010; US FDA, 2004). Contaminated raw material is probably to be the origin of contamination by microbes found in this cosmetic product (Sharif-Abad *et al*., 2015). Gram negative bacteria are most commonly isolated and can survive in several types of environments as a consequence of their diverse metabolic capabilities. They are frequently introduced through water supplies. Contamination of cosmetics by microorganisms may cause product spoilage and when the microorganisms are pathogenic, they show serious risk to health of consumers (Campana *et al*., 2006). The storage condition of cosmetics can also determine whether microorganisms are likely to grow or not. Several environmental factors can judge the fate of microorganisms which enter a product. Examples include temperature, pH, and moisture content, osmotic pressure within the product, the product storage temperature and availability of nutrient materials (Baird and Bloomfield, 1996). For most environmental factors, ranges of values encourage growth of a microorganism, outside this range; the microorganism might cease reproducing but will remain alive for some time (Prescott *et al*., 2008).

MATERIALS AND METHODS

Effect of Temperature and pH on the Growth of Bacterial Isolate

Isolation and identification of bacterial strain

Pseudomonas aeruginosa which was recovered from the sample cosmetic preparation was used throughout this study. Nutrient broth (Lab M, England) was employed for the growth and culturing of *Psudomonas aeruginosa*. The pH values of nutrient broth were adjusted to between 4.5 – 8.0 inclusive using 1M HCl. The broth was dispensed in 330ml amounts and autoclaved at 121^0C for 15 mins.

Inoculums

The microorganism was inoculated into the enriched medium. The enriched medium used was nutrient broth. *Pseudomonas aeruginosa* was grown in nutrient broth and subcultured on 3 successive days. The third subculture was grown for 24h at 37^0C to a stationary phase. Ten-ml aliquots containing the inoculum were standardized using the MacFarland solution up to $5x10^8$ cfu/ml

Experimental procedure

The experimental procedure was adopted by Sutherland *et al*. (1993).The broths were equilibrated overnight to the appropriate incubation temperatures. 20-ml aliquots were then aseptically withdrawn and the pH values recorded but were not readjusted. The remaining 310 ml of the broth was inoculated with 1 ml of the inoculum prediluted to about $5x10^8$ cfu/ml. After inoculation, each broth was properly mixed and aseptically dispensed in 10 ml amounts into 30 sterile screw-capped 'Universal' bottles. The initial inoculum level was determined from one of the bottles and the remaining bottles were incubated at appropriate temperature. This procedure was performed as rapidly as possible to reduce temperature changes. The inoculated bottles were then placed in a controlled temperature water baths (Techmel and techmel, USA), an incubators (Memmert 100-800; 2144 Dartford, England) and oven (Uniscope, England) at temperatures higher than the room temperature, respectively. For non-isothermal, the bottles of inoculated culture were placed in the temperature incubator (Memmert 100-800). After each incubation period at a constant temperature and pH, duplicate sample bottles were removed from the water baths, incubator and oven and cooled in water. Growth was assayed for athourly interval under various pH and temperature conditions. At intervals during incubation, bottles were examined to determine growth in terms of turbidity of the organism. The bottles were discarded after sampling.

Growth evaluation

Growth of *Pseudomonas aeruginosa* was monitored by absorbance measurements through spectrophotometer (Model 6405 UV/Vis Spectrophotometer, Jenway Germany) at optical density of 600 nm at time intervals. The absorbance reading of un-inoculated medium, which served as blank was subtracted to the values of absorbance of the inoculated media. A Jenway 6405 UV spectrometer 1cm path-length cuvettes were used for all spectrophotometric assays.

RESULTS

Effect of temperature and pH on the growth of *Pseudomonas aeruginosa*

Temperature effect on the growth of *Pseudomonas aeruginosa*

The resultant effect of temperature with change in time on the growth of *P. aeruginosa*at varying temperature is illustrated in Fig.1. The result reveals that growth was least at 4^0C and highest at 40^0C. At 4^0C, growth was seen to increase at minimal rate from 1st to 3rd hour and decreased from 4th hour.

Effect of pH on the growth of *Pseudomonas aeruginosa*

The pH effects with changing time on the microbial growth of *P. aeruginosa* are illustrated in Figs. 2 and 3. The result obtained indicated that growth of the organism was highest at pH 7.0 followed by pH 6.0. The pH effect varied across time; for pH 4 and pH 7 growth increased with increase in time.

Combined effects of varying temperatures and pH 5 with change in time on the growth of *Pseudomonas aeruginosa*

The effect of temperature and pH 5 with change in time on *P. aeruginosa* is illustrated in Fig. 4. The result demonstrated that at pH 5 growth of the organism was highest at temperature 30^0C at the 2nd hour. The varying effects of temperature at pH 5.5 with change in time on the microbial growth of *P. aeruginosa* are illustrated in Fig. 5. The result demonstrated that at pH 5.5 the growth of the organism was highest at temperature 35^0C at the 4th hour. The varying temperature

effects at pH 6.0 with change in time on growth of *P. aeruginosa* is illustrated in Fig. 6. The figures obtained demonstrated that at pH 6.0 the growth of *P. aeruginosa* was highest at temperature 40^{0}C at the 4th hour followed by growth at 35^{0}C. The varying temperature effects at pH 6.5 with change in time on the microbial growth of *P. aeruginosa* are illustrated in Fig. 7. The figures obtained demonstrated that at pH 6.5 growth of *P. aeruginosa* was highest at temperature 35^{0}C at the 4th hour followed by growth at 45 ^{0}C, 30 ^{0}C, and 25 ^{0}C.The varying effects of temperature at pH 7.0 with change in time on the growth of *P. aeruginosa* are illustrated in Fig. 8. The result obtained demonstrated that at pH 7.0 growth of *P. aeruginosa* was highest at temperature 35^{0}C followed by growth at 30 ^{0}C, 40 ^{0}C, 45 ^{0}C and 25 ^{0}C. At pH 7.0 microbial growth increased as time increased for the various time intervals except for temperature 40 ^{0}C, and also growth was shown to be highest at the 4th hour. The varying temperature effects at pH7.5 with change in time on the growth of *P. aeruginosa* are illustrated in Fig. 9. The result obtained demonstrated that at pH 7.5 microbial growth was highest at temperature 35 ^{0}C followed by growth at 45 ^{0}C.

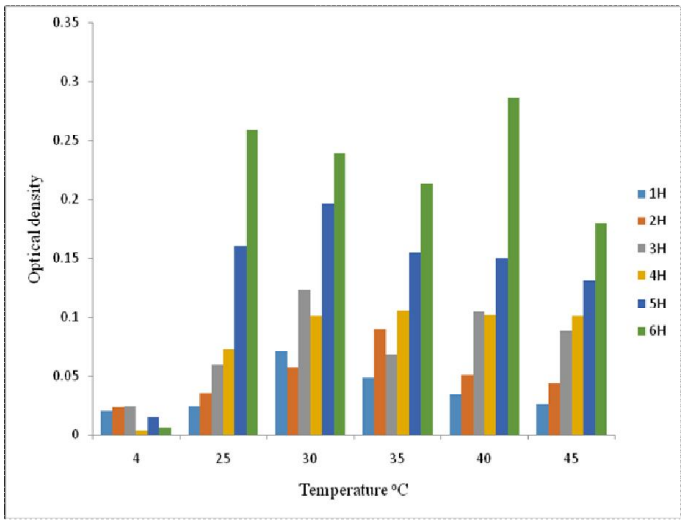

Fig. I: Effect of varying temperatures with change in time on the growth of *P aeruginosa*

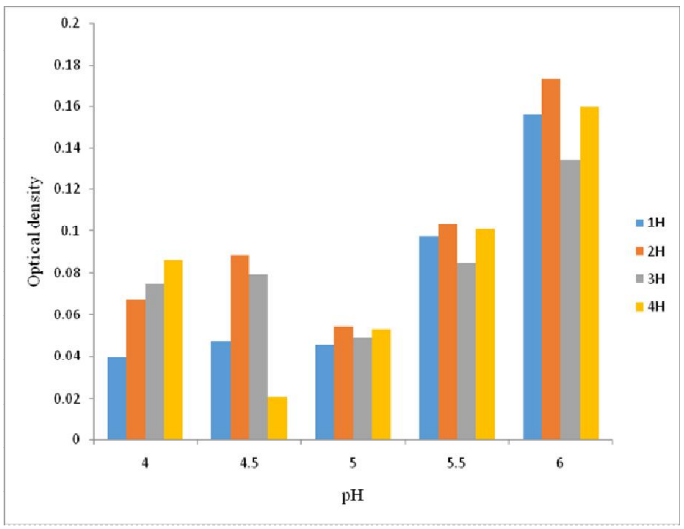

Fig. II: Effect of pH with change in time on the growth of *P. aeruginosa*

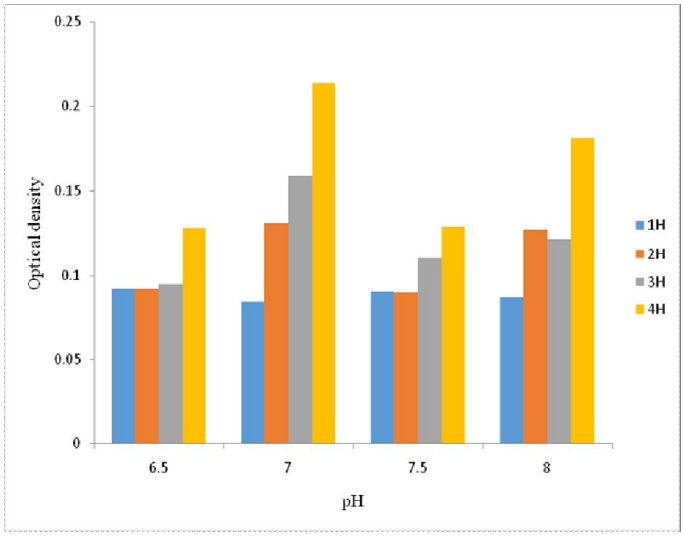

Fig III: Effect of pH with change in time on the growth of *P. aeruginosa*

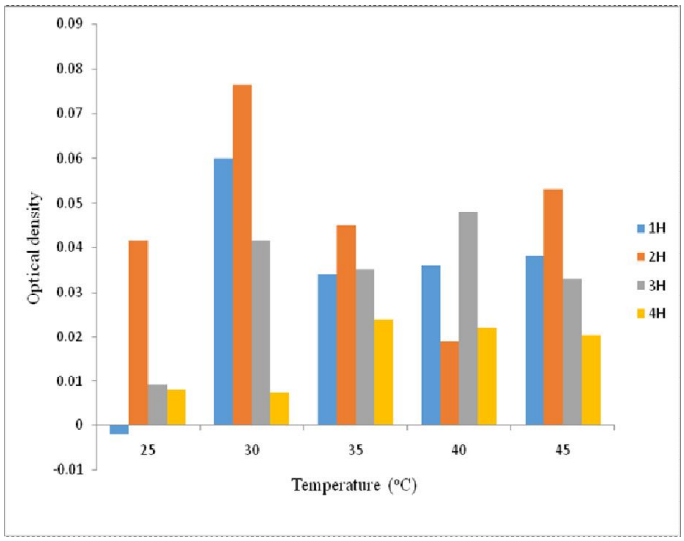

Fig. IV: Effect of varying temperatures with change in time on *P. aeruginosa* at pH 5.

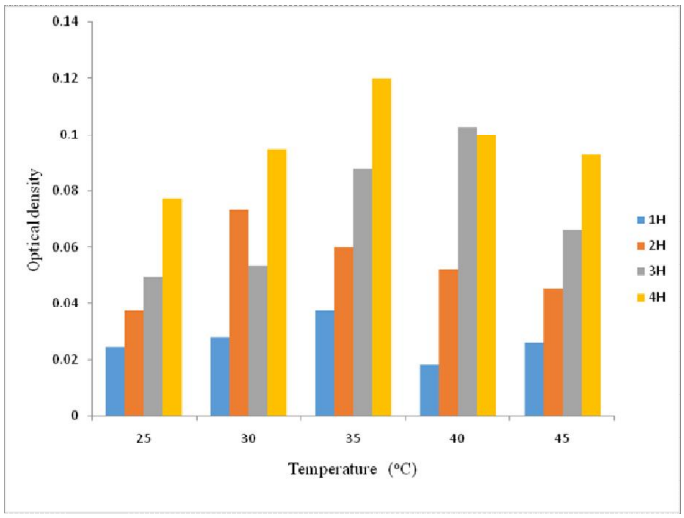

Fig. V: Effect of varying temperatures with change in time on the growth of *P. aeruginosa* at pH 5.5

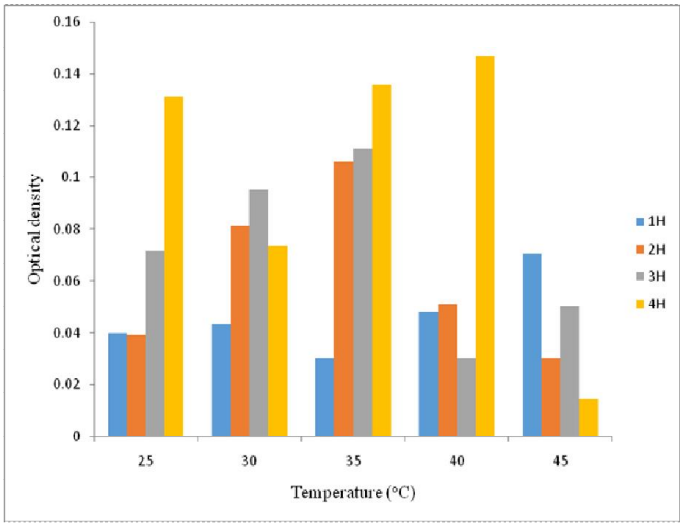

Fig.VI. Effect of varying temperatures with change in time on the growth of *P. aeruginosa* at pH 6

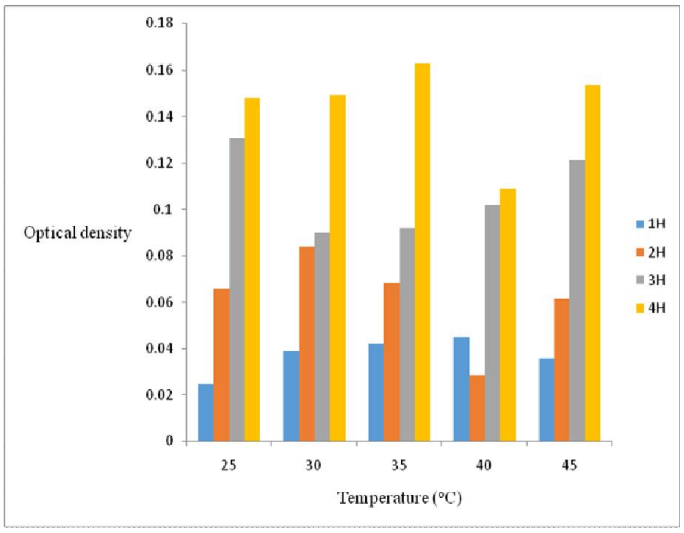

Fig. VII. Effect of varying temperatures with change in time on the growth of *P. aeruginosa* at pH 6.5

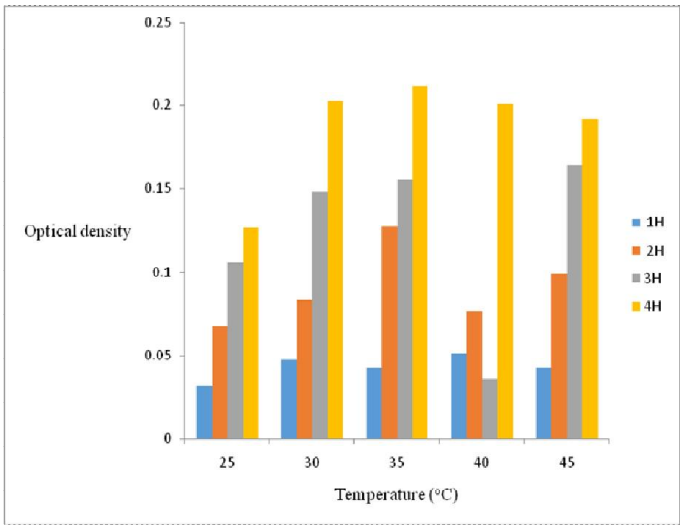

Fig. VIII. Effect of varying temperatures with change in time on the growth of *P. aeruginosa* at pH 7

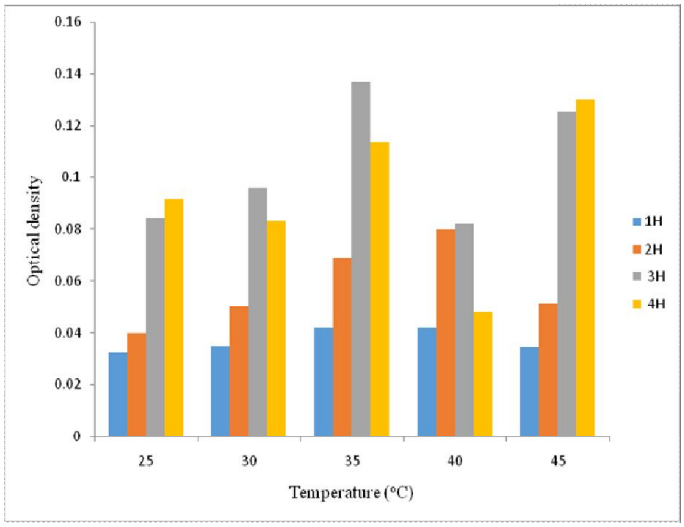

Fig. IX. Effect of varying temperature with change in time on the growth of *P. aeruginosa* at pH 7.5

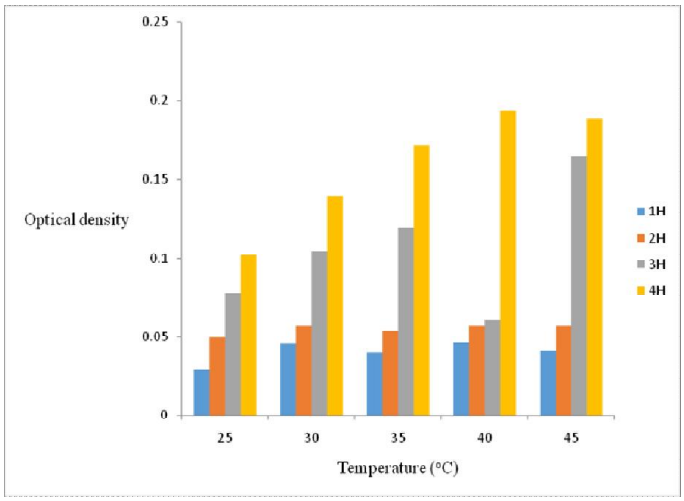

Fig. X. Effect of varying temperature with change in time on the growth of *P. aeruginosa* at pH 8.0

At pH 7.5 growth increased as time increased but declined at the 4th hour for the various temperature except for temperature at 45 ^{0}C, where growth continued to the 4th hour. The varying temperature effects at pH 8.0 with change in time on the growth of *P. aeruginosa* are illustrated in Fig.10. The result indicated that at pH 8.0 growth of the *P. aeruginosa* was highest at temperature 40 ^{0}C followed by growth at 45 ^{0}C, 35 ^{0}C, 30 ^{0}C and 25 ^{0}C. At pH 8.0 growth increased as time increased for the various temperatures; growth was highest at the 4th hour.

DISCUSSION

Whilst a broad limit of factors e.g. pH, temperature, moisture content and inclusion of preservatives are all contributory to controlling bacterial growth, pH and temperature are principal controlling factors. *Pseudomonas aeruginosa* is a critical cause of contamination of cosmetic preparations. The consistent presence of *Pseudomonas aeruginosa* in cosmetics formulations may be as a result of its presence on ingredients and materials used during production. *Pseudomonas*

aeruginosa grows at temperature within the ambit of 30°C to 45 °C depending on other environmental factors with an optimum temperature limit of 35° to 37 °C. The pH growth range is 4.5 - 8.0 with optimum pH of 7.0 to 7.5. But growth at any pH is also influenced by other environmental factors. Growth at any particular pH is can also be influenced by the acid used to modify the pH. These characteristics enable *Pseudomonas aeruginosa* to grow and survive in a broad limit of environmental conditions and also to persist in stressful environments for long periods.

Effect of varying temperature on the growth of *Pseudomonas aeruginosa*

The most leading environmental factor that affects bacterial growth in cosmetic preparation is temperature. The temperature history between the manufacturing and in-use of cosmetic preparations changes with time.

The resultant influence of temperature with change in time on the microbial growth of *P. aeruginosa* at varying temperatures is demonstrated in Fig.1. The result stated that growth was low at 4^0C and highest at 40^0C. At temperature 4^0C growth was seen to accrue at minimal rate from 1st to 3rd hour and decrease from 4th hour. At temperature 4 ^{0}C, *P. aeruginosa* increased in growth as time increased from 1st hour to 3rd hour. Then there was a decrease from the 4th hour. At temperature 4 ^{0}C, the growths of most bacteria are slow. Refrigerator temperatures do not destroy pathogenic or spoilage microorganisms. The lower temperature does, however, reduce the speed of growth of organisms already existing in the cosmetic product. Time and temperature of storage are main factors in brand quality. At temperature 25^0C, *Pseudomonas aeruginosa* increased in growth as time increased from 1st to 6th hour. Increase in growth was slow from 1st hour to 4th hour while from the 4th hour to the 5th hour and from the 5th to 6th hour, increase in growth was prominent. At temperature 30^0C, *P. aeruginosa* fluctuated in growth at the 2nd and 4th hours but increased in growth as time increased from 5th to 6th hour. Increase in growth was gradual from 1stto 4th hour but greater at 25^0C; increase in growth was prominent. At temperature 35^0C, *P. aeruginosa* fluctuated in growth only at the 2nd hour but increased in growth as time increased from 4th to 6th hour. Increase in growth was slow from 1sthour to 4th hour but greater than observed at 25^0C; increase in growth was prominent. At temperature 40^0C, *P. aeruginosa* increased in growth as time increased from 1st to 6th hour, but with a slight decline at the 4th hour. Increase in growth was prominent between the 5th and 6th hour. At temperature 45^0C, *P. aeruginosa* increased in growth as time increased from 1st to 6th hour. Increase in growth was gradually slow from 1st to 6th hour.

Effects of varying pH with change in time on the growth of *Pseudomonas aeruginosa*

The resultant effects of varying pH with change in time on *P. aeruginosa* growth pattern is demonstrated in Figs. 2 and 3. The results stated that the organism's growth was highest at pH 7.0 followed by pH 6. The influence of pH varied across time; at both pH 4 and pH 7 increase in growth occurred as time increased. Bacteria exhibit varying tolerances to pH. Growth of *P.aeruginosa* was seen at a low pH 4, demonstrating the capability of *P.aeruginosa* to grow under acidic pH. However, growth of *P.aeruginosa*at low pH can be affected by the acid used in adjusting the pH. Growth increase was gradual and slowly at pH 4, and increased over time from 1st hour to 4th hour. Most bacteria are inhibited at pH as low as 4. At pH 4.5, growth of *P.aeruginosa* was also seen, showing the capability of *P.aeruginosa* to increase and survive under acidic pH. At pH 4.5, growth increased with time from 1st to 2nd hour and declined minimally to the 3rd hour and 4th hour. At pH 5.0, 5.5 and 6.0 growth of *P.aeruginosa* was observed, but fluctuated with time form 1st hour to 4th hour. *Pseudomonas aeruginosa* increased in growth from 1st hour to 2nd hour, declined at 3rd hour and went up at the 4th hour. At pH 6.5 and 7.5, growth of *P.aeruginosa* was observed. There was no appreciable increase in growth between the 1sthour and 2nd hour, but growth increased slightly to 3rd hour and the 4th hour. For pH 7.0, growth of *P.aeruginosa* was significant. Increase in growth occurred as time increased from 1st hour to 4th hour. At pH 8.0, growth increase of *P.aeruginosa* was observed. Growth increased with time from 1st hour to 2nd hour, with a decline at the 3rd hour and finally increased at the 4th hour.

Combined effect of varying temperatures and pH with change in time on the growth of *Pseudomonas aeruginosa*

The resulting effect of pH over the range 5 - 8 on growth of test organism (*Pseudomonas aeruginosa*) was understudied at varying temperatures of25^0C, 30^0C, 35 ^{0}C, 40^0C and 45^0C. The combining effect of varying temperatures at varying pH with change in time on *P. aeruginosa* growth was observed. A rise in temperature of any solution will result to a decline in viscosity and hence a consequent increase in the mobility of its ions in solution. Also a rise in temperature may result to a rise in the number of ions in solution caused by the dissociation of molecules; this is specifically true for weak acids and bases. During growth process, the microorganism may produce waste matter that cause an alteration in pH of the medium and consequently affects growth of the organism. Barron *et al*. showed that increase in temperature brought about a decline in pH values of a pH buffer. When a several factors are acting on the organism, the growth is also affected. The combination effect produced a growth pattern that is different when the factors are acting independently.

Combined effect of varying temperatures and varying pH (5-8) with change in time on the growth of *Pseudomonas aeruginosa*

The resultant effects of varying temperatures and pH 5 with change in time on growth of *P. aeruginosa* are demonstrated in Fig. 4. The result obtainable from this analysis has demonstrated that pH 5, growth of the organism was highest at temperature 30^0C at the 2nd hour. The combined environmental factors produced a different growth pattern. Growth increased during the 1st hour to the 2nd hour and declined at the 3rd hour and 4th hour at 30^0C, 35^0C and 45^0C. Growth pattern was not ascertained at 25^0C and 40^0C as it fluctuated in between times from 1st to 4th hours. This could be attributable to the low pH medium into which the microorganism was grown. The resultant effects of varying temperatures and pH 5.5 with

change in time on the growth of *P. aeruginosa* is demonstrated in Fig. 5. The result obtainable from this analysis has demonstrated that pH 5.5, organism's growth was greatest at temperature 35^0C at the 4th hour. There was a gradual increase in growth with subsequent increase in time at 25^0C, 35^0C and 45^0C; while growth declined at 3rd hour and 4th hour for temperatures 30^0C and 40^0C respectively. The resulting effect of varying temperatures and pH 6.0 with change in time on the growth of *P. aeruginosa* is demonstrated in Fig. 6. The result obtainable from this analysis has demonstrated that at pH 6.0 *organism's* growth was greatest at temperature 40^0C at the 4th hour followed by growth at 35^0C. For pH 6.0, growth fluctuates in between time in every temperature (25^0C, 30^0C, 40^0C, 45^0C) except for 35^0C temperature where growth of *P.aeruginosa* increased as time increased from 1st hour to 4th hour. Growth was highest at the 4th hour. The resulting effect of varying temperatures and pH 6.5 with change in time on the growth of *P. aeruginosa* is shown in Fig.7. The result obtainable from this test has demonstrated that pH 6.5, the growth *P. aeruginosa* was greatest at temperature 35^0C at the 4th hour followed by growth at 45 ^{0}C, 30 ^{0}C, 25 ^{0}C. Also at pH 6.5 growth increased as time increased from 1st hour to 4th hour for the various temperatures except for temperature 40 ^{0}C where growth fluctuated in between time with a decline at the 2nd hour. Growth was greatest at the 4th hour. The resulting effect of varying temperatures and pH 7.0 with change in time on the growth of *P. aeruginosa* is demonstrated in Fig.8. The result obtainable from this analysis has demonstrated that at pH 7.0, growth of *P. aeruginosa* was greatest at temperature 35^0C followed by growth at various temperatures (30 ^{0}C, 40 ^{0}C, 45 ^{0}C and 25 ^{0}C). Also at pH 7.0, growth increased as time increased from 1st hour to 4th hour for the different time intervals except for temperature 40 ^{0}C where growth fluctuated in between time. Growth was highest at the 4th hour. The resulting effect of varying temperatures and pH 7.5 with change in time on the growth of *P. aeruginosa* is demonstrated in Fig. 9. The result obtainable from this analysis has demonstrated that pH 7.5, growth *P. aeruginosa* was greatest at temperature 35 ^{0}C followed by growth at 45 ^{0}C. At pH 7.5, growth increased as time increased but declined at the 4th hour for the different temperatures (30^0C, 35 ^{0}C, 40 ^{0}C) with exception to temperatures at 25 ^{0}C and 45 ^{0}C, where growth continued to the 4th hour. The resulting effect of varying temperatures and pH 8.0 with change in time on the growth of *P. aeruginosa* is demonstrated in Fig. 10. The result obtainable from this analysis has demonstrated that at pH 8.0, growth of *P. aeruginosa* was greatest at temperature 40 ^{0}C followed by growth at 45 ^{0}C, 35 ^{0}C, 30 ^{0}C and 25 ^{0}C. Also a steady rise occurred as time increased from 1st hour to 4th hour for the various temperatures; growth was greatest at the 4th hour.

Conclusion

Cosmetics and topical products need not be sterile but may comprise of limit levels of contaminating microorganisms during in-use by consumers (Hugo *et al*., 2003). Storage conditions vary with individuals and according to their peculiar environment where they reside. Environmental factors influence significantly the condition of products during in-use by consumers and storage. Product may get contaminated during storage and it is therefore paramount to provide an unfavourable condition that will hinder the growth of contaminants. In this present study, it was shown that different combinations of pH and temperature can influence largely the growth of *P.aeruginosa*. Growth of *P.aeruginosa* can be suppressed at refrigeration temperature (4.4 °C) and by appropriately lowering the pH level. It was observed that the combined effects of varying pH and temperature influenced growth significantly compared with the impact of a single environmental factor. Temperature plays a fundamental role in these effects. Higher temperatures encourage the growth of organisms. There is therefore need to suggest that in the tropics, lotions and creams should be stored in cool place preferably at refrigeration temperature to discourage microbiological contamination. Temperature affects the growth of microbes. Temperature is relatively high in the tropical region therefore temperature factor should be emphasized. The microbial contamination of cosmetic brands may ensue ahead in the course of manufacturing, through ingredients, raw materials, and handling or the contamination of a final product may occur through its continual use by the end-user. Once contaminated, temperature begins to affect the extent of deterioration of the products.

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