Bacterial surface charge in "layers": revealed by wash buffers of different ionic strength

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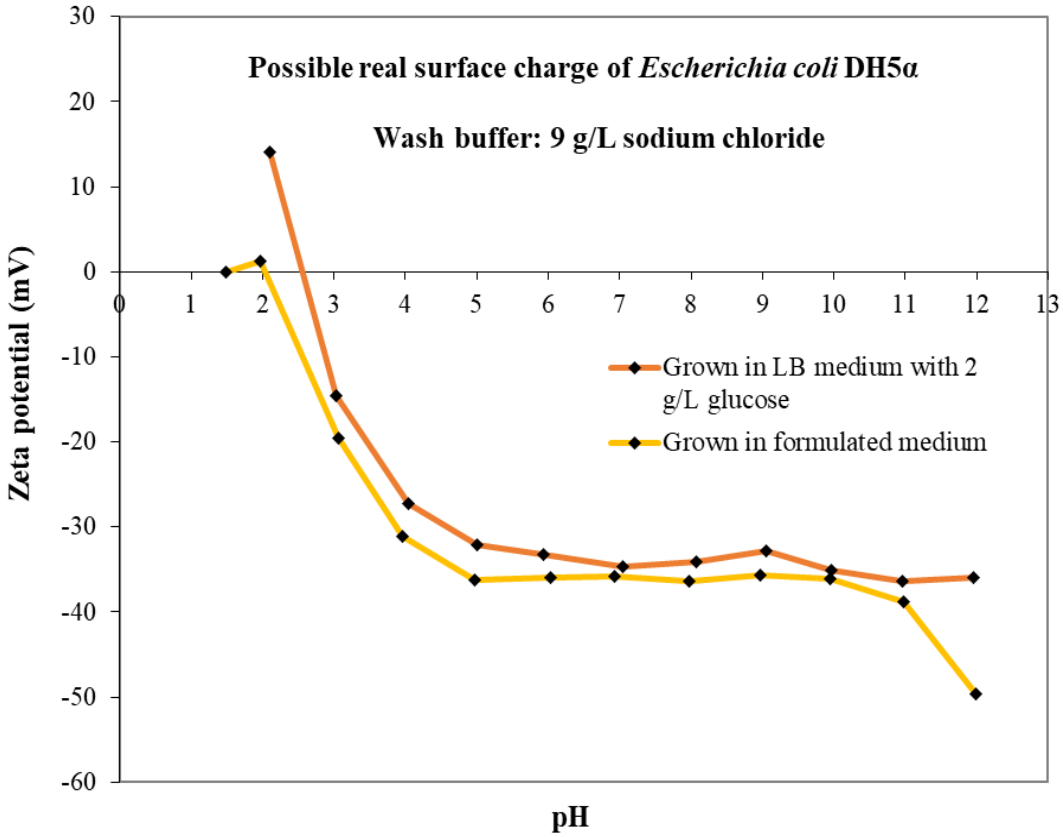
Graphical abstract



Short description

The bacterial cell surface adsorbs and desorbs ions and molecules from its surrounding solution; thus, its surface charge characteristics is dependent on its solution environment. However, there is always a desire to determine the real surface charge of a bacterial cell, which may be shrouded with layers of nonspecifically adsorbed ions and molecules. Using wash buffers of different ionic strength, this study possibly unmasked the real surface charge of *Escherichia coli* DH5α (ATCC 53868) through ionic strength mediated charge screening that removed nonspecifically adsorbed ions and molecules from the cell surface. In addition, the study also suggested that the minimum ionic strength required for complete removal of nonspecifically adsorbed ions and molecules and revealing the real surface charge was 0.15M. Finally, high ionic strength wash buffer such as 0.6M

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sodium chloride and 0.1M sodium citrate could remove ions intrinsic to the cell envelope, and result in altered cell surface characteristics. More importantly, possible adsorption of citrate ions onto the cell surface render 0.1M sodium citrate not suitable as wash buffer for sample preparation for zeta potential analysis.

Abstract

Bacterial surface charge derives its meaning from the cell's environment such as the solution in contact with the cell. Determining the surface charge of bacteria in its native environment requires measuring the proxy variable, zeta potential, using cells obtained from field studies. However, lack of adequate cell mass and concerns over measurement of a mixed species consortia rather than a specific species meant that bacterial surface charge measurement require biomass obtained from pure culture. Often grown in rich medium where myriad proteins and ions nonspecifically adsorbed onto the cell envelope or peptidoglycan layer, standard procedures for preparing the cell mass incorporated repeated steps of washing and centrifugation with various wash buffers, the efficacies of which are poorly understood. This report describes the results of a systematic study on how wash buffers of different composition and ionic strength affect the efficiency of removing nonspecifically adsorbed biomolecules and ions from *Escherichia coli* DH5α (ATCC 53868) cultured aerobically (shake flask, 37 ^{o}C and 230 rpm) in LB Lennox medium with 2 g/L glucose and a formulated medium. Using zeta potential-pH profiles over pH 1 to 12 as readout, the results showed that efficiency of removing nonspecifically adsorbed ions and metabolites positively correlated with wash buffer ionic strength. More importantly, 0.15M ionic strength (i.e., 9 g/L NaCl) seemed to be the minimum below which there was incomplete removal of nonspecifically adsorbed biomolecules. On the other hand, high ionic strength of 0.6M (e.g., 0.1M sodium citrate) significantly changed the point of zero charge (pH_{zpc}), a reference marker for removal of ions intrinsic to the cell envelope. Collectively, results obtained inform wash buffer choice with regards to preserving cell envelope integrity, and avoidance of adsorption of buffer ions such as citrate. But, is there a true cell surface charge? Yes, but how do we define it in number of "layers" of adsorbed biomolecules? Philosophically, cells in culture broth are coated with layers of metabolites, proteins and ions. Hence, desire to reveal the true surface charge is essentially a decoating process, where wash buffers of increasing ionic strength remove each layer via charge screening. However, where is the endpoint? This research offers a different perspective and answer. Imagine a single bacterium suspended in LB medium, where there is constant adsorption and desorption of biomolecules as the cell grows: what is its relevant surface charge? It is the one where the loosely associated ions and metabolites are removed while retaining the nonspecifically adsorbed ions and biomolecules. Thus, deionized water wash provides a good estimate of the bacterial surface charge as grown in specific medium.

Keywords: zeta potential, shear plane, cell surface, bacteria, wash buffer, adsorption, surface charge, point of zero charge, deionized water, resuspension buffer,

Subject areas: microbiology, biochemistry, bioengineering, biophysics, biotechnology,

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Significance of the work

Masked by layers of nonspecifically adsorbed ions and molecules, it is difficult to determine the real surface charge of bacterial cells. Using wash buffer of increasing ionic strength and zeta potential as proxy readout of surface charge, this study illustrated the possibility of revealing the real surface charge characteristics of *Escherichia coli* DH5α (ATCC 53868), as well as providing guidelines on the selection of wash buffers for sample preparation for zeta potential analysis. Specifically, 0.15M sodium chloride wash buffer likely removed almost all of the nonspecifically adsorbed ions and molecules from *E. coli* DH5α cell surface, while 0.6M ionic strength wash buffer such as 0.1M sodium citrate and 0.6M sodium chloride removed ions intrinsic to the cell envelope, thereby, altering cell surface characteristics. Most importantly, citrate ions from 0.1M sodium citrate likely adsorbed to the cell surface. Thus, 0.1M sodium citrate and 0.6M sodium chloride are not suitable as wash buffers for preparing *E. coli* DH5α cells for zeta potential analysis. Collectively, removal of nonspecifically adsorbed ions and molecules increased with increasing ionic strength of wash buffer; however, a limit existed before the removal of ions intrinsic to the cell envelope set in. The threshold ionic strength for completely removing the nonspecifically adsorbed ions and molecules was likely specific to the cell wall structure of the bacterium and growth medium used. Finally, choice of anions played critical roles in ensuring the fidelity of zeta potential measurement, given the propensity of citrate ions in adsorbing to the cell surface and altering cell surface charge. But, in revealing the real surface charge of *E. coli* DH5α, the research also set forth another question: what is the relevant surface charge of cells in a growth medium? It is the one where the loosely bound ions and metabolites are removed from the cell surface, for example, via deionized water wash buffer.

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Highlights

1) Different cell surface charge characteristics were observed for *Escherichia coli* DH5α (ATCC 53868) grown in LB Lennox with 2 g/L glucose medium and a formulated medium; thereby, highlighting possible physiological adaptation to growth in different growth medium.

2) Wash buffer of increasing ionic strength was correlated with more negatively charged zeta potential-pH profiles of *E. coli* DH5α.

3) Wash buffer of 0.15M ionic strength such as 9 g/L sodium chloride likely removed most of the nonspecifically adsorbed ions and molecules, and revealed the real surface charge of *E. coli* DH5α.

4) On the other hand, wash buffer of 0.6M ionic strength likely removed ions intrinsic to the cell envelope of *E. coli* DH5α, resulting in altered cell surface charge characteristics that manifested as altered point of zero charge, pH_{zpc}.

5) 0.1M sodium citrate was not suitable as wash buffer for sample preparation for zeta potential analysis given the adsorption of citrate ions to the cell surface. Its high ionic strength also possibly altered the surface characteristics of *E. coli* DH5α.

Introduction

Conferred by the functional groups present on the cell surface, electrical charge on bacterial cell surface play many roles in mediating interactions of the cell with its environment.[1] For example, cell surface charge mediates the adhesion of cells to minerals and other surfaces,[2 3 4 5] potentiate the binding of metal ions to the cell surface,[6 7] and influence the aggregation of multiple cells into a cluster.[8] Thus, much interest exists in the accurate determination of the bacterial cell surface charge, and instrumented techniques such as microelectrophoresis light scattering play important roles in facilitating the measurement.[9 10 11 12]

Known as electrophoretic mobility analysis or zeta potential measurement, the method involves the forced movement of bacterial cells in a cell suspension under an applied electric field. With light scattering of cells during movement providing the mobility of cells under the electric field, zeta potential of cells (in mV) could be calculated from the raw data, and constituted a proxy parameter for the actual cell surface charge.[3 11] Defined at the shear plane which is a short distance away from the actual cell surface, zeta potential measurement is vulnerable to the nonspecific adsorption of ions and molecules within the inner layer of the electrical double layer surrounding a cell. Thus, the goal of sample preparation for zeta potential measurement should be the removal of the nonspecifically adsorbed ions and molecules from the cell surface. To this end, various wash buffers such as 9 g/L NaCl, 0.1M sodium nitrate and phosphate buffered saline (PBS) are available. However, the relative efficacy of the wash buffers in removing nonspecifically adsorbed ions and molecules remain poorly understood.

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Escherichia coli is a common gut commensal bacterium that could also be found in various matrixes such as water and soil. Gram-negative and facultative anaerobic, *Escherichia coli* is an indicator organism for microbial contamination of groundwater and surface water. Known to adhere to various minerals and soil matrixes, the transport and adhesion of *E. coli* to different environmental matrixes is of special interest from the perspective of modelling its movement and fate within the surface and subsurface environment.[13] Surface charge is thought to play important roles in governing the movement, adhesion and retention of *E. coli* on various matrixes in the environment.[14 15] Conferred by various functional groups such as phosphate groups on the core oligosaccharide of lipopolysaccharide (LPS), as well as amino and carboxyl groups of proteins, the overall surface charge of *E. coli* is likely to be negative at circumneutral pH of 5 and 7. More importantly, the LPS layer of the *E. coli* cell envelope provides many attachment sites for the binding of nonspecifically adsorbed ions and molecules. Hence, given the dense array of LPS molecules on *E. coli* cell surface, difficulty exists in removing the nonspecifically adsorbed ions and molecules entrapped within the LPS layer. Without the desorption of nonspecifically adsorbed ions and molecules from *E. coli* cell surface, the actual surface charge of the cells could not be unmasked.

Using two model growth medium that differed in salt content, this study examined the relative efficacy of different wash buffers in removing nonspecifically adsorbed ions and molecules from *E. coli* DH5α (ATCC 53868) cell surface. Specifically, *E. coli* DH5α was grown in LB Lennox with 2 g/L glucose (LBG) and a formulated medium (FM) for simulating the adsorption of various ions and molecules from the growth medium onto the cell surface. Subsequently, various wash buffers were used in preparing the cell samples for microelectrophoresis light scattering through a sequence of washing and centrifugation steps, where wash buffers of higher ionic strength could possibly help remove nonspecifically adsorbed ions and molecules through the charge screening effect. Thus, the study provided an opportunity for assessing the relative effectiveness of various wash buffers such as 0.1M sodium nitrate, 0.1M sodium chloride, 0.1M sodium acetate, 9 g/L sodium chloride, phosphate buffered saline, 0.1M sodium citrate and 0.6M sodium chloride in removing nonspecifically adsorbed ions and molecules and revealing the real surface charge of *E. coli* DH5α cells.

Materials and Methods

Materials

LB Lennox medium was purchased from Difco and used as is. Composition of LB Lennox medium was in [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0. Composition of LB Lennox medium with 2 g/L glucose was [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0, D-Glucose, 2.0. Composition of formulated medium was [g/L]: K_2HPO_4, 12.54; KH_2PO_4, 2.31; D-Glucose, 6.0; NH_4Cl, 1.5; Yeast extract, 12.0; NaCl, 5.0; $MgSO_4$, 0.24. Composition of phosphate buffered saline was [g/L]: KCl, 0.2; KH_2PO_4, 0.2; NaCl, 8.0; Na_2HPO_4, 1.15. Ionic strength of wash buffer was estimated by the Debye-Huckel theory.

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Growth of E. coli DH5α in growth medium

Stock cultures of *E. coli* DH5α were kept in 80% glycerol at –70 ºC until use. For the seed culture, one glycerol stock culture of *E. coli* DH5α was used in inoculating 100 mL of LB Lennox medium in a 250 mL glass conical flask with incubation conditions of 37 ºC and 230 rpm rotational speed in a temperature controlled incubator (Yih Der LM-570D, Taiwan). After 8 hours of cultivation, 1 mL of the seed culture was used in inoculating 100 mL of either LB Lennox medium with 2 g/L glucose or formulated medium in 250 mL glass conical flasks. Incubation conditions were 37 ºC and 230 rpm rotational shaking in a temperature controlled incubator. Two biological replicates were performed.

Sample preparation for zeta potential analysis

After 15 hours of culture, 2.5 mL of experiment cultures was withdrawn and added to 37.5 mL of non sterile wash buffer in a 50 mL polypropylene centrifuge tube. The contents were vigorously shaken by hand and subsequently centrifuged at 3300 x g for 10 minutes at 25 ºC. The supernatant was carefully decanted and the cell pellet resuspended in 40 mL of fresh wash buffer. The washing and centrifugation process was performed a total of three times. Finally, the cell pellet was resuspended in deionized water. pH of the samples was adjusted with nitric acid and sodium hydroxide prior to zeta potential analysis. pH was measured with an Orion 9156 BNWP pH probe outfitted to a Mettler Toledo Delta 322 pH meter.

Zeta potential analysis

The microelectrophoresis cell was rinsed with deionized water three times prior to analysis. Samples were shaken vigorously and introduced to the plastic microelectrophoresis cell with care taken to avoid bubble formation. Analysis was performed with Malvern's Zetasizer Nano ZS instrument at 25 ºC.

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Results and Discussion

Table 1: Composition of growth media used

Components	LB Lennox + 2 g/L glucose (g/L)	Formulated medium (g/L)
K_2HPO_4		12.54
KH_2PO_4		2.31
D-Glucose	2.00	6.00
NH_4Cl		1.50
Tryptone	10.00	
Yeast extract	5.00	12.00
NaCl	5.00	5.00
$MgSO_4$		0.24

Composition of LB Lennox with 2 g/L glucose (LBG) and formulated medium (FM) was shown in Table 1. Specifically, LBG carries a low salt content compared to FM, which has a high capacity phosphate buffer. Possibilities exist that *E. coli* DH5α grown in FM would have more nonspecific adsorption of ions and molecules on the cell surface compared to cells grown in the low salt LBG medium. Hence, a model system where *E. coli* DH5α had different ensemble of ions and molecules nonspecifically adsorbed to the cell surface after growth in two growth media that differed in salt content was available to understand the relative efficacy of different wash buffers of different ionic strength in removing the nonspecifically adsorbed ions and molecules.

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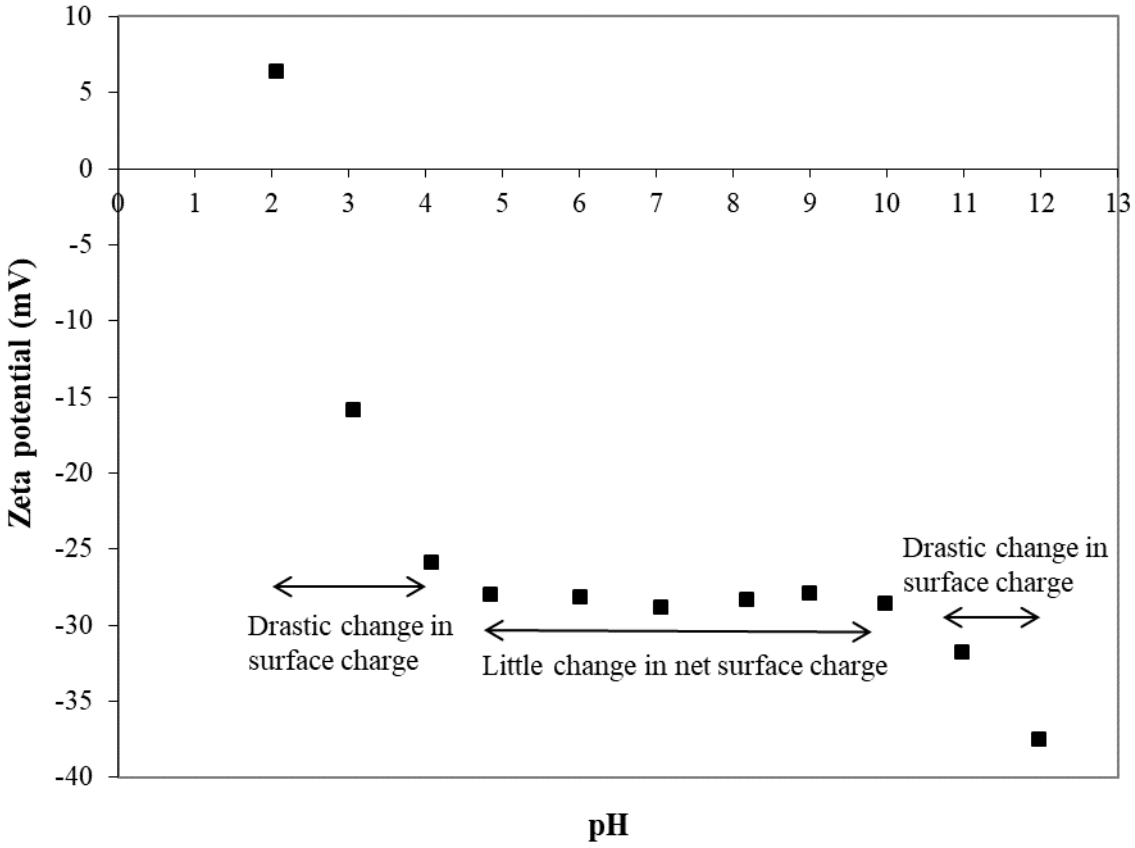

Figure 1: Variation of zeta potential with pH for *E. coli* DH5α grown in LBG medium at 37 ºC. Wash and resuspension buffer used was deionized water.

The zeta potential-pH profile of *E. coli* DH5α is in essence a titration curve where variation in pH revealed changes in zeta potential of the cell surface (Figure 1). The cell surface of *E. coli* DH5α was negatively charged between pH 3 and 12, with drastic decrease in zeta potential between pH 2 and 4, possibly due to deprotonation of $-NH_3^+$ groups on the core lipopolysaccharides.[7] This was followed by a buffering region where there was relatively little change in zeta potential and net surface charge over the pH range from 5 to 10. Finally, drastic decrease in zeta potential was again observed in the pH range from 11 to 12. The point of zero charge (pH_{zpc}) where there was no net surface charge on the cell surface was 2.15 (Figure S1), which highlighted that the cell surface of *E. coli* DH5α was highly negatively charged.

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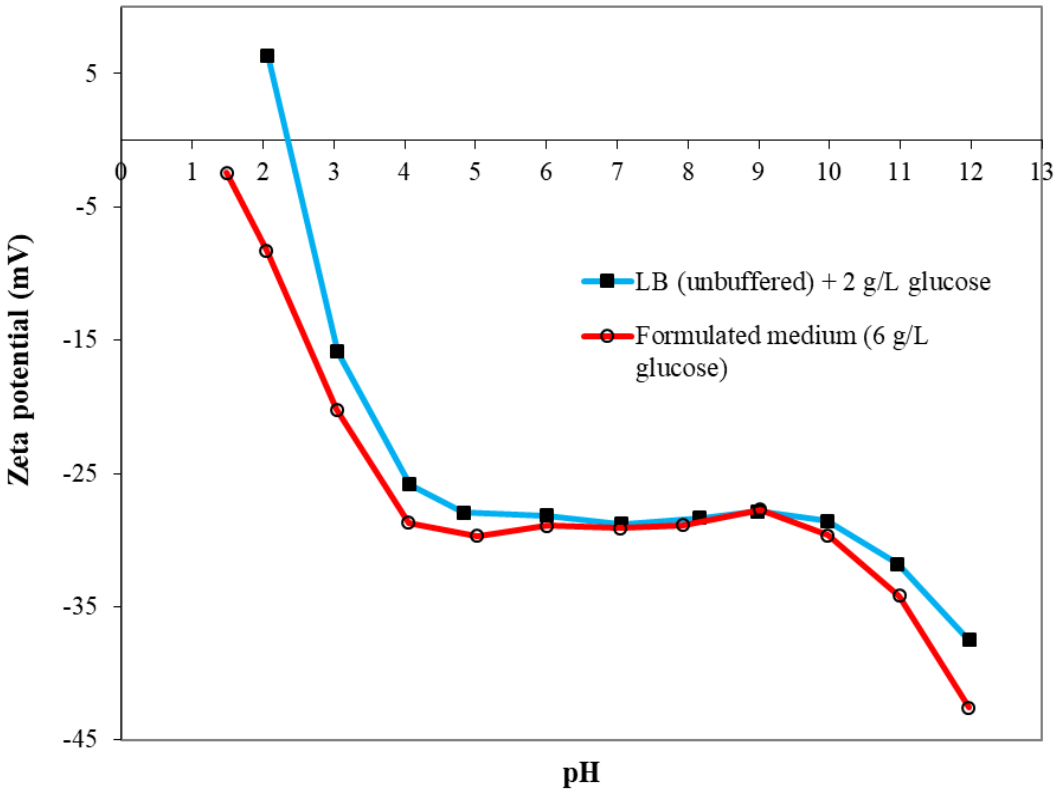

Figure 2: Comparison of zeta potential-pH profiles of *E. coli* DH5α grown in LB Lennox with 2 g/L glucose and formulated medium. Deionized water was used as wash and resuspension buffer.

Zeta potential-pH profiles of *E. coli* DH5α grown in LBG and FM were different, with the profile for *E. coli* DH5α grown in FM being more negatively charged between pH 2 and 4, and between pH 10 and 12 (Figure 2). Additionally, the point of zero charge (pH_{zpc}) for *E. coli* DH5α grown in FM was more acidic compared to that of cells grown in LBG medium. However, cells grown in LBG and FM shared a common buffering region in the zeta potential-pH profile between pH 5 and 10. Observed differences between the zeta potential-pH profiles of *E. coli* DH5α cells grown in LBG and FM could be due to the biosynthesis of different cell surface components at different relative abundances as a result of growth in different growth media. Deionized water was not able to remove the nonspecifically adsorbed ions and molecules from the cell surface of *E. coli* DH5α.

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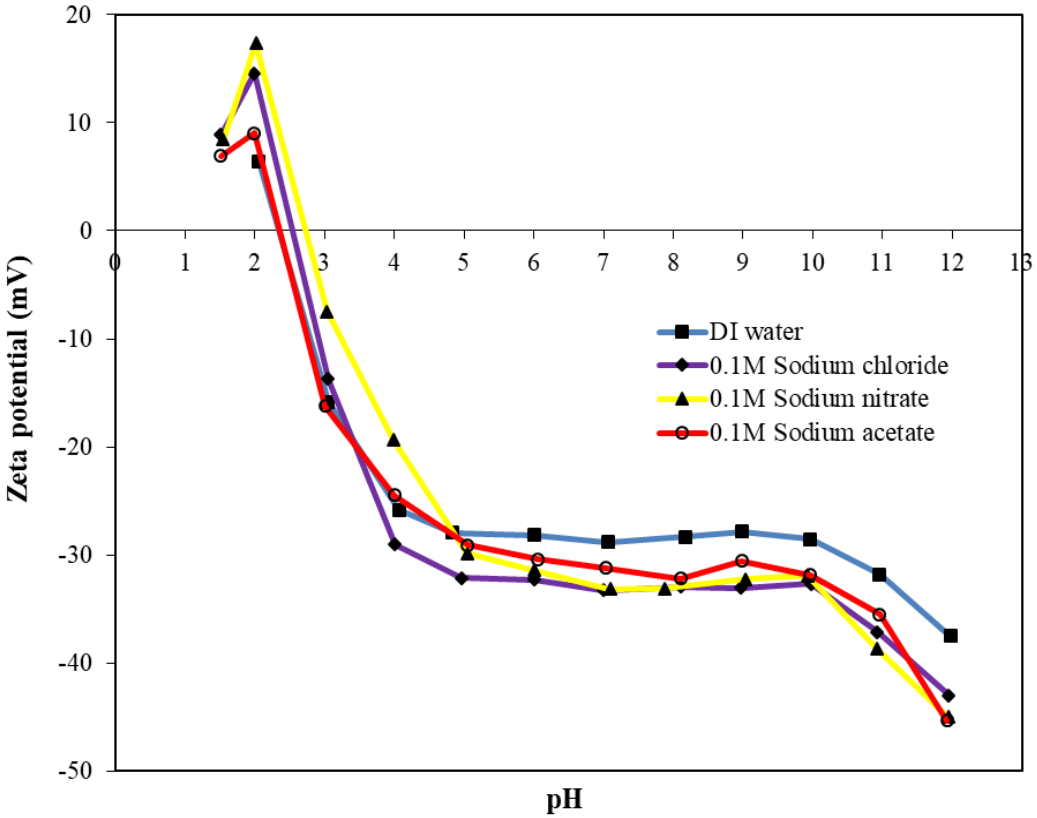

Figure 4: Zeta potential-pH profiles of *E. coli* DH5α cells grown in LBG medium and washed with 0.1M sodium nitrate, 0.1M sodium chloride, and 0.1M sodium acetate. Resuspension buffer used was deionized water.

Comparison of the zeta potential-pH profiles of *E. coli* DH5α grown in LBG medium and washed with 0.1M sodium nitrate, 0.1M sodium chloride, and 0.1M sodium acetate revealed that the profiles coincided with each other within the buffering region between pH 5 and 10 (Figure 4). More importantly, the zeta potential value of the buffering region of cells washed with the three wash buffers was more negative compared to cells washed with deionized water, which indicated that nonspecifically adsorbed ions and molecules could have been removed via ionic strength mediated charge screening. Given that the zeta potential-pH profiles of cells washed with the three wash buffers coincided with each other, it highlighted that similar ionic strength (0.1M) of the wash buffers resulted in similar extent of removal of nonspecifically adsorbed ions and molecules. Additionally, while negatively charged acetate ions could possibly remove cations from the layer of ions and molecules that adsorbed to *E. coli* DH5α surface, experiment results did not highlight that this chelation property contributed to additional removal of nonspecifically adsorbed ions and molecules.

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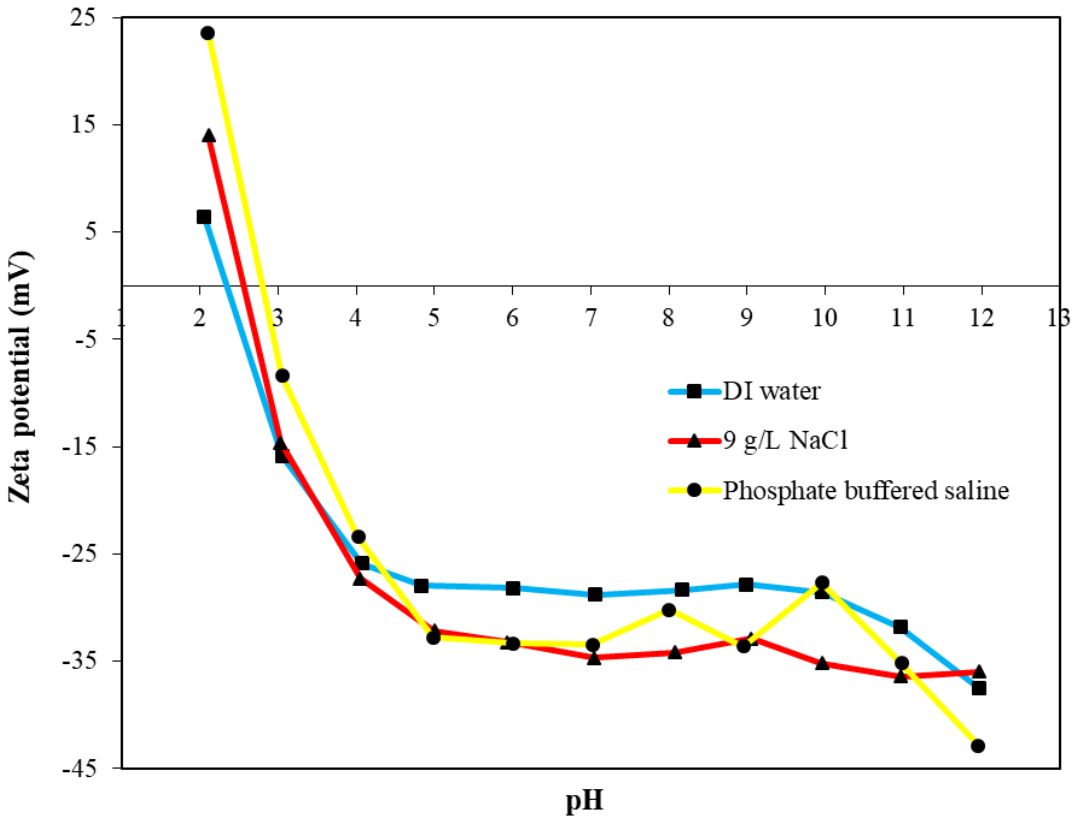

Figure 5: Variation of zeta potential with pH for *E. coli* DH5α cells grown in LBG medium and washed with 9 g/L sodium chloride and phosphate buffered saline (PBS). Cells were resuspended in deionized water prior to zeta potential analysis.

Zeta potential-pH profiles of *E. coli* DH5α cells grown in LBG medium and washed with 9 g/L sodium chloride (0.15M ionic strength) and phosphate buffered saline (0.17M ionic strength) coincided with each other to a large extent, which could be due to similar extent of removal of nonspecifically adsorbed ions and molecules given their similar ionic strength (Figure 5). In general, zeta potential-pH profiles of cells washed with the two wash buffers were more negatively charged in the buffering region from pH 5 and 10 compared to the zeta potential-pH profile of cells washed with deionized water. Additionally, zeta potential-pH profiles of cells washed with PBS had a more alkaline pH_{zpc} compared to the one for cells washed with 9 g/L sodium chloride (Figure S3).

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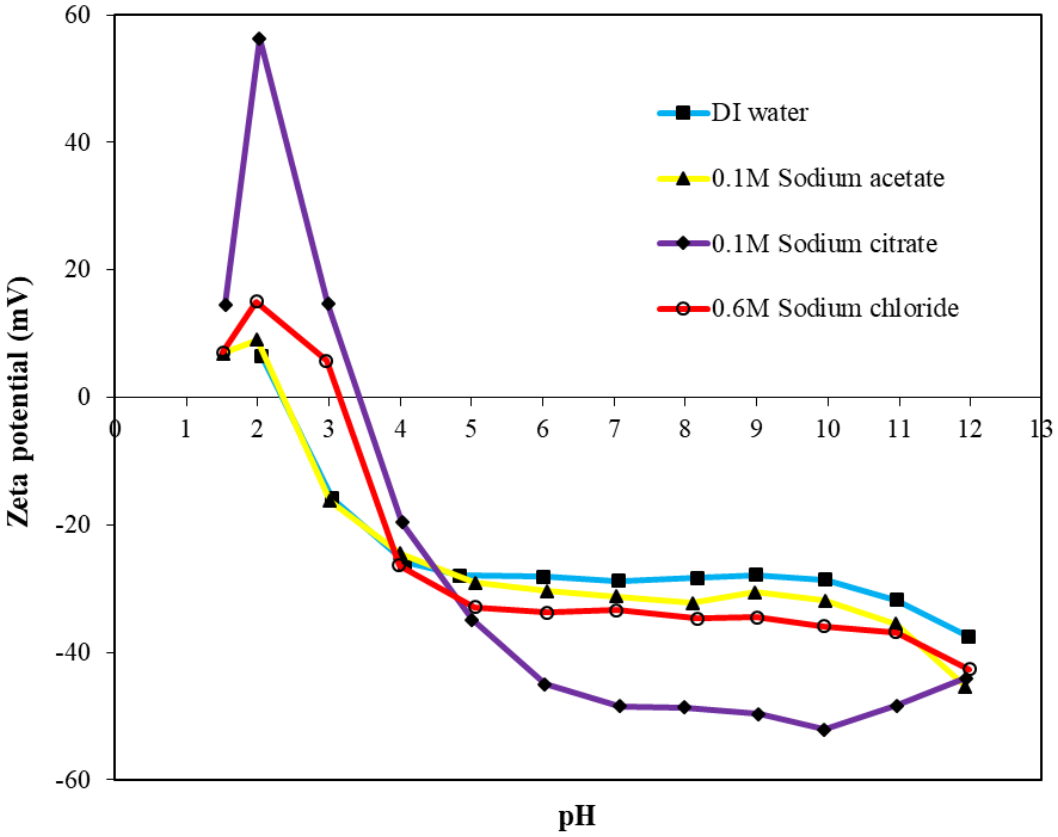

Figure 6: Variation of zeta potential with pH for *E. coli* DH5α cells grown in LBG medium and washed with 0.1M sodium acetate, 0.1M sodium citrate, and 0.6M sodium chloride. Cells were resuspended in deionized water for zeta potential analysis.

Zeta potential-pH profile of *E. coli* DH5α cells grown in LBG medium and washed with 0.1M sodium citrate was significantly more negative between pH 5 and 12 compared to that of cells washed with deionized water, 0.1M sodium acetate, and 0.6M sodium chloride (Figure 6). Additionally, between pH 2 and 3, zeta potential-pH profile of cells washed with 0.1M sodium citrate was more positively charged compared to the other wash buffers, which indicated possible structural changes to the cell surface. More importantly, adsorption of citrate ions onto *E. coli* DH5α cell surface could have resulted in the more negatively charged region between pH 5 and 12. Thus, 0.1M sodium citrate was not suitable as wash buffer for *E. coli* DH5α cells. Given that the ionic strength of 0.1M sodium citrate was 0.6M, experiments were conducted in which 0.6M sodium chloride was used as wash buffer for *E. coli* DH5α cells. Results indicated that the zeta potential-pH profile of cells washed with 0.6M sodium chloride was much less negatively charged compared to that of cells washed with 0.1M sodium citrate. This highlighted that removal of nonspecifically adsorbed ions and molecules could not account for the observed highly negatively charged zeta potential-pH profile of cells washed with 0.1M sodium citrate. Thus, adsorption of citrate ions onto *E. coli* DH5α cell surface might be a strong possibility. On the other hand, the zeta potential-pH profile of cells washed with 0.1M sodium acetate was slightly more positive than

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that of cells washed with 0.6M sodium chloride in the pH range from 5 to 12. This indicated that a 6 fold increase in ionic strength only resulted in a small additional removal of nonspecifically adsorbed ions and molecules that remained on the cell surface.

Thus, in the case of *E. coli* DH5α cells, 0.15M ionic strength seemed to be the threshold at which the nonspecifically adsorbed ions and molecules on the cell surface of the bacterium could be removed after growth in LBG medium. However, given that the amount of nonspecifically adsorbed ions and molecules depended on the cell surface structure and growth medium, wash buffer of different ionic strength may be necessary for removing the nonspecifically adsorbed ions and molecules, and thereby reveal the real surface charge of the cells. On the other hand, 0.1M sodium citrate significantly altered the cell surface charge characteristics of *E. coli* DH5α principally through the adsorption of negatively charged citrate ions on the cell surface as well as removal of ions intrinsic to the cell envelope. Thus, in the use of wash buffer for removing nonspecifically adsorbed ions and molecules from the cell surface, care must be taken not to select anions with the potential for adsorbing to the cell surface and high ionic strength wash buffer must be avoided.

Table 2: Influence of various wash buffers on the point of zero charge (pH_{zpc}) of *E. coli* DH5α grown in LB Lennox + 2 g/L glucose medium

Wash buffer	Ionic strength (M)	Estimated pH_{zpc}
DI water	0.001	2.2
0.1M Sodium chloride	0.100	2.5
0.1M Sodium nitrate	0.100	2.4
0.1M Sodium acetate	0.100	2.5
9 g/L Sodium chloride	0.150	2.8
Phosphate buffered saline	0.170	2.9
0.1M Sodium citrate	0.600	3.7
0.6M Sodium chloride	0.600	Between 3 and 3.5

Table 2 revealed that there was a dependence between point of zero charge (pH_{zpc}) of *E. coli* DH5α grown in LBG medium and the ionic strength of wash buffer used. Specifically, the higher the ionic strength, the more alkaline the pH_{zpc}. For example, the pH_{zpc} of cells washed with deionized water was 2.2, while that for 0.1M ionic strength wash buffer was ~2.5 irrespective of the anion of the wash buffer (i.e., 0.1M sodium chloride, 0.1M sodium nitrate, or 0.1M sodium acetate). On the other hand, 9 g/L sodium chloride and phosphate buffered saline wash buffer resulted in cells having a pH_{zpc} of 2.8 and 2.9, respectively. Finally, 0.1M sodium citrate wash buffer significantly altered the surface charge characteristics of *E. coli* DH5α, with the pH_{zpc} of cells being 3.7 after the washing step. Although with the same ionic strength of 0.6M as 0.1M sodium citrate, 0.6M sodium chloride wash buffer resulted in a smaller shift in pH_{zpc} compared to

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cells washed with deionized water wash buffer. However, the high ionic strength of the wash buffer also possibly removed ions intrinsic to *E. coli* DH5α cell envelope; thereby, resulting in a more alkaline pH_{zpc} of between 3.0 and 3.5.

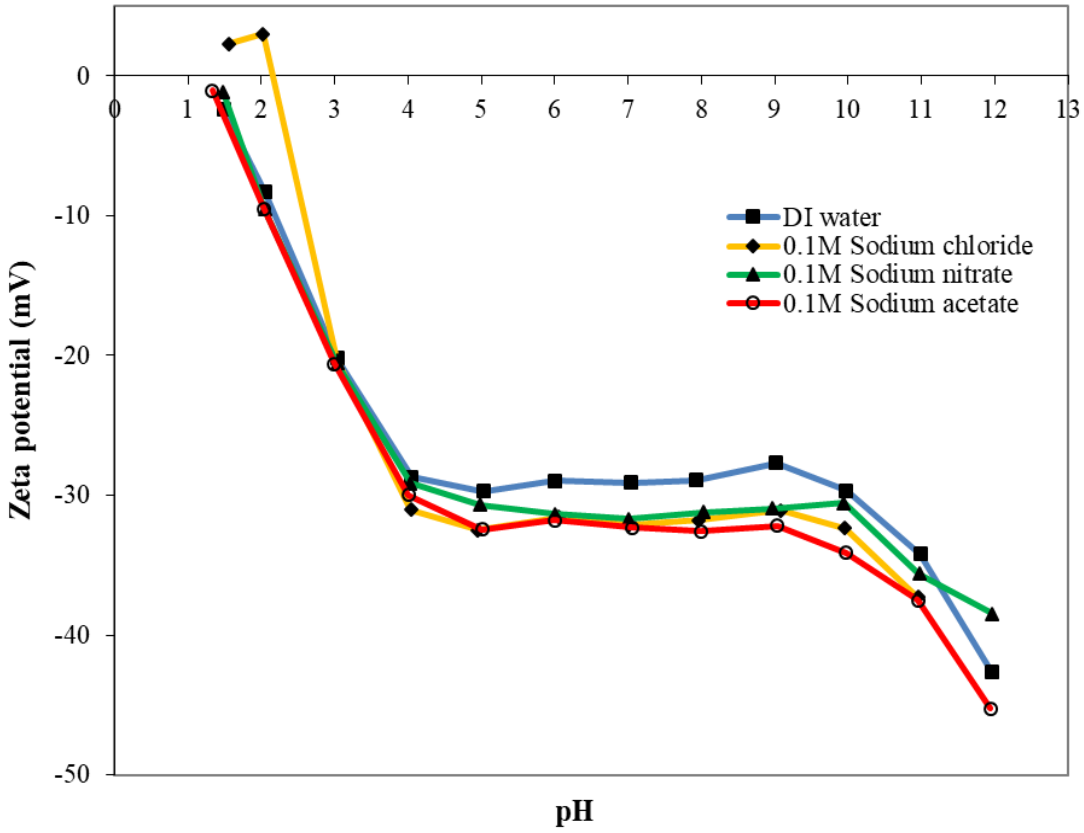

Figure 7: Variation of zeta potential with pH for *E. coli* DH5α cells cultivated in FM and washed with deionized water, 0.1M sodium chloride, 0.1M sodium nitrate, and 0.1M sodium acetate. Cells were resuspended in deionized water for zeta potential measurement.

Zeta potential-pH profiles of *E. coli* DH5α cells grown in FM and washed with 0.1M sodium nitrate, 0.1M sodium chloride, and 0.1M sodium acetate coincided with each other, and were more negatively charged in the pH range from 5 to 12 compared to zeta potential-pH profile of cells washed with deionized water. In the pH range from 1.5 to 3, the zeta potential-pH profiles of cells washed with deionized water, 0.1M sodium nitrate and 0.1M sodium acetate also coincided with each other, which suggested that 0.1M ionic strength wash buffer did not affect the intrinsic surface charge of the cell envelope. On the other hand, 0.1M sodium chloride wash buffer altered the pH_{zpc} of *E. coli* DH5α compared to deionized water, 0.1M sodium nitrate and 0.1M sodium acetate wash buffer. Overall, 0.1M ionic strength wash buffer did remove nonspecifically adsorbed molecules and ions from the surface of *E. coli* DH5α cells to a similar extent; thereby, resulting in a more negative zeta potential-pH profile in the buffering range of pH 5 to 10, where zeta potential did not significantly change with pH variation.

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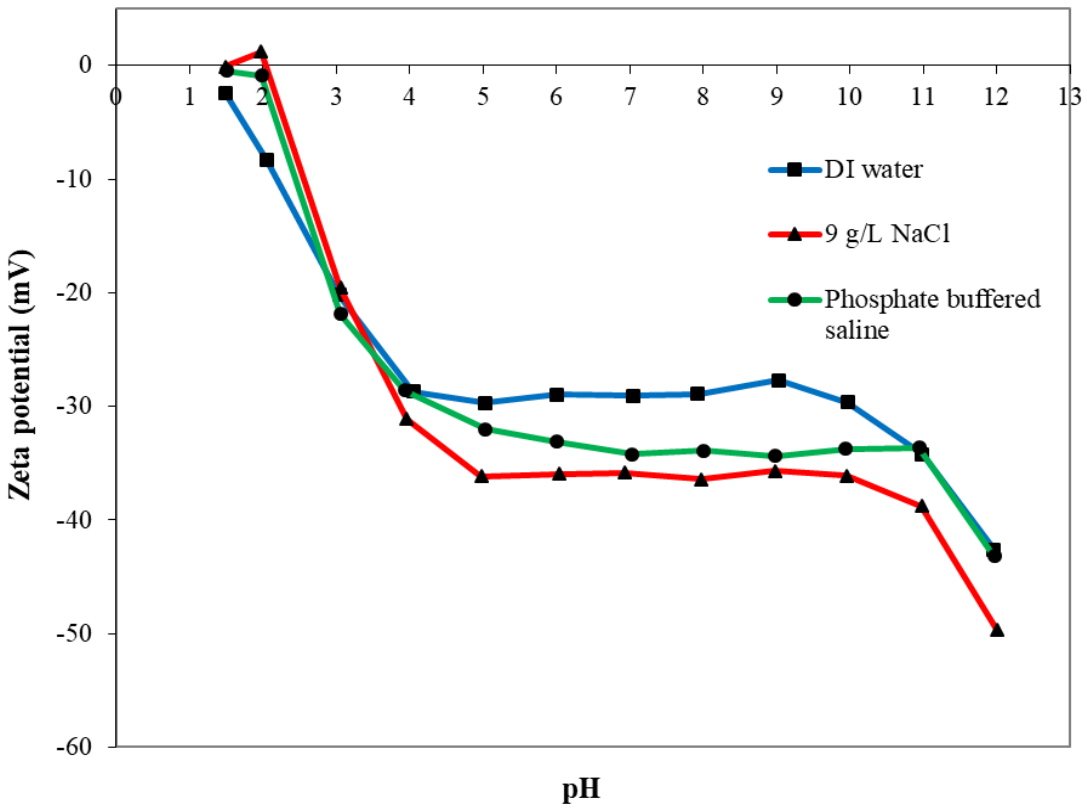

Figure 8: Variation of zeta potential with pH for *E. coli* DH5α cells grown in FM and washed with 9 g/L sodium chloride and phosphate buffered saline (PBS) wash buffer. Cells were resuspended in deionized water for zeta potential analysis.

Comparison of zeta potential-pH profiles of *E. coli* DH5α cells grown in FM and washed with deionized water, 9 g/L sodium chloride and PBS wash buffer revealed that the zeta potential-pH profiles for 9 g/L sodium chloride and PBS wash buffer were more negatively charged in the buffering range from pH 5 to 10 compared to that of deionized water (Figure 8). This indicated that there was removal of nonspecifically adsorbed ions and molecules from the cell surface of *E. coli* DH5α by 9 g/L sodium chloride and PBS wash buffer, which had similar ionic strength. In general, the zeta potential-pH profile of cells washed with 9 g/L sodium chloride was similar to that of cells washed with PBS wash buffer, but was slightly more negatively charged in the pH range from 5 to 12. Additionally, in the pH range from 2 to 4, the zeta potential-pH profiles of cells washed with 9 g/L sodium chloride and PBS wash buffer almost coincided with each other but not with that of cells washed with deionized water. This indicated that 0.15M ionic strength wash buffers such as 9 g/L sodium chloride and PBS was at the threshold of affecting the intrinsic surface charge characteristics of *E. coli* DH5α cells given the changes to the point of zero charge (pH_{zpc}).

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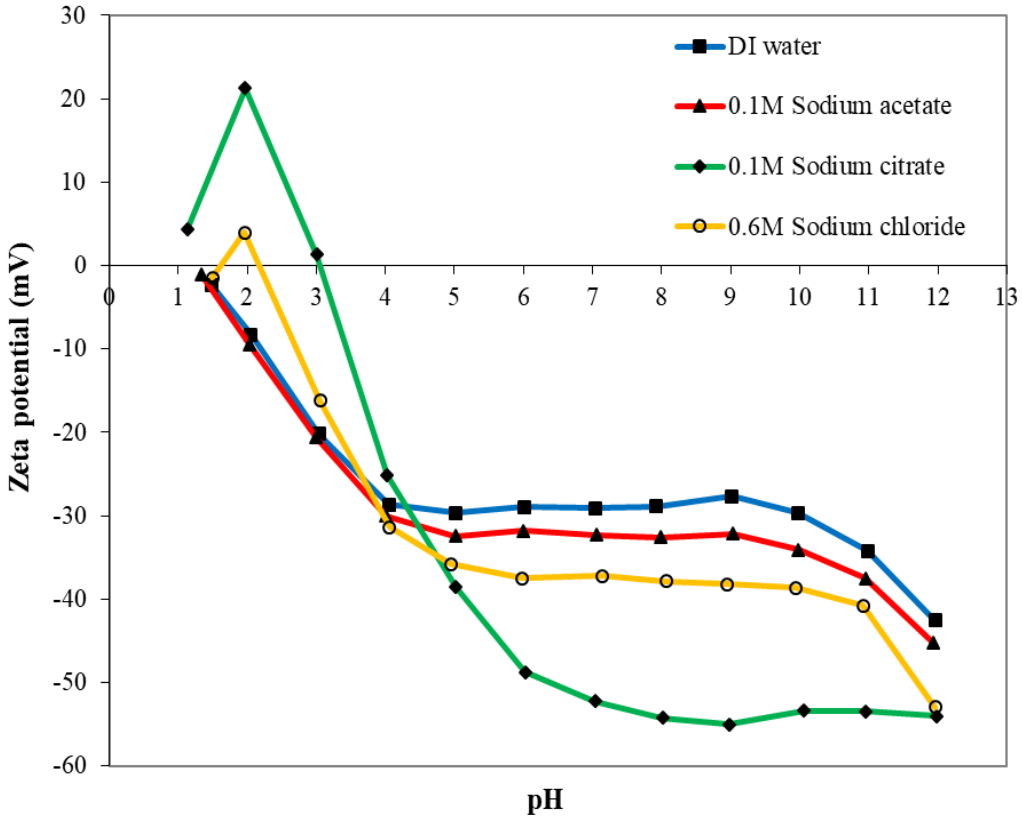

Figure 9: Variation of zeta potential with pH for *E. coli* DH5α grown in FM and washed with 0.1M sodium acetate, 0.1M sodium citrate, and 0.6M sodium chloride. Cells were resuspended in deionized water for zeta potential analysis.

Zeta potential-pH profile of *E. coli* DH5α cells washed with 0.1M sodium citrate was significantly different from that of cells washed with deionized water and other wash buffers (Figure 9). Specifically, the profile was significantly more negatively charged in the pH range from 6 to 12, which suggested that negatively charged citrate ions could have adsorbed to *E. coli* DH5α cell surface. On the other hand, the profile had a pH_{zpc} of pH 3, which was substantially more alkaline compared to that of cells washed with deionized water and other wash buffers. Additionally, between pH 2 and 4, the zeta potential-pH profile of cells washed with 0.1M sodium citrate was more positively charged compared to that of cells washed with other wash buffers. This highlighted that 0.1M sodium citrate could possibly change the surface characteristics of *E. coli* DH5α.

Although with a similar ionic strength of 0.6M compared to 0.1M sodium citrate, 0.6M sodium chloride wash buffer did not generate a zeta potential-pH profile similar to that of 0.1M sodium citrate. This highlighted that ionic strength was not the only factor that impact on the zeta potential-pH profile such as in removing nonspecifically adsorbed ions and molecules. More

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importantly, between pH 2 and 4, the zeta potential-pH profile of *E. coli* DH5α cells washed with 0.6M sodium chloride was substantially different from that of cells washed with deionized water and 0.1M sodium acetate, which indicated possible cell surface changes not related to removal of nonspecifically adsorbed ions and molecules. Hence, ionic strength of 0.6M could potentially remove ions intrinsic to the cell envelope and fundamentally change the surface charge characteristics of the cells beyond the removal of nonspecifically adsorbed ions and molecules.

Thus, nonspecifically adsorbed ions and molecules of cells grown in the two growth media could be removed with 0.15M ionic strength wash buffers such as 9 g/L sodium chloride and PBS. On the other hand, use of 0.1M ionic strength wash buffer such as 0.1M sodium chloride, 0.1M sodium nitrate and 0.1M sodium acetate possibly resulted in the incomplete removal of nonspecifically adsorbed ions and molecules. However, use of wash buffer of higher ionic strength (i.e., 0.6M ionic strength) such as 0.1M sodium citrate or 0.6M sodium chloride could remove ions intrinsic to the cell envelope, and in the former case, results in the adsorption of citrate ions to the cell surface that changed the surface charge characteristics. Hence, solutions with citrate ions should not be used as wash buffers for preparing cell samples for zeta potential analysis.

Table 3: Influence of various wash buffers on the point of zero charge (pH_{zpc}) of *E. coli* DH5α grown in formulated medium

Wash buffer	Ionic strength (M)	Estimated pH_{zpc}
DI water	0.001	1.5
0.1M Sodium chloride	0.100	Between 2 and 3
0.1M Sodium nitrate	0.100	1.5
0.1M Sodium acetate	0.100	1.5
9 g/L Sodium chloride	0.150	2.0
Phosphate buffered saline	0.170	2.2
0.1M Sodium citrate	0.600	3.0
0.6M Sodium chloride	0.600	Between 2 and 3

Table 3 showed the dependence of point of zero charge (pH_{zpc}) of *E. coli* DH5α cells grown in FM and washed with various wash buffers of different ionic strength. In general, as ionic strength of wash buffer increase, the pH_{zpc} of cells washed with the wash buffer became more alkaline. More importantly, wash buffer of similar ionic strength generally resulted in pH_{zpc} of similar value; for example, the pH_{zpc} of cells washed with 0.1M ionic strength wash buffers such as 0.1M sodium nitrate and 0.1M sodium acetate was 1.5. This was in comparison to that of cells washed with 9 g/L sodium chloride (0.15M ionic strength) and PBS (0.17M ionic strength), where the pH_{zpc} was 2.0 and 2.2, respectively. Given that pH_{zpc} could reflect possible intrinsic changes to the cell surface characteristics due to high ionic strength wash buffer, the results highlighted that 0.1M sodium citrate, which resulted in a pH_{zpc} of 3.0, should not be used as wash buffer for sample preparation of zeta potential analysis due to possible induction of cell surface changes.

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Similarly, 0.6M sodium chloride should not be used as wash buffer for preparing cell samples for zeta potential analysis.

Wash buffers of 0.15M ionic strength could remove almost all of the nonspecifically adsorbed ions and molecules from *E. coli* DH5α cell grown in FM medium. However, high ionic strength of 0.6M could potentially result in intrinsic changes to the cell surface that manifested as changes in the point of zero charge (pH_{zpc}). Whether grown in LBG or FM, citrate ions from 0.1M sodium citrate wash buffer could adsorb onto *E. coli* DH5α cell surface; thus, significantly increasing the amount of negative surface charge on the cell surface. More importantly, 0.1M sodium citrate significantly changed the pH_{zpc} of *E. coli* DH5α grown in LBG and FM through possibly removing ions intrinsic to the cell envelope. Thus, 0.1M sodium citrate wash buffer and wash buffer of 0.6M ionic strength should not be used in preparing cell samples for zeta potential analysis.

Thus, by using wash buffer of increasing ionic strength, an approach could be used for revealing the real surface charge of bacterial cells, but is that relevant to the environment of the cells, for example, in the growth medium? The answer is no, since bacterial cells in the growth medium would likely be coated with a layer of nonspecifically adsorbed ions and molecules, as well as loosely bound metabolites and ions. Thus, the surface charge of relevance in this scenario would be that displayed by the cells with a layer of nonspecifically adsorbed ions and molecules. Hence, deionized water wash buffer would be suitable for removing loosely bound metabolites and ions. This is likely to be the cell surface charge sensed by other cells in the environment of the growth medium. The same approach of using deionized water as wash buffer could also be used in revealing the surface charge of bacterial cells in river water through the removal of loosely bound ions and molecules, but where the nonspecifically adsorbed ions and molecules remained in close proximity to the cell surface.

Conclusions

With wash buffer of increasing ionic strength, the zeta potential-pH profiles of cells grown in the two growth media became more negatively charged, highlighting the removal of nonspecifically adsorbed ions and molecules through ionic strength mediated charge screening. Specifically, 0.15M ionic strength wash buffer such as 9 g/L sodium chloride was observed to be useful in removing almost all of the nonspecifically adsorbed ions and molecules; thereby, possibly revealing the real surface charge characteristics of the cells. On the other hand, 0.1M sodium citrate wash buffer was not suitable as wash buffer for preparing cell samples for zeta potential analysis due to a combination of adsorption of citrate ions onto the cell surface as well as changes to the cell surface resultant from exposure to high ionic strength of 0.6M. In general, results from 0.6M sodium chloride wash buffer highlighted that high ionic strength could potentially remove ions intrinsic to the cell surface; thereby, fundamentally changing the cell surface characteristics.

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Collectively, 0.15M ionic strength wash buffer such as 9 g/L sodium chloride was sufficient to remove almost all of the nonspecifically adsorbed ions and molecules on the surface of *E. coli* DH5α, but this is likely to be cell surface structure and growth medium specific. On the other hand, wash buffers with citrate anions should be avoided given their propensity to adsorb to the cell surface. Finally, high ionic strength wash buffer such as 0.6M ionic strength could potentially remove ions intrinsic to the cell envelope of *E. coli* DH5α and should be avoided.

Supplementary materials

Additional experiment data are presented as a supplementary material file appended to this manuscript.

References

1. Ramstedt, M., Leone, L., Persson, P. & Shchukarev, A. Cell Wall Composition of *Bacillus subtilis* Changes as a Function of pH and Zn2+ Exposure: Insights from Cryo-XPS Measurements. *Langmuir* **30,** 4367–4374 (2014).
2. Huysman, F. & Verstraete, W. Effect of cell surface characteristics on the adhesion of bacteria to soil particles. *Biol. Fertil. Soils* **16,** 21–26 (1993).
3. Poortinga, A. T., Bos, R., Norde, W. & Busscher, H. J. Electric double layer interactions in bacterial adhesion to surfaces. *Surf. Sci. Rep.* **47,** 1–32 (2002).
4. Neal, A. L., Bank, T. L., Hochella, M. F. & Rosso, K. M. Cell adhesion of *Shewanella oneidensis* to iron oxide minerals: Effect of different single crystal faces. *Geochem. Trans.* **6,** 77 (2005).
5. Liang, X. *et al. Escherichia coli* attachment to model particulates: The effects of bacterial cell characteristics and particulate properties. *PLOS ONE* **12,** e0184664 (2017).
6. Thomas, K. J. & Rice, C. V. Revised model of calcium and magnesium binding to the bacterial cell wall. *BioMetals* **27,** 1361–1370 (2014).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2086v3 | CC BY 4.0 Open Access | rec: 1 Feb 2018, publ: 1 Feb 2018

7. Shephard, J., McQuillan, A. J. & Bremer, P. J. Mechanisms of Cation Exchange by *Pseudomonas aeruginosa* PAO1 and PAO1 *wbpL*, a Strain with a Truncated Lipopolysaccharide. *Appl. Environ. Microbiol.* **74,** 6980–6986 (2008).
8. Zita, A. & Hermansson, M. Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. *Appl. Environ. Microbiol.* **63,** 1168–1170 (1997).
9. Einolf, C. W. & Carstensen, E. L. Bacterial conductivity in the determination of surface charge by microelectrophoresis. *Biochim. Biophys. Acta BBA - Gen. Subj.* **148,** 506–516 (1967).
10. Matz, C. & Jürgens, K. Effects of Hydrophobic and Electrostatic Cell Surface Properties of Bacteria on Feeding Rates of Heterotrophic Nanoflagellates. *Appl. Environ. Microbiol.* **67,** 814–820 (2001).
11. van der Wal, A., Minor, M., Norde, W., Zehnder, A. J. B. & Lyklema, J. Electrokinetic Potential of Bacterial Cells. *Langmuir* **13,** 165–171 (1997).
12. Dague, E., Duval, J., Jorand, F., Thomas, F. & Gaboriaud, F. Probing Surface Structures of *Shewanella* spp. by Microelectrophoresis. *Biophys. J.* **90,** 2612–2621 (2006).
14. Bradford, S. A., Simunek, J. & Walker, S. L. Transport and straining of *E. coli* O157:H7 in saturated porous media. *Water Resour. Res.* **42,** W12S12 (2006).
14. Redman, J. A., Walker, S. L. & Elimelech, M. Bacterial Adhesion and Transport in Porous Media: Role of the Secondary Energy Minimum. *Environ. Sci. Technol.* **38,** 1777–1785 (2004).
15. Goulter, R. M., Gentle, I. R. & Dykes, G. A. Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Lett. Appl. Microbiol.* **49,** 1–7 (2009).

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Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author designed and performed the experiments, analysed the data, and wrote the manuscript.

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