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RESEARCH ARTICLE

MODEL DEVELOPMENT APPROACH TO PREDICT THE BEHAVIOUR OF E-COLI TRANSPORT ON STATIONARY PHASE IN KHANA, DELTAIC ENVIRONMENT OF RIVERS STATE, NIGERIA

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ABSTRACT

The paper considered the transport of E.coli in stationary phase condition. A mathematical model approach were develop to predict the behaviour of E.coli transport at Khana deltaic environment the model were compared with column experimental result, the model fit in with experimental result displaying the same form of concentration, this explain the behaviour of E.coli on stationary phase condition, both result explain the behaviour of the microbes showing the level of concentration in some distance and time, the result also explain the variation in concentration influenced by the lithology deposition and other environmental factors, finally the model from the result will be applied as a design criteria for the solution of ground water pollution in Khana.

INTRODUCTION

The behaviour of microbes on stationary phase condition varies depending on their types, the migration of e-coli from one strata to another strata down to aquiferious zone also varies including velocity, that is why the condition of stationary condition of e-coli should be studied to find out the influence that cause the migrating to a certain point they find themselves stationing in some region , the study of this microbes behaviour considering this stationary phase condition is a method of understanding the kinetics condition, definitely there should a cause for it, these condition generate a lot of increase in population of microbes in a situation, where microbes station in a region, horizon where there is a deposition of Nitrogen, Phosphorus, Carbon etc, this micronutrients will definitely increase their microbial population, the condition may be in an aquiferious zone , such condition will increase the concentration of e-coli at that region of ground water aquifers , therefore the condition of e-coli in stationary phase is of serious concern, and call for extensive study in other to find a better solution that will be applied in solving the transport of e-coli at stationary phase condition at Khana deltaic environment, because the problem of abstracting quality ground water in some location at Khana in Rivers state has cause a lot of problem of developing potable water at those location affected at Khana the study result will generate a design parameter to be apply in those location the pollution are deposited, the alarming rate of water related diseases from those study location make the study imperative to find a lasting solution to this threat of life, the variation of this microbial on transport, implies that model of solving the transport of e-coli in deltaic environment must be thoroughly studied because of its heterogeneous behavior of its transportation to ground water aquifers in deltaic environment. More so other microbes that pollute ground water aquifers also cause their pollution in different dimension, Pathogenic microorganisms in groundwater are estimated to cause 750,000 to 5 million illnesses per year in the United States (Macler *et al.,* 2000). The fate and transport of these microbes are dependent on their propensity to adhere to mineral surfaces. By studying this phenomenon, we gain insight not only into the mechanisms influencing pathogen transport but also into processes such as the initiation of infection (Prigent *et al.,* 2000), biofilm formation (Espinosa *et al,* 1999), and the colonization of plant roots (Bloemberg *et al.,* 1997).

Escherichia coli, a gram-negative bacterium, is considered an ideal indicator of fecal contamination (Havelaar2001) and was therefore the organism employed in our study. The outer membrane of a gram-negative bacterium a lipid baitlayer primarily contains lipopolysaccharides (LPS) and proteins. The LPS molecule extends into the surrounding medium and is anchored to the outer membrane by a lipid moiety known as lipid A (Macler, 2000). Adjacent to lipid A is the 2-keto-3-deoxyoctonic acid; this molecule links lipid A to the core

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polysaccharide region of the LPS (Coughlin *et al.,* 1983 Macleret al, 2000), which consists of heptose, glucose, galactose, and *N*-acetylglucosamine molecules (Holst, 1996 Madigan,1997). The outermost part of the LPS assembly is a lengthy sugar chain called the O-polysaccharide or O-antigen, for which the exact size and composition are strain specific (Kannenberg Carlson, 2001). The types of proteins that exist on the outer membrane include murein lipoproteins, porins (i.e., OmpC, OmpF, OmpA, and PhoE), diffusion proteins, enzymes, and structural molecules (Nikaido and Vaara 1985). Portions of these proteinaceous molecules are exposed to the external environment. In addition, many gram-negative cells exude extracellular polymeric substances (EPS), which typically consist of proteins, polysaccharides, and nucleic acids (Omoike and Chorover, 2004). Previous studies have addressed the role of these macromolecules on cell adhesion. Specifically, the adhesive nature of bacteria has been attributed to such features as LPS Abu-Lail, , and Camesano. 2003, Flemming *et al.,* 1999 Kannenberg and carlson 2001 Walker and Elimelech 2004), outer membrane proteins (,Navarre and Schneewind 1999 Otto, and Hermansson 1999), fimbriae (Otto *et al.,* 2001), flagella (Deflaun *et al.,* 1990), and EPS (Frank and Belfort 2003; Tsuneda *et al.,* 2003).

MATERIALS AND METHOD

Sample Collection

The method of sample collection was insitu method of sample collection from a point source discharge into a drain at. Khana in Rivers State from Niger Delta Environment

COLUMN EXPERIMENTS

Column experiments were performed to monitor the level of transport of *E. coli* at different deposit of soil formation.

Experiment Set up

The column was set up; the height is 1 metre of 10mm diameter steel pipe, positioned at vertical level, including a funnel of 30cm that contains 4 litres of waste water. Each sample level of average of 2000mg/l of waste water containing *E. coli* was poured inside the column. While the flow was passing through the column, a stop watch was used to monitor the speed level, to determine the level of transport of each sample of aquifer materials. The effluent 1000mg/l from the column were collected and subjected to thorough analysis to determine the level of transport of *E. coli* in each of the aquifer material, which determines the level of transport to aquiferious zone.

DEVELOPED MODELS FOR STATIONARY PHASE CONDITION

C	=	Concentration $[ML^{-3}]$
V	=	Velocity $[LT^{-1}]$
D_A	=	Dispersion coefficient dimension less
T	=	Time [T]
X	=	Distance L

STATIONARY PHASE

$$V^2 D_{A2}\frac{\partial c(x)}{\partial x} - Kc(x)\frac{V(x)}{t} = \frac{V\partial c(x)}{\partial t} \quad \ldots\ldots \quad (1)$$

If $\frac{\partial x}{\partial x} + \frac{V\partial c(x)}{\partial t}$ ………… (2)

and $V^2 D_{A2}\frac{\partial c(x)}{\partial x} = \beta$

We have $\frac{\partial c(x)}{\partial t} - Kc(x)\frac{V(x)}{t} = \beta$ (3)

Such that $\frac{V\partial c(x)}{\partial t} = Kc(x)\frac{V(x)}{t} - \beta$ … (4)

By transformation of eqn (4) we have $C(x) = TX$

This implies that $\frac{\partial c(x)}{\partial x} = T^1 X$

It can be obtained from separation of variables

$$\frac{\partial c(x)}{\partial x} = TX^1$$

Substituting into eqn (4) we get

$$V(T^1X) = KTX^1 - TX\frac{\partial v(x)}{\partial t} \quad \ldots\ldots \quad (5)$$

Expanding further we have

$$VT^1X = KTX^1 - TX\frac{\partial v(x)}{\partial t} \quad \ldots\ldots.. \quad (6)$$

Dividing eqn (6) by TX we have

$$\frac{VT^1X}{TX} = \frac{KTX^1}{TX} - TX\frac{\partial v(x)}{\partial t} \quad \ldots\ldots\ldots \quad (7)$$

This implies that

$$\frac{VT^1}{T} = \frac{KX^1}{X} - \frac{\partial v(x)}{\partial t} \quad \ldots\ldots \quad (8)$$

If $\frac{V\partial c(x)}{\partial t} = \lambda^2$

We have

$$\frac{VT^1}{T} = \frac{KX^1}{X} - \frac{\partial v(x)}{\partial t} = \lambda^2 \ldots\ldots\ldots\ldots\ldots \quad (9)$$

Simplifying it term by term we have

$$\frac{VT^1}{T} = \lambda^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots. \quad (10)$$

$$VT^1 - \lambda^2 T \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

Let $T_{(o)} = 0$

$$VT^1 = \lambda^2 T = 0$$

$$V(ST_{(s)} - T(o) - \lambda^2 T_{(s)} = 0 \quad \ldots\ldots \quad (12)$$

Considering the boundary conditions we have

$$T_{(o)} = Ca_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

Where Ca_1 is the initial concentration

$$V(ST_{(s)} - Ca_1) - \lambda^2 T_{(s)} = 0 \quad \ldots\ldots\ldots \quad (14)$$

$$VST_{(s)} - VCa_1 - \lambda^2 T_{(s)} = 0 \quad \ldots\ldots \quad (15)$$

$$VST_{(s)} - \lambda^2 T_{(s)} = Ca_1 \quad \ldots\ldots\ldots\ldots\ldots \quad (16)$$

$$(VS - \lambda^2)T_{(s)} = Ca_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (17)$$

Then

$$T_{(s)} = \frac{VCa_1}{Vs - \lambda^2} \quad \ldots\ldots\ldots\ldots\ldots \quad (18)$$

$$Vs = \lambda^2 = 0$$

$$S = \frac{\lambda^2}{V} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

Therefore,

$$\boxed{T_{(s)} = VCa_1 \, \ell^{\frac{\lambda^2}{V}t}} \quad \ldots\ldots\ldots \quad (20)$$

$$K\frac{X^1}{X} = \lambda^2$$

where

$$X_{(o)} = Ca_2$$

If we have

$$\boxed{X_{(t)} - \frac{KX^1}{X} \; Ca_2 \, \ell^{\frac{\lambda^2}{KCa_2}}} \quad \ldots\ldots \quad (21)$$

$$\frac{\partial v(x)}{\partial t} = \lambda^2$$

$$SV_{(s)} - V_{(s)} \; C_{(o)} = \lambda^2 \ldots\ldots\ldots \quad (22)$$

Integrating the initial concentration for which

$$V_{(o)} = Ca_3$$

$$SV_{(s)} - Ca_3 = \lambda^2 \ldots \quad (23)$$

$$SV_{(s)} = \lambda^2 + Ca_3 \quad \ldots\ldots \quad (24)$$

Making Vs the subject relation gives

$$V_{(s)} = \frac{\lambda^2 + Ca_3}{S} \quad \ldots \quad (25)$$

Using Laplace inverse where we obtained

$$V_{(t)} = \lambda^2 + C_3 \quad \ldots \quad (26)$$

$$\lambda^2 = \frac{vt}{Ca_3}$$

$$\frac{VT^1}{T} = \frac{KX^1}{X} - \frac{\partial v(x)}{\partial t} = \lambda^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (27)$$

If we let $C_{(x)} = TX$ we obtain

$$\frac{VT^1}{T} = \frac{KX^1}{X} - \frac{\partial v(x)}{\partial t} \quad \ldots\ldots\ldots\ldots \quad (28)$$

Integrating both sides gives

$$\boxed{VCa_1 \, \ell^{\frac{\lambda^2}{v}t} = KCa_2 \, \ell^{\frac{\lambda^2}{-K}t}} \quad \ldots \quad (29)$$

$$\boxed{C_{(x)} = VCa_1 \, \ell^{\frac{\lambda^2}{V}t} = KCa_2 \, \ell^{\frac{\lambda^2}{-K}t}} \quad (30)$$

If $\lambda^2 = \dfrac{Vt}{Ca_3}$

We have

$$\boxed{C_{(x)} = VCa_1 \, \ell^{\frac{Vt^2}{Ca_3}} = KCa_2 \, \ell^{\frac{Vt^3}{Ca_3}t}} \quad \ldots. \; (31)$$

$$C_{(x)} = T_{(x)} = T_{(s)} \; X_{(t)}$$

$$C_{(x)} = \left(VCa_1 \, \ell^{\frac{\lambda^2 t}{V}}\right)\left(KCa_2 \, \ell^{\frac{\lambda^2 t}{K}}\right) \quad \ldots \quad (32)$$

Given the constraint below since

$$t = o, \quad x = C_{(x)} = C_m$$

We have

$$C_m = Ca_1 \; Ca_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (33)$$

Such that

$$Ca_1 = \frac{C_m}{C_2} \quad \ldots\ldots.. \quad (34)$$

Integrating through we have

$$C_{(x)} = \left(\frac{VC_m}{Ca_2} \, \ell^{\frac{vt}{V}}\right)\left(K \, \ell^{\frac{\lambda^2 t}{K}}\right) \quad \ldots\ldots\ldots\ldots\ldots \quad (35)$$

By indices, it simplifies it as

$$C_{(x)} = V^3 \, KC_m \, \ell^{\frac{\lambda^2 t}{V}} + {}^{\frac{\lambda^2 t}{K}} \quad \ldots\ldots\ldots\ldots\ldots \quad (36)$$

If $V = \dfrac{\partial}{t}$ we have

$$C_{(x)_t} = \frac{d^3}{t^3} \, KC_m \, \ell^{\left(\frac{\lambda^2 t}{\frac{d}{t}} + \frac{\lambda^2 t}{\frac{Kd^3}{t^3}}\right)} \quad \ldots\ldots\ldots\ldots\ldots \quad (37)$$

$$C_{x_d} = \frac{d^3}{t^3} \, KC_m \, \ell^{\left(\frac{\lambda^2 t}{\frac{d}{t}} + \frac{\lambda^2 t}{\frac{Kd^3}{t^2}}\right)} \quad \ldots\ldots\ldots.. \quad (38)$$

$$C_{(x)} = \frac{d^3}{t^3} \; KC_m \, \ell^{\left(\frac{\lambda^2 t^2}{d/t} + \frac{\lambda^2 t^3}{Kd}\right)} \quad \ldots\ldots \quad (39)$$

RESULTS AND DISCUSSION

From the figure it shows that the concentration gradually increase with increase in distance in an oscillation form, to an extend were an optimum value were observed at twenty one metres, and suddenly decrease from twenty four to thirty metres.

Table 1. Concentration of *E.coli* at Various Distance versus time

Time	Theoretical model Result Conc. Mg/L(DV}
10	1.41E-03
20	9.60E-04
30	0.024
40	0.1
50	0.41
60	1.71E-03
70	0.42
80	0.023
90	1.00E-14
100	1.86E-16

Table 2. Concentration of *E.coli* at Various Distances versus time

Time	Theoretical model Result Conc. Mg/L(DV}
10	1.41E-03
20	9.60E-04
30	0.024
40	0.1
50	0.41
60	1.71E-03
70	0.42
80	0.023
90	1.00E-14
100	1.86E-16

Table 3. Comparison of theoretical value column experimental Result versus Distance

Distance	Exp Result Conc. Mg/l	Theoretical model Result Conc. Mg/L(DV)
3	0.00013	1.41E-03
6	0.00095	9.60E-04
9	0.021	0.024
12	0.5	0.1
15	0.38	0.41
18	0.00167	1.71E-03
21	0.39	0.42
24	0.019	0.023
27	0.000014	1.00E-14
30	0.000018	1.86E-16

Table 4. Comparison of theoretical value column experimental Result versus Time

Time	Exp Result Conc. Mg/l	Theoretical model Result Conc. Mg/L(DV /SP}
10	0.00013	1.41E-03
20	0.00095	9.60E-04
30	0.021	0.024
40	0.5	0.1
50	0.38	0.41
60	0.00167	1.71E-03
70	0.39	0.42
80	0.019	0.023
90	0.000014	1.00E-14
100	0.000018	1.86E-16

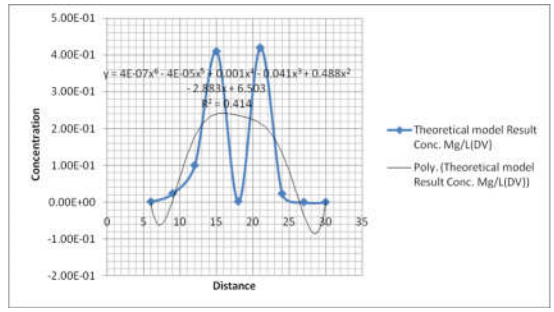

Fig. 1. Comparison of theoretical value with column experimental Result Versus Distance

This condition can be attributed to the level of deposition of the stratum base on the geological formation deposited in the study area. this also explain the level of deposition with respect to microbes migration influenced by the deposition of the formation at khana deltaic environment , which happen to generate a lots of variation in the concentration of microbes , from the study it is discovered that it varies from one soil formation to the other , the level of its heterogeneity in concentration shows that their migration generates a lots of variation that resulted to more accumulation of microbes in some region where it station, if there is deposition of micronutrients, they increase in microbial population. The stationary conditions of microbial transport contribute more to ground water pollution. It produces the best fit line equation displayed on the graph.

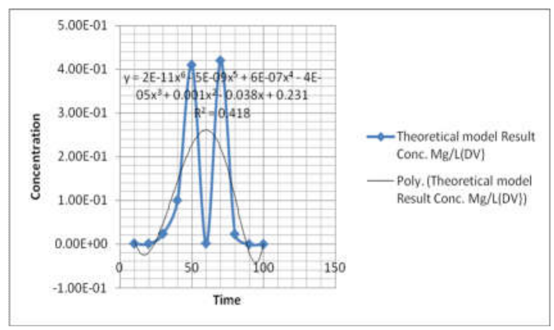

Fig. 2. Comparison of theoretical value with column experimental Result Versus Distance

From the figure presented the concentration of e.coli gradually increase in vacillation form to a point where an optimum value were recorded at seventy days, a sudden decrease was observe from eight days to hundred days, this prove that the migration of the microbes duration varies, it also explain that the ability of migrating fast to ground water aquifers are determined from the lithology from the study area, the issue of stationary condition depend mostly on the soil matrix and deposition of other microelement ,the study area were confirm to generate these type of deposition, therefore the behaviour of e.coli considering stationary phase, become imperative to generate a solution that will solve this type of pollution generating from microbes , the method considered were integrated into the system in formulating a model, it generated the result presented in the figure with best fit equation displayed on the graph.

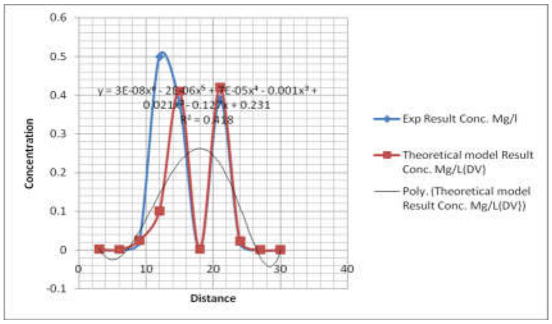

Fig. 3. The comparison of theoretical model with experimental result of column stationary phase versus Time.

Figure 3, the figure presented, shows that the concentration of the microbe increase with distance, until an optimum value was obtained, at a distance range of twelve metres. Similarly, a sudden decrease in concentration of the microbes was observed with increase in distance and the behaviour in the concentration of the microbes is in wave form as presented in figure, similarly to experimental result in the same vein it maintained the same form of concentration, but with little increase in optimum value its was achieved at fifteen metres decreasing in fluctuation form down to thirty metres, at this condition, it implies that both concentration can be attributed to the following reason change in distance, and decrease in microbial population, porosity and other environmental factors.

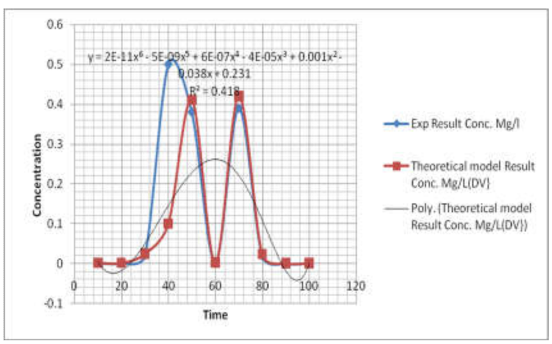

Fig. 4(b). The comparison of theoretical model with experimental result of column stationary phase versus Time

The figure presented shows that the concentration with respect to time from both results, displayed its optimum value at forty days for experimental result and fifty days for theoretical result, but the optimum value of the experiment was higher, the model comparison fit in with the experimental result. This shows that the model can be applied in some study area where is confirm that the migration of microbes e.coli are seen to have such behaviour through the level of concentration The results of both parameters display the best fit line equation $Y = 2E\text{-}11x^6 - 5E\text{-}09x^5 + 6E\text{-}07x^4 - 4E\text{-}05x^3 + 0.001x^2 - 0.038x + 0.231$ with its Root of ($R^2 = 0.418$).

Conclusion

The Study have explain a lots of reason why it is imperative to carry a thorough research ,considering the transport of E.coli in stationary phase condition, the variation of the microbial transport to ground water aquifer were confirm to be very high in some distance and time, which has generated a rapid increase in pollution transport of e.coli at khana in Rivers state, this has generated a lots of water related diseases of which most settlers in those area do not know their sources of ill health, the level of death trap from e.coli transport to ground water aquifers in the study area is of serious concern ,and call for urgent attention, the model formulated with theoretical value result were compared, the model value fit in with the experimental result which implies that the model can be applied in any detail environment as benchmark for solving ground water pollution considering stationary phase condition.

REFERENCE

Abu-Lail, N. I., and T. A. Camesano, 2003. Role of lipopolysaccharides in the adhesion, retention, and transport of Escherichia coli JM109. *Environ. Sci. Technol.,* 37:2173-2183. [PubMed]

Bloemberg, G. V., G. A. Otoole, B. J. J. Lugtenberg, and R. Kolter. 1997. Green fluorescent protein as a marker for *Pseudomonas* spp. *Appl. Environ. Microbiol.,* 63:4543-4551. [PMC free article] [PubMed]

Coughlin, R. T., S. Tonsager, and E. J. McGroarty, 1983. Quantitation of metal-cations bound to membranes and extracted lipopolysaccharide of *Escherichia coli. Biochemistry*, 22:2002-2007. [PubMed]

Espinosa-Urgel, M., A. Salido, and J. L. Ramos, 2000. Genetic analysis of functions involved in adhesion of Pseudomonas putida to seeds. *J. Bacteriol*., 182:2363-2369. [PMC free article] [PubMed]

Frank, B. P., and G. Belfort, 2003. Polysaccharides and sticky membrane surfaces: critical ionic effects. *J. Membr. Sci.,* 212:205-212.

Havelaar, A., U. J. Blumenthal, M. Strauss, D. Kay, and J. Bartram, 2001. Guidelines: the current position, p. 17-42. In L. Fewtrell and J. Bartram (ed.), World Health Organization water quality: guidelines, standards and health. IWA Publishing, London, United Kingdom.

Holst, O., A. J. Ulmer, H. Brade, H. D. Flad, and E. T. Rietschel, 1996. Biochemistry and cell biology of bacterial endotoxins. *FEMS Immunol. Med. Microbiol.,* 16:83-104. [PubMed]

Kannenberg, E. L., and R. W. Carlson. 2001. Lipid A and O-chain modifications cause Rhizobium lipopolysaccharides to become hydrophobic during bacteroid development. *Mol. Microbiol.,* 39:379-391. [PubMed

Lamba, N. M. K., J. N. Baumgartner, and S. L. Cooper, 2000. The influence of thrombus components in mediating bacterial adhesion to biomaterials. *J. Biomater. Sci. Polym.,* 11:1227-1237

Macler, B. A., and J. C. Merkle, 2000. Current knowledge on groundwater microbial pathogens and their control. *Hydrogeol. J.,* 8:29-40.

Madigan, M. T., J. M. Martinko, and J. Parker, 1997. Brock biology of microorganisms, 8th ed. Prentice Hall, Upper Saddle River, N.J.

Nikaido, H., and M. Vaara. 1985. Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.,* 49:1-32. [PMC free article] [PubMed]

Okte, E., N. Sultan, B. Dogan, and S. Asikainen, 1999. Bacterial adhesion of Actinobacillus actinomycetemcomitans serotypes to titanium implants:

SEM evaluation. A preliminary report. *J. Periodontol.,* 70:1376-1382. [PubMed]

Omoike, A., and J. Chorover. 2004. Spectroscopic study of extracellular polymeric substances from Bacillus subtilis: aqueous chemistry and adsorption effects. *Biomacromolecules*, 5:1219-1230. [PubMed]

Otto, J., J. Norbeck, T. Larsson, K. A. Karlsson, and M. Hermansson. 2001. Adhesion of type 1-fimbriated Escherichia coli to abiotic surfaces leads to altered composition of outer membrane proteins. *J. Bacteriol., 183*:2445-2453. [PMC free article] [PubMed]

Otto, K., H. Elwing, and M. Hermansson. 1999. The role of type 1 fimbriae in adhesion of Escherichia coli to hydrophilic and hydrophobic surfaces. *Colloids Surf B.,* 15:99-

Prigent-Combaret, C., G. Prensier, T. T. Le Thi, O. Vidal, P. Lejeune, and C. Dorel. 2000. Developmental pathway for biofilm formation in curli-producing Escherichia coli strains: role of flagella, curli and colanic acid. *Environ. Microbiol.*, 2:450-464. [PubMed]

Sharon L. W, Jane E. H, Jeremy A. R, and Menachem, E. 2005. Influence of Growth Phase on Adhesion Kinetics of Escherichia coli D21g. *Appl Environ Microbiol.,* 71(6): 3093–3099.

Soto, G. E., and S. J. Hultgren. 1999. Bacterial adhesins: common themes and variations in architecture and assembly. *J. Bacteriol.,* 181:1059-1071. [PMC free article] [PubMed]

Tsuneda, S., H. Aikawa, H. Hayashi, A. Yuasa, and A. Hirata. 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. *FEMS Microbiol. Lett*., 223:287-292. [PubMed]

Walker, S. L., J. Redman, and M. Elimelech. 2004. Role of cell surface lipopolysaccharides (LPS) in *Escherichia coli* K12 adhesion and transport. *Langmuir*, 20:7736-7746. [PubMed]

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