Reuse of dredged marine soils as landfill liner: Effect of pH on *Escherichia coli* growth

N M Anuar\(^1\) and C M Chan\(^1\)

\(^1\) Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, Parit Raja, 86400, Batu Pahat, Johor, Malaysia

Corresponding author: chan@uthm.edu.my

**Abstract.** A potential reuse area yet to be explored is the utilization of dredged marine soils (DMS) as geosorbent to retain pathogenic bacteria in landfill leachate. The use of DMS as geosorbent in landfill site could be considered as a new way of environmental friendly solid waste management. By laying DMS at the base of landfill like conventional clay liners, the geowaste could be simultaneously disposed of and act as passive geosorbent for microbes in leachate. DMS are known to serve as a hospitable environment for bacteria growth. Environmental factors such as soil’s pH, salinity and particle size could affect the bacteria growth rate. This study investigated the effect range of pH value on the growth of indicator bacteria, *Escherichia coli* (*E. coli*) isolated from landfill leachate. The results showed that the number of *E. coli* grew higher in alkaline compared to acidic condition. Findings from this study will serve as a base for future studies for removing bacteria in leachate using DMS as geosorbent in a landfill site.

1. Introduction

Dredging operation is an important activity to maintain channel depth and expanding ports and harbours. For these purposes, a large volume of marine soils were dredged out yearly. According to the European Waste Catalogue, the Dredged Marine Soils (DMS) are classified as waste under section 17 05 05 (contaminated marine soils) and section 17 05 06 for other marine soils [1]. Therefore, most of the countries were used to dump back DMS at the disposal facilities (Figure 1). A high demand for dredging exist in the developing countries probably due to the growing maritime trade factor [2]. The project have been undertaken to maintain an adequate ferry and shipping channel depth in ports and harbours. In Malaysia, marine soils are dredged out once in a three years. Marine Department of Malaysia (Jabatan Laut Malaysia, JLM) have been authorized to perform the dredging process which took about six to ten months to be complete [3].

The dredging process in Malaysia commonly ends with the dredged material being disposed offshore in designated dumpsite. Nowadays the disposal of DMS into the sea is gradually being discontinued in developing countries due to concerns for the marine environment. Hence, such large volumes of DMS have created a greater challenge for sustainable disposal practice. Recently, a number of studies have reported the potential of DMS as a raw material for transformation into useful products of economic value. This is especially true for DMS which are marginally contaminated or treatable. For instance, [3] reported that the heavy metal concentrations in the DMS from Kuala Perlis, Malaysia were below the threshold values set by United State Environmental Protection Agency.
(USEPA). Since marine soils such as them are considered harmless, the construction of landfill barriers can be potentially carried out by using this material.

![Figure 1. Dredged marine soils from dredging activities.](image)

As the minimization of the dredged material during dredging operation is impossible, extensive work has been done by researcher to develop various economically favorable for the reuse of DMS. It is a process where dredged material being reuse as raw material in obtaining productive materials such material for road construction, brick production, pavement base material and many more [4][5]. Other researchers have reported the use of marine soils to retain the migration of heavy metals in leachate. Du [6] and Du [7] provided the basis that marine clay soils could be used as landfill liner due to its low conductivity and high heavy metal sorption capability. A study by Chalermyanont [8] also propounded the potential of marine clay soils as landfill liner with better heavy metals retention than lateritic soils. However, the potential of DMS as a geosorbent material in landfill site to retain pathogen bacteria has yet to be thoroughly studied.

The role of marine soils as a reservoir for bacteria have been explored in previous study. Persistence of bacteria was found to be greater in marine soils than water column [9]. Marine soils are known to provide a hospitable environment for bacteria. The soils offer protection from predator, solar exposures, temperature, salinity, and pH that affect bacterial growth and survival. Removal of bacteria in landfill leachate would occur via adsorption when the leachate flow through the geosorbent. A group of researcher [10] investigated the soil properties affecting adsorption of *E. coli*. Thus, this paper aim to determine the influence of pH on the *E. coli* isolated from landfill leachate.

2. Materials and methods

2.1. Materials

The dredged marine soils used in this study was obtained during dredging work from dredging site at Kuala Perlis, Perlis, Malaysia. The soil was packed in sampling bag and kept at 4°C until further analysis. The landfill leachate was collected from a conventional landfill (open dump) located in Johor, Malaysia. The landfill receive municipal solid waste as well as other non-hazardous waste. Prior to restricted issues, the exact location for the conventional landfill is not be mentioned in this paper. The samples were transport to the laboratory and kept at 4 °C until further analysis. Samples collection and preservation was conducted according to Standards Methods for the Examination of Water and Wastewater.
2.2. Characteristic of DMS and landfill leachate
The DMS were mixed before analysis to provide a homogeneity condition. The pH values of sediment were measured by mixing sediment and distilled water in 1:1 weight ratios. The pH value was taken after 60 minutes by inserting the probe in sediments solution. The pH of DMS and leachate was measured using the Eutech Instruments probe. The analysis of particle size, and moisture content of DMS were conducted according to the British Standard 1377:1990. Determination of bacteria from DMS and landfill leachate were conducted based on British Standards [11]. All the apparatus and distilled water used in this experiment underwent the sterilization process using an autoclave to avoid any contamination. Chromocult Coliform Agar (CCA) was used as media agar throughout the study for enumeration and isolation of E. coli in DMS. The use of CCA allows easy detection of E. coli from other coliform colonies.

2.3. Isolation of Escherichia coli
E. coli was isolated from the leachate sample using the streak plate method on the media agar namely Chromocult Agar (CCA). This method is rapid and simple in obtaining pure isolated colonies. Then the plate was incubated in inverted position for 24 hours at temperature 37 °C. To avoid any contamination throughout the streaking process, the wire loop was sterilized by heating till red hot in the blue part of the Bunsen flame heat. Subculture of E. coli was obtained by transfer colonies from the plate culture contain CCA to a growth media. The growth media was sterilized before being inoculate with the bacteria colonies. The fresh inoculate medium allowed for bacteria growth as normal until such time the cells are used for experiments. After incubation period, single colony appeared on the CCA was then brought with sterilized loop into Nutrient Broth (NB). NB was prepared specified by manufacturer. Minimum 10 colonies were transferred into the NB. The incubation period was 18 to 72 hours. Bacteria growth in NB was indicated by turbidity.

2.4. Effect of pH
NB was used as a culture medium in pH effects experiment [12]. The pH of NB was adjusted by dropwise addition of 1M hydrochloric acid (HCl) or sodium hydroxide (NaOH). The pH values obtained were 6, 7, 8 and 9. In order to measure pH effects on the survival of E. coli, the culture medium were inoculated with 10^6 CFU per 1 ml of bacteria. The flasks were then sealed and incubate until Day 5 at 37°C. The concentration of E. coli was counted after 24 h of incubation. Data obtained from the experiments was measured using optical density method and reported in Abs. The number of bacteria can also be counting in CFU/ml using the equation below:

\[ y = 5 \times 10^6 \times 10^{3.0468} \]

where \( y \) = concentration of bacteria in Abs and \( x \) = number of bacteria in CFU/ml. This equation was obtained from the relationship between Abs and colony counting using spread plate method. The relationship is as shown in figure 2.
3. Results and discussion

3.1. DMS and landfill leachate characteristic

The marine soils used in this study contain a mixture of clay, silt and sand. Soil structure depends on the association between soil particles (sand, silt, and clay) in which aggregates of different sizes are formed. According to the Unified Soil Classification System (USCS), the fine-grained soil was classified according to the plasticity. The classification criteria are based on the relationship between Liquid Limit (LL) and Plasticity Index (PI). The relationship was determined from the plastic chart. Based on the USCS, the marine soil from Kuala Perlis was placed in highly plasticity silt categories.

The pH value of the DMS was found to be in the alkaline range with the moisture content of 218.1 %. The concentration of bacteria was 62 times higher in landfill leachate than in DMS. Lower concentration of bacteria was detected in DMS due to the disturbance of marine soils during dredging work. Due to this resuspension event, the bacteria mobilized into water column. The pH of landfill leachate was found to be in acidic while pH of DMS was in alkaline condition. The properties of DMS and landfill leachate was summarized in Table 1.

| Properties          | Kuala Perlis DMS | Landfill leachate |
|---------------------|------------------|-------------------|
| Liquid Limit (%)    | 66.5             |                   |
| Plastic Limit (%)   | 55.8             |                   |
| Plasticity index    | 10.7             |                   |
| Soil classification | MH               |                   |
| pH                  | 8.0              | 6.5               |
| Moisture content (%)| 218.1            | 218.1             |
| E. coli (cfu/g)     | $1.7 \times 10^2$| $1.05 \times 10^4$|
| pH                  |                   |                   |
| E. coli (cfu/ml)    |                   |                   |

Figure 2. Standard curve for bacteria counting in OD and CFU.
3.2. Effect of pH on E. coli growth
In this study, pH of DMS appeared to be in alkaline while leachates’ pH in acidic condition. The growth of E. coli normally range from pH 5 to 9. Despite offered protection from predator and solar exposures, alkalinity of DMS could provide a hospitable environment for the bacteria growth [14]. The survival of E. coli at low and high pH termed as “acid resistance” and “base resistance” respectively. There were differences in growth rate of E. coli among the six pH value tested range of pH 4 to 9. The range of pH were related with leachate and DMS (found in alkaline condition) characterisation. Time series (up to 28 days) results for range of pH effect on bacteria growth were shown in figure 3. In the experiment, the highest growth of E. coli was detected in alkaline compared to the acidic condition. More than 80% of the bacteria was growth in the alkaline condition (pH of 8 and 9). For pH 6 and 7, the growth of bacteria was decreased within 50% compared to the growth in alkaline condition. Lowest growth rate of bacteria was detected in acidic condition. These findings are consistent with the previous report where low pH shown to reduce the growth of E. coli. Ahmad [13] had found increasing bacteria concentration at neutral pH value and reducing bacteria concentration as pH levels dropped below 7. High concentration of bacteria in high pH levels indicated the bacteria are more survive in alkaline conditions as compared to the acidic conditions.

![Figure 3](image_url)

**Figure 3.** Number of bacteria growth in different pH condition.

4. Conclusions
The purpose of the current study was to determine the influence of pH on bacteria isolated from landfill leachate. The isolated bacteria were found to be capable to survive in the range of pH 4 to 9. The results showed that the number of bacteria tend to grew higher in alkaline condition (up to 80%) and vice versa. The findings from this study supported the idea that the bacteria can normally growth in alkaline condition. This will serve as a base for future studies for removing bacteria in leachate using DMS as geosorbent in landfill site.

5. References
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