Journal of Multidisciplinary Engineering Science Studies (JMESS)
ISSN: 2458-925X
Vol. 7 Issue 8, August - 2021

Identification And Characterization Of Micro-Organism From A Domestic Sewer

William-Porbeni, D.[1] Gumus, R.H.[2]
[1,2,] Department of Chemical Engineering, Faculty of Engineering. Niger Delta University.
Wilberforce Island, Bayelsa, Nigeria.

***Abstract*—this study was designed to identify, analyze and characterize the micro-organisms present within a domestic sewer. Heterotrophic bacteria, fungi and acidophilic bacteria were isolated and identified. Results of the study revealed the presence of 9 heterotrophic bacteria strains in the sewer wastewater. It also revealed the presence of fungi of the species Fusarium oxysporium, Alternaria sp, Fusarium sp, aspergillus sp and rhizopus sp. On the basis of maximum sulfate ion production, 4 SOB isolates were selected and phylogenetic analysis was done through 16srRNA sequencing on the basis of maximum sulfate ion oxidizing efficiency. The isolates were identified as serratia, Acidithiobacillus and pseudomonas sp. These micro-organisms play various roles and potentially affect the MICC processes in sewer systems.**

Keywords— SOB, MICC, Sewer, micro-organisms, DNA

1.0 INTRODUCTION

Bacteria are considered the primary colonizers of inanimate surfaces in both natural and man-made environments. Owing to their simple ecological and nutritional needs, they develop easily on outdoor objects, especially where the surface exhibits high water content (Kumar and Kumar, 1999). Microbiologically influenced corrosion (MIC) is an electrochemical process, where the presence and activity of microorganisms accelerate the kinetics of corrosion process (Beech and Sunner, 2004). The MIC process in water and wastewater treatment industries and infrastructures is well documented (Islander et al., 1992, Mori et al., 1992, Grengg et al., 2018, Jiang et al., 2015, Kenneth and Tator, 2003).

A varied population of bacteria may participate in the destruction of materials – both inorganic, such as metals, rocks, concretes, etc., as well as organic, like plastics, leather, textiles, paper, wood and others (Schwermer et al, 2008). The majority of MIC studies have addressed the impact of pure or mixed culture bacterial biofilms on corrosion behavior. The main types of bacteria associated with corrosion in terrestrial and aquatic habitats are sulphate-reducing bacteria (SRB), sulphur-oxidising bacteria (SOB), iron- oxidizing/reducing bacteria, manganese-oxidizing bacteria, and bacteria secreting organic acids and slime (Beech and Coutinho, 2003). The aim of this study was to identify the micro-organisms present in a domestic sewer wastewater used for MICC studies.

2.0 MATERIALS AND METHOD

2.1 Sample Collection

Sewer wastewater used in the study was obtained from a 10 year old sewer chamber within the Edepie community of Yenagoa metropolis. Samples were collected in 10L sterile containers and transported to the laboratory. The pH and temperature were measured as $57^{O}C$ and pH 6.87 at sampling point. Samples were stored at $4^{o}c$ prior to analysis. Experiments were performed in the micro-biology laboratory of the Niger Delta University and samples were

processed as per the procedure outlined in Cheesbrough (2006)

The materials used for this study were either sterilized or disinfected as deemed suitable. The glass wares including petri dishes and the nutrient media, and cotton wool were sterilized by moist heat sterilization method using the autoclave. The glass wares and cotton wool were wrapped with aluminum foil before sterilization. The materials were sterilized at 121^{O}C for 15 minutes to ensure the sterility of the materials and nutrient media. Whilst materials not suitable for sterilization such as the pipettes, were disinfected with ethanol. The bench tops were also disinfected with ethanol before and after work.

2.2 Preparation of nutrient media

The nutrient media used for this study were sterilized by autoclaving. The nutrient media used includes; nutrient agar, iron bacteria medium, API sulfate, and potato dextrose agar. Nutrient agar was used for the cultivation and enumeration of the bacterial population of the samples (total heterotrophic bacterial count), iron bacteria medium was used for the selective cultivation of iron related bacteria, API sulfate agar was used for the selective cultivation and enumeration of Sulphur related bacteria, and the Potato dextrose agar was used for the cultivation of fungal species. The powder media were weighed and dissolved in distilled water according to the manufacturer's instructions. The dissolved media were autoclaved at 121^{O}C for 15 minutes, following standard operation procedures.

2.3 Enumeration of total heterotrophic bacteria

The enumeration of the total heterotrophic bacteria associated with the sample was done on nutrient agar. The samples were diluted in 0.85% normal saline up to the 5th dilution. Plating of the samples was done with the 5th dilution in triplicates using pour plate method. 1ml of the inoculum from the 5th dilution was transferred to the petri dishes aseptically. Thereafter, 20ml of the molten nutrient media (nutrient agar) was poured into the plates. The plates were swirled gently to spread the inoculum evenly in the medium. The plates were allowed to set. Thereafter, the plates were inverted and incubated at 37^{O}C for 24 hours. After the incubation period, the plates were observed for the number of bacteria colonies.

2.4 Isolation of bacteria in pure culture

After the incubation of the agar plates, the colonies were randomly selected and were picked off with sterile wire loop. The colonies were sub-cultured on fresh nutrient agar plates by streaking colonies on the agar surface. The sub cultured plates were inverted and incubated at 37^{O}C under aerobic condition to obtain pure isolates.

2.5 Enumeration of Iron and Sulphur related bacteria

The cultivation and enumeration of iron bacteria was done using" iron bacteria medium". The diluted samples were plated using pour plate method as described in previous section. The cultivation of Sulphur bacteria was done under anaerobic conditions. The inoculated plates were incubated in an anaerobic jar for 3days.

2.6 Calculation of Colony Forming Unit (CFU)

The calculation of CFU was done following the protocol of (Sophia, 2011)

CFU = degree of dilution × aliquot × number of colonies

2.7 Morphological characterization of fungal isolates

The plates were examined for the morphological characteristics of the fungal colonies. The macroscopic observation was aimed at determining the size, shape growth and colour of the plate.

2.8 Microscopic examination of fungal isolates

The examination and microscopic examination of fungal isolates requires the observation of microscopic features such as shape, size of hyphae, shape of sporangia, conidia, conidiophores and spores. Using a flamed inoculating needle, the edge of each colony is picked and slides of the different colonies are made, a drop of lacto phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope using **x100** and **x400** magnification starting from third day of the culture. The microscopic characteristics observed were recorded accordingly.

3.0 DNA extraction and Quantification

Five milliliters of an overnight broth culture of the bacterial isolates in Luria Bertani (LB) were spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro centrifuge tube and stored at -20^oC for other downstream reactions. The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The equipment was initialized with 2ul of sterile distilled water and blanked using normal saline.

3.1 16S rRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Bio systems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

3.2 Sequencing

Sequencing was done using the BigDye Terminator kit on on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

3.3 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

4.0 RESULTS AND DISCUSSION

4.1 Micro-Organism Isolation and Identification

The bacteria isolated and characterized from the raw domestic wastewater are presented in Table 1 below. The isolation and characterization of the bacteria population in the raw wastewater was done to ascertain and specifically identify the bacteria species in the wastewater.

TABLE 1: Enumeration of total micro-Organisms in raw wastewater.

Organism	Media	Mean	Cfu/ml
Sulfur Bacteria	API agar	38	3.8×10^6
Heterotrophic bacteria	Nutrient agar	83	8.3×10^6
Heterotrophic fungi	Potato Dextrose Agar	52	5.2×10^6
Iron bacteria	Iron bacteria medium	56	5.6×10^6

The identification studies was done based on several tests which includes: determination of the morphology of the bacteria isolates, motility, physical and biochemical tests, all according to standard laboratory tests (Cheesbrough 2010). Results obtained from isolation and identification studies on the domestic sewer wastewater identified sulfur oxidizing bacteria (SOB) on API agar, heterotrophic bacteria on nutrient agar, and heterotrophic fungi on PDA and iron bacteria on iron bacteria medium. The total bacteria count of the raw wastewater was $8.3 \times 10^6 cfu/ml$, for heterotrophic bacteria, $5.6 \times 10^6\ cfu/ml$ for iron bacteria, $3.6 \times 10^6\ cfu/ml$ for sulfur bacteria and for total $5.2 \times 10^6\ cfu/ml$ heterotrophic fungal species.

TABLE 2: Biochemical tests and characterization of heterotrophic bacteria

TENTATIVE BACTERIA	*Escherichia coli*	*Pseudomonas Sp.*	*Staphylococcus Sp.*	*Bacillus Sp.*	*Salmonella Sp.*	*Serratia Sp.*	*Streptococci Sp.*	*Proteus Sp.*	*Micrococcus sp*
Gram stain	-ve rod	-ve rod	+ve cocci	+ve rod	-ve rod	-ve rod	+ve cocci	-ve rod	+ve cocci
Catalase	+	+	+	+	+	+	-	+	+
Oxidase	-	-	-	-	-	-	+	-	+
Indole	-	+	+	-	-	+	-	-	-
Glucose in KIA	+	-	+	+	+	+	+	+	+
Lactose in KIA	-	-	-	-	-	-	+	-	-
Gas in KIA	-	-	-	+	-	-	+	+	-
H_2S in KIA	-	-	-	-	+	-	-	+	-
Citrate	-	-	-	+	-	+	+	+	-

Biochemical tests and characterization of bacteria isolates identified 9 bacteria strains in the sewer wastewater. Biochemical tests were performed by standard procedures as outlined in standard laboratory manual (Cheesbrough, 2006, 2010). The isolation procedure was done by using the pour plate method to obtain pure bacteria colonies from form a mixture of colonies to a single colony. To obtain these pure cultures of isolates, it is important that the colonies developed on the plates are not too large. Hence to obtain the appropriate numbers of pure bacteria colonies, samples were diluted in 0.85% normal saline up to the 5^{th} dilution. Plating of the samples was done with the stock bacteria culture in triplicates using pour plate method. The

diluted samples were thereafter transferred onto nutrient agar plates and the bacteria grown on it (Cheesbrough, 2010).

Gram stain study- identified e-coli, pseudomonas sp, salmonella sp, serratia sp and proteus sp were isolated and identified as gram negative bacteria. Identified gram positive bacteria were staphylococcus sp, streptococci sp and micrococcus sp.

Table 3: Physical Characterization of Heterotrophic Fungi

FUNGI IDENTIFIED	MACROSCOPIC FEATURES	MICROSCOPIC FEATURES
Fusarium oxysporum	Floccose, Pink colonies	Hyaline septate hyphae, conidiophores present
Alternaria sp.	Black colour appears on surface of plate.	Conidia in long branched chains, conidiophores ar septate with ovoid to ellipsoidal shape conidia.
Fusarium sp.	White cottony colony	Hyaline septate hyphae, conidiophores present
Aspergillus sp.	Velvety white	Septate hyphae
Rhizopus sp.	Whitish colonies	Non-septate mycelia

Analysis of the wastewater sample isolated, and identified Fusarium Oxysporum, Alternaria sp, Fusarium sp, Aspergillus sp and Rhizopus sp. as fungi isolates in the sewer water. Morphological characterization showed the Fusarium sp, Aspergillus sp and Rhizopus sp as typically whitish in color whlist Fusarium Oxysporum had pink colonies and Alternaria sp. as black. The fungi species aspergillus and Fusarium have been reported as capable of causing corrosion on concrete surfaces (Cwalina, 2014). Fungi secrete a variety of organic acids into sewer environment through a series of metabolic activities. The organic acids produced by these fungi are involved in the complexation of metal ions in the concrete matrix. This process leads to the gradual destruction of stones and concrete structures. (Warschied and Braams, 2000,). The organic acids deep penetrates the concrete matrix causing dissolution of the its components leading to increased solubilization thus stimulating migration of salts into the concrete matrix and thus enlarging the pores and cracks, exfoliating and pulverizing on the surface. (Kumar and Kumar, 1999, Dakal and Cameotra, 2012, Griffin et al, 1991).

4.2 Acidophilic Bacteria Identification and DNA Extraction

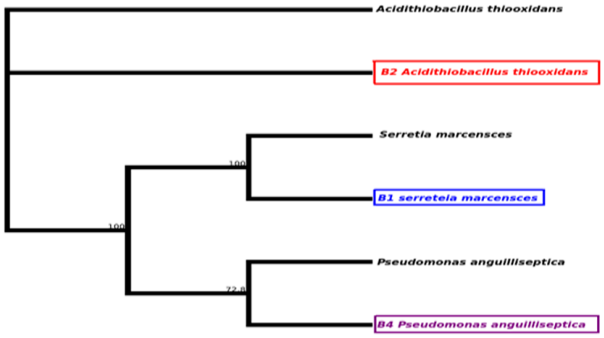

Figure 1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

To further determine the specific acidophilic sulfur oxidizing bacteria species in the wastewater sample, morphological characterization accompanied by microscopic examination of all SOB isolates was done. On the basis of maximum sulfate ions production, 4 SOB isolates were selected and phylogenetic analysis was done through the 16S rRNA gene sequencing. The obtained 16s rRNA sequence from the isolate produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate W1 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate B1, B2 and B4 within the *Serratia*,

Acidithiobaccillus and *Pseudomonas* sp and revealed a closely relatedness to *Serratia marcensces, Acidithiobacillus thiooxidans* and *Pseudomonas anguilliseptica* respectively. Microscopic examination showed that the cells were gram-negative with a short rod shape. Ability to utilize thiosulfate for growth provided a positive identification of SOB. The bacteria that could oxidize sulfur thiosulfate indicated by a drop in pH was termed Sulfur oxidizing bacteria (SOB).

5.0 CONCLUSION

The study emphasized the isolation and characterization of SOB, heterotrophic bacteria and fungi from a domestic sewer chamber. Characterization of bacteria isolates and fungi in domestic sewer water is key in understanding the role of micro-organism in MICC progression in sewer systems. The study showed the presence of SOB Acidithiobacillus thiooxidans, fungi and heterotrophic bacteria. The characterization of the predominant SOB species is key for purposes of sewer corrosion control, specifically in understanding the ion uptake mechanisms of such bacteria. An understanding of the microbial diversity can aid in promoting optimized conditions for control of MICC in sewers.

REFERENCES

[1] Beech I.B. and Coutinho, C.L.M (2003) Biofilms on corroding materials. In Biofilms in Medicine, Industry and Environmental Biotechnology — Characteristics, Analysis and Control. Edited by Lens P, Moran AP, Mahony T, Stoodly P, O'Flaherty V: IWA Publishing of Alliance House; pp:115-131.

[2] Beech, I.B. and Sunner, J. (2004) Biocorrosion: towards understanding interactions between biofilms and metals. *Current Opinion in Biotechnology 2004, 15:181–186.*

[3] Cheesbrough, M. 2006. District Laboratory Practice in Tropical Countries (2nd Edition). London English Language Book Society. pp. 100-194.

[4] Cwalina, B., (2014). 'Biodeterioration of concrete, bricks and other mineral based building materials'. Understanding Biocorrosion. (Available from http://dx.doi.org/10.153319781782421252.3.218).

[5] Dakal, T.C. and Cameotra , S.S. (2012) ' Microbially Induced Deterioration of Architectural Heritages: routes and mechanisms involved ', *Environmental Sciences Europe, 24 – 36.*

[6] Felsentein, J. (1985). Confidence limits on phylogenesis; An approach using bootstrap. *Evolution 39:783-791.*

[7] Grengg, C., Mittermayr, F., Ukrainczyk, N., Koraimann, G., Kienesberger, S. and Dietzel, M. (2018). Advances in concrete materials for sewer systems affected by microbial induced concrete corrosion: A review. *Water Research 134: 341-352.*

[8] Griffin, P.S., Indictor, N. and Koestler, R.J. (1991). The biodeterioration of stone: a review of deterioration mechanisms, conservation case histories and treatment. *Int. Biodeter. 28: 187 – 207.*

[9] Islander, R.L., Devinny, J.S., Mansfield, F., Postyn, A. and Shih, H. (1999). Microbial ecology of crown corrosion in sewers. *J.Environ.Eng. 117:751-770.*

[10] Jiang, G., Sun, X., Keller, J., and Bond, P.L., 2015. Identification of controlling factors for the initiation of corrosion of fresh concrete sewers. *Water. Res. 80:30-40.*

[11] Jukes, T.H. and Cantor, C.R. (1969). Evolution of protein molecules in Munro HN editor, mammalian, protein metabolism..Academic Press, New York. pp 21-132.

[12] Kenneth B. and Tator P.E. (2003). Hydrogen sulfide and microbiologically influenced corrosion of concrete, steel and ductile iron in waste water facilities. *Corrosion paper 03060. NACE International. pp 1-23*

[13] Kumar, R. and Kumar, A.V. (1999) Biodeterioration of Stone in Tropical Environments. An overview. Research in Conservation, Los Angeles, Getty Conservation institute. pp 10-16.

[14] Mori, T. Nonaka, T., Tazaki, K. Koga, M., Hikosaka, Y. and Noda, S. (1992) Interactions of nutrients, moisture,

and pH on microbial corrosion of concrete sewer pipe, *Water Resources 26 (1): 29–37.*

[15] Saitou, N. and Nei, M. (1987). The neighbor-joining method. A new method for reconstructing Phylogenetic Trees. *Molecular Biology and evolution 4:406-425.*

[16] Schwermer, C.U., Lavik, G., Abed, R.M.M., Dunsmore, B., Ferdelman, T.G., Stoodley, P., Gieseke, A. and De Beer, D. (2008)." Impact of nitrate on the structure and function of bacterial biofilm communities in pipeline used for injection of seawater into oil fields". *Appl. Environ. Microbiol. 74:2841-2851.*

[17] Starkey, R.L. (1935). Isolation of some bacteria which oxidize thiosulfate. *Soil Sci., 39: 197-219.*

[18] Warscheid T and Braams J. (2000). Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation. 46(4):343-368.*

Appreciation

The authors thank Prof. Mirabau Tatfeng of the Department of Medical Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria for sending the samples for DNA and RNA analysis.