CHARACTERIZATION OF BACTERIA ISOLATES ON SURFACE OF CORRODED ALUMINIUM COUPON

ABSTRACT:

The study characterized bacteria isolates on the surface of corroded aluminium coupon. The biofilms on the surface of corroded aluminium material was tested by colony, biochemical test and gram staining reaction to know the microorganism(s) responsible for corroding aluminium coupon. The results obtained from the analysis showed that bacteria such as *Bacillus subtilis*, *Micrococcus leteus* and *Staphylococcus* were responsible for corroding the surface of aluminum coupon. Therefore preventive measures to tackle these specific microorganisms on Aluminum surfaces may reduce corrosion of the material.

Keywords: *Bacteria, isolate, aluminum coupon, characterization, corrosion*

1.0 INTRODUCTION

Metals are normally corroded by micro-organisms, particularly bacteria. The bacterial activities on these metal surfaces gradually degrade and eventually damage them. Exposure of a metal usually leads to colonization of its surface by water-borne microorganisms that multiply rapidly to a massive population forming a cohesive structure known as biofilm (Agwa *et al.,* 2017; Maluckov, 2012). This process, referred to as Biocorrosion or Microbiologically Influenced (Induced) Corrosion (MIC) is defined as the corrosion of metal surfaces, influenced by the physiological processes of microorganisms (Pratikno and Titah, 2016). MIC is an electrochemical process in which microrganisms initiate, promote, facilitate, and / or accelerate the corrosion reaction on a metal surface (Zuheir 2014, Garcia *et al.*, 2012; Parande, *et al.,* 2005). Shi *et al*., (2011) reported that MIC could cause stainless steel, carbon steel, copper alloys, zinc and aluminum to corrode. Corrosion is the chemical (or electrochemical) reaction between a metal and its environment, which can cause a change in the characteristics of the metal (Garcia *et al*., 2012). Manga *et al.,* (2012) enumerated the major types of bacteria which can corrode metallic materials to include, Sulphate-Reducing Bacteria (SRB), Iron-Oxidizing Bacteria (IOB), Manganese Oxidizing Bacteria (MOB), and Sulphur Oxidizing

Bacteria (SOB). They act by secreting organic acid and extracellular polymeric substances (EPS). Their habitat is usually stagnant water particularly at bottom of tanks, soil, fresh water, industrial systems and air (Ovri *et al.*, 2013). According to (Garcia *et al*., 2012; Zuo, 2007), the formation of a bacteria biofilm is the first bacteria attack to a surface. Therefore, bacteria adhere to the surface, multiply and form micro-colonies. The interface between the metal surface and the environment is sometimes modified by the biofilms thereby protecting the surface from biocorrosion as is shown by *Pseudomonas Fragi, Escherichia coli* and *Bacillus brevis* (Jayaraman *et al.,* 1999) or by Actinomyes (Valdez *et al.,* 2008). Recent studies have indicated that the aforementioned bacteria species coexist in biofilms forming complex structures on the corrosive metal surfaces (Maluckov, 2012).

The metabolic activities of microorganisms have hitherto affected a wide range of industrial materials particularly those in oil fields, offshore, gas, water pipelines and shipping industries (Manga *et al.*, 2012) as well as pulp and paper industries, ornaments, municipal and industrial waste water treatment, power generation, metal working, chemical process industries and food industries (Gu *et al.,* 2000).

Aluminium and its alloys find applications in aerospace, in some chemical processing industries and in the fabrication of lightweight ratios (Saravanan *et al.*, 2015). Aluminium metal materials are regularly being introduced into the environment from various sources like sludge dumping, industrial effluent and mine tailing (Sani *et al.,* 2001). MIC has the capacity to cause corrosion on aluminum alloy (Shi *et al.,* 2011). MIC of aluminum alloys have been studied by many workers. Jayaraman *et al.,* 1999 studied the Anexnic aerobic biofilms inhibiting corrosion of copper and aluminium. Their study was reported as the first on anexnic aerobic biofilms inhibiting corrosion of copper and aluminum (Zuheir, 2014). Ornek *et al.,* 2002 studied pitting corrosion inhibition of aluminum 2024 by *bacillus* biofilms secreting polyaspartate. This study was carried out in continuous reactors using electrochemical impedance spectroscopy. Filiform corrosion attack on pretreated aluminum alloy with tailored surface of epoxy coating had been reported by Liu 2007. Liu *et al.,* 2010 also carried out a work on reducing microbiologically influenced corrosion of aluminium by using super-hydrophobic surfaces. The results showed that neither anodization nor chemical modification could decrease the bacteria adhesion and corrosion rate individually.

Manga *et al.,* (2012) isolated and characterized bacteria on the basis of colonial morphology, cultural characteristics and biochemical tests. The biochemical tests include gram staining, spore staining, catalase, coagulase, indole, motility, starch hydrolysis, etc, as described by Steve and Dannis (2001); Warren *et al.,* (2005); and Oyeleke and Manga (2008).

The huge economic consequences caused by microbial corrosion are of great concern to many industrial operations. It has been established that 70% of the corrosion in gas transmission is due to problems caused by microorganisms. For instance, the American refinery industry loses $1.4 billion a year from microbial corrosion (Gu *et al.,* 2000). The negative effect of microbial corrosion of metals on the environment and economy has necessitated the identification and characterization of microorganisms involved in corrosion. Consequently, the study is aimed at characterizing bacteria isolate from the surface of corroded aluminum coupon. The specific objectives of the study are; to identify and isolate and characterize the bacteria on the surface of corroding aluminum coupon.

2.0 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Surface scrappings of corroded materials made of Aluminium coupon were manually collected from Dorvibor water side in Bodo city, Gokana, Rivers State, Nigeria and were put in sterile McCartney bottles. The samples were labeled properly and transported aseptically to the Department of Microbiology Laboratory, Rivers State University, Nkpolu, Port Harcourt for bacteriological analysis.

2.2 SAMPLE PREPARATION

The preparation of the stock analytical unit was done by weighing 1g of aluminium coupon. Thereafter, it was dispensed in 9ml of the diluent (normal saline solution) for easy examination to obtain pure cultures.

2.3 MICROBIOLOGICAL ANALYSIS

2.3.1 BACTERIA ENUMERATION

A serial six fold dilution was done on the weighed sample of corroded Aluminium coupon with dilution factor from 10^{-1} to 10^{-4} dilution factor, then the third test tube 10^{-3} and fourth test tube

10^{-4} was used for the incubation.

2.3.2 INOCULATION AND INCUBATION

An aliquot (0.1ml) from two dilutions (10^{-3} and 10^{-4}) was plated in duplicates on Nutrient Agar, using the spread plate technique. The plates were incubated at 37^{o}C for 16 to 24 hours. The colonies on the plates were counted and described morphologically. Colonies formed on Nutrient Agar were used to estimate the total heterotrophic bacterial count (THBC).

2.3.3 IDENTIFICATION OF TEST ORGANISM

Morphological and Biochemical tests were conducted on the isolates for identification of the bacteria associated with corroded aluminium coupon. Biochemical tests such as Indole, Catalase test, Oxidase, Methyl red, Vogesproskaur, Citrate Utilization test were carried out to confirm the isolates (Cheesebrough, 2005; Aditi *et al*., 2017).

2.4 GRAM STAINING

This test was carried out to group bacteria into Gram positive and Gram negative and also shows the cellular morphologies and forms as described by Norris and Swain, (2007). A smear was made from a 24-hours culture on properly labeled grease free glass slide. This was achieved by dropping sterile water on the slide and emulsifying with a loopful of bacteria on the grease free glass slide. The smear was air dried and heated by passing the slide under a Bunsen burner flame three times. Each smeared slide was flooded with the primary stain (Crystal violet) for 60 seconds and rinsed in slow running tap water. The Smears were then flooded with Lugol's iodine for 60 seconds and also rinsed in slow running tap water. The smears were then decolorized with 95% ethanol for 30 seconds and rinsed with slow running tap water and then flooded with a counter stain (Safranin) for 30 seconds and again rinsed with slow running tap water. The slides were allowed to air dry on a slide rack. The stained smear was examined microscopically using oil immersion lens of x100 for better magnification. Purple or violet colour showed gram positive while pink or red colour showed gram negative.

2.5 BIOCHEMICAL TESTS

2.5.1 OXIDASE TEST (FILTER PAPER METHOD)

This test was used to identify whether an isolate contain the enzyme, Cytochrome oxidase. A small portion of the isolate (24 hours culture) was smeared on a filter paper impregnated with freshly prepared oxidase reagent (N, N-dimethyl-p-phenylenediamine). The reaction was observed within 10 seconds to see if there was any colour change. Deep purple colorations appeared within 5-10 seconds, indicating a positive reaction, and a negative reaction was indicated by non-colour change (Shields and Cathcart, 2010).

2.5.2 MOTILITY TEST

The test was used to differentiate between motile and non-motile organisms. Semisolid strength nutrient agar was dispensed into test tubes, autoclaved and allowed to solidify. Using sterilized niddle, each isolate was inoculated by stabbing to half the depth of media and incubated at room temperature for about 48 hours. Growth that appeared away from the line of inoculation was recorded as positive, while growth that confined to the line of stab was negative (Navena and Joy, 2014).

2.5.3 CATALASE TEST (SLIDE METHOD)

Catalase test was carried out to identify the isolates as they produce the enzyme, catalase. The enzyme that detoxifies hydrogen peroxide was broken down into water and oxygen gas by the release of bubbles. A sterile wire loop was used to transfer a loopful of the organism to a grease free slide emulsified with small distilled water. A drop of hydrogen peroxide (6%) was added and observed for effervescence within 3 seconds. The production of bubble indicated a positive result and no bubble indicated negative (Elkins *et al*., 2009).

2.5.4 METHYL RED TEST

This test was used to identify *Escherichia coli,* by producing stable acid with mechanism of mixed acid fermentation of glucose. Seventeen grams (17 g) of methyl red Voges-Proskauer (MRVP) broth was suspended in 100ml distilled water. Five milli liters (5ml) of MRVP broth were distributed into each test tube and autoclaved at 121^{o}C for 15 minutes. A loopful of the test organism was inoculated into the broth and incubated for 48 hours. After incubation, 2-5 drops of methyl red indicator were added to the culture. Positive results indicated red colour as shown by *E. coli* (positive) and negative result indicated yellow colour (Nevena and Joy 2014).

2.5.5 INDOLE TEST

This test was used to ascertain the ability of some isolates to hydrolyze the amino acid tryptophan to produce indole. Tryptophan was made available by tryptone in the medium of 10ml of peptone water and dispensed in test tubes and sterilized by autoclaving. It was allowed to cool before inoculating isolates into the sterile broth. The broth culture was incubated at 37^{o}C for 48 hours after which about 10 drops of Kovac's reagent was added into each of the culture test tubes. The test tubes were shaken and allowed to stand for 5 minutes. Positive result showed a red colour at the surface of the medium and negative result showed no red colour at the surface of the medium.

2.5.6 VOGES-PROSKAUER TEST

This test was used to detect acetone (an important physiological metabolite excreted by many microorganisms) in a bacteria broth culture. A loopful of the test organism was inoculated into MRVP broth and incubated for 24 hours. After incubation, about 10 drops of α–naphthal and 10 drops of potassium hydroxide were dropped into the broth culture and were shaken and allowed to stand for 15 minutes. Positive result indicated a pink or red colour at the surface of the medium and negative result indicated a copper colour at the surface of the medium (Navena and Joy, 2014).

2.5.7 CITRATE UTILIZATION TEST

Citrate utilization test was used to determine the ability of the isolates to utilize sodium citrate as its only carbon source. Simmons citrate agar was prepared according to manufacturer's instructions, transferred into test tubes and autoclaved. The tubes were slanted and allowed to cool and solidify. The slant was inoculated by touching the surface of the slant from 18-24 hours. The tubes were incubated at 35^{o}C for 18 to 24 hours. The development of blue colour denoting alkalinisation was observed and recorded as positive, while negative result showed no blue colour.

2.6 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE ISOLATES

Morphological and biochemical characteristics were used to describe and determine the identities of the isolated bacteria.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

Table 1: Colony/morphological characteristics of isolates from corroded Aluminium (Al) coupon

Isolate code	Colour	Shape	Margin	Transparency	Size	Surface	Elevation

Al 1	Milky	Spherical	Undulate	Opaque	Large	Rough	Flat
Al 2	Milky	Circular	Entire	Opaque	Small	Rough	Raised
Al 3	Light yellow	Ecocenter	Entire	Opaque	Small	Smooth	Raised
Al 4	Light yellow	Circular	Entire	Translucent	Small	Smooth	Raised
Al 5	Yellow	Circular	Entire	Translucent	Small	Smooth	Flat

Key:

Al 1, Al 2, Al 3, Al 4 and Al 5 are codes given to each isolated bacteria

Table 2: Biochemical characteristics of isolates from corroded Aluminium (Biochemical and sugar fermentation tests)

Isolate code	Catalase	Oxidase	Citrate test	Indole	Methyl red	v.p	Motility test	Coagulase	Glucose	Lactose	mannitol	Urease
Al 1	+	-	+	-	-	+	+	-	A	-	AG	-
Al 2	+	+	-	-	-	-	-	-	AG	AG	A	+
Al 3	+	-	+	-	+	+	-	+	AG	AG	AG	+
Al 4	+	-	+	-	+	+	-	+	AG	AG	AG	+
Al 5	+	-	+	-	+	+	-	+	AG	AG	AG	+

Key:

A = Acid

G = Gas

\- = Negative

\+ = Positive

Table 3 Gram staining reaction of isolate from Corroded Aluminium (Al) coupon

Isolate code	Gram Reaction	Shape	Suspected organism
Al 1	+	Rod	*Bacillus Subtilis*
Al 2	+	Cocci	*Micrococcus leteus*
Al 3	+	Cocci	*Staphylococcus*
Al 4	+	Cocci	*Staphylococcus*
Al 5	+	Cocci	*Staphylococcus*

Key + = Positive

Table 4: Morphological and Biochemical Characteristics of isolates from Corroded Aluminium Coupon

	Colony Characteristics						Gram Stain		Biochemical and Sugar Fermentation											
S/N & Isolate Code	**Form/ Shape**	**Elevation**	**Surface**	**Margin**	**Colour**	**Opacity**	**Reaction**	**Shape**	**Catalase**	**Oxidase**	**Citrate test**	**Indole**	**Methyl Red**	**VP**	**Motility test**	**Coagulase**	**Glucose**	**Lactose**	**Mannitol**	**Urease**	**Suspected Organism**
1. Al 1	Spherical	Flat	Rough	Undulate	Milky	Opaque	+	Rod	+		+	-	-	+	+	-	A	-	AG	-	*Bacillus subtilis*
2. Al 2	Circular	Raised	Rough	Entire	Milky	Opaque	+	Cocci	+	+	-	-	-	-	-	-	AG	AG	A	+	*Micrococcus leteus*
3. Al 3	Egocentric	Raised	Smooth	Entire	Light yellow	Opaque	+	Cocci	+	-	+	-	+	+	-	+	AG	AG	AG	+	*Staphylococcus*
4. Al 4	Circular	Raised	Smooth	Entire	Light yellow	translucent	+	cocci	+	-	+	-	+	+	-	+	AG	AG	AG	+	*Staphylococcus*
5.$_{Al\ 5}$	Circular	Flat	Smooth	Entire	Yellow	translucent	+	Cocci	+	-	+	-	+	+	-	+	AG	AG	AG	+	*Staphylococcus*

KEY: A-Acid, G- Gas, (-) Negative, + Positive

3.2 DISCUSSION

The bacteria that are responsible for corroding aluminium coupon were identified through morphological characteristics, biochemical test and gram staining. Tables 1, 2 and 3 show the results of the colonial morphological characteristics of the isolates, biochemical and sugar fermentation test and the gram staining reaction of the isolate respectively.

The isolate with code Al 1, in its morphological characteristics has a milky colour, spherical shape, undulate margin, rough surface, large size and flat elevation and its transparency was opaque (Table 1). The isolate with code Al 1 showed a purple colour and rod shape for its gram staining reaction (Table 3) and was found to be positive (+) to catalase test (Table 2) which means Al 1, was able to degrade hydrogen peroxide to liberate oxygen (O_2) and water (H_2O). It also reacted positively to citrate test, vogesproskauer (VP) test and motility test (Table 2) in the biochemical test and was able to ferment sugar in glucose and mannitol test. Therefore, the bacterium *Bacillus subtilis* was suspected. Zuheir (2014) also obtained similar results.

The isolate with code Al 2 showed a different characteristic from Al 1 for the colony, biochemical and gram staining as shown in Tables 1, 2 and 3 respectively. The isolate with code Al 2 showed a circular shape, entire margin, small size and a raised elevation in the colony/morphological characterization (Table 1). The isolate (Al 2) was positive to oxidase test and it was able to ferment glucose and lactose by the liberation of acid and gas (Table 2).

Al 2 exhibits rod shape and showed a purple colour which indicate gram positive for gram stain reaction as shown in Table 3 and *Micrococcus leteus* was suspected.

The isolate with code Al 3, Al 4 and Al 5 showed the same characteristics with each other for the biochemical test and gram staining as shown in Tables 2 and 3. The three isolates reacted positively to catalase test by the production of bubbles which means they were able to degrade hydrogen peroxide (H_2O_2) to give out oxygen and water. The three isolate (Al 3, Al 4 and Al 5) reacted positively to methyl red test by the exhibition of a characteristic red colour in the biochemical test as shown in Table 2.

Therefore, isolates Al 3, Al 4 and Al 5 had cocci shape each and were all positive to gram stain reaction by the indication of a violet colour, hence, *Staphylococcus* were suspected for isolates Al 3, Al 4 and Al 5 (Table 3). Hence, these results were contrary to the ones recorded by Zuheir (2014).

The microorganism with isolate code Al 2 is a different organism from the isolate with code Al 3, Al 4 and Al 5. This is due to their reaction to oxidase, citrate, methyl red and coagulase test. The isolate with code Al 2 was positive (+) to oxidase test while the isolates with codes Al 3, Al 4 and Al 5 were negative (-) to oxidase test.

The isolate with code Al 2 was found to be negative (-) to citrate, Methyl red and coagulase test while the isolates with codes Al 3, Al 4 and Al 5 were positive (+) to citrate, methyl red and coagulase test (Table 2).

4.0 CONCLUSION

The bacteria on the surface of aluminium coupon have been isolated, identified and characterized as *Bacillus subtilis*, *Micrococcus leteus* and *Staphylococcus.* This study has contributed to the problem of MIC or Biocorrosion on Aluminium surface. Attempts to remove or reduce these bacteria may prevent corrosion on Aluminum surface. This can be another research topic.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

Aditi, F.Y., Rahman, S.S. and Hosain, M.M. (2017). A Study on the Microbiological Status of Mineral Drinking Water. *The Open Microbiology Journal*. 11: 31 – 44.

Agwa, O.K., Iyalla, D. and Abu, G.O. (2017). Inhibition of Biocorrosion of Steel Coupon by Sulphate Reducing Bacteria and Iron Oxidizing Bacteria using Aloe Vera *(Aloe barbadensis)* Extracts, *Journal of Applied Science and Environmental Management,* 21(5):833-838.

Beech, I.B., and Coutinho, C. L.M.(2003). Biofilms on Corroding Materials in Bio Films 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; 115 – 131

Cheesbrough, M. (2005) *District Laboratory Practice in Tropical Countries.* 2nd ed, University Press, University of Cambridge, Edinburgh, Cambridge, United Kingdom, 39: 194 – 201.

Elkins, J.G., Hassett, D.J., Stewart, P.S., Schweizer, H.P. and McDermott, T.R. (2009). Protective Role of Catalase in Pseudomonas aeruginosa Biofilm Resistance to Hydrogen Peroxide. *Applied and Environmental Microbiology* .65 (10): 4594 – 4600.

Garcia, F., Lopez, A. L. R., Guillen, J. C., Sandoval, L. H., Gonzaloz, C. R. and Castano, V. (2012). Corrosion Inhibition in Copper by Isolated Bacteria, *Anti-Corrosion Methods and Materials,* 59 (1): 10-17.

Gu, J.D., Ford, T. E. and Mitchell, R. (2000). Microbiological Corrosion of Metals. Uhlig's Corrosion Handbook, Second Edition, Edited by Revic, R.W. John Wiley and Sons, Inc. pp.915 – 927.

Jayaraman, A., Ornek, D., Duarte, D.A., Lee, C.C, Mansfeld, F.B. and Wood, T.K. (1999). "Axemi Aerobic Biofilms Inhibit Corrosion of Copper and Aluminium". *Applied Microbiology and Biotechnology,* 52 (6): 787-790.

Liu, X.F. (2007). “Filiform Corrosion Attack on Pretreated Aluminium Alloy with Tailored Surface of Epoxy Coating” *Corrosion Science,* 49: 3494-3513.

Liu, B. T., Dong L., Liu, T. and Yin, Y. (2010). “Investigations on Reducing Microbiologically Influenced Corrosion of Aluminum by using Super – hydrophobic Surface”, *Electrochemical Acta,* 55: 5281 – 5285.

Maluckov, B. S. (2012). Corrosion of Steels Induced by Microorganisms, *Association of Metallurgical Engineers of Serbia (AMES)*, 18(3): 223 – 231.

Manga, S. S., Oyeleke, S. B., Ibrahim, A. D., Aliero, A. A.and Bagudo, A. I. (2012). Influence of Bacteria Associated with Corrosion of Metals. *Continental Journal of microbiology*, 6 (1): 19-25.

Misra, T.K. (2000). *Heavy Metals, Bacterial Resistance in Encyclopedia for Microbiology*. Edited by Joshua Lederberg. Vol II. Acedemic Press, California, USA. 361 – 369.

Navena, V. and Joy, P.P. (2014). *Microbiology Laboratory Manual*. Pineapple Research Station (Kerala Agricultural University), 670- 686.

Norris, J.R. and Swain, H. (2007). Staining Bacteria. *Methods in Microbiology.* 5: 105 – 134.

Ornek, D., Wood, T.K., Hsu, C.H., Sun, Z. and Mansfeld. F. (2002). Pitting Corrosion Control of Aluminium 2024 using Protective Biofilms that Secrete Corrosion Inhibitors. *Corrosion.* 58: 761-767.

Ovri, J. E. O., Okeahialam, S. L. and Onyemaobi, O.O. (2013). Microbial Corrosion of Mild and Medium Carbon Steels. *Journal of Engineering, Science and Technology,* 8(5): 639 – 653.

Oyeleke,S.B. and Manga,B.S.(2008). Biochemical Test and Identification of Fungal Isolates. *Essential of Laboratory Practicals In Microbiology*.1st edition. Tobest Publishers, Minna, Nigeria. 28-62.

Parande, A. K., Muralidharan, S., Saraswathy, V. and Palaniswamy, N. (2005). “Influence of Microbiologically Induced Corrosion of Steel Embedded in Ordinary Portland Cement and Portland Pozzolona Cement”, *Anti-corrosion Methods and Materials,* 52(3): 148 – 153.

Pratikno, H. and Titah, H. S. (2016). Microbial Influenced Corrosion on Aluminum by

Pseudomonas Fluorescence in Different Saline Water, International Journal of ChemTech Research, 9(12): 600 – 609.

Sani, R. K., (2001). Copper Induced Inhibition of Growth of *Desulfovibrodesulphuricans* G20: Assessment of its Toxicity and Correlation with those of Zinc and Lead. *Applied Environmental Microbial*, 67: 4765 – 4772.

Saravanan, V., Thyla, P.R., Nirmal, N. and Balajrishnan, S.R. (2015). Corrosion Behaviour of Chemosphere – Aluminum Metal Composite in Seawater Condition. *International Journal of ChemTech Research,* 8(2): 726 – 731.

Shi, X., Xie, N., and Gong, L. (2011). Recent Progress in the Research on Microbial Influenced Corrosion: A Birds Eye View through the Engineering Lens. *Recent Patents on Corrosion Science*. 118-131.

Shields, P. and Cathcart, L. (2010). Oxidase Test Protocol. American Society for Microbiology. Available at: *http://www.microbelibrary.org/library/laboratory-test*oxidase-test-protocol.326

Steve, K.A. and Dannis, S. (2001). Biochemical Tests for the Identification of Bacteria Microbiology. *A Photographic Atlas for the Laboratory*. Benjamin Cummings. Addiction. 20 – 68.

Valdez, S.B., Carrillo, B.M., Zlatev, R., Stoytcheva, M., Schorr, W.M., Cobo, R.J., Perez, L.T. and Bastidas, J.M., (2008). "Influence of Actinomyces Israelii Biofilm on the Corrosion Behavior of Copper IUD", *Anti-Corrosion Methods and Materials,* 55(2):55-59.

Warren,Y.A., Citron,D.M., Merriam,C.V. and Goldstein, E.J.C. (2005). Biochemical Differientiation and Comparison of *Desulphovbrio* species and other Phenotipically Similar Genera. *Journal of Clinical Microbiology*. American Society of Microbioloy.43:4041-4045.

Zuheir, T. K. (2014). Corrosion of Aluminum Alloy in Chloride Medium Containing *Pseudomonas aerginosa* Bacteria, *Nahrain University, College of Engineering Journal*, 16(2): 192 – 207.

Zuo, R. (2007). "Biofilms: Strategies for Metal Corrosion Inhibition Employing Microorganisms", *Applied Microbiology and Biotechnology*, 76 (6): 1245-1253.