Rjeas Research Journal in Engineering and Applied Sciences 1(5) 284-290 Rjeas
© Emerging Academy Resources (2012) (ISSN: 2276-8467)
www.emergingresource.org

MICROBIOLOGICALLY INFLUENCED CORROSION OF S45C MILD STEEL IN CASSAVA MILL EFFLUENT

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ABSTRACT

In the cassava processing mill S45C mild steel is used as the construction material for the grating. The S45C mild steel exposed to the cassava mill effluent faces possible corrosion action due to the microorganisms. In the present study the role of microorganisms in cassava mill effluent on the corrosion of S45C mild steel has been experimentally investigated. Outcome of this investigation provides opportunities for material selection in the construction of cassava mill. Chemical and biological characterises of cassava mill effluent was also experimentally determined. Corrosion rate has been estimated by weight loss measurements. Results indicate the cassava mill effluent exhibits the necessary qualities of an environment suitable for the promotion and sustenance of microbiologically influenced corrosion. The corrosion rates of S45C mild steel in the cassava mill effluent were 1.37, 1.41, 1.60, 1.85, 1.88 mpy at 10, 20, 30 40 and 50 days respectively. *Pseudomonas* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Neisseria* sp. and *Lactobacillus* sp. were identified in cassava mill effluent.

KEYWORDS: Cassava Mill Effluent, S45C Mild Steel, Microbiological Influenced Corrosion, Corrosion Rates, *Pseudomonas* Sp. Microorganisms

INTRODUCTION

Microbiologically influenced corrosion (MIC) is an electrochemical process where activities of microorganisms are able to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature. In the past, participation of microorganisms in a corrosion process is often ignored if an abiotic mechanism can be invoked to explain any observed corrosion. Today, microbiologically influenced corrosion (MIC) has been reported in many systems, for example, underground pipelines (Harris, 1960), drilling operations (Abu, 1992), marine structures (Videla, 1996), cooling water systems (Mittelman, 2003) and waste water treatment facilities (Iversen, 2001). It is estimated that about 20 percent of all corrosion damage of metals are MIC influenced or enhanced (Booth, 1971). Damages resulting from microbial corrosion in production, transport, and oil storage facilities amount to hundred of millions US dollars per year in United States of America (Costerton and Boivin, 1991).

Microorganisms such as sulphate reducing bacteria (*Desulfovibrio, species*), sulphide oxidizing bacteria (*Thiobacillus ferroxidans*), iron bacteria (*Gallionella species*), nitrogen utilizing bacteria (*Pseudomanas species*), and filamentous fungi (*Cladosporium resinae*) are detrimental and can be found in closed systems such as fuel storage tanks, heat exchangers, cooling towers, and pipeline (Characklis, 1981; Pope and Pope, 1998; Videla and Characklis, 1992). Microorganisms add to the corrosion process all the diverse effects derived from microbial interactions with the environment surrounding the metal surface. Microorganisms influence the corrosion by altering the chemistry at the interface between the metal and the bulk fluid (Jones and Amy, 2002; Little and Ray, 2002).

Basic parts of the local cassava processing mill especially in the grating stage are made up of S45C mild steel as a basic material of construction. S45C mild steel is excelling in weldability and machinability, and can be subjected to various heat treatments. In terms of elemental composition, S45C mild steel is reported to consist of Carbon (C) 0.42% – 0.48%; Silicon (Si) 0.15% – 0.35%; Manganese (Mn) 0.6% – 0.9%; Phosphorus (P) 0.030% max and Sulphur (S) 0.035% max (Smith and Hashemi, 2006). The manual grater is usually only a piece of galvanized metal sheet made of S45C mild steel drilled with about 3mm diameter nails leaving a raised jagged flange on the underside. This grating surface is fixed on a wooden frame held together with S45C mild steel and the cassava pieces are pressed against the jagged side of the metal and rubbed vigorously with strong downward movements.

Cassava mill effluent is the water produced after separating starch and fiber during the process of fermentation. On the average, 3.68 m^3 ton^{-1} of

cassava effluent is produced (Horsfall *et al*., 2006; Isabirye *et al*., 2007). It is usually discharged on land or water in an unplanned manner. Cassava mill effluent is reported to contain large amounts of cyanogenic glucosides, tannic acid, lotaustrlin, and high contents of carbohydrate and fats (Oboh and Akindahunsi, 2003). These substances could deteriorate metallic materials if immersed in cassava effluent. In the present study an attempt has been made to investigate the effect of cassava mill effluent on the microbial corrosion behaviour of S45C mild steel. This paper describes the physic-chemical and biological characteristics of cassava mill effluent. The bacterial species are identified up to genus level by employing morphological and biochemical characterization. The microbial corrosion rates on the test material are also assessed by mass-loss technique.

MATERIALS AND METHODS

The study area is Jesse, a rural community of Delta State, Nigeria. The people are mainly farmers with rice, cassava and yam being the main crops grown. The community lies within latitude 5^o43^1N and 5^o30^1N and longitude 6^o20^1E and 6^o12^1E. The area is within the humid tropical zone with defined dry (November – March) and rainy (April – October) seasons (Gobo, 1998). The relative humidity of the area is high with values ranging from 70% in January to 80% in July. Previous study (Gobo, 1998) of the area reveals the average atmospheric temperature to be 25.5^{o}C in the rainy season and 30^{o}C in the dry season months (19.8 - 50.1mm).

Cassava Mill Effluent Sample Collection

Cassava mill effluent sample used for study was collected at the stage of hydraulic pressing of the grated cassava. The effluent was collected in a 10L plastic container pre-treated by washing them with 0.1M dilute hydrochloric acid and sun-dried. At the effluent sample collection point, the plastic container was first of all rinsed with the effluent sample to be collected. Sample for microbiological analysis was collected in sterilized McCartney glass bottles and stored in an ice-chest. Cassava mill effluent sample was transported immediately to Petroleum Training Institute, Consultancy Unit Laboratory, Effurun, Delta State, Nigeria for analysis. Sample of cassava mill effluent was collected on 16th of March 2012 from a local cassava mill.

Preparation of S45C Mild Steel Coupons

Sheets of S45C mild steel were obtained from Tricorr Tech. (Nig) Ltd., Port Harcourt, Nigeria, and cold cut to the dimensions $1.0\text{cm} \times 4.0\text{cm} \times 0.5\text{cm}.$ The cold-cut technique was used so as to maintain the integrity of the steel and avoid probable effect of heat-affected zone (HAZ) on corrosion. The coupons were surface-finished by scrubbing with 80 grit sand papers, sterilized by dipping in pure ethanol, and degreased by washing them in acetone. Average weight of each prepared coupon ranges from 14.30 to 14.50g. The exposed surface area of each coupon is 13.0cm^2 and is calculated as $2(Lw + Lh + hw),$ where $L = 4.0\text{cm}$ is the length of each rectangular coupon, $w = 1.0\text{cm}$ is the width, and $h = 0.5\text{cm}$ is the thickness (or height) of the coupon. Five (5) pieces of S45C mild steel coupons were prepared for the study. The method used in preparing the coupons is consistent with known methods (Avwiri and Tay, 1999). The prepared coupons were weighed before and after each test using a weighing balance (Mettler Balance Model AE 166) with the mass of each coupon determined to the nearest 0.001g.

EXPERIMENTAL METHOD

Cassava Mill Effluent Analysis

The cassava mill effluent sample was analysed for pH, redox potential, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), hydrocyanic acid (HCN), and total microbial count (TMC). These parameters are good environmental impact indicators for microbiologically influenced corrosion problems (Videla, 1996; Stein, 1995). pH, and DO were determined *in-situ* using a multi-parameter water quality monitor (Model 6000UPG). During determination of any of the parameters, the instrument was properly checked and calibrated before and after use. Redox potential of the samples was also determined *in-situ* using Orion multimeter (Model 1260) and combined platinum/silver electrodes. Hydrocyanic acid (HCN) concentrations in the water samples were determined quantitatively using the gravimetric method (Kremers and Schreiner, 1986). TMC was determined using the rapid agar dipstick method. The choice of the rapid agar dipstick method is based on it's ease of application and reliability; it can be used on site and is widely reported in literature (Bloomfield *et al*., 1998; Wang *et al*., 2006; Willinger *et al*., 2005; Nato *et al*., 2003; Olsen *et al*., 2004). Into each sample, an agar nutrient dipstick was dipped into it for 20 minutes. The stick was then retrieved from the system and incubated in a warm oven for 24 hours. The population of microorganisms was determined by comparing it with a calibrated chart provided by the manufactures (Boots Micro – check company, Nottingham, UK). All methods of analysis are consistent with APHA (1992) and DPR (2002) standards.

Bacterial Enumeration and Identification in Cassava Mill Effluent

The collected cassava mill effluent sample was serially diluted (10 fold) using 9 mL of sterile distilled water-blanks and the sample was plated by the pour plate technique. The nutrient agar medium, and lactobacillus medium (Hi-media, Mumbai) was used to enumerate heterotrophic bacteria and lactic acid producing bacteria respectively. The effluent sample was further serially diluted up to 10^{-6} dilution. 1 mL of the sample was poured into sterile petri-dish.

The prepared sterile medium was also poured into the petri- dish. The plate was gently swirled so that the medium might be distributed evenly in the plate. The plate was incubated at room temperature for 24 hours. After 24 hours. to 48 hours. the colonies were counted. The plate containing bacterial colonies with 30-300 numbers were selected for calculation. The bacterial colonies were expressed as colony forming units per mL (CFU/mL). Morphologically dissimilar colonies were selected randomly from the plate and isolated colonies were purified using an appropriate medium by streaking methods. In the streaking method, one loopful of inoculums was placed on the medium near the rim of the plate and spread over a segment in a zigzag horizontal pattern until 1/3 of the plate is covered. The plate was rotated about 60 degrees and spread the bacteria from the first streak in to a second area using the same motion in zigzag horizontal pattern. The lid was replaced and inverted the plate. The plate was incubated at room temperature. The isolated pure cultures were maintained in test tubes as slant culture for further analysis. Six isolates were identified in the cassava mill effluent sample. The strains were maintained at 40°C to keep the microbial strain viable. The isolated bacterial cultures were identified up to genus level by their morphological and biochemical characterization according to the key described in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994).

Corrosion Studies

In the laboratory, four (4) litres of cassava processing effluent from the hydraulic pressing unit of the mill was poured into a batch reactor. Into the batch reactor, 5 prepared S45C mild steel coupons were suspended and completely immersed and the experimental set-up was left for a test period of 10, 20, 30, 40 and 50 days. At the end of each test period, a coupon was retrieved from the reactor, washed, dried, and weighed. Corrosion rate of S45C mild steel coupon was determined using the mass loss technique (Bradford, 1993) as

$$\text{Corrosion rate (mm/year)} = \frac{\Delta M \times 3.45 \times 10^6}{A\rho t} \quad (1)$$

where ΔM is the mass loss (g) of the coupon, A is the total exposed surface area of the coupon (cm^2), ρ is the density of the coupon (g/cm^3), and t is time (hours).

RESULTS AND DISCUSSION

Chemical and Microbial Composition of Cassava Mill Effluent

The results of the chemical and microbial analysis of cassava processing effluent are presented in Table 1.0.

Table 1: Chemical and Microbial Composition of Cassava Mill Effluent

Parameters	Results /Test Periods (days)					
	0	10	20	30	40	50
pH	4.20	3.80	3.60	3.40	3.21	2.50
DO (mg/l)	2.60	2.40	2.10	1.87	1.45	1.10
BOD (mg/l)	73.0	68.0	49.0	33.0	21.0	13.0
COD (mg/l)	360.0	320.0	327.0	330.0	352.0	365.0
Redox Potential (mV)	97	87	82	95	70	61
Hydrocyanic acid (mg/l)	57.40	54.10	55.60	58.50	60.70	63.20
TMC (CFU/ml)	10^6	10^{12}	10^{14}	10^{15}	10^{13}	10^{13}

The pH of the cassava mill effluent is between 4.20 (at the start of the study) and 2.50 (at the end of the test period) indicating an acidic medium. Fermentation of the residues can cause the formation of CO_2 and organic acetic and lactic acids which contribute to its reduction and production of strong odours (Bradbury, 2006; Horsfall, *et al*, 2006, Cumbana, *et al*., 2007). Water bodies receiving untreated cassava water have been reported to be highly acidic, sometimes with pH as low as 2.6 (Zualiya and Muzondo 1993). Acidic environment with pH < 6 or alkaline environment with pH > 8 is more corrosive than an environment with pH in the range 6-8 (Bradford, 1993).

The observed DO levels throughout the study period ranged from 2.60 mg/l at the start of study to 1.10 mg/l at the end of the test period (50days). As the test period progresses, the DO level reduced drastically, and this may be attributed to the presence of microorganisms which utilizes oxygen as a substrate. Similar observations have been made on the rate of oxygen consumption by immobilized nitrate oxidizing bacteria (*Nitrobacteria agilis*) in water environment (Picioreanu, *et al*, 1998). The level of the dissolved oxygen in the study sample indicates an environment that promotes the growth of facultative or anaerobic microorganisms. The level of oxygen in an environment plays an important role in corrosion process especially where oxygen reduction is generally the main cathodic reaction. Further, the reduction of oxygen during fermentation also influences the cathodic reaction and accelerates the corrosion current.

The result also showed a high level of BOD (73 mg/l at the start of the test period) and a low BOD (13mg/l at the end of the test period) in the cassava mill effluent. The value of COD in cassava mill effluent varied from 320mg/l to 365mg/l. These values indicate that the wastewater is highly biodegradable and therefore, there is no need for the supply of external organic carbon source for cyanide-degrading microorganisms. The obtained COD levels in the

cassava mill effluent is low compared with that of waste water from the starch processing companies where COD value is 30,000 mg/l (Cereda and Matos, 1996). The values of BOD and COD suggest that the decreasing of BOD may be due to the fermentation of cassava mill effluent. During decay (fermentation) the microbes present consume organic compounds, degrade the effluent, converting them in to organic acids with the creation of anaerobic environment.

Redox potential (Eh) of cassava mill effluent ranged between 61mV and 97mV. Spectrum of redox potential under which microbial life can be found ranges from -450mV to +850mV, where the negative side of the spectrum favours methanogenic bacteria, and the positive side corresponds to iron bacteria (Newman *et al.*, 1991). Thus, the positive redox potential values obtained for the study sample indicate the presence of facultative microorganisms and indicating a corrosive environment.

The hydrocyanic acid levels in the cassava mill effluent ranged between 57.40 and 63.20 mg/l. Cyanide, which is a major toxic component of cassava that must be removed through processing. In the course of cassava processing (fermentation, pressing and washing) cyanide is released into the environment in the form of hydrocyanic acid (Abiona *et al*. 2004). The high amount of hydrocyanic acid obtained may be related to the high yield varieties of HCN commonly cultivated in the area. A high cyanide concentration of 86mg/l was observed in a similar study and this was attributed to the different variety of cassava that is being treated Ogboghodo, *et al* (2001).

The total microbial count (TMC) in the cassava mill effluent is between 10^6 (at the start of the study) and 10^{13} (at the end of the test period) indicating an environment with adequate bacterial population for effective microbial activity. It has been suggested that an environment with a relative population of 10^6 cells/cm^3 of microorganisms is a concern of potential corrosion problem in an environment (Costello, 1969). The higher levels of TMC observed throughout the period of study can be attributed to the presence of suitable substrate that stimulated bacterial population growth as have been similarly that when cassava processing effluent is released directly into streams and rivers, residual starch can cause rapid growth of bacteria, resulting in oxygen depletion and detrimental effects on aquatic life (Goodley 2004).

Industrial effluents containing low level dissolved oxygen and high level of, and the acidic pH of such effluent are the key factors that influence microbiologically influenced corrosion Gaylarde *et al.,* 1999; Koch, *et al.,* 2001; Rim-Rukeh and Puyate 2007.

Enumeration and Identification of Bacteria

The total viable bacterial activity of hetrotrophic bacteria (HB) is 8.1 X 10^{14}CFU/mL while *Lactobacilli* (LB) is 5.3 X10^8 CFU/mL. Though several isolates have been identified in the effluent, only six predominant bacterial species were characterized up to generic level using standard biochemical characterization methods. The identified genus types are as follows: *Pseudomonas* spp. *Streptococcus* spp. *Micrococcus* spp., *Bacillus* spp. *Neisseria* spp. and *Lactobacillus* spp. It can be seen that out of the six genuses three are rod shaped while three are coccus shaped. Four are gram positive while two belong to gram-negative strains. Among six isolates, four have the ability to move from one place to other. The entire six genus have the ability to secrete the enzyme called oxidase, and to carryout carbohydrate fermentation. In addition, four genuses are able to reduce nitrate and five genuses have the ability to consume citrate. It can also be seen that the entire six genus can produce acid through the consumption of sugar, where as five strains have the ability to produce gas. *Micrococcus* is a chemoorganotroph with a respiratory metabolism often producing little or no acid from carbohydrates. *Streptococcus* is chemo an organotroph requiring nutritionally rich media for growth and 5% carbon dioxide. The cell metabolism is fermentative, producing lactate but no gas. *Bacillus* is a chemolithio organotroph with a fermentative or respiratory metabolism. *Lactobacillus* is also a chemoorganotroph, which requires rich complex media and metabolism is fermentative and saccharoclastic; at least half of the end product carbon is lactate. *Niesseria* is chemoorganotroph, which produces carbonic anhydrase. *Pseudomonas* is also chemolithoorganotrophic, which is able to use hydrogen or carbon monoxide as an energy source. On the basis of biochemical characteristics of microbes it can be concluded that the abovementioned chemoorganotrophs utilize energy from cassava mil effluent and chemolithotrophs accelerates the corrosion process by converting ferros ion to ferric and its oxides (Muthukumar *et al.,* 2003, Jayaraman *et al.,* 1998). It can be assumed that the assimilated sulphate conversion into sulphide by a micro aerophilic organism, namely *Lactobacillus* influences the corrosion rate. The sulphide combines with Fe^{2+} to form FeS. The FeS may then combine with an organic molecule and influence the corrosion process both anodically and cathodically (Williams, *et al.,* 2004). It can also be claimed that during the fermentation process bacteria consume oxygen from the metal surface (O_2 reduction) and take energy from the organic content in the cassava mill effluent.

The identified genus types are similar to the isolates of microorganisms earlier reported Okpokwasili and Nnubia (1999); Nweke and Okpokwasili (2003); Bossert and Bartha (1984)

Corrosion Studies

The corrosion rate of S45mild steel obtained from the weight loss method is shown in Table 2.0. The corrosion rates of mild steel were 1.37, 1.41, 1.60, 1.85, 1.88 mpy at 10, 20, 30 40 and 50 days respectively.

Table 2: Corrosion rates of S45C Mild Steel

Exposure time (days)	Batch Reactor	
	ΔM (g)	Corrosion rate (mpy)
10	0.24	1.37
20	0.37	1.41
30	0.56	1.60
40	0.81	1.85
50	0.99	1.88

Increase in corrosion rates with time was very pronounced in the first 10 days of the test period and minimal increase for the remaining part of the test period (Fig. 1.0). This illustrates the typical behaviour of a metal that demonstrates passivity effects as have been similarly observed (Rim-Rukeh, 2005: Evans, 1968). The behaviour of the S45C steel can be conveniently divided into two regions: active and passive. The minimal increase in corrosion rates could be attributed to the presence of biofilm (biomass) formed on the surface of the metal. Picioreanu *et al*, 2001 had observed that biofilm have the potential of protecting a metal from corrosion.

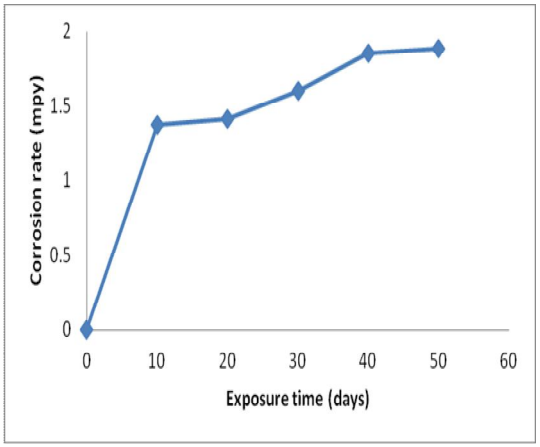

Fig. 1.0: Variation of corrosion rate of coupons with time

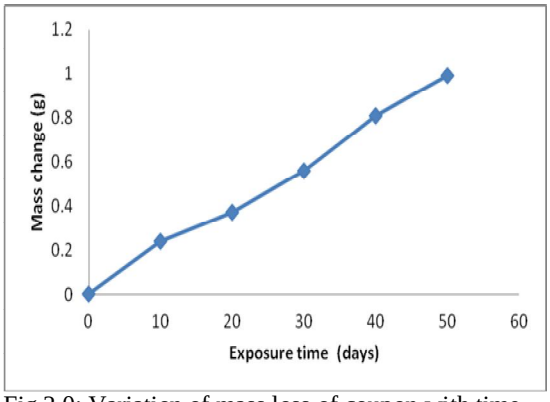

Fig.2.0: Variation of mass loss of coupon with time

Figure 2 illustrates the mass loss of coupons in during the period of the experiment, indicating approximate linear relationship between ΔM and t as obtained by Uhlig (1948) in the form

$$\Delta M = kt \qquad (2)$$

where k is a proportionality constant that depends on the conditions in a specific environment. When the log of mass-loss is plotted against time, an approximate linear relationship is obtained (see Fig. 3), confirming a first-order chemical reaction between the microorganism and the metal. This method of using a linear relationship between log of mass-loss and time in determining the order of a reaction is reported in the literature (Jones, 1988; Omo-Odudu and Oforka, 1999; Rim-Rukeh, 2005).

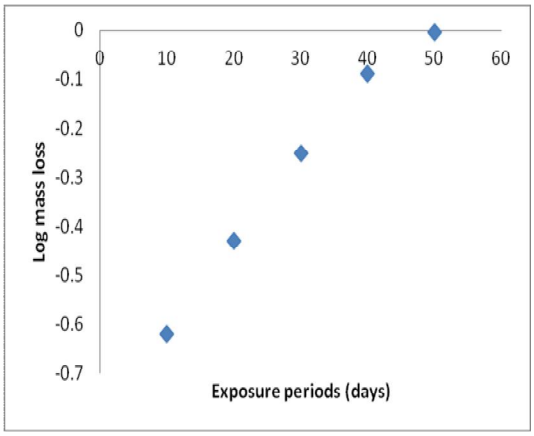

Fig.3.0: log of mass-loss against time

CONCLUSION

Physicochemical and biological characteristics of the cassava mill effluent sample have been presented. It is shown that the levels of measured parameters in the study sample are consistent with the conditions in an environment that favours microbial activity. *Bacillus, Pseudomonas, Micrococcus, Niesseria, Streptococcus* and *Lactobacillus* were found in cassava mill effluent. The cassava mill effluent , therefore, exhibits the necessary qualities for promotion and sustenance of microbiologically influenced corrosion. It is shown that corrosion coupons made of S45C mild steel immersed in the cassava mill effluent sample corroded microbiologically at the rate of 1.37 mpy after 10 days of test period. It was clearly noted that the microbes influence the corrosion by oxygen reduction and fermentation processes. It can be concluded that microbiologically influenced corrosion is responsible for corrosion of S45C steel in cassava mill effluent.

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