

GREEN SYNTHESIS AND CHARACTERIZATION OF IRON OXIDE MICROPARTICLES USING SUGARCANE (*SACCHARUM OFFICINARUM*) BAGASSE AS CAPPING AND REDUCING AGENT AND ITS APPLICATION AS NOVEL BIOSAND FILTER FOR INDUSTRIAL WASTEWATER TREATMENT

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ABSTRACT

Wastewater is a major environmental concern of the modern time. One way to solve this problem is through filtration using microtechnology. In this study, iron oxide microparticles were synthesized using sugarcane bagasse as bio-reducing and capping agent and utilized as biosand filter composite. Physical and chemical properties of the sample confirmed the presence of Iron oxide revealing the following results: greyish red, solid in powder form, insoluble in water, insoluble in alcohol and non-flammable. This was confirmed further by UV visible spectrometer and Auger Electron Spectroscopy (AES) with absorption peak at 260 nm. The particles were also irregular in shape and varied in size with an average diameter of 1.64 μm as revealed by Field Emission Scanning Electron Microscopy (FESEM). The biosand composite with iron oxide microparticles significantly reduced the pH, turbidity, Total Suspended Solids, Total Coliform, Thermotolerant Fecal Coliform and Dissolved Oxygen of the wastewater after the filtration process and were able to pass the accepted range set for Class C water.

Keywords: Sugarcane bagasse; iron oxide microparticles; wastewater.

INTRODUCTION

One of the world's most precious resources is clean water. With the rapid increase in population and reduce in the quality of Philippine waters, the discharge of domestic and industrial wastewater and agricultural runoff has caused extensive pollution of the receiving water bodies (Ahmad and Mahmoud, 2010). A study by the Japan International Cooperation Agency (JICA) conducted in 2010 states that around 700 industrial establishments in the Philippines produce about 273,000 tons of wastewater annually (Greenpeace Southeast Asia, 2010). To address this problem, wastewater treatment plants follow four stages for the wastewater treatment process (Ahmad and Mahmoud, 2012), However, this method is high cost and requires tedious work.

Over the past few decades, microparticles of noble metals such as iron oxide are being used in wastewater treatment (Ammar, 2010) because they exhibited significantly distinct physical, chemical and biological properties from their bulk counterparts. Microparticles are very fine powders, liquids or solids with particle size within the range of 1-500 μm (Carter et. al, 2008). In the process of synthesizing iron oxide microparticles, chemical reduction method is mainly used which involves toxic chemicals which may pose potential environmental and biological risks

(Iravani, 2014). Thus, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols. Biosynthesis approaches include biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity (Iravani, 2014). This motivated the researchers to use sugarcane bagasse (*Saccharum officinarum*), one of the major waste materials of sugar milling in the island of Negros, as capping and reducing agent in producing iron oxide microparticles. It is said that plants rich in antioxidants terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions often used as reducing and capping agents. Sugarcane bagasse, which is a residue obtained after crushing sugarcane to obtain its juice, contains antioxidants including phenolic compounds and anthocyanin, making it a potential reducing and capping agent (Manohar et. al, 2011).

This study was conducted to synthesize iron oxide microparticles using sugarcane bagasse extracts as capping and reducing agent; to confirm the presence of iron oxide microparticles using the UV-Visible (UV-Vis) Spectroscopy, Field Emission Scanning Electron Microscope (FESEM) and Auger Electron Spectroscopy (AES); to evaluate the physical and chemical characteristics of iron oxide microparticles in terms its color, morphology, odor, texture, solubility, pH, flammability and particle size; to determine the chemical oxygen demand (COD), dissolved oxygen (DO) total suspended solids (TSS), total coliform (TC) and thermotolerant fecal coliform (TFC), pH and turbidity of the wastewater before and after being filtered by biosand composite with sugarcane bagasse-synthesized iron oxide microparticles; to determine if there is a significant difference on the COD, DO, TSS, TC, TFC, pH and turbidity of the wastewater after being filtered with biosand composite with sugarcane bagasse-synthesized iron oxide microparticles and sand only (control group).

The result of this study could be an innovation to the traditional application of sand filters as point-of-use water treatment system. Sand filters have been used for several years already to remove pathogens and suspended solids from water (Stauber, 2008) but through the addition of iron oxide microparticles, industrial wastewater treatment could be improved.

MATERIALS AND METHOD

Research Design

This study used the descriptive research method in determining the physical and chemical characteristics of iron oxide microparticles synthesized from the Ferric chloride solution and sugarcane bagasse extracts as capping and reducing agent. On the other hand, experimental research method was used to compare the COD, DO, TSS, TC, TFC, pH and turbidity of the industrial wastewater sample produced from filtration using biosand composite with sugarcane bagasse-synthesized iron oxide microparticles (Set-up A) and sand only (Set-up B). Specifically, the pretest-posttest control group design was used in this study. The quality of the wastewater sample in terms of the aforementioned parameters was tested before and after the application of the filters from both set-ups.

Location and Duration of the Study

The production and characterization of iron oxide microparticles and its application as biosand filter were conducted at the Negros Prawn Producers Diagnostic and Analytical Laboratory, an ISO/IEC accredited Laboratory in Bacolod City. On the other hand, the confirmation of the presence of the iron oxide microparticles and the evaluation of its particle size, surface morphology and chemical composition were done at the Industrial Technology Development Institute (ITDI), DOST Compound, Taguig, Metro Manila.

Materials and Equipment

The materials used in the study were as follows: 6 kg of Ferric chloride (FeCl_3), 5 kg sugarcane bagasse, 500g sand, 3L distilled water, sugarcane bagasse, 500 mL ethanol, nitric acid, and beaker, triple beam balance, pipettes, sieve, aerator, test tubes and test tube rack. The following equipment were used: microwave oven, centrifuge, pH meter, turbidimeter, AES, FESEM and UV-Vis Spectroscope.

General Procedure

A. Preparation of the Sugarcane Bagasse Extracts

The sugarcane bagasse was obtained from the First Farmers Holding Incorporation, Talisay, Negros Occidental. Approximately 50 grams of bagasse was boiled in 100 mL of distilled water for 10 minutes. The suspension was cooled and filtered.

B. Synthesis of Iron Oxide Microparticles

The synthesis of iron oxide microparticles was based on the centrifuge protocol provided by Sun (2004) with some modifications. Eight milliliters (8 mL) of sugarcane bagasse extract was added to 40 mL of ferric chloride solution. The reaction mixture was centrifuged at 300 rpm for 30 minutes. The supernatant was discarded and the particles were washed with distilled water and dried for the evaporation of aqueous phase in an oven. The resulting solution was cooled at room temperature and the attained black product was isolated and washed with ethanol and dried in a vacuum oven at 105 °C for 5 hours and was kept in a stoppered bottle for further use.

C. Physical and Chemical Characterization of the Iron Oxide Microparticles

The particle size, chemical composition, surface morphology and image of the iron oxide microparticles were analyzed using UV-Vis Spectroscope, FESEM (Dual Beam Helios NanoLab 600i at 2.0 kV and beam current of 43 pA) and Auger Electron Spectrometer (JAMP 9500F Field Emission Auger microprobe with Accelerating Voltage of 5kV and Beam Current of 4nA). The pH, color, odor, solubility to water and solubility to alcohol of the iron oxide microparticles were also observed and recorded.

D. Preparation of Biosand Composite with iron oxide Microparticles

Filter media was packed with local quarry sands ranging between 0.85mm and 2.36mm. Before it was used in column and coating, the 400 g sand was soaked in 8% nitric acid solution overnight, rinsed with distilled water to pH 7.0 and dried at 105°C. The iron oxide microparticles were introduced to 100g sand in a conical flask and were manually shaken for 15 minutes.

Two set-ups with 3 replicates were prepared for a total of 6 experimental units. The set-ups were as follows: Set-up A: 100 g Sand coated with 175 mg iron oxide microparticles; Set-up B: 100 g Sand Only (Control Group).

E. Collection of Industrial Wastewater Samples

Water sampling was done at 3:00 pm. Twelve Liters (12) of influent (untreated) industrial wastewater sample were collected at the point of discharge of the wastewater from a local fast food chain in Bacolod City. The temperature of the wastewater was recorded before the sampling took place. The lab-cleaned sample container was dipped into the water and filled from the bottom until full (half-filling the bottle leaves more room for oxygen which will promote degradation of the sample). When the bubbles stop rising to the surface, the container was covered immediately with screw cup to prevent the sample from contacting the air.

F. Pre-Test Water Analysis

For the pre-treatment test, parameters such COD, DO, TSS, TC, TFC, pH and turbidity were immediately measured after the wastewater samples were collected using the approved standard methods of analysis prescribed by the Department of Environment and Natural Resources (DENR) in accordance with “Revised Effluent Regulation of 1990”.

H. Application of Iron oxide Microparticles as Biosand Filter

The filter that was used in this study was made of plastic tubes with 2 cm internal diameters, 14 cm height, and 9 cm medium bed depth. The plastic tube in set-up A contained 100 grams of biosand composite with iron oxide microparticles while the set-up B contained 100 grams of sand only (control group). Three hundred (300) mL of wastewater sample was filtered in each replicate in both set-ups. The samples were obtained from the bottom of the plastic tubes through filtration.

I. Post-test and Analysis

The same procedure employed during the pre-testing was considered for the determination of the final parameter values of the wastewater after it was filtered using the sand only (control group) and biosand composite with iron oxide microparticles.

J. Proper Disposal

After the course of the study, the working area was cleaned and sanitized with disinfectant to avoid contamination of treatments. The equipment used in the study was cleaned thoroughly. On the other hand, the chemicals and other substances used in the study were disposed properly under the guidance of the laboratory chemist.

K. Statistical Tool Analysis

The data of the study were recorded and analyzed using the Statistical Tool for Agricultural Research (STAR). Mean was used to describe the data as computed based on three trials for the wastewater quality parameters. Standard deviation was used to find out how far the values are from the mean. T-test for two independent means at 0.05 α was used to determine if there was a significant difference between the quality of the wastewater filtered using the two set-ups.

RESULTS AND DISCUSSIONS

Table 1. Physical and Chemical Characteristics of Iron Oxide Microparticles

Iron oxide Microparticles Using Sugarcane Bagasse as Reducing and Capping Agent							
Sample	Color	Texture	Odor	Solubility to water	Solubility to Ethanol	Flammability	pH
1	Greyish red	powder form	odorless	insoluble	insoluble	non-flammable	9.10
2	Greyish red	powder form	odorless	insoluble	insoluble	non-flammable	9.10
3	Greyish red	powder form	odorless	insoluble	insoluble	non-flammable	9.10

Physical and chemical properties of the sample confirmed the presence of Iron oxide revealing the following results: greyish red, solid in powder form, insoluble in water, insoluble in alcohol and non-flammable with a pH of 9.10 These results were in agreement with that of Carter (2015) thus, iron oxide microparticles were successfully produced using sugarcane bagasse as capping and reducing agent.

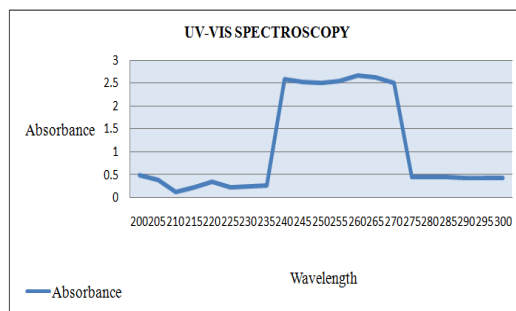


Figure 1. Uv-Visible Spectroscopy of the iron oxide Microparticles

The presence of iron oxide microparticles synthesized from ferric chloride using sugarcane bagasse extract as reducing and capping agent was further validated by UV-Vis spectroscopy. Figure 1, showed the absorption peak at 260 nm. According to Nehru (2015), absorption peak at 260 nm is due to the absorption and scattering of light by iron oxide microparticles. Furthermore, Nehru stated that microparticles have optical properties that are very sensitive on size, shape, agglomeration, and concentration changes.

Table 2. Particle Size of iron oxide Microparticles using Field Emission Scanning Electron Microscopy (FESEM)

Particle No.	Particle size (μm)	Particle No.	Particle Size (μm)
1	1.451	11	0.785
2	1.562	12	0.712
3	1.957	13	1.335
4	0.335	14	3.400
5	1.003	15	2.431
6	4.152	16	1.774
7	4.293	17	1.246
8	0.707	18	1.160
9	0.853	19	0.435
10	0.382	20	2.867
Average			1.643

As illustrated in Table 2 and Figure 2, FESEM showed that the particles were aggregated as irregular sphere shape with rough surfaces and varied in size with an average diameter of 1.64 μm .

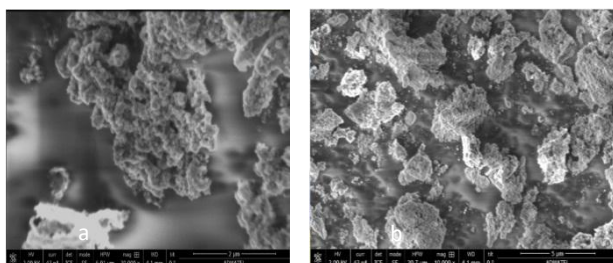


Figure 2. SEM images of the Iron oxide sample taken at intercalated sample taken at (a) 2000x and (b) 10 000x

Moreover, the morphology of the microparticles mostly appeared to be spongy and porous. Therefore, this study effectively produced iron oxide microparticles using sugarcane bagasse as reducing and capping agent. According to Carter, *et al.* (2008) microparticles are very fine powders, liquids or solids with particle size within the range of 1-500 μm . The average particle size of the iron oxide produced in this study was able to meet this criterion.

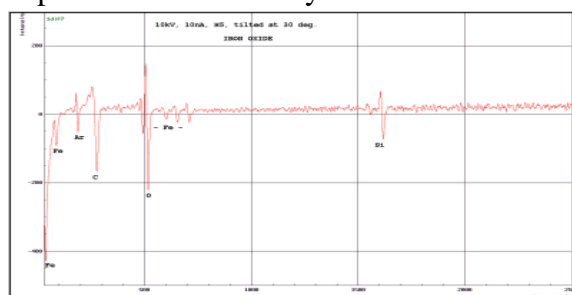


Figure 3. Auger Electron Spectroscopy (AES) Differentiated Spectra of iron oxide Microparticles

Table 3. Quantitative Analysis of iron oxide Samples

Element	Relative concentration (%)
	Good
Si	17
Fe	5
O	29
C	48.9

AES showed that the samples contained iron, carbon, oxygen and silicon, as illustrated in Figure 3. Moreover, Table 3 showed that the iron oxide samples contained the following elements, carbon with relative concentration of 48.9%, oxygen at 29%, silicon at 17% and iron at 5%. The reduction reaction took place when sugarcane bagasse extract was added to ferric chloride solution. Similar to the mechanism of other plant extract, a possible reaction in the formation of iron oxide microparticles is when Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and sugarcane bagasse extract was involved in the reaction of aqueous phase medium.

AES also revealed in Figure 4 that the actual sample had the same spectrum with iron oxide confirming the samples to be iron oxide.

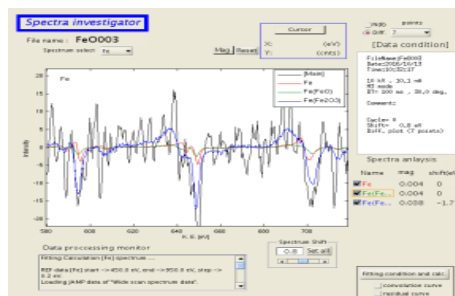


Figure 4. The Spectra of the Iron, Ferrous oxide, Iron oxide and actual sample using AES

Table 4. Pre-test and Post-test Results of the pH of the Industrial Wastewater Sample

Set-Ups	Pre Test Value				Post-Test Value				
	R1	R2	R3	Mean	R1	R2	R3	Mean	SD
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	8.33	8.33	8.33	8.33	7.46	6.36	7.54	7.12	0.80
Set-up B: 100 g of Sand only					8.26	8.20	8.39	8.28	

Table 4 showed that the wastewater sample in set-up A had a greater decrease in the pH level with 7.12 mean post-test value and had a 1.21 difference compared to its pre-test value which was 8.33. On the other hand, the wastewater sample in Set-up B also reduced to 8.28

which had a 0.05 difference from the pre-test value. Moreover, the pH levels of the wastewater sample filtered with biosand composite with iron oxide microparticles and sand alone were still within the acceptable range (6-9) based on the effluent standard set forth by the DENR Administrative Order (2008) for Class C (Inland Water; Old or Existing Industry). The result implies that the wastewater sample is not yet detrimental for the survival of freshwater fish and bottom dwelling invertebrates when disposed to the different bodies of water.

T-test for Two Independent Means showed that there is no significant difference on the mean pH values of the two set-ups. (t-value=-3.67, p-value=0.0914).

Table 5. Pre-test and Post-test Results of the Turbidity of the Industrial Wastewater Sample

Set-Ups	Pre-Test Value (NTU)				Post-Test Value (NTU)				
	R1	R2	R3	Mean	R1	R2	R3	Mean	SD
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	80.2	80.2	80.2	80.2	9.20	5.34	9.33	7.96	13.81
Set-up B: 100 g of Sand only					32.90	30.30	35.40	32.87	

Table 5 shows that the turbidity level of the wastewater sample filtered in set-up A drastically changed as compared to its pre-test value. The turbidity of the wastewater decreased by 59% when filtered using the sand while it decreased by 90% when filtered using the biosand composite with iron oxide microparticles. Interestingly, the turbidity value of the wastewater after being filtered by the biosand composite with iron oxide microparticles reached the acceptable value of the effluent standard for Class C which is 15 NTU (DENR Administrative Order, 2008).

T-test for Two Independent Means showed that 100 g of biosand composite with iron oxide microparticles significantly reduced the turbidity of the wastewater when compared to sand only (t-value= -12.64, p-value=0.0002).

Table 6. Pre-test and Post-test Results of the chemical oxygen demand (COD) of the Industrial Wastewater Sample

Pre-Test Value		Mean	Set-Ups	Post-Test Value (ppm)				
R1	R2			R1	R2	R3	Mean	SD
R1	342	342	Set-up A: 100 g of Biosand composite with Iron oxide microparticles	2	4	7	6.5	2.52
R2	342							
R3	342		Set-up B: 100 g of Sand only	35	44	48	42.33	

The chemical oxygen demand (COD) expresses the total oxygen demand, including the oxidation of all organic matter and reduced inorganic compounds such as ammonium. As shown in Table 6, even though there was a decrease in the percentage of the COD of the wastewater sample filtered in both set-ups, both of them were not able to reach the acceptable range (25-50) required by the effluent standard of DENR Administrative Order (2008) for Class C. However, T-test for Two Independent Means shows that 100 g of biosand composite with iron oxide microparticles significantly reduced the COD of the wastewater sample when compared to 100g of sand only (t-value= -4.02, p-value= 0.0158).

Table 7. Pre-test and Post-test Results of the total suspended solids (TSS) of the Industrial Wastewater Sample

Set-Ups	Pre-test Value				Post-Test Value				SD
	R1	R2	R3	Mean	R1	R2	R3	Mean	
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	854.4	854.4	854.4	854.4	128.2	64.1	106.8	99.7	78.38
Set-up B: 100 g of Sand only					213.6	277.7	192.2	227.83	

The Total suspended solid (TSS) is used to measure the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular (colloidal sol) suspended form. According to Greenpeace Southeast Asia, Water Quality Status Report (2007), the higher the TSS value, the lower is the ability of the water to support aquatic life due to reduced light penetration affecting photosynthesis in aquatic plants, clogging of fish gills which affect respiratory processes, increased absorption of heat that results in higher water temperatures, among others. Table 7 shows that the TSS of the wastewater sample filtered in set-up A drastically changed as compared to the pre-test TSS value of the wastewater sample. In addition, the TSS of the wastewater decreased by 88% when filtered using the sand while it reduced by 98% when filtered using the biosand composite with iron oxide microparticles. Interestingly, the TSS of the wastewater sample filtered with biosand composite with iron oxide microparticles and sand alone reach the acceptable range which is 90 ppm and below the effluent standard (DENR Administrative Order (2008) for Class C.

T-test for Two Independent Means shows that 100 g of biosand composite with iron oxide microparticles significantly reduced the TSS of the wastewater sample when compared to 100g of sand only (t=-9.25, p-value=0.0008).

Table 8. Pre-test and Post-test Results of the dissolved oxygen (DO) of the Industrial Wastewater sample

Set-Ups	Pre-Test Value				Post-Test Value				
	R1	R2	R3	Mean	R1	R2	R3	Mean	SD
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	0.9	0.9	0.9	0.9	6.7	6.5	5.4	6.2	0.7000
Set-up B: 100 g of Sand only					3.8	2.8	2.2	2.93	

Dissolved oxygen (DO) is used to measure the amount of oxygen dissolved in the water. The principal application of DO in the study of water quality for different bodies of water, bacteria in water consumes oxygen as organic matter decays. Table 8 shows that the DO of the wastewater sample filtered in set-up A drastically increased as compared to the pre-test DO value of the wastewater sample. Moreover, the dissolved oxygen of the wastewater increased by 226% when filtered using the sand only while it increased by 588% when filtered using the biosand composite with iron oxide microparticles. Interestingly, the DO levels of the wastewater sample filtered with biosand composite with iron oxide microparticles and sand alone has reached the acceptable range which is 5mg/L and above for the effluent standard (DENR Administrative Order, 2008) for Class C.

T-test for Two Independent Means showed that 100 g of biosand composite with iron oxide microparticles significantly increased the DO of the wastewater sample when compared to 100g of sand only ($t= 5.29$, $p\text{-value}=0.0061$).

Table 9. Pre-test and Post-test Results of the Total Coliform of the Industrial Wastewater Sample

Set-Ups	Pre-Test Value (MPN/mL)				Post-Test Value (MPN/mL)				
	R1	R2	R3	Mean	R1	R2	R3	Mean	SD
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	3670	3670	3670	3670	600	280	920	600	350.43
Set-up B: 100 g of Sand only					2100	1700	2100	1966.67	

Table 9 shows that the total coliform of all the water samples in both set ups decreased after being filtered with biosand composite with iron oxide microparticles (set-up A) and sand alone (set-up B). It was found out that wastewater sample in set-up A had a greater decrease in the total coliform with 620 MPN/100 ml mean post-test value and had a 3050 MPN/100 mL difference compared to the pre-test value which is 3670 MPN/100 mL. On the other hand, the wastewater sample in Set-up B also decreased to 1966.67 MPN/100 mL which had a 1703.33 MPN/100 mL difference only from the pre-test value. Moreover, the total coliform count of the

wastewater sample filtered with biosand composite with iron oxide microparticles and sand alone reached the acceptable value of the effluent standard for Class C which is 5000MPN/100 mL (DENR Administrative Order, 2008). The result indicated that the wastewater sample was not yet detrimental for the survival of freshwater fish and bottom dwelling invertebrates when disposed to the different bodies of water.

T-test for Two Independent Means showed 100 g of biosand composite with iron oxide microparticles significantly reduced the TC of the wastewater sample when compared to 100g of sand only ($t = -5.56$, $p\text{-value} = 0.0051$).

Table 10. Pre-test and Post-test Results of thermotolerant fecal coliform (TFC) of the Industrial Wastewater Sample

Set-Ups	Pre-Test Value				Post-Test Value				
	R1	R2	R3	Mean	R1	R2	R3	Mean	SD
Set-up A: 100 g of Biosand composite with Iron oxide microparticles	2890	2890	2890	2890	920	110	540	523.33	405.26
Set-up B: 100 g of Sand only					1700	1400	1700	1600	

Table 10 shows that the thermotolerant fecal coliform (TFC) of all the water sample in both set ups decreased after being filtered with biosand composite with iron oxide microparticles (set-up A) and sand alone (set-up B). In addition, the wastewater sample in set-up A had a greater decrease in the thermotolerant fecal coliform (TFC) with 523.33 MPN/100 mL mean post-test value and has a 2366.67MPN/100 mL difference compared to the pre-test value which was 2890 MPN/100 mL. On the other hand, the wastewater sample in Set-up B also decreased to 1600MPN/100 mL which had a 1290 MPN/100 mL difference only from the pre-test value. Moreover, the thermotolerant fecal coliform of the wastewater sample filtered with biosand composite with iron oxide microparticles and sand alone reached the acceptable value of the effluent standard for Class C (Inland Water; Old or Existing Industry) which is 5000MPN/100 mL (DENR Administrative Order, 2008). The result indicated that the wastewater sample was not yet detrimental to the survival of freshwater fish and bottom dwelling invertebrates when disposed to the different bodies of water.

T-test for Two Independent Means showed that the 100 g of biosand composite with iron oxide microparticles significantly reduced the TFC of the wastewater sample when compared to 100g of sand only ($t = -4.23$, $p\text{-value} = 0.0134$).

DISCUSSION

As confirmed by the UV-Vis, FESEM and AES iron oxide microparticles were successfully produced in this study using sugarcane bagasse as capping and reducing agent. Reducing agent, which is used in the synthesis of metal microstructures, is the chemical compound which

performs reduction reaction. It transforms the metal ions into elemental metal, which then grows into a particle. The capping agent, on the other hand, prevents that particle from growing beyond the micrometric size of interest. The sugarcane-synthesized iron oxide microparticles were then used as biosand composite due to their unique properties, such as extremely small size, high surface-area-to-volume ratio, surface modifiability, excellent magnetic properties, low toxicity, chemical inertness and great biocompatibility (Xu et al, 2012; Nehru, 2015).

It was also found out in this study that the biosand composite with sugarcane bagasse-synthesized iron oxide microparticles significantly reduced the COD, turbidity and TSS of the wastewater sample as compared to sand alone. Shen *et al.* (2009) explained that the surface charges of iron oxide microparticles are arranged so that atoms in the surface have high reaction capacity and this increase reaction between the microparticles and adsorbents, thus allowing the organic pollutants to adsorb on its surface leads to the reduction of the turbidity and TSS of the wastewater which is also associated with the reduction of COD. Hu et al. (2011) also pointed out that similar to heavy metal adsorption, the adsorption of organic pollutants by iron oxide microparticles took place via surface exchange reactions until the surface functional sites are fully occupied, and thereafter pollutants could diffuse into adsorbent for further interactions with functional groups. The TC and the TFC were also significantly reduced using the biosand composite with sugarcane bagasse-synthesized iron oxide microparticles. Similar results were obtained from the study of Mukherjee and De (2015) wherein the iron oxide microparticle-impregnated ultrafiltration mixed matrix membrane was able to inhibit the growth of *Escherichia coli*. Thukkaram *et al.* (2014) also confirmed in their study that there was a significant reduction in biofilm growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* due to the influence of iron-oxide microparticles on biofilms formed on polymer brush coated biomaterial surfaces. The antibacterial activity of iron-oxide microparticles could be due to the oxidative stress generated by the reactive oxygen species (ROS). ROS includes superoxide radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen, which may cause chemical damage to proteins and DNA in bacteria (Lee *et al.*, 2008). Moreover, electrostatic interactions between microparticles and bacterial cell membranes or cell membrane proteins can result in physical damage, which ultimately leads to bacterial cell death (Ishida *et al.*, 1998). Other studies demonstrated that the small size of microparticles could contribute to their antibacterial effects (Mahmoudi *et al.*, 2011).

CONCLUSIONS

Based on the results of the study, it is concluded that sugarcane bagasse can be used as capping and reducing agent in the production of iron oxide microparticles. Moreover, the biosand composite with iron oxide microparticles has the ability to decrease the pH, turbidity, chemical oxygen demand (COD), total suspended solids (TSS) the total coliform and thermotolerant fecal coliform (TFC) and increase the dissolved oxygen (DO) of the wastewater sample. All of the post-test values of the parameters except for the COD of the wastewater sample filtered in set-up A was also able to pass the accepted range set by the DENR for Class C water. Therefore, the result is a promising route towards the development of a novel filter for industrial wastewater treatment.

RECOMMENDATIONS

To further improve the study, the following are recommended: to add more parameters such as Biological Oxygen Demand (BOD) and Total Dissolve Solids (TDS) and heavy metal adsorption; add more set-ups with varying amounts of iron oxide microparticles to establish the relationship between the amount of iron oxide microparticles and the water quality parameters; to make a specific product which contains biosand composite with iron oxide microparticles; and to provide other sources of industrial wastewater such as those produced in Sugarcane Mills.

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APPENDICES

T-test for Two Independent Means on the Wastewater Quality Parameters of the Industrial Wastewater Samples

Table 11. T-test For Independent Means of the pH of the Industrial Wastewater sample

Variable	Treatment	N	Mean	SD
pH_posttest	Set-up A	3	7.01	0.5981
pH_posttest	Set-up B (Control)	3	8.29	0.0954
pH_posttest	Diff(Set-up A-Set-up B (Control))		-1.28	0.4283
Two Independent Sample t-Test, h₀: mean diff = 0				
Variable	Method	Variances	DF	T Value
pH_posttest	Satterthwaite	Unequal	2.10	-3.67

*2013 further analyzed using Statistical Tool for Agricultural Research (STAR)

Table 12. T-test for Two Independent Means on the Turbidity of the Industrial Wastewater Sample

Variable	Treatment	N	Mean	SD
Turbidity	Set-Up A	3	7.96	2.27
Turbidity	Set-Up B	3	32.87	2.55
Turbidity	Diff (Set-up A-Set-upB)		-24.91	2.41
Two Independent Sample t-Test, h₀: mean diff = 0				
Variable	Method	Variances	Df	t Value
Turbidity	Pooled	Equal	4	-12.64

*2013 Further analyzed using Statistical Tool for Agricultural Research (STAR)

Table 13. Table 9. T-test for Two Independent Means on the chemical oxygen demand (COD) of the Industrial Wastewater Sample

Variable	Treatment	N	Mean	SD
COD_posttest	Set-Up A	3	99.70	32.63
COD_posttest	Set-u B	3	227.83	44.49
COD_posttest	Diff(Set-up A- Set-upB)		-216.58	39.02
Two Independent Sample t-Test, h0: mean diff = 0				
Variable	Method*	Variances	DF	T Value
COD posttest	Pooled	Equal	4	-4.02

Table 14. Pre-test and Post-test Results of the total suspended solids (TSS) of the Industrial Wastewater Sample

Variable	Treatment	N	Mean	SD
TSS_posttest	Set-up A	3	4.33	2.52
TSS_posttest	Set-up B	3	42.33	6.66
TSS_posttest	Diff(set-up A- Set-upB)		-38.00	5.03
Two Independent Sample t-Test, h0: mean diff = 0				
Variable	Method	Variances	DF	T Vaue
TSS_posttest	Pooled	Equal	4	-9.25

Table 15. T-test for Two Independent Means on the dissolved oxygen (DO) of the Industrial Wastewater Sample

Variable	Treatment	N	Mean	SD
DO_posttest	Set-up A	3	6.20	0.7000
DO_posttest	Set-up B (Control)	3	2.93	0.8083
DO_posttest	Diff(Set-up A- Set-up B (Control))		3.27	0.7561
Two Independent Sample t-Test, h₀: mean diff = 0				
Variable	Method	Variances	DF	T Value
DO_posttest	Pooled	Equal	4.00	5.29

Table 16. T-test for Two Independent Means on the Total Coliform (TC) of the Industrial Wastewater sample

Variable	Treatment	N	Mean	SD
TC_posttest	Set-up A	3	600.00	350.43
TC_posttest	Set-up B	3	1966.67	230.94
TC_posttest	Diff(Set-up A- Set-up B)		1346.67	296.76
Two Independent Sample t-Test, h₀: mean diff = 0				
Variable	Method	Variances	DF	T value
TC_posttest	Pooled	Equal	4	-5.56

Table 16. . T-test for Two Independent Means on the thermotolerant fecal coliform (TFC) of the Industrial Wastewater Sample

Variable	Treatment	N	Mean	SD
TFC_posttest	Set-up A	3	523.33	405.26
TFC_posttest	Set-up B	3	1600.00	173.21
TFC_posttest	Diff (Set-up A- Set-upB)		1076.67	311.64
Two Independent Sample t-Test, h0: mean diff = 0				
Variable	Method	Variances	DF	T Vaue
TFC_posttest	Pooled	Equal	4	-4.23

Physical and Chemical Properties of Iron Oxide Microparticles




NEGROS PRAWN PRODUCERS COOPERATIVE

Door No.1 & 2, NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.phCERTIFICATION

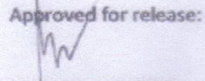
This is to certify that Tricia Ann Mari M. Escordial and John Christian V. Entrata evaluated the Physical and Chemical Properties of the Iron Oxide microparticles from their study entitled Biosynthesis and Characterization of Iron Oxide Mircoparticles using Sugarcane (*Saccharum Offinarum*) Bagasse Extracts as Capping and Reducing Agent and its Application as Filter for Industrial Wastewater treatment. The following results were obtained.

pH	9.10
Solubility in Water	Insoluble
Solubility in Ethanol	Insoluble
Flammability	Non - Flammable

Analyzed by:


Ma. Antonieia C. Lanaca
Laboratory Analyst

Approved for release:


ROSELYN C. USERO
Laboratory Head

Official Results for UV-Visible Spectroscopy of the Iron Oxide Microparticles



NEGROS PRAWN PRODUCERS COOPERATIVE

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Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph


CERTIFICATION

This is to certify that Tricia Ann Marie M. Escordial and John Christian V. Entrata conducted UV - Vis Spectroscopy Analysis of the Iron Oxide microparticles from their study entitled Iron Oxide microparticles from their study entitled Biosynthesis and Characterization of Iron Oxide Mircoparticles using Sugarcane (*Saccharum Offinarum*) Bagasse Extracts as Capping and Reducing Agent and its Application as Filter for Industrial Wastewater treatment. The following results were obtained.

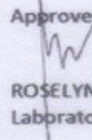
Absorbance	Wavelength
0.481	200
0.385	205
0.121	210
0.229	215
0.347	220
0.226	225
0.253	230
0.258	235
2.575	240
2.517	245

Absorbance	Wavelength
2.49	250
2.543	255
2.66	260
2.62	265
2.49	270
0.446	275
0.443	280
0.438	285
0.433	290
0.428	295
0.423	300

Analyzed by:


Ma Antonietta C. Lariaca
Laboratory Analyst

Approved for release:


ROSELYN C. USERO
Laboratory Head

Report of Analysis for Field Emission Scanning Electron Microscopy (FESEM) and SEM Images of the Iron Oxide Microparticles



Republic of the Philippines
Department of Science and Technology
ADVANCED DEVICE AND MATERIALS TESTING LABORATORY
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
DOST Cpd., General Santos Ave., Bicutan, Taguig City
Tel. Nos. : 837-2071 to 82 (DOST Trunklines), 837-0503 (Direct Line), Telefax No.: 837-3167
<http://www.itdi.dost.gov.ph>, <http://www.admatel.com>



REPORT OF ANALYSIS

Reference No. : ADMATEL 1610-391
Name/Address of Client : Tricia Anne Marie Escordial
Negros Occidental High School
Type/Name of Sample : Iron oxide nanoparticles
Type/Analysis Requested : Imaging using Field Emission Scanning Electron
Microscope (with particle measurement)
Date Received : October 4, 2016
Date Tested : October 10, 2016

RESULTS

The SEM images of the Iron oxide sample taken at 2,000x to 30,000x magnifications are presented in Figure 1. The particle measurement result is summarized in Table 1.

Page 1 of 5

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Particle Size of Iron Oxide Microparticles



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<http://www.itdi.dost.gov.ph>, <http://www.admatel.com>



Table 1. Iron oxide particle size

Particle No.	Particle Size, μm	Particle No.	Particle Size, μm
1	1.451	11	0.785
2	1.562	12	0.712
3	1.957	13	1.355
4	0.335	14	3.400
5	1.003	15	2.431
6	4.152	16	1.774
7	4.293	17	1.246
8	0.707	18	1.160
9	0.853	19	0.435
10	0.382	20	2.867
Average		1.643	

Page 3 of 5

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Revision: 00

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http://www.itdi.dost.gov.ph, http://www.admatel.com



Remarks:

1. As received samples were analyzed.
2. FESEM imaging was conducted using the following parameters:

Instrument	: Dual Beam Helios Nanolab 600i
FESEM Accelerating voltage	: 2.0 kV
Beam Current	: 43 pA

VALIDITY OF THE REPORT: The test results are those obtained at the time of the test and pertain only to the sample(s) received by this laboratory.


KIM CHRISTOPHER C. AGANDA
Laboratory Head

Issued under the authority of:


ARACELI M. MONSADA, Dr. Engg.
Laboratory Manager

Page 4 of 5

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Official Results of Auger Electron Spectroscopy (AES)



PRELIMINARY DATA

Reference No. : **ADMATEL 1610-NOHS**

Name of Client : Negros Occidental High School

Sample/s : One (1) sample labelled as:
• Iron Oxide

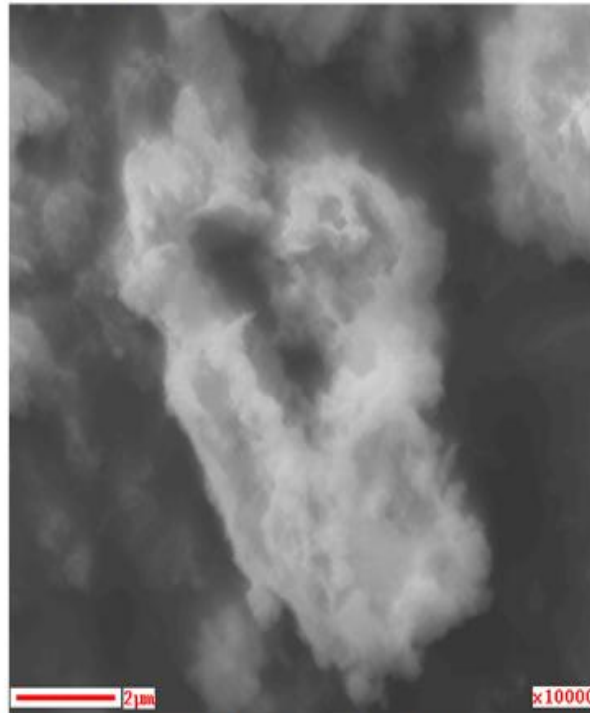
Analysis Requested : Chemical State Analysis by
Auger Electron Spectroscopy (AES)

Date Received : October 13, 2016

Date Analyzed : October 13, 2016

SEM Images of Iron Oxide Samples

SEM Images for Iron Oxide Sample



SEM image of Iron Oxide Sample at 10,000x magnification.

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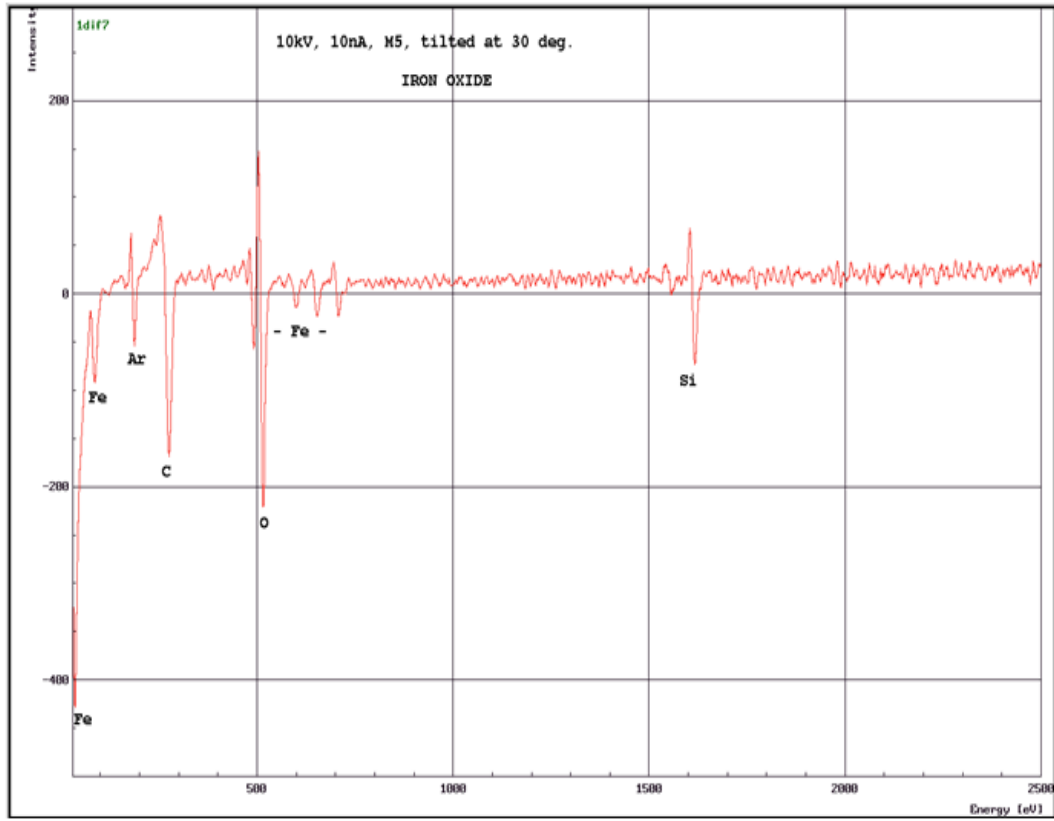
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2

AES Differentiated Spectra of Iron Oxide Samples

AES Differentiated Spectra of Iron Oxide Sample



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3

Quantitative Analysis of Iron oxide Samples

Quantitative Analysis of Iron Oxide Sample

Element	Relative concentration (%)
	Good
Si	17
Fe	5
O	29
C	48.9

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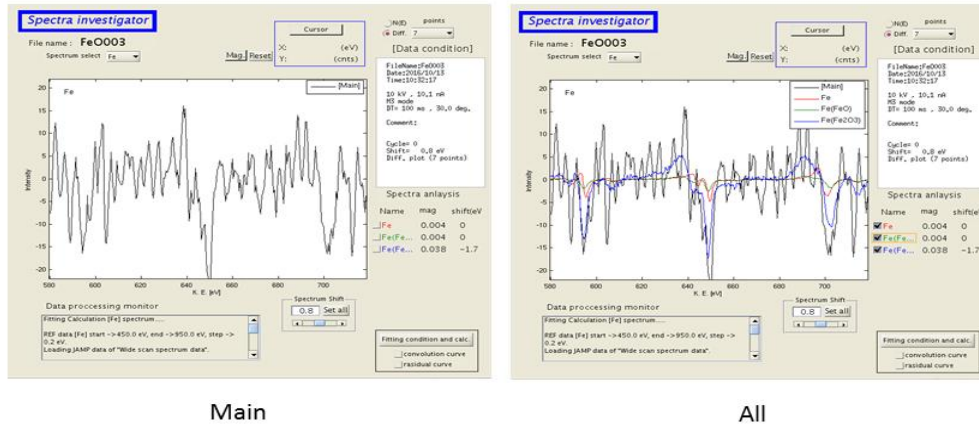
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4

Chemical State Analysis of Iron Oxide Samples

Chemical State Analysis of Iron Oxide Sample



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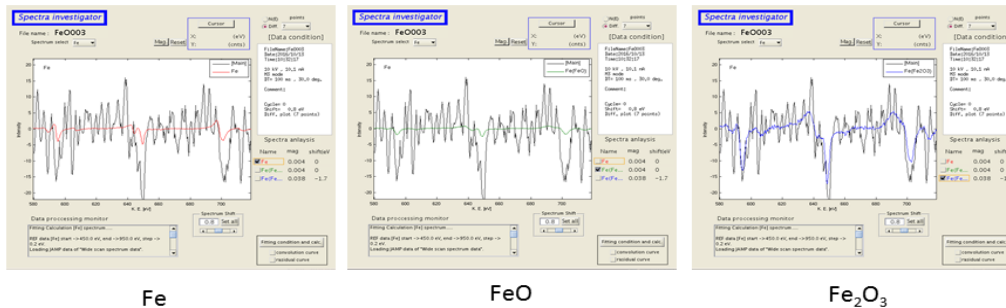


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5

Chemical State Analysis of Iron Oxide Sample



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6

Test Conditions for AES Analysis

- Samples were analyzed as received
 - Chemical State Analysis settings:
0.5 Step, 100 dwell, no. of sweeps = 20
 - Parameters for AES analysis
Equipment: JAMP 9500F Field Emission Auger MicroProbe
Accelerating Voltage and Beam Current: 5kV, 4nA (Imaging and Spectroscopy)
-

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7

Pre-test Results of the Wastewater Samples for Chemical Oxygen Demand, Dissolved Oxygen, pH, Turbidity, Total Coliform, Thermotolerant Fecal Coliform and Total Suspended Solids



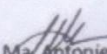
NEGROS PRAWN PRODUCERS COOPERATIVE
Door No.1 & 2., NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph


CLIENT : NEGROS OCCIDENTAL HIGH SCHOOL
ADDRESS : BACOLOD CITY
DATE OF SAMPLE RECEIVED : SEPT. 6, 2016
ANALYSIS REQUESTED : TSS, COD, DO, Turbidity, pH, Total and Thermotolerant Fecal Coliform
SAMPLE DESCRIPTION : WATER
DATE OF ANALYSIS : SEPT. 6, 2016
DATE COMPLETED : SEPT. 6, 2016

LSO NO. : 16-6795

Laboratory Test Results :


Control Number	Turbidity	80.2	TSS	342
16-10278	pH	8.33	Total Coliform	3670
	COD	854.4	Thermotolerant Fecal Coliform	2890
	DO	0.9		

Analyzed by:

Ma. Antonietta C. Lanaca
Laboratory Analyst

Approved for release:

ROSELYN C. USERO
Laboratory Head

Appendix L

Official Results for the pH of the Wastewater treated with Set up A (BioSand Composite with iron oxide Microparticles) and with Set up B (Sand Only)



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Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph


CLIENT : NEGROS OCCIDENTAL HIGH SCHOOL
ADDRESS : Bacolod City
DATE OF SAMPLE RECEIVED : Sept. 6, 2016
ANALYSIS REQUESTED : pH, Total Suspended Solids (TSS)
SAMPLE DESCRIPTION : Water
DATE OF ANALYSIS : Sept. 6, 2016
DATE COMPLETED : Sept. 6, 2016


LSO NO. : 16-6795

Laboratory Test Results :

Control Number	Sample Code	pH	TSS
16-10278	R-1 (Sand Filter)	8.26	35
	R-2 (Sand Filter)	8.20	44
	R-3 (Sand Filter)	8.39	48

NOTE: 1. Result of examination specifically related to samples as received.
2. Test results shall not be reproduced without the approval of the Laboratory Head.

Analyzed by: 
Ma Antoniel C. Lanaca
Laboratory Analyst

Approved for release: 
ROSELYN C. USERO
Laboratory Head

**Official Results for the Chemical Oxygen Demand (COD) of the Wastewater treated with
Set up A (BioSand Composite with iron oxide Nanoparticles)**

**Official Results for the Chemical Oxygen Demand (COD) of the Wastewater treated with
Set up B (Sand Only)**

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Door No.1 & 2, NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph

CLIENT : NEGROS OCCIDENTAL HIGH SCHOOL
ADDRESS : Bacolod City
DATE OF SAMPLE RECEIVED : Sept. 8, 2016
ANALYSIS REQUESTED : C.O.D.
SAMPLE DESCRIPTION : Water
DATE OF ANALYSIS : Sept. 8, 2016
DATE COMPLETED : Sept. 9, 2016

LSO NO. : 16-6795

Laboratory Test Results :

Control Number	Sample Code	C.O.D (g/l)
16-10278	R-1 (Coated Sand Filter)	128.2
	R-2 (Coated Sand Filter)	64.1
	R-3 (Coated Sand Filter)	106.8

NOTE:
1. Result of examination specifically related to samples as received.
2. Test results shall not be reproduced without the approval of the Laboratory Head.


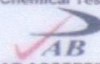
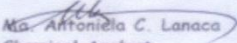
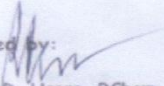
Analyzed by:

Wendy A. Tayson
Laboratory Analyst


Approved for release:

ROSELYN C. USERO
Laboratory Head

Official Results for the Dissolved Oxygen (DO) of the Wastewater treated with Set up A (BioSand Composite with iron oxide Nanoparticles) and with Set up B (Sand Only)

	NEGROS-PRAWN PRODUCERS COOPERATIVE ANALYTICAL & DIAGNOSTIC LABORATORY Door No.1 & 2., NOLKFI Bldg., 6 th Street., Bacolod City Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph	Chemical Testing  AB PAB ACCREDITED TESTING LABORATORY PNS ISO/IEC 17025:2005 LA-2016-289A																												
<p>CLIENT : TRICIA ANNE MARIE ESCORDIAL SAMPLE DESCRIPTION : WATER/SET UP (SAND WITH FERRIC OXIDE) DATE OF SAMPLE RECEIVED : SEPTEMBER 5, 2016 DATE REPORTED : SEPTEMBER 14, 2016 SAMPLE COLLECTED BY : TRICIA ANNE MARIE ESCORDIAL DATE OF SAMPLING : SEPTEMBER 5, 2016 DATE OF ANALYSIS : SEPTEMBER 13, 2016 REFERENCE NO. : 16 - 6795</p>																														
<p>LABORATORY TEST RESULTS:</p> <table border="0"> <thead> <tr> <th></th> <th colspan="3">SAMPLE A</th> <th colspan="3">SAMPLE B</th> </tr> <tr> <th>Sample Code</th> <th>R1</th> <th>R2</th> <th>R3</th> <th>R1</th> <th>R2</th> <th>R3</th> </tr> </thead> <tbody> <tr> <td>Dissolved Oxygen (mg/L)</td> <td>6.7</td> <td>6.5</td> <td>5.4</td> <td>3.8</td> <td>2.8</td> <td>2.2</td> </tr> <tr> <td colspan="7">4500C - Azide Modification - Winkler Method</td> </tr> </tbody> </table>				SAMPLE A			SAMPLE B			Sample Code	R1	R2	R3	R1	R2	R3	Dissolved Oxygen (mg/L)	6.7	6.5	5.4	3.8	2.8	2.2	4500C - Azide Modification - Winkler Method						
	SAMPLE A			SAMPLE B																										
Sample Code	R1	R2	R3	R1	R2	R3																								
Dissolved Oxygen (mg/L)	6.7	6.5	5.4	3.8	2.8	2.2																								
4500C - Azide Modification - Winkler Method																														
<p>Method of Analysis: SMEWW, 21ST Edition*, APHA-AWWA*</p> <p>Note: 1. Result of Examination specifically related to samples as received. 2. Test results shall not be reproduced without the approval of the Laboratory Head. 3. Measurement uncertainty is available upon request.</p>																														
<p>Analyzed by:</p>  Ma. Antonella C. Lanaco Chemical Analyst Approved Signatory	<p>Certified by:</p>  Roselyn D. Usero, RChem, MEE Laboratory Head Registered Chemist, Lic.#6460 Approved Signatory																													
NPPC-ADL LSP 5.10 FO1b Rev. 02/3 Effectivity Date : 05/25/16	- Page 1 / 1 -																													

**Official Results for the Total Suspended Solids (TSS)and pH of the Wastewater treated
and with Set up B (Sand Only)**


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 Door No.1 & 2, NOLKFI Bldg., 6th Street., Bacolod City
 Tele/Fax 034-4332131 email address nppc_adi@yahoo.com.ph

CLIENT : NEGROS OCCIDENTAL HIGH SCHOOL
ADDRESS : Bacolod City
DATE OF SAMPLE RECEIVED : Sept. 6, 2016
ANALYSIS REQUESTED : pH, Total Suspended Solids (TSS)
SAMPLE DESCRIPTION : Water
DATE OF ANALYSIS : Sept. 6, 2016
DATE COMPLETED : Sept. 6, 2016


LSO NO. : 16-6795


Laboratory Test Results :

Control Number	Sample Code	pH	TSS
16-10278	R-1 (Sand Filter)	8.26	35
	R-2 (Sand Filter)	8.20	44
	R-3 (Sand Filter)	8.39	48



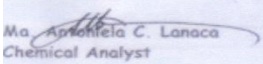

NOTE:

1. Result of examination specifically related to samples as received.
2. Test results shall not be reproduced without the approval of the Laboratory Head.

Analyzed by:

 Mari Antonietta C. Lariaca
 Laboratory Analyst

Approved for release:

 ROSELYN C. USERO
 Laboratory Head

TSS of the Wastewater After It was Filtered in Set-up A (Sand with Iron Oxide Microparticles)

	NEGROS PRAWN PRODUCERS COOPERATIVE ANALYTICAL & DIAGNOSTIC LABORATORY Door No.1 & 2., NOLKFI Bldg., 6 th Street., Bacolod City Tele/Fax 034-4332131 email address nppc_adi@yahoo.com.ph	Chemical Testing  PAB ACCREDITED TESTING LABORATORY PNS ISO/IEC 17025:2005 LA-2016-289A	
CLIENT : SAMPLE DESCRIPTION : DATE OF SAMPLE RECEIVED : DATE REPORTED : SAMPLE COLLECTED BY : DATE OF SAMPLING : DATE OF ANALYSIS : REFERENCE NO. :	TRICIA ANNE MARIE ESCORDIAL WATER/SET UP (SAND WITH FERRIC OXIDE) SEPTEMBER 5, 2016 SEPTEMBER 14, 2016 TRICIA ANNE MARIE ESCORDIAL SEPTEMBER 5, 2016 SEPTEMBER 13, 2016 16 - 6795		
LABORATORY TEST RESULTS:			
Lab. Control No. Sample Code Total Suspended Solids (mg/L) 2540D - Gravimetric Method dried @ 105 °C*	16 - 10278A R1 2	16 - 10278B R2 4	16 - 10278C R3 7
Method of Analysis: SMEWW, 21ST Edition*, APHA-AWWA* Note: <ol style="list-style-type: none"> 1. Result of Examination specifically related to samples as received. 2. Test results shall not be reproduced without the approval of the Laboratory Head. 3. Measurement uncertainty is available upon request. 			
Analyzed by:  Ma. Archifela C. Lanaca Chemical Analyst Approved Signatory	Certified by:  Roselyn D. Usoro, RChem, MEE Laboratory Head Registered Chemist, Lic.#6460 Approved Signatory		
NPPC-ADL LSP 5.10 FOIB Rev. 02/3 Effectivity Date : 05/25/16	- Page 1/1 -		

Official Results for the Thermotolerant Fecal and Coliform of the Wastewater treated with Set up A (Sand with Iron Oxide Nanoparticles)



**NEGROS PRAWN PRODUCERS COOPERATIVE
ANALYTICAL & DIAGNOSTIC LABORATORY**

Door No. 1 & 2, NOLKPI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph

Biological Testing



CLIENT : Escordial Tricia Anne Marie
DATE OF SAMPLE RECEIVED : September 5, 2016
SAMPLE DESCRIPTION : WATER
DATE OF SAMPLING : September 5, 2016
DATE OF ANALYSIS : September 7, 2016
SAMPLING SITE : Alijis, Bacolod
SAMPLE COLLECTED BY : Escordial Tricia Anne Marie
LSO NO. : 16-6795

**LABORATORY TEST RESULTS
(MICROBIOLOGICAL EXAMINATION)**

Lab. Control No.	16-10278A	16-10278B	16-10278C
Sample Code	R1	R2	R3
Total Coliform (MPN/100 mL) Multiple Tube Fermentation Technique*	600	280	920
Thermotolerant (Fecal) Coliform (MPN/100 mL) Multiple Tube Fermentation Technique*	920	110	540
9221 Smew			

** Reference: Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2012

NOTE: Result is based only on sample submitted.

Maria B. Magpantay
Lab Analyst

Wendy A. Jayson
Approved Signatory (Micro)

Certified Correct:

Rosely C. Usero, RCHEM, MEE
Laboratory Head

NPPC ADL
LSP 5.10 F02a
Rev. 01/Issue 2
Effectivity Date: 03/15/16

page 1 of 1

Official Results for the Total Fecal and Coliform of the Wastewater treated with Set up B (Sand Only)



**NEGROS PRAWN PRODUCERS COOPERATIVE
ANALYTICAL & DIAGNOSTIC LABORATORY**

Door No.1 & 2, NOLKFI Bldg., 6th Street, Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph

Biological Testing



PAB ACCREDITED
TESTING LABORATORY
PNS ISO/IEC 17025:2005
LA-2016-288A

CLIENT : Escordial Tricia Anne Marie
DATE OF SAMPLE RECEIVED : September 5,2016
SAMPLE DESCRIPTION : WATER
DATE OF SAMPLING : September 5,2016
DATE OF ANALYSIS : September 5,2016
SAMPLING SITE : Alijis,Bacolod
SAMPLE COLLECTED BY : Escordial Tricia Anne Marie
LSO NO. : 16-6795

**LABORATORY TEST RESULTS
(MICROBIOLOGICAL EXAMINATION)**

Lab. Control No.	16-10278A	16-10278B	16-10278C
Sample Code	R1	R2	R3
Total Coliform (MPN/100 mL) <small>Multiple Tube Fermentation Technique*</small>	2,100	1,700	2,100
Thermotolerant (Fecal) Coliform (MPN/100 mL) <small>Multiple Tube Fermentation Technique*</small>	1,700	1,400	1,700
<small>9221 Sew</small>			

** Reference: Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2012

NOTE: Result is based only on sample submitted.

Marla B. Magpantay
Lab Analyst

Wendy A. Byson
Approved Signatory (Micro)

Certified Correct:

Roselyn L. Usero, RCHEM, MEE
Laboratory Head

NPPC-ADL
LSP 5.10 F02a
Rev.01/Issue 2
Effectivity Date: 03/15/16

page 1 of 1

Turbidity of the Wastewater after It was Filtered with Set-up A (Sand with Iron Oxide Microparticles) and Set-up B (Sand Only)



NEGROS PRAWN PRODUCERS COOPERATIVE
Door No.1 & 2., NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph

CLIENT : NEGROS OCCIDENTAL HIGH SCHOOL
ADDRESS : BACOLOD CITY
DATE OF SAMPLE RECEIVED : SEPT. 6, 2016
ANALYSIS REQUESTED : TURBIDITY
SAMPLE DESCRIPTION : WATER
DATE OF ANALYSIS : SEPT. 6, 2016
DATE COMPLETED : SEPT. 6, 2016

LSO NO. : 16-6795

Laboratory Test Results :

Control Number	Sample Code	Turbidity		
		R1	R2	R3
16-10278	Set-Up A	9.20	5.34	9.33
	Set-Up B	32.90	30.30	35.40

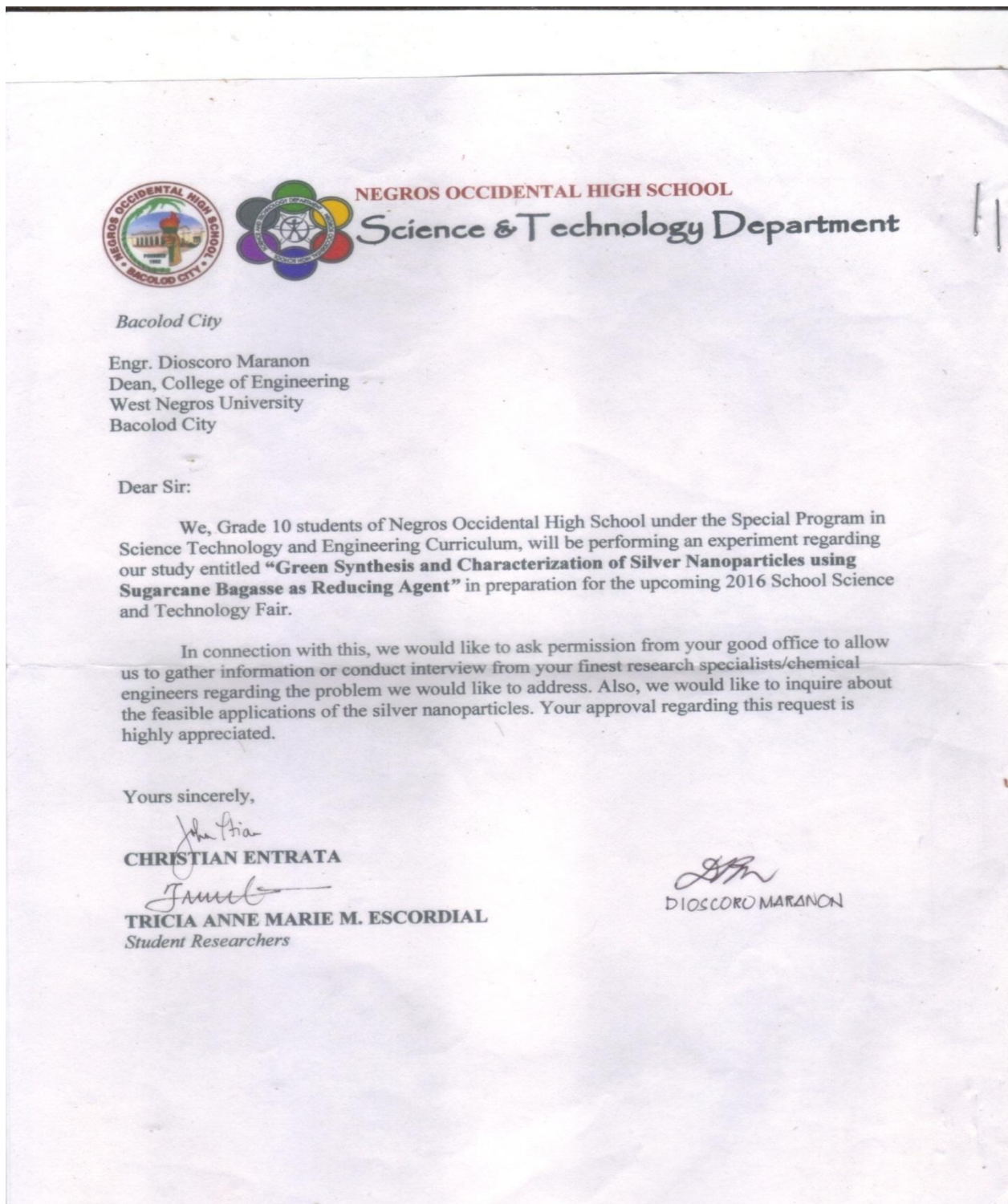
Analyzed by:

Ma Antoniefa C. Lañaca
Ma Antoniefa C. Lañaca
Laboratory Analyst

Approved for release:

Roselyn C. Usero
ROSELYN C. USERO
Laboratory Head

Communication Letter for STI-WNU



Technical Service Request Form for DOST Taguig, ITDI



Republic of the Philippines
Department of Science and Technology
ADVANCED DEVICE AND MATERIALS TESTING LABORATORY
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
DOST Cpd., General Santos Ave., Bicutan, Taguig City
Tel. Nos. : 837-2071 to 82 (DOST Trunklines), 837-0503 (Direct Line) Telefax No.: 837-3167
http://www.itdi.dost.gov.ph, http://www.admatel.com



TECHNICAL SERVICE REQUEST

Clearance box

Laboratory Head	Laboratory Manager	Cashier	Due Date	Request Reference Number
Date:	Date:	Date:	Date:	Date:

This block to be filled up by the Customer:

Company/Institution: Negros Occidental High School
 Address/ Telephone No./Email: Corner Hernaez, Araneta St., Bacolod City / 458-1643
 Requesting Official(name in print): Christian Entrata
 Designation: Student Researcher Signature: [Signature]
 Sample brought by: Rona A Nacino Signature: [Signature]

Sample:
 Description: (Exclude those not evident on sample submitted)
Sand coated with Iron Oxide Nanoparticles
 Identifying Marks:
Sand coated with FeO nps
 Quantity:
1 vial containing sand coated with Iron Oxide Nanoparticles
 Other supplies/ materials/ chemicals submitted

Description	Quantity

Request for INITIAL RESULTS (refer to Non-Disclosure Agreement below)
 I/We agreed that any information obtained from the initial result/s will be considered proprietary and confidential
CHRISTIAN V. ENTRATA
 Printed Name & Signature

Specific Test/ Analysis/ Service required
TEM ANALYSIS

Customer Profile: Government NGO Academe
 Private Mfg. Private Service Company
 Individual Others, Specify STUDENT

REMARKS

This block to be filled up by ADMATEL

Fees/Chargers:	Amount Due	Amount Paid	Official Receipt No.	Date

Job Assigned To: (Please list)

- 1.
- 2.
- 3.

Signature of Laboratory Head:

Date and Time Received:

Form: AL-04-F4
 Issue: April 2014
 Revision: 00

Certifications from Negros Prawn Producers Laboratory



NEGROS PRAWN PRODUCERS COOPERATIVE

Door No.1 & 2, NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adi@yahoo.com.ph

CERTIFICATION

This is to certify that the following students from Negros Occidental High School have conducted their experiment entitled **“Biosynthesis and Characterization of Iron Oxide Microparticles Using Sugarcane (*Saccharum officinarum*) Bagasse as Capping and Reducing Agent and Its Application as a Novel Biosand Filter for Industrial Wastewater Treatment”** from May 27, 2016 - October 17, 2016 at Negros Prawn Producers Analytical Laboratory, Door 1 and 2 NEDF Building, 6th Street, corner Lacson Street, Bacolod City Negros Occidental.

1. Tricia Anne Marie Escordial
2. John Christian Entrata

Approved for release:


ROSELYN C. USERO
Laboratory Head



NEGROS PRAWN PRODUCERS COOPERATIVE

Door No.1 & 2, NOLKFI Bldg., 6th Street., Bacolod City
Tele/Fax 034-4332131 email address nppc_adl@yahoo.com.ph


CERTIFICATION

This is to certify that the wastewater samples used in the study entitled **“Biosynthesis and Characterization of Iron Oxide Microparticles Using Sugarcane (*Saccharum officinarum*) Bagasse as Capping and Reducing Agent and Its Application as a Novel Biosand Filter for Industrial Wastewater Treatment”** were collected by the researchers under the supervision of Negros Prawn Producers Laboratory from a fast food chain in Barangay Alijis, Bacolod City.

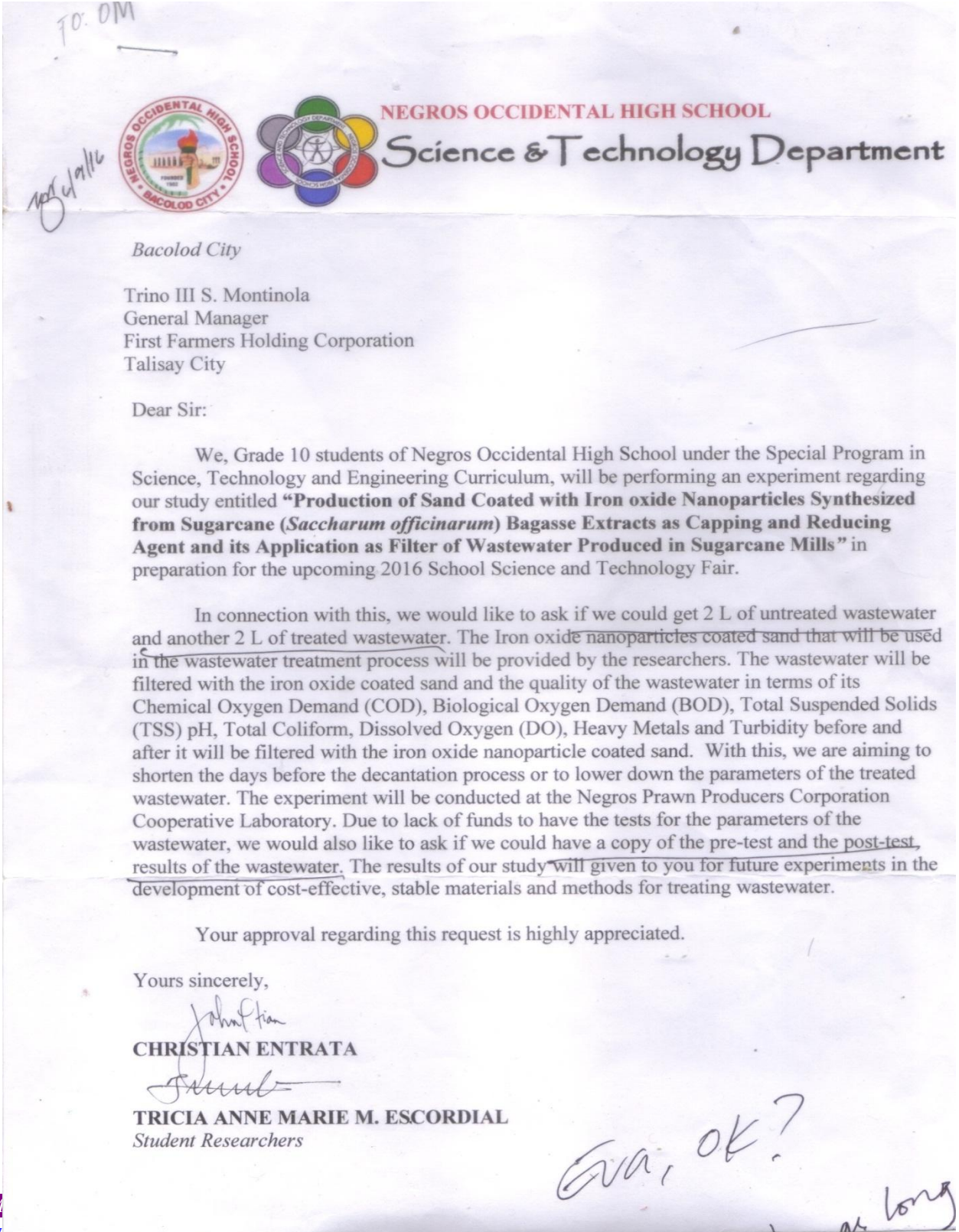
Researchers:

1. Tricia Anne Marie Escordial
2. John Christian V. Entrata

Approved for release:


ROSELYN C. USERO
Laboratory Head

Communication Letter for First Farmers Holding Corporation



Official Receipts from Negros Prawn Producers Laboratory

NEGROS PRAWN PRODUCERS COOPERATIVE (LABORATORY)
DOOR 2, NOLKFI BLDG., 6TH STREET, BCOLOD CITY
TEL. NO. 433-2131
NON-VAT REG. TIN: 417-863-851-000

No. **20285**

OFFICIAL RECEIPT (EXEMPT) Date: 9/5/16

RECEIVED from Escorial, Tricia Anne Marie

Bus. Style: _____ TIN: _____

Address: _____

the sum of PESOS: Two Thousand Only (P. 2,000-)

in full/partial payment of: _____

CASH; CHECK NO. & DATE: _____

BALANCE - P _____

Authorized Signature _____

In settlement of the following:	
PARTICULARS	AMOUNT
<u>bul</u>	
<u>2920</u>	<u>6795</u>
TOTAL P	
Less: Withholding Tax	
NET DUE P	

100 Booklets (50 x 3) 20001-25000
BIR Authority to Print No. 2AU0001589456
Issued: June 27, 2016. Valid until June 26, 2021
MOISES M. MARAVILLA, JR.
Margen Subd., Mansilingan, Bacolod City
VAT Reg. TIN: 104-073-514-000
Printer's Acce'n. No. 077MP2013000000004, issued Dec. 18, 2013

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NEGROS PRAWN PRODUCERS COOPERATIVE (LABORATORY)
DOOR 2, NOLKFI BLDG., 6TH STREET, BCOLOD CITY
TEL. NO. 433-2131
NON-VAT REG. TIN: 417-863-851-000

No. **20006**

OFFICIAL RECEIPT (EXEMPT) Date: 8/12/16

RECEIVED from Tricia Escorial

Bus. Style: _____ TIN: _____

Address: _____

the sum of PESOS: Two Thousand Pesos Only (P. 2,000-)

in full/partial payment of: _____

CASH; CHECK NO. & DATE: pub pay

BALANCE - P _____

Authorized Signature _____

In settlement of the following:	
PARTICULARS	AMOUNT
TOTAL P	<u>2000</u>
Less: Withholding Tax	
NET DUE P	

100 Booklets (50 x 3) 20001-25000
BIR Authority to Print No. 2AU0001589456
Issued: June 27, 2016. Valid until June 26, 2021
MOISES M. MARAVILLA, JR.
Margen Subd., Mansilingan, Bacolod City
VAT Reg. TIN: 104-073-514-000
Printer's Acce'n. No. 077MP2013000000004, issued Dec. 18, 2013

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NEGROS PRAWN PRODUCERS COOPERATIVE (LABORATORY)
DOOR 2, NOLKFI BLDG., 6TH STREET, BCOLOD CITY
TEL. NO. 433-2131
NON-VAT REG. TIN: 417-863-851-000

No. **20320**

OFFICIAL RECEIPT (EXEMPT) Date: 9/7/16

RECEIVED from Escorial Tricia Anne Marie

Bus. Style: _____ TIN: _____

Address: _____

the sum of PESOS: Two Thousand Nine Hundred Twenty Only (P. 2,920-)

in full/partial payment of: _____

CASH; CHECK NO. & DATE: _____

BALANCE - P _____

Authorized Signature _____


In settlement of the following:	
PARTICULARS	AMOUNT
TOTAL P	<u>6795</u>
Less: Withholding Tax	
NET DUE P	

100 Booklets (50 x 3) 20001-25000
BIR Authority to Print No. 2AU0001589456
Issued: June 27, 2016. Valid until June 26, 2021
MOISES M. MARAVILLA, JR.
Margen Subd., Mansilingan, Bacolod City
VAT Reg. TIN: 104-073-514-000
Printer's Acce'n. No. 077MP2013000000004, issued Dec. 18, 2013

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ACCOUNTABLE FORM No. 51-C
Revised January, 1992

(ORIGINAL)

	Official Receipt of the Republic of the Philippines	
	Nº 1444466 N	
GF	Date October 11, 2016	
Agency	INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE (ITDI)	Fund 101
Payor	NEGROS OCCIDENTAL HIGH SCHOOL (NOHS)	
Nature of Collection	Account Code	Amount
TSR No. 1610-391 (ADMATEL)	628-1	P 5,800.00
TOTAL		P 5,800.00
Amount in Words	Five Thousand Eight Hundred Pesos and No Cents	
<input checked="" type="checkbox"/> Cash 5,800.00 <input type="checkbox"/> Check <input type="checkbox"/> Money Order	Drawee Bank	Number Date
Received the amount stated above. <i>Liezl G. Cudapas</i> LIEZL G. CUDAPAS Administrative Officer III Collecting Officer		
NOTE: Write the number and date of this receipt on the back of check or money order received.		

DOCUMENTATION



Figure 12. The Negros Prawn Laboratory



Figure 13. First Farmers Holding Corporation



Figure 14. Department of Science and Technology- ITDI
(Photo Credits: Pinoy Technoguide)



Figure 15. Sugarcane Bagasse collected from the First Farmers Holding Corporation



Figure 16. Measuring 50 grams of bagasse



Figure 17. 50 grams of bagasse



Figure 18. Boiling of 50 grams bagasse in 100 mL distilled water for 10 minutes



Figure 19. After the 50 g of bagasse was boiled



Figure 20. Filtering the mixture using a cloth



Figure 21. The bagasse extract



Figure 22. The residue



Figure 23. Putting sugarcane bagasse extract in each test tube



Figure 24. Adding 40 ml of ferric chloride solution into the test tube

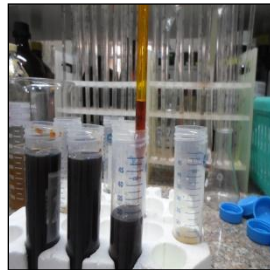


Figure 25. Using the pipette for measuring the ferric chloride solution into the centrifuge tube



Figure 26. Preparing the centrifuge tubes to be placed in the centrifuge



Figure 27. Placing the centrifuge tubes into the centrifuge

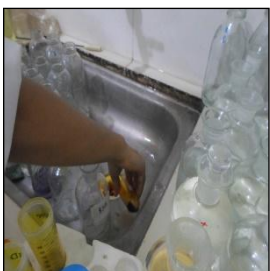


Figure 28. After 15 minutes of centrifugation, the supernatant was discarded

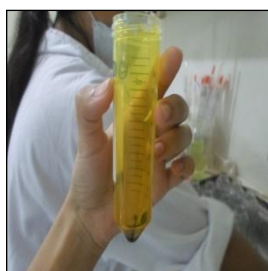


Figure 29. The attained black product



Figure 30. The black product washed with ethanol



Figure 31. The black product after washing with ethanol

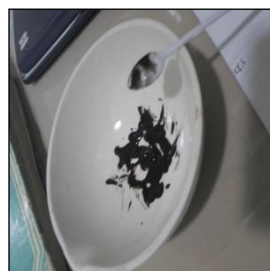


Figure 32. Transferring the black product into the evaporating dish



Figure 33. Placing the evaporating dish in the oven



Figure 34. The oven at 105 degrees Celsius



Figure 35. The evaporating dish inside the oven for 5 hours



Figure 36. The black product after 5 hours



Figure 37. Putting the final black product inside the vial



Figure 38. The Sand with Iron oxide microparticles filter and the sand filter



Figure 39. Sand passing through a sieve



Figure 40. Sieve with holes between 0.85 and 2.36 mm



Figure 41. Preparing the 8% Nitric acid solution



Figure 42. Sand soaked in an 8% Nitric acid solution



Figure 43. Checking the pH



Figure 44. Rinsing the sand soaked with distilled water



Figure 45. Drying the sand in the oven



Figure 46. The oven at 104 degrees Celsius



Figure 47. Evaluating the pH level of the Iron oxide



Figure 48. Evaluating the Iron oxide Microparticles' Solubility to water



Figure 49. Evaluating the Iron oxide Microparticles' Solubility to ethanol.



Figure 50. Evaluating the flammability of Iron oxide Microparticles.



Figure 51. The wastewater samples being filtered using the biosand composite with iron oxide microparticles



Figure 52. The wastewater samples after being filtered using the biosand composite with iron oxide microparticles



Figure 53. The wastewater samples after being filtered using sand only.



Figure 54. Testing the pH of the wastewater



Figure 55. Testing the turbidity of the wastewater



Figure 56. Testing the COD of the wastewater



Figure 57. Testing the DO of the wastewater



Figure 58. Testing the TSS of the wastewater



Figure 58. Testing the TFC of the wastewater