RESEARCH NOTE

Bacterial reduction in river water using nanocellulose membrane from pineapple biomass with ferrous-ferric oxide reinforcement

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BACKGROUND AND OBJECTIVES: Constructing a nanocellulose membrane from biomass waste can lessen harmful environmental effects owing to its ability to absorb chemical and microbiological impurities. Therefore, nanocellulose membranes with magnetic properties were developed as a powerful apparatus for reducing microbials and dyes in water.

METHODS: In this study, bacterial cellulose acetate-based nanocomposite membrane with ferrous-ferric oxide nanoparticle reinforcement was produced from pineapple peel biowaste extract through fermentation and esterification. High-pressure homogenization was used to produce nano properties of cellulose from pineapple. Meanwhile, the ultrasonic homogenizer was used to mix the produced nanocellulose with the ferrous-ferric oxide with various treatment (0.25, 0.50, 0.75, and 1.0 weight percent of cellulose acetate) to produce nanocomposite membrane. The membrane was then applied for the removal of bacteria and dyes. The samples were water from local rivers located near industries such as rubber, cement, and tofu industries. The effectiveness of the nanocomposite membrane at bacteria and dyes reduction was assessed.

FINDINGS: Nano cellulose membrane effectively reduced gram-negative bacteria and anionic dyes in the water samples. The ferrous-ferric oxide reinforcement enhanced the effectiveness of the membrane on bacteria and dye reduction. The addition of ferrous-ferric oxide resulted in a greater amount of dye degradation, and the presence of ≥0.75 percent ferrous-ferric oxide indicated an optimum ability to kill bacteria.

CONCLUSION: Ferrous-ferric oxide yielded good results in reducing the number of microbes and anionic dyes in the water samples tested. The results of this research can be used as basic data to advance the use of nanocellulose membranes as a biomaterial for controlling environmental impacts.

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ABSTRACT

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INTRODUCTION

Cellulose is a natural polymer that is important for the development of environmentally friendly products (Manuel et al., 2020). Wood and other higher plants are the primary sources of cellulose. Algae, tunicates, and certain bacteria can also produce cellulose in relatively large quantities (Thomas et al., 2020). Cellulose has been widely used on various scales, from macroscopic to nanoscale, to increase its function and usefulness. One application of nano-formed cellulose is a paper membrane that is generally used for eco-friendly water treatment technology. The recently developed membrane technology is becoming increasingly important (Deepa et al., 2015). A significant breakthrough for the synthetic membrane industry began in the 1960s, although initial studies on membrane use started in the mid-18th century (Sofiah et al., 2023; Zulaikha et al., 2022, Patil et al., 2021). The use of membranes for efficient ion separation from solutions has become a focus in the field of membrane science over the past few decades owing to the growing demand for more affordable technologies for wastewater treatment. Developing cellulose membranes from biomass waste is very promising because of its environmental impact. Biomass, a renewable organic material from plants and animals, provides a means to reduce waste contaminants through agricultural commodity-based industrial activities (Oliver Paul Nayagam and Prasanna, 2023; Samimi and Nouri, 2023). Such use of biomass is an effort to promote life cycle assessment (LCA) towards a greener environment (Rini et al., 2021; Samimi, 2024). An example of this effort is the production of nanocellulose from pineapple peel waste. Owing to the large variety of aquatic waste in society, the cellulose nanomembrane produced from pineapple peel waste were given a strengthening treatment using ferrous-ferric oxide (Fe$_3$O$_4$). Fe$_3$O$_4$ has hydrophilic property (Azimi et al., 2019); therefore, it facilitates the formation of intermolecular bonds between the oxygen in the Fe$_3$O$_4$ nanoparticles and hydroxyl groups of cellulose, which is reduced by the excess electrons provided by the hydroxyl groups. Another form of intermolecular bonding arises from electrostatic forces between the surface of Fe$_3$O$_4$ and cellulose fibrils. In addition, mechanical interlocking causes Fe$_3$O$_4$ to inhibit the movement of fibrils, thereby boosting the strength of the Fe$_3$O$_4$ nanocomposite system, which has proven effective in killing bacteria and destroying biofilms (Ma et al., 2022; El Sacker et al., 2021). The magnetic effect of metal oxide on waste water treatment has also been studied (Bellebcir et al., 2023; Wu et al., 2023). Reinforcement using metal oxides, specifically magnetite, is known to enhance the adsorption of metals, textile dyes, organic materials, nitrogen, phosphorus and facilitate antibacterial activities (Akbar et al., 2023; Lu Dong et al., 2021; Uchechukwu et al., 2021). This study was conducted to investigate the effectiveness of nanofibrillated cellulose (NCF) reinforcement for reducing bacteria and water waste components such as dyes. Three types of waste in the Padang city area with various suspected pollution were treated with NCF in this study. The information obtained from this research will become basic data for developing widely applicable NCFs. The aim of the current study was to identify the effectiveness of adding Fe$_3$O$_4$ to nanocellulose membrane developed from pineapple peel waste for bacteria and dyes reduction. The obtained data can provide basic information on developing NCF for waste management system. The study was conducted in Malang City, East Java and Padang City West Sumatera, Indonesia. The laboratory analysis was conducted at Agricultural Product Technology laboratories, department of food and agricultural product technology, Andalas University, from May to October 2023.

MATERIAL AND METHODS

Materials

The pineapple waste was collected from Blitar Regency. Acetobacter xylinum, a cellulose-producing microbe, was provided by the Applied Tech laboratory at Malang Muhammadiyah University, Indonesia. The characteristic of pineapple waste was described in the previous report (Suryanto et al., 2019). The chemicals were purchased form Smart-Lab, Indonesia, and the Fe$_3$O$_4$ was imported from Guangzhou Hongwu Co., China. The chemical used were in the pro-analysis grade.

Fabrication of bacterial cellulose

The process for generating nanocellulose membranes can be simplified into four steps:

1. Production of bacterial cellulose through fermentation
2. Nanofibrillation of cellulose
3. Formation of composite of a nanocellulose acetate membrane

4. Addition of metal oxide ($\text{Fe}_3\text{O}_4$) to strengthen membrane characteristics

**Bacterial cellulose production**

The bacterial cellulose was produced in adherence to predetermined procedures described in previous publication (Yanuhar et al., 2022). A mixture of 0.5 litre (L) pineapple peel extract, five grams (g) of glucose, and 2.5 g of ammonia acid were used to create the fermentation medium. Acetic acid ($\text{CH}_3\text{COOH}$) solution was added to the medium to maintain the potential of hydrogen (pH) level at 4.5. One hundred millilitres (mL) of Acetobacter xylinum was added to the medium to trigger the fermentation process. Static fermentation took place at a temperature between 25 degrees Celsius (°C) and 30°C. Bacterial cellulose then floated on the media, and were collected after 10 days and rinsed with aquadest to achieve a pH level of 7.0.

**Bacterial cellulose fibrillation**

The bacterial cellulose was fibrillated using the methods described by (Yanuhar et al., 2022). The pellicles underwent a pre-treatment phase that entailed exposure to a six percent sodium hydroxide (NaOH) alkali solution for two hours at 90°C prior to extraction. The cleaned pellicles were rinsed and divided into smaller pieces. Three hundred grams of these cleaned pellicles were treated in a crushing machine (ICHDS7 type, Fomac, China) at 25,000 revolution per minute (rpm) for five minutes after being soaked in 250 mL of distilled water. The final bacterial cellulose slurry was combined with 750 mL of distilled water before being put through a high-pressure homogenizer (Berkley-Scientific, AH-100D type, China). To collect the fibrillated bacterial cellulose slurry, a filtration procedure was used after the bacterial cellulose was fibrillated under a pressure of 150 bar.

**Synthesis of cellulose acetate**

In a beaker, 50 mL of glacial acetic acid was introduced into 2.50 g of bacterial nanocellulose. The mixture was stirred for thirty minutes. It was then stirred for another twenty-five minutes, while 0.32 mL of sulphuric acid ($\text{H}_2\text{SO}_4$) solution and 18 mL of $\text{CH}_3\text{COOH}$ solution were added. The mixture was then filtered, following which 64 mL of anhydrous acetate was added. Thirty minutes of stirring followed. The solution was vacuum filtered after being laid aside for fourteen hours. The resulting precipitates were then oven-dried for a day at 70°C (Kirin, KBO-190RA) after being washed with aquadest until they were neutral (Cobo et al., 2017).

**Reinforcement nanocomposite membrane production**

A set of recognized procedures were followed for the fabrication of the cellulose acetate (Homem et al., 2020). Fifty millilitres of $\text{CH}_3\text{COOH}$ and 2.5 g of fibrillated bacterial cellulose were combined in a glass beaker. The mixture was stirred for thirty minutes at room temperature. Subsequently, 0.32 mL of $\text{H}_2\text{SO}_4$ and 18 mL of $\text{CH}_3\text{COOH}$ were added to the solution, which was then agitated for twenty-five minutes. Following filtration, 64 mL of acetic anhydride was added to the filtrate, and the mixture was agitated for 30 minutes. The resulting solution was allowed to precipitate for fourteen hours before being neutralized via aquadest-rinsing. It was then suspended in 200 mL of aquadest, agitated for 30 minutes, and sonicated for additional 30 minutes at 20 kilohertz (kHz), 400 watts (ultrasonic homogenizer, UP.400S-type, Lawson Scientific, China). $\text{Fe}_3\text{O}_4$ were added to the dispersion and sonicated for thirty minutes during this procedure. The $\text{Fe}_3\text{O}_4$ addition was set as 0.25; 0.5; 0.75 and 1 g per 100 g cellulose acetate, where the solution with no $\text{Fe}_3\text{O}_4$ was named as control. As previously noted, the dispersion was continuously agitated for two hours to achieve a homogeneous suspension, after which the mixture was baked at 120°C for four hours. The homogenous mixture was then combined with cellulose acetate and ultrasonically homogenized for one hour. Subsequently, the finished product was poured into a mold and baked for 24 hours at 60°C to dry it out.

**$\text{Fe}_3\text{O}_4$ reinforcement morphology observation**

The nanocellulose membrane structure was analyzed using X-ray diffraction (XRD) (PanAnalytical-Expert Pro, USA). The membrane was cut into 20 x 20 square millimetre (mm$^2$), then put in an XRD instrument. Analysis was conducted at a diffraction angle in the range of 10°-70°. For the analysis, CuKα were used as X-ray source with a wavelength of 1.542Å at 40 kilovolt (kV) and 30 mA (Suryanto et al., 2023). At a 25.0 kV acceleration voltage, the scanning
Nanocellulose membrane from pineapple biomass

Electron microscope (SEM) (Inspect S50, Japan) was used to analyze the surface morphology. A coater (Emitech, SC7.620, United Kingdom) was used to apply a 10 nanometer (nm) layer of gold coating to the membrane prior to SEM inspection (Xiao et al., 2015).

Bacterial determination in tested water

Three types of water samples were used in this study. The samples were collected from rivers that flowed around three industries, namely the cement, rubber and tofu industries. The NCF membrane was dipped in the water for ten minutes. Bacteria in water were grown on plate count agar and Luria agar to determine the effect of membrane contact on the membrane’s bactericidal action. The viability of bacteria on the NCF membrane was also measured (Thammawong et al., 2019; Ding et al., 2023).

Pigment decolorization

The ability of the membrane to reduce azo dye components (rhodamine and acid orange) was investigated. Each pigment was made with a concentration of 50 part per million (ppm) and the membrane was dipped in each solution. The absorbance of the pigment solution was measured according to each specific wavelength using a spectrophotometer (Shimadzu-1800, Japan) before and after membrane immersion. All the experiments were repeated, at least, three times. The data were then processed statistically using Microsoft Excel (Fiana et al., 2023; Rini et al., 2023).

Statistical analysis

Statistical analysis was performed using the SPSS package program version 11.5 (SPSS Inc., Chicago, IL, USA). After the data were analyzed using one-way analysis of variance, the Duncan’s multiple range posthoc test was run. The results were expressed using the triple samples’ mean ± SD. P<0.05 was used to denote differences of importance (Samimi et al., 2023).

RESULT AND DISCUSSION

The characteristics of the NCF nanocomposite were identified through XRD analysis (Fig. 1a). These patterns made it possible to identify the membrane’s phase and crystal structures. Three major peaks were visible in membrane control, with values of 14.2°, 16.6°, and 22.5°, which correspond to the crystalline planes of [110], [100], and [200], in that order (Lee et al., 2015). The peaks in question represent natural cellulose (cellulose Iβ). Ferrous-ferric oxide nanoparticles were present in the nanocellulose membrane at peaks of 29.9°, 35.4°, 57.1°, and 62.6°. As the concentration of ferrous-ferric oxide nanoparticles increased, the strong diffraction peaks in the 10°–75° range showed increasing intensity. Owing to their surface growth on the membrane, the ferrous-ferric oxide nanoparticles seemed relatively weak within the nanocellulose membrane, which made it difficult to obtain the cellulose diffractions data during XRD testing. However, more ferrous-ferric oxide nanoparticles led to more self-agglomerated nanoparticles, which produced a more noticeable crystalline structure. The membranes’ crystalline index was roughly 54.3 percent (%). The addition of 0.25, 0.50, 0.75, and 1.0 weight percent of ferrous-ferric oxide nanoparticles yielded crystalline indices of approximately 49.2%, 51.1%, 59.4%, and 68.3%, respectively. The membrane’s crystalline index decreased with the addition of 0.25 wt% and 0.5 wt% ferrous-ferric oxide nanoparticles. The crystalline region of the cellulose membrane appeared to have been disrupted by the modest amount of ferrous-ferric oxide nanoparticles (Kaco et al., 2017); yet, the crystalline index value increased when the ferrous-ferric oxide nanoparticle content was increased to 0.75 wt% and 1.0 wt%. According to some researchers, the addition of magnetic nanoparticles can enhance the mechanical strength, thermal stability, optical transparency, electrical conductivity, and magnetic response of cellulose nanomaterials by enhancing the interactions between the materials (Evans et al., 2023). It can also increase the crystallinity of cellulose nanocrystals (Babaei-Ghazvini et al., 2020). It is conceivable that at the right concentration, ferrous-ferric oxide nanoparticles have a stabilizing effect that promotes the creation of crystalline areas, hence enhancing the crystalline structure of the nanocellulose.

The characteristics of the NCF nanocomposite was also identified through SEM analysis (Fig. 1b). It was inferred from the results that the Fe3O4 nanoparticle successfully integrated with the nanocellulose membrane formed from the pineapple peel. In this study, it was found that the membrane porosity was suboptimal. It was observed that the pores were not
Fig. 1a: Diffractogram of nanocellulose membrane: (a) Control; (b) Fe₃O₄ 0.25 wt%; (c) Fe₃O₄ 0.5 wt%; (d) Fe₃O₄ 0.75 wt%; (e) Fe₃O₄ 1.0 wt%.
Continued Fig. 1a: Diffractogram of nanocellulose membrane: (a) Control; (b) Fe₃O₄ 0.25 wt%; (c) Fe₃O₄ 0.5 wt%; (d) Fe₃O₄ 0.75 wt%; (e) Fe₃O₄ 1.0 wt%.

evenly distributed throughout the cell membranes. Moreover, the Fe₃O₄ treatments also blocked the pores. Therefore, the SEM information was crucial because it provided a starting point for establishing an approach to utilizing the membranes as a material for minimizing bacteria in water samples. However, membrane could not be used for water filtration because its porosity was suboptimal; therefore, the membrane immersion procedure was examined.

The immersion technique was superior to filtration for determining how effectively membranes decimated aquatic microorganisms. Compared to the filtering process, this technique required less effort. Previous researchers have demonstrated how challenging it is to combine nanocellulose combined with metal oxide composites in filtration materials (Ding et al., 2023). A technique for submerging the membrane in a water sample is depicted schematically in Fig. 2. In this study, the number of bacteria that could be absorbed by the membrane and the likelihood of the bacteria surviving were used to determine how effectively bacteria in water might be reduced.

Figure 3 shows the number of bacteria absorbed by the membrane after immersion. The plate count agar media and Luria agar provide information...
Fig. 1b: SEM picture of NCF with zero (a), 0.25% (b), 0.5% (c), 0.75% (d) and 1% Fe$_3$O$_4$ treatment
Nanocellulose membrane from pineapple biomass

Fig. 2: Schematic work for observation of the nanocomposite membrane for bacterial removal in water sample

Water river was collected from the local area in Padang City West Sumatera

The NCF membrane was immersed in the water for 10 min

The water was tested on Plate count agar and Luria Agar

regarding the types of gram-positive bacteria (3a) and gram-negative bacteria (enterobacteria) (3b), respectively. Overall, nanocellulose from pineapple peel effectively absorbed the microbial content in the three types of water samples tested. A reduction of up to 50% of the initial bacterial content in the water samples for the two types of bacteria observed occurred. The use of Fe₃O₄ metal also had a positive effect on the resistance of bacteria present in the sample. The NCF membrane was more effective for gram-negative bacteria than for gram-positive bacteria. The response to the bacterial adsorption process also depended on the type of river water. For water samples from the rivers near the cement and rubber industries, the presence of Fe₃O₄ in the membrane at a concentration of 0.75% did not have a significant effect. In the tofu water samples, membranes treated with Fe₃O₄ gradually decimated both types of bacteria. Two types of mechanisms related to the bacterial reduction process can be proposed from the resulting data (Onyszko et al., 2022; Sivansankari et al., 2019). First, the ability of cellulose to absorb bacterial particles because of the interaction between the hydrogen bonds in cellulose and the bacteria, which makes the bacteria to bond with the cellulose molecules. Meanwhile, the presence of a metal component (Fe₃O₄), a positively charged component, produces an ionic cross-link with bacterial molecules. The influence of the charge was crucial in this phenomenon, where positively charged metals tend to form good bonds with negative charges. The situation might improve the way that NCF interacts with gram-negative bacteria. The surroundings of rivers can have an impact on the polluting elements found in their water. Therefore, different kinds of river water were used in this study. The river water in the vicinity of the cement factory has a comparatively alkaline pH and significant concentrations of insoluble particles. The water in the vicinity of the tofu and rubber industries are somewhat acidic. In addition, the profile of microorganism contamination fluctuates, with companies using organic raw materials typically experiencing higher levels of microbe contamination owing to the overall microbial content (Rini et al., 2021).

For further analysis, the effect of Fe₃O₄ on the membranes’ ability to kill bacteria was described. Figure 4 shows the viability of gram-negative bacteria that survived on the NCF membranes. The likelihood of bacteria adhered to the membrane surviving is demonstrated by these data. These data are
essential because bacteria adhere to membranes following the membrane soaking process. Naturally, it is undesirable if bacteria exhibit strong membrane resistance because of the possibility of cross-contamination. The data show that the higher the concentration of Fe$_3$O$_4$, the lower the survival of bacteria. It was also found that using Fe$_3$O$_4$ concentrations between 0.25%-0.5% did not have a significant bactericidal effect because the bacterial viability value was the same as that of the control membrane. The Fe$_3$O$_4$ concentrations of 0.75% and 1% showed promising results for the viability value of bacteria that survived on the NCF membrane. The bacteria attached to the membrane died owing to the intense interaction between metal charges and hydrogen bonds in cellulose (Gudkov et al., 2021; Hernsdorf et al., 2017). This condition might be attributable to the damage to the protein cell walls of bacteria arising from the metal charge coating the NFC membrane (Janičijević et al., 2022; Muthukumar et al., 2017; Liu et al., 2021). It is recommended that future research be conducted to establish whether increasing the concentration of Fe$_3$O$_4$ would provide a better viability effect or trigger saturation. Bacteria experienced a decrease in viability of up to 26% after incubation on the membrane. Bacteria viability can
be reduced up to 12%–26% by adding magnetite. The addition of magnetite concentration increases the membrane’s ability to reduce the ability of bacteria to survive. Magnetite, such as Fe$_3$O$_4$, is known to have antimicrobial properties and can reduce the viability of bacteria, such as *Pseudomonas aeruginosa*, at the addition of 250 µg/ml. Metal ions can bind to mecapto, amino, and carboxyl groups in proteins (including enzymes) causing total or partial inhibition. Iron oxide ions can also damage the function and integrity of bacterial cell walls. It is known that magnetite tends to concentrate more on the outer and inner membrane surfaces of gram-negative bacteria, thus exhibiting stronger antibacterial capabilities. The results of this research show that magnetite is more effective at killing bacteria (bactericidal) attached to the membrane rather than limiting their growth (bacteriostatic).

The NCF membrane’s capability to reduce the amount of azo pigment in water has also been measured, as additional research data in this study. Azo pigments or dyes can alternatively be categorized as cationic or anionic pigments (Oukebdane et al., 2022; Samimi and Safari, 2022; Vučurović et al., 2014). The pigment solution was simulated using 50-ppm pigment concentration in water. According to the proposed hypothesis, NCF membranes had a better adsorption effect on anionic pigment (Acid Red) than on cationic pigment (Rhodamine). The trend was significantly different. This information
highlights the effectiveness of NFC membranes at reducing a component of water pollution, if it had a negative ionization charge. The river water samples contained both bacteria and domestic waste that were composed of both organic and inorganic components (Samimi and Shahriari Moghadam, 2020). Therefore, different rates of binding were observed for the various materials to the surface of the NFC, with those that had been enhanced with magnetite binding better. It is well known that magnetite possesses strong adsorption properties against metals, organic compounds, textile colors, nitrogen, phosphorus, and microorganisms (Lu Dong et al., 2021).

**CONCLUSION**

In this research, a bacterial nanocellulose membrane was developed. The membrane was produced from agricultural waste, specifically, pineapple peel. The active component from pineapple peel was changed enzymatically to produce cellulose fibers, which were then reduced to nano size and given magnetic properties with Fe$_3$O$_4$. The nanocomposite membrane that was fabricated from bacterial nanocellulose from pineapple peel with Fe$_3$O$_4$ reinforcement effectively removed bacteria and synthetic pigment in water. The Fe$_3$O$_4$ reinforcement of the nanocellulose membrane indicated a better result of material with a negative charge such as gram-negative bacterial and anionic pigment. The adsorption properties of nanocellulose coupled with the magnetic ability of Fe$_3$O$_4$ had a significant effect on reducing gram-negative bacteria and anionic pigments in water media. This research revealed that the adsorption quality of cellulose molecules can be increased by strengthening cellulose with the magnetic properties of Fe$_3$O$_4$. Cellulose is a polysaccharide biopolymer that is widely used as an adsorbent. However, the existence of strong intramolecular and intermolecular hydrogen bonds makes it difficult for cellulose to bond with other compounds. Adsorption was possible because cellulose, which initially has the negative properties of hydroxyl, also has a positive charge because it is coated in metal. This combination of positive and negative charges ultimately made the membrane performance better than when no treatment was applied. Therefore, cellulose must be combined with other materials to enhance its adsorption. The same trend was observed in membrane applications for dyes and bacteria with negative charges. Quite different results were obtained when the membrane was applied to remove positively charged dyes and bacteria. The similar charge as the magnetic properties of Fe$_3$O$_4$ made the membrane adsorption capacity inconsistent. Further research is required to clarify these findings. NCF with Fe$_3$O$_4$ made from pineapple peel can be further improved for more extensive communal water sanitation purposes. It is necessary to examine the distribution of Fe$_3$O$_4$ because, in this study, some of the membrane pores were covered owing to Fe$_3$O$_4$ treatment, such that the membrane could only be applied by immersion. Using a filtration system may provide better economic value in actual application. The use of filtration techniques will make it possible to treat a larger population while not overusing the membrane. In addition, the use of different metals as magnetic infrastructure should be considered to create more powerful NCF membranes. Further research is necessary to determine whether further contamination result from the use of NFC. Although there are no chances of cellulose contamination, it is important to ascertain that the membrane will not release Fe$_3$O$_4$ into water bodies. The potential use of pineapple waste to support mitigation of environmental problems will facilitate cleaner production in the pineapple industry so for LCA optimization.

**AUTHOR CONTRIBUTIONS**

D. Syukri has written the manuscript as well as concepted the research, H. Suryanto has contributed in reviewed the research concept and nanocellulose fabrication, F. Kurniawan was responsible for pigment reduction analysis, P.D. Hari has contributed for data processing, R.M. Fiana was responsible for fermentation process, R. Rini participated for the water sampling process.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of
interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, were observed by the authors.

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ABBREVIATIONS

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<td>%</td>
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<td>Fe₃O₄</td>
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REFERENCES


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