Corrosion Science 112 (2016) 385–392

Contents lists available at ScienceDirect

Corrosion Science

journal homepage: www.elsevier.com/locate/corsci

New advances in copper biomachining by iron-oxidizing bacteria

E. Díaz-Tena[a,*], G. Gallastegui[b], M. Hipperdinger[c], E.R. Donati[c], M. Ramírez[d], A. Rodríguez[e], L.N. López de Lacalle[e], A. Elías[a]

[a] Department of Chemical and Environmental Engineering, Bilbao Faculty of Engineering, University of the Basque Country (UPV/EHU), Alameda de Urquijo s/n, 48013 Bilbao, Spain
[b] Department of Chemical and Environmental Engineering, University College of Engineering of Vitoria-Gasteiz, University of the Basque Country (UPV/EHU), Nieves Cano 12, 01006 Vitoria-Gasteiz, Spain
[c] CINDEFI (CCT La Plata-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115, 1900 La Plata, Argentina
[d] Department of Chemical Engineering and Food Technologies, Faculty of Sciences, University of Cadiz, Puerto Real, 11510Cádiz, Spain
[e] Department of Mechanical Engineering, Bilbao Faculty of Engineering, University of the Basque Country (UPV/EHU), Alameda de Urquijo s/n, 48013 Bilbao, Spain

ARTICLE INFO

Article history:
Received 3 June 2016
Received in revised form 4 August 2016
Accepted 6 August 2016
Available online 8 August 2016

keywords:
Copper
Weight loss
Microbiological corrosion
Oxidation
Potential parameters

ABSTRACT

The machining of copper contained in oxygen-free copper workpieces by extremophile bacteria has been studied. The effect of the main parameters affecting the continuity of the process and which decrease the removal rate were analysed during the incubation, biomachining and regeneration phases. The presence of copper affected the bacterial culture, while the enhancement of process performance due to the simultaneous presence of *A. ferrooxidans* and *L. ferrooxidans* was relatively limited. pH was maintained below 1.80 to avoid Fe^{3+} losses in the form of jarosite precipitates. Measurement of the redox potential allowed a rapid monitoring of the process status.

1. Introduction

The favourable properties of oxygen-free copper (OFC) (high electrical and thermal conductivity, high ductility and corrosion resistance), combined with its homogeneous structure, make OFC the optimum substrate material for numerous components with highly specific requirements, such as spent nuclear fuel canisters, lens moulds, high voltage electrodes in vacuum interrupters and other sophisticated applications in the electronic and electrotechnical industries [1,2].

The manufacturing process for OFC workpieces requires polishing or machining in order to add, remove or shape the material within small tolerances. As the conventional chemical and physical processes that are currently employed increase heat or residual stress during these steps, biomachining has arisen as an alternative sustainable and precise machining tool. Biomachining has been defined as a controlled microbiological technique for the selective formation of microstructures on a workpiece by metal removal or dissolution, and it is characterised by machining components with minimum heat or residual stress and without exerting a cutting force during the process [3]. The microorganisms used in biomachining processes replace the toxic chemical compounds (such as $FeCl_3$) typically employed in chemical processing [4]. In addition, energy consumption is low, thus resulting in a subsequent cost saving in the manufacturing of high quality pieces [5]. Furthermore, the environmental benefit of biomachining is a valuable issue in current industrial policy, as many industries focus their efforts on both improving productivity and complying with environmental regulations [6,7].

Biomachining is a process based on the removal of metallic material by solubilisation through pure chemical reactions in solution. When OFC is the metallic material, chemolithotrophic and acidophilic microorganisms (archaea and bacteria) act as a catalyst by oxidizing ferrous to ferric iron. Fe^{3+} is subsequently reduced back to Fe^{2+} due to copper leaching ($Cu^0 \rightarrow Cu^{2+}$). In a third stage, the Fe^{2+} resulting from this reaction is regenerated to Fe^{3+} by the cells, thus allowing them to obtain energy for growth and bringing the cycle between Fe^{3+} and Fe^{2+} to a close [8].

The main drawback of controlled microbiological corrosion is the progressive decrease in the amount of metal removed per unit

* Corresponding author.
E-mail address: estibaliz.diaz@ehu.eus (E. Díaz-Tena).

http://dx.doi.org/10.1016/j.corsci.2016.08.001
0010-938X/

of time [5,9,10]. Thus, even if a maximum rate is initially achieved, the specific material removal rate (SMRR) has been reported to be very low after several hours. Several factors, such as a decrease in ferrous sulfate concentration, an increase in Cu^{2+} concentration, hydrolysis phenomena or oxygen depletion, have been proposed to have a critical impact on the SMRR. Despite this, Kumada et al. [11] correctly predicted that the machining rate could be controlled by adjusting the Fe^{3+} concentration in the liquid medium. Moreover, although the microbial consortium has proven to be very efficient at regenerating Fe^{3+}, the chemical mechanism of copper dissolution is too fast when compared with the bio-oxidation of Fe^{2+} [12,13]. Given this assumption, a rest period to allow the complete bio-regeneration of Fe^{3+} after each biomachining testing time was included in our previous work (4 or 8 h of rest after 1 or 4 h of biomachining, respectively) using the autotrophic, acidophilic and mesophilic bacterium *Acidithiobacillus ferrooxidans* [14]. Thus, the Cu^0 removal rate increased in a cyclical fashion after each rest period. The discrepancies found during the determination of the iron ion concentration in this study [14], prevented us from developing a continuous, stable and sustainable biomachining process.

The work described herein explores the effect of several process parameters (pure culture *vs* mixed culture, inoculum concentration, jarosite formation, Cu^{2+} and Fe^{3+} concentrations) that might affect the three stages described in our previous study, namely incubation, biomachining, and regeneration period, in an effort to ensure a constant removal rate over time. The mechanism of copper removal during the biomachining process was also evaluated. Finally, a methodology for the correct storage and determination of Fe^{2+} and Fe^{3+} in the bioleaching media is presented and the applicability of the redox potential as a rapid measurement for gaining information about the status of the biomachining process is evaluated.

2. Material and methods

2.1. Microorganisms and culture media

A. ferrooxidans (DSM-14882) was obtained from the Department of Chemical Engineering and Food Technologies of the University of Cádiz (UCA). *Leptospirillum ferrooxidans* (ATCC 29047) (CINDEFI, National University of La Plata, Argentina), a bacterium previously employed in bioleaching activities, was selected to constitute a consortium together with *A. ferrooxidans* [15]. In contrast to *A. ferrooxidans*, *L. ferrooxidans* is less inhibited by ferric iron and sustains higher bio-oxidation activity at higher redox potentials, thus allowing the maintenance of an elevated rate of Fe^{2+} consumption even at very low Fe^{2+} concentrations [16].

Both bacteria were cultured in a modified Lundgren-Silverman 9 K liquor. This medium was composed of mineral salts ($(NH_4)_2SO_4$ 3.0 g/L, K_2HPO_4 0.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KCl 0.1 g/L, $Ca(NO_3)_2$ 0.01 g/L) and 30 g/L of $FeSO_4{\cdot}7H_2O$ (final concentration of 6 g Fe^{2+}/L), which served as the energy source for *A. ferrooxidans* and *L. ferrooxidans*. Bacteria were incubated under agitation conditions (130 rpm) at a temperature of 30 °C until complete oxidation of Fe^{2+} to Fe^{3+} was achieved, which indicated that bacterial growth was satisfactory. The specific bacterial (*A. ferrooxidans* or *L. ferrooxidans)* broth volume mixed with modified 9 K medium for each experiment is detailed in the following sections.

2.2. Preparation of copper samples

OFC workpieces with a minimum purity of 99.99% and a size of $10 \times 15 \times 2$ mm were abraded using an 800-grit abrasive disk wheel (REMET petrographic cleanser LS1) [4,17]. Each workpiece had a hole (2 mm in diameter and 2 mm thick) for suspension in the active medium when performing the tests. Prior to their immersion in 9 K medium, the OFC blocks were rinsed with deionized water and ethanol (96%) and then heated to remove surface moisture.

2.3. Copper biomachining procedure

Biomachining tests were carried out by suspending preweighed OFC blocks into the aforementioned culture medium in conical flasks at 30 °C and 130 rpm (shaking water bath ST 30, Nüve). The metal samples were removed from the solution at user-defined intervals, rinsed with deionized water and ethanol (96%), dried, weighed, and finally reintroduced into the liquid sample. The SMRR achieved was calculated as follows:

$$\text{SMRR (mg/(h cm}^2\text{))} = \frac{\text{Weight loss (mg)}}{\text{Time (h) Area (cm}^2\text{)}} \quad (1)$$

2.4. Effect of inoculum concentration

The effect of the inoculum concentration at 2, 4 and 6% v/v (IN-1:IN-3) on the cultivation period, which corresponded to a bacterial concentration of 3.33×10^6, 6.66×10^6 and 10^7 cell/mL, respectively, was studied. The initial Fe^{2+} concentration in the three 9 K medium samples (IN-1:IN-3) was established at 6 g Fe^{2+}/L. The redox potential and ferrous iron concentration were determined until oxidation of ferrous iron was almost complete (less than 1% of the initial concentration). In all cases, the results showed that the final number of bacteria was 1.3×10^8 cell/mL. Each experiment was performed in duplicate and the mean results were taken.

2.5. Effect of Cu^{2+} concentration

The effect of Cu^{2+} concentration was quantified for the cultivation (C1:C4), biomachining (B1:B4) and regeneration stages (R1:R4). The modified 9 K medium used in all experiments contained 6 g Fe^{2+}/L and the inoculum concentration was established at 6% v/v. Each experiment was performed in duplicate and the mean results were taken.

With regarding to the cultivation period, four culture solutions (C-1:C-4), each of which contained increasing concentrations of Cu^{2+} (Table 1), were prepared. The experiment lasted until the oxidation of ferrous iron was almost complete (less than 1% of the initial concentration).

In the biomachining step, *A. ferrooxidans* broth was initially pre-inoculated at 6% v/v (in the absence of copper); when the culture reached the stationary phase (full oxidation of the ferrous iron), it was divided into four equal aliquots (B-1:B-4) and increasing concentrations of Cu^{2+} were added to each sample (Table 1). A single OFC workpiece was sequentially bio-oxidized for one hour in the four media (B-1 → B-2 → B-3 → B-4) in order to avoid the surface effect of the process [9]. The SMRR was then determined.

In the regeneration step, *A. ferrooxidans* was initially pre-inoculated at 6% v/v (in the absence of copper); when the culture reached the stationary phase, an OFC workpiece was biomachined in the solution for two hours. The resulting leachate, which contained a Cu^{2+} concentration of 1.5 g/L due to the metal released during the biomachining step, was divided into four equal aliquots (R-1:R-4) and additional increasing concentrations of Cu^{2+} were added to each sample (Table 1). The evolution of Fe^{2+} concentration over time was analysed.

2.6. Effect of Fe^{3+} concentration

The influence of Fe^{3+} concentration during the biomachining stage was examined. *A. ferrooxidans* was cultured in three flasks of 9 K medium (inoculated at 6% v/v) containing 0.6, 3 and 6 g Fe^{2+}/L,