An Investigation the Effect of Acidithiobacillus Ferrooxidans Bacteria on Biomachining of Titanium Alloy and Copper

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Abstract
Recent advances in technology have increased the necessity of using components with Micro and Nano dimensions. In recent years, the use of bacteria as a renewable tool has hopeful applications in producing different work-pieces. In this study, the effect of Acidithiobacillus Ferrooxidans (A.F) on Vt20 (Titanium alloy) and Cu were investigated. The results illustrated that in the medium of the Aerobic bacteria A.F, the layer of TiO2 on the surface of Vt20 is formed and this oxide layer impedes Vt20 corrosion. Furthermore, it was observed that Cu in a medium of A.F is corroded in the same condition. According to these results, biomachining by A.F is considered as a new approach that is used for Micro-Bio grooving on Cu, but this method has different effects on Vt20. So that due to improving the hardness of Vt20 and high resistance of this alloy against abrasion, this method can be used for coating space traveling equipment, automotive industries and home appliance. On the other hand the effect of pH and temperature on Vt20 were studied and it was observed that the increase in pH and temperature improve the resistance of Vt20 against the surface corrosion.

Keywords
Biomachining, Acidithiobacillus Ferrooxidans Bacteria, Medium

1. Introduction

These days, using bacteria in the mechanical manufacturing process is developing. This process, known as biomachining, causes microbiological corrosion under controlled conditions, which has a hopeful application in producing a wide range of industrial and home equipment. This innovative process is utilized as a cheap and eco-friendly process [1, 2]. With the advent of microelectronic devices, the various method of the micro-machining process for manufacturing micro or nano devices has been pursued [3-5]. Biomachining has been done by various bacteria such as Acidithiobacillus Thiooxidans [3, 6], Acidithiobacillus Ferrooxidans [7-10]. Eskandarian et al. [11] studied Cu machining by H_2O_2 in the medium of glucose oxidize. Titanium has a wide range of applications due to mechanical and metallurgical properties such as high hardness and high resistance against corrosion. Titanium is being used nowadays as the main substance in manufacturing dental implants, bio-mechanical, oil, and aerospace industries. Also, Cu is being employed with a wide range in producing electronic and communication processors, medical and nano industries due to some great properties such as high electrical and thermal conductivity, high toughness and good antimicrobial activity levels.

The high resistance of titanium and its alloy against corrosion is caused as a result of the ability of this metal in oxidation rapidly. TiO_2 is a stable and dense surface which functions as a protective layer against corrosion. Lorenzetti et al. [12] investigated the influence of surface modification on bacterial adhesion to titanium-based substrates. Visai et al. [13] studied the use of titanium oxide antibacterial surfaces in biomedical devices. Choi et al. [14] observed the Photo-catalytic antibacterial effect of TiO_2 film formed on Ti and TiAg exposed to Lactobacillus acidophilus. Material Removal Rate parameter (MRR) is used to examine the operation of most of the industrial machining process and it is the amount of material that is removed with respect to the time. MRR illustrates the manufacturing efficiency, the necessary time to produce different work-pieces and the operational cost of all stages. In biomachining, specific material removal rate (SMRR) is a suitable parameter to examine the manufacturing efficiency that is calculated as the below equation:

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

For the first time, Johnson et al. [15] calculated the amount of MRR in the biomachining of copper that is done by specific ferroxidan. In this research, it is reported that the amount of MRR is so small. However, at the beginning of the process, MRR had the maximum rate but this parameter by passing the time decreased. It is suggested that the decline of the amount of oxygen is an effective factor that decreases MRR during the process because oxygen is the key factor in the metabolic oxidation process. In other researches, Istiyanto et al. [9, 16] and Jadhav et al. [10] observed that the MRR had the reverse relationship with machining time and after reaching MRR to the maximum rate, it rapidly decreases. Furthermore, Johnson et al. [15] and Istiyanto et al. [9] showed that the decline of the amount of oxygen is not the only factor that decreases MRR because this parameter in the presence of excess oxygen during 6 hours of biomachining decreases from 2.2 mg/h to 1.2 mg/h. So it results that other factors like the decline of the concentration of iron sulfate, the increase of the concentration of Cu^{2+} and another hydrolysis phenomenon. Kumada et al. [17] suggested that the MRR can be controlled by regulating the concentration of Fe^{3+} on the average amount. Chang et al. [3] reported that if we want to have maximum MRR, the amount of microbial tension should be the minimum. Uno et al. [18] and Zhang et al. [19] used a particular kind of ferroxidan for machining of pure copper and pieces of pure iron respectively. In these two studies, the depth of grooves increased linearly by passing the time of machining. Hocheng et al. [20] and Jadhav et al. [10] compared the amount of MRR in machining of copper that is done by ferroxidan bacteria cells and the ferroxidan medium. They showed that the biomachining with medium instead of bacteria cells can increase MRR in a short time and can improve the function of the process.

In this study, the effect of Acidithiobacillus Ferrooxidans Bacteria and specific medium on titanium alloy and copper and the amount of material that is removed in five different conditions were examined.

2. Experimental Procedure

First, the required medium was provided and it was titled 9k. This medium was formed by the solution of different mineral salts which consists of $FeSO_4$ as a source of Fe^{+2}. The combination of the medium

is given as below:
3.0 $(NH_4)_2SO_4$, 0.1 KCl, 0.5 K_2HPO_4, 0.5 $MgSO_4*7H_2O$, 0.01 Ca $(NO_3)_2*4H_2O$ and 30.0 $FeSO_4*7H_2O$

The pH of H_2SO_4 was set to 2 and 3. After the provision of 9k, in the next step, the medium was prepared for growing Acidithiobacillus Ferrooxidans (A.F). For every 10cc of the medium, 300µcc A.F was added. Then 20cc of the medium was poured inside the tubes with 25cc capacity. After that 600µcc A.F was added to the medium by Sampler 1000 tool. As can be observed from the Figure (1), two samples with pH=2, two samples with pH=3 and one tube with 20cc of the medium and without A.F were prepared.

Figure1. Tubes that content 9k culture fluid and Acidithiobacillus Ferrooxidans Bacteria

Then all of the samples were put into the incubator at 30 ºC and were remained under the circumstances for a week until the insemination and the growth of A.F was completed. After a week all of the samples were brought out of the incubator and it can be seen from the Figure (1) that the growth of A.F is evident. In this study, pure Cu and Vt20 (Titanium alloy) were used that the specification of Titanium alloy is given in Table 1. After bringing out the Vt20 and Cu samples of autoclave they were weighed by the scales with five decimal places precision. Then the samples and their medium were put into Erlen with 100cc capacity. Erlens with Cu samples in the presence of A.F and without A.F in different pH (pH=2, 3) and Vt20 samples with the same conditions as Cu were put into incubator shaker with rotation speed= 120 rpm at 25 ºC and 40 ºC respectively.

Table 1. Chemical composition in % for grade VT20-2sv (ВТ20-2св)

Fe	C	Si	Mo	V	N	Ti	Al	Zr
0.25	0.1	0.15	0.5-2	0.8-2.5	0.05	85.15-91.4	5.5-7	1.5-2.5

After 20 hours samples were brought out of the incubator and were washed by alcohol at 70 ºC and then the samples were weighed with precision scales again (Figure 2).

Figure2. Erlens that content 20cc culture fluid with and without Bacteria after one week growing

3. Results and Discussion

Figures (3) and (4) show Vt20 and Cu samples after washing respectively. What stands out from the Vt20 samples is that just the color of Vt20 samples has changed, while the surface of Cu samples has been corroded and their roughness has increased.

Figure3. Vt20 samples after 20 hour

Figure4. Copper sample have been corroded after 20 hour

After measuring the weight of Vt20 samples and comparing them with previous information, Figure (5) has been created.

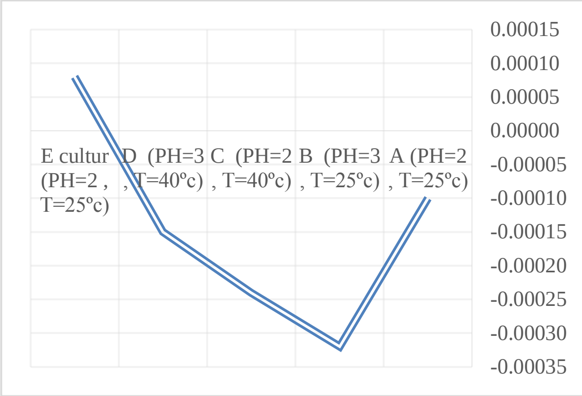

Figure5. Comparison between culture and supernatant in removal rate of titanium Vt20

According to table 2, the weight of Vt20 samples which were put in medium with A.F has increased, while the weight of the samples was put into the medium without A.F has decreased. Sample A which has had pH=2 and was put into the incubator in 25 ºC has 0.001 gr weight difference with the same sample before a test, that it has the minimum amount of weight difference between four experimented samples in the medium with A.F After that sample D has the second least weight difference which has different conditions as sample A. Sample C is the one with pH=2 and T= 40 ºC that ranked third after sample D. Finally, sample B comes fourth which this sample has the highest resistance against corrosion. Moreover, sample E which has the acidic medium in the absence of A. F has the corrosion about 0.0008 at the duration of 20 hours which this amount of corrosion is matched with previous investigations.

Table2. Comparison between weight changing of Vt20 samples in the circumference with and without A.F bacteria

	pH=2, T=25ºc	pH=3, T=25ºc	pH=3, T=40ºc	Culture pH=2, T=25ºc
Titanium (Before)	3.22301	2.83025	3.22145	2.53727
Titanium (after)	3.22311	2.83057	3.2216	2.53719
Difference	-0.00010	-0.00032	-0.00015	0.00008

Figure (6) shows SEM images of the surface of different Vt20 samples under various circumstances. As can be seen by increasing the amount of pH at the same temperature (T=25 ºC or T=40 ºC), the activity of Acidithiobacillus Ferrooxidans Bacteria decreases. So the depth of grooves that are generated by A.F decreases. The reason for this finding is that the A.F bacteria utilize energy that is generated by the oxidation of bivalent iron to trivalent iron according to equation (2) to survive.

$$\begin{aligned} & 2Fe^{2+} + 2H^{+} + 0.5O_2 + \mathit{Bacteria} \\ & \rightarrow 2Fe^{3+} + H_2OM + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} \end{aligned} \quad (2)$$

Where Fe exists in the medium due to $FeSO_4$ and M refers to the metal samples. But bivalent iron must be oxidized spontaneously to trivalent iron in the solution with pH<3. So A.F just survives in a strong acid solution with pH less than 3 [18].

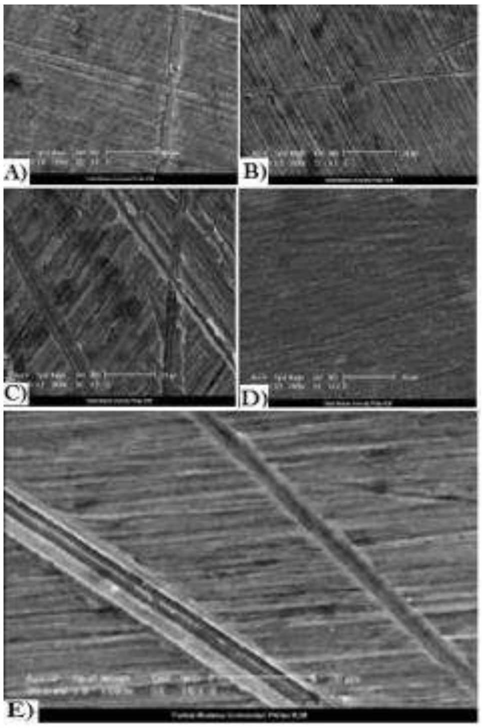

Figure6. The SEM images from the surface of Vt20 in different conditions: A) pH=2, T=25 ºC, B) pH=3, T=25 ºC, C) pH=2, T=40 ºC, D) pH=3, T=40 ºC, E) Culture: pH=2, T=25 ºC

On the other hand by increasing the temperature in the constant pH, the activity of A.F increases and it reaches to the maximum at around 30 ºC. It is the reason that you can see more grooves when sample B is compared with sample A. But it should be mentioned that the oxidation of titanium surface occurs earlier than the oxidation of bivalent iron to trivalent iron. So it can be said that the titanium oxide layer works as a substrate for iron oxidation and this phenomenon can increase the weight of titanium samples. Also, it can be seen that in medium without A.F the MRR value is greater than when existing A.F in the medium. The reason is that the energy generated by oxidation of bivalent iron to trivalent iron plus the energy generated by oxidation of titanium both is used for corrosion of titanium. While in medium with A.F oxidation iron energy is used by bacteria and so suitable energy is not provided for breaking the surface resistance of titanium. In the second step of this research Cu samples under the same conditions as Vt20 samples were studied and the results of these samples were compared with the results of Vt20 samples. Table 3 illustrates these results. This table shows that under this experimental condition the weight of Vt20 samples has increased again, while the weight of Cu samples decreases. This result shows that according to the chemical formulae below, A.F corrodes or machines the material.

Table3. The different behavior of copper and titanium alloy

	pH=2 , T=40ºC	Difference
Titanium (Before)	2.76748	-0.00024
Titanium (after)	2.76772	
Copper (Before)	16.65674	+0.1
Copper (after)	16.55173	

4. Conclusion

In this study, the effect of Acidithiobacillus Ferrooxidans (A.F) and the medium without that on Vt20 (Titanium alloy) and Cu were estimated. It was shown that the medium with low pH and without A.F makes the oxide layer of Vt20 destroy and causes corrosion in it, while in the presence of A.F, this corrosion was not observed. As a result of this, the possibility of corrosion decreases considerably. Generally, by increasing the amount of pH and temperature the resistance of Vt20 against surface corrosion improves. Results have shown in this process, the weight of Cu metal reduces in every condition.

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