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Exploring Biomachining of Copper using *E.coli*

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Abstract

In the widely popular chemical machining method, possibility of burnt or damaged and heat-affected surface is large due to usage of etchants which can also have deteriorating effects on the environment. However, these drawbacks can be totally avoided through the less explored technique of biomachining which is basically a micro-machining process employing micro-organisms for material removal. Various micro-organisms used in the past are *Thiobacillus ferrooxidans*, *Aspergillus niger*, *Staphylococcus species*, etc. for biomachining materials such as copper, iron, nickel, tin, aluminium, palladium, thermobiodegradable plastics, etc. However, in this research, *E.coli* which is a very commonly available and easily isolated micro-organism has been used for the biomachining of copper. Copper is a highly utilized metal in the manufacturing industry and *E.coli* has a selective potential to biomachine it. This research is based on understanding the effects of three crucial process parameters viz. temperature, pH and shaking speed on metal removal rate (MRR) and surface roughness in biomachining of copper by *E.coli*. By analysis of MRR, it is found that 50°C, 1.6 and 150 rpm are the optimum conditions of temperature, pH and shaking speed respectively and by the analysis of surface roughness, it is found that 25°C, 3.2 and 50 rpm are optimum of the same. Further, it is revealed that pH is a highly significant parameter in this process on account of biochemical reactions' dependence on it. Thus along with having better chances of controlling the MRR and getting a better surface finish, biomachining serves as an environment friendly solution.

Keywords: Biomachining, Copper, *E.coli*, Micro-organisms, Machining, Surface roughness, Metal removal rate, Material removal rate.

1. INTRODUCTION

Machining is a metal removal process generally where a cutting tool is used to remove metal parts in order to give the workpiece a desired shape and size. An unfinished workpiece requiring machining will need to have some material removed away to create a finished product. Micromachining is the basic technology for fabrication of micro-components of size in the range of 1 to 500 micrometers. Their need arises from miniaturization of various devices in science and engineering, calling for ultra-precision manufacturing and micro-fabrication. It can typically be classified into physical, chemical and mechanical micromachining. Chemical machining is the subtractive manufacturing process of using baths of temperature-regulated corrosive chemicals, called etchants to remove material to create an object with the desired shape [1, 2]. The etchant reacts with the material in the area to be cut and causes the solid material to be dissolved. However, chemical machining generates heat-affected zones and burnt or damaged surfaces. Thus there arises a need for an alternative technique which eliminates the above disadvantages, and biomachining is hence the solution towards it which is also a more environment friendly technique.

Biomachining is a typical process where bacteria are used to remove the unwanted metal parts to produce a desired shape of the workpiece. Certain bacteria, such as *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, utilize the chemical energy from oxidation of iron or copper to fix carbon dioxide from the air. A metal object, when placed in a culture fluid containing these metal-metabolizing bacteria, will thus lead to material getting removed from its surface over time. Biomachining is thus ideal for micromachining due to its very low material removal rate. In addition, biomachining is less likely to leave an undesirable surface finish; further neither chemical nor physical energy is concentrated on the cutting area, due to which the possibility of a damaged or burnt surface is slim. In biological machining, microorganisms can be produced continuously with low-energy consumption. Moreover, because metabolic processes of microorganisms are utilized, no damage or heat-affected zone is generated in the machined work piece. Thus, the use of microorganisms for the micromachining of metals opens up the possibility of biological machining as an alternative to conventional metal processing methods. In addition, it is easy to control metabolic activities of microorganisms. Hence, it is possible to manufacture the machined part with desired surface finish. This process has been successfully used in the past to cut pure iron, pure copper as well as many other metals [3].

Attempts have been made by previous researchers to conduct biomachining on materials such as copper, iron, nickel, tin, palladium, thermobiodegradable plastic, etc using micro-organisms such as *Acidithiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Staphylococcus aureus*, etc. [4-6]. Microorganisms, particularly bacteria, adopt the electron transport chain for machining copper (Fig. 1). The process of attachment of bacteria on the copper surface is carried out by extracellular polymeric substances (EPS) present in its outer membrane. The Fe^{2+} radical in the culture fluid is transported to the periplasmic space across the bio-membrane where it loses an electron through the catalysis of the iron oxidase. This electron is then transported to oxygen with an electron transport chain (ETC), which couples a chemical reaction between an electron donor and an electron acceptor (O_2) to the transfer of H^+ ions across a membrane, through a set of biochemical reactions. *

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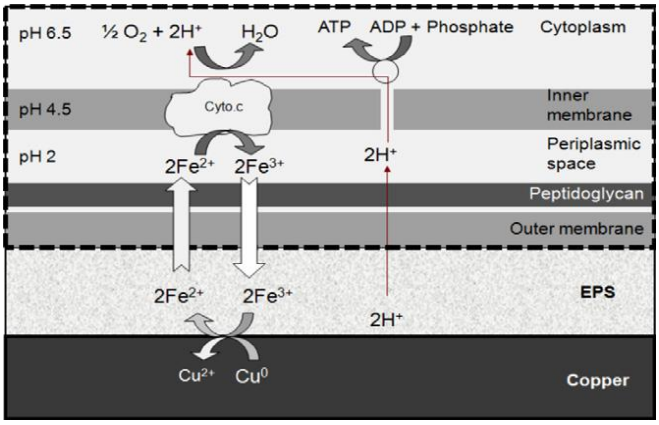

Fig.1. Illustration of a biomachining process on copper workpiece [7]

The overall reaction is as follows [7]:

$$2Fe^{2+} + 12O_2 + 2H^{+} \rightarrow 2Fe^{3+} + H_2O \quad \ldots(1)$$

This reaction generates energy. The Fe^{3+} expelled from the cell is a strong oxidant and is able to oxidize pure copper (Cu) to Cu^{2+}. Therefore, a workpiece can be machined by the Fe^{3+} that is produced by the micro-organism.

$$Cu^{0} + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} \quad \ldots(2)$$

The Fe^{3+} produced is reduced to Fe^{2+} by biomachining. Then Fe^{2+} can be reoxidized to Fe^{3+} by oxygen. Thus, a circulatory system is formed (see Fig. 1) [7]. Thus, the material removal by the microbe is due to oxidation of copper as a result of the biochemical reaction.

Based on previous studies it has been found that the major process parameters that influence biomachining of Copper are temperature, shaking speed, pH, concentration and time. Shikata et al. [8] conducted biomachining of oxygen free copper using staphylococcus species at 25, 30 and 35°C and found that the optimum temperature was 30°C. Ting et al. [9] conducted biomachining of pure copper at shaking speeds of 0-200 rpm and found that increase in shaking speed increases the MRR with 160 rpm being optimum. Eskandarian et al. [10] conducted experiments using *Aspergillus niger* in the pH range of 3-7 and reported that with decreasing pH from 7.0 to 5.5 and then to 4, the rate of material removal increased. Chang et al. [11] conducted biomachining using *Acidithiobacillus thiooxidans* and found that $1x10^8$ cells/ml is the most appropriate concentration of bacteria for machining. Istiyanto et al. [12] used *A. ferrooxidans* for 12, 24, 36, 48 hrs respectively and the average maximum depth wise metal removal was recorded at 48hrs.

From the overall literature review, it is thus concluded that biomachining is a crucial phenomenon and needs to be further explored. Considering the gaps in literature, it was decided to explore biomachining of copper using the micro-organism *E.coli* for the first time. As a primary step towards the same, preliminary experimentation was initially done to inspect the effect of *E.coli* on machining copper in order to ensure practical feasibility of biomachining. It was observed that at a constant concentration of 1×10^8 cells/ml and a time period of 48 hrs, machining was affected by three factors, namely, temperature, pH and shaking speed which were then taken as the process parameters for detailed experimentation, which is actual part of this paper. The response variables considered for evaluating bio-machinability were metal removal rate (MRR) and surface roughness.

2. EXPERIMENTAL

2.1 Work material

Considering the importance from wide application point-of-view, copper was selected as the work material. Copper blocks of dimensions 10 × 10 × 5mm were used as workpieces. These were polished using silicon-carbide waterproof sandpapers so as to achieve a more even surface. The polishing was first carried out systematically, and then specimens cleaned carefully and further average surface roughness was inspected. These steps were repeated until the surface finish on all specimens was almost similar. This was particularly carried out so as to eliminate the variable effects of initial surface on the biomachining phenomenon from sample-to-sample basis. After ensuring similar quality, these copper workpieces were then subjected to biomachining experimentation.

2.2 Culturing of microorganism and preparation of machining medium

For biomachining phenomenon to occur, the basic requirement is occurrence of bio-chemical reaction. Thus it necessitates assistance through micro-organism and hence *E.coli* was chosen. *E.coli* was inoculated on nutrient agar containing 10g/l HiVeg Peptone, 10g/l HiVeg Extract, 5g/l Sodium Chloride and agar 20g/l. Final pH was adjusted to 7.6. Incubation was done for a period of 24hrs at 37°C and the culture was obtained on a nutrient agar plate. Under aseptic conditions, 150ml nutrient broth containing 4.3g/l Meat peptone, 4.3g/l Caesin enzymic hydrolysate and 6.4g/l Sodium Chloride was inoculated with the culture of *E.coli*. Concentration of cells was adjusted to 1×10^8 cells/ml using colorimeter.

2.3 Experimentation strategy and procedure

In order to limit the number of experiments wisely, Taguchi DOE technique [13] by incorporating standard L9 array (3^3) under three process variables viz., temperature, pH and shaking speed, was chosen towards experimental strategy. Each process variable had three levels and the experimentation was hence carried out as per the experimental test matrix shown in Table 1. The metal workpieces after polishing were weighed and then placed in the flasks containing the machining medium (Fig. 2a) which is the nutrient broth and were kept for incubation for a period of 48hrs as per the test conditions so as to favor biomachining.

2.4 Measurement of response variables post-experimentation

After 48 hrs of biomachining as per the specific test conditions, the flasks were removed from the incubator and the metal pieces were placed on a filter paper, cleaned with distilled water and then oven-dried at 60°C for 10mins. The weight of the pieces was then measured and thus, the metal removal rate was calculated by finding the difference between the initial and final weight. After measurement of MRR, the surface roughness of the biomachined pieces was also measured using surface roughness tester Mitutoyo SJ210 (Fig. 2b). All the experiments were replicated and re-measurements were done to validate the data.

Table 1
Experimental test matrix along with observed responses [14]

Expt. No.	Temperature (°C)	pH	Shaking Speed (rpm)	*MRR* (units)	R_a (μm)
1	25	1.6	50	0.027	0.738
2	25	3.2	100	0.02	0.799
3	25	4.8	150	0.003	1.038
4	50	1.6	100	0.255	3.318
5	50	3.2	150	0.045	0.752
6	50	4.8	50	0.0001	0.787
7	75	1.6	150	0.262	2.59
8	75	3.2	50	0.02	0.805
9	75	4.8	100	0.01	0.702

(a) (b)

Fig. 2. (a) Machining medium containing copper workpieces,and (b)Surface roughness tester

3. RESULTS AND DISCUSSION

A statistical analysis of MRR and surface roughness was carried out and the results were analysed by Analysis of Variance (ANOVA). The main effects plots were plotted to see the effect of process parameters. Also the factors which have *P-values* less than 0.05 as from ANOVA are considered highly statistically significant. The next subsections discuss the effect of each individual parameter on surface roughness and MRR.

3.1 Analysis of Metal Removal Rate (MRR)

Fig. 3 shows the main effects plot for MRR and Table 2 shows the ANOVA for the same. The ANOVA reveals that all the three process parameters viz. temperature, pH and shaking speedhave a significant influence on the MRR as their *P-value* is lesser that 0.05 at 95% confidence level. After 48 hours of biomachining, as shown in Fig. 3, at cell concentration of 1×10^8 cells/ml, it is seen that the MRR increases linearly from 25 to 50°C and then slightly decreases from 50 to 75°C. This can be attributed to the inability of the bacteria to survive at very high temperatures. Thus, 50°C is suitable for the biomachining of copper using *E.coli*. The MRR decreases with increase in pH. There is a linear decrease from pH 1.6 to 3.2 and then from 3.2 to 4.8. As the pH decreases, the bio-oxidation rate and bacterial activity increases due to increase in Fe^{2+} radical transportation from the culture media to the periplasmic space of the bacteria, thus supporting the mechanism of biomachining. This indicates that acidic pH is favourable for the process. It is observed that with increase in shaking speed, the MRR continually increases from 50 to 150 rpm. This can be due to increase in contact of the machining media with the metal piece and due to proper mixing of nutrients and aeration. Thus, 150rpm is favourable for the process.

Table 2
ANOVA for Metal removal rate

Source	*DF*	*Adj. SS*	*Adj. MS*	*F-ratio*	*P-value*
Temperature	2	0.021	0.011	7.24	0.010
pH	2	0.084	0.042	28.78	0.000
Shaking speed	2	0.026	0.013	9.11	0.005
Error	11	0.016	0.001		
Pure error	9	0.005	0.001		
Total	17	0.147			

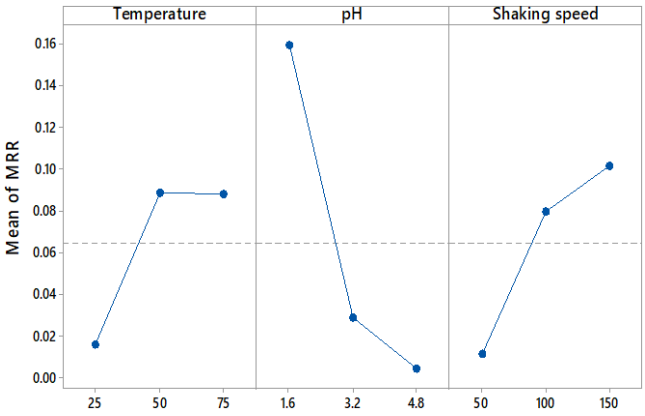

Fig. 3.Main effects plot for metal removal rate

3.2 Analysis of Surface Roughness (R_a)

Fig. 4 shows the main effects plot for surface roughness and Table 3 shows the ANOVA for the same. It revealed from the ANOVA that pH has a highly significant influence on the surface roughness as its *P-value* is lesser that 0.05 at 95% confidence level. This shows that the effect of pH with regards to the surface roughness response particularly on biomachining of copper using *E.coli* is greater than temperature and shaking speed responses. From the main effectsplot for surface roughness (Fig. 4), it is observed that after 48 hours of biomachining, the surface roughness increases from 25 to 50°C and then decreases at 75°C. However, the surface roughness decreases with increase in pH from 1.6 to 3.2 and then slightly increases at pH 4.8. As the shaking speed increases, the surface roughness value increases upto shaking speed of 100rpm and then decreases at 150rpm.

Table 3

ANOVA for Surface roughness

Source	*DF*	*Adj. SS*	*Adj. MS*	*F-ratio*	*P-value*
Temperature	2	1.897	0.948	3.14	0.083
pH	2	7.771	3.885	12.87	0.001

Shaking speed	2	1.972	0.986	3.27	0.077
Error	11	3.320	0.302		
Pure error	9	0.058	0.006		
Total	17	14.96			

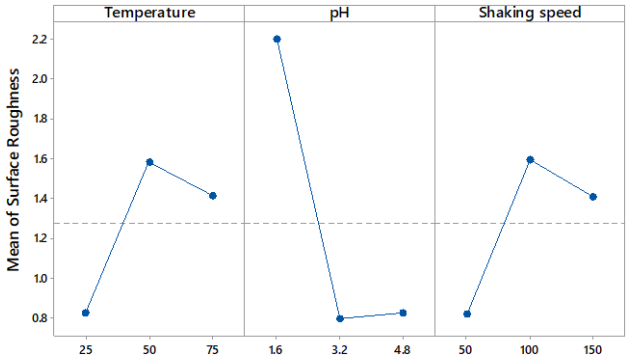

Fig. 4.Main effects plot for surface roughness

4. CONCLUSIONS

The experimental investigation leads to the following conclusions.

- Biomachining of copper particularly by *E.coli* is found feasible for the first time.
- All the three process parameters viz. temperature, pH and shaking speed are very crucial as they appreciably affect the metal removal rate in biomachining of copper particularly by *E.coli*.
- When it comes to surface roughness as a result of biomachining of copper particularly by *E.coli*, pH is the only prime factor affecting it significantly as compared to temperature and shaking speed.
- The optimal combination so as to get higher metal removal rate for biomachining of copper using *E.coli* is the temperature of 50°C, pH of 1.6 and shaking speed of 150rpm.
- For getting better surface finish,temperature of 25°C, pH of 3.2 and shaking speed of 50 rpm are the optimum parametric values in biomachining of copper using *E.coli*.

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