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Selection of Optimum Process Parameters of Biomachining for Maximum Metal Removal Rate

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Micro-manufacturing is one of the growing technologies of near future. Non-traditional machining processes have been found to be beneficial for micro manufacturing using low density of energy for metal removal. To overcome environment related problems of chemical machining, biomachining has been developed over several years by making use of the metabolic activity of microorganisms. Besides many advantages of biomachining such as environmental friendly, low consumption of energy and no heat affected zone generation during machining; one of the common short comings reported by early researchers is a low metal removal rate. In this study, firstly effect of process parameters variation on SMRR and MRR is investigated. Secondly taguchi design of experiment (DOE) approach is used to establish rank of most influential process parameters for maximum metal removal rate. Finally optimal values of selected parameters are predicted and verified. It is observed that process parameters can be optimized to obtain a higher metal removal rate. Micro features are fabricated using optimum process parameters to show the impact of fine tuning on the metal removal rate.

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NOMENCLATURE

SMRR = Specific metal removal rate MRR = Metal removal rate A. Ferrooxidans = Acidithiobacillus ferrooxidans DI = De-Ionized TMR = Total metal removed DOE = Design of experiments

1. Introduction

Non-traditional micro-machining processes are becoming more popular now a day in industries for micro fabrication. Materials can be micro machined by three major methods that use different types of energy for machining: physical, chemical, and biological methods.¹ In micro-machining, high-density of energy is used to remove a very small amount of material which wastes much energy. Although physical and chemical methods of machining are very fast, they have some impacts on the dynamics of processes in biotic and abiotic systems outside the boundaries of the work space. It has been observed that microorganisms attack structures and induce corrosion. If this microbe-induced corrosion process can be controlled and guided in a specific direction, an environmentally benign, energy-efficient, and cost-effective approach can be developed for material processing applications.²

Since last few decades, chemolithotrophs bacteria have been used by many researchers in biomachining for micro fabrication. Uno et al.¹ (1996) reported the first fundamental study on the possibility of biomachining. Ting et al.² (2000) compared metal removal by biomachining and chemical machining. Johnson et al.³ (2007) reported the surface characteristics of biomachining polycrystalline copper for specific initial bacterial concentrations. They described an increase in roughness of the work-piece, which was proportional to the machining time.

Hocheng et al.⁴ (2008) showed the metal removal rate (MRR) by Acidithiobacillus thiooxidans bacteria without pre-secreted metabolite. Istiyanto et al.^{5,6} (2011) reported the profile and surface characteristics of biomachining uni-crystal copper. They reported a U-shaped profile for a rectangular groove with width and depth that increase proportionally to the machining time. Recently, Jadhav et al.⁷ (2013) reported the possibility of biomachining with only ferric ions by removing bacteria from a culture solution and Eskandarian et al.⁸ reported the enzymatic biomachining using glucose oxidase. All these studies reported the capabilities of biomachining for micro fabrication.



One of the common shortcomings reported by previous researchers is the low MRR of biomachining.

This paper aims to shows selection of optimum process parameters using taguchi DOE for increasing the metal removal rate (MRR) of biomachining as response and is structured as follows:

• Firstly, the effect of various process parameters i.e. temperature, shaking rate, pH and FeSO₄ concentration on the MRR and SMRR of biomachining iron is observed.

• Secondly, the ranks of most influential parameters have been calculated using Taguchi DOE.

• Thirdly, the optimum value of process parameters is predicted using taguchi analysis and then validated by experiments.

• Lastly, micro features i.e. micro dimples and micro pillars are fabricated to observe an improvement in the MRR at optimum process parameters.

2. Biomachining Mechanism

A. Ferrooxidans is a non-sporulating rod-shaped bacterium that is about 1 μ m long and 0.5 μ m in diameter.¹ The ability of A. Ferrooxidans to leach and mobilize metals from solid materials possibly involves three principles: (i) redox reactions, (ii) the formation of organic or inorganic acids, and (iii) the excretion of complexing agents.⁹ Zhang et al.¹⁰ explained the chemical kinetics of reactions taking place in biomachining. The reactions involved in biomachining can be characterized as a biological redox reaction and can be described as:

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

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

The first part of these reactions is the continuous conversion of Fe^{2+} to Fe^{3+} by bacterial metabolism, which involves the transfer of electrons from ferrous iron. The second process is completed by the uptake of electrons by oxygen and combining with H⁺ ions, resulting in water. The goal of these two processes is to produce useable energy for the bacteria. The energy-creating process forms a closed system for Fe^{2+} ions as they are continuously converted to Fe^{3+} , exuded from the cell, reduced to Fe^{2+} by their reaction with copper or iron, and then reabsorbed into the periplasmic space for re-oxidation. On the other hand, H⁺ ions are consumed continuously and water is produced. Hence it can be concluded that, if the metabolic activity of bacteria can be improved by tuning process conditions such as temperature, shaking rate, and H⁺ ions (pH), the most crucial shortcoming of biomachining can be eliminated.

3. Experimental Methods and Materials

The experimental procedure of biomachining consists of four major steps, (1) bacteria culturing (2) workpiece preparation (3) bacterial density measurement, and (4) metal removal rate calculation. For culturing A. Ferrooxidans bacteria (ATCC No. 21834) in liquid media, Silverman's media (modified 9K) with 14.75% w/v FeSO₄ solution in



Fig. 1 Ferrous-Ferric cycle in biomachining mechanism

Table 1 Composition of basal salts of modified 9K media

Name	$(NH_4)_2SO_4$	K ₂ HPO ₄	MgSO ₄	KCl	Ca(NO ₃) ₂	H ₂ 0
Qty.	6 g	1 g	1 g	0.2 g	0.02 g	1400 mL

DI water was used except the experiments for observing the effect of $FeSO_4$ variation.¹¹ The composition of basal salts is shown in Table 1.

The FeSO₄ solution was prepared separately and sterilized with 0.2 μ m filter paper. The basal salts were autoclaved at 121°C for 15 minutes, and. both solutions were mixed upon cooling. The pH was adjusted to 2.0 for both solutions using H₂SO₄. The pH of culturing media was adjusted only at the start of culturing and did not maintained during experiments.

Bacteria were cultured at 35°C and shaking speed of 140 rpm in an incubator shaker (Jeio Tech SI 300). Under these culturing conditions (FeSO₄ concentration 14.75% w/v, incubation temperature 35°C, shaking rate 140 rpm and culturing solution initial pH 2.0) bacteria were cultured for 48 hours. Pure iron (22 mm diameter and 10 mm thickness) was used as the work-piece. That were polished using a 1000-grit SiC abrasive disk up to a surface roughness (Ra) of 0.05 - 0.1 μ m before exposing them to the machining solution. Viable cell count method was adopted to calculated living cell density in bacterial solution during culturing process, and at the start of biomachining experiment. For bacterial density measurement, solid media was prepared by solidifying 9K media using agarose solution. The formula used to calculate bacterial density is given below.

$$\frac{\text{Cell density}}{(\text{Cells/mL})} = \frac{\text{number of bacterial colonies}}{\text{dilution factor × vol. of diluted broth inculated}}$$
(3)

The bacterial density calculated at different pH and ${\rm FeSO_4}$ concentrations are shown in Fig. 2.

The bacterial density remains almost constant during 48 hours to 60 hours, so the bacterial solution was used after 48 hours of culturing for all experiments. After culturing for 48 hours, the work-pieces were put into flasks containing 200 mL of bacterial solution. The flasks were then incubated at specific temperatures and shaking speeds. At the end of each machining period i.e. 12, 24, 36, 48, and 60 hours, the work-pieces were removed, rinsed with DI water, air dried, and weighed to



Fig. 2 Bacterial density at different pH, and FeSO₄ concentrations

calculate the MRR and SMRR. The formulae used to measure MRR and SMRR are given below. TMR was calculated by taking total mass removed during full experiment, divided by the corresponding time of biomachining i.e. 48 hours.

MRR =
$$\frac{\text{mass before biomachining} - \text{mass after biomachining}}{\text{biomachining time}}$$
 (4)
SMRR = $\frac{\text{mass before biomachining} - \text{mass after biomachining}}{\text{biomachining time} \times \text{exposed area} \times \text{bacterial density}}$ (5)

A non-contact profilometer was used to observe the micro textures after biomachining.

4. Effect of Various Process Parameters on SMRR and MRR

4.1 Effect of Shaking Rate Variation

First of all, the bacterial samples having $FeSO_4$ concentration 14.75% w/v were cultured at shaking rate of 140 rpm, temperature 35°C and pH 2.0 for 48 hours. Afterwards the effect of shaking rate variation on MRR and SMRR during biomachining experiments was observed by performing experiments at different shaking rates.

The experiments revealed an increasing trend in the MRR and SMRR with increasing shaking rate. The line graph in Fig. 3 shows that from 80 rpm, 20 rpm increases in the shaking rate to 100 rpm and 120 rpm result in 0.103 and 0.328 mg/hr.cm² average increases in the metal removal for the bacterial density of 1×10^8 cells/mL. The bar graph in Fig. 3 shows the TMR for specific shaking rates for a period of 48 hours. At 80 rpm, 700.8 mg of metal is removed in 48 hours of machining. The rate increases steadily and reaches a maximum value of 1129.92 mg at 120 rpm. If the effects of bacterial density and exposed area are neglected, MRRs of 26.69, 28.76, and 40.52 mg/hr. are achieved at 80, 100, and 120 rpm, respectively, in 12 hours of machining.

Increasing the shaking rate can increase SMRR in two possible ways: (i) by increasing the enzymatic production of acid in the direct leaching mechanism due to better oxygen transfer, and (ii) by increasing the indirect leaching mechanism with faster interaction of



Fig. 3 Impact of shaking rate variation on SMRR and TMR of iron

ferric ions with metal.⁹ In non-shaking conditions, there is an issue of clump or colony formation at the bottom of the flask. Shaking helps in making nutrients available everywhere in culture and prevents bacterial colonization at the bottom of the flask. The increase in SMRR is due to an increase in amount of oxygen available to the bacteria at the same pH and temperature. Bacterial metabolism is affected by the oxygen concentration, which is one of the important reactants of the reaction. By shaking, the oxygen concentration becomes almost uniform everywhere in culture, resulting in mass transfer of the oxidized metallic products near the machining point.

An overall decreasing trend of MRR was observed with increasing machining time. The reason can be clearly understood by the charactristic equation of biomachining (see Eq. (1)), Fe^{2+} ions that are converted to Fe^{3+} ions by the bacteria are recycled by reacting with metal workpiece. But the other half reaction, development of water from H^+ ions and oxygen, utilizes H^+ ions continuously. As concentration of H^+ ions is not maintained in these experiments, so due to consumption of these H^+ ions, balance of biomachining process is disturbed resulting in unability of bacteria to further convert Fe^{2+} ions to Fe^{3+} ions. Consequently, the metal removal rate of biomachining reaction starts decreasing.

4.2 Effect of Temperature Variation

In the following experiment, the bacterial samples were cultured at same conditions (FeSO₄ concentration 14.75% w/v, shaking rate 140 rpm, temperature 35°C, and pH 2.0) and the effect of temperature variation on MRR and SMRR during biomachining was observed by performing experiments at different temperatures. Fig. 4 illustrates the effect of temperature variation on the SMRR of pure iron.

Fig. 4 makes it clear that the temperature has a positive effect on SMRR. From 25°C, 5°C increases in temperature result in average increases of 0.110, 0.445 and 0.2205 mg/hr.cm² for bacterial density of 1×10^8 cells/mL. TMRs of 585.6, 734.88, 1129.92 and 1374.24 were measured at the reported temperatures. The MRR increases from 22.68 mg/hr. at 25°C to the highest value of 47.54 mg/hr. at 40°C for 12 hours of biomachining.

The reason for this behavior is that bacteria are made up of certain



Fig. 4 Effect of temperature variation on SMRR of iron

types of proteins, which have a definite optimum temperature. These proteins work well at the optimum temperature, and any increase in temperature within permissible range enhances the metabolic activity of the bacteria. But if the temperature is increased above the optimal temperature, it produces conformal changes in these proteins, resulting in their inactivity. In contrast, if the temperature is decreased below the optimum growth temperature, it does not inhibit bacteria but instead just reduces their growth. The decreasing trend of MRR with increasing machining time was also observed during these experiments. The reason is that, rise in temperature can increase the metabolic activity of bacteria, but cannot compensate the deficiency of H⁺ ions concentration in the solution. Consequently, MRR decreases with the increase in machining time.

4.3 Effect of FeSO₄ Concentration

For these experiments, the bacteria were cultured in culturing media having different FeSO₄ concentration at shaking rate of 140 rpm, temperature 35°C and pH 2.0 for 48 hours. Afterwards the effect of FeSO₄ concentration variation on MRR and SMRR during biomachining experiments was observed by performing experiments with these bacterial solutions having different FeSO₄ concentrations. The results for the concentrations of 10% FeSO₄ (Leathen et al. media) and 14.75% FeSO₄ (Silverman et al. media) are shown in Fig. 5. The graph illustrates that with an increase of 4.75% in FeSO₄ concentration, an average increase of 0.698 mg/hr.cm² in metal removal is obtained for a bacterial density of 1×10⁸ cells/mL. An MRR of 15.89 mg/hr. was obtained in 12 hours of biomachining with 10% FeSO₄, whereas the rate more than doubles with a 4.75% increase in FeSO₄ concentration. An average increase of 150.37% was achieved with a 4.75% increase in FeSO₄ concentration. 505.8 mg of metal in total was removed after 60 hours with 10% FeSO₄ concentration, which increased to 1285.2 mg with 14.75% FeSO₄ concentration.

Considering the direct mechanism, the number of electrons transferred to the cell wall of the bacteria increased with the increase in ferrous ions (Fe^{2^+}), resulting in better SMRR. Whereas if the indirect mechanism is supposed to be responsible for biomachining, the increase in Fe^{2^+} ion concentration would result corresponding increase of Fe^{3^+} ions. In following experiments, a decrease in MRR with increase in machining time was observed due to the consumption of H^+ ions during biomachining reaction. In author perception, if the



Fig. 5 Influence of FeSO₄ concentration variation on SMRR of iron



Fig. 6 Influence of pH variation on SMRR of iron

concentration of the $\rm H^{\scriptscriptstyle +}$ ions is maintained during biomachining experiments, the decreasing trend of MRR can be eliminated.

4.4 Effect of pH Variation

In following experiments, the bacteria were cultured in the culturing media having $FeSO_4$ concentration 14.75% w/v and initial pH values of 2.0, 2.5 and 3.0. After culturing for 48 hours, biomachining experiments were performed using these media at the shaking rate 120 rpm, and temperature 35°C, respectively. Fig. 6 illustrates the effect of pH variation on the SMRR of pure iron.

From pH 2.0 to 2.5, an average increase of 0.265 mg/hr.cm² for bacterial density of 1×10^8 cells/mL in SMRR has been observed, whereas by further changing pH from 2.5 to 3.0, an average decrease of 0.163 mg/hr.cm² has been recorded for bacterial density of 1×10^8 cells/mL. TMRs of 1129.92, 1285.58, and 1273.04 mg were measured at the reported pH values. The MRR increases from 40.52 mg/hr. at pH 2.0 to the highest value of 56.391 mg/hr. at pH 2.5 for 12 hours of biomachining. Then, it decreases to 43.625 mg/hr. at pH 3.0. The decreasing trend of MRR was also seen in following experiments. The reason for this behavior is that even the initial pH values are changed but these values are not controlled during biomachining experiments. If the consumption of H⁺ ions is compensated by adding H⁺ ions in the form of H₂SO₄ during biomachining experiments, this decreasing trend possibly be eliminated.

Factor	Low	Medium	High
Pactor	(-1)	(0)	(1)
Shaking rate (rpm)	80	120	160
pН	2	2.5	3
FeSO ₄ conc. (%w/v)	10	13	16
Temp. (°C)	25	35	45

Table 2 Levels for L9 array (coded and un-coded values)

Table 3 Taguchi DOE for MRR

Shaking rate (rpm)	pН	FeSO ₄ conc. (%w/v)	Temp. (°C)	MRR (mg/hr.)	S/N ratio
80	2.0	10	25	20.3	26.15
80	2.5	13	35	21.7	26.73
80	3.0	16	45	33.0	30.37
120	2.5	16	25	54.6	34.74
120	3.0	10	35	43.0	32.67
120	2.0	13	45	58.6	35.36
160	3.0	13	25	80.2	38.08
160	2.0	16	35	83.8	38.46
160	2.5	10	45	135.1	42.61

5. Selection of Optimum Process Parameters Using Taguchi Design of Experiments

Taguchi methodology was used for design of experiment. An L9 (3^4) orthogonal array (four input variables with three levels as shown in Table 2) dictated 9 tests. The approach was very efficient because instead of doing the 162 (2×3^4) tests required for the factorial method, only 9 tests were required to establish the ranks of the most influential process parameters.¹² The process parameters with their corresponding levels are shown in Table 2.

A series of experiments designed as a result of Taguchi approach are listed in Table 3. The experiments were designed using MINITAB software. Experiments were performed in triplicate and average results were recorded for analysis.

The main effects of selected parameters (which is known as data mean- The main effect plots for means) on MRR of biomachining are shown in Fig. 7. The plot shows that shaking rate has direct relation with MRR within given constraints. An increase in this factor results an increasing MRR accordingly. On the other hand, temperature shows different behavior. From 25°C, an increase of 5°C results a decrease in MRR and further increase in temperature results corresponding increase in MRR. Similarly, FeSO₄ concentration also shows similar trend. On the other hand, pH shows inverse trend that of temperature and FeSO₄, MRR increases with an increase in pH from 2.0 to 2.5, and with further increase in pH it starts decreasing.

The response table for main effect plot for means is shown in Table 4.

The signal to noise ratio provides a measure of the impact of noise factors on performance.¹³ The larger the S/N, the more robust the product is against noise.

$$S/N = -10 \times \log(\Sigma(1/y^2)/n)$$
 (Larger is better)

The results were analyzed using Minitab statistical software. The plots for signal to noise ratio is shown in Fig. 8. Signal to noise ratio, larger is better is used to gain results for maximum metal removal rate.



Fig. 7 Main effect plots for means

Table 4 Response table for means

Level	Shaking rate	pН	FeSO ₄ conc.	Temp.	
1	25.00	54.23	66.13	51.70	
2	52.07	70.47	53.50	49.50	
3	99.70	52.07	57.13	75.57	
Delta	74.70	18.04	12.63	26.07	
Rank	1	3	4	2	



Fig. 8 Main effect plots for SN ratio

Table 5 Response	e table for SN ratio	(Larger is better)
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Level	Shaking rate	pН	FeSO ₄ conc.	Temp.
1	27.75	33.32	33.81	32.99
2	34.26	34.70	33.39	32.62
3	39.72	33.71	34.53	36.11
Delta	11.97	1.37	1.14	3.49
Rank	1	3	4	2

The corresponding response for signal to noise ratio are shown in the Table 5. It can be seen that shaking rate and temperature are the most influential process parameters with ranks 1 and 2, respectively whereas; FeSO₄ has the least impact on MRR with lowest rank.

After analyzing the responses for signal to noise ratio and means, optimum parameters obtained are shown in Table 6.

The mean and S/N ratio at optimum values of process parameters as predicted by Taguchi analysis are 126.1 and 43.3286, respectively. Experiments were performed at optimum values of process parameters to validate predicted values of MRR and S/N ratio. Results are

Drocess parameter	Ontimum laval	Doromator satting
ribeess parameter	Optimum level	I arameter setting
Shaking rate (rpm)	1	160
Temp. (°C)	1	45
pH	0	2.5
FeSO4 conc. (%w/v)	1	16
Table 7 Experimental validation		
Experimenta	l values	Predicted values

126.1

43.3286

149.67

43.503

Table 6 Optimum values of process parameters

recorded in Table 7.

Mean

S/N ratio

Experimentally obtained mean and S/N ratio are 149.67 and 43.503 that are in complete agreement with that of predicted by the taguchi methodology.

6. Micro Feature Fabrication at Optimum Process Parameters

It is clear from the experiments that the maximum SMRR can be obtained at a shaking rate of 160 rpm, temperature of 45°C, FeSO₄ concentration of 16%, and pH of 2.5. After finding optimum process parameters using Taguchi methodology, some micro features were produced on iron using a maskless photolithography system reported by Saragih et al.¹⁴ (2013) to see the efficiency of the biomachining process with these optimum process parameters.

The work-pieces were first polished, and a layer of AZ-P4620 photopolymer was applied via spin coating technique. Then, the work-pieces were soft backed for 60 sec. After that, the photopolymer was cured with a patterned UV light through a maskless photolithography system and then developed for 120 seconds. The patterned work-pieces were then exposed to the bacterial solution. The biomachining was carried out at optimum values of process parameters. After intended period of biomachining work-pieces were isolated from bacterial solution, mask was removed, and micro textures were observed under non-contact profilometer. Nano-map images are shown in Fig. 9.

Fig. 9 shows the experimental results of micro features fabricated by biomachining at optimum values of process parameters. The dimensions are compared in Table 8.

For circular and triangular dimples, biomachining was carried out for 20 min. The maximum depths at the end of the biomachining period were 24.14 and 16.67 μ m, respectively. For circular micro pillars, biomachining was done for 100 minutes, and the maximum height attained was 16.99 μ m. One other aspect revealed that, even with the higher metal removal rate, dimensional accuracy was acceptable. These experiments provide evidence that the MRR and SMRR with the optimum parameters selected by Taguchi methodology is significantly improved, making biomachining a practical technique for micro feature fabrication.

7. Conclusions

In the present study, Taguchi methodology has been used to find out optimum values of process parameters for obtaining maximum metal



Fig. 9 (a) Circular dimple (b) Micro pillar (c) Triangular dimple

Table 8 Feature and mask dimensions comparison

Shape	Average mask dimensions (µm)	Average feature dimensions (µm)	Deviation (µm)
Circular dimple	40.00	43.82	3.82
Circular pillar	40.00	54.18	14.18
Triangular dimple	85.00	97.06	12.06

removal rate. First of all, effects of all of the parameters on MRR and SMRR have been investigated to know a basic trend of each process parameter. All of the process parameters apparently showed direct relationship with MRR and SMRR except pH.

Secondly, Taguchi method was used to design a number of experiments to find out the ranks of most influential process parameters. As a result of Taguchi analysis, shaking rate and temperature have been found to be most influential process parameter, while the FeSO₄ was found to be least influential. Shaking rate of 160 rpm, temperature of 45° C, pH of 2.5, and FeSO₄ concentration of 16% w/v have been calculated as optimum process parameters.

Finally the predicted optimum process parameters were validated with experiments and micro features were fabricated with the optimum values of process parameters. This work provided evidence that metal

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removal rate of biomachining can be significantly improved by optimizing process parameters such as shaking rate, temperature, pH, and FeSO₄ concentration. It also described the basic trends of SMRR for various shaking rates, temperatures, pH, and FeSO₄ concentrations. Dimensional accuracy of micro features was also acceptable even at very high metal removal rate at optimum process parameters.

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