

Article

Micro-WEDM of Ti-29Nb-13Ta-4.6Zr Alloy for Antibacterial Properties: Experimental Investigation and Optimization

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Abstract: Recent developments of orthopedic implant applications have discovered a variety of new metallic biomaterials known as β -type titanium alloys. The μ -WEDM (micro-wire electro discharge machining) surface treatment technique, capable of improving the surface properties of orthopedic implants, was studied in a machining Ti-29Nb-13Ta-4.6Zr alloy. This study aimed to evaluate material removal rate (MRR), kerf width, average surface roughness, microhardness and antibacterial response at different machining parameters which are capacitance (1 nF, 10 nF and 100 nF) and gap voltage (80 V, 95 V and 110 V). The Taguchi method was used to optimize the mentioned output parameters, while ANOVA (analysis of variance) described the significance and contribution of capacitance and gap voltage. Grey relation analysis (GRA) was conducted to perform multiple output optimization. For antibacterial response, cultivations of *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* bacteria on treated surfaces for 72 h were performed. As the results, optimal values of MRR, kerf width, crater area, average surface roughness and microhardness were equal to 0.0637 mm³/min, 93.0 μ m, 21.8 μ m², 0.348 μ m and 442 HV, respectively. Meanwhile, μ -WEDM treatment improved antibacterial properties while the highest antibacterial response was achieved at the lowest average surface roughness resulting in least biofilm formation on treated surfaces.

Keywords: WEDM; beta titanium alloy; TNTZ; biocompatibility; surface treatment; biofilm formation; Taguchi optimization; antibacterial



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1. Introduction

Currently, many people worldwide are suffering from musculoskeletal pathologies which are caused from injuries, inflammations or degenerations. To address this issue, orthopedic implants are commonly used in fixation of fractured bones, their replacements, joints replacements, etc. [1]. However, there are several drawbacks associated with these currently developed metal implants such as the release of metal ions, stress shielding [2] as well as the formation of biofilm on the implant surface [1]. For these reasons, implants have received extensive attention from research aiming to increase their biocompatibility.

In order to address the above-mentioned issues, scientists have developed many metallic alloys as well as surface treatment techniques. Aiming to match the mechanical properties of bone, while maintaining biocompatibility, orthopedic implant material has developed using titanium alloys which are well established due to their excellent corrosion resistance, high fatigue strength, low density and good biocompatibility [3,4]. Meanwhile, commonly used in biomedicine, the Ti-6Al-4V alloy has been shown to have a strong titanium oxide layer which contributes to good corrosion resistance while offering reduced elastic modulus (~110 GPa [4–7]) compared to many other bioimplant materials. In comparison, the elastic modulus of cortical bones is less than 20 GPa where this difference

leads to bone-implant loosening while Al and V elements in the alloy are associated with allergic effects, neurological disorders and carcinogenic effects [8]. As alternatives, a new generation of β -type titanium alloys were developed. Containing non-toxic β -stabilizers, their variation is large.

There are several β -type titanium alloys such as Ti-15Mo, Ti-35Nb-5Ta-7Zr, Ti-13Nb-13Zr, Ti-12Mo-6Zr-2Fe, Ti-29Nb-13Ta-4.6Zr, etc. Their mechanical properties such as ultimate tensile strength (UTS) and elastic modulus are listed in Table 1 with $\alpha+\beta$ type, α type and β type titanium alloys.

Table 1. Ultimate tensile strength and elastic modulus of titanium alloys.

Material	Alloy Type	UTS (MPa)	Elastic Modulus (GPa)	Refs.
Cortical bone	-	119.4–150.6	14–21.8	[9,10]
CP Ti	α	240–500	100–115	[9,11]
Ti-6Al-4V (annealed)	$\alpha+\beta$	895–930	110–114	[9,10,12]
Ti-6Al-7Nb	$\alpha+\beta$	900–1050	105–114	[9,11–13]
Ti-5Al-2.5Fe	$\alpha+\beta$	1020–1033	110–112	[9,11,12,14]
Ti-3Al-2.5V	$\alpha+\beta$	620–690	100–107	[9,11,15]
Ti-6.5Al-3.3Mo-0.3Si	$\alpha+\beta$	911	65	[16]
Ti-15V-3Cr-3Al-3Sn	β	1248	82	[16]
Ti-10V-2Fe-3Al	β	965–1193	109.6	[16]
Ti-29Nb-13Ta-4.6Zr	β	911	65	[11]
Ti-35Nb-5Ta-7Zr	β	590–597	55	[9,11,17]
Ti-15Mo	β	874–921	78–84	[9–11]
Ti-13Nb-13Zr	β	970–1040	77–84	[9,11]
Ti-12Mo-6Zr-2Fe	β	1060–1100	74–85	[11]
Ti-15Mo-5Zr-3Al	β	880–980	75–88	[9,11]

Among these earlier mentioned alloys, the Ti-29Nb-13Ta-4.6Zr alloy presents a β -type titanium alloy which attracted potential interest from biomedicine. Containing non-toxic Nb, Ta and Zr elements, this alloy has a low elastic modulus equal to 60–65 GPa [18,19]. In a relevant study related to aging treatments, Niinomi [20] compared fatigue performance, cyto-toxicity and the elastic modulus of the Ti-29Nb-13Ta-4.6Zr and Ti-6Al-4V alloy. Fatigue limit after 10^7 cycles of the first alloy after solution treatment was equal to 320 MPa. However, after 627 K aging treatment of Ti-29Nb-13Ta-4.6Zr for 259.2 ks (ks = 10^3 s), its fatigue limit increased up to 700 MPa which was also equal for the Ti-6Al-4V alloy. Cyto-toxicity tests showed that the Ti-29Nb-13Ta-4.6Zr alloy had higher cell viability compared to the Ti-6Al-4V alloy. The elastic modulus of the Ti-29Nb-13Ta-4.6Zr alloy was less than that of Ti-6Al-4V. On top of that, the author observed that the value of the elastic modulus of the Ti-29Nb-13Ta-4.6Zr alloy can be increased by using aging treatments. Considering the harsh environment of a human body, the corrosion resistance of Ti-29Nb-13Ta-4.6Zr was studied as well. It showed better resistance to corrosion than microcrystalline Ti grade 2 in phosphate-buffered saline [21]. Meanwhile, Rajabi et al. [22] increased the corrosion resistance of the Ti-29Nb-13Ta-4.6Zr alloy by thermomechanical processing. It resulted from the formation of a martensite phase on the passive layer. Hot compression at 900 °C resulted in smaller resistance compared to warm compression at 300 and 400 °C due to excessive shifts near grain boundaries. Comparing the corrosion resistance of Ti-29Nb-13Ta-4.6Zr and Ti-6Al-4V in a simulated body environment, according to Gunawarman et al. [23], the corrosion rate was 4.5×10^{-9} and 6.4×10^{-8} mm/y, respectively. However, the first alloy witnessed higher pitting corrosion compared to the second which was caused from bigger elemental segregation and higher content of impurities. Implant wear generation, which can result in implant loosening, inflammation, pain, etc., was studied by Li et al. [24]. Observing the wear resistance of Ti-29Nb-13Ta-4.6Zr and Ti-6Al-4V by the pin-on-disk method, the wear generation of both alloys were found to be minor compared to stainless steel. Moreover, the wear resistance of Ti-29Nb-13Ta-4.6Zr is slightly better compared to Ti-6Al-4V because of more brittle TiO₂ oxides in the passive layer than Ti₂O₃ and

Nb₂O₅. For that reason, Ti-6Al-4V generates cracks which increase wear generation, despite almost the same wear loss. From this study, oxidation treatment was also suggested to improve wear resistance while it was stated that heat treatments do contribute to improved wear resistance.

While the choice of implant material is a crucial part of successful implantations, their surfaces play a vital role in biocompatibility. On top of mechanical properties, the surface of implants affects osteointegration [25] as well as bacteria adhesion [26]. For the purpose of increasing the adhesion of body cells and repelling bacteria, different implant surfaces modification techniques were found such as plasma spraying, grit blasting, chemical vapor deposition, and so on. Moreover, these modification techniques are able to change the physical properties of surfaces which can be also beneficial [27].

One of the surface treatment techniques which can alter the surface properties of implants is electro-discharge machining (EDM). This process is based on melting material through electrical discharges between electrodes and a conductive workpiece in a dielectric fluid. The local temperature during the EDM process can reach up to 15,000 °C, which surpasses the melting temperature of all titanium biomedical alloys [28]. This technique removes material through electrical sparks, hence no mechanical force, making it suitable for machining harder materials [29], including nickel alloys, ferrous alloys and titanium alloys [30]. The intense energy elevates the temperature to 15,000 °C in the EDM process leading to the evaporation and melting of both the tool electrode and the work surface. The resulting molten debris is removed from the machining area by a continuously flowing dielectric fluid. The EDM process only changes the surface material properties; the process causes three layers. The recast layer: it is formed due to the re-solidification of melted material along with decomposed hydrocarbon (dielectric) on the surface and it could change the surface properties. Heat effected zone: the second layer is due to the conduction of heat to the successive layer and its thickness can be in the microns meter range; this layer also has different mechanical properties than bulk material. The last layer is known as bulk material; it is the part of the material that is not affected by the heat [31]. High output temperature of EDM is good for machining β -type titanium alloys because of their high melting temperature which increases the preparation difficulty of parts for other conventional machining techniques [32]. The EDM surface treatment of titanium alloys creates a nano-porous surface layer which increases osteointegration [29]. Adding powder particles into dielectric fluid of powder mixed electrical-discharge machining (PMEDM) such as hydroxyapatite facilitates cell growth on the surface [33], while adding silver powder increases antibacterial properties of the surface having no influence on osteoblast function [34].

Researchers exploring the domain of wire electro-discharge machining (WEDM) to machine metallic alloys, including Jayakumar and Suresh [35,36], investigated the effects of current (I), pulse-on time (T_{on}), pulse-off time (T_{off}) and voltage (V) on the machining of SS304 using two different wire materials, brass and zinc-coated brass. Their study focused on machining response variables such as material removal rate (MRR) and kerf width. Additionally, they performed WEDM with both rough and trim cuts to enhance machinability. The electrode used was a 0.25 mm diameter brass wire. They further analyzed the influence of these WEDM parameters on surface roughness (R_a), the hardness of the machined surface and the thickness of the recast layer for both wire materials on SS304. Similarly, Manoj and Narendranath [37] investigate the surface roughness, recast layer and hardness of microfer 4722 superalloy after WEDM machining. They concluded that augmentation in slant angle 0–300 reduces the surface roughness by 15.92% and the recast layer by 62.76%; however, the hardness was enhanced by 4.59%.

Having insufficient research literature at the intersection of the Ti-29Nb-13Ta-4.6Zr alloy and EDM surface treatment motivates conducting this research based on the high research interest in EDM surface modification of a commonly used Ti-6Al-4V alloy. As β -type Ti-29Nb-13Ta-4.6Zr titanium alloy possesses superior properties in biomedical implant applications, EDM surface treatment of the alloy demonstrates a big potential.

This paper aims to investigate the machinability performance of micro-WEDM (wire electro-discharge machining) on a Ti-29Nb-13Ta-4.6Zr alloy and its surface modification for improved biocompatibility.

2. Materials and Methods

2.1. Materials

Surface treatments of titanium alloy were carried out using μ -WEDM. It involves enabling surface treatment processes at micro scales. Figure 1 shows the schematic diagram of the μ -WEDM experimental setup. The workpiece and wire material used in these experiments were Ti-29Nb-13Ta-4.6Zr (TNTZ) and tungsten wire, respectively. The TNTZ alloy plates were procured from “LUOYANG COMBAT TUNGSTEN & MOLYBDENUM MATERIAL CO., LTD.” LuoLong, Luoyang, Henan, China. A scanning electron microscope (SEM, JEOL JSM-IT200(LA)) was utilized to conduct the energy dispersive X-ray (EDX) analysis. The measurement parameters were set to an accelerating voltage of 20.0 kV, a working distance of 12.2 mm and a probe current of 60.0 pA. Analysis on elemental compositions collected through energy dispersive X-ray spectroscopy (EDS) are listed in Table 2. The dimensions of the workpieces were 20 mm $\times$ 20 mm $\times$ 3 mm. μ -WEDM is a stochastic process, so each experiment was repeated trice according to the design of the experiments. The experimental conditions for this study were determined based on the outcomes of initial feasibility tests conducted prior to the main experiment. During these preliminary tests, it was consistently observed that the wire broke under high energy conditions (Capacitance = 400 nF, V = 110 V). This crucial finding led us to refine and optimize the experimental parameters to ensure stable and reproducible results. Table 3 presents the specific experimental conditions for μ -WEDM experiments.

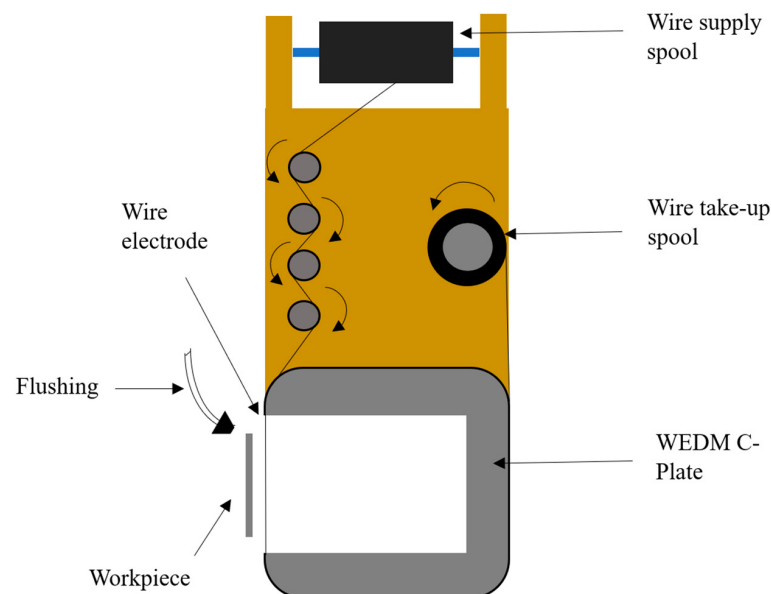


Figure 1. The schematic diagram of μ -WEDM process.

Table 2. EDS analysis of Ti-29Nb-13Ta-4.6Zr alloy before and after machining.

Surface Condition	Ti (wt%)	Nb (wt%)	Ta (wt%)	Zr (wt%)	Si (wt%)	O (wt%)	C (wt%)	W (wt%)
Pure TNTZ *	52.22	30.52	12.49	4.77	-	-	-	-
Not treated	42.19	24.43	10.19	3.81	2.47	8.89	8.03	-
Low-energy treated	33.60	27.80	13.50	3.67	0.56	2.35	16.10	2.42
Medium-energy treated	35.79	27.52	12.89	4.07	0.53	1.79	15.65	1.76
High-energy treated	39.66	26.91	12.47	4.30	0.36	1.54	13.02	1.74

* Calculations for “Pure TNTZ” was based on “Not treated” by excluding Si, O and C elements to show the correlation between the composition and definition of the alloy.

Table 3. Experimental conditions for the μ -WEDM process.

Parameter	Value	Unit
Gap voltage	80, 95, and 110	V
Capacitance	1, 10, and 100	nF
Wire speed	0.8377	mm/s
Feed rate	1.5	$\mu\text{m/s}$
Wire tension	8.25	g-wt
Depth of cut	50	μm
Wire diameter	50	μm
Workpiece polarity	Positive	

2.2. Desing of Experiments

Experimental design was set accordingly to analyze two factors with three levels. To increase the statistical stability of the analysis, the experiments were performed triple for each combination of experiments. The available design that fits the criteria is L27 Taguchi's orthogonal array. The input parameters and their values for each level are shown in Table 4 while the design of experiments is shown in Table 5. In the design, the aim was to maximize MRR and microhardness, and minimize kerf width, crater size and average surface roughness. Therefore, the criteria for MRR and microhardness were chosen as “larger is better”, while the criteria for kerf width, crater size and average surface roughness were set as “smaller is better”. Analysis of means for each factor and ANOVA (analysis of variance) were obtained by analyses of Taguchi's design in Minitab 18.1 software. On top of that, the conditions for optimal multiple output parameters were investigated by using grey relation analysis (GRA).

Table 4. Input parameters and their values for Taguchi design.

Parameters	Factor	Level 1	Level 2	Level 3
Capacitance (nF)	A	1	10	100
Gap Voltage (V)	B	80	95	110

Table 5. L27 orthogonal array and results.

Exp. No.	Factors		MRR (mm^3/min)	Kerf Width (μm)	Crater Size (μm^2)	Average Surface Roughness (μm)	Microhardness (HV)
	A	B					
1	1	1	0.0266	89.6	19	0.457	343
2	1	1	0.0345	94.8	22	0.276	270
3	1	1	0.0305	95.6	24	0.311	313
4	1	2	0.0636	116.6	23	0.635	312
5	1	2	0.0582	104.8	33	0.324	315
6	1	2	0.0546	104.8	24	0.399	349
7	1	3	0.0665	113.2	28	0.504	326
8	1	3	0.0671	113.2	31	0.567	316
9	1	3	0.0663	110.7	34	0.468	385
10	2	1	0.0921	112.4	95	0.611	409
11	2	1	0.0689	95.6	74	0.820	436
12	2	1	0.0670	106.5	66	0.720	409
13	2	2	0.0635	111.5	115	0.778	363
14	2	2	0.0749	111.5	89	0.761	441
15	2	2	0.0721	112.4	93	0.921	423
16	2	3	0.0421	119.9	123	0.931	419
17	2	3	0.0846	119.1	128	0.661	433
18	2	3	0.0645	108.2	133	0.832	425
19	3	1	0.0410	118.3	328	0.908	446
20	3	1	0.0394	115.7	313	0.496	463

Table 5. Cont.

Exp. No.	Factors		MRR (mm ³ /min)	Kerf Width (μm)	Crater Size (μm ²)	Average Surface Roughness (μm)	Microhardness (HV)
21	3	1	0.0593	117.5	308	0.831	459
22	3	2	0.0550	120.1	434	1.142	456
23	3	2	0.0416	117.5	451	0.991	432
24	3	2	0.0411	118.3	392	0.876	416
25	3	3	0.0469	135.0	476	1.339	429
26	3	3	0.0509	121.6	473	1.421	463
27	3	3	0.0578	139.2	480	1.561	435

2.3. Analysis of Kerf Width (KW)

In essence, kerf width is the amount of material that the wire removes throughout the WEDM process. It is the measure of cut width that the wire electrode makes in the workpiece as it passes through it, as shown in Figure 2.

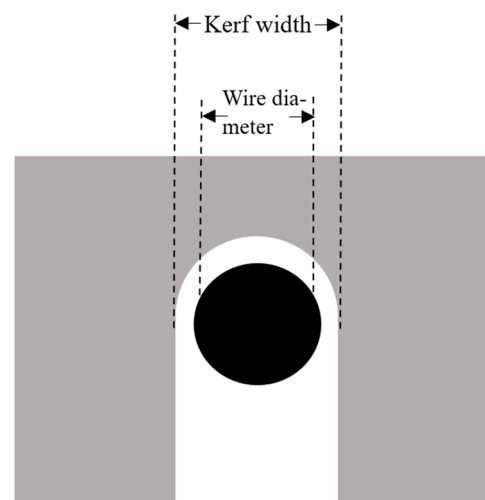


Figure 2. The schematic illustration of kerf width.

2.4. Material Removal Rate (MRR)

Material removal rate is the rate of material volume removed from the workpiece, which is determined using the equation as follows:

$$MRR = \frac{\text{Depth of cut} \times \text{height of cut} \times \text{kerf width}}{\text{machining time}} \quad (1)$$

The depth of cut was equal to 50 μm, height of cut was changed accordingly from 20.03 to 19.79 mm along the workpiece, kerf width and machining time were measured during the experiments.

2.5. Crater Area (CA), Microhardness and Average Surface Roughness (SR)

The surface dimensions for these particular characterizations were 20 mm × 5 mm. The crater size was determined from a scanning electron microscope (SEM) by JEOL JSM-IT200(LA). The surface microhardness of μ-WEDM-ed Ti-29Nb-13Ta-4.6Zr surfaces were measured using a Vickers microhardness tester Shimadzu HMV-G31, with 200 g force indenter load and a 12 s dwell period [38–40] for each experiment. To ensure precision and consistency, three microhardness measurements were taken for each discharge energy setting. Additionally, for the 3D mapping and determination of average surface roughness of μ-WEDM-treated surfaces, the Atomic Force Microscope (AFM) SmartSPM 1000 is

utilized. AC mode (a semi-contact mode) for the surface profiling is conducted with a resolution of $256 \text{ px} \times 256 \text{ px}$ and a scan area of $20 \text{ }\mu\text{m} \times 20 \text{ }\mu\text{m}$.

2.6. Regression Model

To supplement the analysis of means of each factor, the 3D visual representation of the regression model was programmed through MATLAB software. The “combvec” MATLAB built-in function is used to generate all possible combinations of elements from multiple sets of input vectors; it is part of the optimization toolbox. Additionally, the MATLAB “fitlm” function is commonly used to fit regression models, a part of the Statistics and Machine Learning Toolbox. The “fitlm” is used to fit models with single or multiple predictor variables. In this study, the “combvec” is used to generate all possible input vectors for the combination of capacitance-voltage, and “fitlm” is used to fit the quadratic model. The approach allows the surface response to be predicted between process and performance parameters.

Due to A2 being close to A1 and distant to A3, the uneven distribution decreases the accuracy of the regression model. In order to evenly distribute the data across the variable range, capacitance was analyzed according to its power of 10 scale where level 1 is 10^0 nF , level 2 is 10^1 nF and level 3 is 10^2 nF . This allows us to obtain a more accurate model.

2.7. Antibacterial Test

The prevailing hypothesis regarding the influence of surface topography on bacterial attachment suggests that a maximum surface roughness slightly smaller than bacterial cell size is most effective at reducing the contact area between the cell and substrate. This creates a “fakir effect”, resulting in an unfavorable environment for bacterial proliferation. Additionally, these peaks and valleys may induce local mechanical stresses in the bacterial cell wall, potentially altering cellular metabolism [41]. The antibacterial action of the proposed EDM-treated surfaces depends on the final surface roughness. Low-energy-treated surfaces have much lower surface roughness and therefore prevent bacteria from attaching while medium- and high-energy-treated surfaces have higher roughness, leading to increasing bacterial attachment [42]. To analyze bacterial attachment and biofilm formation, separate experiments were conducted using μ -EDM with three different levels of discharge energy, higher energy (100 nF and 110 V), medium energy (10 nF and 95 V) and low energy (1 nF and 80 V). Three $3 \text{ mm} \times 20 \text{ mm}$ treated surfaces for each discharge energy were made on the surfaces of Ti-29Nb-13Zr-4.6Zr. The rest of the machining parameters were kept the same as depicted in Table 3. The goal of the test was to evaluate wire electrical discharge machining’s (WEDM) antibacterial efficacy against multiple strains of bacteria, such as *Escherichia coli* (DH5 α), *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*; the methodology of the experiment is depicted in Figure 3. This was accomplished by assessing the biofilm growth on various metal surfaces following a 72 h broth culture inoculation. Fresh 24 h liquid cultures of every species of bacteria were used to prepare the bacterial inoculum, aiming for an initial optical density (OD 600) of 0.1 or a McFarland turbidity of 0.5. A microplate reader was used to measure the OD 600 of the 16 h culture broth in order to calculate the precise amount of bacteria that needed to be provided to fresh media in a 50 mL Falcon tube, leading to a final volume of 50 mL with an OD of 0.1. Every single specimen of the metal alloy was suspended in a different 50 mL Falcon tube that held a single species of bacteria in the culture medium. After that, these tubes were incubated for 72 h in an aerobic environment at 37 °C and 220 RPM in a shaker incubator.

The formation of biofilms and bacterial cells were then observed on the metal surfaces by staining them with crystal violet (CV) dye for a duration of thirty minutes. Following staining, the specimens were allowed to air dry for an hour at room temperature prior to being imaged. They were then rinsed three times using distilled water. Employing a Zeiss AxioZoom V16 macroscope, bright field and red fluorescent photos were taken with exposure periods of 20 ms and 800 ms, respectively. Using specially created MATLAB programs, a comparative study of the biofilm coverage was carried out. For the purpose of

comparison, only biofilms larger than five times the surface area of a single bacterium and with a signal intensity in excess of 250 were gathered.

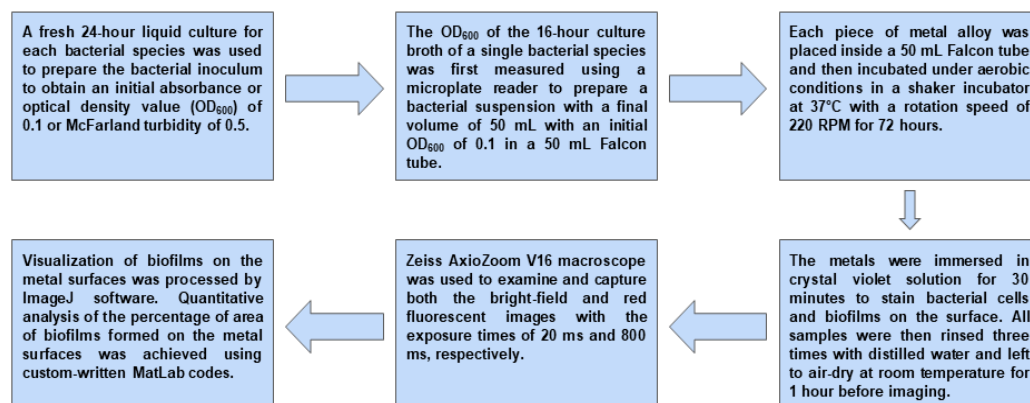


Figure 3. Schematic diagram showing how antibacterial tests were performed, visualized and quantified.

The metals were first submerged in a 30% acetic acid solution for 15 min to eliminate any biofilms on the machined surfaces, ensuring the cleanliness and disinfection of the metals prior to the next investigations. Following a 30 min immersion in a 70% ethanol solution, they were rinsed with distilled water and subjected to a 5 min plasma cleaning process. To guarantee thorough disinfection before to culture them with bacteria, all metals were exposed to UV light for thirty minutes prior to each biofilm development test. With the exception of *Staphylococcus aureus*, which was cultivated in tryptone soy broth (TSB), all bacterial strains were cultured in Luria-Bertani (LB) broth. To avoid cross-contamination, all studies were carried out in an aseptic environment.

3. Results and Discussion

3.1. Taguchi's Orthogonal Array

Taguchi's orthogonal array (L27) as well as the measurements of MRR, kerf width, crater size, average surface roughness and microhardness from μ -WEDM machining of Ti-29Nb-13Ta-4.6Zr alloy are shown in Table 5. According to the measurements in the table, Taguchi analyses were carried out for each output parameter according to "large is better" and "smaller is better" criteria. The plots of means for each output representing the influence of individual input parameters are shown in Figure 4, while Figure 5 shows 3D surface response plots obtained from MATLAB.

Observing Figures 4a and 5a, MRR increased with gap voltage. Its increase contributes to the energy of sparks which increases the amount of material removed. The same as gap voltage, capacitance value is proportional to the value of discharge energy. However, with the change in each level of capacitance, MRR changed more sharply compared to levels of gap voltage. The graph shows that an increase in capacitance from level 1 to level 2 increased the material removed through an increase of spark energy; the analogous trend was observed by Hu et al. [43]. Following, by increasing capacitance from level 2 to level 3, MRR decreased. Increasing spark energy contributes to a bigger volume of removed material; however, for capacitance level 3, the amount of discharge energy was very high which may result in excessive amounts of debris concentration. Due to the presence of debris, a portion of energy was wasted on machining the debris which slowed down the removal rate of workpiece material [44]. This effect is commonly known as secondary sparks [45].

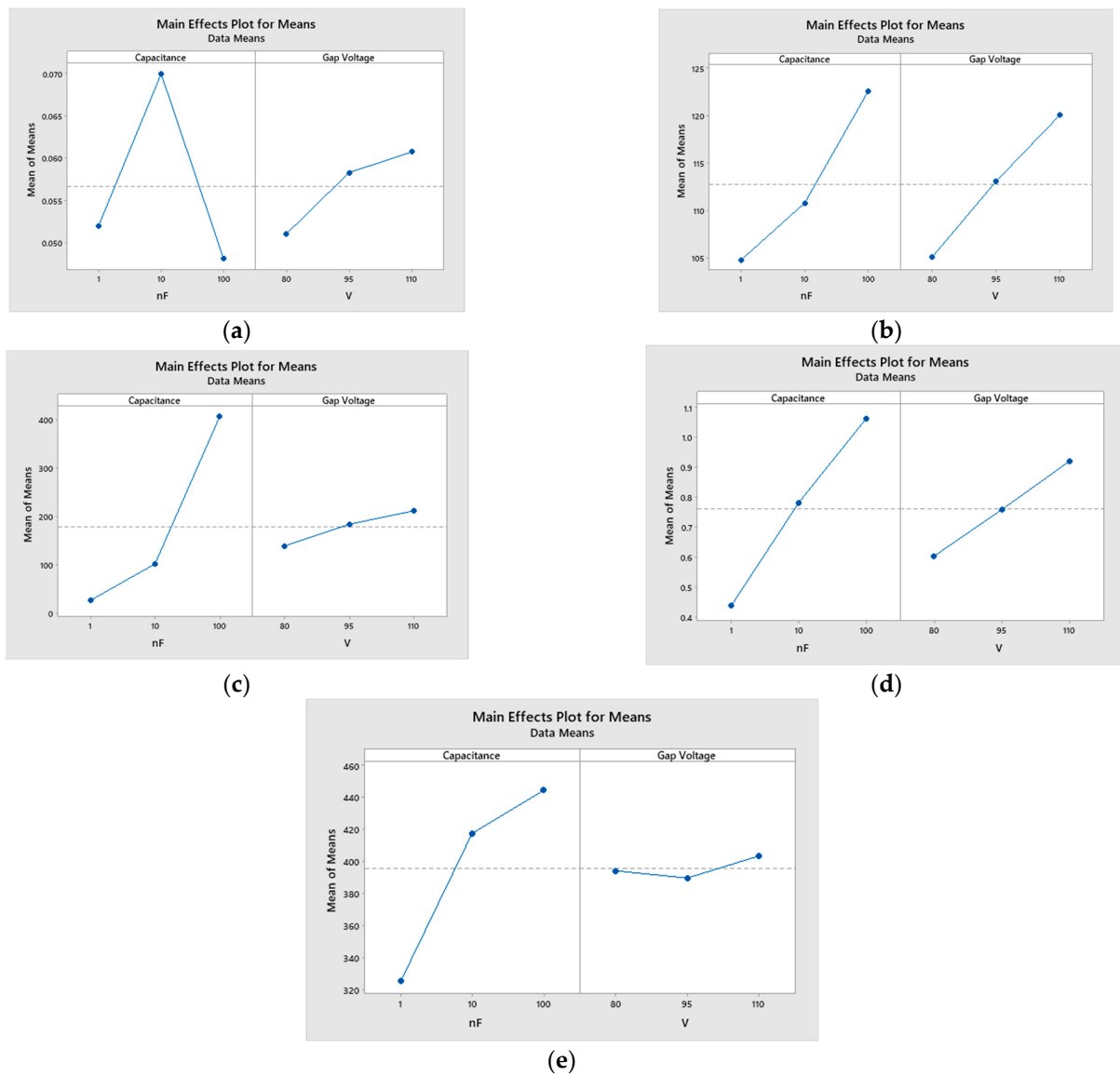


Figure 4. Main effects plot of means for (a) MRR, (b) KW, (c) CS, (d) SR and (e) microhardness.

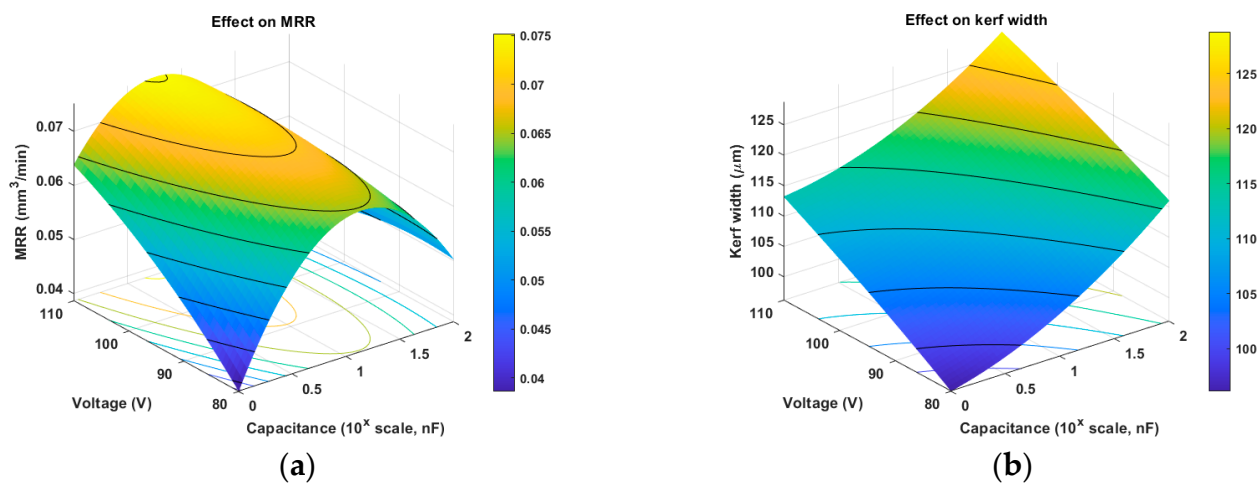


Figure 5. Cont.

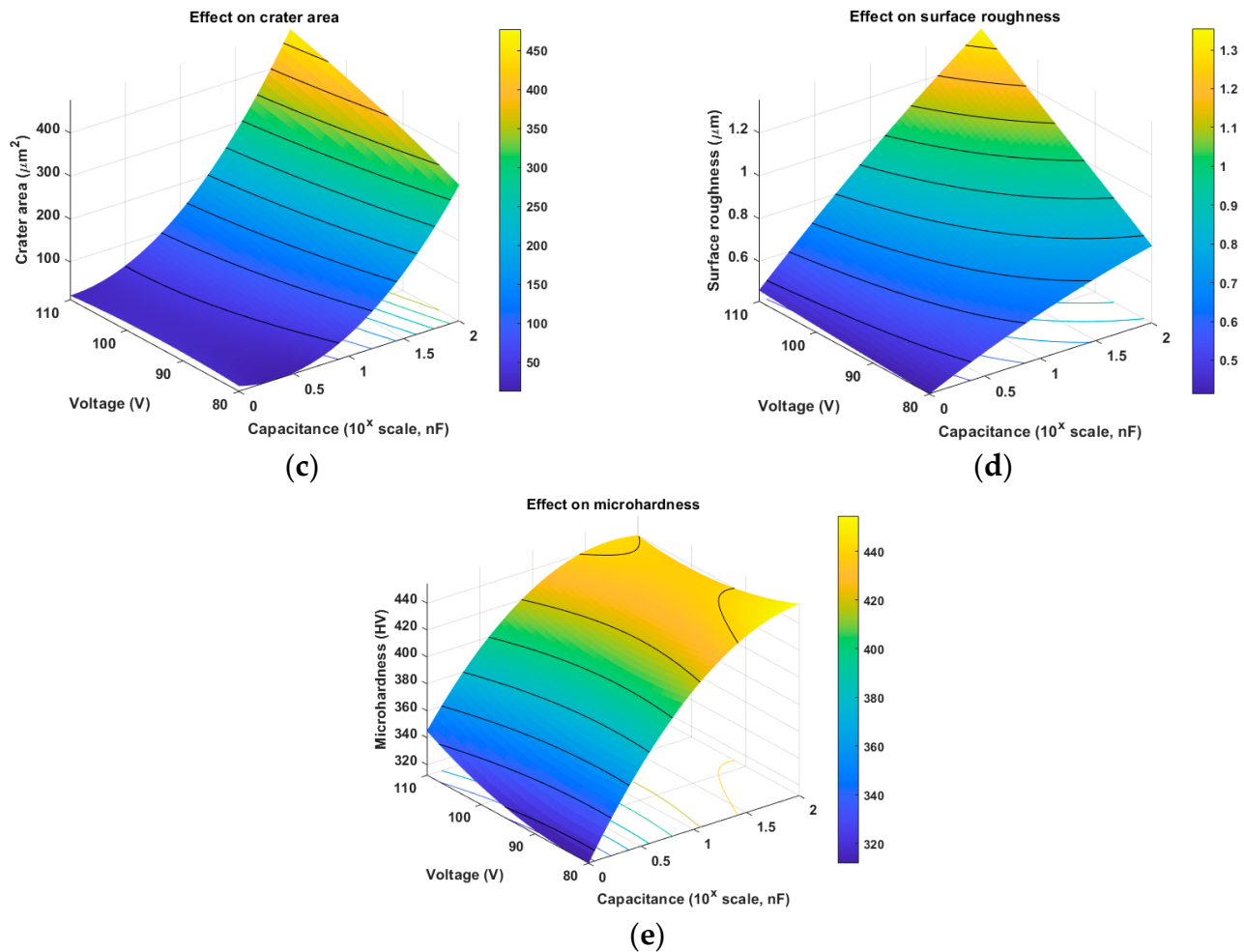


Figure 5. 3D plots of regression models for (a) MRR, (b) KW, (c) CS, (d) SR and (e) microhardness.

From Figures 4b,c and 5b,c, the similar patterns of capacitance are seen on kerf width and crater size. A smaller difference in capacitance value, between level 1 and level 2, resulted in a smaller gain in discharge energy. Comparingly, a bigger difference in capacitance value, between level 2 and level 3, resulted in a bigger gain in discharge energy, as depicted in Figure 6a–c. High discharge energy resulted in bigger sparks which increased kerf width and craters sizes. However, the influence of gap voltage was different for kerf width and crater size; a similar trend was observed by Ali et al. [46]. It is seen that gap voltage had a higher contribution on kerf width, while its effect on crater size was smaller. Comparing the influence of gap voltage on crater size and kerf width, crater size benefits from an increase in gap voltage by its contribution to the amount of discharge energy. Meanwhile, on top of the increased discharge energy, gap voltage can increase the gap distance where a breakdown of dielectric fluid occurs [47] which in turn, increases the distance required for a spark between the workpiece and electrode, which contribute to kerf width.

Figure 7a–c displays the 3D mapping of the average surface-generated WEDM at voltage 95 V and capacitance 1 nF, 10 nF and 100 nF. According to Figure 4d, capacitance and gap voltage had similar trends with respect to average surface roughness. The effect of capacitance on surface roughness value was approximately the same for each level which resulted in the correlation between surface roughness and capacitance value in the logarithmic trend. It should be noted that the scale of capacitance axis in the 3D plot shown in Figure 5d as well as levels of factor A were based on a 10^x scale which resulted in linear behavior of the output. Converting the capacitance to the linear scale will change the output

average surface roughness to the logarithmic trend. The similar empirical approximation between average surface roughness and pulse energy was observed by Jeswani [48].

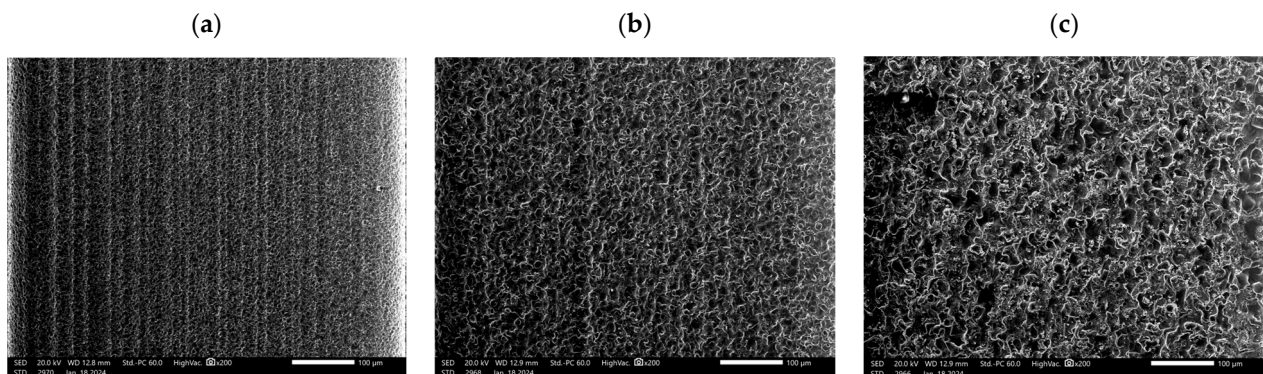


Figure 6. SEM image analysis for the influence of capacitance on the surface morphology at voltage = 95 V and (a) capacitance = 1 nF; (b) capacitance = 10 nF; (c) capacitance = 100 nF.

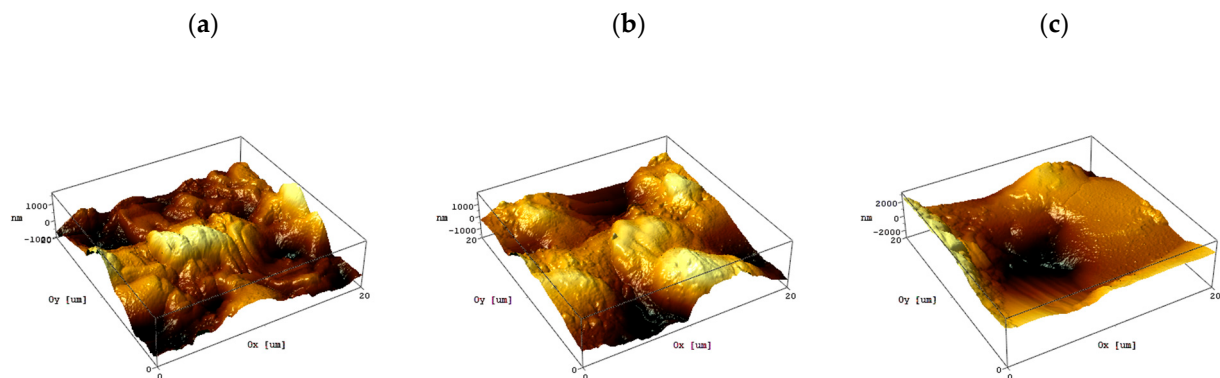


Figure 7. AFM analysis for the influence of capacitance on the surface morphology at voltage = 95 V and (a) capacitance = 1 nF; (b) capacitance = 10 nF; (c) capacitance = 100 nF.

During the micro-EDM process, material removal occurs in the form of craters through melting and evaporation mechanisms. Consequently, the surface undergoes repeated cycles of heating and cooling, potentially altering its hardness. The recast layer, which typically forms as a result of melting of material and re-solidification on the surface, usually exhibits higher surface hardness and may contain micro-cracks and other defects. In biomedical applications like dental implants, the enhanced hardness post-micro-EDM can be beneficial [49]. Figures 4e and 5e depicted the effect of gap voltage on microhardness which was minor; however, the higher the capacitance, the more challenging it was to achieve an Improvement in microhardness. The value of microhardness increased from the formation of the re-melted recast layer. On top of that, the deposition of high hardness tungsten particles from the electrode increases the hardness as well [50].

3.2. ANOVA

The analyses of variance for each output parameter were performed at the 95% confidence level. It shows the significance and contribution of capacitance and gap voltage as well as their interactions on MRR, kerf width, crater area, average surface roughness and microhardness. According to Table 6, capacitance (A) had the most contribution equal to 36.17% followed by interaction of capacitance and gap voltage ($A \times B$), and gap voltage (B) equal to 29.49% and 6.78%, respectively. The p -values showed strong significance ($p < 0.05$) of A and $A \times B$, while B was a non-significant factor in MRR. Therefore, MRR is strongly affected by capacitance while the contribution of gap voltage was minor. Observing Table 7, ANOVA showed strong significance of both A and B from low p -values for kerf width. The

Table 10. ANOVA for microhardness.

Source	DF	SS ($\times 10^3$)	MS ($\times 10^3$)	F	p	Contribution
A	2	70.01	35.00	53.10	0.00	82.66%
B	2	0.89	0.44	0.67	0.52	1.05%
A $\times$ B	4	1.93	0.48	0.73	0.58	2.28%
Error	18	11.87	0.66			14.01%
Total	26	84.69				100%
$R^2 = 85.99\%$		$R^2 (\text{adj}) = 79.76\%$				

From ANOVA, good significance of capacitance was observed in all output parameters which are MRR, kerf width, crater area, average surface roughness and microhardness. On top of that, capacitance had a major contribution on each parameter. Gap voltage was significant for kerf width, crater size and average surface roughness and non-significant for MRR and microhardness. Despite the gap voltage having almost the same contribution with the interaction of two factors for crater size and average surface roughness, it showed the second most contribution after capacitance for kerf width, crater size and surface roughness. Meanwhile, interaction between capacitance and gap voltage was significant for MRR, crater area and average surface roughness. Based on the R^2 values, the capacity of the input factors to explain the variance in the output variables can be ranked as follows: crater area (99.60%), average surface roughness (89.60%), microhardness (85.99%), kerf width (83.55%) and MRR (72.44%). Higher R^2 values for crater area and average surface roughness indicate that the capacitance and gap voltage primarily influence these output factors. In contrast, the lower R^2 values for MRR and kerf width suggest the potential presence of additional input factors affecting these variables.

3.3. Grey Relation Analysis

In order to analyze the most optimal conditions for multiple output parameters, grey relation analysis (GRA) was utilized. Considering “smaller is better” and “large is better” criteria, MRR and microhardness values were normalized according to the “larger is better” formula where high values are desired, while kerf width, crater size and average surface roughness values were normalized according the “smaller is better” formula where low values are desired. The experimental results of each experiment, their grey relation coefficient for each output parameter, grey relation grade and ranks are shown in Table 11. By ranking the average grey relation grade of repeated experiments with the same conditions, the most optimal parameters were A1B1.

Table 11. Calculation of GRG.

No. Exp.	Grey Relation Coefficient					Grey Relation Grade	Average Grey Relation Grade
	MRR	Kerf Width	Crater Area	Average Surface Roughness	Microhardness		
1	0.333	1.000	1.000	0.827	0.446	0.721	0.707
2	0.362	0.827	0.988	1.000	0.333	0.702	
3	0.347	0.805	0.980	0.961	0.391	0.697	
4	0.534	0.479	0.984	0.707	0.390	0.619	0.659
5	0.491	0.619	0.946	0.947	0.395	0.680	
6	0.466	0.619	0.979	0.875	0.458	0.680	
7	0.561	0.512	0.965	0.791	0.413	0.649	0.655
8	0.567	0.512	0.951	0.748	0.396	0.635	
9	0.559	0.540	0.942	0.818	0.553	0.682	
10	1.000	0.521	0.752	0.721	0.641	0.727	0.702
11	0.585	0.805	0.809	0.614	0.781	0.719	
12	0.566	0.594	0.831	0.661	0.641	0.659	
13	0.533	0.531	0.707	0.633	0.491	0.579	0.632
14	0.655	0.531	0.767	0.641	0.814	0.681	
15	0.621	0.521	0.758	0.573	0.707	0.636	

Table 11. Cont.

No. Exp.	Grey Relation Coefficient	Kerf Width	Crater Area	Average Surface Roughness	Microhardness	Grey Relation Grade	Average Grey Relation Grade
	MRR						
16	0.396	0.450	0.690	0.569	0.687	0.558	0.620
17	0.813	0.457	0.679	0.692	0.763	0.681	
18	0.542	0.572	0.670	0.609	0.717	0.622	
19	0.390	0.464	0.428	0.578	0.850	0.542	
20	0.383	0.487	0.440	0.797	1.000	0.621	0.587
21	0.499	0.471	0.444	0.609	0.960	0.597	
22	0.468	0.449	0.357	0.500	0.932	0.541	
23	0.393	0.471	0.348	0.547	0.757	0.503	
24	0.391	0.464	0.382	0.590	0.672	0.500	0.515
25	0.420	0.353	0.335	0.449	0.739	0.459	
26	0.442	0.437	0.337	0.430	1.000	0.529	
27	0.488	0.333	0.333	0.402	0.775	0.466	

By analyzing the variance of grey relation grades (GRG) shown in Table 12, capacitance and gap voltage were significant, showing low p -values. Their contributions to GRG were equal to 64.72% and 18.22%, respectively. The regression model presented in Figure 8 showed big changes at different levels of capacitance, where the peak GRG was between level 1 and level 2 of capacitance. Meanwhile, GRG decreased with an increase in gap voltage level with a comparatively smaller slope having the peak GRG at gap voltage level 1.

Table 12. ANOVA for GRG.

Source	DF	SS ($\times 10^{-3}$)	MS ($\times 10^{-3}$)	F	p	Contribution
A	2	109.50	54.77	36.70	0.00	64.72%
B	2	30.80	15.42	10.33	0.00	18.22%
A $\times$ B	4	2.00	0.50	0.33	0.85	1.18%
Error	18	26.90	1.49			15.87%
Total	26	169.30				100%

$R^2 = 84.13\%$ $R^2 (\text{adj}) = 77.07\%$

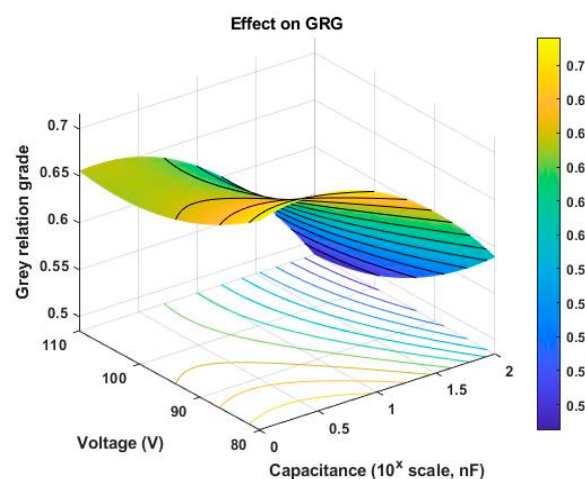


Figure 8. 3D plots of regression models for GRG.

From GRA, the peak GRG for multiple output parameters among performed experiments was A1B1. The means of GRG by Taguchi analysis are shown in Table 13, where analysis showed the most optimal parameter was predicted at A1B1, as well. Comparing the GRG of initial parameters, which are middle factors of capacitance and gap voltage (A2B2), to the most optimal GRG (A1B1), the improvement in GRG was equal to 11.9%.

Table 14 shows the values of MRR, kerf width, crater size, average surface roughness and microhardness at initial parameters (A2B2), at individual optimal parameters and at peak GRG parameters.

Table 13. Means of GRG.

Parameters	Level 1	Level 2	Level 3	Delta	Rank
Capacitance	0.674	0.651	0.529	0.145	1
Gap Voltage	0.665	0.602	0.587	0.078	2
Total mean value of GRG	0.618				

Table 14. Means of GRG.

	Initial Parameters	Taguchi Optimization Levels	GRA Optimization	Initial Parameters
Levels	A2B2			A1B1
MRR (mm ³ /min)	0.0702 *	A2B3	0.0637	0.03056
Kerf width (μm)	111.8 *	A1B1	93.0	93.0
Crater size (μm ²)	99.1 *	A1B1	21.8	21.8
Average surface roughness (μm)	0.820 *	A1B1	0.348	0.348
Microhardness (HV)	409 *	A3B3	442	309
GRG	0.632 *	A1B1	0.707	0.707
		Improvement in GRG = 0.075 or 11.9%		

* Value based on taking average three experiments with the same experimental conditions.

3.4. Antibacterial Response

The effects of various discharge-energy-leveled μ -WEDM treatment of the titanium alloy affected the prevalence of bacteria, *Staphylococcus aureus*, *Escherichia coli*, in addition to opportunistic pathogen *Pseudomonas aeruginosa* found in infections linked to healthcare circumstances. *Bacillus subtilis* will be used as a control, a nonpathogenic strain of bacteria that is commonly present in soil and human gastrointestinal systems. The complete procedure for testing and quantifying the amount of biofilm that forms on the alloy surfaces is depicted in Figure 6. Controlling discharge energy through capacitance and gap voltage, μ -WEDM is able to modify the surface roughness which is one of the most important parameters affecting biofilm formation. It was studied that increasing surface roughness in the micro scale affects bacteria adhesion accordingly due to increasing the contact area and, hence, increasing adhesion force. However, this correlation is opposite when surface roughness is in nanoscale. In that case, bacteria cover only the top pikes of the surface, having less contact area when the surface roughness increases in nanoscale, therefore preferring to adhere on a smoother surface [51]. Here, the correlation between surface roughness and bacteria was discovered for Ti-29Nb-13Ta-4.6Zr. From a previous analysis on regression models, the resulting surface roughness at low (1 nF and 80 V), medium (10 nF and 95 V) and high (100 nF and 110 V) discharge energy levels were equal to 0.348 μ m, 0.820 μ m and 1.440 μ m, respectively.

Figure 9 shows the representative images of biofilm formation developed by four different bacteria strains, visualized through crystal violet (CV) staining, bright field (BF) microscopy and MATLAB biofilm detection, on μ -WEDM-treated surfaces at three different discharge energy levels. Figure 10 illustrates how the discharge energy level affects the amount of biofilm that forms on the alloy for each strain of bacteria. It should be noted that *P. aeruginosa* and *E. coli* showed minimal attachment toward high-energy-treated alloy surfaces which, according to Taguchi regression, had 1.440 μ m surface roughness. The amount of biofilm developed is less than 3% of the total surface area.

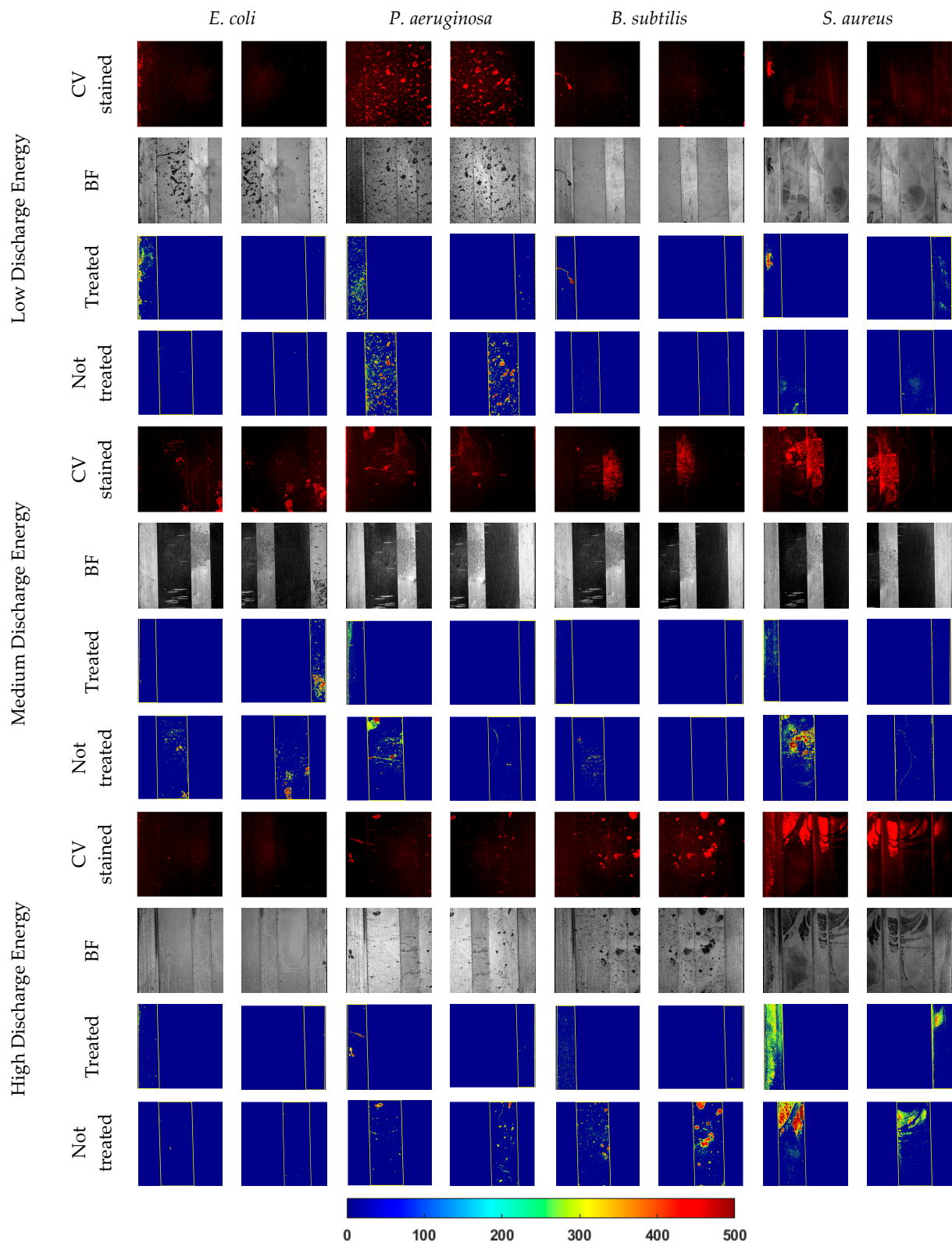


Figure 9. Representative images of crystal violet-stained, bright field (BF) and MATLAB biofilms detection of bacterial biofilms for all bacterial strains on μ -WEDM-treated TNTZ surfaces with different discharge energy levels and untreated surfaces. Quantifications of the percentage of biofilm coverage on machined surfaces was estimated within the yellow box.

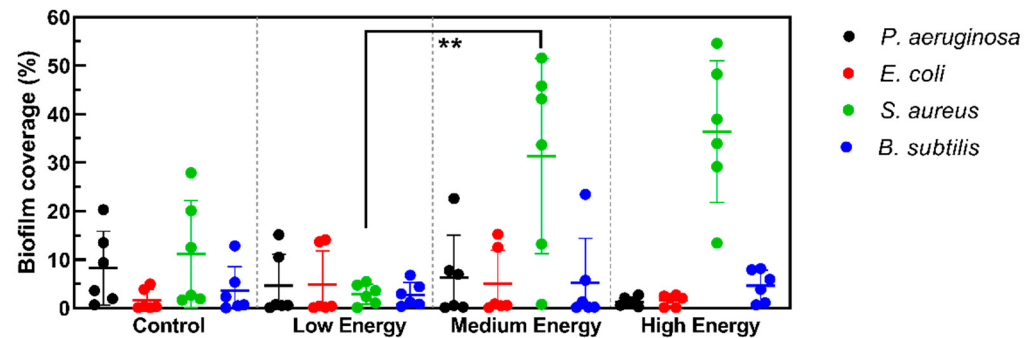


Figure 10. Comparison of the percentage of biofilm coverage for all four bacterial strains on three different energy-level-treated surfaces as well as untreated surfaces.

Figure 10 further illustrates that different μ -WEDM-treated surfaces have varying propensities of attraction to different bacterial species. For example, it clearly shows that the proportion of biofilm coverage for *S. aureus* was lowest at low discharge energy where surface roughness was equal to $0.348\ \mu\text{m}$, while it was much higher at medium and high discharge energies where surface roughness was equal to $0.820\ \mu\text{m}$ and $1.440\ \mu\text{m}$, respectively. Overall, our results seem to suggest that μ -WEDM treatment of Ti-29Nb-13Ta-4.6Zr alloy with the lowest discharge energy (1 nF and 80 V) will minimize the formation of biofilm induced by all four selected strains of bacteria.

4. Conclusions

The performance of μ -WEDM machining of the Ti-29Nb-13Ta-4.6Zr alloy was evaluated in terms of MRR, kerf width, crater size, surface roughness and microhardness by using Taguchi optimization, ANOVA and GRA, while its effect on the bacteria adhesion was investigated for *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* bacteria strain. The following points were summarized from the study:

- Kerf width, crater size, surface roughness and microhardness increased with discharge energy through capacitance and gap voltage. Capacitance had a significant effect for all outputs, gap voltage was significant for kerf width, crater size and surface roughness and their interactions were significant for crater area and surface roughness.
- MRR had an increase from capacitance level 1 to level 2 following a drop at level 3. Meanwhile, increase in gap voltage contributed to a minor increase in MRR. Despite that, capacitance and the interaction of capacitance and gap voltage had a significant effect, while gap voltage was insignificant. The increase in discharge energy can benefit the amount of material removed per spark; however, insufficient debris removal results in poor machinability.
- According to Taguchi optimization, 10 nF and 110 V resulted in MRR ($0.0637\ \text{mm}^3/\text{min}$). The optimal parameters were 1 nF and 80 V for kerf width ($93.0\ \mu\text{m}$), crater area ($21.8\ \mu\text{m}^2$) and surface roughness ($0.348\ \mu\text{m}$). Microhardness was highest at 100 nF and 110 V and equal to 442 HV.
- The rank of output parameters of R^2 were as follows: crater area (99.6%), surface roughness (89.60%), microhardness (85.99%), kerf width (83.55%) and MRR (72.44%). Crater area, having R^2 equal to 99.6%, is strongly affected by capacitance and gap voltage, while MRR, with 72.44% of R^2 , might have other parameters influencing the results than capacitance and gap voltage.
- GRA estimated the most optimal conditions for multiple output parameters. Given the same priority values for every output parameter, the most optimal conditions were at 1 nF and 80 V. Comparing GRG from the middle level parameters (10 nF and 95 V) to optimal (1 nF and 80 V), the total improvement was equal to 11.9%.
- Analysis on biofilm formation of *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* on treated surfaces observed improvement in antibacterial properties compared to untreated surfaces; however, specific observations also investigated a decrease of the antibacterial

properties of a treated surface compared to untreated. It is suggested that the surface of Ti-29Nb-13Ta-4.6Zr can be treated by μ -WEDM with different discharge energy levels to adjust antibacterial properties accordingly against a specific bacterial strain. However, the least amount of total biofilm formation was achieved at the lowest surface roughness value equal to 0.348 μm obtained at 1 nF and 80 V.

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