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Management and Stimulation of High Metabolic Rates of Biomes to Effectively Remediate Mine Drainage

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Abstract

Drainages from mining operations frequently contain elevated levels of contaminants of concern (CoC). Indigenous adapted bacterial communities are characterized and the ability to reduce many CoC is showcased in different treatment implementations. Each contaminated site consists of a distinct prokaryotic community that in turn requires a specific C:N:P balanced environment to contribute to site remediation. This balanced bioremediation strategy is managed both for *in situ* or fix-filmed bioreactors, using electron donor selection and ratios, redox potential, and hydraulic retention times. These communities can effectively treat elevated levels of hexavalent chromium (10 mg/L), nitrate (110 mg/L), and sulfate (1 250 mg/L) in a one-pot balanced system.

Keywords: Bacterial Diversity; C:N:P Stoichiometric Balance; *In Situ* Treatment; Fixed-film Bioreactors; Redox Ladder.

1. Introduction

Mining operations in South Africa, use large amounts of water during extraction, operations, and maintenance of tailing deposits. These tailing facilities often leach or drain, causing the mobilization of elevated contaminants of concern (CoC), including dissolved metals, metalloids, nitrate, and sulfate compounds. In most mine drainages, these CoC are primarily in reducing chemical forms and act as essential oxidants for organic carbon and other reducing chemicals [1], where oxidation states are more toxic to the environment and human receptors.

In recent years in South Africa, concerns have developed around poor management practices to treat these CoC. A myriad of treatment options have been deployed that include filtration, absorption, and chemical reduction. Each treatment has specific benefits, but often capital and operating costs are high, and many could produce additional, often still hazardous, by-products. Biological reduction of CoC using indigenous bacteria can be an environmentally sound alternative technology with lower operational costs and with less or no hazardous by-products. Bacterial metabolic reactions often use CoC as a terminal electron acceptor while precipitating compounds as insoluble hydroxides or metal-sulfides [2-4].

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A better understanding of the biochemical cycles of electron acceptors and electron donor utilisation can identify cooperative metabolic functioning within bacterial communities. These metabolic versatility within bacterial communities can hold the key to developing more sustainable and advanced biological treatment systems. Using the knowledge and characterization of strategic management practices, each specific environment's indigenous microbes can become powerful bioremediation tools [5].

This paper deals with the management and stimulation of selected biomes with high metabolic rates by creating C:N:P stoichiometrically balanced environments, creating dominance within specific species that can naturally attenuate and remediate within these parameters. This is demonstrated both, *in situ* and in fixed-film bioreactors, to treat contaminated mine drainages sites on-site. These remedial strategies can remove several contaminants, including hexavalent chromium (Cr^{6+}), nitrate, and sulfate while improving the overall water quality. Using targeted metagenomics, the microbial community and unique biogeochemical cycles are characterised to balance the C, N and P cycles for environmental geochemical conditions.

2. Methods

2.1. Water Quality Analysis and Site Selection

Two sites with different concentrations of the selected CoC were sampled. Analyses were conducted at accredited laboratories, using anion analysers, ICP-MS/OES, and auto-titration methods, including benchtop physicochemical analysis. The study sites are in the KwaZulu Natal (Site A) and North West (Site B) provinces of South Africa. Site A is a chromium processing facility that produces leather tanning salts, while Site B is a chromium smelter that actively mines and processes chrome ore. Activities on both sites resulted in ground- and surface water contamination with Cr^{6+} and nitrate.

2.2. Microbial Diversity and Metabolic Capabilities

Extracted and purified DNA [6] was analysed using 16S targeted metagenomic sequencing on an Illumina MiSeq system. Sequence reads were analysed using QIIME 2 (<https://qiime2.org>) [7]. Taxonomy was assigned to amplicon sequence variants (ASVs) against the SILVA132 99% OTUs reference sequences (SILVA) [8-10]. The functional potential was predicted using FAPROTAX.

2.3. Microcosm Studies

Using the data from section 2.1 the N (Potassium Nitrate) and P (Di-Potassium Hydro-orthophosphate) supplementation were balanced. The optimal assimilation and utilisation of electron donors, simple - and complex carbon sources, were assessed for the indigenous bacterial communities. These rates and geochemical data sets are used to calculate the specific C:N:P molar ratio, while balancing metabolic function to sustain a chosen redox condition for treatment (Tables 1 and 2). The balanced mixtures were added to site water samples and incubated in 500 mL Scott bottles, at 25 °C, for five days and 20 days. The depletion in dissolved oxygen (DO) concentrations was measured for each incubation period. Refinement of concentration was done with continuous measurement of the reduction of CoC while optimising donor versus acceptor ratios.

Table 1. Calculated molar ratios of stoichiometric electron donor demand for redox reactions of anaerobic metabolic electron acceptors, using acetate and electron donor

Electron acceptor	Balanced reaction	Molar ratio
Oxygen	$\text{CH}_3\text{COOH} + 2\text{O}_2 \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O}$	1/2
Nitrate	$5\text{CH}_3\text{COOH} + 8\text{NO}_3^- + 8\text{H}^+ \rightarrow 10\text{CO}_2 + 4\text{N}_2 + 14\text{H}_2\text{O}$	5/8
Chromate	$3\text{CH}_3\text{COOH} + 8\text{CrO}_4^{2-} + 40\text{H}^+ \rightarrow 6\text{CO}_2 + 8\text{Cr}^{3+} + 26\text{H}_2\text{O}$	3/8
Sulfate	$\text{CH}_3\text{COOH} + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow 2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O}$	1/1

Table 2. Calculated molar ratios of stoichiometric electron donor demand for redox reactions of anaerobic metabolic electron acceptors, using emulsified vegetable oil - EVO

Electron acceptor	Balanced reaction	Molar ratio
Oxygen	$\text{C}_{57}\text{H}_{98}\text{O}_{12} + 75.5\text{O}_2 \rightarrow 57\text{CO}_2 + 49\text{H}_2\text{O}$	1/76
Nitrate	$\text{C}_{57}\text{H}_{98}\text{O}_{12} + 50.34\text{NO}_3^- \rightarrow 57\text{CO}_2 + 25.17\text{N}_2 + 49\text{H}_2\text{O}$	1/50
Chromate	$\text{C}_{57}\text{H}_{98}\text{O}_{12} + 37.75\text{CrO}_4^{2-} \rightarrow 57\text{CO}_2 + 37.75\text{Cr}^{3+} + 49\text{H}_2\text{O}$	1/38
Sulfate	$3\text{C}_{57}\text{H}_{98}\text{O}_{12} + 25.75\text{SO}_4^{2-} \rightarrow 57\text{CO}_2 + 25.75\text{H}_2\text{S} + \text{H}_2\text{O} + 44.5\text{H}$	3/26

2.4. *In Situ* Biostimulation Management

The treatment system for site A was implemented on-site of the contaminant (*in situ*) (Figure 1). Injection - and extraction trenches and boreholes, which is 100 meters apart, were used as the treatment site. Initially, the geological nature of the heterogeneous matrix between the selected boreholes was studied for the treatment implementation design. An EC profile of the injection borehole was created to determine the primary fracture depth. At this depth, a stoichiometrically balanced electron donor (Emulsified vegetable Oil - EVO) mixed with groundwater, was injected. This amended groundwater made a final solution containing 2% by volume. A subsurface groundwater pull was created, by withdrawing groundwater from the bottom extraction boreholes, to control the treatment hydraulic retention time (HRT).



Figure 1. *In situ* treatment implemented at site A, located in the KwaZulu Natal province of South Africa

2.5. iWater Bioreactor Plant

A pump-and-treat semi-active strategy was implemented at site B (Figure 2). iWaters' proprietary mobile treatment units consists of modular fix-filmed bioreactors (5 000 L, each) and treats 40 000 L per day. Inlet site water was continuously dosed with a balanced electron donor (acetate) mixture. All operations, in terms of electron donor dosing concentrations, redox conditions, and HRT were controlled remotely with a Program Logic Controller (PLC). The PLC also allowed for individual parameters (e.g., EC, flow rate and quantity, pH, and ORP) monitoring and recording. Once reducing conditions were established within the bioreactors, the electron donor was incrementally lowered until the minimum donor requirement was determined empirically. The final treatment stage involved removing any residual organic carbon through filtration methods (activated carbon).



Figure 2. Semi-active pump and treatment plant implemented at site B, located in the North West province of South Africa

3. Results and Discussions

3.1. Site Selections

It is crucial to understand each contaminated site’s geochemistry data to design an effective tailored bioremediation strategy [11]. The treatment focuses on creating an optimal environment for the indigenous bacteria to stimulate selected community members to reduce site pollutants. Site A contained fluctuating contamination levels of Cr⁶⁺ and nitrate of around 10 mg/L, and 35 mg/L, respectively. Site B contained similar concentrations of Cr⁶⁺ (8 mg/L) levels, but with additionally elevated concentrations of nitrate (110 mg/L) and sulfate (1 250 mg/L). The solid products obtained from the smelting of ferrochrome are metal, slag, and dust. This slag by-product is often disposed of at tailings storage facilities (TSF). Thus, if the TSF is not properly managed or lined it often result in Cr⁶⁺ leachate into the surface and underground water. In addition, a great environmental risk also lies in the sludge from the gas cleaning system. This is particularly the case for open-top furnaces where chromium is readily oxidised to Cr⁶⁺ in the off-gas dust (Tables 3, and 4).

Table 3. Geochemical dataset of inorganic parameters during *in situ* treatment for site A

Determinants	Units	Initial	30 days after injection		1 year after injection
			Injection borehole	Extraction borehole	Extraction borehole
Total alkalinity as CaCO ₃	mg/L	254	372	298	352
Total dissolved solids	mg/L	595	624	366	600
Total hardness	mg CaCO ₃ /L	171	286	114	314
pH		7.04	7.66	8.29	7.05
ORP	mV	+148	-10	-68	-15
Chromium as Cr ⁶⁺	mg/L	9.87	< 0.02	1.23	< 0.02
Nitrate as N	mg/L	35	< 0.35	< 0.35	< 0.35
Orthophosphate	mg/L	4.0	< 0.03	< 0.03	< 0.03
Sulfate as SO ₄ ²⁻	mg/L	120	129	133	38.7

Table 4. Hydrochemical dataset of inorganic parameters during a pump-and-treat plant on site B. The data represents the final parameters after one year of treatment

Determinants	Units	Plant inlet	Nitrate reduction strategy	Sulfate reduction strategy
			Final sample	Final sample
Total alkalinity as CaCO ₃	mg/L	229	692	1425
pH		7.85	7.95	7.36
Total dissolved solids	mg/L	3406	2798	3486
Total hardness	mg/L	1690	1480	257
ORP	mV	+87	-73	-325
Chromium as Cr ⁶⁺	mg/L	8.19	0.42	< 0.02
Nitrate as N	mg/L	110	0.61	< 0.35
Orthophosphate	mg/L	0.61	5.92	2.26
Sulfate as SO ₄ ²⁻	mg/L	1244	1213	66
Iron - total	mg/L	2.40	< 0.01	< 0.01
Potassium	mg/L	23	3.91	4.3
Zinc	mg/L	4.12	0.2	< 0.01

3.2. Microcosm Studies

It is important to understand the indigenous bacterial community's preferred electron donor type and concentration demand [5]. To determine this balance one can, ensure that enough energy is available for all the desired reductive metabolisms to function. Site A's indigenous bacterial community is more selective toward a complex long-chain carbon source, with minimum phosphate supplementation required, while site B's community prefers a simple carbon source, with balanced phosphate supplementation (Tables 5 and 6). Site B's consortia interestingly can also reach sulfate reduction if the donor concentration is increased, as it can improve this site's water treatment options.

Table 5. Microcosm study results of site A's indigenous bacteria oxygen demand from different electron donors

Electron donor	DO ₀ (mg/L)	DO ₅ (mg/L)	DO ₂₀ (mg/L)	Oxygen demand value
Control	3.40	3.34	-	0
Sodium acetate	3.37	0.99	-	0.48
Sodium acetate + Phosphate	3.48	3.23	-	0.19
EVO	3.48	-	0.08	0.75
EVO + Phosphate	3.45	-	0.23	0.68

Table 6. Microcosm study results of site B's indigenous bacteria oxygen demand from different electron donors

Electron donor	DO ₀ (mg/L)	DO ₅ (mg/L)	Oxygen demand value
Control	5.12	5.08	0
Sodium acetate	5.22	3.28	0.39
Sodium acetate + Phosphate	5.19	3.35	0.29
Glucose	5.17	4.14	0.17
Glycerol	5.17	4.68	0.08

3.3. On-site Treatment and Bacterial Composition

Treatment strategies of both sites were implemented as described in sections 2.4 and 2.5. Before and during the treatments, the change in bacterial diversities was analysed and the metabolic functionality could be correlated to water chemistry changes. At site A, the optimum redox conditions for nitrate, and consequently Cr⁶⁺, the reduction efficiency was reached after 30 days of treatment implementation. Due to the donor's balanced environment, the indigenous bacteria were able to reduce both Cr⁶⁺ and nitrate by 99% and the donor is still sufficient to maintain reduction after one year of treatment. By using the groundwater pull-push method, the electron donor was effectively migrated through the treatment site. In the initial phase of treatment, the extraction borehole showed delayed Cr⁶⁺ reduction due to the slower migration of electron donors, illustrating the importance of contact with the donor to stimulate the indigenous communities. One of the major advantages of using denitrification as a remediation process is the creation of water alkalinity, in the form of calcium carbonate (CaCO₃). Water alkalinity provides buffering capability against pH fluctuations, ultimately improving the hydrochemical balanced of the water source. During denitrification 1 H⁺ is used per 1 mol nitrate reduction to nitrogen gas. Thus 3.57 mg CaCO₃ is recuperated for every 1 mg nitrate reduced [12]. This is evident in the alkalinity generation during treatment of both sites (Table 3 and 4).

Before and after one year, the bacterial composition, up to the genus level, was evaluated between the different boreholes. All boreholes were initially dominated by *Acinetobacter* (39%), *Rhodobacter* (11%), *Prostheobacter* (8%), *Acidovorax* (8%) and other minor representative groups (MRC). Figure 3 shows shifts in bacterial communities throughout the treatment site throughout a one-year treatment. The treatment site is now mainly dominated by (i) *Pseudarthrobacter* (16%) and (ii) *Novosphingobium* (56% - aromatic compound degradation) between the injection, and extraction boreholes, respectively. What is of note is that both the injection and extraction boreholes had a dramatic increase in minor representative groups (54% and 30%, respectively). The data also shows that none of the genera co-exists between the different boreholes, suggesting no flow-through of the dominant bacteria through the site, however, Cr⁶⁺ and nitrate reduction is still evident in all the boreholes.

Interestingly, even though the communities of the separate boreholes are different after treatment, the functional annotation is more closely related, compared to the initial functionality of the communities. Nevertheless, after one year, the nitrate reduction functionality seems to be present in all the boreholes (> 5%).

At site B, optimum operations and CoC reduction were reached at an HRT of 12 h at 40 000 L per day treated. Using sodium acetate continued dosing, effective Cr⁶⁺ and nitrate reduction were achieved, with a 99% reduction of both parameters. Note that only specific redox metabolism is active in this balanced electron donor mixture, for example, nitrate respiration and denitrification.

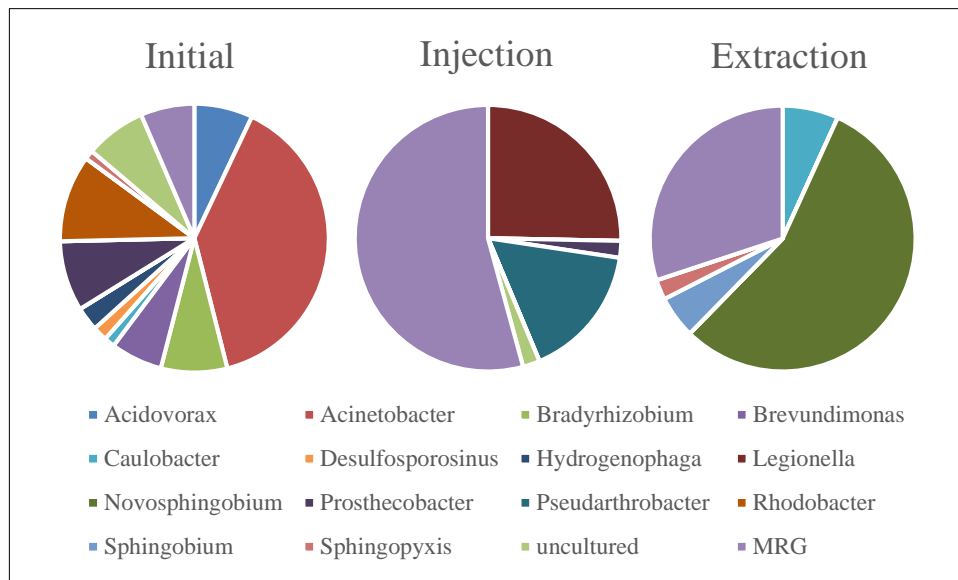


Figure 3. Taxonomic information (genus level) based on 16S rRNA gene sequences of the indigenous bacteria during Site A's treatment

In contrast, electron acceptors like sulfate are not metabolised. As an additional, to showcase the treatment strategy capability, the HRT tempo was lowered, and the electron donor ratio changed to achieve a 95% sulfate reduction from 1 244 mg/L (Table 8). During this treatment strategy, the overall balance of the water chemistry was improved where alkalinity was increased, and parameters such as total hardness, iron, potassium, and zinc were decreased.

Bacterial compositions of the treatment plant inlet water (In) and within the reactors (Out) were studied at a genus level. The inlet dominance of *Pseudomonas* (53%), *Exiguobacteria* (15%), *Rhizobium* (8%), *Stenotrophomona* (6%) change to *Pseudomonas* (65%), *Stenotrophomona* (3%), *Exiguobacteria* (2%), and interesting minor representative groups (13%), within the bioreactors (Figure 4). It is interesting to note that even though Site A and B's indigenous communities are similar, at the phylum level, it demands different electron donor compositions. This indicates the importance of the microcosm study to define treatment strategies for each site. The functional annotation analysis shows that the inlet water's bacterial community and those within the bioreactor are specialised for nitrate metabolic potentials (Figure 5). The fact that this functional annotation is accelerated within the bioreactors illustrates the importance of managing a balanced energy supply and that natural attenuation will struggle without any supplementation.

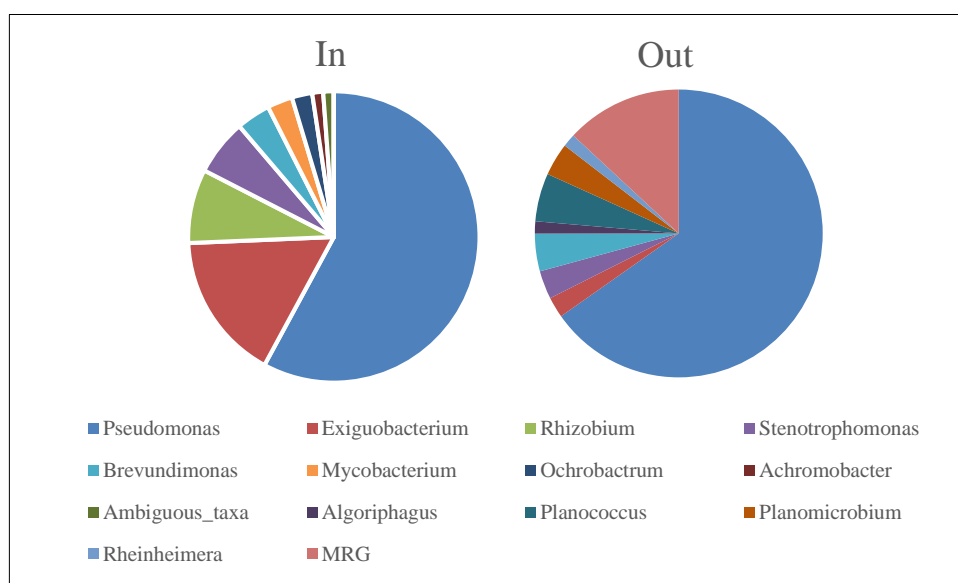


Figure 4. Taxonomic information (genus level) based on 16S rRNA gene sequences of the indigenous bacteria during Site B's treatment

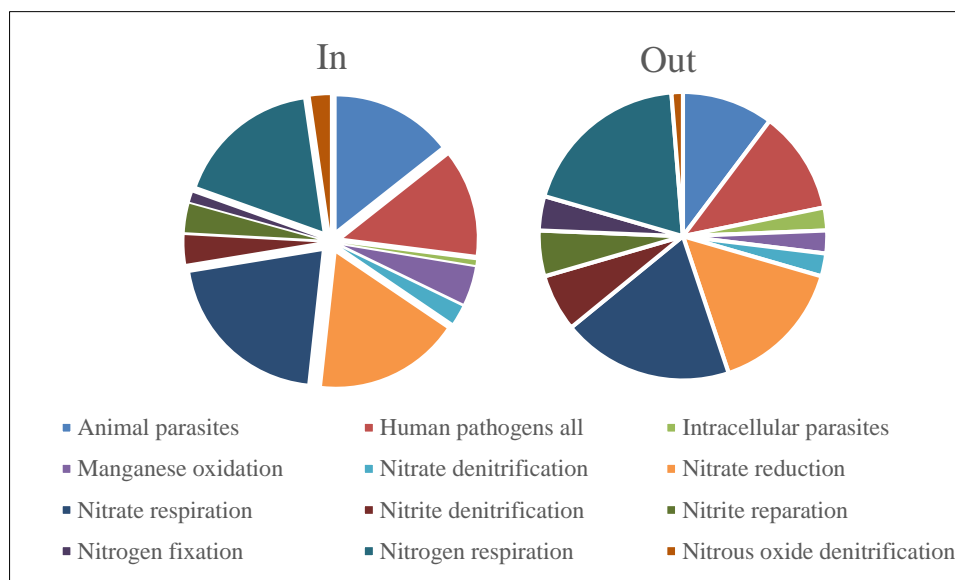


Figure 5. Functionality assignments of the indigenous bacteria during Site B's treatment

At Site A, there appears to be a flow-through of the minor representative groups, causing a specialised functionality towards nitrate, and consequently Cr^{6+} , reduction towards boreholes further away from the injection site. Whereas for site B, the data shows that minor representative groups are only present in the bioreactors. It is essential to mention that several studies confirmed that less dominant bacterial groups support the major communities [13-16] and that they are essential for the successful reduction of CoC. This means the minor representative groups, present at both sites A and B, should not be eliminated from the indigenous community composition and should be carefully managed. It might even indicate novel functions not described previously.

4. Conclusion

Anthropogenic activities on both sites have led to groundwater and surface contamination with CoC, mainly Cr^{6+} , nitrate, and sulfate. The data sets generated in this paper show that even if bacterial community compositions are similar, indigenous bacteria from different environments have different metabolic capabilities and electron donor requirements. This illustrates the importance of understanding the geochemical data and correlating it to the C:N:P ratios of the environment. By creating a stoichiometrically balanced environment that is correlated to the energy requirements of the indigenous bacterial communities, dominant bacterial species were allowed to naturally attenuate and ultimately effectively remediate sites. Interesting minor representative groups showed an important function to ensure that these dominant communities can successfully reduce CoC. This paper illustrates that adapted bacteria acclimate to every change in the physical environment and chemical composition. Thus, it is essential to identify all the informational gaps of each site to tailor the remediation strategy since no aspect of geochemistry, bacterial composition, and metabolic capacities stands alone. All the generated knowledge will optimise bioremediation strategies to extend treatment implementation beyond the CoC levels illustrated in this paper.

5. Declarations

5.1. Author Contributions

Conceptualization, G.P. and E.V.H.; methodology, E.V.H.; software, E.C.; validation, E.C., E.V.H., M.D., and K.J.; formal analysis, G.P.; investigation, G.P.; resources, E.V.H.; data curation, G.P., and E.C.; writing—original draft preparation, G.P.; writing—review and editing, E.V.H., and K.J.; visualization, E.C.; supervision, E.V.H., and M.D.; project administration, G.P.; funding acquisition, E.V.H. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

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5.5. Institutional Review Board Statement

Not applicable.

5.6. Informed Consent Statement

Not applicable.

5.7. Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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