Development of an Enhanced Bioreactor Cell for Coupling of Gas-Liquid Plasma Chemical Reactors with Bioreactors.

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Abstract: This study proposes a possible hybrid system that couples low-temperature plasma with a bioreactor containing *Pseudomonas putida* to achieve efficient pollutant remediation. The plasma reactor generates reactive species which break down complex contaminants into intermediates that are further degraded by the bacteria. The results highlight the potential of this integrated approach for sustainable wastewater treatment.

1. Introduction

Nonthermal plasma (NTP) technologies have garnered significant attention for their ability to generate highly reactive environments conducive to chemical transformations. In gas-liquid systems, such plasmas interact with liquids, often water, to produce reactive species, which can influence microbial activity and survival [1]. Microorganisms, with their metabolic diversity and resilience, present opportunities for novel applications when coupled with these plasma environments. This study explores the potential of integrating NTP with engineered *Pseudomonas putida* to enhance chemical transformations, focusing on the development of plasma-biological systems that combine abiotic plasma processes with biotic metabolic pathways. *P. putida* was selected for its robust oxidative stress resistance and capacity to degrade organic pollutants [2-3].

2. Methods

Three *Pseudomonas putida* strains were engineered: NCC2 (negative control cell) with kanamycin resistance only, EBC13 (engineered bioreactor cell) which overexpresses hydroperoxidase to enhance hydrogen peroxide degradation, and EBC14 which expresses GFP (green fluorescence protein) for visual confirmation of successful transformation and metabolic activity post-plasma reactor exposure. Formic acid and hydrogen peroxide degradation by NCC2 and EBC13 were assessed via HPLC and absorbance assays, while the viability of all three strains after plasma reactor transit at varying flow rates was evaluated using Live/Dead viability assays and flow cytometry. A nanosecond pulsed power supply manufactured by Eagle Harbor Technologies was used at 16kV (input voltage), 40ns (pulse width) and 10 kHz (pulse frequency) to generate nonthermal plasma.

3. Results and Discussion

Both the engineered *P. putida* strain, EBC 13, and the control strain, NCC2, demonstrated efficient degradation of hydrogen peroxide and formic acid. Both strains rapidly degraded ~8 mM hydrogen peroxide and ~25 mg/L formic acid within one minute, with minimal variation between replicates. These results highlight the strains' robust metabolic capabilities under these conditions. Furthermore, exposure to plasma-treated water also led to significant hydrogen peroxide degradation, indicating the strains' ability to handle oxidative stress induced by plasma treatment.

Strain	Transit Type		
	No Transit Live Cell Concentration (cells/mL)	Transit Plasma Off Live Cell Concentration (cells/mL)	Transit Plasma On Live Cell Concentration (cells/mL)
NCC2	7.27E+08	6.99E+08	5.21E+08
EBC13	7.76E+08	8.27E+08	5.60E+08

Table 1. Live cell concentrations for NCC2 and EBC13 at a 2 mL/min flow rate, show high viability maintained after transit with the plasma on.

Both EBC 13 and NCC2 exhibited high cell viability after transit through the plasma reactor, with the number of live cells significantly exceeding dead or mixed cells at both 2 mL/min (Table 1) and 5 mL/min flow rates. These results confirm that the strains can withstand the harsh conditions of plasma exposure while maintaining functionality, underscoring the resilience of *P. putida* in environmental stressors.

4. Conclusion

This study demonstrates the potential of *P. putida* as an effective microbial platform for coupling with plasma reactors to enhance the degradation of persistent pollutants and their byproducts. These results support the integration of *P. putida* with plasma-based technologies as a promising, energy-efficient solution for the degradation of environmental contaminants, offering a sustainable approach to addressing challenge of wastewater treatment.

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