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Metabolic pathway and role of individual species in the bacterial consortium for biodegradation of azo dye: A biocalorimetric investigation

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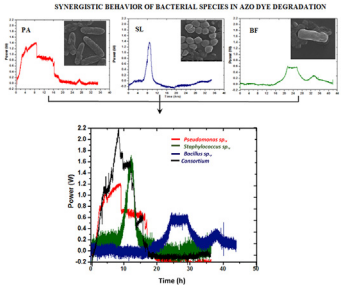
HIGHLIGHTS

- Functional role and metabolic behaviour of monocultures in biocenosis studied.
- Azo dye degradation of individual species and consortia compared.
- Power time profile of individual species was seen in consortium profiles.
- Synergistic relation and division of labour could be identified.
- The most abundant metabolism was palmitic acid and diethyl phthalate.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, an attempt was made to investigate the functional role and metabolic behaviour of the monoculture (*Staphylococcus lentus (SL), Bacillus flexus (BF)* and *Pseudomonas aeruginosa (PA)*) in the bacterial biocenosis for biotransformation of an azo dye. The power-time profile obtained from consortia depicted three distinct peaks, which correlated well with the individual bacterial growth (PA > SL > BF), indicating the synergistic relation and division of labour in the biocenosis. The heat release pattern was used to identify the sequential behaviour of microbial consortia in real time. Yield calculation based on total heat liberated to the complete substrate utilization $Y_{(Q/S)}$ for PA, SL, and BF were 15.99, 16.68, 7.32 kJ/L respectively. Similarly, the oxy calorific values $Y_{(Q/O)}$ for the above species are respectively 386, 375, 440 kJ/mol and indicates the aerobic nature of microorganism employed. Further, the metabolome produced during the biotransformation were identified using Gas Chromatography-Mass Spectrometry (GC-MS), based on which a plausible pathway was predicted. The abundant metabolites were palmitic acid (m/z = 256) and diethyl phthalate (m/z = 222.2). The abundance of diethyl phthalate was much lesser in the consortia compared to the monoculture. Thus, the biocalorimetric heat yield calculation along with the stoichiometry and plausible pathway based biochemical elucidation provides a mechanistic basis for understanding the azo-dye biotransformation by the monocultures in consortia.

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1. Introduction

Natural bacterial community interactions and their metabolism are always found to be interesting and are much to be explored. Bacteria are habitually found as communities, for their effective survival and growth they cooperate or compete with the surrounding bacterial species (Harcombe et al., 2014; Ghosh et al., 2016). Bacteria isolated from the native habitat can effectively perform an identical task in artificial laboratory condition mostly in the presence of a similar (environmental niche) microbial community (Diams et al., 2006; Deepak et al., 2016). Traditional bioprocess involves domesticated, highly engineered single species (superbug) but in nature, most of the reaction occurs in the presence of an another indigenous bacterial species (Stephen et al., 2016). Co-culturing methods hold a significant role in handling difficult tasks that are not possible by monoculture. This phenomenon is due to the symbiotic relationship between the microbial community, division of labour among the species and ability to withstand environmental fluctuations. The metabolic product i.e., a complex compound derived from a species is broken down into simpler biodegradable products by other species in the community (Brenner et al., 2008). In the recent years with the knowledge of synthetic biology and advanced analytical methods, the application of bacterial consortium in the field of industrial, medical and environmental bioremediation are studied (Ghosh et al., 2016; Zafraa et al., 2016). However, the metabolisms involved in co-existing species of microorganisms are not yet clarified (Stephanie et al., 2015). Biotreatment of Industrial dye effluent involving conventional single species is effective in pilot scale but mostly deteriorates in actual treatment scenario. Biotransformation of azo dye (acid blue 113) by the bacterial consortium has been taken as the model process as the reaction is well characterized in our recent paper (Bhuvanesh Kumar and Surianarayanan, 2015) and will be used to compare the result obtained with the monoculture while it metabolizes and degrades the complex azo dye. The challenge of creating a cooperative consortium is avoided, as the bacteria are mostly isolated from the similar ecological niche. It is reported that the mutual and synergetic interactions of the monocultures in consortia contributes for enhanced degradation or improve the efficiency of the bioprocess. Hence, knowledge on the type of interaction between the microorganisms in a consortium has to be understood for process optimization and their consistent performance in industrial applications. However, under optimized reaction conditions, the consortia will outperform its monoculture (Stephen et al., 2016).

Monitoring a bioprocess involving single species is complex, the complexity of the reaction increases with the addition of each species to the bioprocess; hence, a robust and reliable method is to be adopted to study the complex bioprocess. Heat dissipation is the universal factor in most of the biological reactions (Maskow and Harms, 2006: Rohde et al., 2016). Monitoring of heat flux in a bioreactor gives an overall idea of an active bioprocess. Bioreaction calorimeter (BioRC1e) was used to monitor the bacterial monoculture and its community, an accurate and reliable method for instantaneous monitoring and to study the insight happening of the bacterial community (Von Stockar and Marison, 1989). BioRC1e coupled with additional measurements as exhaust gas (CER & OUR), pH and turbidity will be helpful in justification and further correlation of the dissipated heat.

The aim of the present study is to articulate the role of individual species in the natural bacterial community, to identify the plausible metabolic pathway between species, and to study the mechanism involved in the biotransformation of an azo dye. By studying the role of individual species in a community, the reaction can be further engineered to do the desired task efficaciously. The result will be valuable in the identification of relationship among species in the bacterial community and to fingerprint the unique metabolism of monoculture on the consortium. The study will also signify the use of different heat flux pattern in predicting the species responsible for active metabolism in the bacterial consortium. A comparison of the dye degradation pattern and metabolic heat flux of the monoculture with its consortia will throw insights into the existence of mutual and synergetic behaviour in a combined environment.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and solvents used for these experiments were analytical grade (AR) purchased from RANKEM India Pvt Ltd, Bangalore. All the media used for microbiological studies were purchased from Hi-Media Pvt Ltd, Bangalore. The commercial azo dye (Acid Blue 113) ($C_{32}H_{21}N_5Na_2O_6S_2$) with a molecular weight of 681.65 was obtained from Sigma-Aldrich.

2.2. Bacterial strain

Three bacterial strains chosen for the study were *Pseudomonas aeruginosa* (PA), *Bacillus flexus* (BF) and *Staphylococcus lentus* (SL), where PA (MTCC 6458) & BF (MTCC 8487) were obtained from IMTECH Chandigarh, India. SL (NCBI JN673760) was isolated from tannery effluent collected from Central Leather Research Institute. The species PA is a gram-negative rod-shaped bacterium, BF is an endospore-forming rod-shaped gram-positive bacterium, and SL is a gram-positive cocci in the cluster.

2.3. Preparation of inoculum and media

Bacterial cultures were subcultured from 70% glycerol stock; a pure colony was chosen and incubated on a shaker for 24 h at 37 °C and at 100 RPM agitation rate. For monoculture reaction, inoculum concentration of 6% (v/v) was used for the reaction. Exponential phase of inoculum was used (absorbance at 600 nm ≈ 1) in all the reactions. The details of mineral salt media composition used are given in ES − 1, preliminary experiments were done in shaker flask; all the reaction parameters and condition were kept constant for all the three bacterial species, to simulate the experimental conditions of the consortium.

2.4. Biocalorimeter setup

All calorimetric experiments were made using Bioreaction calorimeter (BioRC1e) (Mettler- Toledo AG, Switzerland). A double-jacketed 2.2 L glass reactor with an improved metallic temperature regulated top plate to refrain external heat exchange. The reactor was operated in isothermic condition at 37 °C with 1.5 L of the mineral salt medium in all the experiments. Pure oxygen (0.66 VVM) is filtered through 0.2 μm membrane filtered and purged into the medium with an L-shaped sparger. An effective mixing of media components and uniform dissolved oxygen levels were maintained using a pitched Rushton turbine impeller assisted with four vertical baffles along the side of the reactor. All the experiments were made in duplicate with identical experimental conditions. Sample from the reactor is collected aseptically in even time intervals for offline analysis. The working and measurement principle of BioRC1e is reported previously (Leelaram et al., 2016).