PREPARATION OF CROSS-LINKED LACCASE AGGREGATES USING MILD CROSS-LINKING CONDITIONS AND THEIR APPLICATION TO THE ELIMINATION OF ENDOCRINE-DISRUPTING CHEMICALS

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INTRODUCTION

Enzymes are nature's sustainable catalysts, derived from renewable resources. Fabrication of cross-linked enzyme aggregates (CLEA), developed by Sheldon's group[1] is a valuable method for insolubilisation of enzymes improving storage and operational stability[2] and providing facile separation and re-use (Fig. 1). Laccases have gained increasing attention[3] as an attractive alternative for biodegradation, especially for the transformation of endocrine disrupting chemicals (EDCs). Here, we present the preparation, the optimisation of CLEA from laccase containing culture supernatant (*Coriolopsis gallica* HAI 1184) and its application in a lab-scale membrane reactor to continuously degrade bisphenol A (BPA).

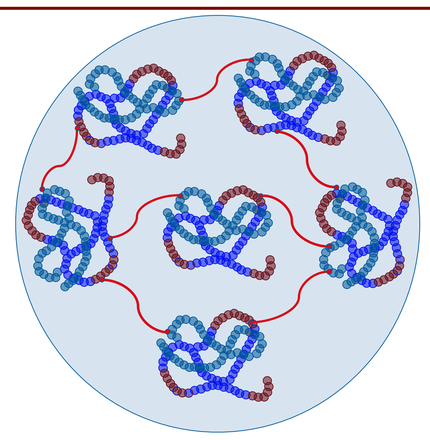

Fig. 1: Schematic representation of a cross-linked enzyme aggregate; the enzymes are covalently X-linked to form a robust and recyclable biocatalyst

OPTIMISATION OF CLEA PRODUCTION

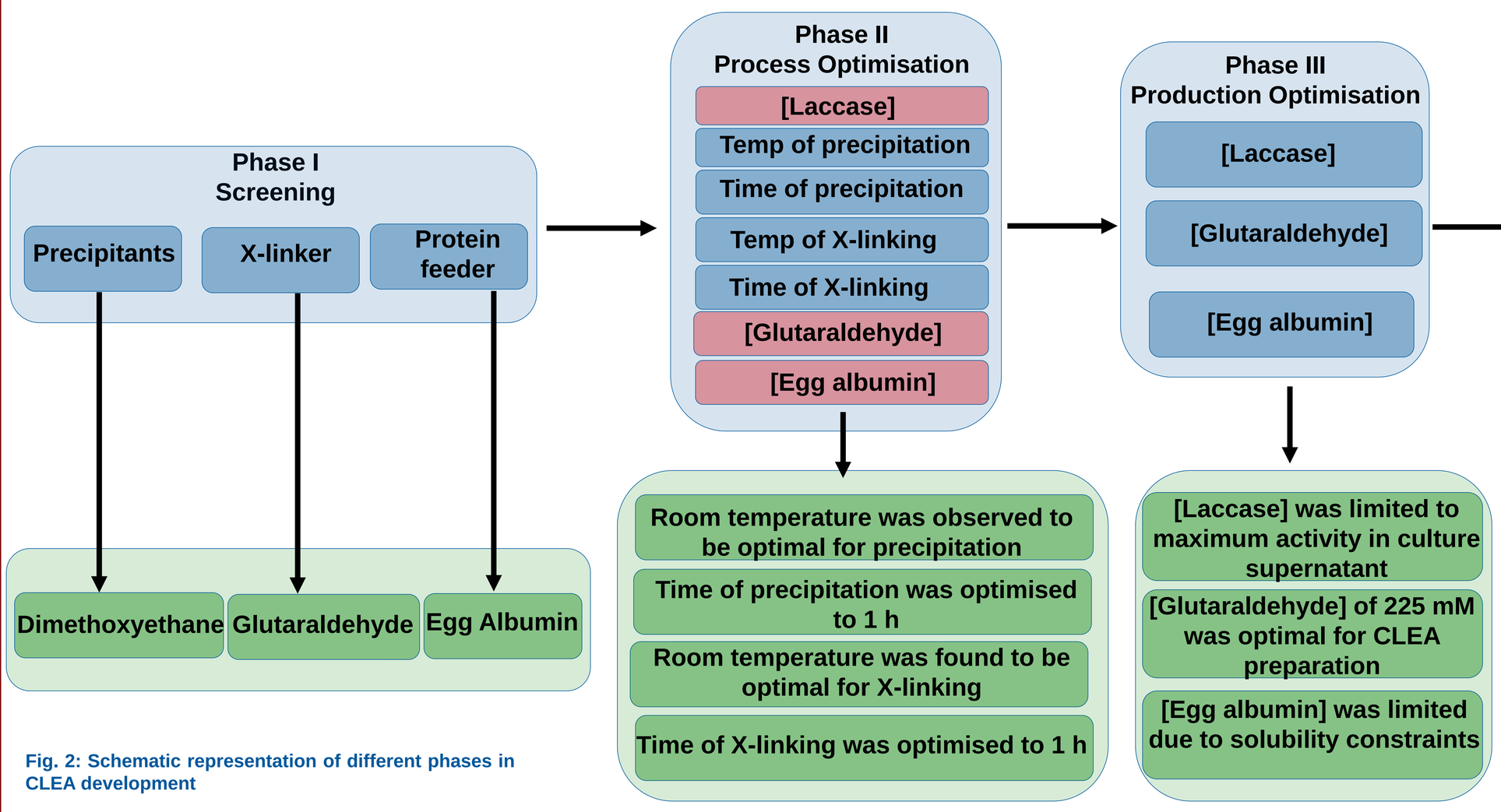

Fig. 2: Schematic representation of different phases in CLEA development

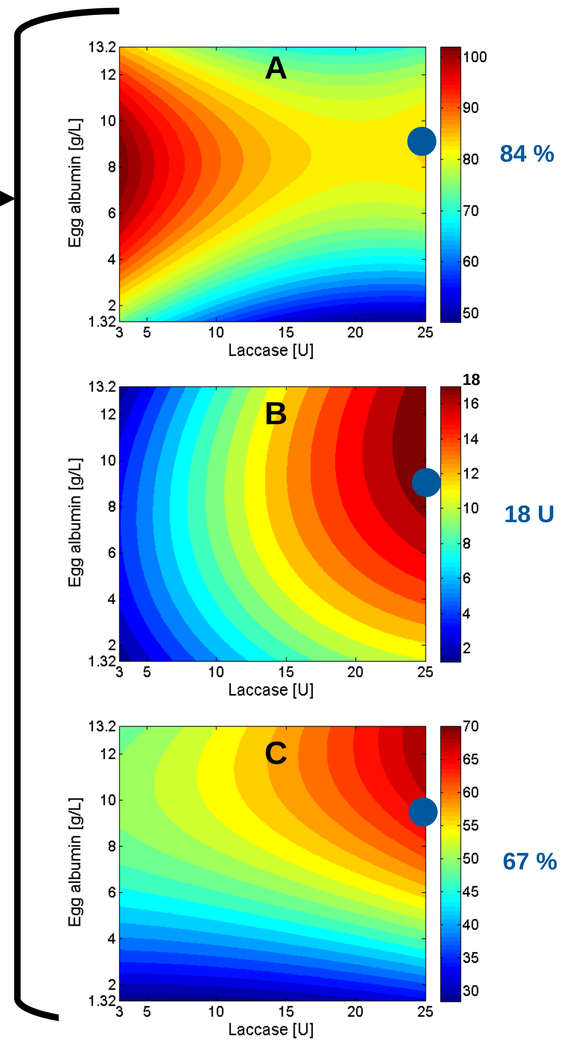

Fig. 3: Response surface plots for the activity recovery (A), CLEA activity (B) and yield (C) The blue spots represent optimised variable combinations obtained from multi-response optimisation. [Glutaraldehyde] (not shown) was set at the optimal level (225 mM).

The preparation of CLEA involves precipitation from aqueous buffer followed by cross-linking with a bifunctional reagent, usually a polyaldehyde such as glutaraldehyde or dextran polyaldehyde. Optimisation of CLEA preparations involves varying parameters (Fig. 2). Activity recovery, CLEA activity and stability was optimised following a central composite precision experiment (Fig. 3).

APPLICATION TO THE ELIMINATION OF BISPHENOL A

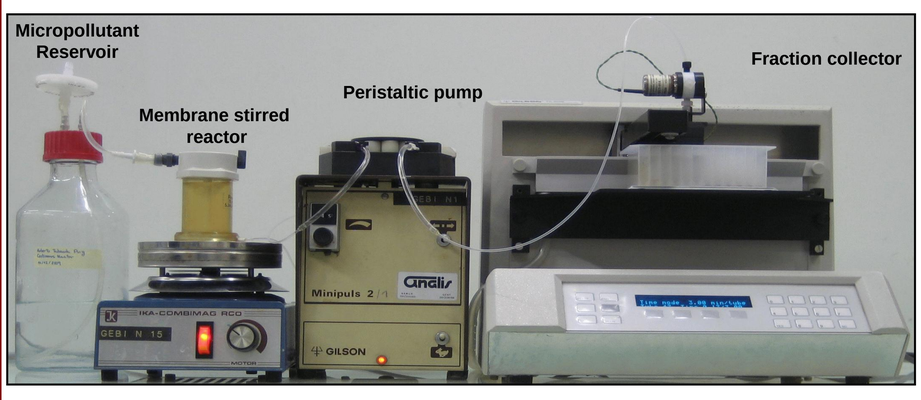

Fig. 4: Lab-scale membrane reactor (Millipore 8000 ultrafiltration cell, 50 mL, 0.2 µm polyvinylidene fluoride membrane) applied for the continuous degradation of BPA (25 µM in McIlvaine buffer pH 5.0). Flow rate was 0.45 mL/min, hydraulic retention time (HRT) was 111 min.

Optimised CLEAs were successfully applied in a membrane reactor (Fig. 4) for the continuous elimination of bisphenol A. Residence time of 1.85 h in the reactor allowed the **elimination of more than 90 % of BPA over 20 reactor volumes**. After this period, the biocatalytic activity was half the initial one and BPA concentration in effluent started to increase (Fig. 5). Less than 2 % of the applied activity was found in the effluent.

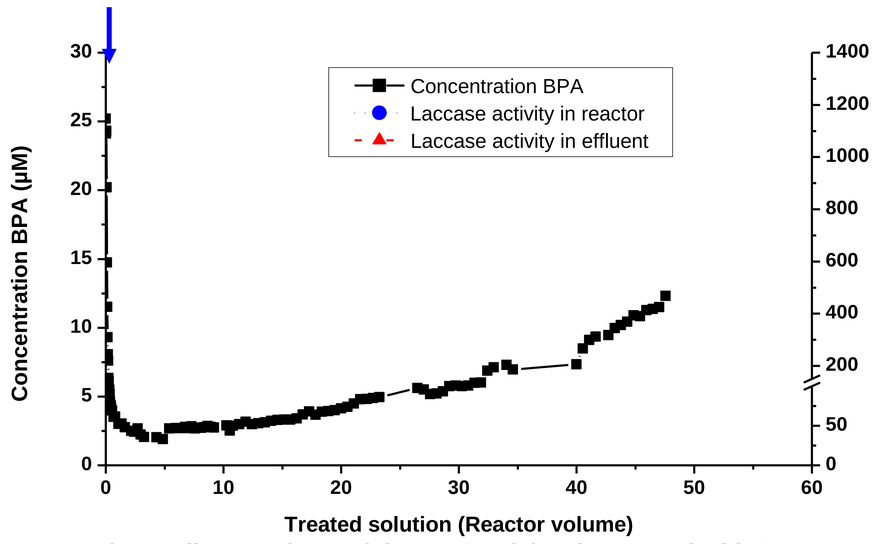

Fig 5. : Illustrated run of the reactor (Fig. 4) operated with 66.45 U of CLEAs, which were added after equilibration of the reactor (indicated by arrow). Please note break of activity axis to show effluent activity and activity in the reactor in sufficient detail. BPA concentrations were determined by HPLC.

SUMMARY AND OUTLOOK

Among other tested factors, concentration of laccase, the protein feeder (egg albumin) and cross-linker (glutaraldehyde) were identified to be most important for CLEA production. Predictive models allowed for discovering mild and optimal process conditions. The application of the optimised cross-linked laccase aggregates demonstrated the capability of these biocatalysts in the continuous degradation of EDCs.

References

1) Sheldon, RA (2011) Organic Process Research & Development, 15, 213-223

2) Matijosyte, I, Arends IWCE, Vries, S, Sheldon, RA (2010) Journal of Molecular Catalysis B: Enzymatic, 62, 142-148.

3) Cabana, H, Jones, JP, Agathos, SN (2007) Journal of Biotechnology, 132, 23-31.

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