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In-situ Product Recovery as a Strategy to Increase Product Yield and Mitigate Product Toxicity

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Abstract: Product inhibition is often the cause limiting the maximum product concentration attainable in fermentation. This study showed the product yield of *p*-cresol could be improved by *in-situ* product recovery (ISPR). *Escherichia coli* transformed with the *hpd BCA* operon from *Clostridium difficile* was shown in this study to express *p*-hydroxyphenylacetate decarboxylase which converted *p*-hydroxyphenylacetate into *p*-cresol under anaerobic fermentation. Toxicity of *p*-cresol found at a concentration as low as 5 mM in a broth spiked with *p*-cresol was shown to have limited the maximum product concentration at 1 ± 0.1 mM after 30 hours of batch fermentation. Product yield was however shown to increase by 51% when activated carbon was used to remove *p*-cresol *in-situ* production. The activated carbon concentrated *p*-cresol on the solid adsorbent which was subsequently separated by sedimentation and *p*-cresol recovered by ultrasonic-assisted solvent extraction. Desorption of *p*-cresol from the spent activated carbon allowed the adsorbent to be regenerated for further product recovery. The ISPR strategy reported here was shown to improve the yield of a toxic product, was sustainable, and when adapted to a continuous process would increase productivity.

Keywords: adsorption, *Escherichia coli*, glycyl radical enzyme, *in-situ* product recovery, *p*-cresol, product toxicity.

1. INTRODUCTION

Para-cresol (*p*-cresol) is an important intermediate for the production of antioxidants in food, personal care products, pharmaceuticals, materials, fuels, oils, herbicides, flavours, fragrances, dyes, resins, disinfectants and preservatives. Production of *p*-cresol by *Clostridium difficile* (*C. difficile*) was via the expression of the *hpd* BCA operon encoding the highly oxygen-sensitive Glycyl Radical Enzyme (GRE) *p*-hydroxyphenylacetate decarboxylase [1]. Although *C. difficile* was able to produce *p*-cresol, it cannot be used in large-scale production due to its virulence in causing life-threatening gastrointestinal infection in humans [2, 3]. This study therefore explored the possibilities of producing *p*-cresol by genetically modifying the Generally Recognised As Safe (GRAS) host *Escherichia coli* (*E. coli*) to express *p*-hydroxyphenylacetate decarboxylase, and to convert *p*-hydroxyphenylacetate (HPA) into *p*-cresol. The *hpd* BCA operon from *C. difficile* was cloned into plasmids which were then used to transform *E. coli* to produce *p*-cresol in whole-cell fermentation. Since *E. coli* was a facultative anaerobe and was most commonly used in recombinant DNA technology [4], it was postulated that the expression as well as the enzymatic activity of the GRE would be possible during anaerobic fermentation.

P-cresol was reported to be toxic to cells whereby the maximum concentration tolerable by *C. difficile* was reported at 35 mM [5]. It was this toxicity that attributed *C. difficile* its virulent characteristics [6], and rendered the bacteria a competitive advantage over other microorganisms in its natural habitat [7]. This study explored the possibilities of mitigating the toxicity imposed by *p*-cresol by implementing an ISPR strategy in order to improve product yield and productivity. The model with *p*-cresol would allow adaptation to other bioproducts, thus providing solutions to product inhibition and improve product yield [8-12].

Solvent extraction using a biphasic extraction system was reported in [13] for product recovery. [14] and [15] suggested the requirement for extraction solvents to have log P values of at least 4 in order to ensure biocompatibility and non-toxicity to the cells during product separation. Alkanes containing 7 carbons and above [16, 17], oleyl alcohol [18], dibutyl phthalate [19, 20] and ethyl laurate [21] were some common organic solvents used for product recovery. Although solvent extraction could be used for product separation, possible formation of emulsion during the product recovery step could result in subsequent difficulty in a clean phase separation. High energy consumption required in the final product purification step due to the high boiling points of extraction solvents, and the reported decrease in the growth rates of cells exposed to toxic solvents [17] rendered solvent extraction to be a non-ideal product separation and purification method.

[22] suggested adsorption to be a more viable separation method for biological products where polymeric resins were reported by [23] to be used for the ISPR of second-generation biofuels. Adsorption of *p*-cresol by different types of adsorbents in the applications of hemodialysis, wastewater

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treatment and as first-principle adsorption study was reported by various authors in Table **1**.

This study explored the feasibility of using activated carbon (AC) adsorption for the separation and recovery of *p*-cresol from fermentation broth which was subsequently recovered by solvent extraction. The decoupling of the solvent extraction process from fermentation via adsorption allowed the flexibility of using an extraction solvent with a lower

Table 1. Adsorption of *p*-cresol by Various Types of Adsorbents

Adsorbent	Adsorption characteristics	Application	Reference
High silica MFI zeolite	The adsorbent was able to remove 80-85% of *p*-cresol from solution, although the removal performance was reduced in the presence of serum.	Hemodialysis	[24]
Hypercross-linked polymer, bituminous coal activated carbon, phartenium-based activated carbon, fly ash, clay and silicalite	Maximum adsorption capacity was achieved with the hypercross-linked polymer at 1.8 mmol/g of polymer.	Adsorption of *p*-cresol from water.	[25]
Polyaniline-modified mesoporous carbon (CMK-1/PANI)	Maximum adsorption capacity for *p*-cresol was 1.5 mmol/g of adsorbent. Acidic-alkaline interaction between the amine groups on the surface of the adsorbent and the phenolic compounds was hypothesised to have improved the performance of the adsorbent.	Adsorption of phenolic compounds including *p*-cresol from aqueous solutions.	[26]
Crosslinked zinc chloride, chloromethylated poly(styrene-co-divinylbenzene) resin (HJ-1), and Amberlite XAD-4 resin	Adsorption capacities of *p*-cresol were reported at 1.3 mmol/g of HJ-1, and 0.6 mmol/g of Amberlite XAD-4.	Adsorption of *p*-cresol from aqueous solutions at 25 to 40 ^{o}C.	[27]
Amberlite XAD-4 and NDA polymeric adsorbents	Maximum adsorbate concentration was at 1.8 mmol/g of adsorbent. The basic functional groups and oxygen groups on the surface of NDA-99 were reported to enhance adsorption.	Adsorption study with different types of adsorbents.	[28]
Fly ash made from wood	Adsorption capacity for *p*-cresol was at 0.49 mmol/g of adsorbent.	The application of low-cost adsorbents for the removal of phenolic compounds from wastewater.	[29]
Sulphuric acid-treated parthenium-based activated carbon	Optimum adsorption capacity of *p*-cresol was reported at 0.56 mmol/g of parthenium-based activated carbon, compared to 0.78 mmol/g of commercial activated carbon.	Adsorption of *p*-cresol from aqueous solutions at pH 6. Maximum concentration of *p*-cresol used in solution was 1000 mg/L.	[30]
Cellulose diacetate and triacetate membranes, synthetic polyamide, polysulfone, polyacrylonitrile, polymethylmethacrylate, zeolite and silicalite	Adsorption performance of silicalite adsorbent was the best, where a 2-min equilibration time was adequate for the adsorbate uptake. The maximum adsorption capacity was 0.98 mmol/g of silicalite.	Hemodialysis	[31]
Spectracarb activated carbon cloth	Adsorption capacities of *p*-cresol were 1.9 mmol/g of activated carbon in water, 1.7 mmol/g of activated carbon in sulphuric acid solution, and 0.65 mmol/g of activated carbon in sodium hydroxide solution.	Adsorption of *p*-cresol at 30 ^{o}C in water, in 1 M sulphuric acid and in 0.1 M sodium hydroxide solution.	[32]
Adsorbents prepared from fertiliser waste	Adsorption capacity of *p*-cresol was 0.38 mmol/g of fertiliser waste adsorbent, compared to 0.85 mmol/g of commercial activated charcoal.	Adsorption of *p*-cresol in water.	[33]
Amberlite XAD-4 and chloromethylated styrene-divinylbenzene copolymer beads (NJ-8).	Adsorption capacities of *p*-cresol were 2.5 mmol/g of NJ-8 and 1.2 mmol/g of XAD-4.	Adsorption of *p*-cresol in water.	[34]

boiling point to separate and recover the product, thus enabling energy savings in subsequent product purification processes. Desorption of *p*-cresol from the spent adsorbent allowed the recovery and reuse of the adsorbent for further product separation and recovery. This study was divided into various phases in order to establish the toxicity levels of *p*-cresol on *E. coli*, to test and establish whether the productivity and yield of *p*-cresol could be improved by ISPR, and to determine the suitability and capacity of the integrated adsorption-cum-solvent extraction ISPR strategy in the recovery of *p*-cresol.

2. MATERIALS AND METHODS

2.1. Cloning

Genomic DNA was extracted from *C. difficile* 630Δerm as described in [35, 36]. The DNA was PCR-amplified, cloned into the modular vectors pMTL84151 and pMTL84251, and were then used to transform Top10 *E. coli*. The transformed cells were cultured aerobically from where the positive clones with chloramphenicol (CM) and erythromycin (EM) resistance were screened and isolated. The clones were maintained aerobically and were switched to anaerobic fermentation for the production of *p*-cresol.

2.2. Production of *p*-cresol Using Transformed *E. coli* Cells

Both CM and EM clones were maintained on Terrific Broth (TB) media containing 12 g/L Bacto-Tryptone, 24 g/L yeast extract, 4 ml/L glycerol, 2 ml/L Vishniac trace element solution, 20 ml/L $FeSO_4$, 4 g/L K_2HPO_4 and 2 g/L KH_2PO_4. The selective media was supplemented with either chloramphenicol at a concentration of 12.5 μg/ml, or erythromycin at a concentration of 500μg/ml, for the CM and EM clones respectively. The Vishniac trace element solution was made up of 0.1 g/L EDTA-disodium salt, 2.2 g/L $ZnSO_4$, 5.54 g/L $CaCl_2$, 5.06 g/L $MnCl_2.4H_2O$, 5 g/L $FeSO_4.7H_2O$, 1.1 g/L $(NH_4).6Mo_7O_{24}.4H_2O$, 1.57 g/L $CuSO_4.5H_2O$, and 1.61 g/L $CoCl_2.6H_2O$. The trace element solution was adjusted to pH 6 with KOH and stored at 4 ^oC until the solution was used. The cell stocks were frozen down in the same culture media formulation with the addition of 100 ml/L glycerol for cryopreservation.

The cells were maintained at 37 ^oC on agar plates and were cultured in liquid broth for the production of *p*-cresol. 100 mM of *p*-hydroxyphenylacetic acid stock solution was neutralised to pH 7 with 10 M of sodium hydroxide solution before it was used as a substrate for the production of *p*-cresol. The neutralised sodium *p*-hydroxyphenylacetate (HPA) stock solution was added to 20 ml of liquid culture media to the required final concentrations for the respective experiments. The culture medium and substrates were autoclaved and placed in the Don Whitley anaerobic workstation while hot. The solutions were left in the anaerobic workstation overnight for equilibration prior to inoculation the next day. Cells that were cultured to the mid-exponential growth phase in liquid culture media were used to inoculate the fermentation broth at 10% (volume by volume) (v/v). The culture universal bottles were capped and taped down with parafilm for anaerobic fermentation at 37 ^oC and 200 rpm. Samples were taken at regular intervals to determine culture growth, product formation and substrate consumption.

2.3. Analytical Methods

Culture growth was quantified by optical density (OD) measurement at 600 nm using the UV Mini 1240 Shimadzu UV-Vis Spectrometer. Colony forming units (CFU) were used to determine culture growth when AC was used in the experiments in order to avoid interference in the OD measurement caused by fines generated from the AC during agitation. CFU was determined by diluting the cell sample and spreading on agar plates. The agar plates were incubated at 37 ^oC overnight and the number of colonies was counted. The values of CFU/ml were then calculated, taking into account the dilution factors used in the experiments.

The concentrations of *p*-cresol and HPA were quantified using the High Performance Liquid Chromatography (HPLC) (Agilent 1200 HPLC) with an Agilent Eclipse XDB-C18 column, a detector at 270 nm, and a temperature controller at 25 ^oC. The mobile phase containing 70% (v/v) acetonitrile, 30% (v/v) water and 0.5% (v/v) acetic acid was used at 0.4 ml/min. The samples were taken from the fermentation broth and were centrifuged repeatedly at 6000 rpm for 10 min at 4 ^oC until no AC fines were left in the sample. 1 μl of the supernatant was injected into the HPLC for analysis. Samples that were not analysed immediately were frozen down to -20 ^oC and were thawed prior to analysis. Internal standards containing 20 μg/ml of nitrobenzene were used in the sample analysis.

The production of *p*-cresol in the fermentation broth was confirmed by qualitative analysis using the Agilent 7890A Gas Chromatography (GC) coupled to the Agilent 5975C Mass Spectrometer Detector (MSD). A HP5MS column with a N_2 mobile phase at 0.5 ml/min was used for the analyses. The GC program was set at 70 ^oC for 2 min, followed by a 10 ^oC/min ramp to 325 ^oC, and was held at 325 ^oC for 10 min. The samples were centrifuged at 6000 rpm for 10 min at 4 ^oC from which 3 mL of the supernatant was acidified with 0.2 mL of 0.1 M HCl. The acid-treated samples were extracted with an equal volume of dichloromethane and the organic phase was injected into the GC-MSD for analysis.

2.4. Recovery of *p*-cresol from the Fermentation Broth Using Activated Carbon

The AC used for the experiments were obtained from Sigma Aldrich. C3014 was wetted and equilibrated with deionised water at 1 g per 10 ml of water in the Certomat BS-1 Sartorius orbital shaker at 200 rpm and 37 ^oC for 24 h before autoclave. The sterilised AC was then neutralised to pH 7 with sterile 0.1 M HCl before it was washed to remove fines. The AC was then coated with Dextran, based on a modification of the method described by [37]. The coating solution consisted of 0.25 M sucrose, 1.5 mM $MgCl_2$, 10 mM HEPES, and 0.05% (weight by volume) (w/v) Dextran T-70 obtained from Sigma Aldrich. The coating was performed at 180 rpm and 30 ^oC for 3 days before the AC was washed and used for experiments.

2.5. Desorption of *p*-cresol from the Activated Carbon Using Ultrasonic-assisted Solvent Extraction

Solvent extraction was used to recover the *p*-cresol concentrated on the AC. Ethanol was selected as the extraction solvent based on its miscibility and good solubility with many chemicals including those containing the hydroxyl functional group. The extraction was performed batch wise with incremental volumes of 5 ml of solvent for the first 4 cycles of extraction, followed by 20 ml each for the subsequent 6 more cycles of extraction. The saturated AC was immersed with the extraction solvent in glass tubes, and was heated to 50 ^{o}C in the U300H Ultra Wave Heated Ultrasonic Bath before sonication at 44 kHz for 15 min. 1.2 ml of sample was taken from the extraction solvent and was analysed for *p*-cresol using the HPLC. The % cumulative recovery was calculated based on the amount of *p*-cresol extracted into the solvent with respect to the original amount of *p*-cresol pre-adsorbed on the AC.

3. RESULTS AND DISCUSSION

The *hpd BCA* operon was cloned from *C. difficile* and expressed in *E. coli*, where the expressed *p*-hydroxyphenylacetate decarboxylase was shown to convert HPA into *p*-cresol under anaerobic conditions (Fig. **1**). The production of *p*-cresol was correlated with the depletion of HPA in the fermentation broth, and reached a maximum concentration of 1 ± 0.1 mM with the EM clone. The CM clone showed a lower product concentration compared to the EM clone, and hence it was not used in subsequent experiments. Toxicity of *p*-cresol was observed to take effect on the cells at 5 mM of spiked *p*-cresol concentration as shown in Fig. (**2**). This toxicity was hypothesised to have caused the low maximum

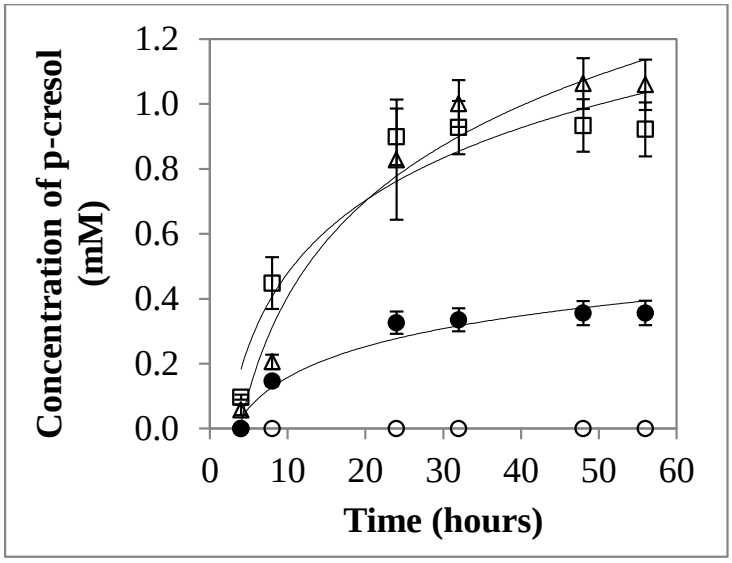

Fig. (1). Production of *p*-cresol by EM clone using various concentrations of HPA without AC. HPA was used at concentrations of: 0 mM (O), 10 mM (□), 50 mM (△) and 90 mM (•).

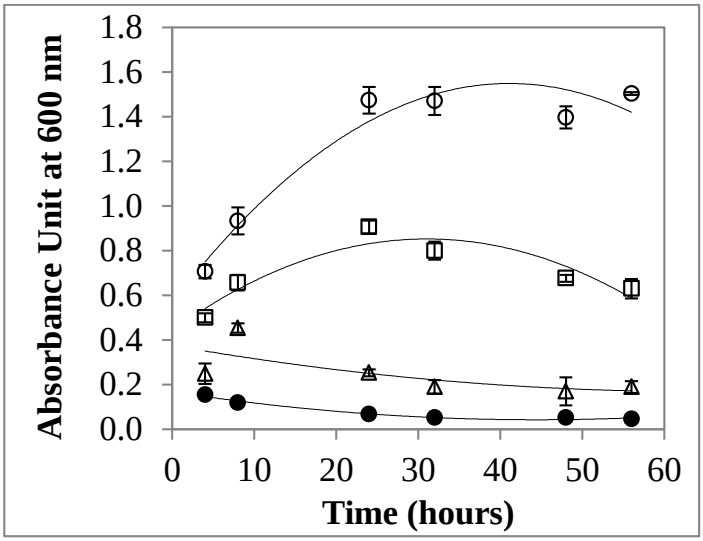

Fig. (2). Toxicity test of *p*-cresol on EM clone at various concentrations of *p*-cresol without HPA and without AC. *P*-cresol was used at concentrations of: 0 mM (O), 2 mM (□), 5 mM (△) and 10 mM (•).

concentration of *p*-cresol attainable in the fermentation as shown in Fig. (**1**).

Granular AC was used in the ISPR strategy to explore the possibility of increasing the productivity of *p*-cresol by removing the accumulation of the toxic product from the fermentation broth. The AC used in these experiments was commercially supplied to treat aqueous solutions, with applications in serum purification to remove steroid hormones. Since the AC was supplied to treat serum, it was postulated to be suitable for microbial cell culture without causing poisoning to the cells. Dextran T-70 was used to coat the AC in order to prevent any possible competitive adsorption imposed by nutrients in the fermentation broth. Experiment was conducted to determine whether there was any effect attributed by the coating on the adsorption of *p*-cresol.

Batch wise adsorption was performed at various concentrations of *p*-cresol in order to determine the performance characteristics and the adsorption capacities. A 500-mM concentration of *p*-cresol was used in the adsorption study, notwithstanding this concentration was above its solubility limit. This was to ensure that the AC has reached saturation during the determination of the adsorption capacity. A 3-phase mixture including a solid phase with 2 aqueous and organic phases was formed at the onset of the experiment with 500 mM of *p*-cresol. The 3-phase mixture became a 2-phase mixture as the adsorption progressed and as the AC adsorbed the *p*-cresol from the liquid phase. The adsorption of *p*-cresol by the AC was observed to be very rapid when 5 and 148 mM of *p*-cresol were used respectively. All of the *p*-cresol in the initial 5 mM concentration was taken up by the AC within the first 5 h of experiment. The adsorption capacities of both dextran-coated and non-coated AC were 2.6 ± 0.088 mmol/g of AC, where the Dextran coating showed negligible effect on the adsorption of *p*-cresol. The AC used in this study was shown to have comparable, if not higher, adsorption capacity compared to those of other adsorbents shown in Table **1**.

Solvent extraction assisted with ultrasonication at low frequency was used to recover *p*-cresol from the AC. The strategy was to exploit the cleaning function of ultrasonication to desorb *p*-cresol from the spent AC. Fig. (**3**) shows ethanol extraction of *p*-cresol with 10 batches of fresh extraction solvent. Extraction cycles 1 to 4 were done with 5 ml of ethanol each, while cycles 6 to 10 were done with 20 ml of the solvent each. 85% of the adsorbed *p*-cresol was shown to be recoverable into the extraction solvent at cycle 6, with a total of 60 ml of solvent used. It was hypothesised that strong interaction of *p*-cresol in the micro pores of the AC could have caused the remaining 15% of *p*-cresol to be non-desorbable.

Figs. (**4** and **5**) show the culture growth curve and the *p*-cresol production curve respectively for the EM clone. The cultures were inoculated with 10% (v/v) inoculum and were fed with 30 and 50 mM HPA respectively. Fermentation broth spiked with 5 mM of *p*-cresol (predetermined to be the lowest concentration of *p*-cresol causing observable cellular toxicity as shown in Fig. **2**), was used with AC to determine whether the presence of AC would mitigate toxicity and enable culture growth. Fermentation broth with AC but without HPA and without *p*-cresol was also used as experimental control to determine whether there was possible toxicity caused by the AC itself.

From the experimental results, the presence of AC in the fermentation broth did not cause toxicity to the cells. On the contrary, the expansion ratio of cells with AC but without HPA and without *p*-cresol was higher at 30.7 times as shown in Fig. (**4**), compared to 10 times when cells were cultured without AC, without HPA and without *p*-cresol as shown in Fig. (**2**). The expansion ratio of 30.7 times with AC in Fig. (**4**) was calculated by dividing the maximum CFU at 1.87×10^9 with the CFU at inoculation at 6.10×10^7, both normalised on per ml basis. The expansion ratio without AC in Fig. (**2**) was calculated by dividing the maximum OD at 1.50 with the OD at inoculation at 0.15, giving an expansion ratio of 10 times. In addition, the specific growth rate of cells with AC but without HPA and without *p*-cresol was observed to be highest at 0.12 /h; compared to the growth rate of cells cultured without AC, without HPA and without *p*-cresol at 0.034 /h; and the growth rate of cells cultured without AC,

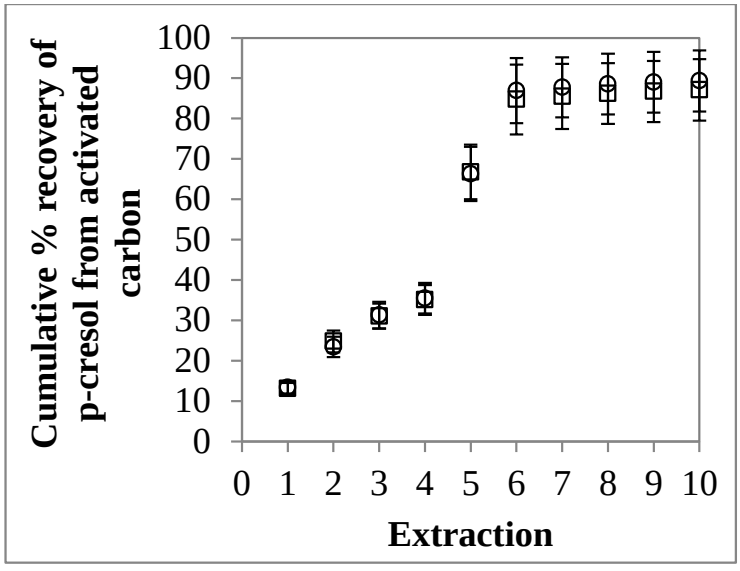

Fig. (3). Ultrasonic-assisted ethanol extraction of *p*-cresol from non-coated AC (O) and Dextran-coated AC (□).

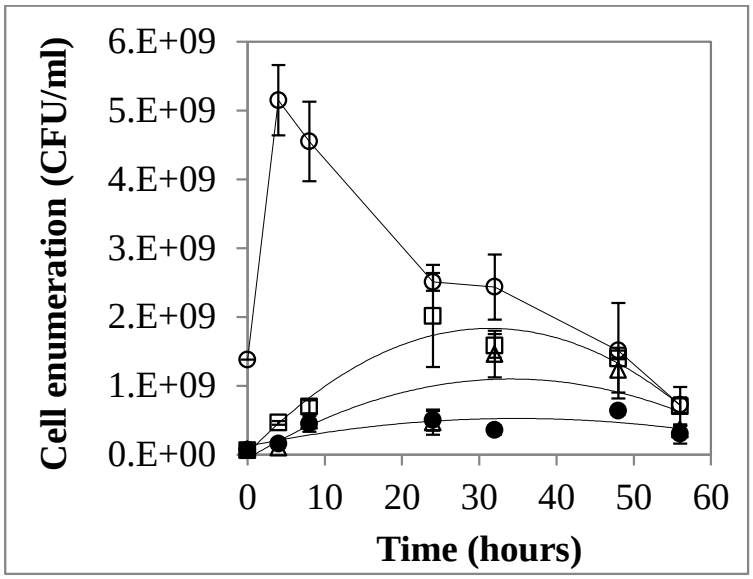

Fig. (4). Growth curves of EM clone at various concentrations of HPA with Dextran-coated AC. 5 mM of *p*-cresol was spiked into the fermentation broth as experimental control without HPA (O). Cells were cultured with various concentrations of HPA at: 0 mM as experimental control (□), 30 mM (△) and 50 mM (•).

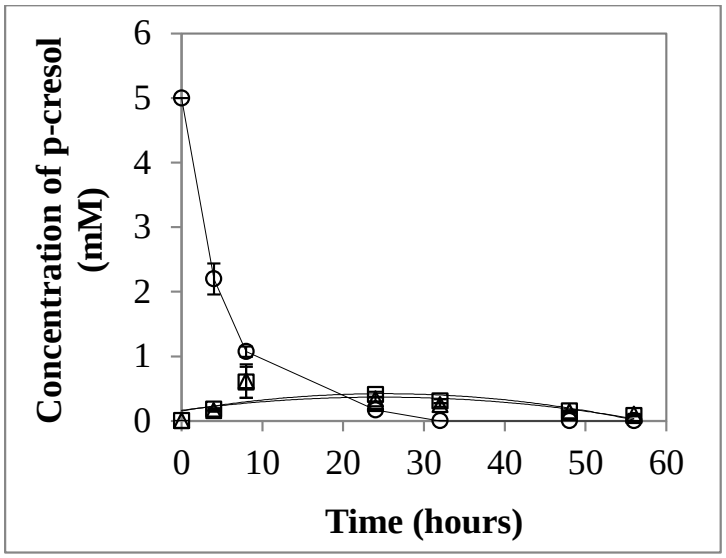

Fig. (5). Production of *p*-cresol by EM clone induced with various concentrations of HPA, and with Dextran-coated AC added to the fermentation broth. 5 mM of *p*-cresol was spiked into the fermentation broth as experimental control without HPA (O). Cells were cultured with various concentrations of HPA at: 30 mM (□) and 50 mM (△).

without HPA and with 2 mM *p*-cresol at 0.027 /h. Toxicity of *p*-cresol was observed to have reduced the specific growth rate of the cells, in agreement with the culture growth curves as shown in Fig. (**2**).

The presence of AC enabled the cells in the fermentation broth spiked with 5 mM of *p*-cresol to multiply, giving a higher CFU count of 4.79×10^9 CFU/ml compared to the initial 1.38×10^9 CFU/ml at inoculation as shown in Fig. (**4**). The concentration of *p*-cresol in the fermentation broth spiked with 5 mM of *p*-cresol was observed to reduce from the initial 5 mM to 0.58 mM by the 4^{th} h, and further decreased to zero by the 32^{nd} h as shown in Fig. (**5**). The removal of *p*-cresol from the fermentation broth by the AC was hypothesised to have reduced toxicity, thus enabled culture growth to reach a maximum CFU count of 4.79×10^9 CFU/ml at the 4^{th} h (Fig. **4**).

Product removal *in-situ* fermentation by AC was observed in this study to have mitigated product toxicity and allowed culture growth and continuous production of *p*-cresol as shown in Figs. (**4** and **5**). *P*-cresol produced by the EM clone was found to be 3605 µg from 50 mM of HPA in 20 ml of fermentation broth added with AC. 3396 µg of the total of 3605 µg of *p*-cresol was recovered from the adsorbent during solvent extraction, while the remaining 209 µg of *p*-cresol was detected in the fermentation broth at the time of sampling. On the other hand, 2386 µg of *p*-cresol was quantified in the fermentation broth without AC. This showed a

51% increase in *p*-cresol production was possible when AC was used to remove *p*-cresol from the fermentation broth. The prevention of the accumulation of the toxic product in the broth by ISPR allowed the cells to survive and continue production, thus enabling a higher yield to be achieved.

The ISPR strategy demonstrated here in batch fermentation constituted a methodology that could potentially be adapted into a continuous process. As the toxic product was removed from the broth, production could be changed from a product-inhibited to a substrate-limited process, thus enabling higher productivity to be feasible for a product that could otherwise be toxic to the cells. As the AC was produced from renewable materials, and was shown to be recyclable by solvent extraction, the ISPR methodology shown here constituted a production process that was low-cost and sustainable.

4. CONCLUSIONS

E. coli cloned with the *hpd BCA* operon from *C. difficile* was shown in this study to be able to express *p*-hydroxyphenylacetate decarboxylase and converted the HPA substrate into *p*-cresol under strict anaerobic fermentation. Product toxicity of *p*-cresol at a concentration as low as 5 mM determined in a separate experiment spiked with *p*-cresol was found to have limited the maximum product concentration at 1 ± 0.1 mM. Product yield was however observed to increase by 51% when AC was used to remove *p*-cresol *in-situ* fermentation. The adsorption capacity for *p*-cresol was determined at 2.6 ± 0.088 mmol/g AC. The accumulated *p*-cresol on the AC was shown to be recoverable by subsequent ultrasonic-assisted ethanol extraction. The decoupling of the solvent extraction process from fermentation via adsorption allowed a solvent with a lower boiling point to be used for product recovery. The ISPR strategy demonstrated here constituted a sustainable production process that could mitigate product inhibition and thus improve product yield.

ABBREVIATIONS

AC	=	activated carbon
C. difficile	=	*Clostridium difficile*
CFU	=	colony forming unit
CM	=	positive clone with chloramphenicol resistance
E. coli	=	*Escherichia coli*
EM	=	positive clone with erythromycin resistance
GC	=	gas chromatography
GRAS	=	Generally Recognised As Safe
GRE	=	glycyl radical enzyme
HPA	=	*p*-hydroxyphenylacetate
Hpd	=	*p*-hydroxyphenylacetate decarboxylase
HPLC	=	High Performance Liquid Chromatography
ISPR	=	*in-situ* product recovery
MSD	=	mass spectrometer detector
OD	=	optical density
TB	=	Terrific Broth
v/v	=	volume by volume
w/v	=	weight by volume

CONFLICT OF INTERESTS

The author(s) confirm that this article content has no conflicts of interest.

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