Production of 3-Hydroxypropanoic Acid Using Calcium Alginate Hydrogel Beads of Compartmentalized Microbial Cells

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ABSTRACT

3-Hydroxypropionic acid (3HP) is an important bio-based molecule and can be used for the conversion of acrylic acid, acrylic ester and amides. In current study, alginate hidrogel beads compartmentalized microbial cells consortia of mono and co-cultures *S. cerevisiae* strains system has been designed for 3-HP production using encapsulation technology. The compartmentalized *S. cerevisiae strains* within alginate hidrogel beads maintained 100% 3-HP production. The hidrogel beads prepared using 3.0% alginate polymer and 2.0% calcium chloride as a cross linking agent encapsulated máximum *S. cerevisiae* cells in term of higher 3-HP production. The co-culture encapsulated strains hidrogel beads showed higher production of 3-HP as compared to mono-culture strains encapsulated hydrogel beads system. The hidrogel beads with smaller beads size of 3.5 mm supported higher production of 3-HP as compared to hidrogel beads with larger beads sizes. The incubation period for 3-HP production was increased after encapsulation and encapsulated co and mono-culture strains produced maximum 3-HP after 48 hours of incubation as compared to free strains which produced maximum 3-HP after 24 hours of incubation. The co-encapsulation of *S. cerevisiae* strains within single bead improved the 3-HP production and co-encapsulated *S. cerevisiae* strains hidrogel beads with 1:2 ratio showed higher 3-HP production as compared to separated hidrogel beads. The encapsulated *S. cerevisiae* strains exhibited good reusability and storage stability properties, and retained 100% 3-HP production after 10 batch cycle fermentation and 30 days of storage, respectively.

Keywords: 3-Hydroxypropionic acid (3HP), *Saccharomyces cerevisiae*, encapsulation, mono and co-culture system.

INTRODUCTION

The evolution from pollution base fossil fuel living standard to a sustainable and fossil fuel free living standard using novel technologies for the production of chemical, fuel and energy is the fundamental requirement to tackle the current climate crises (Peter, 2018). The strategy of exploring the microbial cells for the production of chemicals and fuel from non-food-based biomass is on way for many years (Lee et. al., 2019). 3-Hydroxypropionic acid (3HP) have an important position in bio-based chemical platform which can be converted into acrylic acid, acrylic ester and amides, and can be sustainably used for the production for various materials such as super absorbent polymers, plastics, paints etc (Braga et. al., 2021; Kumar et. al., 2013). 3-HP is the structural isomer of lactic acid and a non-chiral three carbón containing organic compound. 3-HP with its carboxyl and hydroxyl group is very useful for inorganic synthesis chemistry (Jers et. al., 2019). 3-HP can be produced from acrylic acid, 3-propiolactone, 3-hydroxypropionitrile, ally alcohol, vinyl acetate and 1,3-propanediol through various kind of chemical synthetic methods, and among which the acrylic acids was considered as a significant substrate for chemical synthesis of 3-HP. The chemical synthesis of 3-HP is

economically and environmentally not feasible due to high cost of the chemical used for 3-HP synthesis and their toxic effect on human health and environment (Kumar et. al., 2013). Therefore, researchers are taking keen interest in biological production of 3-HP by identification and designing of new biochemical pathway to engineered the microbial strains metabolic pathways for biotechnological production of 3-HP (Fouchécour et. al., 2018; Chen and Nielsen, 2013).

METHODOLOGY

The compartmentalization of *S. cerevisiae* strains was done through encapsulation within calcium alginate hidrogel beads. The encapsulation process was performed by mixing overnight culture of *S. cerevisiae* strains with 1:5 sodium alginate solution (3.0%) and added drop wise into 2.0 M calcium chloride solution.
The following parameters were performed;

i. Mono and co-culture compartmentalized *S. cerevisiae* for the production of 3-HP.
ii. Production of 3-HP using spatial and mixed compartmentalized microbial strains hydrogel beads.
iii. Effect of compartmentalized microbial hydrogel beads size on the production of 3-HP.
iv. Effect of incubation period on the production of 3-HP.
v. Analysis of Solid-liquid interface co-cultures system for 3-HP production.
vi. Re-using of alginate compartmentalized mono and co culture microbial cells system for 3-HP production.

The concentrations of 3-HP were determined using HPLC (Agilent 1290 Infinity II DAD and RID). The samples were centrifuged at 10000 X g for 10 minutes and then the supernatants were filtered using 0.2 µm whatman syringe filters. The samples were eluted through 300 mm x 7.8 mm Aminex HPX-87H column at 65 ºC by using mobile phase of 2.5 mmol/L H_2SO^4.

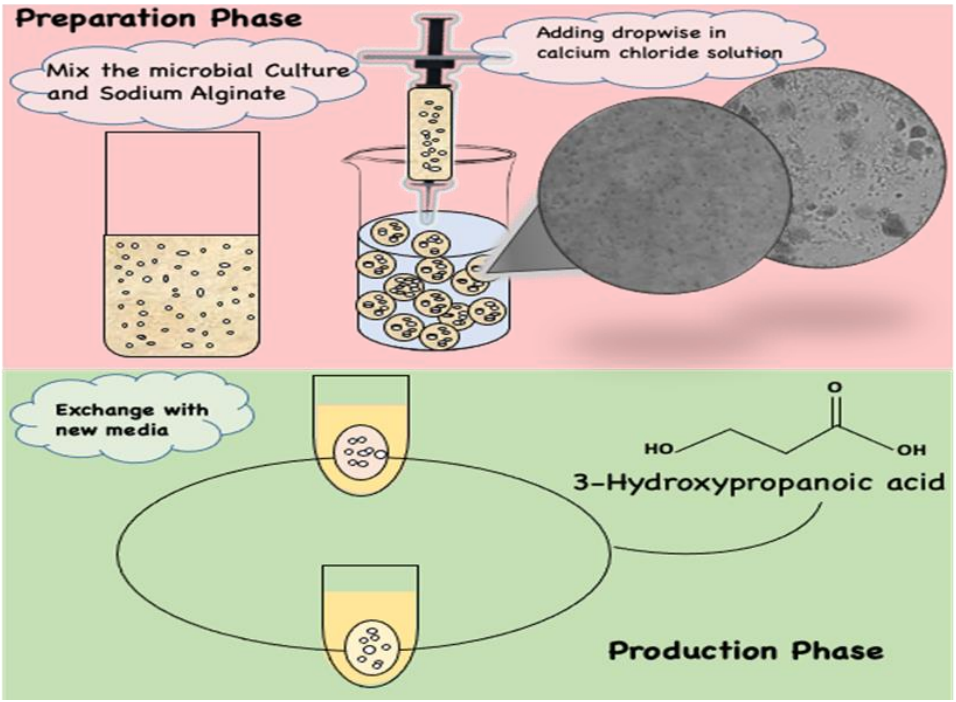

Figure. Overview of compartmentalization of *S. cerevisiae* strains experiments with control and continuous production of 3-HP. (a) encapsulation of microbial cells within alginate hidrogel beads. (b) utilization of these hidrogel beads for 3-HP production.

RESULTS / CONCLUSION

The *S. cerevisiae* strains retained 100 % of their metabolic activity for the production of 3-HP after encapsulation within alginate hidrogel beads with reference to free *S. cerevisiae* cells. The alginate encapsulated *S. cerevisiae* strains hidrogel beads prepared using 3.0% alginate and 2.0% calcium chloride showed high production of 3-HP. The microbial compartmentalized hidrogel beads with smaller sizes gave higher 3-HP production as compared to the hidrogel beads with larger bead size. The encapsulation of hidrogel beads increased the fermentation time of *S. cerevisiae* strains from 24 hours to 48 hours. The co-encapsulated *S. cerevisiae* strains in single hidrogel bead with 1:2 ratio gave 65% of high production of 3-HP as compared to the encapsulated strains in separated hidrogel beads. The co-encapsulated *S. cerevisiae* strains retained 3-HP production in continuous batch fermentation process and showed 100% of their residual activity after 10 time re-using in batch fermentation. The co-encapsulated *S. cerevisiae* strains retained 100% of biological production of 3-HP even after 30 days of storage.

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