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Optimization of critical factors to enhance polyhydroxyalkanoates (PHA) synthesis by mixed culture using Taguchi design of experimental methodology

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HIGHLIGHTS

- ► Taguchi DOE methodology was applied to optimize mixed culture PHA production.
- ▶ Microenvironment (80%) and pH (11%) showed significant contribution in PHA production.
- ► Glucose, phosphorous, nitrogen and VFA had significant interactive effects.
- ▶ Optimization improved PHA production by over 31%.
- ▶ PHA production through wastewater treatment significantly reduces the production cost.

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Optimizing different factors is crucial for enhancement of mixed culture bioplastics (polyhydroxyalkanoates (PHA)) production. Design of experimental (DOE) methodology using Taguchi orthogonal array (OA) was applied to evaluate the influence and specific function of eight important factors (iron, glucose concentration, VFA concentration, VFA composition, nitrogen concentration, phosphorous concentration, pH, and microenvironment) on the bioplastics production. Three levels of factor $(2^1 \times 3^7)$ variation were considered with symbolic arrays of experimental matrix [L₁₈–18 experimental trails]. All the factors were assigned with three levels except iron concentration (2^1) . Among all the factors, microenvironment influenced bioplastics production substantially (contributing 81%), followed by pH (11%) and glucose concentration (2.5%). Validation experiments were performed with the obtained optimum conditions which resulted in improved PHA production. Good substrate degradation (as COD) of 68% was registered during PHA production. Dehydrogenase and phosphatase enzymatic activities were monitored during process operation.

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1. Introduction

Biodegradable plastics production is gaining significant interest to overcome the accumulation of solid waste. Petroleum based synthetic/plastics are produced worldwide to the extent of around 140 million tons per year (Nampoothiri et al., 2010). Research has been developing for production of bioplastics without causing environmental pollution to replace oil based traditional plastics (Nampoothiri et al., 2010). Polyhydroxyalkanoates (PHA) are the biopolymers accumulated as storage granules in many organisms under excess carbon source and nutrient limited condition, and play a role as sink for carbon and reducing equivalents. Because of their production from renewable resources and their 100% biodegradability, PHA production is advantageous both economically and environmentally (Braunegg et al., 1998). Metabolism of PHA

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occurs via a cyclic process in which bacteria simultaneously accumulate and degrade PHA. Under carbon starvation, the accumulated polymers are degraded by intracellular PHA depolymerase. The ability to mobilize PHA is an important factor determining the survival of some bacteria in the absence of exogenous carbon source (Braunegg et al., 1998). Poly-3-hydroxybutyrate, (PHB) is the most common type of PHA studied extensively. The unfavorable characteristics of PHB such as high crystalline nature, stiffness, brittleness and very low strength limit its application as bioplastics (Lee, 1996). In contrast to homopolymer of PHB, copolymer poly(3hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV), is more ductile, easier to mold, tough and have better film forming and mechanical properties similar to polyethylene (Lee, 1996).

PHA production is four to nine times more expensive than conventional plastics due to its high production cost, which also includes substrate cost, cultures procurement and sterilization. PHA has been industrially produced by pure cultures with sugars such as glucose, sucrose and other sugar based compounds such





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