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(Mini Review)

FACTORS AFFECTING THE RHAMNOLIPID BIOSURFACTANT PRODUCTION

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ABSTRACT

Rhamnolipids are the best studied glycolipids having excellent surface activity. Their utilization in various application areas of environment, health, food, cosmetic, oil industry etc., have made it the potential candidates that could replace the chemically synthesized surfactants because these are derived from the natural source, in a pure form and they have low toxicity levels. The production of rhamnolipids dependent on several environmental and nutritional factors and the highest yield of rhamnolipids are estimated at 6 g/L with specific parameters. Effects of multivalent ions, nutritional factors and environmental conditions are described by many researchers to find out its enhanced production (Desai and Banat, 1997). In this mini review, some nutritional, environmental and compositional factors are studied and estimated that how the production of rhamnolipids enhanced and which kind of effects these factors have on its production.

INTRODUCTION

Rhamnolipids, a kind of extra-cellular glycolipids composed of L-rhamnose and 3-hydroxyalkanoic are produced by the *Pseudomonas aeruginosa.* Rhamnolipids were found for the first time in *P. pyocyanea* grown on glucose and was the first report of link between a sugar and a hydroxylated fatty acid (1). There is no any field, which is excluded in the application area of rhamnolipids. Increased demands and high cost of rhamnolipids compelled the scientists to increase its production and to find out the ways which affects its high yield. Researchers are focusing on the factors to enhance its production by changing the environmental conditions and parameters (2). Their wide applications make it an interesting candidate to find out its relationships in its maximum production.

Effect of Nitrogen: Nitrogen or metal ion-dependent regulation plays a prominent role in the synthesis of biosurfactants. The synthesis of rhamnolipids in *P. aeruginosa* is exhausted of nitrogen and commencement of the stationary phase of growth has been observed (3).

Effect of Multivalent: The limitation of multivalent cations also causes over production of biosurfactants. Iron limitation stimulates biosurfactant production in *Pseudomonas aeruginosa* (3).

Effect of Carbon Sources: Micro-organisms utilize a variety of organic compounds as the source of carbon and energy for their growth. Water soluble carbon sources such as glycerol, glucose mannitol and ethanol used for rhamnolipid production by *Pseudomonas aeruginosa* as mentioned in Table-1 (4).

Only glycerol behaved differently, as the rhamnolipid level decreased sharply when

glycerol concentration was over 2%. About 6-7% glycerol concentration yields very less rhamnolipid production. It is reported that 3% glycerol produce 2 g/L rhamnolipid with fermentation (5). Olive oil is excellent for rhamnolipid production with a max of 3.0 g/L at 10 % olive oil conc. The fermentation produce 2 g/L rhamnolipid when sunflower and grape seed oil 6% conc. was used as sole carbon source and 1400-1500 mg/L rhamnolipid produced with 6% glucose and 2 % glycerol. It is also reported that 1.3 and 2.1 g/L rhamnolipids are produced with 6 % and 5% conc. of diesel and kerosene oil used as carbon sources (2).

Nereus reported in 2005 (6), that on using glucose as a sole carbon source for growth, rhamnolipid production was 0.355 g/L. Cultivation of *P. aeruginosa* DAPUPE614 on glycerol and ammonium nitrate produced 3.9 g/L rhamnolipid after 216 hours (5).

Table 1: Effect of carbon sources on rhamnolipid production by *P. aeruginosa* (2, 4, 5)

Carbon sources	Rhamnolipid concentration g/L
Sunflower oil	4.9
Olive oil	5.4
Soy bean oil	4.8
Olein	4.5
Soapstock	12
Waste water	7.2
Glycerol	3.5
Manitol	3.9

Effect of Nitrogen Sources: Nitrates as a nitrogen source supports maximum surfactant production in *P. aeruginosa* (7). It is also observed that the nitrates are the best source of nitrogen for biosurfactant production (8). Addition of nitrogen source caused inhibition of rhamnolipid biosurfactant in the resting cells of *P. aeruginosa* (9). A number of investigators have demonstrated the overproduction of biosurfactant by *Pseudomonas aeruginosa* , when the culture reaches the stationary phase of growth due to limitation of nitrogen and iron (8).

Effect of Agitation: Agitation rate effects the mass transfer efficiency of both oxygen and medium components and is considered crucial to the cell growth and biosurfactant formation of the strictly aerobic bacterium *P. aeruginosa* especially when it was grown in a shake flask. As agitation rate increased from 50-200 rpm rhamnolipid production increased nearly 80 % and cell growth rate was also improved from 0.22-0.72/hr. The dissolved oxygen (DO) level in the batch culture increased from approximately 0.12-0.55 mg/L with an increased in agitation rate from 50-200 rpm, indicating that elevation of DO level seemed to have a positive effect on both cell growth and rhamnolipid production (10).

Effect of Temperature: There are limited researches on the temperature dependence for rhamnolipid production. The strain of *P. aeruginosa* was grown in salt medium at 25-47˚C to explore the influence of culture temperature on rhamnolipid production. Rhamnolipid production increased with temperature from 25 to 30˚C, remained nearly constant from 30 to 37˚C and decreased slightly when temperature was increased to 42˚C. *P. aeruginosa* was unable to grow at 47˚C leading to negligible rhamnolipid production at that temperature (10).

Effect of pH: Metabolism is pH sensitive because pH is the important factor that affects the chemical reactions of the living cells. It was observed that there is maximum production of biosurfactant at

pH range from 6 to 6.8 and decreases sharply when pH increases above 7.0 (3).

Effect of Phosphate: Triphosphate compounds are highly energetic compounds. Phosphate is very important for the growth of microorganisms. Cultivation of gram –ve bacterium on ethanol with low phosphate concentration yields maximum of rhamnolipids (11). A mutant strain of *P. aeruginosa* by using a mutagen, N-methyl-N-nitrosoguanidine on culturing produce 10 time more rhamnolipid than the parental stain at 200 rpm/37˚C (12).

Effect of Metals and Iron: The addition of metals to the medium for biosurfactant production with many bacteria does not effect on the biosurfactant production (13). Iron is the key microelement for biosurfactant production in several microorganisms. In addition iron play an important role as enzyme activator, specifically of isocitrate lyase, an enzyme involved in cell growth on hydrophobic substrates. This enzyme is essential for cell to deal with acetyl-CoA and convert it into a C4 unit during biosurfactant synthesis (14).

Iron is a common cofactor for microbial enzymes and protein and is an essential mineral. The microorganisms have developed a variety of strategies for acquiring iron while simultaneously protecting them from the potential toxicity of iron (15). The main strategies used by bacteria and fungi to acquire iron include producing and utilizing siderophores Ferric specific chelators using host iron compounds such as heme, transferin and lactoferrin (16).

Iron produce acidogenic fermentation behavior and involve in the over production of biosurfactant when used in a dose dependent manner (17). Iron has a dramatic effect on rhamnolipid production resulting in a three folds increase in production when cells were shifted from medium 35μM iron to medium containing 18μM of irons, under these conditions there was no change in biomass yield (3). Growth in high iron conditions represses the synthesis of siderophores iron-chelating agents as well as exotixin A, alkaline protease and elastase in *P. aeruginosa* (18).

Effect of Iron Salts: The highest yield of rhamnolipid of 3.81 g/L was observed when the medium of manitol was varied with 0.008 g/L of ferrous sulfate. The yield of rhamnolipid using 0.004 g/L ferrous chloride was estimated 1.85g/L (19).

FUTURE PERSPECTIVE

Rhamnolipids were discovered in 1949 and till now it is highly purified form 99.9 % and highest yield is achieved. These biosurfactants can work in extreme conditions, are non-toxic, and are themselves biodegradable. It can be used in oil spill management, to treat diseases like skin disorders, immune diseases, respiratory disorders etc. It can be applied in cosmetic industry, food industry and in environmental protection industries. There is no almost a field in which rhamnolipids cannot be applied. The worlds growing largest surfactant industry can be replaced by the rhamnolipids with the huge reduction in diseases. Rhamnolipids can be applied in food industries as emulsifiers, and can be used to prepare gold nano-particles. This can also be used to face and solve the challenges that we are facing presently in health sector.

In this review, the factors affecting the production of rhamnolipids are explained to date indicating that how many parameters are used to enhance its production. The efforts are ongoing for highest yield to overcome the increasing

demands. To open the gates of research on this molecule, this review summarized the factors to increase, decrease or controlled the growth that how can we achieve the highest yield of rhamnolipids in laboratory as well as in industry.

No doubt that rhamnolipids are the molecules of miracle and having tendency to perform almost all functions, which we are searching for our modern industry. This review will be a helpful for the new researches in the industrial biotechnology to apply these parameters and to find out the other undiscovered parameters to enhance the rhamnolipid bioproduction.

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