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Research Paper

Effect of toluene addition on pyocyanin production in the presence of different carbon sources

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Abstract

Pseudomonas aeruginosa is a Gram-negative, opportunistic bacterium and is one of the most biotechnologically important microorganisms. *P. aeruginosa* secretes an extracellular secondary metabolite known as pyocyanin, which is highly functional. In this study, the effects of toluene on pyocyanin production in the presence of different carbon sources in submerged culture of *P. aeruginosa* OG1 were investigated. It was determined that the addition of toluene to all carbon sources increased the production of pyocyanin. Maximum pyocyanin production was achieved when glycerol was used as the sole carbon source. With the addition of toluene, pyocyanin production generally increased, but bacterial biomass decreased. In addition, when glucose was used as the carbon source, the final pH decreased more than the other carbon sources. This study revealed that the addition of toluene to the fermentation medium significantly increased the production of pyocyanin in the presence of different carbon sources. These findings support that the solvent assisted fermentation strategy can be used in microbial fermentations to increase the production of industrially important biotechnological products such as pigments.

Keywords: Pseudomonas aeruginosa, pyocyanin, toluene, carbon sources, glycerol

1. Introduction

Secondary metabolites from microorganisms are useful sources of a diverse range of compounds suited for biotechnological applications. Pyocyanin is one of these compounds derived from the secondary metabolism of *Pseudomonas aeruginosa.* Pyocyanin is a nitrogen-containing ($C_{13}H_{10}N_2O$), water-soluble and phenazine derivative blue-greenish pigment produced mainly by *Pseudomonas aeruginosa* [1].

Pyocyanin is a molecule with both biological and biotechnological importance. Its biological functions include being a virulence factor [2], a quorum-sensing signaling molecule [3], a bio-control agent [4], and having antimicrobial activities against fungi and bacteria [5,6] and antioxidant activity [7]. In terms of biotechnological importance, pyocyanin has the potential to be used in many industries such as food, medicine, and cosmetics. In addition, pyocyanin is a redox active compound, making it useful in electrochemical biosensors [4].

Pyocyanin production is affected by many environmental conditions (temperature, pH, oxygen, and incubation time) and components of the production media (carbon, nitrogen, availability salts). It is also known that oxygen vectors [8], organic solvents [9], and nanoparticles [1] added to the fermentation medium also affect pyocyanin production significantly. The aim of this article is to investigate the effect of toluene on pyocyanin production by *P. aeruginosa* OG1 in the presence of different carbon sources. The effect of toluene addition on bacterial biomass and final pH changes were also determined.

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2. Materials and Methods

2.1. Microorganism and inoculum preparation

For the production of pyocyanin *Pseudomonas aeruginosa* OG1 stain was used. *P. aeruginosa* OG1 is an isolate capable of biodegrading endosulfan [10] and producing rhamnolipid [11]. Bacterial culture was grown on Nutrient agar at 30 ℃ overnight and a single colony was transferred into 50 ml Nutrient Broth medium in 250 ml Erlen Mayer for incubation at 30 ℃, 150 rpm for 24 h. From this preculture, 1 ml bacterial suspension corresponding to OD_{600} of 1 was prepared and used for the inoculation of 50 ml Nutrient Broth medium (Oxoid, USA) containing 1% glycerol, pH 7.2 in 250 ml Erlen Meyers for pyocyanin production.

2.2. Valorization of toluene addition on the production of pyocyanin

After 24 h incubation, 0.2% toluene was added to the production media in order to determine the effect of toluene on pyocyanin production [11]. No toluene (inducer) was added to the control cultures. Incubation was performed at 30 °C for 72 h. The cultures were centrifuged to obtain the bacterial cells at selected time intervals for measurement of the biomass and pyocyanin contents.

2.3. Effect of different carbon sources on the production of pyocyanin in the presence and absence of toluene

To determine the effects of different carbon sources (fructose, glucose, glycerol, lactose, sucrose, olive oil, and sunflower oil) on pyocyanin production, 1% (w/v) carbon sources were added to Nutrient Broth, in the presence and absence of toluene. The pH values of the culture media were adjusted to 7.2 before autoclaving and bacterial suspension was separately inoculated to media and incubated in a shaker incubator at 180 rpm and 30 °C for 72 h.

2.4. Measurement of biomass and pyocyanin

The amount of microbial biomass and pyocyanin were measured at regular intervals throughout the fermentation. Bacterial cultures were centrifuged at 6000 rpm for 10 minutes and the cells were harvested. Cells were washed several times (at least three times) with distilled water, and dried at 60 °C for 24 h and measured. For the measurement of pyocyanin content, cell free supernatant (2.5 mL) was extracted with chloroform (1.5 mL) and of 0.2 N HCl (0.5 mL). The optical density of the extracted supernatant was measured at 520 nm [11, 12] and the amount of pyocyanin was calculated according to the formula below:

$$\text{Pyocyanin (mg/L)} = A_{520} \times 17.072$$

3. Results and Discussion

3.1. Effect of different carbon sources on the production of pyocyanin

The effects of both carbon sources (10 g/L) and toluene (0.2%, v/v) on the production of pyocyanin were investigated and the results are given in Figure 1. As proposed by Ozdal [9], the reason for the increase in pyocyanin is may be due to the increase of protease enzyme as a result of the addition of toluene [9]. When toluene was added to culture media, it was determined that bacterial biomass decreased despite the increase in pyocyanin production (Figure 2). The highest pigment yields were obtained in the presence of glycerol (312%), olive oil (111%), fructose (101%) and sunflower oil (92%), respectively. It is already known that *P. aeruginosa* does not ferment lactose and for this reason pyocyanin production with lactose was low. Glucose exerted an inhibitory effect on pyocyanin production, probably because it lowered the pH of the medium. At the end of the fermentation, the pH of the medium containing glucose was 4.5 and 4.6 in the absence and presence of toluene, respectively (Figure 3). Another reason of reduced pyocyanin production may be the catabolite repression caused by glucose. Similar results also have been reported on prodigiosin production by *Serratia marcescens* [13]. According to these results, glycerol is the most suitable carbon source for pyocyanin production. There are other studies showing that glycerol is a preferable carbon source for the production of pyocyanin [14,15,16]. El-Fouly et al. [15] reported 5.8-9.3 mg/mL pyocyanin production by *P. aeruginosa* U3-R1 cultivated in Nutrient Broth medium supplemented with glycerol.
As a result, it was observed that the nutrient source, especially the type of carbon source, had a significant effect on pyocyanin production. It was determined that the production efficiency increased with the addition of the appropriate toluene concentration in the stationary phase of pyocyanin production. This strategy can be used to increase the productivity of many components produced by fermentation (pigment, biosurfactant, enzymes, etc.).

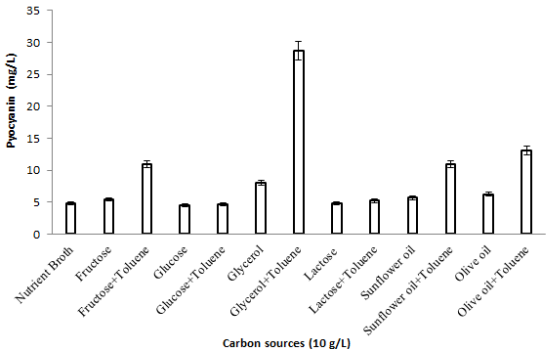

Figure 1. Effect of toluene on pyocyanin production

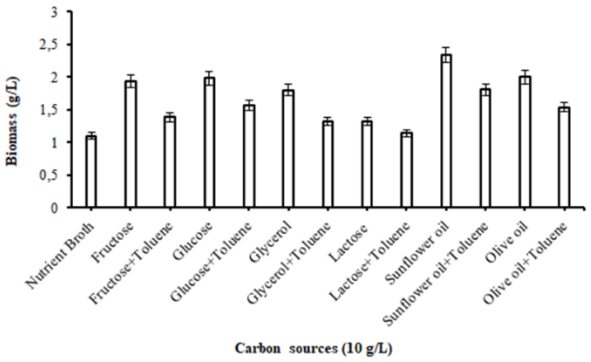

Figure 2. Effect of toluene on bacterial biomass

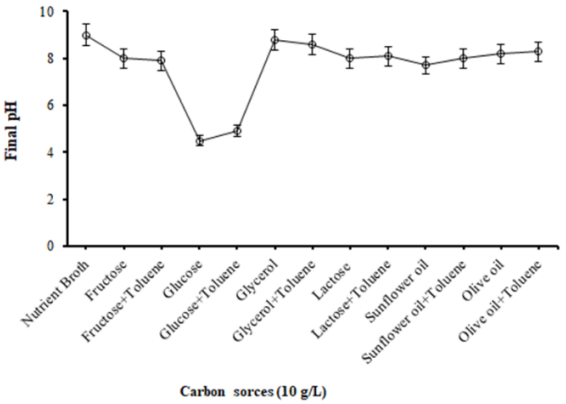

Figure 3. Effect of toluene on final pH

Conflict of Interest

The authors declare no conflict of interest.

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