



Effect of Castor Oil on Bioprocess Parameters of Erythromycin Fermentation by *Saccharopolyspora erythraea*

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Background: Increased economic competitiveness in the biopharmaceutical industry requires continuous improvement of bioprocesses. In this regard compositions of fermentation media have an important role in bioprocesses.

Objectives: The modification of the culture medium has proven effective in enhancing the yield and productivity of fermentation processes. The objective was to investigate the influence of castor oil as the main carbon source for *Saccharopolyspora erythraea*, on the yield of antibiotic fermentative production.

Material and Methods: The titer of erythromycin was evaluated in *Saccharopolyspora erythraea* cultures, containing various concentrations of castor oil, in comparison to the control culture containing rapeseed oil.

Results: The results showed an enhancement in erythromycin production when 50 g.L⁻¹ and 40 g.L⁻¹ of castor oil were added to the fermentation culture instead of rapeseed oil, respectively. The highest amount of production was obtained on the eleventh day of fermentation time in all media.

Conclusion: Erythromycin production in the control medium was relatively less than that of the treatments, indicating that *S. erythraea* consumed castor oil as a rich alternative carbon source. The results show that castor oil was more suitable as a carbon source for erythromycin production than a medium containing rapeseed oil.

Keywords: Castor oil, Erythromycin, Fermentative Production, *Saccharopolyspora erythraea*

1. Background

Antibiotics continue to be one of the major therapies against serious infectious diseases (1, 2). Increasing global competitiveness in the pharmaceutical industry renders continuous productivity improvement crucial for cost-effective delivery of pharmaceuticals, in particular antibiotics (3-5). Biopharmaceutical processing productivity relies on the efficiency of the fermentation process, which in turn is dependent on the composition of the culture medium (6-8). *Saccharopolyspora erythraea* has long been used for industrial production of macrolide antibiotic erythromycin (6, 9-11), which is a standard therapy for many infections (12). Apart from its clinical significance, erythromycin is a precursor for several widely used antibiotics, including Azithromycin, Clarithromycin, Dirithromycin, and

Telithromycin (13, 14).

Erythromycin is biosynthesized as a secondary metabolite by the polyketide synthases (PKS) system of the strain (1, 2) and manufactured through a multistage fermentation process (6,8,15-17). Several attempts have been made to enhance the titer of erythromycin through the optimization of fermentation culture and genetic manipulation of *S. erythraea* (18,19). These include investigating the possible effect of magnetite nanoparticles as an oxygen vector in fermentation media (16) and examining the efficiency of molasses (8), soybean oil (20), rapeseed oil (21), alginate (22), and propanol (23) on erythromycin yield. The present contribution to this ongoing line of research is to characterize the possible impact of Castor oil on erythromycin fermentation. Castor oil is a kind

of vegetable oils obtained from castor seeds (*Ricinus communis*). The motivation for such a study was the reported richness of Castor oil from Ricinoleic, 18-carbon fatty acid (24) which, according to previous literature may provide an efficient carbon source for erythromycin production (25). In addition to being a rich source of carbon, this oil was inexpensive and available in Iran so makes bioprocess more economical.

2. Objectives

In the present study, castor oil was used to optimize the culture medium through changing the carbon source to the more effective one on the growth of *S. erythraea* to increase the production of erythromycin and consequently reduce the cost of raw materials.\

3. Materials and Methods

3.1. Carbon Sources

Pure castor oil was provided from the Dineh Iran Industries Complex. Various concentrations of castor oil (30, 40, 50, 60 g.L⁻¹) were used as treatments. For the control medium pure rapeseed oil was obtained from Oila Food Processing Company in Iran.

3.2. Strains and Culture

S. erythraea PTCC 1685 was provided as a lyophilized form from Persian Type Culture Collection I 124 (Iran) to prepare the appropriate spores of *S. erythraea*. *Micrococcus luteus* ATCC 9341 was employed in the microbiological assay of the antibiotic produced.

3.3. Sporulation Media

Sterile media of corn steep liquor (CSL) Agar (pH =7±0.1) were used as sporulation media (16). The composition of these media is given in **Tables 1** and **2**. After 14 days of incubation, at 30 °C, white and brown spores were observed on the surface of the media. Sporulation suspension was prepared by adding Tween 80% (1 mL Tween 80 per 100 mL of distilled water) to spores.

3.4. Seeding Media

In the next stage, seeding media was used to achieve the mycelial growth of strain and having proper inoculum. The composition of seeding media is given in **Table 3**. One mL of sporulation suspension was added to a 1000 mL Erlenmeyer flask containing 100 mL of sterile seeding media (pH=7±0.1). The seeding flask was placed in a shaking incubator (Shinsaeng-Skir-601) at 30 °C and 0.788×g (6, 16, 19). To have the proper inoculum, after 48 hours some important parameters, including microbial contamination, mycelial growth of strain, biomass, and pH of the media were checked. As shown in **Figure 1**, the young mycelia of *S. erythraea* which were used as inoculum are thin and short.

3.5. Fermentation Media

The compounds used in this culture media are listed in **Table 4**. The control culture was prepared by adding 50 g.L⁻¹ rapeseed oil to the medium. In addition, four treatment cultures were developed by adding 30, 40, 50, 60 g.L⁻¹ of castor oil to the fermentation flask (C1, C2, C3, and C4 respectively). Fermentation was carried out in 1000 mL Erlenmeyer flasks, containing 250 mL of medium (pH=6.8 ± 0.1). After inoculating seeding

Table 1. Composition of CSL Agar Media (16)

Component	Amount
CSL	10 g
Starch [Merck]	10 g
(NH ₄) ₂ SO ₄ [Merck]	3 g
CaCO ₃ [Merck]	2.5 g
NaCl [Merck]	3 g
Agar [Merck]	20 g
Trace elements	2 mL

Table 2. Composition of Trace Elements (16,20,22)

Component	Amount
MgSO ₄ · 7H ₂ O [Merck]	100 g
FeSO ₄ · 7H ₂ O [Merck]	2 g
ZnSO ₄ · 7H ₂ O [Merck]	2 g
CuSO ₄ · 5H ₂ O [Merck]	0.4 g
CoCl ₂ · 6H ₂ O [Merck]	0.1 g
HCL 37% [Merck]	1mL
Distilled water	1000 mL

Table 3. Composition of Seeding Media (6, 10,16, 19,30)

Component	Amount
Soybean Meal [Max Soy]	30 g
Glucose [Merck]	10 g
Glycerol [Merck]	10 g
(NH ₄) ₂ SO ₄ [Merck]	3.5 g
(NH ₄) ₂ HPO ₄ [Merck]	1 g
CaCO ₃ [Merck]	5 g
Distilled Water	1000 mL

Table 4. Composition of Fermentation Media (6,10,16,19,30)

Component	Amount
Soybean Meal [Max Soy]	30 g
Glucose [Merck]	40 g
Starch [Merck]	30 g
(NH ₄) ₂ SO ₄ [Merck]	2.5 g
(NH ₄) ₂ HPO ₄ [Merck]	0.15 g
CaCO ₃ [Merck]	10 g
Rapeseed Oil [oila]	50 g
Distilled Water	1000 mL

culture into the flasks (5% V.V⁻¹) the cultures were placed in the shaking incubator at 33 °C and 0.788×g for 11 days. During the fermentation time, samples were taken for measuring the parameters such as strain morphology, pH, biomass, the erythromycin produced, and its biological activity.

3.6. Downstream Assays

The morphology and growth of *S. erythraea* were investigated with an optical microscope (NikonYS2-T). The pH of the fermentation samples was measured using a pH meter (Laboratory BenchtopAZ 86502). Biomass was estimated by calculating the ratio of the packed-cell weight to the weight of the culture medium, after centrifuging fermentation samples at 13.426×g for 20 min (Sigma3-30KHS) (6, 10, 19).

After removal of biomass from samples of fermentation broth by centrifugation (13.426×g and 20 min),

erythromycin extraction and quantification were carried out by solvent extraction and colorimetric method. The supernatant was diluted with 0.2 M carbonate bicarbonate buffer (pH = 9.6) and mixed with 10 mL of chloroform as a solvent per 10 mL of each sample. The extraction was performed once for each sample. The extracted erythromycin was mixed with the bromophenol blue reagent (bromophenol blue in ethanol and 0.2 M citrate-phosphate buffer, pH = 4.2). The formation of yellow colour at this stage means the production of erythromycin. Samples without microorganisms become colourless at this stage. The absorbance of the organic phase was measured at 415 nm by spectrophotometer (T80+UV/VIS) (6, 10, 16, 19, 22).

The biological activity of the produced erythromycin was evaluated by the agar well diffusion method which is widely used to evaluate the antimicrobial activity of

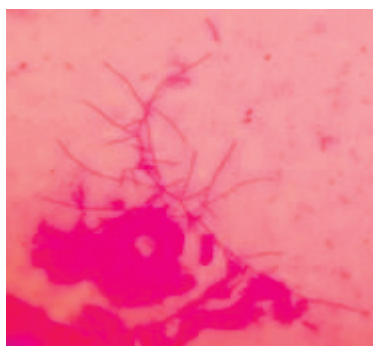


Figure 1. The young mycelia of *S. erythraea* as inoculum, forms after 48 hours incubation in 30 °C.

microbial extracts (2, 26, 27). To this end, *Micrococcus luteus* was cultured on an agar medium in a plate, three holes were punched on the surface of the agar medium with sterile pipettes, and then 100 μL of the produced erythromycin, with the same amount of standard of erythromycin and carbonate bicarbonate buffer, were introduced into three wells separately. After incubating for 24 hours at 35 ° C (10), the appearance of an inhibition zone indicates the biological potency of the produced erythromycin.

3.7. Statistical Analyses

All experiments were carried out three times and the data were the mean of three values. The obtained data were analyzed using the descriptive and inferential statistical method with Graphpad Prism software (version 9.1.0). Differences between mean values were tested by analysis of variance (two-way ANOVA) and Dunnett's multiple comparisons test. Significant

differences between groups and control were shown by asterisks on the graphs. *represents the significance level if any (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

4. Results

4.1. Effect of Castor Oil on Erythromycin Production

In this study, various concentrations of castor oil (30, 40, 50, 60 g.L^{-1}) were used in the fermentation media of *S. erythraea*. **Figure 2** presents the titer of erythromycin against various concentrations of castor oil compared to the control media containing 50 g.L^{-1} rapeseed oil. As shown, the two highest titers of erythromycin were obtained in media containing 50 g.L^{-1} and 40 g.L^{-1} castor oil respectively. All media show an increased production from days 5 to 8 and from days 8 to 11.

4.2. Microbiological Assay of Produced Erythromycin

The inhibitory effect of the produced antibiotic on the

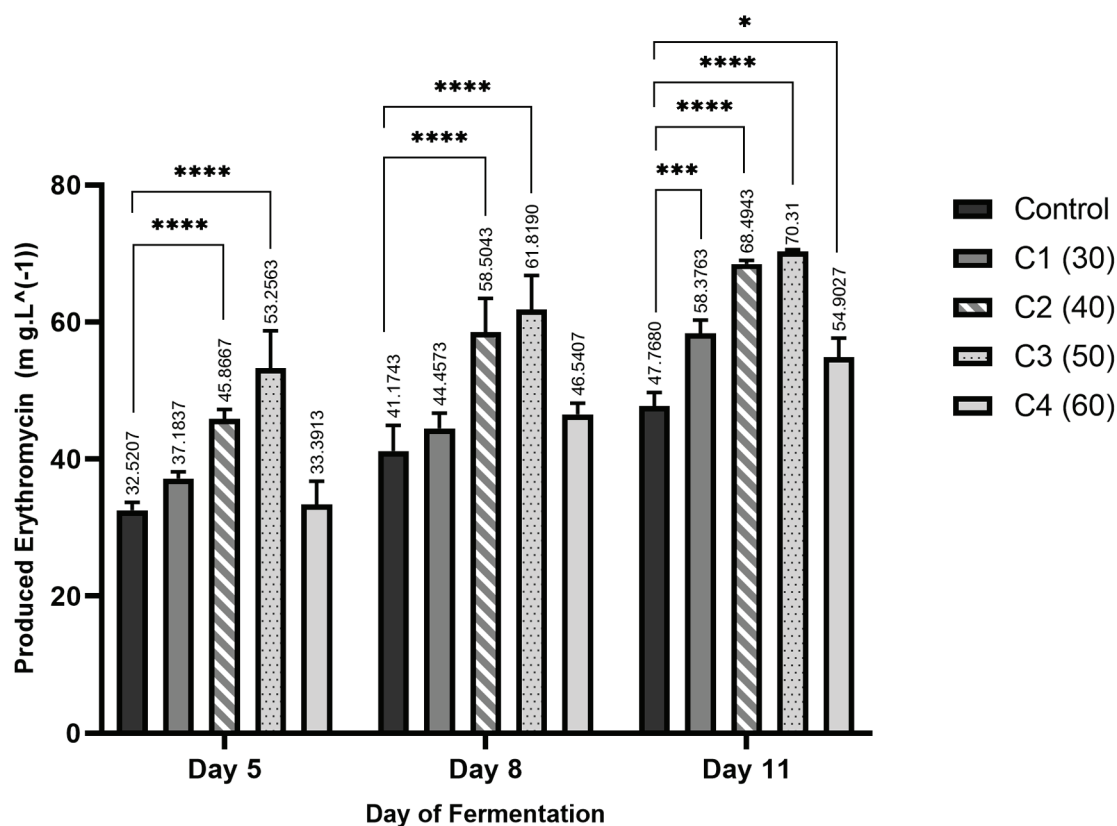


Figure 2. Effect of various concentrations of castor oil on erythromycin production by *S. erythraea* PTCC 1685. Each experiment was performed in three batches and the data given in the graph are the mean of three repetitions. Asterisks indicate significant differences between groups and control. * represents the significance level if any (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

growth of a susceptible strain of *M. luteus* ATCC 9341 was examined by the agar well diffusion method (26, 27). As shown in **Figure 3**, the inhibitory effect of the produced antibiotic is comparable with that of the

standard erythromycin.

4.3. Effect of Castor Oil on pH of Media

The pH of the culture media is an indicator of microbial growth. Initially the pH of fermentation media was

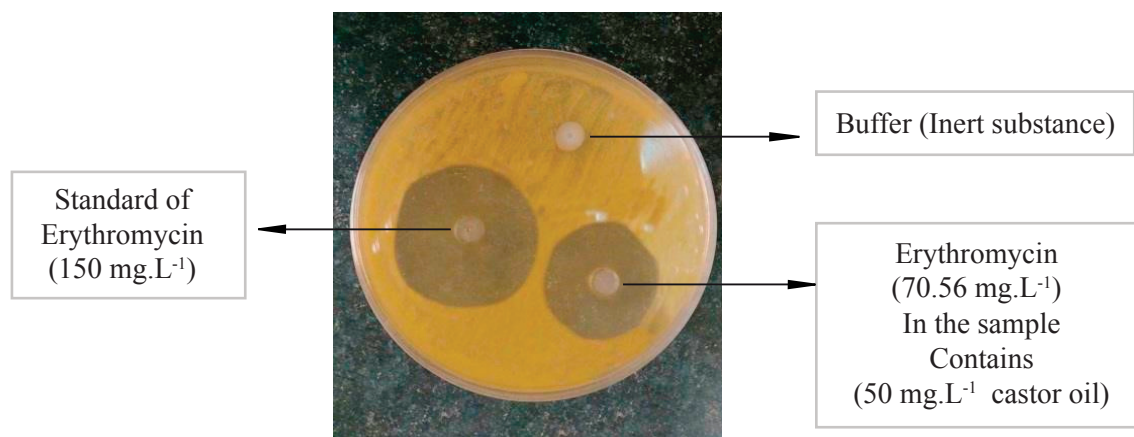


Figure 3. Inhibition zone in agar well diffusion method indicates the biological potency of the produced erythromycin. (The higher the zone, the greater the biological activity of the antibiotic). Around the well filled with buffer, no inhibition zone can be seen.

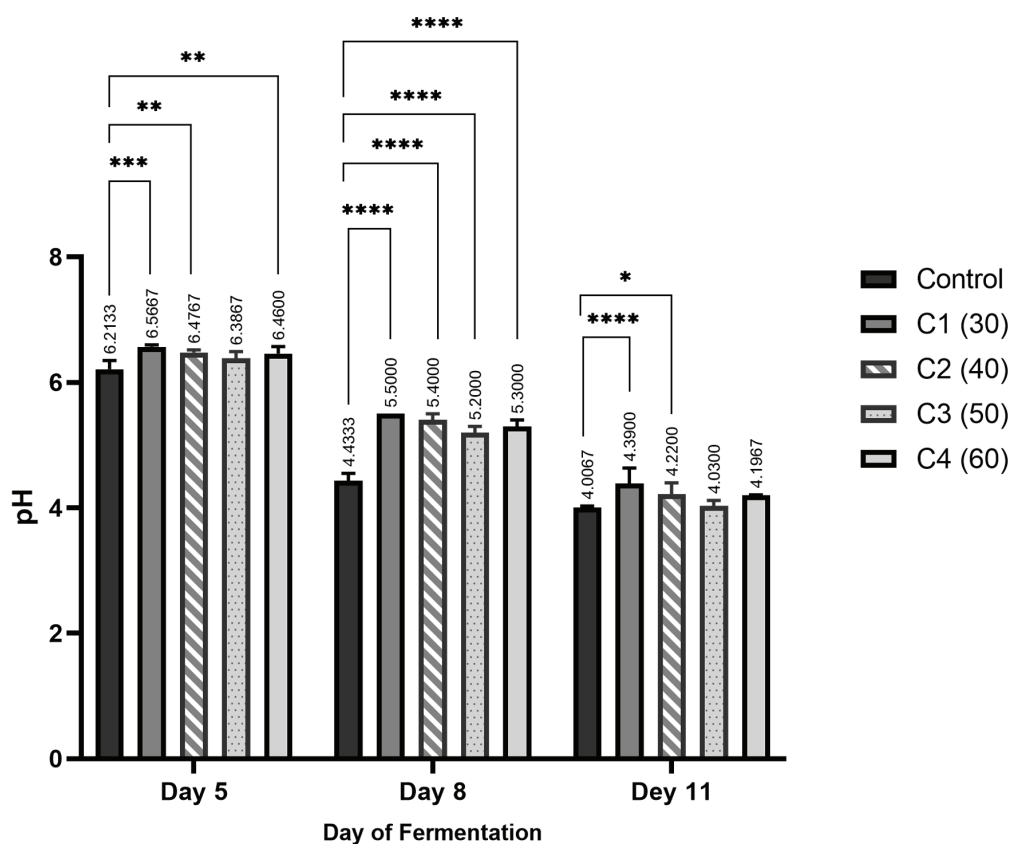


Figure 4. Effect of various concentrations of castor oil on pH of the fermentation media in the process of erythromycin production by *S. erythraea* PTCC 1685. Each experiment was performed in three batches and the data given in the graph are the mean of three repetitions. Asterisks indicate significant differences between groups and control. * represents the significance level if any (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

set to 6.8. As **Figure 4** illustrates, the pH follows a downward trend as fermentation proceeds, independent of the concentration of castor oil. The lowest amount of pH was obtained on day 11 of the process which is significantly lower than that at day 5 in all media, corresponding to the higher titer of erythromycin.

4.4. Effect of Castor Oil on Microbial Biomass

Figure 5 shows the biomass production vs. fermentation time for different treatments. While the highest amount of biomass is observed on day five in all, the difference between different treatments was not significant on day 11, which could be explained by the notion that the corresponding measurement was carried out in the stationary phase of *S. erythraea* growth (28). The decreased biomass production from the fifth to the

eleventh day was corresponding to the reduced length and thickness of the mycelia.

4.5. Morphology of *S. erythraea* During Fermentation

S. erythraea develops mycelium during growth. Morphological changes of *S. erythraea* during the fermentation period are shown in **Figure 6**. Mycelia were observed to be long and convoluted at day five of all treatments. As the fermentation proceeded, mycelia were found to lose their size and density. On day 11, most of the mycelia was broken and separated into small pieces, due to cell wall lysis. As shown in **Figure 6** mycelia in castor oil-containing media were larger and thicker than those in control medium. A higher amount of erythromycin was tittered when the mycelia was shorter and thinner.

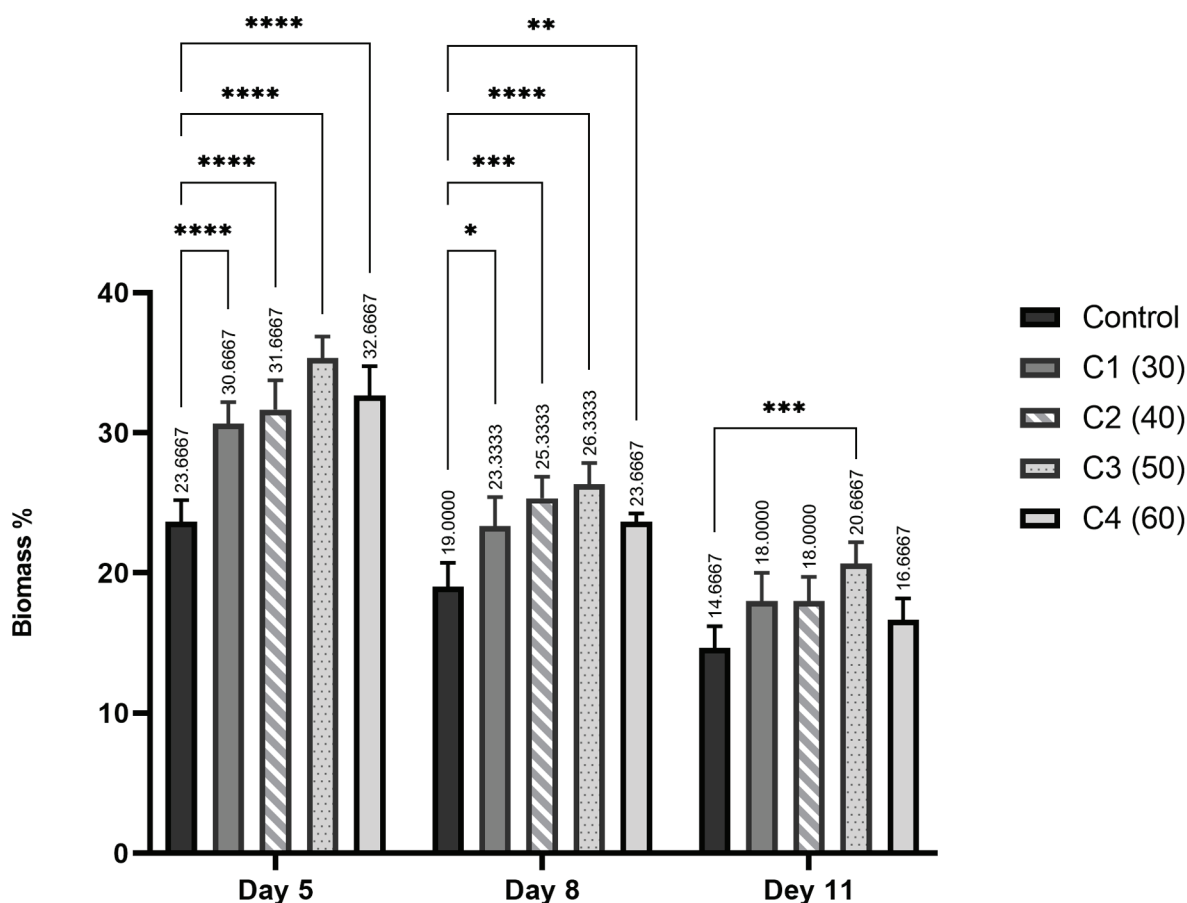


Figure 5. Effect of various concentrations of castor oil on microbial biomass in the process of erythromycin production by *S. erythraea* PTCC 1685. Each experiment was performed in three batches and the data given in the graph are the mean of three repetitions. Asterisks indicate significant differences between groups and control. * represents the significance level if any (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

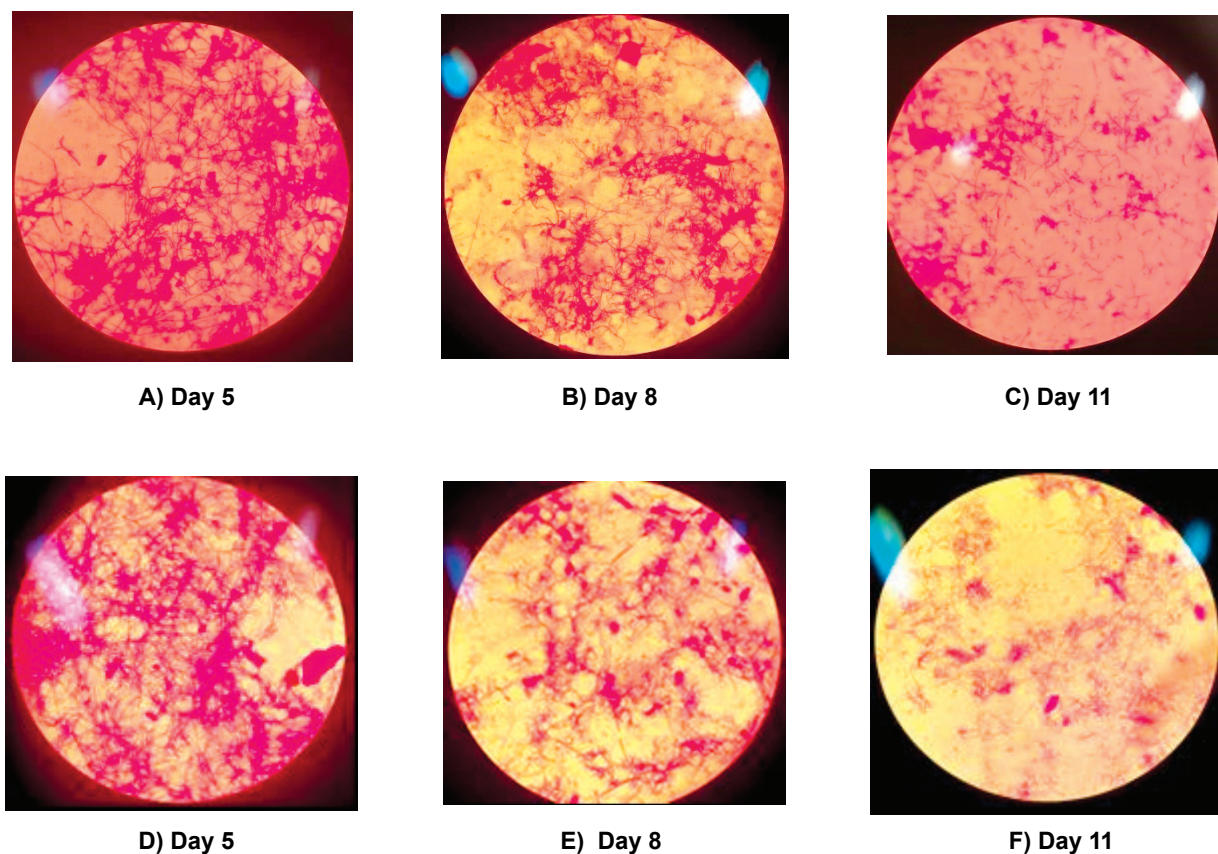


Figure 6. Morphology of *S. erythraea* in control fermentation media (A, B, C) and in fermentation media containing 50 g.L⁻¹ castor oil (D, E, F). The length and thickness of the mycelium indicate the growth of the strain.

The correlation between the growth of mycelia and erythromycin production depends on fermentation conditions and the composition of the culture media (6, 29). Hamedi found a correlation between the growth of mycelia and the production of erythromycin in media containing soybean and rapeseed oil, whereas an inverse result was observed in media containing walnut oil (6). In this study, where castor oil was present in the culture media, no correlation was identified between the increase of biomass and the production of the antibiotic.

5. Discussion

The quality and quantity of microbial production are highly dependent on the operating conditions of fermentation. Among these conditions, the kind and amount of carbon substrates used in the culture media have a great effect on the metabolic pathway and strain yield due to providing energy for cells.

Castor oil is a rich source of carbon, which in turn is a supplier of cellular energy. Hence, the use of castor

oil in microbial culture is expected to positively impact the bioprocess yield. Previous studies have reported the positive effect of various kinds of oil in the production of erythromycin. An enhancement in erythromycin production was reported where the black cherry kernel and watermelon seed oils were used in the culture medium (6). Another study showed a higher production of erythromycin in a culture containing shark oil as compared with an oil-free medium (30). The positive effect of fatty acids such as Lauric, Myristic, Palmitic, Stearic, and Oleic separately on the growth of *S. erythraea*, and also erythromycin production is evident (25). Given that castor oil contains 90% Ricinoleic acid in its composition, the increased titer of erythromycin in oil-containing culture could be possibly explained by the stimulation of antibiotic biosynthesis by the fatty acid. Another study showed a positive effect of fatty oils as an energy source in the fermentative production of Penicillin (31). In addition, oils increase the production of antibiotics through other mechanisms. For example,

the presence of oils seems to reduce foam in the culture (30,31).

6. Conclusion

The composition of fermentation media has an important effect on microorganism's growth and bioprocess's yield, especially in secondary metabolites such as antibiotics. Obtained results in this study indicate that using castor oil as the main carbon source in culture media of *S. erythraea*, enhances erythromycin titer. Hence castor oil is presented as a promising substrate for achieving improved productivity in microbial fermentation processes and provides motivation for future studies aiming to optimize the utilization of castor oil as an alternative carbon source to enhance the productivity of the erythromycin fermentation process.

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