

# EFFECTS OF MEDIUM COMPOSITION ON OXYTETRACYCLINE PRODUCTION BY *STREPTOMYCES RIMOSUS* ATCC 33022

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## ABSTRACT

The economical production of antibiotics to some extent depends on the availability of cheap substrates. The work reported in the present paper deals with the fermentative production of oxytetracycline by *Streptomyces rimosus* ATCC 33022 using commercial high fructose syrup (HFS), vitamin B complex and citric acid of technical grade. The effects of concentration of high fructose syrup (0.5 - 2.5 %, v/v), commercial vitamin B complex (0.03 - 0.07 %, w/v) and the citric acid (0.34 - 1.28 %, w/v) were examined in this study. It was found that fermentation medium (medium- $M_{HFS}$ ) containing high fructose syrup 1.0 % produced maximum activity of oxytetracycline after 4 days incubation period. Fermentation medium (medium- $M_{Bplex}$ ) containing 0.05 % commercial vitamin B complex showed maximum activity after 3 days incubation. While the addition of citric acid (0.64 %) to the fermentation medium (medium- $M_{CA}$ ) was found optimum for production oxytetracycline.

## INTISARI

Produksi antibiotik secara ekonomis bergantung pada tersedianya bahan baku yang murah. Di dalam penelitian ini dilaporkan pengaruh komposisi medium pada produksi oksitetrasiklin secara fermentasi dengan menggunakan *Streptomyces rimosus* ATCC 33022. Pengaruh konsentrasi HFS (0,5 - 2,5 %, v/v), vitamin B kompleks (0,03 - 0,07 %, b/v) dan asam sitrat (0,34 - 1,28 %, b/v) diteliti pengaruhnya terhadap produksi oksitetrasiklin. Hasil percobaan menunjukkan bahwa medium fermentasi ( $M_{HFS}$ ) yang mengandung 1 % HFS menghasilkan oksitetrasiklin maksimum setelah masa inkubasi empat hari. Didapatkan pula bahwa medium fermentasi ( $M_{Bplex}$ ) yang mengandung 0,05 % vitamin B kompleks dapat menghasilkan antibiotik dengan aktifitas maksimum setelah inkubasi tiga hari. Penambahan asam sitrat 0,64 % dalam media fermentasi ( $M_{CA}$ ) adalah optimal untuk produksi oksitetrasiklin.

## INTRODUCTION

Tetracycline and its derivatives are important antibiotics which are widely used in Indonesia. To satisfy the demand of these antibiotics in Indonesia, they are presently imported from PRC, Italy, Eire, France, Germany, or United Kingdom. Oxytetracycline is broad spectrum antibiotic which is effective against Gram-positive and Gram-negative bacteria, as well as rickettsias acting as inhibitor of protein synthesis. (1)

The carbon source has been found to play an important role in the formation of tetracyclines by *Streptomyces spp.* The tetracycline antibiotics display features indicative of an acetate origin in the sense that polyketetomethylene chain applies in the case of oxytetracycline synthesis. The nitrogen source of the medium is

also an important factor in the biosynthesis of tetracycline antibiotics. Glucose is the best carbon source for antibiotic production. (2)

The economical production of antibiotics depends on the availability of cheap substrates. Agricultural by-products from various industries, such as cane molasses, fodder yeast, corn-steep liquor, may be used as raw material for antibiotic production. (3,4) Cane molasses which could be used as carbon source due to its 50% glucose content, also contained heavy metals which could act as inhibition factor for the growth of the microorganism. It was reported by Budiwati (5) that the optimal antibiotic production (0.072 g/L medium) using fermentation medium containing 5% cane molasses was obtained at day-7 of fermentation process. Udin (6) had tried to use another carbon source which has economical value, such as commercial sucrose (sugar) in the production of oxytetracycline. The result showed that the optimal antibiotic production (0.090 g/L medium) was reached at day-3 of fermentation process in the medium containing 2% sucrose. From this experiment, it was obvious that, even though, the period of the optimal production of antibiotic could be reduced, the yield of the antibiotic was still lower. According to Dhanutirto (7), the best yield of the antibiotic production was 0.2 - 4.0 g/L medium.

In this study, high fructose syrup (HFS), which has a high economical value since it was processed from cassava, was used as carbon source for oxytetracycline production using *Streptomyces rimosus* and was expected to give higher yield. In previous studies (3,4), yeast extract was usually used as vitamin source. The use of yeast extract in the production of antibiotic is not economical because of its high price, therefore, the use of commercial vitamin B complex as a substitute for yeast extract was also investigated in this study. Abou-Zeid (3) also reported that citric acid of 1.28 % could be used as co-factor for the growth of *S. rimosus* in the production of antibiotic. The effect of citric acid concentration on the growth of *S. rimosus* for oxytetracycline production was determined.

## MATERIALS AND METHODS

### Chemicals and organisms

High fructose syrup (HFS) and commercial vitamin B complex were purchased from the local market while the other chemicals were of technical grade. *Streptomyces rimosus* ATCC 33022 and *Bacillus cereus* were obtained from the RDCAC-LIPI culture collection.

## Culture media

Composition of maintenance and of growth media were the same as those previously reported. (4,8)

The defined fermentation medium used for production of oxytetracycline from high fructose syrup using *S. rimosus* ATCC 33022 contained the following ingredients (Table 1).

Table 1. Composition of fermentation medium for production of oxytetracycline by *S. rimosus* ATCC 33022

Ingredients	Medium		
	M <sub>HFS</sub>	M <sub>Bplex</sub>	M <sub>CA</sub>
Sucrose 2%	-	+	-
HFS (0.5 - 2.5 %)	+	-	-
HFS 1 %	-	-	+
Ammonium sulfate (0.2 %)	+	+	+
Yeast extract (0.3 %)	+	-	+
Vitamin B-complex (0.03-0.07%)	-	+	-
Citric acid (1.28 %)	+	+	-
Citric acid (0.34 - 1.28 %)	-	-	+
Minerals	+	+	+

Note: The minerals consist of : MgSO<sub>4</sub> (0.025 %), KH<sub>2</sub>PO<sub>4</sub> (0.015%), CaCO<sub>3</sub> (0.1 %), MnSO<sub>4</sub> (0.001 %) and ZnSO<sub>4</sub> (0.04 %)

- M<sub>HFS</sub> = Medium containing HFS  
M<sub>Bplex</sub> = Medium containing vitamin B complex  
M<sub>CA</sub> = Medium containing citric acid  
(+) = added into the medium  
(-) = not added into the medium

Erlenmeyer flasks (250 ml capacity), containing 50 ml of the fermentation medium were sterilised at 121 °C for 15 min. and cooled at room temperature. Each flask was inoculated with 2.5 ml of culture of the *S. rimosus* on growth medium. The inoculated flasks were incubated on a shaker incubation (150 rpm) at 30 °C for 10 days. During the fermentation process, pH of the fermented medium, the contents of biomass, oxytetracycline, glucose and total nitrogen of the medium were determined.

## Biological determination of oxytetracycline

The oxytetracycline content of medium was expressed as zone of inhibition using *Bacillus cereus* as the test organism.

A standard curve was drawn to correlate the various concentration of standard oxytetracycline (SIGMA) and inhibition zones of susceptible bacteria *Bacillus cereus* determined by the diffusion method. (4) The standard curve was used to estimate the concentration of oxytetracycline produced based on the measured inhibition zone.

## Analyses

The biomass produced was determined gravimetrically after centrifugation at 12,500 rpm for 30 minutes, and washing with water and ethanol then followed by drying at 60 °C (4). Glucose

and total nitrogen content were analysed by Nelson-Somogyi Method (9) and Kjeldahl method (4), respectively.

## RESULTS AND DISCUSSION

In this study the optimum concentration of high fructose syrup (HFS) as the carbon source for the production of oxytetracycline was investigated. The range of concentration was 0.5 - 2.5 %. The data obtained was shown in Figure 1. It was found that HFS at concentration of 1.0% was the best for the production of oxytetracycline by *S. rimosus* compared to other concentrations tested. The maximum concentration of antibiotic produced in the fermentation medium M<sub>HFS</sub> containing 1.0% HFS was 0.122 g/L medium at 96 hours incubation period.

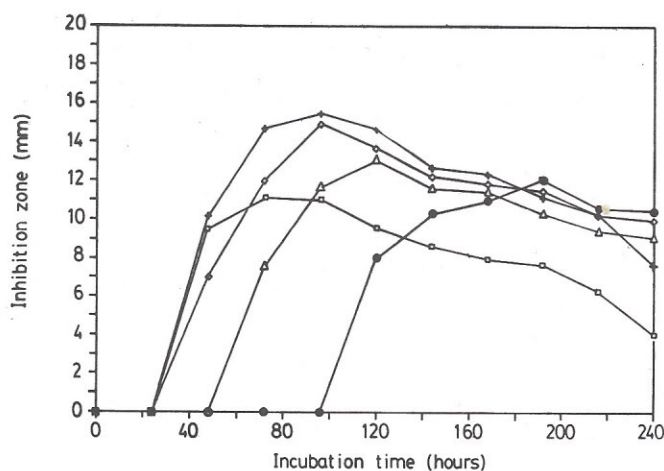


Figure 1. Oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of high fructose syrup (HFS). (□-□) 0.5% HFS, (+-+) 1.0% HFS, (◇-◇) 1.5% HFS, (Δ-Δ) 2.0% HFS, (●-●) 2.5% HFS.

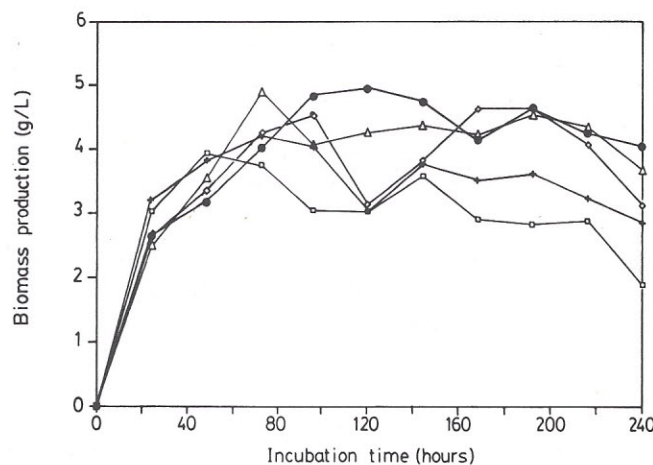


Figure 2. pH changes during oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of high fructose syrup (HFS). (□-□) 0.5% HFS, (+-+) 1.0% HFS, (◇-◇) 1.5% HFS, (Δ-Δ) 2.0% HFS (●-●) 2.5% HFS.

The results given in Figure 1 also show that the activity of oxytetracycline produced increased with the increase of incubation period, and reached the maximum value at 96 hours in the fermentation medium containing 0.5 - 1.5% HFS, 120 hours in the fermentation medium containing 2.0% HFS and 192 hours in the fermentation medium containing 2.5%. A drop in pH was observed during 2 days of fermentation process (Figure 2). This might be correlated with the accumulation of organic acids which were further utilised by the microorganism since at the end of the fermentation process the medium became alkaline. During the fermentation process, the amount of biomass produced fluctuated, this might be due to the aggregation of *S. rimosus* colonies (Figure 3).

In further study, the effect of vitamin B complex on tetracycline production was carried out by omitting the yeast extract from the fermentation medium and replaced with commercial vitamin B complex (fermentation medium  $M_{Bplex}$ ). The result

obtained which was shown in Figure 4, indicated that vitamin B complex at a concentration of 0.05% was found to be optimal and increased the maximum value of oxytetracycline production (0.150 g/L or inhibition zone of 19.20 mm) at 72 hours of incubation. The maximum activity of oxytetracycline produced in the fermentation medium  $M_{Bplex}$  containing 0.03, 0.04, 0.07% vitamin B complex was reached at 96 hours of incubation time, and in the fermentation medium containing 0.06% B complex it was achieved at 72 hours. The optimum period of antibiotic production in the fermentation medium containing 0.06% B complex was the same as the time of fermentation medium containing 0.05% B complex, but the concentration of the yield was lower (0.126 g/L) than of that in the fermentation medium containing 0.05% B complex. Similarity in patterns of the pH medium (Figure 5) and biomass production (Figure 6), with those obtained from the fermentation medium containing yeast extract was also demonstrated.

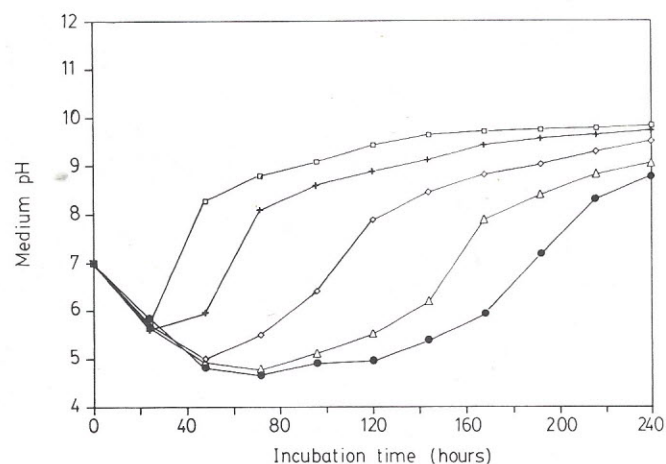


Figure 3. Biomass production of *S. rimosus* during oxytetracycline fermentation using medium containing high fructose syrup. (□-□) 0.5% HFS, (+-+) 1.0% HFS, (◇-◇) 1.5% HFS, (Δ-Δ) 2.0% HFS, (●-●) 2.5% HFS.

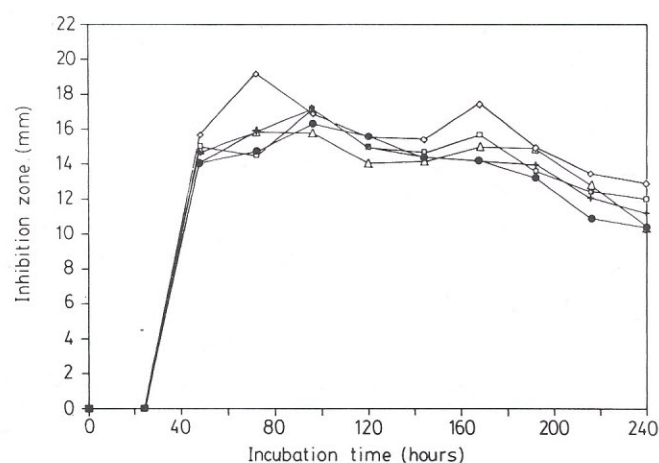


Figure 4. Oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of commercial vitamin B complex (Bplex). (□-□) 0.03% Bplex, (+-+) 0.04% Bplex, (◇-◇) 0.05% Bplex, (Δ-Δ) 0.06% Bplex, (●-●) 0.07% Bplex.

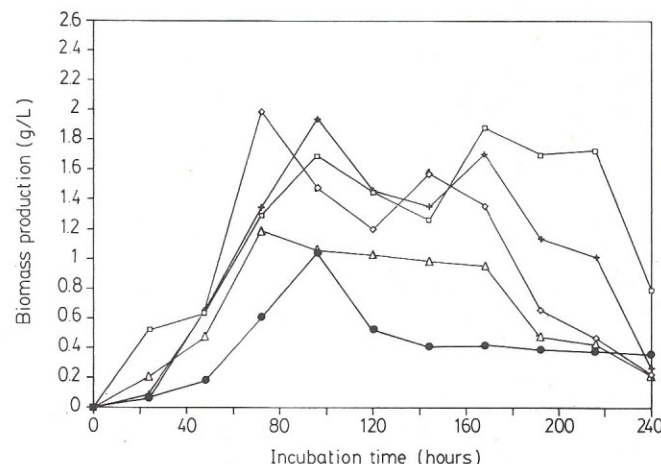


Figure 5. pH changes during oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of commercial vitamin B complex (Bplex). (□-□) 0.03% Bplex, (+-+) 0.04% Bplex, (◇-◇) 0.05% Bplex, (Δ-Δ) 0.06% Bplex, (●-●) 0.07% Bplex.

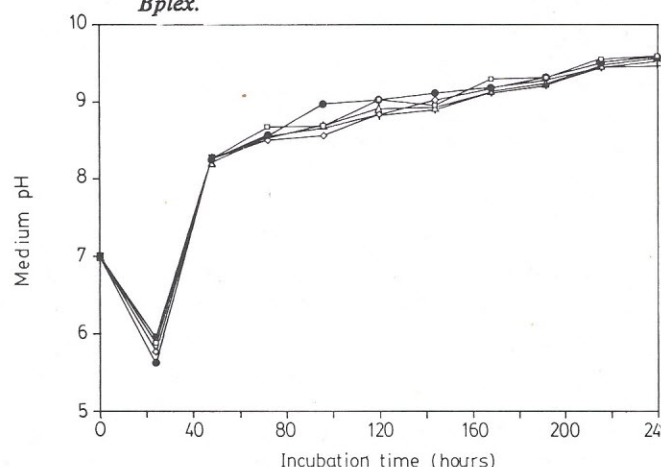


Figure 6. Biomass production of *S. rimosus* during oxytetracycline fermentation using medium containing commercial vitamin B complex (Bplex). (□-□) 0.03% Bplex, (+-+) 0.04% Bplex, (◇-◇) 0.05% Bplex, (Δ-Δ) 0.06% Bplex, (●-●) 0.07% Bplex.

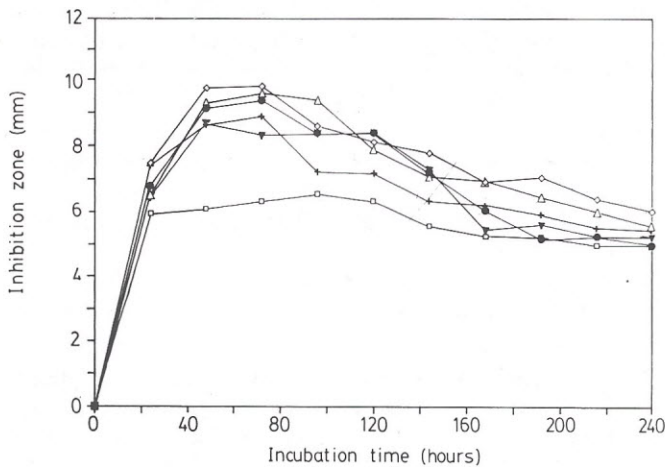


Figure 7. Oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of citric acid (CA). (□ - □) 0.32% CA, (+ - +) 0.48% CA, (◇ - ◇) 0.64% CA, (Δ - Δ) 0.96% CA, (● - ●) 1.12% CA, (▼ - ▼) 1.28% CA.

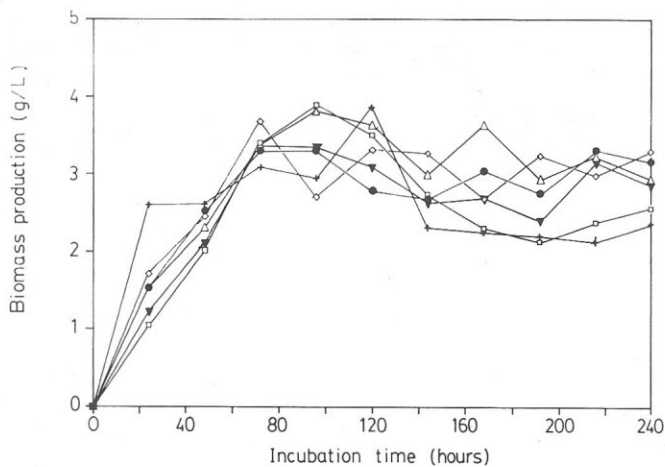


Figure 8. pH changes during oxytetracycline fermentation with *S. rimosus* using medium containing different concentration of citric acid (CA). (□ - □) 0.32% CA, (+ - +) 0.48% CA, (◇ - ◇) 0.64% CA, (Δ - Δ) 0.96% CA, (● - ●) 1.12% CA, (▼ - ▼) 1.28% CA.

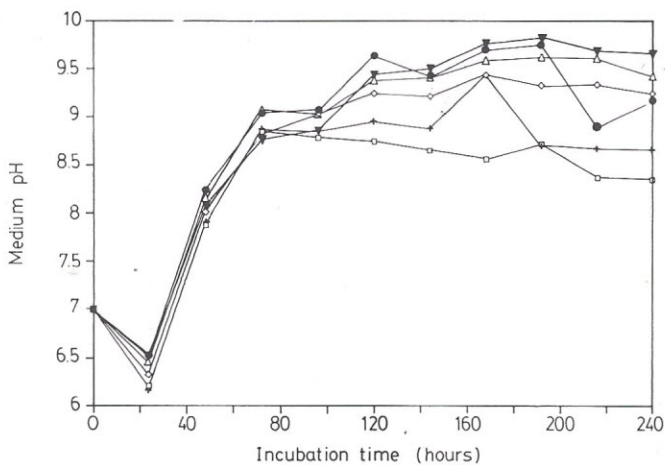


Figure 9. Biomass production of *S. rimosus* during oxytetracycline fermentation using medium containing citric acid (CA). (□ - □) 0.32% CA, (+ - +) 0.48% CA, (◇ - ◇) 0.64% CA, (Δ - Δ) 0.96% CA, (● - ●) 1.12% CA, (▼ - ▼) 1.28% CA.

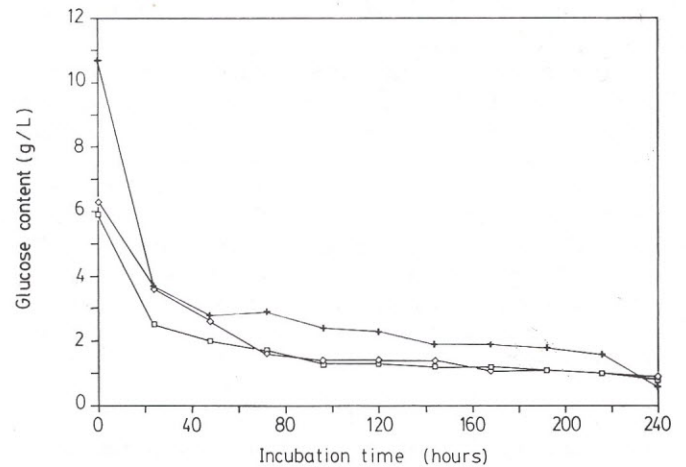


Figure 10. Changes in glucose content during oxytetracycline fermentation with *S. rimosus* at different optimal condition of medium: (□ - □) medium  $M_{HFS}$  containing high fructose syrup 1.0%, (+ - +) medium  $M_{Bplex}$  containing commercial vitamin B complex 0.05%, (◇ - ◇) medium  $M_{CA}$  containing citric acid 0.64%.

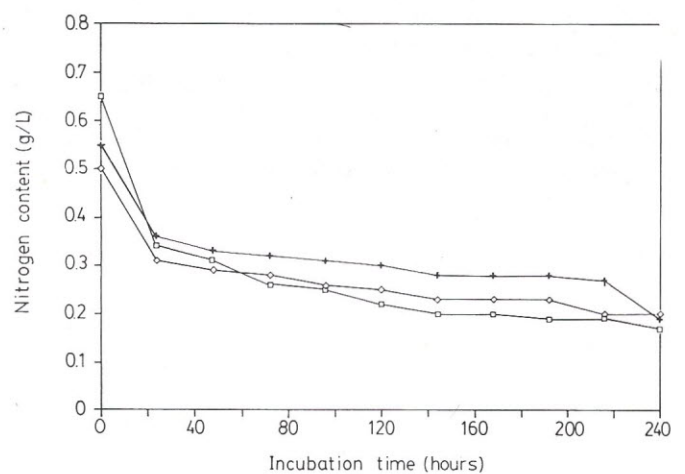


Figure 11. Changes in nitrogen content during oxytetracycline fermentation with *S. rimosus* at different optimal condition of medium: (□ - □) medium  $M_{HFS}$  containing high fructose syrup 1.0%, (+ - +) medium  $M_{Bplex}$  containing commercial vitamin B complex 0.05%, (◇ - ◇) medium  $M_{CA}$  containing citric acid 0.64%.

The influence of citric acid concentration as co-factor on the oxytetracycline production by *S. rimosus* was also studied. The highest activity of oxytetracycline produced, 0.079 g/L (inhibition zone of 9.90 mm), was demonstrated in the fermentation medium with citric acid concentration of 0.64% at 72 hours of incubation. At this point the biomass production was 3.68 g/L and the pH of the medium had increased to 8.83. The chemical changes during the fermentation process using fermentation medium  $M_{CA}$  containing various concentration of citric acid is shown in Figure 7-9.

It was obvious from the results of study that during the first day of fermentation the rapid growth of the microorganism was accompanied by rapid consumption of the principal medium components particularly carbon and nitrogen sources. The carbon and nitrogen sources of the medium decreased further during the fermentation process (Figure 10 and Figure 11). The other medium components, such as fructose and mineral were not analyzed during the fermentation process.

## CONCLUSION

From the results of this study, it was concluded that high fructose syrup (HFS) could be used as carbon source for oxytetracycline production and gave higher yield of the antibiotic than those obtained from the fermentation using either cane molasses or sucrose as carbon source. The optimal production of oxytetracycline, that was 0.12 g/L medium, was obtained after 72 hours of fermentation using medium containing 1% HFS. Commercial vitamin B complex could also be used as a substitute for yeast extract in the production of this antibiotic using *S. rimosus*. The fermentation medium containing 0.05% vitamin B complex was found to be the best medium for the production of the antibiotic, where the maximum concentration of oxytetracycline was 0.15 g/L after 72 hours of fermentation. The use of citric acid as a co-factor for the growth of microorganism for this fermentation might be reduced from 1.28% to 0.64%.

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