

# Effect of different nitrogen source combinations on microbial cellulose production by *Pseudomonas aeruginosa* in batch fermentation

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**Abstract.** Microbial cellulose (MC) is a type of polysaccharide that has high purity properties, which can be produced by different types of microorganism such as *Pseudomonas*, *Komagataeibacter*, *Sarcina* and *Azotobacter*, and widely used in industrial applications. However, the main drawback in microbial cellulose production is low yield, which unable to meet the requirements of the industry. This study aims to determine the effects of different nitrogen sources combinations on microbial cellulose production by *Pseudomonas aeruginosa* and to maximize microbial cellulose production using the best nitrogen sources combination. To improve cellulose production, five different nitrogen sources combinations, which were yeast extract (YE) + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, YE + NH<sub>4</sub>NO<sub>3</sub>, YE + urea, YE + tryptic soy broth and YE + beef extract were added into the media with the composition of 50 g/L glucose, 3/L g KH<sub>2</sub>PO<sub>4</sub> and 0.05 g/L MgSO<sub>4</sub>. The batch fermentation was done in incubator shaker with 150 rpm at 30°C for five days. The samples were harvested every 24 hours, centrifuged, purified and dried for microbial cellulose concentration determination. The results obtained indicated that YE and beef extract combination produced the highest microbial cellulose concentration of 1.7 g/L. To attain the second objective, optimization study was carried out by using different concentrations, which were 5 g/L, 10 g/L, 15 g/L and 20 g/L of YE and beef extract combination. An investigation using 10 g/L YE and beef extract has produced 1.7 g/L of microbial cellulose, which proved to be the potential enhancer for microbial cellulose synthesis. It is recommended that additional morphology analyses should be done to investigate the influence of fermentation conditions and low-cost nitrogen sources media components on microbial cellulose morphology and its mechanical properties. This will help to synthesize desired and reliable microbial cellulose pellicles.

## 1. Introduction

Cellulose is a renewable and biodegradable polysaccharide found richly in the earth. This means it is a kind of energy source, which is not depleted when used and can be decomposed by other living organisms. This undoubtedly can overcome the problems such as energy crisis, population growth and environmental pollutions [1]. These renewable and biodegradable features of cellulose make it being vital topic in the study by many researchers and applied in various aspects. Cellulose has been given an enormous attraction on the common topic of widespread investigations in the field of macromolecular chemistry [1]. It is the major constituent of both about over 50% of the wood and approximately over 94% of the cotton [2]. A French chemist, Anselme Payen, discovered a fibrous substance, which is



called cellulose in early 19th century during the experiment of the plant tissues treated with acids and ammonia [3].

Cellulose is not only can be produced by plants, but also can be synthesized by various bacteria. It can be synthesized by acetic acid bacteria such as *Pseudomonas aeruginosa*. These bacteria are used in this study because it can synthesize microbial cellulose (MC) production. Despite the plant cellulose has the same molecular formula with microbial cellulose, their physicochemical features are significantly different with each other [4]. In fact, the cellulose that is synthesized from the cell wall of the plants is usually not pure because it is commonly contaminated with lignin and hemicelluloses. It is also difficult to purify the plant cellulose from the lignin and hemicelluloses due to the complexity of the cell wall structures [5].

On the contrary, microbial cellulose has a high chemical purify as compared to plant cellulose because it was not associated with the impurities [6,7]. Meanwhile, MC also has high tensile strength, high biocompatibility, high water holding capacity, high crystallinity and high degree of polymerization [8, 9,10,11,12]. Due to these desirable properties of MC, it has been used as an alternative resource instead of plant cellulose in order to synthesize high quality cellulose. Hence, the cellulose can be derived from fermentation process via a microbial system [12].

Although microbial cellulose can be used to replace the plant cellulose, the high processing cost of microbial cellulose had become a great attention in the issues of microbial cellulose synthesis. The expensive nitrogen sources used even increase the cost of microbial cellulose production. Hence, this study is elucidating the effects of different nitrogen sources combinations on microbial cellulose synthesis. The nitrogen sources combination was used as parameter in this study and evaluated with intention to find the optimum concentration to generate the maximum yield of microbial cellulose synthesis [8].

## 2. Methodology

### 2.1 Culture media

Five different nitrogen sources combinations, which were 5 g/L yeast extract (YE) and 5 g/L ammonium sulphate  $(\text{NH}_4)_2\text{SO}_4$ , 5 g/L YE and 5 g/L ammonium nitrate  $(\text{NH}_4\text{NO}_3)$ , 5 g/L YE and 5 g/L urea, 5 g/L YE and 5 g/L tryptic soy broth and 5 g/L YE and 5 g/L beef extract were added into the culture media with the composition of 50 g/L glucose, 3 g/L potassium hydrogen orthophosphate  $(\text{KH}_2\text{PO}_4)$  and 0.05 g magnesium sulphate  $(\text{MgSO}_4)$ . Each 80 mL of culture media were added to 250-mL shake flasks. The pH of the media was adjusted to 6.8 and sterilized at 121 °C for 15 minutes.

### 2.2 Microorganism

*Pseudomonas aeruginosa* provided by Microbiology Laboratory, School of Fundamental Science was used throughout the study for microbial cellulose production.

### 2.3 Fermentation conditions and sampling

Five different nitrogen sources combinations, which were YE and  $(\text{NH}_4)_2\text{SO}_4$ , YE and  $\text{NH}_4\text{NO}_3$ , YE and urea, YE and tryptic soy broth and YE and beef extract used in this study. Eighty mililitre of culture media was added to 250-mL shake flasks. All trials were done at 30°C at pH 6.8 for five days. On each day of fermentation, the broth from shake flasks was harvested and then homogenized at 100 rpm for 5 minutes. The samples were centrifuged at 4000 rpm for 20 minutes. Then, they were washed gently with distilled water and centrifuged again to remove culture broth. The washing procedure was repeated three times. After centrifugation process, the washed pellets were used for microbial cellulose purification and determination. The data analysis was done by plotting the graph.

### 2.4 Microbial cellulose determination and purification

The washed pellets from one sample were treated with NaOH at 90°C for 30 minutes in order to dissolve cells. The cellulose obtained was centrifuged at 4000 rpm for 20 minutes. Next, they were washed with distilled water. After that, cellulose obtained was dried at 80°C for 24 hours and then weighed. Total weight of cellulose on each day of fermentation was measured.

### 2.5 Optimization of cellulose

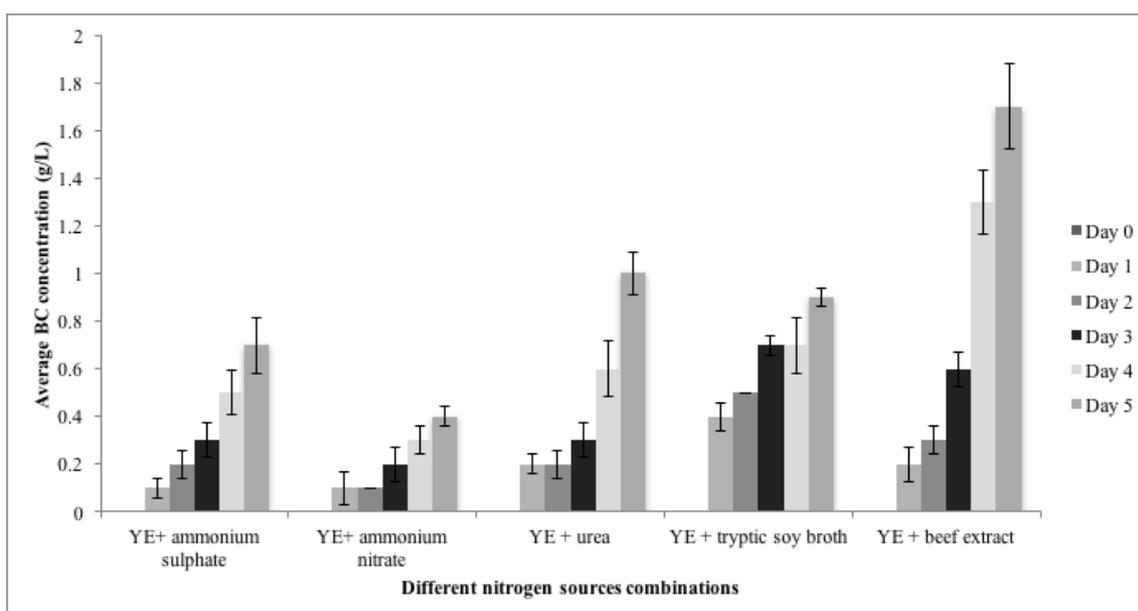
The optimization study was carried out after identifying the best nitrogen sources combination. Different concentrations which were 5 g/L, 10 g/L, 15 g/L and 20 g/L of the best nitrogen sources combination were used in this study. Trials were done in 250-mL shake flasks. Fermentation was done at 30°C with 150 rpm for five days at 6.8. The samples, which taken every 24 hours were centrifuged at 4000 rpm for 20 minutes. Then, they were washed gently with distilled water and centrifuged again to remove culture broth. The washing procedure was repeated three times. After centrifugation, the washed pellets were used for cellulose purification and determination. The data was done by plotting the graph.

## 3. Results

This section explains the results on the effects of different nitrogen sources combinations and also the optimization of MC by varying the best combination of nitrogen source concentrations.

### 3.1 Effect of Different Nitrogen Sources Combinations on Microbial Cellulose Production by *Pseudomonas aeruginosa* After Five Days of Fermentation

Based on figure 1, the bar graph depicted the average cellulose concentration produced for the combination of YE and  $(\text{NH}_4)_2\text{SO}_4$ , YE and  $\text{NH}_4\text{NO}_3$ , YE and urea, YE and tryptic soy broth and YE and beef extract from day 0 until day 5. YE and  $(\text{NH}_4)_2\text{SO}_4$  combination acts as control in this study. For this combination, the amount of microbial cellulose produced was increased gradually from day 0 to day 5 as shown in figure 1. There was the least amount of cellulose of 0.1 g/L on the first day whereas celluloses production reached the highest concentration of 0.7 g/L on the fifth day.



**Figure 1.** Effect of various nitrogen sources combinations on microbial cellulose concentration by *Pseudomonas aeruginosa*

For YE and  $\text{NH}_4\text{NO}_3$  combination, the graph indicated that the microbial cellulose production was increased initially from day 0 to day 1 and then increased steadily from second day to fifth day. There was no increment in the amount of cellulose on second day of fermentation. The combination of YE and ammonium nitrate produced the least cellulose concentration of 0.1 g/L on first day and second day while it synthesized the highest amount of microbial cellulose of 0.4 g/L on fifth day. Although the cellulose concentration was increased constantly until the fifth day, this combination gave negative effect on cellulose synthesis since it produced lower cellulose concentration than the control medium.

According to the graph of YE and urea combination, there was an initial increase in the amount of cellulose from day 0 to day 1 and then the increment of cellulose concentration was continued significantly from second day to fifth day. The amount of cellulose produced on second day was same with the first day with 0.2 g/L of cellulose synthesized. This graph indicated that 0.2 g/L of cellulose produced on first day and second day were the least cellulose concentration whereas 1.0 g/L of cellulose produced on fifth day was the highest cellulose concentration. This combination gave positive effect on the cellulose production since it produced higher cellulose concentration than the control medium. Based on YE and tryptic soy broth combination, MC production was increased greatly from 0 g/L of MC on day 0 to 0.4 g/L of MC on day 1 and then it was rose gradually from first day to fifth day. There was no increment of MC occurred on fourth day in which same MC concentration of 0.7 g/L synthesized on both third day and fourth day. The least amount of MC produced was 0.4 g/L on first day whereas the highest amount of MC produced was 0.9 g/L on fifth day. This combination also gave positive effect on the MC synthesis since it produced more amount of MC than the control medium.

From the graph of YE and beef extract combination, the average MC production was increased significantly from day 0 to day 5. This combination produced the least MC concentration of 0.2 g/L on first day while it reached the maximum MC concentration of 1.7 g/L on fifth day. It could not be denied that this combination also gave positive effect on MC production as it synthesized the maximum MC concentration as compared to the control medium.

Based on Figure 1, the combinations of YE and urea, YE and tryptic soy broth and YE and beef extract produced 1.0 g/L, 0.9 g/L and 1.7 g/L of MC respectively, which were higher than the MC concentration of control medium (0.7 g/L) at the end of fermentation. In contrast, YE and  $\text{NH}_4\text{NO}_3$  combination synthesized only 0.4 g/L of MC on the fifth day of fermentation, which lower than the MC concentration of control medium. Furthermore, the combination of YE and urea produced higher MC concentration than the combination of YE and  $\text{NH}_4\text{NO}_3$ . However, it was lower than the combination of YE and urea and YE and beef extract. From this graph, YE and beef extract combination produced the highest MC concentration of 1.7 g/L of MC and then followed by the combination of YE and urea, YE and tryptic soy broth and YE and  $\text{NH}_4\text{NO}_3$ . Therefore, YE and beef extract combination was considered as the best nitrogen sources combination in this study.

### 3.2 Volumetric Productivity of MC on Various Nitrogen Sources Combinations by *Pseudomonas aeruginosa*

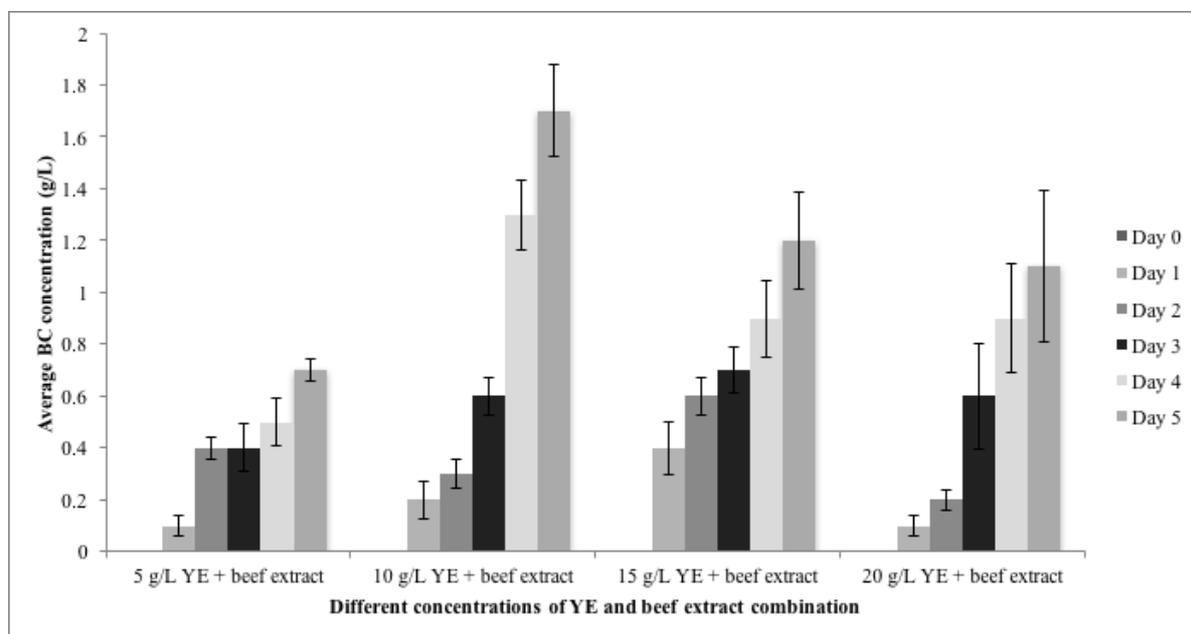
From table 1, it showed the MC volumetric productivity of various nitrogen sources combinations by *Pseudomonas aeruginosa*. The combination of YE and  $\text{NH}_4\text{NO}_3$  yielded the lowest MC productivity with 0.027 g/L/day. Meanwhile, YE and beef extract combination gave the highest MC productivity with 0.113 g/L/day and followed by the combination of YE and urea (0.067 g/L/day), YE and tryptic soy broth (0.060 g/L/day) and YE and  $(\text{NH}_4)_2\text{SO}_4$  (0.047 g/L/day). Hence, the combination of YE and beef extract was considered as the best nitrogen sources combination as it obtained the highest MC productivity as compared to other nitrogen sources combinations.

**Table 1.** Effects of various nitrogen sources combinations on microbial cellulose volumetric productivity by *Pseudomonas aeruginosa*

Various Nitrogen Sources Combinations	MC Volumetric Productivity (g/L/day)
YE + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (control)	0.047
YE + NH <sub>4</sub> NO <sub>3</sub>	0.027
YE + urea	0.067
YE + tryptic soy broth	0.060
YE + beef extract	0.113

### 3.3 Effect of Different Concentrations of YE and Beef Extract Combination on MC Production by *Pseudomonas aeruginosa* After Five Days of Fermentation

According to figure 2, the graph showed the MC concentration produced by different concentrations of YE + beef extract combination, which were 5 g/L, 10 g/L, 15 g/L and 20 g/L of YE and beef extract from day 0 until day 5. For 5 g/L YE and beef extract combination, the average MC concentration was increased initially from day 0 to day 1 and then it was rose significantly from 0.1 g/L on first day to 0.4 g/L on second day. After that, the MC production was continued to increase gradually from third day to fifth day. Although there was an increment on MC concentration until fifth day, the amount of MC was remained constant on third day with 0.4 g/L of MC produced. This combination produced the least MC concentration of 0.1 g/L on first day while it synthesized the highest MC concentration of 0.7 g/L on fifth day.

**Figure 2.** Effects of different concentration of YE and beef extract combination on MC production by *Pseudomonas aeruginosa*

Based on the graph of 10 g/L YE and beef extract combination, the MC production was increased significantly from the initial day to fifth day. This combination synthesized the least amount of MC with 0.2 g/L on first day whereas it produced the highest amount of MC with 1.7 g/L on fifth day.

According to 15 g/L YE and beef extract combination, the MC production was increased greatly from day 0 to day 1 and then it was continued to increase gradually until fifth day. For this combination, the lowest concentration of MC was 0.4 g/L on first day while the highest MC concentration was 1.2 g/L on fifth day.

For 20 g/L YE and beef extract combination, the graph showed that the average MC concentration was rose slowly from day 0 to day 2 and then it increased significantly until fifth day of fermentation. This combination synthesized 0.1 g/L of MC on first day which was the lowest MC production, whereas it produced 1.1 g/L of MC on fifth day, which was the highest MC production.

Based on figure 2, the combinations of 10 g/L, 15 g/L and 20 g/L YE and beef extract produced 1.7 g/L, 1.2 g/L and 1.1 g/L of MC respectively, which synthesized above 1.0 g/L of MC after five days of fermentation. However, the combination of 5 g/L YE and beef extract synthesized only 0.7 g/L of MC, which produced below 1.0 g/L of MC concentration on the fifth day of fermentation. The combination of 15 g/L YE and beef extract synthesized higher MC concentration than the combination of 5 g/L and 20 g/L YE and beef extract but it produced lower MC concentration than the combination of 10 g/L YE and beef extract. According to Figure 2, the combination of 10 g/L YE and beef extract produced maximum MC production of 1.7 g/L, followed by the combination of 15 g/L YE and beef extract, 20 g/L YE and beef extract and 5 g/L YE and beef extract. Hence, 10 g/L YE and beef extract combination was preferred as the best concentration of YE and beef extract combination to maximize the MC production.

### 3.4 Volumetric Productivity of MC on Different Concentrations of YE and Beef Extract Combination by *Pseudomonas aeruginosa*

According to table 2, it showed the MC volumetric productivity of different concentrations of YE and beef extract combination by *Pseudomonas aeruginosa*. The combination of 5 g/L YE and beef extract gave the lowest MC productivity with 0.047 g/L/day. Meanwhile, the combination of 10 g/L YE and beef extract reached the highest MC concentration with 0.113 g/L/day and followed by the combination of 15 g/L YE and beef extract (0.080 g/L/day) and 20 g/L YE and beef extract (0.073 g/L/day). Therefore, the combination of 10 g/L YE and beef extract was considered as the best concentration of YE and beef extract combination as it obtained the highest MC productivity as compared to other concentrations of YE and beef extract combination.

**Table 2.** Effects of different concentrations of YE and beef extract combination on MC volumetric productivity by *Pseudomonas aeruginosa*

Different Concentrations of YE and Beef Extract Combination	MC Volumetric Productivity (g/L/day)
5 g/L YE + beef extract	0.047
10 g/L YE + beef extract	0.113
15 g/L YE + beef extract	0.080
20 g/L YE + beef extract	0.073

## 4. Discussion

The effect of various nitrogen sources on the productivity of MC have been studied by previous researchers. For example, Hungund & Gupta [6] investigated that the effect of different nitrogen sources such as peptone, casein hydrolysate, beef extract, malt extract, sodium nitrate, ammonium chloride, ammonium sulphate, potassium nitrate, ammonium nitrate and urea on the MC production. They concluded that peptone, casein hydrolyzate, beef extract and malt extract gave the MC yield of above

2.0 g/L. A previous study also indicated that the use of different nitrogen sources such as yeast extract, peptone, malt extract, tryptone and sodium nitrate gave different concentration of the MC [13]. Nevertheless, these studies only used a single nitrogen source in the medium to investigate the effectiveness of MC productivity by bacteria. The single nitrogen source exhibited a significant reduction in the MC production [14].

However, there are limited studies on the production of MC by using nitrogen sources combination. In this study, the combination of YE and urea, YE and tryptic soy broth and YE and beef extract were the new nitrogen sources combination being investigated. YE used in all combinations because it was considered as highly potential nitrogen source to enhance the MC synthesis. It increased and enhanced the growth of bacteria [15, 12]. It also provided many nitrogen compounds, carbon and growth factors such as amino acids and vitamins, especially vitamin B complex, which required by the bacteria for growth and also serves to stimulate the productivity of MC [12]. In addition, YE plays an important role in the biosynthesis of essential molecules such as protein and nucleic acids [11]. It was also stated that the combination of both YE and other nitrogen sources improved the MC synthesis effectively [14]. According to figure 1, the average MC concentration was generally increased from day 1 to day 5. This is because the MC concentration was relative to the amount of nitrogen and glucose consumed. When the time taken for fermentation was longer until optimum periods, the amount of nitrogen and glucose consumed was higher and thus increased the MC production by *Pseudomonas aeruginosa* [15]. The bacteria inoculated into the medium containing nitrogen sources, needed certain time to incorporate and respond it for MC production. Thus, there was no MC produced in day 0 because the inoculated bacteria were not responded to the medium within a few minutes to produce MC. Apart from that, there was low MC yield in day 1 in which the combination of YE and  $(\text{NH}_4)_2\text{SO}_4$  and YE and  $\text{NH}_4\text{NO}_3$  were produced 0.1 g/L of MC only while the combination of YE and urea and YE and beef extract were produced 0.2 g/L of MC. This is because the bacteria were adapted at a slow rate to the medium and so they synthesized low amount of MC on initial day. On the contrary, the combination of YE and tryptic soy broth was produced a higher MC concentration of 0.4 g/L on the first day because it contained digests of casein and soybean meal, which supply excessive amino acids and other nitrogenous substances to stimulate the bacteria in order to enhance the MC production at the initial stage.

In figure 1, there was the same average MC production on day 1 and day 2 for the combination of YE and  $\text{NH}_4\text{NO}_3$ . The similar situation also occurred in day 1 and day 2 for the combination of YE and urea and day 3 and day 4 for the combination of YE and tryptic soy broth. This indicated that there was no increase in the amount of MC on the latter day. This is because the media placed in the incubator shaker, were affected by the oxygen transfer rate. The limited amount of incubator shakers in the laboratory were used by many students and the repeated step of opening and closing the incubator shakers influenced the availability of oxygen inside it.

$(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{NO}_3$  used in this study were inorganic nitrogen sources whereas urea, tryptic soy broth and beef extract were organic nitrogen sources. From figure 1, it showed that the combination of both organic nitrogen sources such as YE and urea, YE and tryptic soy broth and YE and beef extract gave higher MC concentration than the combination of both organic and inorganic nitrogen sources such as YE and  $(\text{NH}_4)_2\text{SO}_4$  and YE and  $\text{NH}_4\text{NO}_3$ . Embuscado and colleagues [16] stated that organic nitrogen sources enhanced higher MC concentration than inorganic nitrogen sources. It was similar with the results of the research carried out by Abdelhady and coworkers [17]. Nitrogen sources support rapid growth and high cell yields of bacteria than inorganic nitrogen sources [18]. In addition, it is also indicated that the combination of both inorganic nitrogen sources gave little or no MC produced and vice versa [16]. Therefore, from the result in figure 1, it can be concluded that the combination of both organic nitrogen sources gave the highest MC concentration and then followed by the combination of both organic and inorganic nitrogen sources.

The combination of YE and  $\text{NH}_4\text{NO}_3$  gave negative effect to the MC synthesis since it synthesized lower MC concentration than the control medium because  $\text{NH}_4\text{NO}_3$  provided less nitrogen than

$(\text{NH}_4)_2\text{SO}_4$ .  $(\text{NH}_4)_2\text{SO}_4$  is composed of nitrogen and sulphur whereas  $\text{NH}_4\text{NO}_3$  is the chemical compound of nitric acid and ammonia. Nitric acid is not required by *Pseudomonas aeruginosa* and its acidic property gave effect of significant reduction on MC production. Yeast extract incorporated into the medium containing  $\text{NH}_4\text{NO}_3$  also could not improve much the amount of MC to be produced. In previous study, it has been found that  $\text{NH}_4\text{NO}_3$  produced the least amount of MC as compared to other inorganic nitrogen sources [6]. Apart from that, the combination of YE and tryptic soy broth synthesized lower MC concentration than the combination of YE and urea because many nutrients such as amino acids and other nitrogenous substances in the tryptic soy broth has been utilized at the beginning and there was no much nutrients left to stimulate the bacteria to produce much MC when the fermentation process was continued.

The combination of YE and beef extract produced the maximum MC concentration as compared to other nitrogen sources combinations and it was considered as the best nitrogen sources combination to enhance the MC production. Beef extract provided various sources of nutrients and it was a mixture of minerals, organic acids, nucleotide fractions, vitamins, amino acids and peptides [19]. In addition, its function could be described as complementing the nutritive properties of peptone by providing adequate phosphates, minerals and energy sources. Various nutrients contributed by beef extract became the requirements of the bacteria for cell growth and MC yields. At the same time, these nutrients also enhanced the biosynthesis pathway of bacteria and thus increased the average MC production. According to table 1, the combination of YE and beef extract also gave the highest MC productivity of 0.113 g/L/day as it produced the highest amount of MC during the fermentation.

In further optimization study, based on figure 2, the amount of MC produced also increased gradually from day 0 to day 5 since the MC concentration was directly proportional to the time taken for fermentation. Lina et al. [1] indicated that the fermentation time gave a significant effect on the MC production. There was also low MC synthesized in the first day of fermentation for all the combination of different concentration of yeast extract and beef extract except 15 g/L, because the bacteria needed time to respond with media for MC production. However, the MC concentration was higher in day 1 for the combination of 15 g/L YE and beef extract because the bacteria were adapted well initially in this concentration to produce much MC at the beginning. For 5 g/L YE and beef extract combination, there was same MC concentration in day 2 and day 3 because it was affected by the availability of oxygen in the incubator shaker which had mentioned on above.

Based on figure 2, 5 g/L YE and beef extract combination produced the least MC concentration because the little concentration of beef extract used gave insufficient nutrients and nitrogen for *Pseudomonas aeruginosa* to enhance the production of MC. Meanwhile, the combination of higher concentrations of YE and beef extract such as 15 g/L and 20 g/L also not synthesized the higher production of MC. This is because the excess nitrogen provided by the higher concentration of beef extract could not increase the MC synthesis. The excess nitrogen would disrupt the cell metabolism of bacteria and caused them could not absorbed properly the other vital nutrients such as carbon and salts. Although the higher concentration of beef extract provided excess nitrogen, the insufficient of other nutrients could not enhance the productivity of MC. In addition, Ashjara & Sharabiyani [20] stated that the extra nitrogen favours the biomass production but reduced the MC synthesis. Furthermore, 10 g/L YE and beef extract combination produced the highest MC concentration and it was the potential concentration of beef extract to produce the maximum MC concentration. This is due to the adequate nitrogen provided by optimum concentration of beef extract would not affect the absorption of other nutrients by bacteria. *Pseudomonas aeruginosa* obtained sufficient nitrogen and other crucial nutrients simultaneously and thus eventually improved the MC production. Moreover, Hungund & Gupta [21] investigated that 6 g/L of beef extract produced the highest MC concentration of 5.89 g/L. According to their research and this study, it could be deduced that the range of 6 g/L to 14 g/L of YE and beef extract would be the optimum concentration of beef extract to maximize the MC production. Beyond the optimum concentration, the biosynthesis pathway of bacteria was influenced and thus reduced the

average MC production. Based on table 2, 10 g/L YE and beef extract combination achieved the highest MC productivity of 0.113 g/L/day as this combination synthesized the highest amount of MC during fermentation process.

## 5. Conclusion

In conclusion, YE and beef extract combination gave the highest MC concentration and volumetric productivity. Varying this combination at different concentrations influenced MC production. In addition, 50 g/L of YE + beef extract combination yielded highest MC production. However, increasing YE + beef extract concentration beyond 50 g/L inhibits MC synthesis and its volumetric productivity.

## 6. References

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