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by 41575 41575

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SIGNIFIKANSI KOMPONEN *Nitrogen Limited Medium* (NLM) TERHADAP PRODUKSI LIPID *Lipomyces starkeyi* Y853

SIGNIFICANCE OF NITROGEN LIMITED MEDIUM (NLM) COMPONENTS ON LIPID PRODUCTION *Lipomyces starkeyi* Y853

Abstrak

Khamir *oleaginous* memiliki kemampuan mengubah sumber karbon dan nitrogen menjadi lipid. *Lipomyces starkeyi* merupakan salah satu khamir *oleaginous* yang dapat menghasilkan lipid hingga 50 % dari berat kering sel, yaitu sebesar 4,36 g/L dengan biomassa 8,14 g/L dengan menggunakan *Nitrogen Limited Medium* (NLM) sebagai media pertumbuhan. Komponen dalam NLM yang diduga dapat mengakumulasi lipid tinggi merupakan glukosa sebagai sumber karbon,serta pepton dan *yeast extract* sebagai sumber nitrogen, serta senyawa logam seperti magnesium (Mg^{2+}). Namun, pengamatan mengenai peran dari komponen NLM belum diketahui secara statistik terhadap produksi lipid oleh *L. starkeyi* Y853. Dengan demikian, penelitian ini dirancang dan dilakukan dengan tujuan untuk mengetahui peran komponen NLM terhadap produksi lipid oleh *L. starkeyi* Y853 menggunakan Desain Plackett-Burman (PBD) sebagai metodenya. Hasil penelitian menunjukkan bahwa tidak ada variabel yang signifikan terhadap produksi lipid *L. starkeyi* Y853 berdasarkan uji ANOVA ($P > 0,05$). Namun, efek dari variabel tersebut dapat diamati melalui *main effect plot*. Grafik tersebut menunjukkan bahwa pepton dan *yeast extract* merupakan komponen yang diperlukan dalam konsentrasi tinggi agar memperoleh produksi lipid dan biomassa yang tinggi. Untuk penelitian selanjutnya, penambahan faktor lain di luar komponen medium (pH, suhu, kecepatan *shaker*) diperlukan untuk memperoleh hasil yang lebih signifikan terhadap produksi lipid.

Kata kunci: Akumulasi Lipid; *Lipomyces starkeyi*; NLM; Plackett-Burman

Abstract

Oleaginous yeast has ability to convert carbon and nitrogen sources into lipids. *Lipomyces starkeyi* is an oleaginous yeast that can produce lipid up to 50% of the dry cell weight, namely 4,36 g/L with biomass of 8,14 g/L using Nitrogen Limited Medium (NLM). It is hypothesized that the components of NLM are capable accumulating high levels of lipid are glucose as carbon source, peptone and yeast extract as nitrogen source, and magnesium as metal compounds. However, the statistical analysis of role of NLM components on lipid production has not yet been conducted. Thus, this research was designed and carried out to determine the role of NLM components towards lipid production using Plackett-Burman Design. The results showed that there were no significant variables in lipid production based on the ANOVA test ($P > 0,05$). However, the effects of these variables can be observed through the main effect plot. The graph shows that peptone and yeast extract are components that require high concentrations to obtain high lipid dan biomass production. To obtain more significant results on lipid production by *L. starkeyi* Y853, further research should be conducted with the addition of other factors outside the medium components (pH, temperature, shaker speed).

Keywords: *Lipid Accumulation; Lipomyce starkeyi; NLM; Plackett-Burman*

INTRODUCTION

Microorganisms can obtain a specific compound from other compounds. For example, they can convert carbohydrate compound into lipids. One such microorganism is oleaginous yeast. This is a type of single-cell fungus that capable of accumulating lipid more than 20% of its biomass. Two genera of oleaginous yeast are *Lipomyces* and *Rhodotorula*, which are capable accumulate lipid up to 70% of the weight of the biomass produced (Ratledge & Wynn, 2002). *Lipomyces starkeyi* is one

of the oleaginous yeasts that has the potential to be used in industrial scale for lipid production due to its triacylglycerol (TAG) composition. The TAG composition produced by *L. starkeyi* primarily consists of palmitic and oleic acids, which are also present in vegetable oils. Therefore, TAG accumulated by *L. Starkeyi* can serve as a substitute for vegetable oil as a feedstock for biodiesel production (Yamazaki et al., 2019). The benefits of utilizing microbes as lipid producers are that they have relatively short life cycle, require less labor, and more cost-effective when scaled up (Q. Li et al., 2008).

L. starkeyi has been demonstrated to utilize a diverse range of carbon source for lipid production, including glucose (Oguri et al. 2012), xylose (Zhao et al., 2008), glycerol (L. Liu et al., 2017), starch (J.-X. Liu et al., 2013), and industrial waste (Papanikolaou et al., 2008). Glucose is a typical carbon source utilized in cultivation of *L. Starkeyi* for lipid production. This is due to the fact that *Lipomyces* is capable to convert glucose first into lipids compared to other carbon sources. Moreover, *L. Starkeyi* has been demonstrated to produce highest lipid yields when utilizing a carbon source in the form of D-glucose (Oguri et al., 2012). Mar'atussholihah, (2023) indicates that *L. starkeyi* Y853 can accumulate lipid yield 4,36 g/L at 48th hour with biomass of 8,14 g/L using NLM. The medium contains glucose as the primary carbon source. Hence, glucose was selected as the carbon source to achieve the highest lipid production by *L. starkeyi*.

The medium selected for this experiment is a Nitrogen-Limited Medium (NLM), which are contains of glucose, peptone, yeast extract, KH_2PO_4, $Na_2HPO_4.7H_2O$, dan $MgSO_4$ (Jiru et al., 2016). This medium was selected based on the observation that *L. starkeyi* is capable of accumulating lipid in low nitrogen concentrations, which results in high lipid production (Q. Li et al., 2008). Additionally, the concentration of metal compounds (Mg^{2+}) has shown influence the metabolic rate of yeast when present in low concentrations (Dzurendova et al., 2020). However, deeper investigation of the specific role of each NLM component on biomass and lipid accumulation by *L. starkeyi* Y853, with statistical analysis, had not yet to be conducted.

The Plackett-Burman Design is a quantitative experimental methodology. The objective of the design method is to identify critical factors that impact the production of a given product. The design method uses multitude of factors, with observation from a total of 'n' factors with observations of 'n+1' experiments (Vanaja & Rani, 2007). The objective of this study was to determine the significance of the components in NLM on the lipid production of *L. starkeyi* Y853, utilizing a Plackett-Burman Design (PBD) with six variables.

MATERIALS AND METHODS

Materials

Lipomyces starkeyi InaCC Y853 was obtained from Indonesian Culture Collection (InaCC). Mediums and chemicals which were used were Potato Dextrose Agar (PDA) (Merk Millipore); Glucose Peptones Yeast (GPY) media consist of glucose (Merck Millipore), peptone (Oxoid), and yeast extract (Merck Millipore); Nitrogen Limited Medium (NLM) with composition: glucose (Merck Millipore), peptone (Oxoid), yeast extract (Merck Millipore), KH_2PO_4 (Merck Millipore), $Na_2HPO_4.7H_2O$ (Supelco), and $MgSO_4$ (Supelco); chloroform (Merck Millipore); and methanol (Merck Millipore).

Cell proliferation with liquid GPY Medium

Strain *L. starkeyi* Y853 cultivated into liquid GPY medium composition consisting of (g/L): glucose (100); Peptone (3); and yeast extract (8). Inoculum was taken as much as 10% (v/v) into 45 mL liquid GPY medium, incubate at 28°C for 24h in shaking water bath (*B-One)* at 180 rpm (Jiru et al., 2016).

Cultivation of *L. starkeyi* in liquid NLM

The results of subculture in GPY medium, then measured the optical density (OD) until it reached value of 0.8 using UV-VIS spectrophotometer (*Thermo Scientific*). Subsequently, the effect of the NLM composition in lipid production was observed with six variables at two factor levels (Table 1). Then, *L. starkeyi* cultivated in liquid NLM accordance with the experimental design (Table 2), which was design using Plackett-Burman Design (PBD). The components and levels were

determined by PBD method. The medium was prepared in 50 mL, then incubated in shaking water bath *(B-One)* for 72h at 28°C with agitation at 180 rpm (Jiru et al., 2016).

Table 1 Determination of components and levels using the Plackett-Burman Design (PBD).

No	Component	Variable code	Low Level (g/L)	High Level (g/L)
1	Glucose	A	10	23
2	Peptone	B	0	0.3
3	*Yeast extract*	C	0	0.5
4	KH_2PO4	D	0	7
5	$Na_2HPO_4.7H_2O$	E	0	2
6	$MgSO_4$	F	0	1.5

Table 2 Experiment design using Plackett-Burman Design

Run Order	A*	B*	C*	D*	E*	F*
1	23	0.3	0	7	0	0
2	10	0	0	7	2	1.5
3	10	0.3	0	0	0	1.5
4	10	0.3	0.5	7	0	1.5
5	23	0	0	0	2	1.5
6	10	0	0	0	0	0
7	10	0.3	0.5	0	2	0
8	23	0	0.5	0	0	0
9	10	0	0.5	7	2	0
10	23	0.3	0	7	2	0
11	23	0	0.5	7	0	1.5
12	23	0.3	0.5	0	2	1.5

Note: variable with * symbol is in g/L

Determination of dry biomass weight of *L. starkeyi* Y853

15 mL of the sample was taken from cultivation of NLM, then centrifuged at 5000 rpm for 10 minutes. Sample washed with distilled water (15 mL) twice. Subsequently, the washed sample in conical bottle dried in oven *(Despatch)* at 60°C until the weight reach constant (Amir et al., 2015).

Lipid extraction and determination of lipid weight of *L. starkeyi L. starkeyi Y853*

Lipid extraction was conducted using the modified Bligh and Dyer (1959) method. 35 mL sample was taken from cultivation of NLM, then centrifuged at 5000 rpm for 10 minutes. Sample washed with distilled water (15 mL) twice. Subsequently, 7 mL of 4 M HCl added into the sample and heated in mini water bath for 2h at 60°C. After that, 14 mL mix solution of chloroform:methanol (2:1) was added into the sample and placed at room temperature for 3h. Then, the sample centrifuged at 5000 rpm for 10 minutes in order to separate the liquid phase (upper phase) and organic phase (lower phase), which are contain lipid. The lipid content then extracted with pipette and evaporated in mini water bath *(B-One)* at 80°C in fume hood, until the lipid remains in the bottom of the bottle. Lipid evaporation result was weighed and recorded (Amir et al., 2015). The lipid content was determined using the following formula:

$$Lipid\ content\ (\%) = \frac{lipid\ weight\ (gr)}{dry\ biomass\ weight\ (gr)} \times 100\%$$

(Amir et al., 2015)

Determination of Sugar Consumption in Liquid NLM

35 mL sample was taken from cultivation of NLM, then centrifuged at 5000 rpm for 10 minutes. The supernatant taken using pipette to transfer into a conical tube. Then, 1 mL of the supernatant sample transferred into test tube, followed by adding the 2 mL distilled water and 1 mL of DNS reagent consist of (g/L): 2,3-dinitrosalicylic acid powder (10); Ka-Na tarter (18.2); NaOH (10). The sample was heated with hot water for 2 minutes. Then, the sample cooled with ice water at 4ºC for 1 minute, then removed and transferred to a cuvette. The absorbance value was measured at wavelength of 540 nm using UV-VIS spectrophotometer. The obtained absorbance value was plotted on a standard curve to determine the concentration of glucose in the sample (Julaeha et al., 2016).

Data Analysis

Plackett-Burman Design (PBD) was utilized to optimize the experimental conditions. The PBD employs at two-level design (Table 1) as factor for observing the screening of significant effects on lipid and biomass production by *L. starkeyi* Y853 with six variables. The data will be tabulated using Microsoft Office Excel 2020, then analyzed using MiniTab Version 19. The output will be in the form of ANOVA calculation tables and main plot effects. The calculation of the PBD can be obtained using the formulas showed in Eq. 1 – 4.

$$Diff\ A = \Sigma A\ (H) + \Sigma A(L) \quad \text{(Eq.1)}$$

$$Eff\ A = x_{A(H)} - x_{A(L)} \quad \text{(Eq. 2)}$$

$$Mean\ sq.\ A = \frac{(\Sigma A(H) - \Sigma(L))^2}{total\ trial} \quad \text{(Eq. 3)}$$

$$F\ value = \frac{Mean\ sq.factor}{Mean\ sq.error} \quad \text{(Eq. 4)}$$

(Stanbury, et al. 1995)

RESULTS

Lipid Weight (g/L), Biomass Dry Weight (g/L) and Lipid Percentage (%)

Based on Table 3, the lowest lipid weight in experiment 8 and the highest in experiment 11 yield in 0.0286 g/L and 1.6 g/L, respectively. While the lowest biomass yield in experiment 10 and highest in experiment 12 were 8.533 g/L and 15.767 g/L, respectively. The highest percentage of lipids was obtained in experiment 1 at 12.37%, while the lowest value was observed in experiment 8 at 0.24%. The highest values for lipid weight, lipid content, and biomass dry weight were observed when high level of glucose (variable A) was utilized, while the lowest values is obtained when using

low level of $MgSO_4$ (variable F). The results are then submitted to further analysis using MiniTab version 19, which generates Analysis of Variance (ANOVA) table and main effect plot.

Table 3. Experiment Design with result of lipid weight (g/L), biomass dry weight (g/L), & lipid content (%)

Experiment	A*	B*	C*	D*	E*	F*	Lipid Weight (g/L)	Lipid Content (%)	Biomass dry weight (g/L)
1	23	0.3	0	7	0	0	1.3143	12.37%	10.622
2	10	0	0	7	2	1.5	0.3429	3.44%	9.978
3	10	0.3	0	0	0	1.5	0.6857	5.99%	11.444
4	10	0.3	0.5	7	0	1.5	0.4000	3.01%	13.289
5	23	0	0	0	2	1.5	0.2286	1.96%	11.644
6	10	0	0	0	0	0	0.2286	2.44%	9.356
7	10	0.3	0.5	0	2	0	0.2000	1.43%	14.000
8	23	0	0.5	0	0	0	0.0286	0.24%	11.822
9	10	0	0.5	7	2	0	0.4000	4.28%	9.356
10	23	0.3	0	7	2	0	0.4286	5.02%	8.533
11	23	0	0.5	7	0	1.5	1.6000	11.41%	14.022
12	23	0.3	0.5	0	2	1.5	1.4286	9.06%	15.767

Note: variable with * symbol is in g/L

Effects of NLM Components on Lipid Production by *L. starkeyi* Y853

ANOVA was conducted to determine which variables that influence on lipid production by *L. starkeyi* Y853 in NLM. Based on Table 4, the P-Value associated with the model is not statically significant ($P > 0.05$). The highest value was observed for the yeast extract variable (0.680), while the lowest value was observed for the glucose variable (0.204). Therefore, it can be concluded that none of the variables exhibited a statically significant impact on lipid production. Thus, the effect of each NLM component on lipid production by *L. starkeyi* Y853 will be observed using the main effects plot (Figure 1).

Table 4. ANOVA calculation result for lipid weight (g/L)

Variable Code	Factor	Df	Adj SS	Adj MS	F-Value	P-Value
	Model	6	1,64338	0,27390	0,91	0,550
	Linear	6	1,64338	0,27390	0,91	0,550
A	Glukosa	1	0,6401	0,6401	2,14	0,204
B	Pepton	1	0,221	0,221	0,74	0,430
C	*Yeast Extract*	1	0,0572	0,0572	0,19	0,680
D	KH_2PO_4	1	0,2368	0,2368	0,79	0,415
E	$Na_2HPO_4.7H_2O$	1	0,12577	0,12577	0,42	0,546
F	$MgSO_4$	1	0,36251	0,36251	1,21	0,321
	Error	5	1,49800	0,29960		
	Total	11	3,14138			

df: *degrees of freedom*; SS: *sum of squares*; MS: *mean squares*

Figure 1 illustrates the mean variation of each variable used in the NLM process between low and high level in relation to lipid production. The graph indicated that three variables (peptone, yeast

extract, & $MgSO_4$) have positive effect, two variables (glucose & KH_2PO_4) have minimum impact and one variable ($Na_2HPO_4.7H_2O$) has negative effect.

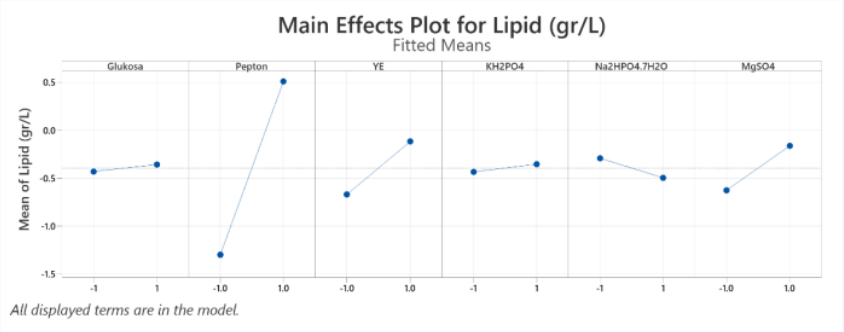

Figure 1 Main effect plot between NLM component with lipid weight (g/L)

Effects of NLM Components on Lipid Production by *L. starkeyi* Y853

Biomass harvesting was conducted through the drying of the biomass using an oven. The objective was to determine the dry weight of the biomass produced by *L. starkeyi* Y853. The result of dry weight biomass will be used as a basis for calculating the lipid content. It is therefore essential to determine which component of NLM are responsible for biomass production. As illustrated in Table 5, the P-value of the model is statically significant ($P \leq 0.05$). The highest value was observed for $Na_2HPO_4.7H_2O$ variable (0.756), meanwhile the lowest value was observed for yeast extract (0.008). The order of the P-values from highest to lowest, is as follows: $Na_2HPO_4.7H_2O$ > glucose > peptones > KH_2PO_4 > $MgSO_4$ > yeast extract.

Table 5 ANOVA calculation result for biomass weight (g/L)

Variable Code	Factor	Df	Adj SS	Adj MS	F-Value	P-Value
	Model	6	48,6254	8,1042	6,41	0,030
	Linear	6	48,6254	8,1042	6,41	0,030
A	Glukosa	1	2,0725	2,0725	1,64	0,257
B	Pepton	1	4,6588	4,6588	3,68	0,113
C	*Yeast Extract*	1	23,1824	23,1824	18,33	0,008
D	KH_2PO_4	1	5,6485	5,6485	4,47	0,088
E	$Na_2HPO_4.7H_2O$	1	0,1359	0,1359	0,11	0,756
F	$MgSO_4$	1	12,9273	12,9273	10,22	0,024
	Error	5	6,3244	1,2649		
	Total	11	54,9498			

df: *degrees of freedom*; SS: *sum of squares*; MS: *mean squares*

Figure 2 depicts the mean variation of each variable utilized in the NLM between low and high levels on biomass production. The result indicates that three variables (peptone, yeast extract, & $MgSO_4$) have positive effect, while the other three variables (glucose, KH_2PO_4, & $Na_2HPO_4.7H_2O$) has minimal effect. The order of factors that have a positive effect, from greatest to least, is yeast extract > peptone > $MgSO_4$ > KH_2PO_4 > $Na_2HPO_4.7H_2O$ > glucose.

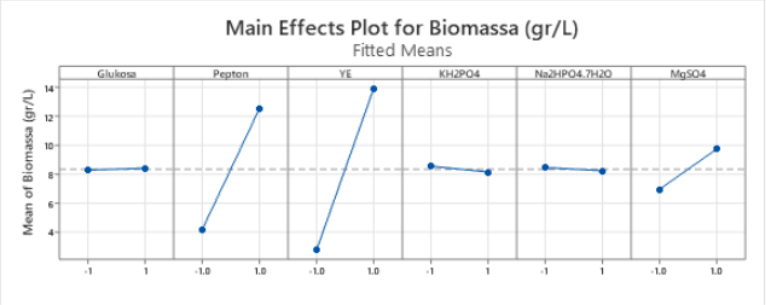

Figure 2 Main effect plot between NLM component with Biomass weight (g/L)

DISCUSSION

The outcomes of the experimental design, which include the result of lipid and biomass weight (g/L) and lipid content (%), are presented in Table 3. The highest values for every variable observed when glucose (Variable A) is in high level, while the lowest results were observed in every variable (Table 3) are when using low levels of $MgSO_4$ (variable F).

The main effect plot (Figure 1 and Figure 2) shows the impact of each factor on the overall performance of the system of which parameters exerts the greatest influence on performance. A horizontal line indicates that the average characteristic is consistent across all parameter variants. If the plot is non-horizontal, it can be concluded that there is a significant effect (Kaur et al. 2023). The effect was observed based on the direction of the sign of the effect to determine whether the average response value increased or decreased. If the effect of parameter is positive, the resulting value indicates that response is high when the levels are high. Conversely, when the effect is negative, it indicates that the average response at the low level resulted in high production outcomes (Antony, 2023).

The two main effect plots (Figure 1 & Figure 2) indicate that yeast extract and peptones had a positive effect on lipid and biomass production. In contrast, the negative effects observed were the result of presence $Na_2HPO_4.7H_2O$, which led to reduction in lipid production, and $MgSO_4$, which had similar effect on biomass production. These findings suggest that the variables displaying negative effects were present at relatively low concentrations, indicating could potentially enhance the lipid and biomass production. Similarly, when variables with positive effects have high levels, lipid and biomass production is correspondingly elevated.

All these results are influenced by several factors, one of them are the presence of carbon source using glucose. Glucose is known to enhance the production of biomass and lipid by *L. starkeyi*. The utilization of glucose by yeast can result in highest biomass and lipid accumulation when compared with other sugar (fructose, sucrose, and lactose) (Liu et al., 2013; VijayaKumar et al., 2010). Moreover, Zhao et al., (2008) indicated that glucose is the primary carbon source utilized, in comparison to other types of sugar. This is evidenced by an observed reduction glucose levels over the initial 72-hour period. Nevertheless, an excessively high glucose concentration can result in a reduction in biomass and lipid. This is due to the fact that elevated glucose concentrations result in diminished biomass and lipid accumulation (Liu et al., 2013)

Nitrogen is a nutrient that plays a pivotal role in cellular growth and metabolism, including processes such as reproduction and lipid accumulation. The nitrogen sources employed were yeast extract and peptone, which demonstrated a beneficial impact on biomass and lipid production by *L. starkeyi* Y853. The impact of varying yeast extract and peptone compositions did not yield statistically significant results for lipid production (Table 4). Nevertheless, the findings of Liu et al., (2013) indicate that yeast extract and peptone exerted a minimal impact on biomass production and the proportion of lipids produced by *L. starkeyi*. This is due to the fact that inorganic nitrogen sources result in higher lipid production than organic nitrogen sources.

It is established that yeast utilizes nitrogen sources derived from ammonia, glutamine, and asparagine to facilitate cellular processes. However, the nitrogen source employed in the form of amino acid represents a secondary nitrogen source, which is utilized in low nitrogen conditions to facilitate the synthesis of specific catabolic enzymes. Therefore, the utilization of nitrogen sources derived from peptone can facilitate positive outcomes in yeast metabolism, thereby enabling the production of elevated biomass and ethanol by *Saccharomyces cerevisiae* (Cruz et al., 2002).

The ANOVA calculation (Table 4) revealed that yeast extract (YE) yields the least favorable results. These finding indicate that YE exerts a notable influence on biomass production. YE is one of the nitrogen sources in NLM and plays crucial role in biomass production. YE contains micronutrients that contribute to the growth of yeast cells. Gao et al., (2013) stated that YE is valuable nitrogen source that stimulate cell growth, leading to high biomass production. This is evidenced by the biomass production of 5.6 g/L with lipid of 4.3 g/L by *Mortierella isabelline* when using YE as the sole nitrogen source.

Inorganic sources and micronutrients also play a role in biomass and lipid accumulation. The inorganic sources use is in the form of $MgSO_4$, KH_2PO_4, and $Na_2HPO_4.7H_2O$. Inorganic elements, including magnesium, sulfur, potassium, sodium, and phosphorus, have been demonstrated to influence lipid production by *L. starkeyi* Y853 (Saenge et al., 2011).

The presence of $MgSO_4$ has been identified as a significant factor influencing the growth of *L. starkeyi* Y853. This compound has been observed to utilize a positive impact on the main effect graph of NLM components on lipid production, when compared to other inorganic compounds (Figure 1). The presence of SO_4^{2-} ions at low concentration has been observed to stimulate lipid production, with excess carbon sources redirected towards the synthesis of lipids. This was demonstrated in study by Morales-Palomo et al., (2023), which yield a low lipid at 0.3 g/g by *Yarrowia lipolytica* at low SO_4^{2-} concentration. As SO_4^{2-} at low concentrations is a crucial compound for oleaginous yeast, functioning as a coenzyme to facilitate lipid production. The process entails the diversion of excess carbon to the lipid synthesis process.

In yeast metabolism, magnesium (Mg^{2+}) has been observed to reduce glucose-6-phosphate production, total phospholipid composition, and oxygen consumption. This allows it to affect the phospholipid membrane of the cell, which will result in diminished cell growth, delayed cell cycles, and other metabolic activities (Dzurendova et al., 2020). Therefore, low concentration of $MgSO_4$ will result in increased biomass and lipid production by *L. starkeyi*.

As illustrated in Figure 1, KH_2PO_4 exerts a minimal impact on lipid production by *L. starkeyi* Y853. The presence of phosphate (PO_4^{3-}) has been demonstrated to affect biomass and lipid production by oleaginous microbes, as evidenced by studies on *Mucor circinelloides* isolates, which produce low levels of lipids in high phosphate concentrations (Dzurendova et al., 2020). Furthermore, the production of lipids is enhanced when of KH_2PO_4 has low concentration, with an output of 11.4 g/L achieved by *Rhodosprodium toruloides* Y4 (Y. Li et al., 2006). The production of lipids in conditions of low phosphate concentration will be regulated by the diacylglycerol (DAG) mechanism, the supply of NADPH, and the activity of isocitrate dehydrogenase, resulting in an increase in lipid production (Morales-Palomo et al., 2023)

The utilization of $Na_2HPO_4 \cdot 7H_2O$ in NLM by *L. starkeyi* Y853 serves as a stimulus for biomass and lipid production. The P-value for $Na_2HPO_4.7H_2O$ is high (Table 5), indicating that this component has a minimal impact on biomass production. This result would be inaccurate since $Na_2HPO_4.7H_2O$ can promote biomass production. This has been demonstrated to induce lipid production by *Rhodosporidium toruloides*, as it enhances the absorption of carbon source for essential metabolic processes and lipid biosynthesis (Osorio-González et al., 2023). Further study by Chang (1986) demonstrated that *Candida tropicalis* is capable of producing high biomass when utilizing Na_2HPO_4. Therefore, $Na_2HPO_4 \cdot 7H_2O$ can be employed as a stimulus for growth and high lipid and biomass production.

CONCLUSION AND SUGGESTION

The analysis using the PBD yielded inconclusive results regarding the impact of the selected factor on lipid production by *L. starkeyi* Y853, with insignificant values ($P > 0.05$). The component that exhibited the most positive effect on lipid production was peptones, while yeast extract demonstrated the greatest positive impact on biomass production. Meanwhile, the components that exhibited the negative impact on lipid production are yeast extract and magnesium sulfate, while magnesium sulfate has negative impact on biomass production. It is necessary to incorporate additional variables beyond the medium components (pH, temperature, & shaker speed) to achieve more significant outcomes to lipid accumulation by *L. starkeyi* Y853.

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