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Research Article

The Effect of Growth Temperature and pH on Lipid Production of *Mucor irregularis* JR 1.1 for Potential Biodiesel Applications

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ABSTRACT
Mucor irregularis JR 1.1 is a filamentous fungus that can produce lipids. The lipids produced by M. irregularis JR 1.1 reach 40-60%, so they can be used as raw material for biodiesel. Temperature and pH are important factors that support M. irregularis JR 1.1 lipid production. Optimum temperature and pH enhance the activity of enzymes involved in lipid production. Based on previous research, the optimization of temperature and pH in the lipid production of M. irregularis JR 1.1 has not been determined. Therefore, this study aims to determine the optimal temperature and pH for lipid production by M. irregularis JR 1.1. The study involved varying temperature and pH treatments using Response Surface Methodology (RSM). Subsequently, lipid production was performed at the optimum temperature and pH under different incubation times. Based on the research results, the highest biomass production was observed at 35°C and pH 4, the highest lipid production was at 35°C and pH 5.5, and the highest glucose consumption rate was at a temperature of 23°C and pH 5.5. The highest lipid yield percentage was 6.2%, and the highest production rate was 0.011g/L/hour at the 96th hour of incubation. Statistical analysis showed high significance, with R^2 values of 95.88% for lipid production and 89.98% for biomass yield. These findings suggest that M. irregularis JR 1.1 has promising potential as a microbial lipid source for sustainable biodiesel production, particularly under optimized culture conditions.

Keywords: *Mucor irregularis JR 1.1; Temperature; pH; Lipids.*

Introduction

Energy plays a crucial role in achieving social, economic, and environmental goals for sustainable development. However, the increasing global demand for energy is not matched by the availability of environmentally friendly and renewable sources. The continued reliance on fossil fuels contributes to environmental degradation and climate change [1]. One solution is the development of renewable energy, such as biodiesel derived from vegetable and animal oils. According to the International Energy Agency (IEA), global biodiesel production is projected to exceed 50 billion liters by 2030, driven by the increasing demand for renewable and sustainable fuels. Biofuel demand is set to expand 38 billion litres over 2023-2028, a near 30% increase from the last five-year period. Although promising, the production of biodiesel from plant or animal sources is considered inefficient since it requires agricultural land, which may reduce land availability for food crops [2].

As an alternative, biodiesel from microbial lipids is more efficient because it requires less land and has higher lipid productivity [1]. Microbial lipids are

produced through biomass extraction from fungi, yeasts, bacteria, and microalgae, with fungi capable of accumulating over 20% lipids from their total dry biomass [3]. Fungi from the genera *Mucor*, *Cunninghamella*, and *Rhizopus* can generate lipid content up to 39.27%-42.6% [4].

Factors such as optimal temperature and pH can enhance lipid accumulation productivity in fungi [4]. Research by Haura and ilmi [5], demonstrated optimal lipid accumulation by *M. irregularis* at 144 hours of incubation with 200 rpm agitation using glucose as a carbon source, yielding 40-60% lipid content. However, further studies are needed to optimize temperature and pH conditions to achieve better lipid production [6].

Materials and Methods

Subculturing the Oleaginous Fungus M. irregularis JR 1.1

The culture used in this research is a stock culture of *M. irregularis* JR 1.1, originally isolated from an orange obtained at Kranggan Market, Yogyakarta, Indonesia. This culture is preserved at the Microbiology Laboratory, Faculty of Biology, Gadjah Mada University. Subcultures of *M. irregularis* JR 1.1 were prepared by inoculating the strain on PDA plates using the streak plate method, followed by incubation for 5 days at 30°C. The resulting colonies then re-inoculated on PDA plates using the same method and incubated for 5 days at 30°C.

Spores Suspensions

The results of subculturing on PDA medium in petri dishes were used to prepare the fungal spore suspension. A total of 9 mL of deionized water mixed with 1 mL of 0.01% Triton was added to the growth medium. Spore harvesting was performed using the scraping method, where the fungal-colonized growth medium was scraped off using a needle until a spore suspension was obtained. A 1 mL sample of the spore suspension was then counted using a Neubauer hemocytometer under a light microscope. Based on the hemocytometer count, the spore concentration in each 1 mL of suspension was determined. Subsequently, the counted suspension was transferred into a sterile 100 mL glass bottle [7].

The Medium of Lipid Production

In this research, the lipid production medium for the fungus is composed by several ingredients, including KH2PO4 2.5 g/L, ZnSO4−7H2O 0.01 g/L, CuSO4−5H2O 0.001 g/L, MnSO4 0.01 g/L, MgSO4−7H2O 0.5 g/L, FeSO4−7H2O 0.02 g/L, CaCl2 0.1 g/L, yeast extract 5 g/L, KNO3 1 g/L, and glucose 30 g/L. These ingredients are then dissolved in a buffer solution at various pH levels. The medium to be used is sterilized using an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes [7].

Variation of Temperature and pH

The variations of temperature and pH used in this research were obtained using Response Surface Methodology (RSM) with a Central Composite Design model, which includes repetitions for these variation choices. The fungal spore suspension was inoculated into the production medium at various pH levels until the spore concentration in the medium reached 107. Each temperature and pH variation was incubated on a rotary shaker for 144 hours at an agitation speed of 200 rpm. Biomass production, lipid production, and the biomass generated in each treatment were observed daily [8].

Calculation of Biomass

After incubating for 144 hours at 200 rpm agitation, biomass harvesting was performed using a centrifuge. The production medium in the Erlenmeyer flask, which had been colonized by the fungus from the shaker, was transferred into conical tubes for subsequent centrifugation to separate the biomass from the residual medium. The residual medium was then transferred into sterile glass bottles with a volume of 100 mL. The biomass was placed

in jars and dried in an oven at 50°C until a constant weight was achieved [9].

Lipid Extraction

Lipid extraction was carried out by homogenizing with acid-washed sand at a 1:2 ratio between biomass and acid-washed sand, so the weight of the acid-washed sand was twice the weight of the biomass. The dried biomass was crushed using a mortar and pestle. Sand and dried biomass were placed in a 15 mL conical tube and then mixed with chloroform and methanol. The chloroform ratio used was 1:2 based on the amount of crushed acid-washed sand and dried biomass, while the methanol ratio was 1:3 based on the amount of crushed acid-washed sand and dried biomass. The solution was centrifuged for 10 minutes at a speed of 4000 rpm. Afterward, a separate layer formed between the pellet and supernatant. The supernatant layer contained chloroform and lipids, while the pellet layer contained acid-washed sand and non-lipid biomass. The supernatant containing lipids was transferred to a weighed glass bottle and dried until a constant weight was reached. Subsequently, the glass bottle containing the dried lipids was weighed again to obtain the dry weight of the lipids [7].

Glucose Residue Estimation

The residual glucose was determined using the Dinitrosalicylic Acid (DNS) method. The DNS reagent was prepared by dissolving 1 gram of powdered DNS, 15 grams of K-Na tartrate, and 20 mL of 2M NaOH, then adjusting the volume to 100 mL. This solution was stored in a refrigerator. A standard curve solution was prepared using glucose solutions with varying concentrations of 0.15, 0.2, 0.25, 0.3, and 0.35 g/10 mL [10].

The residual glucose test was conducted by adding 1 mL of sample, 1 mL of DNS reagent, and 2 mL of distilled water to each test tube containing the glucose solutions. The test tubes were then heated in a water bath for approximately 5 minutes to allow a reaction between the glucose solution and the DNS reagent. The resulting values were plotted on the standard curve to estimate the glucose concentration in the samples used [10].

Yield Lipid

The determination of lipid yield is generally obtained by comparing the lipid production (g/L) at incubation times of 0 hours, 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours against the glucose consumption levels, expressed as a percentage. The higher the lipid yield value, the greater the conversion of glucose into lipids [11].

Growth Curve

The growth curve of *M. irregularis* JR 1.1 was generated at the temperature and pH conditions that resulted in the highest lipid accumulation. The concentration of the inoculum was introduced into the liquid medium at the optimal medium concentration, followed by incubation on a rotary shaker. Samples were collected every 24 hours, resulting in data points at incubation times of 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours. The growth curve was also created to estimate the remaining glucose levels.

Determination of Lipid Production Rate

The determination of lipid production rate is obtained by comparing the total lipid accumulation (g/L) with the incubation time (hours), resulting in a production rate measured in g/L/hour. The higher the lipid production rate, the higher the total lipid produced. In this research, the determination of temperature and pH variations was carried out using Response Surface Methodology (RSM) with a Central Composite Design (CCD) model. Raw data analysis was performed using Microsoft Excel. After obtaining the data for biomass production, lipid content, and glucose consumption, the data was then input into an RSM table for subsequent analysis using Design Experts. Subsequently, the analysis of lipid yield and lipid production rate was

conducted using ANOVA in the SPSS software. The confidence level used in this research was 95% (α = 0.05%), which will be tested using the Duncan Test [12].

Results and Discussion

Biomass of *M. irregularis* was obtained by cultivating the fungus in a lipid production medium for 144 hours with 200 rpm agitation, followed by centrifugation at 5000 rpm for 20 minutes. The resulting biomass was dried in an oven for 24-48 hours to a constant weight, and lipids were then extracted. Temperature and pH variations during incubation significantly affected biomass yield, lipid production, and glucose consumption by *M. irregularis* strain JR 1.1.

Table 1. The Effect of Temperature and pH on Biomass Production, Lipid Yield, and Glucose Consumption by *M. irregularis* JR 1.1

Treatment	Factor 1	Factor 2	Response 1	Response 2	Response 3
	Temperature (°C)	pH	Biomass Production (g/L)	Lipid Production (g/L)	Consumption Glukosa (%)
1	23	4	9.76	5	88.19
2	35	7	18.26	9.3	73.39
3	23	7	10.2	6.22	91.61
4	29	5.5	12.34	6.66	93.64
5	29	5.5	8.9	6	94.49
6	35	4	18.9	10.92	61.11
7	29	5.5	9.8	6.36	87.08
8	29	5.5	9.24	6.96	93.22
9	23	5.5	11.44	6.52	94.7
10	29	5.5	8.9	5.42	93.64
11	29	5.5	9.62	6.82	83.9
12	29	7	9.44	6.36	85.97
13	29	4	13.22	5.8	81.94
14	35	5.5	17.84	11.3	83.9

Before conducting contour plot analysis, a lack of fit test was performed to evaluate whether the ANOVA model was appropriate. Since all p-values were above 0.05, the null hypothesis in ANOVA was accepted, indicating that the model was valid. Thus, the model can be reliably used for further contour plot analysis.

Biomass Production

In the contour plot illustrating the effect of temperature and pH on biomass production by the fungus *M. irregularis* JR 1.1, closely spaced contour lines indicate steeper changes, while widely spaced lines indicate more gradual changes. In the contour plot showing the influence of temperature and pH on biomass, the blue-colored contour area displays widely spaced lines, suggesting that changes in biomass within the temperature range of 23°C to 29°C are relatively gradual. In contrast, the contour lines in the temperature range of 32°C to 35°C are more closely spaced, indicating that biomass changes within this range are steeper (Figure 1).

Lipid Production

The contour plot illustrating the effect of temperature and pH on lipid production by the fungus *M. irregularis* JR 1.1 in Figure 6 shows that each combination of temperature and pH treatments resulted in significantly different lipid production levels. The contour lines in the plot indicate

that the changes in lipid production are quite steep. High lipid production is represented by reddish-orange contour colors, while low lipid production is indicated by blue contour colors (Figure 2).

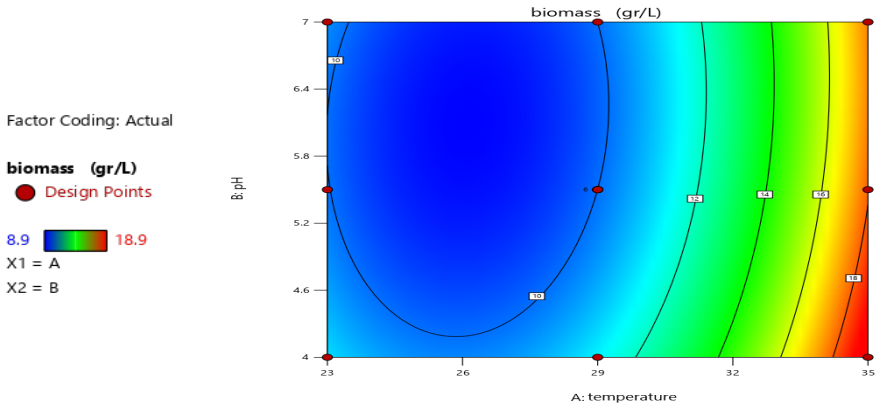

Figure 1. Contour plot graph showing the effect of temperature and pH on biomass production.

The contour plot analysis showed that the highest biomass production was achieved at 35°C and pH 4 with a yield of 18.9 g/L (Figure 1). Comparable biomass yields were observed at 35°C with pH 5.5 (17.84 g/L) and pH 7 (18.26 g/L), with no significant differences among them. The lowest yield was recorded at 29°C and pH 5.5 (8.9 g/L). This suggests that *M. irregularis* JR 1.1 requires temperatures above 30°C for optimal biomass production, as temperatures between 30-35°C align with its incubation needs and enhance its metabolic activity [13], [14].

Optimal pH for fungal growth is acidic, between 4 and 6; alkaline conditions can cause metal ion toxicity, reducing nutrient availability and thus biomass productivity. Biomass production involves nutrient uptake (C, N, P, S, water), entering primary metabolic pathways like glycolysis and the Krebs cycle to synthesize new cell components. Several enzymes, such as acid phosphatase, glucanase, and feroxidase, are more active in acidic conditions, supporting nutrient uptake and biomass production. Enzymes like lipase and protease also function better at low pH, breaking down lipids and proteins for nutrient supply [14].

ANOVA analysis showed a determination coefficient (R^2) of 89.98%, indicating that temperature and pH account for most of the variability in biomass production, with the remaining 10.02% influenced by other factors [15], [16].

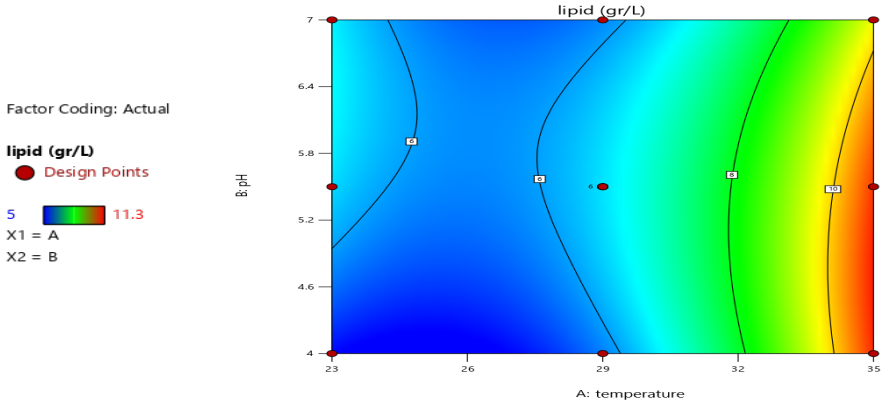

Figure 2. Contour plot graph of the effect of temperature and pH on lipid production by *M. irregularis* JR 1.1.

Lipid production was lower at temperatures between 23°C to 29°C compared to 35°C. At 35°C, lipid accumulation was higher at pH 5.5 (11.3 g/L) than at pH 4 (10.92 g/L), though the contour plot showed them at similar levels (Figure 2). The highest lipid yield occurred at 35°C and pH 5.5, while the lowest was at 23°C and pH 4. This aligns with research by Hashem [13], which suggests that optimal lipid synthesis occurs between 30°C to 35°C due to increased enzyme activity. Specifically, the enzyme acetyl-CoA carboxylase (ACC) plays a critical role in lipid synthesis, with its activity enhanced at higher temperatures due to increased kinetic energy of reacting molecules [17]. Lower temperatures can reduce membrane fluidity, thus limiting biochemical processes and lipid biosynthesis [15].

The optimal pH for lipid production was found to be 5.5, as it maintains a high concentration of intracellular H+ ions, which are essential for the activities of enzymes like glycerol-3-phosphate acyltransferase (GPAT) and diglyceride acyltransferase (DGAT) involved in triacylglycerol (TAG) biosynthesis. These enzymes facilitate the early stages of TAG synthesis by transferring fatty acid groups, leading to increased lipid accumulation. The statistical analysis showed that the coefficient of determination (R^2) for lipid production was 95.88%, indicating a strong correlation between temperature and pH, with only 4.12% of the variance attributed to other factors not captured in the model [8].

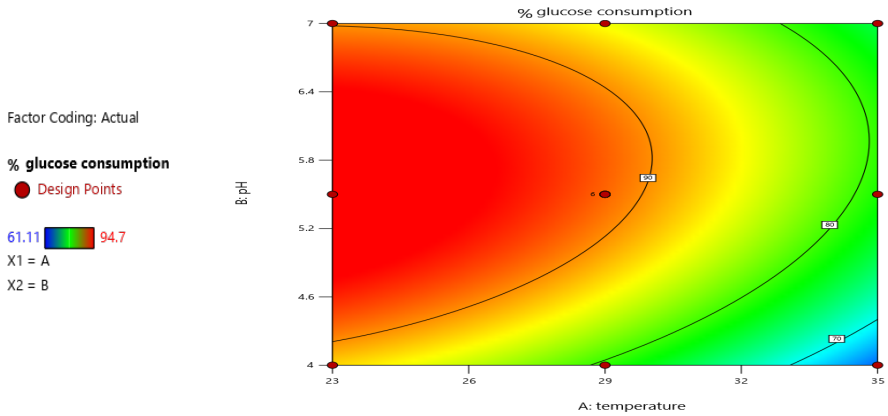

Figure 3. Contour plot graph showing the effect of temperature and pH on glucose consumption by *M. irregularis* JR 1.1.

Glucose Consumption

The glucose levels consumed by *M. irregularis* JR 1.1 during biomass and lipid production under incubation with a shaker at 200 rpm are shown in the contour plot in Figure 7. The graph illustrates that glucose consumption varied significantly across the different treatment combinations. The contour lines indicate that changes in glucose levels were relatively steep; however, compared to the contour plots of biomass and lipid production, the contour plot for glucose consumption appears more gradual. This is evident from the relatively small differences in glucose consumption across treatments. High glucose consumption occurred at lower temperatures, particularly around 23°C. In the graph, high glucose consumption is indicated by reddish-orange contour colors, while low consumption is shown in bluish contour colors (Figure 3).

The glucose levels were measured using the DNS method with a calibrated standard. The contour plot (Figure 3) indicates that the highest glucose consumption occurred at a temperature of 23°C and pH 5.5 (94.7%), while the lowest was at 35°C and pH 4 (61.11%). The data analysis shows that maximum glucose

consumption occurred during biomass and lipid production at 23°C and pH 5.5, which is relatively low for optimal growth of *M. irregularis* JR 1.1. Lipid accumulation is driven by the activity of acetyl-CoA carboxylase (ACC), which peaks at 30°C - 35°C, resulting in lower lipid accumulation at 23°C [1]. At this lower temperature, the fungus tends to consume accumulated lipids for its nutritional needs when glucose in the medium becomes limited [8].

Additionally, enzymes involved in biomass and lipid production are more active in acidic conditions, accelerating metabolism and thereby increasing glucose consumption. The statistical analysis yielded a determination coefficient (R^2) of 85.74%, indicating that 85.74% of the variation in glucose consumption can be explained by temperature and pH, while the remaining 14.26% is influenced by unknown factors.

Optimization of Lipid Production

The optimization of temperature and pH for lipid production by *M. irregularis* JR 1.1 can be seen in Table 4. The optimum point was obtained from lipid production results across various temperature and pH treatments, analyzed using the Design Expert software through the numerical optimization method.

Numerical optimization is a method used to find the optimal point of data through numerical calculations [18]. This method was chosen because the data in this study had not yet reached an optimal point, making other approaches like graphical optimization less suitable. Further research with a broader range of temperatures and pH levels is needed to reach the optimal point. This study used a range of 23°C to 35°C for temperature and pH 4 to 7, aligning with conditions commonly applied in industrial settings.

As shown in Table 2, the optimal point for lipid production was achieved at a temperature of 35°C and pH 4.627. This is because oleaginous fungi, such as *M. irregularis*, prefer warmer conditions around 35°C and slightly acidic pH levels, around 4.627. The selected optimal conditions were then used as a reference for developing the growth curve, which provides insights into the lipid production rate of *M. irregularis* JR 1.1 over time [14].

Table 2. Optimization of lipid production by *M. irregularis* JR 1.1 using the numerical optimization method

Temperature (^{0}C)	pH	Lipid (g/L)	*Desirability Selected*
35	**4.627**	**11.145**	**0.975**
35	4.638	11.145	0.975
35	4.617	11.145	0.975
35	4.653	11.145	0.975
35	4.591	11.145	0.975
35	4.667	11.145	0.975
35	4.691	11.144	0.975
35	4.549	11.144	0.975
35	4.735	11.142	0.975
35	4.838	11.134	0.974
35	4.335	11.123	0.972
35	4.946	11.119	0.971
35	5.296	11.029	0.957
23	6.440	6.584	0.251
23	6.409	6.584	0.251

Growth Curve of Biomass, Lipid, and Glucose Consumption

The growth curves of biomass, lipid production, and glucose consumption by *M. irregularis* JR 1.1 under optimal temperature and pH conditions at different incubation times are shown in Figure 4 and 5. The incubation times used to construct these growth curves include 24, 48, 72, 96, 120, 144, and 168 hours.

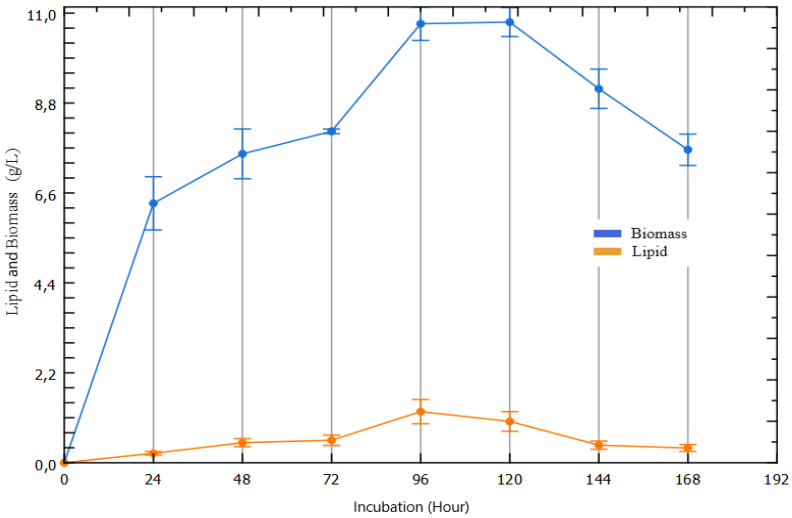

Figure 4. Growth Curve of Biomass and Lipid of *M. irregularis* JR 1.1.

Figure 4 shows that biomass and lipid production significantly differ at different incubation times. Biomass production increases until 120 hours, then declines sharply between 144 and 168 hours, entering the death phase, which causes the graph to drop. A similar decline occurs in lipid production after 120 hours due to the depletion of glucose, the main nutrient. As glucose availability decreases, lipid accumulation decreases and eventually stops. The lipid accumulation process consists of three phases: the first phase (0 to 28 hours) is when the oleaginous cells adapt to the medium. In the second phase (28 to 48 hours), cells reproduce, but lipid accumulation is still low. The third phase (after 48 hours) is when lipid accumulation increases as carbon sources are used for lipid production, until the carbon supply is exhausted [19].

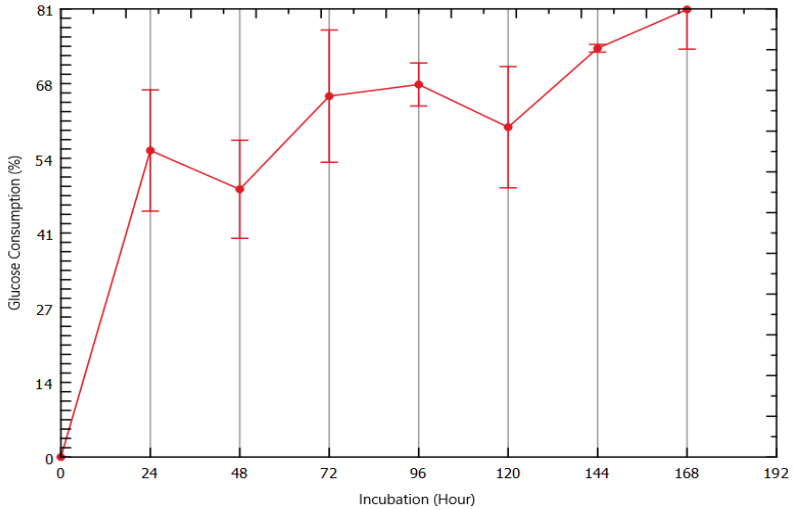

Figure 5. Glucose consumption rate growth curve (%).

Figure 5 shows that glucose consumption was highest at the 168-hour incubation, while biomass production peaked at 120 hours and lipid production at 96 hours. As incubation time increased, glucose consumption also rose, likely due to the depletion of other nutrients in the medium, prompting *M. irregularis* JR 1.1 to consume more glucose as a backup. However, this increase in glucose consumption did not correlate with lipid accumulation, which began to decline after 120 hours. The high glucose consumption at 144 and 168 hours indicates that although glucose was consumed, it was not converted into lipids. This could be due to the crabtree effect, where glucose availability leads to ethanol production instead of lipid synthesis. This effect shifts the metabolism from aerobic respiration to fermentation, allowing *M. irregularis* to produce energy more quickly at the expense of lipid production [19].

Lipid Yield and Lipid Production Rate

Lipid yield is generally calculated by comparing the total lipid produced (g/L) to the amount of glucose consumed (g/L) at each incubation time, multiplied by 100%. Glucose consumption is determined by subtracting the residual glucose in the medium after incubation from the initial glucose concentration. A higher yield value indicates a more efficient conversion of glucose into lipid. The results of glucose consumption at different incubation times are shown in Figure 6.

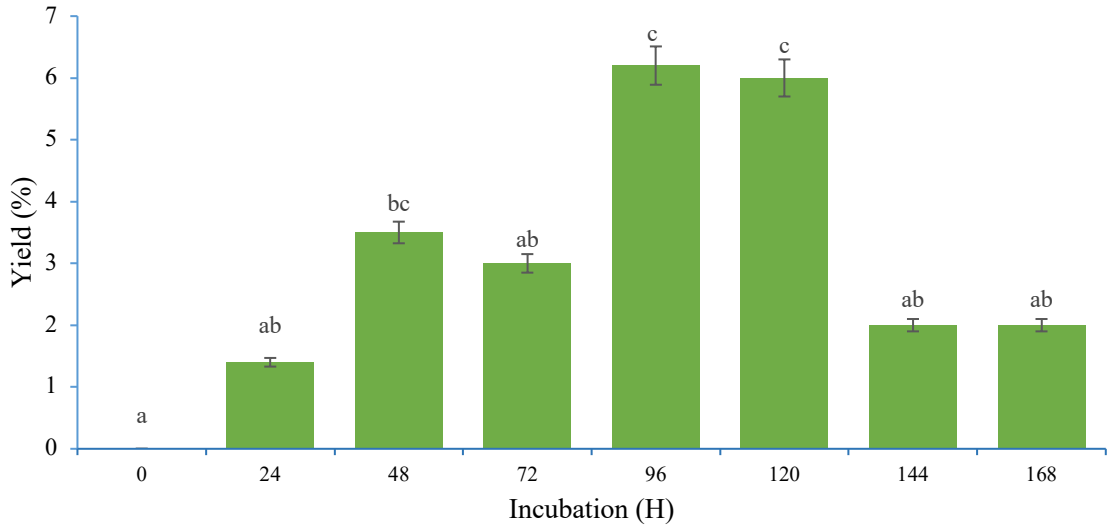

Figure 6. Lipid yield.

The analysis in Figure 6 shows that the highest lipid yield was achieved at 96 hours of incubation, reaching 6.2%. However, this differs from previous findings where *M. irregularis* JR 1.1 achieved optimal biomass and lipid production at 144 hours [19]. This suggests that 96 hours is not yet an efficient incubation time for optimal lipid and biomass production, as the cells are not fully active at that point. The lowest lipid yield was recorded at 24 hours (1.4%) due to the initial adaptation phase, where lipid biosynthesis is still slow.

Although glucose consumption was higher at 144 hours compared to 96 hours, the lipid yield was lower. This is because, after 96 hours, the fungus began utilizing the accumulated lipids to meet its nutritional needs, reducing the total lipid yield. The overall lipid yield in this study was relatively low, as previous studies on Mucorales fungi reported yields as high as 13-14%. The low yield observed may be

due to the cells reusing lipids during the death phase to sustain their nutrition, thereby reducing the overall lipid accumulation [11].

The observed increase in lipid accumulation at 35°C and pH 5.5 can be attributed to enhanced metabolic activity driven by enzyme regulation under favorable environmental conditions. Lipid biosynthesis in oleaginous fungi is closely linked to the central carbon metabolism, particularly the conversion of glucose into acetyl-CoA through glycolysis and the tricarboxylic acid (TCA) cycle. Once nitrogen in the medium becomes limited, excess carbon flux is redirected toward lipid biosynthesis rather than biomass formation [20].

A key enzyme involved in this redirection is acetyl-CoA carboxylase (ACC), which converts acetyl-CoA to malonyl-CoA, the first committed step in fatty acid biosynthesis. This process is further catalyzed by fatty acid synthase (FAS) complexes, enabling chain elongation. At pH 5.5, intracellular proton gradients are optimal for enzyme activity, supporting the function of GPAT (glycerol-3-phosphate acyltransferase) and DGAT (diacylglycerol acyltransferase), which play pivotal roles in triacylglycerol (TAG) assembly TAG being the primary storage lipid in oleaginous fungi [19].

Furthermore, the temperature of 35°C may enhance kinetic energy within the cell, increasing enzyme-substrate interactions and the overall metabolic flux toward lipid storage pathways. This is consistent with previous studies in other oleaginous fungi, such as *Mortierella alpina* and *Yarrowia lipolytica*, where temperatures between 30–35°C and slightly acidic conditions significantly boosted lipid accumulation [15], [17].

In oleaginous fungi such as *M. irregularis* JR 1.1, lipid accumulation is typically triggered under nitrogen-limited conditions with excess carbon supply. This metabolic shift results in decreased protein and nucleic acid synthesis, diverting the carbon flux from growth pathways toward lipid biosynthesis, mainly in the form of triacylglycerols (TAGs) stored in lipid bodies. At high C/N ratios, lipogenesis is favored, but very high carbon levels may induce osmotic stress, disrupt nutrient transport and reducing growth and lipid accumulation [21].

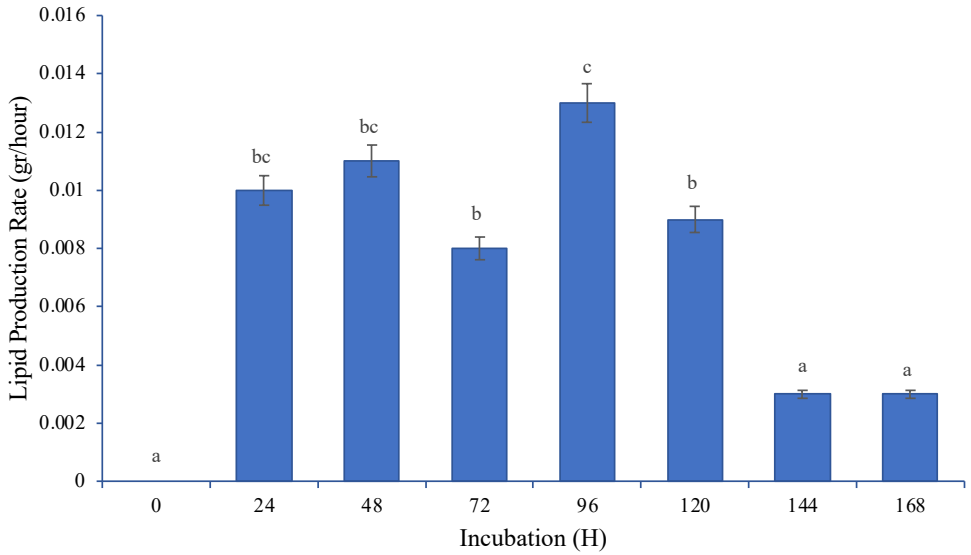

Figure 7. The lipid production rates at different incubation times.

Although glucose consumption was high at 144 and 168 hours, lipid yield declined. This suggests glucose was not efficiently directed into lipid biosynthesis.

One explanation is the Crabtree effect, in which high glucose levels stimulate fermentation even in the presence of oxygen redirecting carbon away from lipid synthesis into ethanol or other metabolites [21].

Moreover, during prolonged cultivation, nutrient exhaustion and entry into the stationary or death phase may trigger lipid catabolism. In this phase, stored lipids are broken down for maintenance energy, explaining the decline in lipid yield despite continued glucose uptake. These results highlight the need to balance pH, temperature, C/N ratio, and incubation duration for optimal lipid productivity in biodiesel applications [21].

Lipid production rate refers to the speed at which *M. irregularis* JR 1.1 produces lipids. It is calculated by dividing the total lipid produced by the incubation time, resulting in a lipid production rate expressed in units of g/L/hour. Figure 7 shows the lipid production rates at different incubation times.

Figure 7 shows that the highest lipid production rate occurs at 96 hours of incubation. In the earlier stages (24, 48, and 72 hours), there are no significant differences in lipid production rates, as *M. irregularis* JR 1.1 is still adapting to the medium. During this phase, the cells focus on reproduction and growth, leading to suboptimal lipid accumulation. At 96 hours, the production rate peaks as carbon sources are used as precursors for lipid accumulation. However, after 96 hours (at 120, 144, and 168 hours), the lipid production rate declines due to depleted carbon sources, and accumulated lipids are re-utilized by the cells to fulfill their nutritional needs. This decline marks the onset of the death phase, where growth and metabolic activity gradually decrease due to limited nutrient availability in the medium [22].

Conclusion

Based on the results of this research, several conclusions can be drawn. First, the highest biomass production by *M. irregularis* JR 1.1 was achieved at a temperature of 35°C and pH 4, reaching 18.9 g/L, while the highest lipid production occurred at 35°C and pH 5.5 with a yield of 11.3 g/L. Second, the highest glucose consumption by *M. irregularis* JR 1.1 was observed at 23°C and pH 5.5, amounting to 94.70%. Lastly, the maximum lipid yield percentage under optimal temperature and pH conditions was 6.2%, while the highest lipid production rate was 0.011 g/L/hour. Both the highest yield and production rate were recorded at 96 hours of incubation.

Acknowledgments

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Conflict of interest

Authors declare no conflict of interest regarding the study.

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