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STANDARDIZATION OF MALASSEZIA FURFUR GROWTH PARAMETERS IN AN OLEIC ACID MEDIUM, A TARGET TOWARDS THE STATIONARY PHASE FOR THE ENHANCED PRODUCTION OF AZELAIC ACID

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1. Introduction

Microbes deliver secondary metabolites in the medium for survival, competitor, and ecological exchanges instead of primary functions crucial for growth and reproduction. The vital role of secondary metabolites is a defence against predators, compete with or inhibit growth, similar to penicillin in the medium inhibiting competitors. Secondary metabolites also serve as signaling molecules in quorum sensing, adaptation to environmental stresses facing fluctuations in growth conditions such as temperature, pH, or nutrient availability, nutrient scavenging similar to Siderophores, fascinating role in facilitating symbiotic relationships as well as byproducts of primary metabolism from the metabolic pathways. The regular release of secondary metabolites typically occurs in the stationary phase of microbial growth, resulting from low nutrient levels in the medium and a decrease in primary metabolism for survival in the critical medium environment.

Yeast biotechnology, to date, holds valuable insight for the production of industrially important metabolites such as ethanol, organic acids, vitamins, and enzymes. Understanding the yeast growth requirements and standardizing the growth parameters uphold organism-friendly practices in producing many products in various sectors, such as industrial and pharmaceuticals. Many metabolites are obtained from plant sources, and the production of such products remains under question due to the climatic conditions of the plant, required soil fertility, seasonal fluctuations, plant pathogen constraints, and the purity of the final product after processing. Azelaic acid, a dicarboxylic acid group found naturally in rye, wheat, and barley, plays a significant

role in the bio-pharmaceuticals and food industries. The yeast *M. furfur*, resident flora of human skin, also produces this natural substance in its natural environment, where the application of the yeast *M. fufur* in Azelaic acid production industrially is still in progress. The investigation of *M. furfur* azelaic acid production holds noteworthy affirmation in the field of industrial biotechnology. By leveraging the metabolic pathways and growth conditions of yeasts, reasonable and endurable product production methods can be materialized, filling existing gaps and unplugging latest avenues for large-scale production of valuable metabolites in bioprocess industries.

Objectives

With the above review concept, the present study was aimed with following approaches:

- Optimizing the Growth Conditions for ***M. furfur*** in Azelaic Acid Production Medium (Oleic Acid Medium)
- Standardization of ***M. furfur*** Culturing Techniques for Consistent Experimental Outcomes

1. Materials and Methods

Using the yeast *M. furfur* MTCC 1374, a thorough technique was used in the current study to systematically synthesis dicarboxylic acid (azelaic acid) from oleic acid. A series of biochemical processes transition oleic acid into azelaic acid during *M. furfur's* in vitro batch culture manufacturing process.

1.1. Maintenance of *M. furfur* (MTCC 1374)

To maintain stability and viability, *M. furfur* (MTCC 1374), obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) at the Institute of Microbial Technology, Chandigarh, was maintained in appropriate medium. In order to support the microorganisms' development and survival, a defined maintenance routine was followed when conserving the culture.

2.1.1. Saline preparation

Saline with 0.9% was prepared by dissolving 0.9 gms of sodium chloride in 100 mL of dis. water and the pH was maintained to 7.0 by adjusting through 0.1 N NaOH or 0.1 N HCl. The saline was filter sterilized (0.22-micron) and kept in a sterile container until usage (Foster and Geyer, 2008).

2.1.2. Reviving of yeast culture

About 10 mL of 0.9% sterile saline solution in a sterile flask was inoculated with 0.1–0.2 g of *M. furfur* granules aseptically and incubated at 35°C for three to five days. The flask containing 100 mL of sterile Sabouraud Dextrose Broth (SDB) was inoculated with 1 to 2 mL of saline-suspended culture and incubated to track the growth of yeast day by day. The growth of yeast in the medium was confirmed by the appearance of turbidity (Kwon-Chung and Bennett, 1992).

2.1.3. Antibiotic supplement preparation

To inhibit bacterial contamination in the yeast growth media, one vial of CC supplement FD035 (CC—Cycloheximide: 200 mg, Chloramphenicol: 25 mg) was rehydrated aseptically with 5 mL of distilled water, mixed well, and kept at room temperature (Foster & Geyer, 2008).

2.1.4. Sabouraud Dextrose Broth (SDB) preparation

About 100 mL of Sabouraud dextrose broth (SDB) was prepared by adding 0.4 g of peptone and 2 g of dextrose (glucose) to 100 mL of distilled water. The pH was adjusted to 5.6 using 0.1 N HCl or 0.1 N NaOH. The broth was sterilized by autoclaving at 121°C for 15 minutes and supplemented with CC supplement to reduce bacterial contamination (Kwon-Chung & Bennett, 1992).

2.1.5. Sabouraud Dextrose Agar (SDA) preparation

Sabouraud dextrose agar (SDA) medium was prepared by mixing 1 g of peptone, 4 g of dextrose, and 1.5 g of agar in 100 mL of distilled water. The pH was adjusted to 5.6, and the medium was autoclaved at 121°C for 15 minutes. After cooling to 45-50°C, CC supplement was added aseptically. The medium was dispensed into Petri dishes, inoculated with *M. furfur*, and incubated at 35°C for 3-5 days (Foster & Geyer, 2008; Kwon-Chung & Bennett, 1992).

2.1.6. Emmons modified medium preparation

To maintain *M. furfur* (MTCC 1374), Emmons modified medium was prepared by suspending 4.7 grams of the medium powder in 100 mL of distilled water and boiling it to dissolve completely (Table 1). The medium's pH was adjusted to 5.6 to suit the lipophilic yeast's preference for acidic conditions, enhancing growth at 37°C. The medium was then sterilized by autoclaving at 121°C for 15 minutes. After cooling to 45-50°C, a CC supplement (Cycloheximide: 200 mg, Chloramphenicol: 25 mg) was aseptically added to prevent bacterial contamination. Sterile Petri plates were prepared by dispensing 15 mL of the medium, which was then overlaid with 0.1 mL of olive oil to enrich it for *M. furfur*. The plates were stored at 4°C until use. Both broth and agar media were prepared for reviving and culturing the yeast strain. A sterile loop was used to subculture the organism onto fresh plates of Emmons modified agar with olive oil to maintain the culture for future assays. This method ensures optimal growth conditions for *M. furfur* and minimizes contamination, providing a stable environment for consistent experimental results.

Table 1. Emmon's modified medium composition		
Ingredients	:	**Gms / Litre**
Dextrose (Glucose)	:	20
Neopeptone	:	10
Agar	:	20
DI Water	:	1000 Ml
Final pH (at 37°C)	:	5.6±0.2 (adjusted)
A selective lipid source is added (overlaid/dissolved) after sterilization of the media to enhance the growth of *M. furfur*.		
Olive oil (lipid source)	:	0.1-2 mL

2.2. Standardization of *M. furfur* growth parameters in Azelaic acid production medium (OaM) (Guillot et al., 1996)

To promote the growth of *M. furfur* yeast, a preliminary qualitative plate culture method was employed to screen active ingredients that enhance optimal growth. An Oleic acid medium (OaM) was formulated with varying ingredients, as detailed in Table 2 (Guillot et al., 1996). The media formulation was then subjected to a thorough analysis of growth parameters to identify and characterize the growth phases of *M. furfur*.

Table 2 Formulation of *M.furfur* growth media		
S.No	**Media Composition**	**Oleic acid Medium (OaM)**
01	Alpha Tocopherol (mL)#	0.1
02	Ghee (mL)#	0.2
03	Linoleic acid (mL)*	0.1
04	Oleic acid (mL)*	1.5
05	Palmitic acid (gm)#	0.1
06	Squalene (mL)*	0.5
07	Distilled Water (mL)	100
	pH	5.6
	Agar (gm)	1.5
#: Ingredients added after sterilization, *: Ingredients added before sterilization		

2.2.1. Evaluation of *M. furfur* optimal growth in Oleic acid Medium (OaM) using the plate culture method

The plate culture method was employed to evaluate the optimal growth conditions of *M. furfur* in Oleic Acid Medium (OaM), formulated per 100 mL as detailed in Table 2. The pH of the medium was adjusted to 5.6 using 0.1 N HCl and 0.1 N NaOH solutions.

After sterilization at 121°C for 15 minutes, the medium was cooled to 45-50°C. To prevent bacterial contamination, CC supplement was added aseptically to the cooled OaM. The prepared medium was then dispensed into sterile Petri dishes and allowed to solidify. Subsequently, log-phase *M. furfur* culture from Sabouraud Dextrose (SD) broth was inoculated onto the plates and incubated at 35±2°C for 3-5 days. Following incubation, yeast growth was evaluated, and the purity of the culture was confirmed through staining methods.

2.2.2. Determination of *M. furfur* growth parameters in Oleic Acid Medium (OaM) using the broth culture method

The Oleic Acid Medium (OaM) was further screened to investigate the growth parameters of *M. furfur* in a broth medium, similar in composition to the plate culture method but excluding agar to facilitate precise examination of the yeast strain's growth phases (Table 3). OaM was prepared and sterilized at 121°C for 15 minutes, then cooled to a temperature range of 45-50°C. Approximately 10 mL of OaM was dispensed into 50 mL conical flasks, with the medium's pH adjusted to values between 5.6 and 7.0 using 0.1 N HCl and 0.1 N NaOH solutions. Two duplicate broth samples were prepared for each pH level to expose them to temperatures of 35°C and 37°C, respectively. pH adjustments were made before sterilization, while heat-sensitive components were filter-sterilized and added after sterilization. Controls were maintained for each pH set to ensure comparative analysis. The OaM with varying pH levels was autoclaved at 121°C and 15 psi for 15 minutes.

Table 3 Formulation of *M.furfur* growth media

S.No	Media Composition	Oleic acid Medium (OaM)
01	Alpha Tocopherol (mL)#	0.1
02	Ghee (mL)#	0.2
03	Linoleic acid (mL)*	0.1
04	Oleic acid (mL)*	1.5
05	Palmitic acid (gm)#	0.1
06	Squalene (mL)*	0.5
07	Distilled Water (mL)	100
	pH	5.6

#: Ingredients added after sterilization, *: Ingredients added before sterilization

Approximately 1 mL of a revived Sabouraud Dextrose (SD) broth culture of M. furfur ($1x10^7$ cells/mL) was inoculated into conical flasks containing Oleic Acid Medium (OaM) adjusted to different pH levels, followed by incubation at temperatures of 35°C and 37°C. Yeast growth was monitored from day 1 (D1) to day 20 (D20), with 2 mL of medium extracted every 24 hours for turbidity analysis using a spectrophotometer at 420 nm and a 1 cm path length. pH measurements were taken in sets to ensure accuracy, and controls were consistently maintained for comparative purposes. Absorbance readings were recorded to construct growth phase graphs, plotting absorbance on the Y-axis against time (days) on the X-axis. This data allowed for comparison of optimal

yeast growth under varying pH and temperature conditions (Guillot et al., 1996; Mayser et al., 2004).

Throughout the study, separate control samples were maintained, including Sabouraud Dextrose broth inoculated with *M. furfur* ($1x10^7$ cells/mL) and uninoculated OaM. Absorbance readings for these control samples were also recorded to establish a baseline for comparison.

3. Experimental observations

3.1. Growth parameters of *M. furfur* (MTCC 1374)

The lipophilic resident yeast *M. furfur* on the skin of humans and animals, heavily depends on lipids for its growth and development. The fondness for lipids, predominantly unsaturated fatty acids such as oleic acid in sebum, stresses yeasts nutritional requirements and role in diverse skin diseases (Gupta et al., 2004; Gaitanis et al., 2012).

Our examination of the growth of M. furfur in diverse conditions closely approximates the existing literature, conveying attention to several notable findings and comparisons:

3.1.1. Growth characteristics in different media

M. furfur's growth characteristics on SDA and Emmons-modified medium confirm previous studies. Creamy to yellowish colonies marked on SDA, indicative of the yeast's growth pattern, are consistent with reports by earlier researchers (Abdillah et al., 2020). Besides, the production of distinguishing pigments on Emmons-modified medium, subsidized by olive oil supplementation, contemplates its appropriateness for mocking lipid-rich environments essential for yeast growth (Sugita et al., 2004; Zhai et al., 2012).

3.1.2. Nutritional and lipid dependence

The yeast growth on Emmons-modified medium with olive oil supplement for excellent growth concur with the reports of Gupta et al. (2004) and Gaitanis et al. (2012). The present examinations highlight the fundamental function of lipids, mainly unsaturated fatty acids, in facilitating metabolic functions and promoting the growth rate of *M. furfur* under controlled laboratory conditions.

3.1.3. Cultural challenges and media adaptation

The present study addresses the slow and sluggish colony nature of *M.furfur* in both SDA and Emmons medium that has been supplemented with specific lipid sources for enhanced growth. The growth promotion of the yeast was identified to be accelerated under the addition of supplements, the major challenge effectively managed in the in vitro growth condition as well as the medium environment and parameters were optimized for the better growth of *M.furfur* (Gupta et al., 2004; Warner et al., 2001).

3.1.4. Relevance to clinical and research applications

Understanding resident yeast *M. fufur* growth needs and cultural characteristics in the present investigation has extended yeast usage in the industrial, clinical and research areas for future novel metabolites. Still, the application of *M. fufur* has not yet been

focused on industrial or pharmaceutical metabolite production. The present pilot scale approach to finding enhanced growth under favorable nutritional requirements and parameters will lead to the application of yeast in producing therapeutic metabolites like Azelaic acid as an alternative to plant-based production. Several investigations related to skin disorders of *M. fufur* has been highlighted Bart et al. (2018) and Guého et al. (1998), and such studies have also explained the usage of Azelaic acid in therapy

3.2. Growth kinetics and stationary phases at 35°C under variable pH

M.furfur (MTCC 1374) growth phases in Oleic Acid Medium (OaM) have been accurately focused by applying constant temperature (35°C) with variable pH (5.6 to 7.0) (Tables 4 and 5, Fig 1 & 2), under the growth acceleration absorbance at 420 nm, for a period of 20 days.

The present investigation screened the growth kinetics, where the optimal temperature and pH influence accelerating the M. furfur in the medium. The findings disclose the exact pH was 6.0, promoting robust growth with the highest absorbance of 2.8395±0.01 (averaged), which denotes the cell density (turbidity) in the medium favorable environment. Furthermore, the entry of the yeast into the stationary phase has been predicted from Day 12 to 15, achieving stability in the growth. This does not mean that other pH levels (5.6, 5.8, 6.2, 6.4, 6.6, 6.8, and 7.0) are not accelerating growth but are still inferior to pH 6.0. It was worth noting that all the pH-induced stationary phase consistently on Day 12, with the exception of pH 5.6, held their stationary phase on Day 14 and terminated on Day 16.

3.2.1. Influence of pH on yeast growth

The present study identified that the pH of growth medium was central to the operations of enzymes in yeast cells. Enzymes would reach and lead their maximum activities in a constant medium environment within a strict or narrow range of pH, holding the efficiency of metabolic pathways (Madigan et al., 2018). It is unquestionable that at pH 6.0, M. furfur may have had optimal enzymatic activity increasing cell division and growth that facilitated lipid metabolism, since M.fufur, an acidophilic yeast with a consistency of lipophilic nature, enabling to ingest nutrients efficiently from conditions in which there is adequate carbon flow (Kurtzman et al., 2011). Similarly, the bioavailability and nutrient uptake at pH 6.0 in the medium could experience the yeast for better growth acceleration, thus explaining the maximum cell density.

3.2.2. Growth at other pH levels

M. furfur's growth was observed throughout the pH range from 5.6 to 7.0, but metabolic efficiency varied within this range. In conditions with pH, which were not optimal, enzymes could be less active leading to slower rate of growth and a lower cellular population density. Yeast experienced a delayed entry into stationary phase starting on day 14 and terminating on day 16 when its pH was lowered to 5.6 (Lodder, 1971). When the yeast cells are exposed to an acidic environment such as in this experiment at pH 5.6, they may need more time to adjust their metabolic activities (Lodder, 1971). The long stationary phase at different pH levels showed that there was a period of balanced nutrient uptake and output of waste materials by the cells in order for them to reach equilibrium through nutrient consumption and waste production rates (Lodder, 1971). However, at pH=6; this steadiness came earlier and remained longer

indicating a better metabolic adaptation.

3.2.3. Role of temperature in cellular functioning

M. furfur's enzymatic kinetics and cellular functions were controlled by temperature, which is crucial for its cellular function. At about 35°C, an optimal temperature, enzyme reactions occurred rapidly so the yeast could get the required nutrients to grow and reproduce. This range of temperatures maintained the desired fluidity of the cell membrane through which metabolic processes like nutrient uptake and waste excretion passed freely (Madigan et al., 2018). The coordination between the best pH and temperature conditions ensured M. furfur's switch to a stationary phase where metabolic activities remained stable while enzymatic activities persisted for sustainable growth and product formation.

The growth study of M. furfur in OaM at 35°C made it clear that a pH level of 6.0 is appropriate for growth till cell density and the growth of the cell is continuous. This was ideal for enhancing enzyme activities and efficiency in the breaking down nutrients, which encouraged the high growth of yeast. These data are of critical importance for fine-tuning the commodity culture conditions in both experimental and clinical practice and elucidating the metabolic plasticity of M. furfur. Enhanced studies should thus zoom in to identify the actual enzymatic and metabolic pathways activated at the different pH levels to understand the mechanisms that affect the growth patterns above. M.furfur growth phase and characteristics at 35°C on an OaM medium have not yet been explored, but the present work has explored the growth pattern at 35 °C.

3.3. Growth kinetics and stationary phases at 37°C under variable pH

3.3.1. Experimental Observations

The study investigated the growth kinetics and stationary phases of M. furfur (MTCC 1374) in Oleic Acid Medium (OaM) at a constant temperature of 37°C under varying pH conditions (5.6- 7.0) (Tables 6 and 7, Fig. 3 & 4). The yeast, known for its association with human skin diseases, showed significant growth and a well-defined stationary phase, particularly at pH 6.0. The observations are as follows:

pH 5.6 **:** The yeast exhibited robust growth, with an average absorbance of 2.604±0.002, peaking by Day 13 and entering the stationary phase from Day 13 to Day 14.

pH 5.8 **:** Growth patterns stabilized with an average absorbance of 3.178±0.004 after the initial log phase, entering the stationary phase from Day 12 to Day 14.

pH 6.0 **:** Demonstrated the highest and most sustained growth, with an average absorbance of 4.4208±0.003, peaking by Day 12 and maintaining stability through Day 15.

pH 6.2 **:** The yeast had an average absorbance of 3.214±0.01, entering the stationary phase from Day 13 to Day 14.

pH 6.4 **:** Exhibited an average absorbance of 2.754±0.01, with the stationary phase from Day 12 to Day 14.

pH 6.6	**:**	Showed an average absorbance of 2.573±0.006, entering the stationary phase from Day 12 to Day 14.
pH 6.8	**:**	The yeast had an average absorbance of 2.279±0.01, with the stationary phase from Day 12 to Day 14.
pH 7.0	**:**	Demonstrated an average absorbance of 2.229±0.01, entering the stationary phase from Day 12 to Day 14.

3.3.2. Influence of pH and temperature on yeast growth

The present study finding revealed that the pH's role in the growth media affects the associated enzymes and metabolic reactions of M. furfur. The enzymes in lipid metabolism are said to be considerably effective at pH 6.0, hence the enhanced nutrient regenerative and storage mechanisms and biomass production (Madigan et al., 2018). At this pH, all the enzymes needed for the yeast's cell division and growth were active at their optimum level. Comparatively, although substantial growth was observed at lower pH levels (5.6-6.2), it was less sustained than at pH 6.0. The stationary phase of the yeast growth was shorter, and peak absorbance values were low under higher pH conditions (6. 4 – 7. 0).

Temperature also became a controlling factor in terms of enzymatic kinetics and other cellular activities as well. The optical densities revealed that yeast activity was highest at 37°C. When the temperature was set at 37°C, the yeast grew optimally and metabolized at its best. This temperature preserved a proper membrane fluidity that is especially important in transporting nutrients, removing wastes, and for unhindered metabolism (Madigan et al., 2018). The combination of pH 6.0 and 37°C provided conditions conducive to rapid proliferation during the log phase and sustained stability in the stationary phase. The pH 6.0 and 37°C allowed for profuse growth in the log phase and stability in the stationary phase.

3.3.3. Growth parameter essentially in the production of azelaic acid

The stationary phase was stressed as vital in metabolite production, especially azelaic acid, synthesized in OaM by M. furfur Azelaic acid, known for its antimicrobial and keratolytic properties, is produced as a byproduct of oleic acid metabolism (Wang et al., 2014). The stationary phase at pH 6. 0 is essential for synthesizing azelaic acid since metabolic energies are only used to form this compound (Lourith K, Kanlayavattanakul, 2014). This makes pH 6. 0 an ideal condition for maximum yield of azelaic acid and serves a tremendous industrial interest. The potential to increase azelaic acid production by optimizing its producer’s growth conditions offers significant applications in industry, particularly dermatology (Pinto et al., 2000).

3.3.4. Significance of the stationary phase

The stationary phase at pH 6. 0 usually indicates a healthy balance between cell division and cell death; nutrient consumption and metabolic activities are ideal. These two characteristics make it essential for industrial employment where relative steadiness in the productivity increase rate is far more significant than its intensity. It was also necessary to explain the changes in growth parameters in case when the pH is 6. The result of this study demonstrates that pH 6 0 supports cell growth and metabolic activity and also supports a longer maintenance phase that would need metabolic and

product stabilization (Madigan et al., 2018; Leeming & Notman, 1987).

3.4. Findings

The pH 6.0 indicates the best improvement of the stationary phase of M. furfur in an Oleic Acid Medium incubated at 37°C. At this pH, the growth is rapid, metabolic rates are high, and the cells are stable in the stationary phase. One of the studies highlights the need to maintain pH 6.0, which helped in having constant and improved production of azelaic acid, an important metabolite. These results are essential for subsequent investigations of M. furfur to understand better the patterns of growth kinetics of this yeast and its potential for use in pharmaceutical and industry fields.

3.4.1. Optimal temperature and pH for *M. furfur* stationary phase

3.4.1.1. Experimental Observations

The key findings of M. furfur (MTCC 1374) growth phases in Oleic Acid Medium (OaM) under two different temperatures (35°C and 37°C) with varying pH (5.6 to 7.0) and absorbance at 420 nm for about 20 days were disclosed below:

A. Growth at 35°C

At optimal pH 6.0 most robust growth with an absorbance peak averaging 2.8395±0.01 was observed. A stable stationary phase from Day 12 to Day 15 was observed where the density of the yeast was stable during this period. The reflection of other pH (5.6, 5.8, 6.2, 6.4, 6.6, 6.8, and 7.0) enhanced growth with lower cell density not comparable to pH 6.0. The onset of stationary phase was delayed and shorter at more acidic or alkaline pH values, with the most significant delay at pH 5.6 (Day 14 to Day 16).

B. Growth at 37°C

At Optimal pH 6.0 highest, most sustained growth, with an absorbance of 4.415 was observed on Day 12 and maintained stability through Day 15. The influence of other pH (5.6, 5.8, 6.2, 6.4, 6.6, 6.8, and 7.0) was analogous to 35°C. Besides the lower pH (5.6, 5.8) entertainment significant growth but less uninterrupted than at pH 6.0. High pH (6.4-7.0) evidenced shortened stationary period and lower absorbance values.

3.4.2. Influence of pH and temperature on growth

A. Enzymatic Activity: Perfect pH (6.0) increases the enzymatic activity of the particular enzymes involved in lipid metabolism, which is crucial for M. furfur for its excellent nutrient use efficiency and biomass accumulation.

B. Temperature: M. furfur has positive development at two temperatures (35°C and 37°C), where 37°C. had a higher absorbance, which states that the growth range and the cell concentration were wider which is not in the case of 35°C.

3.4.3. Optimal Conditions for Industrial Applications

A. Optimal pH: pH 6.0 is the best for the efficient and long-lasting growth of the yeast *M. furfur* in OaM, such change in pH means that maximum levels of the enzymes, sugar, and acids are maintained, leading to maximum growth rates and sustained growth.

B. Optimal Temperature: It could be said that 37°C is a bit better than 35°C for the *M. furfur* population growth. The results include higher peak absorbance, consequently,

the metabolism was more active in the whole experiment.

3.4.4. Significance for industrial production

A. Azelaic acid production: Azelaic acid, a metabolite with antimicrobial and keratolytic properties, is maximally produced when the pH is 6.0 and the temperature is 37°C. During the stationary phase, the metabolic resources are steered in the direction of azelaic acid production.

B. Consistency and Scalability: Consistency and reproducibility of growth rates are achieved by regulating pH to 6.0 and temperature to 37°C as these conditions are necessary for industrial scale- up. These circumstances help secure the expected amount and standard of metabolites, which are important for drug and skin care product manufacturing.

C. Future Research Directions

Further studies should explore:

D. Specific Enzymatic Pathways: Investigating the enzymatic and metabolic pathways at various pH to understand the underlying mechanisms of growth and metabolite production.

E. Environmental Influences: Besides the basic environmental factors, nutrient concentrations and composition of the medium are the other factors to be considered to further optimize the growth conditions. The ideal conditions for the stationary phase of *M. furfur* for production of the product of industrial importance are pH 6.0 and temperature 37°C. Such settings stimulate the proliferation of cells, increase the period of metabolic activities, and the efficient production of the desired metabolites such as azelaic acid.

3.5. Conclusion

3.5.1. Importance for Research and Applications

F. Understanding the synergistic effects of pH and temperature on M. furfur growth and stationary phase attainment is critical for advancing both research and clinical applications. These environmental factors play pivotal roles in regulating yeast physiology, metabolic adaptations, and growth dynamics, offering valuable insights into developing effective strategies for yeast control across diverse environments.

3.5.2. Environmental Influence on Microbial Growth

G. Madigan et al. (2018) emphasized in their study on microbiology that environmental factors profoundly influence microbial physiology and biochemistry. They highlighted how enzymes exhibit peak activity under optimal conditions, crucially impacting microbial growth dynamics. This understanding is particularly relevant to comprehending the robust growth observed in M. furfur under specific pH and temperature conditions.

3.5.3. Taxonomy and Physiological Adaptations

H. Kurtzman et al. (2011) provided an extensive overview of yeast taxonomy, focusing on the physiological properties and environmental conditions favorable for yeast growth. They underscored the importance of environmental factors, including pH and temperature, in modulating lipid metabolism, which is vital for lipophilic yeasts like

M. furfur. This insight helps explain how optimal environmental conditions support enhanced metabolic activities and growth rates in M. furfur.

3.5.4. Growth Dynamics and Stationary Phase

I. Lodder (1971) contributed significantly to understanding yeast taxonomy and growth requirements, highlighting variations in growth rates and stationary phase timings under different environmental conditions. This research contextualized the delayed and extended stationary phases observed in M. furfur at suboptimal pH levels, providing essential insights into the yeast's adaptive strategies and metabolic stability.

3.5.5. Clinical Implications and Disease Pathogenesis

J. Gaitanis et al. (2012) reviewed the clinical implications of Malassezia species in skin diseases, elucidating how microbial growth dynamics correlate with disease pathogenesis. This perspective is crucial for understanding the broader impact of M. furfur in dermatological contexts and underscores the importance of optimizing growth conditions for clinical management strategies.

3.5.6. Biological Activity and Dermatological Applications

K. Lourith and Kanlayavattanakul (2014) explored the biological activity and safety of skin care products containing azelaic acid, a metabolic byproduct of M.furfur. Their findings underscore the relevance of understanding azelaic acid production pathways in M. furfur for dermatological applications, highlighting its potential therapeutic benefits.

3.5.7. Metabolic Pathways and Industrial Applications

Wang et al. (2014) investigated the effects of pH and carbon sources on azelaic acid production by Pityrosporum ovale KCTC 7842, a related yeast species. Their experimental data contribute to understanding metabolic pathways in M. furfur, particularly relevant for optimizing industrial processes aimed at azelaic acid production.

Integrating insights from studies by Madigan et al. (2018), Kurtzman et al. (2011), Lodder (1971), Gaitanis et al. (2012), Lourith and Kanlayavattanakul (2014), and Wang et al. (2014) elucidates how environmental factors, particularly pH and temperature, influence M. furfur's growth and stationary phase dynamics. These findings are instrumental in optimizing culture conditions for industrial applications, including the production of bioactive compounds like azelaic acid, and advancing clinical strategies for managing M. furfur-related skin diseases.

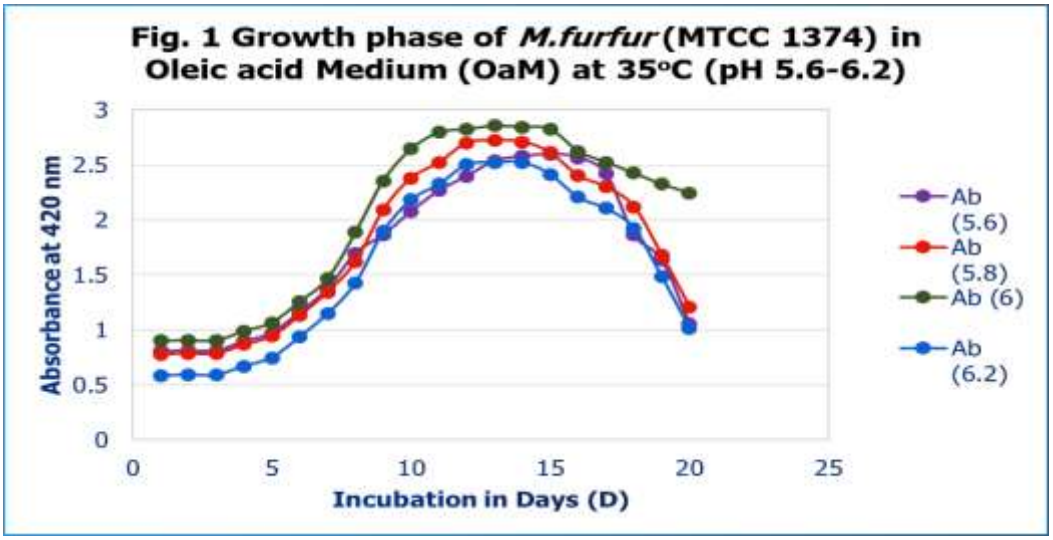
Fig. 1 Growth phase of M.furfur (MTCC 1374) in Oleic acid Medium (OaM) at 35°C (pH 5.6-6.2)
Absorbance at 420 nm
Incubation in Days (D)
Ab (5.6)
Ab (5.8)
Ab (6)
Ab (6.2)

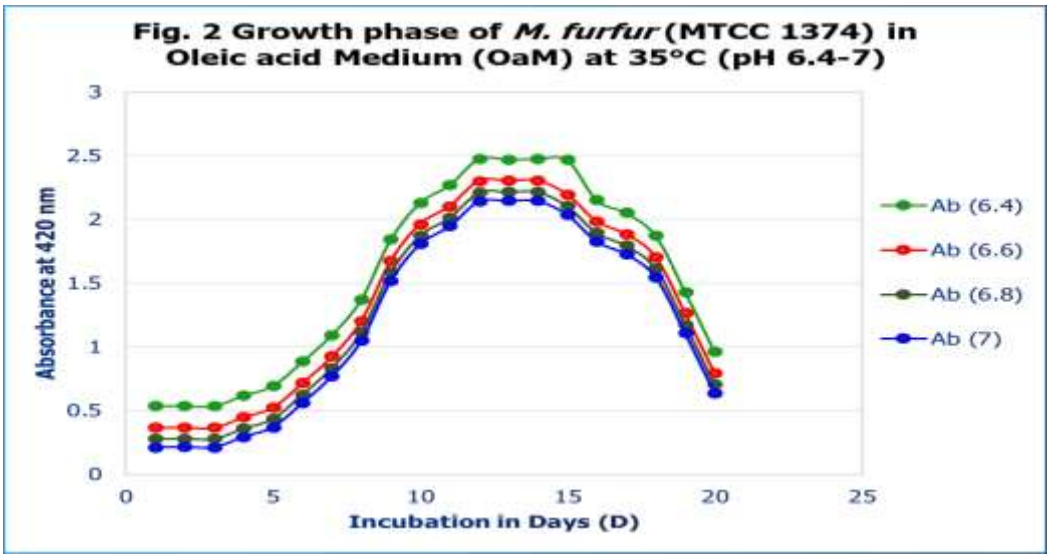
Fig. 2 Growth phase of M. furfur (MTCC 1374) in Oleic acid Medium (OaM) at 35°C (pH 6.4-7)
Absorbance at 420 nm
Incubation in Days (D)
Ab (6.4)
Ab (6.6)
Ab (6.8)
Ab (7)

Table 4 Growth phase of *Malassezia furfur* (MTCC 1374) in Oleic acid medium (OaM) at constant temperature (35⁰ under variable pH (5.6-6.2) and incubation period in days

Absorbance at 420 nm with a path length of 1 cm												
Incubation period in Days	(C)	Ab (5.6)	Ab. at pH 5.6 (Ab-C)	(C)	Ab (5.8)	Ab. at pH 5.8 (Ab-C)	(C)	Ab (6)	Ab. at pH 6 (Ab-C)	(C)	Ab (6.2)	Ab. at pH 6.2 (Ab-C)
Day 1	0.842	1.653	0.811	0.858	1.637	0.779	0.851	1.755	0.904	0.846	1.43	0.584
Day 2	0.842	1.656	0.814	0.858	1.646	0.788	0.851	1.758	0.907	0.846	1.439	0.593
Day 3	0.842	1.656	0.814	0.858	1.644	0.786	0.851	1.758	0.907	0.846	1.437	0.591
Day 4	0.842	1.742	0.9	0.858	1.726	0.868	0.851	1.844	0.993	0.846	1.519	0.673
Day 5	0.842	1.818	0.976	0.858	1.802	0.944	0.851	1.92	1.069	0.846	1.595	0.749
Day 6	0.842	2.008	1.166	0.858	1.992	1.134	0.851	2.11	1.259	0.846	1.785	0.939
Day 7	0.842	2.218	1.376	0.858	2.202	1.344	0.852	2.32	1.468	0.846	1.995	1.149
Day 8	0.842	2.54	1.698	0.858	2.48	1.622	0.853	2.74	1.887	0.846	2.273	1.427
Day 9	0.842	2.71	1.868	0.858	2.95	2.092	0.853	3.21	2.357	0.846	2.743	1.897
Day 10	0.842	2.92	2.078	0.858	3.24	2.382	0.853	3.50	2.647	0.846	3.033	2.187
Day 11	0.842	3.11	2.268	0.858	3.38	2.522	0.853	3.65	2.797	0.846	3.173	2.327
Day 12	0.842	3.24	2.398	0.858	3.56	**2.702**	0.853	3.68	**2.827**	0.846	3.353	**2.507**
Day 13	0.842	3.38	2.538	0.858	3.58	**2.722**	0.853	3.71	**2.857**	0.846	3.373	**2.527**
Day 14	0.842	3.42	**2.578**	0.858	3.57	**2.712**	0.853	3.70	**2.847**	0.846	3.373	**2.527**
Day 15	0.842	3.44	**2.598**	0.858	3.47	2.612	0.853	3.68	**2.827**	0.846	3.263	2.417
Day 16	0.842	3.41	**2.568**	0.858	3.26	2.402	0.853	3.48	2.627	0.846	3.053	2.207
Day 17	0.842	3.26	2.418	0.858	3.16	2.302	0.853	3.38	2.527	0.846	2.953	2.107
Day 18	0.842	2.71	1.868	0.858	2.98	2.122	0.853	3.28	2.427	0.846	2.773	1.927
Day 19	0.842	2.48	1.638	0.858	2.54	1.682	0.853	3.18	2.327	0.846	2.333	1.487
Day 20	0.842	1.9	1.058	0.858	2.07	1.212	0.853	3.1	2.247	0.846	1.863	1.017

C-denotes uninoculated control medium, Ab-Absorbance, Highlights in the matrices indicates the stationary phase of the yeast *Malassezia furfur* in the medium

Table 5 Growth phase of *Malassezia furfur* (MTCC 1374) in Oleic acid medium (OaM) at constant temperature (35⁰C) under variable pH (6.4-7.0) and incubation period in days

Absorbance at 420 nm with a path length of 1 cm												
Incubation period in Days	(C)	Ab (6.4)	Ab. at pH 6.4 (Ab-C)	(C)	Ab (6.6)	Ab. at pH 6.6 (Ab-C)	(C)	Ab (6.8)	Ab. at pH 6.8 (Ab-C)	(C)	Ab (7)	Ab. at pH 7 (Ab-C)
Day 1	0.845	1.379	0.534	0.864	1.23	0.366	0.822	1.099	0.277	0.815	1.025	0.21
Day 2	0.845	1.381	0.536	0.864	1.232	0.368	0.822	1.101	0.279	0.815	1.027	0.212
Day 3	0.845	1.379	0.534	0.864	1.23	0.366	0.822	1.099	0.277	0.815	1.025	0.21
Day 4	0.845	1.461	0.616	0.864	1.312	0.448	0.822	1.181	0.359	0.815	1.107	0.292
Day 5	0.845	1.537	0.692	0.864	1.388	0.524	0.822	1.257	0.435	0.815	1.183	0.368
Day 6	0.845	1.727	0.882	0.864	1.578	0.714	0.822	1.447	0.625	0.815	1.373	0.558
Day 7	0.845	1.937	1.092	0.864	1.788	0.924	0.822	1.657	0.835	0.815	1.583	0.768
Day 8	0.845	2.215	1.37	0.864	2.066	1.202	0.822	1.935	1.113	0.815	1.861	1.046
Day 9	0.845	2.685	1.84	0.864	2.536	1.672	0.822	2.405	1.583	0.815	2.331	1.516
Day 10	0.845	2.975	2.13	0.864	2.826	1.962	0.822	2.695	1.873	0.815	2.621	1.806
Day 11	0.845	3.115	2.27	0.864	2.966	2.102	0.822	2.835	2.013	0.815	2.761	1.946
Day 12	0.845	3.32	**2.475**	0.864	3.164	**2.3**	0.822	3.033	**2.211**	0.815	2.956	**2.141**
Day 13	0.845	3.315	**2.47**	0.864	3.166	**2.302**	0.822	3.038	**2.216**	0.815	2.961	**2.146**
Day 14	0.845	3.321	**2.476**	0.864	3.166	**2.302**	0.822	3.037	**2.215**	0.815	2.959	**2.144**
Day 15	0.845	3.24	2.395	0.864	3.056	2.192	0.822	2.925	2.103	0.815	2.851	2.036
Day 16	0.845	2.995	2.15	0.864	2.846	1.982	0.822	2.715	1.893	0.815	2.641	1.826
Day 17	0.845	2.895	2.05	0.864	2.746	1.882	0.822	2.615	1.793	0.815	2.541	1.726
Day 18	0.845	2.715	1.87	0.864	2.566	1.702	0.822	2.435	1.613	0.815	2.361	1.546
Day 19	0.845	2.275	1.43	0.864	2.126	1.262	0.822	1.995	1.173	0.815	1.921	1.106
Day 20	0.845	1.805	0.96	0.864	1.656	0.792	0.822	1.525	0.703	0.815	1.451	0.636

C-denotes uninoculated control medium, Ab-Absorbance, Highlights in the matrices indicates the stationary phase of the yeast *Malassezia furfur* in the medium

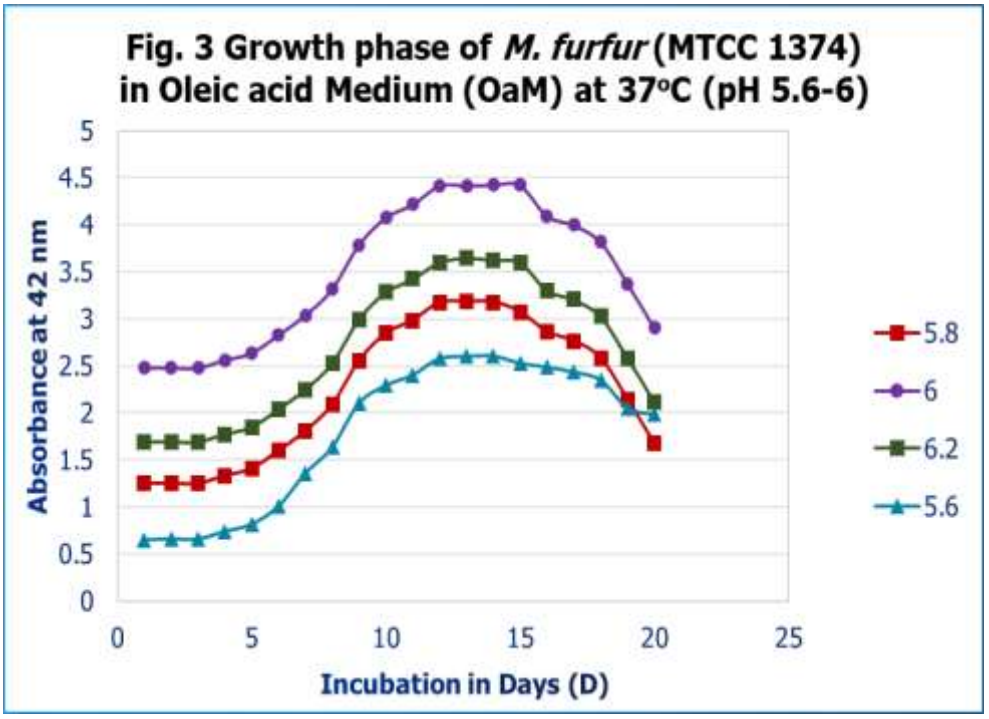

Fig. 3 Growth phase of *M. furfur* (MTCC 1374) in Oleic acid Medium (OaM) at 37°C (pH 5.6-6)

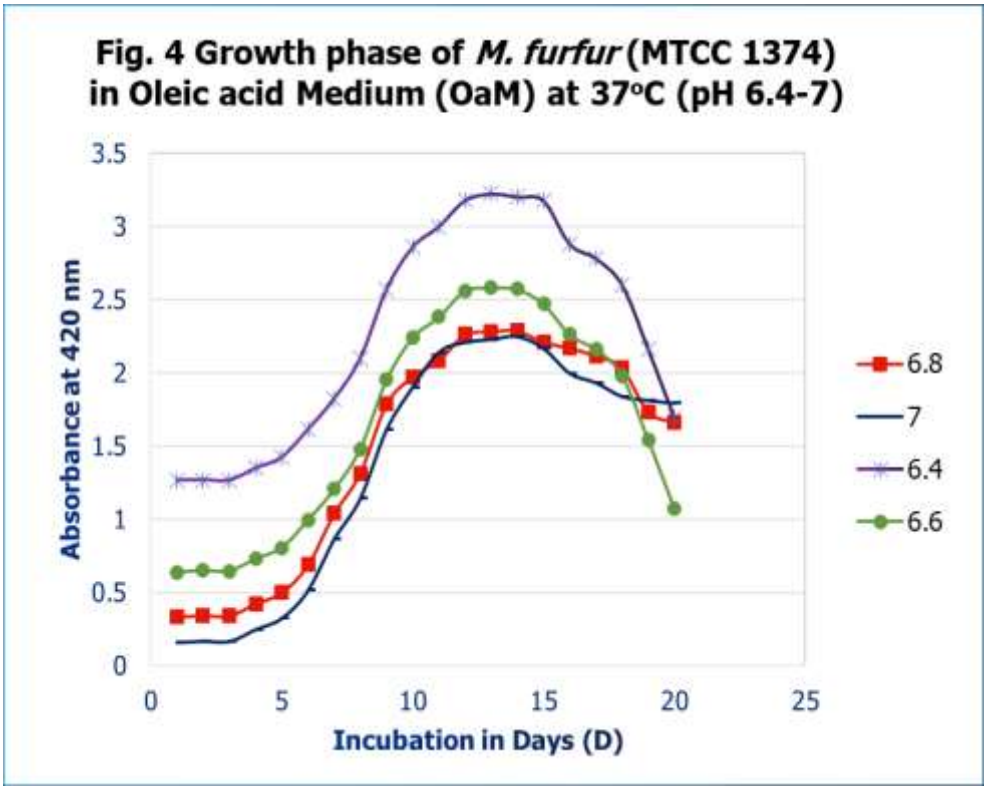

Fig. 4 Growth phase of *M. furfur* (MTCC 1374) in Oleic acid Medium (OaM) at 37°C (pH 6.4-7)

Table 6 Growth phase of *Malassezia furfur* (MTCC 1374) in Oleic acid medium (OaM) at constant temperature (37^{0}C) under variable pH (5.6-7.0) and incubation period in days

Absorbance at 420 nm with a path length of 1 cm												
Incubation period in Days	(C)	Ab (5.6)	Ab. at pH 5.6 (Ab-C)	(C)	Ab (5.8)	Ab. at pH 5.8 (Ab-C)	(C)	Ab (6)	Ab. at pH 6 (Ab-C)	(C)	Ab (6.2)	Ab. at pH 6.2 (Ab-C)
Day 1	0.449	1.099	0.65	0.412	1.66	1.248	0.416	2.897	2.481	0.423	1.689	1.266
Day 2	0.449	1.108	0.659	0.412	1.662	1.25	0.416	2.899	2.483	0.423	1.691	1.268
Day 3	0.449	1.106	0.657	0.412	1.66	1.248	0.416	2.897	2.481	0.423	1.689	1.266
Day 4	0.449	1.188	0.739	0.412	1.742	1.33	0.416	2.979	2.563	0.423	1.771	1.348
Day 5	0.449	1.264	0.815	0.412	1.818	1.406	0.416	3.055	2.639	0.423	1.847	1.424
Day 6	0.449	1.454	1.005	0.412	2.008	1.596	0.416	3.245	2.829	0.423	2.037	1.614
Day 7	0.449	1.804	1.355	0.412	2.218	1.806	0.416	3.455	3.039	0.423	2.247	1.824
Day 8	0.449	2.082	1.633	0.412	2.496	2.084	0.416	3.733	3.317	0.423	2.525	2.102
Day 9	0.449	2.552	2.103	0.412	2.966	2.554	0.416	4.203	3.787	0.423	2.995	2.572
Day 10	0.449	2.742	2.293	0.412	3.256	2.844	0.416	4.493	4.077	0.423	3.285	2.862
Day 11	0.449	2.853	2.404	0.412	3.396	2.984	0.416	4.633	4.217	0.423	3.425	3.002
Day 12	0.449	3.029	2.58	0.412	3.584	**3.172**	0.416	4.831	**4.415**	0.423	3.603	3.18
Day 13	0.449	3.051	**2.602**	0.412	3.596	**3.184**	0.416	4.836	**4.42**	0.423	3.648	**3.225**
Day 14	0.449	3.056	**2.607**	0.412	3.59	**3.178**	0.416	4.841	**4.425**	0.423	3.627	**3.204**
Day 15	0.449	2.979	2.53	0.412	3.486	3.074	0.416	4.839	**4.423**	0.423	3.601	3.178
Day 16	0.449	2.938	2.489	0.412	3.276	2.864	0.416	4.513	4.097	0.423	3.305	2.882
Day 17	0.449	2.884	2.435	0.412	3.176	2.764	0.416	4.413	3.997	0.423	3.205	2.782
Day 18	0.449	2.8	2.351	0.412	2.996	2.584	0.416	4.233	3.817	0.423	3.025	2.602
Day 19	0.449	2.503	2.054	0.412	2.556	2.144	0.416	3.793	3.377	0.423	2.585	2.162
Day 20	0.449	2.43	1.981	0.412	2.086	1.674	0.416	3.323	2.907	0.423	2.115	1.692

C-denotes uninoculated control medium, Ab-Absorbance, Highlights in the matrices indicates the stationary phase of the yeast *Malassezia furfur* in the medium

Table 7Growth phase of *Malassezia furfur* (MTCC 1374) in Oleic acid medium (OaM) at constant temperature (37^{0}C) under variable pH (6.4-7.0) and incubation period in days

Absorbance at 420 nm with a path length of 1 cm												
Incubation period in Days	(C)	Ab (6.4)	Ab. at pH 6.4 (Ab-C)	(C)	Ab (6.6)	Ab. at pH 6.6 (Ab-C)	(C)	Ab (6.8)	Ab. at pH 6.8 (Ab-C)	(C)	Ab (7)	Ab. at pH 7 (Ab-C)
Day 1	0.412	1.22	0.808	0.476	1.114	0.638	0.417	0.75	0.333	0.411	0.57	0.159
Day 2	0.412	1.229	0.817	0.476	1.125	0.649	0.417	0.759	0.342	0.411	0.579	0.168
Day 3	0.412	1.227	0.815	0.476	1.123	0.647	0.417	0.757	0.34	0.411	0.577	0.166
Day 4	0.412	1.309	0.897	0.476	1.205	0.729	0.417	0.839	0.422	0.411	0.659	0.248
Day 5	0.412	1.385	0.973	0.476	1.281	0.805	0.417	0.915	0.498	0.411	0.735	0.324
Day 6	0.412	1.575	1.163	0.476	1.471	0.995	0.417	1.105	0.688	0.411	0.925	0.514
Day 7	0.412	1.925	1.513	0.476	1.681	1.205	0.417	1.455	1.038	0.411	1.275	0.864
Day 8	0.412	2.203	1.791	0.476	1.959	1.483	0.417	1.733	1.316	0.411	1.553	1.142
Day 9	0.412	2.673	2.261	0.476	2.429	1.953	0.417	2.203	1.786	0.411	2.023	1.612
Day 10	0.412	2.863	2.451	0.476	2.719	2.243	0.417	2.393	1.976	0.411	2.313	1.902
Day 11	0.412	2.97	2.562	0.476	2.859	2.383	0.417	2.504	2.087	0.411	2.553	2.142
Day 12	0.412	3.15	**2.738**	0.476	3.039	**2.563**	0.417	2.68	**2.263**	0.411	2.62	**2.209**
Day 13	0.412	3.17	**2.76**	0.476	3.059	**2.583**	0.417	2.702	**2.285**	0.411	2.64	**2.229**
Day 14	0.412	3.18	**2.765**	0.476	3.049	**2.573**	0.417	2.707	**2.29**	0.411	2.66	**2.249**
Day 15	0.412	3.10	2.688	0.476	2.949	2.473	0.417	2.6	2.183	0.411	2.58	2.169
Day 16	0.412	3.059	2.647	0.476	2.739	2.263	0.417	2.589	2.172	0.411	2.409	1.998
Day 17	0.412	3.005	2.593	0.476	2.639	2.163	0.417	2.535	2.118	0.411	2.345	1.934
Day 18	0.412	2.921	2.509	0.476	2.459	1.983	0.417	2.451	2.034	0.411	2.251	1.84
Day 19	0.412	2.624	2.212	0.476	2.019	1.543	0.417	2.154	1.737	0.411	2.224	1.813
Day 20	0.412	2.551	2.139	0.476	1.549	1.073	0.417	2.081	1.664	0.411	2.205	1.794

C-denotes uninoculated control medium, Ab-Absorbance, Highlights in the matrices indicates the stationary phase of the yeast *Malassezia furfur* in the medium

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