

# Determination of Effect of Temperature on Bacteria and Fungi (Salt and Fresh Water)

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## Abstract

*This study investigates the effect of temperature on bacterial and fungal activity in fresh and saltwater environments contaminated with crude oil. Utilizing standard characterization techniques, pure cultures of bacteria and fungi were isolated and identified. A bioreactor setup was employed to assess microbial growth and heat generation at varying temperatures (15°C to 120°C) over a 6-hour period. The results demonstrated that temperature significantly influences microbial activity, with bacterial growth rates increasing with temperature in saltwater, while fungal heat production in freshwater exhibited fluctuating patterns. Mathematical models based on Monod's equation were developed to analyze temperature as both an inhibitor and activator of microbial growth. This research underscores the importance of temperature in bioremediation processes and microbial ecology, contributing valuable insights into managing contaminated aquatic systems.*

**Keywords:** Temperature effect, microbial activity, bioremediation, crude oil contamination, bacterial and fungal dynamics, aquatic ecosystems

## INTRODUCTION

This study explores the influence of temperature on the activity of bacteria and fungi in freshwater and saltwater environments contaminated with crude oil [1]. Understanding how temperature affects microbial dynamics is crucial for effective bioremediation strategies, particularly in aquatic systems facing pollution [2]. We employed standard characterization techniques to isolate and identify pure cultures of bacteria and fungi from contaminated media [3]. A bioreactor setup was utilized to evaluate microbial growth and heat generation across a temperature range of 15°C to 120°C over a 6-hour period [4–6].

The results revealed that temperature significantly impacts microbial activity; specifically, bacterial growth rates in saltwater increased with rising temperatures, indicating a direct activation response [7]. In contrast, fungal heat production in freshwater exhibited fluctuating patterns, suggesting a more complex interaction with temperature. To further analyze these dynamics, we developed mathematical

models based on Monod's equation, treating temperature as both an inhibitor and an activator of microbial growth [8]. This research underscores the critical role of temperature in bioremediation and microbial ecology, providing valuable insights for managing contaminated aquatic ecosystems [9].

## METHODOLOGY

### Characterization and Identification of Bacterial and Fungal Isolates

Pure cultures of bacteria were obtained and subjected to various characterization procedures. The standard characterization tests performed included: Gram stain, motility test, catalase, methyl red and Vogues Proskaver test. Others are urease, indole, protease, nitrate reduction, starch hydrolysis

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and sugar fermentation tests. The isolates were identified on the basis of their cultural, morphological and biochemical reactions, and by referring to Kasai and Yoshikawa [10] and Zeng et al. [11]. ABIS online identification tool was further used to identify bacteria to generic and/species levels.

Fungal isolates were characterized by microscopy by observing the colonial morphology, color of colony, texture, shape, surface appearance, and color on the reverse plates [12]; and microscopy using the wet preparation and slide culture by observing cultural characteristics to reveal the asexual and sexual reproductive structures [13–15].

## Methods

The main bioreactor was set up, which contains the thermocouple, fresh and salt water media and the bioreactor was contaminated with the crude oil. The bioreactor was subjected to temperature effect of the range 15°C for both fresh and salt water media for time period of 6 hours.

### *Model of Temperature as an Inhibitor or Activator on Correlation with Monod's Equation*

For Case I:

The effect of temperature as an inhibitor acting as an activator can be expressed as

$$[\mu] = \frac{[\mu]_{max} [S]}{Km+[S]} [I] \quad (1)$$

If  $[I] = [T]$ , therefore Equation (1) becomes

$$[\mu] = \frac{[\mu]_{max} [S]}{Km+[S]} [T] \quad (2)$$

### *Model of Temperature as an Inhibitor or Activator II on Monod's Equation*

Considering the effect of temperature acting as an inhibitor in the bioreactor system, the mathematical expression can be written as:

$$[I] = \frac{1}{[T]} \quad (3)$$

Therefore, Equation (1) becomes

$$[\mu] = \frac{[\mu]_{max} [S]}{Km+[S]} \cdot \frac{1}{[T]} \quad (4)$$

However, a mathematical model was recalled as stated on Equation (4), thus;

$$[\mu] = \frac{[\mu]_{smax} - [I_s] [S]}{Ks+[S]+[S]^2/K_I} \quad (5)$$

$$\text{Since } [Sa] \alpha [\rho] \alpha \frac{1}{[\mu]_{max}} \quad (6)$$

$$\text{If } P = \rho gh \quad (7)$$

From Equation (7), we have

$$\rho = P / gh \quad (8)$$

Substituting Equation (7) into Equation (6), we have

$$[Sa] \alpha P / gh \alpha \frac{1}{[\mu]_{max}} \quad (9)$$

But recalling a mathematical expression of pressure, volume and temperature relationship, we have

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2} \quad (10)$$

If  $V_1 = V_2$ , Equation (10) becomes

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \quad (11)$$

Simplifying Equation (11), we have

$$P_2 = \frac{P_1 T_2}{T_1} \quad (12)$$

Dividing both sides of Equation (12) by  $P_1$  we have

$$\frac{P_2}{P_1} = \frac{T_2}{T_1} \quad (13)$$

Taking the logarithm of Equation (13), we have

$$\log P_2 - \log P_1 = \log T_2 - \log T_1 \quad (14)$$

or

$$\log P_1 - \log P_2 = \log T_1 - \log T_2$$

$$\rho \alpha \frac{\log T_2 - \log T_1}{gh} \alpha \frac{1}{[\mu]_{max}} \quad (15)$$

$$\log T_2 - \log T_1 = D_T \left( \frac{[\rho]gh}{[\mu]_{max}} \right) \quad (16)$$

where the maximum specific growth rate was obtained and the effect of temperature can be expressed as

$$[\mu]_{max T} = \frac{D_T \rho}{[\mu]_{max T}} \quad (17)$$

Therefore, the mathematical expression for  $D_T$  can be expressed as:

$$D_T = [\mu]_{max}^2 / \rho = I_T \quad (18)$$

where  $D_T$  is the constant for temperature inhibition of maximum specific growth rate (hour)<sup>-1</sup>

$$[\mu] = \frac{([\mu]_{max} - [D_T]) [S]}{K_S + [S] + \frac{[S]^2}{K_I}} \quad (19)$$

## RESULTS AND DISCUSSION

Figure 1 shows the relationship between the velocity (specific rate) of bacteria in a saltwater medium and temperature, highlighting the activation of bacterial growth. An increase in temperature correlates with a rise in velocity (specific rate). The changes in bacterial velocity in the saltwater medium are attributed to temperature variations affecting bacterial activation.

Figure 2 presents the heat generated by fungi in a freshwater medium over time. The results indicate fluctuations in heat production, with both increases and decreases occurring as time progresses. These variations in heat generation are linked to changes in time affecting fungal activity in the freshwater environment.

Figure 3 depicts the relationship between heat generated by fungi in a saltwater environment over time. The results show fluctuations in heat production as time progresses, with both increases and decreases observed. These variations in heat generation are attributed to the temporal changes in fungal activity within the saltwater environment.

### Software Simulation Readings and Code

```
Umax15=500000; % freshwater fungi activator
Umax30=500000;
Umax45=0;
Umax60=0.000638;
```

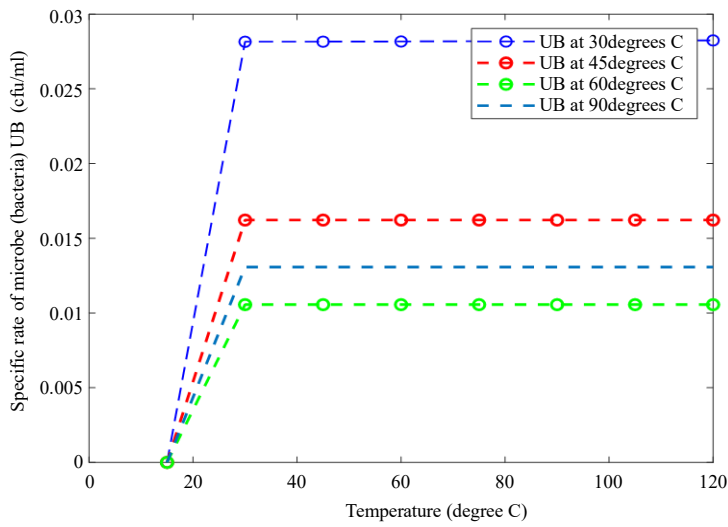


Figure 1. Specific rate of bacteria on saltwater environment versus temperature.

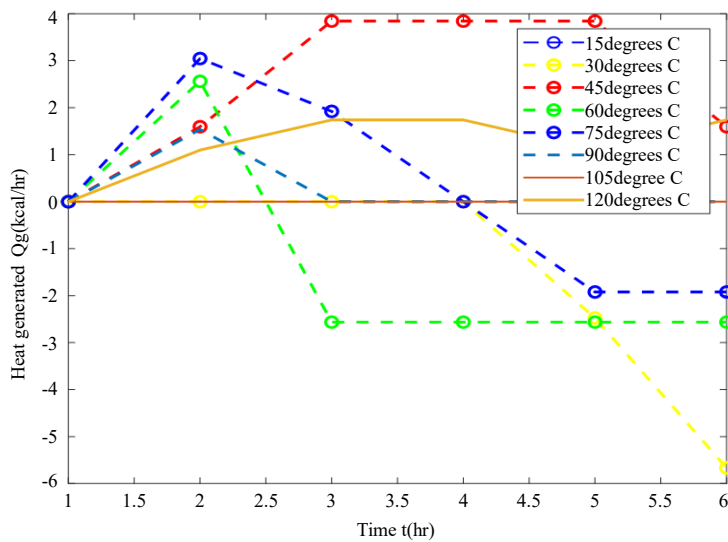


Figure 2. Heat generated by fungi on freshwater environment versus time.

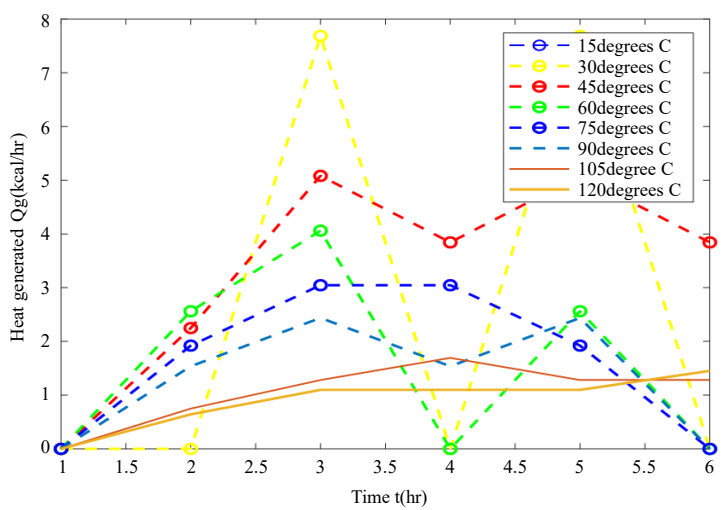


Figure 3. Heat generated by fungi in saltwater environment versus time.

```
Umax75=50000000;  
Umax90=0;  
Umax105=10000;  
Umax120=2.5;  
t= [1 2 3 4 5 6]  
Uo15=50000;  
Uo30= 500000;  
Uo45= 26000;  
Uo60= 300000;  
Uo75=400000  
Uo90= 10000;  
Uo105=10000;  
Uo120=10000;  
Kmax15=38450  
Kmax30=5555.56;  
Kmax45=0;  
Kmax60=0.00004255;  
Kmax75=14.285;  
Kmax90=0;  
Kmax105=3750;  
Kmax120=125;  
s15=[0 50000 100000 150000 220000 300000 350000 470000]  
s30=[0 49000 44000 39000 34000 29000 25000 22000]  
s45=[0 9000 12000 16000 22000 28000 34000 38000]  
s60=[0 10000 9000 7000 6500 6000 5500 5000]  
s75=[0 11000 10000 7000 5000 4000 3500 3000]  
s90=[0 14000 13000 11000 10000 9000 8000 7000]  
s105=[0 8000 7000 6000 5000 4000 3000 2000]  
s120=[0 16000 15000 13000 10000 7000 5000 2000]  
u15=((Umax15.*s15)./(Kmax15+s15))/15  
u30=((Umax30.*s30)./(Kmax30+s30))/30  
u45=((Umax45.*s45)./(Kmax45+s45))/45  
u60=((Umax60.*s60)./(Kmax60+s60))/60  
u75=((Umax75.*s75)./(Kmax75+s75))/75  
u90=((Umax90.*s90)./(Kmax90+s90))/90  
u105=((Umax105.*s105)./(Kmax105+s105))/105  
u120=((Umax120.*s120)./(Kmax120+s120))/120  
%U=[u15' u30' u45' u60' u75' u90' u105' u120']  
T=[15 30 45 60 75 90 105 120]  
figure (1)  
plot(T,u15,'--bo','linewidth',1.0)  
xlabel('Temperature (degree C)')  
ylabel('Velocity (specific rate) of microbe (fungi)Uf(cf/ml)')  
hold on  
plot(T,u30,'--ro','linewidth',1.5)  
hold on  
plot(T,u60,'--go','linewidth',1.5)  
hold on  
plot(T,u75,'--','linewidth',1.5)  
hold on  
plot(T,u105,'--','linewidth',1.5)  
hold on
```

```

plot(T,u120,'--','linewidth',1.5)
legend('Uf at 15degrees C','Uf at 30degrees C',' Uf at 60degrees C','Uf at 75degrees C','Uf at
105degrees C','Uf at 120degrees C')
figure (2)
plot(T,u75)

```

### Computer Program for Determination of Effect of Temperature on Fungi (Freshwater) Activator

```

Umax15=3448.3; % saltwater fungi inhibitor
Umax30=10000000000;
Umax45=13000000000;
Umax60=23000000000;
Umax75=50000000000;
Umax90=0;
Umax105=4081;
Umax120=4347;
t= [1 2 3 4 5 6]
Uo15=50000;
Uo30= 500000;
Uo45= 26000;
Uo60= 300000;
Uo75=400000
Uo90= 10000;
Uo105=10000;
Uo120=10000;
Kmax15=2586
Kmax30=2000;
Kmax45=3084;
Kmax60=8184.5;
Kmax75=9404.7;
Kmax90=0;
Kmax105=0.00000037;
Kmax120=130;
s15=[0 59000 56000 52000 47000 41000 35000 28000]
s30=[0 5000 6000 7000 9000 10000 12000 13000]
s45=[0 41000 39000 36000 31000 27000 22000 18000]
s60=[0 15000 14000 12000 9000 8000 6000 5000]
s75=[0 14000 13000 11000 8000 5000 3000 2000]
s90=[0 24000 23000 21000 18000 16000 13000 12000]
s105=[0 29000 27000 24000 20000 15000 11000 7000]
s120=[0 44000 42000 39000 35000 31000 27000 22000]
u15=((Umax15.*s15)./(Kmax15+s15))*15
u30=((Umax30.*s30)./(Kmax30+s30))*30
u45=((Umax45.*s45)./(Kmax45+s45))*45
u60=((Umax60.*s60)./(Kmax60+s60))*60
u75=((Umax75.*s75)./(Kmax75+s75))*75
u90=((Umax90.*s90)./(Kmax90+s90))*90
u105=((Umax105.*s105)./(Kmax105+s105))*105
u120=((Umax120.*s120)./(Kmax120+s120))*120
%U=[u15' u30' u45' u60' u75' u90' u105' u120']
T=[15 30 45 60 75 90 105 120]
figure (1)

```

```
plot(T,u15,'--bo','linewidth',1.0)
xlabel('Temperature (degree C)')
ylabel('Velocity (specific rate) of microbe (fungi)Uf(cf/ml)')
hold on
plot(T,u30,'--ro','linewidth',1.5)
hold on
plot(T,u45,'--ro','linewidth',1.5)
hold on
plot(T,u60,'--go','linewidth',1.5)
hold on
plot(T,u75,'--','linewidth',1.5)
hold on
plot(T,u105,'--','linewidth',1.5)
hold on
plot(T,u120,'--','linewidth',1.5)
legend('Uf at 15degrees C','Uf at 30degrees C',' Uf at 45degrees C',' Uf at 60degrees C','Uf at
75degrees C','Uf at 105degrees C','Uf at 120degrees C')
Figure (2)
plot(T,u75)
```

## DISCUSSION AND APPLICATIONS

This study highlights the critical role of temperature in influencing bacterial and fungal activities in crude oil-contaminated freshwater and saltwater environments. The data reveal that bacterial growth in saltwater increases with temperature, demonstrating its potential in thermally enhanced bioremediation. Conversely, fungal activity in freshwater exhibits fluctuating heat production, indicating complex temperature-microbe interactions. These findings underline the importance of temperature as both an activator and inhibitor of microbial growth.

### Applications

1. *Bioremediation*: Insights into microbial dynamics can optimize temperature settings in bioreactors for effective crude oil degradation in aquatic environments.
2. *Environmental management*: The study informs strategies for managing microbial populations in contaminated ecosystems, contributing to sustainable environmental practices.
3. *Industrial processes*: Industries dealing with oil spills can leverage these findings to improve microbial-based cleanup systems, enhancing efficiency in diverse temperature conditions.
4. *Aquatic ecology*: Understanding microbial responses aids in maintaining ecological balance in polluted water bodies, fostering healthier ecosystems.

## CONCLUSION

The findings of this study highlight the critical role temperature plays in the microbial dynamics of freshwater and saltwater environments contaminated with crude oil. Bacterial growth rates increased steadily with temperature in saltwater, indicating an activation response to higher thermal conditions. Conversely, fungal activity in freshwater showed a more complex relationship with time and temperature, reflecting variations in metabolic processes. The mathematical models developed provide a framework for understanding temperature's dual role as an inhibitor or activator in microbial growth, which is essential for optimizing bioremediation strategies. Future research should explore long-term temperature effects and the interplay between various environmental factors to further enhance our understanding of microbial responses in contaminated ecosystems. This study demonstrates the significant impact of temperature on microbial activity in crude oil-contaminated aquatic environments, with bacteria thriving in saltwater at higher temperatures and fungi exhibiting variable responses in freshwater. These findings provide valuable insights for optimizing bioremediation processes and managing contaminated ecosystems effectively.

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