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Research Article

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Inhibition kinetics of biodegradation of meat products using bio-preservative from *Murraya Koenigii Spreng*

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ABSTRACT

The effect of bio-preservative extracted from Murraya koenigii spreng (MKS) on biodegradation of meat products was studied. Essential oil (biopreservative) was extracted by steam distillation and analysed by GC-MS. 10.5g of bio- preservative was produced from 1kg of fresh leaves of MKS. The effect of biopreservative concentration, time and initial substrate concentration on microbial growth was studied. Microbial growth decreased as the concentration of the bio-preservative increased from 10mg/l to 30mg/l. However, the specific growth rate increased with increase in the initial substrate concentration from 1g/l to 4g/l but decreased as it increased beyond 4g/l. Four kinetic models such as Matheus, Monod, Modified Monod and Non- Competitive Inhibition models were used to determine the kinetic parameters. The kinetic parameters, maximum specific growth rate (μ_m), Monod's constant (K_s), death rate constant (K_d), and inhibition constant (K_I) were determined as 0.1009hr^{-1}, 0.6786g/l, -0.375hr^{-1} and 69.4 respectively. The activation energy was determined as1089J/mg, 1272J/mg and 2577J/mg at 10mg/l, 20mg/l and 30mg/l of MKS respectively. These results can be used for the correlation of the growth inhibition, the kinetic parameters and the preservative efficacy of the bio-preservative. The result showed that bio-preservative extracted from OG is a good preservative for meat products.

Key words: Biodegradation; Inhibition; Kinetics; *Murraya Koenigii Spreng*; bio-preservative

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INTRODUCTION

Food borne diseases are major concern in the third world and developing countries, and even in developed nations [1]. The consumption of food contaminated with some microorganisms is detrimental to human health. Growth of microorganisms usually leads to spoilage, formation of toxins and quality deterioration of food products. There is therefore scope for new methods of making food safe which have a natural or 'green' image. One of such is the use of essential oils as antibacterial additives [2]. Bailey emphasized that the need to extend the self-life of food products has necessitated the development of new food preservation processes [3].

Food preservation is the process of treating and handling food to stop or slow down spoilage (loss of quality, edibility or nutritional value) and thus allow for longer storage. There are two types of preservatives namely: chemical or synthetic preservatives and bio-preservatives. Chemical or synthetic preservatives are group of synthetic chemical substances that prevent spoilage and contamination of finished products by microorganisms. Although this group of preservatives is used to keep the food fresh and to stop the bacterial growth, yet some chemical preservatives are harmful if taken in more than the prescribed limits. Some of these preservatives are: nitrates, sulfites, sodium benzoate, propyl gallate and potassium sorbate. Although preservatives are used to keep the food fresh and to stop the bacterial growth, most chemical preservatives are harmful to health and as a result are prescribed to be taken in a small quantity [4]. Some of these preservatives cause brain damage, allergies, asthma and skin rashes, kidney and liver malfunctioning. They also cause high blood pressure and cholesterol level.

Bio-preservatives are chemical constituents extracted from natural sources that offer intrinsic ability to protect products against microbial growth. These include essential oil constituents, flavonoids, phenolic compounds etc. Their mode of action is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in the food stuff [5]. The interest of consumers is increasing for natural products due to their awareness towards health issues. Pharmaceutical and food industries are laying emphasis on the use of biopreservatives as an alternative to certain disadvantages associated with chemical preservatives [6].

Murranya koenigii spreng (curry leaves) is used extensively for flavouring and seasoning of dishes. The chemical constituents of the leaf are mainly terpenes and amino acids [7]. They are highly aromatic and possess anti-oxidant, antimicrobial and anti-inflammatory properties. Curry leaves also have a wide spectrum of natural liquid preservative system suitable for food, personal care and cosmetic applications.

The objective of this work is to study the effect of concentration of the bio-preservative, temperature and initial substrate concentration on the microbial growth inhibition and also to determine the kinetics of the reaction.

EXPERIMENTAL SECTION

2.1 Biopreservative extraction and characterization

Steam distillation process was used to extract bio-preservative (essential oil) from MKS according to the method described by [8]. 1kg of the fresh plant sample was placed in an extractor and steam from a steam generator was passed through it for 5hours. The extracted bio-preservative evaporated through a Liebig condenser where it condensed and the bio-preservative collected at the Collector as an emulsion. Aspirator Bucket containing ice block was used to cool the Condenser. The water content of the emulsion was reduced using anhydrous sodium sulphate (Na_2SO_4) and the bio-preservative was stored in a refrigerator at 4^0C until required for use. Gas Chromatographic (GC) analysis of the bio-preservative were performed using GCMS-QP2010 PLUS Shimadzu Japan apparatus equipped with an Rtx-5SIL - MS ('Restek') (30m x 0.25mm i.d; 0.25µm film thickness) fused-silica capillary column.

2.2 Determination of microbial growth kinetics

The procedure stated by Dubey was used for the determination of microbial growth kinetics [9]. Mixed culture spoilt boiled meat was prepared and serial dilution technique was used to dilute 1ml of culture to get 1 x 10^6 cells/ml by serial dilution technique. 100ml of double strength broth medium was dispensed in each of 250ml Erlenmeyer flask. The flask was autoclaved for 30minutes at a pressure of 15psi. One drop (about 0.03ml/drop) of the diluted broth was inoculated into the sterilized medium using a calibrated faster pipette. 10mg/l of the bio-preservative was introduced into the broth and the flask placed on a shaker at 150rpm and a control temperature of 298K for 48 hours. The spectrophotometer was switched on 30 minutes before taking OD (Optimal Density) so that it can get stabilized. 2ml of the broth culture was withdrawn at every 3 hour intervals for 24 hours and measured absorbance (OD) using a spectrophotometer at 600nm wave-length. Control experiment was also done in the absence of Murraya Koenigii Spreng. The procedure was repeated for 20mg/l and 30mg/l of preservative, and also at 308K and 318k.

The equations1-9 were used determine the microbial growth rate [10].

$$\frac{dX}{X} = \mu X \quad (1)$$

$$\int_{Xo}^{X} \frac{dX}{X} = \mu \int_0^t dt$$

$$\ln \frac{X}{Xo} = \mu t \quad (2)$$

$$\mu = \frac{1}{\Delta t} \ln \frac{X}{Xo} \quad (3)$$

A plot of $\ln \frac{X}{Xo}$ against t gives μ as the slope

The Maximum specific growth rate, μ_m and the Monod's constant, Ks at different initial substrate concentrations were calculated using:

$$\mu = \frac{\mu_m S}{K_s + S} \quad (4)$$

Taking reciprocal of equation 4 above gives

$$\frac{1}{\mu} = \frac{K_s}{\mu_m}\frac{1}{S} + \frac{1}{\mu_m} \quad (5)$$

Line-wearver bulk plot of $\frac{1}{\mu}$ against $\frac{1}{S}$ gives

$\frac{Ks}{\mu_m}$ as slope and $\frac{1}{\mu_m}$ as intercept

The modified Monod's equation was used to determine the Death rate constant K_d.

$$\mu = \frac{\mu_m S}{K_s + S} - K_d \quad (6)$$

Reciprocal of equation (6) above gives.

$$\frac{1}{\mu} = \frac{Ks}{\mu_m S} + \frac{1}{\mu_m} - \frac{1}{K_d} \quad (7)$$

A plot of $\frac{1}{\mu}$ against $\frac{1}{S}$ gives

$\frac{Ks}{\mu_m}$ as slope and $\frac{1}{\mu_m}$ - $\frac{1}{K_d}$ as intercept

Non-competitive equation was used to calculate the inhibition constant, K_I

$$\mu = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{I}{K_I}\right)} \quad (8)$$

Taking reciprocal of the equation of (8) and re-arranging gives

$$\frac{1}{\mu} = \left(\frac{K_I + I}{\mu_m K_I}\right)\frac{K_s}{S} + \left(\frac{K_I + I}{\mu_m K_I}\right) \quad (9)$$

A plot of $\frac{1}{\mu}$ against $\frac{1}{S}$ gives

$\left(\frac{K_I + I}{\mu_m K_I}\right)K_s$ as slope and $\left(\frac{K_I + I}{\mu_m K_I}\right)$ as intercept

Determination of activation energy

The equations 10-13 were used to determine temperature effect on microbial growth inhibition at 298K, 308K and 318K [10].

$$\frac{dN}{dt} = kN \quad (10)$$

$$\ln\frac{N}{No} = k\Delta t \quad (11)$$

A plot of $\ln\frac{N}{No}$ against t gives k as slope

$$K = Ae^{-\Delta E/RT} \quad (12)$$

Integrating

$$\ln K = -\left(\frac{\Delta E}{R}\right)\left(\frac{1}{T}\right) + \ln A \quad (13)$$

Plotting ln K against $1/T$ gives $-\Delta E/R$ as slope and ln A as intercept.

Where K = specific growth rate, R = gas constant, ΔE = activation energy,
A = Arrhenius constant.

RESULTS AND DISCUSSION

3.1 Production and characterization of the biopreservatives

10.5g of bio- preservative was produced from 1kg of fresh leaves of *Murraya koenigii spreng* by steam distillation process. This represented a yield of 1.05% of bio-preservative. The bio-preservative produced by steam distillation process was analysed using GC-MS. The GC-MS analysis of MKS bio-preservative showed four distinct peaks representing the four compounds contained in the bio-preservative. They are 2, 6-Dimethyl-2,7-octadien-6-ol(Linalool), n-Decanoic acid, 9- Hexadecenoic acid and 1-pentadecanecarboxylic acid (palmitic acid). The composition of the bio-preservative is shown in Table 1.

Table 1. Constituents of MKS bio-preservative

COMPOUND	%	MOL. WEIGHT	RETENTION INDEX	RETENTION TIME(min)	MOLECULAR FORMULAR
2,6-DIMETHYL-2,7-OCTADIEN-6-OL	28.89	154	1082	8.6	$C_{10}H_{18}O$
N-DECANOIC Acid	20	17.2	1372	21.283	$C_{10}H_{22}O_2$
9-HEXADECENOIC ACID	40	254	1976	23.025	$C_{16}H_{30}O_2$
1-PENTADECANE- CARBOXYLIC ACID	11.11	256	1968	23.183	$C_{16}H_{30}O_2$

3.2 Effect of initial substrate concentration on the specific growth rate at 298k and 30mg/l.

A study of the initial substrate concentration on growth rate showed that the specific growth rate increased as the initial substrate concentration increased from 1g/l to 4g/l. The following specific growth rates at initial substrate concentrations of 1g/l, 2g/l, 3g/l, 4g/l and 5g/l were obtained as $0.078hr^{-1}$, $0.096hr^{-1}$, $0.097hr^{-1}$, $0.099hr^{-1}$ and $0.074hr^{-1}$ respectively for MKS and $0.102hr^{-1}$, $0.115hr^{-1}$, $0.129hr^{-1}$, $0.148hr^{-1}$, and $0.113hr^{-1}$ respectively for the Control broths (Figures 1 – 4). Agarry and his coworkes made a similar observation [11]. Tahere and his coworkers observed that the growth of microorganisms corresponds to the degradation of the substrate [12].

5g/l initial substrate concentration showed a deviation from the trend of the reaction. The decrease in the specific growth rate at 5g/l was an indication of the effect of high initial substrate concentration on the organisms. Figure 1 showed that the highest specific growth rate of $0.099hr^{-1}$ for MKS and $0.148hr^{-1}$ for control was obtained at initial substrate concentration of 4g/l. High initial substrate concentration inhibits microbial growth [13] and high value of K_I indicates that the inhibition effect can be observed only in a high substrate concentration range [14]. The specific growth rate of an organism is controlled at each level of growth by the concentration of the limiting nutrient and the inhibition effects on cell growth stronger at high concentrations than at low substrate concentration [15]. Considering the specific growth rates for MKS and control, it was observed that the control broths showed higher growth than MKS broths. This implies that MKS inhibited growth of the organisms.

In this work, the classical method of linearizing the kinetic models was used to determine the kinetic parameters. Applying line-weaver Bulk plot (figures 5), the maximum specific growth rate of the mixed broth was $0.1621hr^{-1}$. Monod's constant, Ks is inversely proportional to the affinity of an organism to the substrate. That is, high Ks indicate low affinity. The Ks value was determined as 0.6786g/l and 0.6213g/l for MKS and Control respectively. The decrease in the Ks value of MKS is an indication of the effect of the bio-preservative on growth of the organisms. Similar result was obtained by other authors [15, 16]. The Ks value was a direct relation of the preservation efficiency of the bio-preservative (Table 4).

The Inhibition constant, K_I was determined as 69.4g/l using the non-competitive inhibition equation. The higher the K_I the less effective the inhibitor becomes [14]. Also the Decay rate constant, K_d which is a measure of death of the organisms in the presence of the bio-preservative was determined as $-0.3750hr^{-1}$. This result indicates death rate of organisms in the presence of MKS.

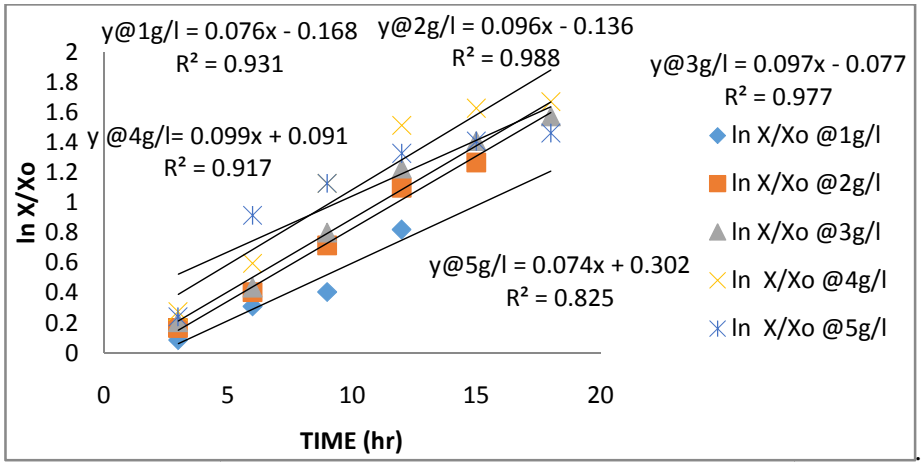

Fig.1. Specific Growth Rate at Initial Substrate Concentrations (1, 2, 3, 4, 5)g/l of 300mg/l of MKS and 298K

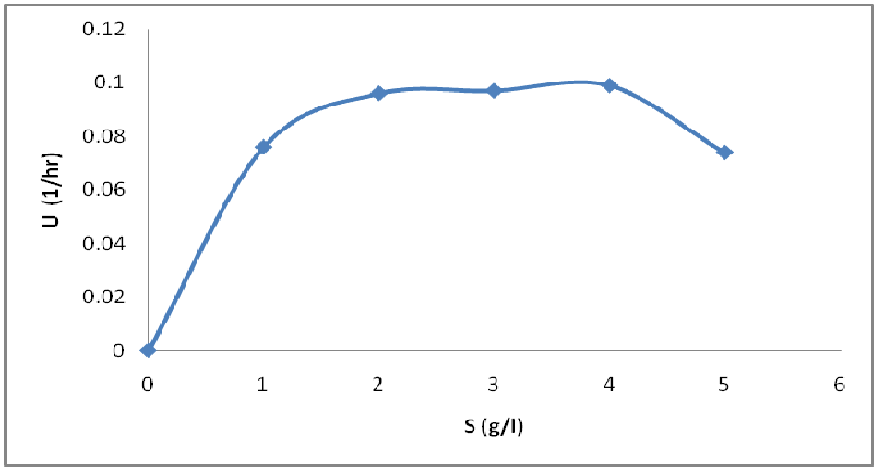

Fig. 2. Effect of Initial Substrate Concentration on microbial growth

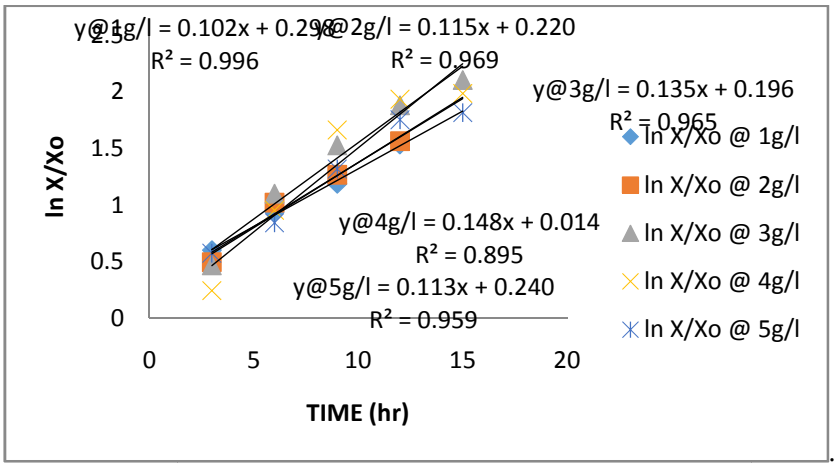

Fig.3. Specific Growth Rate at Initial Substrate Concentrations(1, 2, 3, 4, 5)g/l of 300mg/ml of Control and 298K

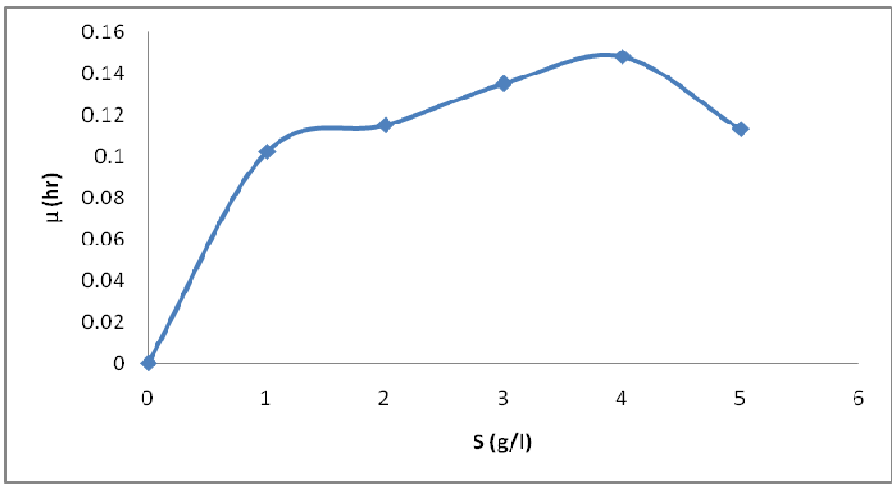

Fig.4. Effect of Initial Substrate Concentration on microbial growth for the control broth

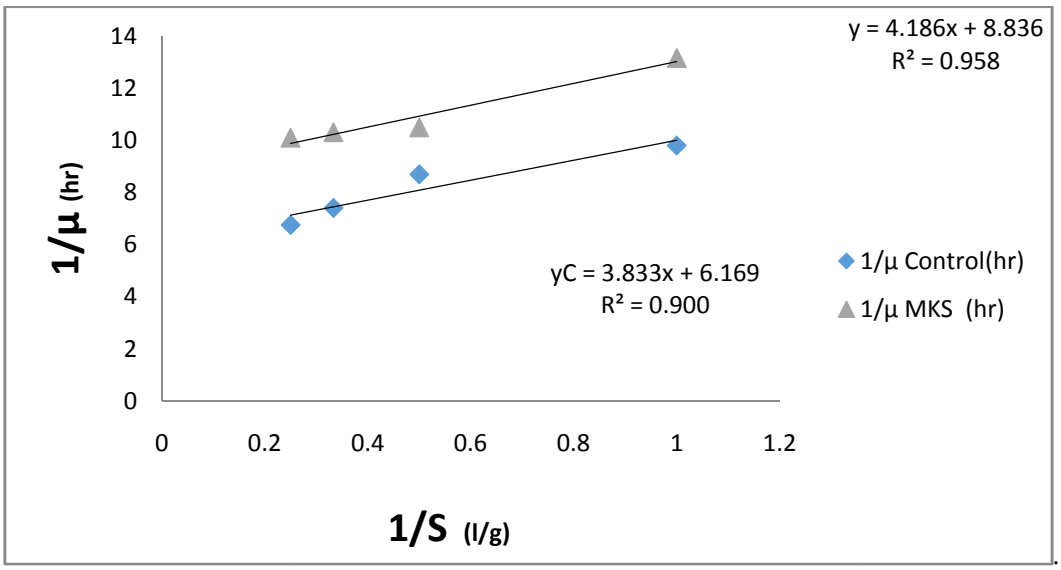

Fig.5. Line-Weaver Bulk plot of effect of Initial Substrate Concentration on the kinetic parameters for MKS and Control

3.3 Effect of concentration on specific growth rate

It can be seen from Figures 6-8 and Table 2 that the specific growth rate of the organisms increased until growth reached the stationary phase and subsequently began to decline. This is as a result of death of some of the organisms which started before growth reached the stationary phase. Dubey made a similar observation [9]. The control broth was seen to have higher average specific growth rate compared to the broths containing MKS bio-preservative. The presence of bio-preservatives decreased the affinity of the enzymes for the substrate thereby inhibiting growth. This is in line with the work of Chime [15]. Decrease in the velocity of the reaction in the presence of inhibitors was because the enzyme inhibitor complex did not break down to yield products. Therefore increasing the value Ks decreases the rate at which substrate binds enzymes and thus the rate of product formation. The degree of inhibition is dependent on the relative concentrations of the substrate and inhibitor [15]. It was observed that the specific growth rate in the absence of MKS was faster and higher than in the presence of MKS.

The average specific growth rate for the control at 298k was 0.1434hr^{-1}, but it decreased in the presence bio-preservatives (Table 3). Increase in concentration of the bio-preservative decreased the average specific growth rate of the organisms. At 10mg/l, 20mg/l and 30mg/l of MKS, the average specific growth rate was 0.1185 hr^{-1}, 0.0910 hr^{-1}, 0.079 hr^{-1} respectively. It was also observed that the average growth rate of the organisms in the absence of bio-preservatives almost doubled the growth in the presence of 30mg/l of MKS. This shows that MKS bio-preservative inhibits microbial growth. This trend was equally observed at temperatures of 308k and 318k.

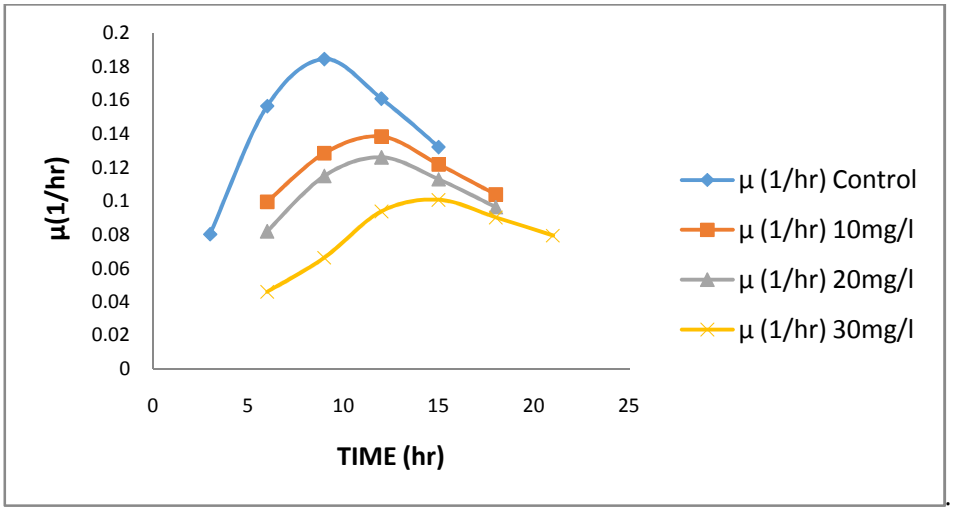

Fig.6. Specific Growth Rate Vs Time At 298k

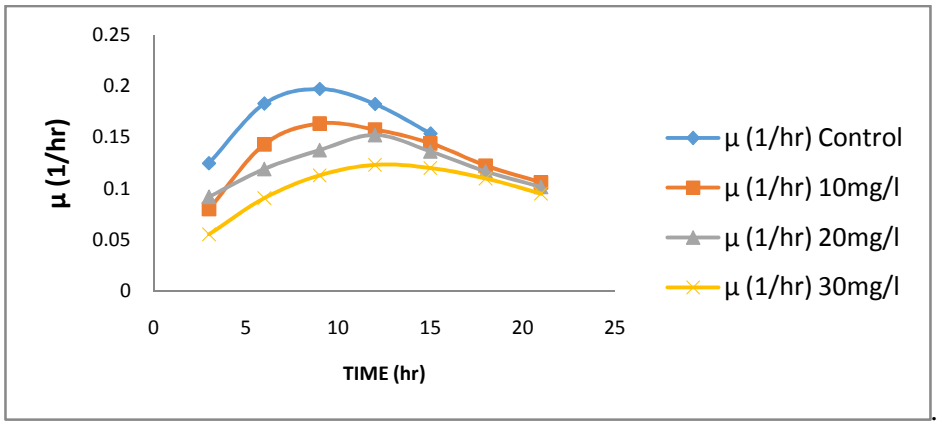

Fig.7. Specific Growth Rate Vs Time at 308k

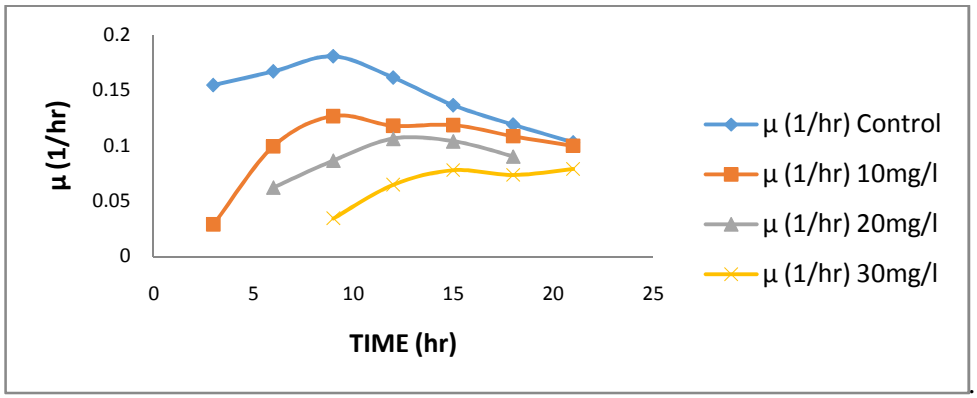

Fig.8. Specific Growth Rate Vs Time at 318k

Table 2. Maximum Specific Growth for the reactions

	Control	10mg/l	20mg/l	30mg/l
μ_m (hr^{-1}) at 298K	0.1847	0.1385	0.1262	0.1009
μ_m (hr^{-1}) at 308K	0.1974	0.1637	0.1524	0.1236
μ_m (hr^{-1}) at 318K	0.1808	0.127	0.1065	0.0792

Table 3. Average specific rate and the doubling time

	CONTROL	MKS		
		10mg/l	20mg/l	30mg/l
$\mu_{AV}(hr^{-1})$ at 298K	0.1434	0.1185	0.1066	0.0796
$\mu_{AV}(hr^{-1})$ at 308K	0.1684	0.1312	0.1223	0.1014
$\mu_{AV}(hr^{-1})$ at 318K	0.1462	0.1002	0.9004	0.0661

3.4 Effect of concentration on microbial growth

It can be seen that the microbial inhibition increased as the concentration of the bio-preservative increased (Figures 9-11 and Table 4). At constant temperature, the bio-preservative exhibited the least inhibition at concentration of 10mg/l and the highest inhibition at concentration of 30mg/l. Microbial growth was very fast with very little lag phase in the absence of bio-preservative. The lag phase increased as the concentration of bio-preservative increased thereby decreasing the growth rate. Lee and his fellow workers stated that eugenol inhibited haxanal oxidation by 32% at 1µg/ml over 30days and the oxidative activity increased to 97% and 100% at 5µg/ml and 10µg/ml respectively [17]. The inhibition of bio-preservative increases with increase in the concentration of the preservation [18]. The maximum specific growth rate (μ_m) at 25°C was $0.1042hr^{-1}$, $0.07hr^{-1}$, $0.0448hr^{-1}$ and $0.1847hr^{-1}$ for 10mg/l, 20mg/l and 30mg/l of bio-preservative and Control respectively.

Figure 9 and Table 2 showed that at constant temperature of 298K, growth in the control broth was rapid with the highest maximum specific growth rate of $0.1847hr^{-1}$. In the presence of bio-preservative, the maximum specific growth rate was $0.1042hr^{-1}$, $0.07hr^{-1}$, $0.0448hr^{-1}$ for 10mg/l, 20mg/l, and 30mg/l respectively. This is an indication of the fact that increase in concentration of MKS bio-preservative increased its preservation value at constant temperature. At 308K, μ_m was $0.1637hr^{-1}$, $0.1524hr^{-1}$, $0.1236hr^{-1}$ for 10mg/l, 20mg/l , 30mg/l respectively and $0.0655hr^{-1}$, $0.127hr^{-1}$, $0.0792hr^{-1}$ for 10mg/l, 20mg/l , 30mg/l respectively at 318k. The microbial density in the presence of MKS was much less than in the absence of MKS (Figure 9-11).

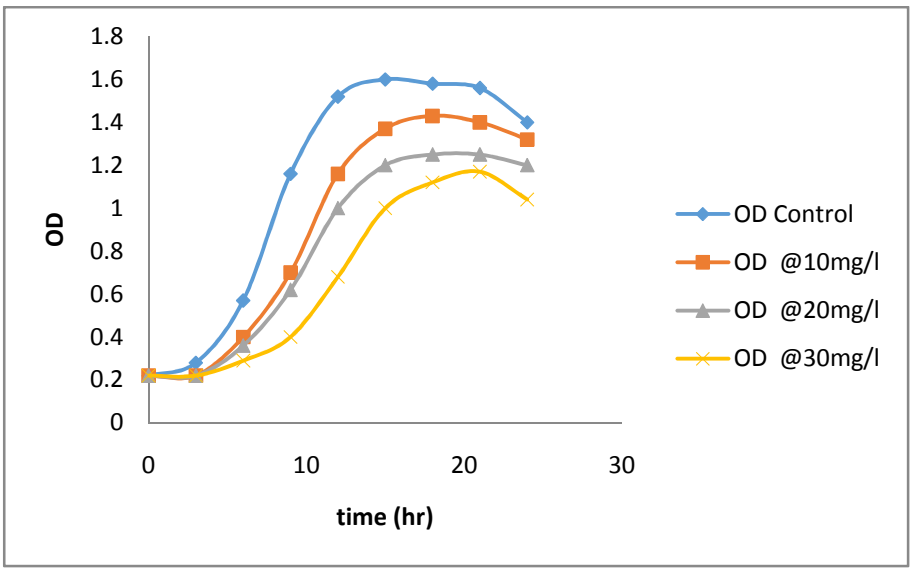

Fig. 9. Effect of Concentration on Microbial Growth at 298K for MKS

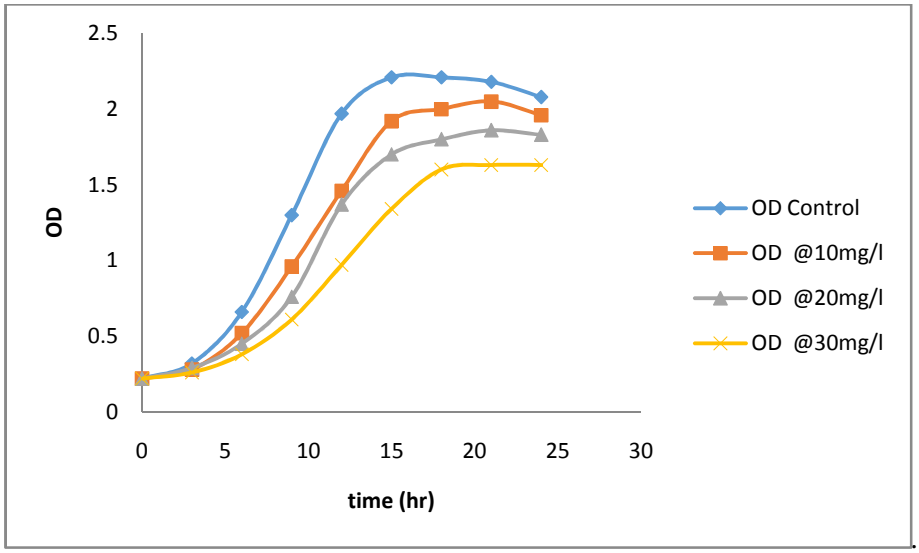

Fig. 10. Effect of concentration on microbial growth at 308K for MKS

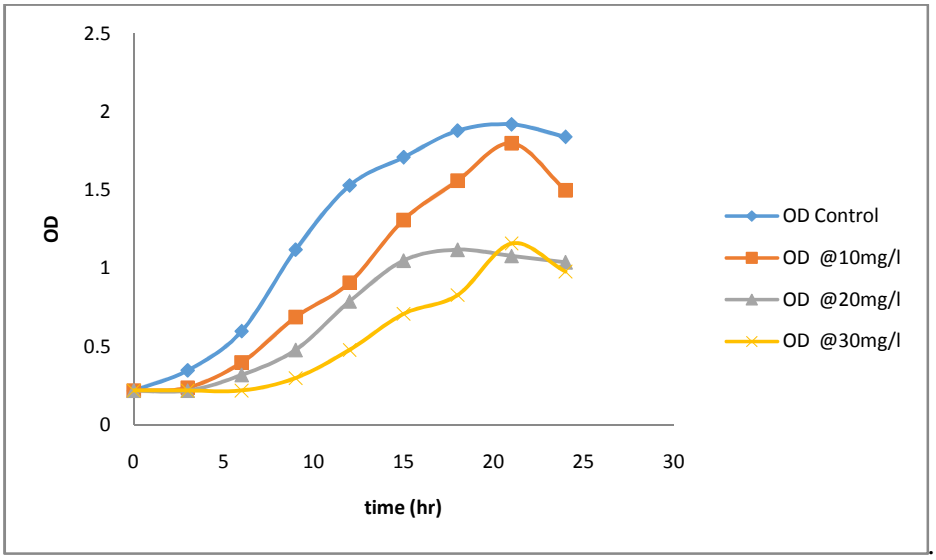

Fig. 11. Effect of Concentration on microbial growth at 318K for MKS

Table 4. % Inhibition of microbial growth rate by MKS

	298K			308K		
TIME(hr)	10mg/l	20mg/l	30mg/l	10mg/l	20mg/l	30mg/l
0	0	0	0	0	0	0
3	21.43	21.43	21.43	12.5	9.38	18.75
6	29.82	36.34	49.12	21.21	31.82	42.42
9	39.66	46.55	65.52	26.15	41.54	53.08
12	23.68	34.21	55.26	25.89	30.46	50.76
15	14.38	24.38	37.5	13.12	23.08	39.37
18	9.49	18.99	29.11	9.5	14.48	21.17
21	10.26	19.87	25	5.96	11.93	19.73
24	5.71	14.29	25.71	5.77	13.02	21.63

3.5 Effect of temperature on microbial growth

It is observed from that the microbial growth increased as the temperature increased (Figures 12 - 14 and Table 7). In the presence of MKS at temperatures of 298K, 308K and 318K, the growth rate was 0.107hr^{-1}, 0.111hr^{-1} and 0.111 hr^{-1} for 10mg/l; 0.105 hr^{-1} , 0.108 hr^{-1} , 0.110 hr^{-1} for 20mg/l; 0.099hr^{-1}, 0.108hr^{-1} and 0.108hr^{-1} for 30mg/l respectively. It was also seen that the specific growth rate decreased as the concentration of the bio-preservatives increased under the same temperature but increased as the temperature increased. This result is in agreement with the work of other authors [10, 15].

In the presence of 10mg/l of MKS, the specific growth rate increased from 0.107hr^{-1} to 0.111hr^{-1} at 298K to 308K and remained constant till 318K. Likewise the specific growth rate at 30mg/l of MKS increased from 0.099hr^{-1} to 0.108hr^{-1} at 298K to 308K and remained constant till 318K. The variation was due to the denaturation of the enzymes at temperatures above the optimal temperature for the organisms. Another author supported this result showing the temperature dependency of growth rate [19].

The Activation energy at 10mg/l, 20mg/l and 30mg/l of MKS was 1089J/mg, 1272J/mg and 2577J/mg respectively (Figure 15 – 17). At constant temperature, increase in concentration of MKS increased the activation energy. Activation energy is very low for very fast reactions and the rate of reaction is less affected by temperature [10]. Consequently, the empirical reaction dependent constant, A at 10mg/l, 20mg/l and 30mg/l of MKS was 0.1676, 0.1766 and 0.2865 respectively. It also increased with increase in concentration.

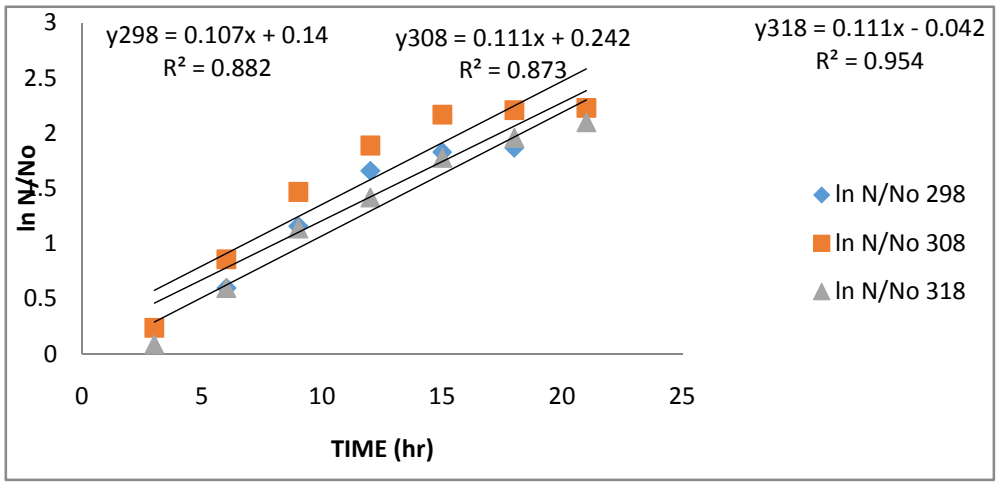

Fig.12. Thermal Growth Rate of the Organisms at 10mg/l of MKS

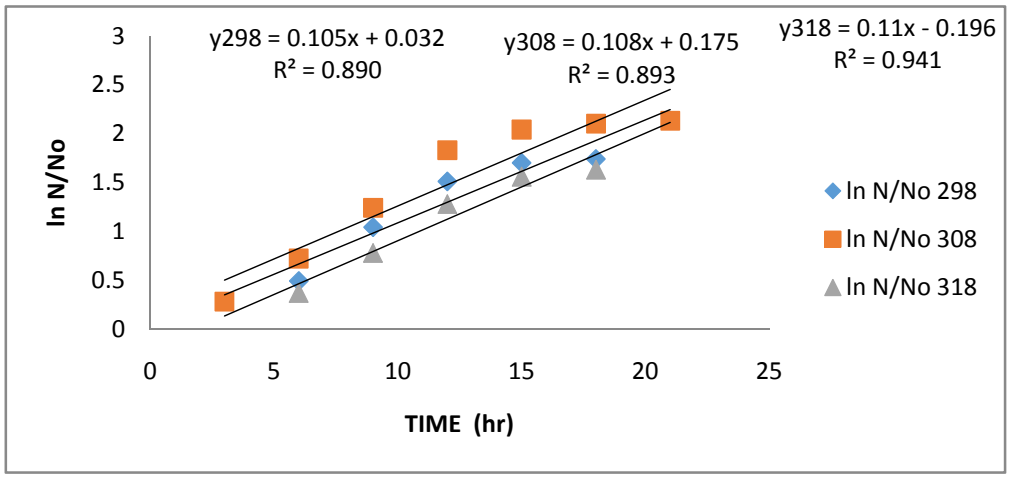

Fig. 13. Thermal growth rate of the organisms at 20mg/l of MKS

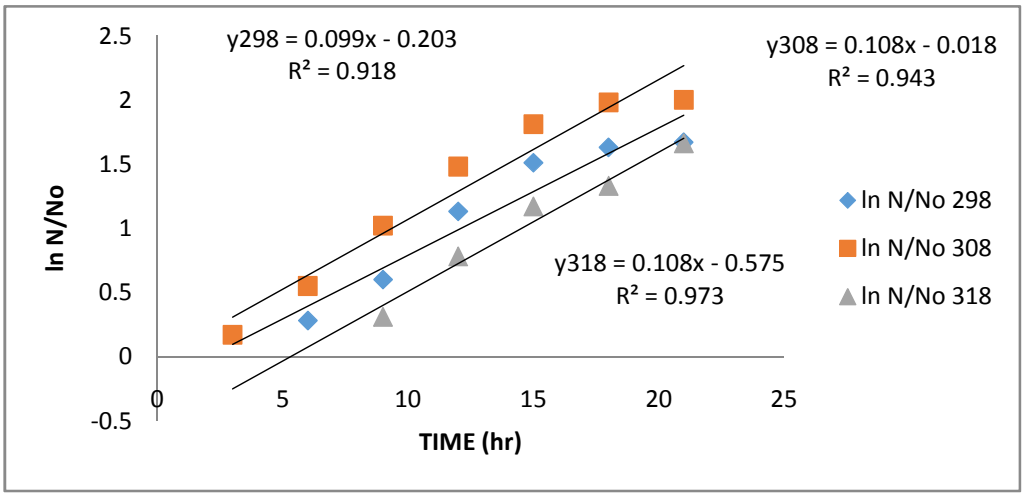

Fig. 14. Thermal growth rate of the organisms at 30mg/l of MKS

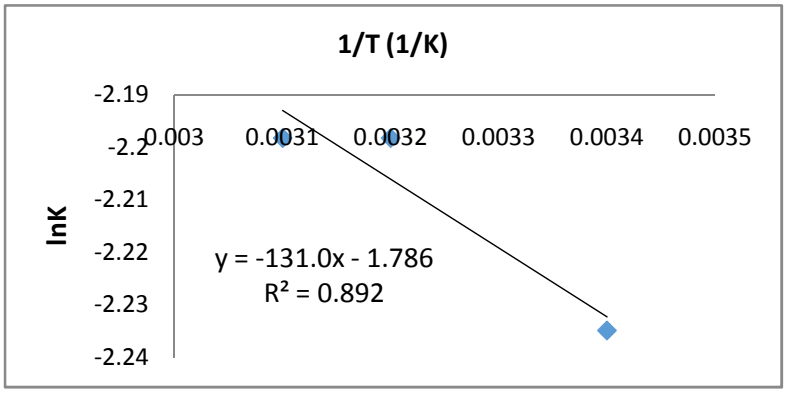

Fig. 15. Plot of lnK vs 1/T for 10mg/l of MKS

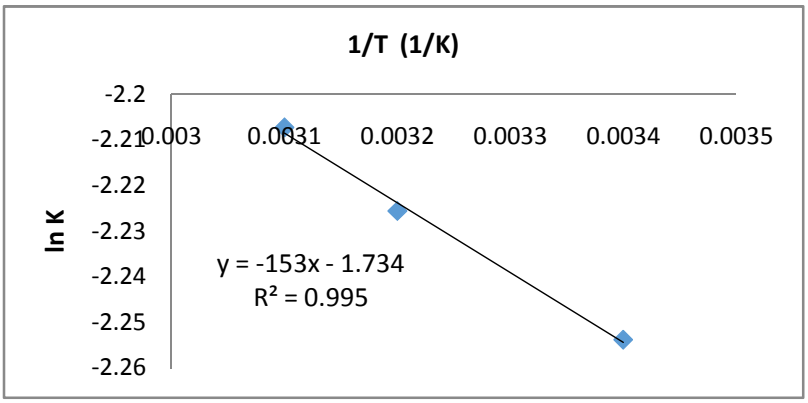

Fig. 16. Plot of lnK vs 1/T for 20mg/l of MKS

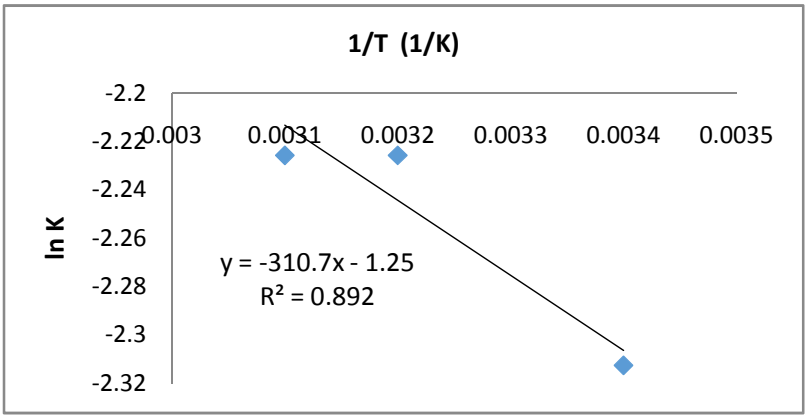

Fig. 17. Plot of lnK vs 1/T for 30mg/l of MKS

CONCLUSION

This study has shown that increase in concentration of bio-preservatives and initial substrate concentration affect microbial growth. Microbial growth decreased with increase in concentration of bio-preservative while it increased with increase in initial substrate concentration. However initial substrate concentration above 4g/l decreased the specific growth rate. This confirmed the inhibitory effect of high initial substrate concentration.

The kinetic constants μ_m, K_s, K_d, K_I were determined. The maximum specific growth rate for the control broth was much higher than that of MKS. This showed that the presence of MKS inhibited the meat degradation by reducing the microbial growth. The high K_s value in the presence of MKS is also a reflection of the inhibitory ability of biodegradation of meat. The death rate, K_d obtained indicated that MKS inhibited the growth of the organisms. Also the inhibition constant, K_I, increased growth rate as it increased.

Change in temperature affected microbial growth. Microbial growth increased with increase in temperature but decreased after the optimal temperature range was reached as a result of denaturation of the organisms. The activation energy, ΔE, of 1089J/mg, 1272J/mg and 2577J/mg was obtained for 10mg/l, 20mg/l and 30mg/l of MKS respectively. The higher the activation energy the higher the energy required to initiate the reaction.

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