Study of The Optimization Condition Of Batch Sterilization Using Genetic Algorithm

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Abstract

The present work is designed to study the parameters interaction of sterilization processes in batch bioreactors (fermentors of volume 120 liter with medium of 56784 kg). he parameters include the effects of sterilization temperatures (117-126 ^{o}C), time of heating, time of holding, and time of cooling on removal of all organisms, and degradation degree of medium. Direct steam was used for heating at different temperatures ranged from 120 ^{o}C to 180 ^{o}C. The *B.Stearothermophilus* was selected as the present contaminants. Another bioreactor of volume (56828) liter was studied at 121 ^{o}C for the same contaminant and compared with actual data.

This study is achieved by designed procedure and simulation program useful for the optimization of batch sterilization cycle in large-scale fermentors. The method of optimization used is Genetic Algorithm (GAs) which uses probability to find the optimum condition for the sterilization cycle and to find Del factor; which is the reduction value of initial to final number of microorganisms, and then evaluate the cost which depend on amount of steam consumed in the sterilization processes

Graphical relations was indicated that as fermentor size increase, the time of heating also increase. For low temperature the time of holding was increased and for high temperature the time of holding was decreased. Also these relations were investigated the best conditions between holding time and Del factor for degradation at different temperatures.

Keywords: Batch Sterilization Reactor, Genetic Algorithm

دراسة واختبار الأفضل في مفاعل التعقيم بالدفعات باستخدام البرمجة الجينية

الخلاصة

صمم عمل البحث دراسة التداخل بين المجموعة من المتغيرات في عملية التعقيم في المفاعلات البيولوجية ذات الدفعات باختيار (حجم 120 لتر مع وزن مادة الوسط 56784 كغم). شملت تأثير المتغيرات كل من درجة حرارة التعقيم (117-126 درجة مئوية)، وزمن التسخين والتبريد وزمن التثبيت عند تلك الدرجة على إزالة جميع الكائنات الحية ومقدار التحلل لكل من الكائنات الحية والوسط المعقم . طريقة التسخين المستخدمة بواسطة البخار عند درجات حرارية تتراوح بين 120-180 درجة مئوية. ثم اختيار البكتريا السبورية B.stearothermphilus على أساس أنها الملوثات الموجودة. كذلك تمت دراسة مخمر أخر ذات حجم (5682 لتر) عند درجة حرارة 121 درجة مئوية وتمت مقارنة النتائج النظرية المتحصل عليها من النتائج العملية.

أنجزت الدراسة بواسطة تصميم طريقة عمل وتمثيلها بواسطة البرمجة الجينية والتي تمثل إحدى طرق الاختيار الأفضل في إيجاد أفضل الظروف لدورة عملية التعقيم وإيجاد عامل إل Del factor الذي يبين اختزال الكتلة الحيوية إلى الحد المطلوب، وكذلك إيجاد الكلفة بالاعتماد على كمية البخار المستخدمة في عملية التعقيم. تم الحصول على علاقات بيانية والتي تشير زيادة الحجم يزداد الزمن اللازم للتسخين والتبريد. استخدام درجة حرارة منخفضة يؤدي ازدياد زمن التثبيت وبالعكس. وكذلك أظهرت العلاقات البيانية أفضل الظروف بين زمن التثبيت والـــ Del factor للتحلل بدرجات حرارية مختلفة.

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Introduction

One of the first and most critical unit operations required for successful fermentation is medium sterilization. The choice of the sterilization method depends upon several factors including effectiveness in achieving an acceptable level of sterility, reliability, effect on medium quality, and cost, including operating and capital expense to achieve sterility *(Cooney, C.L.1985)*[1].

The term "sterilization" is the process designed to produce a sterile state and it is defined as the total absence of living organisms; sterilization in the absolute state cannot be achieved so this term.
indicates a probable conditions of complete freedom from viable microorganisms. The nature of the process of killing microorganisms is similar in its kinetic to a first order reaction (*Perkins, J.J, 1969, Aiba, S.and et.al. 1973)* [2,3].

Mainly there are two methods of seterilization: (A) physical seterilization, which includes (sunlight, drying, dry heat moist heat, filtration, radiation and ultrasonic vibrations) and (B) chemicals, which include (acids, alkalise, salts, halogens, oxidizing agents, reducing agents, formaldehyde, phenol, soap, dyes and erosol). The application of heat, in particular moist heat is practiced more than all others, because it is simple, reliable and (Cooney, C.L., 1985 and Perkins, J.J, 1989).[1,2]

The thermal death point (TDP) is generally defined as the lowest temperature at which a suspension of bacteria or other organisms in an aqueous medium is killed in 10 minutes. A more particular term is the thermal death point (TDT) which is the time required to kill culture at specified temperature *(Lutman, B.F., 1929)* [4]. Therefore, it is important to
optimize the heating process so that a medium is sterile after heating but minimal damage is caused to the ingredients of the medium.

The aim of this work is to find the minimum cost of sterilization process by evaluating different sterilization conditions effect on the batch sterilization cycle, these include:- 1- Temperature of sterilization, and thus (amount of steam needed). 2- Time of heating, holding and cooling for each case at sterilization temperature respectively.

This will be done by using a new method of optimization, Genetic Algorithm, which is search algorithm based on the mechanisms of natural selection and natural genetics

Theoretical Aspects

In batch sterilization process, the medium is placed in the fermentor and the content is heated to the seterilization temperature *(Hawker, L.E. and Linton, A.H., 1974)* [5]. A fermentor may be sterilized empty and then filled with sterile medium from a continuous sterilizer *(Kelsey, J.C., 1959)* [6],. In order to assess the time and temperature relationship, applied equation (1) presenting the kinetic of microbial death.

$$-\frac{dN}{dt} = k_d N \qquad \ldots.(1)$$

which on an integration on a fixed value of K_d yields:

$$\frac{N}{N_O} = \exp.(-K_d t) \qquad \ldots..(2)$$

since K_d is not constant but varies according to the temperature which is given by Arrhenius Eq.(3):

$$K_d = a * \exp(\frac{-E}{RT}) \qquad \ldots.(3)$$

so we get:-

$$\frac{dN}{dt} = A * \exp(-\frac{E}{RT}) * N \qquad \ldots.(4)$$

Temperature is a function of time depends on the type of heating process. An integration of Eq.(4) given:

$$\ln\frac{N_f}{N_o} = A\int_o^t \exp*(\frac{-E}{RT(t)})dt \qquad \ldots.(5)$$

The temperature time profile depends on number of factors. The medium can be heated by direct steam injection, or indirectly by heating coils or jackets, or electrically, or combination of the methods, *(Aiba, S. and et.al 1973, Jaypee, 1995)* [3,7]

The cost of the process can be calculated from Eq.(6) *(Jelen, F.C., 1970)* [8].

$$\cos t = M_e N_e + \sum_i E_i e_i + aL \sum_m I_m \quad \ldots.(6)$$

αL depreciation of equipments taken as (0.3), multiply by lang factor (3.6). the numbers of run can be computed as:

$$N_e = \frac{Anual - working - time}{total - time - of - process} = \frac{7200\,hr}{t_{total}} \quad \ldots.(7)$$

t_{total} represent the time for cleaning the fermentation vessel (hr), time for fermentation (hr), time for sterilization (hr), time for inoculation (hr) and time for discharge the broth (hr).

Genetic Algorithm and Design Procedure Genetic Algorithm

Genetic Algorithms (GAs) are non model based optimization methods with the ability to search along parameter space in highly direct way. The major strength of Gas, namely optimization with no initial guesses, no derivatives of the objective function and scope with local minima. Genetic Algorithms (Gas) are search algorithms based on the mechanisms of natural selection and natural genetics. They combine survival of the fittest among string structures with a structured yet randomized information exchange to form a search algorithm with some of the innovative fair of human search *(David, E.. 1989)* [9]. GAs will exhibit behavior similar to that described in Darwin's evolution theory, relatively high fitness structures have a large chance to survive and to produce even higher fitness off spring. The result will be an increase in the overall fitness of a population in each generation *(Batlett, 1995)* [10]. GAs start by randomly generating a population of individual (chromosome) each representing a point in the search space. The chromosomes are then evaluated to obtain a quantitive measure of how well they perform as possible solutions. The detailed description of GAs with program is shown in reference *(Salah, N.F., 2001)* [11].

Design-Procedure

In the design of a sterilization cycle for ensuring the success of a fermentation process, there are a number of assumption criteria that must be fulfilled. There are important points to be considered:- (1) How to reach such typical cycle, reduce the cost of sterilization, and how can we get use of each temperature, how can we be sure that the time for sterilization specified is enough?

To simplified the problem by considering the population to consist of a single heat resistant spore (Bacillus *stearothermoPhilus*). The thermal deactivations of this spore are well known and have been found to follow the Arrehenius relationship with temperature. This assumption leads to the design of a conservative sterilization cycle. Therefore integration Eq.(5) becomes:

$$\nabla_{total} = Ln\frac{N_o}{N} = A\int_o^t \exp(-E/RT)dt \quad ..(8)$$

where:

R is the universal gas constant = 1.98 $\frac{cal}{mol^{\circ}k}$

E is the activation energy = 67700 $\frac{cal}{mol}$

A is the frequency factor

$= 9.5x10^{37}\ min^{-1}$

T is the temperature (K) (Ashley, and Mooyman, 1982)

The symbol V_{total} (heating, holding, cooling) represent the design criteria of the sterilization process. ∇ is the logarithmic ratio of the initial to the final population, and is called the Del factor. The Del factor provides an indicator of the severity of the sterilization procedure. The detail design procedure and optimization program is shown in reference *(Salah, N.F., 2001)* [11].

The Gas treats an optimization by setting up a population of conditions and then after evaluating those conditions, produces a new population of conditions (next generation) by breeding from the best conditions of the parent populations. After random mutation of some individuals, the whole process continues until a suitable result is achieved. The vector of parameters of one individuals is coded as a bit string; each bit gives the value of part of one parameter. The coded variables are firstly the temperature, which is ranged from 100 ^{O}C to 130 ^{o}C and thus have five bits that simulate this range; secondly. Time of heating ranged from 40 to 100 minute and thus it have six bits that simulate this range; thirdly, time of holding ranged from 1 to 50 minute and thus it have six bits that simulate this range; finally, time of cooling ranged from 30 to 130 minute and thus it have bits that simulate this range ; thus we have final chromosome of length 23 bits as follows:-

Temperature Time heatig Time holdig Tim coolig

0		4	5		10	11		16	17		23

The fitness function is the decimal equivalence of binary string.

Results and Discussion

The results of the optimization was presented by evaluating the effect of varying batch sterilization conditions on the process parameters.

Effect temperature

It is important to asses the effect of optimum seterilization temperature on relevant criteria in order to minimize overall nutrient degradation. Therefore a batch fermentor of volume 120 m^3 with total mass of media of 56784 kg is assumed and using direct steam sparging. For a range of sterilization temperature(117-126 ^{o}C) the optimum result of the time of heating, holding and cooling should be used as shown in Figs.(1 to 3).

The general trends of these Figs. are similar; high temperature requires shorter time than low temperatures. Figure (4) gives a comparison between actual data Diendoerfer *(Deindoerfer,1957)* [13]. and estimated data of temperature and times of heating, holding, and cooling to get the sum reduction in viable organisms at temperature of 121 ^{o}C. one finds small difference between the actual and estimated calculations due to approximation in the equations with regard to conditions.

Effect of Fermentor size

The time taken to reach sterilization temperature and cooling to initial temperature will vary according to the size of vessel as shown in Figs.(5 to 8). These Figs. Show a comparison between the actual data from Deindoerfer *(Deindoerfer, 1957)* [13] and the results obtained from the program when using the same volume and to reach the same reduction in viable organisms (Del factor). The general trends of these Figures are similar to that of actual results.

DeathRate of Microorganisms

Figures (9 a, b, c, d) shows death rate data for spores of bacillus stearothermophilus for the condition used in the optimization proves, the general trend of these Figs. Is similar to the actual data by Aiba and Humphery [5].

Del Factors

Figures (10 to 12) shows the reduction value of microorganisms (Del factor) with temperature of sterilization (for range 117-126 ^{o}C) at different time of heating, holding and cooling. Fig.(13) gives the relation between the Del Factor of the nutrient degradation with time of holding at different temperature, this Fig. allow the selection of acceptable combinations of sterilization temperature without causing nutrient degradation.

Effect of Amount of Steam

Amount of steam can effect the cost of the process. The change of the total mass of steam depends on the enthalpy difference between the temperature of steam and initial temperature of the media. Steam of temperature given total mass of steam less than if we use lower steam temperature as seen in Figs.14 and 15

Conclusions

The following conclusions are drawn from the present study:-

(1) An optimization method based on the natural selection (GAs) is presented which can be used to determine the optimal sterilization temperature and necessary hold time that will minimize overall nutrient damage while ensuring sterility.

(2) The effect of long heat up time, and cool down time have adverse affect on the product yield due to high value Del factor ∇_n , estimate of degradation for a typical nutrient.

(3) The value ∇_m of overall cycle varying from 7.97 to 371.12. It was found that at Del values of about 36-56 give the sterility level and minimum ∇_n value, which mean good productivity.

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Nomenclature

	requency factor for first order reaction	min^{-1}
	ubstrate concentration	g.bio/L.
ΔE	ctivation energy	J/mol.K
i	mount of steam, water	Kg
Ei	Cost of steam, water	$
K	Specific death rate constant	min^{-1}
Mc	Cost of fresh medium per batch	$
N	Final number of microorganisms	No./mol.
No	Initial number of microorganisms	No./mol.
Nc	Number of runs	---
R	Universal gas constant	J/mol.K
T	Temperature	^{o}K
t	Time	min.
α	Absortivity of can	___

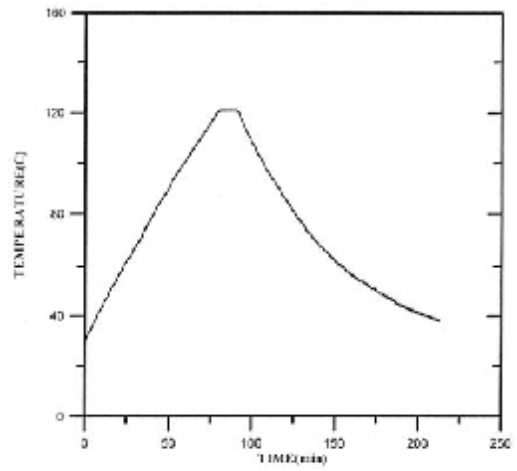


Fig (2) Temperature-time profile at temperature=121 °C

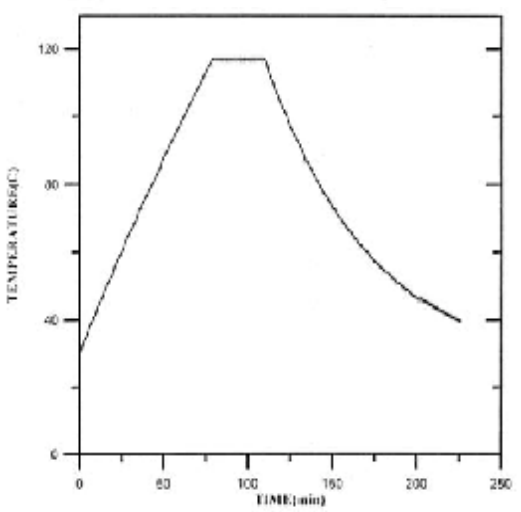


Fig (3) Temperature-time profile at temperature=117°C

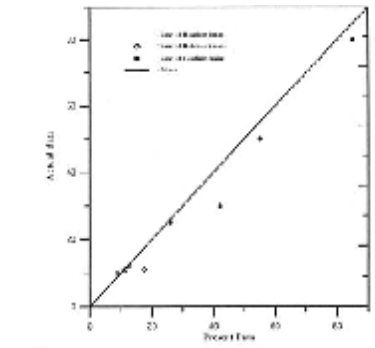


Fig (4) Comparison between actual and present data for time of heating, holding and cooling

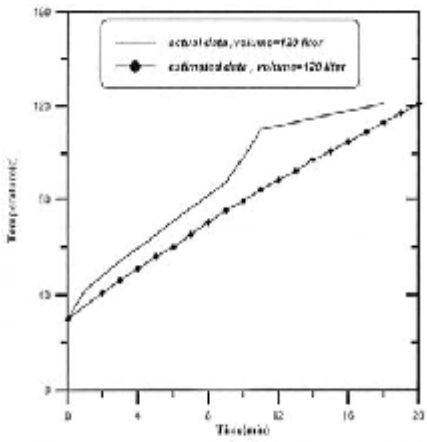


Fig (5) Heating temperature-time profile for volume 120 liter

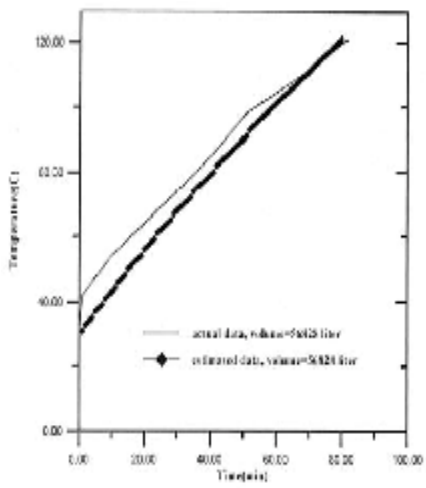


Fig (6) Heating temperature-time profile for volume 56828 liter.

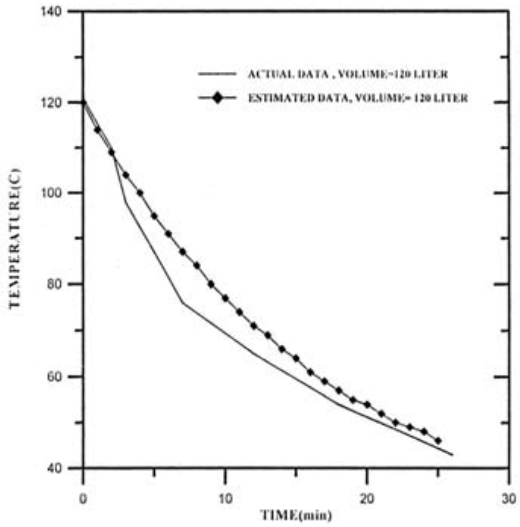


Fig (7) Cooling temperature-time profile for volume 120 liter.

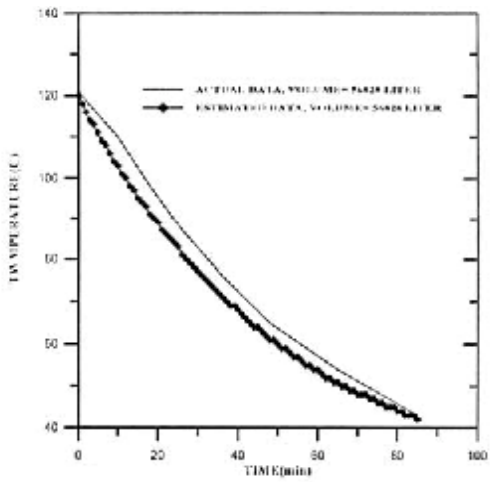
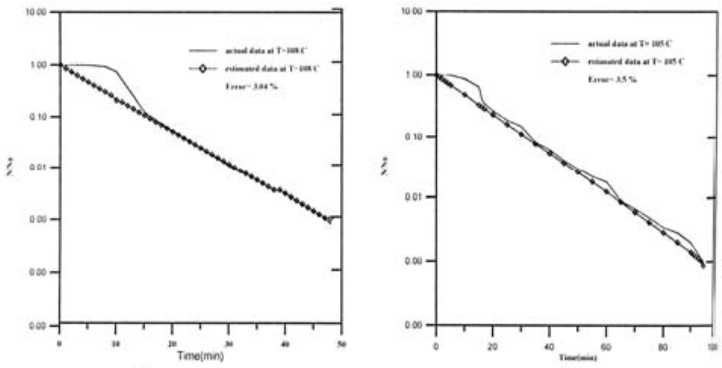
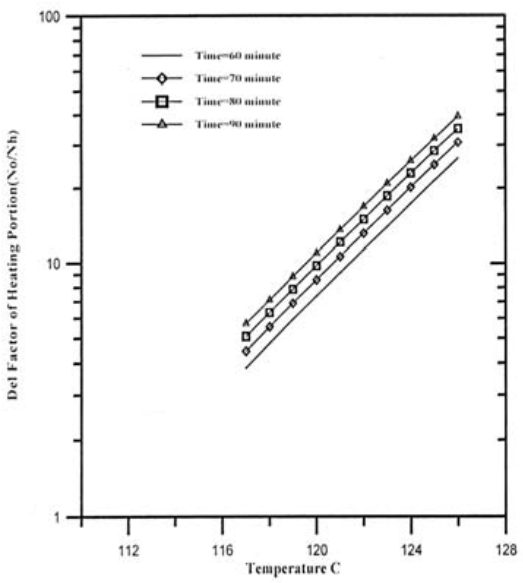


Fig (8) Cooling temperature-time profile for volume 56828 liter.

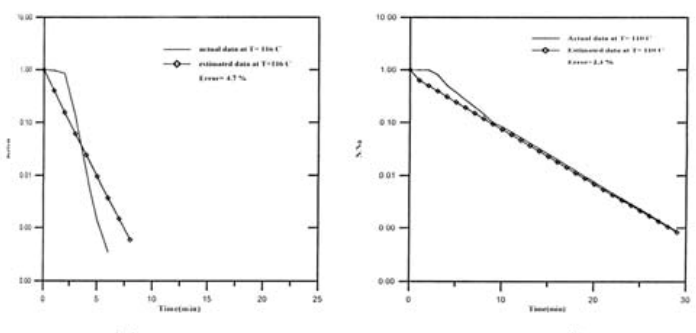


(c) (d)

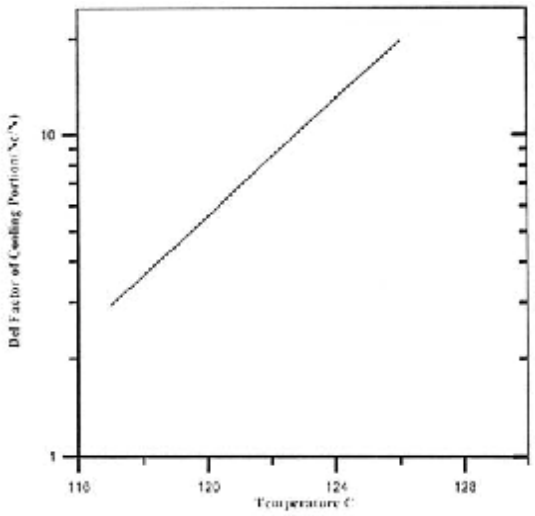
Fig (9) Time with (N/N_0) of Bacillus stearothermophilus (actual and estimated data for different temperatures ((a) for T=116 C^0, (b) for T=110 C^0, (c) for T=108 C^0, (d) for T=105 C^0).



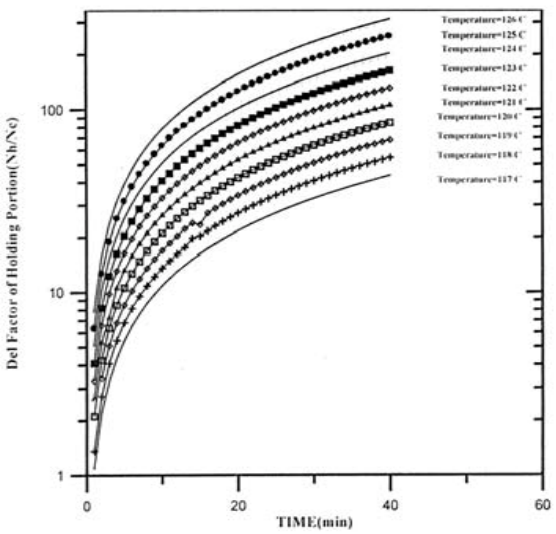
Fig(10)Del factor of heating portion with temperature



(a) (b)



Fig(12) Del factor of cooling portion with temperature



Fig(11) Del factor of holding portion with time at different temperatures

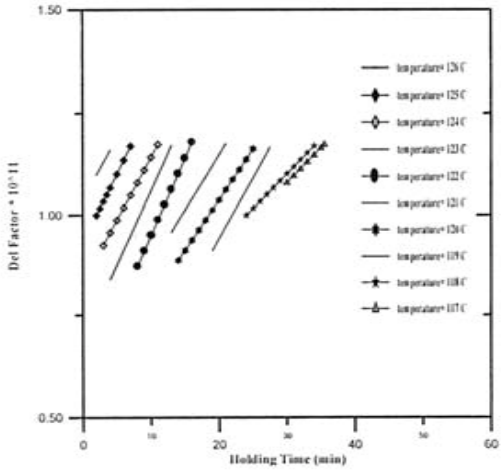


Fig (13) Del factor of degradation with time of holding at different temperature.

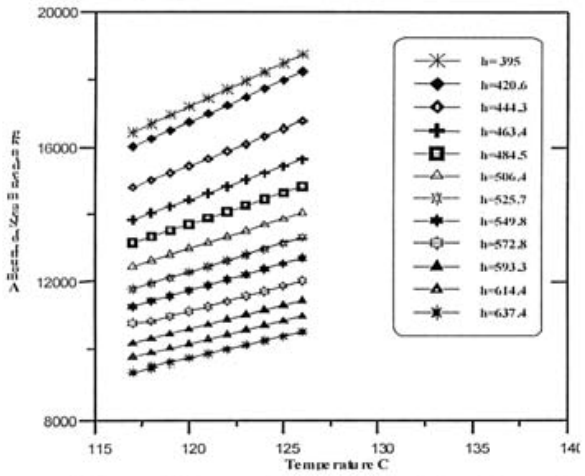


Fig (14) Amount of steam with temperature at different steam temperatures

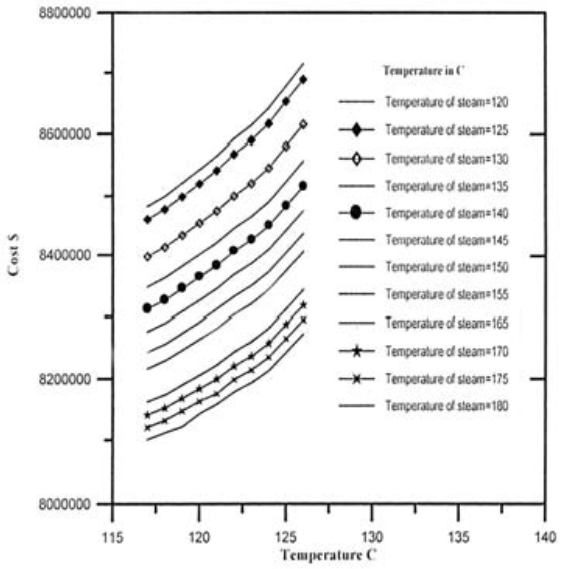


Fig (15) Cost of the process at different steam temperatures