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Quantitative Analysis of Caffeine by Percent Degradation Assay and Iodometric Titration

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Abstract: ***Caffeine, a methyl xanthine molecule which is the most widely consumed psychoactive substance in the world over, most commonly from the beverages coffee, tea and soda. Caffeine is naturally present in different varieties of plants, tea leaves, cocoa beans etc. It acts as stimulant to central nervous system and has various negative withdrawal effects on health. Caffeine can be treated chemically as well as biologically. Biodegradation by bacteria is considered to be the most efficient technique in degrading caffeine within environment. In the present study, four isolates capable of utilizing caffeine as a sole source of carbon were isolated from garden soil. The bacteria were characterized by conventional morphological and biochemical test. To quantify the amount of biodegradation, percent degradation assay was performed in which Isolate 1 and Isolate 4 shows maximum degradation of caffeine. Isolate-1 shows 98, Isolate- 2 shows 88.63, Isolate-3 shows 95.45 and Isolate-4 shows 95.45 after 48 hours of incubation. Iodometric back titration was performed to measure the remaining concentration of caffeine in broth.***

Keywords: Caffeine, Biodegradation, Percent degradation assay, Iodometric back titration

1. Introduction

Caffeine is a methyl xanthine molecule which is the most widely consumed psychoactive substance in the world, most commonly from the beverages coffee, tea and soda. The English word caffeine comes from the French (Spanish and Portuguese) word for coffee: café. Because of its stimulatory nature, it was used as a cardiotonic till the end of 19th century (wijhe, 2002).In the first half of the 20th century; it was used as a stimulant of respiration and circulation in Dutch medicine. The Islamic physician was the first to exploit the medicinal use of coffee well before second millennium A.D, the first documented use as a beverage was by the Sufis of Yemen. With caffeine being increasingly used as a stimulant, it was prohibited from being used as it was thought that caffeine used was a cause for wices and is seditious. Coffee was introduced to England around 1650's and in Holland a decade later. The Dutch introduced the coffee plant to the island of Java in 1688.the Island's association with coffee production led to the use of "Java" as a nickname for high quality coffee[12].

Caffeine is found in about a hundred species of plants, but the most highly cultivated sources are the coffee beans. (*Coffea arabica*or, *Coffea canephora*, variety robusta), the leaves and leaf-buds of tea (*Thea sinensis* or *Camellia sinensis*), cola nuts (*Cola acuminata*) and cacao beans (*Theobroma cacao*) [1][12].

Coffee and tea plants are the major sources of natural caffeine and related compounds such as theophylline and theobromine are produced by a large number of plant species belonging to numerous genera, families, andorders. It is believed that methylxanthine producing plants accumulate these substances as part of a chemical defense system against pests and herbivores [1] [12].

A very large proportion of the non-alcoholic beverages used in social settings contain caffeine. The most important beverages and foods containing caffeine are coffee, tea, guarana, mate, cola nuts, cola drinks, cocoa, chocolate.The amount of caffeine found in these products varies, but is generally high. Based on dry weight, the highest amounts are found in guarana (4-7%).Tea leaves contain approximately 3-5% caffeine, coffee beans 1.1-2.2%(Saldana et.al 2000), cola nuts 1.5%, and cocoa beans 0.03% (Bogo and Mantle, 2000; Kretschmar and Baumann, 1999).Cocoa beans in addition contain about 1.8-2.5% theobromine. Caffeine also occurs in certain soft drinks, and so called "smart "drinks, as well as in medicinal drugs. In these cases, however, purified or synthesized caffeine has often been added to the products. Caffeine is responsible for the stimulant action of coffee (Europaisches, 1978). It stimulates the central nervous system, increases the contraction power of the heart, widens the vessels of heart, kidney and the skin and exhibits broncholytical and diuretical action. (Europaisches, 1978) [1].

In mammals, ingested caffeine is rapidly absorbed, metabolized, and excreted in the urine as methyl xanthine derivative. Apart from being a stimulant to the central nervous system, if consumed in excess it causes mutation; it is teratogenic, causes inhibition of DNA repair, inhibition of cyclic AMP phosphodiesterase activity and inhibits seed germination (Friedman and Waller, 1983 a and b). It is the major cause of cancer, heart diseases, and complications in pregnant woman and aging (Green and Suls, 1996; Infante et. al., 1993; Srisuphan and Bracken, 1986, Dlugosz et.al., 1996; Fenster et.al., 1991) [1][12].

The stimulant effect of caffeine is thought to be due to an increase in adrenaline release, which may stimulate the sympathetic nervous system, but the mechanism is not completely understood(Clarkson, 1993).Caffeine enhances Acetylcholine release in the hippocampus in vivo by a selective interaction with adenosine A1 receptors. Carter, et al, 1995).

It is known that caffeine is responsible for many effects on the human body. Details of the mechanism of caffeine at cellular and organ level can give an insight into how this

molecule affects different functions in the body and the reasons for adverse effects on the body.

Caffeine is 1, 3, 7-trimethylxanthine, meaning it is a xanthine molecule with methyl groups replacing all of the three hydrogen's bound to nitrogen's in the xanthine ring. The molecular weight of caffeine is 194.2 and structurally it is related to uric acid and contains Imidazole and a uracil ring[1][12].

Theobromine and paraxanthine are dimethylxanthines derived from the removal of methyl groups at 1 and 3 N position of xanthine ring of caffeine. Caffeine, paraxanthine and theobromine have stimulatory effects on humans, but caffeine due to it is 3 methyl groups is associated with highest stimulatory activity and other ill effects on health. Dimethyl xanthines are known to possess anti asthmatic, anti-cancer and anti-oxidant properties [1][12].

Recent studies show that excess consumption of caffeine has many health implications in humans. Therefore decaffeination technologies have been developed since 1920's through chemical routes; these chemical decaffeination methods have several disadvantages. Biodecaffeination as an alternative route in the budding stage and knowledge about this area of coffee and tea biotechnology is scarce. Biodecaffeination is the process of removal of caffeine from coffee, tea and other caffeine containing materials by the action of externally added microbial cells or enzymes [12].

Caffeine reacts with excess accurately known amount of iodine in acidic environment, forming insoluble precipitate. Then the insoluble precipitate is removed by filtration.

$C_8H_{10}N_4O_2 + 2\ I_2 + KI + H_2SO_4 \rightarrow C_8H_{10}N_4O_2.HI.I_4 + KHSO_4$
$I_2 + 2Na_2S_2O_3 \rightarrow 2NAI + NA_2S_4O_6$

Using titration by standard Sodium thiosulphate solution with starch solution as indicator, the amount of remaining iodine, and hence the amount of caffeine can be found [4].

2. Materials and Methods

1) Percent degradation Experiment: [10]

Caffeine liquid medium broth containing 0.2gm% caffeine was inoculated with respective isolates isolated from soil samples [12]. After inoculation immediately take OD at 560nm..Flasks were incubated at 37°C.Sample was withdrawn at different time intervals i.e. after 12, 24, 36, 48 hours. Sample were centrifuged and filtered before taking the absorbance. Following formula was used to calculate percent degradation-Uninoculated broth was kept as control.

Caffeine degradation (%) = (a-b)*100/a
Where,
a=Initial caffeine concentration (g/l)
b=Residual caffeine concentration (g/l)

2) Quantitative analysis of caffeine by Iodometric Titration:[4]

Caffeine liquid medium containing 0.2gm% caffeine was inoculated with respective isolates [12]. Flasks were incubated at 37°C for 48 hours. Sulphuric acid added to standard caffeine solution and extract solution separately. Iodine were added to solution, Brown-red precipitate formed. After filtration, filtrates were titrating against sodium thiosulphate solution by adding few drops of starch solution as indicator. The brown-red precipitate solution converts to colourless. Reading was taken, till get 2-3 consistent reading.

3. Result and Discussion

1) Percent degradation Assay

Caffeine degradation (%)= (a-b) * 100/a
Initial caffeine concentration (g/l) = a
Residual caffeine concentration (g/l) = b

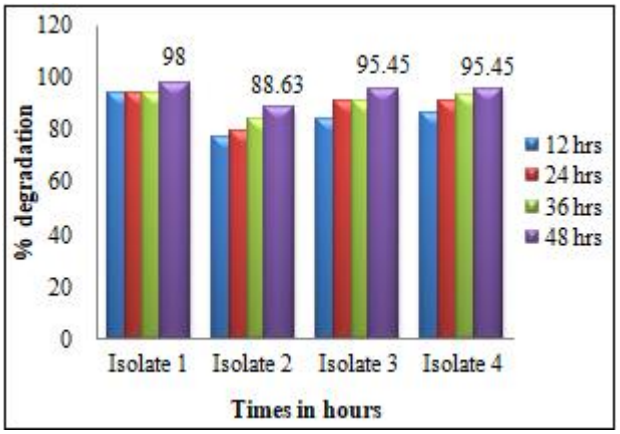

Figure 1: Isolates showed efficient degradation of caffeine. Isolate-1 shows 98, Isolate- 2 shows 88.63, Isolate-3 shows 95.45, Isolate-4 shows 95.45.Uninoculated broth was kept as control.

2) Iodometric back titration

Table 1: Final titration readings for Iodometric back titration

Strain	Reading(Caffeine) Ml
Isolate 1	14
Isolate 2	16
Isolate 3	16
Isolate 4	17
Control (uninoculted)	14

Calculation for Iodometric back titration for an isolate is demonstrated. Accordingly, remaining percentage of caffeine in broth was calculated for all isolates.
Concentration of standard caffeine solution used: 0.421mg/cm^3
Concentration of standard $Na_2S_2O_3$ solution: 0.00595 M
Concentration of standard iodine solution: 0.0028 M
Molecular weight of caffeine: 194.19

Volume of standard caffeine solution used in each titration: 15.00 cm^3
Number of mole of I_2 added = (25/1000)*0.0028 = 7*10^{-5} mol
Number of mole of$Na_2S_2O_3$ added = (14/1000)*0.00595 = 8.33 *10^{-5} mol

By the following equation, $I_2 + Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$

Number of mole I_2 of reacted with $Na_2S_2O_3$ (remaining $I_{2)}$
$= (8.33*10^{-5})*/2$
$= 4.165*10^{-5}$ mol
Number of mole I_2 of reacted with caffeine
$= 7*10^{-5} - 4.165*10^{-5}$
$= 2.835*10^{-5}$ mole
$= 2.835*10^{-5} *194.19$
$= 5.5052*10^{-3}$ g
= 5.5052 mg
By the following equation,

$$C_8H_{10}N_4O_2 + 2I_2 + H_2SO_4 \rightarrow C_8H_{10}N_4O_2.\ HI.\ I_4 + KHSO_4$$

Number of mole of Caffeine = $(2.835*10^{-5}) /2$
$= 1.4175*10^{-5}$
Mass of caffeine found in the 15.00cm^3 of standard solution
$= 1.4175*10^{-5}* 194.19$
$= 2.7526*10^{-3}$ g
= 2.7526mg
Original mass of caffeine contained in 15.00 cm^3 standard solution=5.5052mg
Accuracy of Iodometric Back Titration = (2.7526/5.5052)*100%= 50%

Table 2: Percentage of reaming caffeine in the broth by Iodometric back titration

Strain	% caffeine
Isolate 1	28.42
Isolate 2	27.66
Isolate 3	25.36
Isolate 4	22.87
control (uninoculted)	49.99

Percent degradation assay was performed for quantifying for the degradation of caffeine by all the isolates. Fig no.1 shows Isolate 1, Isolate 3 and Isolate4 degrades caffeine up to 98%, 95.45% and 95.45% respectively after 48 hours of incubation.

Iodometric back titration was performed for determining the remaining concentration of caffeine in percent. Table no.2 shows the percentage of remaining caffeine in the broth. After the treatment by four isolates, 25.36% and 22.87% of caffeine was found to be unutilized in the medium with caffeine when treated by Isolate 3 and Isolate 4 respectively. 28.42% and 27.66% of caffeine was found to be unutilized in the medium treated by Isolate 1 and Isolate 2 respectively. This indicates Isolate 3 and Isolate 4 are efficient degraders than Isolate 1 and Isolate 2.

To determine the amount of caffeine in aqueous solution, the Iodometric back titration is a simple and an accurate method. It requires simple apparatus and common chemicals only. The above results showed that it is an accurate method.

Different applications of caffeine can be studied such as construction of biosensors using these isolates can be constructed to measure the caffeine content in the products such as coffee pulp or husk which contain large amount of caffeine.

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