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Research Article
Immobilisation of *Brevibacterium* sp. for maximal caffeine degradation in various matrices

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Caffeine removal, *Brevibacterium*, Immobilization, Entrapment.

Abstract

Caffeine (1,3,7 trimethyl xanthine) is a purine alkaloid has been identified as an environmental pollutant. Decaffeination thus becomes an important step in disposal of caffeine containing wastes from coffee processing industries. Bio decaffeination has more advantages being economical, less labour and less toxic than Conventional decaffeination techniques. Thus In the present study, caffeine removal by *Brevibacterium* immobilised by various matrices (Agar, Agarose , Sodium alginate, -carrageanen and Gelatin) was investigated using the method of entrapment. The residual caffeine was obtained by HPLC analysis. Caffeine removal was found to be 80 % by alginate immobilised whole cells of *Brevibacterium* species followed by Gelatin (68 %) and Agar (65%).The experiments were performed in batch mode. Effectiveness of the process is to be tested involving continuous- flow column studies.

1.Introduction

Caffeine (1, 3, 7 trimethylxanthine) is a psychostimulant and is consumed worldwide through Coffee, tea and colas. Increased level of intake leads to "caffeinism" which is marked by irritability, insomnia and depression. Headaches and morning lethargy are the most common withdrawal symptoms. There is an increased demand in the market for decaffeinated products.

Conventional decaffeination techniques viz., ethanol, methyl chloride, CO_2 and Swiss water process are solvent based, laborious, lead to flavor reduction and costlier. So Biodecaffeination involving microbes which degrade caffeine is the method of choice.

Harnessing the caffeine-degradation potential of organisms growing in caffeine rich soil is of importance in developing processes for biodecaffeination is of prime importance and immobilization is the primary step in industrial application.

2.Materials and Methods

2.1 Isolation of bacteria

Isolation of bacteria was carried out by spread plate method and pure cultures were obtained by streak plate method. Pure cultures were maintained on nutrient agar medium at 4ºC and were sub-cultured at an interval of every 2 week.

2.2 Bacterial Identification

Various morphological, physiological and biochemical tests were performed to identify the bacteria with reference to Bergey's Manual of Systemic Bacteriology.

2.3 Amplification of the caffeine tolerant bacteria

Solid screening medium (SSM) for isolating the caffeine-tolerant bacteria was prepared by mixing the mineral solution with caffeine (2.5 g/ L) and agar (1.5%) and autoclaved

at 121°C for 10 min. Solid purifying medium (SPM) was also prepared as SSM except different concentrations of caffeine (1 to 10 g/L) was supplemented. Liquid amplifying medium (LAM) was obtained after addition of caffeine (0.5 g /L) and sucrose/glucose (5.0 g /L) in the mineral solution and disinfection.

2.4Immobilisation of whole cells:

2.4.1Alginate:

Sodium alginate solution (3% w/v) and was prepared and sterilized. 10 grams cell by wet weight was mixed thoroughly in the alginate solution. The cell suspension was dropped slowly into a solution of 0.22 M Calcium chloride at 4oC. The beads were kept for primary curing at 4oC for 24 hours and secondary curing was done in 0.02 M calcium chloride for 48 hours at 4oC. Traces of calcium chloride were removed by through washing with distilled water and used for further studies.

2.4.2Gelatin:

Gelatin (porcine skin) was used as the matrix for the immobilization of cells Of *Brevibacterium* . Solutions of gelatin in the concentration range of 3-20% was prepared by dissolving required weight of solid gelatin powder in 100 ml water maintained at 60^{O}C. Cells of *Brevibacterium* (Ten grams by wet weight) were mixed with 100 ml of gelatin maintained at 30^{O}C and mixed thoroughly to obtain a homogeneous suspension. This suspension was then dropped slowly into an aqueous solution of 1.5% v/v glutaraldehyde at 4°C using a surgical syringe. Another approach for achieving good bead characteristics was tried in which the modified curing solution consisted of toluene and chloroform in the ratio of 3:1 with 1.5% glutaraldehyde. Another modification of the curing solution using a mixture of paraffin oil and chloroform in the ratio of 3:1 with 1.5% glutaraldehyde

2.4.3 Agar:

Immobilisation of the cell of *Brevibacterium* in agar was done by dissolving 3 gm of agar in 100ml of distilled water 80^{O}C and then cooling it to 40^{O} C with subsequent mixing of 10 gm of cell equilibrated to the same temperature.After thorough mixing, the suspension was dropped through a syringe into water maintained at 4^{O}C.

2.5 Shake flask experiments:

Immobilized cells of *Brevibacterium* were tested for the ability to degrade caffeine in shake flask. 10 gm of the immobilized beads were incubated with 100 ml of the working medium under agitation on a rotary shaker set at 200 rpm at 30±2°C.The medium were taken at 12 hour intervals for caffeine degradation studies by HPLC.

2.6 Estimation of methylxanthines by high performance liquid Chromatography (HPLC)

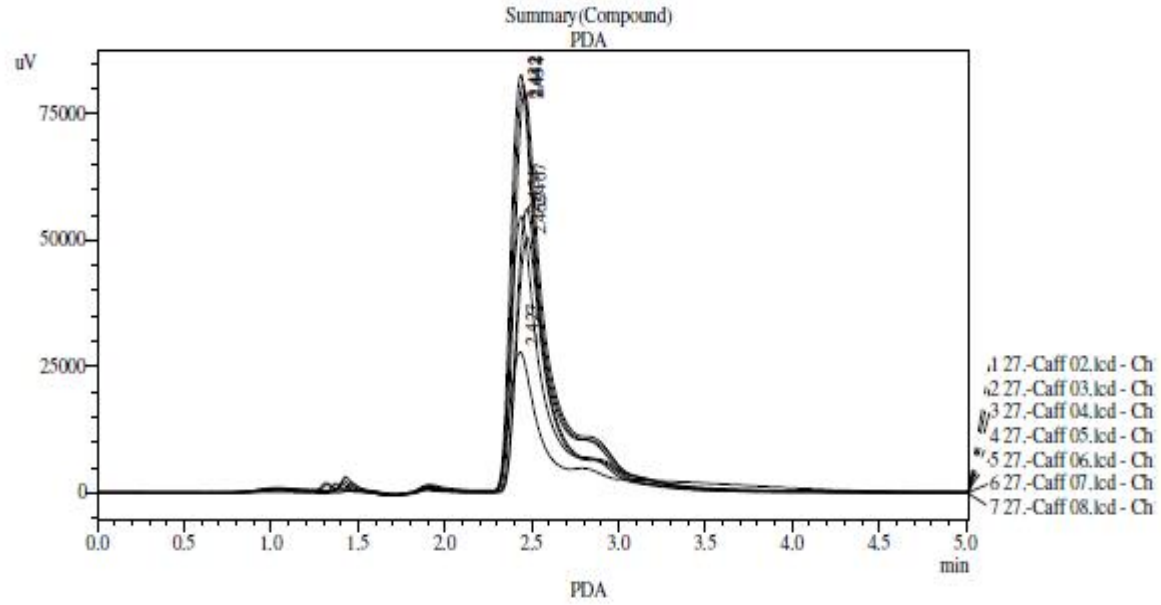
HPLC analysis of caffeine is to be performed in a HPLC System, and the Methylxanthine compounds were separated on a column under isocratic conditions with 15 % acetonitrile in water at a flow rate of 1.0 ml/min.

Compounds eluting from the column were detected at 273 nm, and the peak areas were compared with those obtained with standards of known concentration

3.Results and Discussion

Table 1 Effect of different matrices on immobilization of *Brevibacterium* sp.

CALCIUM ALGINATE	hours	12	24	36	48	72
	CD %	10	20	28	40	80
AGAR	hours	12	24	36	48	72
	CD %	15	25	35	45	65
GELATIN	hours	12	24	36	48	72
	CD %	17	46	76	84	68



The caffeine degradation found to increase with respect to time (Table 1). The bead strength, longevity and caffeine degradation are well observed in Calcium alginate with highest caffeine degradation 80% followed by gelatin and agar. An interesting observation was all the beads performed stable over time.

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