EXTRACTION OF L-ASPARTATE AMMONIA LYASE FROM *Acinetobacter calcoaceticus* BY ULTRASONICATION FOR L-ASPARTATE SYNTHESIS

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Introduction. L-aspartate ammonia lyase (EC 4.3.1.1) catalyzes the reversible conversion of L-aspartate to fumarate and $NH4^+$. This enzyme is mainly used for the production of the amino acid L-aspartic (Asp), one of the ingredients for the production of aspartame, and it also used in the pharmaceutical industry, parental nutrition, and food additives. Until now, most of the production processes of L-aspartate have been focused on the use of immobilized cell in different supports, however immobilized purified aspartasa has been investigated, in recent times, with satisfactory results [(1)].

Our work is aimed at identifying new methods to extraction of aspartase.

Methods. *Acinetobacter calcoaceticus* strain was obtained from a soil sample and identified molecularly on a previously work [(2)]. *A. calcoaceticus* was growing in a liquid medium with the following composition (w / v) 0.1% NaCl, 0.1% KCl, 0.5% casein peptone, 0.25% yeast extract. Cultivation was carried out according to the methodology described on a previously work [(2)]. Cell were harvested by centrifugation (10,000 rpm at 15℃ for 20 minutes) and washed twice with potash phosphate buffer (10 mmol, pH 7.0). The pellet obtained (0.38 g wet weight) was resuspended in 1 ml of Buffer A, Buffer B and Buffer C. [(3)]

Table 1. Composition Sonication buffer

Buffer A	50 mM potassium phosphate buffer (pH 6.8) containing 100 mM KCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol
Buffer B	10 mM potassium phosphate buffer (pH 7.0) containing 0.75% (w/v) of NaCl.
Bufer C	PBS Buffer (pH 7.3) containing 1mg of lizozyme per 1ml of Cell suspensions

Cell suspensions were disrupted by sonication with a Cole Parmer® Utltrasonic Processor model CPX-130 with 3 different treatments of sonication:

Table 2. Sonication treatments.

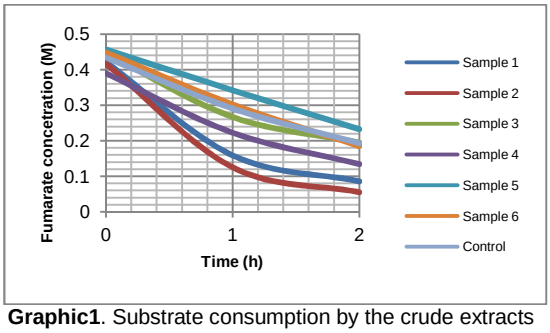
	Sonication Time	Pulses on/off Seconds
Treatment 1	8 minutes	40/10
Treatment 2	5 minutes	40/10
Treatment 3	5 minutes	30/10

After sonication, disrupted cells were centrifuged and the supernatant was used for the enzymatic reaction. As a substrate of this reaction was used 0.5 M ammonium fumarate at pH 8.0 at 37℃. Enzyme activity was determined in batch reactors using the methodology described on a previously work [(2)], and defined as the amount of transformed substrate (µM) per mg of protein each minute.

Results. The sonication treatments, with different buffers, showed a greater activity with respect to whole cells; nevertheless buffer A in combination with longer sonication treatments showed the best activity. These are results to previous process of immobilization of the crude extract.

Table 3. Specific enzyme activity of crude extract of the enzyme

	Buffer A		Buffer B		Buffer C		
	T2	T1	T3	T2	T3	T2	
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Control
AEE µM.mg^{-1}.min^{-1}	2974.76172	3158.99499	2643.26564	2551.76236	2352.08887	2284.72276	2330.41146



Graphic1. Substrate consumption by the crude extracts of *Acinetobacter calcoaceticus*.

Conclusions. Cell disruption by sonication increased activity with respect to free cells, because aspartase is an intracellular enzyme, Buffer A obtained the best results in contrast with the other buffers.

References.

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