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Enzymatic Bio-mining of Balkaya Lignite Coal with Bovine Carbonic Anhydrase Enzyme

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ABSTRACT

Carbonic anhydrase is a metalloenzyme containing zinc in its active center. In this study, the use of carbonic anhydrase enzyme in the bio-mining of Balkaya lignite coal has been matched. Carbonic anhydrase (carbonate hydrolyses: E.C. 4.2.1.1) was purified from bovine erythrocytes using a Sepharose-4B L-tyrosine sulfanilamide affinity chromatography. Hydratase and esterase activity of the enzyme were determined. The purity of the enzyme was recognized by single band on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, Balkaya lignite coal with the size −20 mesh was investigated to break into pieces by using purified enzyme carbonic anhydrase with affinity chromatography. As a result of the bio-mining experiments, the carbonic anhydrase enzyme grounded Balkaya lignite coal. It was understood that the purified enzyme made smaller 1.75-fold of the Balkaya lignite coal within 96 hours using a shaker against the blank. When using a magnetic stirrer in the experiments were shown the enzyme made a smaller 73.75-fold against the blank. The results of these experiments showed that the enzyme carbonic anhydrase can be successfully used in the bio-mining of lignite coal of the same type.

Keywords: Bovine, carbonic anhydrase, Balkaya lignite, coal, bio-mining

INTRODUCTION

Carbonic anhydrase (CA) was first isolated from mammalian red blood cells. It is a zinc-containing metalloenzyme.[1-4] It catalyses in the below reaction (Figure 1).

Carbonic anhydrases are produced in a variety of tissues where they participate in a broad range of physiological processes such as acid–base homeostasis, carbon dioxide and ion transport, respiration, bone resorption, renal acidification, gluconeogenesis, ureagenesis, and the formation of cerebrospinal fluid and gastric acid.[5] The expanding a-CA gene family includes 11 enzymatically active members with different structural and catalytic properties. The cellular distribution and physiological functions of the various CA isozymes have been extensively described in several recent reviews.[6-8]

Preparation of the solid-liquid suspensions of coal carrying, storage, and handling facilities has very great practical importance. Coal-liquid dispersion stability of suspensions of solid particles has been dispersed, or aggregation environment against the resistance of the suspension depends on the size.

In the aggregation of solids such as coal, the grain size of the barrier due to mechanical operations, could not be bellowed by a certain limit. For this, a number of additional procedures were needed, such as wet grinding and chemical breakup. In particular, the chemical breakup has disadvantages such as high pressure and the need for inert gas. The geometries of the lignite particles change during the mechanical process. In addition, high pressure and mechanical friction on the surface of coal due to high temperature caused oxidation.

In this study, it was investigated whether the CA enzyme could be used in the bio-mining of Balkaya lignite coal.

EXPERIMENTAL

Bio-mining Materials

Lignite coal abundantly obtained from the Erzurum-Balkaya excavation area ground with a cell-mill to sizes of 20 mesh as to ASTM.

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$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

Figure 1. The reaction catalysis with carbonic anhydrase.

Purification of Bovine Carbonic Anhydrase

For obtaining the bovine CA enzyme, blood samples taken from Erzurum slaughterhouse were centrifuged (2500 rpm, 15 minutes, 4°C). The plasma was removed carefully. The precipitates were washed with saline 3 times. Then 40-50 mL of red cell pack was obtained from 100 mL of whole blood by this procedure. The resultant red cell pack was mixed with chilled distilled water at a ratio of 1/15 and left for 30 minutes until the completion of hemolysis. To isolate the membrane structure, the hemolysate was centrifuged (20 000 rpm, 30 minutes, 4°C). The supernatant was taken and its pH was adjusted to 8.7 with solid Tris, and then it was then applied to the affinity column.[9]

Affinity Chromatography

An affinity column was used for the separation of CA, which has a matrix composed of Sepharose-4B activated by CNBr and having covalently bound L-tyrosine arms. In the last step of the column preparation, the diazotized sulfanilamide was attached to the L-tyrosine arms. One hundred milliliters of hemolysate mentioned above was applied to the column. Then it was washed with 400 mL of 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.7), giving rise to CA attachment and removal of undesirables. By adding 0.1 M $NaCH_3COO/NaClO_4$ (pH 5.6), bovine carbonic anhydrase (BCA) was eluted. The eluates were collected as parts of 5 mL by a fraction collector. The flow rate was adjusted to 20 mL/h, and it was conducted at a temperature of 10-15°C.[10]

Protein Determination

For finding amount of pure enzyme, after scanning at 280 nm the tubes with significant absorbance were pooled and a quantitative protein determination was performed using the Coomassie Brilliant Blue G-250 method.[10]

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

The purity of the enzyme was determined by applying a discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (3%-10%) as reported by Laemmli.[11]

Determination of Hydratase Activity

Enzim aktivitesinin CO2'yi HCO3-'e dönüştürmesini ölçmek için, 2 mL veronal tampon (pH 8,2), 0,4 mL bromotimol mavisi (%0,004), 0,8 mL seyreltilmiş enzim çözeltisi (1/10 seyreltme) ve 2 mL doymuş CO2 çözeltisi 0°C'de karıştırıldı. The time t_c (for the sample) interval was determined between the addition of CO_2 solution and the occurrence of a yellow-green color. The same interval was recorded without enzyme solution (for blank). The activity was calculated from the following formula[12]:

1 WA Unit: $(t_o - t_c)/t_c$

Table 1. Size Reduction (%) of Balkaya Lignite Coals Using Record of the Light Microscope

Size Reduction (%) of Mixed Enzyme Solution	Size Reduction (%) of Mixed Blank Solution	Size Reduction (%) of Shaken Enzyme Solution	Size Reduction (%) of Shaken Blank Solution
80	6.25	4	2.25

Determination of Esterase Activity

At the same time, CA has esterase activity. This activity can be used for biosynthesis reactions. For this purpose, its activity was measured in this way. The principle of this determination is that the substrate of CA (*p*-nitrophenylacetate) is hydrolyzed to *p*-nitrophenol and acetic acid (Figure 2). The reaction is determined at 348 nm. For this procedure, 1.5 mL of a buffered enzyme solution (0.1 mL enzyme + 1.4 mL 0.05 M Tris–SO_4, pH 7.4), and 1.5 mL of substrate were mixed in a measurement cuvette, and 3 minutes later, the absorbance was measured (348 nm, 25°C). A blank measurement was obtained by preparing with adsorption support material without adding enzyme solution.[13]

Monitoring of Enzymatic Bio-mining

For this examination, 2 reaction vessels (1.74 mg/mL) containing 5 mL of the enzyme at pH 0.05 M Tris–SO_4 were used. In addition, in preparing the blank experiment, only 5 mL (pH 0.05 M Tris–SO_4) buffer was added to the 2 reaction cup. At last, 0.2 g Balkaya lignite coal was added to the vessels. Shaking and mixing effects were also investigated by using a shaker and a magnetic stirrer.

The samples were taken in a reaction mixture to understand the changing of the particle size of Balkaya lignite coal periodically. The size of the samples was measured with a light microscope after these recording images were compared and averaged.

RESULTS AND DISCUSSION

Carbonic anhydrase was purified by Sepharose-4B L-tyrosine sulfanilamide affinity chromatography abundantly from bovine erythrocytes. The purity of the enzyme was determined by SDS-PAGE.

According to the results of the experiments using a shaker, the coal size was reduced 2.25 times in blank solution and 4 times

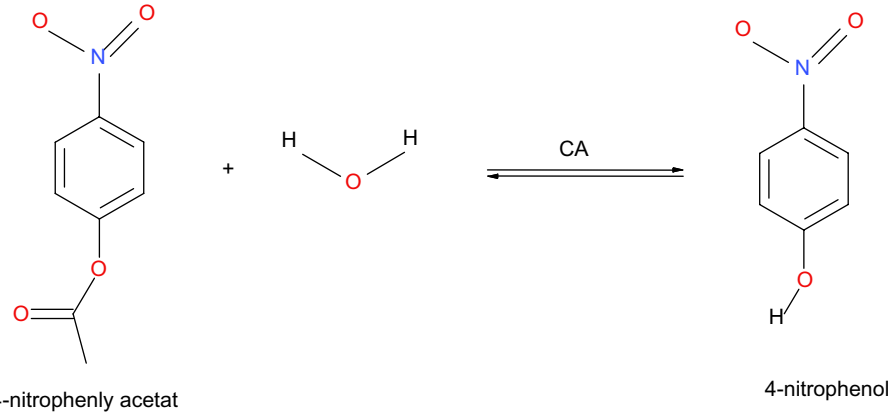


Figure 2. Esterase activity of carbonic anhydrase.

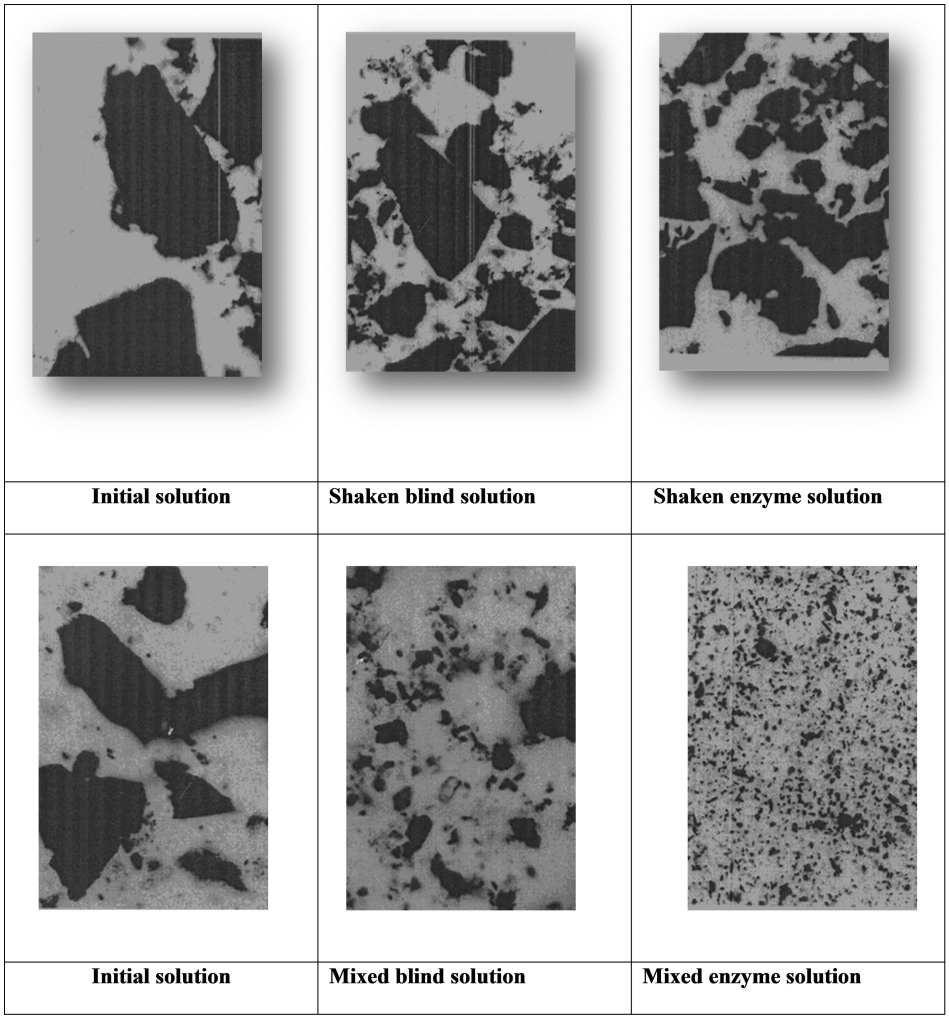


Figure 3. Records of scanning electron microscope (SEM-Jeol 6400).

in enzymatic solution. A shaker was used and the results were compared. It was observed that the minus effect of the enzyme could be seen in 96 hours. In addition, a magnetic stirrer was used and it was observed that the coal size was reduced 6.25 times in blank solution and 80 times in enzymatic solution in 96 hours (Table 1).

Evaluation of the results of enzyme activity to ensure the necessity of using a mechanical mixer was understood (Figure 3).

After enzymatic fragmentation, separation capabilities of dispersion particles were observed. The Balkaya lignite coal was estimated to become maseralls at the level of fragmentation by CA enzyme.

In addition, it was suggested that the BCA enzyme catalyzed the following reactions for coal decomposition using the esterase and hydratase activity of this enzyme (Figure 4).[14]

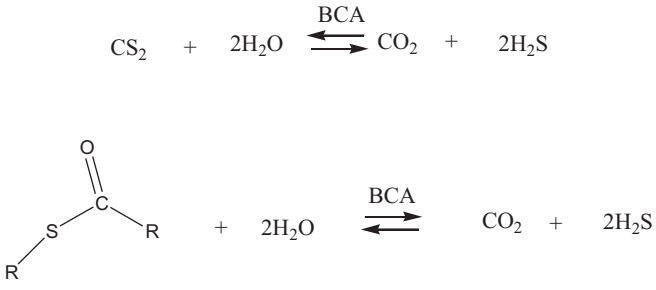


Figure 4. Two different activities of bovine carbonic anhydrase (BCA).

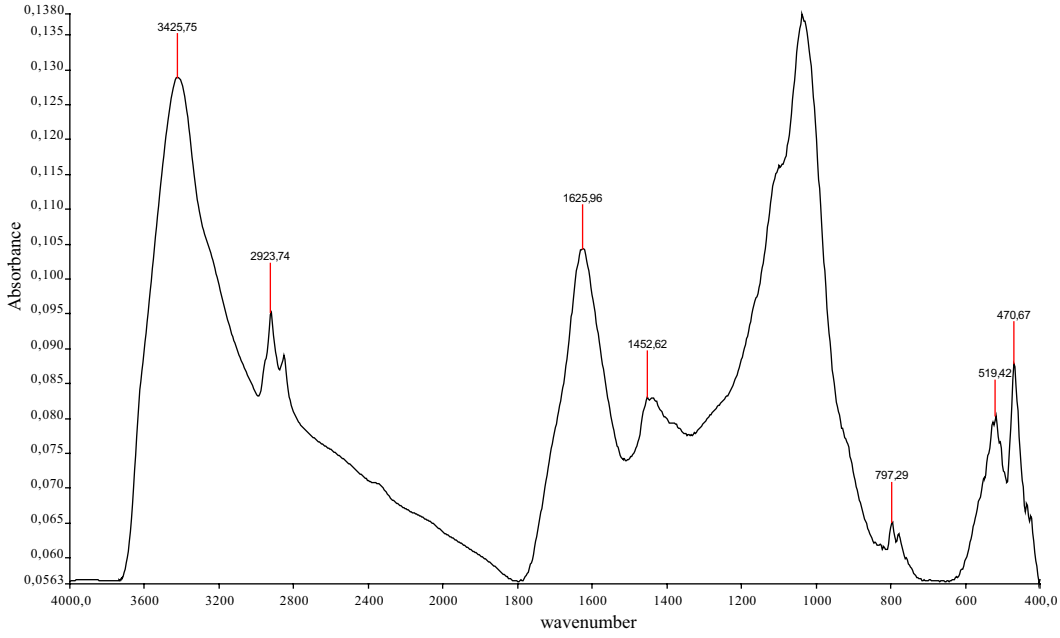
A dried blank and enzyme mixture including Balkaya lignite coal was used to understand whether the enzyme was changed to the structure of the Balkaya lignite coal. . The elemental analysis results are shown in Table 2. The results showed significant changes in the amount of %S and %C of coal structure.

From these results were considered the BCA used the Balkaya lignite coal as a substrate. Decreasing the %S and %C can be explanation of the occurring CO_2 and H_2S which is the results of upper reactions. These analyses were made with LECO CHNS.

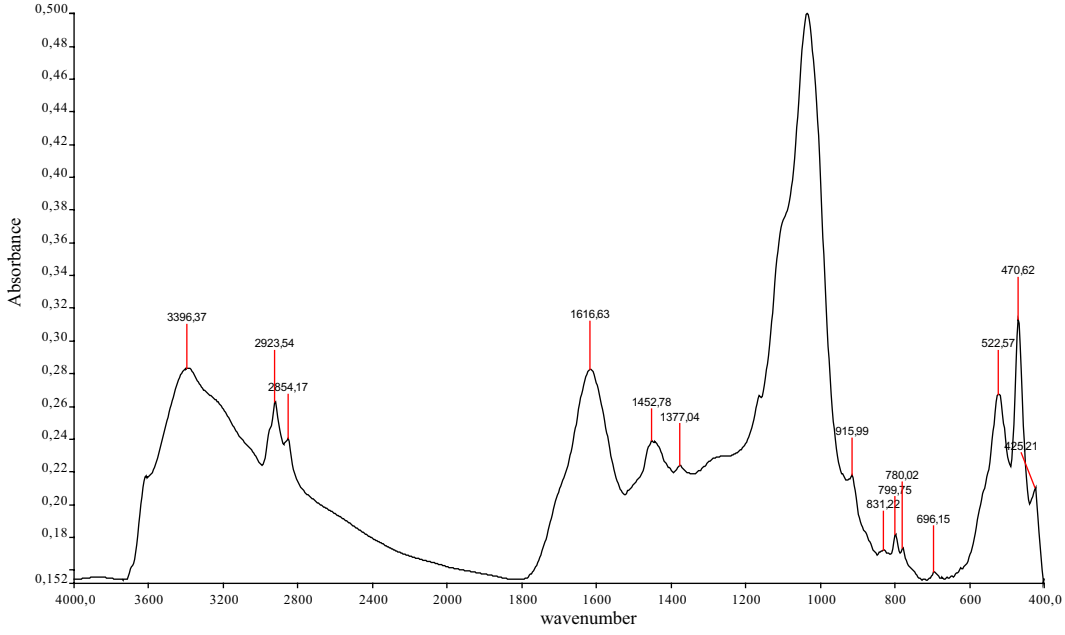
The Balkaya coal, reducing 73.75 times by bovine carbonic anhydrase, was analyzed using an infrared spectroscopy. The FTIR

Table 2. Elemental Analysis of Balkaya Lignite Coal

Dried blank solution including Balkaya lignite coal	C = 38.08%	H = 3.81%	N = 1.367%	S = 2.341%
Dried enzyme solution including Balkaya lignite coal	C = 21.18%	H = 3.962%	N = 1.285%	S = 0.985%



IR(KBr, cm^{-1}): Balkaya lignite coal: 3425.74, 2923.74, 1625.96, 1452.62, 797.29, 519.42,470.67.



IR(KBr, cm^{-1}): Enzymatic disintegrated Balkaya lignite coal: 3396.37, 2923.54, 2854.17, 1616.63, 1452.78, 1377.04, 915.99, 813.22, 799.75, 780.02, 696.15, 522.57, 470.62, 425.21.

Figure 5. The results of the IR spectroscopy.

spectroscopy results show all the different structures, as shown in Figure 5; for this analysis, Fourier-transform infrared spectroscopy (FTIR) spectrophotometer was used. Infrared spectra were obtained from solution in 0.1 mm cells or KBr pellets on a regular instrument.

FTIR analysis of the parts of the coal structure has been understood as a structural change due to enzymatic reaction. Therefore, the broken Balkaya coal compared with Balkaya coal of the fingerprint region of the spectrum shifts for observing changes in the coal structure.

CONCLUSION

Carbonic anhydrase enzyme is abundantly found in natural life. Therefore, it can be easily purified and characterized. In addition, CA enzymes can be easily purified from bovine blood. In this research, we understand that using this purified CA enzyme can be grounded in Balkaya lignite coal. The structural chancing of the Balkaya lignite coal was measured using light microscopy, elemental analysis, and IR spectrum. According to all the data obtained, it was determined that the structure was completely changed. Results of these experiments, CA enzyme can be used for bio-mining of the same kind of lignite coal.

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