



## Converting of Cardboard Waste for Bioethanol Production Using Anaerobic Fermentation

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### Abstract

Many countries are interested in waste management technologies and their use, where these technologies contribute to the disposal of pollutants that affect the environment. As a significant fraction of municipal solid waste, waste paper is a potential source for producing bioethanol. Bioethanol production is a widely studied process for biofuel production, as waste disposal through incineration emits dangerous greenhouse gases (which cause global warming). The current work uses cardboard waste as a raw material for bioethanol synthesis through the physical, chemical, and enzymatic treatments to improve glucose synthesis from cardboard waste by two-stage saccharomyces and fermentation stage, using yeast extract. We relied on Trichoderma is a genus of fungi found in all soils, being the most widely cultured fungi; this fungus is a producer of the cellulase enzyme that breaks down cellulose into fermented sugar and relies on three different media with a carbon source and a vegetable source. (CMC) carboxymethyl cellulose agar the medium was chosen for the growth of enzyme-dissolving fungi, and then the enzymatic filtrate was taken, which contains a high percentage of sugars about (12 mg/l) in the optimum conditions pH (5.5-6) and temperature 28 °C. The purpose of the research exploitation of cellulose in cardboard and production of ethanol by fermentation process for a period of 5-8 days, satisfactory results have been obtained, consumption of 5g cardboard waste produces 1%, which is equivalent to 20ml ethanol yield.

### 1. Introduction

Since ancient times, human has been exploiting natural resources to secure their life and using the land to dispose of their waste, which was not a big problem due to the small size of the population centers and the large areas of vacant land that can be used, Since people began to gather in the form of villages and cities, the problems of waste collection and disposal began to impose themselves, and these problems became more complicated with the increase in population density in cities and villages and the expansion of human economic activity [1]. It is possible to take advantage of some types of waste containing cellulose by converting it into

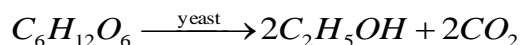
biofuel, as many researchers worldwide have produced biofuel through the biological conversion of corn waste into ethanol. As well as the biological conversion of wood and some agricultural waste and industrial materials, as the value of ready-made cellulosic materials constitute 70%, from the value of the production process, the cellulosic materials present in the waste are used, as they are of little or no price [2]. Another example is the solid waste left by industrial facilities. After the production processes, the accumulation that pollutes the environment is waste paper and cardboard, which the recycling process can treat. Paper and cardboard constitute a high percentage that may reach more than 30% of solid waste. The recycling of cardboard waste is environmental and economic importance because it contributes to reducing the depletion of natural resources such as energy, water and forests, which are vital to the stability of the global climate. Alternative energy sources have grown even more critical in recent years because of the continuing depletion of restricted fossil fuel stocks and the need for a safe and better environment. With the world's energy supply inevitably depleting, there has been an increase in global interest in alternative energy sources [3, 4].

## 2. Experimental Procedure

### 2.1. Materials and Methods

#### 2.1.1. Composition of Cardboard Waste as the Base Material

Cardboard waste papers were collected locally; they were washed to remove suspended matter and colours that may contain and dried in an oven at a temperature of less than 100°C. Then cut it and smash it into as small pieces as it can get, measuring approximately  $2 \times 1$  cm. A small amount of phosphoric or hydraulic acid at a concentration of 1% is added to help break down the cellulose fibres in the carton. NaOH was added at a concentration of 1% to maintain the pH within the range (5-6). The weight of 5 grams of the cut carton was taken and placed inside a glass beaker, and 100 ml of sterile water was added to it and placed on the magnetic stirrer so that the carton was mashed and turned into an emulsion and then filtered the solution. Thus, three models of cartoonish matter are formed. The first form is a liquid (filter), the second is semi-liquid, and the third is a solid (a residue). Producing ethanol takes place in two stages; the first is called Saccharomyces. It converts the cellulose in the carton into fermentable sugars, which is used in the second stage; this process is the aerobic Fermentation of sugars in the presence of yeast to produce ethanol, as in the equation [5].



#### 2.1.2. Saccharification of Cardboard Waste

Cellulose-producing fungi must be available at this stage, so locally isolated microorganisms from the soil have been relied upon. The samples were collected from different locations, where the weight was 2.5g from it, put in test tubes and prepared the growth medium (Nutrient Broth) by weight of 1g with 125ml sterile water; 20 ml of it and added to the isolated soil inside the test tubes [6]. Slides of filter paper are placed inside each test tube to provide the cellulosic source. The samples were incubated for two weeks at a temperature of 28°C. The sample isolates taken from the soil were prepared, and the prepared Tricoderma fungus was isolated and cultivated. With the media prepared above as shown in Figure (1), more than one medium was adopted for comparison in which medium fungi can grow more, i.e., analyze cellulose and produce more cellulose enzymes. The percentage of the sugar yields due to the decomposition of cellulose and the hydrolyzed enzyme activity was measured using a spectrophotometer with (540nm). Standard concentrations of glucose sugar were prepared, based on the Stock Solution at a concentration of 0.2g/100ml, from which other concentrations were prepared.



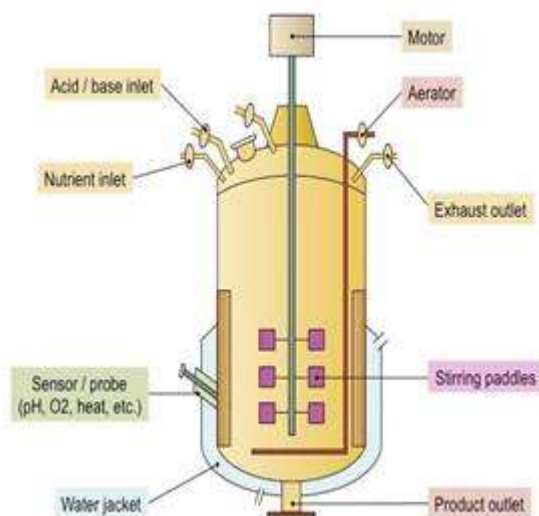
**Figure (1).** The prepared culture media after the growth of fungi on some of them.

### 2.1.3. Fermentation Stage

The enzymatic filter with high glucose content was taken, and yeast extract was added. The fermentation process is carried out on a liquid medium to produce ethanol; the process is at the level of shaking flasks (shaking Fermentation) only because the sample volumes used are few the similar structure for shaking flasks used in Fermentation shown in Figure (2), but if large volumes such as 7 litres or more are obtained, a fermented is used shown in Figure (3). the ethanol yields obtained (1%), compared to the standard solution, which had a concentration of 5%, ethanol, which was prepared from ethanol at a concentration of 98%, and the percentage of ethanol was obtained from only 5 grams carton, under optimal conditions such as pH value (4-5.5) and temperature 28°C. The ethanol formed was diagnosed using gas chromatography [7, 8].



**Figure (2).** A similar structure for shaking flasks used in fermentation.



**Figure (3).** Diagram of large volume fermenter [9].

### 3. Results and Discussion

The growth of fungi was observed on the isolated samples after incubation through the apparent decomposition of the filter paper, where the fungus exploited the cellulose present in the paper, after comparing it to the test tube (the control) containing only a sample of soil with no source of cellulose. To determine which of the three culture media was previously created, the CMC medium and the Czapek Dox medium containing and free of Sucrose, the filter paper was removed and filtered in the media containing the fungus [10-12]. The supernatant solution, which is the fluid that results from the disintegration of the leaf, was also implanted into the three media. Good growth was observed in CMC medium, where the source of cellulose in the medium was exploited for growth, and the growth was lower in Czapek Dox medium containing Sucrose, which does not contain a source of cellulose, only the carbon source contained in Sucrose, a small percentage of mould was observed. White had been formed. This is because the proportion of fungi in the solution may be little compared to the fungi grown on filter paper since good growth was obtained without a notch, and the growth in the medium was weak, so the fungi that grew on the CMC medium were relied upon to form green mould, as shown in Figure (4).



**Figure (4).** The fungi that grew on CMC.

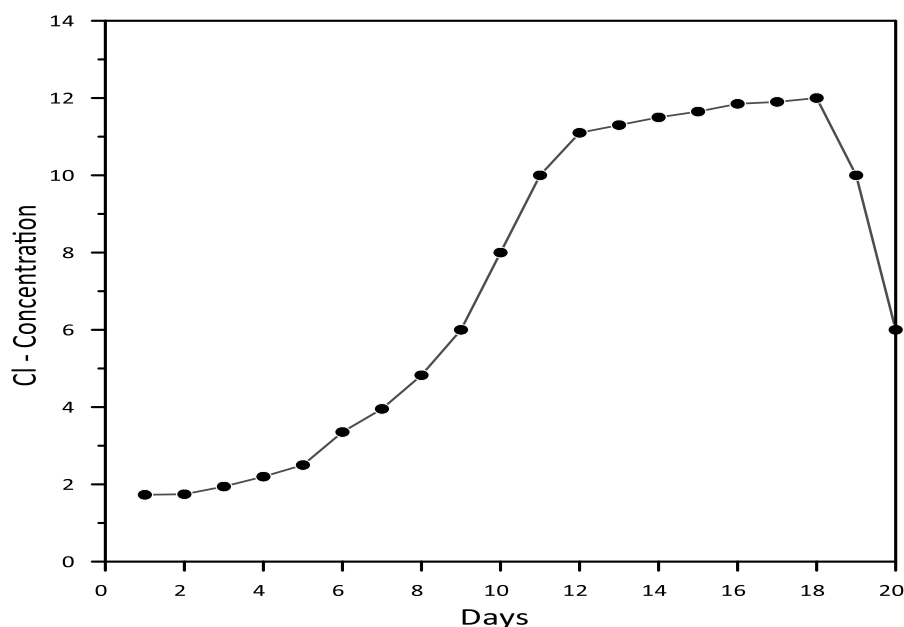
Trichoderma fungi were planted in the culture media to reproduce after establishing the optimal fungal growth and reproduction medium, the fungal solution (vaccine) for the pre-prepared waste carton was made by adding 10 ml of sterile water to the culture medium containing the pre-isolated Trichoderma fungus, then withdrawing 3 ml and carefully distributing it in the waste carton using Micro Pipit. Other fungi's fungicidal solutions (green rot, white rot) behave the same way. After the incubation, fungus grew clearly and well on the cardboard waste,

indicating that the fungi were consuming the cellulose in the carton material and converting it to intermediate sugars like glucose sugar.

On the other hand, a fungal solution is derived from other fungi (green mould, white mould). At a temperature of 28°C, all samples were placed in the incubator for three weeks [13, 14]. Showed a clear and reasonable development of fungus, proof of the fungi consuming the cellulose in the carton and converting it to intermediate sugars like glucose sugar. Filtering the sample, the glucose formed is investigated by using the curve that shows the relationship between the concentration of glucose and the absorptive value of sugar, where (12mg/ml) was obtained, which is the best percentage that can be obtained for glucose, as the concentration of sugar decreased due to the fungi exploiting the glucose present in the sample. That presented in Figure (5) and Table (1).

**Table (1).** The change in glucose concentration with the change in the incubation period.

Days	Glucose concentration [mg/ml]	Days	Glucose concentration [mg/ml]
1	1.7299	11	10
2	1.7446	12	11.1
3	1.9437	13	11.3
4	2.2	14	11.5
5	2.5	15	11.65
6	3.3546	16	11.85
7	3.9533	17	11.9
8	4.8251	18	12
9	6	19	10
10	8	20	6



**Figure (5).** The change in glucose concentration with incubation time.

The fermentation results showed that the percentage of ethanol produced was (1%) from ethanol yield. Compared to the standard solution whose concentration was 5% ethanol which was prepared from standard ethanol with a concentration of 98% using only 5g of Cardboard Waste in the case of using larger quantities of waste, estimated at about a ton of Cardboard waste, it is possible to obtain 4 liters of ethanol as demonstrated in Figures (6 & 7).

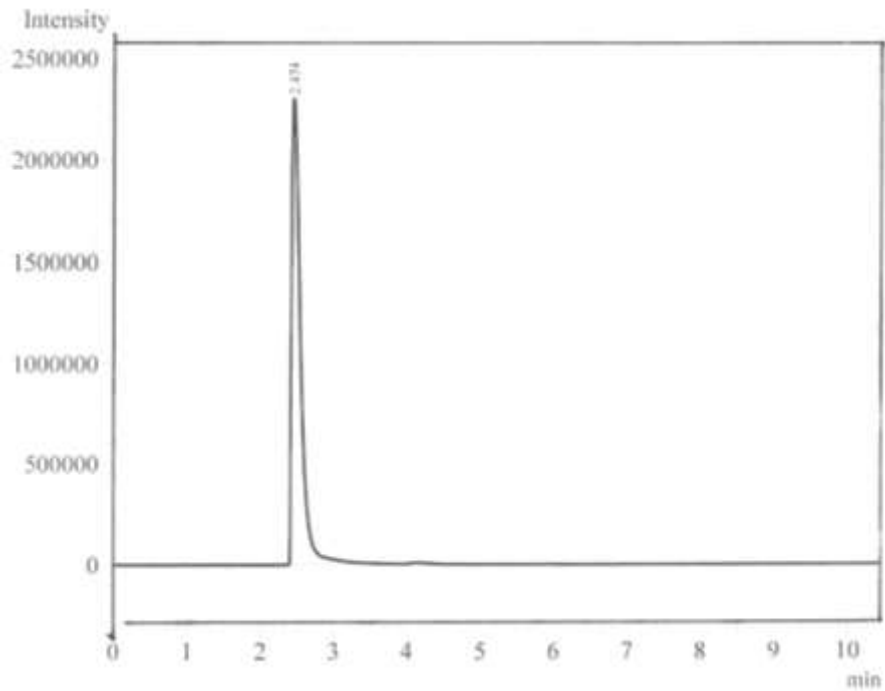


Figure (6). The peak of standard ethanol.

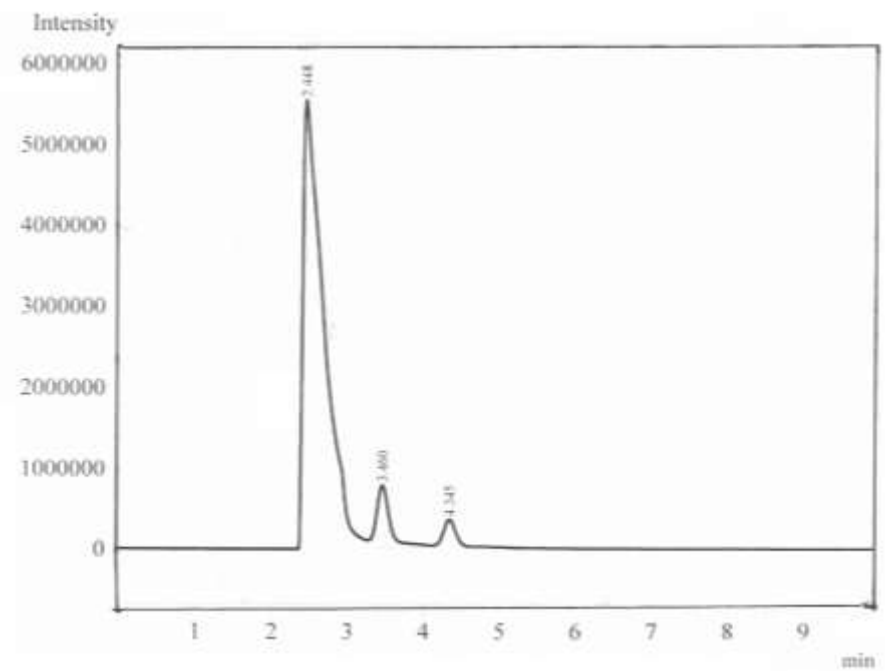


Figure (7). The peak of the ethanol produced.



#### 4. Conclusions

Based on the results obtained in this study, the possibility of using *Trichoderma* fungi in analyzing the cellulose complexes present in the paper waste and converting it into simple sugars that can be exploited by the yeasts to produce alcohol, in addition to the possibility of using some other microorganisms to exploit the cellulose content in the waste is converted biologically into alcohol. We conclude that consuming a small amount of carton waste gave an excellent percentage of ethanol, and it is possible to consume larger quantities of cartons to obtain more percentages of ethanol. If the study is carried out as a pilot project, it is feasible to establish an economic feasibility for the study.

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