

## Screening for Aerobic Fibrolytic Microorganisms to Produce Glucose from Waste Paper

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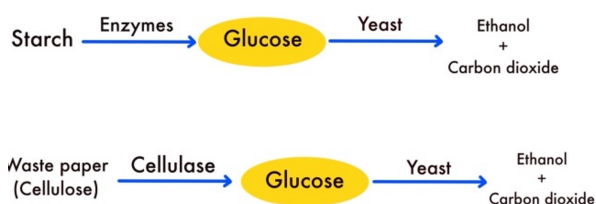
### Abstract

Waste paper, which is produced in large quantities, contains a high percentage of cellulose, which can be converted to glucose by enzymes produced by aerobic fibrolytic microorganisms. Samples from five natural sources: river water, pond water, clay, and dried and fresh cow dung were screened for the presence of these microorganisms. The samples were incubated with filter paper. It was found that the paper is digested by microorganisms in dried and fresh cow dung only. The conversion of cellulose to glucose over time was confirmed by testing with Benedict's reagent, confirming that the cow dung contains aerobic fibrolytic microorganisms.

**Keywords:** Aerobic fibrolytic microorganisms, cellulose, cow dung, waste paper.

## 1. INTRODUCTION

Cellulose is a linear polymer of glucose molecules that is an important structural component in the cell walls of plants. It is classed as a dietary fibre, insoluble in water and resistant to the enzymes found in the digestive systems of humans and animals. For this reason cellulose is used to make paper and various types of apparel. Paper and cardboard contain a high percentage of cellulose and is a major component of landfills. If a way can be found to cheaply digest the cellulose currently being deposited in landfills to glucose, (figure 1) it could partly replace sugar cane as a source of raw material to make ethanol.



**Figure 1:** Ethanol production process using glucose as a product of cellulose digestion by cellulase

Aerobic fibrolytic microorganisms produce cellulase, the enzyme that digests cellulose fibers to obtain glucose. A number of organisms, including species of fungi, bacteria, (actinomycetes), and paenibacillus species, have been found to produce

enzymes that are active in digesting cellulose and hemicellulose (Islam, 2018).

A medium containing one or more of these organisms can be cultured in the laboratory to digest cellulose in the form of filterpaper. Samples from five natural sources are screened for the presence of aerobic fibrolytic microorganisms, which can be cultured for use in converting waste paper into glucose.

## 2. METHODS

### Culturing microorganisms

Samples of 40 ml from five sources that possibly contain microorganisms: river water, pond water, clay, dry cow dung and fresh cow dung were each combined with ten ml of distilled water and put in five sterilised beakers, each with a 0.085 g strip of filter paper. Filter paper was chosen as the test material because it has a cellulose content close to 100%. The five cultures were incubated at 39°C until the paper was found to be digested in some of the beakers. The presence of glucose in the beakers after incubation was tested for with Benedict's reagent.

Following this, new samples of dry and fresh cow dung suspensions were sterilised by boiling. The filter paper from the cow dung in the first

experiment was added to sterilised beakers along with new filter paper and the mixtures were incubated for 18 hours at 39 °C. The final condition of the new filter paper was observed and the solutions were again tested for any increase in the concentration of glucose with Benedict's reagent. This procedure was repeated three times.

The procedure was repeated with sterile shredded paper replacing the new filter paper strips. The condition of the waste paper and any increase in glucose concentration was observed in the same way after incubation.

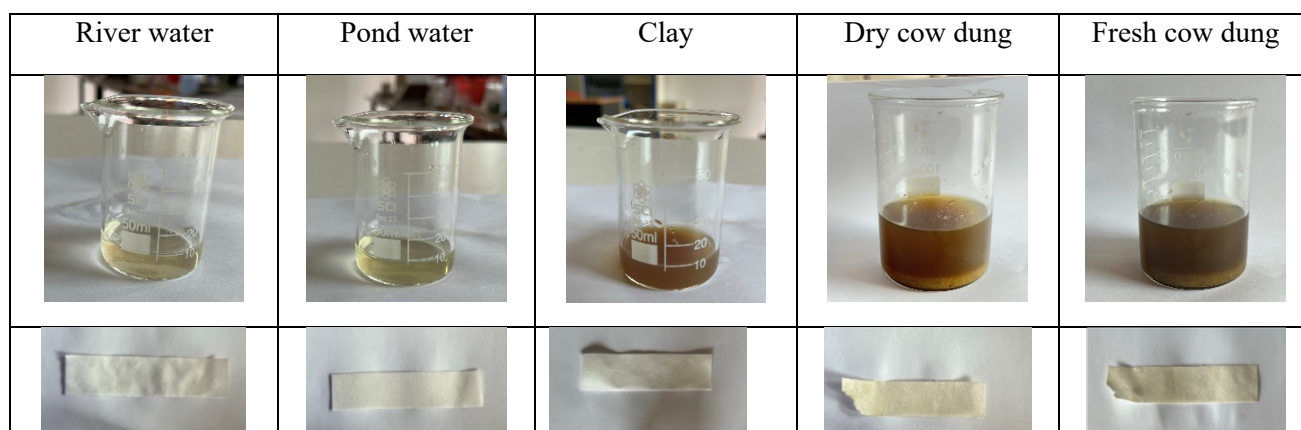
### 3. RESULTS AND DISCUSSION

Figure 2 shows the results for the test for the ability of microorganisms in each of the samples to digest cellulose. The top row of images in figure 2 shows the sample solutions in the beakers after incubating with paper. The first three samples from the left do







not show any evidence of paper residue on the bottom, while in the beakers with dry and fresh cow dung samples there is a clear layer of residue left from the digestion of the filter paper.

The bottom row of images in figure 2 shows the pieces of filter paper after incubation. Again, the three on the left show no signs of degradation, while the paper that was in the dry and fresh cow dung shows clear signs of degradation, with the left end of the paper partially disintegrated. The microorganisms present in river water, pond water, and clay did not digest the paper, while those present in both dry and fresh cow dung clearly were able to digest the cellulose in the paper, resulting in disintegration of the paper and residue on the bottom of the beaker.

When the solutions were tested for the presence of glucose (figure 3) it was found that pre incubation testing of both fresh and dried cow dung gave a weak positive result (light green color), indicating a



**Figure 2.** Digestion of cellulose filter paper by samples from natural sources media. The top row of pictures shows the digested residue from the shredded waste paper added to the beakers of dry and fresh cow dung. The bottom row of pictures shows the pieces of filter paper after incubation, with only the paper left in the dry and fresh cow dung showing evidence of being partially digested on the left end.

	River Water	Pond Water	Clay	Dry Cow Dung	Fresh Cow Dung
<b>Pre incubation</b>	No colour change	No colour change	No colour change	Weak positive 	Weak positive 
<b>Following incubation</b>	No colour change	No colour change	No colour change	Strong positive 	Strong positive 
<b>Following incubation with cellulose residue in boiled cow dung</b>				Strong positive 	Strong positive 

**Figure 3.** Results of testing with Benedict's solution. Only the dung showed the presence of glucose, with the increasing level of glucose after incubation being evidence for the conversion of cellulose to glucose.

low concentration of glucose. This is expected, since some sucrose would be expected in dung, even after sterilization, left from the digestion process. The result of the Benedict's test after incubation, for both the unsterilized and the sterilized condition, (rows 2 and 3 of figure 3) showed a dark green color, indicating that the fibrolytic microorganisms present in the filter paper that had been in the manure continued to break down cellulose during incubation, resulting in higher concentrations of glucose. No glucose was found in the water or clay samples, as expected.

Here, we show that fibrolytic microorganisms derived from cow dung can be used to digest cellulose, converting it into glucose. Whether this mechanism can be scaled up to produce commercial quantities of glucose from waste paper will be the subject of further research.

#### 4. CONCLUSION

It has been demonstrated that cellulose in the form of filter paper is converted to glucose by microorganisms when incubation in either dry or fresh cow dung suspensions, but is not digested in river water, pond water, or clay samples. This indicates the presence of microorganisms in the dung that produce cellulase. Upon further incubation of the microorganisms derived from the cow dung in suspensions of sterilized dry and fresh cow dung, the production of glucose was shown using Benedict's test. This demonstrates the potential for using the microorganisms found in cow dung in the production of glucose from waste paper, for use in the production of ethanol.

#### 5. REFERENCES

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