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Thermophilic cellulolytic microorganisms from western Algerian sources: promising isolates for cellulosic biomass recycling

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Abstract

Cellulosic biomass is considered as one of the most promising sources for the production of alternative renewable bioenergy and other valuable products. The abundance of cellulosic waste such as agricultural, industrial and forest waste, and the need for their biodegradation and their bioconversion into fermentable sugars, has increased the demands for more effective cellulase producing microorganisms. For this purpose, the present study was conducted to isolate thermophilic cellulolytic microorganisms. 111 thermophilic microorganisms (91 bacteria and 20 yeasts) were isolated from 10 western Algerian sources (thermal and non-thermal) and tested for the production of cellulase. The results revealed the presence of 19 thermophilic cellulolytic isolates. Macroscopic and microscopic examination has indicated the presence of 16 thermophilic bacteria and 3 thermophilic yeasts. These isolates were tested for the degradation of cellulosic biomass (printable paper, filter paper and cotton) for 14 days of incubation at 60°C. The obtained results showed a great potential of these thermophilic cellulolytic microorganisms to produce thermostable cellulolytic enzymes, and can be used in the recycling of cellulosic biomass for bioenergy production after optimization studies in the future.

Keywords: cellulosic biomass; recycling; bioenergy; thermophilic microorganisms; thermostable cellulases; Algerian sources.

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1. Introduction

In this rapidly changing world, demand for energy is increasing every year. According to Enerdata[1] world energy consumption in 2012 has increased by 1%, this has led to an augmentation in CO2 emissions by 1.4% [1]. Fossil fuels which are the main sources of energy in the world are an exhaustible resource and threaten our planet with a continued increase in pollution that causes global warming and which may adversely affect our future. This is why finding more renewable energy sources is now no longer something of luxurious.

Biomass which is the oldest source of energy in the world, can be today an effective alternative for the production of bioenergy. Technologies of bioenergy production from biomass require the intervention of microorganisms, these microorganisms have the ability to transform lignocellulosic materials using lignocellulolytic enzymes to fermentable sugars, for finally use them to produce ethanol, methane (gasification), Hydrogen, etc.

A large variety of enzymes are involved in the degradation of lignocellulosic compounds; Laccase, Manganese peroxidase, Lignin peroxidase for lignin, Pectin methyl esterase, pectate lyase, polygalacturonase, rhamnogalacturonan lyase for pectin, Endo-xylanase, acetyl xylan esterase, β-xylosidase, endomannanase, β-mannosidase, α-L-arabinofuranosidase, α- glucuronidase, ferulic acid esterase, α-galactosidase, p-coumaric acid esterase for Hemicellulose, and finally for cellulose bioconversion, three types of enzymes are implicated: exo-1,4-β-glucanases (cellobiohydrolase), endo-1,4-β-glucanases and β-glucosidases (cellobiases) [2].

Enzymes from mesophilic microorganisms are generally characterized by a remarkable instability, and require the use of techniques to reduce the temperature, which increases the cost. In addition to that, the enzymatic treatment carried out at 50°C causes a slow hydrolysis and gives a low yield of sugar (incomplete hydrolysis) [3].

Thermostable enzymes offer a real alternative, these enzymes as their name implies, have a very high stability; a higher specific activity, allowing for extended hydrolysis times and decreasing the amount of enzyme needed for saccharification [4].

The goal of this work is to isolate thermophilic cellulolytic microorganisms from local sources, and test their ability to degrade cellulosic biomass for a possible application in paper recycling field.

2. Materials and methods

All chemicals used in this study are of technical grade and obtained from commercial sources.

2.1. Preparation of colloidal cellulose

Cellulose used in this study to test the cellulolytic activity is a cellulose MN, this type of cellulose don't dissolve in water, that's why it was treated with pure HCl for one hour. After a series of washing with distilled water to remove HCl; the cellulose that is formed in this reaction is called “colloidal cellulose” which dissolves perfectly in distilled water.

2.2. Isolation of total thermophilic microflora

16 Samples were taken from 7 thermal and non thermal western Algerian sources (Table 1).

To induce the growth of total microflora, a method of sources enrichment was followed by adding 1g of soil to 10 ml of nutrient broth solution containing 0.1% yeast extract and 0.1% peptone. For liquid sources 0.1% yeast extract and 0.1% peptone were added directly to the sources, the tubes containing the samples were incubated at room temperature for 24h.

For the thermophilic microflora isolation, 200 ml of each enrichment solution was added to tubes containing 20 ml of an enrichment broth comprising 0.5% peptone, 0.3% beef extract, and 0.02% cellulose MN.

[1] http://www.enerdata.net