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ScienceDirect

Procedia Environmental Sciences 35 (2016) 826 – 832

Procedia Environmental Sciences

International Conference on Solid Waste Management, 5IconSWM 2015

Fungal Enzyme Cocktail Treatment of Biomass for Higher Biogas Production from Leaf Litter

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Abstract

Biogas production from leaf litter (road sweepings of MSW) is difficult under normal conditions requiring longer retention time due to the complex chemistry involving interlinking structures of lignin and cellulose where in lignin is considered recalcitrant under anaerobic conditions while also limiting access to the cellulose and hemicelluloses. Pre-treatment of biomass with single enzymes system is expensive and uneconomic and an alternative option has been tried. P. florida "ready-to-fruit" bags were sprinkled with biogas digester liquid (BDL) from a plug-flow reactor (PFR) type biogas plant fed with leaf litter. Spraying BDL not only increased the mushroom yields from 1.6 to 2.3kg/kg substrate but also gave around ~85ml of leachate rich in lignocellulolytic enzymes. Analysis of this enzyme cocktail at various stages of mushroom growth showed that on the 8d a combination of lignocellulolytic enzymes rich in peroxidase (46.67U/gTS) and laccase (204.38U/gTS) and low in cellulase (8.87U/gTS) could be obtained which can be used for various industrial applications such as softening of biomass for higher biogas production and/or bioethanol production. In this study the enzyme cocktail was used for softening paddy straw, a typical lingo-cellulosic biomass, for increasing biogas yields. This type of an enzyme treatment for paddy straw increased biogas yields by 29% and also enhanced sustainability by recycling end product of one process (mushroom cultivation) in softening biomass for higher biogas production and thereby overall system efficiency.

Peer-review under responsibility of the organizing committee of 5IconSWM 2015

Keywords: Biomass; Biogas; Fungal Enzyme.

1.0 Introduction

Anaerobic digestion of leafy litter under normal conditions without pre-treatment requires longer retention time

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Peer-review under responsibility of the organizing committee of 5IconSWM 2015
doi:10.1016/j.proenv.2016.07.099

due to the complex inter linking structures of lignin and cellulose which prevents the access of cellulose and hemicellulose by the anaerobes (Chanakya and Malayil 2012). Hence for higher biogas yields its necessary to carry out a pre-treatment of leafy biomass to reduce the retention time of the substrate in the reactor. During AD of leaf litter anaerobes attach to the leafy material forming a biofilm and releases various enzymes for hydrolysis of plant polymers like pectin, cellulose and hemicelluloses. The sugars released are later utilized by the overall methanogenic flora for producing biogas. It has been established earlier that hydrolysis forms the rate limiting step in biogas production process (Chanakya and Malayil 2012). Hydrolysis of leaf litter under AD conditions follows a pattern wherein pectic materials are used up and factors that affect the hydrolysis of pectic materials include leaf cuticle layer and surface area exposure. Degradation of pectic materials are followed by breakdown of hemicelluloses and then celluloses. Lignin is generally reported to be recalcitrant under AD conditions and higher lignin content leads to lowered biogas production (Chanakya and Malayil 2012). Various chemical and enzyme treatments have been tried and tested by researchers for higher biogas yields but most of the commercial enzymes and chemicals make the process economically unsustainable. These treatments aim at making the cellulose and hemicelluloses more accessible to the anaerobes by removing the obstructing lignin component. Due to the heterogeneity of leafy biomass and its complex structural features it is not possible to have one type of pre-treatment process for all types of leaf litter. And also in case of enzyme treatment, a cocktail of various lignocellulolytic enzymes would work better than single enzyme systems.

White-rot fungi are characterized by their unique set of lignocellulolytic enzymes responsible for degradation of wood/biomass substrate on which it is grown (Baldrian, 2003). They are the only micro-organism know to degrade lignin completely (Muller and Trosch, 1986). White-rot fungi release a battery of enzymes such as pectinase, xylanase, cellulases and lignanses to degrade the biomass on which it colonizes and utilizes the hydrolyzed products (sugars) for its growth (Kurt and Buyukalaca, 2010). Therefore understanding the enzyme action of these white-rot fungi is important to make the cellulose and hemicellulose component of biomass more accessible for further biotechnological use. Among white-rot fungi producing edible fruiting body, *Pleurotus spp.* is the most widely cultivated and studied species. Many studies have shown that the structure of lignocellulosic materials, mushroom species and cultivation techniques have an important role on the enzyme production potential of *Pleurotus spp.* (Tan and Wahab, 1997; Reddy et al., 2003; Elisashvili et al., 2006; Elisashvili et al., 2008). Studies have shown that supplementing single substrates with additional nitrogen source have increased yields for *P. florida.* For this purpose various organic supplements such as soaking the material in biogas digested slurry or mixing of biogas digester residue (Ganguli and Chanakya 1994) have been tested. Mixing of biogas digester residue in a 1:1 ration with paddy straw increased the biological efficiency by 300% (Ganguli and Chanakya 1994, Chanakya et al. 2015). In a plug flow type biogas plant designed at Astra-CST, like in most biomass fed biogas plants the feedstock is not pulverized and is fed as it. Therefore like in most biogas plant where the output is slurry of biomass in PFR the out consists of a separated liquid phase (biogas digester liquid, BDL) and digested biomass (biogas digester residue, BDR) along with biogas.

Spraying of BDL to mushroom bags have found to increase the yields by 150% but spraying BDL initiates leaching of the nutrients and enzymes from the bags. Since this liquid contains various lignocellulolytic enzymes along with nutrients such as CN&P it can be used for various biotechnological applications for selectively removing biomass components. Recovery of such enzyme cocktail along with edible fruiting body becomes a suitable example which follows the triple bottom line of sustainability where in more than 90% of the nutrients starting from BDL and agro-residue is used in various forms and recycled. The aim of the current study is to determine the ability of *Pleurotus florida* to produce lignocellulolytic enzymes such as pectinase, xylanase, cellulase, laccase and peroxidase and its potential in treating paddy straw for higher biogas yields.

2. Materials and Methods:

Ready to fruit mushroom bags was purchased from IIHR (Indian Institute of Horticulture research) Bangalore. These bags are 1kg paddy straw packed along with the spawn (*Pleurotus Florida*) in perforated plastic bags. The mycelia network was well established and the purchased bags were expected to fruit within a week. Each of these 1kg bags were placed on separate plastic trays. The cultivation was carried out in a humid chamber covered with jute cloth in laboratory conditions. Around 10L of BDL was collected from the outlet of a functioning PFR