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Towards a competitive solid state fermentation: Cellulases production from coffee husk by sequential batch operation and role of microbial diversity

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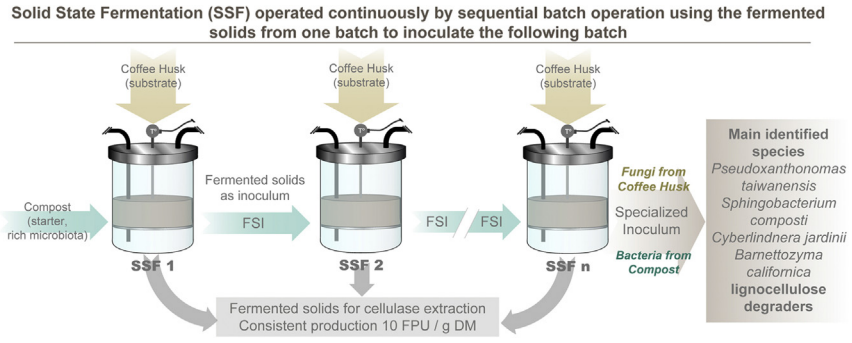
HIGHLIGHTS

- Cellulases were produced by solid-state fermentation (SSF) of coffee husk (CH).
- SSF performed consistently by sequential batch operation to produce 10 FPU/g dry mass.
- Fermented solids from one batch were used to inoculate the following batch.
- Compost was used as initial inoculum to obtain a specialized inoculum.
- Bacteria from compost and fungi from CH thrived through the fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

The cost of cellulases is the main bottleneck for bioethanol production at commercial scale. Solid-state fermentation (SSF) is a promising technology that can potentially reduce cellulases cost by using wastes as substrates. In this work, a SSF system of 4.5 L bioreactors was operated continuously by sequential batch operation using the fermented solids from one batch to inoculate the following batch. Coffee husk was used as lignocellulosic substrate. Compost was used as starter in the first batch to provide a rich microbiota. Two strategies were applied: using 10% fermented solids as inoculum in 48 h batches (SB90) and using 50% solids in 24 h batches (SB50). A consistent and robust production process was achieved by sequential batch operation. Similar cellulase activities around 10 Filter Paper Units per gram of dry solids were obtained through both strategies. Microbial diversity in the starting materials and in the final fermented solids was characterized by next generation sequencing. Microbial composition of both fermented solids was similar but the relative abundance of families and species was affected by the operation strategy used. Main bacteria in the final solids came from compost (families *Sphingobacteriaceae*, *Paenibacillaceae* and *Xanthomonadaceae*), while main fungi families came from coffee husk (families *Phaffomycetaceae*, *Dipodascaceae* and two unidentified families of the class of *Tramellomycetes*). There was a high presence of non-identified mycobiota in the fermented solids. Main identified species were the bacteria *Pseudoxanthonomas taiwanensis* (12.3% in SB50 and 6.1% in SB90) and *Sphingobacterium composti* (6.1% in SB50 and 2.6% in SB90) and the yeasts *Cyberlindnera jardinii* and *Barnettozyma californica* (17.8 and 4.1% respectively in SB50 and 34 and 9.1% in SB90), all four previously described as lignocellulose degraders.

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The development of these operational strategies and further biological characterization of the end product could eventually benefit the process economics by providing a standard and specialized inoculum for a continuous SSF for cellulases production.

1. Background

Increasing global demand for energy has resulted in the need for new and renewable energy forms such as bioethanol. In this sense, the second and third generation of bioethanol has gained great attention over the last years, due to the potential use of lignocellulosic biomass as raw material, avoiding the use of pure substrates such as starch (Behera and Ray, 2016; Kuhad et al., 2016). The use of agricultural by-products or wastes presents great advantages in comparison with pure substrates from the points of view of the energy balance and the environmental impact (Olofsson et al., 2008).

In general, bioethanol production from lignocellulosic materials consists of a pretreatment to hydrolyse lignin and hemicellulose-based materials, followed by an enzymatic hydrolysis of cellulose and a final yeast-based fermentation of the released free sugars. From these stages, enzymatic hydrolysis appears as the main bottleneck for the production of bioethanol at commercial scale. This is mainly due to the cellulases production process, which accounts up to 40% of total costs (Arora et al., 2015). Moreover, some studies performed on the life cycle assessment of this process conclude that cellulase production requires a higher energy input than cellulose to ethanol conversion itself (Heather and Sabrina, 2009).

Cellulases used to break down cellulose into fermentable sugars are commercially produced by submerged fermentation mainly from pure substrates (McMillan et al., 2011). Despite the fact that the use of commercial cellulases potentially provides high sugar yields, its use is far of being a cost-effective alternative. The enzymatic preparations must be purchased continuously and its production process is complex and energy intensive (Lever, 2005). Submerged fermentation normally includes sterilization systems, control and monitoring of different parameters such as temperature, pH and dissolved oxygen, which implies the use of high amounts of energy in the process (Tolan and Foody, 1999). Thus, cellulases production should be optimized to avoid the high cost of commercial enzymes, and to make the bioethanol production a sustainable process. Many aspects have been studied for the improvement of cellulases production, such as substrate (El-Bakry et al., 2015), inoculum selection (Eichorst et al., 2013; Mihajlovski et al., 2015) and alternative fermentation strategies such as solid-state fermentation (Lever, 2005; Olofsson et al., 2008), among others.

Solid state fermentation (SSF) of lignocellulosic biomass presents important advantages over conventional submerged fermentation such as reduced energy requirements, high productivity and less inhibitory effects for enzymatic production (Kuhad et al., 2016). However, its application at the industrial scale appears to be hindered by technology issues, such as reactor design, heat transfer issues or sterilization costs (Mitchell et al., 2006; Pessoa et al., 2016). To overcome some of these constrains, our research group have proposed to work in a composting-like process, under near adiabatic conditions using the native microbiota for the production of hydrolytic enzymes such as proteases (Abraham et al., 2013). However, a successful strategy for enzymatic production includes a proper selection of microorganisms to improve productivity coupled with these engineering aspects of SSF (Kuhad et al., 2016). In this context, we have proved in the same SSF configuration that thermophilic strains inoculated in non-sterile wastes were able to compete with autochthonous microbiota and significantly increase proteases activity (El-Bakry et al., 2016). Furthermore, it has been proven that it is possible to select and adapt compost microorganisms to use cellulosic materials as substrate, which could potentially increase the cellulolytic degradation capacity (Eichorst et al., 2013). These facts suggest the possibility to adapt a complex inoculum to degrade lignocellulosic materials in an easily scalable process to provide a standardized cellulase production process. The obtained enzymatic pool or even the fermented solids obtained by SSF can be potentially used in the hydrolysis step of the bioethanol production as suggested by some authors (Farinas, 2015). Pensupa et al. (2013) found a higher glucose release using cellulase obtained by SSF than commercial preparations while Lever et al. (2010) successfully applied crude lignocellulosic extracts for bioethanol production.

Coffee husk (CH) is a lignocellulosic waste obtained in the roasting process of coffee. More than eight million tons of coffee beans are produced annually world-wide, 18% of which is obtained as CH (Murthy and Naidu, 2012). Currently, coffee husk is disposed as an industrial waste and managed through incineration, landfill or composting in the best case. Shemekite et al. (2014) demonstrated the suitability of the native populations in coffee husk for biodegradation, correlating several enzymatic activities with the fungi communities. The current change of paradigm into the circular economy framework leads to an increasing interest in biomass as feedstock for new processes. Coffee husk has attracted interest of researchers worldwide as the substrate for solid-state fermentation and other processes to obtain valuable products (Mussatto et al., 2011; Narita and Inouye, 2014).

Despite all of the above advances, still the setting up of a long term SSF operation in continuous or semi-continuous regime remains a challenge with few attempts reported at lab scale using restrictive conditions, such as the use of pure strains under initial sterile conditions (Cheirsilp and Kitcha, 2015; Astolfi et al., 2011). Lately, a novel and more suitable approach has been assessed for enzymatic production by SSF using a sequential batch operation with non-sterile wastes, showing promising applications (Cerda et al., 2016).

The aims of this work are: i) to assess two different operational strategies for the development of a continuous SSF using coffee husk as a model substrate and ii) to produce a specialized inoculum for cellulases production and assess its microbial biodiversity. This process of optimization for cellulases production will provide a standardized and cost-effective technology with evident benefits for bioethanol production.

2. Materials and methods

2.1. Raw material

Coffee husk (CH) was kindly provided by Marcilla S.A (Mollet del Vallés, Barcelona, Spain). Compost (C, from source-selected organic fraction of municipal solid waste) was obtained from the municipal solid waste treatment plant Ecoparc de Montcada (Montcada, Barcelona, Spain). All materials were stored frozen (−18 °C) until use. The full characterization of both materials is presented in Table 1.

2.2. Solid-state fermentation

2.2.1. Preliminary experiments at the lab scale

Preliminary SSF experiments were performed in order to assess the inoculum size and the time for maximum cellulase production. Non sterile coffee husk was mixed with compost as inoculum in 0, 10, 20, 50 and 100% (w/w) ratio. Wood chips were added in a 1:1 (v/v) ratio as bulking agent. Fermentation was carried out in triplicates for 4 days