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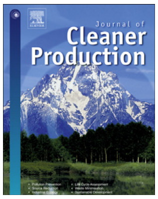

Potential use of pulp and paper solid waste for the bio-production of fumaric acid through submerged and solid state fermentation

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ABSTRACT

Pulp and paper solid waste (PPSW) originating from paper industry, was experimented for the production of fumaric acid (FA) through submerged and solid state fermentation by utilizing the filamentous fungus *Rhizopus oryzae 1526*. Physicochemical characterization, pH and moisture content analysis of PPSW was carried out. Pre-treatment of PPSW by size reduction resulted in particles of different size ranges ($1.7\text{ mm} < x \le 3.35\text{ mm}$, $850\ \mu\text{m} < x \le 1.7\text{ mm}$, $300\ \mu\text{m} < x \le 850\ \mu\text{m}$, $75\ \mu\text{m} < x \le 300\ \mu\text{m}$ and $33\ \mu\text{m} < x \le 75\ \mu\text{m}$). In submerged fermentation with all particle size ranges, a maximum of 23.47 ± 0.70 g/L of FA was obtained with $33\ \mu\text{m} < x \le 75\ \mu\text{m}$ under the fermentation conditions of 30 °C, 200 rpm, 5% pre-cultured inoculum (*v/v*) and at 48 h. Viscosity measurement and analysis of by-product of the fermented broths were performed. Microwave-phosphoric acid mediated hydrolysis of $33\ \mu\text{m} < x \le 75\ \mu\text{m}$ particle size produced hydrolysate with maximum glucose (11.2 ± 0.8 g/L) and xylose (20.22 ± 0.85 g/L) contents. Submerged fermentation with this hydrolysate confirmed the utilization of xylose for both FA production and fungal growth. Solid state fermentation with $75\ \mu\text{m} < x \le 300\ \mu\text{m}$ particle size resulted in highest FA production (41.45 g/kg dry weight) after 21 days. Scanning electron microscopy revealed the morphological features of the fungus grown on the particles. The results of the present study confirmed the utilization of PPSW as a source of carbon and trace elements by the fungus *R. oryzae 1526* and also the bioconversion into FA during fermentation. FA being a high value platform chemical, its bioproduction from the low cost PPSW, is a value addition approach.

1. Introduction

To meet the escalating demand for paper based products; huge amounts (several tonnes per day) of pulp and paper solid waste (PPSW) are produced by paper mills worldwide every year. In fact, PPSW has been found to be the 3rd largest industrial polluter of air, water and soil (www.theworldcounts.com). Proper disposal of PPSW has become a big environmental challenge in many countries, including Canada. There are approximately 130 pulp and paper industry establishments in Canada, mostly located in the provinces of Quebec, British Columbia and Ontario. More than 1/3 of Canada's total waste is PPSW and only 1/4 of PPSW and paperboard is recycled (www.ec.gc.ca). Apart from Canada, the proper disposal and management PPSW is a serious global problem (Oral et al., 2005; Abou-Elela et al., 2006). PPSW accounts for 25% of landfill waste and 33% of municipal waste (www.theworldcounts.com). Although different conventional strategies, such as landfilling, composting, incineration and recycling are being adopted for the management of PPSW, they are not found to be safe for environmental and human health (Fikru, 2014). In terms of microbial susceptibility, PPSW is a very good source of carbon (energy), vital micro and macro nutrients (minerals), high moisture content (60–70%) and easily biodegradable organic load (high BOD and COD values). Dumping of these wastes in the environment can have direct adverse effects on the environment; worth mentioning are the generation of greenhouse gases (GHGs) and secondary pollution (emission of foul smell caused by microbial

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Abbreviations: PPSW, Pulp and paper solid waste; FA, Fumaric acid; BDW, Biomass dry weight; MC, Moisture content; SSF, Solid state fermentation; SmF, Submerged fermentation; TC, Total carbohydrates; AIR, Acid insoluble residues; FAEs, Fumaric acid esters; ASTM, American Society for Testing and Materials.

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putrefaction and contamination of groundwater). Rotten PPSW emits methane (CH_4) gas, which has a global warming potential 72 times greater than CO_2 over a 20 year period (www.global-warming-forecasts.com). PPSW can also provide food and shelter to microorganisms and disease vectors (e.g. rats, insects etc.) that can cause epidemics in nearby localities. Moreover, because of the unavoidable associated cost factor (transportation and labour charges), landfilling option is no more considered profitable by paper industries. The extra cost demands for price rise in the paper industry based products. Another alarming issue in PPSW landfilling is the new trends in regulations, imposed in terms of ban on landfilling of the waste biosolids in different continents (www.epa.gov; Monte et al., 2009; Testa et al., 2014). Thus, new alternatives of safer disposal of PPSW with the intervention of modern techniques can contribute to the solution of this global problem. On the contrary, valorization of PPSW for the production of high value product without much economic inputs will be a welcome approach.

With the advent of biotechnological innovations, mainly in the area of fermentation technology, many new avenues have been opened for the proper valorization of different industrial wastes (Kajaste, 2014). In this regard, PPSW can be subjected to valorization for fumaric acid (FA) production through microbial fermentation. As compared to refined carbon sources such as glucose, PPSW is a low cost carbon source and its utilization can slash the overall fermentative production cost of FA. Recalling the fact that biological FA production was sought to replace the petrochemical route, search for new waste biomass with the potential of serving as a good source of carbon, micro and macro nutrients is actively going on. Moreover, the worldwide demand for FA and its derivatives is growing each year (Goldberg et al., 2006). In 2014, the global FA market demand was around 240, 000 t and the projected market volume is 350,000 t by 2020 (www.grandviewresearch.com). In addition to the conventional uses, FA and its ester derivatives (FAEs) have been explored with a number of newer applications in diverse fields (Yang et al., 2011b; Mrowietz and Asadullah, 2005). The ever-increasing demand for FA mandates the use of alternative fermentation processes using inexpensive raw materials, such as agro-industrial wastes through fermentation. Investigations on FA production from woodchips, dairy manure, crude glycerol, brewery wastewater and lignocellulosic biomass, such as corn straw exhibited high product yield of FA (Xu et al., 2010; Zhou et al., 2014; Das et al., 2015). As literature suggests, different FA producing fungal species of *Rhizopus* have been successfully grown on wastes raw materials without supplement of any nutrients and resulted in good FA product spectrum. The comparison of petrochemical and fermentation routes for FA production suggests that the lower raw material cost of the fermentative production might compensate the higher yields of the petrochemical production from maleic anhydride, and fermentation may become an economically viable alternative (Roa Engel et al., 2008). The fungal strain, *Rhizopus oryzae* 1526 (to be abbreviated as *R. oryzae* afterwards) is highly susceptible to various physico-chemical factors, such as pH, temperature, incubation time, total solids concentration (g/L) of the used substrate, inoculum volume size (*v*/*v*) and has a direct impact on the FA production (Xu et al., 2012). Screening of a novel carbon source for FA production has to be experimented at individual level. Process control and corresponding fungal (FA producing) responses are very specific for a combination of new carbon source and the fungal strain being used. Optimization of all the fermentation conditions can enhance FA production.

Thus, taking all the aforesaid important aspects of FA into consideration, investigations on the suitability of PPSW for improved FA production through fermentation (submerged and solid-state) were carried out.

2. Materials and methods

2.1. Materials

The FA producing fungal strain *R. oryzae* NRRL 1526 was procured from Agricultural Research Services (ARS) culture collection, IL, USA. The PPSW was procured from Resolute Forest Products, Montreal (Quebec) Canada. All the chemicals used were of analytical grade and purchased from Fisher Scientific (Ottawa, Ontario, Canada).

2.2. Methods

2.2.1. Microbial culture and media preparation

The procured fungal strain was first cultured on potato dextrose agar (PDA) slant at 37 °C ± 1 °C for a maximum of 4 days. Spread plate method was used to prepare spore inoculum by propagating the spores on PDA plates (90 mm) at 37 °C ± 1 °C for 72 h. Mycelium free spores were collected in sterile distilled water after filtration through sterile cotton wool. For regular use, the spore suspension was maintained at 4 °C. Spores were also preserved at −80 °C after adding 20% glycerol solution for long term use. A stock of 1×10^8 spores per mL was maintained and used for inoculation.

Glucose–basic salts medium (g/L: glucose 50, urea 2, KH_2PO_4 0.6, $MgSO_4 \cdot 7H_2O$ 0.5, $ZnSO_4 \cdot 7H_2O$ 0.11 and $FeSO_4 \cdot 7H_2O$ 0.0088) was used for preparing the pre-culture of *R. oryzae*. The medium final pH (4.6) was not adjusted, unless specifically indicated. To avoid Maillard reaction, pre-culture medium was prepared in two parts: (a) glucose; and (b) urea + salts and were heat sterilized (20 min, 103421.3594 Pa, 121 ± 1 °C) separately. Room temperature cooled media components were mixed together inside a laminar hood and used for the pre-culture of *R. oryzae*. Pre-culture of *R. oryzae* was prepared by inoculating 50 mL of pre-culture medium with 2% (*v*/*v*) spore suspension in a 250 mL Erlenmeyer flask and then incubating at 30 °C, 200 rpm for 24 h.

2.2.2. Physicochemical characterization of dried pulp and paper solid waste

The estimation of total organic carbon (TOC) and total organic nitrogen (TON) of dried PPSW was done using a carbon:hydrogen:nitrogen:sulphur (C:H:N:S) analyzer (LECO Corporation, USA, Model: CHNS-932). The ash, extractives and acid-insoluble contents of the PPSW was determined using American Society for Testing and Materials (ASTM) standard methods (ASTM Method E 1756-95, ASTM Method E 1755-95 and ASTM Method E 1690-95). Determination of total carbohydrates of the extractive free PPSW was done following the Anthrone method (Hedge and Hofreiter, 1962). For pH measurement, 1 g of PPSW was mixed in 10 mL of distilled water using magnetic stirrer, and the pH was recorded using a pH-meter equipped with glass electrode. The moisture content (MC) of PPSW was analysed by oven-dry method (Reeb and Milota, 1999). About 5 g of PPSW sample was allowed to dry at 60 ± 1 °C until a constant weight was achieved. The MC in PPSW was calculated as per the following formula:

$$\mathrm{MC} = \frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Initial weight}} \times 100 \tag{1}$$

2.2.3. Size reduction and hydrolysis of pulp and paper solid waste

Oven dried PPSW was ground using an electrical grinder and then sieved through a set of USA standard test sieves (140, 200, 50, 20, 12 and 6 mesh) (ASTM, E-II specification) to obtain the initial particle size ranges: $1.7\ \mathrm{mm} < x \leq 3.35\ \mathrm{mm}$, $850\ \mu\mathrm{m} < x \leq 1.7\ \mathrm{mm}$,

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