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Research article

Bioethanol production from recovered napier grass with heavy metals

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ABSTRACT

Using plants to absorb and accumulate heavy metals from polluted soil, followed by the recycling of explants containing heavy metals, can help achieve the goal of reverting contaminated soil to low heavy-metal content soil. However, the re-use of recovered explants can also be problematic. Meanwhile, bioethanol has become a popular energy source. In this study, napier grass was used for the remediation of soil contaminated with heavy metals (artificially contaminated soil). The influence of bioethanol production from napier grass after phytoremediation was also investigated. The concentration of Zn, Cd, and Cr in the contaminated soil was 1000, 100, and 250 mg/kg, respectively. After napier grass phytoremediation, the concentration (dry biomass) of Zn, Cd, and Cr in the explants was 2701.97 ± 173.49, 6.1 ± 2.3, and 74.24 ± 1.42 mg/kg, respectively. Biomass production in the unpolluted soil was 861.13 ± 4.23 g. The biomass production ratio in high Zn-polluted soil was only 3.89%, while it was 4.68% for Cd and 21.4% for Cr. The biomass obtained after napier grass phytoremediation was pretreated using the steam explosion conditions of 180 °C, for 10 min, with 1.5% H_2SO_2, followed by enzymatic hydrolysis. The efficiency of enzymatic hydrolysis for Zn-polluted biomass was 90% of the unpolluted biomass, while it was 77% for Cd, and approximately the same for Cr. The fermentation efficiency of the heavy-metal-containing biomass was higher than the control biomass. The fermentation ethanol concentration obtained was 8.69–12.68, 13.03–15.50, and 18.48–19.31 g/L in Zn, Cd, and Cr environments, respectively. Results show that the heavy metals had a positive effect on bacteria fermentation. However, the fermentation efficiency was lower for biomass with severe heavy metal pollution. Thus, the utilization of napier grass phytoremediation for bioethanol production has a positive effect on the sustainability of environmental resources.

1. Introduction

Heavy metals are trace elements derived from industrial activities. Improper disposal of heavy metals has resulted in the release of large amounts of potentially toxic compounds, increasing human health risks worldwide (Antoniadis et al., 2017). Soil contamination by heavy metals is one of the major environmental problems raising critical concerns for both, human health and ecosystems (Adrees et al., 2015; Jennings, 2013).

Various physical, chemical, and biological processes are currently being used to remediate metal-contaminated soils. Phytoremediation is the *in situ* application of plants and their associated microflora for environmental cleanup (Antoniadis et al., 2017). This technology makes use of the naturally occurring processes, by which plants and their microbial rhizospheric flora degrade and sequester organic and inorganic pollutants (Heckenroth et al., 2016; Ma et al., 2016). Despite its obvious advantages, the effectiveness of phytoremediation must be determined to validate its use in practical remediation. Criteria, such as its ability to remove or neutralize contaminants and time efficiency need to be evaluated on a long-term basis (Salam et al., 2016). The technology depends on the level and types of metals, their chemical forms, the capacity to uptake and tolerate metal accumulation without phytotoxic effects on the plants, the biomass production capacity of the harvested

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crop, and the final level of heavy metals in the soil for it to be considered remediated (Paz-Alberto and Sigua, 2013; Cundy et al., 2016). Different amounts of heavy metals may be stored in different parts of the plants, depending on the plant species (Antoniadis et al., 2017; Pavoni et al., 2017). Although phytoremediation has been regarded as a suitable alternative, the diversity of plant species (Lee, 2013; Laghlimi et al., 2015) continue to impact the efficiency of phytoremediation and competitiveness towards conventional methods of soil and groundwater remediation.

Napier grass (*Pennisetum purpureum*) is a well-recognized animal feed stock, due to its high growth rate and adaptability (Grant et al., 1974). Napier grass cultivar Taishi No. 4 was developed by TLRI (Taiwan Livestock Research Institute, Tainan, Taiwan) for its low maintenance and drought resistance characteristics. Since the ecological impacts of invasive plants have received significant global attention (Carvalho et al., 2016; Mohapatra et al., 2017), native and domesticated species were used in this study.

Ethanol production from lignocellulosic biomass, referred to as second generation biofuel, is highly regarded for the sustainable production of fuels, with social, economic and environmental benefits (Carvalho et al., 2016; Mohapatra et al., 2017). There are four main steps for producing ethanol from lignocellulose: pretreatment, hydrolysis, fermentation and distillation (Ko et al., 2012; Mohapatra et al., 2017). Pretreatment is the most crucial step for increasing the accessibility during hydrolysis and fermentation. The recalcitrant cellulose structure, which includes lignin, hemicelluloses and high cellulose crystallinity, is the main barrier in ethanol production (Hendriks and Zeeman, 2009; Chundawat et al., 2011). Among the pretreatment methods, steam explosion has been regarded as an effective process to increase the accessible surface area to further assist hydrolysis (Hendriks and Zeeman, 2009; Mohapatra et al., 2017).

With regard to fermentation, simultaneous saccharification and fermentation (SSF) was more successful in improving the hydrolysis rate and ethanol conversion yield than separate hydrolysis and fermentation (SHF). Moreover, SSF reduces the end-product inhibition of enzyme complex (Olofsson et al., 2010). Ballesteros et al. (2004) applied steam explosion pretreatment to convert 71.2% and 62.5% ethanol from poplar and eucalypt chips, respectively. Cara et al. (2008) converted olive tree pruning to ethanol with steam explosion method as well, obtaining a yield of 50–69%. SSF increases the final ethanol conversion rate to about 79% from steam-exploded salix biomass (Sassner et al., 2006).

In order to fulfill the goal of green remediation and achieve sustainability, bioethanol production from harvested biomass (Napier grass), after the phytoextraction of soil contaminated with heavy metals, was investigated. Steam explosion was employed to pretreat the recovered Napier grass with Zn, Cd, and Cr uptake, as well as for obtaining optimally fermentable sugars. Subsequently, pretreated biomass was subjected to SSF for bioethanol production. SSF was carried out using *Escherichia coli* KO11. The impact of the heavy metal uptake of the biomass on saccharification and SSF process were assessed.

2. Material and methods

2.1. Phytoextraction by napier grass Taishi No.4

Twenty centimeters high cuttings of napier grass Taishi No. 4, provided by TLRI, with at least two buds, were employed. All the seedlings and cuttings were transferred into pots in the following dosages: 1000, 2000, 4000, and 10000 mg Zn/kg of dried soil; 125, 250, 500, and 1000 mg Cr/kg of dried soil; 10, 20, 40, and 100 mg Cd/kg of dried soil. Sandy loams, with an average organic carbon content of 0.32% (w/w) and pH of approximately 6.5 were fertilized monthly with an N:P:K ratio of 12:3:6 kg/ha/yr.

2.2. Acid steam explosion pretreatment

Napier grass was pretreated by steam explosion in accordance with the published procedures (Ko et al., 2012). Before pretreatment, the biomass was immersed in a 3% H_2SO_4 solution for 24 h. For steam explosion, the reactor was charged with 1 kg (dry matter) of raw materials and heated to 190 °C with saturated steam for 10 min, following which the pressure was released instantly. Samples were subsequently washed with tap water on a 200-mesh screen until the eluants turned neutral. It was subsequently refrigerated at 4 °C.

2.3. Enzymatic hydrolysis

Subsequent to the acid-steam explosion and sulfite pretreatment for overcoming the recalcitrance of lignocelluloses (SPORL), the pretreated biomass was hydrolyzed with a combination of multi-component cellulase (Novozymes 50010, 50013) and xylanase (Pulpzyme HC). Three levels of enzyme loadings were applied: equivalent to 1.68, 5, and 10 IU filter paperase (FPU)/g of dried solids; 0.54, 1.68, and 10 IU cellobiohydrolases (CBU)/g of dried solids; and 100, 300, and 600 IU xylanase/g of dried solids. The enzymatic saccharification of the treated feedstock was performed in 0.1 M, 200 mL tris-buffer, with 2.5% solid content. The reaction conditions were: temperature of 37 °C, pH of 7.0, at 100 rpm for 120 h of hydrolysis, with the samples being analyzed every 12 h.

2.4. Simultaneous saccharification and fermentation (SSF)

Escherichia coli KO11, purchased from American Type Culture Collection (ATCC), was employed. Before SSF, the *E. coli* KO11 was pre-cultured in liquid medium (with 10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, 20 g/L of glucose dissolved in H_2O) at 37 °C for 24 h (optical density, OD_{600} up to 1.4).

The water-insoluble, steam-exploded biomass was subjected to SSF. All SSF processes were prepared by loading 250 mL baffled flasks with 3 g of glucan and pretreated biomass. The flask and samples were subsequently autoclaved at 121 °C for 20 min. Each flask contained 0.5% w/v yeast extract, 1% w/v tryptone, 0.1 M tris-buffer (pH 7), 5 mL *E. coli* KO11 inoculums with the highest enzyme loading. All the flasks were shaken slowly (100 rpm) at 37 °C, with samples being taken at 12 h intervals, for a total of 120 h, after which they were frozen for subsequent analysis. The ethanol yield, expressed as a percentage of the maximum theoretical yield (% cellulose conversion) that can be produced from glucan, was calculated using the following equation (Faga et al., 2010):

Cellulose conversion to ethanol (%):

$$= \frac{[EtOH_t]}{0.511 \times f \times [Biomass] \times 1.11} \times 100 \quad (1)$$

where [EtOHt] is the ethanol concentration produced at time t (g/L), 0.51 is the mass conversion factor of glucose to ethanol (g/g), f is the fraction of glucan in dry solids (g/g), [Biomass] is the initial concentration of solids (g/L), and 1.11 is the mass conversion factor of glucan hydrolysis to glucose (g/g). Samples were analyzed for glucose, xylose, and ethanol by HPLC.

2.5. Analytical methods

Oven-dried biomass, acid-steam-exploded and pretreated samples were analyzed for acid-insoluble lignin (TAPPI T222 om-

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