FULL-LENGTH ARTICLE

Production of Bioethanol from Dried Coffee Pulp Using Pervaporation of the Fermented Broth

Dessalegn Dadi[1*], Antonio Amelio[2], Abebe Beyene[1], Kenneth Simoens[3], Mekonnen Demeke[4], Johan Thevelein[5], Kristel Bernaerts[3], Patricia Luis [6], Bart Van der Bruggen[7, 8]

[1]Department of Environmental Health Sciences and Technology, Jimma University, P.O. Box 378, Jimma, Ethiopia
[2]Department of Environment, Land and Infrastructure Engineering (DIATI), Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[3]Bio- and Chemical Systems Technology, Reactor Engineering and Safety Section, Department of Chemical Engineering, KU Leuven, Celestijnenlaan 200F box 2424, 3001 Leuven, Belgium
[4]KU Leuven Laboratory of Molecular Cell Biology VIB-KU Leuven Center for Microbiology Institute of Botany and Microbiology Kasteelpark Arenberg 31 - Bus 2438, 3001 Heverlee-Leuven, Belgium
[5]Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Department of Biology, KU Leuven and Center for Microbiology, VIB Kasteelpark Arenberg 31, bus 2438, B-3001 Leuven-Heverlee Flanders, Belgium
[6]Materials & Process Engineering (iMMC-IMAP) UCLouvain, Place Sainte Barbe 2, 1348 Louvain-la-Neuve, Belgium
[7]Process Engineering for Sustainable Systems Section, Department of Chemical Engineering, KU Leuven, Celestijnenlaan 200F box 2424, 3001 Leuven, Belgium
[8]Faculty of Engineering and the Built Environment, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

***Corresponding Author**: dessalegndadiolani@gmail.com

ABSTRACT
The present study evaluates the potential of dried coffee pulp for bioethanol production and upgrading the quality of the produced ethanol by using a pervaporation membrane. Coffee pulp was ground and pretreated with different concentrations of diluted H_2SO_4 (0, 1, 2, 3, 4, 5 and 10 vol %) at different solid: liquid ratios (1:1, 1:2, and 1:4). The neutralized hydrolysate was fermented using lignocellulosic yeast GSE16-T18 strain using a solid to liquid ratio of 1:2. Before fermentation of the hydrolysate with the lignocellulosic yeast, the enzyme accellerase was used for hydrolysis. The fermented feed solution was filtered and pervaporated at different temperatures (30, 40, 50 and 60 ℃) using the PolyAn POL_AL_M1 pervaporation membrane. The optimum ethanol yield (7.03 ± 2.63 g/l) was obtained after 12 h of fermentation with lignocellulosic yeast GSE16-T18 strain. Furthermore, pervaporation of the fermented feed concentrated the ethanol to 15.47 ± 0.66 g/l indicating that dried coffee pulp can be a suitable candidate for the production of bioethanol.

Keywords: Bioethanol, Coffee pulp; Enzyme hydrolysis; Lignocellulosic yeast; Pervaporation

INTRODUCTION

As the global economy is highly dependent on fossil energy sources (Uihlein & Schebek, 2009) and its excessive consumption has resulted in the generation of high levels of pollution (Ballesteros et al., 2006), the need to seek new alternatives to cover the future energy demand is considerably increasing (Navia et al., 2011). An alternative fuel shall be technically feasible, economically competitive, environmentally acceptable, and readily available (Demirbas, 2008). In this regard, Uihlein and Schebek (2009) indicated

that biomass is a sustainable alternative to fossil energy carriers, with the potential to produce fuels, electricity, chemicals, and other goods. The development of fuel production processes from renewable resources is one of the most promising ways to replace fossil fuels and reduce greenhouse emissions (Amelio et al., 2016). Coffee residues have a large potential as raw materials for bioethanol and volatile compounds production (Ballesteros et al., 2014).

Coffea arabica L. has its origin and diversity in the Afromontane rainforests of southwestern Ethiopia (Yadessa, 2014). About 80 tropical countries produce and export coffee, generating a significant income (Tesfaye et al., 2014). It plays a significant role in the Ethiopian economy, contributing over 35% of the total export value; 4 to 5% to the national Gross Domestic Product and generating 20% of government revenue (Petit, 2007). The coffee industry is generating large quantities of residues, among which coffee pulp, coffee husk, spent coffee grounds and coffee silverskin are the most significant (Ballesteros et al., 2014). Studies by Murthy and Naidu (2010) indicated that the coffee industry liberates enormous amounts of coffee byproducts, rich in carbohydrates, proteins, pectins and bioactive compounds. However, large-scale utilization and management of coffee waste around the world remain a challenge due to its content of caffeine, free phenols, and tannins (polyphenols) (Fan et al., 2003).

Studies reported that wet coffee processing industries are generating different types of coffee residues like pulp, mucilage, hulls, and residual water (Rojas et al., 2002; Haddis & Devi, 2008; Beyene et al., 2012). Coffee pulp is obtained during the wet processing of coffee and for every two tons of coffee produced, one ton of coffee pulp is obtained (Roussos et al., 1995). The organic components present in coffee pulp (dry weight) include tannins (1.80–8.56%), total pectic substances (6.5%), reducing sugars (12.4%), non-reducing sugars (2.0%), caffeine (1.3%), chlorogenic acid (2.6%), and total caffeic acid (1.6%) (Murthy & Naidu, 2012). The conversion of lignocellulosic biomass to ethanol is more difficult than when using corn or molasses because of the complex structure of the plant cell wall (Menezes et al., 2014). The crucial step in the production of biofuels from lignocellulosic biomass is pretreatment. The choice of the optimum pretreatment process depends on the feedstock and its economic assessment and environmental impact. It has been demonstrated that dilute acid prehydrolysis can achieve high reaction rates in a short time and significantly improve cellulose hydrolysis (Xiang et al., 2003).

Dilute sulfuric acid pretreatment (Teramoto et al., 2009) was used to disorganize the crystalline structure and recalcitrant nature of coffee pulp to release the polymer chains of cellulose and hemicelluloses for facilitating the enzymatic attack. Menon and Rao (2012) also pointed out that the most commonly used acid, dilute sulphuric acid (H_2SO_4), has been commercially used for the pretreatment of a wide variety of lignocellulosic materials. Several recent studies indicate that coffee byproducts can be suitable for bioethanol production using fermentation technology (Gouvea et al., 2009; Shenoy et al., 2011; Choi et al., 2012; Woldesenbet et al., 2016). Though bioethanol production from coffee waste was attempted, no research has been carried out to investigate the potential of the dried coffee pulp for bioethanol production using yeast strain GSE16-T18. Thus, the current study focuses on bioethanol production from dried coffee pulp and its quality upgrading by pervaporating the produced bioethanol using an alcohol selective membrane.

MATERIALS AND METHODS

Coffee waste collection

Dried coffee pulp samples were collected from wet coffee processing industries located in the Manna district of Jimma zone, southwest Ethiopia. Coffee pulp is the byproduct obtained when the washed coffee bean is deshelled using the wet processing method. Fresh samples of coffee pulp were collected and thoroughly mixed to obtain homogeneous samples. The samples were then air-dried and grinding was carried out with a coffee blender with grinder 'Seven 7 star' (Germany). To obtain a particle size < 0.5 mm, the ground coffee waste samples were sieved with a U.S. standard sieve series mesh (ASTM E11-61, Tyler equivalent 32 inches mesh with 0.5 mm pore size). The ground samples were collected in well-dried polyethylene plastic containers and stored in a dry place until use.

Determination of physicochemical properties

The ground and sieved coffee waste samples were characterized as follows. The pH was determined using a Docu pH meter (Sartorius, Germany). For determination of the electrical conductivity (EC), 5 g of air-dried ground coffee pulp was transferred into a bottle and 25 mL of deionized water was added to obtain a suspension at a 1:5 ratio. The bottles were capped and homogenized at 200 rpm for 15 min (D72379 Hechingen, Edmund Buhler GmbH®, Germany). The electrical conductivity (EC) was measured using a pre-calibrated Hach HQ40d dual-input multi-meter. For the determination of the moisture content, oven drying and a gravimetric method was used. Samples of fresh coffee waste were weighed and placed in a forced-air oven at 105 °C for 24 h. After drying, the samples were weighed to determine the moisture content. The volatile suspended solids fraction (VSS) and the ash content of the samples were determined by ashing the samples in a furnace (LH 60/13, Naberthem, Germany) at 550 °C for 1 h. For the determination of biological oxygen demand (BOD) and chemical oxygen demand (COD), 1 g of ground coffee sample was mixed with 10 ml of distilled water and they were determined using BOD HACH dilution method 8043 and dichromate reactor digestion method 8000, respectively (Dadi et al., 2018).

Hydrolysis of coffee waste

Acid hydrolysis is an often proposed pretreatment method for the decomposition of lignocellulose (van der Pol et al., 2014). The dried coffee pulp samples were pre-treated with distilled water, 1%, 2%, 3%, 4%, 5% and 10 % (v/v) H_2SO_4 (99%, Sigma Aldrich) by varying the solid to liquid ratio (1:1, 1:2, and 1:4). The samples were then autoclaved at 121 ºC for 20 min (SystecV-150 autoclave, Systec, Germany). Pretreatment is required to increase the surface area of the feedstock, thereby rendering the lignocellulose accessible for hydrolysis (Klinke et al., 2004). After pretreatment, samples were centrifuged and the supernatant was filtered using a 0.2 µm filter. Acid pre-treated coffee pulp was neutralized with 1molar KOH (VWR International, United States) to a pH of 5.1 before fermentation with lignocellulosic yeast, and then saccharified using the enzyme accellerase® TRIO™ (0.1 mL of the enzyme per gram of biomass). The saccharification was conducted at 50 °C and 160 rpm for 48 h. For the determination of reducing sugar, an Agilent 1200 series High performance liquid chromatography (HPLC) was used.

Fermentation

Before fermentation of the samples using lignocellulosic yeast GSE16-T18, the samples were first further hydrolyzed using the enzyme accellerase® TRIO™ (DuPont). The optimum hydrolysis time was found to be 48 h. Incubation was done in an incubator shaker (Innova 4300, Brunswick Scientific, United States). The fermentation of samples with C5-degrading yeast GSE16-T18 (at a rate of 1 g dry yeast/L of hydrolyzed solution) was carried out at 30 °C with shaking at 100 rpm and stopped after 12 h since from the optimization study, this time was found to be optimal for ethanol production. For sugar and ethanol determination, samples were centrifuged at 14,000 rpm for 10 min and the supernatant was filtered using a 0.2 µm Whatman filter. The samples were stored at -20 °C before analysis. Ethanol and sugar concentrations were measured with an Agilent 1200 series HPLC with a refractive index detector, the column BioRad Aminex HPX-87H (7.8 × 300 mm) was kept at 40 ºC. The eluent was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min (Dadi et al., 2018).

Pervaporation

After fermentation of the samples, the fermented broth was centrifuged and then filtered using a coffee filter to remove the remaining suspended solids, and then pervaporated using two pervaporation instruments: Sulzer Chemtech AG (PERVAP™ LZ 2013 PV Pol.22, Switzerland), and GFT GmbH, Germany operating at different pervaporation pressures. The operating pressure above the active layer of the membrane is atmospheric pressure or slightly higher caused by the pressure drop in the membrane cell but no overpressure was applied. On the other side of the membrane (permeate), a vacuum pressure lower than 20 mbar was achieved using a 2-stage vacuum pump. Permeate was collected every 30 min in a cold trap. At least duplicate permeate samples under steady-state conditions were taken per experiment. The membranes were immersed in the feed solution at least 24 h prior to the experiment and then placed into the membrane test cell (Chovau et al., 2011; Luis et al., 2013).

For pervaporation of the filtered samples, an alcohol selective pervaporation membrane (Vane, 2005) Polyvinyl alcohol type M1 (POL_AL_ M1, PolyAn GmbH, Germany), with a total and active diameter of 75.6 and 70 mm, respectively, were used. The total thickness of the membrane was measured by a fowler ip54 caliper and was found to be 0.171 mm. Pervaporation of the fermented samples was conducted at 30, 40, 50, and 60 °C. The membrane flux J (kg h^{-1} m^{-2}) was determined gravimetrically using a balance with an accuracy of 10^{-4} g by weighing the mass of permeate w (kg) obtained during the collecting time Δt (h):

$J = \frac{w}{\Delta t * A}$; where A is the effective surface area (Steinigeweg & Gmehling, 2004).

The separation factor was calculated as the ratio of the molar component concentrations in the permeate (y_i) and feed (x_i) solutions:

$\beta \frac{i}{j} = \frac{y_i / y_j}{x_i / x_j}$ (Jiménez et al., 2002)

The experimental flux for each component was calculated as:

$Ji = J * y_i * \frac{m_i}{m_t}$, with m_i and m_t being the molecular weight of component i and the mixture, respectively (Švandová & Markoš, 2011).

The partial molar flux for each component ji (cm^3(STP)$cm^{-2}s^{-1}$) was calculated from (ignoring simple conversion terms, m^2 to cm^{-2}, L to cm^3, h to s):

$j_i = \frac{J_i * v_i^G}{m_i}$; where v_i^G is the molar volume of gas i (22.4 l (STP) mol^{-1} and m_i is the molecular weight of component i (Jiménez & Costa-López, 2002).

The permeance was calculated according to the following equation:

$\frac{P_i}{l} = \frac{j_i}{\left(x_i * \gamma_i * p_i^o - y_i * P_p\right)}$; Where, P_i is the permeability coefficient, l is the membrane thickness, P_i^o is the vapor pressure and p_p is the pressure in the permeate side measured experimentally during the experiment and the activity coefficient γi for each component have been calculated using UNIQUAC as the thermodynamic model (Steinigeweg & Gmehling, 2003).

The selectivity of the membrane was calculated from the ratio between permeances:

$\alpha \frac{i}{j} = \frac{P_i / l}{P_j / l} = \frac{P_i}{P_j}$ (Bessling et al., 1998).

RESULTS AND DISCUSSION

Characterization of the coffee waste samples

The air-dried and ground coffee pulp sample used for analysis has the following characteristics: electrical conductivity (EC), pH, biological oxygen demand (BOD), and chemical oxygen demand (COD) results were measured to be 1790 ± 10 µS/cm, 8.76 ± 0.23, 4200 ± 12 g/kg, and 5880 ± 14 g/kg, respectively. Furthermore, the moisture content was 8.05 ± 0.10%; the ash content was 20.14 ± 0.14%, and the volatile solid content was 79.86 ± 0.14%. The study conducted by Woldesenbet et al (2014) on coffee pulp juice and mucilage reported a volatile solid content of 66.5 and 90.2%, respectively.

Hydrolysis of the coffee waste samples

The samples were pretreated with either distilled water alone or using different concentrations of H_2SO_4 (v/v) (1%, 2%, 3%, 4%, 5%, and 10%), and finally autoclaved. Among the samples mixed in a solid to liquid ratio of 1:1, 1:2, and 1:4, the samples mixed in a 1:1 ratio yielded no filtrate at all, and they were excluded from further study. The results of the hydrolysis of the remaining samples are indicated in Table 1. These results indicate that the hydrolysate is composed of more xylose and arabinose than glucose.

Table 1. Mean results of hydrolysis of samples pretreated with distilled water alone and different concentrations of % H_2SO_4 (v/v) and autoclaved.

% H_2SO_4 used	Sample ID	Solid: Liquid ratio	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)	Xylitol (g/l)	Glycerol (g/l)	Acetic acid (g/l)
0 (DW)	A1	1:4	0.35 (0.00)	1.32 (0.23)	0.00	0.00	0.16 (0.01)	0.74 (0.05)
1	B1	1:4	0.39 (0.10)	0.25 (0.00)	2.97 (0.31)	0.00	0.18 (0.00)	0.47 (0.13)
2	C1	1:4	1.04 (0.20)	4.86 (1.03)	5.92 (1.40)	0.09 (0.01)	0.25 (0.01)	1.28 (0.03)
3	D1	1:4	2.25 (0.60)	11.27 (1.50)	6.26 (1.42)	0.19 (0.00)	0.26 (0.00)	2.09 (0.40)
4	E1	1:4	2.76 (0.70)	13.73 (1.46)	6.51 (1.65)	0.29 (0.05)	0.26 (0.10)	2.52 (0.90)
5	F1	1:4	3.15 (1.05)	14.14 (2.60)	6.27 (1.39)	0.37 (0.08)	0.28 (0.03)	2.77 (0.40)
10	G1	1:4	4.15 (0.69)	14.37 (1.82)	5.35 (1.97)	0.67 (0.04)	0.40 (0.07)	3.69 (0.55)
0 (DW)	A2 *	1:2	-	-	-	-	-	-
1	B2	1:2	0.73 (0.01)	1.71 (0.21)	0.82 (0.00)	0.00	0.26 (0.01)	0.26 (0.01)
2	C2	1:2	1.12 (0.10)	4.25 (0.23)	10.07 (1.01)	0.00	0.50 (0.11)	0.35 (0.20)
3	D2	1:2	2.05 (0.32)	11.53 (2.10)	10.52 (1.30)	0.32 (0.10)	0.41 (0.05)	2.42 (0.40)
4	E2	1:2	4.15 (1.01)	20.18 (2.32)	11.09 (1.32)	0.49 (0.02)	0.43 (0.04)	3.70 (0.62)
5	F2	1:2	4.50 (0.71)	22.93 (3.01)	10.93 (2.31)	0.52 (0.01)	0.41 (0.00)	4.19 (0.33)
10	G2	1:2	6.33 (0.54)	24.99 (2.71)	9.61 (2.24)	0.59 (0.22)	0.55 (0.20)	5.27 (0.84)

DW = Distilled water; * = no filtrate was obtained; Values outside the bracket are the mean results and those in brackets are the standard deviations

As shown in Table 1, the amount of glucose, xylose, and arabinose that can be obtained by hydrolysis of the sample using distilled water alone is lower than using H_2SO_4. Furthermore, Table 1 indicates that as the amount of H_2SO_4 is increasing (from 1%, 2%, 3%, 4%, 5% to 10%), the amounts of glucose, xylose, arabinose, xylitol, glycerol, and acetic acid were enhanced for both the 1:4 and 1:2 solid to liquid ratio. Thus, it can be observed that as the yield of fermentable sugars (glucose, xylose, and arabinose) increases, the concentration of byproducts (xylitol, glycerol, and acetic acid) is also enhanced. In comparison, Klinke et al. (2004) confirmed that the decomposition of lignocellulose to acquire monomeric sugars results in the formation of a large amount of byproducts.

The study by Urbaneja et al. (1996) on coffee pulp hydrolysates reported concentrations of xylose in the range between 0.08 and 3.23 g/l, arabinose between 0.23 and 11.26 g/l, glucose between 1.30 and 6.31 g/l, and fructose between 0.90 and 3.00 g/l. Furthermore, the study by Gurram et al. (2016) on coffee pulp from Mexico reported sugar contents, expressed as percentages of dry mass, of 5.8, 5.2, 20.2, 4.2, and 4.7% for arabinose, galactose, glucose, xylose, and mannose, respectively. From these yields, it can be observed that the coffee pulp hydrolysates obtained in this study are different from the studies mentioned above. An explanation for this difference was given by Navia et al. (2011) who reported that the chemical composition of coffee byproducts changes according to the plantation's height above mean sea level. At the same time, the composition also changes according to the type of coffee and the stage of development of the fruit (mature/immature, defected, and ripe/unripe) when it is harvested. Thus, the coffee beans considered in this study are different from the others since it is a dried and ground Arabica coffee bean collected from wet coffee processing industries in Jimma, Ethiopia, at a latitude and longitude of 7°40′N 36°50′E, and it has a tropical rainforest climate.

Impact of drying and neutralization of the autoclaved samples and formation of degradation products

The pH of wet coffee pulp was measured to be 5.69 ± 0.18 (slightly acidic). However, after it is air-dried and ground, the pH was 8.76 ± 0.23 (slightly alkaline). The cause of this pH variation might be the volatilization of organic acids from the samples. The impact of neutralization of the autoclaved samples on the yield of glucose, xylose, arabinose, xylitol, glycerol, and acetic acid was assessed by neutralization of all autoclaved hydrolysates with KOH to a pH value of 5; the results are indicated in Table 2. Knowledge of the inhibitory compounds can provide strategies for efficient fermentation processes. The degradation products formed by pre-treatment of lignocellulose depend on both the biomass and the pretreatment conditions such as temperature, time, pressure, pH, redox conditions, and the addition of catalysts (Klinke et al., 2004). Knowing about the formation of by-products from lignocellulosic material is beneficial when the decomposed lignocellulose is used in a fermentation process. The study of van der Pol et al (2014) indicated that the by-products can result in problems further downstream since they can inhibit the growth and production of micro-organisms during fermentation. In this regard, Taherzadeh et al. (1997) reported that for fermentation at low pH, 3.5 g/l of acetic acid can reduce growth rates by 33% in *S. cerevisiae*, while at higher pH, 9 g/l of acetic acid has the same effect.

Table 2. Mean results of the neutralization of the hydrolysate with KOH to pH 5 and the concentration of hydrolyzed samples; Values outside the bracket are the mean results and those in brackets are the standard deviations.

% H_2SO_4	Sample ID	Solid: Liquid ratio	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)	Xylitol (g/l)	Glycerol (g/l)	Acetic acid (g/l)
1	B1	1:4	0.43 (0.01)	0.28 (0.00)	3.11 (0.25)	0.00	0.17 (0.03)	0.48 (0.06)
2	C1	1:4	1.03 (0.00)	4.39 (0.05)	5.96 (0.61)	0.00	0.23 (0.02)	1.26 (0.11)
3	D1	1:4	2.12 (0.40)	10.50 (1.89)	6.32 (1.37)	0.00	0.25 (0.01)	1.94 (0.50)
4	E1	1:4	2.56 (0.22)	12.56 (2.14)	6.18 (1.63)	0.20 (0.00)	0.24 (0.00)	2.36 (0.15)
5	F1	1:4	3.00 (0.42)	13.17 (1.76)	6.08 (1.59)	0.24 (0.02)	0.26 (0.01)	2.48 (0.53)
10	G1	1:4	3.80 (0.75)	12.65 (2.82)	5.10 (0.62)	0.28 (0.00)	0.38 (0.04)	3.09 (0.17)
1	B2	1:2	0.77 (0.09)	1.73 (0.60)	1.08 (0.04)	0.00)	0.27 (0.03)	0.26 (0.11)
2	C2	1:2	1.18 (0.32)	3.62 (0.70)	9.86 (1.69)	0.00	0.34 (0.05)	1.81 (0.67)
3	D2	1:2	2.22 (0.40)	11.20 (1.52)	10.86 (1.53)	0.00	0.40 (0.06)	2.61 (0.44)
4	E2	1:2	3.97 (0.15)	18.76 (2.11)	11.03 (1.70)	0.00	0.40 (0.00)	3.40 (0.72)
5	F2	1:2	4.37 (0.20)	21.80 (2.35)	11.12 (0.86)	0.00	0.40 (0.05)	3.83 (0.45)
10	G2	1:2	5.93 (0.38)	22.67 (3.55)	9.86 (1.85)	0.00	0.49 (0.07)	4.34 (0.25)

The results in Table 2 indicate that, in general, while the samples are neutralized with KOH, it is observed that the amount of glucose, xylose, arabinose, xylitol, glycerol, and acetic acid increases following the increase of H_2SO_4 used. For solutions mixed in a 1:4 solid: liquid mixing ratio, changing the pH of autoclaved samples (Table 1) to 5 (Table 2), increased the glucose and xylose concentration by 10% and 12%, respectively in 1% H_2SO_4. For the remaining solutions (2%, 3%, 4%, 5%, and 10% H_2SO_4), the yield of glucose was reduced by 1%, 5.8%, 7.3%, 4.8%, and 8.4%, respectively. Hence, bringing the pH to 5 did not enhance the formation of sugar. Xiang et al (2003) indicated that the disappearance of glucose during acid hydrolysis of lignocellulosic biomass was due to a recombination of glucose with acid-soluble lignin in hydrolysates. The xylose concentration was also reduced. The arabinose yield was found to increase only for solutions treated with 1%, 2%, and 3%, and decreases for the other solutions (4%, 5%, and 10%). Furthermore, for solutions mixed in a 1:2 ratio, changing the pH of autoclaved samples (Table 1) to pH 5 (Table 2), led to an increment of the glucose concentration by 5.5%, 5.4%, and 8.3% for solutions mixed in 1%, 2% and 3% H_2SO_4, respectively. For the remaining solutions (4%, 5%, and 10% H_2SO_4), the yield of glucose concentration was reduced by 4.3%, 2.9%, and 6.3%, respectively.

To explain these results, the comprehensive kinetic model prepared for dilute-acid hydrolysis of cellulose by Xiang et al (2003) indicated that once the crystalline structure of the cellulose is disrupted, acid molecules can penetrate the inner layers of the cellulose chains, and once glucose is formed in the hydrolysate, it interacts with acid-soluble lignin, forming a lignin-carbohydrate complex at further treatment. Moreover, the study by Woldesenbet et al. (2014) on hydrolysis of wet coffee pulp and mucilage using different concentrations of H_2SO_4 (0%, 1%, 2%, 3%, and 4%) reported that the total reducing sugar content increases with an increase in acid concentration up to 3%, and then decreases. Davis et al. (2005) also analyzed the concentration of the release of sugars from wheat stillage cellulose and hemicelluloses using (0–4% (v/v)) of H_2SO_4 and found that sugar recovery from both hemicellulose

and cellulose peaked at 2% H_2SO_4. The study conducted by Urbaneja et al. (1996) on oven-dried coffee pulp from Venezuela using diluted H_2SO_4 (0.5, 1, 1.5, and 2%) also reported that the glucose yield increased with higher acid concentration and the highest yield was obtained at 2.0% H_2SO_4.

In fact, with 10 % H_2SO_4 a better sugar yield was obtained in this result. However, the concentration of acetic acid, which is inhibitory for fermentation, would simultaneously increase with increasing H_2SO_4 volume. Thus, mild conditions of H_2SO_4, i.e., 3 % H_2SO_4, are a better choice. Different studies also support using 3% H_2SO_4. On the other hand, glycerol generation is decreasing in both 1:2 and 1:4 mixing ratios after neutralization. This implies that varying the pH of the hydrolysate could affect the solubilization of sugars and byproducts during the pretreatment. Therefore, to proceed with the investigation under optimum conditions depending on the obtained results, the sample was treated with 3 % (v/v) H_2SO_4 in a solid to liquid ratio of 1:2.

Fermentation of treated coffee pulp with lignocellulosic yeast

Table 3. Mean results of initial concentration of hydrolysates taken and their remaining concentration after fermentation with lignocellulosic yeast. Values outside the bracket are the mean results and those in brackets are the standard deviations.

Conc. (g/l)	Glucose	Xylose	Arabinose	Xylitol	Glycerol	Acetic acid
Initial concentration pretreated (with 3% H_2SO_4) and autoclaved hydrolysate	2.22 (0.40)	11.20 (1.52)	10.86 (1.53)	0.00	0.40 (0.06)	2.61 (0.44)
Concentration of hydrolysate using enzymatic hydrolysis	6.84 (2.87)	11.41 (1.78)	10.92 (0.62)	0.75 (0.16)	0.48 (0.19)	2.62 (0.32)
Remaining residual conc. after optimum fermentation time	0.31 (0.02)	1.89 (1.07)	5.24 (0.78)	0.59 (0.06)	1.67 (0.11)	2.82 (0.46)

Different conditions using cellulolytic enzymes accellerase® TRIO™ for hydrolysis and lignocellulosic yeast GSE16-T18 for fermentation were evaluated to select the optimum conditions. Using this enzyme, the optimal hydrolysis time was found to be 48 h. At this hydrolysis time, the yield of glucose, xylose, arabinose, xylitol, glycerol, and acetic acid in the hydrolysate is as shown in Table 3. The results indicate that the enzyme hydrolysis enhanced the glucose yield at least by a factor of 3 (relative to the glucose concentration from hydrolysate after 3% H_2SO4 treatment). Subsequently, the hydrolysates were fermented for up to 48 h, and samples were taken every 6 h. The optimum ethanol yield (7.03 ± 2.63 g/l) was obtained at 12 h of fermentation. The results are indicated in Fig. 1.

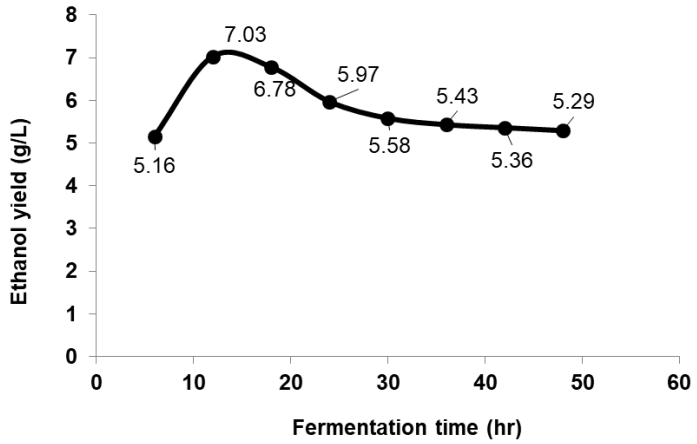

Fig. 1. Variation of ethanol yield with fermentation time using lignocellulosic yeast GSE16-T18 strain (C5 yeast) and enzyme hydrolysis

As shown in Fig. 1, after the optimum ethanol concentration (7.03 ± 2.63 g/l) was obtained, its concentration was found to slightly decrease with time. This might be because the lignocellulosic GSE16-T18 yeasts are shifting to use ethanol as the source of carbon. Another reason might be that ethanol is a poison for yeast. In this regard, Birch and Walker (2000) indicated that ethanol is an inhibitor of yeast growth at relatively low concentrations, inhibiting cell division, decreasing cell volume, and specific growth rate, while high ethanol concentrations reduce cell vitality and increase cell death. Therefore, it is crucial to continuously remove ethanol from the broth. At the end of the optimum fermentation time (12 h), the remaining amount of glucose, xylose, arabinose, xylitol, glycerol, and acetic acid in the fermentation was 0.31 ± 0.00, 1.89 ± 1.07, 5.24 ± 0.78, 0.59 ± 0.06, 1.67 ± 0.11 and 2.82 ± 0.46 g/l, respectively (Table 3). These figures indicate that arabinose was barely consumed by the GSE16-T18 yeast.

These results are in agreement with the earlier studies by Demeke et al. (2013a and b), which confirmed that the yeast GSE16-T18 strain contains a heterologous pathway for arabinose fermentation, but it still has poor functionality. This is the reason why 5.24 ± 0.78 g/l of arabinose is remaining at the optimum fermentation time without being consumed by the yeast (see Table 3). Soares et al. (2016) reported that using the same yeast GSE16-T18, it was possible to obtain a maximum ethanol concentration of 3.73 ± 0.2 % (v/v) from coconut mesocarp husk. This study demonstrates that dried coffee pulp can be used as an alternative substrate for ethanol production, in comparison to different biomass resources as shown in Table 4.

Table 4. Comparison of ethanol yields of various substrates as reported in the literature with the results obtained in the present study

Residue	Ethanol Yield (g/l)	References
Corn stalks (using lime treatment, enzyme hydrolysis, *Saccharomyces* cerevisiae and Pachysolen tannophilus ATCC 32691)	5	Belkacemi et al. (2002)
Barley straw (using lime treatment, enzyme hydrolysis, *Saccharomyces* cerevisiae and Pachysolen tannophilus ATCC 32691)	10	Belkacemi et al. (2002)
Wheat stillage (using 2% H_2SO_4 (v/v), enzyme hydrolysis and *Zymomonas mobilis* ZM4(pZB5))	11	Davis et al. (2005)
Coffee residue waste (using popping pretreatment, enzymatic hydrolysis, *Saccharomyces* cerevisiae)	15.3	Choi et al. (2012)
Sweet sorghum bagasse (using steam explosion pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	16.2	Ballesteros et al. (2004)
Corn Stover (using steam pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	16.8	Öhgren et al. (2007)
Eucalyptus (using steam explosion pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	17	Ballesteros et al. (2004)
Wheat straw (using steam explosion pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	18.1	Ballesteros et al. (2004)
Poplar (using steam explosion pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	19	Ballesteros et al. (2004)
B. carinata residue(using steam explosion pretreatment, enzyme hydrolysis, and *Saccharomyces* cerevisiae)	19	Ballesteros et al. (2004)
Dried coffee pulp hydrolysis with cellulolytic enzymes and fermentation with lignocellulosic yeast GSE16-T18	7.03	This study

Ethanol pervaporation of the fermented waste fraction

Pervaporation of coffee pulp fermentation with lignocellulosic yeast GSE16-T18

The coffee pulp samples were also hydrolyzed with the cellulolytic enzymes and fermented with lignocellulosic yeast GSE16-T18. The fermented broth containing glucose (0.31 ± 0.00), xylose (1.89 ± 1.07), arabinose (5.24 ± 0.77), xylitol (0.59 ± 0.06), glycerol (1.67 ± 0.11), acetic acid (2.82 ± 0.46) and ethanol (7.03 ± 2.63) g/l was filtered and pervaporated at different temperatures (30, 40, 50 and 60 °C). The concentration of xylitol, glycerol and acetic acid in the permeate were 0.01 ± 0.00, 0.01 ± 0.00, and 0.03 ± 0.00 g/l, respectively. The results are shown in Table 5.

Table 5. Permeate levels obtained after pervaporation of coffee pulp hydrolyzed with cellulolytic enzyme and fermented with lignocellulosic yeast GSE16-T18. IC = initial concentration

IC (feed solution) (g/l ethanol)	Pervaporation temperature (°C)	Pervaporation pressure (mbar)	Ethanol concentration (g/l) in the permeate
7.03 ± 2.63	30	16.75	9.10 ± 1.48
	40	12.25	9.45 ± 0.28
	50	10.75	15.47 ± 0.66
	60	10.50	13.04 ± 1.55

The feed ethanol solution concentration was 7.03 ± 2.63 g/l. The maximum concentration of ethanol obtained by pervaporation was 15.47 ± 0.66 g/l and this was obtained with pervaporation of the sample at 50 °C. Hence, it was observed that the bioethanol concentration is more than double after using pervaporation. The pervaporation performance of the membrane is indicated in Fig. 2. In general, the membrane flux and water/ethanol separation factor were found to increase with increasing pervaporation temperature.

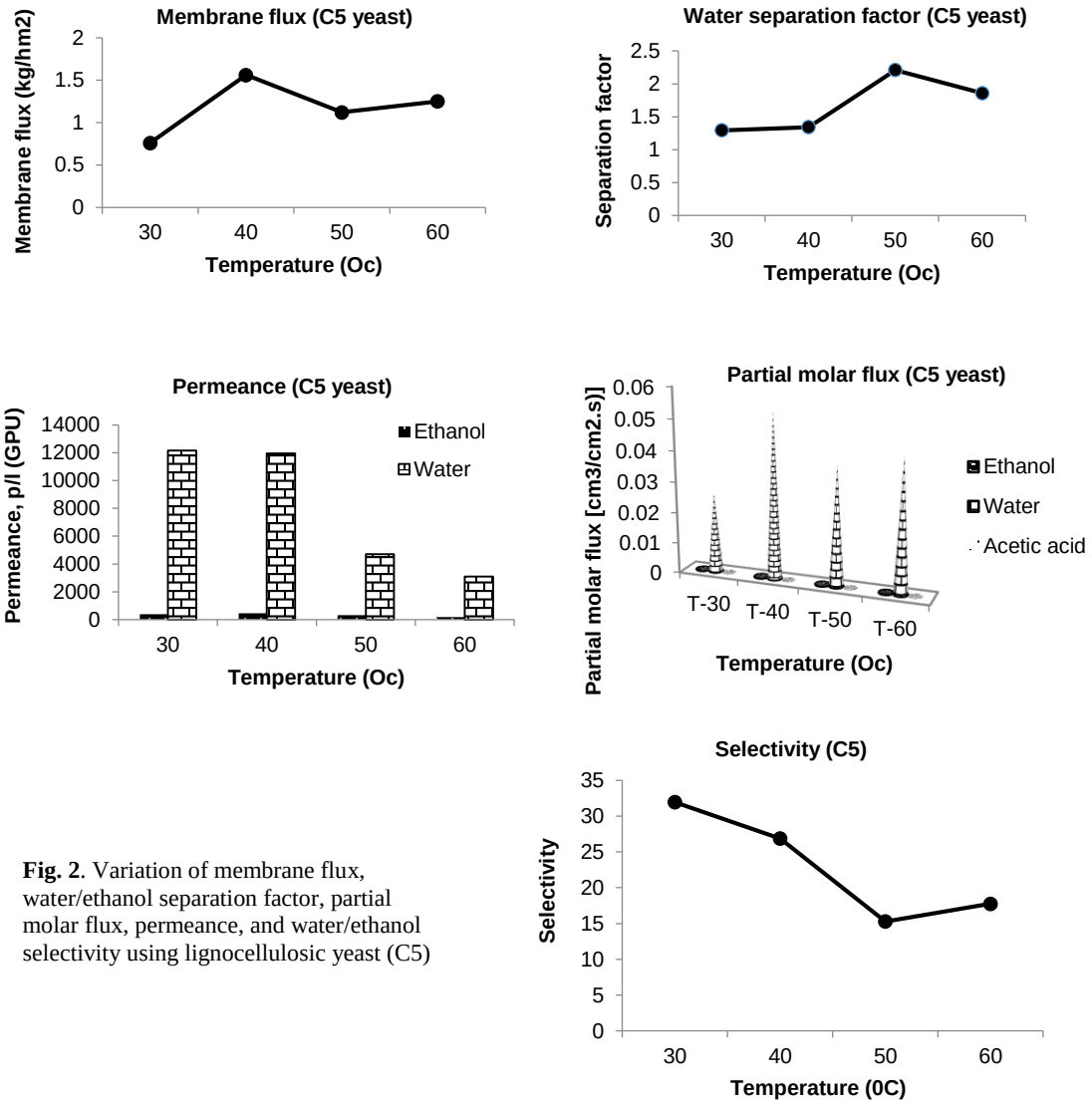

Fig. 2. Variation of membrane flux, water/ethanol separation factor, partial molar flux, permeance, and water/ethanol selectivity using lignocellulosic yeast (C5)

As indicated above, the water separation factor is below 3, and in general, the values were found to increase with increasing pervaporation temperature. The water/ethanol selectivity is calculated to be greater than 15. Besides, the partial molar flux and permeance of water are higher than for ethanol. The study conducted by Shenoy et al (2011) on dried coffee pulp reported that the theoretical ethanol yield that could be obtained from the dried coffee pulp is 9.35%. Similarly, Choi et al (2012) performed enzymatic hydrolysis (after simultaneous saccharification and fermentation) obtaining an ethanol concentration of 15.3 g/l from coffee residue waste. In the study of Davis et al. (2005) on wheat stillage

hydrolysate fermentation supplemented with glucose 10 g/l by recombinant *Zymomonas mobilis* ZM4(pZB5), the possibility of producing 11 g/l ethanol was reported. Although the manufacturer indicates that the membrane is alcohol selective, the results show the difficulty to reach a high concentration of ethanol (since the ethanol/water separation factor and permeance of the membrane were too low, while the ethanol selectivity is even below 1, indicating the ability of separation of water as indicated above in Fig. 2 with polyvinyl alcohol type M1 pervaporation membrane).

CONCLUSION

Coffee pulp is discharged into water bodies and soil near wet coffee processing industries, which causes serious environmental and health concerns. To address this problem, the potential of dried coffee pulp for bioethanol production using the lignocellulosic yeast GSE16-T18 for fermentation and its purification by pervaporation was investigated. Even though the yield of fermentable sugar is increasing as the amount of diluted sulfuric acid is increasing, this increment of acid (diluted sulfuric acid) also increases the concentration of unwanted byproducts such as acetic acid, xylitol, and glycerol. Thus, to obtain an optimum reducing sugar concentration, the final experiment was conducted only with 3% sulfuric acid. The optimization study indicates that 48 h enzyme hydrolysis and 12 h fermentation of the samples with lignocellulosic yeast GSE16-T18 strain is the best fermentation times with better ethanol titer (7.03 ± 2.63 g/l). Pervaporation of the fermented feed concentrated the ethanol to 15.47 ± 0.66 g/l. This indicates that hydrophobic pervaporation is not sufficient to adequately purify ethanol. This is because at the start, the concentration of ethanol in the feed solution is low and the membrane used should be ethanol selective; such membranes have low selectivity. Alternatives such as combinations of hydrophobic pervaporation - hydrophilic pervaporation, and then as a third option, distillation (as in a classical process) followed by pervaporation dehydration should be considered, which yields a sufficient purity of ethanol. The hydrolysis results indicate that the hydrolysate is composed of more xylose and arabinose than glucose. Therefore, to produce more ethanol, future studies should focus on strains that can utilize arabinose effectively.

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