Comparison of Conventional and Novel Pre-treatment Methods for Bioethanol Production from Fruit and Vegetable Wastes

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T. Keskin-Gundogdu*
Faculty of Engineering, Bioengineering Department,
Ege University, Izmir, Turkey

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In this study, novel and conventional techniques for the production of bioethanol from fruit and vegetable wastes (FVWs) by yeast and bacterial fermentation were investigated experimentally. Different pretreatment techniques (acid, heat, acid/heat, and microwave) for yeast fermentation were compared. Maximum ethanol concentrations of 11.7 and 11.8 g L^{-1} were observed from acid/heat and microwave pretreatment, respectively, by using *Saccharomyces cerevisiae*. On the other hand, biochar production from FVWs and syngas fermentation from the waste gas of this process were integrated. From waste gas with 12 % CO content, 5.5 g L^{-1} and 2.5 g L^{-1} ethanol production was observed by using anaerobic mixed culture and *Clostridium ljungdahlii*, respectively. The overall results emphasize the potential of bioethanol production from FVWs by economically feasible and environmentally friendly methods.

Keywords:
bioethanol, fruit and vegetable wastes, pretreatment, syngas fermentation, yeast fermentation

Introduction

The use of renewable energy sources has gained importance because of the continuous increase in energy needs worldwide due to the constant increase in the global population and industrial activities[1]. The main sources of global warming are not only due to human activities but also the depletion of fossil fuel reserves[2]. Biofuels are the most attractive renewable energy sources. Since it is the most commonly used biofuel, bioethanol is gaining more attention among different energy sources[3]. To compete with rising fuel prices, it is very important to reduce the production costs of biofuels[4]. Bioethanol can be produced from three different types of organic sources, and 60 % of fuel ethanol is produced via fermentation processes. Since it can reduce the negative environmental impacts of nonrenewable fuel, bioethanol is a very clean alternative. The use of ethanol started as a replacement for gasoline as E15; the number indicates the percentage of ethanol. In addition, bioethanol can be used in the plastic, beverage, and pharmaceutical industries[5]. The first group is sugar or molasses, which can be converted to bioethanol without pretreatment by microbial fermentation. This technology is easy to apply but the cost of substrate depends on the sugar-producing capacity of countries, which is very limited. The second group comprises starch-based sources such as corn, wheat, and cassava. However, these substrates have a disadvantage of being food sources. The bioethanol production from these sources is called the first generation ethanol production[6]. To overcome these disadvantages, the second generation of bioethanol has gained more attention. Bioethanol can be produced from the fermentation of lignocellulosic biomass with proper pretreatment. Lignocellulosic biomass can include residues of agricultural activities, tree crops or any type of plant-based organic waste. Lignocellulosic biomass is the most promising feedstock because of its low cost, abundance, and availability[7]. However, this biomass has a complex structure that is composed of cellulose, hemicellulose, and lignin. For this reason, the fermentation process needs pretreatment to expose the fermentable sugar[8].

The recalcitrance of lignocellulosic biomass should be reduced to improve ethanol production yields. Ethanol production from lignocellulosic biomass is the combination of hydrolysis, fermentation, separation, and recovery steps. The most problematic step is the hydrolysis step because of the difficulties of revealing fermentable monosaccharide. For this reason, different pretreatment operations have been applied to achieve better yields[9]. Pretreatment methods could be biological, chemical, physical, or physicochemical, etc.

*Corresponding author: tugba.keskin.gundogdu@ege.edu.tr

Biological pretreatment methods are based on using microorganisms, such as brown, white, soft rot fungi to degrade hemicelluloses and lignin. The best degradation yields are found to be by white/rot fungi[10]. Biological pretreatment has the advantages of low energy input, low toxicity and mild environmental conditions, but the economic cost and low yields are the main drawbacks.

The most commonly used physical pretreatment methods are mechanical comminution and extrusion[11]. Mechanical methods have the disadvantage of being energy-intensive. Extrusion is known as a novel and promising technology, but it still requires optimization of the process parameters. Chemical treatment methods include alkali, acids, and organosolv treatment. Alkali treatment performed using NaOH and $Ca(OH)_2$ is known to be the most effective method. A total of 74 % saccharification yields can be achieved by acid pretreatment[12], and acid treatment can be combined with heat treatment for higher yields[13]. Since the use of lignocellulosic biomass is critical for higher yields and is environmentally friendly, studies on bioethanol production methods with different pretreatments are ongoing, such as ultrasound pretreatment, CO_2 explosion, ozonation, and ammonia fiber explosion[9].

Heat pretreatment or steam explosion is the most commonly used method for lignocellulosic biomass. Microwave pretreatment and is one of the best alternatives to heat pretreatment because of lower reaction times for higher yields. Microwaves produce an electromagnetic field that will contact the product directly[14]. Microwave pretreatment could decrease the reaction time for revealing cellulose and lignin[15].

One of the novel approaches for bioethanol production is syngas fermentation, which combines pyrolysis for biochar production and bioethanol production by anaerobic bacteria[16]. Syngas fermentation is a process involving active acetogenesis by microorganisms. These bacteria have the ability to convert CO, CO_2 or bioethanol by the Wood-Ljungdahl pathway.

The stoichiometry of conversion from CO and CO_2 to ethanol is given by[17]:

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$$

$$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O$$

The stoichiometry of conversion from CO and CO_2 to acetate is given by[17]:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

The stoichiometry of conversion during pyrolysis is[18]:

$$C_xH_yO_z \rightarrow CO + H_2 + C + C_mH_n + CO_2 + \text{others}$$

Clostridium ljungdahlii, *Clostridium ragsdalei, Clostridium carboxydivorans* and *Clostridium autoethanogenum* are the most studied acetogenic types for bioethanol production. Syngas fermentation is a very advantageous and promising technology because it can be used to reduce air pollution during bioethanol production[19].

Fruit and vegetable wastes (FVWs) are very important biomass sources for municipalities. They are a combination of proteins, carbohydrates, and complex polysaccharides, and can be a very good source for any type of biofuel. Bioethanol production from a single type of fruit has been performed in different studies. This study aimed to produce bioethanol from FVWs. Several different methods were compared to detect the best yields of ethanol production. Bioethanol production by first-generation and second-generation methods were compared. Different pretreatment methods, such as heat pretreatment, acid pretreatment, acid and heat pretreatment, microwave pretreatment, were combined with yeast fermentation and syngas fermentation after pyrolysis of FVWs, and biochar production by *Clostridium ljungdahlii* and anaerobic mixed culture bacteria were compared for bioethanol production. Many different methods for bioethanol production from FVWs were compared for higher yields.

Materials and methods

Selection of the best yeast culture

Three different yeast cultures were collected from different sources: Wet bakery yeast (Pakmaya, Turkey), dried bakery yeast (Yuvam, Turkey), and beer yeast (Butikbira, Turkey). The three yeast cultures used in this study were *Saccharomyces cerevisiae* in different forms. Since glucose was a reference compound in the phenol-sulfuric acid method used in the determination of total sugar assay, it was decided to use it as a carbon source in pre-culture for selection of the most suitable culture. Firstly, the yeasts were grown in 500-mL Erlenmeyer flasks with 250 mL working volume containing pre-culture medium to determine the fast-growing culture. The composition of growth medium was: 40 g L^{-1} glucose, 10 g L^{-1} yeast extract, 5 g L^{-1} $(NH_4)_2SO_4$, 2 g L^{-1} KH_2PO_4, and 1 g L^{-1} $MgSO_4 \cdot 7H_2O$. The yeasts were inoculated as 10 %. The Erlenmeyer flasks were kept at 30 °C shaking incubator at 120 rpm for 16 h. The OD_{600} values were measured to determine the growth rates to select the best yeast source[20].

All yeasts from growth medium were inoculated to pre-culture medium (40 g L^{-1} glucose, 2.5 g L^{-1} $(NH_4)_2SO_4$, 0.5 g L^{-1} KH_2PO_4 and 0.65 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.65 g L^{-1} $ZnSO_4$)[21] in 500 mL Erlenmayer flasks with 250 mL working volume and kept at 30 °C shaker at 150 rpm. After 16 h, the best yeast (wet *Saccharomyces cerevisiae*) was transferred from pre-culture medium to fermentation mediums with different pretreatments, which are explained in detail in the following sections.

Fruit and vegetable wastes (FVWs)

Fruit and vegetable wastes were collected from a local grocery in Izmir, Turkey. The composition of the wastes by weight was as follows; 7 % potato, 3 % onion, 4 % eggplant, 5 % red apple, 1 % pepper, 2 % cucumber, 4 % orange, 2 % pear, 2 % rucola, 5 % tomato, 2 % bean, 5 % purslane, 2 % green apple, 3 % zucchini, 3 % carrot, 1 % cherry tomato, 33 % watermelon, 1 % strawberry, 1 % mandarin, 2 % banana, 4 % lettuce. The wastes were cut into small pieces with a chopper. The characteristics of the waste were: 10 % dry matter, pH 5, chemical oxygen demand 9500 mg L^{-1}, ammonia 3900 mg L^{-1}. The elemental content of the FVWs was (wt%): 30.3 fixed carbon; 46.70 C, 5.15 H, 2.15 N, 0.16 S, 37.76 O[22].

Bottle batch experiments for yeast fermentation

Schott bottles of 250 mL with 100 mL working volume were used for yeast fermentation. The volume comprised of 50 mL of pretreated FVWs (10 g FVWs), 40 mL of sterilized fermentation medium, and an added 10 mL of yeast. The Schott bottles were kept closed with O-rings. The Schott bottle cap had two outlets. Liquid samples were taken by tubing, which was lowered from one of the bottle outlets. The other outlet was capped with a sterile filter that was left open to air to prevent negative pressure during sampling.

Pretreatment operations

Two Schott bottles were prepared for acid pretreatment. FVWs were immersed into 50 mL of 3 % H_2SO_4 solution for acid pretreatment for 24 h. After 24 h, fermentation medium and yeast were added to pretreated medium. Acid/heat pretreatment was performed in another two Schott bottles. 3 % H_2SO_4 and 50 mL water were added to 10 g FVWs, heat pretreated at 121 °C for 10 minutes. Heat pretreatment was applied to the contents of two serum bottles filled with 50 mL water and 10 g FVWs, using same treatment temperature and time as before. Microwave pretreatment was applied to samples prepared by adding 50 mL water to 10 g FVWs. Microwave pretreatment was performed by CEM MARS 6 reaction system using suitable organic waste program, as follows: 10 °C min^{-1} heating rate up to 200 °C, 5 min holding time, and 10 °C min^{-1} cooling rate. The total duration of the program was 45 min at 400 W power. After MW pretreatment, fermentation medium and yeast were added to the Schott bottles.

Pyrolysis

Pyrolysis experiments were performed in a laboratory fixed bed pyrolysis reactor (V = 1 L). The reactor loaded with about 50 g of dried fruit and vegetable waste was placed in a vertical furnace. After the reactor outlet was connected to water-ice-traps, the reactor was heated to 400 °C at a heating rate of 10 °C min^{-1} and maintained at this temperature for 1 hour. During the pyrolysis experiments, nitrogen gas was passed through the system until the temperature reached 200 °C in order to remove oxygen from the environment. After 200 °C, the nitrogen gas stream was cut off, and pyrolysis was carried out in the gas medium that was produced by the reaction itself. Volatile decomposition products formed during pyrolysis were passed through ice-water-cooled traps, and the liquid product was collected. The gases to be used for ethanol production were collected in a Tedlar bag from the moment the nitrogen gas stream was cut off. At the end of pyrolysis, the percentage of pyrolysis solid residue (biochar) and liquid product accumulated in the traps were calculated[23].

Bioreactors for serum bottle-syngas fermentation

Syngas fermentation with pure culture

Clostridium ljungdahlii DSM 13528 was supplied from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany), DSMZ medium 879 was used as suggested from supplier. The composition of the basal medium was; 1 g L^{-1} NH_4Cl, 0.1 g L^{-1} KCl, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.8 g L^{-1} NaCl, 0.1 g L^{-1} KH_2PO_4, 0.02 g L^{-1} $CaCl_2 \cdot 2H_2O$, 1 g L^{-1} yeast extract, 10 mL trace element solution (Medium 141), 0.5 mL Na-resazurin solution (0.1 %w/v), 1 g L^{-1} $NaHCO_3$, 5 g L^{-1} D-fructose, 10 mL vitamin solution, 0.3 g L^{-1} L-cysteine-HCl·H_2O, 0.3 g L^{-1} $Na_2S_9H_2O$. The composition of medium 141 was 1.5 g L^{-1} nitriloacetic acid, 3 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1} $MnSO_4 \cdot 4H_2O$, 1 g L^{-1} NaCl, 0.1 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.18 g L^{-1} $CoSO_4 \cdot 7H_2O$, 0.2 g L^{-1} $KAl(SO_4)_2 \cdot 12H_2O$, 0.01 g L^{-1} H_3BO_3, 0.01 g L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.01 g L^{-1} $NiCl_2 \cdot 6H_2O$, 0.3 g L^{-1} $Na_2SeO_3 \cdot 5H_2O$, 0.4 g L^{-1} $Na_2WO_4 \cdot 2H_2O$, and the vitamin solution composition was 2 mg L^{-1} biotin, 2 mg L^{-1} folic acid, 10 mg L^{-1} pyrodoxine·HCl, 5 mg L^{-1} thiamine·HCl, 5 mg L^{-1} riboflavin, 5 mg L^{-1} nicotinic acid, 5 mg L^{-1}

D-Ca-panthothenate, 0.1 mg L^{-1} vitamin B_{12}, 5 mg L^{-1} para-aminobenzoic acid and 5 mg L^{-1} lipoic acid. 100 mL serum bottles with 50 mL working volume were used in batch mode. The serum bottles were closed with rubber stoppers and aluminum seals. The fermentation medium was sterilized with autoclave (Hirayama 110 L) at 121 °C for 20 min. Serum bottle medium was inoculated (5 %) with *Clostridium ljungdahlii*, and the headspace was washed out with N_2 to maintain the anaerobic conditions. After 24 h when the culture reached the exponential phase, 10 mL pyrolysis waste gas was injected into the headspace. The serum bottle experiments were prepared as duplicates. A 1-mL sample was removed from medium daily to measure OD_{600} nm value and ethanol analysis.

Syngas fermentation with mixed culture

For syngas fermentation, anaerobic mixed culture was used. The culture was taken from a local energy company's anaerobic reactor and pre-treated at 105 °C for 5 min to remove the methanogens and dominant *Clostridium* types. One-hundred-mL dark serum bottles with 50 mL working volume were used with same basal medium as that used for pure culture. After closing the serum bottles with rubber stopper and aluminum seal, the headspace was washed out with pure N_2 to supply anaerobic conditions. Ten mL of pyrolysis gas was then injected into the headspace for syngas fermentation.

Analytical methods

The growth rates of *Saccharomyces cerevisiae* and *Clostridium ljungdahlii* were measured at 600 nm with a UV spectrophotometer (ThermoScience, Germany). A 1-mL sample was taken from each bottle and, after measuring OD_{600} values, pH values were also checked by a pH meter (Sartorius, Germany). Ethanol, butanol, and volatile fatty acid concentrations in the bottles were measured by gas chromatography (GC) (6890N Agilent Technologies Network GC System) using flame ionization detector (FID) and HP-FFAP 30 m, and 0.25 mm capillary column (Thermoscience). The metabolites detected were alcohols: ethanol, butanol, and volatile fatty acids: acetic, propionic, butyric, isobutyric, isovaleric, valeric, caprionic, isocaprionic, and heptanoic. The method was described previously in our studies[24]. Samples were taken once a week in a volume of 1.5 mL to determine the amount of ethanol by gas chromatography. After the samples were centrifuged at 10000 rpm for 15 minutes, the supernatant was filtered through a 0.22 μm syringe filter and transferred to clean vials.

The quantitative analysis of pyrolysis gases was carried out with the Refinery Gas Analyzer (RGA) with Agilent 7890B model gas chromatography. The RGA system consisted of 5 valves, 7 columns, and 3 detectors. In the flame ionization detector (FID), using He as the reference gas, hydrocarbons from C1 to C5 were analyzed. In the first thermal conductivity detector (TCD_1) with He as reference gas, CO_2, CO, O_2, and N_2 in the gas product were analyzed. In the second thermal conductivity detector (TCD_2), with N_2 as reference gas, only H_2 was detected[23]. Total sugar values were measured according to phenol-sulfuric acid method[25].

Calculation of the process parameters of yeast fermentation

The batch process parameters were calculated at the end of the fermentation, as follows[21].

The volumetric productivity of ethanol: Q_p (g L^{-1} h^{-1})

$$Q_p = \frac{\gamma_{EtOH}}{t} \quad (1)$$

γ_{EtOH}: ethanol concentration (g L^{-1})
t: time (h).

Process yield of ethanol: $Y_{P/S}$ (g g^{-1})

$$Y_{P/S} = \frac{\gamma_{EtOH}}{(S_i - S_f)} \quad (2)$$

S_i and S_f: Initial and final sugar concentrations (g L^{-1}). Theoretical value of $Y_{P/S}$ is Y_{TH} (0.51 g g^{-1}).

Process efficiency: η (%)

$$\eta = \frac{Y_{P/S}}{Y_{TH}} \cdot 100 \quad (3)$$

Results and discussion

Selection of best yeast culture

The best yeast culture to be used in the study was chosen from three different sources. All three yeasts were *Saccharomyces cerevisiae* from different industries: wet bread yeast, dried bread yeast, and dried beer yeast. These yeast cultures were selected by analyzing growth curves (Fig. 1).

For the dried bread yeast, growth started after 60 hours and reached the highest density on 80th hour. For the dried beer yeast, growth was observed between 20 and 60 hours, but then the density decreased. The wet bread yeast started to grow rapidly after 24 hours and reached a maximum density. The dried beer yeast was obtained from a commercial brewing brand. The delayed growth and activation of this strain was due to the inappropriate medium composition used in the study. The growth of the dry bread yeast was slow and continuous. It was understood that the density of dry bread yeast would

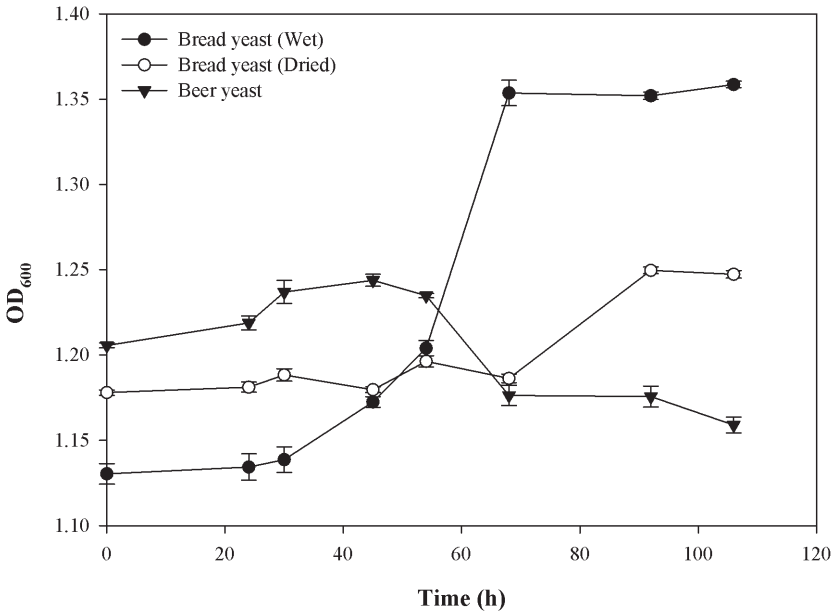

Fig. 1 – *OD_{600} values of different yeast types*

increase gradually and steadily with time. Since time is one of the most important factors in terms of process efficiency, it was decided to use wet bread yeast in this study.

Ethanol production by yeast fermentation

FVWs are high in lignocellulosic content from the vegetables and fruits. In these wastes, fructose from fruits can be used directly in ethanol fermentation, or ethanol yields can be increased by releasing the sugars in the lignocellulosic part by applying a suitable pretreatment. In this study, ethanol yields obtained from heat pretreatment, acid/heat pretreatment, microwave pretreatment, and acid pretreatment applications in yeast fermentation were compared with ethanol yields obtained from FVWs in their raw form. A concentration of 4.5 g L^{-1} ethanol was obtained by direct fermentation of FVWs, while the amount of ethanol produced was increased as a result of the pretreatment applications. The ethanol production after acid pretreatment and heat pretreatment was 5.4 g L^{-1} and 7.5 g L^{-1}, respectively, and the combination of these two methods resulted in 11.7 g L^{-1} ethanol production, while the microwave pretreatment produced 11.8 g L^{-1} ethanol. Most of the ethanol production in all bottle batch experiments was completed within the first 50 hours. After 50 hours, small amounts of daily production were observed. It was observed that acid pretreatment had the lowest efficiency, and heat pretreatment was better than acid pretreatment, but it is better to apply these two methods together. When all pretreatments were compared, the combination of acid and heat pretreatment and microwave pretreatment resulted in very close production values (Fig. 2).

The sugar content of the FVWs was measured and expressed as the total sugar concentration. The amount of sugar in the FVW was approximately 12000 mg L^{-1} in its raw state. Based on the 0 hour values, the pretreatment increased the amount of sugar released. The highest sugar content of 25000 mg L^{-1} was obtained by acid and heat pretreatment. The amount of sugar after microwave pretreatment and acid pretreatment was 22000 mg L^{-1} and 16000 mg L^{-1}, respectively. A rapid decrease in the total sugar concentration was observed with all pretreatments during the first 50 hours, while more gradual decrease was observed between 100 and 300 hours. While 10000 mg L^{-1} residual sugar remained after acid and heat pretreatment, the residual sugar remaining after microwave and acid/heat pretreatment was 5000 mg L^{-1}. The sugar consumption was consistent with ethanol production. The data on the residual sugar concentration indicated the end of *Saccharomyces cerevisiae* activity. This problem can be overcome by continuous reactor systems, and all experiments here presented were performed with batch reactors. The main disadvantage of yeast fermentation is that there is still residual sugar and lignocellulosic waste to be digested at the end of the process (Fig. 3).

Table 1 shows the process parameters of ethanol production by yeast fermentation. According to the process parameters for yeast fermentation, the volumetric productivity of ethanol changed between 0.01 and 0.035 g L^{-1} h^{-1}. The maximum productivity values were obtained with acid/heat treatment and microwave treatment. The lowest productivity was observed with acid treatment. The maximum ethanol yield values were 0.471 and 0.463 g g^{-1} for

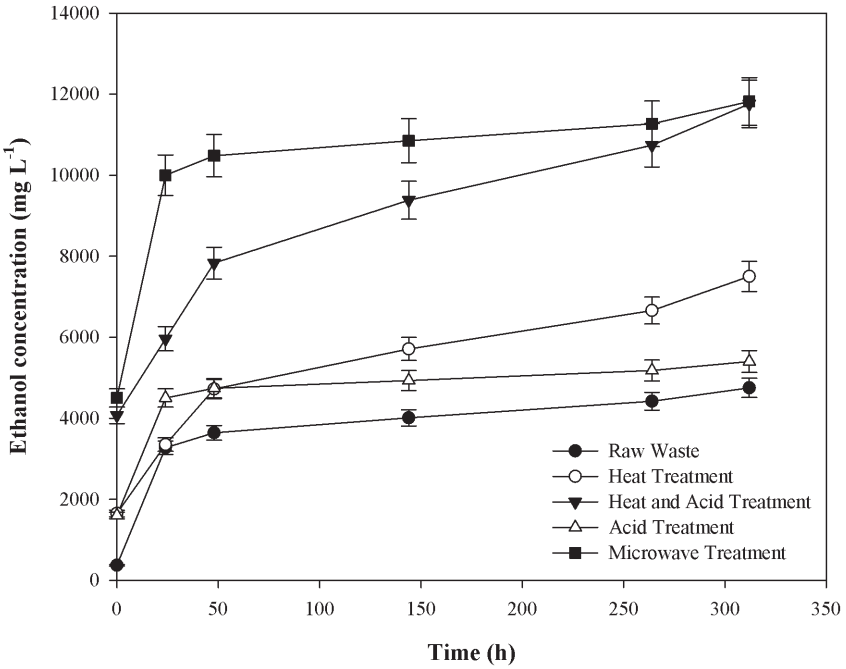

Fig. 2 – *Ethanol production by yeast fermentation from FVWs*

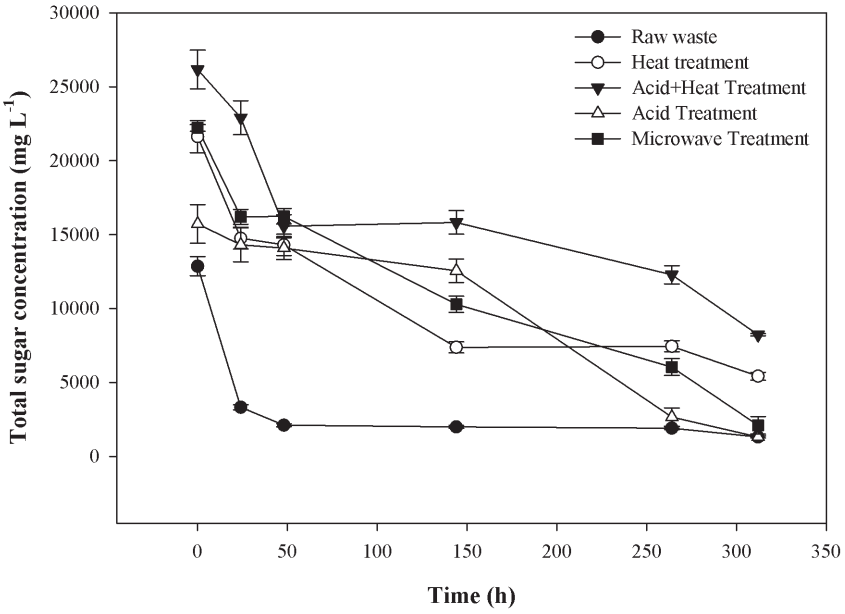

Fig. 3 – *Total sugar consumption during yeast fermentation*

the microwave and heat treatments, respectively. The ethanol yield depends on the consumption of sugar; however, a higher ethanol yield was obtained by a lower amount of sugar consumption as a result of heat treatment. The reason for this situation is the accessible solid content of the biomass. One of the main goals of pretreatment is to increase the amount of fermentable sugars but also promote the digestibility of the solid material[26]. The other factors limiting bioethanol production yield are the degree of polymerization, available surface area, and moisture content of the pretreated waste[27]. Therefore, the ac-

Table 1 – *Process parameters for yeast fermentation*

Pre-treatment	Q_p (g L^{-1} h^{-1})	$Y_{P/S}$ (g g^{-1})	η (%)
Heat treatment	0.022	0.463	90.80
Acid+Heat treatment	0.035	0.421	82.55
Acid treatment	0.016	0.375	73.53
Microwave treatment	0.0352	0.471	92.33
Raw waste	0.0141	0.243	47.65

tivity of yeast can also depend on the digestibility of the waste. In general, pretreatment operations resulted in higher ethanol yields in comparison with yeast fermentation from raw FVWs. The process efficiency values had a similar trend as the ethanol yields. Pretreatment operations increased the efficiency values from 47.65 % to at least 73.53 % with a maximum at 92.33 %.

Ethanol production by syngas fermentation

Syngas fermentation can be conducted with different types of sugars or C gases. Tanner *et al.*[28] studied the effect of different C sources on the growth of *Clostridium ljungdahlii* after its isolation from a chicken yard, and reported that 1 mmol fructose can be converted to 2.44 mol acetic acid with production of no other metabolites. In this study, the only C source in basal medium was fructose, so the ethanol concentration could not have been affected by C originating from sugar; thus, the ethanol produced came only from CO or CO_2 according to Wood-Ljungdahl pathway. Therefore, it can be understood that fructose can affect the growth of *Clostridium ljungdahlii* but its contribution to ethanol production is very limited.

The pyrolysis gas was collected in a Tedlar bag for syngas fermentation. A total of 10 mL gas mixture was injected into serum bottles with *Clostridium ljungdahlii,* and into the serum bottles filled with mixed culture. The first 24 hours of *Clostridium ljungdahlii* growth was the lag phase of production, and ethanol production started after 24 hours (Fig. 4).

The activities of pure culture and mixed culture in ethanol production by syngas fermentation were compared (Fig. 5). *Clostridium ljungdahlii*, which has been widely used in the literature[29], was used as a culture for ethanol production by pure culture in serum bottles. The mixed culture was obtained from an anaerobic reactor. A heat-pretreated sludge was used, since it was shown in our previous studies that various *Clostridium* species are activated by thermal pretreatment, and that many methanogens were suppressed[30]. A different performance was observed in mixed culture used for ethanol production from pyrolysis process waste gas in comparison to the pure culture experiment. Ethanol production started rapidly in mixed culture serum bottles, while production started in pure culture serum bottles only after 24 hours. Production in both mixed culture and pure culture serum bottles showed a rapid increase up to 72 hours, after which it slowed down. Ethanol production of up to 6 g L^{-1} was observed with mixed culture, while ethanol production of up to 2.5 g L^{-1} was observed in serum bottles using pure *Clostridium ljungdahlii*. These values obtained with 12 % CO will further increase with the use of pure CO or higher concentrations of CO. While the use of mixed culture in the production of hydrogen by biogas and dark fermentation is quite common in the literature, studies on its use in syngas fermentation are limited[31].

During syngas fermentation by the Wood-Ljungdahl pathway, another important parameter is acetate production, which shows further capacity for ethanol production in the process. Fig. 6 shows the acetic acid concentrations of the batch fermentation

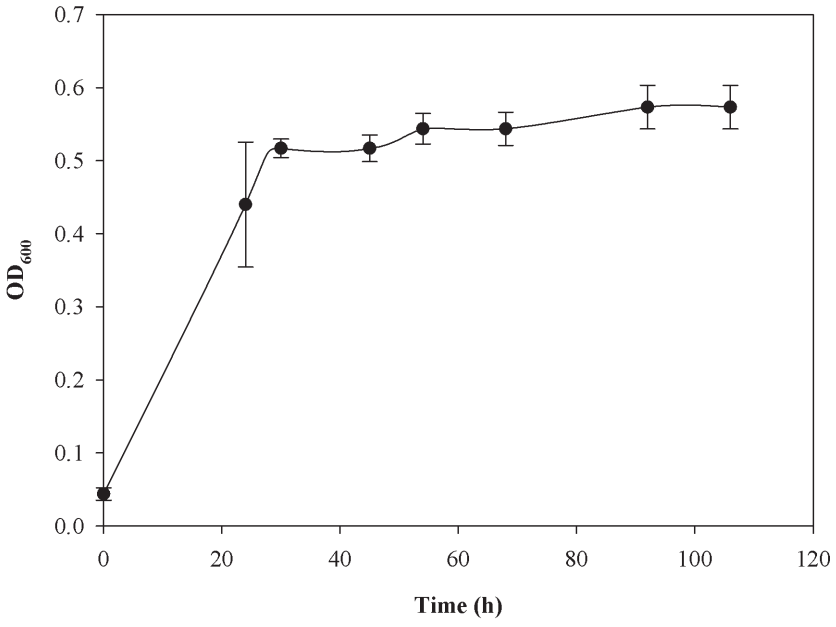

Fig. 4 – *OD_{600} values of Clostridium ljungdahlii*

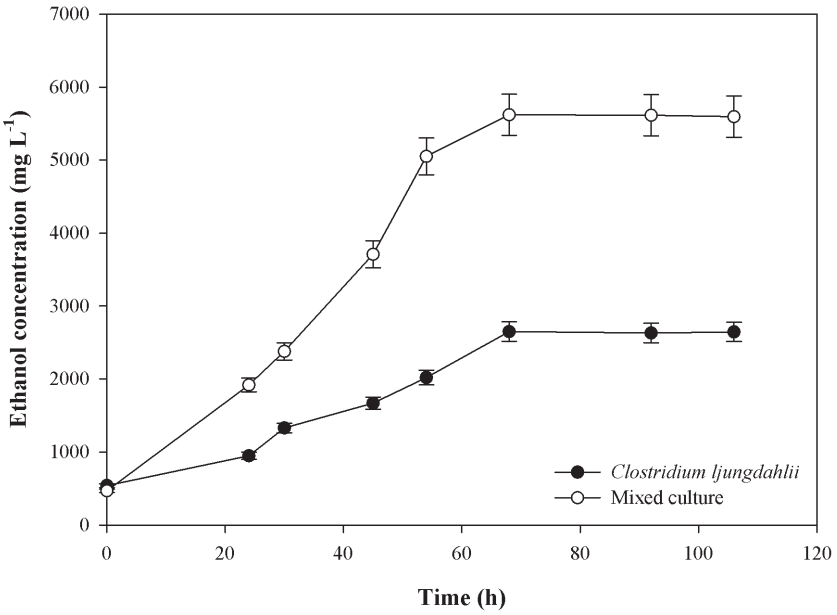

Fig. 5 – *Ethanol production by syngas fermentation from FVWs*

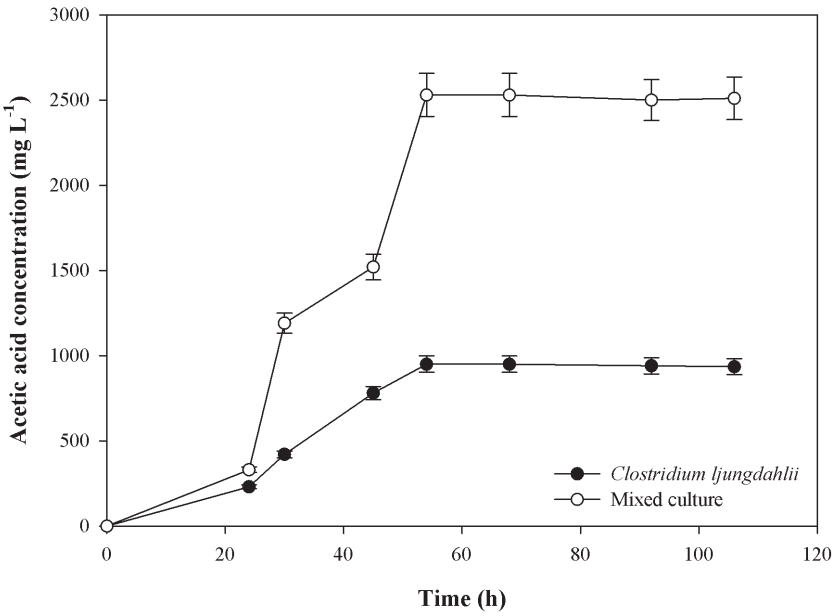

Fig. 6 – *Acetate production during syngas fermentation*

processes. The acetate values increased up to 2.5 g L^{-1} with mixed culture, and up to 1 g L^{-1} with *Clostridium ljungdahlii*. The increase in acetate was related to the increase in ethanol production (Fig. 6).

Acetone-butanol-ethanol (ABE) values of all processes

In recent years, in addition to ethanol production in alcohol fermentation, production of butanol with higher calorific value has started to gain importance. In addition to the use of these solvents as fuel, acetone production is very important, because it can be used as an important raw material, especially in the plastics industry. In this study, the production values of acetone and butanol were investigated in addition to ethanol production (Fig. 7). In terms of ethanol production from FVWs by yeast fermentation, the highest values were obtained by heat/acid pretreatment and microwave pretreatment processes. The production of acetone and butanol as byproducts in yeast fermentation is also quite common. In this study, the acetone values varied between 200–1000 mg L^{-1}, and the butanol values varied between 100–600 mg L^{-1}. When the ABE

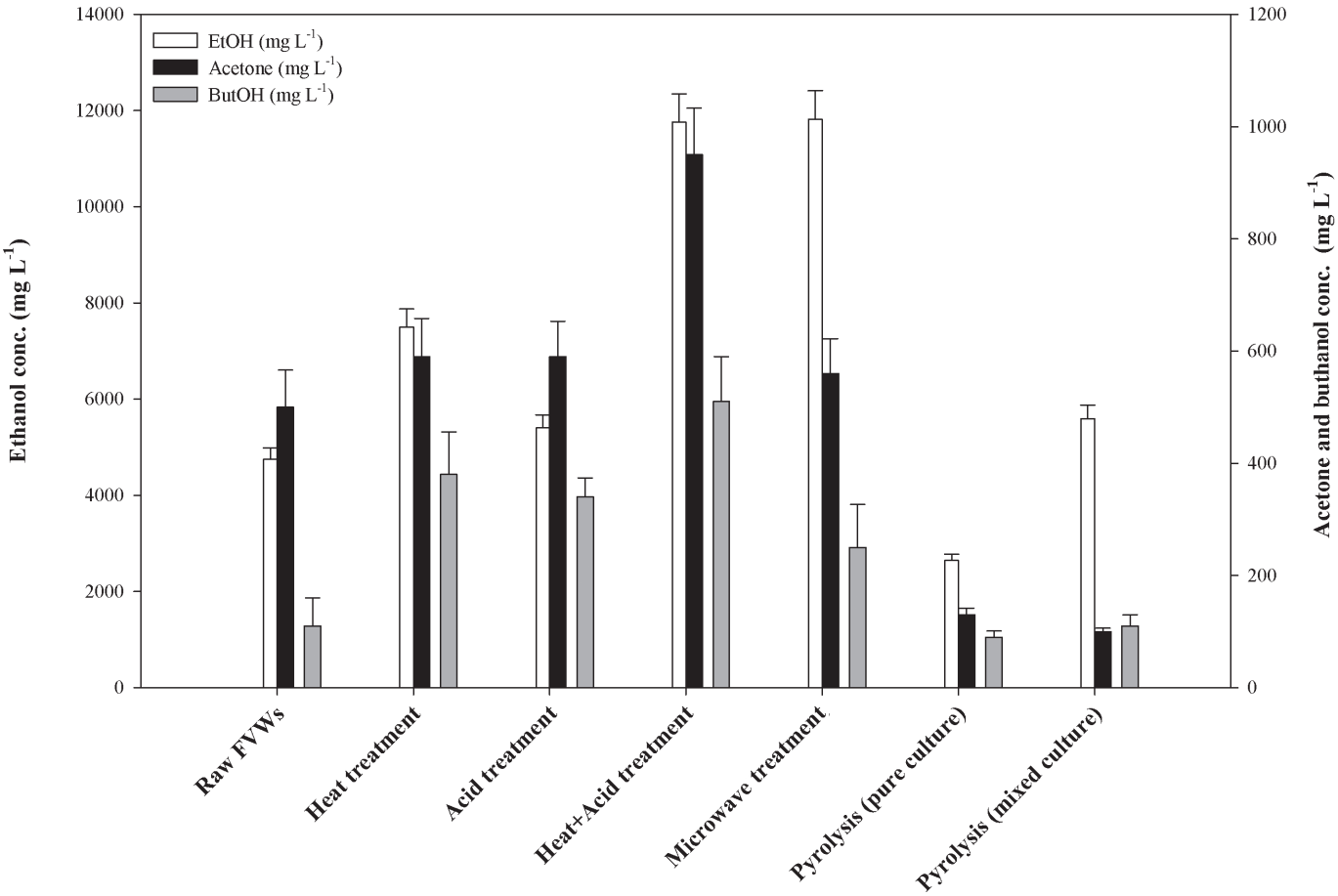

Fig. 7 – *Acetone-buthanol-ethanol values of all processes*

fermentation products were examined together, the highest values were obtained by the microwave pretreatment process.

In general, the ethanol production values were higher in yeast fermentation than syngas fermentation. When the different pretreatments for yeast fermentation were compared, the most suitable pretreatment were heat/acid pretreatment and microwave pretreatment. Although the same yields were provided, microwave pretreatment was preferred. However, the disadvantage of microwave pretreatment are the investment costs to be encountered in scaling up. Since the final residues in ethanol production by the yeast fermentation will still contain some organic content, it will be possible to use the final product for soil enrichment, especially in pretreatments that do not include acid. Ethanol production efficiency was low in syngas fermentation, but the main advantage of this process is the complete treatment of biomass and production of biochar. It is common knowledge that pyrolysis produces three different products: mainly bio-oil, biochar, and gas; therefore – for this integrated approach to ethanol production with FVWs – the final waste product will be bio-oil only[23]. Therefore, syngas fermentation would be more advantageous in terms of process costs. In comparison with the other methods in the syngas fermentation experiments, it can be said that using mixed culture is very advantageous both in terms of high yields and in terms of costs, because no sterilization is required.

Comparison of all processes

The increasing population and needs of people are directly parallel with large amounts of FVWs, and these wastes are one of the greatest problems for municipalities, especially for growing countries. They could be used as cheap sources for renewable energy production. Bioethanol is one of the most common alternatives for biofuel production, such as biogas production. The high amounts of organic matter, especially carbohydrates, in FVWs make them very attractive for bioethanol production. Yeast fermentation using *Saccharomyces cerevisiae* needs to be improved with some pretreatment operations in order to use all the carbohydrate content of FVWs. In this study, different pretreatment methods were used to increase the bioethanol production yields.

Kitchen waste is another major problem for environmental management of municipalities[32]. Kitchen waste is a combination of FVWs and proteins, rice, pasta, etc., and 29.1 g L^{-1} bioethanol production has been reported from kitchen waste[33]. The additional carbohydrate content coming from rice or other foods and enzymatic pretreatment are the reasons for higher ethanol production values in other studies. Similarly, in another study, 32.2 g L^{-1} ethanol production was observed from kitchen waste[34]. Ko *et al.*[8] studied bioethanol production using a mutant bacterium, *E. coli* K011, from napier grass. The carbohydrate content of this lignocellulosic biomass was exposed to a combination of acid (1.5 % H_2SO_4) and heat treatment (at 180 °C for 10 min). Because of the similarity of the cell wall of grass and vegetables, similar ethanol concentrations (11.7 g L^{-1}) were observed in this study.

The immobilization of yeast is another approach for increasing ethanol production yields. Inal and Yigitoglu[35] conducted a study on ethanol production from glucose using *Saccharomyces cerevisiae* immobilized on a modified sodium alginate gel, and up to 69 g L^{-1} ethanol production was achieved from 200 g L^{-1} glucose[34]. The immobilization of yeasts could be a good improvement approach for ethanol production from FVWs. There are many different immobilization materials. For example, alginate-chitosan capsules were used to entrap *Saccharomyces cerevisiae*, and up to 25 g L^{-1} ethanol was produced by batch culture from 30 g L^{-1} glucose. Immobilization of yeast can also be advantageous for the fermentation of lignocellulosic biomass pretreated with toxic substances[35,36].

Sugar beet molasses is a very important organic waste for bioethanol production. The high sugar content of the waste results in higher ethanol production values. In addition, this fermentation process can be enhanced by immobilization. With sugar beet molasses, 60 g L^{-1} ethanol production was achieved using *Saccharomyces cerevisiae* entrapped in alginate-maize stem ground tissue matrix[37]. Therefore, improvement of bioethanol production yields from FVWs could be achieved also by adding sugar beet molasses to the process, which will be a combined treatment approach.

Saccharomyces cerevisiae has the ability to produce ethanol at different temperatures. Lin *et al.*[38] studied a temperature range of 10–50 °C, and 40 g L^{-1} glucose and 17 g L^{-1} ethanol production was observed at 30 °C. In this study, a maximum ethanol production of 11.7 g L^{-1} was observed from 26 g L^{-1} total sugar concentration. An orthogonal design (L_9 3^4) was used to optimize the microwave pretreatment, and 15 g L^{-1} ethanol production was observed with 400 W power[39]. In our study, the application of 400 W power pretreatment resulted in 11.7 g L^{-1} ethanol production.

In general, the ethanol production yields from FVWs can be improved by combining pretreatment with enzymatic hydrolysis. Different wastes were used for bioethanol production by several pretreatment applications[40]. Improvement of bioethanol yields in our study can be achieved by using the residual sugar content and enzymatic hydrolysis. The values reviewed in Teshaw and Assefa[40] showed that it is not necessary to apply enzymatic hydrolysis as an additional pretreatment for FVWs. From an economical perspective, bioethanol production from FVWs can be performed using only one pretreatment step (Table 2).

Syngas fermentation using acetogenic bacteria is an efficient way of producing valuable products, such as acetone, butanol, and ethanol. During syn-

Table 2 – *Comparison of the results with literature (yeast fermentation)*

Waste material	Pre-treatment	Reactor	Microorganism	Ethanol (g L^{-1})	Reference
Recovered napier grass	Acid+Heat pretreatment and enzymatic hydrolysis	Batch	*E. coli* K011	8–18	8
Pine apple industrial waste	Microwave treatment and enzymatic hydrolysis	Batch	*S. cerevisiae*	9.69	15
Wheat straw	Microwave treatment	Fed-Batch	*S. cerevisiae*	15	26
Food waste	Enzymatic pretreatment	Batch	*S. cerevisiae*	29.1	33
Kitchen waste	Enzymatic pretreatment	CSTR	*S. cerevisiae*	32.2	34
Sugar beet molasses	–	Batch	Immobilized *S. cerevisiae*	60	37
40 g L^{-1} glucose	–	Batch	*S. cerevisiae*	17	38
Fruit and vegetable wastes	Heat-treatment	Batch	*S. cerevisiae*	7.5	This study
Fruit and vegetable wastes	Heat and Acid treatment	Batch	*S. cerevisiae*	11.7	This study
Fruit and vegetable wastes	Microwave treatment	Batch	*S. cerevisiae*	11.8	This study

Table 3 – *Comparison of the results with literature (syngas fermentation)*

Microorganism	Reactor	CO (%)	Ethanol (g L^{-1})	Reference
Clostridium ragsdalei	CSTR	40	12	16
Anaerobic granular sludge	CSTR	100	11.1	31
Clostridium ljungdahlii	Batch	32	0.45	41
Clostridium carboxidivorans	Batch	32	0.15	41
Clostridium carboxidivorans	Batch	100	5.55	44
Anaerobic mixed culture	Batch	15	5.5	This study
Clostridium ljungdahlii	Batch	15	2.5	This study

gas fermentation, the optical density can be used to track the growth of bacteria. The OD value of *Clostridium ljungdahlii* reached values of 0.4–0.6 during fermentation in this study, and similar values were obtained by Ramio-Pujol *et al.*[41] using syngas with 32 % CO by batch tests. That study was performed to determine the effect of formate concentration on ethanol production, and a maximum of 0.45 g L^{-1} ethanol production was observed, while acetate production values changed between 0.1–0.65 g L^{-1}. In another study, similar OD values were obtained by using syngas at different ratios of H_2/CO[42]. A H_2/CO ratio of 0.5 resulted in 7.53 mM (0.35 g L^{-1}) ethanol production.

Butanol in particular can be produced by autotrophic acetogens. *Clostridium carboxidivorans* has the natural ability to produce butanol, but there are studies on metabolic engineering applications with *Clostridium ljungdahlii* to increase the production potential[43]. *Clostridium carboxidivorans* was used for gas fermentation of CO, and 5.55 g L^{-1} ethanol and 2.66 g L^{-1} butanol production was observed[44]. Chakraborty *et al.*[31] reported 11.1 g L^{-1} ethanol and 1.8 g L^{-1} butanol production using anaerobic granular sludge in a continuously fed stirred tank reactor by syngas fermentation. Sun *et al.*[16] used poultry litter for biochar production, and by using the waste gas produced during pyrolysis, they observed a maximum of 12 g L^{-1} ethanol production. They also used biochar for process enhancement, which can be tried for FVWs in future studies. In this study, FVWs were used in pyrolysis for biochar production, and the waste gas with 12 % CO was used as a substrate for syngas fermentation. The ethanol production values of 5.5 and 2.5 g L^{-1} and butanol production values of 0.1 and 0.05 g L^{-1} were observed with anaerobic mixed culture and *Clostridium ljungdahlii*, respectively. Syngas fermentation has many advantages, such as the production of valuable biofuels (ethanol and biochar), the ability to treat air polluting gases (especially CO), and mild temperature processes. Syngas fermentation can be a useful, productive, environmentally friendly, and economical alternative for bioethanol production from FVWs (Table 3).

Conclusion

FVWs are important wastes, especially at the municipal level, and their use in ethanol production will be an environmental and economic approach. Selecting the best pretreatment method is very important. In this study, microwave pretreatment and heat/acid pretreatment were considered as the most suitable processes for high ethanol yields. Another important method in ethanol production is synthesis gas fermentation. Although lower ethanol yields were observed than with yeast fermentation, biochar production makes these processes attractive. Most of the studies carried out in syngas fermentation are aimed at pure culture use, but it is seen here that using anaerobic mixed culture results in higher yields. This study has reviewed the simultaneous use of first- and second-generation ethanol production methods from FWVs. Using anaerobic mixed culture for syngas fermentation application from fruit and vegetable wastes is an approach attempted for the first time here. The most important novelty of the study is that the combination of biochar production with syngas fermentation using mixed culture is a very environmentally friendly and cost effective approach with lower amount of waste (bio-oil) and higher amount of energy production (biochar and bioethanol).

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