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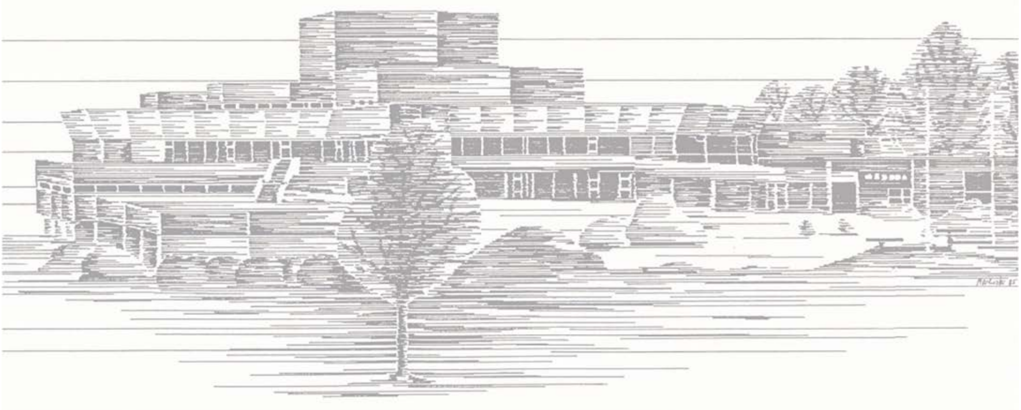

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New feedstocks for bioethanol production

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Abstract

In the last few decades raw material molasses, used in large scale fermentations in the production of bioethanol, citric acid, (baker´s) yeast and yeast extracts, has become more and more expensive. That is why agro-industrial wastes have become an interesting alternative. They are being produced in large volumes every day and represent a serious environmental problem considering its high organic content. The present contribution aims to demonstrate how waste products of wine production can be employed as substrate in bioethanol production. Cultivation of yeast and bioethanol production on molasses and grape pomace extract was studied in flasks in laboratory scale. This work should be regarded as an example of integrated sustainability which demonstrates how the waste from one industrial process is used as feedstock for another.

1. Introduction

Increasing population is the reason of increased energy demand throughout the world. The main source of energy are fossil fuel and non-renewable sources (natural gas, oil and coal), used in the production of transportation fuel, electricity and other goods [1]. 60 % of global utilization of fossil fuels is consumed in transportation sector, which consequently contributes to massive pollution [2]. Consumption of these fuels contributes to the emissions of greenhouse gasses as well as global warming causing climate change, rise in sea level, loss of diversity and urban pollution [3]. This lead to a search for an environmentally friendly, renewable and sustainable source of energy [4, 5], in which priority is given to liquid biofuels [6]. Biofuels are renewable substitutes of fossil fuels [7], defined as transportation fuels derived from biological/agricultural sources, either in liquid form (bioethanol and biodiesel) or in gaseous form (biogas and hydrogen). First generation of biofuels applies raw materials containing sugars, starch, vegetable oils as well as biodegradable waste from agricultural and forestry industries [8]. However, since this generation competes with food and feed materials, increasing ethical concerns encouraged the search for nonedible feedstock alternatives [9]. That is why the second generation of biofuels, the raw materials such as lignocellulosic material as well as waste oil and animal manure, and the third generation applying marine algae as biofuel feedstock, are gaining more and more interest [8, 9].

Bioethanol is eco-friendly oxygenated fuel, commercially produced from starch/sugar based crops [10], most likely to replace gasoline due to its several advantages. Even though one litre of ethanol provides 66 % of the energy provided by the same amount of gasoline, the higher octane number allows it to act as an antiknock agent. Also the power output is improved with ethanol because of its higher heat of vaporization compared with gasoline [3]. After the oil crisis in the 70´s, Brazil launched the Brazilian National Alcohol Program, aiming at large scale ethanol production and the engine-adaptation to consume the E20 mix (20 % ethanol and 80 % gasoline) or even pure anhydrous ethanol. For decades Brazil was the main producer by utilizing cane molasses, but was surpassed by the production of corn-based ethanol in United States [11]. Global ethanol production is presented in Figure 1. Besides United States and Brazil, covering more than 80 % of the world productions, other large ethanol-producing countries are China, Canada, Thailand, Argentina, India and European Union [12].

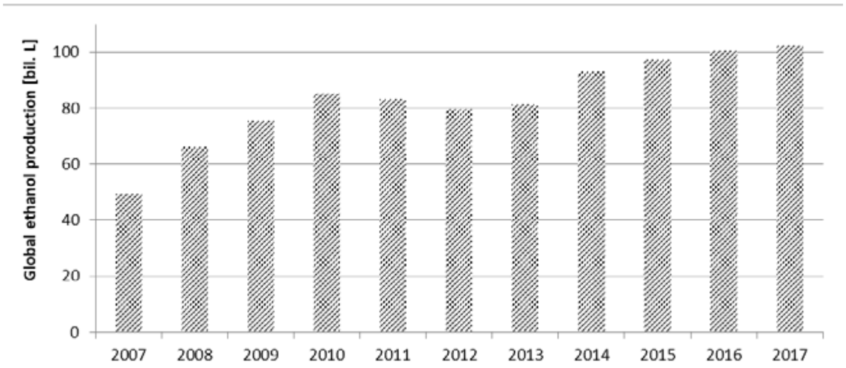

Figure 1. Global ethanol production during the last 10 years in billion litres

Molasses is suitable raw material for ethanol production. However its price has been raising drastically because of the growing demand for this medium. That is why efforts need to be made in order to find alternative raw materials [13]. Ideal raw material for ethanol production would be widely available non-edible feedstock. Although lignocellulosic material appears to be very convenient, the processing steps are energetically and financially costly, which creates a bottleneck in the industrial production [11]. Potential source of raw materials are agro-industrial wastes, which is being produced in large volumes every day and represent a serious environmental problem considering its high organic content [15]. One such potential raw material is grape pomace, which remains after the juice is collected from the pressing of grapes for wine production [14]. When processing grapes, about 75 % is used in wine making and 25 % of the weight of grapes remains as pomace [16]. Its composition depends on grape variety, method of processing, environmental conditions and the ratio of skin:seeds:stem [12]. Traditionally grape pomace is used as fertilizer or animal feed. However, because of the presence of antinutritive compounds that can negatively affect crop yields and animal weight gain, this utilization present some drawbacks [17]. Corbin et al. [14] demonstrated the potential of employing grape pomace as raw material in ethanol production with the theoretical yield up to 270 L/t. In 2017 770 million litres of wine was produced in Germany (Figure 2) [18].

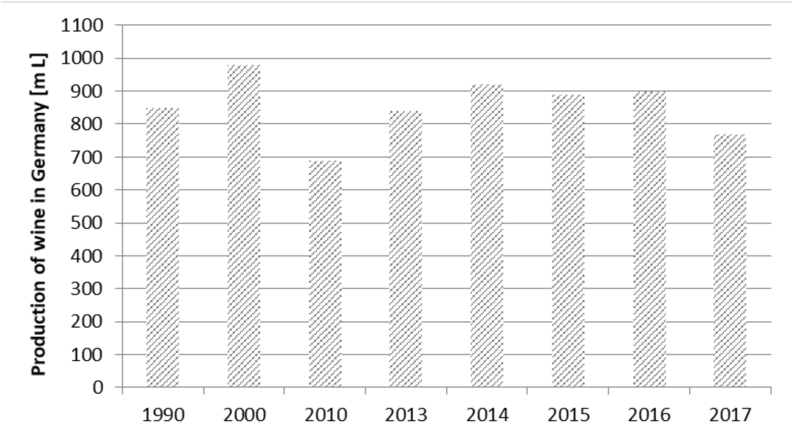

Figure 2. Production of wine in Germany in the last few decades in million litres

The present study aims to access the feasibility of replacing molasses in the production of ethanol by investigating the ethanol production on grape pomace. In this regard, grapes were processed into wine, while waste grape pomace was further processed into grape extract and pellets, therefore creating a concept of zero discharge biorefinery process.

2. Materials and Methods

2.1 Production of wine

84.9 kg of grapes Riesling Mandelberg were washed and pressed by Shark Fruit 1.6 kW (Vares Mnichovice a.s.; Mnichovice, Czech Republic) resulting in 50.4 kg of grape juice and 32.5 kg of grape pomace (grape pomace 1 in Figure 3). Grape juice was supplemented with 10 g of mineral nutrient and inoculated with 10 g of yeast (Alcotec 48 Turbo Yeast Classic). The fermentation was performed at 15 °C and lasted 14 days. Fermentation resulted in 45.2 kg of wine (5.2 kg of CO_2 was evaporated) which was further filtrated (Sheet filter 20x20 FZ 20; Zambelli, Vicenza, Italy; Filter paper MN 540 we, ø 150 mm; Macherey-Nagel GmbH & Co. KG, Düren, Germany) resulting in final product: 32.9 kg of Riesling wine containing alcohol content of 13.3 % vol.

2.2 Production of grape extract and pellets

Grape pomace 1 was mixed with 25 kg of distilled water and boiled for 20 minutes at 90 °C while constantly being stirred. After the extraction the mixture was cooled down to 35 °C and then pressed resulting in 18.8 kg of grape pomace 2 and 28.7 kg of extract. Grape pomace 2 was dried at 60 °C for 24 hours (Vacuum drying oven Heraeus Instruments; Hanau Germany) and then used as raw material for pellet production (EcoWorxx Pelletmaker PM22E; Raddestorf, Germany). Moisture content was determined at 105 °C until constant weight. Durability was determined according to the standard ISO 17831-1:2015(en) [19]. Net calorific value was determined according to ISO 18125:2017(en) [20]. This extract was concentrated to obtain 4.5 kg concentrated grape extract by evaporation in a scraped surface evaporator (Labor- und Prozesstechnik GmbH; Ilmenau Germany) at a temperature of 87 °C, pressure of 134 mbar and rotation speed of 260 rpm. Centrifugation was performed for 10 minutes at 4,000 rpm (Sorvall RC-5B Plus Superspeed Centrifuge, Thermo Fisher Scientific; Waltham, Massachusetts, USA) resulting in 0.103 kg of tartaric acid and 3.8 kg of concentrated grape extract (Figure 3).

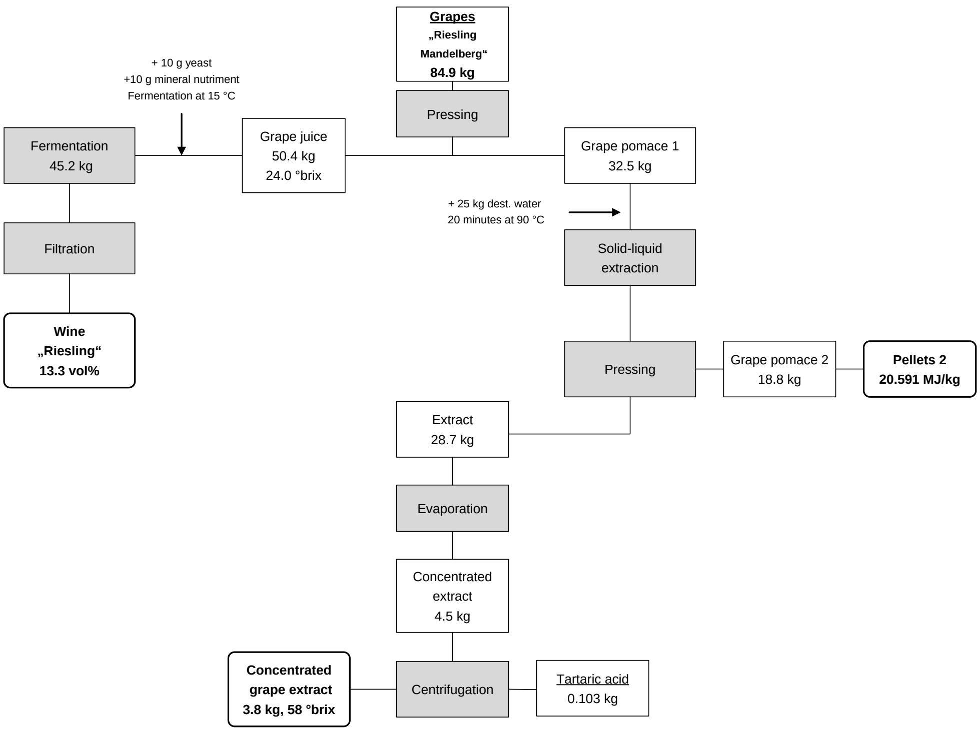

Figure 3. Production of white wine, pellets and concentrated grape extract

2.3 Strain, media and fermentation

Microbial strain used for ethanol production is commercial yeast (Alcotec Turbo Yeast - Classic 48, Hambleton Bard Ltd; Chesterfield, UK).

Molasses mixture, containing beet and cane molasses, and wine extract were adjusted to pH value 3.9 (pH meter PCE-PHD 1, PCE Deutschland GmbH; Meschede, Germany) and 20 °Brix (Refractometer 0-32 %, Greiner Glasinstrumente GmbH; Lemg, Germany) according to Göksungur and Zorlu [21]. Media were sterilized at 121 °C for 20 minutes (Systec DE-150 autoclave, Systec GmbH; Linden, Deutschland).

Erlenmeyer flasks filled with 100 g of the each medium was inoculated with 0.6 g of the commercial yeast and placed on magnetic stirrer (Multiposition magnetic stirrers Variomag

Poly 15; Thermo Fischer Scientific, Waltham, Massachusetts (USA). The flasks were weighed for the determination of ethanol production (Kern PCB 3500, Kern & Sohn GmbH; Balingen, Germany). Fermentation lasted approx. 30 hours. All fermentations were performed in triplicate.

3. Results & Discussion

Grape pellets, with the moisture content of 9.3 %, had the durability index of 92.3 %. Measured net calorific value of 20.6 MJ/kg is very similar to the value obtained with wood pellets [22]. Figure 4 represents the evolution of the ethanol production on molasses mixture and grape extract.

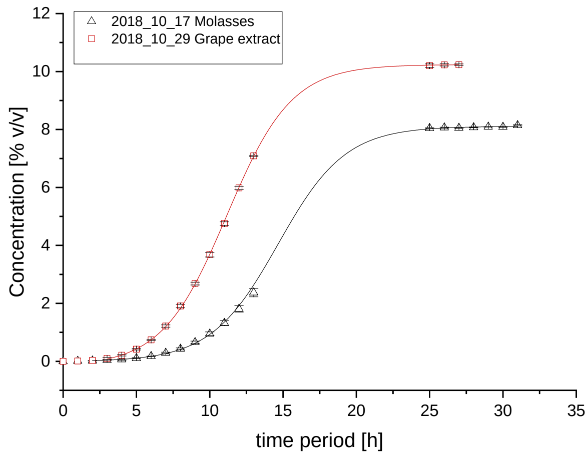

Figure 4. Ethanol production on the molasses medium and grape extract

Adaptation of the yeast on the medium, the lag phase, lasted longer on the molasses medium (approx. 4 hours), than on the grape extract (approx. 3.5 h). After approx. 2 hours from the start of the fermentation, foam formation on the surface of the media occurred because of the CO_2 gas evolution. The grape extract medium discoloured from dark brown colour to orange, while the colour of molasses remained unchanged. After about 30 hours, bubble forming wasn't observed, meaning that the fermentation came to an end, due to the complete utilization of sugars. The fermentations should have been performed at 25 °C. However due to the early start in the morning (approximately at 5:30 am), the room temperature in the laboratory was 15 °C. It took almost 4 hours to reach 25 °C (until 10:00 am). Accordingly, it is possible that this factor could have influenced the duration of the lag phase. Fermentation on molasses medium resulted in the production of 7.88 vol % ethanol, while the fermentation on grape extract has resulted in producing 10.23 vol % ethanol. The fermentation phase of grape extract began earlier than on the molasses medium, therefore the ethanol yield was higher on the grape extract medium. This is demonstrated in the Figure 5; fermentation on grape extract resulted in the release of 7.5 g of CO_2, whilst on molasses medium was up to 5.8 g of CO_2.

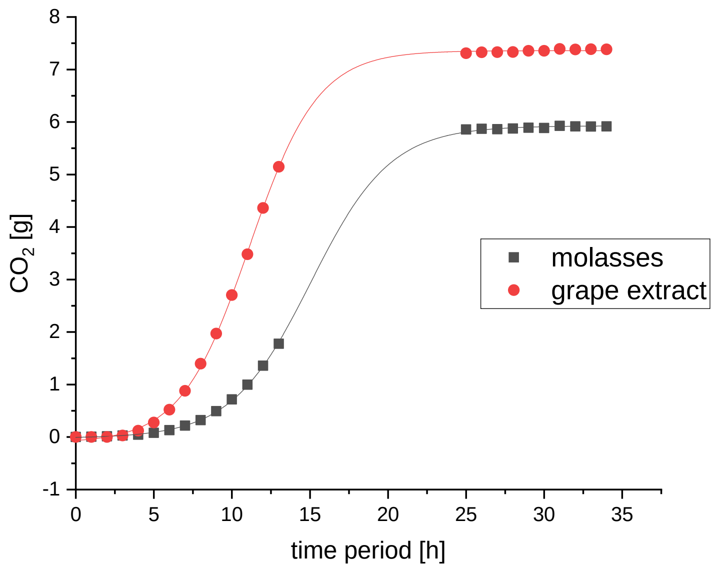

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Figure 5. CO_2 release during the fermentation on the molasses medium and grape extract

Conclusions

In this study, wine production was reshaped into a biorefinery concept, where not only Riesling wine was produced, but also waste stream of grape pomace was directed into the production of pellets, grape extract and the extraction of tartaric acid. Pellets' measured net calorific value of 20.6 MJ/kg is similar to the value obtained with wood pellets. Produced grape extract was tested as the raw material for ethanol production and compared to the production on the molasses medium. Higher yield of ethanol was achieved on the medium containing grape extract, 10.23 vol %, whereas in the molasses medium 7.88 vol % ethanol was achieved. These results confirm the feasibility of applying grape extract as raw material in the production of ethanol. By this strategy the costs for raw material would be reduced as well as energy could be generated from the pellets.

The authors have declared no conflict of interest.

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