Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-019-04894-9

ADVANCES & PROSPECTS IN THE FIELD OF WASTE MANAGEMENT

Non-thermal plasma and ultrasound-assisted open lactic acid fermentation of distillery stillage

Aleksandra Djukić-Vuković[1] · Saša Lazović[2] · Dragana Mladenović[1] · Zorica Knežević-Jugović[1] · Jelena Pejin[3] · Ljiljana Mojović[1]

Received: 3 November 2018 / Accepted: 18 March 2019

Abstract

Stillage is the main by-product of bioethanol production and the cost of its treatment significantly affects the economy of bioethanol production. A process of thermal sterilization before lactic acid fermentation (LAF) is energy demanding and is causing deterioration of valuable compounds in stillage. In this study, ultrasound (UT) and plasma (PT) treatments were used for microbial inactivation, and a significant reduction in the number of viable microorganisms in the stillage after PT and UT was observed. After application of treatment, LAF by *Lactobacillus rhamnosus* ATCC 7469 was initiated. The concentration of LA is used to quantify the efficiency of the stillage revalorization. The highest LA productivity of 1.21 g/Lh and yield of 0.82 g/g were obtained after PT, while UT of 10 min provided productivity of 1.02 g/Lh and LA yield of 0.69 g/g. The results were benchmarked against closed LAF. Around 20% better revalorization of stillage by PT was achieved when compared with conventional sterilization. In addition, an excellent L (+) LA stereoselectivity of 95.5% was attained after PT. From the aspect of energy efficiency, that of PT was three times lower than UT and almost ten times lower than thermal sterilization, but it is the most expensive due to the high consumption of gas which could reduce application of closed Ar atmosphere on larger scales. This way, a simpler and energy efficient process for LA production on stillage was accomplished by "open" fermentation.

Keywords Biorefinery · Pretreatments · Microbial inactivation · Sterilization · Lactic acid bacteria

Introduction

Distillery stillage is the main residue of bioethanol production on renewable feedstock. It remains after distillation of bioethanol, and depending on the feedstock used for bioethanol production, up to 20 L of stillage with COD values as high as 100 g/L remains per liter of bioethanol produced (Noukeu et al. 2016; Wilkie et al. 2000). Stillage has to be treated in order to decrease its organic load, and its revalorization by different routes is increasing competitiveness of bioethanol as a fuel (Baral and Shah 2017; Moestedt et al. 2013).

Stillage from bioethanol production on corn and similar crops is mostly used after drying for distiller's dried grains with solubles (DDGS) production. Energy required for stillage drying in DDGS production is high and smaller bioethanol production plants cannot afford it, so in developing countries, including Serbia, the stillage is often discarded in waterflows without the treatment and cheaper substrates like agri-food wastes are being employed for bioethanol production. Therefore, in some cases, stillage presents a significant environmental and financial burden limiting competitiveness of bioethanol. In 2017, corn stillage was the most represented type of stillage in the EU (ePURE 2017), but the initiative to replace corn and other crops with non-edible feedstocks for bioethanol production is already articulated through an EU Directive (2015/1513) (European Parliament 2015). It sets a goal of at least 0.5% of bioethanol produced on alternative feedstock (waste and non-food) (European Parliament 2015). In the future, the share of advanced bioethanol will rise led by the new policies which promote reduction of "food-/feed-

Responsible editor: Ta Yeong Wu

✉ Aleksandra Djukić-Vuković
adjukic@tmf.bg.ac.rs

[1] Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade 11120, Serbia

[2] Institute of Physics Belgrade, University of Belgrade, Belgrade, Serbia

[3] Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

Published online: 04 April 2019

based" biofuels to 0% and participation of advanced biofuels to at least 3.5% by 2030 (ICCT 2018).

Valorization of the stillage from advanced bioethanol production through DDGS is often not adequate. The stillage from celullosic bioethanol contains potentially toxic compounds and is not suitable for feed (Shan et al. 2016). The stillage remaining after bioethanol production on food waste is high in organic acid content and it was mostly recycled in bioethanol production; however, it often acts as an inhibitory on yeasts as also reported recently (Anwar Saeed et al. 2018). Therefore, finding alternatives for effective utilization of the stillage from advanced bioethanol production is of utmost importance.

Besides anaerobic digestion (Du et al. 2018; Sayedin et al. 2018), nutrients from stillage could be recovered through other processes also, including its utilization as fertilizer (Fuess and Garcia 2014) or as substrates for fermentative production of bacterial cellulose (Wu and Liu 2013), 1,3–propanediol (Kang et al. 2014), fungal biomass, malic acid (West 2011), and lactic acid (LA) (Djukić-Vuković et al. 2015).

Production of LA by lactic acid bacteria (LAB) on cheap substrates like stillage attracts great interest. LA market size was valued at 2.08 billion US$ in 2016 and is expected to grow by an annual growth rate of 16.3% until 2025 (Research Grand View 2017). The demand for LA is mainly driven by increasing utilization of LA as a platform chemical for the production of poly-lactides—biocompatible and biodegradable polymers suitable for pharmaceutical and food applications (Abdel-Rahman et al. 2013a; Research Grand View 2017). Most of the fermentative processes for valorization of stillage and similar substrates like whey are "closed" fermentations which include thermal sterilization of substrate using LA-producing *Lactobacillus* spp. (Djukić-Vuković et al. 2015; Kasmi et al. 2017). Thermal sterilization is an energy demanding and costly treatment; it is difficult to apply in large-scale processes and part of the nutrients is lost during thermal treatment (Pleissner et al. 2017). Open fermentations, performed under unsterile conditions by mixed culture, are simpler and lower in energy consumption than closed fermentations and could be adapted to various renewable and waste substrates due to the evolution capacity of microbiota, if the process is well controlled (Li et al. 2014). However, lower productivities and lower optical purity of LA are often attained in open fermentations while contamination is always a challenge in these processes (Abdel-Rahman et al. 2013a; Li et al. 2014). Currently developed strategies for open LA are performed by thermotolerant *Bacillus* spp., so their growth is favored at elevated temperatures of over 50 °C wherein other microorganisms are less probable to grow, thus decreasing contamination. However, *Lactobacillus* spp. which are the most efficient LA production strains in closed fermentations cannot grow under these conditions. The control of fermentation conditions and possibility to manipulate the substrate microbiota are crucial for efficient open fermentations with *Lactobacillus* spp.

Different non-thermal treatments were used as a technology for control of microorganisms in agricultural and wastewater substrates with varying efficiencies (Yusaf and Al-Juboori 2014). These treatments should enable selective inactivation of undesired microorganisms from complex microbial communities like those in stillage (Sakai et al. 2000; Tang et al. 2016) and preserve the species capable of producing desired chemicals, like LA in this case. Non-thermal plasma treatments (PTs) generate abundance of highly reactive species at low temperatures thus enabling processing of sensitive materials, causing etching of the surface of exposed microorganisms and avoidance of thermal degradation of the compounds present in treated media (Krásný et al. 2014). In media with high dry matter content like stillage, the effects of non-thermal PT on microbial inactivation, selectivity, and applicability for subsequent open LA biorefinery have not been examined previously based on a thorough literature survey. Most of the studies related to microbial inactivation by PT were performed in water, in water-based media with low dry matter content, or on solid surfaces (Liao et al. 2017; Surowsky et al. 2015) with different plasma/substrate interactions.

The high-power ultrasound treatment (UT) can be used for decontamination due to the mechanical disruption and cavitation effects induced in samples but also for disintegration of fresh distillery spent wash (Sangave and Pandit 2004). The microbial inactivation by UT was applied even on larger scales (Leonelli and Mason 2010), but it is highly dependent on substrate composition, types of microorganisms present in media, and treatment conditions (Herceg et al. 2015; Leonelli and Mason 2010).

The main objective of this study was to investigate plasma and ultrasound in LAF on stillage as an alternative to thermal sterilization. The mechanism of non-thermal plasma inactivation of *Lactobacillus acidophilus* and *Escherichia coli* in water and stillage as media was thoroughly studied. Furthermore, non-thermal plasma inactivation of indigenous stillage microbiota was compared with inactivation by UT. The stillage after different treatments was subjected to open or closed LAF to assess its revalorization potential through LA production with high-LA-producing strain of *Lactobacillus rhamnosus* (Djukić-Vuković et al. 2013). The parameters of LAF in terms of LA concentration, optical purity, yield, and productivity were studied and compared for various applied treatments. In addition, all treatments were compared from the aspect of estimated energy efficiency.

Materials and methods

Preparation of distillery stillage

The distillery stillage remained after bioethanol production on wasted bread was obtained from the ethanol-producing

facility (Reahem d.o.o., Serbia) and used for preparation of media for LAF. The pH value in all samples was adjusted to 6.5. The stillage was subjected to various decontamination treatments, PT, UT, and thermal sterilization, and where applicable, it was used as a substrate for LAF. The thermal sterilization was performed in an autoclave (Sutjeska, Serbia, device power 5.25 kW) at 121 °C for 20 min.

Microorganism

Lactobacillus rhamnosus ATCC 7469 and *Lactobacillus acidophilus* ATCC 4356, homofermentative LA-producing strains, and *Escherichia coli* ATCC 25922 were obtained from American Type Culture Collection. The *L. rhamnosus* and *L. acidophilus* cultures were propagated at 37 °C in Man Rogosa Sharpe broth (MRS) under microaerophilic static conditions. *E. coli* culture was propagated at 37 °C in nutrient broth, under aerobic static conditions. Overnight cultures were used as an inoculum in experiments. An overnight culture of *L. rhamnosus* was used as inoculum for LAF on the stillage media.

Non-thermal plasma treatment

Non-thermal plasma treatment

All treatments were conducted using a plasma needle operating at 25 kHz in ambient air. Argon was used as a feed gas (0.5 sLm flow rate) in order to reduce the breakdown voltage through Penning ionization. The operating power was 2 W. The distance between the needle tip and the samples was 1 cm. A detailed description of the plasma device is provided by Zaplotnik et al. (2015).

The effect of non-thermal plasma on G (+) and G (−) bacteria in water and distillery stillage

These experiments were undertaken at the beginning of the study in order to determine how non-thermal plasma acts toward *E. coli* (a representative of G (−) bacteria) and *L. acidophilus* (a representative of G (+) bacteria) in water and stillage media.

In the first set of experiments, the samples of water and stillage (6 mL) were sterilized by autoclaving (Sutjeska, Serbia) at 121 °C for 20 min and inoculated by overnight cultures of *E. coli* and *L. acidophilus* in order to set the initial number of viable cells in samples at around 10^5 CFU/mL. Immediately after inoculation, the samples were transferred in glass Petri dishes and subjected to PT for 30 min (duration of treatment was selected after preliminary studies) by using a plasma needle jet as explained in the section "Non-thermal plasma treatment." The mixing of samples was provided by a magnetic stirrer (IKA®, Germany, device power 9 W). The schematic presentation is shown in Fig. 1a.

The second set of experiments was performed in order to determine the effect of plasma-generated UV photons on microbial inactivation in water and stillage. The samples were prepared and treated in the same way as previously, but quartz glass was placed between the plasma jet and sample to prevent other effects of PT except UV light. The graphical presentation is provided in Fig. 1b. The samples of sterilized water and stillage inoculated with *E. coli* and *L. acidophilus* were subjected to the same procedure, but without PT, as a control.

The number of viable cells in the samples was determined using pour plate counting method on nutrient agar (for *E. coli*) and MRS agar (for *L. acidophilus*). A reduction in the number of viable microorganisms was presented as log reduction, log (N/N_0), where N is the number of viable cells in the samples and N_0 is the number of viable cells in control.

Non-thermal plasma treatment of distillery stillage for lactic acid fermentation

The samples of non-sterile stillage in 6-mL batches were placed in glass Petri dishes and treated by non-thermal plasma needle for 30 min (duration of treatment was selected after preliminary studies). The treatment details are explained in the section "Non-thermal plasma treatment." After the treatments, the samples were first incubated under microaerophilic conditions at 41 °C for 24 h in order to assess the effect of PT on the stillage microbiota. The number of total microaerophilic mesophilic bacteria in samples was determined using pour plate counting method as previously described on MRS agar as a substrate (Issa-Zacharia et al. 2011). The plates were incubated for 48 h at 37 °C. In the second set of experiments, after the PT, samples were subjected to LAF (section "Lactic acid fermentation").

High-power ultrasound treatment of distillery stillage

The stillage samples (60 mL) placed in a 200-mL glass were treated by high-power ultrasound (Sonopuls HD 2200, Germany, device power 200 W) with sonotrode TT 13 for 10 min (duration of treatment was selected after preliminary studies) at actual value of amplitude 75% and frequency of 20 kHz. After the treatment, in the first set of experiments, the samples were incubated under microaerophilic conditions at 41 °C for 24 h in order to examine the effect of treatment on the number of viable bacterial cells in the stillage. The number of total microaerophilic mesophilic bacteria in all samples was determined using pour plate counting method as previously described on MRS agar as a plate substrate (Issa-Zacharia et al. 2011). The plates were incubated for 48 h at 37 °C. In the second set of experiments, after the treatment, samples were subjected to LAF (section "Lactic acid fermentation").

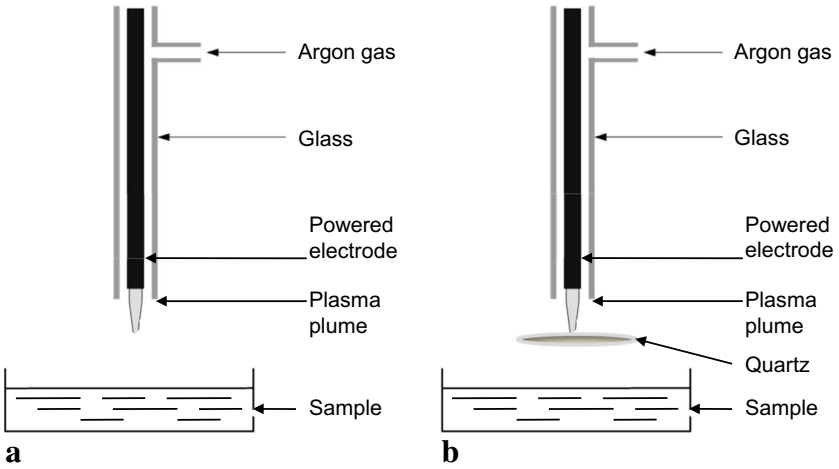

Fig. 1 Schematic presentation of experimental setup for non-thermal PT (**a**) and assessment of contribution of the UV photons generated by plasma (**b**)

Lactic acid fermentation

Stillage samples (60 mL), treated by PT and UT and sterilized, were subjected to fermentation for the production of LA and probiotic biomass. Untreated stillage was also subjected to LAF in the same way as treated samples. Initial glucose concentration in all samples was adjusted at around 35 g/L by addition of a 70% glucose solution and the pH value was adjusted to 6.5. The LAF samples were inoculated with 5% (*v*/*v*) *L. rhamnosus* ATCC 7469 while the untreated stillage was fermented by indigenous microbiota and considered as a control sample. The fermentations were performed as batch cultures with shaking in 200-mL flasks (100 rpm, IKA®, Germany), at 41 °C, under microaerophilic conditions, maintained using a gas-pack system. These conditions were previously selected for the fermentation of stillage by *L. rhamnosus* ATCC 7469 (Djukić-Vuković et al. 2016). During the LAF, the pH value in media was maintained at 6.5 by addition of 30% NaOH, in 4-h intervals.

Energy consumption calculations

The estimation of energy consumption of different treatments was performed by using manufacturers' information for lab-scale equipment applied in the experiments for maximal load. Energy of different treatments was calculated according to the formula (Hulsmans et al. 2010):

$$E = P \times t \tag{1}$$

where E is energy, P is the power of the device used for treatment, and t is the duration of treatment.

For PT, the costs of feed gas (4 €/kg) and energy consumption for mixing were added for capital cost calculation. The price adopted for electric energy was 5.786 euro cents/kWh. For UT, only electric energy was used to run the device. Energy consumption for 60 mL of treated fermentation media was used and benchmarked against per gram of produced LA.

The actual power of ultrasound treatment of stillage was calculated according to the procedure based on calorimetry and the following formula (Herceg et al. 2015):

$$P = m_s \times C_p \times \partial T/\partial t \tag{2}$$

where m_s is the mass of stillage media, C_p is specific heat at a constant pressure (J/gK), and $\partial T/\partial t$ is the slope at the origin of the curve (T is temperature, t is time). This equation is used to calculate the actual power of UT with a presumption that all of the power entering the system is dissipated as heat.

Methods of analysis

Chemical composition of the stillage was determined using methods described in detail previously (Djukić-Vuković et al. 2016). The antioxidative activity of the stillage before and after the treatment against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma Aldrich, USA, CAS No. 1898-66-4) was determined as in the study of Jovanović et al. (2016). The stereoselectivity of produced LA was determined by the enzymatic method L(+)/D(−) LA assay (Megazyme®, Ireland). The LA and glucose concentrations during LAF were determined by HPLC analysis. The samples were withdrawn from fermentation media, filtered through 0.22-μm filters (Minisart® syringe filters, Germany), and analyzed by adapted HPLC method of Srivastava et al. (2014). In brief, the HPLC analysis was performed on the Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) system. A reversed-phase column (Hypersil gold C18, 150 mm × 4.6 mm, 5 μmL; Thermo Scientific, USA) at 65 °C was employed. Mobile phase was 5 mM H_2SO_4 (JT Baker, USA) with an elution rate of 0.6 mL/min. Detection was performed by a UV/VIS detector at 210 nm. All data acquisition and processing were done using Chromeleon Software. All chemicals used in

experiments were of analytical grade and obtained from Sigma Aldrich, USA.

Scanning electron microscopy of stillage

The distillery stillage remaining after bioethanol production on wasted bread obtained from an ethanol-producing facility (Reahem d.o.o., Serbia) and used in all experiments was vacuum dried at 25 °C for 3 h. Dried samples were coated with Au-Pd alloy using a spatter coater. The morphology of the samples was studied by field emission scanning electron microscopy (FESEM) TESCAN Mira3 XMU at 4.0 kV.

Statistical analysis

The experiments were done in duplicate, in three independent experiments. All values are expressed as means ± standard deviation and error bars in figures are standard deviations. Mean values of treatments were compared by the analysis of variance (one-way ANOVA) followed by the Tukey test for mean differences testing. Differences were considered significant at $p < 0.05$.

Results and discussion

Interaction of non-thermal plasma and stillage: effect on chemical composition

The major challenge in processing of a complex medium such as stillage and similar wastewater is to achieve inactivation of different microorganisms while preserving valuable compounds. Uchiyama et al. report that argon cold atmospheric plasma, as the one used in our study, can generate enormous amounts of $^{\bullet}OH$ radicals and H_2O_2—the combination product of $^{\bullet}OH$ radicals in the aqueous phase even at distances of approximately 1 cm from the plasma source nozzle (Uchiyama et al. 2015). Superoxide $^{\bullet}O_2^-$ can be present in the liquid phase in significant amounts, also (Gorbanev et al. 2016; Tresp et al. 2013). Besides reactive oxygen species, the plasma source is generating UV photons (Boudam et al. 2006). UV light has a direct negative effect on bacteria and it can initiate photodissociation of water and additional chemical reactions in treated media; therefore, interaction of non-thermal plasma with a substrate like stillage is rather complex.

The chemical composition of the stillage which was subjected to different treatments and used as a substrate for LAF is given in Table 1.

For effective fermentative processes, the contents of reducing sugars and free amino-nitrogen are crucial. Stillage is very rich in proteins and limited in carbon sources (Table 1). The cost of protein sources such as yeast extract for growth of LAB contributes 38% to total LA production cost (Liu et al. 2010). Stillage is attractive as an alternative to expensive protein substrates, being very cheap, but rich in yeast remaining from bioethanol fermentation. Figure 2 presents scanning electron microscopy of the stillage with clearly visible residual yeast cells. The stillage rich in yeast cells (Fig. 2) is a valuable source of nitrogen for LAB in fermentation, but C:N ratio is most often determining the productivity of processes with LAB (Djukić-Vuković et al. 2016; Koutinas et al. 2014). Since stillage is low in reducing sugars, it has to be supplemented with carbon sources for effective LAF. In this study, we used glucose to keep the media simpler and analyze the effect of different treatments on stillage microbiota as a primary source of contamination in open LAF. Other waste substrates rich in sugars, like molasses (Mladenović et al. 2018) and biomass hydrolysate, are cheaper carbon sources recommended for industrial processes. These sugar-rich substrates are not prone to contamination because of high sugar content, so their addition after the treatments is not expected to significantly alter the microbiota in the fermentation substrate. In our study, PT did not cause significant changes in the contents

Table 1 Chemical composition of distillery stillage

Components	Content
Sugar concentration	11.19 ± 0.83 g/L
Protein concentration	63.91 ± 2.81 g/L
Free amino-nitrogen concentration	290.6 ± 1.5 mg/L
Lipids concentration	17.36 ± 1.84 g/L
Ash concentration	31.2 ± 0.1 g/L
Dry matter	12.79 ± 0.31%

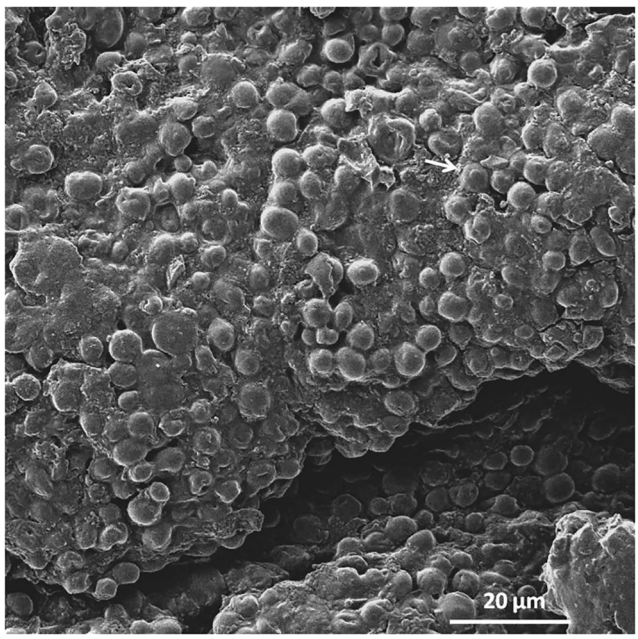

Fig. 2 Scanning electron microscopy of stillage from bioethanol production on wasted bread. The arrow is pointing at a typical yeast cell

of reducing sugars and free amino nitrogen as confirmed by HPLC and spectrophotometric methods (Table 1).

The stillage also contains melanoidins, products of Maillard reaction which occurs in mixtures of proteins and sugars after heating. They are generated in stillage during the distillation of bioethanol and thermal treatments of stillage like sterilization can generate additional amounts (Jake et al. 2016). Maillard reaction products contribute to the antioxidative activity of stillage and can act as scavengers for free radicals (Caderby et al. 2013; Vhangani and Van Wyk 2016). The antioxidant activity (determined by DPPH) of the stillage was followed before and after PT. After the longest studied plasma treatment of 30 min, only a slight decrease in antioxidant activity (from 93.5 ± 1.3% before to 90.1 ± 1.2% after the treatment) was observed. The decrease in the antioxidant activity, as observed in our study, can be explained by the reaction of antioxidants from the stillage with radicals generated during PT. A similar decrease in the concentration of enzymes and molecules associated with oxidative stress (vitamin C, polyphenol oxidase, peroxidase) was reported in other substrates subjected to radical reactions initiated by PT (Surowsky et al. 2015). In other antioxidant-rich substrates exposed to non-thermal plasma, no significant change in antioxidant activity was noticed (Ramazzina et al. 2015) suggesting that it is highly dependent on the compounds present in substrate and overall chemistry of plasma treatment.

Further, we examined the effect of PT on microbial inactivation in stillage as a substrate for LAF.

Inactivation of model G (+) and G (−) bacteria in water and stillage by non-thermal plasma treatment

The effects of non-thermal PT on the survival of *E. coli*, G (−) bacteria, and *L. acidophilus*, G (+) bacteria, were studied first. *E. coli*, being the most studied G (−) bacteria from the sanitary perspective (Puač et al. 2015), was used as a model of undesired G (−) microorganism in substrates. *L. acidophilus* was a representative of G (+) LAB belonging to *Lactobacillus* spp. which are main constituents of indigenous stillage and food waste microbiota and responsible for LAF and silage of these substrates in uncontrolled conditions (Sakai et al. 2000; Tang et al. 2016). PTs were performed in water and stillage inoculated with model G (−) and G (+) microorganisms.

The reduction in the number of viable *E. coli* and *L. acidophilus* in water and stillage by PT is presented in Fig. 3. Reactive oxygen species ($^{\bullet}OH$, H_2O_2, $O_2^{\bullet-}$), reactive nitrogen species, and UV photons created during PT all contribute to the antimicrobial effects of plasma (Gorbanev et al. 2016; Uchiyama et al. 2015). We examined total microbial inactivation by PT and also isolated effects of UV photons generated by plasma on inactivation of bacteria (Fig. 3).

The higher reduction in viable cell number was obtained in water for both studied bacteria, but in general, *E. coli* was more sensitive to PT than *L. acidophilus*, regardless of the media (Fig. 3). The UV-dependent inactivation of *E. coli* in water comprised up to 78% of the total logN reduction, while in the stillage it represented around 50% of the total inactivation. The dissimilarity in inactivation between different media is predominantly due to the UV-dependent inactivation, especially for *E. coli*. Also, the observed presence of scavengers in stillage (section "Interaction of non-thermal plasma and stillage: effect on chemical composition") as well-documented Maillard reaction products could inactivate oxidative species produced by the plasma source leading to lower microbial inactivation than in water. In absolute values, logN reduction mediated by UV photons for *E. coli* decreased three times in stillage in comparison to water. The remaining contribution of reactive species on overall logN reduction has not been altered significantly with a change of media for *E. coli* (Fig. 3).

Because of turbidity of the stillage, the effect of UV radiation was less pronounced in stillage. These results emphasize the importance of penetration depth of UV photons and therefore a significance of the treatment chamber shape and overall treatment conditions, besides the most often reported volume of sample (Boudam et al. 2006).

The inactivation of *E. coli* cells in water obtained in this study was similar to previously published results, but in a more efficient time to volume ratio (Puač et al. 2015; Purevdorj et al. 2002). Generally, G (−) bacteria are more susceptible to both UV light and cold PT than G (+) (Mai-Prochnow et al. 2016). A fine adjustment of plasma operating conditions could increase the participation of UV light or radicals in overall bactericidal activity of PT (Boudam et al. 2006) tuning its efficiency. In this study, *E. coli* was found to be more susceptible to the UV light component of PT compared to *L. acidophilus*. Therefore, PT in stillage enabled a significant decrease in the number of *E. coli*, while growth of *L. acidophilus*, a representative of LAB, was minimally affected. This way, PT provided some selectivity in microbial inactivation in stillage, qualifying it as a promising strategy for treatment of stillage for revalorization in LAF.

The non-thermal plasma and ultrasound treatment of indigenous stillage microbiota

In the next set of experiments, PT was compared with UT for microbial inactivation of indigenous stillage microbiota and the results are presented in Fig. 4. The non-thermal PT caused a higher reduction in viable cell number than UT (Fig. 4). The number of viable cells did not increase within 24 h after the PT, resulting in around 3 logN lower number in PT than in a control, untreated sample (Fig. 4). The possible reason could be a slow growth and recovery of bacteria which survived the

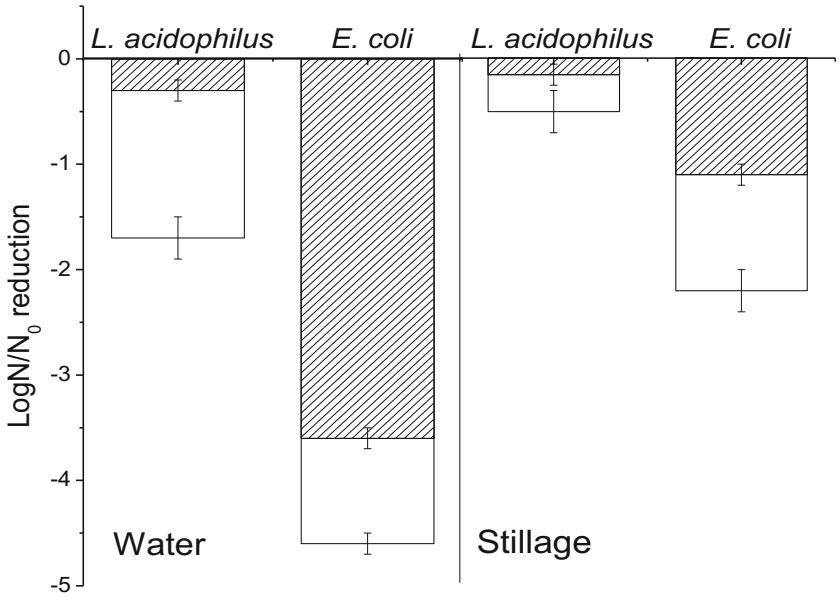

Fig. 3 Reduction in the number of viable *E. coli* and *L. acidophilus* cells in different media. White bars, inactivation by plasma-generated radicals; patterned bars, inactivation by plasma-generated UV photons

treatment. The lifetime of reactive species generated by plasma is extremely short, but they initiate numerous reactions in media causing prolonged effects (Graves 2012). The application of plasma-activated water, water subjected to PT and then used for sanitation purposes, is based on this effect (Ma et al. 2015). The decrease in the number of microorganisms achieved after PT (Fig. 4) could be very useful for the control of contamination of the stillage. This provides a longer storage time of stillage and more versatility in its utilizations. On the other hand, the presence of reactive species in the stillage after PT could negatively affect the growth of LAB responsible for LA production and limit the fermentation productivity. Therefore, we inoculated stillages subjected to different treatments with a high L (+) LA-producing strain *Lactobacillus rhamnosus* ATCC 7469 (Djukić-Vuković et al. 2015) and compared the number of viable bacteria 24 h after different treatments against that of untreated stillage, also inoculated with *L. rhamnosus*. These results are presented in Fig. 5.

When we compare the number of viable microorganisms after 24 h without inoculation with *L. rhamnosus* (Fig. 4) and with inoculation (Fig. 5), the higher number of bacteria was obtained with the addition of *L. rhamnosus*. Also, the growth was enhanced in PT and UT samples over non-treatment or sterilization (Fig. 5). However, LAF performance of bacterial populations in PT and UT samples has to be benchmarked against untreated and thermally treated samples in order to evaluate their potential for effective LA production.

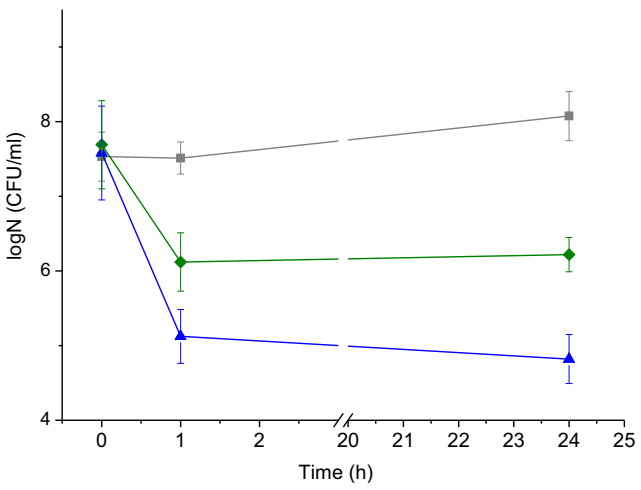

Fig. 4 Number of viable cells of stillage microbiota in time after different treatments. Gray square, untreated stillage; green diamond, ultrasound-treated stillage; blue up triangle, plasma-treated stillage

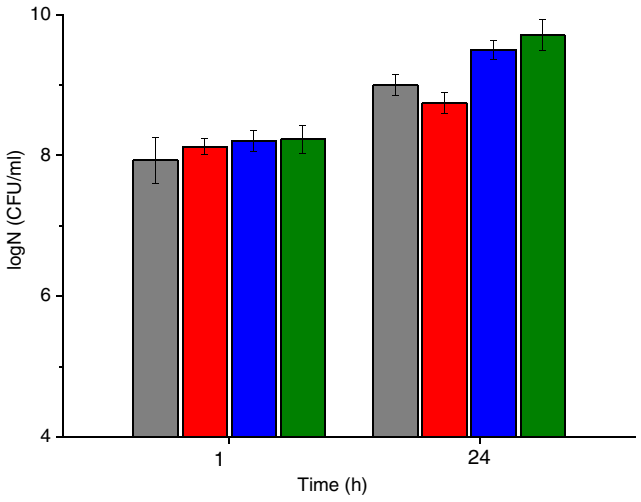

Fig. 5 Number of viable cells in stillage after different treatments and inoculation with high-LA-producing strain *Lactobacillus rhamnosus* ATCC 7469. Gray bars, untreated stillage; red bars, sterilized stillage; blue bars, non-thermal PT stillage; green bars, UT stillage

Lactic acid fermentation of treated stillage by *Lactobacillus rhamnosus* ATCC 7469

The concentration of LA produced in LAF is used to quantify the efficiency of stillage revalorization. Additionally, optical purity of the produced LA is a very important criterion for selection of the most promising process. *L. rhamnosus* ATCC 7469 is a high L (+) LA-producing strain in monoculture, while in open fermentations with mixed populations it can be difficult to achieve the desired optical purity, since different LAB produce different LA isomers. Two key factors have to be taken into account to achieve the best treatment results: a high sugar to LA conversion rate and stereoselective LA production.

After performing the treatments of the stillage (UT, PT, sterilization), all treated stillage samples as well as untreated control were inoculated with *L. rhamnosus* ATCC 7469 and subjected to LAF. The LA concentrations during open LAF, performed on PT, UT stillage and untreated stillage as substrates, and closed LAF, performed on sterilized stillage, are presented in Fig. 6. The most important parameters of all studied LAF are presented in Table 2.

The most productive LAF was achieved in PT samples directly followed by LAF in UT sample (Fig. 6). Obviously, indigenous LAB preserved after the treatments enhanced LA production. The highest LA productivity in PT sample is a result of minimal deterioration of substrate, because of the absence of substrate heating during the treatment (section "Interaction of non-thermal plasma and stillage: effect on chemical composition"), and significant suppression of competition of microorganisms in the substrate (Figs. 4, 5). The competition between indigenous microbiota of stillage and inoculated *L. rhamnosus* in untreated samples resulted in a very low productivity of 0.57 g/Lh (Table 2). However, this is still higher than the values reported for open LAF of kitchen waste (Tang et al. 2016) implying a suitability of the stillage as a substrate for LAF. The higher final LA concentration (in 48 h) after UT can be explained by the presence of a higher number of microorganisms (Fig. 5). Although UT is often considered as a non-thermal technique, ultrasound (10 min) elevated the temperature of stillage to 70 °C, similar to the reported UT of other substrates (Herceg et al. 2015). This plays an additional role in microbial inactivation and deterioration of stillage, and finally results in very similar productivities achieved in closed fermentation on sterilized stillage (0.97 g/Lh) and open fermentation on UT stillage (1.02 g/Lh). Besides the yields and productivities, a stereoselectivity of obtained LA is a very important issue to be addressed.

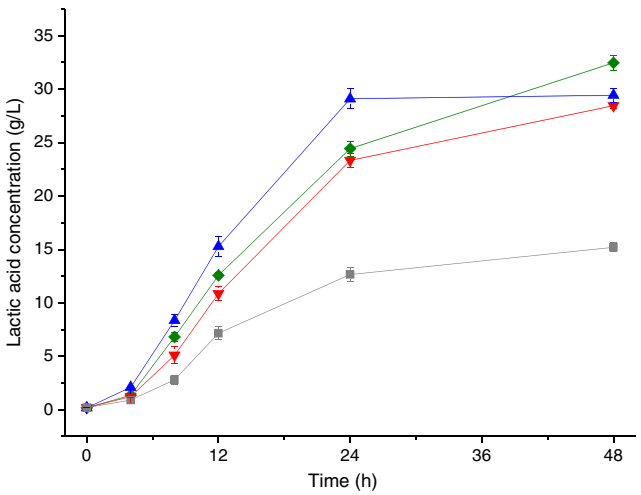

Fig. 6 Kinetics of LA production during closed and open LAF on stillage subjected to different treatments. Gray square, untreated stillage, non-inoculated, open LAF; blue up triangle, non-thermal PT, inoculated by *L. rhamnosus* ATCC 7469, open LAF; green diamond, UT, inoculated by *L. rhamnosus* ATCC 7469, open LAF; red down triangle, sterilized stillage, inoculated by *L. rhamnosus* ATCC 7469, closed LAF

In closed LAF, 97.2% of produced LA was L (+) isomer, while in the open LAF with PT, 95.5% of produced LA was L (+) LA, suggesting the prevalence of L (+) LA-producing species after PT. The stereoselectivity of produced LA was lower in other samples. Although the final concentration was higher after UT, the diversity of LA-producing strains (Fig. 5) resulted in a less stereoselective LA production, which is not desired. The highest values of LAF parameters were obtained in an open LAF performed after PT, being around 20% higher than in closed LAF with sterilized media (Table 2). Therefore, PT could be recommended as a good alternative to sterilization in order to achieve higher overall LA production with still high stereoselectivity. Inoculation with *L. rhamnosus* was necessary at the beginning of fermentation, otherwise the productivities attained solely by stillage microbiota were very low (Table 2).

The interaction of PT and UT with the stillage used for LAF has not previously been studied, especially in the context of substrate pretreatments for open fermentations. The open LAF by *Bacillus* sp., *Lactobacillus* sp., or *Streptococcus* sp. was studied, but without physical pretreatments of substrate (Ouyang et al. 2013; Pleissner et al. 2017; Tang et al. 2016). The highest LA productivities of around 2 g/Lh have been obtained in open LAF on a synthetic substrate by *Enterococcus mundtii* U 25 (Abdel-Rahman et al. 2013b) and on food waste by *Streptococcus* sp. (Pleissner et al. 2017). On substrates more similar to stillage, the average LA productivity of 1.04 g/Lh was obtained in an open fed-batch fermentation on lignocellulosic hydrolysates by *Bacillus* sp. NL01 (Ouyang et al. 2013), while on mixed restaurant food waste, a maximal reported LA productivity by *Lactobacillus* sp. was between 0.27–0.53 g/Lh (Pleissner et al. 2017). These are significantly lower values than the LA productivity of 1.2 g/Lh obtained in a batch LAF on the stillage after PT (Fig. 6, Table 2). The optimal growth temperature of *Bacillus* spp. at 50 °C is significantly higher than the optimal temperature for *Lactobacillus* spp., which are generally the

Table 2 Important parameters of open and closed LAF performed on distillery stillage media

Characteristics of LAF	Distillery stillage treatment	LA concentration[a] (g/L)	LA yield (g/g)	LA volumetric productivity[a] (g/Lh)
Open LAF[b]	Non-thermal PT[c]	29.09 ± 0.94	0.82 ± 0.03	1.21 ± 0.04
	UT[d]	24.43 ± 0.71	0.69 ± 0.02	1.02 ± 0.03
Closed LAF[b]	Sterilization	23.33 ± 0.68	0.66 ± 0.02	0.97 ± 0.03
Open LAF	Untreated	13.66 ± 0.65	0.38 ± 0.02	0.57 ± 0.03

[a] After 24 h of LAF

[b] Samples inoculated by *L. rhamnosus* ATCC 7469 after treatments

[c] 30-min PT

[d] 10-min UT

most exploited for effective LAF (Abdel-Rahman et al. 2013a). A relatively low growth temperature of 30–40 °C, common for many bacteria, is the obstacle for wider application of open fermentations with lactobacilli, leading to contaminations and related problems with productivity and optical purity of obtained LA. Therefore, employing an effective and selective PT for microbial inactivation can bring significant benefit and enable open fermentation by other, already proven, high-LA-producing strains.

L. rhamnosus ATCC 7469, used in our study to produce LA, has a proven probiotic potential (Djukić-Vuković et al. 2015). After the separation of liquid fermentation broth with dissolved LA for extraction, solid remainings with probiotic biomass of *L. rhamnosus* ATCC 7469 could be used as a valuable additive for feed. It is demonstrated that, in open LAF, the selected PT can significantly decrease the number of undesired G (−) microorganisms (Fig. 3). Therefore, the PT could be also a valuable tool to increase the safety of biomass and solid remainings for utilization in animal nutrition.

Comparison of energy efficiency of different treatments

The estimated values of energy inputs of the performed pretreatments, as well as amounts of LA produced, are presented in Fig. 7a. After different treatments, all samples were subjected to LAF in the same way; therefore, only energy consumption of pretreatments was included in the calculation.

The lowest in energy consumption is non-thermal PT while, as expected, sterilization is the highest energy-consuming treatment. The values of energy consumption of PT, UT, and sterilization amounted to 0.0104 kWh, 0.025 kWh, and 0.0875 kWh, while the LA concentrations obtained were 29.09 g/L, 24.43 g/L, and 23.3 g/L, respectively. The productivity of closed LAF solely by *L. rhamnosus* strain did not justify a high cost of energy for medium sterilization. In the case of UT, only part of the energy spent during the treatment was actually delivered to the sample and this part is called actual energy of UT which is calculated based on calorimetric data for every studied sample (Herceg et al. 2015). Hence, although the UT was the second best regarding LA productivity, the actual energy of UT delivered to stillage media was lower than the energy spent for UT (0.025 kWh) and amounted around 0.008 kWh or 32.7%. Therefore, only one-third of spent energy was used in the process for improvement of LAF.

The capital costs for all three studied treatments were calculated. The PT was the most affordable in terms of energy consumption, but the cost of feed gas makes it the priciest (1.04 euro or 59.977 euro cents/g of LA produced), followed by sterilization (0.506 euro cents or 0.362 euro cents/g of LA produced) and UT (0.1446 euro cents or 0.0984 euro cents/g of LA produced). On this scale, the UT was the most cost effective. However, 99.96% of PT cost is due to the cost of feed gas and experimental setup used in our experiments. For application at a larger scale, closed atmosphere of Ar can be

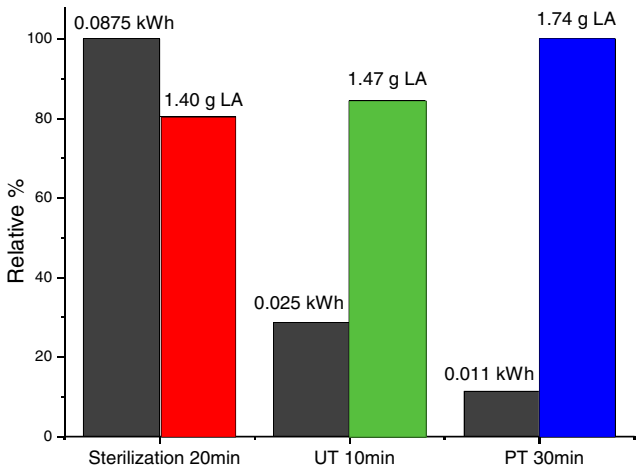

Fig. 7 Estimate of required energy for different processes at laboratory level and mass of LA produced. Dark gray bars, energy consumption for treatment in relative percentage, above bars is actual energy of treatment; red bar, LA produced in sterilized stillage, in relative percentage, above bar is actual mass of LA produced in 60-mL batch LAF; green bar, LA produced in UT stillage, in relative percentage, above bar is actual mass of LA produced in 60-mL batch LAF; blue bar, LA produced in PT stillage, in relative percentage, above bar is actual mass of LA produced in 60-mL batch LAF

applied, which can significantly decrease the cost of PT reducing the use of Ar by two orders of magnitude.

For application of the studied methods at a larger scale, further optimizations of treatments and LAF have to be done. For efficient extraction of LA from the fermentation broth, LA concentrations as high as possible are desired. Alternative and cheaper sources of sugars like molasses (Mladenović et al. 2018) should be combined with the stillage as a nitrogen source to provide higher LA concentration in media. Other strategies such as adaptation of LAB to substrate and process conditions or applying different fermentation modes (Pejin et al. 2017) could additionally improve the productivity and profitability of the process. The application of proposed UT and PT is promising and will be further studied for optimization at a larger scale.

Conclusion

The microbial inactivation by non-thermal PT is highly influenced by substrate. We find that there are two reasons for lower reduction in stillage than in water. The turbidity of the stillage is causing a lower penetration of plasma-generated agents, decreasing their microbial inactivation efficiency. The high concentration of antioxidants in the stillage also explains lower logN reduction, noticed in the stillage in comparison to water.

E. coli was more susceptible to PT than *L. acidophilus*, regardless of substrate. PT has shown selectivity toward G (−) microorganisms and a resistance to G (+) LAB. This recommends the PT as a promising technique for the control of microorganisms present in distillery wastewater.

By comparing PT and UT, we find that a 30-min-long PT shows superior characteristics of up to 2.5 logN reduction, while UT also induced a decrease in the number of viable microorganisms in stillage, but to a lower extent. A 20% higher LA productivity was achieved in open LAF by *L. rhamnosus* ATCC 7469 after PT than in open LAF after UT of distillery stillage. Besides being the most effective, the PT was the lowest in energy consumption and maintains a stereoselectivity of LA production, but in total costs it was a much more expensive technology at lab scale.

The PT provided the most effective revalorization of stillage through LAF with the highest LA productivity and the lowest energy consumption but was the highest in capital cost due to gas consumption in the studied experimental setup. This LA productivity was achieved in the open fermentation mode at a lower temperature than currently applied in processes. Further adaptations for PT in a larger scale could significantly influence effectiveness and improve the economy of processes with integrated PT including reduced cost for feed gas by application of closed Ar atmosphere.

Acknowledgements The authors want to acknowledge Milica Carević, PhD and Prof. Dejan Bezbradica, PhD, Faculty of Technology and Metallurgy, University of Belgrade, for their help in HPLC analysis of samples and Stevan Jovanović, ing., Insitute of Physics Belgrade, University of Belgrade, for his help in calculation of capital costs.

Funding information This work was supported by Serbian Ministry of Education, Science and Technological Development, project number TR 31017, project no. I-1/2018 of Scientific and Technological Collaboration of Republic of Serbia and PR China and III 43007.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdel-Rahman MA, Tashiro Y, Sonomoto K (2013a) Recent advances in lactic acid production by microbial fermentation processes. Biotechnol Adv 31:877–902. https://doi.org/10.1016/j.biotechadv.2013.04.002

Abdel-Rahman MA, Tashiro Y, Zendo T, Sonomoto K (2013b) Improved lactic acid productivity by an open repeated batch fermentation system using *Enterococcus mundtii* QU 25. RSC Adv 3:8437–8445. https://doi.org/10.1039/c3ra00078h

Anwar Saeed M, Ma H, Yue S, Wang Q, Tu M (2018) Concise review on ethanol production from food waste: development and sustainability. Environ Sci Pollut Res 25:28851–28863. https://doi.org/10.1007/s11356-018-2972-4

Baral NR, Shah A (2017) Techno-economic analysis of utilization of stillage from a cellulosic biorefinery. Fuel Process Technol 166:59–68. https://doi.org/10.1016/j.fuproc.2017.05.017

Boudam MK, Moisan M, Saoudi B, Popovici C, Gherardi N, Massines F (2006) Bacterial spore inactivation by atmospheric-pressure plasmas in the presence or absence of UV photons as obtained with the same gas mixture. J Phys D Appl Phys 39:3494–3507. https://doi.org/10.1088/0022-3727/39/16/S07

Caderby E, Baumberger S, Hoareau W, Fargues C, Decloux M, Maillard M-N (2013) Sugar cane stillage: a potential source of natural antioxidants. J Agric Food Chem 61:11494–11501. https://doi.org/10.1021/jf4039474

Djukić-Vuković AP, Mojović LV, Jokić BM, Nikolić SB, Pejin JD (2013) Lactic acid production on liquid distillery stillage by *Lactobacillus rhamnosus* immobilized onto zeolite. Bioresour Technol 135:454–458. https://doi.org/10.1016/j.biortech.2012.10.066

Djukić-Vuković AP, Mojović LV, Semenčenko VV, Radosavljević MM, Pejin JD, Kocić-Tanackov SD (2015) Effective valorisation of distillery stillage by integrated production of lactic acid and high quality feed. Food Res Int 73:75–80. https://doi.org/10.1016/j.foodres.2014.07.048

Djukić-Vuković A, Mladenović D, Radosavljević M, Kocić-Tanackov S, Pejin J, Mojović L (2016) Wastes from bioethanol and beer productions as substrates for l(+) lactic acid production - a comparative study. Waste Manag 48:478–482. https://doi.org/10.1016/j.wasman.2015.11.031

Du J, Chen L, Li J, Zuo R, Yang X, Chen H, Zhuang X, Tian S (2018) High-solids ethanol fermentation with single-stage methane anaerobic digestion for maximizing bioenergy conversion from a C4 grass

(Pennisetum purpereum). Appl Energy 215:437–443. https://doi.org/10.1016/J.APENERGY.2018.02.021

ePURE, 2017. EU ethanol in 2017. https://www.epure.org/media/1763/180905-def-data-epure-statistics-2017-designed-version.pdf Accessed 5 February 2019

European Parliament (2015) Directive (EU) 2015/1513. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015L1513 Accessed 5 February 2019

Fuess LT, Garcia ML (2014) Implications of stillage land disposal: a critical review on the impacts of fertigation. J Environ Manag 145:210–229. https://doi.org/10.1016/j.jenvman.2014.07.003

Gorbanev Y, O'Connell D, Chechik V (2016) Non-thermal plasma in contact with water: the origin of species. Chem Eur J 22:3496–3505. https://doi.org/10.1002/chem.201503771

Graves DB (2012) The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. J Phys D Appl Phys 45:263001–263042. https://doi.org/10.1088/0022-3727/45/26/263001

Herceg Z, Jambrak AR, Vukušić T, Stulić V, Stanzer D, Milošević S (2015) The effect of high-power ultrasound and gas phase plasma treatment on *Aspergillus* spp. and *Penicillium* spp. count in pure culture. J Appl Microbiol 118:132–141. https://doi.org/10.1111/jam.12692

Hulsmans A, Joris K, Lambert N, Rediers H, Declerck P, Delaedt Y, Ollevier F, Liers S (2010) Evaluation of process parameters of ultrasonic treatment of bacterial suspensions in a pilot scale water disinfection system. Ultrason Sonochem 17:1004–1009

International Council on Clean Transportation (ICCT) (2018) Final recast RED II: Renewable Energy Directive for 2021–2030 in the European Union. https://www.theicct.org/sites/default/files/publications/EU_Fuels_Policy_Update_20180719.pdf Accessed 5 February 2019

Issa-Zacharia A, Kamitani Y, Miwa N, Muhimbula H, Iwasaki K (2011) Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. Food Control 22:601–607. https://doi.org/10.1016/j.foodcont.2010.10.011

Jake N, Kwik J, Franko M (2016) System and process for clarifying thin stillage. US patent no. US 20170114293A1. https://patentimages.storage.googleapis.com/eb/e9/ee/3436bb354f66c4/US20170114293A1.pdf Accessed 5 February 2019

Jovanović JR, Stefanović AB, Žuža MG, Jakovetić SM, Šekuljica NŽ, Bugarski BM, Knežević-Jugović ZD (2016) Improvement of antioxidant properties of egg white protein enzymatic hydrolysates by membrane ultrafiltration. Hem Ind 70:419–428. https://doi.org/10.2298/HEMIND150506047J

Kang TS, Korber DR, Tanaka T (2014) Bioconversion of glycerol to 1,3-propanediol in thin stillage-based media by engineered *Lactobacillus panis* PM1. J Ind Microbiol Biotechnol 41:629–635. https://doi.org/10.1007/s10295-014-1403-x

Kasmi M, Hamdi M, Trabelsi I (2017) Processed milk waste recycling via thermal pretreatment and lactic acid bacteria fermentation. Environ Sci Pollut Res 24:13604–13613. https://doi.org/10.1007/s11356-017-8932-6

Koutinas A, Vlysidis A, Pleissner D, Kopsahelis N, Lopez Garcia I, Kookos IK, Papanikolaou S, Kwan TH, Lin CSK (2014) Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. Chem Soc Rev 43:2587–2627. https://doi.org/10.1039/c3cs60293a

Krásný I, Lapčík L, Lapčíková B, Greenwood RWR, Šafářová K, Rowson NA (2014) The effect of low temperature air plasma treatment on physico-chemical properties of kaolinite/polyethylene composites. Compos Part B Eng 59:293–299. https://doi.org/10.1016/j.compositesb.2013.12.019

Leonelli C, Mason TJ (2010) Microwave and ultrasonic processing: now a realistic option for industry. Chem Eng Process Process Intensif 49:885–900. https://doi.org/10.1016/J.CEP.2010.05.006

Li T, Chen X, Chen J, Wu Q, Chen G-Q (2014) Open and continuous fermentation: products, conditions and bioprocess economy. Biotechnol J 9:1503–1511. https://doi.org/10.1002/biot.201400084

Liao X, Liu D, Xiang Q, Ahn J, Chen S, Ye X, Ding T (2017) Inactivation mechanisms of non-thermal plasma on microbes: a review. Food Control 75:83–91. https://doi.org/10.1016/j.foodcont.2016.12.021

Liu B, Yang M, Qi B, Chen X, Su Z, Wan Y (2010) Optimizing l-(+)-lactic acid production by thermophile Lactobacillus plantarum As. 1.3 using alternative nitrogen sources with response surface method. Biochem Eng J 52(2–3):212–219

Ma R, Wang G, Tian Y, Wang K, Zhang J, Fang J (2015) Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. J Hazard Mater 300:643–651. https://doi.org/10.1016/j.jhazmat.2015.07.061

Mai-Prochnow A, Clauson M, Hong J, Murphy AB (2016) Gram positive and gram negative bacteria differ in their sensitivity to cold plasma. Sci Rep 6:38610. https://doi.org/10.1038/srep38610

Mladenović D, Pejin J, Kocić-Tanackov S, Djukić-Vuković A, Mojović L (2018) Enhanced lactic acid production by adaptive evolution of Lactobacillus paracasei on agro-industrial substrate. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-018-2852-x

Moestedt J, Påledal S, Schnürer A, Nordell E (2013) Biogas production from thin stillage on an industrial scale—experience and optimisation. Energies 6:5642–5655. https://doi.org/10.3390/en6115642

Noukeu NA, Gouado I, Priso RJ, Ndongo D, Taffouo VD, Dibong SD, Ekodeck GE (2016) Characterization of effluent from food processing industries and stillage treatment trial with *Eichhornia crassipes* (Mart.) and P*anicum maximum* (Jacq). Water Resour Ind 16:1–18. https://doi.org/10.1016/j.wri.2016.07.001

Ouyang J, Ma R, Zheng Z, Cai C, Zhang M, Jiang T (2013) Open fermentative production of l-lactic acid by *Bacillus* sp. strain NL01 using lignocellulosic hydrolyzates as low-cost raw material. Bioresour Technol 135:475–480. https://doi.org/10.1016/j.biortech.2012.09.096

Pejin J, Radosavljević M, Kocić-Tanackov S, Mladenović D, Djukić-Vuković A, Mojović L (2017) Fed-batch l-(+)-lactic acid fermentation of brewer's spent grain hydrolysate. J Inst Brew 123:537–543. https://doi.org/10.1002/jib.452

Pleissner D, Demichelis F, Mariano S, Fiore S, Navarro Gutiérrez IM, Schneider R, Venus J (2017) Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. J Clean Prod 143:615–623. https://doi.org/10.1016/j.jclepro.2016.12.065

Puač N, Miletić M, Mojović M, Popović-Bijelić A, Vuković D, Miličić B, Maletić D, Lazović S, Malović G, Petrović ZL (2015) Sterilization of bacteria suspensions and identification of radicals deposited during plasma treatment. Open Chem 13:332–338. https://doi.org/10.1515/chem-2015-0041

Purevdorj D, Igura N, Hayakawa I, Ariyada O (2002) Inactivation of *Escherichia coli* by microwave induced low temperature argon plasma treatments. J Food Eng 53:341–346. https://doi.org/10.1016/S0260-8774(01)00174-1

Ramazzina I, Berardinelli A, Rizzi F, Tappi S, Ragni L, Sacchetti G, Rocculi P (2015) Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit. Postharvest Biol Technol 107:55–65. https://doi.org/10.1016/j.postharvbio.2015.04.008

Research Grand View (2017) Lactic acid market & polylactic acid (PLA) market, industry report 2025, Market research report. https://www.grandviewresearch.com/industry-analysis/lactic-acid-and-poly-lactic-acid-market . Accessed 5 February 2019

Sakai K, Murata Y, Yamazumi H, Tau Y, Mori M, Moriguchi M, Shirai Y (2000) Selective proliferation of lactic acid bacteria and

accumulation of lactic acid during open fermentation of kitchen refuse with intermittent pH adjustment. Food Sci Technol Res 6: 140–145. https://doi.org/10.3136/fstr.6.140

Sangave PC, Pandit AB (2004) Ultrasound pre-treatment for enhanced biodegradability of the distillery wastewater. Ultrason Sonochem 11:197–203. https://doi.org/10.1016/j.ultsonch.2004.01.026

Sayedin F, Kermanshahi-pour A, He S(Q) (2018) Anaerobic digestion of thin stillage of corn ethanol plant in a novel anaerobic baffled reactor. Waste Manag 78:541–552. https://doi.org/10.1016/J.WASMAN.2018.06.015

Shan L, Liu J, Yu Y, Ambuchi JJ, Feng Y (2016) Characterization of persistent colors and decolorization of effluent from biologically treated cellulosic ethanol production wastewater. Environ Sci Pollut Res 23:10215–10222. https://doi.org/10.1007/s11356-016-6220-5

Srivastava A, Poonia A, Tripathi AD, Singh RP, Srivastava SK (2014) Optimization of nutritional supplements for enhanced lactic acid production utilizing sugar refinery by-products. Ann Microbiol 64: 1211–1221. https://doi.org/10.1007/s13213-013-0761-3

Surowsky B, Schlüter O, Knorr D (2015) Interactions of non-thermal atmospheric pressure plasma with solid and liquid food systems: a review. Food Eng Rev 7:82–108. https://doi.org/10.1007/s12393-014-9088-5

Tang J, Wang X, Hu Y, Zhang Y, Li Y (2016) Lactic acid fermentation from food waste with indigenous microbiota: effects of pH, temperature and high OLR. Waste Manag 52:278–285. https://doi.org/10.1016/j.wasman.2016.03.034

Tresp H, Hammer MU, Winter J, Weltmann KD, Reuter S (2013) Quantitative detection of plasma-generated radicals in liquids by electron paramagnetic resonance spectroscopy. J Phys D Appl Phys 46:435401. https://doi.org/10.1088/0022-3727/46/43/435401

Uchiyama H, Zhao QL, Hassan MA, Andocs G, Nojima N, Takeda K, Ishikawa K, Hori M, Kondo T (2015) EPR-spin trapping and flow cytometric studies of free radicals generated using cold atmospheric argon plasma and X-ray irradiation in aqueous solutions and intracellular milieu. PLoS One 10:e0136956. https://doi.org/10.1371/journal.pone.0136956

Vhangani LN, Van Wyk J (2016) Antioxidant activity of Maillard reaction products (MRPs) in a lipid-rich model system. Food Chem 208: 301–308. https://doi.org/10.1016/J.FOODCHEM.2016.03.100

West TP (2011) Malic acid production from thin stillage by *Aspergillus* species. Biotechnol Lett 33:2463–2467. https://doi.org/10.1007/s10529-011-0720-7

Wilkie AC, Riedesel KJ, Owens JM (2000) Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 19:63–102. https://doi.org/10.1016/S0961-9534(00)00017-9

Wu JM, Liu RH (2013) Cost-effective production of bacterial cellulose in static cultures using distillery wastewater. J Biosci Bioeng 115:284–290. https://doi.org/10.1016/j.jbiosc.2012.09.014

Yusaf T, Al-Juboori RA (2014) Alternative methods of microorganism disruption for agricultural applications. Appl Energy 114:909–923. https://doi.org/10.1016/j.apenergy.2013.08.085

Zaplotnik R, Bišćan M, Kregar Z, Cvelbar U, Mozetič M, Milošević S (2015) Influence of a sample surface on single electrode atmospheric plasma jet parameters. Spectrochim Acta B At Spectrosc 103–104: 124–130. https://doi.org/10.1016/j.sab.2014.12.004