Efficacy of polyextremophilic *Aeribacillus pallidus* on bioprocessing of beet vinasse derived from ethanol industries

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\textbf{A R T I C L E I N F O}

**Keywords:**  
Beet Vinasse  
*Aeribacillus pallidus*  
Bioprocessing  
Polyextremophilic  
Ethanol Production

\textbf{A B S T R A C T}

This work aimed to evaluate the applicability of *Aeribacillus pallidus* for the aerobic treatment of the concentrated beet vinasse with high chemical oxygen demand (COD 685 g.L\textsuperscript{-1}) that is defined as an environmental pollutant. This bacterium is a polyextremophilic strain and grow aerobically up to 7.5% vinasse at high temperature (50 °C). In the bioreactor and under controlled conditions, *A. pallidus* reduced the soluble COD content of 5% vinasse up to 27% during 48 h and utilized glucose and glycerol, completely. Furthermore, a reduction of manganese, copper, aluminum, and nickel concentrations was observed in the treated vinasse with *A. pallidus*. The obtained results make this strain as an appropriate alternative to be used for the aerobic bioprocessing of the vinasse.

1. Introduction

Nowadays, environmental pollution is an intricate issue and has significant effects on humans, health, wildlife, soil, water, and air. Life-threatening pollutions in the world originate from various sources such as industrial processes producing biohazardous and toxic wastes. In addition, the lack of robust and specific regulations in the countries causes polluting industries to release their untreated wastes in the environment. Moreover, limited resources such as tap water or freshwater are frequently used in the various steps of the manufacturing that exacerbates the global water crisis (Harmsen, 2013; Spellman, 2017). In this regard, cane or beet sugar-based ethanol distillation is one of the noticeable industrial processes produced large amount of wastewater of 9–20 L stillage per liter of produced ethanol, e.g. the estimation of Brazilian Ministry of Agriculture, Livestock and Food Supply showed a growth in the ethanol consumption by 58.8 billion liters that signifies the production of 588 billion liters of vinasse annually (Fernandes et al., 2017).

The stillage that is normally concentrated and named vinasse is considered as a soils and water pollutant due to its high content of chemical oxygen demand (COD), biological oxygen demand (BOD), mineral and organic materials. It is an acidic, odorous slurry that attracts stable flies (Jelvez Serra et al., 2017) with dark brown color containing yeast cells, unfermented sugars, organic acids, glycerol, heavy metals and inorganic ions such as potassium, sodium, calcium, magnesium, sulfate, and chloride (España-Gamboa et al., 2011; Hidalgo, 2009).

In general, vinasse can be applied as a substituted fertilizer, resource for the production of single-cell protein (SCP) used for animal feeding due to its rich composition (Christofoletti et al., 2013; Prado et al., 2013) and high value-added materials such as polyhydroxyalkanoate (Bhattacharyya et al., 2012), biocatalysts (Suhaili et al., 2019), cellulose (Velásquez-Riaño et al., 2013) and xylitol (Salgado et al., 2010) or as an energy source (Dirbeba et al., 2019; Ramos-Hernandez et al., 2016). Nevertheless, utilization of the vinasse is a two-edged sword so that, without any regulation and precaution, continuous discharging at higher dosage or even diluted into the soils gives rise to an irreparable damage resulting in destruction of agricultural products by increasing organic materials, disrupting ions exchange capacity, mobilizing metals, increasing salinity levels and decreasing trapped oxygen in the soil (Fuess et al., 2017; Sousa et al., 2019; Tejada et al., 2006). On the other hand, releasing untreated vinasse into various water bodies like rivers, groundwater, underground reservoirs or lakes is likely to disturb or inhibit aquatic life (Botelho et al., 2012; Christofoletti et al., 2013; Sousa et al., 2019) due to the utilization of dissolved oxygen and, in turn, severely pollutes them because of its phenolic compounds, melanoids, heavy metals and acidic pH (España-Gamboa et al., 2011; Zuurhier & van de Vooren, 2008). Therefore, using affordable and environmentally friendly methods for the treatment of this waste before disposal is indubitably required.
Comprehensively, there are many physicochemical methods to treat vinasse but most of them are expensive due to the high rate of the energy consumption and there are also possibilities for the production of more toxic and hazardous by-products during these treatments (Hoarau et al., 2018). However, biological treatments have been considered as promising and cost-effective approaches that recently improved to reduce the COD, color or phenolic compounds of the vinasse (Campos et al., 2014; España-Gamboa et al., 2012; Krzywonos et al., 2017). Although, the efficiency of anaerobic treatment systems for the high loading of organic materials in the wastes has been confirmed but these systems cannot completely remove all organics and toxic inhibitors like phenols may affect methanogens and reduce the rate of biogas production (López et al., 2018; Wilkie et al., 2000). Therefore, the implementation of an aerobic treatment system seemed to be a necessary pre-step.

Aerobic treatment of the vinasse has been carried out by using fungal, algal or bacterial genera that able to grow under mild conditions (Aparicio et al., 2017; Jiménez et al., 2003; Satyawali & Balakrishnan, 2008). However, the capability of these mesophilic microorganisms to tolerate industrial harsh conditions like high temperature is very limited (España-Gamboa et al., 2011; Seckbach et al., 2013) and this issue could be solved by substituting polyextremophiles. These microorganisms can survive or adapt to two or more than two extreme parameters that are undesirable for other organisms such as the genus Caldalkalibacillus members grow under high temperature condition and tolerate alkalinity at the same time. Recently, some polyextremophilic strains including thermoalkaliphilic have attracted more attention to be applied for the various biotechnological researches and bioprocesses as these microorganisms can resist extreme industrial conditions, reduce capital investment, and be considered as new substitutes for the biological treatments (Seckbach et al., 2013; Chen & Jiang, 2018). One of these fascinating polyextremophilic strains has been isolated recently from a hot spring that showed a wide range of tolerance to the environmental variables. It was able to produce hydrolyzing enzymes, lactic and acetic acids under different conditions and highly heat-resistant spores. This strain was identified and deposited as Aeribacillus pallidus CCUG 72355 and due to its potential applicability was subjected for the vinasse bioprocessing (Harirchi et al., 2020). Moreover, to the best of our knowledge, there are almost a few reports regards to the vinasse bioprocessing by polyextremophilic microorganisms.

The objective of this study was to assess applicability and efficacy of the novel polyextremophilic bacterial strain, A. pallidus CCUG 72355, for the aerobic treatment of the beet vinasse with high COD rate (685 g.L\(^{-1}\)) that could potentially be a perquisite of combined step for the existing anaerobic treatment. The previous study indicated this strain was able to grow under harsh conditions such as high temperature and salinity (Harirchi et al., 2020). Firstly, the effect of culture type (static or agitated), inoculum size and vinasse concentrations on the ability of A. pallidus for the consumption of the vinasse were studied. Subsequently, a bench-scale continuous stirred tank reactor was set up and vinasse biodegradation carried out based on the results obtained from shake flask experiments. Furthermore, a scenario was examined against this background that A. pallidus can grow optimally in the presence of 25 g.L\(^{-1}\) NaCl and therefore, concentrated vinasse could be diluted with the artificial marine water rather than distilled water. Furthermore, the produced extracellular polymeric substances (EPSs) of A. pallidus was characterized and checked for the trapping of suspended solids and decreasing of the heavy metals present in the vinasse.

### Table 1

**Characterization of the beet vinasse.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Sugars/organic acids</th>
<th>Concentration (g.L(^{-1}))</th>
<th>Heavy metals</th>
<th>Concentration (mg.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.7 ± 0.2</td>
<td>Arabinose</td>
<td>3.280 ± 0.113</td>
<td>Manganese (Mn)</td>
<td>4.5</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>31.27 ± 0.005</td>
<td>Cellobose</td>
<td>13.815 ± 0.092</td>
<td>Copper (Cu)</td>
<td>0.6</td>
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<td>Ash (%)</td>
<td>0.64 ± 0.0002</td>
<td>Glucose</td>
<td>7.465 ± 0.262</td>
<td>Aluminum (Al)</td>
<td>2.3</td>
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<tr>
<td>Total Chemical Oxygen Demand (COD) (g.L(^{-1}))</td>
<td>685 ± 21</td>
<td>Sugar Mix</td>
<td>14.790 ± 0.099</td>
<td>Nickel (Ni)</td>
<td>0.9</td>
</tr>
<tr>
<td>Total Soluble Chemical Oxygen Demand (sCOD) (g.L(^{-1}))</td>
<td>615 ± 21</td>
<td>Xylose</td>
<td>9.440 ± 1.004</td>
<td>Iron (Fe)</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>1.520 ± 0.566</td>
<td>Barium (Ba)</td>
<td>0</td>
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Values signify mean ± SD

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Concentrated beet vinasse was obtained from Sepahan Bio-Product Company (Isfahan, Iran) which is an industrial ethanol production unit. It was a dark brown and concentrated liquid with a slight caramel odor. It was produced by concentrating the stillage after distillation to remove c.a. 80% water by evaporation, not to biologically spoil during storage. Its characteristic was shown in Table 1. The chemical analysis of this vinasse was previously reported (Nair & Taberzadeh, 2016). The vinasse was kept at 4 °C before use. No additional nutrients or elements were added to the vinasse otherwise it is mentioned. All the experiments were performed with the same batch of vinasse to avoid the variability in the obtained results. Sterilization of the vinasse was carried out at 121 °C and 103 kPa for 20 min.

### 2. Material and methods

#### 2.1. Vinasse properties

Concentrated beet vinasse was obtained from Sepahan Bio-Product Company (Isfahan, Iran) which is an industrial ethanol production unit. It was a dark brown and concentrated liquid with a slight caramel odor. It was produced by concentrating the stillage after distillation to remove c.a. 80% water by evaporation, not to biologically spoil during storage. Its characteristic was shown in Table 1.

#### 2.2. Bacterial strain and inoculum preparation

The bacterial strain used in this work was A. pallidus strain Lhs-10 isolated from Larijan hot spring (Larijan, Iran). It was deposited as CCUG 72355 in Culture Collection University Of Gothenburg, Sweden and as IBRC-M 11202 in the Iranian Biological Resource Center, Iran. This polyextremophilic spore-forming strain was able to grow at high pH, temperature and salt (Harirchi et al., 2020). The cryopreserved stock of A. pallidus was activated by transferring one vial content to 100 ml fresh tryptic soy broth supplemented with 25 g.L\(^{-1}\) NaCl, pH 8. The cultivated medium was incubated in a shaking water bath (Grant VORSHAKER, Cambridge, UK) at 50 °C and 120 rpm for 18–24 h. The seed inoculum was prepared by growing fresh broth culture of A. pallidus in the diluted vinasse (2.5% v.v\(^{-1}\), pH 8) for 30 h at 50 °C and 120 rpm.

#### 2.3. Shake flask experiments

Initial batch experiments were carried out using 250 ml-Erlenmeyer flasks in the shaking water bath at 50 °C and 120 rpm for 48 h. Each flask contained 100 ml sterilized vinasse (pH 8) and inoculated by A. pallidus has grown in 2.5% vinasse (seed inoculum). The culture type (static or agitated) effect, inoculum size (1, 5, and 10% v.v\(^{-1}\)) and vinasse concentration (1, 2.5, 5, 7.5, 10, 20% v.v\(^{-1}\)) were studied in this series of experiments in triplicate. The abiotic blank was defined as...
the vinasse medium without any inoculum.

2.4. Bioreactor set-up and operation

A 2.0-L continuous stirred tank reactor (CSTR) (B-Braun Biotech International GmbH, Berlin, Germany) equipped with a stainless steel sparger, a radial impeller and a heating jacket was used (working volume 1.5 L). The CSTR was inoculated with 10% v.v\(^{-1}\) seed inoculum of *A. pallidus*. All experiments were carried out in duplicate and under aseptic conditions. The pH was controlled and monitored during the process running not to affect *A. pallidus* growth. Oxygen was supplied through a sterile HEPA filter at a flow rate of 1 vvm (air volume per liquid volume per minute) and medium level in the CSTR checked continuously to prevent liquid losses and retrieved by distilled water when required. The agitation speed and temperature were constant during the running time (48 h) and maintained at 250 rpm and 50 °C, respectively. Moreover, foaming production was controlled by adding 250 mg.L\(^{-1}\) sterile fatty acid ester known as an industrial anti-foaming agent, Sweden. The concentrated vinasse used in this experiment was diluted by distilled water up to 5% v.v\(^{-1}\). To assess the biodegradability of the vinasse under salinity conditions, it was diluted by artificial marine water instead of distilled water. This solution was contained (g.L\(^{-1}\)) FeCl\(_3\) 0.0668, NaCl 19.45, MgCl\(_2\)-6H\(_2\)O 5.9, Na\(_2\)SO\(_4\) 3.24, CaCl\(_2\) 1.8, KCl 0.55, NaHCO\(_3\) 0.16 and KBr 0.08.

2.5. Characterization of *A. pallidus* CCUG 72355 extracellular polymeric substances

The cells of *A. pallidus* after growing on the vinasse produced mucoid and ropy substance that was collected and processed according to the procedures described previously (Au-Felz et al., 2016; Liang et al., 2010). It was also checked for the alginate-like structure and ionic hydrogel formation (Au-Felz et al., 2016).

2.6. Analytical methods

In order to investigate organic acids and soluble carbohydrates concentrations in the vinasse, samples were collected during treatments in the appropriate intervals and supernatants were filtered (filter pore size 0.45 μm, Sartorious, Germany) and analyzed through High-Performance Liquid Chromatography (HPLC) (Waters 2695, Waters Corporation, USA) with a refractive index detector (RID) (Waters 2414, Waters Corporation, USA). A hydrogen-based ion-exchange column (Aminex HPX-87H, Bio-Rad, Hercules, USA) was used in the HPLC unit worked at 60 °C with 5 mM H\(_2\)SO\(_4\) eluent flowing at 0.6 mL.min\(^{-1}\). For the analysis of other sugars such as arabinose, xylose, and galactose a lead-based column (HPX-87P, Bio-Rad, Hercules, USA) as well as two de-ashing and normal Micro-Guards (Bio-Rad, Hercules, USA) were used which operated at 85 °C with water as eluent flowing at 0.6 mL.min\(^{-1}\). Total solids (TS) and ash percentage were measured as defined in the Standard Methods for Examination of Water and Wastewater (A.P.H.A. et al., 2005). The total COD (tCOD) and soluble COD (sCOD) were measured based on the photometric determination via chemical oxidation with potassium dichromate/sulfuric acid at 148 °C for 2 h by using a Nanocolor COD 15,000 kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). To calculate dried biomass weight of samples, 5 ml wet biomass was centrifuged and the pellet was washed three times with deionized water, transferred to the weighted aluminum cup and then dried at 105 °C oven until constant weight. The biomass yield and productivity were calculated according to the equations described previously (Najafpour, 2015; Santos et al., 2019). Moreover, the metals content of the vinasse was determined by the microwave plasma atomic emission spectroscopy (MP-AES 4200, Agilent Technologies, Santa Clara, CA, USA).

3. Results and discussion

3.1. Vinasse consumption by *A. pallidus* CCUG 72355

Many factors may affect bacterial growth on a substrate. The main factors such as temperature, pH, and salinity were previously determined for *A. pallidus* (Harirchi et al., 2020) and were constant during this set of experiments. Other factors including vinasse concentration, culture type, and inoculum size were chosen to be studied here.

The batch shake flask experiments using vinasse were carried out without any extra nutritional supplementation, but the vinasse was diluted with distilled water to 1, 2.5, 5, 7.5, 10 and 20 (% v.v\(^{-1}\)) to be examined for the *A. pallidus* growth. The temperature and initial pH were set at 50 °C and 8, respectively. The most growth of the strain was detected in the presence of 1% v.v\(^{-1}\) vinasse with 0.76 ± 0.5 g.L\(^{-1}\) dried biomass. However, the growth was observed in 2.5% and 5% v.v\(^{-1}\) vinasse by 0.29 ± 0.06 g.L\(^{-1}\) and 0.19 ± 0.02 g.L\(^{-1}\) dried biomass, respectively. No growth was observed on 10 and 20% v.v\(^{-1}\) vinasse that may refer to the high TCOD rate of this vinasse (685 g.L\(^{-1}\)) and other compounds inhibit the growth. Therefore, vinasse dilution is a prerequisite function before applying the vinasse in any aerobic or anaerobic biological treatment process (López et al., 2018; Ryznar-Luty et al., 2008).

The culture type and inoculum size (1, 5, and 10% v.v\(^{-1}\)) affected the vinasse consumption by *A. pallidus*, whereas vinasse was not utilized under static conditions. Moreover, the higher inoculum size, the more rapid vinasse consumption was taken place as the lag phase decreased meaningfully from 12 h to 6 h by 10% inoculum size. So, this size was selected for the subsequent experiments.

3.1.1. HPLC analysis of vinasse

The HPLC analysis of the vinasse showed *A. pallidus* was able to utilize carbohydrates and organic acids existing in the vinasse during the incubation time by this order; firstly, glucose, glycerol, and lactic acid and acetic acid (Fig. 1). As there were not any changes regarding the xylitol and ethanol utilization, these compounds were shown in E-supplementary data. Interestingly, it was observed an increase in the acetic acid concentration in the vinasse profile after 12 h that it referred to the acid production ability of *A. pallidus* from sugars (Harirchi et al., 2020). But, the production of acetic acid in 1% v.v\(^{-1}\) vinasse did not follow the same pattern as other concentrations of the vinasse. It was produced after 6 h and decreased gradually with the increase of incubation time to support the growth due to the low concentration of other utilizable substrates (Fig. 2a). The lactic acid was

![Fig. 1. Carbohydrates and organic acids utilization by *A. pallidus* CCUG 72355 in 2.5% v.v\(^{-1}\) beet vinasse during 48 h at 50 °C. The inoculum size was 10% v.v\(^{-1}\). Fig. characterizes glucose (green), lactate (red), glycerol (purple), and acetate (blue).](image-url)
consumed progressively after 12 h however; in 1% and 2.5% vinasse, it was produced as growth metabolite and then consumed when the carbohydrates existing in the vinasse were utilized by this strain (Fig. 2a and 2b). It should be noted that this strain was able to have a metabolic shift from lactate to acetate when growing in the glucose under aerobic conditions (Harirchi et al., 2020). Moreover, the genus Bacillus and related genera can produce acid(s) via the oxidation pathway (Vos et al., 2011). Fig. 2c and 2d indicated that the utilization profiles of 5% and 7.5% v.v−1 vinasse were relatively similar together but less changes were observed in 7.5% v.v−1 vinasse due to high concentration of the other substrates such as glycerol or other compounds existing in the vinasse. As a result, the vinasse carbohydrates and latterly acids could be utilized by A. pallidus under high temperature and pH (50 °C, 8). Also, at the end of the process, the pH depletion was observed in 2.5, 5 and 7.5% v.v−1 vinasse due to acetic acid production (E-supplementary data). The ability of A. pallidus for the acetic acid production from the vinasse could be investigated more for the making of a di-icing agent called calcium magnesium acetate (Wilkie et al., 2000).

3.1.2. Determination of A. pallidus CCUG 72355 efficacy in the vinasse treatment

The effectiveness of A. pallidus in the aerobic treatment of the vinasse was determined by the magnitude of reduction in tCOD and sCOD content of treated vinasse by the end of the process. The results showed in 1% v.v−1 vinasse, the tCOD and sCOD contents were decreased by 29.2% and 36.6%, respectively, however, in 2.5% and 5% v.v−1 vinasse concentrations, the reduction percent of the tCOD was approximately equal (24.1%). Statistical analysis was done by SPSS V24 and standard deviation (SD) defined ± 25 and ± 12 for the tCOD and sCOD contents, respectively. Error bars were measured and shown in Fig. 3a and 3b, as well. As the figures illustrated there was a significant difference between the control and 1% v.v−1 vinasse (p-value < 0.002; while the reduction percentage was 29.2%). Furthermore, p-value was lower than 0.0001, when the reduction percentage was 36.6% (Fig. 3b). This results showed as the vinasse concentration (7.5% v.v−1 vinasse) raised the tCOD and sCOD contents did not decrease more than 19.7% and 15.3%, respectively and there was not a significant difference between the test and control (Fig. 3a and 3b). Furthermore, in comparison to the previous studies, the COD content of applied vinasses was too lower than the vinasse used in this study that made the COD removal efficiency much higher (Robles-González et al., 2012; Zhang et al., 2013).

As expected, the ability of A. pallidus in acid production during the process imposed a penalty for more COD reduction, whereas this strain could grow in the vinasse without any additional supplementary compounds. Nevertheless, in the previous studies, microbial strains applied for the molasses or vinasse COD reduction required extra supplementations such as glucose or other easily degradable carbon sources for improving growth that could affect the treatment process economically. Moreover, they experienced a longer treatment time compared to what practiced by the application of A. pallidus in this study (Sirianuntapiboon et al., 2004; Zhang et al., 2013). Shorter treatment period lowers energy consumption and increases the economic feasibility of the process (Shin et al., 2010; Stuckey, 2012).

3.2. Cultivation of A. pallidus on the vinasse in the bioreactor under controlled condition

According to the observed results of shake flasks experiments,
where the vinasse was diluted to 1% v.v⁻¹, the concentration of the carbohydrates decreased dramatically and the changes were not accurately detected. Moreover, lactate production was observed in 1 and 2.5% v.v⁻¹ vinasse that resulted in the increasing of COD content and the higher vinasse concentrations (7.5%, 10% and 20% v.v⁻¹) did not support well growth of *A. pallidus*. Therefore, 5% v.v⁻¹ of the vinasse was chosen to be used for the bioreactor experiments. Bacterial growth and bioprocessing that occurred in this set of experiments exhibited comparatively similar results as obtained in the shake flask experiments.

The biomass yield of *A. pallidus* on 5% v.v⁻¹ vinasse was 0.25 g.g⁻¹ during 48 h at 1 vvm of aeration while it was 0.09 g.g⁻¹ when cultivated in the standard glucose broth containing 10 g.L⁻¹ glucose at the same time and conditions. The cellular volumetric productivity was 0.04 g.L⁻¹.h⁻¹ on the vinasse which was twice higher than the value on the glucose broth (data was not shown). These results indicated, even though this vinasse had a high rate of organic compounds but it could be easily utilized by *A. pallidus* in the CSTR system in comparison to the standard glucose broth. Additionally, the carbohydrates and acids profile of vinasse during the bioprocessing with *A. pallidus* was shown in Fig. 4.a. The glucose and glycerol were considerably decreased after 6 to 12 h as the reduction percent was 100%, 61.5%, and 95.9%, respectively at the end of the process. Also, lactic acid amount reduced by 75.7% while acetic acid was increased from 1.6 g.L⁻¹ up to 5.6 g.L⁻¹ as this finding supported by the constant COD reduction rate during 24 to 36 h of the treatment (E-supplementary data). The results showed that the aerobic bioprocessing of the beet vinasse with very high COD content (685 g.L⁻¹) by *A. pallidus* in a bench-scale CSTR could decrease the most of organic materials in a short time (48 h) at 50 °C and high pH value while in a bench-scale up-flow anaerobic sludge bed (UASB) reactor working with Bella Unión vinasse (COD content 36 g.L⁻¹) and an average load of 10.5 Kg COD.m⁻³.d⁻¹ the average removal of this low COD content was 69% during 4 months. However, the UASB reactors may confront the deprived sedimentability of the inoculum or toxic compounds of the vinasse inhibit the biogas production (López et al., 2018). Furthermore, in another study carried out by Del Gobbo et al. COD removal of the sugarcane vinasse (COD content 42.1 g.L⁻¹) was occurred after 12 d by *Aspergillus* sp. V1 under mild conditions (Del Gobbo et al., 2019). Thoroughly, this result indicated the applicability of *A. pallidus* for the fast aerobic bioprocessing of the vinasse under high temperature and alkalinity.

![Fig. 3. COD removal by A. pallidus CCUG 72355 in the various concentration of the beet vinasse at the end of the process at 50 °C. The inoculum size was 10% v.v⁻¹. a) The tCOD removal in different vinasse concentrations in comparison to abiotic control. b) The sCOD removal in different vinasse concentrations in comparison to abiotic control. Error bars indicates significant differences.](image-url)

### 3.3. The effect of artificial marine water in the bioprocessing of the vinasse

When the vinasse is releasing in the environment it is necessary to be diluted to 1–2% (España-Gamboa, et al., 2011) but this consumes limited water resources in the world and it is not cost effective. Regarding to the global water crisis and salt tolerance ability of *A. pallidus* up to 100 g.L⁻¹, the scenario of using marine water instead of distilled water for the dilution of the vinasse was examined. For this purpose, artificial marine water was prepared with the total salts concentration c.a. 3% w/v and the vinasse diluted by this solution up to 5% v.v⁻¹. The CSTR was run under the same conditions used for the diluted vinasse with distilled water and the results indicated that the utilization of the carbohydrates and organic acids existing in the vinasse was similar with previous obtained results (Fig. 4b). However, some differences were observed in the biomass yield, cellular volumetric productivity, vinasse profile and COD reduction (E-supplementary data) as biomass yield and productivity were defined 0.1 g.g⁻¹ and 0.02 g.L⁻¹.h⁻¹, respectively that were approximately twice lower than the obtained values for 5% v.v⁻¹ vinasse diluted with distilled water. Also, carbohydrates consumption rate was slower in this experiment as the glucose was utilized during 24 h in comparison to 12 h when the vinasse was diluted by distilled water. But, the glucose and glycerol were completely reduced by 100% and it was determined 63.8% for the lactic acid. In this condition, acetic acid was continuously increased from 1.5 g.L⁻¹ to 5 g.L⁻¹ that was comparable with the results observed for the diluted vinasse with distilled water (Fig. 4b). As a consequence, vinasse could be diluted with artificial marine water instead of distilled water but it should be mentioned that the raw vinasse has a salty content and when it is diluted with the marine water, it would get more salty and discharging of this treated vinasse into the soil or water should follow the existing regulations.

### 3.4. The role of extracellular polymeric substances produced by *A. pallidus* CCUG 72355 in the treatment of the vinasse

#### 3.4.1. Extracellular polymeric substances extraction

The cells of *A. pallidus* cultivated on the different concentrations of the vinasse showed the ability of slime and ropy material formation. Having analyzed the extracted EPSs including soluble EPSs, loosely bound EPSs and also tightly bound EPSs with HPLC showed that no simple sugars like mannose, glucose, galactose, cellobiose or arabinose were detected; however, the acidified extracted EPSs showed precipitation properties in the presence of sodium hydroxide solution that could be considered as an alginate-like extracellular polymer (ALEP) based on Lin et al., 2010 and Au-Felx et al., 2016. Moreover, this extracted polymeric substance was subjected to the ionic hydrogel formation test and formed stable beads due to its anionic nature when were dipped in the calcium chloride solution without any dispersion up to the one week at 4 °C in deionized water.

![Image of extracellular polymeric substances extraction](image-url)
3.4.2. Heavy metal removal by extracellular polymeric substances in the vinasse

As known, bacterial EPSs play a significant role in the removal and detoxification of heavy metals. It was obviously confirmed in many bacterial species (Gupta & Diwan, 2017; Zhang et al., 2017). Hence, it was assumed EPSs produced by *A. pallidus* can remove the heavy metals existing in the vinasse. The heavy metals profile of this vinasse was characterized in Table 1 and it did not show any special differences with previously reported heavy metals detected in the vinasse (Bernal et al., 2017; Christofoletti et al., 2013). For this purpose, the number of heavy metals was measured by MP-AES at the end of the incubation period (48 h) both in shake flask and bioreactor experiments and the results were compared to the control (untreated vinasse). Among the detected metals by MP-AES, only manganese (Mn), copper (Cu), aluminum (Al), and nickel (Ni) showed a reduction in their amounts in comparison to the control (Fig. 5). These results revealed that the manganese was decreased in the samples with 1, 2.5, 5, and 7.5% (v.v⁻¹) vinasse as its amount was below the detection limit on the MP-AES. In bioreactors, the observed results were similar to the shake flasks samples and showed a reduction related to the four mentioned cations (Mn, Cu, Al, and Ni). But the reduction percent (60%) for the Mn in the bioreactors ran with artificial marine water and 5% vinasse was lower than the same in the shake flask experiments that may be related to the different aeration rate, artificial marine water composition and agitation speed affecting the EPSs production (Moghannem et al., 2018). In addition to heavy metals reduction, this EPSs could trap TS existing in the vinasse at high temperature and pH can be considered as an advantage for the bioprocessing of the vinasse under aerobic condition that may apply as a pretreatment step to decrease toxic metals and particles that may interfere in the anaerobic processing of the vinasse. Moreover, the produced EPSs can be used as a value-added material for the stabilization of soils in the arid and semiarid areas (Costa et al., 2018) though; this potential application requires more investigations and field studies.

4. Conclusion

Having considered a sustainable method with low capital investment and appropriate feasibility, polyextremophilic *A. pallidus* used in this study represented a suitable option for the fast aerobic bioprocessing vinasse. This may reduce SCOD by 27% at 50 °C during 48 h. The growth ability of *A. pallidus* up to 100 g.L⁻¹ NaCl made it possible to use salty water for the dilution of concentrated vinasse. Moreover, produced EPSs of *A. pallidus* seemingly reduced the heavy metals and trapped the TS existing in the vinasse resulting in the mitigation of its impacts when disposed into the environment.

CRediT authorship contribution statement

Sharareh Harirchi: Validation, Formal analysis, Investigation, Writing - original draft, Visualization. Zahra Etemadifar: Methodology, Data curation, Writing - review & editing, Supervision, Funding acquisition. Fatemeh Yazdian: Software, Writing - review & editing. Mohammad J. Tahirzadeh: Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References


