Selected experiences in Chile for the application of UASB technology for vinasse treatment

R. Chamy, C. Pizarro, E. Vivanco, M.C. Schiappacasse, D. Jeison, P. Poirrier and G. Ruiz-Filippi
School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso. General Cruz 34, Valparaíso, Chile (E-mail: *rchamy@ucv.cl*)

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Abstract One of the research areas is the agricultural use of treated wastewaters, because it represents a unique opportunity to solve the problem of water supply for irrigation and at the same time the disposal of treated water. Anaerobic digestion appears as an interesting alternative, since anaerobically treated wastewaters can be used for irrigation purposes. These considerations are applied to the Chilean pisco industry (a traditional alcoholic drink, prepared by distillation of wine made mainly from Muscatel grapes), where high concentrated wastewaters are produced: vinasses originate as a residue from the distillation operation.

Two laboratory reactors fed with wine vinasses, a UASB and an EGSB, were used in order to study the anaerobic treatability of the wastewater. Then, a pilot reactor was built (60 m^3 UASB digester) and treated water was used to irrigate eucalyptus trees. Finally a 300 m^3 reactor, including biogas treatment for its reuse, was developed. Results showed, both at laboratory and full scale, that anaerobic treatment is suitable for pisco's wastewaters, and also that the nutrient content of treated water can be beneficial for plant growth, reducing the need for fertilizers.

Another kind of investigation was carried out in order to study the stability of anaerobic granules and how it can be recovered. UASB and EGSB were fed with low, medium and high load wastewaters, in order to evaluate possible fluctuations in the productive process. From these results, it was possible to propose and to apply recovery techniques to the digesters when they are destabilized.

Keywords EGSB; granule; pisco; stability; UASB; vinasses

Introduction

Water supply represents an important and unfinished task for developing countries that should be solved in the near future. Several challenges, and at the same time, opportunities, for a solution are at hand. Problems like adequate treatment and disposal of agro-industrial wastewaters, and search for water sources for irrigation, are situations that can be related and confronted at the same time, with the selection of the right technology. In the search of this technology anaerobic digestion appears to be an interesting alternative, since it has proven to offer a suitable treatment for a wide spectrum of industrial wastes (Speece, 1996; Lettinga *et al.*, 1997). Besides, anaerobically treated wastewaters can be used for irrigation purposes, because they generally contain important amounts of nutrients (like phosphorus and nitrogen), that can be profitable for agriculture. Thus anaerobic treatment of agroindustrial wastewaters and their utilization for irrigation can solve different kinds of problems:

- an adequate treatment of wastewater is provided, reducing environmental impact of pollutants;
- there is no need for post treatment since the presence of nutrients is desired;
- nutrient content of treated wastewater reduces fertilizer requirements;
- problems related to correct disposition of treated water are solved; and
- important quantities of water are available for irrigation, with no cost.

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Many of these considerations are applied to the Chilean pisco industry. Chilean pisco is an aged drink, prepared by distillation of wine made from mainly Muscatel grapes. As in any distillation process, it produces high concentrated wastewaters: vinasses originated as a residue from distillation operation. Residual process waters from cleaning operations of maceration, sedimentation and fermentation tanks are also generated.

Analyzing Chilean pisco industry characteristics, it is clear that this activity offers a great opportunity for taking advantage of almost all positive properties of anaerobic digestion for wastewater treatment, e.g. biogas can be used in steam production required for distillation; sludge can be employed for soil improvement for agricultural purposes; treated water can be used for agricultural irrigation. This last point is extremely important in the case of Chilean pisco since water availability is low in the region where pisco production is located. Finally, the ability of anaerobic digestion for being stopped for long periods of time is also valuable since pisco industry has almost no activity with non-waste generation for 3–5 months each year.

The granulation phenomenon may represent a crucial issue for the stable performance of anaerobic reactor technologies such as UASB and EGSB. Several factors have been reported as exerting influence on granulation, among which seed characteristics, wastewater type, reactor configuration or hydrodynamics are counted. Microorganisms growing within reactors, e.g. within granules, represent the final recipient for all these effects, acting themselves on both the micro- and macro-environmental conditions inside the reactor, for example, by producing and excreting extracellular polymeric substances (EPS), which are considered essential towards the adhesion between different species present in granules. The EPS production enhancement has been associated, among other factors, to the addition of external polymers (El-Mamouni *et al.*, 1998). The important advance observed during recent years in the application of molecular tools in the field of microbial ecology in anaerobic digesters (Raskin *et al.*, 1994; Godon *et al.*, 1997; Sekiguchi *et al.*, 1998; Wu *et al.*, 2001) offers the potential to correlate operational performance and environmental conditions to microorganisms' diversity and abundance.

This research summarizes the application of anaerobic digestion processes in Chile, using as an example the cumulative experience for the treatment of wastewater from the Chilean pisco industry. This article presents the results obtained from laboratory scale (5 L reactor) to full scale (300 m^3 reactor), focusing on benefits related to reuse of treated water for irrigation purposes and studies on granule stability.

Methodology

Analytical methods

Specific methanogenic activity (SMA) of biomass was determined following the methodology described by Soto *et al.*, (1993). EPS extraction and determination was performed as described in Puñal *et al.* (2003). RNA purification was carried out following the proposal by Summer (1970) and for Dot blot hybridization the methodology presented by Reddy and Gilman (1993) was followed. COD, VSS and CH_4 analysis were made according to *Standard Methods* (1992).

Laboratory study

Two 5 L laboratory reactors, an UASB and a EGSB, were used in order to study the anaerobic treatability of the wastewater. EGSB and UASB reactors were operated at 30 °C, for a period of 8 months, at superficial liquid velocities of 7 and 0.8 m/h, respectively. These values were obtained by applying different levels of recirculation rates. Both reactors were fed with wine vinasses (Table 1), obtained periodically from pisco industry. The vinasses were kept refrigerated at 4 °C until they were used to feed the reactors presents the characterization of wine vinasses. Both digesters were seeded with anaerobic granular sludge from a full scale UASB treating brewery wastewater. pH was controlled by the addition of sodium bicarbonate, at a concentration of 2 g/L.

For granule stability, a synthetic complex-medium-concentrated wastewater (MCW) described elsewhere (Puñal *et al.*, 2003) was supplied during reactors start up (days 0–50) and granule maturation period (days 51–122). Diluted beer was used as a low-concentrated substrate (LCW) during the destabilization (days 123–200) and recuperation (days 201–242) periods. Pectin was added directly to the bottom of reactors in four injections (days 200, 203, 211 and 217) of 1 g in order to study its ability to recover granule stability.

Pilot study

Based on a complete wastewater characterization performed during the grape harvest of 1998, a 60 m^3 UASB digester was designed and built in the smallest production plant of the pisco company. This reactor is considered full-scale for this production plant since it was conceived to treat all wastewater generated, but at the same time as a pilot installation for the company, since positive results can signify the application of treatment technology to the rest of production installations. The reactor was started up with sludge obtained from a full scale UASB digester, treating brewery wastewater. During the start-up period, the reactor was fed only with vinasses. Afterwards it began to treat all wastewater generated in the production plant. pH was controlled on line by the addition of sodium hydroxide. To study the use of treated water for agricultural applications, 3000 eucalyptus were planted in a terrain beside the production plant. Corn was also planted with the purpose of serving as support for growing trees. The growth of this tree plantation is being followed for confirming the results obtained in laboratory experiments.

Full-scale study

A 300 m^3 UASB digester was designed and built using an adapted wine cube as a reactor. An inoculum obtained from the pilot UASB digester was used. This reactor was operated at 20 °C. Afterwards it began to treat all wastewater generated in the production plant. pH was controlled on line by the addition of sodium hydroxide.

Irrigation of treated wastewater study

Several experiments were performed to analyze irrigation properties of treated wastewater. Lemon nursery plants were used as a standard culture. Several irrigation regimes and fertilization degrees were tested, using water, wastewater and anaerobically treated wastewater. Irrigation was determined as a class A pan evaporation percentage (FAO, 1997). Fertilization was performed every two days through the irrigation water and weekly by foliar applications and was determined as a percentage in relation to the

Table 1 Vinasse characterization

Parameter	Units	Value
Total COD	mg/L	37,800
Soluble COD	mg/L	34,400
BOD	mg/L	13,500
Total solids	mg/L	25,226
Volatile solids	mg/L	20,588
Total suspended solids	mg/L	1,526
Volatile suspended solids	mg/L	1,495
Acidity	mg $CaCO_3$/L	1,719
pH		3.0

standard requirements of a nursery plant. Supplementary experiments were carried out using different levels of dilution of the treated wastewater (25, 50, 75 and 100%), in order to evaluate the effect of its salts content. An irrigation of 400% was used in these experiments. Parameters obtained during plant growth were the following: (a) growing of apical shut and diameter of stem at 20 cm height, in reference to the moment in which the plant is ready to be grafted (commercial factor); (b) amount, pH and electrical conductivity of water percolation; and (c) class A pan evaporation (mm/day) to program daily irrigation.

Results

Stability analysis at laboratory scale

In Table 2 a detailed description of operational condition and parameters is presented. Similar performances were observed when treating MCW during start up and granules maturation periods, whereas a slower start up was observed in the UASB reactor as indicated by the lower average for COD removal efficiencies, although biomass SMA at the end of the period was the same in both systems. During the granule maturation period similar performances were obtained in both reactors with higher biomass SMA in the UASB reactor. After substrate shift at day 122, the applied organic loading rate was progressively increased from 5 to 11 kg COD/m^3 d, observing destabilization signs in both systems, more acute in the UASB reactor, as indicated by the final COD removal efficiency, SMA and pH values (Table 3). At day 200, the first pectin injection was performed by supplying 1 g to each reactor (0.38 and 0.39 g/L in UASB and EGSB reactors, respectively). Further injections, supplying the same amount of polymer, were added on days 203, 211 and 217. Both reactors showed a slow recuperation, being necessary, from day 211 to 217, to regulate the influent pH in the UASB reactor to 7.2, when a pH of 5.7 was measured within the reactor. pH regulation was not required in the EGSB reactor. Reactor performances increased progressively until the end of this study attaining 65 and 72% of COD removal capacity in the UASB and EGSB reactors, respectively. However, pH and SMA values still indicate the effect of destabilization.

No effect exerted by the addition of pectin on the content of EPS extracted from granules was detected until day 217 (17 days after the first injection), observing on this day an increase in EPS total concentration to a similar extent in both reactors (from 100 to 300 mg/g$_{VSS}$).

Protein was the main compound of EPS during the whole operation, followed by total polysaccharides, although the ratio between these substances showed a different behavior in reactors UASB and EGSB during the different periods of operation. While in granules from the UASB reactor the ratio remained almost constant around 2 independent of substrate treated or pectin addition, in the EGSB reactor this value showed a constant decrease during the operation treating low concentrated wastewater, observing a relative stabilization around 1.5 when pectin was added.

The effect exerted by maturation, destabilization and pectin addition on microorganism population in granules from the UASB and EGSB reactors was monitored by means of dot blot hybridization, performed on samples retrieved at the end of each operational period (data not shown). Abundance of Bacteria and Archaea in the UASB and EGSB reactors verus operational parameters (OLR and COD removal efficiency) was studied. Although microorganisms belonging to the Bacteria kingdom were predominant in both reactors during the whole operation, a very distinctive proportion related to the Archaea kingdom was detected in each reactor. Thus, more similar relative abundances were measured in the UASB reactor, observing a progressive decrease in Archaea relative abundance during destabilization (40–35%) recovering the previous values at the end of

Table 2 Average of the operational parameters during each period studied and their corresponding achieved values at the end of the period treating medium and low concentrated wastewater in UASB and EGSB reactors. Note. OLR – organic loading rate; SMA – specific methanogenic activity

	Start up		Maturation		Destabilization		Recovering	
COD_{in} (g/L)	5		5		0.5		0.5	
OLR_{in} (kg COD/m^3 d)	0.5–5		5		5–11		8	
Period (days)	0–50		51–122		123–200		201–242	
	UASB	EGSB	UASB	EGSB	UASB	EGSB	UASB	EGSB
% COD removal(max–min)	92.5 – 46.7	97.3 – 73.3	95.9 – 97.9	96.3 – 98.9	81.1 – 22.9	81.0 – 42.2	70.4 – 36.9	74.5 – 48.1
% COD removal (final)	92.5	97.1	97.33	97.87	30.6	47.0	65.5	72.2
pH (max–min)	6.9 – 7.4	8.0 – 7.3	8.0 – 7.3	8.3 – 7.6	6.9 – 6.1	7.2 – 6.5	6.8 – 5.9[a]	6.9 – 6.1
pH (final)	7.4	7.9	7.7	7.9	6.3	6.5	6.4	6.6
SMA (g COD/g VSS d)[b]	0.82[c]	0.82[c]	0.92[d]	0.84[d]	0.30[e]	0.36[e]	0.32[f]	0.39[f]

[a]At day 211 pH was regulated in UASB reactor by adjusting to 7.2 the pH of the influent; [b]Inoculum SMA was 0.77 g COD/g VSS d; [c]day 50; [d]day 120; [e]day 198; [f]day 240

Table 3 Laboratory scale reactors average operational conditions

	UASB	EGSB
Organic loading rate (gCOD/Ld)	20	20
COD removal rate (%)	93	89
Hydraulic retention time (d)	1.8	1.8
pH	6.7–7.0	6.7–7.0
Biogas production (mL/g removed COD)	490	490
Methane concentration in biogas (%)	65	65

the recuperation period after addition of pectin. In the EGSB reactor Bacteria were highly predominant (90–95%) during the operation treating medium and low concentrated wastewater, observing a decrease, as in UASB reactor granules, in Archaea relative abundance during destabilization period and a posterior inversion in relative abundances (35% Bacteria, 65% Archaea) when the pectin was added.

These results were supported by the microscopic observation of granules (SEM) (see Figure 1) where the filamentous morphology of Methanosaetaceae-like microorganisms was predominant during the whole operation, particularly in granules core, finding other diverse morphologies associated to other genus, mainly on the granules surface. The flexibility observed in the EGSB reactor, as given by the sudden shift in relative abundances, likely support the better adaptation and performance of this reactor when compared with the UASB.

Operation of laboratory reactors

Operation of laboratory anaerobic reactors showed the applicability of anaerobic digestion processes for the treatment of Chilean pisco industry's wastewater. After a five week period of start up, the reactors were able to treat an organic loading rate of 20 gCOD/Ld, which was maintained over the duration of the study. Table 3 shows the main operational conditions of both reactors over its operation. High biogas production was observed in both reactors, being close to 500 mL per gram of removed COD.

Considering that the methane concentration in the biogas was 65%, this means that about 90% of the inlet COD was transformed to methane. Growth of acidogenic biomass over EGSB digester granules made reactor operation difficult; granules became less

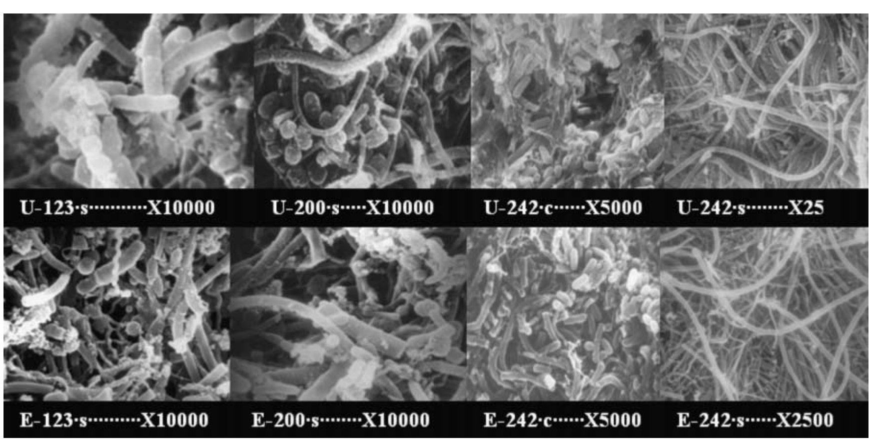

Figure 1 Scanning electron microscopy (SEM) of granules. The notation specifies the reactors UASB (U) and EGSB (E); the days of operation 123, 200 and 242, corresponding to mature granules, unstable granules and recovered granules, respectively, the area from granules (core-c or surface-s) where photos were taken and the augmentation factor

dense, producing a continuous sludge washout. Granules from the EGSB reactor present lower solids content in comparison with UASB granules. This phenomenon seems to be related to content of non acidified organic mater and can seriously affect operation of EGSB reactors (Alphenaar, 1994; van Lier *et al.*,1997; Jeison, 1999). In order to maintain a constant sludge concentration in the EGSB reactor, seed sludge was continuously added during its operation. The UASB reactor presented a much more stable operation. Owing to its stable operation and lower investments and operational costs, the UASB digester was chosen as the most appropriated treatment technology for pilot- and full-scale applications.

Irrigation of treated wastewater

Experiments conducted to establish the possibility of using treated water for agricultural purposes showed good results. The analysis of the measurements suggests that there is an effect of the irrigation level over the diameter and height of the plants. The diameter of the plant shows a tendency to greater increments in those treatments that have a 100% and 150% complement of fertilization and in the endowment of water with 100% and 400% of the pan evaporation.

On the other hand, even though there is a tendency to increment the height of the plant as the water applied increases, a higher accumulation of sodium is also produced, which counterchecks this tendency. A high concentration of this ion is known to produce notorious damage on the plant leaves, a phenomenon that was observed in the experiments. This observation is supported by the fact that the treated water presented an important content of sodium due to the pH control.

Table 4 presents the characterization of the anaerobic granular sludge. Its composition presents an important nutritional content (N, P, Ca, Mg), indicating that it could be used for soil improvement. Preliminary results (not shown) of an investigation that is being carried out in the Agronomy Faculty confirms that hypothesis.

Pilot-scale UASB implementation. The construction of the UASB reactor was finished in November 2000. The start up procedure took place for approximately 75 days. Figure 2 presents the organic loading rate and the COD removal during the start-up of the UASB reactor. From days 36 to 41 there was a decrease in the COD removal due to the discharge of slurry from a sedimentation operation. It produced an increase (not measured) in the total solids content, changing the characteristics of the wastewater. Once this discharge was finished the COD removal recovered. Figure 3 presents the reactor operation for the following eight months. High levels of COD removal were attained during this period (close to 95%).

Table 4 Characterization of the anaerobic granular sludge

Parameter	Units	Value
Nitrogen	%	5.29
Phosphorus	%	1.45
Potassium	%	0.32
Calcium	%	4.5
Magnesium	%	0.42
Zinc	ppm	293.75
Manganese	ppm	182.5
Iron	ppm	13031.25
Copper	ppm	365
Boron	ppm	8.16

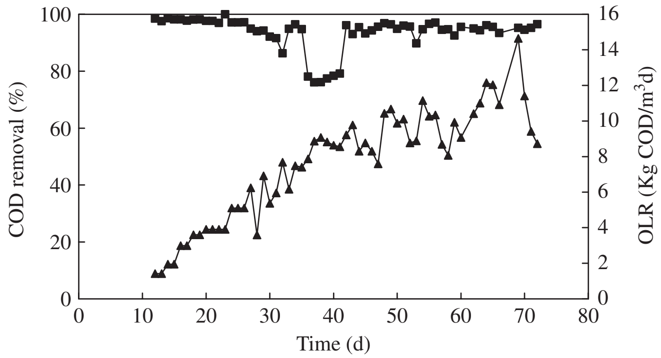

Figure 2 OLR and COD removal rates during start up of UASB reactor

For the first weeks after plantation of trees, some problems were detected on their leaves. As was detected at the laboratory scale nursery's plant growth, this problem was related to the high content of sodium of treated water, due to the use of sodium hydroxide for pH control. Sodium hydroxide utilization was close to 2 g/L, during the first weeks of start-up. At organic loading rates over 6 kg COD/m^3 d, biogas production was large enough to provide an important level of alkalinity by CO_2 dissolution, and therefore the use of NaOH was considerably reduced (to less than 20% of its original value). Exposure of plants to high levels of sodium occurred for a short period of time and did not produce only permanent damage on the trees. Anyway, this is a problem that should be considered seriously, especially during a digesters start-up period, or during operation at low values of organic loading rate.

Development of trees was completely normal. Rate of growth was the expected for a plantation under normal conditions. This is considered a good result, since no fertilizer of any kind was used, and soil conditions were not adequate: the terrain where the tree plantation was placed was being used for several years to dispose of the wastewater by soil infiltration.

Full-scale UASB implementation. The construction of the UASB reactor was finished in October 2004. The start up procedure took place for approximately 90 days. Once a steady state was obtained, the reactor was operated at 20 °C with an OLR of 8 kg COD/(day m^3) and high levels of COD removal (close to 95%).

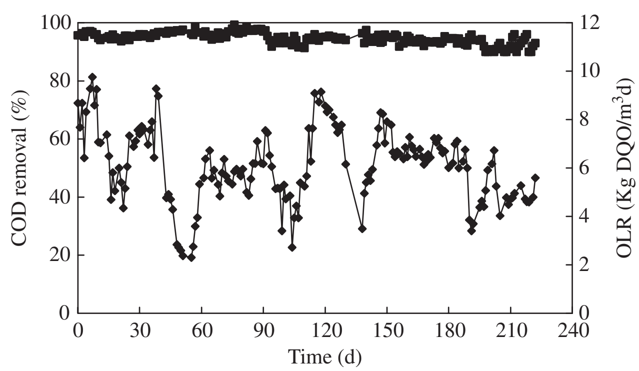

Figure 3 OLR and COD removal rates during operation of UASB reactor

Conclusions

Anaerobic digestion is a suitable technology for the treatment of the wastewater generated during Chilean pisco production. This industry offers the opportunity to exploit all advantages of anaerobic digestion for wastewater treatment. Use of anaerobically treated wastewater for agricultural purposes is not only feasible but also convenient since nutrient content of the treated wastewater can improve plant growth. This is of great importance for agro-industrial activities like pisco production, since they generate high amounts of wastewater and are usually located close to agricultural activities.

Anaerobic sludge also showed good nutritional levels, which means that it could be used for soil improvement. A negative effect of sodium over leaves was observed. This aspect should be taken into consideration in alkalinity control systems on operations of anaerobic digesters.

Regarding granule stability, when medium concentrated wastewater is shifted to a low concentrated substrate, destabilization occurs in UASB and EGSB reactors, associated to granule deterioration characteristics. The effect of adding pectin for performance recovery was studied, and found that granule stability in terms of EPS composition is dependent of the substrate treated and the reactor configuration. A constant ratio of protein: total polysaccharides (2) in the UASB reactor was associated to different granule qualities, whereas a relative maintenance of granule quality in the EGSB reactor was linked to different ratios for medium concentrated wastewater (2.5) and low concentrated wastewater with pectin (1.5).

The destabilization of granule consistency was found to be related to an increase in DNA released from cells to the granule EPS matrix, whereas granule properties recovering when adding pectin was associated to an increase in the mucopolysaccharides content in EPS. Regarding microbial population, destabilization was always related, although to a different extent, to a decrease in Archaea relative abundance, observing recovering or increase of this abundance when pectin was supplied.

From laboratory-scale studies it was conclude that stability, biomass characteristics and reactor performance of UASB reactors were higher than for EGSB reactors. Furthermore, considering investment and operational cost, UASB reactors are preferred, and have been the better alternative for Chilean pisco wastewater treatment.

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