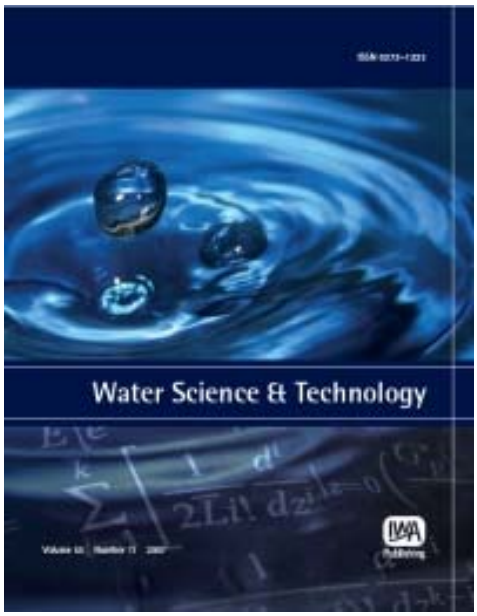

Anaerobic sequencing batch reactor as an alternative for the biological treatment of wine distillery effluents

Andrés Donoso-Bravo, Francisca Rosenkranz, Viviana Valdivia, Michel Torrijos, Gonzalo Ruiz-Filippi and Rolando Chamy

ABSTRACT

The goal of this study was to assess the effect of different modes of operation and configurations of Anaerobic Sequencing Batch Reactors (ASBRs) treating phenolic wastewater. Several lab-scale reactors were used in the mesophilic range. The reactors were fed with synthetic wastewater with a COD of 5 g/L using phenol as a carbon source (variable concentration) and glucose as a co-substrate. One and two-phase (hydrolytic/acidogenic–methanogenic) systems in batch and fed-batch operation were evaluated. The one-stage reactor operated by the fed batch (which was the only configuration using phenol as a sole carbon source), presented better results for the removal of phenol, reaching 100% removal in 10 days at a concentration of 210 mg/L. The two-stage configuration had removal percentages near 100%, but the methanogenic reactor presented greater degradation of the remaining phenol not removed in the hydrolytic/acidogenic reactor. ASBRs might be a feasible alternative to treat this type of effluent due to their operational flexibility.

Key words | acidogenic, anaerobic digestion, hydrolysis, phenol, two-phase

Andrés Donoso-Bravo
Francisca Rosenkranz
Viviana Valdivia
Gonzalo Ruiz-Filippi (corresponding author)
Rolando Chamy
School of Biochemical Engineering,
Facultad de Ingeniería,
Pontificia Universidad Católica de Valparaíso,
General Cruz 34,
Valparaiso,
Chile
E-mail: *gonzalo.ruiz@ucv.cl*

Michel Torrijos
INRA, UR50, Laboratoire de Biotechnologie de l'Environnement,
Avenue des Etangs,
Narbonne F-11100,
France

INTRODUCTION

Chile possesses a large and consolidated wine industry, with 430.5 million litres exported in 2006. Furthermore, an important amount of wine distillate (called "pisco") is produced, having 10,500 hectares of vineyard set aside for wine distillate production. In 2006, 49 million litres of wine distillate was produced, mostly for national consumption (1% of the production was exported). This industry seasonally produces large volumes of effluent (called "vinasse") with high organic strength and low pH (3–4) (Chamy *et al.* 2007).

One of the most complex compounds present in vinasse are polyphenols, with literature values ranging from 290–1,200 mg/l (Borja *et al.* 1993; Strong & Burgess 2006; Melamane *et al.* 2007). Among the most common polyphenols are gallic acid, ρ-coumaric acid and gentisic acid (Borja *et al.* 1993). Polyphenols are responsible for strong inhibitory effects of vinasses on microbial activity, as well as their antibacterial activity, affecting on the anaerobic digester performance used for biological treatment.

Despite the success of aerobic treatment of phenolic wastewater, anaerobic digestion has grown to become a successful technology due to its advantages over aerobic treatment, such as low energy consumption (aeration is not required), less sludge, biogas production (methane and hydrogen) that can be exploited as a source of renewable energy, among others. Most of the anaerobic wastewater treatments of phenolic effluents have been carried out in continuous systems, mainly in Upflow Anaerobic Sludge Blanket (UASB) reactors (Fang *et al.* 2004; Fang *et al.* 2006) and in Expanded Granular Sludge Blanket (EGSB) reactors (Scully *et al.* 2006).

Anaerobic Sequential Reactors (ASBR) work on consecutive cycles of operation, and each cycle consists of the following stages: feeding, reaction, settling, discharge

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and idle time. The most important advantage of this type of reactor is that it allows great flexibility of operation as it can work in different modalities: batch, fed-batch or both, depending on the combination of variables between the feeding and reaction times (Zaiat *et al.* 2001). For example, a sequential fed-batch operation, using the maximum possible feeding time, could avoid reaching inhibitory concentrations of toxic substrates such as phenol.

On the other hand, the separation of the main reactions of anaerobic digestion, acidogenesis and methanogenesis, allows the selection and enhancement of microbial populations in each reactor in terms of temperature and pH, and it also allows for control of the intermediate products (Speece 1996). Some studies have evaluated two-phase ASBRs connected in series (Bouallagui *et al.* 2004; Donoso-Bravo *et al.* 2009), and acidogenic degradation in an ASBR of phenolic wastewater has also been applied (Chin *et al.* 2005).

The aim of this study was to evaluate the treatment of phenolic wastewater, through different configurations and operation modalities in Anaerobic Sequencing Batch reactors.

MATERIALS AND METHODS

Experimental set-up

For the batch (ASBR) and feed batch (ASfBR) operation, 2 glass reactors, with a total of 6 L and 5 L of useful volume, were implemented. For two-phase operation, two reactors of acrylic, both with a total volume of 6 L and effective volume of 5 L, were used (ASBR1, hydrolytic–acidogenic and ASBR2, methanogenic). The temperature was maintained in 35 ± 1°C with a jacket connected to a thermostatic bath. For mixing in the ASBR and ASfBR, magnetic stirrers were used, and in the two-phase system, liquid recirculation. For system automation, a logic controller (Millenium II, SA12 series) was used, where each cycle sequence was programmed and adjusted to last 24 h. Each operation cycle had the following duration: feeding 15 min, reaction 22 h, sedimentation 60 min, download 32 min and dead time 10 min for the ASBR; Feeding/reaction 22 h, sedimentation 60 min, download 45 min, and a dead time of 10 min for the ASfBR.

Wastewater and inoculums

Reactors were fed with a synthetic wastewater composed of glucose (as co-substrate), sodium bicarbonate (to maintain the alkalinity of the system), and macro and micro nutrient solutions. Only phenol was used as substrate, because this represents one the most toxic phenolic compounds and it might be an intermediate in the polyphenols biodegradation; thus, it would be the worst conditions for anaerobic digesters.

ASBR and ASfBR reactors were seeded with non-granular sludge obtained from the bottom of a pilot-scale anaerobic filter treating distillery vinasses. The concentration of biomass in each reactor was 12 g VSS/L with a specific methanogenic activity of 0.12 [g COD_{CH4}/g VSS d]. ASBR 1 and ASBR 2 were seeded with non-granular sludge obtained from a UASB reactor from the tobacco industry. The concentration of biomass in each reactor was 20 g VSS/L with a specific methanogenic activity of 0.6 [g COD_{CH4}/g VSS d].

Experimental design

Four different strategies were evaluated: (1) Batch operation with readily biodegradable co-substrate (ASBR) (2) fed-batch operation with phenol as the only carbon source (ASfBR) (3) two-phase (hydrolytic/acidogenic, ASBR1–methanogenic, ASBR2) batch operation with co-substrate (4) two-phase operation, where the hydrolytic/acidogenic reactor was operated in fed-batch mode. In the two-phase system, the effluent from ASBR1, which corresponds to the carbon source that is hydrolyzed and converted to VFA, was fed to the ASBR2, and the pH was adjusted between 7–8. The experimental conditions for each configuration are shown in Table 1.

Analytical methods

During operation, samples of feeding and effluent were taken for the following analysis: Soluble and total organic matter (tCOD and sCOD) by spectrophotometry, total and volatile suspended solids (TSS and VSS) by gravimetry, volatile fatty acids (VFAs) by chromatography and phenol by colorimetric methods (Folsom *et al.* 1990).

Table 1 | Experimental conditions for each configuration

Configuration	Phenol (g/L)*	Total COD (g/L)	Length (d)	Conditions
ASBR				
1	–	5.207 ± 0.737	22	Only glucose in the feed
2	0.265 ± 0.019	5.294 ± 0.261	8	Increasing phenol concentration
3	0.544 ± 0.088	5.475 ± 0.108	13	
4	0.791 ± 0.066	5.392 ± 0.126	5	
5	0.253 ± 0.035	0.530 ± 0.012	14	Total reinoculation
ASfBR				
1	0.239 ± 0.024	0.491 ± 0.095	12	Increasing phenol concentration
2	0.492 ± 0.024	0.998 ± 0.071	10	
3	0.396 ± 0.053	1.056 ± 0.096	11	
4	0.396 ± 0.049	0.954 ± 0.038	14	Partial reinoculation
5	0.621 ± 0.035	1.384 ± 0.071	11	
Two phase-ASBR1 (batch)				
	0.21	5	7	pH: 4–4.5 glucose and phenol in the feed
Two phase-ASBR1 (fed batch)				
	0.21	5	7	pH: 4–4.5 feeding flow 0.3 [ml/min] glucose and phenol in the feed

*Considering that 1 g phenol is equivalent to 2.38 g COD.

RESULTS AND DISCUSSIONS

One phase-batch operation (ASBR)

Figure 1a shows the results of the operation of the ASBR with glucose as the co-substrate and increasing concentrations of phenol. As the concentration of phenol and glucose increases, the efficiency of removal of total COD steadily decreases, showing little adaptation of the biomass to new operating conditions. The removal of phenol showed variable values throughout the study and were only temporarily increased as the load increased.

No activity on phenol degradation was observed by the 55th day; therefore, a complete reactor reinoculation was made by adding 16 g VSS/L and was completed with the addition of co-substrate (glucose), leaving only phenol as the carbon source at a concentration around 210 mg/L (500 mg COD/L). In order to analyze whether the presence of co-substrates in batch processing conditions promoted the degradation of phenol under the conditions studied, we stopped feeding co-substrate and used a low concentration of phenol that was much less than the reported inhibitory concentration (Lay & Cheng 1998). Again, the sludge showed a low adaptation despite the low concentrations of phenol and achieved a maximum removal of 30%. Under these conditions, the biomass showed sediment problems, and after one week of operation the concentration of the reactor had dropped to 5.3 g VSS/L. This could be due to the nature of the substrate as it exerts an influence on the physico-chemical properties of the biomass. For example,

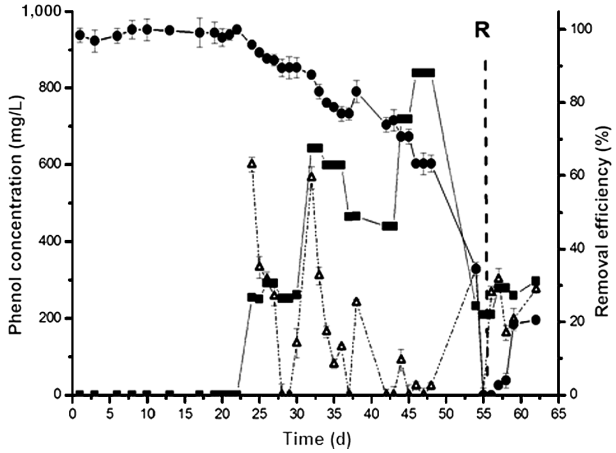

Figure 1 | Operation of the ASBR in the phenol-wastewater treatment using glucose as the co substrate. (●) tCOD removal (■) Phenol concentration and (Δ) Phenol removal. R, reinoculation.

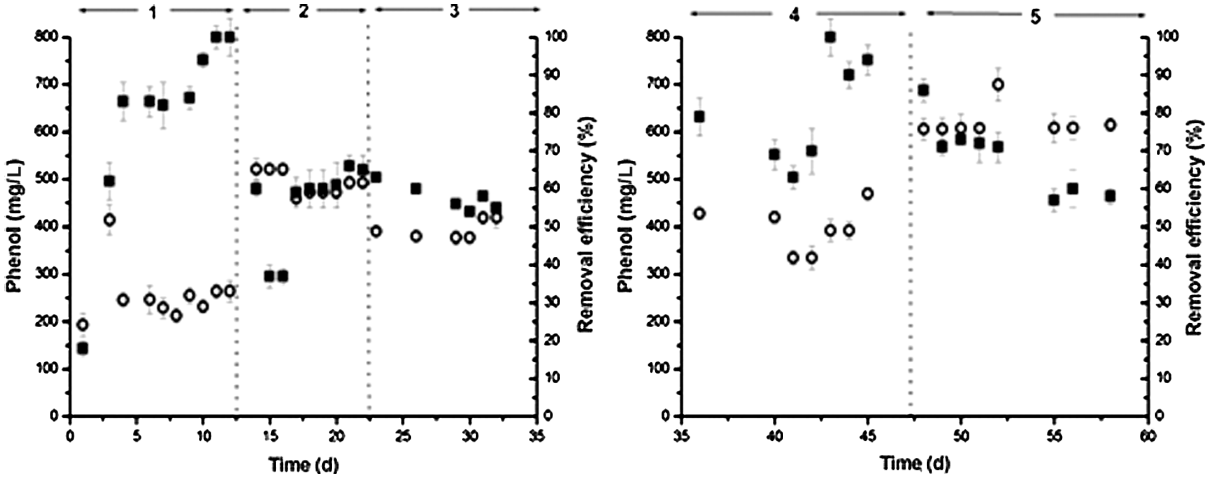

Figure 2 | Performance of the ASfBR (■) Phenol removal and (○) Phenol concentration.

the effect of different carbon sources on biomass settling has been studied by Cuervo-Lopez *et al.* (1999) in a UASB, who found flotation problems using lactate as a carbon source.

One phase-fed-batch operation (ASfBR)

Figure 2 shows the fed-batch operation during five stages of operation in terms of phenol removal and phenol feed concentration. In stage one, with an average concentration of 239 mg/L, a fast adaptation of the biomass to this substrate was observed, reaching 80% removal efficiency at day 4 despite the overload of phenol on day two. At day 10, the removal of phenol was 100%. It is important to highlight the almost immediate acclimation of the biomass to the conditions. To achieve these results, the concentration of phenol was increased by an average of 492 mg/L. There was an immediate decrease in the percentage removal to 40%, and after four days it stabilized at 60%. Because the removal of phenol remained at this value, the phenol feed was diminished to 400 mg/L (stage three) to assess whether the biomass could recover. This did not occur, and the removal decrease maintained. The concentration of the biomass inside the reactor decreased from 12.0 (at the beginning) to 4.0 g VSS/L, indicating that the phenol affected the settling capacity of the biomass.

To begin stage four, the reactor was reinoculated with the biomass needed to achieve a concentration of 15.0 g VSS/L. The phenol concentration was kept at 0.16 g Phenol/L d with almost 100% removal by day 10. Again, the concentration in the reactor had decreased significantly to a value of 5.90 g VSS/L, which confirmed that the phenol affected biomass settling and that the specific activity of the phenol degradation had increased considerably in relation to the low biomass concentration. Subsequently, the concentration was increased by 50% (stage five), and the phenol removal decreased steadily and stabilized at around 60%, with a value of biomass within the reactor of 3.0 g VSS/L.

Two-phase operation

In the hydrolytic/acidogenic reactor (ASBR1), the average percentage of phenol removed after seven days by batch feeding reached nearly 75%, while the fed-batch mode resulted in approximately 84% removal. In both cases, glucose (co-substrate) was consumed quickly, clearly indicating that the microorganisms responsible for the

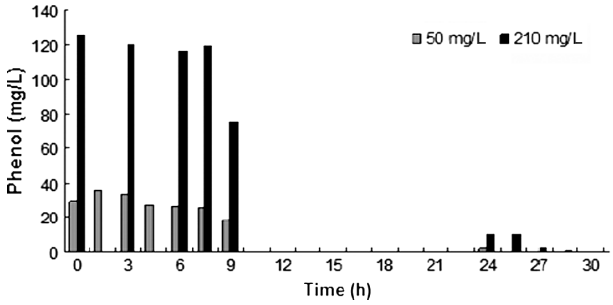

Figure 3 | Kinetics of phenol removal in the methanogenic reactor with 50 and 210 mg/L phenol in the feed.

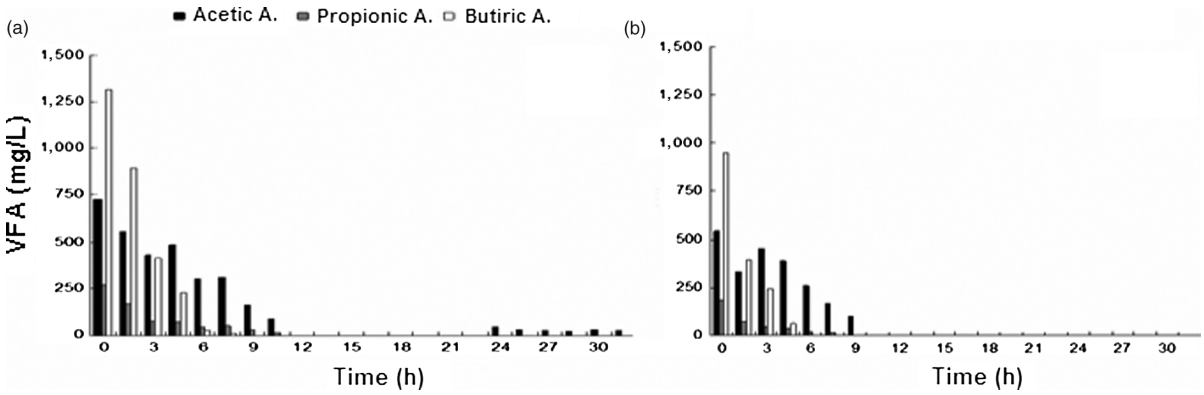

Figure 4 | Kinetics of VFA degradation in a methanogenic reactor with 50 (a) and 210 (b) mg/L phenol in the feed.

acidification of the carbon source preferentially used glucose, whereas phenol is not immediately used as a carbon source. The degradation of phenol requires the appropriate enzymatic machinery, which is not active until it is absolutely needed. To overcome this drawback, the effect of an increase in the HRT to force the microorganisms to activate a new metabolic route that includes phenol as the carbon and energy source, was observed. This was done by removing glucose from the medium, and it resulted in a small decrease in concentration of phenol after seven days of hydraulic residence.

In the methanogenic reactor (ASBR2), once feeding had begun, the reactor with the effluent obtained from ASBR1 reached minimum values of COD in the effluent of this reactor.

The ASBR2 reactor showed a rapid adaptation to degrade phenol (which was not hydrolyzed in ASBR1) on the first cycle. This adaptation is reflected in the final value of COD, which remained virtually the same as the output cycles without phenol.

Due to the success of the phenol degradation, a final assay was performed to determine the behavior of the methanogenic reactor. Phenol was added to ASBR2 at the same concentration as in ASBR1 (0.5 g/L). Figure 3 shows that the concentration of phenol in ASBR2 is negligible after 27 hours, with experimental feeding concentrations of 210 mg/L and 50 mg/L.

We observed that the decrease in the concentration of phenol starts after about 9 hours, which coincides with the total VFA degradation, as shown in Figure 4. This might be explained by the fact that the VFA are the direct precursors of methane, while phenol is first subjected to a series of additional reactions.

These results are seen at very different reaction times, perhaps because these conditions for the hydrolytic reactor operation are most appropriate for the removal of such compounds. Another explanation of these results is that there are two reactors with different bacterial populations and enzymatic activities. This could affect the consumption of phenol under their respective conditions. It could be assumed that methanogenic bacteria are those that possess the appropriate enzymatic machinery for the degradation of phenol.

Despite the good performance in these reactors, especially the ASfBR, anaerobic processes are generally not able to achieve the strict limits for the effluent standards (e.g. COD $<$ 150 mg/L in EU), and aerobic processes are usually required in order to polish the final effluent.

CONCLUSIONS

ASBR reactors are a potential alternative for the treatment of phenolic wastewater with the benefit of operational flexibility. The sequential batch reactor (ASBR) showed little adaptation with low removal of phenol, despite the presence of co-substrate in different concentrations, and low yields with low concentrations of phenol without the co-substrate.

The anaerobic sequential fed-batch reactor (ASfBR) presented a rapid adaptation to reach 100% phenol removal after 10 days of operation at a concentration of 210 mg/L.

The hydrolytic/acidogenic reactor in the two-phase configuration in batch operation showed the inhibitory effect of the phenol on microbial activity.

The reactors that had high populations of bacteria with methanogenic activity showed the best results in the removal of phenol.

In any case, aerobic treatment as a final step to eliminate residual COD is strongly recommended.

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