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Abstract

Fermentative production of alcohols is a promising alternative to petroleum-based fuels industry. However, the development of a competitive biological process implies the achievement of higher production titer and enhanced productivity. In the case of butanol production by solventogenic strains, In Situ Product Recovery (ISPR) techniques help to overcome the inhibition threshold of the microorganism and to reduce the overall cost of downstream process. In this work, gas stripping (GS) was preliminary studied in an abiotic system and high flow rates (vvm - vessel volume minute- > 2 min^{-1}) were needed to achieve higher butanol stripping rate than its production rate. Combined liquid extraction (LE) with pulse Gas Stripping (GS) were applied to batch fermentations resulting in a synergic effect giving a higher glucose consumption rate and a lower aqueous butanol concentration. Moreover, in this hybrid system, biphasic bioreactor acted as a "liquid-liquid equilibrium step" and both phases were stripped concomitantly.

Keywords: IBE; extractive fermentation; gas stripping; *Clostridium beijerinckii.*

Introduction

Production of fuels and chemicals from sugars has attracted much attention during the last decades, becoming a global priority as atmospheric CO_2 levels and oil prices continue to increase. Alcohols obtained by fermentation processes are included among the most promising petroleum substitutes due to their wide range of applications: advanced biofuels, industrial solvents, chemical feedstock, bioplastics production, etc.

The development of an economically competitive biological process implies the achievement of higher production titers of alcohol in the bioreactor. However, microorganisms usually have limited tolerance for certain products and may be inhibited by excessively high concentrations occurring when the threshold concentration is exceeded. Particularly, ABE (acetone, butanol, ethanol) and IBE (isopropanol, butanol, ethanol) fermentations are subject to strong inhibition by products [1], which leads to low final product concentration, high recovery costs and large volumes of wastewater generation. Several options have been reported to produce metabolites from fermentation beyond their inhibition threshold. One possible solution would deal with the improvement of the biocatalyst, in terms of higher selectivity and tolerance towards the main fermentation product. Another alternative focuses on the biocatalyst environment and concerns the decrease of product inhibition by continuous product removal and recovery from the fermentation medium. This can be achieved by an In Situ Product Removal system. The later approach allows the recovery of butanol as fast as it is produced during the fermentation, and thus it would keep the butanol concentration under the inhibition threshold. This allows the generation of more concentrated butanol stream which is more easily recovered [2, 3, 4, 5, 6] and results in several benefits impacting the overall process performance: smaller reactor volume,

lower downstream cost, less wastewater generation. The integrated product recovery of butanol (and other alcohols) from aqueous broth can be based on the differences between physical and chemical properties of the compounds to be separated, or on their interaction with an auxiliary agent or material. In any case, the total recovery of butanol is not necessarily achieved in the pre-concentration step and thus further purification may be required. Separation techniques for *in situ* product recovery that have been explored over the last few decades are adsorption, gas stripping, liquid-liquid extraction, pervaporation, etc.

Among different possibilities, gas stripping (GS) arises as a relatively simple technique that can effectively remove AIBE compounds from the fermentation broth [7]. Gases generated during AIBE fermentation -or external agents like nitrogen - are used in this technique to separate the solvents. These gases are sparged into the bioreactor and volatile compounds are recovered and subsequently condensed. Gas stripping can be used either in situ or in stream configurations, with or without previous removal of solids. Several AIBE configurations (batch, fed batch and continuous mode) have been tested in laboratory scale, and in all cases both the product yield and productivity have improved. The effects of several operating parameter such as the gas recycle rate, the bubble size or the amount of antifoam added to the broth have been studied [8, 9] for a gas stripping system coupled or not to fermentation. Ezeji *et al*., 2005 [10] observed that *C. beijerinckii* was not affected adversely by GS and productivities and yields were increased to 200 and 118% respectively, as compared to control in batch fermentation. Xue *et al*., 2012 [11] applied gas stripping in fed-batch mode to a system combining free and immobilized cells doubling its reference productivity, obtaining highly concentrated condensates and reducing about 90 % of the estimated energy cost for the

overall process. However, even if GS technique improves in all cases fermentation performances, before its industrial application, several aspects will need to be faced up, such as: excessive foaming during fermentation that leads to operational problems, high cost of stripped alcohols recovery, huge compressors needed.

In situ butanol recovery technique by liquid extraction (LE) coupled to a fermentation system, an insoluble extracting compound is added to the fermentation broth. Both phases are then easily separated. The optimal solvent is a compromise between high alcohol distribution coefficient and no toxicity towards microorganisms [6, 12]. In practice, biocompatible solvents present low butanol extraction capacity, therefore high volumetric ratio of solvents to aqueous phase is needed in order to keep butanol below the inhibition threshold concentration, which increases the operational volume necessary in the bioreactor.

Combination of both *in situ* recovery techniques (GS and LE) and their applications for batch and fed-batch ABE fermentations were first proposed by Lu and Li, 2016 [13]. In their work, the authors applied nitrogen stripping directly through the organic phase (oleyl alcohol) in a biphasic reactor. They obtained an ABE productivity of 40 % higher at vvm = 1.6 min^{-1} in batch mode and increased up to 95-105 % in fed batch mode [14] applying 1.6 and 3.3 min^{-1} of gas flow rate, respectively. Moreover, glucose consumption was boosted consequently.

In this work, a hybrid system combining gas stripping and liquid extraction was applied to batch IBE fermentations. Vegetable oil based mixture solvent was used in a biphasic reactor and the integrated technique (GS-LLE) performance was compared to control assays (individual ISPR technique). Previously, gas stripping was studied in an abiotic set up (representative synthetic fermentation medium, no cells) in order to evaluate a

first order striping rate model and to establish the optimal operating window for this system.

1. Material and Methods

2.1 Experimental set-up for abiotic IBE gas stripping

A schematic of the experimental apparatus for gas stripping used in this study is shown in **Fig. 1**. End fermentation representative solutions of 1-butanol-isopropanol-ethanol (11/5/0.5 g/L) (PubChem CID: 263, 3776, 702, respectively) were prepared in demineralized water and placed in 1 L bioreactor (500 mL of working volume) with a Rushton impeller and a sparger placed on the bottom of the vessel below the impeller. Temperature inside the bioreactor, agitation rate and gas flow rate were controlled variables in the system. For collecting condensates, two cold traps were arranged and cooled at 4 and -15 °C, respectively. Sampling was done in the reactor and also in both cold traps, so the performance of total alcohol stripping process could be estimated. Nitrogen (PubChem CID: 947) was sparged through the aqueous solution at a fixed flow rate before it was led to the condenser flasks and then through a flask with water containing ice before it was released to the atmosphere. Temperature set point in the bioreactor was fixed at 37 °C while agitation rate was set at 300 rpm. Gas stripping rate was evaluated at several vvm (1 vvm = 1 L of N_2 per 1 L of liquid volume per minute): 0.5, 2 and 3 min^{-1}. Each experiment was carried out twice.

2.2 Mathematical development

The system was described by means of a simple macroscopic model in order to quantify the stripping process. At gas-liquid interface, the steady state flux balance can be expressed as follows according to Whitman double film theory:

$$\phi_{fluxGL} = k_L\left(C_{i,l} - C^*_{i,l}\right) = k\left(C^*_{i,g} - C_{i,g}\right) = K_{LG}\left(HC_{i,l} - C^*_{i,g}\right) \quad (mol/m^2 s) \quad \text{Eq. A.1}$$

Where k_L, k_G are respectively the individual mass transfer coefficient based on liquid and gas phases (m/s), KL_G is the overall mass transfer coefficient based in gas phase (m/s), $C_{i,l}$, $C_{i,g}$ the bulk liquid and gas composition for compound i (mol/m^3), respectively. $C^*_{i,l}$, $C^*_{i,g}$ are the corresponding equilibrium composition with bulk composition in each phase (mol/m^3). Moreover, H denotes the Henry coefficient (dimensionless). Therefore, the gas phase balance is expressed as follows:

$$V_g \frac{d(C_{i,g})}{dt} = -Q_g(C_{i,g}) + V_T K_{LG} a\left(HC_{i,l} - C^*_{i,g}\right) \quad (mol/s) \quad \text{Eq. A.2}$$

Where V_g is the gas phase volume in the bioreactor (m^3), V_T the total volume (m^3), Q_g denotes the flow gas rate through the bioreactor (m^3/h). Gas composition is assumed to be solute free at the bioreactor inlet. Eq.A.2 may be simplified with a quasi-steady state approximation in the gas phase as following:

$$C_{i,g} = \frac{V_T K_{LG} aH}{Q_g + V_T K_{LG} a} C_{i,l} \quad (mol/m^3) \quad \ldots \text{Eq. A.3}$$

In liquid phase, mass balance for solute *i* being stripped can be expressed as:

$$V_l \frac{d(C_{i,l})}{dt} = (-r_i)V_l + Q(C_{i,l,0} - C_{i,l}) - V_T K_{LG} a\left(HC_{i,l} - C_{i,g}\right) (mol/m^3) \quad \text{Eq.A.4}$$

Where V_l is the liquid phase volume in the bioreactor (m^3), V_T the total volume (therefore $V_T = V_l + V_g$) (m^3), Q denotes the liquid flow rate through the bioreactor (m^3/h) and r_i the reaction rate for compound i inside the bioreactor (mol/Lh). The first two terms are assumed to be equal to zero (closed system for liquid phase, and no reaction inside since abiotic system was employed). Considering these assumptions and

combining previous equations, the simplified expression for the variation of aqueous phase composition during gas stripping for compound I was obtained:

$$\frac{d(C_{i,l})}{dt} = -\frac{K_{LG} a Q_g H}{\frac{V_L}{V_T} Q_g + V_L K_{LG} a} C_{i,l} = -\beta C_{i,l} \qquad (mol/m^3 s) \qquad \text{Eq. A.5}$$

β denotes the stripping factor which finally encloses thermodynamics (*H*) and transfer ($K_{LG}a$) effects. If we assume that the system does not have any mass transfer limitations, previous equation may be simplified to:

$$\frac{d(C_{i,l})}{dt} = -\frac{Q_g H}{V_L} C_{i,l} = -\beta C_{i,l} \qquad (mol/m^3 s) \qquad \text{Eq. A.6}$$

According to Eq. A.6, in a thermodynamic controlled system stripping factor can only be improved by applying higher vessels volume per minute to the system.

In parallel, dynamic simulation of a successive liquid-vapor flash operations occurring inside the bioreactor has been developed with SIMULIS Thermodynamics software. The aim of this model is the prediction of the system thermodynamics, in other words, the maximal attainable stripping rate at operating conditions, in case of no gas-liquid transfer limitations exist. This model also includes liquid-liquid-vapor flash calculus applied to outlet gas flow, in order to simulate cold trap units and to predict condensates composition.

2.3 Microorganism and culture media

C. beijerinckii DSMZ 6423 spores were stored in 150 µL saline suspension cryotubes at -60 °C. All experiments started with a heat-shock of the spores for 1 min at 100 °C to induce germination and subsequently they were used to inoculate 10 ml of potato/glucose preculture medium previously sterilized (121 °C, 20 min). The preculture media was incubated anaerobically at 36 °C, 24 h. The abiotic culture medium was regenerated at 100 °C for 10 min previously and placed inside an Anaerocult jar (Oxoid) for 72 h to guarantee anoxic conditions at the beginning of the fermentation. The potato/glucose preculture media contained the following composition: 250 g/L boiled potatoes; 2 g/L $(NH_4)_2SO_4$ (PubChem CID:6097028); 2 g/L $CaCO_3$ (PubChem CID: 10112), 10 g/L glucose (PubChem CID: 53782692) . The pre-culture medium was transferred to 100 ml of culture medium disposed in 250 mL sealed biphasic bioreactor using Schott bottle, previously purged with nitrogen (PubChem CID: 947) during 20 min. The culture medium composition for IBE fermentations was: 6 mg/L $FeSO_4$ $7H_2O$ (PubChem CID: 62662); 1 g/L $MgSO_4$ $7H_2O$ (PubChem CID: 24083); 1 g/L KH_2PO_4 (PubChem CID: 516951); 0.6 g/L K_2HPO_4 (PubChem CID: 24450); 2.4 g/L CH_3COONH_4 (PubChem CID: 176); 0.1 g/L p-aminobenzoic (PubChem CID: 978); 2.5 g/L yeast extract (PubChem CID: 24973165) and 60 g/L glucose (PubChem CID: 53782692). Biphasic bioreactors were incubated at 36 °C and low orbital agitation (50 rpm).

2.4 Pulse gas stripping, extractive and integrated gas stripping-extractive fermentations

Batch IBE fermentations were performed to study and compare the effect of pulsed gas stripping, liquid liquid extraction and integrated gas stripping-liquid liquid extraction system. All fermentations were carried out in 500 mL schott bottles, filled with 200 mL of culture media and initial glucose concentration of 90 g/L. The culture medium was inoculated with 20 mL of cells in their maximal growth rate (same inoculum for all the bottles). The system was previously purged with N_2. The temperature was fixed at 36 ºC and gentle agitation was kept at 50 rpm. The whole system was autoclaved at 121 °C during 20 minutes before inoculation, pH value was set to 6 at the beginning of the fermentation and then it varied freely through the fermentation according to the acids generation and subsequent consumption.

Gas stripping fermentations were carried out by the application of four nitrogen stripping pulses of 1.5 vvm for 30 min at 25, 46, 51 and 118 h of fermentation, respectively. Schott bottles were adapted with a gas sparger in order to optimize the droplet distribution inside the bioreactor. A vegetable oil base mixture composed of sunflower oil (90 % v/v) and a C_{12} based Guerbet alcohol (2-Butyl -1-Octanol, 2B1O) (PubChem CID: 19800) was tested as extractive agent in biphasic fermentations. The ratio organic: aqueous phase was fixed at 1:1 (v/v). Low agitation allowed maintaining a clear separation between phases in all fermentations. The bioreactor was adapted with a sampling device for both aqueous and organic phases. The integrated system (GS+LLE) was carried out at the operating conditions described above. Two duplicates of each system were carried out at the same time. Only one of the duplicates was sampled periodically and the other one was kept closed and not sampled until the end of fermentation.

2.5 Analytical methods

Samples were centrifuged at 4000 rpm for 20 min, therefore in the supernatant were measured: pH (Toledo mettle; Columbus OH-USA), glucose consumption (YSI 2700 Select; Yellow Springs OH -USA). IBE products in the aqueous phase were quantified by Gas Chromatography (Agilent Technologies 7890B GC System; Santa Clara CA-USA), equipped with an Agilent VF-624ms column using He (PubChem CID: 23987) as the carrier gas and a flame ionization detector (FID), temperature of the oven was 35 °C and it was increased at a gradient of 2 °C/min until 60 °C and subsequently increased up 15 °C/min to 200 °C for 10 min. Alcohols were quantified in the organic phase with a back flush (reversal flow) system consisting of an 10 m HP-PONA precolumn (Agilent Technologies) with a pressure ramp of 38.1 psi for 30, 40, 45 and 50 min followed by 5 psi/min until 10 psi, and 0 psi during 0-10 min. The HP-PONA precolumn was connected to a 45 m HP-PONA column (Agilent Technologies) with a pressure of 34.5 psi and a temperature ramp of 35 °C for 10 min, 1.1 °C/min until 130 °C, then 15 °C/min until 280 °C and finally to 280 °C for 0-15 min. The flame ionization detector temperature was at 300-310 °C. Both columns used He as the carrier gas. In aqueous samples, free growing cell evolution was estimated measuring optical density at 600 nm (Spectrophotometer Shimadzu UV-1240; Kyoto, Japan).

2. Results and Discussion

3.1 Butanol stripping rate

The butanol stripping rate was investigated at three different gas flow rates: 0.25, 1 and 1.5 L/min (corresponding to 0.5, 2 and 3 vvm, respectively). The bioreactor was filled with synthetic aqueous solution representing the final IBE fermentation composition (11, 5 and 2 g/L for butanol, isopropanol and ethanol, respectively) at the beginning of

each experiment. Temperature was first raised up to 37 °C inside the reactor and stirring rate was fixed at 300 rpm. Aqueous solution was sampled each 2 h for calculating alcohol striping rate. At the same time intervals, condensates were collected in the cold traps for further analyses and quantification. Experimental obtained data were first compared with SIMULIS thermodynamic modeling results for alcohols stripping rate inside the bioreactor (butanol aqueous concentration evolution in **Fig. 2**). It can be seen that thermodynamic prediction for aqueous butanol depletion rate is in good agreement with experimental data for 0.5 and 2 vvm. These results confirmed the absence of mass transfer limitation in the bioreactor under experimental conditions. In other words, thermodynamic controlled the process and therefore acquired data will give the maximal attainable butanol stripping rate or the minimal vvm that should be applied for a given extraction rate. Data obtained with 3 vvm showed that butanol was stripped slightly faster than thermodynamic prediction. This can be attributed to non-negligible physical entrainment of water droplets in outlet lines when high flowrates were applied.

Based on these experimental results and previous discussion, Eq. A.7 was regressed for estimation of stripping rate constant (β, h^{-1}) at different conditions (**Table 1**) as follows:

$$\beta = \frac{\ln\left(\frac{C_{i,0}}{Ci}\right)}{t} \qquad \text{…Eq. A.7}$$

Butanol and isopropanol have similar stripping rate constants (slightly higher for butanol), and they are systematically two times higher than ethanol stripping rate constant. These results are in agreement with Vrije *et al*., 2013 [9], which studied the gas stripping with IBE model solutions at fixed vvm = 1 min^{-1}. Stripping rate from aqueous medium will depend not only on stripping coefficient (which encloses

252 thermodynamics – Henry coefficient- and mass transfer –KLG-) but also on
253 concentration ratio of alcohols in aqueous system [9, 11]. Stripping rate order has been
254 kept constant in our experiments: butanol was stripped higher than isopropanol, while
255 stripping rate of ethanol was lower. Relative stripping rate of alcohols is difficult to
256 predict as Henry coefficients from literature present high variability for these
257 compounds in such diluted solutions.

258 Alcohols stripping rates generally diminished with decreasing IBE concentrations in
259 aqueous solution (**Table 2**). In batch process fermentations, butanol sets the inhibition
260 threshold because it is the main inhibitory metabolite and its removal rate should be at
261 least equal or higher than the specific productivity of butanol in the bioreactor, in order
262 to avoid its accumulation. A macroscopic mode inspired in ABE literature was
263 developed (data not shown) in order to estimate instantaneous butanol productivity in a
264 batch fermentation. Maximal butanol productivity was close to 0.5 g/Lh after 30 h of
265 fermentation. These data were compared in **Table 2** and it was observed for each gas
266 flow rate applied (or vvm) a minimal butanol concentration in the aqueous phase inside
267 the bioreactor that it was needed in order to equalize butanol productivity and butanol
268 stripping rate. According to these results, a vvm=0.5 min^{-1} would never be enough to
269 follow biological production in studied conditions (37 °C). Minimal concentration of 5
270 g/L of butanol inside the bioreactor must be reached before applying a gas stripping of
271 vvm=2 min^{-1} in order to strip butanol as fast as its maximal productivity rate. Butanol
272 inhibition threshold imposes the maximal concentration that would be suggested in
273 order to maximize productivity during operation.

274

3.2 Selectivity

Selectivity is defined here as mass unity of stripped alcohol per mass unity of stripped water in the gas outlet of the bioreactor. In a batch operation system, selectivity varies as a function of water and inlet gas stripping composition, since the ratio water/alcohol in the gas stream is governed by system thermodynamics. In this experimental work, only end point selectivity is obtained, as a result of an overall mass balance of water and alcohol in the system; it would be therefore a mean value corresponding to the whole batch assay. In **Fig. 3** selectivity was calculated by thermodynamic simulation (SIMULIS software, using specific in-house thermodynamic model) and was plotted as a function of butanol concentration in the aqueous phase inside the bioreactor at fixed operating conditions (37 °C and 2 vvm). Experimental data corresponding to the end of the batch assay at 2 vvm (at minimal butanol concentration) were higher than the estimated ones, since these data corresponded to the mean total selectivity considering higher concentration of butanol in the aqueous phase from the beginning of the experimental test, as it was stated previously.

Butanol selectivity by nitrogen stripping technique was low: which means that outlet gas left the bioreactor with non-negligible water quantity (even if physical entrainment was not considered here). This behavior will directly impact the operational cost of the process and will determine the recovery system of alcohols in gas loop.

Stripping rate of water scarcely varied in function of butanol aqueous concentration while stripping rate of butanol increased proportionally to its stripping coefficient and the local aqueous butanol composition [8]. Then, the asymptotic diminution of the process selectivity when the medium was increasingly diluted could be explained. These

results showed that gas stripping process became interesting only with a fixed butanol concentration in the aqueous phase [11].

3.3 Condensation rate and condensates composition

In a gas stripping-fermentation coupled industrial process, not only stripping rate of the inhibitory metabolite should be kept at least equal to its production rate inside the bioreactor but also stripped alcohols should be fully recovered from the gas loop before being recycled, in order to renew and maintain their stripping capacity through the operation.

The experimental unit used in these experiments (**Fig. 1**) had two condensers in cascade working at 4 and -15 °C, respectively. Almost the totality of condensates were recovered from the first cold step at 4 °C. Only at the end of each batch assay (when alcohol concentration was lower in the aqueous phase) a mass of condensates could be quantified from cold step at -15 °C. Strong linearity existed between butanol concentration in the condensates and butanol concentration in the aqueous phase (**Fig. 4**). Experimental data corresponding to GC analysis of condensates were obtained from the batch assays carried out at vvm= 2 min^{-1} (represented in **Fig. 4)** experimental data showed good agreement with the simulated ones obtained by SIMULIS model. Besides, if the aqueous butanol concentration was higher than its limit solubility (7.7 % wt. at 20 °C) a phase demixing zone could force an additional separation of the condensate liquid phase (simulated data **Fig. 4**). Moreover, this behavior has been already proved in ABE fermentation-GS coupled technique [11]. Additionally, it would be possible to recover one or two liquid phase alcohols in the condensates collector (cold trap at 4 °C) which were highly concentrated (> 10-100 times the initial concentration in the bioreactor) but the collected volume could represent only the 5% of bioreactor initial volume.

3.4 Suggested operation

The first objective of the gas stripping recovery technique is the end product inhibition alleviation by partial stripping of main inhibitory metabolites in a process. By means of combined abiotic experimentation with synthetic fermentation broth and thermodynamic simulation work, it has been proved that this technique becomes interesting at higher alcohol concentration in aqueous phase (remained under inhibition threshold). Indeed, not only butanol stripping rate was maximized when its concentration was the highest in aqueous phase, but also selectivity of the process (g alcohol stripped/ g water stripped) decreased asymptotically when the medium was diluted. In this work, alcohol concentration in condensates from the outlet gas was linearly dependent on the stripped aqueous phase concentration and above solubility limit of butanol in water (~7.7 % wt. at 20 °C) where additional separation by demixtion zone appeared. From previous statements, it was suggested the application of gas stripping technique for IBE fermentation in a pulse-mode (or intermittent mode): gas stripping would be activated only when butanol attains a predefined concentration in order to boost extraction performances.

3.5 ***Pulse gas stripping, extractive and integrated gas stripping-extractive coupling fermentations***

IBE fermentations coupled to different separation techniques were carried out in batch conditions. GS, LLE and hybrid GS-LLE system are compared to control fermentation (no separation technique). For GS and GS-LLE system, pulse-mode operation for gas

injection (nitrogen) was applied at three predefined time intervals since aqueous butanol was not known on real time.

For extractive fermentations, a vegetable oil based mixture composed of sunflower oil (90% v/v) and a C12 based Guerbet alcohol (2 butyl-1octanol, 2B1O) was used as the extractive agent. **Fig. 5** represents two discriminatory parameters in the performance of IBE fermentation with GS and LLE coupling techniques: the first one represents the glucose consumption and the second one the butanol concentration in aqueous phase. Synergic effect was reached with GS-LLE coupling technique based on higher glucose consumption rate and lower aqueous butanol concentration during the operation because butanol extraction rate from aqueous phase was more increased than individual ISPR techniques and control assays. Overconsumption of 23% glucose was observed when GS or LLE performed individually; while hybrid integrated technique showed 45% of overconsumption of glucose related to control fermentation. Total solvent concentrations were not quantified during these experiences because gas phase was not analyzed in these experiments. Nevertheless, total solvent IBE production could be estimated and ranked from sugar consumption and constant IBE yield of fermentation (0.35 g/g). These data are synthetized in **Table 3**. On the other hand, in **Fig. 5** it is observed that bioactivity was stopped approximately at 60 h for fermentations containing the extracting phase inside the bioreactor, even if non-inhibitory butanol concentration (<4 g/L) was measured in the aqueous phase and remaining glucose concentration could be quantified. This could be attributed to midterm toxicity of the solvent used in LLE-fermentations towards the specific microorganism employed. This needs to be further investigated. This solvent showed biocompatibility at the beginning of the fermentation in previous screening work (data not shown). Glucose consumption

rate and biomass formation were enhanced during the first 50 h (**Fig. 5**), which means that biocompatibility of this solvent is not an issue during the first part of the fermentation. Kollerup and Daugulis (1985) [15] classified the modes of cell inhibition in extractive fermentations into different mechanisms depending on the initial effect of the solvent into the metabolic and enzymes activity during the fermentation. In our case, middle term toxicity was observed with vegetable oil mixture and 2B1O, respectively. These results should be confirmed in future experiments.

Respective theoretical equilibrium concentrations of butanol and isopropanol in organic and aqueous phase are estimated from experimental partition coefficient of the extracting solvent previously determined and considered organic and aqueous volumes at each sampling time. These data were plotted in **Fig. 6** with experimental data corresponding to the evolution and distribution of butanol and isopropanol concentrations in aqueous and organic phases inside the biphasic bioreactor. Both series data (experimental -lines- and theoretical equilibrium distribution -dots-) were in agreement so it could be concluded that GS-LLE system behaved as an equilibrium stage in the experimental conditions studied for this particular set up configuration. In other words, the mass liquid-liquid transfer rate was higher than the main metabolite production rate. This behavior has already been observed for ABE fermentation [12]. On the other hand, butanol in organic phase in hybrid GS-LLE system seemed to be partially stripped when compared to LLE single technique, since its concentration slightly decreased from 50 h of fermentation. This can be explained by a phase transfer phenomena from organic phase to aqueous phase while aqueous phase has been stripped. Butanol in aqueous phase forms butanol/water azeotrope which is more volatile than butanol and water alone. When gas stripping technique was applied, the

393 azeotrope was stripped because of its lower boiling point than water. Interestingly,
394 organic phase acted in this case as a butanol storage to both limit and control butanol in
395 aqueous phase, while gas stripping technique removed the extra water concentration.
396 The concomitant gas stripping with this configuration has already been mentioned [14].
397 The authors applied in their system oleyl alcohol as an extracting agent, which has four
398 times higher partition coefficient for butanol than the vegetable oil based mixture used
399 in this work. As a result, it had a positive effect for liquid extraction but made it more
400 difficult for solvent regeneration (more alcohol affinity in organic phase). Continuous
401 gas stripping technique was applied directly in the organic phase system from 48 h of
402 fermentation in order to boost final glucose consumption, while pulse-fedbatch gas
403 stripping was applied in this work from the early step of fermentation in order to
404 increase glucose consumption and production rates.
405

406 **3. Conclusions**

407 Gas stripping was studied in abiotic representative system for (A)IBE batch
408 fermentation. High flow rates (vvm > 2 min^{-1}) were needed in order to achieve
409 stripping rate of butanol higher than biological production rate. A pulse-GS mode was
410 suggested, allowing to maximize selectivity (g butanol/g water stripped) and alcohol
411 concentration in condensates. Combined separation techniques (pulse –GS-LLE) were
412 then applied to batch fermentations. A synergic effect appeared when using the
413 integrated technique, resulting in the highest butanol extraction rate and productivity.
414 Moreover, biphasic bioreactor acted as an equilibrium step and both phases were
415 stripped concomitantly.
416

4. References

[1] Jones DT, Woods DR, Acetone-Butanol Fermentation Revisited Microbiol Rew 1986;50 (4):484-524.

[2] Oudshoorn A, Van der Wielen LAM, Straathof AJJ. Assessment of Options for Selective 1-Butanol Recovery from Aqueous Solution. Ind Eng Chem Res 2009;48:7325–7336.

[3] Leland MV. Separation technologies for the recovery and dehydration of alcohols from fermentation broths. Biofuels Bioprod Bioref 2008;2(6):553-588.

[4] Woodley JM, Bisschops M, Straathof AJJ. Ottens M. Perspective Future directions for in-situ product removal (ISPR). J Chem Technol Biotechnol 2008;83:121–123.

[5] Qureshi N, Hughes S, Maddox IS, Cotta MA. Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption. Bioprocess Biosyst Eng 2005;27:215–222.

[6] Groot WJ, Van der Lans RGJM, Luyben KCAM. Technologies for Butanol Recovery Integrated with Fermentations. Process Biochem 1992;27:61-75.

[7] Qureshi N, Blaschek HP. Recovery of butanol from fermentation broth by gas stripping. Renew. Energy. 2001;22:557–564.

[8] Liao YC, Lu KM, Li SY. Process parameters for operating 1-butanol gas stripping in a fermentor. Journal Bioscience Bioeng 2014;1-7.

[9] De Vrije T, Budde M, Van der Wal H, Claassen PAM, López-Contreras AM. In situ removal of isopropanol, butanol and ethanol from fermentation broth by gas stripping. Bioresource Technol 2013;137:153–159.

[10] Ezeji TC, Karcher PM, Qureshi N, Blaschek HP. Improving performance of a gas stripping-based recovery system to remove butanol from *Clostridium beijerinckii* fermentation. Bioprocess Biosyst Eng 2005;27:207–214.

[11] Xue C, Zhao J, Lu C, Yang ST, Bai F, Tang IC. High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping. Biotechnol Bioeng 2012;109(11):2746- 2756.

[12] González-Peñas H, Lu-Chau TA, Moreira MT, Lema JM. Solvent screening methodology for in situ ABE extractive fermentation. Appl Microbiol Biotechnol 2014; 98 (13):5915-5924.

[13] Lu KM, Li SY. An integrated in situ extraction-gas stripping process for Acetone–Butanol–Ethanol (ABE) fermentation. J. Taiwan Inst Chem Eng 2014;45:2106–2110.

[14] Lu KM, Chiang YS, Wang YR, Chein RY, Li SY. Performance of fed-batch acetone–butanol-ethanol (ABE) fermentation coupled with the integrated in situ extraction gas stripping process and the fractional condensation. J Taiwan Inst Chem Eng 2016;60:119–123.

[15] Kollerup F, Daugulis AJ. Screening and identification of extractive fermentation solvents using a database. Can J Chem Eng 1985;63:1919-1927.

480 **Legends**

481

482 **Table 1.** Stripping rate constant (β) at 0.5, 2 and 3 vvm for synthetic IBE aqueous
483 solutions.

484

485 **Table 2**. Butanol removal rate from model.

486

487 **Table 3**. Estimated IBE concentration (g/L) in aqueous phase under ISPR (*In situ*
488 *product recovery* technique): gas stripping (GS), liquid-liquid extraction (LLE) and
489 coupling GS-LLE fermentations.

490

Fig.1. Experimental system for abiotic IBE gas stripping used in this study.

Fig.2. Butanol aqueous concentration evolution (experimental data: dots, thermodynamic simulation: lines) vvm=0.5 min^{-1} (■); vvm=2 min^{-1} (▲); vvm=3 min^{-1} (●).

Fig.3. Selectivity variation of the batch abiotic gas stripping process, experimental data (■), thermodynamic simulation (line).

Fig.4. Condensates composition at 4 °C versus aqueous butanol concentration, experimental data (▲), total condensate simulation (line), demixed condensates simulation (o).

Fig.5. Glucose, butanol concentration and absorbance in aqueous phase with ISPR techniques. (▲GS, ♦ LLE, ● GS+LLE, x control).

Fig.6. Total butanol and isopropanol (g/L) concentration in aqueous and organic phases with LLE and GS-LLE coupling fermentations. Calculated butanol (○); calculated isopropanol (Δ); experimental butanol (●); experimental isopropanol (▲).

Tables

Table 1. Stripping rate constant (β) at 0.5, 2 and 3 vvm for synthetic IBE aqueous solutions.

Alcohols	Stripping rate constant, β (h^{-1})		
	vvm 0.5 min^{-1}	**vvm 2 min^{-1}**	**vvm 3 min^{-1}**
Butanol	0.042±0.009	0.107±0.009	0.184±0.010
Isopropanol	0.040±0.01	0.098±0.009	0.170±0.002
Ethanol	0.021±0.005	0.053±0.006	0.108±0.010

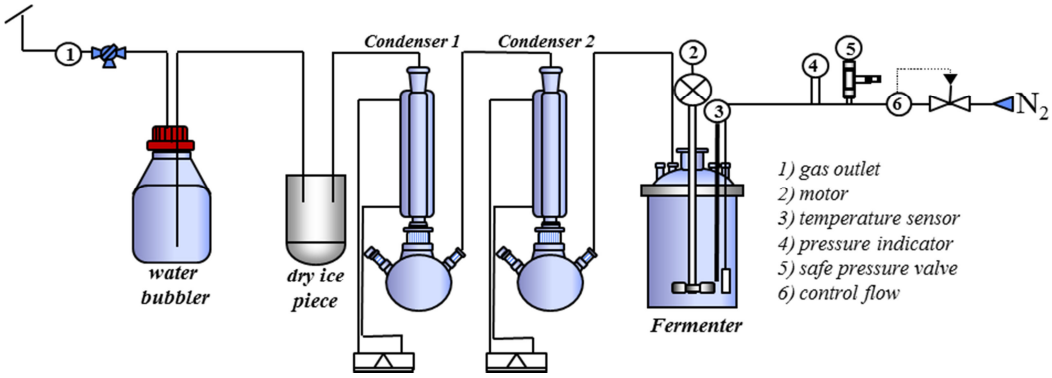
Table 2. Butanol removal rate from model.

Butanol concentration in aqueuse phase (g/L)	Butanol stripping rate (g/Lh)		
	vvm=0,5 min^{-1}	**vvm= 2 min^{-1}**	**vvm=3 min^{-1}**
0	0	0	0
2	0.08	0.21	0.37
4	0.17	0.43	0.74
6	0.25	0.64	1.10
8	0.34	0.86	1.47
10	0.42	1.07	1.84

Table 3. Estimated IBE concentration (g/L) in aqueous phase under ISPR (*In Situ Product Recovery* technique): gas stripping (GS), liquid-liquid extraction (LLE) and coupling GS-LLE fermentations.

	Estimated IBE concentration (g/L)
Control	12.41 ± 0.38
GS	13.01 ± 1.61
LLE	13.82 ± 0.65
GS+LLE	16.46 ± 0.24

Figures



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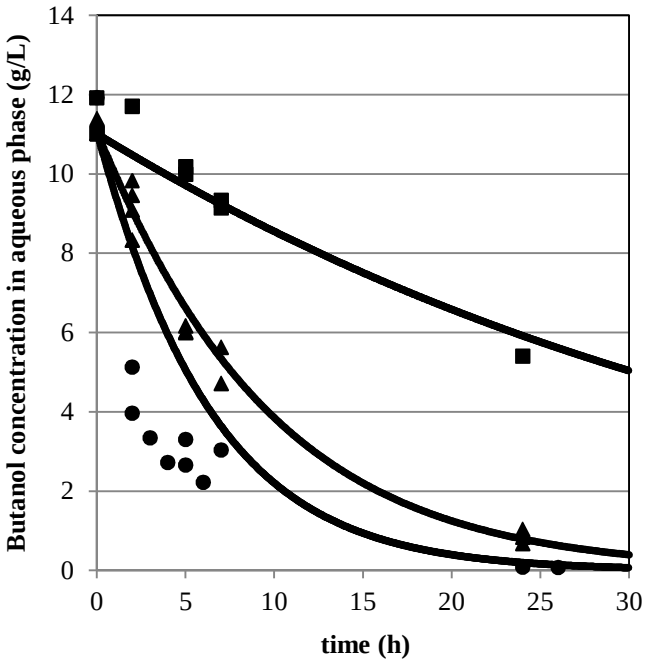

532

533 **Fig. 2.** Butanol aqueous concentration evolution (experimental data: dots,

534 thermodynamic simulation: lines) vvm=0.5 min^{-1} (■); vvm=2 min^{-1} (▲); vvm=3 min^{-1}

535 (●).

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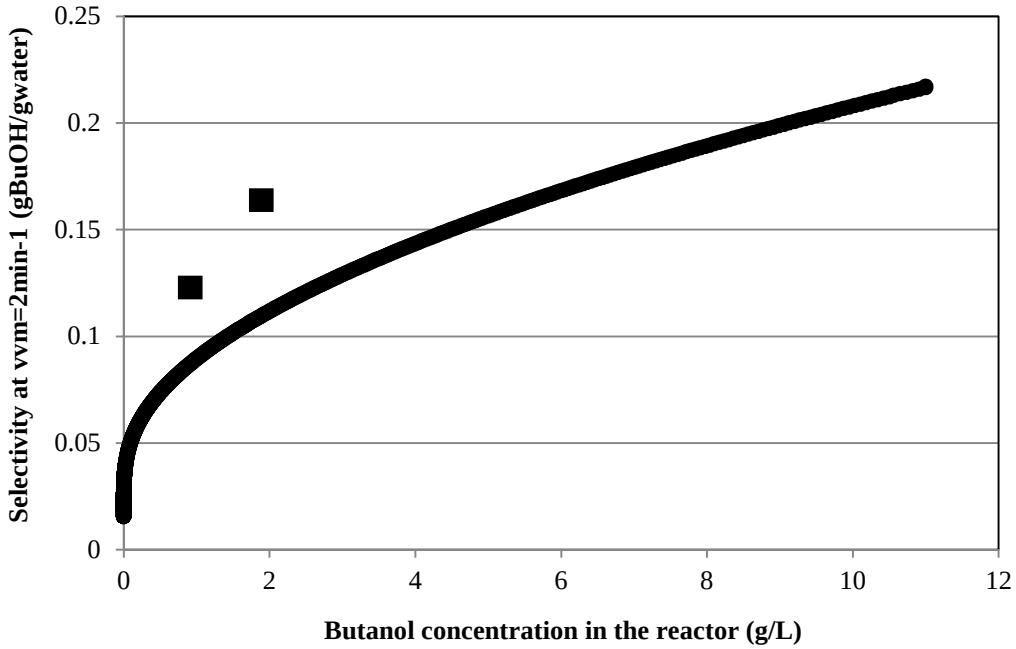

537

538 **Fig. 3.** Selectivity variation of the batch abiotic gas stripping process, experimental data

539 (■), thermodynamic simulation (line).

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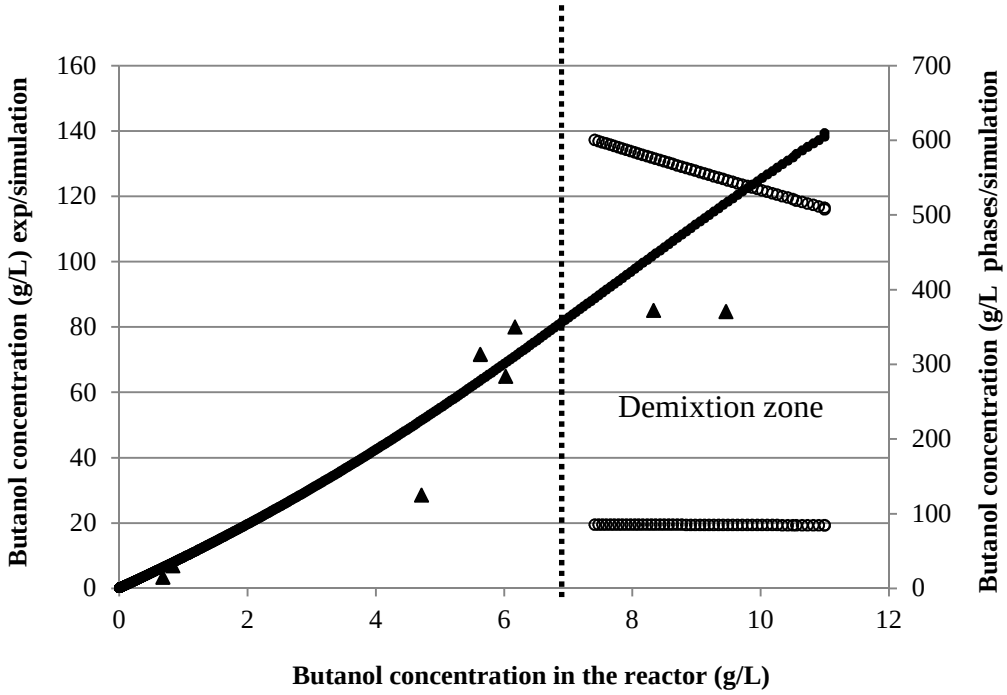

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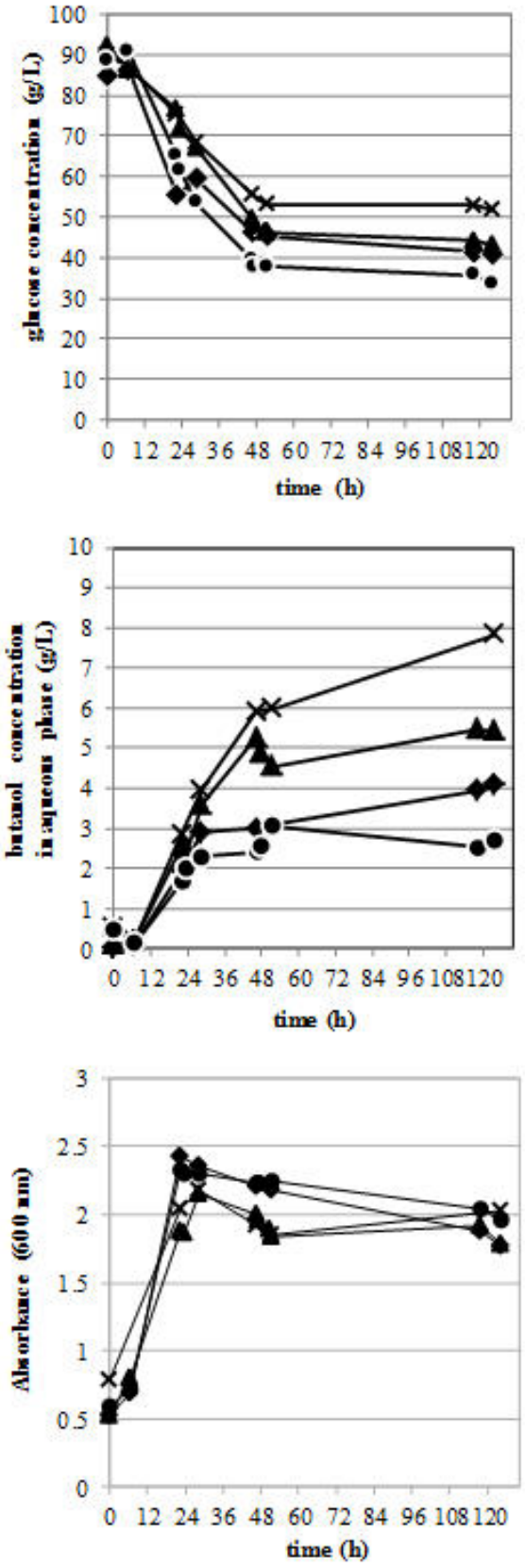


Fig. 5. Glucose, butanol concentration and absorbance in aqueous phase with ISPR techniques. (▲GS, ♦ LLE, ● GS-LLE, x control).

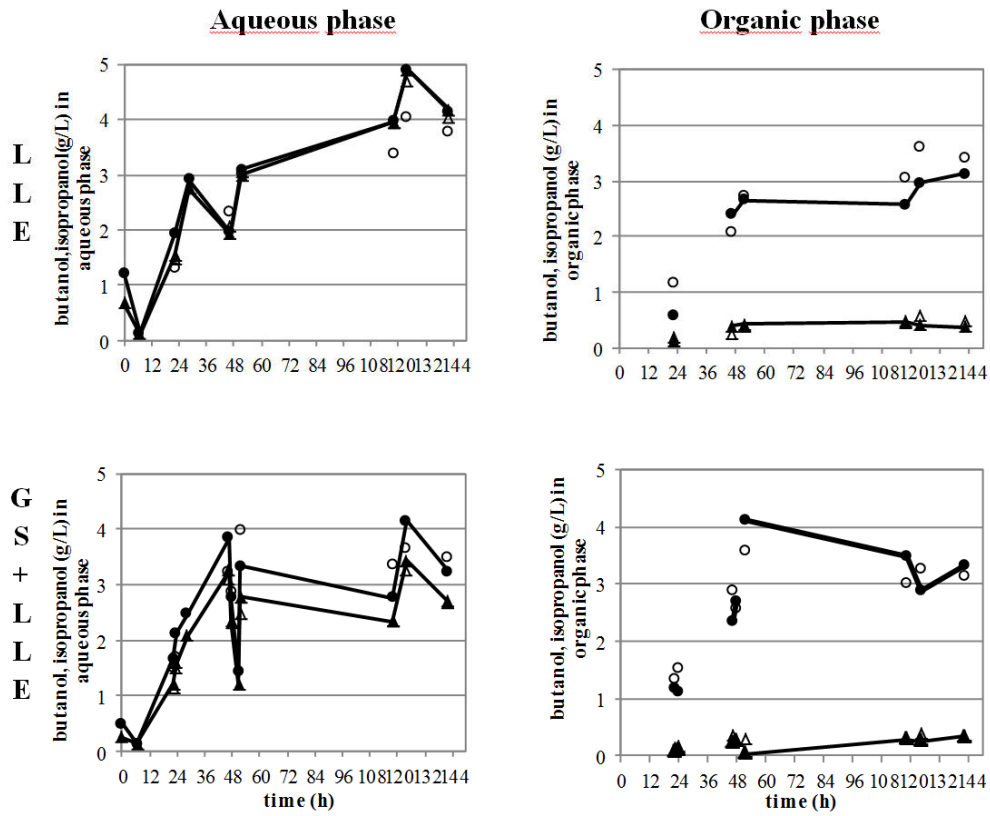
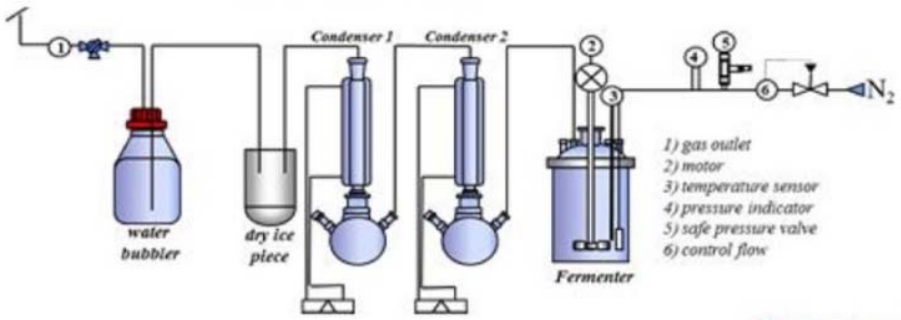
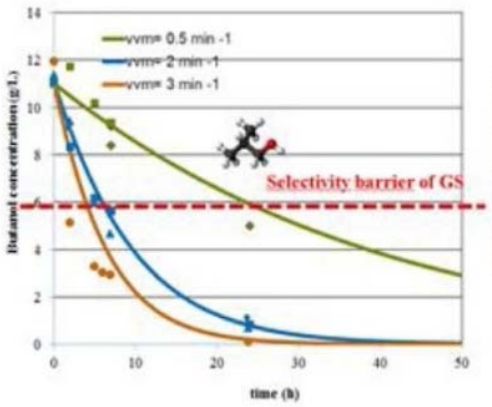
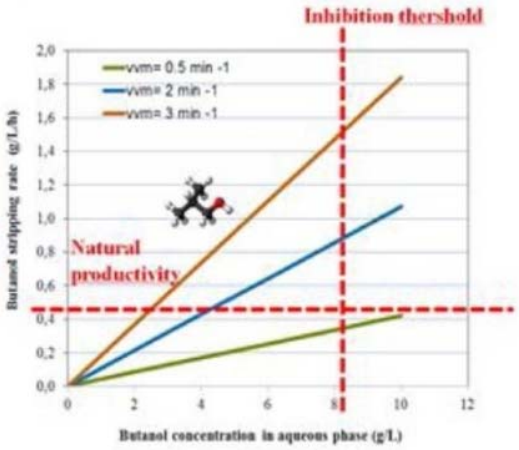


Fig. 6. Total butanol and isopropanol (g/L) concentration in aqueous and organic phases with LLE and GS-LLE coupling fermentations. Calculated butanol (○); calculated isopropanol (Δ); experimental butanol (●); experimental isopropanol (▲).

Abiotic system







Integrated GS+LLE