DEVELOPMENT OF A FAST AND LOW COST METHOD FOR ACETONE-BUTANOL-ETHANOL QUANTIFICATION IN INDUSTRIAL FERMENTATIONS

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***Abstract*— In this work, a method based on mixture density was developed to determine the concentration of acetone-butanol-ethanol solvents (ABE) instead of conventional methods based on gas-chromatography. For this purpose, synthetic solutions diluted in distilled water with different concentrations of ABE solvents were used to develop the method. In a second step, different concentrations of ABE solvents were diluted in sweet sorghum juice at different sugar concentration to simulate the real process conditions. The results demonstrated a linear relation between density and ABE concentration in water. For real fermentation media, it was necessary to take into account the concentration of sugar, where the method presented satisfactory results for synthetic and industrial fermentation media. The main contribution of this work was to determine the ABE concentration in a simple, fast and low cost way.**

***Keywords*— Butanol determination, biofuel, fermentation, industrial media.**

I. INTRODUCTION

Biofuel production is one of the key processes in development of a sustainable economy (Oudshoorn *et al.*, 2009). Bioethanol is the most used biofuel in the world, even though butanol has better proprieties, mainly the energy content, that is higher than ethanol (Al-Shorgani *et al.*, 2012). In this scenario, biobutanol can be attractive to replace gasoline supply due to the great similarity with this fossil fuel (Mayank *et al.*, 2013). Furthermore biobutanol can be obtained from many different raw materials, including residues of food industries and agriculture (Qureshi *et al.*, 2007; Visioli *et al.*, 2014).

Biobutanol production has gain attention in the recent years. According to Qureshi *et al.* (2007), there is a trend to increase biobutanol production, mainly in countries in accelerated development as the case of China and Brazil. On the other hand, research has been carried to industrially produce biobutanol using lignocellulosic biomass. These raw materials can decrease the cost of this biofuel (Jurgens *et al.*, 2012). The importance of biobutanol as alternative fuel can be verified by the great number of patents registered worldwide in the last years (Visioli *et al.*, 2013).

The perspective of expansion in the biobutanol production generated the necessity of an efficient and cheaper method to quantify the products from fermentative broth, which are (mainly) butanol, acetone and ethanol (known as ABE fermentation) in different titers depending of the strain used (Napoli *et al.*, 2010). Nowadays, this determination is made by gas chromatography (Liu *et al.*, 2010; Lépiz-Aguilar *et al.*, 2010). Liquid chromatography can also be used, although less frequently. However, determination of butanol using chromatography is limiting for industrial scale due to high costs associated with equipment, accessories and chemicals.

A cost effective alternative is the determination based on density variation in the medium. The property that is very different between products and the initial broth of fermentation is the apparent density. The ABE solvents have a density near 0.8 g.cm^{-3} and the broth has a density higher than 1.0 g.cm^{-3} (water density + dissolved solids). This shows a difference higher than 20% in the density between the solvents and fermentation medium, in a manner that is possible to associate the decrease in density of broth with solvent production in a simple and fast way.

Based on these aspects, the main objective of this work is to develop and validate a methodology to determine the concentration of ABE solvents based on the variation of density of the fermented medium. In the first step of the work, synthetic solutions diluted in distilled water with different concentrations of ABE solvents were used to develop the method. In the second step, different concentrations of ABE solvents were diluted in sweet sorghum juice at different sugar concentration to simulate the real process conditions.

II. MATERIAL AND METHODS

A. Chemicals

The solvents (acetone, ethanol and butanol) and chemicals used were from Sigma-Aldrich®. The sweet sorghum was kindly provided by a micro distillery of the Federal University of Santa Maria-Brazil.

B. Method development

A linear relation between acetone-butanol-ethanol (ABE) concentration and mixture density was used as base for the method development. In this way, a calibration curve is obtained using several ABE concentrations in different fermentation media, in order to prove the prediction capacity of the method.

C. Determination of ABE in water

Calibration points were prepared at concentrations ranging from 0 to 25 g.L^{-1} for each solvent separately. In a second step, the solvents were mixed in a molar relation of 6:3:1 for butanol, acetone and ethanol, respectively; this proportion of solvents is ordinarily obtained in butanol production by *clostridium sp.* fermentation (Xue *et al.*, 2012). Afterwards, samples at concentrations ranging from 0 to 25 $g.L^{-1}$ of ABE solvents were prepared. For each calibration point, the density of the water and solution containing ABE at specified concentration was determined. The difference between the values of densities was used for calibration purposes, obtaining

$$ABE = a + b \cdot \Delta\rho \quad (1)$$

where *ABE* is the solvent concentration ($g.L^{-1}$), $\Delta\rho$ is the difference of densities between the water and after the addition of solvents ($g.cm^{-3}$), and *a* and *b* are parameters to be estimated.

D. Determination of ABE in RCM medium

To ensure real fermentation conditions, ABE solvents were mixed in a molar relation of 6:3:1 for butanol, acetone and ethanol, respectively, in Reinforced Clostridium Medium (RCM) (Khamaiseh *et al.*, 2012), which contains ($g.L^{-1}$): yeast extract 3.0, meat extract 10.0, peptone 10.0, soluble starch 1.0, L-cysteine hydrochloride 0.5, sodium acetate 3.0, agar 0.5 and NaCl 5.0. Afterwards, samples at concentrations ranging from 0 to 25 $g.L^{-1}$ of ABE solvents were prepared and the density was determined. In this step, the effect of sugar concentration was verified in the problem and observed if it was still possible to determine ABE concentration, due to that the sugar concentration influences greatly the solvent density, since sugar concentration varies during fermentation due to microorganisms growth, being necessary to know its value to predict the ABE concentration by mixture density. For this purpose, tests were carried out using three different sugar concentrations, namely 10.0, 35.0 and 60.0 $g.L^{-1}$, using anhydrous glucose. In the same way as in the previous section, or for each calibration point, the density of the RCM medium and the solution containing ABE at determined concentration, was determined. The difference between the values of densities was used for calibration purposes, taking into account that ABE concentration is dependent of density variation and sugar concentration, leading to

$$ABE = a + b \cdot S + c \cdot \Delta\rho \quad (2)$$

where *ABE* is the solvent concentration ($g.g^{-1}$), *S* is sugar concentration ($g.L^{-1}$), $\Delta\rho$ is the difference of densities between RCM medium and after the addition of solvents ($g.cm^{-3}$), and *a*, *b* and *c* are parameters to be estimated.

E. Determination of ABE in sweet sorghum as fermentation medium

To ensure real fermentation conditions, ABE solvents were mixed in a molar relation of 6:3:1 for butanol, acetone and ethanol, respectively, using sweet sorghum as fermentation medium at different sugar concentrations (10.0, 35.0 and 60.0 $g.L^{-1}$) containing ($g.L^{-1}$): yeast extract 2.0, KH_2PO_4 0.5, $K_2HPO_4.3H_2O$ 0.65, $MgSO_4.7H_2O$ 0.2, $FeSO_4.7H_2O$ 0.01, $MnSO_4.H_2O$ 0.01, NaCl 0.01, sodium thioglycolate 1.0 and tryptone 1.0 (Liu *et al*., 2010). Afterwards, samples at concentrations ranging from 0 to 25 $g.L^{-1}$ of ABE solvents were prepared and density determined. For each calibration point, the density of the sweet sorghum medium and after the addition of ABE at determined concentration, was determined. Equation (2) was used to calibrate the ABE concentration in sweet sorghum medium. In this case, $\Delta\rho$ is the difference of densities between sweet sorghum medium and after the addition of solvents ($g.cm^{-3}$).

F. Measurement of mixture density

The densities of the solutions were measured with an Anton Paar DMA 4500 oscillating U-tube densimeter equipped with automatic temperature correction and operated under static mode. The sample was injected three times to ensure that the strap sample is completely free of contamination, which can affect the density measured. The sample was injected and the syringe was maintained attached to the densimeter inlet in order to prevent the occurrence of air bubbles. The density was set at the temperature of 20 ± 0.01ºC and the time of analyses can be considered as the time to achieve this temperature.

G. Parameter Estimation

All parameters were estimated using the algorithm of Levenberg–Marquardt of software Statistica 7.0 (Statsoft Inc., Tulsa, OK, USA). The estimation of the model parameters consisted in minimizing the sum square residues (SSR) between predicted and experimental data.

III. RESULTS AND DISCUSSION

A. Determination of ABE in water

The results presented in this section will give support for the next step, since we will demonstrate the relation between concentration of solvents and density. Figure 1 shows the influence of the concentration of each solvent separately on the mixture density. A linear relation is observed between the concentration of each solvent and density. The coefficients of determination of the three fits are greater than 0.999, and the probability of the parameter to be significant is very close to 100 % for the parameters of each fit. Furthermore, the visual analyses demonstrate the good agreement between predicted and experimental data.

The results presented in Figure 1 demonstrated that is possible to develop a method based on mixture density to determine the ABE concentration in fermentation media. However, in real fermentation, all compounds will be dissolved in liquid media, being necessary to verify if a linear relation is also maintained in this case. For this reason, the method to quantify ABE solvents was tested considering ABE molar proportion of 6:3:1, and the results are presented in Fig. 2.

The linear relation between ABE concentration and mixture density is maintained for the three solvents. In the same way observed in the use of individual solvents, the coefficient of determination was greater than 0.999 and the probability of the parameter being significant is very close to 100 %. This fact increases the possibility

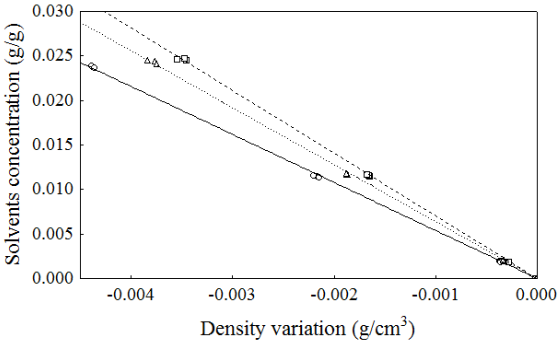

Figure 1. Relation between experimental data of density (g/cm^3) and solvent concentration (g/g) of butanol (Δ), acetone (□) and ethanol (○) solution using distilled water as medium, and its respective linear fits (….), (----) and (──).

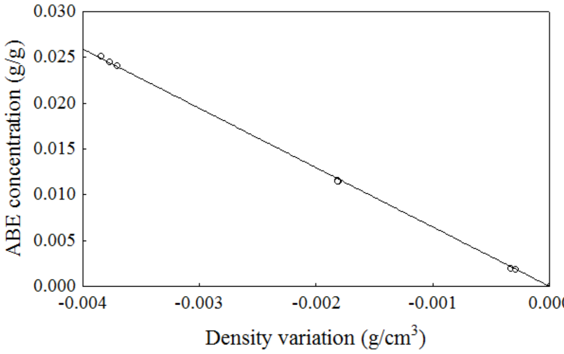

Figure 2. Relationship between experimental data of density (g/cm^3) and solvent concentration (g/g) of butanol, acetone and ethanol (○) in a respective molar proportion of 6:3:1 solution using distilled water as medium, and its respective linear fit (──).

of a good fit to represent the density variation using a real medium during the biobutanol production by fermentation. However, this method does not allow the quantification of these compounds separately, since all of them respond in the same manner to different concentrations. On the other hand, it is possible to obtain an approximated concentration of each compound, since a same microorganism produces a fixed relation between them.

B. Determination of ABE in RCM media

The results presented in Figs. 1 and 2 are related to a mixture of water and ABE solvents in different concentrations. However, real fermentations present other compounds in the medium. For example, if synthetic compounds are used to formulate the culture medium, there is the presence of salts and, mainly, sugar that alters the density of the mixture. For this reason, distilled water was replaced by reinforced clostridium medium (RCM), which is largely applied in butyric fermentation for the growth of clostridium sp., the most used bacteria in butanol production from fermentation (Knoshaug and Zhang, 2009).

Figure 3 shows the results obtained for different ABE and sugar concentrations. A perfect linear fit is verified for all calibration points, indicating that is possible to predict the ABE concentration as a function of density variation and sugar concentration. The determination coefficient is greater than 0.99 and the probability of the parameter been significant is very close to 100%, as well as the calibration of solvents in water. It is evident that the insertion of sugar concentration as a variable to be measured in the analyses is adequate to make this technique applicable. Parameter values, standard errors, significance levels and confidence intervals are presented in Table 1. The p-level of each parameter is very close to zero, indicating that these parameters are statistically significant. This proves that the model proposed can be very useful as a less expensive way for solvents determination in clostridial fermentation for butanol production.

Table 1. Parameters, confidence intervals, standard errors, p-levels, final objective function of the fit to experimental data using RCM as medium and parameters value estimated from Eq. 2.

Par.	**Est.**	**Stand. Error**	**p-level**	**Low conf. limit**	**Up. conf. limit**
a	0.0012	0.0003	0.0002	0.0017	0.0018
b	0.0020	<0.0001	<0.0001	0.0019	0.0020
c	-5.3671	0.0895	<0.0001	-5.5508	-5.1834
Obj. Funct. 0.0000186					

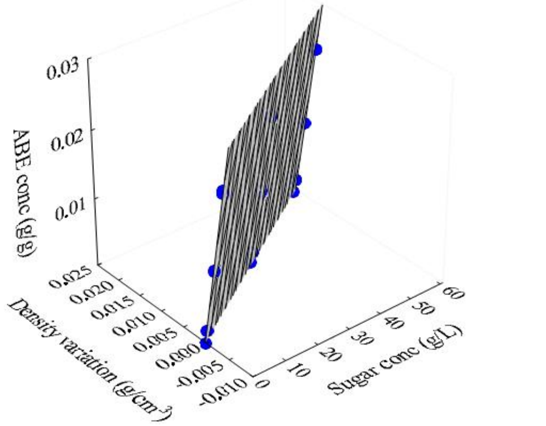

Figure 3. Relationship between experimental data of density variation (g/cm^3), solvent concentration (g/g) of ABE in a respective molar proportion of 6:3:1 solution, and sugar concentration (●) using RCM as medium, and its respective linear fit model.

C. Determination of ABE in sweet sorghum as fermentation medium

Industrial fermentations are carried out using low cost raw material and, due to this reason, the proposed method was applied to determine ABE concentration in fermentation media based on the use of sweet sorghum, since this medium can be used in butyric fermentation (Yu *et al.*, 2012). Calibration points at different ABE and sugar concentrations were prepared in a similar way than for the determination of ABE in RCM medium, and the variation of density was determined in each case. Figure 4 shows the experimental results as well the proposed linear fit. As can be seen, there is a good agreement between experimental and fitted data, showing that the variation of density and sugar concentration can be used to predict ABE concentration in a simple, fast and low cost way. The determination coefficient

Table 2. Parameters, confidence intervals, standard errors and p-levels, and final objective function of the fit of experimental data using sweet sorghum as medium showed in Fig. 4, and parameter value estimated from Eq. 2.

Par.	Est.	Stand. error	p-level	Low conf. limit	Up. conf. limit
a	a	-0.1942	0.0054	<0.0001	-0.2053
b	b	0.0032	<0.0001	<0.0001	0.0030
c	c	-5.4642	0.1414	<0.0001	-5.7561
Obj. Funct. 0.0000323					

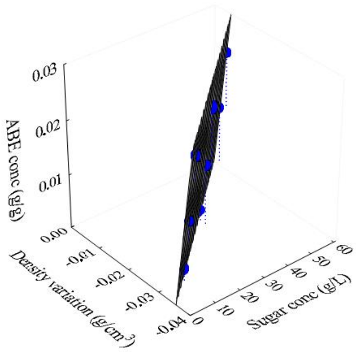

Figure 4. Relationship between experimental data of density variation (g/cm^3), solvent concentration (g/g) of ABE in a respective molar proportion of 6:3:1 solution and sugar concentration (●) using sweet sorghum as medium, and its respective linear fit model.

was greater than 0.99. Parameter values, standard errors, significance levels and confidence intervals are presented in Table 2, where it is confirmed that the linear model satisfactorily represents the experimental behavior. Another important characteristic demonstrated is that the parameters *c* for RCM and sweet sorghum media are statically equal, indicating that the decrease of density in both cases is very similar. This parameter is not dependent of the medium proprieties but it is dependent of the ABE concentration. This is expected and shows that for similar media, it is possible to have a preliminary estimation of ABE production for a fixed sugar concentration.

IV. CONCLUSIONS

In this work, a method for the determination of ABE solvents in fermentation media based on mixture density was developed. The results obtained demonstrated a linear relation between density and ABE concentration in water. For real fermentation media, it was necessary to take into account the concentration of sugar, where the method presented satisfactory results for synthetic and industrial fermentation media. The main contribution of this work was to determine the ABE concentration in a simple, fast and low cost way, unlike classic chromatographic procedures.

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