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Original Article

Aqueous two-phase systems applied to the extraction of syringaldehyde and vanillin from eucalyptus wood residues

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Abstract

This work examines the recovery of phenolic compounds through the autohydrolysis of eucalyptus wood residues. The hydrolysis of pin chips was carried out using hot water under different conditions. This treatment was capable of extracting xylo-saccharides, phenols, furfural (FUR), hydroxymethylfurfural (HMF) and acetic acid (AAc). The highest phenolic contents were attained at 160 ℃ and 110 minutes, where total phenolic compounds (TPC) reached 1.33% gallic acid equivalent (GAE) and syringaldehyde (SA) and vanillin (VAN) were at 0.01% each, in pin chips dry basis (d.b.).

The capacity to extract phenolic compounds from pin chip hydrolysate through aqueous two-phase systems (ATPS) based on PEG 2000 and sodium citrate was demonstrated. High extraction efficiency was obtained (99.8% for the TPC, 82.3% for the SA, 94.9% for the VAN, 89.3% for the FUR and 70.9% for the AAc). The recovery of sugars in the bottom phase was 58.4%.

Keywords: lignocellulosic biorefinery, phenolic compounds, autohydrolysis, aqueous two-phase systems, eucalyptus residues

1. Introduction

There is a growing interest in the conversion of biomass into fuels and chemical products, with the aim of finding new technological solutions to decrease the consumption of non-renewable resources (van Heiningen *et al.*, 2011). Within the sources of biomass, forestry biomass is abundant, can grow in relatively poor soil, requires little energy and nutrients to grow, and does not compete with the production of food (van Heiningen *et al.*, 2011).

Wood biomass is composed basically of cellulose (35-50% dry base (d.b.)), hemicellulose (20-35%), lignin (10-25%), and extractives (<5%) (Sjöström, 1993). The processes involved in so-called integrated forestry biorefinery are as follows. Wood logs are processed to obtain traditional products, such as cellulose, paper, cardboard, veneer, and the like, while by-products, such as sawdust, fines and waste are turned into biofuel, biopolymer, other chemicals, and energy (van Heiningen *et al.*, 2011).

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The hydrolysis of lignocellulosic materials with hot water has already been reported (Garrote, Domínguez, & Parajó, 1999). In the case of biomass containing xylan-type hemicelluloses, a hydrolysate is produced with xylose or xylo-oligomers for the production of bioethanol, xylo-oligosaccharides (XOS), furfural (FUR), xylitol, and others (Gírio, Fonseca, Carvalheiro, Duarte, & Marques, 2010; Moure, Gullón, Domínguez, & Parajó, 2006). This is an environmentally friendly process and easy to perform, but not selective. Although xylo-saccharides are the components produced in the highest proportion, there are components from sugar degradation, organic acids, extractives and phenolic compounds, such as syringaldehyde (SA) and vanillin (VAN) also produced in large proportions (Parajó *et al.*, 2008). Many authors have written about the antioxidant, antimicrobial and anticarcinogenic properties of phenolic compounds (Conde, Moure, Domínguez, & Parajó, 2011a; Kummee & Intaraksa, 2008). SA and VAN are used in the flavour and fragrance industries (Mota, Pinto, Loureiro, & Rodrigues, 2016). Therefore, the extraction of phenolic compounds obtained through hydrolysis is beneficial for two reasons: on the one hand, it purifies the hydrolysate that is rich in sugars, and on the other hand, the extracted compounds are utilized.

Previous investigations, coupled with the increasing interest in green separation chemistry, motivate studying alternative ways of extraction. The most commonly used method reported in the literature is solvent extraction by ethyl acetate, whereas phenolic compounds migrate to the organic phase (Egüés, Sanchez, Mondragon, & Labidi, 2012). Recently, aqueous two-phase systems (ATPS) have been acknowledged as being environmentally friendly, because of their high content of water in both phases as well as their high mass transfer between the phases (Ratanapongleka & Phet som, 2011). ATPS are formed when two water soluble compounds reach a critical concentration leading to the formation of two phases. Particularly, polymer-salt systems have several advantages, namely low price, low viscosity, and a short time required for phase separation. ATPS have been used for the extraction of several biological products (Simental-Martínez, Montalvo-Hernández, Rito-Palomares, & Benavides, 2014; Xavier, Freire, Vidal-Tato, & González-Álvarez, 2015).

In this study, pin chips of eucalyptus from a local pulp mill went through a hydrothermal treatment to fractionate the material. One of the goals of this work was to evaluate the autohydrolysis of pin chips regarding the resulting products (xylo-saccharides, total phenols compounds (TPC), VAN, SA, acetic acid (AAc), FUR, and hydroxymethylfurfural (HMF)), with an emphasis on the phenolic content. To achieve this, we performed three hydrothermal treatments (autohydrolysis). As a second goal, we focused on the extraction of phenolic compounds in the liquor obtained through ATPS based on PEG 2000 and $Na_3C_6H_5O_7$. Extraction efficiency and partitioning of TPC, SA and VAN were analysed for different tie-line lengths (TLL) and volume ratios (V_r) in the system. Extraction of xylo-saccharides, FUR, HMF and AAc was also monitored.

2. Materials and Methods

2.1 Chemicals

All the reagents were of American Chemical Society (ACS) reagents quality or higher. Gallic acid and sodium carbonate were purchased from Panreac (Barcelona, Spain). Polyethylene glycol (PEG) 2000, sulphuric acid, AAc, acetonitrile, methanol, Folin-Ciocalteu reagent and trisodium citrate dihydrate were supplied by Merck (Darmstadt, Germany). HPLC standards were from Sigma-Aldrich (Steinheim, Germany) with a purity higher than 99%. XOS standards: xylobiose, xylotriose, xylotetraose and xylopentaose, for the determination of the molecular weight (MW) distribution, were obtained from Megazyme, Ireland with purities higher than 90%.

2.2 Raw material and biomass pre-treatment

Eucalyptus pin chips were provided by a local pulp mill (UPM, Uruguay). The size of the pin chips ranged between 0.50 and 3.36 mm. The material was dried in a tray drier with air at 40 °C until it reached equilibrium moisture (~8.0%). Finally, it was stored in polyethylene bags.

40 dry grams of eucalyptus pin chips were subjected to autohydrolysis in a silicone oil bath with eight stainless steel 316 rotating cylinder vessels (Fibretec Inc., India). The nominal volume of each reactor was 300 mL. Three treatments were selected with different temperatures and hydrolysis times (Experiment 1: 160 °C, 110 min; Experiment 2: 140 °C, 180 min; Experiment 3: 170 °C, 40 min) with a fixed liquid to solid ratio of 7 g/ g dry solid.

This selection was based on previous experiments, in which the priority was to maximise the XOS yield (Rodríguez-Quinele, Clavijo, & Cabrera, 2015). Once the reaction was completed, the solid material was separated through vacuum filtration and the obtained hydrolysate rich in sugars was analysed as described below. The solid fraction rich in cellulose and lignin may be used as substrate for the production of other value-added products.

2.3 Extraction and separation procedure

Five grams of the liquid obtained from the auto-hydrolysis was neutralised (AHN) and used to carry out the extractions. The composition of the compounds to form the ATPS was selected according to the binodal curves found in the literature (Murugesan & Perumalsamy, 2005) (Table 1).

A known amount of $Na_3C_6H_5O_7$ was dissolved in 5 grams of hydrolysate. Later, in order to form the ATPS, the corresponding amount of PEG 2000 was added. The total mass used to form the ATPS was 20 g.; water was used to replenish as needed. The mixtures were agitated for 30 minutes and left to rest for 12 hours in order to reach the full separation of phases. The extractions were carried out in a glass cell controlled by a thermostat and equipped with a magnetic agitator. Samples of the top and bottom phases were taken simultaneously. The influence of the TLL on the TPC, VAN, SA, FUR, HMF and AAc was analysed. The influence of volume ratio of the phases (V_r=volume top phase/volume bottom phase) on the TPC, VAN and SA was also analysed. The TPC, SA and VAN were determined in the top phase, while the FUR, HMF, sugars and AAc were determined in the bottom phase. Through mass balance the composition of the other phase was obtained. All the experiments were carried out in triplicate.

2.4 Analytical methods

The compositions of the raw materials and solid materials after the pre-treatment (carbohydrates, acetyl groups, lignin, extractives, and ash) were obtained according to NREL protocols (Sluiter *et al.*, 2008, 2008b). Acid soluble lignin was quantified using UV spectroscopy at a wavelength of 205 nm and an absorptivity of 110 L/(g.cm) according to Tappi UM 250, 1991. The results are expressed as g/ 100 g pin chips d.b. (%).

Autohydrolysis liquors are characterised by the determination of sugars (xylose, arabinose and glucose), by-products and degradation products (AAc, HMF, and FUR), based on NREL protocol (Sluiter *et al.*, 2006). The results are expressed as grams of compound / 100 grams pin chips d.b. (%). To determine the MW distribution, the liquid hydrolysate was analysed with a Shimadzu HPLC chromatograph equipped with Bio-Rad Aminex HPX-42A column and a refractive index detector. A calibration curve was made using xylose and XOS standards: degree of polymerisation (DP)= 2 to 5. TPC was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965) as described by Xavier *et al.*

Table 1. Systems selected for evaluating the extraction of phenolic compounds (25 ºC)

System	PEG 2000 (%)	$Na_3C_6H_5O_7$ (%)	H_2O (%)	TLL (%)	V_r	pH_1
1	15.5	13.6	70.9	26.1	0.9	8.2
2	18.5	15.1	66.4	40.4	0.9	8.4
3	20.0	16.0	64.0	46.1	0.9	8.4

(Xavier *et al.*, 2015). The TPC is expressed as g of gallic acid equivalent (GAE)/ 100 g of pin chips d.b. (%). Phenolic compounds were analysed by HPLC (Shimadzu) using a reverse phase C-18 column (5 µm, 4.6 mm×250 mm Waters Spherisorb) and a diode array detector at 280 nm as described by Xavier *et al.* (Xavier *et al.*, 2017). The results are expressed as g of phenol compound (VAN or SA)/ 100 g pin chips d.b. (%).

2.5 Extraction efficiency (%EE) and partition coefficient (K)

The extraction efficiency *(%EE*) is defined as the fraction of the corresponding compound i removed from the hydrolysate into the PEG-rich phase during extraction, that is:

$$\%EE = \frac{C_{TPi} V_{TP}}{C_{0i} V_0} \times 100 \quad (1)$$

where V_{TP} and V_0 are the volume of the top phase and the volume of AHN, respectively, and C_{TPi} (mg/L) is the concentration of the corresponding compound i in the top phase. C_{0i} is the concentration of the corresponding compound i in AHN.

The partition coefficient *K* was determined as the ratio between the concentration of the corresponding compound i in the top phase (C_{TPi} (mg/L)) and that in the bottom phase (C_{BPi} (mg/L)), that is:

$$K = \frac{C_{TPi}}{C_{BPi}} \quad (2)$$

3. Results and Discussion

3.1 Autohydrolysis of eucalyptus pin chips

The chemical composition of the raw material is presented in Table 2. In autohydrolysis treatment, in addition to hemicelluloses, other products are obtained, such as those from sugar degradation, organic acids, phenolic compounds and extractives. It is expected that cellulose and lignin will remain mostly unaltered (Garrote, Cruz, Domínguez, & Parajó, 2008; Parajó *et al.*, 2008). Eucalyptus extractives are mainly hydrolysed tannins and flavonoids. Some of them are water soluble species, so a part of the extractives ends up in the liquor (Conde *et al.*, 2011a). As can be seen in Table 3, the main components in the extracted liquor are the xylo-saccharides, ranging between 11.1 and 12.4% pin chips d.b. and around 80% are in the form of oligosaccharides. These values imply an extraction of 65 – 73% of the initial xylan content in the raw material. There is no difference between the extracted liquors of the three autohydrolysis condition tests, neither in xylo-saccharides content nor in XOS content.

Table 2. Composition of native eucalyptus pin chips

Compound	(% pin chips d.b.)
Ethanol/water extractives	4.3±0.4
Acid insoluble lignin	25.1±0.3
Acid soluble lignin	2.5±0.1
Total lignin	27.6±0.7
Glucan	43.1±0.4
Xylan	14.9±0.3
Acetyl groups	3.6±0.3
Ash	0.9±0.1

Values are presented as mean ± SD (n=3).

Table 3. Sugars and AAc in the liquid hydrolysate. (Results are expressed as g/ 100 g pin chips d.b. (%))

	Experiment 1 160 °C, 110 min	Experiment 2 140 °C, 180 min	Experiment 3 170 °C, 40 min
Xylosaccharides	11.1^{a}±1.5	12.4^{a}±1.8	11.7^{a}±0.8
Xylo-oligomers	9.6^{a}±1.6	10.5^{a}±1.6	9.8^{a}±0.8
Xylose	1.8^{a}±0.4	2.8^{b}±0.3	2.0^{a}±0.4
Glucosaccharides	0.7^{a}±0.1	0.5^{b}±0.1	0.9^{c}±0.1
Gluco-oligomers	0.7^{a}±0.1	0.5^{b}±0.1	0.9^{c}±0.1
Glucose	Not detected	Not detected	<0.1
Acetic acid	2.8^{a}±0.4	3.3^{a}±0.4	2.7^{a}±0.2
MW (Dalton)	624^{a}±12	578^{b}±18	869^{c}±15

Values are presented as mean ± SD (n=3). In each line, values with different letters are significantly different ($p<0.05$).

Garrote *et al.* (1999) reported similar results working with *Eucalyptus globulus* sawdust in similar conditions. However, in the work of Gütsch *et al.* (Gütsch, Nousiainen, & Sixta, 2012) they obtain a lower xylo-saccharides content in the extracted liquor, working with similar conditions, but using *Eucalyptus globulus* chips. A possible explanation for this discrepancy lies in the extracted liquor´s treatment for the analysis. Gütsch *et al.* (Gütsch *et al.*, 2012), centrifuged the extracted liquor before the analysis, eliminating the suspended solids fraction, which also contains hemicellulose derived materials among others.

Glucosaccharides content follows, varying between 0.5 and 0.9% pin chips d.b., and they are attributed to the glucose components of the eucalyptus hemicelluloses (*O*-acetyl-(4-*O*-methylglucurono)xylan). In addition, AAc was generated by the hydrolysis of acetyl groups present in eucalyptus hemicelluloses (Garrote *et al.*, 1999).

The average molecular weight varies from 578 to 869 Da. These values are comparable to the ones obtained by Leschinsky *et al.* (Leschinsky, Sixta, & Patt, 2009) using *Eucalyptus globulus* chips with similar autohydrolysis conditions. However the MW values are much lower than

those reported by Tunc and van Heiningen (Tunc & van Heiningen, 2011), where they found a MW of 4761 - 5464 Da of the liquor extracted at 170 or 160 °C respectively, working with American southern hardwood chips. The authors used another type of hardwood, different from eucalyptus; therefore, a possible explanation of this difference could lie in the species of wood used.

Table 4 shows the phenolic compounds and furans quantified in the hydrolysate. These compounds derive from the degradation of sugars, extractives and a slight degradation of lignin. The TPC varied from 1.10 to 1.33% pin chips d.b. Similar TPC values were previously reported, where autohydrolysis (200 °C, 10 min) was applied to several lignocellulosic materials (*Eucalyptus globulus* wood: 1.31% d.b.; corncobs: 1.53% d.b.) (Conde, Moure, Domínguez, & Parajó, 2011b). It was also reported that on applying autohydrolysis at 210 °C for 10 minutes to olive tree prunings, the TPC was higher than the one reported in this study (2.03% pin chips d.b.). However, this same residue submitted to steam explosion a 200 °C for 5 minutes gave a lower TPC than the one in this study (0.81% biomass d.b.)(Conde *et al.*, 2009).

Among the phenolic compounds, two aldehydes were identified: VAN and SA. An increase in the content of VA, SA and TPC was seen from Experiment 1 to 2, probably because increasing the temperature released phenolic compounds linked to hemicelluloses and sugar oligomers present in the hydrolysate. On the other hand, if we compare the results with Experiment 3, the values of these compounds decrease. The shorter duration and higher temperature of the experiment can account for these results. High temperatures can degrade part of the free phenolic compounds (Conde *et al.*, 2009). The highest VA, SA and TPC contents were attained with Experiment 1 (160 °C, 110 min); consequently the extraction with a two-phase system will be carried out with that treatment.

Regarding the sugar derived compounds, the FUR varied from 0.13 to 1.21% pin chips d.b. and the HMF from 0.02 to 0.09% pin chips d.b. It has been reported in the literature that the compound content of sugar derivates is 0.57% biomass d.b. for *Eucalyptus globulus*; 0.22% biomass d.b. for corncobs residue, and 0.12% biomass d.b. for chestnuts burs (Conde *et al.*, 2011b).

3.2 Separation of phenolic compounds (TPC) by ATPS system

After autohydrolysis treatment of the eucalyptus pin chips, the process of extraction of TPC, SA, and VAN with ATP was then performed at three different TLLs (Table 1). The information in the TLLs is paramount when working with the ATPS, given that the tie-line length has an impact on the composition of the top and bottom phases (Benavides & Rito-Palomares, 2008). Every system was managed with a volume ratio close to 1 and a constant pH. This is advised to avoid any potential concentration effects (Benavides & Rito-Palomares, 2008). The extraction of the FUR, HMF and AAc was also monitored using these systems.

The partition coefficient (ln *K*) and the extraction efficiency (%*EE*) were observed and results are also shown in Figure 1 and Table 5.

Table 4. Phenolic compounds and furans in the liquid hydrolysate. (Results are expressed as g/ 100 g pin chips d.b. (%))

	Experiment 1 160 °C, 110 min	Experiment 2 140 °C, 180 min	Experiment 3 170 °C, 40 min
TPC	1.33^{a}±0.07	1.10^{b}±0.10	1.15^{c}±0.12
SA	0.10^{a}±0.01	0.04^{b}±0.02	0.04^{b}±0.03
VAN	0.10^{a}±0.02	0.03^{b}±0.01	0.04^{b}±0.02
FUR	1.21^{a}±0.03	0.13^{b}±0.01	0.51^{c}±0.21
HMF	0.04^{a}±0.01	0.02^{b}±0.01	0.09^{c}±0.04

Values are presented as mean ± SD (n=3). In each line, values with different superscripts are significantly different ($p<0.05$).

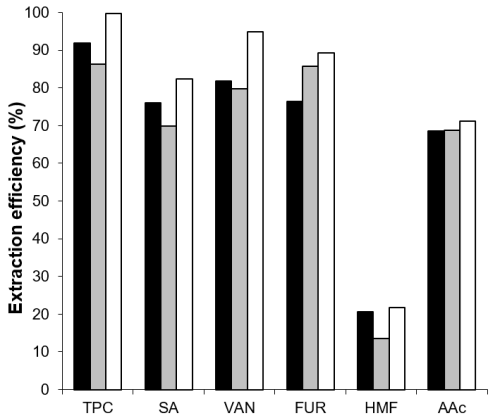

Figure 1. Extraction efficiencies of phenolic compounds, furans and AAc in a system composed of PEG 2000 + $Na_3C_6H_5O_7$ + H_2O for different TLLs. Black, grey and white bars represent System 1, System 2 and System 3, respectively.

Table 5. Partition coefficients of TPC, VA, SA, FUR, HMF and AAc at different tie-line lengths

System	ln *K*					
	TPC	SA	VAN	FUR	HMF	AAc
1	9.39	1.22	1.52	1.18	-1.35	0.96
2	8.83	0.97	1.16	1.80	-1.85	0.97
3	13.41	1.65	2.79	2.12	-1.28	1.06

Phenolic compounds preferentially migrated to the top phase (PEG rich-phase) as expected. The partition of phenolic compounds between the top and bottom phases can be explained considering hydrophobic interactions and hydrogen bonding interactions between the phenolic compounds and the components of the PEG (Willauer, Huddleston, Li, & Rogers, 2000). This has already been mentioned by other authors (Simental-Martínez *et al.*, 2014; Xavier *et al.*, 2017). Both phenols identified are hydrophobic, as indicated by their log P. VAN has log P 1.22 and SA has log P 1.07 ("Chemicalize," n.d.). These values may explain the greater extraction efficiency and ln *K* of VAN versus SA.

FUR and AAc also migrate to the PEG rich phase, with extraction efficiencies above 70%. However, HMF migrates to the salt rich phase (ln K<0). This can be explained by the fact that HMF, being most hydrophilic, has a greater capacity for interacting with water (log P = –0.1) ("Chemi calize," n.d.). Even though the AAc also has a negative log P, it is an amphipathic molecule, which may explain the migration to the most hydrophobic phase.

As shown in Table 5 and Figure 1, higher ln K and efficiency are obtained at higher TLL. As TLL increases, so do the amounts of PEG 2000 and salt, and therefore the water quantity is reduced. The greater the TLL, the greater the extraction efficiency. All this favors hydrophobic interactions between solutes and components of the biphasic system (Simental-Martínez *et al.*, 2014). High extraction efficiency was attained at TLL=46.1 (%) (99.8% total phenols content, 82.3% for the SA, 94.9% for the VAN, 89.3% for the FUR and 70.9% for the AAc). High efficiencies in the extraction of phenolic compounds have been reported in ATPs based on ionic liquids and surfactants (Xavier *et al.*, 2017).

Additionally, the influences of volume ratio (V_r) on TPC, SA and VAN were studied. We worked with three V_r, one close to 1 (V_r =0.9), another above 1 (V_r =4.1) and the third under 1 (V_r =0.4). As shown in Table 6, the %*EE* and the ln K increased with volume ratio.

However, when we worked with volume ratios close to or above 1, the variation in extraction efficiency was not significant. The highest efficiency (100%) is obtained working with a V_r of 4.1, where the phenolic compounds migrate exclusively to the PEG-rich phase (ln K=∞). In an industrial setting, however, it would be necessary to evaluate whether the increased efficiency compensates for working with high volume ratios, as the system's selectivity can decrease due to it promoting the migration of non-desired molecules to the top phase (Gómez-Loredo, Benavides, & Rito-Palomares, 2014). On the other hand, on working with a V_r of 0.4, the extraction efficiency decreases. This implies that the available volume in the top phase is reduced, therefore phenols have less available volume and consequently saturation problems may arise (Benavides & Rito-Palomares, 2008; Gómez-Loredo, Gon zález-Valdez, & Rito-Palomares, 2015).

Another important factor to consider when evaluating the separation method is to estimate the amount of sugars left in the salt rich phase after extraction. The recovery of sugars in the bottom phase was 58.4%. Most methods for separation of these components of sugars reported having a reduction of over 42% (Maiti *et al.*, 2017), which is an indication of efficient separation.

With these results, it can be stated that the system based on PEG2000/ $Na_3C_6H_5O_7$ /H_2O is capable of promoting the extraction and concentration of phenolic compounds, FUR and AAc with high levels of efficiency. Furthermore, these results carry the advantage that both phases generated have great utility. The bottom phase, which is rich in sugars, can be used for fermentation for the production of XOS, and the top phase, rich in phenols, has antioxidant potential (Kummee & Intaraksa, 2008; Parajó *et al.*, 2008).

4. Conclusions

Through the autohydrolysis of eucalyptus pin chips, a hydrolysate rich in XOS was produced. The use of ATPS based on $Na_3C_6H_5O_7$ and PEG2000 for the extraction of phenolic compounds from the hydrolysate obtained revealed high levels of efficiency. High performance levels of extraction were also observed for FUR and AAc. In the process of extracting phenolic compounds, FUR and AAc reacted with TLL. The greater the TLL, the greater the extraction efficiency. It was also noted that the extraction efficiency of phenolic compounds increased with volume ratio. High extraction efficiency was attained at TLL= 46.1 (%). Furthermore, at this TLL the recoveries of sugars in the bottom phase were 58.4%.

Future work includes the assessment of all components in order to benefit from the different components of raw materials and maximize their value, using the concept of forestry biorefinery. The potential of the phenolic compounds to be used as a source of antioxidants and/or to manufacture adhesives should be evaluated. In order to achieve this goal, the possibility of separating these compounds from the polymeric phase, by precipitation or ultrafiltration, should also be evaluated. Moreover, the purification of hemicelluloses could be evaluated.

Table 6. Influence of volume ratio on the recovery of phenolic compounds by system 3. (Results are expressed as grams of the corresponding compound in the top phase/ 100 g pin chips d.b.)

V_r	TPC	ln K	%*EE*
0.4	0.81^a±0.02	8.20	61.0
0.9	1.32^b±0.04	8.83	99.8
4.1	1.33^b±0.05	∞	~100
V_r	SA	ln K	%*EE*
0.4	0.06^a±0.03	1.26	60.4
0.9	0.08^b±0.03	1.65	82.2
4.1	0.08^b±0.06	1.73	86.5
V_r	VAN	ln K	%*EE*
0.4	0.06^a±0.03	1.44	64.6
0.9	0.09^b±0.01	2.79	94.9
4.1	0.10^b±0.01	∞	~100

Values are presented as mean ± SD (n=3). In each column, values with different superscripts are significantly different (p<0.05).

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