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Feasibility Studies on the Effect of Natural Plant Compounds on Sludge Characteristics in a Batch-Type Aerobic Reactor and N-butyryl-L Homoserine Lactone

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ABSTRACT

Quorum quenching using different kinds of inhibitors is a new subject of research that is used for biofilm prevention in wastewater treatment membrane systems. In this study, three natural compounds, namely tea, coffee and vanillin, were tested in order to evaluate their possible effects on wastewater sludge characteristics in a batch type aerobic reactor. The parameters evaluated included the soluble microbial products (total soluble microbial products, protein and carbohydrate fraction of soluble microbial products), extracellular polymeric substance concentrations (total extrapolymeric substances, protein and carbohydrate fraction of the extrapolymeric substances), floc size, zeta potential, sludge volume index and capillary suction time. The effects of these plant based compounds upon the quorum sensing N-butyryl-L-homoserine lactone signal were also investigated using gas chromatography – mass spectrometry (GC-MS) under optimized conditions. The results indicated different effects for each natural compound upon the sludge characteristics. The role of these natural compounds as quorum quenchers in the batch-type aerobic reactor was inconclusive due to their effects on extraction outputs. Understanding the effect of quorum quenchers on sludge characteristics is important and recent studies have been aimed at preventing biofilm formation in membrane bioreactors.

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Introduction

Quorum sensing is defined to be the social communication amongst bacteria (Turan et al. 2017). Bacteria at low-level densities act as individual cells; however, when the microbial community density reaches a threshold level, bacteria begin to communicate using small signal molecules called auto-inducers (Kalia 2013). Quorum sensing has become a research topic of interest in health care, agriculture and environmental science because of its ability to regulate several phenotypes such as virulence factor, biofilm formation, antibiotic production, plasmid conjugal transfer and pigmentation (Galloway et al. 2012; Lade, Paul, and Kweon 2014).

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The inhibition of quorum sensing, known as quorum quenching, was introduced with the aim of inhibiting biofilm formation, which is considered to be a serious problem, primarily in wastewater treatment plants. The use of acyl-homoserine-lactone acylase (AHL-acylase) and procine kidney acylase are two examples of studies related to biofilm inhibition (Yeon, Lee, and Kim 2009). However, each of these studies showed some disadvantages and further studies are needed to improve and overcome these drawbacks. For instance, the instability and short lifetime of the acylase I enzyme used in the previous studies were overcome by immobilization on a magnetic enzyme carrier in other studies (Yeon, Lee, and Kim 2009). However, it is important to note that these studies were carried out in a lab-scale membrane bioreactor and little is known about their applications in a real reactor system with the possible high cost that may be associated with their use (Kim et al. 2011).

There are numerous quorum sensing inhibitors reported that remain under investigation. These inhibitors are generally divided into natural and synthetic compounds as reported by Kalia (2013). The literature lacks a study that presents the efficacy of natural based compounds on activated sludge properties. The importance of choosing non-toxic natural compounds is related to the sensitivity of microorganisms in activated sludge. For example, heavy metals have been shown to be toxic to the protozoan community in activated sludge and it is therefore impossible to employ protozoa in activated sludge systems (Madoni et al. 1996).

This study is only a trial in this context, aiming to determine the efficacy of tea, coffee and vanillin as plant based compounds on activated sludge characteristics and on the quorum sensing signal as N-butyryl-L-homoserine lactone in a batch type aerobic reactor. The reasons behind the selection of tea, coffee and vanillin are their toxicological safety, low cost, and large availability, keeping in mind that tea and coffee contain high concentrations of caffeine and are known to be quorum sensing inhibitors (Norizan, Yin, and Chan 2013). Also, it is important to remember that tea and coffee contain other chemical compounds that may affect quorum sensing signals in different ways.

Many studies has shown the quorum sensing inhibitory effect of the flavoring compound vanillin (4-hydroxy-3-methoxybenzaldehyde), especially upon *Bacillus subtilis, Salmonella enteriditis,* and *Escherichia coli* type of bacteria. Furthermore, vanillin's quorum sensing inhibitory acitivty on biofilm formation was characterized in reverse osmosis membrane that employed *A. hydrophila* as a model organism. The results showed that vanillin is a promoting agent for biofilm prevention that inhibits the activity of different acyl chains, especially acyl-homoserine-lactone acylase short-chains such as N-butyryl-L-homoserine lactone and 3-oxo-octanoyl-homoserine lactone, and long-chain acyl-homoserine-lactone acylase molecules. However, vanillin has not been characterized for its effect on activated sludge characteristics (Choo, Rukayadi, and Hwang 2006; Ponnusamy, Paul, and Kweon 2009; Ponnusamy et al. 2013).

In this study, tea, coffee and vanillin were employed in different batch type aerobic reactors in order to evaluate their quorum quenching capabilities on the bacteria communication signals by N-butyryl-L-homoserine lactone. Different concentrations of these natural compounds were investigated for this reason and their effects upon activated sludge characteristics such as the soluble microbial products, exopolymeric

Table 1. Classification of the reactors used in this study.

Reactor	Natural component	Concentration (g/L)
Control	-	-
*B1	Turkish tea	5.0
B2		10
B3		20
B4	Turkish coffee	5.0
B5		10
B6		20
B7	Vanillin	1.0
B8		1.5
B9		1.8

*B is a batch reactor.

substances, floc size, zeta potential and other parameters of membrane bioreactors were investigated.

Materials and methods

System used and operating conditions

Ten batch type bioreactors having effective volumes of 300 mL each were operated under the same conditions in this study. One reactor was used as a control, and nine reactors were used for each concentration of the investigated natural compounds, as shown in Table 1. The hydraulic retention time was fixed at 24 h, and the organic load was 0.307 kg/m^3 per day. No sludge discharge was carried out during the study.

The duration of the experiments was 4 days. All of the reactors were fed with synthetic domestic wastewater. The composition of the synthetic wastewater was as follows: glucose, 400 mg/L; yeast extract, 14 mg/L; bactopeptone, 115 mg/L; $(NH4)_2SO_4$, 104.8 mg/L; KH_2PO_4, 21.75 mg/L; $MgSO_4$, 15.63 mg/L; $FeCl_3$, 0.075 mg/L; $CaCl_2$, 2.45 mg/L; $MnSO_4$, 1.8 mg/L and $NaHCO_3$, 255.5 mg/L.

All chemicals were obtained from Sigma-Aldrich Chemie GmbH (Germany). Turkish tea (5.0, 10 and 20 g/L), Turkish coffee (5.0, 10 and 20 g/L), and vanillin (1.0, 1.5, 1.8 g/L) spiked to synthetic wastewater were fed to the reactors on a daily basis. Vanillin concentrations were selected to be higher than the minimal inhibitory concentration presented in a previous study (Rupasinghe et al. 2006). However, the tea and coffee concentrations were selected based on the minimal inhibitory concentration of caffeine.

Tea and coffee were prepared by adding an adequate mass to boiling water with brewing for 30 minutes. The concentrations of caffeine were determined to be 0.923 g/L and 0.475 g/L in coffee and tea, respectively. The aliquots of tea and coffee were filtered and added to synthetic wastewater to obtain the desired final concentrations of 5.0, 10 and 20 g/L.

Vanillin was added directly in its powdered form to synthetic wastewater. The inoculum was obtained from a membrane bioreactor system operating with the same synthetic wastewater, having a hydraulic retention time of 6 h and an organic load of 1.667 kg/m^3 per day. Air was supplied to the reactors by a compressor at an average flow rate of 7.5 mL/min.

Sampling and analytical methods

Dissolved oxygen (DO) levels, pH and temperature were measured daily using a WTW Multiline p4 multimeter using a Cellox 325 DO probe and a Sentix 41 pH probe. The sludge volume index and mixed liquor suspended solids were characterized on a daily basis using the 2540D (2005) and 2710D (2004) standard methods. Approximately 100 mL of samples from each reactor were removed daily from the reactor supernatant to measure chemical oxygen demand (COD) and ammonia nitrogen (NH_3-N) using the standard methods (Miner 2006).

The remaining analyses, namely soluble microbial products, extracellular polymeric substances, capillary suction time, protein, carbohydrate, zeta potential, floc size distribution and N-butyryl homoserine lactone determination, were conducted on the last operating day of the reactors. The extraction method introduced by Li et al. was used for the determination of extracellular polymeric substances and soluble microbial products (Li, Bai, and Liu 2008). The carbohydrate fraction was obtained using the phenol-sulphuric acid procedure (Dubois et al. 1956).

The protein fractions were quantified using the Lowry method (Lowry et al. 1951). The capillary suction time was determined according to a standard protocol (2005) using a Triton capillary suction timer with an 18-mm reservoir.

A Mastersizer (Malvern hydro 2000MU) was used for the measurement of the floc size distribution of particles across the range from 0.02 to 2000 µm. The zeta potential was measured using a Zetasizer (Malvern Nano ZS). Three sample replicates were analyzed for each of these measurements.

N-butyryl-Lhomoserine lactone determination

Two series of samples were analyzed in order to determine N-butyryl-L-homoserine lactone in synthetic wastewater samples. The first series consisted of samples from each reactor, which were passed successively through 11 μm filter paper and 0.45 μm syringe filters. The second series consisted of aqueous standards of N-butyryl-L-homoserine lactone treated with tea, coffee and vanillin. The natural compounds were added to the aqueous standards to match their exact concentrations used in reactors B1 through B9. Samples were extracted using the method developed by Bakaraki et al. (2016) and measured by gas chromatography – mass spectrometry (GC-MS) in triplicate for each sample.

Results and discussion

The influence of different concentrations of tea, coffee and vanillin upon the sludge characteristics is presented in the following sections. The average results obtained from each of the measurements is presented in the appropriate section. These effects were evaluated by comparison to a control sample that was not treated with any of the natural compounds but maintained under the same reactor conditions.

Effects upon the extracellular polymeric substance and soluble microbial product concentrations

Extracellular polymeric substances are defined as biopolymers that play an important role in microbial aggregation. Extracellular polymeric substances are composed of carbohydrates, proteins, lipids and nucleic acids. However, only carbohydrates and proteins form the major components, which are usually expressed as the total extracellular polymeric substances (Liu and Fang 2003).

Extracellular polymeric substances may be divided into bound extracellular polymeric substances, which are found inside microbial aggregates, and soluble extracellular polymeric substances or soluble microbial products present in the sludge suspension (Geesey 1982, Lin et al. 2014). The variation of soluble microbial products and extracellular polymeric substances concentrations for sludge samples treated with tea, coffee and vanillin are presented in Table 2.

Total soluble microbial products

The total soluble microbial products concentrations in activated sludge were determined to be 254 mg/L, 640 mg/L and 1737 mg/L for reactors treated with 5.0, 10 and 20 g/L of tea, respectively. However, the sample obtained from the control reactor exhibited a concentration of 51.5 mg/L. It was also observed that by increasing tea concentration in the feed, the protein fraction of soluble microbial products increased from 204 mg/L to 1448 mg/L and the carbohydrate fraction of soluble microbial products increased from 49.7 to 289 mg/L, which are significantly higher than both fractions in the control reactor.

The same trend was not observed for vanillin, for which the highest concentrations of total soluble microbial products, protein and carbohydrate fraction of soluble microbial products were present in the feed with a concentration of 1.5 g/L, in comparison to 1.0 g/L and 1.8 g/L. Similar to tea, 5.0, 10 and 20 g/L concentrations of coffee treated samples resulted in total soluble microbial product values of 536, 1300 and 3616 mg/L, respectively. The protein and carbohydrate fractions of soluble microbial products concentrations were 2516 and 1100 mg/L, respectively, for the 20 g/L coffee treated sample.

In increasing order, the highest protein and carbohydrate concentrations in soluble microbial products were the tea, coffee and vanillin treated samples. The reason for this trend may be related to the differences in the substances added to the reactors and the bacterial reaction following their addition. Each substance probably affected the bacterial activity and soluble microbial products production in different ways. As the substances added were investigated for their effect on quorum sensing, the differences may be related to the bacterial quorum sensing activity.

Another reason may be associated with the nature of these substances. Vanillin was added in its pure powdered form whereas tea and coffee were added in their liquid form, probably affecting the sludge particles and the subsequent soluble microbial products analysis.

Bound extracellular polymeric substances

The highest total extracellular polymeric substance value was observed for the control untreated reactor, which had a 100.23 mg/g mixed liquid suspended solid value,

Table 2. Influence of the concentrations of tea, coffee and vanillin upon the extrapolymeric substance (EPS) and soluble microbial product (SMP) concentrations in the batch reactors.

		Extrapolymeric substances (mg/g mixed liquid suspended solids)			Soluble microbial products (mg/g)		
	Reactor	Protein fraction in extrapolymeric substances	Carbohydrate fraction in extrapolymeric substances	Total extrapolymeric substances	Protein fraction in soluble microbial products	Carbohydrate fraction in soluble microbial products	Total soluble microbial products
	Control	40.66 ± 2.1	59.57 ± 1.9	100.23 ± 3.9	31.5 ± 1.5	20 ± 0.8	51.5 ± 1.5
Tea	B1	33.19 ± 1.9	40.62 ± 1.5	73.82 ± 2.8	204.8 ± 4.0	49.8 ± 1.3	254.5 ± 3.9
	B2	42.99 ± 2.5	41.02 ± 1.3	84.02 ± 3.2	512.5 ± 5.7	128 ± 2.6	640.5 ± 7.1
	B3	53.11 ± 2.3	37.50 ± 0.9	90.61 ± 3.0	1448 ± 6.8	289.5 ± 3.2	1737.5 ± 10
Coffee	B4	37.34 ± 2.0	33.90 ± 0.85	71.25 ± 1.8	414.5 ± 1.55	121.8 ± 6.1	536.3 ± 5.6
	B5	42.33 ± 1.6	33.96 ± 2.1	76.07 ± 1.6	952 ± 12	348 ± 5.0	1300 ± 9.1
	B6	47.76 ± 1.3	38.05 ± 2.4	85.81 ± 3.7	2516 ± 25	1100 ± 8	3616 ± 14
Vanillin	B7	257.12 ± 5.4	35.31 ± 1.6	292.42 ± 6.8	4035 ± 38	217.3 ± 0.9	4252.3 ± 22
	B8	367.67 ± 7.1	74.41 ± 3.1	442.09 ± 5.6	7815 ± 45	615 ± 5.5	8430 ± 25
	B9	429.90 ± 6.5	83.41 ± 3.2	513.32 ± 5.85	6365 ± 27	501.5 ± 5.7	6866.5 ± 16

compared to the 73.82, 84.02 and 90.61 mg/g values for the 5.0, 10 and 20 g/L of tea feed concentrations, respectively. A similar trend in extracellular polymeric substances concentration was observed for increasing coffee concentrations, with the mixed liquid suspended solid values being lower than the control value. Vanillin, on the other hand, had all three concentrations recording total extracellular polymeric substances values that were higher than the value of the control sample. The vanillin added reactors presented the highest total extracellular polymeric substance and total soluble microbial product values compared to the reactors to which tea and coffee were added.

Since the total extracellular polymeric substances in tea and coffee added reactors showed similar results, but the total soluble microbial products for coffee added reactors were higher than total soluble microbial products for the tea added reactors, it may be assumed that tea at a concentration of 5.0 g/L gives lower total extracellular polymeric substance and total soluble microbial product values. It has been reported in the literature that protein concentrations are generally higher than carbohydrates, and the related values vary between 10 and 120 mg/g mixed liquid suspended solids for the protein fraction of the extracellular polymeric substances, and between 6.0 and 40 mg/g mixed liquid suspended solids for the carbohydrate fraction of the extracellular polymeric substances (Le-Clech, Chen, and Fane 2006).

These results indicate that the control reactor fell within the expected range for both the extracellular polymeric substances and the soluble microbial products. On the other hand, the addition of tea, coffee and vanillin caused significant increases in the extracellular polymeric substance and soluble microbial product levels. As reported by Yao, Zhang, and Cui (2010), the protein/carbohydrate ratios in the extracellular polymeric substances may be a direct indication of membrane fouling. Since soluble microbial products are the soluble portion of the extracellular polymeric substances, they do not directly affect fouling, but the soluble microbial products may be transformed into bound extracellular polymeric substances when adhering to the sludge flocs (Lin et al. 2014).

The protein concentration levels of the vanillin treated sludge were significantly higher than for the control, tea and coffee treated samples. These values are probably related to the report in the literature concerning flavoring compounds such vanillin with an aldehydic function. These compounds, under liquid or high-moisture food environments, may bind covalently to the amino groups of proteins (Mikheeva et al. 1998; Dikusar 2006). They may also undergo several types of reactions because of their three reactive functional groups that include the aldehydic group, phenolic hydroxyl functionality, and the aromatic ring (Weerawatanakorn et al. 2015). Therefore, vanillin may retain proteins as they are not as readily digested, resulting in high protein levels.

Influence upon the floc size

Microbial floc size was investigated for the different concentrations of tea, coffee and vanillin. Table 3 shows the average floc sizes for the different activated sludge samples obtained from the related reactors. The mean floc size in the control reactor was determined to be 134.08 µm. The mean floc sizes recorded for the three concentrations of tea and coffee (112 µm and 126 µm) were slightly lower than the control sample.

TABLE 3. Influence of the tea, coffee and vanillin concentrations on the floc size distribution.

	Reactors	Floc size (μm)
	Control	134.08
Tea	B1	126.66
	B2	122.18
	B3	121.35
Coffee	B4	123.62
	B5	115.53
	B6	112.63
Vanillin	B7	126.09
	B8	126.22
	B9	135.72

Table 4. Influence of the tea, coffee and vanillin concentrations upon the zeta potential and electrophoretic mobility.

	Reactor	Zeta potential	Electrophoretic mobility
	Control	−29.5 ± 3.88	−2.31 ± 0.15
Tea	B1	−29.1 ± 2.65	−2.27 ± 0.24
	B2	−38 ± 5.15	−2.98 ± 0.24
	B3	−39.7 ± 4.91	−3.11 ± 0.55
Coffee	B4	−28.1 ± 4.22	−2.51 ± 0.10
	B5	−28.8 ± 4.80	−2.25 ± 0.15
	B6	−28.8 ± 1.70	−2.25 ± 0.09
Vanillin	B7	−29.3 ± 2.85	−2.22 ± 0.14
	B8	−29.5 ± 3.69	−2.31 ± 0.12
	B9	−27.2 ± 4.05	−2.13 ± 0.08

Vanillin showed similar results for the lower concentrations of 1.0 and 1.5 g/L, but the 1.8 g/L vanillin treated reactor produced a mean floc size close to that of the control reactor. This change in floc size may be related to the change in the extracellular microbial substance values discussed in a previous section, in which the total extracellular microbial substances of tea and coffee were at a lower value than the total extracellular microbial substances obtained for the control reactor.

A proportional relationship between the extracellular microbial substances and floc size has also been reported in the literature (Arabi and Nakhla 2008; Erkan et al. 2017). The average floc size did not vary significantly for tea, coffee and vanillin added reactors. For this reason, it may be possible to conclude that tea, coffee and vanillin additions did not significantly affect the floc size.

Influence upon the zeta potential

The results of zeta potential and electrophoretic mobility analysis for tea, coffee and vanillin are presented in Table 4. The zeta potential values were enhanced with an increase in the tea concentration, and decreased with an increase in coffee and vanillin concentrations. The zeta potential is affected by extracellular microbial substances and more specifically by the protein/carbohydrate ratio, since positively charged proteins have the capacity to neutralize negatively charged ions (Liao et al. 2001). The zeta potential values were expected to increase with a decrease in the protein/carbohydrate ratio (Meng et al. 2006). Therefore, vanillin, which had a gradually increasing protein/carbohydrate ratio, induced a decrease in the zeta potential values.

Table 5. Influence of the tea, coffee and vanillin concentrations upon the sludge volume index and capillary suction time.

		Sludge volume index (mL/g)	Capillary suction time
	Control	78.879 ± 0.9	6 ± 0.83
Tea	B1	58.52 ± 1.2	7.9 ± 0.9
	B2	64.34 ± 1.5	9.1 ± 1.1
	B3	66.72 ± 0.7	10.1 ± 1.36
Coffee	B4	69.28 ± 2.1	10.2 ± 1.65
	B5	67.42 ± 0.85	9.9 ± 2.1
	B6	79.90 ± 1.0	13.5 ± 1.24
Vanillin	B7	49.16 ± 0.23	6.3 ± 0.9
	B8	50.70 ± 1.65	5.7 ± 0.12
	B9	23.28 ± 0.5	6.8 ± 0.98

For coffee, a stable zeta potential value close to that for the control reactor was obtained. This phenomenon may be due to the protein values of coffee treated reactors not displaying a significant change compared to the control reactor. In addition, the total extracellular polymeric substances for coffee treated reactors were lower than the total extracellular polymeric substances for the control reactor.

Although the extracellular polymeric substance and total extracellular polymeric substance profiles for tea treated samples were similar to the control sample, their zeta potential results varied greatly, especially for the high concentrations up to −39.7 mV. The reason behind this phenomenon may be related to the extended fermentation of tea leaves which may produce more polymeric compounds, which in turn increases the negative charged ions (Wilén, Jin, and Lant 2003).

Influence upon the sludge volume index and capillary suction time

The variance in sludge volume indices is shown in Table 5. The control reactor provided a sludge volume index equal to 75.89 mL/g while the sludge volume index response varied for the tea, coffee and vanillin treated reactors. The sludge volume indices of the tea and coffee treated samples were less than 67 and 80 mL/g, respectively, with the sludge volume index of coffee samples being more comparable to that for the control reactor.

For the vanillin treated samples, the sludge volume index values decreased significantly to as low as 23.28 mL/g for the 1.8 g/L vanillin concentration. The sludge sedimentation characteristics are evaluated by the sludge volume index, in which values ranging between 40 and 86 mL/g indicate good settling characteristics. Any value lower than 40 mL/g represents a pin-floc sludge, a sludge that is generally old and settles quickly. In addition, a filamentous sludge usually has a sludge volume index value higher than 100 mL/g (Löwén and Piirtola 1998). The results obtained in this study showed that the addition of 1.8 g/L vanillin produced a pin-floc sludge, whereas the additions of tea and coffee did not change the settling characteristics of the sludge.

The measured results for capillary suction time are also shown in Table 5. The capillary suction time value for the control sludge was approximately 6.0 seconds. This value increased to 10.1 and 13.5 seconds for the highest concentrations of the tea and coffee treated reactors, respectively.

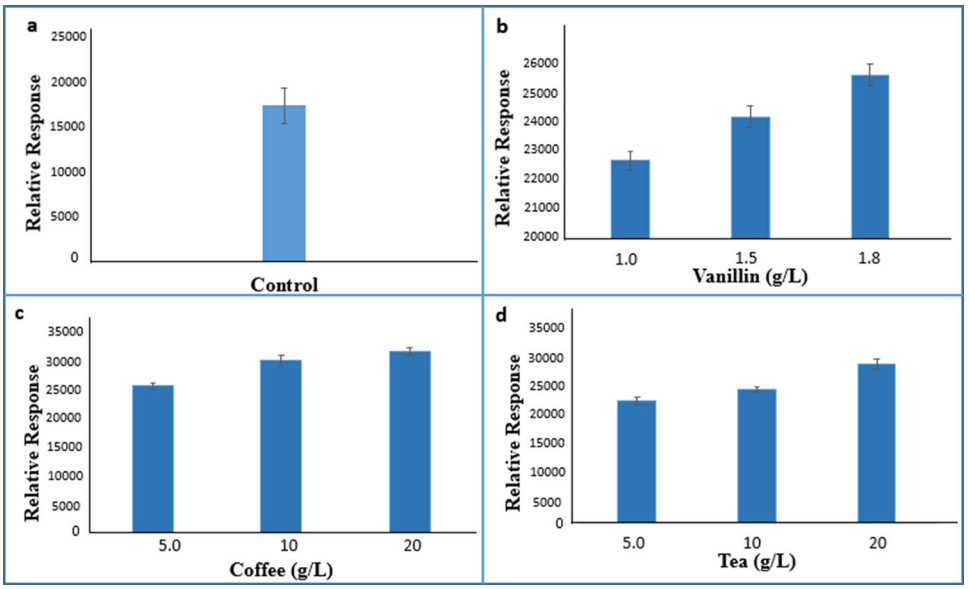


Figure 1. Influence of the natural compound concentrations upon the N-butyryl-L-homoserine lactone signal where V is vanillin, C is coffee, and T is tea. A 250 ng/mL spike concentration was added to all samples with extraction performed using the optimum conditions.

The three vanillin concentrations showed similar capillary suction times as the control sludge. The capillary suction time was originally developed to examine the sludge filterability, especially after chemical pretreatments (Gray 2015). The capillary suction time is the time required for water to be released from a sludge matrix traveling a set distance expressed in seconds.

High capillary suction times are indicative of low filterability properties. Therefore, the addition of tea and coffee were shown to negatively affect the sludge filterability. Vanillin did not have any influence upon the sludge filterability, as similar results were obtained for the sludge samples obtained from the control reactor. Increased levels for the carbohydrate fractions of soluble microbial products for the tea and coffee treated reactors may be correlated with an increase in their capillary suction times, as has been reported by Reid et al. (Reid, Liu, and Judd 2006).

Influence of tea, coffee and vanillin upon the N-butyryl homoserine lactone signal

Samples from the 9 treated reactors and the control reactor were extracted according to the method developed by Bakaraki et al. (2016) and were analyzed by gas chromatography-mass spectrometry (GC-MS). The method was primarily developed to determine N-butyryl homoserine lactone in the complex matrix of wastewater, giving relatively low detection and quantification limits of 12.9 ng/mL and 43.3 ng/mL, respectively. None of the extracted samples from the reactors produced a measurable signal and as such, the effects were studied by spiking with an N-butyryl-L-homoserine lactone standard solution.

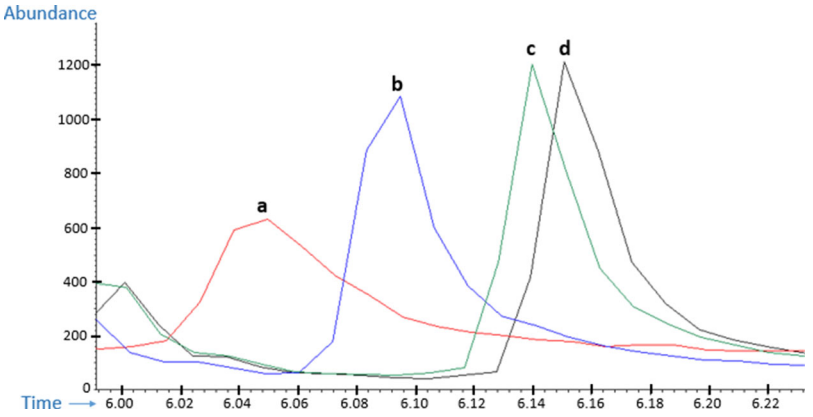


Figure 2. Extract ion chromatograms of (a) N-butyryl-L-homoserine lactone standard, (b) 1.0 g/L vanillin, (c) 1.5 g/L vanillin, and (d) 1.8 g/L vanillin. A variation in the retention time is demonstrated with an increase in the vanillin concentration.

The integrated peak areas obtained from the spiking experiments are presented graphically in Figure 1. All nine of the treated wastewater samples recorded peak areas higher than the control sample, with coffee having the highest results. This observation, however, contradicts the inhibition theory, as lower signals would be expected for tea, coffee and vanillin additions. In order to better understand the effects of these compounds on the extraction output, deionized water containing equivalent amounts of natural compounds were spiked to the same concentration of N-butyryl homoserine lactone standard (250 ng/mL) and isolated for analysis.

An aqueous standard of the same concentration was prepared and extracted as a control sample. The same increasing trend observed for the wastewater samples were observed for the deionized water samples. This result suggests that the natural compounds act as extraction efficiency enhancers and that the high signal values do not directly imply that no inhibition occurred. The enhancement factors increased with the compound concentration but differed from compound to compound. The enhancement factors of coffee and tea in deionized water were slightly higher than in wastewater.

This marginal difference may be attributed to the denser and complex nature of the wastewater matrix used during the study affecting directly the precision, sensitivity and accuracy of the analytical method (Namieśnik and Szefer 2008). Vanillin also recorded an enhancement in the extraction output with increasing concentration. In addition, the extract ion chromatograms of vanillin showed an increase in retention time (6.04 min) with enhanced concentrations (Figure 2).

The enhancement factor of vanillin in deionized water was observed to be lower than in wastewater. An incorrect assumption may have been easily made that lower concentrations of these natural compounds act as better inhibitors. However, the determinations made with deionized water have provided additional information about the extraction enhancement process. Further studies must therefore be performed to determine the inhibition of these compounds while considering these enhancements that must be duly factored in order to obtain representative results. By the use of this protocol, the inhibition role of these natural compounds may be properly addressed.

Conclusion

Three concentrations of tea, coffee and vanillin were added to batch type aerobic reactors in order to evaluate their influence upon the sludge characteristics and upon the N-butyryl homoserine lactone signaling molecule. The results showed that tea and coffee have a positive influence upon the extracellular polymeric substances levels but affect the sludge characteristics in different ways. Vanillin provided good settling and filterability characteristics but caused increases in the extracellular polymeric substance and soluble microbial product levels for the three measured concentrations. The protein and carbohydrate concentrations in soluble microbial products were shown to be highest in the tea treated samples, followed by coffee and vanillin. In addition, the tea, coffee and vanillin additions did not significantly affect the floc size, while vanillin negatively affects the sludge leading to a pin-floc sludge. The inhibition of the plant based compounds upon the N-butyryl homoserine lactone was not be established in this study due to the observation of their direct influence upon the extraction output.

Disclosure statement

The authors declare that they have no conflicts of interest.

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