## Microbial degradation

**Business** 



Page 2

5. 6. 3.

1. Evaluation of CIPC and 3CA Extraction by Rotary Evaporation The methodology for analyte extraction was examined to choose the method that provides consistent 100% extraction. The study included two rotary evaporators. The solutions used for the research were two standard solutions with high concentrations of CIPC and 3CA (standard A: 50 mg/l CIPC; standard B: 500 mg/l 3CA) and standard solutions with low concentrations of analytes (standard C: 10 mg/l CIPC and 3CA in distilled water; standard B: 10 mg/l CIPC and 3CA in distilled water; standard B: 10 mg/l CIPC and 3CA in dichloromethane). Figure 1 describes the results. The rotary evaporator 1 provides consistent results of CIPC and 3CA recovery, and for DCM solution the recovery is close to 100%.

On the contrary, the methodology of 3CA extraction at the rotary evaporator 2 has a systematic fault, which results in inconsistent and low analyte extraction. The extraction of CIPC at the rotary evaporator is efficient and shows 100% recovery for all the studied cases. The rotary evaporator 1 should be used for the further research to ensure the consistent recovery results. 5. 6. 3.

2. Degradation of CIPC and 3CA in Aged Potato Suspensions The suspensions were obtained from potato samples that were stored in a cool place for four months. Apparently, the quantity of bacteria reduced as a result of long-term stay in the unfavorable medium. However, the population of soil bacteria does not die off. As the research of Donsel, Geldreich, and Clarke (1967) proves, the bacteria hibernate at cold temperature. When the environmental conditions become favorable, the population restores. Figure 2 shows the 3ca formation and CIPC & 3CA degradation in aged potato suspensions. The CIPC and 3CA degradation routes show concentration decrease due to adsorption (stage1, day 1), a lag period without significant concentration decrease (stage 2, days 2-15), and a sharp decrease due to microbial degradation (stage 3). The concentrations of CIPC and 3CA are close to zero at the end of the incubation period. As for 3ca formation, it increases after day 1 due to the slow CIPC degradation, remains stable at the stage 2 and then decreases to zero. This confirms the assumption of 3CA microbial degradation in suspension.

Thus, the disproportional quantity of 3ca identified in the previous section is attributed to its microbial degradation in parallel with CIPC. The degradation of industrial potato suspension shows the maximum degradation rate in the aged industrial suspension, 20% for CIPC and 10% for 3CA (Figure 3). CIPC degradation by-product (3ca) is not observed since its generation rate is lower than the biodegradation of 3-chloroaniline. 5. 6.

3. 3 Evaluation of LLE Extraction in Separatory Funnels The extraction of miscible and immiscible solvents from distilled water is meant to validate the separatory funnel method. If high extraction levels and equal results for equal samples are observed, there is no systematic error on the experimentalist's part, e. g. the experimentalist handles the sampling stable conditions (Miller, Miller & Miller 2000).

Extraction by Miscible Solvents The results of the extraction of miscible solvents methanol and acetonitrile (Fig. 4) show 99. 8-100% recovery.

Furthermore, the extraction rates for both solvents are equal due to close values of the dipole moments, 2. 9D and 3.

4D, for methanol and acetonitrile respectively (Burdick & Jackson Laboratories 1997). No significant difference in extraction efficiency is observed for different analytes: solvent ratio. This allows choosing the ratio based on the sample handling convenience. Figures 5 and 6 show extractions from the samples taken after 22 days of incubation in the aged syntetic and industrial potato suspensions. In terms of CIPC and 3CA extraction from the solutions, the samples are equal.

Extraction of different solvents should give similar results. However, extraction from the aged synthetic suspensions (Fig. 5) shows a random variation between the rates received for acetonitrile and methanol recovery. This is a sign of systematic error, which becomes apparent since the concentrations of the analytes are low. The analysis of extraction methodology for the aged industrial potato suspension shows that CIPC acetonitrile extraction results in 1. 4g/l of analyte while it is 2.

5g/l for methanol extraction. Similarly, 3CA methanol extraction shows 2g/l, acetonitrile extraction – about 3 g/l. This is an indication of systematic error either in the methanol or acetonitrile extraction series. The miscible solvent extraction does not provide 100% recovery of analytes and gives inconsistent recovery rates for suspension samples. Thus, the further improvement of extraction methodology is necessary. Extraction by Immiscible Solvents Various immiscible solvents show low efficiency for CIPC and 3CA extraction. The extraction from distilled water is expected to be 10 mg/l, though this value is not observed (Fig. 7). The extraction results from the aged industrial and synthetic potato suspension are different for all solvents. This means that the immiscible solvents are unsuitable for CIPC and 3CA extraction since they are non-polar with low values of dipole moments. Some solvents show up to 50% variance, which is a sign of systematic error.

Thus, the immiscible solvents can not be used for CIPC and 3CA extraction. 5. 7. 2. 1 Extraction with Ethyl Acetate at Different pH and in the Presence of Sodium ChlorideTable 1 presents the data on CIPC and 3CA extraction of ethyl acetate.

Apparently, shaking time has a minor effect on extraction efficiency. Sodium chloride marginally decreases CIPC extraction. The amount of extracted CIPC is equal for acid and alkaline solutions. Generally, CIPC extraction of ethyl acetate remains stable under all the studied conditions. This is explained by CIPC low water solubility of 0. 09 g/l (Occupational Health Services 1992).

On the contrary, 3CA is a weak base with water solubility of 6. 8 g/l (Seidell 2010). The 3CA molecule gets protonated in acid solution, and its water solubility increases. The same is observed for an aniline molecule in acid solution, which forms phenyl ammonium chloride with high water solubility (Patrick 2004). This causes a decrease in ethyl acetate extraction property, which is 21% in acid media. In alkaline solution, electric polarity of the 3CA molecule reduces.

As a result, its water solubility reduces, and ethyl acetate extraction increases. Solubility of 3CA in sodium chloride solutions is lower than in https://assignbuster.com/microbial-degradation/ distilled water due to sodium and chloride ions' hydration. Consequently, solution ionic strength increases the extraction property of ethyl acetate. Although the methodology presented in this section provides the means for 3CA extraction improvement, the maximum recovery is 70%. Ionic strength, pH and shaking time do not have significant influence on the extraction of CIPC.

Thus, further search for extraction methodology is essential. 5. 7. 2. 2 Extraction with Dichloromethane The selection of dichloromethane is determined by its lower water solubility (13 g/l in contrast to 83 g/l for ethyl acetate) and the lower value of dipole moment (1.

6 D to 1. 8 D) (Burdick & Jackson Laboratories 1997). These properties allow predicting higher extraction efficiency for CIPC and 3CA extraction. Table 2 lists the results of the dichloromethane experimental extraction design. For CIPC, there is the increase of DCM: standard ratio generally leads to extraction increase, from 70% to 83 % (2 times shake methhod). Though, if 1 ml of the standard is used, the trend is infringed.

Possibly, this depends on sampling deviation. The number of extraction cycles increases extraction, which is predictable because of its stimulation. The extraction of 3CA with DCM decreases with the increase of DCM: standard ratio since 3CA is more polar (2. 7 D), and, thus, its solubility in DCM is lower than for ethyl acetate. Maximum extraction is observed at the level of 90%, and none of the methods provided full extraction. 5.

8. 2. 1. The Optimized LLE-Vortex Recovery of CIPC and 3CA with DCM The sampling deviation of the previous method was expelled by the use of a https://assignbuster.com/microbial-degradation/

Pyrex test tube instead of a funnel. The data received in the previous sections were used to develop efficient recovery, namely pH, sodium chloride content and the number of extraction cycles. This allowed 99% and 100% extraction of CIPC in DCM: standard ratio as 5 after three and five extraction cycles from the sodium chloride solutions (Table 3 and 4).

However, the method was not effective for 3CA as it was explained in the previous section. The solubility of 3CA in water decreases with the increase of ionic strength. Thus, the extraction of 3CA can be stimulated by the increase of sodium chloride in solution. Table 5 shows data for optimized LLE-Vortex method. The developed method uses DCM: standard ratio as 5, five extraction cycles and the addition of sodium chloride (15 ml). Its undeniable advantage is the simultaneous extraction of CIPC and 3CA with 100% efficiency.

5. 8. 2. 3 Validation of the Optimized LLE-Vortex Method for Incubation StudiesThe optimized LLE-Vortex methodology was developed as an extraction method for CIPC and 3CA recovery from distilled water solution. In order to use this method for the incubation studies, validation is necessary.

Incubation solutions are the suspensions that include the solid particles. The results of methodology validation are presented in Table 6. Apparently, all the experiments provide high extraction rates and low RSD. Thus, LLE-Vortex method can be used for the incubation studies with soil suspensions. 5.

8. 2. 4 Validation of the Optimized LLE-Vortex method for Extraction from High Soil Suspensions The recovery of analytes from the suspensions containing 1 g and 5 g of soil depends on the quantity of colloidal particles https://assignbuster.com/microbial-degradation/ present in the aliquots. The soil samples with high clay content are expected to form colloidal solutions in water media. The solid particles of sandy soils are greater, and they precipitate.

Thus, quantitative extraction is expected to be observed for the sandy samples. Probable divergence from this pattern may appear due to sludge, which can cause the formation of colloidal solutions. Dreghorn A and Quivox B soils exhibit qualitative visual extraction (light turbid soils formed at Quivox B sample) and sufficient quantitative result. However, Dreghorn A clear solution provides lower extraction recovery. Barassie, Subsoil and Arable samples show high recovery rates for CIPC and up to 5% lower rates for 3CA.

In the adsorption study section, Peat 1 proved the highest adsorption capacity for CIPC and 3CA. In this section, the lowest extraction recovery is observed (90% for 1 g of soil and 60% for 5 g). Extraction from the samples with higher soil content is less efficient because the analyte is adsorbed on the soil surface. Generally, CIPC recovery rate is higher than that of 3CA from all samples due to its low solubility and weak adsorption bonds with the soil particles. The LLE-Vortex method is reliable for CIPC extraction from suspensions formed with 1 g of soil and for 5 g of soils with low adsorption capacity.