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EFFECT OF ACRYLAMIDE ON THE DEGRADATION OF PALM-BASED USED COOKING OIL BY SINGLE AND CO-CULTURE ISOLATES OF *Alcaligenes* sp. AQ5-02 AND *Serratia* sp. AQ5-03

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Abstract

Acrylamide is found in the environment, food, and waste products such as palm-based used cooking oil (UCO). The presence of acrylamide is a threat due to its neurotoxic, genotoxic, teratogenic, and carcinogenic characteristics. These negative impacts have sparked an interest in microbe-mediated bioremediation of acrylamide. The aim of this study is to investigate the effect of various concentrations of acrylamide on the degradation of UCO as a sole carbon source by single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03. Using gravimetric analysis, different acrylamide concentrations ranging from 10-200 mg/L were shown to significantly reduce palm oil UCO degradation and bacterial growth in single and co-culture formulations. In the co-culture, there were significant differences between all acrylamide concentrations in terms of UCO degradation. The single and co-culture isolates were able to withstand 10 mg/L of acrylamide while degrading more than 50% of UCO. However, beyond 10 mg/L, there was a significant reduction in the degradation of UCO in both single and co-culture methods. Two-way ANOVA reveals a significant difference in the degradation and bacterial growth between the single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03 with varying acrylamide concentrations ($p < 0.0001$). In conclusion, the co-culture has greater degradation and better tolerance at all concentrations of acrylamide compared to single isolate cultures of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03. The study provides insightful knowledge on the presence of xenobiotics in the bioremediation of hydrocarbons by single and co-culture.

INTRODUCTION

Acrylamide is an aliphatic amide, which is a monomer of polyacrylamide with a chemical formula of C_3H_50. It is a water-soluble, colourless, and odourless crystalline solid [1]. Acrylamide has diverse applications in industries but is classified as a probable human carcinogen [2] that could enter through absorption via the skin, lungs, and digestive tract. Thus, the release of acrylamide into the environment has negative impacts on the ecosystem and human health. Consequently, many researchers have focused on finding solutions to reduce acrylamide concentrations in food and the environment. Despite its poisonousness, selected microorganisms are capable of using acrylamide as a means of energy by generating ammonia and acrylic acid [3]. Microbes are well known for their complex behaviours under

different environmental conditions. The behaviour of microbes varies depending on several environmental factors such as the availability of nutrients, temperature, and the nature of the bacteria that are present in the environment.

Research on acrylamide biodegradation has attracted attention in the last two decades due to its frequent occurrence in food, especially in used cooking oil (UCO) [4, 5]. Acrylamide is formed by frying oil as its composition degrades or when the carbonyl groups interact with other components (such as dietary amino acids) at high temperatures [6, 7]. Since UCO is a significant source of environmental pollution, biodegradation has been identified as a possible means of its disposal. However, the presence of acrylamide in UCO can pose a challenge due to its inhibitory effect on the microbes that are responsible for UCO biodegradation in the environment. A previous study demonstrated that the viability of the bacteria and their degradation of UCO could be impacted by both the concentration of the acrylamide and the bacterial species [8]. Presently, there is little information available on the microbial tolerance of acrylamide in the bioremediation of UCO [9]. As previously mentioned, numerous microorganisms are poisonous to acrylamide. Nevertheless, some soil and water bacteria can degrade acrylamide [10], including those from the genera *Arthrobacter*, *Bacillus*, *Pseudomonas,* and *Rhodococu*s [11]. In addition, fungi, yeast, and plants that take nutrients from the surrounding can also be used to cure acrylamide pollution [11, 12, 13, 14].

In this study, we used single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03 which were previously reported to biodegrade phenol and copper [15,16,17]. The ability of both isolates to break down UCO as a sole source of carbon in the presence of various acrylamide concentrations was investigate. A consortium of microbes can execute sophisticated processes that would be impossible for a single organism to accomplish. To our knowledge, the single and co-cultures of *Alcaligenes* sp. and *Serratia* sp. for the biodegradation of UCO in the presence of acrylamide are yet to be studied. Additionally, no reports have been published demonstrating the biodegradation of UCO using these isolates in the presence of varying levels of acrylamide. Thus, this study aims to investigate the effect of the various concentrations of acrylamide on the degradation of UCO by single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03.

MATERIALS AND METHODS

Chemical

The only carbon source used for this investigation was palm-based UCO, which was gathered from a number of homes in the Kajang district of Selangor, Malaysia. The UCO gathered for this study has been deep-fried numerous times at temperatures between 160 and 200 °C. The oil underwent filtration as a preliminary step to remove any impurities. Sterilised syringe filters were used to filter the oil.

Bacterial Culture and Media Preparation

Previously isolated *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03 were cultured on sterile mineral salt medium (MSM) containing the following composition: 1.0 g/L $(NH_4)_2SO_4$, 0.9 g/L K_2HPO_4, 0.9 g/L KH_2PO_4, 0.2 g/L MgSO4.7H20, and 0.1 g/L yeast extract supplemented with 3% UCO as a sole carbon source [18].

In the co-culture study, equal volumes of equal densities from both isolates were used to prepare the co-culture. The two isolates were cultured separately and mixed in equal proportions as a co-culture consortium. Both bacterial cultures were then centrifuged at 4130 ×g for 10 min after incubation. The bacterial samples' resting cells and inoculum sizes were standardised to an optical density of 1.0 at a wavelength of 600 nm.

Culture Experiments of UCO with Single and Co-culture Isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03

The isolates were cultivated in nutritional broth and kept at 2.26 ×g and 30°C for 4 days while being incubated on a rotary shaker. The experiment was conducted in a conical flask with 100 ml of MSM containing 10% (v/v) of bacterial culture with an initial UCO concentration of 3% (v/v) and incubated for 7 days with agitation at 2.26 ×g and 30°C. The effect of different concentrations of acrylamide was investigated by adding 10–200 mg/L to the medium. At 30°C, pH 7.5, 0.9 g/L (NH4)2SO4, 0.4 g/L yeast extract, and 3% v/v substrate concentration, the media were incubated under optimal conditions. Three duplicates of each experiment were performed. The results acquired from the UCO assessment were analysed to affirm if varying concentrations of acrylamide caused a reduction in oil degradation. A culture with no added acrylamide was used as a control. The percentage of UCO degradation was determined by gravimetric analysis, while the colony forming unit per (CFU/mL) was used to measure bacterial growth by method Hazan *et al.* [19].

Determination of The Bacterial Growth

In 9 mL of normal saline, one millilitre of bacterial culture was serially diluted to concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} and 10^{-7}. 1.0 mL of bacterial suspension (10^{-2} to 10^{-7}) was then aseptically transferred into Petri plates. The bacterial suspensions were allowed to mix and spread, and plating was then performed using molten nutrient agar. The plates were incubated at 30°C for 96 h. After incubation, the plates having colonies ranging from 30 to 300 were counted. The number of CFU/mL was estimated by multiplying the number of colonies by the dilution factor

and dividing by the volume of sample plated on the agar, as shown in equation 1[20]:

$$\frac{CFU}{mL} = \frac{No\ f\ colonies\ counted}{Volume\ of\ sample\ plated} \times dilution\ factor$$

Equation (1)

All assays were performed in triplicates. Bacteria were introduced to assure growth, but the control tubes containing medium, and oil were not.

Determination of Used Cooking Oil Degradation by Gravimetric Method

The amount of residual UCO left behind is calculated by gravimetric analysis method at the end of the 7-day incubation period, n-hexane solvent was added to the single and co-culture mediums in a 1:1 ratio. The flask was then shaken vigorously to separate the mixture of oil and medium into two layers. The petri plates was pre-weighed: top layer contained the oil and the n-hexane was collected into this petri plate. After evaporation process, the remaining UCO was quantified gravimetrically by weighing its mass [21]. The UCO degradation percentage was calculated using the following equation [2]:

$$Degradation\ (\%) = \frac{Mass\ of\ UCO\ degraded}{Mass\ of\ UCO\ added\ in\ the\ Medium} \times 100$$

Equation (2)

Cultures without added oil and incubated in the identical conditions were used as a control.

UCO Degradation in the Varying Concentration of Acrylamide

The effect of acrylamide on UCO degradation was studied. The varying concentrations of acrylamide were used based on information from the literature. The cultures were incubated for 7 days at 30°C in optimal conditions using a rotary shaker at 2.26 ×g [22, 23]. The bacterial community growth and percentage degradation were determined by measuring the oil mass.

Statistical Analyses

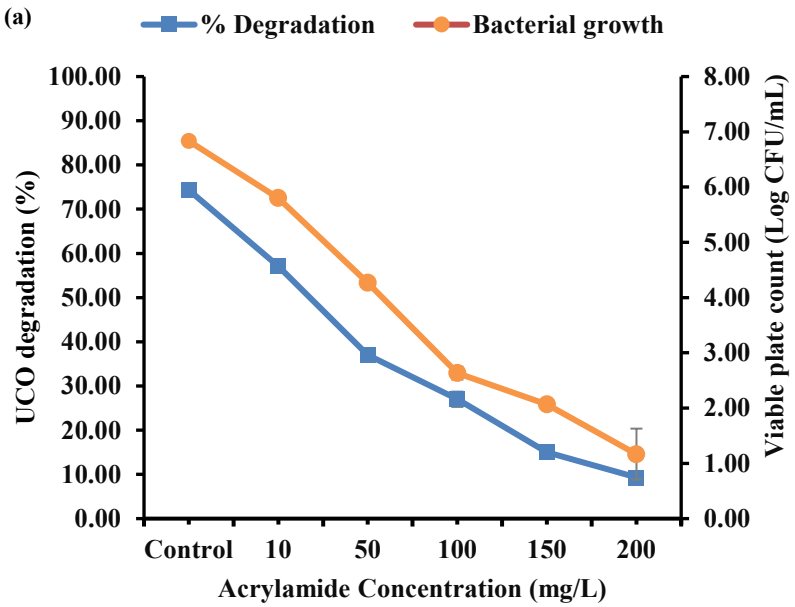
ANOVA was performed using the SPSS Statistics V.24 software package (SPSS Inc., Chicago, Illinois, USA) to differentiate results within treatment groups, and if significant differences were obtained, paired post hoc tests were performed using the Tukey test. Experiment data is reported as mean ± standard deviation and conducted in triplicate.

RESULTS AND DISCUSSION

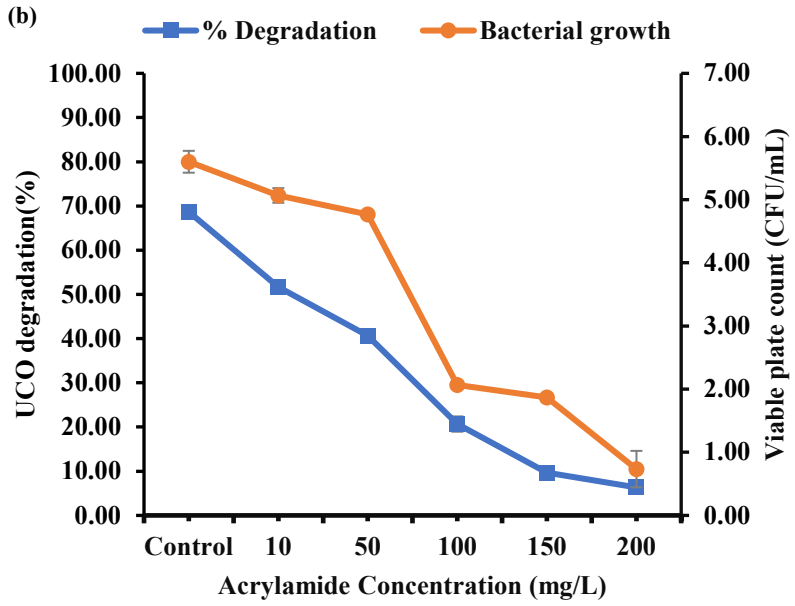
Effect of Acrylamide Concentration on UCO Degradation at Varying Acrylamide Concentrations

The effect of different acrylamide concentrations on the percentage degradation of UCO was studied to ascertain their stimulatory or inhibitory effects. Acrylamide has been discovered in both commercially processed and home-cooked foods. The acrylamide level varies within specific dietary categories. The fluctuation in level of acrylamide is due to precursors in the raw material, differences in food composition, and variations in process parameters such as temperature and time of frying. The formation of acrylamide during frying ranged between 9.25 and 5987.50 µg/kg, depending on the presence of ammonia present in the food to change acrylic acid into acrylamide [24].

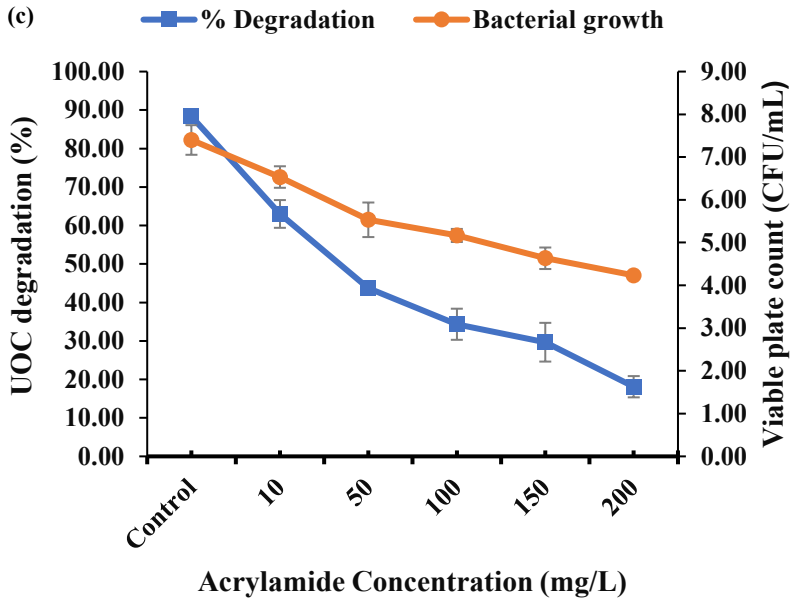
This experiment was conducted to conclude the effects of varying acrylamide concentrations on the percentage degradation of UCO. The study was carried out by using single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03 in 10-200 mg/L of acrylamide over 7 days of incubation, as shown in Figure 1. The results reveal that the bacterial growth decreased when the acrylamide concentration increased from 10-200 mg/L for both single isolates of AQ5-02 and AQ5-03. The degradation and bacterial growth in the co-culture (AQ5-02 + AQ5-03) steadily decreased as the acrylamide concentration rose from 10 mg/L to 200 mg/L. The co-culture was able to degrade 50% of UCO at a maximum tolerated concentration of 10 mg/L of acrylamide. Beyond 10 mg/L of acrylamide concentration, there was a significant reduction in the degradation of UCO. One-way ANOVA as shown in Table 1 demonstrates the overall significant difference in bacterial growth and oil degradation between various acrylamide concentrations: F (5, 12) = 183.168, $p < 0.001$, F (5, 12) = 2778.78, $p < 0.001$, F (5, 12) = 198.870, $p < 0.001$ for single isolates of AQ5-02 and AQ5-03 and co-culture (AQ5-02 +AQ5-03), respectively. Significant differences between all acrylamide concentrations in terms of UCO degradation and bacterial growth were revealed by Post hoc comparison using Tukey's test ($p < 0.05$) in both the single isolates of AQ5-02 and AQ5-03. However, there is no significant difference in terms of the degradation from 100 mg/L to 200 mg/L ($p > 0.05$), $p = 0.53$ for co-culture (AQ5-02 + AQ5-03) isolates.



Alcaligenes sp. AQ5-02



Serratia sp. AQ5-03



Co-culture *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03

Figure 1. Effect of different concentrations of acrylamide on (a) AQ5-02, (b). AQ5-03 and (c) co-culture (AQ5-02+AQ5-03) on UCO degradation after 7 days of incubation. Error bars represent mean ± standard deviation, n = 3.

Table 1. Effect of varying acrylamide concentration on degradation of UCO

Concentration of acrylamide (mg/L)	Degradation (%)		
	Co-culture (AQ05-02+AQ05-03)	*Alcaligenes* sp. AQ05-02	*Serratia* sp. AQ05-03
0	88.3 ± 0.6^{a}	74.3 ± 0.6^{a}	68.7 ± 1.1^{a}
10	63.0 ± 3.6^{b}	57.0 ± 0^{b}	51.7 ± 0.5^{b}
50	43.7 ± 1.5^{c}	37.0 ± 1.5^{c}	40.7 ± 0.6^{c}
100	34.3 ± 4.0^{d}	27.0 ± 1.7^{d}	20.67 ± 0.6^{d}
150	29.7 ± 5.0^{d}	15.7 ± 0^{e}	9.67 ± 0.6^{e}
200	18.0 ± 2.6^{e}	9.0 ± 0^{f}	6.33 ± 1.2^{f}

Described values mean ± SD of three replicates. Different alphabets indicate significance difference ($p < 0.05$) of *Alcaligenes* sp. AQ5-02, *Serrratia* sp. AQ5-03 and co-culture isolate of Alcaligenes sp. AQ5-02 and *Serratia* sp. AQ5-03 for degradation of the UCO.

The biodegradation of acrylamide using bacterial isolates is determined by the nature of the microbes that use acrylamide as their energy source. The enzyme amidase controls the process by breaking down the acrylamide monomer into acrylic acid and ammonia. It is well known that numerous bacteria use acrylamide as a source of carbon, nitrogen, or both. For instance, after 72 hours of incubation, *Burkholderia* sp. strain AQ5-13 demonstrated the capacity to break down 14% of 500 mg/L acrylamide as its carbon and nitrogen source [25].

The proposed mechanism of removal of acrylamide has been connected to specific bacterial strains and types of substrates. The binding affinity to cell walls is coupled to a reduction in acrylamide concentration. Amidase, which is produced by the bacterial species, catalyses the breakdown of acrylamide into ammonia and acrylic acid. Amidase enables microorganisms to thrive in environments where the sole carbon and nitrogen sources are linear aliphatic amides. The primary direct method of lowering acrylamide levels is the integration of acrylamide into microbial cell walls and the formation of amidase, which hydrolyses acrylamide [26]. The process undertaken by microorganisms in the elimination of acrylamide has not been thoroughly investigated or known. Thus, it is essential to investigate the acrylamide removal activity of microorganisms in different settings, such as using single and co-culture isolates with varying pH, temperature, oil, and water activity [27].

Comparison of The Effect of Acrylamide Concentration on UCO Degradation by Single and Co-culture Isolates

The results showed that the presence of acrylamide has a significant effect on UCO degradation and bacterial growth. The comparison of bacterial growth and degradation in single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serratia* sp. AQ5-03 is illustrated in Figure 1. At high concentrations of acrylamide, bacterial growth and degradation dramatically decreased. The percentage of UCO degradation in co-culture was significantly different among all acrylamide concentrations. The isolates can degrade 50% and above of UCO, up to 10 mg of acrylamide concentration. However, there was a significant reduction in the degradation of UCO in single and co-culture isolates (Figure 1) above 10 mg of acrylamide, probably due to its toxicity to the isolates. Two-way ANOVA reveals that there is a significant difference in terms of degradation and bacterial growth between the single isolates of AQ5-02, AQ5-03, and co-culture (AQ5-02 + AQ5-02-03) isolates with varying acrylamide concentrations ($p < 0.0001$). The results suggest that the co-culture has greater degradation and better tolerance at all concentrations of acrylamide compared to single isolates of AQ5-02 and AQ5-02-03 (Table 2).

Table 2. Two-way ANOVA of the isolates AQ5-02, AQ5-03 and coculture (AQ5-02 + AQ5-03)

Source of variation	% of total variation	p-value	p-value summary	Significant	p-value
Interaction	1.127	<0.0001	****	Yes	
Acrylamide concentration (mg/L)	92.85	<0.0001	****	Yes	
Bacterial isolates	5.544	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	
Interaction	341.9	10	34.19	F (10, 36) = 8.471	< 0.0001
Acrylamide concentration (mg/L)	28157	5	5631	F (5, 36) = 1395	< 0.0001
Bacterial isolates	1681	2	840.6	F (2, 36) = 208.3	< 0.0001
Residual	145.3	36	4.036		

**** symbol indicates highly significant.

An elevated concentration of acrylamide has a lethal impact on bacteria, resulting in an inhibitory effect that makes biodegradation in the environment a challenge [11, 28]. The finding demonstrates that the presence of acrylamide has a substantial effect on UCO degradation and bacterial growth. Although the isolates could utilise UCO when acrylamide is present, an increase in the acrylamide concentrations reduced the UCO degradation and bacterial growth. Similar to this study, the hydrocarbon-degrading *Pseudomonas* sp. strain Dr. Y. Kerith was able to grow on 0-1000 mg of acrylamide while showing optimum growth at 300 mg/L and reaching inhibition at 900 mg/L. Another study found that Antarctic *Pseudomonas* sp. strain DRYJ7 observed a comparable decline in bacterial growth at high acrylamide concentrations that exceeded 500 mg/L [29]. Meanwhile, similar to the outcomes of this study, *Rhodococcus* sp. AQ5-07 could breakdown waste canola oil in the presence of up to 10 mg/L of acrylamide [22]. Another study by Gafar and Shukor (2018) [25] demonstrated that 14% of the carbon and nitrogen supply of 500 mg/L of acrylamide could be broken down by *Burkholderia* sp. strain AQ5-13. Bedade and Singhal (2018) [30] demonstrated the degradation of acrylamide by *Cupriavidus oxalaticus* ICTDB921 in acrylamide concentrations ranging from 10 to 100 mM. The highest growth, specific growth rate profiling, and maximum degradation were observed at 60 mM. However, as the acrylamide concentration rose to 70 nM, the growth was inhibited, and the specific growth rate dropped from 0.3906 h−1 at 60 mM to 0.1442 h−1 at 70 mM. At higher concentrations of acrylamide, bacterial growth dramatically decreases. The bacterial growth cessation of the organism could be attributed to the inhibitory impact of acrylamide protein thiol groups [31, 32].

Various species and strains of microorganisms are able to degrade acrylamide in different environments. They are proven to decompose acrylamide in a broad range of concentrations, and bacteria are more efficient in the process compared to yeast [33]. A previous study found that acrylamide was inhibitory to bacterial growth on diesel [34]. The effect of acrylamide on the energy status and survival of *Rhodococcus* sp. and *Alcaligenes* sp. was studied by [35]. This was explained by nitrile-hydrolysing enzymes and the cell wall structures. Toxic compounds additionally hinder the electron transport chain of microorganisms. The majority of the *Rhodococcus* sp. and *Alcaligenes* sp. strains did not grow when acrylamide was present at a concentration of 500 mM. Therefore, a high acrylamide concentration inhibited the growth of the studied strains, which is consistent with our finding [35].

In another study, the probiotic bacteria *Lactobacillus acidophilus* LA-5 (LA5) can breakdown acrylamide and has no harmful influence on high acrylamide concentrations from 7.5 to 100 µg/mL. [33]. Meanwhile, when the concentration of the acrylamide rose to 2000 mg/L, the yeast

*Rhodotorul*a sp. strain MBH23, an effective acrylamide-degrader that can tolerate high concentrations of acrylamide, showed growth inhibition [36].

The introduction of specific microorganisms that are able to degrade harmful contaminants is necessary for successful bioremediation. Biodegradation can be restricted by indigenous predators, parasites, and toxicants. In anthropogenic waste bioremediation, immobilised cells provide numerous advantages over non-immobilised cells. In another study, it was evident that concentrations of acrylamide affect degradation in immobilised and free cells of *Enterobacter aerogenes* [11]. Similarly, two strains of *Alcaligenes faecalis* could utilise acrylamide, and the study demonstrated the highest biodegradation by immobilised cells. The reason could be due to the larger surface area, which allowed for high absorption [37]. Furthermore, cell immobilisation provides defence against harmful acrylamide concentrations that must slowly enter the matrix prior to getting to the cells. This provides a lesser acrylamide toxicity dose than direct contact, as seen in the free-cell condition [10].

Additionally, compared to single isolates, bacterial consortia or mixed cultures are better able to withstand and break down acrylamide. Co-culturing fermentative *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus plantarum* ATCC 20552 could also reduce the acrylamide content in bread rolls [38]. The presence of heavy metals and xenobiotic chemicals at the polluted site is another important barrier to bioremediation. This is because many microorganisms were unable to handle large quantities of such compounds by losing their ability to degrade target compounds since they lost the capability to disintegrate the compounds. Rusnam and Gusmanizar demonstrated that acrylamide degradation by a bacterial consortium and their growth occurred in the range of 300–500 mg/L of acrylamide, though the presence of silver, copper, and mercury slowed down the growth of the consortium [4]. Meanwhile, microbial growth and the influence of acrylamide concentration were studied by Petka et al. [9]. In their study, acrylamide concentrations of 0–10 µg/mL were tested on two bacteria (*Leuconostoc mesenteroides* and *Lactobacillus acidophilus* LA–5) and two yeasts (*Saccharomyces cerevisiae* and *Kluyveromyces lactis* var. *lactis*). The researchers found that acrylamide can exert a positive or negative impact, where only *L. acidophilus* LA-5 demonstrated any growth while the other three tested microorganisms did not. However, the effect of acrylamide on microorganisms is inconsistent. For example, one study found a *Salmonella typhimurium* strain that can grow at an acrylamide concentration of 5 mg/plate without metabolic activation [9]. Meanwhile, in another study, the addition of 1-3% of acrylamide caused a number of changes, such as the inability to divide cells, lengthening of the cells, suppression of DNA synthesis, a reduction in osmatic stability, and changes to the outer membrane`s ultrastructure [12]. Thus, the type of microorganisms that can use acrylamide as a source of energy determines the toxicity of acrylamide and its ability to degrade [10]. Bacteria have the ability to use acrylamide as a source of carbon, nitrogen, or both, though the latter is rarely reported. In this study, UCO was provided as a carbon source, and the bacteria used the oil instead of the toxic acrylamide. The result shows that UCO degradation was inhibited by increasing acrylamide concentrations. Hence, high concentrations of acrylamide are toxic to single and co-culture isolates.

CONCLUSION

Application of particular microorganisms capable of decomposing harmful contaminants is necessary for successful bioremediation. This study was performed to examine the effect of various concentrations of acrylamide on palm-based UCO degradation by single and co-culture isolates of *Alcaligenes* sp. AQ5-02 and *Serrratia* sp. AQ5-03. To the best of our knowledge, this is the first report on the degradation of UCO in the presence of acrylamide by single and co-cultured bacteria isolates of *Alcaligenes* sp. AQ5-02 and *Serrratia* sp. AQ5-03. Our findings demonstrated that the concentration of acrylamide has an inhibitory impact on the bacterial growth and degradation of palm-based UCO, with the single and co-culture managing to degrade 50% of UCO with 10 mg/L of acrylamide, though the degradation and growth dropped off sharply at higher acrylamide concentrations. In addition, the bacterial co-culture demonstrated a better tolerance to an increase in acrylamide concentration. Presently, there is a lack of information in the literature on the influence of acrylamide on the degradation of UCO in single and co-cultured bacterial isolates. Hence, the findings from this study will have a significant impact on future bioremediation of UCO due to the paucity of literature on microbial tolerance to acrylamide.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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