Development Synergy and Optimization of Growth Conditions of Chlorpyrifos Degrading Bacteria Consortium

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Abstract

Chlorpyrifos (CPF) [O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate)] is an organophosphorous used as a house hold and agricultural pesticide in various formulations has adverse toxic effects on human health which has created an environmental concern. In the recent years, degradation studies of chlorpyrifos have greatly increased but only a few biodegradations by consortia have been reported. This study aimed to develop bacteria consortia and optimizing their growth conditions of temperature and pH for effective biodegradation of chlorpyrifos. Experimental research design was used to determine optimum temperature and pH. Five consortia were assembled based on degradation ability. Group I consisted of all of the five bacteria isolates (*Brachybacterium* sp. (CP1), *Exiguobacterium alkaliphilum* (CP2), *Advenella kashmirensis* **(**CP3), *Micrococcus luteus* (CP5), *Pseudomonas protegens* (CP6) and *Lysinibacillus sphaericus* CP7). Group II was composed of high degraders (CP1, CP3 and CP5), Group III are moderate degraders (CP2, CP5 and CP6), Group IV low degraders (CP5, CP6 and CP7) and Group V, a mixture of low and high degraders (CP3, CP7 and CP6). Findings of this study showed that the optimal growth conditions of the bacteria isolates were pH and temperature of 7 and 25^{o}C, respectively. Bacteria consortia had their optimal growth at a temperature of 25^{o}C and 30^{o}C, and pH range between 6 - 8. With Group I, III and IV with highest growth as indicated by high optical density. These results revealed the ability of these bacteria consortia (group I, III and VI) to be used in remediating chlorpyrifos contaminated environment. Further research is required to utilize these three consortia in a bioreactor in a way that is safe, affordable and environmentally friendly.

Key Words: Degradations; Bacterial Consortia; Chlorpyrifos; Condition Optimization

Introduction

Organophosphorus (OPs) compounds, including Chlorpyrifos, have been extensively used as insecticides and pesticides since their development in the late 1930s (Dragun et al., 1984; Singh & Walker, 2006). Despite their effectiveness in pest control, the widespread use of OPs has led to significant environmental pollution and health hazards (Singh & Walker, 2006). These compounds primarily target acetylcholinesterase (AChE), causing the accumulation of acetylcholine (ACh) and resulting in symptoms like sweating, nausea, and respiratory failure (Ganie et al., 2022). Chronic exposure to OPs can lead to severe health issues such as neurological disorders and reproductive defects, with infants being particularly vulnerable due to the importance of ACh in brain development (Ganie et al., 2022). Chlorpyrifos, in particular, is widely used in both household and agricultural settings due to its potency against a broad spectrum of pests (Singh & Walker, 2006). However, its persistence in the environment, along with that of its primary degradation product 3, 5, 6-trichloro-2-pyridinol (TCP), poses serious ecological and health risks (Singh & Walker, 2006; Li et al., 2010).

The accumulation of chlorpyrifos and TCP in various environmental compartments, including water bodies, soil, air, and food chains, has led to ecotoxicological concerns and adverse effects on non-target organisms (Li et al., 2010). Human exposure to chlorpyrifos has been linked to acute and chronic health effects, including liver damage, endocrine disruption, reproductive defects, immune and nervous

system disorders, among others (Naughton & Terry-Jr, 2018). Furthermore, aquatic organisms and soil microbes also suffer from acute toxicity due to chlorpyrifos contamination (Michereff-Filho et al., 2004). Given the environmental and health risks associated with chlorpyrifos, there is a growing need for effective remediation strategies (Fu et al., 2022). Decontamination methods encompass physical, chemical, and biological approaches (Silva *et al.,* 2022). Physical methods, including washing, adsorption, filtration, and sedimentation, aim to reduce exposure to organophosphates-contaminated materials but may not entirely eliminate contamination and pose environmental risks (Silva et al., 2022). Chemical degradation, employing oxidizing or reducing agents like hydrogen peroxide, ozone, and chlorine dioxide, is widely used but may generate hazardous waste and have corrosive properties (Silva et al., 2022). Microbial bioremediation offers a promising approach for decontaminating chlorpyrifos-polluted ecosystems (Fu et al., 2022). Various bacterial species capable of degrading chlorpyrifos have been isolated and characterized, demonstrating their potential for bioremediation applications (Ambreen & Yasmin, 2020).

Enzymes involved in chlorpyrifos degradation have also been identified from microbial sources (Singh, 2009; Singh & Walker, 2005). However, the degradation of chlorpyrifos is influenced by both biotic and abiotic factors (Riyaz et al., 2022). Previous studies have focused on individual bacterial strains rather than synergistic interactions within bacterial consortia. Therefore, this study aims to develop, optimize bacterial consortium capable of degrading chlorpyrifos efficiently and seeks to contribute to the development of effective chlorpyrifos remediation strategies.

Materials and Methods

Study Design

The study used six bacterial isolates identified and characterized for their potential to degrade chlorpyrifos (Atego, 2022). These isolates, included *Brachybacterium* sp., *Exiguobacterium alkaliphilum*, *Advenella kashmirensis*, *Micrococcus luteus*, *Pseudomonas protegens* and *Lysinibacillus sphaericus* (Table 1) and were stored in the Department Pure and Applied Science Laboratories in the school of Applied and Health Sciences at Technical University of Mombasa

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Table 1. Identification of CPF degrading bacteria and their accession numbers adapted from Atego, (2022)

Isolate	Accession	Species (16S rRNA gene analysis)	% Identity
CP1	KU644165.1	*Brachybacterium sp.*	100
CP2	MT225705.1	*Exiguobacterium alkaliphilum*	100
CP3	MT138841.1	*Advenella kashmirensis*	99
CP5	MT383647.1	*Micrococcus luteus*	99
CP6	MT505104.1	*Pseudomonas protegens*	97
CP7	JQ900525.1	*Lysinibacillus sphaericus*	98

This study employed an experimental research design to investigate the optimal conditions (pH and temperature) for chlorpyrifos degradation by these bacterial isolates. A consortium of chlorpyrifos-degrading bacterial isolates was constructed based on their individual chlorpyrifos-degrading abilities as described by Atego, (2022). The isolates were grouped into consortia, with each group comprising three bacterial isolates except group 1 (Table 2).

Table 2. Groups of bacterial isolates used in designing of bacteria consortia for chlorpyrifos degradation

Group	Criteria for grouping	Consortium of bacterial isolates
Group I	All bacteria isolates	*Brachybacterium* sp., *Exiguobacterium alkaliphilum, Advenella kashmirensis, Micrococcus luteus, Pseudomonas protegens* and *Lysinibacillus sphaericus*
Group II	High degraders	*Brachybacterium sp., Advenella kashmirensis* and *Micrococcus luteus*
Group III	Moderate degraders	*Exiguobacterium alkaliphilum, Lysinibacillus* sp., and *Lysinibacillus sphaericus*
Group IV	Low degraders	*Exiguobacterium alkaliphilum, Pseudomonas protegens* and *Lysinibacillus sphaericus*
Group V	A mixture of high, moderate and low degraders	*Advenella kashmirensis*, *Lysinibacillus sphaericus* and *Pseudomonas protegens*

Viability assessment and optimization of growth conditions in chlorpyrifos supplemented media

To revive the isolates, they underwent culturing in nutrient broth by incubating at 30°C in a rotor shaker operating at 150 rpm for 24 hours. Subsequently, the viability and growth curve of the bacterial isolates were evaluated using mineral salt medium (MSM) constituted in grams/L as follows: $MgSO_4$ $7H_2O$, 0.2; K_2HPO_4, 4.8; Fe_2 $(SO_4)_3$, 0.001; NH_4NO_3, 1.0; KH_2PO_4, 1.2 and Ca $(NO_3)_2.4H_20$, 0.4, (Omolo *et al.*, 2012) supplemented with chlorpyrifos (98% purity obtained from Sigma Andrich) at a concentration of 200 ppm and autoclaved. Each culture was inoculated with 1 ml of revived bacterial broth with an optical density at 600 nm (24- hr-old), and then incubated at 30°C in a shaker operating at 150 rpm for 5 days. Growth of bacteria was tracked through the assessment of optical density, utilizing a UV-Vis spectrophotometer (BMS UV-160 Shimadzu, Japan), at 24-hr intervals. The MSM supplemented with CFP but lacking bacterial inoculation served as a control to monitor non-bacterial-related changes in optical density over time. To investigate the effects of temperature and pH on chlorpyrifos utilization, 30 mL of MSM supplemented with 200 ppm chlorpyrifos was inoculated with 500 µL of 24-hr-old bacterial inoculum (with an OD600 of 0.6 and colony-forming units (CFU) $mL^{-1}g^{-1}$ of 10^6) for each isolate. Cultures were incubated at different pH levels (5, 6, 7, 8, and 9) and temperatures (25°C, 30°C, 35°C, and 40°C) for 72 hrs in an orbital shaker set at 100 rpm. Regular sampling involved taking 10 mL samples from each flask, serially diluting them, and spreading them on Nutrient Agar (NA) plates to confirm bacterial growth. Bacterial growth was monitored every 24 hrs by measuring OD600 using a UV-Spectrophotometer. The OD600 readings were converted to colony-forming units per milliliter (CFU/mL) using the following formula established by Kim *et al.* (2012):

Colony-forming units (CFU/mL) = (2 × 10^8 × OD) + 4 × 10^6

Optimization of pH and Temperature of Consortia of Bacterial Isolates

For the optimization of pH and temperature of the developed consortia of bacterial isolates (Table 2), the bacterial isolates, cultured individually in 100 mL of MSM for three days at 30°C, were subjected to centrifugation at 5000g for 10 min to obtain biomass concentration. The resulting cell pellets were washed with distilled water, suspended in 0.9% sterile NaCl to an optical density of 600nm, and proportionally divided for consortium preparation.

In each consortium group, the population of total viable bacterial isolates was determined

by inoculating 1 mL containing approximately 10^6 cells mL^-1 in Nutrient Broth (NB) supplemented with 200 mg/L chlorpyrifos. 10 mL of the prepared and constituted bacterial consortium were cultured under different pH levels (5, 6, 7, 8, and 9) and temperatures (25°C, 30°C, 35°C, and 40°C) and monitoring of growth by measuring the optical density at 600 nm and calculation of colony-forming units per milliliter (CFU/mL performed as describe above. All the experiment was performed in triplicate for each consortium, with MSM without bacterial inoculum serving as a negative control.

Results and Discussion

Revival and Viability of the Stored Bacterial Isolates

As described by Brown & Turne, (2022), among the simplest method of of meassuring the growth of microorganisms is measuring the optical density. However, there are other proposed methods and formulae to convert OD to CFU/mL eliminating the steps of performing continuous plate validation (Begot et al., 1996; Kim et al., 2012; Zhang et al., 2015; Beal et al., 2020; Brown & Turne, 2022). In this study, the measured OD600 were converted to cell count equivalents and CFU/mL were calculated as describe by Kim et al., (2012) during monitoring the growth of *Pseudomonas aeruginosa*, which found the relationship of OD600 to CFU, as described by the equation in the methodology section above. bacterial growth was assessed based on cell denisty as OD600nm converted to CFU/mL. All the six bacterial isolates were successifully revived and showed viability as indicated by presence of colonies in NA after the 24 hours (one day) culturing period (Figure 1) and Nutrient broth (NB) as indicated by increase in optical density 600nm (Table 3).

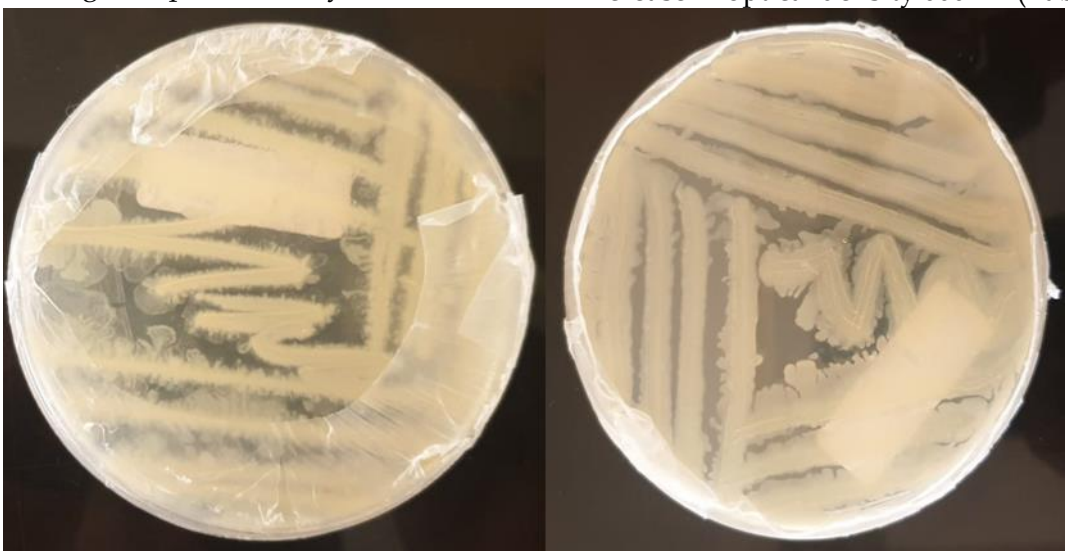

Figure 1. A 24 hours (1-day) culture of stored Chlorpyrifos degrading bacterial isolate on Nutrient agar supplemented with Chlorpyrifos

The growth (Figure 2) and CFU/mL (Table 3) of the stored CPF degrading bacterial isolate after the seven days of culturing in NB indicate successifully revivability of the six bacterial isolates that were used in this study. All the six bacterial isolates had a characteristic sigmoidal curve by showing a slow rate in the increase in biomass (OD600) and CFU/mL starting from day 0 (D0) until day 2 (D2) representing the lag phase of bacterial growth. These bacterial isolates took two days (48 hours) after incubation to aclimatize with condition before the increase in biomass starting in D2. The exponetial or log phase of the bacterial isolates lasted for 24 hours starting from D3 to D4 after incubation upon which gave the onset of stationery phase. The highest growth was observed in *Branchbacterium spp.* and *Pseudomonas protegens*. This was followed by *Exigobacterium alkaliphilion*, *Adenvella kashmirensis*, and *Lysinibacillus sphericus* in that order while the lowest growth rate was

observed in *Micrococcus luteus* (Figure 2). Futher, by converting OD600 to CFU/mL, a significant difference in the rate of growth ($p < 0.05$) was observed between the stored and revived bacterial isolates and also in the entire period lasting for a period of seven days (Table 3).

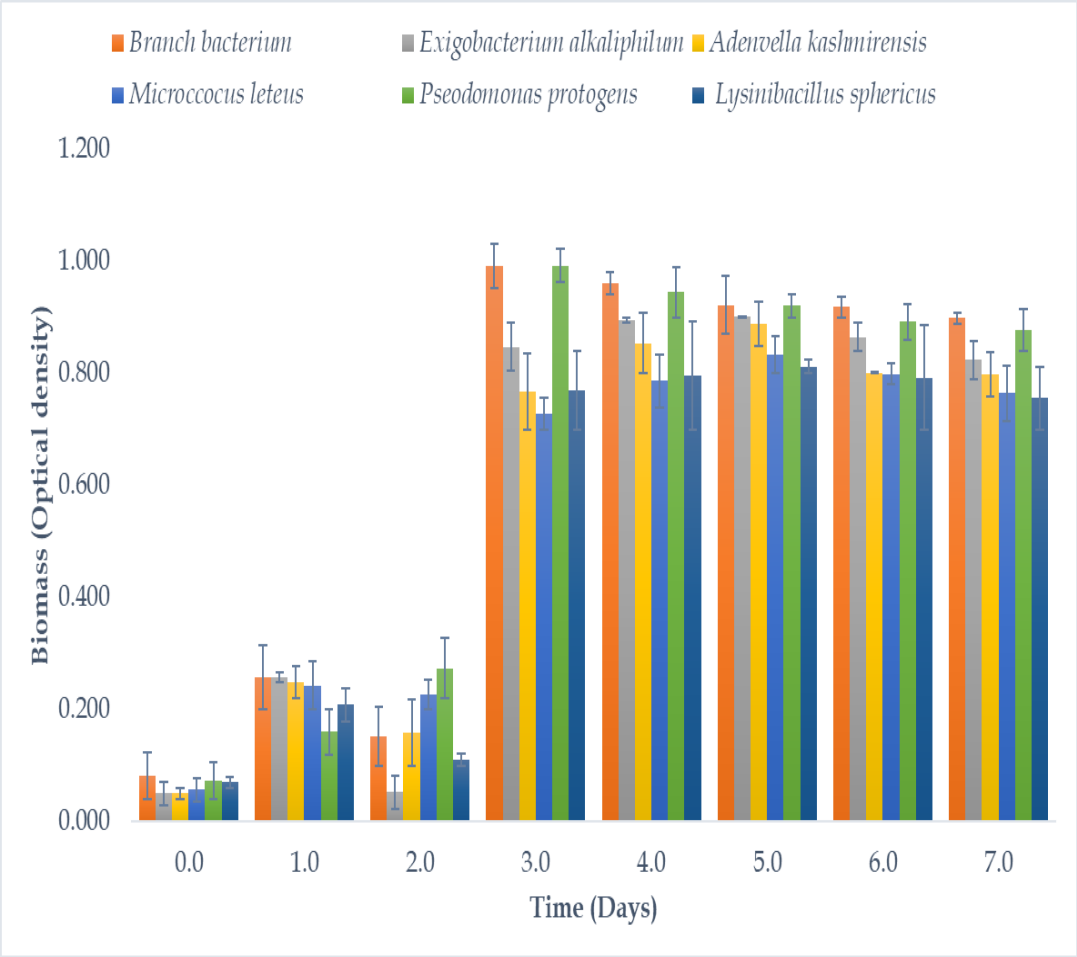

Figure 2. Revival and growth of stored CPF degrading bacterial isolates at 30°C and pH 7.0 in Nutrient Broth

It is noted that the formula used in the study as suggested by Brown & Turne, (2022) need to be verified by cells plating at specific OD and wavelengths with the values confirmed by the formula for each bacterial isolate. This is because the size of the cell and shape may have an effect on the dispersion of light caused by different shapes and sizes of the cells. Further, it should be admitted that cultural differences and turbidiness caused by different bacteria fo instance the six bacterial isolates used in this study and the inability of bacterial isolates to grow in different media such means that converting OD600 to CFU could have resulted in an inaccurate conversion as argued by Brown & Turne, (2022). However, this was performed to maintain conformity among the datasets highlighting the limitations of the conversion factors before verification and modification of the equation empirically for each bacterial isolate (Brown & Turne, 2022).

Table 3: Biomass (OD600) and CFU/mL of stored CPF degrading bacterial isolates after revival lasting for a period of seven days

Bacterial isolate	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day6		Day 7	
	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL
CP1.	0.082	$20.40^{b}x10^{6}$	0.258	$55.60^{a}x10^{6}$	0.153	$34.60^{b}x10^{6}$	0.992	$202.40^{f}x10^{6}$	0.962	$196.40^{c}x10^{6}$	0.922	$188.40^{e}x10^{6}$	0.919	$187.80^{d}x10^{6}$	0.899	$183.80^{c}x10^{6}$
CP2	0.051	$14.20^{c}x10^{6}$	0.258	$55.60^{a}x10^{6}$	0.053	$14.60^{d}x10^{6}$	0.848	$173.40^{d}x10^{6}$	0.895	$183.00^{b}x10^{6}$	0.901	$184.20^{c}x10^{6}$	0.865	$1.77.00^{c}x10^{6}$	0.824	$168.80^{f}x10^{6}$
CP3	0.051	$14.20^{a}x10^{6}$	0.249	$53.80^{b}x10^{6}$	0.159	$38.50^{c}x10^{6}$	0.768	$157.60^{e}x10^{6}$	0.854	$174.80^{a}x10^{6}$	0.889	$181.80^{f}x10^{6}$	0.801	$164.20^{e}x10^{6}$	0.798	$163.60^{a}x10^{6}$
CP5	0.058	$15.60^{e}x10^{6}$	0.243	$52.60^{d}x10^{6}$	0.227	$49.40^{e}x10^{6}$	0.728	$149.60^{a}x10^{6}$	0.787	$161.40^{b}x10^{6}$	0.833	$170.60^{a}x10^{6}$	0.799	$163.80^{b}x10^{6}$	0.765	$157.00^{b}x10^{6}$
CP6	0.073	$18.60^{f}x10^{6}$	0.161	$36.20^{c}x10^{6}$	0.274	$58.80^{f}x10^{6}$	0.993	$202.60^{c}x10^{6}$	0.945	$193.00^{f}x10^{6}$	0.921	$188.20^{b}x10^{6}$	0.892	$182.40^{a}x10^{6}$	0.877	$179.40^{e}x10^{6}$
CP7	0.071	$18.20^{d}x1^{06}$	0.209	$45.80^{e}x10^{6}$	0.111	$26.20^{a}x10^{6}$	0.770	$158.00^{b}x10^{6}$	0.797	$163.40^{e}x10^{6}$	0.812	$166.40^{d}x10^{6}$	0.793	$162.60^{f}x10^{6}$	0.756	$155.20dx10^{6}$

Mean values (n=3) ± SEM. Values appended by different superscript letters within a column are significantly different ($P < 0.05$). Key: *Branchbacterium* sp (P1), *Exigobacterium alkaliphilum* (CP2), *Adenvella kashmirensis* (CP3), *Micrococcus leteus* (CP5), *Pseodomonas protogens* (CP6) and *Lysinibacillus sphericus* (CP7)

Optimization of temperature and pH of chlorpyrifos degrading bacterial isolates

After culturing the stored CPF degrading bacterial isolates under different pH and temperature conditions, the optimal temperature and pH were determined as presented in Table 4 and Figures 3, 4, 5 and 6. A significant increase in biomass (OD600) and CFU/mL ($p < 0.5$) was observed with increase in pH and temperature up to optimum point (Table 4, Figures 3, 4, 5 and 6). However, after the optimum temperature and pH, a significant decrease in biomass (OD600) and CFU/mL ($p < 0.05$) was observed (Table 4 and Figures 3, 4, 5 and 6). Further, as the temperature increased, marked variations in the pH at which maximum growth of the stored CPF degrading bacterial isolates was observed (Table 4 and Figures 3, 4, 5 and 6). The maximum growth as indicated by the highest calculated CFU/mL and biomass (OD600) was observed in the six bacterial isolates at pH 6.0 and temperature of 25°C (Table 4 and Figure 3). It can be concluded from the result obtained in this study as indicated in Table 4 that the optimum conditions for the growth of these stored CPF degrading bacterial isolates are pH 6.0 and temperature of 25°C

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Table 4. Biomass of and CFU/mL of CPF degrading bacterial isolates cultured under different pH and temperature (°C)

Bacterial isolate	Temperature	pH									
		5		6		7		8		9	
		OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL
Branch bacterium	25°C	$0.293^{c}\pm0.000$	6.26×10^{7}	$0.414^{b}\pm0.001$	8.68×10^{7}	$0.233^{a}\pm0.011$	5.06×10^{7}	$0.283^{c}\pm0.001$	6.06×10^{7}	$0.245^{d}\pm0.001$	5.30×10^{7}
Exigobacterium alkaliphilum	25°C	$0.288^{c}\pm0.001$	4.96×10^{7}	$0.816^{d}\pm0.001$	1.672×10^{8}	$0.218^{b}\pm0.005$	4.76×10^{7}	$0.290^{c}\pm0.000$	6.20×10^{7}	$0.130^{f}\pm0.002$	3.00×10^{7}
Adenvella kashmirensis	25°C	$0.289^{c}\pm0.001$	6.18×10^{7}	$0.890^{d}\pm0.001$	1.820×10^{8}	$0.191^{a}\pm0.016$	4.22×10^{7}	$0.197^{a}\pm0.001$	4.34×10^{7}	$0.162^{b}\pm0.002$	3.54×10^{7}
Micrococcus leteus	25°C	$0.279^{d}\pm0.000$	5.98×10^{7}	$0.744^{e}\pm0.001$	1.528×10^{8}	$0.164^{c}\pm0.014$	3.58×10^{7}	$0.239^{b}\pm0.002$	5.18×10^{7}	$0.183^{a}\pm0.001$	4.06×10^{7}
Pseodomonas protogens	25°C	$0.288^{c}\pm0.000$	4.96×10^{7}	$0.795^{e}\pm0.001$	1.63×10^{8}	$0.158^{c}\pm0.003$	3.56×10^{7}	$0.123^{e}\pm0.004$	2.86×10^{7}	$0.247^{g}\pm0.001$	5.34×10^{7}
Lysinibacillus sphericus	25°C	$0.287^{c}\pm0.001$	6.14×10^{7}	$0.941^{c}\pm0.001$	1.922×10^{8}	$0.081^{i}\pm0.005$	2.02×10^{7}	$0.048^{g}\pm0.001$	1.36×10^{7}	$0.095^{f}\pm0.001$	2.30×10^{7}
Branch bacterium	30°C	$0.196^{a}\pm0.002$	4.02×10^{7}	$0.269^{i}\pm0.002$	5.78×10^{7}	$0.109^{i}\pm0.007$	2.58×10^{7}	$0.286^{c}\pm0.002$	6.12×10^{7}	$0.228^{i}\pm0.005$	4.96×10^{7}
Exigobacterium alkaliphilum	30°C	$0.194^{a}\pm0.002$	4.28×10^{7}	$0.237^{i}\pm0.006$	5.14×10^{7}	$0.461^{i}\pm0.055$	9.62×10^{7}	$0.248^{d}\pm0.004$	5.36×10^{7}	$0.263^{g}\pm0.004$	5.66×10^{7}
Adenvella kashmirensis	30°C	$0.191^{a}\pm0.001$	4.22×10^{7}	$0.174^{f}\pm0.010$	4.00×10^{7}	$0.740^{f}\pm0.021$	1.520×10^{8}	$0.227^{i}\pm0.006$	4.94×10^{7}	$0.196^{a}\pm0.003$	4.02×10^{7}
Micrococcus leteus	30°C	$0.178^{a}\pm0.002$	3.96×10^{7}	$0.513^{j}\pm0.026$	1.066×10^{8}	$0.203^{b}\pm0.003$	4.46×10^{7}	$0.172^{a}\pm0.002$	3.84×10^{7}	$0.267^{g}\pm0.002$	5.74×10^{7}
Pseodomonas protogens	30°C	$0.192^{a}\pm0.001$	4.24×10^{7}	$0.196^{a}\pm0.002$	4.02×10^{7}	$0.622^{e}\pm0.012$	1.284×10^{8}	$0.158^{h}\pm0.004$	3.56×10^{7}	$0.199^{a}\pm0.002$	4.38×10^{7}
Lysinibacillus sphericus	30°C	$0.167^{b}\pm0.003$	3.74×10^{7}	$0.196^{a}\pm0.003$	4.02×10^{7}	$0.612^{e}\pm0.019$	1.264×10^{8}	$0.146^{h}\pm0.001$	3.32×10^{7}	$0.246^{g}\pm0.003$	5.32×10^{7}
Branch bacterium	35°C	$0.157^{b}\pm0.004$	3.54×10^{7}	$0.199^{a}\pm0.006$	4.38×10^{7}	$0.140^{h}\pm0.012$	3.2×10^{7}	$0.229^{b}\pm0.006$	4.98×10^{7}	$0.063^{f}\pm0.006$	1.66×10^{7}
Exigobacterium alkaliphilum	35°C	$0.217^{e}\pm0.005$	4.74×10^{7}	$0.157^{h}\pm0.011$	3.54×10^{7}	$0.262^{d}\pm0.001$	5.64×10^{7}	$0.246^{d}\pm0.003$	5.32×10^{7}	$0.084^{f}\pm0.002$	2.08×10^{7}
Adenvella kashmirensis	35°C	$0.157^{b}\pm0.003$	3.54×10^{7}	$0.228^{i}\pm0.005$	4.96×10^{7}	$0.305^{g}\pm0.014$	6.50×10^{7}	$0.186^{a}\pm0.007$	4.12×10^{7}	$0.125^{i}\pm0.010$	2.90×10^{7}
Micrococcus leteus	35°C	$0.223^{e}\pm0.001$	4.86×10^{7}	$0.162^{h}\pm0.014$	3.54×10^{7}	$0.283^{d}\pm0.002$	6.06×10^{7}	$0.173^{a}\pm0.005$	3.86×10^{7}	$0.121^{i}\pm0.002$	2.82×10^{7}
Pseodomonas protogens	35°C	$0.191^{a}\pm0.009$	4.22×10^{7}	$0.146^{h}\pm0.003$	3.32×10^{7}	$0.227^{b}\pm0.002$	4.94×10^{7}	$0.091^{f}\pm0.001$	2.22×10^{7}	$0.048^{f}\pm0.006$	1.36×10^{7}
Lysinibacillus sphericus	35°C	$0.144^{f}\pm0.003$	3.28×10^{7}	$0.198^{a}\pm0.024$	4.36×10^{7}	$0.236^{b}\pm0.005$	5.12×10^{7}	$0.081^{f}\pm0.007$	2.02×10^{7}	$0.091^{f}\pm0.006$	2.22×10^{7}
Branch bacterium	40°C	$0.114^{h}\pm0.001$	2.68×10^{7}	$0.196^{a}\pm0.012$	4.02×107	$0.080^{i}\pm0.001$	2.00×10^{7}	$0.161^{h}\pm0.001$	3.62×10^{7}	$0.078^{f}\pm0.001$	1.96×10^{7}
Exigobacterium alkaliphilum	40°C	$0.072^{g}\pm0.002$	1.84×10^{7}	$0.228^{i}\pm0.001$	4.96×10^{7}	$0.216^{b}\pm0.001$	4.72×10^{7}	$0.217^{b}\pm0.005$	4.74×10^{7}	$0.070^{f}\pm0.001$	1.80×10^{7}
Adenvella kashmirensis	40°C	$0.178^{a}\pm0.004$	3.96×10^{7}	$0.171^{a}\pm0.001$	3.82×10^{7}	$0.175^{a}\pm0.001$	3.9×10^{7}	$0.180^{a}\pm0.003$	4.00×10^{7}	$0.083^{f}\pm0.001$	2.06×10^{7}
Micrococcus leteus	40°C	$0.119^{h}\pm0.006$	2.78×10^{7}	$0.221^{i}\pm0.001$	2.612×10^{7}	$0.215^{b}\pm0.001$	4.70×10^{7}	$0.131^{e}\pm0.006$	3.02×10^{7}	$0.089^{f}\pm0.001$	2.18×10^{7}
Pseodomonas protogens	40°C	$0.144^{f}\pm0.001$	3.28×10^{7}	$0.229^{i}\pm0.001$	4.98×10^{7}	$0.191^{a}\pm0.001$	4.22×10^{7}	$0.107^{e}\pm0.007$	1.472×10^{7}	$0.044^{h}\pm0.001$	1.28×10^{7}
Lysinibacillus sphaericus	40°C	$0.143^{f}\pm0.001$	3.36×10^{7}	$0.078^{g}\pm0.003$	1.96×10^{7}	$0.159^{c}\pm0.001$	3.58×10^{7}	$0.112^{e}\pm0.004$	2.64×10^{7}	$0.062^{h}\pm0.001$	1.64×10^{7}

Mean values (n=3) ± SEM. Values appended by different superscript letters within a row and column are significantly different ($P < 0.05$).

The maximum growth as indicated by the highest calculated CFU/mL and biomass (OD600) was observed in all bacterial isolates at pH 6.0 and temperature of 25°C (Table 4 and Figure 3). The *L. sphaericus* achieved the highest biomass (OD600; 0.941±0.001 and CFU/mL; 1.922 x10^8), followed by *A. kashmirensis* (OD600; 0.890±0.001, CFU/mL; 1.820x10^8), *E. alkaliphilum* (OD600; 0.816±0.001, CFU/mL; 1.672x10^8), *P. protegens* (OD600; 0.795±0.001, CFU/mL; 1.63 x10^8), *M. luteus* (OD600; 0.744±0.001, CFU/mL; 1.5.28 x10^8) and finally *Branchbacterium spp* (OD600; 0.414±0.001, CFU/mL; 8.68x10^7) in that order (Table 4 and Figure 3). Further at 25°C, a significant decrease in biomass (OD600) and CFU/mL ($p < 0.05$) was observed with increase in pH beyond pH 6.0 in each of the bacterial isolates (Figure 3). It can be concluded from the result obtained in this study that the optimum pH for the growth of these CPF degrading bacterial isolates at 25°C is pH 6.0.

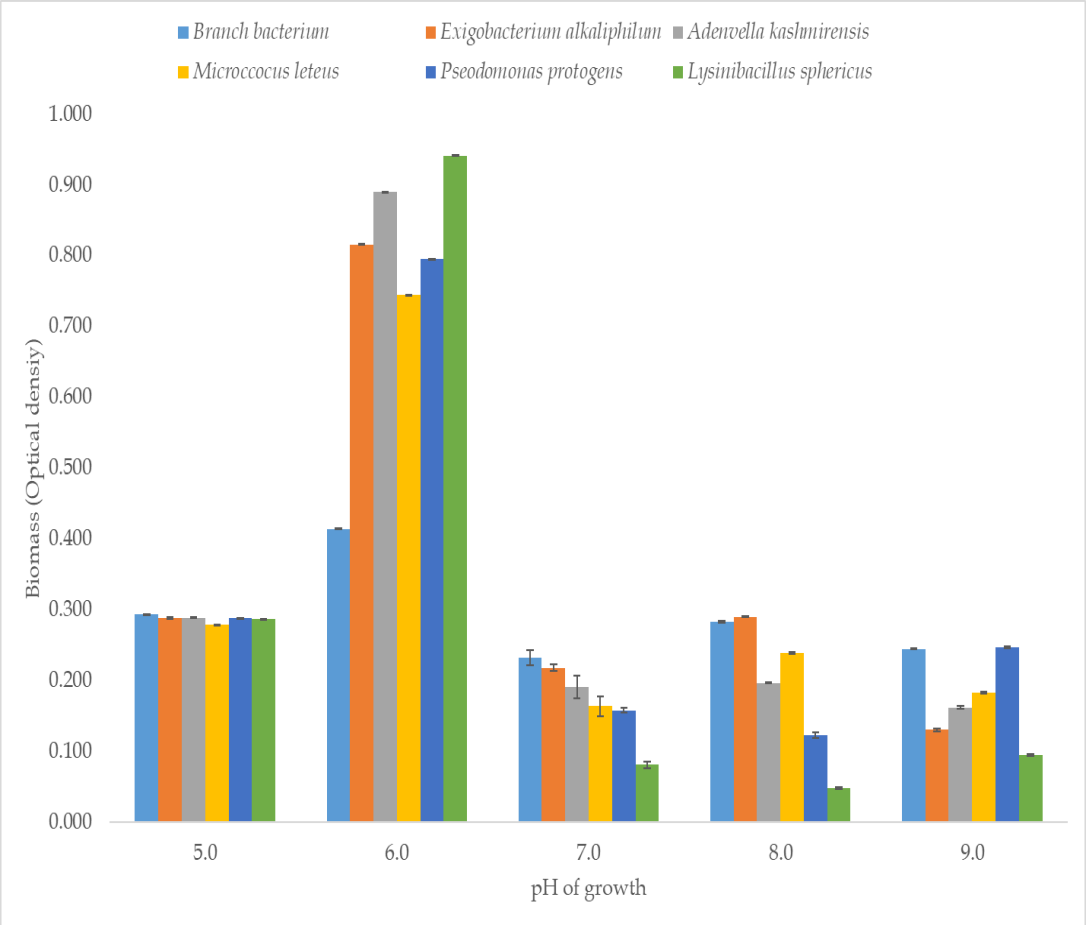

Figure 3. Growth of CPF degrading bacterial isolates at 25°C at different pH

At 30°C, maximum biomass (OD600) and CFU/mL was observed at pH 6 in only for one bacterial isolate *M. luteus* (OD600; 0.513±0.026, CFU/mL; 1.066 x10^8). As the pH was increased to 7.0 at 30°C, the biomass (OD600) and CFU/mL of *A. kashmirensis*, *P. protegens*, *L. sphaericus* and *E. alkaliphilum* increased significantly ($p < 0.05$) in the highest to the lowest that order (Table 4 and Figure 4). The values were; *A. kashmirensis* (OD600; 0.740±0.021, CFU/mL; 1.520x10^8), *P. protegens* (OD600; 0.622±0.012, CFU/mL; 1.284x10^8), *L. sphaericus* (OD600; 0.612±0.019, CFU/mL; 1.264x10^8) and *E. alkaliphilum* (OD600; 0.461±0.055, CFU/mL; 9.62 x10^7). Therefore, the order of growth the stored CPF degrading bacterial isolates cultured at 30°C and pH 7.0 was *A. kashmirensis* > *P. protegens* > *L. sphaericus* > *E. alkaliphilum* > *M. luteus* >

Branchbacterium spp. (Figure 4). Further at 30°C, as the pH was increased to 8.0 and 9.0, a significant decrease in biomass (OD600) and CFU/mL ($p < 0.05$) was observed in all bacterial isolates (Table 4).

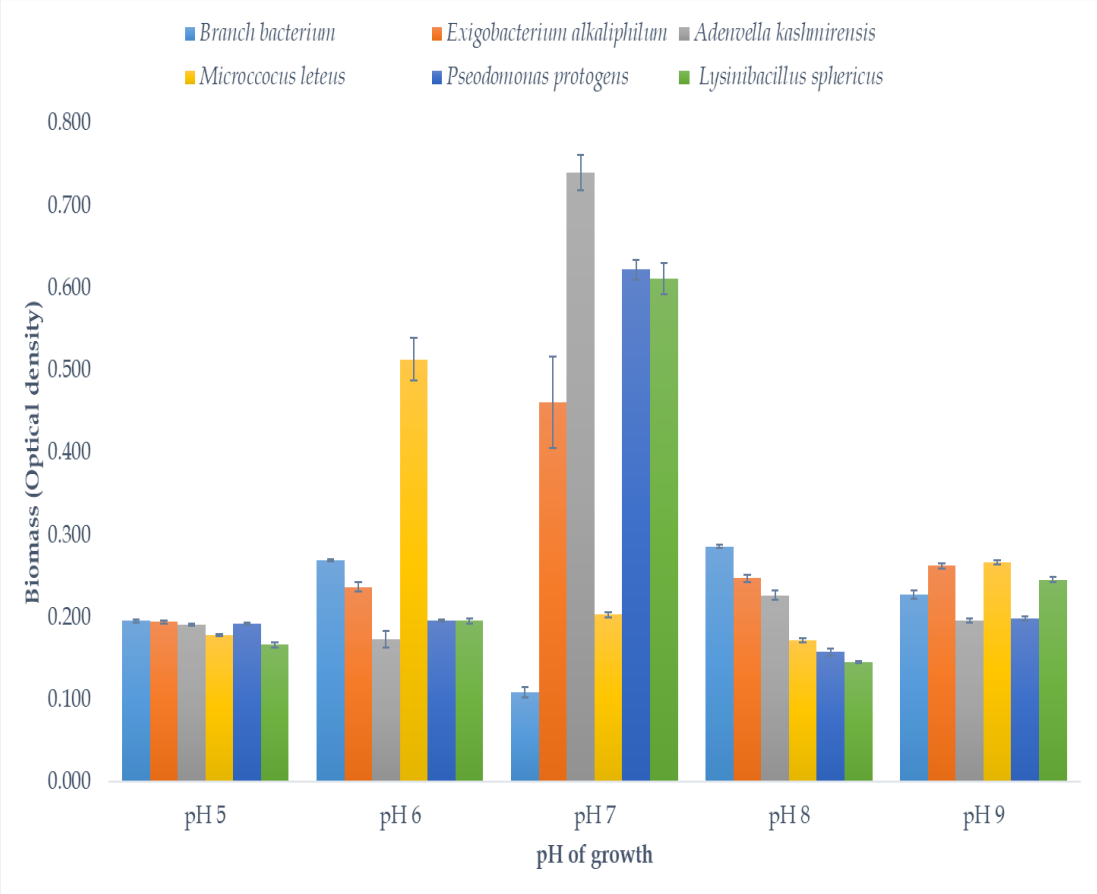

Figure 4. Growth of CPF degrading bacterial isolates at 30°C at different pH

Although the stored CPF degrading isolates showed growth as indicated by the measured biomass (OD600) and CFU/mL at 35°C, it was low compared to that observed at 25°C and 30°C. At 35°C, the maximum biomass (OD600) and CFU/mL was observed at pH 7.0 in all the bacterial isolate except *Branchbacterium spp*. in which the highest biomass was observed at pH 8.0 (Figure 5). The order was *A. kashmirensis* > *M. luteus* > *E. alkaliphilum* > *L. sphaericus* > *P. protegens* > *Branchbacterium spp*. at 35°C and pH 7.0

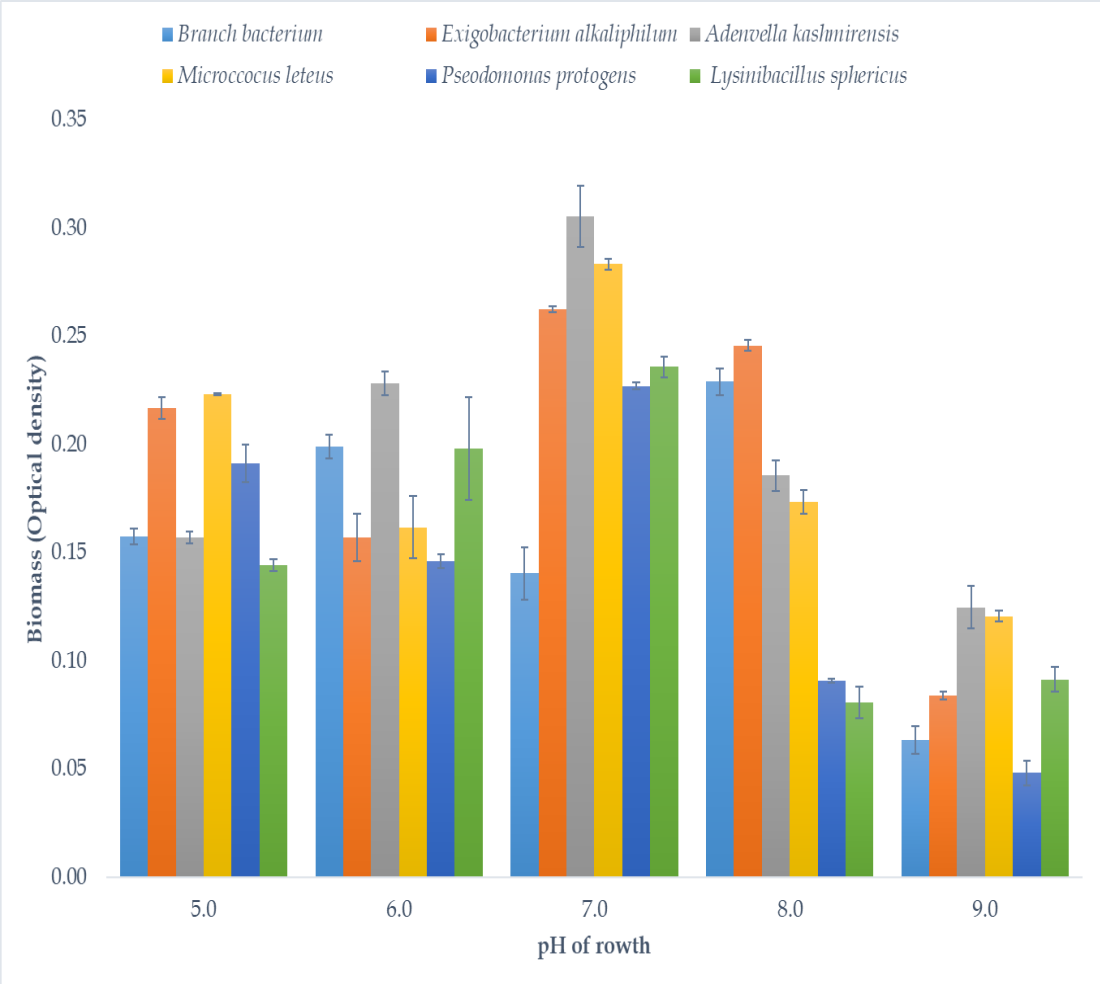

Figure 5. Growth of CPF degrading bacterial isolates at 35oC at different pH

A significant decrease in biomass (OD600) and CFU/mL ($p < 0.5$) was observed as the temperature was increased to 40°C (Table 4, Figure 6). At 40°C, maximum biomass (OD600) and CFU/mL of *E. alkaliphilum*, *P. protegens*, *M. luteus*, and *Branchbacterium spp* were observed at pH 6.0 whereas maximum biomass (OD600) and CFU/mL for *L. sphaericus* and *A. kashmirensis* was observed at pH 7.0 and pH 8.0 respectively (Figure 6). Therefore, the order of growth at 40°C and pH 6.0 was *E. alkaliphilum* >*P. protegens* > *M. luteus* > *Branchbacterium spp* > *A. kashmirensis* > *L. sphaericus*

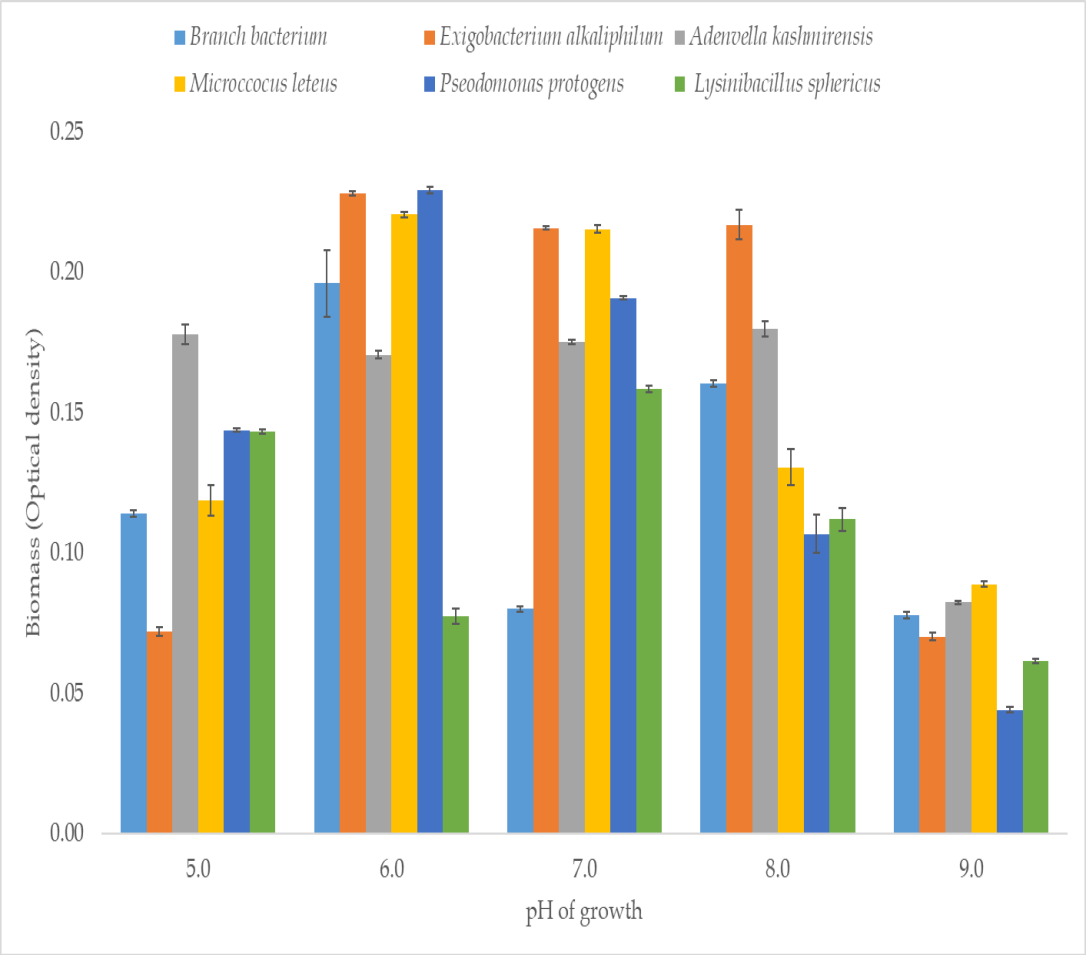

Figure 6. Growth of CPF degrading bacterial isolates at 40°C at different pH

The summary, Biomass (OD600) and CFU/mL at temperature and pH of maximum growth of the stored CPF degrading bacterial isolates is represented in Table 5. Chemical and physical conditions of external environment including temperature, oxygen, pH and osmotic pressure (water availability) may have an effect on microbial growth and survival (Frias et al., 2001; Moral et al., 2017; Rolfe & Daryaei, 2020; Abel & Evivie, 2022; Magar et al., 2022). Magar et al., (2022) noted that the pH has nearly great impact on microbes and their environment as temperature. However, this classification also depends on the environment where the microorganisms grow. Microbes are able to grow in selected range of pH are described by terms analogous to those used for temperature. Acidophilic microbes have been isolated in waters and soils with pH below 5.5, the best pH range of growth for neutrophiles is between 5 to 8 while at the other extreme, alkaliphiles prefer a pH above 8.5 (Magar et al., 2022). As observed in this study (Table 5), the stored CPF degrading bacterial isolates (Atego, 2022) showed growth (OD600 and CFU/mL) between pH 6.0 and pH 7.0 corroborating with study by Magar et al., (2022), thus can be classified as neutrophiles. Furthermore, these bacterial isolates can be regarded as mesophiles as the showed maximum growth (OD600 and CFU/mL) at temperature ranging between 25°C and 40°C (DSMZ, 2015).

Table 5. Biomass (OD600) and colony-forming units (CFU/mL) at temperature and pH of maximum growth of the CPF degrading bacterial isolates

Bacterial isolate	Biomass (OD600nm), CFU/mL, temperature and pH at maximum growth of CPF degrading bacterial isolates											
	25°C			30°C			35°C			40°C		
	pH	OD600	CFU/mL	pH	OD600	CFU/mL	pH	OD600	CFU/mL	pH	OD600	CFU/mL
CP1.	6	0.414^{a}±0.001	8.68x10^{7}	8	0.286^{b}±0.002	6.12 x10^{7}	8	0.229^{b}±0.006	4.98 x10^{7}	6	0.196^{c}±0.012	4.02x10^{7}
CP2	6	0.816^{e}±0.001	16.72x10^{7}	7	0.461^{c}±0.055	9.62 x10^{7}	7	0.269^{d}±0.001	5.78 x10^{7}	6	0.228^{b}±0.001	4.96 x10^{7}
CP3	6	0.890^{c}±0.001	18.20 x10^{7}	7	0.740^{d}±0.021	15.20 x10^{7}	7	0.305^{a}±0.014	6.50 x10^{7}	8	0.180^{e}±0.003	4.00 x10^{7}
CP5	6	0.744^{d}±0.001	15.28 x10^{7}	6	0.513^{e}±0.026	10.66 x10^{7}	7	0.283^{c}±0.002	6.06 x10^{7}	6	0.221^{d}±0.001	2.612x10^{7}
CP6	6	0.795^{f}±0.001	16.3 x10^{7}	7	0.622^{b}±0.012	12.84x10^{7}	7	0.227^{b}±0.002	4.94 x10^{7}	6	0.229^{b}±0.001	4.98 x10^{7}
CP7	6	0.941^{b}±0.001	19.22 x10^{7}	7	0.612^{b}±0.019	12.64 x10^{7}	7	0.236^{e}±0.005	5.12 x10^{7}	7	0.159^{a}±0.001	3.58 x10^{7}

Mean values (n=3) ± SEM. Values appended by different superscript letters within a row and column are significantly different ($P < 0.05$). Key: *Branchbacterium* sp (P1), *Exigobacterium alkaliphilum* (CP2), *Adenvella kashmirensis* (CP3), *Micrococcus leteus* (CP5), *Pseodomonas protogens* (CP6) and *Lysinibacillus sphericus* (CP7)

Microbes are usual selective regarding cultural conditions including carbon and nitrogen source, temperature, pH, salt tolerance and the media (Jamal & Ahmad, 2022). Studies have shown that these conditions greatly influence the growth of different microbial species (Ahmad et al., 2014a; Ahmad et al., 2014b). As described by Atego, (2022), *L. sphaericus* is Gram-positive spore-forming motile bacilli. Further, it is characterized by terminal endosporing, utilization of acetate as the sole source of carbon and lysine and aspartic acid as major component of cell wall peptidoglycan (Ahmed et al, 2007). Growth and isolation of bacterial species in *Lysinibacillus* genus from soil, water, clinical, food samples in several media including Nutrient Agar (NA), Lysogeny broth (LB), Nutrient Broth (NB), Manosa-Rogosa and Sharpe (MRS) Agar, Mineral Salt Medium (MSM) among other media as described by many studies (Smibert & Krieg, 1994; Seiler et al., 2013; Miwa et al, 2009; Wang et al., 2010; Foda et al, 2015; Atego, 2022; Asamba et al., 2022; Wepukhulu et al., 2024). However different growth and development patterns have been observed in these media. As stated by literature sources, *L. sphaericus* can adsorb heavy and toxic metals such as chromium, arsenic lead, mercury and cadmium (Jamal & Ahmad, 2022; Xu et al., 2024; Riaño-Castillo et al., 2024) alongside precious metals (Jarouliya et al., 2024). Functional groups and protein layer are responsible for the initial attachment of these metallic ions followed by binding reactive groups found on the bacterial cell wall and finally internalization of metallic ions inside the bacterial cell (Slavin et al, 2017). The growth of *L. sphaericus* at 25^{o}C and pH 6.0 (OD600; 0.941±0.001, CFU/mL; 1.922 x10^{8}) and at 30^{o}C and pH 7.0 (OD600; 0.612±0.019, CFU/mL; 1.264 x10^{8}) corroborates with previous studies by Temirbekova et al., (2023) where *L. sphaericus* isolated from poultry waste showed growth under similar conditions as describe in this study.

As reported by Jamal & Ahmad, (2022), *Lysinibacillu*s grow maximumly at pH between 6 and 10, contrasting with the findings of this study where maximum growth occurred between pH 6 - 8. Being a mesophilic bacterium, Lysinibacilli has been reported to grow well between 30°C - 37^{o}C, moderately between 20°C - 40^{o}C and least below 20^{o}C and above 40^{o}C (Ahmad et al., 2014a; Ahmad et al., 2014b; Jamal & Ahmad, 2022). The causes of minimal growth at low temperatures may be due to low solubility, nutrients diffusion constrains and slow rate of reaction (Jamal & Ahmad, 2022). Salt tolerance is another cultural characteristic that may have effects on microbial growth since sodium ion has been implicated to play a crucial role in cellular osmoregulation and amino acids transport (Jamal & Ahmad, 2022). Although salt tolerance was not investigated in this study, report by Jamal & Ahmad, (2022) indicate that maximum growth of *Lysinibacillus* can be achieved in culture media supplemented with 1 – 2% sodium chloride (NaCl).

Studies have revealed that *M. luteus* is aerobic with maximal growth at temperature range 25°C to 45°C on a various media such as Agar, Tryptic Soy, Standard Methods Agar, Nutrient Agar, Nutrient Broth and Sheep Blood Agar (Kloos et al., 1974; Sims et al., 1986; Kaprelyants & Kell, 1993; Kocur et al., 2006; DSMZ, 2015). The observed maximum growth of *M. luteus* at pH 6.0 at 25^{o}C (OD600; 0.744±0.001, CFU/mL; 15.28 x10^{7}) and 30^{o}C (OD600; 0.513±0.026, CFU; 10.66 x10^{7}) is in line with DSMZ, (2015) that it is a mesophilic aerobic bacterium. Further, Kaprelyants & Kell, (1993) reported the copiotrophic nature of *M. luteus*. However, Dib et al., (2013) reported that *M. luteus* can survive in oligotrophic conditions. The ability of *M. luteus* to form non-endospore dormant structures enable it to survive hash conditions.

The survival *Micrococcus luteus* in hash environmental conditions including extremely low temperature (4°C), humidity (2.5%), nutrients (starvation) and moisture (dryness) has also been reported (Kaprelyants et al., 1993; Kaprelyants & Kell, 1993; Votyakova et al. 1994; Mukamolova et al. 1995, Mukamolova et al., 2002; Mukamolova et al., 2006; Dib et al. 2008; Ordonez et al., 2009). The formation of non-endospore dorminant structures as reported by Dib et al., (2008) and Ordonez et al., (2009) could be the reason of *M. luteus* adaptation to extreme environments. Although extremely conditions as described by other studies were not investigated in this study, it was able to thrive well in all ranges of temperature (25°C - 40°C) and pH (5 – 9) that were investigated. Due to its ubiquitous in the environment, *M. luteus* has been isolated from several environments (Raju et al., 2023) not

limited to soil (Sims et al., 1986; Biskupiak et al. 1988; kutmutiaet al,2019; Atego, 2022), biofilm of freshwater tank (Rickard et al. 2003), drinking water (Rusin et al., 1997), waste water and contaminated sites (Wieser et al., 2002; López et al. 2005; Zheng et al. 2009; Atego, 2022; Wepukhulu et al., 2024), indoor air (Wieser et al. 2002), sea surface microlayers of polluted waters (Agogué et al. 2005), Surface dust (Wieser et al., 2002; Gu, 2007) food and food products (Addis et al. 2001; Prado et al. 2001; García-Fontán et al. 2007; Pittet et al. 2010; Asamba et al., 2022; Atego, 2022), medical equipments (Marinella et al., 1997; Powell et al., 2003; Purmal et al., 2010; Tambekar et al., 2008), drugs and medical products (US-FDA, 2006) and body surfaces and internal organs of animals and plants (Kloos et al., 1974; Kloos & Musselwhite, 1975; Kloos et al., 1976; Abd El-Rhman et al., 2009; Lampert et al., 2006; Bultel-Poncé et al., 1998; Sezen et al., 2005; Altalhi, 2009). Therefore, *M. luteus* among other bacterial isolates investigated in this study can be a good source of bacterial consortium for bioremediation of CPF.

Optimal conditions of consortia of bacterial isolates for chlorpyrifos degradation

Five consortia groups from the six bacterial isolates (Table 1) isolated and characterized by Atego, (2022) each comprising of three bacterial isolates except Group I which had all the six bacterial isolated were developed based on their CPF degradation capability (Atego, 2022) as outlined in Table 2 and optimal growth temperature and pH determined (Table 6 and Figures 7, 8, 9 and 10). The best combination where maximum biomass (OD600nm) in different temperature and pH was observed in the bacterial isolates in Group I, III and IV (Table 6 and Table 7).

Generally, a significant decrease or increase in the rate of growth or biomass (OD600) and CFU/mL ($p < 0.05$) of the developed bacterial consortia groups with increase in in temperature and pH was observed (Table 6 and 7, Figures 7, 8, 9 and 10). Further, a variation in growth rate (increase or decrease in biomass (OD600) and CFU/mL was observed with increasing temperature and pH. At 25°C the highest biomass (OD600) was observed Group I and IV. Group I bacterial isolates achieved highest biomass (OD600) and CFU/mL of 0.444±0.005 ($9.28x10^7$ CFU/mL) and 0.457±0.006 ($9.54 x10^7$ CFU/mL) at pH 5 and 7 respectively, in Group IV highest biomass (OD600) and CFU/mL was 0.427±0.009 ($8.94x10^7$ CFU/mL) at pH 5 whereas Group II, II and V had poor rate of growth exhibited by lowest biomass (OD600) and CFU/mL in all the pH ranges (5, 6, 7, 8 and 9) that were investigated (Table 6 and Figure 7).

Table 6. Biomass (OD600) and CFU/mL of developed CPF degrading bacterial consortia cultured under different pH and temperature (°C)

Consortium	Temperature	Biomass and CFU of CPF degrading consortia of bacterial isolates at different temperatures and pH different									
		pH 5		pH 6		pH 7		pH 8		pH 9	
		OD600	CFU/mL	OD600	CFU/mL	OD600	CFU/mL		CFU/mL		CFU/mL
Group I	25°C	0.444^{a}±0.005	9.28x10^{7}	0.228^{c}±0.009	4.96 x10^{7}	0.457^{a}±0.006	9.54 x10^{7}	0.339^{d}±0.009	7.18 x10^{7}	0.263^{b}±0.007	5.66 x10^{7}
Group II	25°C	0.193^{h}±0.003	4.26x10^{7}	0.284^{e}±0.002	6.08x10^{7}	0.213±0.009	4.66 x10^{7}	0.264^{e}±0.005	5.68 x10^{7}	0.180^{h}±0.006	4.00 x10^{7}
Group III	25°C	0.325^{c}±0.007	6.90x10^{7}	0.277^{e}±0.009	5.94x10^{7}	0.324^{c}±0.005	6.88 x10^{7}	0.324^{c}±0.003	6.88 x10^{7}	0.282^{b}±0.009	6.04 x10^{7}
Group IV	25°C	0.427^{a}±0.009	894x10^{7}	0.205^{g}±0.007	4.50x10^{7}	0.340^{c}±0.010	7.20 x10^{7}	0.361^{c}±0.005	7.62 x10^{7}	0.235^{a}±0.007	7.40 x10^{7}
Group V	25°C	0.152^{h}±0.008	3.44x10^{7}	0.297^{e}±0.007	6.34x10^{7}	0.244±0.005	5.28 x10^{7}	0.273^{e}±0.008	5.86 x10^{7}	0.220^{a}±0.007	4.80 x10^{7}
Group I	30°C	0.453^{a}±0.005	9.46x10^{7}	0.676^{d}±0.006	13.92x10^{7}	0.297±0.003	6.34 x10^{7}	0.489^{ab}±0.003	11.08x10^{7}	0.261^{b}±0.003	5.62 x10^{7}
Group II	30°C	0.244^{g}±0.004	5.28x10^{7}	0.265^{f}±0.009	5.70x10^{7}	0.228±0.008	4.96 x10^{7}	0.124^{i}±0.007	2.88 x10^{7}	0.310±0.009	6.60 x10^{7}
Group III	30°C	0.314^{c}±0.006	6.68x10^{7}	0.510^{b}±0.006	10.60x10^{7}	0.318^{c}±0.002	6.76 x10^{7}	0.405^{a}±0.002	8.50 x10^{7}	0.270^{d}±0.006	5.80 x10^{7}
Group IV	30°C	0.437^{a}±0.003	9.24 x10^{7}	0.532^{b}±0.006	11.04x10^{7}	0.412^{ad}±0.008	8.64 x10^{7}	0.909^{c}±0.003	18.58x10^{7}	0.237^{e}±0.005	5.14 x10^{7}
Group V	30°C	0.370^{c}±0.002	7.80 x10^{7}	0.432^{a}±0.002	9.04 x10^{7}	0.241^{e}±0.002	5.22 x10^{7}	0.285^{e}±0.002	6.10 x10^{7}	0.310^{c}±0.007	6.60 x10^{7}
Group I	35°C	0.274^{e}±0.005	5.88 x10^{7}	0.130^{i}±0.008	3.00 x10^{7}	0.264^{e}±0.009	5.68 x10^{7}	0.274^{e}±0.007	5.88 x10^{7}	0.185^{h}±0.006	4.10 x10^{7}
Group II	35°C	0.582^{b}±0.006	12.04x10^{7}	0.416^{a}±0.006	8.72 x10^{7}	0.270^{e}±0.009	5.80 x10^{7}	0.243^{f}±0.004	5.26 x10^{7}	0.1540^{d}.004±	3.48 x10^{7}
Group III	35°C	0.370^{c}±0.004	7.80 x10^{7}	0.204^{g}±0.009	4.48 x10^{7}	0.289^{i}±0.007	6.18 x10^{7}	0.243^{f}±0.005	5.26 x10^{7}	0.215^{g}±0.004	4.70 x10^{7}
Group IV	35°C	0.489^{a}±0.003	10.18x10^{7}	0.288^{e}±0.004	6.16 x10^{7}	0.257^{e}±0.005	5.54 x10^{7}	0.216^{g}±0.004	4.72 x10^{7}	0.200^{g}±0.005	4.40 x10^{7}
Group V	35°C	0.534^{b}±0.004	11.08x10^{7}	0.530^{b}±0.009	11.00x10^{7}	0.188^{h}±0.004	4.62 x10^{7}	0.220^{g}±0.005	4.80 x10^{7}	0.166^{d}±0.008	3.72 x10^{7}
Group I	40°C	0.219^{d}±0.007	4.78 x10^{7}	0.195^{h}±0.005	4.30 x10^{7}	0.211^{b}±0.003	4.62 x10^{7}	0.197^{h}±0.003	4.34 x10^{7}	0.054^{e}±0.007	1.48 x10^{7}
Group II	40°C	0.258^{f}±0.008	5.56 x10^{7}	0.251^{f}±0.006	5.42 x10^{7}	0.204^{g}±0.004	4.48 x10^{7}	0.198^{h}±0.003	4.36 x10^{7}	0.090^{e}±0.009	2.20 x10^{7}
Group III	40°C	0.242^{g}±0.004	5.24 x10^{7}	0.185^{h}±0.009	4.10 x10^{7}	0.197^{h}±0.004	4.34 x10^{7}	0.171^{h}±0.006	3.82 x10^{7}	0.023^{i}±0.008	0.86 x10^{7}
Group IV	40°C	0.316^{c}±0.007	6.72 x10^{7}	0.218^{c}±0.003	4.76 x10^{7}	0.176^{h}±0.005	3.92 x10^{7}	0.137^{i}±0.007	3.14 x10^{7}	0.045^{i}±0.006	1.30 x10^{7}
Group V	40°C	0.302^{c}±0.002	6.44 x10^{7}	0.225^{c}±0.007	4.90 x10^{7}	0.204^{g}±0.008	4.48 x10^{7}	0.111^{i}±0.009	2.62 x10^{7}	0.074^{e}±0.007	1.88 x10^{7}

Mean values (n=3) ± SEM. Values appended by different superscript letters within a row and column are significantly different ($P < 0.05$).

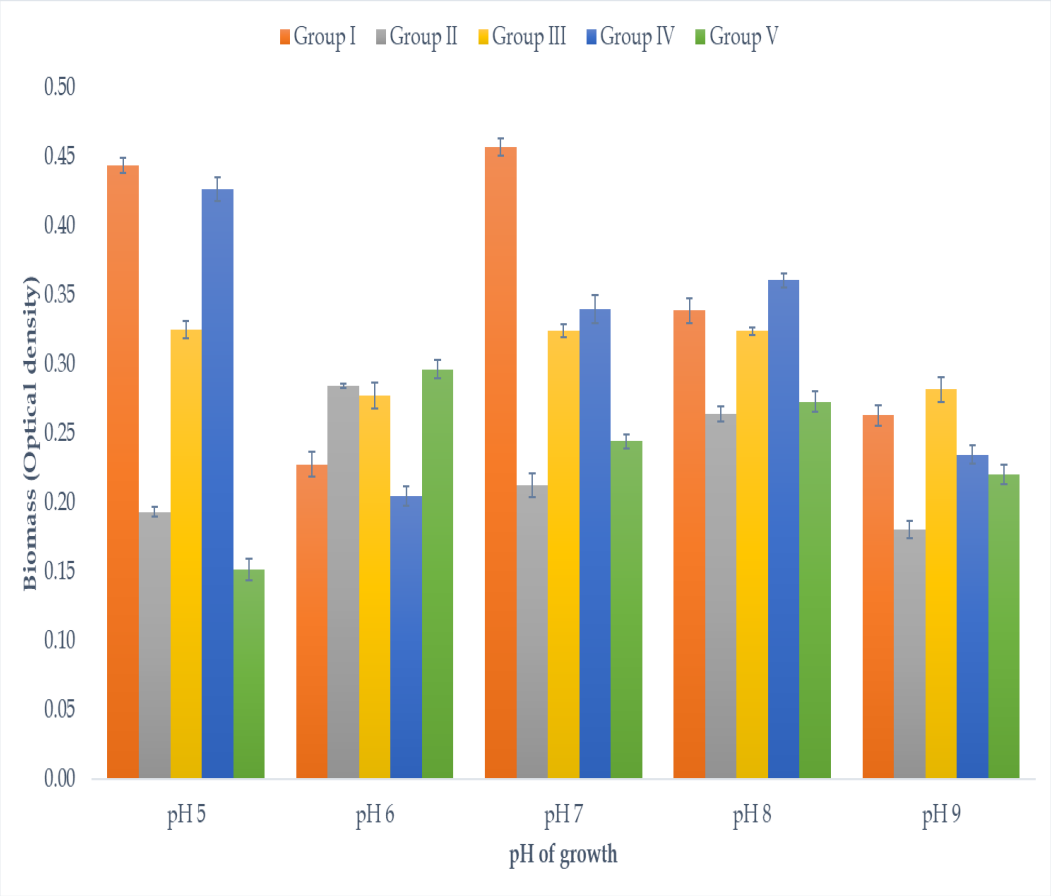

Figure 7. Growth of developed consortium of CPF degrading bacteria at 25°C at different pH

The maximum growth of almost all of the developed CPF degrading consortia groups were observed after incubation at 30°C in different pH conditions. However, Group I, III, IV and V achieved the highest biomass (OD600) and CFU/mL. In Group I, the observed Biomass (OD600) was at 0.453±0.005 (9.46x10^7 CFU/mL), 0.676±0.006 (13.92x10^7 CFU/mL) and 0.489±0.003 (11.08x10^7 CFU/mL) at pH 5, 6 and 8 respectively. Group III bacterial isolates incubated at 30°C grew well at pH 6 and 8 with biomass (OD600) and CFU/mL of 0.510±0.006 (10.60x10^7 CFU/mL) and 0.405±0.002 (8.50 x10^7 CFU/mL) respectively. Bacterial isolates in Group IV had the highest biomass (OD600) and CFU/mL of 0.437±0.003 (9.24 x10^7 CFU/mL), 0.532±0.006 (11.04x10^7 CFU/mL), 0.412±0.008 (8.64 x10^7 CFU/mL) and 0.909±0.003 (18.58x10^7 CFU/mL) at pH 5, 6, 7 and 8 respectively while Group V bacterial isolate had the highest biomass (OD600) and CFU/mL of 0.432±0.002 (9.04 x10^7 CFU/mL) at pH 6 (Table 6 and Figure 8).

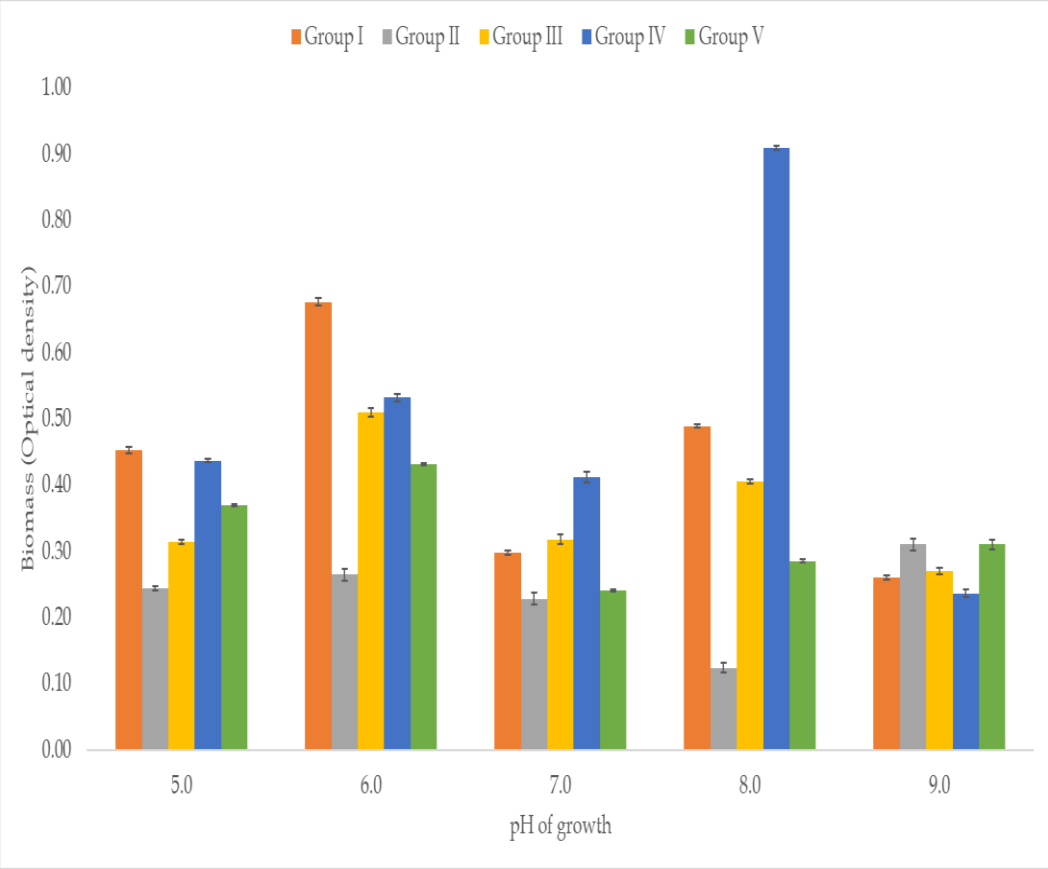

Figure 8. Growth of developed consortium of CPF degrading bacteria at 30°C at different pH

At 35°C bacterial isolates in Group II, IV and V observed the maximum biomass (OD600) and CFU/mL at different pH (Figure 4.9). The bacterial isolates in Group II biomass (OD600) and CFU/mL were 0.582±0.006 ($12.04x10^7$ CFU/mL) and 0.416±0.006 ($8.72 x10^7$ CFU/mL) at pH 5 and 6 respectively. In Group IV the highest biomass (OD600) and CFU/mL was 0.489±0.003 ($10.18x10^7$ CFU/mL) at pH 5 whereas biomass (OD600) and CFU/mL of 0.534±0.004 ($11.08x10^7$ CFU/mL) and 0.530±0.009 ($11.00x10^7$ CFU/mL) were observed in Group V at pH 5 and 6 respectively (Table 6 and Figure 9) At 40°C, a significant decrease in biomass (OD600) and CFU/mL ($p < 0.05$) was observed in bacterial isolate for all groups (Table 6 and Figure 10) with the highest biomass (OD600) and CFU/mL observed only at pH 5 in all groups.

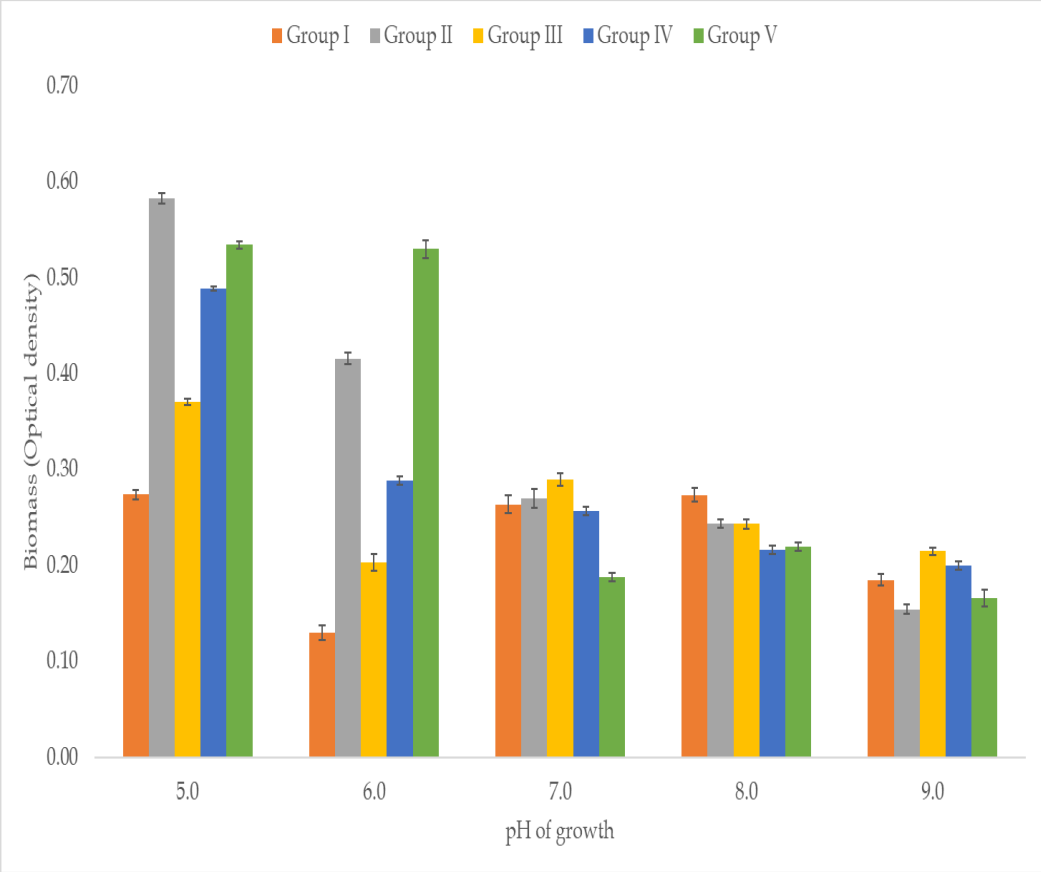

Figure 9. Growth of developed consortium of CPF degrading bacteria at 35°C at different pH

After culturing and growth of the developed consortia of the CPF degrading bacterial isolates at different pH (5, 6, 7, 8 and 9) and temperature (25˚C, 30˚C, 35˚C and 40˚C), the optimum pH and temperature for the growth of each the bacterial consortium were determined and established (Table 7). The summary of biomass (OD600) and CFU/mL at temperature and pH of maximum growth of the developed CPF degrading bacterial consortia is represented in Table 7. From pH and temperature optimization for the growth of the stored and revived CPF degrading bacterial isolates (Table 5) and bacterial consortia (Table 7), Group I, III and IV had the highest biomass (OD600) and CFU/mL at 25ºC and 30ºC culture at pH 6, 7 and 8. Therefore, the general trend indicated that optimal pH 6 and temperature 30ºC this is where bacterial consortia yielded highest growth

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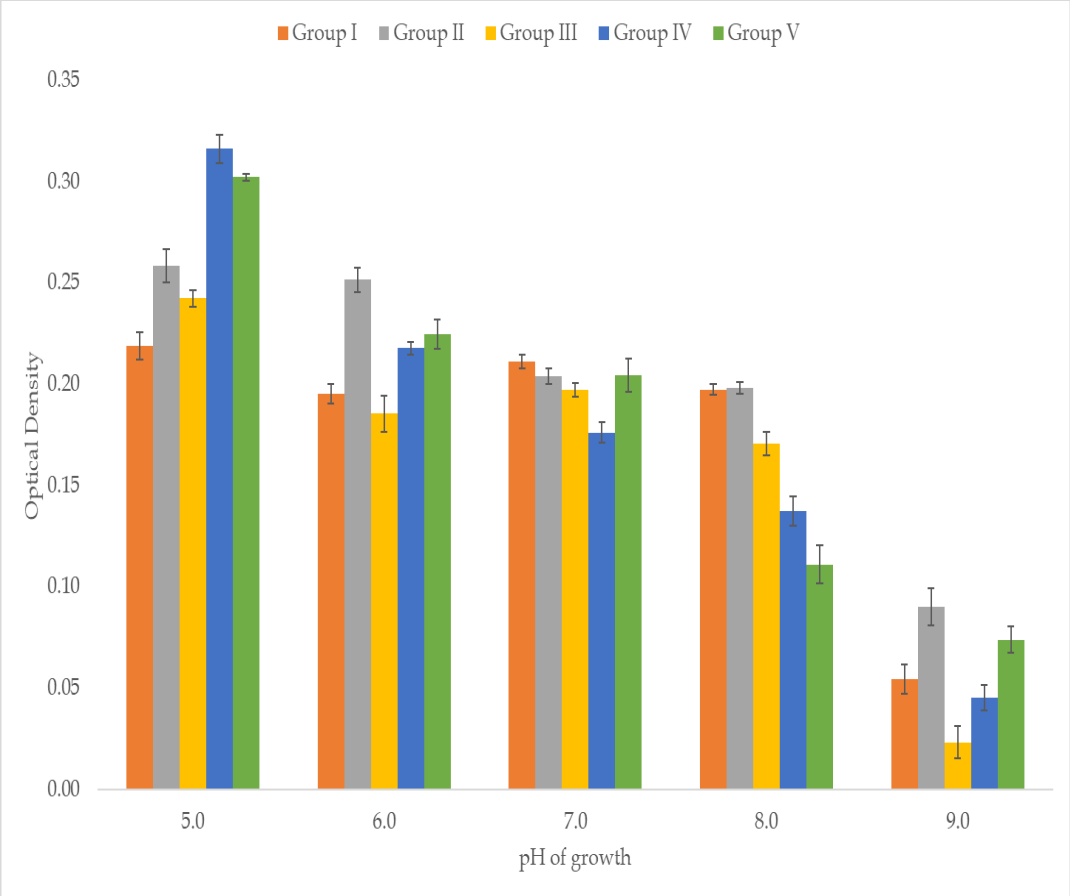

Figure 10. Growth of developed consortium of CPF degrading bacteria at 40°C at different pH.

Table 7. Biomass (OD600) and colony-forming units (CFU/mL) at temperature and pH of maximum growth of the CPF degrading bacterial isolates consortia

Consortium	Biomass (OD600), CFU/mL and pH of maximum growth of bacterial isolates consortia at different temperatures											
	25˚C			30˚C			35˚C			40˚C		
	Ph	OD600	CFU/mL	pH	OD600	CFU/mL	pH	OD600	CFU/Ml	PH.	OD600	CFU/mL
Group I	7	0.457^{b}±0.006	9.54 $x10^{7}$	6	0.676^{c}±0.006	13.92 $x10^{7}$	5	0.274^{a}±0.005	5.88 $x10^{7}$	5		4.78$x10^{7}$
Group II	6	0.284^{a}±0.002	6.08 $x10^{7}$	9	0.310^{a}±0.009	6.60 $x10^{7}$	5	0.582^{b}±0.006	12.04 $x10^{7}$	5	0.219^{d}±0.007	5.56 $x10^{7}$
Group III	5	0.325^{c}±0.007	6.90 $x10^{7}$	6	0.510^{b}±0.006	10.60 $x10^{7}$	5	0.370^{d}±0.004	7.80 $x10^{7}$	5	0.258^{e}±0.008	5.24 $x10^{7}$
Group IV	5	0.427^{b}±0.009	8.94 $x10^{7}$	8	0.909^{d}±0.003	18.58 $x10^{7}$	5	0.489^{c}±0.003	10.18 $x10^{7}$	5	0.242^{a}±0.004	6.72 $x10^{7}$
Group V	5	0.297^{a}±0.007	6.34 $x10^{7}$	6	0.432^{e}±0.002	9.04 $x10^{7}$	5	0.534^{d}±0.004	11.08 $x10^{7}$	5	0.316^{c}±0.007	6.44 $x10^{7}$
											0.302^{b}±0.002	

Mean values (n=3) ± SEM. Values appended by different superscript letters within a row and column are significantly different ($P < 0.05$).

Many studies have reported degradation of pollutants by single bacterial isolates such as azo dyes by *Staphylococcus warneri* (Stingley et al., 2010), chlorpyrifos, endosulfan, OPs nerve agents, p-nitrophenol, TCP among other OPCs using different bacterial isolate not limited to *Branchbacterium sp., Pseudomonas putida*, *Exiguobacterium alkaliphilum, Advenella kashmirensis, Micrococcus luteus, Pseudomonas protegens, Lysinibacillus sphaericus* among othe bacterial genera (Lei et al., 2005, Pradeep & Subbaiah, 2012, Ajaz et al., 2012 Atego, 2022, Asamba et al., 2022, Wepukhulu et al., 2024) and tetrachlorvinphos by *Stenotrophomonas maltophilia* (Barragan-Huerta et al., 2007; Fulekar, 2014, Somaraja et al., 2013, Ortiz-Hernández & Sánchez-Salinas, 2010). The ability of three or more bacteria isolates in pollutants degradation could be harnessed by utilizing them in a consortium. Further, it has been shown that bacteria communities are effective in bioremediation due to diversity of biochemical reactions (Bernstein & Carlson, 2012; Bernstein et al., 2012; Khan et al; .2021). For instance, bacterial consortium made up of *Bacillus pumilus* and *Staphylococcus warneri* has been used in the degradation of polycyclic aromatic hydrocarbons (PAHs) including Pyrene (PYR), Benzo[a]anthracene (BaA) and Phenanthrene (PHE) (Moscoso et al., 2012; Moscoso et al., 2013). Further, ability to degrade pollutants by bacterial consortium has been demonstrated by Pino & Penuela, (2011) and Pino et al., (2011) who constructed a bacteria consortium comprising of *Pseudomonas putida*, *Pseudomonas aeruginosa, Acinetobacter sp.*, *Citrobacter* and *Bacillus* sp., with ability to degrade p-nitrophenol, methyl parathion, and chlorpyrifos.

In addition, bacteria consortia are more resistance to changes in the environment (Burmølle et al., 2006). Stability and functions of bacterial consortia are influenced by microbial interaction such as mutualism and commensalism (Faust & Raes, 2012). These results differ from previous studies on consortia degrading chlorpyrifos, where the optimum temperature was 30°C (Varghes et al., 2021). In the optimization of bacterial consortium PBAC, the optimum temperature was found to be 35°C (Jabeen et al., 2015a). A bacterial consortium ECO-M, was found to have an optimum temperature of 30°C (Uniyal *et al*., 2021), while consortium *Bacillus amyloliquefaciens* CP28 had an optimum temperature of 30°C (Varghes et al., 2021). Another bacterial consortium, BDAM, had an optimum temperature of 40°C (Ahmad et al., 2018).

The optimum pH of all consortia was found to be 6. These results differ from previous studies on the bacterial consortium PBAC, which had an optimum pH of 6.8 (Jabeen et al., 2015b). Consortium C5 had an optimum pH of 7 (John et al., 2016). Another bacterial consortium, BDAM, had an optimum pH of 8 (Ahmad et al., 2018). These results highlight the diverse environmental preferences and adaptability of bacterial species. Microbial consortium are not fully utilized due to inadequate knowledge on optimum growth conditions. Therefore, this provides optimum growth condition for exploiting the potential of consortia for better degradation of chlorpyrifos.

In this study five consortia were constituted from six CPF degrading bacterial isolates namely; *Branchbacterium sp.* (CP1), *Exiguobacterium alkaliphilum* (CP2), *Advenella kashmirensis* (CP3), *Micrococcus luteus* (CP5), *Pseudomonas protegens* (CP6) and *Lysinibacillus sphaericus* (CP7). The composition of the consortia includes Group I (*Branchbacterium sp., Exiguobacterium alkaliphilum, Advenella kashmirensis, Micrococcus luteus, Pseudomonas protegens* and *Lysinibacillus sphaericus*) Group II (*Branchbacterium sp., Advenella kashmirensis* and *Micrococcus luteus*), Group III (*Exiguobacterium alkaliphilum, Lysinibacillus sp.,* and *Lysinibacillus sphaericus*), Group IV (*Exiguobacterium alkaliphilum, Pseudomonas protegens* and *Lysinibacillus sphaericus*) and Group V (*Advenella kashmirensis*, *Lysinibacillus sphaericus* and *Pseudomonas protegens*) based on CPF degradation rate as reported by Atego, (2022).

In all of the five developed consortia, an escalated growth inform of biomass (OD600) and CFU/mL was observed. Although conventional soil enrichment technique regarded the gold standard in isolation of microbes in soil with different physico-chemical conditions (Nair et al., 2014, John et al., 2015; Wepukhulu et al., 2024; Atego, 2022) was used to isolate the bacterial isolates in this study, pH and temperature

optimazation perfrormed before the bacterial consortia were constituted. As reported by Krishna & Philip, (2008), the co-metabolism of toxic compounds can not be achieved by using a single microbial species. In this study, the efficacy of microbial consortia in co-metabolism of CPF, a toxic OPs based compound was conducted. Further, the makeup of the constituted bacterial consortium is believed to play a crucial role in determining the ability to degrade the toxic compounds attributing to the overall degradation percentage (Krishna & Philip, 2008). Compatibility and physiological relationship among individual members of the bacteria consortium allow them to take less time to degrade popllutants when compared to single cultures (John et al., 2016; Uniyal et al., 2021). As reported by Pino et al., (2011), the origin and degree of adaptation of the members of the bacterial consortium highly influences the degradation ability of pollutants. Given that the bacteria used to constitute the bacterial consortion in this study come from different samples; soil, water, spray race residues, dip wash, milk and milk products collected from different environment ranging, they can be regarded as good source of innoculum to degrade toxic compounds. It can be hypothesized that the more types of microbial species with different degradation abilities, the greter the chances of synthesizing a variety of enzymes that released in the medium hence more metabolic capacity to degrade pesticides. Thus, degradation of OPs using consortium of microbes is more effectiently than single cultures cultures (VerBerkmoes, 2009; John et al., 2016; Uniyal et al., 2021).

Conclusion

This study constructed bacteria consortia (group I, II, III, VI, and V) and optimized their growth conditions for bioremediation of chlorpyrifos polluted environment. The result obtained in this study revealed that the optimum temperature and pH for the growth of these chlorpyrifos degrading bacterial isolates are 25^{o}C and pH 6.0. Whereas that of the consortia is 30^{o}C and pH 6.0. Group I, III and IV achieved the highest biomass. If they can be immobilized in a bioreactor at optimum conditions, they can be used as an ideal tool for cleaning up chlorpyrifos contaminated environment as consortia degrade the pesticides completely and more efficiently. Furthermore, they be utilized for enzymes extraction.

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