INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Tools and Techniques for Assessing Recovery Potential of Aerobic Sludge Biomass Stressed with Copper(II), Amoxicillin IV, Chlorpyrifos, and Piggery Wastewater

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Thesis Submitted to the Department/ Center	:	Centre for the Environment
Date of completion of Thesis Viva-Voce Exam	:	25.05.2022
Key words for description of Thesis Work	:	Aerobic sludge biomass; Copper; Amoxicillin; Chlorpyrifos, Piggery wastewater; COD; SVI; ISV; Aerobic sludge biomass activity; Biomass morphology; Enzyme activity; Cell viability; Filamentous biomass; ARDRA

SHORT ABSTRACT

Abstract

The biological processes are widely used for domestic and industrial wastewater treatment to remove the organic matter before its discharge to the natural environment. Various human and industrial activities add undesirable organic and inorganic pollutants (such as heavy metals, pharmaceuticals, agrochemicals, and others) to wastewater, which find their way to the sewage treatment plants. Microorganisms play an essential role in the biological process of sewage treatment. It degrades organic matter present in sewage and utilizes the energy for synthesis of new cells. The undesirable pollutants in wastewater are likely to impart detrimental effects to microorganisms resulting in hindrance in treatment efficiency or sometimes even shut down the operation. Therefore, studies are required to evaluate the behavior, characteristics, and activity of aerobic sludge biomass in presence of undesirable pollutants — such as a heavy metal, an antibiotic, a pesticide, and piggery wastewater. The three fold objective of this experimental investigation are to (i) assess impact of stress imposed by undesirable pollutant on aerobic sludge biomass, (ii) assess the recovery potential of stressed aerobic sludge biomass after discontinuation of stress-causing undesirable pollutant, and (iii) identify the tools and techniques suitable for field application in assessing the health status of aerobic sludge biomass.

The seed biomass has been collected from a facultative lagoon of the sewage treatment plant of IIT Guwahati, Assam, India. It has been used to cultivate aerobic sludge biomass in a growth phase reactor of 1 L capacity for 30 d and thereafter maintained in a mother reactor of 6 L capacity from 31th d onward till the end of the investigation. The experiments have been carried out in batch reactors with a capacity of 1 L by added sufficient amount of aerobic sludge biomass from the mother reactor. The reactors have been fed with stress-causing pollutant along with carbon source. The reactors have been operated at ambient conditions without pH and temperature control for two cycles per day with fill phase of 10-20 min, react phase of 670-690 min, settling phase of 10-20 min, decant phase of 5-10 min, and wash phase of 10-20 min with a total time period of 12 h. The reactors have been labeled as R_{xxyy}, where R stands for reactor; xx stands for stress-causing pollutant [e.g., copper (Cu), amoxicillin IV (AMX), chlorpyrifos (CPS), and screened and raw piggery wastewater (s and r)]; and yy stands for concentration (mg/L) of stress-causing pollutant. For example, R_{Cu10} indicates that reactor has been fed with 10 mg/L of Cu(II), while R_{Cu0} indicates 0 mg/L of Cu(II) in the feed and this reactor is termed as control reactor. The reactor operation has been carried out in two phases. In the first phase, reactors have been fed with varying concentrations of stress-causing pollutants along with the carbon source and operated till steady-state performance has been observed — this phase of operation has been termed as *stressed phase operation*. Thereafter addition of stress-causing pollutants in the feed has been

discontinued and reactors have been fed with only carbon source and operated till steady-state performance has been observed — this phase of operation has been termed as *recovery phase operation*. The duration of both the phases has been chosen based on the steady-state chemical oxygen demand (COD) removal efficiencies. The initial and effluent sample analysis have been carried out to assess environmental related parameters (room temperature, initial pH, and initial concentration of stress-causing pollutant), reactor performance related parameters (effluent pH, residual concentration of stress-causing pollutant, COD, and alkalinity), biomass related general parameters [mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), interface settling velocity (ISV), and aerobic sludge biomass activity (ASBA)] and microbiological parameters [microscopic and photographic observations, filament index (FI); cell viability test; polysaccharides (PS), protein (PN), extracellular polymeric substances (EPS) contents; enzyme activity test viz. catalase ($E_{Catalase}$), dehydrogenase ($E_{Dehydrogenase}$), protease ($E_{Protease}$), urease (E_{Urease}), and amplified ribosomal DNA restriction analysis (ARDRA)].

The impact of copper metal [Cu(II)] stress on aerobic sludge biomass has been studied with four different Cu(II) concentrations (10, 20, 40, and 80 mg/L). The reactors have been operated for 0-25 d with Cu(II) in the feed (termed as stressed phase operation) and then another 29 d without Cu(II) in the feed (termed as recovery phase operation). COD removal of Cu(II) fed reactors have decreased with increase in Cu(II) doses. The reactors R_{Cu10}, R_{Cu20}, R_{Cu40}, and R_{Cu80} have shown 75%, 62%, 49%, and 39% of COD removal, respectively, compared to 91% for control reactor R_{Cu0} during steady-state condition of stressed phase operation. The Cu(II) retention by aerobic sludge biomass of reactors R_{Cu10}, R_{Cu20}, R_{Cu40}, and R_{Cu80} have been observed as 42%, 26%, 10%, and 3%, respectively, at steady-state condition. In addition, presence of Cu(II) in feed has majorly affected SVI and ISV for aerobic sludge biomasses. The Cu(II) exposure to the biomasses has resulted in disintegration of flocs structure, and a significant reduction in filaments has been observed. ASBA and enzyme activities have been drastically inhibited due to Cu(II) stress. The presence of Cu(II) in the feed to the reactor biomasses have caused significant reduction in microbial community structure similarities between control and Cu(II) fed reactors in the range of 38-71%. During recovery phase operation, reactors R_{Cu10} and R_{Cu20} have shown improvement in COD removals with no recovery symptoms observed for reactor R_{Cu40} and R_{Cu80} biomasses. The reactors R_{Cu10}, R_{Cu20}, R_{Cu40}, and R_{Cu80} have shown 82%, 72%, 59%, and 41% of COD removal, respectively, in comparison to 91% for control reactor R_{Cu0} during the steady-state condition of recovery phase operation. A low SVI and high ISV with good COD removal have been obtained for reactor R_{Cu10} and R_{Cu20} biomasses. The increased ASBA values have indicated recovery for reactor R_{Cu10} and R_{Cu20} biomasses, whereas a much slower recovery have been observed for R_{Cu40} and R_{Cu80} biomasses. All four enzymes have shown partial recovery for reactor R_{Cu10} and R_{Cu20} biomasses, whereas $E_{Catalase}$ and E_{Urease} have not recovered at all for reactor R_{Cu40} and R_{Cu80} biomasses. The reactor R_{Cu10} and R_{Cu20} biomasses have shown recovery in microbial community structure with similarity in the range of 80-86%. On the other hand, reactor

R_{Cu40} and R_{Cu80} biomasses have shown slow and partial recovery in the microbial community structure with similarity in the range of 61-76%. The reactor performance, aerobic biomass activity, and biomass morphology have been affected due to stress caused by Cu(II) resulting in incomplete recovery of biomass. Cu(II) doses at 40 and 80 mg/L have caused severe impact on reactor performance and biomass morphology leading irreversible changes.

The addition of amoxicillin IV (AMX) stress to the reactors has influenced the performance, flocs, and microbial community structure along with aerobic sludge biomass activity. The reactor R_{amx0}, R_{amx500}, $R_{amx1000}$, and $R_{amx2000}$ biomasses have been fed with 0, 500, 1000, and 2000 mg/L of AMX, respectively. The reactors have been operated for 43 d with AMX in the feed — termed as stressed phase operation and then another 35 d without AMX in the feed — termed as recovery phase operation. The COD removal for reactors R_{amx500}, $R_{amx1000}$, and $R_{amx2000}$ have been 74%, 38%, and 23%, respectively, whereas control reactor R_{amx0} has shown 93% of COD removal during the steady-state condition of stressed phase operation. AMX has encouraged the increase in filamentous growth, and morphological studies have suggested that AMX has transformed the biomass from denser flocs to weaker filamentous form compared to the control reactor biomass. The ASBA values have decreased with increased AMX concentration in the feed. The microscopic examination has revealed abundance of filamentous biomass during stressed phase operation. The photographic observation has indicated floc sizes between 1.0-7.0 mm for reactor R_{amx500}, $R_{amx1000}$, and $R_{amx2000}$ biomasses. The cell viability has decreased to 17% for reactor $R_{amx2000}$ biomass, and PS, PN, and EPS secretion by aerobic sludge biomasses have increased in response to AMX stress. The AMX stress to aerobic sludge biomass has severely impacted $E_{Catalase}$ (51-85% inhibition) as compared to other enzyme activities. ARDRA has revealed 52-80% similarity in microbial community structure in response to 500-2000 mg/L of AMX in feed as compared to control reactor. During the recovery phase operation, all the reactors have shown COD removal of almost 93% at the steady-state condition. Also, reactors have maintained filamentous biomass with slightly increased SVI. ISV has deteriorated due to abundance of filaments in all reactors during the recovery phase operation. The active portion in aerobic biomass has also increased upto 64% for reactor $R_{amx2000}$ biomass in the recovery phase operation. The discontinuation of AMX has allowed the aerobic sludge biomasses to regain their organic degradation capability. All four enzyme activities have also shown improvement and have indicated almost similar enzyme activity values compared to control reactor R_{amx0} biomass. Stressed reactor biomasses have regained their microbial community structure (68-94% similarity) compared to control reactor biomass during recovery phase operation, indicating almost complete recovery after discontinuation of AMX in the feed. However, increase in filamentous biomass during stressed phase operation needs further in-depth investigation.

The presence of chlorpyrifos (CPS) stress has affected the treatment efficiency of CPS fed reactors. The reactors R_{cps0}, R_{cps50}, R_{cps100}, and R_{cps200} have been fed with 0, 50, 100, and 200 mg/L of CPS, respectively. The reactors have been operated for 34 d with CPS in the feed — termed as stressed phase operation and then another 42 d without CPS in the feed — termed as recovery phase operation. The COD removal for reactors R_{cps50}, R_{cps100}, and R_{cps200} have been observed as 72%, 39%, and 18%, respectively, whereas control reactor R_{cps0} has shown 91% of COD removal during the steady-state condition of stressed phase operation. The biomass growth especially filamentous part in CPS fed reactors has been constrained and influenced a bit by CPS doses. The ASBA values and cell viability have appeared to have decreased with increased CPS concentration in the feed. PS, PN, and EPS contents in reactor biomasses fed with CPS have varied slightly. $E_{Dehydrogenase}$ and E_{Urease} have significantly been affected due to CPS stress. ARDRA has established the relation between control and CPS fed reactors and has confirmed the microbial community changes (42-79% similarity as compared to control reactor) during stressed phase operation. During recovery phase operation, all reactors have shown enhanced COD removal (more than 88% during steady-state condition). The reactors fed with CPS during stressed phase operation have recovered in terms of filamentous part of the biomass and have shown almost similar results as the control reactor during recovery phase operation. SVI has increased for all reactors due to abundance of filamentous biomass, which also appeared to be a reason for decreased ISV. The discontinuation of CPS has allowed the aerobic sludge biomass to regain its organic degradation capability and cell viability. As soon as CPS has been discontinued from the feed during the recovery phase operation, the enzyme activity of aerobic sludge biomass has returned to its original levels. ARDRA has revealed regain in microbial community structure of reactor R_{cps50}, R_{cps100} and R_{cps200} biomasses and has shown 78-93% similarity compared to control reactor R_{cps0} biomass. Overall, it has indicated complete recovery of aerobic sludge biomasses after the discontinuation of CPS stress in the feed.

The addition of piggery wastewater (PWW) stress has affected the treatment efficiency of PWW fed reactors. The reactors R_c, R_s and R_r have been fed with Lactogen 3, screened PWW, and raw PWW, respectively, and operated for 41 d — termed as stressed phase I operation. The adverse effect of PWW on COD removal has been observed. At steady-state condition, the COD removal for PWW fed reactors (R_s and R_r) have been ~76% and 65%, respectively, while the COD removal for control reactor R_c has been ~95%. Alkalinity for reactors R_s and R_r have drastically increased due to addition of PWW. Significant changes have been observed in SVI and ISV. It could be seen that reactor R_s and R_r biomasses have lower SVI values (93 mL/g and 89 mL/g) as compared to control reactor R_c biomass (127 mL/g). On the other hand, PWW fed reactors have shown higher ISVs (3.05 m/h for R_s and 3.16 m/h for R_r) compared to control reactor R_c biomass has exhibited reduced ISV (2.43 m/h). Decreased ASBA values have indicated deterioration in bio-degradation capabilities of biomass. The morphology of flocs have transformed from denser to pinpoint flocs in reactor R_s and R_r biomasses. The active portion of biomass has decreased in the reactor biomasses exposed to PWW stress. PS, PN, and ESP contents have shown minor changes for PWW stressed reactors compared to the control reactor. The PWW stress to aerobic sludge biomass has indicated severe impact on E_{Urease} (11-73% inhibition) as compared to other enzyme activities, which has reflected the changes in biomass characteristics due to imposed stresses. The ARDRA result has indicated minor changes in the microbial community of reactor R_s and R_r biomasses (84-92% of similarity) when compared to control reactor R_c biomass. Aerobic sludge biomasses of reactor R_s and R_r have then been equally divided for the next phase of study. The reactors R_c, R_{sCu40}, R_{sCu80}, R_{rCu40}, and R_{rCu80} have been fed with Lactogen 3, screened PWW+40 mg/L of Cu(II), screened PWW+80 mg/L of Cu(II), raw PWW+40 mg/L of Cu(II), and raw PWW+80 mg/L of Cu(II), respectively, and operated for another 31 d — termed as stressed phase II operation. The addition of Cu(II) (40 and 80 mg/L) along with PWW during stressed phase II operation has a significant impact on performance-related parameters. The reactors R_{sCu40}, R_{sCu80}, R_{rCu40}, and R_{rCu80} have shown a drastic reduction in COD removal (8-29%), whereas control reactor has exhibited 94% of COD removal. The biomass growth has been further inhibited due to addition of Cu(II). The PWW+Cu(II) fed reactor biomasses have exhibited minimum variation in SVI (73.4-76.0 mL/g) when compared to control reactor R_c biomass (111.6 mL/g). In addition, ISV has been observed to be higher for PWW+Cu(II) fed reactor biomasses (3.36-3.46 m/h) than control reactor R_c biomass (2.22 m/h). The ASBA and cell viability have further decreased due to the combined stresses of PWW+Cu(II). The microscopic examination has revealed disintegration of flocs and disappearance of filaments. In addition, PS, PN, and EPS secretion has been enhanced in response to PWW+Cu(II) stress. Aerobic sludge biomass has indicated severe inhibition of $E_{Protease}$ and E_{Urease} in the range of 69-91% and 77-93%, respectively, compared to other enzyme activities due to PWW+Cu(II) stress in the feed. ARDRA has revealed a drastic shift in microbial community structure due to addition of PWW+Cu(II). The similarity in microbial community structure between control and PWW+Cu(II) fed reactor biomasses have been observed in the range of 29-63%. The PWW+Cu(II) feed has been discontinued in reactors R_{sCu40}, R_{sCu80}, R_{rCu40}, and R_{rCu80} and fed with Lactogen 3 and operated for another 48 d — termed as recovery phase operation. When PWW+Cu(II) has been discontinued in the feed, the steady-state COD removal for reactors R_{sCu40}, R_{sCu80}, R_{rCu40}, and R_{rCu80} have been observed as 78.0%, 63.7%, 57.1%, and 49.2%, respectively. The reactor R_{sCu40} and R_{rCu40} biomasses have exhibited increased biomass growth as compared to reactor R_{sCu80} and R_{rCu80} biomasses. Increased floc size and reappearance of filaments has indicated partial recovery of aerobic sludge biomass. Similarly, ASBA and cell viability have shown increase in their respective values suggesting recovery of biomass. PS, PN, and EPS contents in aerobic sludge biomass have been observed to be stabilized during recovery phase operation. $E_{Catalase}$, $E_{Dehydrogenase}$, and $E_{Protease}$ have shown significant recovery for the reactor biomasses exposed to lower doses of Cu(II), while slight improvement has been observed for the reactor biomasses exposed to higher doses of Cu(II). E_{Urease} has not shown any improvement during recovery phase operation. ARDRA results have suggested 47-86% of similarity between control reactor and other reactors after discontinuation of PWW+Cu(II). These results have indicated only partial recovery of aerobic sludge biomasses, while complete recovery appeared to be non-achievable.

This investigation has indicated that solids analysis (MLSS and MLVSS) of biomasses did not provide an alert in degrading health status of sludge biomasses. SVI and ISV of biomasses have been adversely impacted in the presence of Cu(II) and PWW+Cu(II) stresses in the feed, but have marginally affected in the presence of AMX and CPS stresses. It clearly rules out application of SVI and ISV tests as an indicator for deterioration in biomass health. The microscopic examination has revealed floc structure and shape of biomass, which has significantly changed in presence of Cu(II) and PWW+Cu(II) stressed. On the other hand, AMX stress has encouraged increase in filamentous biomass while CPS exposure has a minor impact on floc morphology. However, microscopic examination is a basic technique and may be used as preliminary test to understand the flocs structure. ASBA has also indicated impacts of Cu(II)/AMX/CPS/PWW+Cu(II) stresses during stressed phase operation, revealing deterioration in organic degradation capability of aerobic sludge biomass. Therefore, ASBA seems to have potential to be used as an indicator for aerobic sludge biomass health status. In addition, cell viability has also shown significant variations in aerobic sludge biomass in response to Cu(II)/AMX/CPS/PWW+Cu(II) stresses. As a result, cell viability test might be utilized as an alerting tool by the treatment plant operators. Moreover, it has appeared that $E_{Catalase}$ and $E_{Dehydrogenase}$ have been considerably affected in presence of stress-causing pollutants, and these two enzyme activities have potential to be utilized as a warning indicator for biomass quality. The microbial community structure has also shown changes in aerobic sludge biomass, when exposed to Cu(II)/AMX/CPS/PWW+Cu(II) stresses. Therefore, basic techniques such as microscopic examination and ASBA test have appeared to have potential to be used as preliminary testing for getting an alert on biomass health status. In addition, cell viability tests, enzyme activity assays ($E_{Catalase}$ and $E_{Dehydrogenase}$), and ARDRA might be used as confirmation tests for evaluation of aerobic sludge biomass quality with the help of skilled manpower and laboratory facilities available in nearby institutions of research and higher learning.

Keywords: Aerobic sludge biomass; Copper; Amoxicillin; Chlorpyrifos, Piggery wastewater; COD; SVI; ISV; Aerobic sludge biomass activity; Biomass morphology; Enzyme activity; Cell viability; Filamentous biomass; ARDRA.