

**DEVELOPMENT AND ONSITE VALIDATION OF SEQUENTIAL
MICROBIAL BASED ANAEROBIC-AEROBIC REACTOR
TECHNOLOGY (SMAART) FOR TEXTILE EFFLUENT TREATMENT:
MECHANISM ELUCIDATION AND LIFE CYCLE ASSESSMENT**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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by

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Submitted

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CERTIFICATE

This is to certify that the thesis entitled “**Development and onsite validation of Sequential Microbial Based Anaerobic-Aerobic Reactor Technology (SMAART) for textile effluent treatment: mechanism elucidation and life cycle assessment**” being submitted by **Mr. Saurabh Samuchiwal** to the Indian Institute of Technology Delhi for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for submission of this thesis. To the best of our knowledge the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.



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ABSTRACT

The present study has attempted to develop a microbial-based indigenous pilot-scale technology that can efficiently treat the undiluted textile effluent with minimum additives for supporting microbial growth. To begin with, a visit to leading textile industry was undertaken to understand the functioning of the different wet operational units, types of effluents/wastes generated, and identify the problems associated with the effluent treatment process. Various effluents generated from different wet operations were procured and characterized. A novel microbial consortium enriched from Pre-treatment range (PTR) effluent was used to optimize the process of decolourization under extreme conditions with minimum inputs. With PTR effluent as a carbon source and only 0.5 g L⁻¹ yeast extract as external input, the process enabled 70-73% colour reduction (from 1910-1930 to 516-555 hazen) in dyeing unit wastewater. Unhindered performance at higher temperatures (30 °C-50 °C) and wide pH range (7-12) makes this process highly suitable for the treatment of warm and extremely alkaline textile effluents. No significant difference was observed in the decolourization efficiency for effluents from different batches (Colour: 1647-4307 hazen; pH-11.5-12.0) despite wide variation in nature and concentration of dyes employed. Long term (60 d) continuous mode performance monitoring at hydraulic retention time of 48 h in lab-scale bioreactor showed consistent colour (from 1734-1980 to 545-723 hazen) and chemical oxygen demand (1720-2170 to 669-844 mg L⁻¹) removal and consistently neutral pH of the treated water. This developed anaerobic process shows a significant advancement by uncovering the ability of native microbial consortium to reliably treat dye laden textile wastewater without any dilution or pre-treatment and with minimum external inputs.

Next, the speculation of molecular mechanism for azo dye degradation using the microbial consortium was done by understanding the role of oxido-reductase enzymes followed by deciphering the functional genes and their corresponding proteins. For this, decolourization of 100 mg L⁻¹ reactive blue 13 (RB13) was done using same consortium at optimized condition and the results showed ~92.67% decolourization at 48 h of incubation. The fourier-transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GCMS) analysis were performed to identify the metabolites formed during RB13 degradation, followed by hypothesizing the metabolic pathway. The GC-MS analysis showed formation of 1,4-dihydronaphthalen-1-ol and 1,3,5-triazin-2-amine as the final degraded

compounds after enzymatic breakdown of RB13 dye. The activity of different oxido-reductase enzymes was determined, and the results showed that NADH DCIP reductase and azo reductase had higher activity than other enzymes (veratryl alcohol oxidase, and aldehyde dehydrogenase). It clearly indicated the degradation was initiated with the enzymatic cleavage of azo bond ($-N=N-$) of RB13. Further, the functional genes were annotated against the database of clusters of orthologous groups (COGs) and kyoto encyclopedia of genes and genomes (KEGG). It provided the valuable information about the role of crucial functional genes and their corresponding proteins correlated with dominant bacterial species in degradation of RB13.

Further, an on-site anaerobic biological reactor integrated with activated carbon filter (ACF) and ultra-filtration (UF) unit termed as AN-ACF-UF process was installed and used for the decolourization of fresh textile effluent at the industrial premises. The anaerobic reactor containing the developed microbial consortium was fed with a mixed inlet consisting of coloured and PTR effluents in a ratio of 70:30 (v/v). The anaerobic unit was run in a continuous mode for 32 d with a hydraulic retention time of 2 d. The treated effluent from the anaerobic unit was fed into the ACF unit at 0.7 mL min^{-1} . Finally, the outlet from the ACF unit was fed into the UF unit. The AN-ACF-UF process was effective in decolourizing $91 \pm 3 \%$ of the colour in textile effluent mixture. The phytotoxicity test (germination test) on *Vigna radiata* using the treated effluent did not show any significant difference ($p > 0.05$) between control ($92 \pm 1 \%$ germination) and treated effluent group ($83 \pm 1 \%$ germination). The recovered salt which contained high concentration of sodium salt (349.70 mg g^{-1} of salt) was reused for pad batch process in dyeing unit of textile industry. The results showed a shift towards red-yellow zone of CIELab colour space. However, this colour shift did not interfere with the dyeing process and could be used for darker shade. It creates an inner loop recycling of recovered salts within the industrial operations and eliminates the cost required for the disposal of salt generated from the multi effect evaporator i.e., MEE salt (coloured salt). However, the AN-ACF-UF prototype system was conducted in lab-scale system and still lacked residual COD reduction from the treated effluent. Moreover, it required frequent backwash of ACF and UF due to poor sludge retention in anaerobic bioreactor. To overcome these limitations, an improved pilot scale sequential microbial-based anaerobic-aerobic reactor technology (SMAART) was designed and operated for the treatment of real textile effluent in the industrial premises in continuous mode for 180 days. The sequential treatment technology SMAART consists of specific treatment units, i.e., an anaerobic unit with a membrane module (to improve sludge retention)

integrated with the aerobic unit (for effective COD reduction) followed by the polishing steps (activated carbon columns). The results showed an average ~95% decolourization along with ~92% reduction in the chemical oxygen demand establishing the resilience against fluctuations in the inlet parameters and climate conditions. Moreover, the pH of treated effluent was also reduced from alkaline range (11.05 ± 0.75) to neutral range (7.76 ± 0.22) along with turbidity reduction from 44.16 ± 7.82 NTU to 0.14 ± 0.08 NTU. A comparative life cycle assessment (LCA) of SMAART with the conventional activated sludge process (ASP) showed that ASP caused 41.5% more negative impacts on environment than SMAART. Besides, ASP had 46.15% more negative impact on human health, followed by 42.85% more negative impact on ecosystem quality as compared to SMAART. This was attributed to less electricity consumption, absence of pre-treatment units (cooling and neutralization) and less volume of sludge generation (~50%) while using SMAART. Hence, integration of SMAART within the industrial effluent treatment plant could achieve minimum waste generation in pursuit of sustainability.

सार

वर्तमान अध्ययन ने एक माइक्रोबियल-आधारित इंडिजेनस पायलट-स्केल तकनीक विकसित करने का प्रयास किया है जो माइक्रोबियल विकास का समर्थन करने के लिए न्यूनतम एडिटिव्स के साथ बिना पानी मिला (undiluted) टेक्सटाइल एफ्लुएंट (textile effluent) का कुशलतापूर्वक उपचार कर सकता है। शुरुआत में, प्रमुख कपड़ा उद्योग का दौरा किया गया ताकि विभिन्न पानी पर निर्भर परिचालन इकाइयों के कामकाज, उत्पन्न होने वाले एफ्लुएंट/ कचरे (waste) के प्रकारों को समझा जा सके और एफ्लुएंट उपचार प्रक्रिया से जुड़ी समस्याओं की पहचान की जा सके। विभिन्न पानी पर निर्भर प्रचालनों से उत्पन्न विभिन्न एफ्लुएंट को प्राप्त किया गया और उनका लक्षण वर्णन (characterized) किया गया। प्री-ट्रीटमेंट रेंज (PTR) एफ्लुएंट से समृद्ध एक नावेल माइक्रोबियल कंसोर्टियम का उपयोग न्यूनतम इनपुट के साथ एक्सट्रीम (extreme) स्थितियों में विरंजीकरण (decolourization) की प्रक्रिया को अनुकूलित करने के लिए किया गया था। कार्बन स्रोत के रूप में PTR एफ्लुएंट और बाहरी इनपुट के रूप में केवल 0.5 g L^{-1} यीस्ट एक्सट्रेक्ट डालना के साथ, इस प्रक्रिया ने रंगाई इकाई एफ्लुएंट में 70-73% रंग कमी (1910-1930 से 516-555 hazen तक) को सक्षम किया। उच्च तापमान (30 डिग्री सेल्सियस-50 डिग्री सेल्सियस) और विस्तृत पीएच (pH) रेंज (7-12) पर अबाधित प्रदर्शन, इस प्रक्रिया को गर्म और अत्यंत क्षारीय टेक्सटाइल एफ्लुएंट के उपचार के लिए अत्यधिक उपयुक्त बनाता है। अलग-अलग बैचों (रंग: 1647-4307 hazen; पीएच-11.5-12.0) से निकलने वाले एफ्लुएंट के लिए विरंजीकरण दक्षता में कोई महत्वपूर्ण अंतर नहीं देखा गया था, हालांकि इस्तेमाल किए गए रंगों की प्रकृति और एकाग्रता में व्यापक भिन्नता थी। लैब-स्केल बायोरिएक्टर में 48 घंटे के हाइड्रोलिक अवधारण समय (HRT) पर दीर्घकालिक (60 दिन) निरंतर मोड प्रदर्शन निगरानी ने लगातार रंग (1734-1980 से 545-723 hazen तक) और रासायनिक ऑक्सीजन की मांग (1720-2170 से 669-844 mg L^{-1}) हटाना, पानी के पीएच का नयूत्रलिज़ और लगातार तटस्थ रखना। यह विकसित अवायवीय प्रक्रिया मूल माइक्रोबियल संघ की क्षमता को बिना किसी कमजोर पड़ने या पूर्व-उपचार के और न्यूनतम बाहरी इनपुट के साथ ड्राई से भरे टेक्सटाइल एफ्लुएंट का इलाज करने की क्षमता को उजागर करके एक महत्वपूर्ण प्रगति दिखाती है।

इसके बाद, माइक्रोबियल कंसोर्टियम का उपयोग करके एज़ो ड्राई डिग्रेशन के लिए आणविक तंत्र की अटकलें, ऑक्सीडो-रिडक्टेस एंजाइमों की भूमिका को समझने के बाद कार्यात्मक जीन और उनके संबंधित प्रोटीनों को समझने के द्वारा की गई। इसके लिए, 100 mg L^{-1} रिएक्टिव ब्लू 13 (RB13) का विरंजीकरण अनुकूलित स्थिति में वही कंसोर्टियम का उपयोग करके किया गया था और परिणामों ने ऊष्मायन के 48 घंटे

में ~92.67% विरंजीकरण दिखाया। फोरियर-ट्रांसफॉर्म इन्फ्रारेड स्पेक्ट्रोस्कोपी (FTIR), हाई परफॉर्मेंस लिक्विड क्रोमैटोग्राफी (HPLC) और गैस क्रोमैटोग्राफी-मास स्पेक्ट्रोमेट्री (GCMS) विश्लेषण RB13 डिग्रेडेशन के दौरान बनने वाले मेटाबोलाइट्स की पहचान करने के लिए किया गया था, जिसके बाद मेटाबॉलिक पाथवे की परिकल्पना की गई थी। GC-MS विश्लेषण ने RB13 डार्क के एंजाइमैटिक ब्रेकडाउन के बाद अंतिम अवक्रमित यौगिकों के रूप में 1,4-डायहाइड्रोनाफथलेन-1-ओएल और 1,3,5-ट्रायाज़िन-2-एमीन के गठन को दिखाया। विभिन्न ऑक्सीडो-रिडक्टेस एंजाइमों की गतिविधि निर्धारित की गई थी, और परिणामों से पता चला कि एनएडीएच डीसीआईपी रिडक्टेस (NADH DCIP reductase) और एंज़ो रिडक्टेस में अन्य एंजाइमों (वेरेट्रील अल्कोहल ऑक्सीडेज और एल्डिहाइड डिहाइड्रोजेनेज) की तुलना में उच्च गतिविधि थी। यह स्पष्ट रूप से इंगित करता है कि क्षरण की शुरुआत RB13 के एंज़ो बॉन्ड ($-N=N-$) के एंजाइमैटिक क्लीवेज से हुई थी। इसके अलावा, कार्यात्मक जीनों को ऑर्थोलॉगस समूहों (सीओजी) के समूहों के डेटाबेस और जीन और जीनोम (केईजीजी) के क्योटो विश्वकोश के खिलाफ एनोटेट किया गया था। इसने RB13 के क्षरण में प्रमुख जीवाणु प्रजातियों के साथ सहसंबद्ध महत्वपूर्ण कार्यात्मक जीनों और उनके संबंधित प्रोटीनों की भूमिका के बारे में बहुमूल्य जानकारी प्रदान की।

इसके अलावा, सक्रिय कार्बन फिल्टर (ACF) और अल्ट्रा-फिल्ट्रेशन (UF) इकाई के साथ एकीकृत एक ऑन-साइट अवायवीय (anaerobic) जैविक रिएक्टर को एएन-एसीएफ-यूएफ (AN-ACF-UF) प्रक्रिया के रूप में स्थापित किया गया था और औद्योगिक परिसर में ताजा टेक्सटाइल एफ्लुएंट के विरंजन के लिए उपयोग किया गया था। विकसित माइक्रोबियल कंसोर्टियम वाले अवायवीय रिएक्टर को 70:30 (v/v) के अनुपात में रंगीन और पीटीआर एफ्लुएंट से युक्त मिश्रित इनलेट से भरा गया था। एनारोबिक यूनिट को 2 दिन के हाइड्रोलिक अवधारण समय (HRT) के साथ 32 दिन के लिए निरंतर मोड में चलाया गया था। अवायवीय इकाई से उपचारित प्रवाह को ACF इकाई में 0.7 mL min^{-1} पर डाला गया। अंत में, ACF यूनिट के आउटलेट को UF यूनिट में फीड किया गया। एएन-एसीएफ-यूएफ प्रक्रिया टेक्सटाइल एफ्लुएंट मिश्रण में $91 \pm 3\%$ रंग को विरंजित करने में प्रभावी थी। उपचारित एफ्लुएंट का उपयोग करते हुए *Vigina radiata* पर फाइटोटॉक्सिसिटी परीक्षण (अंकुरण परीक्षण) ने कंट्रोल ($92 \pm 1\%$ अंकुरण) और उपचारित एफ्लुएंट ($83 \pm 1\%$ अंकुरण) के बीच कोई महत्वपूर्ण अंतर ($p > 0.05$) नहीं दिखाया। बरामद नमक जिसमें सोडियम नमक (349.70 mg g^{-1} नमक) की उच्च सांद्रता थी, का कपड़ा उद्योग की रंगाई इकाई में पैड बैच प्रक्रिया के लिए पुनः उपयोग किया गया। परिणामों ने CIELab रंग स्थान के लाल-पीले क्षेत्र की ओर एक बदलाव दिखाया। हालांकि, इस रंग बदलाव ने रंगाई प्रक्रिया में हस्तक्षेप नहीं किया और इसे गहरे रंग की छाया के लिए इस्तेमाल

किया जा सकता है। यह औद्योगिक संचालन के भीतर बरामद नमक का एक आंतरिक लूप रीसाइक्लिंग बनाता है और multi effect evaporator यानी एमईई नमक (रंगीन नमक) से उत्पन्न नमक के डिस्पोजल के लिए आवश्यक लागत को समाप्त करता है। हालांकि, एएन-एसीएफ-यूएफ प्रोटोटाइप सिस्टम लैब-स्केल सिस्टम में आयोजित किया गया था और अभी भी इलाज किए गए प्रवाह से अवशिष्ट सीओडी रिडक्शन की कमी है। इसके अलावा, एनारोबिक बायोरिएक्टर में खराब स्लज रिटेंशन के कारण एसीएफ और यूएफ की लगातार बैकवाश की आवश्यकता होती है। इन सीमाओं को दूर करने के लिए, एक बेहतर पायलट स्केल अनुक्रमिक माइक्रोबियल-आधारित एनारोबिक-एरोबिक रिएक्टर टेक्नोलॉजी (SMAART) को 180 दिनों के लिए निरंतर मोड में औद्योगिक परिसर में वास्तविक टेक्स्टाइल एफ्लुएंट के उपचार के लिए डिजाइन और संचालित किया गया था। अनुक्रमिक उपचार तकनीक SMAART में विशिष्ट उपचार इकाइयां होती हैं, यानी, एरोबिक इकाई (प्रभावी सीओडी कमी के लिए) के साथ एकीकृत एक झिल्ली मॉड्यूल (स्लज रिटेंशन में सुधार करने के लिए) के साथ एक एरोबिक (aerobic) इकाई, जिसके बाद पॉलिशिंग चरण (सक्रिय कार्बन कॉलम) होते हैं। परिणामों ने रासायनिक ऑक्सीजन की मांग में ~92% की कमी के साथ-साथ इनलेट मापदंडों और जलवायु परिस्थितियों में उतार-चढ़ाव के खिलाफ प्रतिरोधक्षमता स्थापित करने के साथ-साथ औसतन 95% डीकोलराइजेशन दिखाया। इसके अलावा, उपचारित एफ्लुएंट का पीएच भी क्षारीय रेंज (11.05 ± 0.75) से न्यूट्रलिज़ रेंज (7.76 ± 0.22) तक कम हो गया था, साथ ही टर्बिडिटी में 44.16 ± 7.82 NTU से 0.14 ± 0.08 NTU तक कमी आई थी। पारंपरिक सक्रिय स्लज प्रक्रिया (ASP) के साथ SMAART के तुलनात्मक जीवन चक्र मूल्यांकन (LCA) से पता चला है कि ASP ने स्मार्ट की तुलना में पर्यावरण पर 41.5% अधिक नकारात्मक प्रभाव डाला है। इसके अलावा, एएसपी का मानव स्वास्थ्य पर 46.15% अधिक नकारात्मक प्रभाव पड़ा, इसके बाद स्मार्ट की तुलना में पारिस्थितिकी तंत्र की गुणवत्ता पर 42.85% अधिक नकारात्मक प्रभाव पड़ा। SMAART का उपयोग करते समय कम बिजली की खपत, प्री-ट्रीटमेंट यूनिट्स (कूलिंग और न्यूट्रलाइजेशन) की अनुपस्थिति और स्लज उत्पादन की कम मात्रा (~50%) को इसके लिए जिम्मेदार ठहराया गया था। इसलिए, औद्योगिक प्रवाह उपचार संयंत्र के भीतर SMAART का एकीकरण स्थिरता की खोज में न्यूनतम अपशिष्ट उत्पादन प्राप्त कर सकता है।

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