

Micro Algae Based Technology for Antibiotic Waste Water Treatment

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Abstract

Antibiotic residues in wastewater pose serious environmental and public health challenges, necessitating sustainable and effective treatment methods. Unfortunately, wastewater including antibiotics is unable to be safely processed by conventional approaches like those employed in wastewater treatment plants. Water shortages throughout the world will be among the main challenges to society and the economy of the twenty-first century. Microalgae based biotechnologies have emerged as promising solutions, offering eco-friendly, cost effective, and solar driven remediation strategies. Multiple mechanisms underpin antibiotic removal by microalgae, including bioadsorption, bioaccumulation, biodegradation, photodegradation, hydrolysis, and surface adsorption. The efficacy of these pathways varies with microalgal strain, operational conditions, and specific antibiotic structure. Many algae such as *Chlorella sorokiniana*, *Monoraphidium contortum*, *Scenedesmus* species can efficiently remove antibiotics from waste water. In conclusion, microalgae-based technologies present a sustainable, multifunctional approach to antibiotic removal coupling water purification with biomass generation and CO₂ mitigation yet their translation from laboratory to full-scale systems hinges on addressing operational constraints and optimizing hybrid designs. This review outlines some microalgae-based technologies and their significant aid in the treatment of antibiotic wastewater which provide information for developing robust, large scale, sustainable systems for the effective removal of antibiotics from wastewater.



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Introduction

Antibiotics are crucial medications which have been extensively employed to prevent infectious diseases, boost public health, and enhance the productivity of domestic and some low-cost animals.¹ Over 100,000 tonnes of antibiotics are utilized globally each year.² Studies have shown that nearly ninety percent of the medications would ultimately end up in different places by means of excretory waste.³ The antibiotic pollution in water primarily stems from the improper disposal of unused medications including antibiotics. They are often disposed of improperly, either flushed down toilets or washed down sinks, leading to direct contamination of water sources. Despite the advances in science and technology, both human health and ecosystems remain at risk as a result of this antibiotic pollution since it can cause bacteria to develop antibiotic resistance. Many countries are currently experiencing a severe water shortage as a result of climate change, excessive water use, and poor waste management systems. The breakdown of contaminants and the safe release and reuse of wastewater depend on cost-effective improvements to wastewater treatment processes. According to studies wastewaters from different sources have been found to contain varying amounts of antibiotics including hospital wastewater (0.1 - 157 μgL^{-1}), domestic wastewater (0.001 - 32 μgL^{-1}) and industrial wastewater (26 ng L^{-1} - 31 mg L^{-1}).⁴⁻⁶ The environment and human health are seriously threatened by the antibiotic pollution in water.⁷ Antibiotic removal from wastewater has therefore attracted increased global public attention and concern in recent years. Selecting the most effective treatment plan is essential for achieving the desired purification objectives since the water gets polluted and cleaning turns into necessary.^{8,9}

Currently, an array of technologies is used for the elimination of antibiotics from aquatic environments, employing biological, physical and chemical methods. To improve the rate of elimination of antibiotics from wastewater, physicochemical techniques have been developed up to this point. These include adsorption, flocculation, coagulation, ozonation, ion exchange, membrane filtration, electrochemical degradation and chemical oxidation.¹⁰ Furthermore, due to its distinctive benefits, such as their affordability and environmental friendliness, biological methods such as ligninolytic fungi,

biochars, and microalgae are frequently suggested for elimination of antibiotics from wastewater.¹¹⁻¹³ Considering numerous advantages such as low cost, wastewater purification, CO_2 sequestration, and biomass production, microalgae-based technology has recently received significant attention among biological treatment techniques.¹⁴

Algae are mostly found in aquatic environments and serve as ecological indicators for pollutant removal, as they possess a limited growth cycle, high sensitivity to aquatic pollutants, and the ability to trigger stress response mechanisms. Techniques based on microalgae have become renowned for their economical ability to simultaneously restore nutrients and antibiotics.^{15,16} Therefore, Microalgae-based biotechnology is regarded as an eco-friendly method of eliminating antibiotics from wastewater and enhancing wastewater quality.

Methodology

The review employed an approach to analyze the current state of microalgae-based technology for antibiotic removal from wastewater. The literature search was conducted using major academic databases (e.g., Scopus, Web of Science, PubMed, Google Scholar). Keywords include: ("microalgae" OR "cyanobacteria"); ("antibiotic" OR "antimicrobial"); ("wastewater treatment" OR "bioremediation" OR "phytoremediation") and ("removal" OR "degradation" OR "biosorption"). The search will be limited to peer-reviewed articles and reviews published in English, with a focus on the last 15 years to capture recent advancements. This methodology will ensure a rigorous, reproducible and insightful review.

Microalgae: Nature's Companion

Microalgae play a vital role in wastewater treatment as they can absorb nutrients, raise pH levels, and help phosphorous precipitation. They are potent source for bioremediation as they flourish in nitrogen and phosphate rich wastewater environments. The ability of several microalgal species, including *Scenedesmus*, *Chlorella*, *Phormidium*, *Botryococcus*, *Limnospira*, and *Chlamydomonas*, to bioremediate nutrients, heavy metals, emerging pollutants and pathogens associated with wastewater has been demonstrated.^{17,18} Microalgae-based technology for treating antibiotics-contaminated wastewater

is an environmental-friendly and cost-effective method. Studies have reported that *Chlorella vulgaris* can remove metronidazole (5 μ M) with 100% removal efficiency through bioadsorption.¹⁹ The mechanisms used to eliminate antibiotics depend on the microalgae species and antibiotic properties, and each mechanism's efficacy differs.²⁰ Commercial research into the efficacy of microalgae has increased due to their ability to absorb nutrients and generate significant volumes of biomass. Microalgae require only limited space for growth and can survive under harsh conditions. Microalgae biomass can double within 13 hours of culture.^{21,22} *Chlorella sorokiniana* was proven to be effective in eliminating painkillers.²³ A recent research investigation, reported the use of microalgae *Chromochloris zofingiensis* for the removal of the antibiotic levofloxacin from waste water.²⁴ Table 1 shows the role of algae in the bioremediation of different classes of antibiotics and their mechanism of action.

Elimination of Antibiotics by Algal Mechanism

Microalgae eliminate antibiotics primarily through biosorption, followed by bioaccumulation and biodegradation.^{15,25,26} Certain types of antibiotics are destroyed by photo-oxidation and volatilization during microalgal growth.²⁷ But some studies reported that photodegradation and volatilization are unusual and generally considered to be irrelevant.²⁸ Micro algae technology comprises three unified processes- Bioadsorption, Bioaccumulation, Biodegradation. In bio adsorption antibiotics are rapidly and passively adsorbed onto the algal cell surface via physical and chemical interactions. In bioaccumulation the compounds slowly diffuse across the cell membrane into the algal interior and in biodegradation the substances gradually accumulate and are metabolized through bioaccumulation pathways.²⁹

Bioadsorption

Bioadsorption is a physico-chemical method used to remove antibiotics from wastewater directly.³⁰ It is a mass transfer mechanism through which a substance moves from the liquid phase and holds itself to the surface of a solid. Since the sorbent is a biological substance that can bind and concentrate contaminants from water, biosorption is regarded as a passive process. The variety of components found in biomass and the spectrum of functional

groups, which are influenced to differing degrees by physico-chemical processes, give biomaterials their structural complexity and diversity.³¹ Microalgae's cell wall is directly responsible for biosorption, its chemical composition is crucial during the process and controls the mechanism of occurrence in. By binding to extracellular polymeric substances (EPS) or components of their cell walls, algae can bioadsorb antibiotics.³² EPS is a mixture of biopolymers produced by microbes. It performs various structural and functional roles, including enhancing adsorption capacity, modifying surface properties, retaining enzymes, maintaining structural stability, and facilitating nutrient transport.³³ Antibiotics interact passively with negative-charged microalgal cellular membranes or discharges.³⁴ Increased area for contact per biomass unit is provided by smaller cell diameters, increasing the adsorbent surface area.³⁵ For instance *Chlorella vulgaris* can bioadsorb metronidazole (initial concentration 5 mM) with 100% removal efficiency.¹⁹ Additionally, it has been shown that nonliving microalgae biomass is a promising biosorbent material for antibiotic removal.

Bio adsorbent loading, initial adsorbate concentration, adsorption duration, pH, temperature, and excretions of extracellular polymeric substances are process parameters that affect the bioadsorption process.²⁷ Furthermore, antibiotic bioadsorption onto the biomass can be influenced by the medium's pH.³⁶ Antibiotics' aggregation, hydrophobicity, electrostatic attraction, and repulsion are all impacted by pH.³⁷ Increased lipophilicity lowers pKa, which in turn affects a substance's lipophilicity and protein-binding ability.^{38,39} Deviations in temperature had a consequence on the rate at which antibiotics are absorbed by microalgal cells during bioadsorption.⁴⁰ The structure of the species and the surrounding environment have a significant impact on the potential of microalgae to adsorb substances.⁴¹ Hydrophilic materials have a lower affinity for bioadsorption and are able to endure longer in growth media.^{27,42}

Bioaccumulation

Bioaccumulation is a dynamic metabolic pathway for the uptake of antibiotics. It is a lively, intracellular process which demands an immense amount of energy.^{43,44} Research indicates that antibiotics enter algal cells by passive diffusion.⁴⁵ Bioaccumulation

is measured using a Bioconcentration factor, which is the ratio of the concentration of a contaminant adsorbent to the medium.⁴⁶ One of the difficulties with bioaccumulation is being aware that some compounds that have collected in the body might release reactive oxygen species. These free radicals may result in oxidative destruction of biomolecules, cellular malfunction, and finally death of cells. They also have a crucial impact on cell metabolism.^{42,47} The antibiotic sulfamethazine bioaccumulated in *C. pyrenoidosa* prior to being eliminated.⁴⁸ Levofloxacin was also removed by *C. vulgaris* by means of accumulation and subsequent intracellular biodegradation.⁴⁹

Biodegradation

One of the most effective techniques for eliminating pollutants from effluents is biodegradation. It involves the metabolic breakdown of complex substances

into smaller.⁵⁰ Biodegradation is a method in which organic compounds break down by means of biotransformation resulting in metabolic intermediates.^{42,51,52} The biodegradation of microalgae can occur in two chief ways. The one is metabolic degradation, in which antibiotics give microalgae a carbon source and act as electron donors or acceptors and the other is co-metabolism, in which enzymes reduce the antibiotics to produce non-toxic product compounds. Studies showed that the degradation potential of several algae, including *C. vulgaris*, *Selenastrum capricornutum*, *Haematococcus pluvialis*, and *S. quadricauda* against various antibiotics, biodegradation was the primary mechanism for antibiotic elimination.⁵³ Algal-based technologies break down antibiotics in three different ways: biodegradation, bioaccumulation, or both; relatively slow molecule transfer through algal cell walls; and rapid adsorption.²⁹

Table 1: Removal of antibiotics by various algal species and its mechanisms

Antibiotic Group	Name of Antibiotic	Algae used	%Removal of antibiotic	Mechanisms
Beta lactum	Amoxicillin	<i>Microcystis aeruginosa</i>	30.5-33.6%	Biodegradation ⁵⁴
		<i>Microcystis aeruginosa</i>	18.5-30.5%,	Biodegradation ⁵⁵
	Cefalexin	<i>Chlorella sp.</i> (lipid extracted dry biomass)	71.2±38.9%,	Biosorption ⁵⁶
		<i>Chlorella pyrenoidosa</i>	96.07%	Bio adsorption and biodegradation ²⁹
	7-amino cephalosporanic acid	<i>Chlorella sp.</i> Cha-1	4.74 mg g ⁻¹ of biomass	Bio adsorption ⁵⁷
		<i>Chlamydomonas sp.</i> Tai-03	3.09 mg g ⁻¹ of biomass	Bio adsorption ⁵⁷
		<i>Mychonastes sp.</i> YL-02	2.95 mg g ⁻¹ of biomass	Bio adsorption ⁵⁷
Tetracyclines	Tetracycline	<i>Chlamydomonas sp.</i> Tai-03	100%,	Biodegradation photolysis and hydrolysis ⁵⁸
		<i>Scenedesmus quadricauda</i> (lipid extracted dry biomass)	48.84 ± 1.4%,	Biosorption ³⁶
	Tetracycline	<i>Tetraselmis suecica</i> (lipid Extract dry	36.71 ± 2.1%,	Biosorption ³⁶

		biomass)		
	Oxytetracycline	<i>Spirogyra sp.</i>	89±2%	Photodegradation ⁵⁹
		<i>Spirogyra sp.</i>	93±2%	photodegradation, biodegradation & hydrolysis ⁵⁹
Sulphonamides	sulfadiazine	<i>Chlorella vulgaris</i>	11-24%	Not discussed ³⁹
		<i>Chlorella ovalisporum</i>	10-20%	Not discussed ³⁹
	sulfamethazine	<i>Chlamydomonas sp. Tai-03</i>	35.60%	Photolysis and biodegradation ⁵⁸
		<i>Scenedesmus obliquus</i>	31.4- 62.3%	Not discussed ⁴²
	sulfamethoxazole	<i>Chlamydomonas sp. Tai-03</i>	20%	Biodegradation ⁶⁰
		<i>Scenedesmus obliquus</i>	46.8%,	Mechanism not reported ⁴²
		<i>Nannochloris sp.</i>	32%	Algae- mediated Photolysis ⁶¹
		<i>Nannochloris sp.</i>	11%	Algae- mediated Photolysis ⁴³
Macrolides	Erythromycin	<i>Chlorella pyrenoidosa</i>	48.5-69.9%,	Accumulation and biodegradation ⁵⁵
		<i>Scenedesmus obliquus</i>	94.18%, 80.59%, and 49.60%	Biodegradation, hydrolysis, and photolysis ⁷
	Roxithromycin	<i>Chlorella pyrenoidosa</i>	80.45%, 76.35% and 64.81%	Photodegradation and Biodegradation ⁶²
		<i>Scenedesmus dimorphus</i>	93%	Bio adsorption and biotransformation Ion ⁶³
		<i>Chlamydomonas mexicana</i>	13%	Biodegradation, bioaccumulation & bio adsorption ²⁶
	Ciprofloxacin	<i>Chlamydomonas sp. Tai-03</i>	65.05%	Biodegradation ⁶⁰
Quinolones	Enrofloxacin	<i>Scenedesmus obliquus</i>	23%,	Biodegradation, bioaccumulation & Bio adsorption ²⁶
		<i>Chlamydomonas mexicana</i>	25%	
		<i>Ourococcus multispurus</i>	18%	
		<i>Micractinium resseri</i>	20%	
		<i>Chlorella vulgaris</i>	53-73%	Bioadsorption and biodegradation ³⁹
		<i>Chlorella ovalisporum</i>	58-79%	Bioadsorption and biodegradation
	Norfloxacin	<i>Chlorella vulgaris</i>	36.9%,	Algae- mediated photodegradat Ion ⁶⁴

Exploring Microalgal Integration for Cleaner Water

Microalgal integrations seem to be a promising wastewater treatment method due to their low cost, large biomass results in significant pollutant removal capacity, and ecological sustainability. Microalgae - bacteria-fungus symbioses offer a potent, integrated approach for treating antibiotic-laden wastewater. Together, these synergistic systems - leveraging biosorption, bioaccumulation, enzymatic biodegradation, and optimized harvesting - demonstrate a sustainable, high-efficiency route for antibiotic wastewater remediation.

Microalgae – Bacteria Consortium

Algae can employ heterotrophic metabolism or extracellular enzymes to entirely eliminate antibiotics. Additionally, they symbiotic interactions with bacteria.⁶⁵ Photosynthetically changing pH levels, or high oxygen formation can all indirectly enhance algae's capacity for biodegradation.⁴¹ Through photosynthetic processes, microalgae generate molecular oxygen, which aerobic bacteria use as an electron acceptor to break down organic pollutants. Microalgae can use the CO₂ released through bacterial mineralization as a carbon source for photosynthesis. Since antibiotics are made expressly to kill bacteria, microalgae are far more resistant to them than heterotrophic bacteria. Using processes like biodegradation, volatilization, photodegradation and sorption, algae-bacteria consortium techniques can break down antibiotics in wastewater. Recent years have seen a lot of research on algal-bacterial systems due to their simple functioning, robustness, and improved removal efficiencies.⁶⁶

Studies demonstrated that the removal of ketoprofen was improved when *Chlorella* sp. was combined with a bacterial consortium.⁶⁷ Combined algal-bacterial systems showed a caffeine removal rate of over 99%, compared to a removal rate of only 17% in microalgal incubation alone.⁶⁸ Bacterial-algal synergy was crucial in the treatment of anthraquinone. Anthraquinone's molecular bonds could be broken by *Chlorella*, transforming it into intermediate molecules. The heterotrophic bacteria fully absorbed the intermediate molecules.⁶⁹ Both bacteria and algae may act as biosorbents in algae-bacteria consortia, and the EPS that the bacteria and algae produce provides vital sites for the biosorption of antibiotics.⁷⁰ For the degradation of cephalosporins,

created a new algae-activated sludge mixed system that demonstrated an exceptional cefradine removal rate with green algae, achieving increased overall removal effectiveness worth 97.91%.³⁴ Algae-bacteria granular sludge reactors (ABGS) combining *Scenedesmus* or *Chlorella* with bacterial sludge have removed tetracycline and sulfadiazine at rates of ~79 % and ~94 %, respectively.¹³

Microalgae – Fungal Consortium

Compared to bacteria in biological wastewater purification, fungi have demonstrated encouraging results and offers several advantages. The microalgal-fungal system's mechanisms that support its exceptional wastewater remediation performance. When it comes to removing nutrients from wastewater, co-approach works better than traditional pure cultivation. Heterotrophic organisms like fungi employ metabolism to convert organic resources into carbon dioxide, whereas autotrophic microalgae use inorganic carbon sources as building blocks to accumulate biomass.⁷¹ In this manner, fungi can be fully supplied with oxygen from microalgal photosynthesis, which returns carbon dioxide to algal cells. Studies discovered that seven pharmaceuticals were removed using *Aspergillus niger*, *Chlorella vulgaris*, and bio-pellets made of both microorganisms.⁷² Systems combining *Chlorella vulgaris* and the fungus *Clonostachys rosea* showed antibiotic removal rates up to ~96 % for tetracycline, ~91 % for oxytetracycline, with moderate efficiency on quinolones and sulfonamides - especially when enhanced with plant hormones like gibberellins (50 mg/L).⁷³

Fungal wastewater remediation, according to Sankaran *et al.*⁷⁴ not only converts organic matter into valuable biochemicals and high-value fungal proteins (like lactic acids, amylase, and chitin), but, it also produces a sizable amount of dewaterable fungal biomass that can be consumed by people or utilized as animal feed. Moreover, fungi have a large number of extracellular enzymes and a high resistance to inhibitory substances, which facilitate the bioremediation of persistent substances. Additionally, it has been discovered that the removal of various pharmaceuticals from wastewater is improved by fungus-assisted algae harvesting.⁷⁵ Certain filamentous fungi have the ability to pelletize, which involves microalgal cells further illustrates the mechanisms by which algae-fungi consortia

remove contaminants. It has been demonstrated that algae-fungus consortia can treat wastewater containing antibiotics. For example, biopellets made of *Aspergillus niger* and *C. vulgaris* have demonstrated a significant ability to remove ranitidine.⁷² Additionally, it has been discovered that the removal of various pharmaceuticals from wastewater is improved by fungus-assisted algae harvesting.⁷⁵ Apart from their potential high removal rate, biopellets enable harvesting through sieve filtration or sedimentation, which drastically lowers treatment expenses. Adding fungi and algae to promote co-pelletization eliminates the need for additional energy or chemical inputs and could be a promising treatment approach.

Microalgae - Genetic Engineering

A desired trait can be introduced into a target organism through genetic engineering. Previously, this method was employed to develop engineered algae with enhanced metabolic activity and functionality.³⁴ Genetic engineering using engineered microbial strains or consortia has also been applied to enhance specific metabolic activities or to enrich microbes with particular functions.³⁴ It has been demonstrated that microalgal clones modified with functional enzyme genes, such as laccase, increase the stability of oxidoreductases, ensuring efficient bioremediation of contaminants.⁷⁶ Zhang *et al.* examined the ability of an enriched bacterial consortium to biodegrade chloramphenicol.⁷⁷ The use of targeted genome editing to modify microalgal strains and introduce functional genes into their genomes is growing in popularity.⁷⁸ For instance, when the *linA* gene from *P. paucimobilis* UT26 was introduced into *Anabaena* sp. PCC7120, lindane removal increased even in the absence of nitrate.⁷⁹ Microalgal ploidy, selection agent sensitivity, and cell wall composition and structure continue to be major barriers to microalgal genetic engineering.⁸⁰ Algal cell walls are very resistant to infiltration because of their complex heteropolymer composition.⁸¹ More research should be done on the utilization of microalgae and genetic engineering methods to expand their usage in wastewater bioremediation.

Microalgae – Nanoparticles

Due to its better degradation efficiency, nanotechnology has replaced traditional methods for breakdown antibiotics in wastewater. Typical characteristics of nanomaterials include a high

specific surface area, size-dependent characteristics, high reactivity, and a high degree of functionalization. Owing to these properties, nanomaterials can be effectively used for water purification and wastewater treatment.⁸² The synthesis of nanoparticles involves a variety of compounds that are produced by microalgae, including proteins, carbohydrates, lipids, nucleic acids, vitamins, and minerals.⁸³⁻⁸⁵ According to some studies, nanofibers are the most effective supportive carriers for encapsulating and immobilizing microalgae. As a result, bio-integrated hybrid materials have been developed that are recyclable, more user-friendly, and more effective in eliminating pollutants.⁵⁵ Using processes like bioadsorption, biodegradation, and the application of novel technologies, microalgae which are frequently supplemented with nanoparticles have demonstrated encouraging efficacy in eliminating antibiotics from wastewater. It has been demonstrated that adding nanoparticles, particularly metal oxides, improves the effectiveness of antibiotic removal.⁸⁶

Factors Affecting Antibiotic Removal by Microalgae

pH affects cell surface charge and antibiotic ionization. For most species, a pH between 6 and 9 is ideal.⁸⁷ Higher temperatures (20–30°C) increase metabolic activity but may decrease thermolabile compound adsorption.⁸⁸ Intensity of Light play a vital role in biodegradation. Studies showed that degradation of β -lactam antibiotics is accelerated by UV light.⁵⁵ Unbalanced ratios, such as a high N/P ratio, can inhibit microalgal growth. Studies revealed that for *Chlorella* C: N:P of 100:16:1 is optimal.⁸⁹ Regarding antibiotic Concentration, Fluoroquinolones and other lipophilic antibiotics (log Kow > 3) adsorb more effectively than hydrophilic ones.¹³ Species-Specific efficiency is crucial in determining the degradation efficiency. For example, *Chlorella* spp. and *Scenedesmus* spp. show high removal rates for tetracyclines and sulfonamides.⁹⁰ In algal-bacterial Synergy, bacteria enhance degradation through co-metabolism e.g., *Pseudomonas* spp. with *Chlorella* for sulfamethoxazole removal.⁹¹

Conclusion

Microalgal technology holds great potential for treating wastewater containing antibiotics since it can efficiently extract antibiotics and other pollutants from wastewater while additionally producing biomass that can be used for other purposes.

Microalgae may effectively eliminate antibiotics from wastewater through a variety of mechanisms, including bioadsorption, biodegradation, and bioaccumulation. The process of bioadsorption by algal cells occurs when antibiotics are adsorbed onto organic substances produced by the cell or to components of the cell wall. The ability of living organisms to bioaccumulate depends on various chemical, physical, and biological processes. Through catalytic metabolic reduction, complexed substrates may be broken down into simpler molecules as part of biodegradation. This reduces the negative environmental effects of antibiotic pollution. An additional economic advantage is that the biomass produced during treatment can be utilized for various applications, such as biofuel production, animal feed, or fertilizer. Overall, microalgal technology shows great promise in addressing the problem of antibiotic contamination in wastewater and advancing environmentally sustainable water treatment methods.

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from Other Sources

Not Applicable

Author Contributions

- **Aswin L:** Data collection, Writing the Original Draft
- **Lincy Davin:** Review and Editing
- **Nitha B:** Visualization, Supervision and Final Editing of manuscript
- **Noha Laj:** Data Collection
- **Harikrishnan Gopalakrishnan:** Data Collection

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