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Preparation of Organic Compost from Kitchen Waste and its Efficiency: Managing Waste at House-Hold Level

Dolon Dhaurya[1], Kinjal Upadhyay[2]

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Abstract

Composting is one of the feasible waste disposal methods used by municipal corporations. This study was aimed to assess the use of kitchen waste in home composting, which can be used in home gardens as plant fertilizer, which is more economical and non-hazardous than commercial chemical fertilizers. Solid waste like vegetable scraps, fruit peels, tissues, newspapers, dry leaves and twigs were used as raw materials for composting in a plastic bag and bucket. A plant growth study was carried out to check the efficiency of compost in plants. Plant with compost from plastic bucket shows the highest efficiency compared to other. We performed different tests to estimate the physio-chemical properties of compost and showed that the prepared compost was a dark brown color. Total C: N ratio was 10.1,13.2 and 3.1 in sample 1, sample 2 and control, respectively. The pH of the compost was alkaline after two months of composting. The results revealed that compost in plastic bag has less nutrient content. Nutrient loss can be a significant drawback of home composting.

Keywords: Composting; Plastic Bag; Plant Growth study; Physio-Chemical Properties; Nutrient Loss.

INTRODUCTION

Solid waste management in metropolitan cities is becoming a challenging task for authorities due to increasing waste generation and its cost of management. Nearly 14-30% of total waste is house-hold waste. There were chances that when one throws this garbage, it ends up in the landfills, taking up rooms and polluting the environment. Wastes are often managed improperly; e.g., wastes were dumped by the roadsides, disposed of in rivers or oceans and directly burnt. These practices draw unwanted breeding of pests and insects, releasing harmful gases and offensive odors that contribute to global warming. This garbage can is used for home composting purposes rather than dumping. Home composting is the process of using the house-hold waste to make compost at home. Materials like food scraps, vegetables or fruit peels, cardboards and tissues were organic waste generated at home which can be biologically decomposed. Home composting can be practiced within house-hold for various advantages, such as reducing the waste disposal cost, a convenient way to handle waste and a free-soil amendment.

Author Affiliation: [1]Student, [2]Assistant Professor Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

Corresponding Author: Kinjal Upadhyay, Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

E-mail: kinjal.upadhyay@sxca.edu.in

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The waste produced doesn't have proper disposal and as of today, solid waste is usually dumped and contains many toxic substances. This problem can be substituted by composting, biodegradation and bioremediation. Biotechnological methods can be used in the advancement of solid waste management. The increased population leads to a drastic increase in waste production, affecting society (Lagerkvist and Dahlen, 2019). The importance of waste management cannot be neglected. Improper waste management and disposal affect humans and the environment. (T. Odonkor *et al.* 2019) a study assessing solid waste management among house-holds in a large Ghanaians district. They selected through random sampling, cluster and systematic sampling of 600 for the study. Results indicate that the communal waste collections bins were far from the homes and some respondents confirmed only a few garbage bins between 11-15 minutes before the refuse site. The distance between houses and the disposal site is around 1-2 hrs. (T. Odonkor *et al.* 2019). In conclusion, bins were few in the community, which leads to improper waste collection.

Sago bark waste is abundant in sago processing; this release of waste cause pollution and harms aquatic life. (Wahi, R., Bidin, E.R., Mohamed Asif, N.M. *et al.* 2019) Sago Bark waste used composting to produce compost for agricultural purposes. The physio-chemical and phytotoxicity of the prepared compost were observed to be evaluated and assessed towards aromatic lettuce via seed germination. (Wahi, R., Bidin, E.R., Mohamed Asif, N.M. *et al.* 2019). The results show the compost color was dark brown. Total C: N observed 21.63, 13.38,4.91 during the first, second and third months respectively. The pH was alkaline after three months. The GI for studies vegetables was more than 100% after five days after germination.

Tiquia, S., Richard, T. & Honeyman, M. 2000) studied the loss of nutrients in composting process in their research article. Hoop manure was composted in windrows with nitrogen mass balance during the feeding process. Treatments include with and without turning and manure spreader. It resulted in greater C, K and Na losses in the turned windrow treatment. C has a higher loss in turning windrows, whereas the nitrogen loss does not affect turning. But the composting in the windrow was faster than without turning. It was found that a low C: N ratio during initial composting affects nutrient content. P, K and Na loss were high due to run-off and leaching from the hoop manure. Nutrient loss due to volatilization, run-off and leaching during compost may significantly contribute to groundwater pollution. Composting is a sustained technology, though it has many shortcomings that have reduced its usage and efficiency, such as low nutrient, odor production, pathogen detection and lengthy process duration. Composting can be helpful in agriculture, but the only challenge is the long duration. They discuss methods of proper waste management, long-duration composting and the reason behind them. The development of odor trapping techniques, extraction of mono-fertilizers from compost, tests for heavy metals and pathogens through strips. Activators and slow degradable raw materials, when added to compost, show improvement in compost's nutrient quality. These aid in the enhancement of composting quality. (Ayilara *et al.* 2020)

MATERIALS AND METHODS

Solid waste like vegetable scarps, fruit pulp and peels, coffee or tea bags, twigs and fresh and dry leaves were collected for raw material in composting.Two different containers were selected; plastic bag and bucket that were available at every house-hold as a garbage bins. It is cheap, requires no tools and is reusable. Photographs of containers are depicted in fig. 1.

a) b)

Fig. 1: container - a) plastic bag b) plastic bucket

Preparation of compost

Step 1: Setting the container plastic bag and bin were selected with desirable size and holding capacity. These two containers were located in a dry and shady spot. Step 2: Material gathering; the next step in home composting was to gather the waste for the compost layers. These were tea bags, shredded papers, tissues, vegetables and fruit scraps. To maintain a proper C:N ratio, collecting 2 parts of brown matter to one part of green compost matter is advisable. Shredding and cutting the materials before adding them to containers aid in faster decomposition. Step 3: Materials were added to the bin. Starting with a layer of dry leaves, some water was added to moisten and then some fresh leaves and top soils were added. Cover the containers (plastic bag and bin) with plastic bag. There were no holes in the bin; thus, holes were created in them to cover for aeration and drainage. Step 4: maintenance since we were following aerobic composting, it requires a large container and essential turning of the pile in intervals.

The efficiency of compost through plant growth

We conducted three separate plant growth studies to compare how the prepared composts from different containers affect plant growth when used as fertilizer for the soil.

Fig. 2: labeled pots

Malabar spinach or Basella alba, commonly known as poi plants seeds, were sown in pots. It is an edible fast-growing vine that tastes somewhat like spinach. In summer, these plants grow well, while in colder climates, growth slowdown and burgundy buds appear. Three separate pots were labeled, such as pot 1, 2, 3 having different prepared compost. In pot 1, we added 75% compost (prepared in the bag) and 25% garden soil; in pot 2, we added 75% compost (prepared in the bucket) and 25% garden soil; last, pot 3 was kept as control which had only garden soil. Seeds were sown 0.25 inches deep; usually, seeds take 10-12 days to germinate. For better growth, it needs a pH between 5.5 to 8.0. they were watering the plant regularly to keep the soil moist. Data were analyzed statistically. Plant height, internodal length and leaf counts were plant growth parameters that weremeasured after germination from different pots-keeping all the pots in the same setting to minimize any variation in temperature, lighting, pests and other environmental factors. The stages of plant growth, from germination to elongation, have been shown in fig. 3.

Fig. 3: Stages of plant growth

Physio-chemical test on prepared compost

Fig. 4: Packaged samples

*-Tests performed on the sample followed standard methods given in Schedule – IV [see clause 2(h) and (q)] Part – A and B FCO-1985

The collected samples performed physiological parameters including odor, nutrient content, bulk density, electrical conductivity, C:N ratio, pH and pathogenicity tests*.

RESULTS

Compost process

In 1st week, Scraps started to produce a foul smell Release some amount of water; In 3rd week, some bugs and mites were observed and pH* was found to be slightly acidic to neutral. 5th weeks Pile was settled down from its original height. Moisture content was high. 7th week, Maggots and worms were observed and pH* was found to be too neutral. 10th week's compost is a little dusty No ammonic smell present, but just a rotten unpleasant odor.12th week compost had a texture like dark soil with no foul odor and small organic pieces observed.

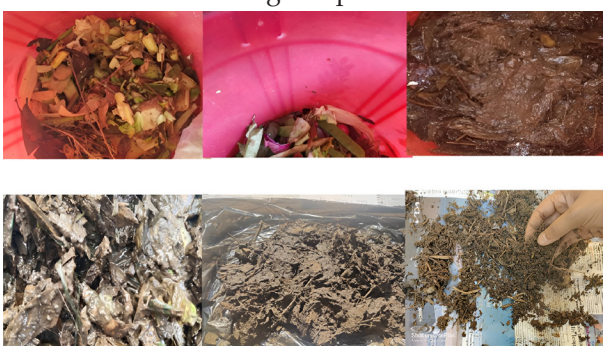
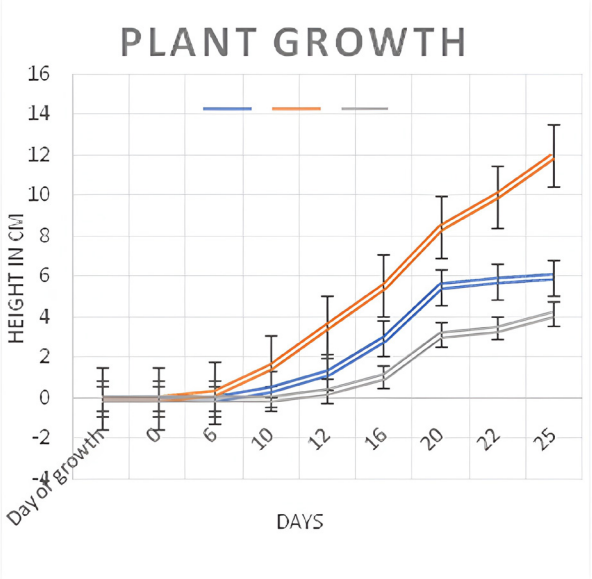


Fig. 5: Weekly observation in compost

*Litmus paper was used for pH determining during weekly records at home. The pH decreased from 6.7 in the first month to 8.3 in the mid-third month of composting. A normal pH measurement for compost is between 6.5-8 because of the low acidification; the microbes will be active as it related to an anaerobic condition inside the compost

Plant growth study

The graph shows how plant height changes with time. (In orange) the straight-line graph obtains for the plant in pot 2, whereas plants in pot 1 and 3 (blue and grey) show straight lines, but it becomes uniform after time. Not any significant difference in height.



Graph 1: Plant rate in the height of Malabar spinach plant

Pot 2, seed germinatefaster than the other two pots.

The growth rate of the plant in pot 2 was best as compared to other plants in pot 3; the leaves were paler and yellowish

Statistical methods studied other parameters such as leaf count, internodal length and plant height. In table 1, Ho: internodal length does not affect plant height. HA: internodal length affects plant height. Table a, b and c shows p-value between $0.01< p> 0.001$. Interference that there is solid evidence against the null hypothesis. The statistically significant value of $p< 0.05$ Elongation of internodes involves many cell divisions followed by cell elongation. At this point, growth

in thickness involves some radial cell division and cell enlargement.

Table 1: Plant growth parameters in the different pots containing compost

Pot - 1		
Day of growth	**Height of plant (cm)**	**Internodal length (cm)**
0	0	0
6	0	0
10	0.5	0
12	1.3	0
16	3	0.2
20	5.5	0.2
22	5.8	0.3
25	6	0.8
27	6.7	0.9
31	7.5	1.5
Mean	**3.36**	**0.39**
test p-value	**0.0032**	
(A)		

Pot - 2		
Day of growth	**Height of plant (cm)**	**Internodal length (cm)**
0	0	0
6	0.3	0
10	1.6	0
12	3.6	0.4
16	5.6	0.5
20	8.5	0.9
22	10	1.5
25	12	1.7
27	12.4	2.5
31	13	2.6
Mean	6.7	1.01
Test p-value	**0.0019**	
(B)		

Table Cont...

Pot 3		
Day of growth	**Height of plant (cm)**	**Internodal (cm)**
0	0	0
6	0	0
10	0	0
12	0.4	0
16	1.1	0
20	3.2	0.6
22	3.5	0.9
25	4.2	0.9
27	5	1.4
31	5.8	1.6
Mean	2.32	0.54
Test p-value	**0.0078**	
(C)		

Results show that compost in pot 2 significantly affects the growth of Basella-producing plants with better height, some leaves, internodes and stems. Thus, compost prepared in the bucket has the highest efficiency.

Physio-chemical analysis

Table 2: Physio-chemical parameters of prepared compost

Tests (Quality characteristics)	S1 (Compost from plastic bag)	S2 (Compost from plastic bucket)	S3 (Soil)	Range
odour	Absence of foul odour	Absence of foul odour	Absence of foul odour	Absence of foul odour
colour	Dark brown to black	Dark brown to black	Dark brown to black	Dark brown to black
Particle size (passes through 4mm sieve)%	92.32	90.12	99.25	Above 90%
Moisture %	24.5	26	18.92	25-40
Bulk density g/cm3	1.09	1.08	1.02	Less than 2.0
Conductivity dsm-1 in 20% sol	1.09	2.04	3.8	Not more than 4.0
pH in 10% sol	8.3	8.7	7.3	5.5-8.5
C:N ratio	10:1	13:1	3:1	5-25:1
Total organic carbon%	2.48	4.76	1.61	12.0
Total nitrogen as N %	0.3	0.4	0.5	0.4-1.5%
Total phosphate as P2O5 %	0.3	0.2	0.3	0.3-0.9%
Total potash as K2O %	0.5	0.2	0.3	0.3-1.9%

The moisture content of all samples was below than range. Excess turning and heat can make the moisture vapourised and make the compost dry and hard. Total organic carbon content was below the range. Sample 2 has less phosphate and potash percentage than other samples. Sample 1 had less nitrogen content. Results show that control had the highest nutrient content followed by sample 1 and lastly by sample 2.

DISCUSSION

Moisture content may be low due to excessive turning, which results in drying out. Below range may limit the activity of microbes and if the high result in anaerobic process and foul smelling. Anaerobic process will produce methane gas and hydrogen sulphide, which were greenhouse gases; thus, they should monitor well. Moisture can be maintained by if compost is too soggy- adding some dry leaves or paper waste and if it is too dry- sprinkle some water and turn it well. Tequila *et al.* found that moisture was less in hoop manure due to moisture soak-up in bedding and N- losses in the deep bedded hoop were the leading cause of gas emission. (Thelosen *et al.,* 1993; Groen estein and Van Faassen, 1996). Dewes (1996) reported that N loss starts immediately after the animal waste was excreted. As the compost was stored in unsealed, there is potential for leaching and volatilization losses. Nutrient loss may be happened due to leaching or drying out since potassium and phosphorus were minimal and vulnerable to volatilization. Nitrogen is especially susceptible to volatilization when ammonium is transformed to ammonia. Some of the N loss can be attributed to microbial denitrification of NO, N2O and N2. (Thelosen *et al.,* 1993). Consistent and extensive application of organic compost may lead to the accumulation of heavy metals and toxicity. Heavy metals can reduce soil microorganisms' growth, morphology and metabolism, consequently affecting soil fertility (Bragato *et al.,* 1998). Chiroma *et al.,* 2014) suggested that regular consuming of heavy metals by plants can affect environment and human due to accumulation through food chain. They suggested that lime, bamboo charcoal and natural zeolite tended to reduce availability and leachability of heavy metals. (Singh & Kalamdhad, 2013) advised that before adding composted manure in agricultural fields; metals should be assessed. Odour is one of the problems in composting, so some odour trapping device can be built in the container.

DNA sequencing techniques enable a proper understanding of microbial behaviour and enzyme functioning in composting. Major function of these enzymes included in waste production, greenhouse gas production and odour production. The addition of bulking agents can prevent nitrogen loss in the manure composting. Peat moss, rice hull was some of bulking agents have been used in past as they have high water and cation absorption capability. (Barrington and Moreno 1995).

CONCLUSION

Home composting may be an effective disposal method, but nutrient loss is a significant drawback of home composting as most of the nutrients, especially N, is lost during the composting period. Among the two composts, compost prepared in a plastic bin shows the highest efficiency in physio-chemical properties and this compost has a significant positive effect on the growth of Basella Alba. It concluded that home composting can help to ensure environmental sustainability since it lowers the waste disposal cost and gives a soil amendment that can be used in home garden, therefore saving the cost of external fertilizers for plants. This compost will increase the land's health, productivity and beauty.

Home composting can be a solution to every community's solid waste management. Our long-term goals include a study on microbial activities in compost, limitations of composing and its solution. Future plans include a society composting plan in which organic waste from every house-hold can be converted into compost that can be reused in home gardens and excess can be sold in the market. Here each house can get 1 rupee per day giving organic waste, but this plan more innovative construction as well as waste management tools and microorganisms.

Conflict of interest

There are no conflicts to declare

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Clinical Identification & Anti-fungal Susceptibility of *Candida Species* by Vitek-2 System

Sheetal Sharma[1], Vipin Kathuria[2], Kuldeep Yadav[3], S.K Datta[4]

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Abstract

Introduction: Among fungal infections, invasive candidiasis is often associated with increased number of morbidity and death rate. Candida species causing so many infections range from non fatal mucocutaneous infections to fatal blood stream infections, so the aim of this study is isolate and speciate the Candida species and their anti-fungal susceptibility to avoid unnecessarily consumption of anti-fungal drugs.

Material & Methods: A Prospective study which was carried out in the Department of Microbiology, Pacific Institute of Medical Sciences Udaipur. Specimen collected from the various sites were collected and cultured on SDA agar and incubated. Identification of Candida species was done through Hi-Crome Candida Differential Agar and anti-fungal susceptibility was evaluated by VITEK-2 automated system and results were calculated through statistical analysis.

Results: Out of 64 *Candida* isolates, 34.38% were *Candida albicans*, followed by *Candida tropicalis* (31.25%) *Candida krusei* (20.31%), *Candida kefyr* (12.50%) and *Candida glabrata* (1.56%). The most common *Candida* species isolated from urine were *Candida albicans* (34.24%) followed by *Candida tropicalis* (31.25%). Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates followed by Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). All the *Candida kefyr* isolates were susceptible to Voriconazole, Amphotericin B and Flucytosine and resistant to Fluconazole. all *Candida glabrata* isolates were susceptible to all the 4 drugs except Micafungin and Caspofungin.

Conclusion: The present study shows the distribution of *Candida species* in various clinical specimens and also revealed that *Non-albicans Candida* species are emerging as the predominant species. The increased resistance of *Candida* isolates towards common anti-fungal drugs which is a concern around all over the world.

Keywords: Composting; Plastic Bag; Plant Growth study; Physio-Chemical Properties; Nutrient Loss.

Author Affiliation: [1]Associate Professor, Department of Microbiology, R.G Medical College & Research Centre, Hathras 204216, Uttar Pradesh, [2]Assistant Professor, Department of Pathology, Sarswati Medical College & Hospital, Hapur 245304, Uttar Pradesh, [3]Associate Professor, [4]Professor & HOD, Department of Microbiology, Pacific Institute of Medical Sciences, Udaipur 313015, Rajasthan, India.

Corresponding Author: Sheetal Sharma, Associate Professor, Department of Microbiology, R.G Medical College & Research Centre, Hathras 204216, Uttar Pradesh, India.

E-mail: ssharmabyc@gmail.com

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INTRODUCTION

The primary cause of the elevated rate of mortality and morbidity is fungus infections in the patients who are immunocompromised and in ICU patients. Among fungal infections, Candida is the commonest pathogenic organism causing invasive infections resulting in increased hospitalization and life threatening conditions.[1] Candida is yeast like fungus that produces pseudo-hyphae. *Candida* species are very common residents of skin, gut and genitals area but occasionally these fungi causes variety of infections, which ranging from the non fatal mucocutaneous infections to invasive Blood Stream Infections (BSI) or systemic infections in immunocompromised patients. From several studies of last few years, it is found out that non-albicans Candida species have now become predominant.[2]

There are about the 20 different species of the *Candida* that are known to cause infections in the humans.[3] Invasive *Candida* (IC) infections are broadly reported in critical patients, admitted in the ICU. The important risk factors for invasive Candidiasis included organ transplants, over consumption of broad-spectrum antimicrobial agents, prolonged Hospitalization, surgery, advanced life support and pugnacious chemotherapy, older age (over 60 years), chronic renal failure and diabetes mellitus, gastrointestinal or cutaneous colonization.[4] In recent years, the epidemiology of invasive candidiasis (IC) has gradually changed all over the world.[5] The *non-albicans Candida* species emphasized the significance of identification of the *Candida* isolate's infecting species for commencement of prompt and efficient therapy, particularly when anti-fungal susceptibility results are not readily available.[6] In these medical conditions, the commensal Candida may convert into opportunistic pathogenic microorganisms causing candidiasis in host.[7,8] The potential of clinical significance in the speciation has been acknowledged as Candida species shows dissimilarity in the expression of putative virulence factors and anti-fungal susceptibility.[9] Surveillance of fungal ecology and the anti-fungal resistance either within patients in ICU or within Candida species is necessary for the superintendence of invasive fungal infections.[10]

MATERIAL AND METHODS

Study place: Department of Microbiology, Pacific Institute of Medical Sciences Udaipur.

Study design: Prospective study.

Study duration: One year

Total of 230 patients were tested for *Candida* infection. Patient's clinical samples including urine sample from mid-stream urine, nasal swab, End tracheal tube, Stool, Central line tip, Pus, Pleural fluid, Throat swab or sputum sample, skin scrapings from the infected part, blood samples and vaginal swabs etc. were collected as per SOP. All the specimens were cultured on Nutrient Agar, Blood Agar and MacConkey Agar for the primary inoculation. After incubation of 24-72 hours, the colonies grown on culture stained with gram stains and microscopy is done. If the mix growth is obtained than the colonies were separated by subculture on Saboraud's Dextrose Agar with antibacterial antibiotics incubated at 25°C and 37° C. Colonies are appeared in 1-3 days. Identification was done on the basis of colony morphology (On Sabouraud's Dextrose agar colonies were creamy white, smooth and with a yeasty odour) and microscopy. In microscopy purple budding yeast cells are seen. Further the identification of Candida species was done with various methods. The method of Candida species identification was Hi-Crome Candida Differential Agar. Inoculation was done for differentiating the Candida species based on the colour pigmentation on Hi-Crome Candida Differential Agar.

Anti-fungal susceptibility testing by automated method VITEK-2 (BioMerieux)

We used the VITEK-2 to check the susceptibility of fungal agents against different fungal drugs. 3 ml of sterile normal saline taken in polystyrene tubes, picked a colony from culture of Candida isolates and it was mixed in the saline. It was mixed properly. The Densichek plus instrument provided by the BioMerieux was used to check the density of prepared inoculums up-to McFarland standard 2.0 for (VITEK-2 YST cards). Now the 280 ml of prepared 2.0 MacFarland suspension was picked with pipettes and mixed it with another tube with 3 ml of saline and mixed it properly by pipetting. VITEK-2 AST cards were placed into tubes. Tubes were placed with VITEK-2 AST cards in the cassettes and after inserting the cassettes into the VITEK-2 device, the corresponding yeast AST cards were loaded, incubated and automatically read by the device. The growth rate in the drug-free control well determined the length of incubation, which ranged from 18 to 20 hours. The outcomes were reported as MICs, or micrograms per millilitre. The density of the inoculums was checked by the

Densichek plus instrument was used to check the density of prepared inoculums.

Fig. 1: Automated Vitek-2 System (BioMerieux)

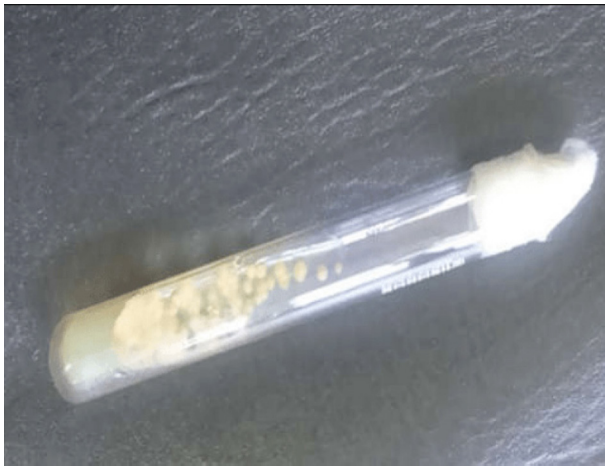

Fig. 2: SDA showing smooth colonies of *Candida albicans*

RESULT

Distribution of Candida species according to sample type

The most common sample from which Candida were isolated was urine (53.12%). Then other samples were sputum (20.30%) and vaginal swab (12.50%). Percentage of Candida species isolated from nasal swab were (4.68%), central line tip (3.12%), blood (1.57%), Et. Tube (1.57%), stool (1.57%) and pus (1.57%).

Table 1: Candida species distribution based on sample type

No.	C. Species	C. albicans	C. tropicalis	C. krusei	C. kefyr	C. glabrata
1	Urine	13 (38.24%)	11 (32.35%)	8 (23.53%)	1 (2.94%)	1 (2.94%)
2	Nasal swab	–	–	–	3 (100%)	–
3	Vaginal swab	4 (50%)	–	3 (37.5%)	1 (12.5%)	–
4	Blood	–	–	1 (100%)	–	–
5	Et. Tube	–	1 (100%)	–	–	–
6	Sputum	5 (38.46%)	5 (38.46%)	1 (7.69%)	2 15.38%)	–
7	Stool	–	1 (100%)	–	–	–
8	Central line tip	–	2 (100%)	–	–	–
9	Pus	–	–	–	1 (100%)	–
10	Pleural fluid	–	–	–	–	–
	Total	**22 (34.38%)**	**20 (31.25%)**	**13 (20.31%)**	**8 (12.50%)**	**1 (1.56%)**

Table 1 shows that the most common *Candida* species isolated were *Candida albicans* (34.38%), followed by *Candida tropicalis* (31.25%). Other Candida species isolated were Candida krusei (20.31%), Candida kefyr (12.50%) and *C. glabrata* (1.56%). It shows the most common *Candida* species isolated were *Candida albicans* (34.28%) from urine (38.24%) followed by sputum (38.46%). The 2nd most common isolated *Candida* species were *Candida tropicalis* (31.25%) from urine (32.35%) followed by sputum (38.46%). The other species isolated were *Candida krusei* most commonly from Urine (23.53%) followed by vaginal swab (37.5%). *Candida kefyr* (12.50%) were isolated most commonly from nasal swabs (100%). *Candida glabrata* were isolated from urine (2.94%). According to results the *non-albicans Candida* species are predominant in all the clinical samples.

Table 2: Antifungal susceptibility pattern of Candida isolates by automated method (VITEK-2)

Drugs	Sensitive	Intermeduate	Resistance	TRM
Fluconazole	40 (62.50%)	0	24 (37.5%)	0
Flucytosine	51 (79.69%)	0	10 (15.63%)	3 (4.69%)
Voriconazole	59 (92.19%)	0	0	5 (7.81%)
Amphotericin B	57 (89.06%)	0	0	7 (10.94%)
Micafungin	45 (70.31%)	0	0	19 (29.69%)
Caspofungin	49 (76.56%)	3 (4.69%)	0	12 (18.75%)

Table 2 shows the total sensitivity pattern by Automated Method and it revealed that Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates. The antifungal sensitivity patterns showed by other drugs were Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). 4.69% isolates show intermediate sensitivity for Caspofungin. Some drugs Micafungin in 29.69% isolates and Caspofungin in 18.75%, Amphotericin B in 10.94%, Voriconazole in 7.81% and Flucytosine in 4.69% isolates were terminated by Machine.

Table 3: Antifungal susceptibility pattern in various Candida species by automated method VITEK-2.

Drugs	Candida Albicans		Candida Tropicalis		Candida Krusei		Candida Kefyr		Candida Glabrata	
	Sensitive	I/R/ TRM	Sensitive	I/R/ TRM	Sensitive	I/R/TRM	Sensitive	I/R/TRM	Sensitive	I/R/TRM
Fluconazole	17 (77.27%)	5 R (22.73%)	19 (95%)	1 R (5%)	2 (18.18%)	11 R (81.82%)	0	8 R (100%)	1 (100%)	0
Flucytosine	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	3 (23.08%)	10 R (76.92%)	8 (100%)	0	1 100%)	0
Voriconazole	17 (77.27%)	5 TRM (22.73%)	20 (100%)	0	13 (100%)	0	8 (100%)	0	1 100%)	0
Amphotericin-B	20 (90.91%)	2 TRM (9.09%)	17 (85%)	3 TRM (15%)	12 (92.31%)	1 TRM (7.69%)	8 (100%)	0	1 100%)	0
Micafungin	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	6 (46.15%)	7 TRM (53.85%)	0	8 TRM (100%)	0	1 TRM (100%)
Caspofungin	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	9 (69.23%)	3 I/1 TRM	0	8 TRM (100%)	0	1 R (100%)

Table 3 shows, the pattern of anti-fungal susceptibility in different *Candida* species using VITEK-2. According to the pattern, Candida albicans was most responsive to fluconazole and voriconazole (77.27%), followed by flucytosine, amphotericin B, Micafungin and capsofungin (90.91%). Candida tropicalis was most sensitive to Flucytosine, Voriconazole, Micafungin, Caspofungin (100%), followed by Fluconazole (95%) and Amphotericin B (85%). Candida krusei was most sensitive to Voriconazole (100%) and Amphotericin B (92.31%), followed by Caspofungin (69.23%) and Micafungin (46.15%). Candida kefyr showed complete sensitivity (100%) against

Flucytosine, Voriconazole and Amphotericin B. Candida glabrata showed complete sensitivity (100%) against Fluconazole, Flucytosine, Voriconazole and Amphotericin B.

DISCUSSION

Candida infections are among the most prevalent causes of the morbidity and mortality in the whole world. Among them *Candida* is also a major concern worldwide due to its increasing incidence and also the increased resistance towards drugs. Fast and very accurate identification of *Candida species* and their anti-fungal susceptibility pattern are of great importance for the selection of appropriate anti-fungal agent and for patient management. The very common *Candida species* isolated were *Candida albicans* (34.28%) from urine (38.24%) followed by sputum (38.46%) shows very similarity with the results of A. Rengaraj. *et al.* study in 2019.The present study reported that the burden of *Candida albicans* species and non-albicans species to be 34.38% and 65.62% respectively. In our study the *Non-albicans Candida* species showed dominance on *Candida albicans* species. Which is similar to Reshma Bhaskaran *et al.* study in 2020, also showed predominance of non-albicans *Candida* species over *Candida albicans* species.

In many previous studies *Candida albicans* was still the most predominant isolated species as showing in some recent studies also Seyoum E. *et al.* study in 2020 comparatively to non *albicans Candida* species which shows quite differences from our study and the reason behind differences occurred in the studies or this shifting the trend of *Candida species* could be the geographical area, age, different hospital settings, different departments, environmental conditions, co-morbidity, immunosuppression, underlying diseases and antibiotic therapies etc.

In our study Vitek-2 is used to evaluate the anti-fungal susceptibility of all candida isolates and it revealed that In Our study all the Candida isolates showed high susceptibility towards Voriconazole (92.19%) which is co-relatable with studies Singh R *et al.*[14], Sundaram M. *et al.*[16] and Seyoum *et al.* Amphotericin B (89.06%) were found the second most sensitive drugs against the Candida isolates and results of our study co-relate with Kaur R *et al.*[14] and Sundaram M. *et al.* For Micafungin and Caspofungin results are correlated well with the study Sundaram M. *et al.*In our study Fluconazole shows less sensitivity against Candida isolates (62.50%) which showed difference with the result in Elias Seyoum *et al.* study in 2020, in which 85.6% of Candida species were susceptible to Fluconazole.

Flucytosine (79.69%), Caspofungin (76.56%) and Micafungin (70.31%) also showed good sensitivity against all the candida isolates. The shifting pattern of Candida species' distribution among isolates and altered anti-fungal susceptibility patterns by the time is the major concern worldwide that's why to prevent these alterations, a diagnosis of the Candida species and an assessment of the susceptibility to antifungal are therefore essential.

CONCLUSION

The current study demonstrated the distribution of different *Candida* species in a range of clinical samples. It also showed that non-albicans *Candida* species are becoming more common and that isolates of *Candida* are becoming more resistant to common anti-fungal medications, which is a global concern. The use of conventional methods for identification and anti-fungal testing of *Candida species,* these methods are not only time consuming but also labour intensive. Commercially available automated systems provide a good option. However their use in a resource constrained setting has not been extensively studied. The VITEK 2 system ensures that each test is performed in a standardized manner and provides quantitative MIC results are very reproducible and so accurate. Use of VITEK 2 will help reduce the turnaround time for identification and susceptibility and reduce labour. When the benefits in terms of cost, labour and ease of performance was considered, VITEK 2 can be a preferable option in resource constrained settings.

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Original Article

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Blood Metagenomic Sequence Analysis for Evaluation of Chronic Systemic Infections

Mohammed Shakeel[1], Ayla Sanjay[2], Zainab Kausar[3], Vadlamudi Nikhil[4], Chand Pasha[5]

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Abstract

Blood systemic infections (BSIs) are major threat in hemodialysis patients. BSIs are diagnosed by blood culturing for bacteria and serology for virus infections. These two methods are time taking and expensive and not covering all BSI microbes. An attempt is made to compare microbial culturing and viral screening with serology by ELISA with blood metagenomic sequencing and sequence analysis. In blood agar media culturing 7 microbes (6 bacterial and 1 fungus), in ELISA screening 0 viruses and in metagenomic sequence analysis 24 microbes (19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma) were detected. Unculturable bacteria are also detected by metagenomic sequence analysis. Hence metagenomic sequence analysis can be best as method for effective detection of BSI's.

Keywords: Meta genomic DNA sequence analysis; Systemic infections; Dialysis; Uncultured Bacteria; Blood infection.

Author Affiliation: [1]Research Student, Department of Microbiology, Mumtaz degree and PG College, Malakpet, Affiliated to Osmania University, Hyderabad, Telangana, [2-4]Research Student, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, [5]Senior Assistant Professor & Head, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, India.

Corresponding Author: Chand Pasha, Senior Assistant Professor & Head, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, India.

E-mail: cpasha21@gmail.com

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INTRODUCTION

Chronic systemic infections or Blood Stream Infections are life-threatening and are responsible for up to 20% of deaths worldwide (Rudd *et al.* 2020). They affect the entire body rather than being localized to one specific area. These infections can persist for months or even years, due to the causative agents such as bacteria, viruses, fungus, or parasites. Prompt and timely diagnosis is essential for effective and timely treatment. Clinical laboratories are crucial in controlling systemic infectious diseases, typically conducting microscopic examinations, blood cultures, blood biomarkers and NAA assay (Yu *et al.* 2024). The limited detecting capabilities and sensitivity of these techniques contribute to loss in identifying pathogens in significant cases (Forbes JD *et al.* 2017).

BSIs can originate from various sources, including surgical incisions, catheter-related, lung, blood coming in contact with equipment and atmosphere like dialysis and peritoneal infections. Blood culturing is primary technique for detecting and identifying bacteria and fungus in sepsis, helping to optimize antimicrobial treatment and assess effectiveness (Garcia RA *et al.* 2015). However, it typically takes twenty-four hours and gives false results as positive and negative. A Current culture methods identify only 30–50% cases within the first 2 days, with some species taking up to five days (Gupta *et al.* 2016) and has lower yield detecting only culturable microorganisms, missing nonculturable bacteria.

In recent times, metagenomic sequencing have gained significant focus on detection of pathogens. Metagenomic shotgun sequencing is culture independent technique which analyses genetic material in sample and has shown promising results in clinical practice by detecting microorganisms undetected by conventional tests and by identifying previously unrecognized pathogens (Vijayvargiya P *et al.* 2019). Since the first use of metagenomic sequence for diagnosing a patient in 2014 suffering from an infection (Wilson MR *et al.* 2014), this innovative method has progressively accepted and incorporated in the clinical practice. The major steps of metagenomic sequence involve preprocessing of sample, extracting nucleic acid, preparation of library, sequencing and bioinformatics evaluation (Li N *et al.* 2021). In patients who are hospitalized and assumed of having sepsis, metagenomic sequence outperformed blood culture, especially in those with moderate symptoms, prior to use of antibiotics and early infection (Zuo YH *et al.* 2023). However, metagenomic sequence has drawbacks (Lamy *et al.* 2020) including complex sample preparation, interference from host DNA and cumbersome sequencing procedures. It is one of the promising methods which detects pathogens from complex samples with a broad spectrum, high sensitivity and minimum time, making it effective to identify rare, unknown and abnormal causes. The current study aims to analyse and for comparing the results of metagenomic sequence for detecting organisms not identified by conventional blood culture methods in chronic systemic infections of dialysis patients.

MATERIALS AND METHODS

Patient and sampling

The male patient of age 40 with fistula for the Dialysis located at Dialysis center, Vikarabad. Patient weighted 66 kg before dialysis and after dialysis 62kg. No abnormalities in vitals like BP, respiration rate, pulse and temperature were noted and diagnosed with diabetes mellitus positive recently. The study was permitted by the institutional ethics committee, written consent was taken from patient for clinical research. During dialysis early 10ml Blood was collected as sample.

Bacterial isolation and growth conditions

Samples

Cultured the blood sample on the blood agar by the spread plate technique and further incubated at 37^{o}C for 24 hours.

Characterization

Morphology: The colony and cellular morphology after gram staining was observed under microscope and further biochemical test were performed to identify the specific organism.

Biochemical test: The following biochemical tests were performed to identify the organisms.

Catalase test, Oxidase test, Coagulase test, IMViC Tests, Bile esculin, Germ tube.

Catalase test: Used to detect catalase enzyme presence in bacteria. Positive result is identified by producing visible oxygen by breaking down hydrogen peroxide.

Oxidase test: Used to detect cytochrome c oxidase presence, It is tested by reacting on N,N,N,N-tetramethyl-p-phenylenediamine dihydrochloride substrate.

Coagulase test: Used to identify organisms based on the ability to produce coagulase, the bacterial suspensions are mixed in plasma to check for coagulase activity of coagulating plasma.

Indole test: Used to test the ability of bacteria to produce indole from tryptophan aminoacid.

Methyl Red test: Detects the production of acids from glucose fermentation.

Voges-Proskauer Test: Used to detect the presence of acetoin, produced by fermentation of glucose in bacterial species.

Citrate Utilization test: Used to test the ability of the organism to use citrate as whole carbon source.

Bile esculin: The test used to identify bacteria based on their ability to hydrolzye esculin in presence of bile.

Germ tube: Test used to differentiate between candida species.

Antibiotic Sensitivity assay

The Antibiotic susceptibility test was carried out on Mueller Hinton agar plates after spreading 8 different bacterial cultures and placing HIMEDIA antibiotic discs. Ampicillin, Cephalosporin, Tetracycline, Carbapenem, Monobactam, Sulfonamide, Nitroimidazole, Macrolide, Chloramphenicol, Rifamycin, Fluoroquinolone, Ceftazidime, Elfamycin, Cefepime, Norfloxacin, Levofloxacin, Methicillin, Streptomycin, Augmentin, Kanamycin, Pencillin – G, Gentamycin and Vancomycin antibiotic discs were used. Augmentin antibiotic solution was prepared by adding 20mg Amoxicillin in 10mg potassium clavulanate, the Whatman discs were suspended in solution and dried. Plates were incubated overnight at 37°C and zone of inhibition were measured in mm.

Viral screening by ELISA

Blood sample for HIV, HBV and HCV viruses were screened by ELISA (Ma *et al.,* 2011).

Isolation of DNA

The collected blood sample of the dialysis patient was undergone 2000 rpm centrifugation for 5 minutes and the supernatant was collected in the sterile Eppendorf. Supernatant (Containing microbial cells and cell free DNA, free of all human cells) was used for genomic DNA isolation using QIAamp BIOstic (QIAGEN Germany) Genomic DNA isolation kits as per manufacturer's instructions.

Library preparation and metagenomic sequencing

Library preparation was carried out by using Nextera XT DNA Library preparation kit (Illumina, USA). DNA was prepared, partial cleaved and tagged using the pUC18 plasmid within the Nextera XT Kit (Moghnia *et al.,* 2015). Separate adapters were assigned to each sample for labelling purposes. A 12-cycle PCR reaction was conducted to amplify DNA fragments, incorporating pUC18 primers and indices for dual-indexed sequencing of pooled libraries. Subsequent to sample normalization, pooling was carried out, followed by 300-base paired-end reads sequencing on the Illumina (Novaseq 6000), 150PE instrument (Moghnia *et al.,* 2015). All steps, from preparation to sequencing, adhered strictly to the manufacturer's instructions.

Organism Identification

The obtained nucleotide sequences from metagenomic sequencing were identified in the NCBI portal by running BLASTn (Chen Y *et al.,* 2015).

Statistical Analysis

Experiments were repeated thrice in triplicate (n=9) and value with standard deviation is presented.

RESULTS

The bacterial cultures isolated from the dialysis patient blood samples were identified by colony morphology, Microscopy and biochemical tests as *Pseudomonas aeruginosa, Salmonella enterica serovar, Staphylococcus aureus, Bacillus paranthracis, Escherichia coli, Streptococcus pyogenes* and *Candida dubliniensis* (Table 1)

Table 1: Morphological, Microscopic and Biochemical results of isolated organisms

S.No	Organism	Colony morphology	Microscopy	Biochemical Tests
1.	*Pseudomonas aeruginosa*	Round with a fluorescent greenish colour	Gram-negative	Oxidase positive, Catalase positive
2.	*Salmonella enterica serovar typhi*	Rod-shaped enterobacterium	Gram- negative	Blackening in (H_2S production) TSI agar
3.	*Staphylococcus aureus*	Circular, smooth, convex	Gram-positive	Beta-hemolysis positive
4.	*Bacillus paranthracis*	Circular colonies	Gram- positive	Voges-Proskauer Test positive,
5.	*Escherichia coli*	Rough or a smooth	Gram- negative	IMViC ++--
6.	*Streptococcus pyogenes*	Dome-shaped, smooth	Gram- positive	Bile esculin-negative
7.	*Candida dubliniensis*	Dark green	Gram-positive	germ tube-positive

Antibiotic susceptibility assay:

All seven cultured microbes were found to be resistant to majority of antibiotics tested.

Table 2: Antibiotic susceptibility assay of isolated organisms against various antibiotics and zone of inhibitions (mm)

Antibiotics	Pseudomonas aeruginosa (mm)	Salmonella enterica serovar typhi (mm)	Staphylococcus aureus (mm)	Bacillus paranthracis (mm)	Escherichia coli (mm)	Streptococcus pyogenes (mm)	Candida dubliniensis (mm)
Ampicillin	06±0.02	04±0.02	15±0.45	13±0.43	06±0.15	16±0.5	18±0.6
Cephalosporin	07±0.16	11±0.37	27±0.84	05±0.09	30±0.8	32±0.9	16±0.6
Macrolide	11±0.45	14±0.2	16±0.8	19±0.7	17±0.5	31±0.85	15±0.57
Monobactam	11±0.3	13±0.4	11±0.47	16±0.6	18±0.7	09±0.45	04±0.03
Carbapenem	27±0.9	14±0.53	12±.0.44	13±0.47	26±0.98	11±0.47	14±0.43
Sulfonamide	05±0.119	04±0.14	11±0.36	04±0.14	02±0.74	13±0.45	15±0.48
Nitroimidazole	16±0.04	18±0.52	11±0.47	13±0.48	15±.0.44	17±0.47	21±0.89
Rifamycin	14±0.11	11±0.21	04±0.07	16±0.5	12±0.4	14±0.3	12±0.4
Fluoroquinolone	26±0.86	06±0.26	18±0.65	12±0.45	06±0.15	14±0.55	13±0.54
Elfamycin	14±0.44	16±0.35	11±0.32	19±0.77	15±0.55	19±0.41	16±0.32
Ceftazidime	28±0.61	05±0.04	14±0.44	18±0.44	16±0.44	14±0.32	15±0.52
Cefepime	18±0.56	14±0.32	04±0.08	17±0.54	06±0.15	20±0.08	19±0.04
Norfloxacin	10±0.32	12±0.42	21±0.52	21±0.31	06±0.15	19±0.14	14±0.65
Levofloxacin	15±0.15	16±0.32	18±0.21	11±0.41	16±0.65	20±0.98	18±0.77
Chloramphenicol	13±0.45	28±0.98	14±0.5	29±0.74	16±0.75	17±0.45	18±0.45
Tetracycline	08±0.12	03±0.065	06±0.22	31±1.12	07±0.14	03±0.06	26±0.885
Streptomycin	14±0.32	04±0.12	15±0.55	17±0.52	15±0.42	19±0.74	12±0.22
Augmentin (Amoxicillin & Potassium Clavulanate)	12±0.35	18±0.52	15±0.62	21±0.74	19±0.69	23±0.52	15±0.23
Kanamycin	15±0.45	15±0.32	14±0.21	16±0.65	19±0.72	11±0.12	09±0.98
Pencillin – G	11±0.32	16±0.2	15±0.15	04±0.06	16±0.95	18±0.85	14±0.52
Gentamycin	18±0.52	14±0.45	14±0.39	30±0.97	18±0.66	19±0.71	16±0.22
Vancomycin	15±0.42	03±0.08	30±0.06	26±0.96	29±0.85	14±0.65	19±0.74
Methicillin	07±0.21	21±0.95	05±0.12	10±0.32	15±0.41	18±0.32	18±0.35

Seven bacterial isolates were tested for antibiotic susceptibility against 23 antibiotics, *Pseudomonas aeruginosa* was sensitive to Carbapenem, fluoroquinolone, ceftazidime. *Salmonella enterica serovar typhi* was sensitive to chloramphenicol. *Staphylococcus aureus* was found to be sensitive for Cephalosporin and vancomycin. Chloramphenicol, Tetracycline, Gentamycin and Vancomycin were effective against *Bacillus paranthracis. E. coli* bacteria was sensitive to Cephalosporin, carbapenem and vancomycin. *S. pyogenes* was sensitive to Cephalosporin and macrolide. *Candida dubliniensis* was resistant to all antibiotics tested. All bacteria were resistant to Ampicilllin, Monobactam, Suldonamide, Nitroimidazole, Rifamycin, Elfamycin, Cefepime, Augmentin, Pencillin-G and Methicillin.

Viral screening by ELISA

Viral Serological screening by ELISA testing for HIV, HbSAg and HCV were not reactive.

Meta genomic sequencing Organism identification

With metagenomic sequencing 19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma consisting of 24 organism sequences were identified (Table 3). Only 4 Un culturable bacteria were identified but on blood agar only 6 bacteria were isolated. Out of 19 bacteria 4 are unculturable hence 15 are culturable. But only 6 bacteria are growing on blood agar in lab conditions. Remaining bacteria can be grown by changing media or growth conditions.

Table 3: Organisms identified by metagenomic sequencing and type of organism

Organism identified by mNGS	Organism type
SARS Cov-2	Virus
Pseudomonas aeruginosa	Bacteria
Corynebacterium pseudokroppenstedtii	Bacteria
Shigella flexneri aerobactin	Bacteria
Salmonella enterica subsp. enterica serovar	Bacteria
Staphylococcus aureus	Bacteria
Bacillus paranthracis	Bacteria
Escherichia coli	Bacteria
Streptococcus pyogenes	Bacteria
Hepatitis C virus	Virus
Uncultured *Citrobacter sp*	Uncultured Bacteria
uncultured *Firmicutes* bacterium	Uncultured Bacteria
uncultured bacterium	Uncultured Bacteria
Caudoviricetes sp.	Virus
Candidatus Enterousia intestinigallinarum	Bacteria
uncultured bacteria	Uncultured Bacteria
Mycobacterium tuberculosis	Bacteria
Candida dubliniensis	Fungus
Mycoplasma conjunctivae	Mycoplasma
Burkholderia pseudomallei	Bacteria
Veillonella parvula	Bacteria
Enterococcus faecium	Bacteria
Moraxella osloensis	Bacteria
Coxiella burnetii	Bacteria

DISCUSSION

In recent advances, blood metagenomics sequencing has been introduced as an effective method for detecting systemic infectious organisms. This study used dialysis patient blood sample to identify pathogens causing BSIs, comparing the result with blood cultures and ELISA viral screening. Peritoneal dialysis-associated peritonitis (PDAP) a complicated issue in dialysis for peritoneum that can impact treatment and endanger patient lives. Its occurrence is linked to factors like improper fluid replacement; infections related to catheter, intestinal bacteria displacement and reduced function of peritoneum (Guo *et al.* 2024). Additionally, high-risk procedures that may lead to peritonitis are common. In current study, investigated systemic infections in dialysis patient. Using metagenomics sequencing 24 microbes were identified, while the blood culture method detected only 7microbes and 0 viruses by ELISA screening. Through this, our findings show that metagenomics sequencing outperforms conventional tests in detecting systemic pathogens. It is demonstrated that metagenomics sequencing has higher rate of detection, greater sensitivity and a wider range of detecting pathogens when comparison with blood cultures and ELISA. In clinical practice, blood is a commonly used sample source for culturing to detect systemic infections but metagenomics sequencing is getting a momentum now. Studies show usage of human blood samples for meta genomic analysis in clinical diagnosis (Yu *et al.* 2024, Qian *et al.* 2023, Moragues-Solanas *et al.* 2024, Lu *et al.* 2023).

The study conducted by Lu *et al.* (2023) found that metagenomics sequencing identified 67 out of 79 patients with positive results, whereas conventional tests only detected 34 positive cases. In another study by Guo *et al.* (2024) showed metagenomic sequencing detected 29 pathogens (which were 24 bacteria, 1 fungus and 4 viruses) while bacterial blood culture identified 10 pathogens (9 bacteria and 1 fungus) and studies by Geng *et al.* (2021) reported 26 positive cases of samples out of 63 while only five were reported in blood culture, whereas our study concludes a total of 24 microbes were detected by metagenomic sequence analysis and 6 bacteria and 1 fungus by blood culture. This indicates that metagenomics sequencing has significant positive rate of detection compared to traditional techniques.

DNA was extracted from blood by cDNA preparation (Yu *et al.* 2024) and Liu *et al.* (2023). The improved DNA extraction procedure of blood samples preserved bacterial DNA at clinical significant amount (Moragues-Solanas *et al.* 2024) and similar results were observed in present study. The blood samples were indused with four primary species responsible for BSI were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Enterococcus faecalis* in the studies of Moragues-Solanas *et al.* (2024). *K. pneumoniae, E. faecium, Pseudomonas aeruginosa* were detected in studies of Yu *et al.* (2024) whereas in our study a total of 24 species were identified, few of which were same as in previous studies. *Candida albicans* was also identified in the studies of Lu *et al.* (2023) along with bacteria, Whereas in present study found *Candida dubliniensis* along with bacteria. Geng *et al.* (2021) reported *Acinetobacter baumannii, S. aureus, K. pneumoniae* and *Candida*. Jerome *et al.* (2019) studied the patients who were diagnosed with hepatitis spp, while in our studies Hepatitis C virus was diagnosed by metagenomics sequencing even it is serologically negative. The samples

were tested positive for HIV virus by Somasekar *et al.* (2017). Grundy *et al.* (2023) reported *Coxiella spp., M. Tuberculosis, Salmonella spp., S. aureus, P. aeruginosa, K. pneumoniae, E. coli,* HIV, Hepatitis B virus, Hepatitis E virus while in the current study *Salmonella enterica subsp. enterica serovar, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, Hepatitis C virus, *Mycobacterium tuberculosis*, *Coxiella burnetii* were reported. Studes by Jerome *et al.* (2019) other strains diagnosed were Dengue virus, Chikungunya virus, mumps virus, Ebola virus, human pegivirus, *Plasmodium falciparum, Plasmodium malariae* whereas in our study the other microbes detected were *Enterococcus faecium, Mycoplasma conjunctivae, Corynebacterium pseudokroppenstedtii,* SARS CoV-2*, Shigella flexneri aerobactin, Veillonella parvula, Burkholderia pseudomallei.* Nie *et al.*(2023) recommends metagenomics sequencing to patients with Peritoneal dialysis in patients who have previously been treated with antibiotics. For individuals who have not undergone treatment with antibiotics, metagenomic sequencing and culture techniques can be used together for detection of pathogens. The positivity rate was 4% with the culture method and 31% with metagenomics sequencing, showing a significant difference between the two methods. Viruses are not detected by culturing and serology methods hence metagenomic sequencing is the best choice of diagnosis.

Summary

To a dialysis patient with systemic infections, compared microbial culturing and viral screening with ELISA with blood metagenomic sequencing and sequence analysis for diagnosis of infective microbe. In blood agar media culturing, 7 microbes (6 bacteria and 1 fungus), in serology by ELISA 0 viruses and in meta genomic sequence analysis 24 pathogens (19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma) were diagnosed. 4 unculturable bacteria were also diagnosed. Hence metagenomic sequence analysis can be one of the best methods for effective detection of BSI's.

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Review Article

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On Earth Harmony: Algae Crafting Environment Revival

Devanshi Trivedi[1], Vivek Pandya[2], Kinjal Upadhyay[3]

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Abstract

In the intricate dance of nature, algae emerge not just as the silent architects of vibrant underwater realms, but as the unsung heroes with the power to breathe life into the natural world. In this comprehensive analysis we go through how algae jack into— carbon's void by sequestering carbon of CO_2 using photosynthesis and mitigates greenhouse emissions, purifies tainted waters by using wastes from wastewater like nutrients, sparks a green revolution as it produces biofuels and being used as food for nutrient enrichment as well as monitors environment as biosensors, thus concluding into algae's ability to transcending conventional boundaries of environmental stewardship. The more emphasis will be on illuminating on major benefits like biofuels, carbon sequestration and wastewater treatment which might be sweet in the citrus.

Keywords: Microalgae; Application; Wastewater treatment; Carbon sequestration; biofuels.

INTRODUCTION

The environment is deteriorating and different pollution is emerging as severe problems around the globe. Thus, rendering it to fundamental concern to ecotoxicologist, environmental biologist, eco-chemists, pathologist and many other fields of research. Therefore, advanced methods redefining approaches meeting the demands of growing world; called 'green agenda'. The Algae being diverse photosynthetic group of eukaryotes diversified in many species and genera like Chlorophyta, dinoflagellates, diatoms, etc. they have emerged as valuable environmental applicators becoming the sustainable solutions across various sectors. Their ability of absorbing nutrients, in wastewater treatment, aiding water purification, forming biofuels and many more. Emerging environmental contaminants present in wastewater are drawing significant awareness as they exhibit several bad qualities such as high polarity, the ability to be bioaccumulated by aquatic organisms and resistance to biodegradation. They harm the aquatic ecosystem and human health as well. The most common contaminants include not only pharmaceuticals products but also several personal care products, perfluorinated compounds, gasoline additives, surfactants, organometallic compounds, disinfection by-products, brominated and organophosphate flame retardants, endocrine-disrupting compounds,

Author Affiliation: [1,2]Student, [3]Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

Corresponding Author: Kinjal Upadhyay, Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

E-mail: kinjal.upadhyay@sxca.edu.in

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nanoparticles and plasticizers (Müller *et al.*, 2007; Morin-Crini *et al.*, 2022). Algae-based technologies have demonstrated greater efficiency in removing emerging contaminants (Morin-Crini *et al.*, 2022) at bothlaboratory scales and in real wastewater.

Bioremediation is basically the use of living biological organisms in order to remove the contaminants. Algae can be used in bio-remediatingindustrial effluent including heavy metal, dye, nitrogen, phosphorus, etc. removal.

Heavy metal are the class with atomic density >4000 kg m³ (Vardhan *et al.*, 2019). They show accumulation and thus are having non-biodegradable properties, toxicity and thus be a critical problem worldwide. A heavy metal being pollutant also causes diseases like nervous system disorder, gastrointestinal and kidney dysfunction, vascular damage, skin lesion, immune system dysfunction and cancer even at low concentrations (Edelstein *et al.*, 2018).

Heavy metals have three categories: 1) radionuclides 2) precious metals 3) toxic metals (Pavithra *et al.*, 2020; Kafil *et al.*, 2022).The heavy metals can be removed by Biosorption and bioaccumulation (Ahmed *et al.*, 2022).

- First method, bioaccumulation works by transporting metal ions across cell membrane using passive and active transport. Method occurs only in live cells.
- Second one, biosorption involves the sorption of metals onto the cell surface which will be helped by Exopolysaccharides (Ahmed *et al.*, 2022).

Applications of algae:

Throughout the last several decades, the world frugality has expanded at a dramatic pace. Extreme population expansion is a major issue, with the world's population cast to reach 8.5 billion in 2030, 9.7 billion in 2050 and 10.9 billion by 2100 (United Nations, 2019). As a consequence of the growing global population, large quantities of energy and coffers have been consumed and pollution situations are high. The necessity of anticipating and preparing for these heads has been honored and appreciated by numerous transnational associations. Promoting a "green frugality" is one similar plan. The green frugality conception was developed during the 2012 United Nations Conference on Sustainable Development in Rio de Janeiro and it's grounded on the idea that environmental protection helps both the frugality and society. The thing of this conception is to empower growers and manufacturers to produce further environmentally friendly product and consumption systems grounded on exercise and recovering for sustainable development (Loiseau *et al.*, 2016).

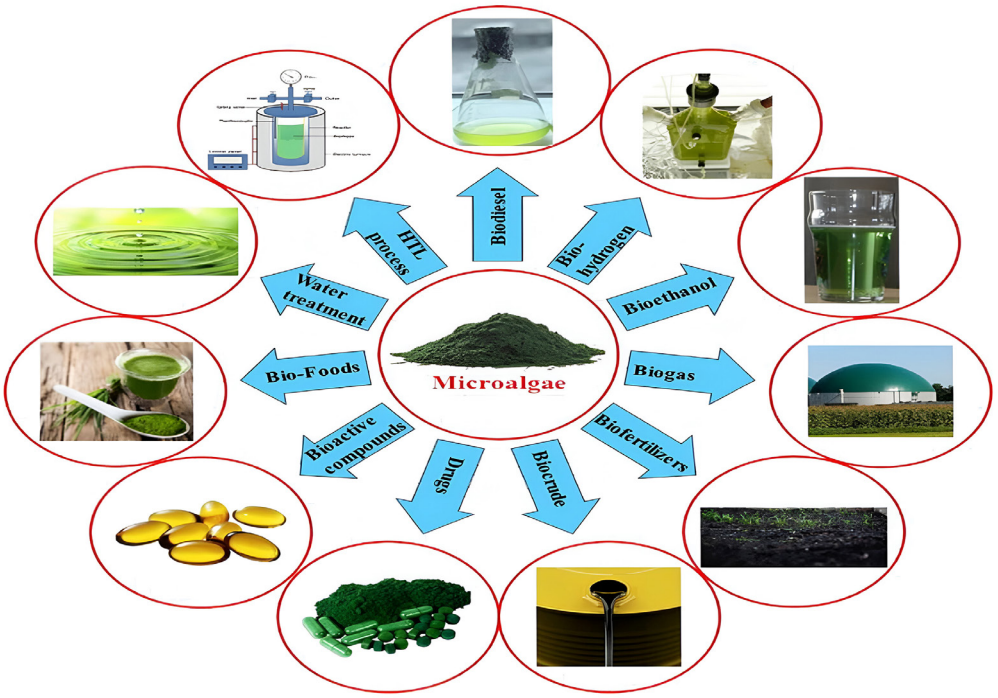

Fig. 1: Applications of algae Adopted from Kandasamy *et al.*, 2022

Nutrients bioremediation by algae

External and agrarian waste water contains a large quantum of nutrients like nitrogen, phosphorus and other minerals. The predominant forms in which they do in wastewater are ammonium ions, nitrite, nitrate and orthophosphate. Phosphate enters the algal cell laboriously through a symporter with H or Na ions furnishing the driving force. Algae also hydrolyze organic phosphorus composites with membrane-bound as well as free phosphatases, releasing bioavailable phosphorus that's latterly taken up by the algal cells (Bolan *et al.,* 2004). Among inorganic nitrogen sources, algae preferentially take up ammonium because of its further stoutly favorable assimilation and direct protein objectification process (Bolan *et al.,* 2004). Algae uptake ammonium by a group of membrane transporter proteins belonging to the ammonium transporter family. On the other hand, nitrate and nitrite are reduced to ammonium, by nitrate reductase and nitrite reductase, independently, for intracellular uptake, which is energy ferocious.

Removal of contaminants by algae

Algae as being able to tolerate toxic elements and require heavy metals as like zinc, molybdenum, manganese, iron, cobalt, copper and boron as trace elementsthus they can grow and metabolize whereas some like cadmium, chromium, lead, arsenic, mercury are harmful to them.

This called hormesis, is a process of requiring toxic heavy metals for growth. Precious metals Ag and Au can be recovered while removing toxic radioactive elements from water and these algae can tolerate toxicity via mechanisms like as gene regulation, heavy metal immobilization, chelation, exclusion and the production of different enzymes that decrease the toxicity of these substances. (Monteiro *et al.,* 2012; Tripathi and Poluri, 2021; Manikandan *et al.,* 2022)

Heavy metal control by algae majorly follows biosorption and bioaccumulation, it can form organometallic compound with heavy metals in cytoplasm and separating vacuoles. The biosorption ismetal ion binding to dead or inactive algal cells via different interaction like electrostatic, chelation, ionic, etc. (Srimongkol *et al.,* 2022).

Instead bioaccumulation involve live algae where first the semi biosorption takes place with metal ion binding functional groups like -OH, -COOH, amine, etc. on wall of cell. Then second phase, intracellular uptake happens, ingesting these toxic elements playing crucial role in detoxification. (Srimongkol *et al.,* 2022).

Some studies show dead algae being better in *wastewater* treatment as for live algae show restricted sorption due to the live cell poisoning; also live cells have complex intracellular mechanism for the uptake of contaminant which makes absorption complex. On the other hand dead ones will act as assemblers of polymers which absorbs metals at extracellular level. (Salam, 2019).

Algal species from family *Phaeophyceae* in which alginate serves mainly for heavy metal binding which directs the influence on biosorption. A weird matter of fact is some contaminant specifically boost rates of other contaminants; for example, sulfamethazine removal gets increase by presence of sulfamethoxazole (Xiong, 2019). Co-metabolism reported in *Chlamydomonas Mexicana* removes ciprofloxacin after adding sodium acetate, here breakdown via enzyme takes place (Xiong, 2019). Temperature effects as higher temperature increases rate of entire process (Vijayaraghavan and Yun, 2007).

Studies show that by product formed can may be proven more toxic than primary compound, one such case is textile water remediation using *Oscilllatoria tenuis, Chlorella pyrenoidosa* and *Chlorella vulgariscan* degrade azo dyes into simple aromatic amines being carcinogenic and persistent (Fazal, 2018). Thus, before using algae systems, the study should be made for effects of environmental factors on monitoring and growth of algae for successful application.

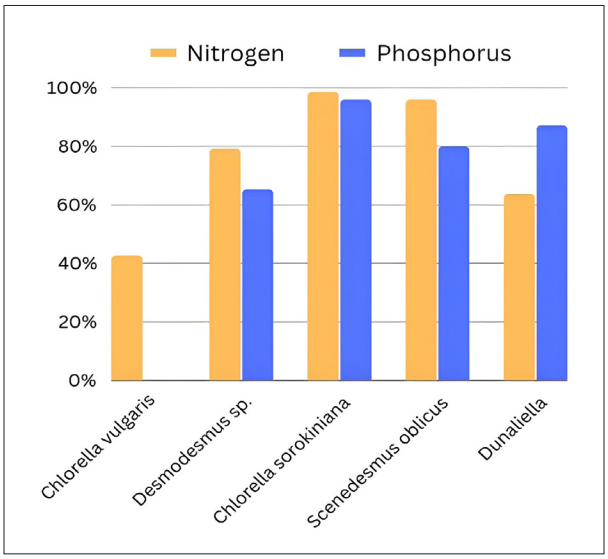

Fig. 2: Percentage removal of Nitrogen and phosphorus by different algal species

Pharmaceutical contaminants can be removed based on their physiological properties using microalgae. A study by De Godos *et al.*, 2012, showed ability of *Chlorella vulgaris* for removing more than 50% tetracycline via adsorption as interaction established between positively charged molecules on cell surface. Not only this but also florfenicol, trimethoprim, sulfamethoxazole and carbamazepine be bioaccumulated by *Chlorella sp*. (Song *et al.*, 2019). An algae *Scedesmus dimorphus* biodegrades 85% of 17a-estradiol in about 7days (Zhang *et al.*, 2014). The biodegradability is determined by how complex structured the compound is, *i.e.* more complex the substrate then less efficiency of degradability is seen and vice versa.

Now when studied for domestic effluent, (Wang *et al.* 2010) reported that sewage sludge (nutrient rich) cultivation of microalgae was more convenient and better than primary and secondary treated wastewater when parameters like COD, PO4-P, NO3-N and NH4-N.Zheng *et al.*, 2018, mixed piggery wastewater with brewery wastewater for determining optimal C/N ratio as to know nutrient Removal and microalgal growth; at an optimum C/N ratio of 7.9, removal efficiency of all the above mentioned parameters reached 93%, 90%, 96% and 100%.

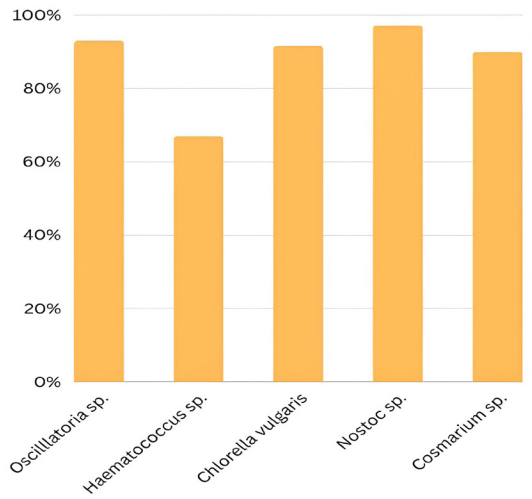

Fig 3: Percentage removal of dye using different algal species

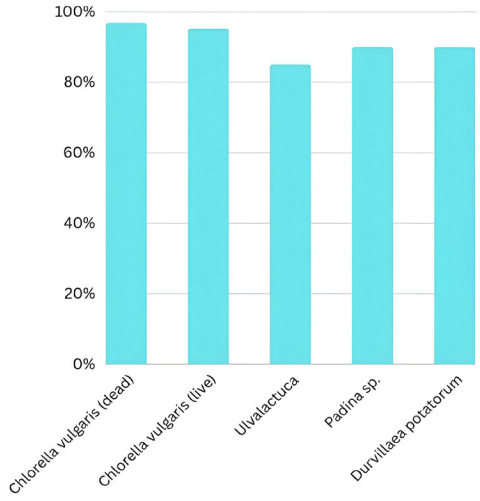

Fig 4: Percentage removal of Cadmium Data Adopted from Bilal *et. al*, 2018

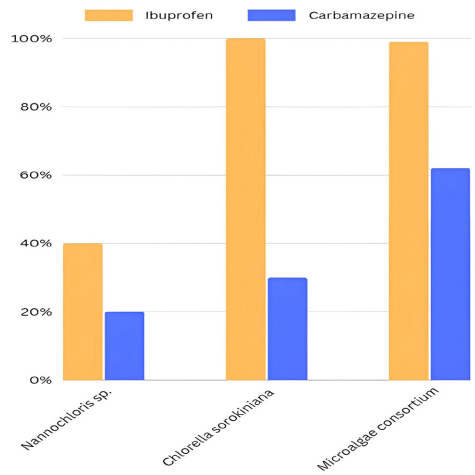

Fig 5: Percentage removal of Ibuprofen and Carbamazepine. Data Adopted from Abdelfattah *et al.*, 2023

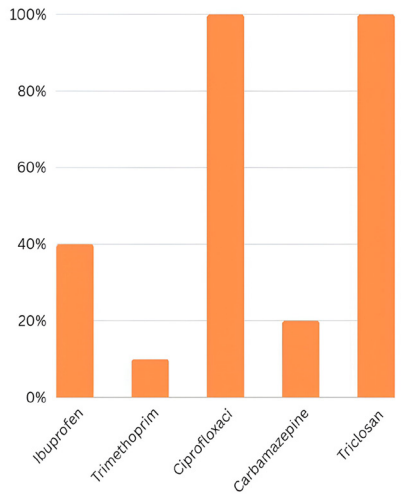

Fig 6: Percentage removal of different pharmaceutical contaminants by *Nannochloris* sp. Data Adopted from Abdelfattah *et al.*, 2023

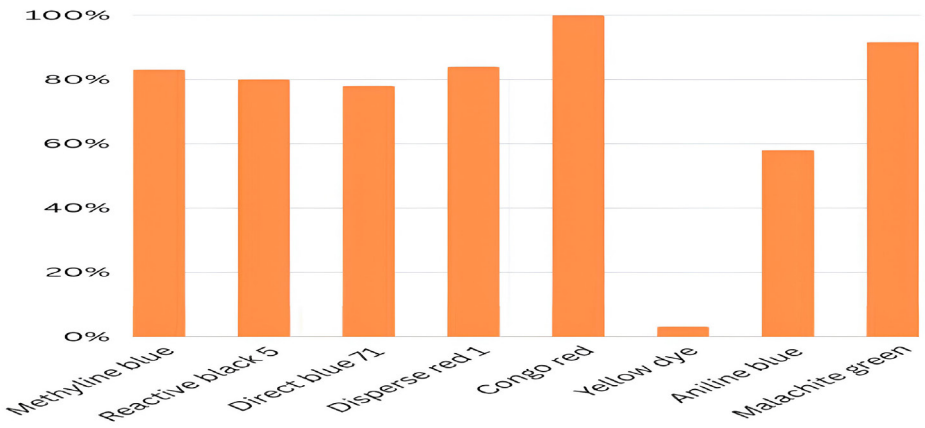

Fig 7: Percentage removal of different dyes by Chlorella sp. Data Adopted from Abdel Fattah *et al.*, 2023

Carbon sequestration

As said by IEA in 2021 that CO_2 amount is to be rebound and increase by 4.8% as 2019 reaches peaks of fossil fuel rebound with increasing demand, which pose the problem of increased green house pollution. Algae being magically active creatures can utilize this CO_2 via photosynthesis and recycle them in form of bioenergy which makes using microalgae a sustainable and environment friendly way (Brilman *et al.,* 2013).

CO_2 assimilation by photosynthesis involves both light dependent and light independent reactions; moreover this CO_2 is converted to carbohydrate precursors and biomass which later synthesizes major biomolecules like lipids, proteins and nucleic acids (Zeng *et al.,* 2011; Gg and Liu, 2021).

A report stated, cost of manufacturing *Chlorella* Biomass is around $4.87 per kg having consumed 0.96kWh per kg of energy (Valdovinos-García *et al.,* 2020).Moreover around 2.2 kilotons of CO_2/ year is sequestered using 4000m³ microalgae growth in pond systems when natural sunlight is available (Stewart and Hessami, 2005).

According to some other studies, algae might reduce CO_2 emissions by 50% (Stepan *et al.,* 2002).

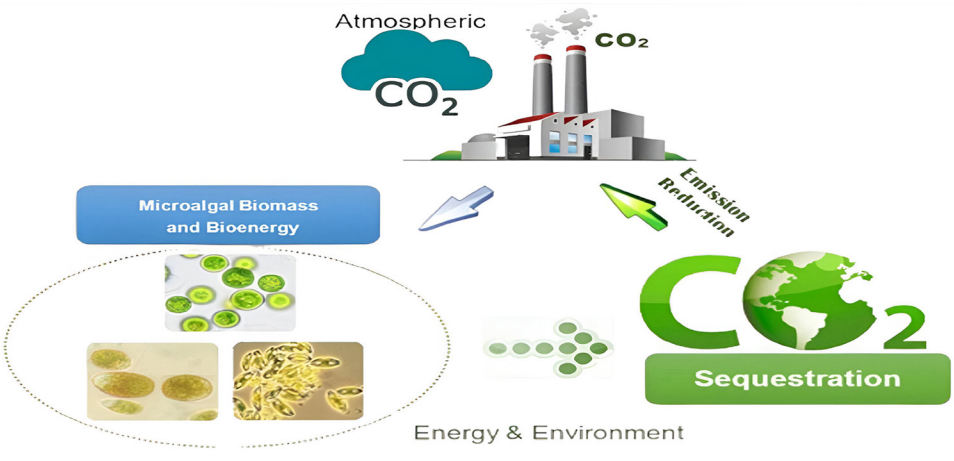

Fig 8: Represents Co_2 sequestration process Adopted from Zhaom *et al.*, 2020

Biofuels

Biofuels are the fuels coming to rescue in the form of renewable energy as the threat of losing these petroleum basedfossil fuels we are using now. Biofuels include ethanol, biodiesel, biohydrogen.

Microalgae have a capacity as a patron similar to that of a land factory, which has been critical to mortal survival for a long time in history considering terms of significant sources of food, drug, erecting accoutrements and energy force. Different photosynthetic unicellular microalgae are arising as new sources of renewable energy that can fulfill the demands of the conditioning. Microalgae lipids can be employed as a raw material for biodiesel conflation and remaining biomass rich in carbohydrates can be used to produce bioethanol or biogas. They generally accumulate lipids between 20 - 50% of their dry weight Yet Some species can be as high as 80% under certain condition (Chisti, 2007). These neutral lipids, substantially in the form of triacylglycerols (mark up to 90 – 95%), which can be converted to adipose acid methyl esters (FAMEs) and converted biodiesel. Algae have advantages over first generation biofuels made from sugar, bounce and vegetable oil because of their high growth rates and productivity, capability to grow on a non-arable land using wastewater, capability to use water pollutants and CO_2 and capability to produce a variety of high-value natural product of biodiesel from microalgae comprises two different way: 1) lipid birth from microalgal cells and 2) transesterification of lipids using alcohol and a catalyst (Mondal *et al.,* 2017). In a primary study of biomass product and ammonium junking in *Synechococcus sp*. VDW (accession number MH393765) insulated from natural seawater in Thailand (Tinpranee*et al.,* 2018), we discovered that at optimum conditions (original pH7.4, inoculum size of 0.17 (OD730) and ammonium of 10.5 mg L −1), maximum ammonium junking and biomass productivity were 95 and 34 mg L−1 d−1, independently. A review study by Pancha *et al.,* 2019, showed colorful microalgae lipid content cultivated in colorful wastewater ranging from 18–79% w/w of biomass. Meanwhile, Chinnasamy *et al.* 2010) set up that > 96% nutrient in treated wastewater containing 85–90% carpet assiduity backwaters with 10–15% external sewage which could be removed by a range of native algal isolates. Biomass product eventuality and lipid content of 92–17.8 tons/h/time and 6.82%, independently. Biohydrogen product from microalgae can take place through in different routes but generally involves turmoil biohydrogen product (e.g., dark turmoil biohydrogen product, print turmoil biohydrogen product and print-dark combined turmoil biohydrogen product) and photosynthesis biohydrogen product (e.g., direct natural photolysis biohydrogen product, circular natural photolysis biohydrogen product) (Wang *et al.,* 2021). Batista *et al.,* 2015, reported that can grow in civic wastewater and also the biomass can be converted into biohydrogen through dark turmoil by *Enterobacter aerogenes* producing 56.8 ml H2 per gvs (Ruiz- Marin *et al.,* 2020)

Table 1: Percentage of Co2 sequestered by different algal species

Algal species	% Co2 sequestered
Cyamodium caldarium	100%
Scenedesmus sp.	80%
Cjalorococcum littorale	60%
Euglena gracilis	50%
Eudorina spp.	20%
Nannochaloris sp.	18%
Tetraselmis sp.	15%

Data Adopted from Sanyal *et al.*, 2019

The Biogas is the end product of anaerobic digestion, Generally, anaerobic digestion is conducted by two processes: 1) simple sugar is instigated by fermentative bacteria and converted into alcohols through anaerobic condensation and 2) metanogenic microorganisms use these composites and synthesize biomethane (Danquah *et al.,* 2011; Choudhary *et al.,* 2016) revealed that native colleges PA6 has good nutrient junking capability from pastoral wastewater with a theoretical methane eventuality of over to 0.79 m 3VS per kg. Kinnunen and Rintala, 2016, showed that the biomethane eventuality of *C. Vulgaris* and mixed culture of native algae species (dominating by *Scenedesmussp*.) varied between 154 and 252 L VS per kg of methanol. Depending on culture media including synthetic medium, wastewater (castrated andnon-sterilized) and digestate from anaerobic digestion of pulp and paper biosludge (castrated and non-sterilized).

CONCLUSION

Algae as a boon can thus be used to clear up the environment by mitigating CO2, reduce heavy metals by adsorption, clearing wastewater and still many others like making biofuels and being the

green gold of earth.

Conflict of Interest

There are no conflicts to declare

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Mobile: 9821671871, Phone: 91-11-79695648
E-mail: author@rfppl.co.in

Review Article

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Eco-Alchemy - Algae Cultivation and Its Application in Bioplastic Production

Vivek Pandya[1], Devanshi Trivedi[2], Kinjal Upadhyay[3]

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Abstract

In this world of rapidly growing techs and steadfast alternatives which rather damage environment than make it flourishing, we need the actual green alternative which will efficiently supersede— the intoxication by petro fuels, waste-treatment machinery, green house effects and many more. This search of alternative has made its way to sleek end through the cultivation of algae and its benefits. As little amounts will do no good to the seeking benefits the major ways found by scientists soaring through early methods like dilution isolation method and use of stencil & gelatine to create patterns to more modern ways of open pond systems to photobioreactors. The review includes various efficient ways of algal cultivation such as use of photobioreactors, open raceways, two-phase hybrid systems and 3D triangular systems by discussing its limitations plus advantages. The photo bioreactors though providing more efficiency than open pond system they still are not much used. The greed for improved systems made discovery of the two-phase hybrid systems which obliterates disadvantage of both systems which are discussed. Though the thirst of improving led to the more different designs in the way of trials like 3D triangular systems which will be honoured in this review. We also go through a brief glimpse of algae's role in replacing synthetic plastics with its bioplastic production as it shows very versatile group of polymers and high degradability.

Keywords: Microalgae; Cultivation-methods; Open system; Closed system; Bioplastic.

INTRODUCTION

The world is now going towards green impact as by alternating the use of non-renewable and polluting sources with the more renewable and environmental friendly source. This involves biofuels like bioethanol, biohydrogen, bio-methane using many of the biological species like plants— *i.e.* their seed, leaves, fruits, etc. and microbes like yeast, *Zymomonasmobilis, Bacillus licheniformis and algae.*

Author Affiliation: [1,2]Student, [3]Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

Corresponding Author: Kinjal Upadhyay, Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

E-mail: kinjal.upadhyay@sxca.edu.in

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Algae are the photosynthetic organisms that are multipurpose for many of the needs for extracting exemplified pigments and vitamins for supplements to treating the waste of industrial lethargy and providing gold standards of eco-fuels in the form of bio-ethanol and biohydrogen, While also using the environmental CO_2 released from petroleum burnt through vehicles in order to make organic carbon for its growth and in turn purifying the environment.

The Algae is divided into groups like green algae, brown algae, red algae, blue green algae, diatoms, golden algae, dinoflagellates, euglenophytes. Out of these mainly blue-green and green algae are used as they are very rich in vitamins and nutrients, also for biofuels too; Others like red and brown algae are majorly used for bioplastic production.

As such of importance are algae that it holds bills of hundreds of millions in dollar for researching it'scultivation, application and products. Almost $200 million were spent in researching and pilot project development by US department of energy (DOE). An investment such high as $600 million is made by an oil company alone in oil production efforts from algae and related advertisements. This led to now blooming many of the experiments revolving around algae cultivation and its fruiting capacity of forming biofuels (Hall and Banemann, 2011)

Salt and wastewater tolerance being a spotlight of algae reduces freshwater use while sometimes wastewater can be considered a hosting nutrient resource in certain circumstances (Schenk *et al.,* 2008). The CO_2 sequestration being cleaning environment property of algae it will not only reduce environmental CO2 but also produce the precious O_2 which accounts 70-80% of the oxygen breath by us (Schenk *et al.,* 2008; Hall, 2011).

There has been report by IMechE, 2009, which told increased CO_2 concentration will increase algae growth rate till other nutrients are in abundance (IMechE, 2009).

The cultivation of algae majorly be done via open system and closed system in which open systems consist of the raceway ponds and other totally open systems and closed systems includes photo-bioreactors.

Cultivation of algae

The algae harvesting is as old as at least 2500 years ago which have been utilized for food and medicine, yet the cultivation began only 300 years ago (Tseng, 1981). As early mentions reverting to 1950's stated their first roles in fuel production (Morton, 1998) and thus began the cultivation from lab to pilot scale.

In algae, they depend on strain specific cultivation criteria so as to increase the yield. Factors include temperature, mixing, hydrodynamics mass transfer, gas transfer, light cycle, water intensity, water quality, pH levels and many others like carbon nutrients availability, cell density/fragility/ growth inhabitation. Thus, there has been more special interest lying in its cultivation system designs.

Algae can be grown in various designed systems from low on tech ponds to highly technical and controlled system of bioreactors. The designs firmly differ in terms of its economic requirements to environmental impact to factors in operating systems.

Major there are two systems that cultivation follows: 1) Open systems and 2) Closedsystems

Open systems

The open ponds are basically the natural pits created with specific dimensions. They can either be simple ponds of desired diameter and depths whereas others called raceway ponds have desired diameter and depth plus circulating paddlewheels (Gundula Proksch, 2013).

Basically the depth of 15 to 20cm or 6 to 8inches is ideal whereas diameter can vary (Gundula Proksch, 2013). At lab scale you can also use flasks, beakers and trays to cultivate this green gold so as they can behave as the mimics.

Advantages: (Schenk *et al.*, 2008)

- There is always a low construction cost.
- They idealise low economic margins.

Disadvantages: (Schenk *et al.*, 2008)

- Large land area.
- Low light utilization.
- High contamination.
- Weather changes.

Solutions: (Gundula Proksch, 2013)

These problems can be overcome by using a transparent or translucent barrier which can be covered on the area and thus can be turned into a greenhouse.

Closed system

The algae need around 1000-10,000 lux of light intensity of indirect sunlight to optimize

photosynthetic reaction as direct sunlight can cause photo inhibition and photo bleaching (Schenk *et al.,* 2008). This can insist that algae can be grown in 3D space instead of growing just on surface like

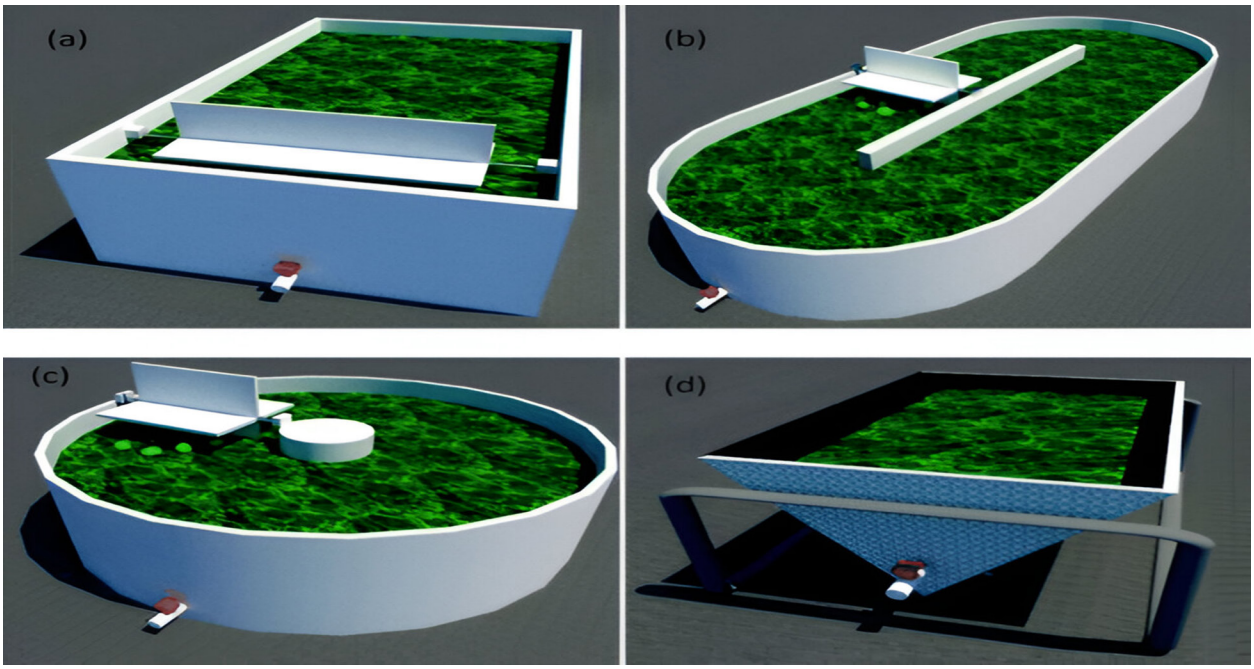

Fig. 1: Open system
Adopted from (Santos *et al.*, 2020)

normal plants (Gundula Proksch, 2013).

Vertical closed system increase productivity as the light will diffuse bitterly. They are made of two components: A feeding vessel through which CO_2 and nutrients can be introduced while second is a solar array through which the mixed solution is pumped being exposed to sunlight (Gundula Proksch, 2013). The system increases yield and contamination is eliminated (Gundula Proksch, 2013).

Ther low cost photobioreactor can be made using poly-ethylene bags which can be hanged through rackssuch that all sides of it can be exposed to the sunlight. Algae can be mixed into solution and pumped mechanically using series of bags. Systems like this can sometime require additional

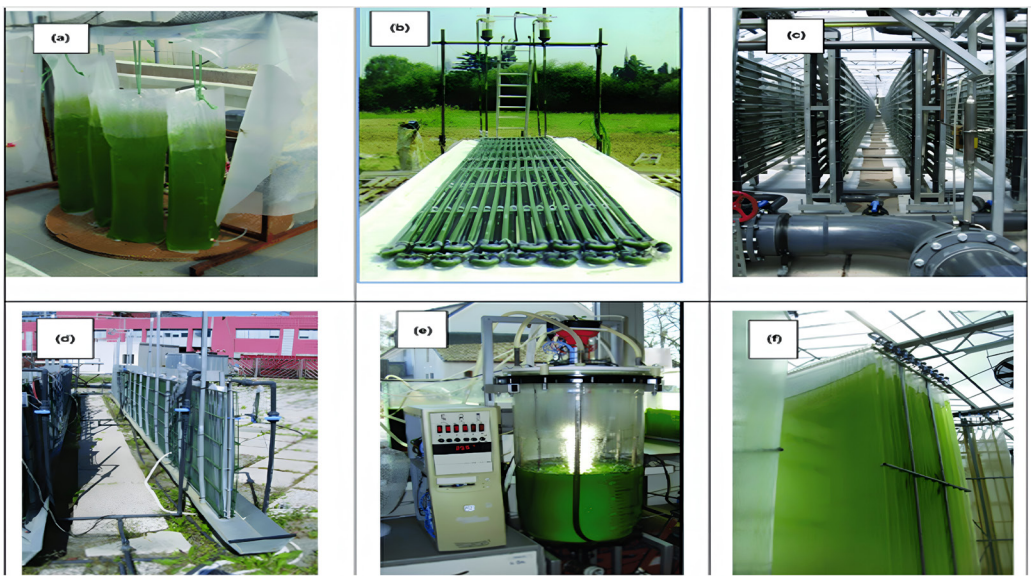

Fig. 2: Closed systems for algal cultivation
adopted from (Masojídek & Torzillo, 2008)

construction in order to cut through the weather fluctuations (Kizililsoley and Helvacioglu, 2008).

The 10 times more costly and producing 5 to 10 times higher yields of areal footprint then conventional methods is the closed photobioreactor, Here minimal volume of water is used for maximizing absorption of nutrients and energy under controlled environment. The surface-to-volume ratio provides light saturations and proper mixing ensures even CO_2 distribution and prevents algal cells from settling down. Glass tubes will efficiently be used to grow algae (Schenk *et al.*, 2008). A German company Algomed operates world's largest photo-bioreactor in Klötze and this consists of 500km of glass tubes in about 12,000m^2 green house being able to produce up to 100t algal biomass per year (Gundula Proksch, 2013).

Two-stage Hybrid system

This system is developed as a result of research carried out to reduce disadvantages of both open pond system and photobioreactor and make a setup that falls somewhere in between having advantages of both (Olaizola, 2000; Schenk *et al.*, 2008; Su *et al.*, 2011). This system is made up of open raceways connected to the photobioreactor

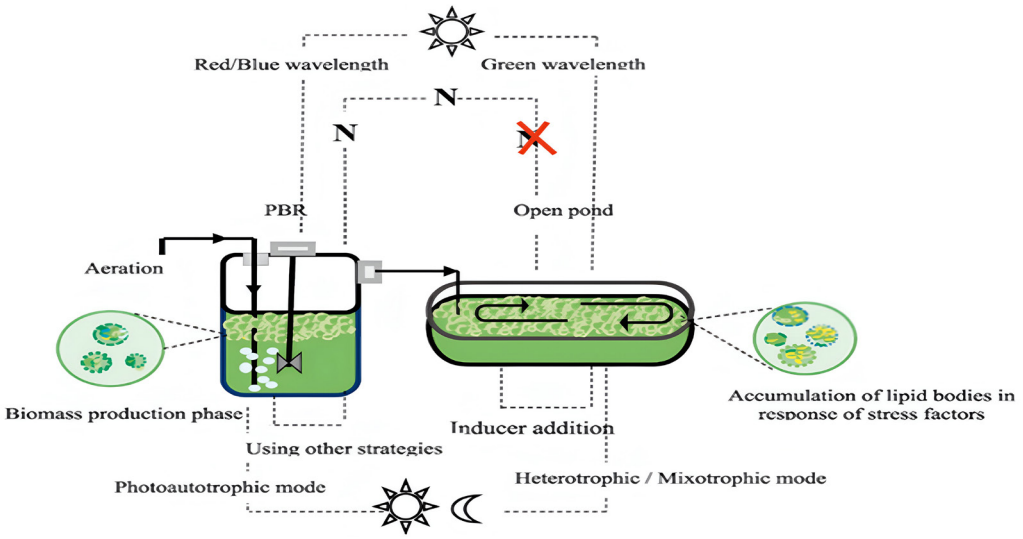

Fig. 3: Two stage hybrid system
Adopted from (Aziz et al., 2020)

and the flow happens from photobioreactor to open raceways pond (Narala *et al.*, 2016).

Comparison between the different systems

A Tetraselmis sp. M8 is used; The photobioreactor as well as the open raceway pond cultivation results three main growth cycleswhereas the fourth harvest seemed possible at 28th and 29th day. The harvest of only that biomass that is rich in lipids is done. If compared the Two-stage system, the hybrid system produced nearly six cycles and harvests. These results suggested that the average of growth

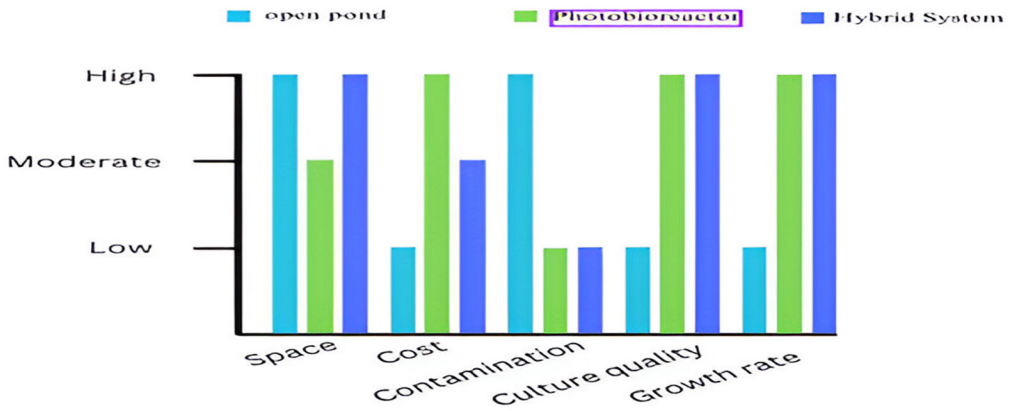

Fig. 4: Comparison between different systems
Data adopted from (Gundula Proksch, 2019)

was higher in the Hybrid system compared to both individual methods (Narala *et al.,* 2016).

This happened because hybrid system has provided biomass growth and lipid inductionan independency as it has two distinct stages. Hybrid system involved higher growth rates and low culture dormancy thanks to the different cultivation system for biomass production and lipid induction.This becomes advantageous as algae can either increase biomass or it can biosynthesize lipid at a single time (Lim *et al.,* 2012; Li *et al.,* 2014; Sharma *et al.,* 2014).

The major disadvantage mostly in open pond system, i.e. contaminating predators or other algae, is overcome by hybrid systems. The issue is solved here as the photobioreactor holds the algae in closed environment for a much of their growth and the open pond systems (prone to contamination) will gain the biomass only few days before harvesting (Moheimani and Borowitzka, 2006; Wang *et al.,* 2013).

Role of algae in bioplastic production

Plastics that are produced from biomass or environment friendly resources like food crops are known as bioplastic and have the same function as petroleum base bioplastic (Mekonnen *et al.,* 2013). Algae makes a preferrable biomass source for the production of bioplastic, which has increased level of lipid content (Khoo *et al.,* 2020; Yew *et al.,* 2020). The biomass of algae consists of protein and carbohydrate-based polymers which can be used as one of the bioplastic constituents. Some of the examples of protein-based polymers are starch, cellulose, PHA (Polyhydroxyalkanoate, PHB (Polyhydroxy butyrate), PLA (Polylactic acid), PE (Polyethylene), PVC (Polyvinyl chloride) from algal biomass which are utilized to develop biodegradable plastics (Karan *et al.,* 2019). Among above mentioned protein-based polymers PHA is a polymer that is most recommended for the production of bioplastic because it can be degraded by enzymatic action (Rasul *et al.,* 2017). Algal biomass can also be blended with other materials in order to prolonged life span, also o provide a better mechanical properties. The materials which can be blended with the algal biomass are petroleum plastics, natural products (cellulose or starch) or polymers, for the production of bioplastic (Rahman & Miller, 2017).

Table 1: Data represents bioplastic products generated by different microalgal species

Algal Species	Product	Bioplastic content (mg ml^{-1})
Microcystis aeruginosa	PHB	0.49±0.5
Haematococcuspluvialis	PHB	0.39 ± 0.42
Botyryococcusbrauni	PHB	247 ± 0.42
Chroococcocusturgidus	PHB	0.1

Data adopted from (Arora *et al.,* 2023)

As shown in Fig 5 the different type of blended materials are used for manufacturing of improved quality of bioplastic. There have been some researchers who manufactured the bioplastics (A. Pownal, 2020).

The few of the bioplastic manufacturing examples by researchers and designers are: Eric Klarenbeek and Maartje Dros, Dutch designers made an algae based bioplastic that can replace the currently used traditional plastics, also establishing lab called Algae Lab for algae cultivation and starch production which can act as raw material for bioplastic production; Austeja Platukyte made biodegradable product mixing agar and algae coated with calcium carbonate having potential to replace petroleum-based plastics; Ari Jonssonmixed water and dried red algae creating plastic bottles that can alternate traditional plastic bottles, these new plastic bottles can retain the form when full and easily biodegrade

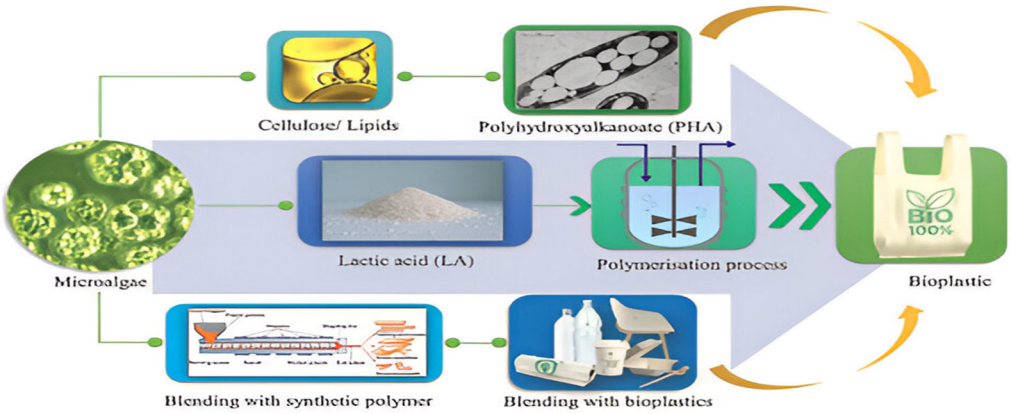

Fig. 5: Bioplastic production

Adopted from (Chong *et al.,* 2022)

when empty;thus, the algae based bioplastic can be promising in replacing the traditional bioplastic very efficiently (Arora *et al*., 2023).

CONCLUSION

Algae being a strong beneficiary can be grown in bulk amounts via the discussed methods out of which bioreactor are more efficient but less used while the hybrid system is very well negating limits of both these systems. Algae being good bioplastic source can also maintain eco-friendly approach.

Conflicts of Interest

There are no conflicts to declare.

Acknowledgements

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Corporate (collective) author

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