Removals of Some High- and Low-Density Polyethylene (HDPE and LDPE), Polypropylene (PP) and Polyvinyl Chloride (PVC) Microplastics Using Some Microalgae Types, Energy Production and Energy Recovery

DELİA TERESA SPONZA*, RUKİYE ÖZTEKİN
Department of Environmental Engineering
Dokuz Eylül University
Tınaztepe Campus, 35160 Buca/Izmir,
TURKEY
* Corresponding Author

Abstract: - Waste plastic conversion involves the treatment of plastic waste to transform in different forms of energy (heat, electricity, liquid fuels). Plastic can be converted into different forms of biofuel via thermochemical conversion methods (gasification, pyrolysis and liquefaction). Algal biomass can be converted into different forms of biofuel (crude bio-oil, bioethanol, biogas, biodiesel and bio-hydrogen) well as value added chemicals. Microalgal cells can accumulate more lipids over a shorter life cycle, they are discussed as a promising feedstock for third-generation biodiesel. The utilization of microalgae as biofuel feedstocks offers an economic, eco-friendly alternative to the use of fossil fuels the aim of microplastics (MPs) removals. Interactions between MPs and microalgal cells could enhance several important features for possible microalgal harvest and MPs accumulation. One hypothesis is microalgal biomass hypothesis can accumulate lipids and carbohydrates under microplastic stress, supporting biomass conversion into biodiesel and bioethanol. In such systems, algal cells act as bio-scavengers for MPs, binding the particles to algal surfaces or incorporating them into their cells; they are filtered from the water body and finally destroyed by further downstream processing of the polluted biomass. In this study, in order to determine biofuel (1-butanol) and methane gas [CH_4(g)] production; High- and low-density polyethylene (HDPE and LDPE), polypropylene (PP), and polyvinyl chloride (PVC) MPs were removed using biomass composed of microalgae *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. The algal inhibition test results proved that small groups of MPs with a size of ≈ 100 nm did not show algal inhibition. According to the algae inhibition test results, the production of 1-butanol from 100 mg/l microalgae biomass under aerobic conditions were determined as 93 ml/g for HDPE, 236 ml/g for LDPE, 387 ml/g for PP and 459 ml/g for PVC. According to the algae inhibition test results, the production of CH_4(g) from 400 mg/l microalgae biomass under anaerobic conditions were measured as 452 ml/g for HDPE, 510 ml/g for LDPE, 529 ml/g for PP and 541 ml/g for PVC. 91.26%, 94.52%, 98.34% and 96.17% energy recoveries were measured for HDPE, LDPE, PP and PVC MPs, respectively, after microalgae biomass experiments, at pH=7.0 and at 35^{o}C. Maximum 98.34% energy recovery was obtained for PP MPs after microalgae biomass experiments, at pH=7.0 and at 35^{o}C.

Key-Words: - Biofuel (1-butanol); *Chlamydomonas reinhardtii*; *Chlorella vulgaris*; Energy recovery; High- and low-density polyethylene (HDPE and LDPE) removal; Methane [CH_4(g)] production; Microalgae; Microplastics; Polypropylene (PP) removal; Polyvinyl chloride (PVC) removal.

Received: June 25, 2022. Revised: October 22, 2023. Accepted: November 24, 2023. Published: December 31, 2023.

1 Introduction

Plastics are widely used in numerous industries, including agriculture, medicine and packaging. Many plastics are thrown into the environment due to the high production volume and difficulty in breaking them down. Once plastic particles are released into the environment, they are broken down by various natural forces and exposed to weathering. Such natural forces include ultraviolet (UV) radiation, mechanical forces of water, as well as biological degradation resulting in the formation of microplastics (MPs) and nano-plastics (NPs), [1]. "MP" is a term used to refer to any synthetic solid plastic polymer with a diameter of ≤ 0.5 mm, formed as a result of primary or secondary processes, [2], [3], [4]. Although, there is no established definition for "NPs", the term is often used to refer to particles of similar origin and composition to MPs, with smaller sizes ≤ 100 nm in size, much smaller than the algal cell diameter, [5], [6], [7]. MPs and NPs (MNPs) are distributed directly into the environment through domestic and industrial wastes from cosmetics, cleaning products and synthetic fibers; By following the food chain between living things in the ecosystem; They can eventually enter the human body and threaten human health.

Biodegradable plastics (BPs) are attracting attention as a replacement for non-degradable plastic materials. It is noted that BPs can be converted to CO_2 and H_2O as final products through naturally occurring microorganism mineralization and may provide new avenues for end-of-life treatment of plastic waste, such as anaerobic digestion and composting [8]. 100% degradation of biodegradable materials cannot be achieved in natural environments, [9]. BPs in natural environments have also been proven to lead to the formation of biodegradable microplastics (BMPs), as do conventional petroleum-based MPs, [10]. Since BPs are more vulnerable to degradation forces; More BMPs can be produced from MPs obtained from non-degradable raw materials. This situation causes much more serious BMP pollution in the soil ecosystem, [11].

According to the United States National Oceanic and Atmospheric Administration (NOAA), microplastics (MPs) are defined as pieces of plastic with particle size < 5 mm. Improper discharge of industrial and subsistence wastewater (ww); It contaminates rainwater, surface water and oceans with large amounts of MPs. Since MPs have a similar density range (0.85 to 1.41 g/cm^3) compared to fresh and ocean water bodies; They are easily distributed worldwide, [12]. Environmental pollutants known to easily adsorb onto MPs include many toxic compounds such as heavy metals (e.g., Cu, Ni, Pb, and Zn) and persistent organic pollutants (POPs) [e.g., polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT) and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDTs)]. MPs may contain chemicals such as bisphenol A (BPA) and phthalates, which are added during the plastic manufacturing process. Through bioaccumulation, these organic pollutants pose significant threats to human health as well as the marine ecological environment. HDPE, LDPE, PP and PVC are the dominant forms of plastic, representing approximately 59% of the total amount of plastic produced worldwide, [13].

The effects of plastic pollution in the aquatic environment are constantly being investigated. So much so that the presence of plastic components is detected even at depths of 7,000-11,000 m in the oceans and plastic waste discharge is increasing every year, [14]. These plastic aggregates, known as MPs, ranging from 0.1 to 5 mm in diameter, are the main pollutant components of long-term environmental pollution, [15]. These MPs can accumulate in aquatic animals through the food chain, affecting their growth and development, reducing their nutritional status, and damaging their ecosystems. As a result of these chain reactions of MPs in the aquatic ecosystem; They pose serious health threats to humans, [16], [17]. In addition, roughness, porosity, polarity and hydrophobicity as a result of the mixing of more than one contaminant; It further increases contamination of MPs, [18], [19]. This causes MPs to adsorb more pollutants in the environment, such as heavy metals, antibiotics, persistent organic pollutants, and other pollutants [16], [20], [21], [22]. Microalgae, the primary producers of aquatic ecosystems, are affected by the toxicity of MP pollution. In addition to its negative effects on microalgae growth, studies conducted include; It proves that MPs affect algal photosynthesis, that chlorophyll content and photosynthesis efficiency decrease with exposure to MPs, and that smaller sizes may be more toxic. However, studies on the effects of mixing MPs with different pollutants are quite limited, [19].

There are many literature studies reporting the interaction of microalgae and MPs; However, these studies mostly examined the effects of microalgae colonization and toxicity, [19]. In recent years, efforts have been made to remove MPs by forming hetero aggregations with microalgae; Preliminary

studies have been carried out in many literature studies, [23], [24], [25], [26], [27], [28], [29]. Applied microalgae mostly include seaweed and freshwater algae; and the tested MP materials mostly consist of PVC, PP, polystyrene (PS) and HDPE. Efficiency of forming hetero aggregates; It is greatly affected by both the algae type, that is, the morphology of the algal cell and the amount of extracellular polymeric substance (EPS) of the algae, and the properties of the plastic, such as the type of MPs material, the size of the MPs, the density of the MPs, and the hydrophobicity property of the MPs. The surface roughness of MP particles has been reported to be positively related to the number of attached microalgae, [30], and high-energy surfaces generally facilitate the growth of biofilms because they are more hydrophilic surfaces, [31]. For this reason, MPs with high surface roughness and hydrophilicity are likely to form hetero aggregations with microalgae more easily. Physicochemical characteristics of MPs for example, surface chemistry, particle size, particle distribution and types affect the toxicity of MPs greatly in aquatic organisms, [32].

HDPE, LDPE, PP and PVC are the most dominant types of plastics, representing approximately 60% of the total amount of plastic produced worldwide, [13]. The remaining 40% is in plastic forms; PS (6.7%), polyethylene terephthalate (PET, 7.4%), polyurethane (PUR, 7.5%), polybutylene terephthalate (PBT), acrylonitrile butadiene styrene (ABS), polymethylmethacrylate (PMMA) and polycarbonate (PC) it consists of other polymers, [13].

Recently, in the search for sustainable and environmentally friendly biofuel sources that can be a truly efficient alternative to fossil fuels; There are many literature studies investigating production from plants, bacteria, yeasts and microalgae. Microalgae stand out as a valuable solution due to their advantages such as higher lipid productivity, faster growth rates, accumulation of biomass in smaller areas, and inability to compete with human food resources. These photosynthetic microorganisms are capable of producing numerous metabolic compounds that can be converted into different forms of biofuel such as biodiesel, biohydrogen, biomethane or bioethanol. Biofuel production from microalgae through various transformation processes were summarized at Fig. 1. Biofuels include biohydrogen, biogas, bioethanol, biodiesel, syngas, bio-oil and bio-char (Fig. 1). The main potential process is to produce triacylglycerides (TAGs), the main component of biodiesel feedstocks, through transesterification into fatty acid methyl esters (FAMEs), derived from the lipid synthesis metabolic pathway in microalgae, [33], [34].

* Fig. 1 can be found in the Appendix section.

Biofuels are divided into four main categories according to the raw material: first generation, second generation, third generation and fourth generation. First and second generations biofuels are made from corn, sugarcane bagasse, wheat starch, soybeans, rapeseed, canola, jatropha, etc. They are traditional biofuels obtained from edible and non-edible terrestrial plants, including, [35]. The biggest disadvantages of first and second generations conventional fuels are; What drives direct competition with agricultural food production is the need for large areas, excess water and excess nutrients for the cultivation of the product, [36]. By using third generation biofuels, which are obtained from the biomass of various microorganisms such as bacteria, yeast, fungi and microalgae, can be grown on smaller lands and have high areal productivity; The disadvantages of first and second generations biofuels can be overcome, [36]. Fourth generation biofuels involve the use of genetically modified microorganisms to increase their biofuel potential, [37]. Fourth generation biofuels include photosynthetic microalgae; They provide superiority over other microorganisms thanks to their ability to utilize CO_2(g) and solar energy to produce biomass, thus eliminating the need for high-cost organic carbon, [38]. The use of microalgae lipids for biodiesel production is a great advantage as they have the ability to naturally survive in the sea, brackish waters or wastewater. Thanks to this advantage, less land and less fresh water usage, faster growth rates, less CO_2(g) emissions from flue gases, reduction of the amounts of nutrients such as nitrogen and phosphorus in wastewater, and continuous production throughout the year are achieved, [39].

Third generation biofuels produced from microalgae against energy crisis and environmental pollution; They offer promising alternatives for sustainable global economic growth and human progress. Microalgae biomass can be processed into biodiesel, bioethanol and biogas; However, high input costs and technical limitations of biodiesel production restrict the further development of biodiesel. Bio-methanation of microalgae biomass via anaerobic digestion; It increases the energy efficiency of biodiesel and is an environmentally friendly and high-efficiency alternative. Most of these include optimization of light delivery to the culture, use of residual glycerol as a heterotrophic carbon source, maximization of triglyceride

accumulation through nutritional supplementation and metabolic engineering, use of direct trans-esterification, especially to prevent desiccation of biomass, cultivation of algae in ww or application of anaerobic digestion, as well as lipid digestion. It is related to additional energy recovery processes from extracted microalgal biomass, [40].

Chlamydomonas reinhardtii is a unicellular green alga ≈ 10 μm in diameter, swimming with two flagella; It has a cell wall composed of glycoproteins rich in hydroxyproline, a large cup-shaped chloroplast, a large pyrenoid, and a light-sensitive eye spot. The typical freshwater alga *Chlamydomonas reinhardtii*, which is widely used as a model aquatic organism in ecotoxicological studies and nutrient removal, is applied as a test species, [41], [42]. Lagarde et al. [25], evaluated the interactions of PP and HDPE with the chlorophyte *Chlamydomonas reinhardtii*, a microalgae species, and found that 400 mg/l microalgae biomass; A significant ≈ 18% reduction in microalgae growth was detected after 78 days of contact with PP. This result was attributed to the formation of hetero-aggregates of microalgae with MP during the 20-day mixing period. The shading effect of the microalgae trap on MPs clusters causes a decrease in photosynthetic efficiency; and this reduces the growth rate of microalgae, [25]. It has been reported that high concentration of MPs with size > 400 μm has no detrimental effect on the freshwater microalgae *Chlamydomas reinhardtii*, [25]. As a typical phytoplankton, *Chlamydomonas reinhardtii* has the potential to be easily cultivated, is considered highly sensitive to environmental pollution, is used as a potential candidate for water pollution assessments, and has many advantages such as high biosorption and removal efficiency in personal care products (PPCPs), [43].

Chlorella vulgaris is a species of green microalgae in the division *Chlorophyta*; It is used as a dietary supplement or protein-rich food additive, especially in Japan. Biodiesel produced from *Chlorella vulgaris* provided the most significant reduction in hydrocarbon, CO and CO_2 gas emissions compared to biodiesel produced from Eruca sativa plant and waste cooking oil, [44]. It was observed that aging MPs inhibited the growth of microalgae *Chlorella vulgaris* to a greater extent than young MPs, with enhanced porosity and adsorption capacity, [45]. PS MPs affected the removal of levofloxacin by altering the adsorption, enrichment, and enzymatic degradation of antibiotics by *Chlorella vulgaris*; On the third day, the levofloxacin (initial concentration=93.8 μg/l) removal rates for the MPs group (35 items/l) and the control group were 23.34% and 46.71%, respectively; however, the combined toxicity on *Chlorella vulgaris* microalgae began to decrease, [46].

Anaerobic digestion of residual lipid-extracted biomass; It is one of the most promising options for improving the economic and environmental sustainability of the process. It allows energy recovery in the form of biogas, allows nutrients to be recycled and reused in microalgae culture, stabilizes waste biomass; thus, reducing the costs associated with waste disposal and management. The high temperatures (49°C-57°C) used during thermophilic anaerobic digestion accelerate biochemical reactions; By intensifying the hydrolysis of the microalgal cell wall, it increases organic matter degradation efficiency and biogas production. Working under thermophilic conditions provides a higher degree of effluent stabilization and hygiene compared to mesophilic conditions, improved sludge dewatering, potentially higher biomethane yield, greater reduction of volatile organics, lower risk of foaming, 2-3 times higher bacterial growth rates and higher It also offers benefits such as allowing for potential organic loading rates (OLRs), [47]. Few and contradictory studies have addressed the thermophilic anaerobic digestion of the residual microalgal biomass up to this date. Both higher, [48], and lower, [49], biogas yields have been reported when compared to mesophilic digestion. It has also been stated that the optimum temperature for anaerobic digestion might be dependent on the microalgae species, [50]. Few studies have evaluated the potential energy contribution of anaerobic digestion to the biodiesel production process from microalgae. The few reports available in the literature indicate that a considerable part of the total energy contained within the biomass can be recovered if anaerobic digestion of lipid-extracted microalgae is implemented, [51].

Microalgae-based biorefinery approach is a system where energy, fuel, chemicals and high-value products (e.g., pigments, proteins, lipids, carbohydrates, vitamins and antioxidants) are produced from biomass through various processes. Microalgae are rich in proteins, lipids and carbohydrates, and the relative amounts of these biochemical components vary among various microalgae species, [52]. They can be used as raw materials in the production of various high-value bio-based products such as production of biodiesel from microalgae lipids, alternative carbon source in fermentation industries of microalgae carbohydrates, healthy food supplements from long-chain fatty acids found in microalgae, and in pharmaceutical applications, [53]. The main focus of microalgae biotechnology for the large-scale application of

microalgae as a sustainable and robust energy feedstock is: (a) increasing their photosynthetic efficiency through metabolic engineering for improved oil yield and improved carbon sequestration in mass cultures, (b) useful as a source of biofuel, energy-rich It is based on increasing carbon flux and energy production into compounds, (c) developing robust and stable algal cells that are low-cost, sustainable in large-scale cultivation, resulting in lower operating costs and a lower carbon footprint of the chemical produced, [54].

In this study, in order to determine biofuel (1-butanol) and CH_4(g) production in *Chlamydomonas reinhardtii* and *Chlorella vulgaris* microalgae species; The use of HDPE, LDPE, PP and PVC MPs has been investigated under anaerobic conditions. Additionally, the energy production processes and energy recovery processes were also investigated after the removal of MPs by microalgae *Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass.

1.1 Originality and Innovation of Our Work

By using biomass consisting of a mixture of *Chlamydomonas reinhardtii* and *Chlorella vulgaris* microalgae; The recovery of energy by producing 1-butanol as an energy source from biodegradable HDPE, LDPE, PP and PVC microplastics under aerobic conditions, followed by the production of CH_4(g) as an energy source under anaerobic conditions and the recovery of energy, shows the originality and innovation of the study. Because using these four microplastics and biomass, which is a mixture of two microalgae, has never been used before to produce and recover energy under aerobic conditions and to produce and recover energy under anaerobic conditions.

An important feature of our study is its accuracy and applicability; It can be tested and compared with a possible new approach [such as artificial intelligence (AI) methods].

2 Materials and Methods

2.1 Microalgae Biomass

Chlamydomonas reinhardtii CC124 strain powder was purchased from Sigma-Aldrich, Germany. *Chlorella vulgaris* CCAP 211/11B (Culture Collection of Algae and Protozoa, Argyll, UK) was purchased from United Kingdom.

Chlamydomonas reinhardtii powder was cultured in a tris-acetate-phosphate (TAP) medium, [55], [56], [57]. Algal cells were cultivated in a constant temperature light incubator at 22 ± 2ºC, at pH=7.0 and at 20 μmol photon/m^2.s illumination. Algae were grown in 250 ml Erlenmeyer flasks and were shaken daily and randomly arranged to reduce any minor differences in photon irradiance. *Chlamydomonas reinhardtii* powder was utilized as substate with 93.1 ± 0.2% total suspended solids (TSS), 84.2 ± 3.6% total volatile suspended solids (TVSS). The elemental composition of *Chlamydomonas reinhardtii* powder were 53.2±0.5% C, 10.4±0.3% N, 6.1±0.1% H, 0.6±0.01% S, C/N=5.1, 65.06±0.2% protein, 17.6±0.8% carbohydrate and 18.9±0.3% lipid of TS, respectively.

Chlorella vulgaris powder was cultured in a bold basal medium (BBM), [58]. All experiments were performed at a temperature-controlled environment at 25 ± 3°C and at optimum pH=7.0. The light was provided by a cool white LED (T5 15W 6400K, 80μmol/m^2.s) with continuous illumination within the experimental period. *Chlorella vulgaris* powder was used as substrate with 93.1 ± 0.2% TSS, 84.2 ± 3.6% TVSS. As for elemental composition of *Chlorella vulgaris* were 47.5 ± 0.3% C, 10.7 ± 0.2% N, 6.9 ± 0.1% H, 0.7 ± 0.02% S, C/N=4.6, 66.9 ± 0.4% protein, 16.2 ± 0.7% carbohydrate and 17.4 ± 0.3% lipid of TS, respectively.

2.2 Lipid Extraction and Characterization of Microalgae Biomass

The lipid extraction was carried out by Soxhlet extraction. 25 g dried microalgae biomass was placed in an extraction thimble (SWISS filter cellulose extraction thimbles, 33 x 80 mm P2, SW3380), which was then placed inside the Soxhlet extraction apparatus. A mixture of 100 ml of hexane and acetone (3/1 v/v) was used as solvent, with a reflux period of 8 h. The lipid extraction yield was determined gravimetrically according to Balasubramanian et al., [59]. The obtained lipid-extracted microalgae biomass was dried to remove any residual solvent at 105ºC.

2.3 Inhibition Test for Microalgae Biomass

Inhibition tests for *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were measured according to Standard Method 8810 and 8813 C, respectively [60].

2.4 Experimental Procedure

To evaluate anaerobic digestion performance: By continuously circulating hot water through the jackets of a 1-liter laboratory-scale continuous anaerobic bioreactor (ABR), keeping the temperature constant under mesophilic (35ºC) conditions; fed with lipid-extracted 400 mg/l of microalgae

(*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass.

CO_2(g) produced was captured by a 100 ml flask containing NaOH. Thymolphthalein was used as indicator to signal the exhaustion of the basic solution. The values of CH_4(g) yields were expressed as volume of gas produced divided by g of VS of substrate fed. ABR was stirred automatically using a mechanical motor connected to a timer. Mixing was performed for 20 min every 3 h. ABR was operated at OLR=0.6 g COD/l.d and at HRT=30 d. The overall operational period was studied for 150 d.

2.5 Analytical Procedures

Chemical oxygen demand-dissolved ($COD_{dissolved}$), total ammonium-nitrogen (total NH_4^+-N), pH, temperature {T[(^oC)]}, TSS, TVSS, chloride ion (Cl^-), volatile fatty acids (VFAs), sodium ion (Na^+), potassium ion (K^+), calcium ions (Ca^{+2}), magnesium ions (Mg^{+2}), copper ions (Cu^{+2}), nickel ions (Ni^{+2}) and zinc ions (Zn^{+2}) were measured according to the Standard Methods (2022); 5220D, 4500-NH_4^+, 4500-H^+, 2320, 2540D, 2540E, 4500-Cl^-, 5560B, 3500-Na^+, 3500-K^+, 3500-Ca^{+2}, 3500-Mg^{+2}, 3500-Cu^{+2}, 3500-Cr^{+2} and 3500-Zn^{+2}, respectively, [60].

CH_4(g) was measured daily through water volume displacement with gas chromatography-mass spectrometry (GC-MS); gas chromatograph (GC) (Agilent Technology model 6890N) equipped with a mass selective detector (Agilent Technology model 5973 inert MSD, mass selective detector). Mass spectra were recorded using a VGTS 250 spectrometer equipped with a capillary SE 52 column (HP5-MS 30 m, 0.25 mm ID, 0.25 μm) at 220°C with an isothermal program for 10 min. The initial oven temperature was kept at 50^{o}C for 1 min, then raised to 220^{o}C at 25^{o}C/min and from 200^{o}C to 300^{o}C at 8^{o}C/min, and was then maintained for 5.5 min. High purity He(g) was used as the carrier gas at constant flow mode (1.5 ml/min, 45 cm/s linear velocity).

During the whole operational period, ABR was monitored by measuring the volume of biogas produced, biogas composition, temperature, pH, TSS, TVSS, $COD_{dissolved}$, carbohydrates, proteins, total NH_4-N and VFAs concentrations, respectively. Concentrations of potentially toxic compounds for anaerobic digestion (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+}, total Cr, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cl^-), carbohydrate, VFAs and the occurrence of residual solvent toxicity, acetone and hexane concentrations in samples from ABR was measured and evaluated by GC-flame ionization detection (GC-FID) (Agilent Technology, Germany) (column 30 m/0.25 mm ID, temperature ramp from 60^{o}Cx2 min, 10^{o}C/min to 190^{o}Cx2.5 min, detector temperature of 250^{o}C and injector temperature of 250^{o}C).

2.6 Biofuel (1-Butanol) Production from MPs with Microalgae Biomass

100 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass, in water contaminated with MPs, surrounds the surface of MPs particles and absorbs them; They grow by creating more lipids in their metabolism. More Lipid provides more energy production. Microalgae biomass produce biofuel (1-butanol) as a result of a series of reactions using these lipids under aerobic conditions (Fig. 2).

***** Fig. 2 can be found in the Appendix section.

2.7 Biomethane Potentials (BMPs) Tests

BMPs tests for microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass were performed under mesophilic (35^{o}C) and thermophilic (55^{o}C) conditions, respectively. BMPs assays were carried out in 120 ml serum bottles containing 50 ml of experimental solutions. An initial substrate concentration of 5 g/l VS was operated. The substrate to inoculum ratio was set at 1/1 (VS/VS), [61]. The BMP medium was supplemented with 200 mg/l yeast extract, 5 g/l sodium bicarbonate ($NaHCO_3$), 65 mg/l ammonium chloride (NH_4Cl), 18.5 mg/l potassium dihydrogen phosphate (KH_2PO_4), 5.7 mg/l magnesium sulphate heptahydrate ($MgSO_4.7H_2O$, Epsomite or Epsom salt) and 4 mg/l calcium chloride dihydrate ($CaCl_2.2H_2O$), respectively.

The CH_4(g) production was determined by monitoring the pressure and composition of the gas contained in the headspace of the bottles. The BMPs value was computed dividing the cumulative CH_4(g) produced by the mass of VS of substrate added at the beginning of the test. The endogenous biogas production from the anaerobic biomass was determined by control assays containing only inoculum. The values of the CH_4(g) yields were normalized at 0^{o}C and atmospheric pressure (1 atm = 101.325 kPa).

2.8 Energy Production and Energy Recovery

Microalgae are a unique biomass feedstock for renewable and sustainable energy production. Energy production; It is released as a result of the breakdown of MPs by using the lipids and biofuels (e.g., 1-butanol) in microalgae biomass structure under anaerobic conditions (e.g., ABR). Energy recovery, ER (%) was calculated from Eq. 1.

$$ER\ (\%) = \frac{HTL_{bio-oil} \cdot m_{bio-oil}}{HTL_{feed} \cdot m_{feed}} \quad (1)$$

where; $HTL_{bio-oil}$: is HTL of the products $m_{bio-oil}$: is the products amount, HTL_{feed}: is HTL of the feedstock and m_{feed}: is the feedstock amount, respectively.

All experiments were carried out three times and the results are given as the means of triplicate samplings. The data relevant to the individual pollutant parameters are given as the mean with standard deviation (SD) values.

2.9 Flux Uncertainties and Limits of Detection (LOD)

The measured flux includes the true flux (F) plus random (∈) and systematic (δ) error components for measurement system (x) at time (t) in Eq. (2):

$$F_{t,x} = F_t + \in_{t,x} + \delta_{t,x} \quad (2)$$

Systematic error can result from (I) incorrect calibration of instrumentation, (II) incomplete sampling of turbulent fluctuations, (III) failure to observe non-turbulent flows during weak mixing conditions, and (IV) potential underestimation of the flow energy used during mixing in the anaerobic digestion process.

The calculations were used to identify the main biodegradable plastics and calculate their biodegradation behavior in various anaerobic digestion processes according to ISO 15985 (simulating high solid and thermophilic anaerobic digestion) and ISO 14853 (simulating semiliquid and mesophilic anaerobic digestion), [62]. While spectral corrections induce uncertainties of their own, we nevertheless assume here that after spectral corrections, remaining $\in_{t,x} >> \delta_{t,x}$.

Before performing experimental error analysis; High-frequency CH_4(g) concentrations were remeasured separately from GC-MS measurements with a low-power open path analyzer (LI-7700, LI-COR Biosciences Inc.) and a closed path tracer gas analyzer (TGA100A, Campbell Scientific). Laser spectroscopy was used in both analyses.

3 Results and Discussions

3.1. Effect of Lipid Extraction Process for Microalgae Biomass Characterization

The results of the proximate analysis for microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass was demonstrated at Table 1.

* Table 1 can be found in the Appendix section.

Low crude fibre proportions (< 3%) may be indicative of low cellulose content in cell walls; and this may facilitate cell lysis. The high ash content (> 17%) indicates that a significant fraction of the total mass of microalgae will not be degraded during digestion and therefore cannot be reduced to CH_4(g) (Table 1). Regarding the presence of possible inhibitors, concentrations of the element Na in the biomass are negligible, while high protein proportions (≈ 50%) in microalgae biomass can lead to inhibition of the digestion process due to the accumulation of free ammonia nitrogen (FAN). This issue needs to be considered when microalgae are used as substrates. The lipid extraction method did not cause lysis of microalgal cells, but only affected the microalgal cell surface.

3.2 Removals of HDPE MPs with Microalgae Biomass

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were examined with 100 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using HDPE MPs during aerobic conditions for 1-butanol production, at pH=7.0 and at 35ºC (Fig. 3). 19 ml/gVS, 40 ml/gVS, 81 ml/gVS and 76 ml/gVS 1-butanol productions from HDPE MPs were observed for 30 days, 60 days, 120 days and 150 days HRTs, respectively, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3). The maximum 93 ml/gVS 1-butanol production from HDPE MPs was measured for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3).

* Fig. 3 can be found in the Appendix section.

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were operated with 400 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using HDPE MPs in ABR during anaerobic conditions for biochemical CH_4(g) production, at pH=7.0 and at 35ºC (Fig. 4). 343 ml CH_4/gVS, 411 ml CH_4/gVS, 286 ml CH_4/gVS and 177 ml CH_4/gVS biochemical CH_4(g) productions from HDPE MPs were obtained for 30 days, 90 days, 120 days and 150 days HRTs, respectively, in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4). The maximum 452 ml CH_4/g VS biochemical CH_4(g) production from HDPE MPs was measured for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4).

* Fig. 4 can be found in the Appendix section.

For the removal of HDPE MPs in wastewater, 400 mg/l *Chlamydomonas reinhardtii* microalgae was applied at 1872 h experimental time, [63]. No significant reduction in growth, no significant change in chloro-plastic genes, and no effect on stress response/apoptosis genes for *Chlamydomonas reinhardtii* microalgae were detected by Qin et al., [63].

3.3. Removals of LDPE MPs with Microalgae Biomass

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were studied with 100 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using LDPE MPs during aerobic conditions for 1-butanol production, at pH=7.0 and at 35ºC (Fig. 3). 33 ml/gVS, 95 ml/gVS, 204 ml/gVS and 172 ml/gVS 1-butanol productions from LDPE MPs were measured for 30 days, 60 days, 120 days and 150 days HRTs, respectively, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3). The maximum 236 ml/gVS 1-butanol production from LDPE MPs was observed for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3).

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were examined with 400 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using LDPE MPs in ABR during anaerobic conditions for biochemical CH_4(g) production, at pH=7.0 and at 35ºC (Fig. 4). 371 ml CH_4/gVS, 424 ml CH_4/gVS, 329 ml CH_4/g VS and 234 ml CH_4/gVS biochemical CH_4(g) productions from LDPE MPs were obtained for 30 days, 90 days, 120 days and 150 days HRTs, respectively, in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4). The maximum 510 ml CH_4/g VS biochemical CH_4(g) production from LDPE MPs was found for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4).

3.4 Removals of PP MPs with Microalgae Biomass

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were examined with 100 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using PP MPs during aerobic conditions for 1-butanol production, at pH=7.0 and at 35ºC (Fig. 3). 118 ml/gVS, 182 ml/gVS, 322 ml/gVS and 248 ml/gVS 1-butanol productions from PP MPs were observed for 30 days, 60 days, 120 days and 150 days HRTs, respectively, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3). The maximum 387 ml/gVS 1-butanol production from PP MPs was measured for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3).

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were studied with 400 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using PP MPs in ABR during anaerobic conditions for biochemical CH_4(g) production, at pH=7.0 and at 35ºC (Fig. 4). 413 ml CH_4/gVS, 433 ml CH_4/gVS, 345 ml CH_4/gVS and 258 ml CH_4/gVS biochemical CH_4(g) productions from PP MPs were obtained for 30 days, 90 days, 120 days and 150 days HRTs, respectively, in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4). The maximum 529 ml CH_4/g VS biochemical CH_4(g) production from PP MPs was observed for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4).

400 mg/l of *Chlamydomonas reinhardtii* microalgae was examined for the removal of PP MPs in wastewater at 1872 h, [64]. 18% of growth decrease, non-significant change in expression of chloro-plastics genes and no effect on stress response/apoptosis genes for *Chlamydomonas reinhardtii* microalgae were evaluated by Sarmah and Rout, [64].

3.5 Removals of PVC MPs with Microalgae Biomass

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were operated with 100 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using PVC MPs during aerobic conditions for 1-butanol production, at pH=7.0 and at 35ºC (Fig. 3). 131 ml/gVS, 274 ml/gVS, 396 ml/gVS and 310 ml/gVS 1-butanol productions from PVC MPs were observed for 30 days, 60 days, 120 days and 150 days HRTs, respectively, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3). The maximum 459 ml/gVS 1-butanol production from PVC MPs was measured for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35ºC (Fig. 3).

Increasing HRTs values (30 days, 60 days, 90 days, 120 days and 150 days) were studied with 400 mg/l of microalgae (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) biomass using PVC MPs in ABR during anaerobic conditions for biochemical CH_4(g) production, at pH=7.0 and at 35ºC (Fig. 4). 417 ml CH_4/gVS, 448 ml CH_4/gVS, 356 ml CH_4/gVS and 265 ml CH_4/gVS biochemical CH_4(g) productions from PVC MPs were obtained for 30 days, 90 days, 120 days and 150 days HRTs, respectively, in ABR during anaerobic conditions, at pH=7.0 and at 35ºC (Fig. 4). The maximum 541 ml CH_4/g VS

biochemical CH_4(g) production from PVC MPs was found for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35^{o}C (Fig. 4).

The different concentrations of *Chlorella vulgaris* microalgae biomass (10 mg/l, 100 mg/l and 1000 mg/l) were applied for the removal of PVC MPs in wastewater at 240 h, [65]. Growth and biomass inhibitions for 10 mg/l of Chlorella vulgaris microalgae were recorded for the removal of PP MPs from wastewater after 240 h, [65].

3.6 Energy Recovery for HDPE, LDPE, PP and PVC MPs after Microalgae Biomass Experiments

Energy recovery for HDPE, LDPE, PP and PVC MPs were determined after microalgae biomass experiments, at pH=7.0 and at 35^{o}C (Fig. 5).

* Fig. 5 can be found in the Appendix section.

91.26%, 94.52%, 98.34% and 96.17% energy recoveries were measured for HDPE, LDPE, PP and PVC MPs after microalgae biomass experiments, at pH=7.0 and at 35^{o}C (Fig. 5). Maximum 98.34% energy recovery was found for PP MPs after microalgae biomass experiments, at pH=7.0 and at 35^{o}C (Fig. 5).

3.7 Results of Inhibition Test

The algae inhibition test results showed that the small MPs groups with sizes of ≈ 100 nm did not exhibit algal inhibition. 1-butanol was produced for HDPE, LDPE, PP and PVC from 100 mg/l of microalgae biomass under aerobic conditions, while CH_4(g) was measured under anaerobic conditions from 400 mg/l of microalgae biomass (Table 2).

* Table 2 can be found in the Appendix section.

The maximum values of 1-butanol productions, biochemical CH_4(g) productions and energy recoveries for HDPE, LDPE, PP and PVC MPs were evaluated after microalgae biomass experiments at pH=7.0 and at 35^{o}C (Table 2).

93 ml/g VS, 236 ml/g VS, 387 ml/g VS and 459 ml/g VS 1-butanol productions were obtained for HDPE MPs, LDPE MPs, PP MPs and PVC MPs, respectively, under aerobic conditions, at pH=7.0, and at 35^{o}C (Table 2). The maximum 459 ml/g VS 1-butanol production was measured for PVC MPs under aerobic conditions, at pH=7.0, and at 35^{o}C (Table 2).

452 ml CH_4/g VS, 510 ml CH_4/g VS, 529 ml CH_4/g VS and 541 ml CH_4/g VS biochemical CH_4(g) productions were measured under anaerobic conditions in ABR, at pH=7.9, and at 35^{o}C (Table 2). The maximum 541 ml CH_4/g VS biochemical CH_4(g) production was found for PVC MPs under anaerobic conditions in ABR, at pH=7.0, and at 35^{o}C (Table 2).

91.26%, 94.52%, 98.34% and 96.17% energy recoveries were determined for HDPE MPs, LDPE MPs, PP MPs and PVC MPs, respectively, after microalgae biomass experiments, at pH=7.0, and at 35^{o}C (Table 2). Maximum 98.34% energy recovery was observed for PP MPs after microalgae biomass experiments, at pH=7.0, and at 35^{o}C (Table 2).

3.8 A Possible New Approach and Its Applicability

In today's technology, as in many areas, a wide variety of methods are used to remove microplastics from the ecosystem with the highest efficiency or to reuse these stubborn and toxic waste materials by converting them into alternative energy forms. Thus, many alternative removal processes emerge when choosing the most suitable process for zero waste management. In recent years, the use of artificial intelligence (AI) methods has been widely preferred.

AI methods allow learning how various components interact and combinations of these components run faster and are much more accurate than running physical experiments for the same amount of time. AI methods are frequently preferred to save both time and financial resources. The most commonly used AI methods are: (1) artificial neural networks (ANN), (2) convolutional neural networks (CNN), (3) long short-term memory network (LSTMs), (4) k-nearest neighbors (k-NN or KNN) and (5) random forest (RF).

In recent years, in order to predict interactions in microalgae cultivation systems; The demand for the use of AI methods is increasing, [66]. In some studies, on this subject, artificial intelligence algorithms such as ANN) and CNN genome interactions, [67], [68], microalgal aggregation, [69], biomass measurement, [70], and most importantly, it provides improvement in processes by reducing the number of experiments and situation optimization, [71]. AI methods predict complex interactions between wastewater treatment and microalgae growth; The accumulation of internal metabolites is an important alternative, especially in better understanding basic factors such as lipid formation, carbohydrates and energy conversion, and in providing energy production at higher yields by converting them into different forms.

As a new approach for this study, we chose to use ANN, one of the AI methods. For a comparative example study; We re-evaluated our data according

to the ANN method. ANNs are increasingly preferred to fill the gaps in CH_4(g) flow time series [72], [73], [74]. The most important advantages of ANNs are (a) their greater capacity to model data with variable temporal periodicity and (b) their independence from previous assumptions regarding the functional relationship between independent and dependent variables [75], [76]. In this ANN approach, established routines were followed; A feed forward network with varying architectural complexity and tan-sigmoid transfer functions was used. A comparative summary of the error analysis results of our experimental study and the ANN method is given in Table 3.

* Table 3 can be found in the Appendix section.

Before network training, the 30-min streaming time series was evenly subsampled into training, validation, and testing subsets. Test subsets were withheld from initialization and validation of individual network trainings and were used only to eliminate uncertainty in the final selected networks. Network training and validation were repeated multiple times with increasing complexity, i.e., increasing the number of hidden layers and neurons per hidden layer. Thus, the ANN network reliability rate has been further increased.

Among the educational variables tested; The 1000 ml laboratory-scale continuous anaerobic bioreactor (ABR) was evaluated for anaerobic digestion temperature (from 20 cm), activated sludge heat flux (from an average of 8 heat flux plates at a depth of 15 cm), ambient active radiation (PAR), location of the water table, active mud moisture and atmospheric pressure were included. The existence of water and steam deficit in anaerobic digestion was tested and observed. First, these variables; were ranked according to their correlation with observed methane flux. These were then added stepwise to the training dataset.

After the training and validation of each neural network was completed, the mean square error (MSE) and coefficient of determination of the modeled data were calculated by comparing them with the stored test data. Among the data found later; We chose the network with the least number of training variables, fewest hidden layers, least number of nodes, lowest MSE, and highest R^2. The ANN routine, including random subsampling, training, and validation, was repeated n = 50 times to calculate the ANN-derived ensemble distribution of space-filled time series. Uncertainty of the ANN approach; It was then evaluated against the ensemble range, and the resulting ensemble mean was used to fill the gap data.

4 Conclusions

The maximum 93 ml/gVS 1-butanol production from HDPE MPs was measured for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35°C. The maximum 452 ml CH_4/g VS biochemical CH_4(g) production from HDPE MPs was obtained for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35°C.

The maximum 236 ml/gVS 1-butanol production from LDPE MPs was found for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35°C. The maximum 510 ml CH_4/g VS biochemical CH_4(g) production from LDPE MPs was observed for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35°C.

The maximum 387 ml/gVS 1-butanol production from PP MPs was measured for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35°C. The maximum 529 ml CH_4/g VS biochemical CH_4(g) production from PP MPs was found for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35°C.

The maximum 459 ml/gVS 1-butanol production from PVC MPs was obtained for 90 days HRTs, during aerobic conditions, at pH=7.0 and at 35°C. The maximum 541 ml CH_4/g VS biochemical CH_4(g) production from PVC MPs was measured for 60 HRTs in ABR during anaerobic conditions, at pH=7.0 and at 35°C.

91.26%, 94.52%, 98.34% and 96.17% energy recoveries were determined for HDPE, LDPE, PP and PVC MPs, respectively, after microalgae biomass experiments, at pH=7.0 and at 35°C. Maximum 98.34% energy recovery was found for PP MPs after microalgae biomass experiments, at pH=7.0 and at 35°C.

Low crude fibre proportions (< 3%) may be indicative of low cellulose content in cell walls; and this may facilitate cell lysis. The high ash content (> 17%) indicates that a significant fraction of the total mass of microalgae will not be degraded during digestion and therefore cannot be reduced to CH_4(g). Regarding the presence of possible inhibitors, concentrations of the element Na in the biomass are negligible, while high protein proportions (≈ 50%) in microalgae biomass can lead to inhibition of the digestion process due to the accumulation of FAN. This issue needs to be considered when microalgae are used as substrates.

Microalgal biomass (*Chlamydomonas reinhardtii* and *Chlorella vulgaris*) can accumulate lipids and carbohydrates under the stress of MPs (HDPE, LDPE, PP and PVC), resulting in microalgal biomass

through anaerobic digestion; were converted to biodiesel, biobutanol, and biogas [e.g., $CH_4(g)$], respectively. Biotransformation results in residues rich in MPs and is the most proposed way to solve the problem of redistribution to the environment; It is the thermochemical transformation of MPs. Microalgae cells are bio-scavengers for MPs; They bind particles to algal surfaces or incorporate them into algal cells, where they are filtered from the water body and eventually destroyed by further processing of the contaminated biomass. Very high energy recovery was achieved with the anaerobic digestion process. Using microalgae biomass as biofuel feedstock for the removal of MPs; It offers a much easier, cleaner, more cost-effective and environmentally friendly alternative to the use of fossil fuels.

The recorded results show that the removal efficiency. Production of MPs by microalgae and its underlying mechanism, it is affected by both the type of plastic and the duration of exposure. Pre-exposure it greatly increased the overall removal efficiency of MPs and proved directly usable in real-life applications.

Microalgal biomass can accumulate, lipids and carbohydrates under MPs stress; It is assumed to play a role in promoting the conversion of biomass to biodiesel and biobutanol. Microalgae biomass can be converted to biogas through anaerobic digestion. Thus, biological transformation results in rich residues. The most recommended method for these rich residues is the thermochemical conversion method; This method can also be applied as a post-treatment process for the transformation of MPs.

This article proposes a new approach that could help eliminate MPs. From contaminated water to the combination of microalgae cultivation and sustainable biofuels to reduce environmental impacts; A net zero waste approach was used. Thus, economic contribution to production is provided by eliminating waste and converting it into energy, and it is also possible to prevent stubborn and toxic environmental pollution on the ecosystem. As an important conclusion, as a possible new approach to this study; Among AI technologies, the ANN Method is safely recommended.

Acknowledgement:
This research study was undertaken in the Environmental Microbiology Laboratories at Dokuz Eylül University Engineering Faculty Environmental Engineering Department, İzmir, Turkey. The authors would like to thank this body for providing financial support.

References:

[1] L. Wang, W.M. Wu, N.S. Bolan, D.C.W. Tsang, Y. Li, M. Qin, D. Hou, Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: Current status and future perspectives, *Journal of Hazardous Materials*, Vol.401, 2021, 123415.

[2] M. Oliveira, M. Almeida, I.A. Miguel, Micro(Nano)Plastic boomerang tale: A never ending story? *TrAC - Trends in Analytical Chemistry*, Vol.112, 2019, pp. 196–200.

[3] C. Schwaferts, R. Niessner, M. Elsner, N.P. Ivleva, Methods for the Analysis of Submicrometer- and Nanoplastic Particles in the Environment, *TrAC - Trends in Analytical Chemistry*, Vol.112, 2019, pp. 52–65.

[4] K. Yin, Y. Wang, H. Zhao, D. Wang, M. Guo, M. Mu, Y. Liu, X. Nie, B. Li, J. Li, M. Xing, A comparative review of microplastics and nanoplastics: Toxicity hazards on digestive, reproductive and nervous system, *Science of the Total Environment*, Vol.774, 2021, 145758.

[5] A.A. Koelmans, E. Besseling, W.J. Shim, Nanoplastics in the aquatic environment, critical review, *Marine Anthropogenic Litter*, Vol.67, 2015, pp 325–340.

[6] I. Ferreira, C. Venâncio, I. Lopes, M. Oliveira, Nanoplastics and marine organisms: What has been studied? *Environmental Toxicology and Pharmacology*, Vol.67, 2019, pp. 1–7.

[7] T. Poerio, E. Piacentini, R. Mazzei, Membrane processes for microplastic removal, *Molecules*, Vol.24, 2019, 4148, pp.1-15.

[8] S. Lambert, C. Scherer, M. Wagner, Ecotoxicity testing of microplastics: Considering the heterogeneity of physicochemical properties, *Integrated Environmental Assessment and Management*, Vol.13, No.3, 2017, pp. 470–475.

[9] J.S.C. Viera, M.R.C. Marques, M.C. Nazareth, P.C. Jimenez, Í.B. Castro, On replacing single-use plastic with so-called biodegradable ones: The case with straws, *Environmental Science and Policy*, V.106, 2020, pp. 177-181.

[10] V.C. Shruti, G. Kutralam-Muniasamy, Bioplastics: Missing link in the era of microplastics, *Science of The Total Environment*, Vol.697, 2019, 134139.

[11] J. Fojt, J. David, R. Přikryl, V. Řezáčová, J. Kučerík, A critical review of the overlooked challenge of determining micro-bioplastics in soil, *Science of The Total Environment*, Vol.745, 2020, 140975.

[12] T.S.M. Amelia, W.M.A.W. Khalik, M.C. Ong, Y.T. Shao, H.-J. Pan, K. Bhubalan, Marine microplastics as vectors of major ocean pollutants and its hazards to the marine

ecosystem and humans, *Progress in Earth and Planetary Science*, Vol.8, 2021, Article number: 12.

[13] Y. Cao, M. Zhao, X. Ma, Y. Song, S. Zuo, H. Li, W. Deng, A critical review on the interactions of microplastics with heavymetals: mechanism and their combined effect on organisms and humans, *Science of the Total Environment*, Vol.788, 2021, 147620.

[14] Z.-L. Zhu, S.-C. Wang, F.-F. Zhao, S.-G. Wang, F.-F. Liu, G.-Z. Liu, Joint toxicity of microplastics with triclosan to marine microalgae Skeletonema costatum, *Environmental Pollution*, Vol.246, 2019, pp. 509–517.

[15] Q. Sun, S.Y. Ren, H.G. Ni, Incidence of microplastics in personal care products: an appreciable part of plastic pollution, *Science of the Total Environment*, 742, 2020, 140218.

[16] R. Coyle, G. Hardiman, K.O. Driscoll, (2020). Microplastics in the marine environment: a review of their sources, distribution processes, uptake and exchange in ecosystems, *Case Studies in Chemical and Environmental Engineering*, Vol.2, 2020, 100010.

[17] G.A. Elizalde-Velázquez, L.M. Gómez-Oliván, Microplastics in aquatic environments: a review on occurrence, distribution, toxic effects, and implications for human health, *Science of The Total Environment*, Vol.780, 2021, 146551.

[18] J. Brandon, M. Goldstein, M.D. Ohman, Long-term aging and degradation of microplastic particles: comparing in situ oceanic and experimental weathering patterns, *Marine Pollution Bulletin*, Vol.110, 2016, pp. 299–308.

[19] V. Nava, B. Leoni, A critical review of interactions between microplastics, microalgae and aquatic ecosystem function, *Water Research*, Vol.188, 2021, 116476.

[20] L.A. Holmes, A. Turner, R.C. Thompson, Adsorption of trace metals to plastic resin pellets in the marine environment, *Environmental Pollution*, Vol.160, 2012, pp. 42–48.

[21] S.L. Wright, R.C. Thompson, T.S. Galloway, The physical impacts of microplastics on marine organisms: a review, *Environmental Pollution*, Vol.178, 2013, pp. 483–492.

[22] C. Barría, I. Brandts, L. Tort, M. Oliveira, M. Teles, Effect of nanoplastics on fish health and performance: A review, *Marine Pollution Bulletin*, Vol.151, 2020, 110791.

[23] P. Bhattacharya, S. Lin, J.P. Turner, P.C. Ke, Physical adsorption of charged plastic nanoparticles affects algal photosynthesis, *Journal of Physical Chemistry C*, Vol.114, No.39, 2010, pp. 16556–16561.

[24] M. Long, B. Moriceau, M. Gallinari, C. Lambert, A. Huvet, J. Raffray, P. Soudant, Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates, *Marine Chemistry*, Vol.175, 2015, pp. 39–46.

[25] F. Lagarde, O. Olivier, M. Zanella, P. Daniel, S. Hiard, A. Caruso, Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type, *Environmental Pollution*, Vol.215, 2016, pp. 331–339.

[26] M. Long, I. Paul-Pont, H. Hegaret, B. Moriceau, C. Lambert, A. Huvet, P. Soudant, Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation, *Environmental Pollution*, Vol.228, 2017, pp. 454–463.

[27] C. Zhang, X. Chen, J. Wang, L. Tan, Toxic effects of microplastic on marine microalgae Skeletonema costatum: Interactions between microplastic and algae, *Environmental Pollution*, Vol.220, pp. 1282–1288.

[28] P. Mohlenkamp, A. Purser, L. Thomsen, Plastic microbeads from cosmetic products: an experimental study of their hydrodynamic behaviour, vertical transport and resuspension in phytoplankton and sediment aggregates, *Elementa: Science of the Anthropocene*, Vol.6, 2018, Art.61, pp. 1-16.

[29] C. Cunha, M. Faria, N. Nogueira, A. Ferreira, N. Cordeiro, Marine vs freshwater microalgae exopolymers as biosolutions to microplastics pollution, *Environmental Pollution*, Vol.249, 2019, pp. 372–380.

[30] H.S. Carson, M.S. Nerheim, K.A. Carroll, M. Eriksen, The plastic-associated microorganisms of the North Pacific Gyre, *Marine Pollution Bulletin*, Vol.75, No.1-2, 2013, pp. 126–132.

[31] C. Dussud, C. Hudec, M. George, P. Fabre, P. Higgs, S. Bruzaud, A.-M. Delort, B. Eyheraguibel, A.-L. Meistertzheim, J. Jacquin, J. Cheng, N. Callac, C. Odobel, S. Rabouille, J.-F. Ghiglione, Colonization of non-biodegradable and biodegradable plastics by marine microorganisms, *Frontiers in Microbiology*, Vol.9, 2018, 1571.

[32] A. Bellingeri, E. Bergami, G. Grassi, C. Faleri, P. Redondo-Hasselerharm, A.A. Koelmans, I. Corsi, Combined effects of nanoplastics and copper on the freshwater alga Raphidocelis subcapitata, *Aquatic Toxicology*, Vol.210, 2019, pp. 179-187.

[33] R. Radakovits, R.E. Jinkerson, A. Darzins, M.C. Posewitz, Genetic engineering of algae for enhanced biofuel production, *Eukaryotic Cell*, Vo.9, No.4, 2010, pp. 486–501.

[34] S. Jeon, J.-M. Lim, H.-G. Lee, S.-E. Shin, N.K. Kang, Y.-Il Park, H.-M. Oh, W.-J. Jeong, B.-R. Jeong, Y.K. Chang, Current status and perspectives of genome editing technology for microalgae, *Biotechnology for Biofuels*, Vol.10, 2017, 267, pp.1-18.

[35] A. Doshi, S. Pascoe, L. Coglan, T.J. Rainey, Economic and policy issues in the production of algae-based biofuels: a review, *Renewable and Sustainable Energy Reviews*, Vol.64, 2016, pp. 329–337.

[36] J.P. Maity, J. Bundschuh, C.Y. Chen, P. Bhattacharya, Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: present and future perspectives – a mini review, *Energy*, Vol.78, 2014, pp. 104–113.

[37] J. Lü, C. Sheahan, P. Fu, Metabolic engineering of algae for fourth generation biofuels production, *Energy and Environmental Sciences*, Vol.4, 2011, 2451.

[38] A.K. Bajhaiya, J. Ziehe Moreira, J.K. Pittman, Transcriptional engineering of microalgae: prospects for high-value chemicals, *Trends in Biotechnology*, Vol.35, 2017, pp. 95–99.

[39] L. Brennan, P. Owende, Biofuels from microalgae — a review of technologies for production, processing, and extractions of biofuels and co-products, *Renewable and Sustainable Energy Reviews*, Vol.14, 2010, pp. 557–577.

[40] P.D. Patil, V.G. Gude, A. Mannarswamy, S. Deng, P. Cooke, S. Munson-McGee, I. Rhodes, P. Lammers, N. Nirmalakhandan, (2011) Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions, *Bioresource Technology*, Vol.102, No.1, 2011, pp. 118-122.

[41] J. Li, X. Zheng, K. Liu, S. Sun, X. Li, Effect of tetracycline on the growth and nutrient removal capacity of Chlamydomonas reinhardtii in simulated effluent from wastewater treatment plants, *Bioresource Technology*, Vol.218, 2016, pp. 1163-1169.

[42] G. Huang, D. Zhao, C. Lan, B. Wu, X. Li, S. Lou, Y. Zheng, Y. Huang, Z. Hu, B. Jia, Glucose-assisted trophic conversion of Chlamydomonas reinhardtii by expression of glucose transporter GLUT1, Algal Research, Vol.62, 2022, 102626.

[43] P. Xie, C. Chen, C. Zhang, G. Su, N. Ren, S.-H. Ho, Revealing the role of adsorption in ciprofloxacin and sulfadiazine elimination routes in microalgae, *Water Research*, 172, 2020, 115475.

[44] S. Tayari, R. Abedi, A. Rahi, Comparative assessment of engine performance and emissions fuelled with three different biodiesel generations, *Renewable Energy*, Vol.147, No.1, 2020, pp. 1058–1069.

[45] D. Fu, Q. Zhang, Z. Fan, H. Qi, Z. Wang, L. Peng, Aged microplastics polyvinyl chloride interact with copper and cause oxidative stress towards microalgae Chlorella vulgaris, *Aquatic Toxicology*, Vol.216, 2019, 105319.

[46] X. Wu, H. Wu, A. Zhang, K. Sekou, Z. Li, J. Ye, (2022). Influence of polystyrene microplastics on levofloxacin removal by microalgae from freshwater aquaculture wastewater, *Journal of Environmental Management*, Vol.301, 2022, 113865.

[47] R.A. Labatut, L.T. Angenent, N.R. Scott, Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance and stability? *Water Research*, Vol.53, 2014, pp. 249-258.

[48] C.G. Golueke, W.J. Oswald, H.B. Gotaas, Anaerobic digestion of algae, *Applied Microbiology*, Vol.5, No.1, 1957, pp. 47-55.

[49] V.H. Varel, T.H. Chen, A.G. Hashimoto, Thermophilic and mesophilic methane production from anaerobic degradation of the cyanobacterium *Spirulina maxima*, *Resources, Conservation and Recycling*, Vol.1, No.1, 1988, pp. 19-26.

[50] C. Zamalloa, N. Boon, W. Verstraete, Anaerobic digestibility of Scenedesmus obliquus and Phaeodactylum tricornutum under mesophilic and thermophilic conditions, *Applied Energy*, Vol.92, 2012, pp. 733-738.

[51] R. Harun, M. Davidson, M. Doyle, R. Gopiraj, M. Danquah, G. Forde, Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility, *Biomass and Bioenergy*, Vol.35, 2010, pp. 741-747.

[52] T. Suganya, M. Varman, H.H. Masjuki, S. Renganathan, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach, *Renewable and Sustainable Energy Reviews*, Vol.55(C), 2016, pp. 909-941.

[53] L.M. Chng, K.T. Lee, D.J.C. Chan, Synergistic effect of pretreatment and fermentation process on carbohydrate-rich Scenedesmus dimorphus

for bioethanol production, *Energy Conversion and Management*, Vol.141, 2016, pp. 410–419.

[54] R. Singh, A.J. Mattam, P. Jutur, S.S. Yazdani, Synthetic biology in biofuels production. In: Meyers, R.A. (Ed.), Rev. Cell Biol. Mol. Medicine: Encyclopedia of Molecular Cell Biology and Molecular Medicine: Synthetic Biology, 2(1), pp. 144-176, Publisher: Wiley-VCH Verlag GmbH & Co. KGaA, New York, NY, United States, 2016.

[55] D.S. Gorman, R.P. Levine, Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of Chlamydomonas reinhardii, *Proceedings of the National Academy of Sciences USA*, Vol.54, 1965, pp. 1665-1669.

[56] E.H. Harris, *The Chlamydomonas sourcebook: a comprehensive guide to biology and laboratory use*, Academic Press, San Diego, United States, 1989, 780.

[57] E.H. Harris, *The Chlamydomonas Sourcebook*, Academic Press, Oxford, United Kingdom, 2009.

[58] H.W. Nichols, H.C. Bold, Trichosarcina polymorpha gen. et sp. nov, *Journal of Phycology*, Vol.1, No.1, 1965, pp. 34-38.

[59] R.K. Balasubramanian, T.T. Yen Doan, J.P. Obbard, Factors affecting cellular lipid extraction from marine microalgae, *Chemical Engineering Journal*, Vol.215-216, 2013, pp. 929-936.

[60] W.C. Lipps, E.B. Braun-Howland, T.E. Baxter, *Standard Methods for the Examination of Water and Wastewater*, (24th. Edition). W.C. Lipps, E.B., Braun-Howland, T.E. Baxter, (editors), American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), Elevate Your Standards. American Public Health Association 800 I Street, NW Washington DC: 20001-3770, United States, December 1, 2022; ISBN:9780875532998.

[61] A. Torres, F.G. Fermoso, P. Neumann, L. Azocar, D. Jeison, Anaerobic digestion as a tool for resource recovery from a biodiesel production process from microalgae, *Journal of Biobased Materials and Bioenergy*, Vol.9, No.3, 2015, pp. 342-349.

[62] O. García-Depraect, R. Lebrero, S. Rodriguez-Vega, S. Bordel, F. Santos-Beneit, L.J. Martínez-Mendoza, R. Aragão Börner, T. Börner, R. Muñoz, Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: Kinetics, carbon fate and particle size effect, *Bioresource Technology*, 2021, 126265.

[63] M. Qin, C. Chen, B. Song, M. Shen, W. Cao, H. Yang, G. Zeng, J. Gong, A review of biodegradable plastics to biodegradable microplastics: another ecological threat to soil environments? *Journal of Cleaner Production*, Vol.312, 2021, 127816.

[64] P. Sarmah, J. Rout, Role of algae and cyanobacteria in bioremediation: prospects in polyethylene biodegradation, *Advances in Cyanobacterial Biology*, Vol.22, 2020, pp. 333-349.

[65] C. Song, Z. Liu, C. Wang, S. Li, Y. Kitamura, Different interaction performance between microplastics and microalgae: the bio-elimination potential of Chlorella sp. L38 and Phaeodactylum tricornutum MASCC-0025, *Science of the Total Environment*, Vol.723, 2020, 138146.

[66] S.Y. Teng, G.Y. Yew, K. Sukacová, P.L. Show, V. Máša, J.S. Chang, Microalgae with artificial intelligence: A digitalized perspective on genetics, systems and products, *Biotechnology Advances*, Vol.44, 2020, 107631.

[67] S. Dutta, S. Madan, H. Parikh, D. Sundar, An ensemble micro neural network approach for elucidating interactions between zinc finger proteins and their target DNA, *BMC Genomics*, Vol.17, 2016, 1033.

[68] J. Linder, N. Bogard, A.B. Rosenberg, G. Seelig, A Generative Neural Network for Maximizing Fitness and Diversity of Synthetic DNA and Protein Sequences, *Cell Systems*, Vol.11, 2020, pp. 49–62.

[69] A.M. Zenooz, F.Z. Ashtiani, R. Ranjbar, F. Nikbakht, O. Bolouri, Comparison of different artificial neural network architectures in modeling of *Chlorella* sp. flocculation, *Preparative Biochemistry and Biotechnology*, Vol.47, 2017, pp. 570–577.

[70] J. Ruiz-Santaquiteria, G. Bueno, O. Deniz, N. Vallez, G. Cristobal, Semantic versus instance segmentation in microscopic algae detection, *Engineering Applications of Artificial Intelligence*, Vol.87, 2020, 103271.

[71] N.J. Treloar, A.J.H. Fedorec, B. Ingalls, C.P. Barnes, Deep reinforcement learning for the control of microbial co-cultures in bioreactors, *PLoS Computational Biology*, Vol.16, 2020, e1007783.

[72] S.H. Knox, C. Sturtevant, J.H. Matthes, L. Koteen, J. Verfaillie, D. Baldocchi, Agricultural peatland restoration: effects of land-use change on greenhouse gas (CO_2 and CH_4) fluxes in the

Sacramento-San Joaquin Delta, *Global Change Biology*, Vol.21, 2015, pp. 750–765.

[73] C. Sturtevant, B.L. Ruddell, S.H. Knox, J. Verfaillie, J.H. Matthes, P.Y. Oikawa, D. Baldocchi, Identifying scale-emergent, nonlinear, asynchronous processes of wetland methane exchange, *Journal of Geophysical Research: Biogeosciences*, Vol.121, 2016, pp. 188–204.

[74] J.P. Goodrich, W.C. Oechel, B. Gioli, V. Moreaux, P.C. Murphy, G. Burba, D. Zona, Impact of different eddy covariance sensors, site set-up, and maintenance on the annual balance of CO_2 and CH_4 in the harsh Arctic environment, *Agricultural and Forest Meteorology*, Vol.228–229, 2016, pp. 239–251.

[75] A.M. Moffat, D. Papale, M. Reichstein, D.Y. Hollinger, A.D. Richardson, A.G. Barr, C. Beckstein, B.H. Braswell, G. Churkina, A.R. Desai, E. Falge, J.H. Gove, M. Heimann, D. Hui, A.J. Jarvis, J. Kattge, A. Noormets, V.J. Stauch, Comprehensive comparison of gap-filling techniques for eddy covariance net carbon fluxes, *Agricultural and Forest Meteorology*, Vol.147, 2007, pp. 209–232.

[76] D. Papale, M. Reichstein, M. Aubinet, E. Canfora, C. Bernhofer, W. Kutsch, B. Longdoz, S. Rambal, R. Valentini, T. Vesala, D. Yakir, Towards a standardized processing of Net Ecosystem Exchange measured with eddy covariance technique: algorithms and uncertainty estimation, *Biogeosciences*, Vol.3, 2006, 571–583.

Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Prof. Dr. Delia Teresa Sponza and Post-Dr. Rukiye Öztekin took an active role in every stage of the preparation of this article.

The authors equally contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself

This research study was undertaken in the Environmental Microbiology Laboratories at Dokuz Eylül University Engineering Faculty Environmental Engineering Department, İzmir, Turkey. The authors would like to thank this body for providing financial support.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

APPENDIX

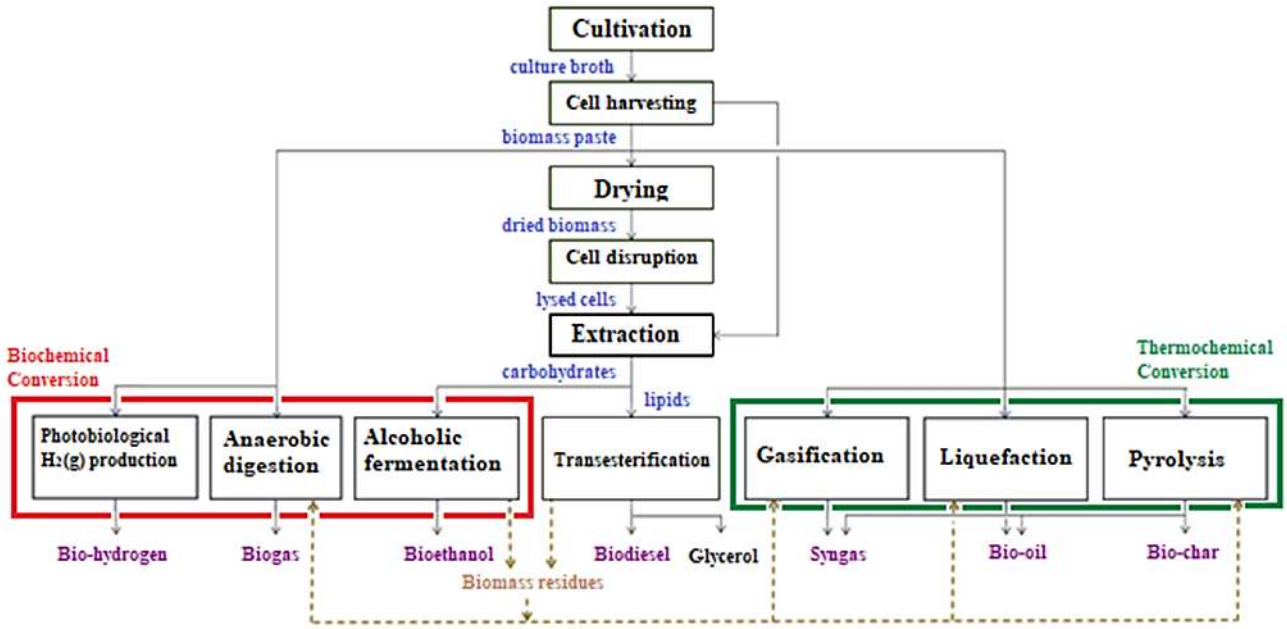

Fig. 1. Biofuel production from microalgae through various transformation processes

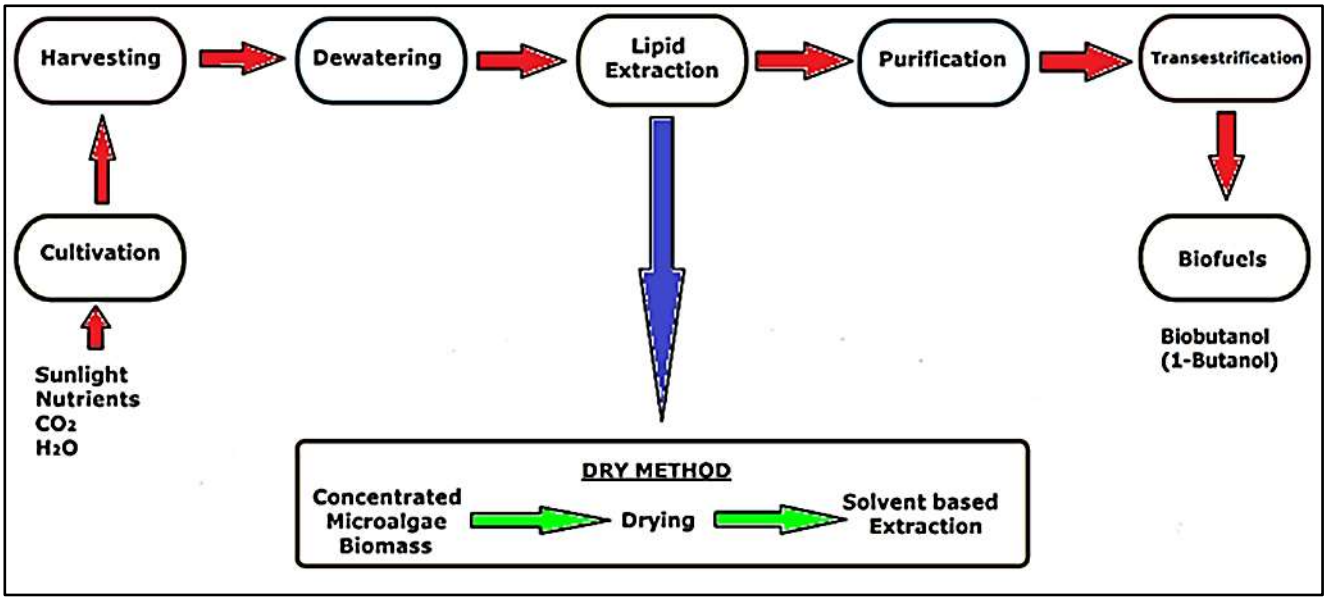

Fig. 2. Schematic diagram of 1-butanol production from MPs with microalgae under aerobic conditions.

Table 1. Results of the elemental analysis of microalgae (*Chlamydomonas reinhardtii* and *Chorella vulgaris*) biomass with oil extracted and dried at 105ºC.

Parameters	**Microalgae Biomass Compositions (%)**
Protein	51.21
Carbohydrates	19.11
Fat	7.23
Moisture	2.47
Na (mg/100 g)	1952
Crude fibre	2.24
Ash	17.64

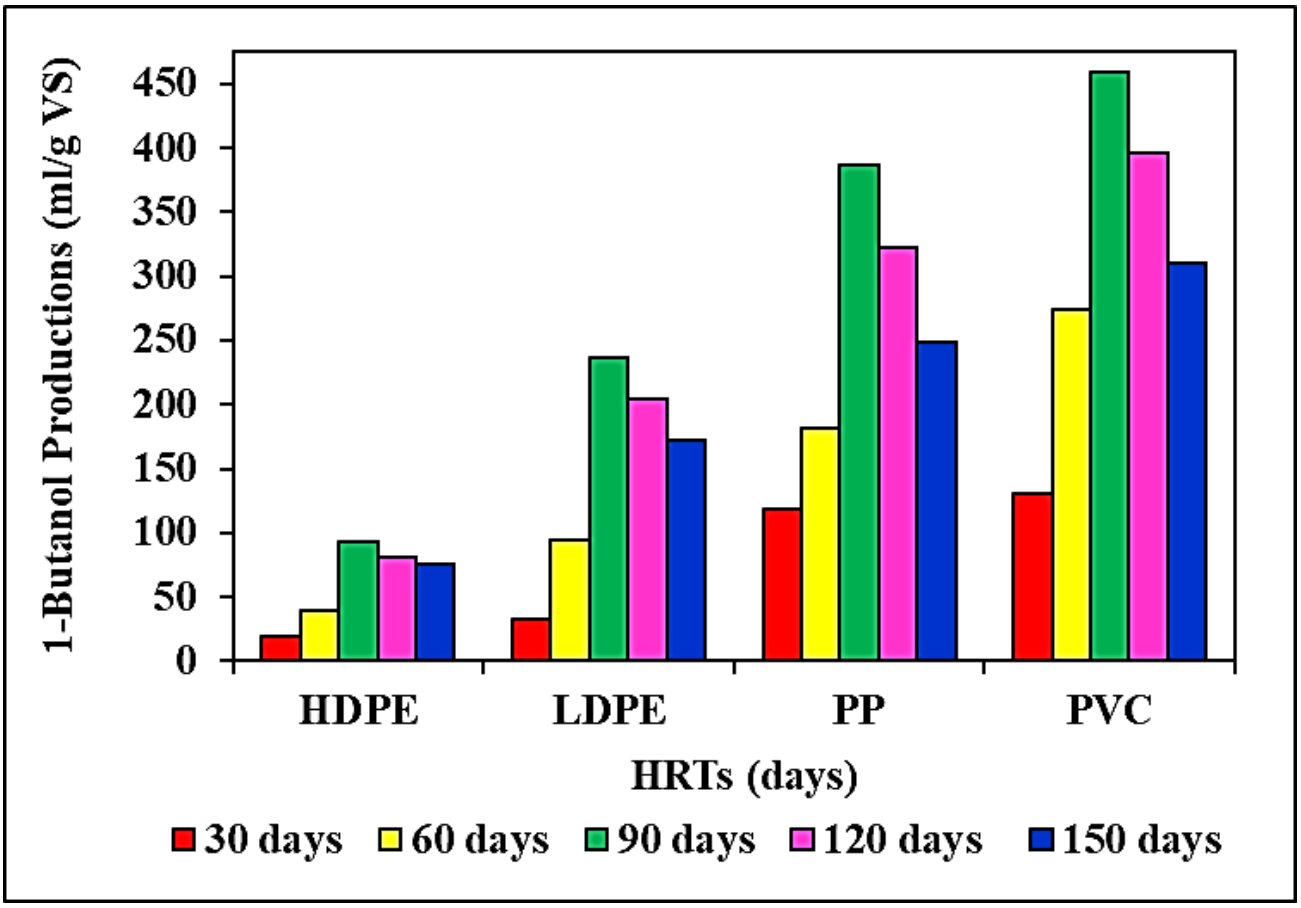

Fig. 3. 1-Butanol productions for HDPE, LDPE, PP and PVC MPs during aerobic conditions after different HRTs, at pH=7.0 and at 35ºC.

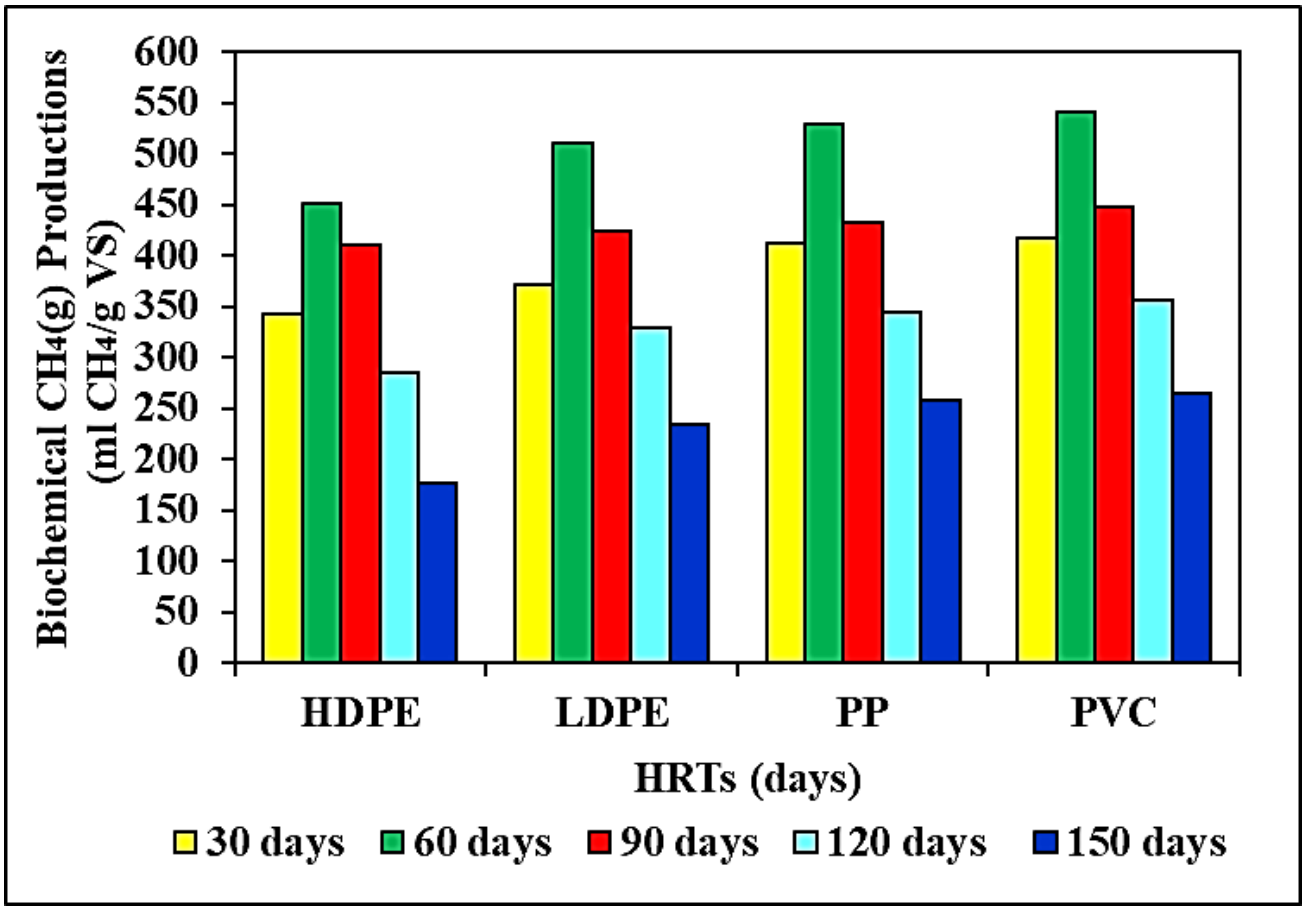

Fig. 4. Biochemical CH_4(g) productions for HDPE, LDPE, PP and PVC MPs in ABR during anaerobic conditions after different HRTs, at pH=7.0 and at 35ºC.

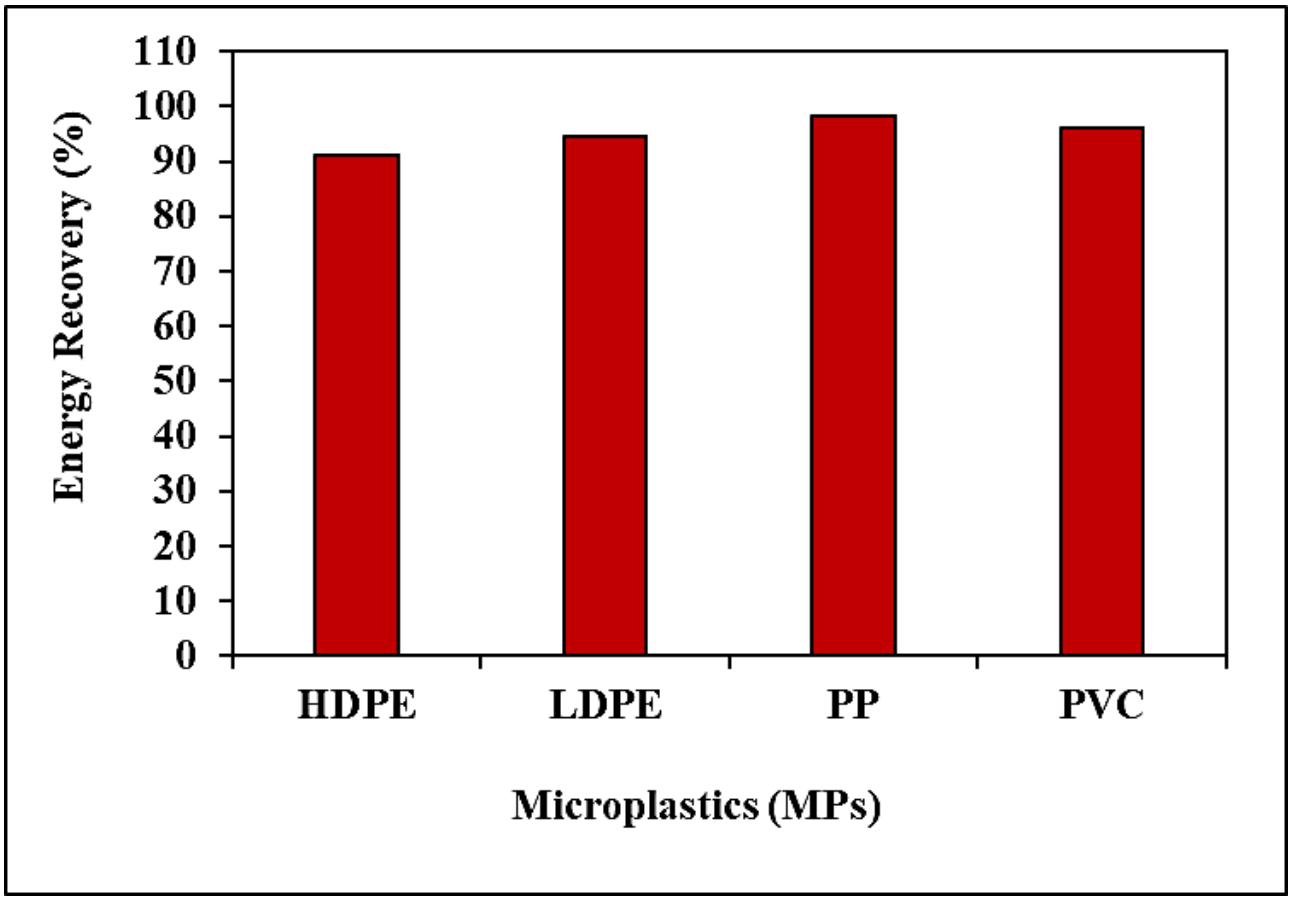

Fig. 5. Energy recovery for HDPE, LDPE, PP and PVC MPs after microalgae biomass experiments, at pH=7.0 and at 35ºC.

Table 2. Results of inhibition test for HDPE, LDPE, PP and PVC MPs after microalgae biomass experiments, at pH=7.0 and at 35ºC.

MPs	1-butanol Productions (ml/g VS)	Biochemical CH_4(g) Productions (ml CH_4/g VS)	Energy Recoveries (%)
HDPE	93	452	91.26
LDPE	236	510	94.52
PP	387	529	98.34
PVC	459	541	96.17

Table 3. The comparative summary of error analysis results with our study and artificial neural networks (ANN) example approach (MAE: mean absolute error, RMSE: root mean square error, BE: bias error, Gap-fill ranges represent the ensemble of budgets derived from bootstrapped datasets using the respective gap-filling method).

Gap-fill method	**CH_4(g) analyser**	**MAE (nmol/m^2.s)**	**RMSE (nmol/m^2.s)**	**BE (nmol/m^2.s)**	**R^2**	**Cumulative flux (g-CH_4/m^2)**	**Gap-fill range**	**Relative gap-fill (%)**
In this study	TGA	9.5	14.1	0.24	0.97	63.0	62.6 – 63.4	2
ANN	TGA	7.8	10.3	0.20	0.99	62.8	62.4 – 63.3	5
In this study	LI-7700	8.7	11.1	0.13	0.96	64.7	64.2 – 72.1	3
ANN	LI-7700	8.4	9.9	0.11	0.99	64.3	64.0 – 64.9	2