COPOLYMERS OF 2,4-DICHLOROPHENYL METHACRYLATE WITH BUTYL METHACRYLATE AND THEIR APPLICATION AS BIOCIDES

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Copolymers of 2,4-dichlorophenyl methacrylate and butyl methacrylate were synthesized with different monomer concentrations using toluene as a solvent and 2,2'-azobisisobutyronitrile (AIBN) as an initiator at 70°C. The copolymers were characterized by IR-spectroscopy. Copolymer compositions were determined by UV-spectroscopy. The reactivity ratios for monomer pairs were determined by Fineman-Ross method. Gel permeation chromatography was employed for determining the average molecular weights and the polydispersity index. The intrinsic viscosities of polymers were also discussed. Thermogravimetric analyses of polymers were carried out in nitrogen atmosphere. The homo- and copolymers were tested for their effect on the growth of various microorganisms.

INTRODUCTION

Contamination by microorganisms is of great concern in several areas, such as medical devices, health-care products, water purification systems, hospital and dental office equipment, food packaging, and food storage. Consequently, biocidal polymers have received much attention in recent years [1−2]. One possible way to avoid the microbial contamination is to develop materials possessing antimicrobial activities. Pleurdean and co-workers [3] reported the synthesis of acrylic esters, useful as biocides by reacting pentachloro phenol, p-chloro-m-cresol, o-phenyl phenol with acryloyl chloride. Cho and

co-workers [4] synthesized 2,4,4′-trichloro-2′-acryloyloxy diphenyl ether and its homo- and copolymers with MMA, styrene and 2-hydroxy ethyl methacrylate and tested bactericidal property against *Staphylococcus aureus* and *Pseudomonas aeruginosa* microorganisms. Nonaka et al. [5] prepared acrylyloxyethyl trialkyl phosphonium chloride based copolymers and they have checked their antibacterial activities against *E. coli* and *S. aureus*. 2,4-Dichlorophenol, a well-known bio cide [6] has been anchored to polymer by copolymerizing 2,4-dichlorophenyl methacrylate (2,4-DMA) with butyl methacrylate (BMA) [7].

The present investigation was carried out to modify acrylics by incorporating an acrylic monomer based on 2,4-dichlorophenyl methacrylate so as to make them useful in pharmaceuticals, pesticides, paints, coatings, inks, adhesives, and cosmetics [8−9]. This article discusses the synthesis and characterization of monomer as well as synthesis of homo- and copolymers using different feed ratios. The copolymer composition was determined by UV-spectroscopy. Molecular weight determination by GPC and thermal analyses of polymers are also included. Homo- and copolymers have been used to screen their antimicrobial activity against microorganisms such as bacteria (*B. subtilis, E. coli, S. citreus*), fungi (*A. niger, S. pulverulentum, T. lignorum*), and yeast (*C. utilis, S. cerevisiae, P. stipitis*).

EXPERIMENTAL

All the chemicals used were of analytical grade reagents. The solvents and monomers were purified by conventional methods [10].

Synthesis of 2,4-Dichlorophenyl Methacrylate [2,4-DMA]

Methacryloyl chloride was prepared by reacting methacrylic acid with benzoyl chloride [11]. The esterification was performed with methacryloyl chloride and 2,4-dichlorophenol (2,4-D). To a three-necked flask, equipped with stirrer, condensor, and thermometer placed in a waterbath, absolute alcohol (400 ml) and NaOH (0.2 mol, 8.0 g) were added and the contents were stirred until all the NaOH dissolved. 2,4-D (0.2 mol, 32.6 g) was added to this. The reaction mixture was kept at 60℃ for 30 min with stirring, cooled to room temperature, and then to 0−5℃ by ice. Freshly prepared methacryloyl chloride (0.21 mol, 20.5 ml) was added dropwise to the cooled reaction mixture and stirred for 90 min. It was then poured into crushed ice-water mixture where a cream colored product separated out. It was filtered, washed thoroughly with cold water, dried at 35℃ in vacuum, and recrystallized from petroleum ether.

The formation of monomer was confirmed by IR and ^{1}H-NMR techniques. IR (cm^{-1}): 2925 (v_{-CH3}); 1743 ($v_{C=O}$), 1642 ($v_{C=C}$), 1333 (v_{C-O}), 1232, and 1160 (v_{C-O-C}), 890 ($-CH$ bending mode of vinyl group), 720 (rocking mode of vinyl group), 670 (v_{C-Cl}), 1590, and 1488 (bands due to phenyl ring).

^{1}H-NMR (ppm) (60 MHz): 2.080 (3H) (Methyl protons), 6.416 (1H) and 5.811 (1H) (Non-equivalent methylene protons), 7.383−7.471 (1H) (Aromatic proton), 7.031−7.236 (2H) (Aromatic proton).

Copolymerization

Homo- and copolymerization were carried out in toluene using AIBN as initiator. Predetermined quantities of 2,4-DMA, BMA, toluene, and AIBN were mixed in round bottom flask equipped with mechanical stirrer and reflux condensor. The reaction mixture was heated at 70℃ for 5 h with constant stirring. After that, it was cooled to room temperature and slowly poured in a large excess of methanol used as a non-solvent with constant stirring. Solid polymers were purified by repeated precipitation by methanol from solution in toluene and finally dried under vacuum.

Characterization

Infrared spectra were recorded with a NICOLET-400D FT-IR spectrophotometer on solid samples in the KBr pellets. Copolymer compositions and reactivity ratios were determined by Shimadzu-160A-recording UV-Visible spectrophotometer. Molecular weights of the polymers were determined in a gel permeation chromatograph (Water 600 E) equipped with a 410-RI detector and calibrated with polystyrene standards. Schott-Gerate viscosity measurement system was used to determine viscosity of polymer samples. Thermogravimetric analysis was performed with Du Pont 951 thermal analyzer at a heating rate of 10℃.min^{-1} in static air atmosphere. Differential thermal analysis was performed with DuPont-9900-differential thermal analyzer at a heating rate of 10℃.min^{-1} in nitrogen atmosphere.

Microbial Screening

The synthesized homo- and copolymers were tested against different microorganisms that are commonly employed for biodegradability tests. Bacterial strains (*Bacillus subtilis, Escheriachi coli,* and *Staphylococcus citreus*), fungi (*Aspergillus niger, Sporotichum pulverulentum,* and *Trichoderma lignorum*) and yeast (*Candida utilis,*

Saccharomyces cerevisiae, and *Pichia stipitis*) were grown in Nutrient broth (N-broth) and Sabouraud dextrose broth medium.

Screening of Acrylic Copolymers for Antibacterial Activity

5% (V/V) inoculum of bacterial culture was used to inoculate 100 ml solution of N-broth (control) and test media (100 ml solution of N-broth + 50 mg polymer sample) and incubated on rotary shaker (200 rpm) at room temperature. 0.5 ml liquid were withdrawn at specified time intervals (24−48 h) from test media. After suitable dilution with DNS reagent (see later) and distilled water, optical density was measured at 660 nm and calculated as optical density per ml (i.e., growth). This method is based on the principle that as the growth proceeds, cell number increases, which leads to an increase in optical density of medium.

Screening of Acrylic Copolymers for Antifungal Activity

Because fungal culture shows filamentous growth, optical method cannot be used to moniter the growth. Gravimetric analysis was carried out to determine dry cell mass. 10% (v/v) inoculum was added to the sterile control medium (without polymer) and test medium (100 ml control + 50 mg polymer sample). Flasks were incubated at room temperature on rotary shaker (200 rpm) for 48 h. Content of the flasks were filtered using cheese cloth and cell pallets were dried to constant weight and supernatant was used to check pH and remaining sugar. Sugar analysis was carried out using DNS reagent [12], which has the following composition (3,5-dinitrosalicylic acid: 10.4 gms, NaOH: 19.8 gms, NaK-tartarate: 306 gms, phenol: 7.6 gms, sodium metabisulphate: 8.3 gms and distilled water: 1416 ml). Suitable diluted supernatant was taken and made to 1.0 ml and to that 1.0 ml DNS was added and boiled for 10 min. After cooling, the final volume was made to 12.0 ml by adding distilled water and optical density was recorded at 540 nm using spectrophotometer.

Screening of Acrylic Copolymers on Yeast

5% (v/v) inoculum of yeast culture was added to the sterile control medium and test medium (100 ml control + 50 mg polymer sample) and the same procedure mentioned in antibacterial activity was followed.

RESULTS AND DISCUSSION

The copolymerization of 2,4-DMA with BMA in toluene solution was studied in a wide composition interval with mole fractions of 2,4-DMA

ranging from 0.2 to 0.8 in the feed. The reaction time was selected to give conversion less than 10 wt% in order to satisfy the differential copolymerization equation.

The IR-spectra confirm the structure of copolymers in all aspects. The two bands at 2980 and 2850 cm^{-1} are due to C-H stretching mode of alkyl group. The C-H stretching vibration of the aromatic ring is assigned to a peak around 3010 cm^{-1}. The absorptions at 1380 and 1467 cm^{-1} may be traced to the in-plane bending vibrations of methyl and methylene groups. The compound has the characteristic strong absorption due to C=O stretching of ester group. In the copolymers two C=O stretching vibrations are observed, one at 1770 cm^{-1} and the other at 1750 cm^{-1}. From a comparison of the spectra of poly(2,4-DMA) and poly(BMA) with those of the copolymers, the 1770 cm^{-1} peak may be assigned to the C=O stretching in the 2,4-DMA moiety of the copolymers and 1750 cm^{-1} may be attributed to the same in BMA moiety. The medium strong absorption in the range 1270–1240 cm^{-1} may have contribution from C-O stretching vibrations of ester group. The band at 740 cm^{-1} is due to the $-CH_2$ rocking mode of n-butyl chain. The prominent sharp band at 670 cm^{-1} is attributed to C–C1 stretching [13]. It is gratifying -to note that as the 2,4-DMA content in the copolymer increases the intensity of 1770 cm^{-1} band also increases, whereas the intensity of the 1750 cm^{-1} peak decreases as the BMA content decreases. The absence of 1640 cm^{-1} peak in the polymer is indicative of the participation of vinyl group in the polymerization.

Copolymer Compositions and Reactivity Ratios

The synthesis of copolymers is represented in Figure 1. The average composition of each copolymer sample was determined from the corresponding UV-spectrum. The assignment of the absorption in the
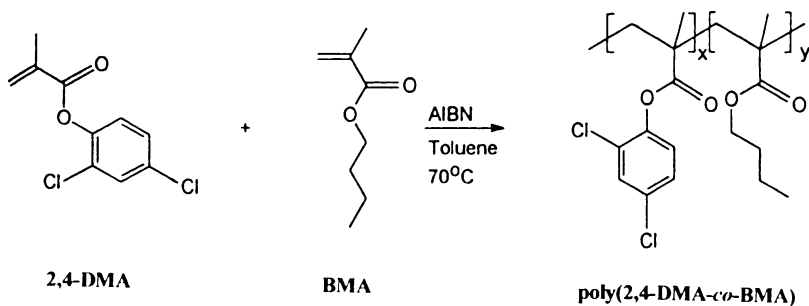

FIGURE 1 Reaction scheme of poly (2,4-DMA-co-BMA).

TABLE 1 Copolymer Compositions Data and Reactivity Ratios of Copolymers of 2,4-DMA and BMA

Sample code no.	Monomer feed compositions		Conversion (%)	Compositions of 2,4-DMA in copolymer [m₁]	Reactivity ratio	
	2,4-DMA [M₁] mol.	BMA [M₂] mol.			r_1	r_2
B-1	1.00	—	—	—		
B-2	0.20	0.80	8.54	0.185		
B-3	0.40	0.60	9.38	0.368		
B-4	0.50	0.50	9.85	0.475	0.75	0.97
B-5	0.60	0.40	8.72	0.583		
B-6	0.80	0.20	9.25	0.766		
B-7	—	1.00	—	—		

UV-spectrum allows for the accurate evaluation of the content of each kind of monomeric unit incorporated into the copolymer chains. From monomer feed ratios and copolymer compositions, the reactivity ratios of 2,4-DMA and BMA were determined by Fineman-Ross method [14] and are presented in Table 1. The values of reactivity ratios for 2,4-DMA(r_1) and BMA (r_2) from F-R plot are 0.75 and 0.97, respectively. When the r_1 and r_2 values are less than 1, the system gives rise to azeotropic polymerization at a particular composition of the monomers, which is calculated using the equation:

$$N_1 = \frac{(1 - r_2)}{(2 - r_1 - r_2)} = 0.107$$

When the mole fraction of the monomer 2,4-DMA in the feed is 0.107, the copolymer formed will have the same composition as that of the feed. When the mole fraction of the feed is less than 0.107 with respect to 2,4-DMA, the copolymer is relatively richer in this monomer unit. When the mole fraction of the monomer 2,4-DMA in the feed is above 0.107, the copolymer is relatively richer in BMA monomeric unit.

Molecular Weights and Viscosity Measurements

The number- and weight-average molecular weight of poly(2,4-DMA), poly(BMA), and five samples of copolymer were obtained from gel permeation chromatography. The polydispersity index of homo- and copolymers varies in the range of 2.48 to 2.93. The intrinsic viscosities [η] were obtained by extrapolating $\eta_{sp}.c^{-1}$ to zero concentration. The

TABLE 2 Average Molecular Weights by GPC and Viscosity Data for the Copolymers of 2,4-DMA with BMA

Sample code no.	$\overline{Mn}$	$\overline{Mw}$	Polydispersity index	Intrinsic Viscosity η (dl.g^{-1})
B-1	7974	19800	2.48	0.028
B-2	7415	21731	2.93	0.026
B-3	6943	20147	2.90	0.023
B-4	6309	17298	2.74	0.021
B-5	6138	16923	2.76	0.020
B-6	5810	16847	2.90	0.018
B-7	7093	20427	2.88	0.025

data of molecular weights and viscosities are presented in Table 2. The values of average molecular weights and polydispersity obtained by GPC indicate the number average molecular weight ranges from 5810 to 7974 and weight average molecular weight varies from 16847 to 21731. These results indicate that with increase in the 2,4-DMA content in the copolymers, average molecular weights and viscosity decrease to a considerable extent whereas polydispersity index changes randomly.

Thermal Analyses

Thermogravimetric Analysis (TGA)

TGA of homo- and copolymers are presented in Table 3. The data clearly indicates that all polymers undergo single step decomposition. Activation energy (E_A) and Integral procedural decomposition

TABLE 3 TGA Data for Homo- and Copolymers of 2,4-DMA and BMA

Sample code no.	% weight loss at various temperature (℃)						Decomposition temperature range (℃)	T_{max}[a] (℃)	T_{50}[b] (℃)	IPDT[c] (℃)	Activation Energy[d] (E_A) (KJ. mol^{-1})
	250	300	350	400	450	500					
B-1	0.0	0.0	7.2	50.1	89.9	100.0	330−495	354	350	358	138
B-2	1.7	5.0	35.7	86.0	93.8	99.9	225−420	359	360	377	126
B-3	1.9	6.9	45.6	90.1	97.8	99.9	225−460	360	358	373	133
B-4	2.0	7.9	54.6	95.2	99.7	—	220−450	345	344	359	140
B-5	3.2	14.3	58.4	92.2	99.9	—	220−435	343	342	356	138
B-6	2.9	19.0	59.5	93.8	99.8	—	219−426	341	339	351	134
B-7	15.5	74.0	86.0	98.0	99.0	100.0	225−400	275	275	285	117

[a]Temperature for maximum rate of decomposition; [b]temperature for 50% weight loss; [c]integral procedural decomposition temperature; [d]by Broido's method.

temperature (IPDT) were determined by Broido's [15] and Doyle's method [16]. Thermal studies show that the polymers undergo decomposition in the range of 219−495℃.

Differential Thermal Analysis (DTA):

DTA of homo- and copolymers are presented in Table 4. The activation energy for thermal degradation and reaction order were determined by Reich's methods [17]. The activation energy of polymers for thermal degradation ranges from 118−144 K.J. mol^{-1}.

Thermal data suggested the homo- and copolymers follow the single step degradation and possess moderate thermal stability. It is also observed that incorporation of 2,4-DMA in certain polymers resulted in slight increase in thermal stability with respect to their individual homopolymers.

Microbial Screening

Microbial screening of poly(2,4-DMA), poly(BMA), and poly(2,4-DMA-*co*-BMA) on bacteria, fungi, and yeast are shown in Figures 2−4, respectively. In the case of poly(2,4-DMA), bacteria shows 20% growth whereas it is 18% for fungi and yeast. The copolymers exhibit 22−40% bacterial growth, whereas in the case of fungi and yeast, the growth varies from 27−45% after 48 h.

All copolymer systems impart almost similar antimicrobial properties against bacteria, fungi, and yeast. It is observed that polymers prepared using 2,4-DMA show strong growth inhibition effect toward microorganisms tested. As the 2,4-DMA content increases in copolymers the growth of microorganisms decreases accordingly.

TABLE 4 DTA Data for Homo -and Copolymers of 2,4-DMA and BMA

Sample code no.	T_1[a] (℃)	T_2[b] (℃)	T_3[c] (℃)	Activation energy[d] (E_A) (KJ. mol^{-1})	Reaction order
B-1	338	494	402	140	1
B-2	285	459	403	131	1
B-3	293	472	407	140	1
B-4	300	487	411	144	1
B-5	322	496	412	135	1
B-6	344	512	416	130	1
B-7	255	428	350	118	1

[a]Starting temperature of DTA trace; [b]ending temperature of DTA trace; [c]peak maxima temperature of DTA trace; [d] activation energy by Reich's method.
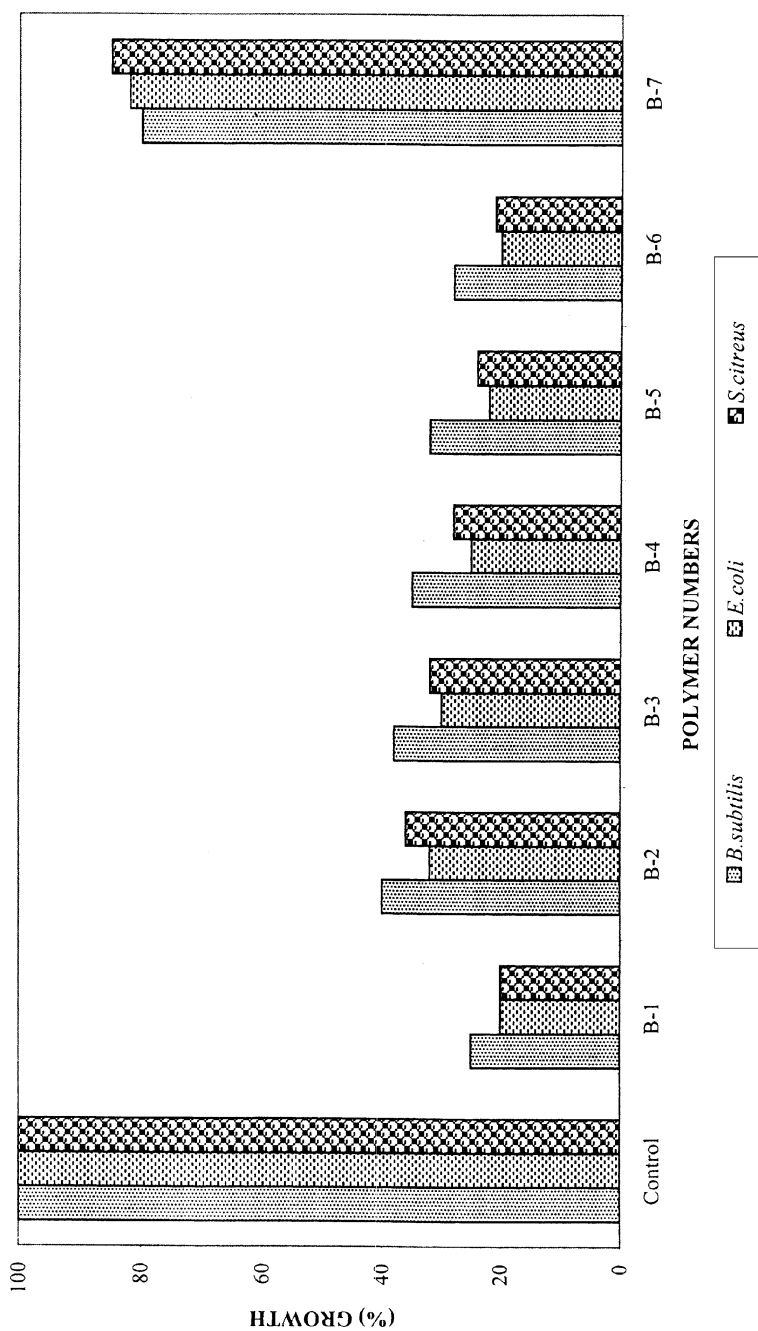
FIGURE 2 Effect of homo- and copolymers on the percentage growth of bacteria.
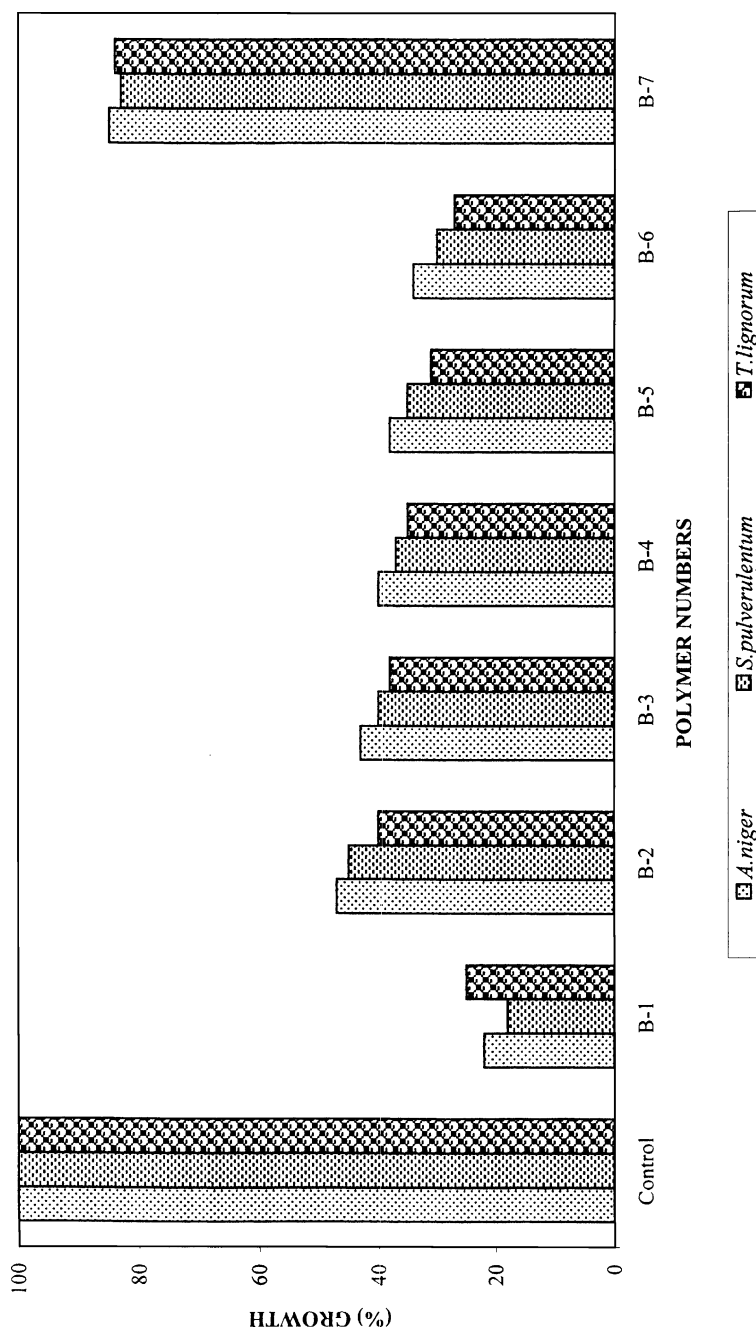
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FIGURE 3 Effect of homo- and copolymers on the percentage growth of fungi.
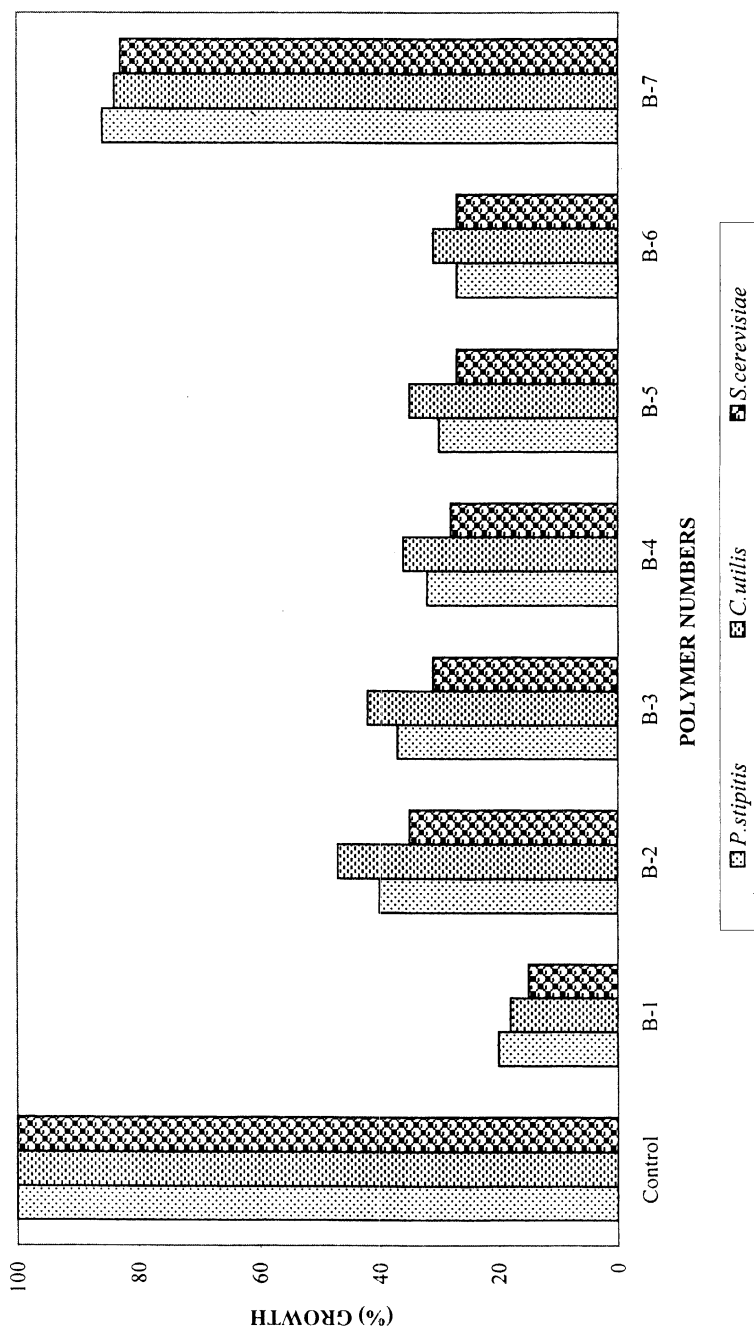
FIGURE 4 Effect of homo- and copolymers on the percentage growth of yeast.

CONCLUSION

A microbial screening on the copolymers of 2,4-DMA with BMA was carried out. It was found that acrylic copolymers containing 2,4-dichlorophenyl methacrylate could be excellent inhibitors against growth of microorganisms. They may find use as polymeric biocide.

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